Transient transformation of Ostreococcus species (OTTH595, RCC809 and RCC802) and Bathycoccus

François-Yves Bouget, Valérie Vergé and Jean-Claude Lozano

Abstract

This protocol describes the preparation of cells and introduction of DNA into the cells by electroporation. For selection of stable transformants or measure of transient gene expression see related protocols.

Citation: François-Yves Bouget, Valérie Vergé and Jean-Claude Lozano Transient transformation of Ostreococcus species (OTTH595, RCC809 and RCC802) and Bathycoccus. **protocols.io**

dx.doi.org/10.17504/protocols.io.g86bzze

Published: 14 Mar 2017

Protocol

Cell preparation

Step 1.

1) Starting from a culture of Ostreococcus tauri, RCC809 or Bathycoccus in stationary phase, innoculate cultures at 1 million cells/ml as determined by flow cytometry (Accuri C6 BD) in 200 ml plastic flasks in Artificial Seawater supplemented with Keller medium supplement (trace metals, vitamins, nitrate and Phosphate as described in Djouani Tahri et al., PloS ONE 2011).For each transformation (including control), you should plan on using 50 ml de culture in exponential phase.

2) Grow cells for 4 to 5 days depending on the light conditions, until they reach densities of 30 to 40.10^6 cells/ml.

3) Count cells by flow cytometry. Check by SyBR Green II straining that bacterial contamination is below 2%.

4) Transfer lcultures to 50 ml Falcon tubes.

5) Centrifuge at 8000g for 10 min at 4°C.

6) Remove the supenatant, resuspend the cell pellet in 1 ml de sorbitol 1M (pH 7.5) in H_2O MQ, at 4°C.

7) Transférer the cell suspension to 1.5 ml eppendorf.

8) Centrifuge at 8000g for 10 min at 4°C.

9) Remove 900 µl of supernatant

10) R**esuspend cells by gently pipeting.**

Electroporation of the transgene

Step 2.

1) Add 5µg of transgene DNA to cell suspension. Keep on ice for 5 minutes. The transgene consist of the high affinity phosphate promoter fused to the firefly luciferase (see Djouani Tahri et al., PloS one 2011).

2) Transfer cells to a 2 mm electroporation cuvette (Biorad).

3) Apply an electric field

For Ostreococcus tauri (OTTH595) : **capacitance:** 25µF, **resistance** 600 Ώ, **voltage** 1.35KV.

For Ostreococcus sp RCC809 : **capacitance:** 25µF, **resistance** 600 Ώ, **voltage** 1.4KV.

For Bathycoccus (RC4222) : **capacitance:** 25µF, **resistance** 600 Ώ, **voltage** 1.5KV.

For Ostreococcus lucimarinus RCC802 : **capacitance:** 25µF, **resistance** 600 Ώ, **voltage** 1.2KV.

4) Add 1ml of fresh culture Medium to resuspend the cells.

5) Add 40 ml of culture medium and transfer to a culture flask.

6) Incubate at 20°C overnight in a light incubator.

At this stage, transient transgene expression can me measured or stable transformants can be selected (see relevant protocols).