Task 3

Screening

IPTG Protocol

We utilized pQE60 plasmid backbones pre-equipped with GFP (pQE60-GFP) for our study. GFP expression is controlled by the lac operon operator present in this plasmid. The lacI gene, encoding the repressor protein of the operon, is situated within the lac operon. When the repressor binds to the lactose metabolite allolactose, an allosteric change occurs, preventing the repressor from binding to the lac operator. Consequently, the lac operator becomes accessible for transcription in the presence of lactose, which is converted to allolactose by proteins within the lac operon.

In our plasmid design, the lac operator governs GFP expression. To confirm GFP expression from our plasmids, we utilized IPTG, an analog of allolactose, to de-repress the lac operator and induce GFP expression.

E. coli cells carrying pQE60-GFP plasmids were initially cultured overnight at 37℃ with shaking at 180 rpm in both tryptone broth (TB) and M9 media. Subsequently, cultures in both media were treated with IPTG to a final concentration of 500 µM, inducing His6-IPTG expression. After IPTG addition, a secondary culture was allowed to grow until reaching an OD of 0.4-0.6 for both media.

From the overnight culture, cells harboring pQE60-GFP plasmids were cultured differently, with various concentrations of IPTG. The final concentrations of IPTG used were: 0, 1 µM, 10 µM, 100 µM, and 1 mM for both M9 and TB cultures. To prepare these working concentrations, IPTG stock solutions were made as follows: 0, 100 µM, 1 mM, 10 mM, and 100 mM, respectively. Two replicates of each concentration were prepared.

The initial plate reading yielded no change, with no fluorescence detected. However, subsequent attempts proved successful, resulting in observable fluorescence. The successful result is detailed in the table below.

The cells were then observed under a fluorescence microscope, and the fluorescence intensity was quantified. We observed a fourfold increase in fluorescence in the tryptone broth cells compared to those in TB.

IPTG Results

Well no.	No_IPTG	IPTG	

	1	2	
A	943	4210	TB Media
B	467	876	
C	63	63	M9 Media
D	64	64	

Upon IPTG induction, a significant increase in fluorescence intensity is observed, indicating successful induction of GFP expression. Higher concentrations of IPTG result in higher fluorescence intensity, indicating a dose-dependent response. Moreover, a notable disparity in fluorescence intensity between TB and M9 media suggests that growth media composition strongly influences GFP expression levels, with TB media fostering substantially higher expression compared to M9 media.

Microscopy Observation of GFP