Protein Expression in *E. coli*

Note: Underlined parameters subject to change depending on cell line/protein construct.

1. Pick a single colony from transformed BL21-DE3 strain *E. coli* (or 15% glycerol stock) using sterile pipette tip, transfer to 50 ml LB media containing appropriate antibiotic in culture flask. (Media should be sterile, transfer by pipetting up and down in media, then eject tip into media)
2. Let culture flask shake at 37°C and 250 rpm overnight until culture is cloudy.
3. Place 10 ml overnight culture (should be extremely cloudy) into 0.5 L of Terrific Broth with appropriate antibiotic and shake at 37°C and 250 rpm. Allow to shake until OD_{600} is 0.8-1.0. This typically takes 3-10 hours. Lower shaker temperature to 25°C for 30 min.
4. Add IPTG to the cells at a final concentration of 0.2 mM (100 ul of 1M stock). Shake at 25°C and 250 rpm overnight.
5. Harvest by centrifuging the cells in centrifuge tubes at 6000 rpm for 15 min.
6. Remove supernatant from cells, make sure that no liquid remains.
7. Transfer pellet into pre-tared 50 ml falcon tube. Weight pellet and label pellet weight, protein construct, researcher name and date on tube.
8. Freeze pellet at -80°C for at least 45 minutes to help gently break up the cell wall. Storing the pellet overnight in a 50ml falcon tube will further assist in this process. Pellet can keep at -80°C for a few months.