Triparental Mating – Conjugation

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Protocol used for 6803, currently on trial for 7120 and 29133.

Materials

- 50 ml thick Cyanobacterial culture
- 10 ml *E. coli* culture with cargo plasmid (eg. pSBXAK1)
- 10 ml *E. coli* culture with conjugal plasmid (pRL443, host strain HB101, Amp^R) for each cargo construct
- Bg11 plates and liquid medium w/o and w/ antibiotics

Anabaena/Nostoc 7120 contains AvaI-III REs, for protection against these, helper plasmid with methylases should be used (like pRL528? Like pRL623!).

For 7120 and 29133: Sonicative filament disruption

To avoid half-conjugated filaments where only a few cells contain the cargo plasmid filamentous strains like 7120 and 29133 should be broken up in very short or individual cells before conjugation. This method has been used successfully by Thorsten Heidorn:

- Pellet 50 ml cells at 3000 rpm for 10 mins at 4 C and remove as much medium as possible. Resuspend in 2 ml.
- Transfer culture to sterile glass tube, place in glass bucket full of ice water.
- Use Amplitude 45 (around 7 W on display), Timer at 0 and Pulse at 1 s. Sonicate for 15 s, let rest for 15 s and repeat for 4 times.
- Check filament integrity by microscopy. If necessary, repeat sonication step.
- Inoculate cells in 50 ml Bg11, protect from light using paper and incubate for at least 4 h at 25 C with light.

Mating protocol

E. coli

- Pellet cultures (10 ml cargo and 10 ml helper) at 3000 rpm for 10 mins.
- Resuspend each in 10 ml LB w/o antibiotics. Combine cargo and helper cultures.
- Pellet mixed cultures (20 ml cargo + helper) at 3000 rpm for 10 mins.
- Resuspend in 200 ul LB for each dilution of Cyanobacteria to be plated (usually 2-3 = 400-600 ul)

Cyanobacteria

Optional: Measure OD750 (one meas. = 2.85) and chlorophyll A (one meas. = 13 ug/ml). This is probably not important as long as the culture is quite dense. Age is not very important either.

- Pellet 50 ml cells at 3000 rpm for 10 mins at RT. Resuspend in 5 ml Bg11.

Optional: Measure OD750 and chlorophyll A.

- Depending on expected numbers of colonies at later stage, perform culture dilutions in Bg11 to a final volume of 1 ml. For a new plasmid, dilutions 1:1, 1:100 and 1:10000 may be suitable. For pSBXAK1 in 6803 and 7120 it seems 1:1 and 1:100 are sufficient, for 29133 1:10 is OK. Or 1:100 and 1:10000 if many colonies.

Mating

- Mix 200 ul *E. coli* cargo/helper mix culture with 100 ul of Cyanobacterial culture in Eppendorf tube. Incubate for 30 min - 1.5 h at 30 C before plating.
- Pour the 300 ul mixture at non-antibiotic Bg11 plate and incubate for about 1 - 3 days at 30 C with light.

Selection of conjugants

- Wash conjugation plates with Bg11 to remove cells. Use enough volume to ensure complete resuspension of cells.
- Concentrate cells down to eg. 200 ul by centrifugation and plate at Bg11 plates with selective antibiotic. For pSBXAK1 50 Km works well.
- Incubate at 30 C with light.

Colonies should appear within 10 days depending on Cyanobacterium and circumstances.