

Total Soil DNA and RNA extraction and purification

Adapted from Eoin Brodie, April 29, 2003

This method can be used to extract and purify total nucleic acids from soil/sediment samples, plant parts, bacterial cultures and fungal mycelia. The method below details the approach for soil or sediment.

Materials

1.5 ml eppendorf tubes	Phenol:chloroform:isoamylalcohol (25:24:1)
Filter tips	Chloroform:isoamylalcohol (24:1)
0.1mm Glass beads & 0.5mm zirconia/silica beads in 2ml screw-top microcentrifuge tubes	Peg 6000 precipitate solution 1.6 M NaCl
Modified CTAB extraction buffer: equal volumes:	30% (wt/vol) polyethylene glycol 6000
• 10% CTAB in 0.7M NaCl	Sterile MQ H ₂ O
• 240 mM phosphate buffer, pH8	70% EtOH
	Tris-EDTA pH7.4

Preparation

- * All vessels and reagents should be DNase/RNase free, or treated with DEPC to denature proteins.
 - * DEPC treatment: Soak in 0.1% DEPC overnight at 37 °C, then autoclave.
- Cool centrifuge to 4°C.

Methods

- 1) Add 0.5 g sieved soil to 2 ml screw-capped microcentrifuge tubes containing 0.5 g each of 0.1 mm glass and 0.5 mm zirconia/silica beads. Add 0.5 ml of modified CTAB (hexadecyltrimethylammonium bromide) extraction buffer and vortex briefly.
- 2) (Incubate tubes at 70°C for 10 min in a waterbath.) This step gives better DNA yield but not as much RNA. For DNA and RNA extraction, skip to (3).
- 3) Add 0.5 ml phenol:chloroform:isoamylalcohol (25:24:1) and shake tubes in the FastPrep Instrument (Qbiogene) at 5.5 m/s for 30 s.
- 4) Following bead-beating, centrifuge tubes at 16000 x g for 5 min at 4°C.
- 5) Remove the aqueous top layer to a new tube and add an equal volume of chloroform:isoamylalcohol (24:1). Mix tubes well and centrifuge at 16000 x g for 5 min at room temperature. Remove the aqueous top layer into a clean 1.5 ml microcentrifuge tube.
(Note: if DNA yield is expected to be low you can increase efficiency by performing this second organic extraction in a pre-spun Phase-Lock gel (Heavy) tube - Eppendorf).
- 6) Add 2 volumes Peg 6000 precipitate solution and incubate at room temp for 2 hours.
- 7) Spin at 18,000xg at for 10 min at 4°C. Wash pelleted DNA/RNA in 70% EtOH and spin again at 18,000xg at for 5 min at 4°C. Dry pellet in dessicator for about 5 minutes (do NOT totally dry). Resuspend pellet in 50 ul DNase/RNase-free TE.

QIAGEN DNA and RNA purification

Use QIAGEN RNA/DNA Mini Kit, #14123.
Your DNA/RNA soil extraction should be in TE.

Important steps before starting:

Prepare reagents

- TE buffer, pH 8.0, DNase and RNase free
- QRL1
 - Warm to redissolve
 - Add 10ul 2-mercaptoethanol per 1ml QRL1 (0.15ml per sample needed). Good for 1 month.
- Heat buffers QRU^R and QF to 45°C
- Isopropanol: Ice-cold and Room-temp, DNase and RNase free
- 70% Ethanol: Ice-cold and Room-temp, DNase and RNase free

Heat water bath or heating block to 60°C.

Cool centrifuge to 4°C.

Follow protocol for isolation of total RNA and genomic DNA from Bacteria with the following exceptions:

- Skip steps 1 and 2.
- Modify step 3 as follows: add 0.15ml QRL1, and vortex vigorously for 10 seconds.
- Follow protocol from step 4 to end as written.