

Chelex Extraction from Dicty cells

This is a quick preparation that does not purify DNA, but makes it useable in PCR. It relies on mechanical breakdown of the spore walls, and chelex to deactivate proteins. (Hillis et al. 1996. Molecular Systematics, Second ed. Sinauer Assoc., Sunderland, MA. pp. 344-345)

- 1) Spin down suspension of cells for 5 minutes at 10,000 rpm in microcentrifuge.
- 2) Pour off supernatant and resuspend by vortexing in 1mL of ddH₂O.
- 3) Spin at 10,000 rpm for 5 minutes.
- 4) Pour off supernatant
- 5)) Resuspend pellet in 200µl Chelex (5%) (Chelex-100 Resin Bio Rad Laboratories Catalog 143-2832) **that has been stirring** and 10 µl proteinase K (20mg/mL) (USB catalog E76230Y).

OPTION 1:

- 6) Transfer to thin wall strip eppendorf tubes. Run this program in thermal cycler:
 - Step 1: 56.0 °C for 4 hours
 - Step 2: 98.0 °C for 30 minutes
 - Step 3: END
 - Preserve at 4 °C

OPTION 2 (alternative to using thermal cycler):

- 6a) Incubate at 56°C for 1 hour.
- 6b) Vortex for 10 seconds.
- 6c) Place sample in boiling water bath for 8 minutes.
- 7) Vortex for 10 seconds and spin at 12,000 rpm for 3 minutes.
- 8) Store samples at -20°C or -80°C for longer term storage

Note: Vortex for 10 seconds and then spin as in step 9 before every use. **Include 0.25 µl BSA (1mg/mL) in any 10 µl PCR.** Try not to store samples longer than one week and use as soon as possible.