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Phage stock preparation

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We use this protocol and it's working

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Abstract

A protocol for phage lambda and phage T7 phage stock preparation

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

2 Prepare and autoclave [M] 1 Molarity (M) CaCl2 and [M] 1 Molarity (M) MgCl2

3 Add 50 µL of [M] 1 Molarity (M) CaCl2 and 50 µL of [M] 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.

8 Pool phage lysate into 50 mL sterile falcon centrifuge tubes and centrifuge at 4,000 rpm for 00:25:00 .

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

10 Determine phage titer using **plaque** or **spot** assay.