C2 Biotechnologies, LLC

Using biotechnology to improve the quality of life

SOP-108

B. subtilis Transformations

Procedure for transforming *B. subtilis* bacteria.

Section 1 Materials and Reagents

LB plates containing antibiotic

37 C Incubators

HS medium:
Spizizen´s medium supplemented with

- 0.5% glucose
- 50 µg/ml DL-tryptophane
- 50 µg/ml uracil
- 0.02% casein hydrolysate
- 0.1% yeast extract [Difco]
- 8 µg/ml arginine
- 0.4 µg/ml histidine
- 1 mM $MgSO_4$

LS medium:
Spizizen´s medium supplemented with

- 0.5% glucose
- 5 µg/ml DL-tryptophane
- 5 µg/ml uracil
- 0.01% casein hydrolysate
- 0.1 % yeast extract [Difco]
- 1 mM $MgSO_4$
- 2.5 mM $MgCI_2$
- 0.5 mM $CaCI_2$

10x Spizizen`s medium:

- 2 g $(NH_4)_2SO_4$
- 14 g K_2HPO_4
- 6 g KH_2PO_4
- 1 g sodium citrate
- add 100 ml distilled water
- autoclave or filter sterilize with 0.25 um filter
- then add 0.1 ml 1 M $MgSO_4$

Section 2 Protocol

Transformation Protocol

Note: All materials that come into contact with bacteria must be sterile.

For transformation of *B. subtilis* we recommend the protocol of Anagnostopoulos and Spizizen (1961), slightly modified:

1. Grow *B. subtilis* overnight at 37 °C in 5 ml HS medium
2. Inoculate 20 ml LS medium with 1 ml of overnight HS culture and let shake for 3 - 4 hours at 30 °C
3. Withdraw 1 ml of his LS culture (late log/early stationary phase; OD_{578}), add 10 µl of 0.1 M EGTA, and incubate at RT for 5 minutes
4. Add 1 - 2 µg of plasmid DNA
5. Shake for 2 hours at 37 °C. The cells will develop antibiotic resistance during this time
6. Plate 200 ul on selective plates (5 µg/ml chloramphenicol)
7. Incubate plates at 37 C overnight.
8. Next day pick colonies and screen for clones that have your plasmid (SOP 104, SOP 105 & SOP 106).

Section 3 References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

Anagnostopoulos, C. and Spizizen, J. (1961). Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. 81:741-746.