Protocol Name: *Pseudomonas* sp. DSM 25356, Electroporation
Category: Endophytic Chassis Development
Date: 12/10/18
Author: Frank Eardley
Source(s): Adapted Martinez-Garcia E, Aparicio T, de Lorenzo V, & Nikel P (2017) Engineering Gram-Negative Microbial Cell Factories Using Transposon Vectors. *Methods in Molecular Biology* 1498:273-293.

Time Required: 4 hours

Materials:

- Overnight culture of *Pseudomonas* sp.
- 1.5 ml Eppendorf tubes.
- 2 ml Eppendorf tubes
- 50ml Falcon Tube
- Electroporation Cuvettes
- Tryptone soy broth
- Tryptone soy agar containing appropriate antibiotic
- 300 mM sucrose solution
- Plasmid DNA solution (50 ng/μl)

Procedure:

1. Inoculate 20ml of sterile tryptone soy broth (TSA) in a 50 ml falcon tube with 200 μl of *Pseudomonas* sp. culture. Incubate overnight at 28 °C with a 220-rpm shake.
2. Centrifuge at 3220 x *g* for 10 minutes at room temperature
3. Discard the supernatant, add 10 ml of filter sterilised 300 mM sucrose and softly resuspended the cell pellet. Then, centrifuge at 3220 x *g* for 10 minutes at room temperature
4. Remove the supernatant and add 1 ml of 300 mM sucrose, resuspended the cells, and transfer suspension to a 2-ml sterile Eppendorf tube. Centrifuge at 7200 x *g* for 3 minutes at toom temperature.
5. Remove the supernatant, add 800 μl of 300 mM sucrose, resuspend the cells, and centrifuge the suspension at 7200 x *g* for 3 minutes at room temperature. Repeat this washing step once more.
6. Remove the supernatant and add 500 μl of 300 mM sucrose to resuspend the pellet.
7. Transfer 100 μl of the electrocompetent cell suspension to a sterile 1.5-ml Eppendorf tube. Add 100ng of plasmid in 2 μl total volume.
8. Pipet the plasmid DNA-cell suspension mix into a 2-mm gap width electroporation cuvette. Avoid forming bubbles which reduce the overall efficiency of the electroporation process.
9. Place the cuvette in the electroporation apparatus, set the electroporation programme to EC2, and proceed to electroporate the cells.
10. Immediately after the electric shock, add 900 μl of TSB to the cuvette and transfer cells to a sterile 1.5-ml Eppendorf tube. Incubate the cells for 3 hours at 28 °C with a gentle shake.
11. Spread cell suspensions onto tryptone soy agar containing the appropriate antibiotic.
12. Incubate at 28 °C overnight.