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RNA extraction protocol (Trizol)

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In 1 collection

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Hebert F.O.[1], Grambauer S.[1], Barber I.[1], Landry C.R.[1], Aubin-Horth N.[1]

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Hébert FO, Grambauer S, Barber I, Landry CR, Aubin-Horth N, Transcriptome sequences spanning key developmental states as a resource for the study of the cestode *Schistocephalus solidus*, a threespine stickleback parasite. GigaScience, 2016, doi: https://doi.org/10.1186/s13742-016-0128-3

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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

Troubleshooting

Safety warnings

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

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.

Phenol extraction

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

Note

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

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

00:03:00

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

00:10:00

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

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

6 Incubate at room temperature for 5 minutes.

00:05:00

Chloroform extraction

7 Add 200 µL of chloroform per tube

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

00:01:00

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

00:15:00

Precipitation

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

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

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

12 Incubate for 15 minutes at room temperature

00:15:00

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

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

00:10:00

Cleaning

14 Discard supernatant

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

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

00:05:00

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

00:05:00

Resuspension

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

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

00:01:00

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