PROTOCOL FOR MAKING CHEMICALLY COMPETENT CELLS

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Solutions	**Sterilize**
2X LB	200 ml of 2X (two times concentrated) LB [6.2g LB Powder + 3.8 g NaCl/200 ml water Bio101 #3001-042] in 1 liter Erlenmeyer Flask
50 ml 10 mMNaCl	250 ul of 2M NaCl + 49.75 ml GD water Milk dilution Bottle (MDB)
2 x 50ml 75mM $CaCl_2$	0.55 g $CaCl_2$-2H20, bring up to 50ml (MDB)
0.488ml 100% glycerol	25 ml Erlenmeyer Flask with aluminum foil on top
100 ml Glass Distilled water	GDB
10ml 1M $CaCl_2$	1.47 g $CaCl_2$ up to 10 ml water (MDB)
LB or SOC sterile	* SOC Medium/Liter: SOB with the addition of 20 ml filter sterilized 1 M glucose per liter of SOB. * SOB Medium/Liter: Tryptone 20 g Yeast extract 5 g NaCl 0.5 g 250 mM KCl 10 ml Add water to 900 ml. Adjust to pH 7.0 and add water to 990 ml. Autoclave, cool to room temperature, and add 10 ml of a sterile solution of 1 M $MgCl_2$ before use.
Materials	
1.5ml microfuge tubes, sterile	Place in 1 L beaker, cover with al. Foil. Autoclave
250 ml GSA bottle	Sterile

Procedure:

Autoclave

200 ml 2X LB in 1 liter Erlenmeyer flask
1 GSA bottle (250 ml)
50 ml 10 mM NaCl in milk dilution bottle (MDB)
2 x 50 ml 75 mM $CaCl_2$ in MDB (1.1 g $CaCl_2$/100 ml water)
0.488 ml 100% glycerol in a 50 ml Erlenmeyer flask
Sterile eppendorf tubes in a 1 liter beaker
1 bottle sterile water
10 ml 1M $CaCl_2$ (1.47 g/10 ml)

Day 1
1. Grow 10 ml of cells overnight at 37˚C

Day 2
2. Inoculate 200 ml 2X LB with 5 ml of overnight cells. Grow to OD_{600} of 1.0 (HB101 takes about 3 hours).

3. Pellet cells in GSA rotor at 3500 rpm, 4˚C, 15 min. Decant

4. Decant completely. Add 50 ml cold 10 mM NaCl. Incubate on ice for 10 min. Swirl around to resuspend cells, do not pipet.

5. Pellet cells in GSA rotor at 3500 rpm, 4˚C, 15 min. Decant

6. Resuspend in 50 ml cold 75 mM $CaCl_2$. Hold on ice for 35 min.

7. Pellet cells in GSA rotor at 3500 rpm, 4˚C, 15 min. Pellet should look white.

8. Resuspend pellet in 3 ml cold 75 mM $CaCl_2$. Transfer cells to a flask that contains 0.488 ml 100% glycerol. Swirl to mix. Leave on ice 1 hour.

9. Aliquot into sterile 1.5 ml eppendorf tubes that have been placed on **dry** ice. Place 50, 100 µl, 200 µl/tube. Transfer to –70˚C freezer.

Basic Transformation Procedure
Set temp block to 42˚C, add water to the block.

1. Thaw on ice one vial of cells (25 µl).

2. Add 5 to 10 ng of DNA in a volume of 1 µl to the cells and mix by tapping gently. **Do not mix cells by pipetting.**

3. Incubate the vial on ice for 30 min.

4. Heat at 42˚C for 30 seconds**. Do not mix or shake**.

5. Remove vial from the 42˚C bath and quickly place on ice.

6. Add 250 µl of pre-warmed SOC (or LB) medium to the vial. Note: SOC is a rich medium, practice good sterile technique to avoid contamination.

7. Place the vial in a microcentrifuge rack and secure with tape. Place the rack on its side in a shaking incubator and shake the vial at 37˚C for 1 hr at 225 rpm.

8. Plate 20 to 200 µl each of the transformation reaction onto two LB plates with appropriate antibiotic. Incubate the plates inverted at 37˚C overnight.

Calculate transformation efficiency, i.e.: 20 µl plated gave 120 colonies, 10 ng DNA used:

120 x 12.5 (plating factor) =1500 colonies/10 ng DNA = 150000 colonies/µg DNA =
Transformation efficiency (TE): 1.5×10^5 /µg DNA**. A good TE is x 10^8 /**µg **DNA**

If the TE is high, you would have to dilute the transformation reaction 1:100, plate 0.1 ml.