Protocol: Making Competent Cells

Making Competent Cells – Bulk Preparation (500 mL)

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Day 1: Inoculate 5 mL of LB media with an appropriate E. coli strain (e.g. DH5alpha) – grow at 37°C

Day 2:

1. Transfer the 5 mL o/n culture into 500 mL of LB media (made in 1 liter flask)
2. Grow cells at 37°C until they reach OD660 = 0.4 - 0.6 (approx 2-5 hours)
3. During Step 2, make transformation buffer (TB) or thaw frozen TB (need 112.5 mL). See separate protocol for TB recipe.
4. Cool cells on ice for 5-10 minutes
5. Spin down cells at 3000 rpm for 7 minutes at 4°C
6. Gently resuspend the cell pellet in 100 ml of ice cold TB – keep on ice during the resuspension procedure.
7. Sping down cells at 3000 rpm for 7 minutes at 4°C
8. Gently resuspend cell pellet in 12.5 mL *ice cold* TB – *keep on ice* during resuspension procedure
9. Incubate on ice for an additional10 min.
10. Add 450ul DMSO(dimethyl sulfoxide) and *mix gently while on ice*
11. Aliquot cells into 1.5 mL eppendorf tubes (100ul or 200ul aliquots) – *keep on ice during aliquoting*
12. Store at -80 °C

To make sure competent cells do not carry any unwanted plasmids, plate out untransformed as well as transformed cells when checking to see if the above protocol has been successful.

Once thawed for use, DO NOT re-freeze cells. Re-freezing cells will lower competency.