

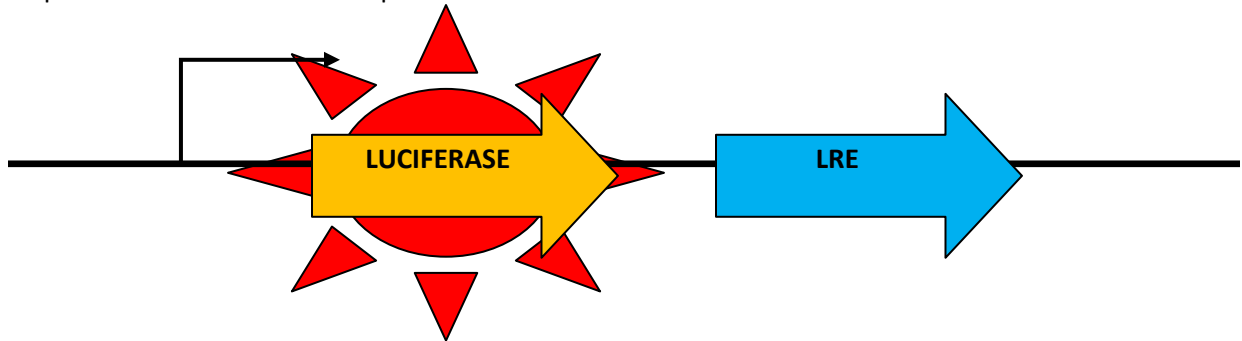
## UNAM\_genomics Mexico Notebook: Mariana

### Goals

\*During my participation in the project, I was focused on constructing the luciferase and Luciferin Regenerating Enzyme (LRE) of the red emission module.

### Objectives

- \*To obtain a functional red luciferase.
- \*To assemble the synthesized LRE.
- \*To prove that LRE worked as expected.



### Methodology and Results

#### Mutated Firefly Luciferase

The original intention was to use the *Photinus pyralis* luciferase biobrick (BBa\_I712019) as a base and to increase its efficiency by using the primer BBa\_K360114 (as registered) due to previous design notes as referred in the part design section of this biobrick. After achieving this, a punctual mutation S284T (also known as S851T) was induced to change its emission spectrum from green to red by means of a PCR with special primers (which are also referred in the biobrick part design). The biobrick name is BBa\_K360115; unfortunately, we were never able to find out why it was not functional.

#### Click Beetle Luciferase

By the middle of the summer, we decided to try the luciferase from another organism: Click Beetle, specifically the Promega Chroma-Luc™ Reporter Vectors pCBG99-Basic Vector Restriction Sites and the pCBR-Basic Vector Restriction Sites, where the first one has a green emission and the second one red. The intention was to work with these vectors as a means of having a previously-

known-to-be functional luciferase, so that once the LRE biobrick was ready, characterization could be feasible. The first step was to extract the coding sequence of the luciferases from these vectors by means of PCR and also to standardize them by adding the prefix and suffix with the following primers:

>Forward Click Beetle luciferase

GAATTCGCGCCGCTTCTAGAGATTAAGAGGAGAAAATGGTGAAGCGTGAGAAAAAT

>Reverse Click Beetle luciferase

CTGCAGCGGCCGCTACTAGTATTATTAACCGCCGGCCTTCT

The second step was to ligate it into the BBa\_J61002 plasmid with a J23101 promoter, and to transform it, having as an output several colonies which were then grown overnight to check whether the luciferases showed any brightness. The protocol followed for the assay is mentioned in BBa\_K216015 biobrick, in the Experience part. In this first assay, no luminometer was used; so eventhough we added 1µL of 100mM luciferin and waited in the dark room for ~30min taking pictures with a SLR camera with high ISO (~400), for about 30secs to 1 min exposure, nothing was observed :'.

After the initial disappointment, I talked to Cambridge team and they help us ever since then by sending us some of their constructions (BBa\_K325909, BBa\_K325109, BBa\_K325209, and 2 other biobricks).

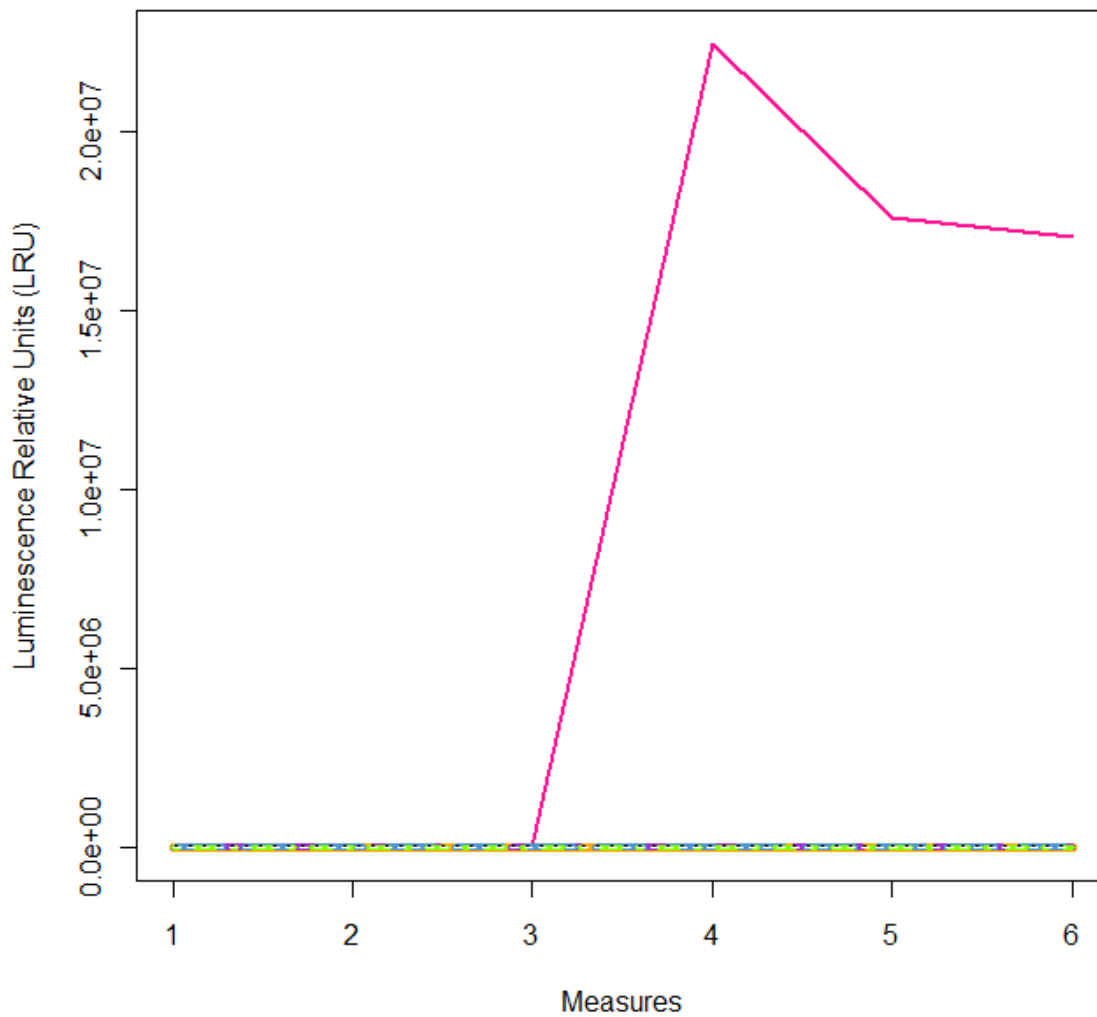
### Luciferase assay

After having worked with luciferases from both firefly and click beetle, another measurement was performed, but this time I was guided by the kind help of Chris Wood Phd., and the willingness of Susana López, Phd. to lend us her luminometer. The measurements were taken as follows (with and without luciferase, triplicate for each one):

|       | control | medium | bacteria | CBG      | CBR | PPlucwt | PPmluc | PPmlucJ23102 |
|-------|---------|--------|----------|----------|-----|---------|--------|--------------|
| 1     | 85      | 151    | 317      | 2181     | 76  | 143     | 231    | 241          |
| 2     | 60      | 116    | 125      | 4144     | 72  | 91      | 102    | 107          |
| 3     | 72      | 97     | 81       | 7748     | 58  | 76      | 92     | 79           |
| Luci1 | 87      | 131    | 111      | 22443367 | 79  | 85      | 128    | 109          |
| Luci2 | 84      | 109    | 93       | 17592397 | 74  | 69      | 91     | 77           |
| Luci3 | 68      | 89     | 94       | 17037342 | 70  | 74      | 78     | 82           |

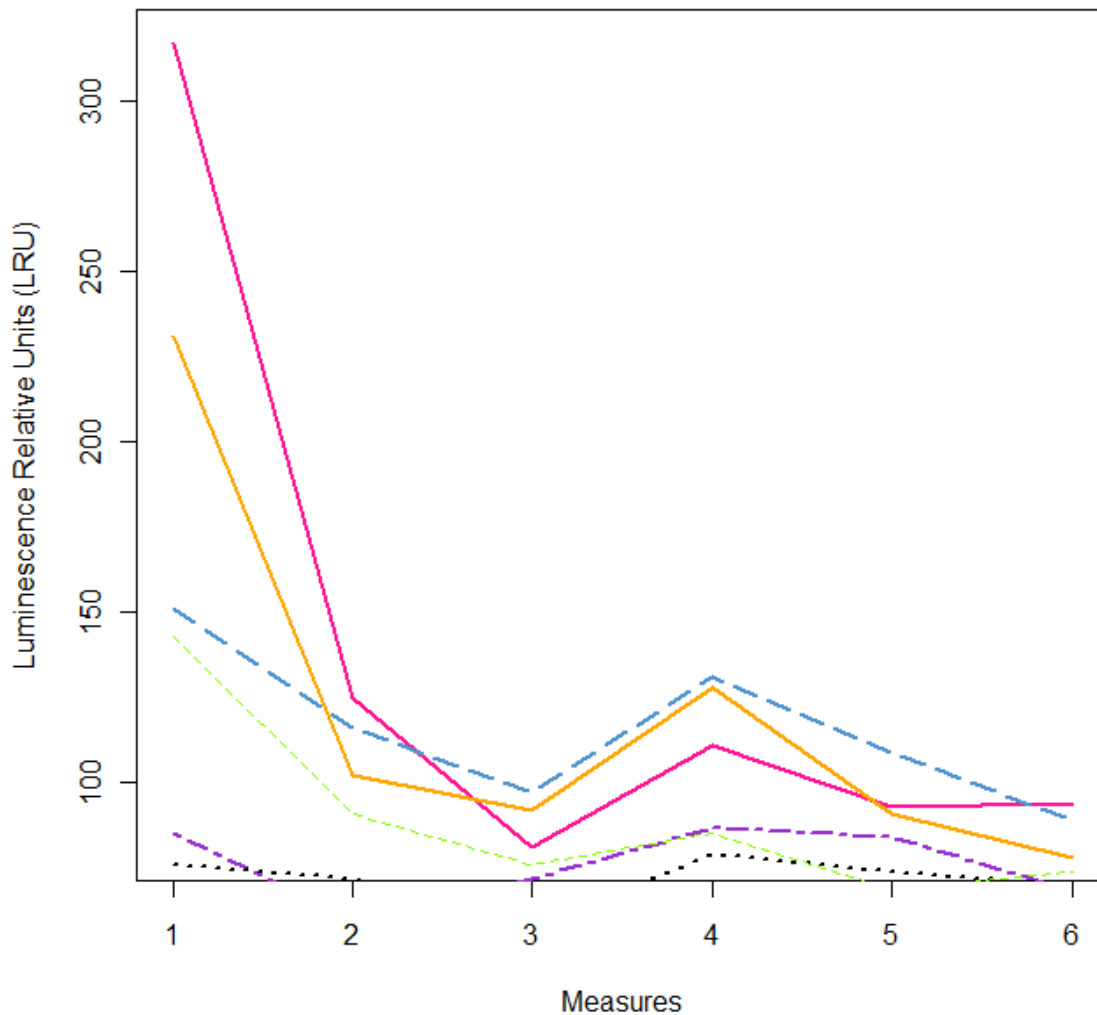
Control = Citratum sodium medium 1mM, Medium = LB, Bacteria = DH5- $\alpha$  without transformation, CBG = Click beetle green, CBR = Click beetle red, PPlucwt = Firefly luciferase (BBa\_I712019), PPmluc = red mutated luciferase, PPmlucJ23102 = red mutated luciferase under J23102 promoter in BBa\_J261002.

### Luciferase functional assay



The image shows the data, where a clear burst of emission can be observed in the CBG (pink) luciferase. As the other data can't be distinguished in this graph, the next one shows the dynamic for every sample with the exception of CBG.

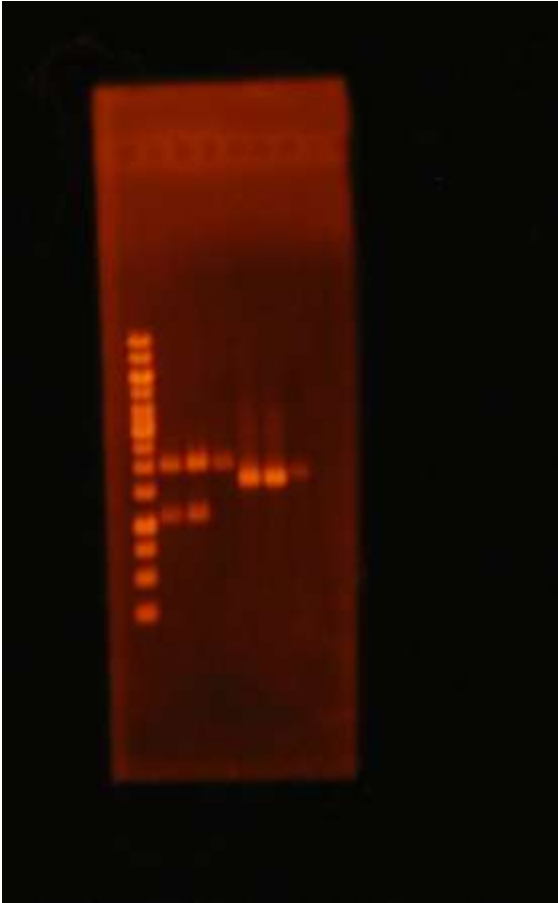
## Luciferase functional assay



As a result, a functional luciferase was obtained, and even though its emission was not among the red spectrum, it was useful for the LRE characterization.

### LRE

Luciferin Regenerating Enzyme (LRE) was first reported by Gomi, K. and Kajiyama, N. [(2001) Oxyluciferin, a Luminescence Product of Firefly Luciferase, Is Enzymatically Regenerated into Luciferin. *The Journal of Biological Chemistry*, Vol. 276, No. 39. This enzyme proved to recycle the luciferase product oxyluciferin. We decided to synthesize LRE with Mr. Gene and then to ligate it



into the DNA submission plasmid pSB1C3 (BBa\_K360113), and also into a constitutive promoter (J23100). The gel shows both, LRE and luciferases in the next order:

1. Ladder.
- 2,3,4. LRE + pSB1C3 restriction.
5. Green CB luciferase from purified PCR product.
6. Red CB luciferase from purified PCR product.
7. Mutated firefly luciferase from purified PCR product.

As the ligation with the BBa\_J61002 went well, the next step was to insert the LRE with the constitutive promoter J23100 into the pSB1C3. The resulting biobrick was the part BBa\_K360111.

### **Vector Containing both Click Beetle Luciferase and LRE**

Our goal here was to couple both, luciferase and LRE, to be able to make the construction autonomous after the first and single input of luciferin. We tried to techniques, and just one of the resulting colonies was used to assess the functionality of LRE.