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Nucleic acids extraction from single cell using MasterPure Complete DNA purification (Epicenter) v.2

Sarah Romac¹

¹UMR7144-Roscoff Marine Station

1 Works for me dx.doi.org/10.17504/protocols.io.bekbjcsn

Ecology of Marine Plankton (ECOMAP) team - Roscoff
Tech. support email: phytopk@sb-roscoff.fr



Sarah Romac
UMR7144-Roscoff Marine Station



ABSTRACT

Radiolaria are protists which can't be cultivated. These microorganisms have to be isolated by single-cell for genetic identification and it can be difficult to get clean DNA.

Here we optimized a DNA extraction protocol from protist single-cell.

It works very well on single-cell Radiolaria, Foraminifers, but also Ciliates, Dinoflagellates, Diatoms.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Biard et al. Protist 2015. <http://dx.doi.org/10.1016/j.protis.2015.05.002>

MATERIALS TEXT

Material and Equipment :

- Glass bent micropipettes.
- Silicone Tubing (Cole-Parmer, Tube silicone platinum LS T17 ref. [FV-96410-17](#))
- StereoMicroscope or binocular microscope.
- Petridishes Diameter 70mm.
- Centrifuge 5417R (Eppendorf)
- Thermomixer Eppendorf

Kit, Reagents and Chemicals :

- **MasterPure Complete DNA and RNA purification kit** (Epicenter Illumina, ref MC85200)

Enzymes (Proteinase K, RNase, DNase) are stored at -20°C

Other reagents from the kit are stored at room temperature.

- Ethanol absolute, molecular biology quality.
- Propan-2-ol quality molecular biology.
- 0,22µm filtered Seawater or artificial Seawater.
- Ice for enzyme storage during labwork.

SAFETY WARNINGS

Wear labcoat, gloves.

Decontaminate all the surface area, and equipments (rotor, racks, pipettes...) with Ethanol 70% and DNA away.

Works with filter tips.

BEFORE STARTING

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 (at least 50µm in length) using a glass bent micropipette under a binocular microscope.
- 2 Wash each cell in three successive baths of 0.22µm-filtered and sterile seawater.
- 3 Transfer subsequently cells in a 1.5mL sterile microtube.
- 4 Add 30 µL of lysis buffer (Tissue and Cell Lysis Solution from MasterPure™ DNA and RNA Purification Kit, Epicenter) and store at -20°C.

2. Cell lysis

- 5 Pellet cells by centrifugation (2 min at Vmax), throw the supernatant, let ~25-30 µL of liquid.
- 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, 1000 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.

4+ 500 µL Propan-2-ol. Mix per inversion.
5-
6
7+ 500 µL d'EtOH 70%. Don't vortex, mix gently the support. Spin 5min at Vmax ;at 12°C.
8-Discard a maximum of supernatant with precaution, without touching the pellet.
9-Let dry 5-10 min at RT.
10-
10 Spin 10 min at 11 000 g, 12°C. If there is no pellet, add more 25 µL MPC buffer and spin again 10 min at 11 000 g, 12°C.
- 11 Transfer the supernatant ion a new clean microtube (1,5 mL), discard the pellet.
(*To keep the squeleton, 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, 12°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 ;at 12°C.

- 15 Discard a maximum of supernatant with precaution, without touching the pellet.
- 16 Let dry 5-10 min at room temperature..
- 17 Elute in 25 µL of TE1x buffer.
Vortex and spin shortly.
Store at - 80°C.



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