Competent cells for expression

A. CaCl2 Buffers Preparation

1M CaCl2 (stock solution, 10x working concentration)

Weigh out 11.1g of anhydrous CaCl2
Add to 80mL of dH2O
Mix solution until CaCl2 is fully dissolved
Top up to 100mL
Filter sterilize through a 0.22µm pore

0.1M CaCl2 (working solution)

Add 10mL of 1M CaCl2 to 90mL of dH2O for a 1:10 dilution
Filter sterilize through a 0.22µm pore

0.1M CaCl2 + 15% glycerol (working solution)

Mix 6mL 1M CaCl2 with 9mL sterile glycerol and 45mL dH2O

B. Overnight Culture(s)

Inoculate 1mL of LB with E. coli
Place in shaking incubator at 37°C and 200rpm
Incubate for 12-16 hours

C. Generation of Competent Cells (CaCl2 wash)

Subculturing overnight culture:

Add 1mL of overnight culture to 199mL of fresh LB (1:200 dilution, no antibiotics)
Shake incubate at 37°C and 200rpm for 3-4 hours or until OD reaches 0.4

CaCl2 wash:

Ensure that all reagents (CaCl2 solutions, tubes, centrifuge) are ice-cold or at 4°C
Separate culture into multiple tubes
Place on ice for 20 minutes
Centrifuge at 4°C at 4000rpm for 10 minutes
Discard the supernatant by tipping tubes over a discard bin and then aspirating any remaining media
Resuspend each pellet with 20mL ice-cold 0.1M CaCl2, incubate on ice for 30 minutes
Centrifuge at 4°C at 4000rpm for 10 minutes
Discard the supernatant and combine pellets by resuspending in 5mL ice-cold 0.1M CaCl2 with 15% glycerol
Store in -80°C freezer