Phenol chloroform extraction of Nucleic acid from Soil

Method of Griffith *et al.* (2000) *AEM*: 66, 5488–5491

1. Preparations for Nucleic acid extraction

## Glassware

1. Glassware for solutions below must be baked at 180°C overnight – remove lids & plastic rims
2. Or use Nuclease free 50ml centrifuge tubes to make solutions in
3. Bake a glass bottle with glass/zirconia beads in too
4. Lids &plastic rims of bottles should be soaked in RNase away
5. Solutions to prepare
6. 5% CTAB / Phosphate buffer (120 mM, pH8) :

Formed by combining equal volumes of 10% CTAB in 0.7M NaCl with 240mM Phosphate buffer (pH8)

Buffering component needs to be prepared fresh **on day of extraction** but separate components autoclaved in advance:

1. Prepare the two components for the phosphate buffer separately (to make 240mM of each):

* K2HPO4.3H2O **5.48 g** in **100ml Nuclease free H2O** (= dibasic K phosphate)
* KH2PO4 **3.27 g** in **100ml Nuclease free H2O** (= monobasic K phosphate)

1. Prepare CTAB (10%) 0.7M NaCl solution:

* NaCl **2.05 g**
* CTAB **5g**
* Nuclease free H2O **Up to 50 ml**

**Autoclave these 3 separate bottles and keep separate until day of extraction** (keep phosphates in fridge and CTAB at room temp *(-> CTAB goes cloudy when cold may need to heat slightly before you use on extraction day*)

**On day of extraction:**

1. prepare **5m**l of 240mM phosphate buffer at **pH 8** by combining:

* **4.7 ml** dibasic potassium phosphate (K2HPO4.3H2O)
* **0.3ml** monobasic potassium phosphate (KH2PO4)

-> (ie 94% Dibasic & 6% monobasic to = ph8)

1. To this, add **5m**l of 10% CTAB buffer

**= final extraction buffer of 5% CTAB, 0.35M NaCl and 120mM phosphate buffer at pH8.**

1. 30% PEG6000 / 1.6M NaCl

* PEG6000 **30g**
* NaCl **9.35 g**
* Nuclease free H2O **Up to 100 ml**

Autoclave

1. 70% Ethanol **Make with RNase free water and put in freezer.**

## 2. Method

Spray all surfaces with RNase away (or RNase zap); change gloves often/spray with RNase zap;

After step 4 **keep samples on ice all the time** to reduce RNA degradation.

If using snap frozen samples stored at -80 see Note 2 at end of method

1. DNA & RNA will be snap frozen at end of protocol so collect liquid N2 at some point in day.
2. 0.1mm glass beads & 0.5mm zirconia/silica beads (0.5 g each) in 2ml screw-top micro centrifuge tubes (ie beads come up to 0.5ml line)
3. Weigh out ~0.50 g of soil into 2ml screw top tubes.
4. Add **0.5 ml of CTAB buffer**, **0.5 ml of Phenol:Chloroform:Isoamyl alcohol** (25:24:1) -> do all phenol work in fume hood & tips/tubes that contact phenol must be put in labelled bag for separate disposal. (*If RNA yield is low, can increase to 600ul CTAB or if protein yield low, increase phenol to >600ul*)
5. Place tubes in Vortex adapter and **vortex cells for 2.5 minutes** – immerse in ice immediately after
6. Centrifuge at **16,000 *g* for 10 minutes at 4oC**.
7. Label tubes for subsequent steps now.
8. Extract the **top aqueous layer** and transfer to a new 1.5 ml microcentrifuge tube
9. Add **0.5 ml of Chloroform:Isoamyl alcohol (24:1)** to top aqueous phase and mix briefly to form an emulsion (this is to remove any residual phenol) → again do in fume hood
10. **Centrifuge for 5 min at 16,000 *g* at 5oC.**
11. Extract the **top aqueous layer** and place in a new microcentrifuge tube.

* Do this carefully to try prevent any phenol carry over. It is usually necessary to leave a small volume of top phase behind to ensure this.

1. Precipitate DNA by adding **two volumes of 30% PEG solution** and **mix well**.
2. Put in fridge for **2 hours**
3. Prepare 1% agarose gel now.
4. Remove tubes from fridge and centrifuge at **16,000 *g* for 20 minutes at 5oC**. [A pellet may be visible at the bottom of the tube *but don’t worry if not at this stage*.]
5. Pour off the supernatant (*very careful as pellet slippery – I leave ~0.5ml PEG in tube to ensure pellet not lost or even better pipette of supernatant keeping tip at surface of PEG and leave ~0.5ml*)
6. Add **1 ml of ice-cold** **70% ethanol** and mix well by inverting.
7. Centrifuge for **25 min at 16,000 *g* at 5oC**. [A small creamy white pellet should be visible at the bottom of the tube.] -> can do 30 mins centrifuge to be sure.
8. Pour off ethanol wash (again take care not to lose pellet) and place tube back in centrifuge and spin for ~5 s to collect residual ethanol. Remove remaining ethanol using a pipette (don’t touch pellet).
9. Leave the pellet to air dry or warm in hot-block at 55oC for ~1-2 min. (*I don’t use hot block as I have so little RNA, just remove ethanol with pipette & air dry off ice for a few mins until no more smell of ethanol*)
10. Re-suspend pellet in **40 ul of RNase free sterile H2O.** (*change this volume depending on how much RNA you have – take down to 30µl if not much RNA*)
11. Examine yield of DNA by agarose gel electrophoresis (*Load 5µl on gel*).
12. Split into aliquots and treat one with DNase = RNA sample and one with RNase = DNA sample. (see DNase treatment protocol) Snap freeze and put at -80°C until DNase/RNase treatment.

**Note 1: Snap frozen samples**

If samples are to be snap frozen prior to extraction then they should be frozen in the tubes that will be used for step one of extraction. This is because when soil defrosts from -80°C it is possible that much of the RNA will degrade. If soil has to defrost before being weighed and put in tubes, then the RNA may be lost. Even samples already in their tubes are at risk of this. To reduce the change of RNA loss, it is best for the sample to defrost *within* the extraction buffer. In order to do this, keep the sample of dry ice to ensure it does not defrost, then once in the lab add extraction buffer (NO beads yet) and push pipette tip through frozen soil to dry and cover all soil with buffer as soon as possible. Vortex *briefly,* if necessary. Then add beads and continue with extraction as normal.