

Propagation of phage vB_EcoM_FV3 (FV3)

Day I

1. Grow *E. coli* K-12 derivative (preferably MH1, K803, XL1 or DH5 α) in LB medium at 37°C to OD₆₀₀ 0.5-0.8 and place the culture on ice.
2. Prepare 10⁻⁶, 10⁻⁷, 10⁻⁸ and 10⁻⁹ serial dilutions of FV3 phage suspension (in LB medium).
3. Plate 3 ml soft LB agar (0.5-0.6%), 500 μ l of bacterial culture and 100 μ l of diluted phage suspension by double-layer agar method.
4. Incubate plates at 37°C overnight.

Day II

1. Pick the content of a single phage plaque and suspend in 100 μ l of LB.
2. Grow the same *E. coli* strain in 10 ml LB at 37°C to OD₆₀₀ ~0.5 and then add the content of suspended phage plaque.
3. Incubate the flask at 37°C with agitation (~180 rpm.) until complete lysis occurs (~1 h).
4. Remove cell debris by centrifugation (20 min at 4000 \times g).
5. Precipitate phage particles from supernatant by centrifugation for 1-2 h at ~15000 \times g.
7. Add to the phage pellet: 1 ml of phage buffer (SM or PB), few drops of CHCl₃ and DNaseI (up to 1 μ g/ml).
8. Incubate at room temperature for 2 h with gentle agitation (or allow to slowly resuspend for 24 h at 4 °C).
9. Centrifuge the content for 10 min at 5000 \times g.
10. Collect the supernatant, which usually is clear and contains ~1-5 \times 10¹¹ phage particles.
11. Titrate phage suspension as described in (Day I).
12. Store at 4°C.

Notes:

- Phage may be further purified by centrifugation in CsCl density gradient.
- Phage may be stored at -80°C in the buffer with 50-80% glycerol.

SM (100 mM NaCl, 8 mM MgSO₄, 50 mM Tris-HCl at pH7.5)

PB (40 mM Na₂HPO₄, 22 mM KH₂PO₄, 70 mM NaCl, 1 mM MgSO₄)

MgSO₄ should be autoclaved or filtered separately and added to the cold autoclaved solutions.

References:

1. Kropinski AM, Mazzocco A, Waddell T, Lingohr E, Johnson RP (2009) Enumeration of bacteriophages by double agar overlay plaque assay. In: Clokie MRJ, Kropinski AM (eds) Bacteriophages: methods and protocols. Humana Press, New York, pp 69–76.
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3. Carlson K., Working with Bacteriophages: Common techniques and Methodological Approaches. In: Kutter E, Sulakvelidze A (2005) Bacteriophages: biology and applications. CRC Press, Boca Raton, pp 437-490.
4. Carlson K, Miller E (1994) Experiments in T4 genetics. In: Karam JD (ed) Bacteriophage T4. ASM Press, Washington D.C., pp 419-483.