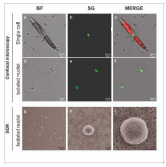

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An optimised method for intact nuclei isolation from diatoms

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1 Works for me dx.doi.org/10.17504/protocols.io.bmj5k4q6

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ABSTRACT

Due to their abundance in the oceans, their extraordinary biodiversity and the increasing use for biotech applications, the study of diatom biology is receiving more and more attention in the recent years. One of the limitations in developing molecular tools for diatoms lies in the peculiar nature of their cell wall, that is made of silica and organic molecules and that hinders the application of standard methods for cell lysis required, for example, to extract organelles. In this study we present a protocol for intact nuclei isolation from diatoms that was successfully applied to three different species: two pennates, Pseudo-nitzschia multistriata and Phaeodactylum tricornutum, and one centric diatom species, Chaetoceros diadema. Microscopy observations confirmed the integrity of isolated nuclei and high sensitivity DNA electrophoresis showed that genomic DNAs extracted by isolated nuclei have low degree of fragmentation. This protocol has proved to be a flexible and versatile method to obtain intact nuclei preparations from different diatom species and it has the potential to speed up applications such as epigenetic explorations as well as single cell ("single nuclei") genomics, transcriptomics and proteomics in different diatom species.

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MATERIALS TEXT

MATERIALS

SYBR™ Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO **Thermo Fisher Catalog #S7563**

NH4F **Sigma Aldrich Catalog #338869 Sigma-Aldrich**

Sea salts **Sigma Aldrich Catalog #S9883**

F/2 Guilliard medium **Sigma Aldrich Catalog #G0154**

Reagents and kits:
1. Artificial sea water (ASW) (Sea salts 3,45% , Sodium bicarbonate 1mM dissolved in distilled water enriched with 1x F/2 Guilliard medium)
2. NH4F (338869 Sigma-Aldrich)
3. NIB (10 mM Tris-HCl pH 7.4; 10 mM NaCl; 3 mM MgCl2; 0.1% IGEPAL CA-630)
4. SYBR Green I Nucleic Acid Stain (S7563 Invitrogen)

Instruments:
1. Refrigerated swinging bucket rotor centrifuge with adapters for 50 mL conical tubes.
2. Refrigerated microcentrifuge for 1.5-2 mL tubes.
3. Tip digital sonifier.
4. Fluorescence microscope.
5. Fluorescence-activated cell sorter (FACS).

DISCLAIMER:

Collection of cells 40m

1 Pellet around 20-30 x 10^6 cells for 15' at 1800 xg at 18°C in 50 mL conical tubes 15m

0.2 Collect pelleted cells in 2 mL tubes and spin 10’ at 1500 xg at 18°C; merge all cells in one 2 mL tube and spin 10’ at 1500 xg at 18°C; 20m

0.3 Wash once with 2 mL of filtered Artificial Sea Water (ASW): spin 5’ at 1500 xg at 18°C 5m

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Dissolving diatom cell walls and isolating nuclei 2h 10m

1 Remove ASW and add 400 µL of NH_4F solution (10 M, pH 5), mix and incubate 10’ at room temperature vortexing periodically. 10m

1.1 Add 1.5 mL of filtered ASW to the sample and centrifuge 5’ at 1500 xg at 18°C 5m

1.2 Remove the supernatant and add 2 mL of filtered ASW, centrifuge 5’ at 1500 xg at 18°C 5m

1.3 Remove completely the ASW and add 200 µL of ice cold NIB, gently pipette to resuspend the cell pellet and put tubes on ice.

1.4 Sonicate on ice applying 5 to 10 repetitions of 15’’ pulses (40 W intensity) with intervals of 10’’. 20m

1.5 Check cell lysis and nuclei morphology: take a small aliquot (5-10 µL) of sonicated cells, add SYBR Green I (1x final dilution), put them on a slide to observe under epifluorescent light.

1.6 Add 1.8 mL of ice cold NIB to the sonicated cells, filter onto 35 µm nylon mesh and add 2µL of SYBR Green I (10x final dilution).

1.7 Dilute the stained sample 10x with ice cold NIB.

1.8 Analyze nuclei at the FAC-sorter; a combination of Side Scatter and Green Fluorescence (530/40 nm, wavelengths of light that are between 510 nm and 570 nm) is used to identify and then sort the nuclei population.

1.9 Sort nuclei using "1 drop pure" sorting criterion and 200 as sorting rate. 1h 30m

2 Sorted nuclei can be kept on ice until downstream applications.

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