PREPARATION OF HIGH TITER PHAGE

(Adapted from Kauffman and Polz 2018)

Introduction

This protocol produces a phage stock of 5 to 10 mL containing 10^8 to 10^{11} CFU /mL. This stock should be stored at 4°C and -80°C (in 25% glycerol), as phages are more or less stable.

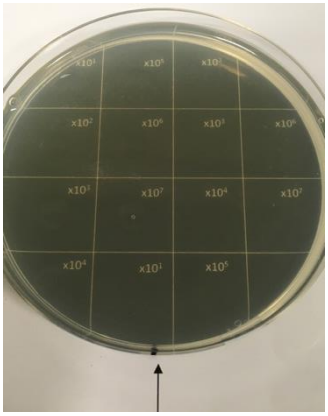
Materials (example for V. crassostreae)

- Marine broth (MB)
- Marine agar (MA)
- Top agar (entre 0.4 or 0.2% depending on the phages)

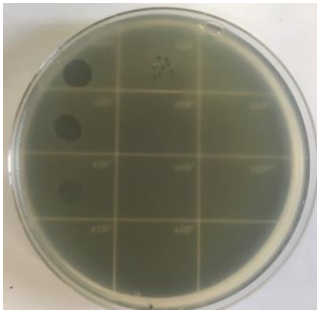
Method

1. Spread a volume of host overnight culture on MA plates with a fresh agar overlay (100 µl host+2,5 ml top agar for small plates; 250 µl host+7,5 ml top agar for large plates). Use small (8,5 cm) or large plates (13,5 cm) depending of the number of phages per host. Dry the plates for a short time on bench.

2. From a purified phage stock prepare decimal dilution series in host-appropriate media (10^{-1} to 10^{-7}) (e.g. 5 µl phage+45 µl media). If you are not interested in knowing the titer of purified stock, dilutions 10^{-1} to 10^{-3} would be enough (recommended). Pipette 10 µl drop spots onto a fresh host agar overlay (use the certain grid as template). After, don´t remove these plates and dilution tubes until you finish the high tittering (if you need a previous enrichment, see below, they are very useful to get good plaques or if you need repeat).



3. Once plaques have developed in the drop series identify the dilution that gives confluent lysis and measure the drop and calculate the drop area. Calculate the volume necessary to achieve confluent lysis on the large plate taking into account the surface of the large plate ($A=\pi r^2=3,14x6,75^2= 143\ cm^2$).



4. Prepare a mixture of 250 μl of overnight host culture and adequate volume calculated previously. Dispense this mixture onto bottom agar in a small or large dish with agar overlay lawn (7,5 ml for large dish), swirl to mix and incubate.

5. Prepare also a negative control in small Petri dish containing 100 μl host and 2,5 ml top agar that serves as a useful reference in assessing plaque development.

6. Check if phages achieve confluence, or near confluence, lysis on the host lawn (see plates against the light). Add 12 ml of MB on the lawn with the phage lysate and incubate the plate at 4ºC overnight to allow phages to elute from top agar.

7. Collect the MB by decanting in Falcon tube (a part of volume elution will be lost during the incubation, final volume approx. 10 ml). Centrifuge at 5000xg for 20 min and filter supernatant by 0,2 μm and store at 4ºC. For long term storage it is recommended that 25% (final volume) glycerol stocks be prepared for storage at -20ºC and -80ºC, as well as raw lysate at 4ºC.

8. Determine the titration of "high titer" stock (titration expected 10^9-10^{10} PFU/ml). Prepare 100x dilution series from 10^0 to 10^{-8} (e.g. 4 tubes 10 µl phage+ 990 µl host appropriate media) and transfer the adequate volume of each tube (e.g. 10 µl) to next tube. Prepare a mixture of 100 μl of overnight host culture and 10 μl phage from dilution 10^{-6} to achieve a dilution 10^{-8} on plate. Prepare another mixture using 100 μl phage from dilution 10^{-8} to achieve dilution 10^{-9} on plate. Dispense these mixtures onto bottom agar in a small Petri dish, swirl to mix and incubate. Finally, calculate the titration of the phage stock.

NOTE: If the volume calculated from point 3 is a limiting factor you could solve this problem creating a primary small-scale enrichment: liquid or using a small plate.

Reference

Kauffman KM, Polz MF. Streamlining standard bacteriophage methods for higher throughput. MethodsX. 2018 Jan 31:5:159-172. doi: 10.1016/j.mex.2018.01.007.