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Working

Selection of stable transformants in Ostreococcus tauri, Ostreococcus lucimarinus and Bathycoccus prasinos

Version 3

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

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ABSTRACT

This protocol describes the selection and growth of stable transformants in semi solid agarose medium. Developped initially for Ostreococcus tauri, it also works for Ostreococcus lucimarinus and Bathycoccus.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

Inclusion of cells in semi-solid agarose medium

1 Autoclave a solution of 2.1% low melting agarose in H2O. Keep at 65-90 °C in a water bath.

2 For each transformation prepare 8 Petri dishes (55 mm diameter) and 8 x 15 ml tubes each containing 9 ml of ASW plus the required selection. (G418 at 1 mg/ml).

3 Add 1 ml of LMP agarose to the 9 ml in one of the tubes. Close the tube, and mix gently by inverting.

4 Add 0.5 ml of overnight transformed cells (see protocol on transient transformation), quickly mix and gently pour into the plate. pay attention to avoid bubles . Repeat this process for all tubes.

5 Let the plates dry open in the flowhood for about one hour, so that the agarose solidifies.

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6 Close the plates and transfer them to large square Petri dishes. Add wet paper towel to keep the chamber humid. Seal the square plates with medical tape. Place the square plates in the incubator for about 10 days.

Selection of transformants

7 Transformant colonies should appear after 10 to 21 days. Pick colonies using with cut-off yellow tips. Suck out the green colony. Take care not to include any cells from neighbouring colonies.

8 Transfer the cells to 0,2 ml of ASW medium containing the selection (G418), in 96 wells microplate. Allow cultures to grow for 7 days in the

culture incubator.

9 After one week transfer to 20 ml culture flasks and grow for 7 to 10 days. Stable integration into the genome by random insertion or homologous recombination can be detected using PCR (see Lozano et al, Plant Journal 2014). When the transgene containsa luciferase reporter, a first screening can be performed directly by measuring luminescene in a microplate luminometer.

10