Lab Notebook

Beta System

1C3+1K3 preparation

05/08/2014

Primer name	**forward/ reversed**	Tm	**Add DMSO?**
Suffix f	forward	61	yes
Prefix r	reversed	62	yes

In the distribution the concentration 25 ng/µl
For final concentration of 2 ng/µl add 4 µl to 46 µl UPW

Reaction mix:

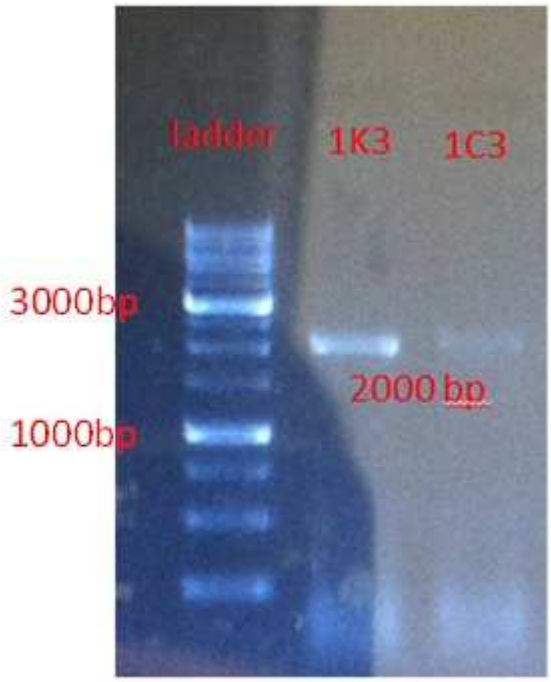
component	Volume[µl]	Mix x2
phusion reaction buffer(x5)	10	20
dNTPs(10 mM)	1	2
Suffix f -forward primer	2.5	5
Prefix r -reverse primer	2.5	5
plasmid (2 ng/µl)	5	-----
Phusion hot start II	0.5	1
DMSO	1.5	3
UPW	27	54
tot	50	-----

PCR program

stage	Temp C^0	time
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	61	30 sec
	72	1.20 min
Final extension	72	10 min
hold	4	

Part name	1C3 (lin)	1K3 (lin)
date	05/08/2014	05/08/2014
concentration	49 [ng/µl)	57 [ng/µl)
-20 ^{0}C storage	iGEM B sys lin.S	iGEM B sys lin.S

Run gel agarose 1% for product validation
Expected product: 1C3: 2070 bp, 1K3: 2204 bp



Blunt ligation

08/08/2014

Reaction content	1C3 49[µl]	1K3 57[µl]
T4 ligase buffer(x10)	2.5	2.5
T4 kinase (PNK)	1	1
Plasmid [ng/µl]	2	2
PEG 4000 (50%)	2.5	2.5
DDW	17	17
total	25	25

Incubate at 37 0C for 1 hr starting at 14:20

Inactivation 65 0C for 20 min
Ligation started at 15:50 for 2 hr at 25 0C in dry both.

Storage in -20 ^{0}C

Part name	1C3 (cir)	1K3 (cir)
date	08/08/2014	08/08/2014
-20 ^{0}C storage		

Transformation to top 10

09/08/2014

20 min frost on ice 100 mL
5 mL of Gibson product (cir)
30 min on ice
1 min heat shock 42 ^{0}C
2 min on ice

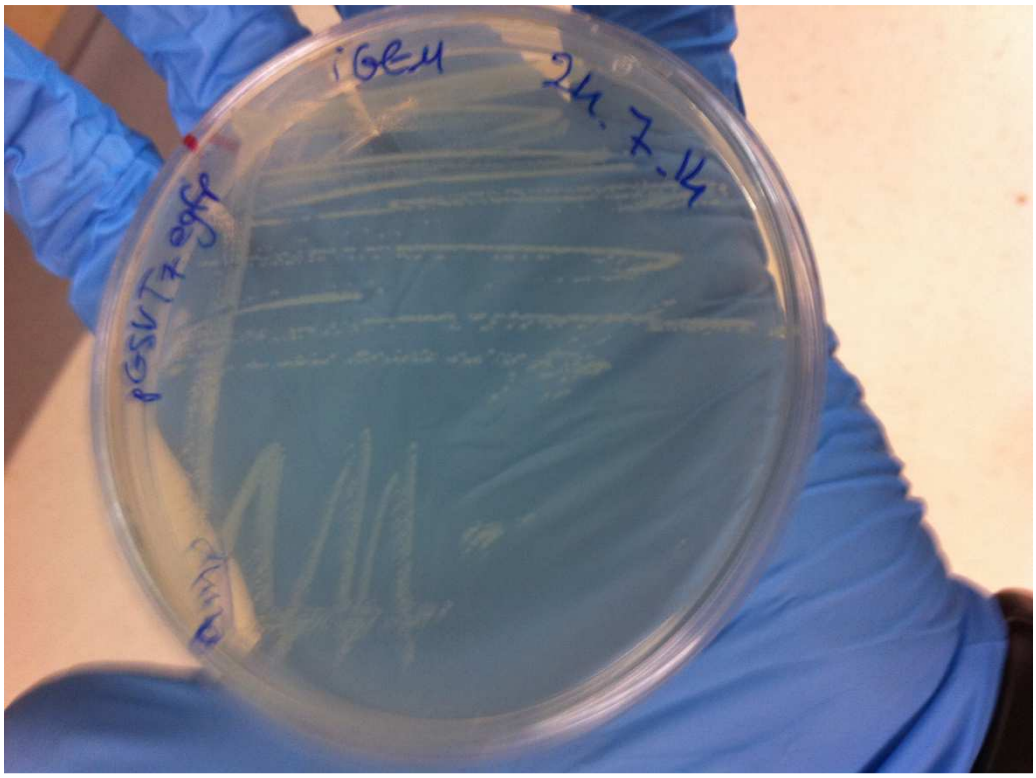
incubation overnight.

T7 poly preparation

In order to accommodate the T7 polymerase with type 2 Gblocks , we will design 2 PCR reaction that will build Gib3-T7 RNA polymerse-Gib4-Gib2 by 3 PCR reaction
Then the linear construct will be verified by sequencing

24/07/2014

Incubation of plate with Top 10, pGSV T7 egfp o\n (from Inbal GS)



25/07/2014

starter incubation in shaker 37 oC 250 rpm o\n at 16:40

26/07/2014

09: 46 GS has been made
10:18 MP has finished

Part name	pGSV T7 egfp (cir)
date	26/07/2014
concentration	703 [ng/µl]
-20 ^{0}C storage	iGEM B system \C1
Part name	pGSV T7 egfp (cir)
date	26/07/2014
Cell type	Top 10
resistance	AMP
-80 ^{0}C storage box name	iGEM 2014 B
Location	B1

Oligo PCR

16/09/2014

name	seq	length	Tm1	Tm2	neb	%GC
Oligo T7 f	CCA ATA TCC TTA GCT GAT CAC CAT TAA AGA GGA GAA ACC ACC AAT GAA CAC GAT TAA CAT CGC TAA GAA CG	71	56	68	St.1:66 St.2:72	40
Oligo T7 r	CCG TGT GAT CAA TTA AGG ATT CCC TTC AAA TTA ACA GGA ATC GGT TAC GCG AAC GCG AAG TCC GAC	66	62	70	St.1:66 St.2:72	47

PCR mix

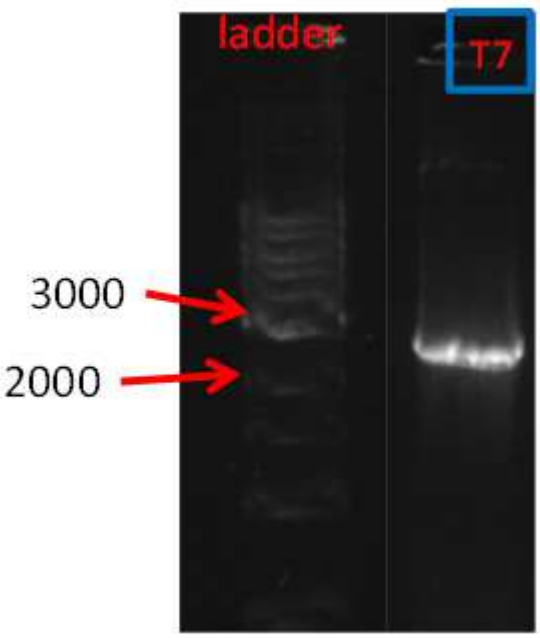
component	Volume[ml]	In?
phusion reaction buffer(x5)	10	
dNTPs(10 mM)	1	
Oligo T7 f (forward primer)	2.5	
Oligo T7 r (reverse primer)	2.5	
T7 poly st.3 (2ng/ml)	5	
Phusion hot start II	0.5	
DMSO	1.5	
UPW	27	
tot	50	

PCR program

stage	Temp C^0	time
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	66	30 sec
	72	1:30 min
30	98	10 sec
	72	30 sec
	72	1:30 min
Final extension	72	10 min
hold		

Part name	3,2 T7 poly (lin)
date	16/9/2014
concentration	228 [ng/ml]
-20 ^{0}C storage	iGEM B system

Run gel agarose 1% for product validation
Expected product: T7 RNA Poly: 2670 bp



PCR

06/08/2014

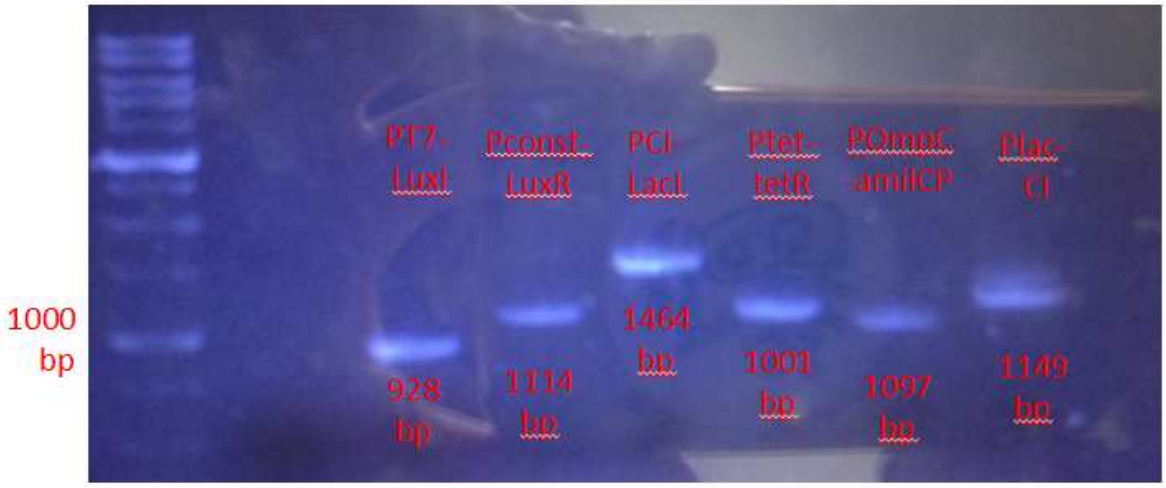
Primer name	**forward/ reversed**	Tm	**Add DMSO?**
Suffix r	forward	61	yes
Prefix f	reversed	62	yes

component	Volume[ml]	Mix x6
phusion reaction buffer(x5)	10	60
dNTPs(10 mM)	1	6
Suffix f -forward primer	2.5	15
Prefix r -reverse primer	2.5	15
template (2 ng/ml)	5	--\--
Phusion hot start II	0.5	--\--
DMSO	1.5	9
UPW	27	162
tot	50	

PCR program

stage	Temp C^0	time
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	61	30 sec
	72	40 sec
Final extension	72	10 min
hold	4	

Run gel agarose 1%



Gibson assembly

08/08/2014

Insertion of plux-ter to pSB1C3 by Gibson assembly
Calculation of the concentrations:

$$\left[\frac{pmol}{\mu l}\right] = \frac{\left[\frac{ng}{\mu l}\right] \times 10^3}{size \times 650}$$

Reaction mix:

name	size (bp)	ng/µl	pmol/µl	Gibson assembly master mix(x2)	pSB1C3 (pmol)	pSB1C3 (ng/µl)	Gblock (0.15pmol)	pSB1C3 (0.05pmol)	UPW	total
Pconst-LuxR	1114	84	0.116	15	0.036	49	1.3	1.4	2.3	20
PT7-LuxI	928	84	0.139	15	0.036	49	1.1	1.4	2.5	20
PCI-LacI	1464	70	0.07	15	0.036	49	2.1	1.4	1.5	20
Ptet-tetR	1001	83	0.127	15	0.036	49	1.2	1.4	2.4	20
Plac-lmmbda CI	1149	97	0.129	15	0.036	49	1.2	1.4	2.4	20
POmpC-amilCP	1097	34	0.047	15	0.036	49	3.2	1.4	0.4	20

Incubation at 50 ^{0}C for 1 hr.

Plux-G.Luc

21/08/2014

PCR

Primer name	**forward/ reversed**	Tm	**Add DMSO?**
Suffix r	forward	61	yes
Prefix f	reversed	62	yes

-

component	Volume[µl]
phusion reaction buffer(x5)	10
dNTPs(10 mM)	1
Suffix f -forward primer	2.5
Prefix r -reverse primer	2.5

Plux-green luciferase (2 ng/µl)	5
Phusion hot start II	0.5
DMSO	1.5
UPW	27
tot	50

PCR program

stage	Temp C^0	time
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	61	30 sec
	72	40 sec
Final extension	72	10 min
hold	4	

-20 storage:

name	size (bp)	date	ng/µl	iGEM B sys lin.s
Plux-green luciferase	1314	21/08/2014	166	

Gibson assembly

Insertion of plux-ter to pSB1C3 by Gibson assembly

Calculation of the concentrations:

$$\left[pmol/\mu l \right] = \frac{\left[ng/\mu l \right] \times 10^3}{size \times 650}$$

Reaction mix:

name	size (bp)	ng/ml	pmol/ml	Gibson assembly master mix(x2)	pSB1C3 (pmol)	pSB1C3 (ng/ml)	Gblock (0.15pmol)	pSB1C3 (0.05pmol)	UPW	total
Plux-green luciferase	1314	166	0.19	15	0.036	49	0.8	1.4	2.8	20

Incubation at 50 ^{0}C for 1 hr.

Store in -20 ^{0}C

Part name	Plux-green luciferase
date	
-20 ^{0}C storage iGEM B system	

Colony PCR

Primer name	**forward/ reversed**	**First Tm**	**Tm**	**Add DMSO?**
prefix f	**forward**	50	63	yes
suffix r	**reversed**	50	63	yes

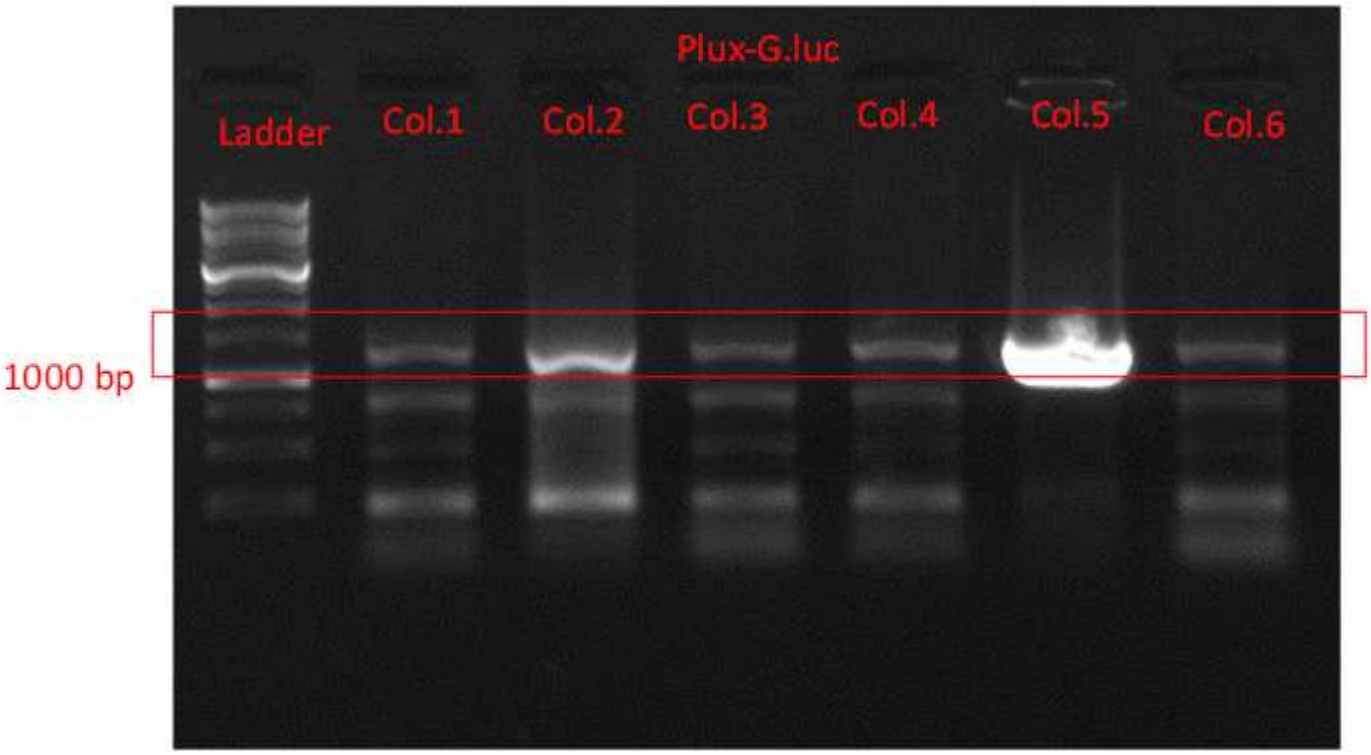
Reaction mix: make total mix and then divide 20 [µl] to each PCR tube

component	Volume[µl] for 1 colony	For total mix 6 reaction	In?
Taq ready mix (x2)	10	60	V
DMSO	0.5	3	V
prefix f (10 ng/µl)	1	6	V
suffix r (10 ng/µl)	1	6	V
colony	colony	----	
UPW	7.5	45	V
tot	20		

PCR program

stage	Temp C^0	time
Initial denaturation	94	3 min
35 cycles	94	30 sec
	57	30 sec
	72	50 sec
Final extension	72	10 min
hold	4	

Run gel agarose 1%, expected product : 1314 bp



Colony PCR

28/08/2014

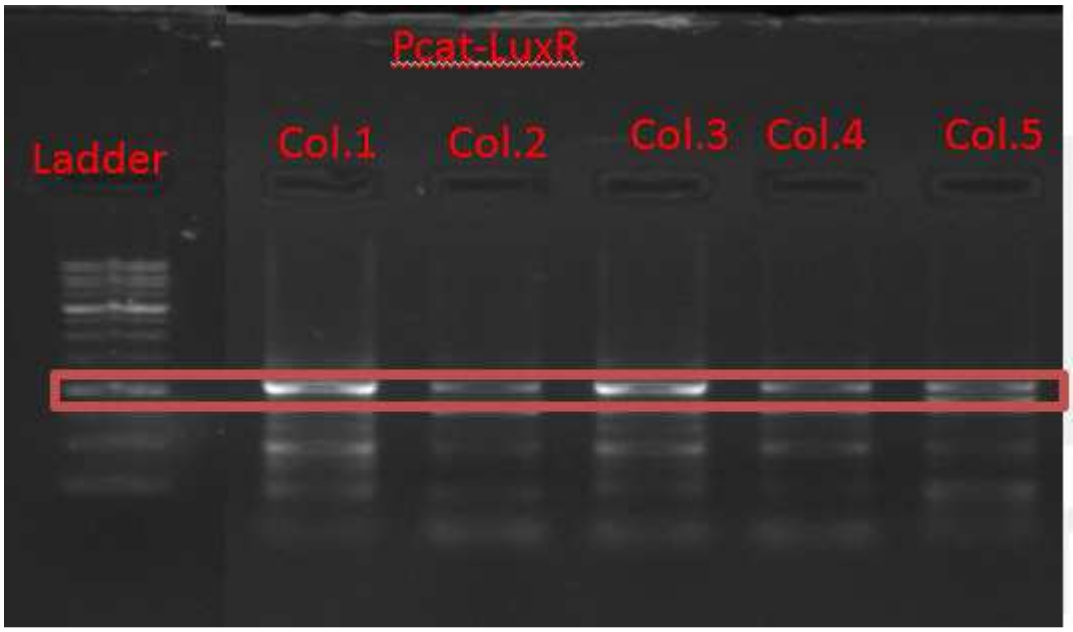
Primer name	**forward/ reversed**	**First Tm**	**Tm**	**Add DMSO?**
prefix f	**forward**	50	63	yes
suffix r	**reversed**	50	63	yes

Reaction mix: make total mix and then divide 20 [ml] to each PCR tube

component	Volume[ml] for 1 colony	For total mix 32 reaction	In?
Taq ready mix (x2)	10	320	V
DMSO	0.5	16	V
prefix f (10 ng/ml)	1	32	V
suffix r (10 ng/ml)	1	32	V
colony	colony	----	
UPW	7.5	240	V
tot	20		

code	name	length
A	Pcat-LuxR	1114
B	PT7-LuxI	928
C	PompC-AmilCP	1097
D	PCI-LacI	1464
E	Plac-CI	1149
F	Ptet-tetR	1001

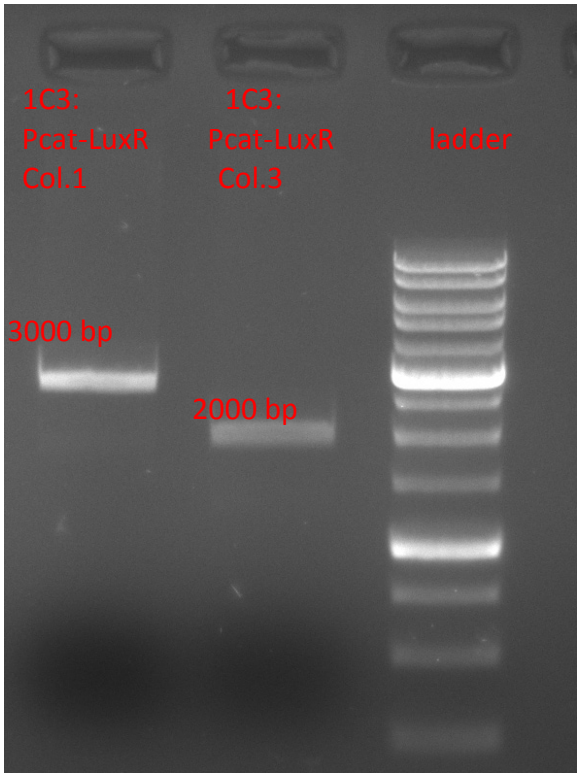
A: Pcat-LuxR, expected product: 1114 bp



Although we have positive result, it seem that the colonies are unpure or the PCR Tm was Low.
In order to support the positive result restriction reaction was made.
Reaction mix:

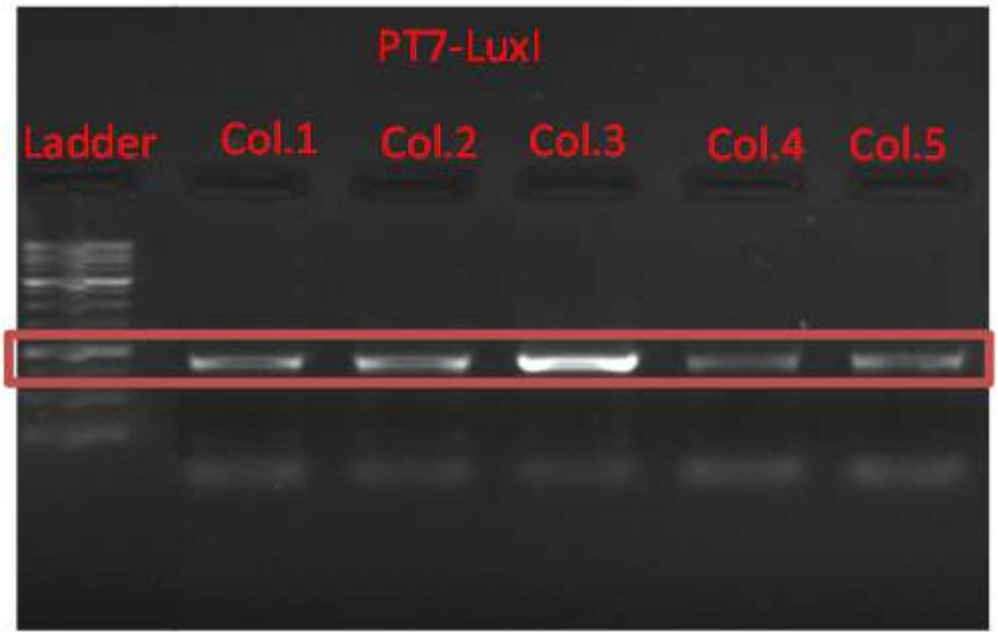
	Volume [ml]	in
1C3:Pcat-LuxR (114 [ng/ml])	5	V
EcoRI HF	0.5	V
Cutsmart buffer (x10)	2	V
MBW	12.5	V
total	20	+

Run agarose gel 1%, positive : 3000 bp negative: 2000 bp



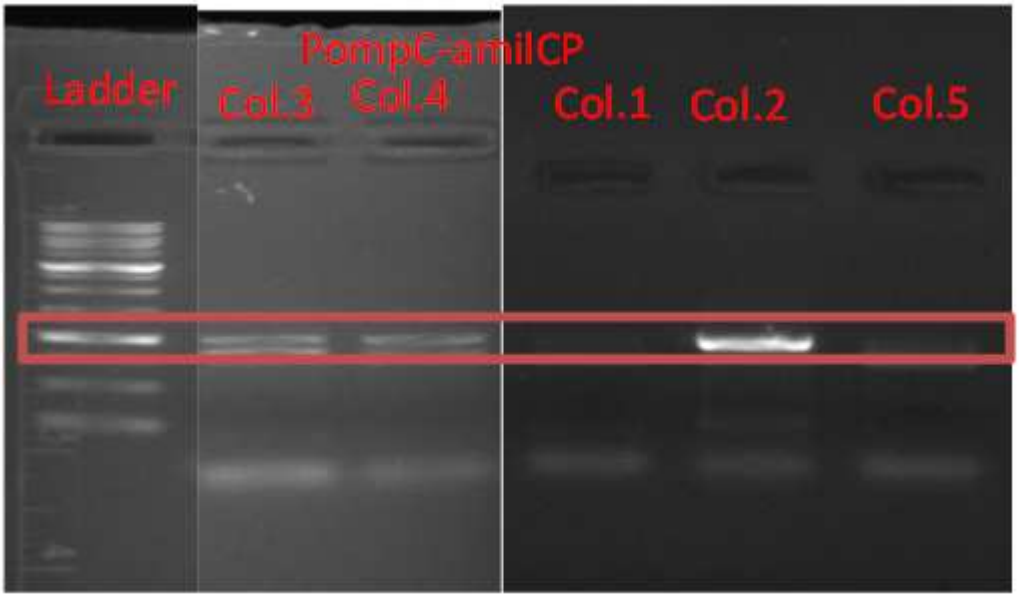
Colony 1 has been selected to be sent to sequencing

B: PT7-LuxI, expected product: 928 bp



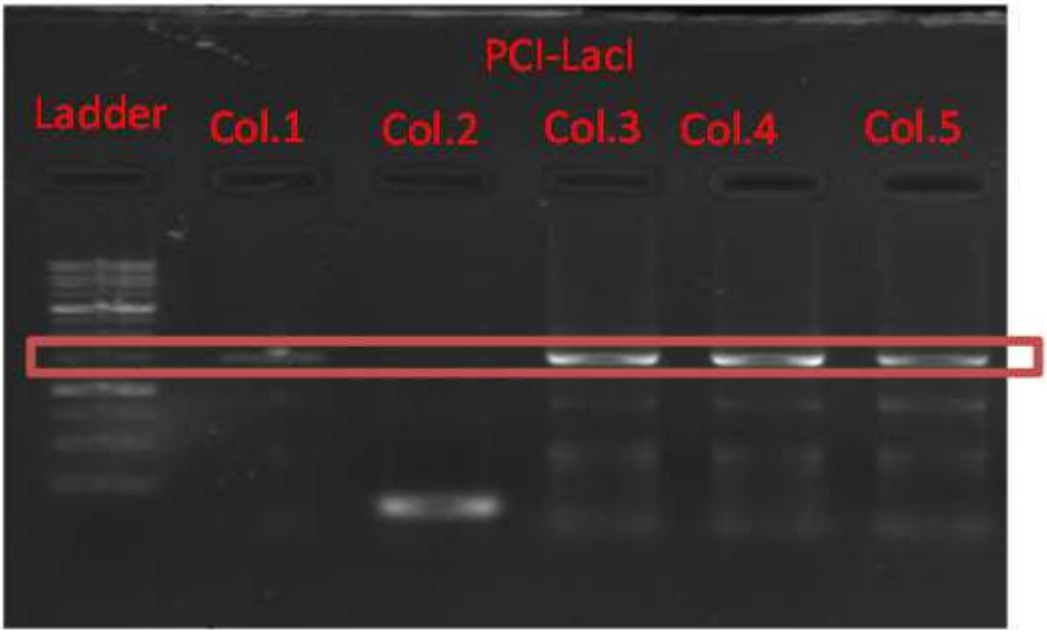
Colony 3 has been selected to be sent to sequencing

C: PompC-AmilCP expected product: 1097 bp



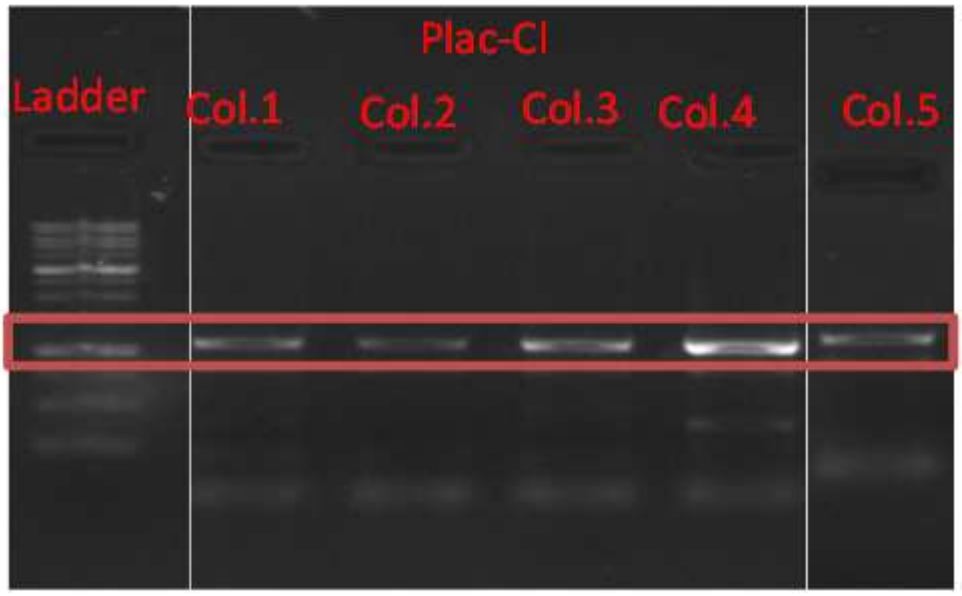
Colony 3 has been selected to be sent to sequencing

D: PCI-LacI expected product: 1464 bp



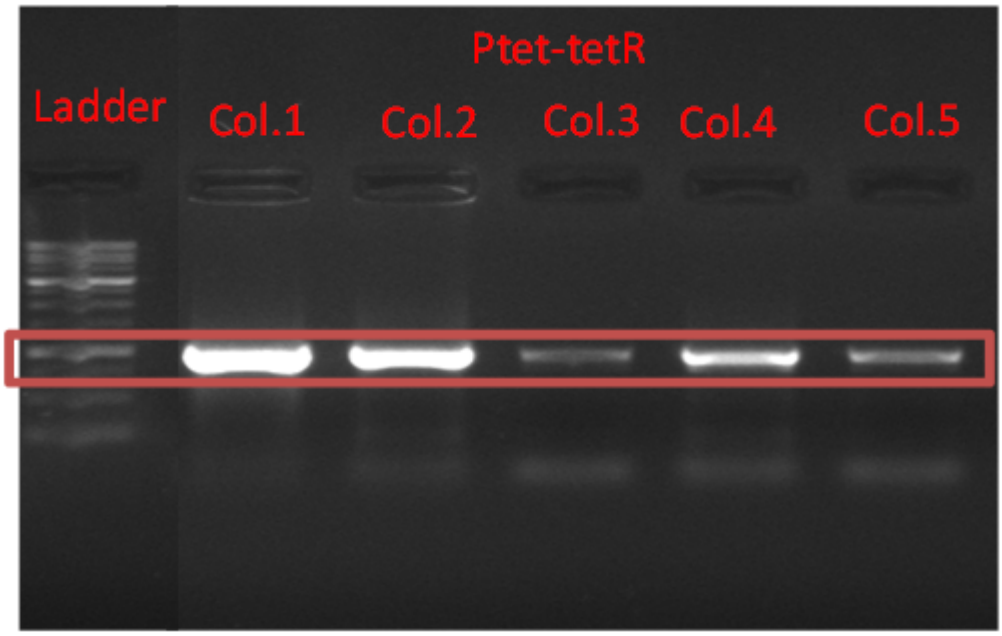
Colony 1 has been selected to be sent to sequencing

E: Plac-CI expected product: 1149 bp



Colony 4 has been selected to be sent to sequencing

F: Ptet-tetR expected product: 1001 bp



Colony 1 has been selected to be sent to sequencing

Colony PCR- 2

01/09/2014

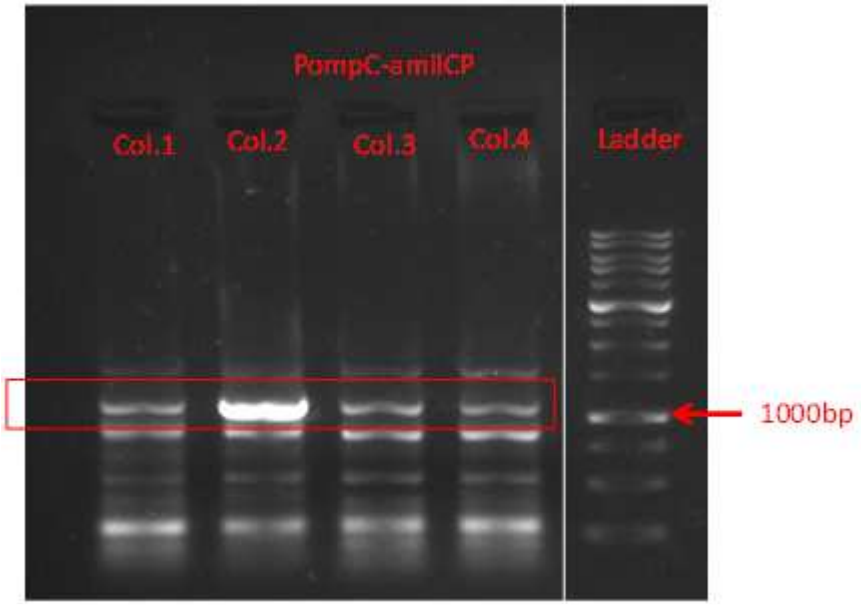
Primer name	**forward/ reversed**	**First Tm**	**Tm**	**Add DMSO?**
prefix f	**forward**	50	63	yes
suffix r	**reversed**	50	63	yes

Reaction mix: make total mix and then divide 20 [ml] to each PCR tube

component	Volume[ml] for 1 colony	For total mix 25 reaction	In?
Taq ready mix (x2)	10	320	V
DMSO	0.5	16	V
prefix f (10 ng/ml)	1	32	V
suffix r (10 ng/ml)	1	32	V
colony	colony	----	
UPW	7.5	240	V
tot	20		

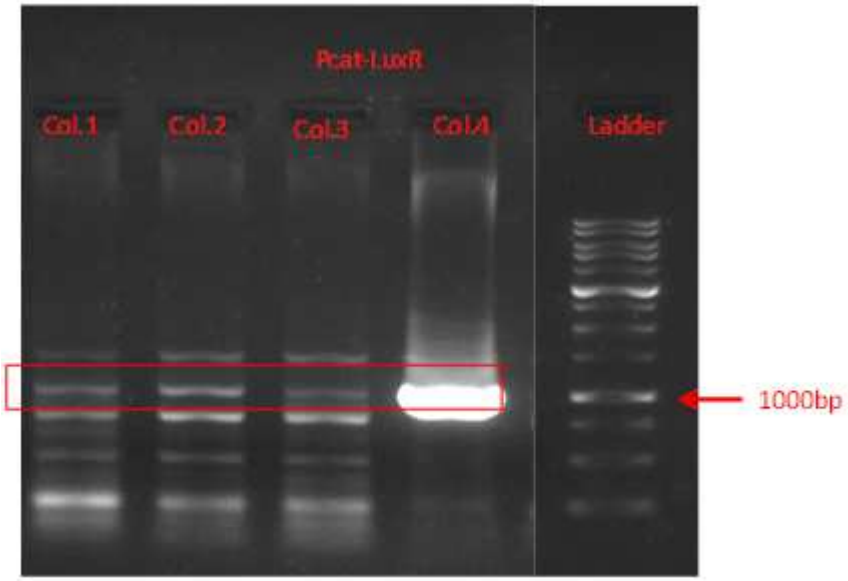
code	name	length
A	PompC-AmilCP	1097
B	Pcat-LuxR	1114
C	PT7-LuxI	928
D	PCI-LacI	1464
E	Plac-CI	1149

A: PompC-AmilCP expected product: 1097 bp



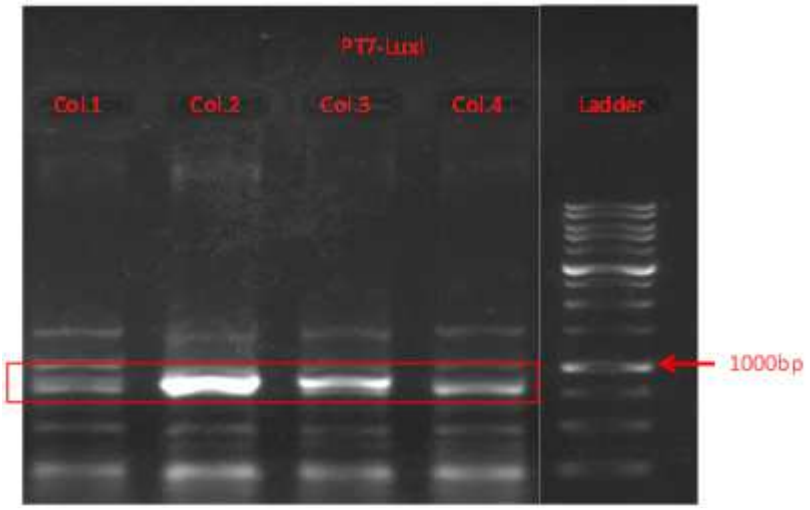
Unspecific band in all colony

B: Pcat-LuxR, expected product: 1114 bp



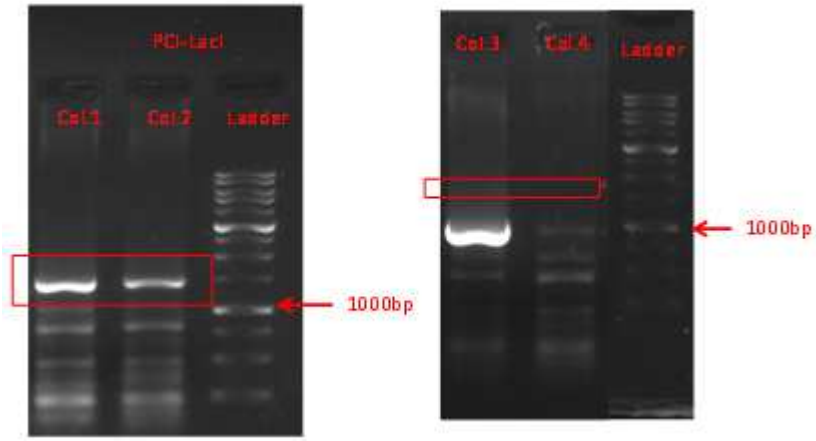
Col 4 seam to be positive and clean

C: PT7-LuxI, expected product: 928 bp



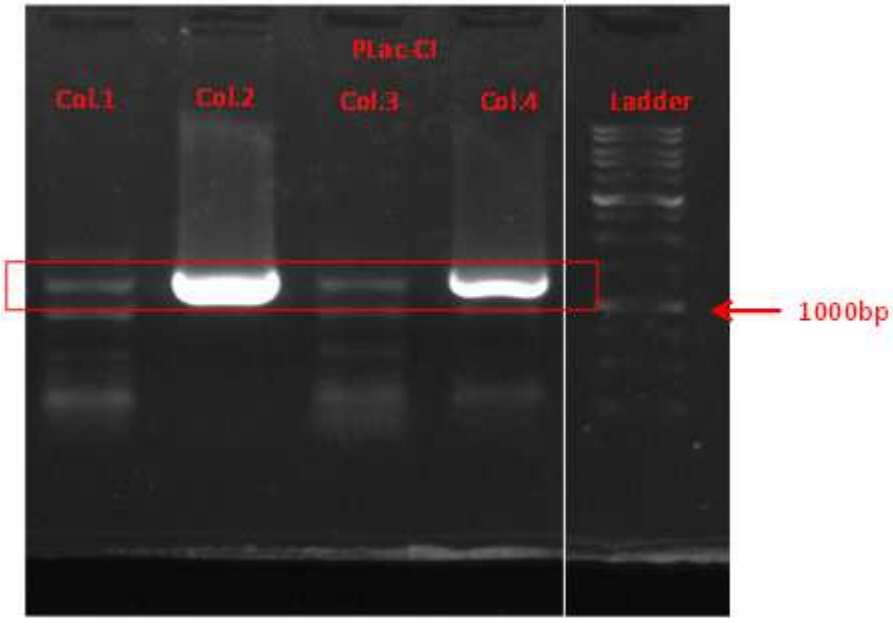
Unspecific band in all colony

D: PCI-LacI expected product: 1464 bp



Colony 1 and colony 2 contains Unspecific band with the positive band
Colonies 3,4 are negative

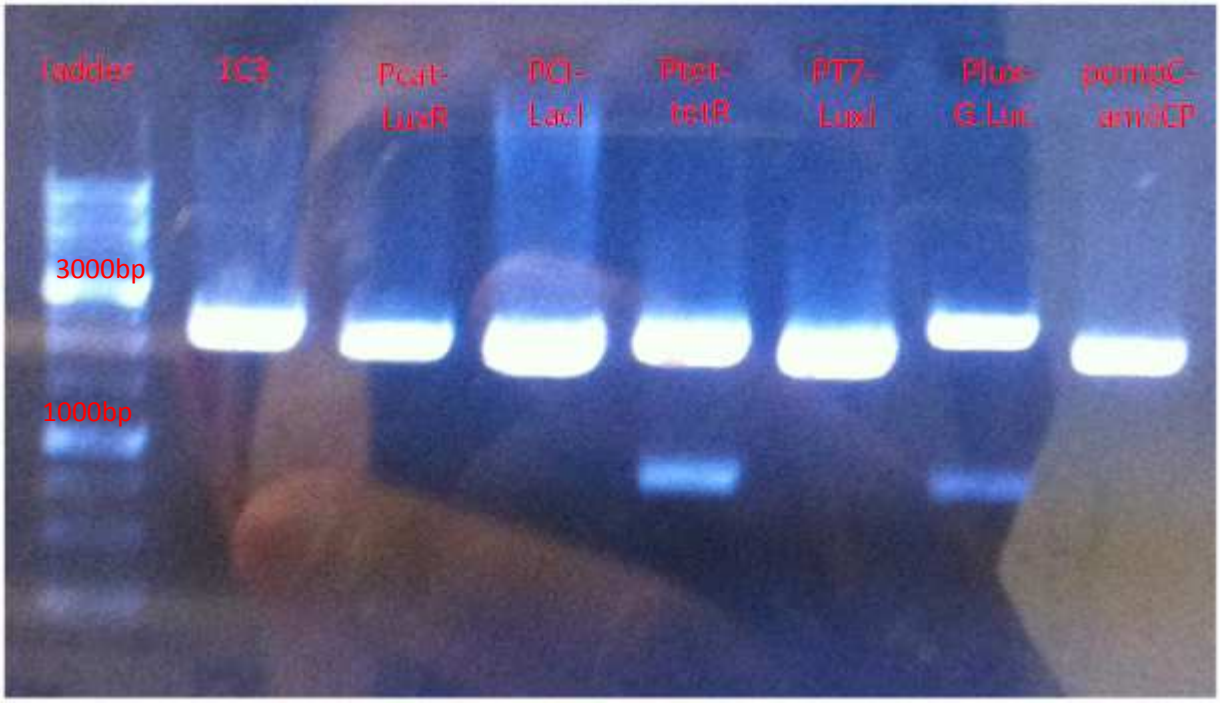
E: Plac-CI expected product: 1149 bp



Colony 2 seam to be positive and clean

Restriction validation

28/08/2014

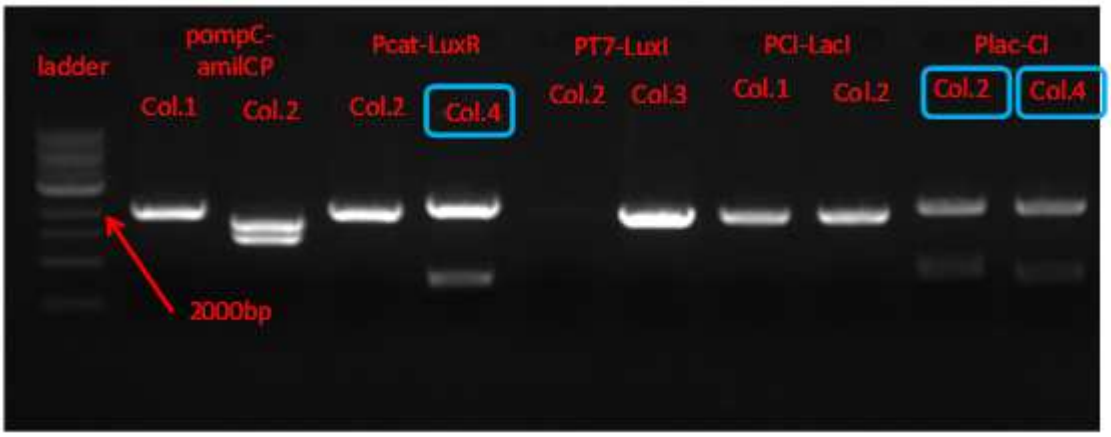


Restriction validation

03/09/2014

code	name	colony	Status
A	PompC-amilCP	1	x
		2	x
B	Pcat-LuxR	2	x
		4	V
C	PT7-LuxI	2	x
		3	x
D	PCI-LacI	1	x
		2	x
E	Plac-CI	2	V
		4	V

Cut with 2 restriction enzyme:
NcoI and BglII
Positive: 2500 bp and 800 bp negative: 2070 bp



Gel map

Ladder	1C3	C9											
Ladder	A5	A6	A7	A8	A9	B5	B6	B7	B8	B9	C5	C6	C7	C8

Restriction validation

07/09/2014

code	name	colony	Status
A	PompC-amilCP	5	x
		6	x
		7	x
		8	x
		9	x
B	PT7-LuxI	5	V
		6	x
		7	x
		8	x
		9	x
C	PCI-LacI	5	x
		6	V
		7	x
		8	x
		9	V
cont	pSB1C3	----	ref

Cut with 2 restriction enzyme:
NcoI and BglII
Positive: 2500 bp and 800 bp negative: 2070 bp

PompC-amilCP - no positive

PT7-LuxI: colony 5 positive

PCI-LacI: colonies 6,9 Positive
Colony 9 will be taken

Gel map

Ladder	1C3	C9												
Ladder	A5	A6	A7	A8	A9	B5	B6	B7	B8	B9	C5	C6	C7	C8


PCI
LacI
1C3
9
2000b
PT7-LuxI
PLtetLacI
5
6
7
8
9
5
6
7
8

Gibson assembly

14/09/2014

Calculation of the concentrations:

$$\left[\frac{pmol}{\mu l}\right] = \frac{\left[\frac{ng}{\mu l}\right] \times 10^3}{size \times 650}$$

code	name	size (bp)	ng/ml	pmol/ml	Gibson assembly master mix(x2)	Plasmid (0.1pmol)	T7 poly (0.1pmol) 2739 bp	UPW	total
X1	1C3: PCI-LacI (lin)	3491	137	0.06	15	1.66	0.78	2.56	20
X2	1C3: Plac-lmmbda CI (lin)	3176	122	0.06	15	1.69	0.78	2.53	20
X3	1C3: Ptet-tetR (lin)	3028	175	0.09	15	1.12	0.78	3.09	20

code	Reaction name	Insert size (bp)	plasmidsize (bp)	Insert ng/ml	Plasmid ng/ml	Insert pmol/ml	Plasmid pmol/ml	Gibson assembly master mix(x2)	Plasmid (0.05pmol)	Insert (0.15pmol)	UPW	total
Y1	1C3: PCI-tetR	777	2163	141	128	0.28	0.09	15	0.55	0.54	3.91	20
Y3	1C3:Plac-tetR	777	2226	141	167	0.28	0.12	15	0.43	0.54	4.03	20
Y5	1C3:Ptet-LacI	1245	2168	76	136	0.09	0.10	15	0.52	1.60	2.88	20
Y4	1C3:Ptet-CI	867	2168	149	136	0.26	0.10	15	0.52	0.57	3.91	20
Y6	1C3:PT7-amilCP	819	2137	63	162	0.12	0.12	15	0.43	1.27	3.30	20
Y2	1C3:Plux-amilCP	819	2296	63	181	0.12	0.12	15	0.41	1.27	3.32	20

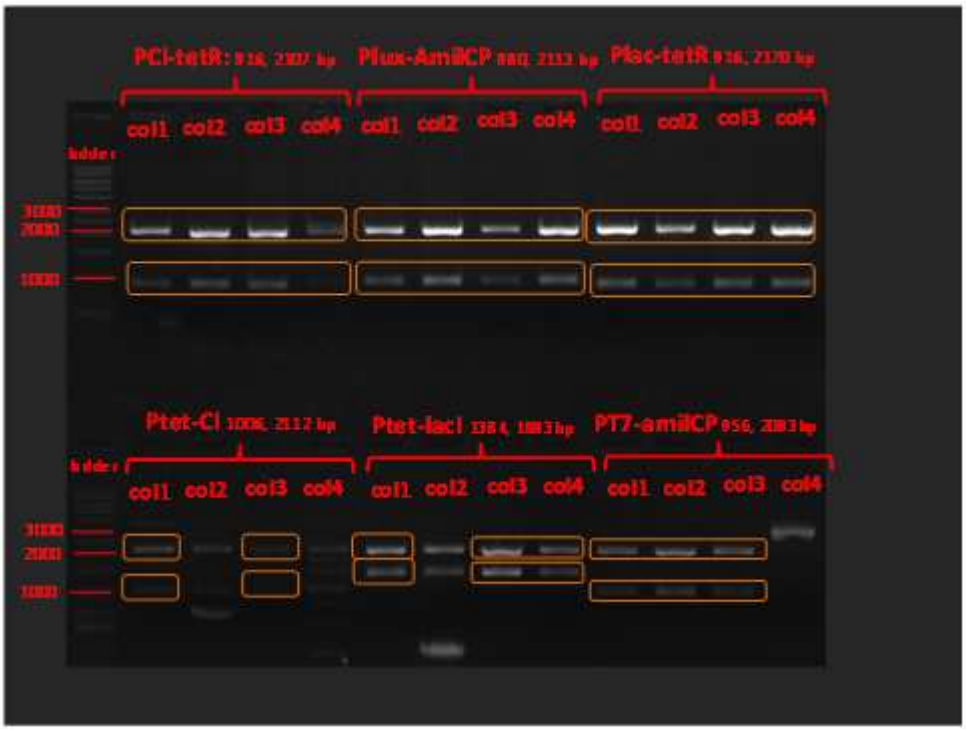
Incubation at 50 ^{0}C for 1 hr.
Store in -20 ^{0}C

Transformation to top 10

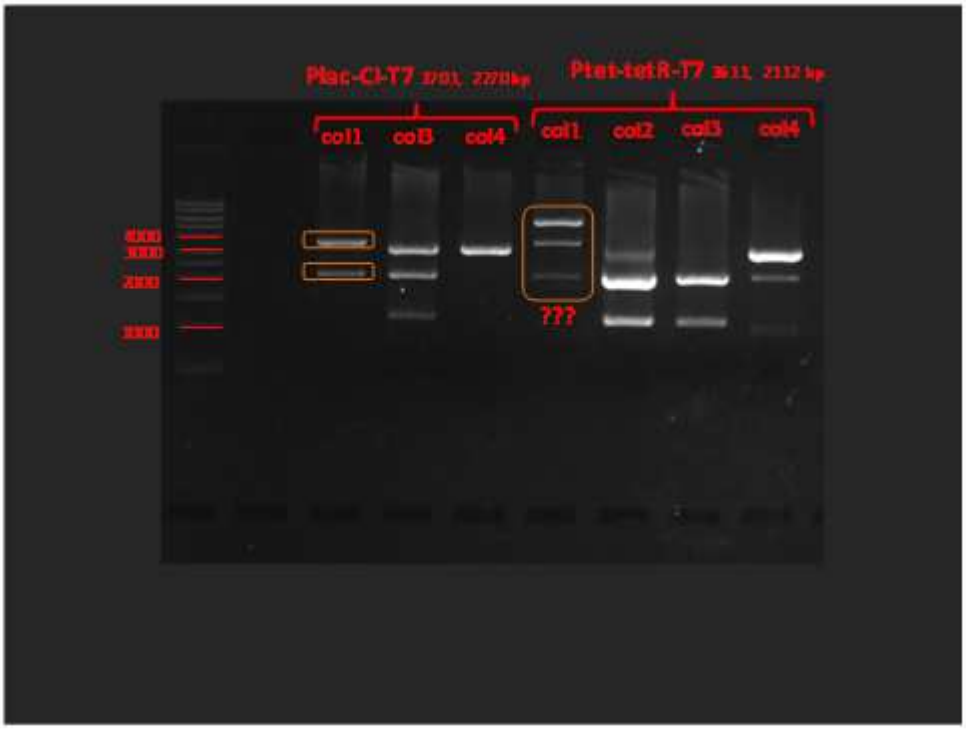
20 min frost on ice 100 mL
5 mL of 1AK3:Plux-ter (cir)
30 min on ice
1 min heat shock 42 ^{0}C
2 min on ice

incubation overnight.

Restriction validation



16/09/2014


Plac-CI-T7
Ptet-tetR-T7
col1
col3
col4
col1
col2
col3
col4
???

Gibson assembly

23/09/2014

Calculation of the concentrations:

$$\left[pmol/\mu l \right] = \frac{\left[ng/\mu l \right] \times 10^3}{size \times 650}$$

code	Reaction name	Insert size (bp)	plasmidsize (bp)	Insert ng/ml	Plasmid ng/ml	Insert pmol/ml	Plasmid pmol/ml	Gibson assembly master mix(x2)	Plasmid (0.05pmol)	Insert (0.15pmol)	UPW	total
Z1	Plux-g.luc-tetR	711	3341	15	82	0.03	0.04	15	1.32	4.62	0.00	20.95
Z2	Plux-g.luc-CI	801	3341	31	82	0.06	0.04	15	1.32	2.52	1.16	20.00
Z3	Plux-g.luc-LacI	1179	3341	27	82	0.04	0.04	15	1.32	4.26	0.00	20.58
Z4	Plux-amilCP-CI	801	3071	31	71	0.06	0.04	15	1.41	2.52	1.07	20.00
Z5	Plux-amilCP-lacI	1179	3071	27	71	0.04	0.04	15	1.41	4.26	0.00	20.66
Z6	Plux-amilCP-tetR	711	3071	15	71	0.03	0.04	15	1.41	4.62	0.00	21.03
M1	Plux-mcherry	727.00	2434	137.00	133.00	0.29	0.08	15.00	1.78	0.17	3.04	20.00

Incubation at 50 ^{0}C for 1 hr.
Store in -20 ^{0}C

Plux-mCherry

Reaction: Che A
PCR mix

component	Volume x1[ml]
phusion reaction buffer(x5)	10
dNTPs(10 mM)	1
che Af-forward primer	2.5
Che A r-reverse primer	2.5
Template (2ng/ml)	5
Phusion hot start II	0.5
DMSO	1.5
UPW	27
tot	50

PCR program

stage	Temp C^0	time
Initial denaturation	98	30 sec
35 cycles	98	10 sec
		sec
	72	1:40 min
Final extension	72	10 min
hold	4	--\--

Part name			
date			
concentration	[ng/ml]	[ng/ml]	[ng/ml]
-20 ^{0}C storage	iGEM B system \	iGEM B system \	iGEM B system \

Primers

name	seq	length	TM 1	Tm 2	Tn neb
Che G F	CCA CCA ATG GTG AGC AAG GGC G	22	64	64	1s: 55 2s: 72
Che G R	CAG GAA TCG GTT ACT TGT ACA GCT CGT CC	29	53	62	1s: 55 2s: 72
Che P F	GGA CGA GCT GTA CAA GTA ACC GAT TCC TGT TAA TTT GAA GG	41	54	64	1s: 66 2s: 72
CheP R	CGC CCT TGC TCA CCA TTG GTG GTT TCT CCT CTT TAA TGG	39	55	67	1s: 66 2s: 72

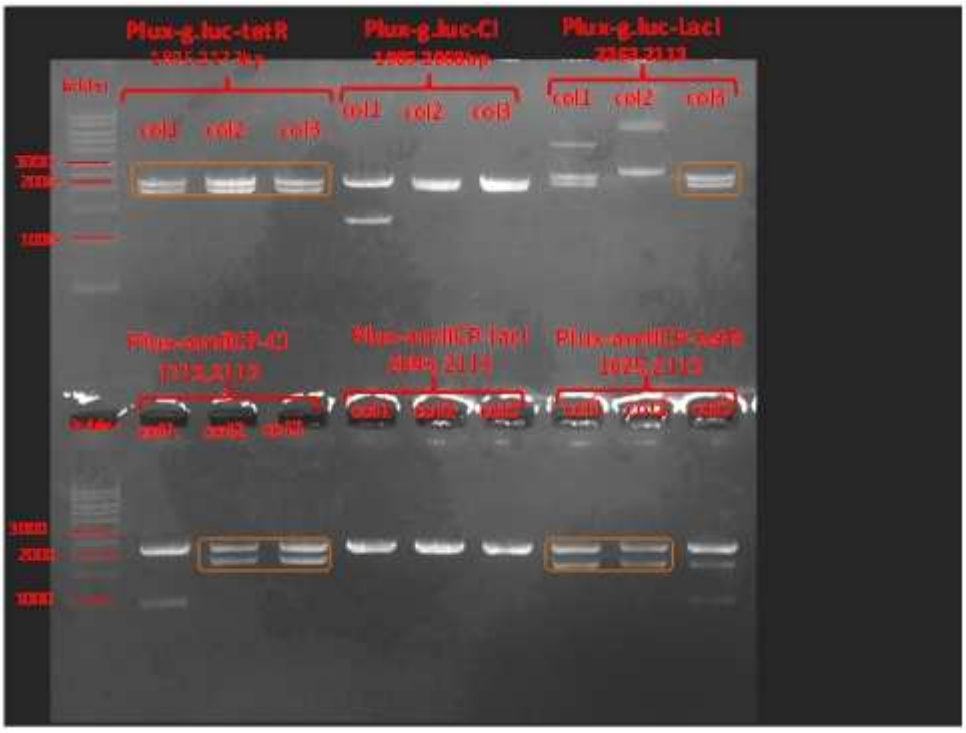
Transformation to top 10

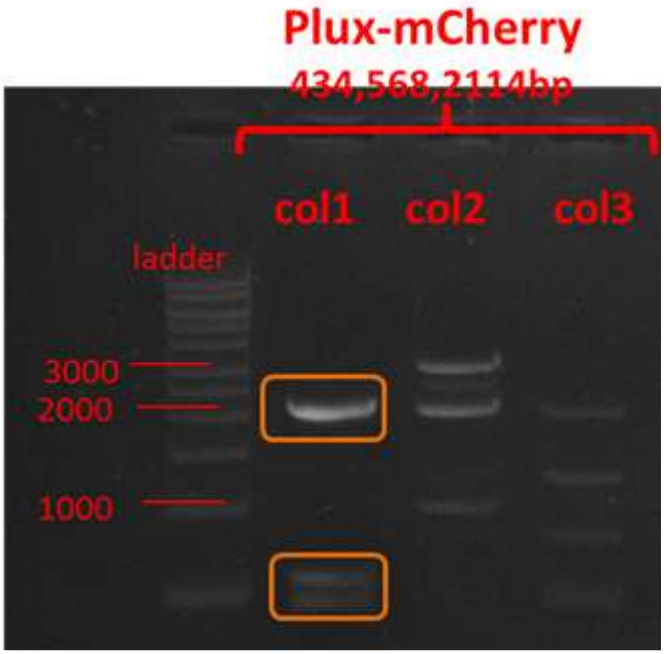
20 min frost on ice 100 mL
5 mL of product (cir)
30 min on ice
1 min heat shock 42 ^{0}C
2 min on ice

incubation overnight.

Restriction validation

24/09/2014




Plux-mCherry
434,568,2114bp
col1
col2
col3
ladder
3000
2000
1000

Gibson assembly

06/10/2014

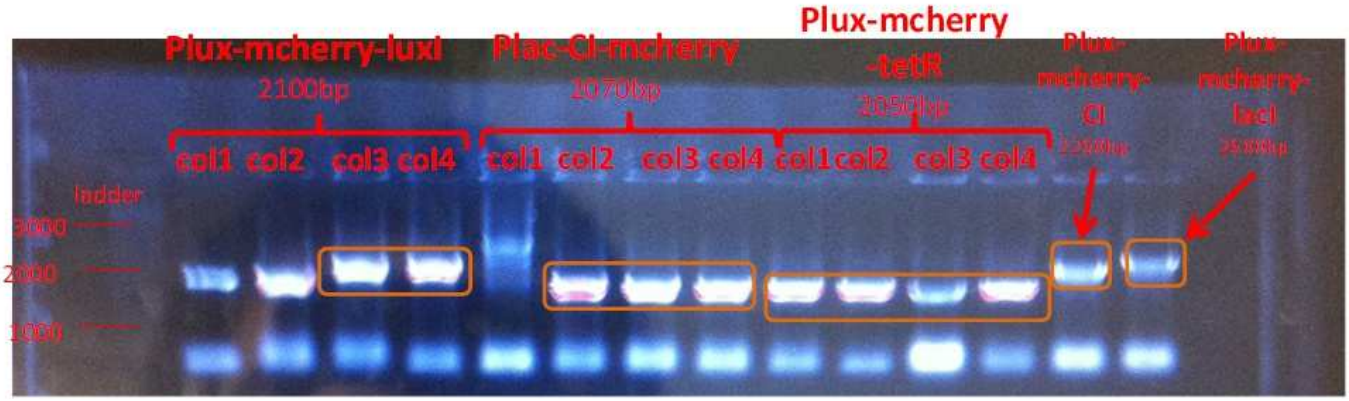
code	Reaction name	Insert size (bp)	plasmidsize (bp)	Insert ng/ml	Plasmid ng/ml	Insert pmol/ml	Plasmid pmol/ml	Gibson assembly master mix(x2)	Plasmid (0.05pmol)	Insert (0.15pmol)	UPW	total
M2	Plux-mcherry-CI	801	3116	31	43.00	0.06	0.02	15.00	7.07	0.84	0.00	22.91
M3	Plux-mcherry-LacI	1179	3116	27	43.00	0.04	0.02	15.00	7.07	1.42	0.00	23.48
M4	Plux-mcherry-tetR	711	3116	15	43.00	0.03	0.02	15.00	7.07	1.54	0.00	23.61
M6	Plux-mcherry-luxI	669	3116	70	43.00	0.16	0.02	15.00	7.07	0.31	0.00	22.38
M8	Plac-CI-mcherry	798	3176	67	48.00	0.13	0.02	15.00	6.45	0.39	0.00	21.84

Colony PCR

Reaction mix: make total mix and then divide 20 [ml] to each PCR tube

component	Volume[ml] for 1 colony	For total mix 15 reaction	In?
Taq ready mix (x2)	10	150	V
DMSO	0.5	7.5	V
1C3 f (10 ng/ml)	1	15	V
1C3 r (10 ng/ml)	1	15	V
colony	colony	----	
UPW	7.5	112.5	V
tot	20		

09/10/2014


Plux-mcherry-luxI
2100bp
Plac-CI-mcherry
2070bp
Plux-mcherry
-tetR
2050bp
Plux-
mcherry-
CI
Plux-
mcherry-
lacI
col1 col2 col3 col4
col1 col2 col3 col4
col1 col2 col3 col4
ladder
3000
2000
1000