

Organic Extractions

Extracting chemicals:

- Add 4ml isoamyl alcohol to 96 ml molecular biology grade chloroform to produce 24:1 mixture
 - Add 6.5ml 1M Tris to 100 ml saturated phenol
 - PCI (25:24:1)
 - CI (24:1)
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- Add samples to 1.5ml centrifuge tubes
- Add equal volume of TE-equilibrated phenol to each sample and mix by inversion for 10 min (wrap tubes in parafilm to prevent phenol leakage)
- Spin at high speed for 5 min to separate phases
- Collect aqueous (top) phase using pipettor
- Add equal volume of PCI (25:24:1) and mix by gentle inversion
- Spin at high speed for 5 min to separate phases
- Collect aqueous (top) phase
- Add equal volume of CI (24:1) and mix by gentle inversion
- Spin at high speed for 5 min
- Collect aqueous (top) phase
- Add equal volume of CI (24:1) and mix by gentle inversion
- Spin at high speed for 1 min
- Collect aqueous (top) phase
- Add equal volume of 100% isopropanol
 - If DNA falls out of solution, pull out with pipette tip and wash (next step)
- Incubate at -80°C for 20 min
- Spin on high for 15 min to form pellet, decant and wash with 0.2ml 70% EtOH
- Spin 5 min and decant
- Air dry at room temperature for 20 min
- Resuspend in 50-150 “low” TE (TLE: 10mM Tris, pH8.0, 0.1mM EDTA pH 8.0) in single tube

Tissue Digestion

- Add tissue to a 1.5ml centrifuge tube and add 500µl SNET and 1µl Prot K (10mg/ml).
- Incubate, with shaking, overnight at 37°C
- SNET: 20mM Tris pH 8.0, 5mM EDTA pH 8.0, 400mM NaCl, 1% SDS