chatter plant

CONTRIBUTION

Universitat Politècnica de València
Valencia UPV iGEM 2017

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

VALENCIA UPV iGEM

INTRODUCTION

Genetic switches allow biotechnological approaches to process information of a determined stimulus in order to produce a biological response. Recombinase-mediated genetic engineering provides an ideal system to allow an easy spatiotemporal manipulation of gene expression that is modular, reversible and repeatable.

Our team is very excited of presenting the characterization of PhiC31 recombinase. Since the serine recombinase PhiC31 (BBa_K1742004) is codon-optimized for Nicotiana benthamiana, it has been characterized under weak and strong plant-specific promotors (Pnos and p35 respectively).

To date, different synthetic and bistable genetic toggle switch was been designed with the ability to flip between stable states. our team chose the recombinase PhiC31 due to its the ability of performing a site-specific recombination between two attachment sites. Therefore, we introduce the PhiC31-recombinate action knowledge in order to provide future iGEM teams with the necessary information to implement this SynBio technology.

GOALS

- We demonstrate that PhiC31 allow an accurate regulation of luciferase expression when it performs the inversion between its attachment sites.
- Recombination performance was demonstrated under weak and strong plant-specific promoters
- Furthermore, a counterintuitive correlation between the recombinase and the luciferase expression has been reported

PARTS USED IN THE CHARACTERIZATION

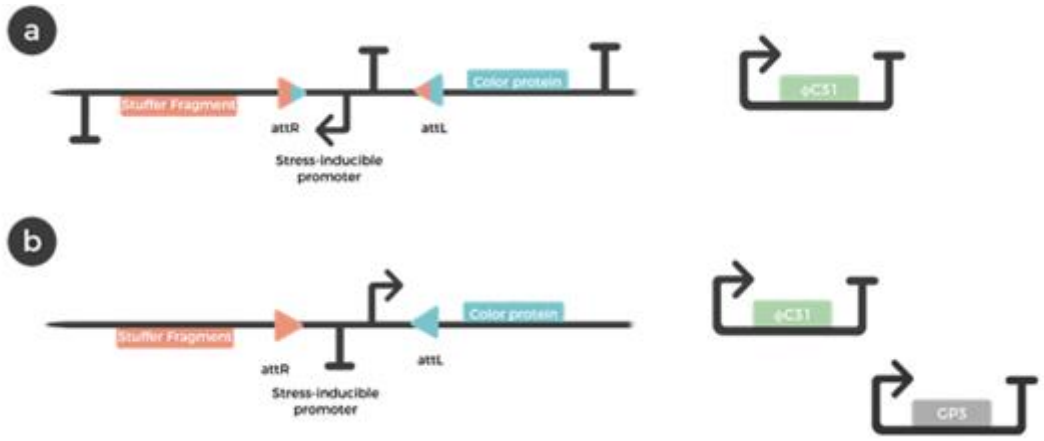

Figure 1. Graphic representation of Plant-Human genetic circuit with the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites. A) In presence of PhiC31 integrase, recombination occurs allowing the expression of a translation of a nonsense sequence or stuffer fragment. In this case, the circuit is in OFF state B) Graphic representation of the toggle switch in ON state only when PhiC31 and gp3 are expressed. Only when the promoter is activated under stress conditions, corresponding color protein will be expressed.

These results showed a counterintuitive correlation between the recombinase and luciferase. The higher the expression of recombinase is, the lower the probability that inversion occurs is. That is, the more concentration of recombinase in plant cells, the fewer inversion efficiency is.

GENETIC PARTS AND RESULTS

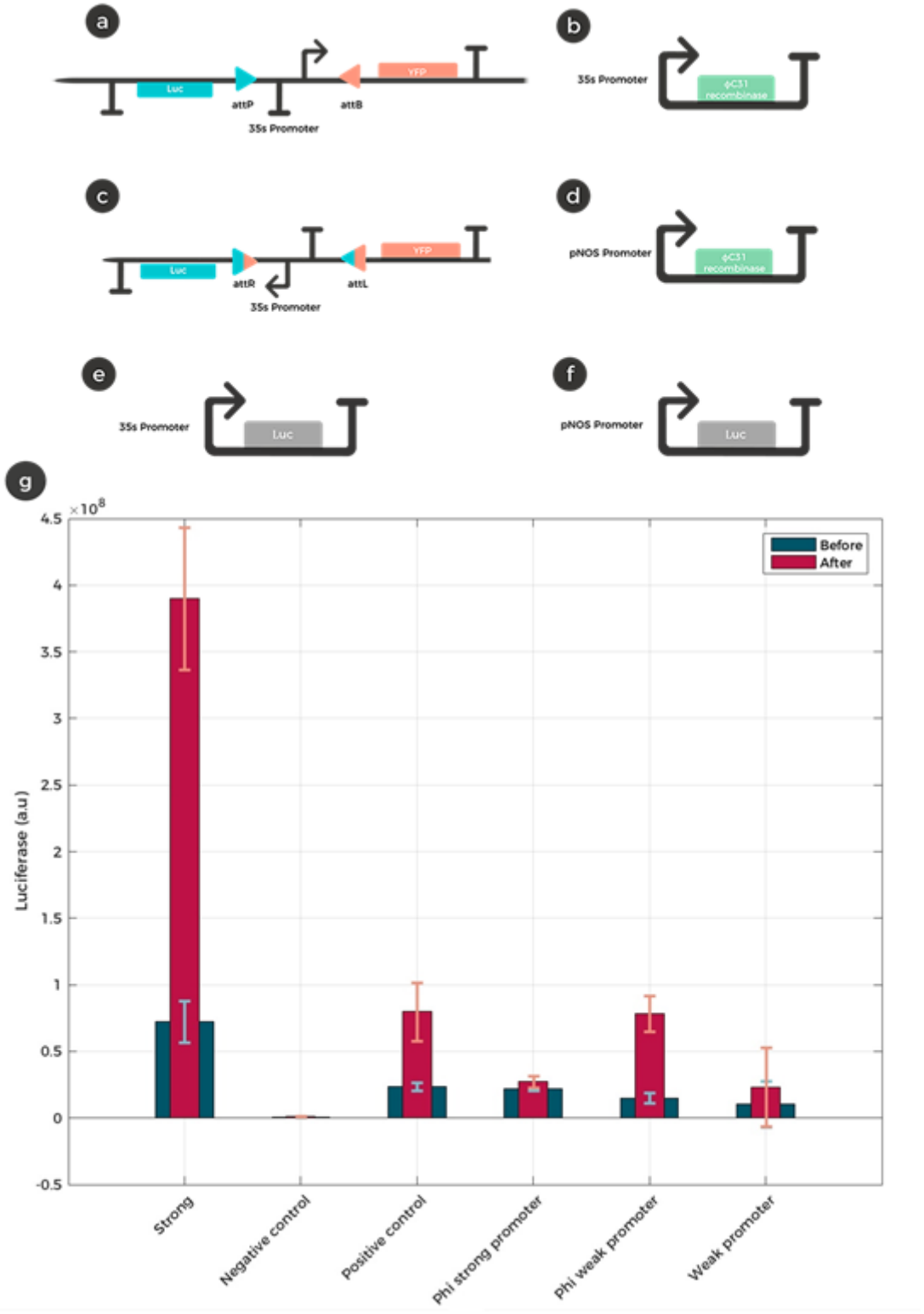

***Figure 2**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed. b) Genetic construct that allows constitutive*

expression of phiC31 in the plant under the control of a strong promoter c) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 recombined attachment sites (attR and attL). In normal basis, the promoter is inverted, allowing the expression of luciferase protein. It represents the positive control of our experiment. d) Genetic construct that allows constitutive expression of phiC31 in the plant under the control of a weak promoter. e) Transcriptional unit for the expression of the Firefly Luciferase under the control of a strong promoter. f) Transcriptional unit for the expression of the Firefly luciferase under the control of a weak promoter. g) Bars chart representing luciferase expression levels before and after induction with PhiC31.

METHOD

An agroinfiltration and subsequent luciferase assay were performed in order to study gene expression at transcriptional level. The final Optical Density of reporter constructs (Figure 2a and Figure 2c) was 0,02 and the optical density of PhiC31 construct (Figure 2b) was 0,01. A triplicate sampling of different plants was performed in order to take account for biological variability due to unknown or uncontrollable conditions.

TIMELINE

Register assembly constructs (Fig. 2a and 2b) and the controls (Fig. 2e and 5f) were agroinfiltrated and after 54h post-infiltration, leaves were sampled. After 48h, all leaves were also sampled. Overall, two points of samples were taken in the assay.

After analyzing the data obtained from luciferase assay, it can be observed at Figure 2:

1) A significantly difference between the OFF and the ON state expression levels.

2) The recombinase expression under a weak promoter (pNOS promoter) shows the same luciferase expression that the constitutive positive control (genetic construct which is expressing constitutively luciferase protein under the strong promoter).

3) However, the recombinase expression under the strong promoter shows the same expression level of the constitutive weak control (genetic construct based on the constitutive expression of luciferase under a pNOS weak promoter)

The likelihood of producing the inversion event should be directly proportional to the luciferase protein concentration inside plant cell. Consequently, more quantity of

phiC31 recombinase entails more probability of activating reporter gene expression. However, this experiment shows that once recombinase expression exceeds a certain threshold, the effect changes so the likelihood of up-regulating the reporter gene expression decreases.

In order to provide robust evidences to the hypothesis explained above, a subsequent experiment was performed. In this case, PhiC31 behavior was analyzed while being regulated by a weak promoter (pNOS) together with an optical density (A.tumefaciens culture concentration) was 10-fold higher than the used in aforementioned experiment (Figure 3).

In order to provide robust evidences to the hypothesis explained above, a subsequent experiment (http://2017.igem.org/Team:Valencia_UPV/Experiments#Phic31Per) was performed. In this case, PhiC31 behavior was analyzed while being regulated by a weak promoter (pNOS) together with an optical density (A. tumefaciens culture concentration) was 10-fold higher than the used in aforementioned experiment (Figure 3).

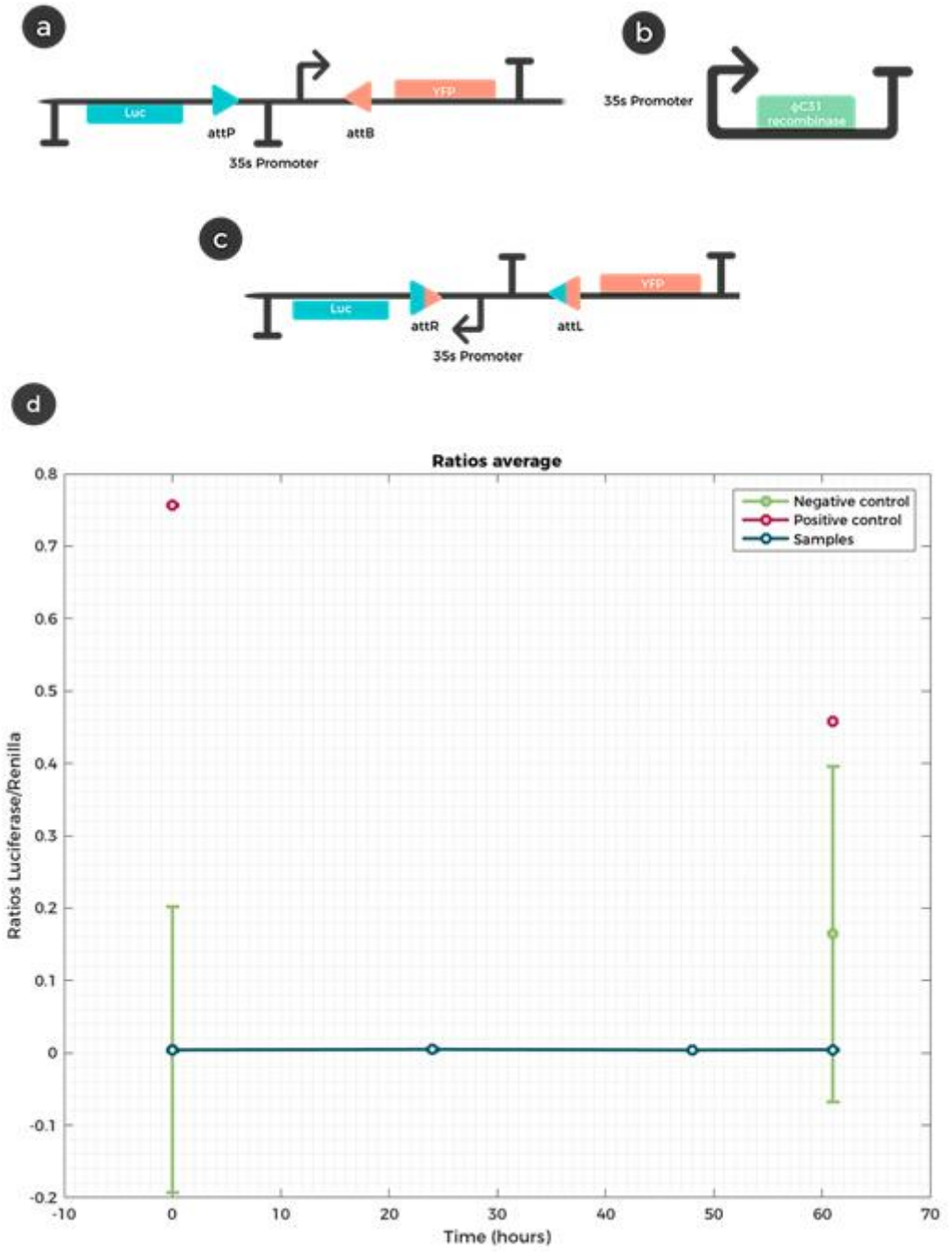

***Figure 3**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed. b) Genetic construct that allows constitutive expression of phiC31 in the plant under a strong promoter c) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 recombined attachment sites (attR and attL). In normal basis, the promoter is inverted, allowing the expression of luciferase protein. It represents the positive control of our experiment.* d) Plot representing PhiC31 behavior when infiltrated at OD 0,1.

Conclusion: It was demonstrated the proper phiC31 functioning and determined its behavior under different protein concentrations and promoter strengths. Although the expected hypothesis may be that the more recombinase concentration the more inversion events, the obtained results suggest the existence of a threshold above which that assumption is not true. Therefore, a low recombinase expression is needed in order to maximize recombinase action.

Our team has improved the characterization of the Phytobricks (PhiC31) documenting our experimental design and the results that were obtained. All data is also documented on the Main Page in the Registry.