

## PREPARATION OF BACTERIOPHAGE STOCKS

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David B. Fankhauser, PhD

17 Feb 1993, latest revision 31 Dec 1995, 3 Jan. '97, 22 July '97, 19 July 98, 11 Aug 03, 18 Aug 04, 23July09  
[http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Phage/Phage\\_Preparation.htm](http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Phage/Phage_Preparation.htm)

Bacterial viruses have proven extremely valuable in the study of the mechanisms of DNA functioning. Their short life cycle (as short as 30 minutes) combined with their large number of progeny from each cell which is lysed (up to 400 phage particles/cell) allows the production of large numbers of progeny in a short amount of time. Their growth is also an excellent model for how mammalian viruses infect and reproduce.

### EQUIPMENT:

sterile capped 13 x 100 mm tubes  
37°C hot block  
0.1 mL micropipettor with sterile tips  
sterile bubbler assemblies  
aerator with humidifier flask  
manifold with spaghetti tubing  
spectrophotometer  
clinical tabletop centrifuge

### SUPPLIES:

sensitive host bacteria (*E. coli* B)  
phage inoculant ( $10^8$ /mL)  
sterile screw capped tubes  
chloroform  
tryptone soy broth\*

### PREVIOUS NIGHT:

1. **START INITIAL HOST CULTURE:** The previous day, inoculate 3 mL of tryptone soy broth with a sensitive host (*E. coli* B for T4 phage). Grow overnight (ON) at 37°C in hot block *without aeration*.

**NEXT MORNING:** (*NOTE:* Read & graph  $A_{660}$  every 30 minutes during steps 2 & 3.)

2. **PREPARE LOG-PHASE HOST CULTURE:** The next AM, add 0.1 mL of the ON culture to about 4 mL of tryptone soy broth (Adding 1% glucose may increase the resulting titer.) Aerate at 37°C in hot block until barely turbid ( $A_{660}$  = about 0.100), about 1 - 1½ hours. (Optional: read  $A_{660}$  every 30 minutes)
3. **INOCULATE WITH PHAGE:** Add 0.05 mL of  $10^8$ /mL of the desired strain of phage. Aerate at 37°C until solution clears, about 1 - 2 hours. (Try ON, it might work?)
4. **PURIFY PHAGE PROGENY:** Spin down the bacteria in the original culture tube for 10 min in a balanced clinical tabletop centrifuge at setting 5. Decant supernatant into screw-capped culture tube, add a few drops chloroform (~0.3 mL), shake, label with name of mutant phage, your initials and the date. Store at 4°C in the refrigerator.
5. **TITER THE PHAGE CULTURE:** To titer, dilute  $10^6$ , plate out 0.1 and 0.01 on tryptone agar using *E. coli* B as indicator bacteria, incubate ON at 37°C. Count plaques and calculate total phage/mL in the suspension. (For details, see protocol *Titering of Phage Viruses*.)