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Selection of stable transformants in *Ostreococcus tauri* and *Bathycoccus prasinos* V.2

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Abstract

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

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 × 15 ml tubes each containing 9 ml of ASW plus the required selection. (CloNat at 2mg/ml or G418 at 1.5 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 bubbles . 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 (CloNat or 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 contains a luciferase reporter, a first screening can be performed directly by measuring luminescence in a microplate luminometer.