Establishment of clonal algal cultures by flow cytometry sorting Version 2

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Abstract

This protocol can be used :

- to isolate novel cultures from natural samples
- to isolate novel cultures from enriched samples
- to purify existing cultures and remove contaminants
- to obtain clonal cultures from a unialgal strain

Reference : Marie, D., Le Gall, F., Edern, R., Gourvil, P. & Vaulot, D. 2017. Improvement of phytoplankton culture isolation using single cell sorting by flow cytometry. J. Phycol. in press.

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Protocol

Prepare sample

Step 1.

Sea water samples can be either unconcentrated or concentrated depending on which population you target. What is critical is to use a sample as fresh as possible. For a cruise, it might be best to concentrate the samples by tangential flow filtration (TFF : https://www.protocols.io/view/tangential-flow-filtration-tff-concentration-of-ph-gpybvpw) and to keep the samples at 100 µE and in situ temperature until they can be sorted.

Prepare sorting plates

Step 2.

- Prepare BSA stock solution in MilliQ water. The percentage of the stock solution will depend on the final concentration used. For example if you use 0.01% final, the stock solution will be 1% BSA (g/v) .
- Sterilize the BSA stock solution by filtration
- Prepare 48 well sorting plates with 0.5 mL of L1, K or f/2 medium with BSA (Bovine serum albumin) concentration ranging from 0.01 to 0.5% (see effect of different BSA concentrations in Figure below reprinted from Marie et al. 2017).

Note : different media and BSA concentration need to be tested for each type of marine sample and target micro-alga

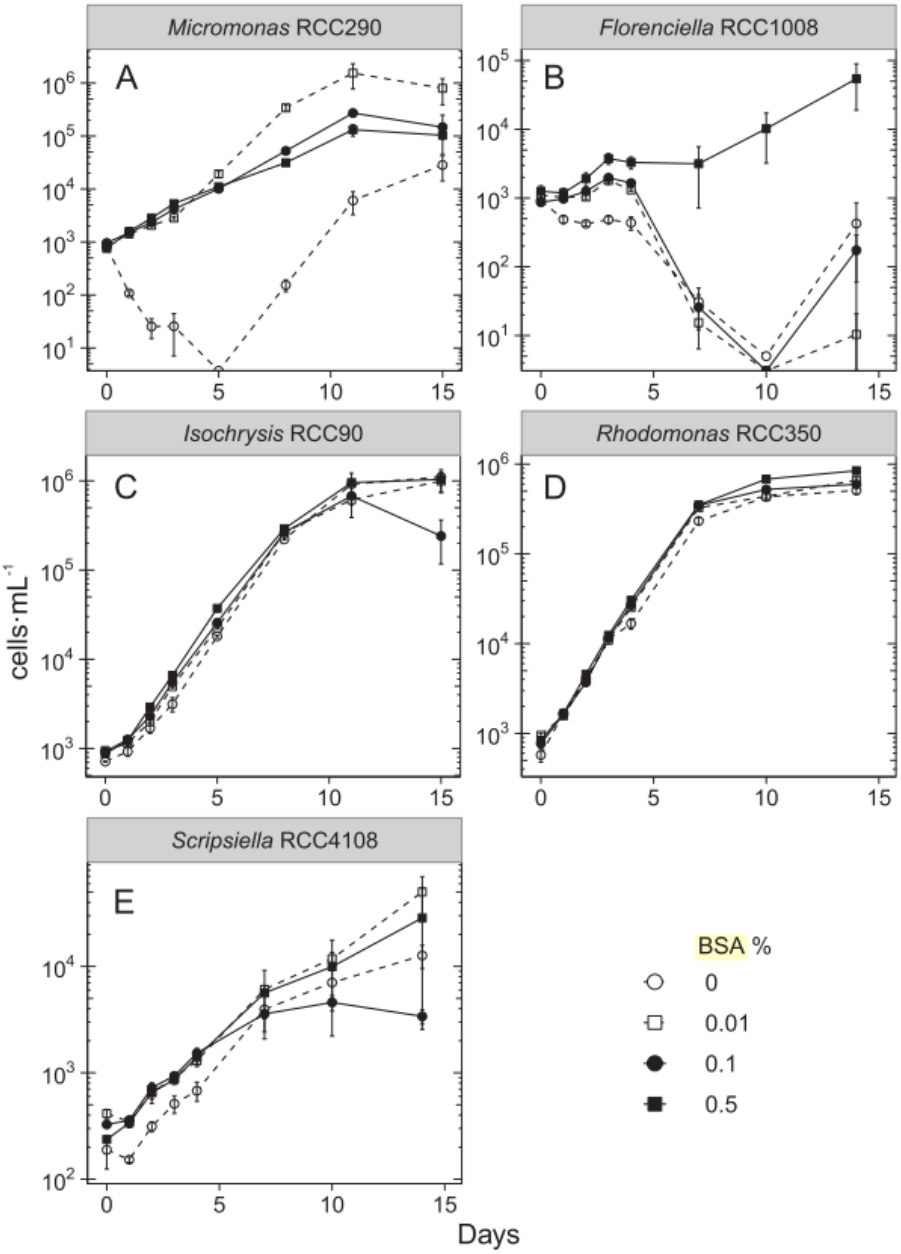

FIG. 2. Effect of the concentration of BSA on the recovery of RCC cultures after sorting of 1,000 cells of *Micromonas pusilla* RCC299 (A), *Isochrysis* sp. RCC90 (B), *Rhodomonas baltica* RCC350 (C), and *Florenciella* sp. RCC1008 (D), and 500 cells of *Scrippsiella* sp. RCC4108 (E) into 1 mL of K medium. Cell concentration was followed by flow cytometry. Error bars correspond to the standard error from three replicates.

REAGENTS

L1 medium MKL150L by NCMA

Bovine Serum Albumin A7030 by Sigma Aldrich

K medium MKK50L by NCMA

f/2 medium MKF250L by NCMA

Sorting

Step 3.

- Sort cells into plates by flow cytometry. Usually we sort a few wells with 100 or 1000 cells and the rest at 1 cell per well.

Incubation

Step 4.

- Incubate at the in situ temperature and at moderate light conditions (typically 100 µE)

Step 5.

- After 3 days, add 0.1% of PNS (Penicillin, Neomycin, Streptomycin), for example, 0.5µl of stock Sigma solution to 500 µL media, to each well (see Figure below reprinted from Marie et al. 2017).

Note : The concentration and timing can be adjusted as a function of the bacterial contamination

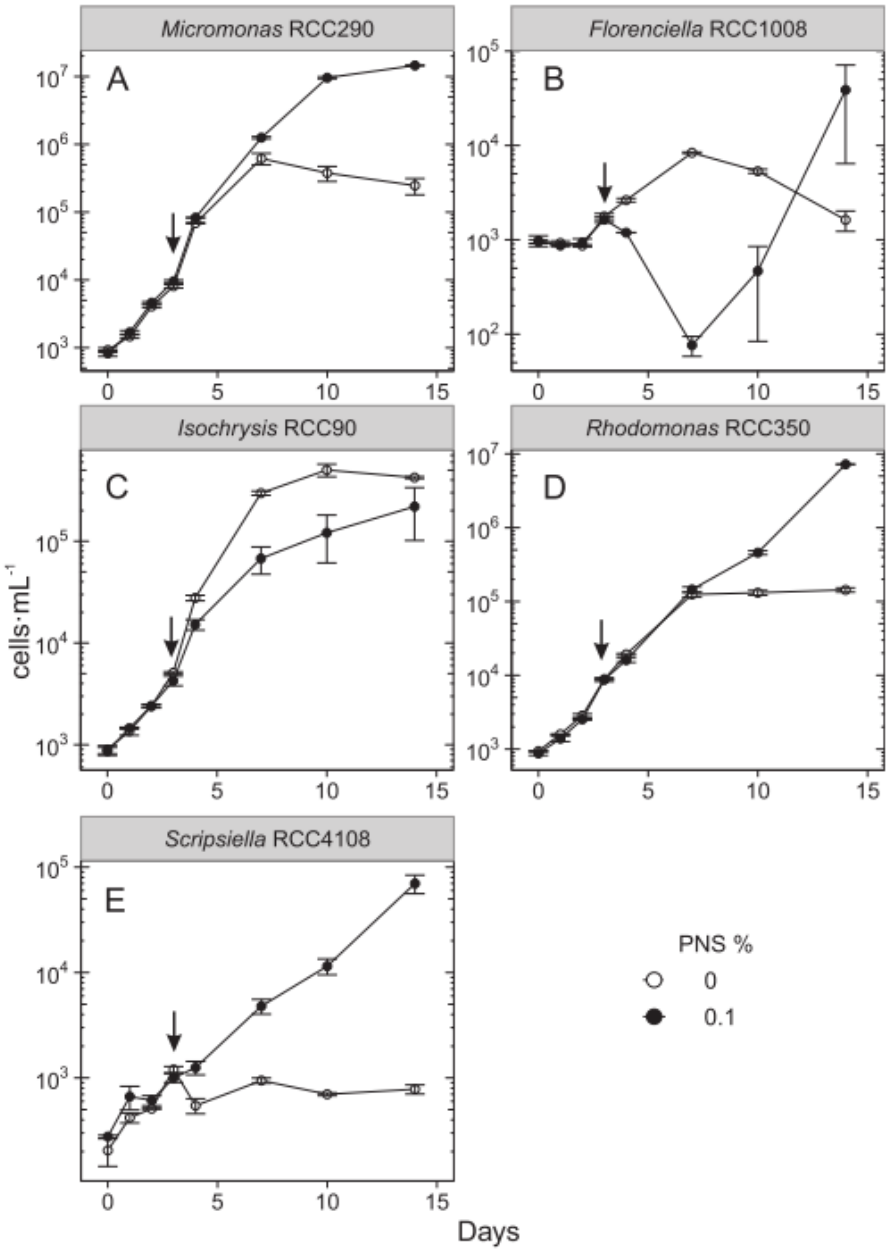

FIG. 3. Evolution of cell concentration for 1,000 cells of *Micromonas pusilla* RCC299 (A), *Isochrysis* sp. RCC90 (B), *Rhodomonas baltica* RCC350 (C), *Florenciella* sp. RCC1008 (D), and 500 cells of *Scrippsiella* sp. RCC4108 (E) sorted into 1 mL K medium containing 0.01% of BSA with and without addition of PNS 3 d after flow cytometric cell sorting (arrow indicates PNS addition). Error bars correspond to the standard error from three replicates.

REAGENTS

PNS - Penicillin, Neomycin, Streptomycin P4083 by Sigma Aldrich

Step 6.

- Continue incubation 5-10 days

Step 7.

- Screen cultures either by microscopy or by flow cytometry to detect presence of cells.
- Transfer positive wells to 50 mL flasks in L1, K or f/2 medium
- Maintain under adequate growth condition.