Electroporation Protocol for *Pseudomonas chlororaphis*

Pseudomonas chlororaphis takes up plasmids not through a heatshock transformation like E.coli (DH5alpha), but through electroporation. This transformation is generally less efficient.

1. Set up an ON culture of P.chlororaphis (scratch glycerol stock (-80°C), eject pipette tip into LB or SOC, incubate ON 28°C, shaking at 180rpm (slower than E.coli!))
2. Measure OD in morning, dilute to OD600 of 0.2. Per electroporation you need 1.4mL of culture, plus 2-3mL for future OD measurements.
3. Grow the culture to an OD600 of 0.4-0.6. This will take about 2-4 hours (180rpm, 28°C)
4. From here on, work in 4°C room or on ice and in cooled centrifuge (this is very important to keep the bacteria happy)
5. Distribute 1.4 ml culture in **cold** 1.5 ml Eppendorf tubes
6. Spin down the cells at 4°C for 1 minute at 7600 rpm and discard supernatant
7. Dissolve pellet in 1 ml **ice cold** ddH2O
8. Spin down the cells at 4°C for 1 minute at 7600 rpm and discard supernatant
9. Dissolve pellet in 1 ml **ice cold** ddH2O
10. Spin down the cells and discard supernatant
11. Dissolve the cells in whatever volume of ddH2O is left in the tube (should be approximately 40µl for later on...)
12. Store on ice and electroporate immediately. What to take with you to the electroporation machine: ice, cell suspension, LB or SOC, DNA/plasmids, electroporation cuvette, pipettes (P2 for DNA, P20 for suspension, P1000 for LB or SOC) and respective tips.
    Add DNA (~100ng) to the cell suspension, dissolved in water or low ionic-strength buffer. Mix well and incubate on ice for 1-5mins
13. Transfer the mixture to a cold electroporation cuvette and tap on table until the suspension is at the bottom (no air bubbles). Place the cuvette in the ShockPod with electrodes to the sides and close the lid. (amount of cell suspension depends on the cuvette: for a 2mm gap you need 40µl of suspension)
14. Pulse once (Control settings with notes below) If there is a flash, something didn't go well (salt or air bubbles) and your cells have probably died :(
    Check and record the pulse parameters. The time constant should be close to 5 milliseconds, The field strength can be calculated as actual volts (kV)/cuvette gap (cm)
15. Remove cuvette and **immediately** add 1ml of LB or SOC (RT) to cuvette. Quickly but gently resuspend the cells
16. Transfer the cell suspension to a glass tube or a 1.5mL Eppendorf tube and incubate at 28°C for 3 hours shaking at 180rpm.
17. Plate on LB or SOB plates with antibiotics (depending on plasmid resistance). Incubate at RT for 2 days, afterwards store at 4°C. Note that P.chlororaphis keeps growing at 4°C although slower, if you need your transformed bacteria for longer than 4 weeks consider making a glycerol stock (1:1 ratio of 100% glycerol and bacterial culture (OD600 0.6) to be stored at -80°C)

Gene Pulser Xcell conditions: Check these in machine

Cuvette gap	0.1cm	0.2cm
Voltage	1.8kV	2.5kV
Field strength	12.5kV/cm	12.5kV/cm
Capacitor	25µF	25µF
Resistor	200Ω	200Ω
Time constant	4.5ms	4.5ms