Preparation of Modular Whole-Cell Bacterial Biosensor

Observing Bacterial Autoaggregation by Spectrophotometry

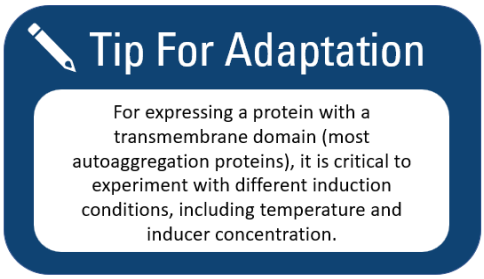
Reagents and Materials	Instruments and Instrument Accessories
Bacterial strain(s) of interest (plated)	Spectrophotometer
Antibody of interest	Cuvettes
Antigen of interest	Timer
1x Phosphate Buffered Saline (PBS)	Shaking incubator
Luria broth (LB), with chloramphenicol (CAM)	
250mL erlenmeyer flask(s)	
Eppendorf tubes	

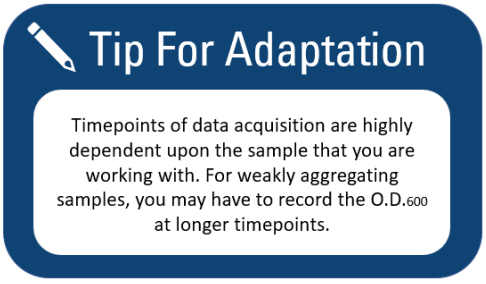
DAY 1: *Prepare an Overnight Culture of EibD E. coli BL21 Strain*

1. Using aseptic technique, transfer 5 mL of LB + CAM into a culture tube.
2. Use a sterile pipette tip to pick one EibD E. coli BL21 colony from an LB + CAM agar plate. Place the tip in the culture tube.
3. Grow at 37 C, 220 rpm, for 12-16 hours.
4. Optional: prepare an overnight culture of a control strain harboring an empty plasmid of the same type, and/or no plasmid

DAY 2: *Induce Culture, Harvest Bacteria, Incubate with Antibody*

5. For each strain: In a 250 mL erlenmeyer flask, add 49.5mL of LB + CAM.
6. Inoculate the media with 500uL of overnight culture.
7. Grow at 37 C, 220 rpm, until the $O.D._{600}$ reaches 0.4-0.6.
8. Induce the EibD culture by adding L-rhamnose (0.001% w/v)
   a. TIP: Also include an uninduced culture as a control
9. Incubate at 30 C, 220 rpm, for 2 hours.
10. Pipette the bacteria into 1-2mL aliquots in eppendorf tubes.
    . TIP: At this point, you may observe autoaggregation, resulting in the bacterial clumps sinking to the bottom of the flask. Be sure to resuspend the culture before and during this step to achieve an equal distribution of bacteria.
11. Centrifuge the aliquots at 5000 rcf for 5 minutes.
12. Remove the supernatant without disturbing the pellet.
13. Resuspend the pellet in 1mL 1x PBS.
14. Repeat steps 11, 12, and 13 two more times.
15. Optional pause point: remove the PBS and flash-freeze the bacterial pellet in liquid nitrogen before transferring to the -80 for storage.
16. Baseline Measurements:
    . Resuspend the pellet in 1mL 1x PBS.
    a. Prepare the spectrophotometer by calibrating with a blank solution of PBS at 600nm.
    b. Transfer 1mL of the bacterial solution to a fresh cuvette and briefly resuspend.





c. Immediately place in the spectrophotometer and record the $O.D._{600}$ at time zero, followed by every 10 seconds for 5 minutes, with additional data points at 6, 7, 10, 20, and 30 minutes.
17. Centrifuge at 5000 rcf for 5 minutes, remove the supernatant and resuspend the bacterial pellet in 600uL 2 mg/mL antibody solution (in PBS).
18. Incubate overnight (12-16 hours) at room temperature with gentle rocking.

DAY 3: *Antibody-Incubated Measurements, Addition of Antigen*
19. Bring up the total volume of the solution to 1mL by adding 400uL 1x PBS.
20. Transfer the antibody-incubated bacteria solution to a fresh cuvette
21. Antibody-Incubated Measurements:
a. Repeat the steps outlined in step 16, for each sample.
22. Centrifuge at 5000 rcf for 5 minutes, remove the supernatant and resuspend the bacterial pellet in 1mL 1x PBS.
23. Repeat step 22 once more.
24. Centrifuge at 5000 rcf for 5 minutes, remove the supernatant, and resuspend in antigen solution:
. The total volume of resuspension as well as concentration is dependent upon the type of antigen being worked with.
a. For antigen of GFP: resuspend in 100uL 0uM GFP, 0.96uM GFP, and 3.85uM GFP
25. Incubate overnight (12-16 hours) at room temperature with gentle rocking.

DAY 4: *Antigen-Incubated Measurements*
26. Bring up the total volume to 1mL using 1X PBS.
27. Transfer the bacterial solution to a fresh cuvette.
28. Antigen-Incubated Measurements:
a. Repeat the steps outlined in step 16, for each sample.