Protein induction and lysis protocol – CT-BPI

Grow Bacteria

1. Using **CT-BPI Pet101D in BL21 cells,** Inoculate 5ml of LB + 1% glucose and 1:1,000 Ampicillin
    a. Glucose stock 36%= 138.8ul
    b. 5ul of ampicillin
2. Incubate overnight shaking at 37'
3. Inoculate 2x15ml of LB + 1:1,000 ampicillin (15ul of AMP) with 500ul of overnight growth
4. Incubate shaking at 37' until A600 (OD) = 0.5-0.8 (~2hr)

Induce

1. Add 7.5ul of 2M IPTG to one of the 15ml growths (induced sample)
2. Incubate 2hr at 37' shaking

Lyse ½ of the sample via freeze/thaw method and ½ via Guanidine lysis buffer

1. Transfer 7.5 ml bacteria into 2 tubes
2. Centrifuge at 12,000 RPM for 5 minutes
3. Wash with 7.5 ml of PBS

    Tube 1:

        Freeze and thaw the bacterial pellet 3 times

        Re-suspend the pellet in 1ml of PBS

        Freeze and thaw 3 more times

        Add another 2ml of PBS

    Tube 2:

        Other tube- resuspend in 3ml guanidine lysis buffer

4. Sonicate 3x 5seconds on ice
5. Measure protein concentration using micro BCA protein concentration assay