MINI PHAGE PREPS.

1. Inoculate 5 ml LB with 0.2 ml plating cells and 300 μl phage stock. Incubate at 37°C overnight.

2. Pour 1.3 ml phage culture into an eppendorf tube, microfuge 1' and transfer supernate to a fresh tube which contains 300 μl of 20% PEG 8000, 2.5 M NaCl. Mix and leave at 4°C for more than an hour.

3. Microfuge for 5' at 4°C. Resuspend pellet in 100 μl 300 mM NaCl, 100 mM Tris pH8, 1 mM EDTA.

4. Add 200 μl phenol:chloroform 1:1, mix and sit for 5'. Spin in microfuge and transfer aqueous layer to a fresh tube.

5. Add 10 μl 4.4 M ammonium acetate pH 5.2 and 400 μl ethanol.

6. Mix and microfuge for 5'.

7. Wash pellet in 70% ethanol, dry and resuspend in 20 μl TE.

8. Use 10 – 20 μl for a restriction digest. (Adding RNAase to the digest).