Title of Experiment

The experiment could be classified into 2 phases

Characterization of pLacI-GFP

1. Preparation
2. Measurement

Personnel

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Materials

Phase I:

IPTG

Lactose

50ml Tubes

LB broth (Amp)

M9 medium with 0.4% glycerol (Amp)

1.5 ml Eppendorf Tubes

Bottle of Distilled water

Phase II:

Absorbance Reader

FLx800™ Fluorescence Microplate Reader

96 well

Protocols/Procedures

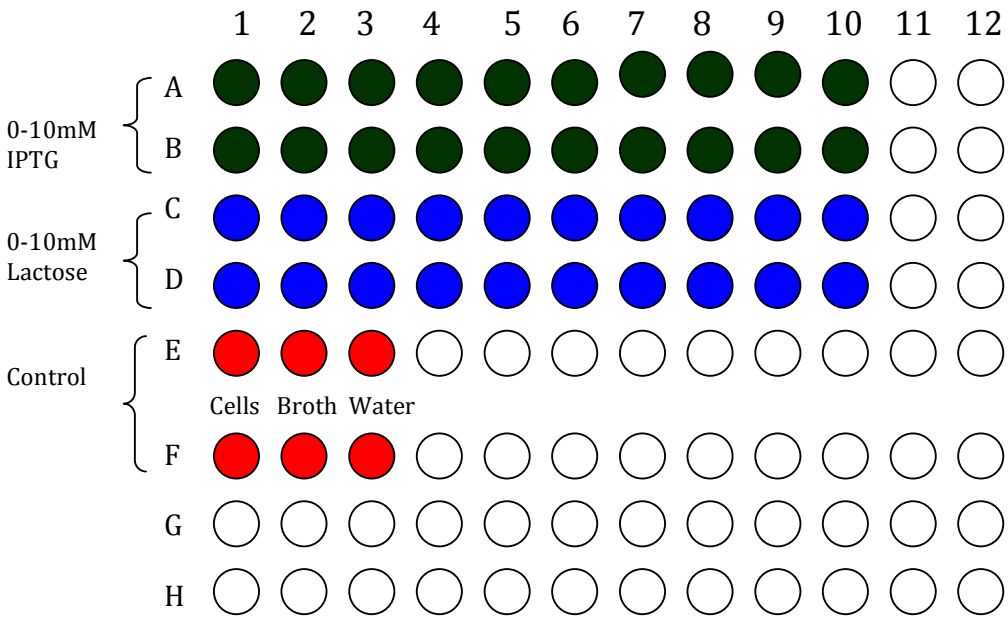
Phase I:

1. Inoculate colonies of interest in 5ml of LB broth with antibiotic resistance (e.g. Amp) for 16 hours in a 37 °C incubator to shake at 235 rpm.
2. From the 100mM stock of Lactose and IPTG prepared and stored in -20°C, the varying concentration of lactose/IPTG were prepared as per table 1 below, from 0 to 10mM range. DO NOT add the cells yet.
3. The labeled 1.5 ml eppendorf tubes were filled with the contents of 1) IPTG/lactose and 2) distilled water as indicated by step 5 for 0 to 10mM range Prepare two samples for each concentration so that an average reading can be measured.

Table 1: 0 to 10mM IPTG/Lactose range

Into Eppendorf tube

	IPTG/Lactose concentration	Amt IPTG/Lactose added (3 samples)	Amt of distilled water	Amt of cells added	Total Amt of contents
1	10mM	25uL (75)	25uL (75)	200 uL	250uL
2	9	22.5 (67.5)	27.5 (82.5)	200	250
3	8	20 (60)	30 (90)	200	250
4	7	17.5 (52.5)	32.5 (97.5)	200	250
5	6	15 (45)	35 (105)	200	250
6	5	12.5 (37.5)	37.5 (112.5)	200	250
7	4	10 (30)	40 (120)	200	250
8	3	7.5 (22.5)	42.5 (127.5)	200	250
9	2	5 (15)	45 (135)	200	250
10	1	2.5 (7.5)	47.5 (142.5)	200	250
11	0 (Control 1) Cells only	0	50 (150)	200	250
12	0 (Control 2) LB Broth only	0	50 (150)	200 of LB broth	250
13	0 (Control 3) Water only	0	250 (750)	0	250 of water
	Total	162.5uL (412.5)		2200uL	



Phase II:

1. Set the program on the PC that is connected to the FLx800™ Fluorescence Microplate Reader. Create a protocol before hand, setting the time interval 600s of measurement for 12 hours, excitation wavelength **485** nm and emission wavelength **525** nm.
2. Zero the Absorbance machine with 1ml of LB broth in a cuvette at wavelength 600 nm, the LB broth must belong to the same batch that was used to grow/inoculate the cells containing LacI-GFP. The cuvette was covered with paraflim tape to prevent contamination
3. Measure the optical density (OD) using a sample of 1ml of cells that was growing to the desired **1.2 OD**, by pippetting it into a cuvette.
4. After overnight growing in phase one, the cells needs to be diluted and re-grew to OD 1.2
5. Diluted cell cultures were prepared in the following mix of 2ml cells: 4ml of fresh LB broth.
6. Measure OD of a 1ml sample immediately. Incubate the remaining 5ml sample at 37 °C with shaking at 235rpm for ~15mins.
7. Measure OD after incubation. Repeat steps 5 & 6 till 1.15 < OD <1.25, with the necessary adjustments. Note down final volume.
8. Centrifuge at 4000rpm for 10 mins at 25 °C. Decant supernatant and re-suspend cell pellet in the appropriate M9 medium (kanamycin / ampicilin resistance). Volume of M9 used should correspond to that noted in Step 7.
9. Incubate at 37 °C with shaking at 235rpm for 10mins.
10. Measure OD of sample and adjust such that 1.15 < OD < 1.25.
11. When the 1.2 (± 0.05) cell density was reached, pipette 200 uL of cells with M9 medium into the wells that was to be used.
12. Induce the GFP production in the fume hood by introducing all the wells with the varying IPTG/lactose and distilled water that was prepared earlier in phase 1.

13. Place the loaded well with the cover into the FLx800™ Fluorescence Microplate Reader.

14. Use the preset protocol and allow the machine to heat up the well to i) **25°C** ii) **37ºC** iii) **42°C**

15. Allow the machine to measure the fluorescence readings for a time period of **12 hrs** at 10 mins intervals.

16. Extract the data and plot a graph of fluorescence versus time for each concentration.

17. Combine the graphs and plot all the different fluorescence versus time plots for all concentration. A 3-D graph would be obtained.

18. To discuss the effect of varying the concentration of the IPTG/lactose on placI-GFP by relating the induction of IPTG/lactose with the lacI promoter activity.