ONPG β-galactosidase Assay (Liquid)

Materials and Solutions

- **Breaking Buffer**
  100 mM Tris-Cl pH 8.0
  20% glycerol
  Roche protease inhibitor cocktail (1 EDTA free mini tablet per 10 mL)
- **Acid washed glass beads** (0.45-0.5 mm diameter)
- **Z Buffer**
  16.1 g of Na₂HPO₄•7H₂O (40mM Na₂HPO₄•7H₂O)
  5.5 g of NaH₂PO₄•H₂O (60mM NaH₂PO₄•H₂O)
  0.75 g of KCl (10mM KCl)
  0.246 g of MgSO₄•7H₂O (1mM MgSO₄•7H₂O)
  2.7 mL of 2-Mercaptoethanol (50mM 2-Mercaptoethanol)
  Distilled H₂O to 1 L
  Adjust pH to 7
  *(Make a 5x stock without the 2-Mercaptoethanol and add 2-Mercaptoethanol to 1x solution right before conducting assay.)*
- **ONPG** (o-nitrophenyl-b-D-galactopyranoside) stock solution
  4 mg/mL in Z buffer
- **1 M Na₂CO₃** in distilled H₂O
- **SC media with appropriate selection**
- **20% (w/v) of appropriate carbon source** (raffinose and galactose for inducible Gal1 promoter, or glucose for all other promoters).
- **Bradford reagent**
- **Whatman 540 filter paper or equivalent**
- **Disposable plastic cuvets**

Procedure

1. Inoculate a 5 mL culture in SC media with appropriate selection + 2% Sugar and grow overnight at 30 °C (~12-18 hours).
2. Determine cell density of overnight cultures by OD₆₆₀. Innoculate new 5 mL cultures in SC media with appropriate selection + 2% Sugar (+2% inducer, i.e. galactose) to starting cell density of 0.7 x 10⁷ cells/mL using overnight cultures and grow overnight at 30 °C (~12-18 hrs).
3. Determine cell density of overnight cultures by OD₆₆₀. Try to get all OD₆₆₀’s
to be between 0.4-0.7 for 10x dilution. If OD\textsubscript{660} of sample is lower, then grow culture longer. If OD\textsubscript{660} is higher, then harvest cells anyways.

4. Harvest cells by centrifuging at 5000 rpm for 10 min at 4 °C.

5. Resuspend cells in 500 mL of sterile water and then harvest them again by centrifuging at 5000 rpm for 10 min at 4 °C. Remove water and resuspend cells in 150-250 mL of Breaking Buffer.

6. Add glass beads to bottom of meniscus. Vortex at max speed 7-10 times for 1-2 min each time with 1-2 min breaks, all at 4°C.

7. Make a hole at the bottom of each tube using a hot syringe needle.

8. Place pierced tube inside an empty tube and spin samples at 2,000 rpm for 1 min at r.t. to collect cell contents.

9. Pellet cell debris by spinning samples at 14,000 rpm for 10 min at 4 °C. Transfer cell extracts (supernatant) to new microfuge tubes and briefly vortex cell extracts.

10. Set up b-gal assays: 10-100 mL cell extract + 990-900 mL Z Buffer. Incubate samples at 30 °C for 10-30 min. then add 200 mL ONPG stock, start timer and continue incubating samples at 30 °C until first sample turns yellow. Stop all reactions by adding 500 mL 1M Na\textsubscript{2}CO\textsubscript{3}. Stop timer when reactions are stopped. Measure OD\textsubscript{420}.

11. Bradford Assay: Add 20mL cell extract to 180mL water. Take 20mL of diluted extract and add it to 980mL Bradford reagent and measure OD\textsubscript{595}. Use protein standard curve to determine protein concentration.