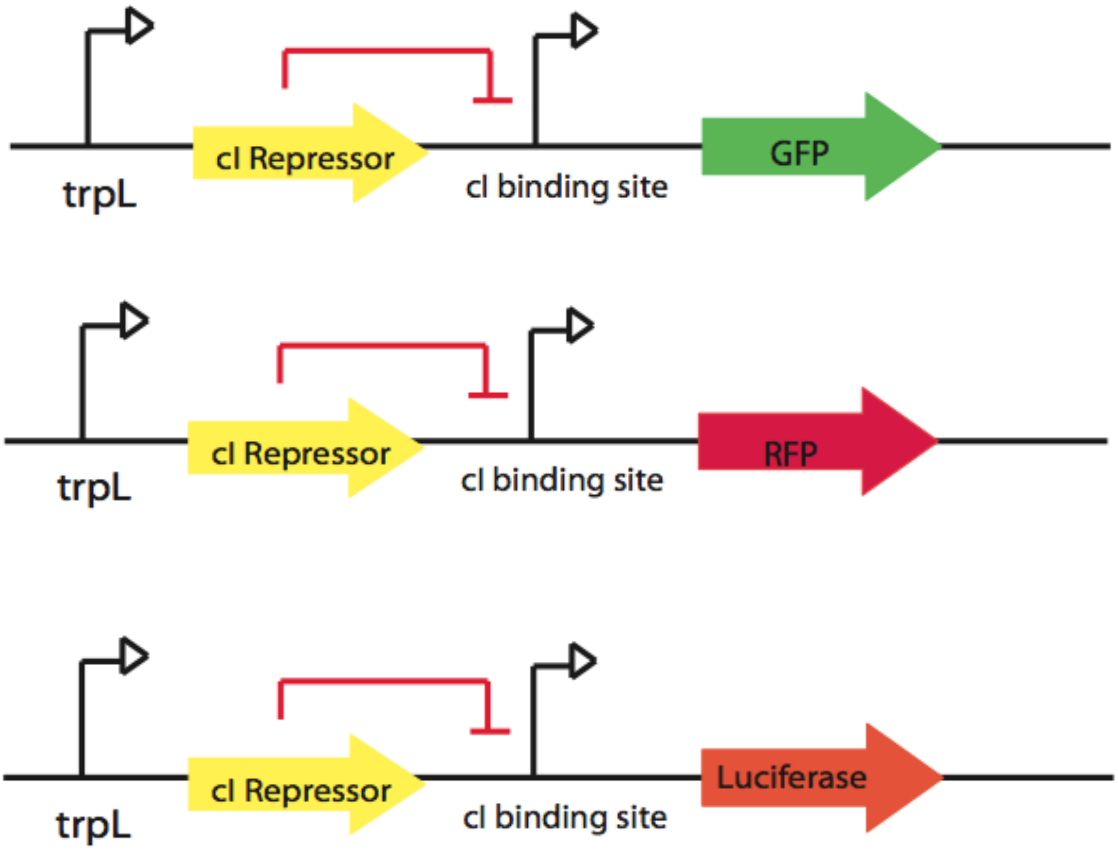
Objectives.

The final aim was the construction of the red light emission control devise both with the red light emitting luciferase, and GFP and RFP as reporters.
The structure of the devise is designed as follows:



Methodology.

The trpL promoter.

The LovTAP system is composed of two modules, the light-sensitive input module is the LOV2 domain of Avena sativa phototropin 1 (AsLOV2). LOV domains absorb light through a flavin cofactor, photochemically form a covalent bond between the chromophore and a cysteine residue in the protein, and proceed to mediate activation of an attached kinase domain.

The output module is the bacterial transcription factor trp repressor (TrpR). TrpR can bind its operator DNA as a homodimer thus repressing transcription.

The DNA operator region where trp repressor binds was annotated in the 2009 EPF-Lausanne's wiki as trpO promoter, but when searching for it in RegulonDB a

very well annotated *Escherichia coli* transcriptional regulation data base, I found that the actually the region where trp repressor binds is trpL promoter not trpO.

Knowing that we synthesized only trpLp's functional elements as an oligonucleotide to introduce by PCR into a selected plasmid as follows:

gaattcgcggccgcttctagag gctgttgacaattaatcatcgaactagttaactagtacgcaag cggaattccg

Preffix Promoter(functional elements only) EcoRI

The primer is the reverse complementary of that sequence so I can use it as a reverse primer, which I used along with a suffix forward to introduce the trpL promoter into pSB3K3 plasmid by PCR suffix.

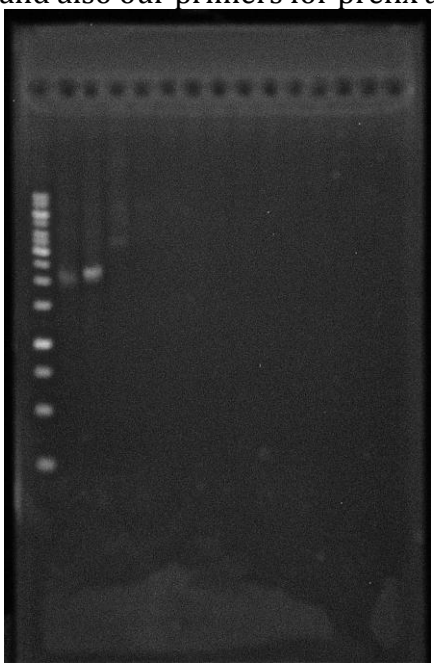
I screw it up twice when doing this, the fist one was placing an EcoRI site instead of an SpeI to use it to ligate to an XbaI site of the cI inverter, but then when I re synthesize it with the SpeI and had successfully ligated it to the cI inverter and GFP, I realized that the promoter itself had two SpeI sites, ergo the ligation could not be used ☹.
In order to overcome this problem Claudia designed a primer to change the SpeI site to an NheI site, which is also compatible with XbaI, the PCR was done on the pSB3C1 plasmid and came out well ready for digestion with NheI/PstI.

The RBS-GFP

I performed a hot start PCR to the Green Fluorescent Protein with LVA (BBa_K145015) to add an RBS (BBa_B0034) and 7 bases before the initial ATG, for this I used Rtth polymerase (hot start).

luxAB and luxCDE from Edimburgh Team.

We received luxAB and luxCDE from the Edinburgh team, so I performed a hot start PCR with Rtth polymerase to extract them, I used both the primers they sent and also our primers for prefix and suffix.



lanes 2 and 3: luxAB
lanes 4 and 5: luxCDE

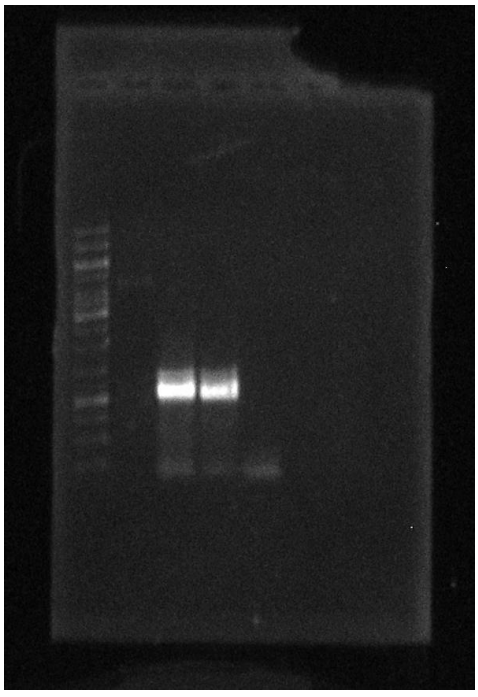
I was never able to amplify luxCDE properly, luxAB was very doubtful and had some unexpected bands (not seen in the picture), so I gave the DNA to our Wet Lab advisor Dr. Miguel Ramirez to try it out, he obtained the only negative results and concluded that there was something wrong with the DNA, therefore we stopped working on that.

The Inverter

I searched in the Registry of Standard Biological Parts for an appropriate inverter which is a repressor followed by its binding site. We chose an inverter because it is going to be constitutively repressing anything downstream of it, but when it gets transcriptionally repressed (by trp repressor), whatever is downstream of it is going to be activated.

I chose the cI inverter from Lambda phage (BBa_Q04510) and proceeded to extract it from the 2009 kit plate 1, I transformed it by heat shock, extracted plasmid and digested it with EcoRI and PstI.

When checking the digestions on an agarose gel, the 1kb band expected from the cI inverter was absent or very thin, so I performed a PCR using the prefix and suffix as primers.
The PCR came out very well with the expected size. As the double digestions were good enough to ligate, we hypothesize that not all plasmids had the inverter that is why it looks very weak on the digestions, but comes out very well in the PCR.



1º lane is the DNA ladder.
2º lane is the double restriction.
3º lane is the cI inverter's PCR.
4º lane is the positive control.
5º lane is the negative control.

As it is clear in the image, the double restriction doesn't shot the 1 kb band it should, only it shows the plasmid, the PCR came out very well.

I did more PCRs for the cI inverter using Rtth polymerase (hot start), purified them and digested with EcoRI and PstI in order to ligate them to GFP.

In order to introduce the GFP with the new RBS I digested the GPF with SpeI/PstI and plasmid pSB1T3 with XbaI/PstI then I ligate them.

I tried several times to ligate the cI inverter to the GFP-PCR with new RBS in the plasmid pSB1T3 with no success.

As non of the attempts to ligate the 2009 cI inverter PCR were not successful and the digestions were doubtful, I decided to extract the cI inverter from the 2010 kit plate.

After the transformation, plasmid extraction and double digestion with EcoRI/PstI I noticed there was something wrong with the plasmid because the in the gel there were always more that the two bands expected, so I extracted the inverter by PCR, digested it with EcoRI/PstI and cloned it into the commercial vector pBluescript II KS + hoping it would work for the ligations.

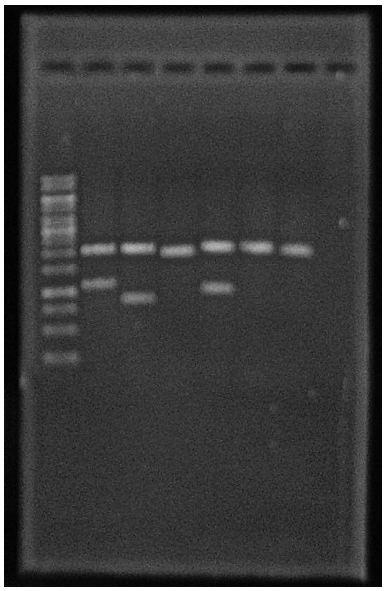
It was successfully cloned in the new vector so I proceeded to ligate it with the GFP (BBa_E0240) into the pSB1T3, then I extracted plasmid and double digested it with EcoRI/PstI, in the gel I can see the two bands expected one for the ligation product about 1900 bp and the other for about 2500 bp corresponding to the plasmid, ergo it seems that it came out well. The only problem is that it doesn't seem to glow when exposed to UV light, but it should as the cI repressor protein is not being transcribed.
Claudia also ligated the cI inverter to the GFP (BBa_E0240) and her ligation did glow in the UV light so I decided to use her ligation instead of mine.

I digested the trpLp into pSB1C3 with NheI/ PstI and ligated to both GFP (BBa_E0240) and RFP.

Results.

TrpLp + RFP and GFP

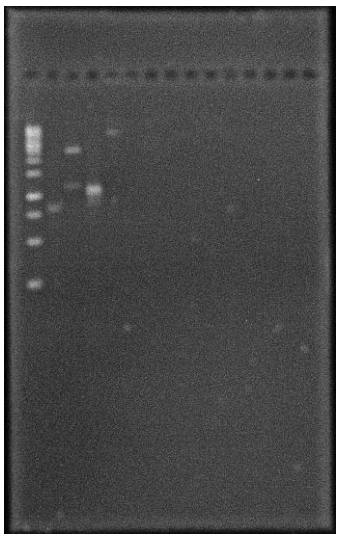
The ligations of trpLp into pSB1C3 to GFP (BBa_E0240) and RFP as seen the double digestions with EcoRI/PstI.



lane 3: trpLp into pSB1C3 + RFP
lane 5: trpLp into pSB1C3 + GFP

GFP + RBS and 7 bases before start codon.

Green Fluorescent Protein with LVA (BBa_K145015) to add an RBS (BBa_B0034) and 7 bases (aacagct) before the start codon.



second lane: GFP with RBS