

Phage genome purification by CsCl:

Day 1

1. Incubate cells until $OD_{600nm}=0.1-0.2$ (dilute ON broth culture 1:100 and incubate at 37 1h15min).
2. Add phages crude lysate at MOI=1 (for EB49) or MOI=10 (for Bas06,07 and 32).
3. Incubate overnight.

Day 2

4. Centrifuge at 7,000 rpm for 25 min at 4 and collect supernatant.
5. Add chloroform (3.3%) and vortex to kill bacteria (1.67mL per 50mL phage crude lysate).
6. Collect supernatant and add 10% PEG and 1M NaCl (100g PEG-8000 and 58g NaCl·H₂O per liter phage crude lysate).
7. Incubate at 4 overnight.

Day 3

8. Centrifuge at 7,000 rpm for 25min at 4.
9. Prepare 13mL phage buffer with CsCl (0.75g/mL).
10. Re-suspend in 13mL phage buffer per liter phage crude lysate.
11. (Optional) Add DNase/RNase (10U per liter phage crude lysate) and incubate for 2.5h at 37.
12. Centrifuge at 30K rpm for 18h at 4.

Day 4

13. Pipette off CsCl mixture above phage band first, then harvest band by syringe.
14. Dialyze mixture 1:1000 in sterilized water overnight to remove CsCl.

Day 5

15. Purify phage genomes by Norgen kit.