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

## Materials

### Recipes listed at end of this protocol:

* extraction buffer
* CTAB solution
* PEG solution
* TE buffer

### Other:

* Soil sample
* phenol (pH 8, Sigma)
* chloroform:isoamyl alcohol (24:1, Sigma)
* 5M NaCL
* 70% v/v ethanol
  + Molecular grade ethanol (Sigma) and RNAse free water
  + ice-cold
* 2 ml microcentrifuge tubes containing 0.5 g heat treated zirconia beads
  + 1 needed for each extraction
* 2 ml microcentrifuge tubes
  + 2 needed for each extraction
* Bucket of ice

## Pre-extraction checklist

* Check the PEG solution to see if it precipitated. The PEG should go back into solution once it has warmed up. This also help with pipetting it.
* Check to make sure the 70% ethanol is in a freezer, so that it is ice-cold when needed.

## Method

1. Weigh 0.25 g soil into screw cap microcentrifuge tube with 2 ml tubes (Cat # 16466-042 VWR) containing 0.5 g heat treated zirconia beads (0.1 mm).
   * 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 the following to each 2 ml tube:
   * 0.5 ml **Extraction buffer**
   * 0.25 ml equilibrated **phenol (pH 8)**
   * 0.25 ml **chloroform:isoamyl alcohol (24:1)**
   * **WARNING:** work in the chemcial hood when handling phenol and chloroform. Also, wear adequate protective equipment (eg., a lab coat).
     + Phenol is corrisive and toxic. It can cause burns that are not immediately painful or visible.
     + Chloroform is an anesthetic and likely a carcinogen.
3. Place tubes in Fast-prep machine to lyse cells for 1 min at 5.5 m s-1.

### CTAB addition:

1. Add 85 ul of 5M NaCl
   * Final conc.: ~0.7M
2. Add 60 ul (~1/10 volume) of CTAB/NaCl solution
   * **Note**: Use a different pipet tip for each addition of CTAB. Since it is viscous, you will need to be slow in pipetting up and down. Flushing the tip multiple times with the extraction solution (in the 2ml tube) will help.
3. Vortex
4. Cool tubes on ice for 1 min.
5. Centrifuge for 5 min at 16 000 x *g* at 4oC
6. Transfer the aqueous layer to a new 2 ml microcentrifuge tube and reserve on ice.
   * ~500-600 ul should be transferred.
   * **Note:** Make sure not to pipette up any of the organic phase.

### Rinsing the soil pellet:

1. Add 85 ul NaCl to soil pellet to the 2ml tube containing beads
2. Add 0.5 ml Extraction Buffer to the 2ml tube containing beads
3. Vortex
4. Centrifuge for soil for 5 min at 16 000 x *g* at 4oC
5. Transfer this new aqueous layer to the first aqueous layer you collected
   * total volume: 0.6-0.8 ml

### Processing the combined aqueous layers:

1. Wash the combined aqueous layers with 0.5 ml volume **chloroform:isoamyl alcohol (24:1)**.
2. Vortex to form an emulsion.
3. Centrifuge for 5 min at 16 000 x *g* at 4oC.
4. Transfer the aqueous layer to a new 2 ml microcentrifuge tube.
   * Approx. volume transferred: ~0.9 ml
   * **Note:** Make sure to not pipette up the organic layer.
   * **Note:** This is the final tube that will contain the purified nucleotides, so label it well.
5. Precipitate the nucleic acids with 1.5-2 volumes of **Polyethylene glycol solution**.
   * Approx. volume of PEG: 1 ml
6. Mix well through inversion by hand.
7. Allow nucleic acids to precipitate on ice for 2 h.
   * **Possible stopping point:** The tubes can be incubated at 4oC over night if needed.
   * **Note:** Make sure to incubate at 4oC and not -20oC; the PEG will solidify at -20oC
8. Centrifuge for 30 min at 16 000 x *g* at 4oC.
9. Gently pour off supernatant while keeping the side of the tube with the pellet up.
10. Wash the pellet by vortexing with 1 ml of ice-cold molecular-grade **70% v/v ethanol**
11. Centrifuge for 10 min at 16 000 x *g* at 4oC
12. Remove any ethanol by pipetting.
13. Wash the pellet again by vortexing with 1 ml of ice-cold molecular-grade **70% v/v ethanol**
14. Centrifuge again for 10 min at 16 000 x *g* at 4oC
15. Remove any ethanol by pipetting and invert onto paper towel to dry.
    * The pellet can be allowed to air dry for 10-20 mins with lid open on ice!
    * **Note:** Make sure to air dry on ice.
    * **Note:** Make sure the tube & pellet are dry before proceeding.
16. Resuspend the pellet in 50 ul 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.

### Optional:

1. Split nucleic acids into DNA aliquot (25 ul) to be stored at -20oC and an RNA aliquot (25 ul) to be stored on ice.
2. For the RNA aliquot: continue directly to DNAse treatment, RNA purification, and cDNA synthesis.

## Reagent recipes

### CTAB (10%), NaCl (0.7M)

* 50 ml Nanopure water
* 5 g hexadecyltriammonium bromide (CTAB)
  + Final conc.: 10% w/v
* 2.04 g NaCl
  + Final conc.: 0.7 M
* Use stir bar to disolve solids
  + **Note:** 10% CTAB is quite viscus
* Autoclave and store at RT

### Extraction Buffer (240 mM phosphate buffer, 0.5% N-lauryl sarcosine)

* Monobasic KH2PO4 solution:
  + 10 ml Nanopure water
  + 0.327 g Monobasic KH2PO4
* Extraction buffer:
  + 50 ml Nanopure water
  + 2.09 g Dibasic KH2PO4
  + Add monobasic phosphate solution (~2.5 ml) to the dibasic phosphate solution until you reach pH 8
* Add sarcosyl detergent (0.5 g per 100 ml)
  + sarkosyl aka: N-lauryl sarcosine Na salt or Na-sarcosyl
  + The current phosphate buffer volume should be ~52-53 ml, so add sarcosyl accordingly
* Autoclave and store at RT

### PEG solution (30% PEG, 1.6M NaCl)

* 200 ml Nanopure water
* 60 g polyethylene glycol (PEG 6000 or 8000)
  + For RNA recovery, use PEG 8000 and not 6000.
  + Final conc.: 30%
* 18.7 g NaCl
  + Final conc.: 1.6M
* Autoclave and store at RT

### TE buffer (10 mM Tris, 1 mM EDTA)

* Make stock solutions in molecular grade water:
  + 1 M Tris
  + 0.5 M EDTA disodium salt
* TE buffer:
  + 40 ml molecular grade water
  + 400 ul of 1 M Tris
    - Final conc.: 10 mM Tris
  + 80 ul of 0.5 M EDTA
    - Final conc.: 1 mM EDTA
* Store at RT