Oct 20, 2019

Transformation of Synechocystis sp. PCC 6803

Anika Wiegard[1], Ilka Maria Axmann[2]

[1]Karolinska Institutet, Department of Cell and Molecular Biology, Stockholm, Sweden, [2]Synthetic Microbiology, CEPLAS, Heinrich Heine University Duesseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.mdrc256

Axmann Lab CyanoWorld view 1 more group

Anika Wiegard
Karolinska Institutet, Department of Cell and Molecular Biol...

ABSTRACT

This protocol describes how to transform naturally competent *Synechocystis* sp. PCC 6803.
Use this protocol, if you aim at integrating a nucleotide sequence in the *Synechocystis* chromosome via homologous recombination. Be aware that *Synechocystis* PCC 6803 strains from different labs vary in their transformation efficiency.

The protocol was modified from
Grigorieva, G. & Shestakov, S. (1982). Transformation in the cyanobacterium *Synechocystis* sp. 6803. FEMS Microbiol Lett 13, 367-370.

GUIDELINES

Work under sterile conditions using a laminar flow hood

SAFETY WARNINGS

Make sure to perform and document your work according to the regulations for GMOs.

1 Grow *Synechocystis* sp. PCC 6803 cells in BG-11 medium (see e.g. Köbler and Wilde https://dx.doi.org/10.17504/protocols.io.wj5fcq6 for recipe) until $OD_{750\ nm}$ =~0.5 -1

should take about 4 days after inoculation

2 Transfer 10 ml of the cell suspension to a sterile 15 ml falcon tube and spin down at ~ 2 200 rcf at room temperature.

3 Discard supernatant, resuspend the pellet in a small volume of residual supernatant (~200-500 µl) and transfer the cells to a sterile 1.5 ml tube (handle cells carefully, don't pipet up and down too often, use large pipet tips to minimize shear forces).

handle cells carefully, don't pipet up and down too often, use large pipet tips to minimize shear forces

4 Add 5 µl plasmid DNA to the cells

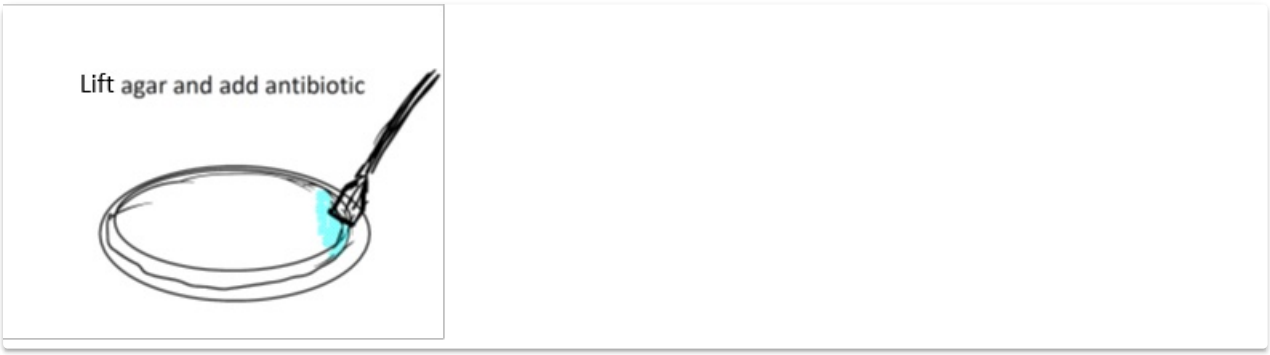
for 'easy' transformations DNA amount seems not to be critical, but 2 µg should be fine

5 Incubate for 30 min at room temperature (in the tube) without shaking.

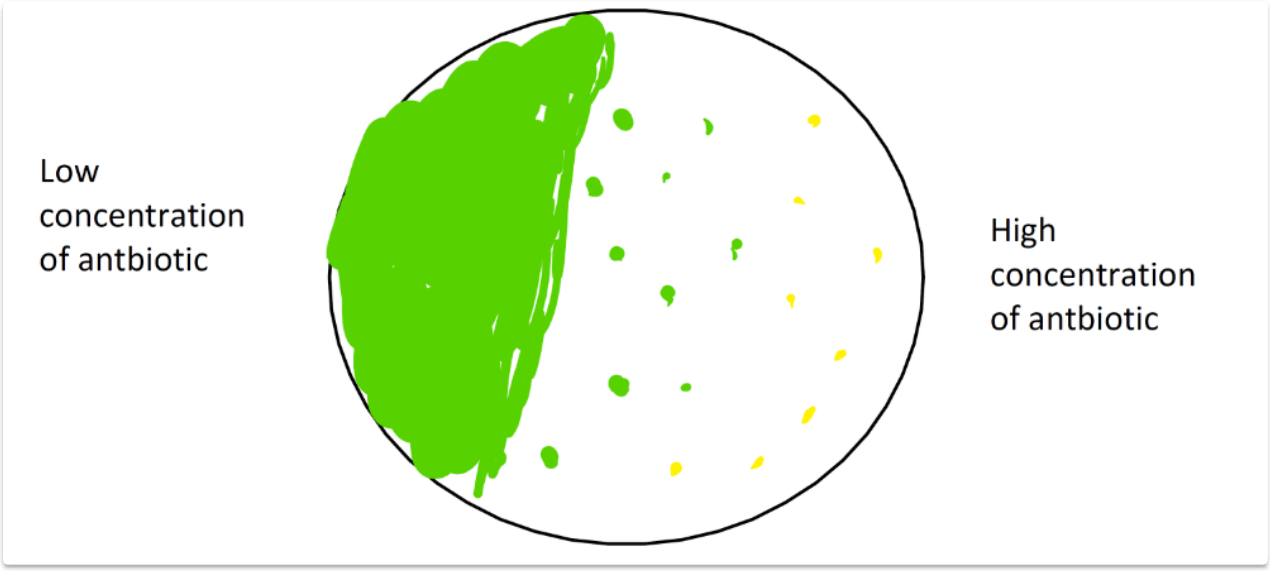
6 Plate on a very thick BG-11 plate (1 % Bacto agar, in 1x BG-11) without antibiotics (~ 50 ml BG-11 agar per plate).

7 Wait for a few hours until residual liquids are absorbed by the agar (in laminar flow hood).

8 Underlay with antibiotics to create a gradient in the plate:
- using a sterile spatula lift the agar at one end of the plate
- Kan: add 300-400 µl of 1mg/ml kanamycin stock solution under the agar at the end of the plate
- Cm: incubate the plate for 2-3 days without antibiotics, then add 400 µl of 1.4 mg/ml chloramphenicol stock solution under the agar at one end of the plate
- place agar back in place
- antibiotic will diffuse trough the plate forming a gradient



9 Incubate at low light intensities (~40 µmol Photonen m^{-2} s^{-1}, optional: cover the plate with a thin sheet of white paper) and 30 °C until first (isolated) green colonies appear (lid facing upwards!!!)



10 When first colonies appear, pick them (only green ones) and streak on a plate with slightly higher antibiotic concentration (for km: e. g. 2 or 4 µg/ml, for Cm: e.g. 1.5 or 2.5 µg/ml). In the beginning, don't streak them over the whole plate, but streak small spots. Incubate at ~ 60 µmol photons m^{-2} s^{-1}, facing the lid down.

11 Increase concentration of antibiotic stepwise (e.g. Kan: 4–8-12-20-40 or more µg/ml, Cm: 2.5-5-7-14 µg/ml etc) until full segregation is achieved (check by colony PCR and/or southern blot).