EXPERIMENTS

PLANT SYNTHETIC BIOLOGY

Universidad Politécnica de Valencia
Valencia UPV iGEM 2017

UNIVERSITAT
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1. PHIC31 PERFORMANCE

Objective: Testing the performance of phiC31 recombinase using the register assembly construct with the attachment sites PxB.

Plant Chassis: *Nicotiana benthamiana*

Parts:

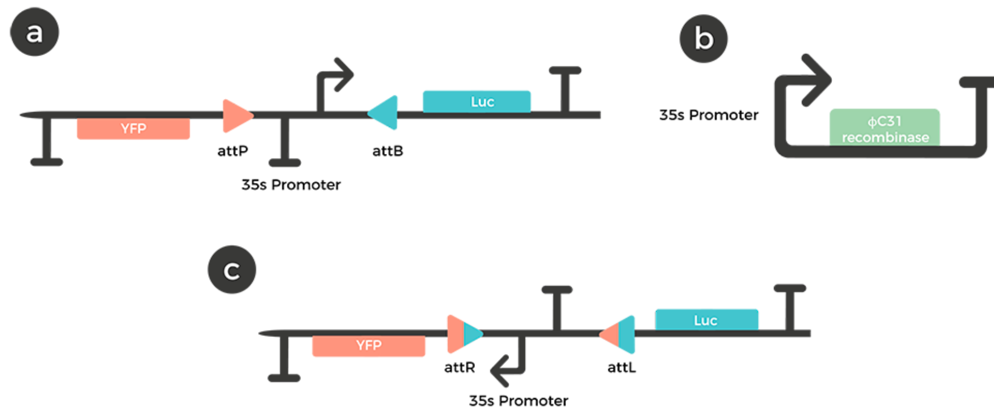


***Figure 1**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed, allowing the characterization of luciferase dynamic. b) Genetic construct that allows constitutive expression of phiC31 in the plant. 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.*

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 1a and Figure 1c) was 0,02 and the optical density of PhiC31 construct (Figure 1b) was 0,05. A triplicate sampling of different plants was performed from 36h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

Timeline

Register assembly constructs (Fig. 1a and 1b) were agroinfiltrated on 12th August at 12:00h. After 48h post-infiltration (14th August), positive and negative control were sampled and the recombinase was agroinfiltrated. After 36h, leaves were sampled every eight hours finishing on 18th August at 16:00h. At this point, positive and negative controls were also sampled. Overall, six points of samples were taken in the assay after recombinase were agroinfiltrated.

2. GP3 PERFORMANCE - REPORTER (L X R) - 1

Objective: Testing the performance of gp3 using the register assembly construct with the recombined attachment sites LxR.

Plant Chassis: *Nicotiana benthamiana*

Parts:

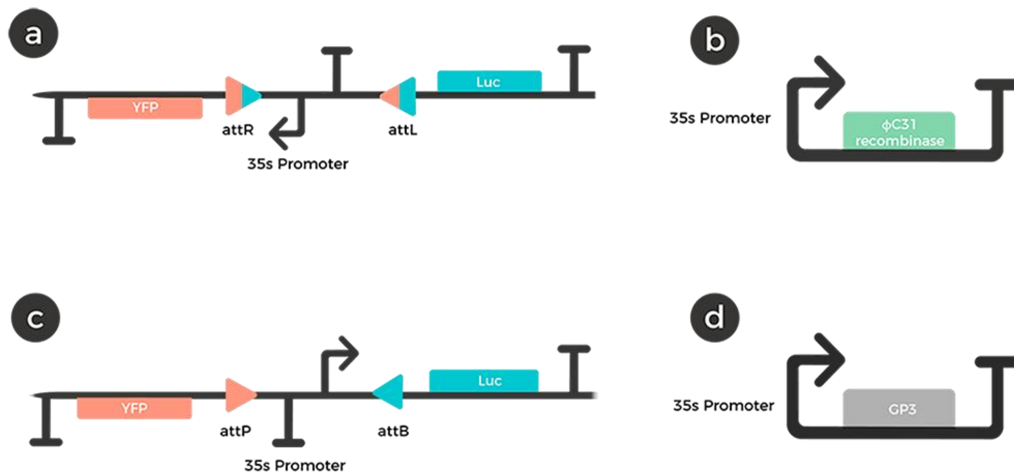


***Figure 2**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 recombined attachment sites (attR and attL). In normal basis, the promoter is inverted, allowing the expression of a fluorescent protein. It represents the negative control of our experiment. b) Genetic construct that allows constitutive expression of phiC31 in the plant. c) 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 positive control of our experiment. d) Genetic construct that allows constitutive expression of RDF under a strong promoter.*

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 (F.2a and c) were 0,02, the optical density of PhiC31 construct (F.2b) was 0,1 and the optical density of gp3 construct (F.2d) was 0,05 and 0,15. A triplicate sampling of different plants was performed from 36h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

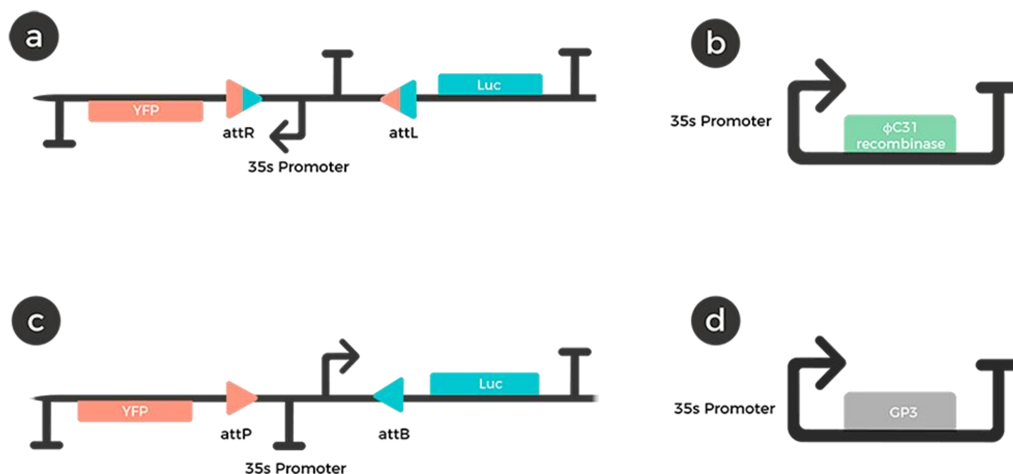
Timeline

Register assembly construct (Fig. 2a) was agroinfiltrated together with recombinase (Fig. 2c) on 21th August at 12:00h. Positive and negative controls (Fig. 2b and Fig. 2a) were also infiltrated. After 48h post-infiltration (23th August), positive and negative control were sampled and RDF (Fig. 2d) was agroinfiltrated. After 36h, leaves were sampled every eight hours finishing on 25th August at 08:00h. At this point, positive and negative controls were also sampled. Overall, ten points of samples were taken in the assay after recombinase were agroinfiltrated.

3. GP3 PERFORMANCE - REPORTER (L X R) - 2

Objective: Testing the performance of gp3 using the register assembly construct with the recombined attachment sites LxR.

Plant Chassis: *Nicotiana benthamiana*



Parts:

Figure 3. *a) 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 a fluorescent protein. It represents the negative control of our experiment. b) Genetic construct that allows constitutive expression of phiC31 in the plant. c) 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 positive control of our experiment. d) Genetic construct that allows constitutive expression of RDF under 35s promoter.

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 (F. 3a and c) were 0,02, the optical density of PhiC31 construct (F.3b) was 0,05 and the optical density of gp3 construct (F.3d) was 0,20 and 0,35. A triplicate sampling of different plants was performed from 36h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions

Timeline

Register assembly construct (Fig. 3a) was agroinfiltrated together with recombinase (Fig. 3c) on 3th August at 12:00h. Positive and negative controls (Fig. 3b and Fig. 3a) were also infiltrated. After 48h post-infiltration (7th September), positive and negative control were sampled and RDF (Fig. 3d) was agroinfiltrated. After 36h, leaves were sampled every eight hours finishing on 11th September at 20:00h. At this point, positive and negative controls were also sampled. Overall, six points of samples were taken in the assay after recombinase were agroinfiltrated.

4. PHIC31 PERFORMANCE (P X B)

Objective: Testing the performance of phiC31 recombinase using the register assembly construct with the attachment sites PxB in order to characterize luciferase dynamics.

Plant Chassis: *Nicotiana benthamiana*

Parts:

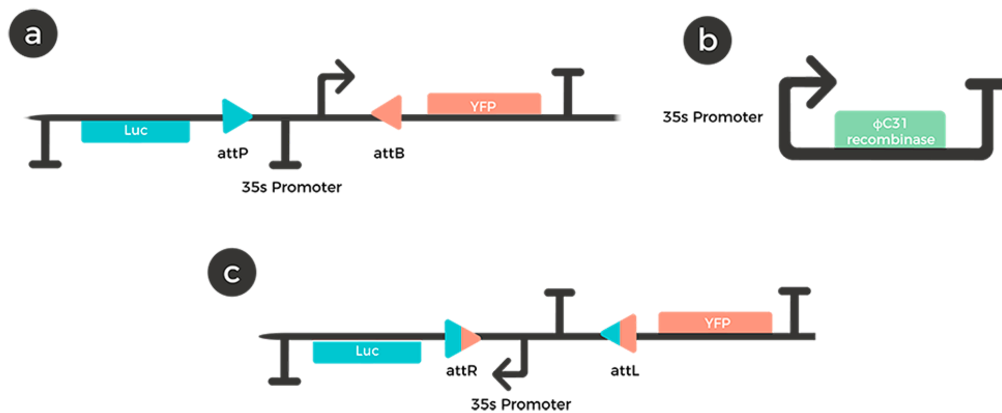


***Figure 4**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed. b) Genetic 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.*

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 4a and Figure 4c) was 0,02 and the optical density of PhiC31 construct (Figure 4b) was 0,1. A triplicate sampling of different plants was performed from 36h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

Timeline

Register assembly constructs (Fig. 4a and 4b) were agroinfiltrated on 3th August at 12:00h. After 48h post-infiltration (7th September), positive and negative control were sampled and the recombinase was agroinfiltrated. After 36h, leaves were sampled every 24 hours finishing on 11th September at 20:00h. At this point, positive and negative controls were also sampled. Overall, four points of samples were taken in the assay after recombinase were agroinfiltrated.

5. PHIC31 PERFORMANCE - PROMOTERS

Objective: Testing the performance of phiC31 recombinase using the register assembly construct with the attachment sites PxB in order to characterize luciferase dynamics.

Plant Chassis: *Nicotiana benthamiana*

Parts:

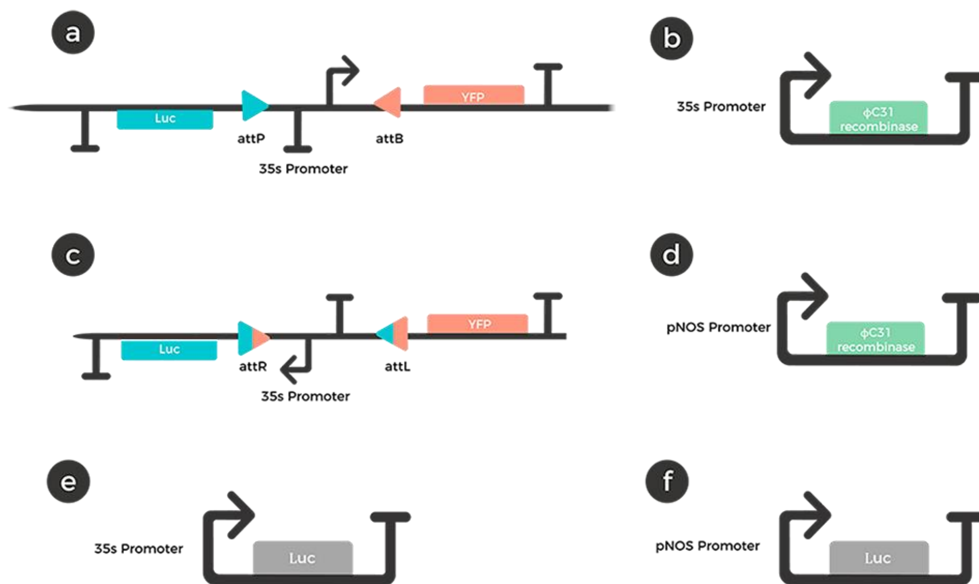


Figure 5. *a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed. b) Genetic 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.*

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 5a and Figure 5c) was 0,02 and the optical density of PhiC31 construct (Figure 5b) 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. 5a and 5b) and the controls (Fig. 5e and 5f) were agroinfiltrated on 19th September at 12:00h. After 54h post-infiltration (21th September), leaves were sampled at 18:00h. After 48h, all leaves were also sampled. Overall, two points of samples were taken in the assay.

6. CHARACTERIZATION OF PHIC31

Objective: Testing the performance of phiC31 recombinase using the register assembly construct with the attachment sites PxB in order to characterize luciferase dynamics under a weak promoter.

Plant Chassis: *Nicotiana benthamiana*

Parts:

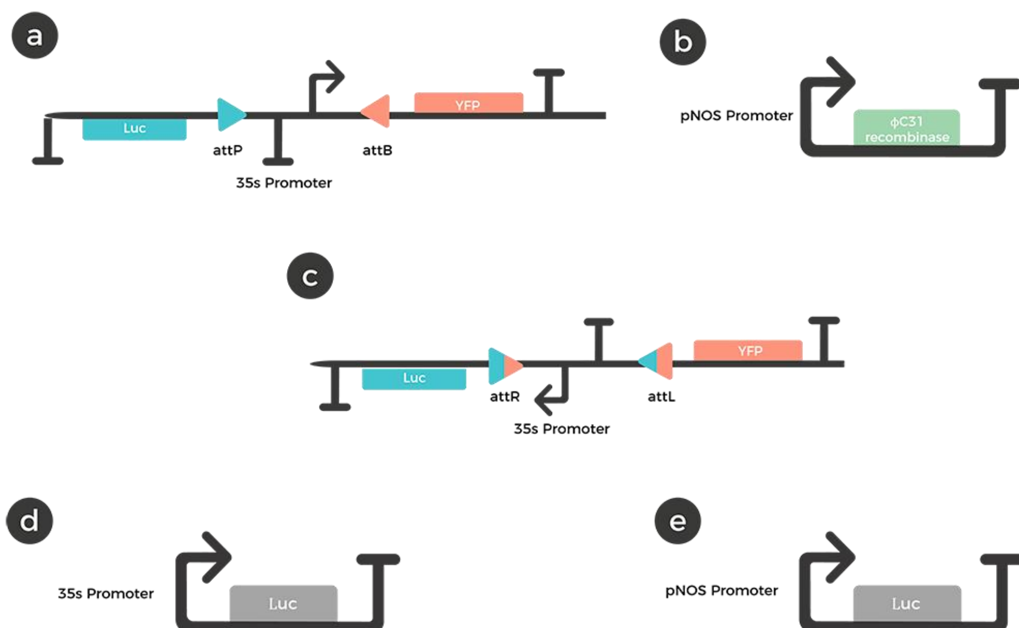


***Figure 6**. a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 attachment sites (attB and attP). It represents the negative control of our experiment. Only when ΦC31 inversion occurs, luciferase protein will be expressed. b) Genetic construct that allows constitutive expression of phiC31 in the plant under the control of a weak 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) Transcriptional unit for the expression of the Firefly Luciferase under the control of a strong promoter. e) Transcriptional unit for the expression of the Firefly luciferase under the control of a weak promoter.*

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 6a and Figure 6c) was 0,02 and the optical density of PhiC31 construct (Figure 6b) was 0,01 AND 0,005. A unique sampling of the same plant was performed.

Timeline

Register assembly constructs (Fig. 6a and 6c) and the controls (Fig. 6e and 6f) were agroinfiltrated on 29th September at 18:00h. After 12h post-infiltration (30th September), leaves were sampled every 6 hours finishing on 1st October at 08:00h.

7. PVX

Objective: Testing the viral systemic movement performed by Potato Virus X (PVX).

Plant Chassis: *Nicotiana benthamiana*

Parts:

***Figure 7**. a) Graphic representation of PVX-dsRED- Express Vector. When the viral vector is being produced, the fluorescent protein (dsRED) will be expressed.*

Method

An agroinfiltration in plant roots was performed in order to test the systemic movement of this viral vector. To do so, the construct containing the fluorescent protein (dsRED) was transformed by Agrobacterium into *N. benthamiana* leaves and roots. (Figure 8a). The final Optical Density of the genetic construct was 0,1.

Timeline

One week after transformation, plants were observed at the magnifier in order to check the fluorescence in the aerial part of the plant.

8. OPTOGENETIC – CONTROLLING THE GENETIC EXPRESSION WITH LIGHT

Objective: the genetic expression control with red-light. The main objective of the following experiment is testing the biological performance of the red-far red switch when it is irradiated with red light.

Plant Chassis: *Nicotiana benthamiana*

Parts:

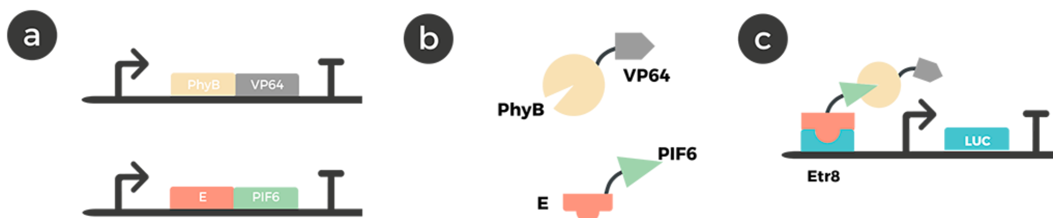


Figure 8*. a) Graphic representation of the first part of the designed genetic circuit Red/ far-red toggle-switch, when activated, will activate luciferase transcription. To do so, PIF6 is fused to a DNA-binding domain (E), which binds to Etr8 operator. Moreover, PhyB is fused to an activator domain (VP64) and a nuclear location sequence (NLS). b) Conformation of the proteins which belong to genetic toggle switch. c) When irradiated with 660nm light, PhyB changes its conformation and this complex is recruited to PIF6 at the promoter site. The polymerase III will recognize the activation domain and the transcription will begin. Only upon absorption of a far-red photon the interaction between PhyB and PIF6 is terminated, resulting in a shut-off of gene expression.*

Method

An agroinfiltration and subsequent luciferase assay were performed in order to study gene expression at transcriptional level. To do so, the construct containing the E-PIF6 and PhyB-VP16 transcriptional units was transformed, together with the chimeric promoter nearby the luciferase gene transcriptional unit, by Agrobacterium into *N. benthamiana* leaves (Figure 8a and 8c). The final Optical Density of optogenetic constructs was 0,1. Several treatments were performed in order to test the toggle switch. Both control and sample discs were incubated in the dark during the first 36h. After that, two samples were illuminated with 660 nm red light (hardware link), two other samples plants and two controls were illuminated with white light and finally, the last two samples and controls were incubated in the dark for the entire experiment.

Timeline

Optogenetic experiment was performed for 24 hours and samples were taken at 0 hrs, 3 hrs, 6 hrs, 9 hrs, 14 hrs and 23 hrs. Sampling of control plants were made at 0 hrs and 23 hrs.

9. OPTOGENETIC 2.0

Objective: the genetic expression control with red-light. The main objective of the following experiment is testing the biological performance of the red-far red switch when it is irradiated with red light.

Plant Chassis: *Nicotiana benthamiana*

Parts:

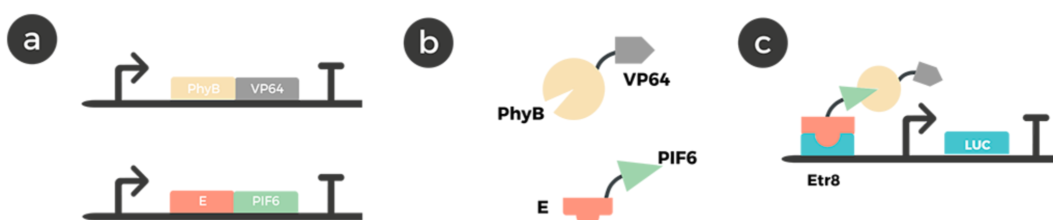


***Figure 9**. a) Graphic representation of the first part of the designed genetic circuit Red/ far-red toggle-switch, when activated, will activate luciferase transcription. To do so, PIF6 is fused to a DNA-binding domain (E), which binds to Etr8 operator. Moreover, PhyB is fused to an activator domain (VP64) and a nuclear location sequence (NLS). b) Conformation of the proteins which belong to genetic toggle switch. c) When irradiated with 660nm light, PhyB changes its conformation and this complex is*

recruited to PIF6 at the promoter site. The polymerase III will recognize the activation domain and the transcription will begin. Only upon absorption of a far-red photon the interaction between PhyB and PIF6 is terminated, resulting in a shut-off of gene expression.

Method

An agroinfiltration and subsequent luciferase assay were performed in order to study gene expression at transcriptional level. To do so, the construct containing the E-PIF6 and PhyB-VP16 transcriptional units was transformed, together with the chimeric promoter nearby the luciferase gene transcriptional unit, by Agrobacterium into *N. benthamiana* leaves (Figure 9a and 9c). The final Optical Density of optogenetic constructs was 0,1. Several treatments were performed in order to test the toggle switch. Both control and sample discs were incubated in the dark during the first 36h. After that, two samples were illuminated with 660 nm red light (hardware link), two other samples plants and two controls were illuminated with white light and finally, the last two samples and controls were incubated in the dark for the entire experiment.

Timeline

Optogenetic experiment was performed for 50 hours and samples were taken at 0 hrs, 3 hrs, 8 hrs, 11 hrs, 13 hrs, 16 hrs, 19 hrs, 23 hrs, 25 hrs, 25hrs, 27 hrs, 29 hrs, 31 hrs, 34 hrs and 50 hrs. Sampling of control plants were made at 0 hrs, 24 hrs, 34 hrs and 50 hrs hrs.

10. GP3 PERFORMANCE

Objective: Testing the performance of gp3 using the register assembly construct with the recombined attachment sites LxR.

Plant Chassis: *Nicotiana benthamiana*

Parts:

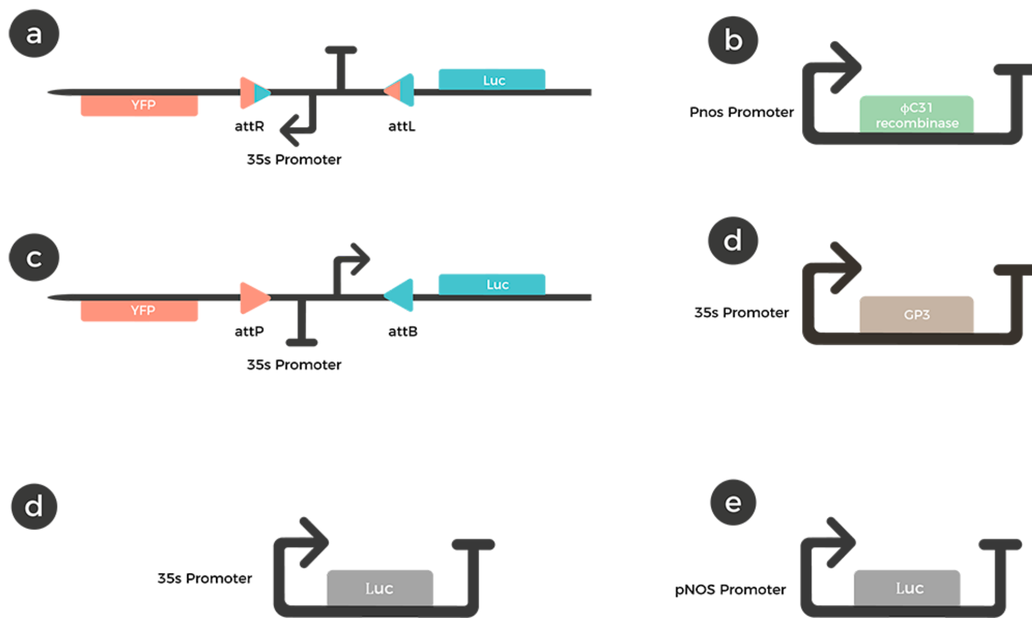


Figure 10. *a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 recombined attachment sites (attR and attL). In normal basis, the promoter is inverted, allowing the expression of a fluorescent protein. It represents the negative control of our experiment. b) Genetic construct that allows constitutive expression of phiC31 in the plant under a weak promoter (Pnos Promoter) c) 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 positive control of our experiment. d) Genetic construct that allows constitutive expression of RDF under 35s promoter (strong 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.*

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 (F. 10a and c) were 0,01, the optical density of PhiC31 construct (F.10b) was 0,005 and the optical density of gp3 construct (F.10d) was 0,10 and 0,20. A unique sampling of the same plant was performed from 36h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

Timeline

Register assembly construct (Fig. 10a) was agroinfiltrated together with recombinase (Fig. 7c) on 12th October at 18:00h. Positive and negative controls (Fig. 10a and Fig. 10c) were infiltrated together with the internal controls (Fig. 10e and 10f). After 48h post-infiltration (14th October), the RDF (Fig. 10d) was agroinfiltrated letting a control plant without being agroinfiltrated. After 36h, leaves were sampled every twelve hours finishing on 18th October at 20:00h. At this point, positive and negative controls were also sampled. Overall, six points of samples were taken in the assay after recombinase were agroinfiltrated.

11. METABOLIC LOAD

Objective: Testing the metabolic load of cells that can affect recombinase performance from the molecular to the organismal level.

Plant Chassis: *Nicotiana benthamiana*

Parts:

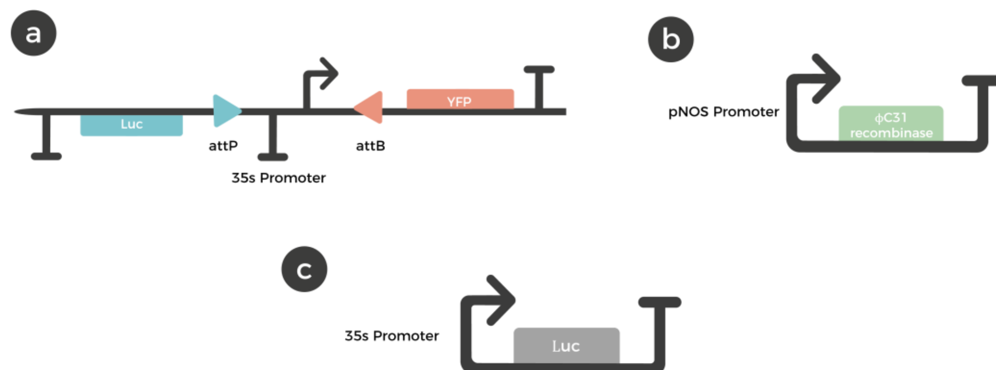


Figure 11. *a) Graphic representation of the construct comprised by a promoter and a terminator in opposite directions flanked by ΦC31 recombined attachment sites (attR and attL). In normal basis, the promoter is inverted, allowing the expression of luciferase protein. b) Genetic construct that allows constitutive expression of phiC31 in the plant under a weak promoter (Pnos promoter)*

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 11a) was 0,02 and the optical density of PhiC31 construct (Figure 11b) was 0,01 and 0,2. A triplicate sampling of different plants was performed from

48h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

Timeline

Register assembly construct (Fig. 11a) was agroinfiltrated together with the recombinase (Fig.11b) letting a control plant without it on 20th October at 12:00h. After 48h post-infiltration (22th October) all plants were sampled. After 96h post-infiltration, leaves were also sampled. Overall, two points of samples were taken in the assay after recombinase were agroinfiltrated.

12. CHROMOPROTEINS

Objective: Testing the performance chromoproteins in plants.

Plant Chassis: *Nicotiana benthamiana*

Parts:

***Figure 12.** a) Graphic representation of the construct comprised by the chromoprotein AmilCP under a strong promoter (35s Promoter) b) Graphic representation of the construct comprised by the chromoprotein eforRed under a strong promoter (35s Promoter).*

Method

An agroinfiltration was performed in order to test chromoprotein gene expression. The final Optical Density of reporter constructs (F. 12a and b) were 0,1.

Timeline

One week after transformation, plants were observed at the magnifier in order to check the color protein in plant leaves.

13. CHROMOPROTEINS PVX

Objective: Testing the performance chromoproteins in plants.

Plant Chassis: *Nicotiana benthamiana*

Parts:

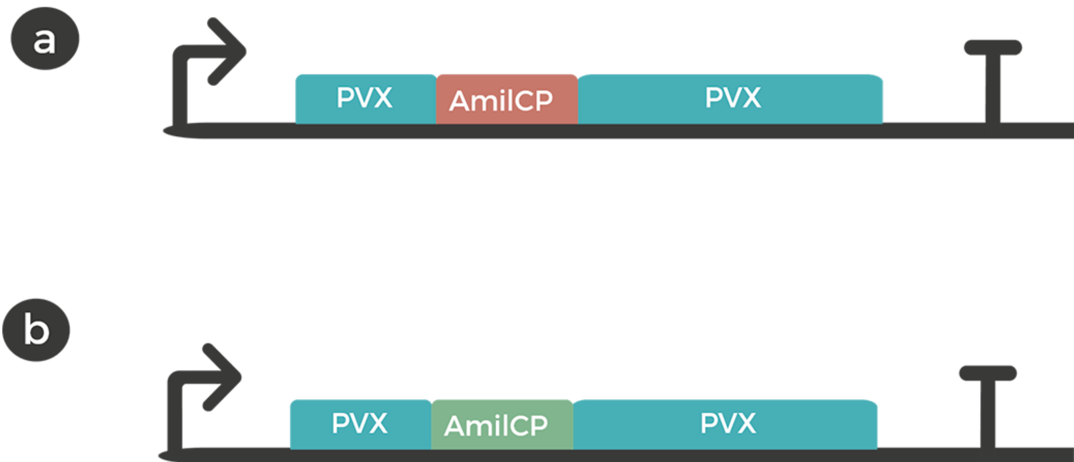


Figure 13. *a) Graphic representation of the viral vector construct comprised by the chromoprotein AmilCP. When the viral vector is being produced, the color protein (AmilCP) will be expressed. b) Graphic representation of the construct comprised by the chromoprotein eforRed. When the viral vector is being produced, the color protein (eforRed) will be expressed*

Method

An agroinfiltration was performed in order to test chromoprotein gene expression. The final Optical Density of reporter constructs (F. 13a and b) were 0,1.

Timeline

One week after transformation, plants were observed at the magnifier in order to check the color protein in plant leaves.

14. STRESS-PROMOTER PR-1A

Objective

Testing the performance of the stress-promoter under several treatments.

Plant Chassis: Nicotiana benthamiana

Parts

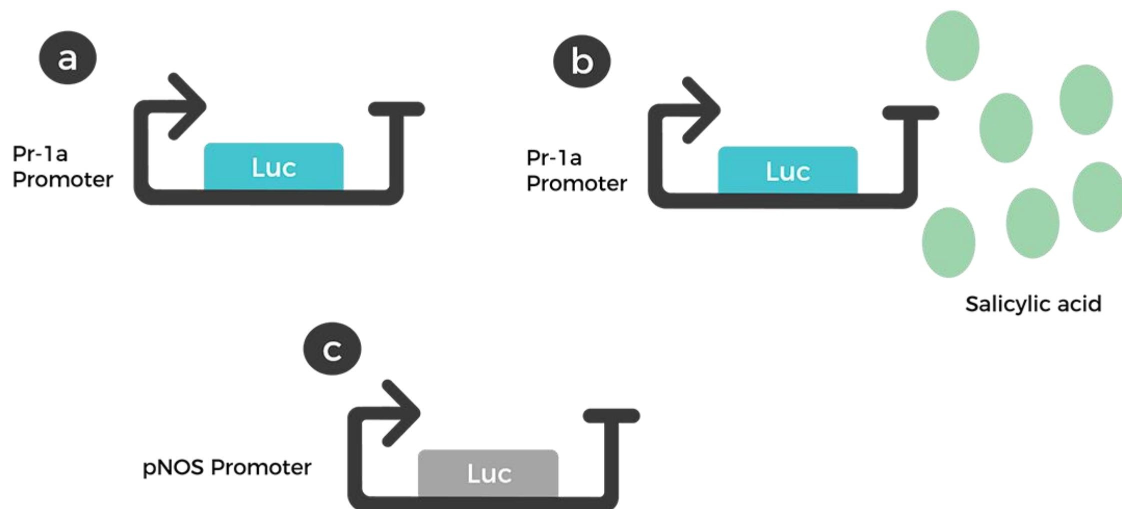


Figure 14. a) Transcriptional unit for the expression of the Firefly luciferase under the control of a stress promoter. b) Transcriptional unit for the expression of the Firefly luciferase under the control of a stress promoter when Salicylic acid is being synthesized. c) Transcriptional unit for the expression of the Firefly luciferase under the control of a weak promoter.

Method

An agroinfiltration and subsequent luciferase assay were performed in order to study gene expression at transcriptional level. The final Optical Density of genetic constructs was 0,1. A triplicate sampling of different plants was performed from 48h post infiltration onwards in order to take account for biological variability due to unknown or uncontrollable conditions.

Timeline

Genetic constructs were agroinfiltrated and after 70h post-infiltration, all plants were sampled.