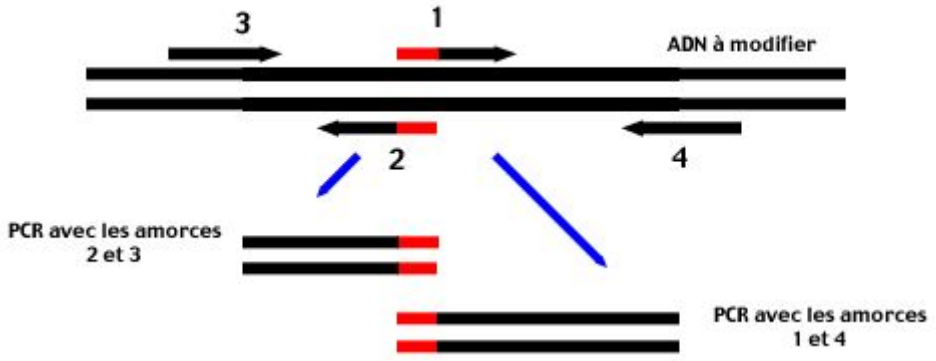
03/08/2017:

Program :
- site-directed mutagenesis to remove all the illegal restriction sites inside our insert.

List of the illegal restriction site :
- WC1 : EcoR1
- WC2 : ajout d'un Xba1, Pst1 (1), Pst1 (2), EcoR1
- Unc119 : EcoR1 (1), EcoR1 (2)

We focused on the illegal sites in RED.

PCR fusion (first step):



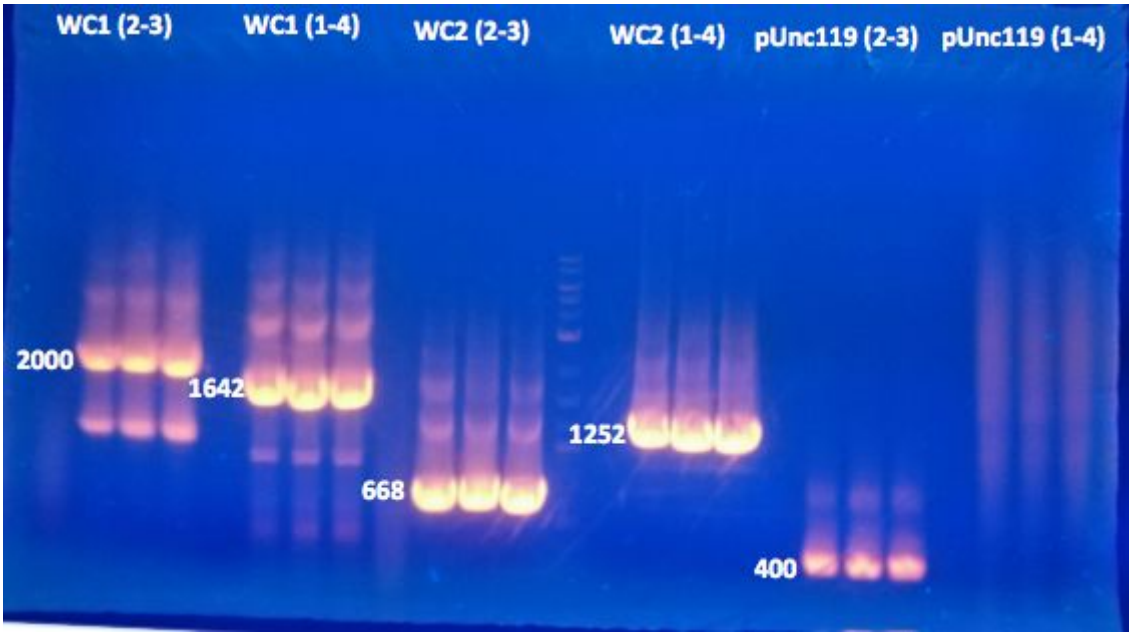
We did 24 tubes:
- 4 (1 control + 1 triplicat) : with the primers n°3 and 2 for WC1 (2000 bp)
- 4 (1 control + 1 triplicat) : with the primers n°1 and 4 for WC1 (1642 bp)
- 4 (1 control + 1 triplicat) : with the primers n°3 and 2 for WC2 (668 bp)
- 4 (1 control + 1 triplicat) : with the primers n°1 and 4 for WC2 (1252 bp)
- 4 (1 control + 1 triplicat) : with the primers n°3 and 2 for pUnc119 (400 bp)
- 4 (1 control + 1 triplicat) : with the primers n°1 and 4 for pUnc119 (1700 bp)

PCR :
T°C : 50
Lenghtening time : 1 ‘

04/08/2017

Program :
1- electrophoresis to see the results from the last PCR for the site-directed mutagenesis.
2- Purification on the amplified products from the last PCR
3- PCR on pUnc119 (1-4) as the last one failed.
4- PCR of Myo-3::unc-60::GFP
5- PCR of Myo-3::unc-60::RFP
6- Purification on the amplified products from the PCR that work.

1- Electrophoresis and Results of the last PCR



We can clearly see the different fragments we wanted to obtain except for the pUnc119 (1-4). However we will have to do some purification on the other amplified products.

2- Purification on the amplified products from the last PCR

According to the protocol inside the SIGMA GenElute ™ PCR Clean-Up KIT (70 purifications).

4- PCR Myo-3::unc-60::GFP:

size=5432pb

elongation time= 3min

Tm = 54°C

PCR was made for 5 PCR with 50µL/tube (4 PCR with DNA Jean and one H2O control with the rest of the mix)

H2O	167,5µL
5x Buffer Phusion	50µL
10nM dNTP	5µL
10µ M Forward	12,5µL
10 µM reverse	12,5µL
DNA polymerase	2,5µL

DNA jean (plasmid)……...1µL/ PCR tube

Tube 1=H2O

Tube 2=DNA GFP

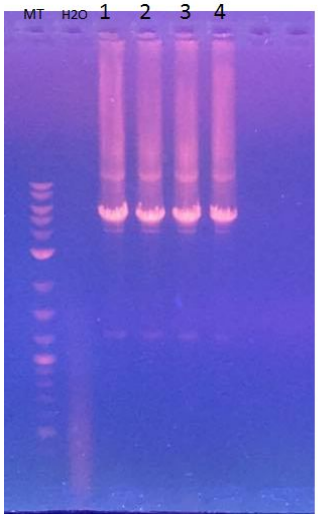
Tube3=DNA GFP

Tube4=DNA GFP

Tube5=DNA GFP

We charged 5µL in every wells. We conserve a stock of 45µL in every PCR tubes.

Results:



We have the desire size of approximately 5,4 kpb in all wells (1,2,3 and 4). We can purify this fragment in order to obtain pure DNA to do aquacloning.

5- PCR Myo-3::unc-60::RFP:

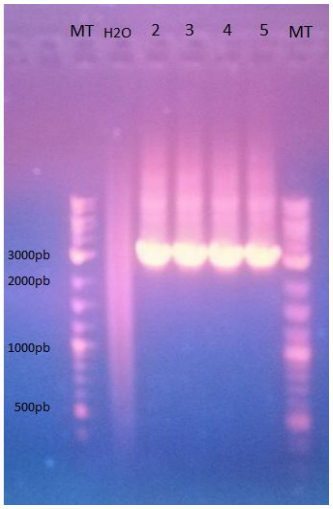
size=3273pb
elongation time= 2min
Tm = 54°C
PCR was made for 5 PCR with 50µL/tube (4 PCR with DNA Jean and one H2O control with the rest of the mix)

H2O	167,5µL
5x Buffer Phusion	50µL
10nM dNTP	5µL
10µ M Forward	12,5µL
10 µM reverse	12,5µL
DNA polymerase	2,5µL

DNA jean (plasmid)……...1µL/ PCR tube

Tube 1=H2O
Tube 2=DNA RFP
Tube3=DNA RFP
Tube4=DNA RFP
Tube5=DNA RFP
We charged 5µL in every wells. We conserve a stock of 45µL in every PCR tubes.

Results:



We amplified pMyo3::unc-60::RFP

Informations about florian promoters that we can used in our ptoject

Florian had amplified many promoters of elegans and we tried to clone them. We obtained a little number of colonies for unc-119', unc-52 and myo3'. To do new aquacloning we have to do new PCR in order to have more DNA. But these PCR are impossible to do for this moment because of less informations like size of the promoter, Tm and forwards and reverses primers used. To have more informations, we studied florian's plasmids and florian's stock primers.

We found these informations:

unc-119:

size=2230pb

Tm =63,5°C

myo-2:

size=1012pb

Tm =62,4

mec-2:

size=2036pb

Tm =65,81°C

gcy-5:

size=

Tm =

gcy-7:

size=1464pb

Tm =61,2°C

unc-52:

size=1845pb

Tm =65,82°C

ida-1:

size=339pb

Tm =69,6°C

PCR Sup-12:

size=561 with prefix and suffix
elongation time=1 min
Tm = 58,9°C
PCR was made for 5 PCR with 50µL/tube (4 PCR with DNA and one H2O control with the rest of the mix)

H2O	167,5µL
5x Buffer Phusion	50µL
10nM dNTP	5µL
10µ M Forward	12,5µL
10 µM reverse	12,5µL
DNA polymerase	2,5µL

DNA sup-12: ……...2µL/ PCR tube 10ng/µL

Tube 1=H2O
Tube 2=DNA
Tube3=DNA
Tube4=DNA
Tube5=DNA
We charged 10µL in every wells because it's the first PCR for this gene. We conserve a stock of 40µL in every PCR tubes.

Results:

04/08/2017

Nanodrop dosage for Myo-3::unc-60::GFP (5432pb) which was purified with the Quiagen quit in 50µL of miliQ water

ng/µL	260/280	260/230	Molarity(nM)

90,4	1,90	2,26	25,54

Nanodrop dosage for Myo-3::unc-60::RFP (3273pb) which was purified with the Quiagen quit in 50µL of miliQ water

ng/µL	260/280	260/230	Molarity(nM)
171,4	1,88	2,30	80,5

Nanodrop dosage for sup-12(561pb) which was purified with the Quiagen quit in 50µL of miliQ water

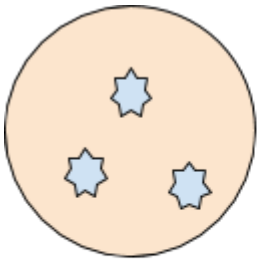
ng/µL	260/280	260/230	Molarity(nM)
175,4	1,87	2,27	Magui?

Aquacloning of Myo-3::unc-60::GFP (5432pb) and Myo-3::unc-60::RFP (3273pb)

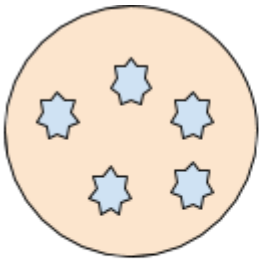
	Plasmid pSB1C3 cut with speI and XbaI 9,92nM	DNA of intersest
Negativ control	10µL water	none
Positiv control	5µL dna stock and 5µL of water	none
Myo-3::unc-60::GFP 25,5nM 3:1	5µL stock	5µL stock without dilution
Myo-3::unc-60::GFP 25,5nM 5:1	5µL stock with 2X dilution 5nM	5µL stock without dilution
Myo-3::unc-60::RFP 30nM 3:1	5µL stock	5µL stock with 2X dilution (3,72µLDNA stock and 6,28µL of water)
Myo-3::unc-60::RFP 5:1	5µL stock	5µL stock with 2X dilution 40,25,nM

9/08/2017

PCR on colony of myo3 unc60GFP



3:1



5:1

Protocol

	20 uL	9 tubes x 20 uL (uL)
H2O	13,4 uL	120,6 uL
5x Buffer Phusion	4 uL	36 uL
10nM dNTP	0,4 uL	3,6 uL
10µM Myo3 Forward	1 uL	9 uL
10 µM Unc60 Reverse	1 uL	9 uL
Phusion DNA polymerase	0,2 uL	1,8 uL

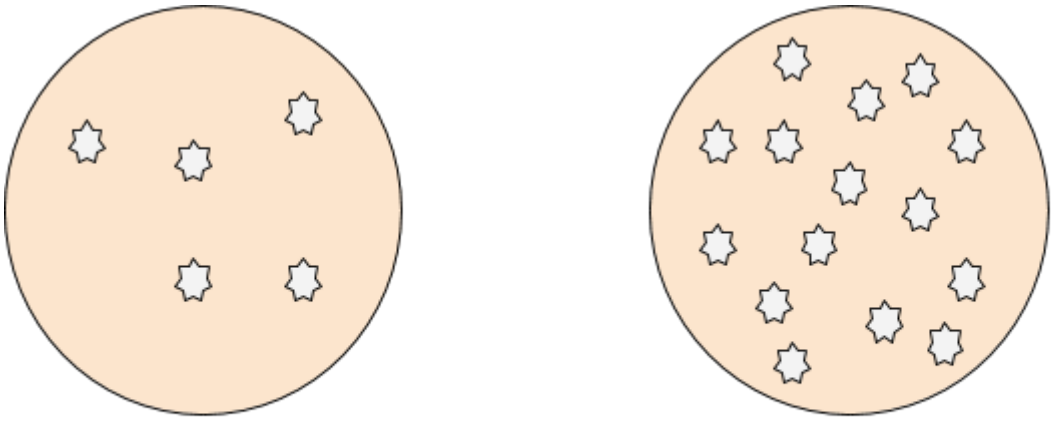
size = 5431 pb
elongation time= 3 min
Tm = 54°C

PCR was made for 9 PCR with 20µL/tube (8 PCR with bacteria and one H2O control with the rest of the mix)

Preparation of 8 bacteriological cultures tubes :

- 5uL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

PCR on colony of myo3 unc60RFP



3 : 1 5 : 1

Protocol

	20 uL	22 tubes x 20 uL (uL)
H2O	13,4 uL	294,8 uL
5x Buffer Phusion	4 uL	88 uL
10nM dNTP	0,4 uL	8,8 uL
10µM Myo3 Forward	1 uL	22 uL
10 µM Unc60 Reverse	1 uL	22 uL
Phusion DNA polymerase	0,2 uL	4,4 uL

size : 3273 pb
elongation time= 2 min
Tm = 54°C

PCR was made for 16 PCR with 20µL/tube (15 PCR with bacteria and one H2O control with the rest of the mix)

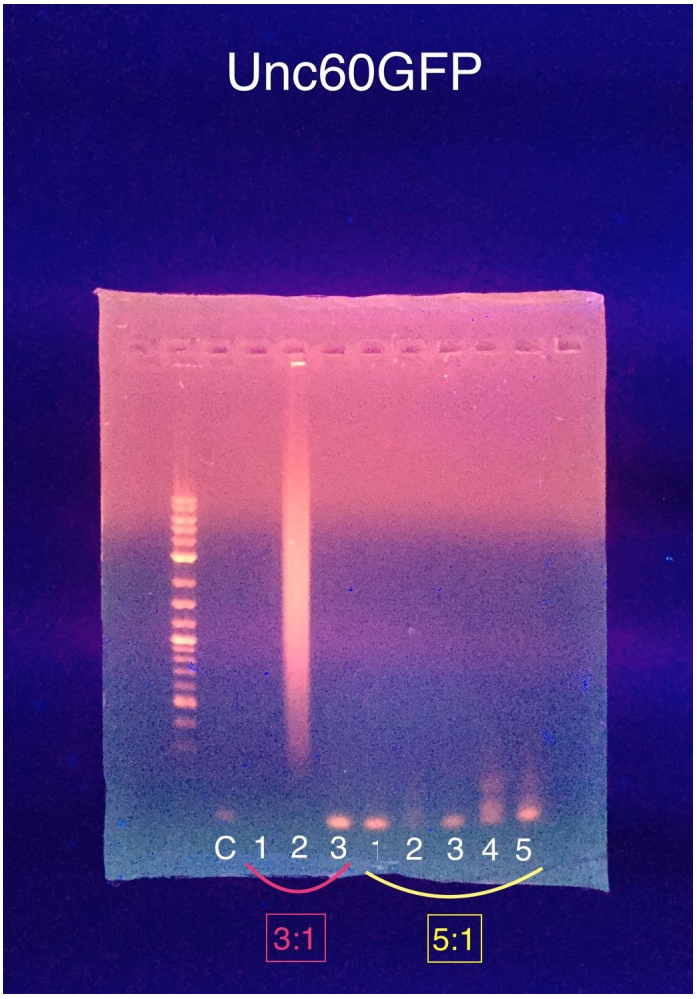
Preparation of 22 bacteriological culture tubes

- 5uL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

10/08/17

Migration, revelation and interpretation

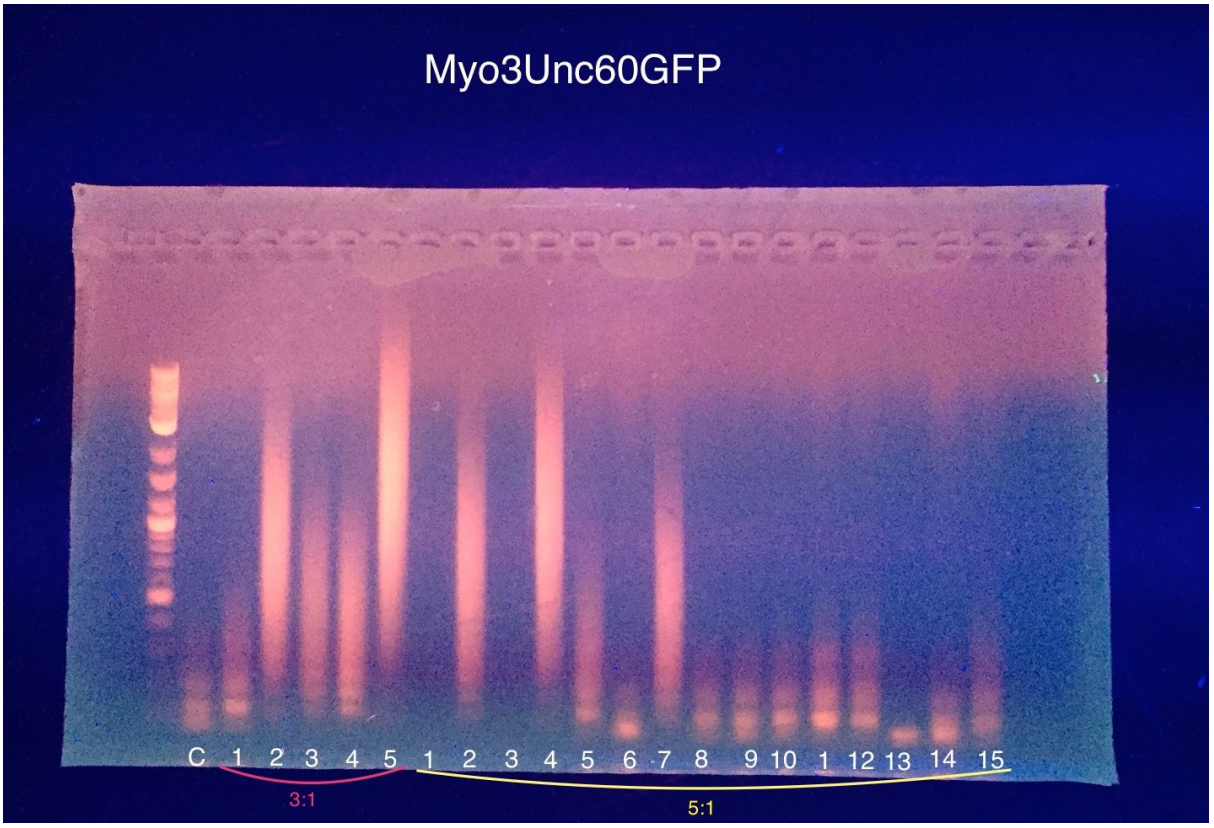
Myo3 unc60GFP



Results

- No amplification

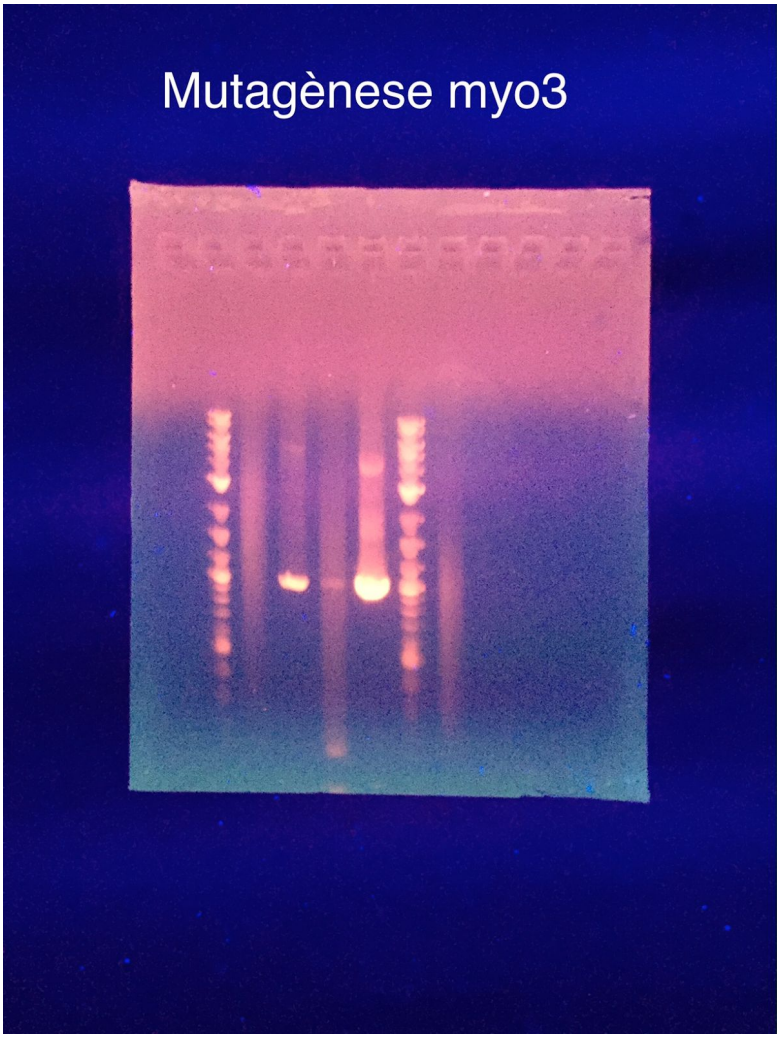
Myo3 unc60RFP



Results

- No amplification

Results of mutagenesis by PCR : myo3

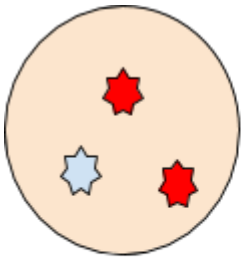


Amplification of tubes 1-2 et tubes 3-4
No amplification of plasmids

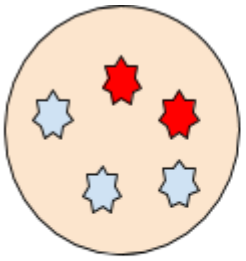
Clonage of sup12 : Colonie rouge

PCR on colony of myo3unc60 GFP and myo3unc60 RFP

Myo3unc60GFP



3:1



5:1

Results :

Protocol

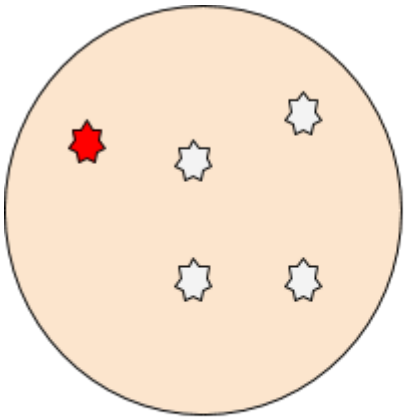
	20 uL	5 tubes x 20 uL (uL)
H2O	13,4 uL	67 uL
5x Buffer Phusion	4 uL	20 uL
10nM dNTP	0,4 uL	2 uL
10µM Myo3 Forward	1 uL	5 uL
10 µM Unc60 Reverse	1 uL	5 uL
Phusion DNA polymerase	0,2 uL	1 uL

size = 5431 pb
elongation time= 3 min
Tm = 54°C
PCR was made for 5 PCR with 20µL/tube (4 PCR with bacteria and one H2O control with the rest of the mix)

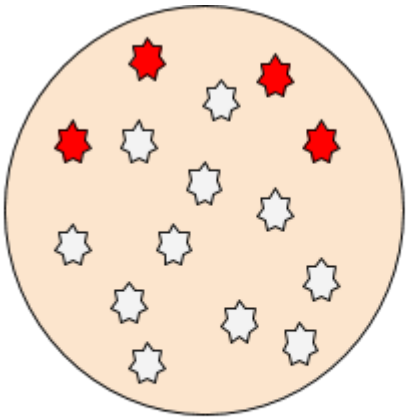
Preparation of 4 bacteriological culture tubes :

- 5uL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

Myo3unc60RFP



3 : 1



5 : 1

Results :

	20 uL	16 tubes x 20 uL (100uL)
H2O	13,4 uL	214,4 uL
5x Buffer Phusion	4 uL	64 uL
10nM dNTP	0,4 uL	6,4 uL
10µM Myo3 Forward	1 uL	16 uL
10 µM Unc60 Reverse	1 uL	16 uL
Phusion DNA polymerase	0,2 uL	3,2 uL

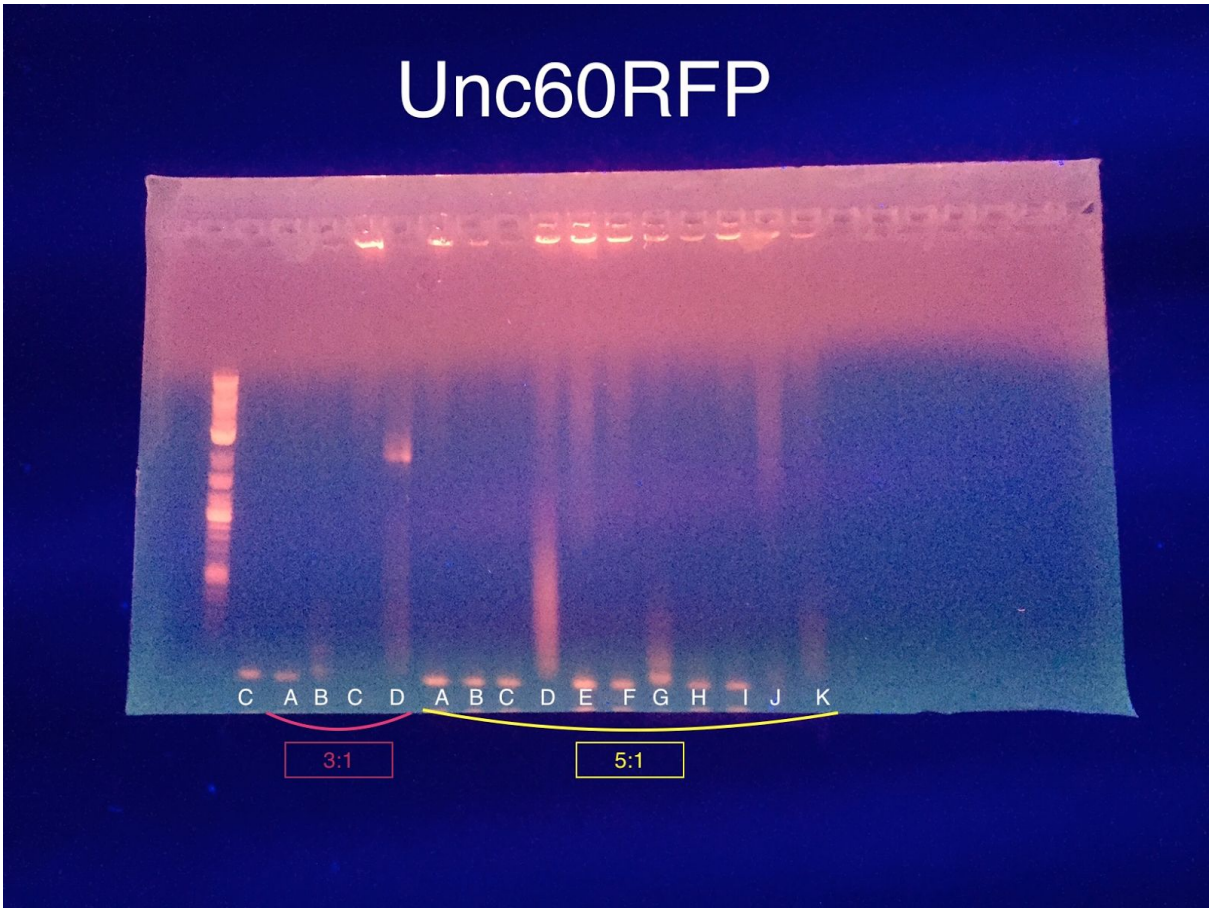
size = 5431 pb
elongation time= 3 min
Tm = 54°C
PCR was made for 16 PCR with 20µL/tube (15 PCR with bacteria and one H2O control with the rest of the mix)

<u>Preparation of 15 bacteriological culture tubes :</u>

- 5uL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

11/08/17

Results of PCR on colony of myo3unc60RFP



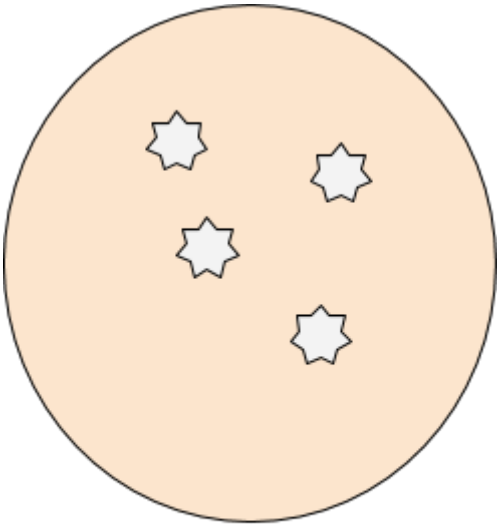
Results :

- We can see an amplification for the colony D. We haven't the desire size of approximately 3273 pb. The next step is the purification with plasmid miniprep kit.
- No amplification for the other colonies
- Contamination of the control

16/08/2017

PCR on colony

Myo3unc60GFP



5 : 1

Protocols

	20 uL	6 tubes x 20 uL (uL)
H2O	13,4 uL	80,4 uL
5x Buffer Phusion	4 uL	24 uL
10nM dNTP	0,4 uL	2,4 uL
10µM Myo3 Forward	1 uL	6 uL
10 µM Unc60 Reverse	1 uL	6 uL
Phusion DNA polymerase	0,2 uL	1,2 uL

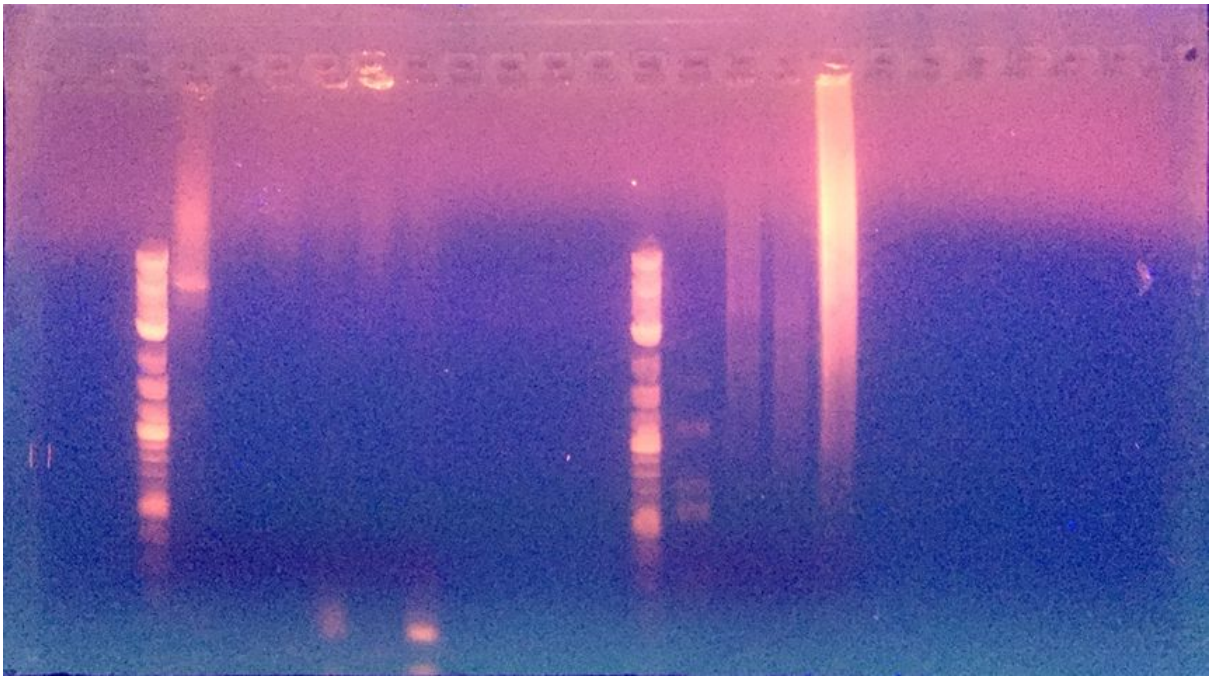
Size = 5431 pb
Elongation time= 3 min
Tm = 54°C

PCR was made for 6 PCR with 20µL/tube (4 PCR with bacteria, one H2O control and one positive control with the rest of the mix)

Preparation of 4 bacteriological culture tubes :

- 5uL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

Results



(Partie de gauche)

there are no amplification.

Plasmid Miniprep : Myo3::unc60::RFP in PSB1C3 backbone (colony D)

1 - Harvest and lyse bacteria

- Pellet cells from 1- 5 mL overnight culture 1 minute (1 mL from TB or 2xYT ; 1-5 mL from LB medium). Discard supernatant.
- Resuspend cells in 200 uL Resuspension Solution. Pipette up and down or vortex.

- Add 200 uL of Lysis Solution. Invert gently to mix. Do not vortex. Allow to clear for 5 minutes.
- Prior to first time use, be sure to add the RNase A to the Resuspension Solution.

2 - Prepare cleared lysate
- Add 350 uL of Neutralization Solution (S3). Invert 4-6 times to mix.
- Pellet debris 10 minutes at max speed

3 - Prepare binding column
- Add 500 mL Column Preparation Solution to binding column in a collection tube.
- Spin at > 12,000 x g, 1 minute. Discard flow-through.

4 - Bind plasmid DNA to column
- Transfer cleared lysate into binding column.
- Spin 30 secondes, 1 minute. Discard flow-through.

5 - Wash to remove contaminants
- Optional (EndA+ strains only) : Add 500uL Optional Wash Solution to column. Spin 30 secondes, 1 minute. Discard flow-through.
- Add 750 uL Wash Solution to column. Spin 30 seconde, 1 minute. Discard flow-through.
- Spin 1 minute to dry column.
- Prior to first time use, be sure to add ethanol to the concentrated Wash Solution

6- Elute purified plasmid DNA
- Transfer column to new collection tube
- Add 50 uL or 30 uL Elution Solution. Spin 5 minutes.
- If a more concentrated plasmid DNA prep is required, reduce the elution volume to a
- minimum of 50 uL.

Nanodrop dosage of Myo-3::unc-60::RFP Miniprep in 100µL of elution buffer

ng/µL	260/280	260/230	Molarity(nM)
33,0	2,07	4,06	

This results shows that one problem occurred during plasmids extraction and 33ng/µL is really less. We should do one enzymatic digestion to see if our plasmid was purified or not.

PCR plasmid

The goal is to ampified our backbones.
Plasmid :
- pSB1A3
- pSB1C3
- pSB1K3

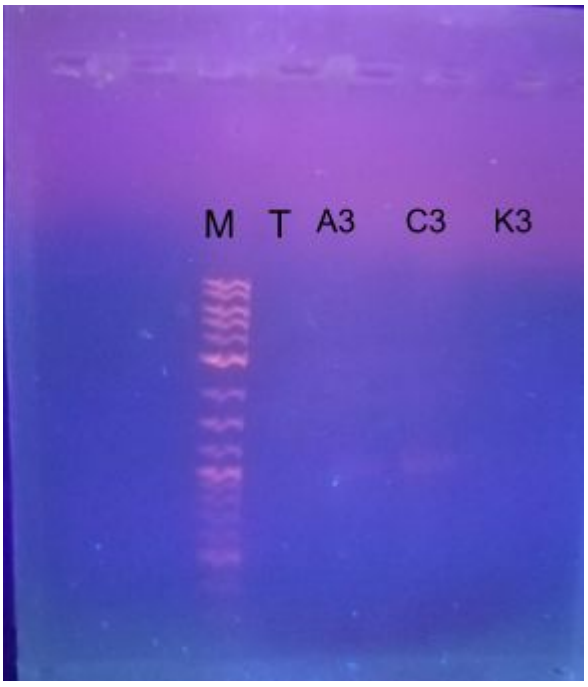
Primer :

- Pre FW
- Suff RV

The mix is done with the same conditions as usual for a volume of 50 µl for 1 PCR.
There will be one control and the 3 PCR.
Tm = 55 °C
Time = 1 minute

Results :

I use wrongs primers.



PCR fusion (mutagenesis)

Gene:

- pMYO3 :
  - Time = 1 minute and 5 seconds
  - Tm = 50 and 55 °C
- WC2 :
  - Time = 1 minute
  - Tmm = 50 and 55 °C
- WC1:
  - Time = 2 minutes
  - Tmm = 50 and 55 °C

Mix for one control and one fusion :

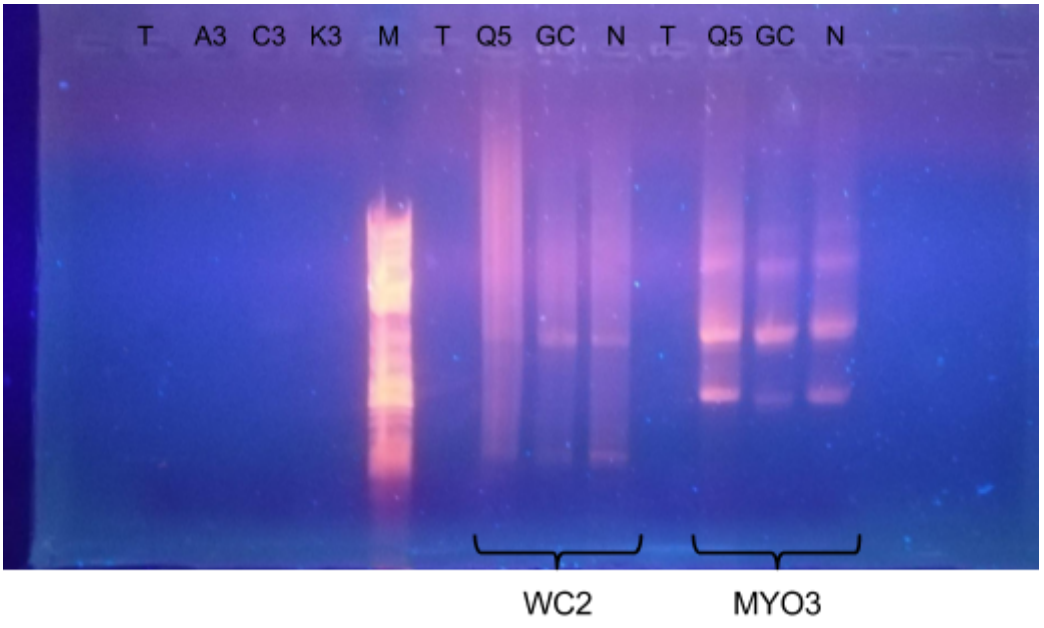
	Normal buffer	Buffer GC	Q5

H20	65 µl		38 µl
Buffer	20 µl	20 µl	50 µl
dNTP	2 µl		
Primer forward (Pre)	5 µl		
Primer reverse (suff)	5 µl		
Polymerase	1 µl		With the mix buffer dNTP
DNA (µl for each fragment)	1		

Primer :

- Pre (Fw)
- Suff (Rv)

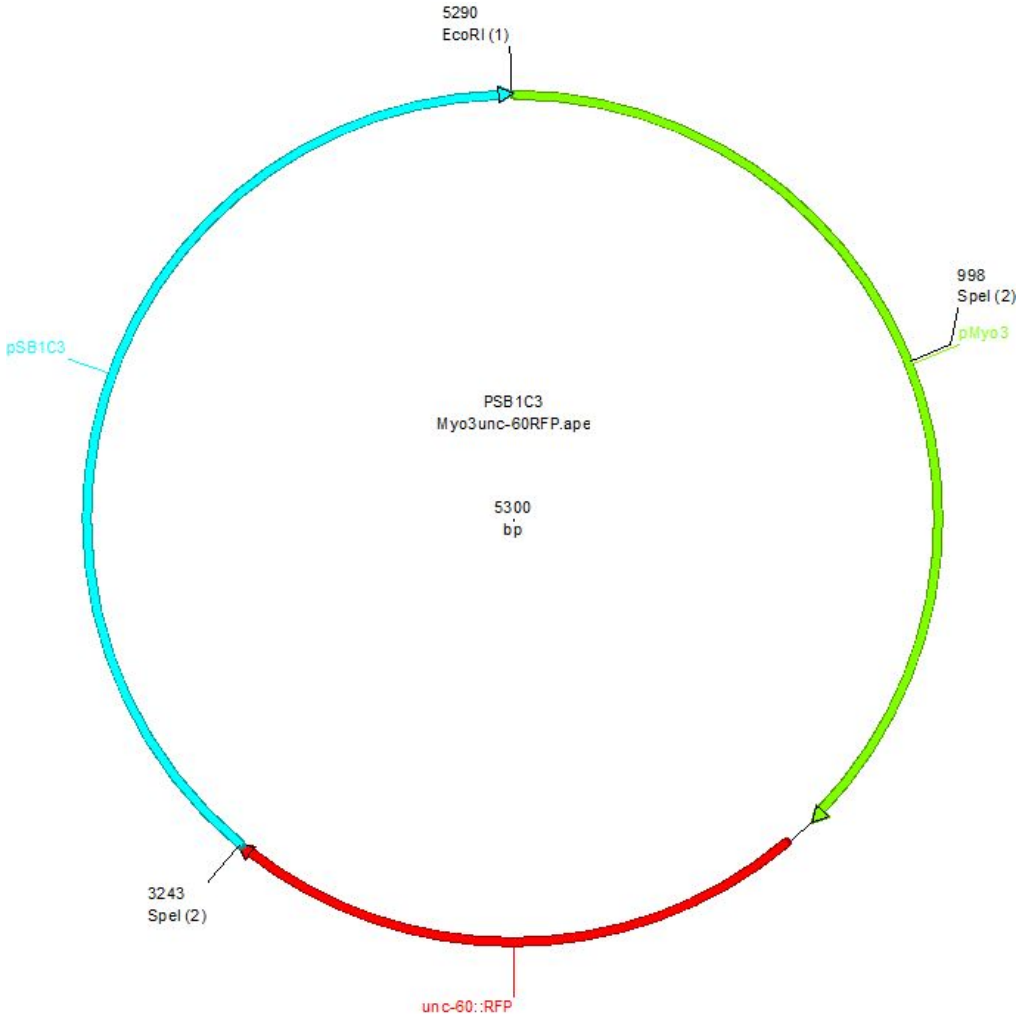
Results:



It seems like the mutagenesis on pMYO3 worked. A digestion by ?? will be done in order to verify the mutation.
The fusion will be done again on WC2.

17/08/2017

plasmid view:



After plasmid purification of pMyo-3::unc-60::RFP in pSB1C3 backbone (5300pb), our result of 33ng/µL which is low could be more due of one contaminant (260/230=4,06). One digestion is necessary to confirm or not our correct plasmid purification.

We decided to digest with EcoRI alone to open our plasmid and EcoRI+SpeI in order to obtain less dna fragments (3 fragments)

Protocol:

1°)Mix of digestion EcoRI alone

-Restriction enzymes……....1µL

-EcoRI……...1µL

-10XNeb Buffer(cutsmart)…….5µL
-DNA (1µg)……..30,3µL
Total Rxn Volume (50µL) qsp H2O.....13,7µL

2°)Incubation time at 37°C

3°) Inactivate at 80°C (EcoRI=65°C)

33ng/µL in 100µL miniprep stock……..3300ng/100µL=3,3µg/100µL
Mix of digestion…….50µL total
DNA concentration of mix……...20ng/µL

In order to observe DNA spots on our gel, we should to charge 0,5µg to1µg.
40µL of digestion mix……...800ng of dna is the correct quantity

1bis°)Mix of digestion EcoRI+SpeI
-Restriction enzymes……....2µL
    -EcoRI……...1µL
    -SpeI……..1µL

-10XNeb Buffer(cutsmart)…….5µL
-DNA (1µg)……..30,3µL
Total Rxn Volume (50µL) qsp H2O.....12,7µL

2bis°)Incubation time at 37°C

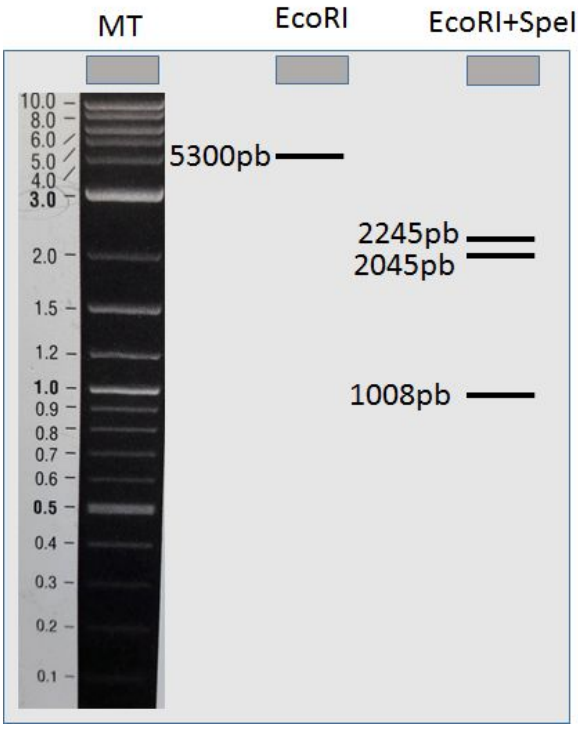
3bis°) Inactivate at 80°C (EcoRI=65°C and **SpeI=80°C**°)

33ng/µL in 100µL miniprep stock……..3300ng/100µL=3,3µg/100µL
Mix of digestion…….50µL total
DNA concentration of mix……...20ng/µL

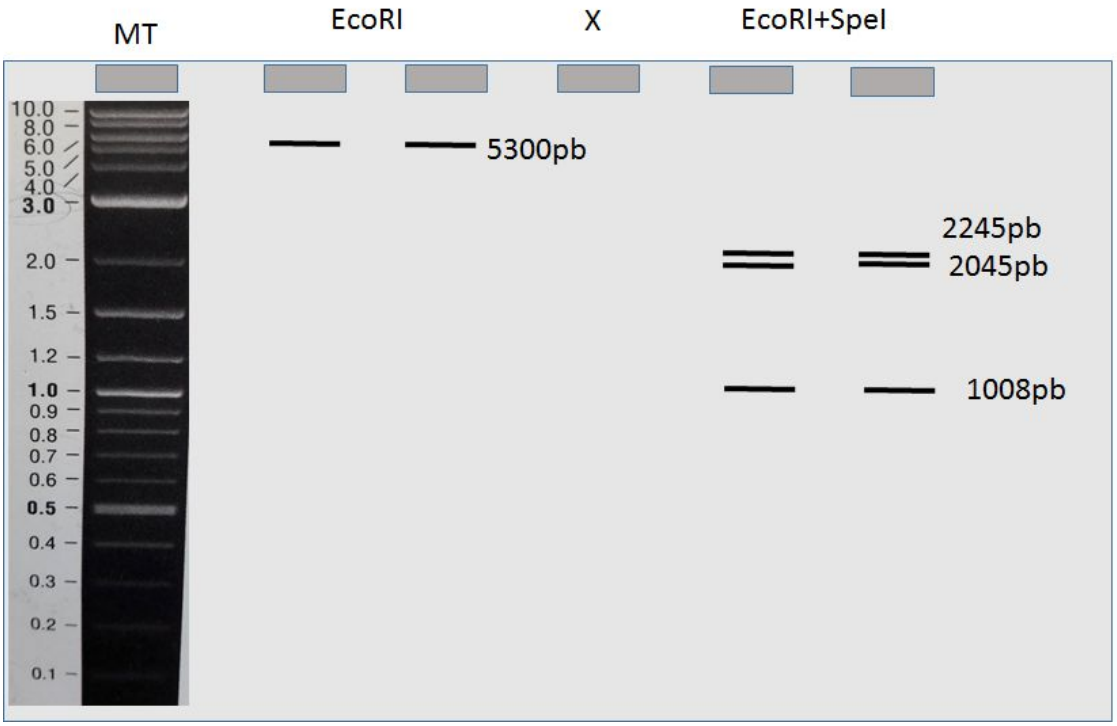
In order to observe DNA spots on our gel, we should to charge 0,5µg to1µg.
40µL of digestion mix……...800ng of DNA is the correct quantity

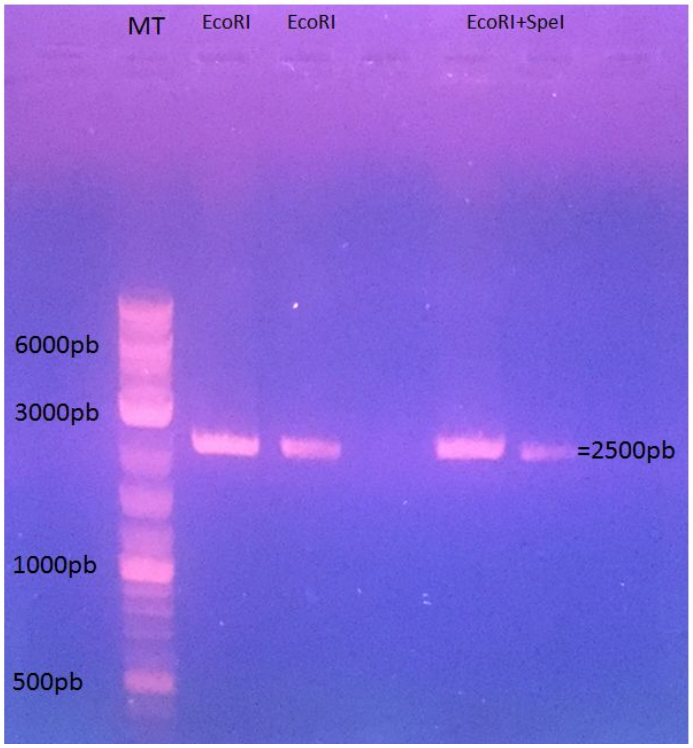
quantity of dna in miniprep stock after digestions and nanodrop
…….1267,2µg of dna in 38,4µL

theoretical gel:



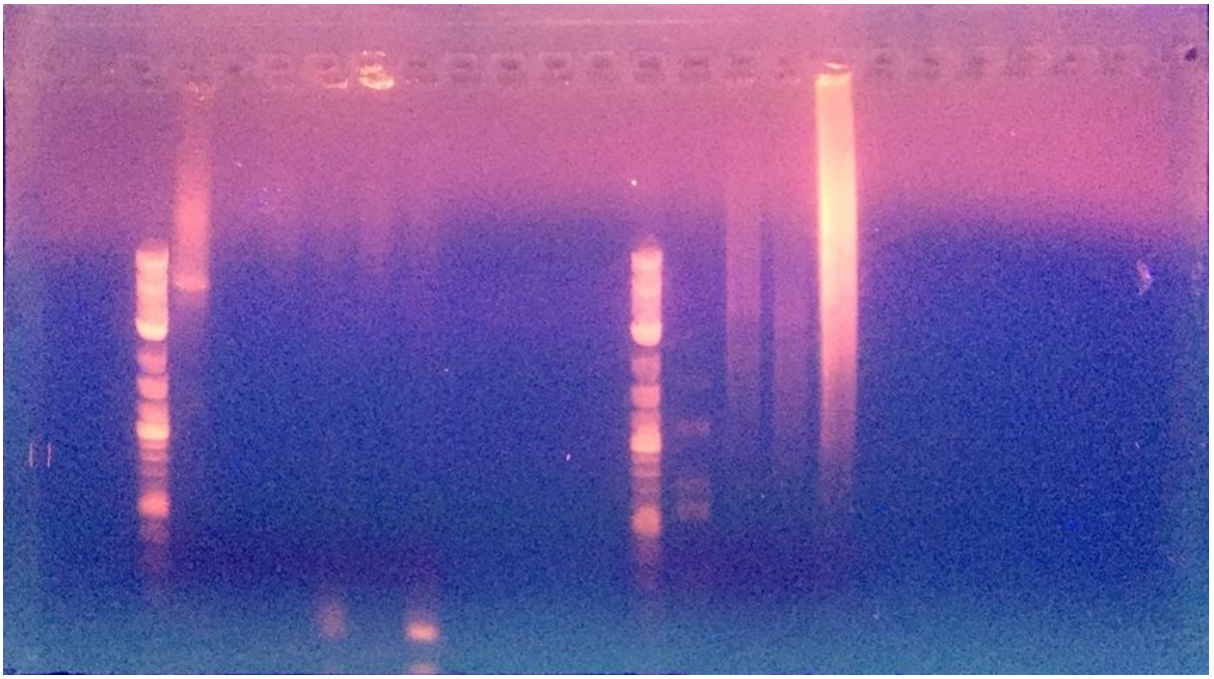
Results: We duplicated EcoRI and EcoRI+SpeI wells due to the fact that we could not charge 44µL in one well but just 25 to 30µL. More we charged diffrent quantity of DNA in order to observe if one little quantity could be detect.





We didn't have the expectations results but in the two cases one spot of DNA with a size of 2500pb. To conclude, we extracted dna and probably our pSB1C3 plasmid but without our fragment of interest which is strange because of our PCR on colony that worked and present or correct fragment

Results PCR fusion WC1

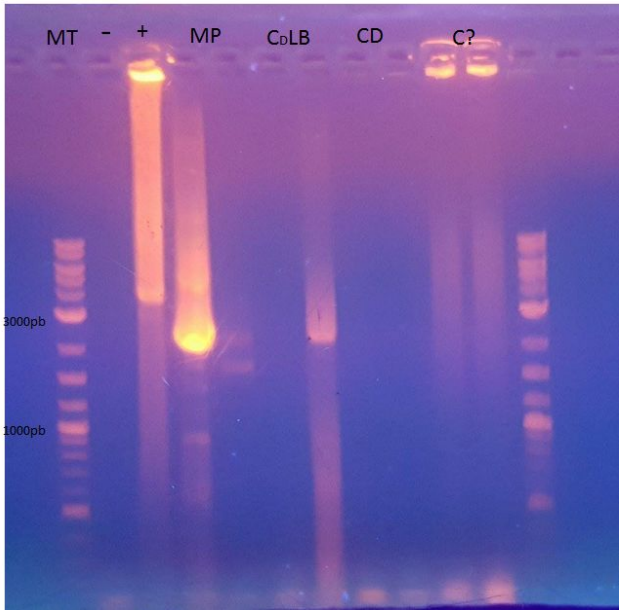


partie droite

Digestion of Myo3

18/08/2017

Many PCR to control one more time our colonies obtained with aquacloning in order to have pMyo3::unc-60::RFP in pSB1C3.



-: negative control
+:positive control
MP: Miniprep DNA extracted with our colony which present one desire spot of DNA -PCR on colony
CDLB: colony D in LB
CD:Colony D used as matrix a nother time
C?: new colony tested

We can observe no DNA amplified in our negative control but one huge band for the positive control which confirm that our PCR conditions are necessary to amplify our desire fragment. We have one spot in well corresponding of the Miniprep DNA matrix tested but with a no desire size (2500pb<3250pb). We could conclude that we amplified something more which is not our fragment. We have exactly the same result the same colony in LB.
Colony tested is a wrong positive.

pSB1C3 digestion

In july we digested a first time pSB1C3 with EcoRI in order to open the plasmid and to have one fragment and with SpeI and XbaI to have the pSB1C3 backbone.

Protocol used to digest 5µg of pSB1C3 miniprep plasmid:

-Restriction enzyme……...10µL
-XbaI……...5µL
-SpeI……...5µL

-DNA (pSB1C3 stock: (321,4ng/µL).....15,56µL
-10XNEB Buffer (cut smart).....25µL
-qsp H2O…….199,44µL

One more digestion should to be done to show if our plasmid is really open.

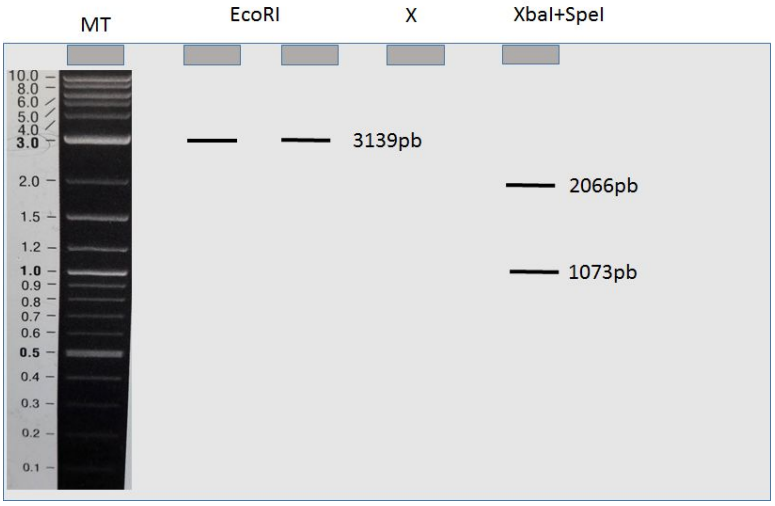
Protocol:

Mix of digestion EcoRI

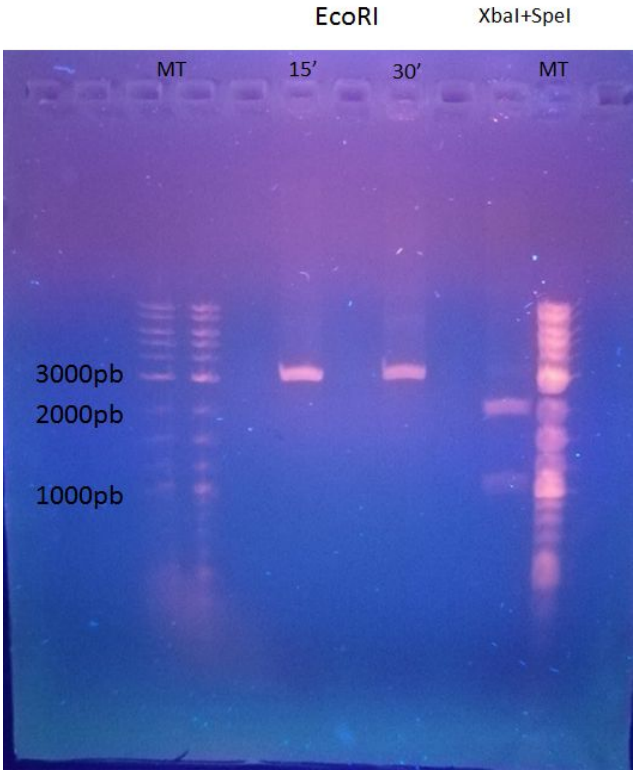
-Restriction enzymes……....1µL
-EcoRI……...1µL

-10XNeb Buffer(cutsmart)…….5µL
-DNA (1µg)……..3,11µL
Total Rxn Volume (50µL) qsp H2O.....40,89µL

theoretical gel:



<u>results:</u>



We have the expectation results with one spot of approximately 3000pb after EcoRI digestion which correspond of the size of pSB1B3 backbone plus RFP (3139pb). And two spots with a size of 2000pb and 1000pb which correspond of our pSB1C3 backbone alone and the RFP fragment alone. We can conclude that if our aquaclonings didn't work, it is not about the fact that pSB1C3 mimiprep digestion was not done.

PCR to amplify more pSB1C3 backbone:

One PCR mix were done for 5 PCR of 50µL

For one mix:

H2O	167,5µL
5x Buffer Phusion	50µL
10nM dNTP	5µL
10µ M Forward	12,5µL
10 µM reverse	12,5µL
DNA polymerase	2,5µL

Tube 1=H2O

Tube 2= 25ng of pSB1C3 backbone matrix was add (Igem stock)

Tube 3=25ng of pSB1C3 backbone matrix was add (Igem stock)

Tube 4=approximately 25ng of pSB1C3 matrix was add (PCR tube stock)

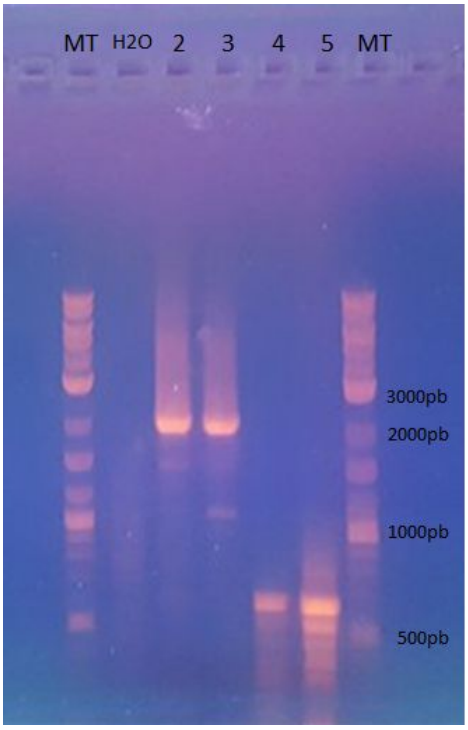
Tube 5=approximately 25ng of pSB1C3 matrix was add (PCR tube stock)

Tm: 60°C

Size=2070pb

Elongation time=1’05

Results:



pSB1C3 was amplified in wells number 2 and 3 with linear pSB1C3 IGEM stock matrix but not with PCR product in wells 4 and 5 which could works because of the same matrix. We obtained in this cases one spot with a size of approximately 600pb which could be dimers of primers which binds together and form many size like 600pb (one ladder).One last PCR should be done to obtain our final stock of linear pSB1C3 in order to do aquacloning.

21/08/2017

Program : 1- PCR to amplify more pSB1C3 2- Aquacloning + cloning with restriction enzymes

PCR to amplify more pSB1C3 backbone:
One PCR mix were done for 5 PCR of 50µL

For one mix:

H2O	167,5µL
5x Buffer Phusion	50µL
10nM dNTP	5µL
10µ M Forward	12,5µL
10 µM reverse	12,5µL
DNA polymerase	2,5µL

Tube 1=H2O
Tube 2= 25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 3=25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 4=25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 5=25ng of pSB1C3 backbone matrix was add (Igem stock)
Tm: 60°C
Size=2070pb
Elongation time=1’05
Results:

We purified PCR products with qiagen PCR purification quit in 100µL of water (for the aquaclonings!!!)

PCR to amplify pSB1A3 backbone:

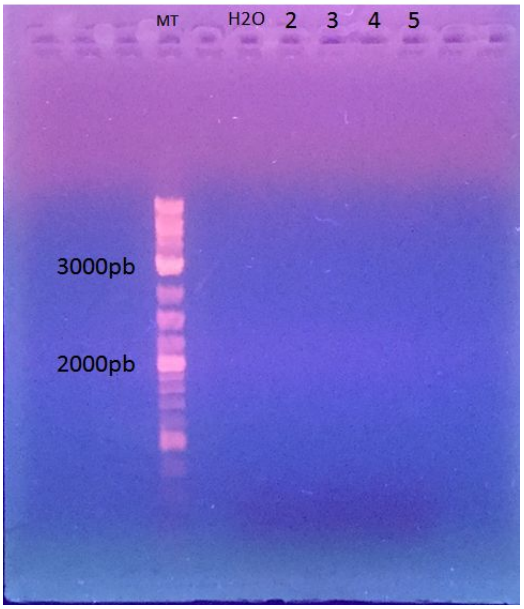
One PCR mix were done for 5 PCR of 50µL

For one mix:

H2O	72µL
5x Buffer Phusion	20µL
10nM dNTP	2µL
10µ M Forward	5µL
10 µM reverse	5µL
DNA polymerase	1µL

Tube 1=H2O
Tube 2= 25ng of pSB1A3 backbone matrix was add (Igem stock)
Tube 3=25ng of pSB1A3 backbone matrix was add (Igem stock)
Tube 4=25ng of pSB1AK backbone matrix was add (Igem stock)
Tube 5=25ng of pSB1K3 backbone matrix was add (Igem stock)
Tm: 64°C
Size=2155pb
Elongation time=1'10''

Results:



No results!

Aquacloning of Myo-3::unc-60::GFP (5432pb) and Myo-3::unc-60::RFP (3273pb)

	pSB1C3 backbone (PCR)	DNA of intersest

Negativ control	10µL water	none
Positiv control	5µL dna stock and 5µL of water	none
Myo-3::unc-60::GFP 25,5nM 3:1	5µL of diluted pSB1C3 (0,625µL stock +4,375H20)	5µL stock without dilution
Myo-3::unc-60::GFP 25,5nM 5:1	5µL of diluted pSB1C3 (1µL stock +13H20)	5µL stock without dilution
Myo-3::unc-60::RFP 30nM 3:1	5µL of diluted pSB1C3 (1,85µL stock +3,15H20)	5µL stock with 2X dilution (3,72µLDNA stock and 6,28µL of water)
Myo-3::unc-60::RFP 5:1	5µL of diluted pSB1C3 (1µL stock +3H20)	5µL stock with 2X dilution 40,25,nM

Purification pMYO3 and WC2

In order to do the aquacloning, the fragment obtained thanks to the PCR fusion are purified.
The purifciation was done with the kit Quiagen.
At the end of the purification, we have :

	Size (bp)	ng/µl	260/280	260/230	nM
WC2-mut	1902	138,4	1,86	2,01	112,12
pMYO3-mut	1985	122,5	1,82	1,69	95,09

Aquacloning

Mix for the incubation for 5:1

Mix	Backbone	Insert
pSB1A3 + WC2	5 µl without dilution	4 µl WC2 + 1µl H20
pSB1C3 + WC2	1,6 µl plasmid + 3µl H20	5 µl without dilution
pSB1A3 + SUP12	1,53 µl plasmid + 3,47 µl H20	5 µl without dilution
pSB1C3 + SUP12	5 µl without dilution	2,55 µL SUP12 + 2,45µl H20

The transformation is done with the same condition as usual.

22/08/2017:

Tableau : ... ?- PCR of WC1

Results transformation

	LB + chloramphénicol	LB + Ampicilline
SUP12	14	23
ASD2	8	1
pMYO3	4	11
pUNC119	0	2
WC1	0	1
WC2	2	8

PCR Colony ASD2, MYO3, UNC119, WC2

We test 4 colony at max for each petri dish.
Primer:

- Prefixe forward
- Suffixe reverse

Mix:

Buffer	230 µl
dNTP	23 µl
Forward	57,5 µl
Reverse	57,5 µl
Phusion	11,5 µl
H2O	770,5 µl

All the bacteria tested are ensemenced in LB + Cloramphenicol or Ampicillin.

In order to clone unc-60::GFP and unc-60::RFP fragments of approximately 5,5kpb and 3,5Kpb in pSB1C3, we digest them and pSB1C3 with EcoRI and SpeI.

unc-60::GFP digestion

Protocol used to digest 500ng of unc-60::GFP produced with PCR (8/08):

-Restriction enzyme……...2µL

-EcoRI……..1µL
-SpeI……..1µL

-DNA (unc-60::GFP stock: (345,2ng/µL).....1,45µL
-10XNEB Buffer (cut smart).....5µL
-qsp H2O…….41,55µL

unc-60::RFP digestion

Protocol used to digest 500ng of unc-60::GFP produced with PCR (8/08):

-Restriction enzyme……...2µL
-EcoRI……...1µL
-SpeI……..1µL

-DNA (unc-60::RFP stock: (92,8ng/µL).....5,39µL
-10XNEB Buffer (cut smart).....5µL
-qsp H2O…….37,61µL

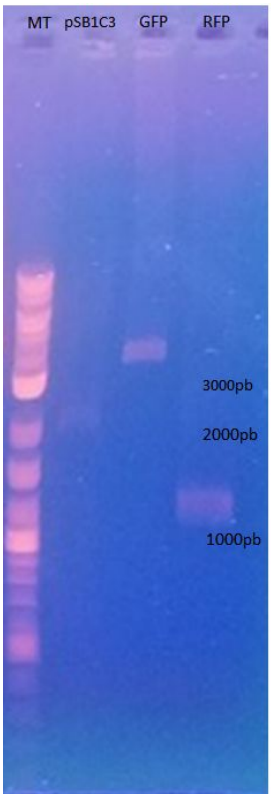
pSB1C3 backbone (PCR product) digestion

Protocol used to digest 500ng produced with PCR (20 and 21/08):

-Restriction enzyme……...2µL
-EcoRI……...1µL
-SpeI……..1µL

-DNA (unc-60::RFP stock: (93,9ng/µL).....5,33µL
-10XNEB Buffer (cut smart).....5µL
-qsp H2O…….37,67µL

Gel control to confirm that dna was digested:



Ligation of unc-60::GFP and pSB1C3:

fragment digested (unc-60::GFP) :.....2µL
pSB1C3 digested:.....2µL
10X T4 DNA Ligase Buffer……...2µL
T4 DNA ligase……1µL
H20……...11µL

1°) Incubate at room temperature for 10minutes and then then heat inactivate at 80°C for 20 minutes.
2°) Transform 2µL of the ligation product into 50µL of competent E.coli cells

Ligation of unc-60::RFP and pSB1C3:

fragment digested (unc-60::RFP) :.....2µL
pSB1C3 digested:.....2µL
10X T4 DNA Ligase Buffer……...2µL
T4 DNA ligase……1µL
H20……...11µL

1°) Incubate at room temperature for 10minutes and then then heat inactivate at 80°C for 20 minutes.
2°) Transform 2µL of the ligation product into 50µL of competent E.coli cells

E.coli DH5alpha transformed with ligation products:

1°)2µL of ligation produts were added with 25µL of bacteria
2°)30min at 4°C
3°C 45 seconds at 42°C
4°) 5min at 4°C
5°) 250µL of LB was added and bacteria were incubated 1 hour at 37°C and 200 RPM
6°) Bacteria were rolled out petri dish with chloramphenicol and incubated overnight at 37°C

PCR to amplify more pSB1C3 and pSB1A3 backbones:

One mix was made for 10 PCR.
tube H20: /
Fort pSB1C3 backbone:

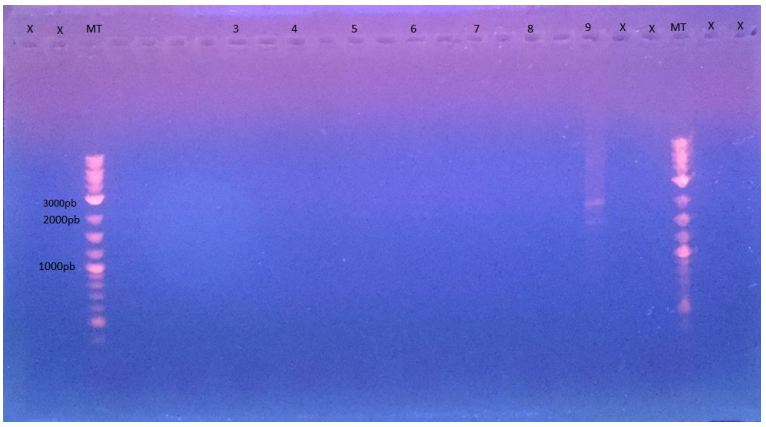
elongation time= 1'10''

tube 1:pSB1C3
tube 1: Tm= 59,8°C
tube 2: Tm= 59,8°C
tube 3: Tm= 60,2°C

tube2:pSB1C3
tube 4: Tm= 60,2°C
tube 5: Tm= 60,2°C
tube 6: Tm= 59,8°C

For pSB1A3 backbone:
tube 7:Tm= 58,1°C
tube 8:Tm= 59,4°C
tube 9:Tm= 60,6°C

Results:



We amplified just pSB1A3 but with a little spot!!
But why no amplification for pSB1C3!! we used the same conditions but just the Tm was changed of 0,2°C!!
Aquacloning Nancy

DIGESTION

psB1C3 avec Ecor1 et pst1

500 ng de pSB1C3	5,33 uL
Ecor1	1 uL
Pst1	1 uL
Cutsmart	5 uL
H20 (50 ul qsp)	37,67 uL

Myo3 avec Ecor1 et Pst1

500 ng de Myo3	4,08 uL
Ecor1	1 uL
Pst1	1 uL
Cutsmart	5 uL
H20	38,92 uL

pSB1C3 avec Xba1 et pst1

500 ng de pSB1C3	5,33 uL
Xba1	1 uL
Pst1	1 uL
Cutsmart	5 uL
H20 (50 uL qsp)	37,67 uL

unc119 avec Xba1 et pst1

500 ng de unc119	10,68 uL
Xba1	1 uL
pst1	1 uL
Cutsmart	5 uL
H20 (50 uL qsp)	32,32 uL

1) Incubation time at 37° for 15 minutes
2) Inactivation at 80° for 20 minutes

LIGATION

Ligation of Myo3 and pSB1C3:

fragment digested (Myo3) :.....3µL
pSB1C3 digested:.....3µL
10X T4 DNA Ligase Buffer……...2µL
T4 DNA ligase……1µL
H20……...11µL

1°) Incubate at room temperature for 10 minutes and then heat inactivate at 80°C for 20 minutes.
2°) Transform 2µL of the ligation product into 50µL of competent E.coli cells

Ligation of unc119 and pSB1C3:

fragment digested (unc119) :.....3µL
pSB1C3 digested:.....3µL
10X T4 DNA Ligase Buffer……...2µL
T4 DNA ligase……1µL
H20……...11µL

1°) Incubate at room temperature for 10 minutes and then heat inactivate at 80°C for 20 minutes.
2°) Transform 2µL of the ligation product into 50µL of competent E.coli cells

?- PCR on WC1

MIX 50µL :

H2O	67 µL
5x Buffer Phusion	20 µL
10nM dNTP	2µL
10µ M Forward	5µL
10 µM reverse	5µL
DNA polymerase	1µL

Hybridation temperature : 55°C
Elongation time : 1’50

Result :

PCR on colony

Sup12

Boite Am : 5 tubes of PCR and ; Boite Cm : 5 tubes of PCR ; and one control (20 uL)

Composant	
5x Phusion GC Buffer	44 uL
dNTPs	4,4 uL
Suffixe (Primer : Reverse)	11 uL
Prefixe (Primer :Foward)	11 uL
Phusion	2,2 uL
H20 (qsp uL)	147,4 uL

™ = 60°

Te = 20''

WC1

Boite : 1 tub of PCR and one control

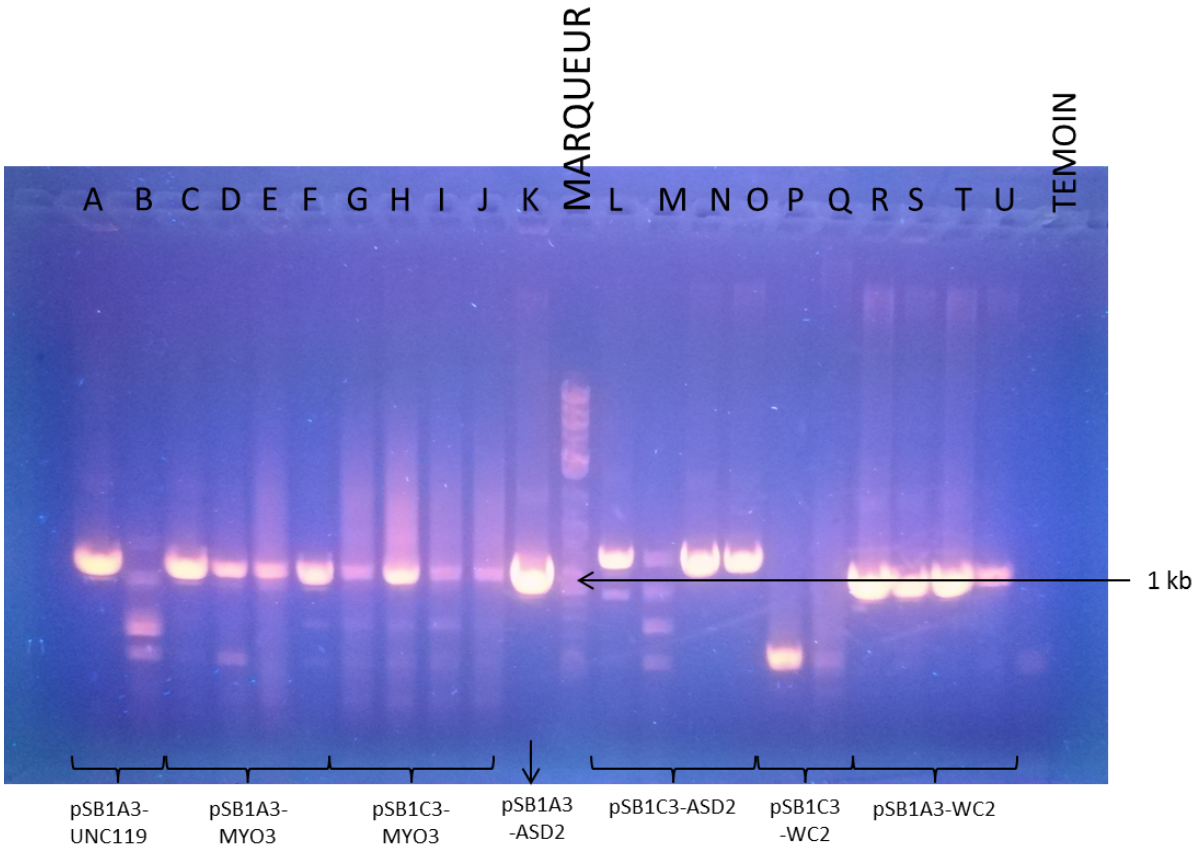
Composant	
5x Phusion GC Buffer	8 uL
dNTPs	0,8 uL
Suffixe (Primer : Reverse)	2 uL
Prefixe (Primer :Foward)	2 uL
Phusion	0,4 uL
H20 (qsp uL)	26,8 uL

™ = 60°

Te = 1'45''

23/08/2017:

Results PCR colony UNC119, MYO3, WC2 et ASD2



The fragment we obtained seems to be at the right size. except for B, M, P and Q. But a miniprep will be done on all the clones.

Miniprep

The condition are the same as usual with the kit GenElute ™ Plasmid Miniprep Kit, Sigma.

Results (Nanodrop)

Clone	Concentration (ng/µl)	260/280	260/230
A	46,5	1,94	1,96
B	36,8	1,94	1,99
C	60,6	1,94	2,10

D	90,5	1,90	2,14
E	37,0	2,01	1,26
F	107,4	1,86	2,16
G	28,5	1,93	1,92
H	40,5	1,94	2,13
I	26,4	1,92	2,00
J	38,3	1,90	1,84
K	64,3	1,92	2,13
L	32,5	1,95	2,16
M	34,8	1,97	2,11
N	45,3	1,95	2,14
O	48,6	1,92	1,99
P	6,5	2,20	1,44
Q	32,1	1,96	2,02
R	70,6	1,90	2,05
S	5,6	2,09	1,12
T	60,9	1,94	2,20
U	52,6	1,92	2,02

The clone P and S are discard due to their concentration and their rapport.

PCR on colony (ligation unc-60::RFP)

Protocols

	20 uL	6 tubes x 20 uL (uL)
H2O	14,4 uL	100,8 uL
5x Buffer Phusion	4 uL	28 uL
10nM dNTP	0,4 uL	2,8 uL

10µM Myo3 Forward	1 uL	7 uL
10 µM Unc60 Reverse	1 uL	7 uL
Phusion DNA polymerase	0,2 uL	1,4 uL

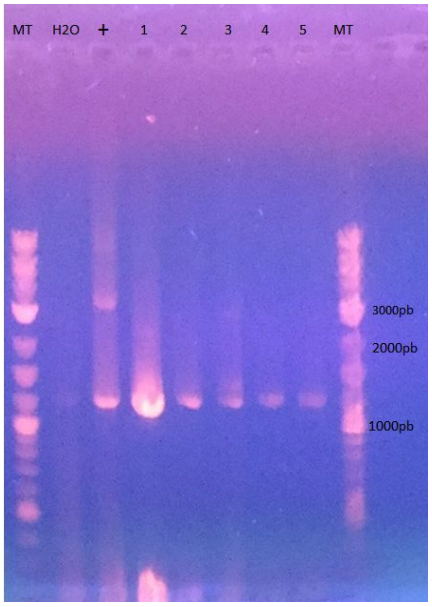
unc-60::RFP:
Size =1217 pb
Elongation time= 1 min
Tm = 54°C

PCR was made for 7 PCR with 20µL/tube (5 PCR with bacteria, one H2O control and one positive control (pmyo3::unc-60::RFP matrix) with the rest of the mix)

Preparation of 4 bacteriological culture tubes :

- 8uL of chloramphenicol antibiotic for each tube.
- 8 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

Results:



We obtained one positive result with our control and for all colonies one strand with the good size of 1200pb. To conclude, unc-60::RFP was cloned in pSB1C3. We have to purify this plasmid with a miniprep quit.

One new aquacloning was made because of no colonies on medium during the night
Aquacloning of Myo-3::unc-60::GFP (5432pb) and Myo-3::unc-60::RFP (3273pb)

	pSB1C3 backbone (PCR)	DNA of intersest

Negativ control	10µL water	none
Positiv control	5µL dna stock and 5µL of water	none
Myo-3::unc-60::GFP 25,5nM 3:1	5µL of diluted pSB1C3 (0,625µL stock +4,375H20)	5µL stock without dilution
Myo-3::unc-60::GFP 25,5nM 5:1	5µL of diluted pSB1C3 (1µL stock +13H20)	5µL stock without dilution
Myo-3::unc-60::RFP 30nM 3:1	5µL of diluted pSB1C3 (1,85µL stock +3,15H20)	5µL stock with 2X dilution (3,72µLDNA stock and 6,28µL of water)
Myo-3::unc-60::RFP 5:1	5µL of diluted pSB1C3 (1µL stock +3H20)	5µL stock with 2X dilution 40,25,nM

24/08/2017:

Digestion of miniprep

What do we expect :

- pMYO3 → SpeI : 2 fragments at 1000 and 3000 bp.
- ASD2 → PstI and XbaI: 2 fragments at 2033 and 1263 bp.
- pUNC119 → EcoRI : 3 fragments at 1100, 400 and 2500 bp.
- WC2 → PstI : 2 fragments at 1885 and 2044 bp.

Mix for pMYO3:

For 1 tube (µl)		For 10 tubes (µl)
1	SpeI	10
5	Buffer Cut Smart	50
5	DNA (1µg)	
39	Water qsp 50 µl	390

Mix for ASD2

For 1 tube (µl)		For 7 tubes (µl)
1	PstI	7
1	XbaI	7
5	Buffer CutSmart	35
33	Water qsp 50 µl	231
10	DNA (1 µg)	

Mix for pUNC119

For 1 tube (µl)		For 4 tubes (µl)
1	EcoRI	4
5	Buffer Cutsmart	20
34	Water qsp 50 µl	204
10	DNA (1µg)	

Mix for WC2

For 1 tube (µl)		For 6 tubes (µl)
1	EcoRI	6
5	Buffer	30
34	Water qsp 50µl	204
10	DNA (1µg)	

Results

PCR on colony (ligation unc-60::GFP)X3 colonies and pMyo3::unc-60::RFP (aqua)X1 conony

Protocols for 4PCR and two H2O control= X6 mix of 20µL per tube

	20 µL	6 tubes x 20 µL (µL)
H2O	14,4 µL	86,4 µL
5x Buffer Phusion	4 µL	24 µL
10nM dNTP	0,4 µL	2,4 µL
10µM Myo3 Forward	1 µL	2µL
10 µM Unc60 Reverse	1 µL	2 µL
Phusion DNA polymerase	0,2 µL	1,4 µL

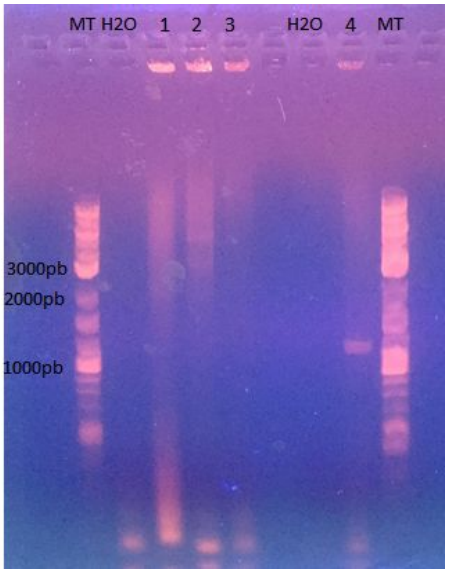
10µM unc-60 Forward	1 µL	2 µL
10 µM Unc60 Reverse	1 µL	2uL

pMyo3::unc-60::RFP:
Size =3273 pb
Elongation time= 2 min
Tm = 54°C
unc-60::GFP:
Size =3376 pb
Elongation time= 3 min 30 sec
Tm = 54°C

PCR was made for 6 PCR with 20µL/tube (4 PCR with bacteria, Two H2O control
Preparation of 5 bacteriological culture tubes :

- 5µL of chloramphenicol antibiotic for each tube.
- 5 mL of *Lysogeny broth* for each tube.
- Colony of bacteria
- Tubes are incubated overnight at 37°C.

Results:



we obtained no results for pMyo3::unc-60::RFP but one positif result for unc-60::GFP but with one abnormal size of 1200pb < 3376pb. We have to do a nother transformation with unc-60::GFP.

Aquacloning of Myo-3::unc-60::GFP (5432pb) and Myo-3::unc-60::RFP (3273pb)

	pSB1C3 backbone (PCR)	DNA of intersest

Negativ control	10µL water	none
Positiv control	5µL dna stock and 5µL of water	none
Myo-3::unc-60::GFP 25,5nM stock 3:1	5µL of diluted pSB1C3 (0,625µL stock +4,375H20)	5µL stock without dilution
Myo-3::unc-60::RFP 80,5nM stock 3:1	5µL of diluted pSB1C3 (1,85µL stock +3,15H20)	5µL stock with 3X dilution (2µL DNA stock and 4µL of water)

Transformation of unc-60::GFP in pSB1C3:

1°)2µL of ligation produts were added with 25µL of bacteria
2°)30min at 4°C
3°C 45 seconds at 42°C
4°) 5min at 4°C
5°) 250µL of LB was added and bacteria were incubated 1 hour at 37°C and 200 RPM
6°) Bacteria were rolled out petri dish with chloramphenicol and incubated overnight at 37°C

Transformation of pMyo3::unc-60::GFP in plasmid of Jean in pSB1C3:

1°)2µL of ligation produts were added with 25µL of bacteria
2°)30min at 4°C
3°C 45 seconds at 42°C
4°) 5min at 4°C
5°) 250µL of LB was added and bacteria were incubated 1 hour at 37°C and 200 RPM
6°) Bacteria were rolled out petri dish with **chloramphenicol!!!!** and incubated overnight at 37°C

This plasmid has one ampicillin resistance. We have to do one transformation one more time!

Transformation of pMyo3::unc-60::RFP in plasmid of Jean in pSB1C3:

1°)2µL of ligation produts were added with 25µL of bacteria
2°)30min at 4°C
3°C 45 seconds at 42°C
4°) 5min at 4°C
5°) 250µL of LB was added and bacteria were incubated 1 hour at 37°C and 200 RPM
6°) Bacteria were rolled out petri dish with **chloramphenicol!!!** and incubated overnight at 37°C

This plasmid has one ampicillin resistance. We have to do one transformation one more time!

PCR to amplify more pSB1C3 backbone:

One PCR mix were done for 10 PCR of 50µL

For one mix:

H2O	335µL
5x Buffer Phusion	100µL
10nM dNTP	10µL
10µ M Forward	25µL
10 µM reverse	25µL
DNA polymerase	5µL

Tube 1=H2O

Tube 1 pSB1C3 igem stock:

Tube 2= 25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 3=25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 4=25ng of pSB1C3 backbone matrix was add (Igem stock)

Tube 2 pSB1C3 igem stock:

Tube 6= 25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 7=25ng of pSB1C3 backbone matrix was add (Igem stock)
Tube 8=25ng of pSB1C3 backbone matrix was add (Igem stock)

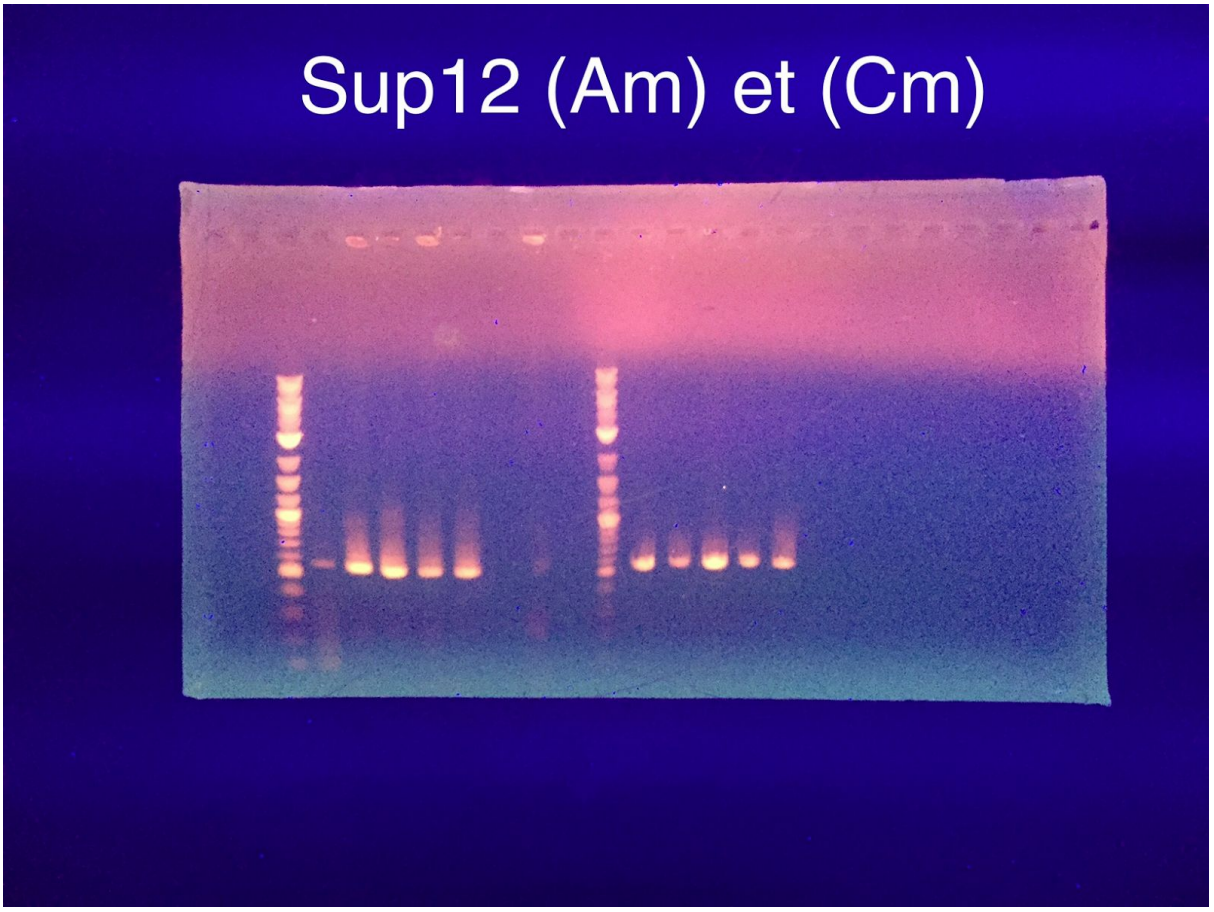
pSB1C3 Miniprem stock:

Tube 9=approximately 321,4ngof pSB1C3 matrix was add
Tube 9=approximately 321,4ng of pSB1C3 matrix was add
Tube10=approximately321,4 ng of pSB1C3 matrix was add

Tm: 60°C
Size=2070pb
Elongation time=1'10

Results:

PCR on colony of sup12 (Am) and sup12 (Cm)



Results

- **Contamination of the control**
- **Amplification**

Plasmid Miniprep : Sup12 in PSB1C3 backbone and PSB1A3 backbone

1 - Harvest and lyse bacteria

- Pellet cells from 1- 5 mL overnight culture 1 minute (1 mL from TB or 2xYT ; 1-5 mL from LB medium). Discard supernatant.
- Resuspend cells in 200 uL Resuspension Solution. Pipette up and down or vortex.
- Add 200 uL of Lysis Solution. Invert gently to mix. Do not vortex. Allow to clear for 5 minutes.
- Prior to first time use, be sure to add the RNase A to the Resuspension Solution.

2 - Prepare cleared lysate

- Add 350 uL of Neutralization Solution (S3). Invert 4-6 times to mix.
- Pellet debris 10 minutes at max speed

3 - Prepare binding column

- Add 500 mL Column Preparation Solution to binding column in a collection tube.
- Spin at > 12,000 x g, 1 minute. Discard flow-through.

4 - Bind plasmid DNA to column

- Transfer cleared lysate into binding column.
- Spin 30 secondes, 1 minute. Discard flow-through.

5 - Wash to remove contaminants

- Optional (EndA+ strains only) : Add 500uL Optional Wash Solution to column. Spin 30 secondes, 1 minute. Discard flow-through.
- Add 750 uL Wash Solution to column. Spin 30 seconde, 1 minute. Discard flow-through.
- Spin 1 minute to dry column.
- Prior to first time use, be sure to add ethanol to the concentrated Wash Solution

6- Elute purified plasmid DNA

- Transfer column to new collection tube
- Add 50 uL or 30 uL Elution Solution. Spin 5 minutes.
- If a more concentrated plasmid DNA prep is required, reduce the elution volume to a
- minimum of 50 uL.

Nanodrop dosage of Sup12 in PSB1C3 backbone and PSB1A3 backbone

Tubes	ng/uL			260/280	260/230
1 : sup12 (Am) colony 1	30,2			2,04	4,17
2 : sup12 (Am) colony 2	76,4			1,96	2,76
3 : sup12 (Am) colony 3	71,8			1,97	2,87
4 : sup12 (Am) colony 4	66,8			1,98	2,94
5 : sup12 (Am) colony 5	58,5			1,96	3,00
6 : sup12 (Cm) colony 1	49,4			2,04	3,64

7 : sup12 (Cm) colony 2	64,4			1,98	2,69
8 : sup12 (Cm) colony 3	142,4			1,90	2,17
9 : sup12 (Cm) colony 4	59,2			2,02	3,09
10 : sup12 (Cm) colony 5	64,7			2,00	3,15

Digestion of Sup-12 minipreps:

Miniprep stock	**Volume of miniprep stock**	**Qsp H2O (50µL)**
1 : sup12 (Am) colony 1	16,55	26,45
2 : sup12 (Am) colony 2	6,54	36,46
3 : sup12 (Am) colony 3	6,96	36,04
4 : sup12 (Am) colony 4	7,49	35,51
5 : sup12 (Am) colony 5	8,55	34,45
6 : sup12 (Cm) colony 1	10,12	32,88
7 : sup12 (Cm) colony 2	7,76	35,24
8 : sup12 (Cm) colony 3	3,51	39,49
9 : sup12 (Cm) colony 4	8,45	34,55
10 : sup12 (Cm) colony 5	7,73	35,27

Protocol used to digest 500ng of unc-60::GFP produced with PCR (8/08):

-Restriction enzyme……...2µL
-EcoRI……...1µL
-PstI……..1µL

-DNA (unc-60::RFP stock: (92,8ng/µL).....XµL

-10XNEB Buffer (cut smart).....5µL
-qsp H2O……XµL

25/08/2017:

Mutagenesis PCR

Plasmid:

- clone D : pMYO3 + pSB1A3
- clone G : pMYO3 + pSB1C3
- clone H : pMYO3 + pSB1C3

Primer :

- MYO3 mut (2) Forward
- MYO3 mut (2) reverse

Mix :

Buffer	50 µl
dNTP	5 µl
Forward	12,5 µl
Reverse	12,5 µl
Water	167,5 µl

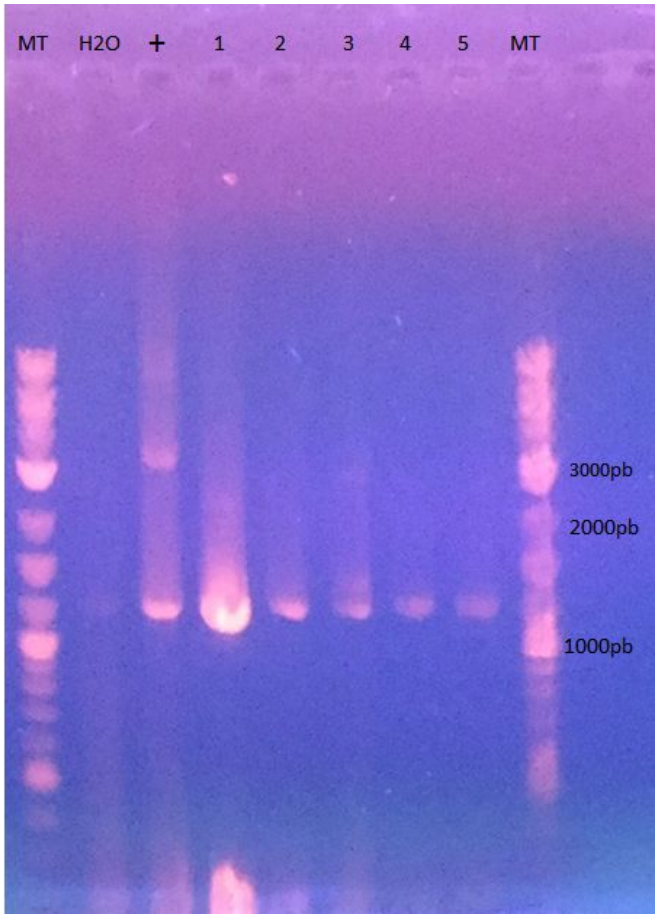
Tm = 60°C
Elongation time = 2'05''

Results

PCR on colonies unc-60::RFP

tube1 : negative control
tube2 : positive control
tube3 : colony 1
tube4 : colony 2
tube5 : colony 3
tube6 : colony 4
tube7 : colony 5

Results



Mimiprep of unc-60::RFP:

All minipreps were done with Sigma miniprep quit and elutions were done in 50µL of water.

Nanodrop dosage of Sup12 in PSB1C3 backbone:

tubes	ng/µL	260/280	260/230
1: unc-60::RFP	36,7	1,93	1,72
2: unc-60::RFP	22,8	1,95	1,57
3: unc-60::RFP	25,9	2,00	1,83
4: unc-60::RFP	26,2	1,96	1,66

5: unc-60::RFP	30,2	2,07	1,88

Mimiprep of Sup-12 in pSB1A3:

All minipreps were done with Sigma miniprep quit and elutions were done in 50µL of water.

tubes	ng/µL	260/280	260/230
sup-12 Am3	83,5	1,91	2,10
sup-12 Am4	78,5	1,93	2,15
sup-12 Am5	75,4	1,90	1,97

28/08/2017:

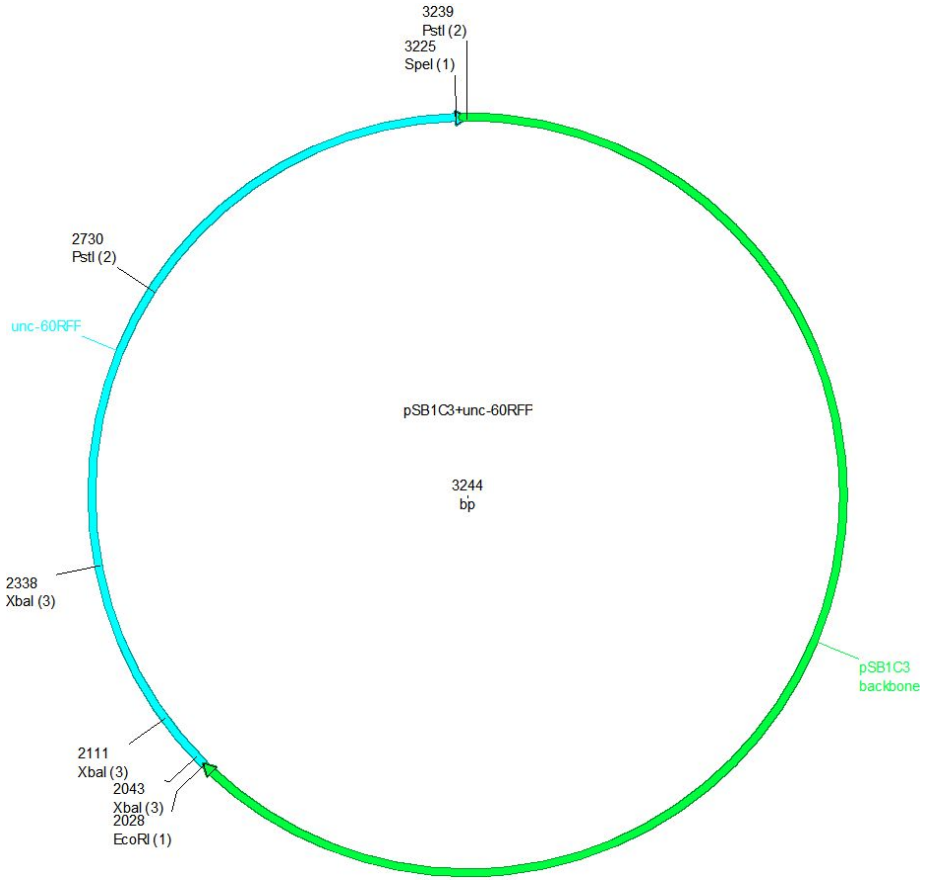
Aquacloning mutagenesis

The transformation is done with the same conditions as usual. We put 5 µl of DNA with the bacteria.
we spread the bacteria on :

- LB + chloramphenicol for H.
- LB + ampicillin for D.

Digestion to control if we have purified the plasmid of interest with unc-60::RFP part:

plasmid view:



Digestions were done with EcoRI alone to open the plasmid and PstI alone to cut inside the part unc-60::RFP and control if the part is present.

Protocol used to digest 300ng produced with PCR :

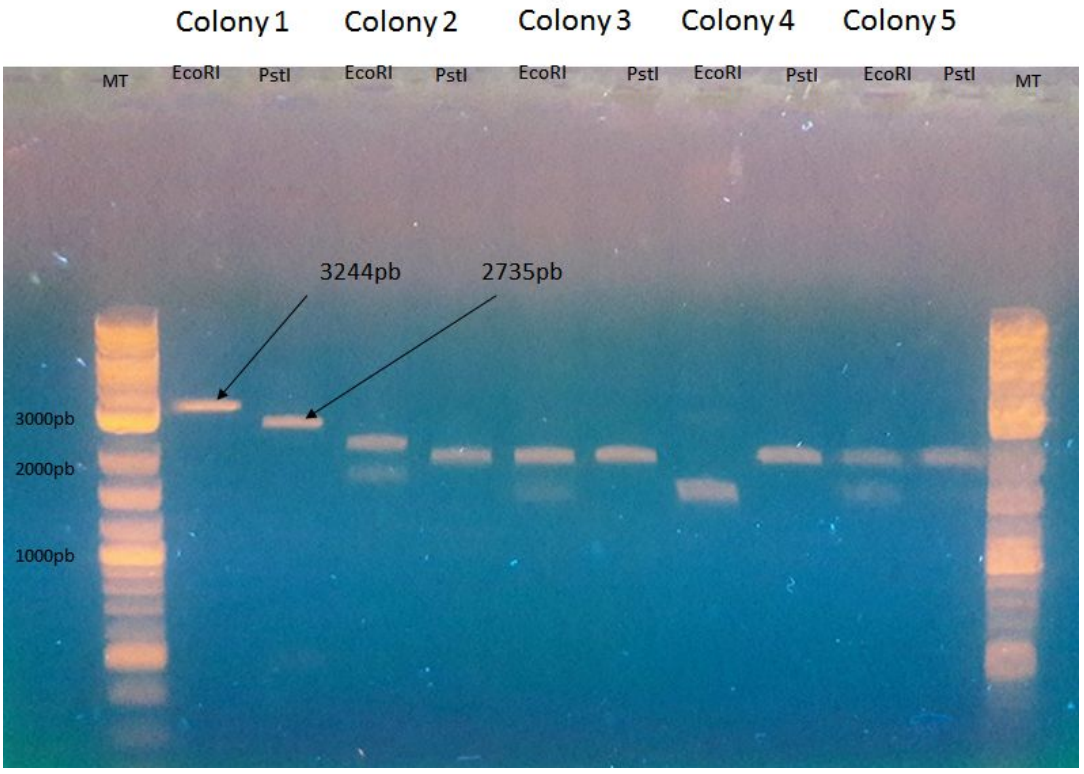
-Restriction enzyme……...1µL

-EcoRI……..0,5µL
-SpeI……..0,5µL

-DNA 300ng.....XµL
-10XNEB Buffer (cut smart).....2µL
-qsp H2O (20µL)…….XµL
1°) Digestion 1 hour at 37°C
2°) Inactivation 20 minutes at 80°C

	DNA miniprep stock	Volume stock to add
1: unc-60::RFP	36,7	8,17
2: unc-60::RFP	22,8	13,16
3: unc-60::RFP	25,9	11,58
4: unc-60::RFP	26,2	11,45
5: unc-60::RFP	30,2	9,93

<u>Gel control to confirm that dna was digested:</u>



PCR on colonies: Aquaclonings of pMyo3::unc-60::GFP, pMyo3::unc-60::GFP, ligation of unc-60::GFP and ligation of unc-60::RFP:

PCR ofpMyo3::unc-60::GFP (5432pb):
One PCR mix were done for 5 PCR of 20µL

For one mix:

H2O	72µL
5x Buffer Phusion	20µL
10nM dNTP	2µL
10µ M Forward	5µL
10 µM reverse	5µL
DNA polymerase	1µL

Tube 1=H2O
Tube 2= colony 1
Tube 3=colony 2
Tube 4=colony 3
Tube 5=positive control

Tm=54°C
Elongation time=3min
size=5432pb

PCR ofpMyo3::unc-60::RFP (3273pb):
One PCR mix were done for 5 PCR of 20µL

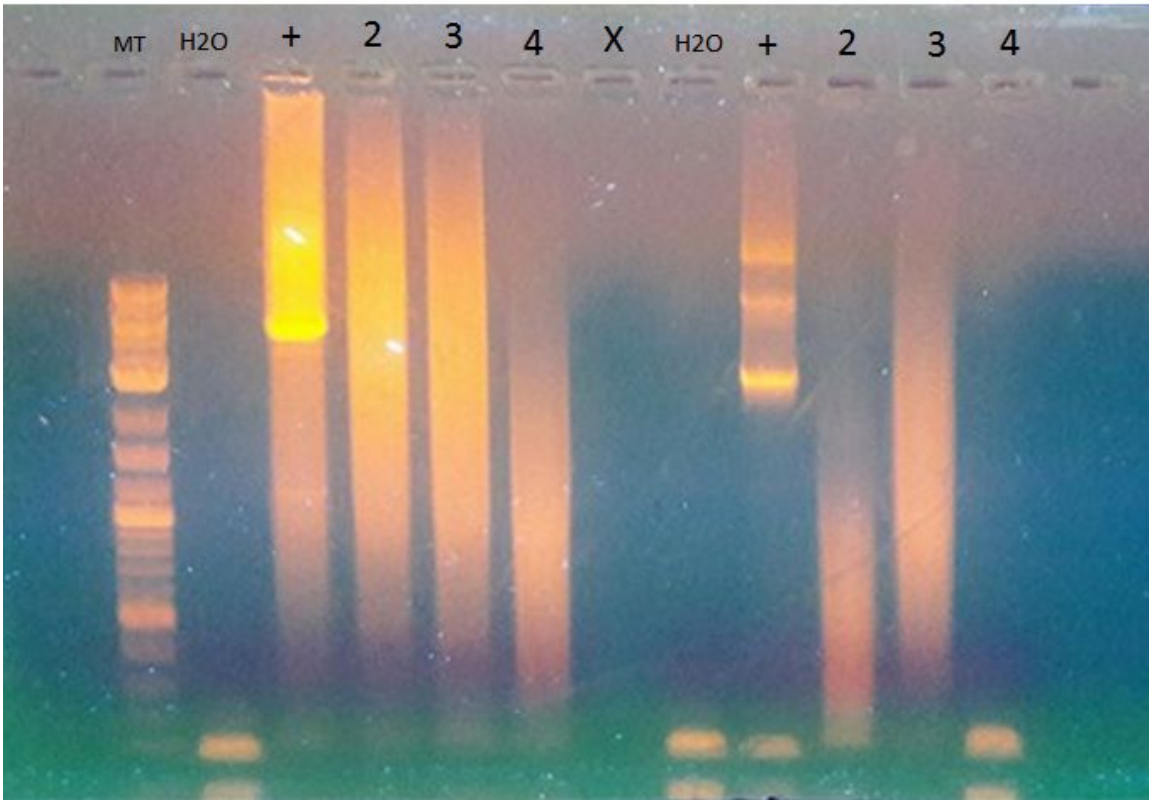
For one mix:

H2O	72µL
5x Buffer Phusion	20µL
10nM dNTP	2µL
10µ M Forward	5µL

10 µM reverse	5µL
DNA polymerase	1µL

Tube 1=H2O
Tube 2= colony 1
Tube 3=colony 2
Tube 4=colony 3
Tube 5=positive control
Tm=54°C
Elongation time=2min
Size=3273pb

Results:



The positives control are correct but no results for all colonies. One hypothisis could be that forward pMYO3 and reverse unc-60 primers could not amplify this part because of the size of approximately 40 bases that don't work with the Tm used. We should used prefix and suffix primers with new PCR.

PCR of pFRQ (1113pb):

One PCR mix were done for PCR of 50µL

For one mix:

H2O	µL
5x Buffer Phusion	µL
10nM dNTP	µL
10µ M Forward	µL
10 µM reverse	µL
DNA polymerase	µL

Tube 1=H2O

Tube 2=

Tube 3=

Tube 4=

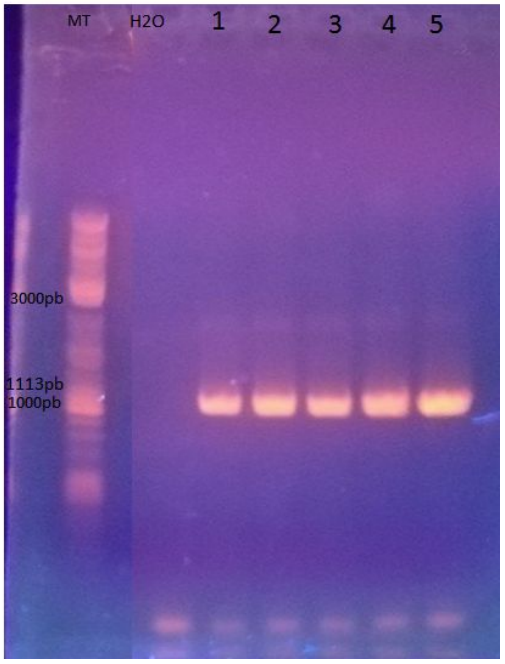
Tube 5=I

Tm=60,2°C

Elongation time=40''

Size=1113pb

Results:



We have the correct size of 1113pb with all conditions. We have to purify them with a qiagen kit diluted in 50µL of H2O.

29/08/2017:

PCR colony of pMYO mut

The condition are the same as usual.
We put the rest of bacteria in liquid cultural medium.

PCR of unc-60::GFP (3376pb):

One PCR mix were done for 17 PCR of 50µL

For one mix:

H2O	244,8µL
5x Buffer Phusion	68µL
10nM dNTP	6,8µL
10µ M Forward	17µL
10 µM reverse	17µL
DNA polymerase	3,4µL

Tube 1=H2O
Tube 2= colony 1
Tube 3=colony 2
Tube 4=colony 3
Tube 5=colony 4
Tube 6colony 5
Tube 7=colony 6
Tube 8=colony 7
Tube 9=colony 8
Tube 10=colony 9
Tube 11=colony 10
Tube 12=colony 11
Tube 13=colony 12
Tube 14=colony 13
Tube 15=positive control

Tm=60°C
Elongation time=3min30sec
Size=3376pb

Results:

PCR of pMyo3::unc-60::GFP (5432pb):
PCR of pMyo3::unc-60::RFP (3273pb):

One PCR mix were done for 20 PCR of 20µL

For one mix:

H2O	288µL
5x Buffer Phusion	80µL
10nM dNTP	8µL
10µ M Forward	20µL
10 µM reverse	20µL
DNA polymerase	4µL

Tube 1=H2O
Tube 2=positive control GFP

Tube 3= colony 1 box GFP
Tube 4= colony 1 LB GFP 1µL
Tube 5= colony 2 boxGFP
Tube 6=colony 2 LB GFP 1µL
Tube 7= colony 3 box GFP
Tube 8=colony 3 LB GFP 1µL

Tube 9=positive control GFP

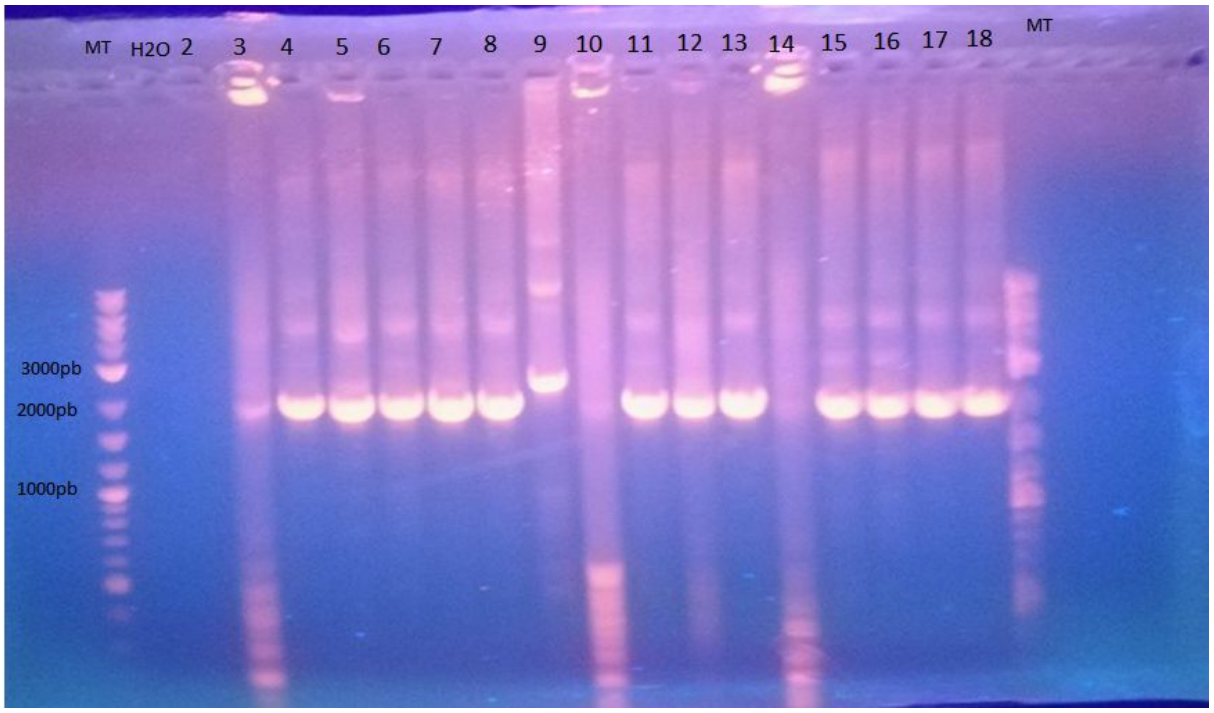
Tube 10= colony 1 BOX RFP
Tube 11= colony 1 LB RFP 1µL
Tube 12= colony 2 BOX RFP
Tube 13= colony 2 LB RFP 1µL
Tube 14= colony 3 BOX RFP
Tube 15= colony 3 LB RFP 1µL

Tube 16= colony 4 LB RFP 1µL
Tube 17= colony 5 LB RFP 1µL
Tube 18= colony 6 LB RFP 1µL

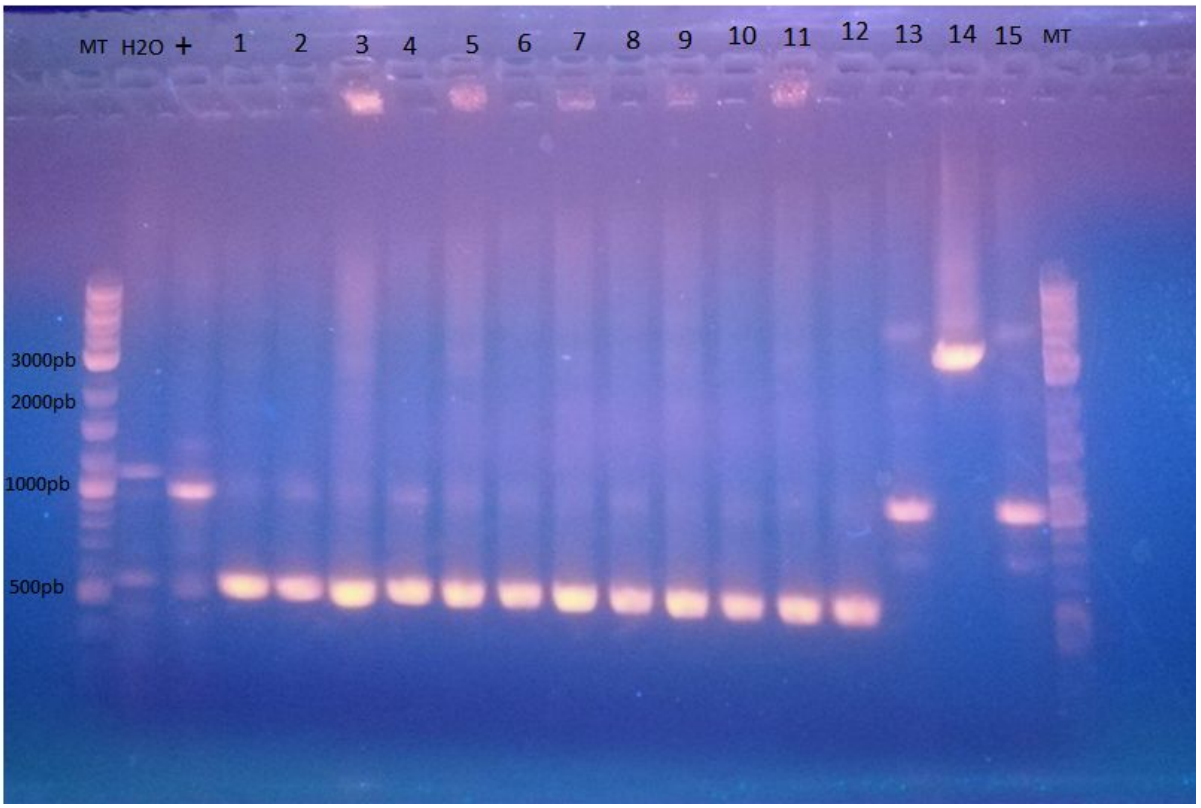
Tm=60°C
Elongation time=3min
size=5432pb

Tm=60°C
Elongation time=2min
Size=3273pb

Results:



New PCR with prefix and suffix primers:



well 1= colony 1 box GFP

well 2= colony 1 LB GFP 1µL

well 3= colony 2 boxGFP

well 4=colony 2 LB GFP 1µL

well 5= colony 3 box GFP

well 6=colony 3 LB GFP 1µL

well 7= colony 1 BOX RFP
well 8= colony 1 LB RFP 1µL
well 9= colony 2 BOX RFP
well 10= colony 2 LB RFP 1µL
well 11= colony 3 BOX RFP
well 12= colony 3 LB RFP 1µL

well 13= colony 4 LB RFP 1µL
well 14= colony 5 LB RFP 1µL
well 15= colony 6 LB RFP 1µL

Tm=60°C
Elongation time=3min
size=5432pb

Tm=60°C
Elongation time=2min
Size=3273 pb

After this positive result in well number 14. The corresponding colony was put one night in culture in 3 more tubes with 8 mL of LB and 8µL of chloramphenicol antibiotic.

30/08/2017

Results of PCR colony MYO3 mut

DNA precipitation

In order to sequence some of our plasmid, we need a concentration of 80-100 ng/µl. Or most of our sample have a concentration of 40 ng/µl. So we need to concentrate to those sample. The miniprep of H and K will be concentrate.

- Add to the sample (35 µl) in order:
    - 1/10 volume of 3M Sodium Acetate (3,5 µl)
    - 3 volume of 100 % ethanol (105 µl)
- Freeze 20 minutes to one hour at -80°C.

- Spin at full speed at 4° for 30 minutes.
- Discard carefully supernatant.
- Dry the pellet.
- Add your desired quantity of water:
    - H (40,5 ng/µl) → 1417 ng : add 14,2 µl => 99,788 ng/µl
    - K (64,3 ng/µl) → 2250,5 ng: add 22,6 µl => 99,579 ng/µl

Nanodrop:

Sequencing

We will sequence :

- ASD2
- pMYO3
- pMYO3 mut

Volume final mix = 10µl

Mix ASD2 :

K	5 µl	
primer prefixe	5 µl at 5µM	5 µl primer + 5 µl H2O

Mix pMYO3:

H (C3) D (A3)	5 µL 5 µl	
Primer prefixe	5 µl at 5 µM	5µl primer + 5 µl H20
Primer suffixe	5 µl at 5 µM	5 µl primer + 5 µl H20

31/08/2017:

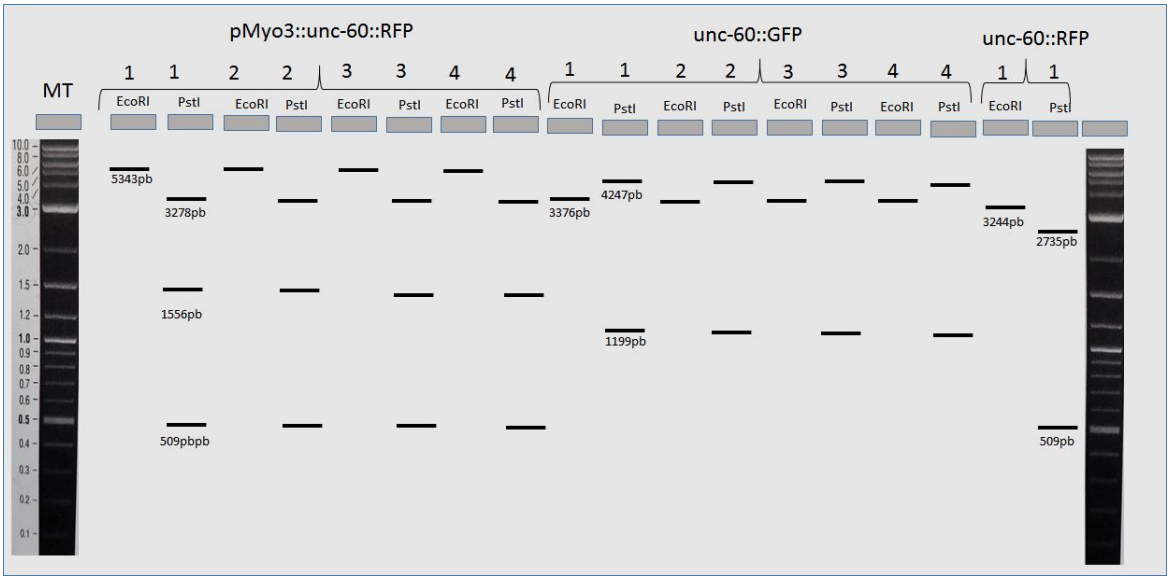
tube	ng/µL	260/280	260/230
pMyo3::unc-60::RFP 1	131,9	1,88	2,60
pMyo3::unc-60::RFP 2	98,9	1,92	2,23
pMyo3::unc-60::RFP 3	117,1	1,88	2,03

pMyo3::unc-60::RFP 4	125,6	1,89	2,12
Lig unc-60GFP 1	51,3	1,92	1,83
Lig unc-60GFP 2	41,2	1,96	1,67
Lig unc-60GFP 3	36,6	1,97	1,83
Lig unc-60GFP 4	30,04	1,96	2,12
Lig unc-60RFP 1	49,8	1,91	1,76
Lig unc-60RFP 5	13,1	1,99	1,47

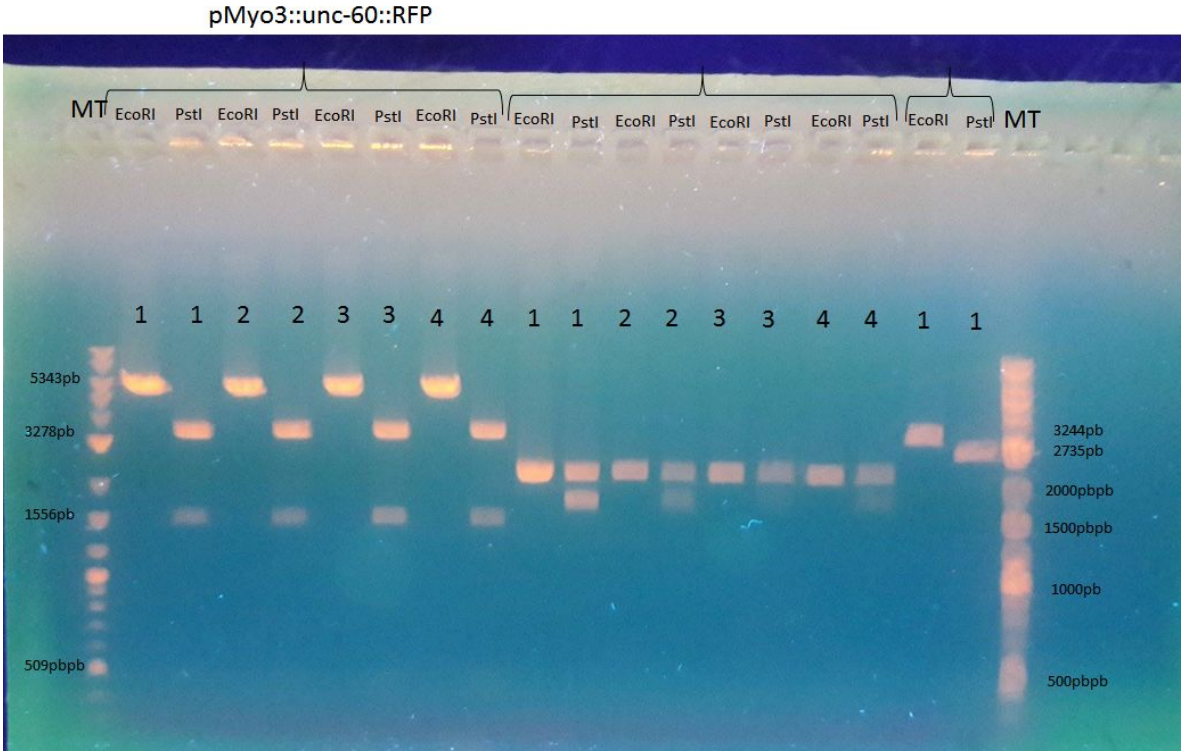
Digestion pMyo3::unc-60::RFP, Ligation unc-60::GFP and unc-60::RFP all in backbone pSB1C3

Digestions were done with EcoRI alone to open the plasmid and PstI alone to cut inside the part pMyo3::unc-60::RFP, unc-60::GFP and unc-60::RFP and control if the part is present.

theoretical gel:



Results:



For pMyo3::unc-60::RFP, we obtained exactly the goods fragments sizes with a linear plasmid of approximately 5300pb and two fragments after digestion which sizes are 3278pb and 1556pb. We could not observ the 509pb fragments maybe because of less DNA quantity that could not less us to see. We have the same phenomenon with the 1556pb fragment which is less bright than the 3278pb fragment. We obtained the good sizes for unc-60::RFP with a linear plasmid od 3244pb and one spot of 2735pb after PstI digestion. We don't observe the 509pb fragment for the same reason that previously. We don't obtain the good DNA spots for unc-60::GFP but one spot with a size of approximately 2000pb and one other of approximately 2500pb and 2000pb with PstI digestion. It means that the plasmid open with EcoRI has a size of approximately 2500pb and It has two PstI restrictions sites. One in the pSB1C3 suffix backbone and one other inside the fragment.