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**Protocol status:** Working
We use this protocol and it's working

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# Sanger Tree of Life RNA Extraction: Manual TRIzol™

In 1 collection

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## ABSTRACT

This protocol describes the manual extraction of RNA from multiple different tissue samples intended for RNA-Seq, using the TRIzol™ Reagent, based on the Thermo Fisher TRIzol™ Reagent User Guide. This process is highly effective for most taxonomic groups covered by the Tree of Life Programme. The output of this protocol is a highly concentrated RNA extract which can be diluted and submitted for both RNA-Seq and IsoSeq.

## GUIDELINES

- All steps can be performed at room temperature unless stated otherwise.
- If samples are not going to proceed to sample lysis immediately, keep samples on dry ice to maintain temperature and prevent nucleic acid degradation.
- An experienced operator can expect to comfortably process up to 20 samples, with approximately 2 hours handling time over a start to finish period of 2.5 hours. This estimation excludes the DNase treatment and subsequent QC checks.

**Additional Notes:**

- FluidX tubes are used throughout the Tree of Life programme in order to track samples, therefore rather than the microcentrifuge tubes which have been mentioned in this protocol for RNA storage, all routine RNA is stored in FluidX tubes.

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**PROTOCOL integer ID:** 86860

**Keywords:** RNA extraction, manual RNA extraction, TRIzol, reference genome, RNA-Seq, IsoSeq

## MATERIALS

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Cat. no. 0030108078)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791A)
- Dry ice
- TRIzol™ reagent
- Chloroform
- 100% absolute isopropanol
- 100% absolute ethanol
- RNase-free water
- Turbo DNA-free™ kit (ThermoFisher Scientific, Cat. no. AM1907)

### Equipment

- Pipettes for 0.5 to 1000 µL and filtered tips
- Diagnocine PowerMasher II tissue disruptor (Cat. no. FNK-891300)
- Eppendorf™ Centrifuge 5425/5425 R (Cat. no. 5405000263)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Timer

**Protocol PDF:** Sanger Tree of Life RNA Extraction_ Manual TRIzol.docx.pdf

## SAFETY WARNINGS

- The operator must wear a coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures, as a number of reagents in this protocol are considered hazardous (irritant) and PPE.
- Phenol and chloroform must be handled in a chemical fume hood. In addition, chloroform-resistant gloves (silver-shield) must be worn.
- Sample lysis must be performed in a fume hood.
- Waste needs to be collected in a suitable container (e.g. plastic-lined glass bottle) and disposed of in accordance with corresponding local regulations. Phenol and chloroform waste bottles should be stored in the fume hood.

## BEFORE START INSTRUCTIONS

- Pre-chill centrifuge to 4 °C.
- Label two 1.5 mL microcentrifuge tubes for each sample – one for transferring the supernatant after TRIzol addition and one for transferring the aqueous phase containing RNA after chloroform addition.

## Sample lysis

1 Place the samples on dry ice.

2 For cryoprepped samples:
a) Label a 1.5 mL microcentrifuge tube for each sample.
b) Add 500 µL of TRIzol into each 1.5 mL microcentrifuge tube.
c) Add 500 µl of TRIzol into the tube containing the sample. Gently mix with a wide-bore tip and transfer the homogenised sample to the sample's corresponding 1.5 mL microcentrifuge tube.

3 For powermashed samples:
a) Label a 1.5 mL BioMasher tube for each sample.
b) Add 50–100 mg of sample tissue to the sample's corresponding BioMasher tube and add 250 µL of TRIzol.
c) Fit a sterile BioMasher pestle into the Diagnocine PowerMasher II tissue disruptor and disrupt the tissue, until no large pieces remain or sample cannot be disrupted further. (For more detailed instructions regarding powermashing, please refer to the Sanger Tree of Life Sample Homogenisation: Powermash protocol.)
d) Discard the pestle and add 750 µL of TRIzol to the 1.5 mL BioMasher tube containing the now powermashed sample.

4 Vortex the samples for 1–2 seconds on maximum setting.

5 If the disrupted samples have any large pieces of debris:
a) Centrifuge sample at 12,000 × *g* at 4 °C for 5 minutes.
b) Transfer the supernatant to a new, labelled, 1.5 mL microcentrifuge tube.

6 Incubate the samples for 5 minutes at room temperature.

7 Add 200 µL chloroform per 1 mL of TRIzol used for lysis, then securely cap the tube.

8 Incubate the samples for 2 to 3 minutes at room temperature.

9 Centrifuge the samples at 12,000 × *g* at 4 °C for 15 minutes. The mixture separates into a lower red phenol-chloroform phase (protein), opaque interphase (DNA), and colourless upper aqueous phase (RNA).

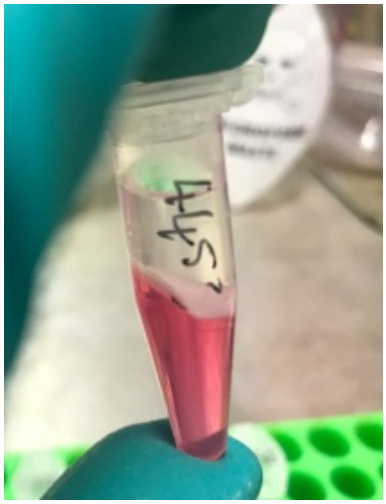

*Figure 1. Sample after centrifugation showing the three phases.*

10 Label a new set of 1.5 mL microcentrifuge tubes for the samples.

11 Transfer the aqueous phase containing the RNA to the newly labelled 1.5 mL microcentrifuge tubes. It may be helpful to hold the sample tubes at 45° whilst removing the aqueous phase. **IMPORTANT: Do not aspirate the interphase or red organic phase. You may wish to leave behind some of the aqueous phase in order to avoid accidentally pipetting the other phases.**

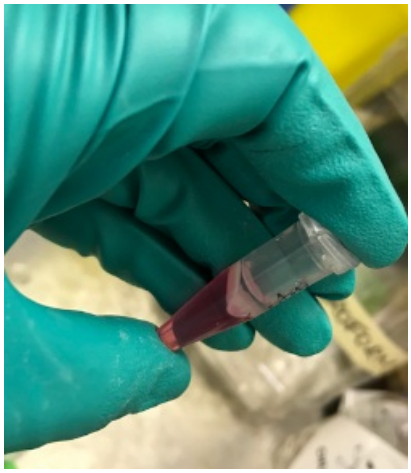

*Figure 2. Sample tube after aqueous phase has been removed – a small amount of the aqueous phase has been left behind to avoid contamination.*

12 Unless they are required for another protocol, dispose of the organic phase and interphase.

## Precipitate and wash RNA

13 Add 500 µL isopropanol per 1 mL of TRIzol used for lysis. Incubate the samples for 10 minutes at 20 °C.

14 Centrifuge the samples at 12,000 × *g* at 4 °C for 10 minutes. The precipitated RNA will form a white, gel-like pellet near the bottom of the tube.

15 Discard the supernatant.

16 Resuspend the RNA in 1 mL of freshly-made 75% ethanol per 1 mL of TRIzol used.

17 Briefly vortex the sample.

18 Centrifuge at 7,500 × *g* at 4 °C for 5 minutes.

19 Discard the supernatant.
**Note: Do NOT allow the pellet to dry to ensure total solubilisation of the RNA. Partially dissolved RNA samples have an A230/280 <1.6. RNA can be stored in 75% ethanol at −20 °C for at least a year, or at 4 °C for 1 week.**

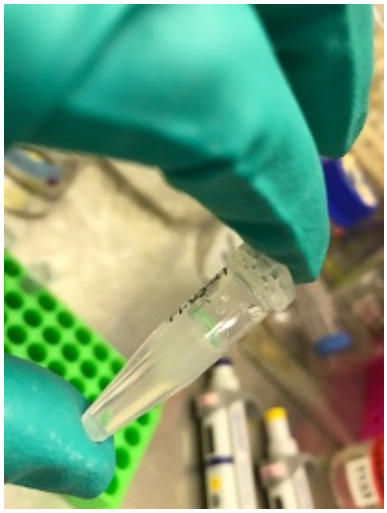

*Figure 3. RNA forms a small, white pellet after precipitation and centrifugation.*

## Resuspend and store RNA

20 Resuspend RNA pellet in 50 µL of RNase-free water. Gently pipette to mix.

21 Incubate the samples at 55–60 °C for 10 to 15 minutes.

22 Proceed directly to DNase treatment, or store samples at –80 °C.

## DNase treatment

23 Remove any DNA from the RNA samples by using Turbo DNase treatment, following the manufacturer's instructions for routine DNase treatment using the Turbo DNA-free™ kit:
a) Add 0.1 volume 10X TURBO DNase™ Buffer and 1 µL of TURBO DNase enzyme to the RNA, then mix gently.
b) Incubate at 37 °C for 20–30 minutes.
c) Resuspend the DNase Inactivation Reagent by flicking or vortexing the tube before use.
d) Add 5 µL of DNase Inactivation Reagent and then mix well.
e) Incubate the sample for 5 minutes at room temperature. Flick the tube 2–3 times during the incubation period to redisperse the DNase Inactivation Reagent.
f) Centrifuge the samples at 10,000 × *g* for 1.5 minutes, then carefully transfer the supernatant containing the RNA to a fresh tube. Do not disturb the pellet of DNase Inactivation Reagent.

**Note: If room temperature cools below 22–26 °C, move the tubes to a heat block or oven to control the temperature. Cold environments can inhibit inactivation of the enzyme, leaving residual DNase in the RNA sample.**

24 Verify the RNA concentration and quality of the samples with the required QC.

25 Store the RNA samples at –80 °C.