RNA extraction protocol (Trizol)

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Abstract

This protocol describes how to extract total RNA from flatworms. It is from:

Hebert, F, O; Grambauer, S; Barber, I; Landry, C, R; Aubin-Horth, N (2016): Reference transcriptome sequence resource for the study of the Cestode Schistocephalus solidus, a threespine stickleback parasite. GigaScience Database. http://dx.doi.org/10.5524/100197

Citation: Hebert F.O., Grambauer S., Barber I., Landry C.R., Aubin-Horth N. RNA extraction protocol (Trizol). **protocols.io**
https://www.protocols.io/view/RNA-extraction-protocol-Trizol-ew7bfhn
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Before start

Pre-heat incubator at 65°C, clean bench surface and all the lab tools that will be used in the protocol (pipettes, tube holders, dissection forceps) with RNAse Zap/Rnase Wipes. Make sure that isopropanol + ethanol are at -20°C.

Protocol

Phenol extraction

Step 1.
Add 1000 µL of Trizol per 50-100 mg of parsite tissues in a 2 mL eppendorf tube (RNAse free) and place the parasite tissues into their respective, labelled tube

NOTES
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If a worm weights more than 100 mg, divide the whole worm into fractions of ~50-100 mg

Phenol extraction

Step 2.
Add 1 metal bead into each tube and place the tubes into a TissueLyzer. Run it at 30 Hz for 3 minutes

DURATION
00:03:00

Phenol extraction

Step 3.
Centrifuge at 12,000 x g for 10 minutes at 4°C

DURATION
00:10:00

Phenol extraction

Step 4.
Discard top layer of liquid formed in the eppendorf after the centrifugation (contains fatty acids)

Phenol extraction

Step 5.

Transfer supernatant into a new and labelled eppendorf tube (2 mL).

Phenol extraction

Step 6.

Incubate at room temperature for 5 minutes.

DURATION

00:05:00

Chloroform extraction

Step 7.

Add 200 µL of chloroform per tube

Chloroform extraction

Step 8.

Mix vigorously by inverting the tubes up and down for 1 minute. **DO NOT VORTEX!**

DURATION

00:01:00

Chloroform extraction

Step 9.

Centrifuge at 12,000 x g for 15 minutes at 4°C

DURATION

00:15:00

Precipitation

Step 10.

Transfer with precaution the aqueous phase (supernatant) into a new 2 mL eppendorf tube

NOTES

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Transfer small volumes (ex. ~7 x 100 µL)

Precipitation

Step 11.

Add 500 µL of RNAse free isopropanol (100%) and mix thoroughly

Precipitation

Step 12.

Incubate for 15 minutes at room temperature

DURATION

00:15:00

NOTES

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If yields are too low, incubate for 30-40 minutes

Precipitation

Step 13.

Centrifuge at 12,000 x g for 10 minutes at 4°C

DURATION

00:10:00

Cleaning

Step 14.

Discard supernatant

Cleaning

Step 15.

Add 1000 µL of 75% ethanol (kept cold at -20°C) per tube

Cleaning

Step 16.

Vortex thoroughly and centrifuge at 7500g for 5 minutes at 4°C.

DURATION

00:05:00

Cleaning

Step 17.

Discard supernatant and let the tubes dry out with the cap opened for 5-10 minutes

DURATION

00:05:00

Resuspension

Step 18.

Add 20-50 µL of DEPC treated water into each tube

Resuspension

Step 19.

Place the tubes into the incubator (65°C) for 1 minute

DURATION

00:01:00

Resuspension

Step 20.

Vortex thoroughly and repeat step 19 until RNA is completely dissolved.

Warnings

Make sure to work under the fume hood for the Phenol and Chloroform extraction sections.