

Preparation of Cyanophage DNA from a Cleared Lysate*

Protocol Developers

Marcia Osburne (mosburne@MIT.EDU), Suzanne Kern, Matt Sullivan, & Penny Chisholm (chisholm@MIT.EDU)

Citing Protocol

Henn MR, Sullivan M, Osburne M, Stange-Thomann N, Suzanne Kern, Berlin A, Weiland M, Young S, Saif S, Nusbaum C, Birren B, Chisholm S (2009) Full-genome sequencing methods for phage from small amounts of template DNA. (in preparation)

Yield : 100 ng – 1 µg DNA

(*protocol omits nuclease and proteinase K to minimize phage DNA degradation)

1. Spin 100 ml of phage lysate for 10 minutes at ~16,500xg to pellet any remaining cells and cell debris. Save supernatant (lysate) and discard the pellet.
2. Put 100 ml phage lysate into centrifuge tube or bottle.
3. Add 4 ml *Phage Precipitant*.
4. Incubate on ice for 1 hour.
5. Centrifuge at 10,000xg for 10 min, then decant and discard supernatant.
6. Resuspend pellet in 500 µl *Phage Buffer*.
7. Transfer supernatant to 2 ml Eppendorf tube.
8. Add 1 ml *Purification Resin* and mix by inverting tube.
9. Attach minicolumn to bottom of syringe and add contents of Eppendorf tube.
10. Use a plunger from a 3-ml syringe to push the slurry through the syringe (what comes through the syringe at this point is waste – DNA remains on the column).
11. Remove minicolumn from syringe and pull out plunger.
12. Reattach minicolumn and add 2 ml 80% isopropanol to syringe.
13. Use the plunger to wash the minicolumn, pushing through the isopropanol.
14. Remove minicolumn from syringe and place minicolumn back in first Eppendorf tube (2ml).
15. Centrifuge at 10,000xg for 2 min to remove any remaining liquid.
16. Place minicolumn in new (fully-labeled) 1.5 ml Eppendorf tube.
17. Add 100µL 80°C TE Buffer and immediately centrifuge at 10,000xg for 20 sec. to elute phage DNA.
18. Discard minicolumn and store purified DNA at 4°C or -80°C.

Materials used:

Phage Precipitant

33% polyethylene glycol (PEG-8000)
3.3M NaCl (molecular weight: 58.44 g/mol)

For 800 ml: 267 g PEG-8000
154.3 g NaCl
H₂O up to 800 ml

Phage Buffer

150mM NaCl
40mM Tris-HCl (pH 7.4)
10 mM MgSO₄

For 100 ml: 12.5 ml NaCl (1.2 M stock)
3.2 ml Tris-HCl (1.25 M stock)
1 ml MgSO₄ (1 M stock)
H₂O up to 100 ml

TE Buffer

10mM Tris-HCl (pH 7.5)
1mM EDTA

For 20 ml: 160 μ l Tris-HCl (1.25 M stock)
40 μ l EDTA (0.5 M stock)

DNA Purification Resin (store in box, away from light)

Promega product A7181 – 250 ml

Minicolumns

Promega product A7211 – 250 columns