Amaxa® General Nucleofection® Protocol

For transformation of bacteria

Application This protocol enables you to optimize the Nucleofection® Conditions for the transformation of different bacteria strains.

Overview

Bacteria alternative programs	Bacteria alternative programs
Program 1	
Program 2	
Program 3	Program 3
Program 4	
Program 5	
Program 6	
Program 7	
Step 1: For each bacteria strain try 7 different Nucleofector® Programs*.	**Step 2:** Select the Nucleofector® Program* yielding the highest colony number for further transformation of this strain.

***Note** Bacteria programs can be only selected via the "Cell Type List" mode. The programs are not suitable for any eukaryotic cell.

Required Material

Transformation solution	Sterile water
Cuvettes	Standard 1 mm electroporation cuvettes (e.g. from BioRad, Eurogentec, Eppendorf, Cole-Parmer, BTX, Epicentre, Genlantis, Sigma, Thermo Electron)
Medium	LB or YT medium
Plasmid DNA	Make sure your plasmid DNA preparation contains low salt concentration. DNA should be solved in distilled water. In case of doubt precipitate DNA with ethanol, wash with 70% ethanol and solve DNA in distilled water after drying

1. Generation of Electro-Competent *E. coli*

Cell culture recommendations

E. coli can be either bought from different sources or prepared as described below:

1.1 Pre-cool approx. 600 ml sterile water and 10 ml sterile water with 10% glycerol to max. 4°C, e.g. by incubation on ice
1.2 Inoculate 500 ml YT with 5 ml of an overnight culture of the bacteria in a large Erlenmeyer flask and strongly shake them at 37°C until the optical density at 600 nm (OD_{600}) reaches 0.5 to 1
1.3 Put the flask on ice for at least 15 minutes. All following steps should be performed at 4°C. Centrifuge *E. coli* at min. 3000xg for 15 minutes
1.4 Discard supernatant and resuspend the *E. coli* pellet in 400 ml pre-iced water. Centrifuge *E. coli* at min 3000 x g for 15 minutes
1.5 Repeat washing of *E. coli* in 100 ml water and centrifuge as above
1.6 Repeat washing of *E. coli* in 8 ml water with 10% glycerol and centrifuge as above
1.7 Resuspend *E. coli* in 1.5 ml water with 10% glycerol
1.8 Freeze aliquots of 30 µl at -80°C

2. Nucleofection®

One Sample contains

20 µl freshly thawed *E. coli*
Up to 5 µl de-salted plasmid DNA

2.1 Thaw required aliquots of electro-competent *E. coli*
2.2 Pre-warm an aliquot of culture medium containing supplements at room temperature in a 50 ml tube (200 µl per sample)
2.3 Mix 20 µl of *E. coli* suspension with a maximum of 5 µl plasmid DNA.
2.4 Transfer a 20 µl sample into a standard 1 mm cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the cap. No cooling of the cuvette is required
2.5 Select the appropriate Nucleofector® Program via the "Cell Type List" mode (for details how to select a program see Nucleofector® II Manual, 2.7.3). Only specialized programs for bacterial transformation will be efficient
2.6 Insert the cuvette into the cuvette holder and press the "X" button to start the program
2.7 Remove the samples from the cuvette immediately after the program has finished (display showing "OK"): Take the cuvette out of the holder. Add 100-200 µl of the pre-warmed culture medium to the cuvette and transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block
2.8 Press the "X" button to reset the Nucleofector®
2.9 Repeat steps 4-8 for the remaining samples

3. Post Nucleofection®

3.1 Incubate the diluted *E. coli* at 37°C for expression of the resistance marker (e.g. for 30 minutes for ampicillin resistance and 60 minutes for tetracycline resistance)

3.2 Plate *E. coli* on plates with selective antibiotics according to standard procedures, e.g. in different dilutions or volumes to insure separated single colony growth

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

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References:

1. Dower, WJ, Miller, JF & Ragsdale, CW, High efficient transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. 16(13): 6127-6145 (1988)

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