(Ref: *Molecular Cloning* A8.12)

Alcohol Precipitation of DNA

The major considerations in using alcohols to precipitate DNA are:

Temperature: -20°C is optimal, but 0°C can be used for >20 ng/mL

2) ***Amount:*** For small amount of DNA (<100 ng, i.e. too little to reliably see a pellet) the use of glycogen (1 µL of a 20 mg/mL stock: Roche) will increase yield and allow visualization of the pellet.

3) ***Time:*** Optimal precipitation requires >1 hr at -20°C.

4) ***Speed of centrifuge*** (>12,000 rpm): Important for small amounts or small oligos.

5) ***Mg*** [10 uM] helps pellet oligos (<100 bp).

6) ***Avoid precipitates:*** EDTA (>10 mM) and Ca (>1 mM) will precipitate at the concentrations indicated in alcohol solutions. SDS will also precipitate with salts other than NaCl.

7) ***Choice of alcohol:*** Isopropanol has the advantage of requiring less volume (from 0.6 vol to 1 vol. of isopropanol is added to 1 volume of DNA/salt soulution compared to 2 vol. of ethanol). However isopropanol has the disadvantage of coprecipitating more salts and is less volatile (so it air dries slower) compared to ethanol.

8) ***Choice of salt:***

NaOAc, pH5.2, [0.3M]: This is the standard. (10x Stock concentration = 3 M)

NaCl [0.2M]: SDS is more soluable in NaCl (Stock conentrations vary 1M - 5M)

NH4OAc [2 - 2.5M]: Less coprecipitation of dNTPs, good for purifying oligos. However, NH4+ inhibits polynucleotide kinase).

LiCl [0.8M]: Soluble in a higher concentration of ethanol which is useful for the precipitation of RNA. However Cl- inhibits the initiation of protein synthesis and RNA will display varying solubility based on size.