iGEM Bordeaux 2015

Lab Notebook of July

01/07/2015

- Verification of PCR of Miniprep of BBa_B0015 (2X-Terminator) on Agarose Gel 3%

0,9 g agarose in 30 mL TAE 0,5X
1µl Blue Loader + 5 µL DNA
3µL of 2-Log DNA Ladder

Problems : we obtained several bands and the troubleshooting of iGEM said that it is normal.

- LB agar + Chloramphenicol

250 mL LB liquid (x2) + 5 g agar.
Autoclave.
Add **74** µL Chloramphenicol (10 µG/mL)
Plate 20 Petri Dishes.

- Resuspend pSB1C3-BBa_J04450 (RFP) of iGEM DNA Distribution Kit

- Resuspend with 10µL dH20 in Plate 4, well 4B.
- Wait 5min
- Transfer in Microcentrifuge Tube
- Storage at 4°C in DNA Vital box.

- Transformation of pSB1C3-BBa_J04450 (RFP) for iGEM BioBrick submission

- Transformation with 1µL of pSB1C3-BBa_J04450
- 50 µL of chimio-competent cells

- Spread 20 µL, 20 µL and 50 µL on agar LB+Chlororamphenicol (10 µg.mL-1)

- Pick selected colony of pSB1C3-BBa_J45992-CrdS + pSB3C5-BBa_J45992-CrdS

- Pick one colony
- Put them in 5 mL LB + Chloramphenicol (10 µg/mL)
- Incubate 37°C overnight

02/07/2015

- Miniprep on selected colony of pSB1C3-BBa_J45992-CrdS + pSB3C5-BBa_J45992-CrdS

Protocol: QIAprep Spin Miniprep Kit

- Verification miniprep with restriction digestion with EcoRI and PstI

Components	1 reaction
DNA	250ng (1μL)
dH2O	15 μL
NEB Buffer 2	2,5 μL
BSA	0,5 μL
EcoRI	0,5 μL
PstI	0,5 μL

Add 1µL DNA on one tube for each restriction digest.
Prepare a mix without DNA.
Mix well by pipetting slowly up and down.
Add 19 µL of Mix in each reaction tubes
Spin the samples briefly to collect all of the mixture to the bottom of the tube.
Incubate the restriction digestions at 37°C for 30 minutes, then 80°C for 20 minutes.

=> Migration on agarose gel 0,8%
60 mL TAE 0,5X + 0,48g agarose
2 µL BET
2 µL DNA Ladder 1 kb
1 µL Blue Loader + 5 µL DNA

- Dosage of Miniprep of pSB1C3-BBa_J45992-CrdS + pSB3C5-BBa_J45992-CrdS by Nanodrop

Clonage	ng/µL
pSB1C3	56,7
pSB1C3	61,3
pSB1C3	78,2
pSB1C3	54,6
pSB1C3	54,0
pSB1C3	63,5
pSB3C5	86,9
pSB3C5	94,4
pSB3C5	216,2
pSB3C5	110,7
pSB3C5	109,2
pSB3C5	54,7

03/07/2015

- Pick 3 Colonies of pSB1C3-BBa_J04450 (RFP) in LB liquid+Chloro

- Miniprep on Pick 3 Colony of pSB1C3-BBa_J04450 (RFP)

Protocol : QIAprep Spin Miniprep Kit

- Dosage on Nanodrop of miniprep pSB1C3-BBa_J04450 (RFP)

1/ 54,6 ng/µL
2/ 58,9 ng/µL
3/ 61,0 ng/µL

- BioBrick Assembly kit

Ligation :

- pSB1C3-BBa_I14033 + CrdS (IDT) PCR : nicknamed (C3 CrdS)
- pSB1C3-BBa_I14033 + BBa_J45992 (IDT) PCR : nicknamed (C3 P92)

06/07/2015

- PCR on colony of Ligation pSB1C3-BBa_I14033 + CrdS (IDT) PCR : nicknamed (C3 CrdS) and pSB1C3-BBa_I14033 + BBa_J45992 (IDT) PCR : nicknamed (C3 P92)

Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

Mix	28 reactions
ddH20	347,2 µL
5X Phusion GC Buffer	112 µL
10 mM dNTPs mix	11,2 µL
10 µM Forward Primer	28 µL
10 µM Reverse Primer	28 µL
Phusion DNApol	5,6 µL
TOTAL	532

5min 95°C

30sec 92°C
30sec 50°C
4 sec 72°C
| X 30

5min 72°C

- 10 colonies of Ligation pSB1C3-BBa_I14033 + BBa_J45992 (IDT)
- 14 colonies of Ligation pSB1C3-BBa_I14033 + CrdS (IDT) PCR
- 1 H20 tube
- 1 pSB1C3-BBa_I14033 (References control)

=> agarose gel 1% (0,6 g agarose + 60mL TAE 0,5X) for pSB1C3-BBa_I14033 + CrdS (IDT) PCR

=> agarose gel 3% (1,8 g agarose + 60mL TAE 0,5X) for pSB1C3-BBa_I14033 + BBa_J45992 (IDT)
GEL THOMAS !!!!!

- Transformation of Chemical-Bacteria with pUC-IDT-CrdS

50µL Chemical-bacteria
2 µL pUC-IDT-CrdS (10ng/µL)
Spread on LB+Amp (50µg/mL) 10µL (x2) and 20µL

- Purification PCR product of CrdS and BBa_J45992 with QIAquick PCR Purification Kit

We follow the Procedure written in the box.

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- Restrict Digestion

- **pSB1C3-BBa_J04450 (RFC)** => Triple digestion : EcoRI + XbaI + PstI

For Destination plasmid and to avoid auto-religation

- **pSB1C3-BBa_J04450 (RFC)** => Double digest : EcoRI + PstI

For Destination plasmid , plus one treatment with Alkaline Phosphatase to avoid auto-religation

- **Purif PCR CrdS** => Double digest : EcoRI + PstI

- **Purif PCR BBa_J45992** => Double digest : EcoRI + PstI

DIGESTION	50 µL reaction	
Plasmid (500ng)	X	µL
10X NEBuffer		5 µL
100 X BSA		0,5 µL
Enzyme 1 (10 units/µL)		1 µL
Enzyme 2 (10 units/µL)		1 µL
H20		50 µL

MIX	4,5 reactions
10X NEBuffer	22,5
10 X BSA	2,25
Enzyme 1 (10 units/µL)	4,5
Enzyme 2 (10 units/µL)	4,5

10µL of pSB1C3-RFP + 31,5 µL H2O
CrdS 28µL of PCR Purif
BBa_J45992 28 µL o PCR Purif

Problems : We don't have Antartica Phosphatase Buffer (NEB) and others bad luck.

07/07/2015

- Pre-culture of yeast cells:

1/ Complementation of YPD
To 1L of YPD medium must be added 55 mL of 2 M glucose.
We do it in 500 mL of medium thus 27.5 mL glc.

2/ Inoculation
Then in 50 mL tubes, is placed 25 mL of YPD medium + glc and yeast collected with a sterile pipette tip.
We put the tip in the tube. We have 3 tubes at the end.

Incubate tubes overnight at 30 ° C in a shaker incubator at 180 rpm.
The plate is stocked at 4°C.

- Pick colony of pYES2
We put 22 mL of LB + 22 µL of ampicillin.
4 tubes were incubated overnight at 37°C in an incubator shaker at 180 rpm.

- Transformation chemical competent cells with pYES3
Follow the protocol on page 9

- Pick a single selected colony of Bacteria with pUC-IDT-CrdS

We take a lot of colony with LB+Amp (22mL LB + 22µL Ampicillin 50mg/mL)

- Purification of Restriction Digest Purif PCR CrdS (EcoRI + PstI) and Purif PCR BBa_J45992 (EcoRI + PstI) on agarose gel

Agarose gel 1% (0,6g agarose + 60mL TAE 0,5X) for migration of Purif PCR CrdS (2030 bp after restrict)
Agarose gel 3% (1,8g agarose + 60mL TAE 0,5X) for migration of Purif PCR BBa_J45992 (264pb after restrict)

After migration : Excise the band of interest from agarose gel
FAIL : WE DON'T SEE BAND for crdS and we see very small band for promoter BBa_J45992 .

So we test, for the first time, the **GenElute Minus EtBr Spin Columns (Sigma)** on only promoter BBa_J45992.
We elute an unexpected volume.

- PCR amplification of crdS on pUC-IDT-crdS and BBa_J45992 (genBlock) 50µL

4 tubes for pUC-IDT-crdS at 1ng/µL (1µL at 10ng/µL in 9µL H20)
4 tubes for BBa_J45992 = 1ng/µL (1µL at 10ng/µL in 9µL H20)
1 tube for Water reference
VR and VF2

Component	50 µL reaction
ddH20	32,5 µL
5X Phusion GC Buffer	10 µL
10 mM dNTPs mix	1 µL
10 µM Forward Primer	2,5 µL
10 µM Reverse Primer	2,5 µL
Phusion DNApol	0,5 µL
DNA	1 µL
TOTAL	50

Mix	9,5 reactions
ddH20	308,75 µL
5X Phusion GC Buffer	95 µL
10 mM dNTPs mix	9,5 µL
10 µM Forward Primer	23,75 µL
10 µM Reverse Primer	23,75 µL
Phusion DNApol	4,75 µL
TOTAL	465,5

5min	95°C	
30sec	92°C	X 30
30sec	50°C	
2min 15sec	72°C	
5min	72°C	

08/07/2015

- Pick of selected colony with pEYS3

Pick 4 colonies in 5 mL of liquid LB + Ampicillin (50µg/mL)
22 mL LB + 22µL Ampicillin (50mg/mL)
Incubation at 37°C 180rpm overnight.

- Miniprep on pUC-IDT-crdS and pYES2

Problems => Centrifuge doesn't open
Protocol : **QIAprep Spin Miniprep Kit** (4 tubes store at 4°C)

- Dosage on nanodrop of miniprep of pUC-IDT-crdS and pYES2

Blank : EB Buffer

1/ pUC-IDT-crdS = 321,4 ng/µL
2/ pUC-IDT-crdS = 410,6 ng/µL
3/ pUC-IDT-crdS = 329,9 ng/µL
4/ pUC-IDT-crdS = 190,3 ng/µL

1/ pYES2 = 10,0 ng/µL
2/ pYES2 = 6,7 ng/µL
3/ pYES2 = 5,7 ng/µL
4/ pYES2 =11,9 ng/µL

- Preparation of stock solution of Agarose gel 1% + BET

200mL TAE 0,5X
2 g agarose
8 µL BET
Stock in 250 mL Flask with aluminium

- Restrict Digestion of pSB1C3-BBa_J04450 (RFP), pSB3C5-BBa_J04450 (RFP) and pUC-IDT-crdS with EcoRI + PstI

2µL of pSB3C5-BBa_J04450 (<300 ng/µL)
2µL of pUCIDT-crdS (<300 ng/µL)
10µL of pSB1C3-BBa_J04450 (50 ng/µL)

DIGESTION	**50 µL reaction**
Plasmid (500ng)	2 µL
10X NEBuffer	5 µL
100 X BSA	0,5 µL
Enzyme 1 (10 units/µL)	1 µL
Enzyme 2 (10 units/µL)	1 µL
H20	40,5 µL
TOTAL	50 µL

MIX	3,5 **reactions**
Add 7,5 µl by reaction tube	
10X NEBuffer	17,5 µL
10 X BSA	1,75 µL
Enzyme 1 (10 units/µL)	3,5 µL
Enzyme 2 (10 units/µL)	3,5 µL

1h at 37°C

09/07/2015

- Restrict Digestion of pSB1C3-BBa_I14033 (pCat) with SpeI+PstI and pUC-IDT-crdS with XbaI+PstI

2µL of pUCIDT-crdS (<300 ng/µL)
10µL of pSB1C3-BBa_I14033 (50 ng/µL)
Incubation 1h at 37°C.

DIGESTION	50 µL reaction
Plasmid (500ng)	2 µL
10X NEBuffer	5 µL
100 X BSA	0,5 µL
Enzyme 1 (10 units/µL)	1 µL
Enzyme 2 (10 units/µL)	1 µL
H20	40,5 µL
TOTAL	50 µL

- Miniprep on pYES3

Protocol : **QIAprep Spin Miniprep Kit**

- Quantification Nanodrop of miniprep of pYES3

Blank = EB Buffer
1/ 16,4 ng/µL
2/ 18,5 ng/µL
3/ 20,6 ng/µL
4/ 22,9 ng/µL

- Verification of First Test gel purification with GenElute Minus EtBr Spin Columns (Sigma) on agarose gel 3%

1µL 2-Log DNA Ladder
40 µL Purification of promoter BBa_J45992 + 4 µL Blue Loading Dye

Result : NOTHING on the gel

- Purification on agarose gel of pSB1C3-BBa_J04450 (EcoRI + PstI), pSB3C5-BBa_J04450 (EcoRI + PstI), pUC-IDT-crdS (EcoRI + PstI) and pUC-IDT-crdS (XbaI + PstI)

Protocol : **GenElute Minus EtBr Spin Columns**
We Keep :
- pSB1C3 (EcoRI + PstI)
- pSB3C5 (EcoRI + PstI)
- crdS (EcoRI + PstI)
- crdS (XbaI + PstI)

+ DNA precipitation of each DNA eluted.

10/07/2015

- Quantification Nanodrop of pSB1C3 (EcoRI + PstI), pSB3C5 (EcoRI + PstI), crdS (EcoRI + PstI)

Blank = dH20
- pSB1C3 (EcoRI + PstI) = 1,2 ng/µL
- pSB3C5 (EcoRI + PstI) = 2,1 ng/µL
- crdS (EcoRI + PstI) = 0,2 ng/µL
- crdS (XbaI + PstI) = 1,6 ng/µL

- Ligation to obtain pSB1C3-CrdS, pSB3C5-CrdS and pSB1C3-BBa_I14033-CrdS (pCat)

Plasmid Destination	Dowstream Part	Result
- pSB1C3 (EcoRI + PstI) purified part	- CrdS (EcoRI + PstI) purified part	pSB1C3-CrdS
- pSB3C5 (EcoRI + PstI) purified part	- CrdS (EcoRI + PstI) purified part	pSB3C5-CrdS
- pSB1C3-BBa_I14033 (SpeI + PstI) No purified part	- CrdS (XbaI + PstI) purified part	pSB1C3-BBa_I14033-CrdS
- pSB1C3 (EcoRI + PstI) No purified part	- CrdS (EcoRI + PstI) No purified part	pSB1C3-CrdS
- pSB3C5 (EcoRI + PstI) No purified part	- CrdS (EcoRI + PstI) No purified part	pSB3C5-CrdS
- pSB1C3-BBa_I14033 (SpeI + PstI) No purified part	- CrdS (XbaI + PstI) No purified part	pSB1C3-BBa_I14033-CrdS

- Add 11 µL of H20 to tube.
- Add 2 µL from each of the digests to the tube. (except for crdS (EcoRI + PstI), we add 4µL because we have a weak concentration)
- Add 2 µL of the 10X T4 DNA Ligase Reaction Buffer to the tube.
- Add 1 µL of the T4 DNA Ligase to the tube

- Transformation of Ligate plasmid with chemical competent cells

Protocol : Heat-Shock Transformation of chemically competent bacteria
2µL of each ligation + 50µL competent cells

- Purification of 2 BBa_J45992 (OsmY) PCR purification with QIAquick PCR Purification Kit (QIAgen)

Protocol : **QIAquick PCR Purification Kit (QIAgen)**

- Restrict Digestion of BBa_J45992 (EcoRI+SpeI), pUC-IDT-CrdS (XbaI+PstI), pSB1C3-BBa_J04450 (EcoRI+PstI) and pSB3C5-BBa_J04450 (EcoRI+PstI)

Protocol : **Digestion using restriction enzyme**

➡ We try to use more DNA for digestion to increase DNA concentration (after purification) at the end for ligation

DNA	Origin	Concentration	Using volume to obtain 1 µg	Restriction Enzyme
BBa_J45992	PCR Purification	100 ng/µL	5 µL	EcoRI + SpeI
pUC-IDT-CrdS	Miniprep	250 ng/µL	4 µL	XbaI + PstI
pSB1C3-BBa_J04450	Miniprep	50 ng/µL	20 µL	EcoRI + PstI
pSB3C5-BBa_J04450	Miniprep	300 ng/µL	4 µL	EcoRI + PstI

- Purification of Restriction Digest on agarose gel

➡ We have 50 µL of digest mix, we use 40 µL on agarose gel (2 well with 20 µL) and we keep 10µL for ligation without purification

Agarose gel 1% (0,6g agarose + 60mL TAE 0,5X) for migration of BBa_J45992
Agarose gel 3% (1,8g agarose + 60mL TAE 0,5X) for migration of the other digestions

FAIL : WE DON'T SEE 2 BANDS for crdS and the other bands are strange (like if the DNA hasn't migrated).

=> We wasted all samples.

- Extraction yeast DNA (YEAST TEAM)

Procedure :

1. 200 µL liquid yeast culture (OD600=0.4). Suspend cells in 100 µL 200 mM LiOAc, 1% SDS solution.

2. Incubate for 5 min at 70°C. To 11h01 16s from 11h06 16s

3. Add 300 µL 96–100% ethanol, then vortex.

4. Spin down DNA and cell debris at 15,000× g for 3 min.

5. Wash pellet with 70% ethanol.

6. Dissolve pellet in 100 µL H2O or TE and spin down cell debris for 15 s at 15,000× g.

surnageant were removed (200uL) in for tubes Y1-4
then tube Y1-4 were dosed in **nanodrop**

Tube	ADN ng/uL	260/280	260/230
Y1	146,7	1,76	0,70
Y2	160,4	1,78	0,73
Y3	157,8	1,77	0,70
Y4	162,6	1,77	0,71

260/280<1,9 slightly protein contamination
260/230>2 No organic molecule protein

- Second DNA yeast

1. 100 µL liquid yeast culture (OD600=0.4). Suspend cells in 100 µL 200 mM LiOAc, 1% SDS solution.

2. Incubate for 5 min at 70°C. To 11h01 16s from 11h06 16s

3. Add 300 µL 96–100% ethanol, then vortex.

4. Spin down DNA and cell debris at 15,000× g for 3 min.

5. Wash pellet with 70% ethanol.

6. Dissolve pellet in 100 µL H2O or TE and spin down cell debris for 15 s at 15,000× g.

surnageant were removed (100uL) in two tubes Y5-6

Tube	ADN ng/uL	260/280	260/230
Y5	72,6	1,86	0,88
Y6	90,5	1,78	0,73

then tube Y1-4 were dosed in nanodrop

260/280 < 1,9 slightly protein contamination
260/230 > 2 No organic molecule protein

- Miniprep of PYES2 and PYES3

from bacteria culure in bacto tube 5mL medium +amph each
each tube were dosed at nanodrop

Tube	ADN ng/uL
PYES2	6,7 ng/uL
PYES2	8,8
PYES2	10,4
PYES2	5,4
PYES3	3,1
PYES3	10,0
PYES3	7,3
PYES3	5,2

13/07/2015

- PCR on colony of Ligation (10/07) pSB1C3-CrdS, pSB3C5-CrdS and pSB1C3-pcat-CrdS

On Ligation of purified and Non purified digestions
29 colonies + 1 H2O
Mix for 32 reactions

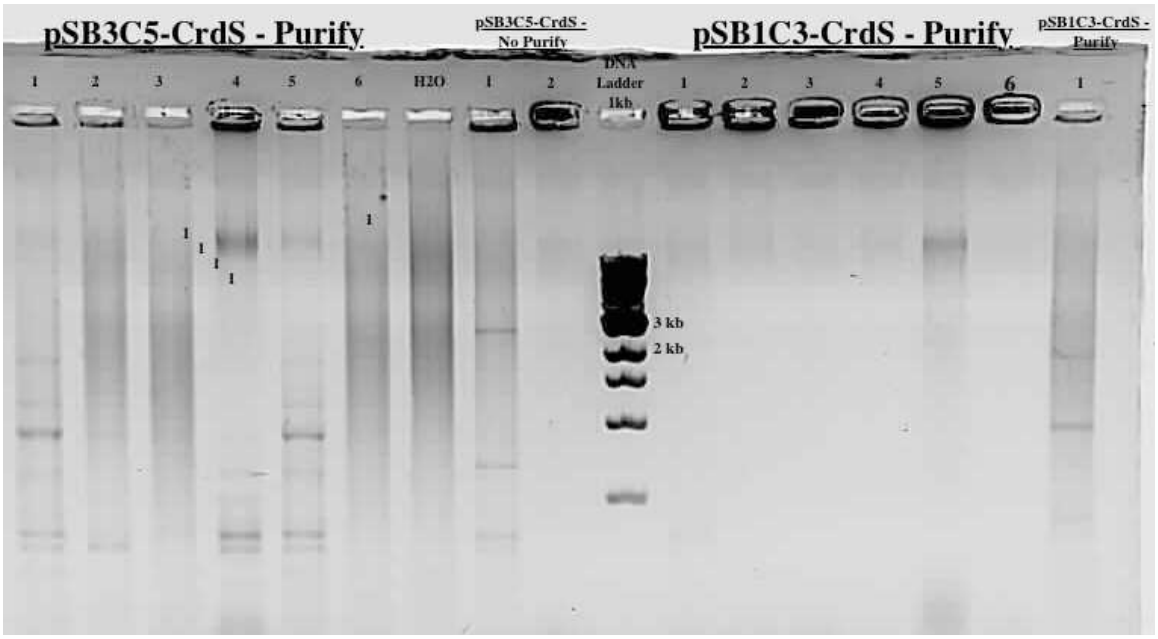
Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

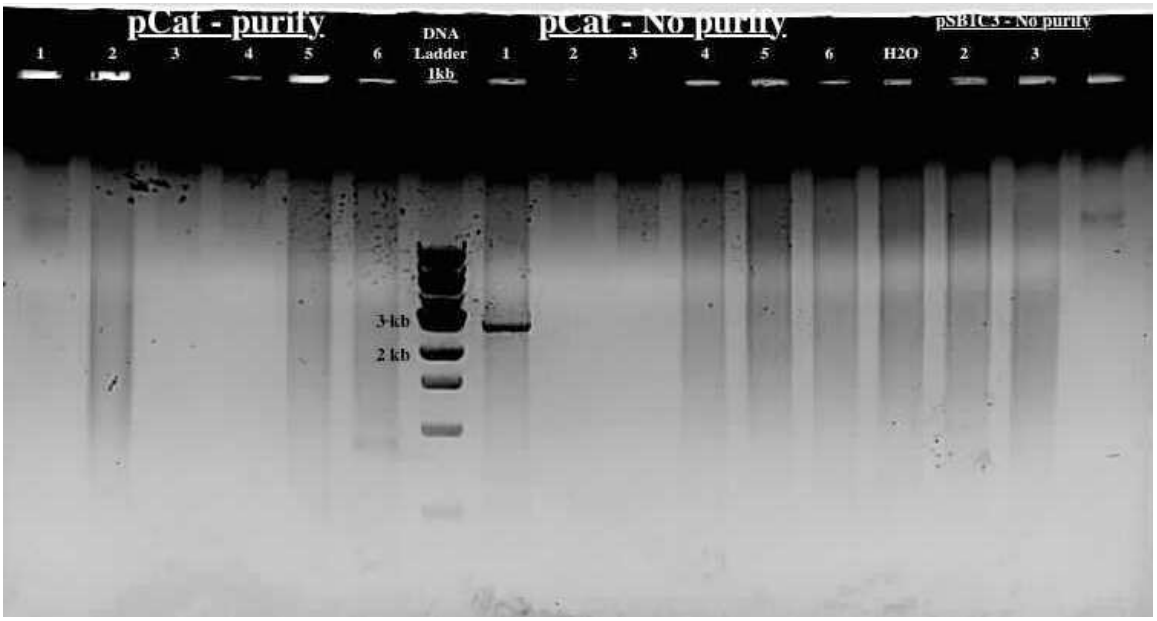
Mix	32 reactions
ddH20	396,8 µL
5X Phusion GC Buffer	128 µL
10 mM dNTPs mix	12,8 µL
10 µM Forward Primer	32 µL
10 µM Reverse Primer	32 µL
Phusion DNApol	6,4 µL
TOTAL	608

5min 95°C

30sec 92°C
30sec 50°C
2min 30sec 72°C
X 30

5min 72°C





- Agarose gel Verification of PCR on colony

Agarose gel 1%
10µL PCR Product + 1µL of 10X Loading Blue Buffer
1µL DNA Ladder 1kb

- Pick « positive ligation » colony in LB+Chloramphenicol

Each colony has been picked in 5mL LB+ Chloramphenicol (Final 10µg/mL)

- Curdlan Extraction of Yeast (YEAST TEAM)

Remarks : yeast conservation 1mL medium with yeast in fridge

Two tubes
100 µL from yeast culture + 100uL 200mM LiOAc, 1% SDS

Dry bath 70°C for 5 min (from 12h12 to 12h18)

+100uL NaOH 0,5M

For two other tube same procedure but we had a step

100 µL from yeast culture + 100uL 200mM LiOAc, 1% SDS

Dry bath 70°C for 5 min (from 12h12 to 12h18)

Add 300 µL 96–100% ethanol, then vortex.

Surnagent were removed and keep for following assay.

- Bacterial culture with pYES3 (YEAST TEAM)

4 tubes with 5 mL medium +ampicilin

14/07/2015

- Yeast Curdlan Assay

Prepare DYE MIX :
630 µL HCl
1770 µL buffer 1M glycine NaOH 1M pH 9,5
1200 µL aniline blue 0,1% 0,1g in 10 mL

1) Diluted sample 10-50-fold with1 N NaOH to a final volume of 300 µL in a 1.5 mL microcentrifuge
tube.
2) Add 30 µL of 6 N NaOH
3) Incubated at 80 °C for 30 min.
4) The tube was immediately put on an ice bath. Then freezer

- Miniprep on picked « positive ligation » colony

Protocol **: QIAprep Spin Miniprep Kit**

- Miniprep verification on agarose gel by restriction digestion with EcoRI+PstI

10 miniprep
+ pSB1C3-RFP : Digestion by EcoRI+PstI
+ pSB1C3-RFP : No digest control
+ pSB3C5-RFP : Digestion by EcoRI+PstI
+ pSB3C5-RFP : No digest control

15/07/2015

- Recovery of petri dishes on the fridge to restart different cultures.

2 mix prepared :
- 22mL of LB containing 22µL of Ampicilline at 50µg/mL
- 42mL of LB containing 12µL of Chloramphenicol at 10µg/mL

Picked some colonies in 5mL of LB+antibiotic

- 4 tubes of LB+Ampicilline containing pUC-IDT crdS
- 4 tubes of LB+Chloramphenicol containing psB3C5-RFP
- 4 tubes of LB+Chloramphenicol containing psB1C3-RFP

=> put on the incubator at 37°C, 180 rpm

- Purification of 2 CrdS PCR products containing crdS (PCR made the 07/07) with QIAquick PCR Purification Kit (QIAgen)

- Recovery in the fridge of 2 PCR products containing BBa_J45992 (OsmY) already purified (purification made the 10/07 / PCR made the 07/07)
- Store at 4°C

- Determination of the concentration in each tube with the Nanodrop

1. crdS : 119,4ng/µL
2. crdS : 118,7ng/µL
1. BBa_J45992 (OsmY) : 132,9ng/µL
2. BBa_J45992 (OsmY) : 163,8ng/µL

- Preparation of DYE Mix for curdlan dosage (YEAST TEAM)

DYE Mix (10 mL) : 1 890 µL HCl 1 N
5 310 µL Glycine NaOH Buffer pH 9,5
2 800 µL Aniline blue
Aniline blue (10 mL) : 0,01 g Aniline blue
10 mL H_2O

- MEGA DIGESTION TEST X2 (Hiba and Jean)

DNA	Digestion Buffer	Dephosphorylation	Extraction Kit	Tube n°
pSB1C3-BBa_I14033 (pCat)	NEBuffer 2.1	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	1
pSB1C3-BBa_I14033 (pCat)	NEBuffer 2.1	No	GelElute Extraction Kit (5PRIME)	2
pSB1C3-BBa_I14033 (pCat)	NEBuffer 2.1	Yes	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	3
pSB1C3-BBa_I14033 (pCat)	NEBuffer 2.1	Yes	GelElute Extraction Kit (5PRIME)	4
pSB1C3-BBa_I14033 (pCat)	CutSmart	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	5
pSB1C3-BBa_I14033 (pCat)	CutSmart	No	GelElute Extraction Kit (5PRIME)	6
pSB1C3-BBa_I14033 (pCat)	CutSmart	Yes	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	7
pSB1C3-BBa_I14033 (pCat)	CutSmart	Yes	GelElute Extraction Kit (5PRIME)	8
pSB3C5-BBa_J04450 (RFP)	NEBuffer 2.1	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	9
pSB3C5-BBa_J04450 (RFP)	NEBuffer 2.1	No	GelElute Extraction Kit (5PRIME)	10
pSB3C5-BBa_J04450 (RFP)	NEBuffer 2.1	Yes	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	11
pSB3C5-BBa_J04450 (RFP)	NEBuffer 2.1	Yes	GelElute Extraction Kit (5PRIME)	12
pSB3C5-BBa_J04450 (RFP)	CutSmart	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	13
pSB3C5-BBa_J04450 (RFP)	CutSmart	No	GelElute Extraction Kit (5PRIME)	14
pSB3C5-BBa_J04450 (RFP)	CutSmart	Yes	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	15
pSB3C5-BBa_J04450 (RFP)	CutSmart	Yes	GelElute Extraction Kit (5PRIME)	16
pUC-IDT-CrdS	NEBuffer 2.1	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	17
pUC-IDT-CrdS	NEBuffer 2.1	No	GelElute Extraction Kit (5PRIME)	18
pUC-IDT-CrdS	CutSmart	No	GenElute Minus EtBr Spin Columns (SIGMA-ALDRICH)	19
pUC-IDT-CrdS	CutSmart	No	GelElute Extraction Kit (5PRIME)	20

16/07/2015

- Purification on Agarose Gel 1% with MEGA DIGESTION

5 Agarose gels
2 wells for each Digestion Product

- We use **GelElute Extraction Kit of 5PRIME**
- We use **GenElute Minus EtBr Spin Columns** of SIGMA-ALDRICH + DNA Precipitation with (without wash with 70% Ethanol).

- Miniprep on Restart colony with pSB1C3-RFP, pSB3C5-RFP and pUC-IDT-CrdS

with QIAprep Spin Miniprep Kit

=> determination of concentrations with nanodrop

psB1C3-RFP 1: 87 ng/µL	psB3C5-RFP 1: 100,4 ng/µL	pUC IDT crdS 1: 228,9 ng/µL
psB1C3-RFP 2: 99,7 ng/µL	psB3C5-RFP 2: 120,2 ng/µL	pUC IDT crdS 2: 150,9 ng/µL
psB1C3-RFP 3: 107,6 ng/µL	psB3C5-RFP 3: 98,5 ng/µL	pUC IDT crdS 3: 195,2 ng/µL
psB1C3-RFP 4: 107,1 ng/µL	psB3C5-RFP 4: 57,8 ng/µL	pUC IDT crdS 4: 251,8 ng/µL

- Digestion with Restriction enzyme of PCR CrdS (purify), PCR OsmY (purify), pSB1C3-RFP (miniprep), pSB3C5-RFP (miniprep) and pUC-IDT-CrdS (miniprep).

5 tubes prepared :

- psB1C3-RFP 1: 87 ng/µL

=> 34,5µL (3µg) + 5µL of NEB buffer + 1µL of EcoRI-HF + 1µL of PstI qsp 50µL H2O

- psB3C5-RFP 4: 57,8 ng/µL

=> 22,9µL (2µg) + 5µL of NEB buffer + 1µL of EcoRI-HF + 1µL of PstI qsp 50µL H2O

- pUC IDT crdS 2: 150,9 ng/µL

=> 39,7µL (6µg) + 5µL of NEB buffer + 1µL of XbaI + 1µL of PstI qsp 50µL H2O

- Purified PCR product of crdS 2: 118,7 ng/µL

=> 42,1µL (5µg) + 5µL of NEB buffer + 1µL of XbaI + 1µL of PstI qsp 50µL H2O

- Purified PCR product of OsmY 1: 132,9 ng/µL

=> 37,6µL (5µg) + 5µL of NEB buffer + 1µL of EcoRI-HF + 1µL of SpeI qsp 50µL H2O

=> put on the incubator at 37°C, overnight

- Purification with the sigma kit of tubes Jean 18, Jean 20 et Hiba 18, Hiba 20

17/07/2015

- Nanodrop quantification of DNA Digest purification

- Ligation of Mega Digestion (Hiba and Jean)

Ligation Reaction in 10µL with Insert:Vector ratio = 4:1
1µL of 10X T4 Ligase Buffer
0,5µL of T4 Ligase
DNA Vector X µL
DNA Insert X µL
H2O qsp 8,5µL

DNA	Tube n°	Volume	DNA Quantity
Purify pSB1C3 Dephosphorylate	4 : Jean	2 µL	6 ng
Purify CrdS	17 : Jean	6 µL	24 ng
Purify pSB1C3 **No** Dephosphorylate	2 : Jean	2 µL	6 ng
Purify CrdS	19 : Jean	6 µL	24 ng
Purify pSB3C5 Dephosphorylate	1		
Purify CrdS			
Purify pSB3C5 **No** Dephosphorylate			
Purify CrdS			

- Nanodrop dosage of tubes Jean18, Jean 20 et Hiba 18, Hiba 20

Jean 18: 9,1ng/µL
Jean 20: 4,3ng/µL
Hiba 18: 10,9ng/µL
Hiba 20: 4,7ng/µL

- Dephosphorylation of digested plasmid samples (digestion done the 16/07)

- psB1C3
- psB3C5

add 5µL of antartic dephosphorylase and 1µL of antartic buffer to samples
put at 37°C during 1h
put at 80°C during 20min

- Curdlan quantification

Protocol :

Diluted sample 10-50-fold with1 N NaOH to a final volume of 300 µL in a 1.5 mL microcentrifuge tube.
Add 30 µL of 6 N NaOH
Incubated at 80 °C for 30 min.
The tube was immediately put on an ice bath.
Add 630 µL DYE MIX* into the tube and mix
Incubated at 50 °C for 30 min
The unbound fluorescent dye was decolorized at room temperature for 30 min
Mesure the fluorescence intensity with a fluorescence spectrophotometer

- emission wavelength of 502 nm (20 nm slit width) / excitation wavelength of 398 nm (20 nm slit width).

10 samples of yeast culture (5 sanitation lysis + 5 chemical lysis)
+ standard range (5 ; 10 ; 15 ;20 ; 30 µg/mL)

Results and analysis :

Best results with chemical lysis : 80 µg/mL vs 750 ng/mL with sonication.

Next step : curdlan purification

20/07/2015

- **Purification on 1% agarose gel Digestion with Restriction enzyme of pSB1C3-RFP (miniprep digest with EcoRI+PstI), pSB3C5-RFP (miniprepdigest with EcoRI+PstI) and pUC-IDT-CrdS (miniprep).**

We use GenElute Minus EtBr Spin Columns of SIGMA-ALDRICH + DNA Precipitation with (without wash with 70% Ethanol).

21/07/2015

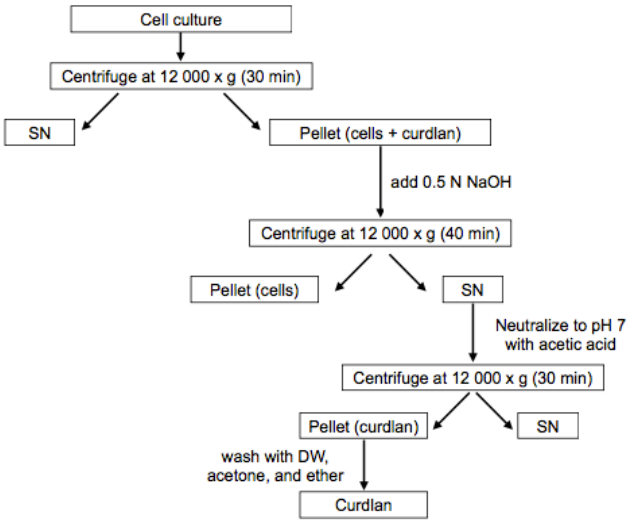
- Curdlan Purification (YEAST TEAM)

Curdlan purification according to 2 protocols with 2 x 2 mLof yeast lysis culture (yeast culture + equal volume of LiOAc 200mM, SDS 1 %)

Protocol 1 :

- The culture was mixed with an equal volume of 1 N NaOH and then centrifuged at 10,000 rpm for 10min to remove the cells.
- The resulting supernatant was neutralized by adding 3 N HCl.
- The precipitate formed was collected by centrifugation at 10,000rpm for 10min.
- It was washed three times with water by centrifugation, and then dehydrated with acetone.
- This precipitated polymer is the curdlan-type polysaccharide.

Protocol 2 :



Results and analysis : We have a precipitate with only protocol 1 so we keep it.

22/07/2015

- Curdlan Purification (YEAST TEAM)

Curdlan purification with protocol 1. 8 x 1 mL eppendorf chemical yeast lysis.

23/07/2015

- Clonage of pUC CrdS + OsmY

1) Digest on Miniprep pUC CrdS (1ng) and purified PCR product of OsmY (1ng)

- Miniprep pUC CrdS : 10µL (150ng/µL)

NEB Buffer 2,1 : 5µL
EcoRI : 1µL
XbaI : 1µL
H2O : 33µL

- Purified PCR product of OsmY : 10µL (150ng/µL)
NEB Buffer 2,1: 5µL
EcoRI: 1µL
SpeI: 1µL

=> 3h at 37°C

2) Dephosphorylation on pUC CrdS

- Add 5µL Antartica Buffer 10X and 1µL Antartica phosphatase

=> 1h at 37°C
=> Deactivation 10min at 80°C

3) Ligation

T4 buffer 10X: 1µL
T4 ligase : 0,5µL
OsmY : 7,25µL
pUC CrdS : 1,25µL

=> Put at 4°C overnight

24/07/2015

- Nanodrop quantification of Mega-Digestion :

NP2 n°1: 2399,9ng/µL	P1 50 n°5: 1398,9ng/µL
NP3 n°1: 6868,3	P1 200 n°3: 32,5ng/µL
NP3 n°2: 9471,7ng/µL	P1 200 n°1: 1217,3ng/µL
NP2 n°2: 869,9ng/µL	P3 50 n°1: 33,3ng/µL
NP2 n°5: 42,7ng/µL	P3 50 n°4: 47,4ng/µL
P3 200 n°2: 21,4ng/µL	psB3C5 n°1: 7580ng/µL
P3 200 n°4: 13,8ng/µL	psB3C5 n°2: 7352ng/µL
P3 200 n°5: 50,4ng/µL	psB1C3 n°1: 561,8ng/µL
P4 50 n°4: 4142ng/µL	psB1C3 n°2: 7980,5ng/µL
P1 50 n°2: 508,3ng/µL	NP4 50 n°2: 4935,7ng/µL
P1 50 n°3: 52,2ng/µL	NP4 50 n°5: 2955,9ng/µL

=> Results unusable because Homemade miniprep contain too many salt = non significant results

- Miniprep verification on agarose gel

- Digestion

Mix prepared for 22,5 reactions :
- 112,5µL of NEB Buffer
- 22,5µL of EcoRI
- 22,5µL of PstI

In 22 tubes =>
Mix: 7,5µL
DNA: 5µL
H2O: 37,5µL

=> 2h at 37°C

- 1% Agarose Gel

Winners are:
Gel 1: NP3 n°1 and NP3 n°2
Gel 2: B1C3 n°1, B1C3 n°2, NP4 50 n°2, B3C5 n°1 and B3C5 n°2

NP3 n°1: non purified psB1C3+CrdS	B1C3 n°1: purified psB1C3+CrdS
NP3 n°2: non purified psB1C3+CrdS	B1C3 n°2: purified psB1C3+CrdS
NP4 n°2: non purified psB3C5+CrdS	B3C5 n°1: purified psB3C5+CrdS
	B3C5 n°2: purified psB3C5+CrdS

- Transformation of pUC CrdS + OsmY

Same procedure that 23/07/2015 (p 23).

- Transformation of pUC IDT CrdS / pcat / psB1C3-RFP / psB3C5-RFP

Same procedure that 23/07/2015 (p 23).

27/07/2015

- PCR amplification on:

- NP3 1
- NP3 2
- NP4 (50µL) 2
- B1C3 1
- B1C3 2
- B3C5 1
- B3C5 2

Each condition is diluted to 1/10, 1/100, 1/1000

21 tubes prepared + 1 control tube (with H2O to replace DNA)

Mix	22	reactions
ddH20	272,8	µL
5X Phusion GC Buffer	88	µL
10 mM dNTPs mix	8,8	µL
10 µM Forward Primer	22	µL
10 µM Reverse Primer	22	µL
Phusion DNApol	4,4	µL
TOTAL	418	

=> 19 µL of mix/tube + 1µL of DNA

5min at 95°C

Cycles (X 30)

30sec at 92°C
30sec at 50°C
2min 30sec at 72°C

5min at 72°C

- PCR purification

- Pick selected colony

- 4 tubes of bacteria containing pUC IDT CrdS + OsmY
- 2 tubes of bacteria containing pUC IDT CrdS

=> In LB medium + Ampicillin

- 2 tubes of bacteria containing B1C3-RFP
- 2 tubes of bacteria containing B3C5-RFP

- 2 tubes of bacteria containing pcat

=> In LB medium + Chloramphenicol

2 mix prepared:
- 32ml of LB medium + 32µL of Ampicillin (50µg/mL)
- 32mL of LB medium + 9,4µL of Chloramphenicol (10µg/mL)

=> 5mL/tube + colonies

=> Put on the incubator at 37°C, 180 rpm for 24h

- Miniprep of Pick selected colony

Protocol: QIAprep Spin Miniprep Kit

- Recovery of 4 tubes containing psB1C3+OsmY+CrdS
- Recovery of 4 tubes containing psB3C5+OsmY+CrdS

- Yeast Curdlan Purification (YEAST TEAM)

Curdlan purification with protocol 1. 8 x 1 mL eppendorf chemical yeast lysis. Neutralization with 38 % HCl for a better precipitation. It's easier to separate the supernatant and precipitate by centrifugation 5 min 10 000 rpm.

28/07/2015

- Nanodrop quantification of psB1C3+OsmY+CrdS and psB3C5+OsmY+CrdS:

Recovery of 7 tubes (miniprep done the 27/07)

B1C3 n°1 : 14,1ng/µL	B3C5 n°1 : 16,7ng/µL
B1C3 n°2 : 12,4ng/µL	B3C5 n°2 : 14,2ng/µL
B1C3 n°3 : 15,3ng/µL	B3C5 n°3 : 12,7ng/µL
B1C3 n°4 : 18,4ng/µL	

- Miniprep of Pick selected colony

Protocol: QIAprep Spin Miniprep Kit

- Recovery of 4 tubes containing pUC-CrdS+OsmY
- Recovery of 2 tubes containing psB1C3-RFP
- Recovery of 2 tubes containing psB3C5-RFP
- Recovery of 2 tubes containing pUC-CrdS
- Recovery of 2 tubes containing pcat

- Nanodrop quantification:

Recovery of 12 tubes (miniprep done the 28/07)

pUC-CrdS+p92 n°1: 49,8ng/µL	pUC-CrdS+p92 n°3: 2,7ng/µL
pUC-CrdS+p92 n°2: 164,4ng/µL	pUC-CrdS+p92 n°4: 52,9ng/µL
psB1C3 n°1: 35,8ng/µL	pUC-CrdS n°1: 274ng/µL
psB1C3 n°2: 53,3ng/µL	pUC-CrdS n°2: 173,6ng/µL
psB3C5 n°1: 17,7ng/µL	pcat n°1: 62,3ng/µL
psB3C5 n°2: 21,1ng/µL	pcat n°2: 37ng/µL

- Miniprep verification on agarose gel

Digestion :

Mix prepared for 11,5 reactions :
- 57,5µL of NEB Buffer
- 11,5µL of EcoRI
- 11,5µL of PstI

In 11 tubes =>
Mix: 7,5µL
DNA: 5µL
H2O: 37,5µL

=> 1h30 at 37°C

1% Agarose Gel :

- Nanodrop quantification of purified PCR products:

-Recovery of 21 tubes (done the 27/07)

Dilution	NP3 1	NP3 2	NP4 2	B1C3 1	B1C3 2	B3C5 1	B3C5 2
1/10	43,2ng/µL	31,3ng/µL	47,6ng/µL	36,7ng/µL	29ng/µL	42,1ng/µL	18,8ng/µL
1/100	17,7ng/µL	32,1ng/µL	51,7ng/µL	64ng/µL	25,4ng/µL	35,2ng/µL	43,2ng/µL
1/1000	50,1ng/µL	47ng/µL	47,2ng/µL	46,8ng/µL	18,3ng/µL	19,4ng/µL	33,4ng/µL

1% Agarose Gel

Put photos

29/07/2015

1% Agarose Gel

=> Because I did shit yesterday!!!!!!!!!

- Miniprep verification on agarose gel

Digestion

Mix prepared for 7,5 reactions :
- 37,5µL of NEB Buffer
- 7,5µL of EcoRI
- 7,5µL of PstI

In 7 tubes :
Mix: 7,5µL
DNA: 5µL
H2O: 37,5µL

=> 2h at 37°C

- PCR amplification on:

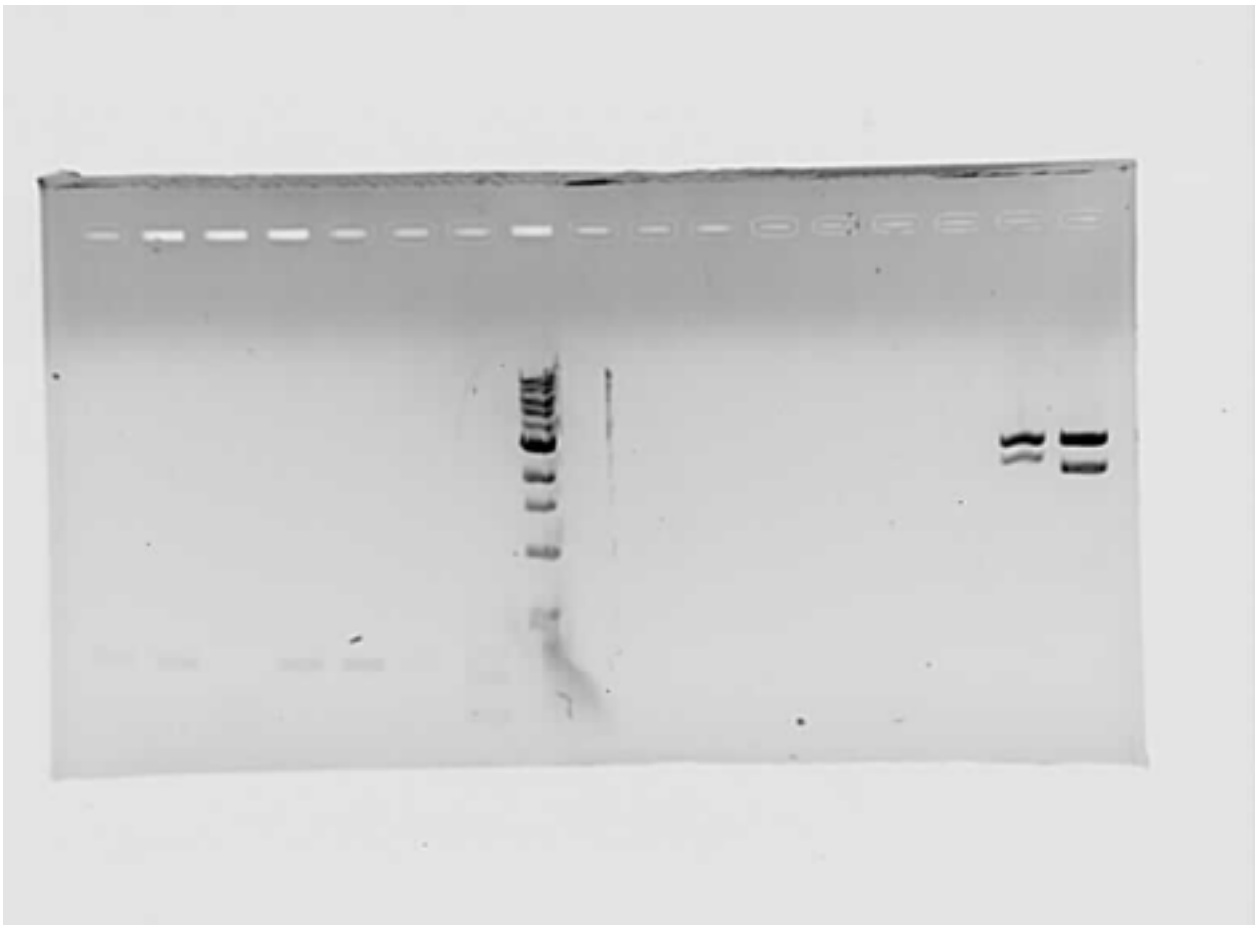
- NP3 1
- NP3 2
- NP4 2
- B1C3 1
- B1C3 2
- B3C5 1
- B3C5 2

=> Recovery of tubes diluted at 1/1000

Mix	8	reactions
ddH20	99,2	µL
5X Phusion GC Buffer	32	µL
10 mM dNTPs mix	3,2	µL
10 µM Forward Primer	8	µL
10 µM Reverse Primer	8	µL
Phusion DNApol	1,6	µL
TOTAL	152	µL

Procedure		
30sec	98°C	
10sec	98°C	
30sec	50°C	X 30
30sec	72°C	
5min	72°C	

- 1% Agarose Gel



30/07/2015

- Transformations:

- Recovery of miniprep tube corresponding to pUC-CrdS+OsmY n°2
- Recovery of miniprep tube corresponding to psB1C3+OsmY+CrdS n°4
- Recovery of miniprep tube corresponding to psB3C5+OsmY+CrdS n°1
- Recovery of miniprep tube corresponding to psB1C3+CrdS n°1
- Recovery of miniprep tube corresponding to psB3C5+CrdS n°1

- Transformation protocol

=> Transformed cells spread on 10 petri dishes containing:
- LB+Chloramphenicol for cells with psB1C3 or psB3C5 plasmid (8 petri dishes)
- LB+Ampicillin for cells with pUC plasmid (2 petri dishes)

Put on the incubator at 37°C overnight

31/072015

- Pick selected colony

- X tubes of 1 colony containing pUC-CrdS + OsmY
=> In LB medium + Ampicillin
- X tubes of 1 colony containing psB1C3+OsmY+CrdS n°4
- X tubes of 1 colony containing psB3C5+OsmY+CrdS n°1
- X tubes of 1 colony containing psB1C3+CrdS n°1
- X tubes of 1 colony containing psB3C5+CrdS n°1
=> In LB medium + Chloramphenicol

Put on the incubator at 37°C for 2 days (weekend)

42 mL de LB
12µL de Chloramphenicol

Hiba remade transformations because we had some dishes problems…

- Yeast Curdlan Purification (YEAST TEAM)

Curdlan purification according to protocol 1 modified : in order to have a larger volume of yeast culture purified, we try the protocol on a different spin down machine. All the previous spin down are now at 15 min, 4 700 rpm and we use 38 % HCl to neutralize the solution with a pH meter.