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Amplifying Bacteriophage

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is for the amplification phage by making webbed plates (Path A; ~3days) or by liquid culture (~2days). This protocol also details how to titer phage through a spot test.

Materials

Phage Buffer

- 100mL ddHO
- 2mL 100mM $MgSO_4$ (or 200µL 1M)
- 1mL 500m$CaCl_2$
- 1mL 1M Tris-Cl
- filter sterilize 0.22µm

T-top

- 10g Tryptone
- 8g NaCl
- 1L ddH2O
- Mix and aliquot 100mL into bottles
- Into bottles, put 0.75g agar
- autoclave

T-broth

- 10g Tryptone
- 8g NaCl
- 1L ddH2O

T-plate

- 10g Tryptone
- 8g NaCl
- 15g Agar
- 1L ddH2O

Day 1 Path A: Plating Bacteria and Phage to make a webbed plate

15m

1 **Incubate Bacteria and Phage**

15m

100µL of overnight culture
100µL of bacteriophage culture
You will need to adjust this accordingly based on the phage titer.
Time of incubation will also be dependent on the phage: ~00:10:00-00:30:00

2 **Making Top agar** (With nutrient deficient T-plates)

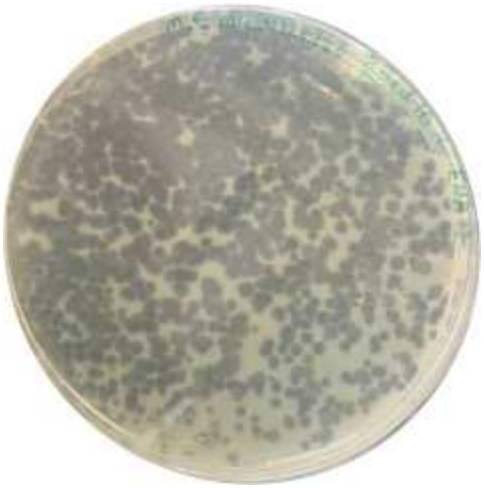
1d

Make melted agar from T-top, T-broth, and 1M $CaCl_2$

Each plate should have a total of 3mL top agar

- 1:1 ratio of T-top and T-broth and for every 10mL 100µL of $CaCl_2$
- Mix 1M $CaCl_2$ and T-broth
- Add melted T-top
- Ex.) if you were making 4 plates (4 * 3 = 12mL). Meaning you would add 6mL T-top, 6mL T-broth, and ~100µL $CaCl_2$

Take the 200µL phage and bacterial mix and mix with 3mL top agar - pour immediately onto T-plate
Sit at room temperature to solidify
Typically 2-3 plates will give a good titer
Note: make sure that the top agar is not too hot that it will immediately kill the bacteria around 40-60˚C depending on bacteria



Webbed Plate

Day 2 Path A: Scrape Method

15m

3 **Prepare items**

- Beaker with ethanol and metal scraping device
- Conical tube with 5mL phage buffer
- 0.22µm filter and syringe
- Sterile conical tube for final filtrate

4 **Scraping** 15m

Sterilize the scraper with ethanol and bunsen burner - wait to cool
Physically scrape off the top bacteria and phage from the T-plate and place in the 5mL of phage buffer. If you have more than one plate combine all scrapings into the same 5mL phage buffer

Centrifuge 4˚C 00:10:00 5,000 RPM

- Filter supernatant with 0.22µm
- Store phage at 4˚C
- To freeze phage in -80˚C: 1mL phage + 1mL 50% Glycerol

Day 1 Path B: Liquid Amplification of phage

4h 45m

5 **Liquid Culture** 45m

In a test tube:
5mL of desired broth (usually LB)
100µL Bacteria
10µL 1M $CaCl_2$
Incubate until log phase (*E. coli* ~00:45:00)

6 **Bacteriophage** 4h

Add Bacteriophage: the amount you add will depend on your titer. For *E. coli* lytic phage, 10µL will result in amplified phage.
Note: incubation times after adding bacteriophage vary depending on host bacteria. For E. coli ~04:00:00 will give you titers of 10^{8+} *phage.*

7 **Filter**

Filter the amplified phage with a 0.22µm filter into a sterile conical tube

- Store phage at 4˚C
- To freeze phage in -80˚C: 1mL phage + 1mL 50% Glycerol

Checking Phage Titer

1d

8 **Making Top agar** (With nutrient deficient T-plates)

Make melted agar from T-top, T-broth, and 1M $CaCl_2$
Each plate should have a total of 3mL top agar

- 1:1 ratio of T-top and T-broth and for every 10mL 100µL of $CaCl_2$
- Mix 1M $CaCl_2$ and T-broth
- Add melted T-top
- Ex.) if you were making 4 plates (4 ∗ 3 = 12mL). Meaning you would add 6mL T-top, 6mL T-broth, and ~100µL $CaCl_2$

- Take the 100µL host bacteria and mix with 3mL top agar - pour immediately onto T-plate

Sit at room temperature to solidify

9 **Serial Dilution** 1d

Perform 10-fold serial dilution of filtered phage out to -8 to -12
Spot 5µL of each serial dilution onto the solidified T-plate
Incubate overnight

Note: if you want to have a more accurate titer, serial dilute and mix onto an entire plate with 100µL host bacteria and 3mL top agar