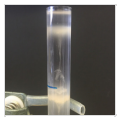

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Density-based removal of bacteria from a culture of the marine heterotrophic flagellate *Cafeteria roenbergensis*

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

Protist Research to Optimize Tools in Genetics (PROT-G)

Sarah Duponchel

ABSTRACT

Heterotrophic flagellates require co-culture with bacteria that serve as a food source. This protocol explains how to reduce the bacterial background of cultures of *Cafeteria roenbergensis*.

MATERIALS

NAME	CATALOG #	VENDOR
Optiprep (Iodixanol)	D1556-250ML	Sigma Aldrich
F/2 medium	MKK50L	
NaCl		
PBS 1X		

Preparation of Cafeteria roenbergensis culture

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

2 Dilute the *Cafeteria* culture to 5 x 10^5 cells/mL in f/2 medium + 0.03% yeast extract and let them grow O/N at 20-25°C

3 Centrifuge the *Cafeteria* cells for 5 min at 4,500 g, 20°C

If it is possible use 50 mL Falcon-types to reduce cell loss

4 Resuspend the cell pellet in 900 µL of 1X Gradient Buffer (0.5M NaCl, 1x PBS).

Pipette several times to break up cellular aggregates

Preparation of OptiPrep density gradient

5 In SW40 Ultra-Clear centrifuge tubes, load not more than 5 ml of the cells suspension in 1X Gradient Buffer.

6 Under-layer the cell suspensions approx. 4 ml of 10% solution of OptiPrep using a syringe and a flat needle.

The OptiPrep stock should be diluted in 1X Gradient Buffer

7 Load approx. 4 ml of 20% solution of Optiprep underneath the 10% solution using a syringe and a flat needle.

The OptiPrep stock should be diluted in 1X Gradient Buffer

Ultracentrifugation

8 Centrifuge the tubes using an ultra-centrifuge at 20000 rpm, 20°C for 20 min **with slow braking**.

Cell recovering

9 Recover Cafeteria cells from the gradient, at the interphase between the 10% and 20% Optiprep layers. You can collect them by pipetting from the top or use a syringe and needle.

10 Checked the samples under the light microscope for the presence of bacteria and to determine flagellate density.

It is easier to dilute the collected samples in at least 10 ml of fresh f/2 media, before observing the cells.

Depending on the bacterial populations, separation can not be as effective. You can use a gradient of OptiPrep (from 30 to 10%) to determine better conditions.

11 It is recommended to remove the OptiPrep from the flagellates by centrifuging them at 4,500 g, 5 min, room temperature.

12 *C. roenbergensis* can be left in f/2 medium (no added yeast extract) overnight without affecting flagellate viability

Account for about 30% of cell loss from the initial stock (from step 4)