Transformation of Synechoccus sp PCC7002

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Abstract

This protocol describes the transformation of Synechococcus sp. PCC7002 with linear DNA fragments with homologous regions.

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Guidelines

Work as sterile as possible.

Protocol

Culture Preparation

Step 1.

- Set up a starter culture of Synechococcus (e.g. from a -80°C stock or from a plate or a liquid culture) in AA+ medium (33°C incubation with bubbling, ca. 150uE)
- Incubate until OD_{730} is above 1

Step 2.

- Use the starter culture to set up fresh culture in the bubbling flasks (cleaned and sterile) with an OD_{730} of 0.07 in 150mL AA+ medium
- Incubate at 33°C with bubbling until OD_{730} of 0.4 (ca. 18 hours in our lab)

Step 3.

- Transfer cells from the bubbling flasks into two sterile 50mL tubes
- Spin down the cells at 2500g (4700rpm) for 8 minutes at room temperature (22°C)
- Carefully discard the AA+ medium

Transformation

Step 4.

- Resuspend cells in AA+ medium to a final OD_{730} of 8
- Place 100 of the resuspended cells in sterile test tubes (15mL volume)
- Add 1µg of linear DNA with 50-500nt homologous regions (e.g. your purified PCR product)

Step 5.

- Place tubes in a rack in a 30°C cabinet with low light (ca. 30uE) for six hours
- In the meantime, place sterile filters (Nucleopore SN 145318) on **non-selective** AA+ plates with sterile tweezers.

Step 6.

- Plate the whole volume of cells onto the sterile filters on the AA+ plates
- Spread the cells carefully
- Leave the plates to dry in the flow cabinet (30 minutes)

Step 7.

- Grow the cells overnight in the 30°C cabinet with low light (ca. 30 uE)

Step 8.

- Transfer the filters with the cells onto AA+ plates with the appropriate antibiotics
- Grow cells in the 30°C cabinet with low light (ca. 30uE) for 2 days.

Step 9.

- Transfer cells to 33°C incubator with higher light (ca. 150uE)
- Place the plates near the door so that they do not get too much light.
- Be careful to seal the plates well with parafilm so that they do not dry out.

Step 10.

- Wait until colonies appear on the filter. A lot of cells will die, so there is most likely a yellowish background on which the green colonies will appear.

Segregation

Step 11.

- Pick single colonies with a sterile pipettip and streak them onto selective AA+ plates.
- Incubate plates in the 33C cabinet the same way as done before with the selective plates.

Step 12.

- Wait until colonies appear and repeat the procedure
- To be sure that complete segregation occurred, perform colony PCR on your colonies.