

Isolation and Purification of DNA

Remove and Lyse Cell Wall

For Eukaryotic cells: Use proteinase K

For Prokaryotic cells: Lysozyme to rupture cell wall

Both lysing procedures require detergents to facilitate disruption of membrane.

Pellet cells via centrifugation (freezing optional) and resuspend in isotonic buffer with 25 % sucrose and TRIS/EDTA @ pH 8.0.

Phenol Chloroform Extraction FYI. Rarely used and newer kits give comparable results with less toxic chemicals. Although P/C extraction can give very pure samples comparatively cheaply.

Dialysis: DNA is collected at P/C interface and dialyzed. Dialysis tubing has a lower weight molecular weight cut off. When in buffers, particles smaller than MW cut off diffuse out of bag.

RNA Digestion: Using clean glassware (procedure documented in other video) conduct RNAase digest.

Isopropanol precipitation: Bring digested solution to 0.4 M NaCl and layer in ice cold isopropanol. DNA will again ppt at interface (digested RNA is too small to do so.)

Suspend in Buffer (often takes overnight to get back into suspension.)

Note: 2 µg pellet is the smallest amount of DNA visible to the naked eye.

Microdialysis: Disc of dialysis paper (2500 MW cut off, 2.5 mm diameter) is floated atop a petri dish 1/2 full of buffer.

Petri dish is covered, placed on paper towel and ring of buffer drizzled onto paper to decrease evaporation.

Commercial kits for DNA isolation are commercially available. They use the same theory, and may use other techniques to filter DNA (e.g. HPLC or gel columns)