EE 425 Lab Report #2

Alexander Hotpants
Stuart Fluffington

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Introduction

Perhaps the most common circuit in synthetic biology is the fluorescent reporter. Fluorescent molecules, which glow when excited by specific wavelengths of light, can be used to monitor cellular conditions and, when observed over a course of time, the dynamics of a system of interest may be characterized. These are typically either constitutively expressed and repressed by a detector or normally expressed only at a basal rate which is elevated by a detector which activates transcription. Figure 1 shows a well known example of a synthetic system whose state is reported by the repression of *gfp*, which codes for a green fluorescent protein [1]. As aspiring synthetic biologists, our present task is to construct a simple fluorescent reporter.

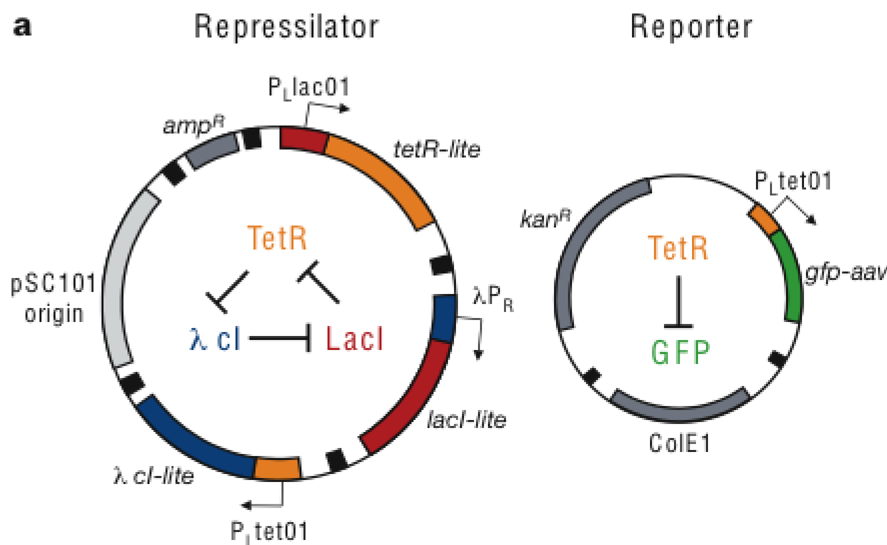

Figure 1: Plasmids implementing a simple negative-feedback loop which generates oscillations. The status of the system is reported by the *gfp* gene, whose expression is repressed by TetR and which fluoresces green.

Methods and Results

As usual, we will dispense with the recapitulation of the protocol presented in the lab handout and merely note that we, to the best of our knowledge, did not deviate from the specified procedure. For the purposes of exposition, we note significant steps and report our results.

Our goal is to implement an IPTG-inducible reporter which fluoresces yellow. In short, we will accomplish this by constructing a *de novo* synthetic circuit consisting of *pLac* (Figure 2; BioBricks part BBa R0011), a transcription factor which inhibits the transcription of a gene by attracting the LacI, which inhibits RNAP binding. The LacI can

be removed by lactose or IPTG, thus activating transcription. Its target is *eyfp* (Figure 3; BioBricks part BBa E0430), a gene that produces a molecule that fluoresces yellow. These parts arrive as BioBrick plasmids which must be extracted from bacteria, cut, and ligated into a plasmid to be inserted into competent bacteria.

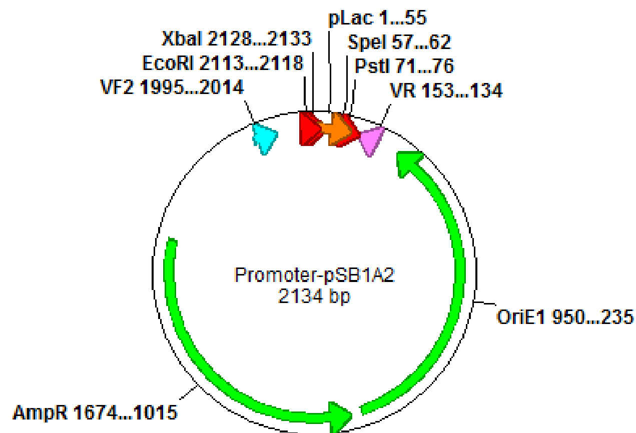

Figure 2: Map of the BioBricks plasmid containing pLac, as constructed using ApE.

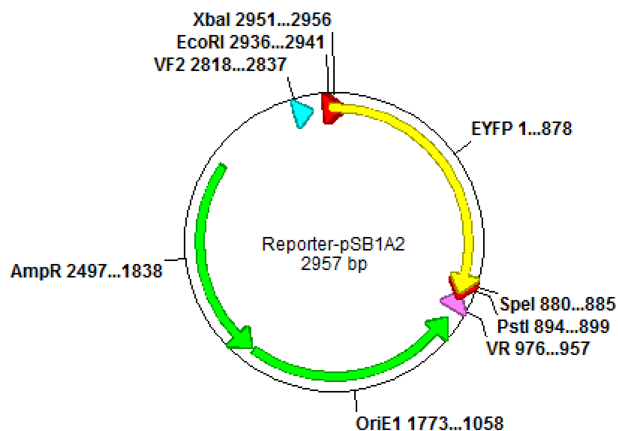

Figure 3: Map of the BioBricks plasmid containing EYFP, as constructed using ApE.

Miniprep

The procedure by which DNA is extracted from bacteria is known as miniprep. In short, the bacteria are lysed then all but plasmid DNA is washed away. We had excellent miniprep yield for both parts: we obtained concentrations of 75.4 ng/μL and 238.2 ng/μL in 50 μL solutions for *pLac* and *eyfp*, respectively. By mass, our minipreps yielded approximately **3.770 μg** *pLac* and **11.910 μg** *eyfp* plasmid DNA.

PCR

Often, BioBrick parts are packaged into plasmids containing different antibacterial resistance genes. This facilitates the use of one as a plasmid template and the other as an insert. The one whose part is extracted and plasmid discarded can be culled from the population by application of the antibiotic to which the other has resistance. Unfortunately, the BioBrick containing the fluorescent reporter arrived in a plasmid with the same antibiotic resistance gene as that of the promoter. To reduce the concentration of the insert's plasmid as much as possible, we amplified the part and surrounding restriction sites using PCR. As with the miniprep, we had very good PCR yield, producing a concentration of 117.1 ng/μL in a 50 μL solution, or **5.855 μg** DNA.

Conclusion

This week, we have completed approximately half the work to construct a simple reporter circuit. In short, we have obtained the parts as BioBrick bacteria containing plasmids and extracted and processed the genetic material. Upon concluding this work, we have the genetic parts of novel circuits which must only be assembled and inserted into bacteria.

In the next lab, we will ligate amplified and digested DNA to form novel IPTG-reporter plasmids. We have already digested the promoter plasmid at the SpeI and PstI restriction sites, as well as the reporter plasmid at the XbaI and PstI restriction sites. In lab #3, we will perform two ligations on these strands of DNA. The first will ligate the SpeI restriction site to the XbaI restriction site and the second will ligate the two PstI restriction sites together. These two ligations will complete the IPTG-reporter plasmid whose structure is depicted in Figure 4. When inserted into LacI-producing bacteria, we should have a colony of IPTG-inducible fluorescent cells.

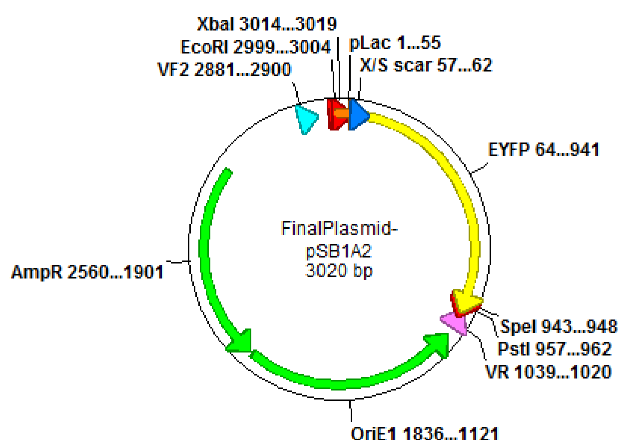

Figure 4: Map of the complete reporter plasmid containing pLac and EYFP.

Bibliography

[1] Elowitz, M. B. and Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335-8, Jan 2000.