Standard Operating Procedure @ ICMAB-CSIC

PROTOCOL	**RNA extraction from *C. elegans*.**
DATE	20/05/2024
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1. Purpose

This procedure describes the materials and protocol used to extract RNA from *C. elegans* after exposure to electromagnetic fields.

2. Background

Environmental changes could alter organisms' genetic expression to adapt to external conditions. We could measure this change by extracting and sequencing the acid ribonucleic (ARN) material. ARN is the translation of specific genes from the organism's acid deoxyribonucleic (DNA) that are transformed into proteins by ribosomes to perform a function. In that way, organisms only produce the proteins required to respond to variable environmental conditions. We previously exposed worms to external electromagnetic waves with their respective control conditions. Worms were exposed for three consecutive generations. They were recollected, cleaned from bacteria and frozen in trizol reagent. Samples were sent from Naples to Barcelona, Spain, for further processing. See

Annex 15 for more information about the exposure conditions. This protocol explains how RNA was extracted and purified for further evaluation.

3. Procedure

3.1. Equipments

- Thermo shaker (Biosan ts-100)
- Table centrifuge (J.P. Selecta. S.A. 7002239)
- Vortex Ibx instruments V05 series)
- -80°C Ultrafreezer (Telstar Boreas)
- Laminar flux hood (ESCO Laminar Flow Cabinet)
- Nanodrop (Nanodrop One C, Thermo Scientific)

3.2. Materials

- Ice
- Polystyrene boxes
- Eppendorf® tubes (Labbox, 1.5 ml, PCRP-015-500)
- Falcon tubes (Labbox, 15 ml, CTPG-E15-050)

3.3. Reagents

- Ethanol (99%) Panreac AppliChem 361086.16153
- N_2 liquid
- Trizol (15596026 Thermo Fisher)
- PureLink™ RNA Mini Kit (12183018A, Invitrogen by Thermo Fisher) Contains spin cartridges with collection tubes, collection tubes, recovery tubes, wash buffer I, wash buffer II, RNAse- and free water.

3.4. Experimental procedure

The experimental procedure is divided into two main steps: worms' lysis and RNA extraction.

3.4.1. Worm's lysis

This step is essential to break all tissues and extract the RNA from the cytoplasm of cells.

1) Defrost worms already in Trizol. The volume of Trizol should be seven times higher than the initial pellet of worms.
2) Vortex for 30 seconds at maximum speed (no units) to resuspend everything.
3) Freeze worms with N2 liquid.
4) Thaw worms in the Thermo shaker at 37°C or in a water bath at 37°C.
5) Repeat steps 3 and 4 five times.
6) Vortex worms for 30 seconds at the maximum speed and let the sample rest for another 30 seconds.
7) Repeat step 6 for five times.
8) Wait 30 seconds at room temperature to allow RNA complexes and proteins to break down.

9) Centrifuge at 2600 g for 5 minutes. Separate the supernatant in sterile falcon tubes. Transfer 1.2 ml of supernatant to these tubes.

Note

- When working with N_2 liquid, take precautions and wear all safety equipment (cold protective gloves, safety glasses, and lab coat). Take a small Polystyrene box and a plastic Eppendorf rack. Remove the Eppendorf rack with tweezers to avoid touching the N_2 liquid.
- For the vortex cycles, divide the tubes into two groups. While one group rests after the vortex, the other is in the vortex. This tip accelerates the process while keeping the optimal conditions.

3.4.2. RNA extraction

After lysis, RNA molecules are mixed with proteins, cellular residues, and other molecules. Therefore, RNA should be purified. PureLink™ RNA Mini Kit is used following the manufacturer's instructions.

1) Add the same volume of 70% Ethanol to these tubes. Add 1.2 ml of 70% EtOH, freshly prepared with sterile water and pure Ethanol.
2) Vortex each tube for 30 seconds to mix everything well to reduce non-homogeneous formation.
3) Transfer 700 µl of the sample (including any remaining precipitate) to the spin cartridge (with the collection tube).
4) Centrifuge at 12000 g for 20 seconds at room temperature. Discard the flow-through and reinsert the spin cartridge into the same collection tube.
5) Repeat steps a-b until the entire sample has been processed.
6) Add 700 µl Wash Buffer I to the spin cartridge.
7) Centrifuge at 12000 g for 20 seconds at room temperature. Discard the flow-through and the collection tube. Insert the spin cartridge into a new collection tube.
8) Add 500 µl of Wash Buffer II to the spin cartridge.
9) Centrifuge at 12000 g for 20 seconds at room temperature. Discard the flow-through, and reinsert the spin cartridge into the same collection tube.
10) Repeat steps 8 and 9 once.
11) Centrifuge at 12000 g for 2 minutes at RT. Discard the flow-through and the collection tube. Insert the cartridge into a recovery tube.
12) Add 50 µl RNAse-free water to the center of the spin cartridge.
13) Incubate at RT for 1 minute.
14) Centrifuge at 12000 g for 2 minutes at RT. Discard the cartridge, close the recovery tube, and store your purified RNA immediately on ice.

Note

- 70% Ethanol was freshly prepared by diluting pure ethanol (99%) with sterile MilliQ® water in the laminar flux cabin.
- Keep samples in ice during this protocol.

- Before using the PureLink™ RNA Mini Kit read carefully the preparation instructions, as you should add pure ethanol to buffer II before using it. Keep them at RT for storage and in the fridge for long periods.
- When centrifuge recovering tubes with the spin cartridge, order them well in the centrifuge to break the tap.

3.4.3. RNA quantification

After purification, RNA quantity should be quantified using a Nanodrop equipment to know the concentration and the purity of these molecules. For quality analysis, two ratios are used:

- Ratio between 260 and 280 nm (A260/A280) represents the contamination by DNA molecules. DNA molecules strongly absorb at 280 whereas DNA molecules absorb at 260 nm. Values between 2-2.2 or higher are considered high-quality levels.
- Ratio between 260 and 230 (A260/A280) represents contamination from chemical reagents used for the extraction procedure. Values around 2.2 or higher indicate good quality.

Procedure

1) Switch on Nanodrop and select the RNA measurement.
2) Clean the measurement area with MilliQ® water and paper.
3) Use the RNA-see free water as a background control for Nanodrop equipment. With sterile tips put 2 µl of water in the area of measurement. Close Nanodrop arm. Measurement starts automatically.
4) Clean the measurement area with MilliQ® water and paper.
5) Introduce the name of the sample you are going to measure.
6) With sterile tips, put 2 µl of the extracted RNA into the measurement area. Close Nanodrop's arm. Measurement starts automatically. Take a picture of the graph on the screen and take notes of the concentration (ng/µl), A260/A280, and A260/230.
7) Repeat steps 4-6 until every sample has been measured.
8) Export data through the pen drive entrance and make a copy of the raw data.
9) Clean the measurement area and switch off Nanodrop.
10) Store your RNA at -80°C until further evaluation.

3.4.4. Sample analysis

Samples should be sent to a company that translates the RNA into complementary DNA (cDNA), amplify the molecules through the PCR technique and sequence each read. Information about the company, how to prepare and send samples, and the quality studies performed by the company.