chatter plant

NOTEBOOK

Universitat Politècnica de València
Valencia UPV iGEM 2017

iGEM UPV VALENCIA

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

04-06-17

- Take from the glycerinates of Goldenbraid Collection

PLASMID	GB CODE
PDGB3 A1	GB0015
PDGB3 A2	GB0017
PDGB3 Ω1	GB0019
PDGB3 Ω2	GB0021
PUPD2	GB0307
35S:VP16:PHYB:TNOS	GB1099
ETR8MINICMV	GB1097
PHIC31	GB1496
TNOS	GB0037
ATTR_AT_T_ATTL	GB1506
35S_YFP_TNOS	GB1483
TNOS:LUC:5'UTR35S	GB1499

05-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of all the GB parts refreshed yesterday. (List above)
- Digestion of minipreps and incubate 1hour at 37°C.
- Prepare infiltration of PVX-dsRED for the experiment we designed to check systemic movement of this viral particle.

  PVX-dsRED 1 → Vi=(0,1*40)/(0,47*5)= 1,7ml culture 38,3ml medium

  PVX-dsRED 2 → Vi=(0,1*40)/(0,44*5)= 1,82ml culture 38,18ml medium

- Take from the glycerinates of Goldenbraid Collection

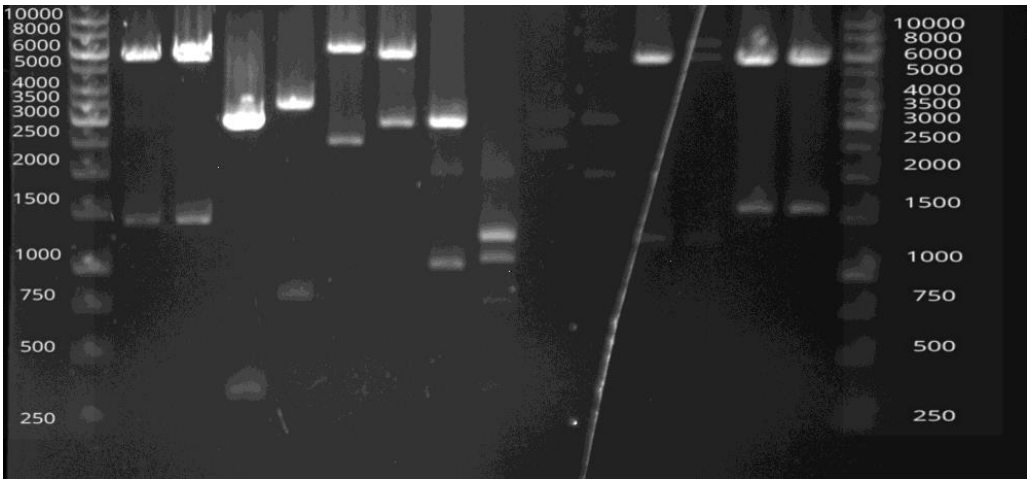
PLASMID	GB CODE
35S:E:PIF6:TNOS	GB0170
E:PIF6	GB0288
PHYB:VP16	GB0289

06-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of:
  - 35S:E:Pif6:Tnos
  - E:Pif6
  - PhyB:VP16
- Digestion of minipreps and other minipreps' plasmids we couldn't do yesterday because enzymes were not available. Incubate 1hour at 37°C.

MINIPREP	ENZYME	FRAGMENTS
ETR8:MINICMV	EcoRI	2997/481
35S:E:PIF6:TNOS	EcoRI	6345/2517
35S:E:PIF6:TNOS	PvuI	5937/2925
E:PIF6	NotI	2981/1038
E:PIF6	BanI	499/345/1097/1283/795
PHYB:VP16	NotI	2981/2481
PHYB:VP16	EcoRI	478/2997/1987
OMEGA 1	PvuI	5867/1438
ALPHA1-R	PvuI	1276/5693
ALPHA2-R	PvuI	1276/5693
OMEGA1-R	PvuI	1577/5722
OMEGA2-R	PvuI	1577/5722

- Run electrophoresis gel of TFL and Ga20ox PCR products. 45 mL agarose gel at 1 % 0.45 g of agarose with 45 mL of TAE 1X. Voltage used is 120 V.



- Ligation using Golden Braid assembly of E:Etr8:PhiC31:Tnos in α1 plasmid.

REAGENT	VOLUME (µL)
A1 PLASMID	1
E:ETR8	1
PHIC31	1
TNOS	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSAI	1
T4 LIGASE	1
H2O MILIQ	3,6

- Take from the glycerinates of Goldenbraid Collection

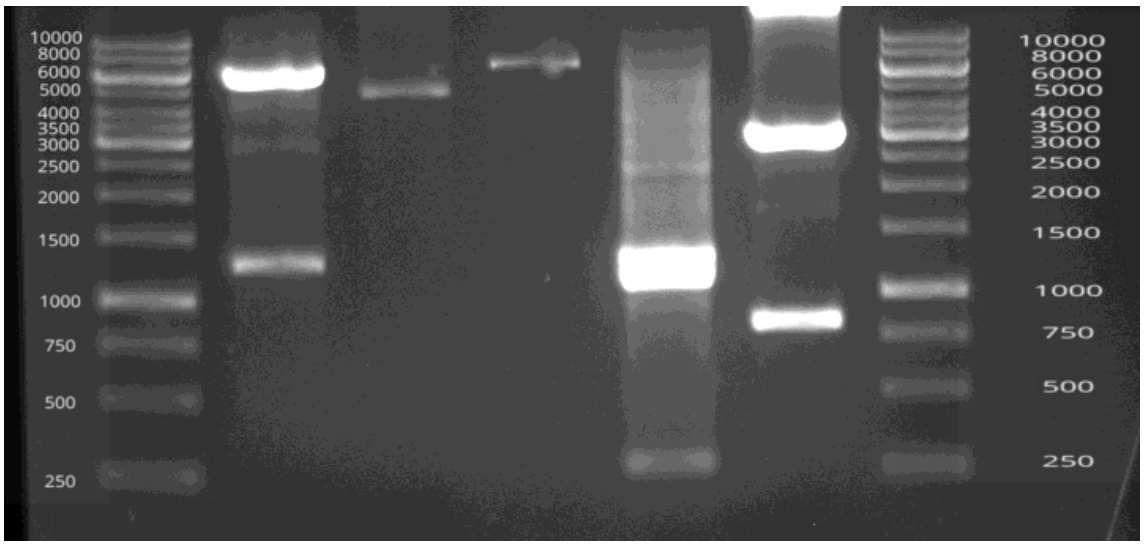
PLASMID	GB CODE
YFP (CDS)	GB0053
PHYB:VP16	GB0289

07-06-17

- Transformation in DH5α E. coli of E:Etr8:PhiC31:Tnos in α1 plasmid.
    - Plating E. coli transformations in plates with LB + agar + IPTG + XGal and incubate it overnight at 37˚C.
- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of:
    - Alpha2-R
    - YFP (CDS)
    - PhyB:VP16
- Digestion of minipreps and incubate 1hour at 37°C.

MINIPREP	ENZYME	FRAGMENTS
ALPHA2-R	PvuI	1276/5693
YFP (CDS)	NotI	2981/807
YFP (CDS)	BanI	919/1097/1283/489
PHYB:VP16	NotI	2981/2481
PHYB:VP16	EcoRI	499/345/1097/1283/795

- Run electrophoresis gel of TFL and Ga20ox PCR products. 45 mL agarose gel at 1 % 0.45 g of agarose with 45 mL of TAE 1X. Voltage used is 120 V.



Note: Digestion of PhyB:VP16 gone wrong, we suppose the glycerinate is contaminated. We took it again from the glycerinate backup.

10-06-17

- Oligos IG17JUN01_FTforward_ACCT and IG17JUN02_FTreverse_GCTT arrived.

13-06-17

- Resuspend gBlocks following pertinent instructions.
- Ligation using Golden Braid assembly of FT in pUPD2, and GmPRP2 in pUPD2.

REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
FT/ GMPRP2	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6

- Pick some E. coli DH5α (PhyB:VP16) colonies from the plate that has been incubated overnight. Inoculate a starter culture of 4 ml of LB medium with 4 µL in a 50 ml tube with the colony and incubate it overnight at 37˚C with shaking.

14-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of PhyB_VP16_NLS in pUPD.
- Digestion of the miniprep. → It is ok.
- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of 35S:dsRed:Tnos and Register Assembly 1/3 YFP.

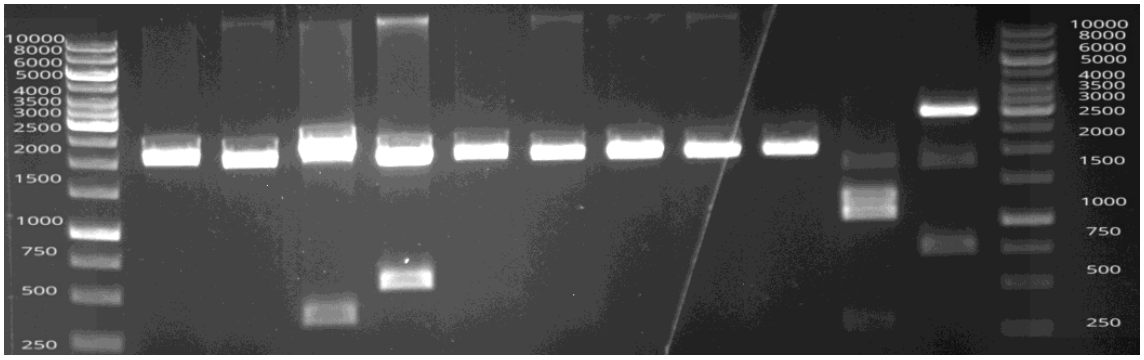
NOTE: Register Assembly 1/3 YFP gone wrong. Sequence is not correct.

19-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of FT in pUPD2, GmPRP2 in pUPD2 and dsRed.
- Digestion of minipreps and incubate 1hour at 37°C.

MINIPREP	ENZYME	FRAGMENTS
FT	EcoRI	2250/384
FT	NotI	2046/588
GMPRP	NotI	2046/915
DSRED	NotI	2981/753
DSRED	BanI	843/1097/1283/511

- Run electrophoresis gel of TFL and Ga20ox PCR products. 45 mL agarose gel at 1 % 0.45 g of agarose with 45 mL of TAE 1X. Voltage used is 120 V.



- Take photos of PVX-dsRed experiment. Plants infiltrated in sleeves, stem, and injection in stem and roots.
- Take from the glycerinates of Goldenbraid Collection
  - 35S:phiC31:Tnos
  - Pless:phiC31:Tnos
  - Pnos:phiC31:Tnos
  - YFP:Luc → Register assembly 2/3

- Ligation using Golden Braid assembly of GmPRP2 in pUPD2, again; 35S:FT:Tnos in alpha1, Etr8:miniCMV:FT:Tnos in alpha2.

REAGENT	VOLUME (µL)
A1 PLASMID	1
35S / ETR8:MINICMV	1
FT	1
TNOS	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSAI	1
T4 LIGASE	1
H2O MILIQ	3,6

20-06-17

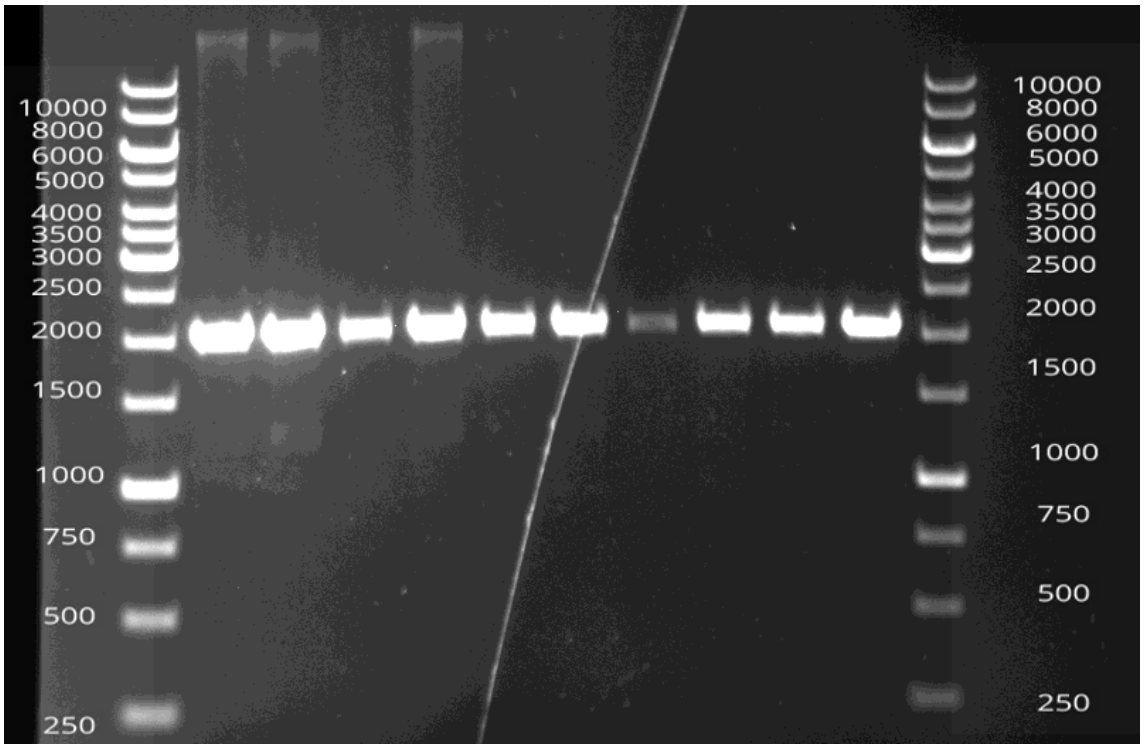
- Ligation using Golden Braid assembly of PhyB:VP16_35S:Pif6:Tnos in omega1.

REAGENT	VOLUME (µL)
Ω1 PLASMID	1
PHYB:VP16	1
35S:PIF6:TNOS	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	4,6

- PCR reaction of FT

REAGENT	VOLUME(ML)	**PROGRAM**		
		Temperature	Time	Repeats
TEMPLATE	1	98˚C	5 minutes	-
BUFFER HF	10	98˚C	30 seconds	35x
DNTPS	2	72˚C	30 seconds	35x
IG17JUN01_FTFORWARD	2.5	72˚C	30 seconds	25x
IG17JUN02_FTREVERSE	2.5	72˚C	10 minutes	-
TAQ PHUSION	0.5	16°C	∞	-
H2O MILLI-Q	31.5			

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of GmPRP2 in pUPD2.
- Digestion of minipreps and incubate 1 hour at 37°C.



- Ligation using Golden Braid assembly of AtAct2 Prom in pUPD2, and FT PCR in pUPD2.

REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
ATACT2 PROM / FT PCR	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6

21-06-17

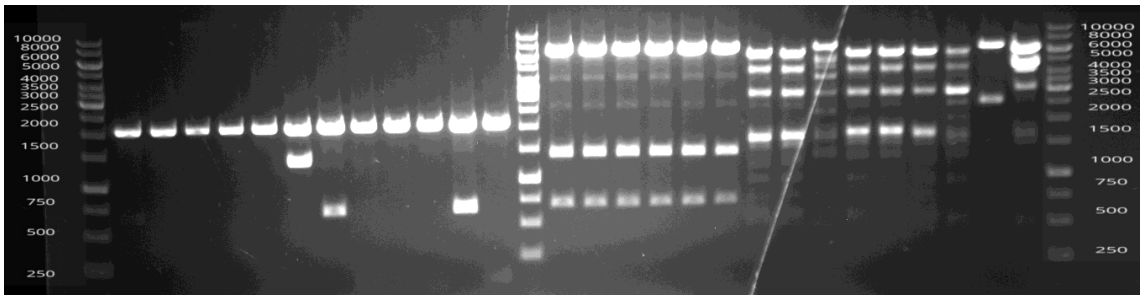
- Ligation using Golden Braid assembly of PhyB:VP16_35S:Pif6:Tnos in omega1, again.
- Pick some E. coli DH5α colonies of 35S:FT:Tnos, Etr8:miniCMV:FT:Tnos and GmPRP2 in pUPD2 y AtAct2 Prom in pUPD2, from the plates that have been incubated overnight. Inoculate a starter culture of 4 ml of LB medium with 4 µL in a 50 ml tube with the colony and incubate it overnight at 37˚C with shaking.
- Transformation in DH5α E. coli of FT PCR in pUPD2 plasmid.
    - Plating E. coli transformations in plates with LB + agar + IPTG + XGal and incubate it overnight at 37˚C.

22-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of 35S:FT:Tnos in alpha1, Etr8:miniCMV:FT:Tnos in alpha2, GmPRP2 in pUPD2 and AtAct2 Prom in pUPD2.
- Digestion of minipreps and incubate 1hour at 37°C.

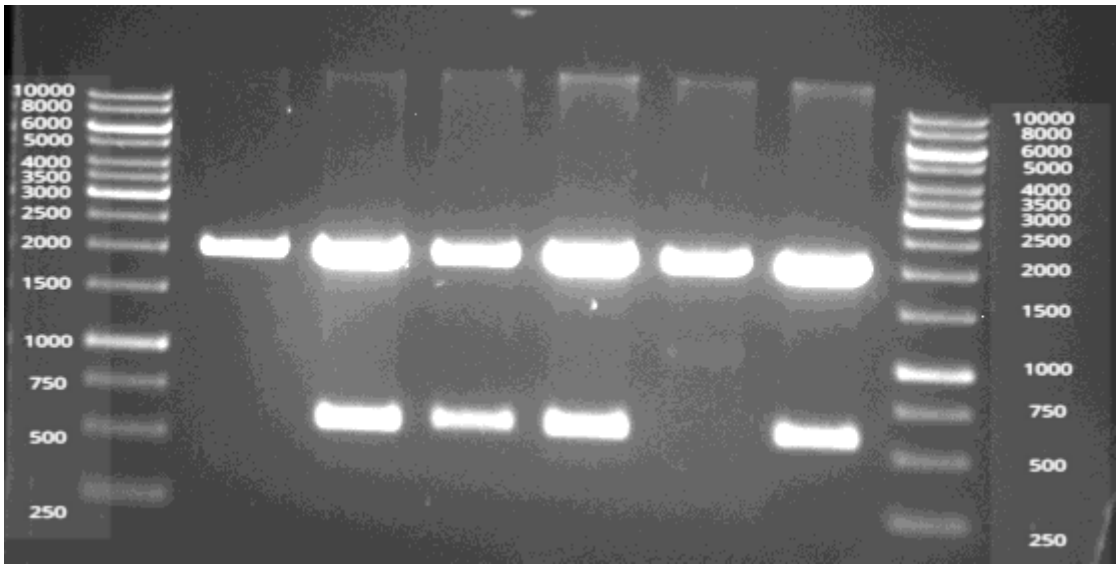
MINIPREP	ENZYME	FRAGMENTS
35S:FT:TNOS	EcoRI	692/1390/6345
ETR8:MINICMV:FT:TNOS	PvuI	5934/1887
GMPRP	NotI	2046/915
ATACT2 PROM	NotI	1405/2046
35S:PIF6:TNOS	PvuI	2925/5937
35S:PIF6:TNOS	EcoRI	2517/6345
35S:PHYB:VP16:TNOS	PvuI	5934/4373

- Run electrophoresis gel of TFL and Ga20ox PCR products. 45 mL agarose gel at 1 % 0.45 g of agarose with 45 mL of TAE 1X. Voltage used is 120 V.



23-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of FT (3/3) PCR in pUPD2.
    - Digestion of minipreps and incubate 1hour at 37°C.



- Take from the glycerinates of Goldenbraid Collection: Reporter YFP:dsRed and P19

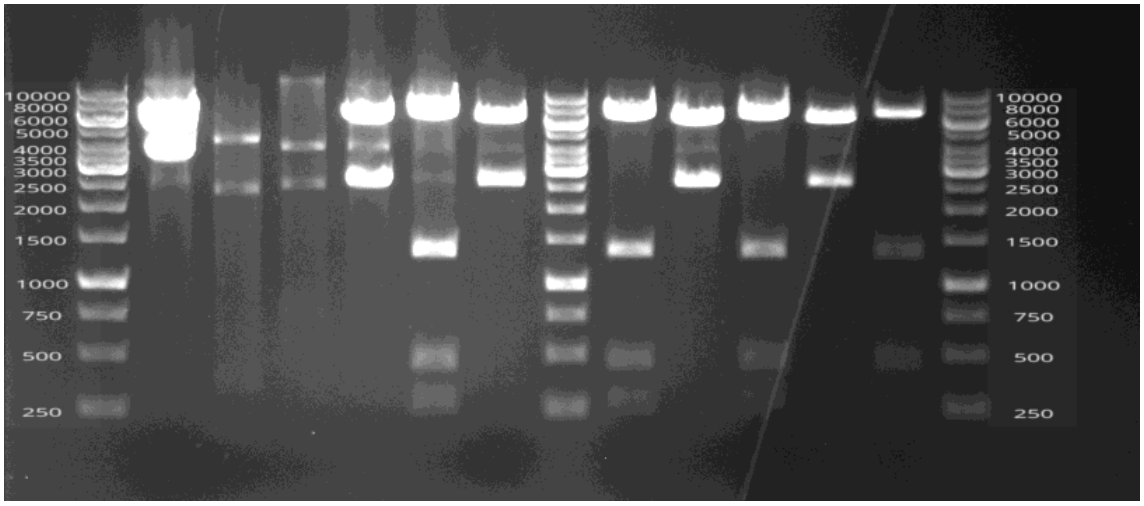
26-06-17

- Follow Agroinfiltration protocol for coinfiltration of Reported + phiC31 with P19.

27-06-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of:
    - Etr8:miniCMV:Luc + phyB+ Pif6 + S in alpha1 → GB1102
    - GB0160
    - E:Etr8:phiC31:Tnos in alpha1 (x4)
- Digestion of minipreps and incubate 1hour at 37°C.

MINIPREP	ENZYME
GB1102	EcoRI
GB0160	EcoRV
E:ETR8:PHIC31:TNOS	EcoRI



- Sequence FT PCR(3/3) miniprep.

  NOTE: After 24h we can't see fluorescence in plants we have infiltrated. So, RT-PCR is aborted.

- Repeat transformation and minipreps of 35S:FT:Tnos in alpha1, Etr8:miniCMV:FT:Tnos in alpha2, GmPRP2 in pUPD2 and AtAct2 Prom in pUPD2.
  - NOTE: GmPRP2 ligation is wrong and AtAct2 Prom digestion does not show the correct fragments.

28-06-17

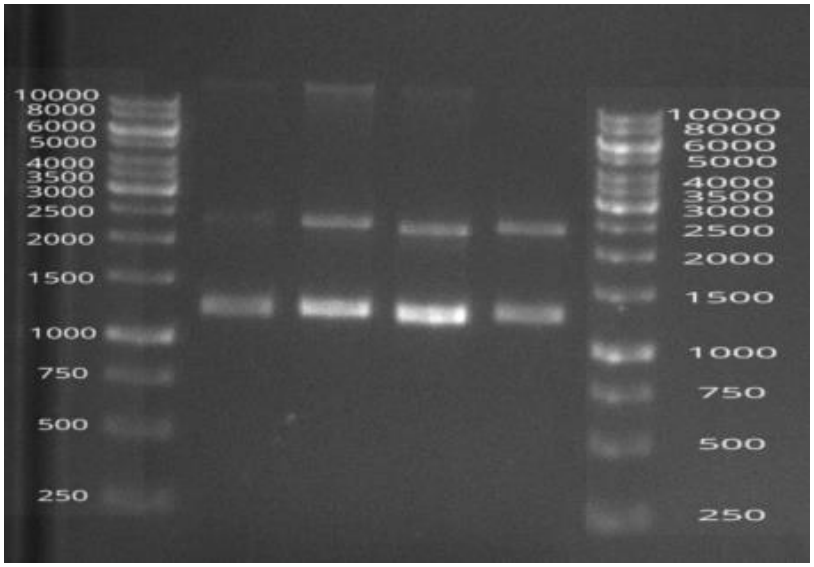
- Ligation using Golden Braid assembly of GmPRP2 in pUPD2, again. Then transformation protocol and plating E. coli transformations in plates with LB + agar + IPTG + XGal and incubate it overnight at 37˚C
- Check switch parts
  - GB1099 – 35S:VP16:PhyB:Tnos
  - GB0170 – Etr8:E:Pif6:Tnos

29-06-17

- Digestion does not work for ITD gBlock, so we run an electrophoresis agarose gel of 1,7%.
- Transformation in Agrobacterium of Luc + Pif6 + PhyB.
  - Plating transformations in plates and incubate it 2 days at 28˚C.
- Pick 4 E. coli DH5α colonies of GmPRP2 ligation.

30-06-17

- Ligation using Golden Braid assembly of AtAct2Prom in pUPD2, again. Then transformation protocol and plating E. coli transformations in plates with LB + agar + IPTG + XGal and incubate it overnight at 37˚C.
- Refresh Renilla
- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of GmPRP2 in pUPD2.
    - Digestion of minipreps and incubate 1hour at 37°C.



02-07-17

- Transformation in DH5α E. coli of 35S:FT:Tnos in alpha1 plasmid, again.
    - Plating E. coli transformations in plates with LB + agar + IPTG + XGal and incubate it overnight at 37˚C.
- Pick Agrobacterium colonies of GB1102.

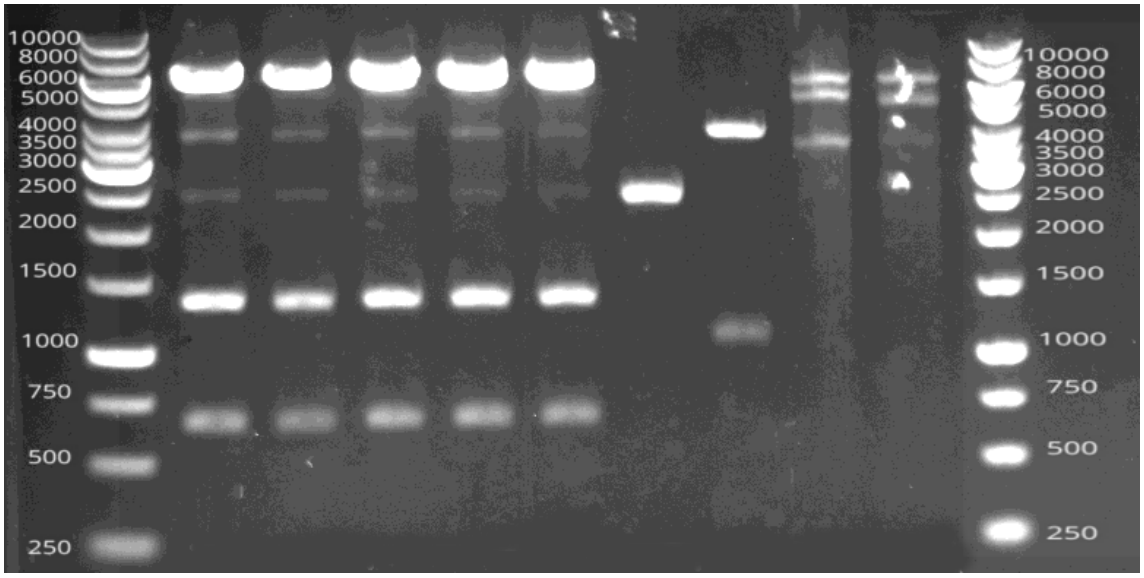
03-07-17

- Refresh Renilla
- Pick E. coli DH5α colonies of 35S:FT:Tnos.
- Sequence GmPRP2 in pUPD2
- Ligation using Golden Braid assembly of GmPRP2:PhyB:Tnos in alpha1 and GmPRP2:Pif6:Tnos in alpha2.

04-07-17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of:
  - 35S:FT:Tnos alpha1
  - Renilla – GB0109
  - GB1102 – Etr8:miniCMV:Luc:Tnos_35S:PhyB:Tnos_35S:Pif6:Tnos_SF alpha1
- Digestion of minipreps and incubate 1hour at 37°C.

MINIPREP	ENZYME	FRAGMENTS
35S:FT:TNOS	EcoRI	1390/692/6345
RENILLA	EcoRI	2574/2493
RENILLA	BanI	1124/3943
GB1102	EcoRI	3618/6345/5587



- Measure concentration in Nanodrop

PLASMID	CONCENTRATION (NG/µL)
PDGB3 A1	101,3
PDGB3 A2	110,2
PDGB3 Ω1	169,1
PDGB3 Ω2	210,8
PDGB3 A1R	49,2
PDGB3 A2R	123,4
PDGB3 Ω1R	124,6
PDGB3 Ω2R	144,5
PUPD2	41,2

05-07-17

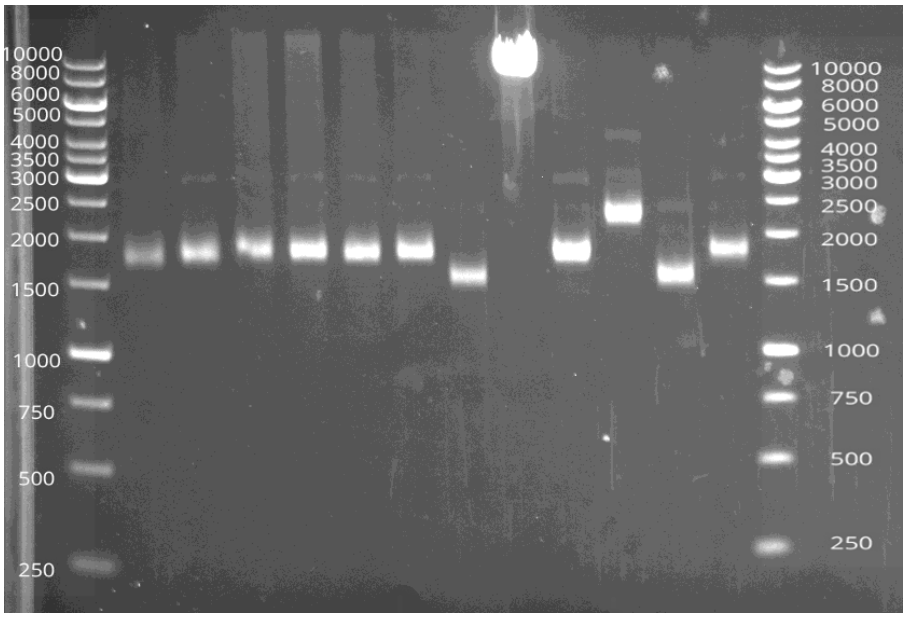
- Sequence 35S:FT:Tnos miniprep
- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of:
    - GmPRP2:PhyB:Tnos
    - GmPRP2:Pif6:Tnos
    - AtAct2Prom in pUPD2
- Digestion of minipreps and incubate 1hour at 37°C.

MINIPREP	ENZYME	FRAGMENTS
35S:FT:TNOS	EcoRI	1390/692/6345
GMPRP2:PHYB:TNOS	EcoRI	2574/2493
GMPRP2:PIF6:TNOS	BanI	1124/3943
GMPRP2:PIF6:TNOS	EcoRI	3618/6345/5587

6-07-17

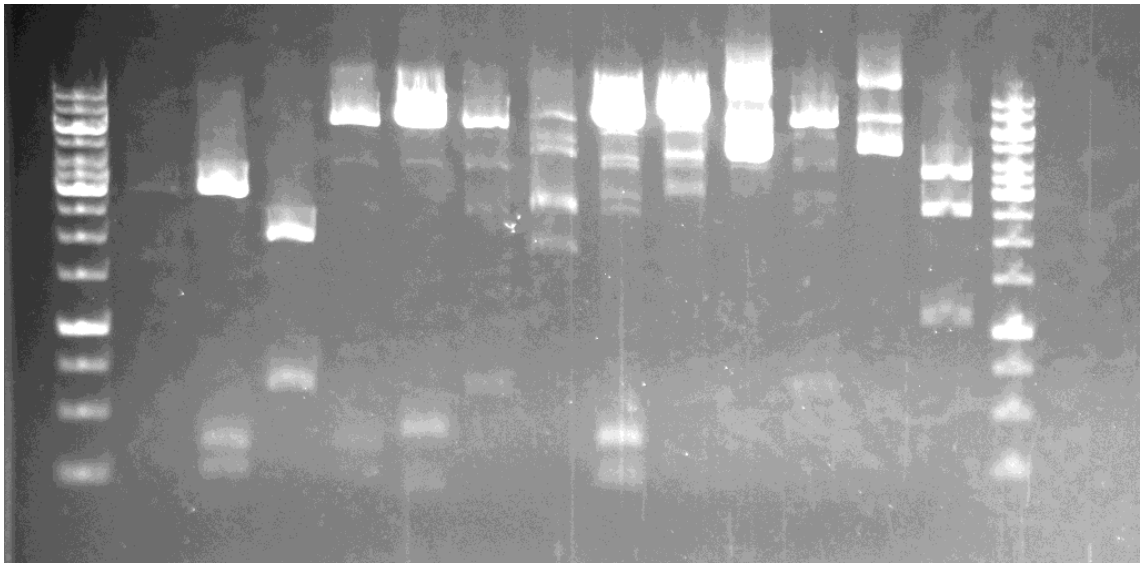
- Take from the glycerinates of Goldenbraid Collection:
    - Luc (pUPD) which its GB code is GB0096.
- Luc is taken from the glycerinate in order to do the construction GmPRP2:Luc:Tnos and to infiltrate in the roots, leafs and stem and quantify the expression.
- The digestion of the past day is repeated because the obtained bands are incorrect.

MINIPREP	ENZYME	FRAGMENTS
GMPRP2:PHYB:TNOS	EcoRI	2574/2493
GMPRP2:PIF6:TNOS	EcoRI	3618/6345/5587

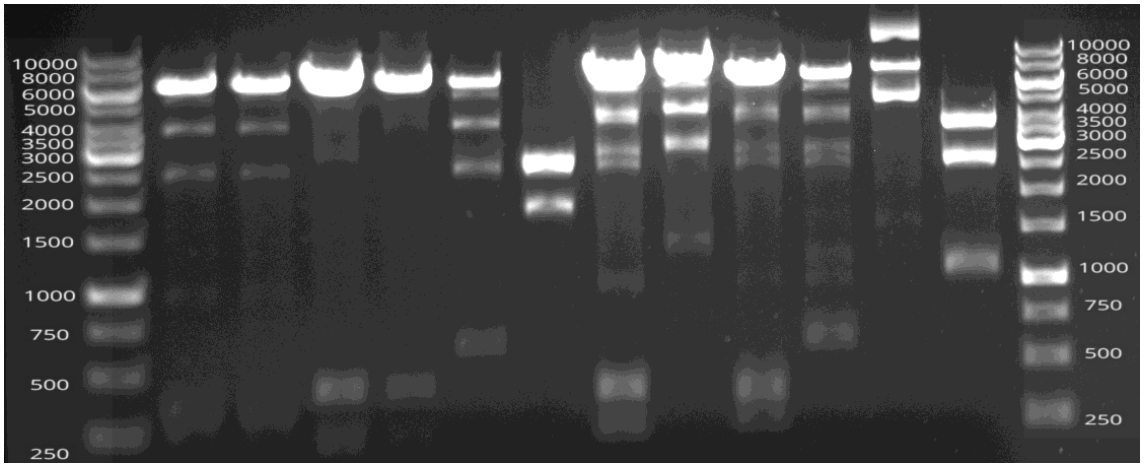


7-07-17

- Minipreps of all the plasmids again. It is supposed that they are contaminated and that is why the constructions are not correct.
- Plasmid digestion and electrophoresis.



- In order to know why our samples are contaminated, a digestion with only water is done, first with the same pipetes and after changing them.
- Digestion of α1, α2, Ω1 and Ω2 corresponding to other samples to determine where the contamination comes from.



10-07-17

- Ligation of GmPRP2 in pUPD:

REAGENT	VOLUME (µL)
PUPD PLASMID	1
GMPRP2	4
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	1,6

- Pick Agrobacterium colonies of 35S:Renilla:Tnos.
- Ligation of AtAct2Prom in pUPD:

REAGENT	VOLUME (µL)
PUPD PLASMID	1
ATACT2	4
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	1,6

11-07-17

- Transform E.Coli with GmPRP2 in pUPD and AtAct2Prom in pUPD.
- Wait 1 hour and plating in places with X-Gal+IPTG+Amp.

12-07-17

- Miniprepts of 35S:Renila:Tnos in Agrobacterium.
- Resuspend and clone Pr-1a in pUPD2.
- Triple streak of 1 GmPRP2 colony in pUPD2.
- Simple streak of AtAct2 prom in pUPD.
- Transformation and plating again GmPRP2 and AcAct2 prom in Pupd (as a second check of the streaks).
- Digestion of 35S:Ren:Tnos which GB code is GB0109, with BanI (obtaining 2 bands, 1124 and 3943) and with EcoRI (obtaining 2 bands, 2493 and 2574). With that, there is no DNA.

- Repeat the above digestion, anything is obtained again.
- With Nanodrop, it is seen that there is no DNA. The minipreps are not correct.
- Refresh the cultures of 35S:Ren:Tnos, in order to repeat the minipreps.

13-07-17

- Ligation of PR-1a in pUPD and in pUPD2.

REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
PPR-1A	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6

REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
PPR-1A	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6

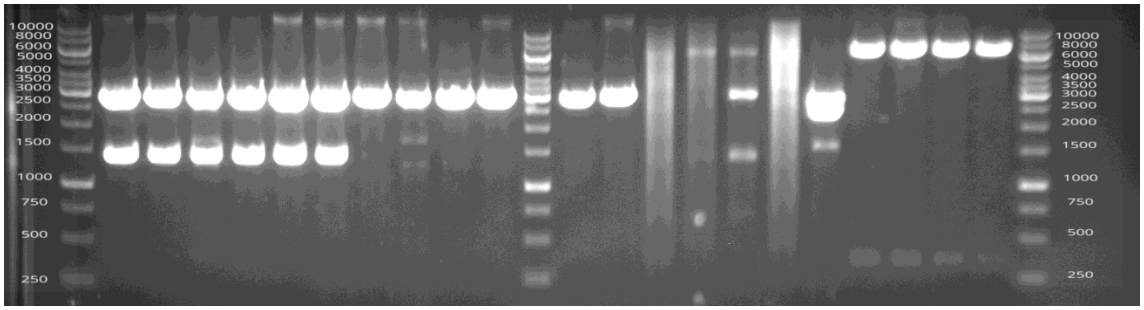
- Pick colonies of AtAct2 Prom and GmPRP2 in pUPD.
- Take from the glycerinates the Tnos:NptII:Pnos (3α1r).
- Preparation of ligation 35S:FT:Tnos(3α2) in order to do a stable with the Kan resistance.

REAGENT	VOLUME (µL)
35S	1
FT	1
TNOS	1
LIGASE BUFFER	1,2
BSA	1
BSA 10X	1,2
T4 LIGASE	1
3A2	1
H2O MILIQ	3,6

14-07-17

- Minipreps 35S:Ren:Tnos (in Agrobacterium) + AtAct2Prom, GmPRP2 and NptII.
- Pick colonies of 35S:FT:Tnos (3α1) + PR-1a (pUPD) and PR-1a (pUPD2).
- Digestions: 35S:Ren:Tnos (Agrobacterium). In order to do the mixes:

ENZYME	H20 (µL)	BUFFER(µL)	ENZYME(µL)
ECORI	25	5	5
NOTI	65	13	13
PVUI	5	13	13



- There is nothing in the pates of PR-1a in pUPD and in pUPD2 all the colonies is blue. The two plates are thrown to the garbage.
- Preparation of the ligations of the 3 promoters with pUPD2, with that, it is seen that pUPD do not have the same OH than pUPD2.

AtAct2 Prom in pUPD2

REAGENTS	VOLUME (µL)
ATACT2PROM	4
PUPD2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	1,6

GmPRP2 in pUPD2:

REAGENTS	**VOLUME (µL)**
GMPRP2	4
PUPD2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	1,6

Pr-1a in pUPD2:

REAGENTS	**VOLUME (µL)**
PPR-1A	4
PUPD2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	1,6

- Digestions:

MINIPREP	ENZYME	FRAGMENTS
35S:REN:TNOS	EcoRI	2493/2574
ATACT2P	NotI	1420/2981
GMPRP2	NotI	930/2981
NTPII	PvuI	1563/2448

15-07-17

- Transformation and plating the ligations of AtAct2Prom, GmPRP2 and pRP-1a in pUPD2.
- Take from the glycerinates the 4 cultures of 35S:FT:Tnos(3α2).
  - GB1102 → Etr8miniCMV:Luc:Tnos-35s:PhyB:VP16-35s:PIF6:Tnos (3α1). This it has to be bounded to Renilla in cis, for that it is taken from the glycerinates in 3α2.
  - GB0243 → Pnos: Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos.
  - GB0164 → 35S:Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos.
  - GB0160→Ren:P19 (E.Coli)
- GB0243 and GB0164 in Agrobacterium.

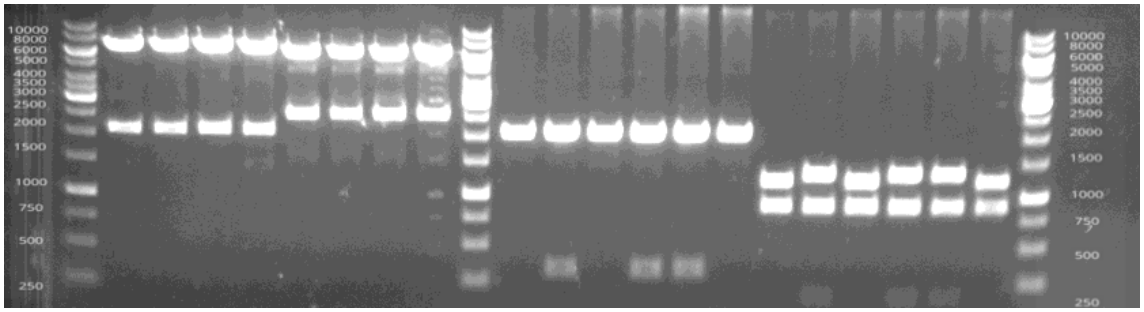
16-07-17

- Pick colonies of 3 pUPD2 and take the glycerinates above.

17-07-17

- Minipreps of FT in 3α2, GmPRP2, AtAct2Prom and pPR-1a in pUPD2 and Renilla and P19 in 3α2.
- Digestion:

MINIPREPS	ENZYMES	FRAGMENTS (BP)
FT	HindIII	2084/6345
	PvuI	2495/5934
REN	EcoRI	2475/381/4424
PR1-A	NotI	326/2046
	XhoI	1311/892/169
GMPRP2	NotI	2046/915
ATACT2	NotI	1405/2046
	XbaI	863/2588



- Electrophoresis following the same order:
  35S:FT:Tnos, Pr-1a, AtAct and GmPRP2
  Everything is correct except AtAct and GmPRP2.
- Preparation of the ligation of Ppr-1a

REAGENTS	VOLUME (µL)
PPR-1A	1
YFP	1
TNOS	1
3A2	1
BSA 10X	1,2
L.BUFFER	1,2
BSAI	1
T4 LIGASE	1
H2O	3,6

18-07-17

- Transformation in E.Coli the ligation of pPR-1a-YFT-Tnos and plating of it.
- Ligation in cis of Renilla and P1a in 3α2 with GB1102. (First, digestion with Renilla and P1a in 3α2, because nothing is obtained before, with 34,4 ng/ µL)
- Minipreps of Agrobacterium (Glycerinates above).
- Sequencing AtAct2Prom in pUPD and pPR-1a in pUPD2.
- Digestion:

MINIPREP	ENZYME	FRAGMENTS
35S:LUC:TNOS-SF-35S:REN:TNOS-35S:P19:TNOS (Ω1)	Bam HI	7904/6674
	EcoRV	3354/2475/381/8368
PNOS:LUC:TNOS-SF-35S:REN:TNOS-35S:P19:TNOS (Ω1)	BamHI	7168/6674
	EcoRV	2475/381/10986
	PvuI	2055/1746/3484

19-07-17

- Ligation of GB1102 (3α1) with Ren and P1a(3α2) in the plasmid 3α1.

REAGENTS	VOLUME(µL)
REN	3
GB1102	1
Ω1	1
BSA 10X	1,2
L.BUFFER	1,2
T4	1
BSMBI	1
H2O	2,6

- Pick colonies of PR-1a-YFP-Tnos in 3α2.

20-07-17

- Minipreps of Pr-1a:YFP:Tnos in 3α2.
- Transformation of GB1102 with Ren and P19 in 3α2 in E.Coli DH5α
- Digestion of Pr-1a:YFP:Tnos:

ENZYME	FRAGMENT
HINDIII	1524/6345
BANHI	1540/6329

- The results of the sequencing of the day 18-07-17 are correct, pPR1-1a in pUPD2 and AtActProm in pUPD.
- Ligation to insert FT in PVX:

REAGENT	VOLUME (µL)
FT	1
PVX	1
BSA10X	1,2
T4 BUFFER	1,2
L. BUFFER	1
BSAI	1
H2O	5,6

21-07-17

- Ligation of Pr-1a:YFP:Tnos, because the previous digestion was not correct

REAGENT	VOLUME (µL)
PR-1A	1
YFP	1
TNOS	1
3A2	1
LIGASE BUFFER	1,2
BSAI	1
BSA 10X	1,2
T4	1
H_2O	3,6

- Ligation of GB0160 and GB1102 (Again) with calculated concentrations of reagents with NEB

REAGENT	CONCENTRATED → DILUTED(NG/ µL)	VOLUME (µL)
GB0160	336,2	1
GB1102	42,4	7,33
Ω1	223 → 75	1
BSA 10X	--	1,5
LIGASE BUFFER	--	1,5
BSMBI	--	1
T4	--	1
H_2O	--	0,67

- Transformation and plating of both ligations

23-07-17

- Pick colonies of Pr-1a:YFT:Tnos, but Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 (GB0160 + GB1102) had just 1 white colony.

24-07-17

- Minipreps of: Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 (Ω1), Pr-1a:YFP:Tnos (3α2) from the old plate, Pr-1a:YFP:Tnos (3α2) from de new ligation and PVX:FT
- Digestion of:
    - Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 (Ω1) with EcoRV (3942, 2823, 2475, 381, 10952) y HindIII(4375,8710, 7488)

  - PVX:FT with EcoRI (2616, 2666, 9168), HindIII(1205,13245) and NotI (1290, 1532, 11628)
  - Pr-1a:YFT:Tnos with HindIII (1524, 6345) and BanII (1540,6329)
  - Everything is ok except Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 (Ω1)
- Transformation of Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 and plating in E. coli
- Ligation of Pr-1a:YFP:Tnos (3α2) and NPTII (3α1) in 3Ω1

REAGENT	VOLUMEN (µL)
PR-1A:YFP:TNOS (3A2)	1
NPTII	1
(3Ω1)	1
BSA 10X	1,2
LIGASE BUFFER	1,2
T4	1
BSMBI	1
H_2O	4,6

- Primers IG17JUN3+ IG17JUN4+ IG17JUN5+ IG17JUN6 arrived
- PCR of PVX:FT

REAGENT	VOLUMEN (µL)
DNTPS	2
POLIMERASE	0,5
PRIMERS F AND R	2,5 each
TEMPLATE	1
BUFFER HF	10
H_2O	31,5

STEP	TEMPERATURE (°C)	TIME (MIN)	CYCLES
1	98	5	1
2	98	10	35
3	72 (68)	0,5	
4	72	2,5	
5	72	10	1
6	16	--	--

- Storage at -80°C these constructions in E. coli:
  - Etr8:minicmv:FT:Tnos (3α1)
  - 35s:FT:Tnos (3α2)
  - 35s:FT:Tnos (3α1)
  - FT (pUPD2)
  - PVX:FT
  - FT 3/3 PCR (pUPD2)
  - Pr-1a (pUPD2)
  - Pr-1a:YFP:Tnos (3α2)
  - AtAct2 prom (pUPD2)
  - E:Etr8:phiC38

25/07/17

- Transformation of Ppr-1a:YFP:Tnos-NPTII in 3Ω1 and plating in E.coli.
- Transformation of Etr8:Luc:tnos + PhyB + PIF6 + Ren + P19 and plating in E. coli again, because there isn't any colonies in the previous plate.
- Previous PCR was correct (we run an electrophoresis digestion)
- Ligation of PVX-FT in pUPD2

REAGENTS	VOLUME (µL)
PVX-FT1	2
PVX:FT2	2
PUPD2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	2,6

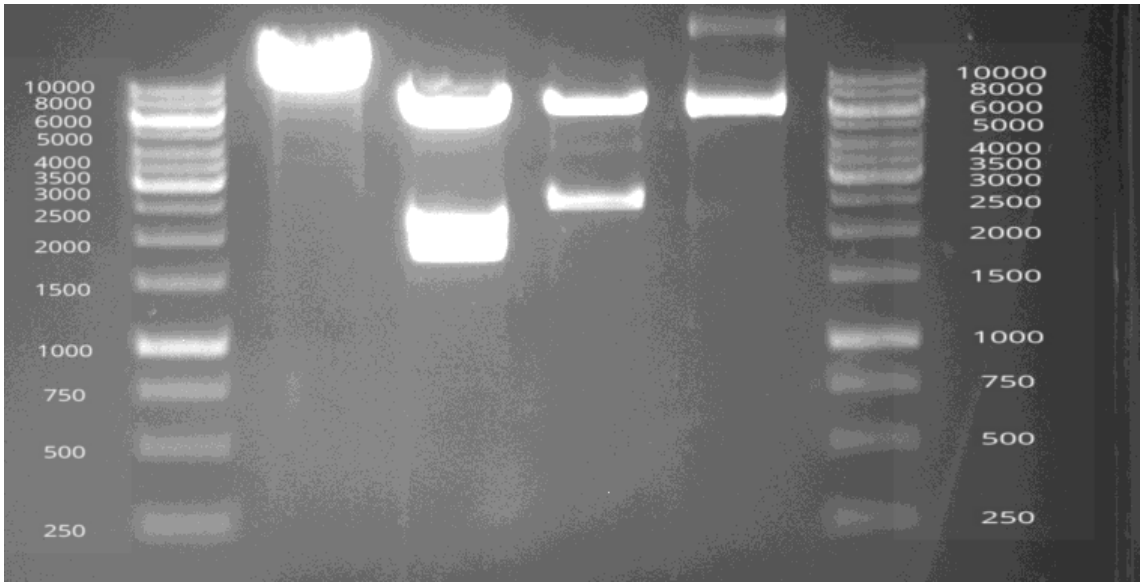
- Transformation of PVX:FT1/2 (pUPD2) and plating in E. coli
- Oligos IG17JUL01 and IG17JUL02 arrived. We will use them to sequence GmPRP2.
- Refresh 35S:Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos (Ω1) and Pnos:Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos (Ω1)

26/07/17

- Sequence GmPRP2 because cloning is going wrong.
- Pick colonies of Pr-1a:YFP:Tnos-Tnos.NPTII:35S (3Ω1)
    - No colonies grew in Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (Ω1) plate neither in PVXFT1:PVXFT2 (pUPD2)
    - Maybe competent cells are not very effective
- Repeat transformation and plating of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (Ω1) and PVXFT1:PVXFT2 (pUPD2), but with Top10 competent cells (other ones were DH5α)

27/07/17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of PR-1a:YFP:Tnos-NPTII (3Ω1)
- Minipreps of 35S:Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos (Ω1) and Pnos:Luc:Tnos-SF-35S:Ren:Tnos-35S:P19:Tnos (Ω1) with agrobacterium special protocol: 27µL Miniprep + 0,5µL Enzime + 3µL Buffer

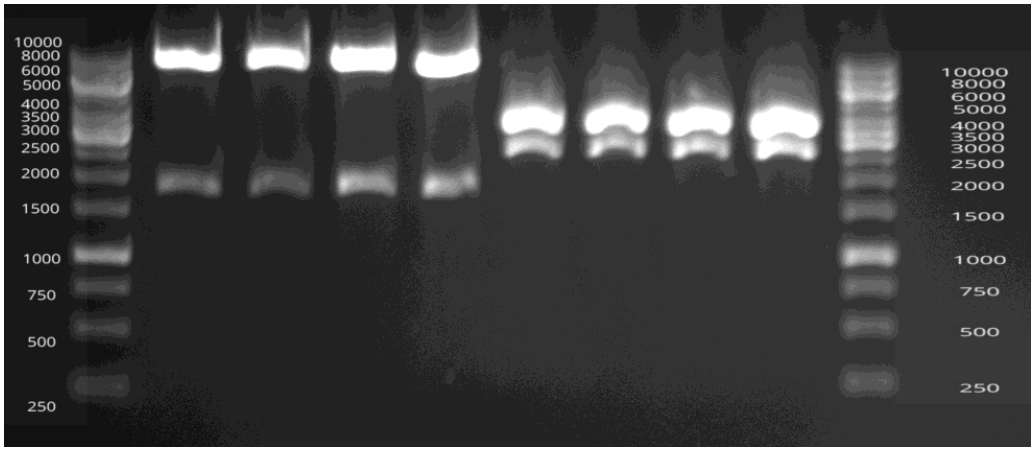


- Pick colonies of PVX:FT
- Repeat ligation of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 but in 3Ω2

REAGENT	CONCENTRATION → DILUTED(NG/ µL)	VOLUME (µL)
GB0160	336,2	1
GB1102	42,4	7,33
Ω1	61,6	1,22
BSA 10X	223 →75	1,5
LIGASE BUFFER	--	1,5
BSMBI	--	1
T4	--	1
H_2O	--	0,45

- Digestion of minipreps and incubate 1hour at 37°C.

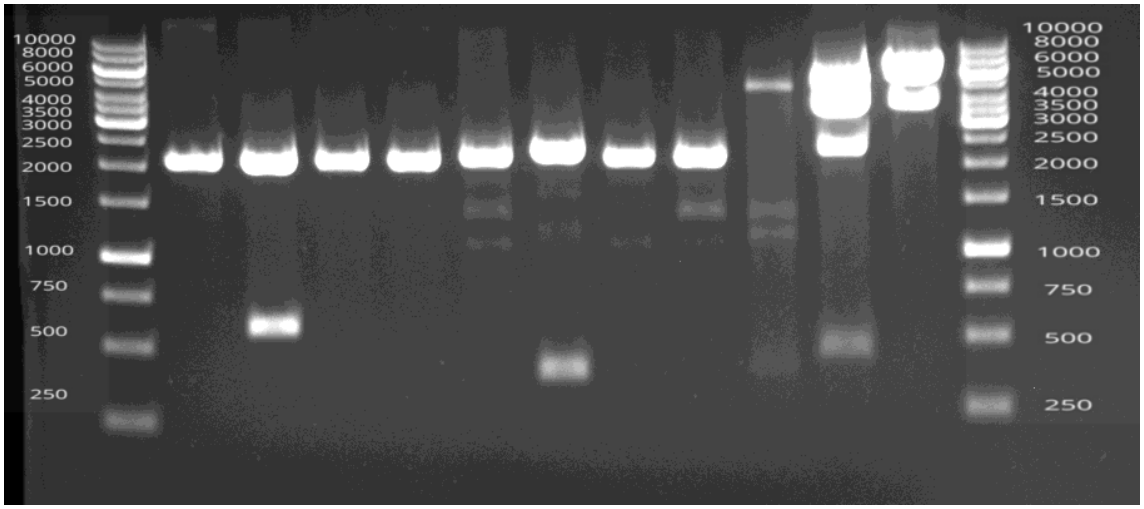
MINIPREP	ENZYME	FRAGMENTS
PR-1A:YFP:TNOS-TNOS:NPTII:35S	BanII	1767/7844
PR-1A:YFP:TNOS-TNOS:NPTII:35S	DraI	3429/3610/2572



- Digestion is correct so Transform in Agrobacterium
- Trasformation of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (3Ω2) in E.coli.

30/07/17

- Miniprep of PVX:FT
- Transformation of NPTII in Agrobacterium (stable strain)
- Digestion of PVX:FT
    - NotI – 2046/7378
    - EcoRI – 2616/2666/3367/775



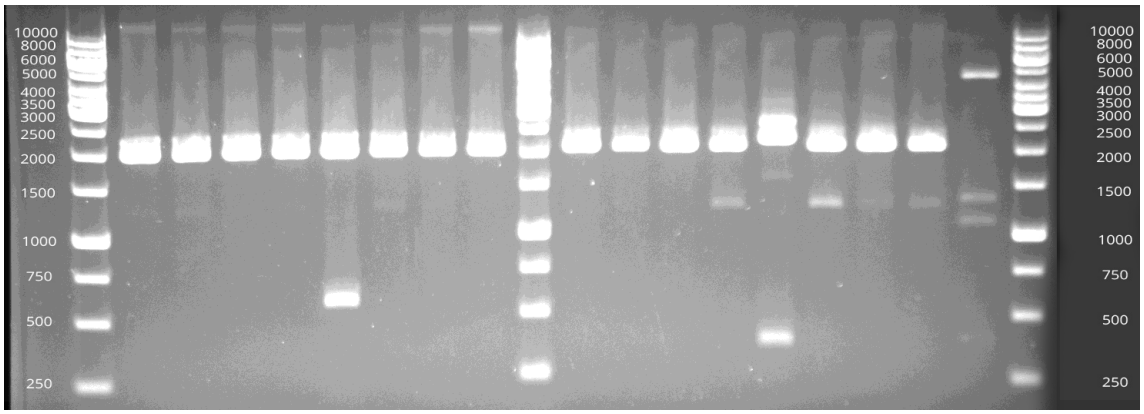
MINIPREP	ENZYME	FRAGMENTS
GB160	BanI	1124/1351/392/4418
GB1102	DraI	5456/3784/3610/2243/457
	EcoRI	3618/6345/5587

- Transformation of Ppr-1a:YFP:Tnos in C58 and plate.
- Pick 8 colonies from PVX:FT (pUPD2) plate.
- Take from the glycerinate GB0160.

31/07/17

- Minipreps of PVX:FT and GB160.
- Digestion of minipreps

MINIPREP	ENZYME	FRAGMENTS
GB160	BanI	1124/1351/392/4418
PVX:FT	NotI	2046/7378
	EcoRI	2616/2666/3367/775



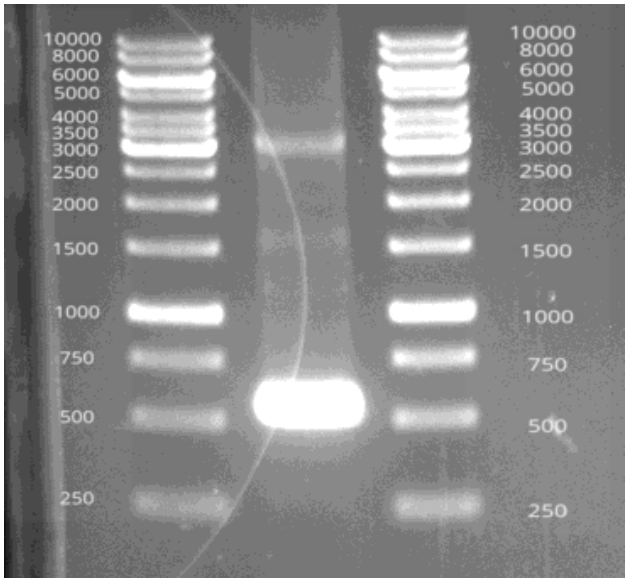
- Transformation of PVX:FT in E.coli Top 10

1/08/17

- Oligos IGJUL03 and IGJUL04 arrived.
- Pick colonies of PVX:FT (pUPD2) and Ppr-1a:YFP:Tnos (agro) and Ppr-1a:YFP:Tnos-NPTII (agro)
- PCR protocol of FT to be fusion with GFP.

REAGENT	VOLUMEN (µL)
DNTPS	2
POLIMERASE	0,5
PRIMERS F AND R	1,25 each
TEMPLATE	1
BUFFER HF	10
H_2O	34

STEP	TEMPERATURE (°C)	TIME (MIN)	CYCLES
1	98	5	1
2	98	10	35
3		15	
4	72	15	
5	72	7	1
6	16	--	--



- Take from the glycerinate GFP (3')
- Ligation of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 in 3Ω2

REAGENT	CONCENTRATION → DILUTED(NG/ µL)	VOLUME (µL)
GB1102	336,2	1
GB010	42,4	7,33
Ω1	61,6	1,22
BSA 10X	223 →75	1,5
LIGASE BUFFER	--	1,5
BSMBI	--	1
T4	--	1
H_2O	--	0,45

- Electrophoresis of the PCR → It's correct
- Ligation of FT (3') in pUPD2

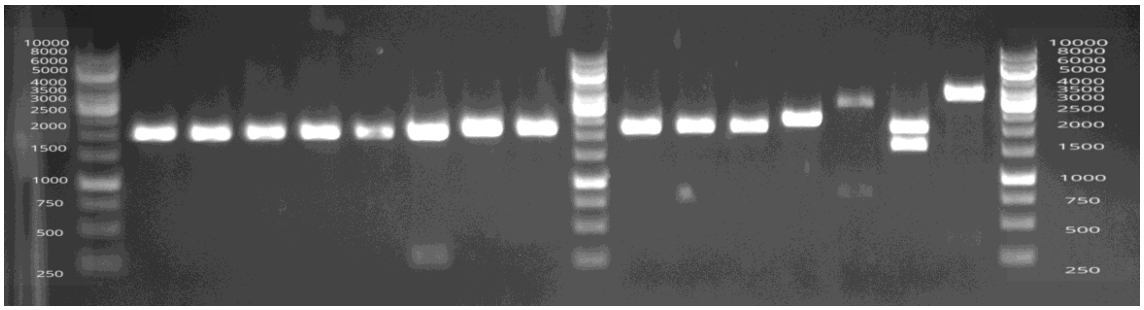
REAGENTS	VOLUME (µL)
FT	1
PUPD2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	5,6

- Transformation of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (3Ω2) in E.coli.
- Prepare special plates for stable transformation.

2/08/17

- Pick 4 colonies of Etr8:Luc:Tnos_35S:PhyB:Tnos
- Transformation of FT (3') (pUPD2) in E.Coli DH5α and plate it.
- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of PVX:FT:PVX, GB1182 and GB1494.
- Digestion and electrophoresis of minipreps.

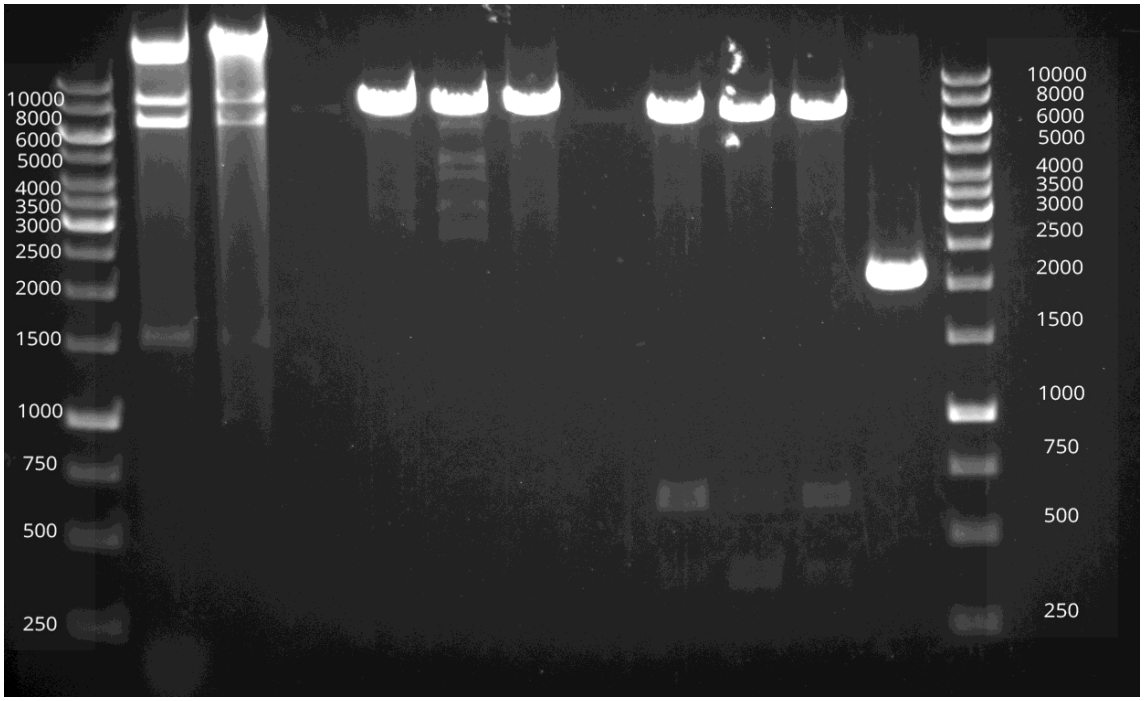
MINIPREP	ENZYME	FRAGMENTS
PVX:FT:PVX	NotI	2046/7378
	EcoRI	2616/2666/3367/775
GB1494	NotI	1573/2046
	BanI	346/3273
GB1182	NotI	2981/798



3/8/17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (3Ω2)
- Pick colonies of FT (3') in pUPD2 and PR-1a:YFP:Tnos_NPTII (Ty culture)
- Minipreps of PR-1a:yfp:Tnos with special protocol QUIAprep for agrobacterium.
- Digestion and electrophoresis of

MINIPREP	ENZYME	FRAGMENTS
PR-1A:YFP:TNOS (3Ω2)	HindIII	1524/6345
ETR8:LUC:TNOS+ PHYB + PIF6 + REN + P19 (3Ω2)	HindIII	1573/2046
	EcoRV	3942/2823/2475/381/10952



NOTE: Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 (3Ω2) is still incorrect.

4/8/17

- Miniprep with E.Z.N.A ®. Plasmid Mini Kit I, Q(capless) Spin of FT (3') pUPD2
- Measure concentration in Nanodrop
    - Ω2 – 52,9 ng/µl
    - GB0160 – 45,3 ng/µl
    - GB1102 – 298,6 ng/µl
- Digestion and electrophoresis of

MINIPREP	ENZYME	FRAGMENTS
Ω2	HindIII	621/6674
FT (3')	NotI	2046/588
	EcoRI	2250/385
GB0160	HindIII	788/2573/3924
	EcoRV	2475/381/4429
GB1102	EcoRI	3618/6345/5587
	BamHI	3756/11794

NOTE: FT (3') 3 is correct and also Ω2, GB0160 and GB1102.

7/8/17

- Repeat ligation with new concentrations of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 but in 3Ω2

REAGENT	CONCENTRATION → DILUTED(NG/ µL)	VOLUME (µL)
GB0160	45,3	6,6
GB1102	298,6	1
Ω1	59,2	1,27
BSA 10X	223 →75	1,5
LIGASE BUFFER	--	1,5
BSMBI	--	1
T4	--	1
H_2O	--	1,13

- Ligation of 35S:FT:GFP:Tnos in 3α2

REAGENTS	VOLUME (µL)
FT (3')	1
GFP (3')	1
TNOS	1
35S	1
3A2	1
BSA 10X	1,2
L.BUFFER	1,2
BSMBI	1
T4	1
H2O	2,6

9/8/17

- Transformation and plate ligations of 35S:FT:GFP:Tnos in 3α2 and of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 in 3Ω2
- Oligos IG17AG01, IG17AG02, IG17AG03 and IG17AG04 arrived. We will use them for Gibson reaction of TEV.
- PCR of TEV with Phussion polymerase.

REAGENT	VOLUMEN (µL)
DNTPS	2
POLIMERASE	0,5
PRIMERS IG17AG01 AND IG17AG02	1,25 each
TEMPLATE	1
BUFFER HF	10
H_2O	34

STEP	Temperature (°C)	Time (min)	Cycles
1	98	5	1
2	98	10s	35
3	62	15s	
4	72	3,4	
5	72	7	1
6	16	--	--

- PCR of Register Assembly 2/3 with Phussion polymerase.

REAGENT	VOLUMEN (µL)
DNTPS	2
POLIMERASE	0,5
PRIMERS IG17AG03 AND IG17AG04	1,25 each
TEMPLATE	1
BUFFER HF	10
H_2O	34

STEP	TEMPERATURE (°C)	TIME (MIN)	CYCLES
1	98	5	1
2	98	10s	35
3	72	15s	
4	72	23s	
5	72	7	1
6	16	--	--

- Band purification of both PCRs reactions.

10/8/17

- Pick colonies of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 in 3Ω2
- GIBSON reaction.
- Measure of concentration with Nanodrop of
    - TEV- 59,7 ng/µl
    - Assembly – 64,7 ng/µl

11/8/17

- Minipreps of Etr8:Luc:Tnos+ PhyB + PIF6 + Ren + P19 in 3Ω2 and 35S:FT:GFP:Tnos.

- Digestion and electrophoresis of

MINIPREP	ENZYME	FRAGMENTS
ETR8:LUC:TNOS+ PHYB + PIF6 + REN + P19 (3Ω2) 35S:FT:GFP:TNOS	HindIII	1573/2046
	EcoRV	3942/2823/2475/381/10952
	HindIII	2819/6345
	PvuI	3230/5934

NOTE: Still incorrect. We would infiltrate in trans instead of in cis. 35S:FT:GFP:Tnos is also incorrect but we realized we have to ligate again but with 35S without ATG, and GFP (instead of GFP (3')).

- Prepare cultures for agroinfiltration assay
  - Register RL YFP:Luc_Ren_P19 + ΦC31 (NEGATIVE CONTROL)
  - Register PB YFP:Luc_Ren_P19 (POSITIVE CONTROL)

12/8/17

- Do the agroinfiltration of the cultures, in order to characterize the reduction of luciferase expression once we add the ΦC31.

13/8/17

- Take from iGEM'16 plate the chromoproteins AmajLime and eForRed. Both are transformed and plated.
- Pick colonies of 35s:FT:GFP:Tnos from the miniprep which it seems to be okay because one of the 2 bands had no DNA but ligate anyway (Ligation of 35s:GFP:Tnos in 3α2)
- Refresh ΦC31 to infiltrate tomorrow

14/8/17

- Digestion of 35s:FT:GFP:Tnos → It's not correct
- Chromoproteins haven't grown, so we transform again. After this we notice that AmajLime has indeed grown so we pick colonies of it and plate anyways.

16/8/17

- Minipreps, digestion and electrophoresis of AmajLime and 35s:FT:GFP:Tnos

MINIPREP	ENZYME	FRAGMENTS
AMAJLIME	PvuII	313/380*
35S:FT:GFP	HindIII	2819/6345
	PvuI	3230/5934

* We didn't notice that the virtual digestion we did was only of the insert and no the plasmid backbone and the insert, so we thought it was wrong. 35s:FT:GFP:Tnos was wrong for sure.

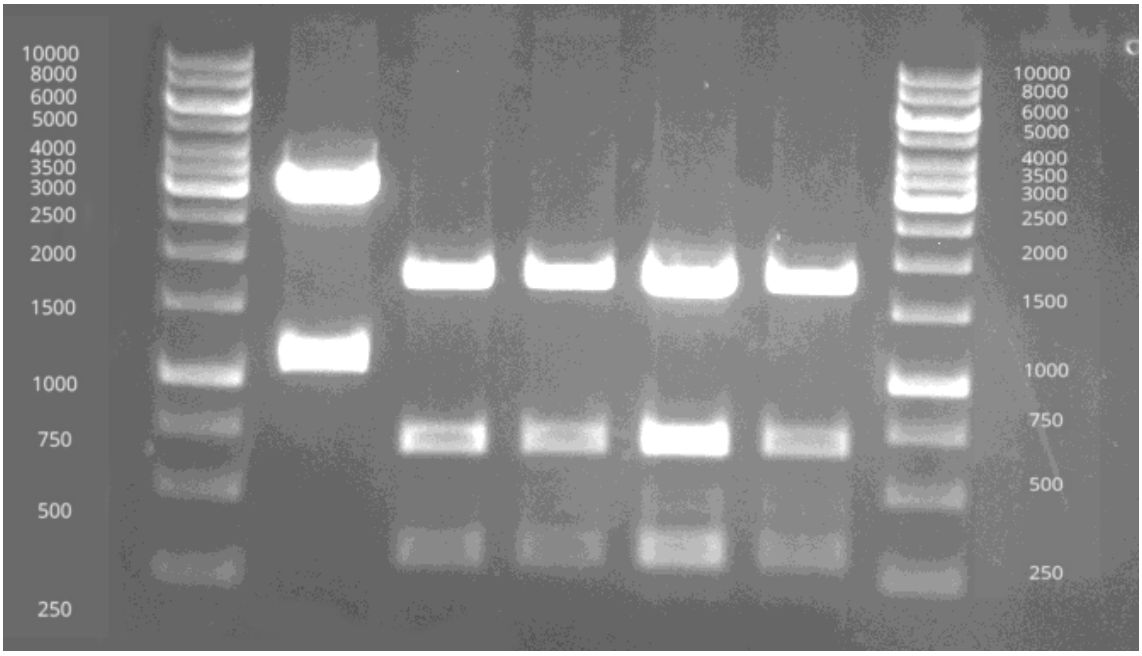
- The plate of AmajLime has grown but eForRed hasn't (again). We read on iGEM DNA distribution kit help pages that the optimal concentration of Chloramphenicol is 25 µg/µL, but in our laboratory we use 34 µg/µL, so we dilute chloramphenicol in the new plates.
- Transform and plate eForRed on the new, diluted plates.
- We finally make the Gibbson assembly of TEV and Register assembly and we purify it.
- Refresh the items needed for next week experiment:
    - Pnos: ΦC31:Tnos
    - RDF
- Take from stock everything else we need for the experiment:
    - Register assembly (PB)
    - Register assembly (RL)
- We notice that the PCR for FT to fuse with GFP was designed with standard GFP (not GFP ready for 3' fusions) and 35s without ATG overhang. So we take from stock GB0552 (35s without ATG)
- PCR with genomic DNA and the primers, to know if agrobacterium integrates the insert in plant's genome

GENOMIC DNA PCR	PLASMID DNA PCR (POSITIVE CONTROL)	TEMPERATURE (°C)	TIME
1 µL TEMPLATE	1 µL Template	98	5 min
1,25 µL IV16ABR01	1,25 µL IV16ABR01	98	35x 10 s
1,25 µL IV16ABR03	1,25 µL IV16ABR03	68	35x 30 s
10 µL BUFFER HF	10 µL Buffer HF	72	35x 20 s
2 µL DNTPS	2 µL dNTPs	72	7 min
0,5 µL PHUSION	0,5 µL Phusion	16	Hold
34 µL H_2O	34 µL H_2O	---	

17/8/17

- Minipreps of GB0552 and AmajLime

GB0552	ECORI	2997/1090
AMAJLIME	PvuII	313/692/1756



- Test the genomic PCR
- Measure AmajLime and eForRed at Nanodrop: 115,3 and 110,0 ng/µL, respectively
- Correct ligation of 35s:FT:YFP:Tnos in 3α2.

1 µL 35S	1 µL TNOS	1 µL T4 LIGASE
1 µL FT	1,2 µL T4 Ligase buffer	1 µL BsaI
1 µL TNOS	1,2 µL Bsa 10x	2,6 µL H_2O

- Transform and plate the ligation
- We made the luciferase assay to finish the experiment

18/08/17

- We refresh the cultures to infiltrate on Saturday at 16:00 ()
- Pick colonies of 35s:FT:GFP:Tnos
- Genomic DNA extraction:
  - 8,4 µL of Mercaptoethanol (20 µL/mL)
  - 6,72 µL of RNAsa (1,6 µL/mL)
  - 4200 µL of CTAB buffer (20 g/L)
- gBlocks of Optimized chromoproteins (AmajLime, AmilCP and eForRed) arrived, so we resuspend it and put a ligation on pUPD2

19/08/17

- Transform and plate optimized Chromoproteins ligations on pUPD2
- Minipreps of 35s:FT:GFP:Tnos on 3α2
- Pick colonies of eForRed from iGEM plate, but TEV has not grown
- Digestion of 35s:FT:GFP:Tnos
    - HindIII: 2819/6345 and PvuI: 3230/5934
    - We though that it was correct but it wasn't, so the next days we tried to transform this construction on A. tumefaciens. Obviously, it didn't worth.

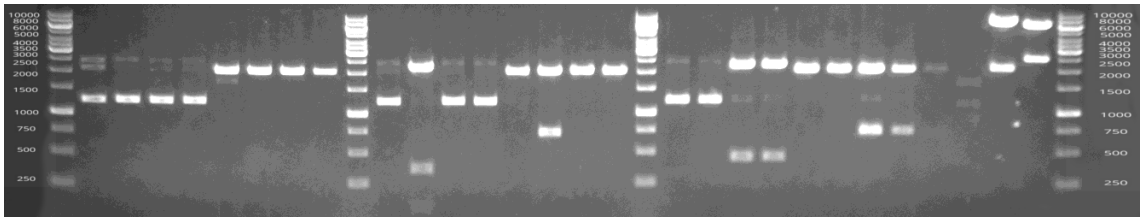
20/08/17

- Refresh cultures for infiltration
- Pick colonies of optimized chromoproteins
- Transform and plate 35s:FT:GFP:Tnos on Agrobacterium

21/08/17

- Minipreps of optimized chromoproteins on pUPD2, 35s:FT:Tnos on 3α2 and eForRed biobrick

AMAJLIME OPT	**NOTI: 753/2046**	**HINDIII: 432/2367**
AMILCP OPT	NotI:729/2046	HindIII: 96/88/363/2228
EFORRED OPT	NotI: 741/2046	HindIII:6/459/2262
EFORRED BIO	Not: 702/2046	EcoRV: 1611/1137
35S:FT:TNOS (3A2)	HindIII: 2084/6345	PvuI: 2495/5934



- AmilCP, eForRed Opt and eforred Bio are correct so we put a ligation with 35s and Tnos.

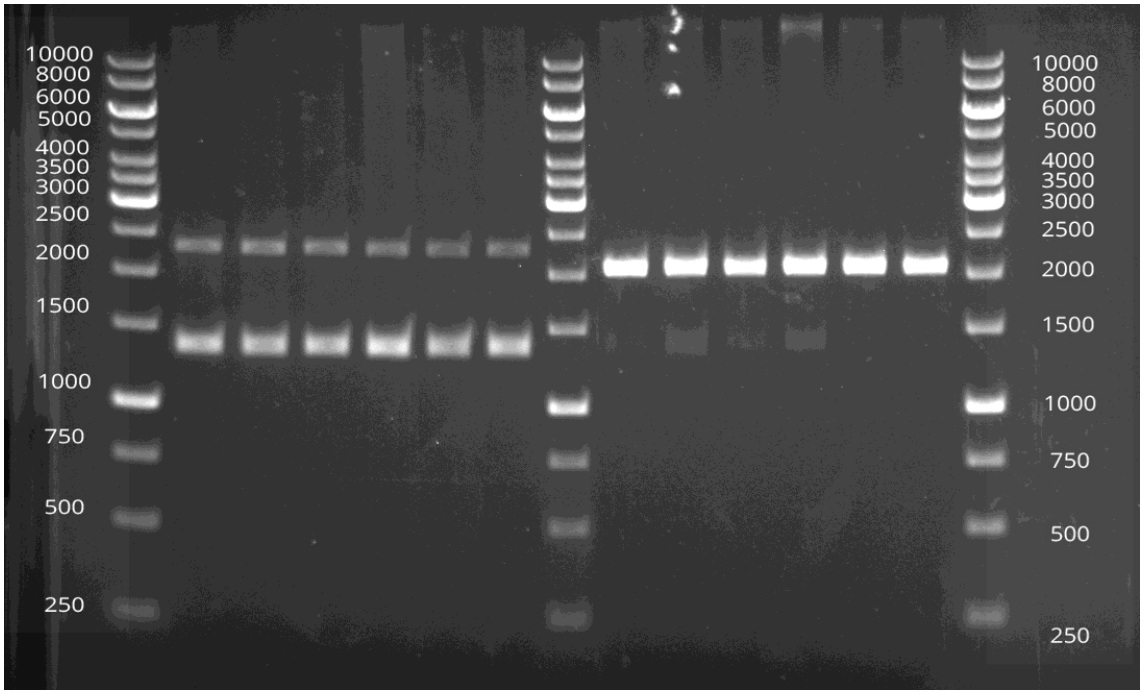
REAGENT	VOLUME
35S	1 µL
TNOS	1 µL
3A2	1 µL
BSA10X	1,2 µL
T4 LIGASE	1,2 µL
BSAI	1 µL
H_2O	2,6 µL
CHROMOPROTEIN	1 µL

- gBlocks of SF 1/3 and amilCP bacteria optimized arrived, so we resuspend it and put a ligation in pUPD2. We also put the ligation we'll send to Cardiff Team (Pr-1a:Luc:Tnos).

REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
SF 1/3 OR AMILCP	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6
REAGENT	Volume
PR-1A	1 µL
TNOS	1 µL
3A2	1 µL
BSA10X	1,2 µL
T4 LIGASE	1,2 µL
BSAI	1 µL
H_2O	2,6 µL
LUCIFERASE	1 µL

22/08/17

- Minipreps of chromoproteins AmajLime (pUPD2)
- Digestion of previous minipreps. → Gone wrong and we repeat the ligation.

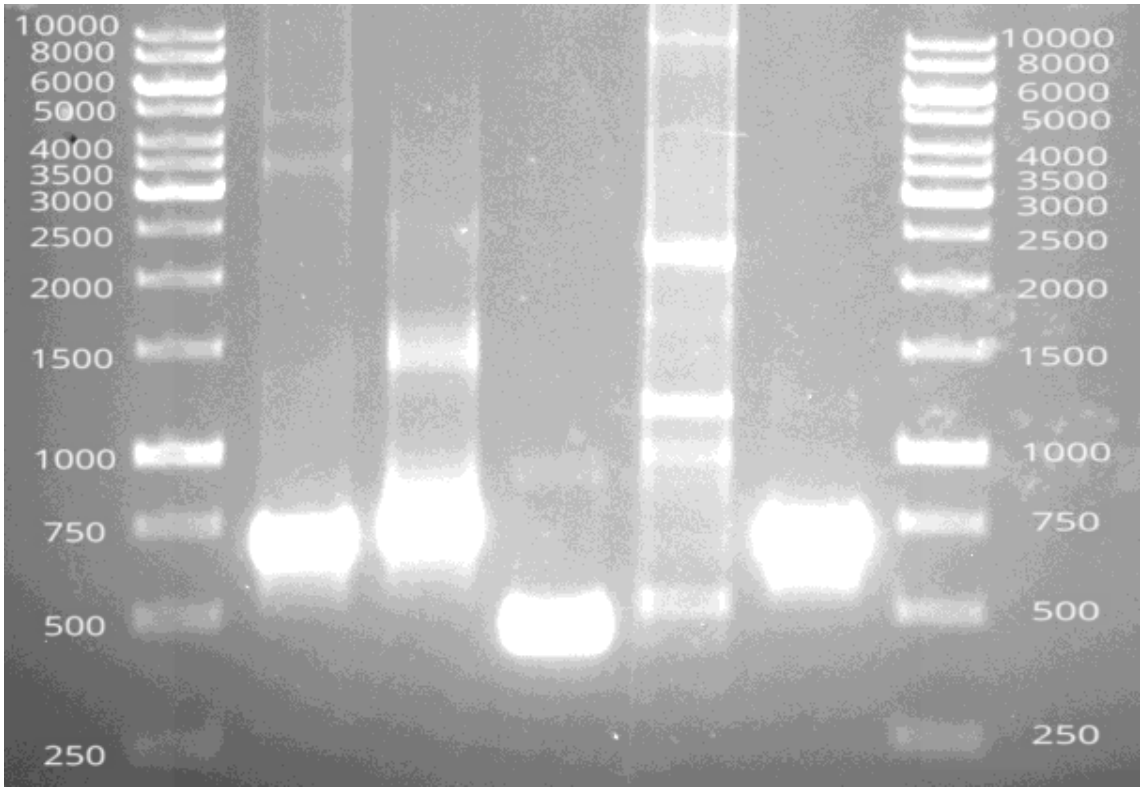


- Pick colonies of AmilCP Opt (pUPD2), eforRED Opt (pUPD2) and 35S:FT:GFP:Tnos (3alpha2) in C58.
- Transformation of SF:amilCPBio in E.coli and plating.
- Gibson reaction of TEV + Register Assembly PB and RL
- Refresh culture for 35S:RDF:Tnos infiltration
- Oligos arrived: IG17AG06, IG17AG07, IG17AG08, IG17AG09, IG17AG10 and IG17AG11.
- PCR reactions with previous oligos.

REAGENT	VOLUMEN (µL)
DNTPS	2
POLIMERASE	0,5
PRIMERS	1,25 each
TEMPLATE	1
BUFFER HF	10
H_2O	34

23/08/17

- Pick colonies of TEV + Assembly PB and RL
- Pick colonies of SF AmilCPbio and PR-1a
- Electrophoresis of PCR products
- Minipreps of eforREDopt and AmilCPopt in pUPD2



- Prepare infiltration of 35S:RDF:Tnos
- Pick colonies 35S:FT:Tnos in (3alpha2) C58
- Pick colonies of AmajLime Opt (pUPD2)
- Infiltration process of RDF
- Repeat PCR of eforRED BIO and TEV2
- Purification process of Amajlime Bio, TEV1 and Ros1 PCRs

- Ligation of AmajLime Bio in pUPD2 and Ros1 in pUPD2

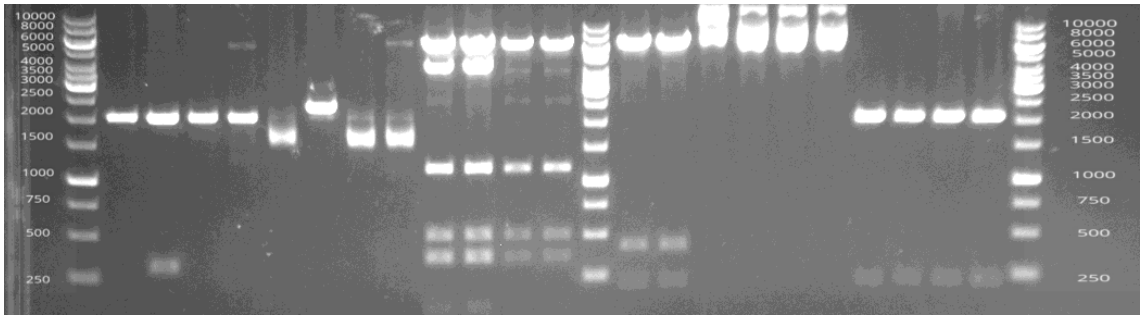
REAGENT	VOLUME (µL)
PUPD2 PLASMID	1
AMAJLIME BIO / ROS1	1
BSA 10X	1,2
LIGASE BUFFER	1,2
BSMBI	1
T4 LIGASE	1
H2O MILIQ	5,6

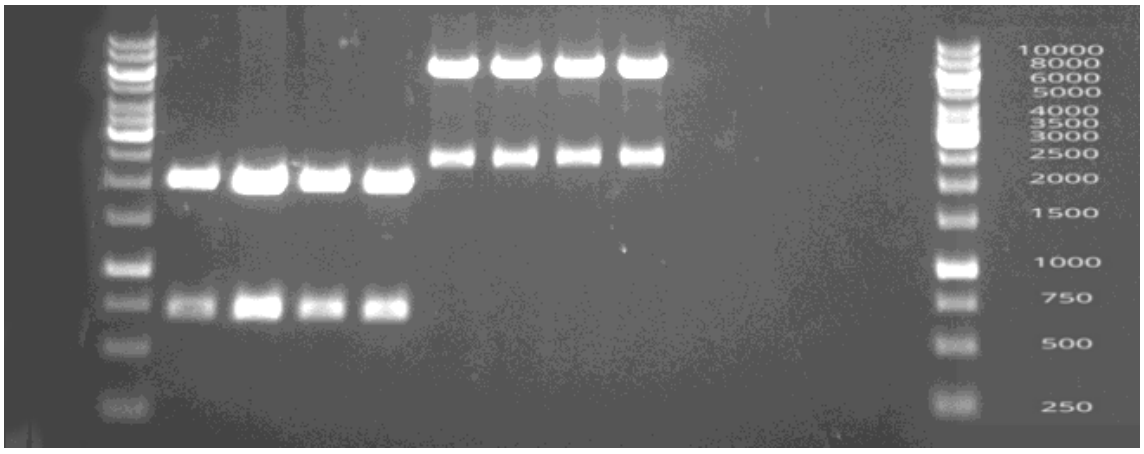
- Transformation of previous ligations y E.coli DH5α and plating.

24/08/17

- Minipreps of AmajLimeOpt (pUPD2), 35s:AmilCP Opt:Tnos, 35S:eforRedOpt:Tnos, TEV:AssemblyPB, SF:RAssem 1/3 (pUPD2), AmilCPbio (pUPD2) and PR-1a:Luc:Tnos.
- Digestion of previous minipreps

MINIPREP	ENZYME	FRAGMENTS
AMAJLIMEOPT (PUPD2)	NotI	2046/753
	HindIII	432/2367
35S:AMILCP OPT:TNOS	HindIII	88/96/363/509/1169/6345
35S:EFORREDOPT:TNOS	HindIII	66/459/633/1079/6345
TEV:ASSEMBLYPB	EcoRi	188/4534/8281
SF:RASSEM 1/3 (PUPD2)	NotI	213/2046
AMILCPBIO (PUPD2)	NotI	729/2046
PR-1A:LUC:TNOS	HindIII	2448/6859
	PvuI	2859/5934





- Pick colonies of Ros1 (pUPD2) and AmajLime Bio (pUPD2)
- Ligation of 35S:AmilCPbio:Tnos

25/08/17

- Minipreps of Ros1 (pUPD2) and AmajLimeBio (pUPD2)
- Transformation of 35d:AmilCPbIO:Tnos and 35S:eforRedOpt:Tnos in E.coli DH5α
- Transformation in Agrobacterium 35S:AmilCPOpt:Tnos
- Gel purification of TEV and eforRed PCRs.
- Electrophoresis of 35s:FT:Tnos (its correct) and 35s:FT:GFP:Tnos (its incorrect)
- Digestion and electrophoresis

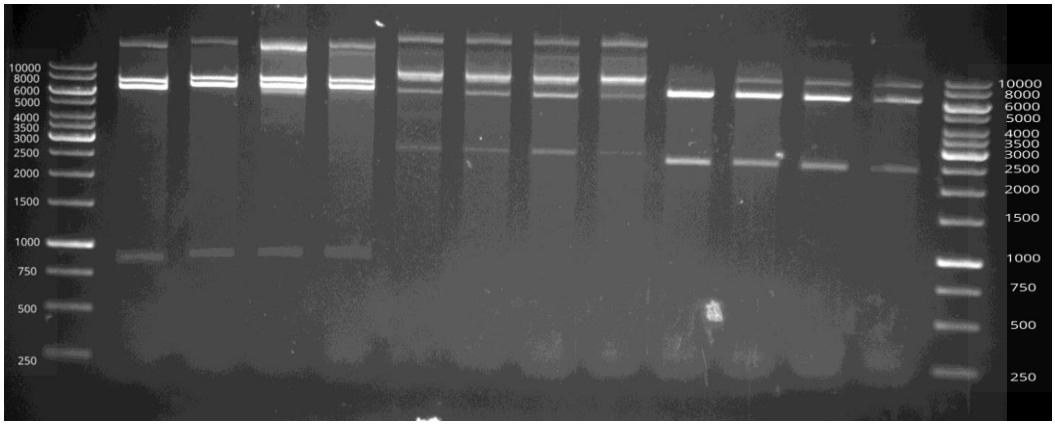
MINIPREP	ENZYME	FRAGMENTS
AMAJLIMEBIO (PUPD2)	NotI	2046/753
	PvuI	710/313/1776

30/08/17

- Pick agrobacterium colonies of SF:RegAssembly RL:Ros1 and 35S:AmilCObIO:Tnos and 35S:eforREDopt:Tnos
- Pick E.Coli colonies 35S:AmajLime:Tnos and 35S:eforREDbio:Tnos
- Optimized ligation reaction od TEV pUPD2
- Minipreps of agrobacterium with 35S:FT:GFP:Tnos and 35S:AmilCPOPt:Tnos and PR-1a:Luc:Tnos

- Digestion of previous agrobacterium minipreps

MINIPREP	ENZYME
PR-1A:LUC:TNOS	PvuI
	HindIII
35S:FT:GFP:TNOS	HindIII
	BanI
35S:AMICPOPT:TNOS	HindIII



31/08/17

- Minipreps of 35S:eforREDBio:Tnos and 35S:AmajLimeBio:Tnos.
- Digestions and electrophoresis

MINIPREP	ENZYME	FRAGMENTS
35S:EFORREDBIO:TNOS	PvuI	633/5934/2015
	HindIII	2237/6345
35S:FT:GFP:TNOS	HindIII	2819/6345
	BanI	3230/5934
35S:AMAJLIMEBIO:TNOS	HindIII	509/1740/6345

- Oligos arrived: IG17AG15, IG17AG16, IG17AG17 and IG17AG18.

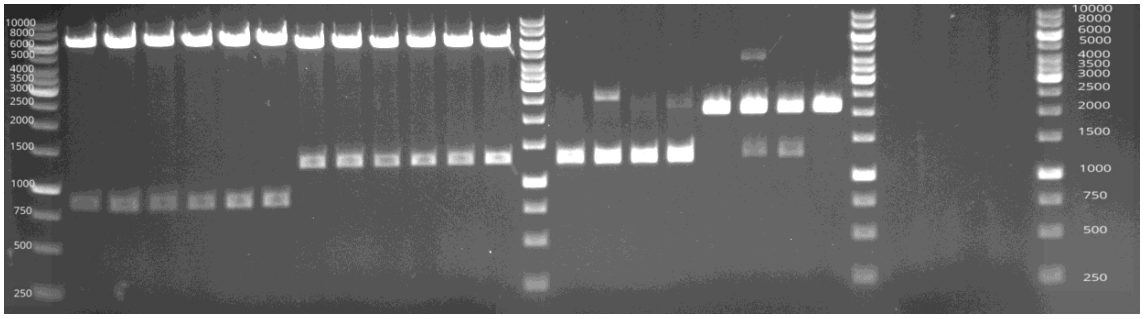
1/09/17

- Ligation reaction of PR-1a:Luc:Tnos + 35S:Renilla:Tnos in omega1

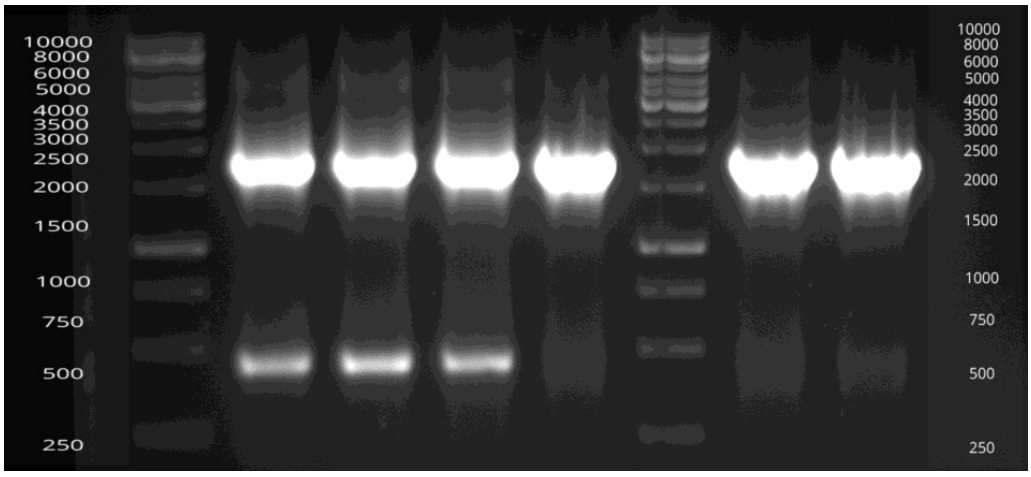
REAGENT	**VOLUME**
PR-1A:LUC:TNOS	1 µL
PR-1A:REN:TNOS	1 µL
3OMEGA1	1 µL
BSA10X	1,2 µL
T4 LIGASE	1,2 µL
BSAI	1 µL
H_2O	2,6 µL

- Minipreps of 35s:AmilCPBio:Tnos + 35S:eforRedOPt:Tnos + SF:RegAssembly.Ros1:Tnos
- Pick colonies of TEV and SF:Reg.Assembly:Ros1
- Digestion of previous minipreps.

MINIPREP	ENZYME	FRAGMENTS
REGISTER ASSEMBLY	NotI	2046/881
2/3 PR-1A (PUPD2)	BanII	1955/972
35S:FT:GFP:TNOS	HindIII	2819/6345
(ALPHA2)	PvuI	3230/5934
TEV (PUPD2)	HindIII	2046/10539
	EcoRI	1881/4534/3756/2423



- Refreseh φC31, Register Assembly RL and Register assembly PB because plants can't be infiltrated.
- Transform 35s:FT:GFP:TNos
- Digestion of every single part of 35s:FT:GFP:TNos → Everything looks right.
- PCR of the genomic extraction and subsequent band purification



- Infiltration of chromoproteins

2/09/19

- Refresh constructions to do the Register assembly with GP3 experiment
- Pick colonies of 35s:FT:GFP:Tnos in E. coli and Chromproteins in Agrobacterium

3/09/19

- Minipreps kept at fridge
- Prepare agroinfiltration for the experiment

4/09/17

- Minipreps of 35s:FT:GFP:Tnos and subsequent digestion.