HHMI DNA ISOLATION II

To purify DNA from tissue, we need to break down cell membranes, inactivate DNases and solubilize proteins. Your notebook should contain much of the information below.

Your presentation next week should explain this procedure.

For this procedure, you will need the following;

A) Digestion Buffer
B) 7.5 M Ammonium Acetate
C) Isopropanol
D) 70% Ethanol
E) TE

A) Digestion Buffer contains 20 mM Tris-HCl pH 8.0, 75 mM NaCl, 25 mM EDTA, 1% SDS (sodium dodecyl sulfate [detergent]) and proteinase K (0.2 mg/ml).

B) Proteinase K (PROK) is a serine endopeptidase with a broad spectrum of action, isolated from the filtrate of the fungus *Tritirachium album limber.* Raising the temperature of the reaction from 37°C to 50°C - 60°C can increase the activity several folds. A special feature of proteinase K is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process. Proteinase K is very useful in the isolation of undamaged DNAs or RNAs since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.5 - 1% SDS.

C) In the presence of SDS, proteinase K efficiently lyses the cells and nuclei and liberates the DNA tightly bound in chromatin.

D) SDS is inhibitory to Taq polymerase at concentrations as low as 0.01%. Thus, SDS will inhibit PCR reactions

E) In the presence of high salt concentrations, SDS will precipitate out of solution.

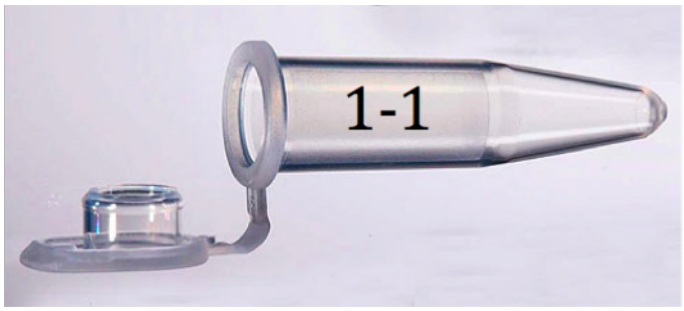
F) Isopropanol is an alcohol. Alcohol with salts dehydrates DNA, making it insoluble in water, and thus it precipitates out

G). T.E. 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA. Tris-HCl is a buffer, and it maintains a constant pH. EDTA chelates divalent cations (like Mg++). Mg

is needed for most DNA enzymes. Thus, because EDTA binds Mg++, it reduces the risk of DNase.

Procedures

Label 6-10 tubes with Group # and sample id.



Label tubes consistently: the same way for each and every tube. Label each tube twice: on the side of the tube and on the top of the tube.

1. Place each of your pieces of tissue into the correctly labeled tube.
2. Add 300 ul of Digestion Mix to the small piece of tissue into a 1.5 ml tube. Tissue pieces should be small and thin (minced).
4. Incubate it at 55°C for approximately 2 hours
5. Add 0.5 volumes of 7.5 M ammonium acetate (150 ul), freeze for 10 minutes and centrifuge for 10 min to get rid of junk.

Remove supernatant to a new, labeled tube and add 0.7 volumes of isopropanol (210 ul) and precipitate on ice (can freeze here forever).

NEXT WEEK

Pellet DNA 20' via centrifugation (max speed)
Carefully remove supernatant (discard). A small pellet of DNA should be at the bottom of the tube.
Carefully add 700 ul 70% ethanol. 70% ethanol washes off any remaining salt.
Spin 10' and carefully remove 70% ethanol

Resuspend DNA in TE (100ul, want ≤ 100 ng/ul). Add TE and let hydrate 5', vortex tube and collect at bottom by quick centrifugation.