

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.

ELECTROPORATION WITH CONTROL DNA	
1.	If the electrocompetent cells are frozen, thaw on ice.
2.	Add 1 µl (= 10 ng) control DNA (e.g. pUC18) 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 µF, 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.
QUALITY CONTROL – TRANSFORMATION EFFICIENCY	
1.	Dilute the transformation mixture 1/10, 1/100 and 1/1000 with non-selective SOC medium.
2.	Spread 100 µl of the transformation mixture and of each dilution on separate plates with the prescribed selective medium (in case of pUC18: LB-Lennox + ampicillin).
3.	Incubate these 4 plates at the required cultivation temperature for 16 up to 24 hours. The specified incubation time applies for standard <i>E. coli</i> strains.
4.	Select a plate where you can easily distinguish between separate colonies (15 to 150 colonies). Calculate the transformation efficiency (TrEf): TrEf = number of colonies x dilution x 10 ³ cfu/µg DNA (≥10 ⁶ – 10 ⁷ cfu/µg DNA dependent on the specific transformation-efficiency of the strain; tolerance: ± 0.5 log-unit)
QUALITY CONTROL – PURITY	
1.	If the competent bacterial host cells are frozen, thaw another tube on ice.
2.	Add 900 µl non-selective SOC medium to 100 µl competent bacterial cells (= 1 tube).
3.	Negative controls: spread 100 µl of the mixture on three different plates with the prescribed cultivation medium, each containing one of three different antibiotics, to which the host strain is not resistant. Positive controls: if applicable, spread 100 µl of the mixture on a plate with the prescribed cultivation medium with any antibiotics to which the host strain is resistant.

4.	Incubate the negative control plates at the required cultivation temperature for at least 48 hours, and any positive control plates for 16 up to 24 hours at the prescribed temperature.
5.	Check the negative control plates visually for contaminants. Check the positive control plate(s) visually for the following aspects: <ul style="list-style-type: none">• Presence of homogeneous colonies• Texture, size and opacity of the colonies correspond with the characteristics of the host strain• No bacterial, yeast or mould contaminants are present

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
KCl 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