WCSB, Wageningen University	EPP-SOP-WU07
	Version 1.0

EPP - Standard Operating Procedure

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

Title: Making electro-competent *E. coli* cells and transformation of them

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Instruction

Making electro-competent *E. coli* cells and transformation of them

1. *Introduction / Purpose*

This protocol describes a method to make *Escherichia coli* cells electro-competent. The method involves one overnight culturing step, followed by several hours of culturing during the day and 1.5 - 2 hours of preparation of electro-competent cells.

The protocol also describes how to do the transformation (electroporation) afterwards.

Keywords: *E. coli* – electro-competent – competent – transformation - electroporation

2. *Equipment and chemicals*

2.1. *Equipment*

<u>For making competent cells:</u>

- Centrifuge for disposable 50 ml tubes that can be cooled to 4°C
- Shaking incubator
- Spectrophotometer

<u>For transformation:</u>

- Thermomixer or water bath at 37°C
- Electroporation machine

2.2. *Chemicals*

- N_2(l)
- Ice

2.3 *Bacterial strains*

- *Escherichia coli*

2.4 *Other materials*

<u>For making competent cells:</u>

- 1 sterile 50 ml tube for culturing (Corning mini bioreactor centrifuge tube 50 ml, #431720)
- 8 sterile 50 ml tubes (generic ones, not the ones for culturing)
- 20-30 sterile 1.5 ml eppendorf tubes that don't pop open when submerged in N_2(l)
- 1 sterile 1 L Erlenmeyer flask

<u>For transformation:</u>

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- Sterile cooled electroporation cuvette with a 2 mm gap
- Sterile 1 ml syringe
- Sterile needle (BD Microlance; 21G 1.5”- Nr. 2; 0,8 x 40 mm; REF 304432; or a thicker needle)

3 Media and Buffers

For making competent cells:

- 500 ml LB w/o salt (per liter: 10 g tryptone, 5 g yeast extract)
- 1 l ice-cold 10% v/v glycerol (per liter: 100 ml glycerol)

For transformation:

- SOC medium, 0.5 ml per transformation: (per liter: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 0,186 g KCl – adjust to pH 7 with NaOH – autoclave – add 5 ml of sterile 2M $MgCl_2$ (which is 19 g $MgCl_2$ in 100 ml) and 4,5 ml of sterile 80% w/v glucose)
- Selective agar plates, depending on the plasmid that will be transformed into *E. coli*

4 Procedures

Making electro-competent *E. coli* cells

- Inoculate 5 ml LB w/o salt in a 50 ml culturing tube with *E. coli* and incubate overnight at 37°C / 250 rpm .
- Next day, first thing in the morning, inoculate 400 ml LB w/o salt in a 1 l Erlenmeyer flask with enough of the overnight culture to reach OD_{600} of 0.2 and incubate for ~3 hours to OD_{600} 0.5-1.0.
- Pre-cool centrifuge to 4°C.
- Transfer culture volume to 8 Greiner tubes of 50 ml and cool on ice for 15 minutes.
- Centrifuge for 10 minutes at 2000g and 4°C.
- Discard supernatant and add 5ml of 10% glycerol, resuspend the pellet in it and then add 45ml of 10% glycerol, shake by hand a few times to mix.
- Centrifuge for 10 minutes at 2000g and 4°C.
- Discard supernatant and add 5ml of 10% glycerol, resuspend the pellet in it and then add 45ml of 10% glycerol, shake by hand a few times to mix.
- Put on ice for 10 minutes.
- Centrifuge for 10 minutes at 2000g and 4°C.
- Discard supernatant and resuspend the pellet in 1 ml ice-cold 10% glycerol, pool the contents of all 8 tubes into one tube.
- Centrifuge for 5 minutes at 1500g and 4°C.
- Discard supernatant and add 400 µl 10% glycerol (this will result in ~800 µl suspension).
- Make aliquots of 40µl in pre-cooled eppendorf tubes and flash freeze with N_2(l). Store at -80°C.

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Transformation of electro-competent *E. coli* cells

- Thaw an aliquot of electro-competent cells on ice.
- Add 1 µl DNA, mix, and transfer to cold cuvette on ice. Make sure that the cells are at the bottom of the cuvette by gently tapping against it.
- Put the cuvette in the electroporation machine and apply a pulse (2.5 kV, 200-400 ohm, 25 µF).
- Immediately add 0.5 ml SOC to the cuvette.
- Empty the cuvette with help of the syringe and needle and put the contents in a 1.5 ml eppendorf tube.
- Incubate it for 1 hour in thermomixer or waterbath at 37°C (in case of thermomixer, shake at 800 – 1000 rpm).
- Spread the cells on a selective plate, incubate at 37°C overnight or until colonies appear.

5. *Remarks / troubleshooting*

- The reason for resuspending the cells in 5 ml glycerol solution first before adding the remainder of the 50 ml is that it is easier to resuspend in a smaller volume than in a completely filled tube.
- Competent cells can be stored for 6 to 12 months at -80°C
- Work sterile during the whole procedure (next to bunsen burner or in flow cabinet)
- After transformation, the cells are spread onto a selective plate. To assure that single colonies appear, two plates can be used instead: one to spread 1/10 of the cells onto, and another one to spread 9/10 of the cells onto. If the transformation was very efficient, the single colonies will appear on the 1/10 plate and a lawn will appear on the 9/10 plate. In the case of a less efficient transformation, single colonies will appear on both plates or only on the 9/10 plate (and nothing on the 1/10 plate).

6. *Biosafety*

- Be careful when using needles.
- Avoid skin contact with N_2(l), it can cause cold burns.
- All materials that came into contact with transformed *E. coli* should be autoclaved before being disposed of.

7. *Acknowledgements*

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