Production of DH5α Competent Cells

$CaCl_2$ Solution:

60mM $CaCl_2$	($CaCl_2 \cdot 2H_2O$)	4.41g
15% (v/v) Glycerol		75mL
10mM MOPS pH 7.0	(1M)	5mL
Final Volume		500mL

*****Filter Sterilize*****

1. Inoculate 5mL LB with a single colony. Grow 37°C overnight shaking.
2. Inoculate 500mL LB with 5mL culture (Inoculate culture with 1:100 dilution of saturated overnight culture). Grow at 37°C shaking until OD_{590} = 0.375.
3. Pellet cells by centrifuging 7,000RPM 10min 4°C

PERFORM ALL BELOW STEPS ON ICE OR IN COLD ROOM

4. Resuspend cells in ½ vol. (250mL if started with 500mL culture) $CaCl_2$ solution by twirling the bottles (NOT by pipetting or vortexing)
5. Incubate on ice 30 min.
6. Centrifuge 7,000RPM 10min. 4°C
7. Resuspend cells in 1/20 vol (12.5mL if starting with 500mL culture) of $CaCl_2$ solution (by twirling, NOT pipetting or vortexing)
8. Aliquot 200µL each into pre-chilled eppendorf tubes (do this in the cold room)

At this point, the cells are competent for transformation but, step 9 should improve their transformation efficiency

9. Leave at 4°C for 12-24 hours
10. Freeze at -80°C (this may be done by first flash freezing in N_2(l) but this is not required due to glycerol content of $CaCl_2$ solution).