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PARTS

PLANT SYNTHETIC BIOLOGY

Universidad Politécnica de Valencia
Valencia UPV iGEM 2017

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

VALENCIA UPV iGEM

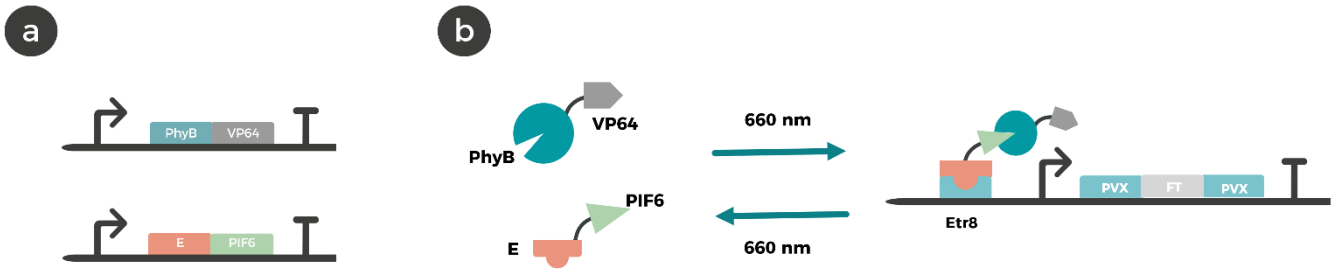
1. INTRODUCTION

ChatterPlant proposes an innovative system to establish a bidirectional communication between plants and humans. Since **Plant Synthetic Biology** started to be a leading field, the Phytobricks collection has continuously been increasing. This year, we provided iGEM registry with the necessary Phytobricks to use ChatterPlant's technology according to the philosophy of our project. Furthermore, we have contributed to the progress of Plant SynBio repository since we have characterized and used many delivered parts at iGEM Registry.

Due to its reusability and exchangeability, we use **GoldenBraid** system assembly. It is based on type II restriction enzymes and can convert single-use Golden Gate multipartite assemblies into reusable composite parts. The major advantages of this system are its speed, accuracy and simplicity (Sarrion-Perdigones et al., 2011).

Essentially, we contributed with a **compilation of Phytobricks** aiming to establish a channel between plants and humans. Here, we report all used parts in this project and finally, we also report a table with the Basic Parts that we submitted to the iGEM Registry.

2. HUMAN – PLANT COMMUNICATION SYSTEM



2.1. BASIC PARTS

Promoter 35s (BBa_K1537015): this constitutive promoter is widely used in transgenic plants to improve the level of the expression of foreign genes effectively.

Tnos (BBa_K1484215): Nopaline synthase terminator of the nopaline synthase gene of Agrobacterium tumefaciens. It is used for gene transfection (Holden, Levine, Scholdberg, Haynes & Jenkins, 2009).

Luciferase (Bba_K322237): this protein is commonly used as a tool to study gene expression at the transcriptional level. In order to quantify gene expression, luciferase reporter assay has been performed in lab experiments.

PhyB: VP64 (BBa_K1936003): Part of a red light-controlled synthetic genetic switch. Chimeric construct comprising the N-terminal fragment of A. thaliana PhyB (amino acids 1-650) fused to the VP16 activation domain and a nuclear localization sequence (Müller et al., 2014)

E:PIF6 (BBa_K1150005): part of a red light-controlled synthetic genetic switch. Designed module for the constitutive expression of a chimeric construct comprising a DNA binding protein fused to A. thaliana PIF6 (amino acids 1-100) and a nuclear localization sequence (Müller et al., 2014).

Etr8: minCMV: Chimeric promoter containing Etr8 (an E-responsive operator motif) fused to the minimal CMV promoter (Müller et al., 2014).

FT (BBa_K797006): Coding sequence corresponding to Flowering Locus T from Arabidopsis thaliana. It has been optimized for Nicotiana benthamiana in order to promote floral induction in response to day length (Andres et al., 2015).

PVX: Gene encoding plasmid of Potato Virus X. This viral vector was used because of systemic movement in plants.

2.2. COMPOSITE PARTS

The human-plant circuit is formed by three close-related constructions: two of them form the optogenetic switch, whereas the third one has the final, desired coding sequence.

35s: E:PIF6: Tnos: Unit transcription for the constitutive expression of a chimeric construct comprising a DNA binding protein fused to A. thaliana PIF6 (amino acids 1-100) and a nuclear localization sequence.

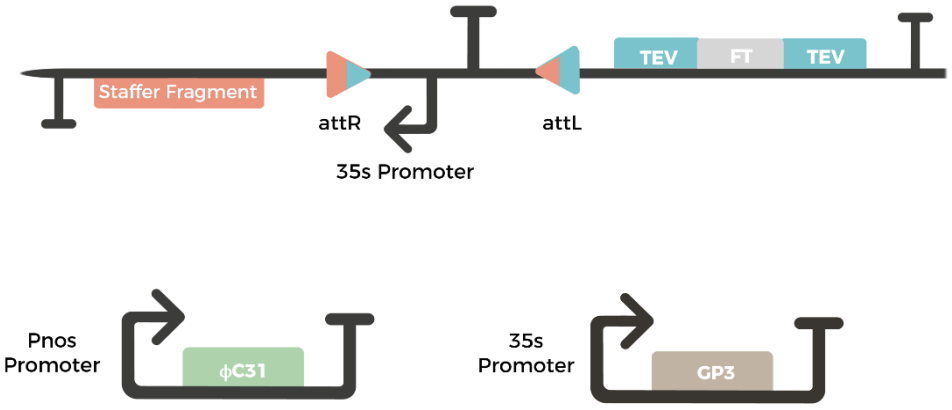
35s: PhyB: VP64: Tnos: Unit transcription for the expression of chimeric construct comprising the N-terminal fragment of A. thaliana PhyB (amino acids 1-650) fused to the VP16 activation domain and a nuclear localization sequence.

Etr8minCMV: Luc: Tnos-35s: PhyB-VP16-NLS: tNos-35s:PIF6: Tnos-SF: Module for the regulated expression of firefly luciferase driven by the synthetic promoter Etr8-minCMV and the constitutive expression of chimeric constructs of A. thaliana PhyB-VP16-NLS and PIF6-NLS

Etr8: minCMV: θC31: Tnos: Unit transcription for regulating θC31 recombinase expression driven by the synthetic promoter Etr8-minCMV.

35s: FT: Tnos: Transcriptional unit coding Flowering Locus T under a strong promoter (35s Promoter).

3. PLANT - HUMAN COMMUNICATION SYSTEM



3.1. BASIC PARTS

Register assembly RL 35s Promoter: middle part for assembly of site-specific recombination based Registers. Composed of the 35S promoter inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attR and attL).

Register assembly RL PR-1a: middle part for assembly of site-specific recombination based Registers. Composed of the PR-1a inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attR and attL).

ϕC31 recombinase (BBa_K1742004): PhiC31 is a site-specific serine recombinase derived from a Streptomyces phage (Keravala et al., 2006). The enzyme recognizes two different attachments sites called also attB and attP, and also excise a sequence flanked with attB and attP sites close to the promoter.

Gp3: Streptomyces phage phiC31-encoded recombination directionality factor (RDF). Upon interaction with PhiC31 Integrase, it promotes site-specific recombination of attR and attL attachment sites to produce PhiC31 attP and attB sites.

Ros1: The Antirrhinum majus transcription factor Rosea1. It's usually used as a visual reporter gene (Majer, Llorente, Rodríguez-Concepción & Daròs, 2017).

TEV: viral vector derived from Tobacco etch virus (TEV) was used since this strategy enables to detect adequately color halo effect. That's because its auto-replicative

capacity and its movement from cell-to-cell to the vascular tissue (Majer, Llorente, Rodríguez-Concepción & Daròs, 2017).

Pr-1a: promoter that regulated the expression of tobacco pathogenesis-related protein 1a. Salicylic acid induce expression of genes under this promoter.

SF: a small intergenic region (Stuffer Fragment) usually used in Goldenbraid assembly. Instead of a composite part, this fragment can be used to perform a binary reaction.

We determined to use three chromoproteins, which were used previously by other teams: two were used by Uppsala in 2011 (eForRed and AmilCP) and the last one in 2013 (AmajLime) while AmajLime was also used by Cambridge-JIC in 2014. In order to test the efficiency of those chromoproteins in a different chassis (because Uppsala used them in bacteria and Cambridge-JIC used it on Marchantia), we've got two version of this construction: one with bacteria codon optimized CDS and other with N. benthamiana codon optimized, so we can compare the efficiency of both sequences in our chassis.

eForRed optimized chromoprotein: this chromoprotein naturally exhibits red/pink color when expressed (BBa_K592012). It was optimized for Nicotiana benthamiana since reporters in plants are highly demanded.

AmilCP optimized chromoprotein: this chromoprotein exhibits strong blue color when expressed (BBa_K592009). It was optimized for Nicotiana benthamiana to increase the number of available reporters in plants.

eforRed chromoprotein: Chromoprotein that exhibits red/pink color when expressed (BBa_K592012). It was reported by Upsala Team and we summit this part in Phytobricks standard. This part has been domesticated using GoldenBraid System Assembly.

AmajLime chromoprotein: chromoprotein that exhibits yellow-green strong color when expressed (BBa_K1033916). It is cloned in Phytobricks standard in order to increase Phytobricks repository of parts. This part has been domesticated using GoldenBraid System Assembly.

AmilCP chromoprotein: chromoprotein that exhibits blue color when expressed (BBa_K592009). It is cloned in Phytobricks standard in order to increase Phytobricks repository of parts. This part has been domesticated using GoldenBraid System Assembly.

3.2. COMPOSITE PARTS

SF: Register Assembly RL PR-1a: Ros1: Transcriptional unit composed of the PR-1a inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attR and attL). In normal basis, a stuffer fragment will be expressing and only when RDF appears, Ros 1 will be expressed.

YFP: Register Assembly PB 35s Promoter: Luc: Transcriptional unit composed of the 35s inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attP and attB). In normal basis, the fluorescent protein will be expressing and only when θC31 appears, luciferase will be expressed.

Luc: Register Assembly PB 35s Promoter: YFP: Transcriptional unit composed of the 35s inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attP and attB). In normal basis, luciferase will be expressing and only when θC31 appears, the fluorescent protein will be expressed.

Luciferase: Register Assembly RL 35s Promoter: YFP: Transcriptional unit composed of the 35s inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attR and attL). In normal basis, the fluorescent protein will be expressing and only when θC31 and RDF appear, the fluorescent protein will be expressed.

YFP: Register Assembly RL 35s Promoter: Luciferase: Transcriptional unit composed of the 35s inverted sequence and the Mtb terminator flanked by two opposing PhiC31 site-specific recombination sites (attR and attL). In normal basis, the fluorescent protein will be expressing and only when θC31 appears, luciferase will be expressed.

Pr-1a: YFP: Tnos: YFP reporter gene with a salicylic acid-inducible promoter (and therefore a pathogen-inducible promoter) to test the efficiency of PR-1a promoter.

Pr-1a: Luc: Tnos: Firefly luciferase reporter gene with a salicylic acid-inducible promoter

35s: AmajLime chromoprotein: Tnos: Transcriptional unit for the expression of the AmajLime yellow chromoprotein, to test a color change in plants.

35s: eForRed chromoprotein: Tnos: Transcriptional unit for the expression of the eForRed chromoprotein, to test a color change in plants.

35s: AmilCP chromoprotein: Tnos: Transcriptional unit for the expression of the AmilCP blue chromoprotein, to test a color change in plants.

35s: eForRed Optimized chromoprotein: Tnos: Transcriptional unit for the expression of an optimized eforRed chromoprotein sequence for *N. benthamiana*.

35s: AmilCP Optimized chromoprotein: Tnos: Transcriptional unit for the expression of an optimized amilCP chromoprotein sequence for *N. benthamiana*.

4. NEW PHYTOBRICKS

NAME	TYPE	DESCRIPTION
BBa_K2269000	Promoter	Pr-1a (stress-inducible promoter)
BBa_K2269001	CDS	Register assembly 3/3 Pr-1a
BBa_K2269002	TU	Pr-1a: Luc: Tnos
BBa_K2269003	CDS	Register assembly 3/3 FT
BBa_K2269004	CDS	Register assembly 1/3 Luc
BBa_K2269006	CDS	Register assembly 1/3 SF
BBa_K2269007	CDS	Register assembly 3/3 YFP
BBa_K2269008	TU	Register assembly RL YFP : Luc
BBa_K2269010	CDS	Register assembly PB 2/3
BBa_K2269011	CDS	Register Assembly RL 2/3
BBa_K2269012	CDS	FT
BBa_K2269014	CDS	Register Assembly 3/3 Ros1
BBa_K2269016	CDS	eforRed chromoprotein
BBa_K2269017	CDS	Gp3
BBa_K2269018	CDS	Amil CP chromoprotein
BBa_K2269019	CDS	AmajLime chromoprotein
BBA_K2269020	TU	Pr-1a:YFP:Tnos
BBa_K2269021	CDS	eforRed Plant Optimized
BBa_K2269022	CDS	AmilCP Plant Optimized
BBa_K2269023	CDS	Register Assembly 1/3 YFP

5. REFERENCES

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