

Transformation of Electro-Competent Cells

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Version: 1.0 - [Print Version \(.PDF\)](#)

This requires a pulse generator using high voltages. Be sure you know how to operate it.

1. Thaw the electro-competent cells on ice
2. Pipet 1 to 3 μ l of DNA solution to the cells
3. Pipet up and down several times to mix
4. Pipet the cell suspension to the pre-cooled cuvet
5. Give a pulse of 2500 volts (12.5 kV/cm)
6. Check pulse time and voltage.
7. Add 700 μ l of LB to the electroporated cells
8. Transfer the solution to a 1.5 ml eppi
9. Incubate for 60-90 minutes at 37 °C
10. Clean the cuvetts!
11. Plate the cells on a LB plate with antibiotic
12. Incubate overnight at 37 °C

Commented Protocol:

1. Thaw the electro-competent cells on ice

2. Pipet 1 to 3 μ l of DNA solution to the cells

3. Pipet up and down several times to mix

4. Pipet the cell suspension to the pre-cooled cuvet

Use electroporation cuvet with a 2 mm electrode distance.

5. Give a pulse of 2500 volts (12.5 kV/cm)

6. Check pulse time and voltage.

Pulse time should be above 4.6. If you hear a bang or the pulse time is too low, you had too much salts in the cuvet. Try to use less DNA solution.

7. Add 700 μ l of LB to the electroporated cells

8. Transfer the solution to a 1.5 ml eppi

9. Incubate for 60-90 minutes at 37 °C

Big plasmids should be left to recover for at least 1.5-2 hours.

10. Clean the cuvetts!

Wash them in 70% ethanol, then with dextran solution, rinse them well with demiwat, rinse them with 96% ethanol and dry them.

11. Plate the cells on a LB plate with antibiotic

You can split the sample. Plate 50 μ l on one plate, spin down the rest 2 min at 8000 rpm, resuspend ca 50 μ l you leave in the tube and plate it on a second plate. Like that you will allways have nice colonies.

12. Incubate overnight at 37 °C

Known Issues:

- If the pulse times are low, the washing was not sufficient.

References and Comments:

This is a very basic protocol. I got to know this protocol in the Bisseling Lab and did it many times. It works well.

How to cite this page in publications:

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<http://www.untergasser.de/lab/protocols/competent_cells_electro_trafo_v1_0.htm>.

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