TITRE OF BACTERIOPHAGE

1. Plate 50æl of SURE cells directly onto an LB plate containing tetracycline. Incubate at 37 C overnight.
2. Pick a colony and onoculate into 5mL LB plus TET. Incubate To saturation at 37 C.
3. Make serial dilutions of stock bacteriophage.
4. Add 10æL of the appropriate phage dilutions to labelled 15mL Falcon tubes.
5. Add 0.3mL if saturated E. Coli to each of the phage suspensions. Let sit at room temperature for 20 min.
6. Move tubes to a 37 C waterbath or heat block for 10 min. At this time also melt some TOP agar in the microwave under the defrost setting for several min. or until completely melted.
7. Let TOP agar cool at room temp. For about 5 min.
8. Keep melted agar in a water bath at 45-50 C.
9. Take 3mL of the TOP agar and add to the E. coli/phage mixture. Quickly vortex and pour the contents of the tube onto a pre-warmed LB plate. Tilt the plate so as to get even distribution of the melted agar. (This step must be done quickly so that the agar doesn't solidify prematurely).
10. Do this for each of the phage dilutions and then place the plates into the 37 C incubator for 10-12 hours.
11. A plague can be picked from the plates which contain less than 100 plaques. Only plaques should be picked which have neighbouring plaques more than 1 cm away.

LARGE SCALE BACTERIOPHAGE PREP BUGS-18 1. Inoculate 500mL of LB with 100æl of SURE cells. Grow at 37 C with agitation for several hours. 2. Pick bacteriophage plaque from a plate and inoculate into 1mL of LB. Let stand at room temp. For 1-2 hours. 3. Inoculate bacterial culture with 1 mL of phage suspension. Grow at 37 C with agitation for 5-6 hours. 4. Spin down the suspension at 12,000g for 10 min. 5. Withdraw the supernatant(make sure not to disturb the bacterial pellet). 6. Add a few drops of Carbon Tetrachloride to the supernatant and store at 4 C. 7. See BUGS-17 to titre phage.