pMCY Enzyme charaterization

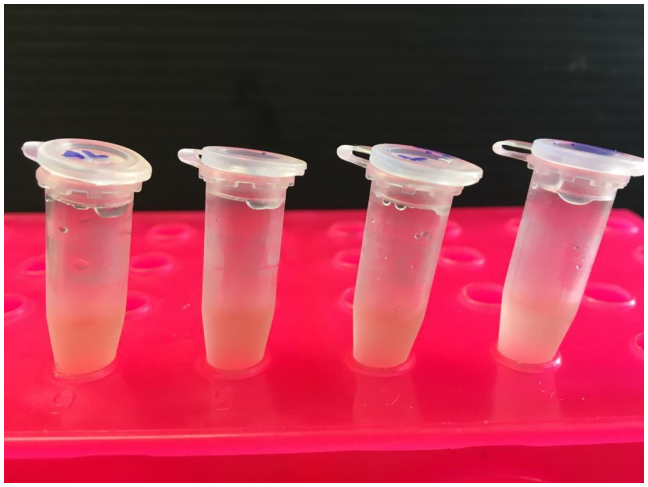
Project: iGEM UANL 2019 Shared Project

Authors: J. Claudio Moreno-Rocha

TUESDAY, 7/23/2019

pMCY30 and pMCY90 DNA miniPrep:

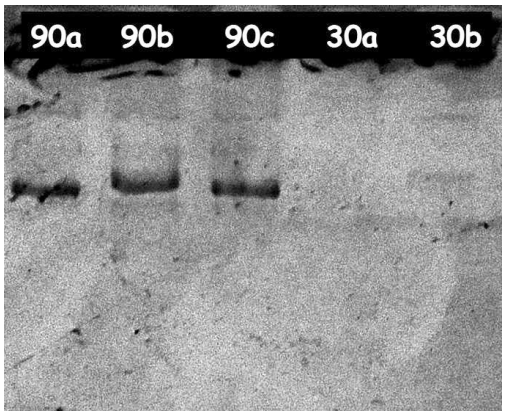
Miniprepstubes.jpeg



LB media plus antibotic

WEDNESDAY, 7/24/2019

Gel1 9030Jul21b.jpg



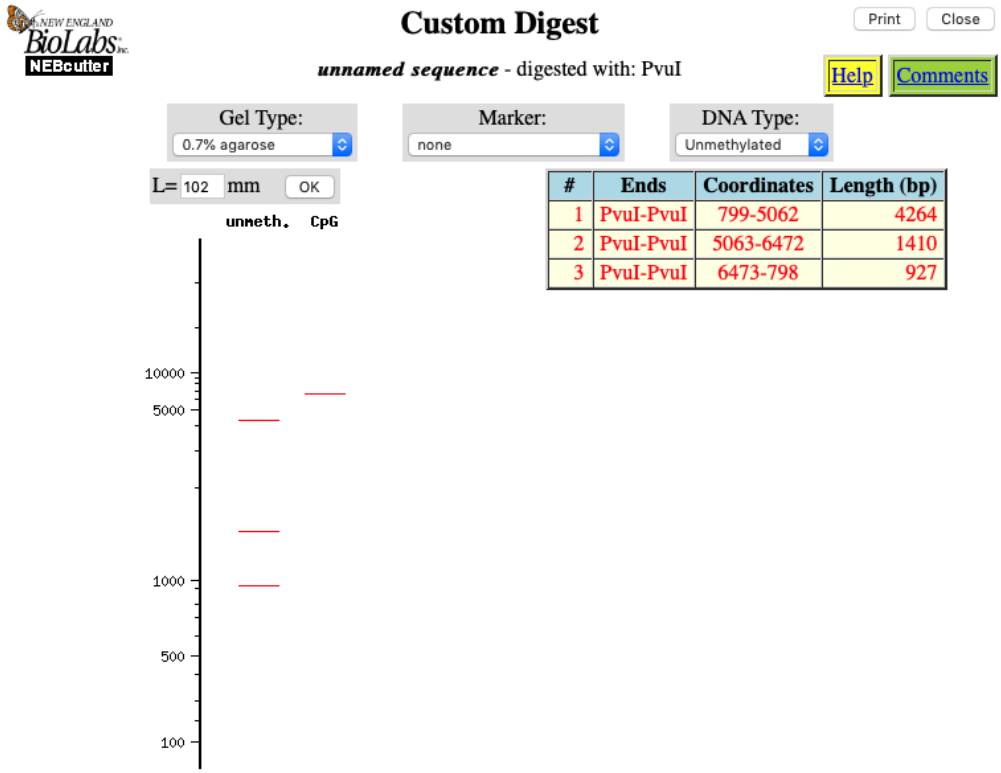
GEL1

For pMCY30 (BMC proteins) can chartarizate with:

*Pvu*I (3 fragment) 4.2 1.4 0.9 Kb

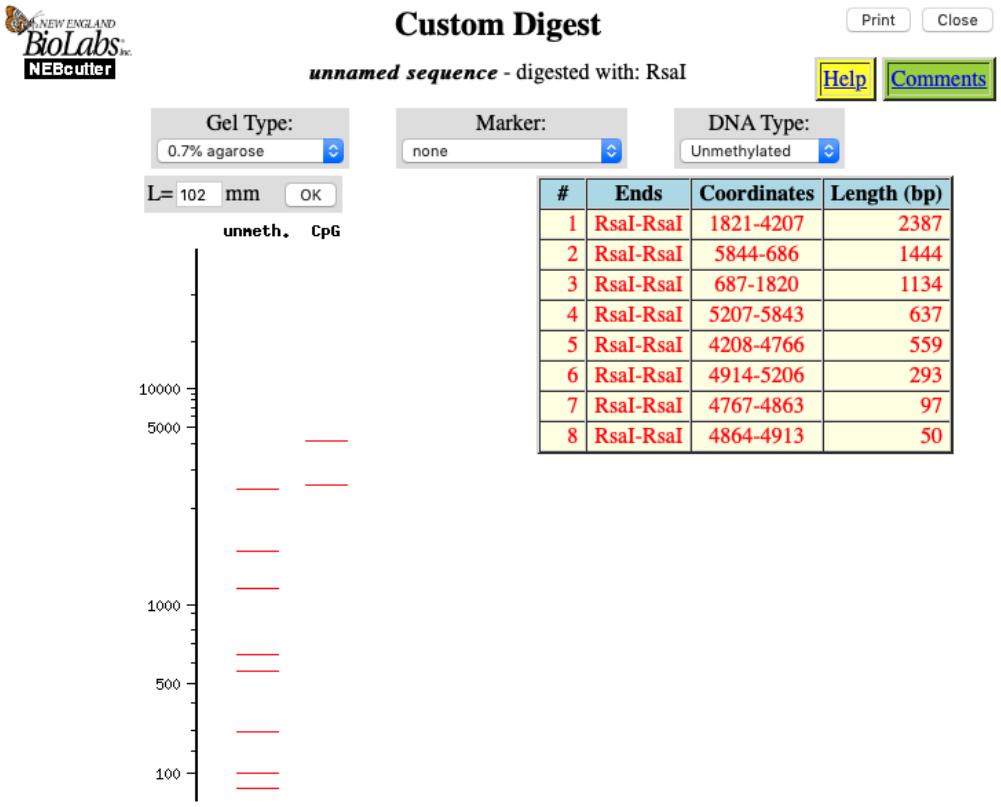
*Rsa*I (8 fragment) 2.3 1.4 1.1 0.6 0.5 0.3 0.09 0.05

pMCY30 PvuI.png



#	Ends	Coordinates	Length (bp)
1	PvuI-PvuI	799-5062	4264
2	PvuI-PvuI	5063-6472	1410
3	PvuI-PvuI	6473-798	927

pMCY30 RsaI.png



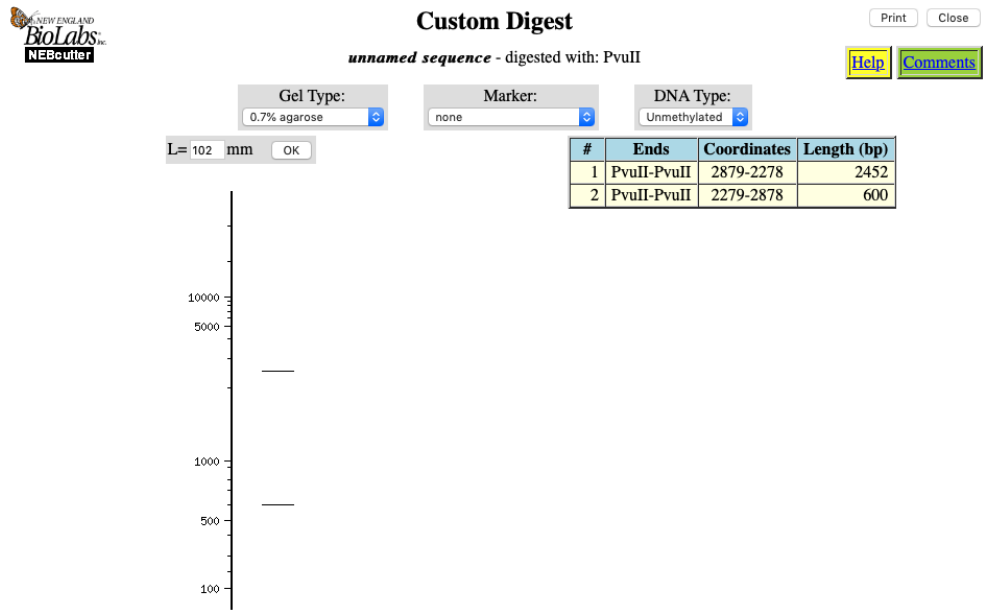
#	Ends	Coordinates	Length (bp)
1	RsaI-RsaI	1821-4207	2387
2	RsaI-RsaI	5844-686	1444
3	RsaI-RsaI	687-1820	1134
4	RsaI-RsaI	5207-5843	637
5	RsaI-RsaI	4208-4766	559
6	RsaI-RsaI	4914-5206	293
7	RsaI-RsaI	4767-4863	97
8	RsaI-RsaI	4864-4913	50

For pMCY90 (mCherry) can chartarizate with:

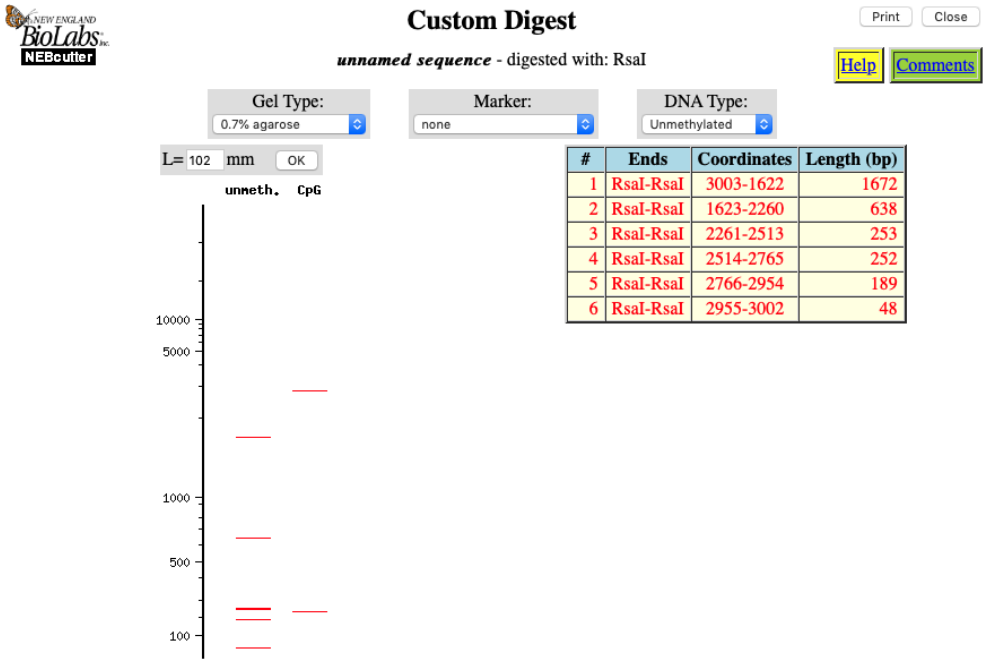
*Pvu*II (2 fragment) **2.4** **0.6** Kb

*Rsa*I (6 fragment) **1.67** **0.63** 0.25 (x2) 0.18 0.048

pMCY90 PvuII.png



pMCY90 RsaI.png



EzDig pMCY90 (mCherry):

*Pvu*II Buffer B2 (Jena BioSciences)

*Rsa*I Buffer B2 (Jena BioSciences)

Table1

		uL	Rx	SubTotal
1	DNA	5.0		15.0
2	Buffer	1.0		3.0
3	Enz RsaI/PvuII	0.5	X3	1.5
4	H2O	3.5		10.5
5		**10.0**		**30.0**

37oC 1-2 hrs

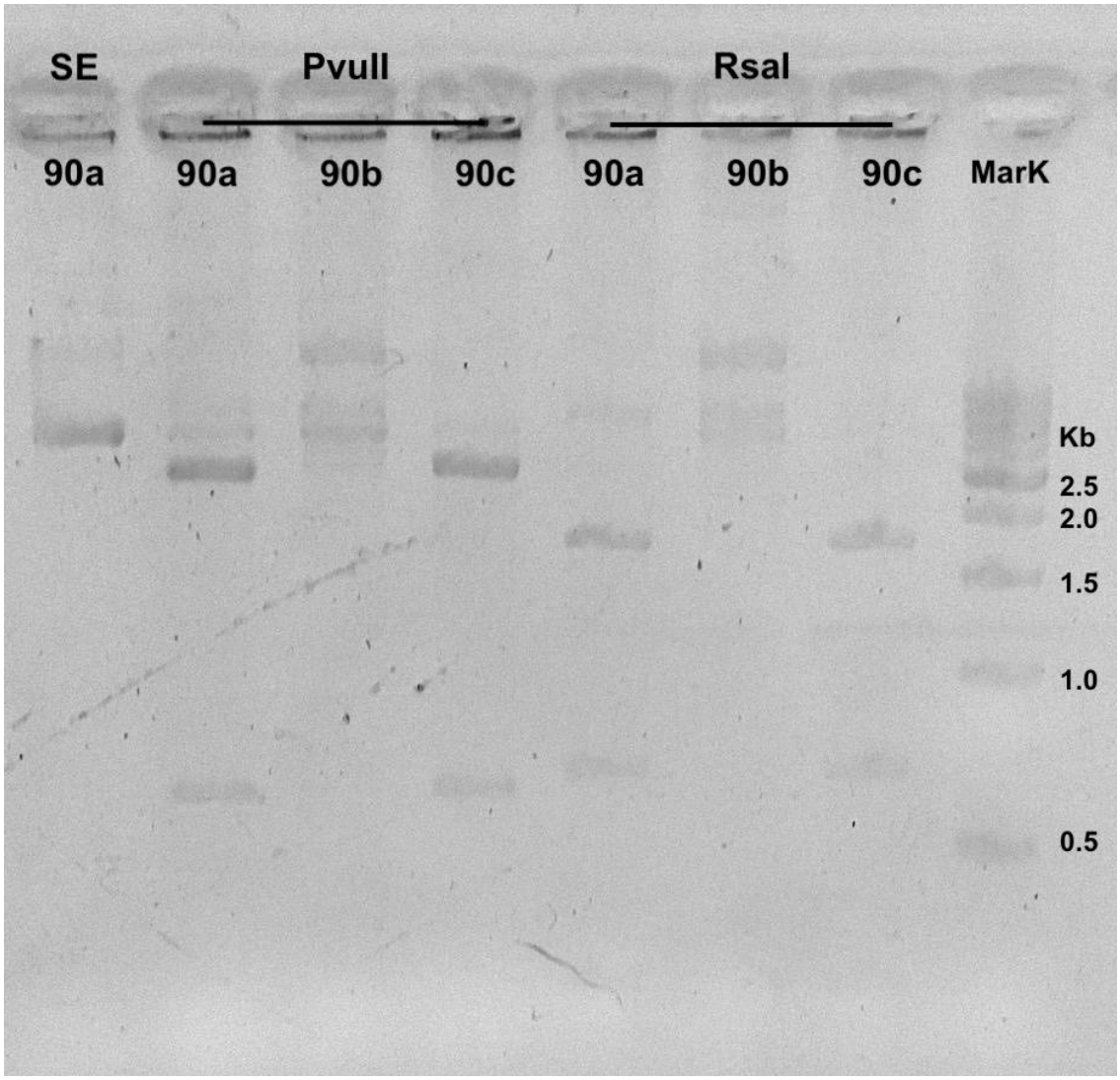
Gel EzDig pMCY90 (mCherry):

*Pvu*I (30a, 30b, 30c)
*Rsa*I (30a, 30b, 30c) (aprox 2hrs)

□□□/---***Pvu*II**---/ /---***Rsa*I**----/
pMCY90a SE 90a 90b 90c 90a 90b 90c Mark

Agarose 0-8%, Buffer SB1X long gel 190V

Gel2 PvuIIRsaI Jul21.jpg



Gel2 (500 bp DNA ladder Jena BioSciences)

THURSDAY, 7/25/2019

pMCY30 Clones

6 Clones from transformation ---> LB media + Amp (50 ng/uL) 37oC 18 hrs 900 rpm thermomixer
No bacteria

Transformation pMCY30 (Filter Paper) plus re-Growth the pMCY30b (Gel1)

Growth **30.1** and **30.3** clones in 600 uL LB and 0.3 uL Amp (50 ug/uL)

5 uL H_2O mQ to filter paper ---> 50 uL Ca++ Cells to transformation ---> Lb Amp plate 37^oC 18 hrs.

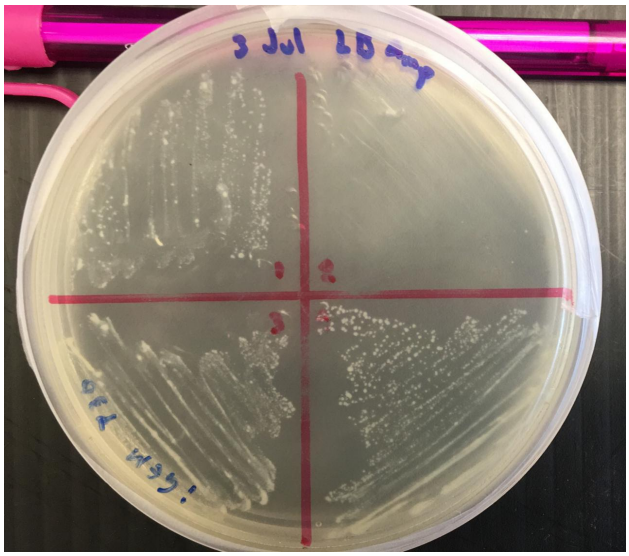
FRIDAY, 7/26/2019

Transformation pMCY30 (Filter Paper)

No bacteria

Re-Growth the pMCY30b

PertiDishJul25.jpeg



(Clone 1, 2, 3, 4)

Growth **two colonies from each clone** in 600 uL LB and without Amp

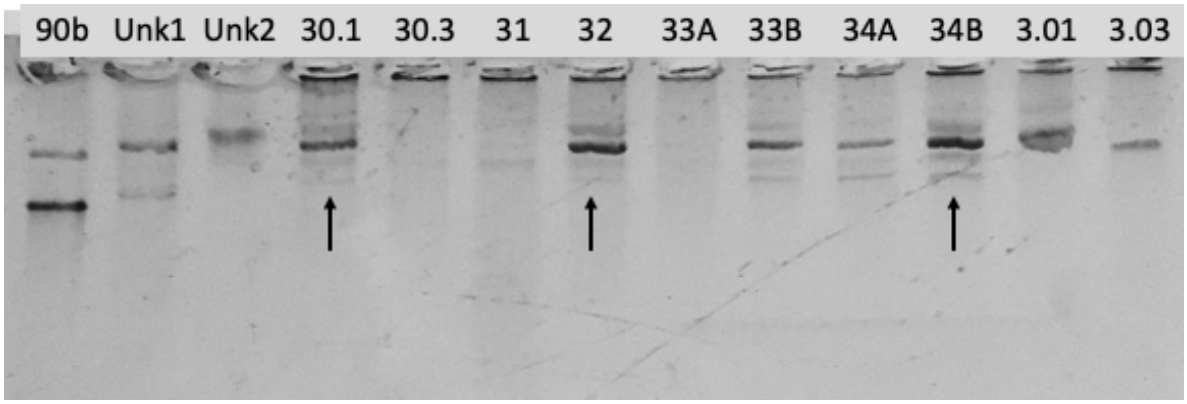
SATURDAY, 7/27/2019

Miniprep from clones pMCY30 (BMC) Clone 1, 2, 3, 4 (from bacteria culture without Amp)

pMCY90b Unk1 Unk2 **30.1** 30.3 31 **32** 33A 33B 34A **34B** 3.01 3.03

Agarose 0.8% SB 1X short gel

Gel3 pMCY90s.png



Gel 3

Clone pMCY90 characterization *Pvu*I + *Rsa*I

*Pvu*I Buffer U (Jena BioSciences)

*Rsa*I Buffer B2 (Jena BioSciences)

Table2

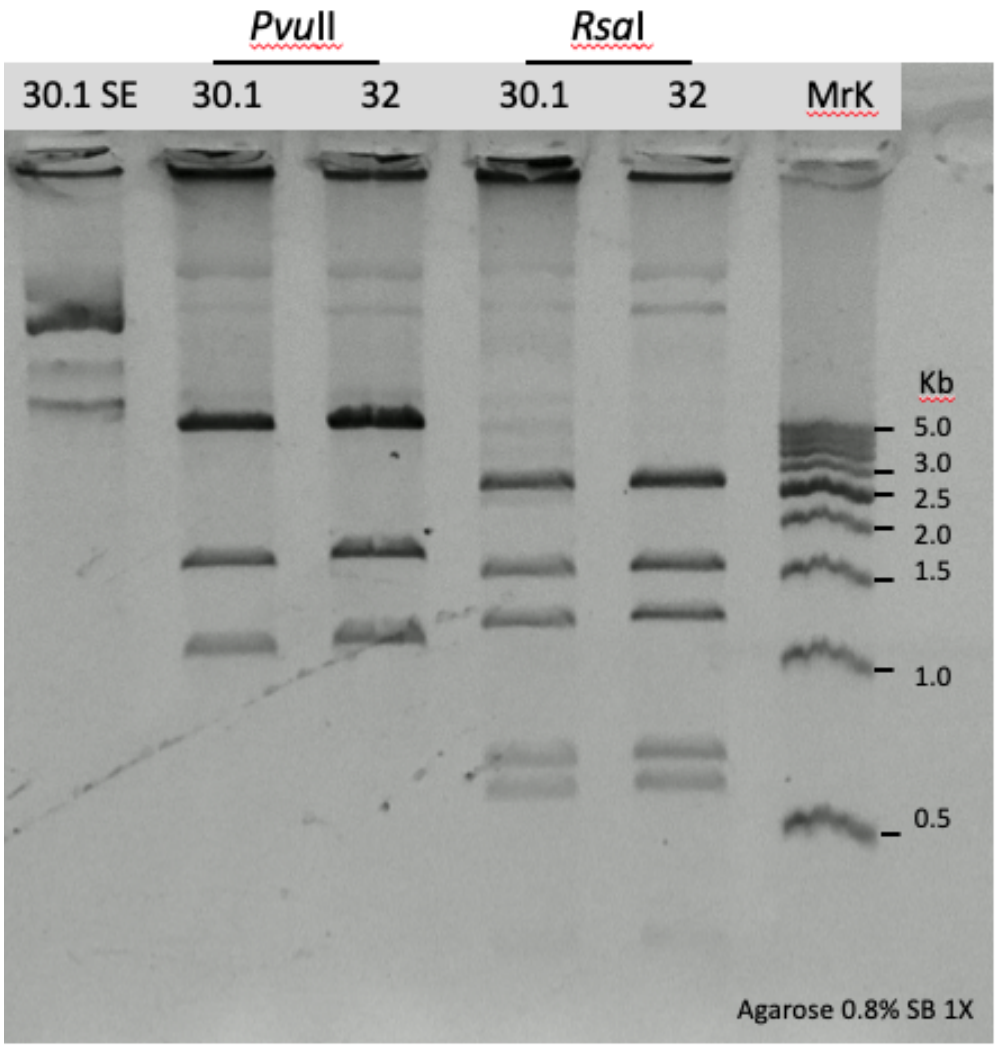
		uL	Rx	SubTotal
1	DNA	3.0		6.0
2	Buffer	1.0		2.0
3	Enz RsaI/PvuI	0.5	X2	1.0
4	H2O	5.5		11.0
5		**10.0**		**20.0**

□□□/--***Pvu*II**--/ /--***Rsa*I**--/

30.1 SE 30.1 32 30.1 32 MarK

Agarose 0.8% SB 1X short gel

Gel4 pMCY30 PvuIIRsaI.png



Gel4 (500 bp DNA ladder Jena BioSciences)

MONDAY, 7/29/2019

pduU modified Synthetic DNA and promoter p3B5C in synthesis (IDT)

Screenshot 2019-07-30 at 13.38.35.png

Estimado/a UANL iGEM 2019,

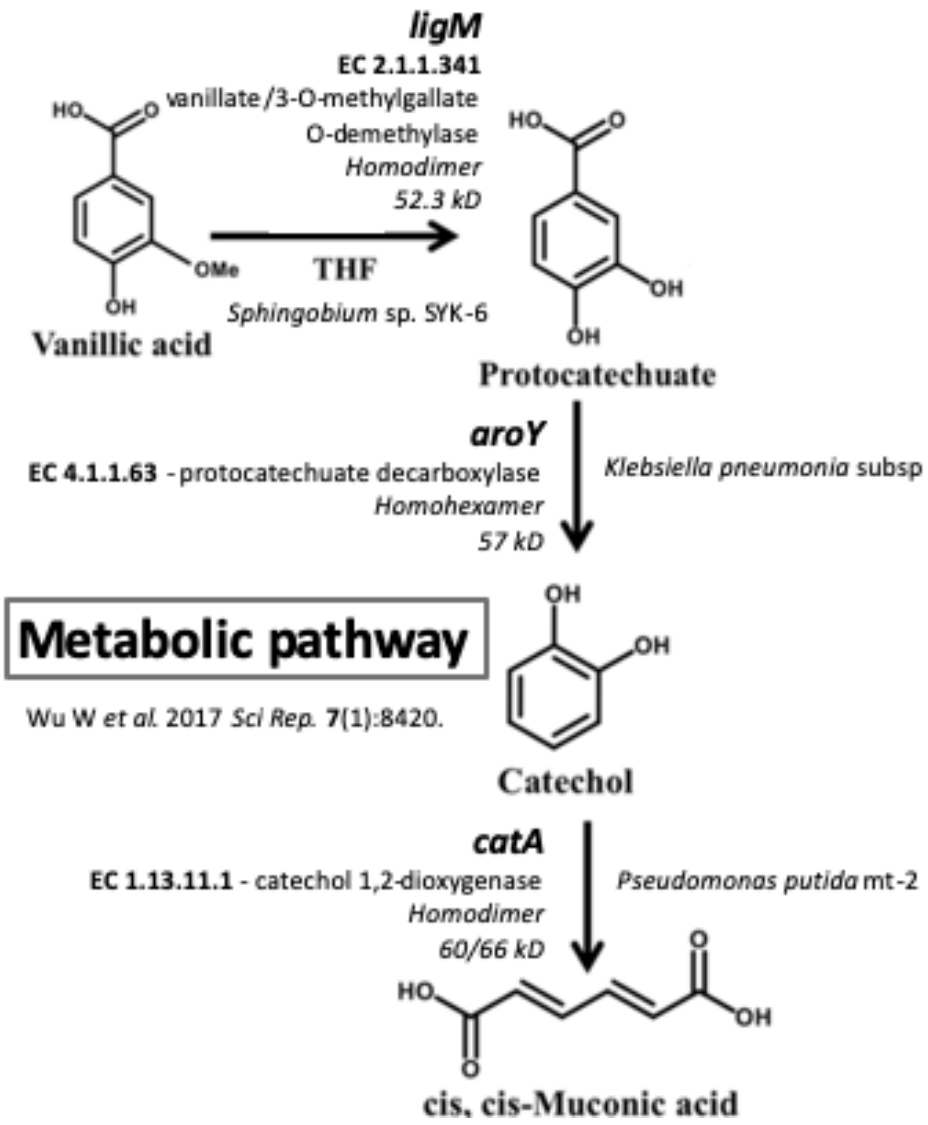
Este es un mensaje automático de Integrated DNA Technologies para informarte que tu pedido ha cambiado de estado en el Uniparts-Mexico Portal de Integrated DNA Technologies. Los detalles de tu pedido se encuentran abajo:

Fecha:7/29/2019 5:43:26 PM
Estado Actual:Approved
Observaciones del Pedido:
Nombre de la secuencia: pduUmod
Productos:gBlocks® Gene Fragments 501-750 bp
Purificación:
Secuencia:GGA TCG AAT TCG GAT TTC TAG AGG GCA TTG TAG ATA CGC TTT CGT GTT AAG AGG CTA GCG AGA ATA TTT GCA CTC CAT CCG AGA TAA GGA ATT TAC CCA TGA AAC AGG TCA CAT TGG CGC ATC TGA TTG CCA ATC CGG GTA AGG ATT TAT TTA AGA AGT TAG GCT TGC AGG ACG CCG TTA GCG CCA TCG GTA TCC TGA CTA TTA CTC CTT CTG AAG CCT CAA TCA TTG CCT GTG ACA TTG CAA CTA AAT CTG GGG CCG TAG AGA TCG GGT TCT TGG ATC GCT TTA CAG GTG CTG TAG TAT TGA CAG GTG ATG TCT CGG CAG TTG AGT ATG CCC TTA AAC AGG TCA CTC GCA CCT TAG GCG AAA TGA TGC AAT TCA CCA CAT GCT CGA TCA CAC GCA CAT AAG AGG CTA GCG AGG GTT GAC CCC AAG GGC GAC ACC CCC TAA TTA GCC CGG GCG AAA GGC CCA GTC TTT CGA CTG AGC CTT TCG TTT TAT TTG ATG CCT GGC AGT TCC CTA CTC TCG GGA CTA GTG GCT GCA GGG
Cantidad:1
Nombre de la secuencia: p3B5C
Productos:gBlocks® Gene Fragments 501-750 bp
Purificación:
Secuencia:GGA TCG AAT TCG GAT TTC TAG AGG TGT CAC CTT CAG CAA CTT TTG TTC GAT TAT CGA ACA AAT TAT TGA AAT ATC GAA CAA AAC CTC TAA ACT ACT GTG GCA CTG AAT CAA AAA ATT ATA AAC CCT GAT CAG ACA TTA CTC ATA AAA ATA CGA GGA GAG CAA CGA TGC AAC AAG AAG CAC TAG GAA TGG TAG AAA CCC GTC CTC GAA GTT CAT CAC GCG CTC CCA CTT GAA GCC CTC GGG GAA GGA CAG CTT CAA GTA GTC GGG GAT GTC GGC GGG GTG CTT CAC GTA GGC CTT GGA GCC GTA CAT GAA CTG ATT CTA CAT GAT GTA TTC GTC TAA GTG CAA TGT TAC AAT CTT AGT CAC CTT CAG CTT GTA TGT CTG GGT GCC CTC GTA GTT ACG GCC CTC GCC CTC GCC CTC GAT CTC GAA CTC GTG GCC GTT CAC GGA GCC CTC CAT GTG CAC CTT GAA GCG CAT GAA CTC CTT GAT GAT GGC CAT GTT ATC CTG GTG TTA TGA GCC ATA TTC AGG GGA CTA GTG GCT GCA GTG ATT TAG
Cantidad:1

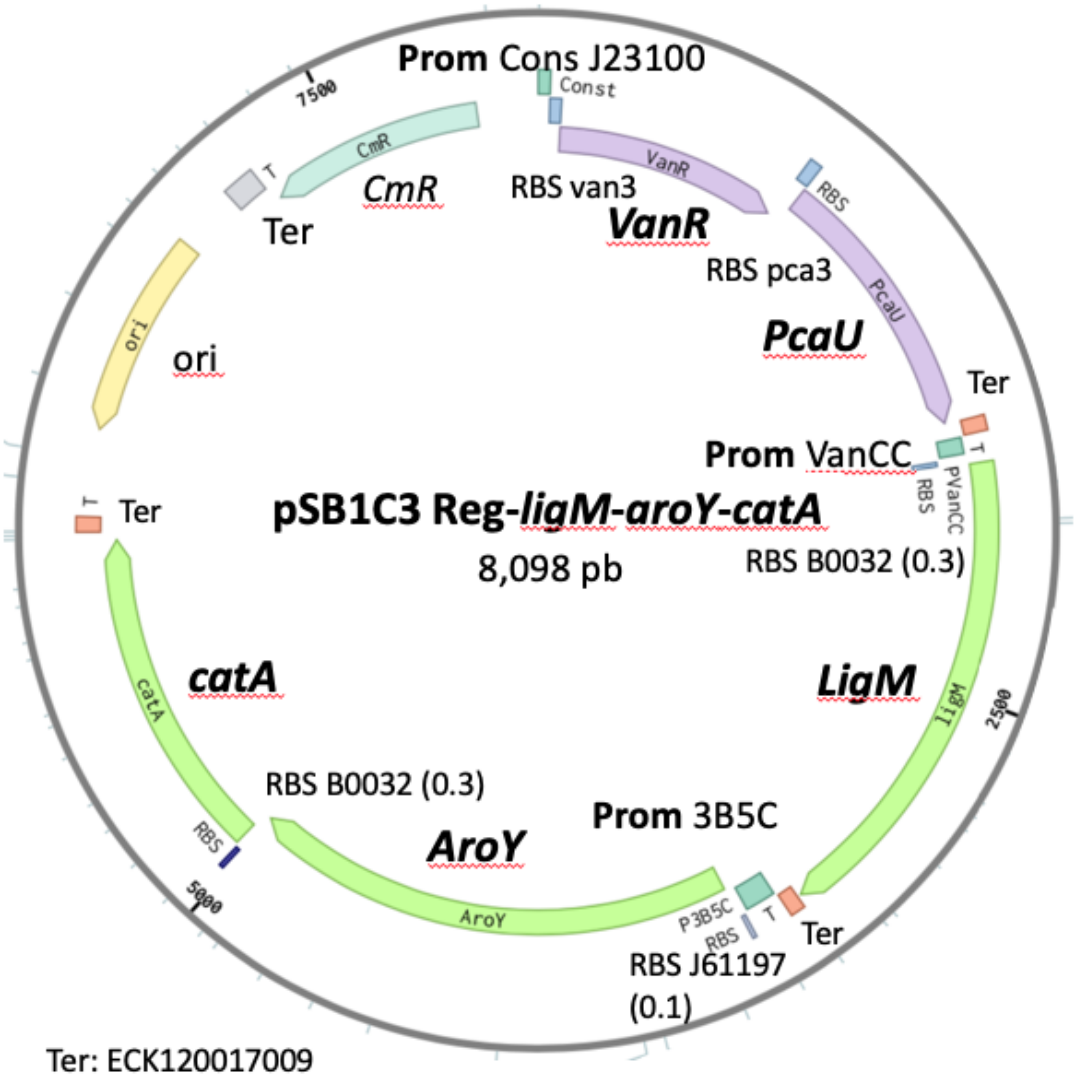
TUESDAY, 7/30/2019

Synthetic DNA Enzimatic Pathway

MetPathway.png



FinalPlasmid.png



Synthetic Parts:

Table3

	A	B	C	D
1	**Name**	**Size pb**	**Prefixx**	**Subfixx**
2	**Pi1VanRPcaU**	1,782	EcoRI/XbaI	SpeI/PstI
3	**Pi2LigM**	1,617	EcoRI/XbaI	SpeI/PstI
4	**Pi3AroY**	1,759	EcoRI/XbaI	SpeI/PstI
5	**Pi4catA**	1,151	EcoRI/XbaI	SpeI/PstI
6	**Pi5LigMpep**	1,593	EcoRI/XbaI	SpeI/PstI

TUESDAY, 8/6/2019

Ca++ Competent Cells *DH5α*

Overnight culture (aprox 1 mL) --> 500 mL flask with 100 mL LB media (37°C 150 rpm)

50 mL Bacteria culture --> 50 mL conic tube (2x) --> 8,000 rpm 8 mins

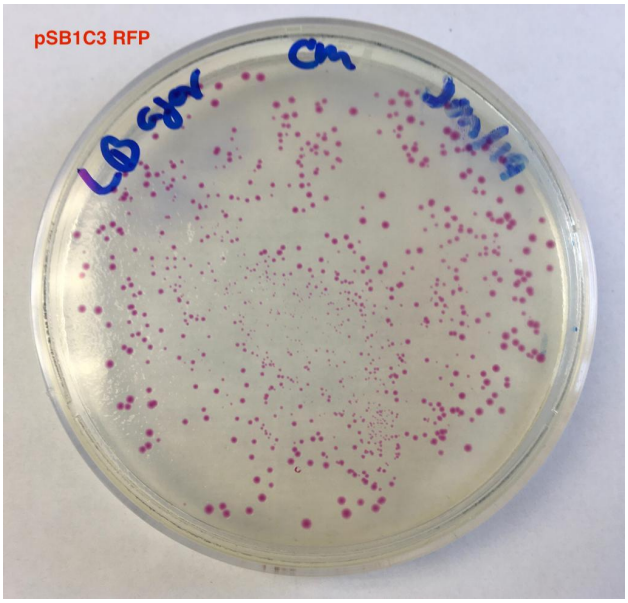
Supernantan out, Bacteria pelet + $CaCl_2$ 0.1 M --> Ice 25 mins --> 8,000 rpm 8 mins

--> Pellet + 2 mL CaCl 0.1 M ready to use

Transformation:

- pSB1C3 0.1 µg (100 ng)
- 2 µL cjBlue
- 2 µL eforRed

TestCaCompJune.jpeg



DNA digestion plasmid pMCY30 (BMCs) (Jena BioSciences Enzyme and Buffer)

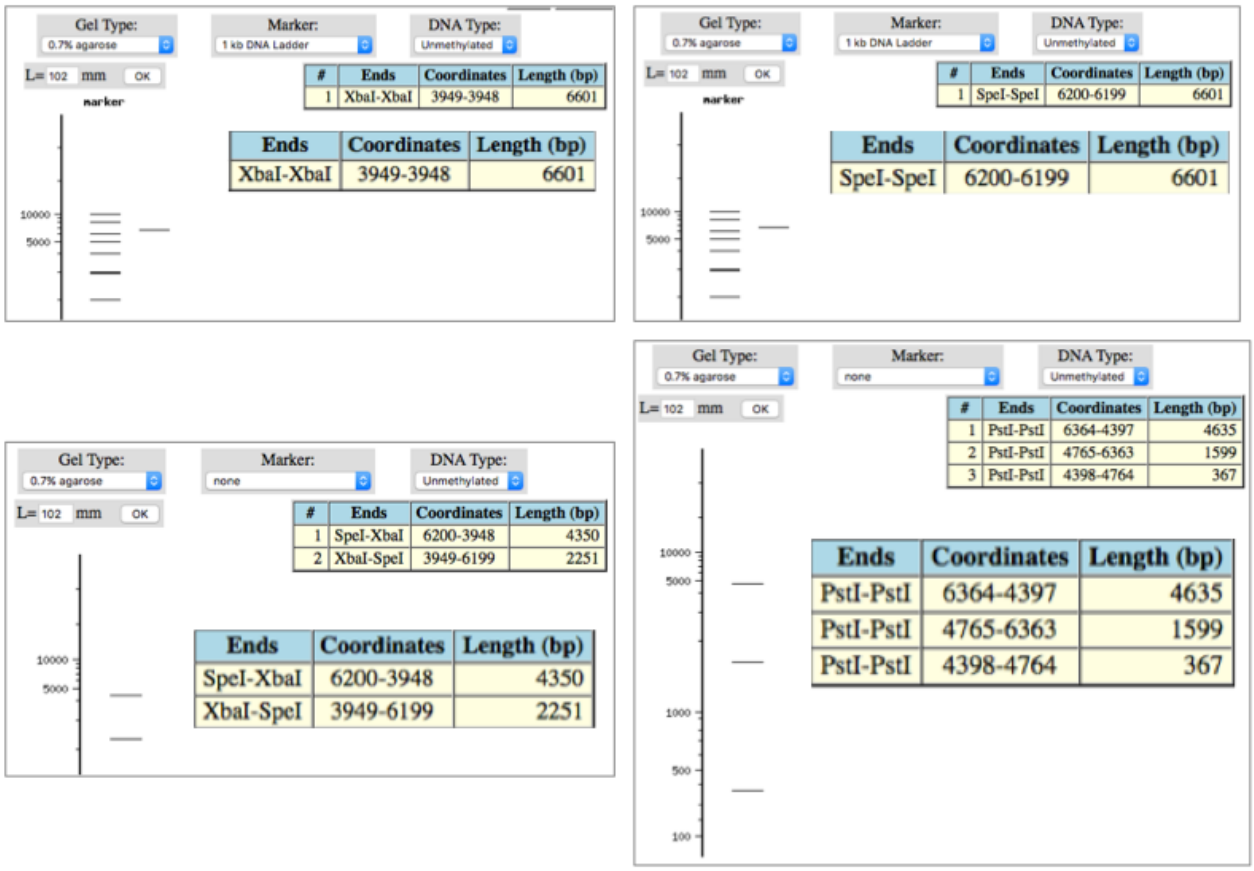
*Xba*I Buffer Universal

*Spe*I Buffer Universal

*Pst*I Buffer Universal

*Xba*I/*Spe*I Buffer Universal

Screen Shot 2019-08-06 at 3.39.57 PM.png

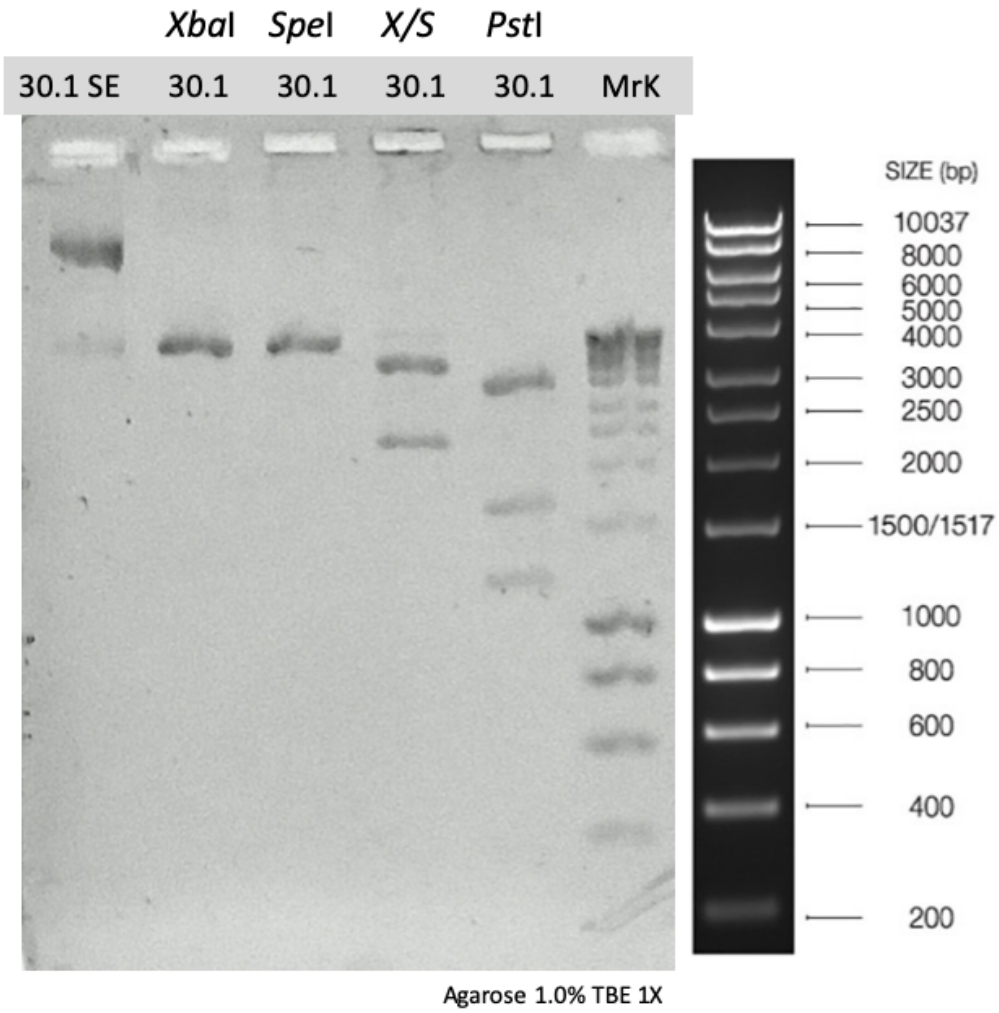


***Xba*I *Spe*I *X/S* *Pst*I**

30.1 SE 30.1 30.1 30.1 30.1 MarK

Agarose 1.0% TBE 1X

Gel5 30.1.png



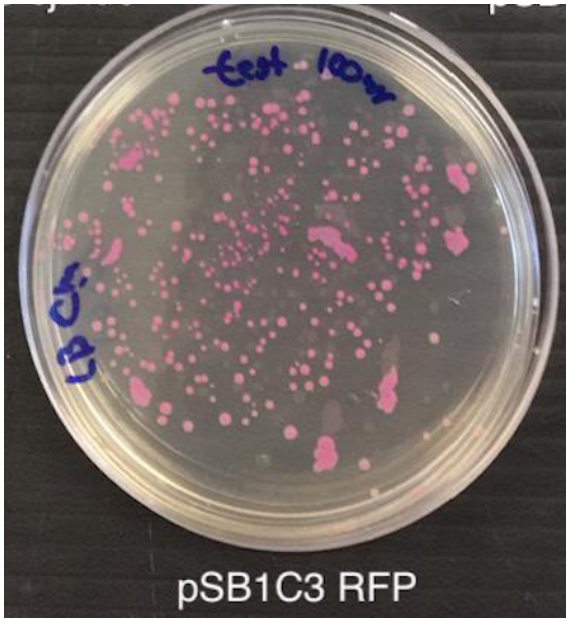
Gel4 (500 bp DNA ladder Jena BioSciences)

WEDNESDAY, 8/7/2019

Transformation pSB1C3 RFP in *E. coli* DH5α Ca++ Competents Cells

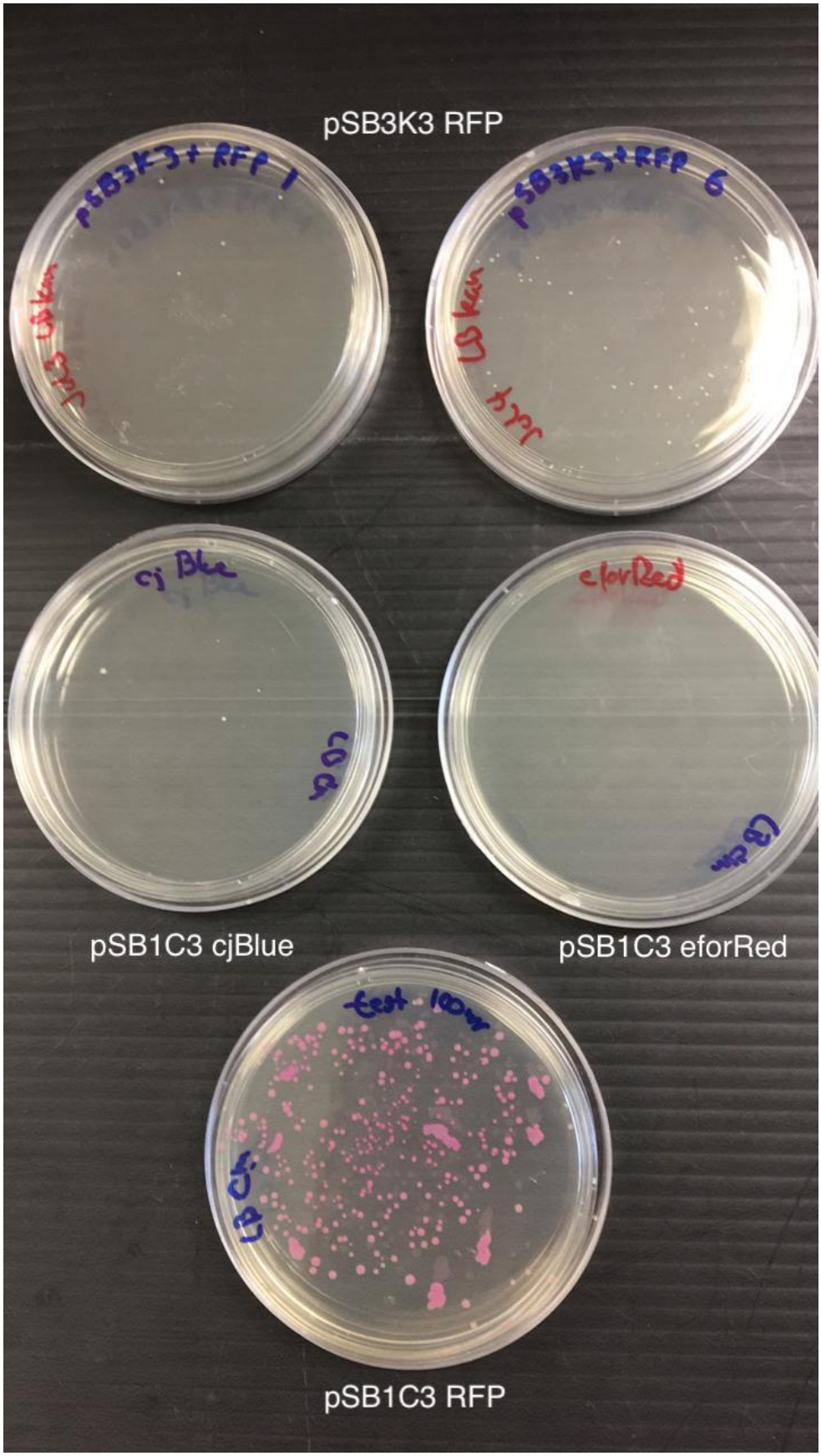
37°C --> 18 hrs

pSB1C3RFP.png



Transformation pSB1C3 *cj*Blue, pSB1C3 *efor*Red and pSB3K3 RFP in *E. coli* DH5α Ca++ Competents Cells 37°C --> 18 hrs.

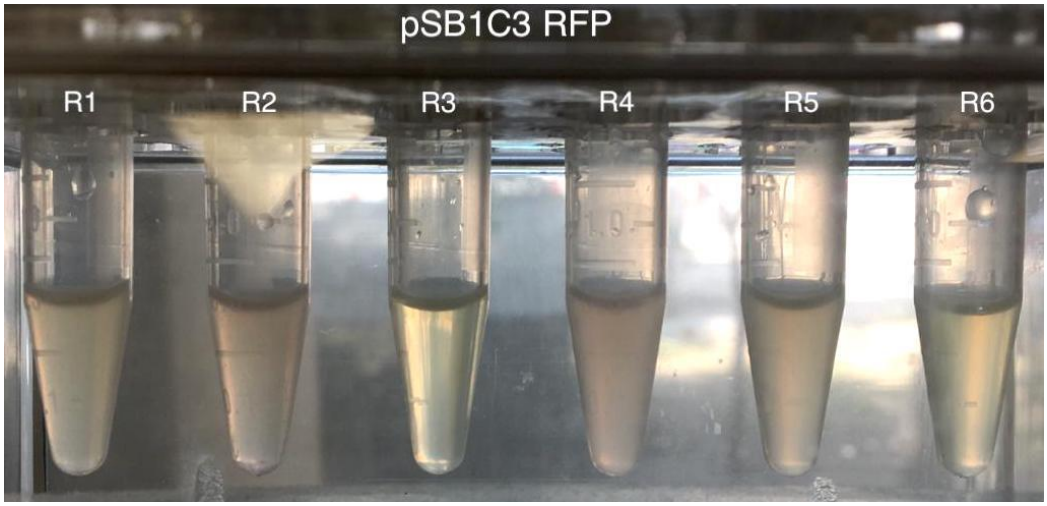
PlatesAug8.jpeg



THURSDAY, 8/8/2019

Tubes pSB1C3 RFP 6 clones

TubesAug8.jpeg

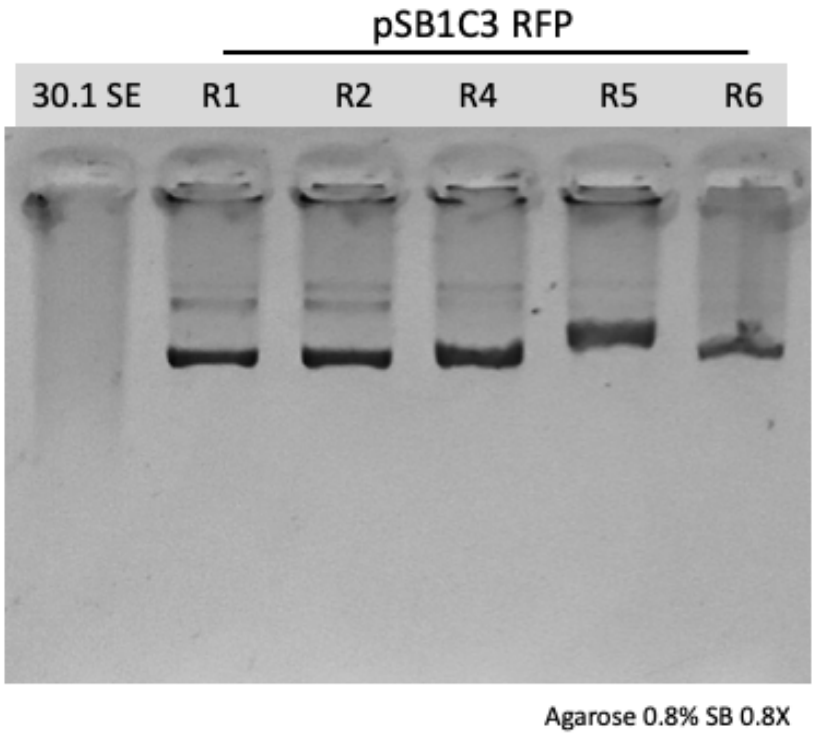


6 Gel clones miniprep

30.1 SE R1 R2 R4 R5 R6

Agarose 0.8% SB 1X

Gel6BW.png



Gel6

FRIDAY, 8/9/2019

pSB3K3 RFP miniprep 6 clones pSB1C3, pSB1C3 cjBlue 4 clones

Clones R1 R2 R4 R5 of pSB1C3 RFP characterization with *Eco*RI + *Pst*I

***Eco*RI/*Pst*I Buffer U (Jena BioSciences)**

Table4

		uL	Rx	SubTotal
1	DNA	1.0		4.0
2	Buffer U	1.0		4.0
3	Enz EcoRI	0.3	X4	1.2
4	Enz PstI	0.3		1.2
5	H2O	7.4		29.6
6		**10.0**		**40.0**

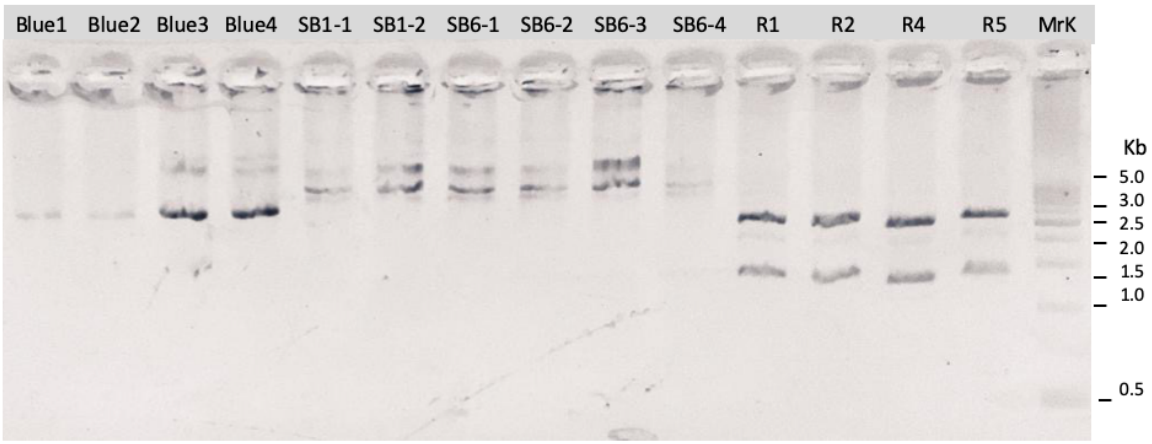
*Eco*RI/*Pst*I

/-- pSB1C3 cjBlue --/ /-- pSB1C3 3K3 --/ /-- pSB 1C3 -/

Blue1 Blue2 Blue3 Blue4 SB1-1 SB1-2 SB6-1 SB6-2 SB6-3 SB6-4 R1 R2 R4 R5 MrK

Agarose 0.8% SB 1X 37°C 2 hrs

Gel7 pSB1C3 charact.png



Gel7

MONDAY, 8/12/2019

Re-growth pMCY30 (BMC) plasmids, 6 tubes (600 µL LB + 0.6 µL Cloramphenicol, 34 mg/mL) --> 37°C-18 hrs

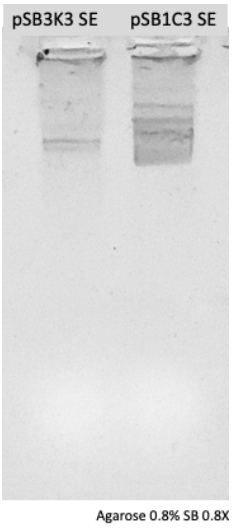
TUESDAY, 8/13/2019

Miniprep for 6 clones same plasmid pMCY30 (BMC)

pSB3K3 pSB1C3

Agarose 0.8% SB 1X

Gel8 pSBC1C3 RFP.png



Gel8

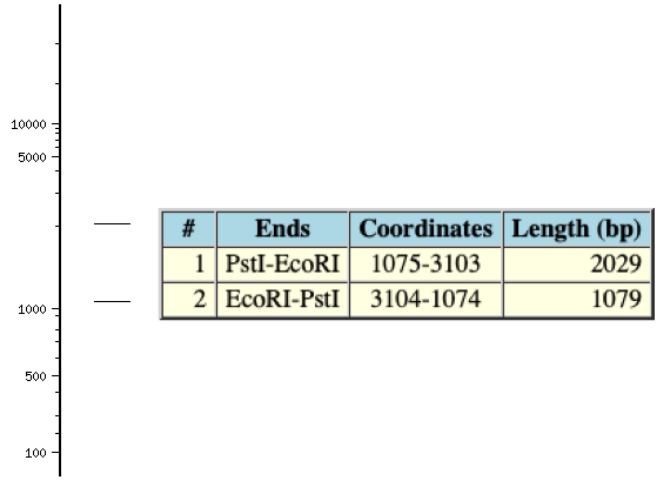
Clones pSB1C3 RFP Enzyme digestion with *Eco*RI + *Pst*I

***Eco*RI/*Pst*I Buffer U (Jena BioSciences) 37°C 2 hrs**

Table5

		uL
1	DNA	4.0
2	Buffer U	4.0
3	Enz EcoRI	1.0
4	Enz PstI	1.0
5	H2O	30.0
6		**40.0**

1C3RFP E:P.png



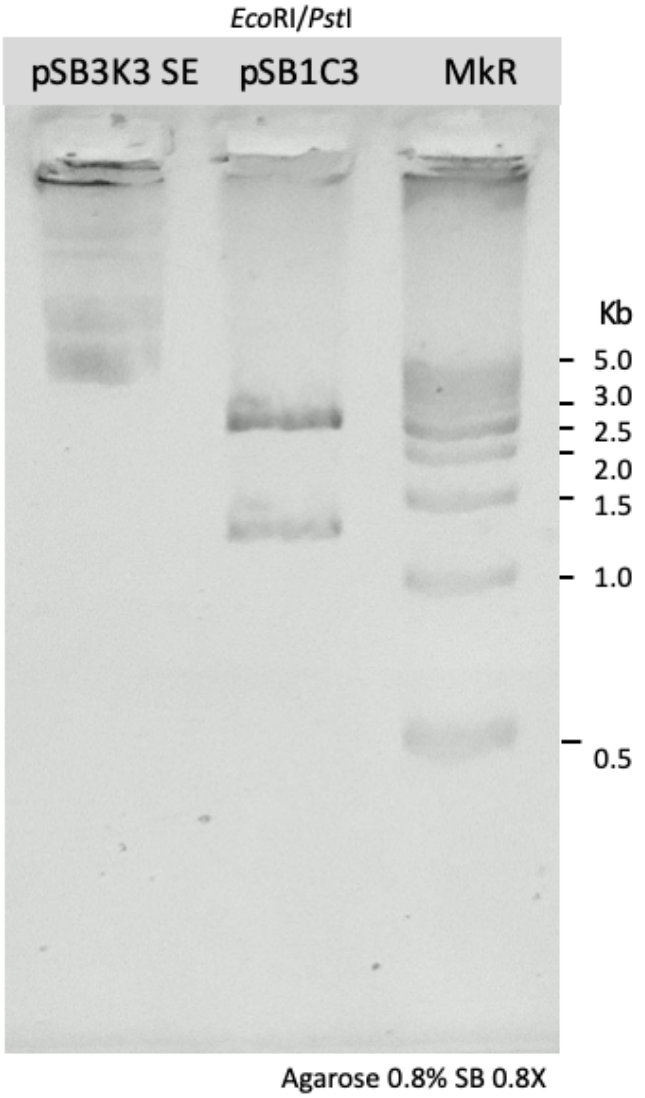
#	Ends	Coordinates	Length (bp)
1	PstI-EcoRI	1075-3103	2029
2	EcoRI-PstI	3104-1074	1079

EcoRI/PstI

pSB1C3 SE (2µL) pSB1C3 (5 µL) MkR (3 µL)

Agarose 0.8% SB 1X

Gel9 pSB1C3 RFP EcoRIPstI.png



Gel9

WEDNESDAY, 8/14/2019

pSB1C3 RFP *Eco*RI/*Pst*I Digestion concentration in NanoDrop

485 ng/µL

Table6

	A	B	C
1		**Direct Sam**	**1:10 Dil**
2	**Abs**	5.815	0.683
3	**260/280**	1.88	1.88
4	**ng/uL**	391.4	48.5

THURSDAY, 8/15/2019

Pippet tips, LB Media and Amp / Cloramphenicol LB plates (30 / 34)

Get Rhamnose (0.5 gr) for encapsulation-Pep-mCherry clone of the pMCY90 plasmid.

Screenshot 2019-08-15 at 16.54.46.png

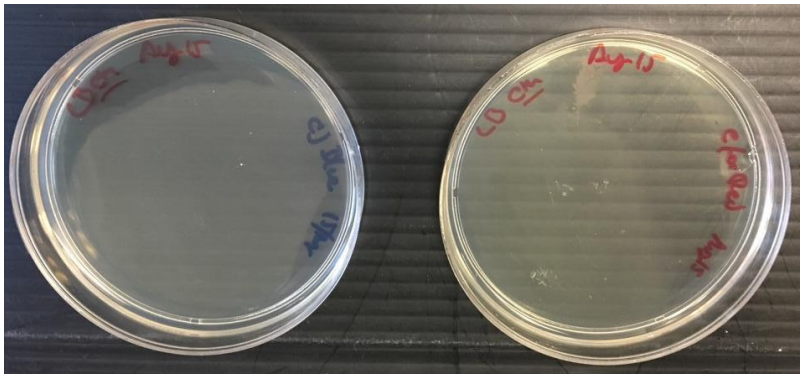
Different BMC systems alter protein expression levels and toxicity of E

To compare E expression, the different *E. coli* strains were grown in LB medium and induced under one of the following conditions: IPTG only (BMC), rhamnose only (E), or rhamnose and IPTG co-induction (E + BMC). IPTG was added to 0.5 mM at the start of growth, while rhamnose was added to 0.1 mM once cells reached an OD_{600} of 0.4, about 3 h after the start of growth. The

Yung *et al. Microb Cell Fact* (2017) 16:71-88

Transformation cjBlue (13D plate 6, 2019 Kit) eforRed (11P plate 6, 2019 Kit) DH5α Ca++ Competent Cell -- LB Agar Plates (New)

eforRedcjBlue2.jpg



No clones

MONDAY, 8/19/2019

Enzyme pathway genes and regulator

Synthetic DNA Disolution, Restriction Enzyme and ligatión with pSB1C3 ligation

10 µL of H_2O to each Synthetic DNA (1 µgr per Synthetic fragment) --> **100 ngr/µL**

Digestion with *Eco*RI/*Pst*I

Table7

	A	B	C	D	E	F
1	DNA	2.5		12.5		
2	Buff U 10X	1.0		5.0		
3	EcoRI	0.5	X5	2.5		
4	PstI	0.5		2.5		
5	H2O	5.5		27.5		
6		**10.0**		**50.0**	Final Con.	**25 ng/uL**

37°C/2 hrs

Ca++ Competent cell preparation

Starter culture of LB (no antibiotics) --> Grow at 37°C in shaker, overnight

(20 µL of DH5α cells in 800 µL LB media X3)

Synthetic DNA concentration **25 ng/µL**

Vector 485 ng/µL --> 1:10 dilution --> 48.5 ng/µL (2,033 pb)

Table8

	A	B	C	D	E	F
1		**Pi1VanRPcaU**	**Pi2LigM**	**Pi3AroY**	**Pi4catA**	**Pi5LigMpep**
2	**2,033 pb Vec**	1,782 pb	1,617 pb	1,759 pb	1,151 pb	1,593 pb
3	**DNA vector**	1.00	1.00	1.00	1.00	1.00
4	**DNA Insert**	5.11	4.64	5.04	3.30	4.57
5	**Buffer Lig 10X**	2.00	2.00	2.00	2.00	2.00
6	**Enzyme T4 Lig**	0.25	0.25	0.25	0.25	0.25
7	**H2O**	11.64	12.11	11.71	13.45	12.18
8		**20.00**	**20.00**	**20.00**	**20.00**	**20.00**
9	*Vector*	*48.5 ng*	*48.5 ng*	*48.5 ng*	*48.5 ng*	*48.5 ng*
10	*Insert*	*127.8 ng*	*116.0 ng*	*126.1 ng*	*82.5 ng*	*114.2 ng*

DNA ligation transformation

Table9

	A	B
1	**pSB1C3 (control)**	2 uL (aprox 0.1 ng)
2	**pi1Van**	5 uL (44.0 ng)
3	**Pi2LigM**	5 uL (41.1 ng)
4	**Pi3AroY**	5 uL (43,6 ng)
5	**Pi4catA**	5 uL (32.7 ng)
6	**Pi5LigM**	5 uL (40.6 ng)

DH5α pre Culture (800 µL LB media no antibiotic) 37°C 900 rpm X2

WEDNESDAY, 8/21/2019

No colonies.

New Ca++ Competen Cells --> Transform pSB1C3RFP as control (aprox 5 ng)

Ca++ Competent Cells from Cellular Biology Lab --> LB Cm Plates

Table10

	A	B
1	**pSB1C3 (control)**	1 uL (aprox 2 ng)
2	**pi1Van**	5 uL (44.0 ng)
3	**Pi2LigM**	5 uL (41.1 ng)
4	**Pi3AroY**	5 uL (43,6 ng)
5	**Pi4catA**	5 uL (32.7 ng)
6	**Pi5LigM**	5 uL (40.6 ng)

THURSDAY, 8/22/2019

Clone in plate Pt2, grow it in LB (Cm) 600 µL (0.6 µL Cm 1000X) 37°C 900 rpm (Thermomixer Eppendorf)

FRIDAY, 8/23/2019

Clone in plate Pt4, grow it in LB (Cm) 600 µL (0.6 µL Cm 1000X) 37°C 900 rpm (Thermomixer Eppendorf)

ClonePt4.jpeg

clone Pi4catA

DNA miniprep and *Eco*RI/*Pst*I digestion (microwave oven)

Table11

	A	B	C	D
1	DNA	2.0		4.0
2	Buff U 10X	1.0		2.0
3	EcoRI	0.3	X2	0.6
4	PstI	0.3		0.6
5	H2O	6.4		12.8
6		**10.0**		**20.0**

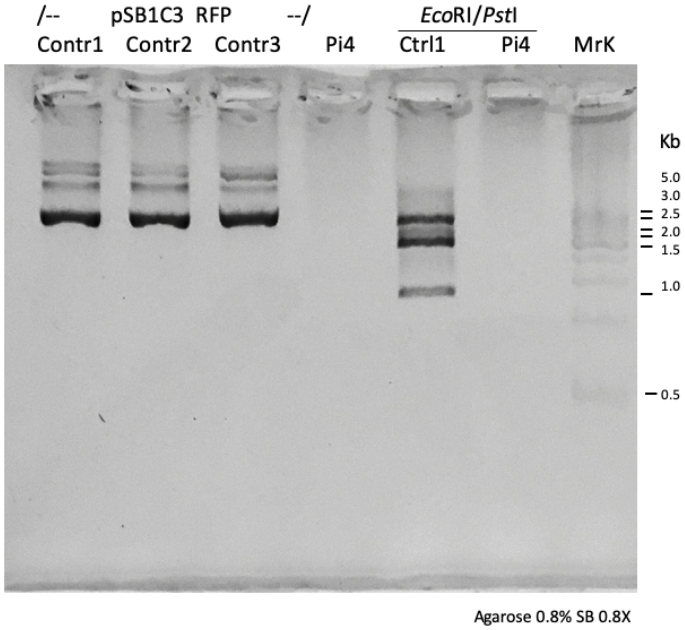
5 seconds in a regular microwave oven

/-- pSB1C3 RFP --/ /*Eco*RI/*Pst*I/

Contr1 Contr2 Contr3 Pi4 Ctrl Pi4 MrK

Agarose 0.8% SB 1X

Gel10 Pi4 miniprepDigestion.png



Gel 10 Pi4 miniprep

SUNDAY, 8/25/2019

Pick colonies from Pi2 (Pi2LigM) and Pi4 (Pi4catA)

LB (Cm) 600 µL (0.6 µL Cm 1000X) 37°C 900 rpm

MONDAY, 8/26/2019

Miniprep Pi2 and Pi4 clones

DNA miniprep and *Eco*RI/*Pst*I digestion (microwave oven)

Table12

	A	B	C	D
1	DNA	1.0		3.0
2	Buff U 10X	1.0		3.0
3	EcoRI	0.3	X3	0.9
4	PstI	0.3		0.9
5	H2O	7.4		22.2
6		**10.0**		**30.0**

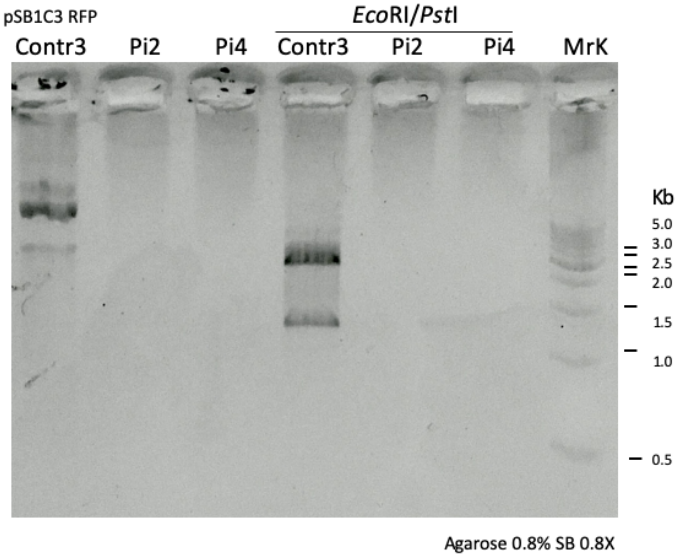
10 seconds in a regular microwave oven

pSB1C3RFP ***/- EcoRI/PstI -/***

Contr3 Pi2 Pi4 Ctrl Pi2 Pi4 MrK

Agarose 0.8% SB 1X

Gel 11 Pi2Pi4miniprep 1b.png



Pick colonies from Pi1 (Pi1Van) and Pi3 (Pi3AroY)

LB (Cm) 600 µL (0.6 µL Cm 1000X) 37°C 900 rpm

TUESDAY, 8/27/2019

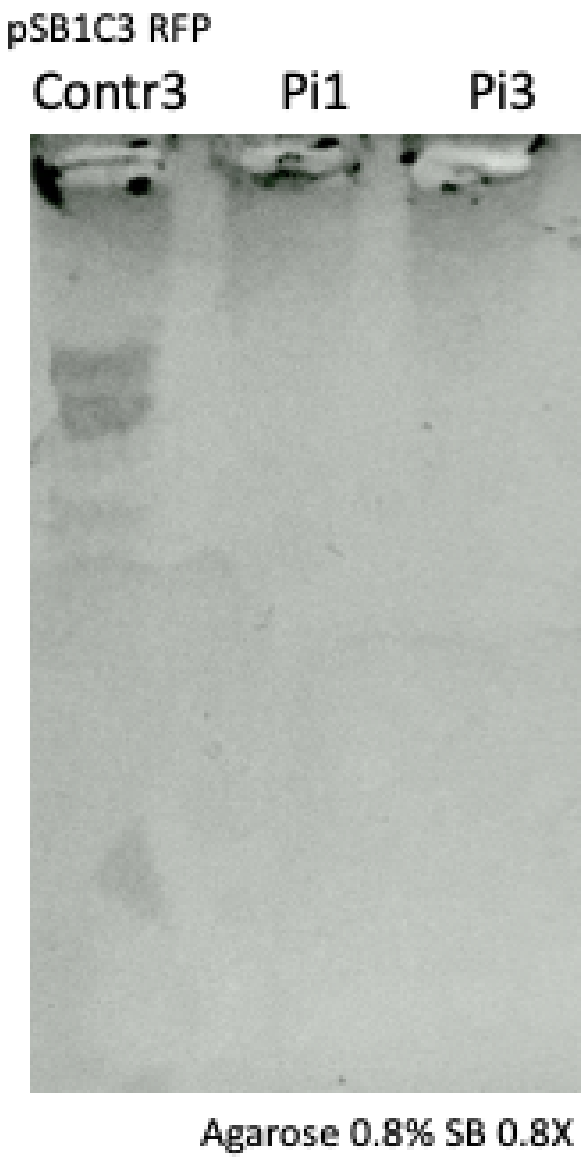
Miniprep Pi1 and Pi3 clones

pSB1C3RFP

Contr3 Pi1 Pi3

Agarose 0.8% SB 1X

Gel 12 Pi1Pi3SE 1b.png



Gel 12 Clones Pi1 and Pi3

iGEM Kit Backbones vector

pSB1C3 backbone --> 25 ng/µL

125 µg Digested with *Eco*RI + *Pst*I --> 37°C 2 hrs

DNA Ligation 2

Table13

	A	B	C	D	E	F
1		**Pi1VanRPcaU**	**Pi2LigM**	**Pi3AroY**	**Pi4catA**	**Pi5LigMpep**
2	**2,033 pb Vec**	1,782 pb	1,617 pb	1,759 pb	1,151 pb	1,593 pb
3	**DNA vector**	2.0	2.0	2.0	2.0	2.0
4	**DNA Insert**	3.3	4.7	5.2	3.4	4.7
5	H2O to 5uL	1.7	0.3	0.0	1.6	0.3
6	**Buffer Lig 10X**	2.00	2.00	2.00	2.00	2.00
7	**Enzyme T4 Lig**	0.5	0.5	0.5	0.5	0.5
8	**H2O**	10.5	10.5	10.5	10.5	10.5
9		**20.00**	**20.00**	**20.00**	**20.00**	**20.00**
10	*Vector*	*131.5 ng*	*119.3 ng*	*129.8 ng*	*84.9 ng*	*117.5 ng*
11	*Insert*	*50 ng*	*50 ng*	*50 ng*	*50 ng*	*50 ng*

25°C overnight

WEDNESDAY, 8/28/2019

DNA ligation 2 transformation in DH5α

Pi1Van -- Pi2LigM -- Pi3aroY -- Pi4catA -- Pi5LigMpep -- Control (100 pg/µL)

--> LB Agar Cm 37°C overnight

Set Seed Culture DH5α, 2 Tubes 800 µL LB (No antibiotic) --> 37°C overnigth

THURSDAY, 8/29/2019

DH5α Ca++ Competent Cells transform a plasmid pSB1C3mCherry (4 ng/µL) as control

FRIDAY, 8/30/2019

No colonies, Try new Competent cells

MONDAY, 9/2/2019

Re growth clones pMCY30 (BMC) and pMCY90 (mCherry-Internalization peptide) bacteria culture in LB.

TUESDAY, 9/3/2019

pMCY30 (BMC) and pMCY90 (mCherry-Pep) growth LB media

--> LB Agar Cm 37°C overnight

Miniprep to 30.1 30.2 90_1 90_4 RFP_1 RFP_2 (pSB1C3RFP)

Pick new clones from LB Plates:

Set 1 30_1 30_2 30_3 30_4 Set 2 30_{11} 30_{12} 30.3 30.4

Miniprep to Set1 --> *Pvu*II Digestion

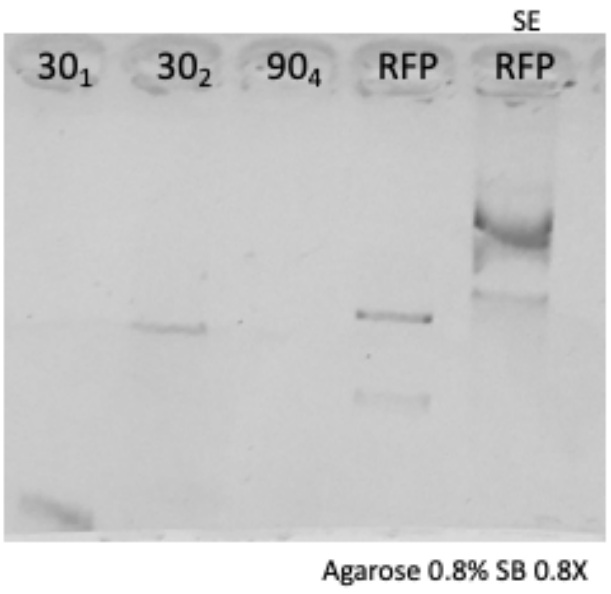
Table14

	A	B	C	D
1	DNA	1.0		4.0
2	Buff U 10X	1.0		4.0
3	PvuII	0.3	X4	1.2
4	H2O	7.7		30.8
5		**10.0**		**40.0**

/-- ***PvuII*** ***--/*** ***E/P*** ***SE***

30_1 30_2 90_4 RFP RFP

Gel13b.png



Gel 13 pMCY digest

No positive digestion

WEDNESDAY, 9/4/2019

Ca++ Competent Cells Test

DNAs used for test:

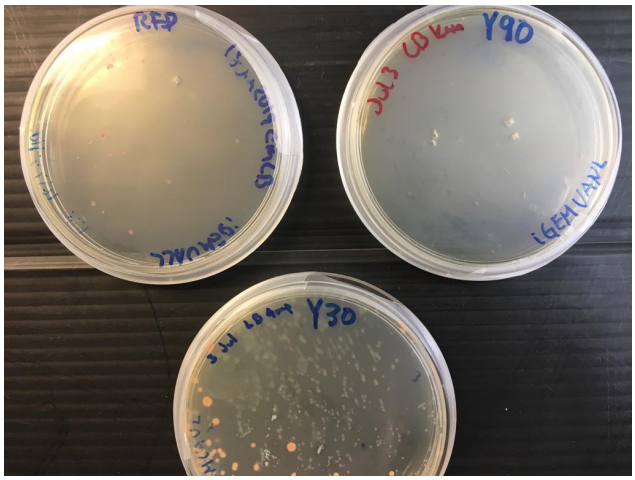
a) pMCY30 (Amp)

b) pMCY90 (Kan) DNA --> 1 : 1000 dil aprox. 4 ng/µL

c) pSB1C3 RFP (Cm)

1 µL (1 : 1000 dil) each plasmid --> 50 µL Bacteria --> ice, 20 min --> 42°C 90 sec --> 200 µL LB media --> 37°C shaking 20 min --> LB Plate --> 37oC overnigth.

RFP3090.jpeg

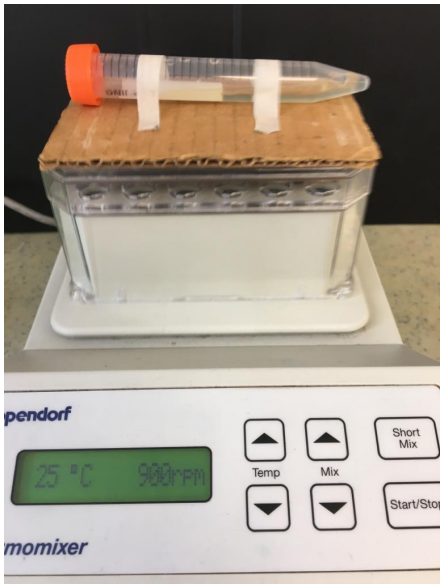


TUESDAY, 10/8/2019

Clone pMCY90 (mCherry-Internalization Pep) induction

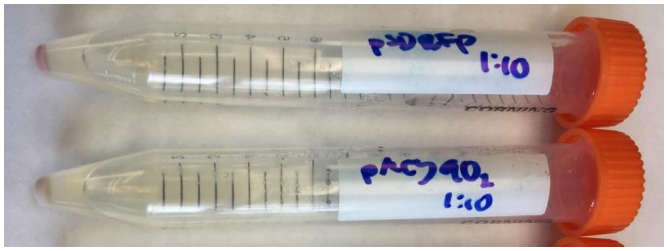
Overnigth growth --> 1:10 dil (2,700 µL LB media + 300 µL Bac) --> 15 mL conic tube/900 rpm/room temp

Clone90Growth.jpeg



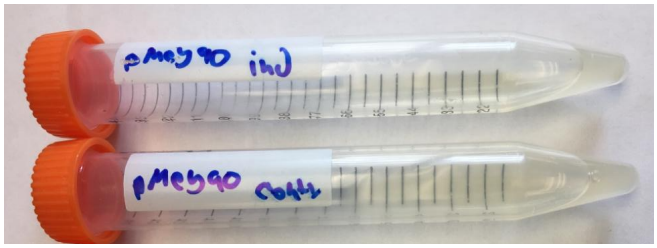
Clone pMCY90 pre-induction

pMCY90RhaTubes.jpeg



Pre-induction

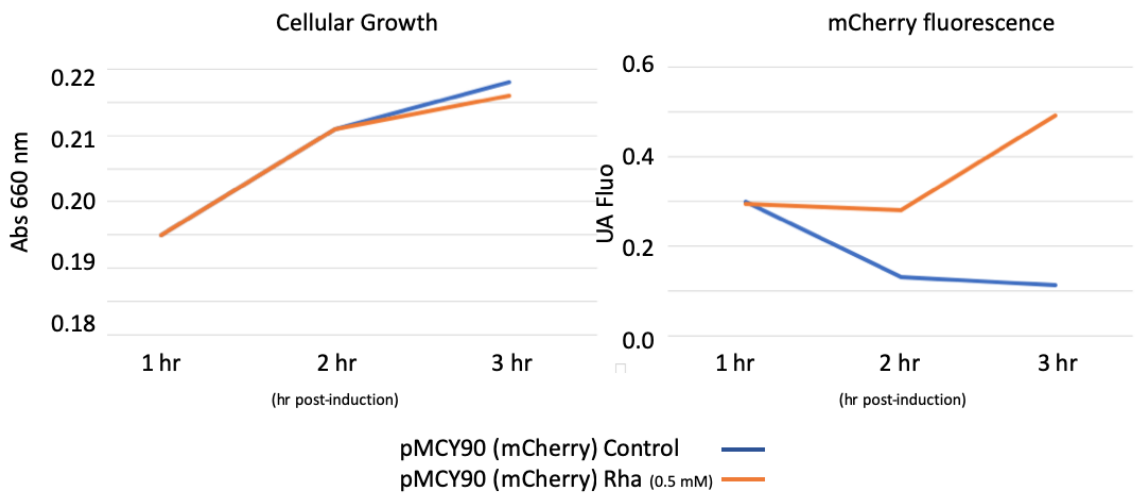
pMCY90RhaTubesb.png



Induction Rha (0.5 mM) + Control

2 hrs growth --> + 3 µL Rhamnose 500 nM (Final conc 0.5 mM) + 3 hrs 900 rpm --> check flourescence (587 nm ext / 610 nm emiss)

pMCY90IndctRes.png



THURSDAY, 10/10/2019

Synthetic DNA design from each circuit construction

All parts of the genetic circuit were synthesized in IDT recibed lyophilized

SyntDNA4.jpeg

The individual parts were dissolved in H_2O molecular grade. They were used to make the digestions and subsequently clones in to pSB1C3 RFP vector.

SUNDAY, 10/13/2019

Clones from Pi's fragments ligation in pSB1C3 RFP vector

Clones from the constructions:

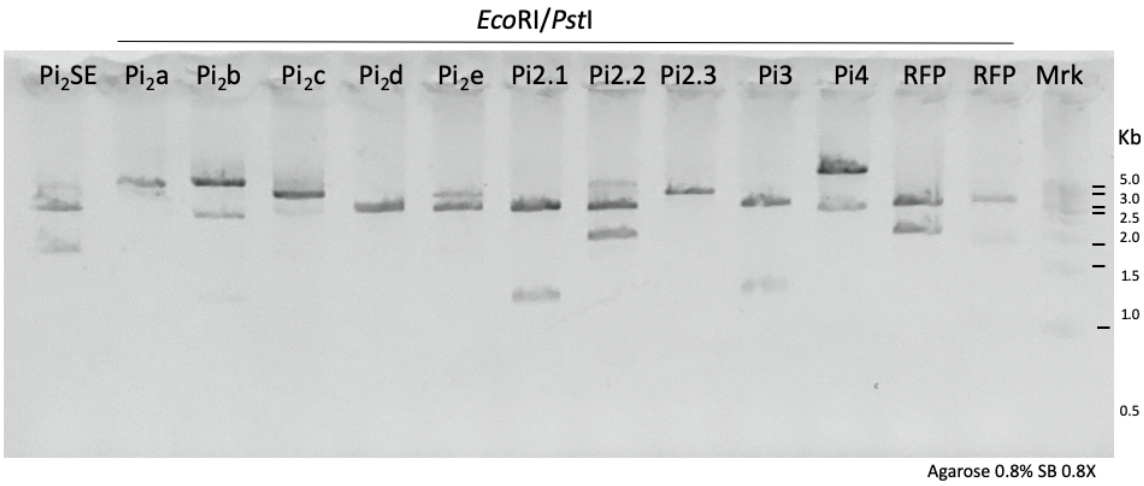
Pi2 LigM no integration peptide

Pi3 aroY + integration peptide

Pi4 catA + integration peptide

*Eco*RI + *Pst*I digestion

Gel14 Pi2Pi3Pi4.png



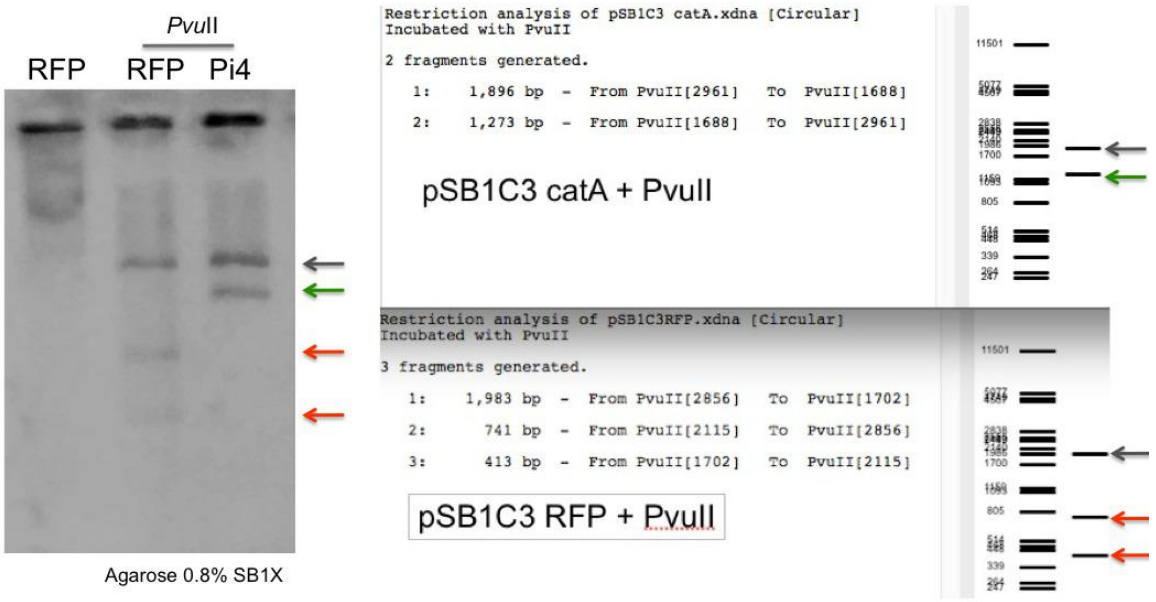
Gel 14 Pi's parts digest

WEDNESDAY, 10/16/2019

Pi4 *catA* clone charaterization.

Clone 4L: enzyme characterization with *Pvu*II enzyme

pi4 catA chart.jpeg



Gel 15 Pi4 catA characterization

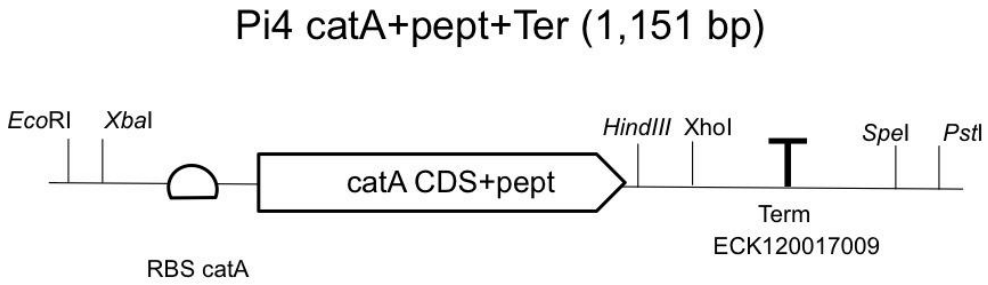
WEDNESDAY, 10/23/2019

catA enzyme expression in *E. coli*

In order to express the catA enzyme in bacteria, the construction was cloned with three constitutive promoters from the Anderson collection.

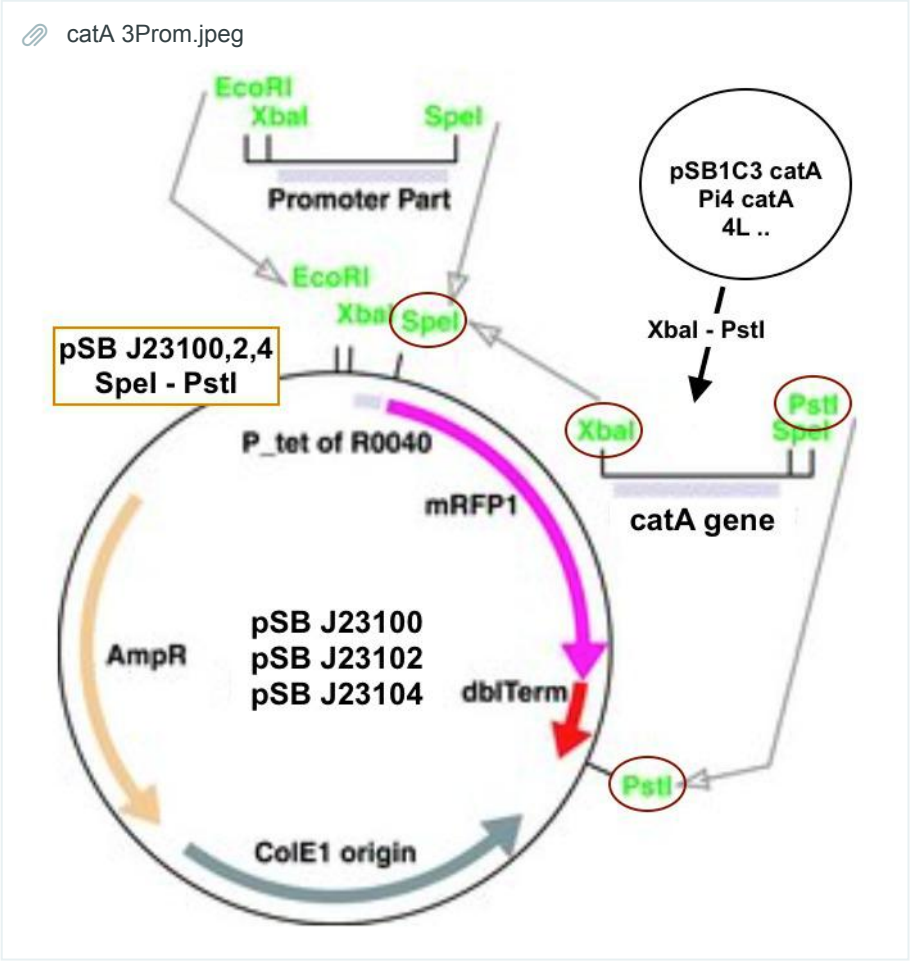
J23100 2547 au
J23102 2179 au
J23104 1831 au

catAmap.jpeg



Cloning strategy

catA 3Prom.jpeg

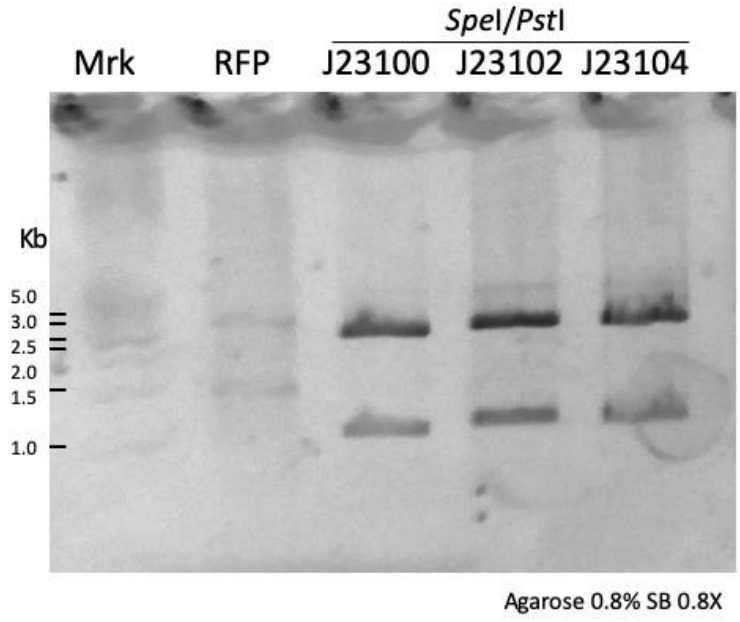


Vectors digestion: J23100, J23102 and J23104 with *Spe*I and *Pst*I

Table15

	A	B	C	D
1	DNA	2.0		6.0
2	Buff U 10X	2.0		6.0
3	SpeI	0.3	X3	0.9
4	PstI	0.3		0.9
5	H2O	15.4		46.2
6		**20.0**		**60.0**

Gel16 3VecSpeIPstI.jpg



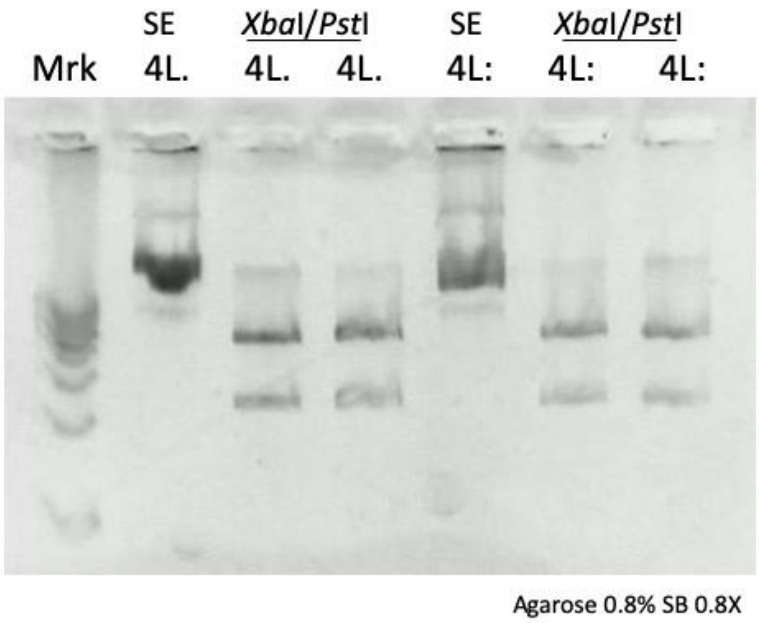
Gel 16 Promoter SpeI/PstI

Vectors digestion: Pi4 catA with *Spe*I and *Pst*I

Table16

	A	B	C	D
1	DNA	2.0		4.0
2	Buff U 10X	2.0		4.0
3	SpeI	0.3	x2	0.6
4	PstI	0.3		0.6
5	H2O	15.4		30.8
6		**20.0**		**40.0**

Gel17 4L catAXbaIPstI.jpg



Gel 17 Pi4 catA XbaI/PstI

Pi4 catA and Constitutive promoters ligation

Table17

	A	B	C	D
1	DNA catA	2.0		6.0
2	DNA vector	1.0		3.0
3	Buffer 10X lig	2.0	x3	6.0
4	T4 Ligase	2.0		6.0
5	H2O	13.0		39.0
6		**20.0**		**60.0**

DNA ligation transformation in Top10

J23100 + catA -- J23102 + catA -- J23104 + catA (100 ng/µL)

--> LB Agar Amp 37°C overnight

Single-temperature Double Digest

Introduction

This is the Double Digest Protocol with Standard Restriction Enzymes, using a common reaction and same incubation temperature for both enzymes.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

Procedure

Single Temperature DD Reaction

1. Set up the following reaction (total reaction volume 50 µl).

Table2

	A	B
1		**Reagent Volumes (µl)**
2	**Buffer (10x)**	5
3	**DNA ***	Input Volume for ng
4	**Restriction Enzyme #1 ****	1
5	**Restriction Enzyme #2 ****	1
6	**Deionized Water (µl)**	48
7	**Total Volume (µl)**	50

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.
** Restriction Enzymes should be added to the mixture last.

- 2. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.
- 3. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- 4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

    Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
    See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.