Removing RNA from DNA/RNA mixtures:

Reagents:

10 X digestion buffer (20 mM $MgCl_2$, 10 mM $ZnCl_2$, 500 mM Tris, pH 7.2) – made with DEPC treated water:

*Note: This buffer is not strictly necessary it is designed for DNase treatment

RNase I

Protein precipitation solution (10 M ammonium acetate)

Ice-cold Isopropanol

Ice-cold Ethanol (70 %)

DEPC treated water

1. *Add 1/10 volume of 10 X digestion buffer and 1 U of RNase I, mix by flicking tubes and spin briefly.

2. Incubate at 37°C for 30 min.

3. To remove enzyme add 1 volume of protein precipitation solution, vortex for 10s on low speed and place in ice-bath for 5 min.

4. Centrifuge at top speed (16,100 x g) at 4°C for 10 min and transfer supernatant to a new tube.

5. Add 1 volume of ice-cold isopropanol and invert tube about 30 times to mix.

6. Centrifuge at top speed (16,100 x g) at 4°C for 10 min and remove supernatant.

7. Rinse pellet twice with 70 % ice-cold ethanol spinning between rinses.

8. Dry pellet slightly in vacuum dessicator (about 2 min).

9. Resuspend pellet either in DEPC treated H_2O.

10. Store at -20°C short term or -80°C long term.