

## Preparing Competent Cells

- Grow a streak isolation plate overnight for TOP10 and DH5 $\alpha$ Pro cells.
- Inoculate single colony from the freshly streaked plate to 2 ml LB media.
- Grow culture for 16 hours at 37 °C and 220 RPM
- Then inoculate 80 ml of media with 800  $\mu$ l of the culture and grow for 2 hours at 37 °C and 220 RPM.
- Shake the culture in an ice bath for 10 minutes while chilling 4 50 ml tubes.
- Transfer the culture into 2 50 ml tubes for both TOP10 and DH5 $\alpha$ Pro
- Spin the culture at 5000 RPM and 4 °C (maintain this temperature as much as possible at this point). Throw the supernatant.
- Add 20 ml of chilled 100mM CaCl<sub>2</sub> to the tube and re-suspend by vortexing.
- Incubate on ice for 40 minutes.
- Spin culture at 4500 RPM and 4 °C for 10 minutes. Throw the supernatant.
- Add 3.5 ml of 100 mM CaCl<sub>2</sub> and 1.5 ml of 50% Glycerol and gently re-suspend by tapping the tube (do not vortex). Pre-chill micro centrifuge tubes.
- Transfer 200  $\mu$ l of the culture to the micro centrifuge tubes, snap freeze and store at -80 °C.