Simultaneous isolation of DNA and RNA from soil or sediment samples

A) Extraction of total nucleic acids (DNA and RNA)

There are two different extraction buffers: Tris or PIPES. Selection of extraction buffer depends on the conditions of the samples. Tris buffer can be used if the samples are not very dirty. PIPES buffer is suggested if the samples contain large amounts of humic acid.

Extraction buffer

1. Tris Buffer: 100mM Tris-HCl pH 7.0, 100 mM sodium phosphate pH 7.0, 100 mM EDTA pH 8.0, 1.5 M sodium chloride, and 1% CTAB.
2. PIPES Buffer: 100mM PIPES salt, 100mM EDTA, 1.5M NaCl and 1% CTAB, final pH=7.0.

Procedure

1. Weigh out 5 g of soil or sediment into a mortar and add 2g of sterilized sand.
2. Immediately add 1 ml of denaturing solution (4M guanidine thiocyanate, 10 mM Tris-HCl, 1 mM EDTA, 0.5% Sarkosyl, and 100 mM 2-mercaptoethanol; this solution is stable for 1 week). Grind to mix soil with denaturing solution. This step is optional. If you chose not to follow this step, proceed immediately to step 3.
3. Flood the mortar with liquid nitrogen. Grind the sample (avoid thawing the sample). Repeat the freezing and grinding step two times (three times total).
4. Transfer sample to a 50 ml polypropylene tube and add 16.5 mL extraction buffer (Either Tris or PIPES) . Mix gently. *Keep the sample frozen and scrap into the tube using a metal spatula.*
5. Incubate at 65 °C for 2-3 min then add 1.83 mL 20% SDS. Mix gently.
6. Incubate at 65 °C for 45 min to 1 hour, gently mix the sample every 20 min by inversion.
7. Centrifuge at 6000 rpm for 10 min at 25 °C.
8. Transfer the supernatant into a new 50 ml polypropylene tube.
9. Wash the supernatant with equal volume of iso-amyl chloroform (24:1 chloroform:isoamyl) by inverting for 5 min.
10. Centrifuge at 8000 rpm for 10 min at 25 °C.
11. Collect the upper aqueous layer and transfer to in a 50 ml OAKRIDGE centrifuge tube and add 0.6 volumes of isopropanol. Mix gently. Incubate in a -20 °C freezer for 30 min. (Incubate at room temperature if Tris buffer was used).
12. Centrifuge at 10,000 rpm for 30 min. Decant the supernatant, and add 1 ml of cold 70% ethanol to wash the pellet. Dry the pellet in the hood for 10 min.
13. Dissolve the pellet in 100 µL of RNse free water.
14. Examine 5 µl of each sample on a fresh 0.8% agarose gel to check the quality of the nucleic acid and estimate the nucleic acid concentration.
15. The nucleic acid is now ready for fractionation.

Fractionation of nucleic acids using the QIAGEN ALLPrep DNA/RNA mini kit (See the kit handbook for the complete protocol.)

1. Add 100 uL RLT buffer into your 100 uL DNA/RNA mix. (Although the RLT buffer is used for lysing pelleted cells, we recommend adding it to the nucleic acid sample based on based on our experimental results).
2. Transfer the homogenized mixture to an AllPrep DNA spin column and place the column into in a 2 ml collection tube (supplied with kit). Close the lid, and centrifuge for 30 s at ≥8000 x g. Repeat if any liquid remains on the membrane. KEEP THE FLOWTHROUGH.

3. Place the AllPrep DNA spin column in a new 2 ml collection tube, and store at 4 ºC (do not freeze; do not store for long periods)until ready for the DNA purification in steps 14–17. Use the flow-through for RNA purification in steps 6–13.

Total RNA purification

4. Add 1 volume of 70% ethanol to the flowthrough from step 3, and mix well by pipetting. Do not centrifuge. Precipitates may be visible after addition of ethanol. This does not affect the procedure.
5. Immediately transfer up to 700 µl of the sample, including any precipitate, to an RNeasy spin column and place into a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through. Repeat centrifugation with any remaining sample using the same column.
6. Add 700 µl Buffer RW1 to the RNeasy spin column and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
7. Add 500 µl Buffer RPE to the RNeasy spin column and centrifuge for 15 s at ≥8000 x g Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.
8. Add another 500 µl Buffer RPE to the RNeasy spin columnand centrifuge for 2 min at ≥8000 x g The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
9. Place the RNeasy spin column in a new 2 ml collection tube, and discard the old collection tube with the flowthrough. Centrifuge at full speed for 1 min.
10. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane and centrifuge for 1 min at ≥8000 x g to elute the RNA.
11. Measure the RNA concentration using the Nanodrop. If the expected RNA yield is >30 µg, repeat step 10 using another 30–50 µl of RNase-free water.

Genomic DNA purification

1. Add 500 µL Buffer AW1 to the AllPrep DNA spin column from step 3and centrifuge for 15 s at ≥8000 x g . Discard the flowthrough.

   Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use.
2. Add another 500 µl Buffer AW2 to the AllPrep DNA spin columnand centrifuge for 2 min at full speed to wash the spin column membrane.
   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

3. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥8000 x g (10,000 rpm) to elute the DNA.
4. Repeat step 13 to elute further DNA. To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 14.