

Phenol-Chloroform Extraction // Large-scale extraction

1. Grow a 25mL culture in LB to late log or O/N
2. In a 50mL conical, spin down cells, wash in 10mL TE.
3. Transfer the 10mL solution to a 15mL conical, spin to pellet again.
4. Resuspend in 4mL TE containing 10mg of lysozyme (2.5mg/mL). Do not vortex. Start by resuspending in a small volume (~1mL) first.
5. Incubate at 37C 15min-1h. Check to see the cells appear lysed (goopy solution).
6. Add 0.3mL 10% SDS; incubate @ RT for 5min (no longer than 5 min!)
7. Add 2mL phenol and invert repeatedly. Mix it well, but do not shake vigorously or vortex. Spin at 10Krpm for 10min at RT.
8. Remove aqueous phase (top) and transfer to a new tube. Dispose of organic phase. Repeat phenol extraction 1-3 times as necessary.
9. Follow with a chloroform extraction to clear phenol—add 2mL chloroform
10. Remove aqueous layer, and transfer to a new tube. Add 2.5mL 100% EtOH at RT. Spool onto a Pasteur pipette.
11. Wash spooled DNA with 100% EtOH. Remove excess liquid.
12. Dissolve DNA in 0.5mL TE (pH 8.0) or in ddH₂O (if enzymes will be used in downstream applications)
13. If using immediately, warm for 20min at 65C. Store at 4C.