Marcus Lehr

Direct Plating Transformation Protocol

Constructed from (Pope & Kent, 1996)

Materials/Notes

- Appropriate pre-warmed plates (2 per transformation, 1 cell only control)
- Ice
- 1 PCR tube per transformation, 1 for cell only control
- 50 µL comp cells per transformation, 50 µL for cell only control
- Chilled sterile DI water
- Unless getting comp cells from the -80 ^{o}C, this entire procedure can and should be done in 1239 ERB, to minimize temperature fluctuations of plates and cells.

Procedure

1. A few hours prior to the procedure (or overnight if convenient), label required plates for transformation and place them in the 37^{o}C incubator in 1239 ERB.
2. Place 2 µL of DNA into a PCR tube on ice. Add 50 µL of comp cells with a chilled pipette and pipette up and down to mix.
3. Keep cells on ice for 1-5 min.
4. Add 50 µL of chilled, sterile DI to cells and pipette up and down, then remove 50 µL and plate. This is a 1:1 dilution plating.
5. Repeat with another 50 µL of chilled water, this will be a 1:3 dilution plating.
6. Immediately place cells in the 37^{o}C incubator in 1239 ERB. Leave plates overnight, colonies should be observed the next day.
7. Proceed to the PCR Screening Protocol.

Pope, B., & Kent, H. M. (1996). High efficiency 5 min transformation of Escherichia coli. *Nucleic acids research*, *24*(3), 536-7. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=145660&tool=pmcentrez&rendertype=abstract.