Transformation of Aureococcus anophagefferens by Electroporation

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Abstract

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Protocol

Concentrating Aureococcus

Step 1.

Filter 100 mL of one week old *Aureococcus* cultures onto a 45-mm diameter, 0.2-µm nominal pore size GTTP membrane filter for each electroporation you are planning to complete.

Concentrating Aureococcus

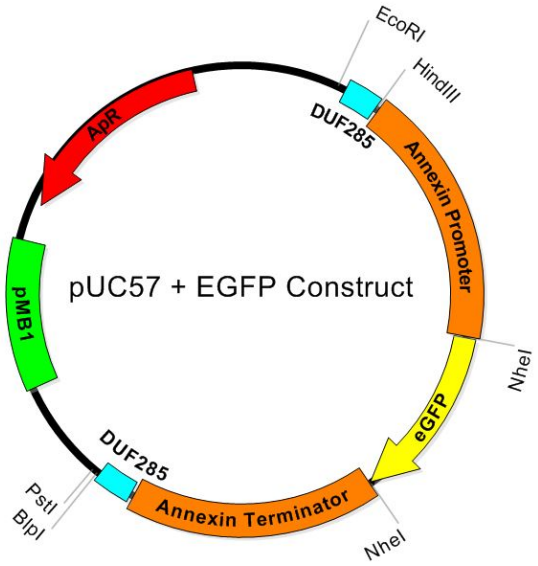
Step 2.

Resuspend filtered *Aureococcus* in 900 µL of 1.0 M sucrose solution by pipetting the sucrose solution against the filter continuously.

Electroporation of Aureococcus

Step 3.

Mix 300 µL of the culture resuspension with 10 µg of pUC57 + EGFP Construct. This plasmid contains EGFP under control of the *Aureococcus* annexin promoter and terminator. Flanking the cassette are DUF285 repeat regions.



Electroporation of Aureococcus

Step 4.

Transfer the *Aureococcus* / DNA mixture to a pre-chilled electroporation cuvette, and electroporate cells. Electroporation conditions are shown below. Place on ice directly after.

Electroporation Conditions					
Voltage	Capacitance	Resistance	Cuvette Size	Field Strength	Transformation Efficiency
400 V	25 µF	200 Ω	0.2 cm	2000 V/cm	$4.3 – 5.3*10^{-8}$

Outgrowth of Aureococcus

Step 5.

Transfer *Aureococcus* into 5 mL of fresh $ASP_{12}A$.

PROTOCOL

. ASP12A Recipe for culturing Aureococcus anophagefferens

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Step 5.1.

Prepare Trace Metal Solutions I - III, Fe-EDTA Sodium Salt Solution, and Vitamin Solution. Add constituents to 750 mL MilliQ water, shake to dissolve. Add MilliQ water to 1.0 L. Filter sterilize. Trace Metal Solutions and Fe-EDTA Sodium Salt Solution are stored at 4ºC in 15 - 50 mL aliquots, while the Vitamin solution is stored at -20ºC in 5 - 10 mL aliquots.

Step 5.2.

Add anhydrous salts, hydrous salts, macronutrients and Tris Base to 75% desired MilliQ water while

stirring to dissolve. Add MilliQ water to final volume, autoclave.

Step 5.3.

Once cooled, add in 1mL of the Trace Metal Solutions, Fe-EDTA Sodium Salt Solution, and Vitamin Solution per 1L of media made.

Transformation of Aureococcus

Step 6.

Concentrate 5 mL of the electroporated *Aureococcus* culture onto a 25-mm diameter, 0.2-µm nominal pore-size GTTP membrane filter disk.

Transformationof Aureococcus

Step 7.

The filter is then placed onto an $ASP_{12}A$ + 0.3% Nobel Agar plate. Incubate at 19ºC with a 14:10 (100 $\mu E/m^2s^2$) light dark cycle.

NOTES

Ashley Humphrey 26 Jan 2017
Growth can be seen after 1 - 2 weeks of growth in the incubator.