Selection of stable transformants in Ostreococcus tauri and Bathycoccus prasinos Version 2

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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 Bathycoccus.

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Protocol

Inclusion of cells in semi-solid agarose medium
Step 1.

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

Inclusion of cells in semi-solid agarose medium
Step 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. (CloNat at 2mg/ml or G418 at 1.5 mg/ml).

Inclusion of cells in semi-solid agarose medium
Step 3.

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

Inclusion of cells in semi-solid agarose medium
Step 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.

Inclusion of cells in semi-solid agarose medium
Step 5.

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

DURATION
01:00:00

Inclusion of cells in semi-solid agarose medium

Step 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

Step 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.

Selection of transformants

Step 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.

Selection of transformants

Step 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.