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HOST GENETICS AND PHENOTYPE ASSOCIATIONS WITHIN THE BOVINE  
RUMEN MICROBIOME

by

Waseem Abbas

A DISSERTATION

Presented to the Faculty of

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Major: Animal Science

(Ruminant Nutrition)

Under the Supervision of Professor Samodha C. Fernando

Lincoln, Nebraska

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# HOST GENETICS AND PHENOTYPE ASSOCIATIONS WITHIN THE BOVINE

## RUMEN MICROBIOME

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University of Nebraska, 2021

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Rumen microbes have a symbiotic relationship with the host where the host provides a suitable environment and microbial fermentation fulfill nearly 70% of the energy needs of the host. Rumen microbes play a central role in host performance and health. To date, most rumen microbiome studies have focused on the influence of diet on microbial species composition. In addition to dietary factors, studies have suggested that host genetic markers could influence the colonization of gastrointestinal microbiota. However, our understanding of the host-microbiome interaction and how host phenotype is influenced by the rumen microbiome is limited. This dissertation demonstrates: (1) host genetic markers select for bacterial species in the rumen. The top associations (1-Mb windows) were located on 7 chromosomes. The annotated genes associated with identified genomic regions suggest the associations observed are directed toward selective absorption of volatile fatty acids from the rumen to increase energy availability to the host, (2) host epithelial gene expression and epimural bacterial community changes in liver abscessed beef cattle. In addition, some epimural bacterial species showed a strong correlation with host epithelial gene expression, (3) Highly marbled beef cattle have different bacterial species than low marbling cattle. The understanding of the host-microbiome interaction and host phenotype association will help the beef researchers and

producers to develop new strategies to raise healthy animals and produce good quality meat.



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## CHAPTER 1

### THE FACTORS AFFECTING THE RUMEN MICROBIOME COMPOSITION AND HOST PHENOTYPE

#### **Overview**

Ruminants have a symbiotic relationship with its rumen microbes that are composed of a wide range of microorganisms belonging to various kingdoms including bacteria, archaea, protozoa, fungi, and viruses. These microbes have different fibrolytic, proteolytic, and lipolytic functions to help breakdown complex carbohydrates present in low quality forages into volatile fatty acids (VFAs) and synthesize microbial crude protein (MCP), which are used by the host to meet their energy and protein needs (Weston, R.H. and Hogan, J.P, 1968; Kempton et al., 1977; Seymour et al., 2005).

The Diet of the animal is one of the main factors that influence the rumen microbial community structure (Tajima et al., 2001; Belanche et al., 2012; Alzahhal et al., 2017).

Dietary changes such as different roughages to concentrate ratio could change the abundance of fibrolytic or proteolytic bacteria in the rumen (Vlaming et al., 2008).

Different dietary manipulation strategies have been used to increase cattle production by improving feed efficiency and lowering methane production but there is always an animal-to-animal variation (Woodward et al., 2001; Vlaming et al., 2008) even on the same diet and under the same environmental conditions (Shi et al., 2014; Henderson et al., 2015; Roehe et al., 2016).

The host-related factors that affect the rumen microbial community could be categorized into two groups: 1) genetic factors, 2) non-genetic factors. Genetic factors explained by host genetics can be described as quantitative trait loci (QTL) or single nucleotide

polymorphism (SNP) associated with microbes (Benson et al., 2010; Goodrich et al., 2016). The host genetics in ruminants also affect the rumen size, salivary secretions, and VFAs absorption. Such factors could affect rumen microbial community structure by changing feed intake, passage rate, and rumen pH (Church, D. C. 1988). In addition, non-genetic factors like age (pre-weaned vs weaned vs adult) and the physiological status of the host can also affect the rumen microbiome.

Researchers have investigated the host specificity by transferring > 95% of rumen content from one dairy cow to another and noticed the bacterial community change was not long-term and host bacterial community composition was returned to its original pre-exchange composition within 14 - 61 days (Weimer et al., 2010b). Similarly, Dairy cattle fed the same diet that have similar milk yield and composition have shown differences in the bacterial community (Weimer et al., 2010a; Welkie et al., 2010). These studies were an early indication that factors other than diet could select for microbial populations within ruminant hosts. In recent years, studies have reported the influence of host genetics on gastrointestinal tract (GIT) microbial communities in ruminants (Roche et al., 2016; Difford et al., 2018; Zhang et al., 2020; Li et al., 2019; Wallace et al., 2019; Saborío-Montero et al., 2020; Sasson et al., 2017; Weimer et al., 2010b; Abbas et al., 2020a), swine (Bergamaschi et al., 2020; Chen et al., 2018) and in humans (Poole et al., 2019; Kurilshikov et al., 2021; Turpin et al., 2016; Qin et al., 2020; Hughes et al., 2020; Goodrich et al., 2014).

The efficiency of beef production has been improved tremendously over the last 50 years. For example, the comparison of beef production from 1977 to 2007 showed that the same amount of beef is produced with 69.9% of animals which indicated an overall



improvement in beef production (Capper 2011). The continued increase in the human population will increase the future beef requirement. Improving feed efficiency of beef cattle will help the beef industry to meet the growing demand while lowering the feeding cost and reducing its impact on environmental sustainability through less methane emission. Understanding the host-microbiome interaction in relation to feed efficiency will provide more genetic markers for animal breeders to select more efficient animals. In this review, I will provide an overview about rumen microbiota and the factors influencing the composition of the rumen microbiome.

### **Rumen Microbiota**

Ruminants belong to class Mammalia and suborder Ruminantia (Vaughan et al., 2013). Ruminant's stomach is divided into four chambers -named rumen, reticulum, omasum, and abomasum (Vaughan et al., 2013). The first three compartments described above do not have any glandular mucus membrane while the abomasum is lined with glandular membrane and functions like a monogastric stomach (Vaughan et al., 2013). Rumen is often described as a “black box” because of its complex nature and limited information about the microbial structure. Rumen microbiota is often described as a metabolic organ with trillions of microbes and a metabolic gene pool which is significantly higher than its host metabolic gene content. Ruminants cannot produce necessary enzymes involved in breakdown of the structural carbohydrates or utilize the non-protein nitrogen (NPN) (Russell, J.B., 2002). However, the microbial genes in the rumen can produce enzymes, such as cellulases, hemicellulases, xylanase, and pectinase, to break down plant cell wall into volatile fatty acids (acetate, butyrate, propionate) which are used by host as energy source (Choudhury et al., 2015). Rumen microbes can degrade the urea into CO<sub>2</sub> and

NH<sub>3</sub> with the enzyme urease. Rumen microbes can utilize NH<sub>3</sub> as nitrogen source and make microbial protein which is digested and used by host (Choudhury et al., 2015).

Rumen is strictly anaerobic environment and there is continuous fermentation and flow of the material (Choudhury et al., 2015). Rumen has 10-13 % dry matter content, temperature ranges between 38-41°C and pH can fluctuate between 5.5 to 6.9 (average 6.4) (Hungate 1966). The rumen microbes include bacteria, archaea, protozoa, fungi, and viruses.

### *Bacteria*

Bacteria are the most abundant microbes in the rumen. Their numbers range from 10<sup>10</sup>-10<sup>11</sup> cells/ml of rumen fluid (McSweeney and Mackie, 2012). Diet is the major factor that affects the bacterial community structure in the rumen (Castillo-González et al., 2014).

Culture-based approaches have revealed that high forage diets favor gram-negative bacteria and high grain diets increase gram-positive bacteria (Hungate, R.E., 2013). Only a small percentage of rumen bacteria can be cultured in the lab with standard methods (Krause et 2013) limiting the ability to fully understand the rumen bacterial population and its role in rumen function. The development of non-culture based methods and sequencing techniques (16S, metagenome, and transcriptome, etc.) have made it possible to identify and characterize a lot more rumen bacteria (Chaucheyras-Durand and Ossa, 2014). The data collected using these techniques have shown that rumen bacteria are dominated by *Prevotella*, *Butyrivibrio*, and *Ruminococcus* (Henderson et al., 2015).

Ruminants could not digest the cellulose and they depend on bacterial species, such as *Fibrobacter succinogenes*, and *Ruminococcus albus*, to degrade cellulose into acetate, butyrate, propionate, and CO<sub>2</sub> (Koike, and Kobayashi, 2009). The change in the diet

from forage to high grains affects the rumen bacteria composition. Grain-based diets are rich in starch which is a substrate for amylolytic bacteria, like *Streptococcus bovis*, and those bacteria produce lactic acid which increases the abundance of *Lactobacillus* species (Chen, and Oba, 2012). Rumen also inhabit pectin degrading bacteria, such as *Lachnospira multiparus*, *Prevotella ruminicola*, and *Butyrivibrio fibrisolvens*, to break down the pectin and produce oligogalacturonides and acetate (Dušková, and Marounek, 2001).

### *Archaea*

Archaea are anaerobic methane-producing microbes present in the rumen (Hook et al., 2010). Their numbers range from  $10^6$ -  $10^8$  /ml of rumen fluid which accounts for <4% of the rumen microbial community (Lin et al., 1997). Archaea uses  $H_2$  and  $CO_2$ , which is an end product of bacterial fermentation, to produce  $CH_4$  (Morgavi et al., 2010).

*Euryarchaeota* and *Crenarchaeota* are two kingdoms under the archaea domain found in the rumen (Cersosimo and Wright. 2015). The Kingdom *Euryarchaeota* has methane-producing methanogens and salt-loving extreme halophiles (Cersosimo and Wright. 2015). The members of the Kingdom *Crenarchaeota* can survive under extreme temperatures ( $>80^\circ C$ ) (Jarrell et al., 1999). In ruminants, the majority of known methanogenic archaea belong to genera *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium* and *Methanothermobacter* (Cersosimo and Wright. 2015). The members of the genus *Methanobrevibacter* produce  $CH_4$  by reducing  $CO_2$  while species belonging to genus *Methanobrevibacter*, such as *M. Stadtmanae* reduce methanol into methane (Hook et al., 2010).

Methanogens depend on other microbes in the rumen for hydrogen transfer (Cersosimo and Wright, 2015). Studies have shown that methanogens have an association with protozoa, such as *Ophryoscolex*, *Epidinium*, *Entodinium*, and *Polyplastron* (Sharp et al., 1998), anaerobic fungi (*Neocallimastix frontalis*) (Bauchop, and Mountfort, 1981), and bacterial species *R. flavefaciens* (Tanner and wolfe, 1988). The methane is considered as energy loss which accounts for 2-12% of gross energy intake (Johnson, and Johnson, 1995). Reducing methane production can improve the feed efficiency (Hook et al., 2010). Small rumen size and higher passage rate decrease the methane production in sheep (Goopy et al., 2014). Johnson and Johnson, (1995) reported that bromochloromethane can inhibit methane production by targeting the synthesis of methyl coenzyme A reductase (McrA), which is a critical enzyme for methane production. Methane production is not desirable due to energy loss and its environmental implications. A low level of H<sub>2</sub> is necessary for continuous fermentation. Methanogens maintain the low level of H<sub>2</sub> in the rumen which is a necessary thermodynamic state for H<sub>2</sub> producing (syntrophic bacteria) bacteria to degrade alcohols and fatty acids into acetate. (Conrad, R., 1999).

### *Protozoa*

Protozoa are either ciliates or flagellates. The ciliates have hair-like structures called cilia, and flagellate has an appendage-like structure called flagella. Ciliates and flagellates have different nucleus structure (Lee and Kugrens, 1992). Ciliates were discovered by Gruby and Delafond, (1843). The ciliates found in wild and domesticated ruminants are also referred to as rumen ciliates. Their length and width ranges are 15-250um and 10-200 um, respectively (Dehority 2003). The abundance of rumen ciliates in cattle and sheep

ranges between  $10^4$ - $10^6$  cells/ml of rumen fluid (Hungate, 1966), and Dehority, 1994 reported a higher concentration of ciliates ( $10^7$  cells/ml in rumen fluid) in wild ruminants. Rumen protozoa account for nearly 50% of the biomass in the rumen (Hungate, 1966). For colonization of the protozoa in the rumen, direct or indirect animal to animal contact is necessary (Fonty et al., 1988). The removal of young calves after birth results in defaunation of the rumen (Fonty et al., 1988), and protozoa start colonizing the rumen once animal to animal contact is restored (Dehority 1993). Protozoa help the ruminants to digest the fiber (Williams and Coleman 1997) and to control the rate of readily fermentable carbohydrate fermentation.

*Isotricha prostoma* is one of the most prevalent species present in almost all ruminants and pseudo ruminants (Wright AD. G, 2015). When ruminants are fed a high grain diet with lots of soluble carbohydrates two rumen ciliates *Isotricha* and *Dasytricha* are important for soluble carbohydrates utilization and the rate of fermentation (Wright AD.G, 2015). *Entodiniomorphid* protozoa can engulf starch granules and slow down starch digestion (Wright AD.G, 2015). The major rumen ciliates families include *Spirodiniidae*, *Ophryoscolecidae*, *Telamodiniidae*, *Amylovoracidae*, *Blepharocorythidae*, *Macropodiniidae*, *polydiniellidae*, *Rhinozetidae*, *Buetschliidae*, *Cycloposthiidae*, *Ditoxidae*, *Troglodytellidae*, and *polycostidae*. The *Ophryoscolecidae* is the largest rumen ciliate family most common genera in this family are *Diplodinium*, *Epidinium*, *Metadinium*, *Elytroplastron*, *Eudiplodinium*, *Polyplastron*, *Ophryoscolex*, *Entodinium*, *Enoploplastron*, *Eudiplodinium*, and *Ostracodinium*. (Wright AD.G, 2015). The rumen ciliates need to be further investigated and their relationship with bacteria and methanogens may provide opportunities for rumen manipulation

through introduction of specific protozoa species. Most protozoa in the rumen are ciliates and they are involved in bacterial predation. Protozoa along with bacteria also break down the polysaccharides. They engulf feed particles and bacteria. The protozoa fermentation produces acetate, butyrate and  $H_2$  (Choudhury et al., 2015). Methanogens attach to the protozoa surface for  $H_2$  and reduce the  $CO_2$  into  $CH_4$ . (Choudhury et al., 2015)

### *Fungi*

Rumen fungi were discovered by Colin Orpin in 1975 (Fliegerova et al., 2015). They are referred to as gut fungi because they are found in rumen and the hindgut of other large herbivores like horses, elephants, and rhinoceros (Fliegerova et al., 2015). Rumen fungi account for 5 to 20% of the microbial mass (Rezaeian et al., 2004). Fungi have the unique ability to break the plant cell wall and increase the surface area for bacterial fermentation (Fliegerova et al., 2015). Unlike other rumen microbes, fungi have motile (zoospore) and non-motile (or vegetative) stages. The Zoospores have flagella and early researchers mistook them as protozoa, but later Orpin classified them as fungi (Orpin 1977a). The moving zoospores attach to plant roughages and encyst, then germinate to make thallus which has two parts rhizoids and sporangium (Orpin and Joblin 1997). Rhizoidal of the thallus penetrate the plant surface and it can be either branched (*Orpinomyces*, *Piromyces*, *Anaeromyces*, and *Neocallimastix*) or spherical (*Cyllamyces* and *Caecomyces*) while sporangia, on the plant surface, have endogenous or exogenous zoosporangial development (Barr et al., 1989; Ho et al., 1993a). The complex life cycle of gut fungi makes it difficult to accurately quantify the fungal population. Obispo and Dehority (1992) reported  $10^3$ -  $10^5$  zoospores/ml of rumen fluid. The sequencing of

internal transcribed spacer 1 (ITS1) showed that cow gut fungi (manure) were dominated by *Cyellamyces/Caecomyces* (67%) and *Piromyces* (24%) (Fliegerova et al., 2015).

Sirohi et al., 2013 reported *Orpinomyces* were dominant in the rumen while Liggerstoffer et al., 2010 reported *Necallimastix* and *Piromyces* were the least abundant in the rumen.

The comparison of gut fungi between different ruminant groups showed that cattle fecal samples have a high abundance of *Caecomyces* (60%), sheep fecal samples have a higher abundance of *Neocallimastix* (44%), and goat fecal samples have a high abundance of *Anaeromyces* (48%) (Fliegerova et al., 2015). Fungi produce a wide range of enzymes, such as esterase, xylanase, glucanase, cellulase, and manage to degrade plant material in the rumen (Fliegerova et al., 2015). The members of the genera *Neocallimastix*, and *Piromyces* are better at degrading the cellulose and hemicellulose (Paul et al., 2010) as compared to the members of the genus *Caecomyces* (Nielsen et al., 2002) in the rumen.

### *Viruses*

Viruses are the least studied microbes in the rumen. Most of the viruses isolated from rumen are bacteriophages (Gilbert and Klieve, 2015). Adams et al., 1966 isolated the first rumen phage from the rumen. The invention of the high-resolution electron microscope helped researchers to investigate viral particles in aquatic, soil, and gut ecosystems (Barnet 1972; Flewett et al., 1974; Torrella and Morita 1979). These studies showed the presence of a large number of viral particles along with other microbial populations. The microscopic examination of rumen fluid showed the presence of a large number of tailed viral particles and a majority of those viruses belong to order *Caudovirales* (Hoogenrad et al., 1967; Paynter et al., 1969; Klieve and Bauchop 1988). Ritchie et al., 1970 reported 40 different phages belonging to families *Myoviridae*,

*Podoviridae*, and *Siphoviridae*. Phages are known to infect the bacteria. Interestingly Ritchie et al., 1970 reported intracellular phages in different bacteria. The electron microscopic estimation reported the concentration of viral particles to range from  $10^9$  viral particles/ml of rumen fluid (Ritchie et al., 1970), and Klieve and Bauchop, 1988 reported  $10^7 - 10^8$  particles/ml of rumen fluid. Rumen viral phages can infect a large number of rumen microbial species. For example, *Podoviridae*, *Inoviridae*, and *Siphoviridae* were isolated from *R. albus* (Klieve et al., 2004), *Myoviridae* and *Siphoviridae* were isolated from *Selenomonas ruminantium*, *Caudovirales*, *Siphoviridae*, were isolated from *Streptococcus bovis* (Klieve et al., 1999), and *Myoviridae* were isolated from *Fusobacterium necrophorum* (Tamada et al., 1985) and *Lactobacillus plantarum* (Nemcova et al., 1993). Viruses can affect the microbial community function through horizontal gene transfer, cell lysis, active lysogeny, and metabolic programming (Suttle, C.A, 2007; Rodriguez-Valera et al., 2009; Hurwitz et al., 2013; Feiner et al., 2015). Viruses can acquire auxiliary metabolic genes (AMGs) from the host for fast self-replication (Thompson et al., 2011). Anderson et al., 2017 reported that rumen bacterial and viral communities can explain the variation in the total digestible nutrient (TDN) in steers. They reported 267 viral communities responsible for driving the rumen viral community. Interestingly, they also identified 14 core viral populations. They identified the AMGs that have a role in glycosidic hydrolases, and in the pentose phosphate pathway to increase energy during viral replication. Rumen viruses potentially have a much larger role in the rumen than already known and more in-depth studies are needed to fully understand their interaction with other microbial communities in the rumen.



## **Factors that shape the rumen microbial community**

Competition for a substrate is considered as one of the main forces that shape microbial community structure in any environment (Foster and Bell, 2012). The availability of substrate or metabolites produced could change the ecological niche and that niche modification can result in a change in the community composition. One example of niche modification was reported by Jamie et al., (2013), where they reported rumen microbiome was changed in the newborn calves after two days (day old vs 3 days old). Those calves were on the same diet and have almost identical physiological conditions. The change in the microbial community was from aerobic or facultative microbes to strictly anaerobic microbes. These changes suggest that change in the niche from aerobic to anaerobic environment caused the major community composition shift in 2 days.

### *The effect of diet on rumen microbiota*

Many factors influence the rumen microbiome. Diet is considered as one of the main factors that affect the rumen microbial community structure (Henderson et al., 2015). The dietary changes (hay vs grains) influence the Firmicutes to Bacteroidetes ratio in cattle (Fernando et al., 2010). High grain diet reduces the abundance of Fibrobacters while it increases the abundance of Prevotella (Fernando et al., 2010), *Streptococcus bovis* (Tajima et al., 2000), and *Lactobacillus* species (Nagaraja and Titgemeyer, 2007). Weng et al., 2013 reported that the type of roughages fed to dairy cows affects the thickness and width of stratum corneum, stratum granulosum, stratum basale, and stratum spinosum of rumen papillae. Similarly, high grain diet has radially available carbohydrate and rumen microbes ferment it into a large quantity of VFAs which reduces the rumen pH that leads to changes in rumen microbial community and host physiological homeostasis (Bevans et

al., 2005). Diet also affects the number of observed species (alpha) and overall bacterial community composition (beta diversity) in cattle. Pitta et al., (2010) [41] reported a decrease in Chao1 and Shannon diversity index with the dietary transition from bermudagrass to winter wheat. Methanogens depend on bacteria for hydrogen availability and Zhou et al., (2010) reported a high abundance of methanogens in low energy diet as compared to a high-energy diet. Wallace et al., (2015) reported higher archaeal abundance in medium grain diet than high grain diet. A high grain diet also affects the abundance of protozoa in the rumen and a diet containing 40-60 % concentrate showed the highest protozoa abundance in the rumen (Dehority and Orpin, 1997). Fungi are known for their role in fiber digestion. Thick wall fibrous forage favors the fungi abundance in the rumen (Clemmons et al., 2018). Other dietary changes like the addition of monensin or essential oil also affect the rumen microbial community. For example, monensin decreases the archaeal abundance in the rumen (Ultee et al., 2002).

#### *The effect of age on rumen microbiome colonization*

Age is also known to affect rumen microbiota composition. Neonatal calves do not have a developed rumen and microbial colonization occurs over time. Adult ruminants have a very dense and complex microbial consortium as compared to pre-weaned calves. At an early age, calves get exposed to microbes during lactation, with feed, and through animal-to-animal contact. Microbes start colonizing the gut over time and these microbes and their fermentation products affect early rumen development (Jami et al., 2013). Jami et al., 2013 reported the bacterial community differences based on age and these differences could largely be explained by changes in the diet over time as calves' transition from milk or milk replacer to a solid diet. However, host maturity and growth also influence

rumen microbial colonization. For example, Li et al., 2012 reported a change in the microbiota composition with the age of cows fed the same diet. Calves fed on milk replacer with calf starter and hay results in early rumen development due to the early microbial inoculation and fermentation end products. Rey et al., (2014) reported the bacterial community change from lactose-consuming bacteria to Bacteroidetes in the reticulorumen after the addition of solid feed to the diet. They also reported a major increase in the abundance of prevotella (11 to >40%) after solid feed addition. Early microbial exposure is also important for the immune system as Spiljar et al., (2017) reported the activation of Toll-like receptors in humans.

### **Host-Microbiome Interaction**

Cattle performance in terms of growth and milk production directly depends on rumen functions and those functions could be influenced by host genes (Zhang et al., 2019). Early feeding of solid feed to calves have shown to have a positive impact on rumen development (Tajima et al., 2000). The microbes present in neonatal calves ferment the solid feed particles and positively impact the expression of host genes involved in rumen development and VFAs absorption (Church, D. C. 1988). The health status of the animal influences the overall physiology of the animal. Clavel et al., 2017 reported healthy individual to have different GIT microbial community as compared to diseased ones. Abbas et al., 2020b reported a dysbiosis of rumen epithelial microbiome in liver abscessed beef cattle. They also reported correlation between epimural bacterial species and epithelial gene expression.

### **Host genetics and association with rumen microbiome.**

A study utilizing more than 1000 dairy cows (Holstein-Friesian and Nordic Red) revealed Holstein-Friesian dairy cows to have 39 heritable ( $h^2 = 0.2-0.6$ ) core OTUs, which mainly belong to bacterial order Bacteroidales and Clostridiales. Additionally, they identified two OTUs belonging to the fungal genus *Neocallimastix* (Wallace et al., 2019). Difford et al., 2018 used linear mixed models to estimate the genetic effect of the host on bacterial and archaeal community abundances in the rumen. They reported a significant association of bacterial and archaeal OTUs with host genetics. The analysis at the genus level reported that bacterial genera *Sporobacter*, Unclassified Proteobacteria, Unclassified Endomicrobia, F16, R4-45b, and Paludibacter to have a significant heritability ( $h^2$ ) ranging from 0.17- 0.25. One archaeal genus (*Methanobrevibacter*) was also identified that had a high heritability estimate ( $h^2 = 0.22$ ) in dairy cows.

Heritability of taxa ranged from 0.08-0.48 in dairy cows. The heritable taxa belong to phylum Firmicutes, Bacteroidetes, Ciliophora, Proteobacteria, Spirochaetes, and Euryarchaeota (*Methanobrevibacter* sp.) (Saborío-Montero et al., 2020). In Holstein-Friesian dairy, Sasson et al., (2017) reported 22 heritable OTUs and they belong to Bacteroidales sp, S24-7 sp, Lachnospiraceae sp, Veillonellaceae sp, [Paraprevotellaceae] sp, Clostridiales sp, Prevotella sp, flavefaciens sp, and Succiniclasticum sp.

For beef cattle, genome-wide association studies have shown the association of host genetic markers with rumen microbiota. Abbas et al., (2020a) reported 7 chromosomes and 8 1Mb regions associated with bacterial and archaeal OTUs, families (BS11, Ruminococcaceae, Succinivibrionaceae, Lachnospiraceae, RFP12, Veillonellaceae, Clostridiaceae, Paraprevotellaceae, Prevotellaceae, S24-7, Fibrobacteraceae, and

Clostridiaceae) and phylum (Firmicutes, Lentisphaerae, Proteobacteria, Verrucomicrobia, Fibrobacteres, Tenericutes). The heritability estimate range for OTUs, families, and Phylum were 0.06-0.82, 0.067-0.722, and 0.075-0.72, respectively (Abbas et al., 2020a). Similarly, in another study on beef cattle Li et al., (2019) reported 59 taxa with heritability estimates higher than or equal to 0.15. The reported 59 taxa belong to the bacterial phylum Firmicutes, Bacteroidetes, and archaeal genus Methanobacterium.

### **Host- microbiome interaction and feed efficiency**

Domesticated ruminants like cattle, goats, and sheep have the unique ability to convert low-quality feed material, which is unfit for human and other livestock consumption, into high quality protein source. Cattle and bison provide nearly 45% of the meat and milk for humans (Mottet et al., 2017). For beef cattle production, beef cattle efficiency is often described in terms of feed efficiency. Feed efficiency can be measured as feed conversion ratio (FCR), which shows the amount of feed consumed to produce 1kg beef, or as residual feed intake (RFI) which is the difference between actual feed intake and predicted feed intake (Koch et al., 1963). Currently, RFI is widely used by beef researchers and producers to identify more efficient animals that consume less than predicted feed amounts while maintaining the same level of gain as other animals (Elolimy et al., 2018).

The host genetic's influence over RFI has been reported by Berry and Crowley., (2013). They reported that the heritability ( $h^2$ ) of RFI range from 0.16 to 0.43 in growing beef cattle. Beef cattle have lower feed efficiency as compared to other livestock species mainly because of fermentation losses. Studies have shown a correlation between low RFI and less methane (CH<sub>4</sub>) production (Elolimy et al., 2018).

Efficient dairy animals have a lower richness of microbial taxa and genes (Shabat et al., 2016). Similarly, low diversity has been reported in obese humans (Turnbaugh et al., 2009; Le chatelier et al., 2013) suggesting that microbial communities were more efficient in terms of harvesting energy from the diet. Paz et al., 2018 reported the bacterial community differences between groups of animals with different average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G: F). They reported bacterial species (OTUs) as predictors for ADFI, ADG, and G: F for heifers (OTUs belong to prevotellaceae and victivallaceae families) and steers (OTUs belongs to prevotellaceae and lachnospiraceae families).

### **American Wagyu Cattle and Marbling**

The word “Wagyu” comes from “Wa” means Japanese and “gyu” means cattle. In general, wagyu refer to all Japanese cattle. Initially, wagyu were selected based on physical toughness which favored the animals to have more muscle and intra-muscular fat (Namikawa, K., 1992).

Currently, there are only four Japanese breeds that are considered as wagyu and those include Japanese Black, Japanese Brown, Japanese Polled, and Japanese Shorthorn. At this time only the first two breeds are successfully raised outside of Japan. The wagyu cattle were imported in the USA during 1975 when two red and two black Japanese bulls were imported by Mr. Morris Whitney. Shortly after that, the American Wagyu Association was established in Taxes on March 14, 1990 and serves to expand the Wagyu herd in the US. USA Wagyu producers exported beef to Japan until 2003. Since 2003, the major market for Wagyu beef has been in the US (Namikawa, K., 1992).

There are two major marbling grading systems i.e., Japanese and USDA systems. The Japanese grading system goes from 3 to 12 and 3 being the minimum amount of marbling. However, the USDA system has three grades i.e., select, choice and prime.

#### *Factors affecting the Marbling*

Genetics is one of the major factors that influence marbling, for example, the Wagyu breed have shown to have the highest marbling followed by Korean Cattle, Angus, Hereford crossbred or Hereford and Brahman breeds (Park et al., 2018). Wagyu cattle have higher heritability (0.40-0.55) for marbling (Oyama, K., 2011). Fat composition is also genetically influenced. The Japanese black Wagyu has the highest oleic acid concentration (52.9%) as compared to Hanwoo (47.3% oleic acid), Australian (39.8% oleic acid) and Angus (32.9-39.8% oleic acid) breed (Smith et al., 2006). Wagyu cattle are genetically disposed to a higher expression of stearoyl-coenzyme A desaturase and as a result have higher oleic acid (Smith et al., 2006). Nogi et al., 2011 reported high heritability for oleic acid in wagyu cattle. Slaughtering age is another factor that affects the IMF deposition in most beef breeds (park et al 2018) and cattle with wagyu genetics have shown an increase in marbling with the increase in age (Zembayashi et al., 1995; Kirkland et al., 2007). Connolly et al., 2020 reported the increase of 21 metabolites related to muscle energy and glucose metabolism in Wagyu crossbred with increasing age during the feedlot period and they attributed this change to mature body conformation. Park et al., (2002) compared the effect of the sex on IMF deposition and they reported Korean steers to have the highest marbling as compared to cows. In addition, castration of the bulls increases the marbling in different breeds (Marti et al., 2013).

Triglyceride (TG) synthesis is the major contributor for IMF deposition (Pethick et al., 2004). Ruminants use glucose or acetate as a substrate to synthesize fatty acids. In ruminants, acetate is the main volatile fatty acid directly used for fatty acid synthesis (Nafikov and Beitz, 2007). The main sources of glucose in ruminants are dietary glucose (absorbed from the small intestine), gluconeogenesis (from propionate, amino acids) and lactate (Hanson and Ballard, 1967). The surplus energy deposits as IMF in the animal and feeding the energy-dense diet (high in grains) is one way to increase the IMF deposition. High grain diet in ruminants can increase volatile fatty acids (VFAs), high concentration of propionate, and glucose absorption for the FA synthesis (Hanson and Ballard, 1967). Propionate is the only gluconeogenic VFA that can increase the supply of glucose and ultimately increase de novo fatty acid synthesis (Hanson and Ballard, 1967). The Angus steers fed a higher proportion of concentrate diet (60% or 75% or 90%) results in a higher marbling score (Gunter et al., 1996). Moreover, different studies involving different breeds (Hereford crossbred, Brahman, Angus) have shown that feed with higher concentrate diet results in higher IMF deposition as compared to feeding a high forage diet (Gunter et al., 1996). The high concentrate diet affects the gene expression of adipogenic factors which increase the subcutaneous and IMF deposition in the animals (Wood et al., 2008).

Fats are incorporated in the ruminant's diet to increase energy density, especially for high producing animals (Zin et al., 2000). Also, other fat sources are used to increase the concentration of n-3 fatty acids and conjugated linoleic acid in meat and milk (Zin et al., 2000). Most dietary fat includes the 18-carbon unsaturated fatty acids and rumen microbes play a role in lipolysis and biohydrogenation which results in saturated fatty



acids (Buccioni et al., 2012). In ruminants, the profile of FAs in the small intestine is different than dietary fats because of rumen digestion and biohydrogenation where, nearly 90% of dietary fat reaches the duodenum as non-esterified fatty acids (NEFAs) (Doreau and Ferlay, 1994). The manipulation of FAs in the small intestine can influence IMF deposition (Doreau et al., 1994). The dietary fat uptake can shift the energy balance of the animal which leads to an increase in fat deposition (Doreau et al., 1994). The feeding oil-rich corn can increase IMF deposition in Angus steers (Andrae et al., 2001). Jeong et al, (2009) studied the effects of emulsifier in Korean cattle and reported that supplementation of sodium stearyl-2-lactylate increases IMF. Irie et al, (2011) reported the supplementation of the bile acid (ursodeoxycholic acid) increases IMF deposition in wagyu cattle.

Genetics and nutrition are considered as two major factors that influence the marbling in beef cattle. A lot of research has been done to see how genetics and nutritional manipulation can change IMF deposition, but we have very limited information about how rumen microbiome influences the marbling. It is possible that Wagyu cattle, known for its increased IMF, have different microbial communities than other breeds that help in IMF deposition by increasing nutrient supply to the animal. Bacterial communities can affect the acetate to propionate ratio and can change the composition of dietary FAs through lipolysis and biohydrogenation, which can affect the IMF deposition. In recent years, many studies have reported the association of rumen microbiota with feed efficiency (Carberry et al., 2012; Shabat et al., 2016; Paz et al., 2018; McGovern et al., 2018). It is possible that Wagyu cattle with high IMF have different microbial communities that help increase marbling compared to animals with low marbling animals

by increasing energy availability to the animal. As such, the rumen microbial communities can affect energy availability to the host, acetate to propionate ratio, and the composition of dietary FAs which can affect IMF deposition. Kim et al., 2020 reported that Hanwoo Korean beef cattle with a high marbling score (marbling score  $\geq 7$ ) have different bacterial taxa compared to cattle with a low marbling score (marbling score  $\leq 4$ ). They reported that Korean cattle with high marbling had higher bacterial species richness and higher abundance of *RFP12*, *Porphyromonadaceae*, *Oscillospira*, *Paludibacter*, and *Verrucomicrobia* as compared to low marbling group.

IMF deposition is affected by multiple factors. Recent advances in culture-independent techniques provide a unique opportunity to investigate the microbial differences between different marbling grades. Studies so far has focused on nutritional and management aspects of Wagyu cattle (Park et al., 2018). As such investigating the rumen microbiome may provide new information to develop strategies to increase marbling in cattle.

### **Liver Abscesses**

Liver abscesses are the liver abnormalities of the feedlot cattle which accounts for >60% liver condemnation at harvest (McKeith et al., 2012). The liver condemnation (>20% annually), carcass quality degradation, and poor beef cattle performance cost the beef producers millions of dollars annually (Amachawadi and Nagaraja, 2016). The liver abscess is categorized based on Elanco Scoring System (Brown et al., 1975) into three groups named A-, A and A+ as A- representing the mild and A+ being the severe case. The prevalence of liver abscesses is highly variable, ranges from 0% to 80% (Brown and Lawrence, 2010; Rezac et al., 2014a). Many factors affect the liver abscess, such as diet,

breed, feedlot period, location, sex of the animal, and season (Nagaraj et al., 1996b; Reinhardt and Hubbert, 2015).

During the feedlot period, cattle are fed high grain diet which could induce acidosis and rumenitis (Amachawadi and Nagaraja, 2016). The incidence of acidosis is associated with liver abscess as Rezac et al., (2014a) reported cattle with mild to severe rumenitis has a 32% chance of having a liver abscess. The availability of the readily fermentable carbohydrate could induce acidosis and prolonged exposure to low ruminal pH could damage the protective rumen epithelial tissue (Elam, 1976). The severity of the rumenitis varies from blunt papillae to ulceration or scar tissue (Rezac et al., 2014b) and damaged epithelial tissue provides the entry point for pathogenic bacteria to enter blood circulation and cause a liver abscess (Amachawadi and Nagaraja, 2016).

Occasionally, rumen epithelial tissue or reticulum wall could be punctured by sharp object ingested by an animal, and it could lead to reticuloperitonitis and liver abscesses (Grohn and Bruss, 1990). Liver Abscess is caused by more than one species (Nagaraja and Chengappa, 1998). The isolates from liver abscess have shown that bacterial species *Fusobacterium necrophorum* is the primary pathogenic organism which is present in all liver abscesses (Amachawadi and Nagaraja, 2016) followed by the second most prevalent bacterial species *Trueperella pyogenes* (Scanlan and Hathock, 1983; Lechtenberg et al., 1988). In addition, isolates from liver abscess of feedlot cattle have several other bacterial species, such as *Streptococcus spp.*, *Pasteurella spp.*, *Streptococcus spp.*, *Mobilincus spp.*, *Staphylococcus spp.*, *Enterobacter spp.*, *Propionibacterium spp.*, *Klebsiella spp.*, *Prevotella spp.*, *Escherichia coli*, *Clostridium spp.*, *Porphyromonas spp.*, *Bacteroides*

*spp.*, and *Peptostreptococcus spp* (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007; Scanlan and Hathock, 1983).

The economic cost of the liver abscess forces the beef producers and researchers to control liver abscess. One way to reduce liver abscess is by managing the number of fermentable carbohydrates in the feed to reduce acidosis and rumenitis and ultimately liver abscess (Amachawadi and Nagaraja, 2016). This approach requires feeding of low energy (more roughages and less grains) diet which could affect weight gain (Amachawadi and Nagaraja, 2016). In the US, beef producers add antibiotics as feed additives to control liver abscess (Lundeen, 2013). Tylosin is the most widely used antibiotic as a feed additive in the beef industry. Each animal receives 60-90 mg of Tylosin each day and it is believed that Tylosin inhibits the *Fusobacterium necrophorum* growth in the rumen and the liver (Nagaraja and Lechtenberg, 2007). Wileman et al., 2009 reported that feeding tylosin reduces the risk of liver abscesses from 30% to 8% in feedlot system. The feedlot period ranges from 90-300 days depending on the producer's objective and continuous use of antibiotics for a long period could result in antimicrobial resistance (Amachawadi and Nagaraja, 2016).

Since January 2017, the use of tylosin is under professional oversight (FDA, 2013). The fear of developing antimicrobial resistance and consumer's pressure to eliminate or minimize the use of antibiotics as a feed additive is forcing researchers to look for new methods to reduce liver abscess.

The researchers are investigating the alternatives to tylosin like essential oils and vaccines (Amachawadi and Nagaraja, 2016). The essential oils (EO) are the plant metabolites with antimicrobial and anti-inflammatory properties (Bakkali et al., 2008).

The EO are extracted from plants with organic solvent (Bakkali et al., 2008) and examples of EO include vanillin, thymol, capsaicin, eugenol, cinnamaldehyde, and limonene (Bakkali et al., 2008). Samii et al., (2016) reported that thymol and limonene can inhibit the *Fusobacterium necrophorum*. Similarly, other studies have reported the use of EO blend could reduce liver abscess and can also improve feed efficiency (Meyer et al., 2009; Geraci et al., 2012). However, some studies also suggested the ineffectiveness of EO to reduce the liver abscess for example Pukrop et al., (2019) reported that the blend of EO did not reduce the liver abscess as compared to tylosin. The development and use of vaccines against *Fusobacterium necrophorum* are another approach to reduce liver abscesses (Nagaraja and Chengappa, 1998). The vaccines based on *F. necrophorum* alone (Fusoguard, Elanco Animal Health) or based on *F. necrophorum* and *T. pyogenes* bacterium (Centurion, Merck Animal Health) have been used with mixed results (Amachawadi and Nagaraja, 2016). Checkley et al. (2005) reported the Fusoguard was not effective in cattle with a higher than 30% prevalence of liver abscess. Similarly, Fox et al. (2009) compared both vaccines on feedlot cattle fed on high steam flaked corn for 238 days. They reported that the total number and severity of the liver abscess were not affected by the vaccine. The researchers are also looking at the possibility of using outer membrane adhesion proteins of *F. necrophorum* and *subsp funduliforme* as a target to develop a new vaccine (Kumar et al., 2003; Amachawadi and Nagaraja, 2016).

Bacteria are the dominant microbes in the rumen. They are found free-floating in the rumen fluid, attached to the feed particles, and attached to the rumen epithelial wall also called epimural bacteria (Zhou et al., 2015). The bacterial community analysis displayed

differences between rumen content (fluid and feed particles) and epimural microbiota (Liu et al., 2016; Wetzels et al., 2016). Liu et al., 2016 reported the rumen epithelial has a higher abundance of *Proteobacteria*, *Butyrivibrio*, and *Campylobacter*, and a low abundance of *Bacteroidetes* as compared to rumen content in Holstein dairy cows. Wetzels et al., 2016 reported the shift in the epimural bacterial communities in response to induced subacute ruminal acidosis. Epimural bacteria are at the interface between the host and rumen ecosystem (Cheng and McAllister, 1997). The epimural bacterial community is important to make a protective biofilm layer against other opportunistic pathogenic bacteria (Kamra, D.N., 2005). Rumen acidosis is one of the major factors that damage the rumen epithelial tissues and pathogenic bacteria could enter the blood circulation and cause a liver abscess in beef cattle (Amachawadi and Nagaraja, 2016). The epimural microbiota is not thoroughly investigated and especially in animals that develop liver abscesses. Dysbiosis of the epimural microbiota may be occurring due to acidosis or rumenitis which provides an opportunity for other pathogenic bacteria to adhere to the rumen epithelial wall or enter blood circulation. The host interaction with epimural microbiota and its response in terms of epithelial gene expression could provide more information about the etiology of the liver abscess. The change in overall microbiota between healthy and liver abscessed animals have not been investigated. Additionally, limited information is available on how host interacts and responds to change in epimural microbiota. The information about changes in the epimural microbiota and epithelial gene expression in the liver abscessed animals could help researchers to develop new methods and strategies to prevent liver abscess without relying on antibiotics.

### **Tools to understand the Rumen Microbiota**

Ruminants depend on microbial fermentation to fulfill their energy requirements and understanding the individual microbial characteristics and overall community composition is important to develop new strategies to increase livestock production. Researchers have used culture-dependent and culture-independent techniques to study the microbial community (Matthews et al., 2019). Culture-based techniques include anaerobic jar techniques, anaerobic glove box, and Hungate's roll tub (Matthews et al., 2019). Hungate developed the roll tube culture system, which uses the test tubes instead of agar plates, to study and isolate different rumen anaerobic microbes and is used to date for isolation of anaerobic bacteria and archaea (Hungate, R.E, 2013). These culture-based techniques are used for population estimation, characterization of pure culture, interactions, and enzyme isolation for industrial applications (Matthews et al., 2019). Rumen microbes are very difficult to culture because it's hard to maintain anaerobic conditions and their growth conditions are complex (Rufener et al., 1963). Scientists have used classical methods to identify different microbial species based on their shape, morphology, and gram stain and they classify them based on substrate utilization and fermentation end products (Matthews et al., 2019).

The culture-dependent methods do not cover the full diversity of the rumen microbes and Creevey et al., (2014) reported that members of the family *Lachnospiraceae* were the most cultured followed by 2 genera of the phylum *Bacteroidetes*. Cultured-dependent techniques can only cover 20% of the total rumen microbiota (Krause et al., 2013) and another estimate by McCabe et al., (2015) reported that <1% of the rumen species are culturable.

Rumen microbiota is a highly integrated system in which dominant and minor species contribute to the overall rumen ecosystem (Cersosimo and Wright, 2015). The culture-based approaches focus on microorganism isolation and pure culture studies which do not provide information about how different microbial species interact at the community level in their natural habitat (Cersosimo and Wright, 2015). In the last decade, metagenomics has emerged as a powerful tool to study rumen microbiota. The development of next-generation sequencing (NGS) technologies, new and improved reference databases and bioinformatics tools addresses the limitations of culture-based techniques (Galbraith et al., 2004).

One metagenomic approach targets the small subunit (SSU) of rRNA genes (16S and 18S) to investigate the microbial community structure at the taxonomy level. The 16S and 18S rRNA genes have been used to study prokaryotes and eukaryotes, respectively (Cersosimo and Wright, 2015). The 16S rRNA gene has 9 (V1-V9) hypervariable regions (Chakravorty et al., 2007) and different primers are used to amplify one or more regions with the polymerase chain reaction (PCR) method (Nossa et al., 2010; Soergel et al., 2012). The targeted region of the 16S is sequenced using next generation sequencing platforms. Illumina sequencing is gaining more popularity because it allows to sequence targeted regions with a length of up to 2x300 base pairs (pair ended) and provides up to 25-2666 million sequence reads (Cersosimo and Wright, 2015).

The whole-genome shotgun sequencing (WGS) approach provides information about the metabolic potential of the microbial community and that information can be used for biological pathway reconstruction (Cersosimo and Wright, 2015). Unlike the SSU method, the short gun sequencing method target all available DNA from a sample. This



method provides more genomic data coverage than the 16S method and after quality control steps the data could be used for metagenomic assembly, binning, and functional gene profiling (Cersosimo and Wright, 2015).

The main limitation of the 16S method is it can only provide information about taxonomy profiling, and it cannot provide any information into the metabolic pathways. Due to the development of the new error correction algorithm, such as DADA2 (Callahan et al., 2016), the 16S data can provide strain-level resolution. Moreover, new processing methods are not dependent on the reference database, and this provides an opportunity to identify new microbial species without any assigned taxonomy. I used the 16S method to investigate the rumen microbiota is explained and discussed in chapters 2-4.

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## CHAPTER 2

INFLUENCE OF HOST GENETICS IN SHAPING THE RUMEN BACTERIAL  
COMMUNITY IN BEEF CATTLE

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**ABSTRACT**

In light of recent host-microbial association studies, a consensus is evolving that species composition of the gastrointestinal microbiota is a polygenic trait governed by interactions between host genetic factors and the environment. Here, we investigated the effect of host genetic factors in shaping the bacterial species composition in the rumen by performing a genome-wide association study. Using a common set of 61,974 single-nucleotide polymorphisms found in cattle genomes ( $n = 586$ ) and corresponding rumen bacterial community composition, we identified operational taxonomic units (OTUs), Families and Phyla with high heritability. The top associations (1-Mb windows) were located on 7 chromosomes. These regions were associated with the rumen microbiota in multiple ways; some (chromosome 19; position 3.0–4.0 Mb) are associated with closely related taxa (*Prevotellaceae*, *Paraprevotellaceae*, and *RF16*), some (chromosome 27; position 3.0–4.0 Mb) are associated with distantly related taxa (*Prevotellaceae*, *Fibrobacteraceae*, *RF16*, *RFP12*, *S24-7*, *Lentisphaerae*, and *Tenericutes*) and others (chromosome 23; position 0.0–1.0) associated with both related and unrelated taxa. The annotated genes associated with identified genomic

regions suggest the associations observed are directed toward selective absorption of volatile fatty acids from the rumen to increase energy availability to the host. This study demonstrates that host genetics affects rumen bacterial community composition.

## **INTRODUCTION**

Complex and diverse microbial communities facilitate the degradation of nutrients within ruminants (Anderson et al., 2016). The composition of this complex microbial community is shaped by the highly dynamic physical, chemical, and predatory conditions within the rumen, and potentially by genetic factors of the host (Anderson et al., 2016; Church, D. C. 1993; Hobson, P. N. 1988; Hungate, R. E. 1966; Li et al., 2019; Sasson., et al 2017). In turn, the microbial community contributes to the environmental conditions within the rumen and the nutrient availability to the host (Church, D. C. 1993). This complex microbial community can covert otherwise unusable organic matter into useable protein and energy and can provide up to 70% of the animal's protein and energy needs (Flint et al., 2004) in the form of microbial cell protein (MCP) or volatile fatty acids (VFAs) for host metabolism. Therefore, ruminal microbial diversity and abundance critically influences nutrient cycling and when inorganic nutrients and carbon are made available for host consumption. As such, differences in the microbial community can change the energy profiles available to the ruminant host and its subsequent performance.

In ruminants, the microbial population is established by successive waves where convergence of microbial populations are seen reaching a more stable population structure (Jami et al., 2013; Furman, et al., 2020; Rey et al., 2014; Wang et al., 2019).

The establishment of a gut microbial community is influenced by multiple factors,

including diet, environment, and host genotype (Church, D. C. 1993; Hobson, P. N. 1988; Hungate, R. E. 1966; Li et al., 2019; Sasson., et al 2017; Benson et al., 2010; Spor et al., 2011). Among these contributors, the influence of diet on gut microbial population structure is well established (Fernando et al., 2010; Fernando et al., 2007; Henderson et al., 2015; Huws et al., 2018). Rumen bacterial species' and functional composition of the rumen microbiota is an important factor that contributes towards animal performance and efficiency (Mizrahi et al., 2018). Additionally, studies in small ruminants have demonstrated the host mucosal innate immune function to affect rumen microbial community composition (Jiao et al., 2017). With studies demonstrating host genetics to influence host immunity (Glass et al., 2012), it is tempting to speculate in ruminants, host genetics may directly or indirectly affect rumen microbial community composition. However, our understanding of how a stable rumen microbial community assembles and what factors affect rumen microbial community composition and function is limited. One such process that is still largely unknown is how the ruminant host genotype effect rumen microbial community assembly. Recent studies have demonstrated that the rumen microbiota is influenced by host genetic factors (Li et al., 2019; Sasson., et al 2017; Wallace et al., 2019). In mammals, Benson et al (2010) demonstrated genome-wide linkages of bacterial taxa abundance in the murine gut using Quantitative Trait Locus (QTL) mapping. While breeds did not have broad representation, a recent study suggested that microbial phylotypes can be influenced by the sire breed and can impact host metabolism (Hernandez-Sanabria et al., 2013). In light of these recent host-microbial association studies, a consensus is evolving that species' composition of the gastrointestinal microbiota is a polygenic trait governed by

interactions between host genetic factors and the environment. As such, genome-wide association studies (GWAS) can be used to identify host chromosomal regions and a subset of single nucleotide polymorphisms (SNP) that influence microbiome composition and function in the rumen. Here we evaluated the effect of host genetic factors in shaping the bacterial species composition in the rumen and the role of such associations on host metabolism by performing a genome wide association study using different cohorts of beef cattle totaling 586 animals. All animals were genotyped using medium to high density single nucleotide polymorphisms (SNP) chips (770 K, 150 K, or 80 K) and the underlying SNPs were used for analysis of genome wide associations using the bacterial community composition as the phenotype. The rumen microbial community of all 586 animals were phenotyped by sampling and characterizing the V4 region of the 16S rRNA gene and was used as a collection of individual traits to perform GWAS to identify host chromosomal regions and subset of SNP that influence bacterial community composition and assembly within the rumen. This study provides detailed and novel insight into how host genotype in ruminants can influence rumen microbial assembly. We also quantify the genomic heritability for bacterial taxonomic traits thus identifying the degree to which they are controlled by host genetic background. This study also demonstrates the potential to utilize associations between rumen microbiota and genetic markers for use in genomic selection and marker-assisted management that can be used to improve feed efficiency, animal health and microbiome manipulation mediated by selecting for favorable microbial taxa within the rumen.

## METHODS

**Experimental design and animals.** All animal procedures implemented in this study were approved by the University of Nebraska—Lincoln and U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee. All experiments were performed using relevant guidelines and regulations described by the Animal Care and Use Committee. The data presented in this study were collected between 2009 and 2015 from different cohorts of animals which included a cohort of heifers ( $n = 127$ ) and a cohort of steers ( $n = 131$ ) from USMARC ( $n = 258$ ) and a cohort of steers ( $n = 328$ ) from University of Nebraska, Lincoln (UNL) research feedlot. Animals within USMARC and UNL were fed in 3 (USMARC1-3) and 5 (UNL1-5) cohort groups, respectively. The number of animals within each cohort ranged from 51 to 127 for animals fed at USMARC and 51 to 111 for animals fed at UNL. The animals from USMARC were part of the USMARC Germplasm Evaluation project (GPE) (Schiermister et al., 2015). The animals from UNL were purchased from Nebraska and surrounding states and were a cross bred population of unknown breed makeup.

The Heifers at USMARC were fed a growing diet at time of sample collection that included 70% corn silage and 30% alfalfa hay on dry matter basis (Paz et al., 2018). The USMARC steers were fed a finishing diet composed of 57.6% dry- rolled corn, 30% wet distillers grains with solubles, 8% alfalfa hay, and 4.4% vitamin and mineral supplement on a dry matter basis (Paz et al., 2018). All UNL animals were fed a common basal diet (UNL-Diet) containing 50:50 blend of Alfalfa and Sweet Bran®. After adaptation to each diet for at least 21 days, rumen samples were collected via esophageal tubing for bacterial community analysis. The diets were formulated to meet or exceed NRC

recommendations for growth and vitamin and mineral supplementation of growing and finishing beef cattle.

**Sample collection for rumen microbiota composition analysis.** Rumen samples were collected after adaptation to each diet via esophageal tubing as described previously (Paz et al., 2016). Briefly, the animal was restrained in the chute, and a stomach tube was inserted through a speculum and passed through the esophagus until it reached the rumen. A vacuum pump was attached to the free end of the tube and the sample was withdrawn from the rumen. The samples collected contained both rumen fluid and feed particles and were a representative sample of the rumen. Samples collected via esophageal tubing have been shown to represent the rumen to contain a similar microbial community composition to a sample collected via a rumen fistula (Paz et al., 2016). The samples collected were snap frozen in liquid nitrogen and were stored in  $-80^{\circ}\text{C}$  until used for DNA extraction and microbial community analysis.

**Microbial DNA extraction.** DNA was extracted from the rumen samples using the PowerMag Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol with a few modifications as described by Paz et al, (2018). The modified protocol also included adding RNase A to the lysis solution to ensure removal of RNA during DNA extraction. The isolated DNA was stored at  $-20^{\circ}\text{C}$  until used for bacterial community analysis.

The V4 region of the 16S rRNA gene was amplified and sequenced on the MiSeq platform as described previously (Paz et al., 2018; Kozich et al., 2013). Briefly, barcoded universal primers specific for the V4 region of eubacteria were amplified using 25  $\mu\text{L}$  PCR amplification reactions (Kozich et al., 2013). Each 25  $\mu\text{L}$  PCR reaction contained



0.75 Units Terra PCR Direct Polymerase Mix, 1X Terra PCR Direct Buffer, 10  $\mu$ M indexed fusion primers, and 5–20 ng of DNA. The cycling conditions contained 98 °C for 2 min, followed by 25 cycles of 98 °C for 10 s, 55 °C for 30 s, and 68 °C for 30 s; and a final elongation step of 68 °C for 4 min (Paz et al., 2018). Following amplification, PCR products were normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturers protocol. The normalized libraries were pooled and were further purified using the MinElute PCR Purification Kit (Qiagen, Valencia, CA, USA) as described by the manufacturer. The resulting pooled sample was subjected to size selection and purification using the Pippin Prep (Sage Science, Inc., Beverly, MA, USA) automated size selection instrument. The resulting sequence ready libraries were further analyzed using the Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and were subjected to 250 bp paired end sequencing using the Illumina Miseq System (Illumina, San Diego, CA, USA) using the V2 500 cycle sequencing kit as described by the manufacturer. Bridge amplification and reversible dye-terminator -based sequencing on the MiSeq was performed as described by the manufacturer. Raw sequences have been deposited at the NCBI Sequence Read Archive (SRA) under the accession no. SRP100776 and PRJNA55259.

**Data processing pipelines for microbial community analysis.** Raw reads generated from Illumina MiSeq sequencing were processed using the quality filter and analysis pipeline described by Paz et al, (2018). Following preliminary quality filtering and read processing, the resulting reads were used for microbial community analysis as described below. Complete information of the bioinformatics pipeline describing data analysis is available at <https://github.com/FernandoLab>. Briefly, forward and reverse reads were

assembled to generate contigs of the V4 region and further quality filtering was performed on subsequent contigs to remove sequences with ambiguous bases, incorrect contig length, or incorrect assembly using MOTHUR v.1.38.1 (Schloss et al., 2009). Following secondary quality filtering, subsequent reads were clustered into operational taxonomic units (OTUs) using the UPARSE pipeline (USEARCH v7.0.1090) (Edgar, 2013) after dereplication, discarding singletons, clustering sequences into OTUs at 97% similarity, and removing chimeric sequences using UCHIME (Edgar, 2011) as described previously (Paz et al., 2018). Representative OTU sequences from each OTU were aligned against the SILVA reference alignment v123 to ensure the OTU reads came from the V4 region of the 16S rDNA gene. The resulting representative OTU sequences that fail to map to the V4 region were discarded. Taxonomy was assigned to each OTU using QIIME v.1.9.1 (Caporaso et al., 2010) as described previously (Schloss et al., 2009). The Greengenes database (gg\_13\_8\_otus) (McDonald et al., 2012) were used as the reference database for taxonomic assignment. OTUs classified as Cyanobacteria were filtered from the data set as cyanobacterial sequences may arise from 16S remnants present in the plant chloroplasts (Giovannoni et al., 1988). However, recent studies have reported Cyanobacterial orders such as YS2, SM1D11, and mle1-12 to be a new class of Cyanobacteria to be present in gut, soil and plants (Soo et al., 2014; Di Rienzi et al., 2013; McGorum et al., 2015). However, these orders were not identified within the cyanobacterial sequences that were removed. A rarefied (7,000 reads) OTU table was used to generate a Bray Curtis dissimilarity matrix that was used for Principal Coordinate Analysis (PCoA) within QIIME v.1.9.1 pipeline. The R package ggplot2 (Wickham, 2009) was used to generate the PCoA plot in R by using first two components of the Bray

Curtis coordinates. The Bray Curtis dissimilarity matrix was used to perform the multivariate analysis of variance (PERMANOVA) (Anderson et al., 2013) within R package *vegan* (Oksanen et al., 2018). The complete information of the bioinformatics pipeline describing data analysis is available at [https:// github.com/FernandoLab](https://github.com/FernandoLab).

**Genotyping the resource population.** All animals (n = 586) were genotyped with either a 770 K, 150 K, or 80 K SNP assay. Blood samples were collected onto blood collection cards and the collection cards were sent to Geneseek (Lincoln, NE) for subsequent DNA extraction and genotyping. The SNP in common across all panels were utilized in the analysis and included 61,974 SNP. Genotypes that were missing were replaced with the mean genotype at that locus across all genotyped individuals.

**Identifying “positional candidate genes”.** The 1 Mb windows that account for the greatest proportion of the genetic variance were used to identify candidate genes using the “positional candidate gene approach” using the *Bos taurus* build UMD\_3.1 assembly (Zimin et al., 2009) and UCSC genome (ars-ucd1.2/*bos taurus*) browser. Due to the limited understanding of the *Bos taurus* genome compared to *Homo sapiens*, human orthologs of beef cattle “positional candidate genes” was identified using the BioMart data mining tool (68) and the Ensembl Genes (release 69). Human gene orthologs to bovine “positional candidate genes” was utilized to identify gene ontology terms, and pathways using the UCSC genome (ars-ucd1.2/*bos taurus*) browser and NCBI gene functions.

**Statistical analysis.** An OTU table generated for the 586 animals was used for subsequent analysis. Rarefaction analysis was performed using QIIME v.1.9.1 (Caporaso et al., 2010) as described previously (Paz et al., 2018). Prior to the analysis, operational

taxonomic unit (OTU), family and phylum read counts were transformed into relative abundance.

The heritability of an OTU, family, or phylum class was estimated using a Bayesian genomic best linear unbiased prediction (GBLUP) model utilizing the BGLR package in R (Pérez et al., 2014). Within each OTU, family, and phylum the following model was fitted:

$$y = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where  $y$  is the proportional abundance,  $\mathbf{b}$  is a vector of fixed effects,  $\mathbf{u}$  is a vector of random additive genetic effects,  $\mathbf{e}$  is a vector of random residuals and  $\mathbf{X}$  and  $\mathbf{Z}$  are incidence matrices relating observations to the fixed and random additive genetic effects, respectively. To determine the extent of and to correct for population structure, a principle component analysis (PCA) on a genomic relationship matrix ( $\mathbf{G}$ ) was utilized. The  $\mathbf{G}$  matrix was constructed as:

$$\mathbf{G} = \frac{\mathbf{M}\mathbf{M}'}{2 \sum p_j(1 - p_j)},$$

where  $\mathbf{M}$  is a genotype incidence matrix that has been centered based on allele frequencies (VanRaden, 2008) and  $p$  is the allele frequency of the second allele at the  $j$ th SNP across all loci.

The fixed effects included the intercept, the first two PC, and contemporary group (concatenation of management type and year). The random additive genetic effect was assumed  $\sim N(0, \sigma_u^2 \mathbf{G})$ , where  $\mathbf{G}$  is a genomic relationship as outlined previously. A chain length of 102 000 iterations was run with the first 2 000 discarded as burn-in and a thinning rate of 10 was utilized. The default priors were utilized within BGLR, which included a flat and bounded prior for the fixed effects and a scaled inverse Chi-squared

distribution for the additive genetic and residual variances. Across both random effects the degrees of freedom were set at their default value of 5 and the scale factors were set based on the rules described by de los Campos and Pérez-Rodríguez (2014) with the assumption that the model explains 20 percent of the phenotypic variance. The posterior mean  $\pm$  PSD heritability estimates for a given OTU, family, or phylum was calculated as the mean of  $\frac{\sigma_u^2}{\sigma_u^2 + \sigma_e^2}$  across all samples that remained after thinning. The number of effective samples across models was estimated using the CODA package in R (Plummer et al., 2006).

**Genome-wide association study.** Across OTU, family, or phylum class the estimated additive genetic effects across animals were decomposed into marker effects for a subset of groups within each class (Wang et al., 2012). The classes investigated are outlined in Table S1. The marker effects (a) were estimated by backsolving using  $G$  and the incidence matrix (i.e.,  $Z$ ) as outlined below:

$$\hat{a} = \frac{M'G^{-1}\hat{u}}{2\sum p_j(1-p_j)},$$

where  $M$  is the genotype incidence matrix as outlined previously,  $G^{-1}$  is the inverse of  $G$  and  $\hat{u}$  is the estimated additive genetic value of an individual which was derived from the Bayesian GBLUP model. After estimating marker effect solutions, the variance of 1 Mb non-overlapping window genomic estimated breeding values (WGE BV) was computed for each window. Within a window the WGE BV for each individual was estimated by multiplying the estimated SNP effects with their respective genotypes and summing across all SNP within the window. The WGE BV variance of a 1-Mb window was calculated as the variance in WGE BV across individuals. All OTUs ( $n = 17$ ) with at least

40,000 reads and present in at least 95% of the animals, all families ( $n = 12$ ) that represented greater than 1.8% total reads and were the top families (12 families were selected as families ranked 11th and 12th had very similar read percentages to the family ranked 10th) and top 10 Phyla ( $n = 10$ ) with the largest variance in WGEV were investigated further. The relative abundances of the identified OTUs, families and phyla were transformed with natural log ( $\log(1 + x)$ ) and then pairwise Pearson correlation analyses were performed. The pairwise correlation among OTUs, families and Phyla associated with chromosome 9 and 27 were plotted using R package GGally (Schloerke et al., 2018) while correlation heatmap among all the identified OTUs, families and phyla was generated by using R package ggplot2 (Wickham, 2009).

### **Data availability**

Raw sequences generated in this study have been deposited at the NCBI Sequence Read Archive (SRA) under the accession no. SRP100776 and PRJNA55259. The complete information of the bioinformatics pipeline describing data analysis is available at <https://github.com/FernandoLab> and appendix A.

## **RESULTS**

**The bacterial populations within the cattle rumen.** The bacterial community within the rumen microbiome of 8 cohorts were phenotyped by sequencing the V4 region of the 16S rDNA gene. The sequencing resulted in 18,992,394 quality filtered reads which included 9,755,502 reads from the USMARC cattle cohorts and 9,236,892 reads from the UNL cohorts. The rarefaction curves and goods coverage tests displayed adequate sampling depth to provide a detailed and quantitative estimate of the rumen bacterial community composition within each animal. The taxonomic classification of normalized OTUs at

phylum, family and OTU level were performed using Naive Bayes classifier (Schloss et al., 2009) using the greengenes database (McDonald et al., 2012). This analysis detected 439 genera, 237 families, 131 orders, 68 classes and 32 phyla from 7,228 OTUs identified from the 586 animals sequenced. The distribution of taxa across all cohorts were similar with the exception of family Succinivibrionaceae which were greater in the USMARC cohorts (Fig. 1). The relative abundance of the major phyla included Bacteroidetes 48.85%; Firmicutes 24.95%; Proteobacteria 13.3%; Verrucomicrobia 3.14% and Tenericutes 2.24%.

The distribution of OTUs across the cohorts displayed wide animal-to-animal variation, therefore to better characterize taxa that are more conserved across animals and variable in abundance, a core measurable micro- biome (CMM) was identified from the total dataset. To ensure robust repeatability of bacterial phenotyping only OTUs present in at least 1% of the animals and was also part of the CMM were used for subsequent analysis. The CMM contained 99.94% of the total reads generated and therefore represent a major portion of the rumen bacterial population. A principal coordinate analysis (PCoA) (supplementary figure S3) and PERMANOVA analysis were performed to identify combined effects of diet, sex, location on microbial community. As all factors (diet, sex, location) cannot be untangled from each other, the PERMANOVA analysis was performed using management type to reflect the collective effect of all above factors. PERMANOVA analysis displayed a significant effect of management type ( $p < 0.001$ ) on microbial community composition. Therefore, in the model used for GWAS analysis, we included contemporary group (concatenation of management type and year) as a fixed

effect to adjust for the variation in the microbial community composition resulting from management type.

**Rumen bacterial community behaves as a “polygenic trait”.** To determine the extent of and to correct for population structure, a principle component analysis (PCA) on a genomic relationship matrix (G) was utilized. The first two principle components annotated by location (UNL and USMARC) are illustrated in Fig. 2. The degree of population differentiation across the two feeding locations was minimal as the first principal component only described 3% of the variation in population structure. We utilized genome wide association analysis using SNP to assess the contribution of host genotype to the variation of bacterial taxa within the rumen. Normalized abundance of each OTU, family and phylum were used as traits to test for associations with 61,974 SNP.

The posterior mean heritability estimates determined using a Bayesian genomic best linear unbiased prediction (GBLUP) model across classes within OTU, family, and phylum level were 0.161, 0.150, and 0.194, respectively. Although posterior mean heritability estimates were low, some taxonomic groups displayed high heritability estimates. The maximum heritability estimates of OTU, family, and phylum level were, 0.820, 0.722, and 0.722, respectively (Fig. 3). Taxa belonging to phyla *Euryarchaeota*, *TM6* and *Proteobacteria* and families, *Methanobacteriaceae*, *Sphaerochaetaceae*, and *Succinivibrionaceae* had heritability estimates greater than 0.5. Additionally, 364 OTUs had heritability estimates higher than 0.5 (supplementary Tables S2, S3, and S4). The effective sample size and summary statistics of heritability estimates are shown in supplementary figure S1.



For genome wide association analysis, the estimated additive genetic effects across animals were decomposed into marker effects across OTU, family, and phylum within each group. Chromosomal regions with the largest WGEV for the selected OTU, family, and phylum are shown in Table 1. The identified SNP in the bovine genome and the phylogenetic relationship of the associated taxa are shown in Fig. 4. The 1-Mb chromosomal regions explained 0.32–3.24% (supplementary Table S5) of the phenotypic variation, where the largest variation was associated with OTU4 and family *Prevotellaceae*. Genomic associations were observed across diverse phylogenetic groups where associations were detected across 7 different phyla including the 4 major phyla that represent more than 90% of the rumen bacterial population (Fig. 4). The SNP associated with bacterial taxa were distributed across 7 autosomal chromosomes with chromosome 9 and 27 demonstrating associations at phylum, family and OTU level. Additionally, some taxa at OTU, family and phylum level were associated with 2 different SNP located on different chromosomes. As described previously (Wallace et al., 2019), this suggests the rumen microbiota is a heritable, polygenic trait.

**Different taxonomic levels are under host genetic control.** The majority of the associations were identified at the OTU level. However, phylum and family level associations were also detected on chromosomes 9 and 27 in addition to OTU associations. As such, to determine if higher taxonomic level associations detected are a result of higher abundance of a single OTU, we performed correlation analysis between taxa on chromosomes 9 and 27 (Fig. 5). Correlation analysis demonstrated that some phylum level associations are driven by one family belonging to that phylum (phylum *Fibrobacter* and family *Fibrobacteriaceae*  $r = 1.0$ ; phylum *Proteobacteria* and family

*Succinivibrionaceae*  $r=0.999$ ; phylum *Verrucomicrobia* and family *RFP12*  $r=0.955$ ).

However, other phyla did not show such correlations with family level associations (*Firmicutes*, *Lentisphaerae*, and *Tenericutes*). A similar trend was seen between OTU level associations and family level associations where a few associations were highly correlated (OTU3 and family *RF16*  $r = 0.994$ , and OTU28 and family *Fibrobacteriaceae*  $r = 0.895$ ) (supplementary Figure S2). However, a majority of the associations detected at OTU level were unique and did not have correlated families.

**Host adapted bacterial species in the rumen.** Relatively large SNP associations were detected between genus *Methanobrevibacter*, *Succinoclasticum*, *Prevotella*, and *Fibrobacter*. Among the major genera associated with host genetics *Prevotella* was the most predominant genera. *Succinoclasticum* was significantly associated with both chromosome 1 and 2 similarly *Prevotella* was associated with chromosomes 2, 6, 9, 19, 23 and 27 (Table 1 and supplementary table S6). *Methanobrevibacter* was associated with chromosome 1 and *Fibrobacter* was associated with chromosome 27 (Table 1 and supplementary table S6). As described previously (Wallace et al., 2019), pleiotropic effects (having multiple effects from a single gene) were observed in many taxa. Including SNP that affect closely related taxa such as the associations observed on chromosome 9 for family *Veillonellaceae* and *Clostridiaceae*; and the association between *Prevotella* and *Paraprevotella* on chromosome 23. These observations suggest that genes in these 1 Mb regions can independently or in unity affect bacterial distribution and structure in the rumen. Additionally, the associated 1 Mb region on chromosome 27 is associated with the bacterial community composition of very diverse bacterial communities including phylum *Fibrobacteres*, *Lentisphaerae* and *Tenericutes*. As such,

this genomic region can affect multiple diverse taxa within the rumen microbiome.

Finally, we also detected genomic regions in multiple chromosomes that control the same bacterial genera. For example, OTU30 belonging to genus *Prevotella* was associated with 1 Mb regions in chromosomes 6, and 23. Additionally, OTU2337 belonging to genus *Succiniclasticum* was associated with 1 Mb regions in both chromosome 1 and 2.

Similarly, OTU53 and OTU72 was associated with 1 Mb regions in chromosomes 6, 9, 23 and chromosomes 19, 23. OTU72 was associated with two different 1 Mb regions in chromosome 23.

## DISCUSSION

The rumen microbiome has recently begun to be explored (Anderson et al., 2016; Brulc et al., 2009; Kim et al., 2009; Anderson et al., 2017). Similar to the mammalian intestinal microbiota, the cattle rumen microbiota is dominated by *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (based on type of diet) (Fernando et al., 2010; Fernando et al., 2007; Brulc et al., 2009; Kim et al., 2009). However, cattle rumen microbiota is distinct from intestinal microbiota of monogastric animals because it has a considerable proportion of members of the Phylum *Fibrobacter*, which are involved in fiber digestion, and because the microbial communities in the rumen utilize the diet before the host digestive system. It is well established that diet is a major contributor of rumen microbial community composition (Anderson et al., 2016; Fernando et al., 2010; Henderson et al., 2015; Huws et al., 2018). However, in the current study cattle originated from two locations, whereby 2 of the 3 diets were only represented at one location. Furthermore, heifers only originated from one location and were fed a diet that differed from that fed to steers. This data structure, and the fact that samples were generated across multiple years, makes

parsing these effects impossible. As such, to identify the collective effect of these factors on the rumen bacterial community, we performed PCoA analysis (supplementary figure S3) and PERMANOVA analysis using management type to reflect the collective effect of diet, location, and sex. PERMANOVA analysis displayed a significant effect of management type on microbial community composition suggesting that collectively these factors affected the rumen microbial community composition. As the objective of this study was to identify association between host genetics and the rumen bacterial community composition, we fitted contemporary group, the concatenation of management type and year, in the model used for GWAS to adjust for the variation in the microbial community composition resulting from management type as described in methods. As such, the association observed in this study has been corrected for variations in the rumen bacterial community that can result from diet, sex, location and year.

Studies investigating the effect of host genetics on shaping the microbial community composition are limited. Recent studies in the bovine rumen have demonstrated that the rumen microbiome is influenced by host genetic factors (Li et al., 2019; Sasson, et al 2017; Wallace et al., 2019). As such, the concept of core taxa within the rumen microbiome being controlled by host genetics is intriguing as it lends to the potential to utilize associations between rumen microbiota and genetic markers for marker-assisted selection and management to improve feed efficiency, animal health and microbiome manipulation mediated by selecting for favorable microbial taxa within the rumen.

In this study, we demonstrate that the relative abundance of the most abundant taxa within the rumen microbiome is a “polygenic trait” (Benson et al., 2010) under host genetic control. The pleiotropic effect of host genetics on the rumen microbiome

demonstrates effects at multiple taxonomic levels. Data in this study demonstrates that the influence of host genetics in shaping the rumen microbiome is more effective at lower taxonomic units and in most cases the effect on the microbiome can be very specific. This is clearly demonstrated by the OTU level associations identified in this study. The association identified with the genus *Methanobrevibacter* on chromosome 1 is the first report of an association identified between an archaeal species in the rumen and the bovine host. We also identified family *Succinovibrionaceae*, which has been associated with chromosome 9. A previous study by Wallace et al. identified *Succinovibrionaceae* to be heritable in dairy cows. Other studies have also reported host genetics and the microbiome to be associated with methane emission (Difford et al., 2018) and this association further supports this notion. In the rumen, archaea play a critical role in recycling NADH back to NAD for glycolysis (Church, D. C. 1993; Geier et al., 2016), so that pyruvate in the rumen is spared for VFA production which becomes an energy source for the host (Church, D. C. 1993; Geier et al., 2016). In the absence of methanogens, recycling of NADH is performed by conversion of pyruvate to lactate or ethanol (Church, D. C. 1993), as such the pyruvate available for VFA production is wasted to recycle NADH making the rumen ecosystem less efficient. Therefore, the association observed between the host and the methanogen is directed toward increased energy to the host animal.

We observed associations on chromosome 1 and 2 for genus *Succiniclasticum*. Only one species named *Succiniclasticum ruminis* has been described in this genus (Van Gylswyk, 1995). *Succiniclasticum ruminis* is a common inhabitant in the rumen specialized in its ability to convert succinate to propionate as its sole mechanism of energy production. In

the rumen, succinate is not accumulated as it is rapidly converted to propionate, and *Succiniclasticum ruminis* is considered as the major organism that is involved in this process. As, propionate is the only gluconeogenic volatile fatty acid in the rumen and also provides more ATP to the host than any other VFA produced in the rumen, it is not surprising that the host animal would prefer selective enrichment of microbes that increase

previously identified to be linked with methane emission (Wallace et al., 2015), energy supply to the host. As such the association between the host and the genus *Succiniclasticum* has important consequences towards host performance and wellbeing. The association observed for *Fibrobacter succinogenes* in chromosome 27 also provides evidence to support the notion that the host genome controls for microbes that help increase nutrient metabolism within the rumen. *Fibrobacter succinogenes* is a characterized as one of the key cellulolytic microbes in the rumen (Wu et al., 2017), that help breakdown cellulose in the rumen. Genome sequence of *Fibrobacter succinogenes* S85 (Suen et al., 2011) has revealed that this organism contains 83 glycosyl hydrolases including 33 cellulases and 24 xylanases, 7 pectate lyases and 14 carbohydrate esterases (Suen et al., 2011; Jun et al., 2007). As such *Fibrobacter succinogenes* is a key microbe in cellulose digestion in the rumen and the association with host genetics suggests that this organism is under genetic control to ensure efficient nutrient metabolism in the rumen and that the host animal's energy requirements can be met from metabolites of microbial fermentation. Wallace et al, (2019) also reported the *Fibrobacter succinogenes* as one of the heritable bacteria in dairy cows.

We observed several loci on different chromosomes (2, 6, 9, 19, 23 and 27) to be associated with the genus *Prevotella*. *Prevotella* is a dominant genus found in the rumen and has been implicated in protein and energy metabolism in the rumen (Anderson et al., 2016; Fernando et al., 2010). It is possible that each of the loci may be affecting the compositional changes of different species of *Prevotella* such as *P. ruminicola*, *P. bryanti*, *P. brevis*, and *P. albinensis* which are all been reported to be members of the rumen microbiome (Avgustin et al., 2001). Although, we are unable to classify the OTUs identified at species level resolution, the fact that different OTUs belonging to genus *Prevotella* are associated with different loci suggests that different *Prevotella* species may be controlled by different loci. Recently, Li et al, (2019) reported 19 SNPs to be associated with 14 microbial taxa in the rumen. This study also identified associations between *Paraprevotellaceae* and bovine chromosome 16, further supporting the notion of genus *Prevotella* may be controlled by many genetic loci. Similarly, family *Prevotellaceae* were reported as highly heritable among Nordic Red dairy cows (Wallace et al., 2019).

Describing factors that shape the rumen microbiome is important to improve animal health and performance and here we see a trend of the host selecting for bacterial species that help in nutrient metabolism in the rumen. To further investigate the associations between the taxa identified and the host, we performed “positional candidate gene analysis”. Positional gene candidate analysis helped identify genes located within the 1 Mb regions associated with rumen bacterial species (supplementary table S1 and S5). Many of the 1 Mb chromosomal regions identified did not contain annotated genes within the bovine genome. The only well annotated region in the bovine genome was in

chromosome 9 (supplementary table S1). The 1 Mb regions between 63 and 67 Mb in chromosome 9 demonstrated association at OTU (63–64 Mb), family (63–64 Mb) and phylum (63–67 Mb) level controlling the abundance of both gram-positive and negative bacteria in the rumen. Positional candidate gene analysis in this region identified genes required for innate immunity, specifically AKIRIN2, a downstream effector of the Toll-like receptor (TLR), TNF and IL-1 beta signaling pathways that results in IL-6 production (Stelzer et al., 2016). As such, it is possible that the association of rumen bacteria to this region of the host chromosome may result in cytokine secretion leading to modulation of host immunity. A similar association was observed on chromosome 9, 66–67 Mb region with the Thymocyte-Expressed Molecule Involved In Selection (THEMIS) gene. This gene has been described to play a critical role in thymocyte development and maturation of T-cells (Leavy, 2009) further implicating the interaction between the host genetics and the rumen microbiome in immune modulation.

Additionally, the rumen epithelium is characterized as a stratified squamous epithelium, and has been described as an organ involved in selective absorption of nutrients in the form of volatile fatty acids (VFAs) from rumen bacterial fermentation (Graham and Simmons, 2005). Therefore, active and secondary active transport systems mediate nutrient absorption (Graham and Simmons, 2005; Sehested et al., 1996). Previous studies have demonstrated Claudin family genes to play a role in the formation of the permeability barrier and to help with tight junction formation in the rumen (Graham and Simmons, 2005). In our GWAS, we identified a significant association with claudin-18 (CLDN18) in chromosome 1 in the 132–133 Mb region. This region was associated with the specialized propionate producer *Succiniclasticum ruminis* that utilizes conversion of



succinate to propionate as its sole energy producing reaction (Van Gylswyk, 1995). As such, it is tempting to speculate that this association between claudin-18 and *Succiniclasticum ruminis* is involved in selective VFA absorption to the host. In addition to the described associations above, associations with GDP-Mannose 4,6-Dehydratase (GMDS) in chromosome 23, Alpha-1,4-N-Acetylglucosaminyltransferase (A4GNT) in chromosome 1, and ANXA5 involved in endocytotic and exocytotic pathways in chromosome 6 all suggests implication of the host in modulating the rumen microbes to increase energy absorption. A majority of the SNPs identified by Li et al, (2019) were present in the non-coding region and thus they were unable to identify how the associated taxa may influence host metabolism or health. The few genes identified in that study suggested, the associated regions to increase nutrient absorption to the host. Results presented in this study is consistent with this notion and the associations observed between the host and the microbial species further suggests that the associations between the microbiota and host genotype is directed toward selective absorption of volatile fatty acids from the rumen to increase energy availability to the host animal.

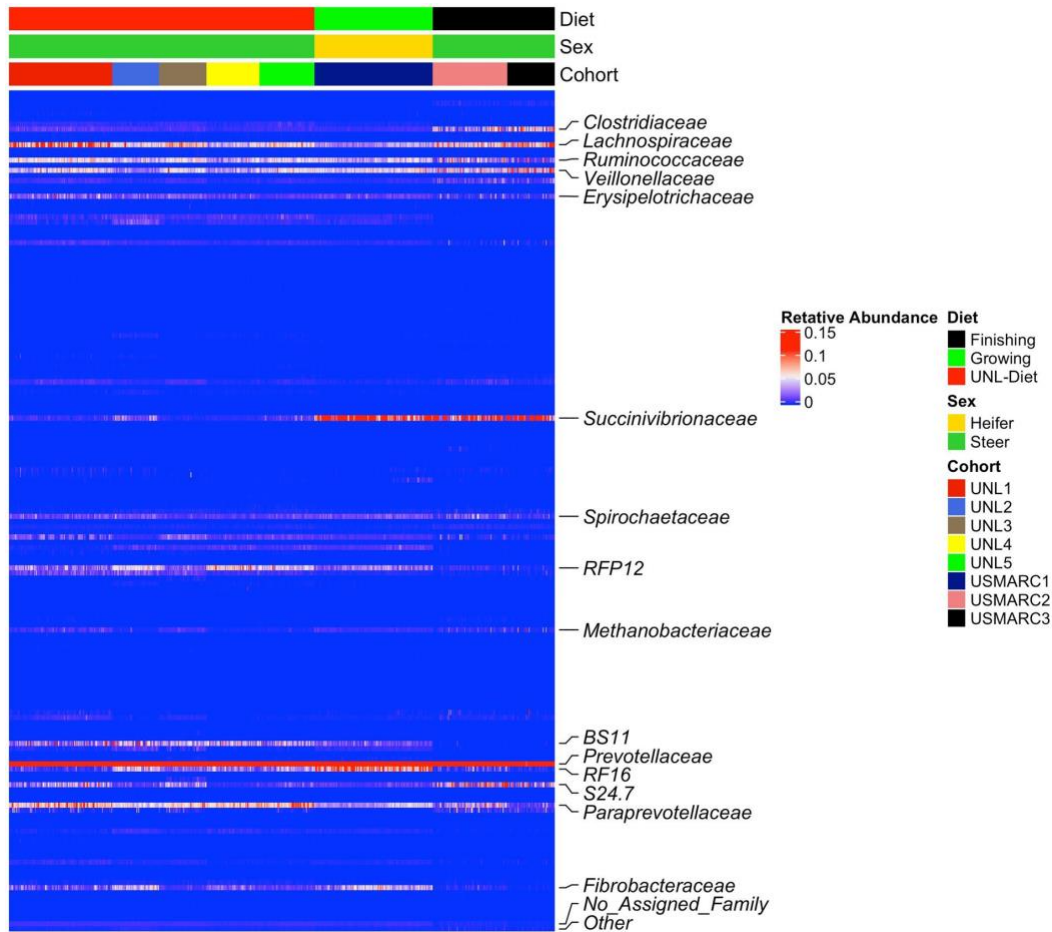
In the ruminant animal, 50–70% of the animal's protein needs and up to 70% of the energy needs (Flint et al., 2004) through the metabolism of rumen microbes. The rumen microbiota can be viewed as an environmental factor that impacts animal health, nutrition and performance. Studies have demonstrated the rumen bacterial species composition to influence feed efficiency, Average Daily Gain (ADG) and intake (Paz et al., 2018). As such demonstrating that heritable trait in the host genome can impact rumen bacterial species composition provides new opportunities to using genome selection to improve animal health and productivity. Future work evaluating the SNP identified herein and

their relative effects in other populations as well as the recruitment of associated bacterial taxa in subsequent generations when parents are selected based on genotypes at these loci would be interesting. Such investigations may lead to the possibility of selection for microbiome manipulation. Additionally, similar associations may exist between the host genotype and the fungal, protozoal and viral populations within the rumen. Future studies focused on other members of the rumen microbiome may shed light into the role of host genetics shaping these microbial populations.

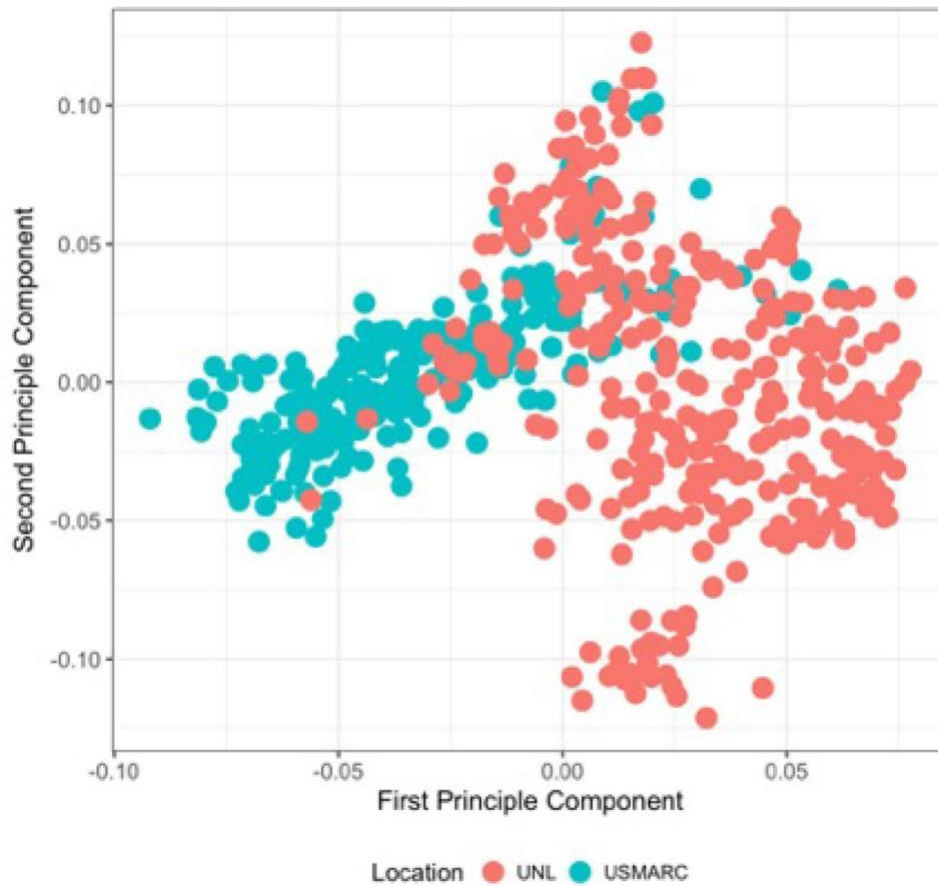
## TABLES AND FIGURES

**Table 1. Chromosomal regions with the largest Window Genomic Estimated Breeding Value (WGEBV) for the selected OTUs, families, and phyla.**

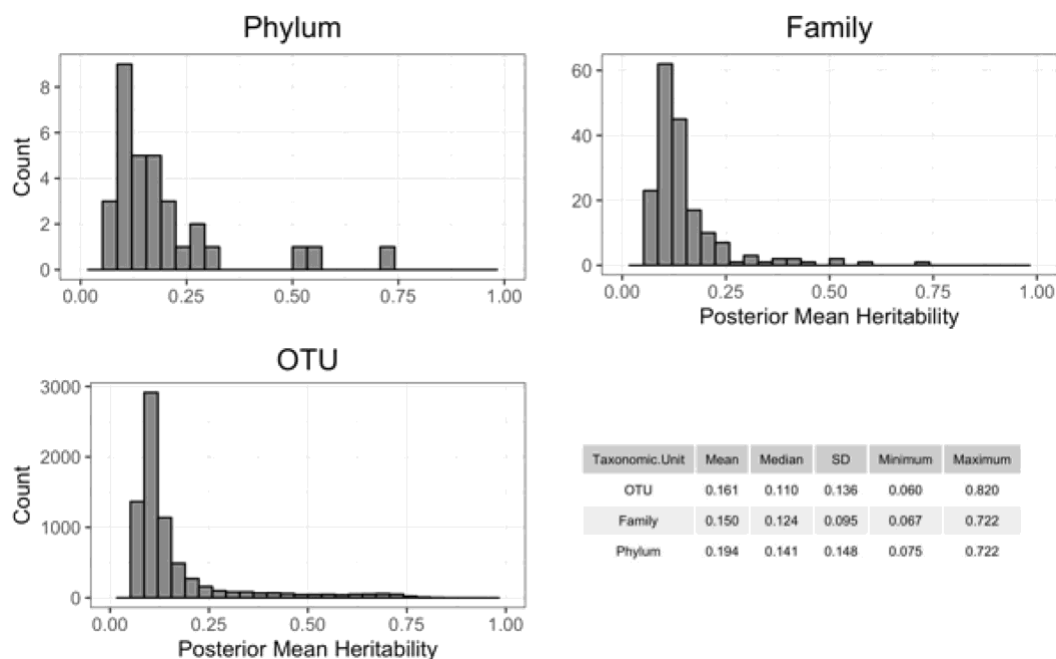
Class	Chromosome	Position (Mb; start–end)	Names
OTU	1	132.0–133.0	41, 57, 2,337
	2	2.0–3.0	21, 2,337, 43
	6	3.0–4.0	27, 30, 53
	9	63.0–64.0	4, 12, 53
	19	3.0–4.0	2,737, 3, 72
	23	0.0–1.0	12, 27, 2,737, 30, 4, 53, 72
	23	51.0–52.5	21, 64, 72
	27	3.0–4.0	19, 28, 3
Family	6	3.0–4.0	<i>BS11, Ruminococcaceae, Succinivibrionaceae</i>
	9	63.0–64.0	<i>Lachnospiraceae, RFP12, Succinivibrionaceae, Veillonellaceae, Clostridiaceae</i>
	23	0.0–1.0	<i>Paraprevotellaceae, Prevotellaceae, RFP12, S24-7, Ruminococcaceae</i>
	27	3.0–4.0	<i>RF16, S24-7, Fibrobacteraceae, Clostridiaceae</i>
Phylum	9	63.0–67.0	<i>Firmicutes, Lentisphaerae, Proteobacteria, Ver-rucomicrobia</i>
	27	3.0–4.0	<i>Fibrobacteres, Lentisphaerae, Tenericutes</i>



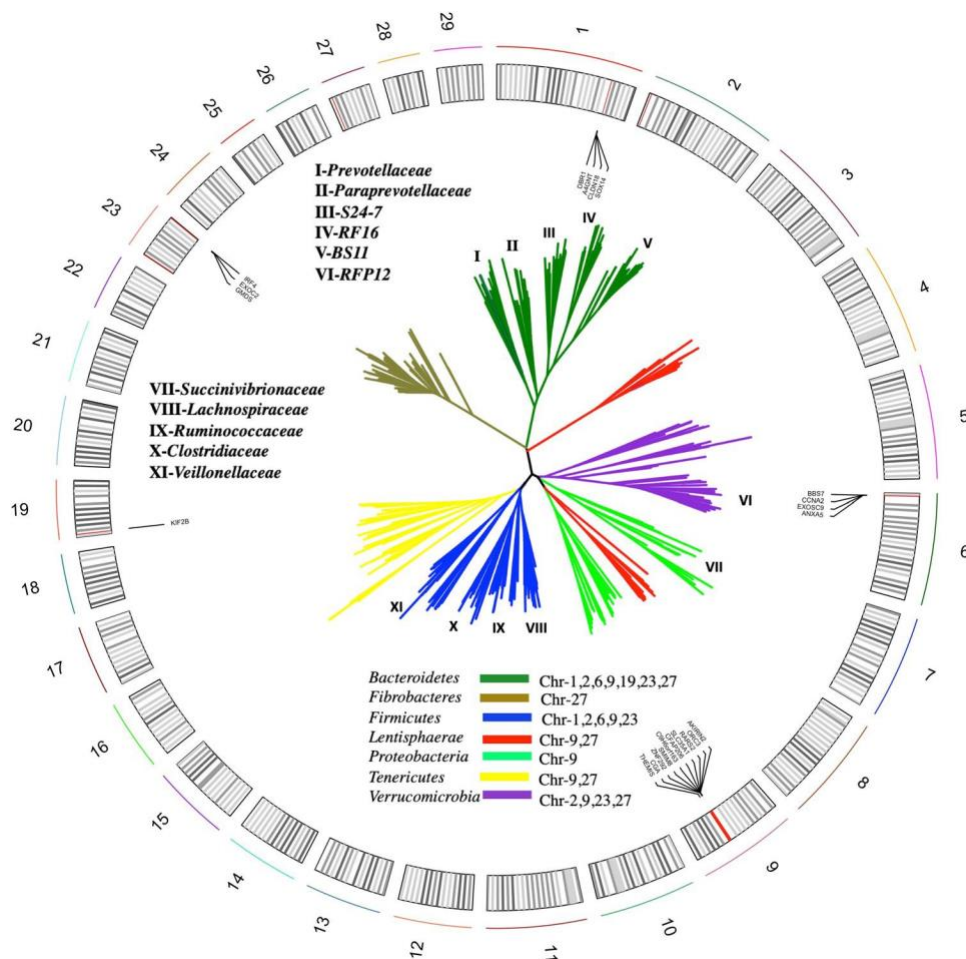
**Figure 1.** Distribution of taxa across all cohorts. The heatmap above displays the abundance and distribution of all taxa identified in at least 1% of the total animals ( $n = 586$ ). The relative abundances of 237 families present within the CMM is shown above. The columns represent the samples and rows represents the relative abundance of each family. Top 17 families which accounts for nearly 90% abundance of all the families are labeled in the heatmap.



**Figure 2.** First and Second principle components of the genomic relationship matrix for USMARC and UNL animals demonstrating limited genomic variation in the two locations. A genomic relationship matrix was constructed for all the animals sampled from USMARC and UNL using the SNP information. The principal component analysis (PCA) was run on genomic relationship matrix and first two principal components (PC1 and PC2) were plotted. USMARC refers to animals that were fed at either the U.S. Meat Animal Research Center and UNL refers to animals fed at the University of Nebraska-Lincoln.

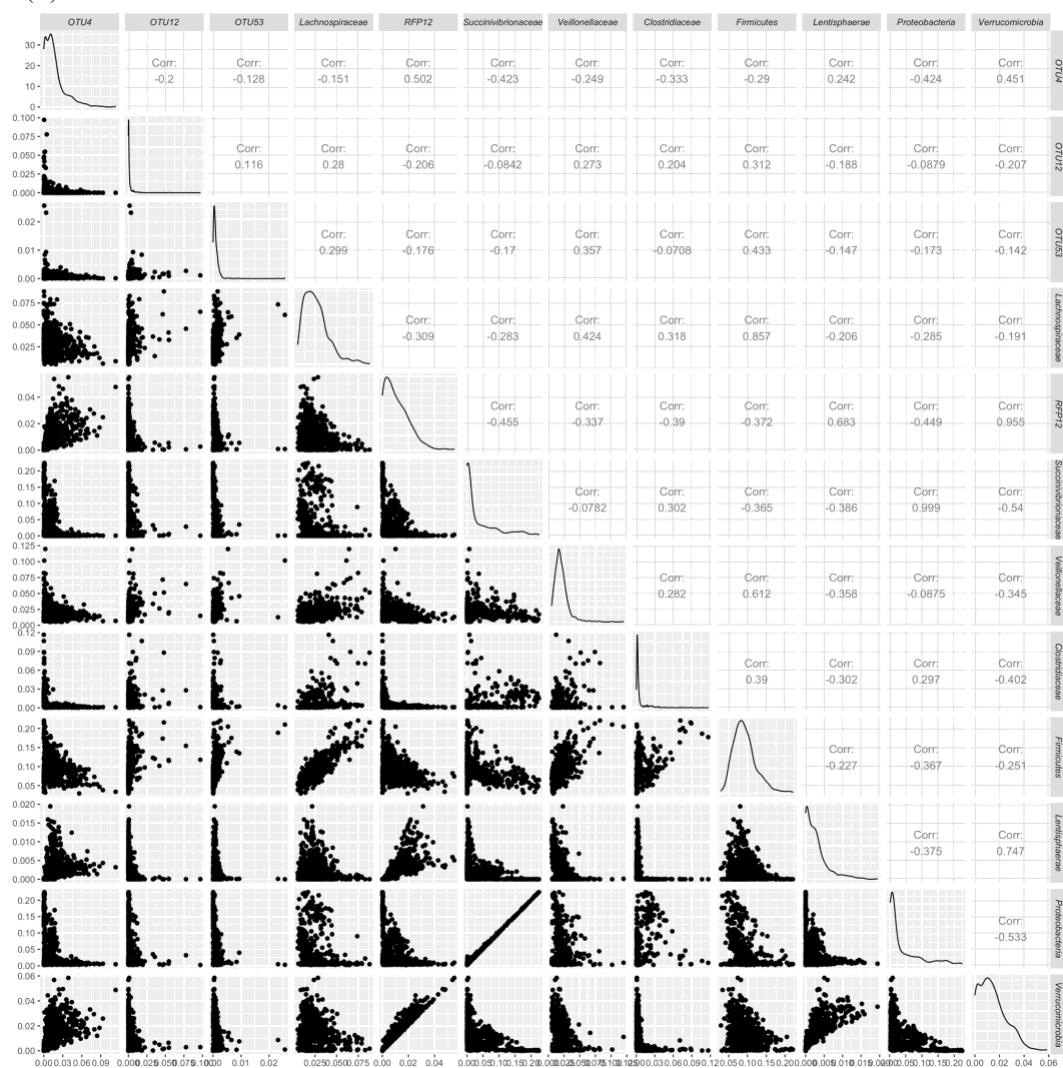


**Figure 3.** Posterior heritability estimates and summary statistics across taxa for phylum, family and operational taxonomic unit (OTU) categories. The relative abundances of Phyla, Families and OTUs were used to estimate the posterior heritability by using the GBLUP model (see “Methods”). The histograms represent the number of Phyla, Families and OTUs that fall into a specific range of posterior heritability. The table shows the mean, median, standard deviation and the minimum and maximum posterior heritability for different taxonomy levels.

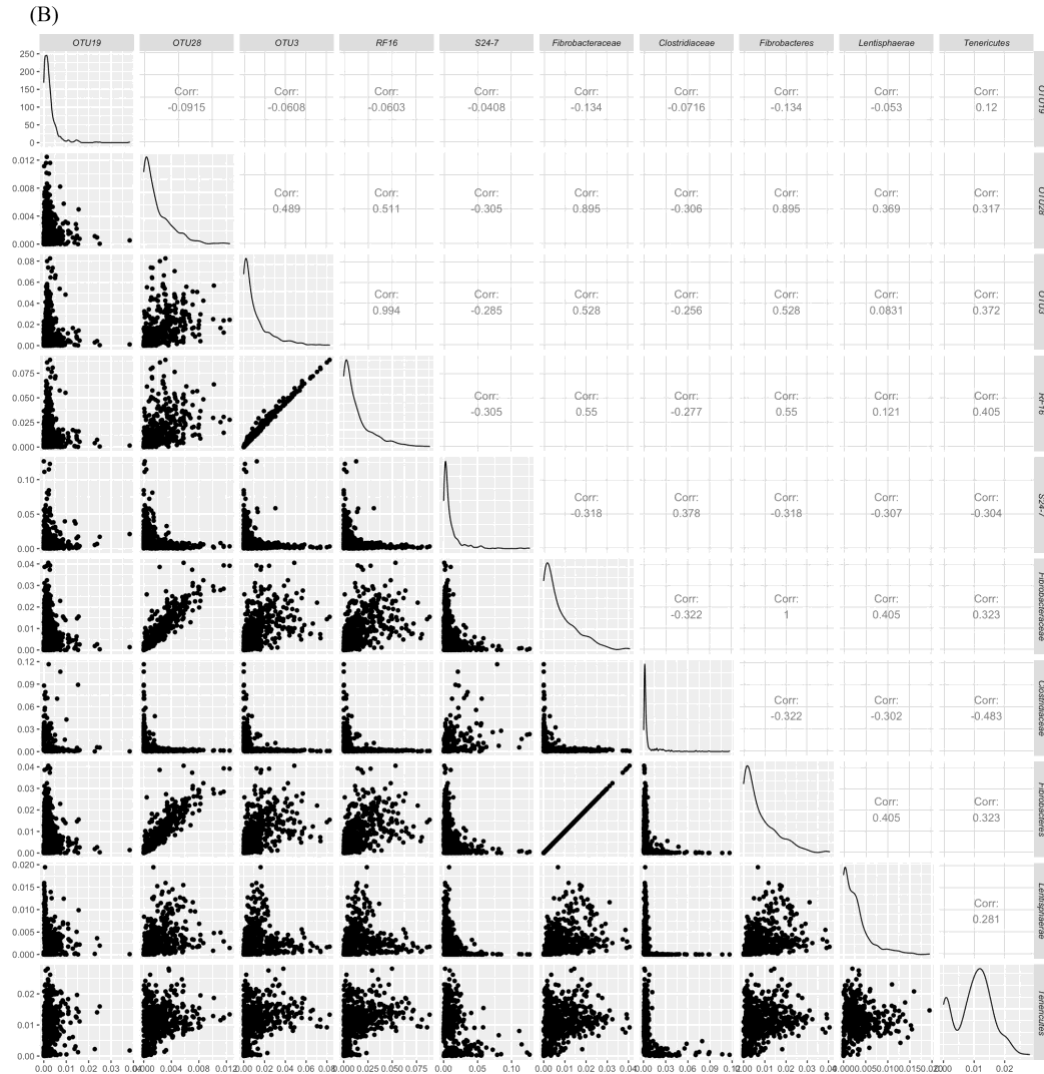


**Figure 4.** SNP mapping of the rumen gut microbiota. The circular diagram depicts the 29 bovine autosomes drawn to scale. Each black line represents 3 Mb region of the chromosome that includes the position of the SNPs used for Genome Wide Association Study. Red lines represent 1 Mb regions (Table 1) that have associations with different bacterial taxa in the rumen. The list of identified genes when annotated in the 1 Mb window are listed close to the region. A representative phylogenetic tree was generated from the rumen bacterial reads using the Interactive tree of life (iTOL). Major phyla are color-coded and associated chromosome(s) are listed with each phyla. The roman numerals represent families as follows; I—*Prevotellaceae*, II—*Paraprevotellaceae*, III—*S24-7*, IV—*RF16*, V—*BS11*, VI—*RFP12*, VII—*Succinivibrionaceae*, VIII—*Lachnospiraceae*, IX—*Ruminococcaceae*, X—*Clostridiaceae*, XI—*Veillonellaceae*. The complete list of annotated genes and their position on each chromosome is listed in supplementary Table S1.

(A)

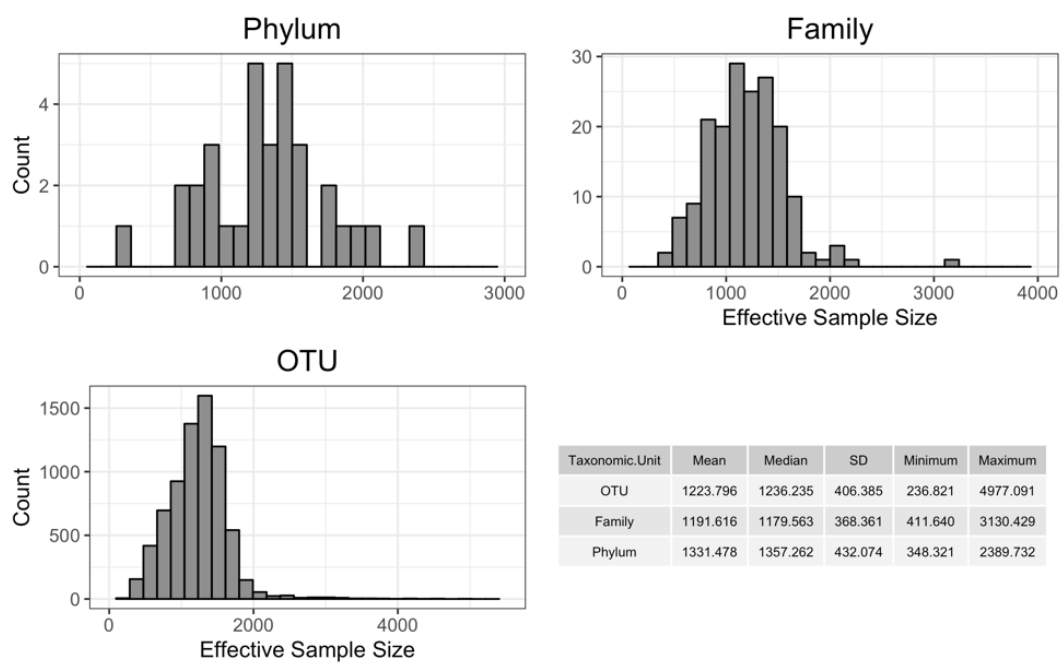




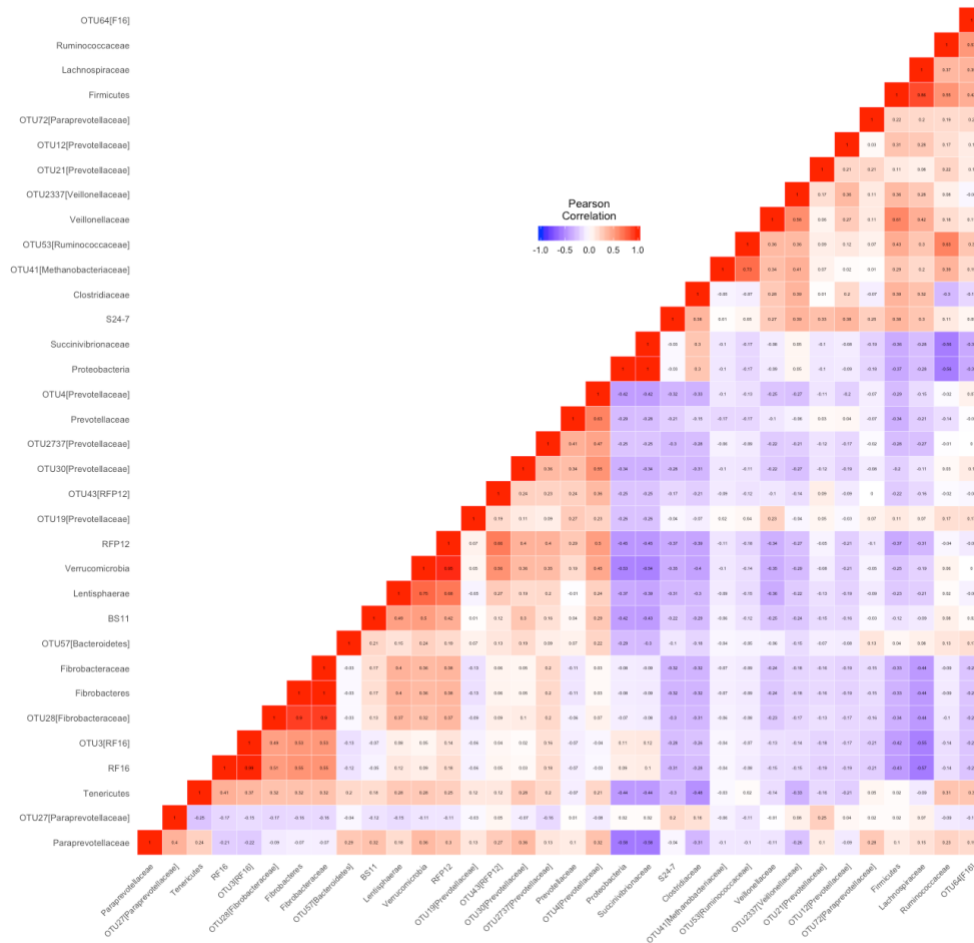


**Figure 5.** Correlations between OTU, Phyla and Family abundance associated with chromosome 9 (A) and chromosome 27 (B). A matrix was generated using  $\log(1 + x)$  transformed relative abundances for OTUs, Phyla and Families associated with each chromosome and pairwise Pearson correlations were calculated and scatter plots and density plots were generated for each chromosome. The pairwise correlations among all the identified taxa on all chromosomes are shown in supplementary figure S3 and further taxonomic information of all identified OTUs can be found in Supplementary Table S1.

**Figure S1: Effective Sample size and summary statistics across taxa for phylum, family and operational taxonomic unit (OTU) categories.**



**Figure S2: Pairwise correlation between all the identified OTUs, Phyla and Families.**



A matrix was generated using  $\log(1+x)$  transformed relative abundances for OTUs, Phyla and Families associated with each chromosome and pairwise Pearson correlations were calculated. The OTUs are listed and classified with Family they belong to. Detailed taxonomy for each OTU can be found in Supplementary Table S2.

**Table S1: The known annotated genes and associated taxa**

<b>Chromosome</b>	<b>chromStart</b>	<b>chromEnd</b>	<b>Gene</b>	<b>Taxonomy</b>
chr1	132002980	132014615	DBR1	OTU41, OTU57, OTU2337
chr1	132063621	132068263	A4GNT	
chr1	132174469	132191685	CLDN18	
chr1	132449821	132450544	SOX14	
chr6	3372938	3411895	BBS7	OTU27, OTU30, OTU53, BS11, Ruminococcaceae, Succinivibrionaceae
chr6	3413085	3419196	CCNA2	
chr6	3419544	3432299	EXOSC9	
chr6	3542634	3575330	ANXA5	
chr9	63127338	63150182	AKIRIN2	OTU4, OTU12, OTU53, Lachnospiraceae, RFP12, Succinivibrionaceae, Veillonellaceae, Clostridiaceae, Firmicutes, Lentisphaerae, Proteobacteria, Verrucomicrobia
chr9	63155999	63225311	ORC3	
chr9	63225446	63299358	RARS2	
chr9	63302745	63334395	SLC35A1	
chr9	63346329	63382321	CFAP206	
chr9	63430800	63458705	C9H6orf163	
chr9	63462320	63474252	SMIM8	
chr9	63520826	63622497	ZNF292	
chr9	63677990	63694859	CGA	
chr9	63762921	63764019	HTR1E	
chr9	64765557	64849621	SNX14	
chr9	64859549	64871772	NT5E	
chr9	64879263	64929945	NT5E	
chr9	65579317	65608088	TBX18	
chr9	66115461	66211054	CEP162	
chr9	66223875	66287509	MRAP2	
chr9	66377195	66503206	CYB5R4	
chr9	66683788	66885925	THEMIS	
chr9	66968685	67594209	PTPRK	
chr19	3876961	3879199	KIF2B	OTU2737, OTU3, OTU72
chr23	51115435	51453575	GMDS	OTU21, OTU64, OTU72, Paraprevotellaceae, Prevotellaceae, RFP12, S24-7, Ruminococcaceae
chr23	51917494	52015703	EXOC2	
chr23	52050984	52063706	IRF4	

**Table S2: Heritability estimates**

Phylum	OTUNumb	h2_mean	h2_sd	ReadCount
Euryarchaeota	32	0.722	0.087	132579
TM6	11	0.561	0.146	32
Proteobacteria	986	0.525	0.096	2539705
Firmicutes	3922	0.300	0.104	4731507
Synergistetes	25	0.277	0.107	21999
Thermi	10	0.263	0.151	75
Bacteroidetes	2605	0.233	0.074	9341349
Spirochaetes	244	0.224	0.088	283694
LD1	1	0.211	0.067	10403
Fibrobacteres	93	0.210	0.070	356499
Nitrospirae	6	0.186	0.069	35
Lentisphaerae	105	0.171	0.048	123215
Verrucomicrobia	354	0.165	0.043	596770
OD1	10	0.156	0.077	67
Tenericutes	595	0.156	0.047	434678
TM7	135	0.152	0.053	144895
Crenarchaeota	6	0.130	0.047	156
Chloroflexi	120	0.130	0.046	24915
Actinobacteria	349	0.129	0.057	48863
Chlamydiae	10	0.124	0.048	34
Acidobacteria	119	0.115	0.042	612
Armatimonadetes	10	0.114	0.041	2500
Planctomycetes	173	0.113	0.052	70521
Gemmatimonadetes	44	0.111	0.045	338
SR1	9	0.108	0.040	35448
GN02	9	0.104	0.043	325
Chlorobi	5	0.095	0.037	79
Elusimicrobia	19	0.095	0.029	14510
Cyanobacteria	5	0.091	0.031	1306
WPS-2	9	0.086	0.026	9880
BRC1	7	0.086	0.033	103
Fusobacteria	9	0.075	0.026	2152

**Table S3: Classification of OTUs Identified**

<b>Chromosome</b>	<b>OTU</b>	<b>OTU Taxonomy</b>
Chromosome 1 [132.0-133.0]	OTU41	OTU41_Genus_Methanobrevibacter
	OTU57	OTU57_Order_Bacteroidales
	OTU2337	OTU2337_Genus_Succiniclasticum
Chromosome 2 [2.0-3.0]	OTU21	OTU21_Genus_Prevotella
	OTU2337	OTU2337_Genus_Succiniclasticum
	OTU43	OTU43_Family_RFP12
Chromosome 6 [3.0-4.0]	OTU27	OTU27_Genus_YRC22
	OTU30	OTU30_Genus_Prevotella
	OTU53	OTU_53_Family_Ruminococcaceae
Chromosome 9 [63.0 – 64.0]	OTU4	OTU4_Genus_Prevotella
	OTU12	OTU12_Genus_Prevotella
	OTU53	OTU53_Family_Ruminococcaceae
Chromosome 19 [3.0-4.0]	OTU2737	OTU2737_Genus_Prevotella
	OTU3	OTU3_Family_RF16
	OTU72	OTU72_Genus_CF231
Chromosome 23 [0.0 – 1.0]	OTU12	OTU12_Genus_Prevotella
	OTU27	OTU27_Genus_YRC22
	OTU2737	OTU2737_Genus_Prevotella
	OTU30	OTU30_Genus_Prevotella
	OTU4	OTU4_Genus_Prevotella
	OTU_53	OTU53_Family_Ruminococcaceae
	OTU72	OTU72_Genus_CF231
Chromosome 23 [51.0–52.5]	OTU21	OTU21_Genus_Prevotella
	OTU64	OTU64_Family_F16
	OTU72	OTU72_Genus_CF231
Chromosome 27 [3.0-4.0]	OTU19	OTU19_Genus_Prevotella
	OTU28	OTU28_Species_succinogenes
	OTU3	OTU3_Family_RF16

**Supplementary Table S4: Heritability estimates**

OTU	h2_mean	h2_sd	ReadCount
4398	0.820	0.038	364
1518	0.814	0.043	561
3362	0.802	0.039	32
4578	0.799	0.046	63
2036	0.781	0.051	178
3671	0.778	0.058	49
2292	0.776	0.056	123
5493	0.776	0.047	59
7156	0.775	0.049	39
9942	0.775	0.056	14
1893	0.775	0.053	2876
2069	0.774	0.061	185
4808	0.774	0.048	549
933	0.771	0.064	964
5203	0.770	0.062	121
4086	0.768	0.048	8539
8251	0.766	0.053	581
417	0.764	0.061	4909
525	0.761	0.062	3122
1220	0.761	0.060	706
3245	0.760	0.051	53
175	0.757	0.060	9634
7456	0.754	0.053	659
6589	0.750	0.064	7526
2283	0.747	0.059	148
41	0.743	0.069	76965

**Table S5: Taxa location and variation explained for each genome wide association.**

Taxonomy	Chromosome	Position (Mb; Start – End)	Percent Variance Explained
OTU41	Chr1	132.0-133.0	0.44
OTU57	Chr1	132.0-133.0	0.38
OTU2337	Chr1	132.0-133.0	0.40
OTU21	Chr2	2.0-3.0	0.55
OTU2337	Chr2	2.0-3.0	0.33
OTU43	Chr2	2.0-3.0	0.37
OTU27	Chr6	3.0-4.0	0.32
OTU30	Chr6	3.0-4.0	0.42
OTU53	Chr6	3.0-4.0	0.55
OTU4	Chr9	63.0 – 64.0	1.17
OTU12	Chr9	63.0 – 64.0	0.62
OTU53	Chr9	63.0 – 64.0	0.36
OTU2737	Chr19	3.0-4.0	0.57
OTU3	Chr19	3.0-4.0	0.44
OTU72	Chr19	3.0-4.0	0.37
OTU12	Chr23	0.0 – 1.0	0.73
OTU 27	Chr23	0.0 – 1.0	0.58
OTU 2737	Chr23	0.0 – 1.0	0.72
OTU 30	Chr23	0.0 – 1.0	0.40
OTU 4	Chr23	0.0 – 1.0	3.24
OTU 53	Chr23	0.0 – 1.0	1.66
OTU 72	Chr23	0.0 – 1.0	2.2



OTU21	Chr23	51.0– 52.5	1.09
OTU64	Chr23	51.0– 52.5	0.39
OTU72	Chr23	51.0– 52.5	0.42
OTU19	Chr27	3.0-4.0	0.85
OTU28	Chr27	3.0-4.0	0.48
OTU3	Chr27	3.0-4.0	1.15
BS11	Chr6	3.0-4.0	0.35
Ruminococcaceae	Chr6	3.0-4.0	0.47
Succinivibrionaceae	Chr6	3.0-4.0	0.44
Lachnospiraceae	Chr9	63.0-64.0	1
RFP12	Chr9	63.0-64.0	0.63
Succinivibrionaceae	Chr9	63.0-64.0	0.65
Veillonellaceae	Chr9	63.0-64.0	1.03
Clostridiaceae	Chr9	63.0-64.0	0.40
Paraprevotellaceae,	Chr23	0.0 – 1.0	0.76
Prevotellaceae	Chr23	0.0 – 1.0	2.06
RFP12	Chr23	0.0 – 1.0	0.37
S24-7	Chr23	0.0 – 1.0	1.71
Ruminococcaceae	Chr23	0.0 – 1.0	0.43
RF16	Chr27	3.0 – 4.0	1.00
S24-7	Chr27	3.0 – 4.0	0.35
Fibrobacteraceae	Chr27	3.0 – 4.0	0.70
Clostridiaceae	Chr27	3.0 – 4.0	0.42
Firmicutes	Chr9	63.0-67.0	1.13
Lentisphaerae,	Chr9	63.0-67.0	0.60

Proteobacteria,	Chr9	63.0-67.0	0.66
Verrucomicrobia	Chr9	63.0-67.0	0.49
Fibrobacteres	Chr27	3.0-4.0	0.71
Lentisphaerae	Chr27	3.0-4.0	0.74
Tenericutes	Chr27	3.0-4.0	0.37

**Table S6: Classification of OTUs Identified**

<b>Chromosome</b>	<b>OTU</b>	<b>OTU Taxonomy</b>
Chromosome 1 [132.0-133.0]	OTU41	OTU41_Genus_Methanobrevibacter
	OTU57	OTU57_Order_Bacteroidales
	OTU2337	OTU2337_Genus_Succiniclasticum
Chromosome 2 [2.0-3.0]	OTU21	OTU21_Genus_Prevotella
	OTU2337	OTU2337_Genus_Succiniclasticum
	OTU43	OTU43_Family_RFP12
Chromosome 6 [3.0-4.0]	OTU27	OTU27_Genus_YRC22
	OTU30	OTU30_Genus_Prevotella
	OTU53	OTU_53_Family_Ruminococcaceae
Chromosome 9 [63.0 – 64.0]	OTU4	OTU4_Genus_Prevotella
	OTU12	OTU12_Genus_Prevotella
	OTU53	OTU53_Family_Ruminococcaceae
Chromosome 19 [3.0-4.0]	OTU2737	OTU2737_Genus_Prevotella
	OTU3	OTU3_Family_RF16
	OTU72	OTU72_Genus_CF231
Chromosome 23 [0.0 – 1.0]	OTU12	OTU12_Genus_Prevotella
	OTU27	OTU27_Genus_YRC22
	OTU2737	OTU2737_Genus_Prevotella
	OTU30	OTU30_Genus_Prevotella
	OTU4	OTU4_Genus_Prevotella
	OTU_53	OTU53_Family_Ruminococcaceae
	OTU72	OTU72_Genus_CF231
Chromosome 23 [51.0– 52.5]	OTU21	OTU21_Genus_Prevotella
	OTU64	OTU64_Family_F16
	OTU72	OTU72_Genus_CF231
Chromosome27 [3.0-4.0]	OTU19	OTU19_Genus_Prevotella
	OTU28	OTU28_Species_succinogenes
	OTU3	OTU3_Family_RF16

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## CHAPTER 3

RUMEN EPITHELIAL TRANSCRIPTOME AND MICROBIOME PROFILES OF  
RUMEN EPITHELIUM AND CONTENTS OF BEEF CATTLE WITH AND  
WITHOUT LIVER ABSCESES

*Portions of this material have previously appeared in the following publication:*

Abbas, W., Keel, B.N., Kachman, S.D., Fernando, S.C., Wells, J.E., Hales, K.E. and Lindholm-Perry, A.K., 2020. Rumen epithelial transcriptome and microbiome profiles of rumen epithelium and contents of beef cattle with and without liver abscesses. *Journal of animal science*, 98(12), p.skaa359. *Used with permission*

**ABSTRACT**

Abscess is the highest cause of liver condemnation and is estimated to cost the beef industry US\$64 million annually. *Fusobacterium necrophorum*, commonly found in the bovine rumen, is the primary bacteria associated with liver abscess in cattle. Theoretically, damage to the rumen wall allows *F. necrophorum* to invade the bloodstream and colonize the liver. The objective of this study was to determine the changes in gene expression in the rumen epithelium and microbial populations adherent to the rumen epithelium and in the rumen contents of beef cattle with liver abscesses compared with those with no liver abscesses. Rumen epithelial tissue and rumen content were collected from 31 steers and heifers with liver abscesses and 30 animals with no liver abscesses. Ribonucleic acid (**RNA**) sequencing was performed on the rumen epithelium, and a total of 221 genes were identified as differentially expressed in the animals with liver abscesses compared with animals with no abscesses, after removal of genes that were identified as a result of interaction with sex. The nuclear factor kappa-

light-chain enhancer of activated B cells signaling and interferon signaling pathways were significantly enriched in the differentially expressed gene (**DEG**) set. The majority of the genes in these pathways were downregulated in animals with liver abscesses. In addition, RNA translation and protein processing genes were also downregulated, suggesting that protein synthesis may be compromised in animals with liver abscesses. The rumen content bacterial communities were significantly different from the rumen wall epimural bacterial communities. Permutational multivariate analysis of variance (PERMANOVA) analysis did not identify global differences in the microbiome of the rumen contents but did identify differences in the epimural bacterial communities on the rumen wall of animals without and with liver abscesses. In addition, associations between DEG and specific bacterial amplicon sequence variants of epimural bacteria were observed. The DEG and bacterial profile on the rumen papillae identified in this study may serve as a method to monitor animals with existing liver abscesses or to predict those that are more likely to develop liver abscesses.

## **INTRODUCTION**

Liver abscesses in feedlot steers have been associated with high- concentrate grain finishing diets. The prevalence of liver abscesses in feedlot steers ranges between 10% and 20% with a slight rise in the prevalence of liver abscesses in beef cattle in the United States over the last decade (Scarath, 2006; Reinhardt and Hubbert, 2015) and liver condemnation rose almost 10% between 2011 and 2016 with abscess accounting for 58% of all liver condemnation (Harris et al, 2017). While the frequency of liver abscesses is mitigated with tylosin phosphate (i.e., Tylan; Reinhardt and Hubbert, 2015), a macrolide

antibiotic, there is pressure to reduce or eliminate the use of prophylactic antibiotics in the United States.

Liver abscesses are the result of bacteria, especially *Fusobacterium necrophorum*, entering the liver through the portal vein as a result of damage to the rumen wall (Nagaraja and Chengappa, 1998). Injury to the rumen wall may be due to acidosis or physical damage from infiltration by ingested hair or debris (Jensen et al., 1954; Amachawadi and Nagaraja, 2016). Large or multiple small liver abscesses are known to reduce cattle body weight gain and feed intake (Brink et al., 1990). The reduction in hot carcass weight of animals with liver abscesses is estimated to reduce producer profitability by US\$38 per animal (Scarth, 2006). Liver abscesses may also affect animal well-being; however, there is currently no effective method available to test whether animals are affected by liver abscesses prior to slaughter.

This study was part of a larger study designed to evaluate whether the essential oil limonene fed as a supplement to cattle would reduce liver abscesses. Essential oils and their components are active against numerous bacterial species, including many Gram-negative pathogens (Dabbah et al., 1970). Limonene is a monocyclic monoterpene found in lemons, orange, and grapefruits (Castillejos et al., 2006) and has been reported to decrease *F. necrophorum* populations in vitro (Samii, 2016). However, the limonene treatment had no effect on liver abscess frequency, microbial bacterial community populations, dry matter intake, average daily gain, or hot carcass weight of cattle on the study (data not published).

The underlying molecular mechanisms involved in liver abscesses have not been well studied, especially those occurring within the rumen. An understanding of the etiology of

liver abscess may aid in the development of tools for the prediction of animals that are more susceptible to liver abscesses. The purpose of this study was to determine whether the expression of various rumen epithelial genes and changes in the microbial populations in the rumen epithelial tissue and content were associated with liver abscesses.

## **MATERIALS AND METHODS**

### **Animal care and use**

The U.S. Meat Animal Research Center (**USMARC**) Animal Care and Use Committee reviewed and approved all animal procedures. The procedures for handling cattle complied with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010).

### **Animal population**

Heifer and steer calves for this study were born in the fall of 2016 and were from the continuous phase of the USMARC Germplasm Evaluation project (Schiermiester et al., 2015). This breeding population includes contributions from the following 18 breeds: Angus, Beefmaster, Brahman, Brangus, Braunvieh, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, Red Angus, Salers, Santa Gertrudis, Shorthorn, South Devon, Simmental, and Tarentaise. Calves were part of a population subset developed to monitor disease susceptibility. In addition, these calves were also part of a larger study to evaluate the use of the essential oil limonene to reduce liver abscesses. Animals were fed a diet of 63% corn, 26.5% wet distillers grain with solubles, 8% corn silage, and either 2.5% mineral and vitamin supplement with monensin (control) or an identical mineral and vitamin supplement with limonene, a generally recognized as a safe essential oil. The experiment included six pens of steers (33 to 34 steers within each pen) and three pens of

heifers (60 to 62 heifers within each pen) fed a control and six pens of steers and three pens of heifers fed the limonene treatment. All cattle were fed once daily at 0800 hours and on dietary treatments for approximately 180 d.

### **Rumen tissue sampling**

A total of 370 heifers and 405 steers were harvested and livers were scored at the packing plant by the same USMARC scientist who had extensive training scoring the livers.

Livers were scored as 0 for no detection of liver abscess, A- for the detection of one small liver abscess or abscesses, A for the detection of multiple small liver abscesses, or A+ for the detection of multiple large lesions. The rumen of animals with liver abscess scores of A+ and control animals with no liver abscesses were tagged for sampling.

Rumens were hung and rumen contents were collected using a sterile tube after incision, and the tube was immediately placed into a bed of powdered dry ice. Two pieces of rumen tissue were sampled from the cranial sac of each rumen after contents were emptied. A 150-cm<sup>2</sup> piece of rumen tissue was removed and placed into a clean resealable bag and placed on ice for rumen wall bacterial microbiome analyses. A 5-cm<sup>2</sup> piece of rumen tissue was placed into a sterile tube and immediately embedded into powdered dry ice for gene expression. Samples remained on dry ice for approximately 4 to 6 h through transport back to the USMARC. Upon arrival, the samples were transferred to an ultracold freezer and stored at -80 °C until further processing.

### **Ribonucleic acid isolation**

Rumen tissue samples were rinsed with sterile water and papillae were clipped from the rumen wall with scissors that were cleaned with RNAZap and RNase/DNase-free water between samples. Total ribonucleic acid (**RNA**) was isolated from the rumen tissue using

the RNeasy Mini Plus kit and QiaShredder columns (Qiagen). Briefly, 800  $\mu$ L of RLT buffer with  $\beta$ -mercaptoethanol was added to 50 to 100 mg of rumen papillae tissue and homogenized for 40 s using an Omni Prep 6-station homogenizer (Omni International, Kennesaw, GA, USA). The homogenate was centrifuged through a QiaShredder column at full speed for 3 min. The RNeasy Mini Plus kit manufacturer's protocol was then followed, and the total RNA was eluted in 50  $\mu$ L of RNase-free water. Total RNA was quantified with a NanoDrop One spectrophotometer (Thermo Scientific, Wilmington, DE). The 260/280 ratios were  $\geq 1.8$ , and the total RNA from each animal was analyzed for quality on a Tapestation 2200 (Agilent, Santa Clara, CA, USA) and produced an average RNA integrity number (**RIN**) of 8.5 with a range of 6.2 to 9.8.

### **RNA sequencing**

Samples were prepared for RNA sequencing (**RNA-Seq**) with the Illumina TruSeq Stranded mRNA High Throughput Sample kit and protocol (Illumina Inc., San Diego, CA, USA). The libraries were diluted to 4 nM in Illumina resuspension buffer. The libraries were sequenced as 75 bp paired-end reads using the 150-cycle high output sequencing kits for the Illumina NextSeq.

### **Processing RNA-Seq data**

The quality of the raw paired-end sequence reads in individual fastq files was assessed using FastQC (version 0.11.5; [www.bioinformatics.babraham.ac.uk/projects/fastqc](http://www.bioinformatics.babraham.ac.uk/projects/fastqc)), and then reads were trimmed to remove adapter sequences and low-quality bases using the Trimmomatic software (version 0.35) (Bolger et al., 2014). The remaining reads were mapped to the Agricultural Research Service-University of California, Davis (ARS-UCD)1.2 genome assembly (Genbank Accession GCA\_002263795.2) using Hisat2

(version 2.1.0) (Kim et al., 2015). The National Center for Biotechnology Information (NCBI) annotation for ARS-UCD1.2 (Release 106) was used to guide the alignment. StringTie (Pertea et al., 2015) was used to determine read counts for each of the 34,624 annotated genes in the ARS-UCD1.2 genome assembly. Genes with low read counts were filtered out of the dataset when there were <15 reads in at least 30 of the samples. This produced a set of 14,288 genes for downstream analyses. Two libraries were removed from the analysis due to low read counts and lower read mapping percentage. The raw sequencing data can be accessed at sequence read archive database with accession number PRJNA555558. The data were analyzed using the DESeq2 package (Love et al., 2014) with the following generalized linear model:

$$Y = \text{Liver Abscess} + \text{Sex} + \text{Limonene} + \text{Liver Abscess} \times \text{Sex} + \text{Liver Abscess} \times \text{Limonene} + \text{Sex} \times \text{Limonene} + \text{Liver Abscess} \times \text{Sex} \times \text{Limonene}$$

Differentially expressed genes (**DEGs**) for the three main effects were identified using DESeq2 ( $P_{\text{FDR}} < 0.05$ ). DEG lists were filtered by removing genes that were significant for any of the four interaction terms.

### **Functional gene annotation and pathway analyses**

Gene functions and pathways of overrepresented DEG ( $P_{\text{FDR}} < 0.05$ ) were determined using the protein analysis through evolutionary relationships (**PANTHER**) classification system (version 13.1) (Mi et al., 2016) and the Database for Annotation, Visualization, and Integrated Discovery (**DAVID**) v6.8 (Huang et al., 2009a, 2009b). Enrichment analysis of gene function was performed using PANTHER's implementation of the binomial

test of overrepresentation. The significance of gene ontology (**GO**) terms was assessed using the default Ensembl *Bos taurus* GO annotation as background for the enrichment analysis. The default parameters for the Kyoto encyclopedia of genes and genomes pathway and gene function analyses in DAVID were used with *B. taurus* annotation and official gene symbols to evaluate genes that were overrepresented in the list of DEG ( $P_{FDR} < 0.05$ ).

Ingenuity pathway analysis (**IPA**; QIAGEN Redwood City, CA, USA; [www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)) was used to identify direct and indirect molecular relationships among DEGs (Krämer et al., 2014). Each of the data sets was imported with a Flexible Format using Gene symbol as the identifier. A core analysis was performed on genes in each set, where a P-value for each network is calculated according to the fit of the user's set of significant genes and the size of the network.

### **Deoxynucleic acid extraction**

Total deoxynucleic acid (**DNA**) was extracted from total rumen content (rumen fluid and solid particles) and rumen papillae samples (0.25 g) using the Mag-Bind Soil DNA 96 Kit (Omega Biotek, Inc., Norwalk, CT, USA) according to the manufacturer's protocol with the modifications described below. During cell lysis, two bead-beating steps were performed in a TissueLyser (Qiagen Inc., Valencia, CA, USA) for 10 min at 20 Hz, and samples were incubated in a 95 °C water bath for 5 min to ensure cell lysis. Following the removal of polymerase chain reaction (**PCR**) inhibitors, nucleic acids were precipitated like the procedure described by Yu and Morrison (2004). Briefly, 850 µL of sample supernatant and 260 µL of sodium acetate (10 mM) were mixed in 1.5 mL Eppendorf tubes, vortexed, and incubated on ice for 5 min followed by a centrifugation at



16,000  $\times$  g for 15 min at 4 °C. One volume (650  $\mu$ L) of supernatant was mixed with one volume of isopropanol and incubated on ice for 30 min followed by a centrifugation at 16,000  $\times$  g for 15 min at 4 °C. The nucleic acid pellet was washed with ice- cold ethanol (70%) and then dried under vacuum for 3 min. The pellet was resuspended in 450  $\mu$ L of Tris (10 mM, pH 8).

### **16S Ribosomal RNA gene amplicon libraries and sequencing**

Amplicon libraries of the 16S ribosomal RNA (rRNA) gene (V4 region) were prepared as described by Kozich et al. (2013). Briefly, each 20  $\mu$ L PCR amplification reaction contained 0.5  $\mu$ L Terra PCR Direct Polymerase Mix (0.625 Units), 7.5  $\mu$ L nuclease- free, sterile water, 10  $\mu$ L 2 $\times$  Terra PCR Direct Buffer, 1  $\mu$ L indexed fusion primers (10  $\mu$ M), and 1  $\mu$ L DNA (20 to 70 ng DNA). The cycling conditions included an initial denaturation of 98 °C for 3 min, followed by 25 cycles of 98 °C for 30 s, 55 °C for 30 s, and 68 °C for 45 s, and a final extension of 68 °C for 4 min. Following amplification, PCR products from each sample were normalized (1 to 2 ng/ $\mu$ L) using the Just-a-Plate 96 PCR Purification and Normalization kit (Charm Biotech, MO, USA) as described by the manufacturer. The normalized libraries were pooled (10  $\mu$ L/ sample) and purified using the Nucleospin Gel and PCR Cleanup kit (Takara Bio USA, Inc., Mountain View, CA, USA) according to the manufacturer's protocol. Libraries were quality controlled using the BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and quantified using the DeNovix QFX Fluorometer (DeNovix dsDNA Fluorescence Quantification Assay). The libraries were sequenced using the Illumina Miseq System (Illumina, San Diego, CA, USA) using the V2 500 cycles kit according to the manufacturer's protocol.

### **16S rRNA gene amplicon sequence data processing**

The Illumina  $2 \times 250$  fastq sequencing files (V4) were processed as described by Callahan et al. (2016a) using R (R Core Team, 2018). The forward and reverse reads were trimmed at a constant length based on their quality score ( $QC > 30$ ), and the reads with more than two expected errors were filtered out from the data (Edgar and Flyvbjerg, 2015). The DADA2 method was used to distinguish the biological variation from PCR or sequencing errors in the filtered reads, and this method infers the sequence variants after removing the substitution errors from the data (Callahan et al., 2016a). The quality-controlled forward and reverse reads were merged and an amplicon sequence variants (**ASVs**) table with abundance was generated. The chimeras were removed from the data (Callahan et al., 2016b), and taxonomy was assigned down to the species level by using the silva (silva\_nr\_v132\_train\_set. fa) database with naive Bayesian classifier method (Wang et al., 2007). The singletons, Archaea, mitochondria, and Cyanobacteria and Rickettsiales taxa were removed from the data. The sequencing depth for each sample was estimated with R package vegan (Oksanen et al., 2017). The R codes used for data processing and analysis are available in the appendix B.

### **Statistical analysis**

The bacterial community differences between rumen content and rumen papillae were tested with nested permutational multivariate analysis of variance (**PERMANOVA**) (Anderson, 2014) with R package BiodiversityR (Kindt and Coe, 2005). For alpha diversity, we calculated the observed species and Simpson's diversity index (1-D), which takes account of the abundances of the species, and differences in diversity between rumen content and rumen papillae were tested with Friedman's test (Friedman, 1937).

The rumen content and rumen papillae microbiome data were analyzed (PERMANOVA) separately to determine whether the differences in the bacterial community were due to changes in health (healthy vs. liver abscess), sex (steers vs. heifers), or diet (control vs. limonene). Bacterial community differences for main effects (diet, sex, and liver abscess) were determined using the Bray–Curtis distance matrix for principal coordinate analysis (PCoA) and PERMANOVA analysis. Alpha diversity (observed species and Simpson's diversity index) was calculated from rumen content and rumen papillae data separately, and its differences for main effects (liver abscess, sex, and diet) were tested with the Wilcox test (Gehan, 1965). The  $P$ -value  $< 0.05$  was considered significant for all the microbiome analysis. The prevalent ASVs ( $n = 194$ ) that were present in at least 50% of the rumen papillae samples were used to run a Pearson correlation with DEG genes ( $n = 221$ ). The  $\log_{10}$ -transformed relative abundances of prevalent ASVs and DEG genes were used to generate a correlation matrix. The correlations lower than 0.5 and higher than  $-0.5$  were considered moderate while correlations higher than 0.5 and lower than  $-0.5$  were considered high correlations. The data visualization and statistical analysis were performed using the R programming language.

## RESULTS

### Liver abscess prevalence and RNA-Seq statistics

A total of 370 heifers and 405 steers were evaluated for liver abscess. Liver abscesses were detected in 12.7% of the heifers and 22.5% of the steers (Table 1). The RNA-Seq libraries from the rumen papillae of a total of 61 steers ( $n = 40$ ) and heifers ( $n = 21$ ) with liver abscess scores of 0 (no abscesses) or A+ (severe) and with or without limonene treatment were prepared and sequenced. We selected 61 crossbred heifers and steers with

and without liver abscesses in order to identify DEGs that are more likely to be robust across sex and population. Fewer heifers were selected because fewer female animals developed liver abscesses. Table 2 presents the number of animals selected by sex, limonene treatment, and liver abscess score. An average of over 42 million 75-bp paired-end reads was generated per animal. Sequence reads were mapped to the *B. taurus* ARS-UCD1.2 genome assembly with an average 98% read mapping rate.

### **DEGs in rumen papillae**

A total of 430 genes were identified as differentially expressed in the comparison between animals with liver abscesses and those with no liver abscess (Supplementary Table S1 in appendix C). The analysis for sex (steers compared with heifers) identified the highest number of DEG with a total of 3,396 ( $P_{FDR} < 0.05$ ; Supplementary Table S2). Only two genes (*PRKCA* and *LOC112441508*) were identified in the analysis for limonene vs. control animals ( $P_{FDR} < 0.05$ ; Supplementary Table S3 in appendix C). After removing genes identified in the analyses of the interaction terms, there were 221 DEGs associated with liver abscesses (Supplementary Table S1). Of these, 96 were upregulated and 125 were downregulated in animals with no liver abscesses.

### **Gene functions and pathways**

Pathways identified by DAVID as overrepresented from the list of DEGs for liver abscess were the transcriptional misregulation in cancer, cyclic adenosine monophosphate (**cAMP**) signaling, ribosome, cardiac muscle contraction, and nuclear factor kappa B (**NFKB**) signaling pathways ( $P < 0.05$ ; Table 3). The PANTHER database search identified the chemokine and cytokine signaling pathway as the pathway with five overrepresented genes (*RELA*, *RELB*, *RGS4*, *TYK2*, and *VWA2*). The genes

identified in this pathway included: *RELA*, *RELB*, *TYK2*, *RGS4*, and *VWA2*. IPA produced the canonical pathways EIF2 signaling, interferon signaling, inducible nitric oxide synthase (iNOS) signaling, hepatic fibrosis/hepatic stellate cell activation, and role of JAK1, JAK2, and TYK in interferon signaling pathways ( $P < 0.05$ ; Table 4).

### **Differences between rumen content and rumen papillae microbiota**

The processing of 3,655,108 reads from all the samples resulted in 4,477 unique ASVs, and 89% and 50% of the identified ASVs were assigned taxonomy at phylum and genus level, respectively. The nested PERMANOVA analysis of the Bray–Curtis distance matrix showed that the bacterial community composition associated with rumen content was different ( $P < 0.001$ ) from rumen papillae. At the phyla level, significant differences were identified in five of the phylogenetic groups. The rumen content had a higher abundance of Bacteroidetes and Proteobacteria, whereas rumen papillae had a higher abundance of *Firmicutes*, *Synergistetes*, and *Epsilonbacteraeota* (*Campylobacterota*). At the genus level, there were significant differences in 15 genera. The rumen content had a higher abundance of 10 of the genera, including *Prevotella\_1*, *Prevotella\_7*, and *Succinivibrionaceae\_UCG-001*, whereas rumen papillae had a higher abundance of 5 genera, including *Prevotellaceae\_UCG-001*, *Desulfovibrio*, and *Butyrivibrio\_2* (Figure 1A). The PCoA analysis plot (Figure 1B) indicated clustering based on sample location (rumen content vs. rumen epithelium) and the first two principal coordinate components (PC1 and PC2) explained 25% and 13.5% of the total variation, respectively. Alpha diversity analysis showed that rumen papillae samples to have a higher number of associated bacterial species ( $P = 0.05$ ) and high diversity index ( $P < 0.001$ ) as compared with rumen content (Figure 1C and Table 5).

### **Bacterial community differences between healthy and liver-abscessed animals**

The rumen papillae samples were further analyzed for phylum and genus level differences between animals without and with liver abscesses. At the phyla level, liver-abscessed animals had a higher ( $P < 0.05$ ) abundance of Proteobacteria and tendency ( $P = 0.053$ ) for a higher abundance of *Epsilonbacteraeota* (*Campylobacterota*) as compared with healthy animals (Figure 2A). At the genus level, there were significant differences in four genera. The epimural microbiota of liver-abscessed animals had a higher abundance of *Succinivibrionaceae\_UCG-001* and, as a note, a strong tendency for higher *Campylobacter* ( $P = 0.053$ ), whereas healthy animals had a significantly higher abundance of *Prevotellaceae\_UCG-001*, *Butyrivibrio\_2*, and *Syntrophococcus* ( $P < 0.05$ ). For rumen papillae data, the PERMANOVA analysis showed that limonene supplementation did not affect the epimural bacterial communities, but a difference in epimural bacterial communities was observed between heifers and steers ( $P = 0.048$ ). The Bray–Curtis distance matrix was used to generate the PCoA plot (Figure 2B), which did not show any clustering between animals with and without liver abscesses, but PERMANOVA analysis showed that healthy and liver-abscessed animals had different ( $P = 0.02$ ) epimural microbiota. For rumen content data, PERMANOVA analysis showed that diet, sex, and health of the animal did not affect ( $P > 0.05$ ) the bacterial communities in rumen content.

### **Interaction between rumen papillae-associated microbiome and rumen epithelial expressed genes**

A wide range of correlations ( $-0.52$  to  $0.67$ ) was observed. There were 165 ASVs that exhibited at least a moderate correlation ( $-0.3 > R > 0.3$ ; 1,433 positive and 640

negative) to at least one of 221 DEG genes, and 34 of these ASVs exhibited moderate correlations to a minimum of 20 genes and were plotted in a Heatmap (Supplemental Figure 1). The ASV51 (*Butyrivibrio\_2*) exhibited the highest number of associations overall with 96 moderate correlations with DEG genes (76 positive and 20 negative), and a significant number of associations were observed with other ASVs, including in order, ASV2 (*Dialister*;  $n = 82$ ), ASV425 (*RBG-16-49-21*;  $n = 77$ ), ASV165 (*U29-B03*;  $n = 68$ ), and ASV213 (*Treponema\_2*;  $n = 65$ ). Collectively, five ASVs that were taxonomically identified as *Butyrivibrio\_2* exhibited 271 moderate correlations. At a higher degree, there were 25 ASVs that exhibited at least a high correlation (Supplementary Table S5,  $-0.5 > R > 0.5$ ; 48 positive and 3 negative) to at least one of 37 DEG genes as shown in a Heatmap (Figure 3), and 10 of these ASVs exhibited a high correlation to more than one of 12 genes. The ASV68 alone was positively correlated with expression differences of nine DEGs, including *EFNA2* and *RELB*, and this ASV identified as the genus *Shuttleworthia*. The most significant five positive and five negative correlations between prevalent ASVs and DEG genes are shown in Figure 4, and correlations between the ASVs that identify with taxonomical groups of bacteria associated with bovine liver abscesses and DEG genes are shown in Figure 5.

## DISCUSSION

Because the etiology of liver abscess is thought to stem from damage to the rumen epithelial tissue, we chose to evaluate the transcriptome and microbiome of rumen papillae from heifers and steers with and without liver abscesses. While we do not know when these animals started developing liver abscesses, it is likely that they began to form during dietary adaptation or during the initial phase of feedlot finishing rations. However,

the evaluation for liver abscesses and the collection of tissue for this study were performed at the time of slaughter. Currently, there is no test or assay to determine when an animal develops liver abscesses; thus, performing this study at harvest seemed a reasonable time point. To our knowledge, this is the first study to evaluate the expression of genes in the rumen, and the rumen epimural bacterial communities for differences associated with liver abscesses detected at slaughter.

The animals used in this study were part of a larger study to evaluate whether limonene was an effective supplement to reduce liver abscesses. The limonene treatment did not reduce the incidence of liver abscesses or have any effect on performance characteristics (data not published). The transcriptome data from this study support these findings with only two genes differentially expressed between limonene- treated and untreated animals. There were approximately 3,400 genes identified as differentially expressed by sex in the rumen papillae in this study. Many of these genes were members of canonical pathways involved in growth and energy production. There are known differences in the finishing production of heifers and steers (Williams et al., 1993) that may offer insight into the pathways identified. Growth curves for heifers and steers on a feedlot diet are different (Sorensen, 1972), and mature body weight has long been associated with sex (Emmans, 1997; Zinn et al., 2008). Growth and body weight gain are complex, polygenic traits, and with additional variation due to sex, it stands to reason that genes involved in cellular energy production, transcription, and protein synthesis would be differentially expressed between steers and heifers.

Animals with liver abscesses exhibited differences in the rumen expression of inflammatory response genes. The wgenes *LY96*, *RELA*, *RELB*, and *TRIM25* were



identified by DAVID as overrepresented in the NFkB pathway and the genes *IFI6*, *RELA*, and *TYK2* were associated with the interferon signaling pathway by IPA. Four of these six genes (*RELA*, *TRIM25*, *IFI6*, and *TYK2*) were downregulated in the cattle with liver abscesses; two (*RELB* and *LY96*) were upregulated. The lower expression of cytokines in the NFkB pathway does not appear to be a common mechanism for disease or tissue damage, so this may be a rather novel finding, as NFkB is a common host response to microbial pathogens. However, some pathogens have mechanisms to inhibit host inflammatory and immune responses by inhibiting the NFkB pathway during various stages of their lifecycle (Rahman and McFadden, 2011). For example, *Bordetella pertussis* produces an adhesin that induces the early activation of the NFkB pathway but longer exposure to the adhesin inhibits NFkB activation (Abramson et al., 2008; Rahman and McFadden, 2011).

Several ribosomal genes were identified as overexpressed in the ribosome pathway by DAVID (*RPL22*, *RPL38*, *RPL39*, *RPS25*, and *RPS27L*) and the EIF2 signaling pathway by IPA (*RPL22*, *RPL38*, *RPL39*, *RPL36AL*, *RPS25*, *RPS27L*, *EIF3A*, and *IGF1R*). All of the ribosomal genes were downregulated in the rumen tissue of animals with liver abscesses compared with those without, while *EIF3A* and *IGF1R* were upregulated. The reduced expression of ribosomal genes may suggest that protein synthesis is compromised in the rumen tissue of animals with liver abscesses. While this is the first report of the transcriptome of the rumen in animals with and without liver abscesses, there is precedence for the involvement of EIF2 phosphorylation in the liver of mice fed a high fructose diet, which causes nonalcoholic fatty liver disease. Mice that were deficient

in EIF2 $\alpha$  phosphorylation showed adverse effects on antioxidant capacity, inflammation, and cell viability (Choi et al., 2017).

While we believe that it may be challenging to visualize small lesions that could allow bacteria to move from the rumen to the liver, we did visually inspect the rumen samples and rumen epithelium at the time of collection, and the tissues were similar in appearance for animals without and with liver abscesses. Not only it is possible that a chronic abscessed liver might alter some cytokine signals in distant tissues like the rumen, but it is also a possibility that the lower level of cytokine genes expressed in the rumen of some animals predisposes calves to the infiltration of *F. necrophorum*, which ultimately causes liver abscesses.

Several bacteria have been associated with liver abscesses in cattle, including *F. necrophorum* and *Trueperella pyogenes* (Amachawadi and Nagaraja, 2016). The rumens of cattle are a reservoir for *F. necrophorum*, and we evaluated the potential relationships between the rumen bacterial communities and liver abscesses. More importantly, the rumen epithelium is a barrier to *F. necrophorum*, and the epimural bacteria that populate the papillae not only could be important to rumen function but might also impact the ability of *F. necrophorum* to infiltrate from the rumen into the blood that goes to the liver. From a community diversity standpoint, the bacterial community structure of the rumen content was distinctly different from the epimural populations found on the rumen papillae in this study and that of Reyes et al. (2019). Although differences in populations between the rumen content and epimural bacteria were expected, there is little available literature reporting these differences (Dinsdale et al., 1980; Reyes et al., 2019). The predominance of the phylum Bacteroidetes in the rumen observed in this current research

is well recognized (Myer et al., 2017; Reyes et al., 2019). In contrast, the epimural populations were predominated by Firmicutes which, although studies are fewer, agrees with previous research (Chen et al., 2011; Petri et al., 2013). The relationships between dietary supplementation of limonene or gender on the community structure of the rumen content bacteria were minimal and not significant relative to diversity analyses or PERMANOVA analysis. Furthermore, the relationship between liver abscess status and bacterial community population structure of the rumen content was not significant. Considering that the rumen content collected for the current study was done at harvest, it would appear that acute population shifts attributed to diet shifts or acidosis may be transient and not reflected in the long-term community structure.

A PERMANOVA analysis of the epimural bacterial communities collected from the rumen papillae did indicate differences in the animals without and with liver abscesses, and significant differences at the phyla level were observed. Animals with liver abscesses had a higher abundance of Proteobacteria, which is a major phylum of phylogenetically related but phenotypically diverse bacteria that includes a variety of opportunistic pathogens (Rizzatti et al., 2017). Considering that this phylum was observed to be more abundant in the rumen content than on the papillae in general, the observation that its higher abundance in the epimural population for the liver-abscessed animal suggests a dysbiosis of the rumen epimural bacteria in the diseased animal. The phylum Epsilonbacteraeota, also recognized as Campylobacterota (Waite et al., 2017), exhibited a strong tendency for greater abundance in the liver-abscessed epimural bacterial populations, and this phylum includes the genera *Campylobacter*, *Helicobacter*, and

*Arcobacter*, all pathogenic species found in food animals that invade epithelial tissue (Chan et al., 1992; Wooldridge and Ketley, 1997; Levican et al., 2013).

At the genus level, the epimural population differed significantly for only a few bacterial groups between the animals with and without liver abscesses. The genus *Butyrivibrio\_2* was found almost exclusively in the epimural population relative to the rumen content, and this same genus was of greater abundance on the papillae of the healthy animals. Furthermore, the genus *Butyrivibrio\_2* had five ASVs that were moderately correlated with transcription of 221 genes, over 13% of the moderate correlations of bacteria and DEGs, and at higher correlations included two ASVs that had significant associations with the transcription of 8 rumen tissue genes. Feeding a concentrate diet has previously been shown to increase the epimural *Butyrivibrio* levels in the rumen of goats and increased expression of Toll-like receptors in the rumen epithelium using quantitative polymerase chain reaction (Liu et al., 2015). In the current study, the epimural abundance of ASV51, a member of *Butyrivibrio\_2* genus, was highly and positively correlated with the rumen epithelial expression of *CREB3L2* and *ETV6*, two transcriptional regulator genes that were observed to be differentially expressed in the animals based on the presence or absence of liver abscesses. The genus *Shuttleworthia* is a bacterial group associated with periodontal disease (Downes et al., 2002). This bacterial group was significantly lower in the epimural population and, within the epimural population, was found to be differentially abundant in the animals with liver abscesses; however, this bacterial group stood out in the analyses as it was highly and positively correlated with nine DEGs, including *EFNA2*, a gene associated with epithelial development, and *RELB*, a gene associated with immune function.

Several disease-associated bacteria were observed in the current study. The genus *Campylobacter* has been previously associated with rumen epimural populations, particularly in animals fed low-grain diets (Wetzels et al., 2016; Petri et al., 2018), and its observation in the current study was not surprising. The microbial composition of liver abscesses in cattle has been described previously and *Campylobacter* was the 15th most abundant genus reported (Weinroth et al., 2017). *Campylobacter* was the only predominant taxa group observed in both the current research with the epimural bacteria and this previous research with liver abscesses. Previous research did not observe changes in *Campylobacter* when animals were subjected to subacute rumen acidosis (Wetzels et al., 2016), so the observed strong tendency for *Campylobacter* to be associated with the epimural population of cattle that exhibited liver abscesses is novel and needs further study. Interestingly, *Campylobacter* is recognized as intestinal invasive bacterial species (Wooldridge and Ketley, 1997), and there were significant associations between *Campylobacter* abundance and rumen epithelial gene expression of *DTX4* and *ICOSLG*, two genes that play a role in immune function. The genus *Bacteroides* was identified as the most abundant taxa in liver abscesses (Weinroth et al., 2017). While no differences were observed of this bacterial group in the epimural bacteria of healthy and liver-abscessed animals, an ASV that taxonomically classified as the species *Bacteroides heparinolyticus* was associated with rumen epithelial expression of the *C3* gene, a gene associated with antimicrobial and inflammation activities. In addition, two ASVs that taxonomically clustered into an order Bacteroidales group were significantly associated with differences in several DEGs, but this bacterial group was specifically associated with significant reductions in the expression of *TYK2*, a gene that plays a role in immune

function and differentially expressed in animals with liver abscesses. The phylum Synergistetes was significantly associated with the epimural population, and this phylum is often associated with soft tissue disease (Vartoukian et al., 2007). However, neither the phyla nor genera members were observed to be differentially abundant in the rumen epimural samples of the cattle with or without liver abscesses. The genus *Pyramidobacter* is a member of the phylum Synergistetes, and this genus was associated with significant reductions in the expression of *TYK2*, a gene associated with cytokine gene expression. There are currently no methods to predict whether an animal is more susceptible to liver abscesses or determine whether the animal currently has liver abscesses prior to viewing the liver at harvest. A method to determine susceptibility or identify animals with liver abscess prior to harvest could be a useful tool for producers to monitor these animals more closely or to manage them differently. This study identified expression differences between inflammatory response genes in the rumen tissue of animals with liver abscesses compared with control animals upon slaughter. To better understand the molecular mechanisms of liver abscesses prior to or during development, samples from live animals must be evaluated. However, the collection of rumen tissue on large numbers of live animals is time-consuming and invasive. There is evidence that animals with liver abscesses can be distinguished from healthy animals by collecting blood. A recent study by Macdonald et al. (2017) showed that there were differences in plasma and blood parameters in cattle with and without liver abscesses. Because inflammatory signals are a means for tissues to communicate, it is possible that the differences we have detected in inflammatory response genes or proteins in the rumen may also be reflected in the circulating blood or may cause changes in the levels of other genes or proteins that could

be detected in the blood, serum, or plasma of animals with liver abscesses. This study was a first step in evaluating the mechanisms in the rumen papillae that may be contributing to or were the result of damage to the rumen wall and the development of liver abscesses in cattle on a finishing diet. This study produced several functional candidate genes and epimural bacterial differences to pursue to gain more insight into the molecular mechanisms underlying liver abscess development in cattle.

### **Supplementary Data**

Supplementary data are available at *Journal of Animal Science* online.

Supplementary Figure S1: Heatmap of the moderately correlated ( $-0.3 > R > 0.3$ ) ASVs and DEG genes present in cattle rumen papillae. The prevalent ASVs ( $n = 194$ ; present in at least 50% of the rumen papillae samples) were used to run a Pearson correlation with DEG genes ( $n = 221$ ). The  $\log_{10}$ -transformed relative abundances of prevalent ASVs and DEG genes were used to generate correlation matrix. The x-axis is representing the ASVs, whereas the DEG genes are on y-axis.

Supplementary Table S1. DEGs associated with the main effect of sex. The expression is presented as the  $\log_2$ -fold change. Green cells are genes that were upregulated in steers compared with heifers, and red represents genes that were downregulated in steers (Appendix C).

Supplementary Table S2. Genes differentially expressed in the rumen papillae of steers and heifers treated with the essential oil limonene vs. control animals (no limonene). The expression is presented as the  $\log_2$ -fold change. Green cells are genes that were upregulated in animals treated with limonene vs. control animals, and red represents

genes that were downregulated. Genes with gray cells indicate those that were excluded because they were also significant for sex.

Supplementary Table S3. Genes differentially expressed in the rumen papillae of steers and heifers with and without liver abscesses. The expression is presented as the log<sub>2</sub>-fold change. Green cells are genes that were upregulated in animals with liver abscess compared with those with no liver abscesses, and red represents genes that were downregulated. Genes with gray cells indicate those that were excluded because they were also significant for sex or limonene (Appendix C).

Supplementary Table S4. List of rumen epimural bacterial ASVs that met criteria for abundance and distribution and used to establish the correlations with genes differentially expressed in the rumen papillae of steers and heifers with and without liver abscesses

Supplementary Table S5. Correlations between most abundant rumen epimural bacterial ASVs and genes differentially expressed in the rumen papillae of steers and heifers with and without liver abscesses.



## TABLES AND FIGURES

### *Abbreviations*

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DAVID	database for annotation visualization, and integrated discovery
DEG	differentially expressed genes
DNA	deoxynucleic acid
GO	gene ontology
IPA	Ingenuity pathway analysis
NFKB	nuclear factor kappa-light-chain-enhancer of activated B cells
PANTHER	protein analysis through evolutionary relationships
PCoA	principal coordinate analysis
PCR	polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
RNA	ribonucleic acid
RNA-Seq	ribonucleic acid sequencing
USMARC	U.S. Meat Animal Research Center

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**Table 1. Liver abscess scores for steers (n=405) and heifers (n=370).**

<b>Liver Score<sup>1</sup></b>	<b>Steers</b>	<b>Heifers</b>
0	313	323
A	18	11
A-	14	18
A+	60	18

<sup>1</sup> Liver abscess scores of 0= no liver abscess present; A= ; A-= ; A+= .

**Table 2. Steers and heifers selected for the study by limonene or control treatment and presence or absence of liver abscess.**

<b>Treatment Group<sup>1</sup></b>	<b>Steers</b>	<b>Heifers</b>
LIM(-) ABS (-)	11	6
LIM(-) ABS (+)	11	5
LIM (+) ABS (-)	8	5
LIM (+) ABS (+)	10	5

<sup>1</sup> LIM: limonene. (+) treated with limonene in the feed supplement. (-) no limonene in the supplement. ABS: Abscess. (+) animals with severe liver abscess (score of A+). (-) animals with no detectable liver abscess (score of 0).

**Table 3. Pathway analysis performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8<sup>2,3</sup> using list of differentially expressed genes for liver abscesses.**

<b>Term</b>	<b>Count</b>	<b>P</b>	<b>Genes</b>
Transcriptional misregulation in cancer	6	0.02	<i>IGF1R, RELA, TSPAN7, ETV6, ETV5, HIST1H3G</i>
cAMP signaling pathway	6	0.03	<i>HCN2, ADRB2, ATP1B3, RELA, CREB3L2, SLC9A1</i>
Ribosome	5	0.03	<i>RPS25, RPL22, RPS27L, RPL38, RPL39</i>
Cardiac muscle contraction	4	0.03	<i>COX7A1, ATP1B3, SLC9A1, UQCRCB</i>
NF-kappa B signaling pathway	4	0.05	<i>LY96, RELA, RELB, TRIM25</i>

**Table 4. Canonical pathways identified by Ingenuity Pathway Analysis (IPA) using list of differentially expressed genes for severe liver abscesses.**

<b>Canonical Pathway</b>	<b>P-value</b>	<b>#Genes</b>	<b>Gene Names</b>
EIF2 Signaling	0.0003	8	<i>EIF3A, IGF1R, RPL22, RPL38, RPL39, RPL36AL, RPS25, RPS27L</i>
Interferon Signaling	0.003	3	<i>IFI6, RELA, TYK2</i>
iNOS Signaling	0.005	3	<i>LY96, RELA, TYK2</i>
Hepatic fibrosis/ hepatic stellate cell activation	0.01	5	<i>COL7A1, IGF2, IGF1R, LY96, RELA</i>
Role of JAK1, JAK2 and TYK2 in interferon signaling	0.01	2	<i>RELA, TYK2</i>

Table 5. Alpha Diversity of rumen content and rumen papillae.

Sample Source	Observed Bacterial Species		Simpson's Diversity Index (1-D)		<i>P</i> - <i>Value</i> <sup>†</sup>
	Rumen Content	Rumen Papillae	Rumen Content	Rumen Papillae	
Sample Source	274±63	303±64	0.95±0.03	0.98±0.01	<0.001
	(n=59)	(n=59)			
Liver Abscesses	282±60	308±71	0.96±0.03	0.98±0.01	>0.05
	265±66	298±56	0.95±0.04	0.98±0.01	
Sex	272±63	301±66	0.96±0.03	0.98±0.01	>0.05
	280±65	308±60	0.94±0.04	0.98±0.01	
Diet	274±73	310±68	0.95±0.04	0.98±0.01	>0.05
	274±53	297±60	0.96±0.03	0.98±0.01	

<sup>†</sup>Alpha diversity differences between different sample sources (rumen content vs rumen papillae) were tested with Friedman's test.

<sup>‡</sup>Alpha diversity differences between different variables (Healthy vs Liver Abscess, Steer vs Heifers and HYE vs HNE) within rumen content or rumen papillae samples were tested with Wilcoxon test.

**Supplemental Table 2: Genes differentially expressed in the rumen papillae of steers and heifers treated with the essential oil limonene versus control animals (no limonene).**

Gene <sup>1</sup>	Log2FoldChange <sup>2</sup>	P-value <sup>3</sup>	Adjusted P-value <sup>4</sup>
CTSK	-0.274855513	2.95E-07	0.004135663
LDLRAD3	2.58548559	8.32E-07	0.004155353
LOC101906347	-0.182983556	8.88E-07	0.004155353
CASP4	0.231789854	3.02E-06	0.010581061
KIAA2013	-0.171172014	4.04E-06	0.011330277
PSAP	-0.133759729	8.55E-06	0.017133712
PCDHGC3	-0.701561509	8.41E-06	0.017133712
GSDMB	0.388074584	1.13E-05	0.017411678
ITIH4	-1.191195159	1.24E-05	0.017411678
CCM2L	-0.580658093	1.21E-05	0.017411678
STRIP1	-0.046189118	2.24E-05	0.028629232
PKN3	-0.54130257	2.66E-05	0.031166302
TMEM127	-0.264972434	3.16E-05	0.033018852
TMEM259	-0.12676328	3.29E-05	0.033018852
PRKCA	1.751905843	5.08E-05	0.041920278
MUSTN1	-0.501715682	4.93E-05	0.041920278
SERPINC1	-0.426415455	4.84E-05	0.041920278
OGN	-0.878906087	5.79E-05	0.042743188
DDX10	-0.129326664	5.48E-05	0.042743188
LOC614208	1.231294743	6.18E-05	0.043398055
RIPOR2	-0.237452713	6.84E-05	0.045739219
LOC112441508	-0.171154151	8.05E-05	0.045775208
TJAP1	-0.229196985	7.67E-05	0.045775208
SMIM19	0.37012665	7.83E-05	0.045775208
LOC112444206	0.097582842	8.15E-05	0.045775208

1 Genes in green are up-regulated in steers vs heifers and genes in red are down-regulated in animals treated with limonene compared to control animals. Genes shown in black were genes identified from an interaction with sex.

2 Log2 fold changes identified in the analysis of animals treated with limonene versus control animals (Limonene vs Control).

3 Nominal P-values.

4 FDR-corrected P-values.

**Supplementary Table 4: List of rumen epimural bacterial ASVs that met criteria for abundance and distribution and used to establish the correlations with DEGs.**

ASVs	Taxonomy
ASV_1	<i>g_Succinivibrionaceae_UCG-001</i>
ASV_2	<i>g_Dialister</i>
ASV_3	<i>Prevotella_7 ruminicola</i>
ASV_4	<i>g_Prevotella_1</i>
ASV_5	<i>g_Prevotella_7</i>
ASV_6	<i>g_Prevotellaceae_UCG-001</i>
ASV_7	<i>f_Family_XIII</i>
ASV_8	<i>g_Prevotella_7</i>
ASV_9	<i>g_Ruminococcaceae_NK4A214_group</i>
ASV_10	<i>f_Lachnospiraceae</i>
ASV_11	<i>f_Prevotellaceae</i>
ASV_12	<i>g_Prevotellaceae_UCG-001</i>
ASV_13	<i>g_Prevotella_1</i>
ASV_14	<i>g_Dialister</i>
ASV_15	<i>g_Prevotella_7</i>
ASV_16	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_17	<i>g_Prevotella_1</i>
ASV_18	<i>f_Prevotellaceae</i>
ASV_19	<i>g_Campylobacter</i>
ASV_20	<i>g_Prevotella_7</i>
ASV_21	<i>g_Oribacterium</i>
ASV_22	<i>g_Prevotella_7</i>
ASV_23	<i>f_Ruminococcaceae</i>
ASV_24	<i>g_Rikenellaceae_RC9_gut_group</i>
ASV_25	<i>f_Veillonellaceae</i>
ASV_26	<i>g_Prevotellaceae_UCG-001</i>
ASV_27	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_28	<i>f_Muribaculaceae</i>
ASV_29	<i>f_p-2534-18B5_gut_group</i>
ASV_30	<i>g_Prevotella_1</i>
ASV_31	<i>Mitsuokella jalaludinii</i>
ASV_32	<i>g_Desulfovibrio</i>
ASV_33	<i>g_Rikenellaceae_RC9_gut_group</i>
ASV_34	<i>g_Shuttleworthia</i>
ASV_35	<i>g_Prevotella_1</i>
ASV_36	<i>g_Succiniclasticum</i>
ASV_37	<i>f_Family_XIII</i>
ASV_38	<i>g_Prevotella_1</i>
ASV_39	<i>o_Clostridiales</i>
ASV_40	<i>f_Muribaculaceae</i>
ASV_42	<i>g_Prevotella_7</i>
ASV_43	<i>Bacteroides heparinolyticus</i>
ASV_44	<i>Desulfovibrio fairfieldensis</i>
ASV_45	<i>f_Christensenellaceae</i>
ASV_46	<i>g_Prevotellaceae_UCG-001</i>
ASV_47	<i>f_Veillonellaceae</i>
ASV_48	<i>g_Shuttleworthia</i>
ASV_49	<i>o_Bacteroidales</i>
ASV_50	<i>g_Succiniclasticum</i>



ASV_51	<i>g_Butyrivibrio_2</i>
ASV_52	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_55	<i>g_Ruminococcaceae_UCG-005</i>
ASV_56	<i>g_Prevotella_1</i>
ASV_57	<i>g_Acidaminococcus</i>
ASV_59	<i>g_Shuttleworthia</i>
ASV_60	<i>g_Lachnospiraceae_NK4A136_group</i>
ASV_63	<i>f_Lachnospiraceae</i>
ASV_64	<i>g_Treponema_2</i>
ASV_65	<i>g_Prevotella_1</i>
ASV_66	<i>g_Prevotella_7</i>
ASV_68	<i>g_Shuttleworthia</i>
ASV_69	<i>g_Treponema_2</i>
ASV_70	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_71	<i>g_Shuttleworthia</i>
ASV_72	<i>g_Ruminococcaceae_UCG-005</i>
ASV_73	<i>g_Succiniclasticum</i>
ASV_74	<i>g_Prevotellaceae_UCG-001</i>
ASV_75	<i>f_Lachnospiraceae</i>
ASV_76	<i>g_Desulfobulbus</i>
ASV_78	<i>g_Rikenellaceae_RC9_gut_group</i>
ASV_80	<i>g_Selenomonas</i>
ASV_81	<i>g_Ruminococcaceae_UCG-014</i>
ASV_82	<i>g_Treponema_2</i>
ASV_84	<i>g_Prevotella_1</i>
ASV_85	<i>g_Desulfovibrio</i>
ASV_86	<i>f_F082</i>
ASV_87	<i>g_Ruminococcaceae_NK4A214_group</i>
ASV_89	<i>g_Prevotella_1</i>
ASV_91	<i>f_p-2534-18B5_gut_group</i>
ASV_92	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_93	<i>g_Ruminococcaceae_NK4A214_group</i>
ASV_94	<i>o_Bacteroidales</i>
ASV_97	<i>k_Bacteria</i>
ASV_98	<i>Selenomonas bovis</i>
ASV_99	<i>g_Prevotellaceae_NK3B31_group</i>
ASV_100	<i>g_Mogibacterium</i>
ASV_102	<i>Ruminococcus_1 flavefaciens</i>
ASV_104	<i>g_Lachnospiraceae_UCG-008</i>
ASV_105	<i>g_Ruminococcaceae_UCG-002</i>
ASV_106	<i>f_Muribaculaceae</i>
ASV_109	<i>g_Candidatus_Saccharimonas</i>
ASV_110	<i>g_Prevotella_1</i>
ASV_111	<i>g_Lachnoclostridium_1</i>
ASV_113	<i>g_Treponema_2</i>
ASV_118	<i>f_Lachnospiraceae</i>
ASV_119	<i>g_Saccharofermentans</i>
ASV_120	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_121	<i>g_Syntrophococcus</i>
ASV_122	<i>g_Lachnospiraceae_UCG-002</i>
ASV_124	<i>Mitsuokella multacida</i>
ASV_127	<i>f_Family_XIII</i>
ASV_128	<i>g_Butyrivibrio_2</i>

ASV_129	<i>f_Erysipelotrichaceae</i>
ASV_133	<i>g_Lachnospiraceae_UCG-010</i>
ASV_134	<i>g_Ruminococcaceae_UCG-002</i>
ASV_138	<i>f_Bacteroidales_RF16_group</i>
ASV_139	<i>g_Butyrvibrio_2</i>
ASV_140	<i>g_Butyrvibrio_2</i>
ASV_141	<i>o_Bradymonadales</i>
ASV_142	<i>g_U29-B03</i>
ASV_143	<i>g_Prevotella_9</i>
ASV_144	<i>g_Prevotella_1</i>
ASV_147	<i>o_Clostridiales</i>
ASV_154	<i>g_Comamonas</i>
ASV_155	<i>g_Butyrvibrio_2</i>
ASV_157	<i>f_Muribaculaceae</i>
ASV_159	<i>f_Rhodocyclaceae</i>
ASV_160	<i>g_Prevotella_1</i>
ASV_161	<i>g_Lachnospiraceae_UCG-008</i>
ASV_162	<i>g_Rikenellaceae_RC9_gut_group</i>
ASV_163	<i>g_Ruminococcaceae_UCG-014</i>
ASV_165	<i>g_U29-B03</i>
ASV_166	<i>f_Neisseriaceae</i>
ASV_167	<i>f_Christensenellaceae</i>
ASV_168	<i>o_Mollicutes_RF39</i>
ASV_169	<i>g_Pyramidobacter</i>
ASV_171	<i>g_Saccharofermentans</i>
ASV_173	<i>g_Erysipelotrichaceae_UCG-009</i>
ASV_176	<i>g_Moryella</i>
ASV_178	<i>f_Veillonellaceae</i>
ASV_181	<i>f_F082</i>
ASV_183	<i>g_Syntrophococcus</i>
ASV_185	<i>g_Bifidobacterium</i>
ASV_186	<i>g_Christensenellaceae_R-7_group</i>
ASV_187	<i>g_Syntrophococcus</i>
ASV_188	<i>f_Veillonellaceae</i>
ASV_189	<i>g_Synergistes</i>
ASV_190	<i>g_Prevotella_1</i>
ASV_191	<i>g_Prevotella_1</i>
ASV_194	<i>o_Clostridiales</i>
ASV_196	<i>g_Ruminococcus_1</i>
ASV_198	<i>g_Howardella</i>
ASV_211	<i>f_Ruminococcaceae</i>
ASV_213	<i>g_Treponema_2</i>
ASV_216	<i>o_Absconditabacteriales_(SR1)</i>
ASV_218	<i>g_Fretibacterium</i>
ASV_219	<i>g_Ruminococcaceae_UCG-009</i>
ASV_223	<i>f_Ruminococcaceae</i>
ASV_228	<i>g_Syntrophococcus</i>
ASV_234	<i>g_Anaerovorax</i>
ASV_237	<i>g_Shuttleworthia</i>
ASV_238	<i>g_Ruminococcaceae_NK4A214_group</i>
ASV_242	<i>f_Prevotellaceae</i>
ASV_244	<i>g_Erysipelotrichaceae_UCG-006</i>
ASV_253	<i>g_Prevotellaceae_UCG-001</i>

ASV_265	<i>f_Lachnospiraceae</i>
ASV_267	<i>Lactobacillus ruminis</i>
ASV_268	<i>o_Bacteroidales</i>
ASV_271	<i>f_Erysipelotrichaceae</i>
ASV_281	<i>o_Mollicutes_RF39</i>
ASV_286	<i>f_Lachnospiraceae</i>
ASV_287	<i>f_F082</i>
ASV_291	<i>g_Rikenellaceae_RC9_gut_group</i>
ASV_292	<i>g_Prevotellaceae_UCG-004</i>
ASV_295	<i>g_Syntrophococcus</i>
ASV_299	<i>g_Butyrvibrio_2</i>
ASV_304	<i>f_Erysipelotrichaceae</i>
ASV_307	<i>g_Selenomonas_3</i>
ASV_309	<i>g_Butyrvibrio_2</i>
ASV_312	<i>g_Christensenellaceae_R-7_group</i>
ASV_318	<i>g_Defluviitaleaceae_UCG-011</i>
ASV_319	<i>f_Erysipelotrichaceae</i>
ASV_322	<i>o_Mollicutes_RF39</i>
ASV_325	<i>f_Ruminococcaceae</i>
ASV_327	<i>g_Ruminococcaceae_UCG-014</i>
ASV_336	<i>g_Butyrvibrio_2</i>
ASV_342	<i>f_Erysipelotrichaceae</i>
ASV_348	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_349	<i>g_Atopobium</i>
ASV_365	<i>f_Muribaculaceae</i>
ASV_395	<i>f_Rikenellaceae</i>
ASV_398	<i>f_Family_XIII</i>
ASV_423	<i>f_Erysipelotrichaceae</i>
ASV_425	<i>g_RBG-16-49-21</i>
ASV_441	<i>g_Christensenellaceae_R-7_group</i>
ASV_442	<i>g_Anaerovibrio</i>
ASV_449	<i>o_WCHB1-41</i>
ASV_482	<i>g_Ruminococcaceae_UCG-009</i>
ASV_488	<i>g_Ruminococcaceae_UCG-003</i>
ASV_514	<i>g_Erysipelotrichaceae_UCG-009</i>
ASV_557	<i>g_Syntrophococcus</i>
ASV_732	<i>g_Lachnospiraceae_NK3A20_group</i>
ASV_847	<i>o_Clostridiales</i>
ASV_1132	<i>o_Clostridiales</i>

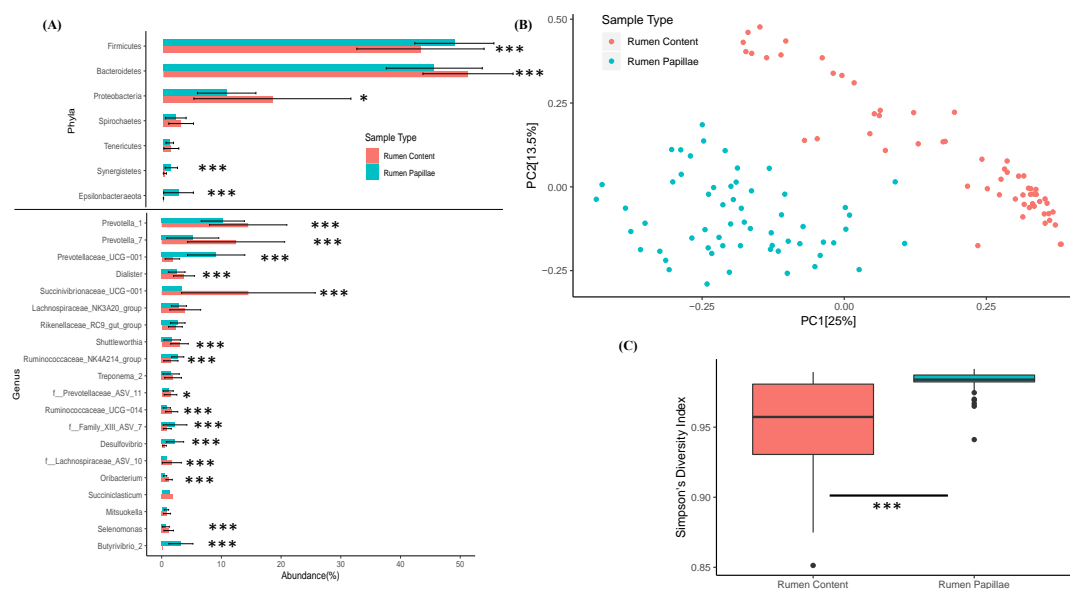
**Figure 1.**

Figure 1. The phylum and genus level differences in relative abundance between rumen papillae and rumen content. (A) Bar plot with error bars is showing the phylum and genus level differences in relative abundance between rumen papillae and rumen content. The y-axis is representing the phyla and genera with at least 1% relative abundance. The Friedman's test was used to see the differences in relative abundances of different phyla and genera between rumen content and rumen papillae samples (#,  $P < 0.1$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ). (B) The PCoA plot is showing the bacterial community differences between rumen papillae and rumen content samples. Bray Curtis distance matrix was used to run the PCoA and PERMANOVA analysis. Each dot represents the bacterial community of individual sample and distance between two dots shows the difference in bacterial community between two samples. (C) This plot is showing the Simpson's diversity index (y-axis) in rumen content and rumen papillae samples (x-axis). The Friedman's test was used to test the difference in alpha diversity between sample sources.

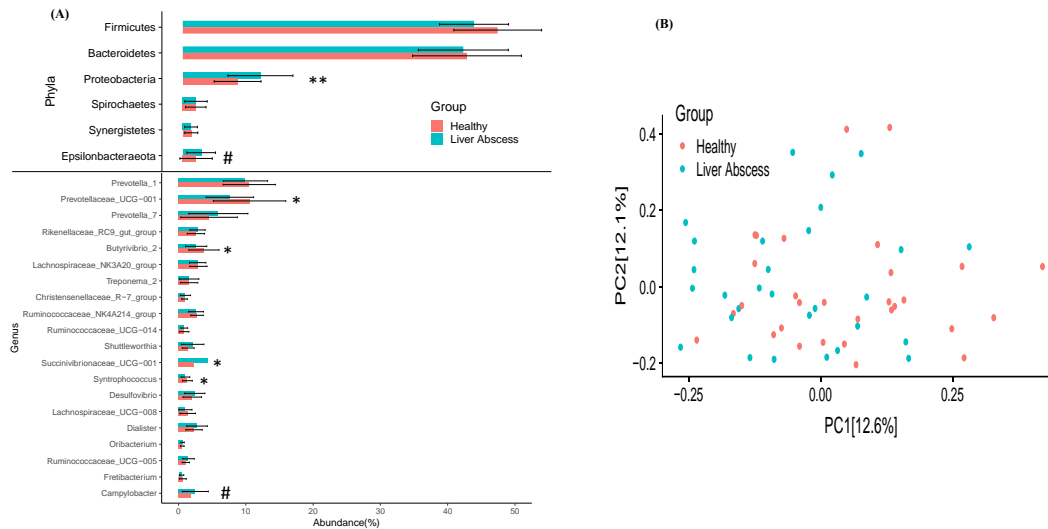
**Figure 2.**

Figure 2. Phylum and genus differences in the rumen papillae of cattle with and without liver abscesses. (A) The bar plot with error bars is showing the phylum and genus level difference in relative abundance between healthy and liver abscessed animals. The phyla and genera with at least 1% abundances are shown on y-axis and their abundances on x-axis. The difference in relative abundance of different phyla and genera between healthy and liver abscessed animals were tested with Wilcoxon test (#,  $P < 0.1$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ). (B) The bray Curtis distance matrix was used to run PCoA and PERMANOVA analysis. The first two components (PC1 and PC2) of PCoA were plotted to see the bacterial community differences between healthy and liver abscessed animals.

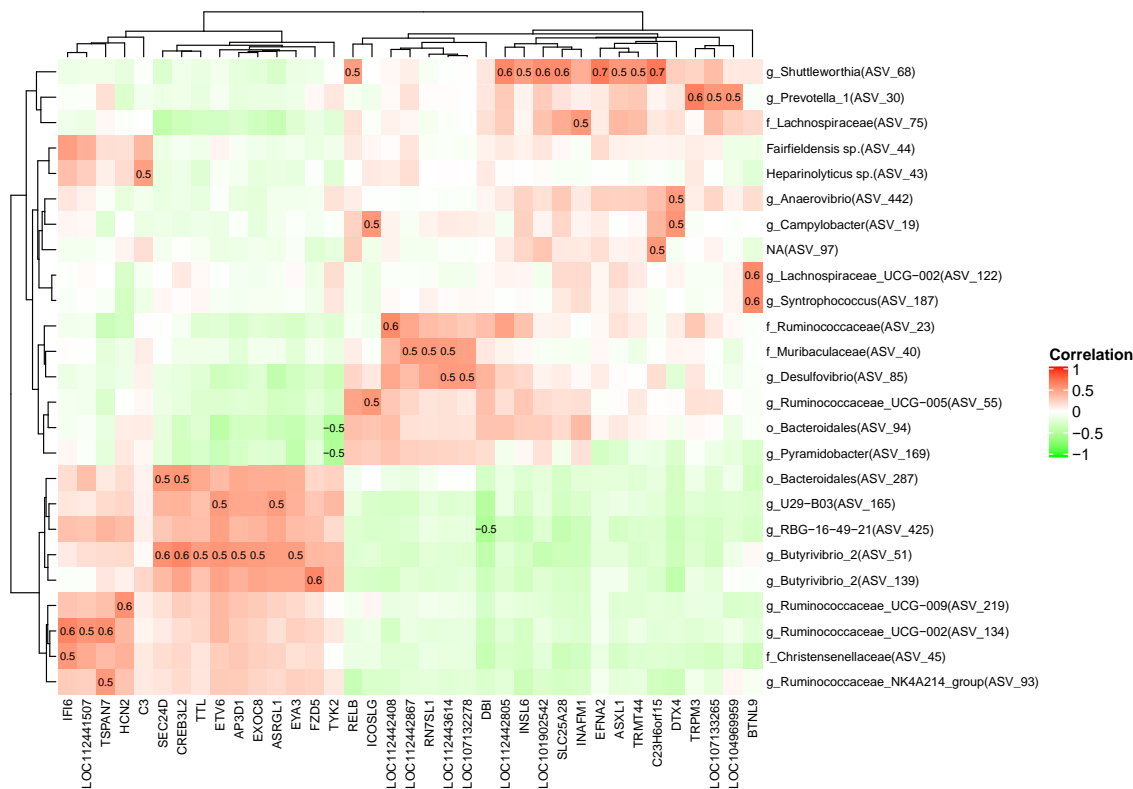
**Figure 3.**

Figure 3. Heatmap of the highest correlated ( $-0.5 > R > 0.5$ ) ASVs and DEG genes present in cattle rumen papillae. The prevalent ASVs ( $n = 194$ ; present in at least 50% of the rumen papillae samples) were used to run a Pearson correlation with DEG genes ( $n = 221$ ). The  $\log_{10}$  transformed relative abundances of prevalent ASVs and DEG genes were used to generate correlation matrix. The x-axis is representing the ASVs whereas the DEG genes are on y-axis.

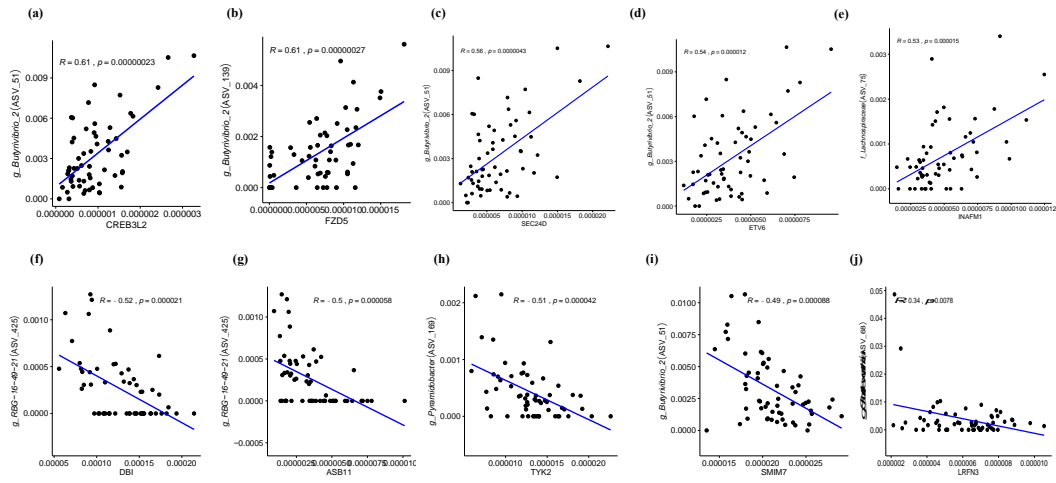
**Figure 4.**

Figure 4. Correlations between the core ASVs and DEG. A-E) illustrate positive and F-J) are negative correlations between core ASVs and DEG genes. The  $\log_{10}$  transformed relative abundances were used to calculate the Pearson correlation. The x-axis is the DEG gene and y-axis is the ASV with taxonomy.

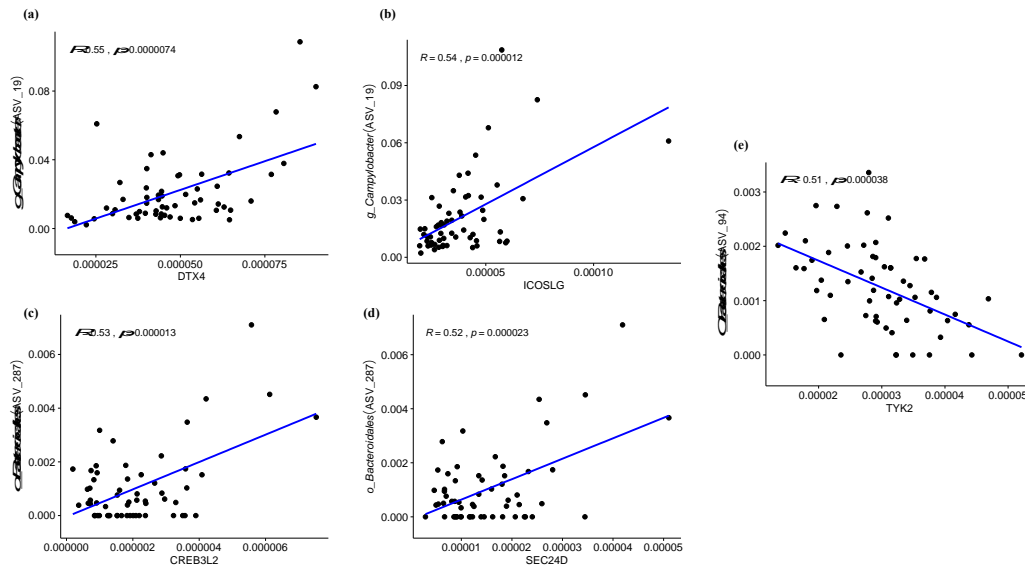
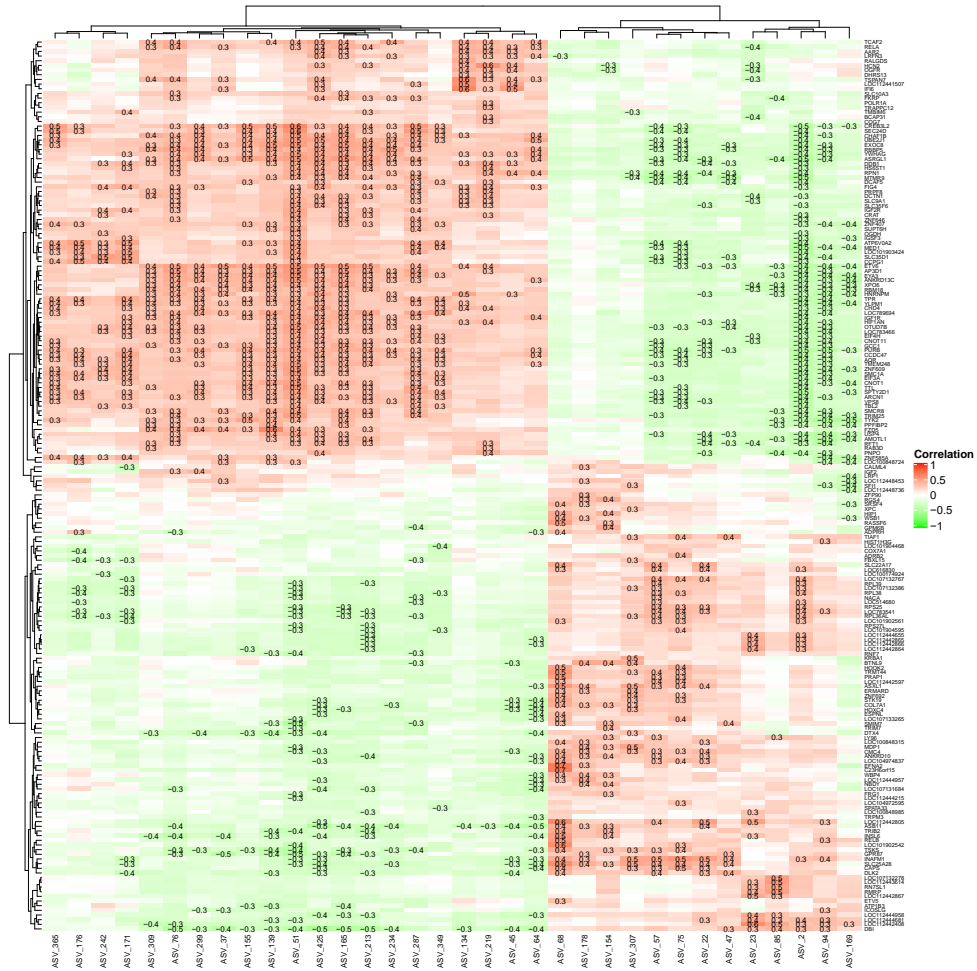
**Figure 5.**

Figure 5. Correlations between the core ASVs and DEG of bacterial ASVs potentially associated with liver abscesses (A-E). The  $\text{Log}_{10}$  transformed relative abundances were used to calculate the Pearson correlation. The x-axis is the DEG gene and y-axis is the ASV with taxonomy.



Supplemental Figure 1



Supplemental Figure 1: Heatmap of the moderately correlated ( $-0.3 > R > 0.3$ ) ASVs and DEG genes present in cattle rumen papillae. The prevalent ASVs ( $n=194$ ; present in at least 50% of the rumen papillae samples) were used to run a Pearson correlation with DEG genes ( $n=221$ ). The  $\text{Log}_{10}$  transformed relative abundances of prevalent ASVs and DEG genes were used to generate correlation matrix. The x-axis is representing the ASVs whereas the DEG genes are on y-axis.

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## CHAPTER 4

INFLUENCE OF RUMEN BACTERIAL SPECIES COMPOSITION ON MARBLING  
IN AMERICAN WAGYU AND ANGUS CATTLE.**ABSTRACT**

Wagyu cattle are known for its high marbling and tender beef. Wagyu beef has a unique fatty acid composition which adds a unique flavor to its meat. Genetics and nutrition are considered as two major factors that influence the marbling in beef cattle. Many studies have investigated how genetics and nutritional manipulation can change intramuscular fat (IMF) deposition. The rumen microbial communities can affect energy availability to the host, acetate to propionate ratio, and the composition of dietary FAs which can affect IMF deposition. However, to date we have limited information about how rumen microbiome influences marbling. We hypothesized that Wagyu cattle with high IMF have unique microbes that help increase marbling compared to animals with low marbling animals by increasing energy availability to the animal. In this study, we collected the rumen samples from full blood (n=17) and crossbred (n=22) Wagyu cattle to investigate the bacterial community differences between different marbling grades. We sequenced the V4 region of the 16S rRNA gene using the illumina MiSeq platform. The data was processed using the DADA2 algorithm. Alpha and beta diversity were not different between marbling grades of full blood (FB) and crossbred (F1) animals. The differential analysis with DESeq2 identified differential ASVs between extreme marbling groups for FB (n=115) and F1 (n=34) animals. The differential ASVs with higher abundance in Excessive Marbling FB animals belong to genera *Prevotella* (n=8 ASVs), *Candidatus\_Saccharimonas* (n=7 ASVs), *Christensenellaceae\_R-7\_group* (n=5 ASVs), *Treponema* (n=5 ASVs), *Rikenellaceae\_RC9\_gut\_group* (n=4 ASVs), *Butyrivibrio* (n=4



ASVs), and *Mogibacterium* (n=3 ASVs). The differential ASVs with higher abundance in High Marbling F1 animals belong to genera *Prevotella* (n=3), *NK4A214\_group* (n=2), *Prevotellaceae\_UCG-001* (n=1), and *Prevotellaceae\_UCG-003* (n=1). The differential ASVs present in highly marbled samples in FB and F1 are known for fermenting polysaccharides, glucose, and other sugars into different volatile fatty acids. The differential ASVs identified in FB and F1 cattle were different (except one common ASV: genus *Prevotella*) but belong to similar genera. The network analysis (SparCC) displayed that the differential ASVs identified may help increase marbling through increased energy flow through the rumen ecosystem and/or by producing unique metabolites that increase marbling directly or indirectly by stimulating other microbes. This study provides preliminary evidence that certain bacterial species affect marbling in Wagyu and Angus cattle. Due to the limitations of the 16S reference database, majority of differential ASVs identified could not be classified at species level and further investigation into metabolic functional differences may reveal how such microbial communities increase marbling.

## INTRODUCTION

Wagyu cattle are known for its high intramuscular fat (IMF) deposition or marbling which is directly linked to meat quality and palatability (Wheeler, et al., 1994). High marbling is preferred by consumers in many countries like the USA, Japan, Australia and Korea, which makes it an economically important trait for beef producers (Hunt et al., 2014).

Genetics is one of the major factors that influence the marbling, for example, the Wagyu breed have shown to have the highest marbling followed by Korean Cattle, Angus,

Hereford crossbred or Hereford and Brahman breeds (Park et al., 2018). Wagyu cattle have higher heritability (0.40-0.55) for marbling (Oyama, K., 2011). Slaughtering age is another factor that affects the IMF deposition in most beef breeds (Park et al 2018) and cattle with wagyu genetics have shown an increase in marbling with the increase in age (Zembayashi et al., 1995; Kirkland et al., 2007). Connolly et al., 2020 reported the increase in 21 metabolites related to muscle energy and glucose metabolism in Wagyu crossbred with the increase in age during the feedlot period and they attributed this change to mature body conformation.

Other than genetics and age, beef producers use different management and nutritional strategies to increase marbling (Park et al., 2018). In ruminants, acetate and glucose (dietary or synthesized from propionate) are the two major substrates that synthesize fatty acids (Hanson and Ballard, 1967) which are used for IMF and subcutaneous fat deposition. Smith and Crouse, 1984 showed that glucose is preferred for marbling whereas acetate is preferred for subcutaneous fat deposition.

Wagyu cattle are fed a high concentrate diet from the age of 11 to 30 months to increase marbling (Gotoh et al., 2014). The fermentation of high concentrate diets produces more propionate, which is gluconeogenic (Nafikov, and Beitz, 2007), additionally, rumen bypassed carbohydrate increases the availability of glucose for absorption through the small intestine leading to increased marbling. Wagyu steers fed a high concentrate diet have shown to have higher IMF deposition and similar subcutaneous fat deposition as compared to animals fed a higher roughage diet (Yamada and Nakanishi, 2012). A high grain diet also increases the production of monounsaturated fatty acids (MUFAs) in IMF by increasing the activity of stearoyl coenzyme A desaturase (SCD) (Smith and Lunt,

2007). The role of different vitamins (A, D and C) in IMF deposition have also been studied (park et al 2018).

Genetics and nutrition are considered as the two major factors that influence marbling in beef cattle (Park et al., 2018). As a result, research has been focused on how genetics and nutritional manipulation can be used to improve IMF deposition. However, we have very limited information about how the rumen microbiome influences the marbling.

In recent years, many studies have reported the association of rumen microbiota with feed efficiency (Carberry et al., 2012; Shabat et al., 2016; Paz et al., 2018; McGovern et al., 2018). It is possible that Wagyu cattle with high IMF have different microbial communities that help increase marbling compared to animals with low marbling animals by increasing energy availability to the animal. As such, the rumen microbial communities can affect energy availability to the host, acetate to propionate ratio, and the composition of dietary FAs which can affect IMF deposition. Kim et al., 2020 reported that Hanwoo Korean beef cattle with a high marbling score (marbling score  $\geq 7$ ) have different bacterial taxa compared to cattle with a low marbling score (marbling score  $\leq 4$ ). Based on the above information we hypothesized that rumen microbial community impacts IMF deposition and identifying differences in bacterial community composition in high and low marbling animals will help identify bacterial species involved in IMF deposition to develop novel direct fed microbials to improve marbling. The objectives of this study were (1) to investigate the differences in bacterial communities between different marbling grades in full blood (FB) and crossbred (F1) American Wagyu cattle. To our best knowledge, this is the first study that investigates the differences in bacterial

communities between different American wagyu genetic types and different marbling grades.

## **METHODS**

### **Animal diet and sample collection**

Samples used in this study were collected in collaboration with Imperial Wagyu Beef (<https://imperialwagyubeef.com/>) during harvesting. The samples were collected from wagyu certified producers who raise full blood (FB) or crossbred (F1, Angus mother, and full-blood Wagyu sire) wagyu cattle. All producers followed approved dietary guidelines suggested by Imperial Wagyu Beef for a minimum of 400 days of feeding before harvest. This study includes the full blood (FB, n=17) and crossbred (F1, n=22) American Wagyu cattle which were raised by two separate producers.

The rumen contents (liquid and feed particles) of each animal were collected at harvest at the slaughtering facility of Imperial Wagyu Beef, Omaha, NE, USA. All the samples were snap-frozen with liquid nitrogen and then placed on ice. Samples were transported to the University of Nebraska-Lincoln and stored at -20 until used for DNA extraction.

The samples were collected from a total of 85 animals and hot carcass weight and marbling grade for each animal were also recorded. The Imperial Wagyu Beef used its marbling grading system based on the Japanese Beef Marbling Score (BMS) system as a reference. The four marbling grades are 1) Market Style (equivalent to 4-5 BMS), 2) Classic (equivalent to 6-7 BMS), 3), Signature (equivalent to 8-9 BMS), and 4) Reserve (equivalent to 10+ BMS). For this study, we named these grades as Low Marbling, Moderate Marbling, High Marbling, and Excessive Marbling, respectively (Figure 1).

## **DNA Extraction**

DNA was extracted with Mag-Bind® Soil DNA 96 Kit (Omega Bio-tek, Inc.) by following the manufacturer protocol with the following changes: The 10 mM sodium acetate was added to each sample tube (0.2 x volumes), and later all the samples were vortexed and placed on ice for 5 minutes. The samples were centrifuged at 16000 g for 15 minutes at 4 °C and the supernatant was used to precipitate the nucleic acids with isopropanol. The ice-cold isopropanol (1 x volumes) was added to each sample and all the samples were incubated on ice for 30 minutes. The nucleic acids were precipitated by centrifuging each sample at 16000 g for 15 minutes at 4°C. The supernatant was discarded, and the DNA pellet was washed twice with ice-cold 70% ethyl alcohol. The DNA pellet was dissolved in Tris solution (10 mM, pH 8.0) and Mag-Bind® Soil DNA 96 Kit reagents were used for further purification by following the manufacturer protocol. The resulting DNA was used to amplify the V4 region of the 16S ribosomal RNA gene by using the universal bacterial primers as described by Kozich et al., 2013. A 20µl PCR reaction (25 cycles) was used to amplify the V4 region as described by Paz et al., 2016. The size of the PCR product was confirmed with 1.5% agarose gel electrophoresis. The samples were normalized for DNA concentration with SequalPrep™ Normalization Plate (96) kit and purified with MinElute® PCR Purification Kit by following the manufacturer's protocol. The BluePippin system was used with a 1.5% agarose cassette to remove the DNA fragments with a length less than 250bp. The quality and quantity of the DNA library were confirmed with Agilent 2100 bioanalyzer with Agilent High Sensitivity DNA kit by following the manufacturer protocol. The resulting DNA library

was used to sequence the V4 region of the 16S rDNA gene with the Illumina Miseq platform with a 2x250 paired sequencing strategy.

### **Processing the Publicly available data**

We also used the publicly available data (Paz et al., 2018) to analyze the rumen bacterial community differences between different marbling grades in Angus Steers. The animals (n=118) were fed on a finishing diet (57.6% dry-rolled corn, 30% wet distillers grains, 8% alfalfa hay and 4.4% vitamins and minerals) for 78 days. The marbling score (MS) was recorded at harvest on a scale of 100 to 1190 as 100 being the beef without any marbling while 1190 for extreme marbling. We divided the marbling score into three grades (1) Select (equivalent to <400 MS) (2) Choice (equivalent to 400-600 MS), and (3) Prime (equivalent to > 600MS) for subsequent analysis.

### *Regression Analysis*

The differential ASVs identified using DESeq2 (Love et al., 2014) between Prime and Select samples were tested as potential predictors for marbling in a Angus population data with stepwise regression. The compositional data was normalized with arcsin square root transformation and predictive ASVs were considered significant at *P-value* < 0.05.

### **16S Data Processing**

The 16S (V4) sequencing data were processed with R (R Core Team, 2019) by following the workflow described by Callahan et al., (2016a). Briefly, the forward and reverse reads for each sample were trimmed at constant length to remove the part of the read with a quality score less than 25. Further, the reads with more than 2 expected errors were also removed. The DADA2 (Callahan et al., 2016b) method was used to differentiate between biological variation and sequencing error. The sequences were inferred by using the

pseudo-pooling method which increases the sensitivity to rare taxa across samples. The forward and reverse reads were merged, and the amplicon sequence variants (ASVs) table was made with default settings, and later chimeras were also removed from the table. The Silva (v138) reference database was used to assign the taxonomy at the species level by using the Bayesian classifier method (Wang et al. 2007). The phylogenetic tree was made with mothur (Schoss et al., 2009) and Silva (silva.nr\_v138.tgz) database was used to align the sequences. The ASVs associated with Archaea, Eukaryota, Rickettsiales, and mitochondria were removed before further analysis. We processed the FB and F1 data separately because 1) F1 animals did not have any Excessive Marbling samples and FB data did not have any Low Marbling samples. As such, to eliminate the breed effect and cohort effect (as the two groups were raised by two different producers) on bacterial community composition and we evaluated each group of animals independent of each other and then looked at common trends between the two groups. The R codes used for data processing and analysis are available in the appendix D.

### **Statistical analysis**

The amplicon sequence variants (ASVs) with less than 5% prevalence (n=4) were removed before statistical analysis. The microbial community data for full blood and crossbred animals were analyzed separately using the R package phyloseq (McMurdie, P.J. and Holmes, S., 2013). Alpha diversity measures (Chao1 and Shannon) were calculated with the estimate\_richness() function for each sample. The effect of change in alpha diversity on marbling score was tested with ordered logistic regression by using polr() function with R package MASS (Venables, W.N. and Ripley, B.D., 2013). Beta diversity was calculated with unweighted UniFrac and bray-Curtis dissimilarity

measures, and differences in beta diversity for different marbling scores were tested with permutational multivariate analysis of variance (PERMANOVA) using the `adonis()` function with R package `vegan` (Oksanen et al., 2013), and subsequently, the principal coordinate analysis (PCoA) plots were generated to visualize the community differences. The DESeq2 (Love et al., 2014) method was used to see the differential ASVs for FB (Moderate Marbling vs Excessive Marbling) and F1 (Low Marbling vs High Marbling) data. The core ASVs for different marbling grades within FB and F1 data were identified. The ASVs present in at least 80% of the samples were considered as core ASV.

### **Co-occurrence network**

Network analysis was used to see the bacterial species co-occurrence and interaction for American Wagyu (FB and F1) and Angus steer data. SparCC (Sparse Correlations for Compositional data) method (Friedman and Alm, 2012) was used to construct the ecological network with R package `SpiecEasi` (Kurtz et al., 2021). For each network, every node was assigned a score based on the number of links with other nodes and the node with highest number of links was considered as a hub or likely a keystone species.

## **RESULTS**

### **1) Bacterial community of American Wagyu full blood (FB) animals**

Days on feed (DOF), average live weight, average hot carcass weight and age at harvest were 661 days, 1108lbs, 694lbs and 29 months, respectively. This group had Moderate (n=4, 6-7 BMS), High (n=9, 8-9 BMS), and Excessive (n=4, 10+ BMS) Marbling scores and did not have any animals with low marbling scores. The 17 FB samples generated 2,402,224 reads that were binned into 1,511 ASVs. The average reads per sample were



141,307. The rarefaction curves showed each sample has adequate sequencing depth (Figure 7). We identified 17 phyla, 26 classes, 49 orders, 61 families, 117 genera, and 25 species within the FB animals. For 1,511 ASVs, 1491 (~99%) were identified at phylum, 1489 (~96%) at class, 1482(~98%) at order, 1297(~86 %) at family, 970(~64 %) at genus and 38(~3 %) at species level. The relative abundances of top 10 phyla and 25 genera across different marbling grades are shown in Figure 2(a and b).

The alpha diversity index Chao1 and Shannon displayed an increase in the diversity with increased marbling (Figure 3). Ordered logistic regression analysis identified the change in the Chao1 to (p-value= 0.05) affect marbling grade, while Shannon diversity (p-value= 0.27) did not affect the marbling grade. Kruskal-Wallis test showed that the mean rank differences between different marbling groups were not different for Chao1(p-value= 0.2) and Shannon (p-value= 0.34) diversity index.

The Permutational multivariate analysis of variance (PERMANOVA) using the Bray Curtis and weighted unifrac dissimilarity matrices displayed no overall bacterial community differences between different marbling grades. This observation was consistent with principal coordinate analysis (PCoA) plots which did not show any clustering of samples based on marbling grades (Figure 3).

The DESeq2 algorithm identified 115 significantly (padj <0.05) differential ASVs between Excessive and Moderate marbling groups (Figure 5, Table3). Out of the 115 differential ASVs identified, 101 ASVs had significantly higher abundance in Excessive Marbling samples while 14 ASVs had higher abundance in Moderate Marbling samples. A majority of 115 ASVs identified as displaying higher abundance (log2Fold change = 8 to 24) in excessive marbling groups belonged to genera *Prevotella* (n=8 ASVs),

*Candidatus\_Saccharimonas* (n=7 ASVs), *Christensenellaceae\_R-7\_group* (n=5 ASVs), *Treponema* (n=5 ASVs), *Rikenellaceae\_RC9\_gut\_group* (n=4 ASVs), *Butyrivibrio* (n=4 ASVs), *Mogibacterium* (n=3 ASVs), *Lachnospiraceae\_ND3007\_group* (n=2 ASVs), *Saccharofermentans* (n=2 ASVs), *Prevotellaceae\_Ga6A1\_group* (n=1 ASVs), *Prevotellaceae\_UCG-001* (n=1 ASVs), *Flexilinea* (n=1 ASVs), *Desulfobulbus* (n=1 ASVs), *Family\_XIII\_AD3011\_group* (n=1 ASVs), *Erysipelotrichaceae\_UCG-009* (n=1), *Acetitomaculum* (n=1), *Coprococcus* (n=1), *Lachnobacterium* (n=1), *Lachnospiraceae\_NK3A20\_group* (n=1), *Syntrophococcus* (n=1), *Mycoplasma* (n=1), *NK4A214\_group* (n=1), *Ruminococcus* (n=1), *Selenomonas* (n=1), *p-1088-a5\_gut\_group* (n=1), *Sphaerochaeta* (n=1), *Fretibacterium* (n=1), and *DNF00809* (n=1).

The abundance of two known differential species *Lachnobacterium bovis*, and *Treponema bryantii* were high in Excessive Marbling samples. The abundances of 14 ASVs were significantly lower (log2Fold change = -14 to -26) in Excessive Marbling group as compared to Moderate Marbling group. The majority of these 14 ASVs belongs to genera *Christensenellaceae\_R-7\_group* (n=3), *Lachnospiraceae\_NK3A20\_group* (n=2), *Prevotella* (n=2), *Shuttleworthia*(n=1), *Roseburia* (n=1), *UCG-001*(n=1) Table 5c.

## **2) American Wagyu crossbred (F1) microbial community**

This data have 22 animals and they were raised by one producer on the same diet. Their days on feed (DOF), average live weight, average hot carcass weight and age at harvest were 409 days, 1482lbs, 946lbs and 25month, respectively. This group has Low (n=7, 4-5 BMS), Moderate (n=11, 6-7 BMS), and High (n=4, 7-8 BMS) Marbling samples and it did not have any Excessive Marbling samples.

The processing of 22 samples generated 1,904,511 reads for 2,005 ASVs. The average reads per sample were 86,568. The rarefaction curves showed each sample has adequate sequencing depth (Figure 7). The F1 bacterial community has 18 phyla, 29 classes, 53 orders, 61 families, 124 genera, and 25 species. For 2,005 ASVs, 1980 (~99%) were identified at phylum, 1975 (~99%) at class, 1958(~98%) at order, 1581(~79 %) at family, 1197(~60 %) at genus and 47(~2 %) at species level. The relative abundances of top 10 phyla and 25 genera across different marbling grades are shown in Figure 2(c and d). The relative abundances of major Phyla include Bacteroidota (41.4%), Firmicutes (47.5%), Spirochaetota (2.4%), Patescibacteria (2.8%), Verrucomicrobiota (1.7%) and Planctomycetota (1%) Spirochaetota (2.4%), and Actinobacteria (1.2%).

The Kruskal-Wallis test showed no significant differences in alpha diversity indices (Chao1 and Shannon) between different marbling grades and ordered logistic regression analysis also concluded that change in alpha diversity did not affect the marbling in F1 American Wagyu.

The overall bacterial community PERMANOVA analysis based on Bray Curtis and weighted Unifrac distance matrices showed that there was no significant difference between marbling grades. This observation was consistent with PCoA analysis plots which did not show any clustering based on marbling grades (Figure 4).

The DESeq2 analysis identified 34 ASVs ( $\text{padj} < 0.05$ ) between High Marbling and Low Marbling groups (Figure 6, Table3). For 34 differential bacterial species, 22 ASVs have higher abundance in High Marbling samples while 12 ASVs have higher abundance in Low Marbling samples.

Majority of 22 ASVs with high abundance in High Marbling group (log2Fold change= 19 to 24) belongs to genera *Prevotella* (n=3), *NK4A214\_group* (n=2), *Prevotellaceae\_UCG-001* (n=1), *Prevotellaceae\_UCG-003* (n=1), *Rikenellaceae\_RC9\_gut\_group* (n=1), *Desulfovibrio* (n=1), *Fibrobacter* (n=1), *Christensenellaceae\_R-7\_group* (n=1), *UCG-001* (n=1), *Candidatus\_Saccharimonas* (n=1), *Treponema* (n=1). The majority of 12 differential ASVs with lower abundance (log2Fold change= -21 to -28) in High Marbling group belongs to genera *Prevotella* (n=1), *Flexilinea* (n=1), *Fibrobacter* (n=1), *possible\_genus\_Sk018* (n=1), *p-1088-a5\_gut\_group* (n=1), *Treponema* (n=1) Table 6c.

### 3) Angus steer microbial community

This data set included 118 Angus steers. The marbling scores ranged from Select (n=6, <400 MS), Choice (n=99, 400-600 MS), and Prime (n=13, >600 MS). The processing of 118 samples generated 5,036,755 reads and was distributed among 1,002 ASVs. The average reads per sample was 42,684. For 1,002 ASVs, 999 (99.7%) were identified at phylum, 995 (~99%) at class, 987(98.5%) at order, 841(~84 %) at family, 637(~64 %) at genus and 42(~4 %) at species level. The alpha diversity (Chao1 and Shannon) analysis with Kruskal-Wallis test and beta diversity (Bray Curtis and weighted Unifrac) differences with PERMANOVA analysis were not significant between marbling grades (Figure S1).

The DESeq2 analysis identified 47 ASVs (padj< 0.05) between Prime and Select marbling groups (Figure 11, Table3). The ASVs (n=13) with significantly higher abundance in Prime samples belong to genera *Prevotella*, *Candidatus\_Saccharimonas*, *Prevotellaceae\_UCG-001* and *U29-B03*. The ASVs (n=34) with significantly lower

abundance in Prime marbling samples belong to genera *Treponema*, *Christensenellaceae\_R-7\_group*, *p-1088-a5\_gut\_group*, *Prevotella*, *Rikenellaceae\_RC9\_gut\_group*, *Succiniclasticum*, *Succinivibrio*, *Erysipelotrichaceae\_UCG-009*, *Lachnospiraceae\_ND3007\_group*, *Moryella*, and species *R. flavefaciens*, and *A. lipolyticus* (Figure 11).

The forward stepwise regression model explains 30.1% of the variation in marbling just using 47 differential bacterial ASVs. The bacterial species (ASVs) belongs to order *RF39*, *Oscillospirales*, and genus *Prevotella* were associated with an increase in marbling. ASVs belong to the family *Ruminococcaceae*, genus *Treponema* and species *lipolyticus* were associated with a decrease in the marbling (Table 1).

### **Co-occurrence network**

The SparCC method (Friedman and Alm, 2012) was used to construct the co-occurrence network of bacterial species (Figure 12). For American Wagyu FB data, a total of 1511 ASVs were used to generate SparCC correlation matrix, and later ASVs with a correlation of 0.8 or higher were used for network analysis (Figure 12a). The top ASVs with highest hub score were ASV\_12 (genus *Lachnospiraceae\_NK3A20\_group*), ASV\_46 (genus *Oribacterium*), ASV\_73 (genus *Erysipelotrichaceae\_UCG-006*), ASV\_23 (order *Oscillospirales*), and ASV\_57 (genus *Lachnospiraceae\_NK3A20\_group*). The network with high correlation contained 50 differential ASVs out of 115 that were identified in Excessive marbling samples. Some of the ASVs had higher edges and were connected to many nodes that represent ASVs. For example ASV\_158 (order *WCHB1-41*), ASV\_164 (genus *NK4A214\_group*), and ASV\_511 (family *UCG-010*) each have 33 links. In contrast, some differential ASVs (ASV\_22: family *F082*, ASV\_796: order *RF39*, and

ASV\_367: genus *Mogibacterium*) have only 1 or 2 links with other ASVs (Figure 12a, Table 2).

For crossbred American Wagyu cattle, the SparCC correlation matrix between 2005 ASVs was made and ASVs with correlation 0.7 or higher were plotted in a network (Figure 12b). The potential keystone species based on hub score in F1 data were ASV\_300 (genus *Rikenellaceae\_RC9\_gut\_group*), ASV\_159 (genus *Butyrivibrio*), ASV\_380 (genus *probable\_genus\_10*), ASV\_298 (family *p-251-o5*), and ASV\_62 (genus *Erysipelotrichaceae\_UCG-009*). The network with high correlation contained 10 differential ASVs that were identified between High and Low marbling grades. A majority of these ASVs have few edges linking them to other nodes (Figure 12b).

For Angus steers data, a total of 1002 ASVs were used for network analysis. The ASVs with a correlation of 0.3 or higher were plotted as a network (Figure 12c). The top ASVs with high hub score were ASV\_1 (genus *Succinivibrionaceae\_UCG-001*), ASV\_14 (genus *Oribacterium*), ASV\_5 (family *Prevotellaceae*), ASV\_31 (genus *Prevotella*), and ASV\_46 (family *p-2534-18B5\_gut\_group*) (Figure 12c). The network analysis contained 42 differential ASVs and a majority of these ASVs have multiple edges inking them to other ASVs (nodes). Some differential ASVs have as many as 70 (ASV\_119), 80 (ASV\_111, ASV\_80) or 102 (ASV\_23) links with other ASVs. However, there were differential ASVs with 1 (ASV\_331, ASV\_522), 2 (ASV\_58), or 3 (ASV\_671) links with other ASVs. The stepwise regression identified predictors of marbling and those ASVs were also present in this network. The predictor ASVs that were associated with an increase in the marbling (Table 1) ASV\_285 (order *RF39*), ASV\_183 (order *Oscillospirales*), and ASV\_102 (genus *Prevotella*) have 1, 80, and 27 links with other

ASVs, respectively. The ASVs that were associated with a decrease in the marbling, such as ASV\_189 (*lipolyticus spp*) and ASV\_263 (family *Ruminococcaceae*), have 38 and 30 links with other ASVs, respectively (Figure 12c).

## DISCUSSION

Wagyu breeds have the highest marbling followed by Korean Cattle, Angus, Hereford crossbred or Hereford and Brahman breeds (Park et al., 2018). Genetics, nutrition, and age are the major known factors that influence marbling (Park et al., 2018). Beef producers mainly rely on nutritional strategies like feeding a high concentrate diet during the feedlot period to increase the marbling. Ruminants use glucose or acetate as a substrate to synthesize fatty acids. Rumen microbial fermentation supply VFAs and fulfill nearly 70% of the energy requirement. The role and differences of rumen microbiome between different marbling grades are understudied. In this study, we investigated the bacterial community differences between different marbling grades in American Wagyu cattle. The rumen bacterial communities of full blood (FB), crossbred (F1) American Wagyu and Angus cattle were investigated to find the differences between marbling grades. For each cohort, the overall bacterial community change (alpha and beta diversity) was not different between different marbling grades. As all animals were fed the same diet and had similar breed compositions therefore this was not surprising as we believe that only a sub-community of the microbes may influence marbling. However, Kim et al., 2020 reported bacterial alpha diversity (Observed and Chao1) was significantly higher in high marbling Korean cattle compared to low marbling group. In addition, they reported significant beta diversity differences (Unweighted Unifrac) between high and low marbling groups. However, in this study we did not observe any

community differences in both weighted and unweighted unifracs distance-based analysis. Krause et al., 2020 has also reported that increase in the Chao1 index was correlated with marbling in Angus steers. Although not significant, our data also displayed a similar trend for Wagyu (FB and F1) data (Figure 3c & d and Figure 4c & d). However, Angus data displayed an increase in the Shannon index and decrease in Chao1 estimates with increase in the marbling grade. It is tempting to speculate that the increased diversity may result in increased metabolic functions within the high marbling animals resulting in unique metabolites that may influence marbling. Metagenome-based shotgun sequencing may provide more information into functional differences in the microbial communities in the different marbling groups that could explain differences in marbling.

The DESeq2 analysis was performed between the highest and lowest marbling groups within each cohort to identify bacterial community differences that may influence marbling. This analysis resulted in 115 significantly different ASVs in FB where 101 were highly abundant in the extreme marbling group (Figure 5). Out of the 34 ASVs identified in the F1 data, 22 ASVs displayed higher abundance in the high marbling group (Figure 6). In Angus steers, 47 differential ASVs were identified, and 13 ASVs have high abundance in Prime marbling samples than Select marbling samples (Figure 11). In FB American Wagyu, the majority of the differential ASVs that were present in high abundance in Excessive Marbling samples are known for fermenting a range of carbohydrates (glucose, hexoses, polysaccharides) into propionate, succinate, acetate, and lactic acid (Choudhury et al., 2015). For example, the members of the *Rikenellaceae* family have shown to convert glucose, lactose, mannose and melibiose into acetic acid, propionic acid and succinic acid (Choudhury et al., 2015), *Flexilinea* genus convert



glucose into acetate, lactate, succinate, propionate, formate and hydrogen, *Butyrivibrio* produce butyrate Choudhury et al., 2015), *Lachnobacterium bovis* produces lactic acid (Choudhury et al., 2015), *Treponema bryanti* produce formic acid, acetic acid, succinic acid and *Selenomonas* produce propionic acid (Choudhury et al., 2015). Other taxa identified are known for their sulfate reducing properties (*Desulfobulbus*) (Choudhury et al., 2015) and as ammonia-oxidizing ability (*Pirellulaceae*) (Choudhury et al., 2015). Similarly, differential ASVs in American Wagyu F1 and Angus animals with high abundance in High Marbling or Prime samples, respectively, were members of the same bacterial taxa that we found in Excessive Marbling (FB) data (Figure 5c, 6c, and 11). This may suggest that the variation in the microbiome of marbling groups may be due to strain level variation in the identified species where unique strains may have genes that help produce metabolites to increase marbling. The ASV-based approach we used to identify bacterial community differences provide the opportunity to identify strain level differences in the microbial community as such may have helped identify this feature. We evaluated the core ASVs present within each marbling group with the intention to identify microbial sub communities that may influence marbling. However, we did not find any common ASVs between core ASVs identified in the Excessive Marbling group (Figure 9) and differential ASVs present in Excessive Marbling group (Figure 5). Nevertheless, majority of the core ASVs identified in the marbling groups of Extreme and High marbling belonged to the same genus, such as *Prevotella*, *Prevotellaceae\_YAB2003\_group*, and *Succinivibrionaceae\_UCG-001*. Similarly, the core ASVs present in High Marbling (Figure 10) samples were different than differential ASVs present in High Marbling (Figure 6) group, and they were member of the common

genus. Kim et al., 2020 reported taxa *RFP12*, *Verrucomicrobia*, *Oscillospira*, *Porphyromonadaceae*, and *Paludibacter* were higher in high marbling group. Similar to Kim et al., 2020 findings we identified ASVs belonging to phylum *Verrucomicrobiota* that were present in high abundance in Excessive Marbling and High Marbling samples. Krause et al., 2020 reported the positive correlation of taxa *Rikenellaceae*, *S24-7*, *Verrucomicrobiaceae*, *Akkermansia*, *Blautia*, *Klebsiella*, *Moryella*, *Peptostreptococcus*, and *Selenomonas* with marbling in Angus steer. In our Angus data, we also identified differential ASVs belong *Rikenellaceae* and *Moryella*. Similar to Krause et al., 2020 the abundance of *Selenomonas* was higher in Excessive Marbling group. The majority of the taxa that were present in high marbling animals were different for Korean cattle (Kim et al., 2020), Angus steer (Kraus et al., 2020). However, the breeds in Our study and the other two studies were different. With studies demonstrating that the host genotype is selecting for rumen bacterial community composition (Abbas et al., 2020), it is possible to speculate that the differences observed in these studies may be due to breed differences.

The majority of the differential ASVs that were present in High Marbling samples in F1 data belong to same genus that has the differential ASVs present in high abundance in Excessive Marbling samples in FB data. To evaluate ASV relatedness between high marbling groups in FB and F1 cohorts, we generated a phylogenetic tree (Figure 8 A&B). The phylogenetic tree displayed that those ASVs belonging to the same genus are closely related (Figure 8A). The ASVs that were present in Extreme Marbling samples (FB) were similar to those ASVs present in High marbling samples (F1). The DADA2 method provides very high resolution which could explain the different bacterial species

identified in the marbling groups. The closely related bacterial species that were present in extreme marbling groups in FB and F1 data may have some common genes to help increase the marbling.

The DESeq2 algorithm identified the differential ASVs that have significantly higher or lower abundance in extreme marbling samples within each dataset. The SparCC correlation networks were used to see the bacterial co-occurrence within each dataset. The correlation cut-off was used for each network to avoid overcrowding and to show highly correlated bacterial species within each dataset. We used the networks to look at the co-occurrence of differential ASVs with other bacterial species. The list of the differential ASVs found in each network along with the number of links or edges are shown in Table2. The number of links for each ASV represents how many other bacterial species are connected (SparCC correlation) to that ASV. Within each network, differential ASVs have a wide range of links with other species.

The differential ASVs with higher number of links are part of the bigger cluster and those bacterial species co-occur with a lot of other species. For example, differential ASV\_511 (family *UCG-010*), ASV\_346 (order *Oscillospirales*), and ASV\_164 (genus *NK4A214\_group*) have high abundance in Excessive Marbling samples (Figure 5) and network (Figure 12a) showed they have 33, 26, and 33 links, respectively. The co-occurrence of these ASVs with high number of bacterial species indicates that those groups of bacterial species might be working together to increase the energy flow by degrading the feed material faster or they might be increasing the overall energy extraction from the feed. However, opposite trends were also observed in this study. For example, In Angus data differential ASV\_119 (genus *p-1088-a5\_gut\_group*) has low

abundance in Prime samples and it co-occurs with 70 other bacterial species which means bigger clusters do not always help the animal to increase the energy flow or marbling. It is possible some bacterial clusters could increase the energy loss and ultimately less energy is available to the animal. In addition, networks also revealed the occurrence of differential ASVs with 1 or 2 other bacterial species (Table 2). It is possible that these bacterial species only metabolize specific metabolites. For example, ASV\_756 (genus *Prevotella*) and ASV\_314 (genus *Butyrivibrio*) have a high abundance in Excessive marbling samples (FB) and they co-occur with 1 and 2 other bacterial species, respectively. It is possible that differential ASVs that co-occur with 1 or 2 other bacterial species and also present in high marbled animals are metabolizing specific metabolites to increase the energy flow and ultimately more intramuscular fat deposition.

The Angus data have the numeric marbling scores, and we used the forward stepwise regression to identify which ASVs within differential ASVs have a bigger role in marbling. The stepwise regression showed the ASV\_285 (order *RF39*), ASV\_183 (order *Oscillospirales*) and ASV\_102 (genus *Prevotella*) were associated with an increase in the marbling. The SparCC network showed ASV\_285 co-occur with only ASV\_114 (genus *UCG-005*), ASV\_183 co-occur with 80 other bacterial species and ASV\_102 co-occur with 27 other bacterial species (Table2). The co-occurrence of these ASVs with either a large number of either bacterial species or with only one bacterial species indicates that animals with higher marbling might have multiple ways to increase the energy availability for marbling.

This study provides evidence that bacterial populations in the rumen affect marbling. However, due to the limitations of the 16S reference databases, the majority of the

differential ASVs were not identified at species level. Future studies focused on metagenome shotgun sequencing of the rumen microbiome may provide information into how the rumen microbiome may affect marbling in beef cattle. Such approaches will provide new information into identification of species for development of direct-fed microbes (DFM) to increase the marbling.

## TABLES AND FIGURES

**Table 1: Stepwise regression model to predict marbling in Angus steers.**

Trait	Predictor	Coefficient	SE <sup>a</sup>	t-statistics	P-value	R <sup>2b</sup>
<b>Marbling</b>						0.3059
	(Intercept)	492.402	8.933	55.120	<2e-16	
	<i>ASV_285:o__RF39</i>	402.114	73.787	5.450	0.000000312	
	<i>ASV_183:o__Oscillospirales</i>	378.137	82.479	4.585	0.000012093	
	<i>ASV_189:lipolyticus</i>	-244.438	75.528	-3.236	0.0016	
	<i>ASV_263:f__Ruminococcaceae</i>	-139.953	75.228	-1.860	0.0655	
	<i>ASV_102:g__Prevotella</i>	158.465	79.125	2.003	0.0477	
	<i>ASV_331:o__RF39</i>	-136.067	90.779	-1.499	0.1368	
	<i>ASV_296:g__Treponema</i>	-101.160	72.053	-1.404	0.1631	

<sup>a</sup> Standard Error

<sup>b</sup> Adjusted R-squared

**Table:2** The list of the differential ASVs present in each network and the number of edges/links for each ASV.

	American Wagyu FB	SparCC correlation ≥0.8
Sr. No	Bacterial Species	No. of links
1	<i>ASV_314:g__Butyrivibrio</i>	2
2	<i>ASV_158:o__WCHB1-41</i>	33
3	<i>ASV_150:g__Prevotella</i>	2
4	<i>ASV_347:g__Prevotella</i>	9
5	<i>ASV_756:g__Prevotella</i>	1
6	<i>ASV_449:g__Prevotella</i>	1
7	<i>ASV_166:g__Prevotellaceae_Ga6A1_group</i>	18
8	<i>ASV_501:f__Prevotellaceae</i>	2
9	<i>ASV_232:f__Prevotellaceae</i>	8
10	<i>ASV_398:f__Lachnospiraceae</i>	2
11	<i>ASV_590:g__Lachnospiraceae_ND3007_group</i>	7
12	<i>ASV_422:g__Lachnospiraceae_ND3007_group</i>	4
13	<i>ASV_47:g__Saccharofermentans</i>	10
14	<i>ASV_164:g__NK4A214_group</i>	33
15	<i>ASV_511:f__UCG-010</i>	33
16	<i>ASV_346:o__Oscillospirales</i>	26
17	<i>ASV_448:g__Flexilinea</i>	26
18	<i>ASV_367:g__Mogibacterium</i>	1
19	<i>ASV_278:g__Mogibacterium</i>	3
20	<i>ASV_1038:g__Family_XIII_AD3011_group</i>	4
21	<i>ASV_321:g__Family_XIII_AD3011_group</i>	31
22	<i>ASV_757:g__Christensenellaceae_R-7_group</i>	1
23	<i>ASV_570:g__Christensenellaceae_R-7_group</i>	16
24	<i>ASV_354:g__Christensenellaceae_R-7_group</i>	12
25	<i>ASV_610:g__Christensenellaceae_R-7_group</i>	26
26	<i>ASV_432:g__Mycoplasma</i>	3
27	<i>ASV_594:g__DNF00809</i>	26
28	<i>ASV_168:bryantii</i>	1
29	<i>ASV_345:g__Treponema</i>	1
30	<i>ASV_615:g__Treponema</i>	1
31	<i>ASV_374:g__Treponema</i>	14
32	<i>ASV_177:g__p-1088-a5_gut_group</i>	5
33	<i>ASV_399:c__Bacteroidia</i>	1
34	<i>ASV_589:c__Bacteroidia</i>	5
35	<i>ASV_344:g__Candidatus_Saccharimonas</i>	7
36	<i>ASV_458:f__Selenomonadaceae</i>	1
37	<i>ASV_931:g__Fretibacterium</i>	6
38	<i>ASV_796:o__RF39</i>	1

39	<i>ASV_508:o__RF39</i>	32
40	<i>ASV_415:g__Ruminococcus</i>	2
41	<i>ASV_22:f__F082</i>	1
42	<i>ASV_193:f__F082</i>	12
43	<i>ASV_356:f__Muribaculaceae</i>	1
44	<i>ASV_279:g__Rikenellaceae_RC9_gut_group</i>	2
45	<i>ASV_621:g__Rikenellaceae_RC9_gut_group</i>	9
46	<i>ASV_202:f__Bacteroidales_UCG-001</i>	3
47	<i>ASV_1007:o__Oscillospirales</i>	2
48	<i>ASV_571:o__Oscillospirales</i>	13
49	<i>ASV_134:o__Oscillospirales</i>	38
50	<i>ASV_237:o__Oscillospirales</i>	32
	American Wagyu F1	SparCC correlation ≥0.7
	Bacterial Species	No. of links
1	<i>ASV_7:g__Prevotella</i>	1
2	<i>ASV_910:g__Prevotellaceae_UCG-003</i>	1
3	<i>ASV_654:f__Prevotellaceae</i>	1
4	<i>ASV_1477:g__Christensenellaceae_R-7_group</i>	1
5	<i>ASV_1185:f__vadinBE97</i>	1
6	<i>ASV_480:g__Treponema</i>	2
7	<i>ASV_703:g__p-1088-a5_gut_group</i>	1
8	<i>ASV_117:o__RF39</i>	2
9	<i>ASV_190:f__F082</i>	1
10	<i>ASV_729:f__Muribaculaceae</i>	1
	Angus Data SparCC Differential ASVs	SparCC correlations ≥ 0.3
	Bacterial Species	No. of links
1	<i>ASV_331:o__RF39</i>	1
2	<i>ASV_285:o__RF39</i>	1
3	<i>ASV_303:o__RF39</i>	25
4	<i>ASV_671:g__Christensenellaceae_R-7_group</i>	3
5	<i>ASV_146:g__Erysipelotrichaceae_UCG-009</i>	22
6	<i>ASV_434:o__WCHB1-41</i>	2
7	<i>ASV_435:g__Succinivibrio</i>	7
8	<i>ASV_326:g__Treponema</i>	6
9	<i>ASV_129:g__Candidatus_Saccharimonas</i>	7
10	<i>ASV_432:g__Flexilinea</i>	10
11	<i>ASV_119:g__p-1088-a5_gut_group</i>	70
12	<i>ASV_405:g__p-1088-a5_gut_group</i>	15
13	<i>ASV_442:g__Pirellula</i>	7
14	<i>ASV_406:o__Absconditabacteriales_(SR1)</i>	5



15	<i>ASV_357:o__Bacteroidales</i>	5
16	<i>ASV_286:g__Succiniclasticum</i>	17
17	<i>ASV_189:lipolyticus</i>	38
18	<i>ASV_259:f__Selenomonadaceae</i>	12
19	<i>ASV_252:g__Moryella</i>	13
20	<i>ASV_470:g__Moryella</i>	7
21	<i>ASV_277:g__Lachnospiraceae_ND3007_group</i>	1
22	<i>ASV_111:g__Lachnospiraceae_ND3007_group</i>	80
23	<i>ASV_93:g__Lachnospiraceae_ND3007_group</i>	24
24	<i>ASV_261:g__Lachnospiraceae_NK3A20_group</i>	53
25	<i>ASV_76:g__Prevotella</i>	9
26	<i>ASV_118:g__Prevotella</i>	14
27	<i>ASV_139:g__Prevotella</i>	40
28	<i>ASV_102:g__Prevotella</i>	27
29	<i>ASV_23:g__Prevotella</i>	102
30	<i>ASV_522:f__Prevotellaceae</i>	1
31	<i>ASV_12:g__Prevotella</i>	17
32	<i>ASV_34:g__Prevotellaceae_UCG-001</i>	10
33	<i>ASV_589:g__Rikenellaceae_RC9_gut_group</i>	7
34	<i>ASV_132:g__U29-B03</i>	12
35	<i>ASV_187:f__Bacteroidales_RF16_group</i>	4
36	<i>ASV_27:f__Muribaculaceae</i>	7
37	<i>ASV_58:f__Muribaculaceae</i>	2
38	<i>ASV_413:f__Oscillospiraceae</i>	7
39	<i>ASV_191:f__Ruminococcaceae</i>	23
40	<i>ASV_263:f__Ruminococcaceae</i>	30
41	<i>ASV_408:g__Ruminococcus</i>	8
42	<i>ASV_183:o__Oscillospirales</i>	80

**Table 3: The Differential ASVs for American Wagyu (FB and F1) and Angus Steers**

American Wagyu Data (FB)		
Excessive Marbling Vs Moderate Marbling		
ASV	Log2FoldChange	Taxonomy
ASV_1075	18.116	<i>Butyrivibrio</i>
ASV_858	16.517	<i>Butyrivibrio</i>
ASV_314	20.491	<i>Butyrivibrio</i>
ASV_337	15.024	<i>Clostridia_UCG-014</i>
ASV_158	21.958	<i>WCHB1-41</i>
ASV_118	-26.266	<i>Prevotella</i>
ASV_150	20.909	<i>Prevotella</i>
ASV_184	20.886	<i>Prevotella</i>
ASV_347	20.350	<i>Prevotella</i>
ASV_756	19.015	<i>Prevotella</i>
ASV_449	17.772	<i>Prevotella</i>
ASV_900	18.218	<i>Prevotella</i>
ASV_1094	-23.881	<i>Prevotella</i>
ASV_934	19.831	<i>Prevotella</i>
ASV_175	17.380	<i>Prevotella</i>
ASV_205	16.819	<i>Prevotellaceae_UCG-001</i>
ASV_166	19.368	<i>Prevotellaceae_Ga6A1_group</i>
ASV_501	18.086	<i>Prevotellaceae</i>
ASV_116	21.082	<i>Prevotellaceae</i>
ASV_232	19.928	<i>Prevotellaceae</i>
ASV_813	19.272	<i>Syntrophococcus</i>
ASV_452	22.540	<i>Butyrivibrio</i>
ASV_826	18.660	<i>Acetitomaculum</i>
ASV_534	-26.061	<i>Lachnospiraceae_NK3A20_group</i>
ASV_1108	-25.270	<i>Lachnospiraceae_NK3A20_group</i>
ASV_398	23.586	<i>Lachnospiraceae</i>
ASV_100	18.690	<i>Lachnospiraceae</i>
ASV_590	19.368	<i>Lachnospiraceae_ND3007_group</i>
ASV_422	21.346	<i>Lachnospiraceae_ND3007_group</i>
ASV_435	20.360	<i>Coprococcus</i>
ASV_733	19.292	<i>Lachnospiraceae</i>
ASV_323	19.179	<i>Lachnospiraceae</i>
ASV_723	17.404	<i>Lachnospiraceae_NK3A20_group</i>
ASV_984	-21.256	<i>Roseburia</i>
ASV_638	-22.835	<i>Shuttleworthia</i>
ASV_124	22.362	<i>L. bovis</i>
ASV_890	18.568	<i>Lachnospiraceae</i>
ASV_47	22.882	<i>Saccharofermentans</i>
ASV_916	19.518	<i>Saccharofermentans</i>
ASV_164	20.192	<i>NK4A214_group</i>
ASV_511	20.579	<i>UCG-010</i>
ASV_555	15.427	<i>UCG-010</i>
ASV_1079	21.266	<i>Oscillospirales</i>
ASV_346	19.975	<i>Oscillospirales</i>
ASV_512	19.986	<i>Oscillospirales</i>
ASV_448	19.852	<i>Flexilinea</i>
ASV_367	21.548	<i>Mogibacterium</i>
ASV_278	21.842	<i>Mogibacterium</i>

ASV_550	19.460	<i>Mogibacterium</i>
ASV_752	16.924	<i>Anaerovoracaceae</i>
ASV_1038	18.368	<i>Family_XIII_AD3011_group</i>
ASV_321	18.790	<i>Family_XIII_AD3011_group</i>
ASV_757	19.994	<i>Christensenellaceae_R-7_group</i>
ASV_1316	15.480	<i>Christensenellaceae_R-7_group</i>
ASV_1249	-23.285	<i>Christensenellaceae_R-7_group</i>
ASV_570	20.788	<i>Christensenellaceae_R-7_group</i>
ASV_569	-24.330	<i>Christensenellaceae_R-7_group</i>
ASV_403	-23.324	<i>Christensenellaceae_R-7_group</i>
ASV_354	21.603	<i>Christensenellaceae_R-7_group</i>
ASV_610	19.633	<i>Christensenellaceae_R-7_group</i>
ASV_432	18.443	<i>Mycoplasma</i>
ASV_1110	19.233	<i>Atopobiaceae</i>
ASV_594	20.302	<i>DNF00809</i>
ASV_616	16.199	<i>Sphaerochaeta</i>
ASV_168	18.458	<i>T. bryantii</i>
ASV_156	18.193	<i>Treponema</i>
ASV_345	19.629	<i>Treponema</i>
ASV_615	17.597	<i>Treponema</i>
ASV_374	17.276	<i>Treponema</i>
ASV_736	20.117	<i>Treponema</i>
ASV_177	19.749	<i>p-1088-a5_gut_group</i>
ASV_399	19.505	<i>Bacteroidia</i>
ASV_560	20.118	NA
ASV_589	20.041	<i>Bacteroidia</i>
ASV_1410	15.734	<i>Candidatus_Saccharimonas</i>
ASV_424	19.523	<i>Candidatus_Saccharimonas</i>
ASV_128	18.473	<i>Candidatus_Saccharimonas</i>
ASV_407	19.997	<i>Candidatus_Saccharimonas</i>
ASV_586	17.191	<i>Candidatus_Saccharimonas</i>
ASV_344	19.862	<i>Candidatus_Saccharimonas</i>
ASV_115	22.964	<i>Candidatus_Saccharimonas</i>
ASV_1176	-21.005	<i>Selenomonadaceae</i>
ASV_328	8.170	NA
ASV_458	21.504	<i>Selenomonadaceae</i>
ASV_1557	21.923	<i>Selenomonas</i>
ASV_931	17.796	<i>Fretibacterium</i>
ASV_1118	18.374	<i>Desulfobulbus</i>
ASV_507	20.210	<i>RF39</i>
ASV_796	18.908	<i>RF39</i>
ASV_387	18.768	<i>RF39</i>
ASV_508	20.067	<i>RF39</i>
ASV_956	19.348	<i>RF39</i>
ASV_623	-14.485	<i>RF39</i>
ASV_779	19.847	<i>Erysipelotrichaceae_UCG-009</i>
ASV_614	20.861	<i>Clostridia_UCG-014</i>
ASV_395	-25.081	<i>Clostridia_UCG-014</i>
ASV_949	20.650	<i>Ruminococcaceae</i>
ASV_1144	-25.990	<i>UCG-001</i>
ASV_415	22.496	<i>Ruminococcus</i>
ASV_22	18.337	<i>F082</i>
ASV_193	21.332	<i>F082</i>

ASV_287	19.862	<i>F082</i>
ASV_356	20.764	<i>Muribaculaceae</i>
ASV_191	22.605	<i>Muribaculaceae</i>
ASV_155	20.039	<i>Muribaculaceae</i>
ASV_1159	-24.402	<i>Muribaculaceae</i>
ASV_279	20.214	<i>Rikenellaceae_RC9_gut_group</i>
ASV_621	18.820	<i>Rikenellaceae_RC9_gut_group</i>
ASV_468	18.746	<i>Rikenellaceae_RC9_gut_group</i>
ASV_947	16.058	<i>Rikenellaceae_RC9_gut_group</i>
ASV_202	19.107	<i>Bacteroidales_UCG-001</i>
ASV_1007	17.104	<i>Oscillospirales</i>
ASV_571	19.964	<i>Oscillospirales</i>
ASV_134	21.857	<i>Oscillospirales</i>
ASV_237	19.888	<i>Oscillospirales</i>
American Wagyu Data (F1)		
High Marbling Vs Low Marbling		
ASV_190	-28.18	<i>F082</i>
ASV_778	23.41	<i>F082</i>
ASV_383	-22.29	<i>F082</i>
ASV_925	21.13	<i>Muribaculaceae</i>
ASV_727	21.75	<i>Muribaculaceae</i>
ASV_1304	20.8	<i>Muribaculaceae</i>
ASV_729	21.53	<i>Muribaculaceae</i>
ASV_307	23.91	<i>Muribaculaceae</i>
ASV_654	21.74	<i>Prevotellaceae</i>
ASV_627	20.31	<i>Prevotella</i>
ASV_449	21.55	<i>Prevotella</i>
ASV_7	19.01	<i>Prevotella</i>
ASV_937	-22.38	<i>Prevotella</i>
ASV_1241	20.19	<i>Prevotellaceae_UCG-001</i>
ASV_910	22.17	<i>Prevotellaceae_UCG-003</i>
ASV_1688	23.09	<i>Rikenellaceae_RC9_gut_group</i>
ASV_1105	-24.97	<i>Flexilinea</i>
ASV_1399	22.22	<i>Desulfovibrio</i>
ASV_1629	23.38	<i>Fibrobacter</i>
ASV_853	-25.58	<i>Fibrobacter</i>
ASV_1477	21.21	<i>Christensenellaceae_R-7_group</i>
ASV_1175	-21.16	<i>possible_genus_Sk018</i>
ASV_117	-23.15	<i>Firmicutes</i>
ASV_919	-22.52	<i>Firmicutes</i>
ASV_676	20.88	<i>NK4A214_group</i>
ASV_652	21.67	<i>NK4A214_group</i>
ASV_938	-22.98	NA
ASV_674	20.96	<i>UCG-001</i>
ASV_732	19.11	<i>Candidatus_Saccharimonas</i>
ASV_703	-22.97	<i>p-1088-a5_gut_group</i>
ASV_480	-22.06	<i>Treponema</i>
ASV_797	21.85	<i>Treponema</i>
ASV_1048	22.47	<i>Verrucomicrobiota</i>
ASV_1185	-22.86	<i>vadinBE97</i>

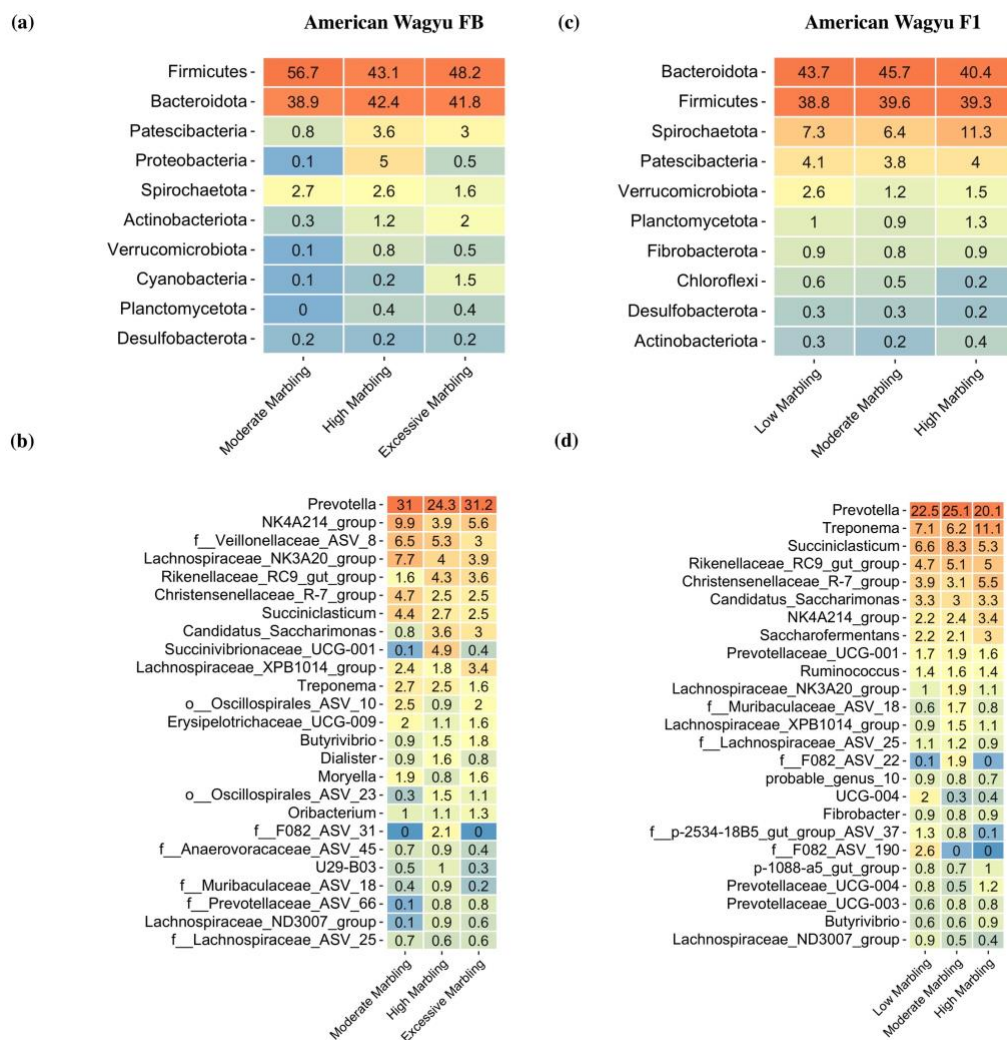
Angus Steer Data (FB)		
Prime Marbling Vs Select Marbling		
ASV_285	25.0442429	RF39
ASV_191	24.6447102	<i>Ruminococcaceae</i>
ASV_102	23.6650058	<i>Prevotella</i>
ASV_161	23.194611	UCG-004
ASV_187	18.1636245	<i>Bacteroidales_RF16_group</i>
ASV_129	9.67091458	<i>Candidatus_Saccharimonas</i>
ASV_331	9.21568615	RF39
ASV_406	8.52308349	<i>Absconditabacteriales_(SR1)</i>
ASV_12	7.55845227	<i>Prevotella</i>
ASV_132	6.13738751	U29-B03
ASV_76	5.43135533	<i>Prevotella</i>
ASV_34	5.04928414	<i>Prevotellaceae_UCG-001</i>
ASV_23	3.43819008	<i>Prevotella</i>
ASV_58	-22.410084	<i>Muribaculaceae</i>
ASV_296	-25.020757	<i>Treponema</i>
ASV_657	-25.22469	<i>Bacteroidia</i>
ASV_671	-25.420028	<i>Christensenellaceae_R-7_group</i>
ASV_405	-25.474465	<i>p-1088-a5_gut_group</i>
ASV_263	-25.56071	<i>Ruminococcaceae</i>
ASV_118	-25.565588	<i>Prevotella</i>
ASV_589	-25.60567	<i>Rikenellaceae_RC9_gut_group</i>
ASV_139	-25.613413	<i>Prevotella</i>
ASV_442	-25.638001	<i>Pirellula</i>
ASV_357	-25.842392	<i>Bacteroidales</i>
ASV_563	-25.852958	<i>Prevotella</i>
ASV_286	-26.101796	<i>Succiniclasicum</i>
ASV_432	-26.179974	<i>Flexilinea</i>
ASV_413	-26.24667	<i>Oscillospiraceae</i>
ASV_303	-26.341928	RF39
ASV_435	-26.381637	<i>Succinivibrio</i>
ASV_522	-26.403983	<i>Prevotellaceae</i>
ASV_146	-26.41823	<i>Erysipelotrichaceae_UCG-009</i>
ASV_111	-26.550731	<i>Lachnospiraceae_ND3007_group</i>
ASV_434	-26.557518	WCHB1-41
ASV_470	-26.633177	<i>Moryella</i>
ASV_27	-26.878849	<i>Muribaculaceae</i>
ASV_386	-27.005911	<i>Ruminococcus</i>
ASV_408	-27.026328	<i>Ruminococcus</i>
ASV_277	-27.415946	<i>Lachnospiraceae_ND3007_group</i>
ASV_183	-27.542272	<i>Oscillospirales</i>
ASV_261	-27.805581	<i>Lachnospiraceae_NK3A20_group</i>
ASV_252	-28.024303	<i>Moryella</i>
ASV_259	-28.26436	<i>Selenomonadaceae</i>
ASV_326	-28.612697	<i>Treponema</i>
ASV_119	-29.127788	<i>p-1088-a5_gut_group</i>
ASV_189	-29.436812	<i>Anaerovibrio</i>
ASV_93	-30	<i>Lachnospiraceae_ND3007_group</i>

**Figure1: Imperial Beef American Wagyu marbling grades**



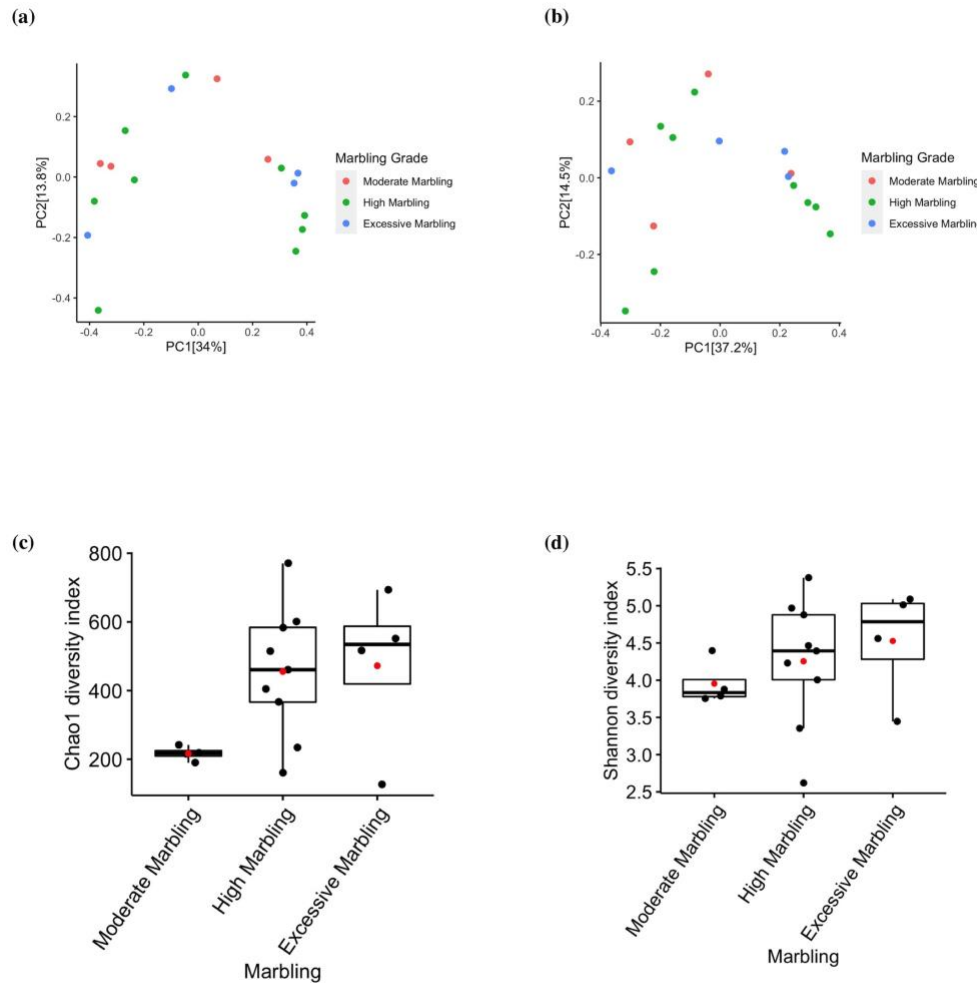
The figure is representing visual example for different marbling grades. Imperial Wagyu Beef uses Japanese Beef Marbling Score (BMS) as a reference and they assign Market Style, Classic, Signature, or Reserve grade based on the marbling. For this study, we divided the marbling grades into four groups named Low Marbling, Moderate Marbling, High Marbling, and Excessive Marbling.

**Figure 2: The relative abundances of top phyla and genera across different marbling grades for FB and F1 American Wagyu cattle**



The graph is showing the relative abundances (percentage) of top 10 phyla and top 25 genera across different marbling grades for FB (a and b) and F1 (c and d) American Wagyu cattle.

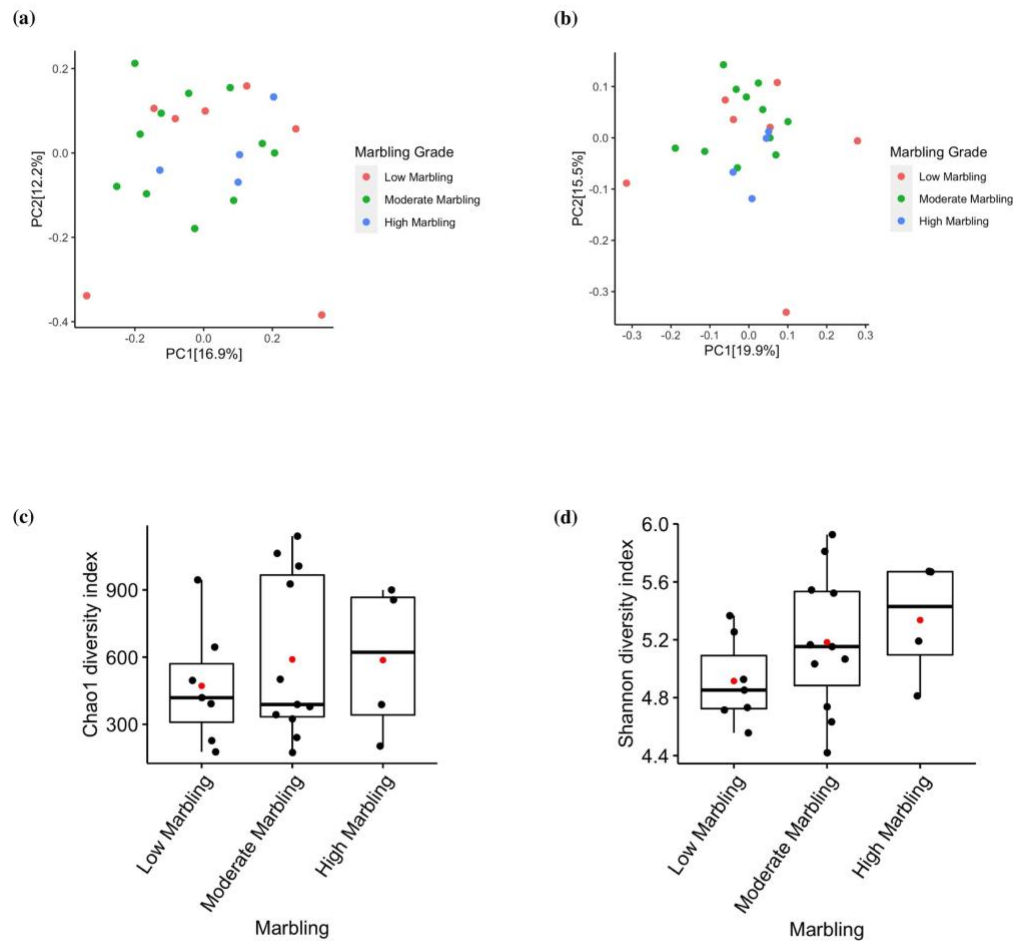
**Figure 3: Bacterial community differences between full blood (FB) American Wagyu cattle with different marbling grades.**



The FB data was used for this figure. Principle coordinate analysis (PCoA) was used to visualize the bacterial community differences between different marbling grades based on Bray-Curtis (a) or weighted unifrac (b) distance matrices. Box plots were used to plot alpha diversity index Chao1 (c) and Shannon (d) for different marbling grades. The red dots (figure c and d) represent the Chao1 and Shannon group average for each marbling grade.

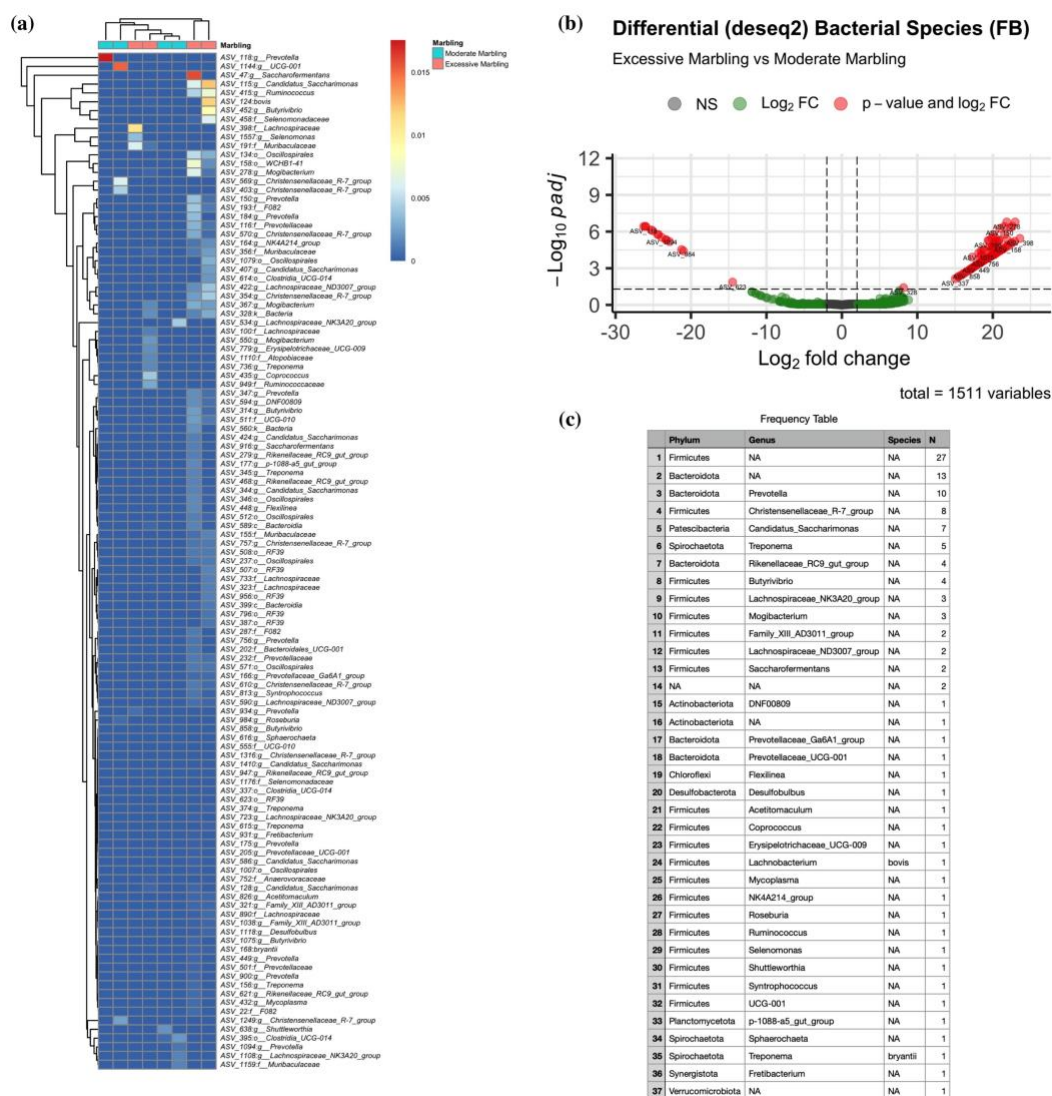


**Figure 4: Bacterial community differences between crossbred (F1) American Wagyu cattle with different marbling grades.**



The F1 data was used for this figure. Principle coordinate analysis (PCoA) was used to visualize the bacterial community differences between different marbling grades based on Bray-Curtis (a) or weighted unifracs (b) distance matrices. Box plots were used to plot alpha diversity index Chao1 (c) and Shannon (d) for different marbling grades. The red dots (figure c and d) represent the Chao1 and Shannon group average for each marbling grade.

**Figure 5: Differential bacterial species between marbling grades for full blood (FB) American Wagyu cattle.**



The DESeq2 analysis identified 115 significantly different ASVs between Excessive and Moderate Marbling groups in FB data. (a) The heatmap is showing the relative abundances of differential ASVs for moderate and excessive marbling samples. (b) The volcano plot is showing the differential DESeq2 analysis output for 1511 ASVs. The black and green dots represent the non-significant ( $p$ -adjusted > 0.05) ASVs whereas red dots represent the significantly different ( $p$ -adjusted < 0.05) ASVs between Excessive and Moderate marbling. (c) The frequency table is showing the number of significantly different ASVs (N) that belongs to a unique genus.

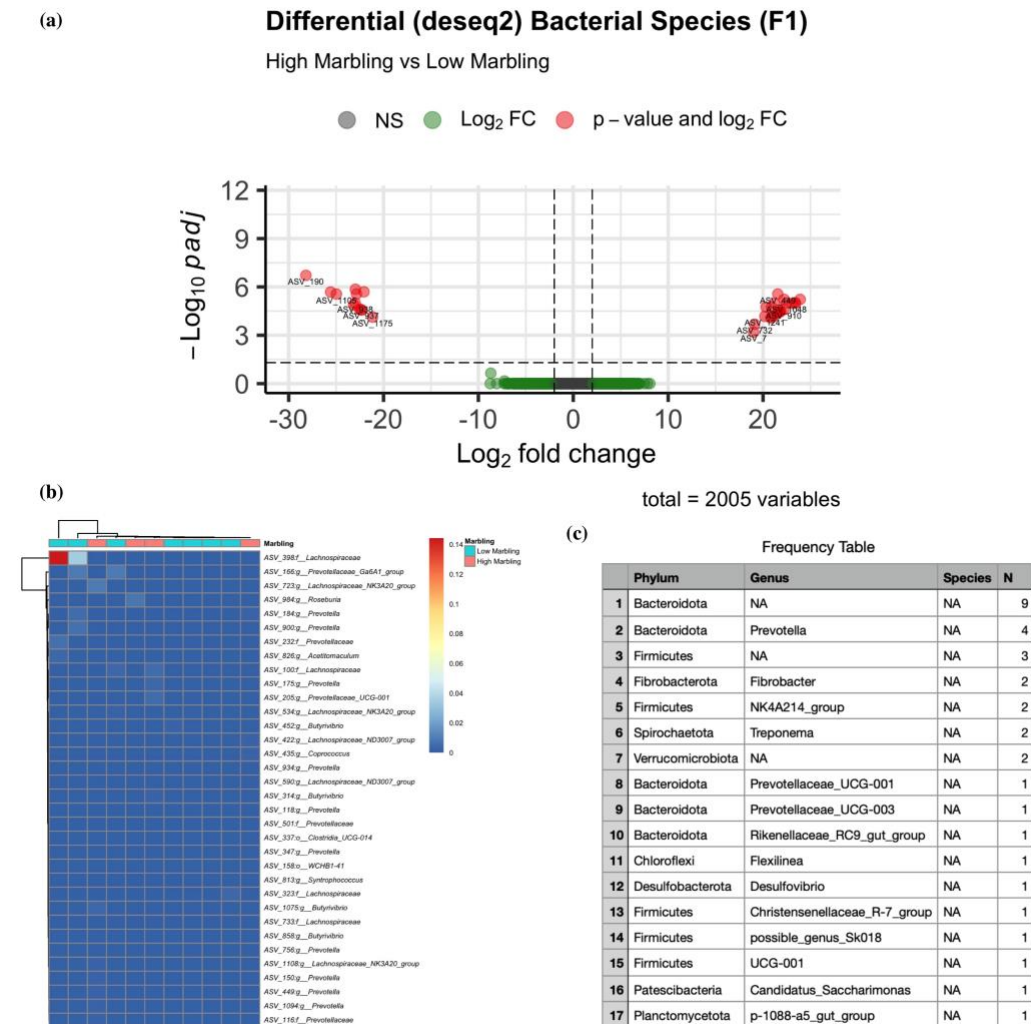
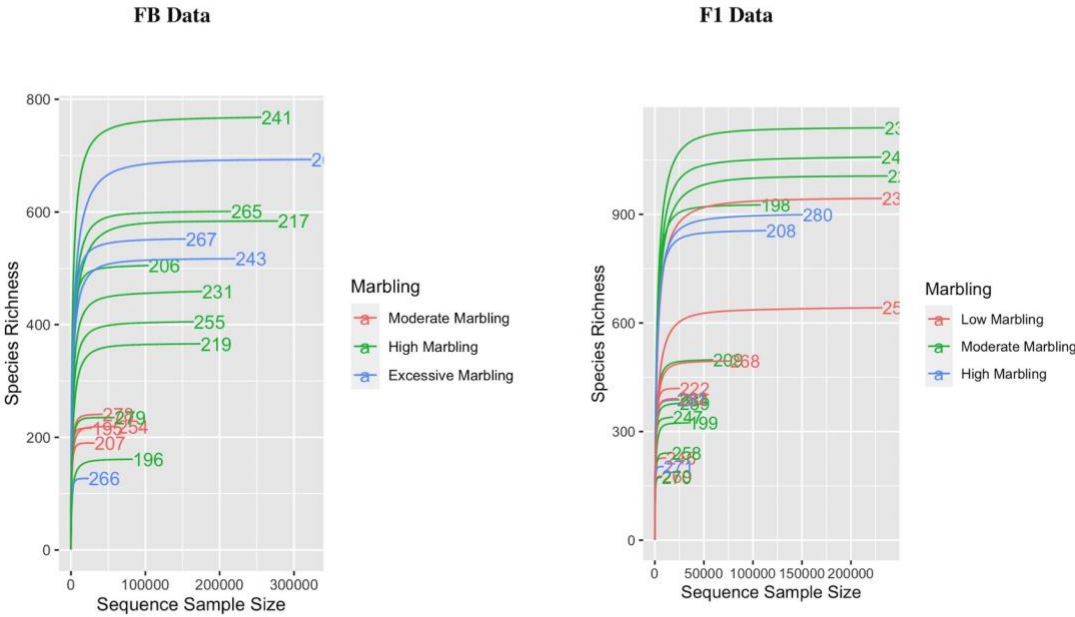
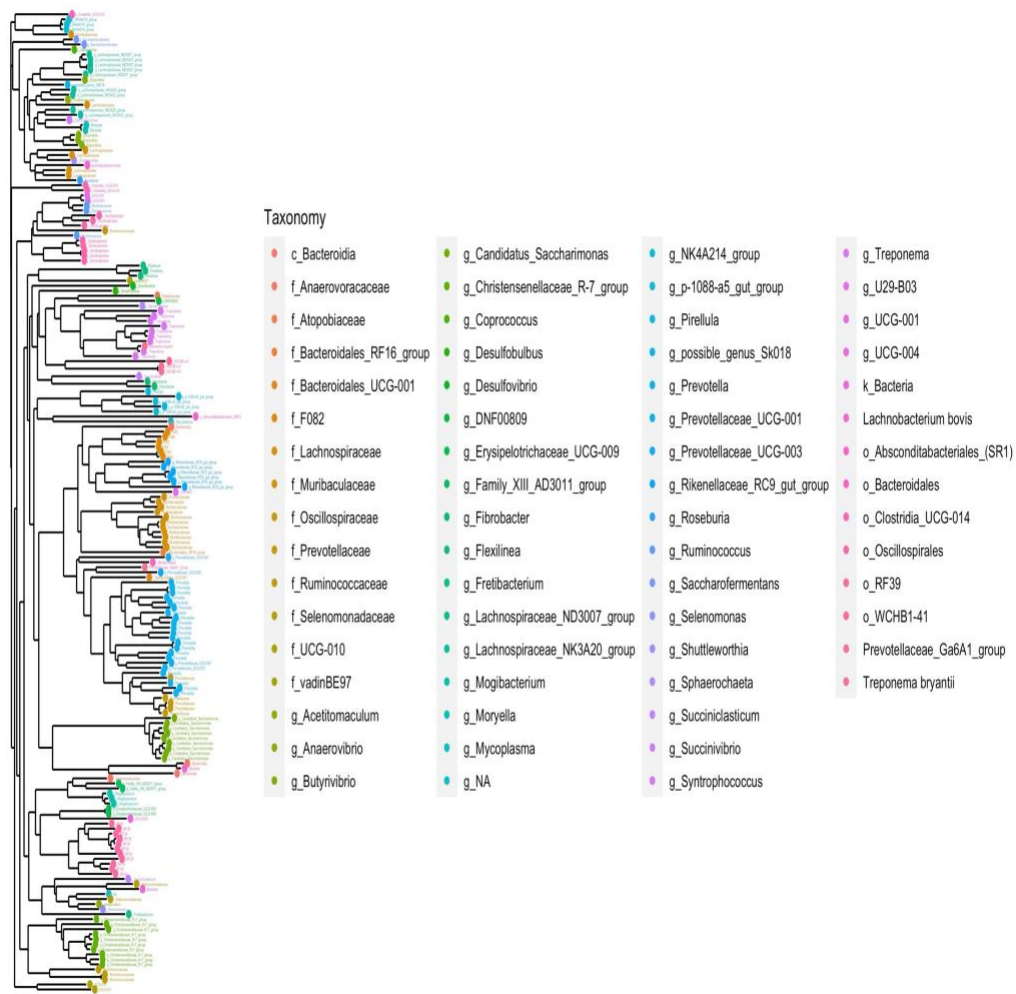


Figure 7: The rarefaction curves for FB and F1 data.



**Figure 8A: The phylogenetic tree of differential ASVs colored based on genus identified in Wagyu and Angus cattle.**



**Figure 8B: The phylogenetic tree of differential ASVs colored based on different categories identified in Wagyu and Angus cattle.**

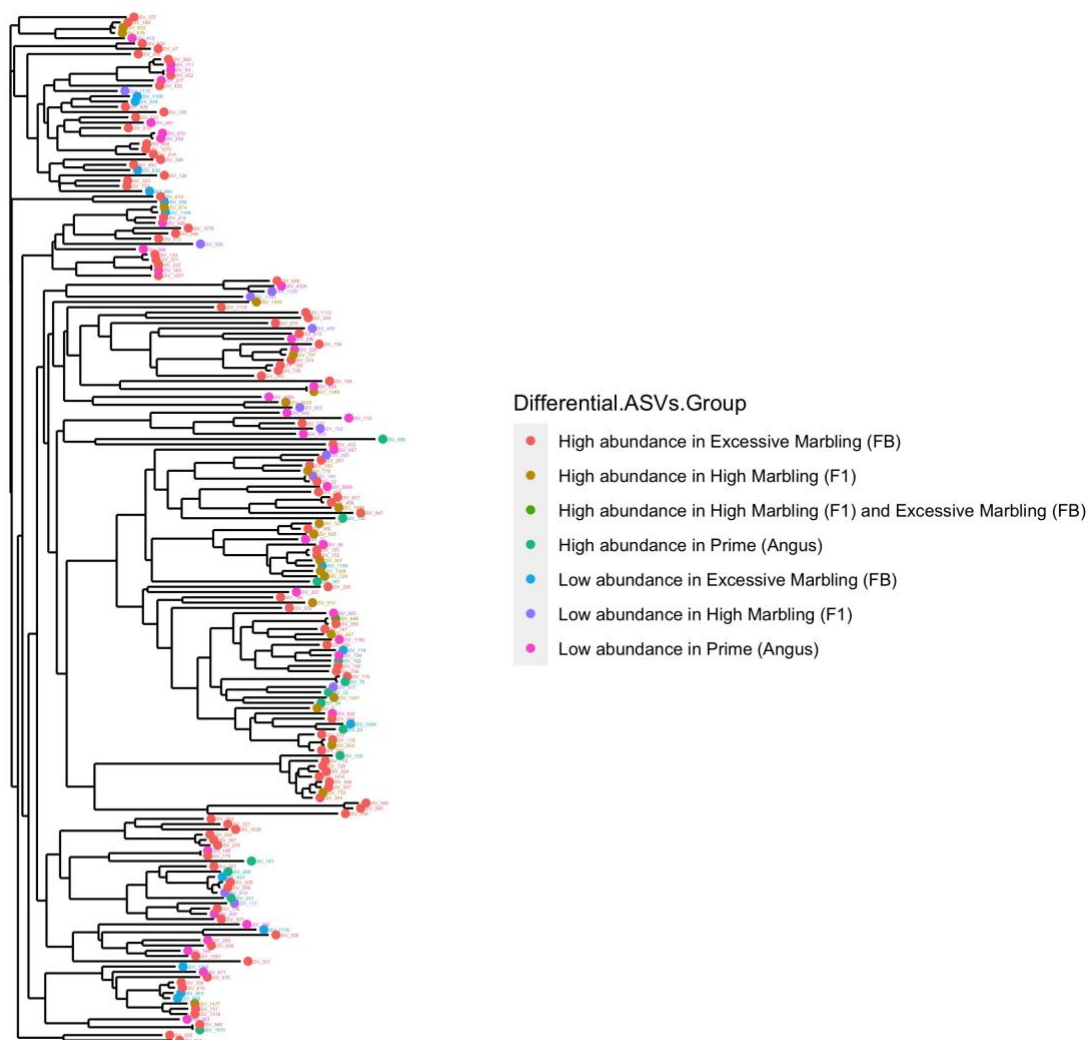
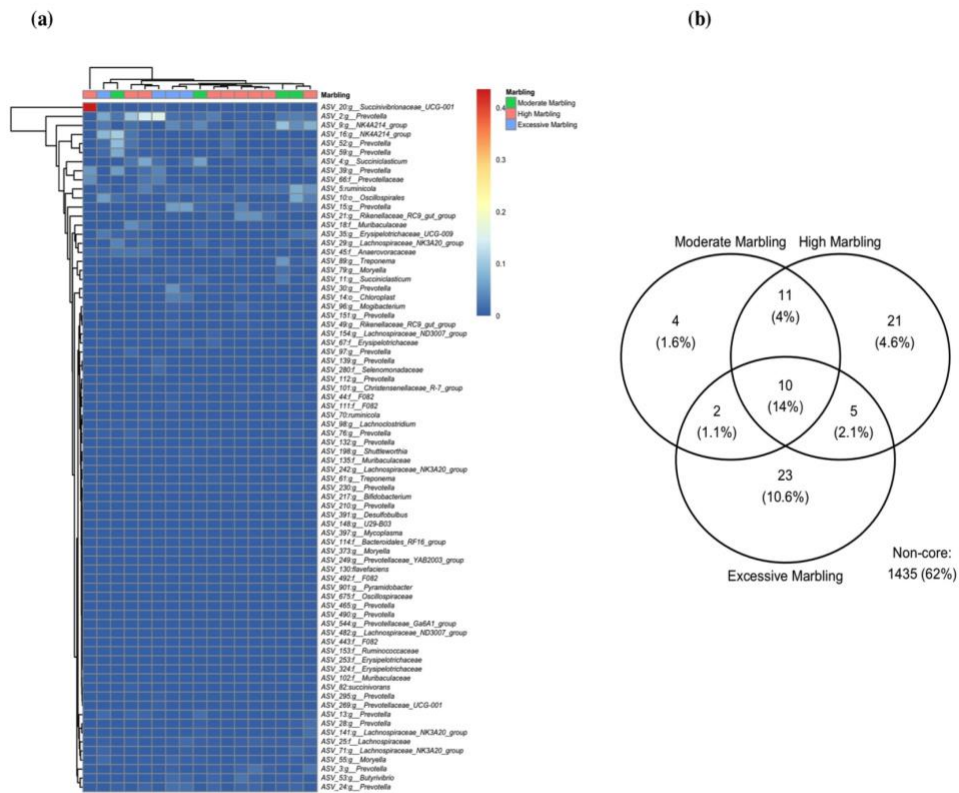


Figure 8 A and B: Each node is representing the ASV, and DNA sequences were used to make phylogenetic tree. The pairwise distance between DNA sequences was calculated with mothur and later a phylogenetic tree was generated. The distance between two nodes represents the differences between their DNA sequences. Figure 8A: the nodes were colored based on their genus. Figure 8B: the nodes were colored based on differential analysis (DESeq2) results. High abundance in Excessive Marbling (FB) category represents the ASV that has significantly higher abundance in Excessive Marbling samples as compared to Moderate Marbling samples in FB data. Low abundance in Excessive Marbling (FB) category represents the ASV that has significantly lower abundance in Excessive Marbling samples as compared to Moderate Marbling samples in FB data. High abundance in High Marbling (FB) category represents the ASV that has significantly higher abundance in High Marbling samples as compared to Low Marbling samples in F1 data. Low abundance in High Marbling (FB) category represents the ASV that has significantly lower abundance in High Marbling samples as compared to Low Marbling samples in F1 data. High abundance in High Marbling (F1) and Excessive Marbling (FB) category represents the ASVs that were present in significantly higher abundance in High Marbling and Excessive Marbling samples in FB and F1 data. High abundance in Prime (Angus) category and Low abundance in Prime (Angus) category represents the ASV that has significantly high and low abundance in Prime marbling samples, respectively.

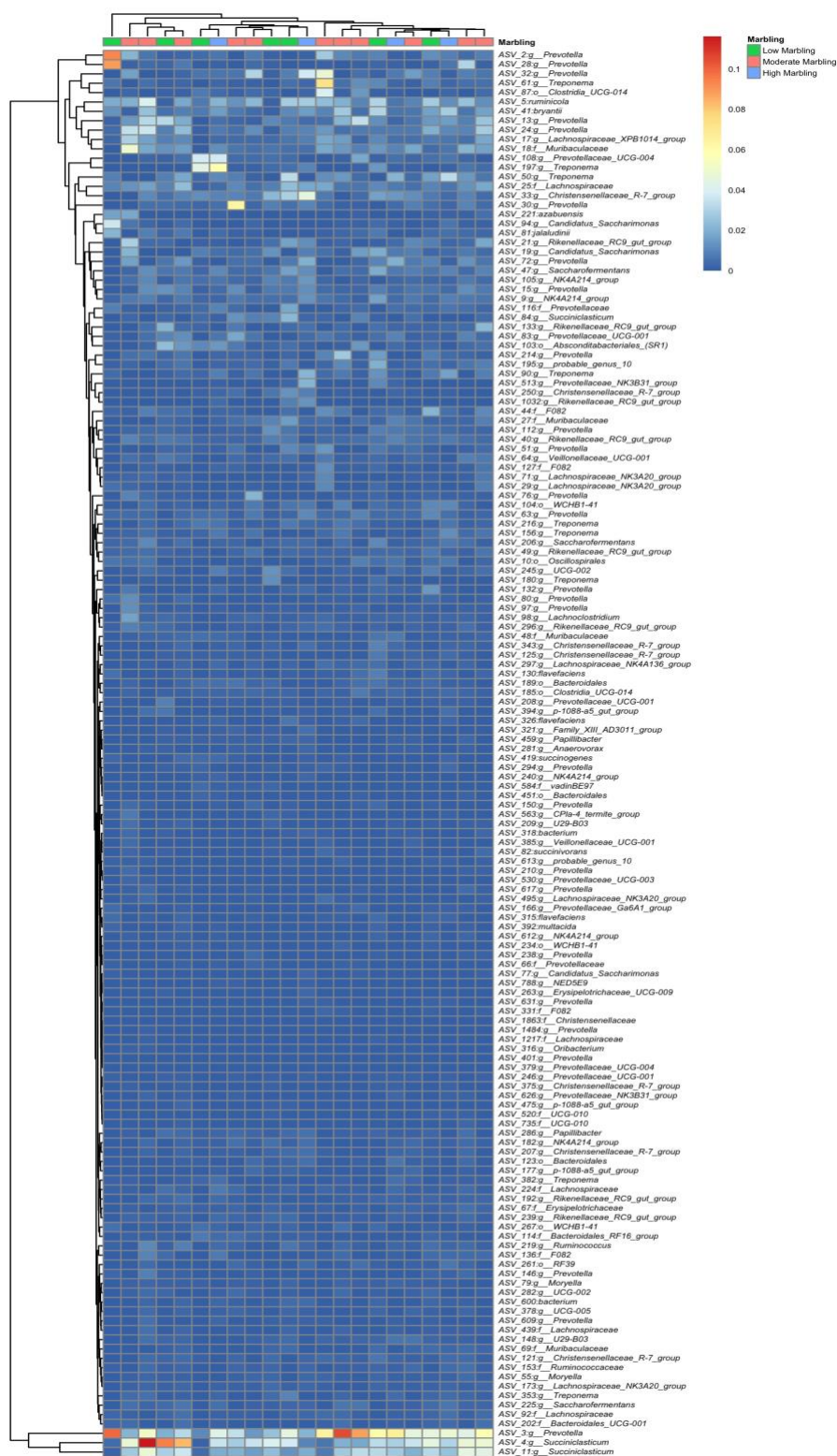


**Figure 9: The core bacteria species for each marbling grade in FB data.**





**Figure 10A: The core bacteria species for each marbling grade in F1 data.**



**Figure 10B: The core bacteria species for each marbling grade in F1 data.**

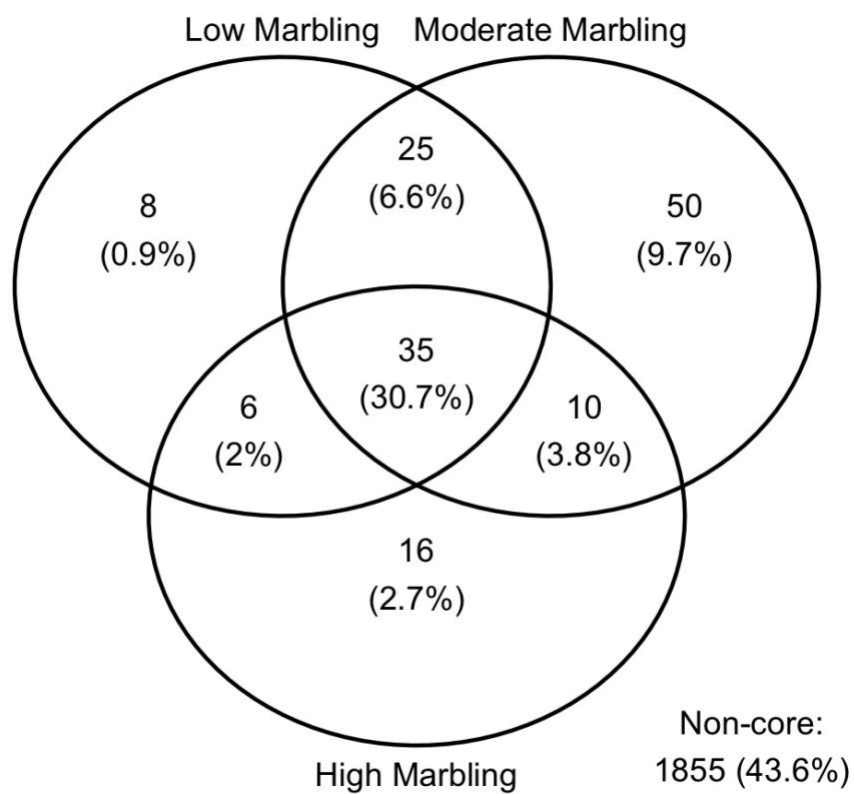
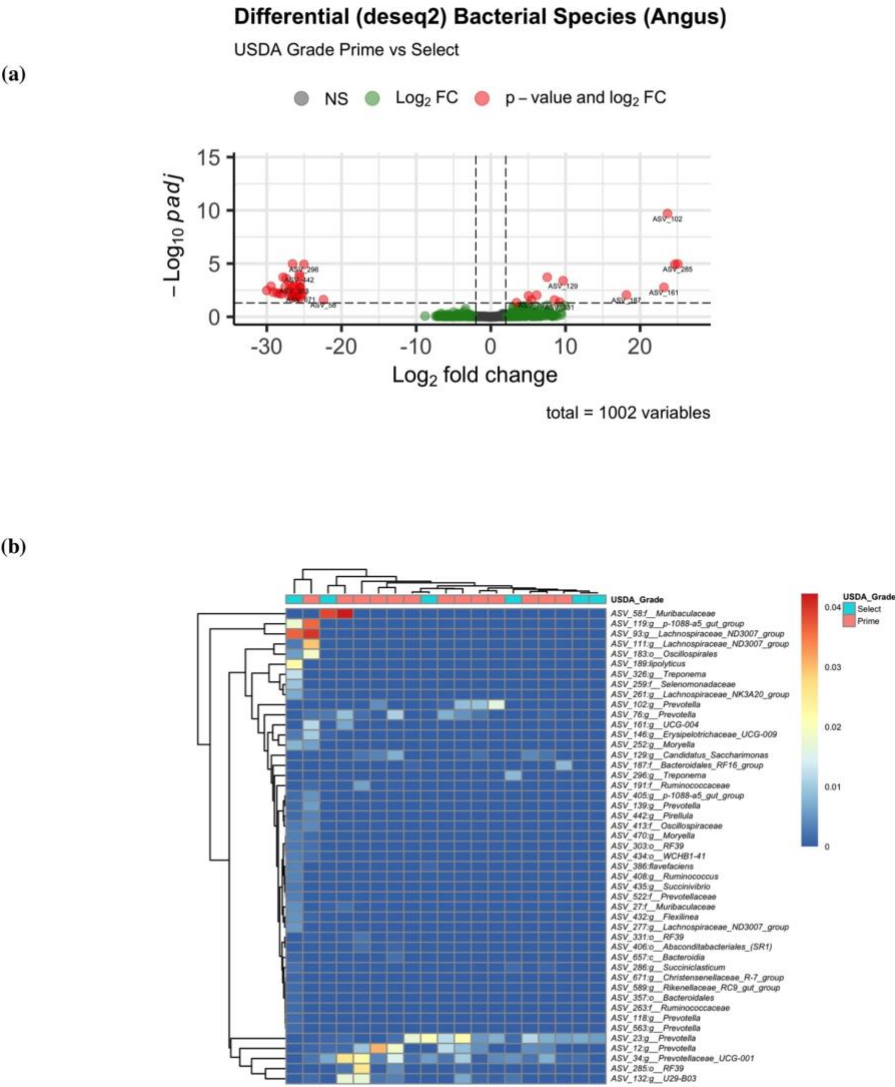
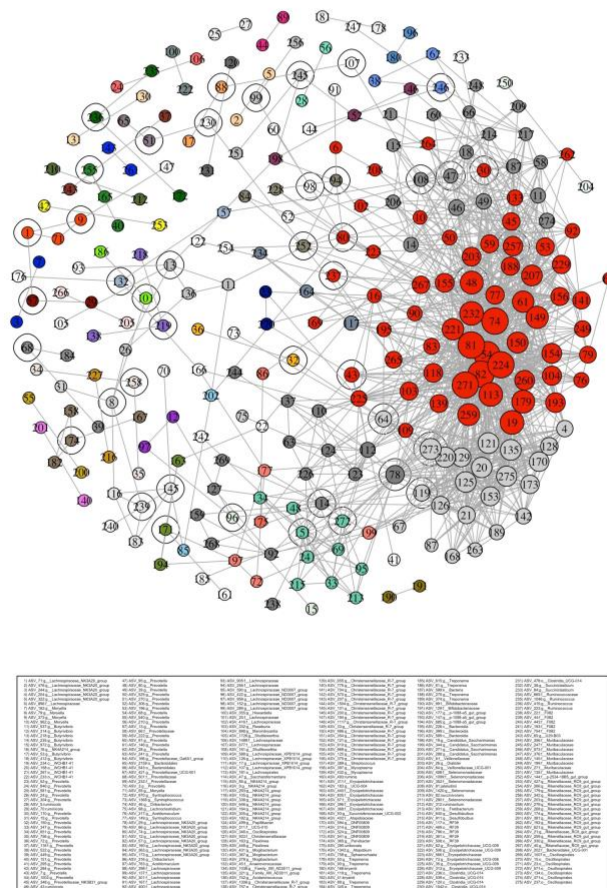


Figure 11: Differential bacterial species between marbling grades for Angus steers.



**Figure 12a: The SparCC based ecological network of bacterial species for full blood (FB) American Wagyu cattle.**

## Wagyu Full Blood

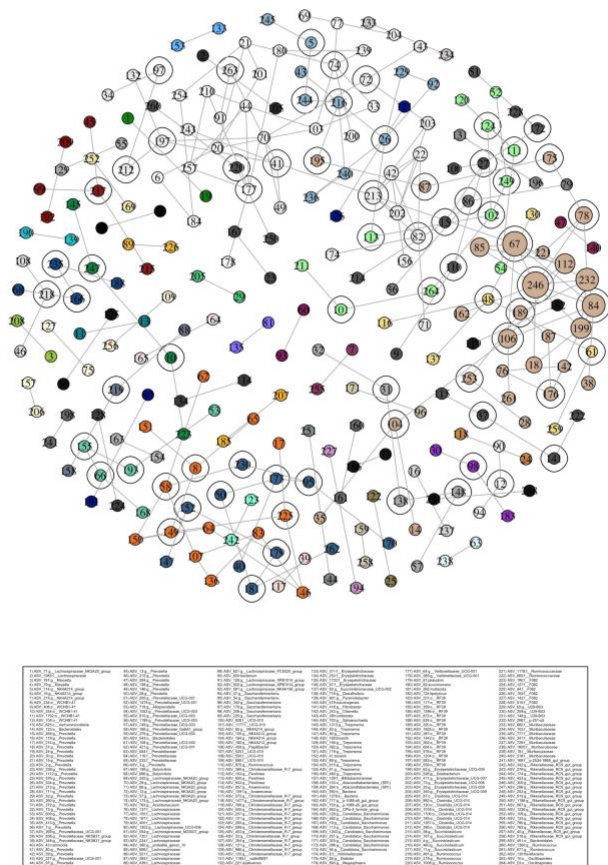


1) ASV_71g_Lachnospiraceae_NK3A20_group	47) ASV_95g_Prevotella	83) ASV_905f_Lachnospiraceae	139) ASV_655a_Christensenellaceae_R-7_group	185) ASV_615g_Treponema	231) ASV_478a_Costridia_UCG-014
2) ASV_478g_Lachnospiraceae_NK3A20_group	48) ASV_80g_Prevotella	84) ASV_256f_Lachnospiraceae	140) ASV_775a_Christensenellaceae_R-7_group	186) ASV_614g_Treponema	232) ASV_38g_Succinacidium
3) ASV_244g_Lachnospiraceae_NK3A20_group	49) ASV_39g_Prevotella	85) ASV_590a_Lachnospiraceae_NK3007_group	141) ASV_704a_Christensenellaceae_R-7_group	187) ASV_598k_Bacteria	233) ASV_84g_Succinacidium
4) ASV_204g_Lachnospiraceae_NK3A20_group	50) ASV_525g_Prevotella	86) ASV_422a_Lachnospiraceae_NK3007_group	142) ASV_570a_Christensenellaceae_R-7_group	188) ASV_273g_Treponema	234) ASV_665f_Ruminococcaceae
5) ASV_322g_Lachnospiraceae_NK3A20_group	51) ASV_270g_Prevotella	87) ASV_559a_Lachnospiraceae_NK3007_group	143) ASV_207a_Christensenellaceae_R-7_group	189) ASV_374g_Treponema	235) ASV_1046g_Ruminococcaceae
6) ASV_886f_Lachnospiraceae	52) ASV_306g_Prevotella	88) ASV_152a_Lachnospiraceae_NK3007_group	144) ASV_101a_Christensenellaceae_R-7_group	190) ASV_96f_Bifidobacteriaceae	236) ASV_415g_Ruminococcus
7) ASV_162g_Moryella	53) ASV_186g_Prevotella	89) ASV_289a_Lachnospiraceae_NK3007_group	145) ASV_101a_Christensenellaceae_R-7_group	191) ASV_129f_Bifidobacteriaceae	237) ASV_223g_Ruminococcus
8) ASV_79g_Moryella	54) ASV_68g_Prevotella	90) ASV_355g_Howardella	146) ASV_200a_Christensenellaceae_R-7_group	192) ASV_177g_P-1088-45_R-7_group	238) ASV_221_F882
9) ASV_352g_Moryella	55) ASV_540g_Prevotella	101) ASV_251f_Lachnospiraceae	147) ASV_194a_Christensenellaceae_R-7_group	193) ASV_147g_P-1088-45_R-7_group	239) ASV_441_F382
10) ASV_922g_Moryella	56) ASV_210g_Prevotella	102) ASV_416f_Lachnospiraceae	148) ASV_117g_Christensenellaceae_R-7_group	194) ASV_443f_F382	240) ASV_443f_F382
11) ASV_539g_Baymbyrio	57) ASV_13g_Prevotella	103) ASV_332g_Roseburia	149) ASV_33g_Christensenellaceae_R-7_group	195) ASV_226c_Bacteroida	241) ASV_193f_F382
12) ASV_314g_Baymbyrio	58) ASV_66f_Prevotellaceae	104) ASV_886g_Marinivibrio	150) ASV_966a_Christensenellaceae_R-7_group	196) ASV_399c_Bacteroida	242) ASV_754f_F382
13) ASV_218g_Baymbyrio	59) ASV_222g_Prevotella	105) ASV_1728g_Stuifvorbijia	151) ASV_354g_Christensenellaceae_R-7_group	197) ASV_589c_Bacteroida	243) ASV_556g_Muribaculaceae
14) ASV_235g_Baymbyrio	60) ASV_91g_Prevotella	106) ASV_1486f_Lachnospiraceae	152) ASV_121a_Christensenellaceae_R-7_group	198) ASV_159g_Candidatus_Saccharimonas	244) ASV_536f_Muribaculaceae
15) ASV_972g_Baymbyrio	61) ASV_146g_Prevotella	107) ASV_677f_Lachnospiraceae	153) ASV_610g_Christensenellaceae_R-7_group	199) ASV_344g_Candidatus_Saccharimonas	245) ASV_247f_Muribaculaceae
16) ASV_169g_NK4A214_group	62) ASV_28g_Prevotella	108) ASV_161g_Stuifvorbijia	154) ASV_669a_Christensenellaceae_R-7_group	200) ASV_271g_Candidatus_Saccharimonas	246) ASV_373f_Muribaculaceae
17) ASV_53g_Baymbyrio	63) ASV_241g_Prevotella	109) ASV_588g_Lachnospiraceae_XPB1014_group	155) ASV_125g_Christensenellaceae_R-7_group	201) ASV_288g_Candidatus_Saccharimonas	247) ASV_376f_Muribaculaceae
18) ASV_412g_Baymbyrio	64) ASV_166g_Prevotellaceae_Ga6A1_group	110) ASV_126g_Lachnospiraceae_XPB1014_group	156) ASV_375g_Christensenellaceae_R-7_group	202) ASV_81g_Veillonellaceae	248) ASV_145f_Muribaculaceae
19) ASV_224g_WCHB1-41	65) ASV_543g_Bacteroides	111) ASV_17g_Lachnospiraceae_XPB1014_group	157) ASV_389g_Streptococcus	203) ASV_261g_Dalziel	249) ASV_196f_Muribaculaceae
20) ASV_158g_WCHB1-41	66) ASV_543g_Bacteroides	112) ASV_107g_Lachnospiraceae_XPB1014_group	158) ASV_397g_Mycoplasma	204) ASV_64g_Veillonellaceae_UCG-001	250) ASV_271f_Muribaculaceae
21) ASV_267g_WCHB1-41	67) ASV_421g_Prevotellaceae	113) ASV_181g_Lachnospiraceae	159) ASV_432g_Mycoplasma	205) ASV_458f_Selenomonadaceae	251) ASV_135f_Muribaculaceae
22) ASV_233g_WCHB1-41	68) ASV_501f_Prevotellaceae	114) ASV_47g_Saccharofermentans	160) ASV_483g_Aminis	206) ASV_1099f_Selenomonadaceae	252) ASV_144f_P-2534-1885_gut_group
23) ASV_52g_Prevotella	69) ASV_232f_Prevotellaceae	115) ASV_85g_NK4A214_group	161) ASV_311f_Erysipelotrichaceae	207) ASV_352f_Selenomonadaceae	253) ASV_427g_Rikenellaceae_RG_gut_group
24) ASV_846g_Prevotella	70) ASV_3g_Prevotella	116) ASV_59g_NK4A214_group	162) ASV_183g_UCG-004	208) ASV_81g_Jaludini	254) ASV_266g_Rikenellaceae_RG_gut_group
25) ASV_581g_Prevotella	71) ASV_55g_Moryella	117) ASV_293g_NK4A214_group	163) ASV_440f_Erysipelotrichaceae	209) ASV_1420g_Selenomonas	255) ASV_179g_Rikenellaceae_RG_gut_group
26) ASV_24g_Prevotella	72) ASV_970g_Syntrophococcus	118) ASV_105g_NK4A214_group	164) ASV_805f_Erysipelotrichaceae	210) ASV_82succinorans	256) ASV_342g_Rikenellaceae_RG_gut_group
27) ASV_344g_Prevotella	73) ASV_138g_Syntrophococcus	119) ASV_338g_NK4A214_group	165) ASV_671f_Erysipelotrichaceae	211) ASV_289f_Selenomonadaceae	257) ASV_203g_Rikenellaceae_RG_gut_group
28) ASV_5ummicola	74) ASV_46g_Oribacterium	120) ASV_646g_NK4A214_group	166) ASV_396f_Erysipelotrichaceae	212) ASV_312truncatum	258) ASV_279g_Rikenellaceae_RG_gut_group
29) ASV_70ummicola	75) ASV_98g_Lachnospiraceae	121) ASV_164g_NK4A214_group	167) ASV_388f_Erysipelotrichaceae	213) ASV_931g_Fretibacterium	259) ASV_268g_Rikenellaceae_RG_gut_group
30) ASV_110g_Prevotella	76) ASV_317g_Acetivibrio	122) ASV_305g_NK4A214_group	168) ASV_93g_Succinivibrionaceae_UCG-002	214) ASV_640g_Desulfohalobium	260) ASV_174g_Rikenellaceae_RG_gut_group
31) ASV_150g_Prevotella	77) ASV_146g_Syntrophococcus	123) ASV_182g_NK4A214_group	169) ASV_402f_Apobacteriaceae	215) ASV_911g_Desulfohalobium	261) ASV_381g_Rikenellaceae_RG_gut_group
32) ASV_150g_Prevotella	78) ASV_29g_Lachnospiraceae_NK3A20_group	124) ASV_499g_Papillibacter	170) ASV_594g_DNF0809	216) ASV_117g_R-79	262) ASV_192g_Rikenellaceae_RG_gut_group
33) ASV_347g_Prevotella	79) ASV_86g_Lachnospiraceae_NK3A20_group	125) ASV_511f_UCG-010	171) ASV_514g_DNF0809	217) ASV_469g_R-79	263) ASV_621g_Rikenellaceae_RG_gut_group
34) ASV_651g_Prevotella	80) ASV_74g_Lachnospiraceae_NK3A20_group	126) ASV_346g_Ocillospirales	172) ASV_524g_DNF0809	218) ASV_796g_R-79	264) ASV_21g_Rikenellaceae_RG_gut_group
35) ASV_756g_Prevotella	81) ASV_12g_Lachnospiraceae_NK3A20_group	127) ASV_933f_Christensenellaceae	173) ASV_541g_DNF0809	219) ASV_361g_R-79	265) ASV_259g_Rikenellaceae_RG_gut_group
36) ASV_72g_Prevotella	82) ASV_57g_Lachnospiraceae_NK3A20_group	128) ASV_163g_Fusiflexa	174) ASV_365g_Papillibacter	220) ASV_508g_R-79	266) ASV_330g_Rikenellaceae_RG_gut_group
37) ASV_1161g_Prevotella	83) ASV_160g_Lachnospiraceae_NK3A20_group	129) ASV_448g_Fusiflexa	175) ASV_380g_Ambatonia	221) ASV_62g_Erysipelotrichaceae_UCG-009	267) ASV_48g_Rikenellaceae_RG_gut_group
38) ASV_522g_Prevotella	84) ASV_363g_Lachnospiraceae_NK3A20_group	130) ASV_431g_Mogibacterium	176) ASV_1343g_Apobacterium	222) ASV_506g_Erysipelotrichaceae_UCG-009	268) ASV_202f_Bacteroides_UCG-001
39) ASV_448g_Prevotella	85) ASV_173g_Lachnospiraceae_NK3A20_group	131) ASV_367g_Mogibacterium	177) ASV_758g_Sphaerochela	223) ASV_566f_Erysipelotrichaceae	269) ASV_1007g_Ocillospirales
40) ASV_151g_Prevotella	86) ASV_316g_Oribacterium	132) ASV_278g_Mogibacterium	178) ASV_90g_Treponema	224) ASV_73g_Erysipelotrichaceae_UCG-006	270) ASV_70g_Ocillospirales
41) ASV_256g_Prevotella	87) ASV_763g_Acetivibrio	133) ASV_457g_Acanthomonas	179) ASV_168g_Bryanti	225) ASV_353g_Erysipelotrichaceae_UCG-009	271) ASV_230g_Ocillospirales
42) ASV_284g_Prevotella	88) ASV_398f_Lachnospiraceae	134) ASV_1038g_Family_XIII_AD3011_group	180) ASV_99g_Treponema	226) ASV_313g_Erysipelotrichaceae_UCG-009	272) ASV_371g_Ocillospirales
43) ASV_7g_Prevotella	89) ASV_137f_Lachnospiraceae	135) ASV_321g_Acanthomonas	181) ASV_119g_Treponema	227) ASV_238g_Costridia_UCG-014	273) ASV_134g_Ocillospirales
44) ASV_1022g_Prevotella	90) ASV_301f_Lachnospiraceae	136) ASV_742g_Acanthomonas	182) ASV_41bryanti	228) ASV_539g_Costridia_UCG-014	274) ASV_471g_Ocillospirales
45) ASV_348g_Prevotellaceae_NK3B31_group	91) ASV_167f_Lachnospiraceae	137) ASV_1306g_Christensenellaceae_R-7_group	183) ASV_89g_Treponema	229) ASV_120g_Costridia_UCG-014	275) ASV_237g_Ocillospirales
46) ASV_43ummicola	92) ASV_400f_Lachnospiraceae	138) ASV_757g_Christensenellaceae_R-7_group	184) ASV_345g_Treponema	230) ASV_1042g_Costridia_UCG-014	



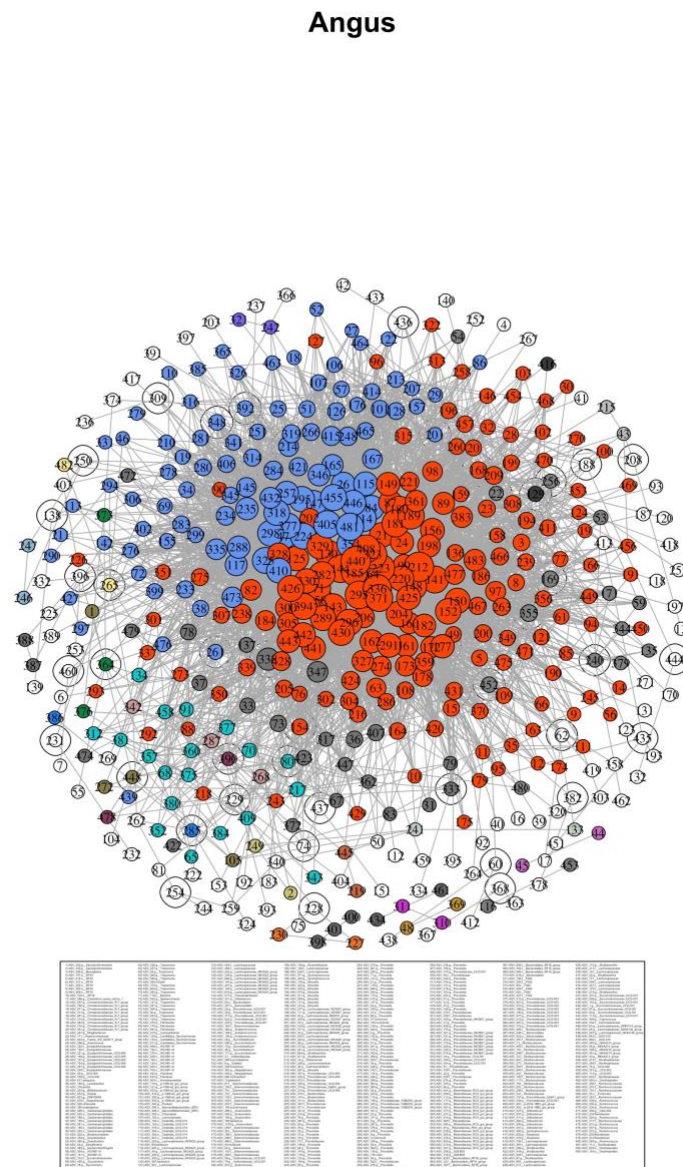
**Figure 12b: The SparCC based ecological network of bacterial species for crossbred (F1) American Wagyu cattle.**

### Wagyu Crossbred



1) ASV_71g_Lachnospiraceae_NK3420_group	45) ASV_13g_Prevotella	89) ASV_821g_Lachnospiraceae_FCS020_group	133) ASV_311f_Erysipelotrichaceae	177) ASV_84g_Verrucomicrobiaceae	221) ASV_1181f_Ruminococcaceae
2) ASV_1983i_Lachnospiraceae	46) ASV_212g_Prevotella	90) ASV_60thacterium	134) ASV_253f_Erysipelotrichaceae	178) ASV_385g_Verrucomicrobiaceae	222) ASV_665f_Verrucomicrobiaceae
3) ASV_761g_Moryella	47) ASV_294g_Prevotella	91) ASV_126g_Lachnospiraceae_XPB1014_group	135) ASV_1737f_Erysipelotrichaceae	179) ASV_81paludini	223) ASV_190f_F082
4) ASV_79g_Moryella	48) ASV_138g_Prevotella	92) ASV_502g_Lachnospiraceae_XPB1014_group	136) ASV_1737f_Erysipelotrichaceae	180) ASV_82sucinonans	224) ASV_127f_F082
5) ASV_714g_NK44214_group	49) ASV_146g_Prevotella	93) ASV_591g_Lachnospiraceae_NK44138_group	137) ASV_33g_Succinivibrionaceae_UCG-002	181) ASV_392multilada	225) ASV_44f_F082
6) ASV_16g_NK44214_group	50) ASV_28g_Prevotella	94) ASV_47g_Saccharofermentans	138) ASV_176g_Desulfotribro	182) ASV_724lipolyticus	226) ASV_193f_F082
7) ASV_215g_NK44214_group	51) ASV_205g_WCHB1-41	95) ASV_54g_Saccharofermentans	139) ASV_301g_Piramidobacter	183) ASV_231o_RF39	227) ASV_142f_F082
8) ASV_234o_WCHB1-41	52) ASV_1475g_Prevotellaceae_UCG-001	96) ASV_340g_Saccharofermentans	140) ASV_19sucinogenes	184) ASV_117o_RF39	228) ASV_510f_F082
9) ASV_406o_Bacteroidales	53) ASV_719g_Alpirevotella	97) ASV_176g_Saccharofermentans	141) ASV_218g_Fibrobacter	185) ASV_65g_U29-B03	229) ASV_65g_U29-B03
10) ASV_254o_WCHB1-41	54) ASV_1052g_Prevotellaceae_UCG-003	98) ASV_206g_Saccharofermentans	142) ASV_343o_Olsenella	186) ASV_209g_U29-B03	230) ASV_209g_U29-B03
11) ASV_1752o_WCHB1-41	55) ASV_910g_Prevotellaceae_UCG-003	99) ASV_225g_Saccharofermentans	143) ASV_390umbonata	187) ASV_148g_U29-B03	231) ASV_148g_U29-B03
12) ASV_104o_WCHB1-41	56) ASV_1188g_Prevotellaceae_UCG-003	100) ASV_238f_UCG-010	144) ASV_700g_Sphaerobactera	188) ASV_624o_RF39	232) ASV_296f_p-251-o5
13) ASV_628c_Verrucomicrobiota	57) ASV_749g_Prevotellaceae_UCG-003	101) ASV_177f_UCG-010	145) ASV_1213g_Treponema	189) ASV_320o_RF39	233) ASV_682f_Muribaculaceae
14) ASV_123o_Bacteroidales	58) ASV_196g_Prevotellaceae_GabA1_group	102) ASV_85g_NK44214_group	146) ASV_50g_Treponema	190) ASV_1825o_RF39	234) ASV_102f_Muribaculaceae
15) ASV_465g_Prevotella	59) ASV_379g_Prevotellaceae_UCG-004	103) ASV_105g_NK44214_group	147) ASV_387o_RF39	191) ASV_387o_RF39	235) ASV_777f_Muribaculaceae
16) ASV_118g_Prevotella	60) ASV_543o_Bacteroidales	104) ASV_164g_NK44214_group	148) ASV_168bryantii	192) ASV_4443o_RF39	236) ASV_341f_Muribaculaceae
17) ASV_108g_Prevotella	61) ASV_108g_Prevotellaceae_UCG-004	105) ASV_182g_NK44214_group	149) ASV_156g_Treponema	193) ASV_362o_RF39	237) ASV_729f_Muribaculaceae
18) ASV_51g_Prevotella	62) ASV_421g_Prevotellaceae_UCG-001	106) ASV_459g_Pegibacter	150) ASV_382g_Treponema	194) ASV_604o_RF39	238) ASV_1805f_Muribaculaceae
19) ASV_24g_Prevotella	63) ASV_654f_Prevotellaceae	107) ASV_520f_UCG-010	151) ASV_719g_Treponema	195) ASV_576o_RF39	239) ASV_181f_Muribaculaceae
20) ASV_30g_Prevotella	64) ASV_116f_Prevotellaceae	108) ASV_555f_UCG-010	152) ASV_41bryantii	196) ASV_1204o_RF39	240) ASV_518f_Muribaculaceae
21) ASV_15g_Prevotella	65) ASV_222f_Prevotellaceae	109) ASV_895f_UCG-010	153) ASV_89g_Treponema	197) ASV_983o_RF39	241) ASV_168f_p-2534-1885_gut_group
22) ASV_2g_Prevotella	66) ASV_3g_Prevotella	110) ASV_875g_Ruminococcus	154) ASV_615g_Treponema	198) ASV_559o_RF39	242) ASV_342g_Rikenellaceae_RC9_gut_group
23) ASV_238g_Prevotella	67) ASV_159g_Butyribrio	111) ASV_389g_Christensenellaceae_R-7_group	155) ASV_61g_Treponema	199) ASV_62g_Erysipelotrichaceae_UCG-009	243) ASV_279g_Rikenellaceae_RC9_gut_group
24) ASV_1112g_Prevotella	68) ASV_699g_Butyribrio	112) ASV_163g_Flexilinea	156) ASV_480g_Treponema	200) ASV_526g_Sobobacterium	244) ASV_174g_Rikenellaceae_RC9_gut_group
25) ASV_576g_Prevotella	69) ASV_242g_Lachnospiraceae_NK3420_group	113) ASV_650g_Flexilinea	157) ASV_129f_Bifidobacteriaceae	201) ASV_411g_Erysipelotrichaceae_UCG-007	245) ASV_453g_Rikenellaceae_RC9_gut_group
26) ASV_334g_Prevotella	70) ASV_29g_Lachnospiraceae_NK3420_group	114) ASV_943g_Flexilinea	158) ASV_103o_Absconditabacteriales_(SR1)	202) ASV_73g_Erysipelotrichaceae_UCG-006	246) ASV_300g_Rikenellaceae_RC9_gut_group
27) ASV_213g_Prevotella	71) ASV_86g_Lachnospiraceae_NK3420_group	115) ASV_657g_Anaerorax	159) ASV_264o_Absconditabacteriales_(SR1)	203) ASV_35g_Erysipelotrichaceae_UCG-009	247) ASV_296g_Rikenellaceae_RC9_gut_group
28) ASV_711g_Prevotella	72) ASV_12g_Lachnospiraceae_NK3420_group	116) ASV_1091g_Anaerorax	160) ASV_592k_Bacteria	204) ASV_350g_Erysipelotrichaceae_UCG-009	248) ASV_252g_Rikenellaceae_RC9_gut_group
29) ASV_32g_Prevotella	73) ASV_57g_Lachnospiraceae_NK3420_group	117) ASV_572g_Christensenellaceae_R-7_group	161) ASV_1278k_Bacteria	205) ASV_87o_Costidia_UCG-014	249) ASV_913g_Rikenellaceae_RC9_gut_group
30) ASV_260g_Prevotella	74) ASV_173g_Lachnospiraceae_NK3420_group	118) ASV_447g_Christensenellaceae_R-7_group	162) ASV_177g_p-1088-a5_gut_group	206) ASV_982o_Costidia_UCG-014	250) ASV_1186g_Rikenellaceae_RC9_gut_group
31) ASV_372g_Prevotella	75) ASV_763g_Acetomaculum	119) ASV_769g_Christensenellaceae_R-7_group	163) ASV_703g_p-1088-a5_gut_group	207) ASV_120o_Costidia_UCG-014	251) ASV_967g_Rikenellaceae_RC9_gut_group
32) ASV_72g_Prevotella	76) ASV_137f_Lachnospiraceae	120) ASV_905g_Christensenellaceae_R-7_group	164) ASV_63g_CPI-a4_ventile_group	208) ASV_1018o_Costidia_UCG-014	252) ASV_1236g_Rikenellaceae_RC9_gut_group
33) ASV_609g_Prevotella	77) ASV_100f_Lachnospiraceae	121) ASV_207g_Christensenellaceae_R-7_group	165) ASV_126g_Candidatus_Saccharimonas	209) ASV_1706o_Costidia_UCG-014	253) ASV_109g_Rikenellaceae_RC9_gut_group
34) ASV_408g_Prevotella	78) ASV_167f_Lachnospiraceae	122) ASV_101g_Christensenellaceae_R-7_group	166) ASV_282g_Candidatus_Saccharimonas	210) ASV_185o_Costidia_UCG-014	254) ASV_133g_Rikenellaceae_RC9_gut_group
35) ASV_413g_Prevotella	79) ASV_400f_Lachnospiraceae	123) ASV_250g_Christensenellaceae_R-7_group	167) ASV_19g_Candidatus_Saccharimonas	211) ASV_1357o_Costidia_UCG-014	255) ASV_780g_Rikenellaceae_RC9_gut_group
36) ASV_7g_Prevotella	80) ASV_1303g_Lachnospiraceae_UCG-008	124) ASV_569g_Christensenellaceae_R-7_group	168) ASV_113g_Candidatus_Saccharimonas	212) ASV_395o_Costidia_UCG-014	256) ASV_765g_Rikenellaceae_RC9_gut_group
37) ASV_268g_Prevotellaceae_UCG-001	81) ASV_554g_Lachnospiraceae_N03007_group	125) ASV_403g_Christensenellaceae_R-7_group	169) ASV_1263g_Candidatus_Saccharimonas	213) ASV_38g_Succinivibrionaceae	257) ASV_40g_Rikenellaceae_RC9_gut_group
38) ASV_838g_Prevotellaceae_NK3831_group	82) ASV_730f_Lachnospiraceae	126) ASV_33g_Christensenellaceae_R-7_group	170) ASV_251g_Candidatus_Saccharimonas	214) ASV_187g_Succinivibrionaceae	258) ASV_516g_Rikenellaceae_RC9_gut_group
39) ASV_348g_Prevotellaceae_NK3831_group	83) ASV_224f_Lachnospiraceae	127) ASV_354g_Christensenellaceae_R-7_group	171) ASV_303g_Candidatus_Saccharimonas	215) ASV_4g_Succinivibrionaceae	259) ASV_451o_Bacteroidales
40) ASV_43truminicola	84) ASV_389g_probable_genus_10	128) ASV_121g_Christensenellaceae_R-7_group	172) ASV_288g_Candidatus_Saccharimonas	216) ASV_460g_Succinivibrionaceae	260) ASV_1380g_Ruminococcus
41) ASV_80g_Prevotella	85) ASV_699f_Lachnospiraceae	129) ASV_666g_Christensenellaceae_R-7_group	173) ASV_94g_Candidatus_Saccharimonas	217) ASV_11g_Succinivibrionaceae	261) ASV_671g_Ruminococcus
42) ASV_39g_Prevotella	86) ASV_521f_Lachnospiraceae	130) ASV_125g_Christensenellaceae_R-7_group	174) ASV_81_Verrucomicrobiota	218) ASV_481g_Ruminococcus	262) ASV_130flavellacis
43) ASV_227g_Prevotellaceae_UCG-001	87) ASV_383f_Lachnospiraceae	131) ASV_1185f_vadriBE97	175) ASV_26g_Dalister	219) ASV_219g_Ruminococcus	263) ASV_10o_Ocillospirales
44) ASV_97g_Prevotella	88) ASV_438f_Lachnospiraceae	132) ASV_221azubiensis	176) ASV_893g_Megasphaera	220) ASV_1008g_Ruminococcus	264) ASV_23o_Ocillospirales

**Figure 12c: The SparCC based ecological network of bacterial species for Angus cattle.**

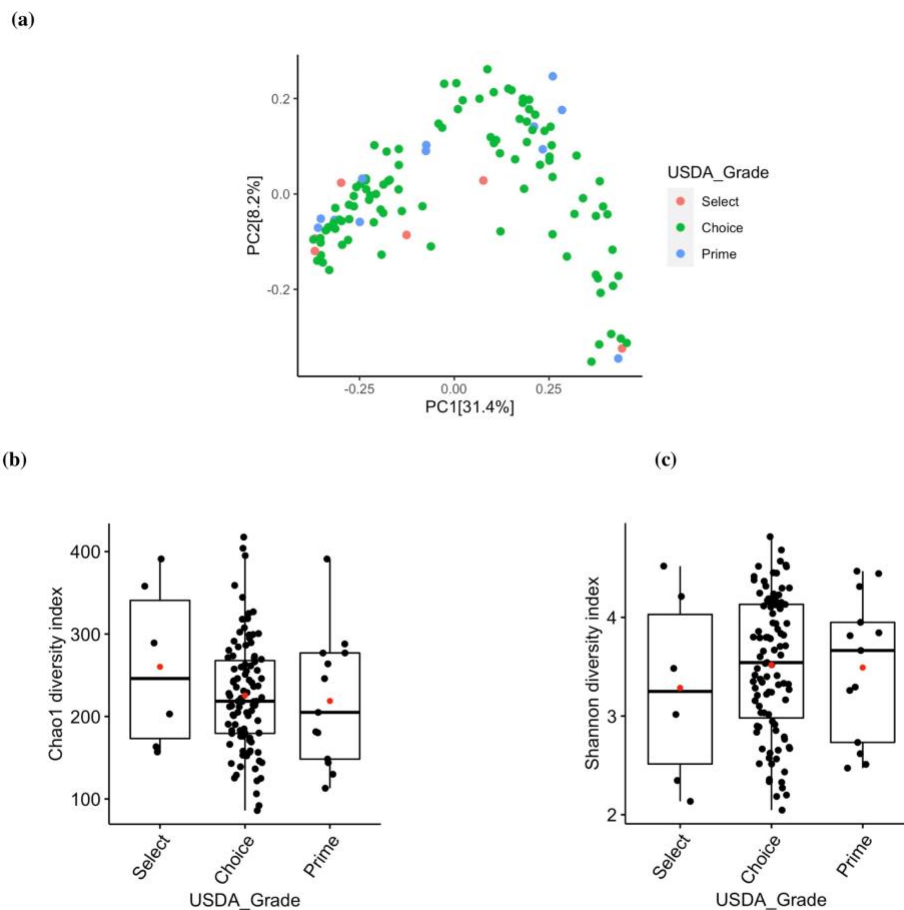






**Figure 12a,b&c:** Bacterial species abundances were used to construct an ecological network using SparCC method. Each node is representing the bacterial species and the size of the node corresponds to the hub score which is based on the number of links with other nodes. Nodes were colored based on the cluster which means nodes with same color belong to same cluster. The nodes with halo represent articulation points. The node labels are provided as a legend with taxonomy. (a) The ASVs with SparCC correlation of 0.8 or higher were used to construct the network from American Wagyu full blood (FB) data. (b) The network is based on American Wagyu crossbred (F1) data and ASVs with SparCC correlation of 0.7 or higher were used to make the network. (c) Angus steers data was used, and network is showing the co-occurrence of ASVs with SparCC correlation of 0.3 or higher.

# Supplementary Figure S1: Bacterial diversity differences for Angus data.



The Angus data was used for this figure. Principle coordinate analysis (PCoA) was used to visualize the bacterial community differences between different marbling grades based on Bray Curtis (a) distance matrices. Box plots were used to plot alpha diversity index Chao1 (c) and Shannon (d) for different marbling grades. The red dots (figure c and d) represent the Chao1 and Shannon group average for each marbling grade.

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## APPENDIX A

**R Codes**

```

title: "GWAS_Beef_Study"
author: "Waseem"
output: html_document
---
```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = TRUE)
knitr::opts_chunk$set(fig.width=12, fig.height=8)
```

# Figure 5 A & B Correlation Plots
```{r, echo=FALSE}
#setwd("working directory")
## Creating Abundance table ####
rarefied_OTUs= read.table("otu_table.tax_final_586Samples_1%_Filtered_Taxa.txt", sep = "\t",
header=T)
row.names(rarefied_OTUs)= rarefied_OTUs$OTUID
rarefied_OTUs = rarefied_OTUs[, -1]
taxonomy_OTUs= subset(rarefied_OTUs, select = taxonomy)
rarefied_OTUs= rarefied_OTUs[, -587] # removing last column of Taxonomy
OTUs_rel= sweep(rarefied_OTUs, 2, colSums(rarefied_OTUs), FUN= "/")
OTUs_abundance_tax = merge(OTUs_rel, taxonomy_OTUs, by= "row.names")
names(OTUs_abundance_tax)[1]= "OTUID"
write.table(OTUs_abundance_tax, sep= "\t",
file="OTUs_abundance_otu_table.tax_final_586Samples_1%_Filtered_Taxa.txt", col.names = T,
row.names = F, quote=F)
OTUs_abundance_Family=
read.table("otu_table.tax_final_586Samples_1%_Filtered_Taxa_L5.txt", sep = "\t", header=T)
OTUs_abundance_Phylu= read.table("otu_table.tax_final_586Samples_1%_Filtered_Taxa_L2.txt",
sep = "\t", header=T)
OTU_abundance=
read.table("OTUs_abundance_otu_table.tax_final_586Samples_1%_Filtered_Taxa.txt", sep= "\t",
header= T)
row.names(OTU_abundance)= OTU_abundance$OTUID
selected_phyla=
OTUs_abundance_Phylu[apply(OTUs_abundance_Phylu,1,function(x){any(c("k__Bacteria;p__Firmicutes", "k__Bacteria;p__Lentisphaerae", "k__Bacteria;p__Proteobacteria", "k__Bacteria;p__Verrucomicrobia", "k__Bacteria;p__Fibrobacteres", "k__Bacteria;p__Tenericutes") %in% x))},)]
selected_family=
OTUs_abundance_Family[apply(OTUs_abundance_Family,1,function(x){any(c("k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae", "k__Bacteria;p__Verrucomicrobia;c__Verruco-5;o__WCHB1-41;f__RFP12",

```



```

"k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae",
"k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae",
"k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae]",
"k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae",
"k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7",
"k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae",
"k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__RF16",
"k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__Fibrobacterales;f__Fibrobacteraceae",
"k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae",
"k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BS11") %in% x)}),]
selecting_OTUs=
OTU_abundance[c("41","57","2337","21","43","27","30","53","4","12","2737","3","72","64","19","28"),]
selecting_OTUs=selecting_OTUs[,-588]
selecting_OTUs$OTUID= paste("OTU",selecting_OTUs$OTUID, sep = "")
selected_phyla_And_family_OTUs= rbind(selected_phyla,selected_family,selecting_OTUs)
write.table(selected_phyla_And_family_OTUs, sep= "\t",
file="selected_phyla_And_family_OTUs.txt", col.names = T, row.names = F, quote=F)
row.names(selected_phyla_And_family_OTUs)= selected_phyla_And_family_OTUs$OTUID
selected_phyla_And_family_OTUs=selected_phyla_And_family_OTUs[,-1]
selected_phyla_family_OTUs_transpose= data.frame(t(selected_phyla_And_family_OTUs))
write.table(selected_phyla_family_OTUs_transpose, sep= "\t",
file="selected_phyla_family_OTUs_transpose.txt", col.names = T, row.names = T, quote=F)
colnames(selected_phyla_family_OTUs_transpose)=
c("Fibrobacteres","Firmicutes","Lentisphaerae","Proteobacteria","Tenericutes","Verrucomicrobia",
BS11","Prevotellaceae","RF16","S24-7",
"Paraprevotellaceae","Fibrobacteraceae","Clostridiaceae","Lachnospiraceae","Ruminococcaceae",
"Veillonellaceae","Succinivibrionaceae","RFP12","OTU41","OTU57","OTU2337","OTU21","OTU43",
"OTU27","OTU30","OTU53","OTU4","OTU12","OTU2737","OTU3","OTU72","OTU64","OTU19",
"OTU28")
write.table(selected_phyla_family_OTUs_transpose, sep= "\t",
file="Correlation_selected_phyla_family_OTUs_transpose.txt", col.names = T, row.names = T,
quote=F)
library(devtools)
library(ggpubr)
library(GGally)
#Correlations of log(data+1)
selected_phyla_family_OTUs_transpose_log_plus1=
log10(1+selected_phyla_family_OTUs_transpose)
selected_phyla_family_OTUs_transpose_log_plus1_chromosome9=
subset(selected_phyla_family_OTUs_transpose_log_plus1, select=

```

```
c("OTU4","OTU12","OTU53","Lachnospiraceae","RFP12","Succinivibrionaceae","Veillonellaceae",
", "Clostridiaceae", "Firmicutes", "Lentisphaerae", "Proteobacteria", "Verrucomicrobia"))
selected_phyla_family_OTUs_transpose_log_plus1_chromosome27=
subset(selected_phyla_family_OTUs_transpose_log_plus1, select=
c("OTU19","OTU28","OTU3","RF16","S24-
7", "Fibrobacteraceae", "Clostridiaceae", "Fibrobacteres", "Lentisphaerae", "Tenericutes"))
ggpairs(selected_phyla_family_OTUs_transpose_log_plus1_chromosome9)
ggpairs(selected_phyla_family_OTUs_transpose_log_plus1_chromosome27)
```

```

### **##Beta\_Diversity Plot (Bray Curtis) [Location,Diet and Sex]**

```
```{r, echo=T}
library(vegan)
mappingfile= read.table("GWAS_586_mapping_573Samples_rarefied7000.txt", sep="\t",
header=T)
data= read.table("bray_curtis_pc.txt", skip= 9, nrow= 573, sep = "\t")
dat_2PCs= data[,1:3]
colnames(dat_2PCs)= c("SampleID", "PC1", "PC2")
mapping_beta_div= mappingfile[, c("SampleID", "Diet", "Cohort", "Sex", "Location")]
PC_and_Mapping= merge.data.frame(dat_2PCs, mapping_beta_div)
library(ggplot2)
ggplot(PC_and_Mapping, aes(x = PC1, y = PC2, color = Sex)) +
  geom_point() + xlab("PC1(28.9.3%)" )+ ylab("PC2(10.5%)" ) + ggtitle("bray_curtis_pc")
ggplot(PC_and_Mapping, aes(x = PC1, y = PC2, color = Location)) +
  geom_point() + xlab("PC1(28.9.3%)" )+ ylab("PC2(10.5%)" ) + ggtitle("bray_curtis_pc")
ggplot(PC_and_Mapping, aes(x = PC1, y = PC2, color = Diet)) +
  geom_point() + xlab("PC1(28.9.3%)" )+ ylab("PC2(10.5%)" ) + ggtitle("bray_curtis_pc")
```

```

### **###Heatmap Families Abundance####**

```
```{r, echo=F}
###Heatmap with complexheatmap#####
library(ComplexHeatmap)
library(stringr)
family_proportions= read.table("otu_table.tax_final_586Samples_1%_Filtered_Taxa_L5.txt", sep=
"\t", header=T)
tax= as.data.frame(str_split_fixed(family_proportions$OTUID, ";", 5))
names(tax) [5]= "family"
tax$family= sub(" ", "", tax$family)
tax$family=sub("f__", "",tax$family)
tax$family=sub("^$", "No_Assigned_Family",tax$family)
tax$family=sub("\\]", "",tax$family)
tax$family=sub("\\[", "",tax$family)
f_p= merge(tax,family_proportions, by= "row.names")

```

```

f_p= f_p[,-c(1,2,3,4,5,7)]
f_p2= f_p[!(f_p$family=="Other" & f_p$family=="No Assigned Family"),]
other= f_p[(f_p$family=="Other"),]
other2= other[,-1]
other2_average= data.frame(colMeans(other2))
colnames(other2_average)= "Other"
other2_average_t= data.frame(t(other2_average))
No_Assigned_Family= f_p[(f_p$family=="No_Assigned_Family"),]
No_Assigned_Family2 = No_Assigned_Family[,-1]
No_Assigned_Family2_average= data.frame(colMeans(No_Assigned_Family2))
colnames(No_Assigned_Family2_average)= "No_Assigned_Family"
No_Assigned_Family2_average_t= data.frame(t(No_Assigned_Family2_average))
remove= c("Other","No_Assigned_Family" )
f_p2= f_p[!(f_p$family) %in% remove, ]
rownames(f_p2)= f_p2$family
f_p3= f_p2[,-1]
f_p4= rbind(f_p3,No_Assigned_Family2_average_t,other2_average_t )
f_p4= as.matrix(f_p4)
f_p4_t= t(f_p4)
heat_map_metadata= read.table("heat_map_families_input_metadata.txt", sep ="\t", header=T)
f_p5= data.frame(t(f_p4))
f_p5$SampleID= rownames(f_p5)
f_p5= f_p5[order(f_p5$SampleID),]
heat_map_metadata= read.table("heat_map_families_input_metadata.txt", sep ="\t", header=T)
heat_map_metadata2= heat_map_metadata[order(heat_map_metadata$SampleID),]
f_p6= merge(heat_map_metadata2,f_p5)
f_p6.1= f_p6[order(f_p6$Cohort2),]
rownames(f_p6.1)= f_p6.1$SampleID
f_p6.1= f_p6.1[,-c(1:4)]
f_p6.1_t= data.frame(t(f_p6.1))
f_p6.1_t= as.matrix(f_p6.1_t)
heat_map_metadata3= heat_map_metadata2[order(heat_map_metadata2$Cohort2),]
ann <- data.frame(heat_map_metadata3$Diet,
heat_map_metadata3$Sex,heat_map_metadata3$Cohort2)
colnames(ann)= c("Diet", "Sex", "Cohort")
colours <- list("Cohort"=c("UNL1"="red2", "UNL2"="royalblue", "UNL3"="burlywood4",
"UNL4"="yellow", "UNL5"="Green", "USMARC1"="darkblue", "USMARC2"="lightcoral", "USMA
RC3"="black"), "Sex"=c("Steer"="limegreen", "Heifer"="gold"), "Diet"= c("Common"="red",
"Finishing"= "black", "Growing"= "green"))
colAnn <- HeatmapAnnotation(df= ann , which="col", col=colours, annotation_width=unit(c(1, 4),
"cm"), gap=unit(1, "mm"))

```

```

R.AN= rowAnnotation(foo = anno_mark(at = c(11,93,64,16,139,131,135,155,14,132,105,162,163,
127,8,21,83), labels = row.names(f_p6.l_t[c(11,93,64,16,139,131,135,155,14,132,105,162,163,
127,8,21,83),])))
Heatmap(f_p6.l_t, name = "Relative Abundance", cluster_rows = F, cluster_columns =
F, show_column_dend = T, show_row_dend = T,
      row_dend_reorder = T, column_dend_reorder = T, top_annotation = colAnn,
      right_annotation = R.AN, show_row_names = F, show_column_names = F)
```

```

This heatmap shows the correlations between all the identified OTUs, Families and Phyla

```

```{r, echo=FALSE}
selected_phyla_family_OTUs_transpose = read.table("selected_phyla_family_OTUs_transpose.txt",
sep="\t", header=T)
colnames(selected_phyla_family_OTUs_transpose) =
c("Fibrobacteres", "Firmicutes", "Lentisphaerae", "Proteobacteria", "Tenericutes", "Verrucomicrobia",
BS11", "Prevotellaceae", "RF16", "S24-
7", "Paraprevotellaceae", "Fibrobacteraceae", "Clostridiaceae", "Lachnospiraceae", "Ruminococcaceae",
"Veillonellaceae", "Succinivibrionaceae", "RFP12", "OTU41[Methanobacteriaceae]", "OTU57[Bact
eroidetes]", "OTU2337[Veillonellaceae]", "OTU21[Prevotellaceae]", "OTU43[RFP12]", "OTU27[Par
aprevotellaceae]", "OTU30[Prevotellaceae]", "OTU53[Ruminococcaceae]", "OTU4[Prevotellaceae]",
"OTU12[Prevotellaceae]", "OTU2737[Prevotellaceae]", "OTU3[RF16]", "OTU72[Paraprevotellaceae
]", "OTU64[F16]", "OTU19[Prevotellaceae]", "OTU28[Fibrobacteraceae]")
selected_phyla_family_OTUs_transpose_log_plus1 =
log10(1+selected_phyla_family_OTUs_transpose)
library("ggplot2")
library("scales")
library("grid")
cormat = round(cor(selected_phyla_family_OTUs_transpose_log_plus1), 2)
library(reshape2)
melted_cormat <- melt(cormat)
head(melted_cormat)
# Get lower triangle of the correlation matrix
get_lower_tri <- function(cormat){
  cormat[upper.tri(cormat)] <- NA
  return(cormat)
}
# Get upper triangle of the correlation matrix
get_upper_tri <- function(cormat){
  cormat[lower.tri(cormat)] <- NA
  return(cormat)
}
upper_tri <- get_upper_tri(cormat)
reorder_cormat <- function(cormat){

```

```

# Use correlation between variables as distance
dd <- as.dist((1-cormat)/2)
hc <- hclust(dd)
cormat <-cormat[hc$order, hc$order]
}
# Reorder the correlation matrix
cormat <- reorder_cormat(cormat)
upper_tri <- get_upper_tri(cormat)
# Melt the correlation matrix
melted_cormat <- melt(upper_tri, na.rm = TRUE)
# Create a ggheatmap
ggheatmap <- ggplot(melted_cormat, aes(Var2, Var1, fill = value))+
  geom_tile(color = "white")+
  scale_fill_gradient2(low = "blue", high = "red", mid = "white",
    midpoint = 0, limit = c(-1,1), space = "Lab",
    name="Pearson\nCorrelation") +
  theme_minimal()+ # minimal theme
  theme(axis.text.x = element_text(angle = 45, vjust = 1,
    size = 8, hjust = 1))+
  coord_fixed()
# Print the heatmap
print(ggheatmap)
ggheatmap +
  geom_text(aes(Var2, Var1, label = value), color = "black", size = 1.5) +
  theme(
    axis.title.x = element_blank(),
    axis.title.y = element_blank(),
    panel.grid.major = element_blank(),
    panel.border = element_blank(),
    panel.background = element_blank(),
    axis.ticks = element_blank(),
    legend.justification = c(1, 0),
    legend.position = c(0.6, 0.7),
    legend.direction = "horizontal")+
  guides(fill = guide_colorbar(barwidth = 7, barheight = 1,
    title.position = "top", title.hjust = 0.5))
...

##Circular Graph#####
```{r}
setwd("/Users/waseem/Desktop/Genetic_Microbiom/circos/A_R.file/RCircos")
library(RCircos)
library(readxl)

```

```

#out.file <- "RCircosDemoHumanGenkome5.pdf"
#pdf(file=out.file, height=13, width=13, compress=TRUE)
#png(file=out.file, height=15, width=15, compress=TRUE)
RCircos.Set.Plot.Area(margins = 0)
f= read_excel("Circos_Input2.xlsx")
chr.exclude= NULL
cyto.info= f
track.inside= 3
track.outside= 0
RCircos.Set.Core.Components(cyto.info, chr.exclude, track.inside, track.outside)
rcircos.params <- RCircos.Get.Plot.Parameters()
rcircos.params$char.width= 350
rcircos.params$text.size= 0.39
rcircos.params$text.color= "gray0"
rcircos.params$plot.radius= 1.8
rcircos.params$chrom.paddings= 550
rcircos.params$track.out.start= 0
RCircos.Reset.Plot.Parameters(rcircos.params)
RCircos.Set.Plot.Area()
t=RCircos.Get.Plot.Parameters()
RCircos.Chromosome.Ideogram.Plot()
g= read_excel("refGene122_only_on_1mb_window2.xlsx")
g= as.data.frame(g)
RCircos.Gene.Label.Data= g
name.col= 4
side= "in"
track.num= 1
RCircos.Gene.Connector.Plot(RCircos.Gene.Label.Data,track.num,side)
track.num = 2
RCircos.Gene.Name.Plot(RCircos.Gene.Label.Data,name.col,track.num, side)
#dev.off()
```

```

## APPENDIX B

**R Codes**

```

---
title: "Liver_Abscess"
---
```{r, echo= F, message= F, warning= F}
#setwd("/Users/waseem/Desktop/Liver_Absces_USDA_study")
#setwd("/Volumes/Waseem_Personal/PD")
#setwd("Volumes")
library("import")
library("knitr")
library("BiocStyle")
library("ggplot2")
library("gridExtra")
library("dada2")
library("phyloseq")
library("DECIPHER")
library("ape")
library("phangorn")
library("knitr")
library("BiocStyle")
library("ShortRead")
---

```{r, echo= F, message= F, warning= F}
library(microbiome) # data analysis and visualisation
library(phyloseq) # also the basis of data object. Data analysis and visualisation
#library(microbiomeutilities) # some utility tools
library(RColorBrewer) # nice color options
library(ggpubr) # publication quality figures, based on ggplot2
library(DT) # interactive tables in html and markdown
library(data.table) # alternative to data.frame
library(dplyr) # data handling
library("ampvis2")
library("tibble")
---

```{r, echo= F, message= F, warning= F}
knitr::opts_chunk$set(echo = F, warning=FALSE)
knitr::opts_chunk$set(fig.width=15, fig.height=8)
---

```{r, echo= F, message= F, warning= F}
load("Liver_Absces_Gene_expression_Analysis.RData")
---

### OCT 25, 2020
```{R}
p0 #Figure1 (b)
box #Figure1 (c)
phylum #Figure1 A
pg2 #Figure1 A
beta_papillae=p0_papi_2+theme(legend.position = c(0.15, 0.9))+ geom_point(size = 1)+theme(text =
element_text(size=15)) #Figure2 B
beta_papillae #Figure2 B
phylum_papillae #Figure2 A
Genus_papillae #Figure2 A
sp3 #Figure4 a

```

```

sp6 #Figure4 b
spp5 #FIGURE4 C
spp14 #FIGURE4 D
spp15 #FIGURE4 E
sp10 #FIGURE F
sp11 #FIGURE G
sp13 #FIGURE H
sp12 #FIGURE I
```

#I removed the singletons (and zero sums) from the final_ps before further analysis
```{r}
# Removing singleton
###removing singleton from rumen papillae data
rumenPappilae
ASVs_sums= data.frame(taxa_sums(rumenPappilae))
ASVs_sums$ASVs= row.names(ASVs_sums)
singleton= ASVs_sums[which(ASVs_sums$taxa_sums.rumenPappilae. < 2) , ]
length(singleton$ASVs)
singleton_taxanames= singleton$ASVs
All_Taxa= taxa_names(rumenPappilae)
taxa_after_singleton= All_Taxa[!(All_Taxa %in% singleton_taxanames)]
rumenPappilae_no_singleton= prune_taxa(taxa_after_singleton, rumenPappilae )
rumenPappilae
rumenPappilae_no_singleton
#Removing singleton from rumen Fluid data
rumenFluid
ASVs_sums= data.frame(taxa_sums(rumenFluid))
ASVs_sums$ASVs= row.names(ASVs_sums)
singleton= ASVs_sums[which(ASVs_sums$taxa_sums.rumenFluid. < 2) , ]
length(singleton$ASVs)
singleton_taxanames= singleton$ASVs
All_Taxa= taxa_names(rumenFluid)
taxa_after_singleton= All_Taxa[!(All_Taxa %in% singleton_taxanames)]
rumenFluid_no_singleton= prune_taxa(taxa_after_singleton, rumenFluid )
rumenFluid
rumenFluid_no_singleton
```

#Core microbiome Analysis
```{r}
final_ps_Proportions =transform_sample_counts(final_ps, function(OTU) OTU/sum(OTU))
final_ps_Proportions_rumenPappilae= subset_samples(final_ps_Proportions, Sample.Source== "Rumen
Papillae")
final_ps_Proportions_rumenFluid= subset_samples(final_ps_Proportions, Sample.Source== "Rumen
Fluid")
core.taxa.standard_papillae = core_members(final_ps_Proportions_rumenPappilae, detection = 0,
prevalence = 50/100)
core.taxa.standard_papillae
core.taxa.standard_rumenFluid = core_members(final_ps_Proportions_rumenFluid, detection = 0,
prevalence = 50/100)
core.taxa.standard_rumenFluid
unique(core.taxa.standard_papillae, core.taxa.standard_rumenFluid)
# With compositional (relative) abundances
det <- c(0, 0.1, 0.5, 2, 5, 20)/100
prevalences <- seq(.05, 1, .05)

```



```

p<-plot_core(final_ps_Proportions_rumenPappilae, prevalences = prevalences, detections = det, plot.type =
"lineplot") + xlab("Relative Abundance (%)")
p
# Core with compositionals:
prevalences <- seq(.05, 1, .05)
detections <- 10^seq(log10(1e-5), log10(.2), length = 10)
# Also define gray color palette
gray <- gray(seq(0,1,length=5))
p1 <- plot_core(final_ps_Proportions_rumenPappilae, plot.type = "heatmap", colours = gray,
  prevalences = prevalences, detections = detections, min.prevalence = .8) +
  xlab("Detection Threshold (Relative Abundance (%))")
print(p1)
library(viridis)
print(p1 + scale_fill_viridis())
library(RColorBrewer)
library(knitr)
# Core with absolute counts and vertical view:
# and minimum population prevalence (given as percentage)
detections <- 10^seq(log10(1), log10(max(abundances(final_ps_Proportions_rumenPappilae))/10), length =
10)
healthycore <- plot_core(final_ps_Proportions_rumenPappilae, plot.type = "heatmap",
  prevalences = prevalences,
  detections = detections,
  colours = rev(brewer.pal(5, "Spectral")),
  min.prevalence = .90, horizontal = F)
# get the data used for plotting
df <- p1$data
# get the list of OTUs
list <- df$Taxa
# check the OTU ids
# print(list)
# get the taxonomy data
tax <- tax_table(final_ps_Proportions_rumenPappilae)
tax <- as.data.frame(tax)

# add the OTUs to last column
tax$OTU <- rownames(tax)
# select taxonomy of only
# those OTUs that are used in the plot
tax2 <- dplyr::filter(tax, rownames(tax) %in% list)
head(tax2)
# We will merge all the column into one except the Domain as all is bacteria in this case
tax.unit <- tidyr::unite(tax2, Taxa_level, c("Phylum", "Class", "Order", "Family", "Genus", "Species",
"OTU"), sep = "_", remove = TRUE)
tax.unit$Taxa_level <- gsub(pattern="[a-z]_", replacement="", tax.unit$Taxa_level)
# add this new information into the plot data df
df$Taxa <- tax.unit$Taxa_level
# you can see now we have the taxonomic information
knitr::kable(head(df))
# replace the data in the plot object
p1$data <- df
plot(p1 + theme(axis.text.y = element_text(face="italic"))))
```


###bray curtis plot



Relative Abundances


```

```

```{r, echo= F, message= F, warning= F}
library(phyloseq)
library(vegan)
library(ggplot2)
library(grid)
library(BiodiversityR)
final_ps_rel_abun= transform_sample_counts(final_ps, function(OTU) OTU/sum(OTU))
bray_litdir = ordinate(final_ps_rel_abun, "PCoA", "bray")
#p0 = plot_ordination(final_ps_rel_abun, bray_litdir, type = "sample", shape = "Liver.Abscess", color =
"Sample.Source", axes = 1:2)+ labs(shape= "Liver Abscess", color= "Sample Source")
p0 = plot_ordination(final_ps_rel_abun, bray_litdir, type = "sample", color = "Sample.Source", axes =
1:2)+labs(color= "Sample Source", y= "PC2[13.5%]", x = "PC1[25%]")+theme(legend.position = c(0.15,
0.9))+theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
panel.background = element_blank(), axis.line = element_line(colour = "black"))+ geom_point(size = 1)+
scale_color_discrete(name="Sample Type",
breaks=c("Rumen Fluid", "Rumen Papillae"),
labels=c("Rumen Content", "Rumen Papillae"))

#permutation
final_ps_bray= phyloseq::distance(final_ps_rel_abun, method = "bray")
sampledf <- data.frame(sample_data(final_ps_rel_abun))
adonis(final_ps_bray ~ Sample.Source+Trt+Sex+Liver.Abscess, data = sampledf)
adonis(final_ps_bray ~ Trt+Sex+Liver.Abscess+Sample.Source, data = sampledf,
strata=sampledf$Red_Tag_Number)
my_metadata_new= my_metadata[,c(1,2,10)]
row.names(my_metadata_new)= my_metadata_new$SampleID
nested.npmanova(otu.table_t ~ Trt+Red_Tag_Number, data = my_metadata_new, method="bray")
my_metadata_new$Red_Tag_Number= as.factor(my_metadata_new$Red_Tag_Number)
class(my_metadata_new$Red_Tag_Number)
```

### richness Plot
rarefied at 8000 reads
```{r, echo= F, message= F, warning= F}
set.seed(1278)
final_ps_farefied_8000= rarefy_even_depth(final_ps, sample.size = 8000, replace = F)
final_ps_farefied_8000
ps0.rar=final_ps_farefied_8000
#hmp.div <- alpha(ps0.rar, index = "all")
Alpha_Diversity= amp_alphadiv(ps1.av2, measure = c("observed", "shannon", "simpson"), rarefy= 8000 )
#write.table(Alpha_Diversity, file = "Alpha_Diversity_rarefy8000.txt", sep = "\t", col.names = T,
row.names = F)
##bell curve
a= mean(Alpha_Diversity$ObservedOTUs)
sd= sd(Alpha_Diversity$ObservedOTUs)
x= Alpha_Diversity$ObservedOTUs
y= dnorm(x, mean = 288.6864, sd = 64.56597)
plot(x,y)
plot(Alp)
####Observed OTUs
Alpha_Diversity2= Alpha_Diversity[,c(8,11,16)]
div_df_melt <- reshape2::melt(Alpha_Diversity2)
tr= ggboxplot(div_df_melt, x= "Sample_Source", y= "value", fill = "Liver_Abscess", palette = "jco",
legend= "right", scales= "free", ylab = "Observed Species", xlab = "Sample Source")
Ad1=tr+stat_compare_means(method = "wilcox.test", aes(group = Liver_Abscess), label = "p.format")
Ad1

```

```

tr2= ggboxplot(div_df_melt, x= "Sample_Source", y= "value", fill = "Sample_Source", palette = "jco",
legend= "right",scales= "free", ylab = "Observed Species", xlab = "Sample Source")
Ad2=tr2+stat_compare_means(method = "wilcox.test",aes(group = Sample_Source), label = "p.format",
label.x.npc=0.5)
Ad2
####Simpson's diversity Index boxplot
Alpha_Diversity3= Alpha_Diversity[,c(8,11,18)]
div_df_melt3 <- reshape2::melt(Alpha_Diversity3)

box= ggplot(div_df_melt3, aes(x=Sample_Source, y=value, fill=Sample_Source)) +
  geom_boxplot(show.legend = F)+labs(y = "Simpson's Diversity Index", color= "Sample Source")+
  theme(legend.position = c(0.5, 0.3))+
  theme(axis.title.x=element_blank())+
  scale_x_discrete(labels= c("Rumen Fluid"= "Rumen Content", "Rumen Papillae"="Rumen Papillae"))+
  theme(panel.grid.major = element_blank(),panel.grid.minor = element_blank(),
        panel.background = element_blank(),axis.line = element_line(colour = "black"))

box
###Friedman's test
library(rstatix)
ft= Alpha_Diversity %>% friedman_test(ObservedOTUs ~ Sample_Source|Red_Tag_Number)
ft
ft2= Alpha_Diversity %>% friedman_test(Simpson ~ Sample_Source|Red_Tag_Number)
ft2
###Table alpha diversity
library(dplyr)
Alpha_Diversity_mean1= Alpha_Diversity %>% group_by(Sample_Source) %>%
  summarise(average_div= mean(ObservedOTUs),
            SD= sd(ObservedOTUs),
            N= n())
Alpha_Diversity_mean11= Alpha_Diversity %>% group_by(Sample_Source) %>%
  summarise(average_div= mean(Simpson),
            SD= sd(Simpson),
            N= n())
Alpha_Diversity_mean2= Alpha_Diversity %>% group_by(Sample_Source,Liver_Abscess) %>%
  summarise(average_Obsdiv= mean(ObservedOTUs),
            SD= sd(ObservedOTUs),
            N= n())
Alpha_Diversity_mean3= Alpha_Diversity %>% group_by(Sample_Source,Liver_Abscess) %>%
  summarise(average_Obsdiv= mean(Simpson),
            SD= sd(Simpson),
            N= n())
Alpha_Diversity_mean2= Alpha_Diversity %>% group_by(Sample_Source,Sex) %>%
  summarise(average_Obsdiv= mean(ObservedOTUs),
            SD= sd(ObservedOTUs),
            N= n())
Alpha_Diversity_mean3= Alpha_Diversity %>% group_by(Sample_Source,Sex) %>%
  summarise(average_Obsdiv= mean(Simpson),
            SD= sd(Simpson),
            N= n())
Alpha_Diversity_mean2= Alpha_Diversity %>% group_by(Sample_Source,Trt) %>%
  summarise(average_Obsdiv= mean(ObservedOTUs),
            SD= sd(ObservedOTUs),
            N= n())
Alpha_Diversity_mean3= Alpha_Diversity %>% group_by(Sample_Source,Trt) %>%

```

```

summarise(average_Obsdiv= mean(Simpson),
          SD= sd(Simpson),
          N= n())
###Alpha Diversity Subsetting and analysis
Alpha_Diversity_RumenFluid= Alpha_Diversity[Alpha_Diversity$Sample_Source=="Rumen Fluid",]
Alpha_Diversity_RumenPapillae= Alpha_Diversity[Alpha_Diversity$Sample_Source=="Rumen
Papillae",]
wilcox_test(ObservedOTUs ~ Trt ,data = Alpha_Diversity_RumenFluid)
wilcox_test(Simpson ~ Trt ,data = Alpha_Diversity_RumenFluid)

wilcox_test(ObservedOTUs ~ Sex ,data = Alpha_Diversity_RumenFluid)
wilcox_test(Simpson ~ Sex ,data = Alpha_Diversity_RumenFluid)
wilcox_test(ObservedOTUs ~ Liver_Abscess ,data = Alpha_Diversity_RumenFluid)
wilcox_test(Simpson ~ Liver_Abscess ,data = Alpha_Diversity_RumenFluid)
#rumen papillae
wilcox_test(ObservedOTUs ~ Trt ,data = Alpha_Diversity_RumenPapillae)
wilcox_test(Simpson ~ Trt ,data = Alpha_Diversity_RumenPapillae)
wilcox_test(ObservedOTUs ~ Sex ,data = Alpha_Diversity_RumenPapillae)
wilcox_test(Simpson ~ Sex ,data = Alpha_Diversity_RumenPapillae)
wilcox_test(ObservedOTUs ~ Liver_Abscess ,data = Alpha_Diversity_RumenPapillae)
wilcox_test(Simpson ~ Liver_Abscess ,data = Alpha_Diversity_RumenPapillae)
...

#taxa graph for All data
```{r, echo= F, message= F, warning= F}
library(ampvis2)
eee3= amp_boxplot(ps1.av2,
  group_by = "Sample_Source",
  tax_show = 10,
  tax_aggregate= "Phylum",
  sort_by = "total",
  detailed_output = T
)
data_Phyla_boxplot= eee3$data
####making phyla bar plot with error bar in ggplot for the paper
library(dplyr)
data_Phyla_boxplot_average= data_Phyla_boxplot %>%
  group_by(Display,Group) %>%
  summarise(average_abundance= mean(Abundance),
            SD= sd(Abundance))
data_Phyla_boxplot_average2= data_Phyla_boxplot_average[-c(1,2,5,6,9,10),]
library(ggplot2)
p= ggplot(data_Phyla_boxplot_average2, aes(x= Display, y= average_abundance, fill=Group))+
  geom_bar(stat = "identity", width = 0.5, position= "dodge", show.legend = T ) +
  theme(axis.text.x = element_text(angle = 0))+
  geom_errorbar(aes(ymin= average_abundance- SD, ymax=average_abundance+SD), alpha=0.9,
size=0.2,width= 0.2, position= position_dodge(0.45))+
  coord_flip()+theme(aspect.ratio = 2/5)+theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour = "black"))+labs(y =
"Abundance(%)", x= "Phylum")+theme(legend.position = c(0.66, 0.3))+ scale_fill_discrete(name="Sample
Type",
          breaks=c("Rumen Fluid", "Rumen Papillae"),
          labels=c("Rumen Content", "Rumen Papillae"))+ theme(axis.title.x=element_blank(),
axis.text.x=element_blank(),
axis.ticks.x=element_blank(),

```

```

axis.line.x = element_blank())
phylum= p+theme(text = element_text(size=15))
p+stat_compare_means(method = "wilcox.test",aes(group = Group), label = "p.format")
compare_means(len ~ supp, data = data_Phyla_boxplot_average2)
ert= dcast(data_Phyla_boxplot_average2, Group ~ Display, mean)
#+theme(axis.text.y = element_text(hjust = -0.1))
p2=p+ theme_classic()
theme(text = element_text(size=20))+ theme(legend.position = c(0.66, 0.3))+
scale_fill_discrete(name="Sample\nType",
                    breaks=c("Rumen Fluid", "Rumen Papillae"),
                    labels=c("Rumen Content", "Rumen Papillae"))
#Supplimantry Figure
eee3_Supp= amp_boxplot(ps1.av2,
  group_by = "Sample_Source",
  tax_show = 20,
  tax_aggregate= "Genus",
  detailed_output = T
)
data_Genus_boxplot= eee3_Supp$data
eee3_Supp$plot
###making bar plot for Genus for paper in ggplot
data_Genus_boxplot_average= data_Genus_boxplot %>%
  group_by(Display,Group) %>%
  summarise(average_abundance= mean(Abundance),
            SD= sd(Abundance))
data_Genus_boxplot_average2= data_Genus_boxplot_average[-c(5,6),]
pg= ggplot(data_Genus_boxplot_average, aes(x= Display, y= average_abundance, fill=Group))+
  ylim(0, 50)+
  geom_bar(stat = "identity", width = 0.5, position= "dodge",show.legend = F) +
  theme(axis.text.x = element_text(angle = 0))+
  geom_errorbar(aes(ymin= average_abundance- SD, ymax=average_abundance+SD), alpha=0.9,
size=0.2,width= 0.2, position= position_dodge(0.45))+
  coord_flip()+theme(panel.grid.major = element_blank(),panel.grid.minor = element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour = "black"))+labs(y =
"Abundance(%)", x= "Genus")
pg+theme(aspect.ratio = 4/5)
pg2=pg+ theme_classic()
pg2
ggplot(data_Phyla_boxplot, aes(x= Display, y= Abundance, fill=Group))+
  ylim(0, 50)+geom_bar(stat = "identity", width = 0.5, position= "dodge",show.legend = F)+coord_flip()
### if you want to merge two graphs in one
library("gridExtra")
library("cowplot")
plot_grid(p, pg, labels=c("A", "B"), ncol = 1, nrow = 2)
plot_grid(p, pg, labels = "AUTO", rel_heights = c(1, 1.5), ncol = 1, rel_widths = c(1,1))
ggdraw() +
  draw_plot(pg2, 0, 0, 1, 0.58) +
  draw_plot(p2, 0.07, 0.6, 1, .3)
ggdraw() +
  draw_plot(pg2, 0, 0, 1, 0.58) +
  draw_plot(p2, -0.048, 0.5, 1, .5)
library(gridExtra)
grid.arrange(p, pg, nrow=2,ncol= 1, heights=c(2, 4), widths= 5 )
grid.arrange(bp, vp, legend, ncol=3, widths=c(2.3, 2.3, 0.8))
```

```

```

### stat on the relative abundances of phyla and genera (bar plots) For all data Friedmans test
```{r}
##phyla level between rumen papillae and rumen fluid
data_Phyla_boxplot_average_sample= data_Phyla_boxplot %>%
  group_by(Sample,Display,Group) %>%
  summarise(average_abundance= mean(Abundance))
ert2= dcast(data_Phyla_boxplot_average_sample, Sample~Display, mean)
colnames(ert2)[1]= "SampleID"
data_Genus_boxplot
data_Genus_boxplot_average_sample= data_Genus_boxplot %>%
  group_by(Sample,Display,Group) %>%
  summarise(average_abundance= mean(Abundance))
ert3= dcast(data_Genus_boxplot_average_sample, Sample~Display, mean)
colnames(ert3)[1]= "SampleID"
metadata_all= as.tibble(sample_data(final_ps))
metadata_all2= metadata_all[,c(1,2,8,9,10,11)]
#merging the metadata and phyla

metadata_phyla= merge(metadata_all2, ert2)
metadata_phyla_Genera= merge(metadata_phyla, ert3)

library(rstatix)
library(dplyr)
metadata_phyla_Genera %>% friedman_test(metadata_phyla_Genera$Planctomycetes ~
Sample.Source|Red_Tag_Number)

metadata_phyla_Genera %>% friedman_test(metadata_phyla_Genera$`Prevotellaceae_UCG-001` ~
Sample.Source|Red_Tag_Number)
metadata_phyla_Genera[[7]]
columns = colnames(metadata_phyla_Genera[,c(7:36)])
ttt= lapply(metadata_phyla_Genera[columns],function(x) friedman_test(x~
Sample.Source|Red_Tag_Number, data = metadata_phyla_Genera))
ttt$`Prevotellaceae_UCG-001`
ttt$Planctomycetes
ttt2= as.data.frame(ttt)
ttt3= ttt2 %>% select(contains(".p"))
```

### state on the relative abundances of phyla and genera (bar plots) For rumen papillae data wilcox test
```{r}
data_Phyla_boxplot_papillae= eee4$data
## Phyla papillae data
library(dplyr)
data_Phyla_boxplot_papillae_average_sample= data_Phyla_boxplot_papillae %>%
  group_by(Sample,Display,Group) %>%
  summarise(average_abundance= mean(Abundance))
Papi_ert3= dcast(data_Phyla_boxplot_papillae_average_sample, Sample~Display, mean)
colnames(Papi_ert3)[1]= "SampleID"
#Genera
data_Genus_boxplot_papillae= eee4$Supp$data
data_Genus_boxplot_papillae_average_sample= data_Genus_boxplot_papillae %>%
  group_by(Sample,Display,Group) %>%
  summarise(average_abundance= mean(Abundance))
Papi_ert4= dcast(data_Genus_boxplot_papillae_average_sample, Sample~Display, mean)
colnames(Papi_ert4)[1]= "SampleID"
###metadata

```

```

metadata_papi= as.tibble(sample_data(rumenPappilae_no_singleton))
metadata_papi2= metadata_papi[,c(1,2,8,9,10,11)]
###merging all 3 data
metadata_phyla_papi= merge(metadata_papi2, Papi_ert3)
metadata_phyla_Genera_papi= merge(metadata_phyla_papi, Papi_ert4)
###wilcox test
wilcox.test(metadata_phyla_Genera_papi$Planctomycetes ~ metadata_phyla_Genera_papi$Liver.Abscess)
columns = colnames(metadata_phyla_Genera_papi[,c(7:36)])
ttt_papi= lapply(metadata_phyla_Genera_papi[columns],function(x) wilcox.test(x~
metadata_phyla_Genera_papi$Liver.Abscess))

lapply(metadata_phyla_Genera_papi[columns],function(x) wilcox.test(x~
metadata_phyla_Genera_papi$Liver.Abscess))
ttt_papi$Planctomycetes
ttt_papi2= as.data.frame(ttt_papi)
ttt4= ttt_papi2 %>% select(contains(".p"))
ttt_papi$
```

#Permutation Rumen Papillae and rumen fluid
```{r}
rumen_proportions = transform_sample_counts(rumenFluid, function(OTU) OTU/sum(OTU))
rumen_proportions = transform_sample_counts(rumenFluid_no_singleton, function(OTU)
OTU/sum(OTU))
fluid_bray= phyloseq::distance(rumen_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumen_proportions))
adonis(fluid_bray ~ Sex+Liver.Abscess+Trt, data = sampledf)
sample_variables(rumen_proportions)
##pcoa
bray_litdir = ordinate(rumen_proportions, "PCoA","bray")
p0_fluid = plot_ordination(rumen_proportions, bray_litdir, type = "sample", color = "Liver.Abscess", axes
= 1:2)+ labs(color= "Liver Abscess")
p0_fluid
####
rumenFluid_no_singleton_proportions = transform_sample_counts(rumenFluid_no_singleton,
function(OTU) OTU/sum(OTU))
rumen_proportions = transform_sample_counts(rumenFluid_no_singleton, function(OTU)
OTU/sum(OTU))
fluid_bray= phyloseq::distance(rumen_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumen_proportions))
adonis(fluid_bray ~ Sex+Liver.Abscess+Trt, data = sampledf)
sample_variables(rumen_proportions)
##pcoa
bray_litdir = ordinate(rumen_proportions, "PCoA","bray")
p0_fluid = plot_ordination(rumen_proportions, bray_litdir, type = "sample", color = "Liver.Abscess", axes
= 1:2)+ labs(color= "Liver Abscess")
p0_fluid
```

#Permutation Rumen Papillae
```{r}
library(vegan)
rumenPappilae_proportions= transform_sample_counts(rumenPappilae, function(OTU) OTU/sum(OTU))
rumenPappilae_proportions
papi_bray= phyloseq::distance(rumenPappilae_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumenPappilae_proportions))
adonis(papi_bray ~ Trt+Sex+Liver.Abscess, data = sampledf)

```

```

sample_variables(rumenPappilae_proportions)
##pcoa
bray_litdir = ordinate(rumenPappilae_proportions, "PCoA", "bray")
p0_papi = plot_ordination(rumenPappilae_proportions, bray_litdir, type = "sample", color =
"Liver.Abscess", axes = 1:2)+ labs(color= "Liver Abscess")+labs(color= "Group",y= "PC2[12.1%]", x =
"PC1[12.6%]")+ theme_classic()
p0_papi +theme(legend.position = c(0.015, 0.9))
####
rumenPappilae_no_singleton
rumenPappilae_no_singleton_proportions= transform_sample_counts(rumenPappilae_no_singleton,
function(OTU) OTU/sum(OTU))
rumenPappilae_no_singleton_proportions
papi_bray= phyloseq::distance(rumenPappilae_no_singleton_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumenPappilae_no_singleton_proportions))
set.seed(423)
adonis(papi_bray ~ Sex+Trt+Liver.Abscess, data = sampledf)
sample_variables(rumenPappilae_no_singleton_proportions)
##pcoa
bray_litdir = ordinate(rumenPappilae_no_singleton_proportions, "PCoA", "bray")
p0_papi_2 = plot_ordination(rumenPappilae_no_singleton_proportions, bray_litdir, type = "sample", color
= "Liver.Abscess", axes = 1:2)+ labs(color= "Liver Abscess")+labs(color= "Group",y= "PC2[12.1%]", x =
"PC1[12.6%]")+ theme_classic()
p0_papi_2+theme(legend.position = c(0.15, 0.9))
...

#####Permutation Rumen Fluid
```{r}
rumenFluid_proportions = transform_sample_counts(rumenFluid, function(OTU) OTU/sum(OTU))
fluid_bray= phyloseq::distance(rumenFluid_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumenFluid_proportions))
adonis(fluid_bray ~ Trt+Sex+Liver.Abscess, data = sampledf)
sample_variables(rumenFluid_proportions)
bray_litdir = ordinate(rumenFluid_proportions, "PCoA", "bray")
p0_fluid = plot_ordination(rumenFluid_proportions, bray_litdir, type = "sample", color = "Liver.Abscess",
axes = 1:2)+ labs(color= "Liver Abscess")
p0_fluid
###rumenFluid_no_singleton
rumenFluid_no_singleton
rumenFluid_no_singleton_proportions = transform_sample_counts(rumenFluid_no_singleton,
function(OTU) OTU/sum(OTU))
fluid_bray= phyloseq::distance(rumenFluid_no_singleton_proportions, method = "bray")
sampledf <- data.frame(sample_data(rumenFluid_no_singleton_proportions))
adonis(fluid_bray ~ Trt+Sex+Liver.Abscess, data = sampledf)
sample_variables(rumenFluid_no_singleton_proportions)
bray_litdir = ordinate(rumenFluid_no_singleton_proportions, "PCoA", "bray")
p0_fluid_2 = plot_ordination(rumenFluid_no_singleton_proportions, bray_litdir, type = "sample", color =
"Liver.Abscess", axes = 1:2)+ labs(color= "Liver Abscess")
p0_fluid_2
...

###Papillae data Phyla and Genus abundance plots

```{r}
library("ampvis2")
library("dplyr")
library("tibble")
# Load data into ampvis

```



```

ps1=rumenPappilae
otu.table <- (otu_table(ps1, taxa_are_rows = FALSE))
otu.table <- as.data.frame(otu.table)
otu.table <- rownames_to_column(otu.table, var = "OTU")
tax.table <- as.data.frame(tax_table(ps1))
tax.table <- rownames_to_column(tax.table, var = "OTU")
my_otu_table <- left_join(otu.table, tax.table, by = "OTU")
my_otu_table <- column_to_rownames(my_otu_table, var = "OTU")
my_metadata <- as.tibble(sample_data(ps1))
#my_tree <- phy_tree(ps1)
ps3.av2 <- amp_load(otutable = my_otu_table, metadata = my_metadata)
ps3.av2
#making ampv object without with rumenPappilae_no_singleton
ps1=rumenPappilae_no_singleton
otu.table <- (otu_table(ps1, taxa_are_rows = FALSE))
otu.table <- as.data.frame(otu.table)
otu.table <- rownames_to_column(otu.table, var = "OTU")
tax.table <- as.data.frame(tax_table(ps1))
tax.table <- rownames_to_column(tax.table, var = "OTU")
my_otu_table <- left_join(otu.table, tax.table, by = "OTU")
my_otu_table <- column_to_rownames(my_otu_table, var = "OTU")
my_metadata <- as.tibble(sample_data(ps1))
rownames(my_metadata)= my_metadata$SampleID
#my_tree <- phy_tree(ps1)
rumen_Papillae_no_singleton_ps3.av2 <- amp_load(otutable = my_otu_table, metadata = my_metadata)
eee4= amp_boxplot(rumen_Papillae_no_singleton_ps3.av2,
  group_by = "Liver_Abscess",
  tax_show = 10,
  tax_aggregate= "Phylum",
  sort_by = "total",
  detailed_output = T
)
eee4$plot
data_Phyla_boxplot_papillae= eee4$data
## bar plot for papillae data with ggplot2
library(dplyr)
data_Phyla_boxplot_papillae_average= data_Phyla_boxplot_papillae %>%
  group_by(Display,Group) %>%
  summarise(average_abundance= mean(Abundance),
    SD= sd(Abundance))
data_Phyla_boxplot_papillae_average2= data_Phyla_boxplot_papillae_average[-c(1,2,5,6,9,10,11,12),]
library(ggplot2)
p= ggplot(data_Phyla_boxplot_papillae_average2, aes(x= Display, y= average_abundance, fill=Group))+
  geom_bar(stat = "identity", width = 0.5, position= "dodge", show.legend = T ) +
  theme(axis.text.x = element_text(angle = 0))+
  geom_errorbar(aes(ymin= average_abundance- SD, ymax=average_abundance+SD), alpha=0.9,
size=0.2,width= 0.2, position= position_dodge(0.45))+
  coord_flip()+theme(aspect.ratio = 2/5)+theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour = "black"))+labs(y =
"Abundance(%)", x= "Phylum")
#+theme(axis.text.y = element_text(hjust = -0.1))
phylum_papillae=p+ theme(legend.position = c(0.66, 0.3))+
  theme(axis.title.x=element_blank(),
    axis.text.x=element_blank(),

```

```

    axis.ticks.x=element_blank(),
    axis.line.x = element_blank())
p2=p+ theme_classic()
#Genus abundance bar plot
#Supplimantry Figure
eee4_Supp= amp_boxplot(rumen_Papillae_no_singleton_ps3.av2,
  group_by = "Liver_Abscess",
  tax_show = 20,
  tax_aggregate= "Genus",
  sort_by = "total",
  detailed_output = T
)
eee4_Supp$plot
data_Genus_boxplot_papillae= eee4_Supp$data
###bar plot for Genus with ggplot2
data_Genus_boxplot_papillae_average= data_Genus_boxplot_papillae %>%
  group_by(Display,Group) %>%
  summarise(average_abundance= mean(Abundance),
    SD= sd(Abundance))
data_Genus_boxplot_papillae_average2= data_Genus_boxplot_papillae_average[-c(5,6),]
pg= ggplot(data_Genus_boxplot_papillae_average, aes(x= Display, y= average_abundance, fill=Group))+
  ylim(0, 50)+
  geom_bar(stat = "identity", width = 0.5, position= "dodge", show.legend = F) +
  theme(axis.text.x = element_text(angle = 0))+
  geom_errorbar(aes(ymin= average_abundance- SD, ymax=average_abundance+SD), alpha=0.9,
    size=0.2,width= 0.2, position= position_dodge(0.45))+
  coord_flip()+theme(panel.grid.major = element_blank(),panel.grid.minor = element_blank(),
    panel.background = element_blank(),axis.line = element_line(colour = "black"))+labs(y =
    "Abundance(%)", x= "Genus")
Genus_papillae=pg+theme(aspect.ratio = 4/5)
pg2=pg+ theme_classic()
pg2
```


```

#beta diversity box plot papillae
```{r}
library(microbiome)
b.st <- as.data.frame(divergence(subset_samples(rumenPappilae_proportions, Liver.Abscess == "Liver
Abscess"))))
colnames(b.st)= "Liver Abscess"
rt= data_frame("Liver Abscess"= 0.490761)
b.st2= rbind(b.st,rt)
b.bx <- as.data.frame(divergence(subset_samples(rumenPappilae_proportions, Liver.Abscess ==
"Healthy"))))
colnames(b.bx)= "Healthy"
div_df <- data.frame(b.st2, b.bx)
dif.g <- reshape2::melt(div_df)
ggpubr::ggboxplot(dif.g, "variable", "value",
  ylab = "Divergence",
  xlab = "Sample Type",
  add = "jitter",
  fill = "variable",
  palette = c("#a6cee3", "#b2df8a"))
```


```

#beta diversity box plot fluid
```{r}

```


```


```

```

library(microbiome)
b.st <- as.data.frame(divergence(subset_samples(rumenFluid_proportions, Liver.Abscess == "Liver
Abscess"))))
colnames(b.st)= "Liver Abscess"
mean(b.st$`Liver Abscess`)
rt= data_frame("Liver Abscess"= 0.45898)
b.st2= rbind(b.st,rt)
b.bx <- as.data.frame(divergence(subset_samples(rumenPappilae_proportions, Liver.Abscess ==
"Healthy"))))
colnames(b.bx)= "Healthy"
div_df <- data.frame(b.st2, b.bx)
dif.g <- reshape2::melt(div_df)
ggpubr::ggboxplot(dif.g, "variable", "value",
                  ylab = "Divergence",
                  xlab = "Sample Type",
                  add = "jitter",
                  fill = "variable",
                  palette = c("#a6cee3", "#b2df8a"))
...

#Correlations
Deseq2-> alpha = 0.01 >
"ASV_12","ASV_37","ASV_390","ASV_407","ASV_584","ASV_624","ASV_692","ASV_805"
Table 4 Gene list
Gene= ("IFI6", "RELA", "TYK2", "AARS", "FARSB", "GARS", "WARS", "LY96", "RELA",
"TYK2","PRDX6","S100A12","RN18S1","LOC112444681","KRT17","LOC100847874")
```{r, echo= F, message= F, warning= F}
rumenPappilae_proportions= transform_sample_counts(rumenPappilae, function(OTU) OTU/sum(OTU))
ASVs_0.01=c("ASV_12","ASV_37","ASV_390","ASV_407","ASV_584","ASV_624","ASV_692","ASV
_805")
ASV_tax= prune_taxa(ASVs_0.01, rumenPappilae_proportions)
ASV_tax_table= t(otu_table(ASV_tax, taxa_are_rows = FALSE))
ASV_tax_table=as.data.frame(ASV_tax_table)
ASV_tax_table=rownames_to_column(ASV_tax_table, var = "SampleID")
# Gene Data
ps_gene
#making proportions
ps_gene_proportions= transform_sample_counts(ps_gene, function(OTU) OTU/sum(OTU))
genes_0.01=c("IFI6", "RELA", "TYK2", "AARS", "FARSB", "GARS", "WARS", "LY96", "RELA",
"TYK2","PRDX6","S100A12","RN18S1","LOC112444681","KRT17","LOC100847874")
#genes_0.01= c("PRDX6","S100A12","RN18S1","LOC112444681","KRT17","LOC100847874")
genes_filtered= prune_taxa(genes_0.01, ps_gene_proportions)
genes_filtered_table= (otu_table(genes_filtered, taxa_are_rows = T))
genes_filtered_table= as.data.frame(genes_filtered_table)
genes_filtered_table= rownames_to_column(genes_filtered_table, var = "SampleID")

data_ASV_Gene= merge(ASV_tax_table, genes_filtered_table, by= "SampleID" )
...

```{r, echo= F, message= F, warning= F}
selected_phyla_family_OTUs_transpose= data_ASV_Gene[,-1]
selected_phyla_family_OTUs_transpose_log_plus1= log10(1+selected_phyla_family_OTUs_transpose)
library("ggplot2")
library("scales")
library("grid")
cormat= round(cor(selected_phyla_family_OTUs_transpose_log_plus1),2)
library(reshape2)

```

```

melted_cormat <- melt(cormat)
head(melted_cormat)
# Get lower triangle of the correlation matrix
get_lower_tri<-function(cormat){
  cormat[upper.tri(cormat)] <- NA
  return(cormat)
}
# Get upper triangle of the correlation matrix
get_upper_tri <- function(cormat){
  cormat[lower.tri(cormat)]<- NA
  return(cormat)
}
upper_tri <- get_upper_tri(cormat)
reorder_cormat <- function(cormat){
  # Use correlation between variables as distance
  dd <- as.dist((1-cormat)/2)
  hc <- hclust(dd)
  cormat <-cormat[hc$order, hc$order]
}
# Reorder the correlation matrix
#cormat <- reorder_cormat(cormat)
upper_tri <- get_upper_tri(cormat)
# Melt the correlation matrix
melted_cormat <- melt(upper_tri, na.rm = TRUE)
# Create a ggheatmap
ggheatmap <- ggplot(melted_cormat, aes(Var2, Var1, fill = value))+
  geom_tile(color = "white")+
  scale_fill_gradient2(low = "blue", high = "red", mid = "white",
    midpoint = 0, limit = c(-1,1), space = "Lab",
    name="Pearson\nCorrelation") +
  theme_minimal()+ # minimal theme
  theme(axis.text.x = element_text(angle = 45, vjust = 1,
    size = 8, hjust = 1))+
  coord_fixed()
# Print the heatmap
finalHeat= ggheatmap +
  geom_text(aes(Var2, Var1, label = value), color = "black", size = 1.5) +
  theme(
    axis.title.x = element_blank(),
    axis.title.y = element_blank(),
    panel.grid.major = element_blank(),
    panel.border = element_blank(),
    panel.background = element_blank(),
    axis.ticks = element_blank(),
    legend.justification = c(1, 0),
    legend.position = c(0.6, 0.7),
    legend.direction = "horizontal")+
  guides(fill = guide_colorbar(barwidth = 7, barheight = 1,
    title.position = "top", title.hjust = 0.5))
finalHeat
```


```

#Correlations table
```{r}
install.packages("remotes")
remotes::install_github("vmikk/metagMisc")

```


```

```

library(metagen)
phyloseq_prevalence_plot(rumenPappilae)
rumenPappilae_proportions= transform_sample_counts(OTU OTU/sum(OTU))
phyloseq_prevalence_plot(OTU OTU/sum(OTU))
rumenPappilae_proportions_0.5= phyloseq_filter_prevalence(OTU OTU/sum(OTU),prev.trh = 0.5,
abund.trh = 0.000000001, threshold_condition = "AND", abund.type = "total")
rumenPappilae_proportions_0.5
rumenPappilae_proportions_0.5_table= (otu_table(rumenPappilae_proportions_0.5))
rumenPappilae_proportions_0.5_table= as.data.frame(t(rumenPappilae_proportions_0.5_table))
rumenPappilae_proportions_0.5_table= rownames_to_column(rumenPappilae_proportions_0.5_table, var
= "SampleID")
#write.table(rumenPappilae_proportions_0.5_table, file = "rumenPappilae_proportions_0.5_table.txt", sep
= "\t", row.names = F, col.names = T,quote = F)
rumenPappilae_proportions_0.5_Taxa_table= as.data.frame(tax_table(rumenPappilae_proportions_0.5))
rumenPappilae_proportions_0.5_Taxa_table=
rownames_to_column(rumenPappilae_proportions_0.5_Taxa_table, var = "TAXONOMY")
#write.table(rumenPappilae_proportions_0.5_Taxa_table, file =
"Correlations_ASV_Taxa_table_rumenPappilae.txt", sep = "\t", row.names = F, col.names = T,quote = F)

rumenPappilae_proportions_0.5_table_SampleID_Order=rumenPappilae_proportions_0.5_table[order(rum
enPappilae_proportions_0.5_table$SampleID),]
rumenPappilae_proportions_0.5_table_SampleID_Order_C=
rumenPappilae_proportions_0.5_table_SampleID_Order[,-1]
rumenPappilae_proportions_0.5_table_SampleID_Order_C=as.matrix(rumenPappilae_proportions_0.5_tab
le_SampleID_Order_C)
ps_gene_proportions= transform_sample_counts(ps_gene, function(OTU) OTU/sum(OTU))
#ps_gene_proportions_0.5= phyloseq_filter_prevalence(ps_gene_proportions,prev.trh = 0.5, abund.trh =
NULL, threshold_condition = "OR", abund.type = "total")
tb=read.table("Supplemental Table 3 for Waseem.txt", sep = "\t", header = T)
significant_genes= as.vector(tb$Gene1)
significant_genes_filtered= prune_taxa(significant_genes, ps_gene_proportions)
significant_genes_filtered_table= (otu_table(significant_genes_filtered))
significant_genes_filtered_table= as.data.frame(significant_genes_filtered_table)
significant_genes_filtered_table= rownames_to_column(significant_genes_filtered_table, var =
"SampleID")
significant_genes_filtered_table_SampleID_Order=significant_genes_filtered_table[order(significant_gene
s_filtered_table$SampleID),]
significant_genes_filtered_table_SampleID_Order_C=
significant_genes_filtered_table_SampleID_Order[,-1]
x= significant_genes_filtered_table_SampleID_Order_C
y= rumenPappilae_proportions_0.5_table_SampleID_Order_C
x_log_plus1= log10(1+x)
y_log_plus1= log10(1+y)
library("lineup")
yt=corbetw2mat(x, y, what = "all",corthresh = 0.9)
nrow(x)
nrow(y)
ncol(x)
ncol(y)
yu= (cor(x,y))
#write.table(yu, file = "Correlation_SignificantGenes_And_ASVs0.5.txt", sep = "\t", row.names = T,
col.names = T)
yu_log= (cor(x_log_plus1,y_log_plus1))
library(ComplexHeatmap)
Heatmap(yt)

```

```
hp_cor=Heatmap(yu_log, row_names_gp = gpar(fontsize= 5),column_names_gp = gpar(fontsize= 6)
,row_km = 5, column_km = 3)
```

```
hp_cor2=Heatmap(yu_log, row_names_gp = gpar(fontsize= 5),column_names_gp = gpar(fontsize= 6)
,row_split = 3, column_split = 3)
```

```
#### Individual Correlation Scatter plots
```

```
# Positive Correlations
```

```
ScatterPlots_data= data.frame(x_log_plus1[,
c("LOC112442408","TRPM3","CREB3L2","C23H6orf15","IFI6","FZD5","EFNA2","LOC112442805","L
OC107133265","DBI","ASB11","SMIM7","TYK2","TYK2") ], y_log_plus1[,
c("ASV_23","ASV_134","ASV_139","ASV_30","ASV_68","ASV_68","ASV_51","ASV_68","ASV_144"
,"ASV_425","ASV_51","ASV_169","ASV_94"))])
```

```
library(ggpubr)
```

```
sp1 <- ggscatter(ScatterPlots_data, x = "LOC112442408", y = "ASV_23",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE,# Add confidence interval
  ylab= expression(italic("f_Ruminococcaceae") (ASV_23)))+ stat_cor(method = "pearson")
sp1
```

```
sp2 <- ggscatter(ScatterPlots_data, x = "TRPM3", y = "ASV_30",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Prevotella_1") (ASV_30)))+ stat_cor(method = "pearson")
sp2
```

```
sp3 <- ggscatter(ScatterPlots_data, x = "CREB3L2", y = "ASV_51",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Butyribrio_2") (ASV_51)))+ stat_cor(method = "pearson")
```

```
sp4 <- ggscatter(ScatterPlots_data, x = "C23H6orf15", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68)))+ stat_cor(method = "pearson")
```

```
sp5 <- ggscatter(ScatterPlots_data, x = "IFI6", y = "ASV_134",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Ruminococcaceae_UCG-002") (ASV_134)))+ stat_cor(method = "pearson")
sp5
```

```
sp6 <- ggscatter(ScatterPlots_data, x = "FZD5", y = "ASV_139",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Butyribrio_2") (ASV_139)))+ stat_cor(method = "pearson")
```

```
sp6
```

```

sp7 <- ggscatter(ScatterPlots_data, x = "EFNA2", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68)))+ stat_cor(method = "pearson")

sp8 <- ggscatter(ScatterPlots_data, x = "LOC112442805", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68)))+ stat_cor(method = "pearson")

sp9 <- ggscatter(ScatterPlots_data, x = "LOC107133265", y = "ASV_144",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Prevotella_1") (ASV_144)))+ stat_cor(method = "pearson")

#Negative Correlations

sp10 <- ggscatter(ScatterPlots_data, x = "DBI", y = "ASV_425",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_RBG-16-49-21") (ASV_425)))+ stat_cor(method = "pearson", label.x.npc
=0.3)

sp11 <- ggscatter(ScatterPlots_data, x = "ASB11", y = "ASV_425",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_RBG-16-49-21") (ASV_425)))+ stat_cor(method = "pearson", label.x.npc
=0.3)

sp12 <- ggscatter(ScatterPlots_data, x = "SMIM7", y = "ASV_51",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Butyrivibrio_2") (ASV_51)))+ stat_cor(method = "pearson", label.x.npc=0.3)

sp13 <- ggscatter(ScatterPlots_data, x = "TYK2", y = "ASV_169",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  legend= "right",
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Pyramidobacter") (ASV_169)))+ stat_cor(method = "pearson", label.x.npc
=0.3 )

sp14 <- ggscatter(ScatterPlots_data, x = "TYK2", y = "ASV_94",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("o_Bacteroidales") (ASV_94)))+ stat_cor(method = "pearson", label.x.npc =0)

### more positive correlations

```

```

ScatterPlots_data= data.frame(x_log_plus1[,
c("LOC112442408","TRPM3","CREB3L2","C23H6orf15","IFI6","FZD5","EFNA2","LOC112442805","L
OC107133265","DBI","ASB11","SMIM7","TYK2","TYK2","SLC25A28","LOC101902542","TSPAN7",
"DTX4","SEC24D","HCN2","LOC112441507","LOC104969959") ], y_log_plus1[,
c("ASV_23","ASV_134","ASV_139","ASV_30","ASV_68","ASV_51","ASV_68","ASV_144","ASV_42
5","ASV_51","ASV_169","ASV_94","ASV_219","ASV_19"))
genes_ASVs_logPlus1= cbind(x_log_plus1,y_log_plus1)

spp1= ggscatter(ScatterPlots_data, x = "SLC25A28", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68)))+ stat_cor(method = "pearson", label.x.npc =0)

spp2= ggscatter(ScatterPlots_data, x = "LOC101902542", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68)))+ stat_cor(method = "pearson", label.x.npc =0)
spp3= ggscatter(ScatterPlots_data, x = "TSPAN7", y = "ASV_134",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Ruminococcaceae_UCG-002") (ASV_134)))+ stat_cor(method = "pearson",
label.x.npc =0)

spp4= ggscatter(ScatterPlots_data, x = "DTX4", y = "ASV_19", xlim= c(0.00001, 0.000041),
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Campylobacter") (ASV_19)))+ stat_cor(method = "pearson", label.x.npc =0.1)

spp5= ggscatter(ScatterPlots_data, x = "SEC24D", y = "ASV_51",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Butyrvibrio_2") (ASV_51)))+ stat_cor(method = "pearson", label.x.npc =0)

spp6= ggscatter(ScatterPlots_data, x = "HCN2", y = "ASV_219",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Ruminococcaceae_UCG-009") (ASV_219)))+ stat_cor(method = "pearson",
label.x.npc =0)

spp7= ggscatter(ScatterPlots_data, x = "LOC112441507", y = "ASV_134",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("o_Ruminococcaceae_UCG-002") (ASV_134)))+ stat_cor(method = "pearson",
label.x.npc =0)

spp8= ggscatter(ScatterPlots_data, x = "LOC104969959", y = "ASV_30",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line

```



```

conf.int = TRUE, # Add confidence interval
ylab= expression(italic("g_Prevotella_1") (ASV_30))) + stat_cor(method = "pearson", label.x.npc = 0)
spp9= ggscatter(genes_ASVs_logPlus1, x = "CREB3L2", y = "ASV_287",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("f_F082") (ASV_287))) + stat_cor(method = "pearson", label.x.npc = 0)

sppP= ggscatter(genes_ASVs_logPlus1, x = "LRFN3", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68))) + stat_cor(method = "pearson", label.x.npc = 0)

spp10= ggscatter(genes_ASVs_logPlus1, x = "TRMT44", y = "ASV_68",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Shuttleworthia") (ASV_68))) + stat_cor(method = "pearson", label.x.npc = 0)

spp11= ggscatter(genes_ASVs_logPlus1, x = "LOC107133265", y = "ASV_30",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("o_Prevotella_1") (ASV_30))) + stat_cor(method = "pearson", label.x.npc = 0)

spp12= ggscatter(genes_ASVs_logPlus1, x = "ICOSLG", y = "ASV_19",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Campylobacter") (ASV_19))) + stat_cor(method = "pearson", label.x.npc = 0)

spp13= ggscatter(genes_ASVs_logPlus1, x = "ICOSLG", y = "ASV_55",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("o_Ruminococcaceae_UCG-005") (ASV_55))) + stat_cor(method = "pearson",
label.x.npc = 0)

spp14= ggscatter(genes_ASVs_logPlus1, x = "ETV6", y = "ASV_51",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Butyrivibrio_2") (ASV_51))) + stat_cor(method = "pearson", label.x.npc = 0)

spp15= ggscatter(genes_ASVs_logPlus1, x = "INAFM1", y = "ASV_75",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("f_Lachnospiraceae") (ASV_75))) + stat_cor(method = "pearson", label.x.npc = 0)
##### Heatmaps for selected data
G1=
c("TPR", "YLPM1", "CHD4", "LOC101903424", "ZNF609", "EIF3A", "MED1", "CNOT1", "SMC1A", "TTL",
"AQR", "TMEM248", "ARCN1", "SPTY2D1", "VPS8", "UBE2J1", "CCDC47", "PURB", "IGF1R", "HIF1AN",
"EIF4H", "LOC783466", "EXOC8", "OTUD7B", "GCC1", "RBBP5", "YWHAG", "LOC789694", "CREB3L2",

```

```
"SEC24D","CCPG1","ATP6V0A2","SLC35D1","DCAF5","HNRNPM","ETV6","CHAF1B","SLC35F6","RBM18","XPO6","ANKRD13C","ASRGL1","EYA3","AP3D1","TRIM25","FZD5","DDB1","RPN1","MTMR9","IGF2R","HS6ST1","CRAT","TBL2","SUPT6H","IGSF3","OGDH","CNOT11","USP4","SMCR8","AMOTL1","RFT1","FKRP","ZNF646","SLC10A3","PRPF8","DCTN1","SLC9A1","RAB3D","PNPO")
G2=
```

```
c("TYK2","METTL5","LOC782938","LOC112441868","LOC783838","LUZP6","DUT","HIGD1B","COX7A1","ADRB2","FBXL15","SLC22A17","LOC616830","LOC101902787","HIST1H3G","TIAF1","LOC107132767","RPS25","RPL39","LOC783541","LOC107132386","RPL36AL","LOC514680","RPL38","LOC101902561","LOC101904595","LOC112444655","LOC112442865","LOC112442866","LOC112442864","LOC104972595","SPINK9","LY96","RGS4","NBDY","LOC101904468","LOC112442597","RPS27L","NACA","LOC101902907","RPL22","PFDN4","FRG1","SPATA33","LOC112444215","LOC112444958","LOC100848315","LOC104976274","LOC112444957","UQCRB","LOC100174924","LOC100848985","LOC107131948","MLLT11","ITGB1BP2","SRSF4","XPC","ERMARD","ZNF692","KRBA1","VWA2","LOC104969959","BTNL9","CMC4","MDP1","HOXC4","INAFM1","CAPS","LOC104974837","ANKRD10","SLC25A28","ASB11","ASXL1","STK19","COL7A1","HOOK2","TRMT44","PRAP1","ESPNL","TSKS","GPR87","TRIB2","SMIM7","RNF7","LOC101902542","TRPM3","LOC107133265","DBI","DLK2","LOC112442805","INSL6","LOC112444681","LOC112442408","LOC112442867","LOC107132278","LOC112443614","RN7SL1","RMRP","ATP1B3","ETV5","RELB","ICOSLG","WBP4","HIP1","WSB1","LOC107131684","RASSF6","EFNA2","GPM6B","C23H6orf15","ADPRH","TRIM7","DTX4")
A1=
```

```
c("ASV_228","ASV_238","ASV_133","ASV_299","ASV_309","ASV_37","ASV_155","ASV_318","ASV_325","ASV_336","ASV_93","ASV_327","ASV_92","ASV_65","ASV_84","ASV_13","ASV_181","ASV_6","ASV_342","ASV_1132","ASV_50","ASV_365","ASV_176","ASV_253","ASV_82","ASV_18","ASV_218","ASV_12","ASV_128","ASV_348","ASV_488","ASV_196","ASV_223","ASV_46","ASV_242","ASV_171","ASV_141","ASV_118","ASV_119","ASV_60","ASV_51","ASV_139","ASV_165","ASV_213","ASV_76","ASV_425","ASV_234","ASV_287","ASV_349","ASV_134","ASV_219","ASV_167","ASV_78","ASV_45","ASV_64")
A2=
```

```
c("ASV_68","ASV_154","ASV_286","ASV_178","ASV_31","ASV_307","ASV_98","ASV_188","ASV_25","ASV_109","ASV_30","ASV_144","ASV_514","ASV_80","ASV_122","ASV_187","ASV_319","ASV_129","ASV_120","ASV_265","ASV_4","ASV_16","ASV_27","ASV_295","ASV_21","ASV_34","ASV_70","ASV_99","ASV_291","ASV_49","ASV_23","ASV_85","ASV_312","ASV_57","ASV_75","ASV_22","ASV_47","ASV_2","ASV_281","ASV_55","ASV_185","ASV_267","ASV_94","ASV_9","ASV_24")
")
```

```
G1= as.vector(G1)
```

```
Heatmap(yu_log)
```

```
significant_genes_filtered_G1 = prune_taxa(G1, ps_gene_proportions)
```

```
significant_genes_filtered_G2 = prune_taxa(G2, ps_gene_proportions)
```

```
library(phyloseq)
```

```
ASVs_filtered_A1 = prune_taxa(A1, rumenPappilae_no_singleton_proportions)
```

```
ASVc_filtered_A2 = prune_taxa(A2, rumenPappilae_no_singleton_proportions)
```

```
otu.table <- (otu_table(ASVs_filtered_A1, taxa_are_rows = T))
```

```
otu.table <- as.data.frame(otu.table)
```

```
A1_otu.table2= rownames_to_column(otu.table, var = "OTU")
```

```
tax.table= as.data.frame(tax_table(ASVs_filtered_A1, ps_gene_proportions))
```

```
tax.table= rownames_to_column(tax.table, var = "OTU")
```

```
A1_my_otu_table_ASVs_filteredA1 <- left_join(A1_otu.table2, tax.table, by = "OTU")
```

```
A1_my_otu_table_ASVs_filteredA1$OTU2= gsub("_", "",
```

```
paste(A1_my_otu_table_ASVs_filteredA1$OTU))
```

```
A1_my_otu_table_ASVs_filteredA1$OTU3=
```

```
paste(A1_my_otu_table_ASVs_filteredA1$OTU2,A1_my_otu_table_ASVs_filteredA1$Genus, sep = "_")
```

```
rownames(A1_my_otu_table_ASVs_filteredA1)= A1_my_otu_table_ASVs_filteredA1$OTU3
```

```
A1= A1_my_otu_table_ASVs_filteredA1[,c(1,61:69)]
```

```
A1_t= as.data.frame(t(A1))
```

```

A1_t= rownames_to_column(A1_t, var = "SampleID")
A1_t_order= A1_t[order(A1_t$SampleID),]
rownames(A1_t_order)= A1_t_order$SampleID
A1_t_order_F= A1_t_order[,-1]
#A2 data preparation
otu.table <- (otu_table(ASVc_filtered_A2, taxa_are_rows = T))
otu.table <- as.data.frame(otu.table)
A2_otu.table2= rownames_to_column(otu.table, var = "OTU")
A2_my_otu_table_ASVs_filteredA2 <- left_join(A2_otu.table2, tax.table, by = "OTU")
A2_my_otu_table_ASVs_filteredA2$OTU2= gsub("_", "",
paste(A2_my_otu_table_ASVs_filteredA2$OTU))
A2_my_otu_table_ASVs_filteredA2$OTU3=
paste(A2_my_otu_table_ASVs_filteredA2$OTU2,A2_my_otu_table_ASVs_filteredA2$Genus, sep = "_")
rownames(A2_my_otu_table_ASVs_filteredA2)= A2_my_otu_table_ASVs_filteredA2$OTU3
A2= A2_my_otu_table_ASVs_filteredA2[,-c(1,61:69)]
A2_t= as.data.frame(t(A2))
A2_t= rownames_to_column(A2_t, var = "SampleID")
A2_t_order= A2_t[order(A2_t$SampleID),]
rownames(A2_t_order)= A2_t_order$SampleID
A2_t_order_F= A2_t_order[,-1]
##G1 data preparation
otu.table <- (otu_table(significant_genes_filtered_G1, taxa_are_rows = F))
otu.table <- as.data.frame(otu.table)
G1_otu.table2= rownames_to_column(otu.table, var = "SampleID")
G1_order= G1_otu.table2[order(G1_otu.table2$SampleID),]
rownames(G1_order)= G1_order$SampleID
G1_order_C= G1_order[,-1]
##G2 data preparation
otu.table <- (otu_table(significant_genes_filtered_G2, taxa_are_rows = F))
otu.table <- as.data.frame(otu.table)
G2_otu.table2= rownames_to_column(otu.table, var = "SampleID")
G2_order= G2_otu.table2[order(G2_otu.table2$SampleID),]
rownames(G2_order)= G2_order$SampleID
G2_order_C= G2_order[,-1]

...

```{r}
library(tidyverse)

library(ComplexHeatmap)
Heatmap(yu_log)
yu_log_selected_0.5=
yu_log[,c("ASV_442","ASV_43","ASV_97","ASV_94","ASV_287","ASV_51","ASV_139","ASV_19","
ASV_45","ASV_44","ASV_85","ASV_75","ASV_122","ASV_425","ASV_40","ASV_30","ASV_169","
ASV_165","ASV_23","ASV_93","ASV_134","ASV_55","ASV_219","ASV_68","ASV_187","ASV_144"
)]
library(reshape2)
yu_log_selected_0.5_melt= melt(yu_log_selected_0.5)
yu_log_selected_0.5_melt_input1= yu_log_selected_0.5_melt %>% filter(value>=0.5)
yu_log_selected_0.5_melt_input2= yu_log_selected_0.5_melt %>% filter(value<=-0.5)
ffff= as.vector(yu_log_selected_0.5_melt_input1$Var1)
sss= as.vector(yu_log_selected_0.5_melt_input2$Var1)
DEG_cor0.5= c(ffff,sss)

```

```
DEG_cor0.5= unique(DEG_cor0.5)
```

```
yu_log_selected_0.5=
yu_log[c("IFI6","ICOSLG","SEC24D","LOC112443614","ETV6","TSPAN7","CREB3L2","DTX4","BTN
L9","C23H6orf15","EFNA2","INAFM1","LOC107132278","RELB","LOC112442408","EYA3","RN7SL1
","SLC25A28","AP3D1","TTL","ASXL1","EXOC8","INSL6","LOC101902542","HCN2","ASRGL1","C3
","LOC104969959","LOC112442805","LOC112442867","LOC112441507","TRMT44","FZD5","LOC107
133265","TRPM3"),c("ASV_19","ASV_23","ASV_30","ASV_40","ASV_43","ASV_44","ASV_45","AS
V_51","ASV_55","ASV_68","ASV_442","ASV_97","ASV_94","ASV_287","ASV_139","ASV_85","AS
V_75","ASV_122","ASV_425","ASV_169","ASV_165","ASV_93","ASV_134","ASV_219","ASV_187")
]
dd=
yu_log[DEG_cor0.5,c("ASV_19","ASV_23","ASV_30","ASV_40","ASV_43","ASV_44","ASV_45","AS
V_51","ASV_55","ASV_68","ASV_442","ASV_97","ASV_94","ASV_287","ASV_139","ASV_85","AS
V_75","ASV_122","ASV_425","ASV_169","ASV_165","ASV_93","ASV_134","ASV_219","ASV_187")
]
dd= as.matrix(dd)
colnames(dd)
colnames(dd)[1]= "g_Campylobacter(ASV_19)"
colnames(dd)[2]= "f_Ruminococcaceae(ASV_23)"
colnames(dd)[3]= "g_Prevotella_1(ASV_30)"
colnames(dd)[4]= "f_Muribaculaceae(ASV_40)"
colnames(dd)[5]= "Heparinolyticus sp.(ASV_43)"
colnames(dd)[6]= "Fairfieldensis sp.(ASV_44)"
colnames(dd)[7]= "f_Christensenellaceae(ASV_45)"
colnames(dd)[8]= "g_Butyrvibrio_2(ASV_51)"
colnames(dd)[9]= "g_Ruminococcaceae_UCG-005(ASV_55)"
colnames(dd)[10]= "g_Shuttleworthia(ASV_68)"
colnames(dd)[11]= "g_Anaerovibrio(ASV_442)"
colnames(dd)[12]= "NA(ASV_97)"
colnames(dd)[13]= "o_Bacteroidales(ASV_94)"
colnames(dd)[14]= "o_Bacteroidales(ASV_287)"
colnames(dd)[15]= "g_Butyrvibrio_2(ASV_139)"
colnames(dd)[16]= "g_Desulfovibrio(ASV_85)"
colnames(dd)[17]= "f_Lachnospiraceae(ASV_75)"
colnames(dd)[18]= "g_Lachnospiraceae_UCG-002(ASV_122)"
colnames(dd)[19]= "g_RBG-16-49-21(ASV_425)"
colnames(dd)[20]= "g_Pyramidobacter(ASV_169)"
colnames(dd)[21]= "g_U29-B03(ASV_165)"
colnames(dd)[22]= "g_Ruminococcaceae_NK4A214_group(ASV_93)"
colnames(dd)[23]= "g_Ruminococcaceae_UCG-002(ASV_134)"
colnames(dd)[24]= "g_Ruminococcaceae_UCG-009(ASV_219)"
colnames(dd)[25]= "g_Syntrophococcus(ASV_187)"

dd_t= t(dd)
dd_t= as.matrix(dd_t)
Heatmap(dd)
library(circlize)
col_fun = colorRamp2 (c(-1, 0, 1), c("green", "white", "red"))
Figure3_heatmap= Heatmap(dd_t, row_names_gp = gpar(fontsize = 8),column_names_gp =gpar(fontsize =
8),name = "Correlation", col = col_fun,cell_fun = function(j, i, x, y, width, height, fill) {
  if(dd_t[i, j] >= 0.5)dd_t[i, j] <= -0.5)
    grid.text(sprintf("%.1f", dd_t[i, j]), x, y, gp = gpar(fontsize = 7))
})
```

```

#write.table(yu_log_selected_0.5, file = "Dr_Jim_May27.2020/yu_log_selected_0.5.txt", quote = F,
col.names = T, row.names = T, sep = "\t")
dfff= yu_log_selected_0.5 %>% filter_all(all_vars(>=0.5))
ee= yu_log_selected_0.5 %>% filter_all(any_vars(>=0.5))
vvv= yu_log_selected_0.5 %>% filter(ASV_19>=0.5)
yu_log_selected_0.5= as.data.frame(yu_log_selected_0.5)
is.numeric(yu_log_selected_0.5)
###heatmap for cor >0.3 and < -0.3
yu_log_selected_0.3=
yu_log[,c("ASV_57","ASV_234","ASV_349","ASV_94","ASV_287","ASV_51","ASV_139","ASV_155",
"ASV_299","ASV_309","ASV_45","ASV_154","ASV_76","ASV_85","ASV_2","ASV_37","ASV_75","
ASV_176","ASV_365","ASV_22","ASV_242","ASV_169","ASV_425","ASV_23","ASV_134","ASV_21
9","ASV_171","ASV_307","ASV_68","ASV_64","ASV_213","ASV_165","ASV_47","ASV_178")]

library(reshape2)
yu_log_selected_0.3_melt= melt(yu_log_selected_0.3)
yu_log_selected_0.3_melt_input1= yu_log_selected_0.3_melt %>% filter(value>=0.3)
yu_log_selected_0.3_melt_input2= yu_log_selected_0.3_melt %>% filter(value<=-0.3)

ffff0.3= as.vector(yu_log_selected_0.3_melt_input1$Var1)
sssN0.3= as.vector(yu_log_selected_0.3_melt_input2$Var1)
DEG_cor0.3= c(ffff0.3,sssN0.3)
DEG_cor0.3= unique(DEG_cor0.3)
dd0.3=
yu_log[DEG_cor0.3,c("ASV_57","ASV_234","ASV_349","ASV_94","ASV_287","ASV_51","ASV_139",
"ASV_155","ASV_299","ASV_309","ASV_45","ASV_154","ASV_76","ASV_85","ASV_2","ASV_37","
ASV_75","ASV_176","ASV_365","ASV_22","ASV_242","ASV_169","ASV_425","ASV_23","ASV_134
","ASV_219","ASV_171","ASV_307","ASV_68","ASV_64","ASV_213","ASV_165","ASV_47","ASV_1
78")]

dd0.3= as.matrix(dd0.3)

library(circlize)
col_fun = colorRamp2 (c(-1, 0, 1), c("green", "white", "red"))
supp_heatmap=Heatmap(dd0.3, row_names_gp = gpar(fontsize = 5),column_names_gp =gpar(fontsize =
7),name = "Correlation", col = col_fun,cell_fun = function(j, i, x, y, width, height, fill) {
  if(dd0.3[i, j] >= 0.3|dd0.3[i, j] <= -0.3)
    grid.text(sprintf("%.1f", dd0.3[i, j]), x, y, gp = gpar(fontsize = 7))
})
###Scatter Plots
DEG_ASVs=
merge(significant_genes_filtered_table_SampleID_Order,rumenPappilae_proportions_0.5_table_SampleID
_Order)
DEG_ASVs_cor_input= DEG_ASVs[,-1]
DEG_ASVs_cor_input_log_plus1= log(1+DEG_ASVs_cor_input)
library(ggpubr)
jw1 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "C3", y = "ASV_43",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE)+ stat_cor(method = "pearson")

jw2 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "SEC24D", y = "ASV_287",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line

```

```

    conf.int = TRUE, ylab= expression(italic("o_Bacteroidales") (ASV_287))) + stat_cor(method =
"pearson")

jw3 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "CREB3L2", y = "ASV_287",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, ylab= expression(italic("o_Bacteroidales") (ASV_287))) + stat_cor(method =
"pearson")

jw4 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "ICOSLG", y = "ASV_19",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, ylab= expression(italic("g_Campylobacter") (ASV_19))) + stat_cor(method =
"pearson")

jw5 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "DTX4", y = "ASV_19",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, ylab= expression(italic("g_Campylobacter") (ASV_19))) + stat_cor(method =
"pearson")

jw6 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "TYK2", y = "ASV_94",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, ylab= expression(italic("O_Bacteroidales") (ASV_94))) + stat_cor(method =
"pearson")
#ylab= expression(italic("")) (ASV_))
# Add confidence interval
#ylab= expression(italic("f_Ruminococcaceae") (ASV_23)))+ stat_cor(method = "pearson")
sp2 <- ggscatter(DEG_ASVs_cor_input_log_plus1, x = "TRPM3", y = "ASV_30",
  add = "reg.line", # Add regressin line
  add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
  conf.int = TRUE, # Add confidence interval
  ylab= expression(italic("g_Prevotella_1") (ASV_30)))+ stat_cor(method = "pearson")
sp2
...

```

## APPENDIX C

**Supplemental Table 1. Genes differentially expressed in the rumen papillae of steers versus heifers.**

Gene <sup>1</sup>	Log2FoldChange <sup>2</sup>	P-value <sup>3</sup>	Adjusted P-value <sup>4</sup>
TDRD9	10.61613343	2.26E-123	3.17E-119
GPR143	3.580005505	7.53E-57	5.28E-53
EIF2S3	-0.856536145	2.37E-24	1.11E-20
KDM6A	-0.848221001	2.74E-16	9.61E-13
USP9X	-0.82415134	6.91E-15	1.94E-11
TXLNG	-0.92860761	5.23E-13	1.22E-09
C7H5orf15	-0.263553892	1.36E-12	2.73E-09
LOC782293	-0.775802476	2.89E-12	5.07E-09
HIF1A	-0.677416541	1.97E-11	3.07E-08
ROBO1	-0.313232203	2.71E-11	3.80E-08
SEC24A	-0.378198161	3.26E-11	4.16E-08
CDO1	-1.013676062	5.01E-11	5.86E-08
ZRSR2	-0.596452001	5.68E-11	6.13E-08
ARMC6	0.52528668	6.45E-11	6.46E-08
NBEAL1	-0.718546628	9.23E-11	8.64E-08
SECISBP2L	-0.381514946	2.82E-10	2.47E-07
BMPR2	-0.374389311	4.88E-10	4.02E-07
HMGH5	-0.700292509	5.16E-10	4.02E-07
ZFR	-0.28355509	6.85E-10	5.06E-07
FBXL15	0.520565182	7.74E-10	5.43E-07
IL1RAP	-0.483098442	1.18E-09	7.90E-07
GALNT4	-0.31263538	1.27E-09	8.11E-07
CLCA2	-0.212713964	1.36E-09	8.33E-07
GSK3B	-0.125948559	1.58E-09	8.53E-07
DNAJB14	-0.606541068	1.55E-09	8.53E-07
EXOC6	-0.296853334	1.50E-09	8.53E-07
N4BP2	-1.262221432	1.68E-09	8.75E-07
YIPF6	-0.029354719	2.05E-09	1.03E-06
LOC783421	-0.770793281	2.92E-09	1.41E-06
DDX3X	-0.519238144	3.20E-09	1.50E-06
TARBP2	0.153838147	3.46E-09	1.57E-06
LBR	-0.167072393	4.34E-09	1.90E-06
SEC62	-0.254097248	5.06E-09	2.15E-06
STXBP3	-0.310631424	6.71E-09	2.77E-06
ROMO1	0.622957935	7.48E-09	3.00E-06
ANOS1	1.059523031	9.65E-09	3.76E-06

TBC1D15	-0.135919999	1.08E-08	3.99E-06
SEN7	-0.493714361	1.08E-08	3.99E-06
DMXL1	-0.559241878	1.13E-08	4.06E-06
TOP1	-0.252735613	1.20E-08	4.16E-06
GSR	-0.555305791	1.21E-08	4.16E-06
NABP2	0.126331497	1.32E-08	4.41E-06
STK26	-0.46816126	1.40E-08	4.58E-06
C1H3orf58	-0.17776388	1.62E-08	5.18E-06
ARID2	-0.269939665	1.79E-08	5.58E-06
GALNT3	-0.562455873	2.06E-08	6.27E-06
PDS5B	-0.306034684	2.11E-08	6.30E-06
C7H19orf70	0.630149852	2.16E-08	6.30E-06
TP53INP1	-0.140019198	2.20E-08	6.30E-06
TNKS2	-0.391620453	2.48E-08	6.88E-06
MED13	-0.412549688	2.50E-08	6.88E-06
PDS5A	-0.23284936	2.74E-08	7.39E-06
IPMK	-0.672642469	2.96E-08	7.85E-06
LOC534742	-0.291137224	3.11E-08	8.09E-06
USP32	-0.205910727	3.23E-08	8.24E-06
LOC615792	-0.599974912	3.57E-08	8.66E-06
ATP5ME	0.774739434	3.55E-08	8.66E-06
LOC104972118	0.864125455	3.58E-08	8.66E-06
ABHD18	-0.596529089	3.69E-08	8.77E-06
TMTC3	-0.412032391	4.70E-08	1.10E-05
ATP6V0A2	-0.497992601	4.92E-08	1.12E-05
PCYOX1	-1.051416201	4.96E-08	1.12E-05
YTHDC2	-0.525170324	5.23E-08	1.17E-05
WWC2	-0.334952077	5.68E-08	1.21E-05
ATP5MG	0.455379215	5.69E-08	1.21E-05
KLF3	-0.46682764	5.61E-08	1.21E-05
C8H9orf3	0.436870248	5.83E-08	1.22E-05
PRKAA1	-0.261011048	6.14E-08	1.27E-05
ZBTB41	-0.405708196	6.58E-08	1.34E-05
IFNAR1	-0.420122776	7.27E-08	1.46E-05
UBL7	0.316681136	7.59E-08	1.50E-05
ACTR2	-0.235804346	7.69E-08	1.50E-05
LRBA	-0.083721199	7.82E-08	1.50E-05
DMAC2	0.282615304	8.33E-08	1.58E-05
ADSS	-0.228525458	8.59E-08	1.58E-05
ITPA	0.461103187	8.49E-08	1.58E-05
SAR1B	-0.501997695	8.67E-08	1.58E-05
TMED5	-0.747049106	9.28E-08	1.65E-05



TRAM1	-0.242017925	9.24E-08	1.65E-05
ACSL3	-0.261810912	9.40E-08	1.65E-05
FRMD6	-0.28856707	9.85E-08	1.71E-05
SNX9	-0.011858513	1.09E-07	1.75E-05
NEMF	-0.355893799	1.09E-07	1.75E-05
MAGT1	-0.089857717	1.04E-07	1.75E-05
CCPG1	-0.051996249	1.06E-07	1.75E-05
AEBP2	-0.16973672	1.08E-07	1.75E-05
SMNDC1	-0.104615905	1.04E-07	1.75E-05
BMP2K	-0.422751609	1.12E-07	1.79E-05
FUBP3	0.020803596	1.16E-07	1.83E-05
WDR13	0.07994936	1.18E-07	1.84E-05
ZBTB21	-0.208219539	1.20E-07	1.86E-05
NOSIP	0.359736352	1.26E-07	1.93E-05
PRRG4	-0.26669079	1.29E-07	1.94E-05
MEX3C	-0.379128036	1.41E-07	2.10E-05
RAB21	0.057665664	1.46E-07	2.16E-05
ARHGEF12	-0.156398843	1.51E-07	2.17E-05
COMMD5	0.231046743	1.49E-07	2.17E-05
CTR9	-0.466272851	1.51E-07	2.17E-05
TOB1	-0.284734203	1.58E-07	2.24E-05
ETNK1	-0.316243618	1.71E-07	2.35E-05
DMAC1	0.515006117	1.69E-07	2.35E-05
LRRC40	-0.298505846	1.68E-07	2.35E-05
NEK7	0.027003568	1.76E-07	2.37E-05
ARL5B	-0.296450377	1.74E-07	2.37E-05
FAM91A1	-0.431767534	1.78E-07	2.38E-05
PBRM1	-0.512576198	1.83E-07	2.42E-05
RP2	0.096369092	1.87E-07	2.45E-05
BAZ1A	-0.386509648	2.05E-07	2.66E-05
HSPA4L	-0.514466252	2.16E-07	2.76E-05
LGALS7	0.768273152	2.16E-07	2.76E-05
ZMYM1	-0.733857992	2.26E-07	2.86E-05
MARCH6	-0.125290308	2.36E-07	2.95E-05
REEP3	-0.423433661	2.37E-07	2.95E-05
SPTLC1	-0.350312603	2.45E-07	3.01E-05
NDFIP2	-0.101586063	2.64E-07	3.23E-05
BRWD3	-0.528251713	2.70E-07	3.26E-05
LACTB2	-0.345917902	2.76E-07	3.31E-05
ATP5MPL	0.543154602	2.79E-07	3.32E-05
RPS15	0.383013761	2.98E-07	3.46E-05
MED31	0.397984629	2.94E-07	3.46E-05

TMPRSS11A	-0.433807943	2.98E-07	3.46E-05
UBR3	-0.450555599	3.06E-07	3.52E-05
LEMD3	-0.408517008	3.11E-07	3.55E-05
GNPTAB	-0.553408369	3.21E-07	3.56E-05
NDUFS1	-0.145521722	3.18E-07	3.56E-05
MRPL41	0.483533956	3.21E-07	3.56E-05
COX7A1	1.212083358	3.22E-07	3.56E-05
TMEM41B	-0.260219015	3.31E-07	3.63E-05
LOC101906779	0.786511775	3.34E-07	3.63E-05
CAND1	-0.230379407	3.39E-07	3.63E-05
PAPD5	-0.196404199	3.42E-07	3.63E-05
ETNPPL	-1.301925899	3.41E-07	3.63E-05
THAP3	0.413967481	3.49E-07	3.68E-05
TM9SF2	-0.124461775	3.80E-07	3.92E-05
RNF139	-0.269184714	3.78E-07	3.92E-05
PRPF40A	-0.222164258	3.75E-07	3.92E-05
KCNG3	-0.746616407	3.86E-07	3.96E-05
EDEM1	-0.196650507	4.01E-07	4.07E-05
MAP3K2	-0.171051005	4.10E-07	4.14E-05
DCUN1D4	-0.196720647	4.49E-07	4.50E-05
FOXN2	-0.409193147	4.65E-07	4.63E-05
SOS1	-0.50260053	4.69E-07	4.64E-05
CPSF6	-0.503666154	4.76E-07	4.68E-05
ROBO2	-0.724151038	4.89E-07	4.73E-05
VCPIP1	-0.461279118	4.88E-07	4.73E-05
PIK3CA	-0.100181744	5.04E-07	4.84E-05
SSFA2	-0.185592076	5.24E-07	4.97E-05
TMEM192	-0.522567012	5.21E-07	4.97E-05
PIK3C2A	-0.448162969	5.33E-07	4.99E-05
PDIK1L	-0.337844588	5.30E-07	4.99E-05
UQCC3	0.172858705	5.52E-07	5.08E-05
SNRPE	0.364470949	5.48E-07	5.08E-05
NUFIP2	-0.177331998	5.54E-07	5.08E-05
MPP5	-0.365477748	5.76E-07	5.08E-05
XRN1	-0.523993503	5.67E-07	5.08E-05
PEX13	-0.543549597	5.60E-07	5.08E-05
ATP11C	-0.471130111	5.62E-07	5.08E-05
PRRG1	-0.156941267	5.81E-07	5.08E-05
BTBD7	-0.130652972	5.83E-07	5.08E-05
B3GNT2	-0.127607145	5.80E-07	5.08E-05
LARP4	-0.329437759	5.71E-07	5.08E-05
GOPC	-0.258598161	5.92E-07	5.13E-05

OSTM1	-0.165475881	6.05E-07	5.19E-05
ATAD2B	-0.353156472	6.06E-07	5.19E-05
SLF2	-0.556351178	6.12E-07	5.20E-05
HMBS	0.174519223	6.22E-07	5.23E-05
TMEM65	-0.339194958	6.21E-07	5.23E-05
CMTR2	-0.443116481	6.53E-07	5.45E-05
TWSG1	-0.115360286	6.65E-07	5.52E-05
SLC30A5	-0.350584886	6.73E-07	5.56E-05
SMIM15	-0.003459578	6.83E-07	5.57E-05
MTG1	0.261074119	6.83E-07	5.57E-05
STRN3	-0.292409172	6.96E-07	5.64E-05
HSDL2	-0.356755605	7.08E-07	5.71E-05
PIGK	-0.192611624	7.14E-07	5.73E-05
STYX	-0.330936485	7.19E-07	5.73E-05
NUBP2	0.354683544	7.91E-07	6.27E-05
TMEM170A	-0.19636123	8.20E-07	6.36E-05
RPL10	0.52192876	8.20E-07	6.36E-05
RPP25L	0.236998908	8.20E-07	6.36E-05
POF1B	-0.48931876	8.19E-07	6.36E-05
EPB41L5	-0.346413836	8.31E-07	6.37E-05
IGFBP6	0.887762527	8.29E-07	6.37E-05
GSPT1	-0.101322614	8.46E-07	6.45E-05
PKN2	-0.729835261	8.53E-07	6.47E-05
ELOB	0.840801881	8.72E-07	6.58E-05
SMCHD1	-0.276104912	8.95E-07	6.72E-05
RICTOR	-0.211031391	9.06E-07	6.77E-05
ZSWIM6	-0.14975758	9.13E-07	6.78E-05
POU2F1	-0.180177213	9.68E-07	7.11E-05
SPPL2A	-0.61847571	9.65E-07	7.11E-05
COL4A3BP	-0.272156852	9.93E-07	7.26E-05
HDHD5	0.489551649	1.02E-06	7.40E-05
SLC38A9	-0.839039338	1.03E-06	7.45E-05
KIF3A	-0.553378413	1.05E-06	7.54E-05
ABCD3	-0.357863188	1.06E-06	7.56E-05
LOC781692	-0.632372511	1.07E-06	7.60E-05
ZFYVE16	-0.342516504	1.07E-06	7.60E-05
LMO7	-0.504870938	1.12E-06	7.85E-05
LOC107132386	0.644634579	1.12E-06	7.85E-05
TDG	-0.291973033	1.14E-06	7.92E-05
ZC3H12C	-0.040630422	1.14E-06	7.92E-05
U2AF1L4	0.32753995	1.21E-06	8.35E-05
VPS13A	-0.57475413	1.26E-06	8.64E-05

PPP2R1B	-0.399429847	1.26E-06	8.64E-05
MAP3K21	-0.338713161	1.27E-06	8.64E-05
NFAT5	-0.349832373	1.31E-06	8.83E-05
MRPL27	0.246768672	1.31E-06	8.83E-05
PDF	0.5141952	1.34E-06	9.01E-05
ALDH6A1	-0.411459366	1.37E-06	9.19E-05
PSMD7	-0.389890568	1.44E-06	9.57E-05
DCAF1	-0.319796006	1.46E-06	9.59E-05
PPIL4	-0.27836776	1.45E-06	9.59E-05
MCCC2	-0.210557513	1.49E-06	9.78E-05
CHD1	-0.189703517	1.50E-06	9.78E-05
NMD3	-0.373905751	1.51E-06	9.80E-05
GNAI1	-0.187715029	1.52E-06	9.84E-05
CPEB4	-0.37283163	1.57E-06	0.000100851
TJAP1	0.317278064	1.59E-06	0.000101643
COPS9	0.458761583	1.62E-06	0.000102788
IREB2	-0.326118144	1.65E-06	0.000102788
RC3H1	-0.143587879	1.62E-06	0.000102788
MOB1B	-0.389758854	1.64E-06	0.000102788
UBL3	-0.217803808	1.64E-06	0.000102788
LAMTOR4	0.352020297	1.63E-06	0.000102788
ALCAM	-1.284633392	1.66E-06	0.000103332
UQCRB	0.242105753	1.68E-06	0.000103951
YIPF4	-0.097860462	1.72E-06	0.000105921
USP15	-0.310426273	1.75E-06	0.000106927
CHM	-0.366070185	1.76E-06	0.000106927
ANKRD44	-0.343226262	1.76E-06	0.000106927
FMNL2	-0.552048242	1.79E-06	0.000107827
CHCHD2	0.39282575	1.80E-06	0.000107827
RPS19	0.569762141	1.80E-06	0.000107827
BDP1	-0.406098115	1.92E-06	0.00011404
PTOV1	0.331497143	1.93E-06	0.00011404
RPL38	0.617704303	1.93E-06	0.00011404
OSBPL8	-0.427228249	1.91E-06	0.00011404
FGFR1OP2	-0.320936044	2.00E-06	0.000117319
CYFIP1	-0.300049921	2.03E-06	0.000118909
LOC785386	0.767388593	2.07E-06	0.000120762
ZXDB	-0.441489929	2.10E-06	0.000121562
BACH1	-0.31436485	2.16E-06	0.000124904
RAP2C	-0.279459485	2.22E-06	0.000127504
RPS5	0.440978016	2.25E-06	0.00012755
ADSL	0.120729011	2.25E-06	0.00012755

LOC614424	0.357630321	2.27E-06	0.00012755
UBE2G1	-0.348246922	2.25E-06	0.00012755
HCFC2	-0.527321976	2.27E-06	0.00012755
MOB1A	-0.474074157	2.26E-06	0.00012755
KPNA1	-0.261477404	2.30E-06	0.000128433
TMX1	-0.240649877	2.34E-06	0.000130349
SLC25A16	-0.638432953	2.35E-06	0.00013035
LIN7C	-0.236485076	2.39E-06	0.000131454
NPRL2	0.056717834	2.39E-06	0.000131454
PHKB	-0.162271756	2.49E-06	0.00013668
ITGAV	-0.561013942	2.51E-06	0.000137074
MAPK1IP1L	-0.163843284	2.54E-06	0.000138207
THAP12	-0.38052026	2.59E-06	0.000140279
STAG2	-0.159391544	2.64E-06	0.000142256
PSME4	-0.075891049	2.67E-06	0.000142969
CPSF2	-0.205772406	2.67E-06	0.000142969
FAM173A	0.64074238	2.71E-06	0.00014409
TRIP6	0.321976855	2.71E-06	0.00014409
SLC33A1	-0.208583279	2.74E-06	0.000145119
SLC35D1	-0.925805633	2.75E-06	0.000145262
LOC112446882	0.561084795	2.83E-06	0.000148943
PLS3	-0.3763163	2.93E-06	0.000153643
LOC100848208	0.16048787	2.95E-06	0.000154154
MCRS1	-0.049388665	3.11E-06	0.000161682
HERPUD2	-0.244866011	3.13E-06	0.000162209
COBLL1	0.008781714	3.15E-06	0.000162669
FAM49B	-0.210849233	3.17E-06	0.000162921
JAG2	0.324303048	3.26E-06	0.000167152
FNIP1	-0.296801013	3.28E-06	0.000167178
ACTR3	-0.249341375	3.29E-06	0.000167178
COX8A	0.407593981	3.49E-06	0.000177024
CHSY1	-0.173815101	3.55E-06	0.000178376
CPNE8	-0.521179346	3.54E-06	0.000178376
PCGF5	-0.120915246	3.62E-06	0.000181354
TMEM30A	-0.33672665	3.65E-06	0.00018246
S100A13	0.807613757	3.67E-06	0.00018246
ACVR2A	-0.300223962	3.71E-06	0.000182584
ATP5F1E	0.520362916	3.72E-06	0.000182584
LOC100847143	0.313939101	3.72E-06	0.000182584
FBXL17	-0.263383618	3.71E-06	0.000182584
RPUSD3	0.174169608	3.80E-06	0.000185139
TRMT10C	-0.040850923	3.79E-06	0.000185139

CPEB3	-0.383249033	3.88E-06	0.000188424
ZMAT3	-0.416673791	4.03E-06	0.000194142
ARHGAP5	-0.274784181	4.03E-06	0.000194142
GADD45GIP1	0.579430572	4.04E-06	0.000194142
C2H2orf69	-0.297091237	4.10E-06	0.000196446
ADAM17	-0.21331619	4.12E-06	0.000196446
FBXW11	-0.233565165	4.17E-06	0.000197907
RPL7	0.368419477	4.16E-06	0.000197907
OXLD1	0.290833734	4.25E-06	0.000197951
CDK3	0.399615852	4.19E-06	0.000197951
PIGX	0.117046551	4.22E-06	0.000197951
ARL6IP6	0.013857835	4.25E-06	0.000197951
GMFB	-0.324016461	4.26E-06	0.000197951
UBE2D1	-0.148436069	4.26E-06	0.000197951
NDUFA11	0.557636159	4.40E-06	0.000201866
POLR2L	0.695740263	4.41E-06	0.000201866
AHCTF1	-0.204107736	4.42E-06	0.000201866
XRCC3	0.30956555	4.41E-06	0.000201866
IFIH1	0.11022649	4.39E-06	0.000201866
DYNC1I2	-0.200206533	4.51E-06	0.000205713
RAB33B	-0.504364398	4.60E-06	0.000208957
LOC514680	0.711075976	4.69E-06	0.000212138
PPIP5K2	-0.083283786	4.75E-06	0.000214372
ZBTB18	-0.087495736	4.88E-06	0.000219512
TOPORS	-0.075404225	4.91E-06	0.00022018
EXOC5	-0.46248893	4.97E-06	0.000220906
PTPRK	-0.265565844	4.96E-06	0.000220906
RNF141	-0.458156899	4.96E-06	0.000220906
CLIP1	-0.26169417	5.00E-06	0.000221475
YES1	-0.534225941	5.05E-06	0.000223034
POLR2J	0.559701321	5.10E-06	0.000224077
ATL3	-0.173037433	5.11E-06	0.000224077
LCOR	-0.318084491	5.17E-06	0.000224544
KLHL32	-0.184442819	5.14E-06	0.000224544
SGK3	-0.194457528	5.17E-06	0.000224544
PPM1B	-0.066578298	5.20E-06	0.000225235
SEC24B	-0.218269467	5.30E-06	0.000227972
SHARPIN	0.377613434	5.29E-06	0.000227972
KDM7A	-0.789904076	5.36E-06	0.000230036
ARFGEF1	-0.299093588	5.39E-06	0.000230444
FAM20B	-0.141773359	5.53E-06	0.000235706
CISD2	-0.021078763	5.56E-06	0.00023634

RPL36AL	0.385933578	5.66E-06	0.000240144
CPEB2	-0.348781641	5.69E-06	0.000240371
DUS3L	-0.159999832	5.74E-06	0.000241931
FAM212A	0.515853454	5.76E-06	0.000242153
RRAS2	-0.397309409	5.82E-06	0.000242522
SMC6	0.002743339	5.82E-06	0.000242522
NDUFA4	0.128244298	5.79E-06	0.000242522
SHOC2	-0.214088549	5.91E-06	0.000245353
LIMS1	0.065824756	6.00E-06	0.000248105
MRPL22	0.435715183	6.03E-06	0.000248105
ZNF654	-0.456070519	6.02E-06	0.000248105
RPL34	0.49671486	6.05E-06	0.000248162
NDUFS7	0.239149451	6.08E-06	0.000248481
PNKP	0.033412304	6.09E-06	0.000248481
STARD10	0.550412477	6.16E-06	0.000250411
DSG3	-0.325148182	6.19E-06	0.000251055
SUCLA2	-0.196977117	6.25E-06	0.000252892
RNF11	-0.04084317	6.30E-06	0.000254218
ZHX1	-0.423419709	6.37E-06	0.000256139
COX7A2	0.310212	6.43E-06	0.000257881
ADGRG6	-0.114618652	6.49E-06	0.000259358
OPA1	-0.159746537	6.64E-06	0.000264573
EIF2AK2	-0.57066086	6.70E-06	0.000266134
ADAM9	-0.770117051	6.73E-06	0.000266134
RPS13	0.466319772	6.79E-06	0.000266134
RCN2	-0.325941695	6.79E-06	0.000266134
SELENOI	-0.471892149	6.77E-06	0.000266134
MRPS14	0.634619952	6.79E-06	0.000266134
JMJD1C	-0.128345007	6.82E-06	0.00026682
SEZ6	0.168503954	6.91E-06	0.000269297
ANKRD12	-0.14023011	7.07E-06	0.000273641
RANBP2	-0.194700674	7.06E-06	0.000273641
PFDN5	0.512524972	7.08E-06	0.000273641
USF3	-0.228702276	7.20E-06	0.000277593
LOC780968	0.078164017	7.32E-06	0.000280583
MAP3K7	-0.185695462	7.33E-06	0.000280583
DPH7	0.398131436	7.34E-06	0.000280583
POLK	-0.301977366	7.36E-06	0.000280871
ASH1L	-0.302053861	7.56E-06	0.000287392
ABTB1	0.517925497	7.59E-06	0.000288096
TM2D1	-0.103399735	7.77E-06	0.000294054
RAB8B	-0.476278578	7.86E-06	0.000296728

NUDT12	0.119205039	7.94E-06	0.000297219
FNDC3A	-0.220874006	7.91E-06	0.000297219
SPTSSA	-0.034623605	7.94E-06	0.000297219
FZD3	-0.508751185	8.12E-06	0.000302544
ING4	0.280573953	8.13E-06	0.000302544
STT3B	-0.244390891	8.40E-06	0.000310465
PRKAA2	-0.613869961	8.41E-06	0.000310465
ATL2	-0.585644757	8.39E-06	0.000310465
ITPR2	-0.53679383	8.44E-06	0.000310798
SCYL2	-0.221200189	8.48E-06	0.00031148
MPZL2	-0.415096641	8.74E-06	0.000317682
ZNF146	-0.127462729	8.73E-06	0.000317682
LOC614208	0.287664508	8.68E-06	0.000317682
LYSMD3	-0.08423691	8.71E-06	0.000317682
LOC112441868	0.719734797	8.86E-06	0.000321452
MED13L	-0.224376607	8.93E-06	0.000323192
TMEM131	-0.364045275	9.11E-06	0.000328811
NAA15	-0.467345956	9.18E-06	0.000330417
SLC41A3	0.373376976	9.22E-06	0.000330827
ACBD3	-0.067852005	9.36E-06	0.000335195
ECSIT	0.363488008	9.39E-06	0.000335352
SP3	-0.242932895	9.72E-06	0.000346257
DDX39B	0.169415004	9.83E-06	0.000349106
LOC100297402	0.116741902	9.96E-06	0.00035287
RPS9	0.59592724	1.00E-05	0.000354557
MORN2	0.401079668	1.02E-05	0.000359307
KLHL15	-0.491153955	1.03E-05	0.000359307
CLTC	-0.070575776	1.02E-05	0.000359307
ZBTB33	-0.338356293	1.03E-05	0.000359307
UGCG	-0.976014151	1.03E-05	0.000359307
MTDH	-0.420292136	1.03E-05	0.000359425
PPP3CA	-0.336744158	1.04E-05	0.000361383
UBA6	-0.355073953	1.07E-05	0.000368416
LCAT	0.485993729	1.06E-05	0.000368416
BOC	0.59371372	1.08E-05	0.000373164
ZNF217	-0.078467142	1.09E-05	0.000373863
FGD4	-0.607456831	1.10E-05	0.0003775
ARMC7	0.316018283	1.11E-05	0.000378892
MFSD13A	0.433471987	1.12E-05	0.0003815
CCDC157	0.167470482	1.13E-05	0.000384964
DCAF10	-0.216997276	1.14E-05	0.000386209
KTN1	-0.178379171	1.14E-05	0.00038711



PDZD8	-0.41643962	1.15E-05	0.000387313
CARNMT1	-0.30315532	1.15E-05	0.000387313
SEL1L	-0.159000144	1.18E-05	0.000393642
RAB13	0.429201504	1.17E-05	0.000393642
ROCK1	-0.289963056	1.17E-05	0.000393642
UQCRH	0.370647654	1.19E-05	0.000396514
LOC112444655	0.507869556	1.20E-05	0.000399843
U2AF2	0.201112391	1.21E-05	0.000401282
RALGPS2	-0.246574795	1.21E-05	0.000401282
EML4	-0.290532406	1.21E-05	0.000401282
RPL37A	0.197479445	1.22E-05	0.000401629
RNF128	-0.264959018	1.23E-05	0.000404822
TMEM33	-0.722171964	1.23E-05	0.000404822
SP4	-0.128420589	1.24E-05	0.000407801
ZNF574	0.168479401	1.25E-05	0.000409116
ZNF507	-0.378604762	1.27E-05	0.000410199
TK2	0.17964708	1.27E-05	0.000410199
INSIG2	-0.119021395	1.27E-05	0.000410199
RPL10A	0.151728183	1.27E-05	0.000410199
MED25	0.194708135	1.26E-05	0.000410199
RPL31	0.455074343	1.26E-05	0.000410199
LOC101907544	-0.637544756	1.27E-05	0.000410199
TTL	-0.41427747	1.28E-05	0.000410535
LOC516108	0.511140356	1.29E-05	0.000412384
H19	0.617493934	1.29E-05	0.000412384
PRR14	0.382070191	1.29E-05	0.000412384
GMPS	-0.224388312	1.30E-05	0.000412792
ABI3BP	-0.16601625	1.31E-05	0.000416711
LOC616200	1.125549286	1.32E-05	0.000417343
NDUFA1	0.293294789	1.32E-05	0.000417343
WDR75	-0.397420739	1.33E-05	0.000417904
COPB1	-0.182089609	1.33E-05	0.000418048
MYO6	-0.074947087	1.34E-05	0.000421777
SERF2	0.47435467	1.36E-05	0.000424938
ZNF800	-0.426017305	1.37E-05	0.000427736
FMO5	-0.26651834	1.37E-05	0.000428698
GRPEL2	-0.130116135	1.38E-05	0.000428957
CHD9	-0.375694774	1.38E-05	0.000428957
TMEM248	-0.251036752	1.41E-05	0.000437582
LOC101905686	0.68736522	1.43E-05	0.000440595
SMG8	-0.43285285	1.44E-05	0.000440983
LOC100294792	-0.070579273	1.43E-05	0.000440983

KLF5	0.093950764	1.44E-05	0.000440983
OST4	0.25490513	1.43E-05	0.000440983
LIG4	-0.406513076	1.44E-05	0.000440991
RPS21	0.528375353	1.45E-05	0.000441495
GMCL1	-0.205544341	1.45E-05	0.000441495
ABHD17B	-0.213056932	1.46E-05	0.000444649
PIGA	-0.912247976	1.51E-05	0.000456971
COQ7	-0.069929276	1.52E-05	0.000459434
TSNAX	-0.141619532	1.58E-05	0.000477448
DXO	0.290222961	1.59E-05	0.000480011
JAK1	-0.301992102	1.62E-05	0.000486863
CHML	-0.273199062	1.63E-05	0.000488788
THOC2	-0.51441976	1.64E-05	0.000491478
EFR3A	-0.309068003	1.66E-05	0.000495746
TRAPPC10	-0.171485385	1.67E-05	0.000495746
SYAP1	-0.280934689	1.66E-05	0.000495746
PGRMC2	-0.308035982	1.71E-05	0.000508078
SCO2	0.5472801	1.73E-05	0.000511411
MDP1	0.126677316	1.78E-05	0.000526397
MAP3K20	-0.048516572	1.79E-05	0.000527324
LOC112445029	-0.02201991	1.81E-05	0.000530379
LOC104970908	0.714482005	1.80E-05	0.000530379
GLI4	0.265380847	1.83E-05	0.000534898
SLC25A36	-0.356823072	1.84E-05	0.000536744
LOC514189	1.551425992	1.86E-05	0.000541764
LAMTOR2	0.464748887	1.87E-05	0.000544196
LOC101902106	0.635550122	1.87E-05	0.000544196
CHCHD10	0.557665732	1.88E-05	0.000544757
KIAA2026	-0.255848514	1.90E-05	0.000547548
PIGM	-0.273717049	1.90E-05	0.000547548
RIC1	-0.275556098	1.90E-05	0.000548765
LPAR6	-0.049222171	1.92E-05	0.000552086
TMED10	0.000494403	1.93E-05	0.000553469
OTUD4	-0.265324812	1.96E-05	0.00056013
PIK3CB	-0.137321748	1.98E-05	0.000565122
NDUFA7	0.535638509	2.00E-05	0.000568804
UQCQRQ	0.616954977	2.00E-05	0.000568804
LRP6	-0.396103169	2.00E-05	0.000568804
SLC52A2	0.36618703	2.01E-05	0.000570894
HELB	-0.499187018	2.03E-05	0.000574708
SOCS4	-0.413020712	2.06E-05	0.000582474
TSPYL1	-0.207787706	2.08E-05	0.000585977

USP38	-0.296653866	2.10E-05	0.000591753
LMBRD2	-0.057846345	2.12E-05	0.000592779
LOC789018	0.722948559	2.11E-05	0.000592779
USP12	-0.212509064	2.13E-05	0.000593807
RPS29	0.608507812	2.13E-05	0.000593807
CDH1	-0.766477173	2.14E-05	0.000595725
EEA1	-0.594236263	2.15E-05	0.000598301
RAB27A	-0.348304323	2.18E-05	0.000604183
ARID4A	-0.418965001	2.20E-05	0.000607947
ITFG1	-0.345361965	2.21E-05	0.000610705
TAOK3	-0.215492289	2.23E-05	0.000612852
FMC1	0.54505502	2.22E-05	0.000612852
SPOPL	-0.374888756	2.24E-05	0.000613981
ACAD11	-0.869823807	2.24E-05	0.000613981
CAND2	0.948420494	2.24E-05	0.000613981
RPS28	0.491082758	2.25E-05	0.000615002
EXOSC7	-0.047868098	2.26E-05	0.000615256
MCRIP2	0.86765617	2.29E-05	0.000622228
DENND4A	-0.304852634	2.30E-05	0.000624438
RAD21	-0.062597843	2.31E-05	0.000625086
MIER3	-0.237730441	2.31E-05	0.000625086
TRMT112	0.559556524	2.32E-05	0.000626248
LOC101903478	0.282985764	2.33E-05	0.000626578
RPL28	0.528680103	2.35E-05	0.000631028
KIF21A	-0.298272108	2.36E-05	0.00063392
FAR1	-0.404166407	2.37E-05	0.00063392
DR1	-0.103294076	2.39E-05	0.000639028
PRMT7	-0.002451777	2.40E-05	0.000641265
ZBTB48	0.461502325	2.43E-05	0.000647626
TOMM40L	0.066139186	2.44E-05	0.000647626
GCC2	-0.614203246	2.44E-05	0.000647626
YAP1	0.00504603	2.48E-05	0.000655653
ATF5	0.533383223	2.48E-05	0.000655653
ATP6V0A1	-0.435416764	2.49E-05	0.000656639
FRK	-0.39059093	2.50E-05	0.000657443
USP8	-0.302020384	2.50E-05	0.000657443
LOC784488	-0.180447762	2.52E-05	0.000659845
FAM8A1	-0.776535898	2.54E-05	0.0006618
LGALS7B	0.522365028	2.53E-05	0.0006618
ATP2A2	-0.517344681	2.53E-05	0.0006618
PPP4C	0.227421518	2.56E-05	0.00066677
DHX36	-0.2265998	2.58E-05	0.000670944

TNKS	-0.206917572	2.60E-05	0.000671209
DCUN1D1	-0.250103705	2.60E-05	0.000671209
ABI1	0.063187419	2.60E-05	0.000671209
CASC4	-0.131059132	2.59E-05	0.000671209
KRAS	0.015933593	2.66E-05	0.000685823
LOC112442597	0.526687834	2.68E-05	0.000688978
G2E3	-0.523282793	2.69E-05	0.000688978
MIEN1	0.475681231	2.71E-05	0.000690031
LOC101904468	0.769550128	2.71E-05	0.000690031
GALNT7	-0.357697061	2.70E-05	0.000690031
CRELD1	0.152058616	2.70E-05	0.000690031
LAMP2	-0.203230476	2.73E-05	0.00069336
ATP5IF1	0.427508614	2.73E-05	0.00069336
GTF2H5	0.345357199	2.81E-05	0.000710851
CNEP1R1	-0.124810688	2.83E-05	0.000712957
STAU2	-0.271057903	2.83E-05	0.000712957
LOC112447652	-1.822132863	2.83E-05	0.000712957
DCP1A	-0.337552206	2.84E-05	0.000712957
FBXO3	-0.272173533	2.84E-05	0.000712957
FAM3C	0.232158668	2.86E-05	0.000715499
SNHG4	0.636801281	2.86E-05	0.000715499
CHD4	-0.279095732	2.87E-05	0.000715729
GABPA	-0.261703316	2.87E-05	0.000715729
ARFGEF3	-0.544485961	2.89E-05	0.000718483
RABAC1	0.189345366	2.89E-05	0.000718483
RAPGEF6	-0.514106218	2.93E-05	0.000726556
STK17B	-0.192764877	2.98E-05	0.000736929
ADPRH	-0.95133304	3.00E-05	0.000737014
YTHDF3	-0.137274507	2.98E-05	0.000737014
NUS1	-0.088413441	2.99E-05	0.000737014
NAMPT	-0.25743346	2.99E-05	0.000737014
BROX	-0.307897135	3.00E-05	0.000737244
C25H7orf26	0.202038733	3.02E-05	0.000739774
STK4	-0.620535415	3.03E-05	0.000740414
UBA52	0.512717993	3.03E-05	0.000740414
FUNDC2	0.182095465	3.11E-05	0.000757958
EIF3K	0.460951712	3.13E-05	0.000760085
DCAF13	-0.11796825	3.13E-05	0.000760085
TOP2B	-0.042886068	3.14E-05	0.000761015
PCGF1	0.052509125	3.25E-05	0.000786903
HSPB1	0.351637301	3.27E-05	0.00078986
NFE2L2	-0.045443366	3.29E-05	0.000793366

XPO1	-0.195426044	3.30E-05	0.000793987
PYCR3	0.317084757	3.32E-05	0.000798036
NDUFB1	0.623320742	3.33E-05	0.000798036
TSEN54	0.189569648	3.33E-05	0.000798036
LOC574091	-0.21761794	3.35E-05	0.000800472
NEDD8	0.466118475	3.39E-05	0.000809402
TMEM238	0.625372301	3.42E-05	0.000813831
DYNLRB1	0.204984841	3.43E-05	0.000817059
DTX4	-0.171172599	3.45E-05	0.000820387
MGC148714	0.369045756	3.52E-05	0.000834813
DTX3	0.51322584	3.60E-05	0.000851879
FNDC3B	-0.313524076	3.64E-05	0.000860202
APIAR	-0.460376899	3.65E-05	0.000861017
ZBTB11	-0.344090434	3.66E-05	0.000861017
ZNF692	0.115831947	3.66E-05	0.000861017
CEP350	-0.264278116	3.68E-05	0.000864859
FAM160B1	-0.936542223	3.71E-05	0.000868383
GGNBP2	-0.203551341	3.76E-05	0.00087844
SMPD4	0.239921889	3.80E-05	0.000887276
NDUFA6	0.251898992	3.81E-05	0.000887276
UFD1	0.278767841	3.82E-05	0.000888272
ZNF609	-0.317164376	3.89E-05	0.000901207
MRPL21	0.393365564	3.88E-05	0.000901207
OXR1	-0.616631742	3.89E-05	0.000901207
GLMP	0.402363435	3.90E-05	0.000902331
APPBP2	-0.142629339	3.93E-05	0.000908323
CBFB	-0.326632444	3.96E-05	0.000911956
WASHC4	-0.483821356	3.98E-05	0.000914821
ALKBH4	0.357438669	4.01E-05	0.000920963
PDCL	-0.088938145	4.03E-05	0.000920963
COX6B1	0.462384265	4.03E-05	0.000920963
LOC112442866	0.422596559	4.02E-05	0.000920963
FAM102B	-0.225178082	4.04E-05	0.000921073
ATP5F1D	0.506382079	4.05E-05	0.00092338
MMS19	0.018046885	4.07E-05	0.00092338
SEC23IP	-0.250083556	4.06E-05	0.00092338
FXVD3	0.453121757	4.13E-05	0.000937169
ABHD13	-0.55463906	4.18E-05	0.000946395
ALG12	0.335138231	4.21E-05	0.000951331
ATE1	-0.11388952	4.25E-05	0.000957186
RSRC1	-0.005909329	4.25E-05	0.000957186
TMEM19	-0.327073184	4.27E-05	0.000959585

CEP70	-0.607566369	4.29E-05	0.000963839
PDP2	-0.048652581	4.33E-05	0.000969319
NECTIN1	-0.67458813	4.34E-05	0.000969319
LOC112442865	0.485613075	4.34E-05	0.000969319
CWF19L2	-0.165191786	4.35E-05	0.000970061
RPS11	0.419641796	4.35E-05	0.000970061
NDUFB8	0.36149718	4.37E-05	0.000971299
NUCKS1	-0.095424799	4.43E-05	0.000982753
RPL18A	0.499308053	4.45E-05	0.000983546
SHPRH	-0.396692659	4.45E-05	0.000983546
TRIM33	-0.561558769	4.44E-05	0.000983546
CENPV	0.242887702	4.46E-05	0.0009842
NDUFAF3	0.480843193	4.50E-05	0.000989138
FMR1	-0.517001706	4.50E-05	0.000989138
RSPRY1	-0.237533922	4.49E-05	0.000989138
TFRC	-0.494230192	4.52E-05	0.0009906
EXOSC5	0.552867247	4.53E-05	0.000991796
LOC101902907	0.520529369	4.55E-05	0.000994434
NRAS	-0.184977622	4.65E-05	0.001013366
IGF2	0.646953458	4.65E-05	0.001013366
NAB1	-0.046034488	4.66E-05	0.001013366
CCDC186	0.352792756	4.68E-05	0.001015874
MED23	-0.431355941	4.68E-05	0.001015874
PARPBP	-0.083696259	4.70E-05	0.001017618
SLC44A1	-0.361613659	4.72E-05	0.00102017
PPCDC	0.17797545	4.79E-05	0.001031296
ATF1	0.078277006	4.79E-05	0.001031296
LOC112444539	-1.69668103	4.78E-05	0.001031296
PPP6R3	-0.131337602	4.83E-05	0.001036637
LOC786614	0.579667547	4.83E-05	0.001036637
RPS23	0.360651233	4.89E-05	0.001046751
HOMER1	-1.173846437	4.98E-05	0.001066366
FBXL6	0.488240972	5.04E-05	0.001072463
SKIL	-0.341833035	5.04E-05	0.001072463
PSPH	0.395166751	5.03E-05	0.001072463
SLC38A2	-0.264155901	5.04E-05	0.001072463
ZNF664	-0.165741433	5.09E-05	0.00108062
TMEM38B	-0.358126158	5.18E-05	0.001097162
NCK1	0.077512788	5.21E-05	0.001097353
TBK1	-0.492370906	5.20E-05	0.001097353
SRP54	-0.168840576	5.18E-05	0.001097353
NTMT1	0.098425545	5.21E-05	0.001097353

ZRANB2	-0.233010603	5.25E-05	0.001104357
RPS25	0.511077379	5.34E-05	0.001121335
TMEM147	0.259777223	5.38E-05	0.001126245
SLC25A46	-0.094505561	5.41E-05	0.001126245
RPL11	0.206928585	5.39E-05	0.001126245
MFSD3	0.370536293	5.42E-05	0.001126245
CAB39	-0.160360456	5.43E-05	0.001126245
TOMM7	0.359467927	5.43E-05	0.001126245
ATF4	0.463547078	5.41E-05	0.001126245
AP5S1	0.23262735	5.41E-05	0.001126245
CCAR1	-0.134520946	5.43E-05	0.001126245
AGAP3	0.210325131	5.53E-05	0.001144737
TMA7	0.673980127	5.54E-05	0.001145547
LOC615271	-0.047217081	5.56E-05	0.001148372
MIF	0.776043244	5.57E-05	0.001148904
FAM117B	-0.265143917	5.59E-05	0.00114938
SLC39A10	-0.642993351	5.61E-05	0.001151928
LOC107132228	0.766666405	5.61E-05	0.001151928
CXADR	-0.638188157	5.64E-05	0.001153053
IL6ST	-0.184620876	5.64E-05	0.001153053
SMAD5	-0.31317736	5.64E-05	0.001153053
CD164	-0.271148738	5.72E-05	0.001159819
ADIRF	1.445023851	5.70E-05	0.001159819
EHMT2	0.10114155	5.70E-05	0.001159819
LOC782987	0.721054439	5.72E-05	0.001159819
ILVBL	-0.181904464	5.72E-05	0.001159819
CRIP2	0.335137631	5.75E-05	0.001162877
QTRT1	0.168210832	5.75E-05	0.001162877
EREG	-0.963460021	5.81E-05	0.001172627
COMMD1	0.489346487	5.82E-05	0.001172627
RNASEL	-0.645414454	5.85E-05	0.001175036
LOC107132767	0.751444165	5.84E-05	0.001175036
LGR4	-0.258549726	5.85E-05	0.001175036
SAMD8	-0.325694186	5.98E-05	0.001197144
XRN2	-0.209723305	5.99E-05	0.001197144
IBTK	0.007957334	5.99E-05	0.001197144
NACA	0.37240975	6.00E-05	0.001197144
PRPF38A	-0.222661567	6.03E-05	0.001200724
PRNP	-0.519552898	6.04E-05	0.001200724
CFAP298	0.076966916	6.04E-05	0.001200724
SMIM15	0.040434631	6.05E-05	0.001201563
LOC784054	0.901820133	6.07E-05	0.001202503

EAF1	-0.43242134	6.07E-05	0.001202503
PPP1R14A	0.920560144	6.10E-05	0.001205558
POLD4	0.354193403	6.12E-05	0.001208463
CCDC84	0.524674804	6.23E-05	0.001228212
SNU13	0.210853741	6.26E-05	0.001231819
CASP8	-0.193610427	6.32E-05	0.001239742
TMEM11	0.334523235	6.31E-05	0.001239742
LOC112446645	0.804828985	6.33E-05	0.001240312
NDUFA2	0.594894295	6.41E-05	0.001255093
DMXL2	-0.252465865	6.53E-05	0.00127564
NDUFA8	0.285959006	6.62E-05	0.001290946
DDX46	-0.270488202	6.62E-05	0.001290946
SEC24D	-0.368905707	6.67E-05	0.00129564
CPNE5	1.881922045	6.67E-05	0.00129564
EME2	0.061377938	6.67E-05	0.00129564
LIN37	0.195734722	6.69E-05	0.001296837
SERINC1	-0.109048575	6.75E-05	0.00130505
CANX	-0.009685024	6.75E-05	0.00130505
ZNF644	-0.327197952	6.77E-05	0.001305551
TMEM123	-0.585658893	6.78E-05	0.001305551
CRIM1	-0.035689456	6.79E-05	0.001305551
GOLIM4	-0.524667402	6.79E-05	0.001305551
LOC101904595	0.569494259	6.88E-05	0.001313498
EXOSC3	0.286177044	6.85E-05	0.001313498
MBNL2	-0.639523751	6.86E-05	0.001313498
RFT1	-0.408275571	6.90E-05	0.001313498
PIK3C3	-0.349934892	6.90E-05	0.001313498
TMEM179B	0.121699814	6.89E-05	0.001313498
POLR2G	0.221322422	6.87E-05	0.001313498
UXT	0.592947108	6.98E-05	0.00132773
CCDC126	-0.249883845	7.06E-05	0.001340932
PRDX2	0.462029717	7.08E-05	0.001342131
LOC112444206	0.450670271	7.10E-05	0.001344594
MUS81	0.026993376	7.17E-05	0.001355813
MSL2	-0.200693677	7.19E-05	0.001358864
AQR	-0.23029738	7.25E-05	0.001366841
PSEN1	-0.708227914	7.27E-05	0.001368198
RNFT1	-0.362916398	7.27E-05	0.001368198
IGF2R	-0.018728971	7.28E-05	0.001368716
LOC514011	0.570242964	7.36E-05	0.00138067
GOLGA4	-0.014309034	7.37E-05	0.00138067
MTCH1	0.315765065	7.38E-05	0.001381395



SCAND1	0.566345719	7.39E-05	0.001381924
ASPSCR1	0.372948213	7.41E-05	0.001382573
NAA25	-0.589212514	7.42E-05	0.001382915
KLHL7	-0.553080198	7.49E-05	0.001391526
HNRNPH2	0.048531532	7.49E-05	0.001391526
TMEM267	-0.476904784	7.53E-05	0.001397164
DSC3	0.000957916	7.55E-05	0.001397279
CKM	0.204695317	7.56E-05	0.001397279
USP33	-0.267034708	7.54E-05	0.001397279
COPB2	-0.093314731	7.58E-05	0.001399435
UHRF1BP1L	-0.203917155	7.60E-05	0.001401743
ATP7A	-0.160207774	7.62E-05	0.001402973
EIF3H	0.469422821	7.63E-05	0.001403519
CUL5	-0.417423563	7.76E-05	0.001425701
ARFIP1	-0.200478267	7.79E-05	0.001428786
SEC63	-0.257781876	7.81E-05	0.00143172
MINPP1	-1.279057297	7.84E-05	0.00143388
RPL37	0.462834373	7.85E-05	0.001434218
AIP	0.038596814	7.88E-05	0.001436451
LOC100847363	0.266500419	7.87E-05	0.001436451
SCAI	-0.424690764	7.89E-05	0.001437017
TM4SF5	0.678990617	7.92E-05	0.001440022
MAP1LC3A	0.386938275	7.98E-05	0.001447472
NUDT14	-0.107026949	7.98E-05	0.001447472
JTB	0.187905967	8.00E-05	0.001447758
VPS50	-0.346695578	8.00E-05	0.001447758
RPL39	0.464637571	8.04E-05	0.001448059
TANGO2	0.715946712	8.03E-05	0.001448059
PCID2	0.340613268	8.06E-05	0.001448059
SDR39U1	0.144821239	8.05E-05	0.001448059
PSMB10	0.602058112	8.03E-05	0.001448059
SSNA1	0.43091873	8.09E-05	0.001449708
AMFR	-0.052184308	8.08E-05	0.001449708
MEF2A	-0.274590791	8.12E-05	0.001452347
SASH1	-0.203793205	8.12E-05	0.001452347
SMUG1	0.1047255	8.13E-05	0.001452347
RPL30	0.127850894	8.18E-05	0.001458592
PSAP	-0.330396596	8.19E-05	0.001459016
LOC100139115	0.441412713	8.21E-05	0.001459773
ATP6V1A	-0.320210543	8.25E-05	0.001465248
SLIRP	0.52936442	8.26E-05	0.001465248
MED29	0.82653116	8.27E-05	0.001465882

LOC783541	0.732454342	8.29E-05	0.0014676
CNOT6	-0.362346771	8.33E-05	0.001468574
FBXO30	-0.462106411	8.31E-05	0.001468574
DALRD3	0.295556363	8.32E-05	0.001468574
WAPL	-0.279435392	8.36E-05	0.001471368
BABAM1	0.09315903	8.42E-05	0.001481214
SEC61B	0.522435027	8.46E-05	0.001486617
LOC104975663	0.789020639	8.48E-05	0.001487989
MTM1	-0.360667894	8.53E-05	0.001494415
LOC100847759	0.517557556	8.54E-05	0.001494803
MUTYH	0.393910118	8.56E-05	0.001496569
FAM120A	-0.062263031	8.59E-05	0.001500205
PAK2	-0.24323204	8.62E-05	0.001503679
TADA3	0.174825076	8.64E-05	0.001504082
COX7B	0.439632691	8.69E-05	0.001507383
MRPS33	0.47327791	8.69E-05	0.001507383
GNAQ	-0.574685761	8.68E-05	0.001507383
HERC5	-0.784217829	8.74E-05	0.001514195
DDX41	0.092025966	8.83E-05	0.001524836
PHACTR2	-0.43102806	8.81E-05	0.001524836
RCE1	0.376461051	8.84E-05	0.001524836
RNF6	-0.180478899	8.84E-05	0.001524836
YY1	0.118232406	8.90E-05	0.001533241
TMEM87A	-0.322129577	8.92E-05	0.001533896
RPS16	0.430219202	8.95E-05	0.001537731
MZT1	-0.205559154	8.96E-05	0.001537812
SNF8	0.39511666	9.00E-05	0.001542493
CDS1	-0.199589713	9.02E-05	0.00154324
COG3	-0.395434089	9.04E-05	0.001545503
GLYCTK	0.176799875	9.10E-05	0.001554086
COIL	-0.171833387	9.14E-05	0.001558558
ZNF148	-0.169296309	9.20E-05	0.001567223
RAD52	0.858077238	9.25E-05	0.001574168
THNSL1	-0.312189553	9.28E-05	0.001576066
SON	-0.428062804	9.32E-05	0.001581636
PMS1	-0.282595577	9.36E-05	0.001587207
NDUFAF8	0.596540106	9.44E-05	0.001597453
ZCCHC9	0.205315103	9.50E-05	0.001605871
LOC510454	-0.548115739	9.52E-05	0.001605871
HACD2	-0.356496953	9.53E-05	0.001605871
ITCH	-0.216828984	9.53E-05	0.001605871
BMPRI1A	-0.242490008	9.55E-05	0.001607033

PIN4	0.437803793	9.59E-05	0.001612326
WASL	-0.203047597	9.67E-05	0.001622804
POLL	0.015266842	9.73E-05	0.00162521
ANKRD50	0.450542103	9.72E-05	0.00162521
BTAF1	-0.177815602	9.70E-05	0.00162521
CAMK2D	-0.466377901	9.74E-05	0.00162521
FNDC10	0.627319748	9.71E-05	0.00162521
CD2AP	-0.313585361	9.77E-05	0.00162936
TRMT44	-0.012305076	9.80E-05	0.001631495
TMEM127	-0.30834742	9.82E-05	0.001633473
DIAPH2	-0.278335416	9.87E-05	0.001638909
PFDN6	0.605608401	9.89E-05	0.001641008
PCDHGC3	-0.472810542	9.96E-05	0.001651071
RMND5A	0.08185441	0.000100791	0.00166622
RPS27L	0.360490764	0.000100792	0.00166622
SGSM2	0.48698904	0.000101504	0.001673259
ARL4A	0.030130035	0.000101576	0.001673259
ATRX	-0.250626467	0.000101372	0.001673259
NIPBL	-0.197055284	0.000101834	0.001675543
RNF7	0.222348234	0.000102986	0.001690536
DDHD1	-0.560247888	0.000102871	0.001690536
LIN54	-0.559167935	0.000103483	0.001696705
TEPSIN	0.27474605	0.000104098	0.001704129
PSMD12	-0.215383871	0.000104178	0.001704129
BAP1	0.171026583	0.000104551	0.001708241
DMPK	0.035918917	0.000104969	0.001713072
LIFR	-0.377983549	0.000105344	0.001717188
ATP5MF	0.46463644	0.000105495	0.001717659
UBL5	0.528586068	0.000106185	0.001723896
LOC100300938	0.133118169	0.000106247	0.001723896
SMS	-0.278869412	0.000106023	0.001723896
TEN1	0.03214601	0.000107523	0.001740972
CEP97	-0.398764245	0.000107642	0.001740972
AP3M1	-0.203236814	0.000107671	0.001740972
TMED7	-0.028381344	0.000107802	0.001741076
ASCC3	-0.310171824	0.000108606	0.001752058
SPRED1	-0.430758501	0.000108758	0.001752492
WASHC5	-0.129069126	0.000109308	0.001759339
MTX1	0.461545359	0.000109544	0.00176031
RAPGEFL1	0.384595427	0.00010962	0.00176031
CRIP1	1.559095826	0.000110072	0.001765561
HAX1	0.293824871	0.000110677	0.001773226

C3H1orf123	0.019633566	0.000111871	0.001790313
SBNO1	-0.461723763	0.000112036	0.001790914
LDB1	0.243213887	0.000112949	0.00180345
SEPSECS	-0.293341287	0.000113149	0.001804599
EXOC1	-0.443697697	0.000113885	0.001814279
SERINC5	-0.293961535	0.000114026	0.001814454
CYBC1	0.130167052	0.000114219	0.001815468
SUPT16H	-0.479224917	0.000115801	0.001838532
NCAPH2	0.402025044	0.000116059	0.001840551
WDR91	0.02957564	0.000116814	0.001850441
EIF3A	-0.274398158	0.000117547	0.00185994
RPL17	0.485080928	0.000117711	0.001860277
NRADD	0.317388802	0.000117833	0.001860277
BCAP29	-0.326169997	0.000118906	0.001875113
EIF5B	-0.282762121	0.000119305	0.001879285
CDPF1	0.187745545	0.000119781	0.001884677
ERLEC1	-0.170317604	0.000121717	0.001912983
RPS18	0.579995993	0.000122304	0.001920065
FASTK	0.471692401	0.000122459	0.001920348
LOC107132196	0.46502416	0.000124958	0.001957344
SMDT1	0.244786809	0.000125651	0.001966012
CHMP2B	-0.11133918	0.000126264	0.001973398
KLF13	-0.738349987	0.000126763	0.001979
POGLUT1	-0.534910455	0.00012803	0.001996565
FNTB	0.316956582	0.000128274	0.001996566
NUP153	-0.368511634	0.000128315	0.001996566
CALCRL	-0.190820668	0.000128733	0.001998636
RPL21	0.337372766	0.000128644	0.001998636
AJUBA	0.226506517	0.000129118	0.002002407
S100A10	0.278895477	0.000129265	0.002002461
PCM1	-0.267984633	0.000129735	0.002007533
MYO18B	-0.39808808	0.000129924	0.002008219
NSA2	0.454253747	0.000130066	0.002008219
LOC614522	0.004654069	0.000130477	0.002010451
UBE3A	-0.182681582	0.000130497	0.002010451
SH2D5	-0.458194462	0.000130697	0.002011331
LOC101906230	-0.405335495	0.000130841	0.002011333
ZNF326	-0.344379677	0.000132523	0.002034971
SERINC3	-0.167314701	0.00013324	0.002043743
MPP7	-0.54773453	0.000133618	0.0020473
C20H5orf51	-0.192219781	0.000134042	0.002051552
TRIR	0.460517687	0.000135366	0.002064368

KATNBL1	-0.397299613	0.000135035	0.002064368
NDUFB11	0.333617872	0.000135467	0.002064368
PCNX1	-0.629423611	0.000135334	0.002064368
MRPS16	0.496696782	0.000136432	0.002072119
SNHG12	0.259453323	0.000136467	0.002072119
NCOA1	-0.023606303	0.000136566	0.002072119
SMIM14	-0.15366927	0.000136566	0.002072119
SRA1	0.305347344	0.000137171	0.002077415
RPL18	0.475107231	0.000137246	0.002077415
RPL22	0.448336837	0.00013736	0.002077415
ATP2B1	-0.715137426	0.000137781	0.002081235
CHUK	-0.235383943	0.000137909	0.002081235
JARID2	-0.606767123	0.000138186	0.002081592
SIGLEC15	-0.923075348	0.000138229	0.002081592
CD47	-0.214415393	0.000138866	0.002088944
PAFAH1B1	-0.047456116	0.000139614	0.002092615
TAX1BP1	-0.455391078	0.000139706	0.002092615
NUP43	-0.166558936	0.00013926	0.002092615
ODF2L	-0.45949247	0.000139544	0.002092615
CENPX	0.566536779	0.000140305	0.002097111
FZD5	0.474280917	0.000140258	0.002097111
WRAP73	0.041585224	0.0001409	0.00210376
SMIM37	0.531415437	0.000141583	0.002111709
SLC25A38	0.314002042	0.000142107	0.002117278
LOC101906024	0.08383832	0.000142992	0.002128206
TMEM160	0.624725335	0.000143168	0.002128569
ARHGEF1	0.183924662	0.000143329	0.002128704
FAM241B	0.480709183	0.000143969	0.002131446
VWA2	-0.67561732	0.000143837	0.002131446
LOC100848315	0.202530873	0.000143966	0.002131446
ASPH	-0.345415463	0.000144156	0.002131962
CLINT1	-0.466517671	0.000145752	0.0021533
MRI1	0.188760317	0.000145916	0.002153454
LIN7B	0.29201141	0.000146577	0.002160935
LOC112442863	0.985722737	0.000147126	0.00216675
NNT	-0.189449402	0.000148193	0.002174575
LOC112443614	-1.916924929	0.000149207	0.002174575
C5H12orf10	0.528718766	0.000148274	0.002174575
CSNK1G3	-0.213165875	0.00014887	0.002174575
INAFM1	0.50825427	0.000149053	0.002174575
ELF1	-0.151901185	0.000148233	0.002174575
TSNARE1	0.267121511	0.000148554	0.002174575

LOC112443840	0.448260008	0.000148701	0.002174575
PMM1	0.158632695	0.000149076	0.002174575
SGK1	0.00124251	0.000148919	0.002174575
GRSF1	-0.302264751	0.000149676	0.002179157
TIAF1	1.143309907	0.000150649	0.002191052
FAM213B	-0.144687419	0.000150826	0.002191348
ZNF345	-0.188426982	0.000152592	0.002212427
COA6	0.560377039	0.000152472	0.002212427
CPD	-0.285211046	0.00015277	0.002212726
CNOT6L	0.267098959	0.000153018	0.002214023
MINOS1	0.565492064	0.000153354	0.002216599
RB1	0.133653525	0.000153607	0.002217978
DHX15	-0.250399662	0.000153804	0.002218536
RPL9	0.215232082	0.000154271	0.002222994
TELO2	0.038332786	0.000155175	0.002233728
DLG1	0.044540936	0.000155703	0.002239035
ITFG2	0.229369386	0.000155976	0.002240653
RPS7	0.441150345	0.000156467	0.002245409
OSBPL11	-0.491796658	0.000157087	0.002249706
GCNT2	-0.972506744	0.000157058	0.002249706
AURKAIP1	0.384127495	0.000157439	0.002252458
COX7C	0.457251473	0.00015775	0.00225461
PTGFRN	-0.132071955	0.000158819	0.00226757
RAB3GAP1	-0.094976104	0.000159504	0.002273687
POLR2B	-0.218591177	0.000159571	0.002273687
NIN	-0.66507762	0.000160419	0.002283452
ISY1	0.199592512	0.000161281	0.002293392
CNN3	-0.011853309	0.000161731	0.002297467
ERMP1	-0.091059984	0.000162516	0.00230628
SYPL1	-0.551078017	0.000164946	0.002338394
KPNA6	-0.187963593	0.000165448	0.002343145
NDUFA3	0.670666701	0.000167156	0.002364947
SLC35E3	0.104832395	0.000167815	0.002367301
LOC783797	0.399938566	0.000167828	0.002367301
CEP55	-0.136570899	0.000167685	0.002367301
TRIM59	-0.071533898	0.000168893	0.002379937
COQ4	0.458248777	0.000169266	0.002382794
TRPM7	-0.470681908	0.000169597	0.002383945
OAT	-0.657418737	0.000169687	0.002383945
NUDT9	-0.181803009	0.000171207	0.002402884
CDK10	0.203078459	0.000172239	0.00241397
STX10	0.391863991	0.000172453	0.00241397

SLC35F5	-0.384236368	0.000172512	0.00241397
NUDT5	0.220876617	0.000172926	0.002417352
HACD3	-0.337997144	0.000174195	0.002432664
CD151	0.402119151	0.000174457	0.002433897
SWSAP1	0.528917528	0.000174909	0.002437784
ATP11B	-0.389454268	0.000175449	0.002442886
LOC112442347	0.185969647	0.000175947	0.002447391
SNRPC	0.285388833	0.000176265	0.002449387
KREMEN2	-0.191807544	0.000177039	0.002457714
PPM1D	-0.289279364	0.000177634	0.002461863
NDUFB7	0.622266049	0.000177689	0.002461863
NEIL1	0.396071146	0.000178105	0.002463335
CDC27	-0.667606679	0.000178146	0.002463335
PIH1D1	0.259981223	0.000178858	0.002470746
IARS2	-0.29315757	0.000179694	0.002477418
LAD1	0.339438384	0.000179611	0.002477418
POP4	-0.049077612	0.000180218	0.002482194
MMACHC	0.101880955	0.000180996	0.002490468
DAAM1	-0.436187788	0.000181575	0.002495988
HINT3	-0.211882819	0.000183999	0.002516987
DENND6B	0.46826282	0.000183957	0.002516987
ORMDL1	-0.240323089	0.000183954	0.002516987
PDCD6IP	-0.149080403	0.000183298	0.002516987
MAPK6	-0.347491342	0.000183782	0.002516987
TMEM256	0.682760595	0.000185827	0.002533927
ARL1	-0.095126478	0.00018596	0.002533927
TULP3	0.304592024	0.0001859	0.002533927
SMARCB1	0.171383965	0.000185456	0.002533927
MOB4	-0.125769575	0.000186592	0.002540074
AMT	0.448803317	0.000186991	0.002543047
IGF1R	-0.156515378	0.000188285	0.002558158
RORA	-0.257941824	0.00018852	0.002558877
C3H1orf50	0.078733099	0.000189626	0.002571408
EEF1AKMT1	0.409489703	0.000190515	0.002580962
FAM69C	0.386122447	0.00019143	0.002588357
SLC10A7	-0.236988967	0.000191271	0.002588357
RNF103	-0.12071295	0.000193231	0.002608142
TAF13	-0.235921776	0.000193265	0.002608142
MFSD1	-0.200659937	0.000195279	0.002632795
TRIM28	0.2708364	0.00019552	0.002633511
AKAP11	-0.379481544	0.000196825	0.002648556
LOC101902854	-0.132689987	0.000197526	0.002649082

CARMIL1	-0.504049186	0.000197619	0.002649082
LATS1	-0.56274251	0.000197616	0.002649082
MOB2	0.509658281	0.000197167	0.002649082
INTU	-0.84410886	0.000198275	0.002652804
ZNF784	0.5170955	0.000198231	0.002652804
RBM7	-0.175434525	0.000198497	0.002653247
TMEM219	0.309282306	0.000199711	0.002666935
ITM2B	0.024017087	0.000200129	0.002669971
SLC36A4	-0.363831899	0.000200354	0.002670435
USO1	-0.126547263	0.0002008	0.002671309
CDK20	0.208077397	0.000200656	0.002671309
CCL25	0.125428309	0.000202171	0.002684461
C26H10orf143	0.327671683	0.00020209	0.002684461
LAGE3	0.398993858	0.000204227	0.002709163
MRPL28	0.276985507	0.000204418	0.002709163
HSD17B12	-0.221224243	0.000205237	0.00271746
LOC112446037	-0.600401258	0.000206179	0.002724784
DGAT1	0.278054954	0.000206108	0.002724784
SRP9	-0.022387098	0.000207574	0.002738069
TOGARAM1	-0.352163053	0.000207456	0.002738069
RNF152	0.173440958	0.000208421	0.002746662
APBA3	0.15066511	0.000209381	0.002756723
LOC786914	0.775762463	0.000209747	0.00275895
PHPT1	0.344041423	0.000210354	0.002764343
RPL36A	0.356589073	0.000211476	0.002773898
SNRPD1	0.485970122	0.000211461	0.002773898
LOC112442864	0.544388933	0.000211746	0.002774842
PAWR	-0.113255704	0.000212033	0.002776014
SLK	-0.400226937	0.0002126	0.002780843
RTN3	0.024284046	0.000212819	0.002781113
SNRPD2	0.511611175	0.000213168	0.00278198
ATP5PF	0.507667454	0.000213282	0.00278198
C7H19orf53	0.328758074	0.000215088	0.002801314
LOC100139990	-0.076565684	0.000215163	0.002801314
PPP6R1	0.685838775	0.000216327	0.00281386
RHOT1	-0.175911214	0.000217164	0.002822124
CBR3	0.331496371	0.000217661	0.002823352
LACC1	-0.634414233	0.000217558	0.002823352
LOC100299705	-0.61388392	0.000218379	0.002830059
CHI3L1	1.590822162	0.000218876	0.002832721
SNX18	-0.072526418	0.000218988	0.002832721
LOC101904796	0.0441812	0.000221874	0.002864771



RFNG	0.311288691	0.000221744	0.002864771
PLA2R1	-1.068603776	0.000224403	0.002894759
LOC112443479	-0.569841756	0.000226674	0.002921372
MTF2	-0.070831166	0.000227972	0.002935405
PTPRZ1	-0.304891171	0.000229261	0.002948945
TSEN34	0.083613104	0.000229444	0.002948945
TSACC	0.270222693	0.000230304	0.002950733
LOC783202	0.241251053	0.000230004	0.002950733
SMC1A	-0.551562046	0.000230009	0.002950733
LGI4	0.470601656	0.000230424	0.002950733
JMY	-0.175184947	0.00023269	0.002974874
SMIM7	-0.020980009	0.000232733	0.002974874
MIIP	0.149883013	0.000233203	0.002978171
CHCHD8	0.108947209	0.000233954	0.002985041
SLC17A5	-0.292885163	0.000234417	0.002988232
GSDMD	0.483911539	0.000234741	0.002989646
LOC784358	-0.821805722	0.000235	0.002990237
PCNP	-0.109903966	0.000235829	0.002998064
COG5	-0.277933729	0.000236484	0.003001136
LOC101906195	-0.3206888	0.000236498	0.003001136
GAPDH	0.744635518	0.000237692	0.003013553
MRPL18	0.264625255	0.000239366	0.003032045
RPL29	0.461979082	0.000239619	0.003032507
DBF4	-0.331077536	0.000241251	0.003050406
KIF1B	-0.192321794	0.000242079	0.003058124
CLPP	0.301236342	0.000244118	0.003081118
DCTN1	-0.015397665	0.000244526	0.003083485
DCTN3	0.312826572	0.000247034	0.003112321
DOCK7	-0.359395308	0.000248484	0.003122466
PYGO1	-0.390988443	0.000248678	0.003122466
NUP58	-0.045831691	0.000248334	0.003122466
MRPS18B	-0.032285888	0.000248729	0.003122466
TM7SF3	-0.32808057	0.00024905	0.003123695
UBQLN1	-0.157795215	0.000250221	0.003135583
GNB1L	-0.256805563	0.000252267	0.003158404
IMPAD1	-0.320358126	0.0002531	0.003166003
LOC112446004	0.301786958	0.000253818	0.003172161
MVB12A	0.351799036	0.000254072	0.003172515
LOC784254	0.104534602	0.000254847	0.003179359
NCKAP1	-0.34225199	0.000255412	0.003183571
LAMTOR5	0.403736795	0.000256229	0.00319093
RIF1	-0.317738006	0.000257475	0.003203361

B4GALT1	-0.575581485	0.000257684	0.003203361
BANF1	0.251037738	0.000259532	0.003223481
MRPS17	0.38696689	0.000259975	0.003226131
ZNF614	-0.506895153	0.000260286	0.00322713
CDKN1B	-0.029164809	0.00026066	0.003228922
REEP6	-0.592853151	0.000261267	0.003233579
SEPT7	-0.476270451	0.000261763	0.003234571
CDKN2AIP	-0.284014561	0.000261808	0.003234571
ZCCHC6	-0.253406225	0.000264069	0.003259642
HPGD	-0.177045601	0.000264343	0.003260157
PCDH19	-0.352753128	0.000266062	0.003275602
EPN1	0.534902567	0.000266012	0.003275602
DTX3L	0.071499655	0.000266322	0.003275923
RBM26	-0.422243355	0.000266582	0.003276246
MRPS6	0.526747974	0.00026721	0.0032811
REV3L	-0.317786399	0.000267769	0.003285089
TMEM134	0.040722019	0.000268505	0.003291236
TCAP	0.296311471	0.000269065	0.003292347
RPL24	0.364619062	0.000268853	0.003292347
CCDC167	0.39371999	0.000269546	0.003293927
FBXO34	-0.084948482	0.000269663	0.003293927
LOC100174924	0.124671704	0.000270445	0.003297648
PUS1	0.177034912	0.000270478	0.003297648
TRIM47	0.566551156	0.000270673	0.003297648
MARCH7	-0.415061072	0.000272106	0.003312235
PICALM	-0.165340913	0.000274529	0.003338837
RPL36A	0.335710118	0.000275575	0.003348651
UBA5	-0.069637579	0.000277261	0.003366231
BRMS1	0.21302954	0.000277646	0.003367987
TRPT1	0.236371082	0.000279247	0.003381558
TRIM41	-0.032963535	0.000279247	0.003381558
REST	-0.257031584	0.000280363	0.003389233
RAB2A	0.092317522	0.000280242	0.003389233
SMO	-0.102295553	0.000281376	0.003389801
C1H21orf58	0.879911349	0.000281201	0.003389801
NAA35	-0.335520427	0.000280915	0.003389801
MATR3	-0.359232912	0.000281128	0.003389801
LOC112443328	0.78230404	0.000281813	0.003390138
PJA2	-0.47067253	0.000281888	0.003390138
PUF60	0.109472049	0.000282371	0.003390682
ZC3H7A	-0.335649706	0.000282416	0.003390682
IL17RD	-0.052938119	0.000283113	0.003396143

KRBA1	0.09536448	0.000283919	0.003402908
SDK1	-0.714524283	0.000286057	0.0034256
RPS27	0.327177292	0.000287886	0.003444566
TMEM237	-0.680624268	0.000289034	0.003451509
CMC4	0.228623654	0.000289859	0.003451509
RAB10	-0.217455128	0.000289942	0.003451509
SAXO2	0.839520134	0.000288943	0.003451509
CLDN1	-0.692650256	0.000289541	0.003451509
RSRP1	0.347047767	0.000289692	0.003451509
LOC101906837	-0.035716156	0.00029051	0.003455341
PCED1A	0.40719036	0.000292695	0.003478388
CLOCK	-0.348592687	0.00029324	0.003481914
METAP2	-0.139928177	0.000293676	0.00348414
DNAJB9	0.134622261	0.000294035	0.003485453
NT5DC1	-0.438945469	0.00029551	0.00349704
WBP1	0.396892998	0.000295397	0.00349704
CLCC1	0.81937376	0.000298144	0.003525235
U2SURP	-0.34915015	0.000299645	0.003540001
TANC1	-0.424428947	0.000301409	0.003557848
AAMDC	0.616965675	0.000303668	0.003575483
C17H22orf39	0.374247181	0.000303222	0.003575483
CDC37L1	-0.017279528	0.000303565	0.003575483
CYB561D2	0.585946687	0.000305256	0.003591167
LOC783497	-0.37389995	0.000305967	0.003596519
RPL4	0.342910837	0.000307514	0.003611677
SLC30A9	-0.1898783	0.000308048	0.003613051
LDLRAD3	2.597905802	0.000308146	0.003613051
ATP8A1	-0.160895222	0.000308725	0.003616825
PTAR1	-0.245607827	0.000310622	0.003636014
USP49	-0.799355815	0.000313626	0.00366812
PLSCR2	-0.853894066	0.000314657	0.003674765
NDUFB4	0.325070273	0.000314768	0.003674765
RLF	-0.290927948	0.00031498	0.003674765
MACC1	-0.382654943	0.000316691	0.003691657
RAD54L2	-0.441751812	0.000317274	0.003695382
DARS2	0.030001911	0.000317814	0.003696244
SAP130	-0.143264824	0.000317874	0.003696244
KLHDC4	0.05958639	0.000318181	0.003696749
ID1	0.952478842	0.000318773	0.00370056
TMOD3	0.563837928	0.00031909	0.003701181
OTUD7B	-0.50838622	0.000320007	0.003703068
KIAA0100	-0.443177422	0.000320044	0.003703068

ATP5PO	0.232169495	0.000319869	0.003703068
SPAG9	-0.366324162	0.000320622	0.0037067
SNRPF	0.437304791	0.000322138	0.003710423
CCDC61	-0.155375723	0.000321881	0.003710423
KLHL9	-0.414342558	0.000322403	0.003710423
LOC783461	0.610006272	0.00032253	0.003710423
CMC1	0.557451611	0.000321803	0.003710423
SMIM19	0.224492294	0.000322389	0.003710423
PDLIM7	0.50396915	0.000323034	0.003713169
BGN	1.084997336	0.000323453	0.003714939
ATP6V1H	-0.060103738	0.000323944	0.003717539
IKBKG	0.137386772	0.00032535	0.003730628
RPS27A	0.248844738	0.000327311	0.00374532
SARS2	0.114663124	0.000327083	0.00374532
ACVR1	0.02394046	0.000327432	0.00374532
HRAS	0.595264829	0.000328327	0.003750656
UBL4A	0.282631344	0.000328433	0.003750656
SLC30A7	-0.308904841	0.000329782	0.003763005
GPT	0.358321544	0.00033027	0.003765502
ATP6AP2	-0.118506904	0.000330572	0.003765893
RAC3	0.134029699	0.000331312	0.003771256
LOC101904039	0.110748329	0.000332292	0.003779355
CAVIN3	0.483861626	0.000333392	0.003787851
GALE	-0.159112494	0.000333579	0.003787851
SUB1	0.232941306	0.000335305	0.003801299
BLOC1S1	0.446055801	0.000335079	0.003801299
MRPS26	0.625265154	0.000335935	0.003802297
BORCS8	0.300411003	0.000335805	0.003802297
LOC112443528	-1.685447261	0.000338481	0.003828026
MRPL11	0.26704847	0.000339383	0.003831264
EFCAB14	-0.41694406	0.000339808	0.003831264
TAZ	0.233930267	0.000339859	0.003831264
C25H16orf91	0.271484953	0.000339667	0.003831264
RNF145	-0.232741978	0.000340213	0.003832173
ADCK5	0.091259416	0.000341719	0.003845346
MIOS	-0.20565173	0.00034193	0.003845346
LMAN1	-0.439431481	0.000343717	0.00385925
FAU	0.457965858	0.000343635	0.00385925
PPP4R2	0.084686571	0.000346179	0.003883789
VAMP8	0.418049208	0.000347531	0.003895843
BPTF	-0.39471269	0.00034836	0.003902022
PUSL1	0.564310495	0.000349008	0.003906167

PEX16	0.15864218	0.000349351	0.003906891
HECTD1	-0.506470061	0.000349927	0.003910211
C5H12orf57	0.515303601	0.000352021	0.003930485
AHCYL2	-0.251732612	0.000352337	0.003930883
TRAPPC8	-0.251570108	0.000354227	0.003948824
SPTBN1	-0.221652208	0.000354537	0.003949143
FNBP1L	-0.523471713	0.000357079	0.003974307
NDUFS6	0.235788208	0.000358292	0.003984645
WWTR1	-0.278747066	0.000360089	0.004001467
FAM89B	0.497226251	0.000361496	0.004013917
DGKQ	0.153998017	0.000364138	0.004040057
ARHGEF38	-0.843727587	0.000365969	0.004057163
ANKRD39	0.182779807	0.000367756	0.00406438
METTL5	0.288509339	0.000367254	0.00406438
TMEM135	-0.075348796	0.000367136	0.00406438
DNAJC1	-0.097079699	0.000367778	0.00406438
RBM41	-0.371176883	0.000368427	0.004068346
PANK3	-0.453691244	0.000368921	0.004070602
MRPL52	0.500674456	0.000370541	0.004083882
MT2A	0.750768687	0.000370998	0.004083882
LOC112441655	0.352566962	0.000370837	0.004083882
EIF4EBP3	0.357223682	0.000372653	0.004095682
TCEANC	0.227736439	0.000372575	0.004095682
FBXO11	-0.013172852	0.000373742	0.004104438
SNRPD3	0.460142274	0.000374443	0.004106962
C25H16orf72	0.090114648	0.000374557	0.004106962
LOC101902490	0.345838077	0.000375109	0.004109804
SSSCA1	0.281304269	0.000375432	0.004110136
NR1D2	0.098161198	0.000378116	0.004136286
MORC3	-0.442169119	0.000379604	0.004146099
GPR155	-0.290623964	0.000379451	0.004146099
CIDEB	0.263016024	0.000380977	0.00415786
STX7	-0.257247228	0.000381296	0.004158113
GPATCH2	-0.114980984	0.00038435	0.004184915
FMN1	-0.356524072	0.000384265	0.004184915
ZNF24	-0.621826689	0.000384967	0.004188376
HPS1	0.003480136	0.000385602	0.00419204
C14H8orf76	-0.01040164	0.000386842	0.004202264
LOC112444653	0.893807905	0.000388332	0.004211937
RYBP	-0.084717679	0.000388049	0.004211937
ATP5MD	0.341815884	0.00038999	0.004226652
SLC25A11	0.213933915	0.000392311	0.004248527

UCKL1	0.08118415	0.000393712	0.004260405
CD302	-0.200671306	0.000394242	0.004262853
MRPL23	0.423217291	0.000395157	0.004269464
C14H8orf59	0.201047548	0.000396734	0.004283198
ATXN1	-0.088293965	0.000398277	0.004293252
MFSD4B	-0.794664513	0.000398226	0.004293252
MYEF2	-0.802869415	0.000398669	0.004294186
LRFN3	0.191764048	0.000399674	0.004301707
RANBP17	-0.056289411	0.000400655	0.004308958
ACD	0.2199884	0.000401233	0.004311869
SIGIRR	0.156945401	0.000403683	0.004326656
OTUB1	0.402961102	0.000403805	0.004326656
MLLT11	0.051340271	0.000403842	0.004326656
BRK1	0.155893031	0.000403778	0.004326656
ENTPD4	-0.556760372	0.000405164	0.004337512
CHRNA3	0.688705252	0.000406505	0.004347042
KAT2A	0.094786084	0.000406674	0.004347042
PCBD2	0.418153662	0.000407126	0.004348562
LOC104974330	0.517143244	0.000410125	0.00437727
LOC101906818	0.45640761	0.000410489	0.004377825
SPART	-0.088642796	0.000412727	0.004395359
ATP8B1	0.047589118	0.00041307	0.004395359
LOC784768	1.317235323	0.000413073	0.004395359
PPP1R35	0.417409616	0.000414835	0.004407425
RPS14	0.438452925	0.000414797	0.004407425
HEATR5B	-0.543023529	0.000415636	0.004412597
RPL13A	0.326896591	0.000421101	0.004467238
LOC112444920	-0.258839498	0.000423636	0.004490728
SMC4	-0.325807029	0.000424782	0.004496085
ADD3	-0.658409956	0.000424732	0.004496085
SNAP23	-0.348536944	0.000427066	0.004513459
MBLAC2	-0.490577919	0.000427029	0.004513459
BCORL1	0.000657205	0.000431809	0.004560149
TMF1	-0.224583497	0.000432805	0.004567227
CXXC1	0.130566356	0.000433902	0.004573493
LCORL	-0.502683205	0.00043405	0.004573493
SNAPC4	0.015377408	0.000440459	0.004637536
THOC5	0.29292909	0.000441101	0.004640821
EIF2AK3	-0.049424018	0.000443288	0.004647293
RBBP8NL	0.216236476	0.000443372	0.004647293
LOC100336734	-1.693414376	0.000443285	0.004647293
IBA57	0.221956122	0.000443022	0.004647293

EIF4E	-0.0908822	0.000442702	0.004647293
STAT5A	-0.66256381	0.000443945	0.004649831
TLN1	0.1825909	0.000444765	0.004654945
LOC107131607	0.773060108	0.000446723	0.004671954
LOC104968435	0.165227598	0.000447485	0.004676433
CWC22	-0.265714267	0.000449435	0.004693318
SLU7	-0.304447846	0.000451172	0.004702989
COMMD3	0.007037447	0.000452372	0.004702989
LOC107132278	-1.807177241	0.000451441	0.004702989
TPRG1L	0.14965606	0.000452103	0.004702989
BCAS2	0.452725712	0.000452175	0.004702989
COPE	0.387854565	0.000451515	0.004702989
MANEA	-0.085530055	0.000453254	0.004708677
ETV6	-0.430188126	0.000454246	0.004715492
KXD1	0.038367878	0.000455574	0.004725784
TDRD7	0.007803529	0.000456199	0.004726813
RBX1	0.177512386	0.000456347	0.004726813
ROGDI	0.424152763	0.000456695	0.00472693
SPAG7	0.109022926	0.000457903	0.004735938
PHIP	-0.295257613	0.000459116	0.004739812
LOC112448582	-0.13151496	0.000459163	0.004739812
ZNF518A	-0.359821422	0.000459291	0.004739812
TMEM187	0.027891356	0.000461292	0.004756961
TMUB2	0.220105215	0.00046163	0.004756961
JAK2	-0.387222227	0.000463172	0.004769343
TPGS1	0.177029537	0.000464281	0.004777259
RFXANK	0.395703023	0.000467459	0.00480644
AP1M1	0.092705867	0.000469244	0.004819109
RPL7L1	0.130166715	0.000469378	0.004819109
AAK1	-0.575793985	0.000470408	0.004826151
IMP3	0.36273885	0.000472912	0.004848302
TMEM170B	-0.440054956	0.000475236	0.004868572
DNAJC10	-0.292407588	0.0004762	0.004874881
MOSPD3	0.203379193	0.000478025	0.004890003
UQCR11	0.29423821	0.000478484	0.004891132
RBM25	-0.110677613	0.000480037	0.004903436
CPOX	-0.574131745	0.000481348	0.004913252
SCARB2	-0.031330326	0.000483151	0.004924604
ITGA7	0.20122199	0.000483162	0.004924604
PNCK	0.25325596	0.0004838	0.004927531
LOC107132247	0.468780203	0.000485943	0.004945768
OSBPL7	-0.275519348	0.000487331	0.004956294

CLPX	-0.251432112	0.000488756	0.004967187
KIFC2	0.487021331	0.000490039	0.004976629
MAN1A2	-0.620502295	0.000493645	0.00500927
FCHSD2	-0.541982469	0.000493967	0.00500927
TOMM6	0.41805482	0.000498353	0.005050096
TMEM64	-0.363603349	0.000502248	0.005085895
HOXC4	0.191960056	0.000505342	0.00511354
NAA30	-0.382213635	0.000506258	0.005119119
ABL2	-0.252862716	0.000513329	0.005183919
TRIM7	-0.467689203	0.000513406	0.005183919
RNF43	0.108364821	0.000518314	0.005229722
DPM3	0.407059481	0.000519153	0.005234422
APPL1	-0.409484447	0.00052037	0.005242924
RALGAPA1	-0.256868094	0.000520811	0.005243606
RBCK1	0.37420965	0.000523867	0.00527059
DDT	0.44912713	0.000524575	0.005273936
RSAD1	-0.131813966	0.000526787	0.00529238
CREB3L2	-0.760843285	0.000528274	0.005297741
QSOX2	0.334280777	0.000528453	0.005297741
CHCHD5	0.41886017	0.000527701	0.005297741
FUCA2	-0.464164166	0.000529404	0.005303489
COMMD4	0.345399966	0.000531315	0.005314731
RALY	0.298913257	0.000532041	0.005314731
GSTCD	-0.283590884	0.00053163	0.005314731
STAMBP	-0.255675259	0.000531834	0.005314731
C1D	-0.078837917	0.00053333	0.005323198
LOC112441507	1.898706189	0.000533647	0.005323198
ZDHHC20	-0.528758555	0.000535516	0.005334132
ELMOD1	-0.955529822	0.000535884	0.005334132
SPSB3	0.252784379	0.000535303	0.005334132
CEMIP2	-0.432637145	0.000538892	0.00534512
CD55	-0.415507703	0.000537681	0.00534512
GPS2	0.580144244	0.000537758	0.00534512
CASP3	-0.062557421	0.000538522	0.00534512
C21H14orf28	-0.246138626	0.000538705	0.00534512
GPN3	-0.253822472	0.000539624	0.005348605
RNF40	-0.20580214	0.000542901	0.005373498
SNRPB	0.421579882	0.000542652	0.005373498
GFM2	-0.286917842	0.000544912	0.005389601
MTBP	-0.562578405	0.000547111	0.005407538
LSM8	0.329346516	0.000549372	0.005426061
LYRM4	0.344327425	0.000552057	0.00544875



LOC510362	-0.263495956	0.000553417	0.005458333
TUBGCP6	0.260528504	0.000553906	0.005458475
SUGP1	0.205839235	0.000554209	0.005458475
ZNF414	0.214509709	0.000557119	0.005483288
SCCPDH	-0.455628493	0.00055875	0.00549548
MCC	-0.780247615	0.000559531	0.005499314
RHBDL1	0.981457961	0.000560497	0.005504948
RIC8A	0.084826405	0.000561817	0.005511758
LOC101902561	0.587957254	0.000561975	0.005511758
MAPK15	0.427866629	0.000565094	0.005538469
NDUFB2	0.101831172	0.000565827	0.005541791
LOC101903758	0.400120373	0.00056844	0.005559622
SLC9A1	-0.196465604	0.000568307	0.005559622
RPL27A	0.488132725	0.000569169	0.005562177
GOLT1B	-0.210605448	0.000569494	0.005562177
COX6A1	0.323878139	0.000572314	0.005585835
SERPINB12	-0.33692647	0.000574421	0.005602499
ZFAND2B	0.154803287	0.000576259	0.005616525
PLEK2	0.234616308	0.000577291	0.005622677
KIAA2012	0.401551465	0.000578069	0.005626356
NDOR1	0.123829019	0.000582964	0.005668257
LOC104975593	-0.214738948	0.000583182	0.005668257
EXOC4	0.166885406	0.000585496	0.005686808
PEX3	-0.450981772	0.00058598	0.005687576
ERH	0.440375843	0.000586458	0.005688275
RPS3	0.242262643	0.000587491	0.005694365
COA1	0.307353015	0.000589841	0.005709257
CCNQ	0.493160723	0.000589468	0.005709257
CAPS	0.284153918	0.000591386	0.005720265
LOC789192	-0.248658525	0.000591994	0.005722198
SMAD2	0.073756551	0.000594947	0.005746784
ATXN3	-0.100318651	0.0005973	0.005765548
RPL15	0.375185667	0.000598633	0.005774439
CACUL1	-0.163941491	0.000601554	0.005790484
GAPVD1	-0.323352315	0.000601946	0.005790484
FBXW9	0.889604586	0.000601301	0.005790484
ARF4	-0.176119905	0.000601184	0.005790484
GOLPH3	-0.084445892	0.000610303	0.005866853
CEBPZ	-0.183828596	0.000611022	0.005869742
URM1	0.241251116	0.000611774	0.005872951
CCDC47	-0.169165027	0.000613886	0.005889198
QPCT	-0.475105917	0.000618116	0.005925719

YEATS4	-0.243232623	0.000619212	0.00593218
DHX9	-0.019493229	0.000620815	0.005940589
SCNM1	0.386117696	0.000620937	0.005940589
MTMR10	-0.695673742	0.000622107	0.005947737
LOC112444473	0.478394819	0.000626911	0.005985506
MED15	0.291760864	0.000626628	0.005985506
EXOC8	-0.571360075	0.0006288	0.005999466
CUL2	-0.300682965	0.000631736	0.006023377
HSPB6	0.898910331	0.000632286	0.006024529
N4BP2L2	-0.265611718	0.000632995	0.006027192
AVEN	-0.197230394	0.00063378	0.006030576
LOC100337507	0.385445696	0.000634317	0.006031598
ARL15	-0.581501518	0.0006408	0.006089122
LOC104971101	0.549100033	0.000645928	0.006132813
FBXL5	-0.131545908	0.000646272	0.006132813
WDR90	0.322636303	0.0006471	0.006135367
RAB18	-0.058341933	0.000647416	0.006135367
KLHL42	-0.705555609	0.000647932	0.006136115
C1QTNF5	0.052711917	0.000649021	0.006138147
LOC112443181	0.05888536	0.000648973	0.006138147
FKBP8	0.4697232	0.000650372	0.006146779
GTF2H4	0.138498243	0.000651278	0.006151205
LAPTM4A	0.010071586	0.000653418	0.006163115
ERO1B	-0.187955992	0.000653228	0.006163115
ANXA4	-0.321873537	0.000655533	0.006178916
CLN5	-0.411927089	0.000658764	0.006205207
NIPAL1	-0.790674279	0.000661898	0.006230544
REX1BD	0.24823048	0.000662464	0.00623169
NUP50	-0.10556959	0.000663113	0.006233622
NUDT22	0.439904356	0.000664068	0.006234245
LAMB2	0.215250428	0.00066369	0.006234245
PSMD1	-0.3861965	0.000671808	0.006302691
PDPK1	-0.052146133	0.000672395	0.006303985
PTPN18	0.212050161	0.000673958	0.006314418
GPX1	0.09054305	0.000674488	0.006315171
LOC100196901	0.82943023	0.000675784	0.006323088
USP11	0.022515491	0.00067968	0.006351173
LOC112442867	0.846449629	0.000680143	0.006351173
SNAPIN	0.229616183	0.000679926	0.006351173
MYL6	0.519494359	0.000681033	0.006355255
DDX6	-0.038264388	0.000687673	0.006412948
HYPK	0.391911939	0.000688224	0.006413828

C23H6orf136	0.059740012	0.000688894	0.006415814
DDB1	0.049748086	0.000692115	0.006441532
MPST	0.374689386	0.000694332	0.006457883
ZNF524	0.261576239	0.000695411	0.00646364
FAM135A	-0.569756806	0.00069801	0.0064835
TMEM260	-0.248074027	0.000701034	0.006507287
AFF4	-0.514054259	0.00070298	0.006521036
PRKCA	1.496127306	0.000704767	0.006528983
MT1E	1.327895944	0.00070475	0.006528983
ATPAF1	0.053220565	0.000706628	0.006541903
TMEM265	0.430140744	0.000711932	0.006586665
TTC39B	-0.490915623	0.000720145	0.006658257
MITF	-0.190080892	0.000720879	0.006660657
ZNF579	0.463702703	0.000723561	0.00668104
INTS2	-0.277716491	0.000724469	0.006685028
GCH1	-0.255406382	0.000725744	0.006692386
LOC509006	0.444639632	0.000728324	0.006711771
SLC25A26	0.124054515	0.00073314	0.006751715
MACROD1	0.641612927	0.000734435	0.006759209
ARRDC4	-0.627994935	0.000735941	0.006768634
PDLIM4	0.842074818	0.000736766	0.006771782
SNUPN	-0.127686115	0.000738873	0.006782263
RPS20	0.329998989	0.000738816	0.006782263
LOC101902787	0.828832732	0.000740653	0.006794163
ZNF711	-0.333328212	0.000744642	0.00682629
VARs2	0.018245802	0.000745749	0.006831972
BCL2L12	0.341858785	0.000747388	0.006842522
PLEKHF2	0.037337611	0.000749546	0.006857809
CCNC	0.12270843	0.000751702	0.006861378
C15H11orf74	0.598522993	0.000752869	0.006861378
SPATA33	0.079148353	0.000750765	0.006861378
GNPDA2	-0.093629981	0.000752786	0.006861378
ILKAP	0.200422617	0.000752263	0.006861378
OGDH	-0.273659661	0.000752004	0.006861378
LOC112444681	0.6864737	0.000754087	0.006863559
RFXAP	0.018163213	0.000753643	0.006863559
SNX17	0.27655588	0.000755674	0.006873545
ERMARD	0.104793684	0.000757337	0.006879755
PPP2R3A	-0.23105485	0.000757261	0.006879755
AKIRIN1	-0.116483792	0.00075897	0.006890128
TANK	-0.188642701	0.000761745	0.006910855
CUTA	0.287532178	0.000763513	0.006922417

HIGD1B	0.44381296	0.000767135	0.006950766
P3H2	0.457080475	0.000768448	0.006958176
TPR	0.013913715	0.000770072	0.00696838
PLEKHH3	0.349746328	0.000771351	0.006975459
PUM1	0.318090739	0.000774095	0.006995765
VPS8	-0.255829211	0.000775158	0.00700086
LOC104976232	-0.638500526	0.000778214	0.00702012
YTHDF2	-0.211595237	0.00077829	0.00702012
CHMP4A	0.095027478	0.000779234	0.007024112
RGL2	0.307174186	0.000780377	0.007029908
PUM2	-0.164796652	0.000782092	0.007040836
C25H7orf43	0.238531477	0.000782864	0.007043265
EEF1D	0.30249122	0.000784918	0.007057226
UBN2	-0.438204634	0.000787762	0.007078258
PMAIP1	-0.85879525	0.000788362	0.007079119
RSBN1	-0.5490889	0.000793941	0.007124659
NAT9	0.338618804	0.000798237	0.007136915
RHOT2	0.094057992	0.000797007	0.007136915
NR2C2AP	0.181649341	0.000797883	0.007136915
MED14	-0.302788156	0.000798687	0.007136915
LOC100847284	0.378061428	0.000796718	0.007136915
URAD	0.997289668	0.000798396	0.007136915
HNRNPL	0.479087372	0.000798867	0.007136915
VANGL1	-0.240598572	0.000800605	0.00714335
IKZF5	-0.525753933	0.000800484	0.00714335
MRPL2	0.252975617	0.000808561	0.007209755
UBE2W	-0.394319969	0.000809545	0.007213943
HERC6	-0.13637311	0.000812648	0.007236999
ELP1	-0.19648246	0.00081347	0.007239728
LOC107132589	0.078046562	0.000816381	0.007261028
ELMOD2	-0.207659867	0.000818508	0.007275336
STK25	0.371462571	0.000819869	0.007282669
HDAC10	0.486490935	0.000820889	0.007282669
ALDH1A1	0.029532698	0.000820804	0.007282669
SH3YL1	-0.103967024	0.000822135	0.007289111
POLR2I	0.169640762	0.000823	0.007292177
MAT2B	-0.02614356	0.000825198	0.007298014
MAGI3	-0.387792849	0.000824526	0.007298014
LOC100141168	0.296847728	0.000825739	0.007298014
SLC6A9	0.33904621	0.000825564	0.007298014
RNH1	0.533487316	0.000826472	0.007299896
LUC7L3	-0.156995609	0.000828821	0.007316036

PGM2	-0.474940476	0.000834302	0.00735517
LRAT	-0.401535227	0.000834186	0.00735517
NDUFS8	0.529874831	0.000835353	0.007359808
ANKRD52	-0.773265574	0.000836122	0.007361962
AK3	-0.209595903	0.000836806	0.007363367
PYGO2	0.241999614	0.000839014	0.007375981
PARL	0.08964491	0.00083929	0.007375981
S100A4	1.019110529	0.000841495	0.007390725
HSPA13	-0.582557474	0.000843834	0.007406634
SAMD1	0.008696495	0.000847589	0.007427502
FXVD1	0.941181242	0.000846939	0.007427502
TMX3	-0.355251856	0.000847799	0.007427502
ANKLE2	-0.164726302	0.00084953	0.007438028
GNG10	-0.087389813	0.000852287	0.00745751
MPV17	0.313872152	0.000855405	0.007461532
PRPF31	0.272957613	0.000855345	0.007461532
ADO	-0.175601263	0.000853736	0.007461532
BAZ2B	-0.263949411	0.000854372	0.007461532
LOC100848985	0.014258623	0.000854913	0.007461532
CAPZA2	-0.240503054	0.000856955	0.007470408
PTPN13	-0.137435066	0.000860245	0.007494436
HCFC1R1	0.823080252	0.000862392	0.007508481
CENPI	-0.202493129	0.000864764	0.007524464
LRRC14	0.089319759	0.00086745	0.007540597
LOC781381	0.315246844	0.000867692	0.007540597
RFX7	-0.437238208	0.000870591	0.007556427
CSRP1	0.889561147	0.000870129	0.007556427
ENKD1	0.334921602	0.000871741	0.007561731
RNF25	0.069413294	0.000872608	0.007564577
RRAS	0.785685957	0.000874241	0.007569388
KIAA1614	-0.01410256	0.000873735	0.007569388
PIGP	0.402254126	0.000875757	0.007577833
HNRNPF	0.04513742	0.000877016	0.007584055
RNF2	-0.152226599	0.000880042	0.007605533
RAP1A	-0.123101634	0.000881342	0.007612085
UGDH	0.111774227	0.000883319	0.007624463
TIMM17B	0.212819304	0.000887753	0.007658028
PEX11G	0.363440559	0.00088911	0.007665024
TMEM50B	-0.19539602	0.000890084	0.00766871
EMC9	0.217755228	0.000891308	0.007674548
VPS9D1	0.117477576	0.00089668	0.007716062
EBAG9	-0.192466509	0.000898386	0.007726007

LOC101904529	0.513331472	0.000899424	0.007730198
MFSD8	-0.436853098	0.000904124	0.007765837
PLXNA3	0.309994239	0.000908184	0.007791173
TST	0.635832382	0.000907678	0.007791173
SSR2	0.422518432	0.000912591	0.007824199
FAM98A	-0.189718996	0.000920304	0.007885509
DUT	0.39358625	0.000921225	0.007888587
RNGTT	-0.292938146	0.000923737	0.007905272
TIMM13	0.206338786	0.000924475	0.007906764
INPP5F	-0.357693754	0.000925141	0.007907645
PARS2	-0.516918688	0.000930883	0.007951884
RPL26L1	0.479456947	0.000931619	0.007953328
TSPAN7	0.905317718	0.000932959	0.007959929
BORCS5	0.205383577	0.000937066	0.007980413
C1H3orf70	-0.736131754	0.000936705	0.007980413
EIF2D	-0.011577741	0.000936397	0.007980413
HERC4	-0.20644205	0.000939288	0.007994482
SLC22A17	0.8990316	0.000942937	0.008000144
LSM7	0.642711755	0.000943085	0.008000144
SLC25A28	-0.094280745	0.00094261	0.008000144
PTPN12	-0.599060701	0.00094109	0.008000144
RPS8	0.213873867	0.000941839	0.008000144
OAZ1	0.258805751	0.000943373	0.008000144
MYSM1	-0.587249113	0.000949333	0.008045825
PET100	0.667979109	0.000952589	0.008063685
GJA4	0.051973214	0.000952325	0.008063685
SOX15	0.858246337	0.000955113	0.008069015
YIF1A	0.278840508	0.000955519	0.008069015
SLC35A3	-0.115780803	0.000954976	0.008069015
HSD17B13	0.034908254	0.000955042	0.008069015
SELENOW	0.343149536	0.000956575	0.008073081
CYSTM1	0.412550903	0.000957252	0.008073941
TMPO	-0.635692841	0.000961025	0.008100889
JKAMP	-0.023417544	0.000963288	0.008110466
PAIP1	0.023089645	0.000963316	0.008110466
PRKCI	-0.249806855	0.000969192	0.008155039
MCL1	-0.093129977	0.000971279	0.008167709
NDUFA13	0.221680845	0.000972439	0.008172566
LOC100296952	0.028656042	0.000979968	0.008227435
GJA1	-0.262456682	0.000981216	0.008227435
LOC787257	0.531997595	0.000981899	0.008227435
TWF1	-0.204141133	0.000981224	0.008227435

CLPB	-0.372039591	0.000981883	0.008227435
NUBP1	-0.047946281	0.000982655	0.008228855
SLC31A1	-0.136680336	0.000984801	0.008236994
PLXDC2	-0.451704521	0.000984385	0.008236994
GNA11	-0.338372394	0.000985655	0.008239234
TNFRSF1B	-0.43433577	0.000990985	0.008278853
GCLC	-0.105060328	0.000994693	0.008304887
CCDC124	0.223249297	0.000996033	0.008311133
TFPI	0.066350772	0.001001938	0.008350478
CNBP	0.300293215	0.001001531	0.008350478
LOC101902527	-0.088688465	0.001007837	0.00839465
CYP27C1	-1.136704224	0.001010118	0.008408667
LOC782437	0.23983444	0.001013455	0.008431441
LOC100139996	0.759159431	0.001016358	0.008450579
DEPDC1	-0.439039339	0.001020045	0.008476216
LY75	-0.488677264	0.00102379	0.008497275
LOC514181	0.848722978	0.001023638	0.008497275
MIA2	-0.420609874	0.001027761	0.008525191
YLPM1	-0.15394823	0.001028738	0.008528256
TMX4	-0.734705581	0.001035776	0.008571416
DNASE1	0.073610744	0.001035268	0.008571416
SMU1	-0.09796863	0.001035229	0.008571416
MRPL4	0.055984648	0.001037771	0.008577804
LOC112442023	-1.010763049	0.001037741	0.008577804
KDF1	0.362350528	0.001043014	0.008611184
LZTR1	0.083791467	0.001043036	0.008611184
VPS45	-0.13257304	0.001045388	0.00862553
THAP8	0.21244458	0.001047282	0.008636075
DHX30	0.01195584	0.001048019	0.008637083
MFSD14B	-0.275382193	0.001050981	0.008656405
NIPSNAP2	-0.163150997	0.001055211	0.008679568
RABEP2	0.092951319	0.001055484	0.008679568
PPP1R11	-0.061477467	0.001056267	0.008679568
PPP1R12A	0.130042887	0.001056143	0.008679568
DENND4B	0.102246211	0.001059736	0.008702977
PHF2	-0.137650473	0.001065583	0.00874085
WRAP53	0.164057956	0.001065593	0.00874085
ANKRD65	0.182763255	0.001067339	0.008750061
LOC784208	0.161357423	0.001068874	0.008752952
SNX16	-0.181483627	0.001068939	0.008752952
NDUFB6	0.171219371	0.001071408	0.008767424
LTC4S	0.568885221	0.001071956	0.008767424

JMJD8	0.483378921	0.001075851	0.00879416
EIF2B4	-0.082519981	0.001080352	0.008825807
ZFPM1	0.849543665	0.001085186	0.008860145
TBC1D8B	-0.450032201	0.001086044	0.008861994
RPS15A	0.429359533	0.00109041	0.008887545
ZNF48	0.021871488	0.001090442	0.008887545
ZNF408	0.362752215	0.001095307	0.008920353
AAR2	0.033890509	0.001095738	0.008920353
PLSCR3	0.147288702	0.001097395	0.008920812
RECQL5	0.225452378	0.001098337	0.008920812
SPRN	-0.35198636	0.001098157	0.008920812
FAM83B	-0.321460494	0.001097279	0.008920812
EHF	0.042950972	0.001099483	0.008924954
ITSN2	-0.077969074	0.001101243	0.008934009
DCK	-0.329693993	0.001101872	0.008934009
SETD7	0.00969925	0.001102574	0.008934538
LOC112447303	-0.935131381	0.001103926	0.008940337
ESF1	-0.185357848	0.001105307	0.008946357
IQGAP1	-0.162578898	0.001108887	0.008968944
LOC112447329	0.535336064	0.001109376	0.008968944
PIGG	-0.630520349	0.00111006	0.008969308
SSR4	0.308485562	0.00111626	0.009010328
TEAD1	-0.177061104	0.001117062	0.009010328
GPCPD1	-0.162451626	0.00111667	0.009010328
RFK	-0.45355408	0.001117855	0.009011546
FBXO38	-0.138260222	0.00111868	0.009013014
FIG4	-0.101637311	0.001126786	0.009067912
CTNND1	-0.244444019	0.001126194	0.009067912
MTF1	-0.519527482	0.001130778	0.009094826
ZDHHC4	0.39146989	0.001136576	0.009132134
UTP15	-0.379922052	0.001136718	0.009132134
DENND4C	-0.374076612	0.001137886	0.009136289
SNRNP27	0.097791849	0.001141458	0.00915973
SMARCD3	0.14014354	0.001144066	0.009166873
PSMG4	0.55245309	0.001144308	0.009166873
S100A2	0.34948408	0.001143161	0.009166873
LOC504858	0.225401274	0.001145332	0.009169841
NAA38	0.19159722	0.001146839	0.009176675
ZFAND4	-1.053316638	0.001150087	0.009197421
RASAL2	-0.211823744	0.00115151	0.009203558
HS6ST1	-0.284836928	0.001170244	0.009347967
PARP14	0.012014041	0.001172861	0.009363538



SPTBN4	0.063345908	0.001174405	0.009370535
LOC616830	0.978017314	0.001175257	0.009372004
TET3	-0.487469837	0.001177711	0.009386246
HAUS3	-0.079851749	0.001184724	0.009436776
STK19	0.163301037	0.001186087	0.009442277
LTN1	-0.4339211	0.001191012	0.009476108
MT1E	1.343667858	0.001195387	0.009499106
NPRL3	0.229799934	0.001195168	0.009499106
NT5C3A	0.092975497	0.001195933	0.009499106
SULF2	-1.188576799	0.001196944	0.009501761
NLGN2	-0.502296013	0.001199266	0.009514811
RPL35A	0.305339321	0.001208498	0.009571822
CACNA2D1	-0.527053363	0.001207973	0.009571822
LOC112445052	0.606535582	0.001208163	0.009571822
FCER1G	0.402771532	0.001211544	0.009590539
HIPK1	-0.21693862	0.00121444	0.009607154
MBTPS1	-0.054553738	0.001215122	0.009607154
DSG2	-0.242203807	0.001215697	0.009607154
HMGCR	0.034444713	0.001217487	0.009615888
YME1L1	-0.083046175	0.001221602	0.009642959
ALG10	-0.225089013	0.001223056	0.009649009
LMBR1L	-0.220758872	0.001224492	0.009654911
DUSP1	-0.275617732	0.001226379	0.00966436
STOML2	0.270548104	0.001229873	0.009686458
LOC101906565	0.539478903	0.001231427	0.009693254
PSMC6	-0.122111584	0.001233222	0.009701945
DNAJC4	0.316657086	0.001235134	0.009711548
HACE1	-0.335292454	0.00123593	0.009712361
LOC519132	-0.075507208	0.001239729	0.009736761
TRAPPC5	0.285337057	0.001240555	0.009737802
TBC1D23	-0.193330937	0.001252365	0.009823958
C15H11orf96	0.685737779	0.001252931	0.009823958
DSP	-0.222879323	0.001254596	0.00983152
TSTD1	0.354649703	0.001257384	0.009845208
KIAA1551	-0.409227554	0.001257745	0.009845208
SMIM4	0.447888707	0.001265498	0.009900368
FAM185A	-0.780027484	0.001267117	0.009901995
MTFP1	0.599446636	0.001266514	0.009901995
TAF1C	0.260907846	0.001272126	0.009935606
PSMG3	0.529111555	0.001274751	0.009944932
RPLP2	0.582104933	0.001274812	0.009944932
ARCN1	-0.339046758	0.001275446	0.009944932

ARHGDIG	1.090528922	0.001284429	0.010009416
MTMR6	-0.165936498	0.001285503	0.010012228
TOP2A	-0.273478086	0.001290574	0.010024131
SOCS5	-0.122009907	0.001290602	0.010024131
SLC1A3	0.01271821	0.001289812	0.010024131
P4HA1	-0.085700008	0.001289903	0.010024131
CEPT1	-0.202768498	0.001288177	0.010024131
IGSF8	-0.015628579	0.001292671	0.010034647
LTA4H	-0.130953711	0.001294204	0.010040993
HSPBP1	0.268159611	0.001296344	0.010049459
TMEM181	-0.168894405	0.001296727	0.010049459
KIAA2013	-0.212985654	0.001303449	0.010095972
KLHL28	-0.575083071	0.001304439	0.01009807
DGCR8	0.288878527	0.001309291	0.01012899
TNNT3	-0.083554188	0.001309877	0.01012899
ABCC10	-0.199365058	0.001313253	0.010135491
ZNF212	0.149068519	0.001313118	0.010135491
CDK4	0.255217353	0.001313606	0.010135491
XPR1	-0.977873905	0.001313149	0.010135491
ECPAS	-0.306503551	0.001320878	0.010183492
SLC35A1	-0.011085173	0.001321278	0.010183492
PGAP1	-0.946281757	0.00132215	0.010184619
ZNF32	0.105156154	0.001331158	0.010209182
CEP76	-0.609405669	0.001330372	0.010209182
PTRHD1	0.062594933	0.001327362	0.010209182
SH3BGRL3	0.435601138	0.001330669	0.010209182
FHOD1	-0.019134553	0.001328255	0.010209182
RPS3A	0.154594211	0.00132927	0.010209182
ZNF131	-0.486017335	0.001330183	0.010209182
MARCH5	-0.253314291	0.001329462	0.010209182
SNRPG	0.29920934	0.001332644	0.010214998
MED30	0.239092725	0.001335386	0.010230424
MMADHC	-0.045250536	0.0013365	0.010233376
ARPIN	0.230073351	0.001339545	0.010251096
MED1	-0.48113763	0.001348643	0.010315096
AUP1	0.281467846	0.001352069	0.010335668
PRKAR1A	0.050504337	0.001353356	0.010339877
INAVA	0.294598915	0.00135817	0.01037101
GCFC2	-0.63995323	0.001358979	0.010371544
TJP1	-0.028262738	0.001360108	0.01037452
KANSL1L	-0.231300242	0.001369475	0.010434623
AGO3	-0.708600626	0.001369072	0.010434623

RAB3D	-0.429433641	0.001373829	0.010462125
MROH6	-0.033243867	0.001375907	0.010472263
AP1S2	-0.316030424	0.00137827	0.010484563
NUMB	0.08797176	0.00138151	0.01050352
LOC112441508	0.914973606	0.001385161	0.010525577
CNIH1	0.045624176	0.001389197	0.010550527
LOC107131494	0.281765415	0.001393308	0.010570744
DENND6A	-0.251005611	0.001393365	0.010570744
UBE2V1	0.195471171	0.00139449	0.010573564
FASTKD2	-0.292326045	0.00139616	0.010574797
PCBP4	0.193187419	0.001396074	0.010574797
SLC9A9	-0.400533564	0.001397299	0.010577718
TP53RK	0.252898905	0.001399614	0.01058953
LNPEP	-0.286942713	0.001401627	0.010599046
MAP3K5	-0.164270561	0.001403332	0.010606224
ESCO1	-0.146140223	0.001407885	0.010634915
GBA3	0.590674297	0.001408811	0.010636179
CDC14B	-0.05218159	0.001409827	0.010638128
SMIM5	0.203955741	0.001412409	0.010651885
OCIAD1	0.048333437	0.001414858	0.010664623
STRADA	0.055596541	0.001417993	0.010682518
PEBP1	0.219230622	0.001423707	0.010717438
SENP6	-0.186789207	0.001424155	0.010717438
CDK17	-0.42138377	0.001433306	0.010780521
NUDT16	0.480193884	0.001437988	0.010798377
FAM96B	0.157807626	0.001437324	0.010798377
MYNN	-0.370784325	0.001436839	0.010798377
OTUD1	0.238504213	0.001442027	0.010819086
LOC614617	0.168112455	0.001442288	0.010819086
PHLDA3	0.314768221	0.001443883	0.010819488
POLR2C	0.227434212	0.0014432	0.010819488
NDUFC1	0.125214831	0.001447935	0.010834313
METTL26	0.476724641	0.001448114	0.010834313
DPP7	0.701834679	0.001448822	0.010834313
SMC5	-0.407186336	0.001448949	0.010834313
GORASP2	-0.108337504	0.001452996	0.010858787
PPP1R15B	-0.227525368	0.001457297	0.010879346
NOTCH2	-0.193748515	0.001456832	0.010879346
SNRK	-0.064013785	0.00146226	0.010910588
WDR6	0.086851808	0.001466325	0.01093511
MPP6	-0.794591036	0.001468832	0.010942173
KPNA5	-0.445125871	0.00146862	0.010942173

TP53BP2	-0.49263265	0.001470831	0.010951256
GAS2L3	-0.507193536	0.001471764	0.010952391
TIMM50	0.358210762	0.001475899	0.010977343
KAT2B	-0.095848609	0.001478174	0.010988437
LOC101905228	-0.39970785	0.001484261	0.011027846
PPIA	0.345383311	0.001486273	0.011036952
MTMR9	-0.248712927	0.001494133	0.011089453
MOCOS	-1.284763292	0.001499054	0.011120094
CNTNAP1	0.054279944	0.00150046	0.011124644
RILP	-2.11409583	0.001511885	0.011203438
INTS3	-0.375851674	0.001514477	0.011210777
CSTB	0.242254817	0.001515272	0.011210777
LOC104968522	-1.279472598	0.001513907	0.011210777
SMG9	0.228069426	0.001518676	0.011222906
SEMA3B	-0.012219515	0.00151931	0.011222906
ATP5S	-0.606722075	0.001518751	0.011222906
ALS2CL	-0.216707507	0.001521389	0.011232352
MON2	-0.358379401	0.001524491	0.01124933
MDH2	0.278484637	0.0015258	0.011253077
NR1H2	0.236994979	0.001528022	0.011263547
CPNE3	0.088149718	0.001529346	0.01126411
ZNF688	0.029894493	0.001529704	0.01126411
MCRIP1	0.10928869	0.001533567	0.011286633
ACAP1	0.17482305	0.001544359	0.011360102
MRPS2	0.475341802	0.001545881	0.011365342
ALKBH7	0.179072944	0.001547286	0.011368495
TLR4	-0.045119384	0.00154793	0.011368495
LOC781989	0.740474972	0.001551394	0.011387977
MZT2B	0.568756672	0.001553123	0.011394714
NEO1	-0.310827765	0.001556931	0.011410721
GLULP	-0.03098202	0.001556465	0.011410721
FGFR3	0.682186113	0.00156153	0.01143845
CTNNBIP1	0.251344284	0.001565867	0.011464237
ALS2	-0.402863702	0.001568306	0.011476107
LOC107131623	0.641711116	0.001570298	0.011484696
LOC101906178	0.256015481	0.001574799	0.011511619
CLDN12	-0.332228941	0.001576781	0.011520107
IMP4	0.210337296	0.001579981	0.011537476
B4GALT7	-0.125723312	0.00158256	0.011550302
PHGDH	0.463828059	0.001586974	0.011570931
SOD3	1.062105436	0.001587035	0.011570931
FAM174A	-0.127323151	0.001588868	0.011572271

SELENOT	-0.593374754	0.00158869	0.011572271
ULK4	-0.048513441	0.001594659	0.01160842
SGPP1	-0.182497274	0.001598948	0.011633612
TUBG2	-0.038687628	0.001600333	0.011637652
RHOBTB3	-0.238155134	0.001605772	0.011671158
C1GALT1C1	-0.220039104	0.001609869	0.011694879
ZKSCAN1	-0.307425575	0.001612787	0.011710022
SOCS6	-0.177552596	0.001613953	0.011712425
PIAS4	-0.312793736	0.001619092	0.011731631
GINM1	-0.364141909	0.001619107	0.011731631
IRF3	0.182491871	0.001618655	0.011731631
LOC514507	-0.283777036	0.001624436	0.011764167
SMKR1	-0.652741768	0.001627864	0.011776842
CASP4	0.407851824	0.001627389	0.011776842
DBP	0.157991082	0.001633722	0.011813135
PDCD7	0.058749501	0.001637644	0.011835392
TMEM246	-0.539555216	0.001639003	0.011839116
NPM1	0.145961055	0.001640233	0.011841907
MBLAC1	0.127662692	0.001641343	0.011843828
AQP3	-0.264116396	0.001650874	0.011906485
ARL5A	-0.343800707	0.001653388	0.011912407
MSTO1	0.202894926	0.001653393	0.011912407
PHF3	-0.155431571	0.001654965	0.011917613
SRP19	0.116376525	0.001657888	0.011926426
AKIRIN2	0.110366815	0.001657554	0.011926426
LOC104968964	0.119206521	0.001661165	0.011942456
EIF4H	-0.18238271	0.001661818	0.011942456
TBPL1	0.052352872	0.00166276	0.011943113
MRPL34	0.238612144	0.00166607	0.011960764
LOC112444152	0.449637265	0.001667921	0.011967931
PDCD10	-0.069421609	0.001669845	0.011975616
LPGAT1	-0.270729853	0.001672689	0.011983826
ATG4A	-0.579136188	0.001673539	0.011983826
SLC44A2	-0.252247255	0.001674406	0.011983826
C8G	0.909897718	0.001673664	0.011983826
RNF19A	-0.457498859	0.001705544	0.012194937
FAM162A	0.343368889	0.00170564	0.012194937
NFXL1	-0.414464414	0.00171228	0.012236175
PRPF39	-0.209756962	0.00171998	0.012284949
RNASEH2C	0.319073641	0.001721985	0.012287637
ARMT1	-0.242072039	0.001722108	0.012287637
ZNF513	-0.131777473	0.001725987	0.012309056

MRPL24	0.366624547	0.001729603	0.012328582
ZNRF1	0.330680042	0.001731528	0.012336037
ISCU	0.124213638	0.001739841	0.012388978
MANBAL	0.037514615	0.001747491	0.012437137
GDPD1	-0.123493958	0.001750671	0.012453457
PRPF38B	-0.132889184	0.001754412	0.012469107
RANGRF	0.060166338	0.001754648	0.012469107
DNM1L	-0.232581842	0.001756769	0.01247786
MED17	-0.27039604	0.001758771	0.012485761
MRPS18C	0.358340294	0.001764403	0.012519415
GGA3	0.132822156	0.001770251	0.012548219
RPS4X	0.137781542	0.001769518	0.012548219
SNRNP40	0.141416487	0.001776495	0.01257775
PPDPF	0.412379118	0.001777106	0.01257775
RNF4	0.211244782	0.001776277	0.01257775
PFDN2	0.253688499	0.001784472	0.012618678
TBL2	-0.144559817	0.001784687	0.012618678
RPL26	0.418592779	0.001787998	0.012635726
ASCL4	0.597381031	0.001791558	0.012654515
APTX	0.158999092	0.001802503	0.012725416
PLA2G12A	0.091312619	0.001804932	0.012736159
PIN1	0.33033705	0.001806732	0.012742452
NCBP1	-0.06768538	0.001812305	0.012775343
MRPL36	0.322775456	0.001814033	0.012781101
NME3	0.523674003	0.0018188	0.012808261
KIF2A	-0.334550173	0.001821679	0.012822101
COPA	-0.108116719	0.00182446	0.01283524
TMEM68	0.018055416	0.00182909	0.01286136
LOC112446667	0.334921784	0.001833959	0.01288914
PEF1	0.091075789	0.001838398	0.01291387
MST1R	0.099582551	0.0018407	0.012923573
TAF1	-0.118962215	0.001843443	0.012936362
ATG2A	-0.058783033	0.001846719	0.012946404
TRPM3	0.494229818	0.001846057	0.012946404
RUNX1	0.016783514	0.001849148	0.01295579
FRMD4B	-0.382944113	0.001849904	0.01295579
CCP110	-0.311970227	0.001854462	0.012981237
MAP4K3	-0.140518563	0.001855948	0.012985158
PITPNM1	0.36697705	0.001863383	0.013030679
RIOK3	-0.450993576	0.001865658	0.013033601
SNX4	-0.193351994	0.001865258	0.013033601
VPS4B	-0.090882477	0.001869369	0.013053034

ADAMTSL5	0.442726154	0.001870812	0.013056611
MCFD2	-0.265661046	0.001872051	0.013058762
CREBL2	0.001036055	0.001876683	0.01308457
SDAD1	-0.444075934	0.001879271	0.013096114
METAP1	0.053898708	0.001884261	0.013124372
PPP1R12B	0.237777048	0.001890208	0.013159261
DCAF5	0.83511626	0.001899464	0.013217143
ZNF638	-0.021929727	0.001904243	0.013243827
MIGA2	0.108970909	0.001910146	0.013278306
ELF2	-0.089221689	0.001911947	0.013283347
TTC21B	-0.377767476	0.001912764	0.013283347
MYO1H	-0.019426602	0.001915721	0.013297303
PKD1	0.06078388	0.001917669	0.013304242
RPL35	0.336842184	0.001924994	0.013348461
LOC112447392	-0.786000654	0.001939575	0.013442934
NPEPPS	-0.2523259	0.001942585	0.013450509
NT5C3B	0.119499656	0.00194204	0.013450509
PPP1R7	0.113042594	0.001958252	0.013552298
MEA1	0.216134156	0.00195962	0.013554085
RPL3	0.182095418	0.00196047	0.013554085
ESPNL	0.050848772	0.001961407	0.013554085
MUT	-0.443104664	0.001965593	0.013576324
ERCC1	0.156674459	0.001968723	0.013581143
CAMSAP2	-0.077851012	0.001969193	0.013581143
PURB	-0.303351055	0.001967431	0.013581143
MRPS11	0.341899788	0.001975165	0.013615636
MRM1	0.006534583	0.001977444	0.01361617
SLF1	-0.550044585	0.001980093	0.01361617
MAPK1	-0.082888871	0.001978309	0.01361617
SMIM11A	0.241193193	0.001977175	0.01361617
NISCH	0.18282615	0.001979272	0.01361617
XPOT	-0.151662355	0.00198283	0.013624211
ZGPAT	0.19887786	0.001983204	0.013624211
TRPM4	0.100833733	0.00198425	0.01362473
KBTBD2	-0.038947997	0.001987796	0.013642406
EXOSC4	0.437798893	0.001990092	0.013651484
MAN2C1	0.19425515	0.001996506	0.013675431
OCLN	-0.35712283	0.001995695	0.013675431
LOC787858	-0.655097143	0.001995929	0.013675431
ARAF	0.039160833	0.002017638	0.013799972
FZD6	-0.492779552	0.002017453	0.013799972
PMF1	0.382953289	0.00201609	0.013799972

RPLP1	0.451469293	0.002020232	0.01381099
PRRC2C	-0.018464177	0.002025605	0.013840976
AP5Z1	0.001588408	0.002028029	0.013848086
VKORC1	0.444716115	0.002028619	0.013848086
IMPA2	0.310988436	0.002048368	0.013972339
KIF18A	-0.507130595	0.002048812	0.013972339
PRRT1	0.679574495	0.002051626	0.013984737
PLA2G4A	-0.302646329	0.002060793	0.014036538
LOC104974444	-0.173891253	0.002061226	0.014036538
RPS6KB2	-0.095204257	0.002063587	0.014045805
N4BP1	-0.190439255	0.002064613	0.014045973
H3F3A	0.287246817	0.002068952	0.014068672
FAM3A	-0.037861926	0.002077401	0.014119283
CASTOR1	0.289501898	0.00207918	0.014124536
FIZ1	0.176694779	0.002080821	0.014128847
WDR83OS	0.293102554	0.002082389	0.014132457
PACSIN2	0.518104379	0.002083367	0.014132457
G3BP2	-0.355354468	0.002087211	0.014134501
ACLY	-0.398523134	0.002087697	0.014134501
PTPN23	0.193929174	0.002086208	0.014134501
RDX	-0.335508933	0.00208568	0.014134501
BMT2	-0.235070789	0.002092001	0.014156814
UBAC1	0.127684817	0.002094759	0.014168644
LOC783838	0.407902592	0.00210215	0.014211791
NUDT4	-0.141896572	0.002107425	0.014240593
AP1G1	-0.502530666	0.00211057	0.014254981
COX17	0.294142187	0.002113447	0.014266724
CTSS	-0.295132699	0.002114342	0.014266724
C7H5orf24	-0.475322336	0.002115552	0.014268029
INPP5K	0.269272554	0.002118515	0.014274297
ZNF451	-0.337182007	0.002117801	0.014274297
RNF181	0.338699267	0.002122554	0.014294649
TMEM88	0.325446748	0.002125568	0.01430808
USP14	-0.215611511	0.002131545	0.014337452
PNRC1	-0.197615253	0.002132741	0.014337452
C23H6orf226	0.335614917	0.002132996	0.014337452
UNC13B	-0.260690884	0.002145676	0.014415782
TRMU	0.184052906	0.00214736	0.014420192
HIVEP2	0.077951116	0.002149892	0.01442835
SMC3	-0.205471972	0.002150631	0.01442835
ZCCHC11	-0.50608815	0.002153214	0.014438776
PLPP6	-0.619791784	0.002154255	0.014438855



TTC7A	-0.342396397	0.002157336	0.014452609
NUP155	-0.272171421	0.002163395	0.014486284
EMG1	0.424358247	0.002164547	0.014487085
ZBED1	0.365154151	0.002174583	0.014540387
ERAL1	0.07453887	0.002174437	0.014540387
KIFAP3	-0.30692532	0.00217696	0.014549352
AGTPBP1	-0.232133071	0.002180962	0.014569158
CENPS	0.123114486	0.00218626	0.014597605
CLASP2	-0.132005456	0.002188303	0.014601536
SRFBP1	-0.210791688	0.00218893	0.014601536
LOC523461	-0.021518319	0.002192564	0.014618831
PEX1	-0.361099924	0.002193803	0.014620146
VPS36	-0.344030497	0.002195574	0.014624887
LNK2	-0.16726886	0.002196599	0.014624887
EFNA4	0.396906273	0.002204387	0.014669785
UBE2J1	-0.230531334	0.002209228	0.014677985
RAB14	0.001312239	0.00220787	0.014677985
PLPP1	-0.227070032	0.00220896	0.014677985
HSD11B1L	0.544411607	0.002209803	0.014677985
VAMP2	-0.220843794	0.00221176	0.014684035
HSF2	-0.238981184	0.002213534	0.01468541
COPS7A	0.162787128	0.002214434	0.01468541
RPL5	0.301579663	0.002215106	0.01468541
IGSF3	-0.594783165	0.002223589	0.014734689
BBS5	-0.422594925	0.002225368	0.014739516
GAN	-0.492355446	0.002227236	0.014744932
SKP1	0.16677741	0.002231807	0.014768231
RRAGC	-0.230240811	0.002234399	0.014774231
COA5	0.145845826	0.00223482	0.014774231
MRPS21	0.127373679	0.002236601	0.014774778
CRYBG3	-0.348544149	0.002237008	0.014774778
SUCO	-0.984852696	0.002240305	0.014789593
NT5C	0.375346515	0.002243012	0.014800504
RAB11B	0.186062656	0.002244255	0.014801748
GTF3C3	-0.497396505	0.002252776	0.014850966
LOC100847567	0.157731739	0.002254666	0.014856449
EMSY	-0.2836077	0.00225619	0.014859513
AP4M1	0.183066548	0.002264036	0.014904193
RFX3	-0.628838479	0.002270605	0.014940429
LOC100847604	-0.196249676	0.002281181	0.015001944
ZBTB38	-0.2933637	0.002282091	0.015001944
CUL3	-0.246654652	0.002289189	0.015041556

KRTCAP2	0.572534576	0.00229054	0.015043392
LOC100847122	1.085037512	0.002298173	0.015086463
TMEM30B	-0.421120415	0.002301011	0.015098029
SP6	-0.947218806	0.002304436	0.015113437
INO80C	0.074929743	0.002308508	0.015126006
MRPL9	-0.020431288	0.002308477	0.015126006
GATD3A	0.346492624	0.002319729	0.015166116
RTKL1	0.359786535	0.002319916	0.015166116
NUDCD1	-0.251129614	0.002316817	0.015166116
SLC49A3	0.078918271	0.002316908	0.015166116
CSE1L	-0.243150832	0.002320032	0.015166116
YTHDC1	-0.211875491	0.002326366	0.015193366
VTI1B	0.20688413	0.002325914	0.015193366
INTS1	-0.040578117	0.002329067	0.015203935
SLC30A1	-0.38860396	0.002335807	0.015228026
CTC1	0.050806421	0.002337098	0.015228026
CASP2	-0.371772921	0.002337019	0.015228026
STRN4	0.059616272	0.002336678	0.015228026
PNPLA8	-0.204668491	0.002338307	0.015228833
RPL19	0.40831068	0.002341666	0.015243638
EGFR	-0.222766405	0.002345955	0.015257404
FANCG	0.25715703	0.002345211	0.015257404
LOC782938	0.191244497	0.002353795	0.015294528
BRAT1	0.057496178	0.002354888	0.015294528
RSBN1L	-0.283221449	0.002354932	0.015294528
MRS2	-0.425282988	0.002359823	0.015314423
RSL24D1	0.034596924	0.002360178	0.015314423
CTSK	0.266775577	0.00236347	0.015328696
CYP39A1	-0.659475288	0.002368422	0.01535372
BCAS4	0.800812576	0.002370952	0.015363023
EAPP	-0.182133158	0.002376907	0.015394503
RPAP1	-0.091616416	0.002380958	0.015399425
CARD19	0.069742916	0.002380059	0.015399425
FAM84B	0.157944477	0.002378987	0.015399425
SIRT1	-0.332941776	0.002390466	0.015453794
LOC101905779	0.36886342	0.002392562	0.015454212
SLC2A4RG	0.427384577	0.002392733	0.015454212
TMSB4X	0.431524242	0.002396044	0.01546848
MED11	0.436704397	0.002400925	0.015492863
RMND5B	0.221001401	0.002407974	0.015524079
FBXO28	-0.23458336	0.002407355	0.015524079
SLC4A9	0.185713107	0.00241019	0.015531227

IGSF6	-0.936297726	0.002411567	0.015532968
OSGEP	0.071356242	0.002418183	0.015568438
GTPBP10	-0.095493483	0.002419944	0.015572632
LOC104975635	-0.201408989	0.002422888	0.015584436
CEP290	-0.064259025	0.002433356	0.015644593
FSD1L	-0.213841882	0.002437417	0.015660268
SAMSN1	0.265680096	0.002438025	0.015660268
MYO1B	-0.529680456	0.002441446	0.015675065
TROAP	0.283352039	0.002443654	0.015682071
TVP23B	0.154946509	0.002445982	0.015689835
TMED2	0.010915461	0.00245223	0.015718292
NAP1L3	1.472437531	0.002452658	0.015718292
LUC7L	0.342573105	0.002454014	0.0157198
SH2B1	0.147151068	0.002470503	0.015818207
RPL13	0.45962498	0.002475729	0.01584444
UAP1	-0.346748284	0.002483646	0.015887862
THADA	0.5180948	0.002499418	0.015981473
CYP21	-0.285778388	0.002512622	0.016052026
EIF3I	0.15558481	0.00251274	0.016052026
NRG2	0.471791606	0.002518271	0.016080041
DOC2G	0.595221233	0.002534258	0.016174767
SOAT1	-0.426305229	0.002538092	0.016184514
PEX10	0.047404608	0.002537417	0.016184514
CCDC91	-0.181561313	0.002542275	0.016203831
SUZ12	-0.50633586	0.002550705	0.016250182
FANCM	-0.451091635	0.002554298	0.016265688
TECPR1	0.169106349	0.002572995	0.016377318
SLMAP	-0.300463735	0.002575766	0.01638752
MBTPS2	-0.257674247	0.002579867	0.016406179
RPAP3	-0.353142477	0.002583778	0.01642361
LOC101903301	0.38985726	0.002587711	0.016433722
ME1	0.072758594	0.002587187	0.016433722
LOC507930	-0.305973103	0.002592199	0.016454775
ARFGEF2	-0.374918957	0.00259348	0.016455464
LOC101903649	-0.145658227	0.002602532	0.01650544
ARID4B	-0.083133457	0.002610366	0.016535604
C18H16orf87	-0.284334598	0.002612001	0.016535604
RRM2B	-0.422713237	0.002611704	0.016535604
C7H19orf57	0.35173456	0.002608581	0.016535604
PAFAH1B2	-0.164690804	0.002619329	0.016552635
HSCB	0.165601911	0.002618123	0.016552635
MLST8	-0.138917611	0.002619409	0.016552635

DHCR24	-0.511289702	0.002616003	0.016552635
LOC112444958	0.493714446	0.002626706	0.016591275
LOC101905167	0.11593897	0.002631086	0.016611464
ARFGAP1	0.084078569	0.0026338	0.016621125
SCAPER	-0.358947434	0.002642378	0.016667766
LOC783612	0.835667724	0.002648491	0.016698822
AFMID	0.234464787	0.002653327	0.016721796
RASA2	-0.133998543	0.00265737	0.016739763
TMCO6	0.2437569	0.002669085	0.016806015
LOC104976274	0.216970621	0.00267509	0.016832594
STON1	0.04744383	0.002675705	0.016832594
AGA	-0.286110493	0.002677713	0.01683768
GSKIP	-0.182516634	0.002694551	0.016928392
OVOL2	0.783425827	0.002693763	0.016928392
TXNDC17	0.403095267	0.002697721	0.01694072
SMIM8	0.217584447	0.002699418	0.0169438
IRGQ	-0.002242862	0.002709688	0.017000656
RAB34	0.529064292	0.002712155	0.017008532
SH3BGRL	-0.01842737	0.002715475	0.017021744
RBM42	0.33288888	0.002720215	0.01704385
IER3IP1	0.122375096	0.00272715	0.017079672
TPT1	0.238014524	0.002739797	0.017151226
UTRN	-0.294781319	0.002757731	0.017255798
TMEM59	-0.149744779	0.00276404	0.01728375
CISD3	0.529852679	0.002764661	0.01728375
SNX14	-0.049830826	0.002779078	0.017358417
GLA	-0.464713251	0.002778489	0.017358417
ATG3	0.020513576	0.002782312	0.01736316
BCL2L2	0.11945297	0.002781437	0.01736316
AKAP9	-0.254521731	0.002799243	0.017437803
LYRM7	-0.136836872	0.002798175	0.017437803
SDHAF2	0.311479168	0.002798068	0.017437803
ASCC1	0.941745902	0.00279874	0.017437803
FAF2	-0.357316336	0.002801173	0.017442089
CKS1B	0.323562296	0.002804547	0.017455353
TMEM161B	-0.348927244	0.002807228	0.017456944
FAAP20	0.496929572	0.00280729	0.017456944
TIMM10	0.826719057	0.002813648	0.017488726
LOC619029	0.01379285	0.002816718	0.01749232
EDRF1	-0.407538282	0.002816172	0.01749232
LOC512953	0.190945326	0.002821915	0.017514243
DHX35	-0.468317785	0.002822744	0.017514243

SNX13	-0.07628183	0.002827667	0.017529288
PRAP1	0.188709325	0.002826935	0.017529288
IQANK1	0.181349219	0.002834447	0.017548063
UBE2Q2	-0.277680869	0.002832972	0.017548063
LOC505972	0.403451301	0.002834322	0.017548063
ADRB2	0.16824096	0.002840857	0.017579994
VPS72	0.166800446	0.002843129	0.017586302
MFSD14A	-0.232624951	0.002846268	0.017597963
CCNT1	-0.956876726	0.002849878	0.01761252
TMEM168	-0.623454847	0.002855074	0.01763687
FCHO2	-0.584194108	0.00286642	0.017684394
GPS1	0.074649216	0.002865968	0.017684394
DHRS7B	0.459098996	0.002866548	0.017684394
COL8A2	2.006898166	0.00287382	0.017721468
HOOK1	-0.648284372	0.002881583	0.01775374
LOC104974554	-0.449133457	0.002880972	0.01775374
OXSRI	-0.34331478	0.002888299	0.017779969
HLTF	0.018591298	0.002888374	0.017779969
TLR2	-0.503950212	0.002897031	0.017825443
ERCC6L2	-0.52359001	0.002898733	0.017828099
TBC1D12	-0.1591206	0.002902631	0.017844249
DRG2	0.134463073	0.002906046	0.017857425
ATG2B	-0.321333326	0.002909702	0.017864246
COL16A1	0.199168542	0.002909596	0.017864246
SLC12A2	-0.204624692	0.002914618	0.017886601
CBR4	0.050375114	0.002921047	0.017918226
LOC112449563	0.042317647	0.002924812	0.01793348
RASSF3	-0.464475362	0.002932333	0.017971745
SSH3	0.159979139	0.002943182	0.018030363
LOC104973803	0.221808338	0.002949091	0.018058676
SURF2	0.423519151	0.002951333	0.018063921
RPS24	0.31911767	0.002952521	0.018063921
NQO2	0.5448635	0.002969831	0.018161907
PLXNA2	-0.33628894	0.002975442	0.018188295
ST8SIA4	-0.840423007	0.002982285	0.018215157
CASD1	-0.255125621	0.002982432	0.018215157
KDSR	-0.421224234	0.002984157	0.018217768
L3MBTL3	-0.353036055	0.002995661	0.018280042
ZNF407	-0.161341347	0.002997341	0.018282347
LOC782560	-0.532129165	0.003002899	0.018308289
NCOA4	-0.032730272	0.00300973	0.018341973
DLD	-0.242431509	0.003012571	0.018351314

MGMT	0.383051435	0.003017629	0.018374154
COL18A1	0.308779511	0.003023153	0.018399803
TAF6L	0.128342393	0.003032457	0.018448433
IDH3G	0.241888702	0.003035749	0.018460461
C2H2orf76	-0.206813995	0.003042695	0.018494684
TCEAL8	0.094515959	0.003045889	0.018506081
MAP3K6	0.010827242	0.003051435	0.018531759
CASKIN1	0.096334619	0.003057422	0.018560087
XYLT1	0.231796295	0.003060814	0.018572644
LOC784735	0.559205821	0.003062706	0.01857609
SEC61G	0.344067045	0.003064857	0.018581111
VPS29	0.149235156	0.003072285	0.018618098
USP45	0.02204933	0.003073675	0.018618482
RPP21	0.471889084	0.003075108	0.018619132
GTPBP8	-0.524385234	0.003078682	0.018632732
DBNL	0.241243097	0.003086021	0.018669095
RPS6KA6	-0.520449909	0.003091537	0.018694408
ALG11	-0.22351024	0.003095688	0.018711446
PKP4	-0.040641422	0.003103599	0.018751189
LRRC24	0.212353995	0.003106681	0.018754017
ARFRP1	0.41226131	0.00310674	0.018754017
H2AFX	0.56720496	0.003118631	0.01879705
LOC112449063	0.624518105	0.003119226	0.01879705
LOC112443001	-0.009146067	0.003116684	0.01879705
EPRS	-0.142488907	0.003117249	0.01879705
FANCA	-0.08969514	0.0031223	0.018807503
LOC101904057	-1.048557122	0.003133832	0.018868866
SHROOM2	-0.744188822	0.003136406	0.01887627
EHD4	-0.42319978	0.003137893	0.018877121
LOC101906200	0.585853624	0.00314442	0.018890204
NUDT16L1	0.03511336	0.003143159	0.018890204
TOP3B	0.158438981	0.003144513	0.018890204
IDH1	-0.326571864	0.003145451	0.018890204
RABEPK	0.328466311	0.003148055	0.018897753
STUB1	0.171380984	0.003153019	0.018919464
WEE1	-0.1134083	0.003159129	0.018944827
MIA	0.69386516	0.003160589	0.018944827
NUAK2	0.271131936	0.003161296	0.018944827
TREX1	0.752411463	0.003166385	0.018967225
RANBP3	0.234612415	0.003169662	0.018978759
WDR44	-0.515007479	0.003180569	0.019035944
AMPH	1.046718994	0.003189168	0.019079274

RPS10	0.382341315	0.003192217	0.019089378
IFT20	0.229261077	0.003193833	0.019090905
ID3	0.566528084	0.003197044	0.019093838
EVL	0.426872124	0.003196408	0.019093838
NSD3	-0.500180316	0.003199887	0.019102683
LOC101907853	0.214104553	0.003201315	0.019103084
PEAR1	0.224084642	0.003212946	0.019164342
RWDD4	-0.204331181	0.003216695	0.019178552
DCAF15	0.000431381	0.003220973	0.01919279
APOM	-0.061970609	0.003222639	0.01919279
SEC23A	-0.360420223	0.003223185	0.01919279
MYL9	0.776285181	0.003229634	0.019214884
SLC9A3R2	0.437306368	0.003228816	0.019214884
CAMLG	-0.058671858	0.003232894	0.019226129
MTMR12	-0.652429679	0.003234648	0.019228416
SLC19A2	-0.557478841	0.003239714	0.019250375
C17H4orf46	-0.231756293	0.003263801	0.019377091
LZTS2	0.379412285	0.003263424	0.019377091
PLP2	-0.063138312	0.003265816	0.019380858
OTOP1	0.526675354	0.003281912	0.019458195
USP6NL	-0.256999515	0.00328267	0.019458195
NLK	-0.155791634	0.003283007	0.019458195
UHMK1	-0.817949859	0.003297382	0.019527169
TMEM220	0.270358718	0.003297427	0.019527169
TMEM203	0.15501035	0.003301368	0.01954226
MRPL49	0.257020105	0.003305483	0.019558371
LRRC58	-0.835437556	0.003308078	0.019565475
LOC112441456	0.207157864	0.003309981	0.019568485
TFE3	0.003828862	0.003317848	0.019606736
SELENOH	0.420864688	0.003326724	0.019650912
THOC6	0.266389526	0.003334421	0.019688093
TEX264	0.191892805	0.003349796	0.019769942
CINP	0.597835787	0.0033511	0.019769942
NSUN5	-0.052708069	0.003366446	0.019852132
MGAT2	-0.041270849	0.003372857	0.019881584
PRKAB1	0.059089851	0.003376459	0.019890168
F8A1	0.265286319	0.003377148	0.019890168
XPO6	-0.106079918	0.003386703	0.019938075
LOC783376	0.392216919	0.003392397	0.019963225
EIF1	0.34524636	0.003399418	0.019982895
OGFOD2	0.0570725	0.003398022	0.019982895
DOCK4	-0.430759126	0.003400011	0.019982895

LOC112447079	-0.508058892	0.003401541	0.019983519
MRNIP	0.197009802	0.003406641	0.020005104
LOC101904642	-0.536356961	0.003420597	0.020078664
MRPL12	0.369812294	0.003426806	0.020106701
CPM	-0.413667063	0.003431439	0.020125466
SDC1	-0.210543599	0.003441397	0.020175442
PSMB3	0.228652926	0.00344444	0.020184853
LRWD1	0.035425843	0.00345451	0.020235412
ACTN4	-0.17696278	0.003462866	0.020260122
HOOK2	0.16946033	0.003464313	0.020260122
PSMB7	0.252612443	0.003462275	0.020260122
PIEZO1	-0.322146422	0.003464503	0.020260122
H2AFJ	0.375567218	0.003472519	0.020290096
SNRPN	0.291730328	0.003472127	0.020290096
LAMA5	0.265876497	0.003480396	0.020327657
PFN1	0.597376149	0.003498578	0.020416858
HECA	-0.018549424	0.003498532	0.020416858
E4F1	0.122114893	0.00351823	0.020523008
COX4I1	0.209508339	0.003524819	0.020536077
ERCC4	-0.583823337	0.00352486	0.020536077
HNRNPU	-0.10880585	0.00352343	0.020536077
ZNF852	0.558830357	0.003532294	0.020570852
ARHGAP35	-0.089881341	0.003537512	0.02059269
RPL36	0.554772282	0.003540273	0.020600218
DEF6	0.055073163	0.003546468	0.020627713
AR	-1.241145647	0.003548815	0.020632815
CHMP1B	-0.139417945	0.003552318	0.020644629
NCOA7	-0.493485864	0.003559161	0.020654377
PNPO	-0.162191918	0.003559882	0.020654377
CNOT3	0.06690621	0.003559109	0.020654377
C11H9orf16	0.652929368	0.003556274	0.020654377
BLOC1S5	-0.192949961	0.003576006	0.020739357
SNAPC2	0.283908916	0.003578077	0.020742798
SHROOM3	-0.117536047	0.003580048	0.020745651
ZNF263	0.202949746	0.003582211	0.020749619
CPSF1	-0.031914155	0.003591822	0.020796709
C23H6orf15	-3.510933491	0.003593501	0.020797852
ZNF576	0.236279138	0.003596641	0.020807444
CARD10	0.586854986	0.003605349	0.020849225
TPM2	0.664945871	0.003616778	0.020906706
PATZ1	-0.052283552	0.003623725	0.02093824
MRPL58	0.22173374	0.003635997	0.021000501



LOC112446036	-0.462510232	0.003638538	0.021006533
NGEF	0.351935667	0.003646284	0.021042595
GET4	0.063166221	0.003650526	0.021058417
MDFIC	-0.250916572	0.003655271	0.021077127
EMP3	0.540908623	0.003661115	0.021102158
COX5B	0.369171721	0.003674175	0.02116874
LOC783255	0.397694175	0.003691959	0.021259413
EXOC6B	-0.167561898	0.003692943	0.021259413
MYH9	0.033830096	0.003701149	0.021297919
IFIT5	-0.075001004	0.003704145	0.021306424
HDAC2	-0.079249755	0.003706316	0.021310178
NDUFS5	0.284043097	0.003709629	0.02131264
LOC505199	0.183142572	0.003709781	0.02131264
ATP5PD	0.385313471	0.003717907	0.021341853
FBXO21	0.014579265	0.003716404	0.021341853
ZNF76	0.186628869	0.003722444	0.021359161
ICMT	-0.023977554	0.003727863	0.021381513
DGCR6L	0.503627684	0.003735657	0.021417463
DNLZ	0.18186035	0.003737988	0.021422073
NDUFV1	0.278735622	0.00374235	0.021438321
VMA21	-0.006489364	0.003751941	0.021484492
MRPL55	0.505561942	0.003763081	0.021539495
BRAP	-0.463017836	0.00376873	0.021543811
NOL11	-0.316861505	0.003767954	0.021543811
PNRC2	-0.084715626	0.00377151	0.021543811
THYN1	-0.126577569	0.00377008	0.021543811
STX18	-0.006531241	0.003768795	0.021543811
RPAIN	0.122721201	0.003774546	0.021552379
LOC101905668	0.478132304	0.003778743	0.021567569
LOC781256	-0.26213861	0.003794608	0.021640522
LOC100848991	-0.42152561	0.003793665	0.021640522
HIGD2A	-0.08433437	0.003801142	0.021668981
LOC101903385	-0.173923271	0.003808566	0.021702485
WDR73	0.190970232	0.003810387	0.021704054
DDX52	-0.321328803	0.003819681	0.021748162
RPL32	0.348128863	0.003827566	0.021784221
NPAT	-0.287076145	0.00383261	0.021804084
STAT1	-0.365340322	0.003842622	0.021830038
COX4I2	0.543950591	0.003843393	0.021830038
RAPGEF3	0.375942413	0.003840497	0.021830038
CALML4	0.282123927	0.003840424	0.021830038
LOC100335936	0.26639431	0.003849461	0.021844626

APAF1	-0.454161244	0.003850631	0.021844626
LOC112441881	0.280857955	0.003848741	0.021844626
ZFX	-0.527775502	0.003854237	0.021856249
ARL3	0.650722926	0.003858593	0.021872114
PLEKHA5	-0.298933012	0.003867554	0.021914058
ELP5	0.207892113	0.003869556	0.021916554
NPTXR	0.500384716	0.003892032	0.022034959
NDUFB3	0.354528884	0.003893979	0.022037097
UQCR10	0.38693663	0.003900472	0.022042527
HMGN1	-0.169704211	0.00390248	0.022042527
TSHZ1	0.170600536	0.003898985	0.022042527
FADD	0.224196385	0.003902792	0.022042527
AVL9	-0.54117123	0.003897756	0.022042527
C2CD5	-0.24075859	0.003922501	0.022144934
CATSPERD	-0.727391757	0.00393308	0.022195727
HEXDC	-0.005266782	0.003936985	0.022199913
LOC101902037	0.626400287	0.003935437	0.022199913
COX14	0.501209857	0.003940383	0.022210152
PSMA3	0.208374115	0.003944096	0.022222152
PCDHA13	-0.033998327	0.003946429	0.022226378
LOC101903424	-0.1660616	0.003951084	0.022243665
CA13	-0.378024493	0.003955555	0.022255668
UBLCP1	-0.210782483	0.003956387	0.022255668
USP37	-0.128303598	0.003963454	0.02228649
LOC100847890	0.649229491	0.003970528	0.022301426
SNX10	-0.626811574	0.003971985	0.022301426
TBRG4	0.355862226	0.003972466	0.022301426
VPS41	-0.23997082	0.003970484	0.022301426
KANSL2	0.188755417	0.003980053	0.022335084
HNRNPA2B1	0.002338451	0.003984403	0.022350555
DIRC2	-0.308252334	0.0040038	0.022450391
ABCE1	-0.312081828	0.004009515	0.022464488
RASSF9	-0.588176549	0.004008891	0.022464488
LOC101907920	0.837668109	0.004018124	0.022503742
PSMG1	0.323714996	0.004019758	0.02250391
RNF208	0.238286899	0.004030437	0.02255237
IFT80	-0.346050063	0.004033384	0.02255237
CHORDC1	-0.26762163	0.004032352	0.02255237
RNF111	-0.286656214	0.004034842	0.02255237
DDX58	0.094444538	0.004036627	0.022552507
DZIP3	-0.224830566	0.00403808	0.022552507
LOC101904449	0.393841047	0.004042752	0.022560648

NTPCR	0.129097827	0.004041382	0.022560648
NFKBID	0.088368112	0.004057288	0.022632766
TRIM44	0.519684985	0.004063567	0.022658787
PPP1R14B	0.463474992	0.004069587	0.022683344
HSPG2	0.204374744	0.004073323	0.022695153
OSBP	-0.246653951	0.004075519	0.022698377
PLEKHH2	-0.074585372	0.004082732	0.022729528
ENPP3	-0.392224926	0.004087208	0.022745429
TIAM1	-0.109306573	0.00408994	0.022751606
ADGRL2	-0.450479257	0.004093453	0.022762131
SETBP1	-0.141489749	0.004095256	0.022763139
PARK7	0.25741483	0.004101308	0.022787752
RIN1	-0.106015777	0.004103264	0.022789596
EDEM3	-0.71102694	0.004111742	0.022827652
TSC22D2	-0.081368177	0.004128018	0.02290895
ZBTB1	-0.538066401	0.004141644	0.022975482
RAB11FIP2	-0.851926685	0.004144257	0.022980898
AP3S2	0.353779864	0.004148586	0.022995815
RPL23	0.344096397	0.004159218	0.02304152
HTRA2	0.388841532	0.004160115	0.02304152
TMEM210	0.787010663	0.004170672	0.023090881
CHMP2A	0.45104443	0.004175359	0.023104654
TRAF3	1.355618043	0.004176452	0.023104654
TTC37	-0.190928029	0.004191396	0.023178186
TSPO	0.419387016	0.004216341	0.023306949
OXCT1	-0.730242973	0.004220353	0.023319943
KDM8	-0.466067766	0.004234132	0.023359691
SNX7	-0.159578675	0.004233208	0.023359691
USP42	-0.392841303	0.004234204	0.023359691
LANCL1	-0.153006593	0.004233557	0.023359691
PBX1	-0.089371125	0.004250467	0.023440199
TCAF2	0.631352421	0.004258185	0.023473536
RGS11	0.43000474	0.004261186	0.023480859
CDCA4	0.645555127	0.004268241	0.023510502
AP1G2	0.119511928	0.004274703	0.023536861
LOC617475	0.169314008	0.004279593	0.023553053
EEF1G	0.18430469	0.004282678	0.023553053
NBDY	-0.241466938	0.004281939	0.023553053
ZC3HC1	0.079650425	0.004285812	0.023561053
SET	-0.227094665	0.004308702	0.023673661
MCCC1	-0.088239661	0.004309669	0.023673661
CD109	-0.256231045	0.004312861	0.023681924

ELOVL6	-0.204306686	0.004315587	0.023687627
AGPAT3	-0.287735785	0.004327737	0.023745032
ANKIB1	-0.05357318	0.004329643	0.023746207
PREB	0.02425965	0.004351906	0.023858984
HEYL	0.630299525	0.00435361	0.023859008
SEL1L3	-0.289963254	0.004366146	0.02391837
JRK	-0.108464653	0.004369132	0.023925388
SERPINC1	-0.037702716	0.004391728	0.024039744
KBTBD8	-0.745966961	0.004394702	0.024046647
ZNF646	0.000879389	0.004409015	0.024115561
RGP1	-0.073417494	0.004412275	0.024123989
WBP4	0.003892349	0.004423313	0.02417492
MMAB	0.499342738	0.004425063	0.024175073
RASL12	0.281988941	0.004447426	0.024287793
NFATC2IP	-0.547229923	0.004451595	0.024292586
LOC107132302	0.711766459	0.004451766	0.024292586
GABRE	0.852530498	0.004463339	0.024346274
TAF10	0.447109269	0.004467576	0.02435992
RMI1	-0.04704123	0.004500033	0.024508328
RAD50	-0.259409947	0.004499619	0.024508328
VKORC1L1	-0.215522071	0.004499733	0.024508328
RPS2	0.263688335	0.004503772	0.02451918
TTC30A	-0.170977812	0.00450625	0.024523155
NME2	0.022094985	0.004522857	0.024603994
PTPN13	-0.361801054	0.004531189	0.024630222
IAH1	-0.161364773	0.004531013	0.024630222
CAST	-0.115760297	0.00454106	0.024674323
LOC112449099	1.533692947	0.004544229	0.024681988
KIAA1211L	0.566106697	0.004549509	0.024701105
LOC784914	-0.130603645	0.004554973	0.024721211
HIPK2	-0.130707539	0.00456414	0.024722571
VAMP5	0.529845603	0.004569078	0.024722571
PWWP2B	0.700522553	0.004569043	0.024722571
MRPL40	0.633757281	0.00456192	0.024722571
TSKS	-0.386853374	0.004558574	0.024722571
LOC789587	0.643629924	0.004569316	0.024722571
PI4K2B	-0.054758729	0.00456261	0.024722571
LOC107131728	0.589453601	0.004564239	0.024722571
TMBIM6	-0.041643902	0.004574937	0.024743445
CDC73	-0.080754162	0.00458487	0.024787614
DLK2	-0.055092846	0.004589729	0.024804332
DYNLT3	0.09126468	0.004595	0.024823257

TNRC6C	-0.167211419	0.004599024	0.024830511
ARSK	-0.287084629	0.004599881	0.024830511
IWS1	-0.160381282	0.00460423	0.024844432
FIS1	0.242618091	0.004615495	0.024895648
NMNAT1	-0.41326611	0.004624969	0.024937166
TMEM200B	0.195268244	0.004638683	0.025001506
FARS2	0.277601675	0.004679116	0.025209748
LOC537017	-0.880351411	0.004684577	0.025229485
KLHL11	-0.111785329	0.00468894	0.025243297
TIMM8A	0.317120503	0.004712261	0.025359121
DNAJC19	0.297249271	0.004714164	0.025359638
RPS12	0.107151813	0.004742937	0.025504642
LRRC69	-0.799762835	0.004753107	0.025549542
SIRT4	0.047379664	0.004756282	0.025556822
HINFP	0.203399052	0.004768595	0.025613179
GPR107	-1.096164078	0.004776964	0.025648311
PHLDB1	0.501463455	0.004788953	0.025700466
TXNRD1	-0.300713656	0.004792347	0.025700466
LOC101906131	0.399851968	0.004792908	0.025700466
LOC614614	0.4645393	0.004794002	0.025700466
BLOC1S6	-0.339533836	0.004796673	0.025704964
DNAJC30	0.241309392	0.004799355	0.025709522
SIRT7	0.033622256	0.004801266	0.025709947
CEP120	-0.204813345	0.004807404	0.025732995
LOC781064	0.178019771	0.004814587	0.025761622
TRIM4	-0.287916387	0.004831663	0.025829318
ARAP1	0.170011596	0.00483276	0.025829318
JMJD7	0.140798575	0.004831739	0.025829318
FBXL4	-0.328220169	0.004840638	0.025861571
ZNF260	0.221853027	0.004853773	0.025910953
LOC107131489	-0.336652493	0.004854165	0.025910953
RAD23A	0.235784047	0.004855419	0.025910953
NELFB	0.257470208	0.004859477	0.025922751
GIN1	-0.279289343	0.004872159	0.025975854
LNPK	-0.232310316	0.004873133	0.025975854
RPLP0	0.229467	0.004875773	0.025980056
TSPYL4	-0.077360309	0.004880111	0.025993304
VWA8	-0.178143631	0.004882255	0.025994858
MIER1	-0.048104823	0.004885644	0.026003041
AMMECR1L	0.032891264	0.004892545	0.0260299
QDPR	0.219644731	0.004907603	0.026100118
OTX1	0.094556916	0.004921118	0.026152171

NUTF2	0.448139908	0.004920086	0.026152171
ZNF750	-0.495304713	0.00492499	0.026162846
SYCE1L	0.483205911	0.004928392	0.026171012
RAB3GAP2	-0.279160523	0.00493569	0.026199853
OPHN1	-0.158588256	0.004937913	0.026201742
NUDT2	0.467210146	0.004941168	0.026209105
GNAI3	-0.227775705	0.004945857	0.026224067
KLHDC8A	-0.420432484	0.004962376	0.026301717
JPH1	-0.122770921	0.004971987	0.026342708
CRAT	-0.017375151	0.004982444	0.026388153
HSD17B8	0.364537333	0.004985444	0.026394079
KBTBD7	-0.087074792	0.005003767	0.026476428
MTMR9	0.40358645	0.005004771	0.026476428
MRPL16	0.216591467	0.005009147	0.026487478
TMEM258	0.346329259	0.005010634	0.026487478
GPAM	-0.365182475	0.005025973	0.026558556
ECHDC1	-0.212537945	0.005033663	0.026585433
MFF	0.188983979	0.005034847	0.026585433
CSTF3	-0.068230617	0.005038039	0.026592281
LOC509155	-0.028547654	0.005052044	0.026632284
LOC104969097	0.107301205	0.005048315	0.026632284
NELFA	0.006425845	0.005053208	0.026632284
XRCC6	0.051197743	0.005049688	0.026632284
ANKHD1	-0.2777901	0.005057453	0.02663795
SF3B6	0.374631062	0.005058079	0.02663795
EDEM2	-0.222050502	0.005077116	0.026728179
LOC783466	-0.069505097	0.005086528	0.026744456
ZNHIT1	0.266824825	0.005084856	0.026744456
AIMP2	0.066800292	0.005087731	0.026744456
RN7SL1	0.220715589	0.00508783	0.026744456
SRRM1	-0.48364344	0.005095641	0.026758601
NPAS2	0.291728874	0.005096241	0.026758601
WDR7	-0.158019642	0.005096035	0.026758601
ZNF12	-0.827831478	0.005102849	0.026783277
H2AFY2	0.961663366	0.005117279	0.026846472
NR2C1	-0.229533802	0.005118715	0.026846472
LOC100848114	3.380451787	0.005125581	0.026862406
CREG2	0.31340143	0.005124103	0.026862406
TRADD	0.11630114	0.005130197	0.026876564
ZNF354A	-0.278350951	0.005134564	0.026887007
LOC101905894	0.113256348	0.005136022	0.026887007
SULT1B1	-0.253602001	0.005149659	0.026948347

ZNF292	-0.290296697	0.005154806	0.026965224
CASK	-0.594579938	0.005161784	0.026991667
AKAP7	-0.532960879	0.005168986	0.02701926
ETF1	-0.055840512	0.005180227	0.02706794
LOC782787	-2.618944016	0.005184396	0.02707964
RPL22L1	0.137335905	0.005187249	0.027084466
ASB11	-0.145588363	0.005198628	0.027133581
HDDC3	0.161501023	0.005200522	0.027133581
SLC25A40	0.245890724	0.005208408	0.027164624
IMMP1L	0.249898941	0.00521394	0.027183374
GRHPR	0.136446624	0.005225819	0.027235191
NPTN	-0.006143844	0.005235608	0.027276082
CLDN5	0.636422715	0.005238876	0.027282978
LOC101908535	0.714789292	0.005244547	0.02730238
ATP5MC2	0.252800098	0.005294016	0.027529276
LOC101907518	0.368383643	0.005293472	0.027529276
DICER1	-0.357108363	0.005292203	0.027529276
LIN9	-0.067593849	0.005306942	0.02758627
B9D1	0.71912578	0.005318278	0.027624736
KLF10	-0.344577887	0.005316615	0.027624736
LYPLAL1	0.037552905	0.005322204	0.027630352
STAR	0.52639528	0.005323297	0.027630352
LOC101906455	0.385942412	0.005335754	0.027684774
MRM2	-0.43497324	0.005341227	0.02770293
LYST	-0.282427538	0.005348894	0.027722206
TRAPPC6A	0.366780432	0.005347696	0.027722206
ACCS	-0.438614991	0.005355282	0.027740704
COPS6	0.265272992	0.005358393	0.027740704
AP2S1	0.342428126	0.005357709	0.027740704
APOO	0.027101139	0.005361898	0.027748614
MAP2K2	0.312878279	0.005370618	0.027773257
H2AFV	0.190343496	0.005369233	0.027773257
BCKDHB	-0.082011079	0.005378619	0.027804388
CRACR2B	0.641382027	0.00538238	0.027813588
DDAH2	0.524044255	0.005386785	0.027824292
UPK1B	-0.639860846	0.005388416	0.027824292
API5	-0.132340105	0.005391232	0.027828594
SOCS7	-0.078416917	0.005393757	0.027831392
MTX3	-0.996222173	0.005405291	0.027880656
RNF220	0.244894213	0.005407853	0.027883619
LOC783686	-0.373892612	0.005411231	0.027890791
COPS8	0.049835887	0.005416047	0.027905367

SMARCA2	-0.237906609	0.005427981	0.027956591
PREPL	-0.428735535	0.005430358	0.027958573
TXN2	0.406781013	0.00543266	0.02796017
F2RL1	-0.299402007	0.00543924	0.02797352
BPNT1	-0.159664939	0.005438443	0.02797352
CFAP97	-0.357635397	0.005457163	0.028055417
MPG	0.491432321	0.005461515	0.028064763
ACPP	-0.994085508	0.005462981	0.028064763
COQ10B	-0.307049282	0.005467014	0.028075208
AAAS	0.153393158	0.005494989	0.028208549
DEFB4A	3.148286179	0.005513182	0.028291596
RECQL	-0.147039951	0.005520298	0.028317757
TYROBP	0.280312965	0.005530021	0.02835727
PPP4R3A	-0.228630899	0.00553529	0.028373921
SGPP2	-0.344245781	0.005551017	0.028444151
RDH12	-1.263110227	0.005558302	0.028465656
VPS26A	-0.181435608	0.005559271	0.028465656
ZDHHC6	0.154837656	0.00556705	0.028495091
BIRC3	-0.295509838	0.005574618	0.028522023
CEP89	-0.833896562	0.005576376	0.028522023
WRN	-0.077526582	0.005581843	0.028529196
GTF2E1	-0.331617773	0.005581276	0.028529196
LOC112444215	0.467177167	0.005596366	0.028582603
PARN	-0.147120355	0.005595317	0.028582603
ZFYVE19	0.056581414	0.005625676	0.028721849
KMT2C	-0.030475304	0.005646007	0.028815168
ECSCR	0.376560251	0.005656485	0.02885815
IKBIP	-0.208899581	0.005665929	0.028895824
KDEL2	0.134751469	0.005668145	0.028896627
MRPL13	0.256726526	0.005684425	0.028969101
IPP	-0.351427629	0.005693667	0.029005667
DBI	0.190849625	0.005707133	0.029063718
COPZ2	-0.293466756	0.005735416	0.029189672
ZSCAN4	0.741159857	0.005736025	0.029189672
CAT	-0.060006068	0.005753063	0.029265763
CD46	-0.354835948	0.005758089	0.029280719
EIF4G1	-0.139437646	0.005761461	0.029287254
LOC112448166	0.617259008	0.005773663	0.029338651
MIS18BP1	-0.204622303	0.005790749	0.029414824
ABRACL	0.506943178	0.005796306	0.0294324
KCNE3	0.070070678	0.005803344	0.029457481
COMTD1	0.396143847	0.005811433	0.029487877



ZNF207	-0.168398497	0.00581589	0.029499823
LOC786348	0.306634698	0.005839491	0.029598143
GPR137	-0.076145834	0.005837455	0.029598143
RPS26	0.187270083	0.005841828	0.029599298
GFM1	-0.179018277	0.005854363	0.029652108
CENPC	-0.42192947	0.005883339	0.029788116
OSBPL1A	-0.017036761	0.005895101	0.029836908
GCC1	-0.351751859	0.005901682	0.029839934
PPP2R2A	-0.260309368	0.005902077	0.029839934
RACK1	0.318783882	0.005898264	0.029839934
LOC784251	0.388299281	0.005922022	0.02992999
TMEM209	-0.059977458	0.005935841	0.029989032
ATP5MC1	0.396311506	0.005958895	0.030083847
LOC112448834	0.386282534	0.0059571	0.030083847
MBD6	0.128869337	0.005963126	0.03009438
NCOA2	-0.760717638	0.005967424	0.030105245
RPL6	0.321558158	0.00597126	0.030113777
CYHR1	0.235519546	0.005986734	0.030180966
LBHD1	1.053778092	0.005991362	0.030182615
KIN	-0.413135676	0.005989935	0.030182615
DUSP23	0.310174566	0.005997383	0.030191275
LOC101908759	0.372988431	0.00599557	0.030191275
BTBD8	-0.430103557	0.006018997	0.030289217
PDE4DIP	-0.402570255	0.006028281	0.030325063
SNRPA	0.114064035	0.006048456	0.030415648
FAM212B	0.345752746	0.006055366	0.030439493
CRYZL1	-0.294505479	0.006059695	0.030450346
MYO15B	0.201984648	0.006068165	0.030481995
GLMN	-0.183063734	0.006070442	0.030482525
PDGFB	0.570792486	0.006075892	0.030498976
TMEM79	-0.303342276	0.006086773	0.030509951
NEURL4	-0.17117837	0.006083317	0.030509951
XPC	0.221514406	0.006086058	0.030509951
PARP9	0.279453693	0.006083453	0.030509951
RPTOR	-0.330421837	0.006090669	0.030518578
LOC101902937	0.298676168	0.006101778	0.030563333
LOC112448832	0.664467629	0.006111459	0.030600904
ANO10	-0.073278362	0.006126157	0.030630787
MAGED1	-0.040804212	0.00612327	0.030630787
NAPA	0.249899328	0.006124885	0.030630787
CDK5RAP3	-0.116947532	0.006120527	0.030630787
LOC100297616	0.46709424	0.006176566	0.030871831

CCHCR1	0.250787132	0.006179341	0.030874706
SUPT6H	-0.121035796	0.006185432	0.03089414
RALGDS	0.260934371	0.006190955	0.030910725
RNF215	0.241859957	0.006195837	0.0309241
LOC104971613	-1.200103581	0.006206843	0.030968022
SLC12A9	0.107573822	0.006225287	0.031047561
ODF2	-0.228395082	0.00622721	0.031047561
BRWD1	-0.349247395	0.006232923	0.031053987
MARCH2	0.102364483	0.006231377	0.031053987
VPS35	-0.126421275	0.006240269	0.03107955
ZBTB8OS	1.128613587	0.006243421	0.031084221
FAAP24	0.139253227	0.006256166	0.03113663
GPAA1	0.251982081	0.006258529	0.031137348
UFSP1	0.243178409	0.006263414	0.031150606
ASCL2	0.214126139	0.006270511	0.031174857
LOC112442057	-0.203378526	0.006273536	0.03117885
LOC101902449	0.594226136	0.006298827	0.031293466
TOMM5	-0.218529708	0.006328168	0.031428108
FTL	0.804314892	0.006349034	0.031520584
MOSPD1	-0.431681618	0.00635842	0.03155602
CD2BP2	0.231911852	0.006376482	0.031623297
SSR1	0.02618219	0.006375546	0.031623297
CORO1C	-0.343594542	0.006380227	0.031630692
MARVELD2	0.006682088	0.006384441	0.031640408
LOC112443325	0.092173338	0.006392867	0.031670982
PHF5A	0.25178238	0.006407831	0.031733914
BICRA	0.267124995	0.006421115	0.031788484
FBXL3	-0.152099626	0.006426016	0.031801529
ARHGEF12	-0.512608306	0.006432078	0.031820307
ARAP2	-0.386129692	0.006458573	0.031940123
WNT5A	-0.65553353	0.006463389	0.031941431
GYG1	0.294257203	0.006462335	0.031941431
LOC100847870	-0.002940624	0.006473273	0.031979017
RALBP1	0.022832536	0.00648023	0.031990864
LOC112445943	0.291322781	0.006479816	0.031990864
LOC101902705	-0.263666078	0.006489008	0.032022937
RPL12	0.288851869	0.006498419	0.032051674
TSFM	0.248547587	0.006501683	0.032051674
LOC101903806	-0.322578851	0.006499639	0.032051674
SAAL1	-0.6861096	0.006510134	0.03207766
TRAPPC4	0.128096119	0.006511525	0.03207766
BTNL9	-0.542942955	0.00651884	0.032091167

PKN3	0.427785321	0.006516897	0.032091167
STAT2	-0.280999401	0.006559466	0.032279841
LRRN4CL	0.294846441	0.006568584	0.032290745
NT5DC2	0.095954103	0.00656474	0.032290745
TMEM216	1.520501348	0.006568296	0.032290745
LOC507787	-0.357569038	0.006571086	0.032291736
CDKN2AIPNL	0.219500348	0.00657631	0.032306095
POP5	0.198962007	0.006597382	0.032398268
PLCXD2	-0.355166313	0.00660815	0.032439801
SELENOP	-0.210034245	0.006616534	0.032467789
M6PR	0.015042605	0.006618478	0.032467789
FOXRED1	0.2034997	0.00663343	0.032529765
PFDN4	0.131044037	0.006652297	0.032605304
SEMA6D	-1.22660993	0.006653506	0.032605304
DOK4	0.529432024	0.006655803	0.032605304
GUK1	0.408271131	0.006662213	0.032625316
RAD23B	0.018635574	0.006675391	0.03267845
MYL12A	0.29008771	0.006684414	0.032699806
HS2ST1	-0.519828449	0.006682998	0.032699806
VAPA	0.153306393	0.00668997	0.032715586
C9H6orf120	0.009849469	0.006703519	0.032770422
DYRK3	-0.227534995	0.006712194	0.032801406
ABCB7	-0.059783594	0.006725031	0.0328527
MINDY3	-0.281267153	0.006730733	0.032869115
KRT14	-0.133533758	0.006735635	0.032881615
LOC112442408	0.436143346	0.006739145	0.032887307
CIZ1	0.137179923	0.006744733	0.032903139
KPNB1	-0.221554826	0.006757857	0.032955706
COL7A1	-0.252645292	0.006763533	0.032971931
FAM214A	-0.255639331	0.006769666	0.03297892
ST7L	-0.544566743	0.006768571	0.03297892
GNMT	0.071941566	0.006772625	0.032981887
NHLRC2	-0.296378861	0.006784188	0.033026736
LOC107131864	0.508038754	0.006806809	0.033125371
LOC107133032	0.321277668	0.006827147	0.033212827
PPP1R13L	0.000788561	0.006836513	0.033239895
CCNT2	-0.481439073	0.006837448	0.033239895
PHAX	-0.007844409	0.006842028	0.033250645
DHRS13	0.315219845	0.006862368	0.033337951
CSNK2B	0.211355645	0.006872219	0.033374255
ZNF529	-0.190345272	0.006886906	0.033423263
C21H15orf40	0.135200796	0.006887102	0.033423263

TEDC2	0.366861469	0.006889455	0.033423263
LOC100848775	-0.726384062	0.006900619	0.033463732
MARC1	-0.433781077	0.006902565	0.033463732
CFAP53	-0.324210703	0.006930657	0.033567878
GPR63	-0.013033777	0.006931223	0.033567878
OSGIN2	-0.206234802	0.006931063	0.033567878
LOC616304	-0.006414628	0.006936315	0.033580954
MAGI1	0.036333225	0.00694166	0.03358366
MTX2	0.187136456	0.00694034	0.03358366
APOOL	0.178276493	0.006954039	0.033620371
PCF11	-0.229164713	0.006953225	0.033620371
SMIM10L1	0.444911999	0.006967212	0.033672457
PAIP2B	-0.15573921	0.006973828	0.033681239
LOC107131695	0.521941691	0.006971434	0.033681239
LOC112443447	0.379591993	0.006996455	0.033778894
RRAGD	-0.076185547	0.007009431	0.033818274
BAG6	0.02167103	0.007007782	0.033818274
TLE2	-0.106690072	0.007011868	0.033818407
LOC104974837	0.191336088	0.007014922	0.033821517
SLC27A3	0.005368583	0.00701975	0.033824197
TCF25	0.066737323	0.007020298	0.033824197
EBNA1BP2	0.120916595	0.007028516	0.03385217
NPY1R	0.400399107	0.007032353	0.033859029
PSMB4	0.233883671	0.007041521	0.03388039
IDI1	0.220261657	0.007041718	0.03388039
GOT1	-0.068525603	0.007044031	0.03388039
GTPBP3	0.104099474	0.007050878	0.033901705
LOC112442625	0.502901035	0.007056928	0.033919172
PAIP2	0.099257313	0.007064432	0.033943615
PEMT	-0.257414097	0.007086421	0.034037619
TSTA3	0.232618025	0.007094082	0.034062759
SHISA9	1.204832145	0.007116086	0.034156728
AFG3L2	-0.046773377	0.007136344	0.034214497
DCXR	0.427712189	0.007140311	0.034214497
SPATA24	-0.004483321	0.00713472	0.034214497
SYNGR3	0.975243476	0.007130993	0.034214497
CHTOP	0.086030264	0.007140174	0.034214497
IGFBP2	0.818312214	0.007166767	0.034329548
TTI2	-0.27718664	0.007174759	0.034356103
TOB2	0.076736767	0.007177365	0.034356859
CNOT11	-0.34792063	0.007183444	0.034368674
MLX	0.131610629	0.00718473	0.034368674

CBL	-0.279594807	0.007194774	0.034404992
FIBP	0.292429557	0.007207395	0.034453608
LOC786783	-0.096366521	0.007216169	0.034483806
ABHD3	-0.341400879	0.007234115	0.034546036
CROT	-0.340943186	0.007231795	0.034546036
GPSM2	-0.13417241	0.007259792	0.034633048
C7H5orf30	-0.183569067	0.007257297	0.034633048
ACBD5	-0.08574866	0.007256939	0.034633048
BARD1	-0.108718319	0.007262206	0.034633048
PLEKHJ1	0.128482298	0.007271462	0.034665411
NME4	0.647279119	0.007277101	0.034680514
OGFR	0.25483304	0.007287528	0.034718418
NSMF	0.321731812	0.00729884	0.034756814
RNF217	-0.024399788	0.007300541	0.034756814
ADGRL4	-0.268611359	0.007305063	0.034762178
CHCHD4	0.299884076	0.007306621	0.034762178
COQ9	0.448868155	0.007318736	0.034808016
LOC112446454	0.821026075	0.007327045	0.034835729
POMP	0.167697416	0.007335783	0.034865464
HOXC8	0.055238185	0.007366138	0.034997885
KDM5A	-0.627784219	0.007374327	0.035024934
USP21	-0.046595283	0.007378631	0.035033522
CEP135	-0.391609077	0.007385785	0.035055627
LOC112446758	0.542612403	0.007393394	0.035079881
LOC104971427	-0.800395918	0.007402158	0.035109593
CCDC34	-0.068632048	0.007410906	0.03513921
FAM207A	0.424624216	0.007431159	0.035223341
USF2	0.292668948	0.007444948	0.035276785
MISP3	0.35640447	0.007455735	0.035315979
EFS	0.024701974	0.007465002	0.035347944
POLR3GL	0.247096864	0.007469271	0.035350445
CDC14A	-0.392609319	0.007470568	0.035350445
PPOX	-0.137527957	0.0074758	0.035363283
LOC112447360	-0.409118985	0.007482839	0.035384649
NFATC4	-0.047839208	0.007489142	0.035390608
CD68	0.179359122	0.007488945	0.035390608
HTT	0.057138199	0.007500449	0.035423628
LOC614643	0.500444679	0.007501177	0.035423628
TLK2	-0.106272203	0.007506938	0.035438908
ZNF428	0.418359613	0.00750998	0.035441346
LRRC74B	0.464150262	0.007519872	0.03546418
PIK3C2G	-1.444715695	0.007518963	0.03546418

TM9SF3	-0.550478488	0.007529374	0.035491719
NAGS	-0.458668626	0.007530769	0.035491719
PITX1	0.514344681	0.007533811	0.035494138
NR1H3	0.175995335	0.007545809	0.035538733
SAT1	-0.102500025	0.007593359	0.035750687
LOC100336368	0.708083122	0.007596142	0.035751797
QPCTL	-0.207699546	0.007603234	0.035765666
ATP1A2	0.385900284	0.007604186	0.035765666
HCK	-1.397052899	0.007610764	0.035784613
FAM204A	0.285114652	0.007615615	0.035795431
USE1	0.362832205	0.007629891	0.035847565
RAD51C	-0.106349298	0.007631815	0.035847565
LONRF2	-1.01162868	0.007640308	0.035875453
LOC787057	-1.022888855	0.007643744	0.035877336
ACSL4	0.042276487	0.007648122	0.035877336
LOC100847874	-0.069510768	0.007650935	0.035877336
SPG11	-0.45422664	0.0076496	0.035877336
KDM1B	-0.407443282	0.007660558	0.035886491
WDFY1	-0.327962148	0.007659918	0.035886491
TIMM44	0.073265289	0.00766025	0.035886491
DEPDC7	-0.663517422	0.007667296	0.035906073
NFKBIZ	-0.275776923	0.007679424	0.035950871
ZNF532	-0.566823467	0.007690108	0.035976889
LOC112443177	0.22014161	0.00768864	0.035976889
DNTTIP1	0.051720207	0.007698361	0.036003499
NMI	0.297817086	0.007713346	0.03602556
LOC101907965	0.431416499	0.007712092	0.03602556
NIPA2	-0.023072139	0.007708824	0.03602556
DYNLL2	0.478187577	0.007708434	0.03602556
SNAI2	0.258950827	0.007733207	0.036106306
METTL8	-0.261664056	0.0077399	0.036125537
BOLA1	0.106459644	0.007742588	0.03612607
RC3H2	-0.292898911	0.007751556	0.036155893
ACTR10	-0.202183579	0.007769654	0.03622827
PARP3	-0.134178723	0.007775647	0.036237547
ECT2	-0.367102041	0.007776807	0.036237547
NMRK1	-0.344780817	0.007808126	0.036371405
PYGB	0.545464341	0.007813445	0.036384108
NT5M	0.110771323	0.007824442	0.036423233
DOCK1	-0.084117342	0.007831434	0.036443692
SPHK2	0.121350068	0.007841749	0.036479596
GGCX	-0.672651954	0.007858485	0.036545342

LOC781001	-0.053013709	0.007878372	0.036589331
RIMKLB	-1.064957895	0.007877528	0.036589331
LOC112446676	0.089738476	0.007872304	0.036589331
C20H5orf22	-0.329997573	0.007877114	0.036589331
LYPLA1	-0.230157466	0.007881319	0.036590908
LOC518437	-0.372050718	0.007897255	0.036652768
ANGEL2	-0.209274315	0.007907543	0.036676262
NUDCD3	0.369854848	0.007907467	0.036676262
CCDC28B	0.533021771	0.007915787	0.03670237
NDUFS3	0.045450917	0.007950409	0.036850726
DPY19L3	-0.203787032	0.007973971	0.036941718
NOX1	-0.166768553	0.007975305	0.036941718
FDX2	0.222718846	0.007981598	0.036946479
CISH	0.100995091	0.007980759	0.036946479
ABCA1	-0.730822106	0.007996112	0.037001462
DDIT3	0.423008436	0.008006118	0.037035553
CYB5RL	0.564718077	0.008009676	0.037039805
MRPL10	0.149112745	0.008018799	0.037069778
DCPS	0.219611662	0.008027144	0.037096135
TYSND1	0.049182604	0.008041352	0.037149565
C7H19orf67	0.355074913	0.008050576	0.037179939
PRODH	0.220762614	0.008062607	0.037223255
GRAMD4	-0.55327103	0.008079144	0.037287334
TLR5	0.427768547	0.008087841	0.037315202
TRIM3	0.03365676	0.008093744	0.037330165
LOC107131975	-0.482259022	0.008107205	0.037379968
ACO2	0.096473038	0.008115018	0.037403705
RASA1	-0.232073559	0.008158862	0.037585063
DNTTIP2	-0.360860501	0.008159721	0.037585063
AHCY	0.201814764	0.008192294	0.037722717
SLC6A1	-0.027359281	0.008207633	0.037756188
TMEM234	0.131414744	0.008206574	0.037756188
APRT	0.491702589	0.008203411	0.037756188
WDR83	0.485668591	0.008211194	0.037760193
MAP3K8	-0.144295557	0.008216118	0.037770462
ADAT2	0.065212226	0.008219398	0.037773167
ZNF93	0.162332709	0.008252591	0.037913293
THAP4	0.325631476	0.008277832	0.038016812
TIMM29	0.264463502	0.008297605	0.038095153
EFL1	-0.567764068	0.008304767	0.038115567
C14H8orf33	0.435218978	0.008347424	0.038298824
TRIM23	-0.532318918	0.008395502	0.038506819

CD1E	-0.878886691	0.008400366	0.038516541
SPON1	-1.646350843	0.008407108	0.038534869
SCOC	-0.173236642	0.008413471	0.038551442
KDM3B	-0.156615563	0.008418479	0.038561799
TRPV3	-0.326570631	0.008427897	0.038592343
HIF1AN	-0.228058788	0.008430752	0.038592828
TAF2	-0.293146401	0.008434727	0.038598432
TPRA1	0.111044376	0.008441196	0.038609293
IFRD2	0.577497645	0.008442602	0.038609293
GK	-0.157030592	0.008470358	0.038698398
LOC100848212	0.291004891	0.008468803	0.038698398
DNAJB5	0.251952474	0.008466335	0.038698398
ZNF746	0.86273075	0.008479012	0.03872533
CCNDBP1	0.208932958	0.008504402	0.038828655
CMC2	0.406619942	0.008528183	0.038911914
SPTLC2	-0.273887842	0.008526015	0.038911914
TAF9B	-0.401517368	0.008535526	0.038932761
RHBDD2	0.11923868	0.008541508	0.038934738
DOCK11	-0.269173901	0.008539612	0.038934738
PTRH1	0.118655772	0.00858834	0.039135506
EGFL8	0.375678981	0.00859738	0.039155221
CGRRF1	-0.305281074	0.008598247	0.039155221
SEC13	0.190148525	0.008618429	0.039221676
ANKRD17	-0.297480431	0.008616706	0.039221676
MALSU1	0.320516761	0.008629951	0.03924866
LOC104975749	0.720541462	0.00862906	0.03924866
LOC782479	0.503865677	0.008694806	0.039518006
ZNF317	-0.254684258	0.008694484	0.039518006
ZBED6CL	-0.518450591	0.008739407	0.039707859
STK11IP	0.130016424	0.008775055	0.039856926
LOC784052	3.942786942	0.008782995	0.039880082
RPL23A	0.359861608	0.008813597	0.040006091
GTF2A2	0.246146446	0.008829503	0.040065333
SLC5A1	-0.763604853	0.008860483	0.040185046
BLVRB	0.481822838	0.008868052	0.040185046
TPPP3	1.880766149	0.008870201	0.040185046
POLR2F	0.346669685	0.008865065	0.040185046
SYTL1	0.002258051	0.008866497	0.040185046
CEBPZOS	0.169512286	0.00888732	0.040236625
SPTY2D1	-0.25650053	0.008886268	0.040236625
LRRFIP1	-0.172480933	0.008895681	0.040261493
AOX1	-1.09134822	0.008902177	0.040277904



COMMD7	0.263456178	0.008912305	0.040310733
YDJC	0.008235778	0.008936977	0.040409303
ARL6IP4	0.267521494	0.008963072	0.04051424
THAP7	0.167828205	0.00899216	0.040632637
ANO6	-0.395582271	0.008996509	0.040639205
AP1S3	-0.095651674	0.009003064	0.040655728
VIM	0.573435528	0.009040735	0.040799589
ARHGAP39	0.032339026	0.009039678	0.040799589
EFNA2	-0.382075141	0.009045784	0.04080925
MFN1	-0.369842206	0.009054091	0.040833602
KBTBD3	-0.382451283	0.009065349	0.04087124
BLOC1S2	0.074713831	0.009088957	0.040935246
SPSB2	-0.092032712	0.009088228	0.040935246
TRMT2A	0.154752592	0.009087819	0.040935246
MYO1E	-0.009634941	0.009091212	0.040935246
SNAPC1	-0.452121905	0.009102584	0.040973304
FKRP	0.139263466	0.009129639	0.04108191
ELMO3	-0.018877543	0.009158646	0.041199229
MAST3	-0.199094301	0.009167967	0.041227944
TFPT	0.357578586	0.009180297	0.041256952
PSMF1	0.138611292	0.009179276	0.041256952
NSUN3	-0.170109483	0.009191899	0.041295869
CSGALNACT2	-0.179197429	0.009203726	0.041317424
PGM3	0.286789074	0.009202342	0.041317424
ACTR1B	0.230477416	0.009205528	0.041317424
HP1BP3	-0.100528604	0.009221535	0.041376038
CD9	-0.141302484	0.009228092	0.041392223
DEPP1	0.350800409	0.009245172	0.041455586
TMEM82	0.614310573	0.009253933	0.041468373
GGTA1	-0.327485222	0.009253494	0.041468373
OGG1	0.029575933	0.009267736	0.041516974
RTRAF	0.186114336	0.009280925	0.041549534
PHF20L1	-0.569446832	0.009278322	0.041549534
MEGF9	0.059591686	0.00930058	0.041610978
MINDY2	-0.45142551	0.009299111	0.041610978
ASB12	-0.265332769	0.009345905	0.041800439
ZMYM4	-0.344073502	0.009352157	0.041815077
BCL7C	0.23963618	0.009361928	0.041845431
ABRAXAS2	-0.247625053	0.009365895	0.041849838
CDAN1	0.127627358	0.009369306	0.041851751
SLC25A6	0.242820364	0.00937882	0.041880923
AASDHPPT	-0.066173354	0.009398081	0.041900275

ANKRD10	0.100062856	0.009394764	0.041900275
PHLPP2	-0.225764283	0.009395729	0.041900275
LOC100139548	-0.169617156	0.009390724	0.041900275
DVL1	0.032872406	0.009389522	0.041900275
CLEC3B	0.168235265	0.009404284	0.041906734
AMOTL1	-0.348714036	0.009405501	0.041906734
DOCK8	-0.439819223	0.00941993	0.041931088
DHX34	-0.022330079	0.009416718	0.041931088
DPP8	-0.146967258	0.009419669	0.041931088
LOC112442226	0.171259532	0.009426735	0.041948076
BAX	0.414695017	0.009437936	0.041984604
MNF1	0.180928917	0.009444801	0.042001834
RAB25	0.024753293	0.009450659	0.042014568
LOC533597	-0.28227726	0.009466899	0.042050179
SCAMP2	-0.035321014	0.009466048	0.042050179
LOC107132556	0.134327062	0.009467657	0.042050179
C29H11orf68	0.225092341	0.009476492	0.042069148
RBM12B	-0.347635353	0.009477923	0.042069148
GPR183	-0.02558507	0.009490331	0.042110904
PTTG1	0.295941779	0.009493651	0.042112324
LOC100847613	0.055738667	0.009511983	0.042176094
PHB2	0.189045362	0.009514037	0.042176094
SFT2D1	0.106077726	0.009520277	0.042190428
TMEM41A	-0.316935952	0.009525641	0.042200875
MRPL14	0.40884943	0.009537689	0.042240917
DNAJB2	0.298047176	0.00954833	0.042261372
RPS6KL1	0.304360109	0.00954585	0.042261372
PPP3R1	-0.360266879	0.009564523	0.042319699
LOC112448304	0.577844614	0.009572528	0.042341769
ZFYVE21	0.396601159	0.009578734	0.042355868
TBCB	0.318186568	0.009607204	0.042468379
MED22	0.39064338	0.009633466	0.042571065
LOC104974529	-0.060590424	0.009661813	0.042672837
NDUFC2	0.023722067	0.009662578	0.042672837
TTF1	-0.108359223	0.009681585	0.042743332
LOC101905951	0.408305865	0.009702261	0.042821143
FAM193B	-0.051402463	0.009709899	0.04284138
PSME2	0.304395499	0.009727269	0.042904531
RNF167	0.147103237	0.009731983	0.04291184
DEK	-0.041765834	0.00974411	0.042951818
LOC100299201	0.400379517	0.009747747	0.042954358
APIP	0.366309759	0.009782042	0.043091954

DGKH	-0.469447931	0.009791791	0.043107839
GNL1	-0.093523192	0.009790852	0.043107839
POLD1	0.362628631	0.009825708	0.043193701
FPGS	0.213555281	0.009826682	0.043193701
PMS2	-0.038068253	0.009818222	0.043193701
MUSTN1	0.7215095	0.009825838	0.043193701
MTIF3	0.083803269	0.009820751	0.043193701
CRNN	-2.281214412	0.009867326	0.043358773
TBC1D16	0.938482929	0.009875383	0.043369337
C17H12orf43	0.316239096	0.00987591	0.043369337
GLG1	0.007575928	0.009890096	0.043418049
PSMB2	0.217534525	0.009894814	0.043425175
ARMCX3	-0.24564357	0.009911372	0.043484247
MYO10	-0.020679252	0.009916518	0.043493228
TP73	0.59009001	0.009923524	0.043510357
ZNF580	0.911311863	0.009971696	0.043700661
CUEDC2	0.248786002	0.009973154	0.043700661
PRR19	0.275174785	0.009979512	0.043711926
RIT1	-0.183489745	0.009981954	0.043711926
SNAPC5	0.219855512	0.010014057	0.043797847
LOC789829	0.689686979	0.010008052	0.043797847
MANSC4	-0.194848821	0.010012233	0.043797847
SESN3	-0.533618802	0.010010557	0.043797847
LOC112442610	0.435513023	0.010019532	0.043808141
EIF3F	0.023618303	0.010050945	0.043915428
LOC112444842	-0.80870585	0.010053457	0.043915428
CCDC189	0.248660214	0.010049042	0.043915428
CHAF1B	-0.072904029	0.010063436	0.043945342
LOC101905997	0.319960211	0.010079964	0.044003823
ATXN7L2	-0.077432578	0.010094067	0.044024309
RABL2B	0.004673871	0.010093932	0.044024309
LOC534181	0.102257321	0.010092984	0.044024309
SUDS3	-0.482670627	0.010106622	0.044047771
MTPAP	0.033950861	0.010108862	0.044047771
PAG1	0.128447694	0.010108653	0.044047771
SLC39A13	0.392436053	0.010113529	0.044054432
RPS27P	0.106176705	0.010121869	0.044077082
LOC618256	0.654923449	0.01012553	0.044079347
ATP2C2	0.280481134	0.010132972	0.044084397
RPS6KB1	-0.202157295	0.010132746	0.044084397
PIP5K1B	-0.073345376	0.010150781	0.044140617
EWSR1	0.18720793	0.010152185	0.044140617

TBC1D32	0.261394212	0.010162494	0.044171758
LOC100848339	0.403252807	0.010166466	0.044175339
LOC788541	0.163027529	0.010177083	0.044207787
NAXE	0.177068501	0.010181335	0.044212571
RSPH3	-0.191791223	0.010226144	0.044393422
BOLA3	0.322454828	0.010241273	0.04444535
MGRN1	0.160299878	0.010255714	0.044494264
FAXDC2	0.437662035	0.010267069	0.044529761
GUCY2D	-1.604536669	0.010276088	0.04455511
H3F3C	0.302109237	0.010290561	0.044604083
CCDC120	0.374354546	0.010297226	0.044619193
CDHR4	0.231198593	0.010304065	0.044635047
ATG5	0.055112353	0.010316342	0.044674439
LOC100616098	0.259208405	0.01032634	0.044703943
OGFOD3	0.109577097	0.010335149	0.044714495
ZNF322	0.029733847	0.010334178	0.044714495
NFIC	0.201096677	0.01034239	0.044732033
NOXA1	0.442615655	0.010362621	0.04479233
RANBP9	-0.053598438	0.010362714	0.04479233
C10H14orf119	0.262203614	0.010378331	0.04483222
CD63	0.366998128	0.010376874	0.04483222
PROSER3	0.295803212	0.010387166	0.044851953
DGKA	-0.002122904	0.010389291	0.044851953
TMEM165	-0.104577902	0.010395123	0.044863333
RNF168	-0.346925791	0.010427859	0.044990777
EXTL2	-0.174520589	0.010443203	0.045043133
SNRNP200	-0.102138283	0.01044675	0.045044588
SOS2	-0.247173715	0.010450144	0.045045386
ASAP2	-0.695351814	0.01046713	0.045104751
MOK	-0.037967263	0.010481001	0.045142706
YPEL1	0.421032564	0.010482371	0.045142706
KLHDC2	-0.064694843	0.010493534	0.045163065
SLC16A1	0.155660211	0.010490478	0.045163065
ELL2	-0.521761727	0.010508449	0.045213389
PKP2	-0.278038801	0.010512108	0.045215275
TATDN1	0.307030491	0.010531639	0.045257669
TMEM94	0.097138547	0.01053145	0.045257669
LSM5	-0.007399534	0.010526885	0.045257669
FLII	-0.11598692	0.010551097	0.045299678
LOC101905053	-0.267805869	0.010547461	0.045299678
TRMT1	0.242004607	0.01054997	0.045299678
ANAPC13	0.334647551	0.010571751	0.045374474

HINT1	0.231908155	0.010581284	0.045401505
ATM	-0.416355655	0.010593405	0.045439619
CLU	-0.177890176	0.010599301	0.045449256
TMEM50A	0.11337564	0.010602128	0.045449256
SPEF1	0.636199015	0.010622644	0.0455233
DTNBP1	0.209821665	0.010638151	0.04557584
BRAF	-0.124203238	0.010652367	0.045622816
MFSD6	-0.09266935	0.010655954	0.045624255
RBM8A	0.175335832	0.010666996	0.045657607
MAMDC4	0.490459064	0.010680883	0.045703108
HNRNPLL	-0.284562748	0.010699311	0.045768006
BBIP1	0.142290691	0.010706768	0.04578595
TRAPPC6B	-0.27697385	0.010728277	0.045863958
LRRC75A	0.492184545	0.010741531	0.045898831
UBR7	-0.0624241	0.010742975	0.045898831
LOC782706	-0.158127031	0.010771799	0.04600104
PPP1CC	-0.06821061	0.010773453	0.04600104
KATNB1	0.157075378	0.010786061	0.046040867
GHR	-0.697370375	0.010801401	0.046064316
XYLT2	-0.312957662	0.01079909	0.046064316
RWDD3	0.11121274	0.010798271	0.046064316
INPP4B	-0.03925935	0.010867436	0.046331855
RTL8C	0.371614225	0.010882127	0.046366785
SMG6	0.2711274	0.010882236	0.046366785
LOC521224	0.176689299	0.010891217	0.046389935
DERL1	-0.263271443	0.01089428	0.046389935
C23H6orf223	0.450184871	0.01090075	0.046403405
ETV2	0.343348648	0.010926825	0.046500301
LOC112446127	0.21784354	0.010932083	0.046508573
SELP	-0.325468381	0.010947808	0.046547253
ZKSCAN5	0.00878917	0.010946455	0.046547253
NYAP2	-0.171161108	0.010963829	0.046601255
MRPL57	0.163958983	0.010980549	0.046629958
ITGB4	0.083180145	0.010979948	0.046629958
LOC100848883	0.354514822	0.010975865	0.046629958
TBXA2R	0.560911933	0.010984129	0.046631051
ATG4C	-0.042794368	0.011042768	0.046865815
ZNF385A	0.627431296	0.011061847	0.046932594
ZBTB3	-0.272554966	0.011065893	0.046935572
DDX39A	0.265649473	0.011081154	0.046986103
EEFSEC	0.217823978	0.011085163	0.046988903
SAMD4B	0.025123807	0.01108957	0.046993393

LACTB	-0.217413154	0.011096726	0.047009522
TMEM243	-0.018616221	0.011116361	0.047078494
TRMT13	-0.199026903	0.011120267	0.04708083
ANK3	-0.465564672	0.011133693	0.047109251
HPRT1	-0.279523323	0.011132109	0.047109251
MAML2	-0.430184128	0.011144748	0.047141814
SGF29	0.033392177	0.011174462	0.04725326
PELI1	0.072715324	0.011211111	0.047393959
LOC100300881	-0.494184024	0.011246728	0.047530208
GKAP1	0.302299187	0.011265345	0.047594556
TRPS1	-0.05519435	0.011281146	0.047635796
ZMPSTE24	-0.002276787	0.011281894	0.047635796
LMO1	0.867959157	0.011292248	0.047665171
PPP2R5B	0.091016438	0.011310596	0.047728267
LOC112447506	-0.940591507	0.011316515	0.047738891
TMEM177	0.216252369	0.011357847	0.047898852
AHR	-0.429645179	0.011362561	0.047904337
MRPS23	0.262353211	0.011411173	0.04809484
MTREX	-0.266131168	0.011437848	0.048151
LRP11	-0.735428422	0.011450767	0.048151
PKIB	-0.565523083	0.011443061	0.048151
CAV2	-0.39529801	0.011432671	0.048151
ZXDC	0.019497334	0.011442795	0.048151
ABCB10	-0.37926159	0.011431306	0.048151
LRRC28	0.146259602	0.011447726	0.048151
LOC101905979	0.376363522	0.011451944	0.048151
ELMOD3	0.319910352	0.011488027	0.048288246
SZT2	0.017241682	0.01149517	0.048303805
C23H6orf106	-0.125697119	0.011504329	0.048313362
TROVE2	-0.314944043	0.011503111	0.048313362
LOC515358	0.237151078	0.01153787	0.048439727
MRPL38	0.203038662	0.011542557	0.048444912
GPR27	-0.755429947	0.011548547	0.048455564
ARHGEF25	0.49354985	0.011555308	0.048469441
G3BP1	-0.027491285	0.011576992	0.048545888
G6PC3	0.637161111	0.011591077	0.048590432
LOC511386	0.169119648	0.011598677	0.048607773
ARL6	-0.061744879	0.01160973	0.048639569
MRPS18A	0.340192925	0.011622148	0.048677064
EMC6	0.337747455	0.011662367	0.048801826
KEAP1	0.178373256	0.011659656	0.048801826
RBBP6	-0.042650829	0.011659186	0.048801826

RNF126	0.179435629	0.011681306	0.048851945
HSPE1	0.547721663	0.011678129	0.048851945
ZNF862	-0.353996485	0.01171036	0.048944283
LOC511695	-0.04849948	0.011708163	0.048944283
PMEPA1	0.696880251	0.011722089	0.048978719
C15H11orf34	-2.123648192	0.011725713	0.048979281
EOGT	-0.155474865	0.011735859	0.049007075
TRIQQ	-0.340564271	0.011739906	0.049009392
SPECC1	0.437228255	0.011765131	0.049074109
PARD6B	-0.487933881	0.011762465	0.049074109
RSRC2	0.144825511	0.011767207	0.049074109
EDARADD	0.837328042	0.011769394	0.049074109
SPRYD4	0.095470354	0.011780545	0.049106015
DCTN4	-0.123848287	0.011797876	0.049149064
UPF2	-0.09182462	0.011794429	0.049149064
LOC101902172	0.315835275	0.011814297	0.04917602
AK1	0.430298299	0.011814859	0.04917602
ZNF205	0.155787588	0.011814257	0.04917602
EPS8	-0.313661313	0.011826142	0.049208389
SND1	0.109856287	0.011839582	0.049249714
MPZL3	0.01255429	0.011845454	0.04925954
GLUD1	0.090287221	0.011874782	0.049366875
RAB6A	-0.050069785	0.011881031	0.049378227
ABHD14A	-0.451605119	0.01188808	0.049392894
BTBD3	-0.323871012	0.011907757	0.049445375
TAGLN	0.712392184	0.011904855	0.049445375
HES2	0.475057719	0.011913414	0.049454235
INTS14	-0.070145947	0.011922613	0.049477786
CA9	0.188725914	0.011941753	0.049542568
GALK1	0.303135516	0.011972306	0.049625316
LOC101905499	0.176823259	0.011967121	0.049625316
SAMD4A	0.22972911	0.011970758	0.049625316
SEM1	0.333922317	0.011977936	0.049633992
LOC787530	0.033515721	0.011986627	0.049655344
PPP2CB	0.021633042	0.011990823	0.049658068
IL13RA1	-0.069719757	0.012033015	0.049803412
SLC25A10	0.250167008	0.012029951	0.049803412
SIVA1	0.552047167	0.012059045	0.049896433
EVI5	-0.219865037	0.012067961	0.049918607
RCAN3	0.386611012	0.012087974	0.049971935
NUDT8	0.299791332	0.012086471	0.049971935
LOC782812	0.496485793	0.012092132	0.049974404

LOC101906676      0.057312057      0.012096699      0.04997856

1 Genes in green are up-regulated in steers vs heifers and genes in red are down-regulated in steers.

2 Fold changes for steers compared to heifers (Male vs Female).

3 Nominal P-values.

4 FDR-corrected P-values.

**Supplemental Table 3. Genes differentially expressed in the rumen papillae of steers and heifers with and without severe liver abscesses.**

Gene <sup>1</sup>	Log2FoldChange <sup>2</sup>	P-value <sup>3</sup>	Adjusted P-value <sup>4</sup>
CDO1	-1.312957696	8.87E-09	0.000124491
CTSK	-0.362293043	2.45E-07	0.001721008
KIAA2013	0.288655292	4.50E-07	0.001958386
TMEM127	0.684763545	5.58E-07	0.001958386
KPNA6	0.697971183	1.31E-06	0.003058902
PSAP	0.529124861	1.21E-06	0.003058902
CMC4	-0.805990604	3.27E-06	0.004589823
NECTIN1	1.102625782	3.23E-06	0.004589823
PCDHGC3	0.076508038	2.44E-06	0.004589823
ACO2	0.352117296	3.00E-06	0.004589823
LDLRAD3	1.193660178	4.38E-06	0.005594216
GSDMB	0.293009252	4.95E-06	0.005784114
ITIH4	-0.121524124	6.15E-06	0.006334217
STRIP1	0.243331063	6.32E-06	0.006334217
ULK4	0.946103445	6.82E-06	0.006375229
CASP2	0.706579724	7.27E-06	0.006375229
CDH1	0.894205277	8.49E-06	0.007006949
DDX10	0.379242505	9.06E-06	0.007066846
UQCRB	-0.538423011	9.62E-06	0.00710381
AFG3L2	0.446591932	1.07E-05	0.00749544
TJAP1	-0.432739891	1.30E-05	0.00784965
SEC24C	0.606768285	1.34E-05	0.00784965
CASP4	-0.053024288	1.24E-05	0.00784965
GCNA	-0.957794013	1.28E-05	0.00784965
CHD4	0.534597906	1.51E-05	0.008146562
LY96	-0.549918138	1.49E-05	0.008146562
CCM2L	-0.48448308	1.57E-05	0.008146562
CHRNA3	-0.797250089	1.69E-05	0.008193098
MAPK1	1.048298929	1.69E-05	0.008193098
CTNND1	0.399101667	2.27E-05	0.010430114
LOC112444206	-0.423876505	2.38E-05	0.010430114



HESX1	-0.684729602	2.34E-05	0.010430114
DHCR24	1.131041336	2.58E-05	0.010625122
LOC112442867	-1.918740691	2.65E-05	0.010625122
EIF4G1	0.645194293	2.63E-05	0.010625122
CARMIL1	0.472160031	2.76E-05	0.010778243
B4GALT7	0.492968384	3.13E-05	0.010798309
C14H8orf76	-0.446603852	2.92E-05	0.010798309
SEZ6	-0.917569932	3.02E-05	0.010798309
SERPINC1	-0.385499355	2.98E-05	0.010798309
EAFL	0.613650434	3.15E-05	0.010798309
FLII	0.330326991	3.74E-05	0.011236453
MTMR9	-0.217761401	4.17E-05	0.011236453
OGFOD3	0.280688306	4.24E-05	0.011236453
AP3D1	0.521970674	3.60E-05	0.011236453
LOC614208	-0.444417692	3.74E-05	0.011236453
EFCAB8	-0.38139165	4.06E-05	0.011236453
SGPP2	0.40843467	4.07E-05	0.011236453
HOXD3	-0.717167441	3.82E-05	0.011236453
SDC1	0.283287378	3.86E-05	0.011236453
IGIP	-0.956242307	4.06E-05	0.011236453
SUPT16H	0.734017093	3.73E-05	0.011236453
TMEM259	0.166546159	4.24E-05	0.011236453
EFNA2	-1.501907733	4.48E-05	0.011296543
LOC112444681	-1.180392075	4.45E-05	0.011296543
LOC112441868	-0.417425235	4.51E-05	0.011296543
ACTN4	0.563390972	4.90E-05	0.011665238
RNF7	-0.527912214	4.77E-05	0.011665238
RNF40	0.539046742	4.82E-05	0.011665238
AMT	-0.960526525	5.17E-05	0.012099452
SLC9A1	0.736275527	5.33E-05	0.012252471
B4GALT1	0.775129644	5.65E-05	0.01278806
YKT6	0.428230511	5.82E-05	0.012973681
SMCR8	0.523039058	5.98E-05	0.013103717
RMRP	-2.629938593	6.12E-05	0.013203784
TIAF1	-3.426734815	6.25E-05	0.013285292
CLPB	0.25098013	6.44E-05	0.013352103
LOC100848315	-0.794557935	6.47E-05	0.013352103
TTC7A	0.438391314	7.05E-05	0.014134131
CDKN1C	-1.256556585	7.05E-05	0.014134131
PKN3	-0.235434461	7.31E-05	0.014440947
VWA2	-0.550953709	7.46E-05	0.014546869
DPP3	0.281028892	7.67E-05	0.014748117

ICMT	0.424745789	7.94E-05	0.015059242
KPNB1	0.589856765	8.85E-05	0.016504246
TBL2	0.649128668	8.94E-05	0.016504246
DCTN1	0.319831294	9.50E-05	0.017100442
RN7SL1	-2.991649645	9.46E-05	0.017100442
SRP68	0.269387832	9.82E-05	0.017386793
SLC39A3	0.733480411	0.000100226	0.017386793
FZD5	1.493336995	0.000100344	0.017386793
PSMB10	-0.812147356	0.000105461	0.018050492
HIF1AN	0.777196502	0.000107893	0.018244396
PFDN4	-0.701248576	0.000119234	0.018389504
HIST1H3G	-0.351859864	0.000113766	0.018389504
LOC101906230	0.039845966	0.000117542	0.018389504
CLPTM1	0.330314209	0.000113209	0.018389504
MUSTN1	-0.134534751	0.000112242	0.018389504
PCYOX1	0.673550279	0.000116755	0.018389504
LOC112442610	-0.244177633	0.000116672	0.018389504
RPL22	-0.663558504	0.000118421	0.018389504
TNFRSF1B	0.61484303	0.000122746	0.018725364
INSL6	-1.927071422	0.000125248	0.018901619
SNRNP200	0.334543105	0.000135889	0.019175751
BTBD9	0.369276441	0.000136967	0.019175751
AGPAT3	0.527580793	0.00014416	0.019175751
METTL5	-0.443593297	0.000140676	0.019175751
LOC112442598	-0.625755215	0.000141367	0.019175751
CDK5RAP1	0.128210806	0.000147558	0.019175751
CNOT1	0.640687948	0.00014651	0.019175751
SPATA33	-1.081471999	0.000142505	0.019175751
LOC104970537	-0.517277024	0.000146762	0.019175751
DDB1	0.481670542	0.000130051	0.019175751
LOC101902787	-0.618929001	0.000146471	0.019175751
SCAMP2	0.255050867	0.000141269	0.019175751
LAMB2	-0.302821612	0.000146845	0.019175751
AMOTL1	0.296793989	0.000141664	0.019175751
BCAP31	0.355931995	0.000133356	0.019175751
DOLK	0.123446636	0.000154062	0.019400919
AAMP	0.165228943	0.000153376	0.019400919
ZNF263	-0.433675596	0.000151773	0.019400919
TPR	0.547571837	0.00015482	0.019400919
ZCCHC9	-0.169220106	0.000157681	0.01958453
ATP6V0A1	0.775034377	0.000165628	0.01968442
TMEM79	0.51734189	0.0001669	0.01968442

LOC112444655	-1.23023546	0.000162585	0.01968442
IGF1R	0.672647875	0.000165696	0.01968442
BTNL9	-2.121392353	0.000160268	0.01968442
LOC112442865	-1.149733347	0.000166763	0.01968442
ZNF609	0.872728843	0.000174009	0.01970833
ETV6	0.531028461	0.000173093	0.01970833
FASTKD2	0.171091509	0.000175528	0.01970833
NQO2	-0.469016062	0.000175188	0.01970833
LOC112444473	-0.795914297	0.000170803	0.01970833
LOC101906347	-0.362039641	0.000168874	0.01970833
TSPAN7	0.685388685	0.000178466	0.019722649
LOC112442863	-0.922845147	0.000177576	0.019722649
WDR7	0.364054527	0.000181144	0.019862119
PEX10	-0.905466007	0.000183873	0.019981841
AAR2	0.409099484	0.000185083	0.019981841
LRFN3	0.391064455	0.000191029	0.020466366
NFATC4	-0.627254207	0.000198168	0.021070407
RPTOR	0.437932938	0.000204552	0.021585623
NAPB	-0.483208748	0.000218935	0.022257391
COX7A1	-0.446287384	0.000217757	0.022257391
TTL	0.699402794	0.000220477	0.022257391
NBDY	-0.66021656	0.000223605	0.022257391
COPB2	0.443512525	0.000219365	0.022257391
KIAA2012	0.204117425	0.000220147	0.022257391
LOC112442866	-1.223730812	0.000215012	0.022257391
LOC112442864	-1.184243124	0.000222913	0.022257391
WBP4	-0.394901291	0.000236443	0.022758017
MDP1	-0.793576755	0.000234981	0.022758017
TMEM88	-0.441575678	0.000234438	0.022758017
MLLT11	-0.348708578	0.00023164	0.022758017
COPG1	0.273206838	0.000236742	0.022758017
LOC112444958	-1.257285153	0.000240177	0.022885989
PLXNA2	0.168758207	0.000241334	0.022885989
LOC101902907	-0.717415419	0.000244367	0.023018091
DBI	-0.549324522	0.00024624	0.023039897
VPS8	0.240685649	0.000250349	0.023269236
LMAN1	0.528947737	0.00025528	0.023563125
TRIB2	-0.757615967	0.000256869	0.023563125
ZNF93	-0.295751441	0.000260512	0.023595463
ARHGAP30	-0.176410994	0.000261716	0.023595463
DTX4	-1.191383612	0.000262265	0.023595463
ERMARD	-0.604750993	0.000266785	0.023698265

TRAPPC9	0.29766067	0.000266175	0.023698265
C2CD2	0.45703333	0.000268657	0.023714436
POGLUT1	0.323443623	0.00027875	0.024001566
POU6F1	-0.911783309	0.0002772	0.024001566
CNOT11	0.601118024	0.000273905	0.024001566
SLC25A28	-0.993503596	0.000277157	0.024001566
AQR	0.450175812	0.000285946	0.024420483
AARS	0.375378596	0.000287095	0.024420483
HDGF	0.312286551	0.00028951	0.024477513
RIPOR2	-0.853566844	0.000298293	0.025069124
SPINK9	-3.482089885	0.000300101	0.025070929
TCAF2	1.413446471	0.000305436	0.025216473
MARC1	-1.066667893	0.000305202	0.025216473
EGFL8	-1.006783626	0.000307577	0.02524473
ARCN1	0.550308344	0.000313404	0.025425551
PPM1G	0.253537898	0.00031211	0.025425551
ETV5	-1.19968822	0.000322009	0.025921524
COL16A1	-0.621897156	0.000323211	0.025921524
PDE12	0.540588493	0.000331207	0.026411841
PHF11	-0.69262946	0.000350391	0.026582382
IGF2R	0.66045463	0.000337185	0.026582382
LAMA5	-0.184052215	0.000348637	0.026582382
RBM18	0.502785216	0.000348813	0.026582382
LOC112442597	-0.860794655	0.000340339	0.026582382
EPRS	0.457087116	0.000346047	0.026582382
AJUBA	-0.02717705	0.000346162	0.026582382
EXOC8	0.855718526	0.000339332	0.026582382
S100A13	-0.377781042	0.000347149	0.026582382
LOC112441507	2.444237333	0.000355197	0.02680207
PDGFB	-0.090371847	0.000358109	0.026854589
NAA35	0.094197925	0.000359719	0.026854589
LUC7L	-0.57985012	0.000362869	0.026946394
LOC101904468	-0.791519239	0.000376737	0.02706852
TSNARE1	-0.785149114	0.000376119	0.02706852
XPNPEP1	0.188001595	0.000369332	0.02706852
IGF2	-0.730382726	0.000372273	0.02706852
GART	0.341218668	0.000378014	0.02706852
M6PR	0.13278569	0.000368208	0.02706852
ADAMTSL5	-0.41103267	0.00037773	0.02706852
HS6ST1	0.631324876	0.000383749	0.027339667
NTRK3	-1.283843692	0.000387714	0.027482686
DLK2	-1.363331959	0.000397977	0.027766878

SLC22A17	-0.856747974	0.00040075	0.027766878
LOC100848985	-0.65030543	0.000399565	0.027766878
TMEM86B	-0.59748881	0.000403594	0.027766878
EIF4H	0.729737611	0.000396027	0.027766878
RSRP1	-0.069021503	0.000403244	0.027766878
RASGRF2	-0.627742581	0.000412727	0.028096166
ESPNL	-0.948027242	0.000414386	0.028096166
ITGA7	-1.060103334	0.00041379	0.028096166
SLC35F6	0.464792956	0.000417689	0.028164747
RPUSD3	-0.339088162	0.000419544	0.028164747
LOC104974837	-1.094939781	0.000421418	0.028164747
FRG1	-0.554965929	0.000432292	0.028219591
TCAP	-0.725538463	0.000431081	0.028219591
MED1	0.547710256	0.000425469	0.028219591
PPP2R1B	0.437804302	0.000430639	0.028219591
SPTY2D1	0.810531932	0.00043033	0.028219591
DCAF5	1.873634886	0.000438566	0.028235203
NOA1	0.088038193	0.000436584	0.028235203
WDR18	0.322039858	0.000434964	0.028235203
INAFM1	-0.920220218	0.000442048	0.028329434
LOC112447381	-1.394989462	0.000446837	0.0283772
PSMD1	0.28395698	0.000445896	0.0283772
SLC5A1	1.008521204	0.000452807	0.028498393
RPL38	-0.62667254	0.000450954	0.028498393
SMIM7	-0.523829201	0.000455457	0.028537241
LOC100848724	0.314285415	0.000467092	0.029136192
COG7	0.167650298	0.00047623	0.029574757
APOE	-0.403306214	0.000480222	0.029591765
ABCF2	0.184085404	0.000480721	0.029591765
ASRGL1	0.719224972	0.00048305	0.029605289
LRP1	0.210208236	0.000487822	0.029767774
WSB1	-0.585991642	0.000496892	0.029930783
LMAN2	0.265553604	0.000492908	0.029930783
SYNE1	-0.042869399	0.00049478	0.029930783
OGFR	0.388091381	0.000500233	0.030003274
CCPG1	0.764696933	0.000508773	0.030161898
TSKS	-1.633956726	0.000509325	0.030161898
ZNF585A	0.574185897	0.00050756	0.030161898
SGSM2	0.260142411	0.000513465	0.030279335
COMMD3	-0.39541144	0.000517328	0.030295011
CREB3L2	1.064295321	0.000518048	0.030295011
CAPS	-0.815762831	0.000526474	0.030316374

RBBP5	0.576435677	0.00052695	0.030316374
LOC101904595	-0.763022137	0.000523307	0.030316374
AKAP8L	-0.455488043	0.000527053	0.030316374
SLC6A1	-0.397613673	0.000530969	0.030416915
LOC112448736	-0.257752224	0.000539732	0.030544935
EYA3	0.592610719	0.000538912	0.030544935
ZFPL1	0.107422859	0.000535604	0.030544935
GCC1	0.700815852	0.000565528	0.031496782
FIG4	0.248049002	0.000563331	0.031496782
LOC783466	0.782697115	0.000563703	0.031496782
TRMT44	-0.944858589	0.000559878	0.031496782
PNPO	0.286676089	0.00057175	0.031717409
SPRY1	-0.161239708	0.000577799	0.031926821
ICOSLG	-0.161757854	0.000585816	0.032242867
OTUD7B	0.68890397	0.000602335	0.032766568
BDNF	-0.482597116	0.000600221	0.032766568
ADRB2	-0.883966885	0.000601551	0.032766568
LOC112448373	-0.087722283	0.000605175	0.032793937
PEAR1	-0.373717584	0.000608342	0.03283877
SERTM1	-0.232433109	0.000617999	0.033232247
CHAF1B	0.841175943	0.000635356	0.033943067
DUT	-0.487814379	0.000636055	0.033943067
CNTNAP1	-0.944986149	0.000639767	0.034011874
PKD1	-0.706659952	0.000650171	0.034434524
RFT1	0.035307268	0.000657756	0.034705273
EFL1	-0.061856361	0.000663275	0.034865405
YLPM1	0.461184525	0.000685438	0.035817694
C15H11orf96	-0.79794545	0.000687718	0.035817694
EDEM1	0.647607695	0.000689047	0.035817694
LOC107132386	-0.791755955	0.000694651	0.035843465
LOC107132767	-0.781528086	0.000694253	0.035843465
LIG3	0.144720023	0.000702936	0.036138097
IGSF3	0.744049511	0.000709741	0.036354822
HNRNPM	0.494835514	0.000714425	0.036461662
FKRP	0.081403682	0.000717996	0.036511128
LOC107131695	-0.826858595	0.000721647	0.036564333
CCDC47	0.400472338	0.000724929	0.036598493
LOC616830	-0.087176955	0.0007309	0.036636352
TYK2	0.222283324	0.00073054	0.036636352
LOC112448453	-0.481186485	0.000740773	0.036999105
LOC783838	-0.729020708	0.000751735	0.0373255
C3	0.480742377	0.000752627	0.0373255

DHRS13	0.324102693	0.000780697	0.038454528
HOXC4	-0.485125156	0.000780872	0.038454528
SOX4	-0.659598373	0.00081047	0.039692686
PNN	-0.229842576	0.000814499	0.039692686
DCP1A	0.348547726	0.000814406	0.039692686
MAST3	-0.345757608	0.000833314	0.04010386
NOL11	0.224248312	0.000840827	0.04010386
FBXL15	-0.480331645	0.00082836	0.04010386
SDF2	-0.009201204	0.000828897	0.04010386
KIFC2	-0.521502539	0.000840938	0.04010386
ANKRD10	-0.699977918	0.000842651	0.04010386
RPS25	-0.513435627	0.000843931	0.04010386
ZKSCAN5	-0.451187717	0.000845796	0.04010386
PPP1R12B	-0.577636619	0.000857457	0.040519907
RAB3D	0.256147983	0.000862888	0.040639694
LOC112442805	-1.069134696	0.00086619	0.040658775
RERGL	-0.551956682	0.000882687	0.041295027
TMEM200B	-0.454911119	0.000890017	0.041499638
POLR1A	0.341338924	0.000894822	0.041585505
UBE2J1	0.494543313	0.000902045	0.04178284
RELA	0.346539928	0.000926549	0.042034445
GPR87	-0.551372982	0.000997324	0.042034445
ASB11	-1.13718635	0.000992708	0.042034445
STK19	-0.689057744	0.000988306	0.042034445
CPOX	0.221905255	0.000978043	0.042034445
LOC112444215	-1.130439155	0.00098891	0.042034445
EIF3A	0.571038565	0.000932141	0.042034445
LOC100174924	-0.694564652	0.00097498	0.042034445
LOC783541	-0.745305632	0.000924293	0.042034445
GPR151	-0.371765721	0.000944535	0.042034445
LOC784358	0.370118614	0.000938388	0.042034445
MTHFR	0.121604431	0.000925011	0.042034445
LOC107132278	-2.773924297	0.000980927	0.042034445
RELB	-1.279721953	0.000925407	0.042034445
LOC112446667	-0.614977726	0.000973093	0.042034445
INPP5K	-0.372052775	0.000979315	0.042034445
ZFP90	-0.35620667	0.000938666	0.042034445
VWCE	-0.584656886	0.000946243	0.042034445
TMEM132A	0.104686583	0.000959541	0.042034445
EGFL7	-0.445374046	0.000985156	0.042034445
LOC519132	0.889192819	0.00091948	0.042034445
LOC107131684	-0.474748759	0.000979262	0.042034445

AQP3	0.465853202	0.000957016	0.042034445
IMMP1L	-0.152108535	0.000919804	0.042034445
KREMEN1	0.19447903	0.00099075	0.042034445
LOC512684	-0.487396465	0.000976457	0.042034445
PLCB2	-0.578023624	0.000987714	0.042034445
ENKD1	-0.430737174	0.000997326	0.042034445
ZNF692	-0.676450791	0.000971514	0.042034445
CACNA1H	-0.648209717	0.000991449	0.042034445
LETM1	0.347020308	0.001021435	0.042292906
GPM6B	-1.336314194	0.001017116	0.042292906
MANEA	0.422622012	0.001021539	0.042292906
TRAPPC12	0.224643911	0.00100838	0.042292906
LOC782938	-0.516671118	0.001019282	0.042292906
XPC	-0.139773301	0.001009521	0.042292906
SLC10A3	0.420719917	0.001030462	0.042341688
PIGK	0.268498339	0.001031768	0.042341688
TRPM3	-0.819464507	0.001031391	0.042341688
LOC107131948	-0.489853282	0.001043072	0.042348056
LOC104976274	-0.875511205	0.001038814	0.042348056
ZNF461	-0.340461737	0.001043992	0.042348056
PDGFA	-0.279456413	0.00103749	0.042348056
HCN2	0.432472124	0.001049427	0.042445852
KRBA1	-0.563365117	0.001053521	0.042488984
ADPRH	-0.858272746	0.001071175	0.042589066
USP4	0.344021357	0.001069745	0.042589066
ANKRD13C	0.88036601	0.00106223	0.042589066
RPS27L	-0.487690711	0.001066395	0.042589066
TMA7	-0.205438093	0.001070996	0.042589066
LOC101902542	-1.392737925	0.001076252	0.042670043
SEC23B	0.296658461	0.001080175	0.042680497
SRSF4	-0.324304906	0.001082598	0.042680497
OGDH	0.612774771	0.001092341	0.042943975
SFI1	-0.301133785	0.001101772	0.043073471
HOOK2	-0.54803778	0.001099419	0.043073471
TMBIM6	0.06127577	0.001112751	0.043381825
CRAT	0.569652759	0.001143045	0.044177452
COG5	0.219955935	0.001141857	0.044177452
RPL36AL	-0.563078128	0.001142179	0.044177452
LOC104969959	-0.185739008	0.001145749	0.044177452
LOC789694	0.753312359	0.001153367	0.044349337
STT3A	0.198512962	0.001159861	0.044477201
YTHDF2	0.300283596	0.001171052	0.044711501



TLE2	-0.545545824	0.001178908	0.044711501
ITGA10	-0.523021539	0.0011819	0.044711501
LOC112448454	-0.270664032	0.001174206	0.044711501
RFTN2	-0.040227081	0.001177425	0.044711501
LOC511695	0.884159849	0.001193244	0.044841437
GPR63	-0.580944821	0.001190007	0.044841437
FGF2	-0.936096448	0.00119492	0.044841437
SCO1	0.245532391	0.001200399	0.044926939
RASSF6	-1.177314117	0.001204885	0.044974905
EFNB1	0.202245047	0.001215973	0.045148643
ARHGEF25	-0.527695152	0.00121314	0.045148643
TRIM25	0.919100584	0.001235373	0.045272805
SLC35D1	0.567576583	0.001251149	0.045272805
LOC112441777	-1.033907157	0.001249081	0.045272805
YWHAG	0.58659436	0.001232901	0.045272805
LOC112444957	-0.925765926	0.001243192	0.045272805
TMEM248	0.411208791	0.001238862	0.045272805
TRIM7	-1.201861338	0.001236965	0.045272805
ZNF283	-0.047815325	0.001251575	0.045272805
RGS4	-1.214715089	0.001236106	0.045272805
C23H6orf15	-4.513736566	0.001243205	0.045272805
STAT5A	-0.073751077	0.001262004	0.045279114
PPFIBP2	0.496563118	0.001263037	0.045279114
ZNF646	0.257121873	0.001280025	0.045279114
PRPF8	0.39972965	0.001283873	0.045279114
ASXL1	-0.698193982	0.001280577	0.045279114
PFN2	-0.362627972	0.001281828	0.045279114
LOC514680	-0.671719754	0.001265663	0.045279114
CALML4	-0.205713538	0.001273589	0.045279114
WDR19	-0.17841865	0.00128401	0.045279114
LOC107133265	-1.588456947	0.001261109	0.045279114
TRIM3	-0.383328934	0.001288986	0.04529208
XPO6	0.386595358	0.001290832	0.04529208
HIP1	-0.826797966	0.001306863	0.045429209
SEC24D	0.931422859	0.001307688	0.045429209
LOC112443614	-3.112392944	0.001298842	0.045429209
LOC101902561	-0.605371349	0.001306304	0.045429209
CANT1	0.039387227	0.001311119	0.045435243
ITGB1BP2	-0.673584543	0.001314888	0.045435243
LOC104972595	-0.944271605	0.001317573	0.045435243
WDR12	0.177372987	0.001333199	0.045861408
LOC112442408	-1.091796271	0.001349073	0.04629398

RPL39	-0.567040485	0.001368731	0.046853995
TPM2	-0.634978831	0.00137321	0.046879943
GNG11	-0.156510031	0.001376169	0.046879943
RANGAP1	0.161641355	0.001389314	0.047213117
RPN1	0.304450536	0.001415555	0.047988681
ZNF407	0.311113677	0.001424308	0.048023175
LOC101903424	1.148579223	0.001426213	0.048023175
RALGDS	0.501698458	0.001426837	0.048023175
IFI6	1.248324318	0.001441421	0.048125904
SMC1A	1.074049909	0.001442015	0.048125904
COL7A1	-1.068810862	0.001438666	0.048125904
SUPT6H	0.496103812	0.001443606	0.048125904
INTS5	0.097630736	0.001486395	0.04864812
ATP1B3	-0.563090706	0.001481941	0.04864812
HIGD1B	-0.773404087	0.001473174	0.04864812
ATP6V0A2	0.555877193	0.001478248	0.04864812
PURB	0.518325252	0.001469269	0.04864812
LUZP6	-0.444718149	0.001487	0.04864812
PRAP1	-0.92795715	0.001478822	0.04864812
NACA	-0.446745708	0.001466297	0.04864812
NMT1	0.063473249	0.00149634	0.04883985

1 Genes in green are up-regulated in animals with no liver abscess compared to those with severe liver abscesses and genes in red are down-regulated . Genes shown in gray are genes identified from an interaction with sex or limonene treatment.

2 Fold changes for control animals (no liver abscess) compared to animals with severe liver abscesses (Control vs Abscess).

3 Nominal P-values.

4 FDR-corrected P-values.

## APPENDIX D

**R Codes**

```

---
title: "Wagyu Full Blood (FB) and F1 AND Angus data Deseq2 Network Analysis"
output:
  html_document:
    df_print: paged
---
```{r ,echo=F, message= F}
library("import")
library("knitr")
library("BiocStyle")
library("ggplot2")
library("gridExtra")
library("dada2")
library("phyloseq")
library("DECIPHER")
library("ape")
library("phangorn")
library("BiocStyle")
library("ShortRead")
library("ampvis2")
library("ggpubr")
library("metagMisc")
library("vegan")
library("plyr")
library("dplyr")
library("MASS")
library("tibble")
library("vegan")
library("microbiomeutilities")
library("microbiome")
library("reshape2")
library("ggplot2")
library("viridis")
library("hrbrthemes")
library("SpiecEasi")
library("igraph")
```

load("Wagyu_Phyloseq_FB_ONLY.RData")

deseq2_output_FB_F1= read.csv("Deseq2_Tree/deseq2_ASVs_FB_F1_label_marbling.csv")
deseq2_output_henry= read.csv("Deseq2_Tree/deseq2_ASVs_henry_label_marbling.csv")
deseq2_tree_labels= rbind(deseq2_output_FB_F1,deseq2_output_henry)
ot= as.data.frame(deseq2_tree_labels$ASV)
ot$A= seq(1:195)
ot= column_to_rownames(ot, var = "deseq2_tree_labels$ASV")
deseq2_tree_labels= column_to_rownames(deseq2_tree_labels, var = "ASV")
deseq2_tree_labels= as.data.frame(deseq2_tree_labels)
otu= otu_table(ot, taxa_are_rows = T)
tax= tax_table(as.matrix(deseq2_tree_labels))
tree= read_tree("Deseq2_Tree/deseq2_ASVs_wagyu_FB_F1_henry_fasta_449.phylip.tre")
phh= merge_phyloseq(otu,tax,tree)

```

```

deseq_tree= plot_tree(phh, color = "Differential.ASVs.Group", label.tips="OTU")
deseq_tree2= plot_tree(phh, color = "Differential.ASVs.Group", label.tips="Differential.ASVs.Group")
tree2= plot_tree(phy, color = "Genus", label.tips="OTU") +theme(legend.position = "bottom")
jpeg("Deseq2_Tree/eseq_tree2.jpeg", width = 8, height = 8, units = 'in', res = 300)
deseq_tree2
dev.off()
### Co-Occurrence network for FB and F1
# SparCC
```{r}
  load("Wagyu_Phyloseq_FB_ONLY.RData")
  load("Muhlbauer_breed_FB_formated_ASVs_sparcc.matrix.RData")
  #load("Sparcc_matrix_FB_F1.RData")
  #save(sparcc.matrix,sparcc.matrix_f1,wagyu_breed_FB, wagyu_Producer_Muhlbauer,file
  ="Sparcc_matrix_FB_F1.RData" )
  wagyu_breed_FB= wagyu_breed$FB
  wagyu_Producer_Muhlbauer = wagyu_Producer$Muhlbauer
  library(microbiome)
  library(microbiomeutilities)
  wagyu_Producer_Muhlbauer2=format_to_besthit(wagyu_Producer_Muhlbauer)
  Muhlbauer_otu= as.data.frame(t(as.data.frame(otu_table(wagyu_Producer_Muhlbauer2))))
  #Sparcc
  Muhlbauer_sparcc.matrix <- sparcc(Muhlbauer_otu)
  #save(Muhlbauer_sparcc.matrix, file = "Muhlbauer_sparcc.matrix.RData")
  wagyu_breed_FB2=format_to_besthit(wagyu_breed_FB)
  breed_FB_otu= as.data.frame(t(as.data.frame(otu_table(wagyu_breed_FB2))))
  #Sparcc
  breed_FB_sparcc.matrix <- sparcc(breed_FB_otu)
  save(Muhlbauer_sparcc.matrix, breed_FB_sparcc.matrix,file =
  "Muhlbauer_breed_FB_formated_ASVs_sparcc.matrix.RData")
  sparcc.matrix_FB_cor=as.data.frame(sparcc.matrix$Cor)
  write.csv(sparcc.matrix_FB_cor, file = "sparcc.matrix_FB_cor.csv")
  rownames(sparcc.matrix_FB_cor) <- colnames(fb_otu)
  colnames(sparcc.matrix_FB_cor) <- colnames(fb_otu)
  sparcc.matrix_FB_cor_deseq2_ASVs= sparcc.matrix_FB_cor[deseq2_ASVs_wagyu_FB]
  write.csv(sparcc.matrix_FB_cor_deseq2_ASVs, file = "sparcc.matrix_FB_cor_deseq2_ASVs.csv")
  deseq2_ASVs_wagyu_Muhlbauer
  write.csv(FB_cor_matrix, file = "FB_cor_matrix_Sparcc.csv")
  #####Newwork breed_FB_sparcc
  sparcc.cutoff <- 0.8
  sparcc.adj <- ifelse(abs(breed_FB_sparcc.matrix$Cor) >= sparcc.cutoff, 1, 0)
  # Add OTU names to rows and columns
  rownames(sparcc.adj) <- colnames(breed_FB_otu)
  colnames(sparcc.adj) <- colnames(breed_FB_otu)
  #write.csv(sparcc.adj, file = "sparcc.adj_FB_0.5.csv")
  # Build network from adjacency
  sparcc.net <- graph.adjacency(sparcc.adj,
                              mode = "undirected",
                              diag = FALSE)
  clean.net <- delete.vertices(sparcc.net, which(degree(sparcc.net, mode = "all") == 0))
  plot(clean.net)
  net= clean.net
  #####Network
  #hub
  # Hub detection
  library(igraph)

```

```

net= clean.net
net.cn <- closeness(net)
net.bn <- betweenness(net)
net.pr <- page_rank(net)$vector
net.hs <- hub_score(net)$vector
#Sort the species based on hubbiness score
net.hs.sort <- sort(net.hs, decreasing = TRUE)
# Choose the top 5 keystone species
net.hs.top10 <- head(net.hs.sort, n = 10)
# Get clusters
wt <- walktrap.community(net)
ml <- multilevel.community(net)
# Get membership of walktrap clusters membership(wt)
# Get clusters using MCL method
adj <- as_adjacency_matrix(net)
#install.packages("MCL")
library(MCL)
mc <- mcl(adj, addLoops = TRUE)
#Isolated nodes that are not connected to any other node in the network can be removed as follows.
#clean.net <- delete.vertices(net, which(degree(net, mode = "all") == 0))
# Network components
net.comps <- components(net)
# Largest component
largest.comp <- V(net)[which.max(net.comps$size) == net.comps$membership]
# Second component
second.comp <- V(net)[net.comps$membership == 2]

# Degrees
deg <- degree(net, mode = "all")
# Degree distribution
deg.dist <- degree_distribution(net, mode = "all", cumulative = T)
# Plot degree distribution
plot(deg.dist, xlab = "Nodes degree", ylab = "Probability")
lines(deg.dist)
# qgraph method
library(qgraph)
qgraph::centralityPlot(net)

# Find articulation points
AP <- articulation.points(net)
# Function 3: Plot network with clusters and node size scaled to hubbiness
plot.net.cls <- function(net, scores, cls, AP, outfile, title) {
  # Get size of clusters to find isolated nodes.
  cls_sizes <- sapply(groups(cls), length)
  # Randomly choosing node colors. Users can provide their own vector of colors.
  colors <- sample(colours(), length(cls))
  # Nodes in clusters will be color coded. Isolated nodes will be white.
  V(net)$color <- sapply(membership(cls),
    function(x) {ifelse(cls_sizes[x]>1,
      colors[x], "white")})
  # Convert node label from names to numerical IDs.
  node.names <- V(net)$name
  col_ids <- seq(1, length(node.names))
  V(net)$name <- col_ids
  # To draw a halo around articulation points.

```

```

AP <- lapply(names(AP), function(x) x)
marks <- lapply(1:length(AP), function(x) which(node.names == AP[[x]]))
# Define output image file.
outfile <- paste(outfile, "jpeg", sep=".")
# Image properties.
jpeg(outfile, width = 10, height = 17, res = 300, units = "in")
#par(mar= c(1, 0.5, 0, 0.5))
# Customized layout to avoid nodes overlapping.
e <- get.edgelist(net)
class(e) <- "numeric"
l <- qgraph.layout.fruchtermanreingold(e, vcount=vcount(net),
                                     area=4*(vcount(net)^2),
                                     repulse.rad=(vcount(net)^2.5))

# Main plot function.
plot(net, vertex.size = (scores*5)+4, vertex.label.cex=0.9,
      vertex.label.color = "black", mark.border="black", mark.groups = marks,
      mark.col = "white", mark.expand = 10, mark.shape = 1, layout=l)
title(title, cex.main=2)
# Plot legend containing OTU names.
labels = paste(as.character(V(net)), node.names, sep = " ")
legend("bottom", legend = labels, xpd = F, ncol = 6, cex = 0.3)
dev.off() }
# Execute this command after running Function 3
set.seed(4)
y=plot.net.cls(net, net.hs, wt, AP,outfile = "Network_figures//wagyu_FB_sparcc_0.8cutof.jpeg", title =
"Wagyu Full Blood")

labels = paste(as.character(V(net)), node.names, sep = " ")
l1= labels[1:138]
l2= labels[139:275]
legend= cbind(l1, l2)
write.csv(legend, file = "Network_figures/ FB_legend_0.8.csv")
deseq2_ASVs_wagyu_FB
node.names <- V(net)$name
deseq2_ASVs_wagyu_FB
deseq2_ASVs_nw=intersect(deseq2_ASVs_wagyu_FB,node.names)
write.csv(deseq2_ASVs_nw, file = "Network_figures/deseq2_ASVs_nw_FB.csv")
ASV.edges <- incident_edges(net, deseq2_ASVs_nw, mode = "all")
#####Newwork Muhlbauer_sparcc
Muhlbauer_otu= as.data.frame(t(as.data.frame(otu_table(wagyu_Producer_Muhlbauer2))))
#Sparcc
Muhlbauer_sparcc.matrix <- sparcc(Muhlbauer_otu)
sparcc.cutoff <- 0.7
sparcc.adj <- ifelse(abs(Muhlbauer_sparcc.matrix$Cor) >= sparcc.cutoff, 1, 0)
# Add OTU names to rows and columns
rownames(sparcc.adj) <- colnames(Muhlbauer_otu)
colnames(sparcc.adj) <- colnames(Muhlbauer_otu)
#write.csv(sparcc.adj, file = "sparcc.adj_Muhlbauer_0.5.csv")
# Build network from adjacency
sparcc.net <- graph.adjacency(sparcc.adj,
                             mode = "undirected",
                             diag = FALSE)
clean.net <- delete.vertices(sparcc.net, which(degree(sparcc.net, mode = "all") == 0))
net= clean.net
length(V(net)$name)

```

```

plot(clean.net)
#####BOOK
#hub
# Hub detection
library(igraph)
net= clean.net
net.cn <- closeness(net)
net.bn <- betweenness(net)
net.pr <- page_rank(net)$vector
net.hs <- hub_score(net)$vector

#Sort the species based on hubbiness score
net.hs.sort <- sort(net.hs, decreasing = TRUE)
# Choose the top 5 keystone species
net.hs.top10 <- head(net.hs.sort, n = 10)
# Get clusters
wt <- walktrap.community(net)
ml <- multilevel.community(net)
# Get membership of walktrap clusters membership(wt)
# Get clusters using MCL method
adj <- as_adjacency_matrix(net)
#install.packages("MCL")
library(MCL)
mc <- mcl(adj, addLoops = TRUE)
#Isolated nodes that are not connected to any other node in the network can be removed as follows.
#clean.net <- delete.vertices(net, which(degree(net, mode = "all") == 0))
# Network components
net.comps <- components(net)
# Largest component
largest.comp <- V(net)[which.max(net.comps$size) == net.comps$membership]
# Second component
second.comp <- V(net)[net.comps$membership == 2]
# Degrees
deg <- degree(net, mode = "all")
# Degree distribution
deg.dist <- degree_distribution(net, mode = "all", cumulative = T)
# Plot degree distribution
plot(deg.dist, xlab = "Nodes degree", ylab = "Probability")
lines(deg.dist)
# qgraph method
library(qgraph)
qgraph::centralityPlot(net)
# Find articulation points
AP <- articulation.points(net)
# Function 3: Plot network with clusters and node size scaled to hubbiness
plot.net.cls <- function(net, scores, cls, AP, outfile, title) {
  # Get size of clusters to find isolated nodes.
  cls_sizes <- sapply(groups(cls), length)
  # Randomly choosing node colors. Users can provide their own vector of colors.
  colors <- sample(colours(), length(cls))
  # Nodes in clusters will be color coded. Isolated nodes will be white.
  V(net)$color <- sapply(membership(cls),
    function(x) {ifelse(cls_sizes[x]>1,
      colors[x], "white")})
}

```

```

# Convert node label from names to numerical IDs.
node.names <- V(net)$name
col_ids <- seq(1, length(node.names))
V(net)$name <- col_ids
# To draw a halo around articulation points.
AP <- lapply(names(AP), function(x) x)
marks <- lapply(1:length(AP), function(x) which(node.names == AP[[x]]))
# Define output image file.
outfile <- paste(outfile, "jpeg", sep=".")
# Image properties.
jpeg(outfile, width = 10, height = 17, res = 300, units = "in")
#par(mar= c(1, 0.5, 0, 0.5))
# Customized layout to avoid nodes overlapping.
e <- get.edgelist(net)
class(e) <- "numeric"
l <- qgraph.layout.fruchtermanreingold(e, vcount=vcount(net),
                                     area=4*(vcount(net)^2),
                                     repulse.rad=(vcount(net)^2.5))

# Main plot function.
plot(net, vertex.size = (scores*5)+4, vertex.label.cex=0.9,
      vertex.label.color = "black", mark.border="black", mark.groups = marks,
      mark.col = "white", mark.expand = 10, mark.shape = 1, layout=l)
title(title, cex.main=2)
# Plot legend containing OTU names.
labels = paste(as.character(V(net)), node.names, sep = " ")
legend("bottom", legend = labels, xpd = F, ncol = 6, cex = 0.3)
dev.off() }
# Execute this command after running Function 3
set.seed(4)
plot.net.cls(net, net.hs, wt, AP, outfile = "Network_figures//wagyu_Muhlbauer_sparcc_0.5cutof.jpeg", title
= "Wagyu Crossbred")
net.hs.top10
deseq2_ASVs_wagyu_Muhlbauer
node.names <- V(net)$name
deseq2_ASVs_wagyu_Muhlbauer
deseq2_ASVs_nw_Muhlbauer=intersect(deseq2_ASVs_wagyu_Muhlbauer,node.names)
write.csv(deseq2_ASVs_nw_Muhlbauer, file = "Network_figures/deseq2_ASVs_nw_Muhlbauer.csv")
ASV.edges_Muhlbauer <- incident_edges(net, deseq2_ASVs_nw_Muhlbauer, mode = "all")
deseq2_ASVs_nw
ASV.edges
str(ASV.edges) # list
ASV.edges[ 1:length(ASV.edges) ]
...

##stepwise regression
```{r}
#Muhlbauer
wagyu_Producer_Muhlbauer2=format_to_besthit(wagyu_Producer_Muhlbauer)
Muhlbauer_otu= as.data.frame(t(as.data.frame(otu_table(wagyu_Producer_Muhlbauer2))))
wagyu_Producer_Muhlbauer2_mapping= as.data.frame(sample_data(wagyu_Producer_Muhlbauer2))
#FB
wagyu_breed_FB2=format_to_besthit(wagyu_breed_FB)
breed_FB_otu= as.data.frame(t(as.data.frame(otu_table(wagyu_breed_FB2))))
##
Muhlbauer_otu_proportions= sweep(Muhlbauer_otu, 2, colSums(Muhlbauer_otu), FUN = "/")
trans.arcsine <- function(x){ asin(sign(x) * sqrt(abs(x)))}

```



```

Muhlbauer_otu_proportions[, 1:2005] <- as.data.frame(lapply(Muhlbauer_otu_proportions[, 1:2005], FUN
= function(x) {sapply(x, FUN = trans.arcsine)}))
library(MASS)
mod= lm(as.numeric(wagyu_Producer_Muhlbauer2_mapping$Grade) ~., data
=Muhlbauer_otu_proportions )
summary(mod)
library(tidyverse)
library(caret)
library(leaps)
full.model <- lm(Fertility ~., data = swiss)
# Stepwise regression model
step.model <- stepAIC(full.model, direction = "both",
                      trace = FALSE)
summary(step.model)
tibble(data("swiss"))
data("swiss")
```

###**Full Blood FB Data Analysis**

```

```{r}
metadata_All[metadata_All$Breed=="FB",]
wagyu_breed= phyloseq_sep_variable(final_ps_filtered_0.04,"Breed", drop_zeroes = T)
phy_FB= wagyu_breed$FB
p_rare_FB <- ggrare(wagyu_breed$FB, step = 10, color = "Marbling", label = "SampleID", se = FALSE)
p_rare_FB
jpeg("Figures_May17_2021/p_rare_FB.jpeg", width = 4.5, height = 4.5, units = 'in', res = 300)
p_rare_FB
dev.off()
names(sample_data(phy_FB))[1]= "SampleIDD"
library(ampvis2)
devtools::source_gist("8d0ca4206a66be7ff6d76fc4ab8e66c6")
ampvis2_obj_FB <- phyloseq_to_ampvis2(phy_FB)
FB_Phyla= amp_heatmap(ampvis2_obj_FB, group_by = "Marbling")
FB_Phyla= FB_Phyla+ theme(axis.text.x = element_text(angle = 45,hjust = 1, vjust = 1))
FB_Genus= amp_heatmap(ampvis2_obj_FB, group_by = "Marbling", tax_aggregate = "Genus",tax_show
= 25)
FB_Genus=FB_Genus+ theme(axis.text.x = element_text(angle = 45,hjust = 1, vjust = 1))
jpeg("Figures_May17_2021/FB_Phyla.jpeg", width = 4, height = 4, units = 'in', res = 300)
FB_Phyla
dev.off()
jpeg("Figures_May17_2021/FB_Genus.jpeg", width = 4, height = 6, units = 'in', res = 300)
FB_Genus
dev.off()
FB_Phyla_breed= amp_heatmap(ampvis2_obj_FB, group_by = "Breed")
FB_Phyla_breed= FB_Phyla_breed+ theme(axis.text.x = element_text(angle = 0,hjust = 1, vjust = 1))
FB_Genus_breed= amp_heatmap(ampvis2_obj_FB, group_by = "Breed", tax_aggregate =
"Genus",tax_show = 25)
FB_Genus_breed=FB_Genus_breed+ theme(axis.text.x = element_text(angle = 0,hjust = 1, vjust = 1))
FB_Species_breed= amp_heatmap(ampvis2_obj_FB, group_by = "Breed", tax_aggregate = "Species",
tax_show = 25)
FB_Species_breed=FB_Species_breed+ theme(axis.text.x = element_text(angle = 0,hjust = 1, vjust = 1))
jpeg("Figures_May17_2021/FB_Phyla_breed.jpeg", width = 4, height = 4, units = 'in', res = 300)
FB_Phyla_breed
dev.off()
jpeg("Figures_May17_2021/FB_Genus_breed.jpeg", width = 4, height = 6, units = 'in', res = 300)
FB_Genus_breed

```


```

```

dev.off()
jpeg("Figures_May17_2021/FB_Species_breed.jpeg", width = 4, height = 6, units = 'in', res = 300)
FB_Species_breed
dev.off()
FB_taxonomy= as.data.frame(tax_table(phy_FB))
length(unique(FB_taxonomy$Phylum))
unique(FB_taxonomy$Phylum)
unique(FB_taxonomy$Class)
unique(FB_taxonomy$Order)
unique(FB_taxonomy$Family)
unique(FB_taxonomy$Genus)
unique(FB_taxonomy$Species)
#Core Venn
FB_venn= amp_venn(ampvis2_obj_FB, group_by = "Marbling", cut_a = 0.001, cut_f = 80, detailed_output
= T, text_size= 3)
jpeg("Figures_May17_2021/FB_venn.jpeg", width = 3.5, height = 3, units = 'in', res = 300)
FB_venn$plot
dev.off()
FB_venn_Otutable= as.data.frame(FB_venn$Otutable)
FB_venn_Otutable= FB_venn_Otutable[!(FB_venn_Otutable$Shared %in% "Non-core"), ]
write.csv(FB_venn_Otutable, file = "FB_venn_Otutable.csv")
##heat map for Venn

Venn_ASVs_FB= c(FB_venn_Otutable$OTU)
wagyu_breed_FB_proportions
Venn_ASVs_ph_proportions_FB= prune_taxa(Venn_ASVs_FB,wagyu_breed_FB_proportions )
#Venn_ASVs_hp= plot_heatmap(Venn_ASVs_ph,sample.label = "Marbling", sample.order = "Marbling" )
library(microbiome)
library(microbiomeutilities)
Venn_ASVs_phy_FB=format_to_besthit(Venn_ASVs_ph_proportions_FB)
otu.mat= abundances(Venn_ASVs_phy_FB)
meta.tab <- meta(Venn_ASVs_phy_FB)
select.meta <- subset(meta.tab, select = c("Marbling"))
newnames <- NULL
newnames <- lapply(
  rownames(otu.mat),
  function(x) bquote(italic(. (x))))
Venn_ASVs_phy_FB_hp <- pheatmap::pheatmap(otu.mat,
  labels_row = as.expression(newnames),
  annotation_col = select.meta,
  #annotation_colors = annotation_colors,
  #annotation_row = row_df,
  fontsize = 5,
  cellwidth = 10,
  cellheight = 6,
  treeheight_col= 10,
  show_colnames = F)
jpeg("Figures_May17_2021/Venn_ASVs_phy_FB_hp.jpeg", width = 6, height =8 , units = 'in', res = 300)
Venn_ASVs_phy_FB_hp
dev.off()
####Alpha Diversity
library("sjstats")
Alpha_Diversity_FB= estimate_richness(wagyu_breed$FB, measures=c("Observed", "InvSimpson",
"Shannon", "Chao1"))
Alpha_Diversity_FB= rownames_to_column(Alpha_Diversity_FB,var = "SampleID")

```

```

Alpha_Diversity_FB= lapply(Alpha_Diversity_FB, function(x) as.numeric(gsub("[X,]", "", x)))
metadata= as.tibble(sample_data(wagyu_breed$FB))
metadata_Alpha_Diversity_FB= merge(metadata,Alpha_Diversity_FB)
metadata_Alpha_Diversity_FB$Marbling
metadata_Alpha_Diversity_FB$HCWgt= as.numeric(metadata_Alpha_Diversity_FB$HCWgt)
Marbling_grouped= group_by(metadata_Alpha_Diversity_FB, Marbling)
Marbling_Summary= summarize(Marbling_grouped,
                             mean_Chao1= mean(Chao1),
                             se_Chao1= parameters::standard_error(Chao1),
                             sd_Chao1= sd(Chao1),
                             mean_Shannon= mean(Shannon),
                             se_Shannon= parameters::standard_error(Shannon),
                             sd_Shannon= sd(Shannon))
Marbling_Chao1_FB=ggboxplot(metadata_Alpha_Diversity_FB, x= "Marbling", y= "Chao1", legend=
"right", ylab = "Chao1 diversity index", xlab = "Marbling", add= "jitter")+stat_summary(fun.y=mean,
geom="point", shape=20, size=2, color="red", fill="red")+ theme(axis.text.x = element_text(angle = 55,
hjust = 1))
Marbling_Shannon_FB=ggboxplot(metadata_Alpha_Diversity_FB, x= "Marbling", y= "Shannon", legend=
"right", ylab = "Shannon diversity index", xlab = "Marbling", add= "jitter")+stat_summary(fun.y=mean,
geom="point", shape=20, size=2, color="red", fill="red")+ theme(axis.text.x = element_text(angle = 55,
hjust = 1))
#dir.create("Figures_May17_2021")
jpeg("Figures_May17_2021/Marbling_Chao1_FB.jpeg", width = 3.2, height = 3.7, units = 'in', res = 300)
Marbling_Chao1_FB
dev.off()
jpeg("Figures_May17_2021/Marbling_Shannon_FB.jpeg", width = 3.2, height = 3.7, units = 'in', res = 300)
Marbling_Shannon_FB
dev.off()
library(stats)
###kruskal.test
kruskal.test(Chao1 ~ Marbling, data = metadata_Alpha_Diversity_FB)
kruskal.test(Shannon ~ Marbling, data = metadata_Alpha_Diversity_FB)
library(dunn.test)
dunn.test()
###Pairwise.Wilcox Test
P.W= pairwise.wilcox.test(metadata_Alpha_Diversity_FB$Chao1,
metadata_Alpha_Diversity_FB$Marbling,p.adjust.method = "none")
P.W_P=P.W$p.value
#install.packages("rcompanion")
library(rcompanion)
P.W_T= fullPTable(P.W_P)
library(multcompView)
multcompLetters(P.W_T,compare="<",
                threshold=0.05,
                Letters=letters,
                reversed = FALSE)
###Dunn Test
library(FSA)
dunnTest(Chao1 ~ Marbling, data = metadata_Alpha_Diversity_FB)

library("MASS")
#interpreting anova(fit1,fitChao1)
#https://stats.stackexchange.com/questions/127509/how-to-determine-whether-a-variable-is-significant-
using-pr-chi-and-df

```

```

#Ordered Logistic Or Probit Regression
polr(Marbling~ Chao1, data= metadata_Alpha_Diversity_FB)
fit1=polr(Marbling~ 1, data= metadata_Alpha_Diversity_FB)
fitChao1=polr(Marbling~ Chao1, data= metadata_Alpha_Diversity_FB)
summary(fitChao1)
anova(fit1,fitChao1)
##Shannon
#Ordered Logistic Or Probit Regression
polr(Marbling~ Shannon, data= metadata_Alpha_Diversity_FB)
fit1=polr(Marbling~ 1, data= metadata_Alpha_Diversity_FB)
fitShannon=polr(Marbling~ Shannon, data= metadata_Alpha_Diversity_FB)
summary(fitShannon)
anova(fit1,fitShannon)
##### Beta Diversity #####
wagyu_breed_FB_proportions= transform_sample_counts(wagyu_breed$FB, function(OTU)
OTU/sum(OTU))
plot_net(wagyu_breed_FB_proportions, maxdist = 0.7, point_label = "SampleID",color =
"Marbling",distance = "bray")
ordu_bray_FB = ordinate(wagyu_breed_FB_proportions, "PCoA", "bray")
ordu_Wt_Unifrac_FB = ordinate(wagyu_breed_FB_proportions, "PCoA", "unifrac", weighted= T)
#####Marbling
beta_div_marbling_bray_FB= plot_ordination(wagyu_breed_FB_proportions, ordu_bray_FB, type =
"sample", color = "Marbling", axes = 1:2)+
  labs(title = "",shape= "Breed", color= "Marbling Grade",y= "PC2[13.8%]", x =
"PC1[34%]")+theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line = element_line(colour ="black"))+
geom_point(size = 2)
beta_div_marbling_bray_FB
jpeg("Figures_May17_2021/beta_div_marbling_bray_FB.jpeg", width = 5, height = 3.7, units = 'in', res =
300)
beta_div_marbling_bray_FB
dev.off()
beta_div_marbling_wunifrac_FB= plot_ordination(wagyu_breed_FB_proportions, ordu_Wt_Unifrac_FB,
type = "sample", color = "Marbling", axes = 1:2)+
  labs(title = "",shape= "Breed", color= "Marbling Grade",y= "PC2[14.5%]", x = "PC1[37.2%]")+
  theme(panel.grid.major = element_blank(),panel.grid.minor = element_blank(),
    panel.background = element_blank(),axis.line = element_line(colour ="black"))+ geom_point(size =
2)
beta_div_marbling_wunifrac_FB
jpeg("Figures_May17_2021/beta_div_marbling_wunifrac_FB.jpeg", width = 5, height = 3.7, units = 'in',
res = 300)
beta_div_marbling_wunifrac_FB
dev.off()
#PERMANOVA
library("vegan")
wagyu_breed_FB_proportions_bray= phyloseq::distance(wagyu_breed_FB_proportions, method = "bray")
sampledf <- data.frame(sample_data(wagyu_breed_FB_proportions))
adonis(wagyu_breed_FB_proportions_bray ~ Marbling, data = sampledf)
##Weighted Unifrac
wagyu_breed_FB_proportions_wunifrac= phyloseq::distance(wagyu_breed_FB_proportions, method =
"wunifrac")
sampledf <- as.data.frame(sample_data(wagyu_breed_FB_proportions))
sampledf <- (sample_data(wagyu_breed_FB_proportions))
sampledf= as.data.frame(sampledf)
adonis(wagyu_breed_FB_proportions_wunifrac ~ Marbling, data = sampledf)

```

```

##uWeighted Unifrac
wagyu_breed_FB_proportions_unifrac= phyloseq::distance(wagyu_breed_FB_proportions, method =
"unifrac")
sampledf <- as.data.frame(sample_data(wagyu_breed_FB_proportions))
sampledf <- (sample_data(wagyu_breed_FB_proportions))
sampledf= as.data.frame(sampledf)
adonis(wagyu_breed_FB_proportions_unifrac ~ Marbling, data = sampledf)
pairwise_wUnifrac= adonis_pairwise(sampledf, wagyu_breed_FB_proportions_wunifrac,group.var
="Marbling", permut = 999, all_results = T, comparison_sep = "." )
#####DeSeq2
library(DESeq2)
wagyu_breed_FB2= format_to_besthit(wagyu_breed$FB)
diagdds = phyloseq_to_deseq2(wagyu_breed_FB2, ~ Marbling)
diagdds$Marbling= releval(diagdds$Marbling, ref = "Moderate Marbling")
diagdds = DESeq(diagdds, test="Wald")
resultsNames(diagdds)
res = results(diagdds, cooksCutoff = FALSE)
res
alpha = 0.05
sigtab = res[which(res$padj < alpha), ]
sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(wagyu_breed$FB)[rownames(sigtab), ], "matrix"))
sigtab
deseq2_ASVs_wagyu_FB= rownames(sigtab)
#write.csv(sigtab, file = "sigtab_deseq2_wagyu_breed_FB.csv")
library(dplyr)
deseq_FB_freq= sigtab %>% group_by(Phylum,Genus,Species) %>% summarise(freq=n())%>%
arrange(desc(freq))
write.csv(deseq_FB_freq, file = "deseq_FB_freq.csv")
library('EnhancedVolcano')
volcano_plots_FB= EnhancedVolcano(res, lab = rownames(res),
  x = 'log2FoldChange',
  y = 'padj',
  pCutoff = 0.05,
  FCcutoff = 2,
  pointSize = 3.0,
  labSize = 2.0,
  title = "Differential (deseq2) Bacterial Species (FB)",
  subtitle = "Excessive Marbling vs Moderate Marbling",
  ylab = bquote(~Log[10]~italic(padj)),
  )
jpeg("Figures_May17_2021/volcano_plots_FB.jpeg", width = 6.5, height =5 , units = 'in', res = 300)
volcano_plots_FB
dev.off()
##Heat map for significant ASVs
deseq2_ASVs_wagyu_FB_ps_proportions= prune_taxa(deseq2_ASVs_wagyu_FB,
wagyu_breed_FB_proportions)
sample_data(deseq2_ASVs_wagyu_FB_ps_proportions)$Marbling
deseq2_ASVs_wagyu_FB_ps_proportions2= subset_samples(deseq2_ASVs_wagyu_FB_ps_proportions,
Marbling%in%c("Moderate Marbling", "Excessive Marbling"))
# format the taxonomy to include unique names
library(microbiome)
library(microbiomeutilities)
phyobj2=format_to_besthit(deseq2_ASVs_wagyu_FB_ps_proportions2)
otu.mat= abundances(phyobj2)
meta.tab <- meta(phyobj2)

```

```

select.meta <- subset(meta.tab, select = c("Marbling"))
newnames <- NULL
newnames <- lapply(
  rownames(otu.mat),
  function(x) bquote(italic.(x)))
heatmap_deseq2_FB <- pheatmap::pheatmap(otu.mat,
  labels_row = as.expression(newnames),
  annotation_col = select.meta,
  #annotation_colors = annotation_colors,
  #annotation_row = row_df,
  fontsize = 5,
  cellwidth = 10,
  cellheight = 6,
  treeheight_col= 10,
  show_colnames = F)
jpeg("Figures_May17_2021/heatmap_deseq2_FB.jpeg", width = 5, height = 10.5, units = 'in', res = 300)
heatmap_deseq2_FB
dev.off()
#####box plots of the selective differential ASVs
deseq2_ASVs_wagyu_FB_ps_proportions= prune_taxa(deseq2_ASVs_wagyu_FB,
wagyu_breed_FB_proportions)
# format the taxonomy to include unique names
library(microbiome)
library(microbiomeutilities)
phy_obj_FB=format_to_besthit(deseq2_ASVs_wagyu_FB_ps_proportions)
otu.mat_FB= abundances(phy_obj_FB)
otu.mat_FB_t= as.data.frame(t(otu.mat_FB))
otu.mat_FB_t= rownames_to_column(otu.mat_FB_t, var = "SampleID")
meta.tab_FB <- meta(phy_obj_FB)
deseq2_ASVs_wagyu_FB_proportions= merge(meta.tab_FB,otu.mat_FB_t)
deseq2_ASVs_wagyu_FB_proportions2= deseq2_ASVs_wagyu_FB_proportions[,c(17,20:134)]
lapply(otu.mat_FB_t,function(x) boxplot(x~ deseq2_ASVs_wagyu_FB_proportions2$Marbling))
ASV_118:g__Prevotella
ASV_158:o__WCHB1-41
names(otu.mat_FB_t[,c(23,47,53,59,87,99,96,103)])
otu.mat_FB_t[,96]
deseq2_FB1=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_826:g__Acetivibrio", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
deseq2_FB2=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_367:g__Mogibacterium", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
deseq2_FB3=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_757:g__Christensenellaceae_R-7_group", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
deseq2_FB4=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_354:g__Christensenellaceae_R-7_group", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
deseq2_FB5=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_1118:g__Desulfovibrio", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
deseq2_FB6=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_415:g__Ruminococcus", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))

deseq2_FB7=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_395:o__Clostridia_UCG-014", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))

```

```

deseq2_FB8=ggboxplot(deseq2_ASVs_wagyu_FB_proportions2, x= "Marbling",
y="ASV_356:f__Muribaculaceae", add= "jitter")+ theme(axis.text.x = element_text(angle = 55, hjust = 1))
jpeg("Figures_May17_2021/boxplots_deseq2/deseq2_FB5.jpeg", width = 3, height = 4, units = 'in', res =
300)
deseq2_FB5
dev.off()
jpeg("Figures_May17_2021/Marbling_Chao1_FB.jpeg", width = 3.2, height = 3.7, units = 'in', res = 300)
Marbling_Chao1_FB
dev.off()
####CrossBred F1 from one producer Muhlbauer Data Analysis** *Thesis*
```{r}
summary(metadata_All$Producer)
metadata_All[metadata_All$Producer=="Muhlbauer",]
wagyu_Producer= phyloseq_sep_variable(final_ps_filtered_0.04,"Producer", drop_zeroes = T)
wagyu_Producer$Muhlbauer
phy_F1= wagyu_Producer$Muhlbauer
names(sample_data(phy_F1))[1]= "SampleIDD"
p_rare_F1 <- ggrare(wagyu_Producer$Muhlbauer, step = 10, color = "Marbling", label = "SampleID", se =
FALSE)
p_rare_F1
jpeg("Figures_May17_2021/p_rare_F1.jpeg", width = 4.5, height = 4.5, units = 'in', res = 300)
p_rare_F1
dev.off()
ampvis2_obj_F1 <- phyloseq_to_ampvis2(phy_F1)
F1_Phyla= amp_heatmap(ampvis2_obj_F1, group_by = "Marbling")
F1_Phyla= F1_Phyla+ theme(axis.text.x = element_text(angle = 45,hjust = 1, vjust = 1))
F1_Genus= amp_heatmap(ampvis2_obj_F1, group_by = "Marbling", tax_aggregate = "Genus",tax_show =
25)
F1_Genus=F1_Genus+ theme(axis.text.x = element_text(angle = 45,hjust = 1, vjust = 1))
jpeg("Figures_May17_2021/F1_Phyla.jpeg", width = 4, height = 4, units = 'in', res = 300)
F1_Phyla
dev.off()
jpeg("Figures_May17_2021/F1_Genus.jpeg", width = 4, height = 6, units = 'in', res = 300)
F1_Genus
dev.off()
F1_Phyla_breed= amp_heatmap(ampvis2_obj_F1, group_by = "Breed")
F1_Phyla_breed= F1_Phyla_breed+ theme(axis.text.x = element_text(angle = 0,hjust = 1, vjust = 1))
F1_Genus_breed= amp_heatmap(ampvis2_obj_F1, group_by = "Breed", tax_aggregate =
"Genus",tax_show = 25)
F1_Genus_breed=F1_Genus_breed+ theme(axis.text.x = element_text(angle = 0,hjust = 1, vjust = 1))
jpeg("Figures_May17_2021/F1_Phyla_breed.jpeg", width = 4, height = 4, units = 'in', res = 300)
F1_Phyla_breed
dev.off()
jpeg("Figures_May17_2021/F1_Genus_breed.jpeg", width = 4, height = 6, units = 'in', res = 300)
F1_Genus_breed
dev.off()
F1_taxonomy= as.data.frame(tax_table(phy_F1))
length(unique(F1_taxonomy$Phylum))
unique(F1_taxonomy$Phylum)
unique(F1_taxonomy$Class)
unique(F1_taxonomy$Order)
unique(F1_taxonomy$Family)
unique(F1_taxonomy$Genus)
unique(F1_taxonomy$Species)
#Core Venn

```

```

F1_venn= amp_venn(ampvis2_obj_F1, group_by = "Marbling", cut_a = 0.001, cut_f = 80, detailed_output
= T, text_size= 3)
jpeg("Figures_May17_2021/F1_venn.jpeg", width = 3.5, height = 3, units = 'in', res = 300)
F1_venn$plot
dev.off()
F1_venn_Otutable= as.data.frame(F1_venn$Otutable)
F1_venn_Otutable= F1_venn_Otutable[!(F1_venn_Otutable$Shared %in% "Non-core"), ]
write.csv(F1_venn_Otutable, file = "F1_venn_Otutable.csv")
##heat map for Venn
Venn_ASVs_F1= c(F1_venn_Otutable$OTU)
wagyu_breed_Muhlbauer_proportions
Venn_ASVs_ph_proportions_F1= prune_taxa(Venn_ASVs_F1,wagyu_breed_Muhlbauer_proportions )
#Venn_ASVs_hp= plot_heatmap(Venn_ASVs_ph,sample.label = "Marbling", sample.order = "Marbling" )
library(microbiome)
library(microbiomeutilities)
Venn_ASVs_phy_F1=format_to_besthit(Venn_ASVs_ph_proportions_F1)
otu.mat= abundances(Venn_ASVs_phy_F1)
meta.tab <- meta(Venn_ASVs_phy_F1)
select.meta <- subset(meta.tab, select = c("Marbling"))
newnames <- NULL
newnames <- lapply(
  rownames(otu.mat),
  function(x) bquote(italic.(x))))
Venn_ASVs_phy_F1_hp <- pheatmap::pheatmap(otu.mat,
  labels_row = as.expression(newnames),
  annotation_col = select.meta,
  #annotation_colors = annotation_colors,
  #annotation_row = row_df,
  fontsize = 5,
  cellwidth = 10,
  cellheight = 6,
  treeheight_col= 10,
  show_colnames = F)
jpeg("Figures_May17_2021/Venn_ASVs_phy_F1_hp.jpeg", width = 7, height =13 , units = 'in', res = 300)
Venn_ASVs_phy_F1_hp
dev.off()
####Alpha Diversity
library("sjstats")
Alpha_Diversity_Muhlbauer= estimate_richness(wagyu_Producer$Muhlbauer, measures=c("Observed",
"InvSimpson", "Shannon", "Chao1"))
Alpha_Diversity_Muhlbauer= rownames_to_column(Alpha_Diversity_Muhlbauer,var = "SampleID")
Alpha_Diversity_Muhlbauer= lapply(Alpha_Diversity_Muhlbauer, function(x) as.numeric(gsub("[X,]", "",
x)))
metadata= as.tibble(sample_data(wagyu_Producer$Muhlbauer))
metadata_Alpha_Diversity_Muhlbauer= merge(metadata,Alpha_Diversity_Muhlbauer)
metadata_Alpha_Diversity_Muhlbauer$Marbling
Marbling_grouped= group_by(metadata_Alpha_Diversity_Muhlbauer, Marbling)
Marbling_Summary= summarize(Marbling_grouped,
  mean_Chao1= mean(Chao1),
  se_Chao1= parameters::standard_error(Chao1),
  sd_Chao1= sd(Chao1),
  mean_Shannon= mean(Shannon),
  se_Shannon= parameters::standard_error(Shannon),
  sd_Shannon= sd(Shannon))

```



```

Marbling_Chao1_Muhlbauer=ggboxplot(metadata_Alpha_Diversity_Muhlbauer, x= "Marbling", y=
"Chao1", legend= "right", ylab = "Chao1 diversity index", xlab = "Marbling", add=
"jitter")+stat_summary(fun.y=mean, geom="point", shape=20, size=2, color="red", fill="red")+
theme(axis.text.x = element_text(angle = 55, hjust = 1))
Marbling_Shannon_Muhlbauer=ggboxplot(metadata_Alpha_Diversity_Muhlbauer, x= "Marbling", y=
"Shannon", legend= "right", ylab = "Shannon diversity index", xlab = "Marbling", add=
"jitter")+stat_summary(fun.y=mean, geom="point", shape=20, size=2, color="red", fill="red")+
theme(axis.text.x = element_text(angle = 55, hjust = 1))

jpeg("Figures_May17_2021/Marbling_Chao1_Muhlbauer.jpeg", width = 3.2, height = 3.7, units = 'in', res
= 300)
Marbling_Chao1_Muhlbauer
dev.off()
jpeg("Figures_May17_2021/Marbling_Shannon_Muhlbauer.jpeg", width = 3.2, height = 3.7, units = 'in',
res = 300)
Marbling_Shannon_Muhlbauer
dev.off()
library(stats)
###kruskal.test
kruskal.test(Chao1 ~ Marbling, data = metadata_Alpha_Diversity_Muhlbauer)
kruskal.test(Shannon ~ Marbling, data = metadata_Alpha_Diversity_Muhlbauer)
library(dunn.test)
###Pairwise.Wilcox Test
P.W= pairwise.wilcox.test(metadata_Alpha_Diversity_Muhlbauer$Chao1,
metadata_Alpha_Diversity_Muhlbauer$Marbling,p.adjust.method = "none")
P.W_P=P.W$p.value
#install.packages("rcompanion")
library(rcompanion)
P.W_T= fullPTable(P.W_P)
library(multcompView)
multcompLetters(P.W_T,compare="<",
threshold=0.05,
Letters=letters,
reversed = FALSE)
###Dunn Test
library(FSA)
dunnTest(Chao1 ~ Marbling, data = metadata_Alpha_Diversity_Muhlbauer)
library("MASS")
#Ordered Logistic Or Probit Regression
polr(Marbling~ Chao1, data= metadata_Alpha_Diversity_Muhlbauer)
fit1=polr(Marbling~ 1, data= metadata_Alpha_Diversity_Muhlbauer)
fitChao1=polr(Marbling~ Chao1, data= metadata_Alpha_Diversity_Muhlbauer)
summary(fitChao1)
anova(fit1,fitChao1)
##Shannon
#Ordered Logistic Or Probit Regression
polr(Marbling~ Shannon, data= metadata_Alpha_Diversity_Muhlbauer)
fit1=polr(Marbling~ 1, data= metadata_Alpha_Diversity_Muhlbauer)
fitShannon=polr(Marbling~ Shannon, data= metadata_Alpha_Diversity_Muhlbauer)
summary(fitShannon)
anova(fit1,fitShannon)

##### Beta Diversity #####

```

```

wagyu_breed_Muhlbauer_proportions= transform_sample_counts(wagyu_Producer$Muhlbauer,
function(OTU) OTU/sum(OTU))
plot_net(wagyu_breed_Muhlbauer_proportions, maxdist = 0.7, point_label = "SampleID",color =
"Marbling",distance = "bray")
ordu_bray_Muhlbauer = ordinate(wagyu_breed_Muhlbauer_proportions, "PCoA", "bray")
ordu_Wt_Unifrac_Muhlbauer = ordinate(wagyu_breed_Muhlbauer_proportions, "PCoA", "unifrac",
weighted= T)
#####Marbling
beta_div_marbling_brays_Muhlbauer= plot_ordination(wagyu_breed_Muhlbauer_proportions,
ordu_brays_Muhlbauer, type = "sample", color = "Marbling", axes = 1:2)+
  labs(title = "", color= "Marbling Grade",y= "PC2[12.2%]", x = "PC1[16.9%]")+theme(panel.grid.major =
element_blank(),panel.grid.minor = element_blank(),panel.background = element_blank(),axis.line =
element_line(colour ="black"))+ geom_point(size = 2)
jpeg("Figures_May17_2021/beta_div_marbling_brays_Muhlbauer.jpeg", width = 5, height = 3.7, units =
'in', res = 300)
beta_div_marbling_brays_Muhlbauer
dev.off()

beta_div_marbling_wunifrac_Muhlbauer= plot_ordination(wagyu_breed_Muhlbauer_proportions,
ordu_Wt_Unifrac_Muhlbauer, type = "sample", color = "Marbling", axes = 1:2)+
  labs(title = "", color= "Marbling Grade",y= "PC2[15.5%]", x = "PC1[19.9%]")+
  theme(panel.grid.major = element_blank(),panel.grid.minor = element_blank(),
  panel.background = element_blank(),axis.line = element_line(colour ="black"))+ geom_point(size =
2)

jpeg("Figures_May17_2021/beta_div_marbling_wunifrac_Muhlbauer.jpeg", width = 5, height = 3.7, units
= 'in', res = 300)
beta_div_marbling_wunifrac_Muhlbauer
dev.off()

#PERMANOVA
library("vegan")
library(reshape2)
wagyu_breed_Muhlbauer_proportions_brays= phyloseq::distance(wagyu_breed_Muhlbauer_proportions,
method = "bray")
sampledf <- data.frame(sample_data(wagyu_breed_Muhlbauer_proportions))
adonis(wagyu_breed_Muhlbauer_proportions_brays ~ Marbling, data = metadata)

##Weighted Unifrac
wagyu_breed_Muhlbauer_proportions_wunifrac=
phyloseq::distance(wagyu_breed_Muhlbauer_proportions, method = "wunifrac")
sampledf <- as.data.frame(sample_data(wagyu_breed_Muhlbauer_proportions))
sampledf <- (sample_data(wagyu_breed_Muhlbauer_proportions))
sampledf= as.data.frame(sampledf)
adonis(wagyu_breed_Muhlbauer_proportions_wunifrac ~ Marbling, data = sampledf)

pairwise_wUnifrac= adonis_pairwise(sampledf, wagyu_breed_Muhlbauer_proportions_wunifrac.group.var
="Marbling", permut = 999, all_results = T, comparison_sep = "." )

##uWeighted Unifrac
wagyu_breed_Muhlbauer_proportions_unifrac= phyloseq::distance(wagyu_breed_Muhlbauer_proportions,
method = "unifrac")
sampledf <- as.data.frame(sample_data(wagyu_breed_Muhlbauer_proportions))
sampledf <- (sample_data(wagyu_breed_Muhlbauer_proportions))
sampledf= as.data.frame(sampledf)

```

```

adonis(wagyu_breed_Muhlbauer_proportions_unifrac ~ Marbling, data = sampledf)

#####DeSeq2
library(DESeq2)

wagyu_Producer_Muhlbauer2=format_to_besthit(wagyu_Producer$Muhlbauer)
diagdds = phyloseq_to_deseq2(wagyu_Producer$Muhlbauer, ~ Marbling)
diagdds$Marbling= releval(diagdds$Marbling, ref = "Low Marbling")
diagdds = DESeq(diagdds, test="Wald")
res = results(diagdds, cooksCutoff = FALSE)
res
alpha = 0.05
sigtab = res[which(res$padj < alpha), ]
sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(wagyu_Producer$Muhlbauer)[rownames(sigtab), ],
"matrix"))
sigtab
deseq2_ASVs_wagyu_Muhlbauer= rownames(sigtab)
#write.csv(sigtab, file = "sigtab_deseq2_wagyu_Producer_Muhlbauer_F1.csv")
deseq_Muhlbauer_freq= sigtab %>% group_by(Phylum,Genus,Species) %>% summarise(freq=n())%>%
arrange(desc(freq))
#write.csv(deseq_Muhlbauer_freq, file = "deseq_Muhlbauer_freq.csv")
library('EnhancedVolcano')
volcano_plots_Muhlbauer= EnhancedVolcano(res, lab = rownames(res),
  x = 'log2FoldChange',
  y = 'padj',
  pCutoff = 0.05,
  FCcutoff = 2,
  pointSize = 3.0,
  labSize = 2.0,
  title = "Differential (deseq2) Bacterial Species (F1)",
  subtitle = "High Marbling vs Low Marbling ",
  ylab = bquote(~Log[10]~italic(padj)),
  )
jpeg("Figures_May17_2021/volcano_plots_Muhlbauer.jpeg", width = 6.5, height =5 , units = 'in', res =
300)
volcano_plots_Muhlbauer
dev.off()
## Heatmap
deseq2_ASVs_wagyu_Muhlbauer_ps_proportions= prune_taxa(deseq2_ASVs_wagyu_Muhlbauer,
wagyu_breed_Muhlbauer_proportions)
sample_data(deseq2_ASVs_wagyu_Muhlbauer_ps_proportions)$Marbling
deseq2_ASVs_wagyu_Muhlbauer_ps_proportions2=
subset_samples(deseq2_ASVs_wagyu_Muhlbauer_ps_proportions, Marbling %in% c("Low Marbling",
"High Marbling"))
library(microbiomeutilities)
library(microbiome)
Muhlbauer_heatmap_data= format_to_besthit(deseq2_ASVs_wagyu_Muhlbauer_ps_proportions2)
Muhlbauer.tab= abundances(Muhlbauer_heatmap_data)
Muhlbauer.meta= meta(Muhlbauer_heatmap_data)
Muhlbauer_meta_marbling2= subset(Muhlbauer.meta, select=c("Marbling"))
newnames <- NULL
newnames <- lapply(
  rownames(otu.mat),
  function(x) bquote(italic(. (x))))
heatmap_deseq2_Muhlbauer <- pheatmap::pheatmap(Muhlbauer.tab,

```

```

        labels_row = as.expression(newnames),
        annotation_col = Muhlbauer_meta_marbling2,
        #annotation_colors = annotation_colors,
        #annotation_row = row_df,
        fontsize = 5,
        #cellwidth = 5,
        #cellheight = 6,
        treeheight_col= 8,
        treeheight_row = 15,
        show_colnames = F
    )
jpeg("Figures_May17_2021/heatmap_deseq2_Muhlbauer.jpeg", width = 5, height = 5, units = 'in', res =
300)
heatmap_deseq2_Muhlbauer
dev.off()
---
title: "Angus Samples Analysis"
output: html_document
---

```{r ,echo=TRUE}
library("import")
library("knitr")
library("BiocStyle")
library("ggplot2")
library("gridExtra")
library("dada2")
library("phyloseq")
library("DECIPHER")
library("ape")
library("phangorn")
library("BiocStyle")
library("ShortRead")
```

# Load packages into session, and print package version
sapply(c(.cran_packages, .bioc_packages), require, character.only = TRUE)
```

```{r}
load("Henry_Phyloseq_rarefaction.RData")

load("Henry_phyloseq_ONLY.RData")
deseq2_output= read.csv("sigtab_deseq2_Angus_Henry_Data.csv")
deseq2_ASVs_henry= as.vector(deseq2_output$X)
library("seqinr")
fastafile= read.fasta(file = "Tables/ASVs.fa", seqtype = "DNA", as.string = T, set.attributes = T,
forceDNAtolower = F, whole.header=T)
NameS= names(fastafile)
deseq2_ASVs_henry_fasta=fastafile[c(which(names(fastafile) %in% deseq2_ASVs_henry)]]
names_henry_fa= names(deseq2_ASVs_henry_fasta)
dir.create("Deseq2_files")
write.fasta(deseq2_ASVs_henry_fasta, file = "Deseq2_files/deseq2_ASVs_henry_fasta.fa", names =
names_henry_fa)
deseq2_ASVs_henry_taxa= prune_taxa(deseq2_ASVs_henry, Hn_final_ps_filtered_0.04)

```

```

deseq2_ASVs_henry_taxa_tb= tax_table(deseq2_ASVs_henry_taxa)
write.csv(deseq2_ASVs_henry_taxa_tb, file = "Deseq2_files/deseq2_ASVs_henry_taxa_tb.csv")
```
```{r}

load("Henry_Phyloseq_rarefaction.RData")
ps
final_ps
sample_names(final_ps)
new_variable= read.table("mapping_file_finished_diet_only_final_new_variable.txt", sep = "\t", header =
T)
new_variable$SampleID= gsub("S*", "", new_variable$SampleID)
rownames(new_variable)= new_variable$SampleID
new_variable$USDA_Grade
new_variable$USDA_Grade= factor(new_variable$USDA_Grade, levels = c("Select", "Choice", "Prime",
""))
new_variable$USDA_Grade_Ad
new_variable$USDA_Grade_Ad= factor(new_variable$USDA_Grade, levels = c("Select",
"Choice", "Choice+", "Prime", "Prime+", ""))
new_var= sample_data(new_variable)
sample_variables(final_ps)
final_ps= merge_phyloseq(final_ps, new_var)
Hn_final_ps_filtered_0.04= phyloseq_filter_prevalence(final_ps, prev.trh = 0.04, abund.trh =
1, threshold_condition = "AND", abund.type = "total")
Angus_mapping= as.data.frame(sample_data(Hn_final_ps_filtered_0.04))
sample_NO_Marbling= Angus_mapping[Angus_mapping$Marbling_group=="",]
sample_NO_Marbling_N= (sample_NO_Marbling$SampleID)
Hn_final_ps_filtered_0.04= subset_samples(Hn_final_ps_filtered_0.04,
!(sample_names(Hn_final_ps_filtered_0.04) %in% sample_NO_Marbling_N))
#####
names(sample_data(Hn_final_ps_filtered_0.04))[1]= "SampleIDD"
library(ampvis2)
devtools::source_gist("8d0ca4206a66be7ff6d76fc4ab8e66c6")
ampvis2_obj_Angus <- phyloseq_to_ampvis2(Hn_final_ps_filtered_0.04)
Angus_Phyla= amp_heatmap(ampvis2_obj_Angus, group_by = "Sex")
Angus_Phyla= Angus_Phyla+ theme(axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1))
Angus_Genus= amp_heatmap(ampvis2_obj_Angus, group_by = "Sex", tax_aggregate = "Genus", tax_show
= 25)
Angus_Genus=Angus_Genus+ theme(axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1))
jpeg("Figures_May17_2021/FB_Phyla.jpeg", width = 4, height = 4, units = 'in', res = 300)
FB_Phyla
dev.off()
#####
Hn_final_ps_filtered_0.04_besthit= format_to_besthit(Hn_final_ps_filtered_0.04)
sample_variables(Hn_final_ps_filtered_0.04)
sample_data(Hn_final_ps_filtered_0.04)$USDA_Grade
Henry_Angus_otu= as.data.frame(t(as.data.frame(otu_table(Hn_final_ps_filtered_0.04_besthit))))
#sparcc.matrix_Angus <- sparcc(Henry_Angus_otu)
sparcc.matrix_Angus_cor= as.data.frame(sparcc.matrix_Angus$Cor)
rownames(sparcc.matrix_Angus_cor) <- colnames(Henry_Angus_otu)
colnames(sparcc.matrix_Angus_cor) <- colnames(Henry_Angus_otu)
sparcc.matrix_Angus_cor_deseq_ASVs= sparcc.matrix_Angus_cor[deseq2_ASVs_Angus]
###
deseq2_ASVs_Angus
sparcc.matrix_Angus.Cor= as.data.frame(sparcc.matrix_Angus$Cor)

```

```

rownames(sparcc.matrix_Angus.Cor) <- colnames(Henry_Angus_otu)
colnames(sparcc.matrix_Angus.Cor) <- colnames(Henry_Angus_otu)
sparcc.matrix_Angus.Cor_deseq= sparcc.matrix_Angus.Cor[deseq2_ASVs_Angus]
sparcc.cutoff <- 0.3
sparcc.adj <- ifelse(abs(sparcc.matrix_Angus.Cor_deseq) >= sparcc.cutoff, 1, 0)
# Add OTU names to rows and columns
rownames(sparcc.adj) <- colnames(Henry_Angus_otu)
colnames(sparcc.adj) <- colnames(Henry_Angus_otu)
# Build network from adjacency
sparcc.net <- graph.adjacency(sparcc.adj,
                             mode = "undirected",
                             diag = FALSE)
clean.net <- delete.vertices(sparcc.net, which(degree(sparcc.net, mode = "all") == 0))
plot(clean.net)
otus.edges
#####
#write.csv(sparcc.matrix_Angus_cor_deseq_ASVs, file = "sparcc.matrix_Angus_cor_deseq_ASVs.csv")
sparcc.cutoff <- 0.3
sparcc.adj <- ifelse(abs(sparcc.matrix_Angus$Cor) >= sparcc.cutoff, 1, 0)
# Add OTU names to rows and columns
rownames(sparcc.adj) <- colnames(Henry_Angus_otu)
colnames(sparcc.adj) <- colnames(Henry_Angus_otu)
# Build network from adjacency
sparcc.net <- graph.adjacency(sparcc.adj,
                             mode = "undirected",
                             diag = FALSE)
clean.net <- delete.vertices(sparcc.net, which(degree(sparcc.net, mode = "all") == 0))
plot(clean.net)
net= sparcc.net
#####BOOK
#hub
# Hub detection
library(igraph)
net= clean.net
net.cn <- closeness(net)
net.bn <- betweenness(net)
net.pr <- page_rank(net)$vector
net.hs <- hub_score(net)$vector
#Sort the species based on hubbiness score
net.hs.sort <- sort(net.hs, decreasing = TRUE)
# Choose the top 5 keystone species
net.hs.top10 <- head(net.hs.sort, n = 10)
# Get clusters
wt <- walktrap.community(net)
ml <- multilevel.community(net)
# Get membership of walktrap clusters membership(wt)
# Get clusters using MCL method
adj <- as_adjacency_matrix(net)
#install.packages("MCL")
library(MCL)
mc <- mcl(adj, addLoops = TRUE)
#Isolated nodes that are not connected to any other node in the network can be removed as follows.
#clean.net <- delete.vertices(net, which(degree(net, mode = "all") == 0))

# Network components

```

```

net.comps <- components(net)
# Largest component
largest.comp <- V(net)[which.max(net.comps$size) == net.comps$membership]
# Second component
second.comp <- V(net)[net.comps$membership == 2]
# Degrees
deg <- degree(net, mode = "all")
# Degree distribution
deg.dist <- degree_distribution(net, mode = "all", cumulative = T)
# Plot degree distribution
plot(deg.dist, xlab = "Nodes degree", ylab = "Probability")
lines(deg.dist)
# qgraph method
library(qgraph)
qgraph::centralityPlot(net)
# Find articulation points
AP <- articulation.points(net)
# Function 3: Plot network with clusters and node size scaled to hubbiness
plot.net.cls <- function(net, scores, cls, AP, outfile, title) {
  # Get size of clusters to find isolated nodes.
  cls_sizes <- sapply(groups(cls), length)
  # Randomly choosing node colors. Users can provide their own vector of colors.
  colors <- sample(colours(), length(cls))
  # Nodes in clusters will be color coded. Isolated nodes will be white.
  V(net)$color <- sapply(membership(cls),
    function(x) {ifelse(cls_sizes[x]>1,
      colors[x], "white")})
  # Convert node label from names to numerical IDs.
  node.names <- V(net)$name
  col_ids <- seq(1, length(node.names))
  V(net)$name <- col_ids
  # To draw a halo around articulation points.
  AP <- lapply(names(AP), function(x) x)
  marks <- lapply(1:length(AP), function(x) which(node.names == AP[[x]]))
  # Define output image file.
  outfile <- paste(outfile, "jpeg", sep=".")
  # Image properties.
  jpeg(outfile, width = 10, height = 15, res = 300, units = "in")
  #par(mar= c(1, 0.5, 0, 0.5))
  # Customized layout to avoid nodes overlapping.
  e <- get.edgelist(net)
  class(e) <- "numeric"
  l <- qgraph.layout.fruchtermanreingold(e, vcount=vcount(net),
    area=4*(vcount(net)^2),
    repulse.rad=(vcount(net)^2.5))
  # Main plot function.
  plot(net, vertex.size = (scores*5)+4, vertex.label.cex=0.9,
    vertex.label.color = "black", mark.border="black", mark.groups = marks,
    mark.col = "white", mark.expand = 10, mark.shape = 1, layout=l)
  title(title, cex.main=2)
  # Plot legend containing OTU names.
  labels = paste(as.character(V(net)), node.names, sep = " ")
  legend("bottom", legend = labels, xpd = F, ncol = 4, cex = 0.35)
  dev.off() }
# Execute this command after running Function 3

```

```

set.seed(4)
plot.net.cls(net, net.hs, wt, AP, outfile = "Figures//Henry_sparcc_0.5cutof.jpeg", title = "Angus")
node.names <- V(net)$name
deseq2_ASVs_Angus
deseq2_ASVs_nw=intersect(deseq2_ASVs_Angus,node.names)
ASV.edges <- incident_edges(net, deseq2_ASVs_nw, mode = "all")
ASV.edges
write.csv(deseq2_ASVs_nw, file = "deseq2_ASVs_nw.csv")
#####
stepwise reg
Henry_Angus_otu= as.data.frame(t(as.data.frame(otu_table(Hn_final_ps_filtered_0.04_besthit))))
Henry_Angus_mapping= as.data.frame(sample_data(Hn_final_ps_filtered_0.04_besthit))

Henry_Angus_otu_proportions= sweep(Henry_Angus_otu, 2, colSums(Henry_Angus_otu), FUN = "/")
trans.arcsine <- function(x){ asin(sign(x) * sqrt(abs(x)))}
Henry_Angus_otu_proportions[, 1:1002] <- as.data.frame(lapply(Henry_Angus_otu_proportions[, 1:1002],
FUN = function(x) {sapply(x, FUN = trans.arcsine)}))
library(MASS)
class(Henry_Angus_mapping$Marbling)
data =Henry_Angus_otu_proportions[deseq2_ASVs_Angus]
full.model= lm(Henry_Angus_mapping$Marbling ~., data
=Henry_Angus_otu_proportions[deseq2_ASVs_Angus] )
summary(full.model)
step.model <- stepAIC(full.model, direction = "both",
trace = FALSE)
summary(step.model)
#####
null_Angus_Marbling=lm(Henry_Angus_mapping$Marbling~1, data
=Henry_Angus_otu_proportions[deseq2_ASVs_Angus])
summary(null_Angus_Marbling)
full_Angus_Marbling=lm(Henry_Angus_mapping$Marbling~., data
=Henry_Angus_otu_proportions[deseq2_ASVs_Angus])
summary(full_Angus_Marbling)
step(null_Angus_Marbling, scope=list(lower=null_Angus_Marbling, upper=full_Angus_Marbling),
direction="forward")
#Model Marbling
model_Angus_Marbling=lm(Henry_Angus_mapping$Marbling~ `ASV_285:o__RF39` +
`ASV_183:o__Oscillospirales` + `ASV_189:lipolyticus` + `ASV_263:f__Ruminococcaceae` +
`ASV_102:g__Prevotella` + `ASV_331:o__RF39` + `ASV_296:g__Treponema`, data
=Henry_Angus_otu_proportions[deseq2_ASVs_Angus])
summary(model_Angus_Marbling)
library(car)
anova(model_Angus_Marbling)
#Test multi-collinearity (variance inflation factor)
vif(model_Angus_Marbling)
sqrt(vif(model_Angus_Marbling)) > 2
#Homoscedasticity & Normality
layout(matrix(c(1,2,3,4),2,2))
plot(model_Angus_Marbling)
#Extract observed and predicted values
OP_Angus_Marbling <- data.frame(Observed=Henry_Angus_mapping$Marbling,
Predicted=fitted(model_Angus_Marbling))
#Plot observed vs predicted
par(mfrow=c(1,1))

```



```

plot(OP_Angus_Marbling$Predicted, OP_Angus_Marbling$Observed, xlab="Predicted Marbling",
ylab="Observed Marbling")
OP=lm(Observed~Predicted, data=OP_Angus_Marbling)
#summary(OP)
abline(OP)
#####
####alpha
library("sjstats")
Alpha_Diversity_Angus= estimate_richness(Hn_final_ps_filtered_0.04, measures=c("Observed",
"InvSimpson", "Shannon", "Chao1"))
Alpha_Diversity_Angus= rownames_to_column(Alpha_Diversity_Angus,var = "SampleID")
Alpha_Diversity_Angus= lapply(Alpha_Diversity_Angus, function(x) as.numeric(gsub("[X,]", "", x)))
metadata= as.tibble(sample_data(Hn_final_ps_filtered_0.04))
metadata_Alpha_Diversity_Angus= merge(metadata,Alpha_Diversity_Angus)
Marbling_Chao1_Angus=ggboxplot(metadata_Alpha_Diversity_Angus, x= "USDA_Grade", y= "Chao1",
legend= "right", ylab = "Chao1 diversity index", xlab = "USDA_Grade", add=
"jitter")+stat_summary(fun.y=mean, geom="point", shape=20, size=2, color="red", fill="red")+
theme(axis.text.x = element_text(angle = 55, hjust = 1))
Marbling_Shannon_Angus=ggboxplot(metadata_Alpha_Diversity_Angus, x= "USDA_Grade", y=
"Shannon", legend= "right", ylab = "Shannon diversity index", xlab = "USDA_Grade", add=
"jitter")+stat_summary(fun.y=mean, geom="point", shape=20, size=2, color="red", fill="red")+
theme(axis.text.x = element_text(angle = 55, hjust = 1))
#dir.create("Figures_May17_2021")
jpeg("Figures/Marbling_Chao1_Angus.jpeg", width = 3.2, height = 3.7, units = 'in', res = 300)
Marbling_Chao1_Angus
dev.off()
jpeg("Figures/Marbling_Shannon_Angus.jpeg", width = 3.2, height = 3.7, units = 'in', res = 300)
Marbling_Shannon_Angus
dev.off()
library(stats)
###kruskal.test
kruskal.test(Chao1 ~ USDA_Grade, data = metadata_Alpha_Diversity_Angus)
kruskal.test(Shannon ~ USDA_Grade, data = metadata_Alpha_Diversity_Angus)
#####beta
Hn_final_ps_filtered_0.04_proportions= transform_sample_counts(Hn_final_ps_filtered_0.04,
function(OTU) OTU/sum(OTU))
"USDA_Grade"
ordu_bray_Angus = ordinate(Hn_final_ps_filtered_0.04_proportions, "PCoA", "bray")
ordu_Wt_Unifrac_Angus = ordinate(Hn_final_ps_filtered_0.04_proportions, "PCoA", "unifrac",
weighted= T)

beta_div_marbling_bray_Angus= plot_ordination(Hn_final_ps_filtered_0.04_proportions,
ordu_bray_Angus, type = "sample", color = "USDA_Grade", axes = 1:2)+
labs(title = "",shape= "Breed", color= "USDA_Grade",y= "PC2[8.2%]", x =
"PC1[31.4%]") + theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line = element_line(colour = "black"))+
geom_point(size = 2)
beta_div_marbling_bray_Angus
jpeg("Figures/beta_div_marbling_bray_Angus.jpeg", width = 5, height = 3.7, units = 'in', res = 300)
beta_div_marbling_bray_Angus
dev.off()
#PERMANOVA
library("vegan")
sampledf = as.tibble(sample_data(Hn_final_ps_filtered_0.04_proportions))
##bray

```

```

wagyu_breed_FB_proportions_bray= phyloseq::distance(Hn_final_ps_filtered_0.04_proportions, method =
"bray")
adonis(wagyu_breed_FB_proportions_bray ~ USDA_Grade, data = sampledf)
##Weighted Unifrac
wagyu_breed_FB_proportions_wunifrac= phyloseq::distance(Hn_final_ps_filtered_0.04_proportions,
method = "wunifrac")
adonis(wagyu_breed_FB_proportions_wunifrac ~ USDA_Grade, data = sampledf)
##uWeighted Unifrac
wagyu_breed_FB_proportions_unifrac= phyloseq::distance(Hn_final_ps_filtered_0.04_proportions,
method = "unifrac")
adonis(wagyu_breed_FB_proportions_unifrac ~ USDA_Grade, data = sampledf)

#####DeSeq2
library(DESeq2)
Hn_final_ps_filtered_0.04_besthit
sample_variables(Hn_final_ps_filtered_0.04_besthit)
sample_data(Hn_final_ps_filtered_0.04_besthit)$USDA_Grade=
factor(sample_data(Hn_final_ps_filtered_0.04_besthit)$USDA_Grade, levels = c("Select",
"Choice", "Prime", ""))
diagdds = phyloseq_to_deseq2(Hn_final_ps_filtered_0.04_besthit, ~ USDA_Grade)
diagdds$USDA_Grade= relevel(diagdds$USDA_Grade, ref = "Select")
diagdds = DESeq(diagdds, test="Wald")
resultsNames(diagdds)
res = results(diagdds, cooksCutoff = FALSE)
res
alpha = 0.05
sigtab = res[which(res$padj < alpha), ]
sigtab
sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(Hn_final_ps_filtered_0.04_besthit)[rownames(sigtab),
], "matrix"))
sigtab
deseq2_ASVs_Angus= rownames(sigtab)
write.csv(sigtab, file = "sigtab_deseq2_Angus_Henry_Data2.csv")
sigtab %>%
  count(Phylum, Genus, Species) %>%
  arrange(desc(n))
library('EnhancedVolcano')
volcano_plots_Angus_hn= EnhancedVolcano(res, lab = rownames(res),
  x = 'log2FoldChange',
  y = 'padj',
  pCutoff = 0.05,
  FCcutoff = 2,
  pointSize = 3.0,
  labSize = 2.0,
  title = "Differential (deseq2) Bacterial Species (Angus)",
  subtitle = "USDA Grade Prime vs Select",
  ylab = bquote(~-Log[10]~italic(padj)),
)
jpeg("Figures/volcano_plots_Angus_hn.jpeg", width = 6.5, height = 5, units = 'in', res = 300)
volcano_plots_Angus_hn
dev.off()
##Heat map for significant ASVs
deseq2_ASVs_Angus_ps_proportions= prune_taxa(deseq2_ASVs_Angus,
Hn_final_ps_filtered_0.04_proportions)
sample_data(Hn_final_ps_filtered_0.04_proportions)$USDA_Grade

```

```

deseq2_ASVs_Angus_ps_proportions2= subset_samples(deseq2_ASVs_Angus_ps_proportions,
USDA_Grade%in%c("Select", "Prime"))
# format the taxonomy to include unique names
library(microbiome)
library(microbiomeutilities)
phyobj2=format_to_besthit(deseq2_ASVs_Angus_ps_proportions2)
otu.mat= abundances(phyobj2)
meta.tab <- meta(phyobj2)
select.meta <- subset(meta.tab, select = c("USDA_Grade"))
newnames <- NULL
newnames <- lapply(
  rownames(otu.mat),
  function(x) bquote(italic.(x))))
heatmap_deseq2_Angus <- pheatmap::pheatmap(otu.mat,
  labels_row = as.expression(newnames),
  annotation_col = select.meta,
  #annotation_colors = annotation_colors,
  #annotation_row = row_df,
  fontsize = 5,
  cellwidth = 10,
  cellheight = 6,
  treeheight_col= 10,
  show_colnames = F)

jpeg("Figures/heatmap_deseq2_Angus.jpeg", width = 6.5, height =6 , units = 'in', res = 300)
heatmap_deseq2_Angus
dev.off()

```