Establishment of clonal algal cultures by flow cytometry sorting

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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.

LINK: https://www.protocols.io/view/tangential-flow-filtration-tff-concentration-of-ph-gpybvpw

Prepare sorting plates

Step 2.

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 concetrations in Fig. 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

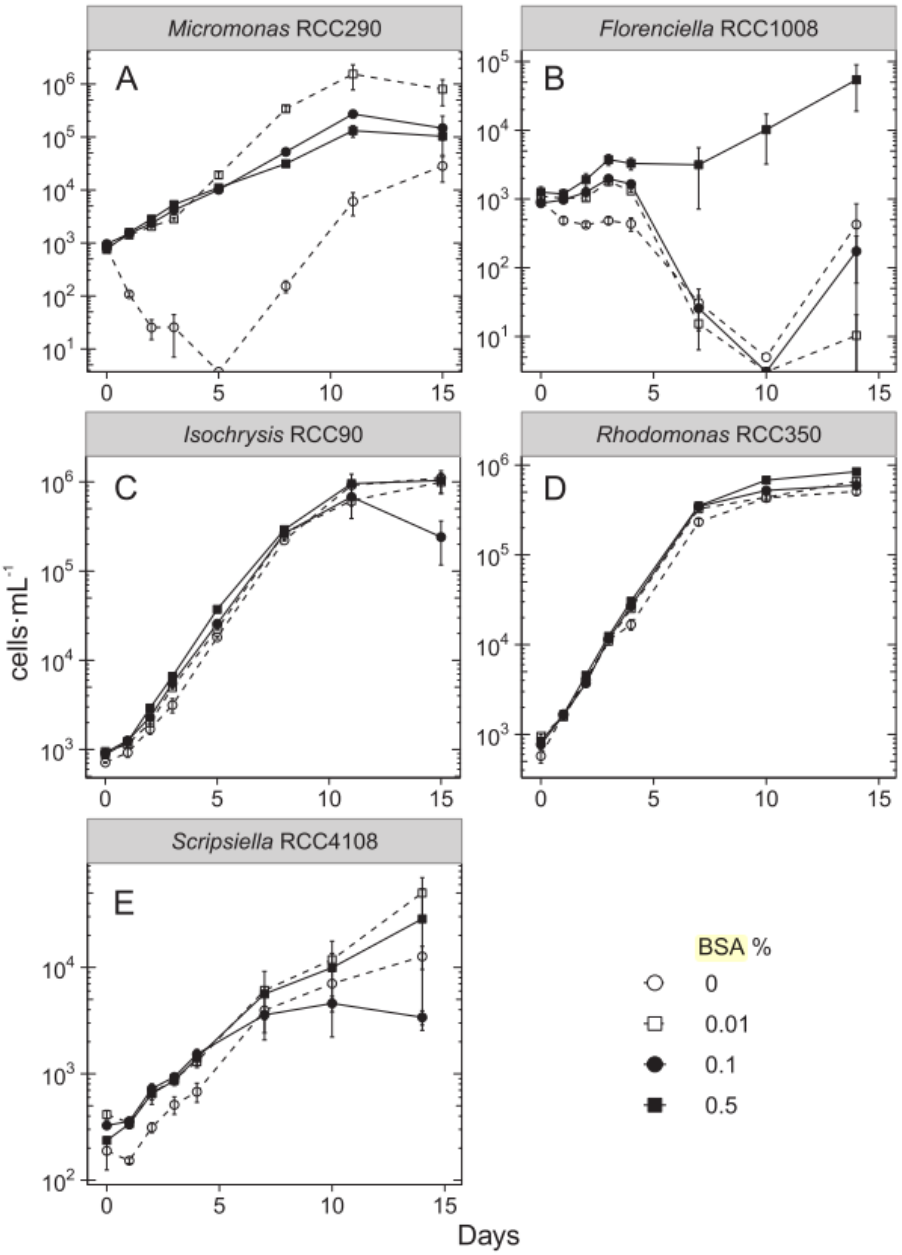

FIG. 2. Effect of the concentration of BSA on the recovery of RCC cultures after sorting of 1,000 cells of *Micromonas pusilla* RCC299 (A), *Isochrysis* sp. RCC90 (B), *Rhodomonas baltica* RCC350 (C), and *Florenciella* sp. RCC1008 (D), and 500 cells of *Scrippsiella* sp. RCC4108 (E) into 1 mL of K medium. 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

ANNOTATIONS

Adriana Lopes dos Santos 19 Feb 2018

BSA stock solution

1% BSA (g/v) in MilliQ water and sterilized by filtration

Sort cells into plates by flow cytometry

Step 3.

Step 4.

ANNOTATIONS

Adriana Lopes dos Santos 16 Jan 2018

It seems that there is a problem with this protocol since I cannot see points 3 and 4.

Step 5.

Add 0.1% of PNS (Penicillin, Neomycin, Streptomycin) to each well (see Figure below reprinted from Marie et al. 2017).

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

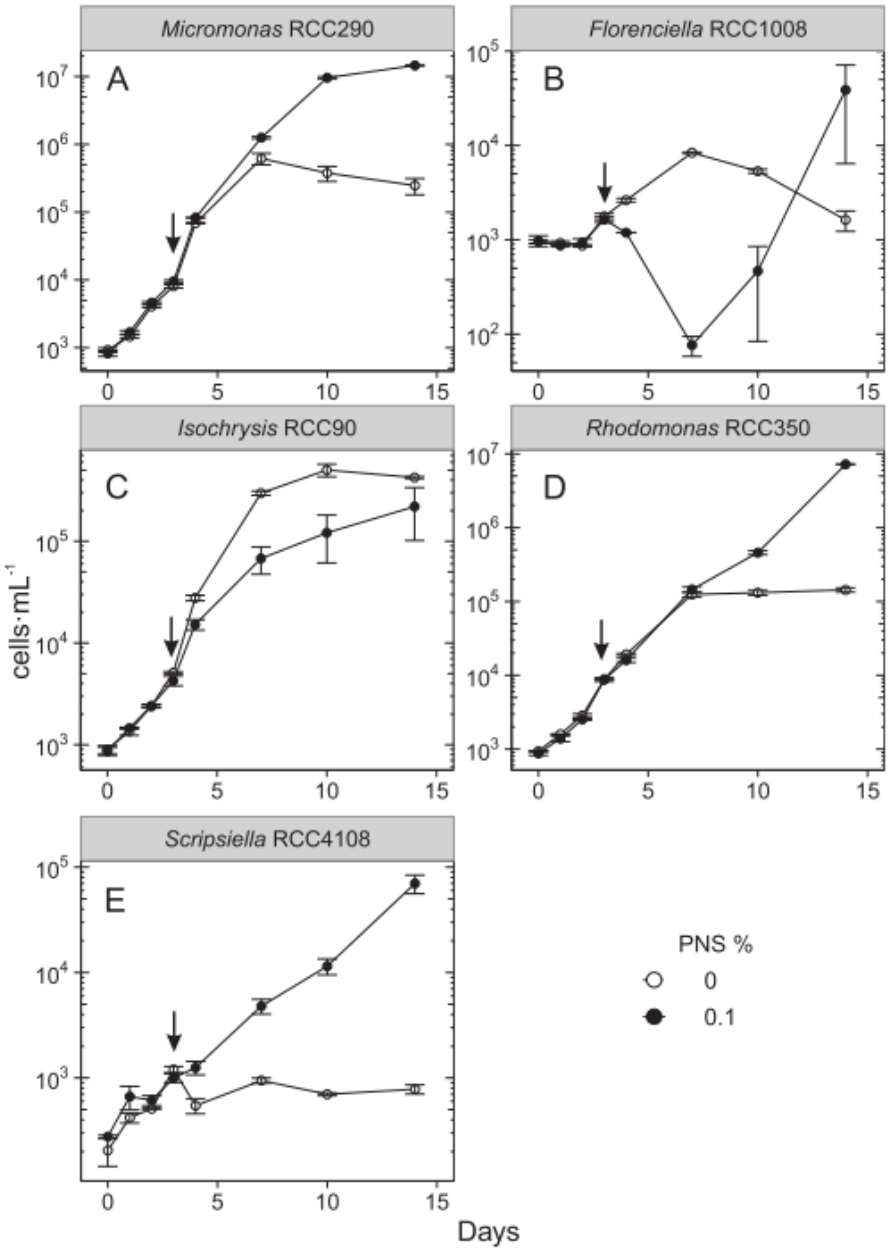

FIG. 3. Evolution of cell concentration for 1,000 cells of *Micromonas pusilla* RCC299 (A), *Isochrysis* sp. RCC90 (B), *Rhodomonas baltica* RCC350 (C), *Florenciella* sp. RCC1008 (D), and 500 cells of *Scrippsiella* sp. RCC4108 (E) sorted into 1 mL K medium containing 0.01% of BSA with and without addition 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

ANNOTATIONS

Adriana Lopes dos Santos 19 Feb 2018

I would change to ...

Add 0.1% of PNS (1000X diluted, example, 0.5µl of stock sigma solution in 500 µL media)

Incubate 5-10 days

Step 6.

Screen cultures and transfer to 50 mL flasks

Step 7.