Chemically Competent Cells

Note: Work under sterile conditions, keep all samples on ice in closed tubes to avoid contamination. Prepare buffers and autoclave prior to preparing competent cells.

Buffers (sterilize prior to use):
80 mM $MgCl_2$ 20 mM $CaCl_2$
100 mM $CaCl_2$

1. Inoculate from DMSO stock and grow cell culture overnight in 2 x 5 mL LB tubes shaking at 37°C.
2. From the 5 mL cultures, inoculate 2 x 50 mL LB flasks to an OD_{600} of 0.1.
3. Grow to an OD_{600} of approximately 0.6 in a shaking incubator at 37°C.
4. Centrifuge cells at 2700 x g for 7 minutes at 4°C.
5. Pour off supernatant being careful not to disturb the pellet. Tap gently on paper towel to remove any remaining supernatant.
6. Add 30 mL ice cold 80 mM $MgCl_2$ 20 mM $CaCl_2$ to each tube. Resuspend by pipetting gently up and down. DO NOT VORTEX.
7. Centrifuge for 5 minutes at 2700 x g.
8. Pour off supernatant.
9. Resuspend cell pellet in 2 mL ice cold 100 mM $CaCl_2$.
10. Combine both tubes for a final volume of 4 mL.
11. Add 1.2 mL sterile glycerol and mix gently.
12. Aliquot samples (20 – 100 µL each), flash freeze with liquid nitrogen and store at -80°C.