OGRE in Bacillus subtilis 168

OGRE cycle 1

1. Add 100 ul of electroporation competent cells to ice cold electroporation cuvettes.
2. Add 5uM oligo to electroporation cuvette and was mixed gently with pipetting or flicking.
3. The sample was Incubated for 5 - 10min.
4. Prepare 1 sterile plastic tube for each sample with 1 ml recovery medium, put them on ice
5. Sample was added to electroporation's cuvette and electroporated with 2.2kV (for 0.2 cm cuvettes). Runtime should be 5-6ms. Optimal runtime is 5.5.
6. Immediately after the electroporation add 1 ml of recovery medium to the electroporation cuvette and pipette this to a sterile plastic tube. Repeat this 2 times. Now the total volume should be about 3 ml in each plastic tube.
7. Incubate the samples for 4h at 30 degC shaking at 220rpm.
8. Optional: measure OD_{600} of sample and use following equation to calculate the a suitable dilution.
    a. $d = log_{10}(1.33 * X_{OD} * 10^5)$, where the right dilution will be 10^d.[1]
    b. This equation will over estimate the dilution, so dilution of 10^{d-1} should be used as well.
9. Plate the samples on appropriate antibiotics and in appropriate dilutions.
10. If doing more cycles save the rest of the sample and continue to "More cycles of OGRE".

More cycle of OGRE

11. Centrifuge samples at 5000g for 10 min. at 4 degC
12. Dispose the supernatant and resuspend in 1/20 (of original volume) cold electroporation medium. Repeat this 4 times. Keep everything cold.
13. Go to step 1.

References

This is a modified version of the protocol from Sun et. al, 2015 [1]. Modifications was inspired from Lu et al, 2012 [2] and Carr et al, 2012 [3].

[1] See http://2015.igem.org/Team:DTU-Denmark/Project

[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.

[2] Y. P. Lu, C. Zhang, F. X. Lv, X. M. Bie, and Z. X. Lu, "Study on the electro-transformation conditions of improving transformation efficiency for Bacillus subtilis," *Lett. Appl. Microbiol.*, vol. 55, no. 1, pp. 9–14, 2012.

[3] P. a. Carr, H. H. Wang, B. Sterling, F. J. Isaacs, M. J. Lajoie, G. Xu, G. M. Church, and J. M. Jacobson, "Enhanced multiplex genome engineering through co-operative oligonucleotide co-selection," *Nucleic Acids Res.*, vol. 40, no. 17, 2012.