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Lysozyme-based removal of bacteria from cultures of the marine heterotrophic flagellate Cafeteria roenbergensis V.2

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

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Abstract

This protocol allows to remove bacteria from cultures of the marine heterotrophic nanoflagellate Cafeteria roenbergensis without affecting flagellate viability. Bacterial removal is essential to reduce contamination and background signal in subsequent microscopy analysis, protein extraction, DNA extraction or other applications. With some changes, this protocol could be adapted to different types of protists.

Materials

MATERIALS

- PBS
- MgCl2
- NaCl
- BD Bacto™ Yeast Extract **Becton Dickinson (BD)** **Catalog #**212750
- OptiPrep™ 250 mL **STEMCELL Technologies Inc.** **Catalog #**7820
- 2 mL Eppendorf
- Falcon tube (50 mL)
- DNAse I (100 mg) **Roche** **Catalog #**1284932
- Thermomixer
- Lysozyme **Thermo Fisher Scientific** **Catalog #**89833
- High speed centrifuge
- Phase-contrast microscope
- F/2 artificial seawater medium
- Lugol's acid iodine solution
- Haemocytometer
- SW28 tubes 25×89 mm **Beckman Coulter** **Catalog #**344058

Preparation of *Cafeteria roenbergensis* culture

1 Determine the cell density of *Cafeteria roenbergensis* : stain 10 µL of a Cafeteria culture with 1 µL of Lugol's acid iodine solution and count the cells on a haemocytometer (Neubauer Chamber)

2 Dilute the *Cafeteria* culture to 5 × 10^5 cells/mL with f/2 medium + 0.03% (w/v) yeast extract.
Incubate overnight at 22-25 °C.

3 Determine the flagellate concentration and use approx. 8 × 10^7 *Cafeteria* cells/sample

4 Centrifuge flagellates at 4,500 g, 5 min, room temperature in 50 mL Falcon tubes. Flagellates will be in the pellet.

5 Resuspend the cell pellets in 1.8 mL of 1X PBS and transfer them into 2 mL microfuge tubes.

Lysozyme and DNaseI treatment

6 Add 40 µL of 50 mg/mL **freshly prepared** lysozyme in 1X PBS (1 mg/mL final concentration).
Incubate for 1 hour at room temperature.

Note

Optionally, you can also add 5 mM EDTA here. EDTA increases the activity of lysozyme against Gram negative bacteria, but also negatively affects *Cafeteria* mobility.

7 Add 200 µL of 10 mg/mL **freshly prepared** DNaseI in 1X PBS (1 mg/mL final concentration, 2 U/µL) and 11 µL of 1M $MgCl_2$ (5 mM final concentration).
Incubate for 30 min at 34°C in a thermomixer with shaking.

8 Centrifuge at 5,000 g, 6 min, 20°C

Optiprep purification

9 Resuspend the pellets in 100 µL of 1X gradient buffer (0.5 M NaCl in 1X PBS). Pipette up and down several times to break up aggregates.

10 Up to 1 × 10^9 *Cafeteria* cells can be loaded per SW28 centrifuge tube.
Add 1X gradient buffer up to 0.5 - 1 mL final resuspended cell volume.

11 Load the sample on top of a two-step Optiprep cushion cushion, 20% (w/v) Optiprep at the bottom of the tube and 10% (w/v) Optiprep on top of the 20% layer (both Optiprep solutions are prepared in 1X gradient buffer).

12 Centrifuge the tubes in an ultracentrifuge, Beckman SW28 rotor, at 20,000 g, 20 min, 20°C, **with slow braking.**

13 Recover *Cafeteria* cells from the gradient, at the interphase between the 10% and 20% Optiprep layers.

Recovery of *Cafeteria* culture

14 Dilute the recovered *Cafeteria* cells at least twofold with F/2 medium (no yeast extract)

15 Diluite the culture 25fold or 50fold with F/2 and stain 10 µL of this dilution with 1 µL of Lugol's acid iodine solution

Load the sample on a haemocytometer to quantify *Cafeteria* cells and remaining bacteria.

16 Dilute *Cafeteria* cells to the desired density. If no or little dilution is required, you can centrifuge again for 6 min at 5,000 g, 20°C and resuspend the cells in F/2 to remove all the remaining Optiprep.