Electroporation - Transformation of *B. subtilis*

Introduction

This protocol follows the instructions given by the IGEM 2016 collaboration Bonn and Freiburg *Bacillus subtilis* guide:
http://2016.igem.org/wiki/images/7/74/T--UBonn_HBRS--How-To-Bacillus-Subtilis.pdf
"Electroporation is a transformation method that relies on sending an electrical current through the cell and creating holes or pores in the cell membrane. Through those pores, the plasmid will enter the cell before they are getting closed."
-description from the guide.

Materials

› Electrocompetent cells
› Competency Medium
› Plasmid you want to transform
› Electroporator
› Electroporation cuvettes

Procedure

Electrocompetent cells

1. Inoculate a liquid culture of *Bacillus subtilis* and let it grow overnight
2. Vortex the culture gently and give 500 µl each in 3x 20 ml competency medium
3. Grow the bacteria in the flasks at 37°C 250 rpm shaking till you reach an OD600 between 0.5 and 0.7/ml
4. Add 1 ml of a 20% glycine solution to the first 1.25 ml to the second and 1,5 ml to the third flask to reach
total glycine concentrations of 1%, 1.25% and 1.5%
5. Keep shaking for 1 h (because of the glycine the optical density should not change significantly)
6. Cool down the cells on ice for 15 mins (if not done already, transfer cells in 50 ml Falcon tubes)
7. Centrifuge at 8500 rpm for 10 min at 4°C for getting bacteria pellets
8. Pour off the supernatant and wash the cells three times with an ice-cold washing buffer (20 ml/10 ml/5 ml).
Centrifuge the cells down at 8500 rpm for 10 min at 4°C between each washing procedure and decant the supernatant
9. Resuspend the cells in 1 ml ice cold washing-buffer (all three different cultures are supposed to get in it!)
10. Make aliquots (recommended: 120 µl → enough for two transformations)
11. Freeze in liquid nitrogen and store at -80°C

Transformation

12. Mix electrocompetent cells with the plasmid and acquire a total volume of 60 µl with a final DNA concentration of 10 ng/µl
13. Place the cell-plasmid-suspension, the electroporation-cuvettes and competency-medium on ice for 10
min (you need 1 ml competency medium & 1 electroporation cuvette per transformation, but it is recommended to take more)
14. Pipet the cold cell-plasmid-suspension in the prechilled electroporation cuvette and tap the cuvette multiple times (this way you get rid of bubbles and spread your suspension equally
15. Make sure that the electroporation-cuvette is dry (take care that you don't touch the metal sides anymore!)
16. Electroporate at 2100 Volt (the electroporator will give out a "time constant". A time-constant from 3.0 to
5.5 is a positive indicator although the transformation could also be successful at a lower time constant)
17. Flush the electroporated mixture out of the electroporation-cuvette with 1 ml of competency-medium
18. Let the cells grow for 3 h at 37°C 300 rpm shaking
19. Pellet cells by centrifugation at RT 5 min and decant the supernatant (do not throw away)
20. Resuspend the pellet in 100 µl of the supernatant
21. Plate on selective agar