Chemically Competent Cells

BE SURE TO PERFORM THIS IN A COLD, STERILE ENVIRONMENT. READ AND PREPARE BEFORE PERFORMING.

- Grow overnight culture of desired strain you want to make Chemically Competent. This is your seed stock.
- Inoculate 10 ml of SOB medium with seed stock and grow at 37°C to an OD600nm of ~0.5
- Place inoculum on ice for 30 minutes
- Shake frequently to maintain homogeneous solution
- Centrifuge at 4000rpm at 4°C for 10 minutes.
- Discard supernatant by pouring out slowly and pipetting gently or mix by "flicking" remaining supernatant.
- Gently re-suspend in 10 ml of ice cold CCMB80 buffer.
- Incubate on ice 20 minutes
- Centrifuge again at 4°C and discard supernatant as described above.
- Re-suspend in ice cold CCMB80 buffer to an OD of ~3.0
- Aliquot into 2.0 mL graduated tubes
- Label appropriately and store at -80°C.

Heat Shock Transformation SOP

1. Pre-chill 15mL sterile **culture tube** on ice.
2. Place Competent Cells on wet ice until just thawed.
3. Gently transfer 50 µl of Competent Cells to pre-chilled 15mL culture tube.
4. Add 1-2 µl of Ligated DNA (either Registry BioBricks from distribution kit or ligated parts). Move pipette tip through the cells while dispensing. Gently (VERY GENTLY) tap the tube once or twice.
5. Immediately return tube to ice for 30 min.
6. Place tube in 42^0C water bath for 45-50 sec (DO NOT SHAKE).
7. Immediately place the tube on ice for 2 min.

or LB + glucose ↳ 100 uL of 20% g/L solution

8. Add 950 µl of cold SOC medium and incubate for ~~60~~ 90 minutes at 37^{0}C with shaking. 60-120
9. Centrifuge tubes @4000rpm for 3 minutes and discard supernatant. Resuspend cells in remaining solution and use 50µL to spread onto LB with appropriate antibiotic.
10. Incubate the plates overnight at 37^{0}C.