WCSB, Wageningen University	IBISBA-SOP-WU14
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: *Preparation of P. putida electrocompetent cells and transformation*

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Instruction

Preparation of P. putida electrocompetent cells and transformation of them

1. Introduction / Purpose

This protocol describes how to make electrocompetent *P. putida* cells. The electrocompetent cells can't be stored for long. Transformation of the cells is described as well

Keywords: *P. putida – electrocompetent - transformation*

2. Equipment and chemicals

2.1. Equipment

- Centrifuge
- Microcentrifuge
- Electroporation machine
- Thermo block (heating block)

2.2. Chemicals

2.3 Other materials

Special consumables

- Electro cuvettes with 2 mm gap
- 1 ml syringe with large-gauge needle

3. Media and Buffers

- Sucrose 300 mM (1 L): 102.7 g Sucrose (Sterilization by autoclaving)
- Growth medium of choice
- SOC medium (0.25 L): 7.75 g SOC powder (MPbio) (Sterilization by autoclaving)

4. Procedures

Preparation of electrocompetent cells

- Prepare a 20 ml overnight liquid culture of the strain of *Pseudomonas putida* that we want to make electrocompetent.

- The next day, when the cells have reached the stationary phase, centrifuge the culture for 10 minutes at 4700 rpm and room temperature.

- Discard the supernatant completely (first by pouring the supernatant in a waste container and later by collecting the remaining liquid with the pipette) and resuspend the pellet gently in 10 ml of Sucrose 300 mM.

- Centrifuge the culture for 10 minutes at 4700 rpm and room temperature.

- Discard the supernatant completely and resuspend the pellet gently in 1 ml of Sucrose 300 mM.
- Transfer the volume to a 1.5 ml Eppendorf tube.
- Centrifuge the culture for 2 minutes at >12000 rpm and room temperature.
- Discard supernatant completely and resuspend the pellet gently in 500 µl of Sucrose 300 mM.
- Distribute in 100 µl aliquots.

Transformation of electrocompetent cells

- Mix one aliquot of *P. putida* electrocompetent cells with 1 µl of plasmid (approx. 100 ng/µl).
- Transfer the mix to a pre-chilled electro-cuvette. Make sure that the mix is on the bottom of the electro-cuvette.
- Dry the electro-cuvette before placing it in the Electroporation machine.
- Set up the conditions for the electroporation (2.5 kV, 200 ohm, 25 µF).
- Push the button for Pulse.
- Once the pulse has finished, immediately, add 0.9 ml of SOC into the electro-cuvette.
- Collect the volume with the needle and the syringe and transfer it into a 1.5 mL Eppendorf tube.
- Incubate for 2 hours in the thermo-block at 30°C and 650 rpm.
- Centrifuge the culture for 2 minutes at >12000 rpm and room temperature.
- Discard the supernatant and resuspend the pellet gently in 100 µl of LB or SOC.
- Collect the 100 µl of culture and spread them on a selective plate (LB-agar, MM-agar, etc. with antibiotic) by using an inoculation hook/L-spreader.
- Place the plate upside down in the 30°C incubator and incubate overnight until colonies appear.

5. Remarks / troubleshooting

- Work under sterile conditions all the time.
- The electrocompetent cells are prepared directly before use since they should not be stored for long.
- Cold conditions are not required for this protocol.

6. Biosafety

No biosafety issues are associated with this protocol.

7. Acknowledgements

IBISBA (grant agreement No 730976) and EmPowerPutida (grant agreement No 635536) projects have received funding from the European Union's Horizon 2020 research and innovation programme.

