ETH Zurich	EPP-SOP-ETH02
	Version 1.1

EPP - Standard Operating Procedure

Title: Preparation and transformation of electrocompetent bacterial cells

Describes the cultivation-, washing- and electroporation-steps required in order to transform bacterial cells with DNA.

distribution list

EPP consortium

changes to prior version:

Section ‘6. Biosafety’ added.

	name	signature	date
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Instruction

Preparation and transformation of electrocompetent bacterial cells.

1 Introduction/Purpose

Preparation of microbial cells for the introduction of pieces of purified DNA (plasmids, PCR products and the like) by electroporation followed by selection of transformed cells.

2 Equipment and chemicals

2.1 Equipment

Plastic tubes, 15 mL (Greiner Bio-One)
Erlenmeyer flask, wide neck, e.g. 1 L (Schott)
Incubation shaker (Infors HT Multitron Pro)
Bench-top centrifuge, refrigerated (Eppendorf 5920R and 5424R)
Electroporator (BioRad Micropulser)
Paper towels
Electroporation cuvettes, 1 mm or 2 mm gap (Cell projects)
Plastic tubes, 1.5 mL (Greiner Bio-One)
Petri dishes, (Greiner Bio-One)
Incubator (Binder)

2.2 Chemicals

Pure water (autoclaved)
1000x streptomycin stock (50 mg mL^{-1}; AppliChem)
Antibiotics as required for selection

2.3 Bacterial strains

Escherichia coli strain 10β (NEB, $Strep^R$) or
Pseudomonas putida KT2440

3 Media and buffers

3.1 LB

BD Difco LB broth, Miller (Luria-Bertani)
for LB agar, add 15 g L^{-1} BD Bacto agar

4 Procedures

4.1 Preparation of electrocompetent cells

Inoculate 5 mL of LB medium in a 15 mL tube with a single colony from a LB agar plate or from a monoclonal cryostock. In case of *E. coli* 10β, add 5 µL of streptomycin

stock solution to the culture. Grow the culture over-night (o/n) shaking at 200 rpm at 37°C or 30°C for *E. coli* or *P. putida* KT2440, respectively. Next, inoculate 100 mL LB with 5 mL of the o/n culture and grow to an OD_{600} of 0.6 in a 1L Erlenmeyer flask. Meanwhile, pre-cool the centrifuges to 4°C and chill sterile water close the freezing point, then keep on ice. Split the culture into 10 mL aliquots in tubes and keep on ice for 10 min. Next, centrifuge for 10 minutes at full speed and 4°C. Discard the supernatant and resuspend each cell pellet in 1.4 mL of ice-cold water, transfer into 1.5 mL tubes. Spin down the cells for 30 sec at 10'000 rcf and 4°C. Repeat the washing step three more times and finally resuspend the pellet in 50 µL of ice-cold water. If the cells should be stored at -80°C, resuspend in ice-cold 10% (v/v) glycerol and snap-freeze on dry ice or in liquid nitrogen.

4.2 Transformation of electrocompetent cells

Thaw an aliquot of cells on ice, add purified DNA (few ng of plasmid or assembly/ligation mix in less than 5 µL volume). Wait about 30 sec, then add the mixture to a pre-chilled electroporation cuvette. Tap the cuvette on a hard surface in order to make sure the cells are placed between the electrodes, then blot dry with a paper towel and electroporate. Immediately add 1 mL of LB medium to the cells, then transfer cells into a 1.5 mL tube and recover for 1 h at 30°C or 37°C (*P. putida* or *E. coli*, respectively), shaking at 200 rpm. Plate 50 µL of the cell suspension on LB agar plates containing the corresponding antibiotics and grow o/n at the appropriate temperature. For *E. coli*, dilute about 10^4-fold before plating in order to obtain single colony forming units (cfu) or streak out with an inoculation loop, if only a few single colonies are required.

5 Remarks/Troubleshooting

Electroporation should reach pulse times ≥ 5.0 ms, preferably about 6.0 ms. For *E. coli*, cuvettes with a 1 mm gap were used at 1.8 kV, for *P. putida* cuvettes with a 2 mm gap at 2.5 kV. Transformation efficiency should be around 10^8 per µg of plasmid (depending on the strain and the DNA type), for *P. putida* this efficiency is usually significantly lower. If *E. coli* cells are transformed with DNA from ligations, isothermal assemblies and the like, use about 100 ng of purified DNA (e.g. by column purification or desalting over a membrane). If pulse times are low, reduce the amount of DNA used and/or use low salt LB medium while preparing the cells. For the preparation of cell libraries, do not dilute and plate all cells (preferably 200 µL aliquots on larger agar plates). Let grow only until cfu become barely visible, harvest the library by scraping the cell off with a Drigalski spatula using about 5 mL of LB medium. In order to estimate the library size, also plate 50 µL of a dilution series and incubate until cfus are properly countable. In order to optimize transformation efficiency, cells can be recovered in pre-warmed SOC medium instead of LB.

6 Biosafety

No biosafety issues are associated with this protocol and the microbiological strains used (BSL1). Please note that the transfer of virulence factor gene(s) could increase the biosafety level to the corresponding BSL of the original pathogen.