

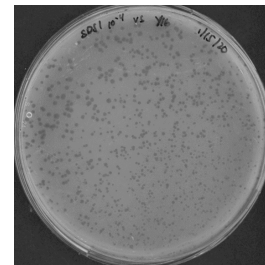
Apr 15, 2020

🌐 Plaque Assay

📁 In 1 collection

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Protocol status: Working

We use this protocol and it's working

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Abstract

Bacteriophages (phage) are viruses that infect bacteria and can be found anywhere that bacteria is found. Performing plaque assays is a technique to purify a population of viruses and can also be used to determine the viral concentration of a given sample. This protocol will outline the steps to carry out plaque assays.


Materials

MATERIALS

 Brain Heart Infusion Broth **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53286-500G**

 UltraPure LMP Agarose **Invitrogen - Thermo Fisher Catalog #16520-050**


 Agar **Catalog #BP9744-2**

 SM Buffer

Snap Cap Tubes, Disposable Inoculating Loops, Shaker, 37C incubator, Petri Plates, Glass Tubes, Serological Pipette, 5mL Disposable Serological Pipette Tips, 1.5uL tubes, Microwave

Troubleshooting

Safety warnings

-  - PPE should be worn at all times (i.e. gloves, lab coat, safety glasses)
- Work with phages should be performed in BSL2

Before start

- Make BHI plates
- Make BHI broth media
- Make BHI + 0.35% LMP agarose
- Make SM buffer

Inoculating a liquid bacterial culture overnight

13h

1 The day before performing the plaque assay experiment, grow the bacteria associated with the phage of interest.

13h

1.1 Take out an already streaked bacteria plate from the $4\text{ }^{\circ}\text{C}$ room.

1m



This is an example of how a streak plate of your bacteria should look like.

1.2 Take a snap cap tube and place it on a rack. Open the cap and aseptically transfer 2 mL of BHI media into the tube.

2m

1.3 Take a disposable inoculating loop and scrape a colony from your bacteria streak plate and transfer into the snap cap tube with 2 mL of BHI media. Recap the snap cap tube and throw the used inoculating loop in the red biohazard bin.

3m

1.4 Transfer the snap cap tube to the $37\text{ }^{\circ}\text{C}$ incubator room and place it on the shaker. Then turn the shaker on to 200 rpm . When leaving the room, remember to turn off the light switch on the outside of the room.

5m







1.5 Grow the bacteria on $37\text{ }^{\circ}\text{C}$ shaker overnight.

12h



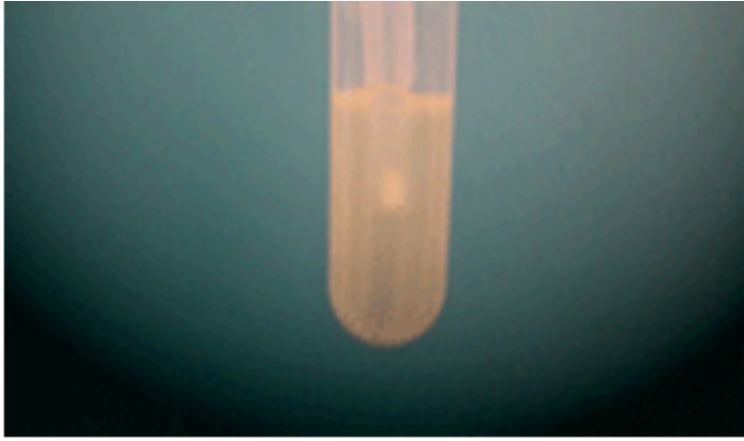
Inoculating liquid bacterial culture day of experiment

3h

- 2 The day of the experiment, take the overnight grown bacteria and transfer to fresh media to grow so that we will be working with log-phase bacteria.
 - 2.1 Take a new snap cap tube and aseptically add  2 mL of fresh BHI media into the tube.
 - 2.2 Take the overnight grown bacteria from the  37 °C incubator. Aseptically transfer  100 µL of the overnight culture into the new tube with  2 mL of BHI media.
 - 2.3 Transfer this snap cap tube to the  37 °C incubator room and place it on the shaker. Then turn on the shaker to  200 rpm .
 - 2.4 Incubate the sample on the shaker for ~3 hours or until the sample has become cloudy indicating bacterial growth.

Media without growth (top) and with growth (bottom)





Preparing BHI plates

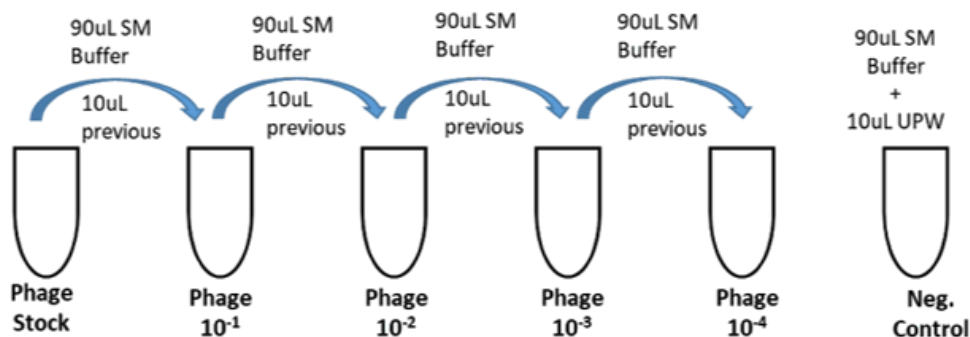
20m

- 3 Take out the required number of BHI plates from the $4\text{ }^{\circ}\text{C}$ incubator room. Wipe down the hood with 70% ethanol. Place the plates inside the hood with the lid halfway open. This will dry out the liquid droplets from the plate.

Phage Serial Dilution







30m

- 4 In order to determine the concentration of the phage stock, you will need to perform a serial dilution of the stock.



Example of a 10-fold dilution series.

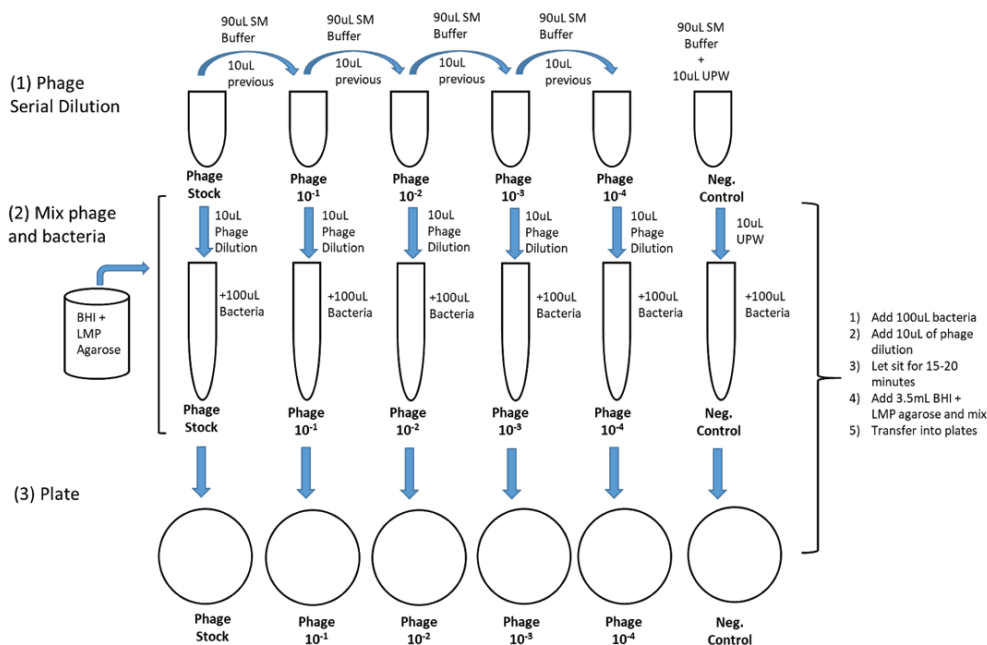
- 4.1 Take the phage stock tube from the $-80\text{ }^{\circ}\text{C}$ freezer and let it thaw at room temperature. The location of the phage stocks can be determined by looking at the MissionControl document on Google Drive.

- 4.2 To perform a 1:10 dilution of the stock sample, add  90 μL of SM buffer into a 1.5mL tube then add  10 μL of the stock phage into the tube. Mix well.
- 4.3 To perform a 1:100 dilution of the stock sample, add  90 μL of SM buffer into a 1.5mL tube then add  10 μL of the 1:10 phage dilution from Step 4.2 into the tube. Mix well.
- 4.4 Continue performing the serial dilutions for how many samples you will be observing.
- 4.5 Create a tube for the negative control by adding  90 μL of SM buffer and  10 μL of Ultra Pure Water (UPW) into a 1.5mL tube.

Plaque Assay


2h

- 5 Combine the log phase bacteria and serially diluted phages then plate them.





- 5.1 Take a bottle of BHI + 0.35% LMP agarose and microwave until boil. The microwave is located in BSL1 so make sure to remove all BSL2 PPE before entering BSL1.


10m

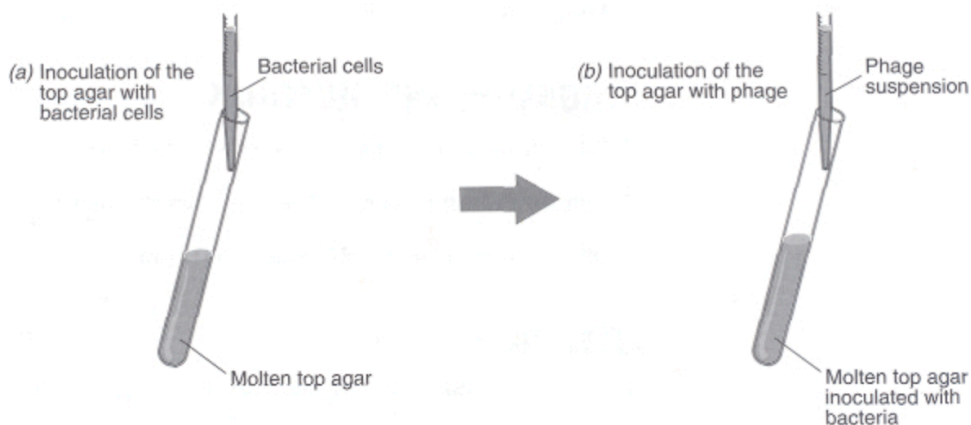
Start off with  00:00:30 then gradually add more time to prevent boil over of the media onto the microwave. Use paper towels or an autoclave glove to handle the glassware and transfer it back to BSL2.

5.2 Ensure that the BHI + 0.35% LMP agarose cools down before using it or it may heat shock the bacteria and kill it. 15m

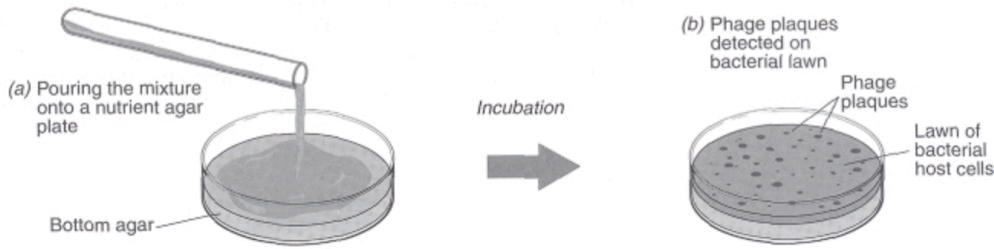
5.3 Take a glass culture tube and label it appropriately with the sample.

5.4 Add  100 μL of log-phase bacteria from Step 2 into the glass tube. Then add  10 μL of the phage dilution into the same glass tube. Let sit for 15-20 minutes. 20m

5.5 Use a serological pipette and measure  3.5 mL of cooled down BHI + 0.35% LMP agarose. Aseptically transfer to the glass tube and mix by pipetting up and down but not quickly to prevent any bubble formation.



5.6 Transfer the contents of the glass tube onto a dried BHI plate and swirl the plate around to properly disperse the contents.



- 5.7 There is a green taped X mark on the sink counter which is leveled. Place the plate on top of the X mark and let it solidify.
- 5.8 Continue for the rest of the samples. Don't forget to always perform a negative control plate.
- 5.9 After the plates have solidified, store in the 🌡️ 37 °C incubator upside down. Incubate overnight.

Determine the phage concentration

30m

- 6 The next day, observe the plates to determine the concentration of the viral sample from the stock.
 - 6.1 Look at the plates and find one where there are enough phage forming units (PFU) that can be counted. Count the number of plaques on the plate.
 - 6.2 Use this formula:

$$\text{PFU/uL} = \text{Number of Plaques Counted} / (\text{Dilution} \times \text{Volume of Diluted Virus Added})$$

Example: There are 28 plaques on a 10^{-2} phage dilution plate. You had plated 10uL of this along with 100uL of bacteria.

$$28/10^{-2} \times 10\text{uL} = 280 = 2.8 \times 10^2 \text{ PFU/uL}$$