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## Evaluation of gene expression in pigs selected for enhanced reproduction using differential display PCR: II. Anterior pituitary<sup>1</sup>

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**ABSTRACT:** The objective of this study was to identify differentially expressed genes in the anterior pituitary (AP) of sows selected for enhanced reproductive phenotypes. Selection in the Index (I) line was based on an index of ovulation rate and embryo survival, whereas random selection was used in the Control (C) line. Average numbers of fully formed piglets at birth were 12.5  $\pm$  1.5 and 9.9  $\pm$  2.0 for Line I and C sows used in this study, respectively. In order to induce luteolysis and synchronize follicle development, sows were injected (i.m.) with 2 mL of prostaglandin  $F_{2\alpha}$  analog between d 12 and 14 of the estrous cycle. Tissue was harvested 2 d (d2) or 4 d (d4) after injection, resulting in four experimental groups: Cd2 (n = 6), Cd4 (n = 4), Id2 (n = 6), and Id4 (n = 7). Differential display PCR (ddPCR) was used to search for transcriptional changes between selection lines in the AP, using samples within line but pooled across days. Northern hybridization was used to confirm ddPCR results. For ddPCR, two pools were used from each line (C and I). Three genes were confirmed to be differentially expressed between Lines I and C: *G-beta like protein, ferritin heavy-chain,* and *follicle stimulating hormone beta subunit,* whereas many other expressed sequence tags were observed to be differentially expressed but still require confirmation. Our findings indicate that long-term selection to increase ovulation rate and decrease embryo mortality has altered transcriptional patterns in the anterior pituitary, most likely as correlated responses.

Key Words: Gene Expression, Pig, Pituitary, Reproduction, Selection

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#### Introduction

Differential display PCR (**ddPCR**) was first described (Liang and Pardee, 1992) as a way of conducting gene expression studies by comparing eukaryotic messenger RNA (mRNA) levels from different sources. This technique enables the large-scale screening of the transcriptome without prior knowledge of existing information regarding sequence and identification of transcripts.

Gene expression analysis is a functional genomics approach that is useful for discovering novel expressed genes and for comparing mRNA levels of transcripts

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regulating fertility. The ddPCR method has been used for transcriptional analysis to understand reproductive processes in mouse (Lee et al., 2001; Minami et al., 2001), cow (Robert et al., 2001), human (Xu et al., 1999), Rhesus monkey (Ace and Okulicz, 1999), and pig (Li et al., 1996). Gene expression analysis is also useful for understanding the biological basis that underlies polygenic traits and the response to long-term genetic selection. Such traits as heat loss in mice (Allan et al., 2000) and reproduction in pigs (Gladney et al., 2004) have been investigated using ddPCR analysis in selection lines. These studies enabled the identification of a variety of differentially expressed genes, demonstrating that selection for polygenic traits can cause detectable changes in the transcriptome at various temporal and spatial coordinates related to the physiology of the trait.

The anterior pituitary is an important reproductive gland that intermediates communication between hypothalamus and ovaries. We investigated the effect of selection for enhanced reproduction on gene expression in the anterior pituitary of pigs. Differential display PCR was used to search for differences in transcription levels between lines, under the hypothesis that gene expression in the anterior pituitary gland of females has changed during the period of ovarian

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follicular development, as either a direct or correlated response to selection. Northern hybridization was used to confirm ddPCR results.

#### Materials and Methods

#### Resource Population, Animals, and Treatments

A general description of selection lines and sows used in this experiment is described in the companion article (Gladney et al., 2004) for a concurrent study evaluating the ovarian transcriptome. Briefly, 23 second-parity sows from the 16th generation of selection in the Index (Line I; n = 13) and Control (Line C; n = 10 lines were used. Sows were checked daily after weaning for estrus, and the average weaning-to-estrus interval was  $6.4 \pm 0.7$  d for Line C sows and  $8.9 \pm 4.4$ d for Line I sows. All sows from both lines were injected (i.m.) with 2 mL of the prostaglandin  $F_{2\alpha}$  analog Lutalyse (Upjohn, Kalamazoo, MI) between d 12 and 14 after detection of estrus in order to induce luteolysis and synchronize follicle development. Anterior pituitaries from sows were harvested at d 2 or d 4 after Lutalyse injection, resulting in four treatment groups: Line C, **d2** (n = 6); Line C, **d4** (n = 4); Line I, **d2** (n = 6); and Line I, **d4** (n = 7).

#### Tissue Collection and RNA Extraction

Sows were weighed before harvest. Anterior pituitary lobes were collected, within 20 min after stunning of the animal, by opening the cranial cavity, and the lobes were snap-frozen in a cryovial using liquid nitrogen. Tissues were stored at -80°C. The anterior pituitary was weighed before extraction of total RNA using Trizol LS (Gibco Life Technologies, Grand Island, NY). Poly(A) RNA was purified using the Qiagen (Valenica, CA) Oligotex mRNA midi kit. Total RNA and poly(A) RNA were quantified using the TD-700 fluorometer (Turner Designs, Sunnyvale, CA).

#### Differential Display PCR (ddPCR)

Differential display PCR (ddPCR) was used to search for differences in AP gene expression between lines, and Northern hybridization was used to validate selected ddPCR results. Two pools of poly(A) RNA (2.2 ng/ $\mu$ L) were used from each line, each having samples from one d2 sow and from one d4 sow. Pools were designated as CA and CB (Line C) and IA and IB (Line I). Although evaluation of line × day interaction would have been appealing, insufficient sample was available to afford adequate power for such an analysis.

Methods for ddPCR are described in the companion article (Gladney et al., 2004). Briefly, first strand complementary DNA (cDNA) synthesis was performed using 2.2 ng of poly(A) and 4 pmol anchor primer to a final volume of 10  $\mu$ L. Template cDNA representing the four experimental groups were used for fluorescent

ddPCR employing 10 anchor primers combined with 20 arbitrary primers, resulting in a total of 200 different primer combinations and PCR reactions per sample pool.

#### Evaluation of ddPCR Gels

Products of ddPCR (4  $\mu$ L) were mixed with 1.5  $\mu$ L of loading buffer, denatured at 95°C for 5 min, loaded in a 5.8% polyacrylamide denaturing gel, and electrophoresed at 3,000 V (100 W) and at  $50^{\circ}$ C for 2.5 to 5 h. Gels were dried and washed before scanning for fluorescence. Images were evaluated using Adobe (San Jose, CA) Photoshop 4.0 software. Analysis of banding patterns was based on subjective visual inspection, taking into account background intensities, consistency within pools of each line, and intensity of the differences between lines. A scoring system of 1 to 6 was used to rank and prioritize bands for further investigations, with score 1 representing marginal differences between selection lines and increasing values representing increasing robustness and consistency of differences. Selected bands were excised and placed in 50 µL of TE buffer (10 mM Tris-Cl, pH 7.4), incubated at 37°C for 30 min and stored at -40°C.

#### Reamplification, Cloning, and Sequencing

Bands with scores of 5 and 6 (n = 168) and a few bands with similar expression between lines (n = 12)were sequenced (n = 179) as previously described (Gladney et al., 2004). Sequences were managed using BioEdit version 5.0.0 (jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html). Expressed sequence tags (EST) were searched for similarity against the nr databases using the BLAST nr database (www.ncbi.nlm.nih.gov/ BLAST/). Threshold of the expected value used for significant similarity was less than 0.001. Sequences were characterized regarding the presence of repetitive elements using RepeatMasker (repeatmasker.genome.washington.edu) and were submitted to Gen-Bank (accession numbers BE231450 to BE231460, BE231489 to BE231492, BE241013 to BE241061, and BE344516 to BE344576). Sequences in GenBank were incorporated by TIGR (the Institute for Genomic Research) to help construct the Porcine Gene Index (www.tigr.org/tdb/tgi/ssgi).

#### Northern Hybridization Validation of Gene Expression Differences

Three EST with putative gene expression differences between selection lines were chosen for further validation of ddPCR results using Northern hybridization. Total RNA from the same animals used in the ddPCR was used to form pools for Northern blots. One pool of total RNA (15  $\mu$ g) was used for each line, representing equal amounts of RNA from two d2 sows and one d4 sow. The small number of EST confirmed with Northern analysis, and the use of RNA pooled across days in the validation process, were the necessary result of the very limited amounts of pituitary samples available.

Asymmetric PCR was utilized for probe preparation with 10  $\mu$ L of a specific PCR product, 0.012  $\mu$ M arbitrary primer,  $0.12 \ \mu M$  anchor primer, 5 U of Tag polymerase (Promega, Madison, WI),  $1 \times$  enzyme buffer, 1.5 mM MgCl<sub>2</sub>, 0.2 mM each d(C,G,T)TP, 0.006 mM dATP, and 1  $\mu$ Ci of  $[\alpha$ -<sup>32</sup>P]dATP in a volume of 50  $\mu$ L. The PCR mixture was incubated in a PTC-200 thermal cycler (MJ Research, Waltham, MA) as follows: initial denaturation for 2 min (94°C), followed by 41 cycles of 30 s at 92°C, 30 s at 60°C, and 2 min at 72°C, with a final extension period of 7 min at 72°C. Probes were denatured and hybridized with membranes overnight at 42°C. Membranes were washed and exposed to a PhosphoImager cassette (Molecular Dynamics, Sunnyvale, CA) and scanned in a PhosphoImager SF. The level of expression of the gene under investigation was quantified using the software ImageQuant version 3.3 (Molecular Dynamics). Membranes were then stripped by two successive washes at 95°C (20 min each) in a solution of  $0.1 \times SSC$  with 0.5% SDS, and probed with G3PD (glyceraldehyde-3-phosphate dehydrogenase) to normalize expression of the gene under investigation.

#### Statistical Analysis

Anterior pituitary weight was analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with the model  $Y_{ijk} = \mu + L_i + T_j + (LT)_{ij} + BW_{ijk} + e_{ijk}$ , where  $Y_{ijk}$  is the response of sow k in line i injected with the prostaglandin analog on d j;  $\mu$  is the constant; Li is the effect of the ith line;  $T_j$  is the effect of the jth day of slaughter after prostaglandin analog injection;  $LT_{ij}$  is the interaction between line and day; B is the regression on body weight, Wijk; and  $e_{ijk}$  is the residual.

#### Results

#### Anterior Pituitary Mass

Weights of anterior pituitaries were significantly different (P < 0.01) between Control (351.0 mg) and Index (453.8 mg) lines. No difference was observed between anterior pituitary weights at d 2 (407.4 mg) and d 4 (410.9 mg), and the line × day interaction was not significant. Body weights of sows were not different between lines or day of tissue collection (Gladney et al., 2004).

#### Differential Display and Sequence Characterization

A total of 372 bands was extracted from differential display PCR and assigned quality scores based on the prioritization system previously described. Bands scored as 5 (n = 100) and 6 (n = 68) amounted to 45.2% of the extracted bands. Examples of bands scored as

6 are in Figure 1. The majority of extracted bands originated from Index line samples (n = 246; 66.1%) based on stronger intensity. Some bands with similar expression between lines (n = 12) were also cloned and sequenced.

From the 179 bands processed, sequences were obtained for 162 clones. Sequence analysis indicated that 125 of the 162 (77.2%) anterior pituitary expressed genes characterized had distinct sequences, being either singletons or part of an EST cluster. Long interspersed elements, short interspersed elements, long terminal repeats, or DNA elements were found in 10.4% (13/125) of these, and simple repeats or sequences with low complexity were found in 4.8% (6/ 125). Table 1 displays information on 125 EST with their GenBank accession numbers and TIGR tentative cluster (**TC**) ID.

These EST represent clones with total or partial similarity to known genes (n = 73; 58.4%), to genes with an uncharacterized biological function (n = 43; 34.4%), or are novel (n = 9; 7.2%). Among the EST that matched sequences of known biological function, we observed EST related to mitochondrial function, signal transduction, splicing, endocrine activity, cell growth, and translation. Most (110 of 125) of the anterior pituitary EST are included in the TIGR Porcine Gene Index (March, 2003); 89 are clustered into 85 TCs, and 21 represent singletons.

#### Northern Hybridization Validation of Gene Expression Differences

Evaluation of gene expression using Northern hybridization was performed for the *G-beta like protein*, *ferritin heavy-chain*, and *follicle stimulating hormone beta subunit* genes. Differential expression of each gene was validated with line direction of expression changes in agreement with those observed in differential display. Results for two genes are presented (Figure 1). Signal observed for *G3PD* control probe showed that similar amounts of RNA were loaded for each line (data not shown). Selection for the reproduction Index decreased expression relative to Line C (*G-beta like protein* and *ferritin heavy-chain*) by 56 and 42%, respectively, and increased expression of *follicle stimulation hormone beta subunit* by 10%.

#### Discussion

We employed a thorough differential display evaluation to identify gene expression changes in the female anterior pituitary as a result of long-term selection for increased ovulation rate and embryo survival. Many differentially expressed transcripts were identified and characterized, and a sample of these was confirmed using Northern hybridization (*G-beta like protein, ferritin heavy-chain, and follicle stimulation hormone beta subunit*). In general, the range of mRNA expression ratios observed was small, as might be ex-



**Figure 1**. Differential display PCR and Northern hybridization results showing reduced expression (mRNA levels) of *G-beta like protein* and *Ferritin heavy chain* in anterior pituitaries of the Index selection line (I) relative to the Control line (C). Arrows point to the ddPCR bands that were excised from gels for sequence characterization. Two pools of samples were used to represent each line.

pected when evaluating selection response at the transcriptional level, and in agreement with a concurrent study using ovarian follicles (Gladney et al., 2004).

The Index line of pigs exhibits significant phenotypic improvements in ovulation rate and embryo survival (Johnson et al., 1999), which were the direct targets of selection. Increases in the number of pigs born alive have been partially limited to some extent by an increase in stillbirths (Johnson et al., 1999). Stillbirth in pigs can be caused by a variety of factors and has a genetic basis with several QTL localized in the Index and Control selection lines (Cassady et al., 2001). It has been suggested that iron deficiency is associated with an increased incidence of stillbirths in both pigs (Moore et al., 1965) and humans (Batu et al., 1972). Ferritin is a protein expressed in many tissues that plays an important role in iron storage and metabolism. Ferritin subunits (light and heavy chains) can be regulated at the transcriptional or translational levels (White and Munro, 1988). Ferritin Heavy Chain (FTH) transcription can be increased by transcription factors (Bevilacqua et al., 1994), thyrotropin (Chazenbalk et al., 1990), and progesterone (Zhu et al., 1995).

A negative correlation exists in boars between ferritin and iron concentrations and testis size, associated with changes in FSH levels (Ford et al., 2001; Wise et al., 2003). Iron overload is a genetic condition in humans caused by a mutation in the hemochromatosis gene (*HFE*; Feder, 1999), which encodes a membrane protein that can form a complex with endogenous transferrin receptor (TfR; Ikuta et al., 2000). Mice that have had *HFE* knocked-out have the iron overload phenotype (Zhou et al., 1998). Iron overload can result in lower levels of circulating LH and FSH leading to hypogonadotropic hypogonadism (Cundy et al., 1993), probably due to iron deposition in pituitary cells impairing their function (Bergeron and Kovacs, 1978; Charbonnel et al., 1981). In contrast, boars with elevated testicular ferritin have elevated FSH secretion (Ford et al., 2001).

We demonstrate that selection for increased ovulation rate and embryo survival has decreased mRNA levels of *FTH* in the anterior pituitary of sows. Although no evidence exists for a link between reduced FTH and increased incidence of stillbirths, it is possible that alterations in iron homeostasis are involved. Alternatively, it has recently been demonstrated that *FTH* is involved in the folate pathway by enhancing expression of serine hydroxymethyltransferase and de novo thymidine biosynthesis (Oppenheim et al., 2001). Supplementation of folic acid during gestation leads to increases in litter size, primarily due to improved embryo or fetal survival (Lindemann, 1993).

Expression of the *follicle-stimulating hormone beta* subunit (FSH $\beta$ ) gene is up-regulated by selection for enhanced reproduction. This result is consistent with the role of FSH in regulation of ovarian follicle development (Hadley, 2000). Higher plasma levels of FSH have been found in both females and males (Cassady et al., 2000) from the Index line. Although large differences in allele frequencies were found for a genetic marker within the FSH $\beta$  locus between the Index and Control lines (Linville et al., 2001), influences of potential genetic drift were confounded with those results

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**Table 1.** GenBank accession numbers, the Institute for Genomic Research (TIGR) cluster information, gene identification, and direction of expression change between selection lines for anterior pituitary EST characterized in this differential display study

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$\begin{split} & \text{BE231463}  \text{TC477890} \\ & \text{CALD delydrogenase subunit 4 (ND4)^d} \\ & \text{BE231463} \\ & \text{Singleton} \\ & Hom appiers chromosome 15, chen RP11-36104^s \\ & \text{C} < 1 \\ & \text{BE231465} \\ & \text{Singleton} \\ & Hom a spices chromosome 15, chen RP11-361015 map 15q22^s \\ & \text{C} < 1 \\ & \text{BE231465} \\ & \text{Singleton} \\ & Hom a spices chromosome 15, chen RP11-361015 map 15q22^s \\ & \text{C} < 1 \\ & \text{BE231466} \\ & \text{Singleton} \\ & \text{Hom a spices chromosome 15, chen RP11-361015 map 15q22^s \\ & \text{C} < 1 \\ & \text{BE231467} \\ & \text{Singleton} \\ & \text{Hom a chromosome 164, 407010^s \\ & \text{C} & \text{L} \\ & \text{BE231468} \\ & \text{Singleton} \\ & \text{Hom a chromosome 164, 407010^s \\ & \text{C} & \text{L} \\ & \text{BE231469} \\ & \text{TC63344} \\ & \text{TC63344} \\ & \text{NA DH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} & \text{C} \\ & \text{BE231469} \\ & \text{TC63544} \\ & \text{TC47580} \\ & \text{NADH delydrogenase subunit 1 (CO11)^d \\ & \text{C} & \text{C} & \text{C} \\ & \text{BE231469} \\ & \text{TC63744} \\ & \text{Cychrome 6 cidase subunit 1 (CO11)^d \\ & \text{C} & \text{C} & \text{C} \\ & \text{E2210141} \\ & \text{TC47580} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 1 (ND4)^d \\ & \text{C} & \text{C} & \text{C} \\ & \text{E2210147} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E2210147} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E2210147} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E2210161} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E221017} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E221018} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 4 (ND4)^d \\ & \text{C} & \text{C} \\ & \text{E221019} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 5 (ND6)^d \\ & \text{C} & \text{C} \\ & \text{E2210102} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 5 (ND6)^d \\ & \text{C} & \text{C} \\ & \text{E2210102} \\ & \text{TC477580} \\ & \text{NADH delydrogenase subunit 5 (ND6)^d \\ & \text{C} & \text{C} \\ & \text{E2210102} \\ & \text{TC57677} \\ & \text{Hom a spices, similar to theraronosome 14 open readingframe 3^* \\ & \text{C} & \text{C} \\ & E2210$	BE231451	TC47390	Cytochrome c oxidase subunit I (COI) <sup>d</sup>	C < I
BE231463 TC47699 Calmodulia 2 (CALM2) <sup>4</sup> C - 1 BE231464 Singleton Horos aspirate chromosome 15, clone RP11-361D15 map 15q22* C - 1 BE231465 TC60706 Not available <sup>4</sup> C - 1 BE231465 Singleton U4/06-associated RNA splicing factor URT3' C - 1 BE231467 Singleton U4/06-associated RNA splicing factor URT3' C - 1 BE231469 Singleton Sos arryfs clone RNA-splicing factor URT3' C - 1 BE231469 Singleton Nos arryfs clone RNA-splicing factor URT3' C - 1 BE231469 Singleton Human DNA sequence from clone RP11-360H5 onchromasome 6° C - 1 BE231490 Singleton Human DNA sequence from clone RP11-360H5 onchromasome 6° C - 1 BE231491 TC50847 Not available <sup>4</sup> C - 14 BE231492 TC47841 Neuronation UNA7t <sup>1</sup> C - 1 BE231492 TC47841 Veuronation UNA7t <sup>1</sup> C - 1 BE231492 TC47841 Cytochrome coidase subunit 11 (CO11 <sup>4</sup> C - 1 C - 1 BE231492 TC47854 Cytochrome coidase subunit 10 (CO3, CO11) <sup>4</sup> C - 1 BE24101 TC47504 Cytochrome coidase subunit 10 (CO3, CO11) <sup>4</sup> C - 1 BE24101 TC47750 NADH dehydrogenase subunit 6 (ND57 <sup>4</sup> ) BE24101 TC47750 NADH dehydrogenase subunit 6 (ND67 <sup>4</sup> ) BE241010 TC47750 NADH dehydrogenase subunit 6 (ND67 <sup>4</sup> ) BE241010 TC47750 NADH dehydrogenase subunit 6 (ND67 <sup>4</sup> ) BE24102 TC57464 Hydrogenase subunit 6 (ND67 <sup>4</sup> ) BE24102 TC57474 Horos saptens, similar to KIA0689 gene product(JOC116284) <sup>6</sup> C - 1 BE24102 TC57474 Horos saptens, similar to KIA0689 gene product(JOC116284) <sup>6</sup> C - 1 BE24102 TC57474 Horos saptens, similar to the Integrove and as available of C - 1 BE24102 TC57474 Horos saptens, similar to KIA0689 gene product(JOC116284) <sup>6</sup> C - 1 BE24102 TC574757 Horos saptens, similar to KIA0689 gene product(JOC116284) <sup>6</sup> C - 1 BE24102 TC57477 Horos saptens, similar to the finger protein 302 <sup>4</sup> C - 1 BE24102 TC57477 Horos saptens, similar to the finger protein 302 <sup>4</sup> C - 1 BE24102 TC57477 Horos saptens, similar to the finger protein 302 <sup>4</sup> C - 1 BE24102 TC57477 Horos saptens, similar to the finger protein 302 <sup>4</sup> C - 1 BE24102 TC57484 Hydrosenase clone RP1443215 fs <sup>4</sup> C - 1 BE24102 TC57459 Hydrosenase clone RP144	BE231452	TC47530	NADH dehydrogenase subunit 4 (ND4) <sup>d</sup>	C < I
BE231454 Singleton Horo sapiers chromosome 15, clone RP11-363L4" C < 1 BE231456 Singleton Horo sapiers chromosome 15, clone RP11-361D15 mup 15q22 C < 1 BE231456 Singleton Human chromosome 14 DNA sequence BAC R 124D2 of library RPCI-11" C = 1 BE231458 Singleton Human chromosome 14 DNA sequence BAC R 124D2 of library RPCI-11" C = 1 BE231458 Singleton Human thromosome 14 DNA sequence BAC R 124D2 of library RPCI-11" C = 1 BE231459 TC63344 Not available <sup>4</sup> C = 1 BE231450 TC63350 NADH dehydrogenase abunit 1 (CO11 <sup>4</sup> C = 1 BE241015 TC63364 NADH dehydrogenase abunit 5 (ND5) <sup>4</sup> C = 1 BE241015 TC63364 NADH dehydrogenase abunit 5 (ND5) <sup>4</sup> C = 1 BE241019 TC637374 ATP synthase abunit 6 (NT47F6) <sup>6</sup> C = 1 BE241021 TC57459 NADH dehydrogenase abunit 6 (NT47F6) <sup>6</sup> C = 1 BE241021 TC57459 Horo sapiens, similar to KIAA0663 gene productIAC1162845 <sup>4</sup> C = 1 BE241023 Singleton Alexcer face:ultra's brain CDA, clone.QBA-11460 <sup>4</sup> C = 1 BE241024 TC56854 Horo sapiens, similar to train finger protein 302 <sup>4</sup> C = 1 BE241025 Singleton Alexcer face:ultra's brain CDA, clone.QBA-11467 <sup>4</sup> C = 1 BE241025 Singleton Alexcer face:ultra's brain CDA, clone.QBA-11467 <sup>4</sup> C = 1 BE241025 TC56867 Sus served, similar to thermosome TF294011 ondernovance 22 <sup>4</sup> C = 1 BE241037 TC56454 Horo sapiens, similar to thermosome TF294011 ondernovance 22 <sup>4</sup> C = 1 BE241038 TC568954 Horo sapiens, similar to thermosome TF294011 ondernovance 22 <sup>4</sup> C = 1 BE241039 TC56844 Horo sapiens, similar to thermosome TF294011 ondernovance 22 <sup>4</sup> C	BE231453	TC47699	Calmodulin 2 (CALM2) <sup>d</sup>	C < I
BE231455 TC67076 Not available <sup>6</sup> C < 1 BE231457 Singleton Home septime dromosome 15, due RP11-361D15 map 15q22° C < 1 BE231458 Singleton Home Arronosome 4 DNA sequence BAC R124D2 of Brary RPC1-11° C < 1 BE231459 Singleton Sus script clene RP4-397B10° C < 1 BE231469 Singleton Sus script clene RP4-397B10° C < 1 BE231490 TC47530 NADH dekydrogenase submit 4 (ND4) <sup>4</sup> C < 1 BE231491 TC47530 NADH dekydrogenase submit 4 (ND4) <sup>4</sup> C < 1 BE231491 TC47530 NADH dekydrogenase submit 4 (ND4) <sup>4</sup> C < 1 BE231491 TC47534 (C + 10 + 10 + 10 + 10 + 10 + 10 + 10 + 1	BE231454	Singleton	Homo sapiens chromosome 15, clone RP11-358L4 <sup>e</sup>	C < I
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	BE231455	TC60706	Not available <sup>f</sup>	C < I
BE221467SingletonU4/06-associated RNA splicing factor (PRP3) <sup>6</sup> C < 1BE231469SingletonSus acrofa clone RP44-397B10"C < 1	BE231456	Singleton	Homo sapiens chromosome 15, clone RP11-361D15 map 15q22 <sup>e</sup>	C < I
$\begin{array}{llllllllllllllllllllllllllllllllllll$	BE231457	Singleton	U4/U6-associated RNA splicing factor (PRP3) <sup>d</sup>	C < I
BE231469 Singleton NADH dehydrogenase subunit 4 (ND4) <sup>d</sup> C 4 BE231469 TC47530 NADH dehydrogenase subunit 4 (ND4) <sup>d</sup> C 4 BE231491 TC69847 Not available <sup>d</sup> C 5 BE231492 TC47541 Not available <sup>d</sup> C 5 BE231492 TC47541 Neuronalii (NNAT <sup>d</sup> C) BE231491 TC69847 Not available <sup>d</sup> C 5 BE231491 TC47540 Not available <sup>d</sup> C 5 BE231492 TC47541 Neuronalii (NNAT <sup>d</sup> C) BE241013 TC47412 Cytochrome oxidase subunit II (CO17 <sup>d</sup> C 5 BE241014 TC47500 C ytochrome oxidase subunit II (CO33, COIII) <sup>d</sup> C 5 I C 4 BE241015 TC47530 NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C 4 BE241016 TC47530 NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C 5 I C 4 BE241017 TC47530 NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C 4 BE241018 TC47574 ATP synthase subunit 6 (ND6) <sup>d</sup> BE241019 TC47574 ATP synthase subunit 6 (ND6) <sup>d</sup> BE241021 TC57168 HADH dehydrogenase subunit 6 (ND6) <sup>d</sup> BE241021 TC57168 HADH dehydrogenase subunit 6 (ND6) <sup>d</sup> BE241021 TC57171 Hono sapiers, similar to KIAA0663 gene product/LOC1162847" C 4 BE241021 TC57171 Hono sapiers, similar to cinc finger protein 302 <sup>d</sup> C 5 I BE241023 Singleton Maccae fusicialaris brain cDNA, clone-QIA.11466° C 5 I BE241024 Singleton Hono sapiers, similar to cinc finger protein 302 <sup>d</sup> C 5 I BE241025 Singleton Hono sapiers, similar to cinc finger protein 302 <sup>d</sup> C 5 I BE241025 Singleton Hono sapiers, similar to cinc finger protein 302 <sup>d</sup> C 5 I BE241027 TC66467 Human DNA sequence from clone CITF22-49(11 onehromosome 2 <sup>d</sup> C 5 I BE241028 TC66974 Splicing factor 3b, subunit 1 (SFB1) <sup>d</sup> C 5 I BE241029 TC66674 Splicing factor 3b, subunit 1 (SFB1) <sup>d</sup> C 5 I BE241030 TC59967 Sub servid scince RP14-45301500 chromosome 61 <sup>d</sup> C 5 I BE241031 TC5944 Hono sapiers, similar to taberin 1 <sup>d</sup> C 5 I BE241031 TC5944 HONO sapiers, similar to taberin 1 <sup>d</sup> C 5 I BE241031 TC5944 HONO sapiers, Similar to taberin 1 <sup>d</sup> C 5 I BE241031 TC5947 Splicing factor 3b, subunit 1 (SFB1) <sup>d</sup> C 5 I BE241031 TC5947 Splicing factor 3b, subunit 1 (SFB1) <sup>d</sup> C 5 I BE241031 TC5947 HONO sapiers, Similar to taberin 1 <sup>d</sup> C 5 I C 5 I BE241041 TC56274 Splicing factor 3b, Subunit 1 (SFB1) <sup>d</sup>	BE231458	Singleton	Human chromosome 14 DNA sequence BAC R-124D2 of library RPCI-11 <sup>e</sup>	C > I
BR2214460 TCd7320 NADH dehydrogenase subunit 4 (NDd) <sup>4</sup> C - 1 BR221429 Singleton Human DNA sequence from clone RP11-260H5 onchromosome 6" C - 1 BR231491 TCG8947 Not available <sup>6</sup> C - 1 BR231492 TCG7541 Neuronatin (NNAT) <sup>6</sup> C - 1 BR231492 TCG7541 Veurone oxidase subunit II (COII) <sup>4</sup> C - 1 C - 1 BR231492 TCG7542 Cytochrome coxidase subunit II (COII) <sup>4</sup> C - 1 BR241015 TCG7543 Cytochrome oxidase subunit II (COII) <sup>4</sup> C - 1 BR241015 TCG7530 NADH dehydrogenase subunit 4 (ND4) <sup>4</sup> BR241017 TCG7530 NADH dehydrogenase subunit 6 (ND5) <sup>4</sup> C - 1 BR241018 TCG7530 NADH dehydrogenase subunit 6 (ND5) <sup>4</sup> C - 1 BR241018 TCG75468 165 ribosomal RNA <sup>4</sup> BR241017 TCG75468 165 ribosomal RNA <sup>4</sup> BR24102 TCG75468 165 ribosomal RNA <sup>4</sup> BR24102 TCG75468 165 ribosomal RNA <sup>4</sup> BR24102 TCG75468 165 ribosomal RNA <sup>4</sup> C - 1 BR24102 TCG75474 ATP synthase subunit 6 (NTATP6) <sup>4</sup> BR24102 TCG75468 165 ribosomal RNA <sup>4</sup> C - 1 BR24102 TCG75476 Human DNA sequence from clone (CFL312610) <sup>6</sup> BR24102 TCG75471 Homo sagines, similar to KIA0666 gene product/LOC116284) <sup>a</sup> C - 1 BR24102 TCG56967 Human DNA sequence from clone (CTFL224) <sup>c</sup> 1466 <sup>c</sup> C - 1 BR24102 TCG56967 Human DNA sequence from clone (CTFL224) <sup>c</sup> 146 <sup>c</sup> C - 1 BR24102 TCG56967 Sus serofa clone RP44-331021 <sup>a</sup> C - 1 BR24102 TCG56967 Sus serofa clone RP44-331021 <sup>a</sup> C - 1 BR24102 TCG56967 Sus serofa clone RP44-331021 <sup>a</sup> C - 1 BR24103 TCG56967 Sus serofa clone RP44-331021 <sup>a</sup> C - 1 BR24103 TCG56967 Human DNA sequence from clone CTFL224-9611 acdromosome 22 <sup>c</sup> C - 1 BR24103 TCG56974 Homo sagines, similar to theorie-inking protein f <sup>4</sup> C - 1 BR24103 TCG56974 Homo sagines shall ruberi-alke protein f <sup>4</sup> C - 1 BR24103 TCG56974 Homo sagines, similar to theorie-inking protein f <sup>4</sup> C - 1 BR24103 TCG56974 Splicing factor 3, 6 folloge protein f <sup>4</sup> C - 1 BR24103 TCG56974 Human DNA sequence from clone CTFL224-9611 acdromosome 22 <sup>c</sup> C - 1 BR241040 TCG56974 Splicing factor 3, 6 folloge protein f <sup>4</sup> C - 1 BR241041 TCG56974 Human DNA sequence from clone CTFL224-9611 acdromosome 62 <sup>c</sup> C - 1 BR241045 TCG5677 Splicing factor 3, 6 fo	BE231459	Singleton	Sus scrofa clone RP44-397B10 <sup>e</sup>	C < I
BE231489 TC63344 Not available <sup>4</sup> C - 1 BE231491 TC69847 Not available <sup>6</sup> C - 1 BE231492 TC47841 Neuronalin (NNAT <sup>d</sup> COID <sup>4</sup> C - 1 BE231492 TC47841 Neuronalin (NNAT <sup>d</sup> COID <sup>4</sup> C - 1 BE241013 TC47412 Cytochrome oxidase subunit II (COID <sup>4</sup> C - 1 C - 1 BE241014 TC47506 VADH oxit (COX3, COIID <sup>4</sup> C - 1 C - 1 BE241015 TC47858 Cytochrome oxidase subunit II (COX3, COIID <sup>4</sup> C - 1 C - 1 BE241015 TC47858 NADH dehydrogenase subunit 5 (ND6) <sup>4</sup> C - 1 BE241015 TC47578 NADH dehydrogenase subunit 5 (ND6) <sup>4</sup> C - 1 BE241017 TC47530 NADH dehydrogenase subunit 6 (ND6) <sup>4</sup> BE241019 TC47374 ATP synthase subunit 6 (ND6) <sup>4</sup> BE241021 TC57817 Home sajers, similar to KIAA0663 gase product/LOC1162847 <sup>4</sup> C - 1 BE241021 TC57817 Home sajers, similar to KIAA0663 gase product/LOC1162847 <sup>4</sup> C - 1 BE241023 Singleton Maccac fascicularis brain cDNA, clone-QfA-11466 <sup>6</sup> C - 1 BE241025 Singleton Home sajers, similar to clone CUTF22-49(11 onchromosome 2 <sup>se</sup> C - 1 BE241025 Singleton Home sajers, similar to clone CUTF22-49(11 onchromosome 2 <sup>se</sup> C - 1 BE241027 TC66467 Home sajers, similar to clone CUTF22-49(11 onchromosome 2 <sup>se</sup> C - 1 BE241028 TC66674 Splicing factor 3h, subunit 1 (SPB1) <sup>4</sup> C - 1 BE241029 TC66677 Sus scroß clone RP44-31(22) for 1 <sup>d</sup> BE241029 TC66677 Sus scroß clone RP44-31(22) for 1 <sup>d</sup> BE241029 TC66677 Sus scroß clone RP44-31(22) for 1 <sup>d</sup> BE241029 TC66677 Sus scroß clone RP44-31(22) for 1 <sup>d</sup> BE241030 TC59678 Home sajers, similar to taberin 1 <sup>d</sup> BE241030 TC65978 Home sajers, similar to taberin 1 <sup>d</sup> BE241031 TC65978 Home sajers, Similar to taberin 1 <sup>d</sup> BE24104 TC65674 Splicing factor 3h, subunit 1 (SPB1) <sup>d</sup> C - 1 BE24103 TC65978 Home sajers, Similar to taberin 1 <sup>d</sup> BE24104 TC65674 Splicing factor 3h, subunit 1 (SPB1) <sup>d</sup> C - 1 BE24103 TC6	BE231460	TC47530	NADH dehydrogenase subunit 4 (ND4) <sup>d</sup>	C < I
BE231490 Singleton Human DNA sequence from Jone RP11-260H5 onchromosome 6° C < 1 BE231492 TC59847 Not available <sup>f</sup> C > 1 BE231492 TC59847 Not available <sup>f</sup> C < 1 BE241013 TC47411 Cytochrome oxidase subunit II (COII) <sup>d</sup> C > IC < 1 BE241014 TC47504 Cytochrome oxidase subunit III (COII) <sup>d</sup> C > IC < 1 BE241015 TC47384 Cytochrome b (CTB) <sup>d</sup> C < 1 C < 1 BE241017 TC47530 NADH dehydrogenase subunit 4 (ND4) <sup>d</sup> BE241017 TC47530 NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C < 1 C < IC < 1 BE241018 TC47530 NADH dehydrogenase subunit 6 (ND6) <sup>d</sup> C < IC < 1 BE241017 TC47530 NADH dehydrogenase subunit 6 (ND6) <sup>d</sup> C < IC < 1 BE241017 TC47530 NADH dehydrogenase subunit 6 (ND6) <sup>d</sup> C < IC < 1 BE24102 TC57468 ISS ribosonal RNA <sup>d</sup> BE24102 TC57468 Homo sepiens, similar to XIA 0666 gene product/LOC116284/ <sup>o</sup> C < I BE24102 TC57468 Homo sepiens, similar to zinc finger protein 302 <sup>d</sup> C > I BE24102 TC55697 Human DNA sequence from colmo C1792-449G11 onchromosome 22 C > I BE24102 TC55697 Human DNA sequence from colmo C1792-449G11 onchromosome 22 C > I BE24102 TC55697 Sus scroft clone RP44-331C21 <sup>s</sup> BE24103 TC55967 Sus scroft clone RP44-331C21 <sup>s</sup> C < I BE24103 TC55967 Human DNA sequence from clone C1792-449G11 onchromosome 22 C > I BE24103 TC55967 Human DNA sequence from clone C1792-449G11 onchromosome 22 C > I BE24103 TC55967 Human DNA sequence from clone C1792-449G11 onchromosome 22 C > I BE24103 TC55967 Human DNA sequence RP14-331C21 <sup>s</sup> C < I BE241044 TC55084 PGP5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C < I BE241045 TC55957 Human DNA sequence RP14-3510 <sup>s</sup> C < I BE241045 TC55957 Human DNA sequence RP14-3510 <sup>s</sup> C < I BE241045 TC55967 Human DNA sequence RP14-3510 <sup>s</sup> C < I BE241045 TC45797 Human Seqiens Similar to tab	BE231489	TC63344	Not available <sup>t</sup>	C > I
BE231491TC59847Not available'C < 1BE231492TC47412Cytachrome oxidase subunit I(COII) <sup>d</sup> C < I	BE231490	Singleton	Human DNA sequence from clone RP11-260H5 onchromosome 6 <sup>e</sup>	C < I
BR221192TC47541Neuronatin (NNAT) <sup>d</sup> C < IBR241013TC47742Cytochrome oxidase subunit II (COII) <sup>d</sup> C > IC < I	BE231491	TC59847	Not available <sup>f</sup>	C > I
BR241013TC47412Cytochrome oxidase subunit II (COII) <sup>d</sup> C > IC < IC < IBR241015TC47384Cytochrome oxidase subunit II (COXI) <sup>d</sup> C > IBR241016TC47384Cytochrome o tidase subunit II (COXI) <sup>d</sup> C < I	BE231492	TC47841	Neuronatin (NNAT) <sup>d</sup>	C < I
BE24101 BE241015TC47304 TC47384Cytochrome o cxidase subunit III (COX3, COIID <sup>4</sup> C > 1BE241015TC47384 TC47530Cytochrome b (CTB) <sup>d</sup> C > I/C < 1	BE241013	TC47412	Cytochrome oxidase subunit II (COII) <sup>d</sup>	C > I/C < I
BE241015TC47334Cytochrome b (CYTB) <sup>d</sup> C < 1 C > 1/C < 1BE241017TC47530NADH dehydrogenase subunit 5 (ND6) <sup>d</sup> C > 1/C < 1	BE241014	TC47504	Cytochrome c oxidase subunit III (COX3, COIII) <sup>d</sup>	C > I
BE241016TC47530NADH dehydrogenase subunit 4 (ND4) <sup>d</sup> C<1BE241017TC47530NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C<1	BE241015	TC47384	Cytochrome b (CYTB) <sup>d</sup>	C < I
BE241017 TC47530 NADH dehydrogenase subunit 4 (ND4) <sup>4</sup> C < I BE241017 TC47530 NADH dehydrogenase subunit 5 (ND5) <sup>4</sup> C < I C > UC < I BE241018 TC47530 NADH dehydrogenase subunit 6 (ND6) <sup>4</sup> C < I BE241020 TC57468 16S rubosmal RNA <sup>4</sup> C > I BE241021 TC57217 Homo sapiens, similar to KIAA0663 gene product(LOC116284) <sup>6</sup> C < I BE241022 TC48933 Hypothetical protein FLJ2610 (FLJ2610) <sup>6</sup> C > I BE241023 Singleton Macaca fascicularis brain CDNA, clone:QlA-11466 <sup>6</sup> C > I BE241023 Singleton Macaca fascicularis brain CDNA, clone:QlA-11466 <sup>6</sup> C > I BE241024 Singleton Homo sapiens, similar to chromesome 14 open readingframe 3 <sup>6</sup> C > I BE241025 Singleton Homo sapiens, similar to chromesome 14 open readingframe 3 <sup>6</sup> C > I BE241026 TC47371 Homo sapiens, similar to zint floper protein 302 <sup>4</sup> C > I BE241027 TC56467 Human DNA sequence from clone CITP22-49(E1) onchromosome 22 <sup>s</sup> C > I BE241029 TC56674 Splicing factor 3b, subunit 1 (SP3B1) <sup>4</sup> C > I BE241029 TC56674 Splicing factor 3b, subunit 1 (SP3B1) <sup>4</sup> C > I BE241030 TC55067 Sus scr6a clone RP44-331021 <sup>6</sup> de in <sup>4</sup> C > I BE241030 TC55067 Sus scr6a clone RP44-331021 <sup>6</sup> de in <sup>4</sup> C > I BE241031 TC51044 Homo sapiens, similar to taberin-like protein 1 <sup>4</sup> C > I BE241031 TC55048 Immunoglobulin-like variable motif-ontaining protein(BIVM) <sup>d</sup> C > I BE241033 TC55973 Homo sapiens 3 BAC RP11-436A20 <sup>o</sup> C > I BE241034 TC56934 PGP5 m RNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > I BE241035 — Not available <sup>d</sup> BE241035 — Not available <sup>d</sup> BE241036 — Regulatory factor X, 5 (influences HLA class Ilexpression) (RPX5), mRNA <sup>d</sup> C < I BE241037 — Regulatory factor X, 5 (influence RP3-4530150n chromosome 6q16 <sup>o</sup> C < I BE241041 TC569231 Suppression of tumorigenicity 13 (colon carcinoma/Hsp70-Interacting protein) <sup>d</sup> C < I BE241045 TC61558 Gene dJ198K11.1 <sup>e</sup> BE241045 TC61558 Gene dJ198K11.1 <sup>e</sup> BE241045 TC61558 Gene dJ198K11.1 <sup>e</sup> BE241045 TC61558 Gene dJ198K11.1 <sup>e</sup> BE241045 TC61549 Homo sapiens clone RP3-4530150n chromosome 6q16 <sup>o</sup> C < I BE241045 TC61549 Ricken DNA sequence from clone arelyon-34150. <sup>d</sup> C < I BE2				C > I/C < I
BE241017TC47530NADH dehydrogenase subunit 5 (ND5) <sup>d</sup> C < IBE241018TC47530NADH dehydrogenase subunit 6 (ND6) <sup>d</sup> C > UC < I	BE241016	TC47530	NADH dehydrogenase subunit 4 (ND4) <sup>d</sup>	
C > $JC < I$ BE241018TC47530NADH dehydrogenase subunit 6 (ND6) <sup>d</sup> C < I	BE241017	TC47530	NADH dehydrogenase subunit 5 (ND5) <sup>d</sup>	C < I
$\begin{array}{l c c c c c c c c c c c c c c c c c c c$				C > I/C < I
$\begin{array}{l c c c c c c c c c c c c c c c c c c c$	BE241018	TC47530	NADH dehydrogenase subunit 6 (ND6) <sup>d</sup>	
BE24102TC5746816S ribosonal RNA <sup>4</sup> C > IBE24102TC57217Home sapiens, similar to KIAA0663 gene product(LOC116284)*C < I	BE241019	TC47374	ATP synthase subunit 6 (MTATP6) <sup>d</sup>	C < I
BE241021TCS7217Home sapiens, similar to KIA40663 gene productIOC116284)*C < IBE241023SingletonMacaca fascicularis brain cDNA, clone:QfA-11466*C > IBE241024SingletonHome sapiens cDNA FLJ34215 fis*C > IBE241025SingletonHome sapiens, similar to zinc finger protein 302 <sup>d</sup> C > IBE241026TC47371Home sapiens, similar to zinc finger protein 302 <sup>d</sup> C > IBE241027TC56647Human DNA sequence from clone CTF22-49G11 onchromosome 22*C > IBE241028TC56935Home sapiens, similar to melanoma antigen, family D,1 <sup>d</sup> C < I	BE241020	TC57468	16S ribosomal RNA <sup>d</sup>	C > I
BE241022TC48993Hypothetical protein FJJ12610 (FJJ12610)*C > IBE241023SingletonMacca fascicularis brain cDNA, clone-QfL-11466*C > IBE241024SingletonHomo sapiens, similar to xine finger protein 302dC > IBE241025SingletonHomo sapiens, similar to xine finger protein 302dC > IBE241026TC47371Homo sapiens, similar to xine finger protein 302dC > IBE241027TC56467Human DNA sequence from clone CITF22-49GI1 onchromosome 22*C > IBE241028TC56695Homo sapiens, similar to thenons and igen, family D,1dC > IBE241029TC56674Splicing factor 3b, subunit 1 (SF3B1)dC > IBE241030TC55967Sus scröfa clone RP44-331621*C > IBE241031TC51044Homo sapiens, similar to tuberin-like protein 1dC > IBE241032TC52948Immunoglobulin-like variable motif-containing protein/BIVM)dC > IBE241033TC56973Homo sapiens 3 BAC RP11-436A20*C < I	BE241021	TC57217	Homo sapiens, similar to KIAA0663 gene product(LOC116284) <sup>e</sup>	C < I
BE241023SingletonMacaca fascicularis brain cDNA, clone:QfA-11466°C > IBE241024SingletonHomo sapiens, similar to zinc finger protein 302 <sup>d</sup> C > IBE241025SingletonHomo sapiens, similar to zinc finger protein 302 <sup>d</sup> C > IBE241027TC56467Human DNA sequence from clone CITF22-49G11 onchromosome 2°C > IBE241028TC56935Homo sapiens, similar to nelanoma antigen, family D,1 <sup>d</sup> C < I	BE241022	TC48993	Hypothetical protein FLJ12610 (FLJ12610) <sup>e</sup>	C > I
BE241024SingletonHomo sapiens cDNA FLJ34215 fisteC > IBE241025SingletonHomo sapiens, similar to zinc finger protein $302^d$ C > IBE241026TC47371Homo sapiens, similar to chromosome 14 open readingframe $3^e$ C > IBE241027TC56467Human DNA sequence from clone CITF22-49G11 onchromosome $22^e$ C > IBE241028TC56955Homo sapiens, similar to relanoma antigen, family D, 1 <sup>d</sup> C < I	BE241023	Singleton	Macaca fascicularis brain cDNA, clone:QflA-11466 <sup>e</sup>	C > I
BE241025SingletonHomo sapiens, similar to zine finger protein $302^d$ C > IBE241026TC47371Homo sapiens, similar to chromosome 14 open readingframe 3"C > IBE241027TC56467Human DNA sequence from clone CITF22-49G11 onchromosome $22^e$ C > IBE241028TC56935Homo sapiens, similar to melanoma antigen, family D, 1 <sup>d</sup> C < I	BE241024	Singleton	Homo sapiens cDNA FLJ34215 fis <sup>e</sup>	C > I
BE241026TC47371Homo sapiens, similar to chromosome 14 open readingframe 3°C < IBE241027TC566467Human DNA sequence from clone CITF22-49G11 onchromosome 22°C < I	BE241025	Singleton	<i>Homo sapiens</i> , similar to zinc finger protein $302^d$	C > I
BE241027TC56467Huma DNA sequence from clone CITF22.49G11 onchromosome $22^{\circ}$ C > IBE241028TC56935Homo sapiens, similar to melanoma antigen, family D,1 <sup>d</sup> C < I	BE241026	TC47371	Homo sapiens, similar to chromosome 14 open readingframe 3 <sup>e</sup>	C > I
BE241028TC56935Homo sapiens, similar to melanoma antigen, family D,1dC < IBE241029TC56967Suls cord alone RP4-331G21cC > IBE241030TC55967Suls scrofa clone RP4-331G21cC > IBE241031TC51044Homo sapiens, similar to tuberin-like protein 1dC > IBE241032TC52948Immunoglobulin-like variable motif-containing protein(BIVM)dC > IBE241033TC59793Homo sapiens 3 BAC RP11-436A20cC > IBE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolasedC < I	BE241027	TC56467	Human DNA sequence from clone CITF22-49G11 onchromosome 22 <sup>e</sup>	C > I
BE241029TC56574Splicing factor 3b, subunit 1 (SF3B1) <sup>d</sup> C > IBE241030TC55967Sus scrofa clone RP44-331G21°C > IBE241031TC51044Homo sapiens, similar to tuberin-like protein 1 <sup>d</sup> C > IBE241032TC52948Immunoglobulin-like variable motif-containing protein(BIVM) <sup>d</sup> C > IBE241033TC59793Homo sapiens 3 BAC RP11-436A20°C > IBE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > IBE241035-Not available <sup>f</sup> C < I	BE241028	TC56935	Homo sapiens, similar to melanoma antigen, family D,1 <sup>d</sup>	C < I
BE241030TC55967Sus scrofa clone RP44-331G21°C > IBE241031TC51044Homo sapiens, similar to tuberin-like protein 1 <sup>d</sup> C > IBE241032TC52948Immunoglobulin-like variable motif-containing protein(BIVM) <sup>d</sup> C > IBE241033TC59793Homo sapiens 3 BAC RP11-436A20°C > IBE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > IBE241035-Not available <sup>f</sup> C < I	BE241029	TC56574	Splicing factor 3b, subunit 1 (SF3B1) <sup>d</sup>	C > I
BE241031TC51044Homo sapiens, similar to tuberin-like protein $1^d$ C > IBE241032TC52948Immunoglobulin-like variable motif-containing protein(BIVM) <sup>d</sup> C > IBE241033TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > IBE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C < I	BE241030	TC55967	Sus scrofa clone RP44-331G21 <sup>e</sup>	C > I
BE241032TC 52948Immunoglobulin-like variable motif-containing protein(BIVM)dC > IBE241033TC 59793Homo sapiens 3 BAC RP11-436A20°C > IBE241034TC 56394PG P9.5 mRNA for ubiquitin C-terminal hydrolasedC > IBE241035-Not availablefC < I	BE241031	TC51044	<i>Homo sapiens</i> , similar to tuberin-like protein 1 <sup>d</sup>	C > I
BE241033TC59793Homo sapiens 3 BAC RP11-436A20°C > IBE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > IBE241035-Not available <sup>t</sup> C < I	BE241032	TC52948	Immunoglobulin-like variable motif-containing protein(BIVM) <sup>d</sup>	C > I
BE241034TC56394PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup> C > IBE241035—Not available <sup>f</sup> C < I	BE241033	TC59793	Homo sapiens 3 BAC RP11-436A20 <sup>e</sup>	C > I
BE241035—Not available <sup>4</sup> C < IBE241036SingletonGlycoprotein GPIIIa (CD61) <sup>d</sup> C < I	BE241034	TC56394	PGP9.5 mRNA for ubiquitin C-terminal hydrolase <sup>d</sup>	C > I
BE241036SingletonGlycoprotein GPIIIa (CD61) <sup>d</sup> C < IBE241037-Regulatory factor X, 5 (influences HLA class IExpression) (RFX5), mRNA <sup>d</sup> C < I	BE241035	—	Not available <sup>f</sup>	C < I
BE241037—Regulatory factor X, 5 (influences HLA class IIexpression) (RFX5), mRNAdC < IBE241038—Tudor repeat associator with PCTAIRE 2(PCTAIRE2BP) $^{\rm c}$ C < I	BE241036	Singleton	Glycoprotein GPIIIa (CD61) <sup>d</sup>	C < I
BE241038—Tudor repeat associator with PCTAIRE 2(PCTAIRE2BP) °C < IBE241039—Mus musculus strain 129/SvJ BAC clone citb10i1from the MHC region °C < I	BE241037	—	Regulatory factor X, 5 (influences HLA class IIexpression) (RFX5), mRNA <sup>d</sup>	C < I
BE241039—Mus musculus strain 129/SvJ BAC clone citb10i1from the MHC regioneC > IBE241040—RIKEN cDNA 0610040D20 gene6C > IBE241041TC59231Suppression of tumorigenicity 13 (colon carcinoma)(Hsp70-interacting protein)dC < I	BE241038	—	Tudor repeat associator with PCTAIRE 2(PCTAIRE2BP) <sup>e</sup>	C < I
BE241040RIKEN cDNA 0610040D20 geneC > IBE241041TC59231Suppression of tumorigenicity 13 (colon carcinoma)(Hsp70-interacting protein)dC < I	BE241039	—	Mus musculus strain 129/SvJ BAC clone citb10i1from the MHC region <sup>e</sup>	C > I
BE241041TC59231Suppression of tumorigenicity 13 (colon carcinoma)(Hsp70-interacting protein)d $C < I$ BE241042TC53537Human DNA sequence from clone RP3-453D15on chromosome 6q16° $C < I$ BE241043TC47711Heterogeneous nuclear RNA ( hn-RNA)d $C > I$ BE241044TC56074Synaptic vesicle glycoprotein 2 b (Sv2b)° $C < I$ BE241045TC51558Gene dJ198K11.1° $C < I$ BE241046SingletonHomo sapiens clone RP11-444D15° $C < I$ BE241047TC47599RIKEN cDNA 2610003J06 gene° $C < I$ BE241048TC48755Homo sapiens cDNA: FLJ22518 fis, clone HRC12216° $C < I$ BE241050TC51491Uveal autoantigen with coiled-coil domains andankyrin repeats (UACA)d $C < I$ BE241052SingletonHomo sapiens 12p BAC RP11-114G22° $C > I$ BE241054SingletonHypothetical protein FLJ13213 (FLJ13213)° $C < I$ BE241055TC47786Pituitary glycoprotein hormone alpha subunitd $C > I$ BE241056TC53496Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748° $C > I$ BE241058SingletonHomo sapiens BAC clone RP11-242C19° $C > I$	BE241040	—	RIKEN cDNA 0610040D20 gene <sup>e</sup>	C > I
BE241042TC53537Human DNA sequence from clone RP3-453D15on chromosome $6q16^e$ C < IBE241043TC47711Heterogeneous nuclear RNA ( hn-RNA) <sup>d</sup> C > IBE241044TC56074Synaptic vesicle glycoprotein 2 b (Sv2b) <sup>e</sup> C < I	BE241041	TC59231	Suppression of tumorigenicity 13 (colon carcinoma)(Hsp70-interacting protein) <sup>d</sup>	C < I
BE241043TC47711Heterogeneous nuclear RNA ( hn-RNA)dC > IBE241044TC56074Synaptic vesicle glycoprotein 2 b (Sv2b)eC < I	BE241042	TC53537	Human DNA sequence from clone RP3-453D15on chromosome 6q16 <sup>e</sup>	C < I
BE241044TC56074Synaptic vesicle glycoprotein 2 b (Sv2b)eC < IBE241045TC51558Gene dJ198K11.1eC < I	BE241043	TC47711	Heterogeneous nuclear RNA (hn-RNA) <sup>d</sup>	C > I
BE241045TC51558Gene dJ198K11.1°C < IBE241046SingletonHomo sapiens clone RP11-44D15°C > IBE241047TC47599RIKEN cDNA 2610003J06 gene°C < I	BE241044	TC56074	Synaptic vesicle glycoprotein 2 b (Sv2b) <sup>e</sup>	C < I
BE241046SingletonHomo sapiens clone RP11-444D15°C > IBE241047TC47599RIKEN cDNA 2610003J06 gene°C < I	BE241045	TC51558	Gene dJ198K11.1 <sup>e</sup>	C < I
BE241047TC47599RIKEN cDNA 2610003J06 geneC < IBE241048TC48755Homo sapiens cDNA: FLJ22518 fis, clone HRC12216C < I	BE241046	Singleton	Homo sapiens clone RP11-444D15 <sup>e</sup>	C > I
BE241048TC48755Homo sapiens cDNA: FLJ22518 fis, clone HRC12216eC < IBE241049TC62883Six-transmembrane epithelial antigen of prostate $2(STEAP2)^d$ C > IBE241050TC51491Uveal autoantigen with coiled-coil domains andankyrin repeats (UACA) <sup>d</sup> C < I	BE241047	TC47599	RIKEN cDNA 2610003J06 gene <sup>e</sup>	C < I
BE241049TC62883Six-transmembrane epithelial antigen of prostate $2(STEAP2)^d$ C > IBE241050TC51491Uveal autoantigen with coiled-coil domains and ankyrin repeats (UACA)^dC < I	BE241048	TC48755	Homo sapiens cDNA: FLJ22518 fis, clone HRC12216 <sup>e</sup>	C < I
BE241050TC51491Uveal autoantigen with coiled-coil domains andankyrin repeats (UACA) <sup>d</sup> C < IBE241051TC52924Proteasome regulatory particle subunit p44S10 <sup>d</sup> C < I	BE241049	TC62883	Six-transmembrane epithelial antigen of prostate 2(STEAP2) <sup>a</sup>	C > I
BE241051TC52924Proteasome regulatory particle subunit p44S10d $C < I$ BE241052SingletonHomo sapiens 12p BAC RP11-114G22e $C > I$ BE241053TC52950Putative acid phosphatase F26C11.1 (LOC118924)d $C > I$ BE241054SingletonHypothetical protein FLJ13213 (FLJ13213)e $C < I$ BE241055TC47786Pituitary glycoprotein hormone alpha subunitd $C > I$ BE241056TC53496Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748e $C > I$ BE241057TC63728Homo sapiens X BAC RP11-242C19e $C > I$ BE241058SingletonHomo sapiens BAC clone RP11-533I8e $C < I$	BE241050	TC51491	Uveal autoantigen with coiled-coil domains andankyrin repeats (UACA) <sup>d</sup>	C < I
BE241052SingletonHomo sapiens 12p BAC RP11-114G22eC > IBE241053TC52950Putative acid phosphatase F26C11.1 (LOC118924)dC > IBE241054SingletonHypothetical protein FLJ13213 (FLJ13213)eC < I	BE241051	TC52924	Proteasome regulatory particle subunit p44S10 <sup>d</sup>	C < I
BE241053TC52950Putative acid phosphatase F26C11.1 (LOC118924) <sup>d</sup> C > IBE241054SingletonHypothetical protein FLJ13213 (FLJ13213) <sup>e</sup> C < I	BE241052	Singleton	Homo sapiens 12p BAC RP11-114G22 <sup>e</sup>	C > I
BE241054SingletonHypothetical protein FLJ13213 (FLJ13213) <sup>e</sup> C < IBE241055TC47786Pituitary glycoprotein hormone alpha subunit <sup>d</sup> C > IBE241056TC53496Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748 <sup>e</sup> C > IBE241057TC63728Homo sapiens X BAC RP11-242C19 <sup>e</sup> C > IBE241058SingletonHomo sapiens BAC clone RP11-53318 <sup>e</sup> C < I	BE241053	TC52950	Putative acid phosphatase F26C11.1 (LOC118924) <sup>a</sup>	C > I
BE241055TC47786Pituitary glycoprotein hormone alpha subunitd $C > I$ BE241056TC53496Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748e $C > I$ BE241057TC63728Homo sapiens X BAC RP11-242C19e $C > I$ BE241058SingletonHomo sapiens BAC clone RP11-533I8e $C < I$	BE241054	Singleton	Hypothetical protein FLJ13213 (FLJ13213) <sup>e</sup>	C < I
BE241056TC53496Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748° $C > I$ BE241057TC63728Homo sapiens X BAC RP11-242C19° $C > I$ BE241058SingletonHomo sapiens BAC clone RP11-533I8° $C < I$	BE241055	TC47786	Pituitary glycoprotein hormone alpha subunit <sup>a</sup>	C > I
BE241057TC63728Homo sapiens X BAC RP11-242C19e $C > I$ BE241058SingletonHomo sapiens BAC clone RP11-53318e $C < I$	BE241056	TC53496	Homo sapiens cDNA FLJ13930 fis, cloneY79AA1000748 <sup>e</sup>	C > I
BE241058 Singleton Homo sapiens BAC clone RP11-533I8 <sup>e</sup> C < I	BE241057	TC63728	Homo sapiens X BAC RP11-242C19 <sup>e</sup>	C > I
	BE241058	Singleton	Homo sapiens BAC clone RP11-53318 <sup>e</sup>	C < 1

**Table 1** (*Continued*). GenBank accession numbers, the Institute for Genomic Research (TIGR) cluster information, gene identification, and direction of expression change between selection lines for anterior pituitary EST characterized in this differential display study

Accession No.			
GenBank	TIGR <sup>a</sup>	Identification or clone information <sup>b</sup>	DD <sup>c</sup>
BE241059	Singleton	Sus scrofa clone RP44-123F10 <sup>e</sup>	C < I
BE241060	Singleton	Mouse DNA sequence from clone RP23-419J18 onchromosome X <sup>e</sup>	C < I
BE241061	TC47523	Polyubiquitin (UBC) <sup>d</sup>	C > I
BE344516	TC58697	Not available <sup>f</sup>	C < I
BE344517	—	Not available <sup>t</sup>	C < I
BE344518	_	Not available <sup>r</sup>	C > I
BE344519	TC47674	NADH dehydrogenase subunit 3 (ND3) <sup>a</sup>	C < I
BE344520	Singleton	Homo sapiens chromosome 8, clone RP11-324E17 <sup>e</sup>	C < I
BE344521	TC57464	Homo sapiens hypothetical protein FLJ10330(FLJ10330) <sup>e</sup>	C < 1
BE344522	Singleton	Human DNA sequence from clone 879J18 onchromosome 11p13 <sup>e</sup>	C > I
BE344523	TC56725	Sorcin (SRI) <sup>a</sup>	C < 1
BE344524	— —	Mus musculus chromosome 5 clone RP24-486D23°	C < 1
BE344525	TC50478		C < I
BE344526	—	Not available.	C < I
BE344527	— —	Homo sapiens chromosome 10 clone RP11-564D11°	C < I
BE344528	1C55980	Steroidogenic acute regulatory protein gene"	C < I
BE344529	_	Homo sapiens clone RP11-296014 on chromosome 1°	C > I
BE344530		Human DNA sequence from clone RP13-46M24 onchromosome Ap11.4-21.2°	C < I
BE344531	TC94996	Uroplakin II gene" Uroplakin II gene"	C < I
BE344532	1047034	Homo sapiens CDNA from clone DKFZp434C0118°	C < I
BE344533	Singleton	Sus scrola DNA for SINE sequence SSPRE	C = I
BE344534	TC48023	Small actors protein (SMAP) <sup>2</sup>	C = I
DE344939	TC49801	Homo sapiens hypothetical gene supported byAL8333556 (LOC283778) <sup>2</sup>	C = I
DE344330 DE944597	TC50172	Component of alignmenia galai complex 2 (COC2) <sup>d</sup>	C = I
DE044007	TC50175	Dretain phagnhataga 2 (formarky 2P), actalytic gubunit alpha iceform	C = I
DE344556	1C50166	(calcineurin A alpha) (PPP3CA) <sup>d</sup>	0 = 1
BE344539	TC53759	Homo sapiens chromosome 8, clone CTD-2544N14 <sup>e</sup>	C = I
BE344540	TC47874	Ribosomal protein S23 (RPS23) <sup>d</sup>	C > I
BE344541	TC56277	Ribosomal protein S8 (RPS8) <sup>d</sup>	C < I
BE344542	TC56559	Ribosomal protein L31 (RPL31) <sup>d</sup>	C < I
BE344543	TC47353	Eukaryotic translation elongation factor 1 alpha 1 <sup>d</sup>	C < I
BE344544	TC47550	Ribosomal protein S3a <sup>d</sup>	C < I
BE344545	—	Ribosomal protein S12 (RPS12) <sup>d</sup>	C < I
BE344546	TC56336	Ribosomal protein S24 (RPS24) <sup>d</sup>	C < I
BE344547	TC47583	Ribosomal protein L11 (RPL11) <sup>d</sup>	C < I
BE344548	TC56928	Ribosomal protein L12 pseudogene 4 (RPL12P4) <sup>d</sup>	C > I
BE344549	_	Ribosomal protein S25 (RPS25) <sup>d</sup>	C > I
BE344550	TC56335	Follicle stimulation hormone beta subunit <sup>d</sup>	C < I
BE344551	TC47383	Preprolactin <sup>d</sup>	C < I
BE344552	TC47694	Steroid membrane binding protein <sup>d</sup>	C < I
BE344553	TC49402	Schwannomin interacting protein 1 (SCHIP-1) <sup>d</sup>	C > I
BE344554	TC47179	Peptidyl-prolyl isomerase G (cyclophilin G)(PPIG) <sup>a</sup>	C > I
BE344555	TC56361	Superoxide dismutase <sup>a</sup>	C = I
BE344556	TC47497	Ferritin heavy-chain"	C > 1
BE344557	Singleton	Scinderin (SCIN) <sup>a</sup>	C < 1
BE344558	TC61710	GI-related zinc finger protein (GIrp)"	C < I
BE344559	TC52167	Homo sapiens CDNA FLJ39000 fis, cionely 12R12022468°	C < I
DE344500	1048003	1-complex 1 (1cp1) <sup>-</sup>	C < I
DE344301 DE944569	TC56221	MADS/MEF2-lamily transcription factor (MEF2C) <sup>*</sup>	C > I
DE344502	TC50521 TC57604	Activating transcription factor 4 (ATF4) <sup>2</sup>	C > I
DE344303 BE344564	TC56584	Protain inhibitor of neuronal nitrie oxide synthese(DIN) <sup>d</sup>	
DE044004	1000004	A probability of the theorem in the transformation $(ADD)^d$	C>1
BE344500	Singlaton	Illiquitin conjugating enzyme E2 II (URC6 homolog yeast) (URE2 I) <sup>d</sup>	0>1
BE344500	TC57000	Conserved ATPase domain protein 44 (CADr44) Snormanhilus	
01011007	1001000	tridecemlineatus 26S Proteasome S.U. <sup>d</sup>	0 > 1
BE344568	TC58868	Secretogranin III (SCG3) <sup>d</sup>	C = I
BE344569	TC56220	Beta 2-microglobulin <sup>d</sup>	C = I
BE344570	TC56366	Heterogeneous nuclear ribonucleoprotein F (HnRPF) <sup>d</sup>	C = I
BE344571	TC47243	Calpain I light subunit <sup>d</sup>	C = I
			Continued

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Accessi	on No.		
GenBank	TIGR <sup>a</sup>	Identification or clone information <sup>b</sup>	DDc
BE344572	TC49039	F1F0-ATP synthase complex Fo membrane domain f subunit <sup>d</sup>	C > I
BE344573	TC47591	G-beta like protein <sup>d</sup>	C > I
BE344574	TC57182	Homo sapiens BAC clone RP11-746I5 from 2 <sup>e</sup>	C < I
BE344575	TC52833	Rapamycin associated protein (FRAP2) <sup>d</sup>	C > I
BE344576	TC63812	SPARC-like 1 (mast9, hevin) (SPARCL1) <sup>d</sup>	C < I

**Table 1** (*Continued*). GenBank accession numbers, the Institute for Genomic Research (TIGR) cluster information, gene identification, and direction of expression change between selection lines for anterior pituitary EST characterized in this differential display study

<sup>a</sup>TIGR, March 2003 (www.tigr.org/tdb/tgi/ssgi).

<sup>b</sup>GenBank BLAST Nucleotide, March 2003 (www.ncbi.nlm.nih.gov/Genbank/index.html).

<sup>c</sup>Direction of differential display difference in expression. For example, C > I indicates that greater banding intensity was observed in the Control line. Both C > I and C < I indicates that in one instance the EST was found to be up-regulated in the Control line (C > I; band was excised and sequenced), whereas in another instance, the EST was found to be up-regulated in the Index line (C < I; band was excised and sequenced). Only after analysis of sequence data was it determined that these EST had similarity to the same gene.

<sup>d</sup>Total or partial similarity to known genes.

<sup>e</sup>Total or partial similarity to uncharacterized genes.

<sup>f</sup>Novel.

because the additive and dominance effects of the alleles did not differ from zero for any trait. Rohrer et al. (2001) identified several *trans*-acting QTL regulating plasma FSH levels in boars, but no evidence for a *cis*acting QTL within or near the  $FSH\beta$  locus was found. In follicles of sows from the Index line, *follistatin* (*FST*) was found to be down-regulated (Gladney et al., 2004).

Taking into account these findings along with those of others studies conducted with these selection lines (Cassady et al., 2000), it is possible that low levels of follicular *FST* mRNA could be related to the elevated levels of *FSH* $\beta$  pituitary mRNA in the Index line. However, Schneyer et al. (2003) recently found that follistatin has lower affinity for activin B, the pituitary form, than for activin A, the ovarian form. Also, Li et al. (1998) determined that *FST* mRNA concentration in the pituitaries of boars is not associated with FSH secretion.

Levels of mRNA for the *G-beta like protein* transcript were lower as a response to Index selection. G-Proteins are coupled with different receptors, and the beta subunit can be coupled with alpha subunits, which could stimulate or inhibit a given signaling pathway (Garrett and Grisham, 1999). Given the potential involvement of *G-beta like protein* in many important physiological processes, it will be difficult to assign relevance to the down-regulation of this gene in the Index line until a global gene expression analysis is conducted and cluster analysis is performed.

A novel finding of this study was that selection for the reproductive Index led to significantly increased weight of anterior pituitary in sows. There may be a correlation between this finding and the fact that twice as many differentially expressed bands were isolated from Line I vs. Line C, with the increased pituitary weight representing a physiological response to an increased metabolic demand in the Index line pituitary.

Many EST with known functions in reproductive physiology were isolated, including *pituitary glycopro*-

tein hormone alpha subunit, steroidogenic acute regulatory protein, follicle stimulating hormone beta subunit, preprolactin, and steroid membrane binding protein. In addition, many EST with undefined functions or that are novel were isolated. Future characterization of this latter subset of genes may contribute to understanding the molecular architecture of litter size in swine.

In summary, selection for increased ovulation rate and embryo survival has acted in part by altering gene expression of many transcripts in the anterior pituitary during a period of follicular development. Concurrent alterations in the ovarian follicle transcriptome have also been identified in the same animals (Gladney et al., 2004). The use of gene expression analysis represents a new vista to dissect polygenic traits and the nature of long-term selection response in livestock species. Although many potentially significant gene expression differences were uncovered using ddPCR, only a small sample of these was confirmed using Northern hybridization due to limited availability of anterior pituitary samples. This limitation also led to pooling of results across the two time points of follicular development used in this study, potentially confounding temporal differences that may have existed between the selection lines. Further confirmation of differential expression on a larger scale, as well as a deeper analysis of the anterior pituitary transcriptome, will be made possible by future largescale expression profiling using microarrays.

Although application of transcriptional analysis to understanding selection response is a powerful tool, it is not possible to determine whether genes differentially expressed between selection lines represent QTL or the downstream consequences of QTL actions. The integrated approach of expression profiling in QTL mapping populations (e.g., Schadt et al., 2003a,b) would help determine the nature of transcriptional changes and their relationship to genetic variation that contributes to complex traits and long-term response to selection. Furthermore, such integration would help partition gene expression results into those that are true responses to selection and those that result from genetic drift.

#### Implications

Differential display PCR was used to analyze gene expression differences in anterior pituitaries between pigs selected for increased ovulation rate and embryo survival and pigs of a randomly selected control line. Combined with concurrent analysis of the ovarian follicle transcriptome, our results indicate that a large number of gene expression changes may be involved in long-term selection response for a complex trait such as reproduction. Several individual gene expression changes were confirmed and present novel insights into pathways regulating reproductive phenotypes and the nature of the genetic architecture of complex traits with low heritability such as ovulation rate.

#### Literature Cited

- Ace, C. I., and W. C. Okulicz. 1999. Identification of progesteronedependent messenger ribonucleic acid regulatory patterns in the rhesus monkey endometrium by differential- display reverse transcription-polymerase chain reaction. Biol. Reprod. 60:1029-1035.
- Allan, M. F., M. K. Nielsen, and D. Pomp. 2000. Gene expression in hypothalamus and brown adipose tissue of mice divergently selected for heat loss. Physiol. Genomics 3:149–156.
- Batu, A. T., U. Hla-Pe, T. Than, and K. K. Nyunt. 1972. Iron deficiency in Burmese population groups. Am. J. Clin. Nutr. 25:210-217.
- Bergeron, C., and K. Kovacs. 1978. Pituitary siderosis. A histologic, immunocytologic, and ultrastructural study. Am. J. Pathol. 93:295-309.
- Bevilacqua, M. A., M. C. Faniello, T. Russo, F. Cimino, and F. Costanzo. 1994. Transcriptional regulation of the human H ferritin-encoding gene (FERH) in G418-treated cells: role of the Bbox-binding factor. Gene 141:287–291.
- Cassady, J. P., R. K. Johnson, and J. J. Ford. 2000. Comparison of plasma FSH concentration in boars and gilts from lines selected for ovulation rate and embryonal survival, and litter size and estimation of (co)variance components for FSH and ovulation rate. J. Anim. Sci. 78:1430–1435.
- Cassady, J. P., R. K. Johnson, D. Pomp, G. A. Rohrer, L. D. Van Vleck, E. K. Spiegel, and K. M. Gilson. 2001. Identification of quantitative trait loci affecting reproduction in pigs. J. Anim. Sci. 79:623-633.
- Charbonnel, B., M. Chupin, A. Le Grand, and J. Guillon. 1981. Pituitary function in idiopathic haemochromatosis: Hormonal study in 36 male patients. Acta Endocrinol. 98: 178-183.
- Chazenbalk, G. D., H. L. Wadsworth, and B. Rapoport. 1990. Transcriptional regulation of ferritin H messenger RNA levels in FRTL5 rat thyroid cells by thyrotropin. J. Biol. Chem. 265:666-670.
- Cundy, T., J. Butler, A. Bomford, and R. Williams. 1993. Reversibility of hypogonadotrophic hypogonadism associated with genetic haemochromatosis. Clin. Endocrinol. 38:617–620.
- Feder, J. N. 1999. The hereditary hemochromatosis gene (HFE): a MHC class I-like gene that functions in the regulation of iron homeostasis. Immunol. Res. 20:175–185.

- Ford J. J., T. H. Wise, D. D. Lunstra, and G. A. Rohrer. 2001. Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. Biol. Reprod. 65:906-912.
- Garrett, R. H., and C. M. Grisham. 1999. Biochemistry. Saunders, Essex, England.
- Gladney, C. D., G. R. Bertani, R. K. Johnson, and D. Pomp. 2004. Evaluation of gene expression in pigs selected for enhanced reproduction using differential display PCR and human microarrays: I. Ovarian Follicles. J. Anim. Sci. 82:17–31.
- Hadley, M. E. 2000. Endocrinology. Prentice Hall, Upper Saddle River, NJ.
- Ikuta, K., Y. Fujimoto, Y. Suzuki, K. Tanaka, H. Saito, M. Ohhira, K. Sasaki, and Y. Kohgo. 2000. Overexpression of hemochromatosis protein, HFE, alters transferrin recycling process in human hepatoma cells. Biochim. Biophys. Acta 1496:221–231.
- Johnson, R. K., M. K. Nielsen, and D. S. Casey. 1999. Responses in ovulation rate, embryonal survival, and litter traits in swine to 14 generations of selection to increase litter size. J. Anim. Sci. 77:541–557.
- Lee, K. F., J. F. Chow, J. S. Xu, S. T. Chan, S. M. Ip, and W. S. Yeung. 2001. A comparative study of gene expression in murine embryos developed in vivo, cultured in vitro, and cocultured with human oviductal cells using messenger ribonucleic acid differential display. Biol. Reprod. 64:910–917.
- Li M. D., G. J. Macdonald, T. Wise, and J. J. Ford. 1998. Positive association between expression of follicle-stimulating hormone beta and activin betaB-subunit genes in boars. Biol. Reprod. 59:978–982.
- Li, M. D., R. L. Matteri, G. J. Macdonald, T. H. Wise, and J. J. Ford. 1996. Overexpression of beta-subunit of thyroid-stimulating hormone in Meishan swine identified by differential display. J. Anim. Sci. 74:2104-2111.
- Liang, P., and A. B. Pardee. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257: 967–971.
- Lindemann, M. D. 1993. Supplemental folic acid: A requirement for optimizing swine reproduction. J. Anim. Sci. 71:239–246.
- Linville, R. C., D. Pomp, R. K. Johnson, and M. F. Rothschild. 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. J. Anim. Sci. 79:60–67.
- Minami, N., K. Sasaki, A. Aizawa, M. Miyamoto, and H. Imai. 2001. Analysis of gene expression in mouse 2-cell embryos using fluorescein differential display: comparison of culture environments. Biol. Reprod. 64:30–35.
- Moore, R. W., H. E. Redmond, and C. W. Livingston, Jr. 1965. Iron deficiency anemia as a cause of stillbirths in swine. J. Am. Vet. Med. Assoc. 147:746–748.
- Oppenheim, E. W., C. Adelman, X. Liu, and P. J. Stover. 2001. Heavy chain ferritin enhances serine hydroxymethyltransferase expression and de novo thymidine biosynthesis. J. Biol. Chem. 276:19855–19861.
- Robert, C., D. Gagne, D. Bousquet, F. L. Barnes, and M. A. Sirard. 2001. Differential display and suppressive subtractive hybridization used to identify granulosa cell messenger RNA associated with bovine oocyte developmental competence. Biol. Reprod. 64:1812–1820.
- Rohrer, G. A., T. H. Wise, D. D. Lunstra, and J. J. Ford. 2001. Identification of genomic regions controlling plasma FSH concentrations in Meishan-White Composite boars. Physiol. Genomics 6:145–151.
- Schadt, E. E., S. A. Monks, T. A. Drake, A. J. Lusis, N. Che, V. Colinayo, T. G. Ruff, S. B. Milligan, J. R. Lamb, G. Cavet, P. S. Linsley, M. Mao, R. B. Stoughton, and S. H. Friend. 2003a. Genetics of gene expression surveyed in maize, mouse and man. Nature 422:297–302.
- Schadt, E. E., S. A. Monks, and S. H. Friend. 2003b. A new paradigm for drug discovery: integrating clinical, genetic, genomic and molecular phenotype data to identify drug targets. Biochem. Soc. Trans. 31:437–443.

- Schneyer A., A. Schoen, A. Quigg, and Y. Sidis. 2003. Differential binding and neutralization of activins A and B by follistatin and follistatin like-3 (FSTL-3/FSRP/FLRG). Endocrinology 144: 1671–1674.
- White, K., and H. N. Munro. 1988. Induction of ferritin subunit synthesis by iron is regulated at both the transcriptional and translational levels. J. Biol. Chem. 263: 8938-8942.
- Wise, T., D. D. Lunstra, G. A. Rohrer, and J. J. Ford. 2003. Relationships of testicular iron and ferritin concentrations with testicular weight and sperm production in boars. J. Anim. Sci. 81:503-511.
- Xu, B., L. Lin, and N. S. Rote. 1999. Identification of a stress-induced protein during human trophoblast differentiation by differential display analysis. Biol. Reprod. 61:681–686.
- Zhou, X. Y., S. Tomatsu, R. E. Fleming, S. Parkkila, A. Waheed, J. Jiang, Y. Fei, E. M. Brunt, D. A. Ruddy, C. E. Prass, R. C. Schatzman, R. O'Neill, R. S. Britton, B. R. Bacon, and W. S. Sly. 1998. HFE gene knockout produces mouse model of hereditary hemochromatosis. Proc. Natl. Acad. Sci. USA 95:2492–2497.
- Zhu, L. J., M. K. Bagchi, and I. C. Bagchi. 1995. Ferritin heavy chain is a progesterone-inducible marker in the uterus during pregnancy. Endocrinology 136:4106–4115.