Bacteriophage Enrichment

Introduction

This protocol describes the P.larvae bacteriophage enrichment process in order to reproduce bacteriophages with a high titer (>10^9 PFU/mL).

Materials

- BHI Media
    - BHI Powder 37 g/L
    - Yeast Extract 3 g/L
- 1x TM Buffer
- sterile H_2O

Procedure

- **Day 1** - *Paenibacillus larvae* (*P.larvae*) Bacterial Lawn Preparation

    1. Take one row of the bacteria lawn from a fresh (max 2 days old) *P.larvae* streak plate and dissolve them in 200 µL sterile dd.H_2O
    2. Plate out those 200 µL of the bacterial suspension on a MYPGP Agar plate
    3. Incubate overnight, 37 °C

- **Day 2**

- 5 mL *P.larvae* ONC:

    1. Resuspend the *P.larvae* ONC Plate with 2 mL ddH_2O (sterile) and pipette it in a 1.5 mL reaction tube
    2. Centrifuge 5,000 x g, 5 min in the eppifuge
    3. Discard the supernatant, and dissolve the Pellet in ca. 250 µL ddH_2O (sterile)

4. Create 1:50 and 1:500 dilutions from the Probe and measure OD_{600}

5. Set the OD_{600} of the bacterial probe to be OD_{600} = 15

6. Pipette 5 mL BHI Media and 50 µL of bacterial suspension (OD_{600} = 15) into a 15 mL falcon tube

7. Add 500 µL Phage lysate to the falcon tube

8. Incubate overnight at 37 °C, 200 rpm

- 50 ml *P.larvae* ONC

1. Take one row of the bacteria lawn from a fresh (max 4 days old) *P.larvae* streak plate and dissolve them in 200 µL sterile dd.H_2O

2. Pipette the bacterial suspension into 50 mL BHI Media

3. Incubate overnight at 37 °C, about 200 rpm

- **Day 3**

1. Add 500 µL $CHCl_3$ (Chloroform) to the Probe (5 mL ONC), vortex and let it sit for 5-10 min

2. Centrifuge the $CHCl_3$-free suspension (2 mL is enough) at 12,000 x g, 15 min

3. 50 mL *P.larvae* ONC + 1 mL Phage lysate

4. Incubate at RT, 15 min

5. Add the Probe into 400 mL BHI Media

6. Incubate at 37 °C, ca. 120 rpm, 4 days

- **Day 7**

1. Cool the ultracentrifuge rotor at 4 °C

2. Give up to 0.5 M NaCl and 5-10 % $CHCl_3$ to the Phage culture

3. Incubate 5-10 min, ca. 200 rpm
4. Transfer the $CHCl_3$ free solution to the 25 mL ultracentrifuge tubes

5. Centrifuge 12,000 x g for 15 min

6. Transfer the Phage-supernatant in a sterile 500 mL Media bottle
    - Store at 4 °C
    - If not using that day, put 1 drop of $CHCl_3$ in it in order to prevent bacterial growth

7. Wash the ultracentrifuge tubes with soap, and let them dry

8. Take 1 mL Aliquot for the phagetiter determination

9. Centrifuge the Phage-supernatant at 100,000 x g, 4 °C, 1.5 h

10. Discard the supernatant and fill up the tubes with the rest of the Phage lysate

11. Centrifuge and repeat at above until there is no more phage lysate

12. Discard the supernatant

13. Pipette about 3 mL of sterile 1x TM Buffer into the tubes

14. Put the tubes (45° angle) into a shaker, overnight, 16 °C, 140 rpm in order to dissolve the phage pellet

- ## Day 8

15. Dissolve the undissolved phage pellet with a inoculating loop

    - store at 4 °C

16. Unite all the phage pellet solutions into one sterile 50 mL falcon tube

17. Determine the phage titer via Plaque drop assay

18. If the phage titer is >10^9 PFU/mL, than proceed to the purification step with a caesium density gradient centrifugation.