Saptasense Protocol

Protein Induction and Measuring Expression

Background: Now that we have gotten our biobricks successfully transformed, we can move on to generating large amounts of our proteins of interest. This will be done via induction by IPTG. We will first perform small-scale tests to determine the conditions for optimal expression. Once these have been determined, we will perform large-scale protein expression and purification.

I. Small Scale Induction

Reagents
Transformed bacteria
Selective LB media
IPTG (MW = 238.30g/mol)
dH_2O

Instruments/Materials
Shaking incubator
125 mL Erlenmeyer flask
Four 50 mL Erlenmeyer flasks

Protocol:

Day 1:

- ☐ Prepare overnight culture of bacteria of interest
- ☐ If necessary, prepare 1M IPTG by dissolving 4.76g IPTG in 20 mL H_2O. Aliquot and store in the -20 freezer

Day 2:

- ☐ Add 500 uL overnight culture to 49.5 mL selective LB in a 125 mL Erlenmeyer flask
- ☐ Grow at 37°C until the OD_{600} is between .4 and .6
- ☐ Remove 1 mL as an uninduced control and store at room temperature
- ☐ Split each culture equally into four 50 mL Erlenmeyer flasks
- ☐ Add 1M IPTG in varying amounts to the remaining samples to get the following concentrations, where X is the volume in each of the 4 culture tube in mL:

Final Concentration IPTG	Volume IPTG (uL)
.2 mM	.2 * X
.3 mM	.3 * X
.5 mM	.5 * X
1 mM	X

- ☐ Shake at 37°C for at least 2 hours
- ☐ Spin down 1mL of each sample at 6k rpm for 3 minutes and discard supernatant

- [ ] Resuspend in 25 uL SDS PAGE sample buffer and 25 uL dH_2O
- [ ] Boil at 95°C for 5-10 minutes
- [ ] Centrifuge at 14k rpm for 10 minutes
- [ ] Run each sample on a 12% polyacrylamide gel
- [ ] The optimal concentration of IPTG for protein induction is the concentration that produces the thickest band at the expected protein size in the induced samples

II. Large Scale Induction

<u>Reagents</u>
Transformed bacteria
Selective LB media
IPTG (MW = 238.30g/mol)
dH_2O

<u>Instruments/Materials</u>
Shaking incubator
2L Erlenmeyer flask

Protocol:
Day 1:
- [ ] Prepare overnight culture of bacteria of interest
- [ ] If necessary, prepare 1M IPTG by dissolving 4.76g IPTG in 20 mL H_2O. Aliquot and store in the -20 freezer

Day 2:
- [ ] Add 6 mL overnight culture to 600 mL selective LB in a 2L Erlenmeyer flask
- [ ] Grow at 37°C until the OD_{600} is between .4 and .6
- [ ] Remove 1 mL as an uninduced control
    - [ ] Spin down at 6k rpm for 3 minutes and discard supernatant
- [ ] Add 1M IPTG to produce the optimal IPTG concentration for induction
- [ ] Shake at 37°C for at least 2 hours
- [ ] Spin down the culture into a single pellet. Store at -80°C until needed.
- [ ] Proceed to the protein purification protocol