Lab book

LUNDI 18/06/2018

- **Casting petri dish (LB agar +ATB)**

Kan	250ml LB - agar + 250 µL kan
Cam	250ml LB - agar + 250 µL CAM
Tet	250ml LB - agar + 250 µL Tet

- **Recovery of plasmids from the iGEM kit**

Locating Plasmids in Plates Using the Registry (http://parts.igem.org/Help:2018_DNA_Distribution)
Drill the cover over the well
Add 10 µL of ELIX water, homogenize by pipetting
Incubate for 5 minutes at room temperature
Store at -20 ° C

Transformation of pSB3T5 and pSB4K5

Defrost 25µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it 100µL on petri dish LB +kan or LB+Tet
Centrifuge the rest of the culture 1 min.
Remove the supernatant
Resuspend in 100µL of LB
Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium
Incubate at 37 ° C overnight

MARDI 19/06/2018

- **Results of the transformation of monday :**

- E.coli DH5α + pSB3T5 did not grow
- E.coli DH5α + pSB4k5 OK

- **Transformation of pSB3T5**

Defrost 25µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it 100µL on petri dish LB +kan or LB+Tet
Centrifuge the rest of the culture 1 min.
Remove the supernatant
Resuspend in 100µL of LB
Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium

Incubate at 37 ° C overnight

- **Liquid culture of E. coli DH5α + pSB4k5 and DH5α +pSB1C3**

Add 4mL LB-agar + 4 µL Kan or CAM (10000X) in 2 falcon tubes of 15mL
Put one colony in the medium
Incubate at 37°C, overnight

MERCREDI 20/06/2018

- **Result of the transformation of pSB3T5 :**

E .coli DH5a + pSB3T5 it's still didn't worked

- **Transformation of pSB3T5 from the plaques of iGEM 2014 and 2017; with DH5a**
  Defrost 25µL of E. coli (DH5alpha) on ice
  Add 4 µL of DNA
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 60″
  Incubate again 5′ on ice
  Add 300µL of LB
  Incubate 1h at 37°C
  After that put it 100µL on petri dish LB +kan ou LB+Tet
  Centrifuge the rest of the culture 1 min.
  Remove the supernatant
  Resuspend in 100µL of LB
  Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium
  Incubate at 37 ° C overnight

- ***Résultats des cultures***

pSB1C3 (A40+ A29) OK
pSB4k5 (duplicat) OK

- ***Extraction et purification of the plasmids* pSB1C3 and pSB4k5**

Miniprep on the overnight culture according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

- ***Nanodro of the minipreps***

pSB1C3 (A40) : 142
pSB1C3 (A29) : 36.8
PSB4k5 : 43
pSB4k5 (duplicat) : 53.5

JEUDI 21/06/2018

E .coli Dh5a + pSB3T5 finally grow weakly

- **Liquid culture of E. coliDh5a + pSB3T5**

Add 4mL LB-agar + 4 µL Tet (10000X) in 2 falcon tubes of 15mL
Put one colony in the medium
Incubate at 37°C, overnight

- ***Transformation of pSB3T5***
  Defrost 25µL of E. coli (DH5alpha) on ice
  Add 4 µL of DNA
  Incubate 30′ on ice

Then make a thermic choc at 42°C during 60''
Incubate again 5' on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it 100µL on petri dish LB +kan ou LB+Tet
Centrifuge the rest of the culture 1 min.
Remove the supernatant
Resuspend in 100µL of LB
Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium
Incubate at 37 ° C overnight

VENDREDI 22/06/2018

- **Liquid culture of E. coliDh5a + pSB3T5**

Add 4mL LB-agar + 4 µL Tet (10000X) in 2 falcon tubes of 15mL
Put one colony in the medium
Incubate at 37°C, overnightcultures liquides 3T5

- ***Extraction of the purification des plasmids* pSB1C3 and pSB4k5**

Miniprep on the overnight culture according to Thermo scientific '' GeneJet Plasmid Miniprep Kit #K0503''

- ***Nanodrop of the mini of ^SBrep 3T5)***

pSB3T5 : 23 ng/µL
pSB3T5 (duplicat) : 20 ng/µL

LUNDI 25/06/2018

PCR on pSB1c3 and psB4k5 in order to add Bsa1 sites

- PCR Mix

Table1

	A	B
1	Compound	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG102 (Fwd)	2.5
5	iG103 (Rvs)	2.5
6	DNA (0.2ng/µl)	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

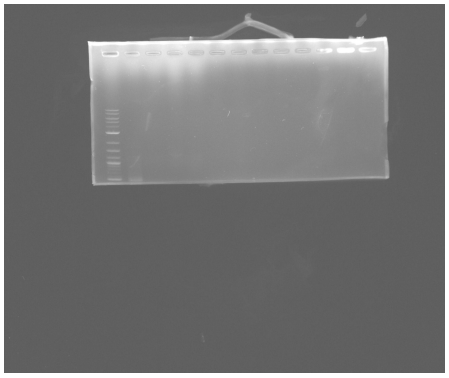
- PCR Programme :

Table2

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*2
4	64	30″	
5	72	2'30″	
6	98	10″	*3
7	68	30″	
8	72	2'30″	
9	98	10″	*30
10	phusion	30″	
11	72	2'30″	
12	72	2'	
13	4	-	

Electrophoresis gel of the PCR (1% agarose) :

pcr 625.jpg



MARDI 26/06/2018

- PCR on pSB1c3 and psB4k5 in order to add Bsa1 sites

Table3

	A	B
1	Compound	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG102 (Fwd)	2.5
5	iG103 (Rvs)	2.5
6	DNA (0.2ng/µl ou directement après Miniprep)	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

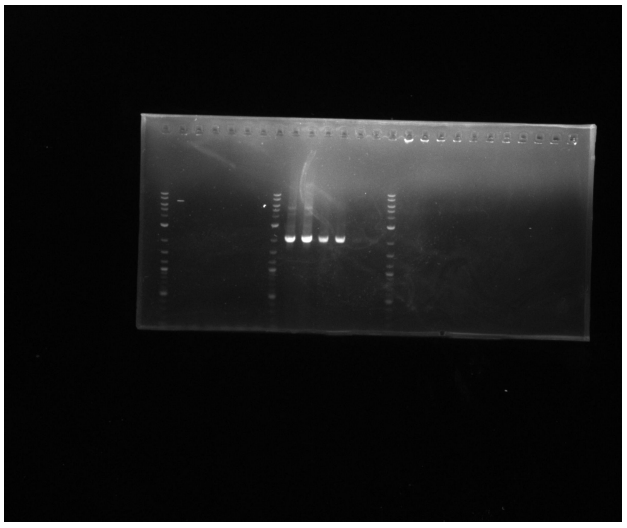
Programme :

Table4

	A	B	C
1	Temperature (°c)	time	Cycles
2	98	30″	
3	98	10″	*30
4	63	30″	
5	72	1′ (psb1C3) ou 2′ (psb4K5)	
6	72	2′	
7	4	-	

- Electrophoresis gel of the PCR :

pcr626.jpg



pSB1C3 is good in all of the conditions
Absence of bands for pSB4k5

MERCREDI 27/06/2018

- Miniprep on the overnight culture of psB4K5 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"
- Nanodrop of these minipreps

pSB4K5 : 66.2ng/µl
pSB4K5 (duplicat) : 95.2ng/µl

- PCR sur la miniprep

Table5

	A	B
1	Reactifs	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG102 (Fwd)	2.5
5	iG103 (Rvs)	2.5
6	DNA (0.2ng/µl)	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

Program of the thermocycler :

Table6

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30"	
3	98	10"	*2
4	62	30"	
5	72	2'30"	
6	98	10"	*3
7	65	30"	
8	72	2'30"	
9	98	10"	*30
10	70	30"	
11	72	2'30"	
12	72	2'	
13	4	-	

One band at the expected size

- PCR clean-up pSB1C3 according to the kit Nucléospin, validé par électrophorèse (~2kb)
- Ligation o of the pSB1C3 cleanup.n

- Digestion of the Quick Cbyge par Dpn1

JEUDI 28/06/2018

- PCR clean-up pSB4k5 according to the kit Nucleospin.

Denaturation of pSB3T5's digested strands
Put the digestion product 5min at 95°c to dnaturate the neo-synthetised strands and the matrix fragments
Turn off the machine to allow a slow hybridation, favoring neo-synthetised strands.

- Transformation de pSB3T5.
  Defrost 50µL of E. coli (DHalpha) on ice
  Add 4 µL of DNA
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 45″
  Incubate again 5′ on ice
  Add 300µL of LB
  Incubate 1h at 37°C
  After that put it all on petri dish LB +tet
  Overnight at 37°C

VENDREDI 29/06/2018

PCR colony for pSB1C3 et pSB3T5 and creation of Backup plates.

LUNDI 02/07/2018

Liquide cultures of pSB1C3 Golden Gate Ready (GGR), pSB3T5 QC, pSB3T5 et pSB4K5
in 5ml LB + 5 µl ATB, pick a colony, seed the medium.

MARDI 03/07/2018

Glycerol stock of :
pSB1C3 GGR, pSB3T5 and psb4K5

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of the overnight culture of DHa5+:
- psb1C3_GG
-psb3T5 QC
-psb4K5

PCR: in order to add the golden gate site in psb4K5

Table184

	A	B
1	Compound	quantity (µl)
2	Q5 Master Mix	25
3	iG024 (Fwd)	2.5
4	iG025(Rvs)	2.5
5	DNA (0.2ng/µl): psb4K5	1
6	H2O MQ	qsp 50

Tm60°c; 2′30″
Visualization by electrophoresys, no band

PCR pSB4K5 (Master Mix Q5 2X; qsp 25)
Tm = 5*55+10*60+30*70; 2′30″
Quantity DNA : 200;20;2;0.2 ng
Visualization by electrophoresys, no band

Digestion of 60ng of pSB3T5 QC, pSB3T5, pSB1C3 GGR using Bsa1
Visualization by electrophoresys, normal bands for controls. Weak digested band in QC

Quick Change on pSB3T5
Digestion using Dpn1

MERCREDI 04/07/2018

- Denaturation of pSB3T5′s digested strands

Put the digestion product 5min at 95°c to dnaturate the neo-synthetised strands and the matrix fragments
Turn off the machine to allow a slow hybridation, favoring neo-synthetised strands.

Digestion of 60ng of pSB3T5 QC, pSB3T5, pSB1C3 GGR using Bsa1 & EcoR1
Visualization by electrophoresys, no band

Digestion of 60ng of pSB3T5 QC, pSB3T5, pSB1C3 GGR using Bsa1 & EcoR1
Visualization by electrophoresys, no band

PCR pSB4K5 (primers 10->100mM; dNTPs 10mM ->2.5mM)
Tm = 58;58.3;59.3;60.8;62.5;64.2;65.8;67.5;69.2;70.7;71.7;72
Visualization by electrophoresys, no band

- PCR pSB4K5 (Phusion)

ADN 1ng;10ng
1.25µl primers (10mM)
5µl buffer
0.5 dNTP 10mM
0.75µl DMSO
0.25µl phusion
qsp 25µl MQ

hybridation : 5*55+10*60+35*70

JEUDI 05/07/2018

Digestion of 60ng of pSB3T5 QC, pSB3T5, pSB1C3 GGR using Bsa1
Visualization by electrophoresys, no band

Transformation of the new pSB3T5 QC
Defrost 25µL of E. coli (MH1) on ice
Add 5 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it 100µL on petri dish of LB+Tet
Centrifuge the rest of the culture 1 min.
Remove the supernatant
Resuspend in 100µL of LB
Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium
Incubate at 37 ° C overnight

Visualization by electrophoresys of pSB4K5, weak bands

PCR pSB4K5 (Phusion)
on the PCR product, 1ng et 10ng
2.5µl primers (10mM)
10µl buffer
1µl dNTP 10mM
1.5µl DMSO
0.5µl phusion
qsp 50µl MQ
hybridation : 5*55+10*60+35*70

VENDREDI 06/07/2018

psB1C3

Golden Gate

- 0,5µL T4DNA ligase
- 2µL 10X T4 DNA ligase Buffer
- 0,5µL Type II Restriction enzyme
- 100ng vector (psB1C3)
- 100ngxxx of insert
- qsq 20µL

Insertion done for :
- aimP
- ompA
- pelB
- TAT
- aimR

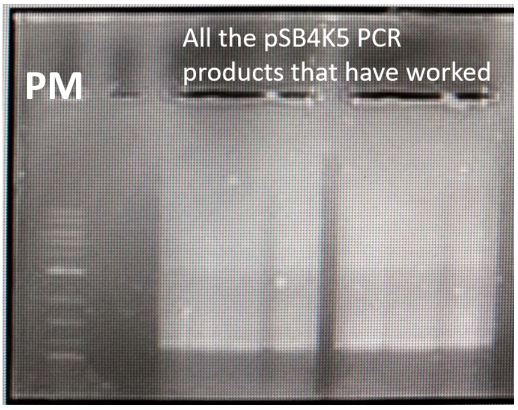
psB3K5

Observation of amplicons of 1.2kb et 200pb probably due to aspecific amplification.

Gel electrophoresys of all PCR products of psB3K5 with the right size (3.2kb)
Gel extractionof the PCR products With the kit : "Monarch DNA Gl Extractin Kit (NEB #T1020)"
Nanodrop of the gel extraction product : 64,6ng/µL

Gel electrophoresysfor verification

Image21.png



LUNDI 09/07/2018

pSB1C3 GG

PCR on the vaious GG of 1C3

Mix :

25µL DreamTaq

2,5 µL VR

2,5 µL VF2

2µL psB1C3 GG (+ insert)

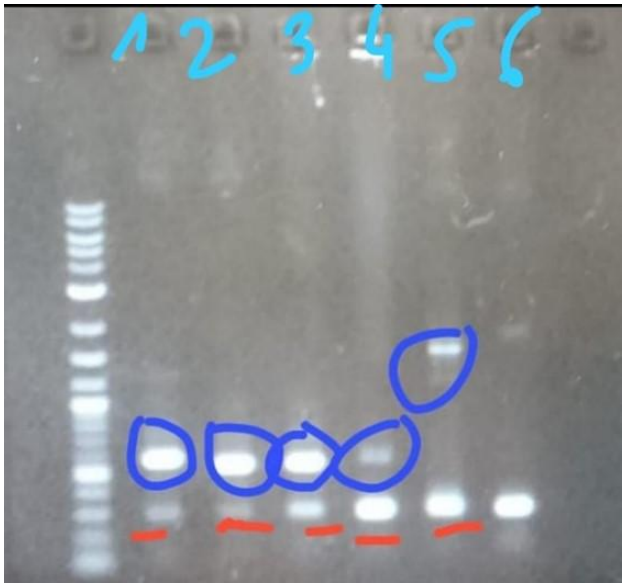
- Program for the Thermocycleur

Table7

	A	B	C
1	Temperature (°c)	Time	Cycles
2	95	3′	
3	95	30″	*25
4	50	30″	
5	72	45″	
6	72	5′	
7	4	-	
8			

- Gel electrophoresys Agarose 1%

Image3.jpg



Well 1 : PCR product on the plasmid which integrated AimP
Well 2 : PCR product on the plasmid which integrated ompA
Well 3 : PCR product on the plasmid which integrated pelB
Well 4 : PCR product on the plasmid which integrated TAT
Well 5 : PCR product on the plasmid which integrated AimR
Well 6 : PCR product on the empty plasmid (controle)

We see bands corresponding to the expected size and a control-like band for the various PCR products, meaning that the GG efficiency was not of a hundred percent and that empty plasmids are still present.

pSB3K5
PCR to amplify gel extration products
Mix PCR Phusion 50µL

Thermocycleur Program

Table8

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*5
4	55	30″	
5	72	2′30″	
6	98	10″	*10
7	60	30″	
8	72	2′30″	
9	98	10″	*30
10	70	30″	
11	72	2′30″	
12	72	2′	
13	4	-	

- Gel electrophoresys Agarose 1%

Well 1 : 1ng of PCR product with phusion buffer
Well 2 : 1ng of PCR product with GC buffer

Observation of asmear for both PCR products

- Ligation psB3T5
  - Mix ligation

2µL T4 DNA buffer
1µL T4 DNA Ligase
10µL psB3K5 linear (no RFP)
qsp H2O

Incubate 15 min at room temperature

Transformation psB3K5
Defrost 25µL of E. coli (MH1) on ice
Add 3 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it 100µL on petri dish of LB+Tet
Centrifuge the rest of the culture 1 min.
Remove the supernatant
Resuspend in 100µL of LB
Spread 100µL in a Petri dish containing LB + Agar + Antibiotic medium
Incubate at 37 ° C overnight

psB3T5
Miniprep with the kit : "GeneJET KIT" sur psb3T5

Nanodrop 114,1ng/µL

Digestion of psB3T5 with BsaI
Mix digestion
0,5µL BsaI
60ng of psB3T5 (0,52µL)
qsp H2O 25µL (21,48µL)
Put the Mix at 37°C during 2h

MARDI 10/07/2018

Transformation of psB1C3 GG AimP/ompA/pelB/TAT/AimR

Defrost 50µL of E. coli (DHalpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + CAM
Overnight at 37°C

psB3K5

- Results of the transformation of monday09/07/2018

there are some clones

- Liquid culture of 2 clones from the transformation of psb3T5
  - (5ml) LB + 5µL KAN
  - At 37°C overnigth

MERCREDI 11/07/2018

psB1C3 GG AimP/ompA/pelB/TAT/AimR

After the transformation of the tuesday we obtained:
1 colony for psb1C3_GG_AimR
0 Colony for psb1C3_GG_pelB
At least 10 colonies for psb1C3_GG_OmpA, psb1C3_GG_TAT and psb1C3_GG_AimP

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table9

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	25
3	Primer : VR	2,5
4	Primer : VF2	2,5
5	Water qsp	19
6	Boiling bacteria	1

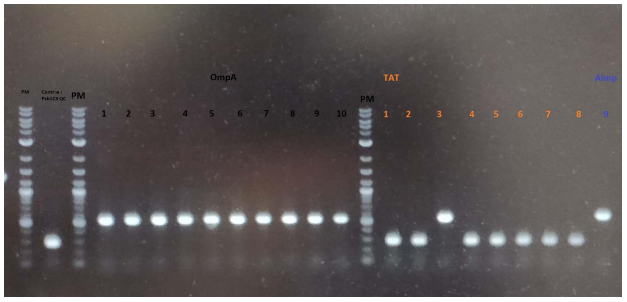
- Thermocycler program

Table10

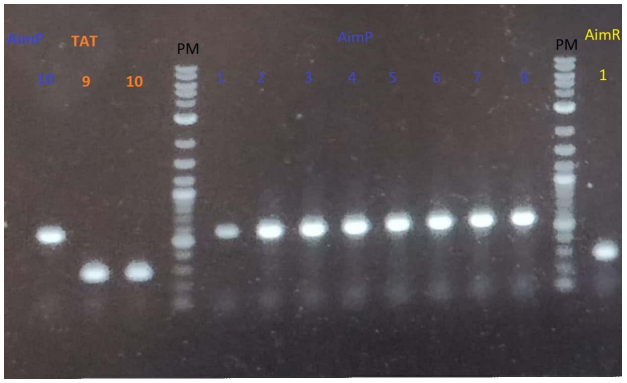
	A	B	C
1	Température (°c)	Temps	Cycles
2	95	3′	
3	95	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	
8			

- Electrophoresis gel of the PCR colony (Agarose 1%)

Nouveau document 2018-07-23_1.jpg



Nouveau document 2018-07-23_2.jpg



Amplification of the shares: sfGFP and AimeR by PCR

- Mix PCR

Table11

	A	B
1	Composition	Volume en µL
2	Q5 Buffer	10
3	Primer : iG024	2,5
4	Primer : iG025	2,5
5	Q5 pol	0.5
6	GC enhancer	10
7	*Insert (shares)*	1
8	qsp water(50µL)	23

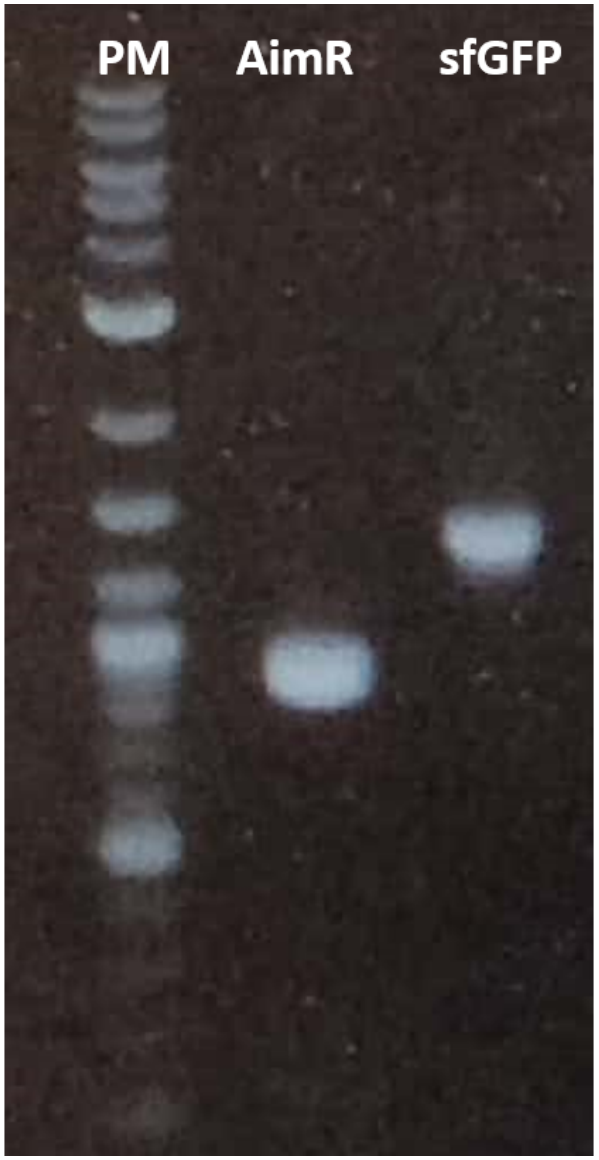
- Thermocycler program

Table12

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30''	
3	98	10''	*30
4	72	20''	
5	72	50''	
6	72	2'	
7	4	-	
8			

- Electrophoresis gel of the PCR products (Agarose 1%)

Image6.png



Digestion of the psb3T5 in order to verify the enzymes PstI and XbaI

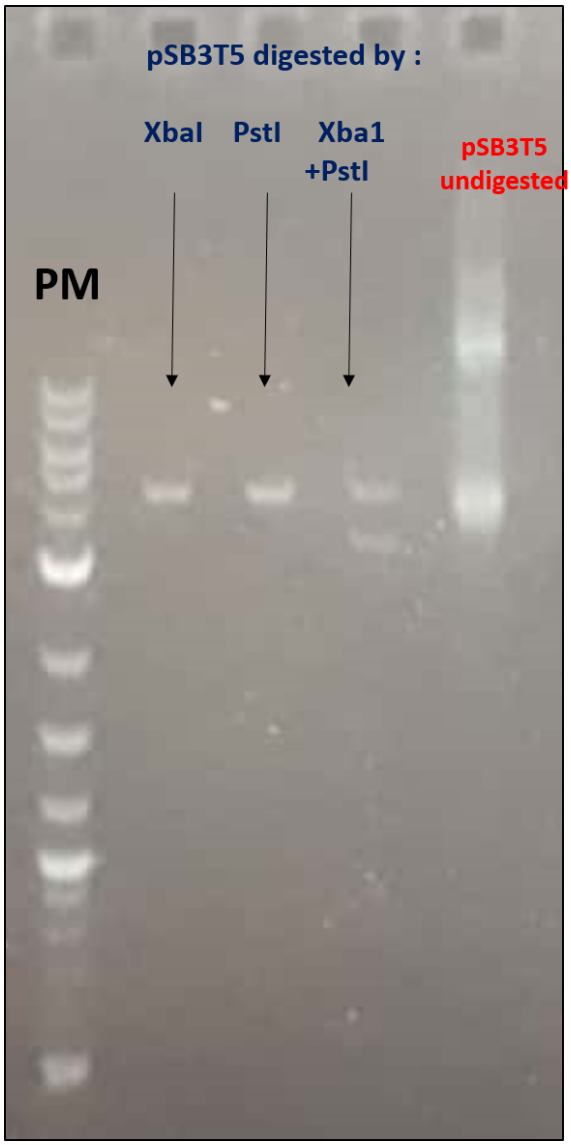
- Digestion Mix

Table13

	A	B	C	D	E	F	G	H
1	**For XbaI only**			**For PstI only**			**For PstI + XbaI**	
2	Composition	Volume en µL		Composition	Volume en µL		Composition	Volume en µL
3	Buffer fastDigest Green 10X	2		Buffer fastDigest Green 10X	2		Buffer fastDigest Green 10X	2
4	plasmidic DNA : psb3T5	2		plasmidic DNA : psb3T5	2		plasmidic DNA : psb3T5	2
5	Enzyme : XbaI	1		Enzyme : PstI	1		Enzyme : XbaI	0.5
6	H2O qsp (20µL)	15		H2O qsp (20µL)	15		Enzyme : PstI	0.5
7							H2O qsp (20µL)	15
8								

- Electrophoresis gel of the digestion (Agarose 1%)

Image10.png



Transformation of pSB1C3_AimR and pSB1C3_pelB

Defrost 50µL of E. coli (DHalpha) on ice

Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 300µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + CAM
Overnight at 37°C

Liquid culture of the colonies positif after the colony PCR :
1-2-3 of the tranformation psb1C3_AimP
2-3-4 of the tranformation psb1C3_OmpA
3 of the tranformation psb1C3_TAT

- liquid culture : LB (5mL) + CAM (5µL) and incubate overnight at 37°C

JEUDI 12/07/2018

Miniprep on the overnight culture of the colonies to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503":
1-2-3 of the tranformation psb1C3_AimP
2-3-4 of the tranformation psb1C3_OmpA
3 of the tranformation psb1C3_TAT

- Nanodrop
  - psb1C3_AimP :
    colony 1 : 93.1 ng
    colony 2 : 80.9 ng
    colony 3 : 92.9 ng
  - psb1C3_ompA :
    colony 2 : 106.4 ng
    colony 3 : 17.8 ng
    colony 4 : 71.3 ng
  - psb1C3_TAT :
    colony 3 : 32.7 ng

Sends to sequencing of these purified plasmids

Table14

	A	B	C	D	E	F	G	H
1	Plasmid	psb1C3_AimP colonie n°1	psb1C3_AimP colonie n°2	psb1C3_AimP colonie n°3	psb1C3_OmpA colonie n°2	psb1C3_OmpA colonie n°3	psb1C3_OmpA colonie n°4	psb1C3_TAT colonie n°3
2	Sequencing code	Seq.ID 68EH86	Seq.ID 68EH85	Seq.ID 68EH84	Seq.ID 68EH89	Seq.ID 68EH92	Seq.ID 68EH91	Seq.ID 68EH90

Result of the transformation of psb1C3_pelB and psb1C3_AimR :
No colonies on the petri dish for psb1C3_AimR
At lest 10 colonies on the petri dish for psb1C3_pelB

Colony PCR on the colonies obtained after the transformation of psb1C3_pelB :

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them

Put each colony in 20µL of water
Then put them at 95°C during 5′
In the same time we make the back up for each colony on petri dish LB + CAM

- MiX PCR

Table15

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	25
3	Primer : VR	2,5
4	Primer : VF2	2,5
5	Water qsp	19
6	Boiling bacteria	1

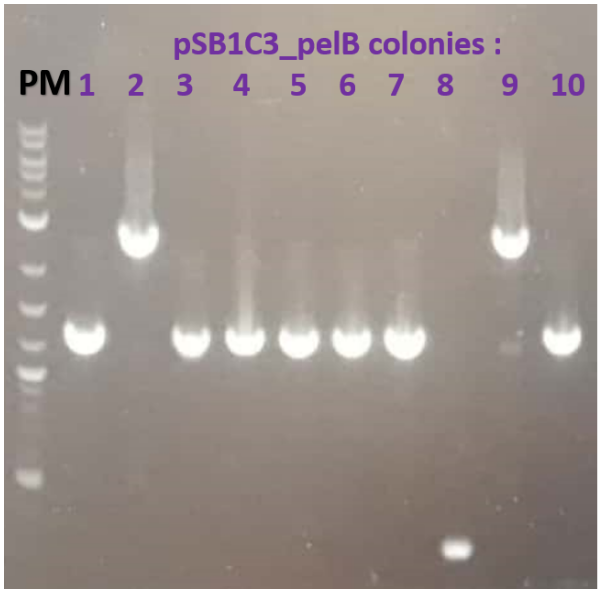
- PCR progam

Table75

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Electrophoresis gel of the PCR colony products (Agarose 1%)

Image11.png



The colonies 9 and 2 are correct

Liquid culture of :

- A46 in order to give the plasmid to a other iGEM team : in LB (5mL) + CAM (5µL)
- G87 : pSB3T5 RFP: LB (5mL) + Tet (5µL)
- G86 : pSB4K5 RFP : LB (5mL) + Kan (5µL)
- colonies : 1-2-3 of psb1C3_AimP for make a glycreol stock
- colonies : 2-3-4 of psb1C3_OmpA for make a glycerol stock
- colony 3 of psb1C3_TAT for make a glycerol stock

Transformation of pSB1C3_AimR, pSB3T5 and SB4K5

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + CAM for pSB1C3_AimR ; LB+Tet for pSB3T5 and LB+ Kan for pSB4K5
Overnight at 37°C

VENDREDI 13/07/2018

Result of the transformation of pSB1C3_AimR, pSB3T5 and pSB4K5 :

4 colonies on the petri dish for psb1C3_AimR
Many colonies on the petri dish for psb3T5 and pSB4K5

Colony PCR on the 4 colonies obtained after the transformation of psb1C3_AimR :

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

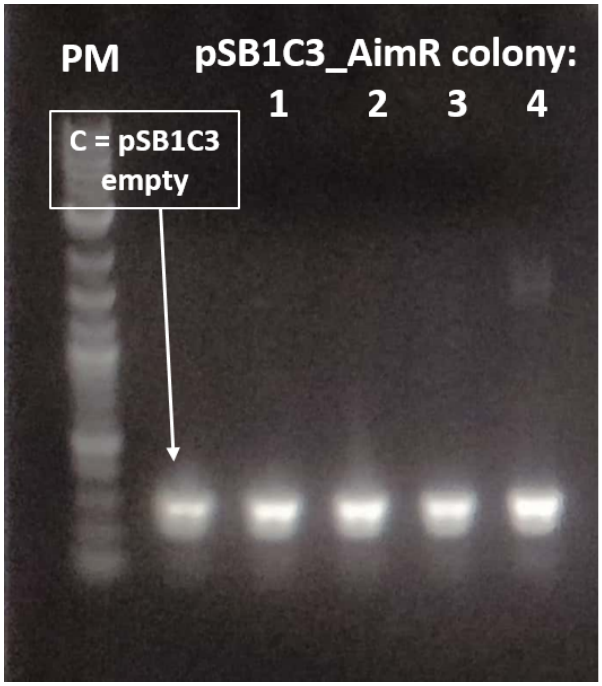
  - MiX PCR

Table16

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	25
3	Primer : VR	2,5
4	Primer : VF2	2,5
5	Water qsp	19
6	Boiling bacteria	1

- Electrophoresis gel of the PCR colony products (Agarose 1%)

Image2.png



Colony PCR on the colonies obtained after the transformation of psb1C3_pelB :

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

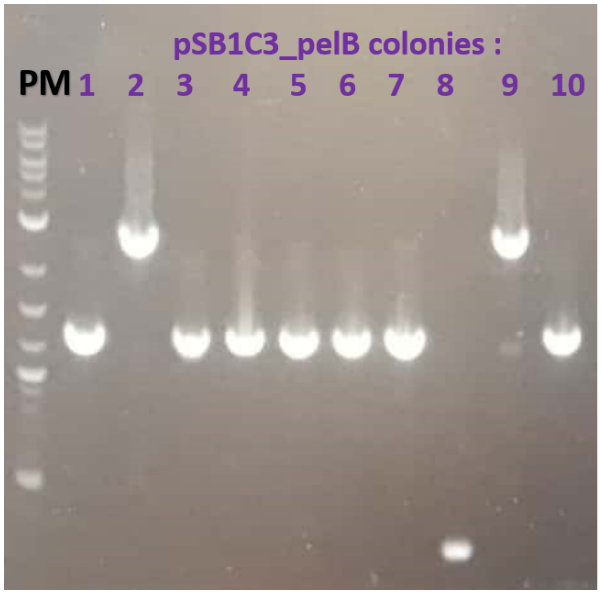
  - MiX PCR

Table17

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	25
3	Primer : VR	2,5
4	Primer : VF2	2,5
5	Water qsp	19
6	Boiling bacteria	1

- Electrophoresis gel of the PCR colony products (Agarose 1%)

Image11.png



Miniprep on the overnight culture according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" :

- A46
- G87 : pSB3T5 RFP
- G86 : pSB4K5 RFP

- Nanodrop : A46 =159.9 ng

Digestion of A46 by thes enzymes XbaI, NotI and XhoI in order to verify the plasmid :

- Digestion mix

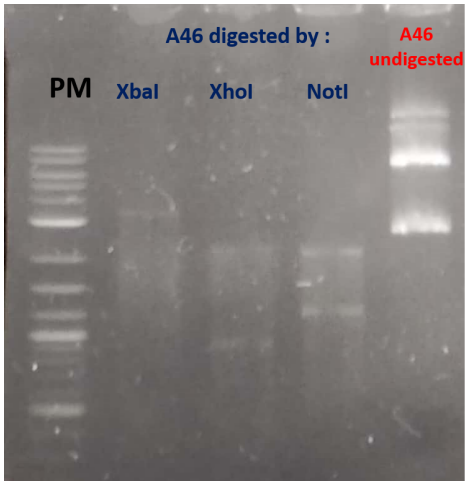
Table19

	A	B	C	D	E	F	G	H
1	**By XbaI only**			**By PstI only**			**By PstI + XbaI**	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	Buffer FastDigest Green 10X	2		Buffer FastDigest Green 10X	2		Buffer FastDigest Green 10X	2
4	Plasmid DNA: A46	2		Plasmid DNA: A46	2		Plasmid DNA: A46	2
5	Enzyme: XbaI	1		Enzyme: NotI	1		Enzyme: XhoI	1
6	Water qsp (20µL)	15		Water qsp (20µL)	15		Water qsp (20µL)	15

- Expected size for the digestion by :
  - XbaI : 1 band at 3229pb
  - NotI : 2 bands , one of 1183pb and the other of 2046pb
  - XhoI : 3 bands ; 207 pb , 892pb and 2130pb

- Electrophoresis gel of the digestion (Agarose 1%)

Image9.png



We have the bands that we expected, but we couldn't see the band of 207pb our the digestion by XhoI probably due to a low quantity of DNA.

Cloning :

Prepare the vectors for cloning :

- Digestion of the vectors by XbaI an PstI
  - Digestion Mix

Table18

	A	B	C	D	E
1	**For pSB4K5**			**For pSB3T5**	
2	Composition	Volume in µL		Composition	Volume in µL
3	Buffer FastDigest Green 10X	5		Buffer FastDigest Green 10X	5
4	Plasmid DNA: pSB4K5 (2000µg)	26		Plasmid DNA: pSB3T5 (2000µg)	17
5	Enzyme: XbaI	2.5		Enzyme: XbaI	2.5
6	Enzyme: PstI	2.5		Enzyme: PstI	2.5
7	Water qsp (20µL)	14		Water qsp (20µL)	15

- Electrophoresis gel of the digestion of the vectors (Agarose 1%)

(rajouter le gel)
We have
And nothing for pSB3T5 digestion

- Gel extraction of the backbone without RFP according to " Monarch Gel extraction Kit #T1020L NEB"
    - Nanodrop: pSB4K5 : 55,5 ng

Prepare the inerts for cloning

- Digestion of the inserts (parts) AimR and sfGFP by XbaI and PstI
    - Digestion Mix

Table20

	A	B	C	D	E
1	**For AimR**			**For sfGFP**	
2	Composition	Volume in µL		Composition	Volume in µL
3	Buffer FastDigest Green 10X	2		Buffer FastDigest Green 10X	2
4	Purifed PCR product : AimR	18		Purifed PCR product : sfGFP	18
5	Enzyme: XbaI	1		Enzyme: XbaI	1
6	Enzyme: PstI	1		Enzyme: PstI	1
7	Water qsp (20µL)	9		Water qsp (20µL)	9

- Purification of the digest inserts according to " Monarch DNA and PCR cleanup kit" NEB
    - Nanodrop :
      AimR : 82.5 ng
      sfGFP : 87.8 ng

Ligation :

- Ligation of sfGFP and pSB4K5
    - Ligation Mix :

Table21

	A	B
1	Composition	Volume in µL
2	Buffer T4 DNA ligase	2
3	T4 DNA ligase	1
4	Insert : sfGFP	10
5	vector: pSB4K5	4

Then incubate 10′ at room temperature
After that incubate on the weekend at 16°C

LUNDI 16/07/2018

Cloning:

Transformation :

- Tranformtion of the ligation product pSB4K5_sfGFP
  Defrost 25µL of E. coli (DHalpha) on ice
  Add 4 µL of ligation product of pSB4K5 _sfGFP
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 60″
  Incubate again 5′ on ice
  Add 200µL of LB
  Incubate 1h at 37°C
  After that put 100µL on petri dish LB+ Kan
  Overnight at 37°C

Digestion of pSB3T5 by NotI in order to verify the plasmid :

- Digestion Mix

Table22

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA: pSBT5 (or control plasmid : pSB4K5)	2
4	Enzyme: NotI	1
5	Water qsp (20µL)	15

Put the mix at 37°C during 1h

- Expected Sizes for the digestion by NotI for :
    - pSB3T5 : 2 bands = 3.2kb and 119pb

- the control : pSB4K5 : 2 bands = 3.4kb and 1.1 kb

- Electrophoresis gel of the digestion (Agarose 1%)

(gel)

No Bands for pSB3T5
2 bands for the contol at the good size

Liquid culture :

- From the pétri dish of the transformation of pSB3T5 (12.07.18) : LB (5mL) + Tet (5µL)
- From colony 2 et 9 of the transformation of pSB1C3_pelB (12.07.18) : LB (5mL) + CAM (5µL)

MARDI 17/07/2018

Golden Gate :

- Golden gate on pSB1C3_TAT and pSB1C3_AimR
  - Golden Gate Mix

Table23

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	Vector : pSB1C3	
6	Insert : AimR or TAT	
7	Water qsp (20µL)	

  - Golden gate Program :

Table24

	A	B	C
1	Température (°c)	Temps	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Miniprep of the liquid culture of pSB1C3_PelB colonie 2 , 9 and of pSB3T5 according to " GeneJet Plasmid Miniprep Kit #K0503" :

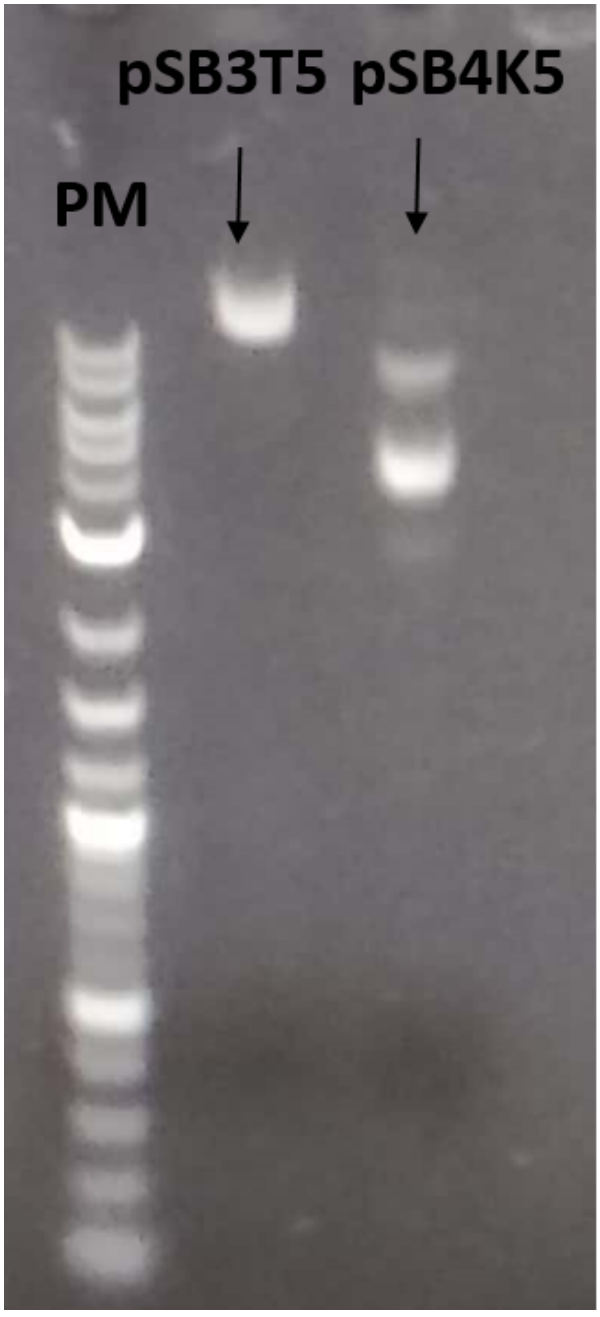
- Nanodrop :

MERCREDI 18/07/2018

Electrophoresis gel of pSB3T5 in order to see if we really have some DNA (Agarose 1%):

We use pSB4K5 like control.

Image8.png



We observe a band for pSB3T5 and also for our control.

Coloning:

Prepare the vectors for cloning :

- Digestion ogf pSB3T5 by XbaI and PstI

Table25

	A	B
1	**For pSB3T5**	
2	Composition	Volume in µL
3	Buffer FastDigest Green 10X	5
4	Plasmid DNA: pSB3T5 (2000µg)	40
5	Enzyme: XbaI	2.5
6	Enzyme: PstI	2.5

Then incubate 1h at 37°C

- Electophoresis gel of the digestion product (1% agarose)

(rajout gel)

We observed a band at 3.2kb corresponding to the backbone psb3T5 without RFP

- Extraction gel of the band of 3.2kb according to " Monarch DNA Extraction Gel Kit"
    - Nanodrop : pSB3T5 digest (coloning ready) = 64.1 ng

Liquid culture pSB3T5 from the transformation of the 12.07.18:

LB (5mL) + Tet (5µL)

Transformation of ligation product : pSB3T5_sfGFP in DH5alpha

- Defrost 50µL of E. coli (DHalpha) on ice
  Add 5 µL of ligation product : pSB3T5_sfGFP
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 30′′
  Incubate again 5′ on ice
  Add 1mL of LB
  Incubate 1h at 37°C
  After that put 100µL on petri dish LB+ Tet
  Overnight at 37°C

JEUDI 19/07/2018

Transformation of GG proucts : pSB1C3_TAT and pSB1C3_AimR

- Defrost 50µL of E. coli (DHalpha) on ice
  Add 3µL of pSB1C3_TAT or pSB1C3_AimR
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 30′′
  Incubate again 5′ on ice
  Add 1mL of LB
  Incubate 1h at 37°C
  After that put 100µL on petri dish LB+ CAM
  Overnight at 37°C

LUNDI 23/07/2018

Amplification by PCR of sfGFP :

- PCR Mix

Table26

	A	B
1	Composition	Quantity
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG024	2.5
5	iG025	2.5
6	DNA : sfGFP stock	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

- PCR program

Table27

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30''	
3	98	10''	*30
4	72	20''	
5	72	50''	
6	72	2'	
7	4	-	

Colony PCR on the clonies obtained after the transformation of pSB1C3_AimR and pSB1C3_TAT (19.07.18) :

- Boiling bacteria to kill them

  Put each colony in 20µL of water

  Then put them at 95°C during 5'

  In the same time we make the back up for each colony on petri dish LB + CAM

- PCR Mix

Table28

	A	B
1	Composition	Volume in µL
2	One taq Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp (12,5)	3.75
6	Boiling bacteria	1

- PCR program

Table29

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

Liquid culture of :

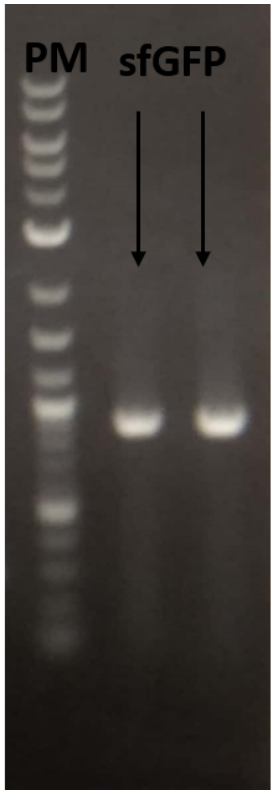
- psb3T5 from the transformation of the 12.07.18
- psb3T5 from the transformation of the plasmid of 2014
- psb3TX from manish

LB (5mL) + Tet (5µL)

MARDI 24/07/2018

Electrophoresis gel of the PCR product of the amplification of sfGFP (1% agarose)

Image4.png



Purification of the amplifiction of sfGFP according to "Monarch DNA and PCR clean up kit" (NEB)

- Nanodrop : sfGFP = 142 ng

Cloning

Prepare the inerts for cloning

- Digestion of sfGP by XbaI and PstI for the cloning:
  - Digestion Mix

Table33

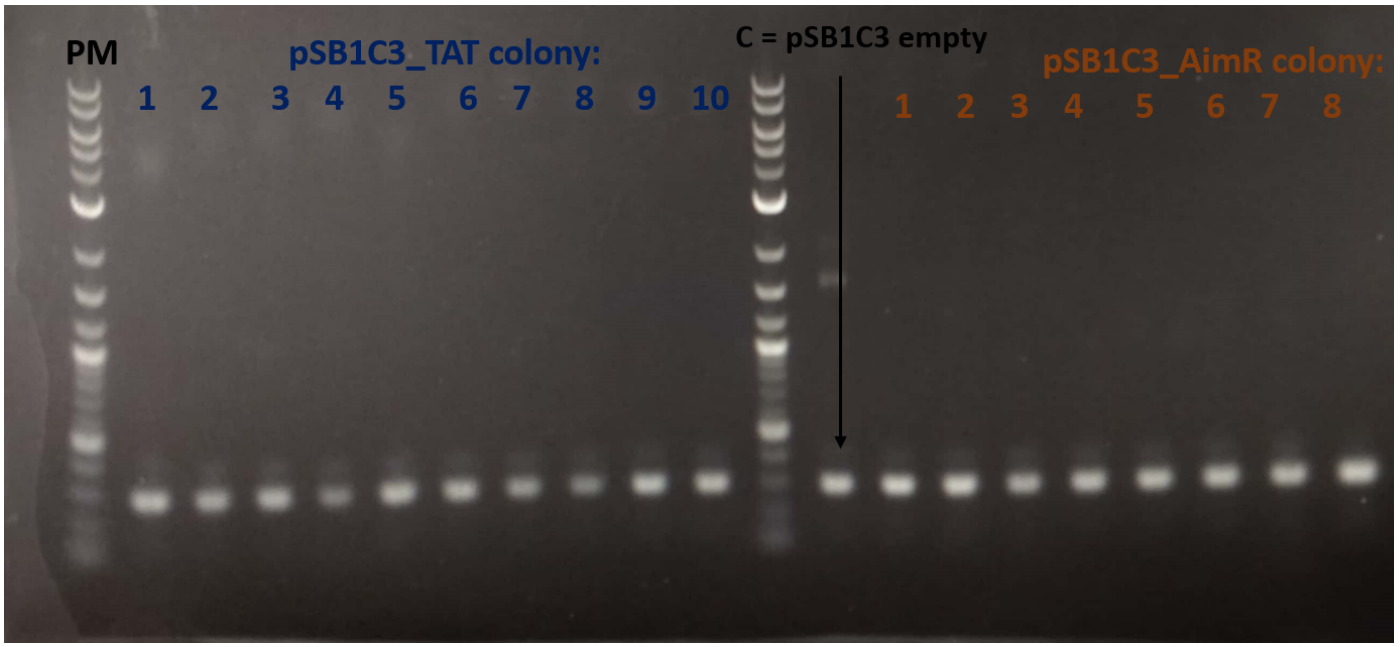
	A	B
1	**For sfGFP**	
2	Composition	Volume in µL
3	Buffer FastDigest Green 10X	2
4	Purifed PCR product : sfGFP	10
5	Enzyme: XbaI	1
6	Enzyme: PstI	1
7	Water qsp (20µL)	6

  - Electrophoresis gel in order to verify if we still have the inert after the cleanup

Image15.png

Electrophoresis gel of PCR colony product (1% agarose)

Image13.png



No colony seem good.

Owing to this results we decided to test other colonies of pSB1C3_AimR and pSB1C3_TAT (19.07.18) :

- Boiling bacteria to kill them
  Put each colony in 20µL of water
  Then put them at 95°C during 5′
  In the same time we make the back up for each colony on petri dish LB + CAM

- PCR Mix

Table31

	A	B
1	Composition	Volume in µL
2	One taq Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp (12,5)	3.75
6	Boiling bacteria	1

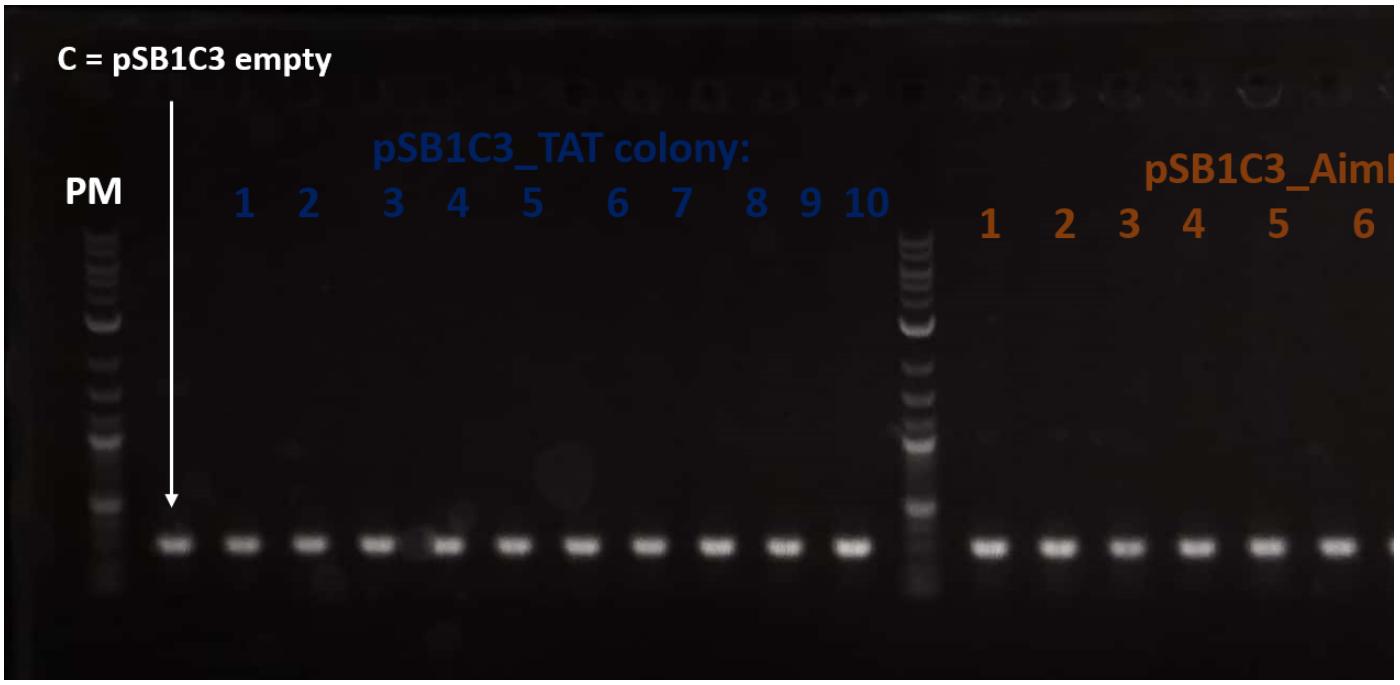
- PCR program

Table32

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

- **Electrophoresis gel of PCR colony product (1% agarose)**

Image14.png



Miniprep accordint to "GeneJet plasmid Miniprep Kit" (Thermo scientific) of :

- psb3T5 from the transformation of the 12.07.18
- psb3T5 from the transformation of the plasmid of 2014
- psb3TX from manish

    - Nanodrop
      psb3T5 (the transfo 12.07) =149.6 ng
      psb3T5 (2014) = 294.4 ng
      psb3TX (manish)= 136.7 ng

Verification of this different plasmids above by digestion with NotI:

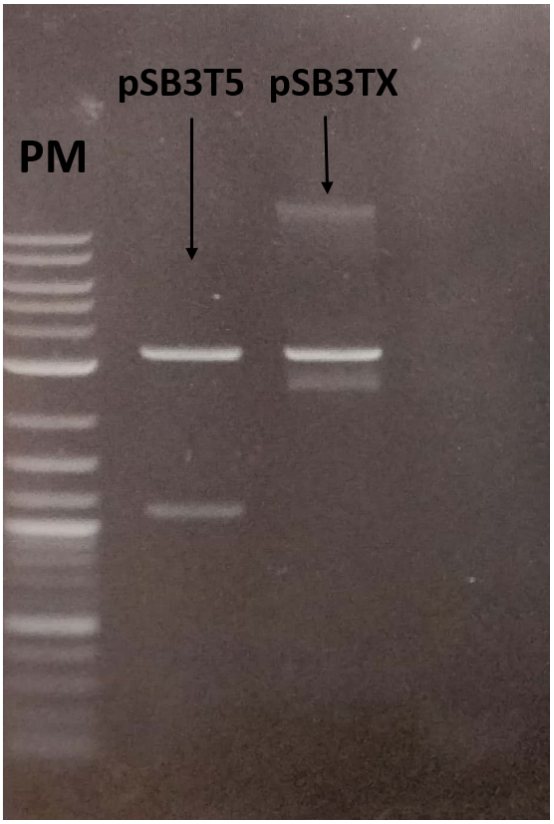
- Digestion Mix

Table30

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA: psb3T5 (the transfo 12.07)/ or psb3T5 (2014)/ or psb3TX (manish)	2
4	Enzyme:NotI	1
5	Water qsp (20µL)	15

- Electrophoresis gel of the digestion products (1% agarose)

Image12.png



Verification of the plasmid psb3TX by EcorI :

We use the pSB4K5 like control

- Digestion Mix

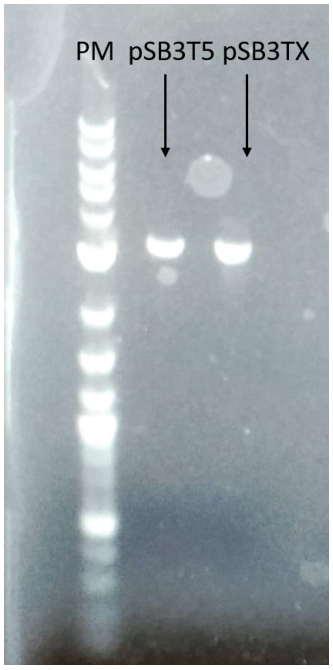
Table34

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA: psb3T5 (the transfo 12.07)/ or psb3T5 (2014)/ or psb3TX (manish)	2
4	Enzyme:NotI	1
5	Water qsp (20µL)	15

MERCREDI 25/07/2018

- Electrophoresis gel of the digestion products (1% agarose)

Imag2.png



Classing Cloning

Prepare the vectors for cloning :

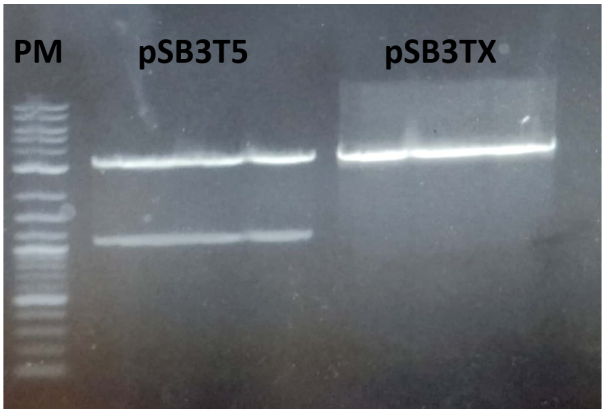
- Digestion of pSB3T5 (transfo 12.07) and pSB3TX (manish)
  - Digestion mix

Table35

	A	B	C	D	E
1	**For pSB3TX**			**For pSB3T5 (transfo 12.07)**	
2	Composition	Volume in µL		Composition	Volume in µL
3	Buffer FastDigest Green 10X	5		Buffer FastDigest Green 10X	5
4	Plasmid DNA: pSB3TX (2000µg)	13.4		Plasmid DNA: pSB3T5 (2000µg)	14.63
5	Enzyme: XbaI	2.5		Enzyme: XbaI	2.5
6	Enzyme: PstI	2.5		Enzyme: PstI	2.5
7	Water qsp (20µL)	26.6		Water qsp (20µL)	25.37

  - Electrophoresis gel of the digestion products (1% agarose)

Image1.png



- Gel extraction of backbone of psb3T5 (transfo 12.07) and pSB3TX according to " Monarch Gel extraction kit " (NEB)
    - Nanodrop :
      pSB3T5(transfo 12.07) = 27.5 ng
      pSB3TX = 30.5 ng

    - Electrophoresis gel in order to verify if we have thee good backbone
  (gel)

Ligation :

- Ligation of pSB3T5_AimR/ pSB3TX_AimR/ pSB4K5_sfGFP)
    - Ligation Mix

Table36

	A	B	C	D	E	F	G	H
1	For pSB3T5_AimR			For pSB3TX_AimR			For pSB4K5_sfGFP	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	Buffer T4 DNA ligase	2		Buffer T4 DNA ligase	2		Buffer T4 DNA ligase	2
4	T4 DNA ligase	1		T4 DNA ligase	1		T4 DNA ligase	1
5	Insert : AimR (99.7 ng)	1.36		Insert : AimR	1.21		Insert : sfGFP (140ng)	3.45
6	vector: pSB3T5 (82.5ng)	3		vector: pSB3TX (91.5 ng)	3		vector: pSB4K5 (166ng)	3
7	H2O qsp (20µL)	12.64		H2O qsp (20µL)	12.79		H2O qsp (20µL)	

incubate 10′ at room temperture.

Transformation

- Transformation of : pSB3T5_AimR/ pSB3TX_AimR/ pSB4K5_sfGFP/ pSB4K5_sfGFP (of 13.07.18)
  Defrost 25µL of E. coli (DHalpha) on ice
  Add 4 µL of ligation product
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 60′′
  Incubate again 5′ on ice
  Add 200µL of LB
  Incubate 1h at 37°C

After that put 100µL on petri dish LB+ Kan or Tet
Overnight at 37°C

Amplification by PCR of the inserts : AimR/TAT/pelB

- PCR Mix

Table37

	A	B
1	Composition	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG024	2.5
5	iG025	2.5
6	DNA: Aimr or TAT or pelB from the stock	0.5
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

- PCR Program

Table39

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30''	
3	98	10''	*30
4	72	20''	
5	72	50''	
6	72	2'	
7	4	-	

Golden Gate ready pSB1C3 by PCR

- PCR Mix

Table38

	A	B
1	Composition	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG101	2.5
5	iG026	2.5
6	DNA: pSB1C3	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

- PCR program

Table40

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*2
4	62	30″	
5	72	2′30″	
6	98	10″	*3
7	65	30″	
8	72	2′30″	
9	98	10″	*30
10	70	30″	
11	72	2′30″	
12	72	2′	
13	4	-	

Liquid culture of the plasmid pSB3T5 of manish : LB (5mL) + Tet (5µL)

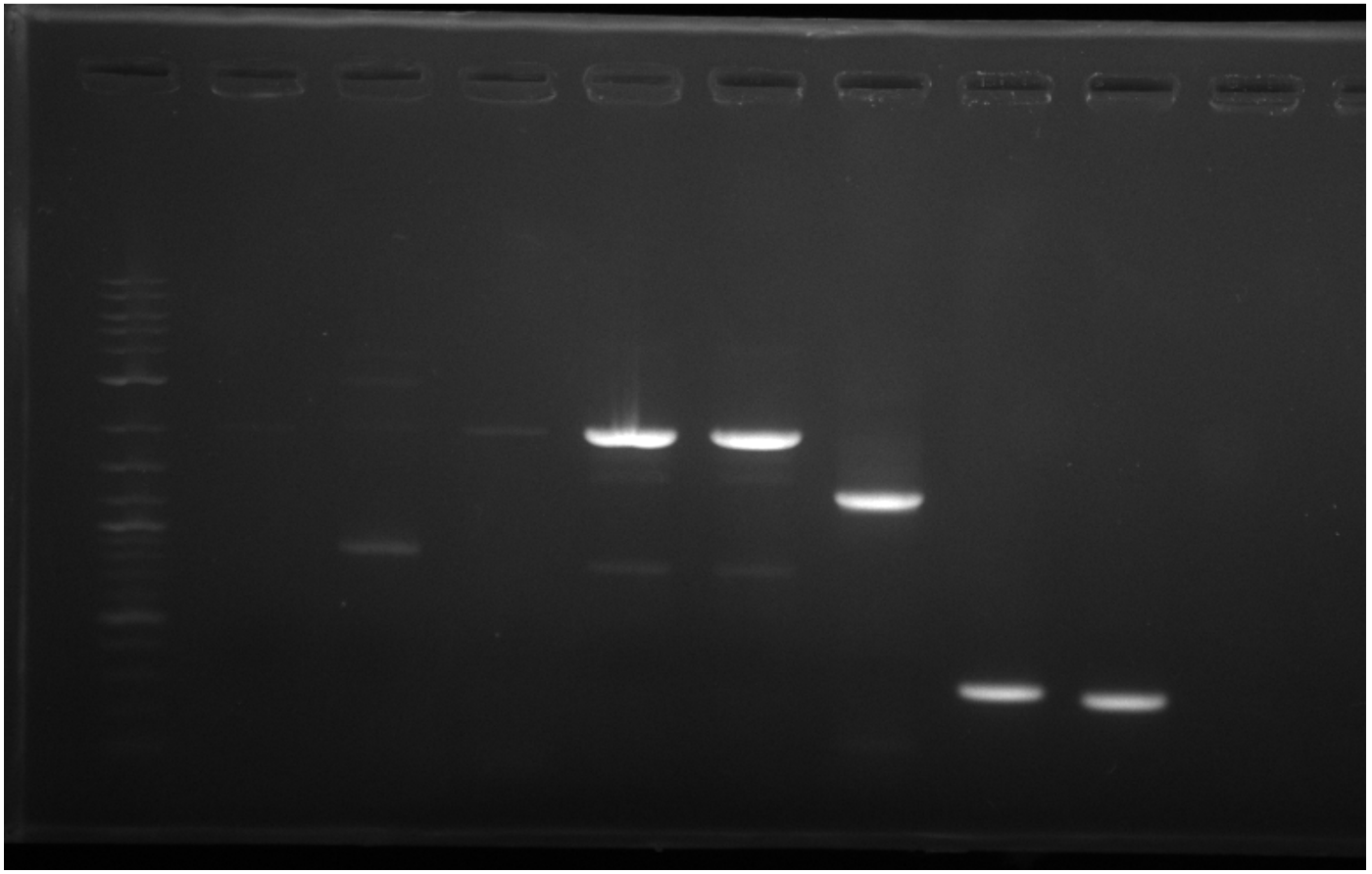
- Two culture from isolated colonies

JEUDI 26/07/2018

Electrophoresis of PCR and Golden Gate cloning

M NTC pSB1C3+ sfGFP NTC pSB1C3+ GG sites pSB1C3+GG + GG sites AimR+GG TAT+GG PelB+GG

image.png

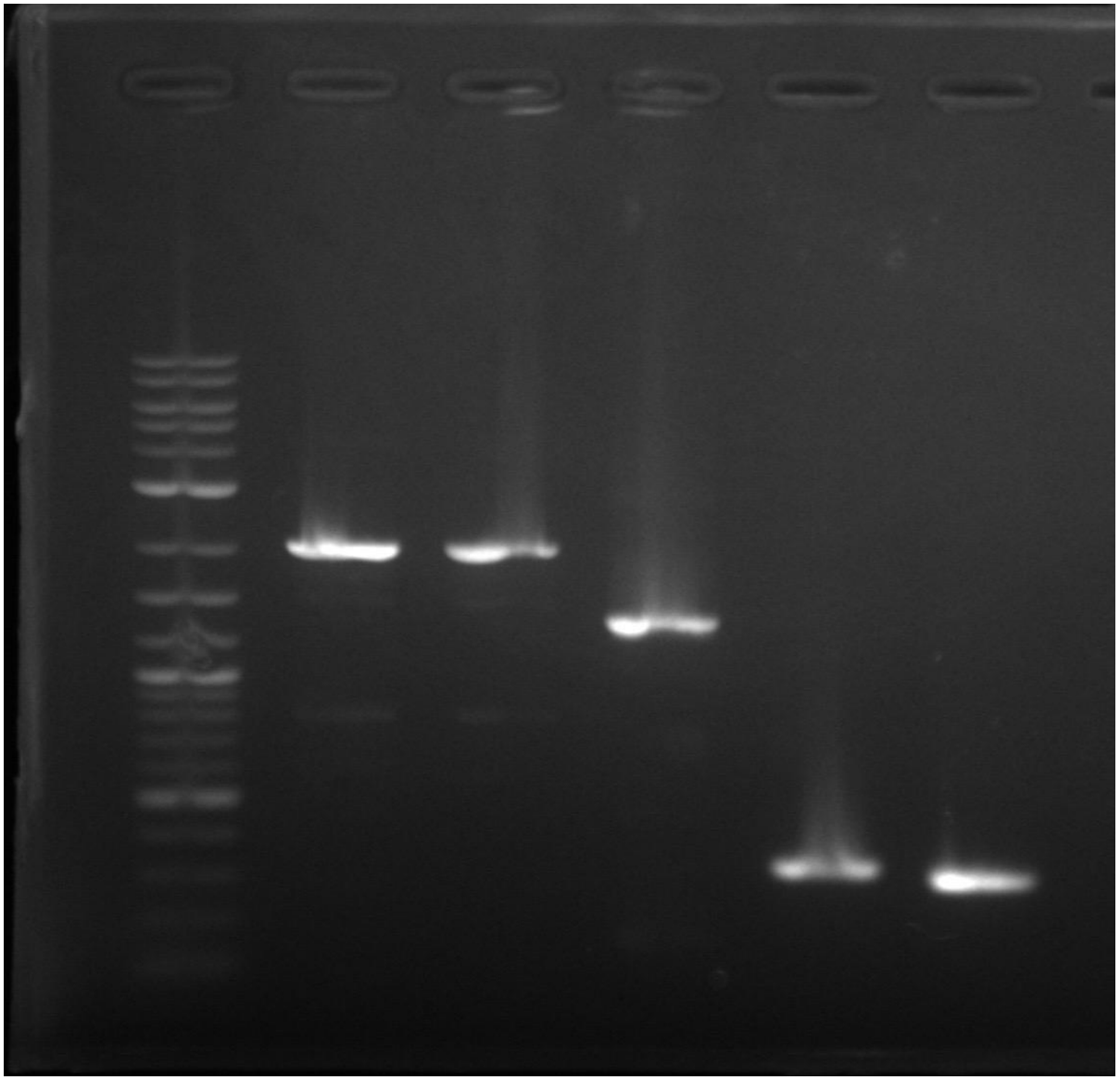


We have gotten a good result for PCR amplifications of AimR (approx. 1.3 kb), TAT_AimP (approx. 300 bp), PelB_AimP (approx. 287 bp) and both backbones pSB1C3 samples (approx. 2 kb). However, the NTC exhibied and delighted band in the NTC well, a possibly cross contamination when adding samples in the gel. Additionally, the golden gate cloning sample did not show a reliable result. A second golden gate is going to be made by using these amplicons.

PCR Clean-up

M	pSB1C3	pSB1C3	AimR	TAT	PelB

26.07.18 purification of PCR products AimR, TAT, PelB, pSB1C3.jpg



The purification appears to work well and these samples will be used for futher experiments.

Miniprep accordint to "GeneJet plasmid Miniprep Kit" (Thermo scientific) of :

- psb3T5 of manish
    - Nanodop
      psb3T5 C1 = 39 ng
      psb3T5C2 = 54.7 ng

Verifiction of psb3T5(manish) C1 and C2 by digstion with NotI

- Digestion Mix

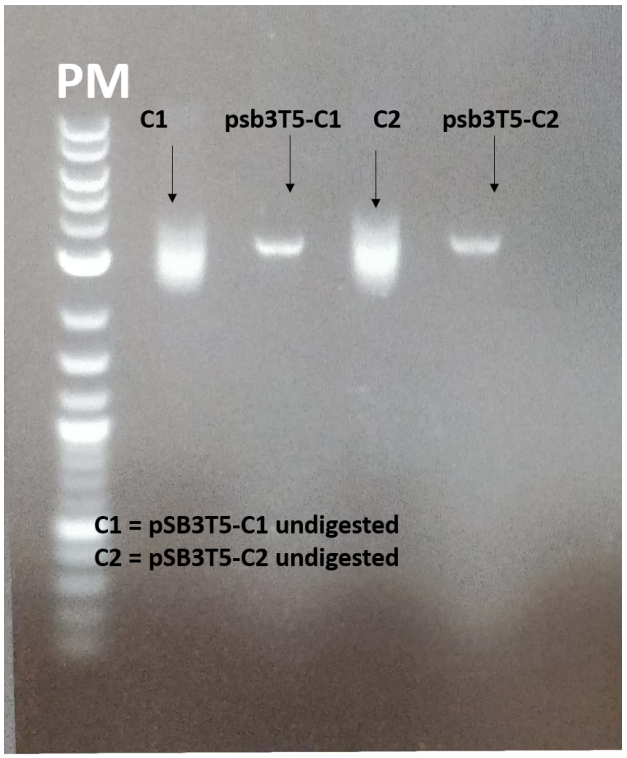
Table41

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA: pSBT5 (Manish) C1 or C2	2
4	Enzyme: NotI	1
5	Water qsp (20µL)	15

Incubate 1h at 37°C

- Electrophoresis gel of the digetion (1% Agarose)

Image20.png



VENDREDI 27/07/2018

P**CR colony on pSB3T5_AimR Nazim competent cells (1 colony) / pSB4K5_sfGFP Nazim competent cells (3 colonies)/ pSB3TX_AimR Nazim competent cells (10 colonies)/ pSB4K5sfGFP (13.07.18) (10 colonies) / psb4K5_sfGFP (10 colonies)**

- Boiling bacteria to kill them
  Put each colony in 20µL of water
  Then put them at 95°C during 5′
  In the same time we make the back up for each colony on petri dish LB +Tet or KAN

- PCR Mix

Table42

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp (12,5)	3.75
6	Boiling bacteria	1

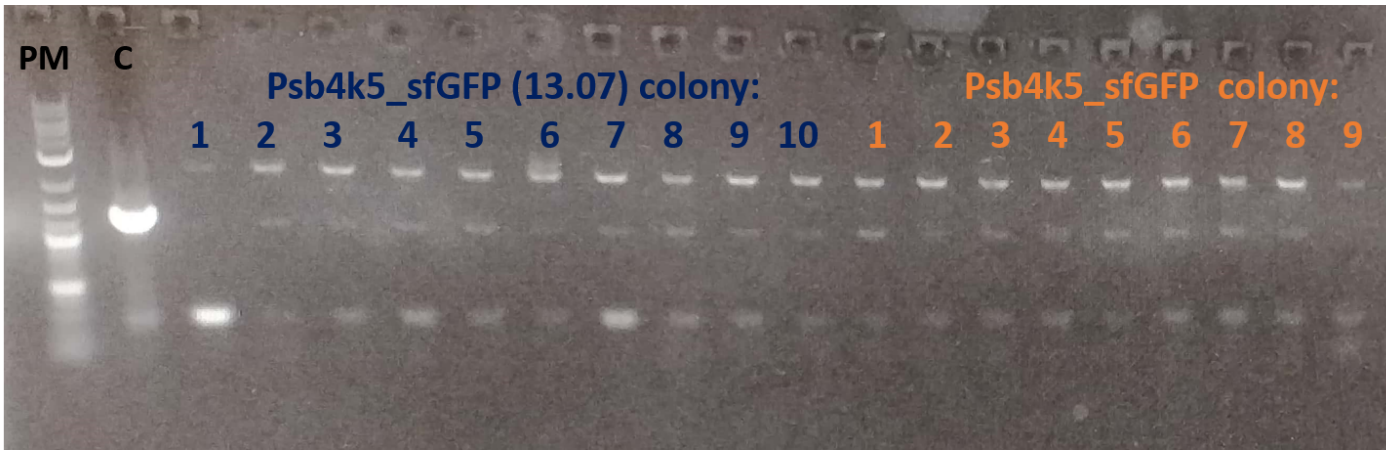
- PCR program

Table43

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

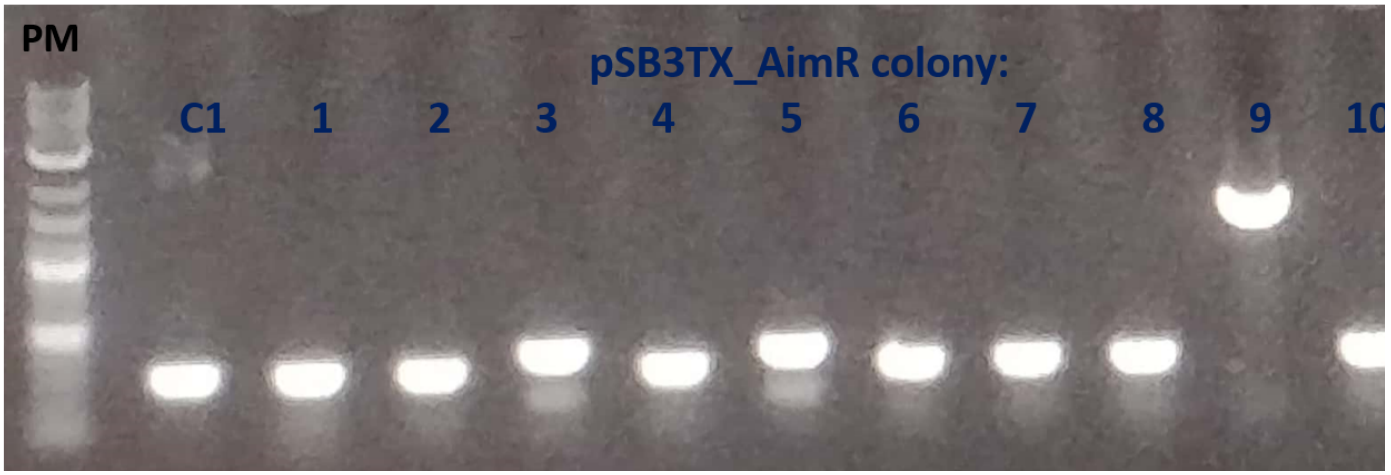
- **Electrophoresis gel of PCR colony product (1% agarose)**

Image19.png



C = controle = amplicafation of pSB4K5 with RFP instead of the insert

Image18.png



C1 = controle = amplicafation of pSB3TX without insert
C2 = controle = amplicafation of pSB3T5 with RFP instead of the insert

Classic Cloning

Transformation

- Transformation of : pSB3T5_AimR/ pSB3TX_AimR/ pSB4K5_sfGFP/
  Defrost 25µL of E. coli (DHalpha) on ice
  Add 4 µL of ligation product
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 60′′
  Incubate again 5′ on ice
  Add 200µL of LB
  Incubate 1h at 37°C
  After that put 100µL on petri dish LB+ Kan or Tet
  At room temperature doing the weekend

Golden Gate Cloning

Golden Gate assembly for : pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP

- Golden Gate Mix ration de 5:2 (insert:vector)

Table48

	A	B	C	D	E	F	G	H	I	J	K
1	Mix for pSB1C3_AimR			Mix for pSB1C3_TAT			Mix for pSB1C3_pelB			Mix for pSB1C3_sfGFP	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Vector : pSB1C3	1		Vector : pSB1C3	1		Vector : pSB1C3	1		Vector : pSB1C3	1
7	Insert : AimR	2		Insert : TAT	3		Insert : pelB	3		Insert : sfGFP	1
8	Water qsp (20µL)			Water qsp (20µL)			Water qsp (20µL)			Water qsp (20µL)	
9											

- Golden gate Program :

Table49

	A	B	C
1	Température (°c)	Temps	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

LUNDI 30/07/2018

Classic Cloning

Results of the transformation of the 28.07.18

No colonies for pSB3T5_AimR

Really small colonies for pSB4K5_sfGFP

At least 10 Colonies for pSB3TX_AimR

Classic Cloning

PCR colony on the colonies obtained after the tranformation for pSB3TX_AimR

- Boiling bacteria to kill them

  Put each colony in 20µL of water

  Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + Tet

- PCR Mix

Table44

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp (12,5)	3.75
6	Boiling bacteria	1

- PCR program

Table45

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

- Electrophoresis gel of PCR colony product (1% agarose)

Image24.png

Classic Cloning and Classing Cloning

Amplification of the inserts sfGFP and AimR

- PCR Mix

Table46

	A	B
1	Composition	Quantity
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG024	2.5
5	iG025	2.5
6	DNA : sfGFP stock	1
7	Q5 polymerase	0.5
8	Q5 High GC enhancer	10
9	H2O MQ	qsp 50

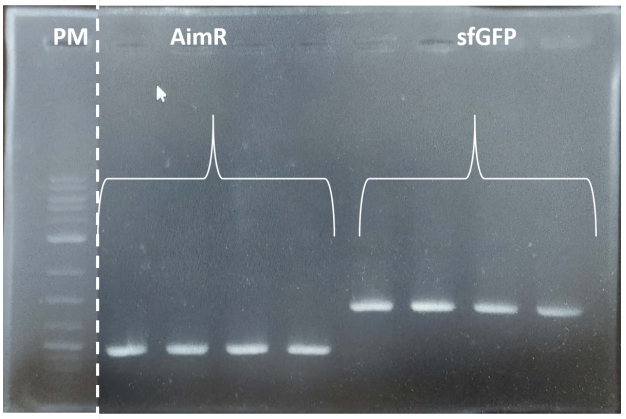
- PCR program

Table47

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30''	
3	98	10''	*30
4	72	20''	
5	72	50''	
6	72	2'	
7	4	-	

- Electrophoresis Gel of the amplification of sfGFP and AimR (1% agarose)

Image25.png



- PCR cleanup of AimR and sfGFP according to " Monarch DNA and PCR cleanup kit" NEB
  - Nanodrop :
    - sfGFP =128.4 ng
    - AimR = 118.2 ng

Golden Gate Cloning

PCR to check if the Golden Gate assembly for : pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP are correct.

- PCR Mix

Table50

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	25
3	Primer : VR	2,5
4	Primer : VF2	2,5
5	Water qsp	18
6	ADN : product of the goden gate assembly	2

- PCR program

Table51

	A	B	C
1	Température (°c)	Temps	Cycles
2	95	3′	
3	95	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	

Preparatio of the plasmids pSB3T5 (12.07) , pSB3K3, pSB4A3, psB3T5 (manish) C1 and C2 for the GG

- PCR Mix

Table52

	A	B	C	D	E
1	For pSB3T5 (12.07) pSB3T5 (manish) C1 and C2			For pSB3K3 and pSB4A3,	
2	Composition	Quantity		Composition	Quantity
3	Q5 buffer	10		Q5 buffer	10
4	Mix dNTPs (10mM each)	1		Mix dNTPs (10mM each)	1
5	iG026	2.5		iG102	2.5
6	iG101	2.5		iG103	2.5
7	DNA : plasmids	1		DAN : plasmids	1
8	Q5 polymerase	0.5		Q5 polymerase	0.5
9	Q5 High GC enhancer	10		Q5 High GC enhancer	10
10	H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR program

Table53

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*2
4	62	30″	
5	72	2′30″	
6	98	10″	*3
7	65	30″	
8	72	2′30″	
9	98	10″	*30
10	70	30″	
11	72	2′30″	
12	72	2′	
13	4	-	

Digestion of AimR and sfGFP by XbaI and PstI for cloning

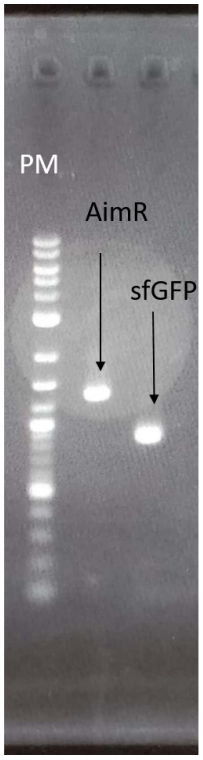
Table69

	A	B
1	Composition	Volume en µL
2	Buffer fastDigest Green 10X	4
3	DNA: AimR or sfGFP	30
4	Enzyme : XbaI	1.5
5	Enzyme : PstI	1.5
6	H2O qsp (20µL)	3

MARDI 31/07/2018

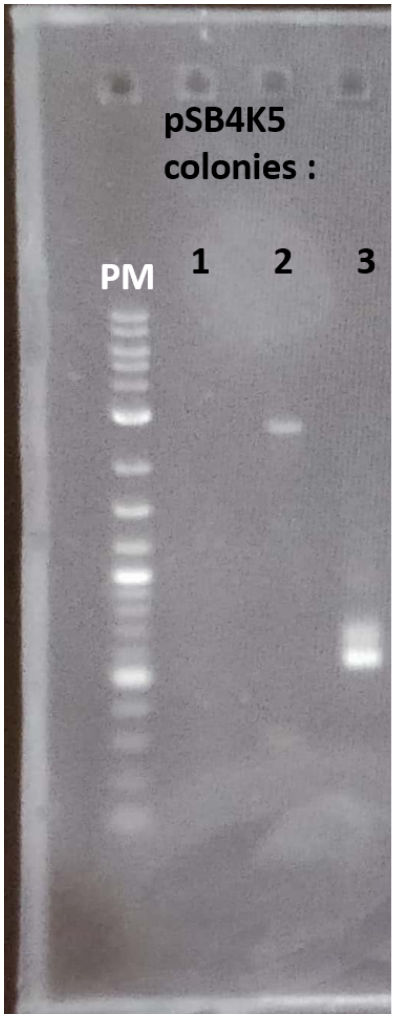
Electrophoresis Gel of the digestion AirmR and sfGFP (1% agarose)

Image26.png



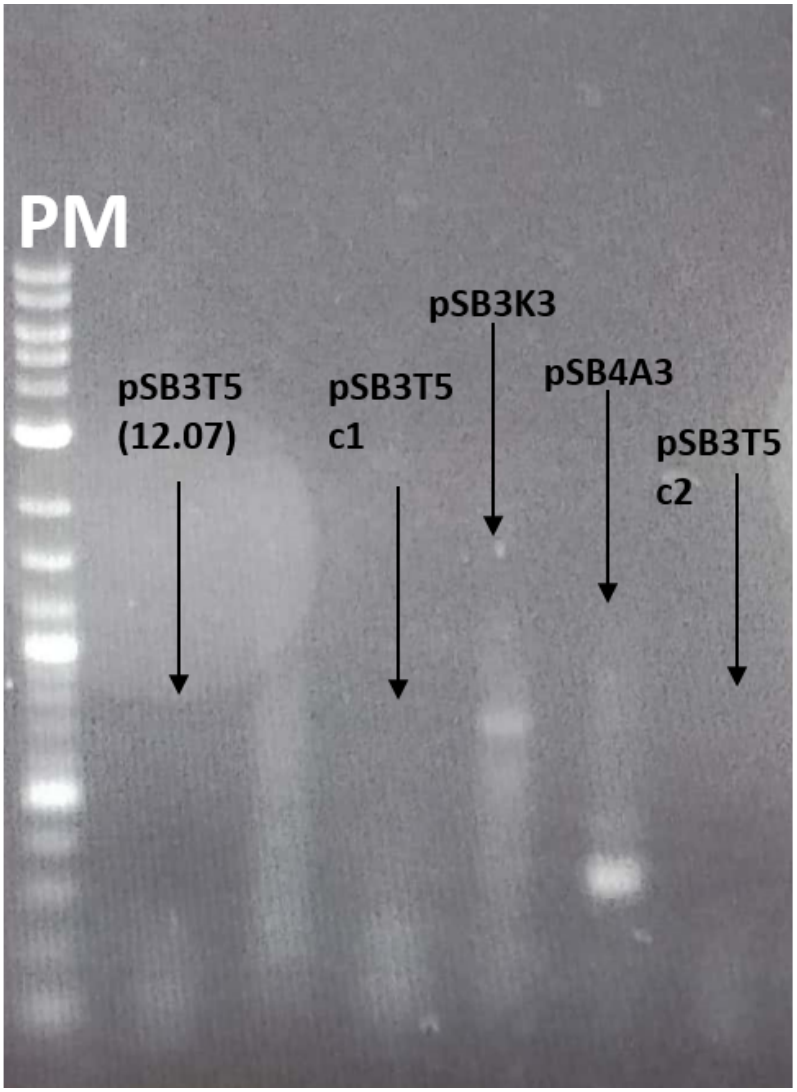
Electrophoresis Gel of the PCR colony of pb4K5_AimR (1% agarose)

Image27.png



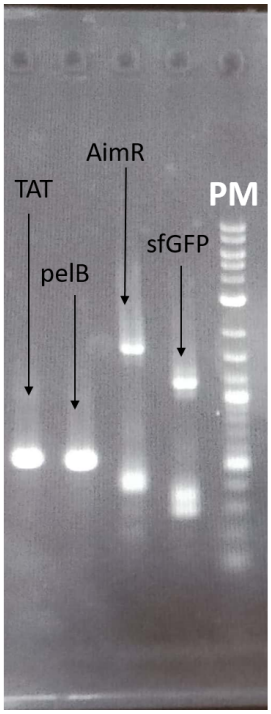
Electrophoresis Gel for the golden gate ready of : psb3T5 (12.07) ; psb3T5 (manish)C1 and C2 ; psb3K3 (1% agarose)

Image29.png



Electrophoresis Gel of the verification of the Golden Gate assembly of : pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP (1% agarose)

Image28.png



Miniprerp psb3TXAimR C°1;2;4;9 and 10 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" :

- Nanodrop
  - psb3TXAimR C°1 =234.4
  - psb3TXAimR C°2 = 338.9
  - psb3TXAimR C°4 = 289.6
  - psb3TXAimR C°9 = 209
  - psb3TXAimR C°10 =

Transformation of pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP

- Transformation of : pSB3T5_AimR/ pSB3TX_AimR/ pSB4K5_sfGFP/ pSB4K5_sfGFP (of 13.07.18)
  Defrost 25µL of E. coli (DHalpha) on ice
  Add 3 µL ofgolden gate product
  Incubate 30′ on ice
  Then make a thermic choc at 42°C during 60′′
  Incubate again 5′ on ice
  Add 1mL of LB
  Incubate 1h at 37°C 300rpm
  centri 5′ at 5000rpm
  Resuspend the cells in 200µL of LB
  After that put 100µL on petri dish LB+ CAm (two time)
  Overnight at 37°C

Digestion of pSB3TX C° 1 ;2 ;4 ;9 and 10 by XBaI and PstI

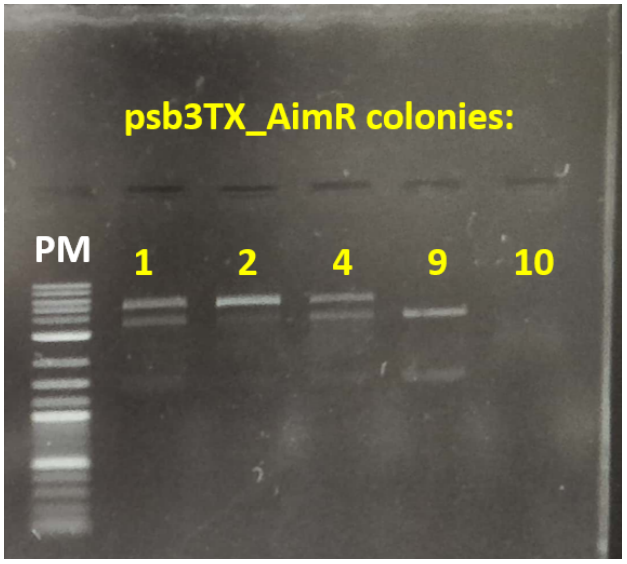
- Digestion Mix

Table54

	A	B
1	Composition	Volume en µL
2	Buffer fastDigest Green 10X	2
3	plasmidic DNA : psb3T5	2
4	Enzyme : XbaI	1
5	Enzyme : PstI	1
6	H2O qsp (20µL)	14

- Electrophoresis Gel of th dgestion of pSB3TX C° 1 ;2 ;4 ;9 and 10 by XBaI and PstI (1% agarose)

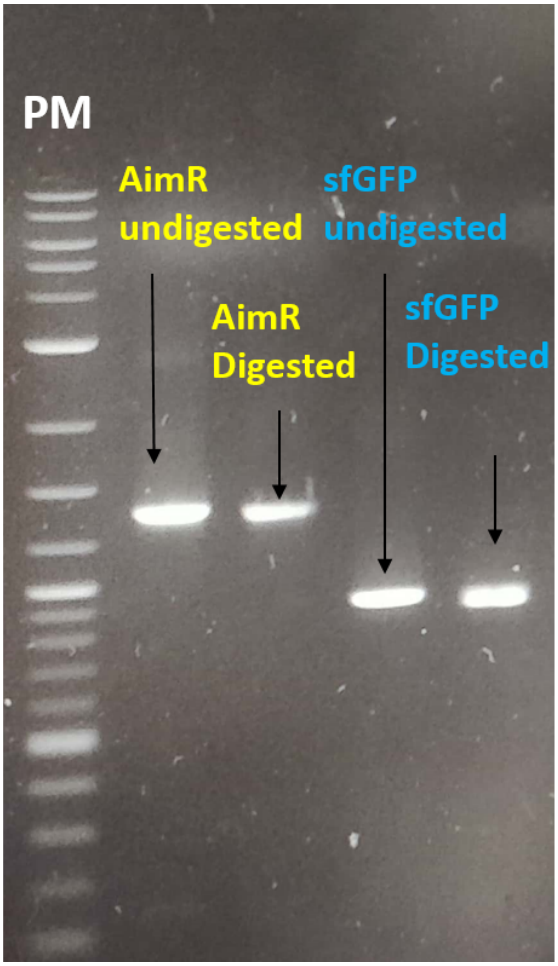
Image35.png



I think we made some mistake on the digestion mix.

Electrophoresis gel of AimR and sfGFP digest and undigest at 100Volt during 1h10 (1% agarose)
We made that elecrophoresis in order to see if the digestion worked.

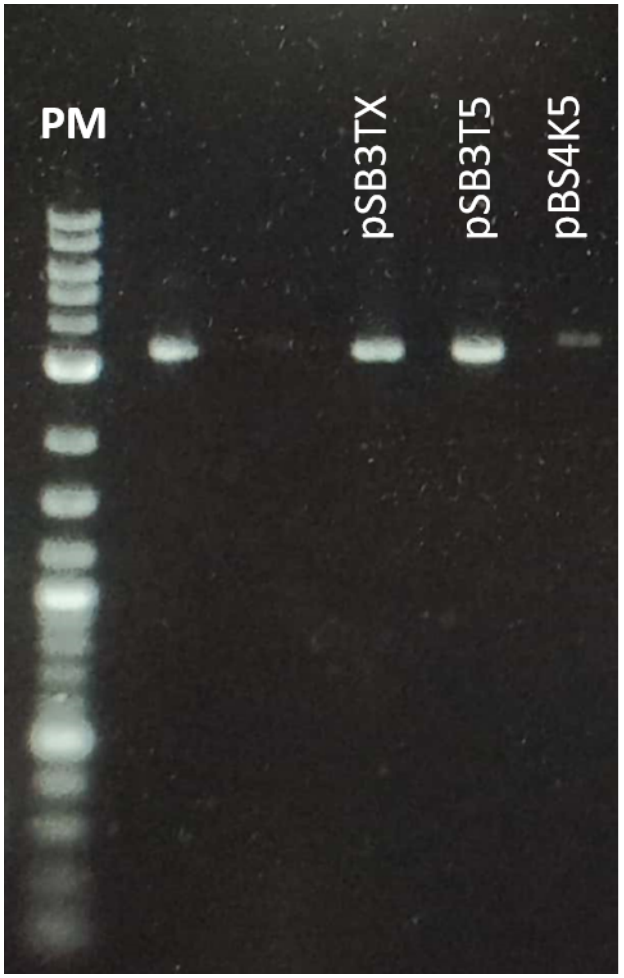
Image31.png



MERCREDI 01/08/2018

Electrophoresis gel of pSB3TX; pSB3T5(12.07) and pSB4K5 after the extraction gel (1% agarose)

Image32.png



PCR in order to prepare the backbone for the goden gate with Q5 and done by Nazim

Backbone used: psb3T5 (manish); pSB3T5 (12.07) and pSB4K5

Quantity : 0.78ng 2.992ng 2.02ng

Table55

	A	B
1	composition	quantity (µl)
2	Q5 buffer	10
3	Mix dNTPs (10mM each)	1
4	iG102 (Fwd)	2.5
5	iG103 (Rvs)	2.5
6	DNA (0.2ng/µl)	1
7	Q5 polymerase	0.5
8	H2O MQ	qsp 50

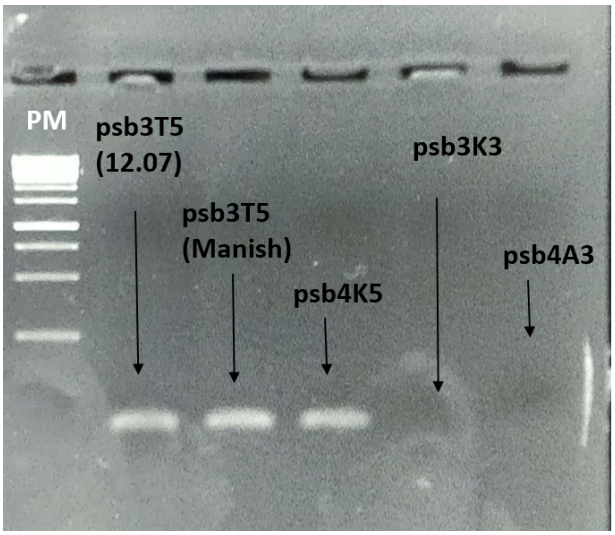
- PCR program

Table56

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	64	30″	
5	72	3′	
6	72	2′	
7	4	-	

- Electrophoresis gel of the ampfifcation

Image33.png



PCR in order to prepare the backbone for the goden gate with phusion

- PCR Mix

We used differents concentration of backbone used: psb3T5 (manish); pSB3T5 (12.07) and pSB4K5:
Quantity : 100ng ; 10ng ;1ng ; 0.1ng

Table58

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	0.4
4	iG102 (Fwd)	1
5	iG103 (Rvs)	1
6	DNA : psSB4K5; or pSB3T5(manish) or pSB3T5(12.07)	1
7	DMSO	0.6
8	phsuion	0.2
9	H2O MQ	qsp 20

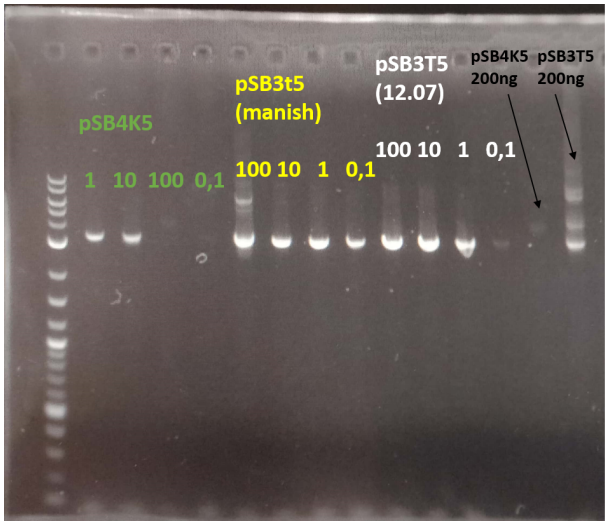
- PCR program

Table57

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*5
4	64	30″	
5	72	2′30″	
6	98	10″	*10
7	68	30″	
8	72	2′30″	
9	98	10″	*30
10	70	30″	
11	72	2′30″	
12	72	2′	
13	4	-	

- Eletrophoresis gel of the amplification

Image34.png



Digestion in order to verifiy pSB3TX_AimR colonies C°1; C°2;C°4;C°9;C°10

- Digestion Mix

Table67

	A	B
1	Composition	Volume en µL
2	Buffer fastDigest Green 10X	2
3	plasmidic DNA : psb3TX	2
4	Enzyme : XbaI	1
5	Enzyme : PstI	1
6	H2O qsp (20µL)	14

- Electrophoresis gel of the digestion

Image30.png

Results of the transformation pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP
we have at last 10 colonies on each petri dish

PCR colony on the colonies obtined for the transformation the Golden gate of : pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP

- Boiling bacteria to kill them
  Put each colony in 20µL of water
  Then put them at 95°C during 5′
  In the same time we make the back up for each colony on petri dish LB + Tet

- PCR Mix

Table59

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp (12,5)	3.75
6	Boiling bacteria	1

- PCR program

Table60

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

Transformation of pSB3TX; SB3K3 and pSB4A3

Defrost 25µL of E. coli (DHalpha) on ice
Add 2 µL of pSB3K3 and pSB4A3; and 1µL of pSBETX
Incubate 30' on ice
Then make a thermic choc at 42°C during 60''
Incubate again 5' on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
centri 5' at 5000rpm
Resuspend the cells in 200µL of LB
After that put 100µL on petri dish LB+ KAN pour pSB4K5 / LB+ Tet Psb3TX/ LB+ Amp pSB4A3
Overnight at 37°C

JEUDI 02/08/2018

Electrophoresis gel of PCR colony product of pSB1C3_AimR/pSB1C3_TAT/pSB1C3_PelB/pSB1C3_sfGFP

Image37.png

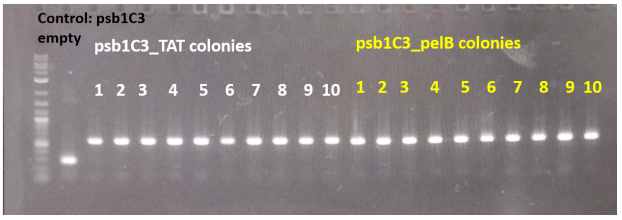
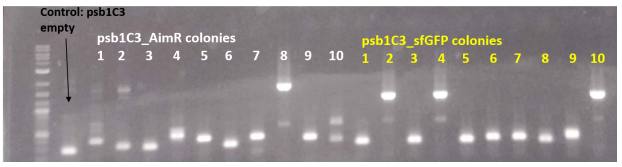


Image36.png



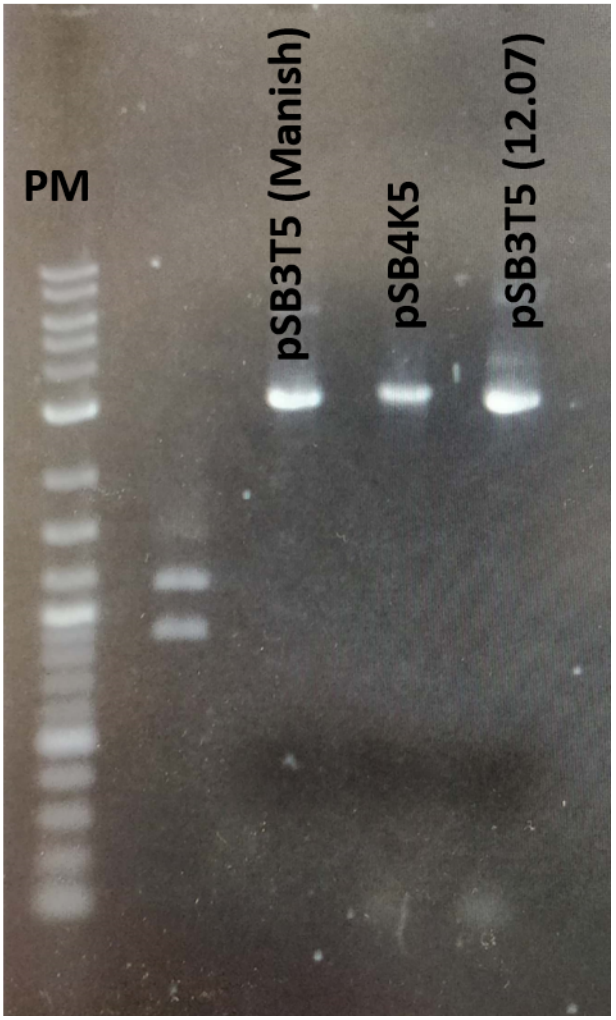
Digestionby DnpI of PCR product for psb3T5 (manish); pSB3T5 (12.07) and pSB4K5 golden gate

- Add 1µL of DnpI in each PCR tubes
- Then incubate 1h at 37°C

PCR cleanup of pSB4K5 ; pSB3T5(manish) and pSB3T5 (12.07) according to" Monarch DNA and PCR cleanup kit" NEB

Electrophoresis of of pSB4K5 ; pSB3T5(manish) and pSB3T5 (12.07)

Image40.png



Miniprep of pSB1C3 GG ready according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" :

- Nanodrop
  - psb1C3 GG Ready = 174 ng

Digestion of pSB1C3 GG in order to verify the plasmid

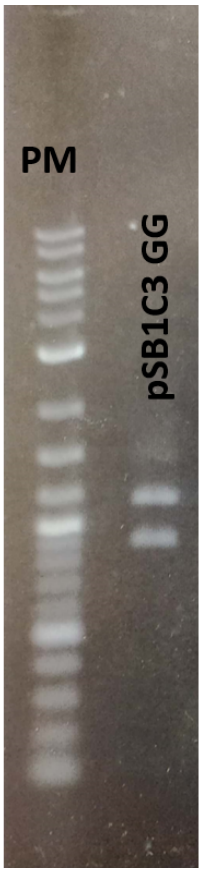
- Digestion Mix

Table68

	A	B
1	Composition	Volume en µL
2	Buffer fastDigest Green 10X	2
3	plasmidic DNA : psb1C3 GG	2
4	Enzyme : XhoI	1
5	H2O qsp (20µL)	15

- Electrophoresis gel of the digestion product

Image38.png



Transformation of pSB3TX; SB3K3 and pSB4A3

Defrost 25µL of E. coli (DHalpha) on ice
Add 2 µL of pSB3K3 and pSB4A3; and 1µL of pSBETX
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
centri 5′ at 5000rpm

Resuspend the cells in 200µL of LB
After that put 100µL on petri dish LB+ KAN pour pSB4K5 / LB+ Tet Psb3TX/ LB+ Amp pSB4A3
Overnight at 37°C

Ligation of pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG

- Ligation Mix

Table61

	A	B
1	Composition	Volume in µL
2	Buffer T4 DNA ligase	2
3	T4 DNA ligase	1
4	vector: pSB4K5	10
5	H20 qsp (20µL)	7

Ligation of pSB4K5 with sfGFP using different quantiity of the insert

- Ligation mix

Table62

	A	B	C	D	E	F	G	H
1	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
2	Buffer T4 DNA ligase	2		Buffer T4 DNA ligase	2		Buffer T4 DNA ligase	2
3	T4 DNA ligase	1		T4 DNA ligase	1		T4 DNA ligase	1
4	Insert : sfGFP (128ng)	0.2		Insert : sfGFP (128ng)	1		Insert : sfGFP (128ng)	2
5	vector: pSB4K5	2.5		vector: pSB4K5	2.5		vector: pSB4K5	2.5
6	H2O qsp (20µL)	14.3		H2O qsp (20µL)	13.5		H2O qsp (20µL)	12.5

Overnight at 4°C

Golden Gate assembly of pSB3T5(Manish)_AimR , pSB3T5(12.05)_AimR and pSB4K5_sfGFP

- Golden gate mix

Table63

	A	B	C	D	E	F	G	H
1	**pSB4K5_sfGFP**			**pSB4K5_sfGFP**			**pSB4K5_sfGFP**	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Vector : pSB4K5	2		Vector : pSB3T5(Manish)	1		Vector : pSB4K5	1
7	Insert : sfGFP	0.70		Insert : AimR	0.85		Insert : AimR	1.12
8	Water qsp (20µL)	14.3		Water qsp (20µL)	15.15		Water qsp (20µL)	14.88

- Golden gate assembly program

Table64

	A	B	C
1	Température (°c)	Temps	Cycles
2	37	5′	*75
3	16	5′	
4	4	-	

Liquid culture of : pSBTX _AimR colonies C°1; C°2;C°4;C°9;C°10 ;pSB3TX ; pSB1C3_pelB C°1; C°2; C°3;pSB1C3_TAT C°1; C°2; C°3;pSB1C3_sfGFP C°2; C°4; C°10;pSB1C3_AimRC°1; C°9;

LB + antibio

Send to sequencing :

Table72

	A	B	C	D	E
1	Plasmid	pSBTX _AimR colonies C°1	pSBTX _AimR colonies C°2	pSBTX _AimR colonies C°4	pSBTX _AimR colonies C°9
2	Sequencing code	68FB64	68FB61	68FB58	68FB66

VENDREDI 03/08/2018

PCR of verfication of the golden gate assembly of pSB3T5(Manish)_AimR , pSB3T5(12.05)_AimR and pSB4K5_sfGFP

- PCR Mix

Table65

	A	B
1	Composition	Volume in µL
2	One Taq MM	12.5
3	Primer : VR	0.5
4	Primer : VF2	0.5
5	Water qsp (25µL)	10.5
6	DNA	1

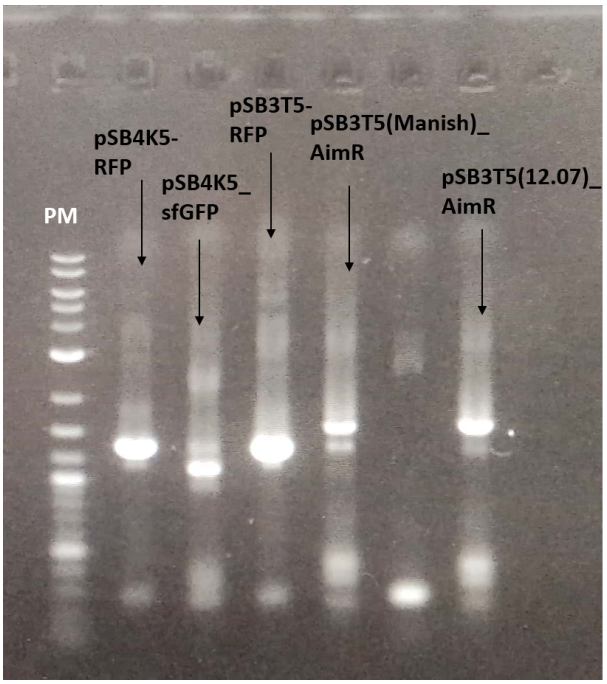
- PCR program

Table66

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	30''	
3	94	30''	*30
4	50	30''	
5	72	2'	
6	72	4'	
7	4	-	

- Electrophoresis gel of the PCR product

Image41.png



Miniprep of pSBTX _AimR colonies C°1; C°2;C°4;C°9 ;pSB3TX ; pSB1C3_pelB C°1; C°2; C°3;pSB1C3_TAT C°1; C°2; C°3;pSB1C3_sfGFP C°2; C°4; C°10;pSB1C3_AimR C°8 according to according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503":

- Nanodrop

Table71

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Plasmid	pSBTX_AimR colonies C°1	pSBTX_AimR colonies C°1	pSBTX_AimR colonies C°2	pSBTX_AimR colonies C°2	pSBTX_AimR colonies C°4	pSBTX_AimR colonies C°4	pSBTX_AimR colonies C°9	pSBTX_AimR colonies C°9	pSB1C3_pelB C°1	pSB1C3_pelB C°2	pSB1C3_pelB C°3	pSB1C3_TAT C°1	pSB1C3_TAT C°2	pSB1C3_TAT C°3	pSB1C3_sfGFP C°2	pSB1C3_sfGFP C°4	pSB1C3_sfGFP C°10	pSB3TX	pSB3TX
2	Nanodrop en ng/µl	239	29.4	202.5	249.2	218.4	29.6	205.6	123	140.1	220.8	157.4	198	171.9	180.7	198.4	312.1	263.4	183	149

Digestion by XbaI and PstI of : pSBTX _AimR colonies C°1; C°2;C°4;C°9;C°10 ;pSB3TX ; pSB1C3_pelB C°1; C°2; C°3;pSB1C3_TAT C°1; C°2; C°3;pSB1C3_sfGFP C°2; C°4; C°10;pSB1C3_AimRC°8

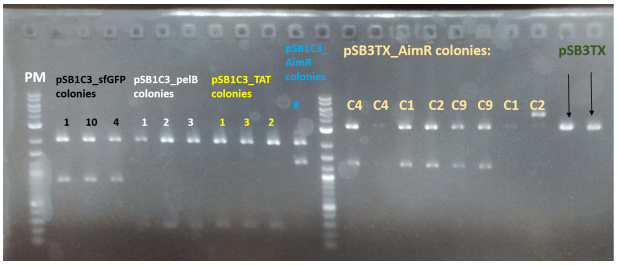
- Digestion Mix

Table70

	A	B
1	Composition	Volume en µL
2	Buffer fastDigest Green 10X	2
3	plasmidic DNA : psb3TX	2
4	Enzyme : XbaI	1
5	Enzyme : PstI	1
6	H2O qsp (20µL)	14

- Electrophoresis gel of the digestion producs (1%agarose)

Image39.png



LUNDI 06/08/2018

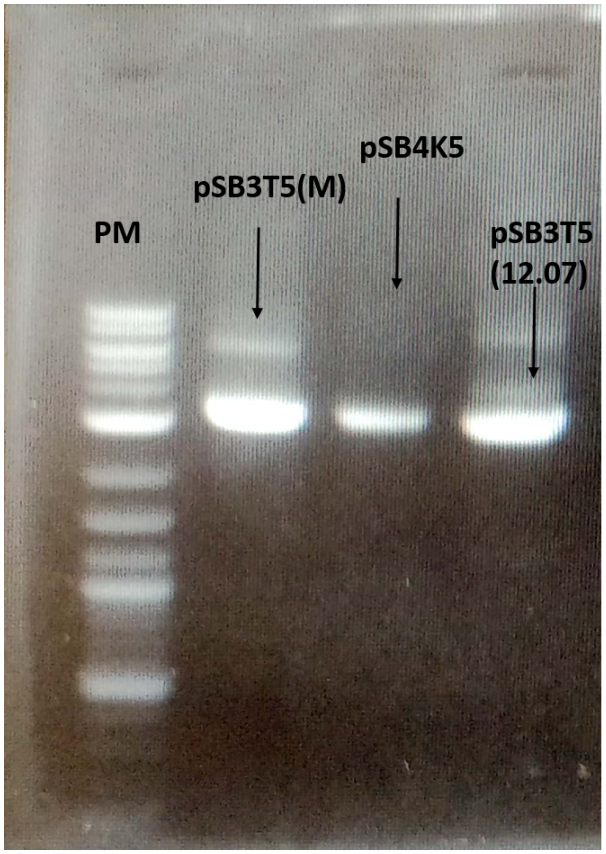
Send to sequencing :

Table73

	A	B	C	D	E	F	G	H	I	J	K
1	Plasmid	pSB1C3_pelB C°1	pSB1C3_pelB C°2	pSB1C3_pelB C°3	pSB1C3_TAT C°1	pSB1C3_TAT C°2	pSB1C3_TAT C°3	pSB1C3_sfGFP C°2	pSB1C3_sfGFP C°4	pSB1C3_sfGFP C°10	pSB1C3_AimR C°8
2	Sequencing code	68FB60	68FB57	68FB56	68FB59	68FB62	68FB65	68FB52	68FB49	68FB46	68FB55

Electrophoresis gel of pSB3T5(manish) GG ; pSB3T5(12.07) GG ; pSB4K5 GG for the gel extraction (agarose 1%)

Image42.png



Gel Extraction of pSB3T5(manish) GG ; pSB3T5(12.07) GG ; pSB4K5 GG according to " Monarch DNA Extraction Gel Kit"

- Nanodrop (ng/µL)
  pSB3T5(manish) GG =
  pSB3T5(12.07) GG =
  pSB4K5 GG =

Classic Cloning

Colony PCR on the colonies obtained after the transformation of pSB4K5_sfGFP :

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5'

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table74

	A	B
1	Composition	Volume in µL
2	Dream Taq MM	6.25
3	Primer : VR	0.625
4	Primer : VF2	0.625
5	Water qsp	4
6	Boiling bacteria	1

- PCR program

Table76

	A	B	C
1	Température (°c)	Temps	Cycles
2	95	3′	
3	98	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Electrophoresis Gel of the PCR colony products

Transformation of the golden gate products pSB3T5(Manish)_AimR , pSB3T5(12.05)_AimR and pSB4K5_sfGFP; Ligation products of pSB4K5 with sfGFP (0.2µL;1µL;2µL); and ligation products of pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG

Defrost 25µL of E. coli (DHalpha) on ice
Add 2 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
centri 5′ at 5000rpm
Resuspend the cells in 200µL of LB
After that put 100µL on petri dish LB+ KAN or LB+ Tet
Overnight at 37°C

Liquid culture of pSB3K3: : LB +Kan

MARDI 07/08/2018

Colony PCR on the colonies obtained after the transformation of the GG pSB3T5(Manish)_AimR , pSB3T5(12.05)_AimR and pSB4K5_sfGFP and pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG.

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table77

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3126
5	Water qsp	4
6	Boiling bacteria	1

- PCR program

Table78

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	3′	
3	94	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Electrophoresis Gel of the PCR colony products

Image50.png

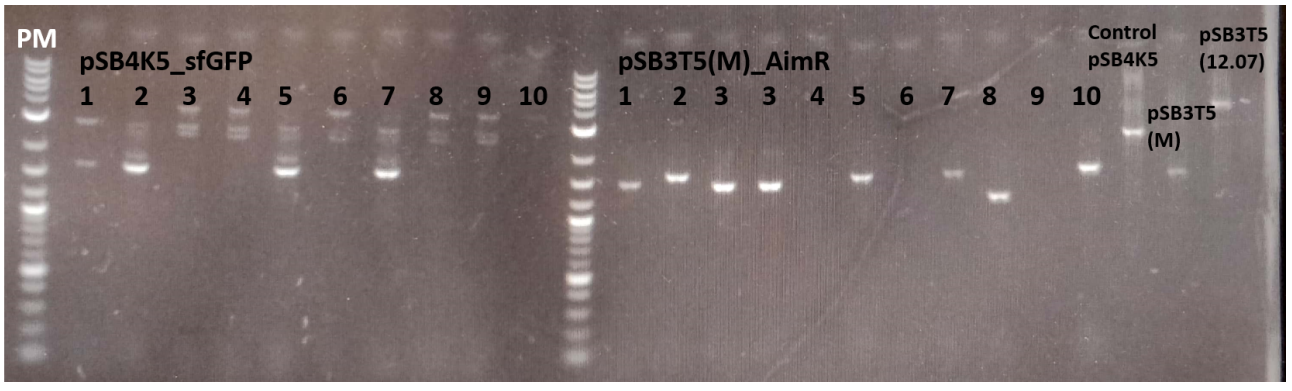
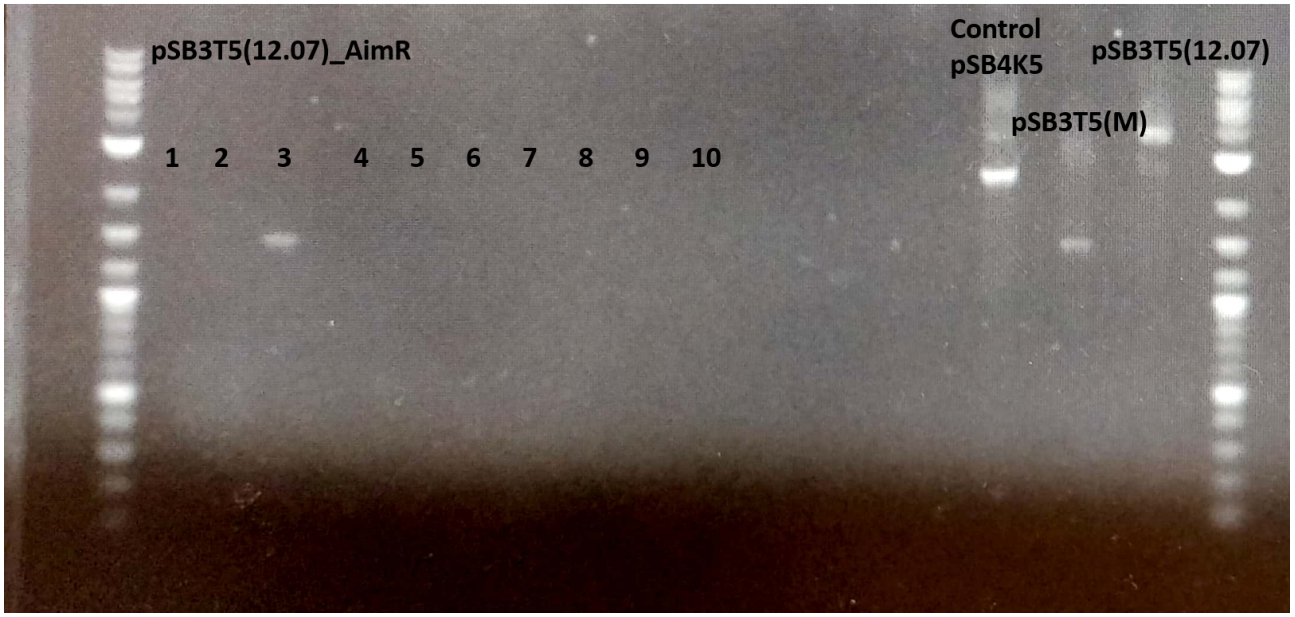


Image51.png



Miniprep on the overnight culture of the colonies to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503":
- pSB3K3 C°1 and C°2

- Nanodrop:
  pSB3K3 C°1 = 225ng/µL
  pSB3K3 C°2 = 298ng/µL

Digestion of pSB3K3 by XhoI:

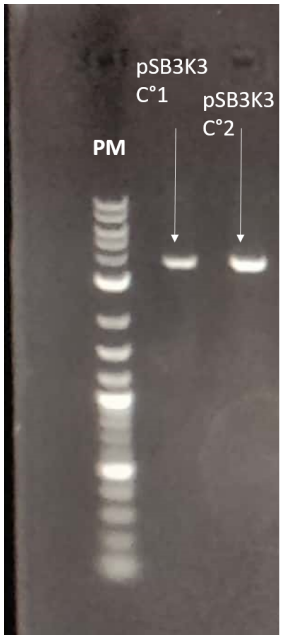
- Digestion Mix

Table79

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA: pSB3K3 C°1 or C°2	2
4	Enzyme: XbaI	1
5	Water qsp (20µL)	15

- Electrophoresisgel of the digestion products

Image43.png



MERCREDI 08/08/2018

Colony PCR on the colonies obtained after the transformation of the GG pSB3T5(Manish)_AimR , pSB3T5(12.05)_AimR and pSB4K5_sfGFP and pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG.

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table80

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3126
5	Water qsp	4
6	Boiling bacteria	1

  - PCR program

Table81

	A	B	C
1	Température (°c)	Temps	Cycles
2	94	3′	
3	94	30″	*30
4	50	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Electrophoresis Gel of the PCR colony products

Image46.png

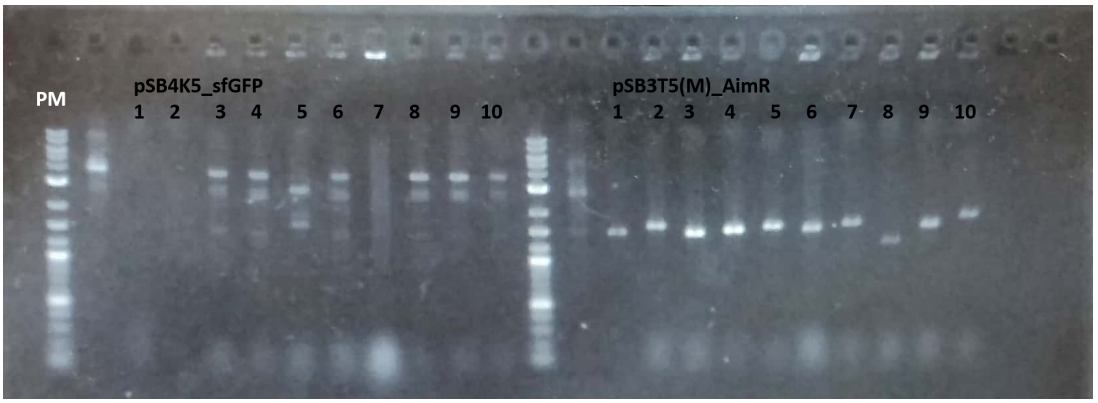


Image47.png

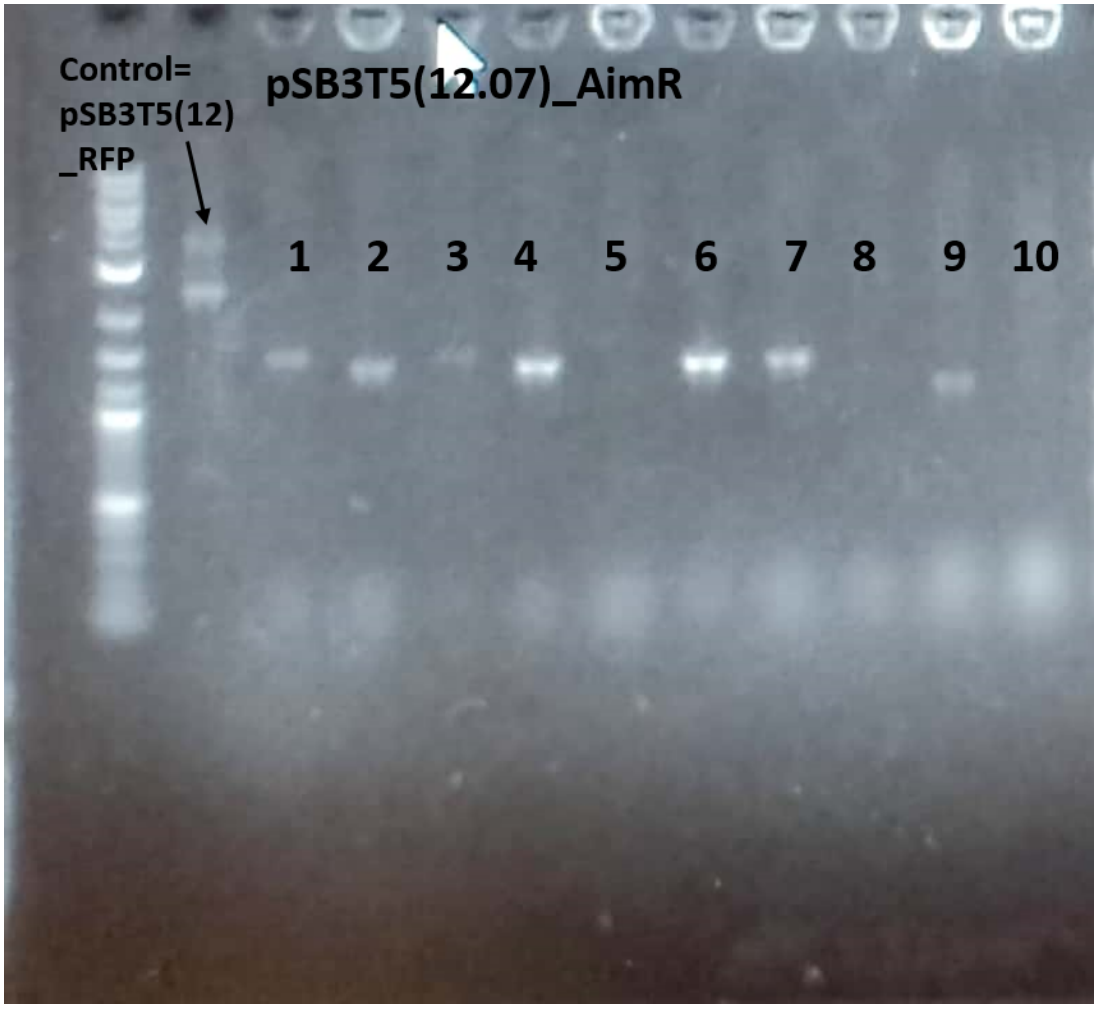


Image48.png

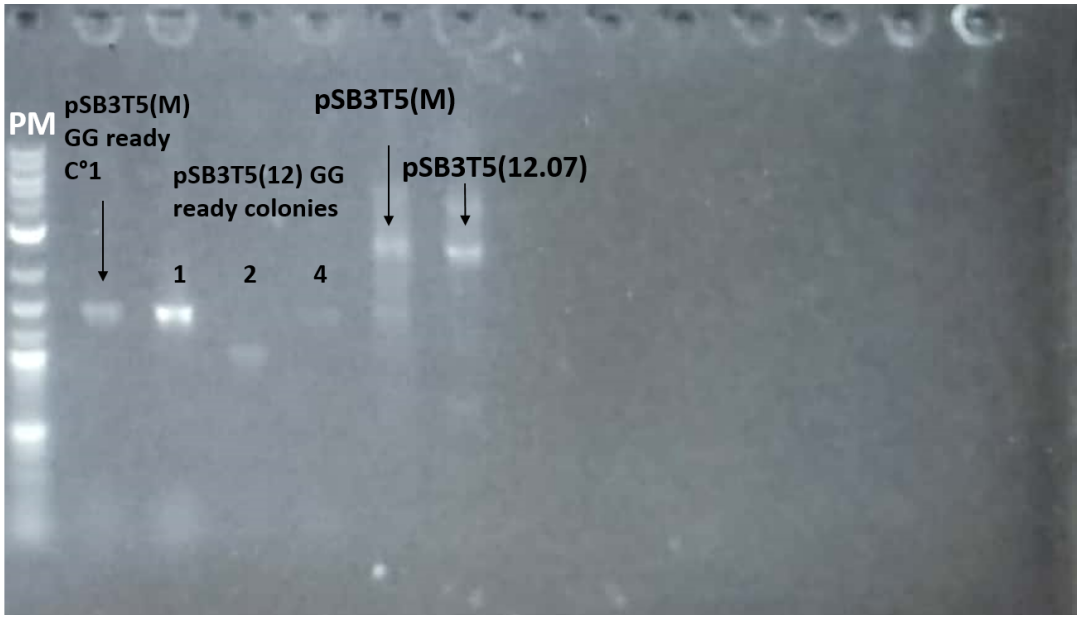
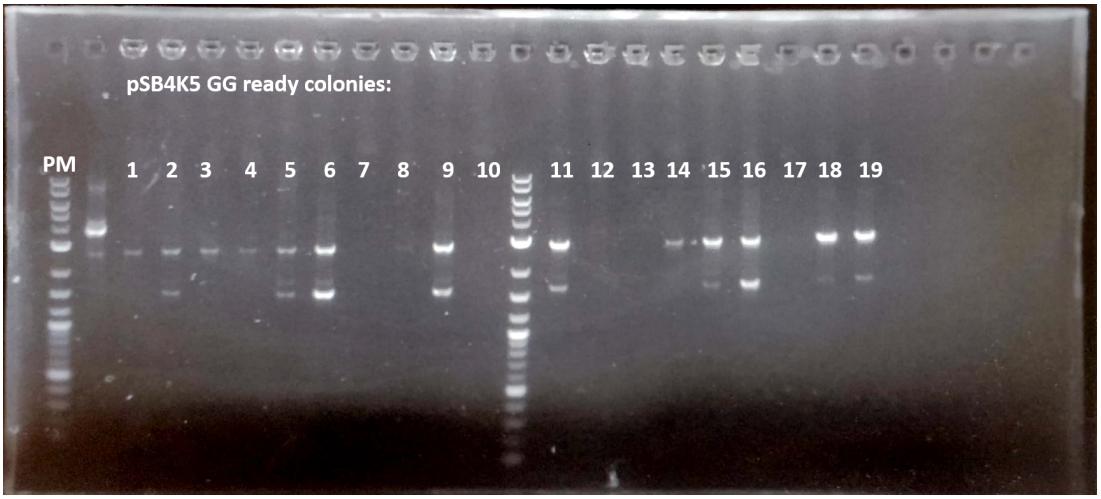


Image49.png



Liquid culture of the GG pSB3T5(Manish)_AimR C°1;3;4;5 / pSB3T5(12.05)_AimR C°2;4;6;7 / pSB4K5_sfGFP C°2;5;7 /pSB3T5(Manish) GG C°1 /pSB3T5 (12.07) GG C°1;4;3 / pSB4K5 GG C°6;9;18;19

10mL LB + Antio (Tet or Kan)

JEUDI 09/08/2018

Stock glycerol of the liquid culture of the GG pSB3T5(Manish)_AimR C°1;3;4;5 / pSB3T5(12.05)_AimR C°2;4;6;7 / pSB4K5_sfGFP C°2;5;7 /pSB3T5(Manish) GG C°1 /pSB3T5 (12.07) GG C°1;4;3 / pSB4K5 GG C°6;9;18;19

1mL of Liquid culture + 400µL of glycerol 80%

Miniprep on the liquid culture of the pSB3T5(Manish)_AimR C°1;3;4;5 / pSB3T5(12.07)_AimR C°2;4;6;7 / pSB4K5_sfGFP C°2;5;7 /pSB3T5(Manish) GG C°1 /pSB3T5 (12.07) GG C°1;2;4 / pSB4K5 GG C°6;9;18;19

- Nanodrop

Table86

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Plasmid	pSB3T5(Manish)_AimR C°1	pSB3T5(Manish)_AimR C°3	pSB3T5(Manish)_AimR C°4	pSB3T5(Manish)_AimR C°5	pSB3T5(12.07)_AimR C°2	pSB3T5(12.07)_AimR C°4	pSB3T5(12.07)_AimR C°6	pSB3T5(12.07)_AimR C°7	pSB4K5_sfGFP C°2	pSB4K5_sfGFP C°5	pSB4K5_sfGFP C°7	pSB3T5(Manish) GG C°1	pSB3T5 (12.07) GG C°1	pSB3T5 (12.07) GG C°2	pSB3T5 (12.07) GG C°4	pSB4K5 GG C°6	pSB4K5 GG C°9	pSB4K5 GG C°18	pSB4K5 GG C°19
2	Nanodrop en ng/µl	208.6	121.3	140.2	205.1	214.7	161.4	171.5	197.9	107.5	100	87.7	142.2	162.5	186.3	140	81.9	91.6	81.9	85.1

Digestion of GG pSB3T5(Manish)_AimR C°1;3;4;5 / pSB3T5(12.05)_AimR C°2;4;6;7 / pSB4K5_sfGFP C°2;5;7 /pSB3T5(Manish) GG C°1 /pSB3T5 (12.07) GG C°1;4;3 / pSB4K5 GG C°8;9;18;19 by XbaI and PstI

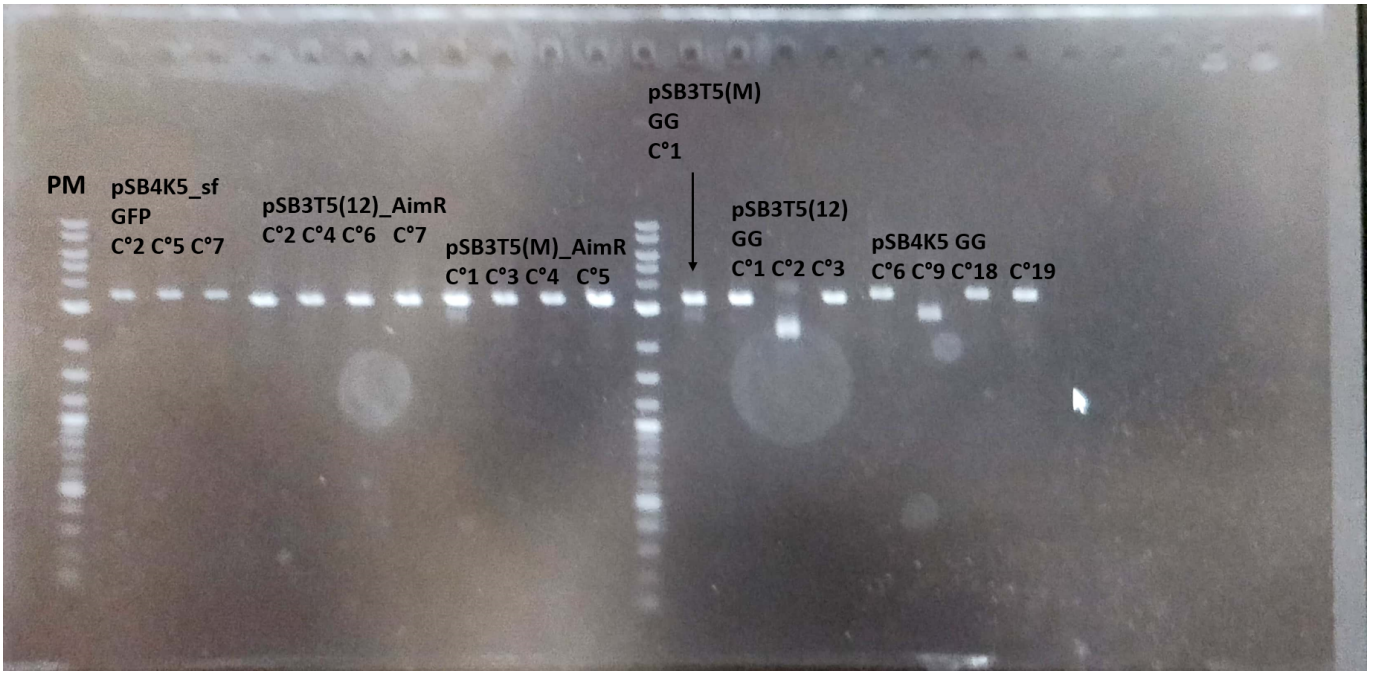
- Digestion Mix

Table82

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA	2
4	Enzyme:PstI	1
5	Enzyme:XbaI	1
6	Water qsp (20µL)	14

- Electrophoresis gel of the digetion

Image44.png



Colony PCR on the colonies obtained after the transformation of pSB4K5_sfGFP and pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG.

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5'

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table83

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3126
5	Water qsp	4
6	Boiling bacteria	1

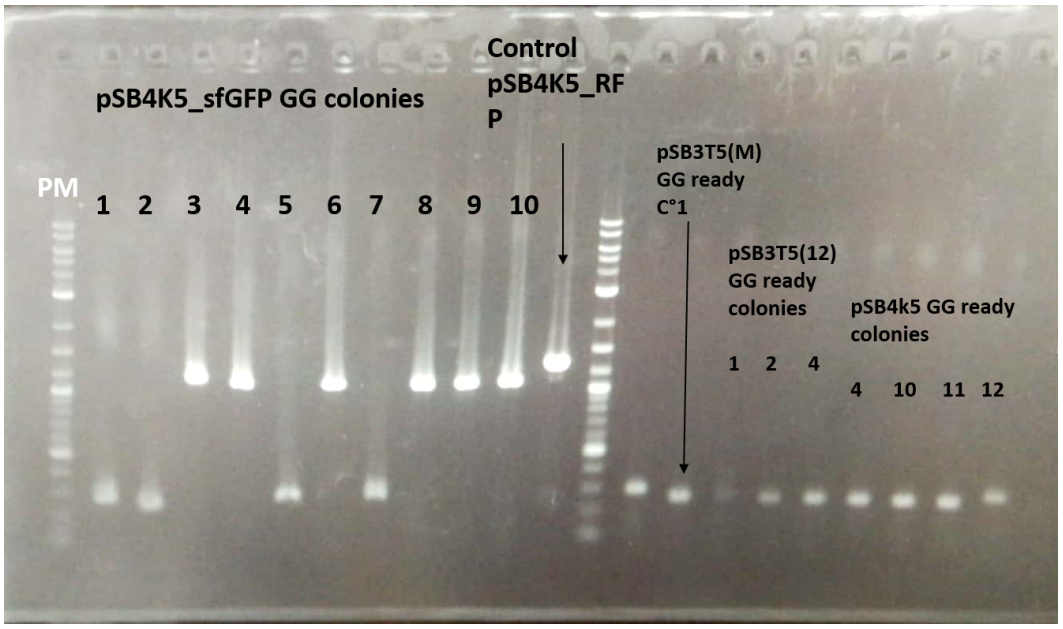
- PCR program

Table84

	A	B	C	D	E	F	G
1	**Template for pSB4K5_sfGFP**				**Template for pSB3T5(Manish) GG ; pSB3T5 (12.07) GG ; pSB4K5 GG**		
2	Température (°c)	Temps	Cycles		Température (°c)	Temps	Cycles
3	94	3′			94	3′	
4	94	30″	*30		94	30″	*30
5	**54**	30″			**54**	30″	
6	72	2′			72	**40″**	
7	72	5′			72	5′	
8	4	-			4	-	

- Electrophoresis gel of PCR colony products

Image45.png



Liquid culture of pSB3T5(Manish)_AimR C°2;5;7 and pSB3T5(12.07)_AimR C° 1;3

VENDREDI 10/08/2018

Miniprep of the liquid culture of pSB3T5(Manish)_AimR C° andpSB3T5(12.07)_AimR C°pSB3T5(12.07)_AimR C°according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

Nanodrop

Table85

	A	B	C	D	E	F	G
1	Plasmid	pSB3T5(Manish)_AimR C°2	pSB3T5(Manish)_AimR C°5	pSB3T5(Manish)_AimR C°7	pSB3T5(Manish)_AimR C°10	pSB3T5(12.07)_AimR C°1	pSB3T5(12.07)_AimR C°3
2	Nanodrop en ng/µl	156.1	143.6	251.6	143.3	204.5	191.4

Golden Gate

Golden gate assembly of pSB1C3_PAimX(full)_sfGFP and pSB1C3_PAimX(small)_sfGFP

- Golden gate assembly Mix

Table87

	A	B	C	D	E
1	Mix for pSB1C3_PAimX(full)_sfGFP			Mix for pSB1C3_PAimX(small)_sfGFP	
2	Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BbsI	0.5		Enzyme: BbsI	0.5
6	Vector : pSB1C3_sfGFP (2906bp)	0.5		Vector : pSB1C3_sfGFP (2906bp)	0.5
7	Insert : PAimX(full) (190bp)	1.5		Insert : PAimX(promoter) (115bp)	0.388
8	Water qsp (20µL)	15		Water qsp (20µL)	16.1

- Golden gate assembly program

Table88

	A	B	C
1	Température (°c)	Temps	Cycles
2	37	5′	*198
3	16	5′	
4	55	15′	
5	85	20′	
6	4	-	

Liquid culture of pSB4K5_sfGFP GG C°3;4;6;8;9;10 /pSB3T5(Manish)_AimR C°2; 5;7;10/ pSB3T5(12.07)_AimR C°1;2

LB + antibio (10mL and 10µL)

LUNDI 13/08/2018

Stock glycerol of the Liquid culture of pSB4K5_sfGFP GG C°3;4;6;8;9;10 /pSB3T5(Manish)_AimR C°2; 5;7;10/ pSB3T5(12.07)_AimR C°1;3

1mL liquid culture + 400µL glycerol 80%

Miniprep of the liquid culture of pSB4K5_sfGFP GG C°3;4;6;8;9;10 /pSB3T5(Manish)_AimR C°2; 5;7;10/ pSB3T5(12.07)_AimR C°1;2 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

Nanodrop

Table89

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Plasmid	pSB3T5(Manish)_ AimR C°2	pSB3T5(Manish)_ AimR C°5	pSB3T5(Manish)_ AimR C°7	pSB3T5(Manish)_ AimR C°10	pSB3T5(12.07) _AimR C°1	pSB3T5(12.07) _AimR C°3	pSB4K5_sfGFP GG C°3	pSB4K5_sfGFP GG C°4	pSB4K5_sfGFP GG C°6	pSB4K5_sfGFP GG C°8	pSB4K5_sfGFP GG C°9	pSB4K5_sfGFP GG C°10
2	Nanodrop en ng/µl	196.7	179.1	156.9	184.8	221.8	189.2	119.2	220.9	254.1	297.8	229.3	297..2

Transformation of pSB1C3_PAimX(full)_sfGFP /pSB1C3_PAimX(small)_sfGFP/ pEXA128pAimXPhi3T(full)

Defrost 50L of E. coli (DHalpha) on ice
Add 2 µL ofgolden gate product
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
centri 5′ at 5000rpm
Resuspend the cells in 200µL of LB
After that put 100µL on petri dish LB+ CAm (two time) or LB +Amp
Overnight at 37°C

Send to sequencing :

Table90

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Plasmid	pSB4K5_sfGFP GG C°3	pSB4K5_sfGFP GG C°4	pSB4K5_sfGFP GG C°6	pSB3T5(Manish)_ AimR C°2	pSB3T5(Manish) _AimR C°7	pSB3T5(Manish) _AimR C°10	pSB3T5(12.07) _AimR C°1	pSB3T5(12.07) _AimR C°3	pSB4K5 GG C°6	pSB4K5 GG C°18	pSB4K5 GG C°19	pSB3T5(Manish) GG C°1	pSB3T5 (12.07) GG C°1	pSB3T5 (12.07) GG C°4		
2	Sequencing code	68FB35	68FB36	68FB37	68FB38	68FB42	68FB41	68FB40	68FB39	68FB45	68FB44	68FB48	68FB47	68FB50	68FB53		

Golden gate

Golden gate assembly of pSB4K5 _PAimX(full)_sfGFP

Golden gate assembly Mix (ration 3:1)

Table01

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Mix for pSB4k5_PAlmX(full)_sfGFP C*3			Mix for pSB4k5_PAlmX(small)_sfGFP C*3			Mix for pSB4k5_PAlmX(full)_sfGFP C*4			Mix for pSB4k5_PAlmX(small)_sfGFP C*4			Mix for pSB4k5_PAlmX(full)_sfGFP C*6			Mix for pSB4k5_PAlmX(small)_sfGFP C*6	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BbsI	0.5		Enzyme: BbsI	0.5		Enzyme: BbsI	0.5		Enzyme: BbsI	0.5		Enzyme: BbsI	0.5		Enzyme: BbsI	0.5
6	Vector : pSB4k5_sfGFP (4255bp)	0.84		Vector : pSB4k5_sfGFP (4255bp)	0.84		Vector : pSB4k5_sfGFP (4255bp)	0.45		Vector : pSB4k5_sfGFP (4255bp)	0.45		Vector : pSB4k5_sfGFP (4255bp)	0.39		Vector : pSB4k5_sfGFP (4255bp)	0.39
7	Insert : PAlmX(full) (1908bp)	1.06		Insert : PAlmX(promoter) (115bp)	0.79		Insert : PAlmX(full) (1908bp)	1.06		Insert : PAlmX(promoter) (115bp)	0.79		Insert : PAlmX(full) (1908bp)	1.06		Insert : PAlmX(promoter) (115bp)	0.79
8	Water qsp (20µL)	15.10		Water qsp (20µL)	15.37		Water qsp (20µL)	15.49		Water qsp (20µL)	15.75		Water qsp (20µL)	15.55		Water qsp (20µL)	15.81

- Golden gate assembly program

Table92

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	55	15′	
5	85	20′	
6	4	-	

MARDI 14/08/2018

Colony PCR on the colonies obtained after the transformation of pSB1C3_PAimX(full)_sfGFP /pSB1C3_PAimX(small)_sfGFP

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + CAM

  - MiX PCR

Table93

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3125
5	Water qsp	4.625
6	Boiling bacteria	1

  - PCR program

Table94

	A	B	C
1	Temperature (°c)	time	Cycles
2	94	3′	
3	94	30″	*30
4	**54**	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Eletrophoresis gel of the colony PCR

Image52.png

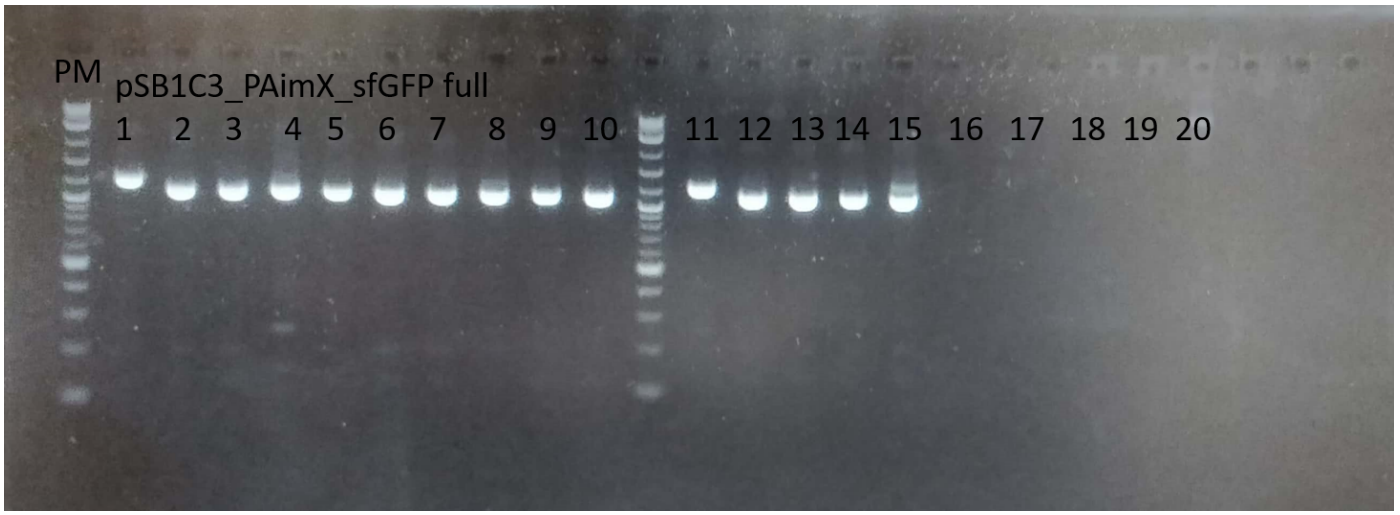
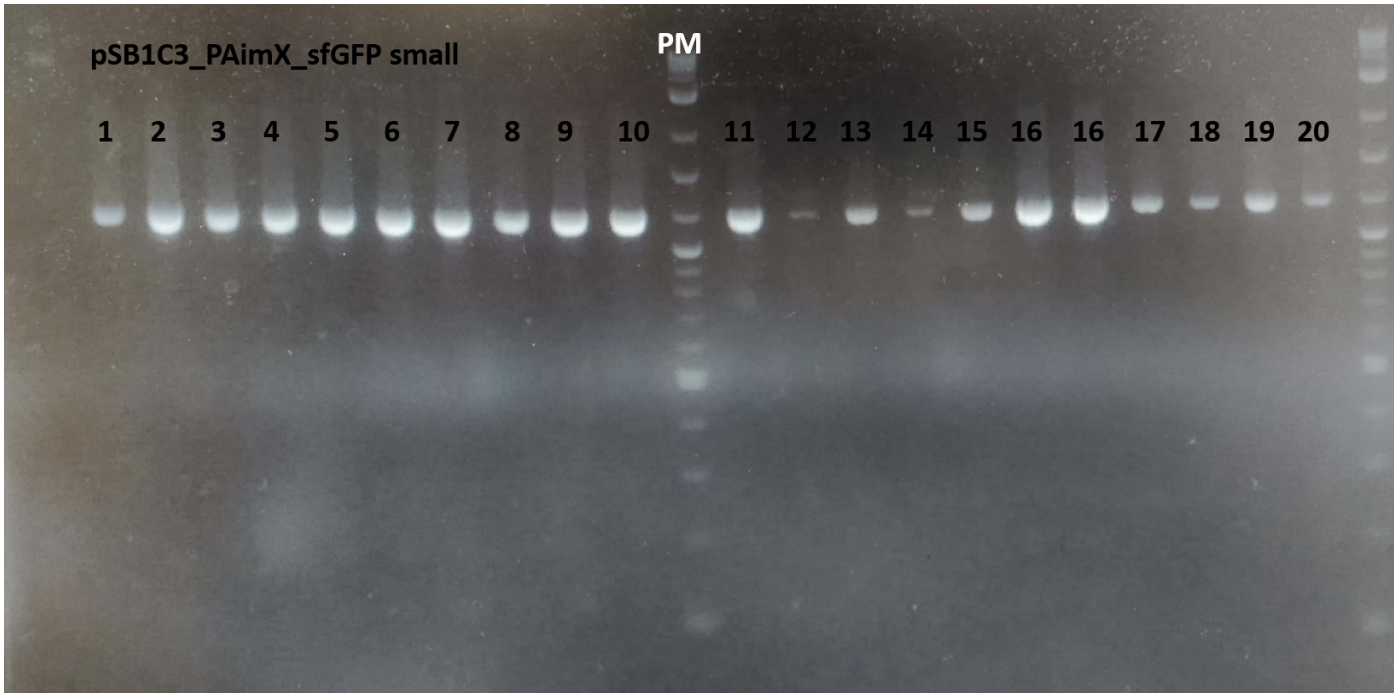


Image53.png



Transformation of pSB4K5 _PAimX(full)_sfGFP C°3;4;6

Defrost 50L of E. coli (DHalpha) on ice
Add 2 µL of golden gate product
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN
Overnight at 37°C

Liquid culture of pSB1C3_PAimX(full)_sfGFP C°1;11 /pSB1C3_PAimX(small)_sfGFP C°1;8;12;14;18;20/ pEXA128pAimX-Phi3T(full) C°1;2;3

LB (10mL)+ Antibio (10µL)

JEUDI 16/08/2018

Colony PCR on the colonies obtained after the transformation of pSB4K5_PAimX(full)_sfGFP /pSB4K5_PAimX(small)_sfGFP

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′

  In the same time we make the back up for each colony on petri dish LB + Kan

  - MiX PCR

Table95

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3125
5	Water qsp	4.625
6	Boiling bacteria	1

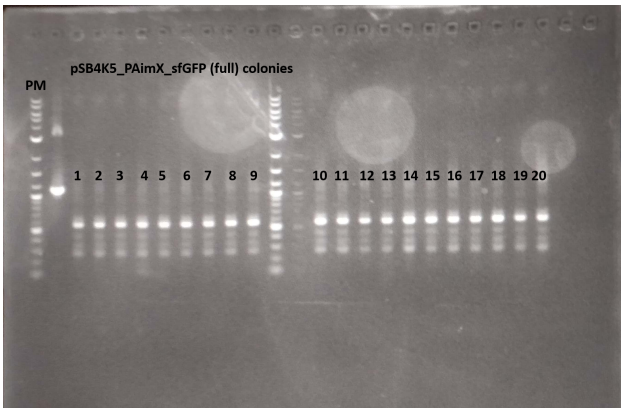
- PCR program

Table96

	A	B	C
1	Temperature (°c)	time	Cycles
2	94	3′	
3	94	30″	*30
4	**54**	30″	
5	68	2′	
6	68	5′	
7	4	-	

- Eletrophoresis gel of the colony PCR

Image59.png



Miniprep of the liquid culture of pSB1C3_PAimX(full)_sfGFP C°1 /pSB1C3_PAimX(small)_sfGFP C°1;8;14;18;20/ pEXA128-pAimXPhi3T(full) C°1;2;3 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

Nanodrop

Table97

	A	B	C	D	E	F	G	H	I	J
1	Plasmid	pSB1C3_PAimX(full)_sfGFP C°1	pSB1C3_PAimX(small)_sfGFP C°1	pSB1C3_PAimX(small)_sfGFP C°8	pSB1C3_PAimX(small)_sfGFP C°14	pSB1C3_PAimX(small)_sfGFP C°18	pSB1C3_PAimX(small)_sfGFP C°20	pEXA128-pAimX-Phi3T(full) C°1	pEXA128-pAimX-Phi3T(full) C°2	pEXA128-pAimX-Phi3T(full) C°3
2	Nanodrop en ng/µl	42.3	163.8	120.7	171.5	190.8	95.4	269.3	232.9	249.8

Digestion Mix of pEXA128pAimXPhi3T(full) C°1;2;3

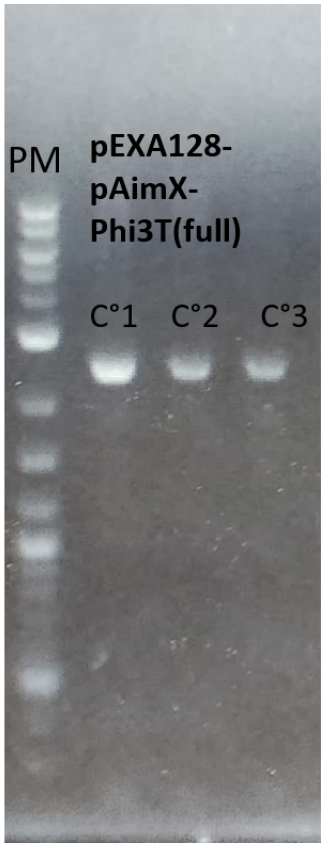
- Digestion Mix

Table98

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	2
3	Plasmid DNA	2
4	Enzyme:NotI	1
5	Enzyme:BamHI	1
6	Water qsp (20µL)	14

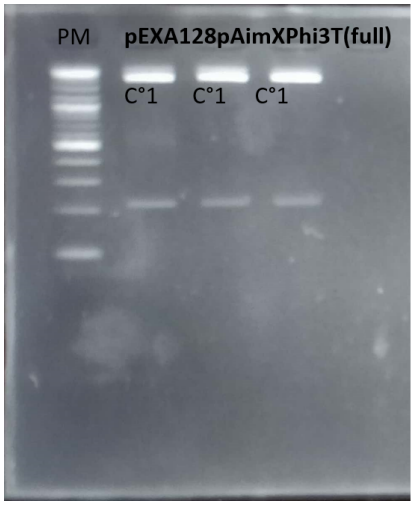
- Electrophoresis gel of the digetion (1% agarose)

Image58.png



- Electrophoresis gel of the digetion (2% agarose)

Image57.png



Send to sequencing :

Table99

	A	B	C	D	E
1	Plasmid	pSB1C3_PAimX(full)_sfGFP C°1	pSB1C3_PAimX(small)_sfGFP C°1	pSB1C3_PAimX(small)_sfGFP C°20	pSB1C3_PAimX(small)_sfGFP C°
2	Sequencing code	68FB34	68FB33	68FB32	68FB31

VENDREDI 17/08/2018

Colony PCR on the colonies obtained after the transformation of pSB4K5_PAimX(full)_sfGFP /pSB4K5_PAimX(small)_sfGFP

- Colony PCR (on the colony obtained by these transformations)
  - Boiling bacteria to kill them
    Put each colony in 20µL of water
    Then put them at 95°C during 5′
  - MiX PCR

Table100

	A	B
1	Composition	Volume in µL
2	One Taq MM	6.25
3	Primer : VR	0.3125
4	Primer : VF2	0.3125
5	Water qsp	4.625
6	Boiling bacteria	1

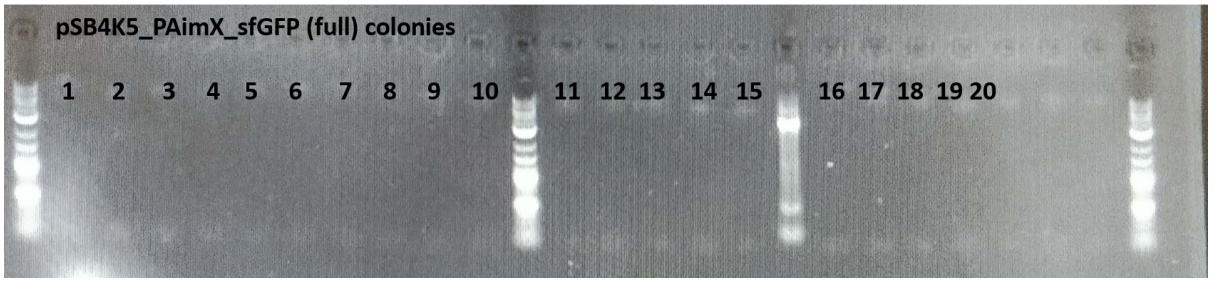
- PCR program

Table101

	A	B	C
1	Temperature (°c)	time	Cycles
2	94	3′	
3	94	30″	*30
4	**54**	30″	
5	72	2′	
6	72	5′	
7	4	-	

- Electrophoresis gel of the digetion (1% agarose)

Image54.png



Liquid cultue of pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20

LUNDI 20/08/2018

Miniprep of the liquid cultue of pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

Nanodrop

Table102

	A	B	C	D	E	F	G	H	I
1	Plasmid	pSB4K5_pAimX_sfGFP small C°1	pSB4K5_pAimX_sfGFP small C°7	pSB4K5_pAimX_sfGFP small C°14	pSB4K5_pAimX_sfGFP small C°20	pSB4K5_pAimX_sfGFP full C°1	pSB4K5_pAimX_sfGFP full C°7	pSB4K5_pAimX_sfGFP full C°14	pSB4K5_pAimX_sfGFP full C°20
2	Nanodrop en ng/µl	200	132.7	109.4	115.3	126.2	217.9	32.6	91.9

Digestion Mix of pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20

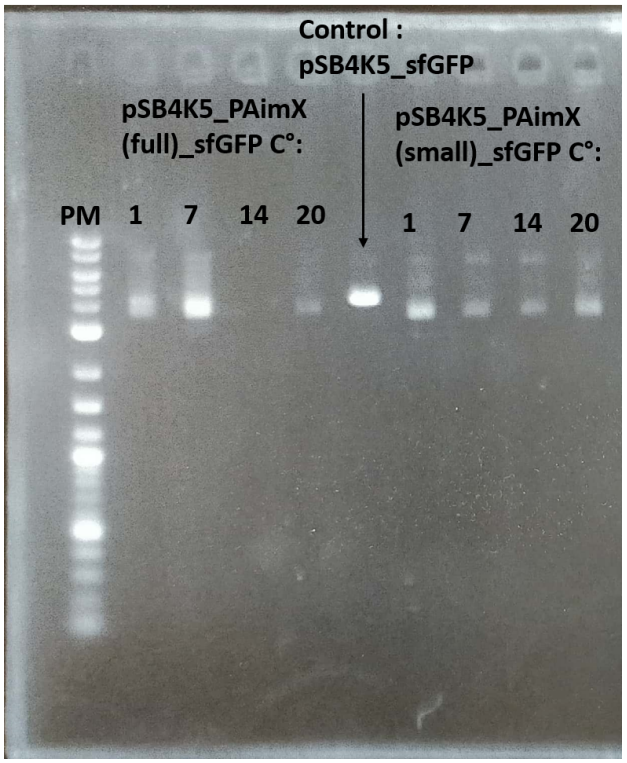
- Digestion Mix

Table103

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	1
3	Plasmid DNA	2
4	Enzyme:BbsI	0.5
5	Water qsp (10µL)	6.5

- Electrophoresis gel of the digetion

Image55.png



Digestion Mix of pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20

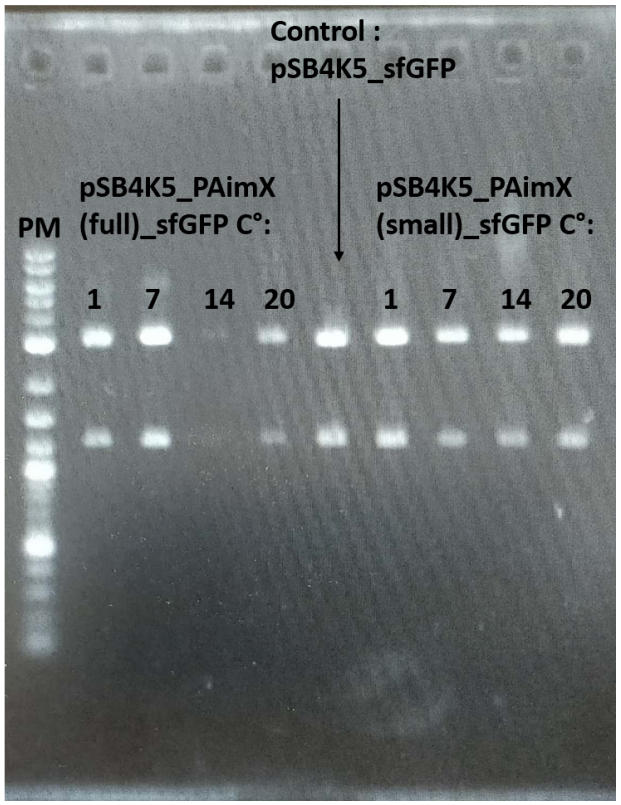
- Digestion Mix

Table105

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	1
3	Plasmid DNA	2
4	Enzyme:BcuI	0.5
5	Enzyme:NdeI	0.5
6	Water qsp (10µL)	6

- Electrophoresis gel of the digetion

Image56.png



Send to sequencing :

Table104

	A	B	C	D	E	F	G	H
1	Plasmid	pSB4K5_pAimX_sfGFP small C°1	pSB4K5_pAimX_sfGFP small C°7	pSB4K5_pAimX_sfGFP small C°14	pSB4K5_pAimX_sfGFP full C°7	pSB4K5_pAimX_sfGFP full C°14	pSB4K5_pAimX_sfGFP full C°20	pSB1C3_pAimX_sfGFP full C°1
2	Primer	VF2	VF2	VF2	VF2	VF2	VF2	VR
3	Sequencing code	68FB24	68FB25	68FB26	68FB27	68FB28	68FB29	68FB30

Liquid cultue of pSB1C3_pAimX_sfGFP full C°1 and small C°1/ pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20

MARDI 21/08/2018

Glycerol stock of pSB1C3_pAimX_sfGFP full C°1 and small C°1/ pSB4K5_pAimX_sfGFP small C°1;7;14;20 and full C°1;7;14;20

1ml liquid culture + 400µL of glycerol 80%

Miniprep of the liquid cultue of pSB1C3_pAimX_sfGFP full C°1 and pSB4K5_pAimX_sfGFP full C°7;14 according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503"

Nanodrop

Table106

	A	B	C	D
1	Plasmid	pSB1C3_pAimX_sfGFP full C°1	pSB4K5_pAimX_sfGFP full C°7	pSB4K5_pAimX_sfGFP full C°14
2	Nanodrop en ng/µl	137.2	212.9	246.6

JEUDI 23/08/2018

Double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag

Defrost 50L of E. coli (DH5alpha) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN+Tet
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN +Tet
Overnight at 37°C

Double transformation of psb3T5_AimR with psb4K5_PAimX(small)_sfGFP_LVAtag

Defrost 50L of E. coli (DH5alpha+ **psb1C3_GG**) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice

Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN+CAM +Tet
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN +Tet+CAM
Overnight at 37°C

VENDREDI 24/08/2018

Double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag or psb4K5_sfGFP_LVAtag
Defrost 50L of E. coli (DH5alpha+**psb1C3_GG**) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN+tet+CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN +Tet+CAM
Incubate on bench on the weekend

LUNDI 27/08/2018

Liquid culture of colonies :

- From the pétri dish of the Double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag in DH5a (23.08.18) : LB (5mL) + Tet (5µL)+ kan (5µL)
- From colony of the double transformation of psb3T5_AimR with psb4K5_PAimX(small)_sfGFP_LVAtag in DH5a+psb1C3_GG (23.08.18) : LB (5mL) + CAM (5µL)+Tet (5µL)+ kan (5µL)
- From the pétri dish of the double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag or psb4K5_sfGFP_LVAtag in DH5a+psb1C3_GG (24.08.18) : LB (5mL) + CAM (5µL)+Tet (5µL)+ Kan Glycerol stock of the culture liquide of the

MARDI 28/08/2018

Glycerol stock

Glycerol stock of the culture liquide of the double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag in DH5a (23.08.18)/ the double transformation of psb3T5_AimR with psb4K5_PAimX(small)_sfGFP_LVAtag in DH5a+psb1C3_GG (23.08.18)/ the double transformation of pSB4K5 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag or psb4K5_sfGFP_LVAtag in DH5a+psb1C3_GG (24.08.18)

Characterization

Liquid culture of colonies :

MERCREDI 29/08/2018

Classic cloning: In order to add a constitutifs promoter (Bba J23100) front of the SfGFP_LVAtag

- Digestion of psb1C3_BbaJ23100 by BcuI and psb1C3_sfGFP_LVATag byBcuI and XbaI

Table107

	A	B	C	D	E
1	**Digestion miX of psb1C3_BbaJ23100**			**Digestion miX of psb1C3_sfGFP_LVAtag**	
2	Composition	Volume en µL		Composition	Volume en µL
3	Buffer fastDigest Green 10X	5		Buffer fastDigest Green 10X	5
4	plasmidic DNA : psb1C3_BbaJ23100	10		plasmidic DNA : psb1C3_sfGFP_LVATag	7
5	Enzyme : BcuI	2.5		Enzyme : XbaI	2.5
6	H2O qsp 50µL			Enzyme :	2.5
7				H2O qsp 50µL	
8					

JEUDI 30/08/2018

Digestion of the insert sf_GFP_LVAtag by XbaI and BcuI

Table108

	A	B
1	**Digestion miX of sfGFP_LVAtag**	
2	Composition	Volume en µL
3	Buffer fastDigest Green 10X	5
4	plasmidic DNA : sfGFP_LVAtag	10
5	Enzyme : XbaI	2.5
6	Enzyme : BcuI	2.5
7	H2O qsp 50µL	

DNA clean up of the digestionproduct, according to the kit " Monarch DNA and PCR cleanup kit" NEB

Electrophoresis gel of the digested insert didn't show anything : no band

LUNDI 03/09/2018

Digestion of psb4K5_sfGFP_LVAtag by XbaI and BcuI

- Digestion Mix

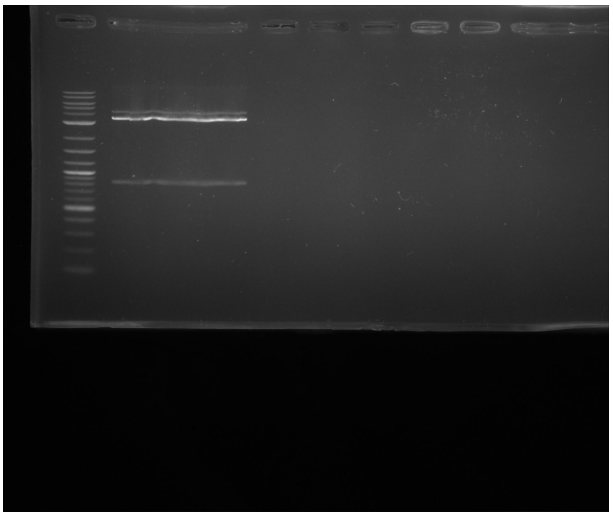
Table109

	A	B
1	**Digestion miX of psb4K5_sfGFP_LVAtag**	
2	Composition	Volume en µL
3	Buffer fastDigest Green 10X	5
4	plasmidic DNA : psb4K5_sfGFP_LVAtag	10
5	Enzyme : XbaI	2.5
6	Enzyme : BcuI	2.5
7	H2O qsp 50µL	

Incubate 1h at 37°C

Electrophoresis gel of the digestion product

03.09.18 digestion psb4k5_sfGFP par xbaI et bcuI.jpg



- Gel extraction of the sfGFP_LVAtag according to " Monarch Gel extraction Kit #T1020L NEB"
  - Nanodrop :15 ng

- Ligation of sfGFP_LVAtag and pSB1C3_BbaJ23100
  - Ligation Mix :

Table110

	A	B
1	Composition	Volume in µL
2	Buffer T4 DNA ligase	2
3	T4 DNA ligase	1
4	Insert : sfGFP _LVAtag	10
5	vector: pSB1C3_BbaJ23100	4
6	H2O qsp 20µL	

Then incubate 10′ at room temperature
After that incubate on the weekend at 16°C

Double transformation of pSB4K5 _GG with psb1C3_GG/psb1C3_AimR or psb1C3_GG with psb4K5_PAimX(full)_sfGFP_LVAtag or psb4K5_PAimX(small)_sfGFP_LVAtag

Defrost 50L of E. coli (DH5alpha) on ice
Add 2 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN+ CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN +CAM
Overnight at 37°C

Double transformation of pSB1C3 _GG with psb3T5_GG/psb3T5_AimR or psb3T5_GG with psb1C3_PAimX(full)_sfGFP_LVAtag or psb1C3_PAimX(small)_sfGFP_LVAtag

Defrost 50L of E. coli (DH5alpha) on ice
Add 2 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+Tet+ CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+Tet +CAM
Overnight at 37°C

Liquid culture of :

- psb4K5_sfGFP_LVAtag

MARDI 04/09/2018

Double transformation of psb3T5_GG with psb1C3_GG/ psb1C3_pAimX(full)_sfGFP_LVAtag with psb3T5_AImR/psb1C3_pAimX(small)_sfGFP_LVAtag with psb3T5_AImR/psb1C3_pAimX(small)_sfGFP_LVAtag with psb3T5_GG/psb1C3_pAimX(full)_sfGFP_LVAtag with psb3T5_GG

Defrost 50L of E. coli (DH5alpha) on ice
Add 2 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+Tet+ CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+Tet +CAM
Overnight at 37°C

Double transformation of psb1C3_GG with psb4K5_GG/ psb4K5_pAimX(full)_sfGFP_LVAtag with psb1C3_AImR/psb4K5_pAimX(small)_sfGFP_LVAtag with psb1C3_AImR/psb4K5_pAimX(small)_sfGFP_LVAtag with psb1C3_GG/psb4K5_pAimX(full)_sfGFP_LVAtag with psb1C3_GG

Defrost 50L of E. coli (DH5alpha) on ice
Add 2 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+Kan+ CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dishLB+Kan+ CAM
Overnight at 37°C

Transformation of psb1C3_BbaJ23100_sfGFP_LVAtag (ligation product)

Defrost 50L of E. coli (DH5alpha) on ice
Add 2 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+CAM
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+CAM
Overnight at 37°C

No clones on plate

VENDREDI 07/09/2018

Protocol verification of the secretion of the peptide

Preparation of the sample for de Mass Spec
Make a liquid culture in LB of the strain :

- Dh5a+pSB1C3_AimP_SAIRGA
- Dh5a+pSB1C3_OmpA_SAIRGA
- Dh5a+pSB1C3_Tat_SAIRGA
- Dh5a+pSB1C3_pelB_SAIRGA
- Dh5a+pSB1C3_GG

Incubate overnight at 37°C

100X dilution of the culture in minimal medium supplemented with chloramphenicol
Incubate at 37°C and 200 rpm for 3h

After this 3h
Very 2h take :

- 1ml of the culture for the Do
- 1mL for the treatment A
- 1mL for the treatment B

Treatment A
Centrifuge during 15min at 5000rpm
Then recover the supernatant
Centrifugation during 15min at 5000rpm
Recover the supernatant and filter it with a 2µm filter
Then filter with a 3K biomolecular filter
Transfer all the liquid into a tube for mass spectro
Well annotated and placed at -80

Treatment B
Centrifuge during 15min at 5000rpm
Then recover the supernatant
Centrifugation during 15min at 5000rpm
Recover the supernatant and filter it with a 2µm filter
Then filter with a 3K biomolecular filter
Concentrate the sample by dehydration
Add 100µL of water and well resuspend by agitation
Transfer all the liquid into a tube for mass spectro
Well annotated and placed at -80

LUNDI 10/09/2018

PCR in order to remove the degradation tag "LVAtag" of: psb1C3_sfGFP_LVAtag/psb1C3_pAimX(small)_sfGFP_LVA_tag/psb1C3_pAimX(full)_sfGFP_LVA_tagpsb4K5_sfGFP_LVAtag /psb4K5_pAimX(small)_sfGFP_LVA_tag/psb4K5_pAimX(full)_sfGFP_LVA_tag

- PCR mix

Table111

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG106	2.5
5	iG107	2.5
6	DNA	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

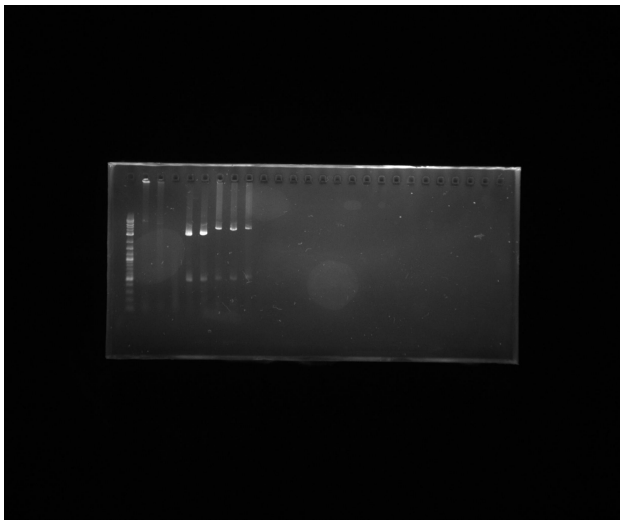
- PCR program

Table112

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

Electrophoresis gel of the PCR products (1% agarose)

10.09.18 Migration PCR k32,k27,ctrl,1C3 S, 1C3 F, 4k5.jpg



- Gel extraction of the backbone without LVAtag according to " Monarch Gel extraction Kit #T1020L NEB"

Golden Gate :

- Golden gate of the PCR product
    - Golden Gate Mix

Table113

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	psb1C3_sfGFP/psb1C3_pAimX(small)_sfGFP/psb1C3_pAimX(full)_sfGFP/psbK5_sfGFP/psb4K5_pAimX(small)_sfGFP/psb4K5_pAimX(full)_sfGFP	2
6	Water qsp (20µL)	

    - Golden gate Program :

Table114

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Improve a part : Experiment 3

PCR in order to clone the parts: Bba_K274002 and Bba_K274003

Table115

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG024	2.5
5	iG025	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table116

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

- Gel extraction of the PCR products, according to " Monarch Gel extraction Kit #T1020L NEB"

Golden Gate :

- Golden gate of the PCR product
    - Golden Gate Mix

Table117

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	psb1C3 or psb4K5 or psb3T5	2
6	Insert : Bba_K274002 or Bba_K274003	5
7	Water qsp (20µL)	

    - Golden gate Program :

Table118

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

    - Gel extraction of the PCR products, according to " Monarch Gel extraction Kit #T1020L NEB"

Lab book jouy

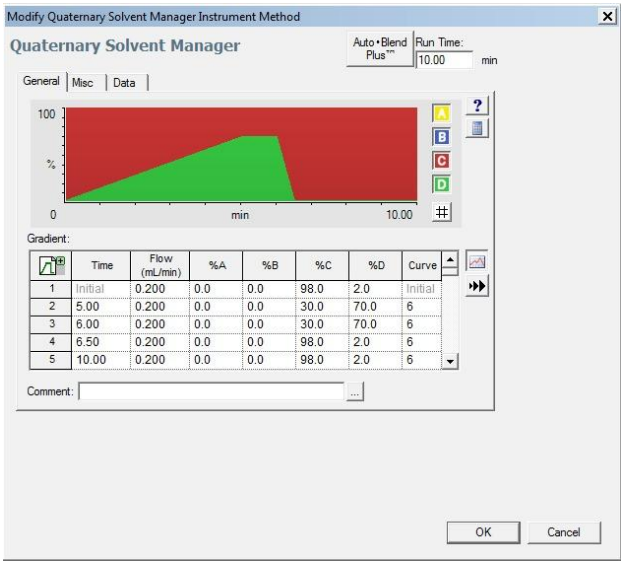
Oligos (ssDNA) in both directions (5'-3'and 3'-5') of J23110 promoter were dimerized in thermocycler. After, dimerization, oligos were phosphorylated by PNK reaction (T4 Polynucleotide Kinase) for one hour.
Meanwhile, AimX(short) and AimX(full) sequences in both plasmids (pSB1C3 and pSB4K5) were amplified by PCR (Q5 pol). Subsequently, PCR products were purified and used in AnP reaction

Both insert J23110 promoter oligo and backbones containing AimX(short) and AimX(full) were ligated overnight.

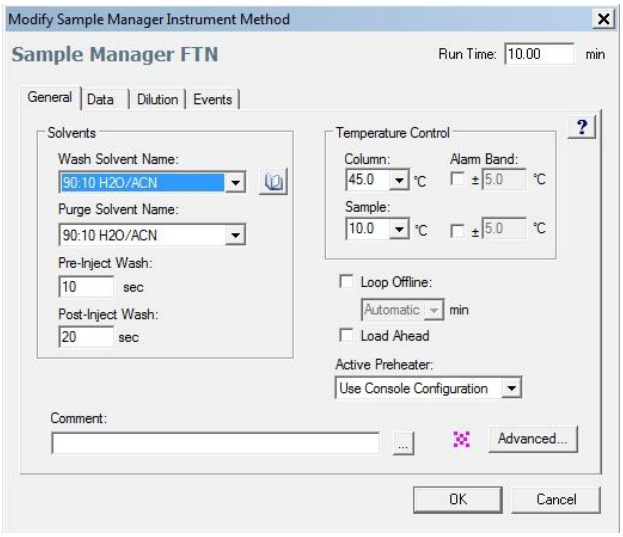
MARDI 11/09/2018

Parameter of the mass spec

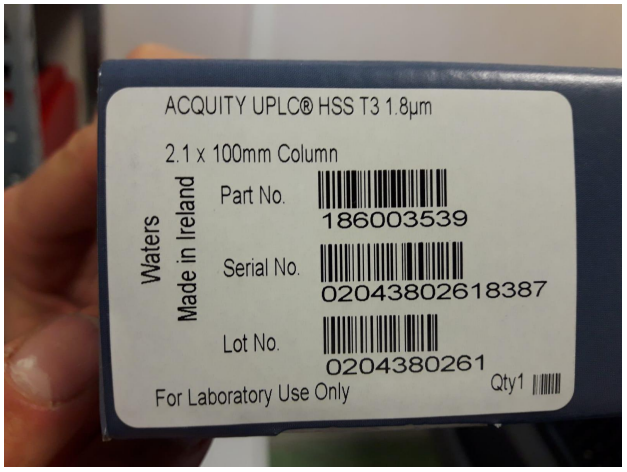
WhatsApp Image 2018-10-10 at 4.40.23 PM.jpeg



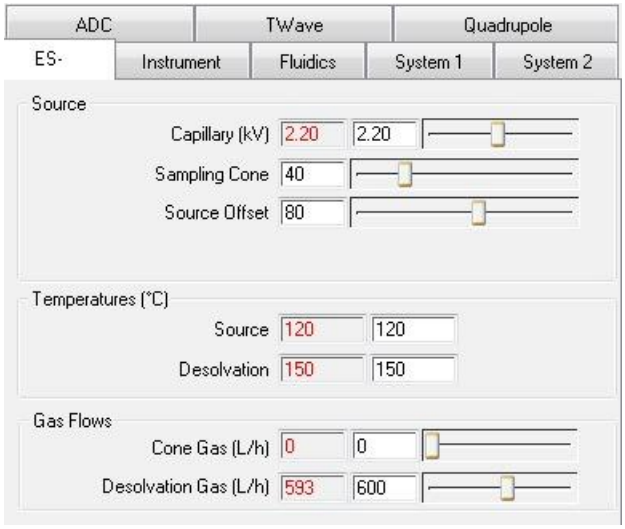
WhatsApp Image 2018-10-10 at 4.40.23 PM (1).jpeg



WhatsApp Image 2018-10-10 at 4.40.24 PM.jpeg



WhatsApp Image 2018-10-10 at 4.40.24 PM (1).jpeg



Lab book Jouy

Product of ligation comprising AimX(short) and AimX(full) in both pSB1C3 and pSB4K5 backbones with J23110 promoter sequence were transformed into TOP10 cells.

MERCREDI 12/09/2018

Lab book jouy

From the cloning reaction of AimX(short) and AimX(full) in both pSB1C3 and pSB4K5 with J23110 promoter sequence through phosphorylation/ligation just AimX(Short)_ pSB4K5 has shown colonies. Colony PCR has shown 3 positive colonies which were inoculate in LB for verification by sequencing the day after.

JEUDI 13/09/2018

Improve a part : Experiment 1 and 2

- Experiment 1

Table119

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG110	2.5
5	iG111	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table120

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

- Experiment 2

Table121

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG112	2.5
5	iG113	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

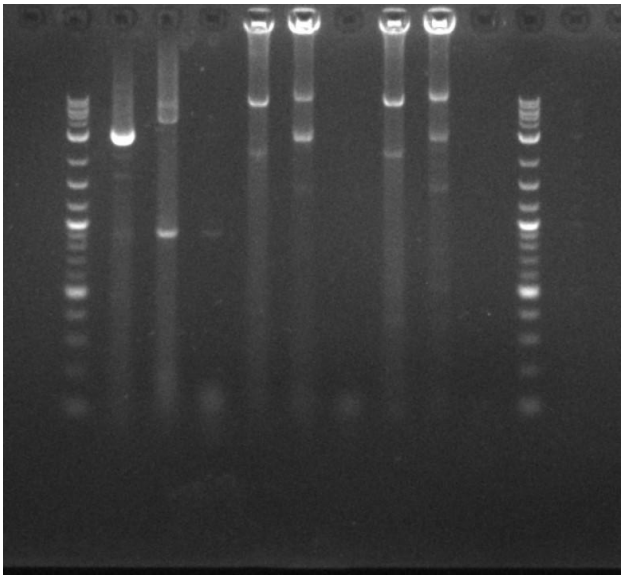
- PCR program

Table122

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

Electrophoresis gel of PCR product for improve a part experiment 1 and 2

2018-09-13 6 PCR.jpg



- Gel extraction of the PCR products, according to " Monarch Gel extraction Kit #T1020L NEB"

PCR in order to add a constitutif promoter J23110 in front of sfGFP : for psb1C3_pAimX_sfGFP and psb4K5_pAimX_sfGFP

Table123

	A	B
1		
2	composition	quantity (µl)
3	Phusion buffer	4
4	Mix dNTPs (10mM each)	1
5	iG112	2.5
6	iG113	2.5
7	DNA : psb4K5_pAimX_sfGFP or psb1C3_pAimX_sfGFP	2
8	DMSO	1.5
9	phsuion	0.5
10	H2O MQ	qsp 50

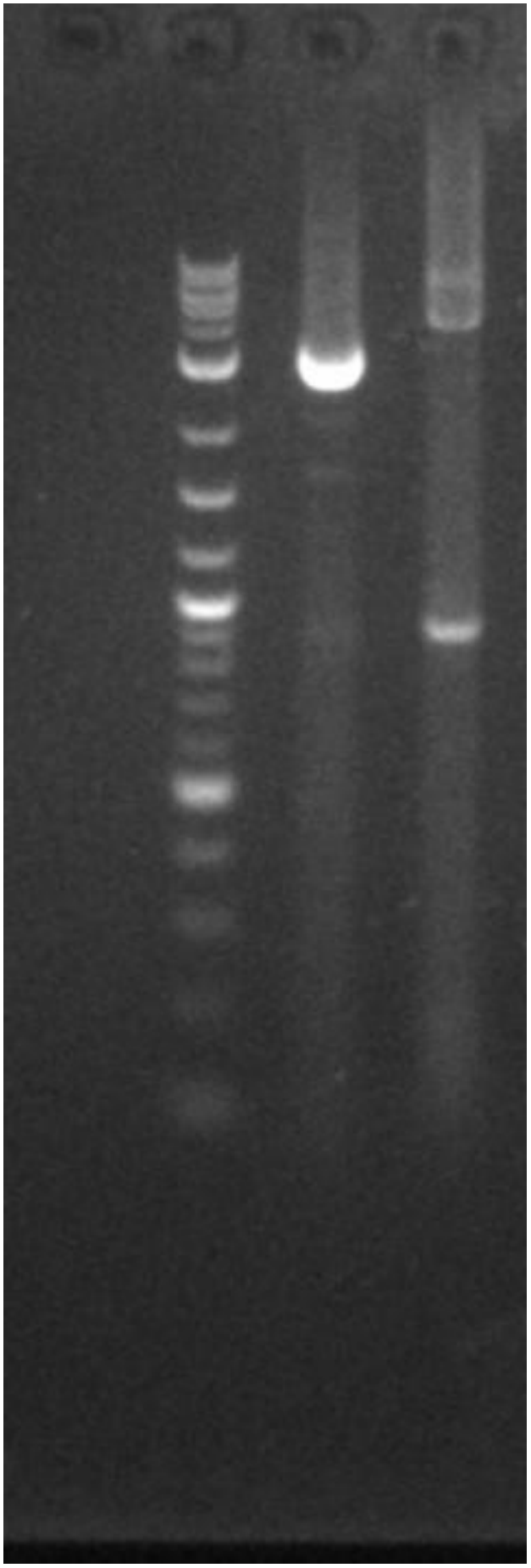
- PCR program

Table124

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

Electrophoresis gel of the PCR product (Agarose1%)

2018-09-13 6 PCR.jpg



- Gel extraction of the PCR products, according to " Monarch Gel extraction Kit #T1020L NEB"

Lab book Jouy

The DNA extracted from AimX(short) in pSB4K5 with J23110 promoter was sent for sequencing with VF2 primers.

VENDREDI 14/09/2018

Golden Gate : Improve a part experiment 1 and 2

- Golden gate of the PCR product
  - Golden Gate Mix

Table125

	A	B	C	D	E	F	G	H
1	Experiment 1			Experiment 2			psb4K5_pAimX_sfGFP	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: PnK	0.5		Enzyme: BsaI	0.5
6	k274002 or K274003	2		k274002 or K274003	2		PCR product	2
7	Water qsp (20µL)			Water qsp (20µL)			Water qsp (20µL)	

  - Golden gate Program :

Table126

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

LUNDI 17/09/2018

Transformation of pSB1C3_J23110_sfGFP_LVAtag/pSB4K5_J23110_sfGFP_LVAtag/ i*mprove a part (exp 1) psb1K3_k274002 and psb1K3_k274003/*improve a part (exp 2) psb1K3_k274002 and psb1K3_k274003*

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

PCR in order to add a constitutif promoter in front of psb4K5_pAimX(small)_sfGFP and psb1C3_pAimX(small)_sfGFP

Table127

	A	B
1		
2	composition	quantity (µl)
3	Phusion buffer	4
4	Mix dNTPs (10mM each)	1
5	iG112	2.5
6	iG114	2.5
7	DNA : psb4K5_pAimX(small)_sfGFP or psb1C3_pAimX(small)_sfGFP	2
8	DMSO	1.5
9	phsuion	0.5
10	H2O MQ	qsp 50

- PCR program

Table128

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

Improve a part : Experiment 3
PCR in order to clone the parts: Bba_K274002 and Bba_K274003

Table131

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG024	2.5
5	iG025	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table132

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

MARDI 18/09/2018

Liquid culture psb1C3_sfGFP(no tag) : LB (5mL) + CAM(5µL)

- Two culture from isolated colonies

Lab book jouy

A PCR reaction to isolate and integrate golden gate sites upon the sequences AimX(full).

MERCREDI 19/09/2018

Lab book jouy

A DpnI treatment on PCR products of AimX(full) was made for 1 hour, followed by a reaction purification. The purified products were used for a Golden Gate reaction with equimolar concentration of insert:backbone (100fmol of each DNA) with gg-ready_pSB1C3 or gg-ready_pSB4K5 backbone.

JEUDI 20/09/2018

Gel extraction of the PCR products, according to " Monarch Gel extraction Kit #T1020L NEB" of the PCR product of :

-"Improve a part : Experiment 3" (17.09.18)

- PCR in order to add a constitutif promoter in front of psb4K5_pAimX(small)_sfGFP and psb1C3_pAimX(small)_sfGFP (17.09.18)

Golden Gate : Improve a part experiment 3 / psb1C3_J23110_pAimX(small)_sfGFP_LVAtag/psb4K5_J23110_pAimX(small)_sfGFP_LVAtag

- Golden gate of the PCR product
    - Golden Gate Mix

Table129

	A	B	C	D	E	F	G	H
1	Experiment 3			psb1C3_J23110_pAimX(small)_sfGFP_LVA tag			psb4K5_J23110_pAimX(small)_sfGFP_LVAtag	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Insert: k274002 or K274003	2		PCR product of psb1C3_pAimX(small)_sfGFP with the primers iG114-115	2		PCR product of psb4K5_pAimX(small)_sfGFP with the primers iG114-115	2
7	Vector: psb3T5 or psb1C3 or psb4K5	3		Water qsp (20µL)			Water qsp (20µL)	
8	Water qsp (20µL)							

- Golden gate Program :

Table130

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Liquid culture of :

- psb3T5_AimR C°1
- pSB4K5_J23110_sfGFP_LVAtag
- i*mprove a part (exp 1) psb1K3_k274002 and psb1K3_k274003*
- i*mprove a part (exp 2) psb1K3_k274002 and psb1K3_k274003*

LB (5mL) + Right antibiotic (5µL)

Double Transformation of psb3T5_AimR with psb1C3_pAimx(small)_sfGFP/psb3T5_AimR with psb1C3_pAimx(full)_sfGFP/psb3T5_AimR with psb4K5_pAimx(small)_sfGFP/psb3T5_AimR with psb4K5_pAimx(full)_sfGFP

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Lab book jouy

Transformation of golden gate reaction product of AimX(full) on TOP10 cells.
Additionally, a PCR reaction on AimX(short) sequence to insert golden gate sites. Subsequently, a DpnI treatment was performed and the reactions was purified. The eluted DNA was used for a golden gate cloning into gg-ready_pSB1C3.

VENDREDI 21/09/2018

Remake PCR for: **Improve a part : Experiment 1 and 2**

- Experiment 1

Table133

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG110	2.5
5	iG111	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table134

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

- Experiment 2

Table135

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG112	2.5
5	iG113	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table136

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	4′	
6	72	10′	
7	4	-	

PCR : in order to modify the "-35" site of the full promoter

- PCR MIX

Table137

	A	B	C	D	E	F	G	H
1	Full 1			Full 2			Full 3	
2	composition	quantity (µl)		composition	quantity (µl)		composition	quantity (µl)
3	Phusion buffer	4		Phusion buffer	4		Phusion buffer	4
4	Mix dNTPs (10mM each)	1		Mix dNTPs (10mM each)	1		Mix dNTPs (10mM each)	1
5	iG124	2.5		iG126	2.5		iG128	2.5
6	iG125	2.5		iG127	2.5		iG129	2.5
7	DNA : psb1C3_pAimX(full)_sfGFP_LVAtag or psb1C3_pAimX(full)_sfGFP or psb4K5_pAimX(full)_sfGFP_LVAtag or psb4K5_pAimX(full)_sfGFP	2		DNA : psb1C3_pAimX(full)_sfGFP_LVAtag or psb1C3_pAimX(full)_sfGFP or psb4K5_pAimX(full)_sfGFP_LVAtag or psb4K5_pAimX(full)_sfGFP	2		DNA : psb1C3_pAimX(full)_sfGFP_LVAtag or psb1C3_pAimX(full)_sfGFP or psb4K5_pAimX(full)_sfGFP_LVAtag or psb4K5_pAimX(full)_sfGFP	2
8	DMSO	1.5		DMSO	1.5		DMSO	1.5
9	phsuion	0.5		phsuion	0.5		phsuion	0.5
10	H2O MQ	qsp 50		H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR programme

Table138

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

***Extraction et purification of the plasmids* pSB3T5_AimR**

Miniprep on the overnight culture according to Thermo scientific ″ GeneJet Plasmid Miniprep Kit #K0503″

Digestion of psb1C3_J23110_sfGFP Not

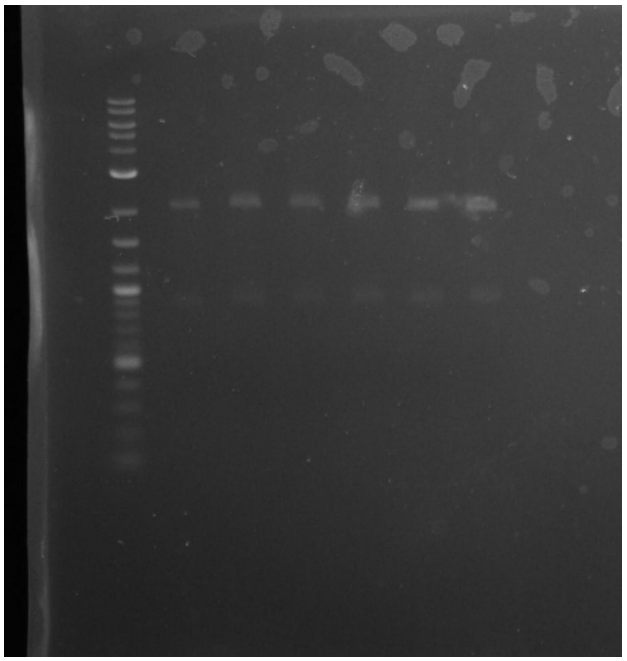
- Digestion Mix

Table139

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	1
3	Plasmid DNA:	2
4	Enzyme: NotI	1
5	Water qsp (10µL)	6

- Elelctrophoresis of the digestion (agarose 1%) : Good clone

21.09.18 Digestion pSB1C3_J23110_sfGFP-LVAtag par AvrII et PstI..jpg



Lab book jouy

A colony PCR was done on transformant TOP10 cells with AimX(full) only in pSB4K5, because none cell with AimX(full) + pSB1C3 has grown on chloramphenicol plates.
We have noted AimX(full) was not showing any green phenotype as it's short version in the same backbone. We run some analysis and we found a predicted terminator upstream the short promoter AimX(short).
Additionally, the product of golden gate cloning between AimX(short) and gg-ready_pSB1C3 was used for transformation on TOP10 cells.

LUNDI 24/09/2018

Remake PCR : in order to modify the "-35" site of the full promoter

- PCR mix

Table140

	A	B	C	D	E
1	Full 2			Full 3	
2	composition	quantity (µl)		composition	quantity (µl)
3	Phusion buffer	4		Phusion buffer	4
4	Mix dNTPs (10mM each)	1		Mix dNTPs (10mM each)	1
5	iG126	2.5		iG128	2.5
6	iG127	2.5		iG129	2.5
7	DNA : psb1C3_pAimX(full)_sfGFP_LVAtag	2		DNA : psb4K5_pAimX(full)_sfGFP	2
8	DMSO	1.5		DMSO	1.5
9	phsuion	0.5		phsuion	0.5
10	H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR programme

Table141

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30"	
3	98	10"	*30
4	72	30"	
5	72	1'30"	
6	72	10'	
7	4	-	

Transformation of psb1C3_J23110_pAimX(small)_sfGFP_LVAtag/psb4K5_J23110_pAimX(small)_sfGFP_LVAtag/psb1C3_J23110_pAimX(small)_sfGFP/psb4K5_J23110_pAimX(small)_sfGFP/Improve a part (exp 3) psb1C3_K274002, psb1C3_K274003,psb4K5_K274002, psb4K5_K274003, psb3T5_K274002, psb3T5_K274003

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30' on ice
Then make a thermic choc at 42°C during 60"
Incubate again 5' on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Golden Gate : of the PCR product of " PCR to modify the "-35" site of the full promoter"

- Golden gate of the PCR product

- Golden Gate Mix

Table142

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	Vector : psb1C3_pAimX(full1)_sfGFP_LVAtag or psb1C3_pAimX(full1)_sfGFP or psb4K5_pAimX(full1)_sfGFP_LVAtag or psb4K5_pAimX(full1)_sfGFP psb1C3_pAimX(full2)_sfGFP_LVAtag or psb1C3_pAimX(full2)_sfGFP or psb4K5_pAimX(full2)_sfGFP_LVAtag or psb4K5_pAimX(full2)_sfGFP psb1C3_pAimX(full3)_sfGFP_LVAtag or psb1C3_pAimX(full3)_sfGFP or psb4K5_pAimX(full3)_sfGFP_LVAtag or psb4K5_pAimX(full3)_sfGFP	3
6	Water qsp (20µL)	

- Golden gate Program :

Table143

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Remake PCR for: Improve a part : Experiment 1 and 2

- Experiment 1

Table144

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG110	2.5
5	iG111	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table145

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

Remake : PCR in order to add J23110 promoter before sfGFP

- PCR Mix

Table148

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG108	2.5
5	iG109	2.5
6	DNA : psb4K5_pAimX(small)_sfGFP or psb1C3_pAimX(small)_sfGFP	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table149

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

MARDI 25/09/2018

Transformation of psb1C3_pAimX(full1)_sfGFP_LVAtag ; psb1C3_pAimX(full1)_sfGFP; psb4K5_pAimX(full1)_sfGFP_LVAtag ; psb4K5_pAimX(full1)_sfGFP psb1C3_pAimX(full2)_sfGFP_LVAtag ; psb1C3_pAimX(full2)_sfGFP ; psb4K5_pAimX(full2)_sfGFP_LVAtag ; psb4K5_pAimX(full2)_sfGFP psb1C3_pAimX(full3)_sfGFP_LVAtag ;psb1C3_pAimX(full3)_sfGFP ; psb4K5_pAimX(full3)_sfGFP_LVAtag ;psb4K5_pAimX(full3)_sfGFP; psb1C3_pAimX(small)_sfGFP; psb1C3_pAimX(full)_sfGFP; psb4K5_sfGFP; psb4K5_pAimX(small)_sfGFP; psb4K5_pAimX(full)_sfGFP ; psb1K3_Bba_K274002 ; psb1K3_Bba_K274003; psb4K5_Bba_K274002 ; psb4K5_Bba_K274003; psb3T5_Bba_K274002 ; psb3T5_Bba_K274003

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic

Overnight at 37°C

Double transformation of pSB3T5_AimR with psb4K5_pAimX(small)_sfGFP/pSB3T5_AimR with psb4K5_pAimX(full)_sfGFP/pSB3T5_AimR with psb1C3_pAimX(small)_sfGFP_LVAtag/pSB3T5_AimR with psb1C3_pAimX(full)_sfGFP/pSB3T5_AimR with psb4K5_sfGFP_LVAtag/pSB3T5_AimR with psb1C3_pAimX(full)_sfGFP/pSB3T5_AimR with psb1C3_pAimX(small)_sfGFP/pSB3T5_AimR with psb4K5_sfGFP

Defrost 50L of E. coli (DH5alpha) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put 100µL on petri dish LB+KAN+Tet
centri 5′ at 5000rpm
Resuspend the cells in 100µL of LB
After that put 100µL on petri dish LB+KAN +Tet
Overnight at 37°C

Golden Gate of the PCR product of :

PCR in order to add J23110 promoter before sfGFP (24.08.18)
Improve a part : Experiment 1 and 2 (24.08.18)

- Golden gate of the PCR product
  - Golden Gate Mix

Table146

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	vector	3
6	Water qsp (20µL)	

  - Golden gate Program :

Table147

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Lab book jouy

A colony PCR was made on AimX(small) in pSB1C3 vector where all colonies were positive, thus, these colonies were inoculated.
Additionally, the DNA from 4 positive-PCR colonies for AimX(full) in pSB4K5 were extracted.

MERCREDI 26/09/2018

Transformation of psb1K3_Bba_K274003; psb4K5_Bba_K274002 ; psb4K5_Bba_K274002 ; psb4K5_Bba_K274003;psb4K5_pAimX(small)_sfGFP; psb4K5_pAimX(full)_sfGFP;psb1C3_pAimX(small)_sfGFP ;psb1C3_pAimX(full)_sfGFP

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Remake PCR : In order to remove the LVAtag of psb1C3_sfGFP_LVAtag

- PCR mix

Table150

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG106	2.5
5	iG107	2.5
6	DNA	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table151

	A	B	C
1	Température (°c)	Temps	Cycles
2	98	30″	
3	98	10″	*30
4	72	30″	
5	72	1′30″	
6	72	10′	
7	4	-	

- Gel extraction of the backbone without LVAtag according to ″ Monarch Gel extraction Kit #T1020L NEB″

Golden Gate of the PCR product of : psb1C3_J23110_sfGFP/psb4K5_J23110_sfGFP_LVAtag/psb4K5_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP_LVAtag/psb4K5_pAimX(small)-to-_J23110_sfGFP/psb4K5_pAimX(small)-to-_J23110_sfGFP_LVAtag

- Golden Gate Mix

Table152

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	vector	3
6	Water qsp (20µL)	

- Golden gate Program :

Table153

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Liquid culture of all transformations make the 25.09.18:
psb1C3_pAimX(full1)_sfGFP_LVAtag ; psb1C3_pAimX(full1)_sfGFP; psb4K5_pAimX(full1)_sfGFP_LVAtag ; psb4K5_pAimX(full1)_sfGFP psb1C3_pAimX(full2)_sfGFP_LVAtag ; psb1C3_pAimX(full2)_sfGFP ; psb4K5_pAimX(full2)_sfGFP_LVAtag ; psb4K5_pAimX(full2)_sfGFP psb1C3_pAimX(full3)_sfGFP_LVAtag ;psb1C3_pAimX(full3)_sfGFP ; psb4K5_pAimX(full3)_sfGFP_LVAtag ;psb4K5_pAimX(full3)_sfGFP; psb1C3_pAimX(small)_sfGFP; psb1C3_pAimX(full)_sfGFP; psb4K5_sfGFP; psb4K5_pAimX(small)_sfGFP; psb4K5_pAimX(full)_sfGFP ; psb1K3_Bba_K274002 ; psb1K3_Bba_K274003; psb4K5_Bba_K274002 ; psb4K5_Bba_K274003; psb3T5_Bba_K274002 ; psb3T5_Bba_K274003
pSB3T5_AimR with psb4K5_pAimX(small)_sfGFP/pSB3T5_AimR with psb4K5_pAimX(full)_sfGFP/pSB3T5_AimR with psb1C3_pAimX(small)_sfGFP_LVAtag/pSB3T5_AimR with psb1C3_pAimX(full)_sfGFP/pSB3T5_AimR with psb4K5_sfGFP_LVAtag/pSB3T5_AimR with psb1C3_pAimX(full)_sfGFP/pSB3T5_AimR with psb1C3_pAimX(small)_sfGFP/pSB3T5_AimR with psb4K5_sfGFP

- LB(5ml) + the right antibiotics (5µL)

Lab book jouy
DNA extract from 4 different colonies of AimX(full) in pSB4K5 vector and from 4 positive-PCR colonies for AimX(small) in pSB1C3 were extracted and sent for sequencing with VF2 primers

JEUDI 27/09/2018

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :
psb4K5_pAimX(full3)_sfGFP

Digestion of:

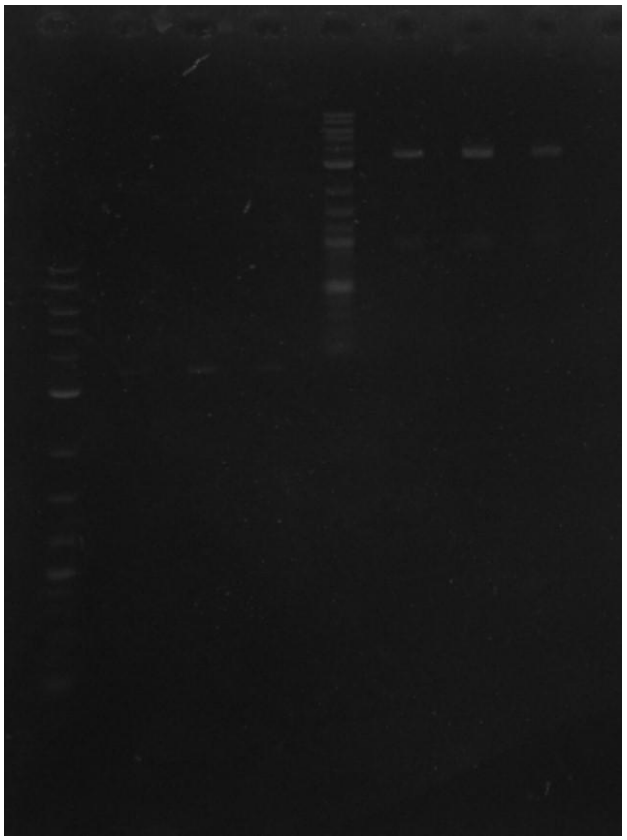
psb4K5_pAimX(full3)_sfGFP

- Digestion Mix

Table154

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	1
3	Plasmid DNA:	2
4	Enzyme: NotI	0.5
5	Water qsp (10µL)	6.5

2018-09-27 digestion minipreps.jpg



VENDREDI 28/09/2018

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :

psb1C3_pAimX(full1)_sfGFP_LVAtag ; psb1C3_pAimX(full1)_sfGFP; psb4K5_pAimX(full1)_sfGFP_LVAtag ; psb4K5_pAimX(full1)_sfGFP psb1C3_pAimX(full2)_sfGFP_LVAtag ; psb1C3_pAimX(full2)_sfGFP ; psb4K5_pAimX(full2)_sfGFP_LVAtag ; psb4K5_pAimX(full2)_sfGFP psb1C3_pAimX(full3)_sfGFP_LVAtag ;psb1C3_pAimX(full3)_sfGFP ; psb4K5_pAimX(full3)_sfGFP_LVAtag ; psb1C3_pAimX(small)_sfGFP; psb1C3_pAimX(full)_sfGFP; psb4K5_sfGFP; psb4K5_pAimX(small)_sfGFP; psb4K5_pAimX(full)_sfGFP ; psb1K3_Bba_K274002 ; psb1K3_Bba_K274003; psb4K5_Bba_K274002 ; psb4K5_Bba_K274003; psb3T5_Bba_K274002 ; psb3T5_Bba_K274003

Digestion of:

psb1C3_pAimX(full1)_sfGFP_LVAtag ; psb1C3_pAimX(full1)_sfGFP; psb4K5_pAimX(full1)_sfGFP_LVAtag ; psb4K5_pAimX(full1)_sfGFP psb1C3_pAimX(full2)_sfGFP_LVAtag ; ; psb4K5_pAimX(full2)_sfGFP_LVAtag ; psb4K5_pAimX(full2)_sfGFP psb1C3_pAimX(full3)_sfGFP_LVAtag ;psb1C3_pAimX(full3)_sfGFP ; psb4K5_pAimX(full3)_sfGFP_LVAtag ;psb1C3_pAimX(small)_sfGFP; psb1C3_pAimX(full)_sfGFP; psb4K5_sfGFP; psb4K5_pAimX(small)_sfGFP; psb4K5_pAimX(full)_sfGFP ; psb1K3_Bba_K274002 ; psb1K3_Bba_K274003; psb4K5_Bba_K274002 ; psb4K5_Bba_K274003; psb3T5_Bba_K274002 ; psb3T5_Bba_K274003

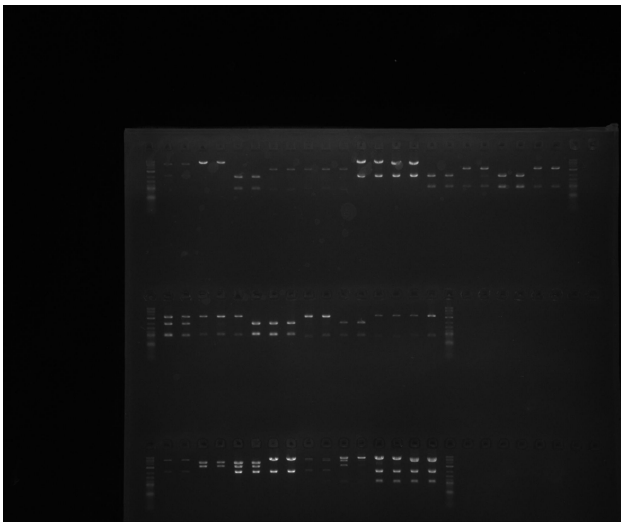
- Digestion Mix

Table155

	A	B
1	Composition	Volume in µL
2	Buffer FastDigest Green 10X	1
3	Plasmid DNA:	2
4	Enzyme: NotI	0.5
5	Water qsp (10µL)	6.5

Electrophoresis Gel

28.09.18 Digestion minipreps.jpg



Transformation of psb1C3_J23110_sfGFP/psb4K5_J23110_sfGFP_LVAtag/psb4K5_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP_LVAtag/psb4K5_pAimX(small)-to-_J23110_sfGFP/psb4K5_pAimX(small)-to-_J23110_sfGFP_LVAtag

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Lab book jouy

PCR reaction to amplify AimX(full) and to insert 1T and 2T mutations into the the stem-loop structure of a predicted terminator in this region. Additionally, for this PCR product a DpnI reaction was incubated

SAMEDI 29/09/2018

Liquid culture of new clones of DH5a+psb1C3_K274002 and DH5a+psb1C3_K274003 :

LB(5mL) + CAM (5µl)

ReTransformation of psb1C3_J23110_sfGFP/psb4K5_J23110_sfGFP_LVAtag/psb4K5_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP_LVAtag/psb4K5_pAimX(small)-to-_J23110_sfGFP/psb4K5_pAimX(small)-to-_J23110_sfGFP_LVAtag, psb1C3_pAimX(full2)_sfGFP

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Remake PCR for: **Improve a part : Experiment 1 and 2**

- Experiment 1

Table156

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG110	2.5
5	iG111	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table157

	A	B	C	D	E	F	G
1	psb1K3_Bba_K274002				psb1K3_Bba_K274003		
2	Température (°c)	Temps	Cycles		Température (°c)	Temps	Cycles
3	98	30″			98	30″	
4	98	10″	*30		98	10″	*30
5	60	30″			64	30″	
6	72	4′			72	4′	
7	72	10′			72	10′	
8	4	-			4	-	

- Experiment 2

Table158

	A	B
1	composition	quantity (µl)
2	Phusion buffer	4
3	Mix dNTPs (10mM each)	1
4	iG112	2.5
5	iG113	2.5
6	DNA : psb1K3_Bba_K274002 or psb1K3_Bba_K274003	2
7	DMSO	1.5
8	phsuion	0.5
9	H2O MQ	qsp 50

- PCR program

Table159

	A	B	C	D	E	F	G
1	psb1K3_Bba_K274002				psb1K3_Bba_K274003		
2	Température (°c)	Temps	Cycles		Température (°c)	Temps	Cycles
3	98	30″			98	30″	
4	98	10″	*30		98	10″	*30
5	60	30″			64	30″	
6	72	4′			72	4′	
7	72	10′			72	10′	
8	4	-			4	-	

LUNDI 01/10/2018

Gel extraction according to " Monarch Gel extraction Kit #T1020L NEB" of :

the PCR product of the experiment 1 for psb1K3_K274002 and psb1K3_K274003

the PCR product of the experiment 2 for psb1K3_K274002 and psb1K3_K274003

Golden Gate of the PCR product of :

the PCR product of the experiment 1 : for psb1K3_K274002 and psb1K3_K274003

the PCR product of the experiment 2 : for psb1K3_K274002 and psb1K3_K274003

- Golden Gate Mix

Table160

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	vector	3
6	Water qsp (20µL)	

- Golden gate Program :

Table161

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

PCR:

- PCR Mix

Table162

	A	B
1	Compound	quantity (µl)
2	Q5 Master Mix	25
3	iG024 (Fwd)	2.5
4	iG025(Rvs)	2.5
5	DNA (0.2ng/µl): psb1C3_pAimX(small)_sfGFP_LVAtag or psb1C3_pAimX(full)_sfGFP_LVAtag or psb1C3_pAimX(small)_sfGFP or psb1C3_pAimX(full)_sfGFP	1
6	H2O MQ	qsp 50

- PCR Programme :

Table163

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	1′30″	
6	72	2′	
7	4	-	

Miniprep according to Thermo scientific ″ GeneJet Plasmid Miniprep Kit #K0503″ of :

DH5a+psb1C3_pAimX(full2)_sfGFP

DH5a+psb4K5_pAimX(full3)_sfGFP_LVAtag

Re-Golden Gate of the PCR product of : psb1C3_J23110_sfGFP/psb4K5_J23110_sfGFP_LVAtag/psb4K5_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP/psb1C3_pAimX(small)-to-_J23110_sfGFP_LVAtag/psb4K5_pAimX(small)-to-_J23110_sfGFP/psb4K5_pAimX(small)-to-_J23110_sfGFP_LVAtag

- Golden Gate Mix

Table164

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	vector	3
6	Water qsp (20µL)	

- Golden gate Program :

Table165

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Transformation of psb1C3_pAimX(full2)_sfGFP_LVAtag and psb4K5_pAimX(full2)_sfGFP_LVAtag

Defrost 50µL of E. coli (DH5alpha) on ice

Add 4 µL of DNA

Incubate 30′ on ice

Then make a thermic choc at 42°C during 60″

Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Lab book jouy

A PCR purification was done to clean up the fragment of 2 variants (1T or 2T) of disruption of terminators.
The cleaned up products were used for a golden gate reaction of 2 variants (1T or 2T) of disruption of terminators of 4 colonies each (total of 8 individual reactions) with pSB4K5 backbone. After golden gate cloning, the product was used for a transformation on TOP10 cells.

MARDI 02/10/2018

Gel extraction according to " Monarch Gel extraction Kit #T1020L NEB" of :

the PCR product realise on psb1C3_pAimX(small)_sfGFP_LVAtag with iG024-025
the PCR product realise on psb1C3_pAimX(full)_sfGFP_LVAtag with iG024-025

PCR : in order to amplify the inser Bba_J23110_sfGFP_LVAtag

- PCR Mix

Table166

	A	B
1	Compound	quantity (µl)
2	Q5 Master Mix	25
3	iG024 (Fwd)	2.5
4	iG025(Rvs)	2.5
5	DNA (0.2ng/µl): psb1C3_J23110_sfGFP_LVAtag	1
6	H2O MQ	qsp 50

- PCR Programme :

Table167

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	45″	
6	72	2′	
7	4	-	

PCR : in order test the specificity of primers for Q-RT-PCR

- PCR Mix

- PCR Programme :

Table168

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	iG116-117			iG118-119			IG120-121			IG122-123				
2	Compound	quantity (µl)		Compound	quantity (µl)		Compound	quantity (µl)		Compound	quantity (µl)		Compound	quantity (µl)
3	Q5 Master Mix	25		Q5 Master Mix	25		Q5 Master Mix	25		Q5 Master Mix	25		Q5 Master Mix	25
4	iG116(Fwd)	2.5		iG118 (Fwd)	2.5		iG120 (Fwd)	2.5		iG122 (Fwd)	2.5		iG024 (Fwd)	2.5
5	iG117(Rvs)	2.5		iG119(Rvs)	2.5		iG121(Rvs)	2.5		iG123(Rvs)	2.5		iG025(Rvs)	2.5
6	DNA (0.2ng/µl): psb4K5_pAimX(small)_sfGFP	1		DNA (0.2ng/µl): psb4K5_pAimX(small)_sfGFP	1		DNA (0.2ng/µl): psb1C3_AimR	1		DNA (0.2ng/µl): psb1C3_AimR	1		DNA (0.2ng/µl): psb1C3_J23110_sfGFP_LVAtag	1
7	H2O MQ	qsp 50		H2O MQ	qsp 50		H2O MQ	qsp 50		H2O MQ	qsp 50		H2O MQ	qsp 50

Table169

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	30″	
6	72	2′	
7	4	-	

Golden Gate of the PCR product of :

the PCR product of the experiment 2 : for psb1K3_K274002 and psb1K3_K274003

- Golden Gate Mix

Table172

	A	B
1	Composition	Volume in µL
2	T4 DNA Ligase	0.5
3	T4 DNA ligase Buffer (already aliquot)	2
4	Enzyme: BsaI	0.5
5	vector	3
6	Water qsp (20µL)	

- Golden gate Program :

Table173

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Transformation of psb1C3_K274002 C°1 to 5; psb1C3_K274003 C°1 to 5; psb1C3_J23110_sfGFP; psb4K5_J23110_sfGFP_LVAtag; psb4K5_J23110_sfGFP; psb1C3_sfGFP; psb1C3_pAimX(short) -to-J23110_sfGFP_LVAtag; psb1C3_pAimX(short) -to-J23110_sfGFP; psb4K5_pAimX(short) -to-J23110_sfGFP_LVAtag; psb4K5_pAimX(short) -to-J23110_sfGFP/ improve a part exp 2 psb1K3_K274002 and psb1K3_K274003/improve a part exp 1 psb1K3_K274002 and psb1K3_K274003

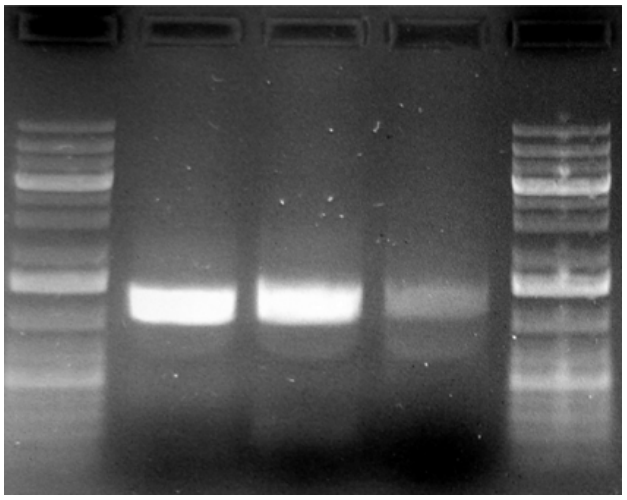
Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice

Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Lab book jouy

Aimx(full), Aimx(short) (truncated version) and Aimx(short) inserts were amplified by PCR with Q5 pol kit.
Reaction was cleaned up and set up a digestion with EcoRI and PstI for 4 hours at 37°C. After digestion a gel was used to confirm the fragmented.

image.png



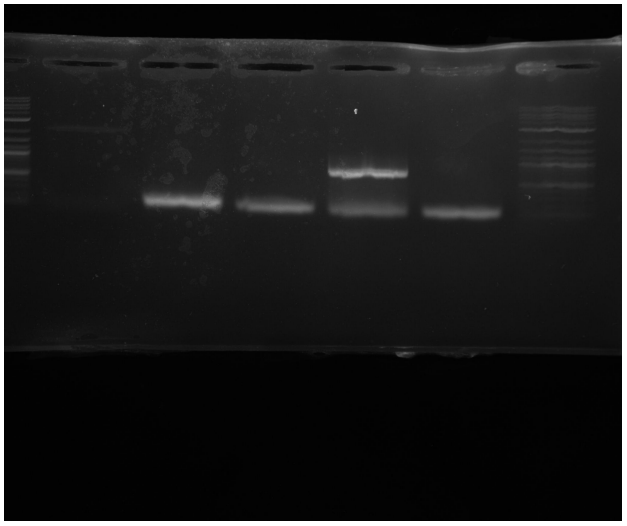
DNA Ladder: GeneRuler 1Kb Plus ready-to-use (Thermo).
The digestion product was treated with DpnI enzyme for an hour, thus, the product was cleaned up a second time and used for ligation.
A ligation was performed with the three versions of PJ23110_AimX and pSB1C3 backbone where a ration of 20:100 of backbone to insert was used. The reaction was incubated 1 hour and 20 minutes and used for a transformation with 4µL.

MERCREDI 03/10/2018

Gel extraction according to " Monarch Gel extraction Kit #T1020L NEB" of :

the PCR product "**PCR : in order test the specificity of primers for Q-RT-PCR**"
Nanodrop : iG118-119 with psb4K5_pAimx(small)_sfGFP = 24.6 ng/µL
iG116-117 with psb4K5_pAimx(small)_sfGFP = 28.7 ng/µL
iG120-121 with psb1C3_AimR = 20.5 ng/µL
iG122-123 with psb1C3_AimR = 24.4 ng/µL

03.10.18 Extraction sur gel - Test qPCR.jpg



Liquid culture of :

psb3T5_AimR
psb4K5_pAimX(small)_sfGFP
psb4K5_pAimX(small)_sfGFP
psb1C3_pAimX(small)_sfGFP
psb1C3_pAimX(small)_sfGFP
psb1C3_AimR
psb1C3_K274002 C°1 to 5;
psb1C3_K274003 C°1 to 5
psb1C3_J23110_sfGFP;
psb4K5_J23110_sfGFP_LVAtag;
psb4K5_J23110_sfGFP;
psb1C3_sfGFP;
psb1C3_pAimX(short) -to-J23110_sfGFP_LVAtag;
psb1C3_pAimX(short) -to-J23110_sfGFP;
psb4K5_pAimX(short) -to-J23110_sfGFP_LVAtag;
psb4K5_pAimX(short) -to-J23110_sfGFP
/ improve a part exp 2 psb1K3_K274002 and psb1K3_K274003
/improve a part exp 1 psb1K3_K274002 and psb1K3_K274003

Lab book jouy

Transformation of Aimx(full), Aimx(short) (truncated version) and Aimx(short) product of ligation with pSB1C3 backbone

JEUDI 04/10/2018

Glycerol stock of :

psb1C3_K274002 C°1 to 5;
psb1C3_K274003 C°1 to 5

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :

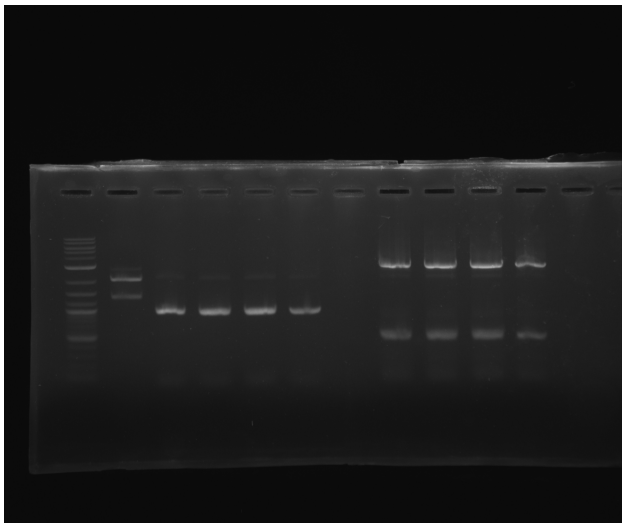
psb4K5_J23110_sfGFP_LVAtag; ; psb1C3_pAimX(short) -to-J23110_sfGFP_LVAtag; ; psb4K5_pAimX(short) -to-J23110_sfGFP_LVAtag; psb4K5_pAimX(short) -to-J23110_sfGFP/ improve a part exp 2 psb1K3_K274002 improve a part exp 1 psb1K3_K274002

Transformation of the golden gate product of the experiment 2 : for psb1K3_K274002 and psb1K3_K274003

Defrost 50µL of E. coli (DH5alpha) on ice

Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 400µL of LB

04.10.18 Vérification pSB1C3_aimR + extraction sur gel S6, S7.tif



Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

PCR: in order to transfert (iG024-25) the insert or remove the LVAtag (iG106-107)

- PCR Mix

Table170

	A	B	C	D	E
1	iG024-25			iG106-107	
2	Compound	quantity (µl)		Compound	quantity (µl)
3	Q5 Master Mix	25		Q5 Master Mix	25
4	iG024 (Fwd)	2.5		iG024 (Fwd)	2.5
5	iG025(Rvs)	2.5		iG025(Rvs)	2.5
6	DNA (0.2ng/µl): psb1C3_J23110_sfGFP_LVAtag	1		DNA (0.2ng/µl): psb1C3_J23110_sfGFP_LVAtag	1
7	H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR Programme :

Table171

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	1′30″	
6	72	2′	
7	4	-	

Golden Gate of the PCR product :

- Golden Gate Mix

Table174

	A	B	C	D	E
1	The PCR on psb1C3_J23110_sfGFP_LVAtag with iG024-025			The PCR on psb1C3_J23110_sfGFP_LVAtag with iG106-107	
2	Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Insert: PCR product	3		vector: PCR product	3
7	vector: psb4K5_GG	3		Water qsp (20µL)	
8	Water qsp (20µL)				

- Golden gate Program :

Table175

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

PCR :

Table176

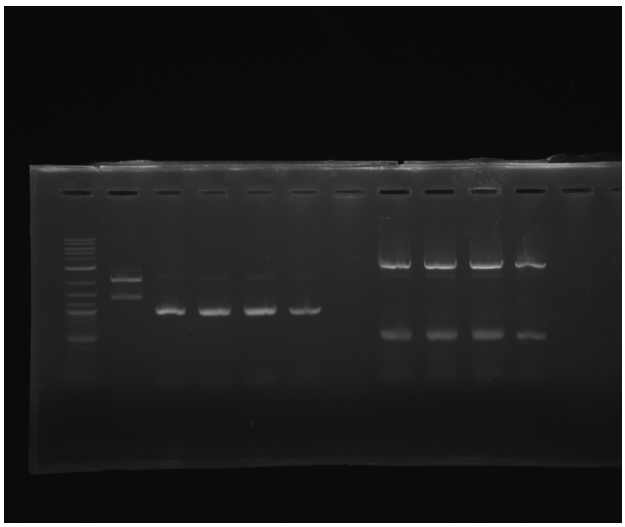
	A	B	C	D	E
1	iG024-25			iG106-107	
2	Compound	quantity (µl)		Compound	quantity (µl)
3	Q5 Master Mix	25		Q5 Master Mix	25
4	iG024 (Fwd)	2.5		iG024 (Fwd)	2.5
5	iG025(Rvs)	2.5		iG025(Rvs)	2.5
6	DNA (0.2ng/µl): psb4K5_sfGFP or improve a part exp 1 psb1K3_k274002 and psb1K3_274003 or psb1C3_pAimX(small)_sfGFP_LVAtag	1		DNA (0.2ng/µl): psb1C3_sfGFP_LVAtag or psb1C3_pAimX(small)_sfGFP_LVAtag or psb4K5_pAimX(small)_sfGFP_LVAtag	1
7	H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR Programme :

Table177

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	1′30″	
6	72	2′	
7	4	-	

04.10.18 Vérification pSB1C3_aimR + extraction sur gel S6, S7.tif



Lab book Jouy

An overnight golden gate cloning on AimR insert with pSB1C3 backbone.
Colony PCR was made on cells transformed with Aimx(full), Aimx(short) (truncated version) and Aimx(short) product of ligation with pSB1C3 backbone. The colony PCR was made with DreamTaq kit.

image.png

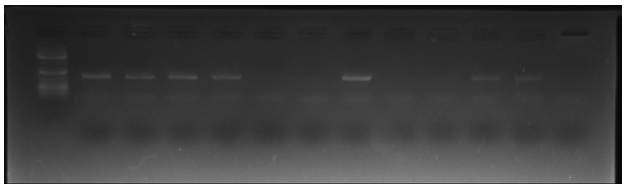
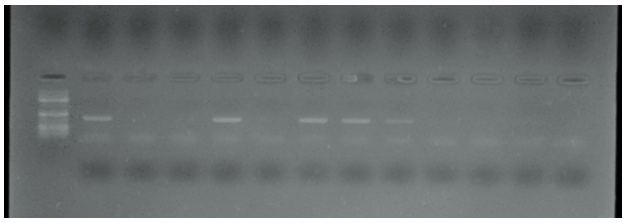


image.png



An experiment was performed to screen the effect of the promoter J23110 upstream the full and both short (normal and truncated) versions of AimX upon sfGFP expression. Additionally, the effect of AimR was also analyzed to verify its capacity as a repressor. Thus, cells were co-transformed with the following combination of plasmids:
AimX(short)_ pSB4K5 + pSB3T5;
AimX(short)_ pSB4K5 + AimR_pSB3T5;
AimX(full)_ pSB4K5 + pSB3T5;
AimX(full)_ pSB4K5 + AimR_pSB3T5;
J23110_AimX(truncated short)_ pSB4K5 + pSB3T5;
J23110_AimX(truncated short)_ pSB4K5 + AimR_pSB3T5;
J23110_AimX(short)_ pSB4K5 + pSB3T5;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5;

J23110_AimX(full)_ pSB4K5 + pSB3T5;
J23110_AimX(full)_ pSB4K5 + AimR_pSB3T5;
Cells were incubated at 37°C and analyzed in its log phase of growing.

Day 03

VENDREDI 05/10/2018

PCR :

- PCR Mix

Table180

	A	B	C	D	E
1	iG024-25			iG106-107	
2	Compound	quantity (µl)		Compound	quantity (µl)
3	Q5 Master Mix	25		Q5 Master Mix	25
4	iG024 (Fwd)	2.5		iG024 (Fwd)	2.5
5	iG025(Rvs)	2.5		iG025(Rvs)	2.5
6	DNA (0.2ng/µl): psb4K5_sfGFP or improve a part exp 1 psb1K3_k274002 and psb1K3_274003 or psb1C3_pAimX(small)_ sfGFP_LVAtag	1		DNA (0.2ng/µl): psb1C3_sfGFP_ LVAtag or psb1C3_pAimX(small)_sfGFP_L VAtag or psb4K5_pAimX (small)_sfGFP_ LVAtag	1
7	H2O MQ	qsp 50		H2O MQ	qsp 50

- PCR Programme :

Table181

	A	B	C
1	Temperature (°c)	Time	Cycles
2	98	30″	
3	98	10″	*30
4	56	30″	
5	72	1′30″	
6	72	2′	
7	4	-	

Golden Gate of the PCR product :

- Golden Gate Mix

- Golden gate Program :

Table182

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	improve a part exp 1 psb1K3_k274002 and psb1K3_274003			psb4K5_sfGFP										
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Insert: PCR product	3		Insert: PCR product =sfGFP	3		vector: psb1C3_pAimX(small)-to-J23110_sfGFP	3		vector: psb4K5_pAimX(small)-to-J23110_sfGFP	3		vector: psb1C3_sfGFP	3
7	vector: psb1C3	3		vector: psb1C3_GG	3		Water qsp (20µL)			Water qsp (20µL)			Water qsp (20µL)	
8	Water qsp (20µL)			Water qsp (20µL)										

Table183

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

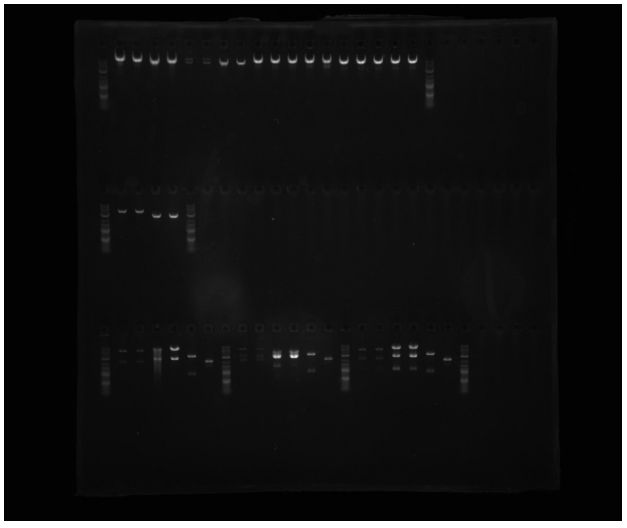
Transformation of the golden gate product of psb1C3_J23110_sfGFP;psb4K5_J23110_sfGFP_LVAtag

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :

The violaceine clones

05.10.18 Digestion minipreps violacein and others.jpg



Lab book jouy

Plasmids of 1T1 to 1T4 and 2T1 to 2T4 constructs of disrupted terminator of Aimx(full) were sent to be sequenced with VF2 primers. Additionally, plasmids of Aimx(full), Aimx(short) (truncated version) and Aimx(short) in pSB1C3 backbone were sent to sequencing with VF2.

Co-transformation of P23110_Aimx(short)_GFPlva (pSB4K5) with the version of peptide expressing plasmids in pSB1C3: OmpA_AimP. Besides, the co-transformation also contained AimR plasmid (pSB3T5). Cells were left on the bench for the whole weekend.

An experiment was performed to screen the effect of the SAIRGA hexapeptide on AimR activity. Thus, cells were co-transformed with the following combination of plasmids:
J23110_AimX(truncated short)_ pSB4K5 + pSB3T5;
J23110_AimX(truncated short)_ pSB4K5 + AimR_pSB3T5;
J23110_AimX(short)_ pSB4K5 + pSB3T5;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5;
Then, serial dilution of SAIRGA peptide was added into LB inoculation media consisting on the following order: 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0 and 10.0 µM of peptide.
Cells were incubated at 37°C and analyzed in its log phase of growing.

SAMEDI 06/10/2018

Transformation of psb1C3_sfGFP; psb1C3_AimR; psb1C3_pAimX(small)-to-J23110_sfGFP;psb4K5_pAimX(small)-to-J23110_sfGFP; improve a part exp 1 psb1C3_k274002 and psb1C3_k274003

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

DIMANCHE 07/10/2018

Golden Gate of the PCR product :

- Golden Gate Mix

Table178

	A	B	C	D	E	F	G	H
1	psb1C3_AimR			psb1C3_sfGFP			psb4K5_pAimX(full3)_sfGFP_LVAtag	
2	Composition	Volume in µL		Composition	Volume in µL		Composition	Volume in µL
3	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
4	T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2		T4 DNA ligase Buffer (already aliquot)	2
5	Enzyme: BsaI	0.5		Enzyme: BsaI	0.5		Enzyme: BsaI	0.5
6	Insert: AimR	3		Insert: sfGFP	3		vector: PCR product	3
7	vector: psb1C3_GG	3		vector: psb1C3_GG	3		Water qsp (20µL)	
8	Water qsp (20µL)			Water qsp (20µL)				

- Golden gate Program :

Table179

	A	B	C
1	Temperature (°c)	time	Cycles
2	37	5′	*150
3	16	5′	
4	4	-	

Liquid culture of :
psb1C3_pAimX(small)-to-J23110_sfGFP;
psb4K5_pAimX(small)-to-J23110_sfGFP;
improve a part exp 1 psb1C3_k274002 and psb1C3_k274003

LUNDI 08/10/2018

Transformation of psb1C3_sfGFP; psb1C3_AimR; psb4K5_pAimX(full3)_sfGFP_LVAtag

Defrost 50µL of E. coli (DH5alpha) on ice
Add 4 µL of DNA
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60″
Incubate again 5′ on ice
Add 400µL of LB
Incubate 1h at 37°C
After that put it all on petri dish LB + right antibiotic
Overnight at 37°C

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :
psb1C3_pAimX(small)-to-J23110_sfGFP;
psb4K5_pAimX(small)-to-J23110_sfGFP;
improve a part exp 1 psb1C3_k274002 and psb1C3_k274003

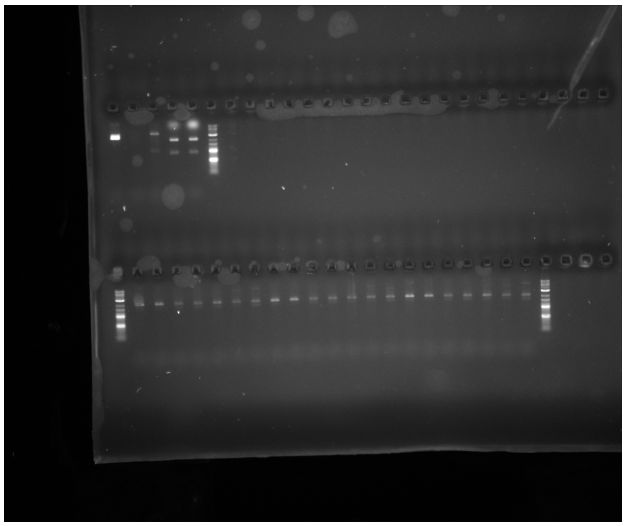
Digestion of psb1C3_pAimX(small)-to-J23110_sfGFP;psb4K5_pAimX(small)-to-J23110_sfGFP; improve a part exp 1 psb1C3_k274002 and psb1C3_k274003

- Digestion Mix

Table185

	A	B	C	D	E
1	**For NotI only**			**For NotI +BamHI**	
2	Composition	Volume en µL		Composition	Volume en µL
3	Buffer fastDigest Green 10X	1		Buffer fastDigest Green 10X	1
4	plasmidic DNA	2		plasmidic DNA	2
5	Enzyme : NotI	0.5		Enzyme : BamHI	0.5
6	H2O qsp (10µL)	6.5		Enzyme : NotI	0.5
7				H2O qsp (10µL)	6
8					

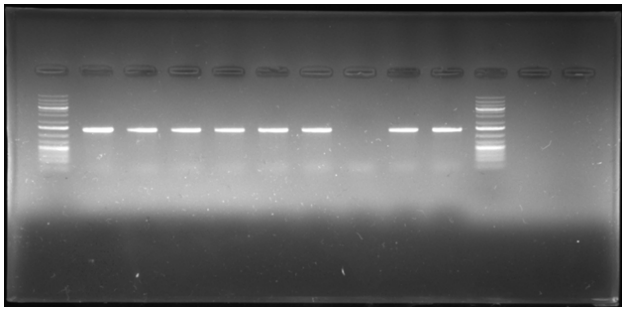
Digestion miniprep m3;4;5;6 n1;2;8 s7 (1;2) et l1; 2;3 08.10.18.tif



Liquid culture of :
psb1C3_sfGFP;
psb1C3_AimR;
psb4K5_pAimX(full3)_sfGFP_LVAtag

Lab book jouy
Colony PCR of AimR (pSB1C3) transformants revealed 7 positive colonies. They were inoculated to be verified by sequencing the day after. Colony PCR were performed with DreamTaq kit (thermo).

image.png



DNA Ladder: GeneRuler 1Kb Plus ready-to-use (Thermo).
All disrupted terminators were confirmed by sequencing to have different mutated sequencings. We will test all mutants.
Recovery of co-transformation P23110_Aimx(short)_GFPlva (pSB4K5) + OmpA_AimP (pSB1C3) + AimR (pSB3T5).

MARDI 09/10/2018

Miniprep according to Thermo scientific " GeneJet Plasmid Miniprep Kit #K0503" of :
psb1C3_sfGFP;
psb1C3_AimR;
psb4K5_pAimX(full3)_sfGFP_LVAtag

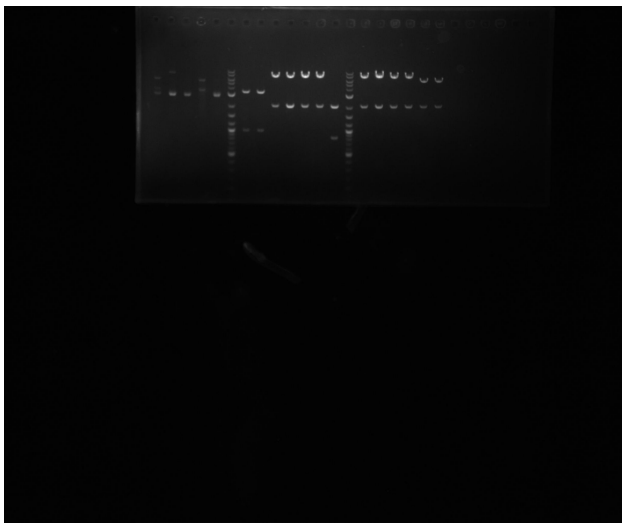
Digestion of psb1C3_sfGFP; psb1C3_AimR; psb4K5_pAimX(full3)_sfGFP_LVAtag

- Digestion Mix

Table186

	A	B
1	**For NotI only**	
2	Composition	Volume en µL
3	Buffer fastDigest Green 10X	1
4	plasmidic DNA	2
5	Enzyme : NotI	0.5
6	H2O qsp (10µL)	6.5
7		
8		

Digestion miniprep m1;m6 n1;n8 et l;2;3 09.10.18.jpg



Liquid culture of :
psb1C3_sfGFP;
psb1C3_AimR;
psb4K5_pAimX(full3)_sfGFP_LVAtag
psb1C3_pAimX(full2)_sfGFP
psb4K5_pAimX(full2)_sfGFP

Improve a part
Dilution of the liquide culture to eighty thousandths and six hundred and forty thousandths
then sending of 125µL on petri dish

Lab book jouy
Co-transformations of P23110_Aimx(short)_GFPlva (pSB4K5) with all versions of peptide expressing plasmids in pSB1C3: AimP, TAT_AimP, PelB_AimP. Besides, the co-transformations also contained AimR plasmid (pSB3T5). This transformation had an incubation time of 2 hours for recovering time.
Extracted plasmids of AimR (pSB1C3) were sent for sequencing with VF2 and/or VR primers.

MERCREDI 10/10/2018

Double transformation

Table187

	A	B	C
1	Double transformation		
2	pSB3T5_AimR	With	pSB1C3_empty
3			pSB1C3_sfGFP
4			pSB1C3_pAimX(short)_sfGFP
5			pSB1C3_pAimX(full)_sfGFP
6			pSB1C3_pAimX(full-1)-to-J23110_sfGFP
7			pSB1C3_pAimX(full-2)-to-J23110_sfGFP
8			pSB1C3_pAimX(full-3)-to-J23110_sfGFP
9			pSB1C3_J23110_sfGFP
10			pSB1C3_sfGFP-LVAtag
11			pSB1C3_pAimX(short)_sfGFP-LVAtag
12			pSB1C3_pAimX(full)_sfGFP-LVAtag
13			pSB1C3_pAimX(short)-to-J23110_sfGFP-LVAtag
14			pSB1C3_pAimX(full-1)-to-J23110_sfGFP-LVAtag
15			pSB1C3_pAimX(full-3)-to-J23110_sfGFP-LVAtag
16			pSB1C3_J23110_sfGFP-LVAtag
17			pSB1C3_J23110_pAimX(full)_sfGFP-LVAtag
18			pSB1C3_J23110_pAimX(short)_sfGFP-LVAtag

19			pSB1C3_J23110trunc_pAimX(short)_sfGFP-LVAtag
20			pSB4K5_empty
21			pSB4K5_sfGFP
22			pSB4K5_pAimX(full)_sfGFP
23			pSB4K5_pAimX(short)_sfGFP
24			pSB4K5_pAimX(full-1)-to-J23110_sfGFP
25			pSB4K5_pAimX(full-2)-to-J23110_sfGFP
26			pSB4K5_pAimX(full-3)-to-J23110_sfGFP
27			pSB4K5_sfGFP-LVAtag
28			pSB4K5_pAimX(full)_sfGFP-LVAtag
29			pSB4K5_pAimX(short)_sfGFP-LVAtag
30			pSB4K5_pAimX(short)-to-J23110_sfGFP-LVAtag
31			pSB4K5_pAimX(full-1)-to-J23110_sfGFP-LVAtag
32			pSB4K5_pAimX(full-2)-to-J23110_sfGFP-LVAtag
33			pSB4K5_J23110_pAimX(full)_sfGFP-LVAtag
34			pSB4K5_J23110_pAimX(short)_sfGFP-LVAtag
35			pSB4K5_J23110trunc_pAimX(short)_sfGFP-LVAtag

Defrost 50L of E. coli (DH5alpha) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put all in a liquid culture of 5ml LB + the right antibiotic
Incubate at 37°C overnight

Lab book JOUY

Confirmation by sequencing our AimR in pSB1C3 (colonies 2, 5, 7, and 8).
An experiment was performed to screen the effect of the SAIRGA hexapeptide on AimR activity. Thus, cells were co-transformed with the following combination of plasmids:
J23110_AimX(truncated short)_ pSB4K5 + pSB3T5;
J23110_AimX(truncated short)_ pSB4K5 + AimR_pSB3T5;
J23110_AimX(short)_ pSB4K5 + pSB3T5;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5;
Then, serial dilution of SAIRGA peptide was added into LB inoculation media consisting on the following order:
16.625, 31.25, 62.5, 125, 250, 500, 1000µM of SAIRGA hexapeptide.
Cells were incubated at 37°C and analyzed in its log phase of growing

JEUDI 11/10/2018

Glycerol stock of the double transformation of the 10.10.18

Double transformation

Table188

	A	B	C	D	E	F	G	H	I	J	K
1	Double transformation				Double transformation				Double transformation		
2	pSB3T5_GG	With	pSB1C3_empty		psb1C3_GG	with	pSB4K5_empty		psb1C3_AimR	with	pSB4K5_empty
3			pSB1C3_sfGFP				pSB4K5_sfGFP				pSB4K5_sfGFP
4			pSB1C3_pAimX(short)_sfGFP				pSB4K5_pAimX(full)_sfGFP				pSB4K5_pAimX(full)_sfGFP
5			pSB1C3_pAimX(full)_sfGFP				pSB4K5_pAimX(short)_sfGFP				pSB4K5_pAimX(short)_sfGFP
6			pSB1C3_pAimX(full-1)-to-J23110_sfGFP				pSB4K5_pAimX(full-1)-to-J23110_sfGFP				pSB4K5_pAimX(full-1)-to-J23110_sfGFP
7			pSB1C3_pAimX(full-2)-to-J23110_sfGFP				pSB4K5_pAimX(full-2)-to-J23110_sfGFP				pSB4K5_pAimX(full-2)-to-J23110_sfGFP
8			pSB1C3_pAimX(full-3)-to-J23110_sfGFP				pSB4K5_pAimX(full-3)-to-J23110_sfGFP				pSB4K5_pAimX(full-3)-to-J23110_sfGFP
9			pSB1C3_J23110_sfGFP				pSB4K5_sfGFP-LVAtag				pSB4K5_sfGFP-LVAtag
10			pSB1C3_sfGFP-LVAtag				pSB4K5_pAimX(full)_sfGFP-LVAtag				pSB4K5_pAimX(full)_sfGFP-LVAtag
11			pSB1C3_pAimX(short)_sfGFP-LVAtag				pSB4K5_pAimX(short)_sfGFP-LVAtag				pSB4K5_pAimX(short)_sfGFP-LVAtag
12			pSB1C3_pAimX(full)_sfGFP-LVAtag				pSB4K5_pAimX(short)-to-J23110_sfGFP-LVAtag				pSB4K5_pAimX(short)-to-J23110_sfGFP-LVAtag
13			pSB1C3_pAimX(short)-to-J23110_sfGFP-LVAtag				pSB4K5_pAimX(full-1)-to-J23110_sfGFP-LVAtag				pSB4K5_pAimX(full-1)-to-J23110_sfGFP-LVAtag
14			pSB1C3_pAimX(full-1)-to-J23110_sfGFP-LVAtag				pSB4K5_pAimX(full-2)-to-J23110_sfGFP-LVAtag				pSB4K5_pAimX(full-2)-to-J23110_sfGFP-LVAtag
15			pSB1C3_pAimX(full-3)-to-J23110_sfGFP-LVAtag				pSB4K5_J23110_pAimX(full)_sfGFP-LVAtag				pSB4K5_J23110_pAimX(full)_sfGFP-LVAtag
16			pSB1C3_J23110_sfGFP-LVAtag				pSB4K5_J23110_pAimX(short)_sfGFP-LVAtag				pSB4K5_J23110_pAimX(short)_sfGFP-LVAtag
17			pSB1C3_J23110_pAimX(full)_sfGFP-LVAtag				pSB4K5_J23110trunc_pAimX(short)_sfGFP-LVAtag				pSB4K5_J23110trunc_pAimX(short)_sfGFP-LVAtag
18			pSB1C3_J23110_pAimX(short)_sfGFP-LVAtag								
19			pSB1C3_J23110trunc_pAimX(short)_sfGFP-LVAtag								
20			pSB4K5_empty								
21			pSB4K5_sfGFP								
22			pSB4K5_pAimX(full)_sfGFP								
23			pSB4K5_pAimX(short)_sfGFP								
24			pSB4K5_pAimX(full-1)-to-J23110_sfGFP								
25			pSB4K5_pAimX(full-2)-to-J23110_sfGFP								
26			pSB4K5_pAimX(full-3)-to-J23110_sfGFP								
27			pSB4K5_sfGFP-LVAtag								
28			pSB4K5_pAimX(full)_sfGFP-LVAtag								
29			pSB4K5_pAimX(short)_sfGFP-LVAtag								

30			pSB4K5_pAimX (short)-to-J23110_sfGFP-LVAtag								
31			pSB4K5_pAimX (full-1)-to-J23110_sfGFP-LVAtag								
32			pSB4K5_pAimX (full-2)-to-J23110_sfGFP-LVAtag								
33			pSB4K5_J23110_pAimX(full)_sfGFP-LVAtag								
34			pSB4K5_J23110_pAimX(short)_sfGFP-LVAtag								
35			pSB4K5_J23110 trunc_pAimX(short)_sfGFP-LVAtag								

Defrost 50L of E. coli (DH5alpha) on ice
Add 3 µL of each plasmids
Incubate 30′ on ice
Then make a thermic choc at 42°C during 60′′
Incubate again 5′ on ice
Add 1mL of LB
Incubate 1h at 37°C 300rpm
After that put all in a liquid culture of 5ml LB + the right antibiotic
Incubate at 37°C overnight

Lab book Jouy

Transformation of 1T1 to 1T4 and 2T1 to 2T4 constructs of disrupted terminators of Aimx(full) into TOP10 cells.
Since, other screenings haven't shown any activity/intaking of SAIRGA on sfGFP expression; an experiment was performed to test if SAIRGA peptide could trigger sfGFP expression if produced intracellularly. Thus, cells were co-transformed with the following combination of plasmids:
J23110_AimX(short)_ pSB4K5 + pSB3T5 + pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + OmpA_AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + PelB_AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + TAT_AimP_pSB1C3;
Cells were incubated at 37°C and analyzed in its log phase of growing.

VENDREDI 12/10/2018

Glycerol stock of the double transformation of the 11.10.18

Lab book Jouy

Cells grown in M9 supplemented with 100µg/mL accordingly to the Maio et al., 2016 (https://www.mdpi.com/2079-6382/5/2/17/htm). However, cells did not grow.

LUNDI 15/10/2018

Lab book Jouy

A second screening was made with co-transformation of Plasmids containing promoter AimX, AimR repressor and Peptide expressing plasmids. Additionally, all disrupted terminators plasmids were analyzed in comparison to Aimx(full) and Aimx(short).
Data were measured on the log phase of growth.

LB with the respective antibiotics was used for this experiment.
Another experiment was performed to test if SAIRGA peptide could trigger sfGFP expression if produced intracellularly. Thus, cells were co-transformed with the following combination of plasmids:
J23110_AimX(short)_ pSB4K5 + pSB3T5 + pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + OmpA_AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + PelB_AimP_pSB1C3;
J23110_AimX(short)_ pSB4K5 + AimR_pSB3T5 + TAT_AimP_pSB1C3;
Additionally, to test the disrupted terminators, a screening was performed on all 4 mutants comprising the two types of terminator disruption (1T or 2T). Cells were transformed and analyzed accordingly to the following groups of transformants:
J23110_AimX(full)_ pSB4K5;
J23110_AimX(short)_ pSB4K5;
J23110_AimX(full)_1T(1)_ pSB4K5;
J23110_AimX(full)_1T(2)_ pSB4K5;
J23110_AimX(full)_1T(3)_ pSB4K5;
J23110_AimX(full)_1T(4)_ pSB4K5;
J23110_AimX(full)_2T(1)_ pSB4K5;
J23110_AimX(full)_2T(2)_ pSB4K5;
J23110_AimX(full)_2T(3)_ pSB4K5;
J23110_AimX(full)_2T(4)_ pSB4K5;
Cells were incubated at 37°C and analyzed in its log phase of growing.

MARDI 16/10/2018

Cell-free screening of pAimX(short) + AimR with a gradient concentration of hexapeptide was analyzed. Additionally, the ration between the transcription factor plasmid (AimR_pSB4K5) and the regulated promoter with a reporter [J23110_pAimX(short)] were tested in order to test the best ratio between the 2 components .

Table189

	A	B	C	D	E	F	G	H
1	10nM AimR 5nM J23110_pAimX(short) Ø peptide	10nM AimR 5nM J23110_pAimX(short) 0.01µM peptide	10nM AimR 5nM J23110_pAimX(short) 0.03µM peptide	10nM AimR 5nM J23110_pAimX(short) 0.1µM peptide	10nM AimR 5nM J23110_pAimX(short) 0.3µM peptide	10nM AimR 5nM J23110_pAimX(short) 1µM peptide	10nM AimR 5nM J23110_pAimX(short) 3µM peptide	10nM AimR 5nM J23110_pAimX(short) 10µM peptide
2	10nM AimR 2nM J23110_pAimX(short) Ø peptide	10nM AimR 2nM J23110_pAimX(short) 0.01µM peptide	10nM AimR 2nM J23110_pAimX(short) 0.03µM peptide	10nM AimR 2nM J23110_pAimX(short) 0.1µM peptide	10nM AimR 2nM J23110_pAimX(short) 0.3µM peptide	10nM AimR 2nM J23110_pAimX(short) 1µM peptide	10nM AimR 2nM J23110_pAimX(short) 3µM peptide	10nM AimR 2nM J23110_pAimX(short) 10µM peptide
3	10nM AimR 1nM J23110_pAimX(short) Ø peptide	10nM AimR 1nM J23110_pAimX(short) 0.01µM peptide	10nM AimR 1nM J23110_pAimX(short) 0.03µM peptide	10nM AimR 1nM J23110_pAimX(short) 0.1µM peptide	10nM AimR 1nM J23110_pAimX(short) 0.3µM peptide	10nM AimR 1nM J23110_pAimX(short) 1µM peptide	10nM AimR 1nM J23110_pAimX(short) 3µM peptide	10nM AimR 1nM J23110_pAimX(short) 10µM peptide
4	Blank (lysate)	5nM J23110_pAimX(short)						

Cell-free protocol was adapted from Sun et al., 2013 protocol.
A co-culture experiment was performed with OmpA_AimP cell + (pAimX(short) + pSB1C3 +/- AimR) in oder to check if Ompa could induce GFP expression.

All three cell types (7A+Tetx+Cmx, 7A+AimR_Tet+Cmx, and ompA-SAIRGA) were grown to OD600 of 0.8 to 1.2. They were then cooled down on ice for 30 min to stop growth. Next, all three cultures were diluted on ice to OD600 of 0.1, and used to create co-cultures by mixing them in different ratios in LB media (Effective antibiotic concentration: Cm 8.75 mg/mL). Finally, 200 uL of the mixed cultures were added to each well and the cultures grown at 37C in the plate reader for 12 hours. The table below describes the effective starting density of the two cell types in the wells.

Table190

	A	B	C	D	E	F
1	Effective Starting OD600 of these cells	7A+Tetx+Cmx	ompA-SAIRGA		7A+AimR_Tet+Cmx	ompA-SAIRGA
2		Row C			Row E	
3	Col 1	0.025	0.075		0.025	0.075
4	Col 2	0.025	0.0375		0.025	0.0375
5	Col 3	0.025	0.01875		0.025	0.01875
6	Col 4	0.025	0.009375		0.025	0.009375
7	Col 5	0.025	0.0046875		0.025	0.0046875
8	Col 6	0.025	0.00234375		0.025	0.00234375
9	Col 7	0.025	0.00117188		0.025	0.00117188
10	Col 8	0.025	0.00058594		0.025	0.00058594
11	Col 9	0.025	0.00029297		0.025	0.00029297
12	Col 10	0.025	0.00014648		0.025	0.00014648
13	Col 11	0.025	7.3242e-5		0.025	7.3242e-5
14	Col 12	0.025	3.6621e-5		0.025	3.6621e-5

The data collected was analyzed for GFP/ OD600.

MERCREDI 17/10/2018

Unfortunately both cell-free and co-culturing experiments were extremely inconclusive and rejected for the study.