Phage stock preparation

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ABSTRACT

A protocol for phage lambda and phage T7 phage stock preparation

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Protocol status: Working
We use this protocol and it's working

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28019

**Keywords:** bacteriophage, E. coli, lambda, T7, propagation

1. Grow E. coli bacterial host (for example LE392, DH10B or DH5alpha) in LB medium overnight at 37 °C.

2. Prepare and autoclave 1 Molarity (M) CaCl2 and 1 Molarity (M) MgCl2.

3. Add 50 µL of 1 Molarity (M) CaCl2 and 50 µL of 1 Molarity (M) MgCl2 to 50 mL of LB medium. Make aliquots of 10 mL and inoculate with 0.1 volumes of overnight bacterial host.

4. Incubate with agitation for 01:00:00 at 37 °C.

5. Add 100 µL of high titer phage lysate (> 10^8 PFU/ml).

6. Incubate at 37 °C with agitation for ~ 05:00:00 or until lysate clears.

7. Collect phage lysate and store at 4 °C until clean up.
Pool phage lysate into 50 mL sterile falcon centrifuge tubes and centrifuge at 4,000 rpm for 00:25:00.

Filter-sterilize the phage supernatant using a 0.22 μm filter to yield a bacterial cell-free phage lysate.

Determine phage titer using plaque or spot assay.