

Total DNA Prep from Salmonella or E. coli

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- 1) Grow 2 ml overnight in 2XYT
- 2) Harvest a microfuge tube's worth of culture
- 3) Resuspend in 800 ul 50 mM Tris-HCl, 50 mM EDTA, pH 8.0
- 4) Add 100 ul lysozyme (20 mg/ml in TE), room temp (RT) 10 min
- 5) Add 20 ul 10% SDS + 100 ul protease (Pronase, 10 mg/ml), 1 h at 37°C with light agitation
- 6) Add Tris-buffered phenol to top of tube, 1 h 37°C with light agitation
- 7) Spin 5 min, transfer aqueous phase to new tube
- 8) Add chloroform to top of tube, let stand at RT 2 min
- 9) Spin 1 min, transfer aqueous phase to new tube
- 10) Add 35 ul 3 M NaAcetate pH 4.8 + isopropanol to top of tube, rock gently at RT 5 min
- 11) Swirl DNA onto glass rod (flamed capillary tube)
- 12) Optional: Resuspend DNA in 300-500 ul TE. Treat with 3 ul RNase (1 h 37°C), then precipitate again as in steps 10-11.
- 13) Rinse DNA while on rod with 95% ethanol (I use a squirt bottle)
- 14) Resuspend DNA in 300-500 ul TE