Electroporation Protocol

1. Inocualte 2-5 days prior for a disperse exponential AA/4 ammonia grown culture. Grow in in a shaking incubator (26°C, 250RPM, 500ml culture in 1 l flask).

2. Prepare *Nostoc*.

a. Morning of EP, take a Chla reading of culture and concentrate. Concentrate a volume of culture enough for a total Chla of [# of EP's X volume of EP (usually 0.4ml) X 100 μg Chla/ml] down to a volume of 3-5 ml in growth medium. Sonicate*Nostoc* culture gently (10 bursts, 50% duty at 1.5 in 3-5ml). Place into 50 ml AA/4-ammonia mops for >4 hours recovery in New Brunswick or under bench.

b. Just prior to EP, sterily wash cells 3X with 20 ml room temperature ddwater, vortexing between washes. After third wash, resuspend to a volume that gives a concentration of 50-100 μg/ml Chla. Cool on ice immediately before use.

3. Prepare DNA. Run minipreped DNA through a mini column prep protocol (Quiagen) omitting the incubation times. Elute with water. Use 4-20μl/EP.

4. Electroporation. Aliquot DNA to sterile microfuge tube and set on ice. Chill EP cuvettes on ice. Add 400μl concentrated, washed cells to microfuge tube, mix by pipetting up and down, and wait 30-60 seconds to allow DNA adsorption to cells. Transfer to cold 0.2cm cuvette, cover and electroporate.

EP parameters which work: 600 Ω
1.6 kEV
25μF

Expected time constants (MSec):

Volumes which work: 400μl with 20μl DNA ~12.5-13.2
120μl with 4μl DNA ~11.5-12.5

5. Electroporation recovery. IMMEDIATELY following electroporation dilute cells in cuvette with AA/4-ammonia mops containing 20mM $MgCl_2$ and remove to a 50 ml flask of the same media. Incubate overnignt in dim light with gentle shaking.

6. Selection. Concentrate overnight recovered culture and resuspend in ~1ml Vortex vigorously to break up clumps. Plate 100μl of this undiluted culture, as well as 1:5, and 1:10 dilutions onto selective media. Place remaining cells under liquid selection for backup. Incubate under low light until colonies are visible at ~11 days.

Selection which work for *Nostoc punctiforme*:

PSCR119/pSUN119 based plasmids 10 μg/ml Nm
PSCR202/pSUN202 based plasmids 5 μg/ml Ap