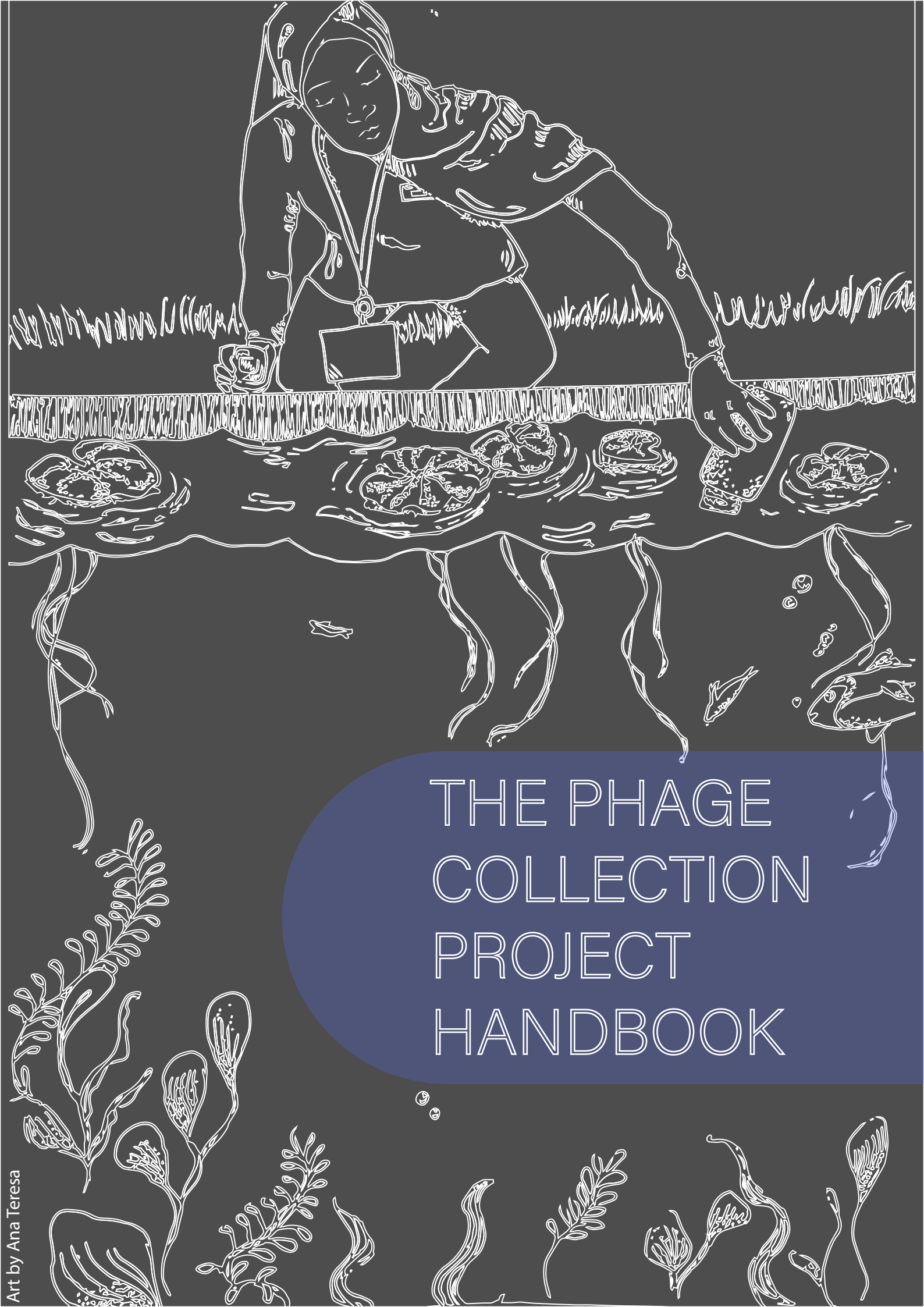


# THE PHAGE COLLECTION PROJECT HANDBOOK

Art by Ana Teresa


University of
Southampton

THE PHAGE COLLECTION PROJECT

# WELCOME

Welcome to the Phage Collection Project. A project designed by the Microbial Interactions Lab at the University of Southampton to engage you in University-level research.

At the Microbial Interactions Lab we focus on the arms-race between bacteria and their viruses, the bacteriophages. Our goal is to understand bacteriophages from a biological, ecological, and therapeutic perspective and to eventually develop innovative phage therapy approaches to fight antibiotic-resistant bacterial infections.

This project is not only designed to increase your knowledge in phage biotechnology, but also give you the skills to isolate your own phages and take part in an active scientific research community.

This handbook was created by Franklin Nobrega and Ruweyda Sayid.

THE PHAGE COLLECTION PROJECT

# MATERIALS NEEDED

Water sample for phage isolation

LB media

LB agar plates (LBA)

0.7% LB Top Agar

Escherichia coli K-12

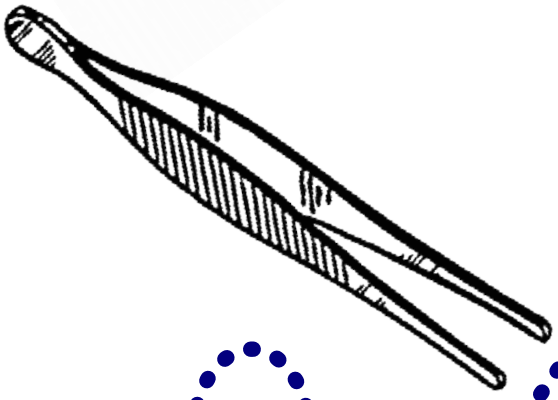
0.22 µM filters

20 mL syringes

Sterile paper strips

Sterile toothpicks

Sterile tweezers



THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 1*

# SAMPLE PREPARATION

We must first prepare the samples of water for phage isolation by removing debris. For this, we will use centrifugation and filtration.

1. Centrifuge the water sample a 3000 x g for 5 minutes at 4 °C, collecting the supernatant into a clean sterile tube.

Centrifugation spins samples at high speeds. This causes denser particles in a mixture to settle at the bottom of the tubes (the pellet), while lighter particles stay at the top (supernatant). Our phages are very small (between 20 and 200 nanometers) and therefore will stay in the supernatant after centrifugation.

2. Filter the supernatant into a clean tube using a 0.2 µm PES filter.

Polyethersulfone (PES) membranes of 0.2 µm pore size will remove finer particles, bacteria, and fungi that were not fully removed by centrifugation. These particles will be stuck at the filter, while phages, which are smaller than the pore size of the filter, can go through. This creates your filtered water sample.

THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 1*

# PHAGE DISCOVERY

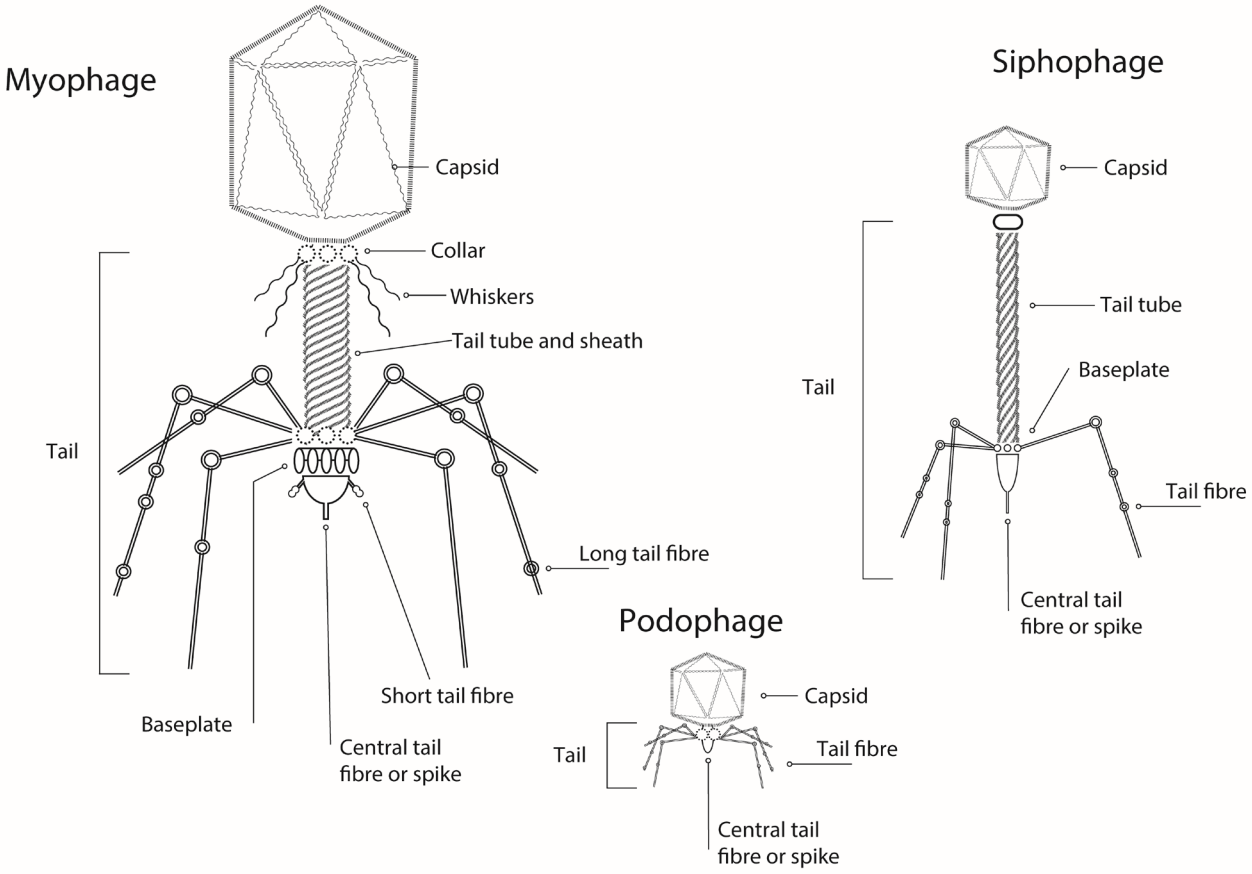
1. Add 10 mL of LB broth to a clean sterile tube.

2. Add 100 µL of the Escherichia coli culture grown overnight to the tube with LB broth.

3. Add 1 ml of the filtered water sample to the same tube.

4. Incubate this flask overnight at 30 °C, with rocking.

If a phage that targets Escherichia coli K12 is present in your sample, the phage will replicate and you will be able to detect it next week.

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THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 2*

# PHAGE TEST

1. Centrifuge your culture at 5000 x g for 15 minutes at 4 °C.

2. Collect the phage-containing supernatant and filter using a 0.2 µm PES filter.

In the supernatant that you just recovered, you may have a mixture of many different phages, and probably in high quantities.
To be able to get individual phages in a plate, you will need to dilute your sample first.

3. To prepare serial dilutions, first add 180 µL of LB broth to a row of wells on a 96-well plate.

4. Add 20 µL of the supernatant to the first well and mix by pipetting up and down.

5. Pipette 20 µL from the first well into the second well and mix well by pipetting up and down.

6. Repeat this procedure until you reached the final well.

Important! Change tip between each dilution and do this carefully to avoid cross contamination between wells.

7. Store the plate with the dilutions at 4 °C, to test next week.

THE PHAGE COLLECTION PROJECT

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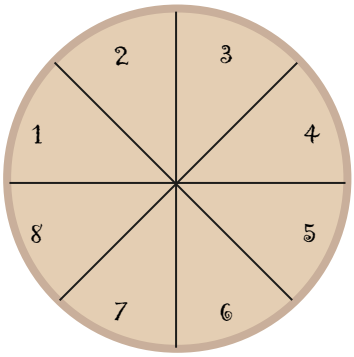
*Week 3*

# PHAGE SPOTTING




1. Prepare your LBA plate by drawing lines to create 8 equal segments on the bottom of the plate with a sharpie, and label the segments 1 to 8.

2. Mix 100 µL of bacterial culture and 3 mL of warm Top Agar in a tube.

The Top agar should be at around 50 °C, so that it does not harm the bacteria when you mix them. Do steps 2 and 3 quickly, before the Top Agar cools down and starts to solidify.

3. Pour the mixture onto an LBA plate to form a bacterial lawn.

4. Let the plate dry for 10 min.

5. Spot 5 µL of each phage dilution onto the bacterial lawn you prepared. Start with the most diluted sample.

6. Let the plate dry for 10-20 min.

7. Incubate the plate at 30 °C overnight.

If your sample contains phages, you will see plaques (clearing zones) formed on the top agar - where phages have lysed the bacteria.

THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 4-6*

# PURIFYING PHAGES

Inspect your agar plate from the previous week to see if you have any phage plaques. Select one (or more, if they look different) to purify.

1. Mix 100 µL of bacteria and 3 mL of Top Agar in a clean tube.

2. Pour this mixture onto an LBA plate to form a bacterial lawn.

3. Let the plate dry for 10 minutes.

4. Prick one phage plaque of your previous plate with a sterile toothpick and use this toothpick to prick the Top Agar in the freshly prepared plate 8 times in a row.

5. Using sterile tweezers, slide a strip of sterile paper over the row of punctures to spread the phages across the agar plate in a straight line.

6. Slide another strip of sterile paper half across the previous line, and across the agar plate in a straight line.

8. Repeat the previous step until you have covered the agar plate.

9. Incubate the plate overnight at 30 °C.

Repeat the purification for two additional weeks, or until you see that the morphology of the phage plaques is consistent.

THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 7*

# PRODUCING PHAGES

Now that you purified your phage, let's produce it in high amounts, so that it can be used to test host range (i.e., how many different bacteria it can infect) and other properties in the laboratory.

1. Add 100 mL of LB broth and 1 ml of the bacterial culture to a sterile 500 mL flask.

2. Incubate the culture at 30 °C with rocking until it reaches an optical density (OD) at 600 nm of 0.2.

This will allow us to infect the bacterial cells at their exponential phase, when the phage will achieve higher replication rates.

3. Using a sterile toothpick, select one individual phage plaque from your last round of purification and add to the culture flask.

4. Incubate the culture overnight at 30 °C, with rocking.

The phage will infect some of the bacterial cells, generating new phages that will burst from the cell and into the surrounding medium. Here, the new phages will encounter new bacterial cells to infect, generating more phages at each round of infection.

THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 8*

# PHAGE TITRATION

1. Transfer the culture into 2x 50 mL tubes and centrifuge at 5000 x g for 15 minutes at 4 °C.

2. Filter the supernatant with a 0.2 µm PES filter into 2 sterile 50 mL tubes.

Now you are going to check the amount of phage that you produced.

3. Add 180 µL of LB broth to a row of wells on a 96-well plate.

4. Add 20 µL of your filtered phage to the first well and mix by pipetting up and down.

5. Pipette 20 µL from the first well into the second well and mix well by pipetting up and down.

6. Repeat this procedure until you reached the final well.

Important! Change tip between each dilution and do this carefully to avoid cross contamination between wells.

7. Store the filtered phage and the plate with the dilutions at 4 °C, to test next week.

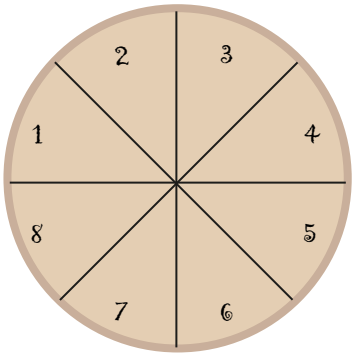
THE PHAGE COLLECTION PROJECT

# NOTES

THE PHAGE COLLECTION PROJECT

*Week 9*

# PHAGE TITRATION




1. Prepare your LBA plate by drawing lines to create 8 equal segments on the bottom of the plate with a sharpie, and label the segments 1 to 8

2. Mix 100 µL of bacterial culture and 3 mL of warm Top Agar in a tube.

3. Pour the mixture onto an LBA plate to form a bacterial lawn.

4. Let the plate dry for 10 min.

5. Spot 10 µL of each phage dilution onto the bacterial lawn you prepared. Start with the most diluted sample.

6. Let the plate dry for 10-20 min.

7. Incubate the plate at 30 °C overnight.

THE PHAGE COLLECTION PROJECT

# *Week 10*

# PHAGE TITRATION

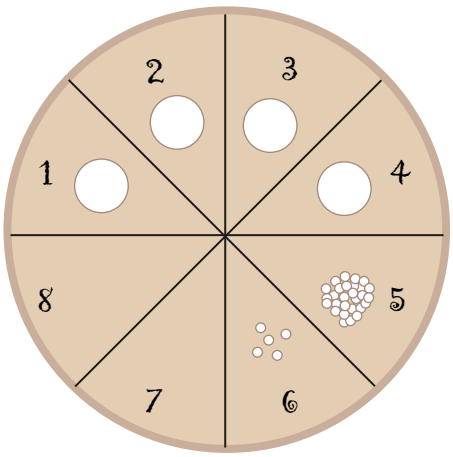
You are now going to check how well your phage replicated, by determining the concentration of the phage stock you produced.

1. Look at the agar plate where you spotted the phage dilutions. Look for the dilution where you can count between 3-30 individual phage plaques.

2. Determine the concentration of your phage using the formula:

$$\text{Phage concentration} = \frac{\text{number of phage plaques} \times 10^{\text{dilution}}}{\text{volume of sample plated (in mL)}}$$

Let's see an example. In the plate below, you can count the individual phage plaques in dilution 6. Also, you know from the protocol that you plated 10 µL of sample (= 0.01 mL). So, the formula becomes:




$$\text{Phage concentration} = \frac{5 \times 10^{6}}{0.01} = 5 \times 10^{8} \text{ pfu/mL}$$

3. Write the concentration of your phage in the sample tubes.

The phages will be stored at the biobank of the Microbial Interactions Lab for future research.

THE PHAGE COLLECTION PROJECT

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THE PHAGE COLLECTION PROJECT

# CONGRATULATIONS!!

## You have isolated your first phage!

We would like to thank you for taking part in the phage collection project.

Through your involvement with the project, you have participated in an active research community. The phages you have collected and isolated will help us understand phages better and lead to innovative solutions in phage therapy. We look forward to conducting further experimentation on your phages and sharing these findings with you. We hope you have enjoyed this project as much as we have enjoyed creating it.

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