Inducible Repressible System: gadA Promoter Testing Procedure

Purpose:

This is a procedure to test stationary phase and acid induction of the gadA promoter. It should be easily adaptable to other situations where automated measurement (without dilution) is inappropriate as cells must be grown OD_{600}'s outside of the linear range while measuring fluorescence.

Equipment:

- Corning 96 Well Flat Bottom Black Polystyrol Plate [COS96fb.pdfx]
- Aspirator with attached liquid trap
- LB agar plates, LB broth, tubes, appropriate antibiotics, a shaker-incubator, and appropriate laboratory supplies
- Acidic LB preparation:
  Each prep of LB broth is different (it is an undefined media) so for every bottle, a new amount of acid to add must be determined. To prepare 5.5 pH LB broth, we used a pH meter and 5M HCl to carefully bring a 30 mL sample of LB to pH 5.5. Then, as pH meters are difficult to complete sterilize, we disposed of this sample and used the HCl ratio : LB broth we determined to create sterile media (it was usually about 50 µL 5M HCl : 30 mL LB broth).
- Tecan Infinite 200 plate reader set to the following parameters:
  1) Shake samples (linear 4.5mm amplitude) for 60 seconds.
  2) Cycle (x4):
  3) Gather OD_{600}

Mode	Absorbance	
Wavelength	600	nm
Bandwidth	9	nm
Number of Flashes	25	
Settle Time	0	ms

  4) Gather RFU (excitation and emission were **not** set to the exact values for mRFP as our device had too wide of a bandwidth and caused detection of the excitation; more sensitive devices may be set to directly obtain mRFP excitation and emission).

Mode	Fluorescence Top Reading	
Excitation Wavelength	583	nm
Emission Wavelength	614	nm
Excitation Bandwidth	9	nm
Emission Bandwidth	20	nm
Gain	110	Manual
Number of Flashes	25	
Integration Time	20	µs
Lag Time	0	µs
Settle Time	0	ms

  5) Shake samples (linear 4.5mm amplitude for 60 seconds).

6) Return to step 2.

Procedure:

1) gadAp (w/RBS) + mRFP + TT (BBa_K318513) pSB1A3 plasmid was transformed into MG1655 with appropriate controls in the same strain.
2) After streaking and growth, colonies were picked with a sterile pipette tip and grown in small (10mL) LB cultures at 250 RPM and 37 C.
3) After reaching an OD_{600} of at least 0.5, experimental and control samples were used to inoculate small LB cultures (10mL) of appropriate pH to the same OD_{600} (within the range of 0.01 to 0.05). Care was taken to assure that samples to be considered independent from each other came from independent colonies and small cultures. These were also grown at 250 RPM and 37 C.
4) Samples were taken directly from the growths at 0.5 to 3 hour increments and diluted directly onto the plate (into a total volume of 200µL) as direct OD_{600} measurements from the stationary phase (>~1.5) do not exhibit a linear ratio between cell count and OD_{600}. Effort was made to keep final dilution OD_{600} values between 0.1 and 0.9. Three samples were taken per data point to minimize the effect of pipetting and dilution error. After measurement, each sample was removed from the 96 well plate via aspirator.
5) Samples were graphed directly from Tecan output via Excel spreadsheet. OD_{600} was normalized by subtracting a blank (LB with no cell growth) and multiplying by 4 (an experimentally-determined factor we used to correct for the shorter pathlength of the Tecan instrument). RFU/(corrected OD_{600}) was assumed to be proportional to mRFP produced per cell.
6) Samples were collected until growth curve exhibited little growth and RFU/OD_{600} count was steady.
7) Curve features such as relative (to control, earlier points in growth curve, or different pH media) induction of mRFP, OD of maximum induction, etc were determined by a (primary) OD_{600} vs. time, (secondary) RFU/OD_{600} plot.