Ethanol Precipitation of Nucleic Acids[1]

Nucleic acid precipitation is used to concentrate and/or purify nucleic acids. The below protocol is based on the fact that nucleic acids are less soluble in alcohol than in more polar water. Addition of salt further decreases solubility by competing for water dipoles; as does low temperature.

Note: if you have proteins in your sample, you should first clean up your sample with phenol/chloroform extraction (adding equal volumes of phenol/chloroform to your aqueous sample, vortexing, spinning down, and saving the aqueous layer, then repeating with chloroform.)

1. To your sample (in aqueous solvent), add:
    a. 10 µL glycogen (5 mg/mL) (*optional*)
    b. 1/10 vol 3 M NaOAc[2]
    c. 2-3 vol 100% ice-cold EtOH[3] (we keep a 50 mL conical of EtOH in the -20 freezer)
2. Place the tube in crushed dry ice, for at least 30 min (this can go several hours, if you'd like)
    a. Completely submerge the tube – only the top of the tube should be visible
    b. It is important that the dry ice be well crushed – to create the best contact with the eppendorf tube
3. Spin the tube, full speed, 4 °C, for 30 min
4. Decant
    a. You may or may not see the pellet
    b. Depending on the situation, your pellet may or may not stick to the tube – be observant and do the best you can to not lose the pellet!
5. Add 500 µL ice-cold 70% EtOH (also in the -20 freezer)
6. Spin the tube, full speed, 4 °C, for 10 min
7. Decant
8. Dry the pellet by inverting the tube onto a Kimwipe / paper towel, 5-15 min
9. Resuspend pellet in 1 mM TRIS pH 8.0, MQ-H2O, or DEPC-H_2O, depending on your needs
    a. The final volume will depend on how concentrated you want your sample
    b. Recommended: 10-50 µL

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[1] Useful website: http://bitesizebio.com/articles/dna-precipitation-ethanol-vs-isopropanol/

[2] Sometimes, people use ½ vol 5M NH_4OAC

[3] Sometimes, people use 1 vol room temp isopropanol here; but still wash with 70% EtOH

Working with Glycoblue (add in place of glycogen)

Pros: Helps achieve higher yields of RNA and paints RNA blue (making it easy to see!)

Cons: We've had some trouble getting it to go back into solution

Some tips from the web:

A. Once you add buffer to your pellet, let it sit for 15 minutes (room temp)

OR

B. Once you add buffer, heat at 65 °C, 800-900 rpm on the thermo shaker, vortex 2-3 times in between

Try one! And let me know how it goes!!