Laboratory Report

UE 2.2 Genome Engineering: Implementation of logic gates in E. coli

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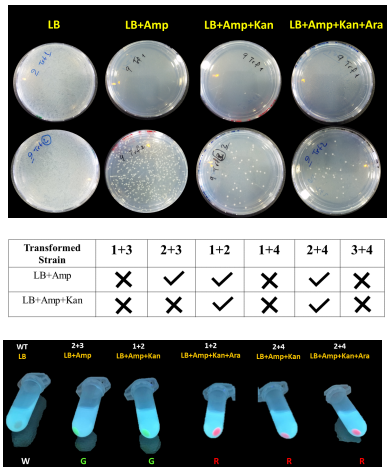
A. Introduction and Objectives

Boolean genetic logic gates can be designed using promoters and terminators flanked by integrase recognition sites (IRS) with different combinations of their orientations. Thanks to the importance of the directionalities of the promoters, terminators and IRSs, various types of logic gates with different desired outcomes are created. In this work, in order to explore an "If" gate genetic circuit using the mentioned elements, we analysed and characterized a circuit in E. Coli which depending on the expression of an integrase (either induced by arabinose in plasmid #87 or constitutively in plasmid #75), a switch in the expressed fluorescent protein is expected. In the design of the plasmid #261 which carries two fluorescent proteins; GFP (Green Fluorescence Protein) and mKate (Red Fluorescence Protein), a promoter is flanked by two IRSs which its direction in the basal state result in GFP expression. Upon its expression, the integrase inverts the direction of the promoter leading to the switch to mKate expression. Here, we were able to identify three plasmids by exploring the growth rate, fluorescence observation and colony PCR of the E. coli strains transformed with different combinations of the plasmids.

B. Results and discussion

To explore the identity of the four given unknown samples, we observed the growth of transformed strains on different mediums (LB, LB+Amp, LB+Amp+Kan and LB+Amp+Kan+Ara). We considered that plasmid #75 and #87 bring ampicillin resistance and #261 codes for kanamycin resistance. Based on the observations shown in the **Up** image in Figure 1 and the table shown below, we conclude that sample number 2 contains plasmid #261, sample number 3 contains water and sample number 1 and 4 contain #75 or #87.



Transformed Strain	1+3	2+3	1+2	1+4	2+4	3+4
LB+Amp	✗	✓	✓	✗	✓	✗
LB+Amp+Kan	✗	✗	✓	✗	✓	✗

Figure 1: Transformed E. coli in different liquid mediums. **Up:** Bacteria in the first row was not able to resist ampicillin and the ones in the second row survived in the 4 mediums. **Middle:** Summary results of growth for each strain, where **x** represents not growth. **Down:** The fluorescence of the different strains allows the discrimination of the inserted plasmid.

To discriminate between the samples 1 and 4, we must address their difference, which is based on the expression of the integrase with or without induction. To do so, we cultivated our bacteria in different liquid medium and then, visualized the respective cell pellet colors in the bottom of each tube as is shown in Figure 1 **Down**. Taking a look at the transformed strain with 1+2 plasmids in the LB + Amp + Kan, we observed green color. But, in the presence of Arabinose in the medium, the switch in color from green to red is appreciable implying that plasmid 1 is #87 due to the fact that it encodes the expression of the integrase under the control of the Arabinose inducible promoter. Consequently, plasmid 4 is #75.

However, the colony PCR results could not help us fully to discriminate the content of the samples 1 and 4. In the basal state of plasmid #261, using primers 2 and 3, there should be a product of 566 bp. Whereas, using primer 1 and 2 should only give us a product of 688 bp only when the switch in the sequence is occured. As is shown in the Figure 2 **(2)** in blue and red boxes, strains transformed with plasmids 1+2 and 2+4, both have a band of 688 bp (the product of primer 1 and 2) in the absence of arabinose. Thus, based on this we can not conclude about the identity of

plasmid #1 and #4. But, we know their identities from fluorescence colors results meaning that plasmid #87, which should only express the integrase in the presence of the arabionse, was expressed in its absence indicating the leakiness of this promoter. As the control, we see in Figure 2 **(1)** in purple box that strain 2+3 which was not transformed with any integrase, does not showed a band when primers 1 and 2 were used which implies that the switch was not occurred spontaneously or by a contamination. This leakiness was only observed in colony PCR results and not in the color observation due to much higher sensitivity of PCR.

To find out if the microplate reader was sensitive enough to show the leakiness of the of the promoter, the signal of mKate channel in the wells containing strains 1+2 and 2+3 (control strain with no mKate signal) was studied. As is illustrated in the **First** graph in Figure 3, no significant difference between the red lines in each respective plate (1 or 2) are observed. Thus, we conclude that the sensitivity of the microplate reader was not enough to detect an increase in the leaky expression of mKate that was observed by PCR results. A fact observed in the data is the high basal fluorescence level of mKate in 2+3 strain in which mKate is not expressed. This can be due to high gain in the setting of the instrument (plate 1 line) this amount of signal is just basal noise. However, in plate 2 line, a little increase in the mKate channel is seen as the GFP expression level increases in time. The reason for this is that perhaps some GFP signal was dectected in mKate channel as mKate signal and the reader can not fully distinguish between two proteins. However, due to difference between plate 1 and plate 2 graphs, a clear conclusion can not be made.

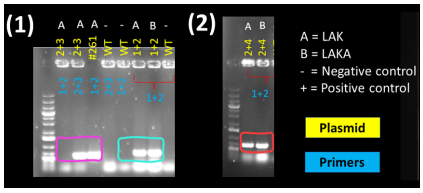
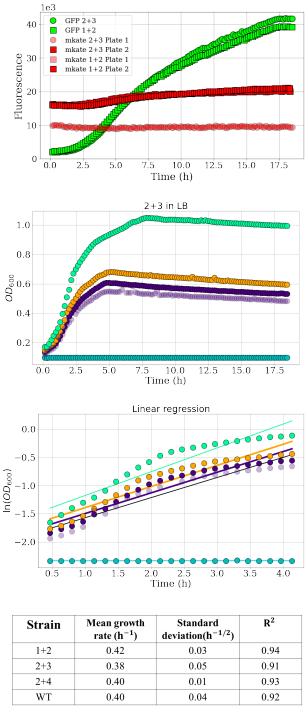


Figure 2: PCR results. Despite of the different combinations carried out in the experiment with different primers and plasmids, the PCR results were not enough to identified the plasmids. Nevertheless, we were able to observed the leakiness on the arabinose promoter.

To explore toxicity of the fluorescent proteins on the strains during exponential growth, the growth rates of the strains were measured by a microplate reader. We compared the growth rate of the strain 2+3 (who contains the expression of the eGFP) and 2+4 (who express mKate protein) in LB medium with the Wild type untransformed E.coli in LB. Furthermore, to find the toxicity of integrase expression, we compared the growth rate of 2+4 and 2+3 strains. We restricted our analysis to the data in the exponential phase of the growth. The growth rate can be computed between the range of 0.5h and 4h where is seem to behaves exponentially (Figure 3 **Second**). Then, by taking the logarithm and performing a linear regression we were able to find the exponential growth rate for each realization, as is displayed in the **Third** graph of Figure 3.

Once the values for the growth rates were obtained, we discarded the outliers for each strain and performed a statistical T test to compare the strains 2+3 and 2+4 with the wild type, considering that the standard deviations of the samples are different (Welch's correction) (Figure 3 **Fourth**). The values obtained are displayed in the following table.

Comparative Strains	T-test	p-value	Δ mean (h^{-1})
2+3 with WT	-0.73	0.49	0.02
2+4 with WT	0.12	0.90	0.02
2+4 with 2+3	1.29	0.27	0.02



Strain	Mean growth rate (h^{-1})	Standard deviation($h^{-1/2}$)	R^2
1+2	0.42	0.03	0.94
2+3	0.38	0.05	0.91
2+4	0.40	0.01	0.93
WT	0.40	0.04	0.92

Figure 3: Bacterial growth. **First:** GFP and mkate fluorescense of the strains 1+2 and 2+3. **Second:** Each curve represent one realization of the experiment. **Third:** Linear regression for the exponential phase of growth in bacteria in LB medium. **Fourth:** Summary results of growth rate and standard deviation of each strain.

Based on these results, although there is a very slight decrease in the value for the growth rate in the strain 2+3 compared with the WT (Figure 3 **Fourth**) the difference is not significant due to the high p-value. The same occurs for other comparisons. Hence, in our experiment, the GFP, RFP and integrase did not seem to be toxic to the cells. Although we did all comparisons, these comparison are not completely reliable due to lack of right controls. For instance, the right control for assessing the toxicity of GFP expression is not untransformed WT, but the WT transformed with the same vector lacking the CDS coding for GFP.