4D-Nucleofector® 96-well Unit Guideline for Bacteria Transformation

1. Kit for Bacteria Transformation:
We do not offer a kit specialized for bacteria transformation. Please contact your local sales representative or our Scientific Support Team for a special Sales Agreement to access 96-well Nucleocuvette® Plates as custom product.

2. Protocol
Programs:

- Start your respective Nucleofector® System and create or upload the experimental parameter file (see system manual for details)

- This special program definition file contains the standard 96-well programs for eukaryotic cells plus **17 different programs for bacteria** which can be accessed most easily via the “cell type code” drop down:

Table 1:

Cell type code	Program Code
X_Bacteria 1	GA-100
X_Bacteria 2	GB-100
X_Bacteria 3	GC-100
X_Bacteria 4	GD-100
X_Bacteria 5	GE-100
X_Bacteria 6	GF-100
X_Bacteria 7	GG-100
X_Bacteria 8	GH-100
X_Bacteria 9	GI-100
X_Bacteria 10	GJ-100
X_Bacteria 11	GK-100
X_Bacteria 12	GL-100
X_Bacteria 13	GM-100
X_Bacteria 14	GN-100
X_Bacteria 15	GO-100
X_Bacteria 16	GP-100
X_Bacteria 17	GQ-100

We recommend following optimization strategies:

- If you work with an *E.coli* strain please try programs GM-100, GN-100 und GO-100
- For any other strain we recommend an initial screen using all 17 programs
- Programs GO-100, GP-100 and GQ-100 may lead to error 8, depending on the sample background

Bacteria stocks:

- The Nucleofection® Procedure (see below) should be the same for any strain. Nevertheless the competence (e.g. # of colonies/µg pUC19) for electroporation as well as for chemical/heat shock transformation differs significantly for different strains
- Bacteria stocks should not contain more than 4–5% glycerol as pipetting of small volumes with higher glycerol amounts could lead to use of imprecise volumes.

DNA stock:
Ideally, the plasmid DNA should be dissolved in water. Use of any buffers may lead to arcing or splashing.

Nucleofection® Procedure:

1. Thaw electro-competent bacteria on ice
2. Pre-warm SOC medium to 37°C
3. Optionally: Pre-cool 96-well Nucleocuvette® Plate on ice for at least 15 minutes. With pre-cooled vessels you may increase transformation efficiencies
4. Dispense desired amount of plasmid DNA (e.g. 10 – 50 pg pUC19; in 2 – 5 µl of H_2O) in each well of the 96-well Nucleocuvette® Plate
5. Add 20 µL of bacteria suspension to each well of the 96-well Nucleocuvette® Plate
6. Carefully remove any ice-water from the plate

7. Place the 96-well Nucleocuvette® Plate into the 4D-Nucleofector® 96-well Unit and start the Nucleofection® Process as described in the system manual
8. Post Nucleofection®, remove the 96-well Nucleocuvette® Plate and add 200 µL SOC medium (or your established medium) to each well
9. Transfer 200 µL bacteria suspension to a 10 ml bacteria tube containing 300 µL SOC (total volume 500 µL)
10. Incubate at 37°C and 225 rpm for 1 h
11. Plate 25 µL or 50 µL of the bacteria suspension of each tube on an agar plate (containing appropriate antibiotics and 40 µg/ml X-gal and 100 µM IPTG for blue/white screen (if desired)
12. Incubate at 37°C for additional 24 h

Additional Information

For additional information and an up-to-date list of Nucleofector® References, please visit the Lonza Knowledge Center:
https://knowledge.lonza.com

For more technical assistance, contact our Scientific Support Team:

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