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Bacteriophage That Infect Gordonia Species Show Varying Host Specificity and Infection

Lindsay Birge
University of Nebraska - Lincoln

BACTERIOPHAGE THAT INFECT *GORDONIA* SPECIES SHOW VARYING HOST SPECIFICITY AND INFECTION

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Lindsay Birge, BS

Biological Sciences

College of Arts and Sciences

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Faculty Mentors:

Catherine Chia, PhD, Biological Sciences

John Osterman, PhD, Biological Sciences

ABSTRACT

Bacteriophage (phage) are an incredibly abundant species of virus that infect bacteria. One major characteristic of phage is their small host range- many phage are known to infect only one or a few hosts. This phage and host relationship has led to phage therapy recently becoming highlighted as a possible alternative to traditional antibiotics in light of the growing antibiotic resistance crisis. The study of phage host specificity has risen in line with this concept, as a phage with a more extensive host range can provide more opportunities for use as an antibiotic. Samples of phage previously isolated on a single *Gordonia* host were serially diluted and plated on both *Gordonia rubripertincta* and *Gordonia terrae*. The calculated titer and nature of plaques were recorded following two trials. Three main types of infection were identified: Types A, B, and C; and the phage were sorted according to their performance. Type A is characterized by little to no infection and plaque formation. Type B shows a fading infection with no countable plaques. Type C infection is seen in phage that showed a nearly identical or identical level of infection on both the isolation host and test host strains. The phage that exhibited Type C infection had a similarity in genes, specifically those related to tail proteins, which may indicate the role tail proteins play in host specificity. The genomes of sequenced phage in the PhagesDB database were compared to investigate similarities among phage with the same infection type. These findings indicate that even phage that infect the same host have different effects on other hosts. A similarity in tail proteins may indicate the ability to infect new hosts other than the original. More testing is necessary to investigate the extent of host range amongst known phage and what this might mean for further phage-based scientific research.

KEY WORDS

Biology, Bacteriophage, Bacterial Virus, Host Specificity, Host Range, Host Infection

INTRODUCTION

Bacteriophage (phage) are some of the most abundant organisms on the planet and have an extremely diverse and biologically-significant population (1, 2). Phage are viruses that infect bacteria by injecting its own DNA into the host and use the host to replicate and subsequently lyse it (3, 4). The study of bacteriophage has become an area of increasing interest, especially in regards to their possible uses as cellular defense systems (5, 6), an alternative to antibiotics (7), and control systems for bacterial growth (8). One major characteristic of bacteriophage is their narrow host range, with a single phage infecting only one or a few related host species (5, 3). The Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program focuses on phage which infect bacteria in the phylum *Actinobacteria* (8). There are currently seven major hosts: *Arthrobacter globiformis, Arthobacter sp., Gordonia rubripertincta, Gordonia terrae, Microbacterium foliorum, Microbacterium testaceum,* and *Mycobacterium smegmatis* (4). *Gordonia rubripertincta* (*G. rubripertincta*) and *Gordonia terrae* (*G. terrae*) are the bacterial hosts of interest in this study. *G. rubripertincta* is a gram-positive bacterium usually found in soils and is characterized by the smooth glossy tan-to-orange colonies it forms when growing (4). *G. terrae*, like *G. rubripertincta*, is also gram-positive and often found in soils. It forms drier and rougher tan-to-orange colonies when grown (4). Both bacteria, as well as the rest of the *Gordonia* species, are known for their ability to break down hydrocarbons, thus giving them an essential role in bioremediation (4).

The study of bacteriophage host specificity has increased in recent years, especially with the growing antibiotic resistance crisis, as a phage with a broader host range could be a better candidate for phage therapy (3, 9). However, this field of study is still relatively small and

underdeveloped, with aspects that determine phage specificity still unknown (9, 10). A majority of phage (59%) in the SEA-PHAGES database have been found to infect *Mycobacterium* species of bacteria, so much of the present research on phage host specificity and host range has been focused on this bacteria and its phage (8, 10). A previous study of many phage infecting *Mycobacterium smegmatis* has indicated distinct infection properties on a similar bacterial host, *Mycobacterium tuberculosis*, based on their cluster, an organizational method of grouping phage that share strong genetic similarities (10).

Gordonia phage make up 12% of the identified phage in the SEA-PHAGES database and have great diversity in genome length and gene organization, thus presenting an area of great interest for study (8,11). In addition, a study of this scale has not been conducted with phage that infect *Gordonia* species, although one sequenced phage was reported to show equal infection on multiple *Gordonia* host strains (12). There is a noticeable gap in knowledge regarding *Gordonia* phage, so this study is unique in its examination of multiple *Gordonia* phage and their relative infection properties of *G. rubripertincta* and *G. terrae*. Based on previous host specificity literature, plating phage on both its isolation host and a test host may reveal a reduction in its infection ability on the test host (10).

One theory on the source of host specificity in bacteriophage lies with the tail protein. Bacteriophage tails are a significant factor in cell recognition and the transportation of phage DNA into bacterial hosts, as they interact with the cell surface and binding proteins (13). The importance of these tail proteins in major steps of host infection has caused them to become a forefront in host specificity studies (13, 14). The engineering of tail proteins and genes that control properties of the tail has been a significant target for expanding the host specificity of

phage (15, 16). Comparative analyses of major and minor tail proteins may reveal patterns of host specificity.

METHODS

G. rubripertincta B16540-SEA (SEA-PHAGES Program, Univ. Pittsburgh) and *G. terrae* 3612 (Doane University, Crete, NE) were used for host range testing. Fresh cultures of each host bacteria were grown weekly from single colonies on streak plates following protocols described in the Phage Discovery Guide (PDG) (4). A single colony was first used to inoculate a mini-culture of PYCa liquid and incubated on a shaker at 30˚C for 48 hours. Then, the mini-culture was used to inoculate a larger volume of PYCa, which was then incubated on a shaker at 30˚C for 48 hours. Cultures used to prepare lawns for spot titer plates were no greater than five days old at use. A spot titer of each phage was initially carried out on its isolation host bacteria to get an approximation of the phage concentration in the lysate (Protocol 6.4 – Spot Titer, PDG). If the titer was below the minimum recommended titer of 5×10^9 Plaque Forming Units/Milliliter (pfu/mL), fresh lysates were prepared following Protocol 7.1 – Making Webbed Plates and the titer approximated using Protocol 6.4 – Spot Titer. Host infection capability was tested on *G. terrae* and *G. rubripertincta* (Protocol 11.5 – Host Range Assay, PDG). Each phage was tested twice. The titer from each spot titer on each host, Efficiency of Plating (EOP) (Protocol 11.5 – Host Range Assay, PDG), as well as the nature of the plaques, such as “fading” or “host-like,” were recorded.

RESULTS

Table I. Tested Phage Lysates.

Phage	Host	Cluster	Source	Lysate Year
Aang	*G. rubripertincta*	Unknown	University of Nebraska-Lincoln	2021
BearsBeets	*G. rubripertincta*	Unknown	UNL	2021
Big Ed	*G. rubripertincta*	Unknown	UNL	2020
Chidiebere	*G. rubripertincta*	DQ	University of Pittsburgh, PA	2018
Chikenjars	*G. rubripertincta*	DJ	UNL	2018
DoubleDipper	*G. rubripertincta*	Unknown	UNL	2020
Dre3	*G. rubripertincta*	CT	UNL	2018
Feastonyeet	*G. rubripertincta*	CT	UNL	2021
Gibbous	*G. rubripertincta*	CT	UNL	2019
GrootJr	*G. terrae*	CR (CR2)	Doane University, Crete, NE	2021
Monty	*G. terrae*	CS (CS2)	University of Pittsburgh, PA	2019
Nithya	*G. rubripertincta*	DJ	UNL	2020
Norvs	*G. terrae*	CQ	Doane University, Crete, NE	2021
ObLaDi	*G. rubripertincta*	DZ	UNL	2020
Poptart2	*G. rubripertincta*	Unknown	UNL	2019
Vine	*G. rubripertincta*	CT	UNL	2020

Table II. Type A Infection Results.

Cluster	Phage	Isolation Host *Gordonia rubripertincta* Titer (pfu/mL)	Test Host *Gordonia terrae* Titer (pfu/mL)	Efficiency of Plating (EOP)
CT	Gibbous Trial 1	1.66×10^{11}	0	$< 1/(1.66 \times 10^{8})$
	Gibbous Trial 2	1.33×10^{11}	1.00×10^{4}	7.52×10^{-8}
CT	Dre3 Trial 1	4.30×10^{11}	0	$< 1/(4.30 \times 10^{8})$
	Dre3 Trial 2	3.33×10^{10}	0	$<1/(3.33 \times 10^{7})$
Unknown	BigEd Trial 1	3.33×10^{11}	0	$< 1/(3.33 \times 10^{8})$
	BigEd Trial 2	2.33×10^{11}	1.00×10^{5}	4.29×10^{-7}

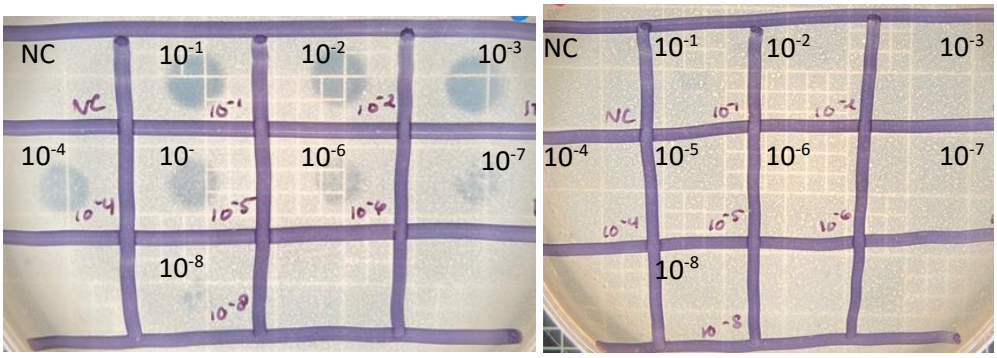


Figure 1. Type A Infection. A spot titer of ten-fold serial dilutions of Gibbous phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C. EOP was found to be 7.52×10^{-8}. Titer is markedly reduced and there is very little evidence of plaques on the test host species. NC: negative control (phage buffer).

Table III. Type B Infection Results.

Cluster	Phage	Isolation Host *Gordonia rubripertincta* Titer (pfu/mL)	Test Host *Gordonia terrae* Titer (pfu/mL)	Efficiency of Plating (EOP)
CT	Feastonyeet Trial 1	6.67×10^{10}	1.00×10^{5}	1.50×10^{-6}
	Feastonyeet Trial 2	6.67×10^{10}	1.00×10^{5}	1.50×10^{-6}
CT	Vine Trial 1	1.00×10^{12}	1.00×10^{5}	1.00×10^{-7}
	Vine Trial 2	1.00×10^{11}	1.00×10^{5}	1.00×10^{-6}
DQ	Chidiebere Trial 1	2.00×10^{10}	1.00×10^{5}	5.00×10^{-6}
	Chidiebere Trial 2	3.33×10^{10}	1.00×10^{3}	3.00×10^{-8}
Unknown	Double Dipper Trial 1	3.30×10^{11}	1.00×10^{5}	3.03×10^{-7}
	Double Dipper Trial 2	1.33×10^{11}	1.00×10^{4}	7.52×10^{-8}
Unknown	Aang Trial 1	8.30×10^{11}	1.00×10^{6}	1.20×10^{-6}
	Aang Trial 2	1.00×10^{10}	1.00×10^{5}	1.00×10^{-5}
Unknown	BearsBeets Trial 1	2.67×10^{11}	1.00×10^{6}	3.75×10^{-6}
	BearsBeets Trial 2	1.33×10^{11}	1.00×10^{5}	7.52×10^{-7}
Unknown	Poptart2 Trial 1	2.30×10^{10}	1.00×10^{7}	4.35×10^{-4}
	Poptart2 Trial 2	3.00×10^{10}	1.00×10^{6}	3.33×10^{-5}
		Isolation Host *Gordonia terrae* Titer (pfu/mL)	Test Host *Gordonia rubripertincta* Titer (pfu/mL)	
CR (CR2)	GrootJr Trial 1	1.67×10^{10}	1.00×10^{4}	5.99×10^{-7}
	GrootJr Trial 2	1.00×10^{10}	1.00×10^{3}	1.00×10^{-7}
CS (CS2)	Monty Trial 1	6.67×10^{9}	6.00×10^{4}	9.00×10^{-6}
	Monty Trial 2	3.33×10^{10}	1.00×10^{4}	3.00×10^{-7}

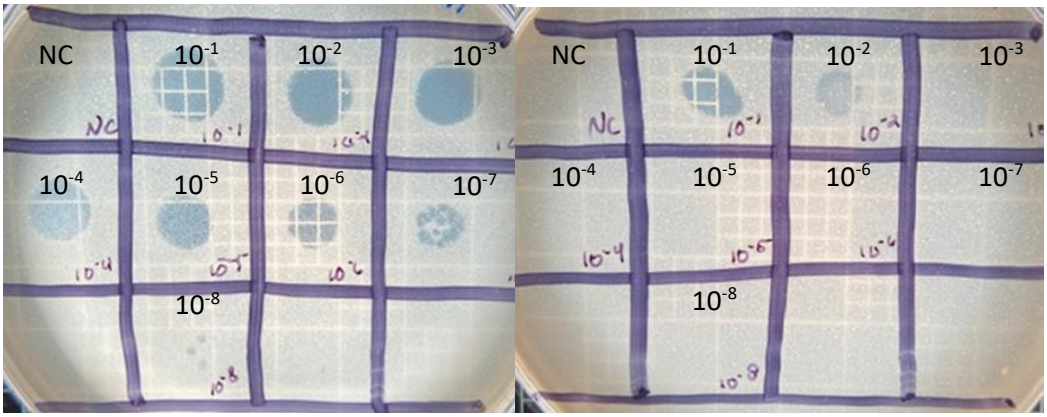


Figure 2. Type B Infection. A spot titer of ten-fold serial dilutions of Vine phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C. EOP was found to be $1.00x10^{-6}$. Titer is obviously reduced and there is some clearing but no countable plaques on the test host species. NC: negative control (phage buffer).

Table IV. Type C Infection Results.

Cluster	Phage	Isolation Host *Gordonia rubripertincta* Titer (pfu/mL)	Test Host *Gordonia terrae* Titer (pfu/mL)	Efficiency of Plating (EOP)
DJ	Nithya Trial 1	1.33×10^{11}	1.00×10^{7}	7.52×10^{-5}
	Nithya Trial 2	6.67×10^{10}	4.33×10^{10}	0.649
DJ	Chikenjars Trial 1	3.00×10^{10}	1.00×10^{7}	3.33×10^{-4}
	Chikenjars Trial 2	3.33×10^{10}	3.33×10^{10}	1.00
DZ	ObLaDi Trial 1	2.67×10^{11}	1.30×10^{9}	0.005
	ObLaDi Trial 2	3.33×10^{9}	3.00×10^{7}	0.009

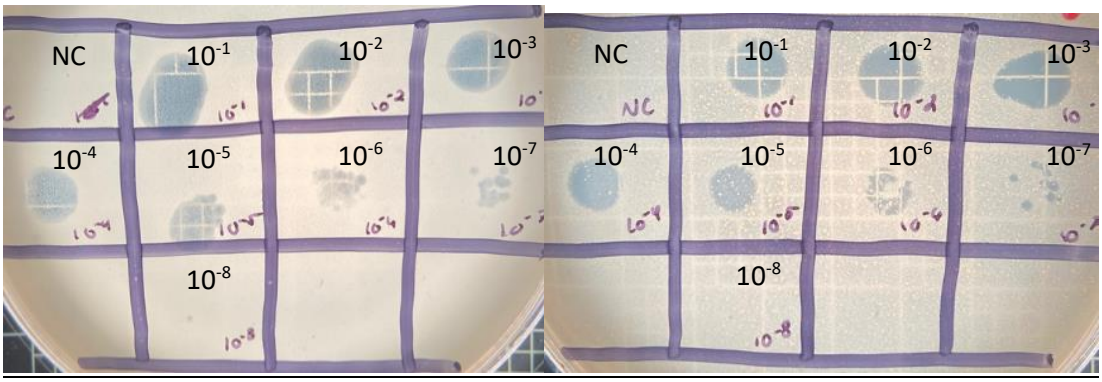


Figure 3. Type C Infection. A spot titer of Chikenjars phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C. EOP was found to be 1, indicating identical performance on both hosts. There are countable plaques on both the host species and the test host species and plaques look nearly identical. NC: negative control (phage buffer).

Titer and plaque appearances from each trial were grouped into three infection categories. The first type (type A) is characterized by little to no infection on the test host. This type of infection can be seen in Gibbous (Figure 1), Dre3, and BigEd, which all had at least one "0" titer on the test host across the two trials (Table II). Plaques were not visible or barely visible at the smallest dilutions. A "fading" type infection characterizes the second type (type B). Phage with this type of infection showed a range of titers on the test host, but most had a markedly reduced titer (Table III). On the test host strain, each phage could produce a completely cleared plaque at smaller dilutions. However, as the dilutions became greater, the single clearing became increasingly fainter until it was completely absent. There were no individual, countable plaques at the greatest dilutions like a traditional spot titer, so titers for these phage were approximated. This type of infection is seen in 9 of the 15 phages tested, including Vine (Figure 2) and

PopTart2. The final type of infection (type C) is the infection type of interest. Phage with this type of infection showed a titer on the test host that was very similar or identical to its titer on the isolation host species (Table IV). Plaques on the test host species looked just like those on the isolation host species and showed individual, countable plaques at greater dilutions. This type of infection can be seen in Chikenjars (Figure 3), ObLaDi, and Nithya.

DISCUSSION

Titer results and visual observations indicate three different outcomes when a phage is plated on a bacterial host different from the host it was initially isolated with. Type A is identified by little to no evidence of clearing and a highly reduced titer on the test host species. Generally, a performance of this type by a phage could be caused by a low titer of the original lysate or a possible inherent incompatibility with the test host. In this case, due to the initial spot titer testing to confirm a high titer, this performance can be inferred as an inability to infect the test host species by the phage. Two of the phage that produced this type of infection, Dre3 and Gibbous, are inferred to be very similar phages due to their placement in the same cluster, indicating nucleotide and gene similarity (8, 11). This similarity likely contributes to their similar performances. BigEd, the third phage that produced this type of infection, has not been sequenced, so its relationship to the other phage is unknown at this time.

Fading clearings are a trademark of Type B infection, in which a large, round clearing fades with increased dilution. This type of infection noticeably lacks one of the hallmarks of a bacteriophage spot titer, which are countable individual plaques. Countable plaques are imperative in determining the titer of a phage lysate; the fact that this characteristic is missing in this type of infection poses difficulties with determining titer. Titers of phage that showcased this type of infection were approximated, but may be an over or underestimation due to this

difficulty. These phage belong to varying clusters (Table I) and thus have quite different genes, including tail proteins, so not many conclusions can be drawn from this infection type (8). This infection type may not even be an infection at all; it could be caused by "lysis from without," a phenomenon that has been observed in other studies of bacteriophage (3, 17, 18). This phenomenon has different subtypes, but at its basis, it involves an overwhelming of bacterial host cells without replication of phage. Rather than infecting the cells, replicating, and expelling from the host ("lysis from within"), phage bind to the surface and cause immediate lysis (17). This phenomenon can be with large multiplicities of phage populations, in which the host cells are simply unable to complete normal life-sustaining processes and die (18). This phenomenon may be what is seen with Type B infection.

Type C infection is of greatest interest, in which bacteriophage infected the test host strain with close to or identical potency. This type of infection produced countable individual plaques at greater dilution levels, allowing for precise titer calculations from spot titer trials. Only a few phage showcased this type of infection: Nithya, Chikenjars, and ObLaDi, raising questions about what connects these specific phage and why they were showing this type of infection. Nithya and Chikenjars are in the same cluster, cluster DJ, indicating genomic similarity (8, 11). 98% of their genes are in common, most notably their major and minor tail proteins (Figure 4). ObLaDi is in a cluster DZ and bears some genetic similarities to phage VanLee (Figure 4), which has been published as being able to infect multiple *Gordonia* strains of bacteria with a Type C-like infection style (12). Most interestingly, a lot of their similarity lies within the genes responsible for tail proteins: ObLaDi and VanLee have six of the ten tail-associated proteins' Phams, a grouping system for similar genes, in common (Figure 4). This similarity is more interesting because VanLee is a singleton and not in ObLaDi's cluster,

indicating wider genomic dissimilarity (8). Type C phage and VanLee have many unique characteristics which indicate a possible correlation between tail proteins and host range: tail proteins that are in the same Pham, the ability to infect multiple *Gordonia* phage in similar manners, and a lack of genomic similarity to Type A and B phage (8).

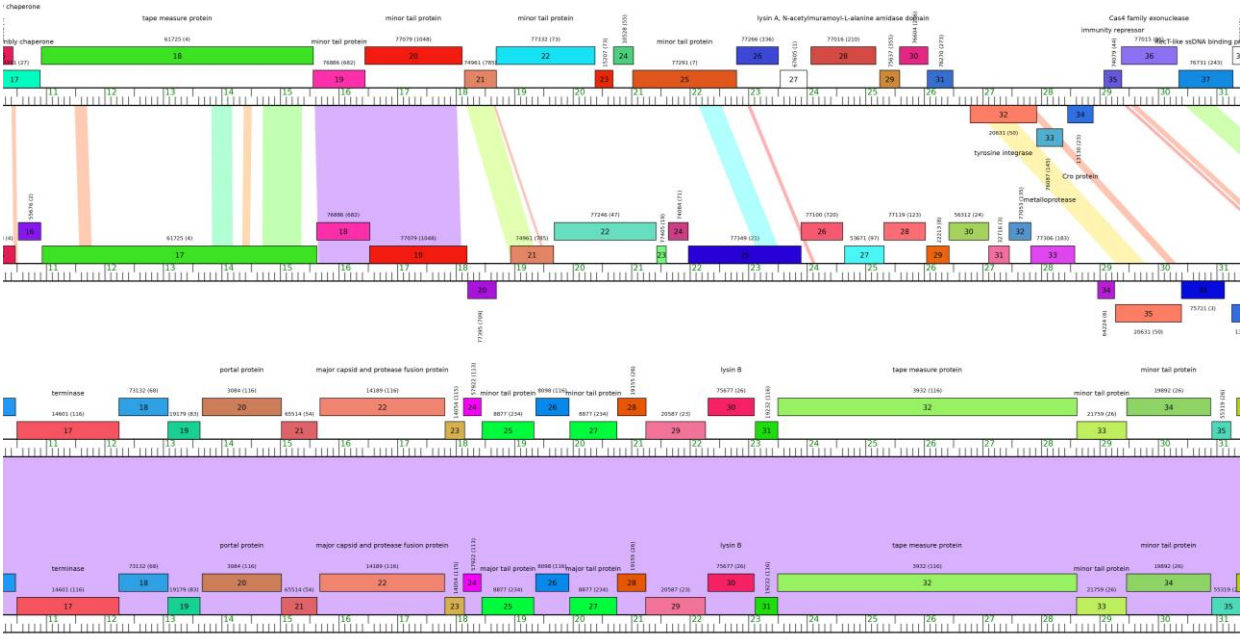


Figure 4. Phamerator maps comparing tail proteins of (from top to bottom) VanLee, ObLaDi, Chikenjars, and Nithya. Nithya and Chikenjars are in the same cluster, DJ, and have tail proteins that are in the same Phams. ObLaDi is in cluster DZ and VanLee is a singleton (no assigned cluster), and share many tail proteins that are in the same Phams. The full Phamerator (19) maps are in the Appendix (Appendix Figure 1).

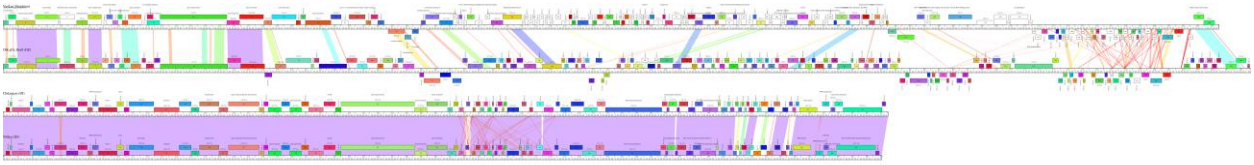
Regardless, host specificity is a complex property of phage that most likely involves many different parts on both the phage and bacterial sides. Tail protein interactions appear to be

one part of a vast and dynamic network that culminates in host interaction. Host range does not appear to be a fixed property and has recently been thought of as something that is malleable and can change over time (3). However, findings from this study do provide interesting insight into the possible relationship between genome and host specificity, as well as levels of infection amongst different phage. There appears to be a stark difference in infection ability amongst phage, with only a few being able to truly infect a new host like their original host. This property of equally infecting a test host seems to be rare and must arise from something that these phage have in common. This commonality could be their tail proteins or could come from some other combination of genetic similarities arising from Phams or clusters (8). These findings have the possibility to be used in future studies of host range. The number of unique phage used in this study were limited to what was available at the local institutions, especially when compared to the total number of unique phage known to the SEA-PHAGES program (8). Some of the phage samples came from other locations, but most came from the Lincoln, Nebraska, area. A small sample size leads to trouble generalizing. However, the methods and findings from this paper serve as a stepping stone for the expanding field of phage host range and host specificity studies. Replication of these methods with many more phage could provide more insight into the rarity of Type C infection and the possible genetic relationship to these infection types (10). Maximizing usage of sequenced phage via the SEA-PHAGES program would be ideal, as more analysis could go into genomic similarities. This could lead to more insight into whether tail proteins are similar amongst phage with Type C infection. Similarly, the use of only two host types provides a small view of the full host range of phage from this study. Repeating the same methods on other *Gordonia* strains or close relatives of *Gordonia*, such as *Mycobacterium*, could provide more insight into how broad a host range Type C phage may have (11). In addition, the method

of spot testing used in this paper, while quick and efficient and incredibly common within the SEA-PHAGES program, can be misleading, as seen with the Type B infection (3). Further analysis of the best methods for the study of host range is needed to ensure a proper and trustworthy result. Nevertheless, with the knowledge of bacteriophage methods at this time, the results from this study can undoubtedly be used to inform future methods.

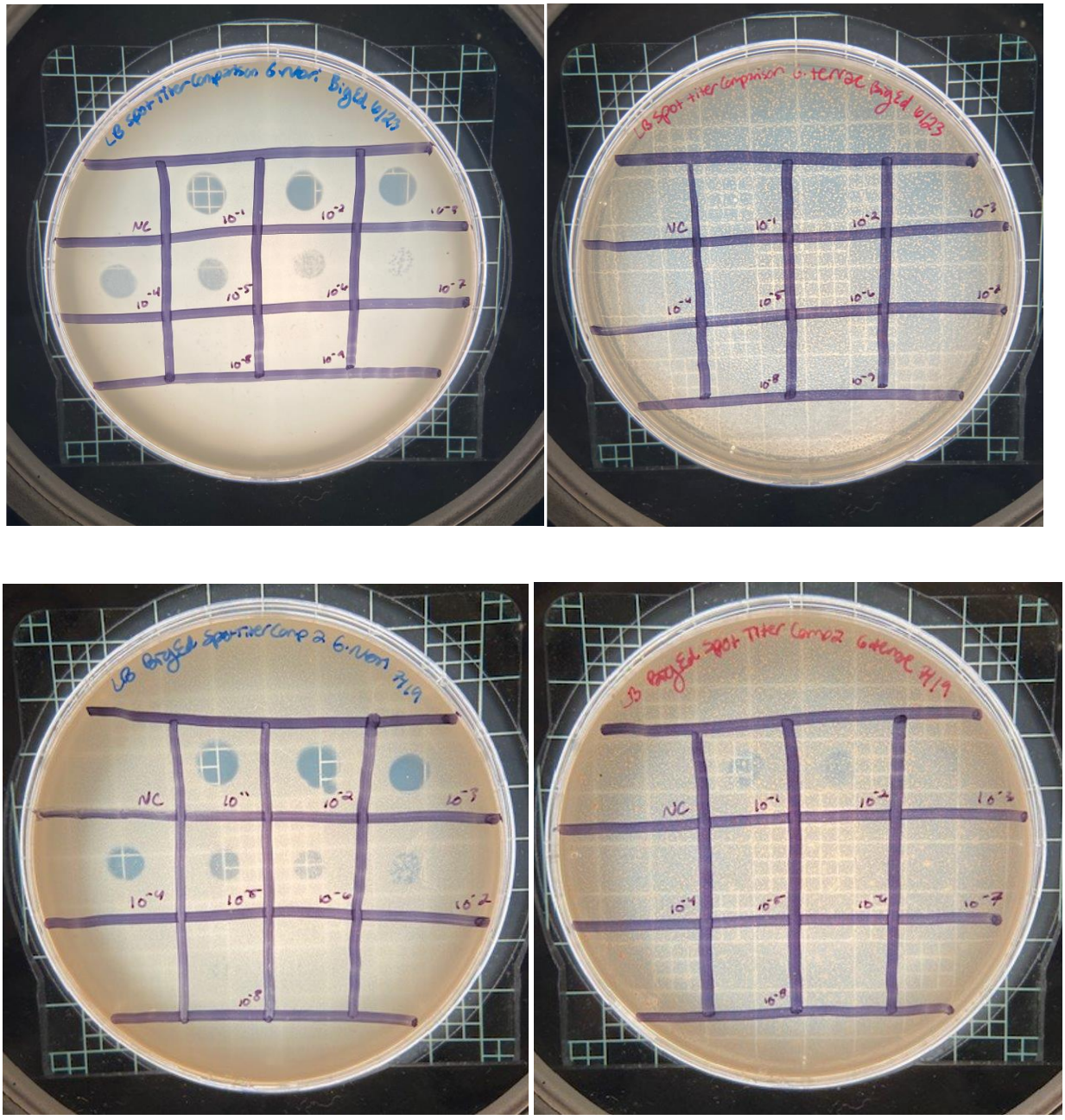
APPENDIX

Figure A1

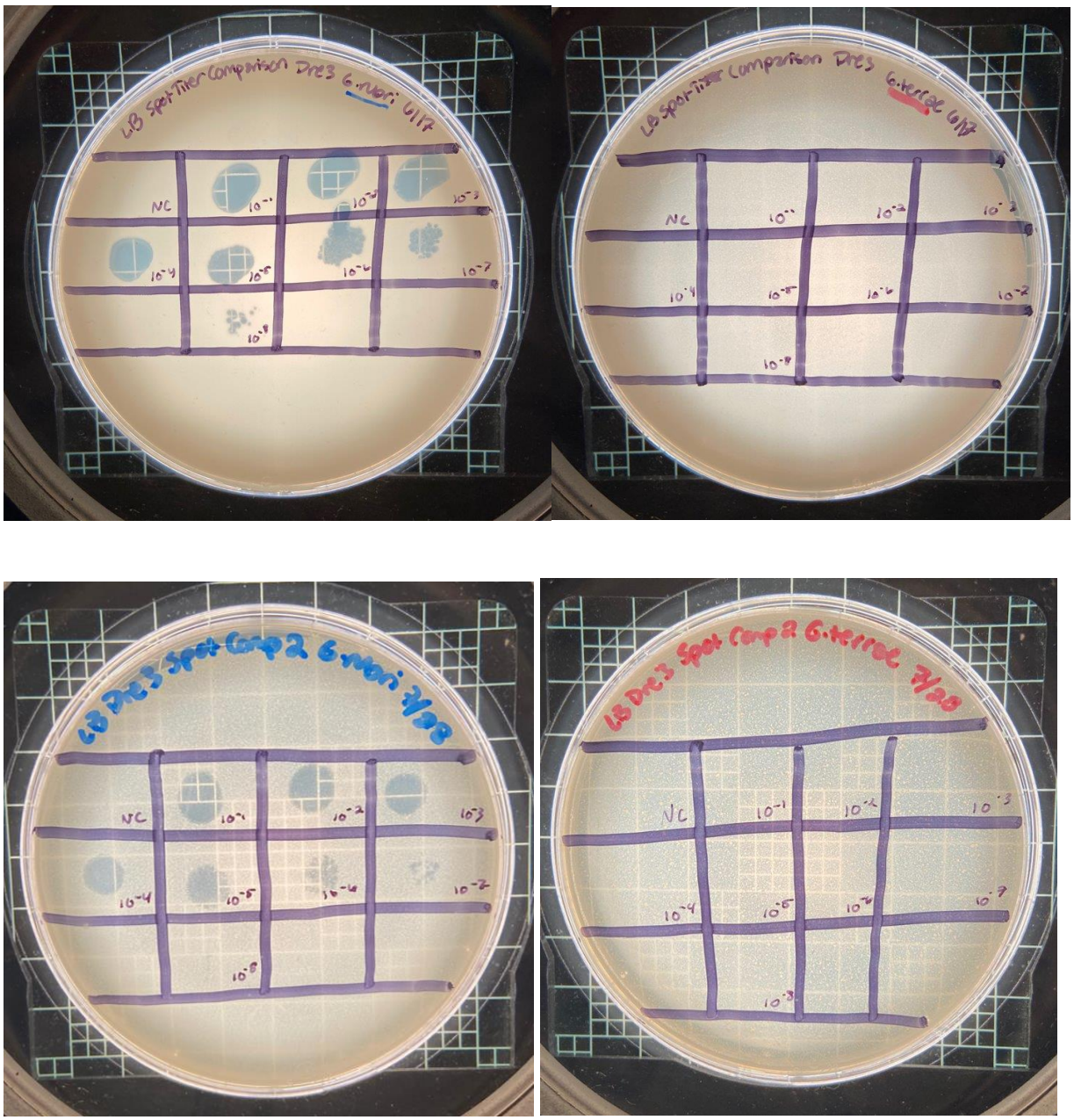


Appendix Figure A1. Full Phamerator Map comparing tail proteins of (from top to bottom) VanLee, ObLaDi, Chikenjars, and Nithya.

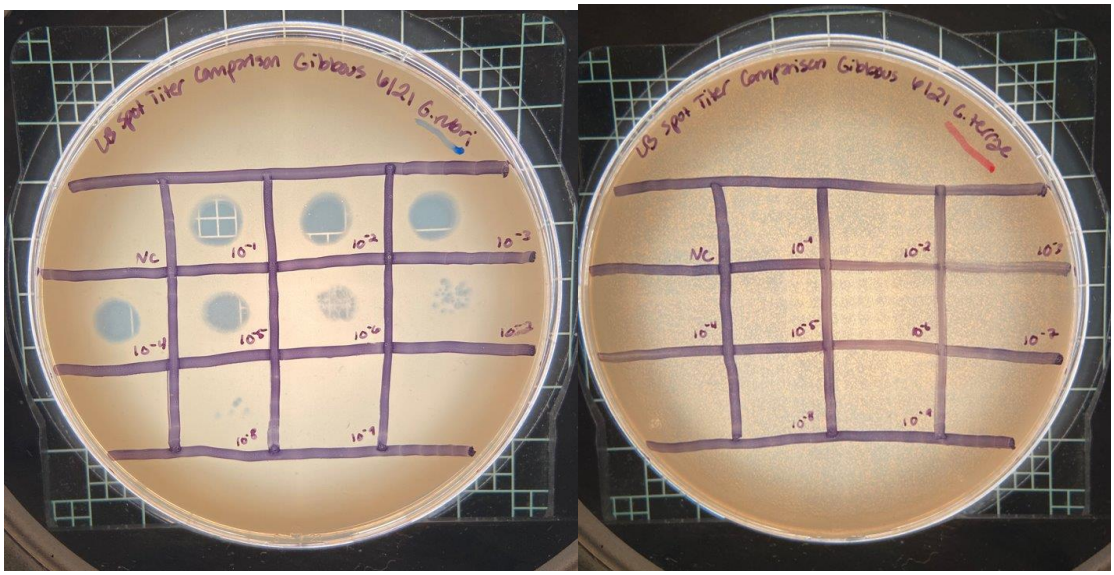
Trials of Type A Infection (See Table II and Figure 1)



Appendix Figure A2. BigEd. A spot titer of ten-fold serial dilutions of BigEd phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.

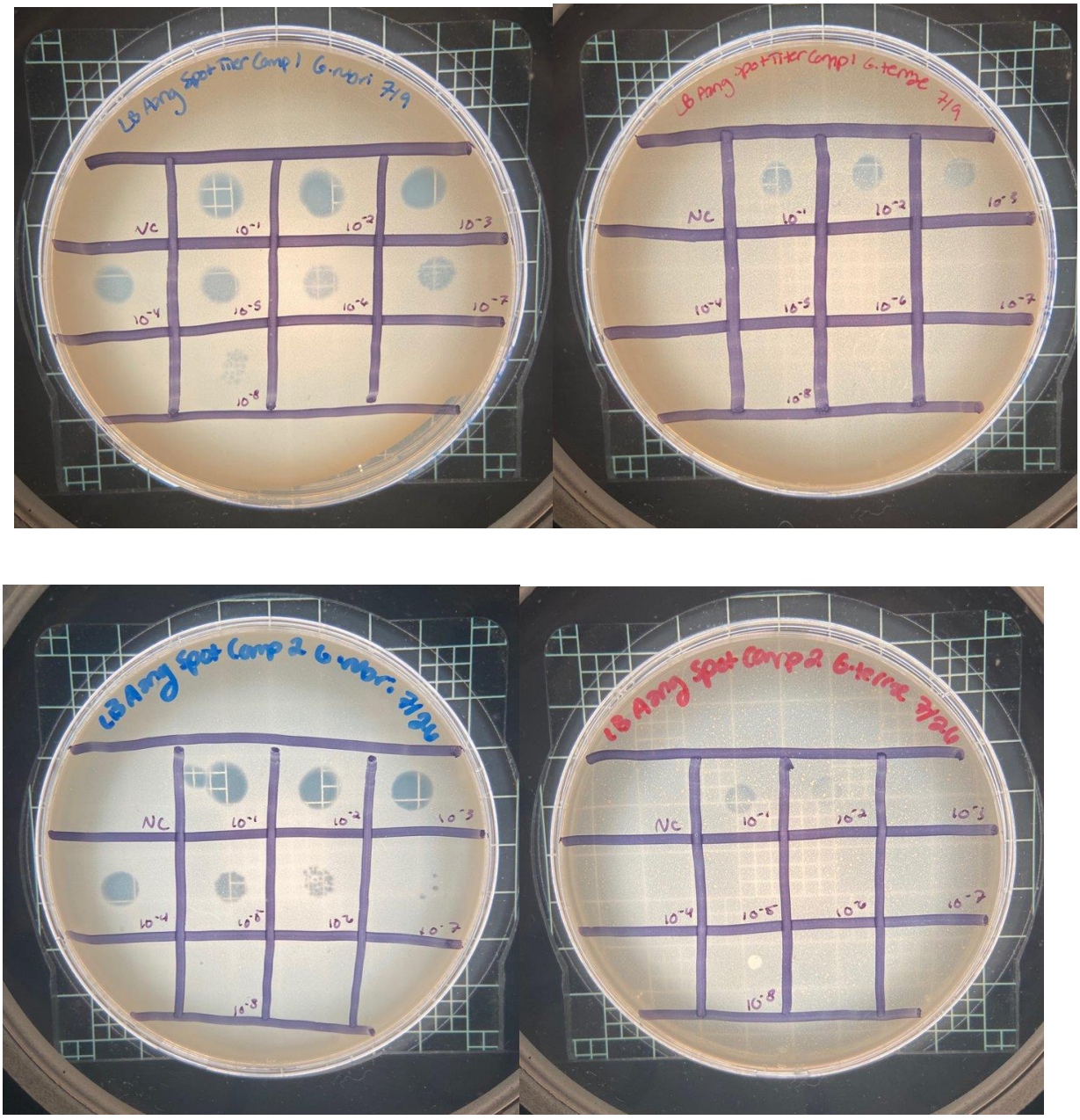


Appendix Figure A3. Dre3. A spot titer of ten-fold serial dilutions of Dre3 phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.

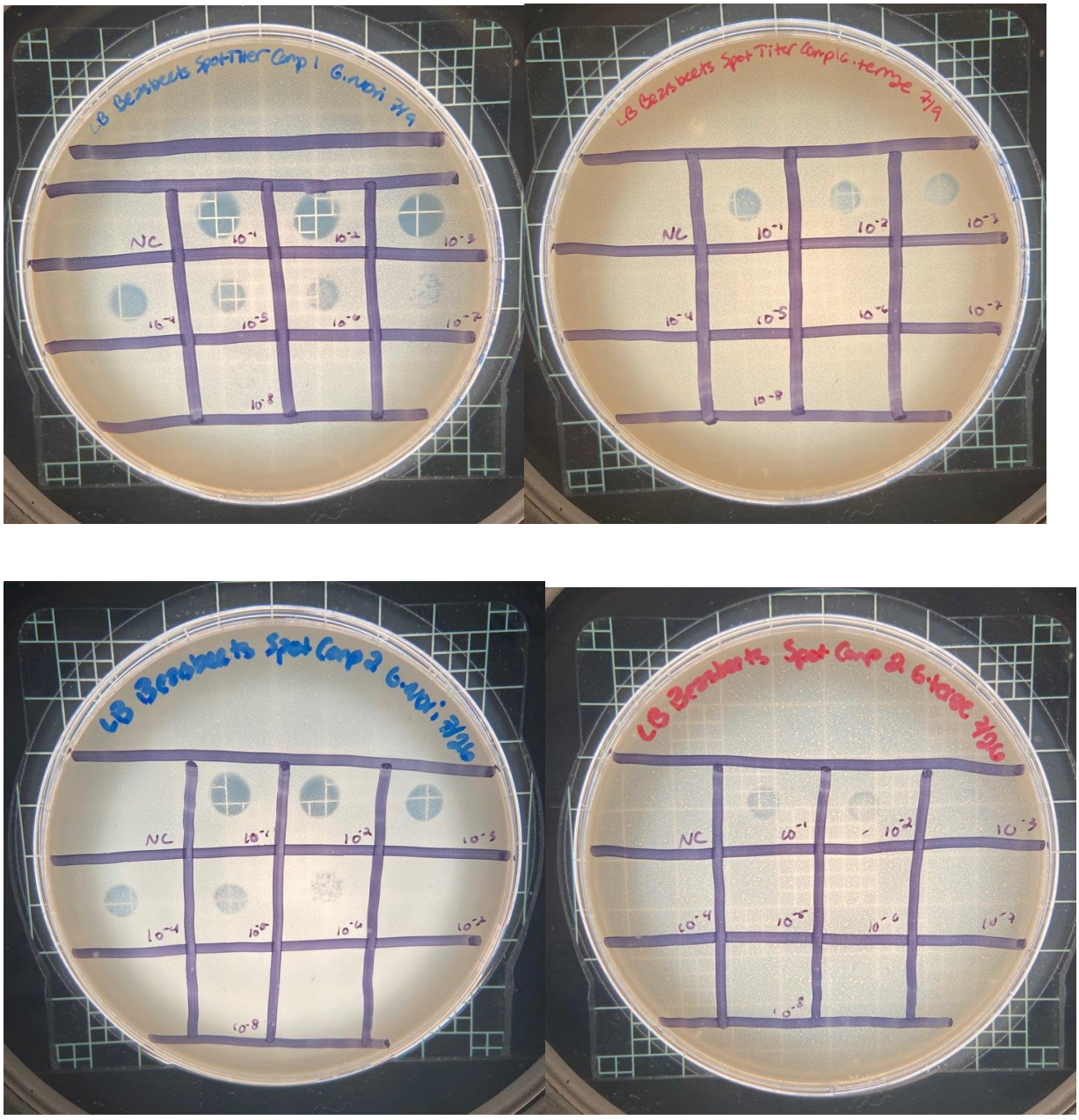


Appendix Figure A4. Gibbous. A spot titer of ten-fold serial dilutions of Gibbous phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.

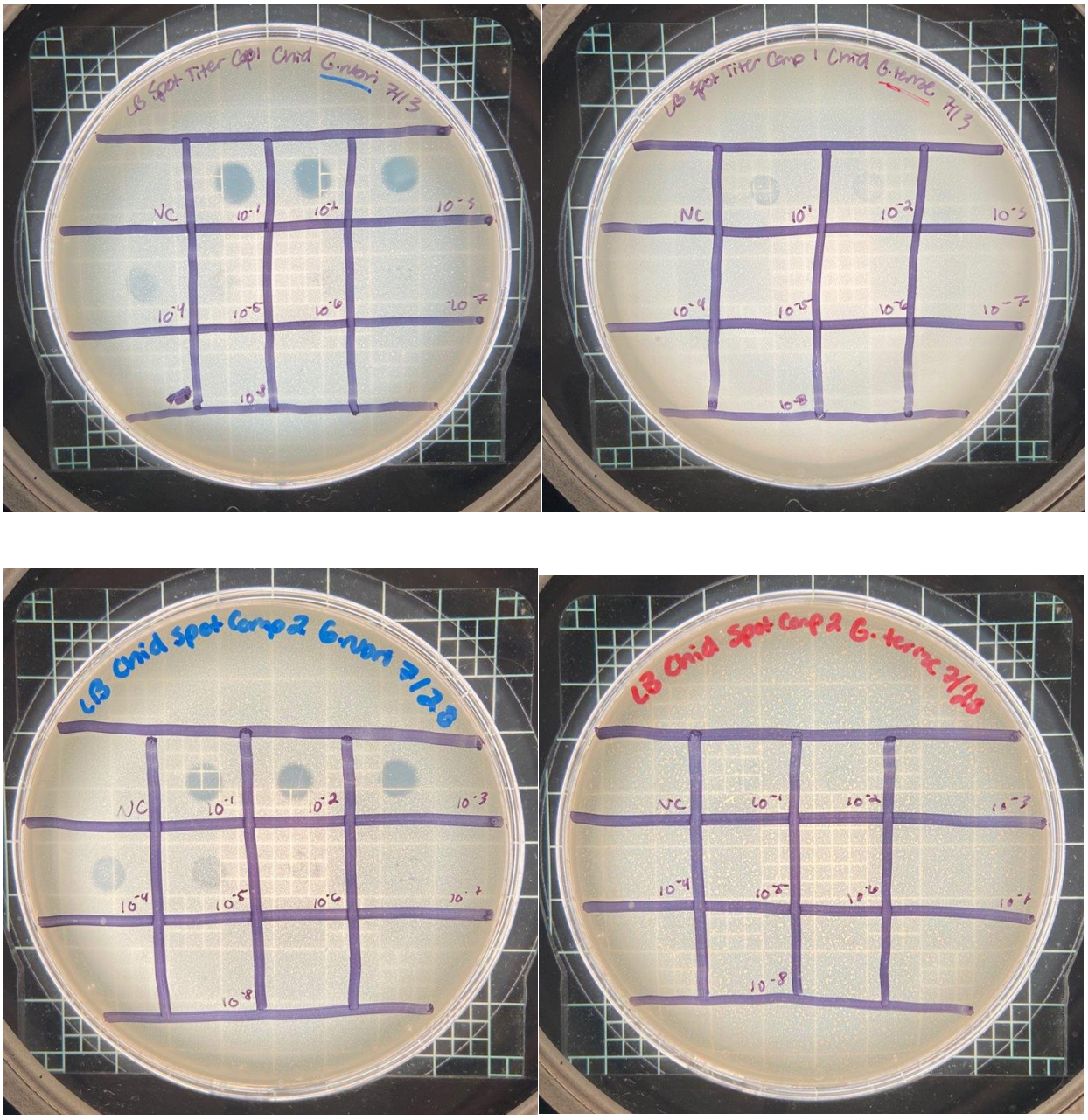
Trials of Type B Infection (See Table III and Figure 2)



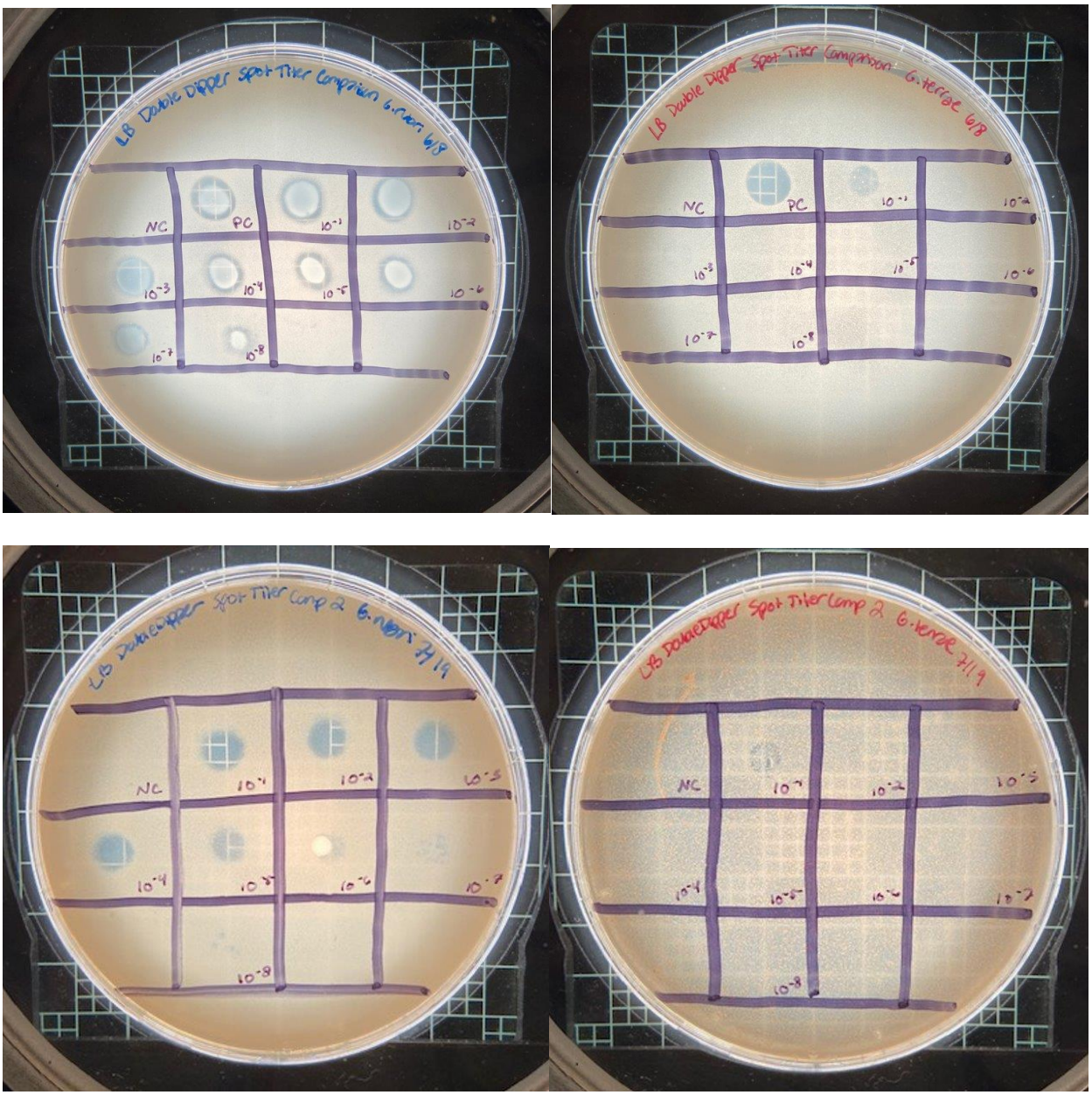
Appendix Figure A5. Aang. A spot titer of ten-fold serial dilutions of Aang phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



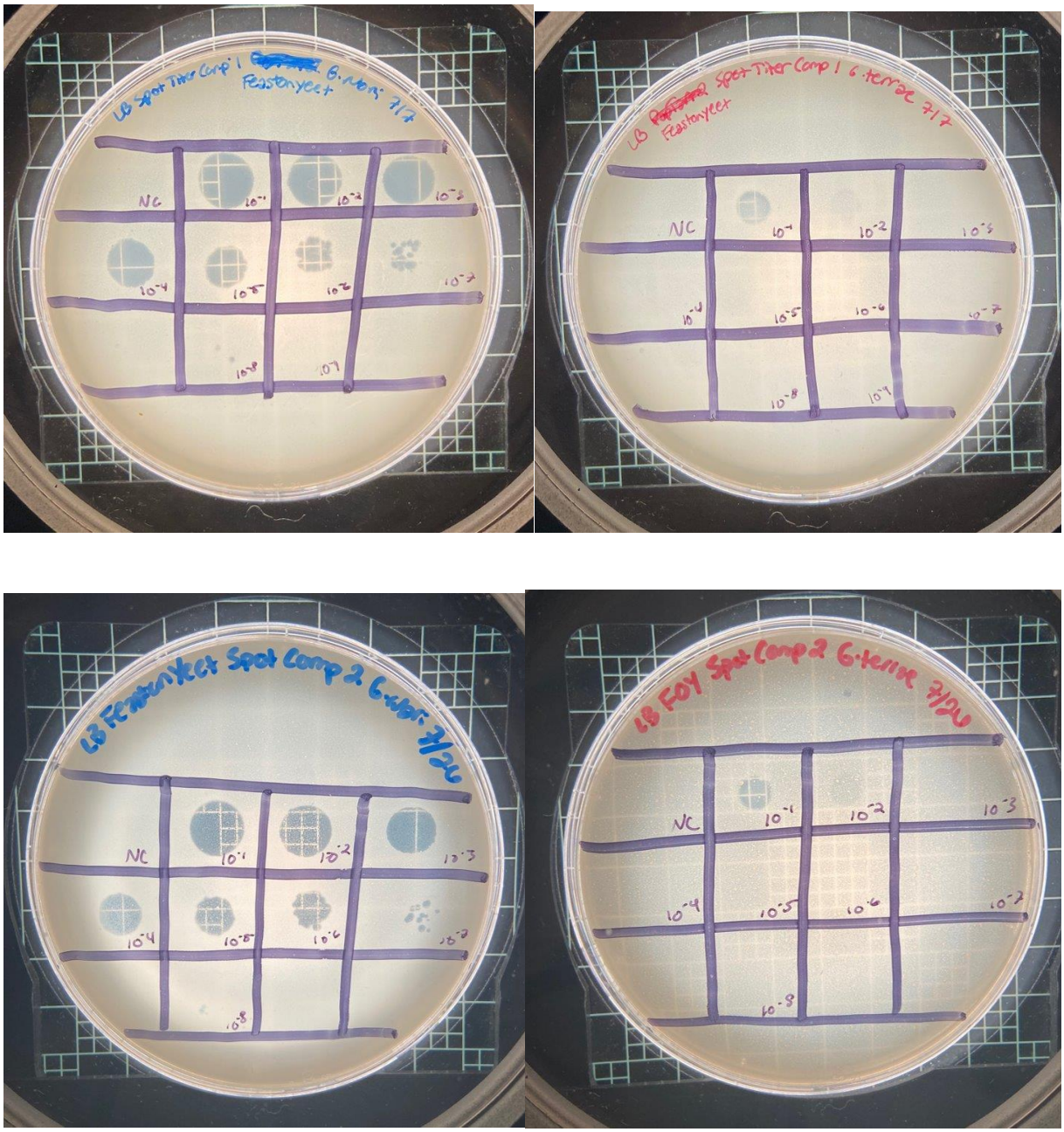
Appendix Figure A6. BearsBeets. A spot titer of ten-fold serial dilutions of BearsBeets phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



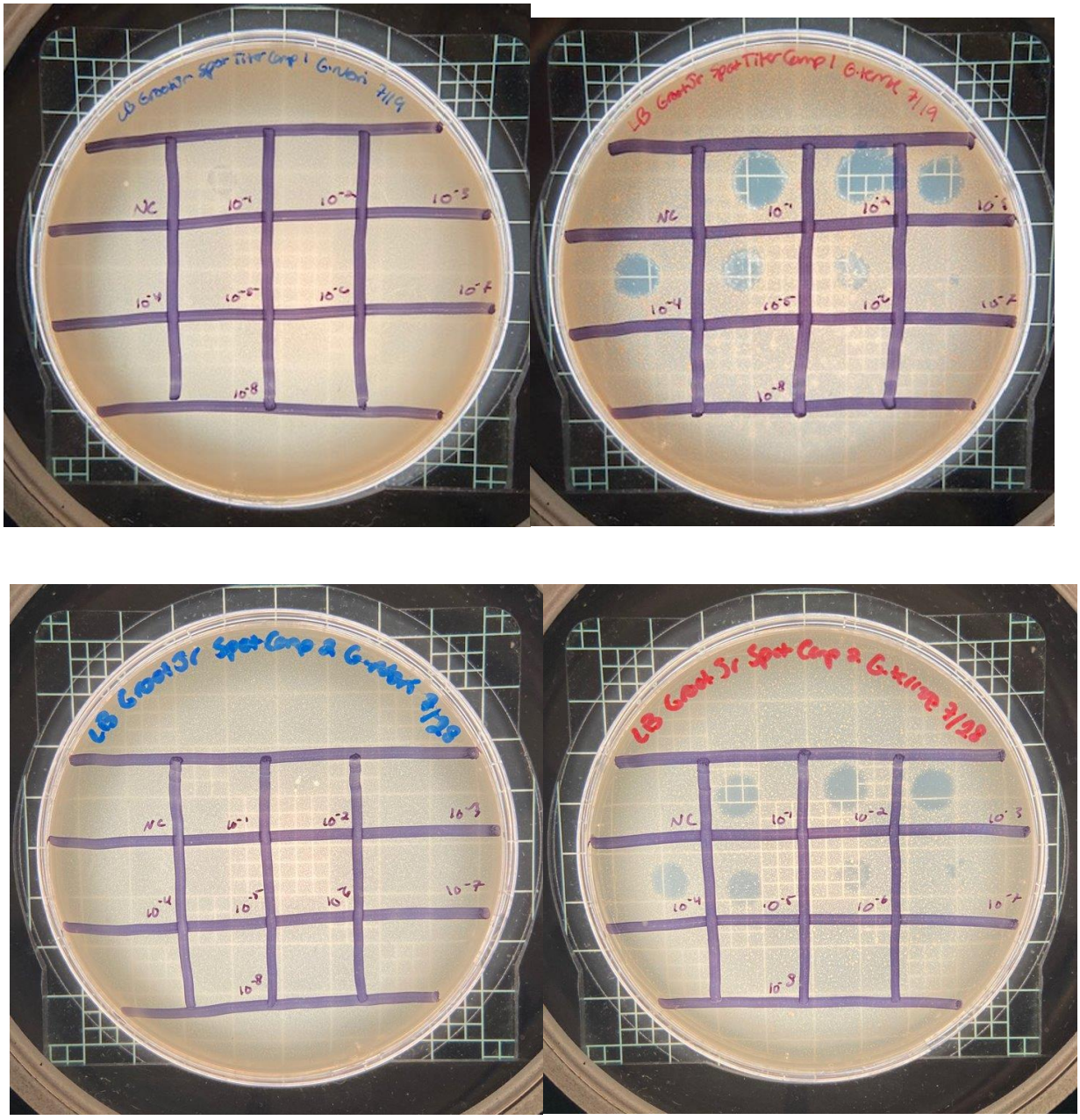
Appendix Figure A7. Chidiebere. A spot titer of ten-fold serial dilutions of Chidiebere phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



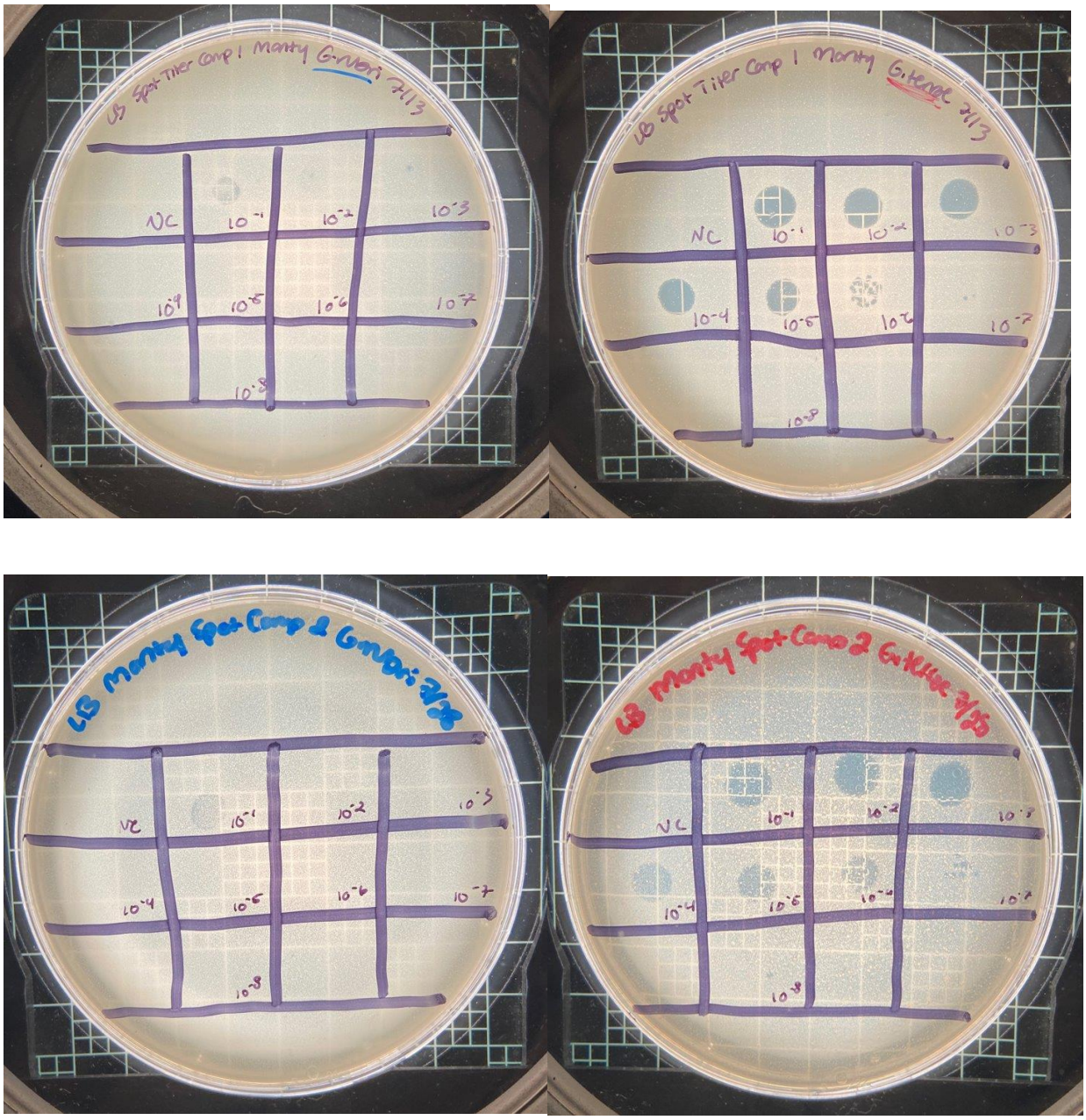
Appendix Figure A8. DoubleDipper. A spot titer of ten-fold serial dilutions of DoubleDipper phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C. The top set of plates experienced a “fogginess” contamination that was possibly caused by old bacterial culture.



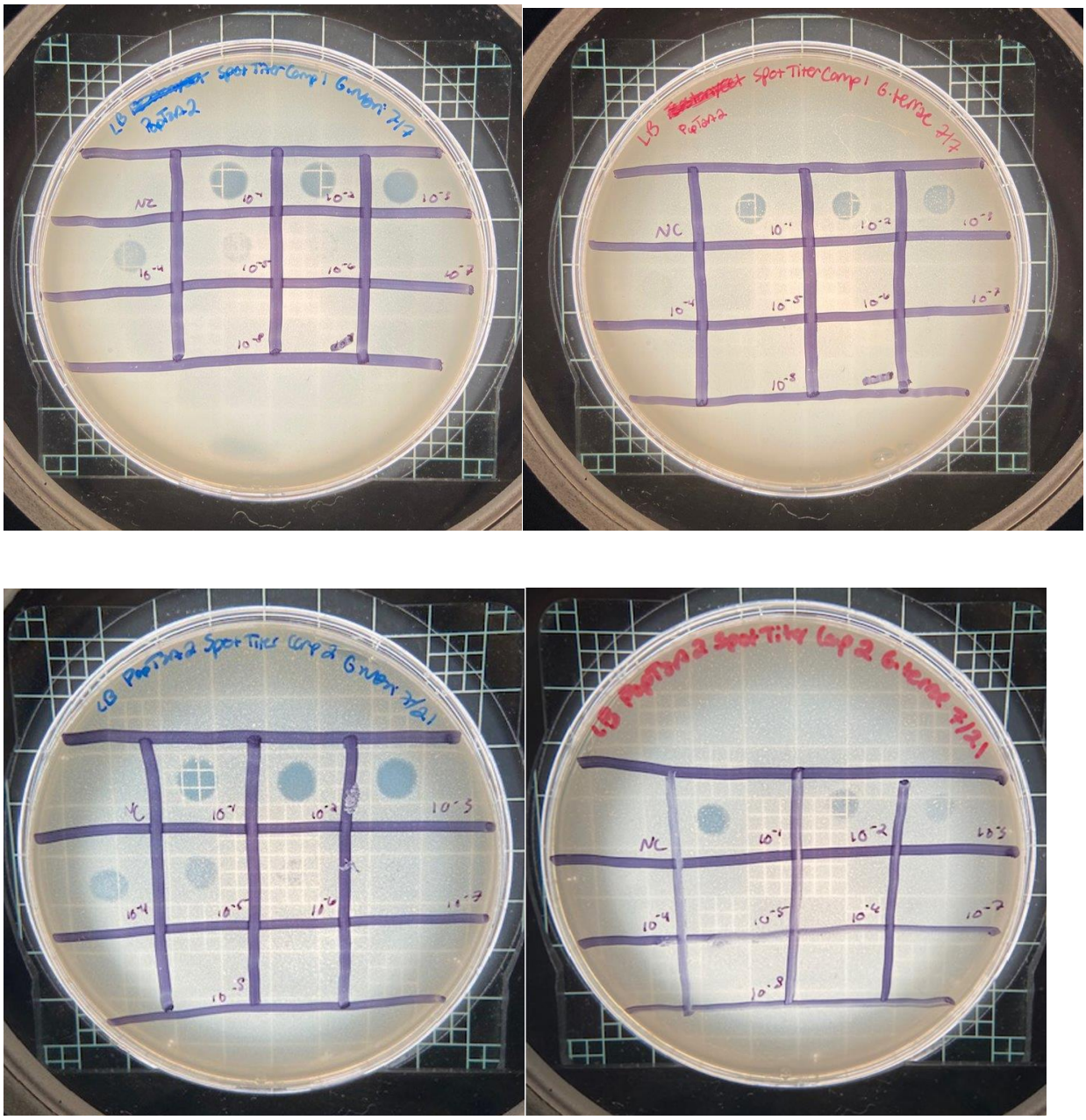
Appendix Figure A9. Feastonyeet. A spot titer of ten-fold serial dilutions of Feastonyeet phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



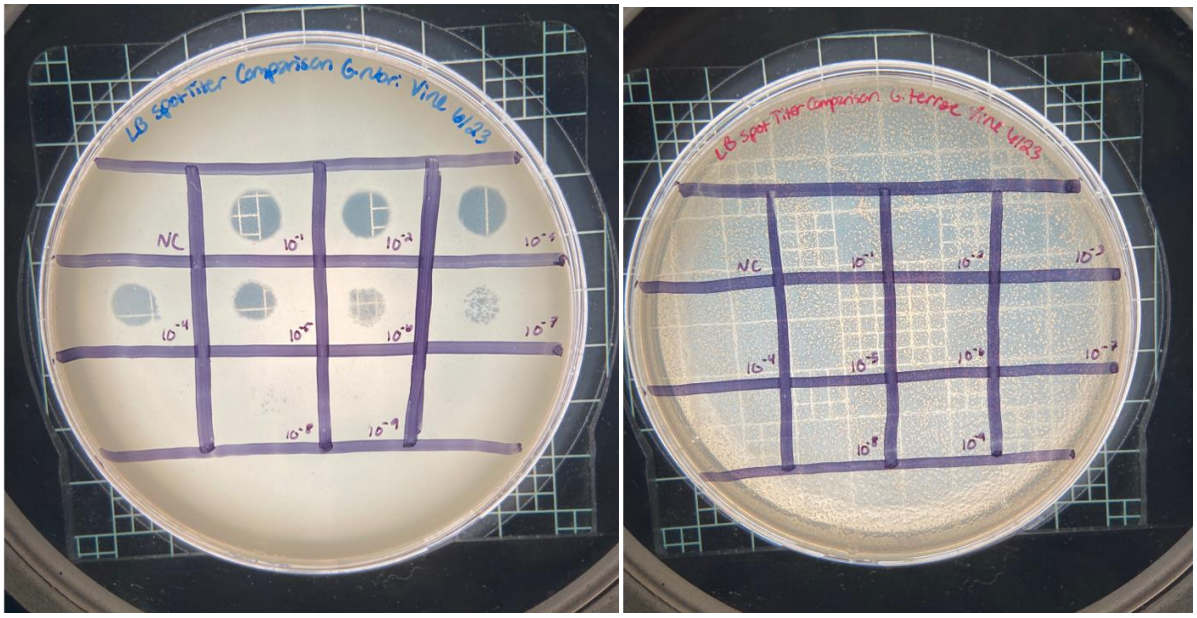
Appendix Figure A10. GrootJr. A spot titer of ten-fold serial dilutions of GrootJr phage lysate on test host *G. rubripertincta* (left) and isolation host *G. terrae* (right) after 48 hours of incubation at 30˚C.



Appendix Figure A11. Monty. A spot titer of ten-fold serial dilutions of Monty phage lysate on test host *G. rubripertincta* (left) and isolation host *G. terrae* (right) after 48 hours of incubation at 30˚C.

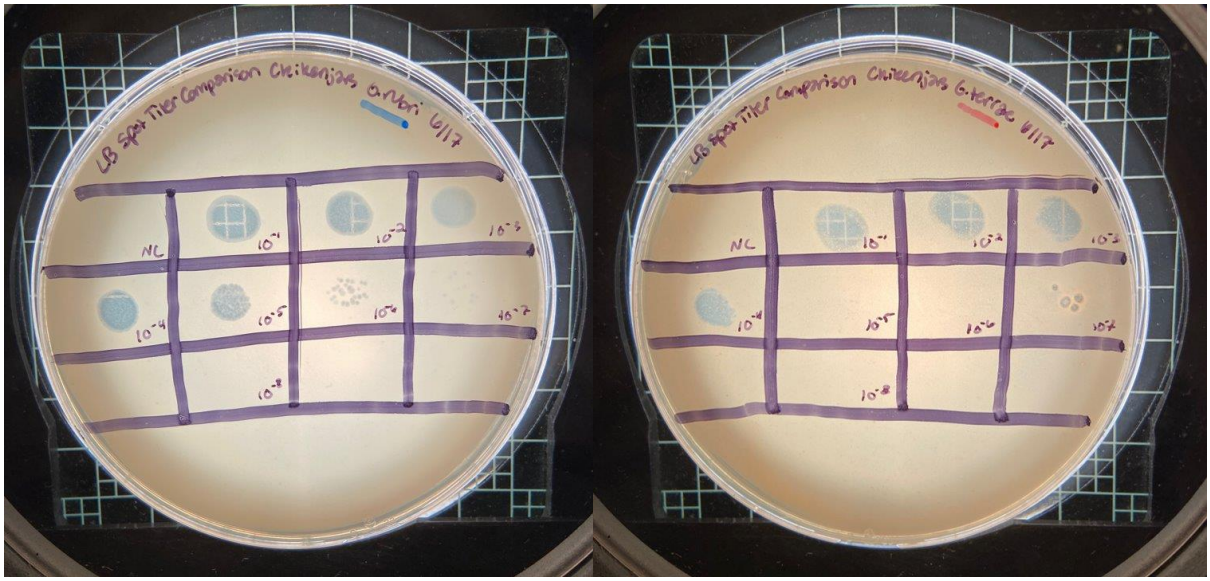


Appendix Figure A12. Poptart2. A spot titer of ten-fold serial dilutions of Poptart2 phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.

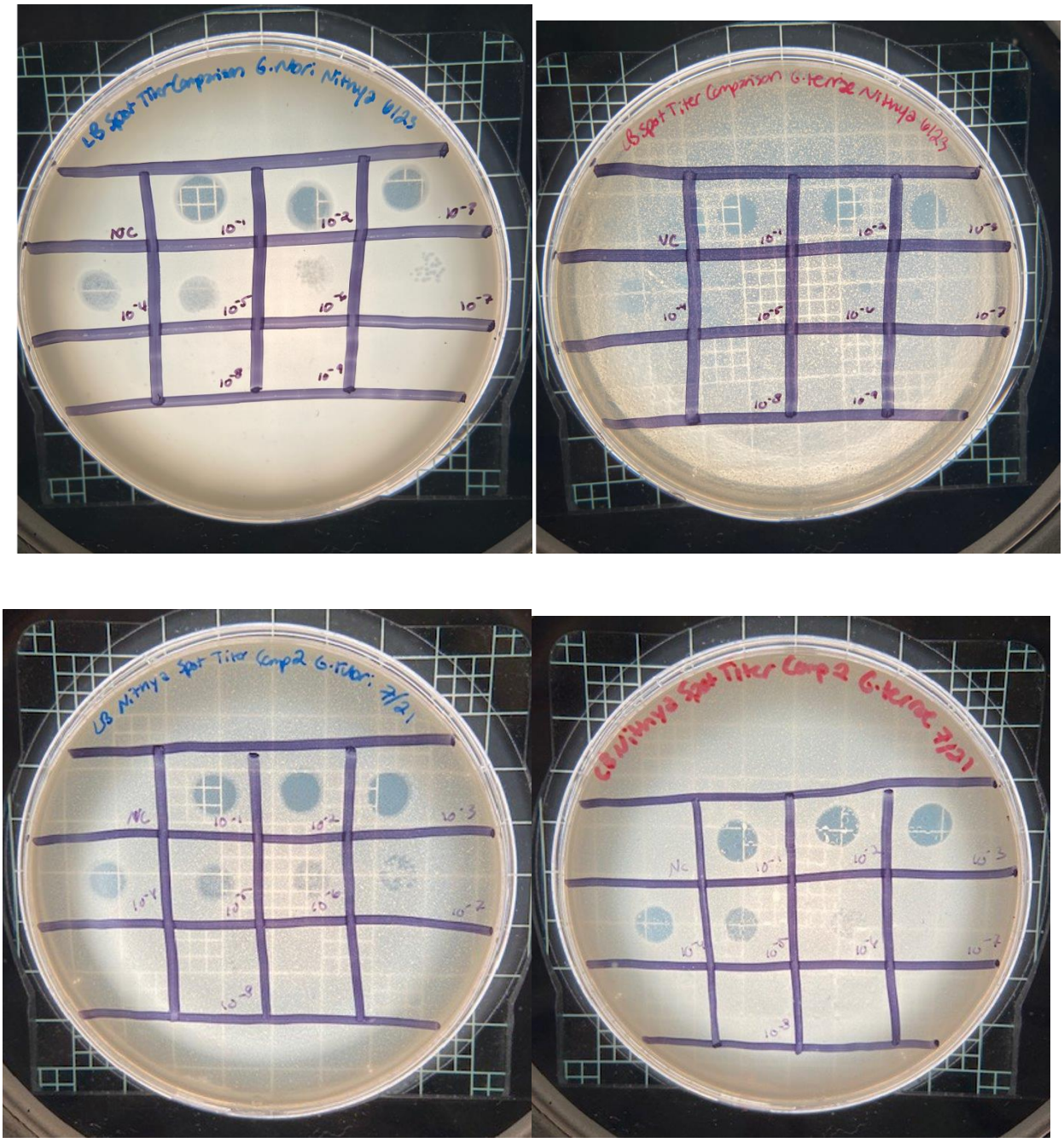


Appendix Figure A13. Vine. A spot titer of ten-fold serial dilutions of Vine phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.

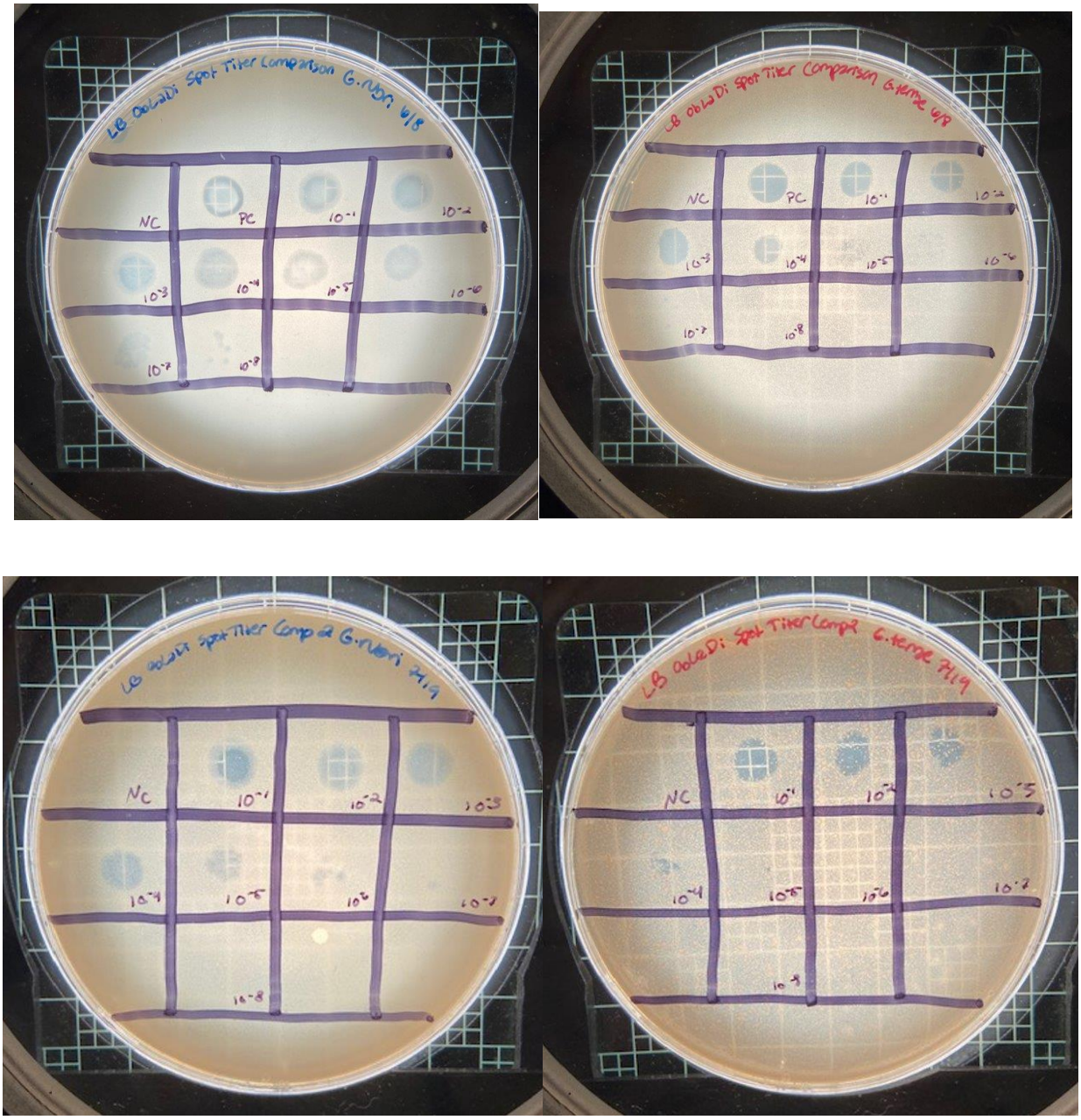
Trials of Type C Infection (see Table IV and Figure 3)



Appendix Figure A14. Chikenjars. A spot titer of ten-fold serial dilutions of Chikenjars phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



Appendix Figure A15. Nithya. A spot titer of ten-fold serial dilutions of Nithya phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C.



Appendix Figure A16. ObLaDi. A spot titer of ten-fold serial dilutions of ObLaDi phage lysate on isolation host *G. rubripertincta* (left) and test host *G. terrae* (right) after 48 hours of incubation at 30˚C. The top set of plates experienced a “fogginess” contamination that was possibly caused by old bacterial culture.

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