Biol 207x Phage Discovery - Kevin Ayala Pineda
Ayala Pineda Notebook

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Kevin Ayala Pineda

on

Dec 31, 2017 @06:40 PM PST

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Protocol 5.1: collecting Environmental Sample

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:37 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Nov 14, 2017 @11:25 AM PST

Environmental sample Information:

09/04/2017

Aims and purpose:

For this protocol students had to collect an environmental sample. In this process they will strive to find a bacteriaphage that can infect the host bacteria (M. smegmatis $mc^2 - 155$) they are working with. Samples can vary from soil, compost, or water. It is advised to learn about the host bacteria and seek out where it grows. Wherever your host bacteria lives there will be bacteriaphages. In this case we are hunting for M. smegmatis $mc^2 - 155$.

Protocol Procedures:

- Arrived at location with 3 sandwich bags, 3 spoons, a ruler, a notebook, a pen, and a backpack.
- Went hunting for an area with shade, water, and plants.
- Once location was found, the spoon was used to dig into the soil.
- Ruler was used to indicate depth. 9.3 cm
- The next step was to collect the sample with the sandwich bag.
- The sandwich bag was turned inside out and right hand was inserted so it can act like a glove.
- After this a handful of soil was collected and the plastic bag was inverted.
- Bag was then sealed.
- Data was collected as follows.

Data and Results:

Environmental Sample Location Data

	Description	Notes
Date	09/04/2017 at 5:58 pm	
Location	Wetlands park	The wetlands park is located on the east side of Las Vegas. This is a nature preserved area. Permission for soil sampling was granted by park officials. (702) 455-7522
Street address	7050 Wetlands Park Ln, Las Vegas, NV 89122, USA	
GPS Coordinates	Latitude: 36.1039607 Longitude: -155.02022340000002	Coordinates were obtained through android phone.
Temperature	104 degrees Fahrenheit / 40 degrees Celsius	
Precipitation	0%	
Humidity	17%	
Wind	10 kilometers per hour (km/h)	
Soil	• Near small pond, several bushes, and several plants • Soil was damp • Found under shade • Dug 9.3 cm into soil	Soil sample was collected in a sandwich bag.

Conclusion:

After studying about our host bacteria, M. smegmatis $mc^2 - 155$, it was concluded that it enjoys living near water and plants. The wetlands park seems like a perfect home for M. smegmatis $mc^2 - 155$. Wherever bacteria is there will be phages with it. The goal is to extract a phage. This experiment is the 1st step in our bacteria isolation section. Now that we have a bag filled with soil, enriched in bacteria, we are now ready to proceed to the next step. We will extract possible phages from our soil sample by either the enriched isolation experiment or the direct isolation experiment.

1 revisions print ▪ Kevin Ayala Pineda ▪ Sep 12, 2017 @09:22 PM PDT

20170904_182327_1_.jpg(3.7 MB) - download This picture shows the overall area of my environmental sample collection. Pond and plants near the hole might be important for the bacteria growing in this area.

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20170904_175543_1_.jpg(2.9 MB) - download This is a picture of the area where experiment was conducted. These plants, in the picture, might be a valuable asset for the bacteria that was growing inside the hole.

20170904_175556_1_.jpg(2.8 MB) - download This is a close up picture of where my soil sample was collected.

Protocol 5.2: Direct Isolation

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:37 PM PST

2 revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @05:16 PM PDT

Direct Isolation

09/05/2017

Aims and Purpose:

This protocol is aimed to extract phages from the environmental soil. We will separate phages from unnecessary and dangerous matter in soil sample. Once this is completed we will have a small sample of bacteriaphages that may infect the host bacteria (M. smegmatis $mc^2 - 155$).

Changes in Protocol Procedures:

Procedures for extracting phage from the soil sample was changed. **Originally** the class was suppose to shake the conical tubes, which held soil and enrichment broth, for **2 hours**. Due to the time restriction, the amount of time shaking the tubes was **changed to 90 minutes at 250 amp.**

Centrifugation was changed. **Originally** the tubes were suppose to be centrifuged **at 2,000 g for 10 minutes**, but due the environment in Las Vegas, Nevada the samples were centrifuged at **4,000 x g for 15 minutes** and stored at 5 degrees Celsius.

Data and Results:

This Protocol was done by lab partner. These are the observations.

Data for Direct isolation Experiment

Main procedure	Steps involved	Results
Preparing soil sample for phage extraction	15 ml conical tube was filled with 1/3 of soil. 2-3 ml of enrichment broth was then added to the conical tube.	A 15 ml conical tube with soil and enrichment broth was present after this procedure.
Centrifugation	The 15 ml Conical tube was put in a shaking incubator at 250 rpm for 90 minutes. The conical tube with mixture was then centrifuged at 4,000 x g for 15 min.	A 15 ml conical tube with phage and bacteria liquid at the top (supernatant), and unnecessary soil matter at the bottom.
Filtration	2 ml of the Supernatant was sucked with a pipettor, and then was dispensed into a 15 ml sterile syringe. Solid material was avoided, because it can contain dangerous bacteria that could contaminate the sample. The syringe contained a 0.22 um filtrate. 0.7 ml of Liquid was then dispensed into a clean sterile microcentirfuge tube.	A microcentrifuge tube filled with 0.7 ml of clear, light yellow liquid from partners environmental sample was present at the end of this protocol. We were then allowed to proceed to protocol 5.3 in the next class lecture.

Conclusions:

My partner successfully extracted any possible phages from his soil. With his microcentrifuge tube he will have to undergo the plaque assay protocol which will be conducted next class. This next experiment will determine if phages are present in the filtered environmental sample.

Extra Notes:

This type of isolation preserves the diversity of the phages in the environmental samples. However, it is much harder to successfully complete this type of experiment due to not having enough phage particles.

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Direct_Isolation_3_1_.jpg(178.3 KB) - download This is a picture of my partners soil inside the 15 ml conical tube.

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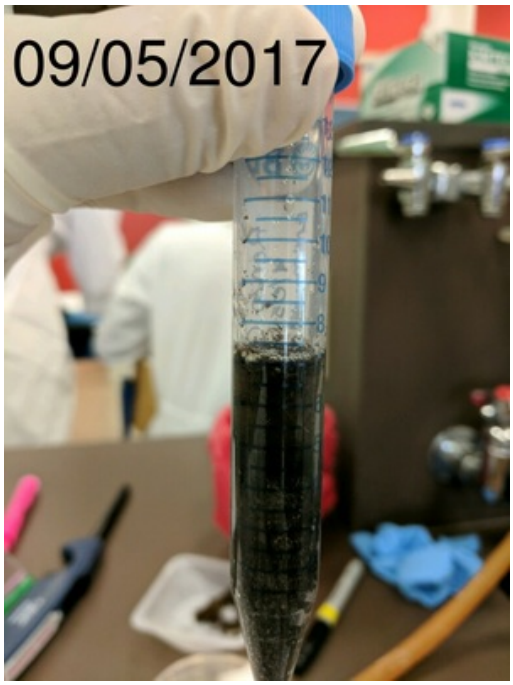

Direct_isolation_1_.jpg(165 KB) - download This is a picture of his soil with 2-3 ml of enrichment broth. This still contains the soil.

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Direct_Isolation_2_1_.jpg(163.4 KB) - download This is a picture after it shook for 90 minutes and centrifuged at 4,000 x g for 15 minutes. This tube has soil and unwanted waste in the bottom and phage liquid at the top.

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Direct_Isolation_1_.jpg(787.1 KB) - download This is the final result for lab partners direct isolation protocol. This microcentrifuge tube contains only the bacteriaphages from my partners soil sample.

Protocol 5.3: Plaque Assay

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:37 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @08:49 PM PDT

Plaque Assay for Direct Isolation

Date: 09/09/2017

Aims and Purpose:

This protocol confirms presence of phages on bacterial lawns. This experiment proceeds several protocols such as 5.2, 5.5, 5.6, 6.1, 6.4, 6.5, and 7.1. The goal is to obtain a plate present with plaques, purify, or make webbed plates.

Change in Protocol Procedures:

500 ul of host bacteria is used instead of 250 ul

4.5 ml of top agar is used per sample. Not 3 ml.

Data and Results

Main Procedure	Important Notes	Results
Gathered plates needed for protocol	A positive control plate was not made. Only a negative control plate	2 plates
Mix phage sample with host bacteria.	With a micropipettor 500 ul of the direct isolation sample was dispensed into the host bacterial tube. The tube was gently mixed and sat for 10 minutes undisturbed.	Tube with host bacteria and direct isolation sample

This experiment was conducted twice due to putting both the negative and positive top agar bacteria mixture in the same plate.

Conclusions:

These plates were the first attempt at isolating a phage. Unfortunately, no plaques were present in the following lab. From here the enrichment sample will be tested to see if any plaques form.

revisions print ▪ Kevin Ayala Pineda ▪ Oct 14, 2017 @02:34 PM PDT

20170912_092116_1_.jpg(1.4 MB) - download This is a picture of the Direct Isolation sample plate. No plaques were present. The observation was conducted on 09/12/2017

revisions print ▪ Kevin Ayala Pineda ▪ Oct 14, 2017 @02:35 PM PDT

20170912_092403_1_.jpg(1.4 MB) - download This is a picture of the negative control plate. No plaques were present as expected. The observation was conducted on 09/12/2017

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Protocol 5.4: Picking a Plaque

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:37 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @05:25 PM PDT

Picking a plaque

09/14/2017

Aims and purpose:

This protocol is aimed to retrieve phages from a plaque present in one of the plated bacterial lawns. Choosing a well isolated plaque will give us the best results. By picking a plaque we will be retrieving phage particles and then resuspending them in phage buffer. This liquid will be needed for follow up experiments to confirm that a phage or phages are present.

Changes in protocol will

This protocol was performed 3 times due to unsuccessful dilutions. .

Data and results

Main Procedure	Important Notes	Results
Label a plaque plaque	The plaque that was chosen was well isolated. There were only 3 plaques near it.	Marked location of desired plaque
Prepare microcentrifuge tube	Obtained 1 microcentrifuge tube Labeled microcentrifuge tube as 10^0 Added 100 ul of phage buffer	1 microcentrifuge tube with 100 ul of phage buffer.
"Pick" the plaque	A thin sterile micropipette tip was used to gently stab the location of marked plaque. The end of the micropipette tip was placed into the microcentrifuge tube with the phage buffer. The tip was tapped on the wall. The sample was also pipetted up and down.	1 microcentrifuge tube with 100 ul of phage buffer and phage particles.

Conclusion

After this test, dilutions will be made to further confirm that a bacteriophage was successfully picked. Both protocol 5.6 (spot test) and protocol 6.2 (serial dilutions) will be the next step to confirm the presents of phage. If no plaques show up in plates then this protocol will be repeated.

Protocol 5.5: Enriched Isolation

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

2 revisions print

▪ Kevin Ayala Pineda ▪ Oct 21, 2017 @02:05 PM PDT

Enriched Isolation Experiment

09/05/2017, 09/07/2017, 09/12/2017

Aims and Purpose

This experiment is aimed to amplify phages from our environmental samples. We will be using enrichment broth to allow phages and bacteria to replicate. This will give us a high quantity amount of phages.We will then remove any dangerous matter from our sample through filtration. Once our samples are filtered we will take a small sample of our host bacteria and put it in our filtered sample. This will allow the phage to undergo infection with either a lysis or lysogenic life cycle.

Changes in Protocol Procedures:

Procedures for extracting phage from the soil sample was changed. **Originally** the class was suppose to shake the conical tubes, which held soil and enrichment broth, for **2 hours**. Due to the time restriction, the amount of time shaking the tubes was **changed to 90 minutes**.

Centrifugation was changed. **Originally** the tubes were suppose to be centrifuged **at 2,000 g for 10 minutes**, but due the environment in Las Vegas, Nevada the samples were centrifuged at **4,000 x g for 15 minutes** and stored at 5 degrees Celsius.

Procedure for **filtration was changed**. Filtration was **attempted through a 0.22 um filter** but it repeatedly **got clogged**. To solve this problem the class **filtered** their enriched sample **through** a **0.45 um filter first then a 0.22 filter.**

Data and Results

Part 1

Date: 09/05/2017

Data for Part 1 of Enriched Isolation Protocol

Main procedure	Important Notes	Results
Preparing soil sample for phage extraction.	50 ml sterile conical was filled with 15 ml of soil. 20 ml of enrichment broth was then added to the conical tube.	A 35 ml mixture conical tube with soil and enrichment broth.
Centrifugation	The 50 ml conical tube was put in a shaking incubator at 250 rpm for 90 minutes. The conical tube with mixture was then centrifuged at 4,000 x g for 15 min.	A 35 ml conical tube with phage and bacteria liquid at the top (supernatant), and unnecessary soil matter at the bottom.
Filtration	Supernatant was sucked up with a 15 ml sterile syringe. Filtration was conducted through two filters. • A 0.45 um filter was used first. Liquid was dispensed into a new sterile 50 ml conical tube. • A 0.22 um filter was then used. Liquid was dispensed into a new sterile 50 ml conical tube.	After filtration was conducted, a 50 ml conical tube should have 20-25 ml of bacteriaphage sample. **However, we were only able to obtain 17.5 ml after filtration.**
Adding host bacteria (M. smegmatis $mc^2 - 155$)	The last step of the procedure was to add 0.5 ml of bacterial host culture into conical tube. **Experiment ended here due to class lecture concluding.**	NONE

Date: 09/07/2017

Data for Part 1 of Enriched Isolation Protocol

Main Procedure	Important Notes	Results
Adding host becteria (M. smegmatis $mc^2 - 155$)	The last step involved adding 0.5 ml of bacterial host culture into 50 ml conical tube containing filtered phage sample.	The final physical result of part 1 of the enriched isolation protocol is a 50 ml conical tube with bacteriaphages from environmental sample and host bacteria (M. smegamatis $mc^2 - 155$).
Incubation	Instructors incubated conical tube at the correct temperature and shook at 220 rpm for 2 days.	After the conical tube was incubated, enriched isolation protocol continued to part 2.

Part 2

Date: 09/12/2017

Data for Part 2 of Enriched Isolation Protocol

Main Procedure	Important Notes	Results
Transfer enriched culture into microcentrifuge tube.	1.4 ml of the enriched culture was transfered into a microcentrifuge tube. Process was done twice.	The results were two microcentrifuge tube each with 1.4 ml of enriched culture.
Centrifugation	Tubes were centrifuged at high speed for one minute.	Two microcentriuge tubes each with phage supernatant liquid at the top and bacteria at the bottom.
Filtration	With a pipette, 1 ml of the supernatant was transfered into sterile syringe with a 0.22 um filter. Liquid was dispensed into a new sterile microcentrifuge tube. **This process had to be conduced twice due to spilling the phage liquid from the syringe.**	After filtration was concluded, two microcentriuge tubes filled with enriched bacteriphages were present. This now allowed us to proceed to protocol 5.6 (Spot Test).

Conclusion:

After this experiment two microcentrifuge tubes filled with bacteriophages were present. Throughout the experiment the phages in the environmental sample were amplified. This leaves a higher chance of success for a phage to infect the host bacteria M. smegmatis $mc^2 - 155$. The next step is to proceed to the spot test to determine if any phages are present.

Extra Notes:

The enriched isolation experiment has a higher success rate compared to direct isolation. This is because we are culturing a large concentrated amount of phages specific to the bacterial host. However, this experiment does yield a biased sample due to growing phages not particulate to your sample.

 ▪ Kevin Ayala Pineda ▪ Sep 12, 2017 @09:41 PM PDT

20170907_102838_1_.jpg(1.4 MB) - download This is a picture of the 50 ml conical tube containing bacteriaphages. This was obtained after the filtration protocol was conducted.

 ▪ Kevin Ayala Pineda ▪ Sep 12, 2017 @09:40 PM PDT

20170912_101022_1_.jpg(1.3 MB) - download This picture was taken after centrifuging the two tubes containing the enriched culture of bacteriaphages.

 ▪ Kevin Ayala Pineda ▪ Sep 12, 2017 @09:40 PM PDT

Microcentrifuge_tubes_1_.jpg(155.7 KB) - download This picture contains the two filtered microcentrifuge tubes that were concluded at the end of the enriched isolation protocol. With these samples we are now ready to proceed protocol 5.6 (spot test).

Protocol 5.6: Spot Test

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @05:41 PM PDT

Spot Test from Enriched Isolation Sample

09/12/2017

Aims and purpose

This protocol checks any presents of phage in a sample. This is done by adding a small drops of different dilutions onto the agar. This protocol was done for the enriched isolation sample obtained from protocol 5.5.

Change in protocols

This protocol was done 3 times due to messing up the dilutions. .

Data, Results, and Observations

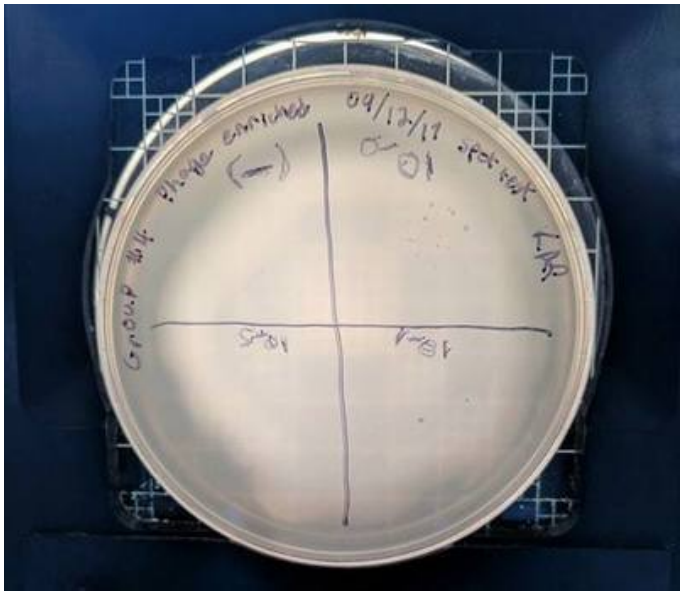
Object	Observation
Plaque assay from the direct isolation sample .	No plaques were present on the plate that were made with the direct isolation sample.

Main Procedure	Important Data	Results
Labeling plat	Plate was divided up into 4 quadrants • 10^-5 • 10^-1 • 10^-0 • negative control	Plate and other materials were set and ready
Made bacteria lawn	A 5 ml serological tube was used to transfer 4.5 ml of agar to the tubes containing 500 ul of host bacteria. The plate sat undisturbed for 25 minutes	Plate with bacteria and agar
Performed spot test	10 ul of each sample was placed onto the plate	One plate with 3 different spot dilutions and a control.

Conclusion

This spot test will confirm whether or not a phage is present in the enriched isolation sample. If plaques are present in the next lab, then the next step is to proceed to protocol 6.2. Here we will start to purify the phage present. If no plaques are present next lab then protocol 5.5 will be conducted again in hopes of finding a phage.

▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @09:57 PM PDT



received_1823860994320870_1_.jpeg(25 KB) - download This is a picture of the spot titer for the enrichment sample. This test confirmed the presence of phage in the enrichment sample.

Protocol 6.1 Plaque Assay for Purification

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @05:45 PM PDT

Plaque Assay for Purification Round 1

09/14/2017

Aims and purpose:

This protocol is aimed to create well isolated plaques. This protocol involves 3 different protocols. These include protocol 5.4 picking a plaque, protocol 6.2 serial dilutions, and protocol 5.3 plaque assay. Three rounds of purification will be conducted to make sure plaques are consistent and uniformed.

Changes in protocols

No changes to the protocol were made.

Data and results

Object	Observation
Plaque assays from enriched sample. Both spot test and Dilutions 10^0 through 10^-5	The plates came out positive with plaques. This means we are allowed to continue to the purification protocols. The plaques have a clear feature. They all seem to have the same morphology. They have a dark shade on the outer circumference of the plaques.

Main procedure	Important Notes	Results
Protocol 5.4 Picking a plaque	Chose the plate 10^0. from the Enriched dilutions made on 09/12/2017.	A microcentrifuge tube with 100 ul of phage buffer and "phage sample"
Protocol 6.2 Dilute liquid phage sample	Sample were diluted from 10^-1 to 10^-5 Transferred 10 ul of phage sample from the 10^-0 tube to the 10^-1 microcentrifuge tube.	6 microcentrifuge. 5 of them with 90 ul and the last dilution had 100 ul
Protocol 5.3 Plaque assay	4.5 ml of top agar was added to 500 ul of host bacteria.	6 Plates were present at the end.

Conclusion

This is the first of three rounds of purification that will be conducted. Six plates were made today and each are expected to have a 10 fold reduction in plaques. If plates come out successful next lab, this protocol will be repeated.

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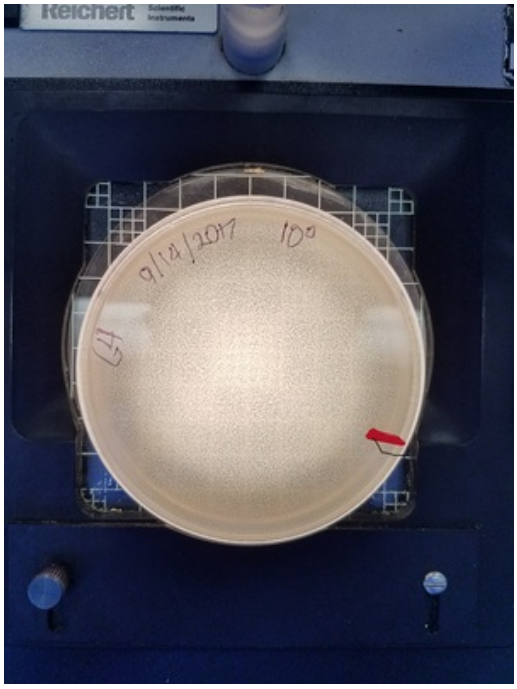

20170919_095454_1_.jpg(1.6 MB) - download 10^0 first round of purification plate

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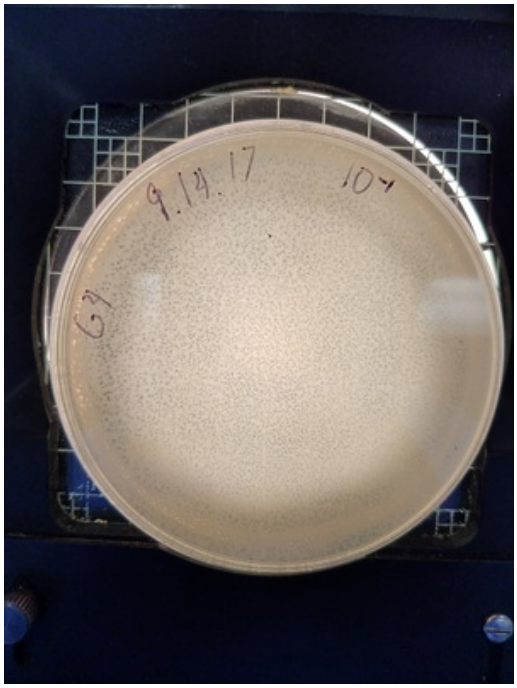

20170919_095556_1_.jpg(1.3 MB) - download 10^-1 first round of purification plate

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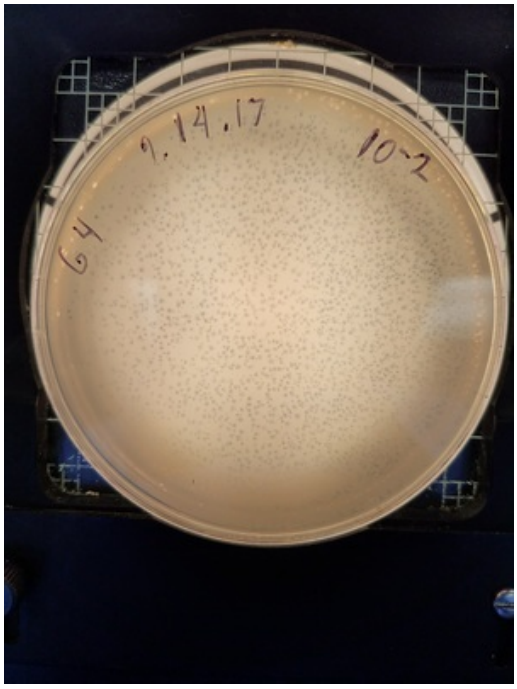

20170919_095607_1_.jpg(1.1 MB) - download 10^-2 first round of purification plate

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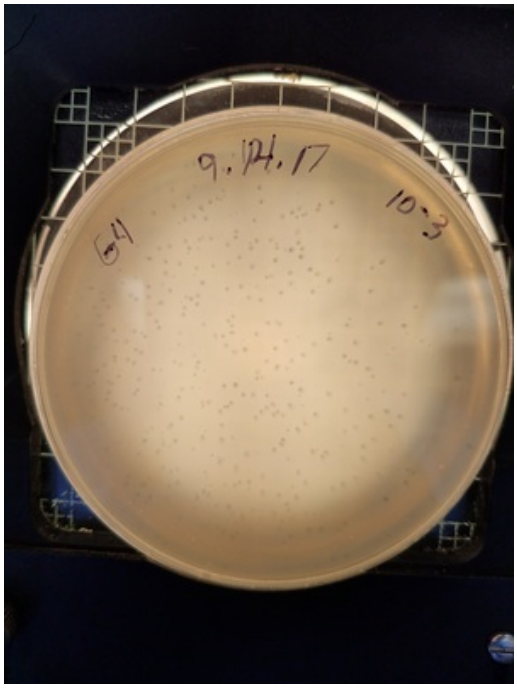

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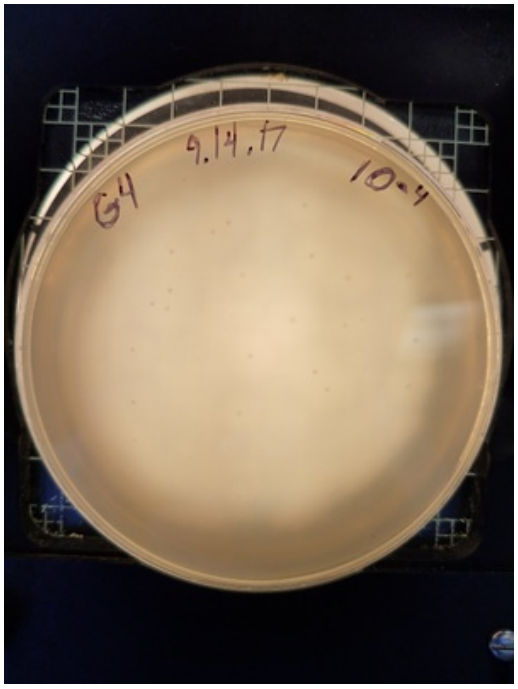

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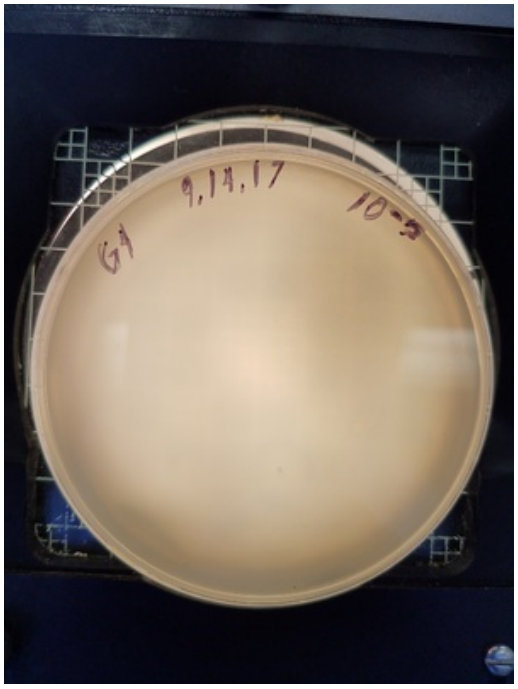


20170919_095658_1_.jpg(1.1 MB) - download 10^-5 first round of purification plate

Plaque Assay for Purification Round 2

09/19/2017

Aims and Purposes

This is the second round of purification for the plaques. In this lab we will continue purifying the plaques. Lab partner was absent today. Protocol was done solo.

Changes in protocol

No changes were done to this protocol.

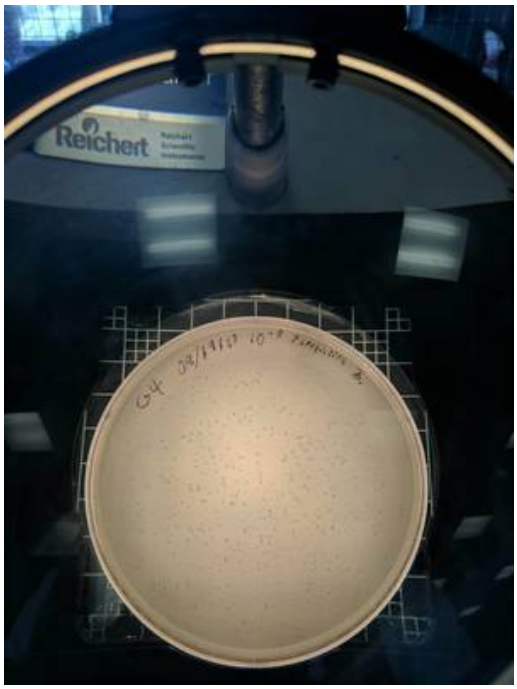
Data and results

Object	Observation
1st round of purification of plates	The plates follow the expected pattern of serial dilutions. The 10^0 plate is almost filled with plaques. As the plates go from 10^-1 through 10^-5 there is less and less plaques. All plaques are clear and seem to have the same characteristics.

Main Procedure	Important notes	Results
Protocol 5.4 Picking a plaque	Chose the plate 10^-3 made on 09/14/2017	Microcentrifuge tube with 100 ul of phage buffer and plaque from 10^-3

Conclusion

Today the 2nd round of purification was done. Next lab the plates will be observed for plaques. If present another round of purification will be made.



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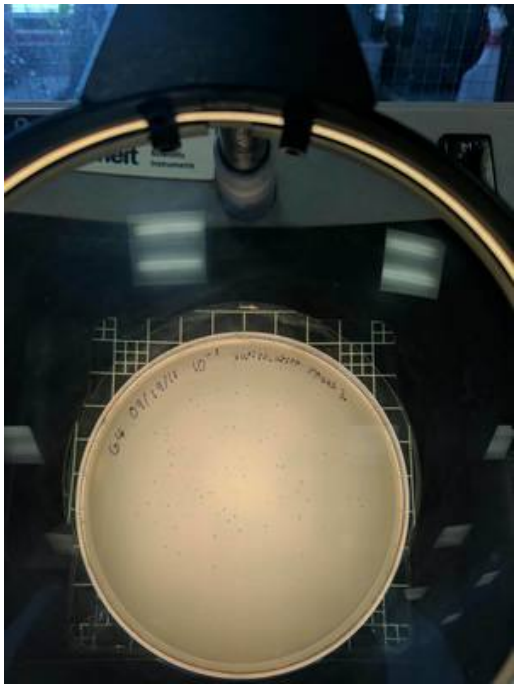

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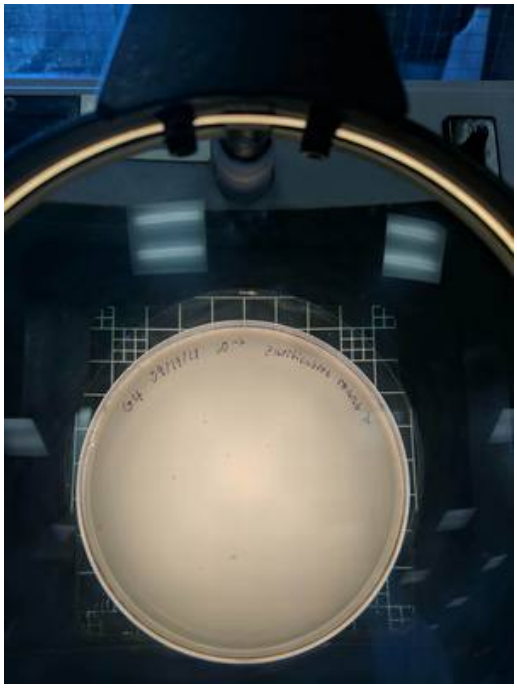

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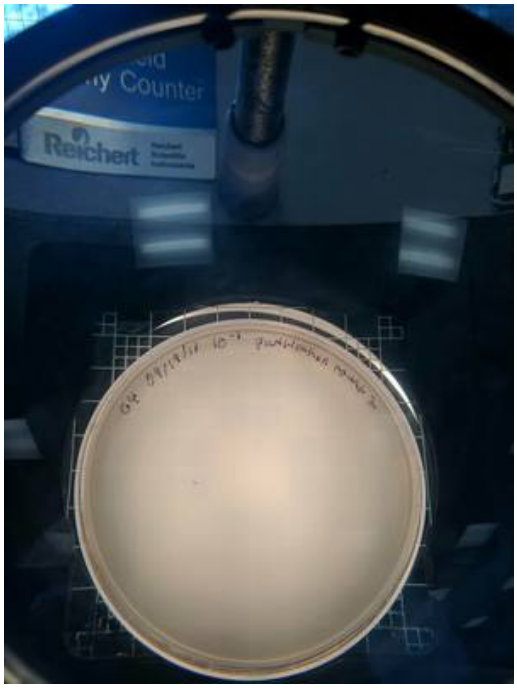

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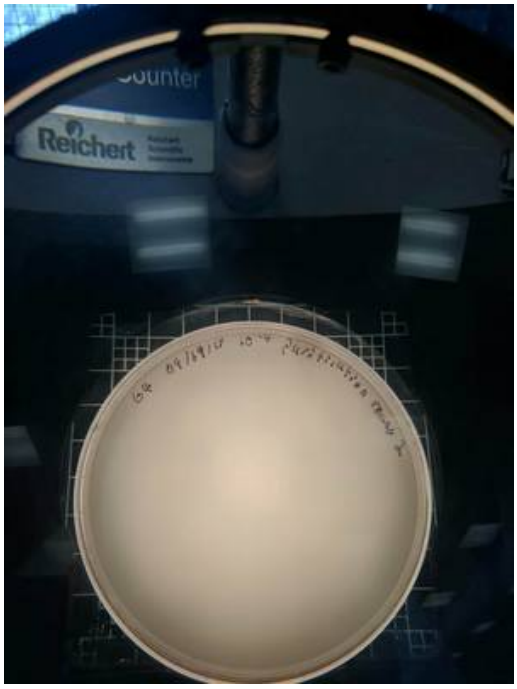

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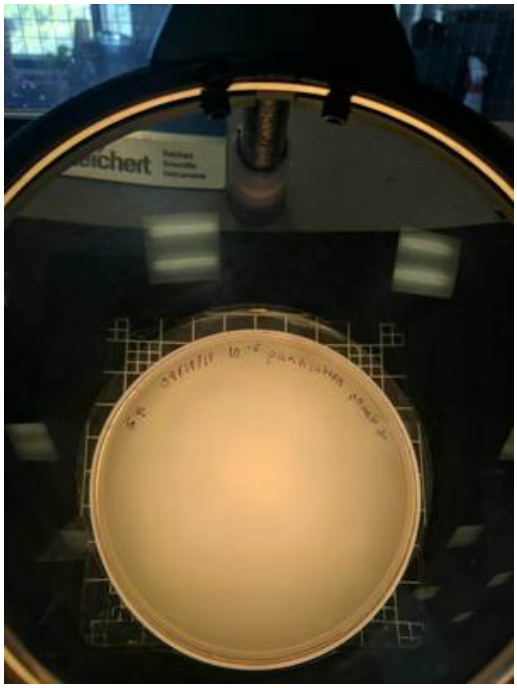

received_1823868240986812_1_.jpeg(99 KB) - download 10^-5 second round of purification plate

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Plaque Assay for Purification Round 3

09/21/2017

Aims and purposes

This last round of purification is to make sure the phage present is well isolated. Six more plaque assay plates will be made.

Changes in Protocol

No changes in protocol were made.

Data and Results

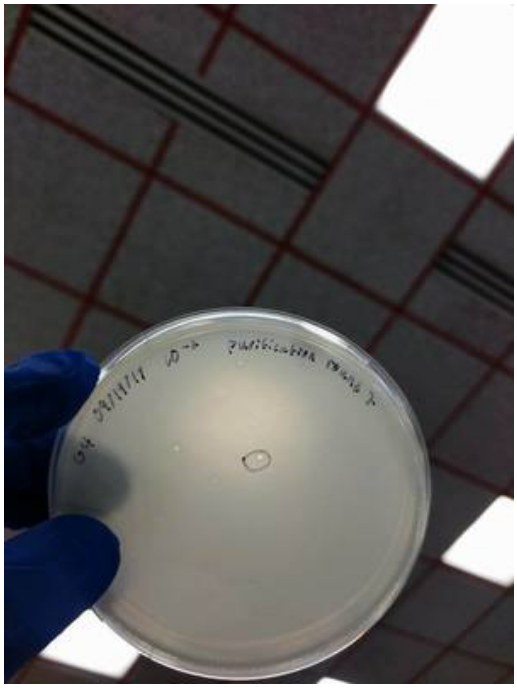
Object	Observation
2nd round of purification of plates made on 09/19/2017	The plaques from each plate follow the expected dilution pattern. All the plaques are looking similar. To be safe it was advised to continue through one more purification.

Main Procedure	Important Note	Results
Protocol 5.4 Picking a plaque	Chose the plate 10^-2 for plate made on 09/19/2017	Microcentrifuge tube with 100 ul of phage buffer with picked plaque virus particles from 10^-3 plate.

Conclusion

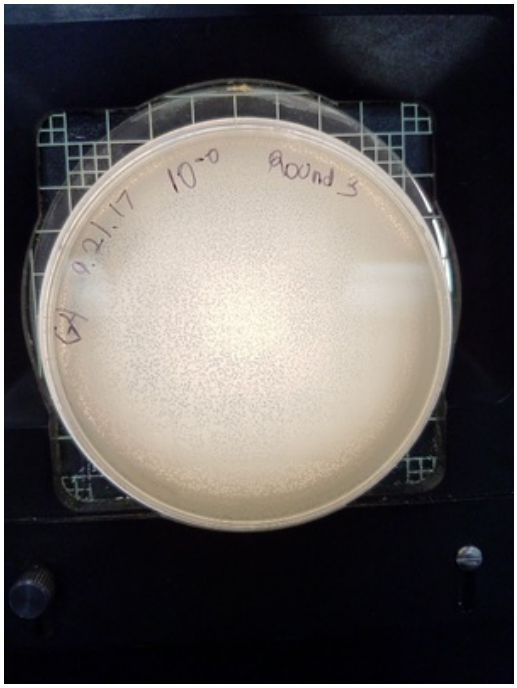
This was the last round of purification. Next week the plates will be observed to make sure plaques are present, consistent, and diluted correctly. If everything comes out as expected, the next step is to proceed to protocol 6.3, collecting plate lystate, and 6.4, spot titter.

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @06:00 PM PDT



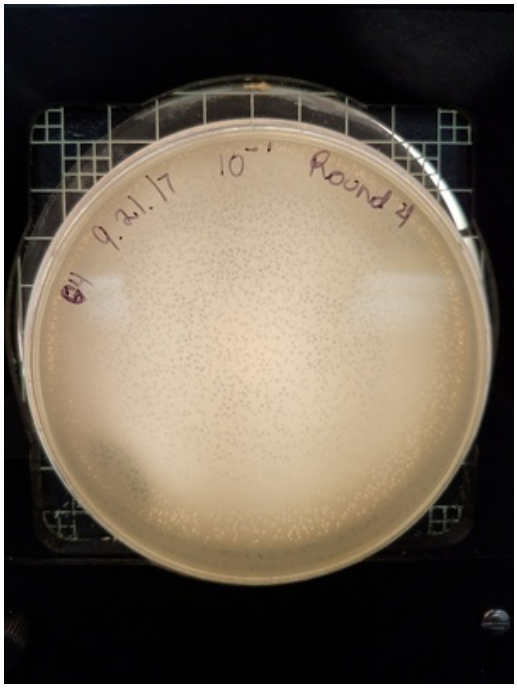
received_1823868230986813_1_.jpeg(98.8 KB) - download 10^-2 plate used for "picking" the plaque on 09/21/2017 for the third round of purification.

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @06:01 PM PDT



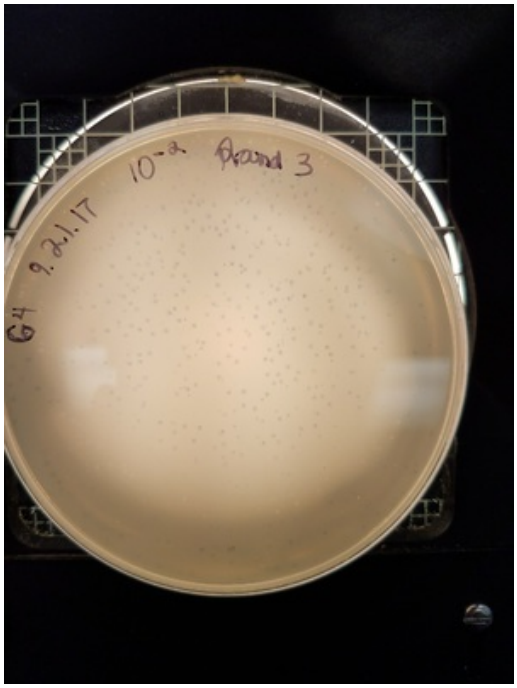
20170926_084744_1_.jpg(1.3 MB) - download 10^0 third round of purification plate

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @06:02 PM PDT



20170926_084757_1_.jpg(1 MB) - download 10^-1 third round of purification plate

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @06:02 PM PDT



20170926_084807_1_.jpg(977.4 KB) - download 10^-2 third round of purification plate

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20170926_084820_1_.jpg(923 KB) - download 10^-3 third round of purification plate

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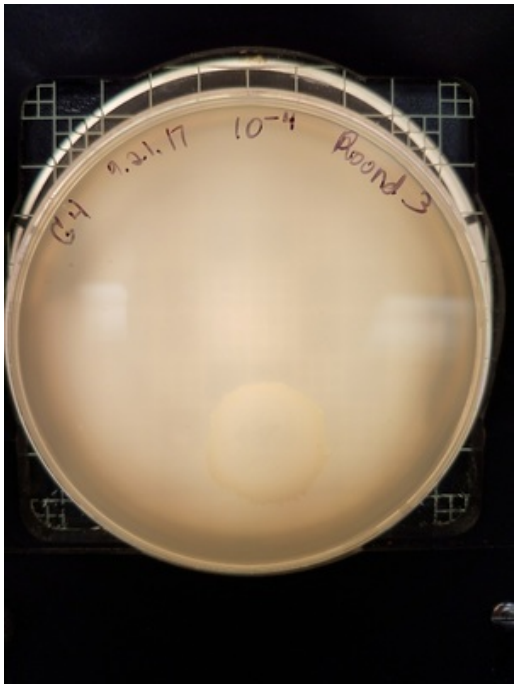
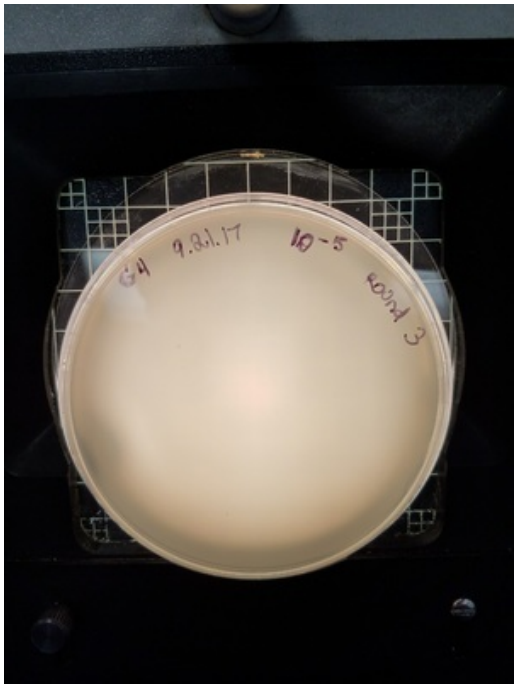


20170926_084832_1_.jpg(983.8 KB) - download 10^-4 third round of purification plate

▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @06:03 PM PDT



20170926_084847_1_.jpg(983.6 KB) - download 10^-5 third round of purification plate

Protocol 6.2 Serial Dilutions

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

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▪ Kevin Ayala Pineda ▪ Oct 17, 2017 @09:00 PM PDT

Serial Dilution Protocol for Enrichment sample

09/12/2017

Aims and purpose

The purpose of this protocol is to prepare liquid phage samples of decreasing concentrations. This protocol is performed several times throughout other experiments. This protocol uses 10 fold dilutions, meaning that the concentration of phage in each tube will have 10 times less than the previous tube. This gives us easy mathematical calculations.

Changes in Procedures

No changes in the protocol were made.

Data and results

Main procedure	Important Notes	Results
Add buffer to microcentrifuge tubes	Six total microcentrifuge tubes were needed. Tubes were labeled from 10^0 to 10^-5 90 ul of buffer was transferred to each tube.	6 labeled microcentrifuge tubes each with 90 ul of phage buffer
Transferred enriched sample into microcentrifuge tube	10 ml of the enrichment sample was transferred into the 10^0 microcentrifuge tube	10^0 Microcentrifuge tube with 100 ul solution of phage buffer and enrichment sample
Performed dilutions	Started by transferring 10 ul Sample was vortexed.	6 microcentrifuge tubes each having a 10 fold serial dilution.

Conclusion

This protocol gives the dilutions needed for the first round of plaque assays for the enrichment sample. After these dilutions were plated it was confirmed that a phages were present. This protocol will be performed several more times. Next lab dilutions will be needed for protocol 6.1, plaque assay for purification.

Serial Dilutions for Plaque Purification

09/14/2017, 09/19/2017, 09/21/2017

Aims and purpose

The purpose of this protocol is to purify the phage present in the plates. First the a plaque will be "picked" then a 10 fold serial dilution will me done.

Changes in Protocol

No changes in protocol were made

Data and Results

Main Procedure	Important Notes	Results
Performing dilutions	A total of 6 microcentrifuge tubes were needed. The phage samples for plaque purification were diluted down to 10^-5	6 microcentrifuge tubes each having a 10 fold reduction in phage concentration

Conclusion

The samples that were diluted were used to make plaque assays. These dilutions were made for purification. This will help ensure that a clonal phage population is present. These dilutions were then used to make plaque assays (Protocol 5.4.)

Serial Dilutions for Webbed Plates

09/28/2017, 10/03/2017, 10/05/2017

Aims and purposes

The purpose of this protocol is to make sure the dilutions needed for webbed plates are properly made.

Changes in Protocol

Instead of "picking a plaque" and mixing it with phage buffer. for the 10^0 sample, the lysate, collected on 09/26/2017, was used.

Data and Results

Main Procedure	Important Notes	Results
Dilutions	The first round of webbed plates did not come out as expected. It could be possible that the dilutions were incorrectly diluted. This however, is only one out of many possibilities that could have gone wrong.	Unsuccessful webbed plates.

Conclusion

These dilutions were made for webbed plates. The 10^0 sample is not from a "picked plaque". It is directly from the lysate that was collected on 09/26/2017. This will make plates with a much higher concentration of phage.

Protocol 6.3 Collecting Plate Lysates

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

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Collecting Plate lysates

09/26/2017

Aims and purpose

In this protocol phages are extracted from a highly concentrated plate. Phage buffer is used to extract the phages. Then we will be collect the sample and then filter it to get bacteria and agar residue out. .

Changes in Protocol Procedures

4 ml of phage buffer was used to flood the plate instead of 8 ml of phage buffer.

Data and Results

Main procedure	Important Notes	Results
Flood a webbed plate with phage buffer	4 ml of phage buffer was added to the plates made on the last round of purification. 10^-0 and 10^-1 were flooded The plate sat for two hours.	A plate flooded with phage buffer
Collecting, Filtration, and Combine in lysate liquid	Two plates were flooded. Each were filtered through a 0.22 um filtrate. The filtered samples was placed in a 15 ml conical tube.	A 15 ml sterile conical tube with 7 ml of lysate
Store	The conical tube was then stores at 4 degrees Celsius.	Stored lystate for future experiments.

Conclusions

The highly concentrated phage sample that was collected will be used next week for making webbed plates. The goal is to have a highly concentrated phage lysate. Once we collect the proper amount and concentration of the lysate we will proceed to extracting phage DNA.

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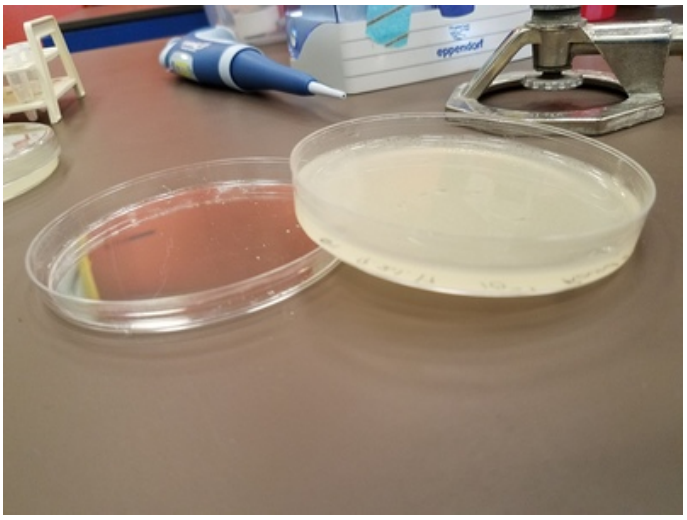

20170926_112744.jpg(4.6 MB) - download This is a picture of the tilted plate before the lysate was collected.

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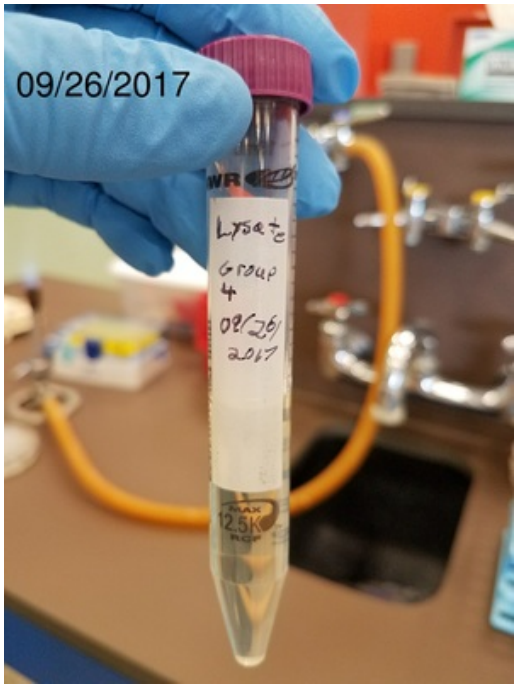

20170926_112801_0_1_.jpg(1.5 MB) - download This is the 15 ml conical tube that stored the filtered lysate. 7 ml of phage lysate was filtered.

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revisions print ▪ Kevin Ayala Pineda ▪ Oct 21, 2017 @02:21 PM PDT

Collecting Webbed Plate Lysate

10/12/2017

Aims and Purposes

In this protocol the plates that are webbed will be flooded, then will be collected, filtered, and then placed in a conical tube. The purpose of this protocol is to retrieve a highly concentrated phage sample. This sample will be needed for many other protocols.

Changes in Protocol

No Changes in Protocol were made

Data and results

Main Procedure	Important Notes	Results
"Flooding" the plates with phage buffer	All of the 10^-3 plates and 10^-2 plates came out highly concentrated with plaques. It was advised to flood all six plates.	Two lysates in a 15 ml conical tube. 12 ml of sample was collected from the 10^-3 plates. 10 ml of sample was collected from the 10^-2 plates

Conclusions

Two lysates were collected on this date. The next step is to calculate the titer of both the samples from a spot test. The goal is to have at least one lysate with a titer of 5.0 x 10^-9.

Protocol 6.4 Spot Titer

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

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▪ Kevin Ayala Pineda ▪ Oct 19, 2017 @03:30 PM PDT

Spot Test

09/26/2017

Aims and Purpose

In this protocol a spot test plate will be made with the lysate collected from protocol 6.3. The purpose of this plate is to give an approximation titer calculation for the lysate. From the results we will see which dilutions need to be plated to give a more accurate titer calculation. At the end of this protocol we will have only one plate with 8 different dilution spots. Today a new person joined the team! Vennessa!

Changes in Protocol

No change in the protocol was made

Data and Results

Main Procedure	Important notes	Results
Labeling plate, and preparing bacterial lawn	Labeled a plate in a square format Sample will be diluted to 10^-8 The plate sat for 25 minutes after top agar was added.	Labeled plate with bacterial lawn
Added phage lysate to microcentrifuge tubes.	Used a serological tube to collect lysate from the conical tube and was then placed in microcentrifuge tube	Microcentrifuge tube with pure lystate sample.
Make dilutions	Obtained 8 microcentrifuge tubes and labeled them from 10^-1 to 10^-8 Each tube was filled with 90 ul of phage buffer. Performed dilutions	8 microcentuge tubes each having a 10 fold reduction in phage concentration from initial lysate sample
Perform spot test	3 ul of each dilution was added to the agar plate into the correct labeled location	An agar plage each with dilution spot from 10^-0 to 10^-8

Conclusion:

The spot test had spots that were consistently diluted down. There was a 10 fold reduction in number of plaques as the plaques moved through the spots. The 10^0 through 10^-5 spots were too concentrated. The calculation of the titer was performed on the 10^-6 spot which yielded 52 plaques.. Next step is to calculate a full plate titer. From the spot test we will make plates with 10^-6, 10^-7, and 10^-8 dilutions.

Titer calculations

The titer is calculated by dividing the number of plaques by the volume of sample plates. Then multiply that number by the reciprocal of the dilutions used to make that plate and convert ul to ml to obtain the titer in pfr/ml.

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$

$$Titer(pfu/ml) = (52pfu/3ul) \times (10^3/ml) \times (10^6)$$

$$Titer(pfu/ml) = 1.73 \times 10^{10} pfu/ml$$

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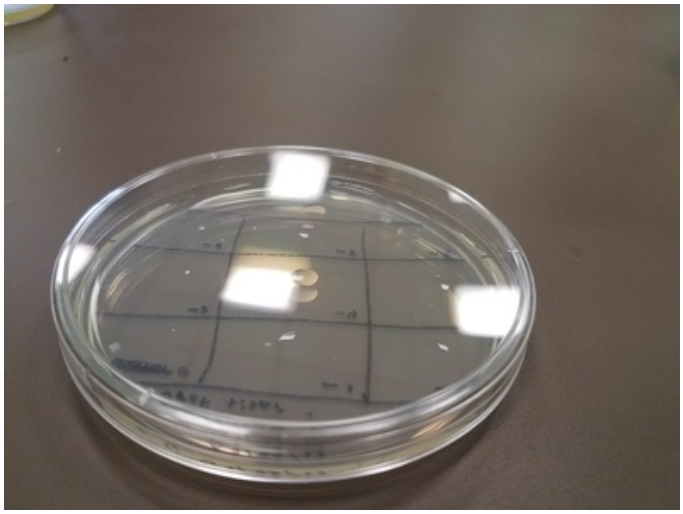

20170926_114543_1_.jpg(5 MB) - download Picture of spot test on 09/26/2017. Day of spotting.

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20170928_093339.jpg(1.6 MB) - download This is a picture of the spot titer plate. The dilutions follow the expected 10 fold reduction of plaques. The titer concentration was calculated off the 10^-6 spot which yielded 52 plaques.

Protocol 6.5 Full Plate Titer

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:38 PM PST

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @09:50 PM PDT

Full Plate Titer

09/28/2017

Aims and purpose

A full plate titer is aimed at retrieving a more accurate titer calculation. The spot test made from last lab will determine which plates will give an accurate phage count. The spot test gave an approximation of which dilution would be best fit for making full plates. This lowers the amount of plates that need to be made

Change in Protocolwi

No changes in Protocol were made.

Data and results

Main procedure	Important Notes	Results
Choosing the concentration to "bracket"	Plates that needed to be plated were estimated off the spot test. Dilution 10^7 was the spot that calculated the estimated titer.. The dilution concluded for plating was 10^-6, 10^-7, and 10^-8.	Dilutions for the full plate titer.
Plaque assay was performed.	Refer to Protocol 5.3.	3 Plates each with different dilutions.
Calculate the titer in pfu/ml	Calculations are at the bottom of the page.	A more accurate titer calculation.

Conclusion

Three plaque assay plates were present at the end of this protocol. Each plate has different phage concentration. Recall that a spot test only gives an approximation of its titer. These full plates gave a more accurate titer for the lysate. From here, the next step is to make webbed plate so more lysate can be collected. The goal is to collect 8-10 ml of lysate with titer of 5 x 10^9 pfu/ml or higher.

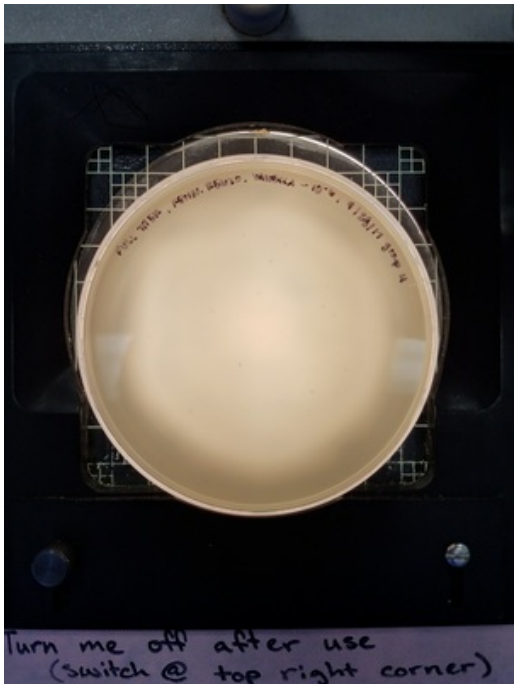
Titer Calculation

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$

$$Titer(pfu/ml) = (31pfu/10ul) \times (10^3/ml) \times (10^6)$$

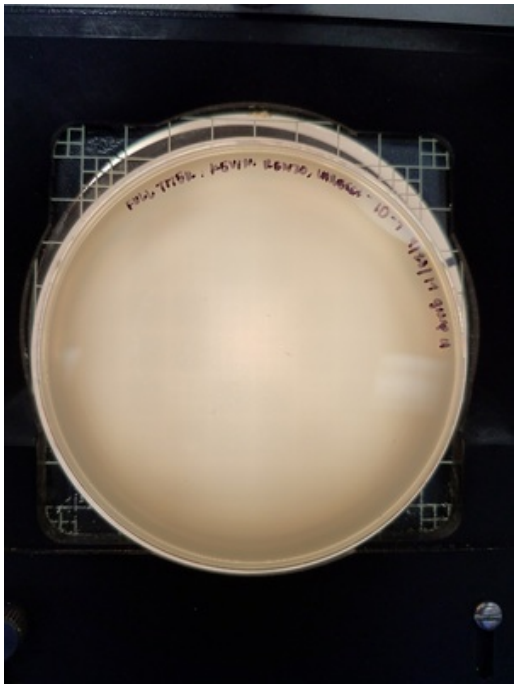
$$Titer(pfu/ml) = 3.1 \times 10^9 pfu/ml$$

1 revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @09:42 PM PDT



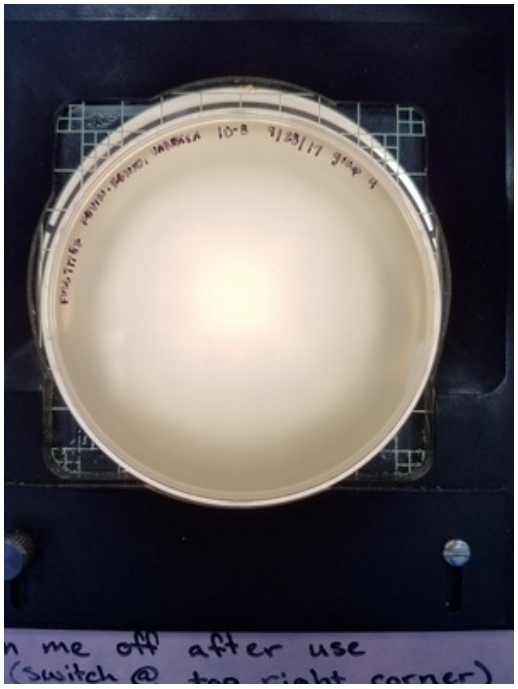
20171003_085714_1_.jpg(1009.6 KB) - download This is a picture of the 10^-6 plate. This is the one that was used to calculate the titer. It had 31 plaques.

revisions print ▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @09:41 PM PDT



20171003_085734_1_.jpg(1 MB) - download This is a picture of the 10^-7 plate

▪ Kevin Ayala Pineda ▪ Oct 18, 2017 @09:43 PM PDT



20171003_085747_1_.jpg(1.2 MB) - download This is a picture of the 10^-8 plate.

Protocol 7.1 Making Webbed Plates from a Lysate of Known Titter

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @04:42 PM PST

Making Webbed Plate First Attempt

09/28/2017

Aims and Purpose

This protocol involves creating plates that have high concentration of plaques. These plates are called webbed plates. In this experiment the initial lysate will be used to make these plates. The goal is to obtain a lysate concentration of at least 10^-9

Changes in Protocol

No changes in the protocol were made.

Data and Results

Main Procedure	Important step	Results
Estimated webbed plate	Calculations	4500 plaques was the amount estimated to obtain a webbed plate.
Making the webbed plates	After calculations the 10^-4 plate was bracketed. We pippetted 26.0 ul of a 10^-3 dilution 26.0 ul of a 10^-4 dilution 26.0 ul of a 10^-5 dilution	One of each plate was made.

Calculations

$$VolumeNeeded(ml) = \frac{NumberOfPlaquesNeeded}{TiterOfLysate} \times \frac{ul}{ml}$$

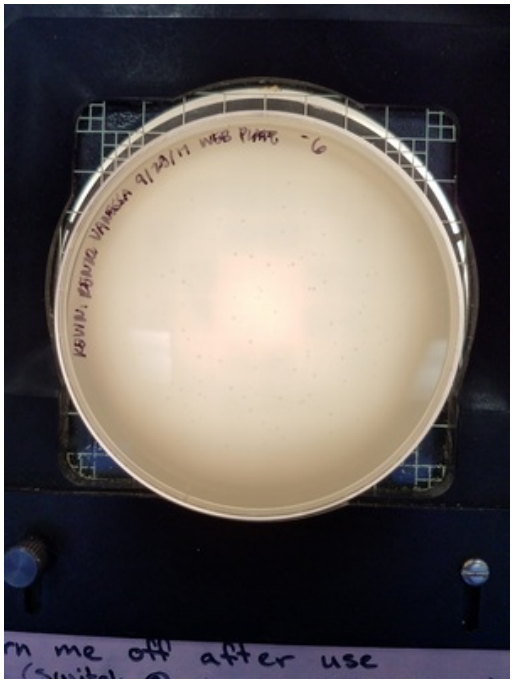
$$VolumeNeeded(ml) = \frac{4.50\times10^3 pfu}{1.73\times10^{10} pfu/ml} \times \frac{1.0\times10^3 ml}{ul}$$

$VolumeNeeded(ml) = 2.60ul \times 10^-3$ or pipette $26ul$ of a 10^-4 dilution

Conclusions

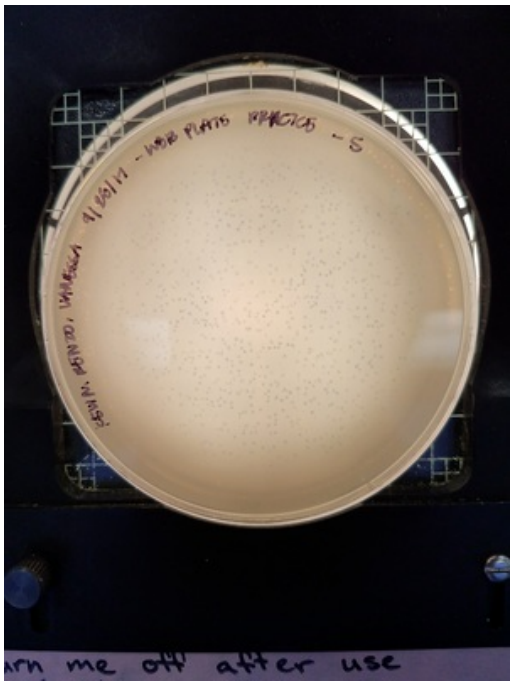
Next lab observation will be made to see if the plate came out webbed. If they are not webbed, calculations will be altered and the procedure will be repeated.

1 revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:21 AM PST



20171003_085804_1_.jpg(1.2 MB) - download This is a picture of the 10^-6 webbed plate. The picture was taken on 10/03/2017.

1 revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:20 AM PST



20171003_085826_1_.jpg(1 MB) - download This is a picture of the 10^-5 webbed plate. Picture was taken on 10/03/2017.

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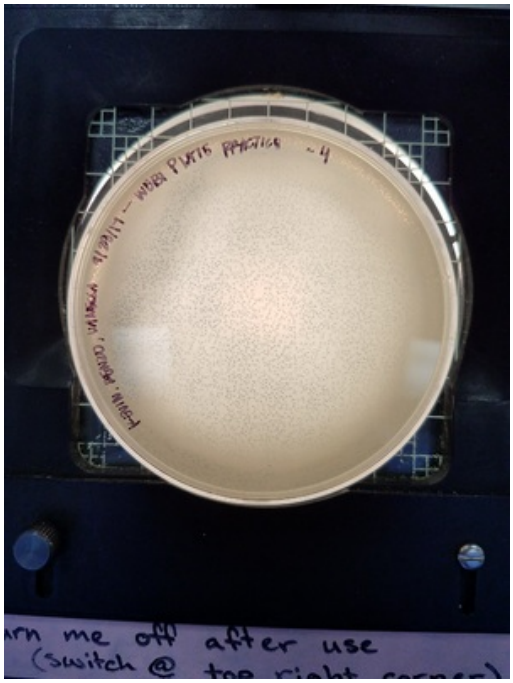


'

20171003_085836_1_.jpg(1.3 MB) - download This is a picture of the 10^-4 webbed plate. Picture was taken on 10/03/2017.

Webbed plate Second Attempt

10/03/2017

Aims and Purpose

The goal of this protocol is to achieve webbed plates. Since the last attempt did not yield the correct amount calculations will be manipulated to improve the probability of achieving webbed plates.

Changes in Protocol

No changes in protocol were made only the calculations from last lab were altered.

Data and Results

Object	Observation
Webbed plates made on 09/29/2017	Three webbed plates were made last lab. The plates did not have enough phage. Calculations will be more accurately made this time to give a better probability of obtaining webbed plates.

Main Procedure	Important Step	Results
Producing Webbed Plates	1. Find 90% of Surface Area of plate 2. Find Surface Area of Plaque 3.Find the amount of plaques needed to cover 90% of plate. Divide surface area of plate by the surface area of plaque. 4. Find volume needed to obtain a webbed plate. Divide the number of plaques needed (answer from previous step) and divide it by the titer of the lysate.	Calculations Below
Making webbed plates	After making the calculation the 10^-4 plate was bracketed. We pippetted 49.6 ul of a 10^-3 dilution 49.6 ul of a 10^-4 dilution 49.6 ul of a 10^-5 dilution	Three plates of each were made

Calculations

Radius of Plate $= 42.5mm$

Surface Area of Plate $= \pi \times (42.5mm)^2 = 15,675mm^2$

90% Of Surface Area of Plate $= 0.90 \times 15,674.5mm^2 = 5107.05mm^2$

Diameter of Plaque $= 0.65mm$

Radius of Plaque $= \frac{diameter}{2} = \frac{0.65mm}{2} = 0.325mm$

Surface Area Of Plaque $= \pi \times r^2 = \pi \times (0.325mm)^2 = 0.33183mm^2$

Number of Plaques required to cover 90% of plate's surface area

$= \frac{90 Percent Of Surface Area Of Plate}{Surface Area Of Plaque} = \frac{5107.05 mm^2}{0.33183 mm^2} = 1.539 \times 10^4 Plaques$

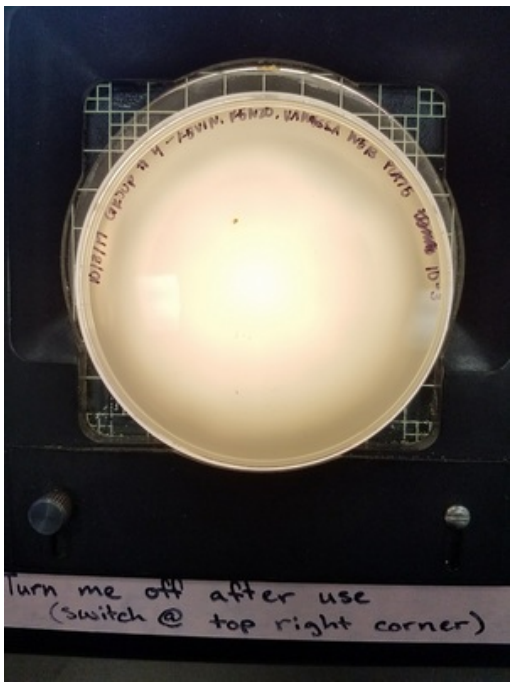
Volume(ml) of lysate needed for webbed plate

$= \frac{Number Of Plaques Needed}{Titer Of Lysate} \times \frac{ul}{ml} = \frac{1.539 \times 10^4 pfu}{3.1 \times 10^9 pfu/mL} = 4.9647 \times 10^6 mL = 4.96 \times 10^3 uL Or\ Pippette : 49.6 ul\ of\ a\ 10^-4\ Dilution$

Conclusion

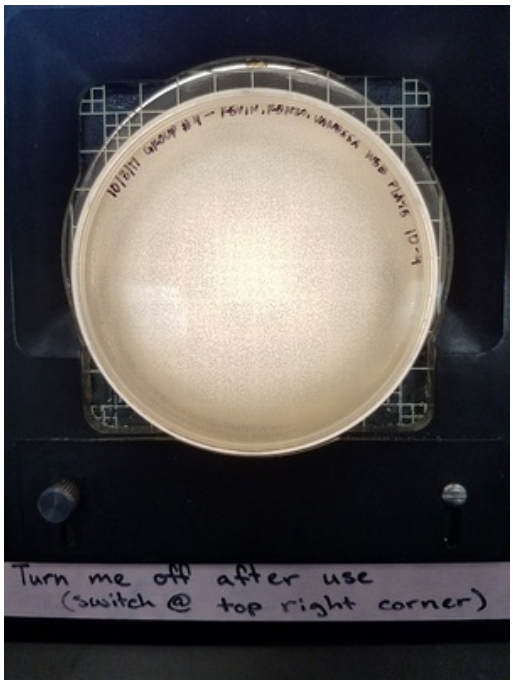
Three different plates with different dilutions were made. Next lab we will observe if any plates came out webbed.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:27 AM PST



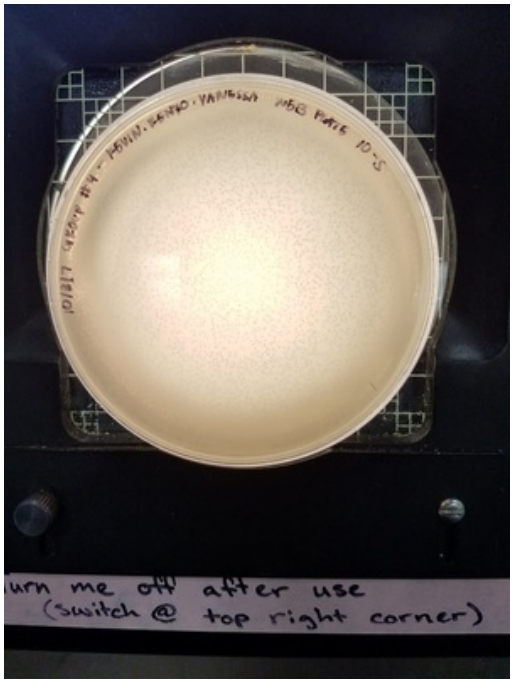
20171005_083441_1_.jpg(1.3 MB) - download This is a picture of the 10^-3 webbed plate. Picture was taken on 10/05/2017.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:25 AM PST



20171005_083402_1_.jpg(1.5 MB) - download This is a picture of the 10^-4 webbed plates. Picture was taken on 10/05/2017.

2 revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:26 AM PST



20171005_083419_1_.jpg(1.3 MB) - download This is a picture of the 10^-5 webbed plate. Picture was taken on 10/05/2017.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @04:51 PM PST

Webbed Plate Third Attempt

10/05/2017

Aims and Purpose

The purpose of this protocol is to make webbed plates. One out of the three plates made last lab came out webbed, and the goal for this lab is to reproduce three more exactly similar to it.

Changes in Protocol

No changes in protocol were made

Data and Results

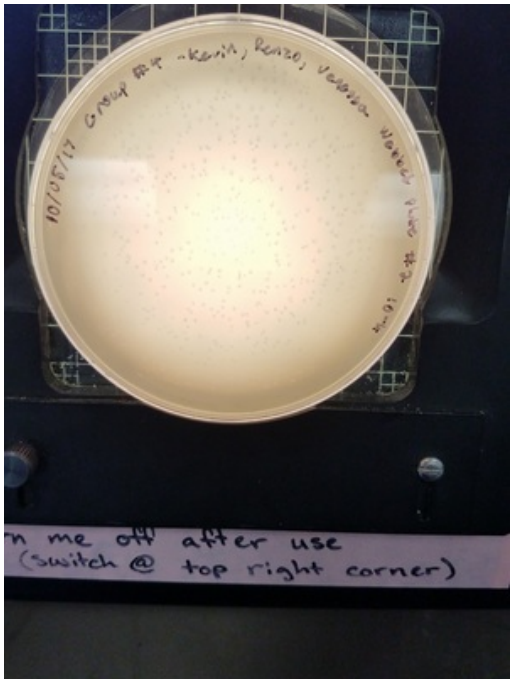
Object	Observation
10^-3 webbed plate from last lab	Only 3 plaques were present. Something probably went wrong making the dilution. More plaques were expected for this plate.
10^-4 webbed plate from last lab	This plate was perfectly webbed. This plate was flooded and the lysate was collected.
10^-5 webbed plate from last lab w	This plate had too much phage. There were no plaques.

Main Procedure	Important Steps	Results
Producing webbed plates	Since the 10^-4 plate came out successful the same calculations from last week were used to make the webbed plates.	We pippetted 49.6 ul of a 10^-4 dilution to make three more webbed plates.

Conclusion

Three plates were made this lab. Next week we will observe if they came out webbed. If they are not, then calculations will be redone or protocol will be attempted one more time with the same estimated webbed calculations.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:29 AM PST



20171010_102500_1_.jpg(1.1 MB) - download This is a picture of one of the 10^-4 plates. Picture was taken on 10/10/2017.

Webbed plate Fourth Attempt

10/10/2017

Aims and Purpose

The purpose of this protocol is to obtain webbed plates. Since the webbed plates from last lab did not come out successful another attempt will be made.

Changes in Protocol

No changes in protocol were made.

Data and Results

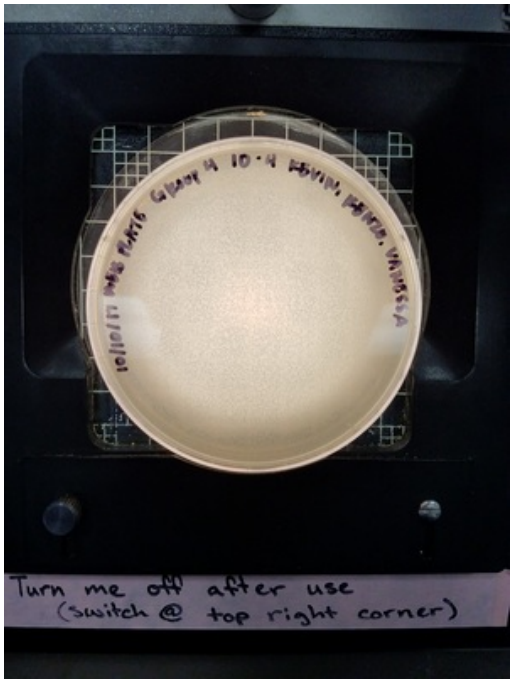
Object	Observation
Three 10^-4 plates from last lab	The plates were not webbed. There were only a few plaques on every plate. This could have occurred due to the phage lysate being stored for too long. The phage lysate could now have less phage then what it started with. Another reason for this could be due to incorrectly preparing the dilutions. One more attempt with the calculations from two labs ago will be used.

Main Procedure	Important notes	Results
Producing Webbed plates	The same calculations from last week were used to make a webbed plate, but this time we bracketed the 10^-3 plate.	49.6 ul of a 10^-4 dilution was used to make three plates 49.6 ul of a 10^-3 diltuion was used to make three plates 49.6 ul of a 10^-2 dilution was used to make three plates

Conclusion

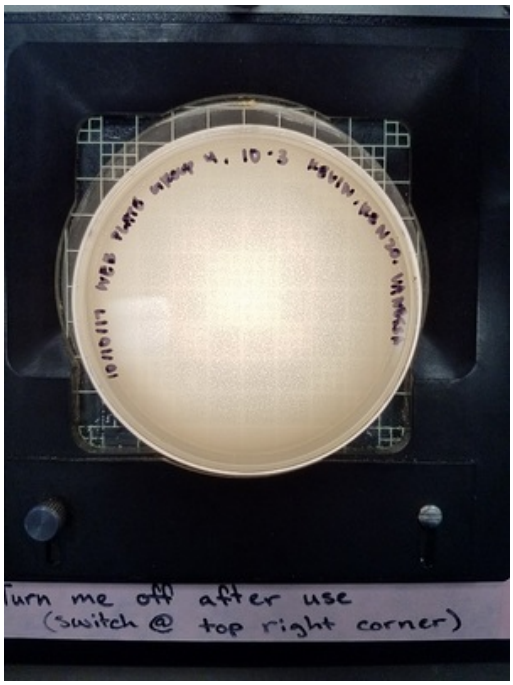
Nine plates were made with the same dilutions from two labs ago. If the plates do not come out webbed then calculations will be altered. If they do come out webbed the plates will be flooded with buffer and the lysate will be collected.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:38 AM PST



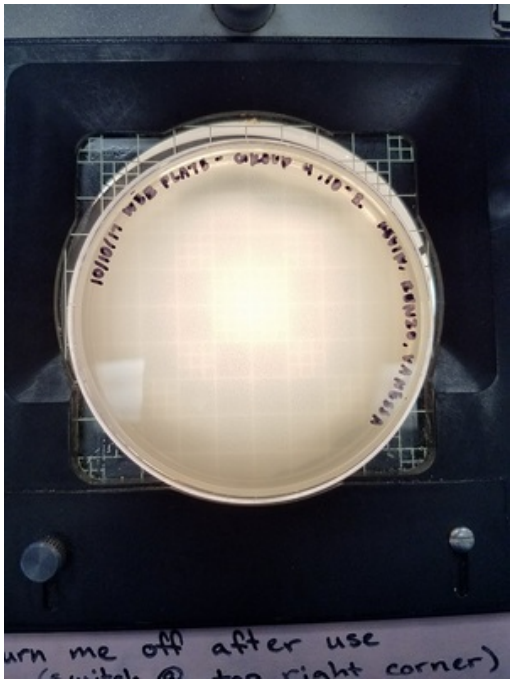
20171012_083756_1_.jpg(1.2 MB) - download This is one of the 10^-4 webbed plates. This picture was taken on 10/12/2017.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:38 AM PST



20171012_083828_1_.jpg(1.5 MB) - download This is one of the 10^-3 webbed plates. The picture was taken on 10/12/2017.

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:40 AM PST



20171012_083946_1_.jpg(1.5 MB) - download This is one of the 10^-2 webbed plates. Picture was taken on 10/12/2017.

Spot Test From lysate Obtained from Webbed plate

10/12/2017

Aims and purposes

The purpose of this protocol is to predict which dilutions should be used for the full plate titer. Now that there is a two lysates, from the webbed plates, the next step is calculate its titer. The goal is to obtain a titer that is 5.0 x 10^-9 or higher for DNA extraction. There will be two spot test plates at the end of this protocol and an approximation of its titer.

Changes to Protocol

No changes in protocol were made

Data and Results

Object	Observation
Nine webbed plates made on 10/10/2017 3 plates of a 10^-2 dilution 3 plates of a 10^-3 dilution 3 plates of a 10^-4 dilution	All plates came out highly concentrated with phage. We ended up flooding only the 10^-2 and the 10^-3 plates because plaques were more visible.

Main Protocol	Important Notes	Results
Labeling Plates	There were two lysates collected on this date. One is from webbed plates of 10^-2 and the other from 10^-3. Since these lysates can have different titer it is important to make two spot test to get an appropriate concentration.	Two labeled plates with nine quadrants on each plates. 10^0 through 10^-9
Dilutions	Dilutions were made up to the 10^-8 for each lysate.	Both lysates had 8 microcentrifuge tubes Each with a 10 fold serial dilution

Conclusion

Both plates came out with different dilution patterns. The 10^-3 spot test was more concentrated than the 10^-2. The next step is to calculate the full plate to have a more accurate titer.

Titer calculation

Calculations made on 10/17/2017

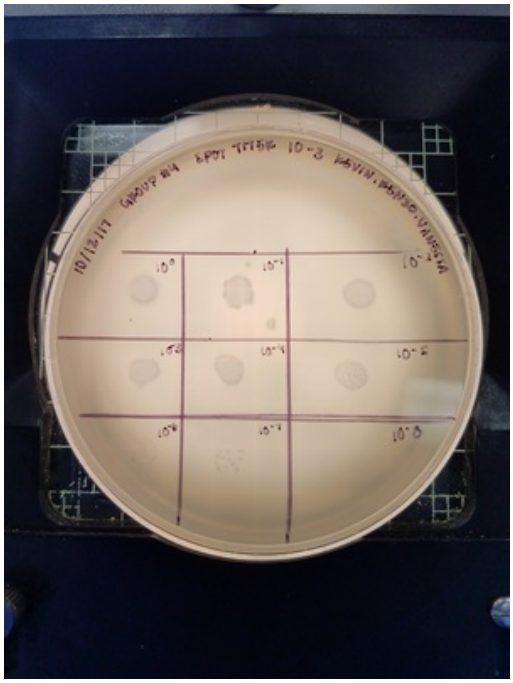
Titer Calculation for the 10^-2 spot plate

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = (45pfu/3ul) \times (10^3/ml) \times (10^4)$$
$$Titer(pfu/ml) = 1.5 \times 10^8 pfu/ml$$

Titer Calculation for the 10^-3 spot plate

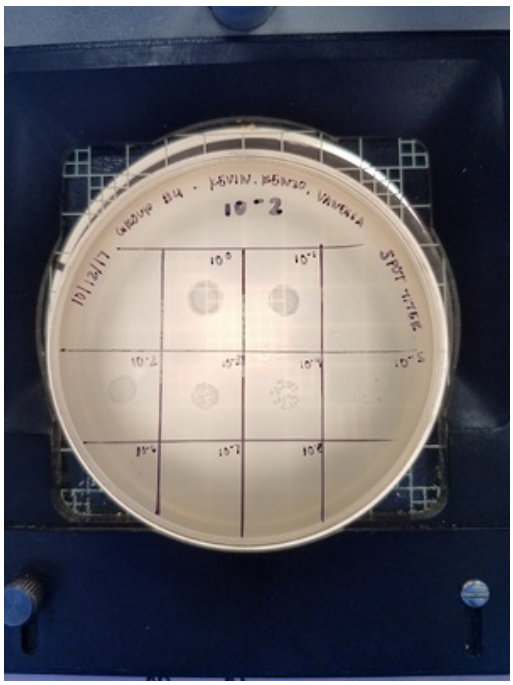
$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = (16pfu/3ul) \times (10^3/ml) \times (10^7)$$
$$Titer(pfu/ml) = 5.33 \times 10^{10} pfu/ml$$

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @09:39 AM PST



20171017_092146_1_.jpg(1.1 MB) - download This is a picture of the spot titer made with the lysate that was extracted from three 10^-3 webbed plates. Titer approximation is 5.33 x 10^10 pfu/ml. The 10^-3 titer is more concentrated than the 10^-2 titer.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @09:39 AM PST



20171017_092128_1_.jpg(1.4 MB) - download This is a picture of the spot titer made with the lysate that was extracted from three 10^-2 webbed plates. Titer approximation is 1.5 x 10^8 pfu/ml.

Full plate Titer from Webbed plate lysates

10/17/2017

Aims and purposes

The protocol is the most accurate method of determining the number of infectious phage in a liquid sample. After this protocol there will be several plates from our two different lysate samples. The spot test performed on 10/12/2017 determined which dilutions were plated.

Changes in Protocol

No changes in Protocol were made.

Data and Results

Main Protocol	Important Notes	Results
Dilution for plating	The 10^-3 lysate had 8 dilution and 8 plates the 10^-2 lysate had 5 dilutions and 5 plates	Two different set of dilutions for plating

Conclusion

Both titer calculations for the lysates came out highly concentrated. These calculations were made on 10/19/2017. The next step is to confirm these calculations next week when we observe and calculate the titers of the full plates.

Titer Calculations

Calculations made on 10/19/2017

Full Plate from 10^-2 lysate

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = (1253 pfu/10ul) \times (10^3/ml) \times (10^5)$$
$$Titer(pfu/ml) = 1.25 \times 10^{10} pfu/ml$$

Full plate from 10^-3 lysate

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = (31 pfu/10ul) \times (10^3/ml) \times (10^7)$$
$$Titer(pfu/ml) = 3.1 \times 10^{11} pfu/ml$$

Full Plate Titer from Webbed lysate second calculations

10/19/2017

Aims and Purpose

The goal is to get a highly concentrated lysate sample for DNA extraction protocol. The full plates from last lab determined that both lysates are highly concentrated. In this lab, full plates will be made again so these calculations can be confirmed.

Changes in protocol

No Changes in Protocol were made.

Data and Results

Main Protocol	Important Notes	Results
Transferring bacteria and phage mixture to plate	In this protocol there was a mistake. Our group accidentally transferred two mixtures in one plate.	10^ plate with two mixtures.

Conclusions

The titer for both of the lysates are high enough to go forwards with DNA extraction. However,the plates came out contaminated. For next lab, this protocol will be repeated.

Titer Calculations

Calculations made on 10/24/2017

Full plate from 10^-2 lysate

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$

$$Titer(pfu/ml) = 4.8 \times 10^{10} pfu/ml$$

Full Plate from 10^-3 lysate

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$

$$Titer(pfu/ml) = 2.6 \times 10^{10} pfu/ml$$

Full Plate Titer From Webbed Lysate Third Calculation

10/26/2017

Aims and Purposes

For this protocol the lysates will be filtered through a 0.22 um filtrate, because there is a possibility of contamination. Then full plates will be made once more to confirm that there is no contamination present and that the titer for the lysate is high enough.

Changes in Protocol

No changes in the Protocol were made.

Data and Results

Main Protocol	Important Notes	Results
Dilution for plates	The 10^-3 lysate had 8 dilution and 3 plates • $10^6, 10^7, 10^8$ were plated The 10^-2 lysate had 5 dilutions and 4 plates • $10^5, 10^6, 10^7, 10^8$ were plated	Two different set of dilutions for plating

Conclusion

The plates came out with no contamination. The titer for both lysates were above 10^9. The next lab will involve practicing DNA extraction.

Titer Calculations

Calculations made on 10/31/2017

Full Plate 10^-2

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = 2.31 \times 10^{10} pfu/ml$$

Full Plate 10^-3

$$Ttier(pfu/ml) = (NumberOfPlaques/VolumeUsed) \times (10^3 ul/ml) \times (DilutionUsed)$$
$$Titer(pfu/ml) = 4.5 \times 10^{10} pfu/ml$$

Protocol 7.2 Entering a Phage into the Actinobacteriophage Database

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

2 revisions print

▪ Kevin Ayala Pineda ▪ Dec 01, 2017 @06:19 AM PST

Entering a Phage into the Actinobacteriophage Database

11/14/2017

Aims and Purpose

In this protocol our phages information was entered into a database so its information is accessible to everyone.

Changes in Protocol

No changes in protocol were made.

Data and Results

Important Information	Notes
Name of phage: Riparian	The phage was found in a riparian location, which is an interface between land and a river or stream. This name seemed best fit for the phage.

Information submitted to Phagesdb.org

Discovery Information

Isolation Host	Mycobacterium smegmatis mc^2-155
Found By	Cotton Grove
Year Found	2017
Location Found	Las Vegas, NV USA
Finding Institution	University of Nevada Las Vegas
Program	Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science
From enriched soil sample?	Yes
Isolation Temperature	Not entered
GPS Coordinates	36.103961 N, 155.020223 W
Discovery Notes	The phage was discovered at the wetlands park near a pond. There were several plants and bushes near the location it was dug up. The soil was damp and conditions were near 90 degrees Celsius 9.3cm of soil was dug up.
Naming Notes	A riparium is a cluster of plants found along the edges of lakes, rivers, pond and streams. Since the area of phage discovery had several plants and it was next to a pond, Riparium is a perfect name.

Sequencing Information

Cluster	Unclustered
Subcluster	--

Plaque Notes	Riparium plaques are small. The plaques have a dark shade on the outer circumference
Final annotation complete?	No
Has been Phamerated?	No

Publication Information

Uploaded to GenBank?	No
GenBank Accession	None yet
Refseq Number	None yet

Conclusions

Now that the phage is in the actinobacteriophage database, it's genome is capable of being sequenced and is available for other scientists.

Protocol 7.3 Archiving Your Phage Sample

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Dec 01, 2017 @06:22 AM PST

Archiving Your Phage sample

11/16/2017

Aims and Purpose

The purpose of this protocol is to prepare lysate for long term storage. By doing this the phage sample will remain viable for decades. Three samples will be made. Two will go to the University of Pittsburgh, and the other will be stored at UNLV.

Changes in Protocol

No changes in Protocol were made.

Data and Results

Main Procedure	Important Notes	Results
Preparing tubes	Three tubes were prepared for storage. The two being sent to the University of Pitsburgh had beads. The one stored at UNLV did not. The three tubes were filled with a mixture of 2.8 mL of high-titer lysate and 200 µL of DMSO.	Three tubes
Labeling Tubes	The three tubes were labeled as follows: • Riparian • University of Las Vegas • 11/16/2017	Three labeled tubes prepared for storage

Conclusion

Our phages sample is now accessible for other researches and held in for long term storage.

Protocol 8.1: Modified Mounting Phage Samples for SEM and staining with Phosphotungstate

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

▪ Kevin Ayala Pineda ▪ Dec 13, 2017 @02:47 PM PST

Modified Mounting Phage Samples for SEM and staining with Phosphotungstate

11/09/2017

Aims and Purpose

In this protocol the phages lysate is prepared on a EM grid for shipment to BYU. The concept of negative staining is used in this protocol. The phage is mixed in an electron dense solution so it can appear white in a black background during SEM.

Changes in Protocol

This protocol was handed to us by our instructor.

Data and Results

Main Procedure	Steps
1	Obtain 10 ul of phage lysate and place on parafilm
2	Place the dark side of the grid on top of phage lysate, and let sit for two minutes if concentration is higher than 10^9. If concentration is less, then more time will be needed for absorption.
3	15 seconds before timer is up pippette 10 ul of stain and place on parafilm. Once timer is up rinse the grid with 300 ul of water.
4	Carefully wick the film with paper. Do not let the grid dry. Plce it on the 10 ul stain for 1 minute
5	After this let it dry for four to five minutes.
6	Place the grid in designated area.

Conclusion

Samples were sent to BYU and have not returned yet.

scaled tail size: $\frac{300nm \times 51.66mm}{54mm} = 287nm$

scaled capsid size: $\frac{300nm \times 12.75mm}{54mm} = 70.83nm$

Protocol 9.1 Phage DNA Extraction

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

1 revisions print ▪ Kevin Ayala Pineda ▪ Nov 30, 2017 @06:02 PM PST

Phage DNA Extraction

11/07/2017

Aims and Purpose

In this protocol phage DNA will be extracted from lysate. DNA is necessary for proceeding to further experiments. The goal is to have a non contaminated phage DNA sample .

Changes in Protocol

No changes in protocol were made.

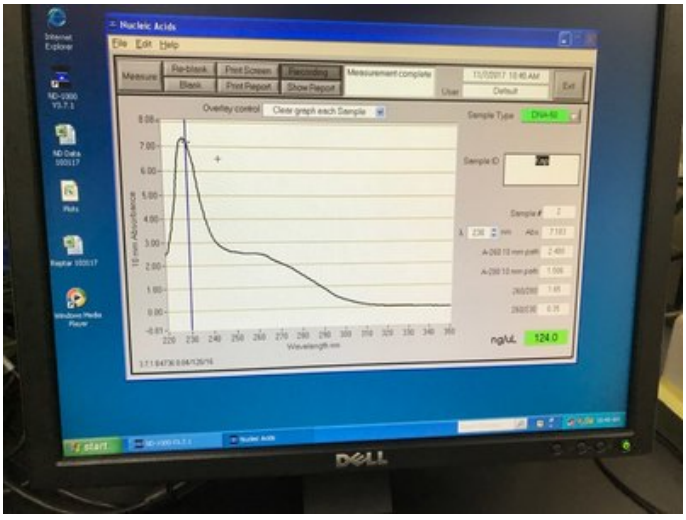
Data and Results

Main Procedure	Important Notes	Results
Degrading Bacterial DNA/RNA in high titer phage lysate	There are two highly concentrated lysates,10^-2 and 10^-3. Only one was chosen for DNA purification protocol.	The 10^-3 lysate was used in this protocol.
Determining the concentration of Phage DNA	Nanodrop machine determined the phages DNA concentration	129.48 ng/ul was calculated

Conclusion

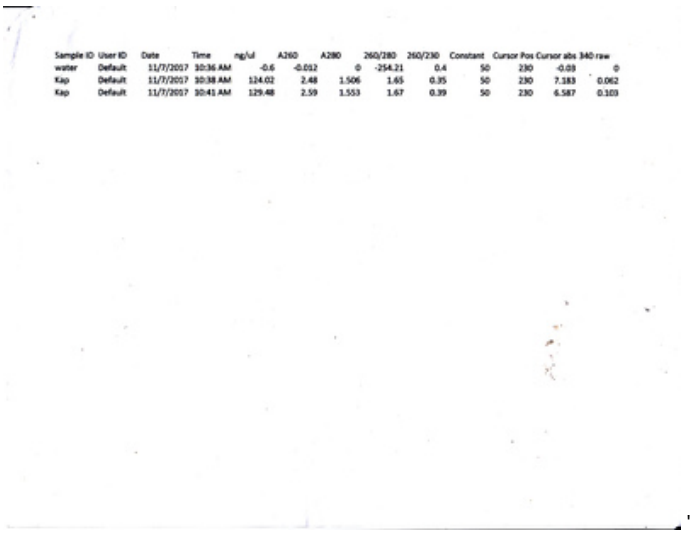
There was a 230 contamination peak with the DNA, but this is normal. Our Instructors found a phage protocol from 2013 stating that it is normal to have this contamination. Because of this our group was allowed to proceed to restriction enzyme digests.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:43 AM PST



IMG_9537_1_.jpg(74.7 KB) - download This is the graph from the nanodrop procedure. Picture was taken on 11/07/2017.

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:42 AM PST



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos	Cursor abs	340 raw
water	Default	11/7/2017	10:36 AM	-0.6	-0.012	0	-254.21	0.4	50	230	-0.03	0
Kap	Default	11/7/2017	10:38 AM	124.02	2.48	1.506	1.65	0.35	50	230	7.183	0.062
Kap	Default	11/7/2017	10:41 AM	129.48	2.59	1.553	1.67	0.39	50	230	6.587	0.103

IMG_0002.jpg(668.8 KB) - download This is our data from the nanodrop procedure. Picture was taken on 11/07/2017.

Protocol 10.1: Setting up Restriction Enzyme Digests

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

revisions print

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @06:01 AM PST

Setting Up Restriction Enzyme Digests

11/09/2017

Aims and Purpose

In this protocol the phages genome will be cut into fragments of different sizes with restriction enzymes. The amount of fragments will depend on the number and locations of the restriction sites the DNA holds.

Changes in Protocol

No changes in protocol were made

Data and Results

Main Procedure	Important Notes	Results
Setting up the restricting enzyme digest reaction	Eight different tubes were prepped for this protocol 1. Uncut DNA 2. Bam HI 3. ClaI 4. EcoRI 5. HaeIII 6. HindIII 8. NciI 9. SalI	Each tube had 25 ul of diH2O, 10X rection buffur and phage genomic DNA

Calculations

Amount of DNA in ul needed to obtain 0.5 ug of DNA. The concentration of our phages DNA was used in this calculation.

$$\frac{129.48ng}{ul} \times \frac{ug}{1000ng} \times \frac{1000ul}{ml} = 129.48\ \frac{ng}{ml}$$

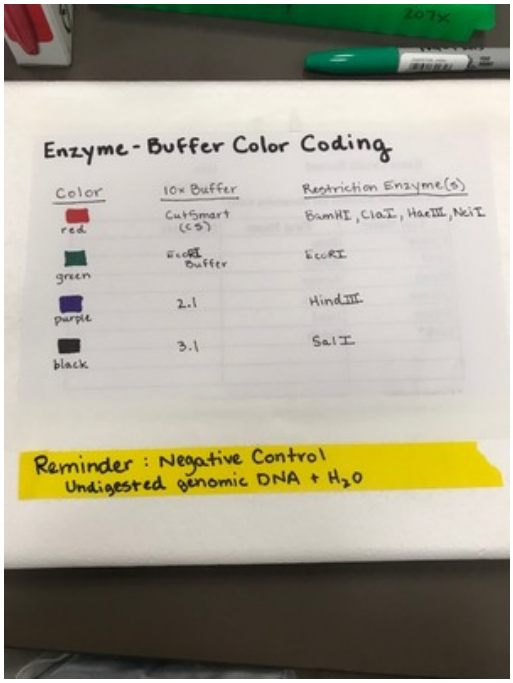
The DNA sample contained 129.48 ug/ml

$ulDNA = 0.5 \times \frac{ml}{124.48} \times \frac{1000ul}{1ml} = 3.861ul$ needed. to obtain 0.5 ug of DNA

Conclusions

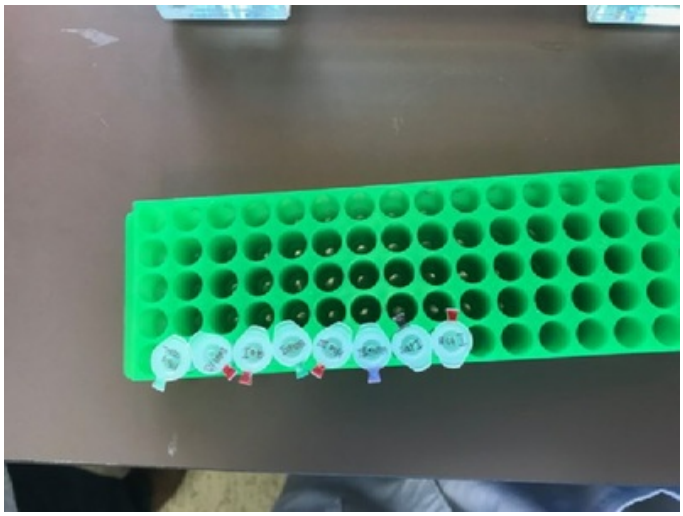
The seven different enzymes used on the phages DNA will recognize and cleave specific sequences of base pairs. After this protocol the DNA, placed in enzymes, will be in fragments and ready for gel electrophoresis.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @07:11 AM PST



IMG_8978_1_.jpg(73 KB) - download This is a picture of enzymes used on the phages DNA. Photo was taken on 11/09/2017.

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @07:12 AM PST



IMG_8079_1_.jpg(61.1 KB) - download These are the microcentrifuge tubes that held the solution before the enzymes were added. Photo was taken on 11/09/2017

Protocol 10.2: Casting Agarose Gels

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

3 revisions print ▪ Kevin Ayala Pineda ▪ Dec 01, 2017 @06:48 AM PST

Casting Agarose Gels

11/14/2017

Aims and purposes

The purpose of this protocol is to prepare a agarose gel for electrophoresis. The gel is made by melting agarose in buffer and then it is poured into an electrophoresis apparatus where it will solidify. This will prepare the group for protocol 10.3.

Changes in Protocol

Changes in protocol were made. Instead of preparing a 0.8% agarose gel, a 1% mixture was made instead.

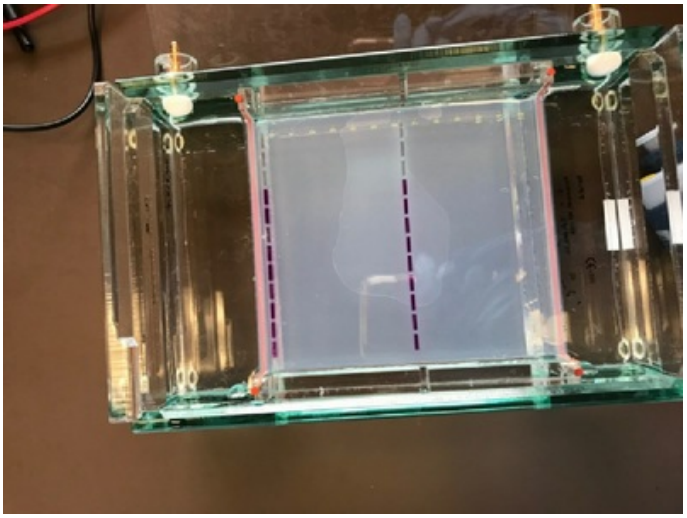
Data and Results

Main Procedure	Important Notes	Results
Adding buffer and agarose gel	Amount of buffer added: 200 mL Amount of powdered agarose: 2g Calculations: $Concentration\ of\ solution(\frac{w}{v}) = \frac{Mass Agarose(g) Needed}{Volume Agarose(ml)} \times 100$ $1\% = \frac{X(g) Needed}{200ml} \times 100$ $\frac{1 Percent \times 200}{100} = X(g)$ $X(g) = 2g$	After mixture was made it was then heated
Heating the mixture	The mixture was placed in a microwave and heated for 1 minute and 30 seconds, with 2 interval breaks. After one minute in the microwave the mixture was removed and was swirled carefully then placed back into the microwave for another 30 seconds.	This procedure was done to get rid of any clumps in the solution.
Adding ethidium bromide	$(200ml)(0.5ug/ml EtBr) = (x)(10mg/ml)$ $(200)(0.5ug) = x(10ug/ul)$ $x = \frac{(100)(9.5ug)}{(10ug/ul)}$ $x = 10uL$	10 uL of ethidium bromide was added to the agarose solution
Allowing the gel to cool	We let the gel sit for 20 minutes before proceeding to protocol 10.3	After the gel solidified and was placed into the gel box, 1X TBE buffer was poured into the apparatus.

Conclusions

After the gel solidifies we proceeded to protocol 10.3.

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:46 AM PST



IMG_1150_1_.jpg(113.9 KB) - download This is a picture of the solidified agarose gel. Picture was taken on 11/14/2017.

Protocol 10.3: Gel Electrophoresis of Restriction Enzyme Digests

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @09:55 AM PST

Gel Electrophoresis of Restriction Enzyme Digests

11/14/2017

Aims and Purpose

The aim of this protocol is to separate the phages DNA fragments with gel electrophoresis. The phage/restriction enzyme sample will be pippetted in wells created in the agarose gel. After this an electric current will be conducted on the gel electrophoresis apparatus to separate the fragments of phage DNA.

Changes in Protocol

No changes in protocol were made.

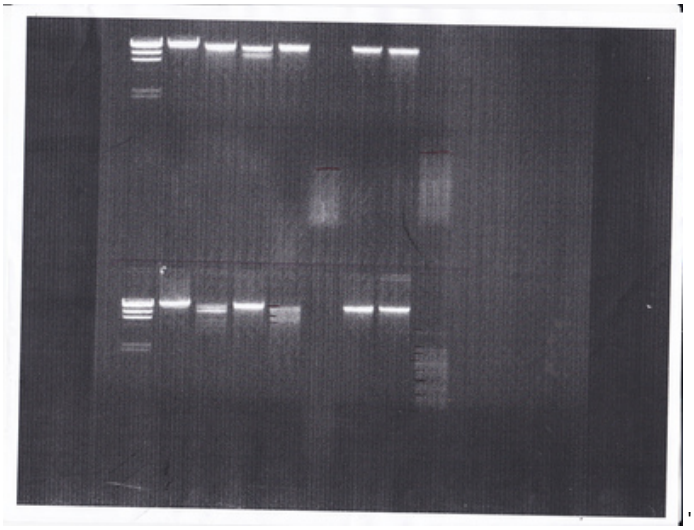
Data and Results

Main Procedure	Important Notes	Results
Loading gel into wells	Seven different enzymes were used in this protocol. Each lane had a specific enzyme assigned to it. **Lane number and enzyme assigned** 1. Ladder 2. Uncut DNA 3. BamHi 4. ECoRi 5. HaeIII 6. HindIII 7. SaeII 8. NciI	Each lane was successfully

Conclusions

The gel electrophoresis was successful. The phages DNA segments were visibile after illuminating it with specific wavelengths of light. The next step is to analyze this data in protocol 10.4.

▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @08:51 AM PST



IMG_0001.jpg(10.2 MB) - download This is a picture of the gel electrophoresis inside the UV light machine. Picture taken on 11/14/2017.

Protocol 10.4: Analyzing Restriction Enzyme Gels

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

revisions print ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @12:51 PM PST

Analyzing Restriction Enzyme Gels

11/21/2017

Aims and Purpose

In this protocol the restriction enzyme gel data was analyzed.

Changes in Protocol

No changes in protocol were made.

Data and Results

Object	Observation
Restriction Enzyme Gel	The gel reflects the sizes of each DNA fragment by showing different series of bands. Each lane has multiple fragments that migrated down the lane. The enzymes cleaved the phages DNA in different areas of its genome. The enzyme NciI, which was in lane eight, cut the most. The enzyme ClaI, which was in lane two, cut it the least. Nothing was wrong with the gel.

Conclusions

This protocol characterized the uniqueness of the phages genome. We can now compare this data with other phages and observe the similarities or differences within their genetic fingerprints.

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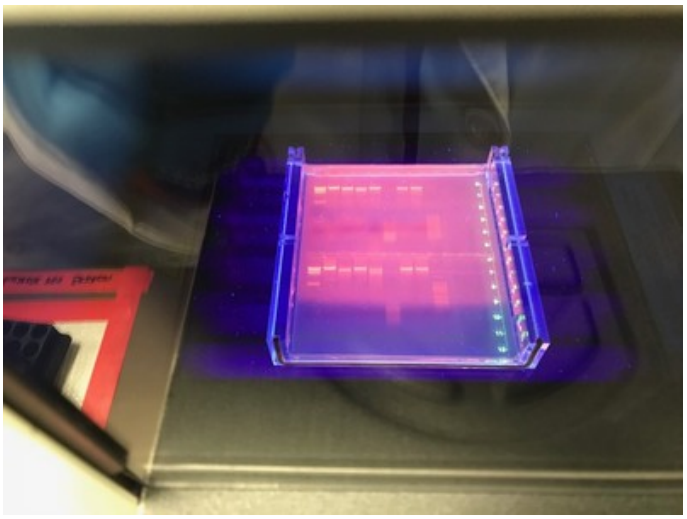
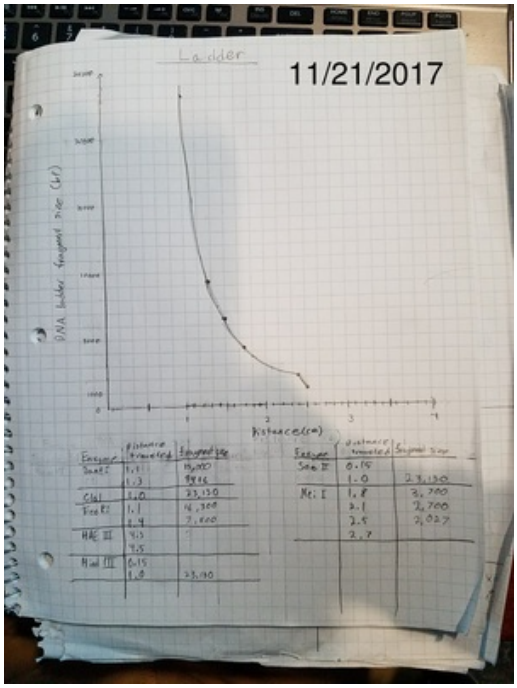


IMG_4257_1_.jpg(76.2 KB) - download This is a picture of the gel electrophoresis inside the UV light machine. Picture taken on 11/14/2017.

 ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @12:47 PM PST



20171121_121832_1_.jpg(1.7 MB) - download This is the graph of the ladder, and it was used to obtain the fragment sizes of the DNA.

 ▪ Kevin Ayala Pineda ▪ Nov 21, 2017 @12:35 PM PST

Enzyme used on phage DNA	Distance Traveled (cm)	Fragments size (bp)
BamHI	1.1 1.3	15,000 9,416
ClaI	1.0	23,130
EcoRi	1.1 1.4	16,300 7,500
HaeIII	4.3 4.5	smears
HindIII	1.0	23,130
SaeII	1.0	1.0
NciI	1.8 2.1 2.5	3,700 2,700 2,027

This data displays the size of the DNA fragments. This information was derived from the graph of the ladder.

Contamination issue 10/24/2017

SIGNED by Kevin Ayala Pineda Dec 31, 2017 @06:39 PM PST

revisions print ▪ Kevin Ayala Pineda ▪ Nov 20, 2017 @01:19 PM PST

Contamination issue

10/24/2017

Aims and purposes

Everyone in class came out with a contamination issue. The aim of this lab is to figure out where the contamination came from. Several procedures will be done.

Changes in Protocol

No changes were done to a protocol

Data and Results

#	Procedure
1	New plate + New Agar
2	Old Plate + New Agar
3	New Plate
4	Old Plate
5	New Plate + Old Smegmatis
6	Old Plate + Old Smegmatis

Conclusions

It was the bacteria that caused the contamination issues in the class. The old sample of bacteria was thrown away and a new colony M. Smegmatis MC^155 was made.

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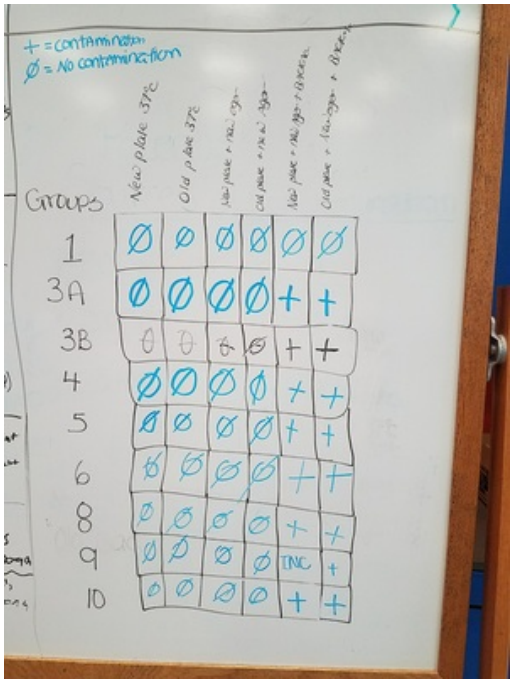

20171026_105735_1_.jpg(1.5 MB) - download This is the class data on the contamination issue. This showed that the bacteria was contaminated. Picture taken on 10/26/2017

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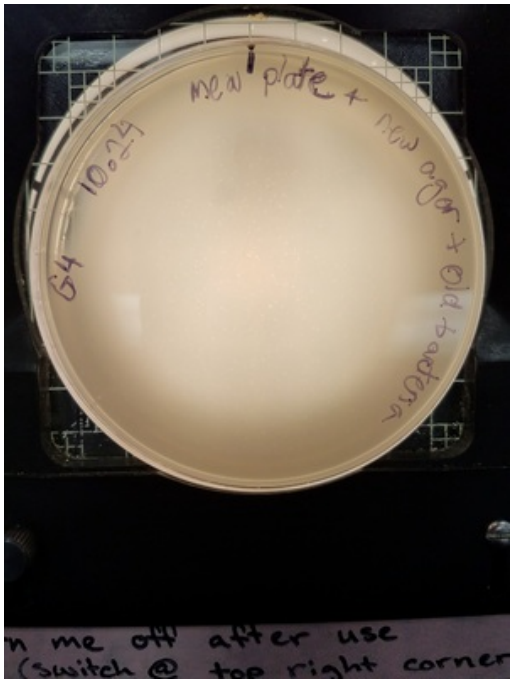

20171026_085514_1_.jpg(1 MB) - download This is a picture of new plate, new agar, and old bacteria. Picture taken on 10/26/2017.

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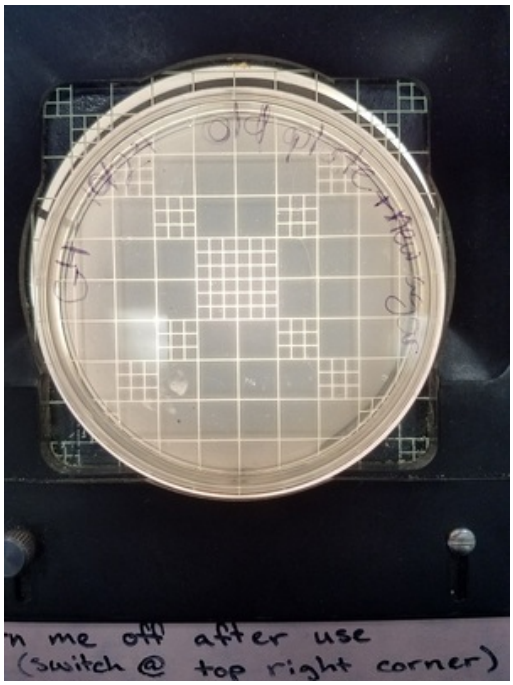

20171026_085524_1_.jpg(1.5 MB) - download This is a picture of a an old plate with new agar. Picture taken on 10/26/2017.

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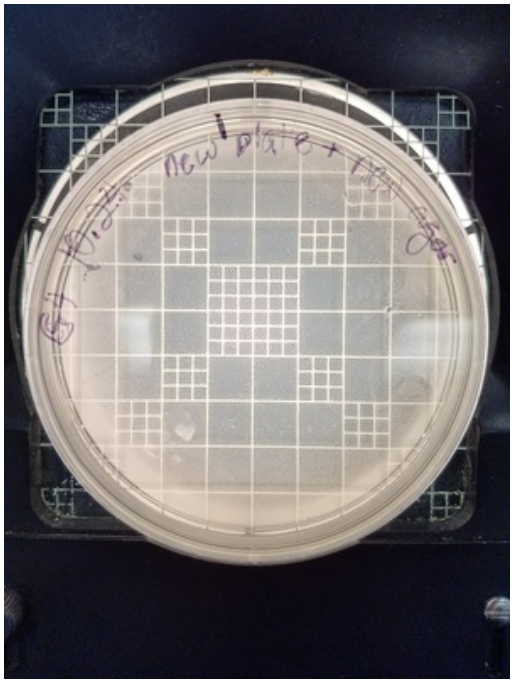

20171026_085543_1_.jpg(1.7 MB) - download This is a picture of a new plate with new agar. The picture was taken on 10/26/2017.

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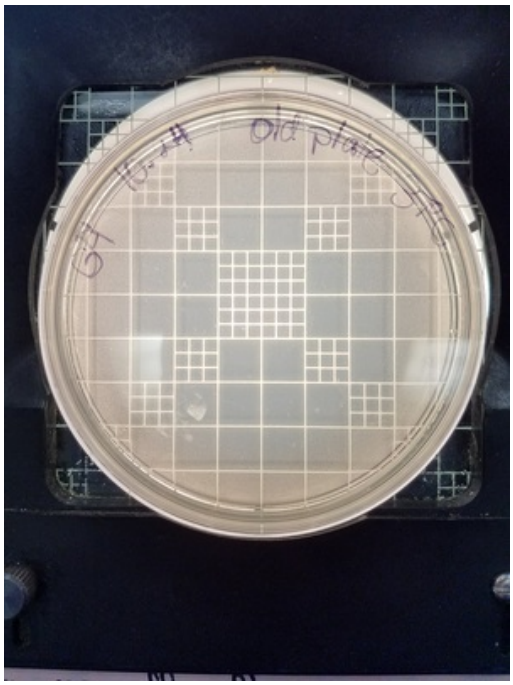

20171026_085533_1_.jpg(1.5 MB) - download This is a picture of an old plate which was incubated. The picture was taken on 10/26/2017.

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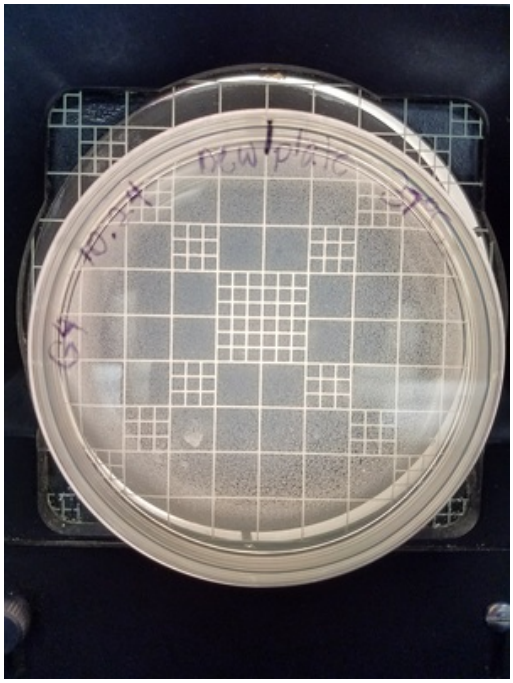

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20171026_085553_1_.jpg(1.7 MB) - download This is a picture of a new plate which was incubated. The picture was taken on 10/26/2017.