Fablab Lab Book 2

LUNDI 15/07/2019

Site-directed mutagenesis on pSR658 and pSR659 (1)

Aim: to introduce an XbaI restriction site in pSR658 and pSR659

Design of the primers

Gibson MS2 PP7

Forward primer:
- 5′ CAC CCA CGG GGC GTC TAG AAT GAA AGC GTT AAC 3′
- Tm = 65.9°C
- Amount of oligo received: 0.38mg

Reverse primer:
- 5′ GTT AAC GCT TTC ATT CTA GAC GCC CCG TGG GTG 3′
- Tm = 65.9°C
- Amount of oligo received: 0.25mg

PCR

Site-directed mutagenesis Taq'Ozyme

Hybridation temperature: 61°C
Primers quantity: 1µl of 20µM
Plasmids quantity: 10ng
The protocol was used on pSR658 and pSR659 with the same primers and the same cycles

Results from Qubit with master mix HS:
- pSR658: 15ng/µl
- pSR659: >50ng/µl (too high for the master mix used)

MARDI 16/07/2019

Site-directed mutagenesis on pSR658 and pSR659 (1)

Digestion by DpnI

In order to remove the original, methylated plasmid, the PCR product was digested by DpnI.
1µl DpnI 20U/µl in 50µl PCR products - 1h 37°C

Agarose gel eletrophoresis

Digestion of plasmids by XbaI (20U/µl)
We kept the PCR buffer as XbaI works in most of PCR buffers (Activity of restriction enzymes in PCR buffers_NEB)
- For pSR658: we used 2µl (30ng) of PCR product + 0.5µl XbaI 0.4U/µl (= XbaI 20U/µl diluted at 1/50)
- For pSR659: we used 0.6µl (> 30ng) of PCR product + 0.5µl XbaI 0.4U/µl (= XbaI 20U/µl diluted at 1/50)
  we used 0.6µl (> 30ng) of PCR product + 0.5µl XbaI 2U/µl (= XbaI 20U/µl diluted at 1/10)

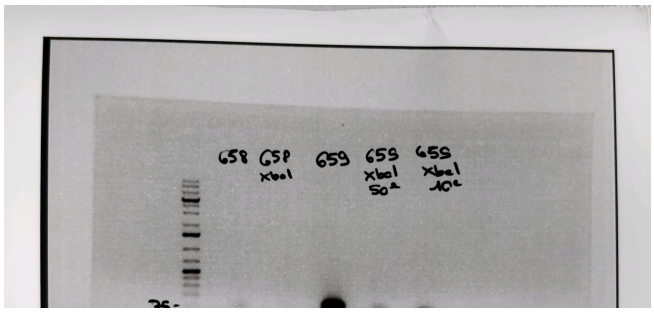
Vtot = 10µl
1h20 - 37°C

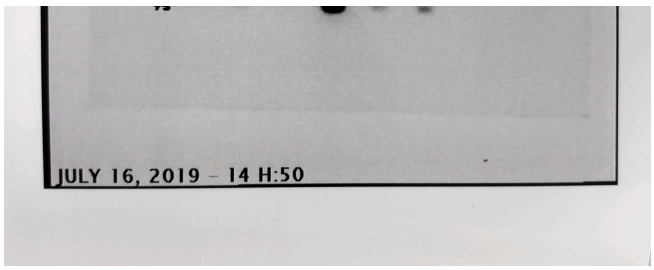
Then agarose gel electrophoresis:

Agarose gel electrophoresis

We used 12µl for digested plasmids (10µl + 2µl load buffer 6X) and 6µl for non digested plasmids (5µl + 1µl load buffer 6X)

1607_gel_mutagenese_image.jpg





Gel electrophoresis after site directed mutagenesis and DpnI digestion - with and without XbaI digestion. We only amplified the primers.

JEUDI 18/07/2019

Site-directed mutagenesis on pSR658 and pSR659 (2)

We did the PCR for site-directed mutagenesis again in different conditions:

PCR conditions

	A	B	C	D	E	F
1		Probes		Plasmids	Mix PCR	
2		Qty (µl)	Ci (µM)	Qty (ng)	Vol (µl)	Vtot (µl)
3	1	1	20	10	25	50
4	2	1	20	100	25	50
5	3	1	20	200	25	50
6	4	1	2	10	25	50
7	5	1	2	100	25	50
8	6	1	2	200	25	50
9	7	2	2.5	8	25	50
10	8	0.5	2.5	2	6.25	12.5

Hybridation temperature: 63°C
Because we lacked plasmid pSR659, the conditions 3 and 6 could not be done for this plasmid.
Because of a pipetting mistake, the condition 7 for pSR658 was not done either.

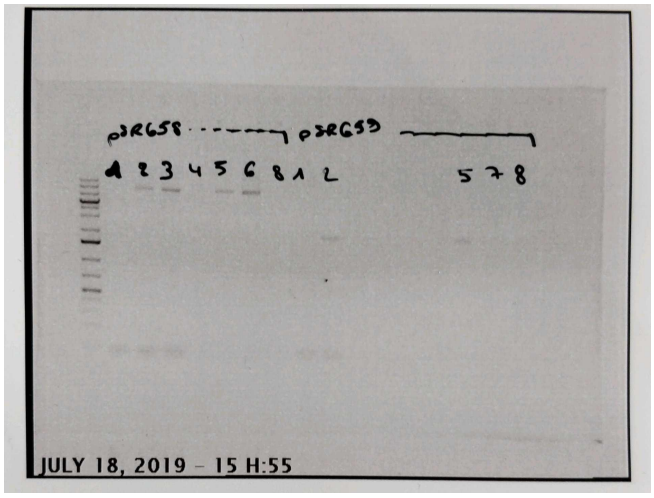
We obtained the following results from Qubit HS:

Results from Qubit HS

	A	B	C
1	ng/µl	pSR 658	pSR 659
2	1	1.64	0.876
3	2	1.29	5.32
4	3	3	
5	4	0.3	TOO LOW
6	5	0.15	0.186
7	6	0.278	
8	7		0.654
9	8	0.21	0.130

We then performed an agarose gel electrophoresis with 10µl PCR product + 2µl loading buffer.

1807_gel_mutagenese_image.jpg



The conditions 2 and 3 worked but with a contamination coming from the primers and the conditions 5 and 6 worked without any visible contaminations. The bands were stronger for conditions 3 and 6.

Transformation of pSR659 in Top10 bacteria

Because we did not have enough plasmid, we performed a transformation to amplify it.

CaCl2 Competent cells / Transforming

We used 10ng of pSR658 plasmid from two conditions (the plasmid at 28.6ng/µl and the one at 16.2ng/µl).

VENDREDI 19/07/2019

Transformation of pSR659 in Top10 bacteria

There was no colonies from yesterday transformation. This might be due to the transportation of the competent bacteria from one lab to another.

Transformation SU202 with pSR658*, pSR659* and pSR659

*= afer the mutagenesis

Transformations

	Bacteria	Plasmid	Plasmid origin	Antibiotic
1	SU202	pSR658*	1808PCR, n°3	Tetracycline
2	SU202	pSR658*	1808PCR, n°6	Tetracycline
3	SU202	pSR659*	1808PCR, n°2	Ampicilline + chloramphenicol
4	SU202	pSR659*	1808PCR, n°5	Ampicilline + chloramphenicol
5	SU202	pSR659	0407Miniprep (c = 6.4ng/µl)	Ampicilline + chloramphenicol
6	SU202	pSR659	2606Miniprep (c = 28.6ng/µl)	Ampicilline + chloramphenicol

Transformation (Protocol iGEM)

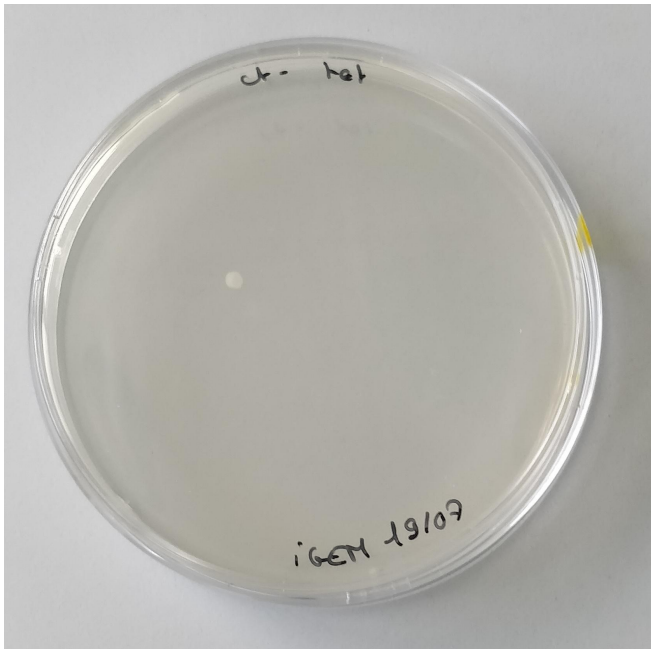
Quantity of plasmid : 250pg/µl and 10ng/µl (for pSR658* and 659*, 1µl was used for the 250pg condtions and all the PCR product left was used in the 10ng condition)
The petri dishes which were plated before the centrifugation stayed in the incubator at 37°C for the weekend whereas the petri dishes which were plated after were left of the bench.

LUNDI 22/07/2019

Results from transformations SU202 with pSR658*, pSR659* and pSR659

pSR658*

2207_ct tet.jpg



Control: SU202 on tetracylin plate -> 1 colony

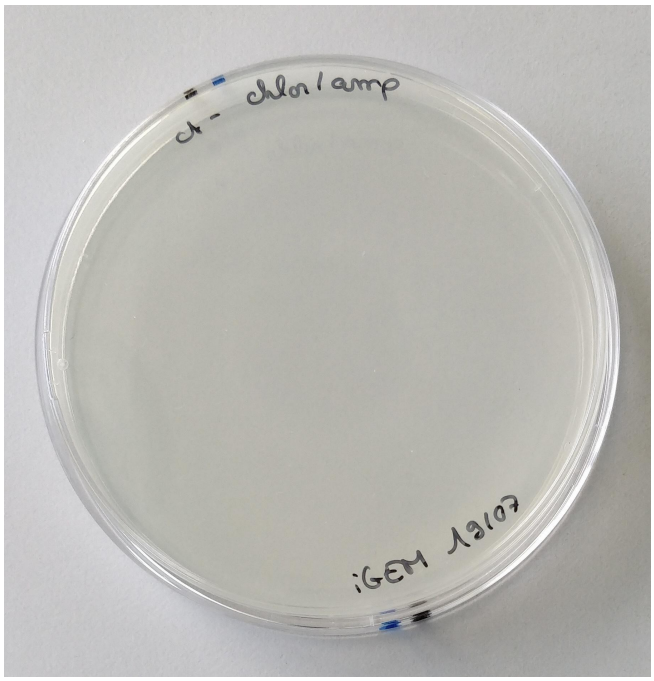
For SU202 + pSR658*: only one colony on one condition (PCR n°3, 10ng from PCR product, plated after centrifugation). A PCR on colony (Taq'Ozyme, 30 cycles, T°hybrid = 51°C) was performed and the colony was put in liquid LB + tetracyclin.

pSR569*

No colonies on any conditions
The bad resultats of transformation after mutagenesis might be due to the non-purification of the PCR product.

pSR659

2207_ct chlor+amp.jpg



Control: SU202 on chlor + amp -> no colonies

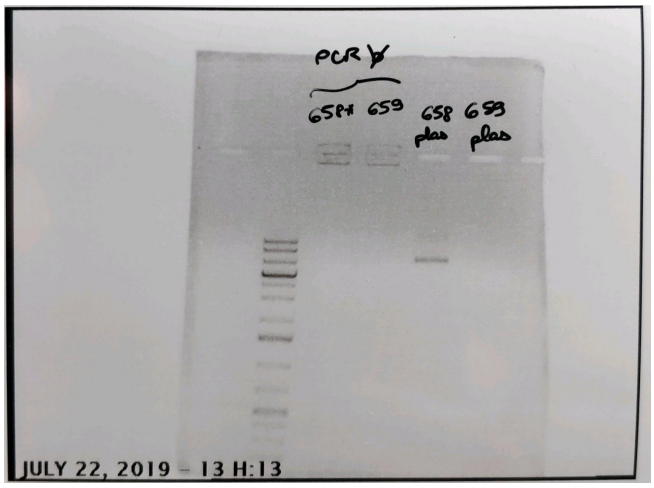
Every conditions gave single colonies.
We chose plasmids from 26/06, 10ng from Miniprep, plated directly after the transformation to perform a PCR on colony (Taq'Ozyme, 30 cycles, T°hybrid = 51°C) and to make it grow in liquid LB + ampicillin.

PCR on colony

Protocol Taq'ozyme polymerase HS mix:
https://www.ozyme.fr/gammes/ozy/taq-ozyme-hs-mix-pcr-demarrage-a-chaud-manuel.asp

PCR bacteria: from PCR on colony

2207_gel_PCRcolo.jpg



658 and 659 plas: from original plasmids (very small quantity for 659)

No plasmid visible in the colonies.

MARDI 23/07/2019

Site-directed mutagenesis on pSR658 (3)

Site-directed mutagenesis Taq'Ozyme

In duplicates.
PCR + digestion with Dpn I.

PCR on MS2 and PP7 (1)

To have the DNA ready for Gibson, the anchored-PCR was perform on pKB989 (2) and pKB1081 (2). It was done in duplicates (1) and (2).
The plasmids were diluted at 1:20 first.
Protocol Taq'ozyme polymerase HS mix: https://www.ozyme.fr/gammes/ozy/taq-ozyme-hs-mix-pcr-demarrage-a-chaud-manuel.asp

PCR Conditions

	A	B	C	D	E	F	G	H	I	J	K
1		Probes			Plasmids			Mix PCR		Hybridation temperature	
2		Vol (µl)	Ci (µM)	Cf (µM)	Vol (µl)	Ci (µM)	Qty (ng)	Vol (µl)	Vtot (µl)	5 cycles	20 cycles
3	MS2	10	2	0.4	1.12	8.95	10	25	50	51°C	69°C
4	PP7	10	2	0.4	1.78	5.62	10	25	50	61°C	70°C

Miniprep of pSR658*, pSR659 and MS2 (trial)

Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010)

The miniprep was performed on the liquid cultures from 22/07.
In order to purify MS2 after the PCR, we tried to use the last steps of the miniprep kit on MS2 (2).

Results (from Qubit):

DNA quantity after miniprep

	A	Qty (ng/µl)
1	pSR659	26.0
2	MS2 (2)	1.36
3	pSR658*	460

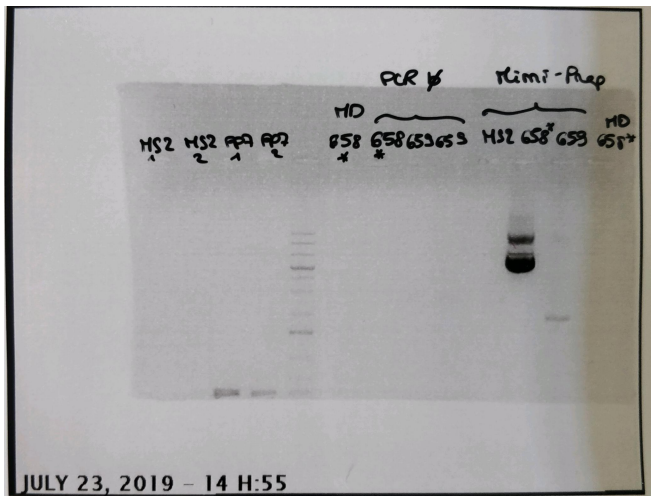
PCR on liquid culture

PCR on pSR658* and pSR659 liquid cultures from 22/07
Protocol Taq'ozyme polymerase HS (https://www.ozyme.fr/gammes/ozy/taq-ozyme-hs-mix-pcr-demarrage-a-chaud-manuel.asp) mix with 1µl of liquid culture in the mix.

Gel electrophoresis

5µl of PCR products or miniprep products. The gel was run for too long (40 min) and some of the samples went out.

2307_gel.jpg



MS2 (378+93 pb=471): may have went out
PP7 (384+91 pb=475): band around 400pb
MD (mutagénèse dirigée/site-directed mutagenesis): no bands -> did not work
PCR on liquid culture: did not work
Mini-prep: no MS2 (same as before, may have run out) ; big band for 658* ; small band for 659

MERCREDI 24/07/2019

Freezing of competent SU202

CaCl2 Competent cells / Transforming

Growth of liquid cultures: OD600

	Culture volume	12h34	14h15
1	40ml (1)	0.10	0.38
2	40ml (2)	0.09	0.38
3	10ml (1)	0.09	0.30
4	10ml (2)	0.12	0.34

10 aliquots of 100µl, 6 aliquots of 200µl and 1 with the rest (around 400µl) at OD 15

Site-directed mutagenesis of pSR658 and pSR659 (4)

PCR

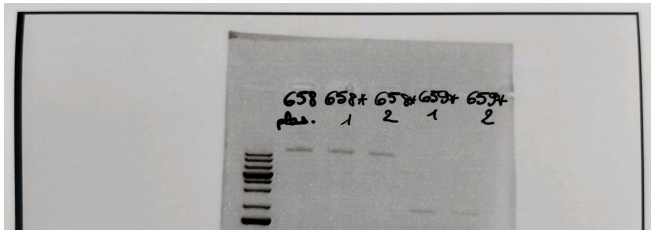
Done in duplicates - hybridation temperature: 63°C

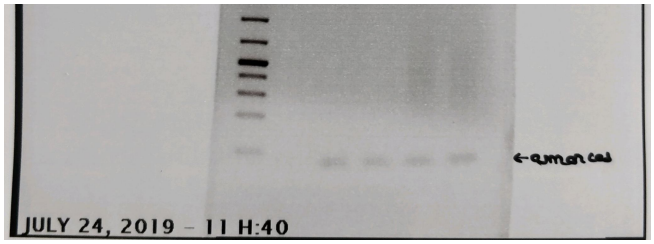
PCR conditions 23/07

	A	B	C	D	E	F	G	H	I
1		Probes			Plasmids			Mix PCR	
2		Vol (µl)	Ci (µM)	Cf (µM)	Vol (µl)	Ci (ng/µl)	Qty (ng)	Vol (µl)	Vtot (µl)
3	pSR658	1	20	0.4	7.9	25.3	200	25	50
4	pSR659	1	20	0.4	7.69	26	200	25	50

Gel electrophoresis

2407_gel_SDmut.jpg





Bands for the plasmids
Contamination by the primers (was anticipated but these PCR conditions were the only one to give a result for transformation)

DpnI digestion

1µl, 2h, 37°C

Transformation

Transformation (Protocol iGEM)

*= afer the mutagenesis

Transformation plan

	Bacteria	Plasmid	Plasmid origin	Plasmid quantity	Antibiotic
1	SU202	pSR658*	2307PCR	1µl in 50µl bacteria and what's left in 50µl bacteria	Tetracycline
2	Top10	pSR658*	2307PCR	1µl in 50µl bacteria and what's left in 50µl bacteria	Tetracycline
3	SU202	No plasmid			Tetracycline
4	Top10	No plasmid			Tetracycline
5	SU202	pSR659*	2307PCR	1µl in 50µl bacteria and what's left in 50µl bacteria	Ampicilline
6	Top10	pSR659*	2307PCR	1µl in 50µl bacteria and what's left in 50µl bacteria	Ampicilline
7	SU202	No plasmid			Ampicilline
8	Top10	No plasmid			Ampicilline

What's left = around 40µl

Quantification of MS2

Because there was no band for MS2 on the gel, it was quantified by Qubit.

Quantification of MS2

	A	Qty (ng/µl)
1	MS2 (1)	5.82
2	MS2 (2) eluate	0.852

It seems that there is some DNA in MS2 samples even if it is not visible on the gel.

Digestion of pSR658* with XbaI

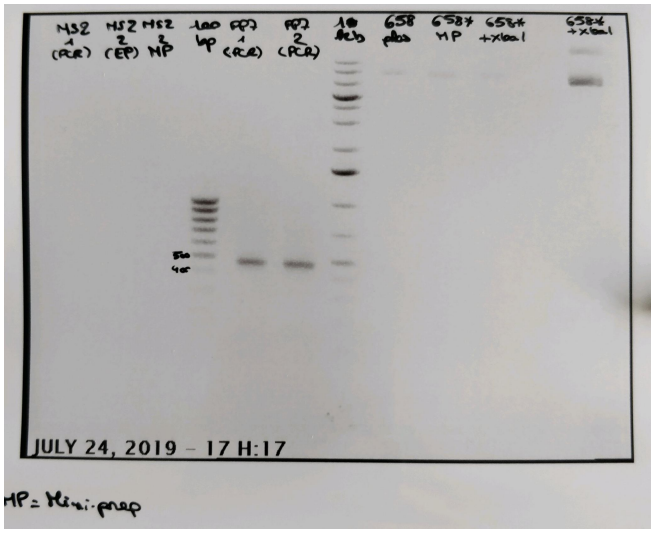
In order to verify the presence of the mutation in pSR658* after the miniprep from 23/07, it was digested with XbaI.

	Compound	Volume (µl)	Quantity tot
1	Plasmid pSR658*	1	460ng
2	CutSmart buffer 10X	1	1X
3	XbaI 2U/µl	1.15	2.3U
4	Water	6.85	

Gel electrophoresis

5µl for MS2 and PP7
1µl 1:10 for the first three 658 deposits
All digestion left (9µl) for the last 658 deposit
100bp DNA ladder: lyophylized Roth DNA ladder (Art N° T834.1) + 500µl sample buffer Roti-Load 1X (Art N° 0100.1)

2407_gel.jpg



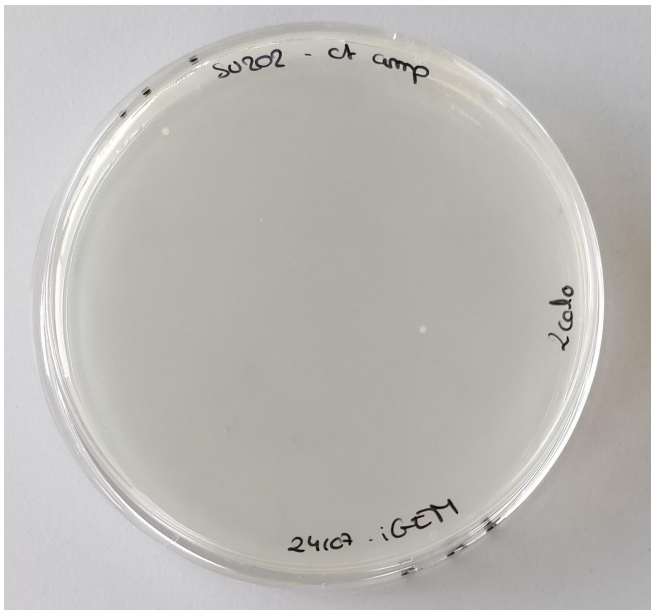
No bands for MS2 (even if the Qubit measured some DNA!)
Bands around 470pb as planned for PP7
Not enough plasmid to compare before and after the digestion of 658* by XbaI but it seems that it did not work well

JEUDI 25/07/2019

Results from 24/07 transformation

Controls

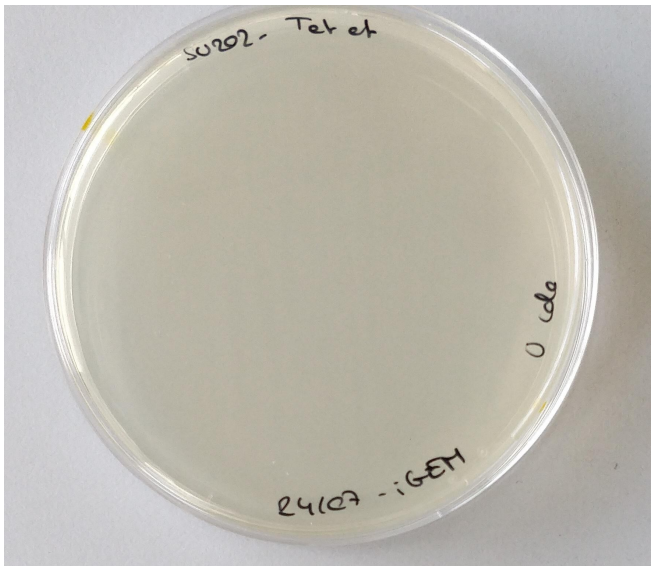
2407_ct amp.jpg



2 colonies on ampicilline plates

2407_ct tet.jpg

No colonies on tetracycline plates



pSR658*

One colony on SU202 - 1µl - after centrifugation
One colony on SU202 - what's left (around 40µl) - after centrigugation
Thoses colonies had a weird shape
They were put in liquid LB + tetracycline
Nothing on the other plates

pSR659*

Many colonies (around 15) on SU202 - what's left (around 40µl) - after centrifugation
One colony on SU202 - 1µl - after centrifugation
They had the expected shape, size and colour.
Three colonies from the plate 40µl and the one from the plate 1µl were put in liquid LB + ampicilline
Nothing on the other plates

Mini-prep pSR659

With kit Monarch (NEB)

Quantification (ng/µl) after mini-prep

	A	Qty (ng/µl)
1	pSR659 colo3	14.2
2	pSR659 colo4	11.9

Very small amount of plasmid -> the colonies were put in liquid medium from a plate stored at +4°C, it may have affected it.

Site-directed mutagenesis of pSR658 and pSR659 (5)

PCR

Hybridation temperature: 62°C

PCR conditions 24/07

	A	B	C	D	E	F	G	H	I
1		Probes			Plasmids			Mix PCR	
2		Vol (µl)	Ci (µM)	Cf (µM)	Vol (µl)	Ci (ng/µl)	Qty (ng)	Vol (µl)	Vtot (µl)
3	pSR658	1	20	0.4	7.9	25.3	200	25	50
4	pSR659	1	20	0.4	14	14.2	200	25	50

Quantification (Qubit HS)

Quantification (ng/µl)

	PCR product	DNA qty (ng/µl)
1	pSR658*	6.7
2	pSR659*	7.8

XbaI digestion

In order to verify the presence of the mutation in pSR658* and pSR659* after the PCR, it was digested with XbaI.

Mix composition

	Compound	Volume (µl)	Quantity tot
1	DNA from pSR658* or pSR659* PCR	5	around 35ng
2	XbaI 0.2U/µl	1	0.23U
3	Water	4	

It was also done again on pSR658* from the mini-prep (23/07)

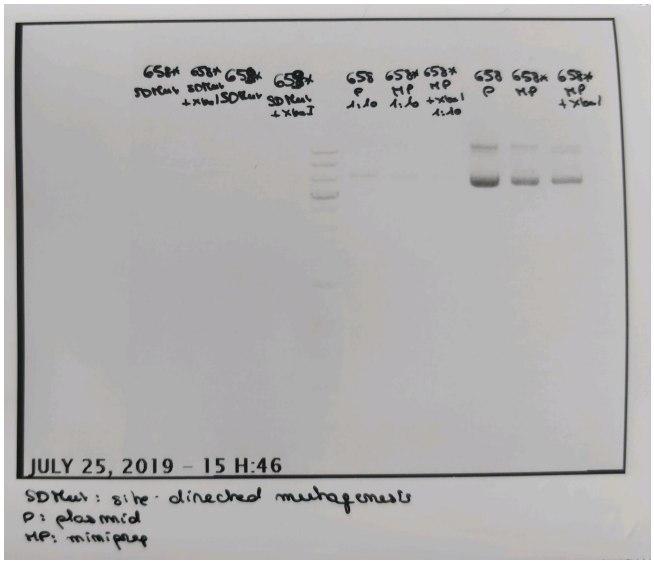
Mix composition for pSR658* after mini-prep

	Compound	Volume (µl)	Quantity tot
1	Plasmid pSR658*	1	460ng
2	CutSmart buffer 10X	1	1X
3	XbaI 2U/µl	1.15	2.3U
4	Water	6.85	

Gel electrophoresis

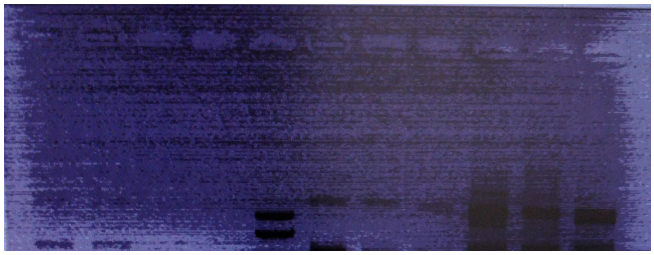
For SD Mut (site-directed mutagenesis): 5µl of PCR product

2507_gel_658+-XbaI.jpg

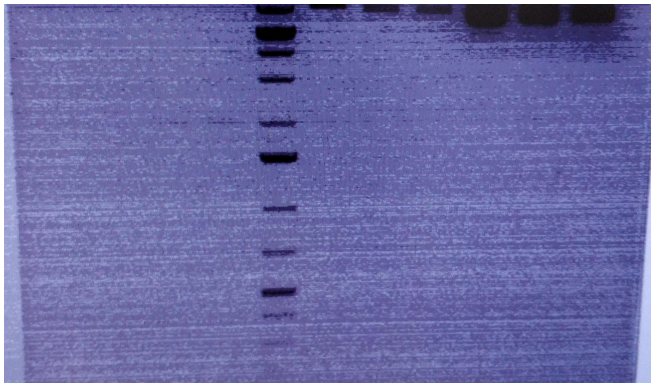


XbaI does not cut the plasmid.

2507_gel_658+-XbaI_écran.jpg



Same gel but the picture is taken from the screen, where we see better tne first two bands XbaI did not cut either this plasmid.



VENDREDI 26/07/2019

Anchored-PCR on pKB989 (MS2)

PCR with the Gibson primers from this file:

Gibson MS2 PP7

According to Serial Cloner the Tm for MS2 primers are 59.5°C/71.6°C and 60.5°C/77.7°C
Protocol from Taq'Ozyme HS Mix with 5 cycles with an hybridization temperature of 55°C and 25 cycles with an hybridization temperature of 68°C.

Miniprep on pSR659*

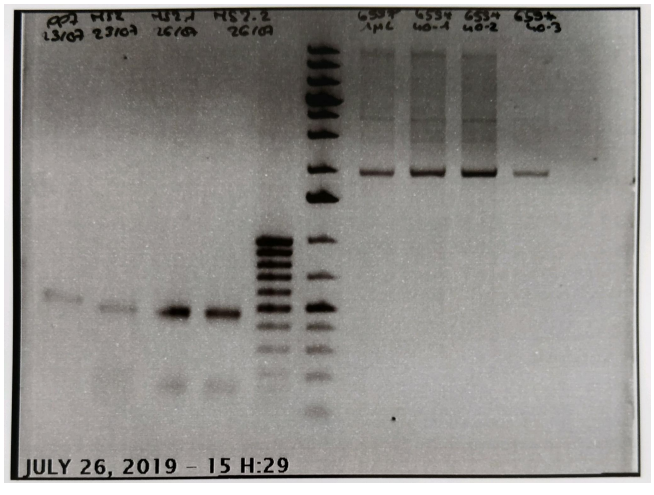
From bacteria transformed the 24/07 and put in liquid cultures the 25/07.
The liquid culture from pSR658* did not grow so no miniprep was done with them.

Because of lack of material, only one miniprep was quantified (from plate 40µl, colony 2): 9ng/µl

Gel electrophoresis

5µl of each sample was used. For the PP7 sample, it went out of the well so the quantity seen on the gel is far less from what it was.

2607_gel_MS2+659mut.jpg



There is more MS2 from 26/07 PCR than 23/07 PCR.
All minipreps had 659* in them.

Transformation of pSR658* and pSR659* in Top10

Rebecca did the transformation of pSR658* and pSR659* from 25/07 site-directed mutagenesis on Top10. She also used the kit Competent cells from iGEM.
PROTOCOL

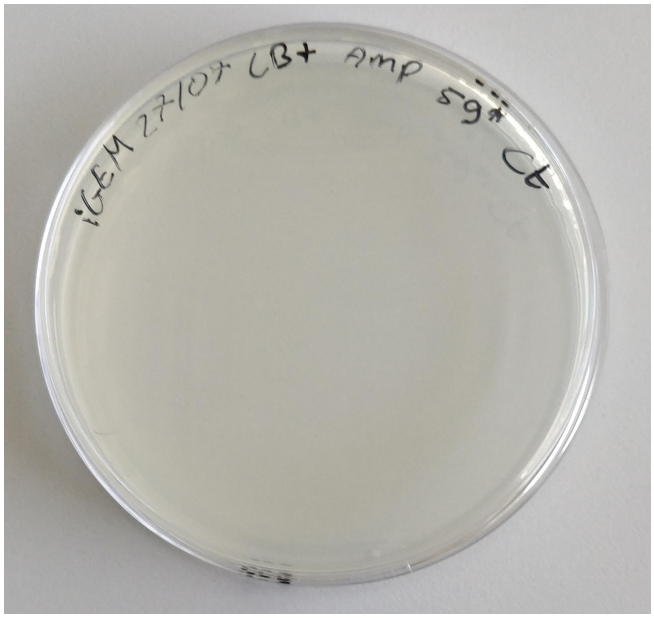
LUNDI 29/07/2019

Transformation of pSR658* and pSR659* in Top10 - Results

Controls

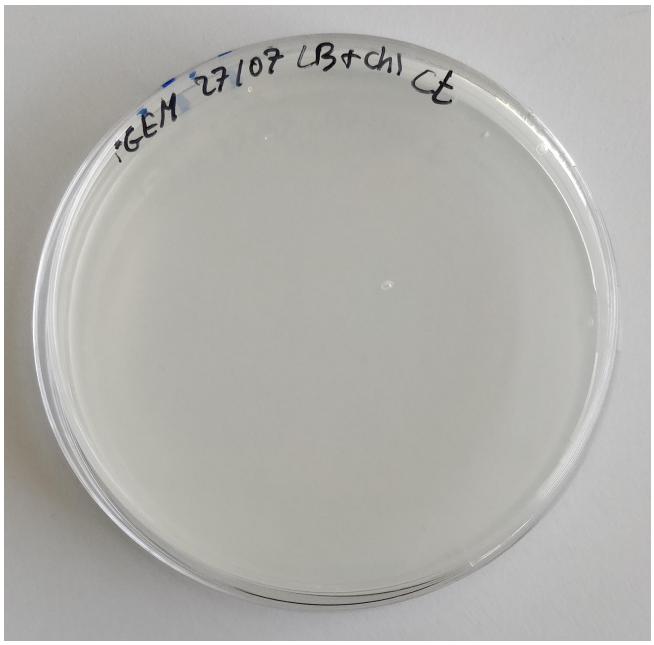
There was no colony on the negative controls.

2607_ct amp.jpg



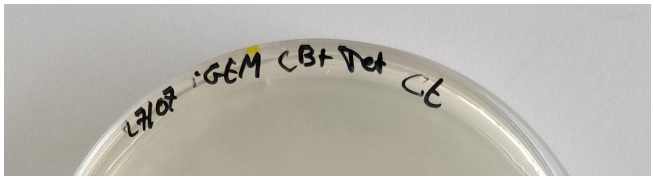
Control ampicillin

2607_ct chlor.jpg

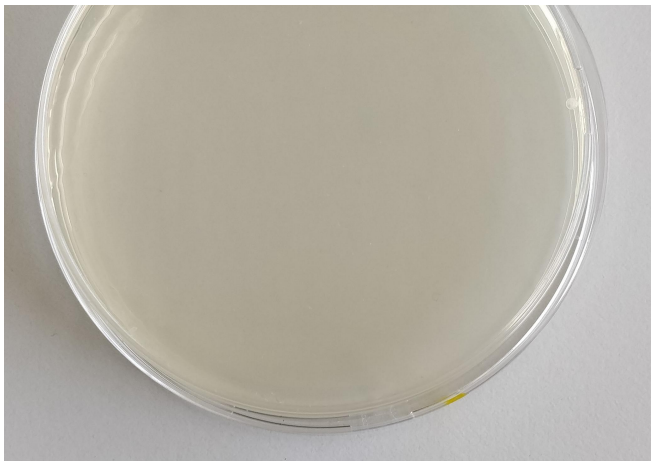


Control chloramphenicol

2607_ct tet.jpg



Control tetracyclin



With the competent cell kit from iGEM there were 6 colonies with 10pg/µl DNA and 24 colonies with 100pg/µl DNA which leads to an average transformation efficiency of 4.2x10^6.

pSR658*

There were many colonies on all conditions.

pSR659*

There were many colonies on all conditions.

Site-directed mutagenesis on pSR658 and pSR659 (6)

PCR

It seems that the PCR does not amplify the original plasmid. Another polymerase was used with its protocol.

Site-directed mutagenesis Pfu pol

PCR mix 29/07

	A	B	C	D	E	F	G	H	I	J	K
1		Probes			Plasmids			Pfu buffer 10X	dNT mix 10mM	Pfu pol 2.5U/µl	
2		Vol (µl)	Ci (µM)	Cf (µM)	Vol (µl)	Ci (ng/µl)	Qty (ng)	Vol (µl)	Vol (µl)	Vol (µl)	Vtot (µl)
3	pSR658	0.75	20	0.3	1.98	25.3	50.1	25	1µl	1µl	50
4	pSR659	0.75	20	0.3	4.20	11.9	50.0	25	1µl	1µl	50

Program on thermocycler: 1 min @ 95°C, (1 min @ 95°C, 1 min @ 62°C, 10 min @ 72°C) x 12, 30 min @ 72°C, ∞ @ 4°C

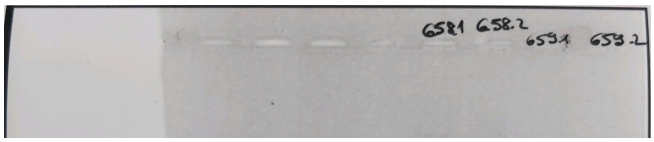
MARDI 30/07/2019

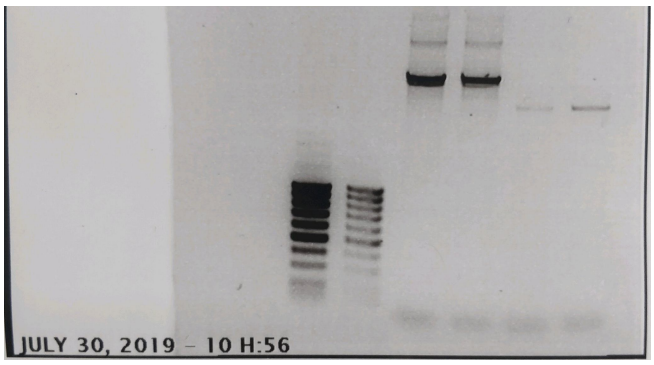
Site-directed mutagenesis on pSR658 and pSR659 (6)

Agarose gel electrophoresis

5µl of each PCR product
DNA ladder: 100bp

3007_gel_658 et 659 Pfu.jpg





There are strong bands for pSR658 but the bands are quite weak and inequal for pSR659.

DpnI digestion

Add *Dpn*I 20U/µl, 2µl per PCR product 2h @ 37°C in the thermocycler

Transformation

The PCR products were purified with NucleoSpin Gel and PCR Clean-up from MN and eluted in 30µl.
https://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/DNA%20clean-up/UM_PCRcleanup_Gelex_NSGelPCR.pdf

Quantification after the purification (ng/µl)

	PCR product	DNA qty (ng/µl)
1	pSR658*	19.10
2	pSR659*	3.96

5µl of those purified plasmids were used to transform 50µl of DH5α competent cells. They were incubated 30 min on ice, 1 min @ 42°C, 5 min on ice. 1ml of LB was then added and they were incubated 1h @ 37°C.
The same was done for control - cells (without plasmids) and control + (competent cells kit from iGEM).
They were centrifuged at 2000rpm, 3 min, most of the supernatant was removed and they were plated on LB/agar petri dishes with apropriate antibiotic (tetracycline for pSR658, ampicillin for pSR659 and chloramphenicol for controls +). They were incubated at 37°C ON.

Amplification of pSR659

We amplified SU202 - pSR659 from plates from 22/07 in 2.5ml LB + ampicillin.

MERCREDI 31/07/2019

Results from the DH5α transformation

Controls

No colonies grew on negative controls (plates with tetracyclin, ampicillin or chloramphenicol).

With the competent cell kit from iGEM there were 4 colonies with 10pg/µl DNA and 31 colonies with 100pg/µl DNA which leads to an average transformation efficiency of 3.6x10^5.

pSR658*

164 colonies grew (transformation efficiency: 8.6x10^2). 6 of them were amplified in liquid LB + tet.

pSR659*

169 colonies grew (transformation efficiency: 4.3x10^3). 6 of them were amplified in liquid LB + amp.

Amplification of pSR659

The two cultures from 30/07 were plated.

They had reached an OD600 of 2.37 and and 1.38.

JEUDI 01/08/2019

MS2 and PP7 amplification for Gibson

Anchored PCR

The anchored PCR was performed with Pfu polymerase to obtain a better copy than with Taq polymerase.

Anchored-PCR

	A	B	C	D	E	F	G	H	I	J	K
1		Probes			Plasmids			Pfu buffer 10X	dNT mix 10mM	Pfu pol 2.5U/µl	
2		Vol (µl)	Ci (µM)	Cf (µM)	Vol (µl)	Ci (ng/µl)	Qty (ng)	Vol (µl)	Vol (µl)	Vol (µl)	Vtot (µl)
3	MS2	7.5	2	0.3	1.12	8.95	10	25	1µl	1µl	50
4	PP7	7.5	2	0.3	1.78	5.62	10	25	1µl	1µl	50

Program on thermocycler for MS2: 1 min @ 95°C, (1 min @ 95°C, 30 sec @ 55°C, 2 min @ 72°C) x 5, (1 min @ 95°C, 30 sec @ 68°C, 2 min @ 72°C) x 25, 30 min @ 72°C, ∞ @ 4°C

Program on thermocycler for PP7: 1 min @ 95°C, (1 min @ 95°C, 30 sec @ 61°C, 2 min @ 72°C) x 5, (1 min @ 95°C, 30 sec @ 70°C, 2 min @ 72°C) x 25, 30 min @ 72°C, ∞ @ 4°C

Purification

The PCR products were then purified with NucleoSpin Gel and PCR Clean-up from MN and eluted in 25µl.
https://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/DNA%20clean-up/UM_PCRcleanup_Gelex_NSGelPCR.pdf

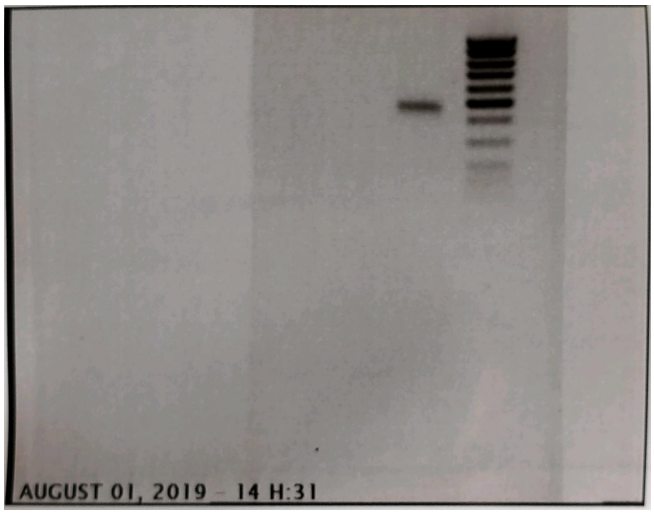
Quantification of MS2 and PP7 after purific...

	PCR product	DNA qty (ng/µl)
1	MS2	1.78
2	PP7	48.8

Gel electrophoresis

1µl + 9µl water + 2µl loading buffer were loaded on an agarose gel 1%.

0108_MS2 et PP7.jpg



We do not see any band for MS2.
We see a band around 470bp for PP7.
DNA ladder: 100bp equimolar from Roth.

Purification of pSR658* and pSR659*

Miniprep of pSR658* and pSR659*

The plasmids amplified in liquid cultures from yesterday were purified with Monarch kit (NEB).
Because of lack of material, only one miniprep for each plasmid was quantified.

	Plasmid	Concentration (ng/µl)
1	pSR658*, 3	24.1
2	pSR659*, 3	23.4

XbaI digestion

The purified plasmids were digested with XbaI to open them.

Mix composition for XbaI digestion

	Compound	Volume (µl)	Quantity tot
1	Plasmid pSR658*	15	around 375ng
2	CutSmart buffer 10X	1.8	1X
3	XbaI 2U/µl	1	2U

Gel electrophoresis

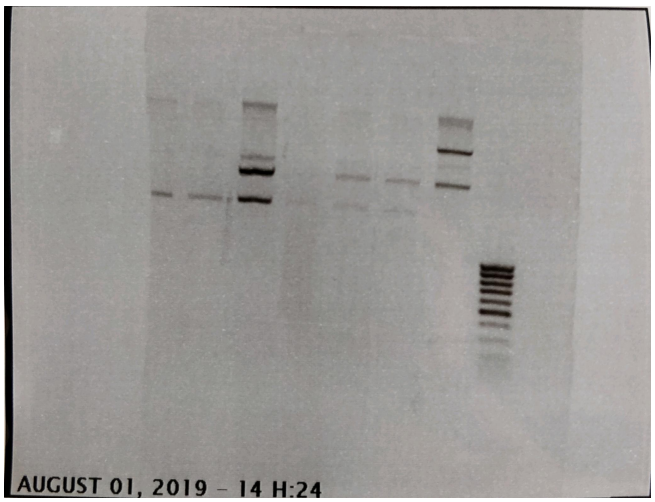
To the 18µl, 3.6µl of loading buffer 6X was added and loaded on a 1% agarose gel. It was then run at 120V for 40min. The band corresponding to the opened plasmid was cut and purified with NucleoSpin Gel and PCR Clean-up from MN and eluted in 25µl.

0108_pSR658.jpg

Colonies 1-3 of pSR658* / plasmid pSR658 without any mutation or digestion / colonies 4-6 of pSR658*
A strong band appears in digested plasmids which is not present in the plasmid not digested. This band must correspond to linearized plasmid.

The band correspondind to linearized plasmid in colonies 1 and 4 was cut and purified.

0108_pSR659.jpg



Colonies 1-3 of pSR659* / plasmid pSR659 without any mutation or digestion / colonies 4-6 of pSR659* / 100bp DNA ladder
A band appears in digested plasmids 1-5 which is not present in the plasmid not digested. This band must correspond to linearized plasmid.
For colony 6, a higher band appears.

The band correspondind to linearized plasmid in colonies 3 and 5 was cut and purified.

Gibson cloning

The Gibson cloning was done with the kit from NEB (https://www.neb.com/products/e5510-gibson-assembly-cloning-kit#tabselect0)

PP7 was inserted in pSR658 and MS2 in pSR659.
Because there was no band for MS2 on the gel done earlier today, the cloning was performed with MS2 from Pfu PCR (done today, called MS2 (Pfu)) and from Taq PCR (from 26/07, called MS2 (Taq)).
A positive control was performed with the control in the kit.

Gibson mix

	A	pSR658 - PP7	pSR659 - MS2 (Taq)	pSR659 - MS2 (Pfu)
1	Plasmid from miniprep	2.5µl	5µl	3µl
2	DNA from anchored PCR (MS2 or PP7)	4µl	5µl	7µl
3	Gibson mix from NEB	10µl	10µl	10µl
4	Water	3.5µl	0µl	0µl

It was incubated at 50°C in a thermocycler for 15 minutes.
2µl of the assembly reaction was then transformed in DH5α provided with the kit (30 min on ice, 45 sec @ 42°C, 5 min on ice, + 950µl LB, 60 min @ 37°C and 250 rpm, centrifuged 3 min @ 2000g, 100µl of pellet plated on LB agar + antibiotic)

In parallel, SU202 were transformed with the same protocol by 2µl of the the following plasmids:

- pSR658-PP7
- pSR659-MS2
- pKB822 or pKB1094 empty or pKB1094 theophylline+ or pKB1094 guanosine+

They were plated on LB agar + chloramphenicol + tetracycline + ampicilline + spectinomycine.
One positive control was also done.

VENDREDI 02/08/2019

Transformation results

Many DH5α bacteria grew for every transformations. They were stocked in the fridge.
No SU202 grew, except for the positive control.

pSR659 miniprep

Liquid cultures of pSR659 were purified with with Monarch kit (NEB).

Quantification of pSR659 miniprep b...

	A	B
1	Culture 1	10.2ng/µl
2	Culture 2	7.2ng/µl