Triparental mating of Synechocystis

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Abstract

Protocol for genetic manipulation of *Synechocystis* sp. PCC 6803 using the triparental mating method described in Thiel *et al*., 1988.

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Guidelines

Thiel T, Wolk CP: **Conjugal Transfer of Plasmids to Cyanobacteria**. *Methods in Enzymology* 1988, **153**:232–243.

Before start

Pour BG11 agar plates with 5% LB.

Protocol

Materials required:

Step 1.

- HATF filters (Millipore), ∅ = 5 cm
- LB media
- 2xBG11
- Bacto Agar, 1.5 %

Step 2.

Always work under sterile conditions, even when working with *E. coli*.

Inoculate **50-100 mL** BG11 with *Synechocystis* strain of choice. Grow for **7 days** - culture should be

in **exponential** phase.

Inoculation

Step 3.

The day before performing mating reaction, inoculate one over night culture **each** for strains carrying moblilizable plasmid, as well as the J53 strain carrying RP4.

Prepare BG11 agar plates containing **5 % LB (no antibiotics)**. Place sterile HATF filter on top of agar.

Growth

Step 4.

In the morning, fresh cultures are inoculated by dilution. **4.875 mL of LB (no antibiotics)** are inoculated with **125 µL** of each over night culture and incubated for two and a half hours at **37˚C**. For RP4, **5 mL** fresh culture is required for each mating step.

DURATION
02:30:00

Step 5.

The cultures are centrifuged at **RT** and **2500 rpm for 8 min** and each pellet is resuspended in **1 ml LB.**

DURATION
00:08:00

Mating step 1

Step 6.

For each mating reaction, **1 mL** RP4 is gently mixed with **1 mL** of conjugative plasmid strain.

Step 7.

The mixtures are centrifuged at **RT**, **2500 rpm, for 5 minutes**, resuspended in **100 µL LB** and incubated at **30˚C for 1 hour** (no shaking).

DURATION
01:00:00

Mating step 2

Step 8.

Depending on the cell density, add **0.9 or 1.8 mL** of *Synechocystis* culture to each mating reaction, mix gently, centrifuge at **RT** and **2500** rpm for **5 min**, resuspend in **30 µL BG11.**

DURATION
00:05:00

Step 9.

Spot **30 µL** of mating mixture on top of HATF filter. Cover plates and incubate in light chamber for **24-48 hours.**

Step 10.

Carefully resuspend dried cyanobacteria from HATF filter with **100 µL BG11.** Plate mixture on BG 11 containing appropriate antibiotics. Note: Do not plate excessive amounts of cell material, as mobilization is very efficient and colonies should be sufficiently separated on the plate.. After 10-14 days, should colonies become visible.

Step 11.

Transfer colonies on agar plates containing twice the amount of antibiotics.

Verify correct clones by colony PCR with universal BioBrick or NeoBrick prime

Warnings

Always work under sterile conditions, even when working with E. coli.

Plasmids are mobilized with very high efficiency; make sure not to plate too much cell material to ensure proper spacing of colonies.