RNA extraction using TRIzol

Adapted from www.youtube.com/watch?v=RmPsLoIPRwc, www.thermofisher.com, and https://www.youtube.com/watch?v=MgNicWbANkA

Basic principle

RNA extraction can be performed using TRIzol, also known as the Guanidinium Thiocyanate phenol-chloroform extraction method. This isolation also enables the separation of DNA and proteins from a biological sample and is characterized by high-yield extraction. TRIzol promotes cell lysis and protein denaturation because it is a monophasic solution of phenol and guanidine isothiocyanate. Exposing cells to TRIzol promotes the release of cellular content by disrupting cellular membranes and denaturing proteins. These processes protect RNA from RNase degradation and enable RNA separation for downstream processing.

Required equipment and consumables

2 mL RNAse-free tubes
70% ethanol
A frozen cooler or ice
Chloroform
Isopropanol
Laboratory hood
Pipettes, tips, and racks
Refrigerated centrifuge
RNase decontamination solution
RNase-free towels
RNase-free water
Scientific scale
Three Erlenmeyer flasks
Tissue homogenizer and pestle
TRIzol
Vortex

Procedure

1. Perform all procedures inside a laboratory hood.
2. Clean all surfaces involved in the RNA extraction procedure using an RNase decontamination solution, including pipettes and tissue homogenization pestle.

3. Prepare two containers with RNase water and one with 70% ethanol to perform the initial rinse of the pestle and additional rinse between samples.
4. Clean the tissue homogenization pestle using the first RNase-free water container to remove any residuals of the RNase decontamination solution (first container). Rinse again with 70% ethanol (second container) and then with RNase water (third container) for a final wash. These washes are performed by inserting the pestle into each solution container and allowing it to run for 20 seconds.
5. Dry the pestle using RNase-free towels.
6. Label as many 2 mL RNAse-free tubes as samples you are planning to process. Label a total of two sets of tubes.
7. Weight 40 mg of tissue if working with muscle or liver. Use the first set of labeled tubes and place each sample in the corresponding tube.
8. Add 1 mL of TRIzol to each tube.
9. Grind the tissue using the tissue homogenizer and pestle, keeping the sample on ice or a frozen cooler. Do not let the sample warm up, as this will damage the RNA's molecular integrity. Perform homogenization by using 10-second pulses.
10. Clean the homogenizer’s pestle between each sample to prevent sample cross-contamination. Use the same procedure described in point 4.
11. Dry the pestle using RNase-free laboratory towels.
12. Add 200 µL of Chloroform to the solution. This reagent will promote the phase separation.
13. Vortex vigorously.
14. Incubate on ice or a frozen cooler for 15 minutes.
15. Centrifugate to get phase separation at 12,000 g for 15 minutes at 4°C.
16. There will be a phase separation in the tube. Take the second set of labeled tubes and transfer only the aqueous phase (upper layer). The RNA will remain suspended in the supernatant (aqueous phase).
17. Add 500 µL of Isopropanol to the mixture and pipette up and down. This will allow RNA precipitation.
18. Incubate on ice or the frozen cooler for 10 minutes.
19. Centrifuge for 10 minutes at 12,000 g at 4°C.
20. Remove the supernatant by inverting the tube, keeping the pellet inside the tube.
21. Wash the pellet with 1 mL of 70% ethanol by pipetting. Do not forget to change the tip between samples.
22. Centrifuge at 7,500 g for 10 minutes at 4°C.
23. Remove the supernatant by inverting the tube, keeping the pellet inside the tube.
24. If some residual supernatant is still present, remove it by pipetting.
25. Air-dry the pellet for 10 min by leaving the tube open at room temperature.
26. Dissolve the RNA pellet in 100 µL of RNase-free water.

Precautions

1. Do not invert tubes containing TRIzol and make sure all tubes are completely closed because contamination of phenol (present in the TRIzol reagent) in the centrifuge can cause the centrifuge lid to loosen.
2. Do not over-dry the RNA pellet because it will make it difficult to dissolve.

Applications

The extracted RNA can be used for qPCR amplification or sequencing.

Bibliography

1. www.youtube.com/watch?v=RmPsLoIPRwc
2. www.thermofisher.com
3. www.youtube.com/watch?v=MgNicWbANkA