

DNA “Plasmid” Alcohol Precipitation (DRG)

Materials:

Sodium Acetate Stock (3M, pH 5.2): MW 136.08 → 4.08 g/10 ml autoclaved Water

Isopropanol – 100%, stock

Ethanol – 70%, stock

DEPC Water stock

TE Buffer 1x (Amresco)

Protocol:

***** Be sure to centrifuge with the tube in the same orientation at every step *****

1. Add 1/10 volume of Sodium Acetate (3M, pH 5.2).
(I.e. If DNA sample is 900 ul add 90 ul salt)
2. Add 0.6 – 0.7 x volume (calculated after addition of sodium acetate) of at least Isopropanol.
(I.e. $990 \times 0.7 = 693$ ul, so add 693 ul Isopropanol to 990 ul salted DNA sample)
3. Centrifuge at $> 14,000 \times g$ for 30 minutes at room temperature or 4°C .
4. Carefully decant the supernatant being careful not to throw out DNA pellet which may or may not be visible. **YOU MAY NEED TO LEAVE 50-100 ul SUPERNATANT IN THE TUBE.**
5. Rinse with 1 – 1.5 ml 70% Ethanol
6. Centrifuge at $>14,000$ again for 15 minutes.
7. Carefully decant the supernatant being careful not to throw out DNA pellet.
8. Air-Dry the pellet: by placing the tube with the cap open on a paper towel on the bench for 30 minutes.
9. Resuspend the pellet in 100 ul DEPC Water or TE Buffer.
(Make sure the buffer comes into contact with the whole surface of the tube since a significant portion of DNA may be deposited on the walls instead of in the pellet.)