Simultaneous DNA/RNA extraction and purification from soil
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 or yeast cultures and fungal mycelia. The method below details the approach for soil or sediment. Part I is a modification of the method of Griffiths et al (2000) and Part II is from the Qiagen DNA/RNA Handbook.

Materials

1.5 ml eppendorf tubes
Filter tips
0.1mm glass beads & 0.5mm zirconia/silica beads (0.5 g each) in 2ml screw-top microcentrifuge tubes
Modified CTAB extraction buffer
    Equal volumes of 10% CTAB in 0.7 M NaCl and 240 mM potassium phosphate buffer [pH8]
Phenol (pH>7.8):chloroform:isoamylalcohol (25:24:1)
Chloroform:isoamylalcohol (24:1)
PEG/NaCl solution
    1.6 M NaCl
    30% (wt/vol) polyethylene glycol 6000
Sterile MilliQ H_2O
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: Solutions: Add DEPC to a final conc. of 0.1% and incubate overnight at 37 ^{o}C, then autoclave. Equipment: Soak in a DEPC solution (0.1% in H_2O) overnight at 37^{o}C and autoclave.
  Cool centrifuge to 4^{o}C.

Methods

1) Add 0.5 g 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) 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.

3) Following bead-beating, centrifuge tubes at 16000 x g for 5 min at 4^{o}C.

4) 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: Yield may be increased by performing this second organic extraction in a pre-spun Phase-Lock gel (Heavy) tube - Eppendorf). Spin phase-lock gel tube with aqueous phase and chloroform:isoamyl alcohol (24:1) at 12,000 x g for 5 min at 4^{o}C. Remove aqueous phase into fresh tube and continue at step 5.

5) Add 2 volumes PEG/NaCl precipitate solution and incubate at room temp for 2 hours.

6) Spin at 18,000xg at for 10 min at 4^{o}C. Wash pelleted DNA/RNA in ice-cold 70% EtOH and spin again at 18,000xg at for 5 min at 4^{o}C. Dry pellet in vacuum dessicator for about 5 minutes (do NOT totally dry). Resuspend pellet in 50 ul DNAse/RNAse-free TE (pH 7.4 or

8.0) if using the Qiagen kit for further purification. Alternatively you can resuspend in DEPC treated water.

If you require maximum yield of both DNA and RNA from your sample, or if your sample contains high amounts of organic matter (yellow to brown pellet) then proceed with Qiagen purification. If sample is low in organic matter or you only require either DNA or RNA not both then treat resuspended pellet with either DNase or RNase to remove unwanted fraction.

QIAGEN DNA and RNA purification

Use QIAGEN RNA/DNA Mini Kit, #14123.
Your DNA/RNA soil extraction should be in TE pH 7.4 or 8.0.

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 so only make enough for immediate work.
- Heat buffers QRU^R and QF to 45ºC
  If the working solution of buffer QRU^R was made more than two weeks previously then test the pH and adjust to pH 7.0 with HCl if necessary.
- 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 60ºC.
Cool centrifuge to 4ºC.

Skip steps 1 and 2 of kit method.

3. Modified as follows: add 0.15ml QRL1 to DNA/RNA extract from Part I, and vortex vigorously for 10 seconds. Ensure that ß-ME is added to Buffer QRL1.

4. Add 1.35 ml Buffer QRV2. Mix thoroughly by vortexing or shaking, and centrifuge at 5000 x *g* for 5 min at 4°C.

Dilution with Buffer QRV2 creates optimal conditions for binding RNA to QIAGEN Resin. During centrifugation, proceed with step 5.

Note: Any undissolved particles must be removed by centrifugation prior to loading the QIAGEN-tip otherwise low yield of RNA and DNA and/or clogging of the column may occur.

5. Pipet 1 ml Buffer QRE into the QIAGEN-tip to equilibrate, and allow the buffer to enter the column by gravity flow.

Place QIAGEN-tips either over tubes using the tip holders provided (see Figure 2, page 15 of manual) or in a QIArack over the waste tray. The buffer will begin to flow automatically due to detergent in the equilibration buffer which reduces the surface tension. Allow the QIAGEN-tip to drain completely. The flow of buffer will stop when the meniscus reaches the upper frit in the QIAGEN-tip. This prevents drying out so QIAGEN-tips can be left unattended. Do not force out the remaining buffer.

Note: After equilibration, place collection tubes (2-ml, 12–15-ml, or 30–50-ml, not supplied) underthe QIAGEN-tip to collect DNA-containing flow-through in step 6 if DNA is required.

6. Apply supernatant from step 4 to the QIAGEN-tip, and allow it to enter the resin by gravity flow. Collect the flow-through for later DNA isolation and place at room temperature. If DNA is not desired, discard the flow-through.

Due to the high RNA/DNA ratio in cultured bacteria, slight RNA contamination may occur in the subsequently isolated DNA. If this RNA contamination is unacceptable, add 1.5 µl RNase A stock solution (100 mg/ml, QIAGEN Cat. No. 19101) to the flow-through. Mix and keep flow-through at room temperature for later DNA extraction. RNase A solution is not supplied with the kits.

7. Pipet 2 ml (2 x 1 ml) Buffer QRW onto the QIAGEN-tip. Allow it to enter the resin by gravity flow.

The QIAGEN-tips are almost completely filled by these volumes. Nucleic acids remain bound to the QIAGEN Resin, while contaminants such as proteins, polysaccharides, carbohydrates, and cellular metabolites are washed away. Do not force out residual wash buffer. Traces of buffer will not affect the elution step.

8. Pipet 1 ml (or 2 x 1 ml*) preheated (45°C) Buffer QRUR onto the QIAGEN-tip, and elute by gravity flow into a 2-ml collection tube (supplied).

Using a QIArack or a tip holder, place the QIAGEN-tip over the collection tube. RNA is specifically eluted, while DNA remains bound to the QIAGEN Resin.

Note: A working solution of Buffer QRUR must be prepared by dissolving 29 g of urea in 60 ml of Buffer QRU (both supplied with the kit).

**A second elution step with 1 ml of preheated (45°C) Buffer QRUR will increase yields by 10–15%.*

9. Add 1 volume ice-cold isopropanol. Mix thoroughly by vortexing or shaking, and place on ice.

Note: If the total RNA concentration is expected to be less than 2 µg/ml, addition of 5–10, 20–30, or 50–100 µg of a carrier such as tRNA or an RNA homopolymer (not supplied) is recommended.

<u>If both RNA and DNA are needed, continue with steps B10–B18. If only total RNA is required, continue with steps A10–A13.</u>

To complete isolation of total RNA without simultaneous isolation of genomic DNA

A10. Incubate the sample on ice for 10 min, then centrifuge at 15,000 x *g* for 30 min at 4°C to precipitate the RNA. Carefully remove the supernatant.

Pellets from isopropanol precipitation may be difficult to see. It is helpful to mark the expected location of the pellet on the tube before centrifugation.

A11. Add up to 0.5 ml, 2 ml, or 4 ml of 70% ethanol to the RNA pellet. Vortex, centrifuge at 15,000 x *g* for 15 min at 4°C, and carefully remove the supernatant. Repeat this step once.

A12. Air-dry the RNA pellet for approximately 10 min at room temperature with the tube

resting upside down on a paper towel.

Note: Overdrying the pellet will make it difficult to dissolve the RNA.

A13. Dissolve the RNA in a small volume of RNase-free water by heating the tube for 3 min at 60°C followed by vortexing for 5 sec and sharply flicking the tube. Repeat at least twice.

Both vortexing and flicking the tube are important to completely dissolve the RNA. Large RNA molecules will not be quantitatively recovered if the RNA pellet is not completely dissolved. The RNA should be stored frozen at –20°C or –70°C.

To complete simultaneous isolation of total RNA and genomic DNA

B10. Pipet the flow-through from step 6 onto the same QIAGEN-tip, and allow it to enter the resin by gravity flow. Save the flow-through for the next step.

B11. Pipet the flow-through onto the same QIAGEN-tip. Allow it to enter the resin by gravity flow.

Omission of this step may result in less than quantitative recovery of genomic DNA.

B12. Pipet 3 ml (3 x 1 ml) of Buffer QC onto the QIAGEN-tip, and allow it to enter the resin by gravity flow.

It is particularly important not to force out residual Buffer QC. Traces of Buffer QC will not affect the elution step.

B13. Elute the genomic DNA with 1 ml (2 x 1 ml)* of preheated (45°C) Buffer QF. Using a QIArack or a tip holder, place the QIAGEN-tip over a 2-ml collection tube (supplied).

B14. Add 0.7 volumes room-temperature isopropanol. Mix thoroughly by vortexing or shaking, and incubate for 10 min at room temperature.

Note: If the DNA concentration is expected to be less than 2 µg/ml, addition of 10, 30–40, or 75–100 µg of a carrier such as tRNA or an RNA homopolymer (can use glycogen also) (not supplied) is recommended.

**A second elution with 1 ml of preheated (45°C) Buffer QF will increase yields by10–15%. Elute into a 10–15-ml sterile polypropylene or glass centrifuge tube if elution volume is 2 x 1 ml.*

B15. Centrifuge RNA from step 9 and DNA from step B14 at 15,000 x *g* for 30 min at 4°C to precipitate. Carefully remove the supernatants.

Pellets from isopropanol precipitation may be difficult to see. It is helpful to mark the expected location of the pellet on the tubes before centrifugation.

B16. Add up to 0.5 ml of ice-cold 70% ethanol. Vortex briefly, and centrifuge at 15,000 x *g* for 20 min at 4°C. Carefully remove the supernatant. Repeat this step once.

B17. Air-dry the RNA and DNA pellets for approximately 10 min at room temperature with the tubes resting upside down on a paper towel.

Note: Overdrying the pellets will make the RNA and DNA difficult to dissolve.

B18. Dissolve the DNA in a suitable volume (0.1–2 ml) of the buffer of choice (e.g., TE, pH 8.0) by heating the tube for 3 min at 60°C followed by vortexing for 5 sec and sharply flicking the tube. Repeat at least twice.

Alternatively, dissolve the DNA overnight at room temperature on a shaker or at 55°C for 1–2 h. DNA dissolves best under slightly alkaline conditions (pH 8.0–8.5).

B19. Dissolve the RNA in a small volume of RNase-free water by heating the tube for 3 min at 60°C followed by vortexing for 5 sec and sharply flicking the tube. Repeat at least twice.

Both vortexing and flicking the tube are important to completely dissolve the RNA. Large RNA molecules will not be quantitatively recovered if the RNA pellet is not completely dissolved. The RNA should be stored frozen at –20°C or –70°C.

References

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., and M.J. Bailey. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl. Environ. Microb. 66:5488-5491.

Qiagen DNA/RNA handbook available at:
http://www1.qiagen.com/literature/handbooks/INT/rnalit.aspx