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High-quality RNA extraction from zebrafish tissue

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol can be used to extract high-quality RNA from zebrafish tissue or cells, at embryonic and larval stages, for applications such as qRT-PCR or bulk RNAseq.

Materials

Trizol reagent

Chloroform

Ethanol (96 or 99%)

3M Sodium Acetate, pH5.2



Sample collection and preparation

- 1 Collect embryos and grow to the desired developmental stage. It is best practice to ensure closeness of age by only allowing pairs of fish to mate for 5 minutes at a time before collecting embryos. This makes the greatest difference at the earliest developmental stages, where development is most rapid i.e. 0–1 dpf.

You can either extract RNA from whole animals or perform dissection/FACS etc. as required for your experiment.

Extraction

1h 50m

- 2 Keep samples on ice throughout the extraction process wherever possible.

Place the samples in Eppendorf tubes and add  500 μL of Trizol reagent. At this point samples can be frozen at  $-80\text{ }^{\circ}\text{C}$ for later extraction.

- 3 Homogenise samples by aspiration through a G30 needle and 1 ml syringe. Be as vigorous as possible without causing the tube to overflow. Lots of foaming is good!

- 4 Follow this with a standard chloroform extraction followed by ethanol precipitation.

1h 50m

Note

Perform all centrifugations at 12000 g. Faster can cause more contaminants to pellet with the RNA, such as the guanidinium thiocyanate and phenol.

Add  100 μL of chloroform to your samples and shake vigorously. Incubate for

 00:05:00 on ice and subsequently centrifuge at 12000g and  $4\text{ }^{\circ}\text{C}$ for

 00:15:00 .

Remove the aqueous phase, being careful not to touch the intermediate or organic phases to avoid carrying over DNA or other contaminants. You will likely have to leave some of the aqueous phase behind.

Add 0.1 volumes of  3 Molarity (M) sodium acetate, pH 5.2 and 2.5 volumes of ice-cold 96–99% ethanol. Incubate samples at  $-20\text{ }^{\circ}\text{C}$ for at least  01:00:00 .

Longer incubations (up to overnight) precipitate more RNA but can also lead to more



contamination. In general, more concentrated RNA precipitates faster. Centrifuge at 12000 g and 4 °C for 00:20:00 .

Note

I filter my 99% and 70% ethanol solutions through a 0.2 um filter and store them in the freezer, ready for use when needed.

Carefully dispose of the supernatant and perform two washes with 500 µL ice-cold 70% ethanol, again spinning at 12000 g and 4 °C for 00:10:00 each.

Air dry the pellet until all ethanol has evaporated (drying for too long impairs resuspension). Resuspend in 30-50 µL of distilled water. Store at -80 °C .

The RNA can now be used for QC, RNAseq or other applications.