MEASUREMENT

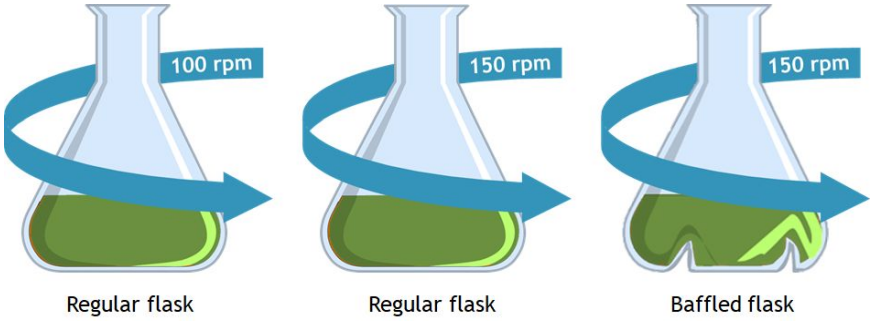
Description : For our characterization experiment, our team has chosen to characterize the part BBA_S03452. The iGEM registered part BBA_S03452, EGFP(Enhanced green fluorescence protein) is a type of fluorescent protein whose fluorescence measurement can be used to quantify protein. Our team used the cell line BL21(DE3) for this project and focused on altering the aeration condition for EGFP (BBA_S03452) expression in three different conditions as described in the method section to investigate the effect of aeration on *E.coli* growth and protein expression.

Method : We divided three Experimental Group for EGFP expression.

1. Group # 1. Regular Flask with 100 RPM
2. Group # 2. Regular Flask with 150 RPM
3. Group # 3. Baffled Flask with 150 RPM

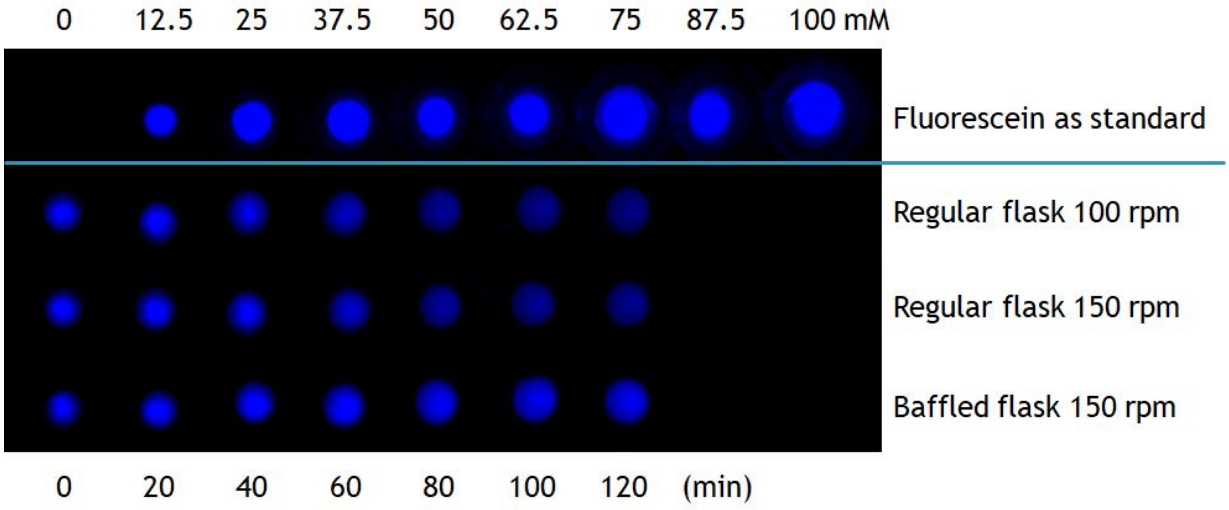
Protocol :

1. Transform the eGFP gene contained plasmid to *E.coli* (BL21(DE3)) and plated on LB plates with antibiotics(carbenicillin). Grow overnight at 37℃.
2. Starter culture preparation: Prepare 100ml of LB with antibiotics and inoculate a single colony. Grow overnight at 37℃ with shaking.
3. Prepare 2 regular flasks and 1 baffled flask with 500ml of LB. Seed 30ml of starter culture at each flask.
4. Grow at 37℃ with using shaking incubator with different rpm. Keep measuring OD_{600}.
5. When OD_{600} reaches 0.4~0.6, add 500$\mu\ell$ of 1M isopropyl-β-thiogalactopyranoside(IPTG) to the cultures to induce expression.
6. Measure OD_{600} and fluorescence intensity of *E.coli* cells every 20 minutes.



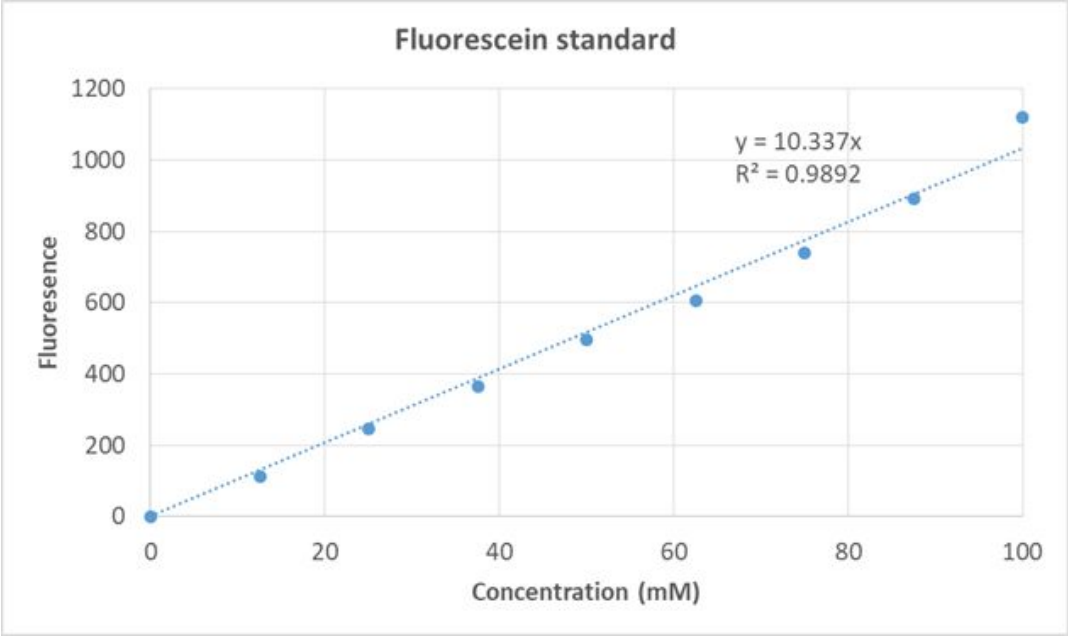
Results :

Fluorescence Measurement of fluorescein standard and *E.coli* expressing EGFP



Making calibration curve using fluorescein standard from iGEM

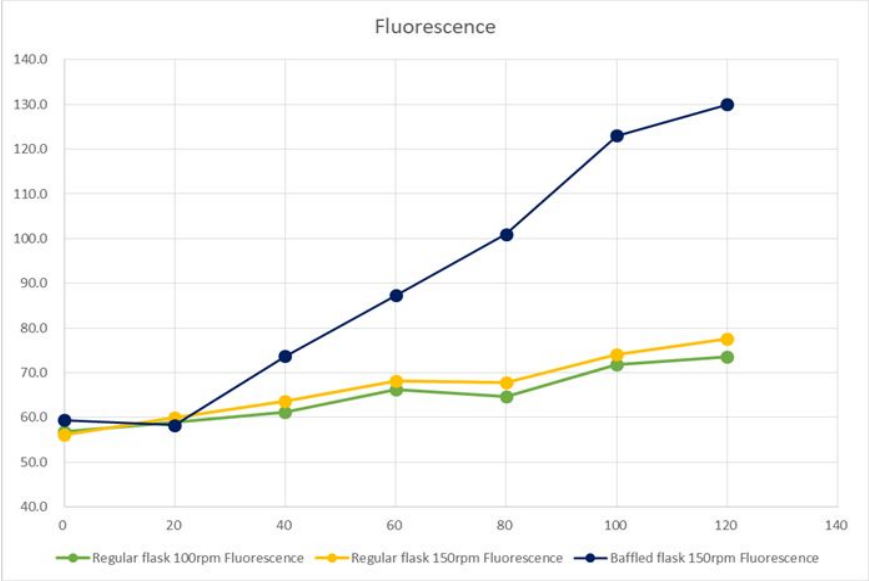
Fluorescein (mM)	Fluorescence
0	0
12.5	111
25	245
37.5	365
50	496
62.5	607
75	740
87.5	892
100	1120

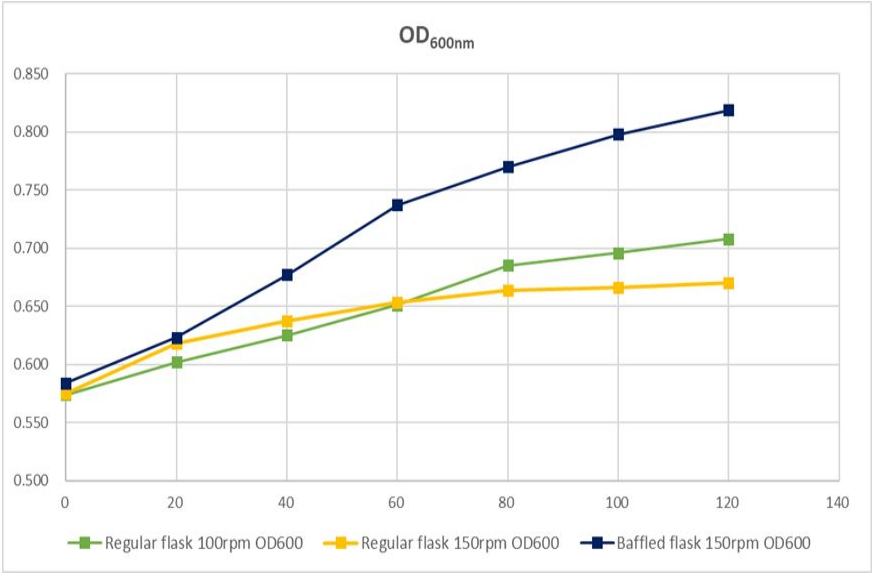


Fluorescein provided by iGEM was used a standard for fluorescence measurement. The near linear response of fluorescence signal with increasing concentrations of fluorescein (0 - 100 mM) shows that our instrument (Odyssey FC) can measure fluorescence signals between 0 and 1000 reliably.

Measurements of OD600 and fluorescence signal of *E.coli* cells expressing EGFP

	Regular flask 100rpm		Regular flask 150rpm		Baffled flask 150rpm	
Time(min)	Fluorescence	OD600	Fluorescence	OD600	Fluorescence	OD600
0	56.8	0.574	56.2	0.575	59.4	0.584
20	59.0	0.602	60.0	0.618	58.3	0.623
40	61.1	0.625	63.6	0.637	73.7	0.677
60	66.2	0.651	68.1	0.653	87.3	0.737
80	64.6	0.685	67.8	0.664	101	0.770
100	71.8	0.696	74.0	0.666	123	0.798
120	73.5	0.708	77.6	0.670	130	0.819





Overall, the results showed that aeration condition affects cell growth and protein expression represented by fluorescence signal. The first group (green) with standard flask and 100 rpm resulted in the lowest fluorescence and cell growth after 120 minutes. The second group (yellow) with regular flask with 150 rpm showed a slight increase of fluorescence compared to the 1st group at every time interval. Lastly, the group with baffled flask and 150 rpm (blue) showed the highest fluorescence for all time.

The significant figures of all measurements (fluorescence and OD600) are 3 digits. Replicate experiments were not performed due to time- and space-limitations of the laboratory.

Analysis: Higher RPM and usage of baffled flask represents higher aeration condition. Thus, we can derive from the result that higher aeration condition is optimal for EGFP expression and cell growth, particularly on the BL21 cell line.

Limitation: Because we did not directly measure the concentration of dissolved oxygen in the flask, it is not conclusive if the increased fluorescence signals and OD600 in the baffled flask are dues to increased aeration. Also, the effect of RPM variations was not very evident from our experiment. To check the effect of RPM difference, we would need more trials with wider RPM ranges (50 ~ 200 rpm, for example). Also, exploring other methodology such as use of a fermentor for more controlled aeration change would be beneficial to discover the exact effect of aeration on the expression of eGFP and growth of *E.coli*.