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Extracting total RNA from *Caenorhabditis elegans* using phase-lock gel separation tubes V.2

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Protocol status: Working

We use this protocol and it's working

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Keywords: RNA extraction, RNA, rna, phase-lock gel separation, phase-lock tube, phase-lock, extraction, worm, worms, *C. elegans*, nematode, *Caenorhabditis elegans*

Abstract

This protocol describes how to extract total RNA from *C. elegans* worms starting with 3300 worms on a 90mm NGM plate



Materials

MATERIALS

- ✕ liquid nitrogen
- ✕ NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**
- ✕ NanoDrop™ 2000c Spectrophotometer **Thermo Fisher Scientific Catalog #ND-2000C**
- ✕ RNaseZap **Ambion Catalog #AM9780**
- ✕ DNA LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108051**
- ✕ DEPC water
- ✕ TRISure **Bioline Catalog #BIO-38033**
- ✕ Qubit RNA BR Assay Kit **Thermo Fisher Scientific Catalog #Q10211**
- ✕ Qubit 4 Fluorometer **Thermo Fisher Scientific Catalog # Q33226**
- ✕ M9 solution for nematode culture
- ✕ 0.1% SDS solution
- ✕ Phase Lock Gel Separation tube Heavy QuantaBio **VWR International (Avantor) Catalog #733-2478**
- ✕ manual repeater pipette
- ✕ 10ml combitip
- ✕ Ethanol absolute ≥99.8% AnalaR NORMAPUR® ACS Reag. Ph. Eur. analytical reagent **VWR International (Avantor) Catalog #20821.330P**
- ✕ Isopropanol Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog #P/7500/17**
- ✕ Chloroform 99.8 % Certified AR for Analysis Stabilised with Amylene Fisher Chemical **Fisher Scientific Catalog #C/4960/PB08**


Safety warnings


- ⚠ Trisure and chloroform are toxic and should be handled in a fume hood with gloves, goggles and a lab coat. They should be disposed in a special way.



Before start



Turn on heat block to 80 C.






- 1
-  phaseLockTubes.docx

 PLG.pdf

 trisure_fact_sheet.pdf
-  trisure_product_manual.pdf

 trisure.jpg
-  MAN0017209_Qubit_4_Fluorometer...

 MAN0017210_Qubit_4_Assays_QR.p...
-  QRC10210.pdf

 Qubit_RNA_BR_Assay_UG.pdf

- 2 Info on phase lock tubes: Quantabio, 5PRIME, Phase Lock Gel Heavy, 2ml – 200, catalog number: 2302830, store at 15-25 degrees.

Ordered more from VWR International Ltd., ref. 733-2478

- 3 Start with a 90mm NGM plate containing 3300 worms.
- 4 Add M9 to the plate and collect the worms into a 14 ml Falcon tube
- 5 Centrifuge at 2000 rpm in big centrifuge.
- 6 Remove supernatant.
- 7 Add M9 to Falcon to resuspend worm pellet.
- 8 Repeat steps 5-7 three times.
- 9 Remove supernatant.

- 10 Add 750 ul TRIsure (Bioline, catalog number BIO-38033) to samples and store in 80 C freezer.
- 11 Decontaminate the working area with RNaseZap or 0.1% SDS in DEPC water.
- 12 5 times freeze-thaw cycles in the TRIsure in liquid nitrogen. Dip the tube in liquid nitrogen for 3 minutes. Dip the tube in water at room temperature until no ice can be seen anymore. For 24 samples, 3 tanks of liquid nitrogen are useful.
- 13 Make sure there are very few carcasses left in the Trisure.
- 14 If after 5 freeze-thaw cycles, there are still many carcasses in the TriSURE, consider adding 750 ul TriSURE and splitting each sample into 2.
- 15 pre-spin phase-lock tubes at 21,130 for 1 min at 4 C.
- 16 Transfer Trisure/worms to a Heavy phase lock gel tube (pipet),
- 17 Add 150 ul chloroform, shake by hand (until emulsion) and incubate for 10 min at RT. After 10 minutes, there are 3 phases. The gel is at the bottom, the green phase is in the middle and a pale/clear phase is at the top. Do not store chloroform in plastic! Chloroform penetrates plastic very efficiently and gets out of falcon tubes. Use chloroform straight out of the glass jar.
- 18 Centrifuge at 21,130 x g for 5 minutes at 4 C.
- 19 Add 400 ul chloroform using the manual repeater pipette with a 10 ml combitip. I experienced no spillage from the 10 ml combitip as opposed to normal tips/P1000. However, the combitip pipette can be splashy if the sliding mechanism is not smooth.
- 20 Centrifuge at 21,130 x g for 5 minutes at 4 C.
- 21 Now, the positions of the gel and the green phase have exchanged. The gel is in the middle, with the pale phase at the top and the green phase at the bottom.

- 22 Collect upper phase (decant) into a DNA LoBind tube (leave some in order to avoid taking gel). Add 500 ul isopropanol (meaning 1:1 aqueous phase-isopropanol) and 1 ul glycogen (optional for abundant RNA = no need to add if RNA is abundant). Mix the tube by inversion. Incubate for 5 minutes at room temperature (RT for abundant RNA, -80 C for one hour for low qty RNA). A postdoc in the lab suggests to not incubate it on ice or in the freezer because that might precipitate salts. The isopropanol/aqueous phase solution can appear granular. The granules are the precipitated RNA.
- 23 Centrifuge at maximum speed for 30 min at 4 C (=21,130 x g in Eppendorf Centrifuge 5424 R).
- 24 Heat DEPC water at 80 C in heat block.
- 25 Throw away the phase-lock tubes into their own waste bin.
- 26 Remove isopropanol.
- 27 Wash 4 times with 900 ul of cold (keep on dry ice) freshly prepared 70% EtOH (make sure to remove as much supernatant as possible but do not let the pellets dry). Prepare the 70% EtOH fresh each time because ethanol solutions are hygroscopic and can thus decrease in ethanol content over time. The washing works as follows: add 900 ul ethanol, centrifuge 5 minutes at 4 C 21,000 x g and remove ethanol. Note: for small RNA extraction, 80% EtOH is better. However, the Sanger small RNA seq facility receives total RNA and then does a size selection to isolate small RNA, so use 70% EtOH.
- 28 Note: for small RNA extraction, 80% EtOH is better. However, the Sanger small RNA seq facility receives total RNA and then does a size selection to isolate small RNA, so use 70% EtOH.
- 29 Centrifuge 5 minutes at 4 C top speed (21,130 x g) and remove supernatant.
- 30 First remove supernatant with P1000 on 1000 ul. Then, remove it with the P20 at 20 ul. If the pellet detaches, centrifuge again for 2 minutes. Keep the eppendorfs with the opening in the inside to locate the pellet more easily.
- 31 Air-dry in the hood for 10 minutes at room temperature. A tiny drop of ethanol left after drying does not correlate with low RNA or contamination.



- 32 Resuspend in 50 ul 80 C DEPC water (because the Sanger Institute facility accepts a volume of up to 50 ul).
- 33 Flick tube for 1 minute. If many samples are being processed at once, take 3-4 in a hand and scrape the bottom of the tubes on a rack back and forth.
- 34 Heat tube in heat block for 1 minute at 80 C.
- 35 Spin down.
- 36 Transfer tubes on water ice. A PhD student in the lab advises to transfer on ice from aqueous phase onwards but the time I did not use ice, the RNA extraction worked and the RNA was good quality (Bioanalyser Nano).
- 37 Continue with Nanodrop and RNA Broad Range Qubit. Info on phase lock tubes: Quantabio, 5PRIME, Phase Lock Gel Heavy, 2ml – 200, catalog number: 2302830, store at 15-25 degrees. Ordered more from VWR International Ltd., ref. 733-2478