

Electroporation of E. coli cells

Page 1 of 1

Sterilize all material before use.

Put plasmid DNA, electrocompetent cells and electroporation cuvette on ice before use.

Pre-heat petri dishes and SOC¹ medium to 37°C before use.

Only open your flasks or tubes in a flow bench.

1.	If the electrocompetent cells are frozen, thaw on ice.
2.	Add 100 ng of plasmid DNA to 100 µl electrocompetent bacterial cells (= 1 tube).
3.	Transfer the cell suspension to an electroporation cuvette of 0.2 mm.
4.	Set electroporation parameters to 25 $\mu\text{F},$ 2.5 kV and 200 Ω and activate.
5.	Remove the cuvette immediately after electroporation and add 1 ml pre-heated SOC medium. Mix by pipetting up and down and transfer the cell suspension to a sterile Eppendorf tube.
6.	Incubate the tube at the required cultivation temperature for 1 hour.
7.	Dilute the transformation mixture 1/10, 1/100 and 1/1000 with non-selective SOC medium.
8.	A 4x4 dilution ent is made on a plate with the prescribed selective medium.
9.	Incubate the plates at the required cultivation temperature for 16 up to 24 hours. The specified incubation time applies for standard E. coli strains.
10.	Single colonies are obtained and can be used for sub-cultivation in liquid media.

¹ Using SOB (Super Optimal Broth) or SOC (SOB + glucose) medium can increase the transformation efficiency.

SOB broth:	Tryptone	20 g
	Yeast extract	5 g
	NaCl	0.5 g
	Purified water	950 ml
	KCI 250 mM	10 ml

Set to pH 7.0 and add purified water to a total volume of 1 liter

Sterilize

Add 5 ml of a sterile 2 M MgCl₂ solution

SOC broth: Prepare 1 liter SOB broth (including MgCl₂ solution)

Add 20 ml of a sterile 1 M glucose solution