LabPats Modelling Report

By NUS-GEM

Overview

NUS-GEM has completed modelling of Plasmid 1, and Plasmid 2 from the LabPats iGEM. Below you can find the related methodology, results, and discussion. Results indicate the expression levels in increasing order from RBS33, RBS31, RBS30, RBS29, RBS34, RBS35. The modelling results should serve to guide your experiment: you can use the predictions ascertained from modelling results to guide your choice of RBS to obtain the correct expression level of sfGFP in the experiment phase which in turn saves you time and resources.

Methodology

Given the genetic circuits provided by LabPats team, we modelled the response functions of Plasmid1 and Plasmid 2. After generating the functional response models for both models, we ran a combinatorial analysis that tested the response of sfGFP expression across a range of RBS from RBS29-35, and performed sensitivity analysis. A combinatorial analysis requires changing one part and measuring the changing response of the model. In contrast, a sensitivity analysis measures the impact each part contributes to the response of the mode. The RBS in Plasmid 2 that was changed in the combinatorial analysis was the RBS downstream of J23117, as requested. Plasmid 1 and Plasmid 2 models were both simulated for 3600s. This timeframe is suitable as the models capture the transient and steady state responses of sfGFP.

Due to a lack of data regarding the promoter strength and molecular interaction kinetics of PLsr (Plasmid 1), and the molecule interaction kinetics between PLasR and LasR molecules (Plasmid 2), we have assigned approximations for these two values. Plasmid 1 model assumes PLsR to have a promoter strength of 1 RPU (relative promoter unit), and sets the initial conditions of LsrK and LsrR to be 0 a.u. The Plasmid 2 model assume all LasR molecules induce the PLasR promoter with no degradation.

In addition, the RBS kinetic values used were determined experimentally from characterisation experiments conducted by AdvanceSyn using the PBbE8K vector with E. Coli MG1655 host. We can expect discrepancies in the strength of RBS across different experimental conditions such as when testing in different strains and mediums.

Table 1 RBS strengths determined from AdvanceSyn experiments

RBS	Relative RBS Strength
RBS29	0.00001
RBS30	0.000009184
RBS31	0.0000042
RBS32	0.0001
RBS33	3e-8
RBS34	0.00045
RBS35	0.00051

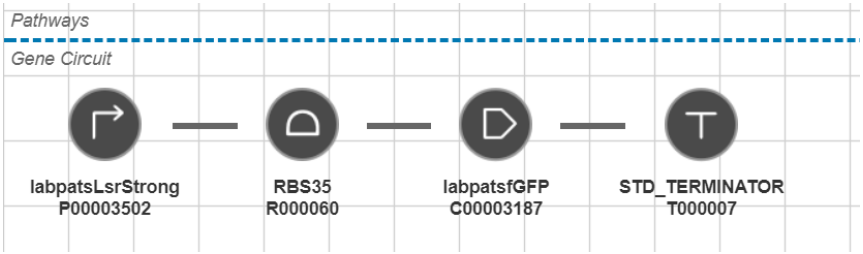


Figure 1 (Above) LabPats Plasmid 1 modelled genetic circuit with RBS35

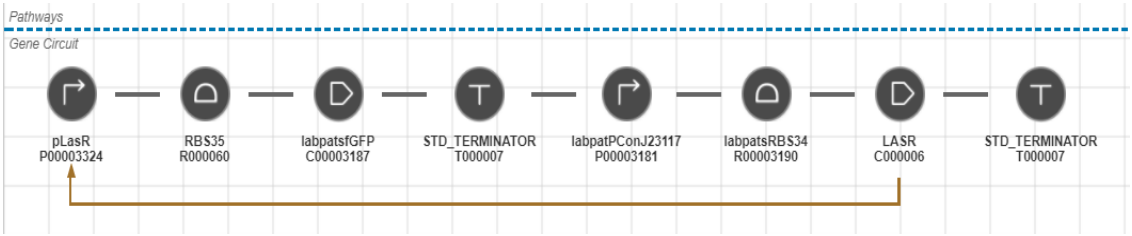
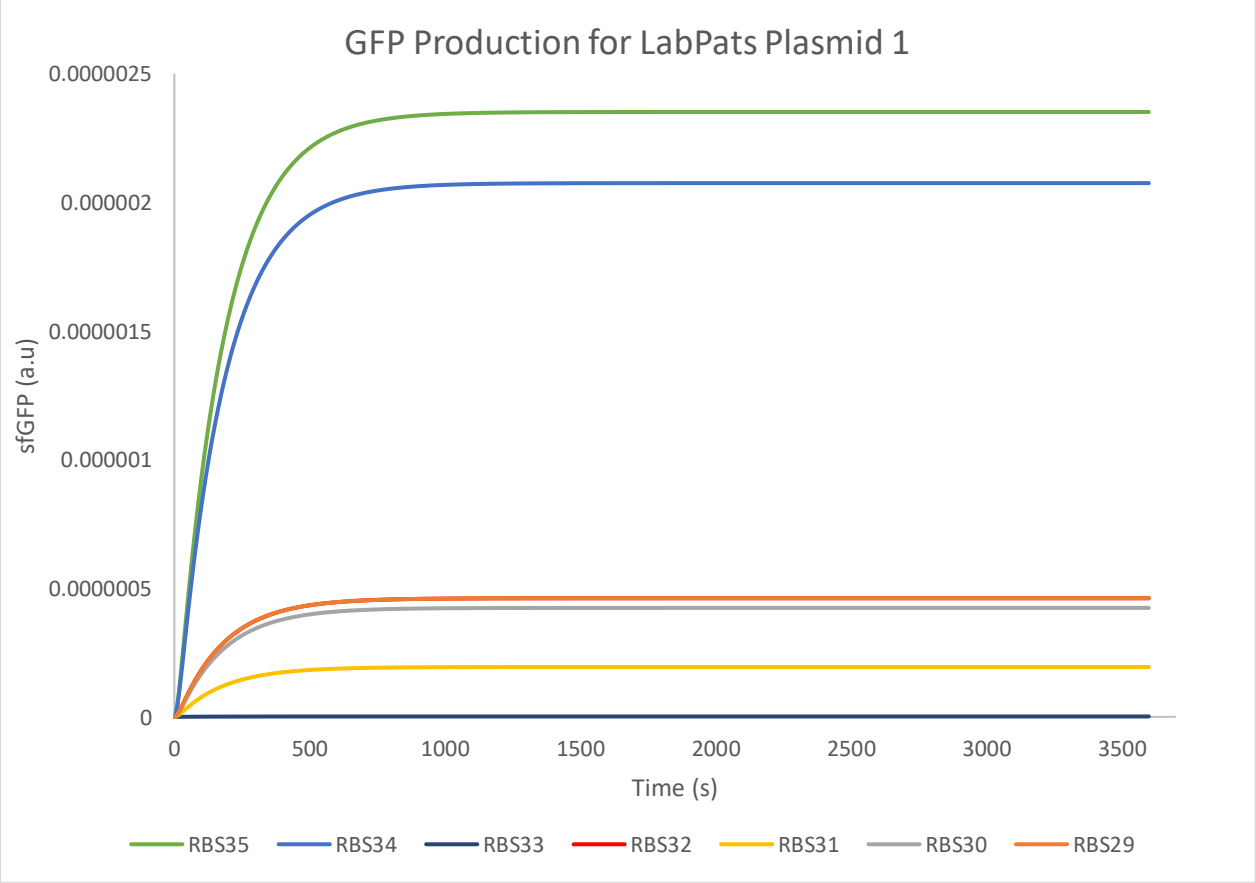


Figure 2 (Above) LabPats Plasmid 2 genetic circuit modelled using RBS 34

Results

LabPats Plasmid #1

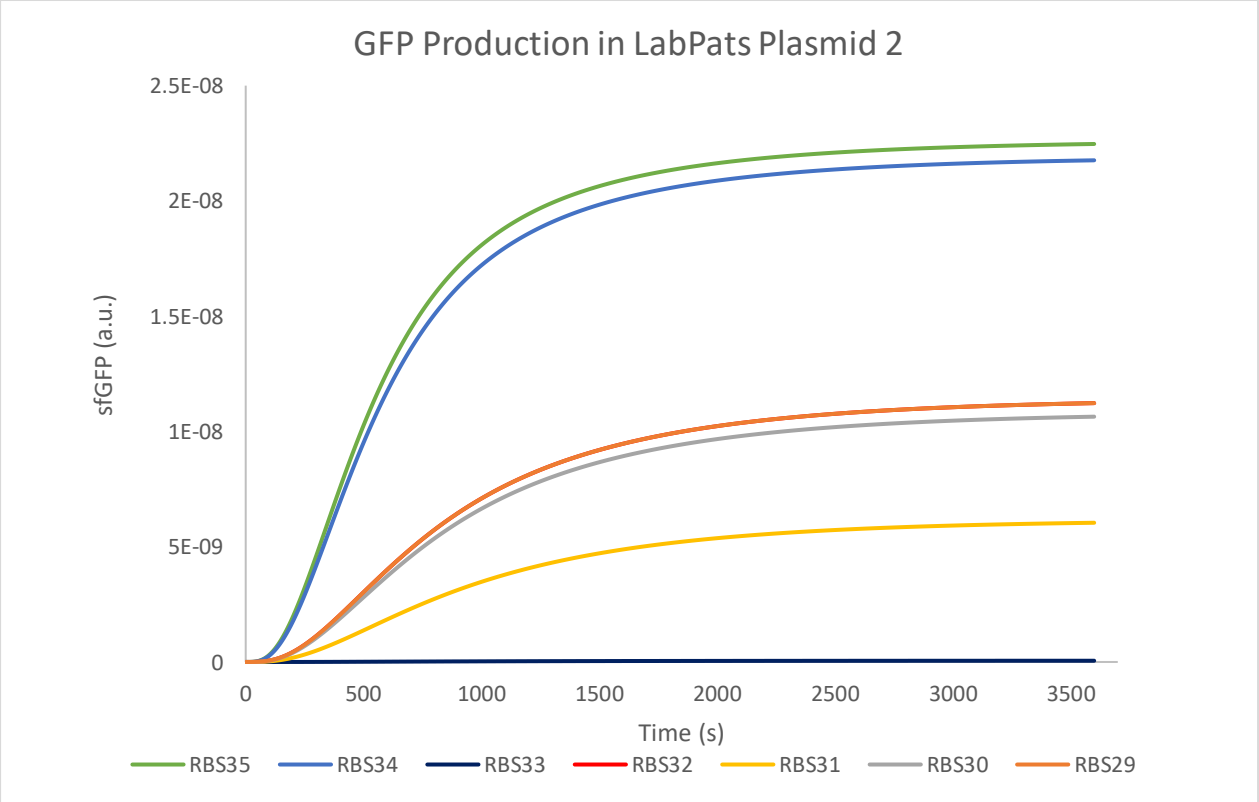


Graph 1: sfGFP expression is greatest when using RBS 34,35. Medium sfGFP expression is achieved when using RBS 29,30. Low sfGFP expression is achieved with RBS 31. Very low sfGFP expression is achieved with RBS33.

Most Sensitive Variables (Descending Order)
PLsr Vmax
PLsr Hill Coefficient
Initial condition of LsrR molecule

Table 2 The maximum velocity rate of PLsr and the Hill Coefficient (measure of binding between molecule and binding site) are the most sensitive parameters of LabPats Plasmid 1. Therefore, changing the type of promoter or the kinetics of the promoter, will have the greatest effect on sfGFP response. To a lesser extent, increasing the amount of LsrR repressor molecule will also impact the response.

LabPats Plasmid #2



Graph 2 The expression patterns from using different RBS is identical to the results obtained in LabPats Plasmid 1.

Most Sensitive Variables (Descending Order)
PLasR Vmax
J23117 Vmax
Relative RBS strength
PLasR Km

Table 3 Changing the promoter or kinetics of PLasR and J23117 will have the greatest effect on the response of sfGFP. Fine tuning of the system can be achieved by modifying the RBS used.

Discussion

Results indicate that the greater the RBS strength, the greater the expression level of sfGFP. Therefore, from RBS29-35, RBS 35 and RBS34 offer the greatest levels of expression. They are followed by RBS29 and 30 which offer medium levels of expression, then RBS31 which offers low levels of expression, and finally RBS33 which offers a very low level of expression.

In addition, the sensitivity analysis show that the kinetic characteristics of the promoter have the greatest effect on sfGFP response. If substantial changes are needed to modify expression levels, the model strongly recommends experimenters to change the promoter used, or modify its kinetic values. However, as promoter engineering is a complex process, the next best solution is change the RBS which can offers less impact in changing response than changing the promoter. Therefore, for reasons of convenience or fine-tuning of response, the model suggests changing the RBS can achieve the required results.

In practice, let us consider a case in Plasmid 2 where 1. the expression levels of sfGFP is not high enough despite using RBS34; and 2. PLasR is fixed because of its application in the circuit. Experimenters could change RBS34 to RBS35 to increase expression levels, however the resultant increase in sfGFP expression may not be enough. From the model we can identify that promoter strength has the greatest effect on response, therefore by changing J23117 to a stronger constitutive promoter such as J23114, we should be able to increase sfGFP expression levels significantly.

Limitations of the Model

Models of Plasmid 1 and Plasmid 2 represent a general understanding of the system; however, they are not without their limitations. As stated previously, the models are limited due to assumptions in Plasmid 1 about promoter strength and molecule interaction kinetics between LsrR and PLsr; and in Plasmid 2 assumptions about the molecule interaction kinetics between PLasR and LasR. To rectify these limitations further characterisation of PLsR and LsrR molecule, and PLasR and LasR molecule are needed.

Finally, modelling results are not true representations of results but rather only general representations; models only offer a guide for experimenters. Parameters such as humidity, pH, medium, strain type and temperature are only a few of the many parameters known to affect results. However, as current modelling systems cannot capture all possible parameters, they cannot match experimental results in terms value. Therefore, the purpose of modelling is to guide the experimenter by presenting them with insight and understanding of their system. This advantage, saves the experimenter time and resources during the experiment phase. For example, the models produced from LabPats Plasmid 1 and LabPats Plasmid 2 identify the effect different RBS may have on the system as well as identify the most sensitive parts in the genetics circuits.

We hope you find these models helpful. Do let us know if you have any questions. For collaboration purposes, if you can display the graphs on your website or mention our contribution that would fantastic. We have also participated in the Flat Stanley project initiative. Great stuff LabPats! All the best with your iGEM.

See you in Boston!

NUS-GEM