MYXOCOCCUS **ELECTROPORATION PROTOCOL**

Adapted from Youderian and Hartzell Protocol (1992) by Vinh Pham.
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MATERIALS:

CTTYE Liquid Medium (CTT is ok)
CTTYE + Antibiotic plates
CTTYE Soft Agar
Invitrogen #65-0030 0.1cm cuvettes (one per electroporation)
Erlenmeyer flasks (50ml or larger)

PROCEDURE:

1. Grow cells in 25ml CTTYE to about 80-120 Klett units, at 33ºC and 300rpm.

2. Pellet at 8Krpm for 8' in SS34 or SA600 rotor at 12-18ºC.

3. Resuspend pellet in 1.5ml distilled water (dw) and transfer to 2ml _fuge tube.

4. Pellet at 8Krpm for 2-3'.

5. Repeat wash 4 times, then resuspend in 1.5ml dw.

6. Mix 200_l of cells with 3-20 □l of plasmid DNA (resusp. in dw) in a _fuge tube, then transfer to a 0.1cm cuvette.

Note: Optimal transformation efficiencies can be obtained with more plasmid DNA, cleaner plasmid DNA (dialysis rids plasmid preps of salts that can lower time constant values), quicker and cleaner washes of cells, and the use of Invitrogen cuvettes.

7. Electroporate at 400□, 25□FD, 0.65kV at room temperature. Ideal time constant values are 9.0 or higher.

8. Immediately add 1ml of CTTYE to cuvette, and transfer to a 50ml Erlenmeyer flask containing 1.5ml of CTTYE.

9. Grow cells 4-8 hours at 33ºC to allow for phenotypic lag.

Note: Cells can also be incubated overnight if the arising of sibling colonies is not a concern.

10. Add desired volume of cells to CTTYE soft agar + selective antibiotic, mix, and plate onto CTTYE (hard) agar + selective antibiotic.

11. Allow transformants to grow through the soft agar for 4-7 days at 33ºC.