

Procedure for growing λ gt10 with onion cDNA inserts

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1. Two nights before growing phage, streak *E. coli* strain C600 Hfl on LB Agar with tetracycline at 15 μ g/ml. Grow overnight at 37 C.
2. The night before growing phage, pick a single colony of C600 Hfl from LB + tet plate and place in 5 ml liquid LB with 50 μ l 1 M $MgSO_4$ and 50 μ l 20% Maltose (Do not add tetracycline). Grow overnight in shaking incubator at 37 C and 225 rpm.
3.
 - A) Aliquot 90 μ l phage diluent into sterile microfuge tube;
 - B) Add 10 μ l phage from microtiter plate to the above tube. It may be necessary to do additional 10 and 100 fold dilutions;
 - C) Add 100 μ l fresh C600 Hfl bacteria from LB liquid culture and vortex;
 - D) Place in 37 C water bath for 30 minutes;
 - E) Add culture + phage to 5 ml of top agar with 50 μ l of 1 M $MgSO_4$ at 50 C;
 - F) Pour top agar and bacteria onto LB plate without antibiotic;
 - G) Place at 37 C overnight.
4. Harvest a single plaque using a sterile pasteur pipette and place in tube containing 600 μ l phage diluent. Phage may be stored long term at -70 C.

PHAGE DILUENT:

10 mM Tris-Cl (pH 7.5)

8 mM $MgSO_4$

For long term storage add:

50 mM NaCl

0.01 % gelatin

1 M $MgSO_4$ (F.W. 246.47):

24.65 g $MgSO_4$

~75 ml H_2O

Dissolve and H_2O to 100 ml

Sterilize by autoclaving

LB, 500 m

5.0 g Bacto-Tryptone

2.5 g Yeast Extract

5.0 g Sodium Chloride

Adjust pH to 7.5 with sodium hydroxide

7.5 g Agar for plates

3.75 g Agar for top-agar tubes

Tetracycline

Prepare a 12.5 mg/ml solution of tetracycline hydrochloride in 50% ethanol. Sterilize by filtration, and store in aliquots at -20°C in the dark. For agar plates: Allow autoclaved media to cool to 50°C before adding tetracycline, to a final concentration of 15 μ g/ml (1.0 to 1.2 ml stock solution per liter of media). Because tetracycline is light sensitive, store plates in the dark at 4°C.

