Coral TRIZOL RNA Extraction

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Abstract

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Protocol

Step 1.
Prep TRIzol® Plus RNA Purification Kit (Ambion 12183-555) in advance:

- **TRIzol®** included in Kit - roughly 1ml per 100uL tissue slurry or 1cm chunk of skeleton

- **Chloroform** - need 200 uL/sample (or per 1ml TRIZOL)

- **96–100% ethanol** - need 60ml to make up Wash Buffer II for 50 samples - Before beginning lysis, add 60 mL 96–100% ethanol to Wash Buffer II. Check the box on the Wash Buffer II label to indicate that ethanol was added. Store Wash Buffer II with ethanol at room temperature.

- **70% ethanol** (in RNase-free water) - need 600 uL/sample

- **Prepare PureLink on-column DNAse treatment:**

1. **Reconstitute the PureLink™ DNase** (Invitrogen 12185-010) by dissolving the lyophilized DNase in 550 µl RNase-free water (included). (For short-term storage, store the reconstituted PureLink™ DNase at 4°C. For long-term storage, prepare aliquots of the PureLink™ DNase and store at -20°C. Avoid repeat freezing and thawing. Thawed PureLink™ DNase stocks may be stored at 4°C for up to six weeks.)
2. **Pre-Mix 80uL/sample DNAse Treatment Before Extractions**: Combine 8uL of 10X DNAse I Reaction Buffer with 10 uL of DNAse (3U/uL) and 62 uL RNAse-free water for a total of 80uL/sample.

Step 2.
Prep Equipment Required:
Microcentrifuge capable of centrifuging at 12,000 ×g at 4degC
Bead Beating Tubes for RNA Extractions (MoBio 13123-50/vwr 101672-312) OR
Bead Beating Tubes for RNA Extractions (BioSpec Zirconia Beads 11079107ZX)
DNA Extraction Tubes with buffer (MoBio 12888-100-PBT)
1.5 mL RNase-free microcentrifuge tubes
RNase-free pipette tips
Two full ln2 dewars
Cooler of Dry Ice

Scissors
Two Styrofoam coolers with lids - for working with nubbins out of LN2.
Log sheet with nubbin code, datetime, bag label, 3 caliper measurements, slurry volume
Tin snips or other snips
Flame and rough ethanol
Calipers
Storage cups for inside cooler
Foil for counter cover
Freezer boxes for DNA and DNA tubes
Needle for flame sterilizing (heavy gauge syringe)
Agilent Bioanalyzer RNA and DNA 1000 Kits
QuBit and Qubit RNA Broad Range Kit with tubes
Microplate centrifuge
Magnetic stand-96 with beads to spare
High-Speed Microplate Shaker (need good seals for the plates if used)
2 MIDI plate inserts for heating systems (Illumina BD-60-601) or PCR machine
Either an Illumina TruTemp Heating System or ScieGene Hybex Microsample Incubator (or equivalent - PCR Machine or incubator)

Step 3.
Pre-Select Nubbins for RNA analysis.

		DNA	RNA
Scenario Ia	No TPAIN Levels 0,4	84	28
Scenario Ib	No TPAIN Levels 0,2,4	126	42
Scenario Ic	No TPAIN 0,1,2,3,4	210	70
Scenario II	TPAIN	280	112

Step 4.
Select Sample Set to Fragment

- Pull out one stocking, comprising a single species from a single tank at a single nutrient level.
- Cut stocking to remove SW nubbins
- pile into styrofoam cooler with Dry Ice, close lid.
- These will be your 'working samples'

Step 5.
Select Sample to Fragment
Noting sample number and recording bag label, select one whirlpacked sample from the cooler. Unwhirl it, you may need warm plastic with your hand or cut the whirlpack if it's taking too long.

Pull whole sample out with gloved hands holding the plastic base, identify target branch/clip for nucleic acid extraction.

Step 6.
Clip target branch/section, with flamed, ethanol sterilized tin-snips, over tin-foil covered bench - cover nubbin with gloved hand to prevent flying bits, . Replace the rest of the nubbin into dry ice cooler in separate cooler section.
Measure resulting piece using calipers: take three measurements to be able to estimate surface area and volume [need more explicitness here]

Step 7.

Fracture piece in 'two' and allocate to RNA and DNA Extraction Tubes

Fragment (1) drop into ready tube with 1 ml trizol and 0.2 ml beating beads for RNA extraction. The sample volume should not exceed 10% of the volume of TRIzol® Reagent used for homogenization, however in this case we consider the tissue to be the 'sample' so if the nubbin is covered by Trizol that is fine.

Fragment (2) drop into ready DNA extraction buffer bead tube for DNA extraction and freeze -80C. The remainder of this protocol focuses only on RNA Extraction Fragment (1).

Step 8.
Lyse the Tissue:
Immediately Bead beat for 45s with FastPrep at max speed.
Note: FastPrep must cool 5-10 minutes between runs.

Incubate the lysate with TRIzol® Reagent at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.

Sample is stable in TRIzol at room temperature after bead beating.

Step 9.
Remove the Lysate:
Either pipette TRIZOL mix out to a clean tube,
OR
Tom's method...:
-ethanol-clean bottom of beat beat tube
-flame sterilize syringe needle
-poke bottom of tube
-nest into new, clean tube (5ml spin tubes?)
-spin fluid out... --> coral tissue/TRIZOL slurry in new clean tube.

Step 10.
Add 200 µL chloroform per 1 mL TRIzol® Reagent used. **Vortex 15s and Incubate at room temperature** for 2–3 minutes.

Note: If you are not doing a DNAse step below, then instead of vortexing shake the tube vigorously by hand for 15 seconds. Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA or perform a DNase-digestion step during RNA purification (as below) or after purification.

Step 11.
Centrifuge the sample at 12,000 x g for 15 minutes at 4°C. Note: After centrifugation, the mixture separates into a lower, red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is 600 µL.

Step 12.
Transfer 600 µL of the colorless, upper phase containing the RNA to a fresh RNase-free tube. **Add an equal volume of 70% ethanol** to obtain a final ethanol concentration of 35%. Mix

well by vortexing. **Invert the tube** to disperse any visible precipitate that may form after adding ethanol.

Step 13.
***BINDING*: Transfer up to 700 µL of sample (prepared as described above) to a Spin Cartridge** (with a Collection Tube). **Centrifuge at 12,000 × g for 15** seconds at room temperature. Discard the flow–through and reinsert the Spin Cartridge into the same Collection Tube. **Repeat until the entire sample has been processed (in other words until all sample has passed through the spin column and RNA bound to the silica frit).**

Step 14.
Add 350* µL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through and the Collection Tube. Insert the Spin Cartridge into a new Collection Tube.

*Without DNAse treatment in the subsequent step there would be only one step with addition of 700 µL Wash Buffer I.

Step 15.
Add 80 µL PureLink® DNase mixture directly onto the surface of the Spin Cartridge membrane. **Incubate at room temperature for 15 minutes.**

Step 16.
Add 350* µL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through and the Collection Tube. Insert the Spin Cartridge into a new Collection Tube.

*Without DNAse treatment in the previous step there would be only one step with addition of 700 µL Wash Buffer I, effectively eliminating this step.

Step 17.
Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge. **Centrifuge at 12,000 × g for 15 seconds** at room temperature. Discard the flow-through, and **reinsert the Spin Cartridge into the same Collection Tube.**

Repeat with a second 500 µL Wash Buffer II with ethanol to the Spin Cartridge. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through, and **reinsert the Spin Cartridge into the same Collection Tube.**

Step 18.
Centrifuge the Spin Cartridge and Collection Tube at 12,000 × g for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.

Step 19.
Add 30 µL–3 × 100 µL (3 sequential elutions with 100 µL each) RNase-Free Water to the center of the Spin Cartridge and **Incubate** at room temperature for 1 minute.

Step 20.
Centrifuge the Spin Cartridge with the Recovery Tube for 2 minutes at ≥12,000 × g at room temperature. Discard the Spin Cartridge. The recovery tube contains the purified total RNA. **Store the purified RNA** on ice if used within a few hours. For long-term storage, store the purified RNA at –80°C.

Note: If you are performing sequential elutions, collect all eluates into the same tube

Step 21.
Quantify and Quality Check RNA

Bioanalyzer?
Qubit?