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RNA extraction from Drosophila embryos

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

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Abstract

A simple protocol for RNA extraction and cDNA synthesis from Drosophila embryos

Materials

Invitrogen (Thermo Fischer)
TRIzol (cat number: 15596026)

Chloroform

Isopropanol

RNAse-free water

Sigma-Aldrich
DNAse I (AMPD1)

Applied Biosystems (Thermo Fischer)
High-Capacity RNA-to-cDNA Kit (Cat Number: 4387406)

RNA extraction

1h 3m 30s

1 Collect 100-200 staged embryos in a 1.5 ml eppendorf tube. Wash with PBT twice. Either centrifuge them at a low speed (<= 500 g) for a minute or let them precipitate by themselves. 10m

2 Remove all the liquid, add 50 µL of Trizol. Homogenize the embryos using a plastic pestle. Depending on the amount of material, add 50-150 µL of Trizol trying to wash the pestle. The protocol proceedes with the assumption that the total volume of Trizol is now 200 µL. Incubate for 00:05:00 at room temperature. 10m

3 Add 1/5 volume of chloroform (for 200 µL of Trizol it would be 40 µL of chloroform). Vortex for 00:00:30 and incubate at RT for 00:03:00 3m 30s

4 Centrifuge for 00:15:00 at 12000 x g at 4 °C 15m

5 Transfer the aqueous phase to a new tube and add 0.8-1 volume of isopropanol. Incubate for 00:10:00 at RT if expected yield is high, or at -20 °C for 02:00:00 if you want RNA to precipitate better. 10m

6 Centrifuge for 00:10:00 at 12000 x g at 4 °C 10m

7 Discard the supernatant and wash the pellet one time with 200 µL of cold 70% EtOH. Try to dislodge the pellet from the tube side but do not suck it into the pipette tip when mixing.

8 Centrifuge for 00:05:00 at 10000 x g at 4 °C. Discard the supernatant. 5m

9 (Optional) Dry the pellet at 55 °C for 00:01:00

10 Resuspend the pellet in 10-20 µL of RNAse-free water.

11 (Optional) Dissolve at 55 °C for 00:01:00

12 Measure the RNA concentration at NanoDrop spectrophotometer.

DNAse treatment and reverse transcription

1h 35m

13 Use 1 µg of RNA from the previous step (the total volume shouldn't exceed 6.4 µL .

14 Add 0.8 µL of DNAse buffer and 0.8 µL of DNAse I from Sigma DNAse I Kit to your RNA, if the volume is less than 8 µL , add RNAse free water until it is equal to 8 µL . Mix by pipeting. Leave for 00:20:00 on RT. (Adding 1 µL of buffer and DNAse would not result in the failure of the experiment, so one can opt to do that, if their pipette is not very precise).

20m

15 Add 1 µL of Stop solution, mix by pipeting and put on 65 °C for 00:10:00 .

10m

16 Transfer everything to a 200 µl PCR tube. Add 10 µl of RT buffer and 1 µl of RT enzyme from High-Capacity RNA-to-cDNA Kit. Mix by pipeting and put in the PCR machine for RT reaction. Set the program like this:

1. 37 °C for 01:00:00
2. 95 °C for 00:05:00

1h 5m

17 Add RNAse-free water to the final volume of 200 µl. Your cDNA is ready.