Objectives

The final aim was the construction of the blue light emission devise with the blue light emitting bacterial luciferase from *Vibrio fischeri* coded by luxA and luxB genes.
The structure of the devise was designed as follows:

Because the luciferase coded by luxA and luxB needs a substrate (an aldehyde synthesized by enzymes into the lux operon) and we were not planning the design of a bio-brick containing the genes needed for its synthesis we would add the aldehyde to the reaction in an exogenous way.

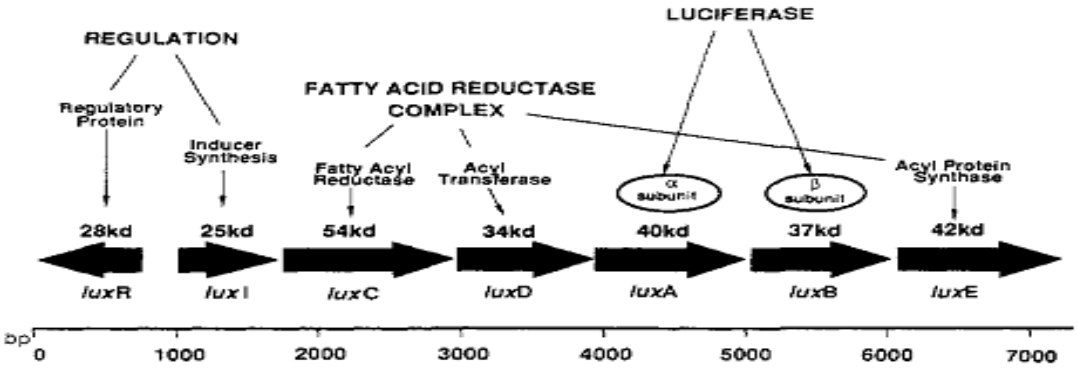
This is the light emission reaction.

$$FMNH_2 + O_2 + RCHO \rightarrow h\nu + FMN + H_2O + RCOOH$$

Methodology

The lux operon

Vibrio fischeri's lux operon is composed of several genes, all of them involved in bioluminescence and its regulation.
This is the structure of *Vibrio fischeri*'s lux operon:



As it is shown in the diagram luxA and luxB genes, those coding for the luciferase, are placed together into the operon so the strategy was amplify them by means of PCR and then ligate PCR product with a strong constitutive promoter; the exogenously aldehyde added to the strain containing luxAB genes would be enough to trigger the luminescence reaction.

In the first place I purified genomic DNA from *Vibrio fischeri* strain MJ11 and then I ran a PCR with the following primers.
The primers designed to extract luxAB region were:

Forward primer

CGG AAT TCG CGG CCG CTT CTAG AGGA A ACA GCT ATG AAG TTT GGA AAT ATT TGT TTT TCG TAT CAA CC

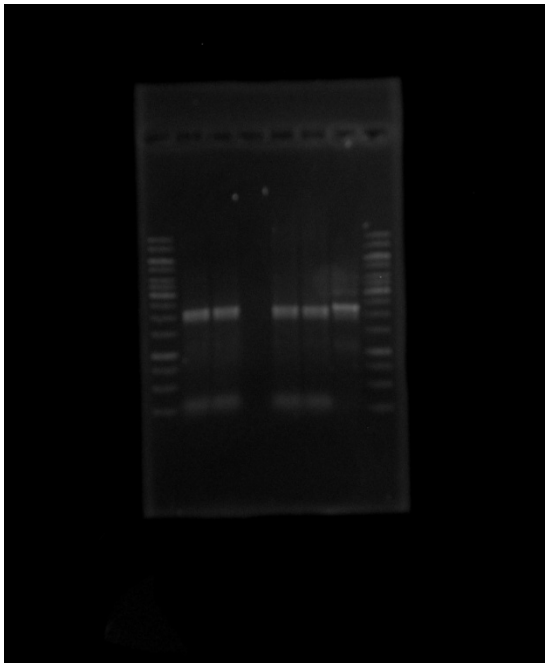
Standard iGEM prefix RBS Coding Region

Reverse primer

CTG CAG CGG CCG CTA CTA GTA TTA TTA GGG TAG ATT CTT TTC AAT TTT TTG GTT CAA C

Standard iGEM suffix Coding Region

In general I did not have too many problems getting luxAB genes from *Vibrio fischeri*'s genome by PCR. (We sent luxAB from *V. fischeri* to Edinburgh as a PCR product)



This gel shows a DNA band of the expected size luxAB genes are supposed to be (~2100bp) as PCR product, template DNA was *V. fischeri*'s genomic DNA using primers shown above.
It is also worth to notice some resulting unspecific amplification products.

Once I got luxAB genes from genomic DNA I purified PCR product with *High Pure PCR Product Purification Kit* by *Roche* and then I tried to ligate them with different plasmids, all of them containing constitutive promoters. The general protocol I followed is this:

- Digestion of the plasmid with SpeI and PstI in order to ligate luxAB genes in front of the promoter carried by the plasmid.
- Digestion of the PCR product with XbaI and PstI.
- Dephosphatation of plasmids to avoid re-annealing and false positives after bacterial transformation.
- Overnight ligation

- Bacterial transformation with the ligation left overnight and culturing on selective media.

The different plasmids I used as vectors are the following ones:

- J61002 carrying constitutive promoter J23101
- J61002 carrying constitutive promoter J23102
- J61002 carrying constitutive promoter J23106

I also used plasmids without constitutive promoters (of course these plasmids were digested with EcoRI and PstI or SpeI) such as:

- pSB1C3
- pSB1T3
- BBa_I51020

Because none of these ligations were successful we thought that it could be due to issues with our plasmids from the iGEM DNA submission so we decided to ligate luxAB genes into a commercial plasmid, I tried the ligation with

- pBluescript II KS (+/-) restricted with EcoRI and PstI

However none of these ligations was successful, whenever they resulted in probable colonies, an analysis revealed they were always false positives, it seemed that some other smaller alternative PCR product was being ligated into these vectors as can be seen in the following gel.

This gel shows the digestion with EcoRI and PstI of a plasmid extracted from a colony resulting from a transformated ligation of luxAB and pBluescript II KS (+/-).
The first lane is the ladder, in the 3rd and 4th lanes we can observe two bands, that of ~3000bp is the linear plasmid whereas the smaller band of ~800bp is an alternative unspecific PCR product (If we had the actual luxAB genes we would expect a band of ~2100bp).

Because we thought there was some smaller unspecific PCR product with bigger chances of ligation than actual luxAB genes, we decided to make a PCR and then a gel band purification; the resulting purification would contain only luxAB genes so it should avoid the ligation of unspecific

products to the vector, nonetheless these ligations never resulted so at the end we were unable to get luxAB genes from *Vibrio fischeri*.
(It is worthy to note that these ligations were carried out several times by several people, some of them with a lot of experience and it never was successful).

Our collaborator team from Cambridge University sent us a plasmid carrying the whole operon lux (except the regulatory genes) this is luxA, luxB, luxC, luxD, luxE, which means that an *E.coli* transformated with this plasmid would glow without the need of any exogenous input because it has the genes coding for the bacterial luciferase as well as the genes coding for the enzymes needed for substrate synthesis.
This operon was under control of PBAD promoter depending on arabinose to be active so the bacteria transformated with this plasmid had to be grown on arabinose medium in order to observe the blue glowing phenotype.

Other Activities

Although the main line I followed was that of luxAB, I also performed other tasks such as:

luxY

luxY is a gene coding for YFP, a protein that shifts the light emission wavelength of *Vibrio fischeri*'s luciferase from 484nm (blue) to 534nm (green - yellow); because our system needed a green or yellow emission module we looked in the literature for a protein capable of provoke this phenotype.
The sequence was taken from the following article:

Cloning and expression of the luxY gene from Vibrio fischeri strain Y-1 in Escherichia coli and complete amino acid sequence of the yellow fluorescent protein.
Thomas O. Baldwin, Mary L. Treat, S. Colette Daubner
Biochemistry 1990 *29* (23), 5509-5515

And then the sequence was synthesized by Mr.gene.
This is the composition of the synthesized sequence, "Gene" stands only for luxY coding region.

Promoter + Prefix + RBS + Gene + Double Terminator + Suffix

Promoter: BBa_J23100
RBS: BBa_B0034
Gene: *Vibrio fischeri* yellow fluorescent protein (luxY)
Double Terminator: BBa_B0015

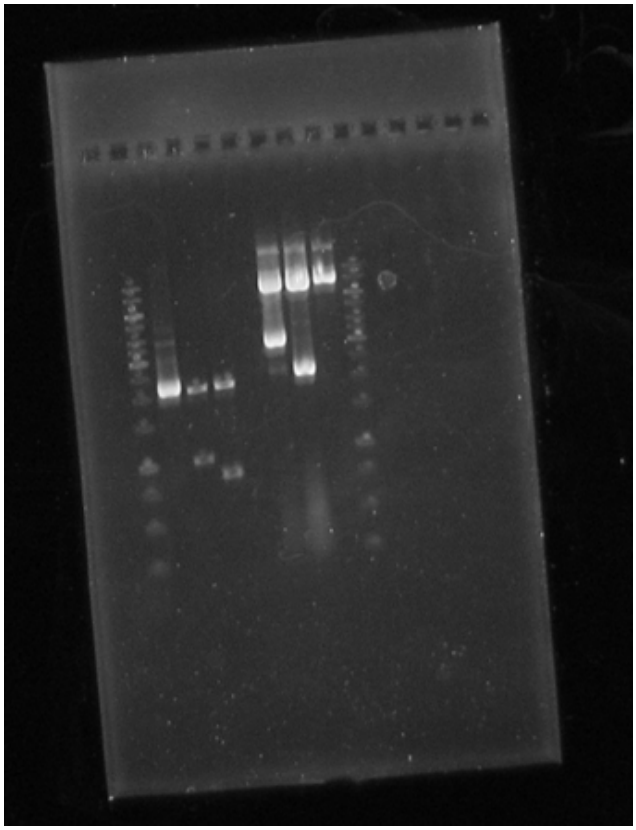
I cotransformated luxY containing operon sent by Mr.gene with the plasmid sent by Cambridge containing luxABCDE genes. The cotransformation was successful as the following gel shows but we have not been able to see the yellow glowing phenotype yet.

Lumazine protein

Lumazine protein of *Photobacterium phosphoreum* (lumP) is a small protein which shifts the wavelength of light emitted by the *P.phosphoreum* luciferase LuxAB from 495 nm to about 475 nm, we needed it to tune the blue light emission wavelength towards another one closer to that of LovTap system reception.

Lumazine protein was sent to us by Edinburgh Team.

It was also contransformated with plasmid carrying LuxABCDE, although cotransformation was successful we have not been able to measure the desired wavelength shifting.



In the sixth and seventh rows we can see a plasmid extraction from a cotransformation resulting colonies, we can see they contain two plasmids, the bigger one is luxABCDE and the smaller one is in the sixth row luxY and in the seventh row lumP.

The eighth row shows luxABCDE as a control.

The first and last rows shows DNA ladder.

DNA extraction from iGEM Kit Plate

I also extracted several bio-bricks from DNA submission and I tranformated them, nonetheless some of them did not work maybe due to DNA bad quality or low quantity, as reported in parts registry.

The extracted bio-bricks were the following ones:

- BBa_B0015: Double terminator – Successful transformation.
- BBa_K098010: HO-PcyA – Unsuccessful transformation.
- BBa_M30109: Pbad regulated phycobilins (ho1 and pcyA) followed by Ptet regulated light sensor (Cph8) – Unsuccessful transformation.

Some Help

I also helped Claudia doing some stuff of his work with LovTap System (transformations, ligations, digestions, plasmid extractions, etc.)

Results

- I did not success in getting Vibrio fischeri's luxAB as a biobrick.
- Strains carrying Cambridge luxAB showed luminescence.
- Cotranformations of luxABCDE from Cambridge with lumP and luxY were both successful but we have not been able to see the desired phenotypes, however we expect to see some results during the week.
- BBa_B0015 was used by Mariana as part of her work with firefly luciferase.
- Claudia has been able to do some experiments with LovTap system (see Claudia's results)