# Modified Griffith et al (2000) nucleic acid extraction method:

1. Weigh 0.25 g soil into screw cap microcentrifuge tube with 2 ml tubes (Cat # 16466-042 VWR) with 0.5 g heat treated zirconia beads (0.1mm). Beads baked at >250oC for >2 h which should be sufficient to destroy RNAses and DNA. Alternatively, lysing matrix E tubes (MP Biomedicals Cat # 16) can also be used.
2. Add 0.5 ml **Extraction buffer** , 0.25 ml equilibrated **phenol (pH 8, Sigma)** and 0.25 ml **chloroform:isoamyl alcohol (24:1, Sigma).**
3. Place tubes in Fast-prep machine to lyse cells for 1 min at 5.5 m s-1.
4. Add 85 ul of 5M NaCl (to about 0.7M final) and vortex
5. Add 60 ul (about 1/10 volume) of CTAB/NaCl solution and vortex
6. Cool tubes on ice for 1 min and centrifuge for 5 min at 16 000 x *g* at 4oC
7. Transfer the aqueous layer to a new 2 ml microcentrifuge tube and reserve on ice
8. Add 85 ul NaCl to soil pellet (tube with beads), vortex to mix well
9. Add 0.5 ml Extraction Buffer to soil pellet, vortex to mix well
10. Centrifuge for soil for 5 min at 16 000 x *g* at 4oC
11. Transfer this new aqueous layer to the first aqueous layer you collected (total volume 0.6 to 0.8 ml)
12. Wash the combined aqueous layers with 0.5 ml volume **chloroform:isoamyl alcohol (24:1, Sigma)** . Mix to form an emulsion by vortexing. Centrifuge again for 5 min at 16 000 x *g* at 4oC.
13. Transfer the aqueous layer to a new 1.5 ml microcentrifuge tube and precipitate the nucleic acids with 1.5-2 volumes of **Polyethylene glycol solution (PEG 6000)** . Mix well by inversion by hand and allow nucleic acids to precipitate on ice for 2 h.
14. Centrifuge for 30 min at 16 000 x *g* at 4oC and gently pour off supernatant keeping the side of the tube with the pellet up.
15. Wash the pellet by vortexing with 1 ml of ice-cold **70% v/v ethanol** (Molecular grade ethanol, Sigma and RNAse free water) and centrifuge again for 10 min at 16 000 x *g* at 4oC
16. Wash the pellet again by vortexing with 1 ml of ice-cold **70% v/v ethanol** (Molecular grade ethanol, Sigma and RNAse free water) and centrifuge again for 10 min at 16 000 x *g* at 4oC
17. Remove any ethanol by pipetting and invert onto paper towel. Can allow to air dry for 10-20 mins with lid open on ice! Resuspend the pellet in 50 ol TE buffer by pipetting up and down. Be sure to wash the sides with the buffer by pipetting of the tube since the RNA pellet can smear half way up the side of the tube.
18. Split nucleic acids into DNA aliquot (25 ul) to be stored at -20oC and RNA aliquot (25 ul) stored on ice. Continue directly to DNAse treatment, RNA purification and cDNA synthesis steps with RNA aliquot.

## CTAB/NaCl

* 5 g of 10% w/v hexadecyltriammonium bromide (CTAB)
* 2.04 g of 0.7 M NaCl
* Make up to 50 ml
* autoclave and store RT

## Extraction Buffer

* 240 mM Phosphate buffer
* 0.5 % N-lauryl sarcosine (aka: N-lauryl sarcosine Na salt, aka: sarkosyl or Na-sarcosyl)
* 0.3266 g Monobasic KH2PO4 make up to 10 ml
* 2.09 g Dibasic KH2PO4 make up to 50 ml
* Add monobasic phosphate solution (approx. 2.5 ml) to the dibasic phosphate solution till you reach pH 8
* Add sarcosyl detergent (0.5 g per 100 ml)
* autoclave and store RT

## PEG solution

* 30% polyethylene glycol/1.6M NaCl
* 60 g polyethylene glycol (PEG 6000)
  + I made mine with PEG 8000 not 6000. It's better known for RNA recovery
* 18.7 g 1.6M NaCl
* Make up to 200 ml and autoclave.

## TE buffer

* Make up stocks of 1M Tris and 0.5M EDTA disodium salt
* 1 ml (100 mM tris)
* 200 ul (1mM EDTA)
* Make up to 100 ml with sterile water