

***Nostoc* Plasmid Prep**

This prep takes approx. 1-2 hours the first day and 1.5 hours the second day.

1. Harvest a *Nostoc* culture in 50ml disposable centrifuge tube and wash 2 times with 25ml 5M NaCl.

2. Suspend cell pellet in 2 ml TE and add:

10 mg lysozyme (C_f=5mg/ml)

0.5 ml 20% TritonX-100 (C_f=5%)

Incubate at 37° on a very very slow roller drum for 30 min-1.5 hr.

3. Cool clear lysate on ice and add 0.625 ml cold 5M NaCl (1M final conc.). Incubate overnight at 4°.

4. Centrifuge 10 min clinical centrifuge, high. Discard the viscous pellet.

5. Extract with an equal volume phenol/chloroform (1:1) one or two times, spinning 4 min in clinical to separate phases. Remove top aqueous phase to Corex centrifuge tube and add an equal volume room temperature isopropanol, mix and let sit at room temperature 20 min. Centrifuge at 20° in SS-34 rotor 10,000RPM, 15 min. Wash pellet with 4 ml 70% ethanol and vacuum dry. Resuspend in 50µl. Use 2-4µl per lane in digests.