Preparation of electro-competent DH10B cells

The day before inoculation, prepare following:

1. Autoclave 3 liter of water and leave in cold room;
2. Autoclave 100 ml of water (for making 10% glycerol);
   Add 10 ml glycerol from a new clean bottle to 90 ml of sterile water (do not autoclave)
3. Sterilize 4 GS3 centrifuge bottles and 2 boxes Eppendorf tubes.
4. Autoclave 900 ml of LB without Ampicillin.
5. Streak competent stock cells (from -70ºC freezer) onto LB plate (no amp), Incubate O/N at 37ºC.

Next day at 5 PM, pick a colony and inoculate into 100 ml LB (no amp). Grow O/N at 37ºC with shaking.

1. Next morning, add the O/N 100 ml of culture into 900 ml LB medium and grow at 37ºC with shaking until OD600 reaches ~ 0.7 (this takes ~ 2 h);
2. Cool cells in cold room on ice for ~ 20 min;
3. Spin centrifuge rotor for 5 min to pre-cool to 4ºC;
4. Pour cells into 4 pre-cooled GS3 bottles, 250 ml each. Spin at 5 krpm for 15 min at 4ºC;
5. Decant supernatant and resuspend cells in 1 liter of sterile ice-cold water;
6. Spin at 5,000 rpm for 15 min at 4ºC. Decant supernatant and resuspend cells in 500 ml of sterile ice-cold water and transfer cells to two GS3 bottles;
7. Centrifuge at 5,000 rpm for 15 min at 4ºC. Decant supernatant and resuspend cells in 500 ml of sterile ice-cold water;
8. Spin at 5,000 rpm for 15 min at 4ºC. Decant and resuspend cells in 20 ml ice-cold 10% glycerol. Transfer cells into a 50 ml centrifuge tube and centrifuge on table-top centrifuge, at max speed for 15 min at 4ºC. Decant supernatant and resuspend cells in 2 ml sterile 10% glycerol. Transfer cells to small sterile beaker.
9. Aliquot 40 µl of cells into each pre-cooled Eppendorf tubes on ice.
10. Freeze down cells with liquid N2 and store at -70ºC.

To test competent cells:

Take 10 pg of a closed plasmid (1 µl of 10 ng/ml pUC19), mix with 20 µl of cells and do electroporation. Resuspend cells in 980 µl of LB. Plate 100 µl on LB/amp. Count number of colonies next day. Expected efficiency is 4,180 colonies/10 pg DNA.