Electroporation competent Bacillus subtilis 168

1. Prepare an O/N culture of bacillus subtilis 168 LB media with appropriate antibiotic incubate at 37degC.
2. Make a 16 fold dilution of the O/N culture Growth medium (GM), incubate in 37 degC shaken at 200rpm.
3. Grow till the OD reaches 0.85-0.95, then add threonine, glycine and Tween 80 to an final concentration of 1.0, 2.0, 0.03% respectively.
4. Incubate for 1 hour
5. Cool the culture on ice on ice for 5 min.
6. Centrifuge the culture at 5000g for 10 min and resuspend in 1/20 of original volume in electroporations medium.
7. The cells can now be stored at -80 degC (for up to one month) or directly used for electroporation.

Media:

Growth medium

- LB
- 0.5M sorbitol

Recovery medium

- LB
- 0.5M sorbitol
- 0.38M mannitol

Electroporations medium

- 0.5M sorbitol
- 0.5M mannitol
- 10% glycerol
- mixed in ddH2O

References

This protocol is following the article sun et al, 2015 [1].

[1] Z. Sun, A. Deng, T. Hu, J. Wu, Q. Sun, H. Bai, G. Zhang, and T. Wen, "A high-efficiency recombineering system with PCR-based ssDNA in Bacillus subtilis mediated by the native phage recombinase GP35," *Appl. Microbiol. Biotechnol.*, pp. 5151–5162, 2015.