PROTOCOL FOR ETHANOL PRECIPITATION OF DNA SAMPLES

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1. Dissolve DNA in LoTE, or ddH_2O with a total volume of 200ul.

2. Add 3-5ul of diluted SeeDNA [**Note**: If you have >10ug/ml DNA, you do not need carrier; you can also use glycogen (2. 5ul per sample; 20 mg/ml) if for some ridiculous reason you don't like seeDNA or you **need to read A_{260}** after precipitation].

2. Add 100ul 7.5M ammonium acetate (final concentration ammonium acetate =2.5M).

3. Add 600-700ul (or two volumes of aqueous part) of 100% ice-cold ethanol (**Note**: if DNA is large, like genomic DNA from mammalian cells, 1 volume ethanol is adequate; if DNA is small, e.g., <100-200bp, use 3 volumes ethanol; if DNA is <100 bp, use 4 volumes ethanol). Vortex hard and invert tubes to completely mix.

4. Microfuge 5 minutes at top speed in room temperature. (**Note**: There is absolutely no reason to spin your DNA for >5 min, rather than to annoy your benchmate. If you are really concerned, you may prechill your DNA/ethanol mix in a dry ice-methanol bath for 5 min and then spin down for 5min).

5. Carefully aspirate out liquid in the tube using pasteur pipette w/ pipette tip on end attached to vacuum (**Note**: Watch your pellets).

7. Add 500ul of 70% ethanol and vortex hard, followed by spinning for 2 minutes.

8. Repeat steps 6 and 7. (Note: do not need 2nd 70% ethanol wash for some procedures, e.g. if loading directly on gel).

9. Re-centrifuge a few seconds to collect any residual liquid to bottom of tube. Remove the liquid with pipette tips. (**Note**: this is the most effective way to dry your DNA. Air drying or using Speed Vac is totally B.S., and waste your time!).

10. Dissolve DNA pellet in ddH_2O at < 1 mg/ml for plasmid DNA, < 200 ug/ml for genomic DNA (**Note**: it may take several hours for large genomic DNA to dissolve. Heating at 37°C water bath may be helpful).

GENERAL COMMENTS:

A) The most common mistake in ethanol precipitation is to not mix the stuff together adequately before centrifuging. There is no need to vortex until after adding everything, but vortex hard. Vortexing does not degrade DNA to less than 50 kb, so vortexing is fine for most purposes.

B) This protocol will precipitate all DNA > ~8 bases. Oligonucleotides will generally precipitate quite well. Ethanol will also precipitate protein, so you must use phenol to get rid of proteins.