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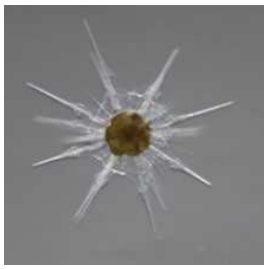
Isolation and RNA extraction from Marine Protist Single Cells for Transcriptomics (sc-RNAseq) V.4



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Ecology of Marine Plank... AD2M

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Manuscript citation:
Rizos I, **Romac S**, Juery C, Berthelier C, Decelle J, Bernardes J, Corre E, Bittner L, Not F. Single-cell transcriptomics highlights sexual cues among reproductive life stages of uncultivated Acantharia (Radiolaria). DOI: 10.1101/2024.07.02.601653.

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

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Abstract

It is difficult to get clean RNA from uncultivated protists (phyto and zooplankton), as they have to be isolated by micromanipulation and very sensitive to exogenous contaminations.
Here we optimized an extraction protocol to get clean DNA-free RNA from protist single-cells for transcriptomic studies.

Image Attribution

Romac S.

Materials

STEP 1 : Isolation

Equipment and supplies :
- Area decontaminated using DNAaway;
- Stereomicroscope or inverted microscope (depending of the size of microorganisms) equipped with a camera;
- Glass Pasteur pipettes or micropipette P10;
- Silicone tubing LS17 (Masterflex™ , ref MFLX9641017);
- Petridishes or 6-well plates, or 3-well glass slides
- Freezer -20°C or -80°C;
- Racks, filter tips 10µL, sterile microtubes 1.5mL and box for storage.

Kit, Reagents and Chemicals :
- TC buffer from **MasterPure Complete DNA and RNA purification kit** (Lucigen, ref MC85200, distributed by Euromedex in France);
- Prefiltered <0.2µm Seawater;
- nuclease-free H2O;
- Ethanol absolute, molecular biology grade;

Misceallenous :
- nitrile gloves;
- paper towell;
- DNA away and RNase away;
- Technical Ethanol 70%;
- Tape and Markers.

STEP2 : RNA extraction

Equipment and supplies :
- Nucleic acid extraction lab (= pre-PCR lab)
- Centrifuge 5427R (Eppendorf, ref 5429000010);
- Thermomix - magnetic stirrer (Eppendorf – ref 5382000015) equipped with thermoblock 1.5-2mL (Eppendorf - ref 3880000151);
- Vortex, Microcentrifuge;
- IsoSafe and IsoPack (Eppendorf, ref 3880001042);
- Micropipettes P1000, P100, P20.
- Thermocycler for DNase incubation;
- Racks, filter tips, sterile Flacon 15mL, sterile microtubes 1.5mL and boxes for storage.

Kit, Reagents and Chemicals :
- MasterPure Complete DNA and RNA purification kit (Lucigen, ref MC85200, distributed by Euromedex in

France)
- Enzymes (Proteinase K, RNase, DNase) are stored at -20°C;
- Other reagents from the kit are stored at room temperature;

- Ethanol absolute, molecular biogy grade;
- Propan-2-ol molecular biology grade;
- TURBO DNAfree 50 reactions (Invitrogen, ref AM1907, distributed fy Fisher Scientific, ref 10792877), stored at -20°C;
- RNA Clean & Concentrator-5 w/ Zymo-Spin IC Columns (Capped) 50 preps (Zymo, ref ZR1015, distributed by Ozyme)

Misceallenous :
- nitrile gloves;
- paper towell;
- DNA away and RNase away;
- Technical Ethanol 70%;
- Tape and Markers.

Safety warnings

- Always wear a labcoat and gloves during all RNA extraction steps.
- Decontaminate all the surface areas, and equipments (rotor, racks, pipettes...) with RNase away.
- Work only with filter tips and sterile microtubes.

Before start

Prepare Ethanol 70% from absolute Ethanol : Mix 35 mL of absolute Ethanol with 15 mL nuclease-free water in a Falcon 50mL Store at -20°C.

1. Cell Isolation

1 Isolate individually protist cells (5-500µm in length) using a glass bent micropipette (or a micropipette adapted to the length of the microorganism) under a binocular microscope or an inverted microscope.

2 Wash each cell in three successive baths of 0.22µm-filtered and sterile seawater on a glass slide.

Wash glass bent in H2O Nuclease-free water, and EtOH96%, and then filtered seawter <0,2µm between each bath.
Image the isolated cell.

3 Add 30 µL of lysis buffer (Tissue and Cell Lysis Solution from MasterPure^{TM} Complete DNA and RNA Purification Kit, Epicenter) in a 1.5mL sterile microtube.

4 Transfer subsequently single-cell in the 1.5mL microtube, flash-freeze in liquid Nitrogen and store at -80°C for RNA/DNA extraction.

2. Cell lysis

5 Pellet cells by short-spin centrifugation.

6 Dilute 1 µL of Proteinase K in 300 µL de lysis solution Tissue et Cellule for each sample. Vortex 10 sec for resuspending cells (facultative).

7 Add 300 µL of mix Proteinase K + lysis solution Tissue et Cellule in each sample. Vortex.

8 Incubate 15 min at 65°C , 600 rpm. Put samples in ice 3-5 min.

3. Total nucleic acids precipitation

9 Add 150 µL MPC reagent to 300 µL of lysed sample. Vortex.

10 Spin 10 min at 11 000 g, 4°C. If there is no pellet, add more 25 µL MPC buffer and spin again 10 min at 11000 g, 4°C.

11 Transfer the supernatant ion a new clean microtube (1,5 mL), discard the pellet.
(To keep the squeletton, keep the tube with the pellet, add 500 µL MilliQ Water and store at -20°C).

12 Add 500 µL of Isopropanol. Mix per inversion. Spin 10min at Vmax (~20 000 g), 4°C.

13 Discard the supernatant with precaution, without touching the pellet.

14 Add 500 µL of Ethanol 70%. Don't vortex, mix gently the support. Spin 5min at Vmax (~20 000 g), 4°C.

15 Discard a maximum of supernatant with precaution using both P1000 and P100, without touching the pellet.

16 Let dry 5-10 min at room temperature until the pellet becomes transparent.

17 Elute in 33 µL of TE1x buffer. Vortex and spin shortly.
1- RNA : Take an aliquot of 30 µL and transfer it in a 0.2mL
sterile microtube. Proceed immediately to the TurboDNase treatment.
2- DNA : You should have 3µL left for barcoding (PCR 18S1, 18S2, 28S). Store at -20°C.

4. TurboDNase treatment (Work on ice)

18 To 30µL of each RNA extract, add :
- 3 µL TurboDNase buffer
- 1µL (2u) of TurboDNase
Do a molecular flick.

Incubate 25min at 37°C in the thermocycleur.
Replace on ice and let incubate on ice for 2min.

19 Add again 1µL (2u) of TurboDNase.
Do a molecular flick.
Incubate 25min at 37°C in the thermocycleur.
Replace samples on ice. Let incubate on ice for 2min and proceed immediately to RNA concentration.

5. RNA purification and concentration using RNA Clean and Concentrator-5 kit (ZymoResearch)

20 Adjust volume of RNA extract to 50µL (Add 15 µL TE1x MP buffer to the 35µL of TurboDNase product).

21 Add 2v (100µL) of RNA binding buffer. Mix thoroughly.
Add 1v (150µL) of EtOH 96-100%. Mix thoroughly.

22 Transfer into a Zymo-Spin IC Column in a Collection Tube.
Centrifuge 30sec at 11 000g, at room temperature.

23 Add 400µL RNA Prep buffer to the column. Centrifuge 30sec at 11 000g.
Discard the flow-through and replace the column into the Collection Tube.

24 Add 700 µL RNA Wash Buffer. Centrifuge 2min at 11 000g.

25 Transfer the column into a RNA-DNafree microtube (1,5 mL) correctly annotated (sample-template-date).

26 Add 11.5µL DNase/RNase free water directly to the column (and 1 µL of RiboGuard if you store at -80°C). Centrifuge 30sec at 11 000g.
Store at -80°C or proceed directly to cDNA synthesis.