Sugden Lab.

Ethanol precipitation

1. Add the appropriate volume of salt stock solution and mix well. Add 2.5~3.0 volume of ice-cold 100% ethanol and mix well.
   -Note: Addition of 1 µl of 20~25 µg/µl glycogen should increase the precipitation efficiency.
2. Store the ethanolic solution on ice for 15~30 min (or at –80˚C for 5~10 min) to allow the precipitaiton to form.
3. Centrifuge mixture for 10 min at 4˚C.
4. Aspirate the supernatant and wash the pellet in 1 ml 70% ethanol.
5. Centrifuge at 15,000 rpm for 10 min at 4˚C.
6. Aspirate supernatant and dissolve pellet in the desired volume of the appropriate buffer or dH_2O.

-Note: One volume of isopropanol may be used in place of ethanol.

Salt Solution

	Stock (M)	Final concentration (M)
Ammonium acetate	10.0	2.0~2.5
Lithium Chloride	8.0	0.8
Sodium chloride	5.0	0.2
Sodium acetate	3.0 (pH 5.2)	0.3

Extraction of DNA with Phenol/Chloroform

1. Add an equal volume of phenol:chloroform (1:1) to the nucleic acid sample in a 1.5 ml microcentrifugation tube.
2. Mix the contents of the tube vigorously until an emulsion form.
3. Centrifuge the mixture at 12,000 x g for 15 sec. at r.t.
4. Transfer the aqueous phase (the upper phase) to a fresh tube.
5. Repeat step 1 through 4 until no protein is visible at the interface of the organic and aqueous phases.
6. Add an equal volume of chloroform and repeat step through 4.
7. Recover the nucleic acid by ethanol precipitation.

Equilibration of phenol

1. Allow phenol to r.t., melt it at 68˚C and add hydroxyquinoline to a final concentration of 0.1%.
2. To a melted phenol add an equal volume of 0.5 M Tris-HCl pH 8.0, and stir the mixture on a magnetic stirrer for 15 min. When two phases have separated, aspirate as much as possible of the upper aqueous phase.

Last Modified 07/19/05

3. Add an equal volume of 0.1 M Tris-HCl pH 8.0 to the phenol. Stir for 15 min and remove the upper aqueous phase. Repeat the extractions until the pH of the phenolic phase is >7.8.
4. Remove the final aqueous phase and add 0.1 volume of 0.1 M Tris-HCl pH 8.0 contains 0.2% β-mercaptoethanol. The solution may be stored in a light-tight bottle at 4˚C up to 1 month.

Phenol:Chloroform (1:1)

1. Allow phenol to r.t., melt it at 68˚C and add hydroxyquinoline to a final concentration of 0.1%.
2. Mix the equal volume of chloroform to a melted phenol.
3. Add an equal volume of 0.5 M Tris-HCl pH 8.0, and stir the mixture on a magnetic stirrer for 15 min. When two phases have separated, aspirate as much as possible of the upper aqueous phase.
4. Add an equal volume of 0.1 M Tris-HCl pH 8.0 to the phenol. Stir for 15 min and remove the upper aqueous phase. Repeat the extractions until the pH of the phenolic phase is >7.8.
5. Remove the final aqueous phase and add 0.1 volume of 0.1 M Tris-HCl pH 8.0 contains 0.2% β-mercaptoethanol. The solution may be stored in a light-tight bottle at 4˚C up to 1 month.

Phenol:Chloroform:Isoamyl Alcohol (25:24:1)

To the equilibrated phenol, add the appropriate volume of chloroform and isoamyl alcohol so that the ratio of volume becomes 25:24:1.