Bacteriophage culture and titer assay

Cultivation of Bacteriophage

A. liquid culture protocol

1. Prepare an actively growing broth culture of the recommended host strain. Host should be 16 to 24 hours old.

2.Mix 100µl of original phage nutrient broth with the desired indicator bacterium broth culture 300µl. Allow to infected for 15 minutes.

3. Add the mixture liquid into a test tube filled with 10ml fresh culture medium, incubated in vibrating culture under the appropriate conditions depends on different phages.

4. Spin down the bacteria in the original culture tube for 10 min 10,000 rpm (5,000g).

5. A clear supernatant liquid was filter through a 0.22µm filter to remove residual bacterial cells in order to obtain the phage without host strain. Because of the liquid is clear and will not able to see the growth of the bacteria; therefore, we need to using potency assay to determine the growth of the bacteria.

B. Agar overlay culture protocol

1. Prepare an actively growing broth culture of the recommended host strain. Host should be 16 to 24 hours old.

2.Mix 100µl of original phage nutrient broth with 300µl desired host bacteria broth culture. Allow to infected for 15 minutes.

3.Add the mixture into 5ml 45°C , 0.7% agar, mix well then pour out onto 1.8% agar plate.

4.When fully gelled, incubated cultures under the appropriate conditions for about 8 to 24 hours, depends on different strain.

5. Repeat steps 1 through 4 to produce culture without phage for comparison.

6. Compare two plates, one with phage will be more clear and the one with host strain will be more turbid.

7. Put the phage plate in -20℃ for 4 to 5 hours then move out to thaw at room temperature.

8.Spin down the bacteria in the original culture tube for 10 min 10,000 rpm (5,000g).

9. A clear supernatant liquid was filter through a 0.22μm filter to remove residual bacterial cells in order to obtain the phage without host strain.

C. Agar surface culture protocol

1. Prepare an actively growing broth culture of the recommended host strain. Host should be 16 to 24 hours old.

2. Take 3ml host bacteria broth overlay onto 1.8% agar plate for 2 hours.

3. Suck out the exceeded host bacteria broth then overlay 0.3ml original phage nutrient broth onto the agar.

4. Incubate cultures under the appropriate conditions usually around 16-24 hours depends on different phages.

5. Add 5ml fresh culture medium onto the plate and using L-shaped glass rod to scrape the phages off the plate.

6. Harvested all the cells into a centrifuge tube and spin down the host bacteria at 10,000 rpm (5,000g) for 10 minutes.

7. A clear supernatant liquid was filter through a 0.22μm filter to remove residual bacterial cells in order to obtain the phage without host strain.

Bacteriophage potency assay

A. Titrating

1. Prepare an actively growing broth culture of the recommended host strain. Host should be 16 to 24 hours old.

2. Add the mixture into 5ml 45°C , 0.7% agar, mix well then pour onto 1.8% agar plate for about 30 minutes.

3. 3. Prepare a 10-fold serial dilution of the phage by using 1% peptone, usually a dilution of 107

4. Divided the plate for eight areas then add 1µl of each dilution to each area using pipetman.

5. Incubate cultures under the appropriate conditions usually culture for 8 to 24 hours. Count the plaques and calculate the phage titer. The high concentration of phage will produce a big plaque and the right concentration will produce plaque for eye observation.

6. The phage titer (pfu/ml) =number of plaques on a plate X the dilution factor X the dilution number

For example: if there were 15, 18, 16 plaques on each plate made from a 106 dilution, 0.001ml of dilution was plated (the dilution number is 1000), there are **$1/3(15+18+16)\times10^{6}\times1000=1.63\times10^{10}$ (pfu/ml)**

B. Agar overlay culture protocol

1. Prepare an actively growing broth culture of the recommended host strain. Host should be 16 to 24 hours old.

2. Prepare a 10-fold serial dilution of the phage by using 1% peptone, usually a dilution of 107

3. Mix 100µl of original phage nutrient broth with the desired indicator bacterium broth culture 300µl. Allow to infected for 15 minutes.

4. Add the mixture into 5ml 45°C , 0.7% agar, mix well then pour out onto 1.8% agar plate.

5. Incubate cultures under the appropriate conditions usually culture for 8 to 24 hours. Count the plaques and calculate the phage titer.

6. The phage titer (pfu/ml) =number of plaques on a plate X the dilution factor X the dilution number

For example: if there were 275, 252, 263 plaques on each plate made from a 107 dilution, 0.1ml of dilution was plated (the dilution number is 10), there are **$1/3(275+252+263)\times10^{7}\times10=2.63\times10^{10}$ (pfu/ml)**