LAB NOTEBOOK

USP_UNIFESP-Brazil
iGEM 2016

- Full version -

São Paulo, Brazil - 2016

CHEAT SHEET - USP_UNIFESP-BRAZIL - OLIGONUCLEOTIDES/PRIMERS

	Sequence 5' - 3'	Amplicon	Primer type	Used for plasmid	NOTES	Notes 2	Length bp	
iGP0001	tctgGAATTCGCGGCCGC	tctg + iGEM prefix	Forward	IDT sequences	Tm - 60.7		18	
iGP0002	gccggaCTGCAGCGGC	iGEM sufix + tccggc	Reverse	IDT sequences	Tm - 61.0		16	
iGP0003	ggcttaauCTCGAGGGTCGCGGGCAGGGG	USER cassete adapter + XhoI + Ea Masp1	Forward	pUSER	Tm - 59.8		29	
iGP0004	ggtttaaUggatccTTATGCCGCCGCGGCGGC	USER cassete adapter + BamHI + STOP + Ea Masp1	Reverse	pUSER	Tm - 62.5		32	
iGP0005	aGGGCAGGGuGGCTACGGTCAGGGCTC	Ea MaSp1 polymerizing DNA amplicon	Forward	pUSER	Design annotation		27	
iGP0006	aCCCTGCCCuCGACCTGCCGCCGCGGCGGC	Ea MaSp1 polymerizing DNA amplicon	Reverse	pUSER	Design annotation		30	
iGP0007	ggcttaauCTCGAGgagagtgctggtcgtGGGGGCGCAGGACAAGG	USER cassete adapter + XhoI + GGAGR + Lh MaSp1 Modified Type2	Forward	pUSER	Tm 62.0	Adding a GGAGR for next USER	46	
iGP0008	ggtttaaUggatccTTAtgctcctcgtgcgcAGCCGCGGCAGCCGCTG	USER cassete adapter + BamHI + STOP + AAGGA + Lh MaSp1 Modified Type2	Reverse	pUSER		Adding a AAGGA for next USER	49	
iGP0009	aGCCGCGGCuGGGGGCGCAGGACAAGG	Lh MaSp1 Modified Type2 polymerizing DNA amplicon	Forward	pUSER	Design Annotation	Tm 62	27	
iGP0010	AGCCGCGGCuGCCGCTCGCTGCTCCTCC	Lh MaSp1 Modified Type2 polymerizing DNA amplicon	Reverse	pUSER	Design Annotation		27	
iGP0011	ggcttaaUctcaggagaagttatggtcaaggaggtgctggtcgtGG	USER cassete adapter + XhoI + GGYGQ + Lh MaSp1 Modified Type2	Forward	pUSER		Adding a GA for next USER	46	
iGP0012	ggtttaaUggatcctTaaagcagctgctgcagctgctcctctgctgcAGC	USER cassete adapter + BamHI + STOP + AAAAA + Lh MaSp1 Modified Type2	Reverse	pUSER		Adding a AA for next USER	51	
iGP0013	agcaggaggugctggtcgtGGGGGCG	Lh MaSp1 Modified Type2 Polimerizante - 2ªRodada	Forward	pUSER			26	
iGP0014	acctcctgcucctcctgctgcAGCCGC	Lh MaSp1 Modified Type2 Polimerizante - 2nd round	Reverse	pUSER			27	
iGP0015	ggcttaaUctcgagGGGGGtGCGGGCCAAG	USER cassete adapter + XhoI + Lh MaSp1 Silwa1	Forward	pUSER			30	
iGP0016	ggtttaaUggatccTTAAGCAGCTGCCGCAGCAG	USER cassete adapter + BamHI + STOP + Lh MaSp1 Silwa1	Reverse	pUSER			34	
iGP0017	aGCTGGGGGUGCGGGCCAAGGCGGTC	Lh MaSp1 Silwa1 polimerizante	Forward	pUSER	Design Annotation		26	
iGP0018	ACCCCCAGCUGCTGCCGCAGCAGCACG	Lh MaSp1 Silwa1 polimerizante	Reverse	pUSER	Design Annotation		27	
iGP0019	GGCTTAAUCTCGAGGGGGAGCTGGGCAGG	USER cassete adapter + XhoI + Lh MaSp1 Silwa2 4 mer	Forward	pUSER	t overhang for enabling RE function	OBS: small primers with less complem	1530	
iGP0020	ggtttaaUggatccTTACGCGACAGCGACAGCTAC	USER cassete adapter + BamHI + STOP + Lh MaSp1 Silwa2 4 mer	Reverse	pUSER	gcc overhang for enabling RE function	later, tm = 58 °C (http://tmca	1635	
iGP0021	AGCTGTCGCUGTCGCGGGGGAGCTGGGCAGG	Lh MaSp1 Silwa2 4mer polimerizante	Forward	pUSER	Design Annotation		32	
iGP0022	AGCGACAGCUACCGCTACGCGTCCCAG	Lh MaSp1 Silwa2 4mer polimerizante	Reverse	pUSER	Design Annotation		27	
iGP0023	GGCTTAAUCTCGAGGGCGGTGCTGGACAGGG	USER cassete adapter + XhoI + Lh MaSp1 Silwa3 4 mer	Forward	pUSER			31	
iGP0024	ggtttaaUggatccTTAAACGACAACTACAACGACTACG	USER cassete adapter + BamHI + STOP + Lh MaSp1 Silwa3 4 mer	Reverse	pUSER			39	
iGP0025	AGTTGTCGTU GGCGGTGCTGGACAGGG	Lh MaSp1 Silwa3 4mer polimerizante	Forward	pUSER	Design annotation		28	
iGP0026	AACGACAACU ACAACGACTACGCGGCCC	Lh MaSp1 Silwa3 4mer polimerizante	Reverse	pUSER	Design annotation		29	
iGP0027	ATTAAACCutactagtagcggccgctgc	pSB1C3 + USER cassete adapter	Forward	pUSER	Amplificar os Plasmídeos com Prefix e Sufixo		28	
iGP0028	ATTAAGCCuctctagaagcggccgcg	pSB1C3 + USER cassete adapter	Reverse	pUSER	Amplificar os Plasmídeos com Prefix e Sufixo		26	
iGP0029	tCTGGAATTCGCGGC	t + gBlocks(CTGGAATTCGCGGC)	Forward	gBlocks				
iGP0030	gccGGACTGCAGCGGC	gcc + gBlocks(GGACTGCAGCGGC)	Reverse	gBlocks				
iGP0031	ggcttaauCTCGAGgagagtgctggtcgtGGGGGCGCAGGACAAGG	same as iGP0007	Forward				46	
iGP0032	ggtttaaUggatcctgctcctcctgctgcAGCCGCGGCAGCCGCTG	same as iGP0008, but without stop codon	Reverse	TTA removed			46	
iGP0033	aGCCGCGGCuGGGGGCGCAGGACAAGG	same as iGP0009	Forward				27	
iGP0034	AGCCGCGGCuGCCGCTCGCTGCTCCTCC	same as iGP0010	Reverse				27	
iGP0035	ggcttaaUctcgaggagagttatggtcaaggaggtgctggtcgtGG	same as iGP0011	Forward				46	
iGP0036	ggtttaaUggatccaagcagctgctgcagctgctcctctgctgcAGC	same as iGP0012, but without stop codon	Reverse	TTA removed			48	
iGP0037	agcaggaggugctggtcgtGGGGGCG	same as iGP0013	Forward				26	
iGP0038	acctcctgcucctcctgctgcAGCCGC	same as iGP0014	Reverse				27	
iGP0039	ATTAAACCutactagtagcggccgctgc	same as iGP0027	Forward				28	
iGP0040	ATTAAGCCuctctagaagcggccgcg	same as IGP0028	Reverse				26	
iGP0041	ggcttaaUCTCGAGgagagtgctggtcgtggtggatatggtcctggag	USER cassete adapter + XhoI + GGAGR + MaSp2 Biobrick BBa_K844008	Forward	pUSER	Tm 59.5	Adding a GGAGR for next USER	48	
iGP0042	ggtttaaUggatcctgctcctcctgctgcTGCctgctgctgctgctgc	USER cassete adapter + BamHI + AAGGA + Lh MaSp1 Modified Type2	Reverse	pUSER	Design Annotation	Adding a AAGGA for next USER	48	
iGP0043	agcagcagcg Uggatatggtcctggagcag	MaSp2 Biobrick BBa_K844008 polymerizing DNA amplicon	Forward	pUSER			29	
iGP0044	acctgctgc Ugctgctgctgcaccttggtc	MaSp2 Biobrick BBa_K844008 polymerizing DNA amplicon	Reverse	pUSER			29	
iGP0045	ggcttaaUCTCGAG ctcgaggagagttatggtcaaGgaggtgctggtcgtgg	USER cassete adapter + XhoI + GGYGQ + MaSp2 Biobrick BBa_K844008	Forward	pUSER			52	
iGP0046	ggtttaaUggatcc agcggctgctgcagc tgctcctcctgctgctgc	USER cassete adapter + BamHI + AAGGA + Lh MaSp1 Modified Type2	Reverse	pUSER			47	
iGP0047	aGgaggtgctgg Ugctggtggatatggtcctg	MaSp2 Biobrick BBa_K844008 polymerizing DNA amplicon - 2nd round	Forward	pUSER			32	
iGP0048	Accagcacc UcCtgctcctcctgctgctgc	MaSp2 Biobrick BBa_K844008 polymerizing DNA amplicon - 2nd round	Reverse	pUSER			30	
iGP0049	ATCGGTTGCGCATGCG *ctcgag atta ggatcc* CACCATCACCACCACCATTAG	SP2 + XhoI + Spacer + BamHI + His Tag + Homol 3'UTR	Forward	Slice		Expression vector with His-tag	69	86,25
iGP0050	CATTTACACGGAGCGGC TAATGGTGGTGGTGATGGTG *ggatcc taat ctcgag* CGCCGCTGCCGCGGCTGC	SP2 + XhoI + Spacer + BamHI + His Tag + Homol 3'UTR	Reverse	Slice			69	86,25
iGP0051	tgcccaagtcaggaat **ctTcag** tcagccctgggcttcg	Homol 5'UTR + Point Mutation + Homol 3'UTR	Forward	Slice		Removal of PstI site	38	47,5
iGP0052	cgaaagcccaggctga ctgAag attcctgacttgggca	Homol 5'UTR + Point Mutation + Homol 3'UTR	Reverse	Slice			38	47,5
iGP0053	ct***tctagag*** GAcggcggggagctcg	Close to END + XbaI + Annealing	Forward	Cloning	Tm 62,1	iGEM Biobrick Vector	25	31,25
iGP0054	ct***actagt***aCGCTTCAAATACGCCCAGC	Close to END + SpeI + Annealing	Reverse	Cloning	Tm 57,2	iGEM Biobrick Vector	28	35
iGP0055	ct***actagt*** aCAtggccatcctgcaaatgg	Close to END + SpeI + Annealing	Reverse	Cloninig Biobrick	Use with 53 Tm 57,2	Promoter + Intron 1	29	36,25
iGP0056	ct***tctag*** ATgcatatggccaagctgacc	Close to END + XbaI + Annealing	Forward	Cloninig Biobrick	Use with 57 Tm 58,1	Ble + 2A + SP	28	35
iGP0057	ct***actagta*** CTcgaGCGCATGCGCAAC	Close to END + SpeI + Annealing	Reverse	Cloninig Biobrick	Use with 56 Tm 60,4	Ble + Intron 2+ 2A + SP	27	33,75
iGP0058	ct***tctagag*** GGatccCCGCTCCGTGTA	Close to END + XbaI + Annealing	Forward	Cloninig Biobrick	Use with 59 Tm 59,4	3'UTR + intron 3	27	33,75
iGP0059	ctactagtaGGTACCCGCTTCAAATACGC	Close to END + SpeI + Annealing	Reverse	Cloninig Biobrick	Use with 58 Tm 56,3	3'UTR + intron 3	29	36,25
iGP0060	GAATTCGCGGCCGCTTCTAG	**Prefix-F Biobricks**	Forward	Cloning			20	25
iGP0061	CTGCAGCGGCCGCTACTAGTA	**Suffix-R Biobricks**	Reverse	Cloning			21	26,25
iGP0062	ct *tctaga* g CtcgaG *AGATCT* GGGGGCGCAGGACAAGGG	Type 2 Ligation Approach (XbaI, XhoI, BglII)	Forward	Cloning	BglII and BamHI	Use with pJP22 Type2 MaSp1	44	55
iGP0063	ct actagt a ggatcc AGCCGCGGCAGCCGCT	Type 2 Ligation Approach (SpeI, BamHI)	Reverse	Cloning	BglII and BamHI		35	43,75
iGP0064	GACctgctgaagctggcg	Ble2A annealing	Forward	Cloninig Biobrick	59,3 °C (IDT calc) / 60,7 °C (Serial Cloner calc.)			
iGP0065	CTCCATTTACACGGAGCGGg	rbsc 3UTR Piece 1	Reverse	Cloninig Biobrick	58,1 °C (IDT calc) / 58,6 °C (Serial Cloner calc.)			
iGP0066	cttcg GAATTC CtcgaG ATGGTGTCCAAGGG	Close to END + EcoRI + (XhoI)mCherry Annealing	Forward	Cloninig Biobrick	57,4 (IDT calc) / 62,5 (Serial Cloner calc.)	mCherry pJP22	34	42,5
iGP0067	gaa CTGCAG ctcgatcc TTACTTGTACAGCTCG	Close to END + PstI + (BamHI)mCherry Annealing	Reverse	Cloninig Biobrick	54,8 (IDT calc) / 62,1 (Serial Cloner calc.)	mCherry pJP22	34	42,5
iGP0068	ct tctaga CtcgaG AGATCT ATGGGTGGATATGGTCCTGG	MaSp2 Ligation Approach (XbaI, XhoI, BglII)	Forward	Cloninig Biobrick	56,4 °C (IDT calc) / 60,5 °C (Serial Cloner calc.)		44	55
iGP0069	ct actagt a ggatcc TGCTGCTGCTGCTGCTGC	MaSp2 Ligation Approach (SpeI, BamHI)	Reverse	Cloninig Biobrick	60,7 (IDT calc) / 62,4 (Serial Cloner calc.)		37	
iGP0070	gaa AGATCT TTA ggatcc TTACTTGTACAGCTCG	Close to END + BglII + (BamHI)mCherry Annealing	Reverse	Adding a Stop codon pJP22, using pJP22 mCherry				
iGP0071	ctctaga atcgat	Close to END + XbaI + ClaI + Annealing	Forward	Clonning Biobrick	gB2			
iGP0072	g TCTAGA a ATGGTGTCCAAGGG	Close to END + XbaI + (XhoI)mCherry Annealing	Forward	Cloninig Biobrick				
iGP0073	gaa ACTAGT CCatcc TTACTTGTACAGCTCG	Close to END + SpeI + (XhoI)mCherry Annealing	Reverse	Cloninig Biobrick				

CHEAT SHEET - USP_UNIFESP-BRAZIL ORDERED SEQUENCES / PUTATIVE BASIC PARTS

1550	LysK	Synthesized by IDT, phage K	Enzybiotic	Bacterial lysis
1508	MV-L	Synthesized by IDT, phage phiMR11	Enzybiotic	Bacterial lysis
803	Lysotaphin	Synthesized by IDT, *Staphylococcus simulans*	Enzybiotic	Bacterial lysis
1547	b-galacto	Synthesized by IDT	Gene reporter, beta galactosidade	
872	Lip_Thela	Synthesized by IDT	Control prortein for immobilization test , lipase	
569	gLuc	Synthesized by IDT	Gene reporter, luciferase	oxidative enzyme involved in bioluminescence phenomena.
252	Ea MaSp1	Synthesized by IDT	Immobilization matrix	spider silk protein domain
267	Lh MaSp1 Type2	Synthesized by IDT	Immobilization matrix	spider silk protein domain
231	Lh MaSp1 Silwa1	Synthesized by IDT	Immobilization matrix	spider silk protein domain
444	Lh MaSp1 Silwa2	Synthesized by IDT	Immobilization matrix	spider silk protein domain
1593	gblock 1	Synthesized by IDT	Device for C. reinhardtii expression	Not applied (synthetic device)
1478	gBlock 2	Synthesized by IDT	Device for C. reinhardtii expression	Not applied (synthetic device)
141	USER Cassete	Synthesized by IDT	Cassete for USER Cloning	Not applied (synthetic device)
	Promoter	Chimeric gene partially derived from Chlamydomonas reinhardtii in a plasmid	Promoter region for Chlamydomonas reinhardtii protein expression	Transcription regulator
	Resistance gene	Artificial antibiotic resistance gene in a plasmid	Resistance cassete for bleomycin resistance in Chlamydomonas reinhardtii protein expression	Resistance gene
	Terminator	Terminator region in a plasmid	Terminator region for Chlamydomonas reinhardtii protein expression	Terminator gene
	Codon-optimized mCherry	Genetically-modified fluorophore in a plasmid	Fluorescent protein with codon optimized for Chlamydomonas reinhardtii protein expression	Fluorophore

----------**11/07/16**----------

Second trial transformation DH5α with pSB1C3 containing gBlocks
Vivi and Tiago
The ligated pSB1C3 with LysK and gBlock1 were transformed a second time into DH5α cells using the following volumes of ligation reaction:

Ligation product **LysK** (tube 56)	6 µL
Ligation product **gBlock1** (tube 67)	6 µL
RFP	1 µL

Incubation of new appeared colonies in LB medium
Vivi and Tiago
All sequences from the IDT tubes were digested with EcoRI and PstI and ligated to the pSB1C3 plasmid (see 07/04-10/04). *E. coli* X were heat-shock transformed with these plasmids. A few colonies transformed with gLuc, Ea MaSp1 and Silwa1 appeared.

(~18h30)

- gLuc (tube 61)
- Ea MaSp1 (tube 62)
- Lh MaSp1 Silwa1 (tube 64)

----------**12/07/16**----------

Miniprep of colonies and DNA quantification
Tiago and Brayan

Item	Concentration	260/280 ratio
gLuc (tube 61)	125.7 ng/µL	2.01
Ea MaSp1 (tube 62)	96.2 ng/µL	1.95
Lh MaSp1 Silwa1 (tube 64)	104.7 ng/µL	1.90

Probably the Miniprep tubes gLuc (tube 61) and Lh MaSp1 Type2 (tube 64) were inverted.

Digestion
Vivi and Brayan

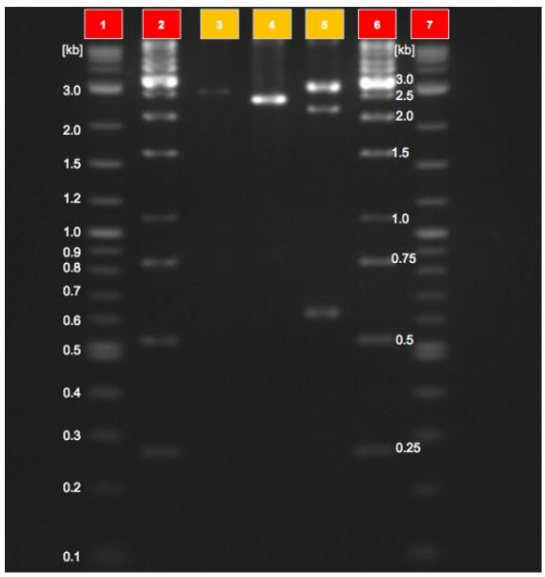
Item	Volume	Final concentrations
EcoRI 5000 U/mL	0.5 µL	0.25 U/µL (25 U)
PstI 5000 U/mL	0.5 µL	0.25 U/µL (25 U)
Buffer H 10x	1.0 µL	1.0 µL
H_2O	3.0 µL	-
DNA (~100 ng/µL)	5.0 µL	50 ng/µL (500 ng)
Total	10 µL	-

----------13/07/16----------

Agarose electrophoresis

Vivi and Brayan

Agarose gel 2%, TAE 1X
1h 30min a 100V



Digestion with EcoRI and PstI of pSB1C3 with inserted gene Blocks (3-5).
3: gLuc (tube 61),
4: Lh MaSp1 Type2 (tube 62) and
5: Lh MaSp1 Silwa2 (tube 64). *see annotation on 12/07/16 1 and 7: Quick-Load 1 kb. 2 and 6:GeneRuler.

12/07/16: Probably the Miniprep tubes gLuc (tube 61) and Lh MaSp1 Silwa2 (tube 64) were inverted by accident.

Preparation of a digestion mix:

Item	Volume	Concentration
Buffer H 10x	5 µL	2x
BSA	0.5 µL	-
EcoRI	0.5 µL	0.1 U/µL (1 U)
PstI	0.5 µL	0.1 U/µL (1 U)
H2O	18.5 µL	-
Total	25 µL	-

This digestion mix is ready to use in the “reagentes gerais” box, in the freezer.

----------14/07/16----------

Dilution of IDT sequences
Brayan and Eduardo

Of each stock solution (see 06/07/16), 1 µL was added to 19 µL TE buffer, getting 12 tubes with 20 µL 5 ng/µL DNA solution.

MaSp1 Silwa1 50 ng/µL in stock solution

PCR of IDT sequences
Brayan and Eduardo

Reaction composition using **Taq polymerase**

Sequences: Lysk, MV-L, Lysostaphin, b-galacto, Lip-Thela, gLuc, Ea Masp1, Lh Masp1 Type 2, Lh Masp1 Siwa1, Lh Masp1 Silwa2, gBlock1, gBlock2

Item	Initial concentration	Final concentration	1Rx	10 Rx
Buffer 10X	10X	X	2,5 µL	25 µL

Betaína	5M	1M	5 µL	50 µL
Primer foward	10µM	0,2µM	0,5 µL	5 µL
Primer reverse	10µM	0,2µM	0,5 µL	5 µL
Taq polymerase	5U/L	1,25U/50µL	1 µL	10 µL
dNTPs	10mM	0,2mM	0,5 µL	5 µL
H2O	-	-	10 µL	100 µL
DNA	5ng/µL	20-25 ng	5 µL	-
Total			**25 µL**	**250 µL**

Reaction conditions using **Taq polymerase**

96°C 1 min	98°C 15 sec 72°C 2 min 60°C 30 sec ---------30 cycles---------	72°C 5 min	4°C ∞

Reaction composition using **X7 home made polymerase**

Sequences: Lysostaphin Ea Masp1, gBlock2

Item	Initial concentration	Final concentration	1Rx	15 Rx
Buffer 10X	10X	X	2.5 µL	37.5 µL
Betaine	5M	1M	5 µL	75 µL

For´ward primer	10µM	0.2µM	0.5 µL	7.5 µL
Reverse primer	10µM	0.2µM	0.5 µL	7.5 µL
X7 home made	5U/L	1.25U/50µL	0.3 µL	4.5
dNTPs	10mM	0.2mM	0.5 µL	7.5 µL
H2O	-	-	10.7 µL	-
DNA	5ng/µL		5 µL	160.5
Total			**25 µL**	**375 µL**

Reaction conditions using **X7 home made polymerase**

96°C 1 min	98°C 15 sec		
	72°C 1 min	72°C 5 min	
	60°C 30 sec		
	---------30 cycles---------		4°C ∞

Tubes:

C: commercial taq polymerase
P: produced phusion polymerase
c(-): negative control, containing everything except DNA
H_2O: H_2O + loading buffer + Gel Red

C 56	C 57	C 58	C 59	C 60	C 61	C 62	C 63	C 64	C 65	C 67	C 68

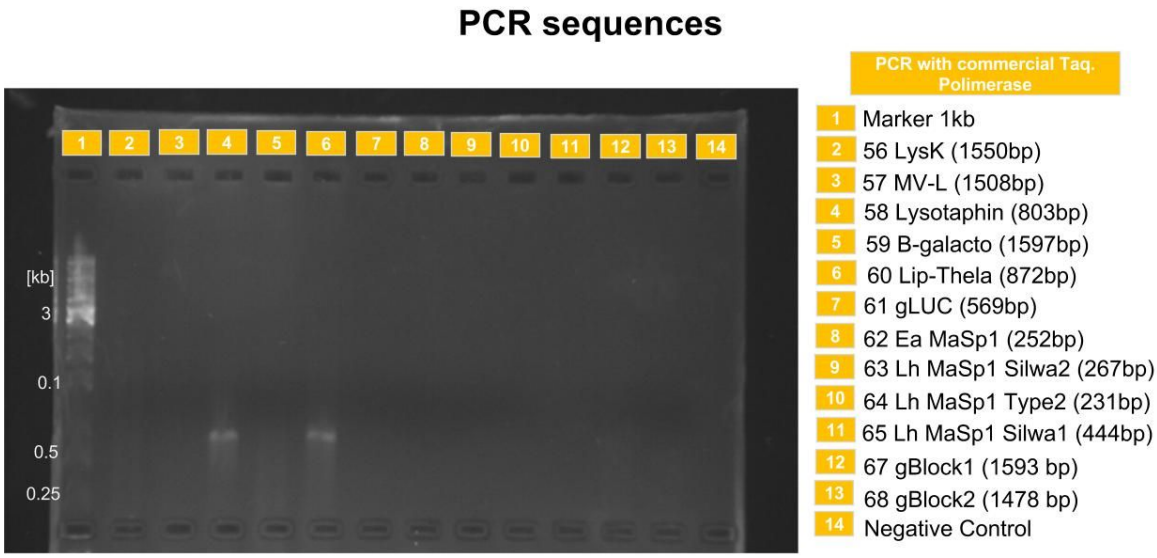
P 68	P 58	P 61	P c(-)	P H_2O	C c(-)	C c(-)	C H_2O

Agarose electrophoresis

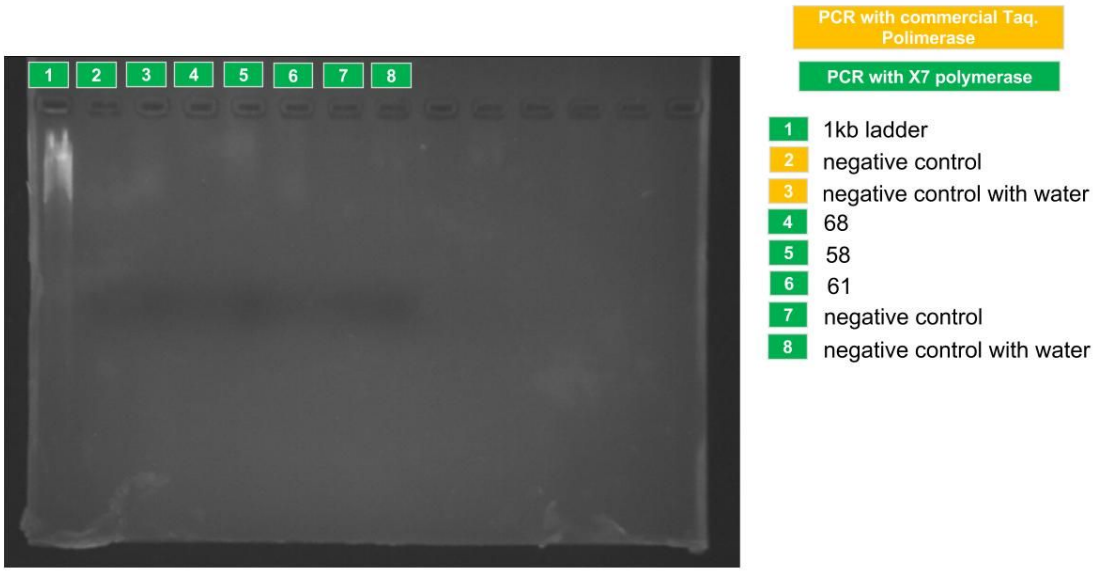
Item	Volume
Loading Buffer 6X	1 µL
GelRed 6X	1 µL
DNA	4 µL
Total	6 µL

Ladder: 4 µL

80 V, 1h30 caderno vs 100 V 2h resultado ppt



PCR



Primer dilution (iGP0001 and iGP0002)

Brayan and Eduardo

iGP0001 (285 µg, 51.9 nmol, MW 5487)

103.8 µL H_2O were added to get a 500 µM stock solution.
An aliquot of 10 µL of the stock solution was added to 490 µL DEPC H_2O to get a 10 µM solution and distributed into five tubes with 100 µL solution each.

iGP0002 (319 µg, 65.1 nmol, MW 4902)

130.2 µL H_2O were added to get a 500 µM stock solution.
An aliquot of 10 µL of the stock solution was added to 490 µL DEPC H_2O to get a 10 µM solution and distributed into five tubes with 100 µL solution each.

----------15/07/16----------

-80°C glycerol stock of new colonies that appeared on the initial pSB1C3 with IDT sequences transformation plaques
Tiago

Colonies were supposed to contain: gBlock1, gBlock2 and Lip-Thela sequences.

Miniprep of pSB1C3 plasmid + insert (new colonies that appeared)

Tiago

Item	Concentration	260/280 ratio
gblock 1 (tube 67)	94,3 ng/µL	1,79
Lh MaSp1 S2 (tube 65)	35,5 ng/µL	2,07
gblock 2 (tube 68)	117,4 ng/µL	1,86
Lip (tube 60)	56,2 ng/µL	1,90

Touchdown PCR

Sequences: Lh MaSp1 Type2 (267 bp), Lh MaSp1 Silwa 2 (444 bp), Lh MaSp1 Silwa 1 (231 bp)

Reaction composition using **Taq polymerase**

Samples were pre-heated for 10 minutes.

Item	Initial concentration	Final concentration	Initial Volume	Final Volume
Buffer Pol	10X	1X	1,25 µL	6,25 µL
Betaine	5 M	1 M	2,5 µL	12,5 µL
Primers (forward/reverse)	10 µM	0,2 µM	0,25 µL Forward + Reverse	1,25 µL
Template	5 ng/µL	25ng/µL	5 µL	25 µL
Taq polymerase	5 U/µL	1,25U/50 µL	0,20 µL	1 µL
dNTPs	10 mM	0,2 mM	0,25 µL	1,25 µL

H2O			3,05 µL	15,25 µL

Reaction conditions using **Taq polymerase**

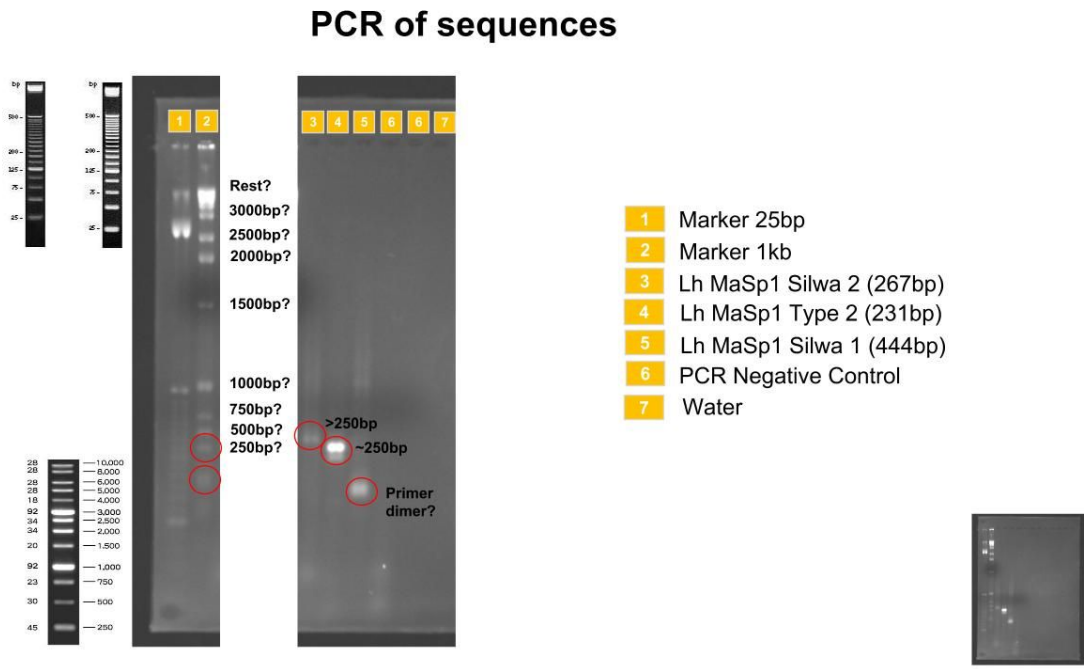
Touch-down PCR (each cycle 63°C to 56°C + 25 cycles at 60°C)

	Temperature	**Time**				
Initial denaturation	98°C	1 min				
Touchdown cycling	98°C	15 seg	98°C	15 seg	98°C	15 seg
	63°C	30 seg	60°C	30 seg	57°C	30 seg
	72°C	30 seg	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg	98°C	15 seg
	62°C	30 seg	59°C	30 seg	56°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg		
	61°C	30 seg	58°C	30 seg		
	72°C	2 min	72°C	2 min		
Rest of cycling	98°C	15 seg				
	60°C	30 seg	25 additional cycles			
	72°C	2 min				
Final extension	72°C	5 min				
Hold	4°C	Hold				

Tube labeling:

Lh MaSp1 Type 2	Tube 1
Lh MaSp1 Silwa 1	Tube 2
Lh MaSp1 Silwa 2	Tube 3

See [gel photo “PCR das nossas seequências 16/07/16”](#) .



----------16/07/16----------

PCR of sequences LysK, MV-L and b-galacto
The probes were pre-heated at 70°C for 15 min.

PCR reactions:

Item	Volume for 1 reaction	10 reactions
Buffer 10X	1.25 µL	12.5 µL
dNTPs 10 mM	0.375 µL	3.75 µL
Forward primer 10 µM	0.5 µL	5 µL
Reverse primer 10 µM	0.5 µL	5 µL
Polymerase	0.3 µL	3 µL
Betaine 5 M	2.5 µL	25 µL
H_2O	2.075 µL	20.75 µL
Template (5 ng/µL)	5 µL	0.5 µLx 10
Total	12.5 µL	125 µL

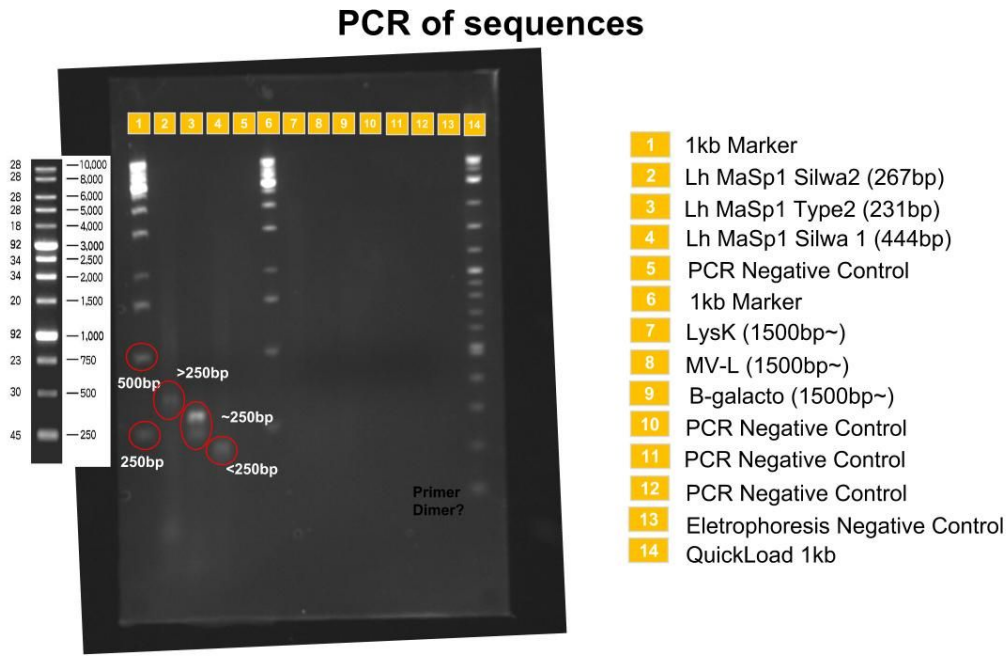
Tube labeling:

LysK	MV-L	b-galacto	c(-)	c(-)	c(-)

PCR program:

98°C 2 min	98°C 30 sec			
		72°C 2 min	72°C 5 min	
	58°C 30 sec			
	---------35 cycles---------			4°C ∞

See gel photo “PCR das nossas seequências 18/07/16” .



----------20/07/16----------

“La revancha” PCR of sequences LysK, MV-L and b-galacto

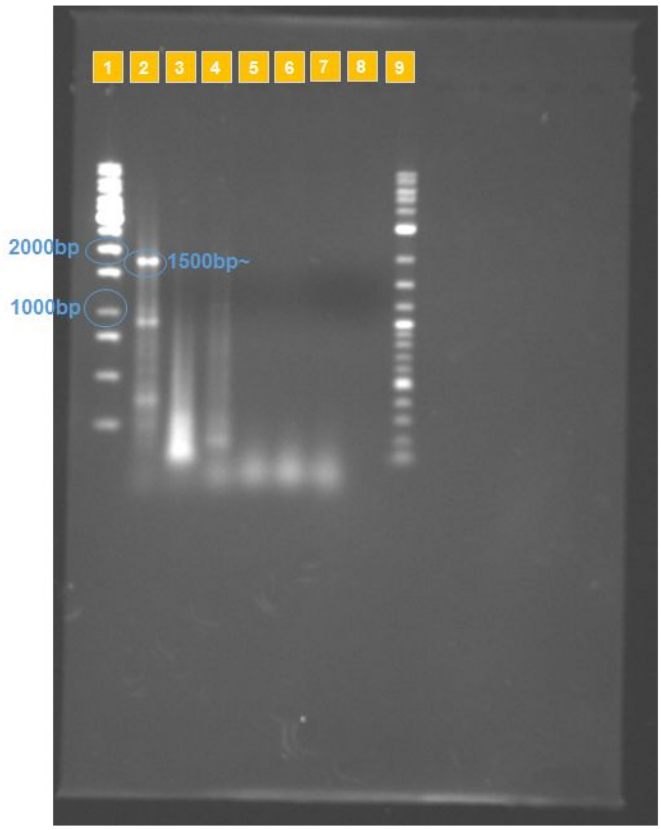
Brayan

The probes were pre-heated at 65°C for 15 minutes.

PCR reactions:

Item	Volume for 1 reaction	10 reactions
Buffer 10X	1.25 µL	12.5 µL
dNTPs 10 mM	0.25 µL	2.5 µL
Forward primer 10 µM	0.25 µL	2.5 µL
Reverse primer 10 µM	0.25 µL	2.5 µL
X7 Polymerase	0.3 µL	3 µL
Betaine 5 M	2.5 µL	25 µL
H_2O	2.7 µL	27 µL
Template (5 ng/µL)	5 µL	5 µLx 10
Total	12.5 µL	125 µL

98°C 1 min	Touchdown 63°C 55°C ---------10 cycles---------	98°C 15 s 60°C 30 s 72°C 2 min ---------25 cycles---------	75°C 5 min 4°C ∞



1 GeneRuler 1kb
2 LysK (1550bp)
3 MV-L (1508bp)
4 B-galacto (1597bp)
5 Controle negativo de PCR
6 Controle negativo de PCR
7 Controle negativo de PCR
8 Água
8 QuickLoad 1kb

Dilution of IDT sequences Lh MaSp1 Type2, Lh MaSp1 Silwa1 and Lh MaSp1 Silwa2
Brayan

Sequences: Lh MaSp1 type2, Lh MaSp1 Silwa2
20 fold-times dilution
1 uL DNA+ 19 uL H2O DEPC

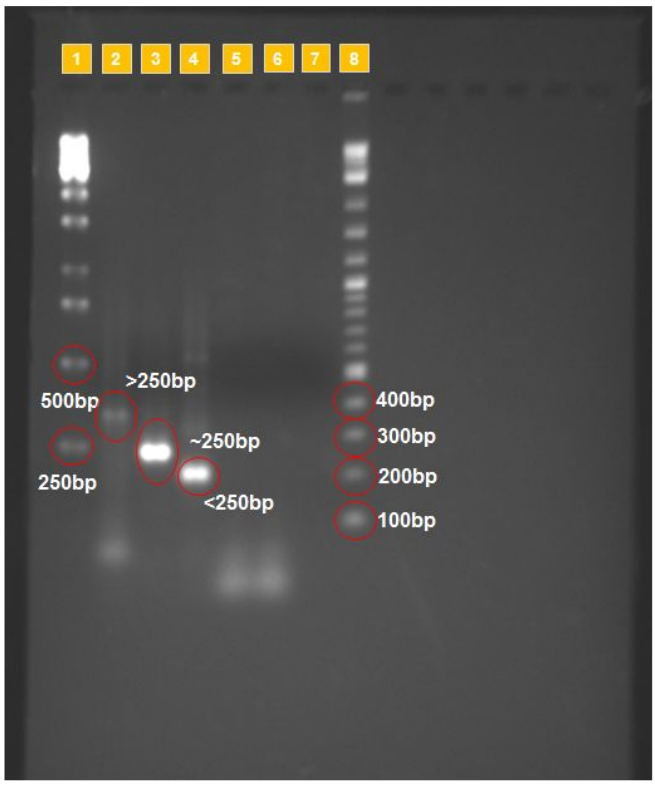
Sequences: Lh MaSp1 Silwa1 (50 ng/uL)
10 fold-times dilution
1 uL + 9 uL H2O

The probes were pre-heated at 65°C for 15 minutes.

PCR reactions:

Item	Volume for 1 reaction	10 reactions
Buffer 10X	1.25 µL	12.5 µL
dNTPs 10 mM	0.25 µL	2.5 µL
Forward primer 10 µM	0.25 µL	2.5 µL
Reverse primer 10 µM	0.25 µL	2.5 µL
Polymerase	0.3 µL	3 µL
Betaine 5 M	2.5 µL	25 µL
H_2O	2.7 µL	27 µL
Template (5 ng/µL)	5 µL	5 µLx 10
Total	12.5 µL	125 µL

98°C 1 min	Touchdown 63°C 55°C ---------10 cycles---------	98°C 15 s 60°C 30 s ---------25 cycles---------	72°C 2 min	75°C 5 min 4°C ∞



1 Marcador 1kb
2 Lh MaSp1 Type 2 (267bp)
3 Lh MaSp1 Silwa 1 (231bp)
4 Lh MaSp1 Silwa 2 (444bp)
5 Controle negativo de PCR
6 Controle negativo de PCR
7 Água
8 QuickLoad 1kb

Results: We have checked that:
Lh Masp1 Type 2 is actually Silwa 2
Lh Masp1 Silwa 1 is actually Type 2
Lh Masp1 Silwa 2 is actually Silwa 1

----------24/07/16----------

PCR of sequences Lip-Thela (60) and Lysostaphin (58)
Brayan

Item	Volume for 1 reaction	10 reactions
Buffer 10X	10 µL	100 µL
dNTPs 10 mM	2 µL	20 µL
Forward primer 10 µM	2 µL	20 µL
Reverse primer 10 µM	2 µL	20 µL
X7 Polymerase	1.6 µL	16 µL
Betaine 5 M	20 µL	200 µL
H_2O	61.4 µL	614 µL
Template	1 µL	10 x 1 uL
Total	100 µL	1000 µL

ANNOTATION GAP

During this period, the documentation was done on a physical notebook and we were not able to transfer it online.

TIP for anyone reading this : orient iGEM teams towards annotating
EVERYTHING DIRECTLY ONLINE.
EVERYTHING.

ANNOTATION GAP

During this period, the documentation was done on a physical notebook and we were not able to transfer it online.

TIP for anyone reading this : orient iGEM teams towards annotating
EVERYTHING DIRECTLY ONLINE.
EVERYTHING.

Notebook organization template:

What was done:
Who did it:
Brief resume
Protocols:
Results:

----------08/11----------

USER primers dilution:
Tiago and Brayan

Two primers for amplifying our IDT sequences, 8 primers specific for the USER multimerization and 2 primers for constructing USER cassettes from “pSB” plasmids were resuspended to a final concentration of 100µM and working solutions (10mM) were prepared.

The primer list is available on the Cheat Sheet.

DEPC treated water was added to a final concentration of 100µM

Lh MaSp1 Type 2 monomer touchdown PCR for USER and additional amplicon production
Brayan and Tiago Lubiana

The standardized PCR was repeated to generate more fragments for downstream applications, as well as the first PCR step for the USER multimerization of the “Lh MaSp1 Modified Type2” blocks. Reaction was left overnight.

The iGP0001 and iGP0002 primers were used for the amplification steps, while the following pairs were used instead for the USER construction:

USER MaSp1 Type2 A (UMT2-A) - iGP0007 and iGP0010
USER MaSp1 Type2 B (UMT2-B) - iGP0009 and iGP0010
USER MaSp1 Type2 C (UMT2-C) - iGP0009 and iGP0008

USER Primers sequence in the cheat sheet:

Master mix preparation for MaSp fragments (USER downstream applications):

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume “n” PCR reactions

Buffer GC	5X	X	2,5	n*2,5
dNTPs	10 mM	0,5 mM	0,625	n*
Primer forward*	10 uM	0,5 uM	0,625	n*
Primer reverse*	10 uM	0,5 uM	0,625	n*
Betaine	5 M	M	2,5	n*
X7 HomeMade polymerase	100X		1,0	n*
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			2,625	n*
			12,5	n*12,5

*Based on primer combination for each fragment (UMT2-(X)), separated reactions were prepared.

Master mix preparation for MaSp fragments amplification:

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume "n" PCR reactions
Buffer GC	5X	X	2,5	n*2,5
dNTPs	10 mM	0,5 Mm	0,625	n*
Primer iGP0001*	10 uM	0,5 uM	0,625	n*
Primer iGP0002*	10 uM	0,5 uM	0,625	n*
Betaine	5 M	M	2,5	n*
X7 HomeMade polymerase	100X		1,0	n*
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			2,625	n*
			12,5	n*12,5

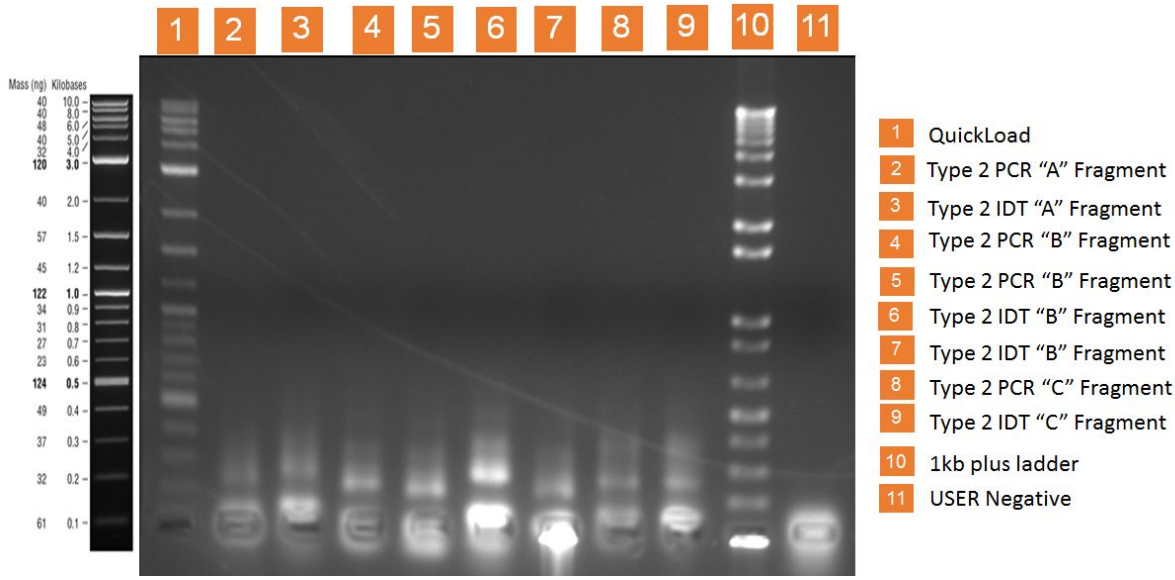
*Primer used for these reactions were from Eton Biosciences Inc (new ones that arrived last week).

Cycle conditions:

Touch-down PCR (each cycle 63°C to 56°C + 25 cycles at 60°C)

	Temperature	Time
Initial denaturation	98°C	1 min
Touch-down cycling	98°C	15 seg
	63-56ºC	30 seg
	72°C	2 min
Final extension	72°C	5 min
Hold	4°C	Hold

USER Results



*Preliminary comments: Seems like USER primers are annealing elsewhere amplifying unspecific regions. It may be due to touch down PCR conditions that primarily aims to yield regardless the primers specificity.

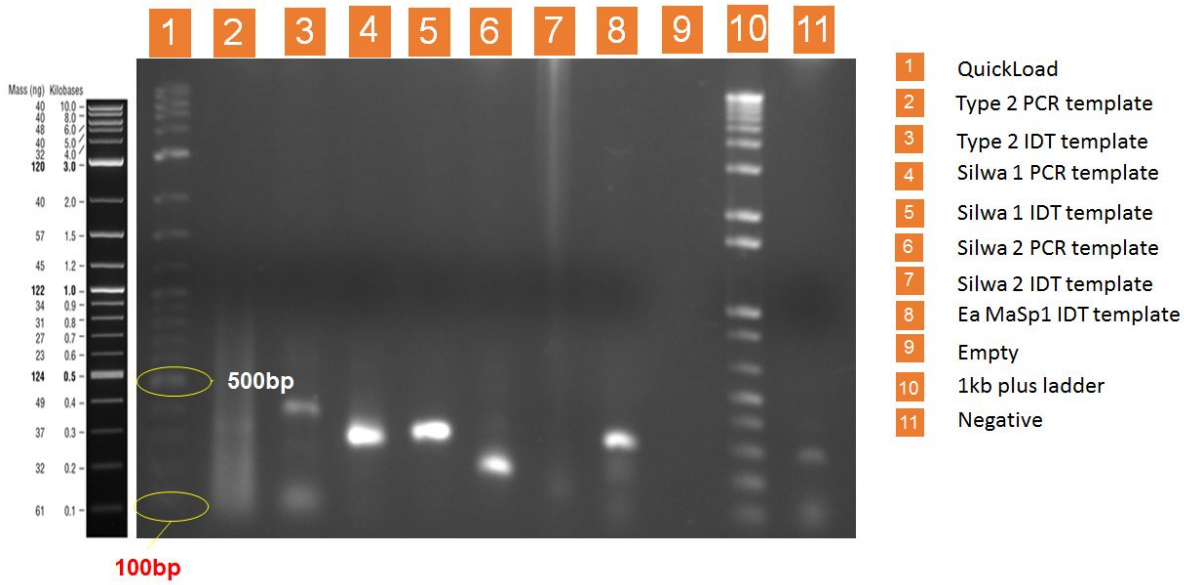
*This also demonstrates that low temperature annealing (56 ~ 63°C) may not be the best for this reaction.

Troubleshooting:

1° Try annealing at 60°C or 71°C or gradient?. See how it goes.

2° Try one-primer-at-time cycling step for each USER primer within each USER fragment amplicon (Sair's suggestion when primer dimer occurs). Find a good annealing temperature first.

MaSp1 amplicon production



As the first primers that we ordered came wrongly, with 'T' instead of 'U', we had to wait a long time before we got working primers.

----------**12/08/2016**----------

Viviane

Analytical digestion of plasmid (transformants without pcr) - 2nd attempt

*gblocks (1 and 2) were excluded of this digestion because they didn't have insert according to the last gel

60 - Lip Thela 56,4 ng/ul
61 - GLuc 125 ng/ul
62 - Ea Masp 1 96,2 ng/ul
64 - Lh Masp 1 Silwa 1 104,7 ng/ul
65 - Lh Masp 1 Silwa 2 35,5 ng/ul

	60	61	62	64	65
EcoRI	1 ul	0,5 ul	0,5 ul	0,5 ul	0,5 ul
PstI	1 ul	0,5 ul	0,5 ul	0,5 ul	0,5 ul
Buffer H 10x	1,5 ul	1 ul	1 ul	1 ul	1 ul
H2O	1,5 ul	4 ul	2,8 ul	3 ul	3 ul
DNA	10 ul	4 ul	5,2 ul	5 ul	5 ul (it was the total of sample: <500 ng)
Total	15 ul	10 ul	10 ul	10 ul	10 ul

Cycle conditions: (file "iGEM")

37°C 6h	80°C 20 min	4 °C For ever

Summary

PCR reactions for purification

Sequence	Observation	Proceed?
07/14/2016		
58 Lysotaphin	Specific	PCR purifcation
60 Lip-thela	Specific	PCR purifcation
07/20/2016		
LysK	Unspecific amplicons	Gel purification
07/28/2016		
Ea MaSp1 TD	Unspecific amplicons	Gel purification?
Ea MaSp1 71°C	Specific	PCR purifcation
LysK 60°C	Unspecific amplicons	Gel purification
08/12/2016		
Type 2	Primer dimer	Gel purification?
3 x Silwa 1	Specific	PCR purifcation
2 x Silwa 2	Specific	PCR purifcation
3 x Ea MaSp1	Unspecific	Gel purification?
08/08/2016		
58 Lysothapin A	Unspecific 25ul	Gel purification?
60 Lip-Thela A	Unspecific 25ul	Gel purification?
3 x Silwa 1 (E+B)	Specific	PCR purifcation
Silwa 1 B Lane 9	Unspecific uncommon product	?
58 Lysothapin B	Specific	PCR purifcation
60 Lip-Thela B	Unspecific	Gel purification?
58 Lysothapin G	Unspecific	Gel purification?
60 Lip-Thela G	Specific	PCR purifcation
58 Lysothapin C	Specific	PCR purifcation
60 Lip-Thela C	Specific	PCR purifcation

Pending activities

1° Reagents for ICB group (on going)

(PART 1)
*USER fragment amplification implies ----
USER primers 007, 008, 009, 010
X7 HomeMade polymerase 100X (work solution)
iGEM primers (001, 002), in case of insufficient amplicon...
Lh MaSp1 Type 2 amplicon
*pSB1C3 & pSB1A3 amplification
pSB1C3 + RFP aliquots (ready)
pSB1C3 purified solution (ready)
pSB1A3 (where?)

Aim: Produce enough fragments for USER cloning

(PART 2)
*Enzybiotics production ----
LysK IDT dilution
Lysosthapin IDT dilution
Rest of materials gonna be covered by the team.

2° Purify enough Type 2 amplicon by PCR (done). Confirm by gel electrophr.

3° Confirm analytical digestion (Gel electrophoresis) (Done)

4° USER PCR. Testing appropriated sequence (Done). Confirm by gel electrophr.

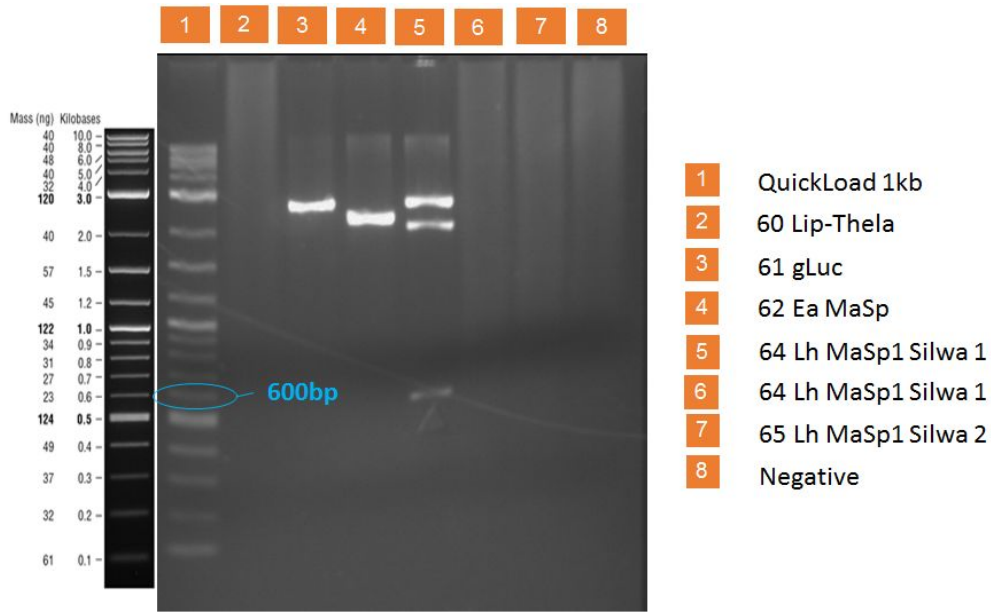
5° Purify rest of sequences (Priority)

6° IDT PCR identification for: gBlock 1, 2, MV-L, b-gal, gLUC, Cassette USER
For gblock 1 & 2, Q5 polymerase may be a solution?
Long PCR troubleshooting…. Primer concentration to 1uM, dNTP to 1uM. Template range from 3 to 5ul.

----------14-08-2016----------

Gel electrophoresis: IDT Seqs + pSB1C3 direct ligation

Brayan



Seems like lane 5 contains a ~600bp-like product.
Probably, tube #61 and #64 were unfortunately exchanged while doing miniprep extraction.
This could explain why gLUC amplicon is appearing as a band around 600bp ~

PCR production of MaSps

Brayan

To start with USER and BioBrick final construction, we will produce more product
It was prepared 40 PCR reactions for our four MaSp1 sequences:

- Lh MaSp1 Type 2
- Lh MaSp1 Silwa 1
- Lh MaSp1 Silwa 2
- Ea MaSp1

Master mix preparation for MaSp production:

Item	Initial concentration	Final concentration	Initial volume (ul)	Final volume
			1 PCR reaction	40 PCR reactions

Buffer GC	5X	X	2,5	n*2,5
dNTPs	10 mM	0,5 Mm	0,625	n*
Primer iGP0001*	10 uM	0,5 uM	0,625	n*
Primer iGP0002*	10 uM	0,5 uM	0,625	n*
Betaine	5 M	M	2,5	n*
X7 HomeMade polymerase	100X		1,0	n*
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			2,625	n*
			12,5	n*12,5

*Primer used for these reactions were from Eton Biosciences Inc (new ones that arrived last week).

2 negative controls were made, which left 38 reactions for:

10 tubes for Lh MaSp1 Type 2
10 tubes for Lh MaSp1 Silwa 1
10 tubes for Lh MaSp1 Silwa 1
8 tubes for Ea MaSp

Reaction was left running overnight.

Cycle conditions:

Touch-down PCR (each cycle 63°C to 56°C + 25 cycles at 60°C)

	Temperature	**Time**				
Initial denaturation	98°C	1 min				
Touch-down cycling	98°C	15 seg	98°C	15 seg	98°C	15 seg
	63°C	30 seg	60°C	30 seg	57°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg	98°C	15 seg
	62°C	30 seg	59°C	30 seg	56°C	30 seg

	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg		
	61°C	30 seg	58°C	30 seg		
	72°C	2 min	72°C	2 min		
Rest of cycling	98°C	15 seg	25 additional cycles			
	60°C	30 seg				
	72°C	2 min				
Final extension	72°C	5 min				
Hold	4°C	Hold				

USER PCR optimization for MaSp1 sequences
Brayan

This reaction was made once again due to codification mistakes.
We shall remember that IDT has mistakenly named our MaSp's sequences:
Lh MaSp1 Type 2 actually is Silwa 2
Lh MaSp1 Silwa 1 actually is Type 2
Lh MaSp1 Silwa 2 actually is Type 1

This time, we picked Silwa 1 IDT sequence (which is actually Type 2) for USER PCR amplification

USER MaSp1 Type2 A (UMT2-A) - iGP0007 and iGP0010
USER MaSp1 Type2 B (UMT2-B) - iGP0009 and iGP0010
USER MaSp1 Type2 A (UMT2-C) - iGP0009 and iGP0008

USER Primers sequence:

Master mix preparation for MaSp fragments (USER downstream applications):

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume 5 PCR reactions
Buffer GC	5X	X	2,5	5*2,5
dNTPs	10 mM	0,5 mM	0,625	5*0,625
Primer forward*	10 uM	0,5 uM	0,625	5*0,625
Primer reverse*	10 uM	0,5 uM	0,625	5*0,625
Betaine	5 M	M	2,5	5*2,5
X7 HomeMade polymerase	100X		1,0	5*1,0
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			2,625	5*2,625
			12,5	5*12,5

*Based on primer combination for each fragment (UMT2-(X)), it was prepared separated reactions.
Reaction was left working overnight

Cycle conditions:

Touch-down PCR (each cycle 63°C to 56°C + 25 cycles at 60°C)

	Temperature	**Time**				
Initial denaturation	98°C	1 min				
Touch-down cycling	98°C	15 seg	98°C	15 seg	98°C	15 seg
	63°C	30 seg	60°C	30 seg	57°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg	98°C	15 seg

	62°C	30 seg	59°C	30 seg	56°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg		
	61°C	30 seg	58°C	30 seg		
	72°C	2 min	72°C	2 min		
Rest of cycling	98°C	15 seg				
	60°C	30 seg	25 additional cycles			
	72°C	2 min				
Final extension	72°C	5 min				
Hold	4°C	Hold				

----------08/15----------

PCR amplification of gBlocks 1 and 2
Tiago

After a lot of trouble that mainly Brayan had with amplifying the genes, trying many different conditions, the PCR conditions were reanalyzed. A problem with the Tm of the primers was spotted and new primers will be designed specifically for gBlocks 1 and 2.
A gradient PCR was nevertheless tried, without betaine this time, varying from 53 to 68 degrees the annealing temperature.

gBlock1 - 1593 bp - 61,7% GC
gBlock2 - 1478 bp - 62,0% GC

Component	20 µl Reaction
Nuclease-free water	to 20 µl
5X Phusion HF or GC Buffer	4 µl
10 mM dNTPs	0.4 µl
10 µM Forward Primer	1 µl
10 µM Reverse Primer	1 µl
Template DNA	1 µl (5ng)
Phusion DNA Polymerase 100x stock	2µl

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 53-68°C (gradient, in different lanes) 72°C	10 seconds 30 seconds 1 min
Final Extension	72°C	5 minutes
Hold	4 10°C	

PCR purification: eletrophoresis
Viviane

All PCR products we have prepared till now were pooled together into one tube for each MaSp sequence

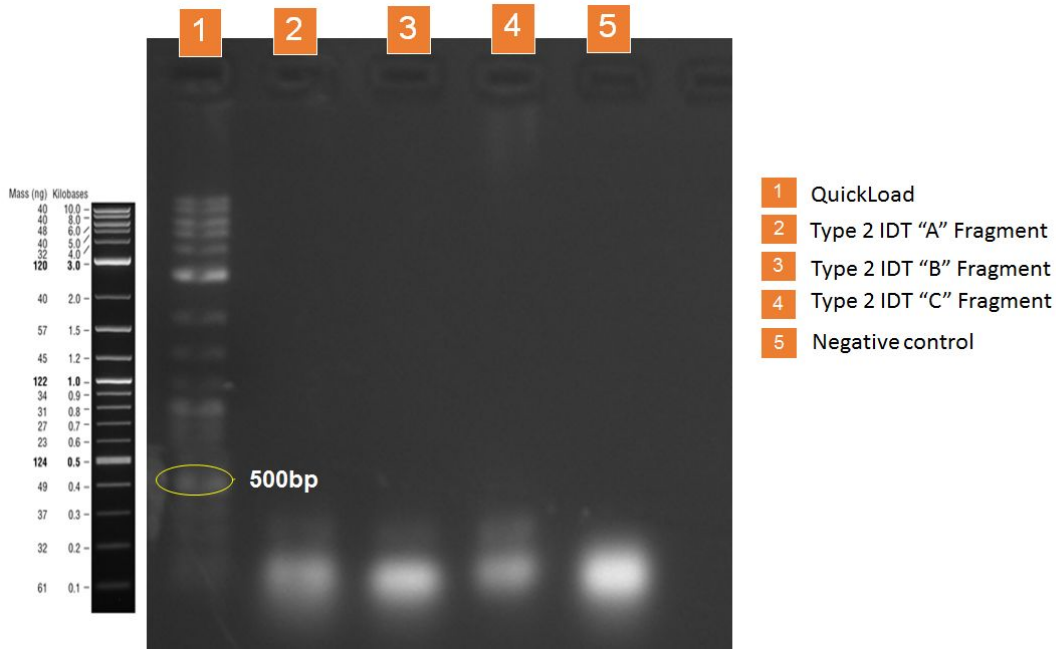
We rendered about 100ul of pooled sequences:
Lh Masp 1 Silwa1 (S1)
Lh Masp 1 Silwa2 (S2)
Ea Masp (Ea)
Masp 1 type 2 (T2)

User amplicon (Primers iGP0007 + iGP0010) (A)
User amplicon (Primers iGP0009 + iGP0010) (B)
User amplicon (Primers iGP0009 + iGP0008) (C)

Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Row 1	X	ldd	T2	T2	T2	S1	S1	S1	S2	S2	S2	Ea	Ea	Ea
Row 2	ldd	A	B	C	(-)									

ldd: ladder
X: empty well
(-) negative control

Gel electrophoresis



Wrong codification
*In spite of wrong codification, we continued to use those names until the very end of the gel purification process
For gel purification, it was used HiYield GelPCR DNA minikit according to the manufacturers' instructions. Based on these recommendations, 500ul of DF buffer was used with Ea sequence, and proportional volumes were calculated for the other sequences.

20ul of buffer TE was used for final elution.

Sequences	T2	T2D	S1	S2	EA
Gel mass	367mg	393,7mg	355,5mg	518,5mg	231,5mg

Abbreviation	Sequence	Concentration	Purity A260/280nm
T2	Lh MaSp Type 2	13,1 ng/μL	1,48
S2D	Lh MaSp Silwa 2 Dimer	12,9 ng/μL	1,38
S1	Lh MaSp Silwa 1	16,4 ng/μL	2,19
S2	Lh MaSp Silwa 2	4,7 ng/μL	0,99
EA	Ea MaSp	9,1 ng/μL	1,92

All sequences were renamed according to what we discovered was correct

Lh MaSp Silwa 2 was mistakenly sent by IDT as Silwa 1
Lh MaSp Silwa 1 was mistakenly sent by IDT as Type 2
Lh MaSp Type 2 was mistakenly sent by IDT as Silwa 2

----------08/16----------

Preparation of Masp digestion (biobricks)

Viviane

3) Masp 2 (80,5 ng/µL) - 2/7B
4) Masp 2 (186,5 ng/µL) - 2/7D
5) Masp 2 (172,8 ng/µL) - 2/7F

Item	3	4	5
EcoRI 15 U/µL	0.5 µL	0.5 µL	0.5 µL
PstI 15 U/µL	0.5 µL	0.5 µL	0.5 µL
Buffer H 10x	1 µL	1 µL	1 µL
H_2O	1,75 µL	5,3 µL	5,1 µL
DNA	6,25 µL	2,7 µL	2,9 µL
Total	**10 µL**	**10 µL**	**10 µL**

Cycle programming:

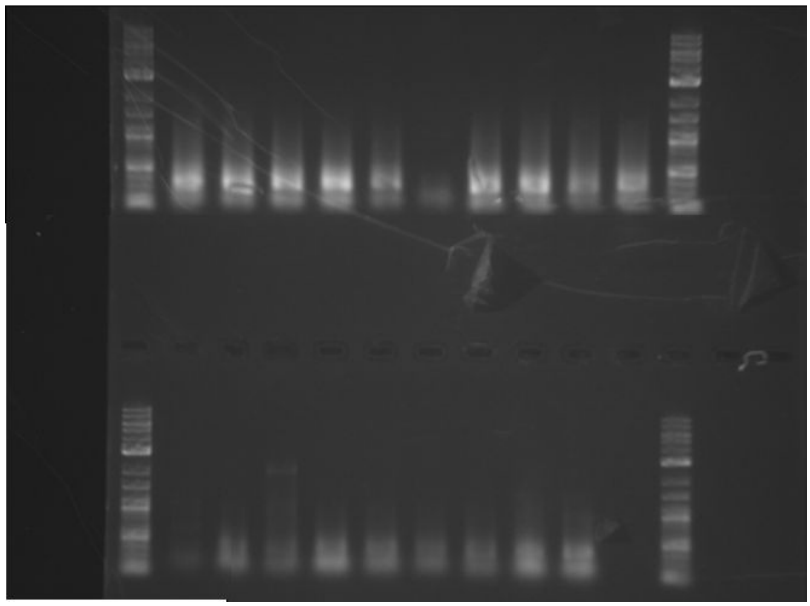
	80°C 20 min	
37°C 6h		4 °C For ever

----------08/16----------

Electrophoresis of gBlocks 1 and 2

Allan

Well	1	2	3	4	5	6	7	8	9	10	11	12
row 1	ldd	gb1 53	gb1 53	gb1 57	gb1 57	gb1 61	CN	Gb1 64	Gb1 64	gb1 68	gb1 68	ldd
row 2	ldd	CN	gb2 53	gb2 57	gb2 57	gb2 61	gb2 61	gb2 64	gb2 64	gb2 68	CN	ldd



----------08/16----------

PCR production of LysK sequences

Felipe

It was prepared 17 tubes for LysK production

Master mix preparation for MaSp fragments amplification:

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume "20" PCR reactions
Standard Buffer	10X	X	1,25	20*2,5
dNTPs	10 mM	0,2 Mm	0,25	20*0,25
Primer iGP0001	10 uM	0,2 uM	0,25	20*0,25
Primer iGP0002	10 uM	0,2 uM	0,25	20*0,25
Betaine	5 M	M	2,5	20*2,5
Commercial taq polymerase	5U/ul	1.25U/50 uL	0,3	20*0,3
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			5,7	20*5,7
			12,5	250

----------08/17----------

Gel electrophoresis of MaSp1 analytical digestion & LysK production
Brayan

Purification of Enzybiotics sequences
Tiago, Fabio, Brayan
LysK (Gel purification)
Lysostaphin (PCR-product purification)

Sequence		Concentration	Purity
Lysostaphin		22,7 ng/µl	1,93
Lysk		8,2 ng/µl	1,27
T2	Lh MaSp Type 2	13,1 ng/µL	1,48
S2D	Lh MaSp Silwa 2 Dimer	12,9 ng/µL	1,38
S1	Lh MaSp Silwa 1	16,4 ng/µL	2,19
S2	Lh MaSp Silwa 2	4,7 ng/µL	0,99
EA	Ea MaSp	9,1 ng/µL	1,92

Cycling programming:

	Temperature	**Time**				
Initial denaturation	98°C	1 min				
Touch-down cycling	98°C	15 seg	98°C	15 seg	98°C	15 seg
	63°C	30 seg	60°C	30 seg	57°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
	98°C	15 seg	98°C	15 seg	98°C	15 seg
	62°C	30 seg	59°C	30 seg	56°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min

	98°C	15 seg	98°C	15 seg	98°C	15 seg
	61°C	30 seg	58°C	30 seg	55°C	30 seg
	72°C	2 min	72°C	2 min	72°C	2 min
Rest of cycling	98°C	15 seg	25 additional cycles			
	60°C	30 seg				
	72°C	2 min				
Final extension	72°C	5 min				
Hold	4°C	Hold				

----------08/18----------

Storage buffer preparation for X7 Polymerase
Prepared around 20ml
Composition:
50mM Tris pH 8.3
50% Glycerol
0.1mM EDTA
1.0mM DTT
0.1% Tween
OBS: All these materials should be previously sterilized

----------08/17----------

Inoculation of BL21DE3 cells and BL21DE3 cells containing pETDuet-
~endolysin expression by *E. coli* for activity test~
Edmar and Mireia

BL21DE3 cells stored at -80°C were inoculated in 3 mL LB medium and BL21DE3 pETDuet-1 were inoculated in 3 mL LB with ampicillin (100 ug/mL). The cultures were incubated at 37°C and 150pm o.n..

The BL21DE3 cells will be used to make electrocompetent cells and of the ones containing pETDuet-1, the plasmids will be extracted.

08/18
XhoI/BamHI digestion of enzybiotics for cloning
Tiago

LysK, Lysostaphin and MV-L IDT sequences and Lysostaphin PCR product were digested overnight (10 hours) with XhoI and BamHi for cloning in the lab's *Chlamydomonas* expression vector.

BamHI-HF and XhoI from NEB, lab stock,
4U for 100ng of LysK (LK), Lysostaphin (LS) and MVL sequences(10 microliters)
4U for 330ng of Lysostaphin PCR product (L*, 20 microliters)
CutSmart Buffer

EcoRI/PstI digestion of gBlocks 1 and 2 (Seq #67 and #68)
Tiago
pSB1C3, pSB1A3, gBlock1 and gBlock2 were also double digested with EcoRI and PstI, in order to try cloning the IDT genes in a high-copy plasmid.

OBS: pSB1A3 was already ressuspended with a concentration of 50 ng/µL.

PstI and EcoRI from invitrogen, iGEM 2013,
5U for 100 ng of gBlocks 1 and 2 (10 microliters)
10U for 500 ng of pSB1A3 (linearized, distribution kit, 20 microliters)
10U for 200 ng of pSB1C3 (linearized, distribution kit, 20 microliters)
H Buffer

All double-digested material was stored at -20°C in a red rack. Remember all double-digested material bears a "X" symbol on the tube.

Transformation with another plasmids for improved product recovery
Tiago

Colony PCR of electroporated cells ~endolysin expression by *E. coli* for activity test~

Result: no amplification.

----------08/19----------

Tiago Lubiana

pJP22 verification with sodium borate buffer
Tiago

Even though no plasmid was found on the gel, our new running buffer (substitutingTAE/TBE) resolve the ladder perfectly and in a higher voltage (200V, power supply max) without heating or melting.
We tried using anyway, the quantification yielded about 50ng/microliter.

Enzybiotic XhoI/BamI ligation in pJP22
Tiago

The enzybiotics digested in 08/18 were ligated directly (without purification) with XhoI/BamHI digested pJP22 (a expression plasmid for microalgae with Amp resistance and E. coli ori).

Ligation reaction

9,5µL H2O
1,5µL CutSmart Buffer
2µL ATP 10mM
1µL (50ng) DNA vector (pJP22)
1µL T4 Ligase (NEB)
5µL digested inserts

Insert preparation

5µL (50ng) of LysK and MV-L (separately), 30ng (3µL of IDT Lysostaphin) and ~ 40 ng (3µL of Lysostaphin PCR product) were used.

1 hour and 40 minutes at RT before transformation (and left for several hours at RT after, before freezing).

Transformation of enzybiotic XhoI/BamI ligation in pJP22
Tiago

5-DHalpha cells were transformed by the standard heat-shock protocol. The following amounts of ligation products were used:

1- Negative control
2- Lysostaphin IDT 3.5µL
3- Lysostaphin PCR 3.5µL
4- LysK 3.5µL
5- MV-L 3.5µL
6- Positive control (BBa_J04450 in pSB1A3 from distribution kit) 1µL
7- LysK 1µL

We also transformed 2 ligation products from the first batch, ligated to pSB1C3, that were frozen:

8- 58 (lysostaphin in pSB1C3) 1µL
10- 63 (Lh MaSp Type2) 1µL

The petri dishes were left on 37ºC O/N in 250 rpm agitation (due to other inoculations also growing in the incubator)

Extraction of pETDuet-1 and preparation of electrocompetent cells ~endolysin expression by *E. coli* for activity test~
Edmar and Mireia

The plasmid pETDuet was extracted from BL21DE3 cells with the extraction kit SD Wizard (Promega). It was obtained at a concentration of 180 ng/µL and stored at -20°C.

In order to get electrocompetent BL21DE3 cells, an overnight culture in LB medium was centrifuged at 6000 rpm, 15 min and 4°C, and washed with water and glycerol 20% with the following washing steps (between them the probe was centrifuged at the mentioned conditions):

1) 50 mL sterile miliQ water
2) 25 mL sterile miliQ water

3) 12.5 mL sterile glycerol 20%
4) Resuspended in 1 mL glycerol 20% and distributed competent cells in Eppendorf tubes (600 mL) ~100 µL/tube.

The tubes were stored at -20°C overnight and at -80°C.

----------08/20----------

LB-agar plates preparation and pouring
Viviane, João

LB-agar plates were prepared according the antibiotics concentrations below:

500 ml of LB agar/ampicilin at 100µg/ml concentration (stock ampilicin at 100mg/ml)
500 ml of LB agar/chloramphenicol at 25µg/ml concentration (stock ampilicin at 25mg/ml)

Digestion of LysK, lysostaphin and pETDuet ~endolysin expression by *E. coli* for activity test~
Mireia

Item	Volume
H_2O	2.7 µL
Buffer H	1 µL
LysK (8 ng/µL)	5 µL
pETDuet (180 ng/µL)	0.3 µL
EcoRI	0.5 µL
PstI	0.5 µL
Total	10 µL

Item	Volume
H_2O	6.6 µL
Buffer H	1 µL
lysostaphin (22.7 ng/µL)	1.1 µL
pETDuet (180 ng/µL)	0.3 µL
EcoRI	0.5 µL
PstI	0.5 µL
Total	10 µL

Reaction conditions: 37°C 16h, 80°C 20min, 4°C hold. (The probes were kept about 2h at 4°C).

pSB1C3 and pJP22 gel extraction and purification

Previously miniprepped RFP in PSB1C3 were digested with EcoRI and PstI and pJP22 with XhoI and BamHI. The plasmids were then extracted from the agarose gel (run with SB buffer, 0.5 % agarose gel).

CONCENTRAÇÕES OBTIDAS

----------08/21----------

Ligation of LysK and lysostaphin to different pETDuets and electroporation of BL21DE3 cells ~endolysin expression by *E. coli* for activity test~
Mireia

Item	Volume
Digestion reaction	10 µL
ATP (10 mM)	2 µL
DTT (100 mM)	2 µL
H_2O	4,5 µL
T4 Ligase (5U)	1 µL
Volumen total	20 µL

Item	Volume
Digestion reaction	10 µL
ATP (10 mM)	2 µL
DTT (100 mM)	2 µL
H_2O	4,5 µL
T4 Ligase (5U)	1 µL
Volumen total	20 µL

Conditions

Temperature °C	**Time**
25	1 h
On ice	30 min

Electroporation:

2 uL ligation reaction was pipetted into the electroporation cuvette. 100 uL electrocompetent cells were added. The cuvette was shaken horizontally.
Conditions: 1 mm cuvette (-20°C for 30 min before use), 1800 V.
After electroporation cells were incubated for 1h in LB at 37°C and 150 rpm and plated on LB Amp agar plates.

Ligation of digested gBlocks to purified pSB1C3
Brayan & João
The EcoRI/PstI digested gBlocks (07/06) were ligated to the digested pSB1C3 according to the standard protocol.

Miniprep of enzybiotic XhoI/BamI ligation in pJP22
Brayan &João
The colonies resulting from the ligation products transformation on friday (08/19) were grown in LB media overnight and miniprepped. Some tubes showed abnormal growth, perhaps due to protein “leaking” between system, as pJP22 is an expression plasmid for *Chlamydomonas reinhardtii.*

----------22/08----------

Colony PCR of electroporated BL21DE3 cells ~endolysin expression by *E. coli* for activity test~
Mireia & Karent :)

Step	Temperature °C	Time
Initial Denaturation	98	30 seconds
35 Cycles	98 60 72	10 seconds 30 seconds 1 min
Final Extension	72	5 min
Hold	4	

Component	1 reaction (μL)	21 reaction (μL)
Nuclease-Free water	6,6	138
5X phusion HF or GC Buffer	4	84

10 mM dNTPs	0,4	8,5
10 μM Forward Primer	1	21
10 μM Reverse Primer	1	21
Template DNA	1	---
X7 DNA- Polymerase 100X stock	2	42
Betaine 5M	4	84
Volumen total	20	398,5

Several electroporated cells were obtained on the ampicillin LB agar plates of both LysK and lysostaphin transformations.

Transformation of digested gBlocks to pSB1C3

Tiago & João

The ligations performed on sunday were used to transform DH5-alpha bacteria by heat shock. The heat-shocked bacteria were grown in LB liquid media for 1 hour and plated.

----------08/23----------

Primers dilution
Brayan

New primers have arrived.
iGP0029
iGP0030

500uM Stock solution was prepared. Five 100uL-tubes of working solution (10uM) were also prepared.

All primers were stored at -20°C in our Primers box.

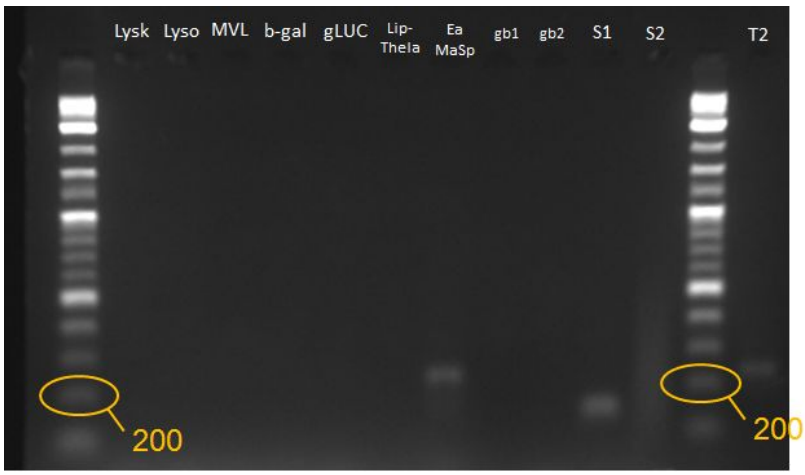
PCR of gBlocks (#56 ~ #67)
Fábio

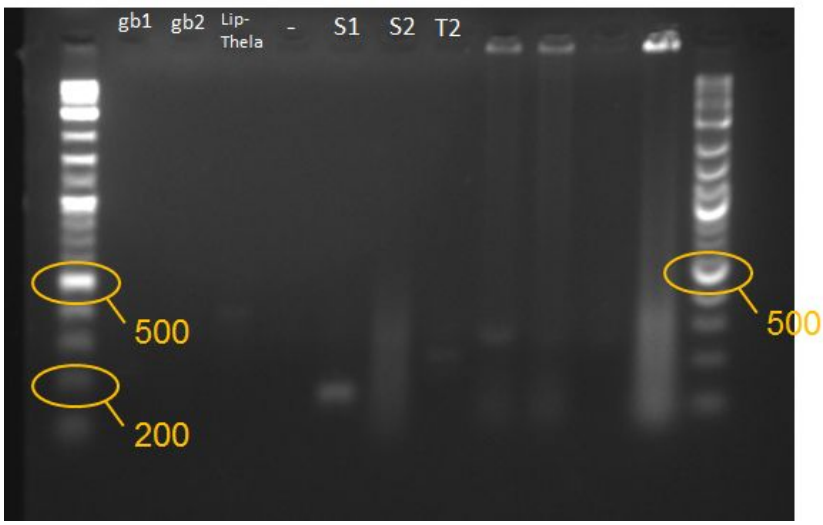
Master mix preparation

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume (ul) "20" PCR reactions
GC Buffer	10X	X	10	200
dNTPs	10 mM	0,5 mm	2,5	50
Primer iGP0029	10 uM	0,5 uM	2,5	50
Primer iGP0030	10 uM	0,5 uM	2,5	50
Betaine	5 M	M	10	200
Phusion	5U/ul	1.25U/50 uL	2,5	50
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			18	360
			50,0	1000

STEP	TEMP	TIME
Initial Denaturation	98°C	1 min 30 seconds
35 Cycles	98°C 53-68°C (gradient, in different lanes) 72°C	20 seconds 35 seconds 1 minute
Final Extension	72°C	5 minutes
Hold	4°C	

19 PCR tubes were prepared





Transformation by electroporation of digested gBlocks to purified pSB1C3
João, Fábio and Tiago

As the efficiency of transformation was rather low, we tried to electroporate electrocompetent *E. coli* with the ligations performed on sunday (08/21). The cells were grown on freshly made SOC media for 1 hour before plating. The cells were plated in the same petri dishes that didn't grow anything today (due to lack of prepared chloramphenicol LB agar dishes)

Digestion of pSB1A3, pSB1C3 and analytic digestion of pSB1C3 + 63 (MaSp1 type2)
Tiago

50 microliters (about 15 micrograms of DNA) of MIDI prep'd pSB1A3 or pSB1C3 + RFP were mixed with 15 U of PstI + 15 U of EcoRI in Buffer H 1x and left overnight in a water bath at 37ºC. (total 60microL)

4 microliters (about 600 ng) of the pSB1C3 + 63 (MaSp1 type2) midi prep'd plasmids were also incubated with 5U of of PstI + 5 U of EcoRI in Buffer H 1x and left overnight in a water bath at 37ºC. (total 10microL)

Second trial: Colony PCR of electroporated cells ~Endolysine expression by *E. coli* for activity test~
Mireia

This time taq master mix was used. Colonies were further diluted 10x.

Reaction composition:

Item	Volume for 1 reaction	Volume for 25 reactions
Taq Master Mix (2x)	5 uL	125 uL
Template	1 uL	25 uL
GP0001	1 uL	25 uL
GP0002	1 uL	25 uL
Betaine 5M	1 uL (0.5M)	25 uL
DMSO 50%	1 uL (10%)	25 uL
Total	10 uL	250 uL

Reaction conditions:

	Temperature	Time
Initial denaturation	95°C	1 min

35 cycles	95°C	15 sec
	60°C	30 sec
	72°C	45 sec
Final extension	72°C	5 min
Hold	4°C	Hold

Result: No amplification.

----------08/24----------

Digestion of enzybiotic sequences and/or pET-DUET (2)

Mireia

Reaction composition:

Item	Volume for 1 reaction	Volume for 3 reactions
pETDuet (180 ng/uL)	6 uL	18 uL
EcoRI	0.5 uL	1.5 uL
PstI	0.5 uL	1.5 uL
Buffer H	2 uL	6 uL
H_2O	11 uL	33 uL
Total	20 uL	60 uL

Reaction conditions:

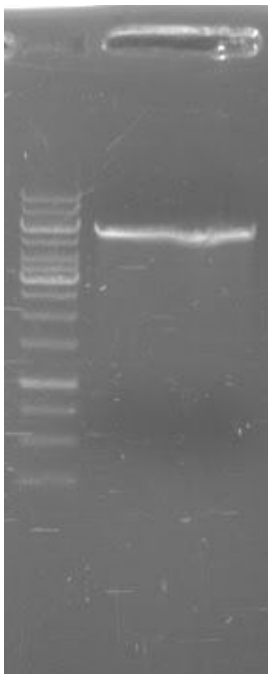
37°C (agar plate incubator) for 16 h.

08/25

Gel purification of digested pETDuet ~Endolysine expression by *E. coli* for activity test

Mireia

The total 60 uL digestion were loaded on a 40 mL, 0.8% agarose gel. It was run at 90 V 45 min. The 5 kb band was purified using Wizard SV Cleaner (Promega). It was obtained 50 uL of 18.0 ng/uL double digested pETDuet.



Miniprep plasmid extraction of Electroporated plasmids+gBlocks
João

Some colonies appeared in some LB dishes, showing that ligated products were successfully transformed by electroporation. Plasmid extraction was done.

	Concentration	A260-280nm (Purity)
pSB1C3 + gBlock 1.2	389.4	2.06
pSB1C3 + gBlock 1.1	260.6	2.01
pSB1C3 + 68	174.4	1.92
pSB1C3 + gBlock 1.3	214.8	1.98
pJP22 + Lysk 2	314.5	1.82
pJP22 + MV-L 2	11.3	1.49
pJP22 + MV-L 1	16.0	1.10
pJP22 + Lysk 1	188.0	

Analytic digestion of transformed gB1, gB2 and enzybiotics
Tiago
Standard digestion protocol. About 600ng of DNA, EcoRI, PstI 2U each/reaction and Buffer H.

pSB1C3+gb1.2
pSB1C3+gb1.1
pSB1C3+68 (gB2)
pSB1C3+gb1.3
pJP22+LysK 2
pJP22+LysK 1

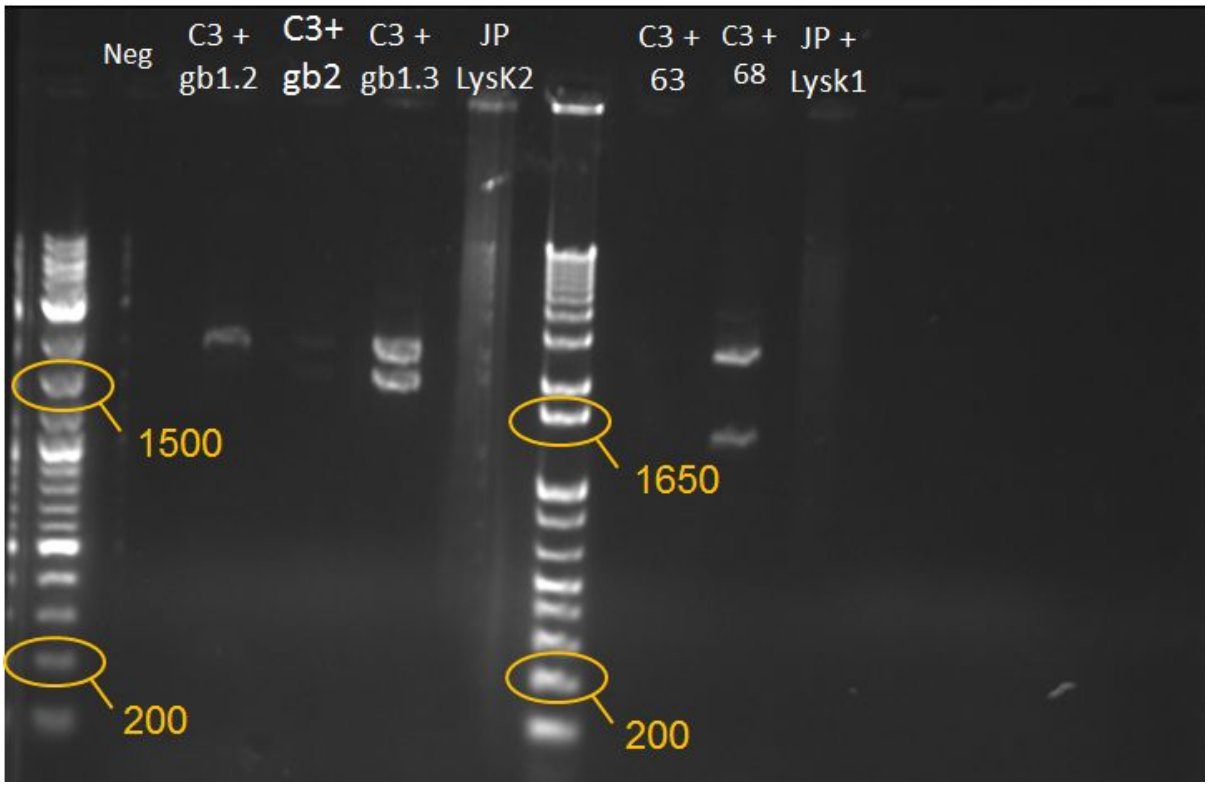
Gel purification of pSB1A3, pSB1C3, RFP
Fabio

These three sequences were gel purified from double-digested products made by Tiago yesterday **08/23.**

	Concentration
pSB1A3	5,7 ng/ul
pSB1C3	29,0 ng/ul
RFP	27,0 ng/ul

----------08/25----------

Electrophoresis of previously isolated plasmids (08/24)
Brayan



Results: Habemus gBlocks! (1)

MaSp PCR production
Viviane

Ea Masp
Silwa 1
Type 2

Master mix preparation

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume (ul) "20" PCR reactions
GC Buffer	10X	X	10	200
dNTPs	10 mM	0,5 mm	2,5	50
Primer iGP0029	10 uM	0,5 uM	2,5	50
Primer iGP0030	10 uM	0,5 uM	2,5	50

Betaine	5 M	M	10	200
Phusion	5U/ul	1.25U/50 uL	2,5	50
DNA template	-	-	2,0	Each reaction needs the proper sequence
DEPC water			18	360
			50,0	1000

STEP	TEMP	TIME
Initial Denaturation	98°C	1:30 minute
35 Cycles	98°C 53-68°C (gradient, in different lanes) 72°C	20 seconds 35 seconds 1 min
Final Extension	72°C	5 minutes
Hold	4 10°C	

19 PCR tubes were prepared

Digestion of pSB1C3+68 (gB2) and pSB1C3+gb1.3 and analytic digest of enzybiotics
Tiago
The plasmids were digested as follows, at 37º C overnight:
pSB1C3+68 (gB2) (40uL) with 15U of ClaI and 15U PstI , H buffer, total 50 uL
pSB1C3+gb1.3 w (40uL) with 15U of ClaI and 15U EcoRI, H buffer, total 50 uL
pJP22+LysK 2 (3uL) with 3 U of XhoI and 3 U of BamHI, CS buffer, total 10 uL
pJP22+LysK 1 (3uL) with 3U of XhoI and 3 U of BamHI, CS buffer, total 10 uL

MaSp1 (Type 2, Silwa 1 and Ea MaSp1) production

Brayan

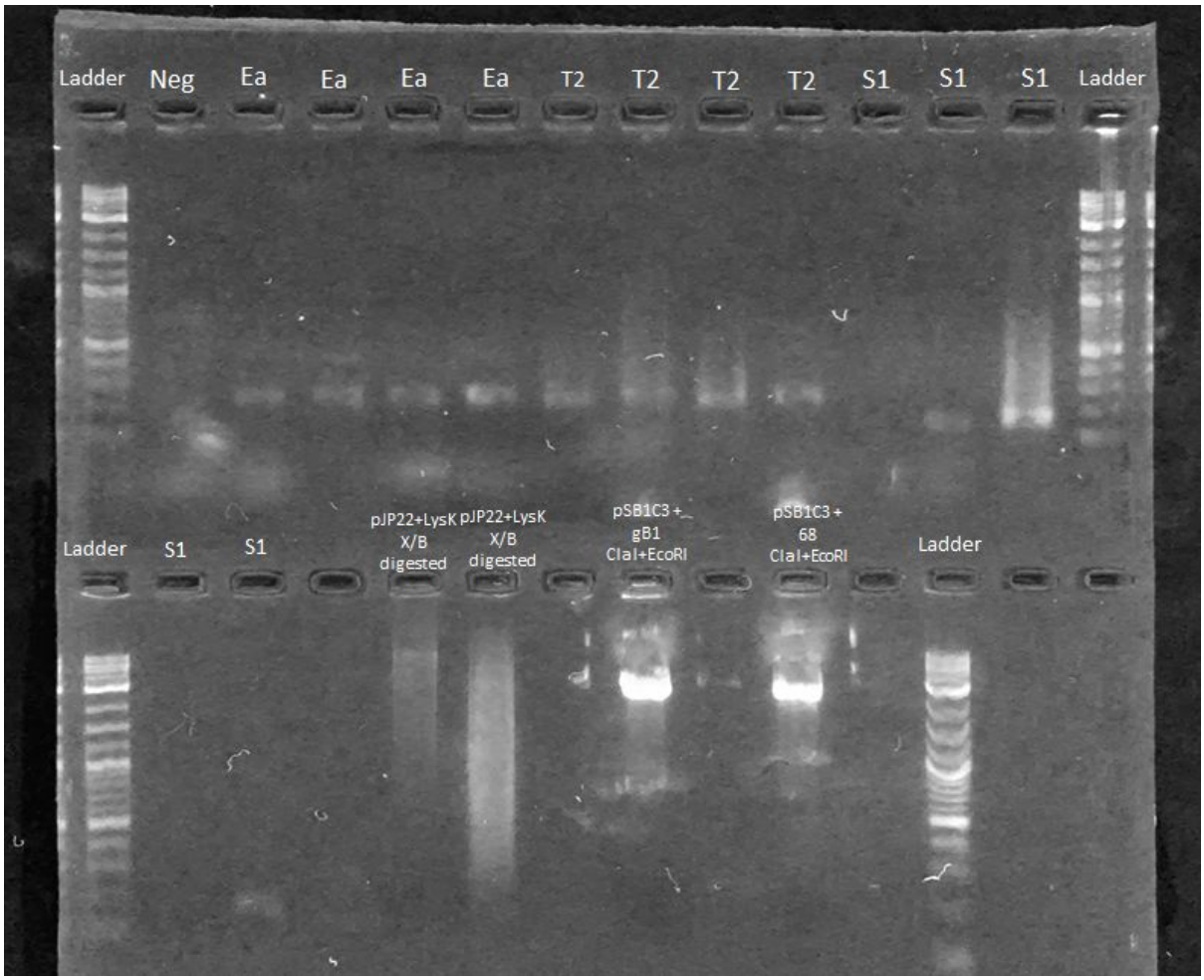
Several tubes were prepared for PCR production of sequences (50ul) based on standardized protocol for MaSp production.

----------08/26----------

Electrophoresis of PCR products and digestion products:

João and Brayan

MaSps PCRs worked (top lanes). Endolysins produced a weird smear (bottom, both left) and the plasmid digestion yielded only one strong band.



Growth of stored 68 and gB1.3 in glycerol in LB/Chlo :

Tiago

68 and gB1.3 stored bacteria were inoculated in ~10ml of LB media with chloramphenicol overnight, in order to miniprep for further digestion

Digestion of pSB1C3+68 (gB2) and , pSB1C3+gb1.2 pSB1C3+gb1.3

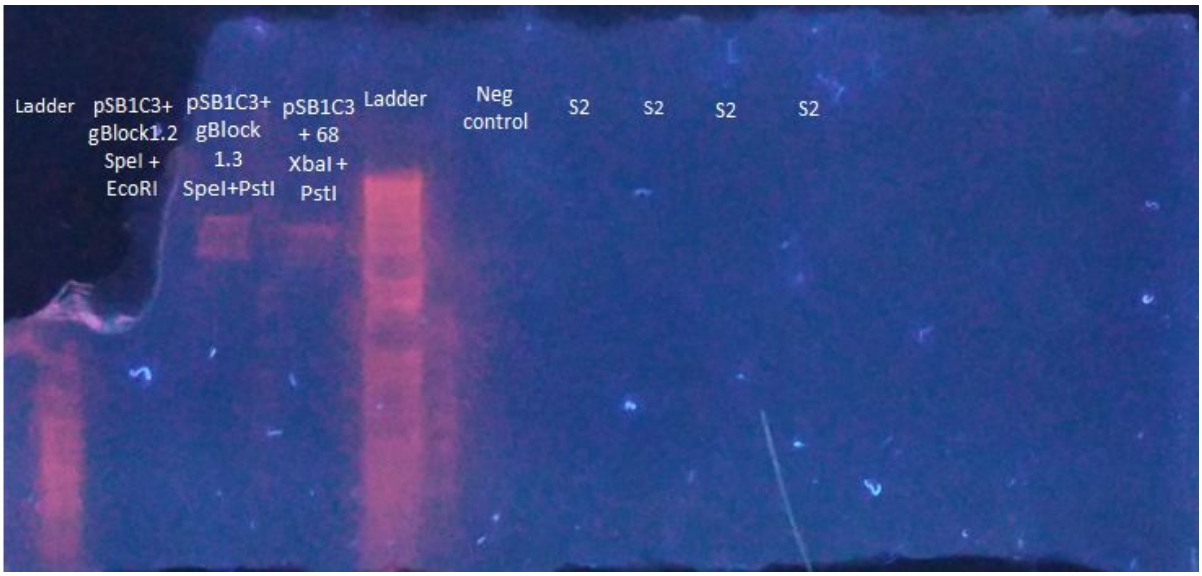
Tiago

pSB1C3+68 (gB2) (analyt.) - 3uL + 3U XbaI, 6U PstI, Tango buffer, total 10 uL
pSB1C3+gb1.3 (analyt,) - 3uL + 3U SpeI, 6U PstI, Tango buffer, total 10 uL
pSB1C3+gb1.2 - 40uL (~15,6 ug) + 15U SpeI, 30U PstI, Tango buffer, total 50 uL

----------**08/27**----------

Digestion of IDT sequences (From #56 to #75)
João
100ng of each sequence was digested with EcoRI and PstI to try electroporation next day.

Gel purification of digested plasmids
Viviane
Regardless of the result, both pSB1C3+gBlock 1.3 SpeI + PstI & pSB1C3+68 XbaI + PstI were gel purified



----------08/27----------

Gel purification of MaSp
Viviane

PCR amplified sequences were purified and their concentrations are below:

Sequences	Concentration	
Ea Masp	35,5	3,51
Silwa 1	22,1	1,57
Type 2	70,0	4,65

Gel purification of digested sequences with XbaI PstI SpeI PstI

pSB1C3 + 68 XbaI + PstI	4,6	1,25
pSB1C3 + gb1,3 SpeI + PstI	4,3	1,03

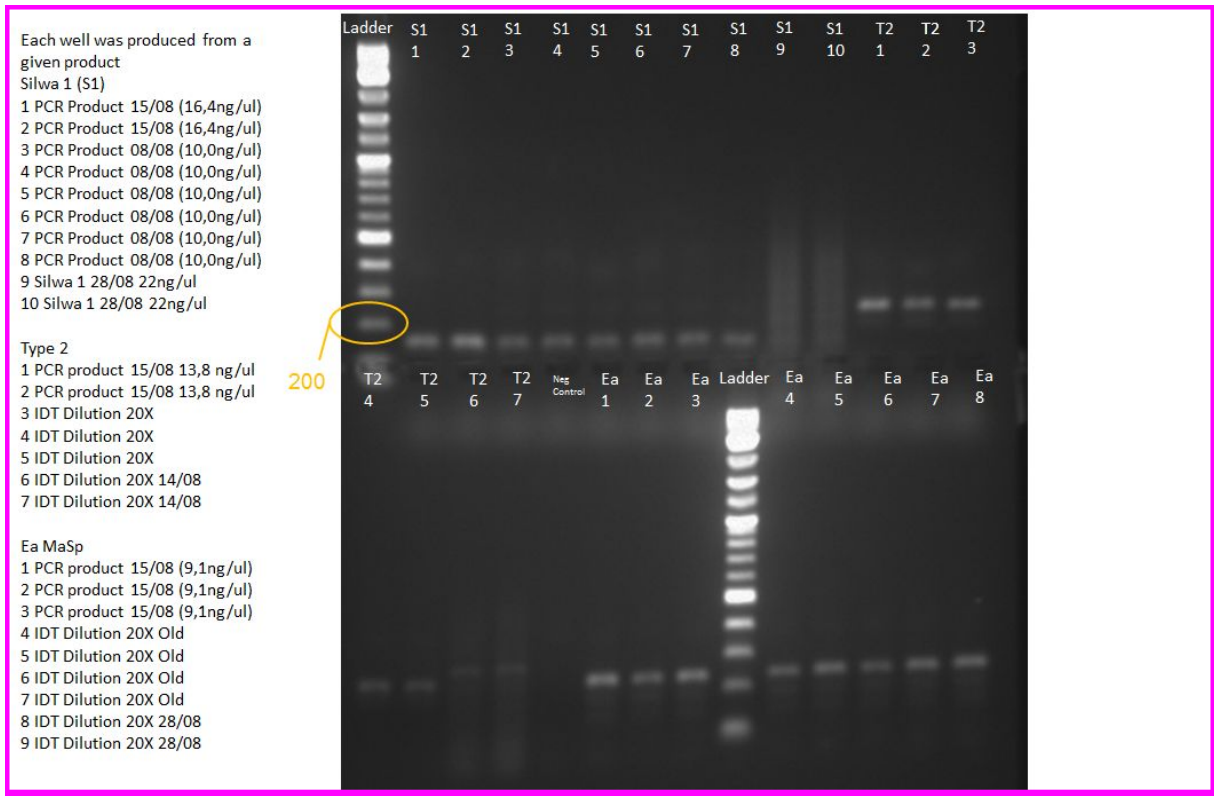
Miniprep
Viviane

pSB1C3 + gb2RFP (really reddish)	82,7 ng/ul	1,12
pSB1C3 + gb2RFP (not that red)	112,1 ng/ul	1,41
pJP22 + Lysostaphin	142,9 ng/ul	1,63
pSB1C3 + Lysostaphin	165,5 ng/ul	1,80
pSB1C3 + gb1.3	82,7 ng/ul	1,35
BBa_K146701	106,9 ng/ul	1,74
BBa_K1467104	56,8 ng/ul	1,80

----------08/28----------

MaSp1 (Type 2, Silwa 1 and Ea MaSp1) production
Brayan

Several additional tubes were prepared for PCR production of sequences (50ul) based on standardized protocol for MaSp production.



Electroporation of IDT sequences in DH5(Alpha) E. Coli electrocompetent cells
João & Brayan

All sequences were digested and ligated once again in pSB1C3. Molar ratios were cautiously calculated so as to have no trouble with concentrations.

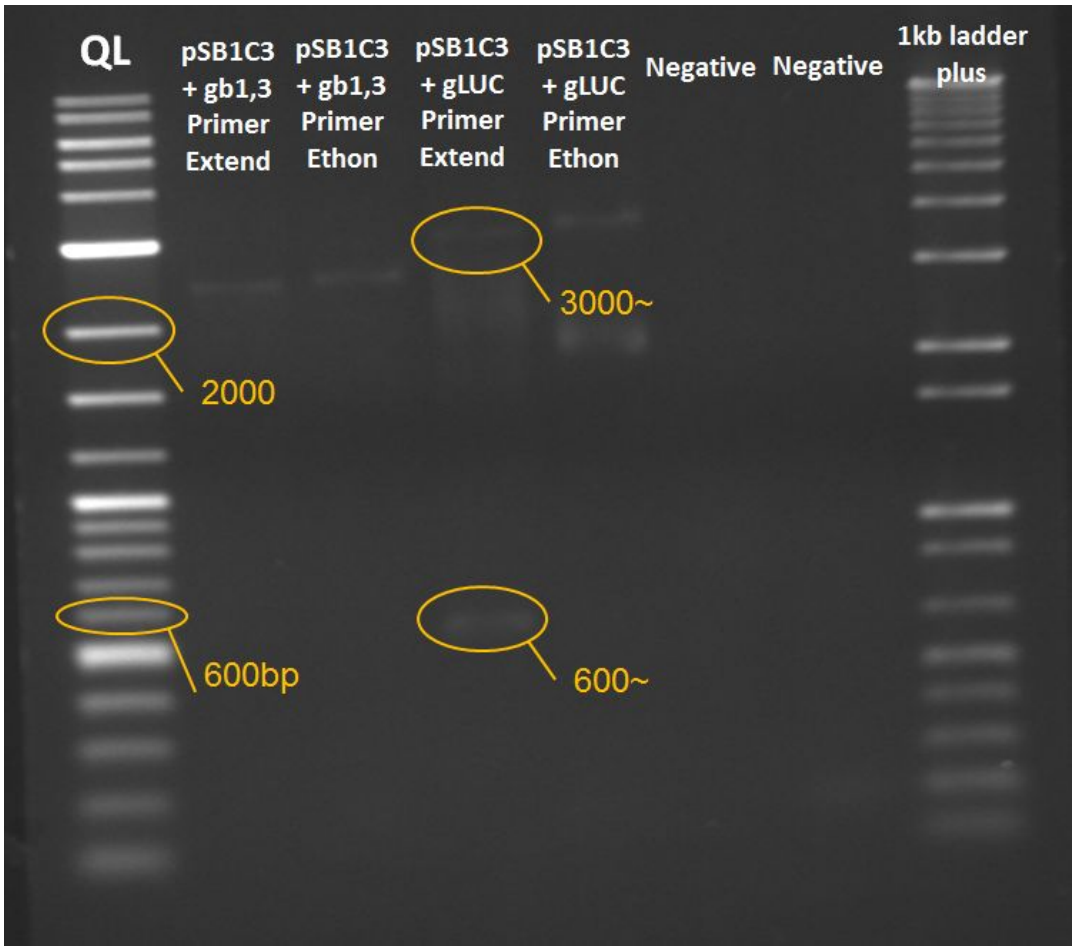
Transformants were plated in LB+Chloramphenicol dishes and incubated for 24hrs at 37°C.

----------08/29----------

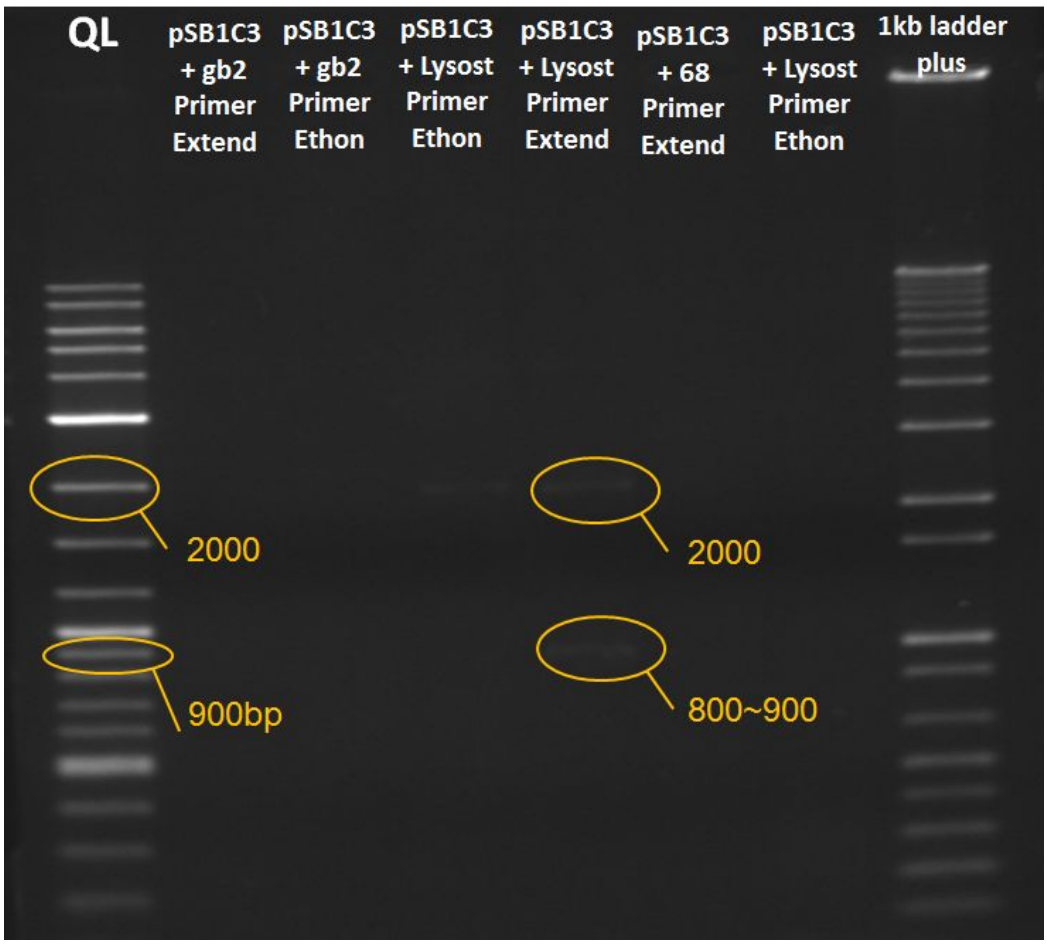
Electrophoresis of some pSB1C3 + IDT inserts

Brayan

Some produced plasmids were screened in order to find if standard PCR protocol is working with bigger constructs (>2kb). It also was tested which primer (Extend vs Ethon) performed better



Results: Habemus pSB1C3 + gLUC (Lane 4).



Results: pSB1C3 + Lysostaphin (Lane 5)

Miniprep of 54 transformed colonies
João and Fabio

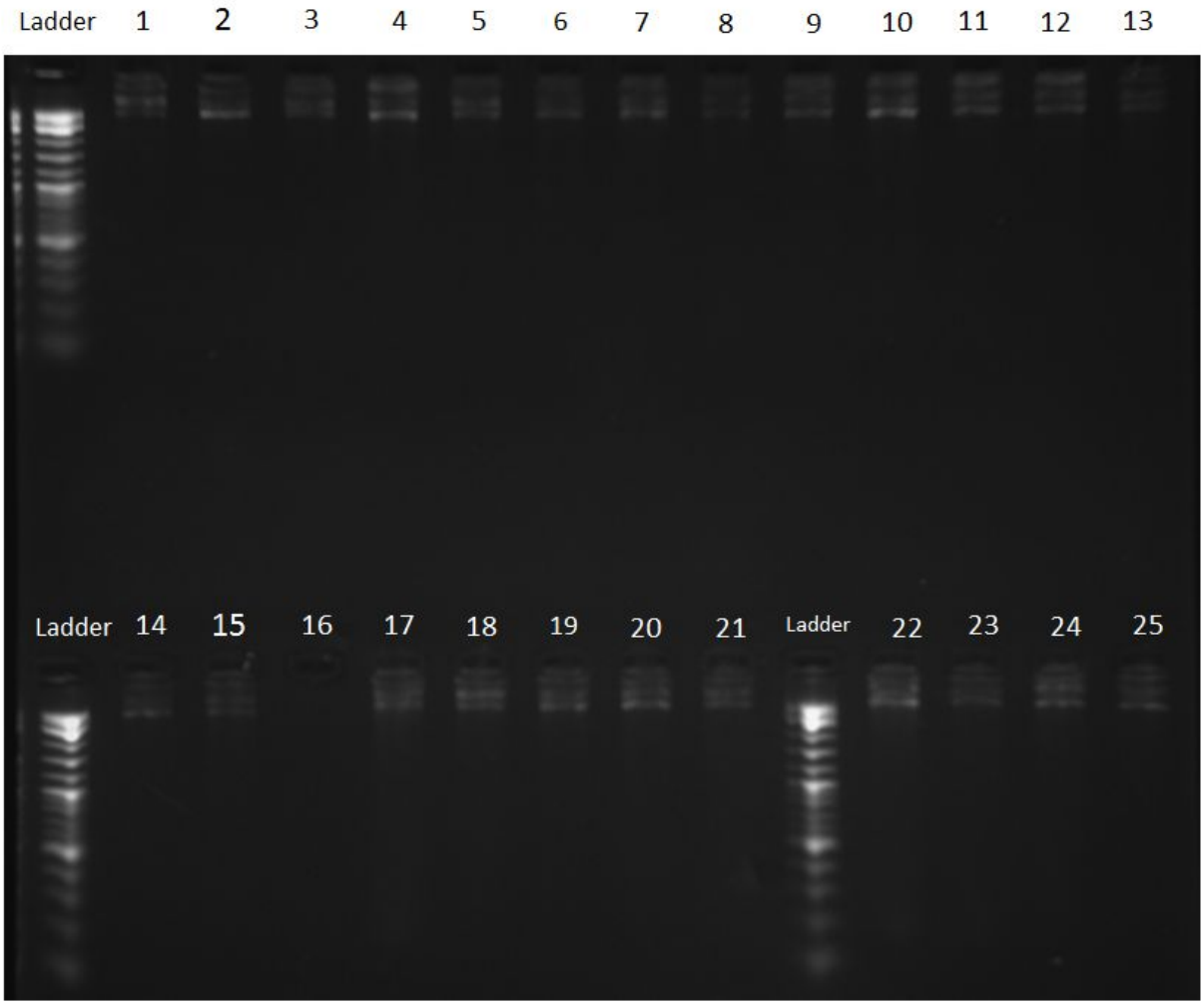
54 colonies were picked from electroporated transformants (from IDT #56 to #75, the day before) and inoculated in 8 ml LB broth. Today about 2 pm, miniprep will be performed.

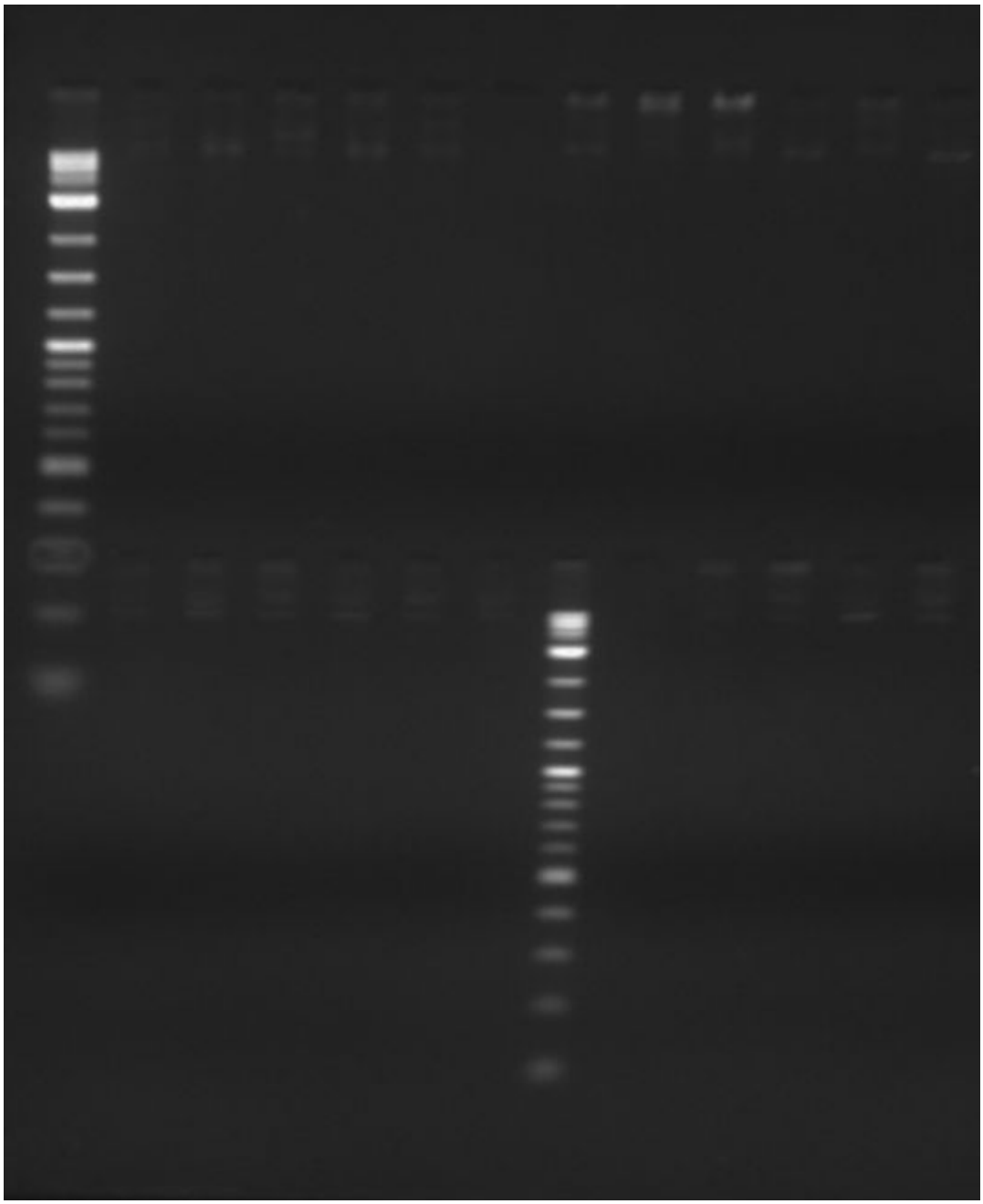
	Sample	Meaning	ng/µL
1	-1	Negative Colony 1	22.5
2	-2	Negative Colony 2	80.2
3	-3	Negative Colony 3	63.7
4	-4	Negative Colony 4	61.8
5	-5	Negative Colony 5	51.1
6	-6	Negative Colony 6	77.2
7	56.1	LysK Colony 1	61.8
8	56.2	LysK Colony 2	48.3

9	56.3	LysK Colony 3	49.9
10	56.4	LysK Colony 4	52.5
11	56.5	LysK Colony 5	82.8
12	56.6	LysK Colony 6	63.1
13	57.1	MV-L Colony 1	86.1
14	59.1	b-galacto Colony 1	86.4
15	59.2	b-galacto Colony 2	24.7
16	59.3	b-galacto Colony 3	51.7
17	59.4	b-galacto Colony 4	44.7
18	59.5	b-galacto Colony 5	49.8
19	59.6	b-galacto Colony 6	65.7
20	60.1	Lip_Thela Colony 1	50.5
21	60.2	Lip_Thela Colony 2	74.6
22	60.3	Lip_Thela Colony 3	84.5
23	60.4	Lip_Thela Colony 4	67.4
24	61.1	gLUC Colony 1	80.3
25	61.2	gLUC Colony 2	34.5
26	62.1	Ea MaSp1 Colony 1	31.6
27	62.2	Ea MaSp1 Colony 2	36
28	62.3	Ea MaSp1 Colony 3	75.9
29	62.4	Ea MaSp1 Colony 4	44.2
30	62.5	Ea MaSp1 Colony 5	71.5
31	62.6	Ea MaSp1 Colony 6	69.1
32	63.1	Type2 Colony 1	42.7
33	63.2	Type2 Colony 2	29.1
34	Fail		
35	63.4	Type2 Colony 4	42.2
36	63.5	Type2 Colony 5	125.5
37	63.6	Type2 Colony 6	98.6
38	64.1	Silwa 1 Colony 1	34.1
39	65.1	Silwa 2 Colony 1	108.7
40	65.2	Silwa 2 Colony 2	50.9
41	65.3	Silwa 2 Colony 3	27.8
42	65.4	Silwa 2 Colony 4	73
43	65.5	Silwa 2 Colony 5	23.4
44	67.1	gBlock 1 Colony 1	128.1
45	67.2	gBlock 1 Colony 2	54.6

46	68.1	gBlock 2 Colony 1	86.9
47	68.2	gBlock 2 Colony 2	81.1
48	68.3	gBlock 2 Colony 3	8.6
49	75.1	USER Colony 1	31.9
50	75.2	USER Colony 2	174.8
51	75.3	USER Colony 3	32.2
52	Fail		
53	75.5	USER Colony 5	63.1
54	75.6	USER Colony 6	29.6

EcoRI and PstI digestion from previously miniprepped plasmids
Tiago and Brayan





No positive clones were found by restriction digestion ON with EcoRI and PstI at standard conditions

E. Coli DH5 (Alpha) Electrocompetent cell preparation
500ml Glycerol 10% was prepared

Digestion of pSB1C3-Lysostaphine
Mireia

Item	Volume for 1 reaction	Volume for 3 reactions

pSB1C3-Lysostaphine (165,5 ng/uL)	6 uL	18 uL
EcoRI	0.5 uL	1.5 uL
PstI	0.5 uL	1.5 uL
Buffer H	2 uL	6 uL
H_2O	11 uL	33 uL
Total	20 uL	60 uL

Reaction conditions:
37°C (agar plate incubator) for 19 h.

----------09/01----------

Gel purification of digested pSB1C3-Lysostaphine ~Endolysine expression by *E. coli* for activity test
Mireia

The total 60 uL digestion were loaded on a 25 mL, 0.75% agarose gel. It was run at 70 V 30 min. The 0.8 kb band was purified using Wizard SV kit (promega). It was obtained 50 uL of 18 ng/uL double digested Lysostaphine.

PCR of pJP22 parts in order to make separated biobricks
Viviane

Master mix

Item	Volume for 1 reaction	Volume for “15 reactions”
CG buffer	2.5 uL	37.5 uL
dNTPs	0.250 uL	3.75 uL
Foward primer	0.250 uL	-
Reverse primer	0.250 uL	-
Homemade polymerase	1 uL	15 uL
Betaine 5M	2.5 uL	37.5 uL
H2O	5.25 uL	78.75 uL
Template DNA	0.5 uL	-
Total	12.5 uL	172.5 uL

Primers x Template	Digested pJp22 with XhoI and BamHI	pJp22 with MV-L (from the ligation attempt)	pJp22 mCherry
Promoter primers (53 and 55)	P1	P2	P3
Resistance (Ble) primers (56 and 57)	R1	R2	R3
Terminator primers (58 and 59	T1	T2	T3

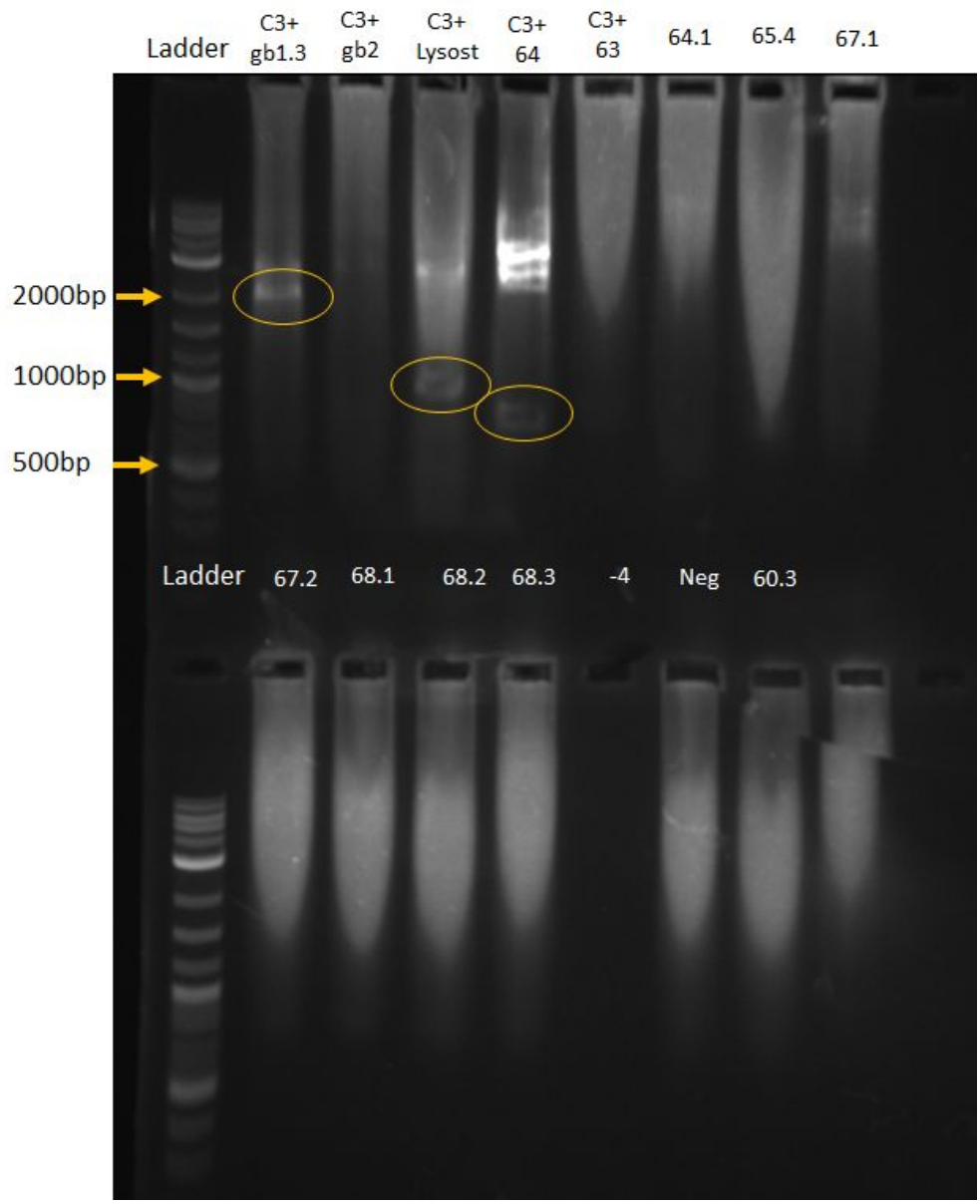
Promoter: ~680 bp
Resistance (ble): ~870 bp
Terminator: ~490 bp

STEP	TEMP	TIME
Initial Denaturation	98°C	1 min
35 Cycles	98°C 60°C 72°C	15 seconds 30 seconds 45 seconds
Final Extension	72°C	5 minutes
Hold	4°C	

----------09/02----------

Results of analytical digestion/PCR of gBlocks and MaSp/PCR of pJP22 parts
Tiago, Vivi, Brayan

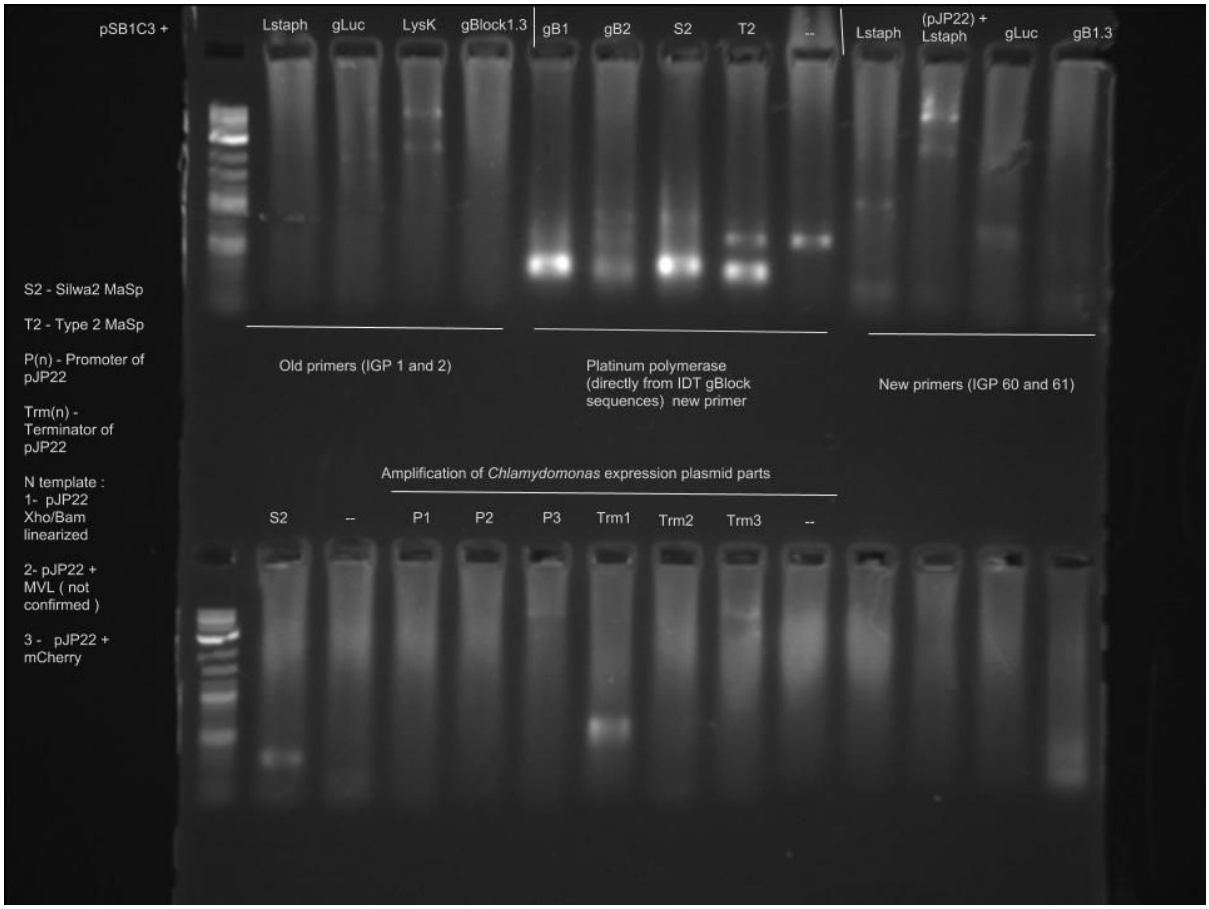
pSB1C3 with gLuc and pSB1C3 with Lysostaphin were properly digested.



RESULT:
pSB1C3 + gb1.3 **reconfirmed**
pSB1C3 + Lysostaphin confirmed for the **first time w analytical digestion**
pSB1C3 + 64 wrong **codification confirmed** (instead of 64, it's gLUC (#61)) and analytical digestion just reassure previous results

The gBlocks PCRs with Platinum polymerase showed an unexpected band pattern. Silwa 2 e Type 2 were amplified. T1 also appears to show a band in the desired size. All the others showed only a smear.

S2 PCR is robust for IGP60 and IGP61 primers both with X7/Phusion and Platinum.
Touch-Down PCR worked here.



NEB PRODUCTS
Stored by Tiago

Q5 MasterMix, Biobrick assembly Kit and QL 2l log ladder were stored in the -20ºC freezer. 6x0.2ml competent DH5-alpha cells were stored in the -80ºC freezer

Test of Q5 2X Master Mix (expired on 2014 on the -20ºC without use)
Tiago

Master mix preparation

Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume (ul) "20" PCR reactions
Q5 Master Mix	2X	X	12,5	
Primer iGP0001	10 uM	0,5 uM	2,5	50
Primer iGP0002	10 uM	0,5 uM	2,5	50

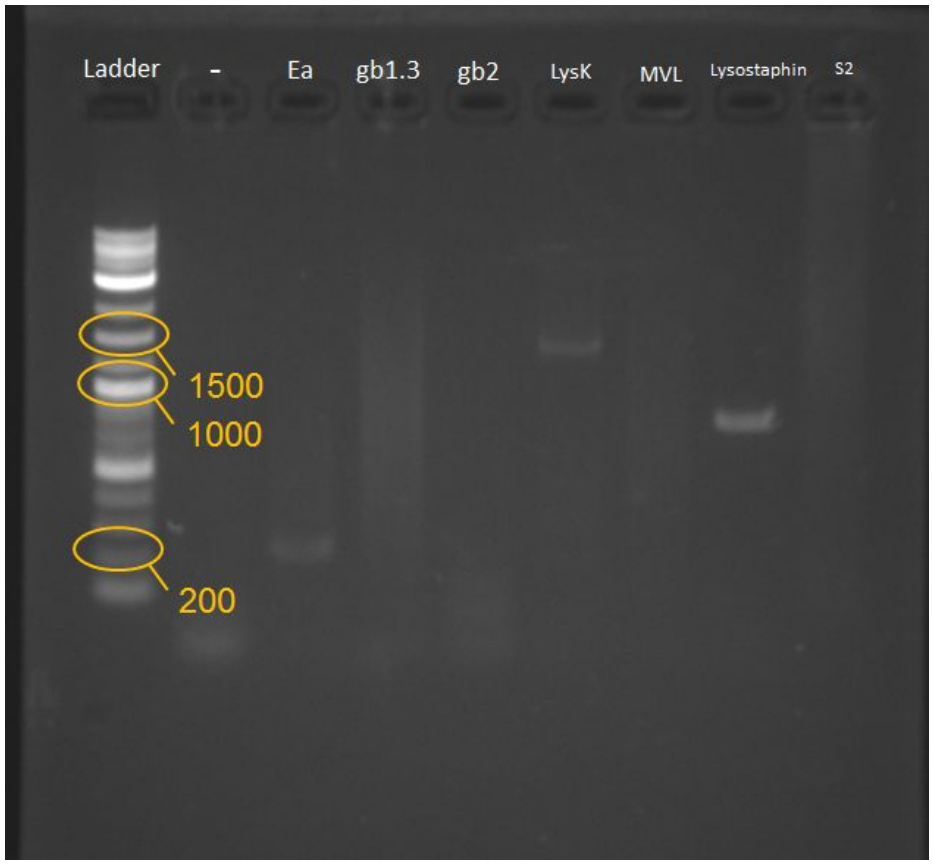
DNA template	-	-	1	Each reaction needs the proper sequence
			25,0ul	1000

STEP	TEMP	TIME
Initial Denaturation	98°C	1 min 30 seconds
35 Cycles	98°C 53-68°C (gradient, in different lanes) 72°C	20 seconds 35 seconds 1 minute
Final Extension	72°C	5 minutes
Hold	4°C	

2-step PCR

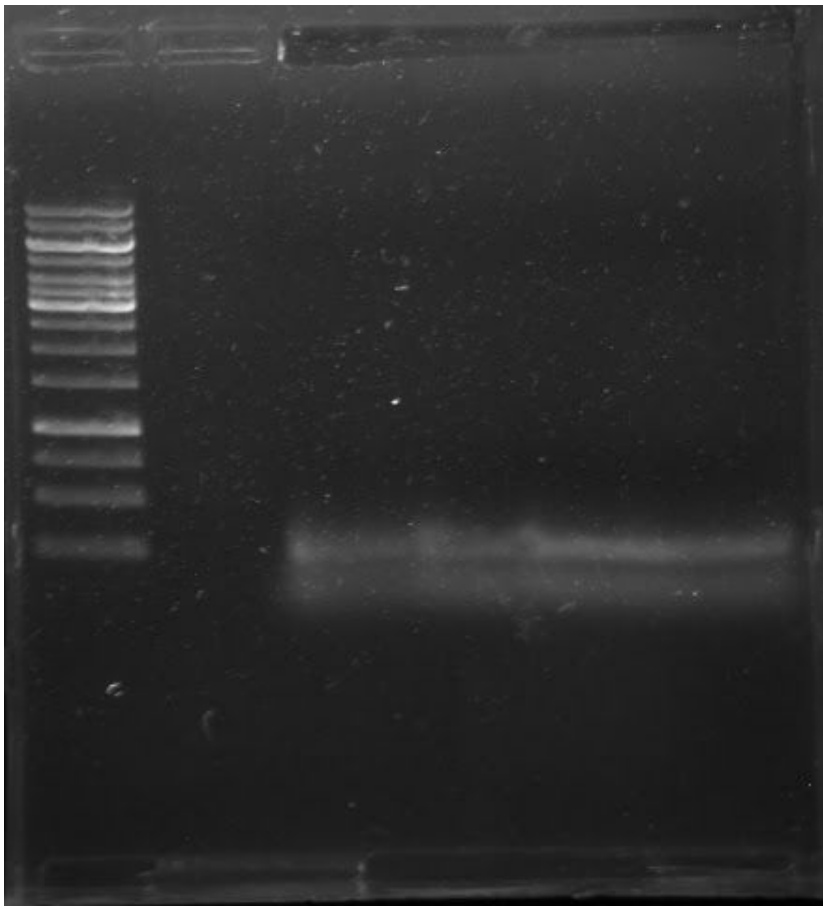
No annealing step (72°C for both annealing & extension)
50 seconds of extension time
Primers IGP001 and IGP002

Templates
5ng of IDT seqs of gBlock1, gBlock2, MVL and Ea MaSp. Ligation products of LysK and Lysostaphin + pSB1C3



MaSp1 type2 PCR product gel purification ~MaSp1 gene polymerization~
Mireia

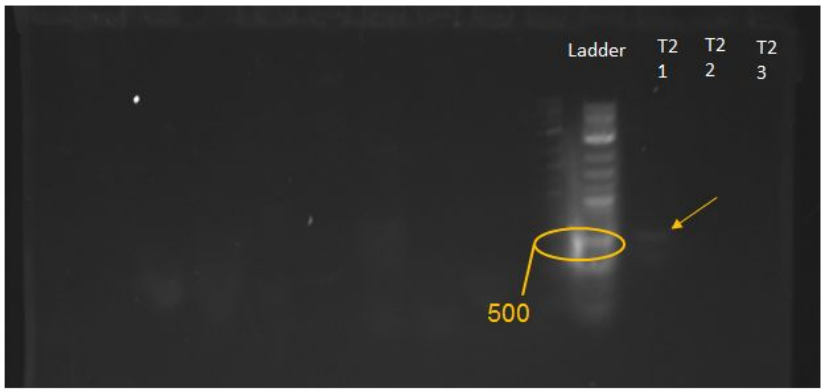
350 uL of MaSp1 type 2 PCR product were run on a 0.8% agarose gel (80 V, ~45 min)

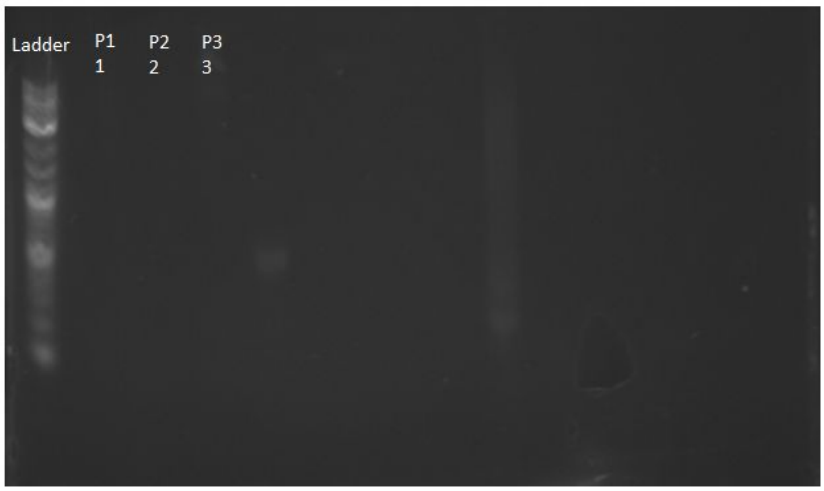


----------09/03----------

Confirming previous gel (09/02)
Brayan

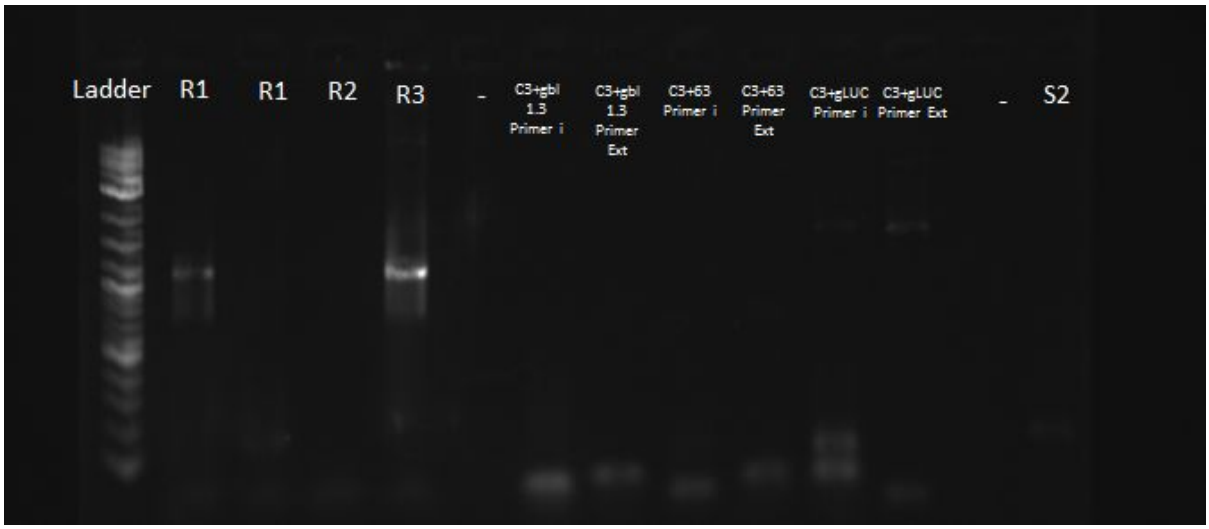
1 = pJP22 Xho/BamHI digested (29ng/ul ~)
2 = pJP22 + MVL (15ng/ul)
3 = pJP22 + mCherry (>100ng/ul)

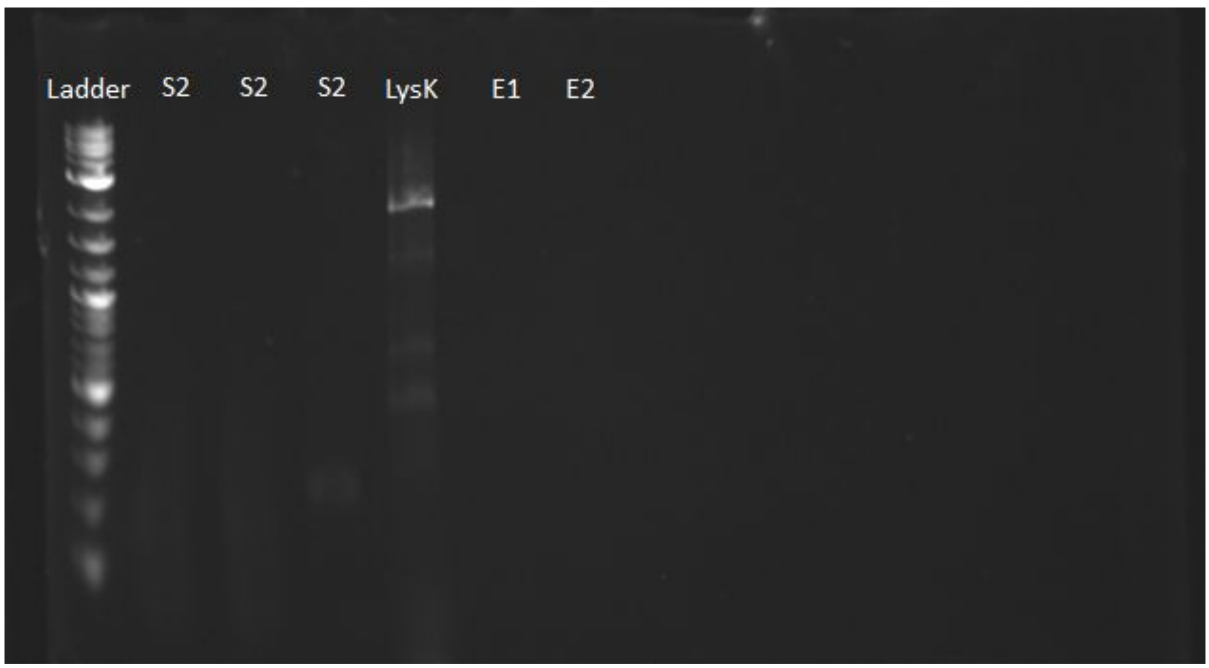




Electrophoresis
Viviane

Resistance gene from pJP22 is working with both template 1 and 3.
Terminator gene from pJP22 is working with template 1.
No promoter region amplified.
Silwa 2 (probably Type 2) is working with 60° Hybridization temperature.





Transformation of ligation products in NEB High Efficiency Competent Cells
Viviane and Brayan

Ligation products, which demonstrated being efficient for gBlock1 and Lysostaphin, from **08/20** were selected: gBlock 1 (**Positive control**) - gBlock 2 - LysK - MVL - Type 2 MaSp*
*Selection of these products were based on medal criteria and priority.

Positive control from NEB BioBrick Assembly Kit: pUC19 (FTW!)

Standard transformation protocol was used, and 10-fold dilution principle from NEB protocol was also performed for: gBlock 1, LysK, Type 2 MaSp, pUC19 (2 dishes for these ligation products).

----------09/04----------
Brayan

About 18 hours later, some colonies were picked for inoculation from petri dishes.
2 colonies from gBlock 1 (gBlock 1.1, gBlock 1.2)
1 colony from gBlock 2 (A reddish colony also grew, indicating RFP presence)
1 colony from LysK
1 colony from MV-L
1 colony from Type 2
1 colony from pUC19
No colonies in negative control until now.

At last, seven 15-mL falcon were prepared for 16~20 hours growing phase. Tomorrow is miniprep day.

----------09/05----------

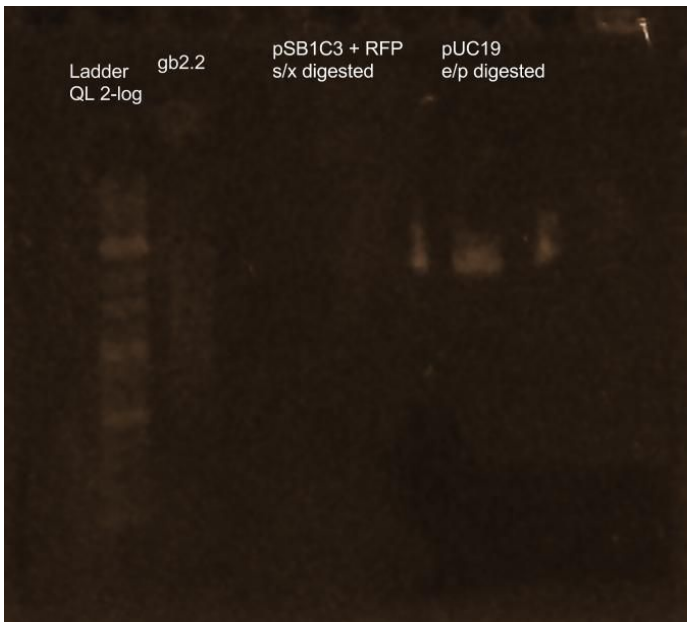
Miniprep and quantification of pSB1C3 + IDT constructs in NEB DH5-alpha cells
Tiago and Brayan

10ml of overnight LB liquid cultured were used. The DNA was diluted in 30microliters of elution buffer
The first 2 were measured with a different elution buffer as blank and the 3rd

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
puc19 eb1	Default	9/5/2016	7:15 PM	124.09	2.482	1.254	1.98	2.09	50.00	230	1.189	-0.743
puc19 eb1	Default	9/5/2016	7:17 PM	153.94	3.079	1.629	1.89	2.47	50.00	230	1.247	0.040
puc19 eb1	Default	9/5/2016	7:23 PM	-4.25	-0.085	-0.063	1.36	0.33	50.00	230	-0.258	-0.009
psb1c3 + puc19	Default	9/5/2016	7:24 PM	160.01	3.200	1.730	1.85	2.04	50.00	230	1.571	0.015
psb1c3 + mvl	Default	9/5/2016	7:25 PM	7.68	0.154	0.080	1.92	1.14	50.00	230	0.135	0.039
psb1c3 + mvl	Default	9/5/2016	7:26 PM	8.23	0.165	0.108	1.53	1.03	50.00	230	0.160	0.013
psb1c3 + gb2.1	Default	9/5/2016	7:27 PM	39.74	0.795	0.414	1.92	1.57	50.00	230	0.505	0.032
psb1c3 + gb2.1	Default	9/5/2016	7:28 PM	39.81	0.796	0.436	1.83	1.58	50.00	230	0.505	-0.018
psb1c3 + gb2.2	Default	9/5/2016	7:29 PM	183.12	3.662	2.040	1.80	2.01	50.00	230	1.825	0.081
psb1c3 + gb2.2	Default	9/5/2016	7:30 PM	182.89	3.658	2.001	1.83	2.02	50.00	230	1.815	0.009
psb1c3 + RFP	Default	9/5/2016	7:31 PM	31.86	0.637	0.351	1.81	1.71	50.00	230	0.373	-0.012
psb1c3 + lysK	Default	9/5/2016	7:33 PM	9.90	0.198	0.112	1.77	1.14	50.00	230	0.173	0.100
psb1c3 + lysK	Default	9/5/2016	7:33 PM	14.23	0.285	0.180	1.58	0.64	50.00	230	0.445	0.056
psb1c3 + type2	Default	9/5/2016	7:34 PM	4.53	0.091	0.045	2.02	0.62	50.00	230	0.146	0.017
h2o	Default	9/5/2016	7:35 PM	3.35	0.067	0.065	1.03	0.78	50.00	230	0.086	-0.013
h2o	Default	9/5/2016	7:36 PM	2.64	0.053	0.052	1.02	0.72	50.00	230	0.074	-0.002
h2o	Default	9/5/2016	7:37 PM	2.38	0.048	0.055	0.86	0.75	50.00	230	0.063	-0.004

Analytic digestion and total digestion
Tiago

pSB1C3 + gb2.2, +lysK and pUC19 were digested with EcoRI and PstI
pSB1C3 + RFP (old tube, 220 ng/microliter) was digested with XbaI and SpeI



Digestion was inconclusive

LB + Chloramphenicol dishes preparation

Brayan & Fábio

28 plates were prepared.

LB + Kanamycin dishes preparation

Brayan & Fábio

9 plates were prepared

LB Browth

Brayan

250ml of LB browth was prepared

Analytical digestion of plasmids

pSB1C3+gblock2.2, pJP22+Lysostaphin and other two pSB1C3 were digested again for confirmation. Rest of plasmids did not have enough concentration to run another digestion.

PCR of pJP22 parts

Viviane

It was an attempt to amplify P1 and R3 of 09/01 to bind to pSB1C3.

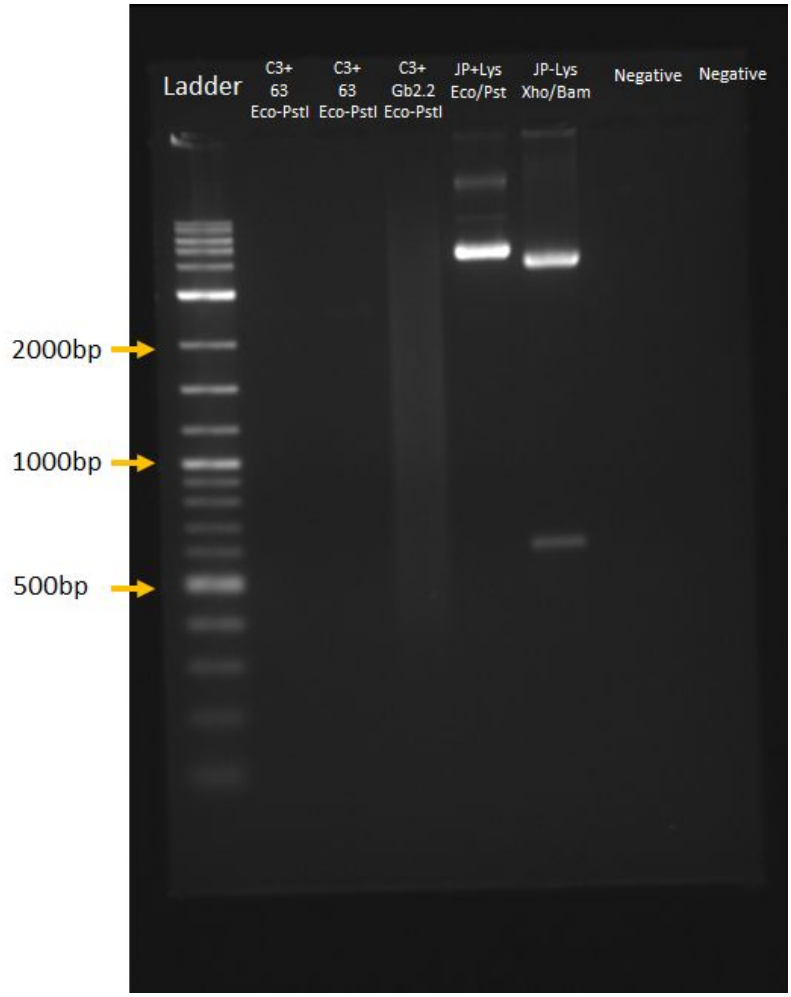
Resistance primers: 56 and 57
Terminator primers: 58 and 59

Item	Volume for 1 reaction	Volume for 3 reactions
CG buffer	10 uL	30 uL
dNTPs	2.5 uL	7.5 uL
Foward primer	2.5 uL	-
Reverse primer	2.5 uL	-
Homemade polymerase	2.5 uL	7.5 uL
Betaine 5M	10 uL	30 uL
H2O	18 uL	54 uL
Template DNA	2 uL	-
Total	50 uL	150 uL

----------09/06----------

Electrophoresis of digested plasmids

Brayan



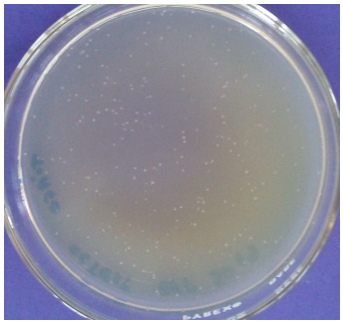
Abnormal smear for pSB1C3+gb2.2 colony.
We shall plate again gblock 2.1 and 2.2 to test correctly.

Transformation and competent cell test
Tiago

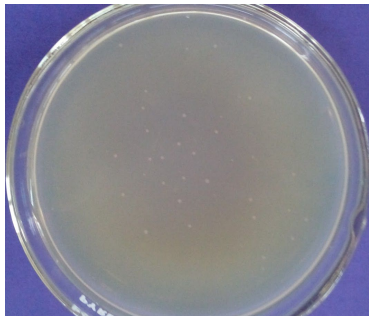
The following DNA templates were transformed into DH5-alpha “in-house” competent cells by standard heat shock protocol (with 1hour incubation in LB medium before plating) :
Ligation from 08/28/2016(2 microliters):
-56, 57, 63, 64, 65
RFP +pSB1C3 from iGEM test kit
-0.5pg
-5pg
-50pg

JM110 (not competent cells) + BBa_J04450 were used as negative control/test

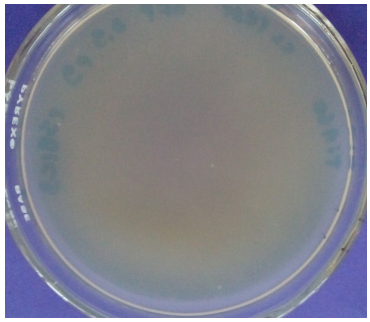
Results: The efficiency of the chemocompetent cell transformation is quite low)



10ng of DNA plated
~240 colonies



1ng of DNA plated
~38 colonies



0,1 ng of DNA plated
3 colonies

Colonies were counted with OpenCFU (Geissmann 2013). An average of 30 colonies/ pg of DNA plated (3x10^7 / microgram) was found. That's far from the recommended 1.5 - 6 x 10^8 CFUs per microgram of DNA

----------09/07----------

Digestion
Viviane and Brayan

The goal of this digestion was to compare our old enzymes with the new enzymes.

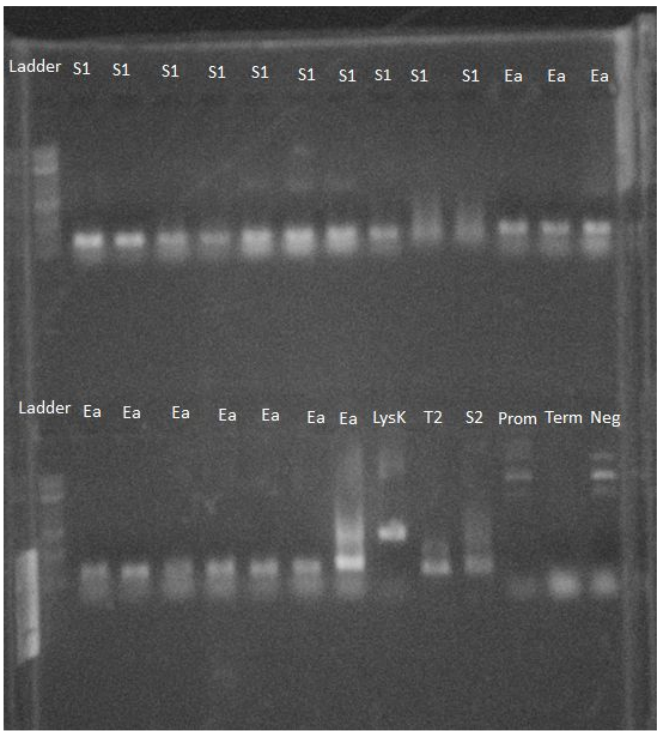
1. pSB1C3 + RFP 1
2. pSB1C3 + RFP 2
3. pSB1C3 + gb1.3
4. Lip Thela

Master Mix

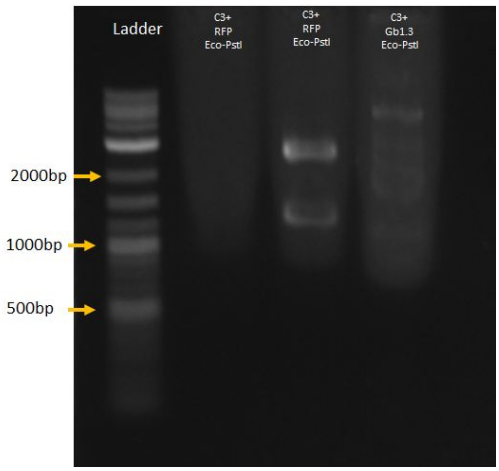
Item	1, 2, 3	4
EcoRI	0.5 ul	0.5 ul
PstI	0.5 ul	0.5 ul
Buffer H 10x	1 ul	1 ul
H2O	1 ul	7 ul
DNA	7 ul	1 ul
Total	10 ul	10 ul

Electrophoresis of S1 & Ea MaSp/PCR of pJP22 parts
Viviane

PCR of Silwa 1 and Ea Masp were confirmed and gel was cut.
pJP22 parts were not amplified correctly.



Plasmid confirmation
Brayan and Viviane



New NEB-arrived enzymes were tested.
One of the pSB1C3 + RFP plasmids is not actually present. It should be discarded.
pSB1C3 + gBlock1.3 seems sort of weird, but it must contain the gBlock 1 band.

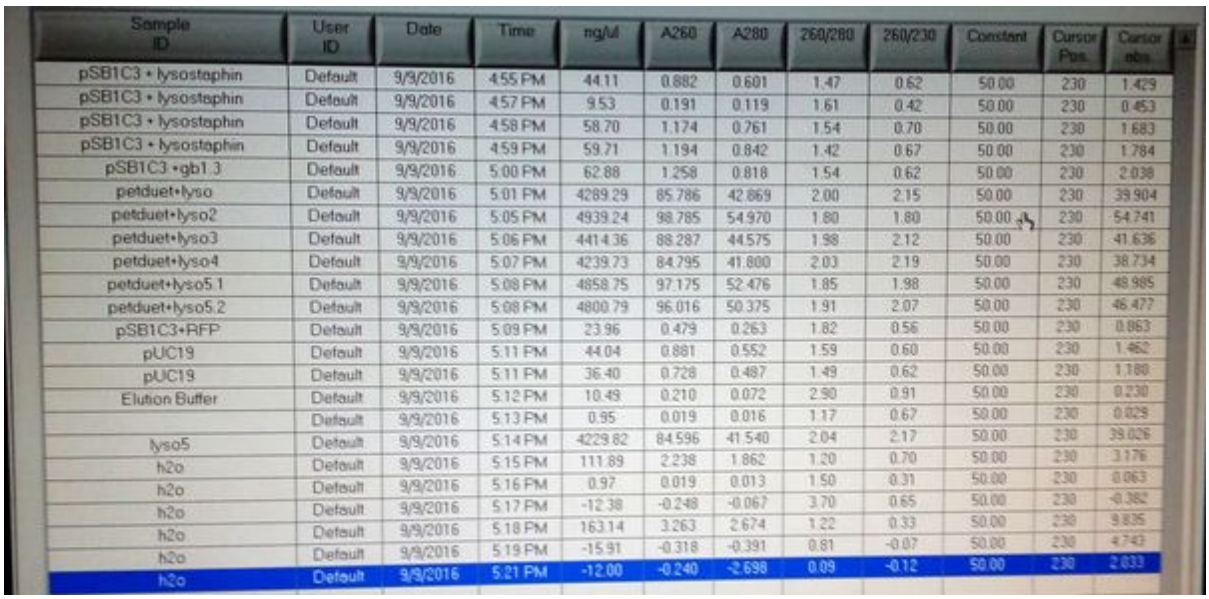
----------09/08----------

Alkaline lysis of pETDUET+ lysostaphin
Tiago and Mireia

The DNA of BL21 cells transformed with pETDUET+ lysostaphin and grown overnight was extracted through a standard alkaline lysis + ethanol extraction protocol. 1.4 ml of a 5 uL overnight culture in LBCb medium was used for each extraction.

----------09/09----------

Quantification of alkaline lysis of pETDUET+ lysostaphin and other colonies
Tiago



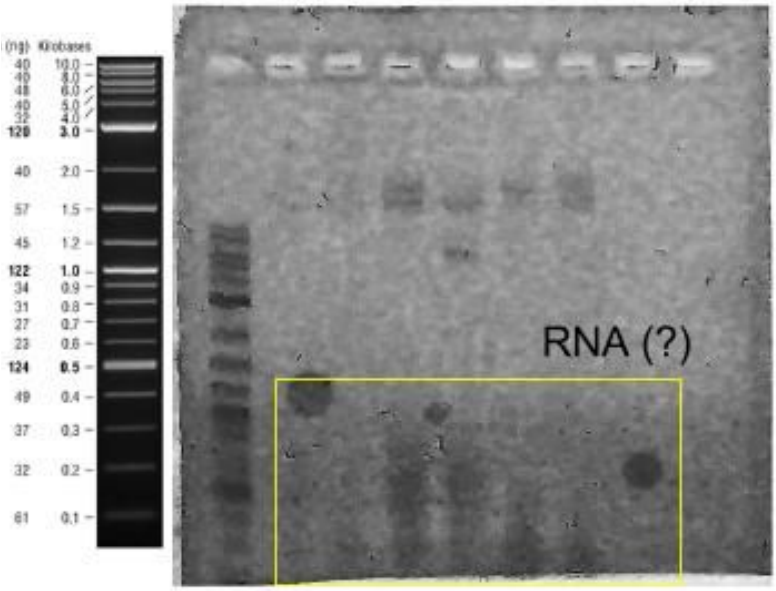
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.
pSB1C3 + lysostaphin	Default	9/9/2016	4:55 PM	44.11	0.882	0.601	1.47	0.62	50.00	230	1.429
pSB1C3 + lysostaphin	Default	9/9/2016	4:57 PM	9.53	0.191	0.119	1.61	0.42	50.00	230	0.453
pSB1C3 + lysostaphin	Default	9/9/2016	4:58 PM	58.70	1.174	0.761	1.54	0.70	50.00	230	1.683
pSB1C3 + lysostaphin	Default	9/9/2016	4:59 PM	59.71	1.194	0.842	1.42	0.67	50.00	230	1.784
pSB1C3 +gb1.3	Default	9/9/2016	5:00 PM	62.88	1.258	0.818	1.54	0.62	50.00	230	2.038
petduet+lyso	Default	9/9/2016	5:01 PM	4289.29	85.786	42.869	2.00	2.15	50.00	230	39.904
petduet+lyso2	Default	9/9/2016	5:05 PM	4939.24	98.785	54.970	1.80	1.80	50.00	230	54.741
petduet+lyso3	Default	9/9/2016	5:06 PM	4414.36	88.287	44.575	1.98	2.12	50.00	230	41.636
petduet+lyso4	Default	9/9/2016	5:07 PM	4239.73	84.795	41.800	2.03	2.19	50.00	230	38.734
petduet+lyso5.1	Default	9/9/2016	5:08 PM	4858.75	97.175	52.476	1.85	1.98	50.00	230	48.985
petduet+lyso5.2	Default	9/9/2016	5:08 PM	4800.79	96.016	50.375	1.91	2.07	50.00	230	46.477
pSB1C3+RFP	Default	9/9/2016	5:09 PM	23.96	0.479	0.263	1.82	0.56	50.00	230	0.863
pUC19	Default	9/9/2016	5:11 PM	44.04	0.881	0.552	1.59	0.60	50.00	230	1.462
pUC19	Default	9/9/2016	5:11 PM	36.40	0.728	0.487	1.49	0.62	50.00	230	1.186
Elution Buffer	Default	9/9/2016	5:12 PM	10.49	0.210	0.072	2.90	0.91	50.00	230	0.230
	Default	9/9/2016	5:13 PM	0.95	0.019	0.016	1.17	0.67	50.00	230	0.029
lyso5	Default	9/9/2016	5:14 PM	4229.82	84.596	41.540	2.04	2.17	50.00	230	39.026
h2o	Default	9/9/2016	5:15 PM	111.89	2.238	1.862	1.20	0.70	50.00	230	3.176
h2o	Default	9/9/2016	5:16 PM	0.97	0.019	0.013	1.50	0.31	50.00	230	0.063
h2o	Default	9/9/2016	5:17 PM	-12.38	-0.248	-0.067	3.70	0.65	50.00	230	-0.382
h2o	Default	9/9/2016	5:18 PM	163.14	3.263	2.674	1.22	0.33	50.00	230	9.835
h2o	Default	9/9/2016	5:19 PM	-15.91	-0.318	-0.391	0.81	-0.07	50.00	230	4.743
h2o	Default	9/9/2016	5:21 PM	-12.00	-0.240	-2.698	0.09	-0.12	50.00	230	2.033

The quantification of the grown colonies was extremely high, perhaps due to genomic and tRNA.

Electrophoretic analysis of alkaline lysis of pETDUET+ lysostaphin
Tiago

Bands were found clearly in samples 3, 4 and 5. Probably corresponding to different forms of the plasmid (circular, less mobile, and supercoiled, more mobile)

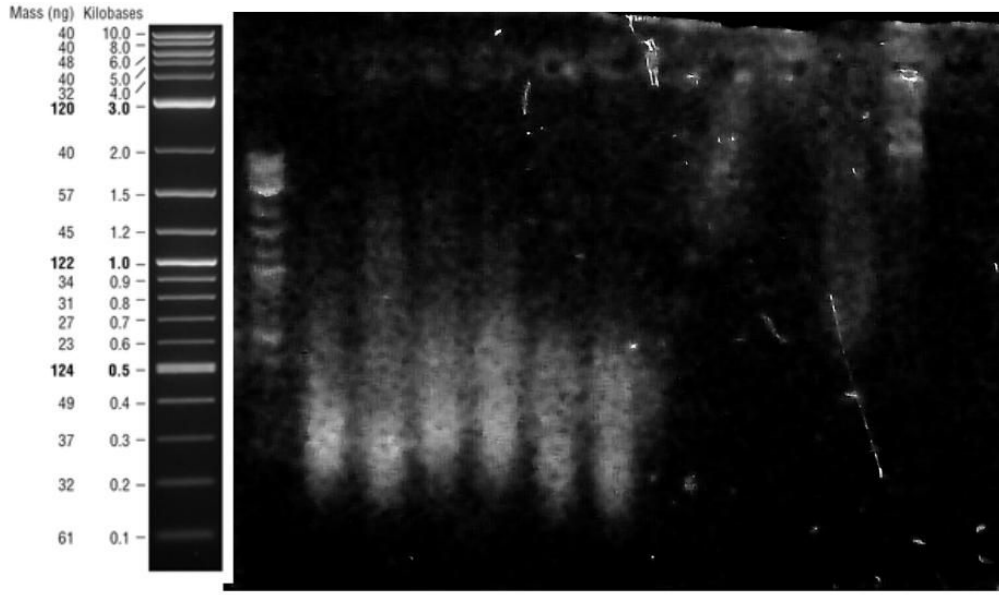
Ladders - lyso1 - lyso2 - lyso3 - lyso4 - lyso5 - lyso5



Analytic digestion of alkaline lysis of pETDUET+lysostaphin
Tiago

- 5 microliters of DNA
- 0.3 microliters EcoRI
- 0,3 microliters PstI
- 2 microliters Buffer H
- H2O to 20

Wells 1 to 6 - pETDUET + lyso 1, 2, 3 ,4 5.1 and 5.2
Wells 8 to 11 - DNA from failed extraction (silica column was extracted by accident with the DNA, probably
Well 12 - Two bands from pSB1C3 + gBlock2.1 undigested (from 09.05, there was a problem with the previous gel.



Scaled plasmid preparation
Brayan

40ul of glycerol stock was used for plasmid recovery of the following backbones:
pSB1C3 + Lysostaphin (Chlo)
pSB1C3 + gBlock1.3 (Chlo)
pJP22 + Lysostaphin (Amp)
pUC (Amp) x2 plates

Furthermore, two 8ml-falcon with pSB1C3 + RFP was prepared for miniprep and pSB1C3 recovery.

LB + Chloramphenicol/Ampicilin preparation
Allan

15 plates for each antibiotic. Our stock was restored with this (In total, we'll have 28~ Ampi and 25~Chlo plates).

----------09/10----------

PCR of pJP22 parts
Viviane

It is new attempt to amplify P1 and R3 of 09/01 to bind to pSB1C3.

Resistance (Ble) primers: 56 and 57
Terminator primers: 58 and 59

Item	Volume for 1 reaction	Volume for 3 reactions
CG buffer	5 uL	15 uL
dNTPs	0.5 uL	1.5 uL
Foward primer	0.5 uL	-
Reverse primer	0.5 uL	-
Homemade polymerase	2 uL	6 uL
Betaine 5M	5 uL	15 uL
H2O	10.5 uL	31.5 uL
Template DNA	1 uL	-
Total	25 uL	75 uL

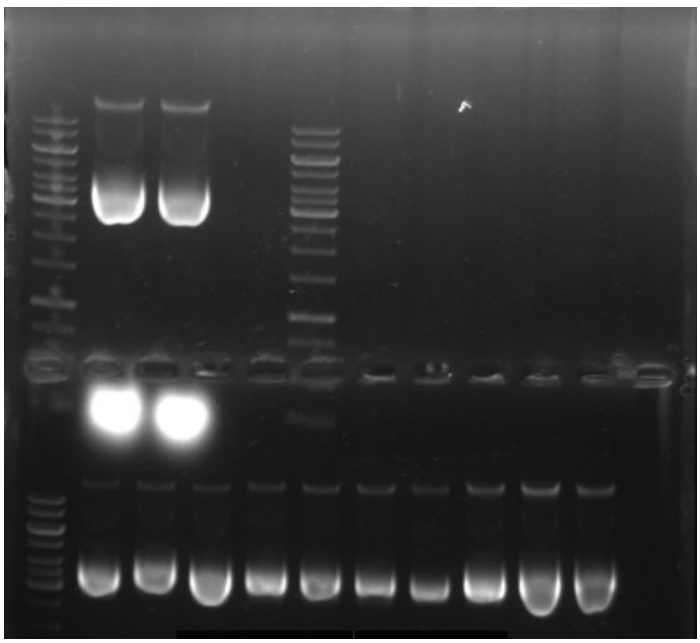
After the mix were prepared, I decided to divide the negative control at 2 tubes with 12.5 uL to test the PCR of promoter again (it failed at 09/01). Then, I added DNA template (pJp22 mCherry => P3) and its primers as described below.

Promoter primers: 53 and 55

Item	Volume for 1 reaction
CG buffer	2.5 uL
dNTPs	0.250 uL
Foward primer	0.250 uL
Reverse primer	0.250 uL
Homemade polymerase	1 uL
Betaine 5M	2.5 uL
H2O	5.25 uL
Template DNA	0.5 uL
Total	12.5 uL

Inoculum for plasmid recovery
Brayan
Growing colonies from yesterday were picked for plasmid recovery.

Alkaline lysis of DH5alfa transformed with pJP22-MaSp1-2X
Mireia

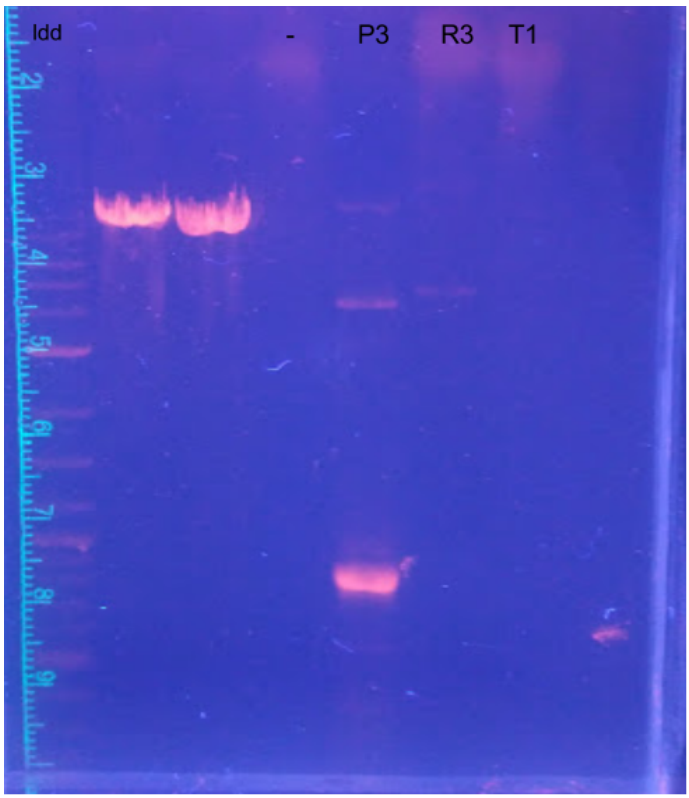


----------09/12----------

Electrophoresis of PCR

Viviane

This PCR was done at 09/10. P3 was amplified, R3 and T1 were not amplified.
Note: I think I put 1 µL of template at P3, not 0.5 µL like I usually do => repeat!



----------09/13----------

Miniprep of previously prepared inocules

João

Plasmid	Concentration (ng/µL)	Purity	230nm
pSB1C3 + Lysostaphin (1)	659,5	1,98	6,344
pSB1C3 + Lysostaphin (2)	702,4	2,00	6,635
pSB1C3 + gBlock 1.3 (1)	571,6	1,95	5,189
pSB1C3 + gBlock 1.3 (2)	690,4	2,03	6,538
pJP22 + Lysostaphin (1)	590,5	1,85	5,5
pJP22 + Lysostaphin (2)	981,2	1,91	9,069
pSB1C3 + RFP (1)	275,3	2,03	2,409

pSB1C3 + RFP (2)	375,4	1,93	3,599
pUC 1	377,1	1,92	3,421
pUC 2	334,2	1,72	3,443

Digestion of plasmids
Allan

Code	Plasmid
1 and 4	pJP22 + Lysosth
2 and 3	pUC
5 and 8	pSB1C3 + Lysosth
6 and 7	pSB1C3 + gB1.3
9 and 10	pSB1C3 + RFP

The plasmids 1 and 4 were digested using Xho/BamHI in CutSmart Buffer. The other plasmids were digested using EcoRI/PstI in 10XH buffer. 1 uL of the plasmids 5,6,7 and 8 and 2 uL of the plasmids 2,3,9,10 were used in the reactions.The reactions were carried out in a thermocycler for 8h at 37ºC, 20 minutes at 80ºC and forever at 4ºC.

PCR of pJP22 parts
Viviane

The ideia here is to obtain the parts in large quantity to bind with pSB1C3. The templates were chosen according to previous PCRs that work. Some of the samples were placed on a different thermocycler to find out if there is some difference.

Item	Volume for 1 reaction	Volume for 20 reactions
CG buffer	2.5 µL	50 µL
dNTPs	0.250 µL	5 µL
Foward primer	0.250 µL	-
Reverse primer	0.250 µL	-
Homemade polymerase	1 µL	20 µL
Betaine 5M	2.5 µL	50 µL
H2O	5.25 µL	105 µL

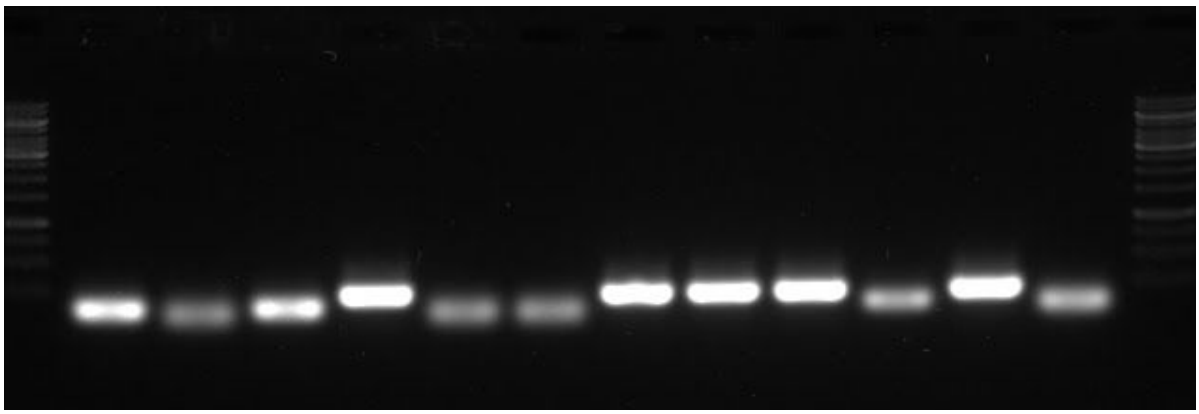
Template DNA	0.5 µL	-
Total	12.5 µL	230 µL

4x P1 (12.5 µL)
4x P3 (12.5 µL)
4x R1 (12.5 µL)
4x R3 (12.5 µL)
4x T1 (12.5 µL)

Inoculum of pJP22 mcherry
Viviane

Glicerol stock was placed in 50mL of LB medium at 37ºC and 250 rpm.

Alkaline lysis PCR of DH5alfa pJP22-MaSp1-2X
Mireia

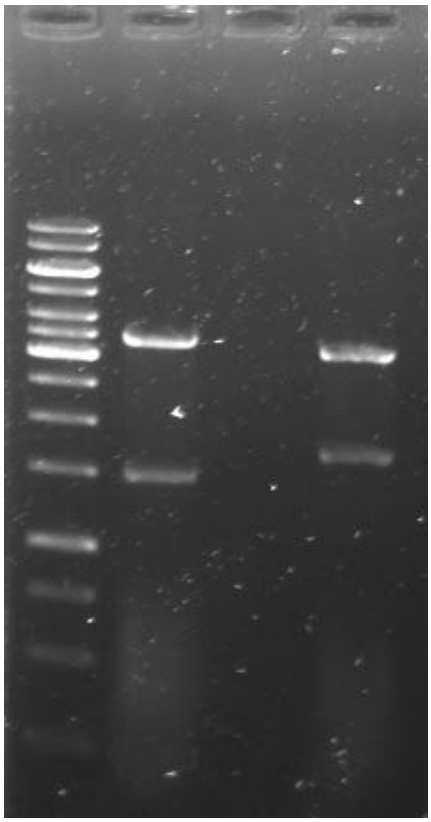


----------09/14----------

Digestion of pJP22-MaSp1-2X with BamHI and ScaI (A) / BglII and ScaI (B)
Mireia

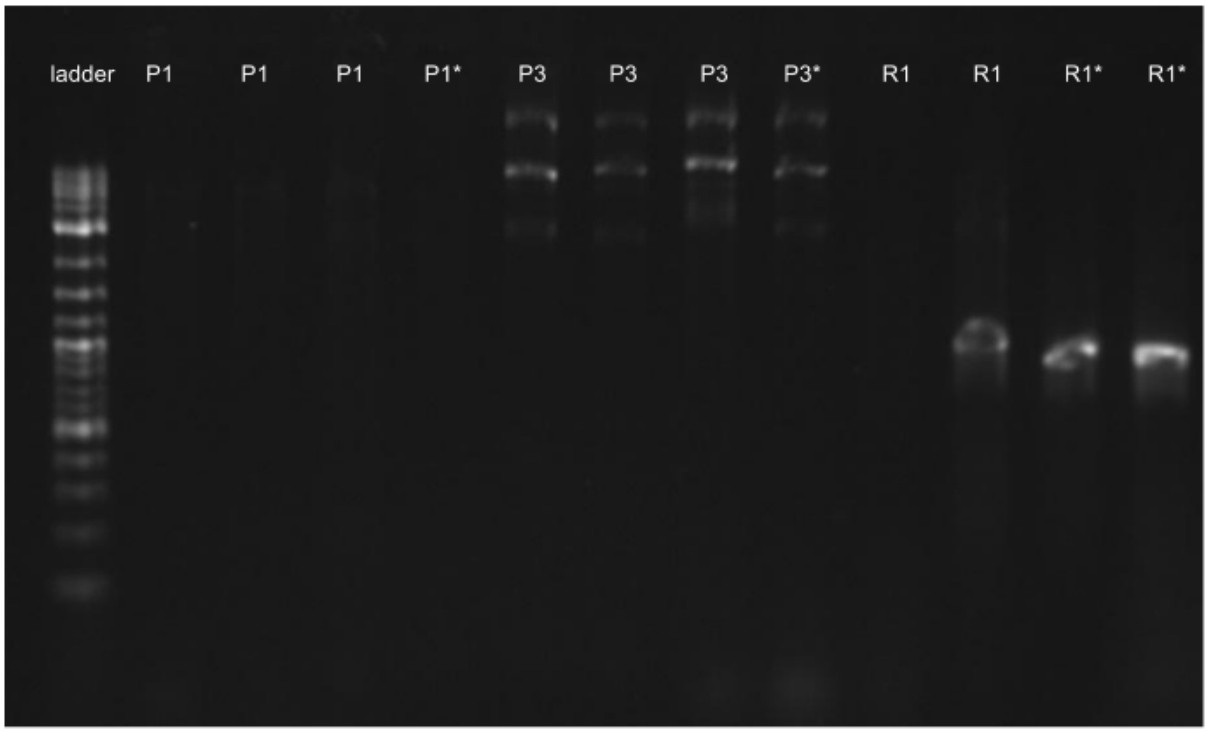
A: **3531 bp** + 1570 bp
B: 3333 bp + **1768 bp**

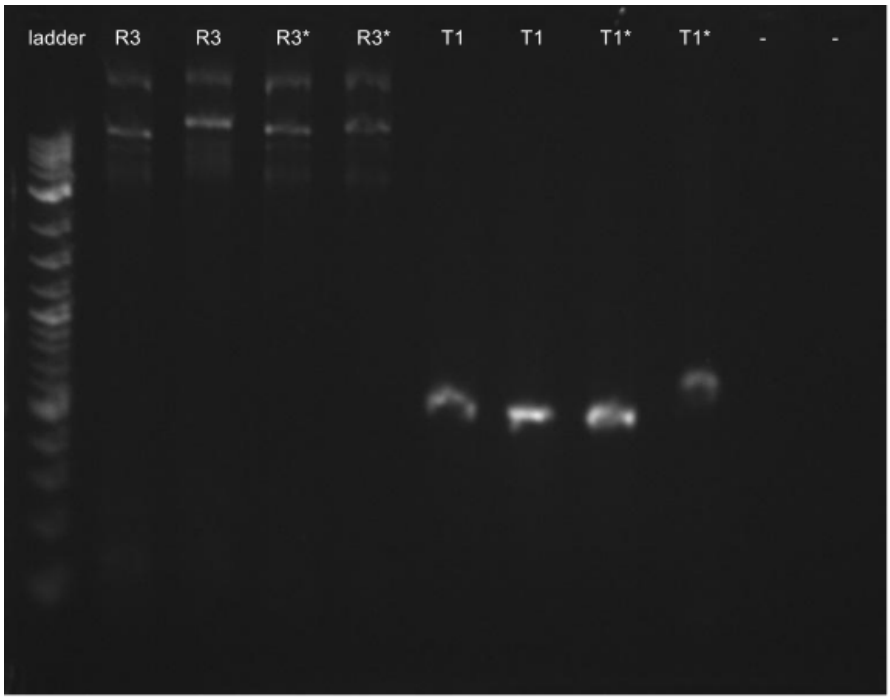
Bold number fragments contain a copy of the MaSp1 insert and are to be ligated to duplicate that insert and regenerate the Amp resistance gene.



Electrophoresis of PCR
Viviane

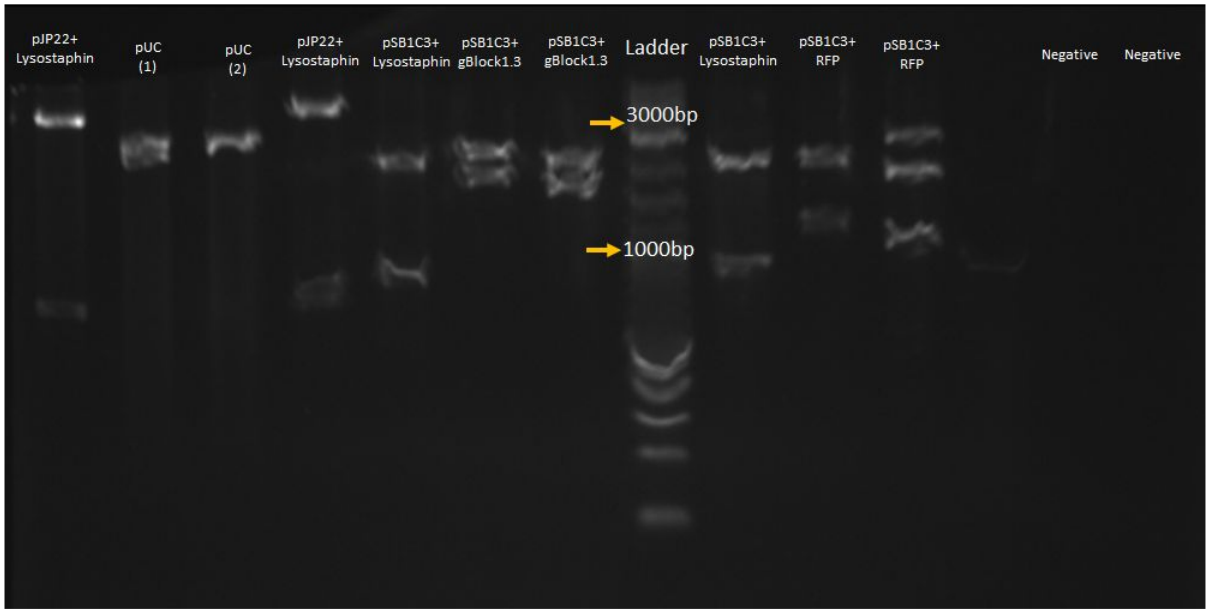
The promoter was not amplified like at 09/12. Resistance and terminator were amplified. I think I forgot to put primer at the first lane of R1.





(*): different thermocycler

Electrophoresis of digested plasmids



Result: All plasmids were successfully digested. It's important to note that pJP22+Lysostaphin is probably not cloned with lysostaphin; rather it seems more like mCherry (700bp ~)

Check this gel made on 09/06 to verify this hypothesis
https://docs.google.com/document/d/1o0yALTXrikHfnB2aBJHc0En-0OF9AnapT0wp_TT7IQE/edit

Note: is it the most beautiful gel you have ever seen?

Inoculation of pSB1C3+RFP
70ml (aprox.) were prepared for pSB1C3 preparation

----------**09/15**----------

Digestion
Viviane and Brayan

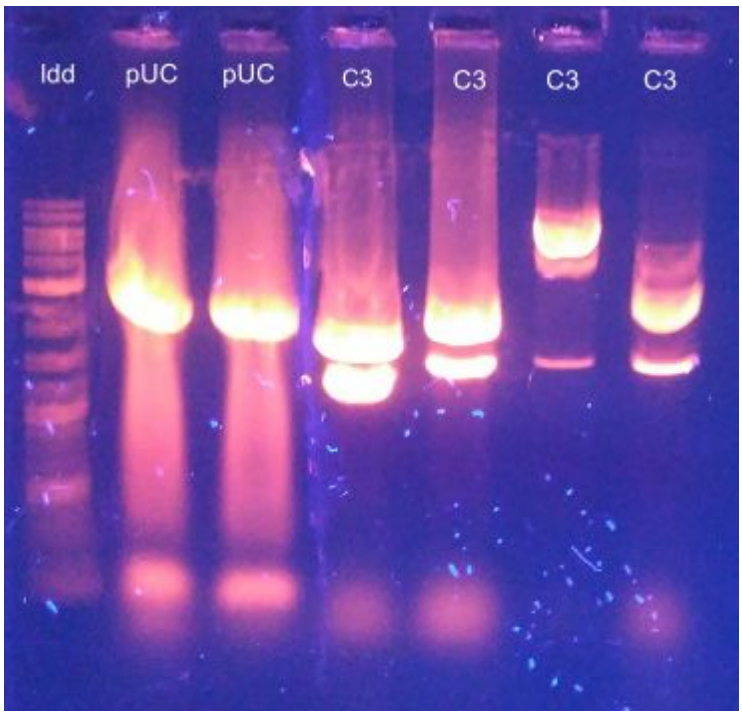
Plasmids were digested to bind with inserts.

1	pUC 1	377 ng/µL
2	pUC 2	334.2 ng/µL
3	pSB1C3	373.6 ng/µL
4	pSB1C3	275.3 ng/µL
5	pSB1C3	150.6 ng/µL
6	pSB1C3	75.9 ng/µL

Item	1, 2, 3, 4	5, 6
EcoRI	1 µL	0,5 µL
PstI	1 µL	0,5 µL
Buffer 2.1 NEB	5.5 µL	5.5 µL
H2O	2.5 µL	2.5 µL
DNA	~45 µL (all of the sample)	~45 ul (all of the sample)
Total	55 µL	54 µL

Electrophoresis
Viviane

pUC (NEB) and C3+RFP were digested. The third lane of C3 is very weird, I didn't get it to purify.



Ligation of pJP22-MaSp1-2X A and B to get pJP22-MaSp1-4X
Mireia

Item	Total
T4 Ligase Buffer	2 uL
pJP22 MaSp 1-2X (A)	10 uL
pJP22 MaSp 1-2X (B)	5 uL
T4 Ligase	1 uL
H_2O	2 uL

----------09/16----------

Gel purification
Viviane

Digested pUC (NEB), digested pSB1C3, PCR of Silwa 1 and PCR of Ea Masp were purified with the new kit of Thermo.

Item	Concentration
pUC	55 ng/µL
pSB1C3	10.4 ng/µL
Silwa 1	4.4 ng/µL
Ea Masp	33.9 ng/µL

Digestion
Viviane

All positive PCR of 09/14 were digested with EcoRI & PstI, it includes R1 and T1. João suggested do not purify PCR before digestion because we got a little quantity of DNA, then I did it. I put enzymes and buffer in the PCR reaction.

EcoRI	0.5 µL
PstI	0.5 µL
Buffer 2.1	1 µL

PCR reaction	~8.5 µL
Total	11.5 µL

Transformation of DH5alfa cells with pJP22-MaSp1-4X
Mireia

The ligation that should create the MaSp1 type2 4-mer was transformed via heat shock into DH5alfa cells.

50 uL of chemocompetent cells (on ice) were pipetted on 2 uL ligation and chilled on ice for 30 min. A heat shock was performed for 1 min at 42°C using a heat block. After that, the cells were chilled on ice for 2 min. 500 uL LB were added and the eppendorf tube was incubated for 1h at 37°C and 150 rpm and finally plated on LB Cb.

No colonies were obtained.

Chemical transformation in E. Coli
Brayan
In order to recover some plasmids, they underwent chemical transformation:

Plasmid	ng/ul	Dilution	Final [pg/ul]	Electroporated volume (ul)
gLUC	100	0,5ul ---> 100ul	500	2
pJP22+LysK 70 ng/ul	7	1,0ul ---> 100ul	70	2
pJP22+LysK 42 ng/ul	42	1,0ul ---> 100ul	420	2
pJP22+MVL	70	0,5ul ---> 100ul	350	2
pJP22+MVL	80	0,5ul ---> 100ul	400	2
BL21 1 (pETDUET+Lysos)	? Too high	0,5ul ---> 100ul	?	1
BL21 3 (pETDUET+Lysos)	? Too high	0,5ul ---> 100ul	?	1

Results: All plates showed colonies. Next day, colonies from gLUC and BL21 1 & 3 (pETDUET+Lysos) were inoculated in 8ml-falcon tubes. Rest of colonies in the other plates were put on a 50-ml erlenmeyer for better recovery. Then, João, miniprepped everything.

----------09/17----------

Slice pJP22
Viviane

The goal of this reaction is to obtain pJP22 with His-tag. First, 25 µL of each primer at 10 µM were placed in a tube, then it was warmed at 98 ºC for 5 minutes. So I mixed it with slice extract, template and H2O. After, I placed the reaction in the thermocycler at 37 ºC for 1 hour. Now, it is necessary to transform cells using this product.

Item	1:5 (vector:insert)	1:10 (vector:insert)
pJP22 digested (28.8 ng/µL)	2 µL	2 µL
Slice extract	1 µL	1 µL
Slice buffer 5X	2 µL	2 µL
Primer forward and reverse (49+50)	0.63 µL	1.26 µL
H2O	4.37 µL	3.74 µL
Total	10 µL	10 µL

PCR of pJP22 parts
Viviane

Its a another attempt of to amplify the promoter. The template is pJP22 digested with BamHI and XhoI, so I named it P3, although, it is not the same tube of 09/12.

Item	Volume for 1 reaction
Q5 master mix 2X (NEB)	12.5 µL
Foward primer	1.25 µL
Reverse primer	1.25 µL
H2O	10 µL
Template DNA	1 µL
Total	25 µL

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 65°C 72°C	10 seconds 30 seconds 5 minutes (ops)
Final Extension	72°C	2 minutes
Hold	4°C	

PCR of pJP22 parts
Viviane and Brayan

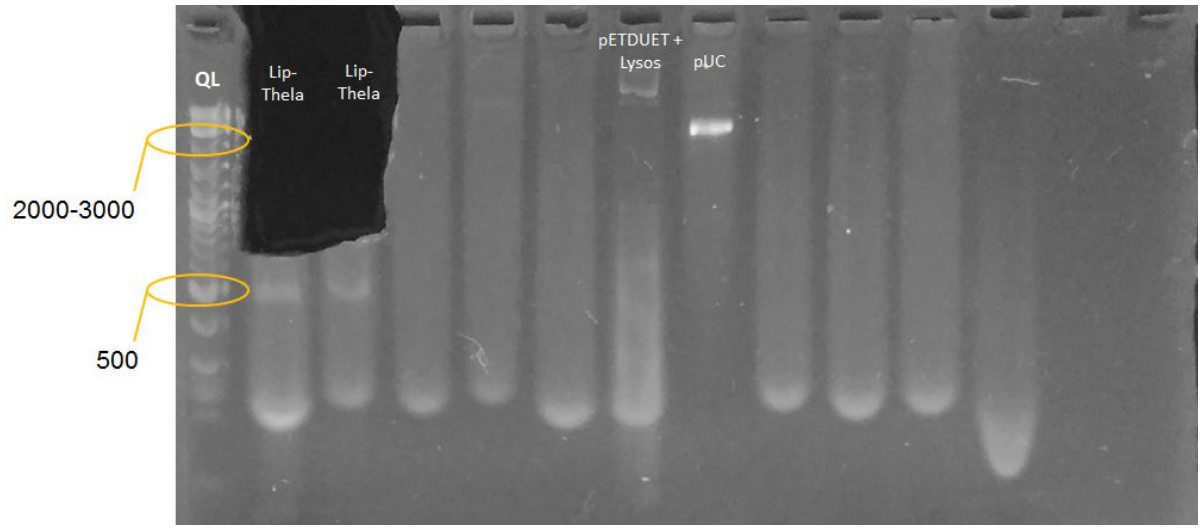
In an attempt to reutilize previous failed PCR reactions (09/14), same tubes were used for a new PCR cycling. It was taken into account the addition of template (0.5 µL) and polymerase (0.5 µL).

Thought: Touch-down cycling for recovering every kind of template and 44°C to guarantee that everything binds to the template.

STEP	TEMP	TIME
Initial Denaturation	98°C	1 min
Touch Down Cycling	From 64°C to 56°C (Annealing temperature)	30 seconds
44°C annealing condition	44°C	30 seconds
22 Cycles	98°C 60°C 72°C	10 seconds 30 seconds 30 seconds
Final Extension	72°C	2 minutes
Hold	4°C	For ever

Gel purification of Lip-thela
Brayan

PCR product of Lip-Thela (800bp) was sliced from the gel. pETDUET+Lysostaphin (isolated by Alkalyne lysis) and pUC were also tested to check presence of plasmids.



Results: pUC is present. pETDUET+Lysos seems positive. Digestion of Lip-Thela gel was sliced and treated with reagents till column purification.

----------09/18----------

Electroporation of *E.Coli* with pJP22-MaSp1-4X

Felipe and Mireia

As no colonies were obtained after the chemical transformation (09/16), we electroporated electrocompetent *E. coli* DH10B with the ligation performed on 09/15 (4-mer). 50 uL of DH10B were pipetted on 2 uL of ligation. A shock of 1800 V was applied using a 1 mm cuvette. The cells were grown in LB media at 37°C and 150 rpm for 1 hour before plating on LBCb.

No colonies were obtained.

Quantification of recovered plasmids from 09/16

João

50-ml erlenmeyer cultures were divided in several tubes to facilitate the miniprep process.

Terminator X (double digested)	13,6 ng/ul	1,78
Resistance X (double digested)	7,5	2,21
pUC 1	314,8	1,97
pUC 2	313,3	1,97
gLUC 3	98,6	1,98
gLUC 4	77,3	2,05

pSB1C3 + Lysostaphin (5)	766,4	1,86
pSB1C3 + Lysostaphin (6)	-	-
pETDUET + Lysos (7)	123,7	1,86
gBlock 2.1 (8)	106,6	2,17
pJP22 + Lysk (9) Seemed like gBlock 2.2	161,3	1,64
pJP22 + Lysk (10)	116,9	1,69
pJP22 + Lysk (11)	101,1	1,67
pJP22 + Lysk (12)	125,1	1,84
pJP22 + Lysk (13)	119,6	1,76
pJP22 + Lysk (14)	127,5	1,61
pJP22 + Lysk (15)	71,8	1,71
pJP22 + Lysk (16)	168,2	1,98
Dilution 1:10 pSB1C3 + Lysostaphin For sequencing	98,2	2,04

Preparation of competent and electrocompetent cells
João

João has prepared 4 batches:
DH5A Electrocompetent + chemocompetent
JM110 Electrocompetent + chemocompetent

These cells were tested for efficiency test to check if they were suitable for further transformations.

----------09/19----------

Digestion of pJP22 mCherry
Viviane

Slice reaction did not perform as expected. So, we decided to digest pJP22 again.
To obtain 1500 ng in 30 µL after recovery (~30%), we started the digestion from ~7000 ng of DNA.

BamHI	1 µL
XhoI	1 µL
Cutsmart	5 µL
H2O	23 µL
DNA	20 µL
Total	50 µL

Electroporation (Transformation efficiency test)
Brayan

Result: Growing DH5Alpha cells in Ampicilin plates shows that our stock could be cross-contaminated.

PCR of the 4-mer ligation (pJP22-MaSp1-4X)
Mireia

To find out whether the ligation worked or not, a PCR was performed following the protocol of PCR1 in which the overhangs were added to the MaSp1 t2 sequence to perform the polymerization technique.

Result: The MaSp1 T2 insert wasn't duplicated in the ligation reaction, so we assumed the 4-mer ligation was not successful.

09/20
Pre-sequencing PCR
Allan

Test was done with Taq Platinum, 1 uM of primers and 0.2 uM of dNTPs
pSB1C3 + gLUC
pSB1C3 + Lysostaphin
pSB1C3 + gBlock 1,3

Item	Volume for 1 reaction
10X buffer	2.5 µL
Platinum Taq	0.1µL
50mM MgSO4	1 µL
dNTPs	0.5 µL
Pf	2.5 µL
Pr	2.5 µL
Template	2 µL (~200 ng)
H2O	13.9 µL
Total	25 µL

STEP	TEMP	TIME
Initial Denaturation	94°C	1 min
35 Cycles	94°C 60°C (53°C for gBlock1,3) 68°C	10 seconds 30 seconds 60 seconds (1:45 for gBlock1,3)
Final Extension	68°C	5 minutes
Hold	4°C	For ever

Cell lysis in 96 PP deep well plates with glass beads - pilot
Tiago

We tried to perform lysis of DH5-alpha cells in PP deep plates using ~0.5g of 0.5-0.9mm glass beads and shaking at 1400rpm for 10 minutes.
However, we were not able to detect any lysis as measured by OD600 reading.
Probably using smaller beads or different incubation conditions could improve the results, but we opted for sonication for the following experiments

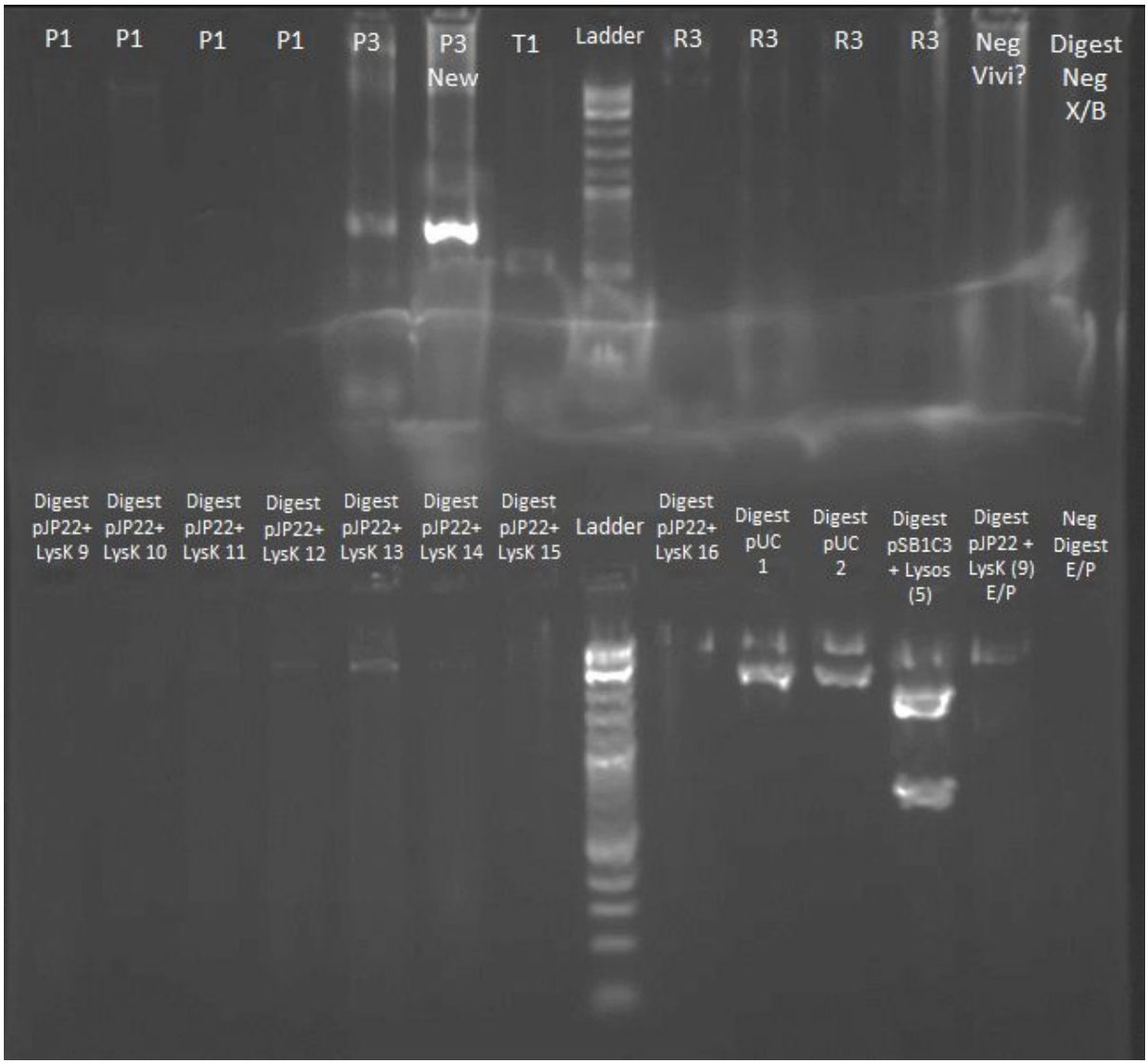
Enzybiotic activity screening - pET-DUET + lysostaphin growing in PP deep well plates
Tiago

92 colonies of BL21 cells transformed with pET-DUET + lysostaphin previously by Mireia were picked individually with toothpicks and deposited in 92 wells in 1 ml of LB medium each. 4 wells were inoculated with DH5-alpha + pUC19 as controls.
The cells were put in a shaker to grow at 37ºC ON.

Gel electrophoresis (Promoter - Resistance - Analytical digestions recovered plasmids from 09/16)
Brayan

Promoter & Resistance PCRs from 09/17 made by Viviane & Analytical digestion (from recovered plasmids from 09/16).

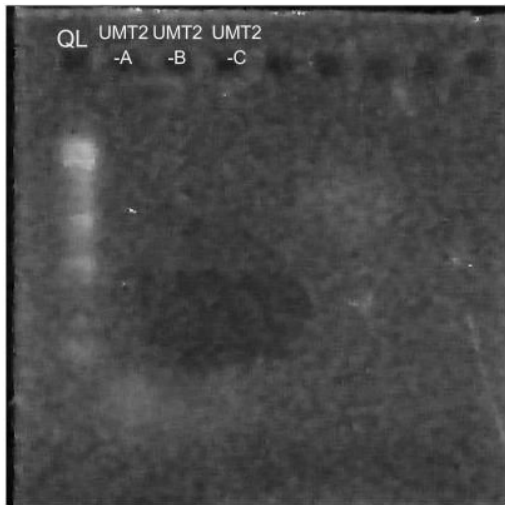
Results: Throw away Lysk pJP22+LysK 11, 14, 15. Rest of plasmids are OK.
pJP22+LysK 9 should be tested for E-P digestion, alongside 10, 11.



USER fusion of MaSp1 type2 monomers - PCR
Tiago

Using primers with uracil (Cheat Sheet, IGP0031 - IGP0034) a 2step PCR was performed with NEB's Q5 High fideity 2x master mix. The template used was 4ng of MaSp1 type2 PCR product per 25 microliter reaction.

USER MaSp1 Type2 A (UMT2-A) - iGP0031 and iGP0034
USER MaSp1 Type2 B (UMT2-B) - iGP0033 and iGP0034
USER MaSp1 Type2 C (UMT2-C) - iGP0032 and iGP0033



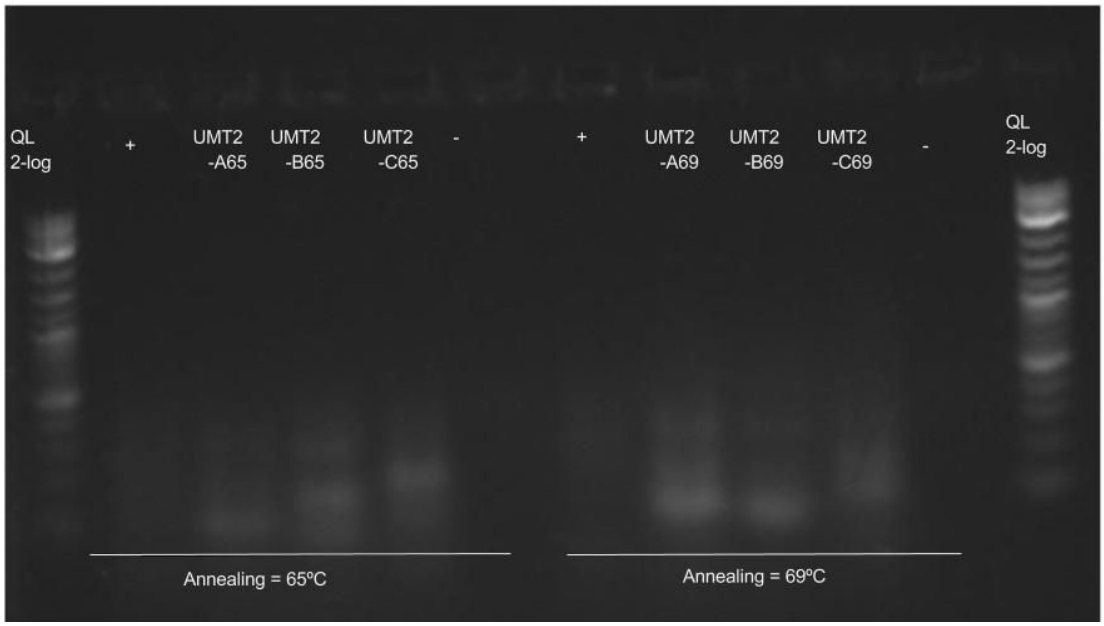
Annealing/extension time was 20 seconds.

OBS: We didn`t realize that NEB does not recommend the use of Q5 polymerase for reactions with uracil containing primers. The PCR will be re-done with the homemade Pfu X7 polymerase.

USER fusion of MaSp1 type2 monomers - PCR
Tiago

Using primers with uracil, X7 home made polymerase and a PCR product as a template

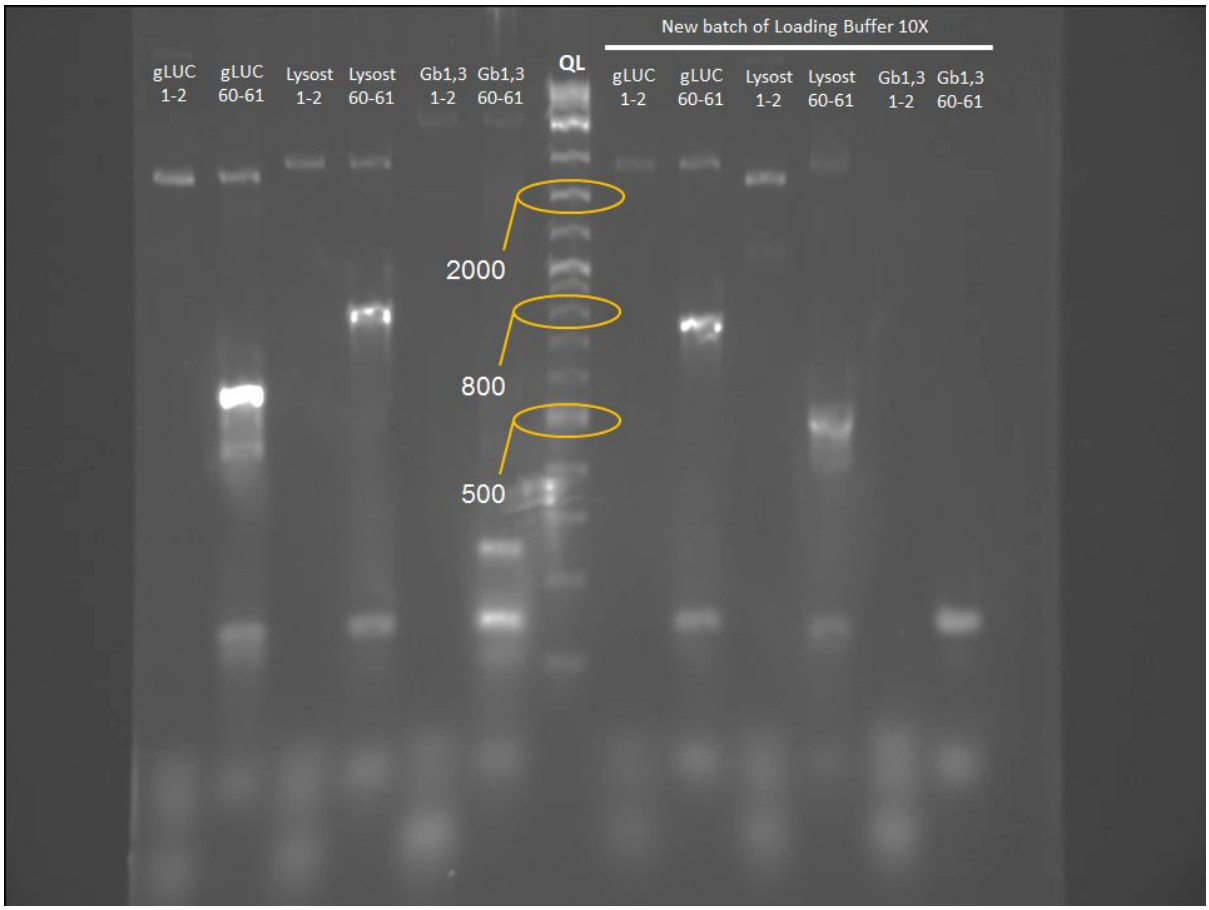
USER MaSp1 Type2 A (UMT2-A) - iGP0031 and iGP0034
USER MaSp1 Type2 B (UMT2-B) - iGP0033 and iGP0034
USER MaSp1 Type2 C (UMT2-C) - iGP0032 and iGP0033



Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume "n" PCR reactions
Buffer GC	5X	X	5	n*5
dNTPs	10 mM	0.5 mM	1.5	n*
Primer forward*	10 uM	0.5 uM	1.25	n*
Primer reverse*	10 uM	0.5 uM	1.25	n*
Betaine	5 M	M	5	n*
Phusion polymerase	100X		2.0	n*
DNA template	-	-	1.0	Each reaction needs the proper sequence
DEPC water			to 25	n*
			25	n*25

Pre-sequencing PCR for pSB1C3 + gLUC & pSB1C3 + Lysostaphin
Brayan

PCR products made on 09/20 by Allan



Expected result: 1-2 will not perform as good as expected, and 60-61 should amplify everything clearly.
Results: iGP0060 and 061 are working. Anyways, another PCR will be performed with new prepared primers pair (iGP0060-061). Seem like a 100-bp contaminant was present (Lane 2, 4, 6, 8, 10, 12, primer aliquot was the contaminant?) but expected PCR products appeared, though.

Digestion of pJP22
Viviane

XbaI	1 µL
KpnI	1 µL
Cutsmart	5 µL
H2O	26 µL
DNA	17 µL
Total	50 µL

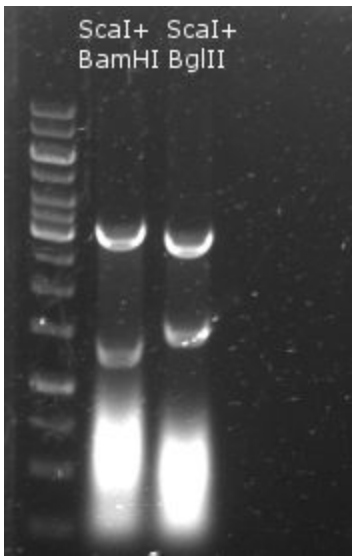
Transformation
Viviane and Brayan

In order to verify if the pSB1C3, pUC and pJP22 double-digested plasmids will perform well in further ligations, transformation was done.
It was a test of digested plasmids for future experiments.
Expected result: No colonies should grow.
Result: No colony grew on the plates.

Sample	When was it digested?
C3.1	09/16
C3.2	08/21
C3.3	08/22
C3.4	08/24
pUC	09/16
pJP22	08/22

Digestion of pJP22-MaSp1-2X with BamHI and ScaI (A)/BglII and ScaI (B) (2nd time)
Mireia and Felipe

As the PRC done on 9/19 showed the previous ligation wasn't successful, we decided to digest the 2X plasmid once again to try another ligation later. Digestion electrophoresis in agarose gel showed bands in the right spots:



OBS: band curviness due to putting too much DNA in the wells, what makes middle DNA travel faster through gel. Ends of the bands travel correctly and should be used for reference.

Ligation of pJP22-MaSp1-2X fragments A and B (2nd time)
Mireia and Felipe

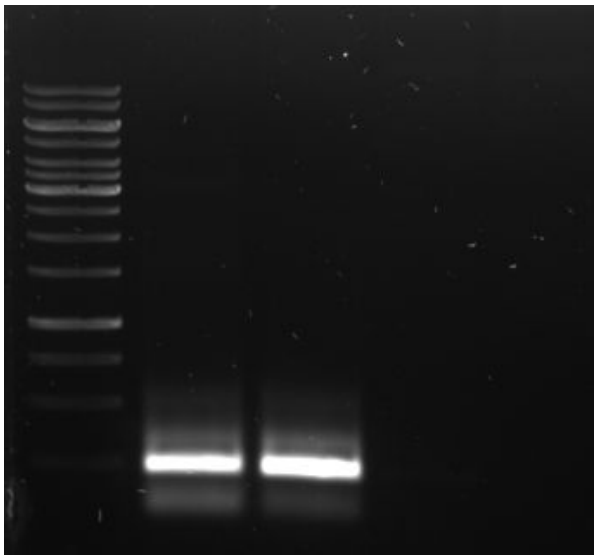
Item	Volume
H_2O	1 uL
A (55,5ng/uL)	4 uL (~222 ng)
B (43ng/uL)	3 uL (~132 ng)
T4 Buffer 10X	1 uL
Ligase	1 uL
Total	10 uL

Reaction conditions:

	TEMP	TIME
30X	22°C	3 min
	37°C	3 min
	4°C	hold

----------09/21----------

PCR of MaSp1 4-mer ligation (2nd time)
Mireia



Result: 2-mer amplicon band.
Hypothesis: The primers can attach at the ends of the 4mer but also at the center, so probably we are obtaining only the 2mer amplicon. All primers attach and the polymerase is not able to amplify through the whole 4mer, because it finds an attached primer on its way.
So: transform and check for colony growth, miniprep and digest for 4mer confirmation.

Transformation of DH10B with the 2nd 4-mer ligation
Mireia

Electroporation of 50 uL DH10B with 2 uL ligation. (1 mm cuvette, 1800 V)
Result: 5 colonies grew on the LBCb plate.

----------09/22----------

Alkaline lysis of 5 MaSp1t2 4mer colonies
Mireia

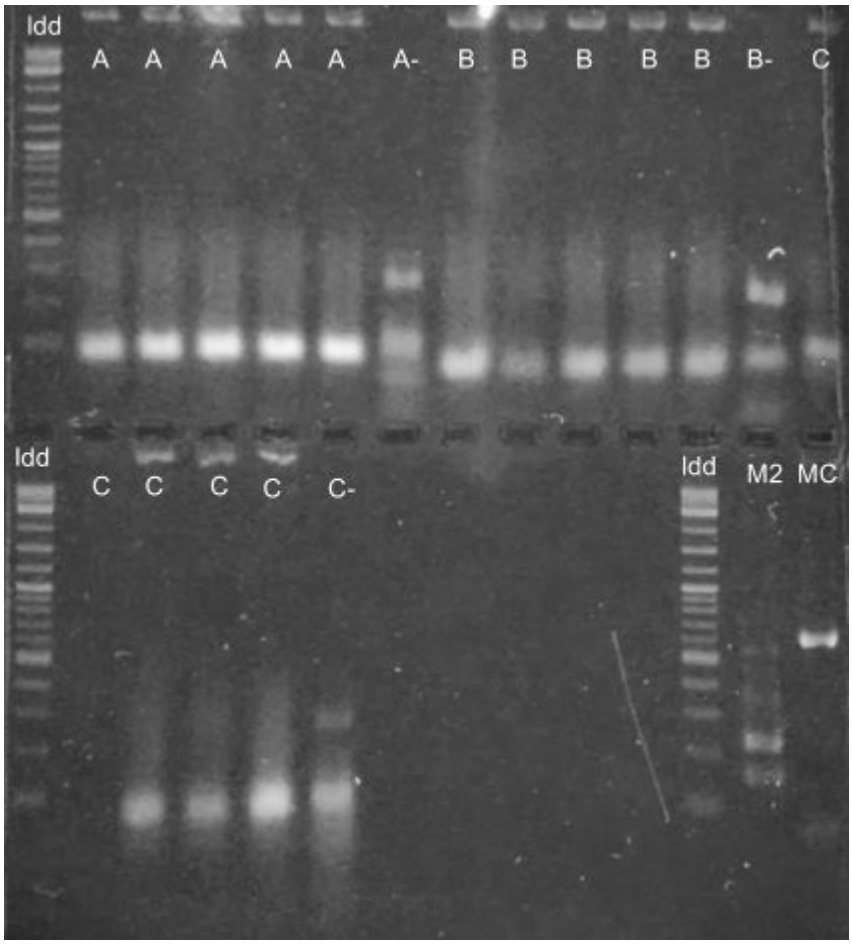
USER fusion of MaSp1 type2 monomers - Gradient PCR
Tiago

As we were not able to amplify it sucessfully, we tried again the USER PCR with the primers with uracil, X7 home made polymerase and, now, a Alkaline lysis of pJP22 + MaSp1 type2 cells as a template

USER MaSp1 Type2 A (UMT2-A) - iGP0031 and iGP0034
USER MaSp1 Type2 B (UMT2-B) - iGP0033 and iGP0034
USER MaSp1 Type2 C (UMT2-C) - iGP0032 and iGP0033

Temperatures for the gradient were 59, 60, 61, 63, 66 and the negative control was run at 66.
The negative control showed a band, while all the others didn't. Perhaps this was due to some kind of contamination and excess template in the reaction

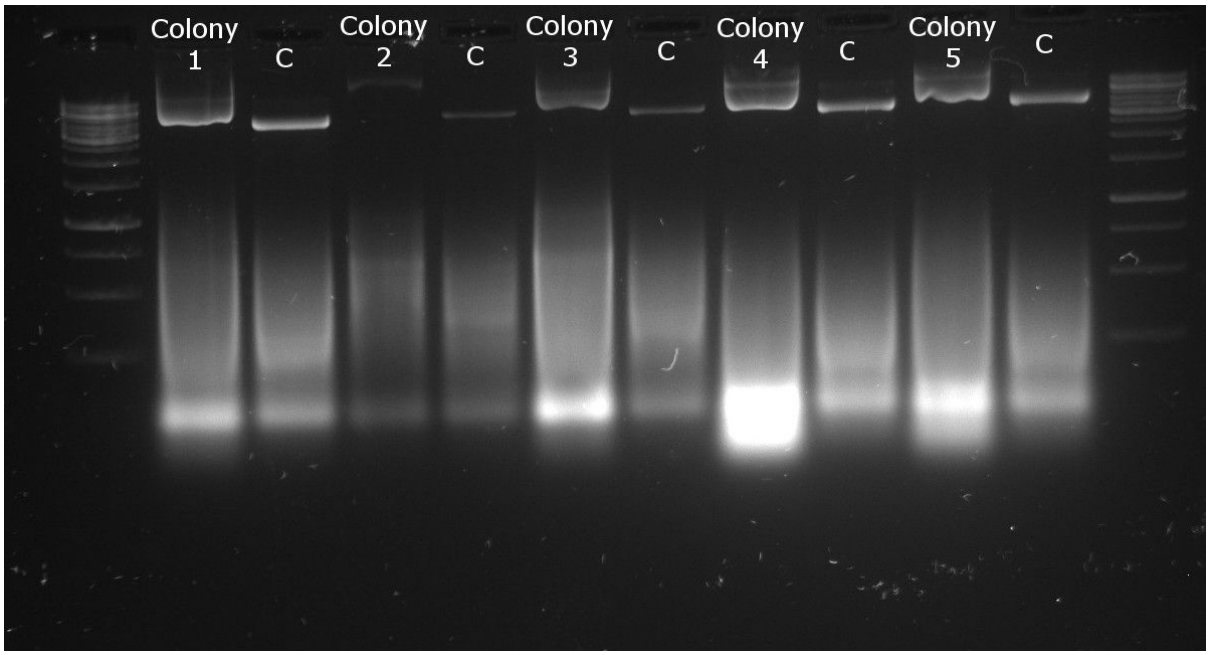
Item	Initial concentration	Final concentration	Initial volume (ul) 1 PCR reaction	Final volume "n" PCR reactions
Buffer GC	5X	X	5	n*5
dNTPs	10 mM	0.5 mM	1.5	n*
Primer forward*	10 uM	0.5 uM	1.25	n*
Primer reverse*	10 uM	0.5 uM	1.25	n*
Betaine	5 M	M	5	n*
Phusion polymerase	100X		2.0	n*
DNA template	-	-	1.0	Each reaction needs the proper sequence
DEPC water			to 25	n*
			25	n*25



----------09/23----------

Digestion of pJP22-4mer with XhoI and BamHI

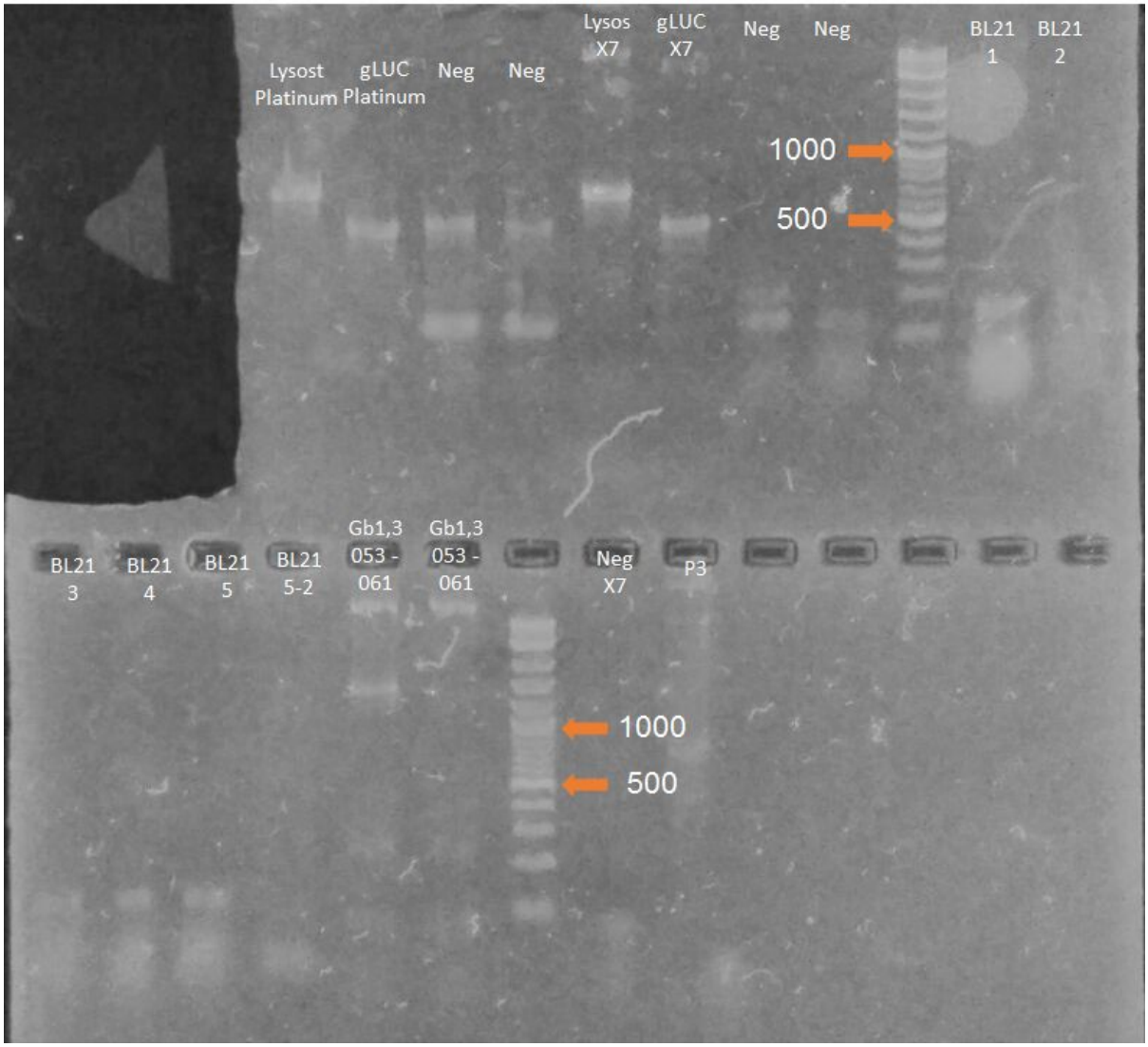
Mireia and Felipe



Top bands represent the backbone of the plasmid. Insert bands are mixed with the RNA drag due to being 200~400 bases long. We will repeat the digestion with other enzymes to better separate the elements. C= cut with XhoI and BamHI.

Repeated PCR of Lysos + gLUC
New primers were prepared (iGP0061 - 0062) with fresh DEPC Water.

Conditions were the same as the used **by Allan on 09/20**
1uM Primers; Taq Polymerase; 200ng~ of template.



Expected result: Get rid of any kind of contaminant. It seems like using Platinum facilitates the production of unspecific products or primer-dimer. Meanwhile, using X7 polymerase demonstrate that our reaction was well prepared.

Result: We are finally ready for sequencing. BL21 plasmids does not contain any kind of insert. gBlock1.3 was finally amplified with different primers (iGP0053 - 0061) but those do not really help for cloning. P3 sequence also appeared (Last lane). It was saved on the **“PCR purification products” box**

Ligation of double-digested Terminator sequence made by Vivi on 09-16
Brayan

	pSB1C3	pUC
Buffer 10X	1	0,5
T4	0,5	0,5
Vector	5 uL	1 uL
Terminator sequence	2,7 uL	2,1 uL
H2O	0,8	5,4
Total	10 uL	10 uL

Step 1	22 °C	10 min	
Step 2	16 °C	10 min	Repeat 5 times from Step 1
Step 3	4 °C	30 s	
Step 4	27 °C	30 s	
Step 5	13 °C	30 s	Repeat 50 times from Step 3
Step 6	4 °C	30 s	
Step 7	16 °C	1h	Repeat 2 times from Step 1
Step 8	22 °C	1h	
Step 9	18 °C	3h	
Step 10	4 °C	Infinite	

OBS: This product was not used for further transformations (yet!).

PCR of pJP22 parts
Viviane

PCR of promoter (P3) using Q5 performed as expected at 09/17, so I repeated it once again and I did it for resistance gene too. Now, the pcr of resistance gene was done with gBlock 1

as template (Rgb1) because it does not have an undesirable restriction site like pJP22.

Item	Volume for 5 reactions of each one
Q5 master mix 2X (NEB)	62.5 µL
Foward primer	6.25 µL
Reverse primer	6.25 µL
H2O	50 µL
Template DNA	-
Total	125 µL

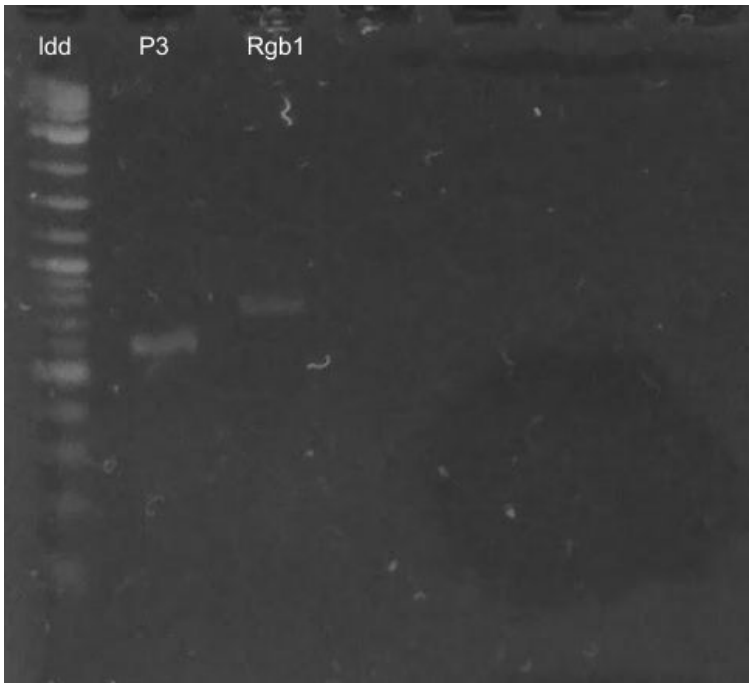
5x P3 (25 µL)
5x Rgb1 (25 µL)

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 65°C 72°C	10 seconds 30 seconds 30 seconds
Final Extension	72°C	2 minutes
Hold	4°C	

----------09/24----------

Electrophoresis
Viviane

Pcr of promoter and resistance sequences using Q5 was confirmed.



Gel purification
Viviane

pJP22 digested with XbaI and KpnI was purified using kit of Thermo, concentration at 26.5 ng/µL.

PCR purification
Viviane

Promoter and resistance sequences were purified.

Sample	Concentration
P3	274.9 ng/µL
Rgb1	181.7 ng/µL

Alkaline lysis
Viviane

pSB1C3 with type 2 was extracted. Nanodrop did not quantify it.
A new wash step might solve this. Tube is still stored in a rack

----------09/25----------

Plates preparation: LB+Ampi - LB+Chlo
João and Brayan

6L of LB was prepared. It rendered about 70 plates for each LB+antibiotic (Ampicilin or Chloramphenicol). Our stock should survive for two or three weeks more.

----------09/26----------

Digestion

Brayan

Two parts of our device were digested for further ligation on C3 and pUC. Two additional plasmids were tested for final confirmation.

1. P3
2. Rgb1
3. pJP22 + LysK (9)
4. pJP22 + LysK (10)

Item	1, 2	3, 4
EcoRI	1 µL	0.5 µL (Thermo enzyme)
PstI	1 µL	0.5 µL (Thermo enzyme)
Buffer 2.1	2.9 µL	1.0 µL Buffer H (Thermo)
H2O	0	3 µL
DNA	24~25 µL	5 µl
Total	29.9 µL	10 µl

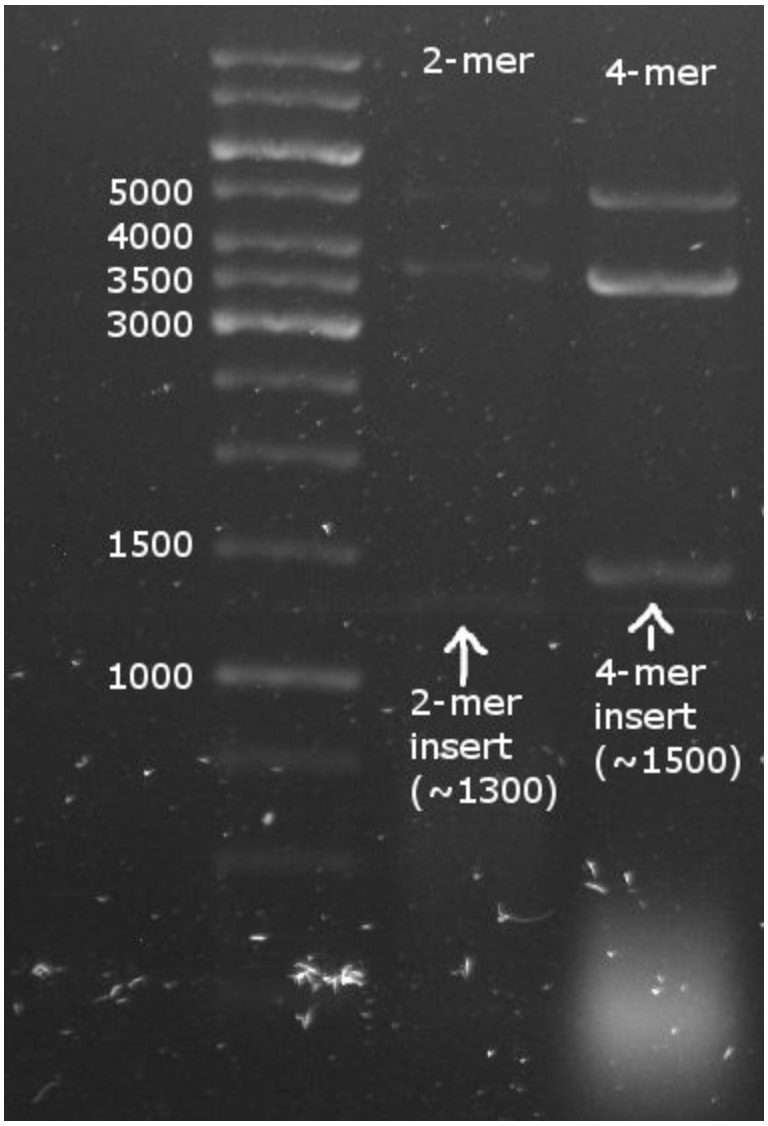
Digestion of pJP22 MaSp1t2 4mer with PstI and KpnI for 4mer confirmation

Felipe and Mireia

As we couldn't see the insert bands due to RNA on the XhoI/BamHI digestion, we decided to try again with PstI/KpnI because that would form more discernable bands, at ~1300 (2mer) and ~1500 (4mer)

Item	Volume for 1 reaction	Volume for 7 reactions
PstI NdeI	1uL	7 uL(used 5)
KpnI	1uL	7 uL(used 5)

H_2O	11uL	77 uL(used 77+4)
Buffer	2uL	14 uL
Total	15uL	105 uL



Result: A ~1500 band showed we have the 4mer insert.

----------**09/27**----------

Ligation

Viviane

The values were based on the molecular weight of plasmids and inserts, so that their molecular proportion was 1:3.

Item	pSB1C3			pUC19		
	P3	Rgb1	T1	P3	Rgb1	T1
Vector (~50ng)	1.72 µL	1.72 µL	1.72 µL	1.1 µL	1.1 µL	1.1 µL
Insert	0.28 µL	0.20 µL	2.67 µL	0.22 µL	0.16 µL	2.05 µL
Buffer	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL
T4 ligase	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL
H2O	6 µL	6.08 µL	3.61 1 µL	6.68 1 µL	6.74 µL	4.85 µL
Total	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL

Note: quantity of promoter and terminator were changed when I did, but it must not to be a problem.

Competent cells + transformation
Brayan

On-day electrocompetent cells were prepared in order to defy our standard method. This new method is based on http://www.nature.com/articles/srep24648. Briefly, it highlights the advantage of preparing your electrocompetents cell on the same day you gonna use and using room temperature (avoiding the fact that everything should be kept on ice or cold).

8 tubes with 60ul of cells were prepared

Viviane's ligations from the same day (**09/27**) were used for this test.
Selected plasmids:
C3+P3
C3+Rgb1
C3+T1
pUC+P3
pUC+Rgb1
pUC+T1
10pg/uL of C3 from transformation efficiency test (iGEM)

Additional plasmids
pJP22+Type 2

----------09/28----------

Inoculum
Viviane

Some plates of Brayan have colonies.

pUC+P3
pUC+Rgb1
pUC+T1
pSB1C3+P3
pSB1C3+T1

Pcr of pJP22+Type 2/ parts of USER/ Masp 2 of distribution kit (2-7F)
Viviane

Primers of mCherry and Masp2 arrived today, so I tried to amplify them.

Item	Volume for 1 reaction
Q5 master mix 2X (NEB)	12.5 µL
Foward primer	1.25 µL
Reverse primer	1.25 µL
H2O	10 µL
Template DNA	0.5 µL
Total	25.5 µL

Plasmid recovery
Brayan

Aim: Produce more pSB1C3 digested with XhoI and BamHI and with EcoRI and PstI to have enough material for future experiments

Expected: “mom” glycerol stock came from a positive ligation done before 24/08. This means that a successful ligation product was used for transformation. If we are supposed to use “successful” ligations for our experiments to clone into pSB1C3, maybe using from 07/07 or from 08/20. Perhaps the second one was better.
pSB1C3+Lysostaphin (24/08) “mom”

pSB1C3+Lysostaphin (07/09) “daught”
pJP22+mCherry (14/09)
pJP22+mCherry (22/09)

Result: Everything grew.

Ligation
João

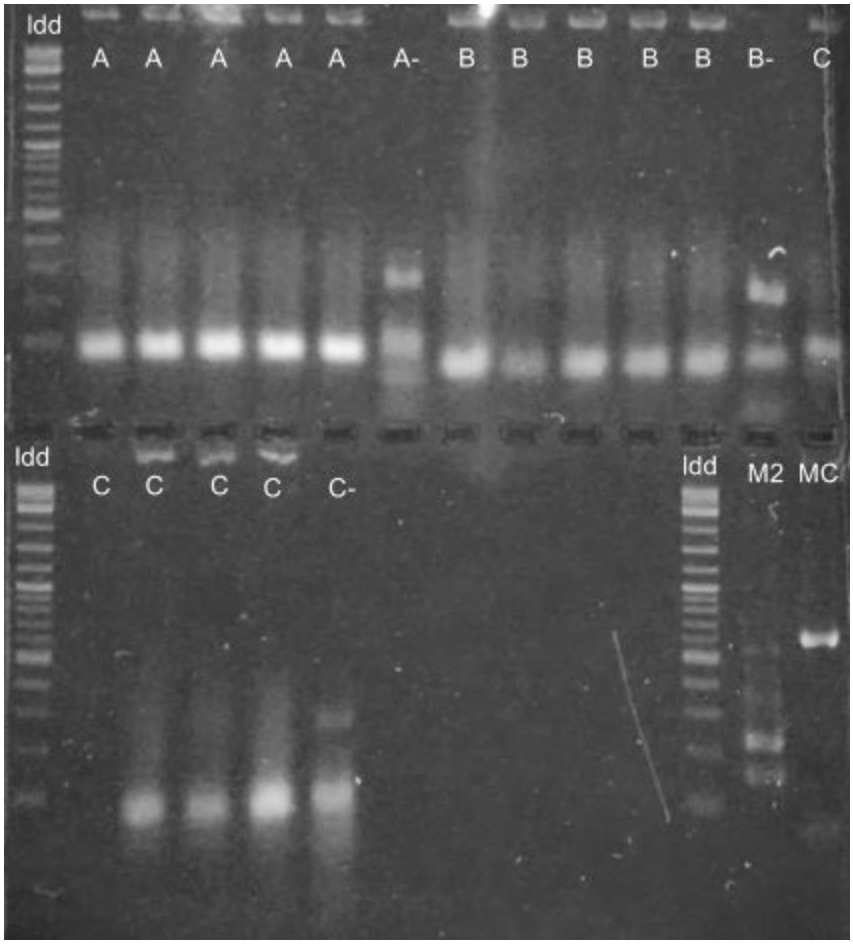
----------09/29----------

Slice
João

Item	1:5 (vector:insert)
pJP22 digested (28.8 ng/µL)	2 µL
Slice extract	1 µL
Slice buffer 5X	2 µL
Primer forward and reverse (49+50)	0.63 µL
H2O	4.37 µL
Total	10 µL

Electrophoresis of USER A, B and C parts PCR
Viviane

MC: mCherry
M2: Masp 2



Result: Time to produce more MaSp2 and to start 3-assembly approach to polymerize with BgIII and SacI. mCherry will also be produced for direct digestion.

USER fusion of MaSp1 type2 monomers

The A, B and C parts of the USER fusion PCR were incubated in a thermocycler as follows:
1 microliter of USER enzyme (NEB)
1 microliter of USER cassete (from Stephen Mayfield's lab at UCSD)
1.5 microliters of UMT2-A65 PCR product (not purified)
1.5 microliters of UMT2-C65 PCR product (not purified)
5 microliters of UMT2-B65 PCR product (not purified)

15 minutes 37ºC
5 minutes 25ºC

All the volume was used to transfec on-day electrocompetent cells.

Competent cells on day and transformation
João and Viviane

pSB1C3 + selected Biobricks (#56 to #68)
pJP22 + selected Biobricks (#56 to #68)

USER fusion of MaSp1 type2 monomers - Ethanol precipitation dilution as template
Tiago

As we were not able to amplify it sucessfully, we tried onceagain the USER PCR with the primers with uracil, X7 home made polymerase and, now, a alkaline lysis and ethanol precipitation of pJP22 + MaSp1 type2 cells as a template BUT diluted 100 times.

USER MaSp1 Type2 A (UMT2-A) - iGP0031 and iGP0034
USER MaSp1 Type2 B (UMT2-B) - iGP0033 and iGP0034
USER MaSp1 Type2 C (UMT2-C) - iGP0032 and iGP0033

The temperature used for the annealing was 65.

Item	Initial concentration	Final concentration	Initial volume (ul)	Final volume
			1 PCR reaction	"n" PCR reactions
Buffer GC	5X	X	5	n*5
dNTPs	10 mM	0.5 mM	1.5	n*
Primer forward*	10 uM	0.5 uM	1.25	n*
Primer reverse*	10 uM	0.5 uM	1.25	n*
Betaine	5 M	M	5	n*
Phusion polymerase	100X		2.0	n*
DNA template	-	-	1.0	Each reaction needs the proper sequence
DEPC water			to 25	n*
			25	n*25

Digestion of pJP22 parts
Brayan

Every plasmid was quantified (see below) and was used for digestion in order to confirm insert presence.

Plasmid	Concentration	260nm-280nm ratio
pUC + P3 1	181,3	1,95
pUC + P3 2	144,3	1,89
pUC + P3 3	24,8	1,82
pUC + T1 1	33,3	1,82
pUC + T1 2	72,4	1,94
pUC + T1 3	37,8	1,89
pUC + Rgb1 1	78,4	2,01
pUC + Rgb1 2	89,2	1,94
pUC + Rgb1 3	22,5	1,91
C3+P3 1	22,9	1,95
C3+P3 2	25,0	2,14
C3+P3 1	12,8	1,83
C3+T1 1	24,8	2,00
C3+T1 2	14,2	2,08
C3+T1 3	33,8	2,03

Item	1Rx	10Rx
Buffer 2.1	1	10ul
Eco	1.0	10ul
Pst	1.0	10ul

DNA	50 (all reaction)	-
Total	60ul	600ul

----------09/30----------

Gel electrophoresis of digested plasmids
Brayan

pUC + P31	pUC + P32	pUC + P33	pUC + T11	pUC + T12	pUC + T13	pUC + R1	pUC + R 2	Leakage	1kb plus	pUC + R 3	Negative	C3+ P31	C3+ P32	C3 + P33
C3 + T11	C3+ T1 2	C3+ T1 3	1kb plus	mCh 1	mCh 2	mCh 3	mCh 4							

Result: Seems like one-side digestion was achieved, regardless the fact that it was supposed to be a double digestion. mCherry is ok.

Proceed to: Confirm by PCR.

Transformation (Chlamydomonas)
Brayan

Main goal: Transform and obtain *C. reinhardtii* expressing mCherry.
One of objectives of this experiment was to test the usefulness of Saphire buffer or water as resuspension buffer for transformation
With cc1010 strain
pJP22 (3) + mCherry with Saphire buffer
pJP30 (6) + mCherry with Saphire buffer
pJP30 (6) + mCherry with Water
Negative with Saphire buffer
Negative with Water

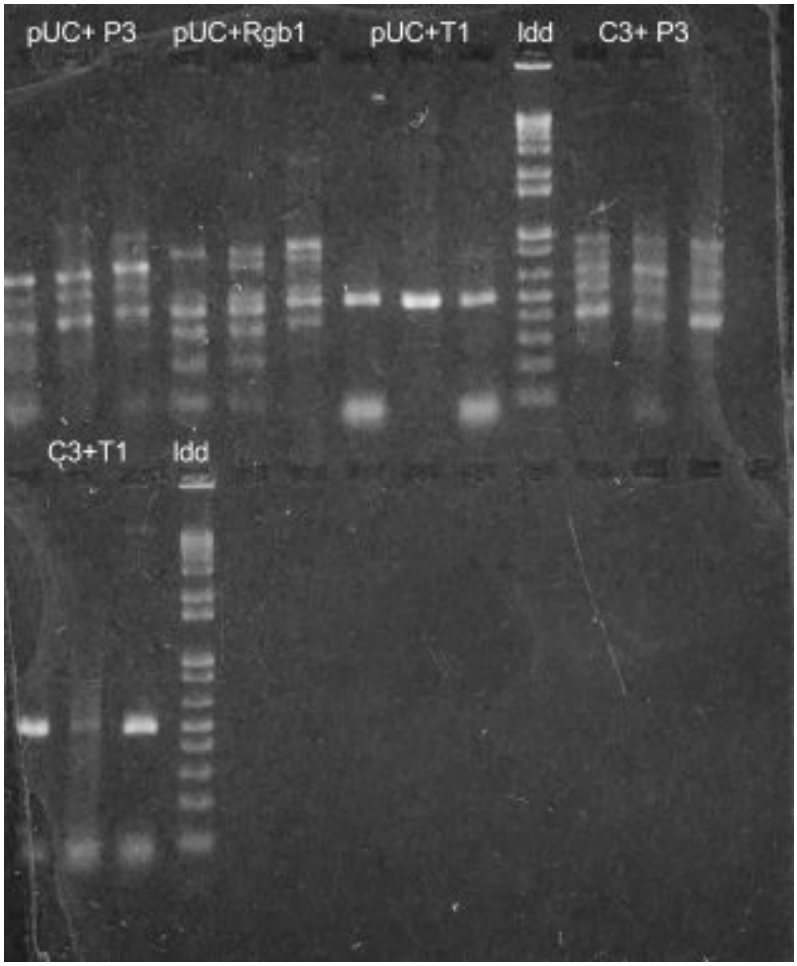
With cc1690 strain
pJP22 (3) + mCherry with Saphire buffer
Negative with Saphire buffer
Negative with Water

Competent cells on day and transformation (E. Coli)
João

PCR of pJP22 plasmids coming from 09/27
Viviane

Digestion seemed inconsistent to confirm presence of device parts, so I prepared a PCR reaction with rest of plasmids that were left after almost-total digestion

Item	Volume for 1 reaction
Q5 master mix 2X (NEB)	6.25 µL
Foward primer	0.625 µL
Reverse primer	0.625 µL
Template DNA + H20	5 µL
Total	12.5 µL



Digestion
Brayan

Pcr products of mCherry were digested to bind with pSB1C3 and pUC.

PCR purification
Viviane

After digestion, PCR products of mCherry were column purified.
Quantification: around 100 ng/ul

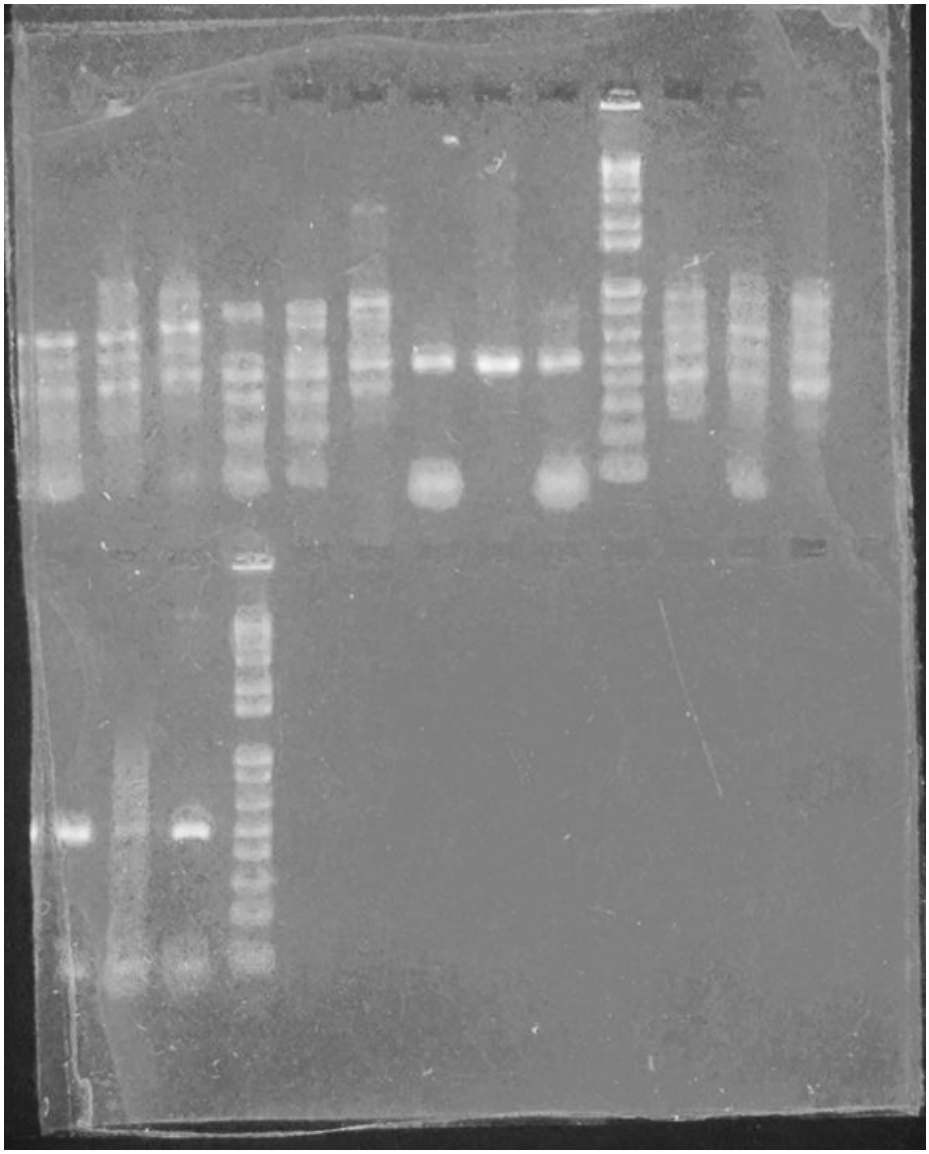
Ligation
Viviane

Item	pUC19	pSB1C3
	mcherry	

Vector (~50ng)	1 µL	5 µL
Insert	0.4 µL	0.5 µL
Buffer	1 µL	1 µL
T4 ligase	0.5 µL	0.5 µL
H2O	7.1 µL	3 µL
Total	10 µL	10 µL

10/02
Gel confirmation of pJP22 parts PCR
Brayan

pUC +P3 1	pUC +P3 2	pUC +P3 3	pUC+ Rgb1 1	pUC + Rgb 1 2	pUC + Rgb 1 3	pUC + T1 1	pUC + T1 2	pUC + T1 3	1kb ladder plus	C3+ P3 1	C3+ P3 2	C3+ P3 3
C3+ T1 1	C3+ T1 2	C3+ T1 3	1kb ladder plus									



Colony PCR
Brayan
Growing colonies from **09/30 Transformation made by João & Vivi**

It was used Primers iGP0060 - iGP0061
All PCR tubes were named with alphanumeric code (A1, A2, A3 and so on…)

A: pSB1C3 + Lysostaphin	2 reactions with Q5 pol.	----	5 reactions with X7 Polymerase
B: pSB1C3 + LysK	2 reactions with Q5 pol.	----	8 reactions with X7 Polymerase
C: pSB1C3 + MV-L	2 reactions with Q5 pol.	----	8 reactions with X7 Polymerase
D: pSB1C3 + B-gal	2 reactions with Q5 pol.	----	8 reactions with X7 Polymerase
E: pSB1C3 + gLUC	2 reactions with Q5 pol.	----	3 reactions with X7 Polymerase
F: pSB1C3 + Lip-thela	2 reactions with Q5 pol.	----	8 reactions with X7 Polymerase

G: pSB1C3 + Ea 2 reactions with Q5 pol. ---- 8 reactions with X7 Polymerase
H: pSB1C3 + S1 2 reactions with Q5 pol. ---- 8 reactions with X7 Polymerase
I: pSB1C3 + gb2 1 reaction with X7 Polymerase
J: pSB1C3 + - 2 reactions with Q5 pol. ---- 8 reactions with X7 Polymerase
Positive PCR colony controls:
pSB1C3 + Lysostaphin (3 reactions)
pSB1C3 + RFP (2 reactions)
Positive isolated plasmids controls
pSB1C3 + Lysostaphin 700ng/ul
pSB1C3 + Lysostaphin 100ng/ul
Some random tests
pJP22 + Type 2 (Primers for pJP22 MCS iGP0064 - 65)
pJP22 + mCherry (Primers for pJP22 iGP0066 - 67)

Traditional PCR conditions for Q5 polymerase
60°C annealing temperature

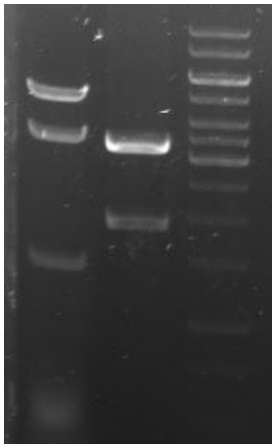
Expected result: If insert was cloned correctly, everything but gBlock2 & MV-L should amplify (remember that PCR for gBlock2 was not well standardized). Therefore, do not desperate! Do analytical digestion on this growing inoculum.
OBS: As Joao said, it's possible that I and J plate were changed during plating or transformation. 'Cuz "I plate" (C3+gb2) did not have any colony (I just picked up something that seemed as a colony, but i'm not sure). So it seems that "I plate" was actually our negative control.
Priority to run on gel electrophoresis:
Tubes named as "1 and 2" 'cuz both were inoculated in 8-ml culture. If some of them contents insert, go ahead with miniprep and forget the rest of PCR tubes.

Digestion of pJP22-MaSp1-4X with BamHI and ScaI (A) / BglII and ScaI (B)
Mireia

A: BamHI + ScaI (Fast Digest Thermo) 1h ~37ºC
B: BglII + ScaI (Pharmacia) overnight ~37ºC

Result:
A: **3759** and 1570 (Not completely cut fter 1h at ~37ºC, after the gel it was left for 30 min at 37ºC)
B: 3333 and **1966**

bold = contains the 4X MaSp1t2 insert.
5 uL restriction check. 45 uL were run afterwards to purify the bold fragments:

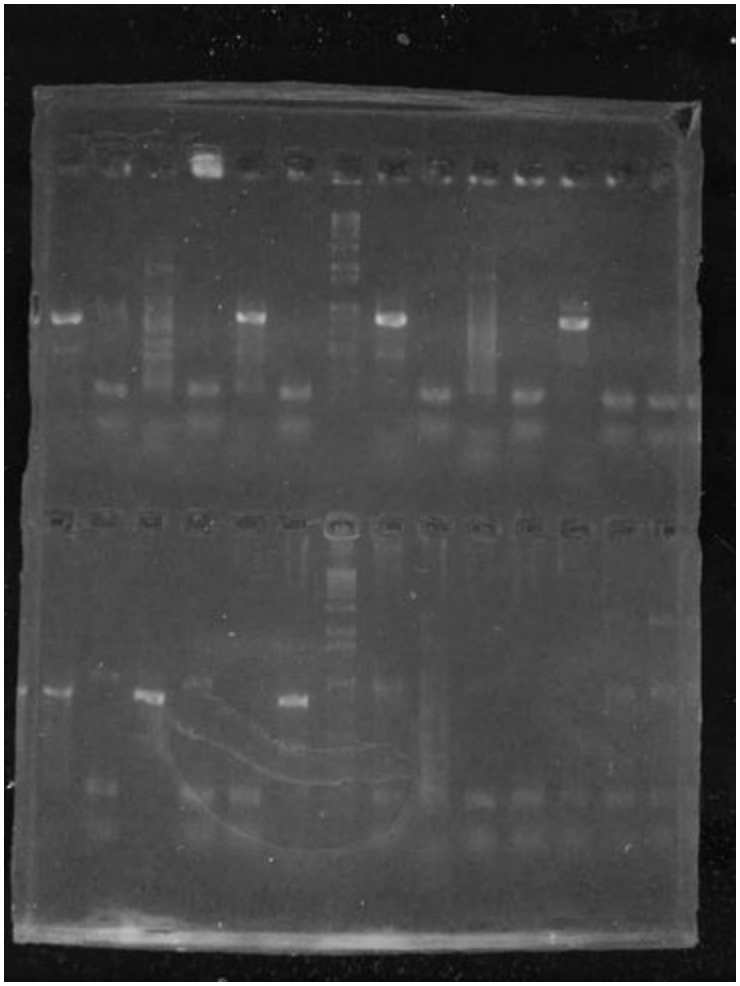


----------10/03----------

Gel electrophoresis of colony PCR
Allan

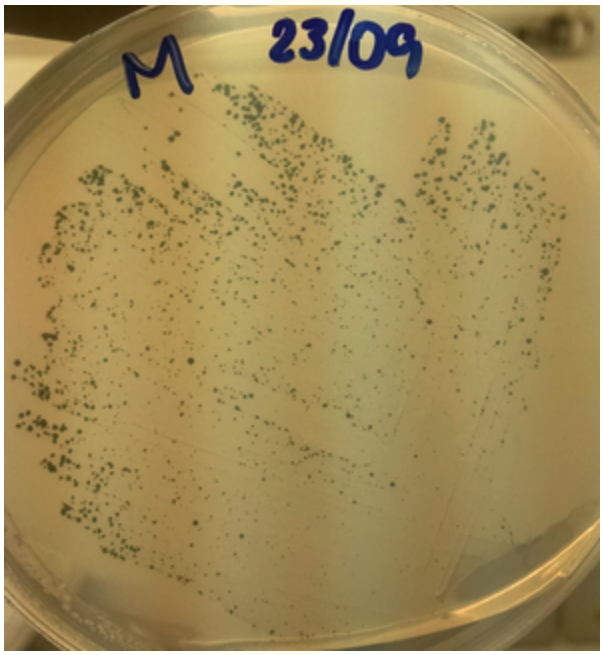
It was used 6,25 uL of DNA for 1 uL of 8x Loading Buffer + 5x Gel Red. The electrophoresis was run at 110V in SB 1x buffer.

Sample location in gel													
A1	**A2**	**B1**	**B2**	**C1**	**C2**	**Ldd**	**D1**	**D2**	**E1**	**E2**	**F1**	**(-)**	**F2**
G1	**G2**	**H1**	**H2**	**I1**	**J1**	**Ldd**	**J2**	**Col Lys**	**(-)**	**1 C3 Lys**	**2 C3 Lys**	**LYS [Conc = 700ng]**	**LYS [Templ conc = 100ng]**

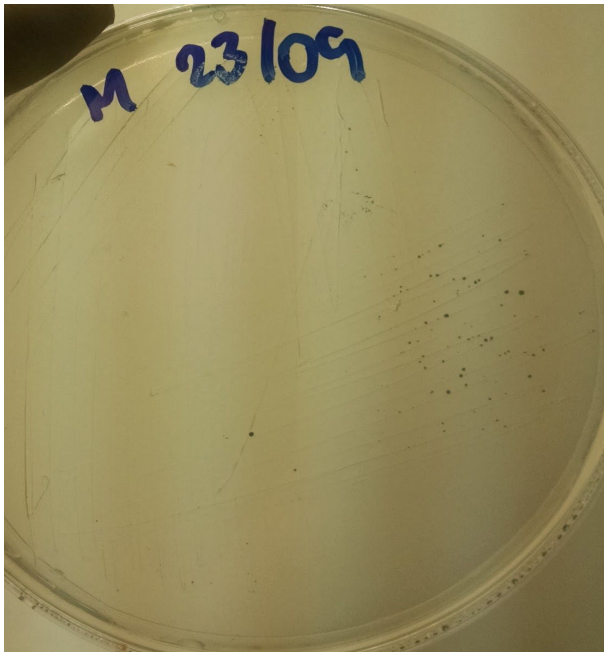


Colony Screening - pJP22mCherry

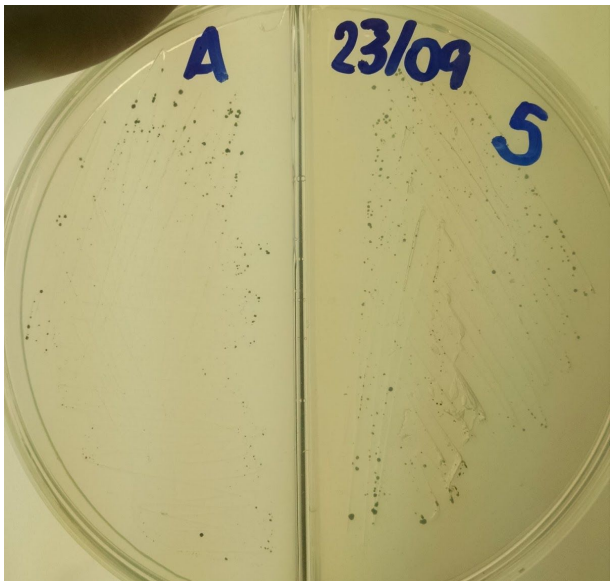
Cells transformed from 09/23.
Photo Colonies



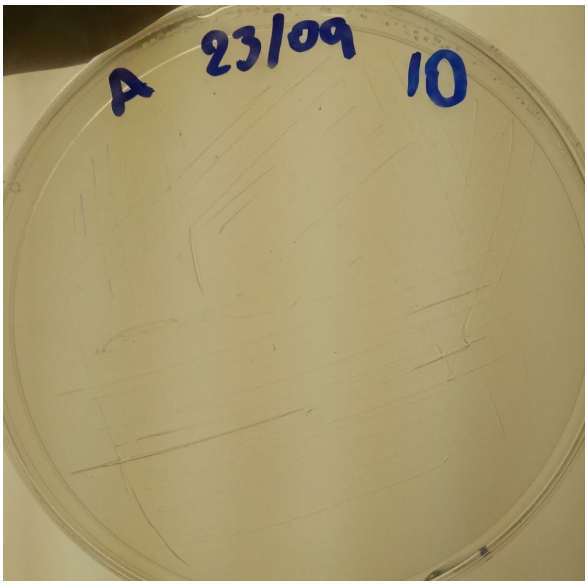
pJP22 mCherry transformants - Zeocin 5 ug/mL



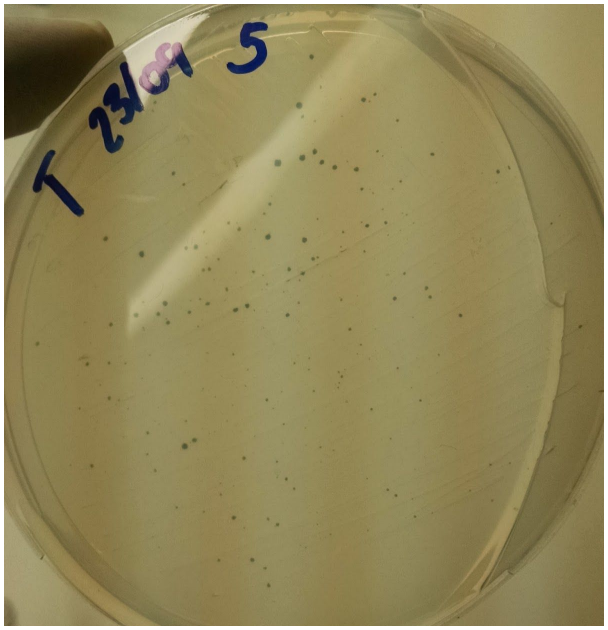
pJP22 mCherry transformants - Zeocin 10 ug/mL



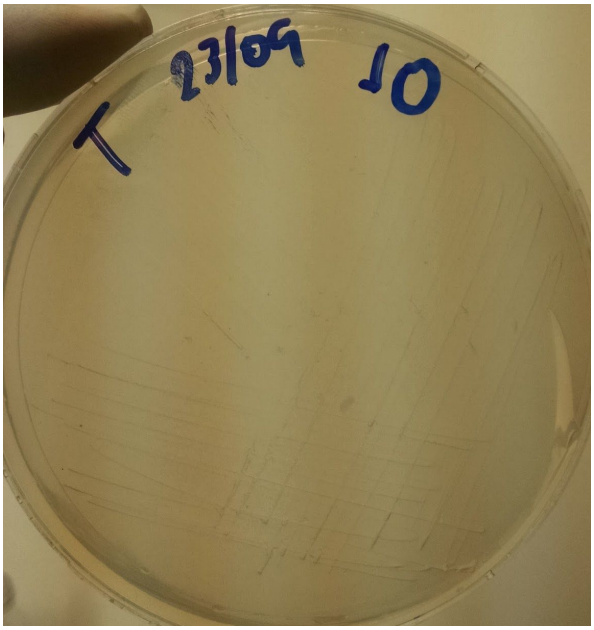
pJP22 mCherry transformants - Z5 (Water Transformed)



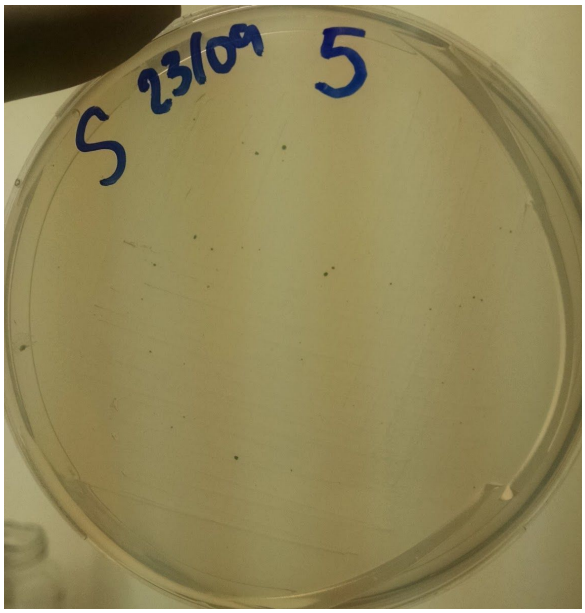
pJP22 mCherry transformants - Z10 (Water Transformed)



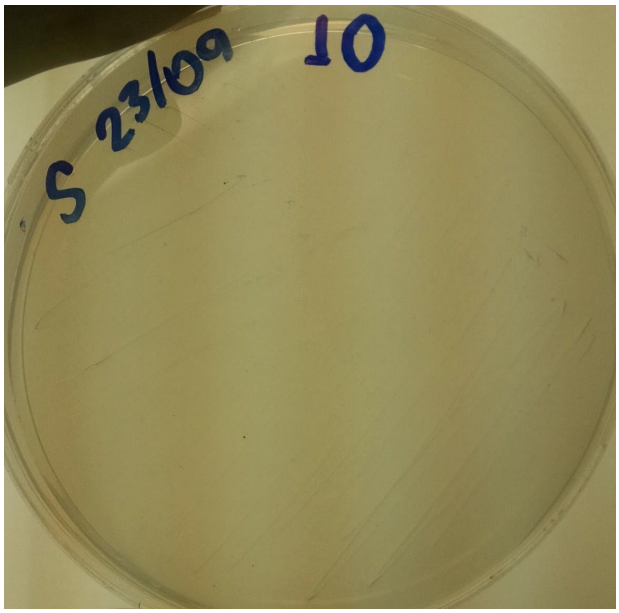
pJP22 mCherry transformants - Z5 (TAP Transformed)



pJP22 mCherry transformants - Z10 (TAP Transformed)



pJP22 mCherry transformants - Z5 (Sapphire Transformed)



pJP22 mCherry transformants - Z10 (Sapphire Transformed)

Screening by Colony picking and growing in a Black Clear Bottom 96 well plate.

200 uL TAP per Well
Agitation of 800 RPM
25ºC +-1ºC
80 µE m^{-2} s^{-1}
Clear sealing film

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M	Z10M
B	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M	Z5M
C	Z10A	Z10A	Z10A	Z10A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A
D	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A	Z5A
E	Z10T	Z10T	Z10T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T
F	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T	Z5T
G	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S	Z5S
H	CC1690 WildTyp	cc1690	cc1690	cc1690	cc1690	cc1690	TAP	TAP	TAP	mCherry	mCherry	mCherry

Mode mCherry Reading	**Fluorescence Top Reading**	
Excitation Wavelength	**575**	**nm**
Emission Wavelength	**608**	**nm**
Excitation Bandwidth	**9**	**nm**
Emission Bandwidth	**20**	**nm**
Gain	**200**	**Manual**
Number of Flashes	**10**	
Integration Time	**20**	**µs**
Lag Time	**0**	**µs**
Settle Time	**0**	**ms**
Z-Position (Manual)	**18141**	**Mm**

Label: Label4

Mode - Density Chlamy	**Absorbance**	
Wavelength	**750**	**nm**
Bandwidth	**9**	**nm**
Number of Flashes	**25**	
Settle Time	**0**	**ms**
Start Time:	**03/10/2016 21:17:06**	

Label: Label3

Mode - Chlorophyll Reading	**Fluorescence Top Reading**	
Excitation Wavelength	**440**	**nm**
Emission Wavelength	**680**	**nm**
Excitation Bandwidth	**9**	**nm**
Emission Bandwidth	**20**	**nm**
Gain	**100**	**Manual**
Number of Flashes	**10**	
Integration Time	**20**	**µs**
Lag Time	**0**	**s**
Settle Time	**0**	**ms**
Z-Position (Manual)	**18141**	**µm**
Start Time:	**03/10/2016 21:19:03**	

Miniprep and digestion of produced plasmids
Viviane

Plasmid	Concentration	260-280nm ratio
pAH04+IFN	384,0	1,85
pSB1C3 + Ea	48,1	1,75g
pJP22 + Type 2	234,8	1,88
pSB1C3 + B-gal	90,8	1,96
pSB1C3 + Lip	56,4	1,88
pSB1C3 + gLUC	72,0	198
pSB1C3 + S1	88,3	189
pSB1C3 + MaSp2	113,8	1,93
pSB1C3 + Neg J1	70,5	1,97
pSB1C3 + LysK	58,6	1,93
pSB1C3 + Lysos	160,8	1,87
pSB1C3 + MVL	72,1	1,88
USER	131,1	1,83
USER pool	558,5	1,82
USER 1:20 dilution	26,1	1,99

EcoRI/PstI digestion for C3 plasmids

	1Rx	10Rx
Buffer 2.1	1ul	10
EcoRI	0.5ul	5
PstI	0.5ul	5
H20	-	-
Template*	8ul	
	10ul	100ul

*Except for C3+Ea - C3+LysK, it was rather used 12 ul.

Xho/Bam digestion for pJP22 + Type 2 and USER

Item	Type 2	USER
Buffer 2.1	1	2.5
XhoI	0.5	2
BamHI	0.5	2
H20	4.0	2.5
Template	4ul	16 ul (all reaction)
	10ul	25ul

Results: No positive bands
Pending photo

Ligation and transformation of pJP22-MaSp1-4X fragments A and B to get the 8mer
Mireia

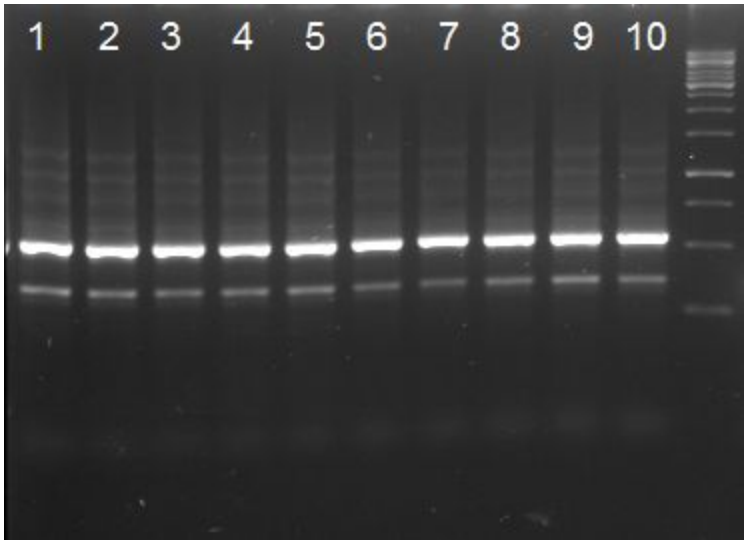
The described fragments of the A and B ligation were purified from a 0.7% agarose gel and resuspended in 100 uL H20. This samples were concentrated and resuspended in 10uL H20 to reach a concentration of 18 and 20 ng/uL. They were further concentrated to ~4 uL. The 4 uL of each fragment were ligated together in a 10 uL reaction using 1 uL T4-ligase.

4 uL of the 8mer ligation were electroporated into 100 uL DH10B (2mm cuvette, 2500V). The transformed cells were left in 1 mL (15 mL falcon) LB at 37ºC, 150 rpm for 45 min, centrifuged at 5000xg for 3 min and plated on LB Cb.

Result: >100 colonies were obtained on the transformation plate.

----------10/04----------

Colony PCR of the pJP22-MaSp1 8X transformation
Mireia



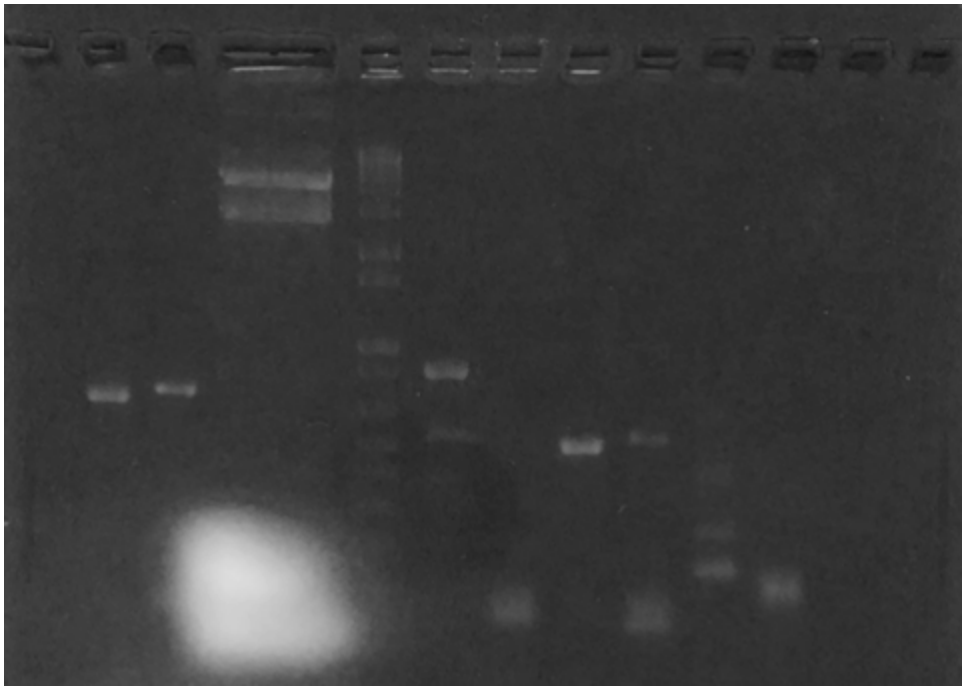
No 8mer band (~950b) was obtained.

PCR of MaSp2 with primers 58 and 59
Mireia

OK

PCR of pJP22 parts
Joao

Template
pJP22 type2 10/03
C3 gb1.3 09/13



	Promoter	Neg	USER	1kb ladder plus	Ressitance	Neg1	Terminator	Neg	MaSp2	Neg			

Digestion
Viviane

	pSB1C3 (153 ng/µL)	Promoter (3x25 µL) Reistance (3x25 µL) Terminator (3x25 µL)
XbaI	1 µL	0.5 µL
SpeI	1 µL	0.5 µL
Cutsmart	5.17 µL	2.89 µL
H2O	0	0
DNA	45 µL	25 µL
Total	52.17 µL	28.89 µL

Item	Masp 2 (5x25 µL)
XhoI	0.5 µL
BamHI	0.5 µL
Cutsmart	2.89 µL
H2O	0
DNA	25 µL
Total	28.89 µL

----------10/05----------

Electrophoresis of PCR USER 09/28

Allan

25 ul of sample were runned in 0.8% agarose gel in SB buffer.

Sample location in gel						
B Ly	B PCR	B Ly	Ldd	B Ly	A Ly	A PCR
A Ly	A Ly	C PCR	Ldd	C Ly	C Ly	C Ly

USER fusion of MaSp1 type2 monomers -2nd try

Tiago

The A, B and C parts of the USER fusion PCR were incubated in a thermocycler as follows:
1 microliter of USER enzyme (NEB)
1 microliter of USER cassete (from Stephen Mayfield's lab at UCSD)
1.5 microliters of UMT2-A69 PCR product (not purified)
1.5 microliters of UMT2-C65 PCR product (not purified)
5 microliters of UMT2-B65 PCR product (not purified)

15 minutes 37ºC
5 minutes 25ºC

This time, a negative control without USER enzyme (total 9 microliters) was also prepared. All the volume was used to transform on-day-made electrocompetent cells.

10/06
PCR purification
Viviane

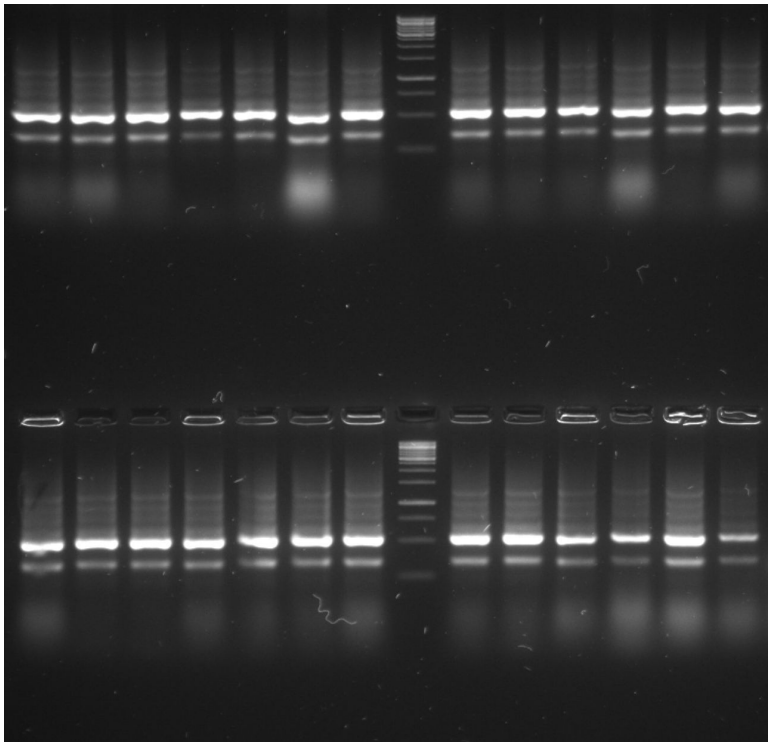
Promoter	129.5 ng/µL
Resistance	143.4 ng/µL
Terminator	86.8 ng/µL
Masp 2	90 ng/µL

Ligation
Joao

USER
Promoter + C3
Resistance + C3
Terminator + C3
C3 negative

Colony PCR 2 of the pJP22-MaSp1 8X transformation
Mireia

PCR done with more colonies this time (26) to confirm 8mer plasmid absence


hhhh

No 8mer band (~950b) was obtained.

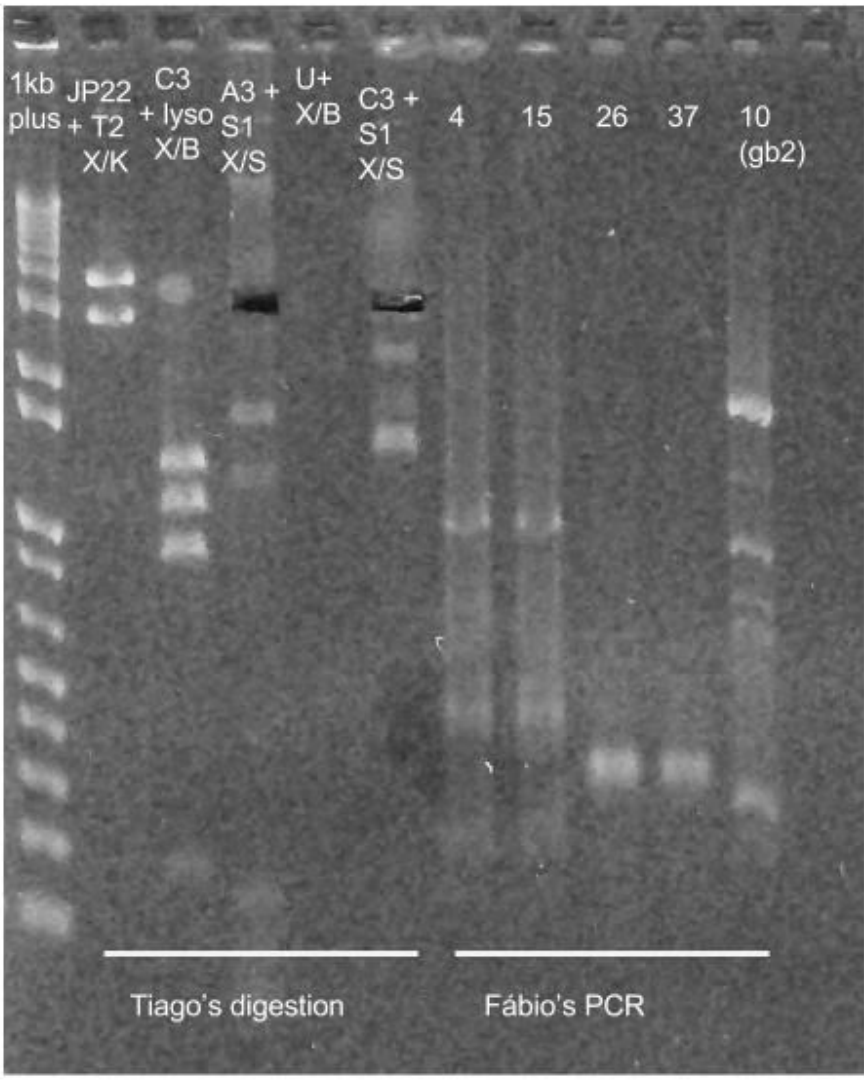
Transformation

Joao & Fabio

C3+Insert ligations from 20/08 batch
C3+Device parts purified from PCR purification made by Viviane
Negative C3
Results (next day): C3+Device parts grown but seems as contamination.
Recommendation: Re-do this parts with NEB cells

Overnight digestion - USER, pJP22+ MaSp1 type 2., pSB1C3+lysostaphin, pSB1A3 +Silwa1, pSB1C3+ RFP.

Tiago
Standard digestion procedure was followed with the following enzymes:
pJP22 + MaSp1 type 2 2mer → XbaI/KpnI
pSB1C3 + lysostaphin → XhoI / BamHI
pSB1A3 +Silwa1 → XbaI/SpeI
USER cloning after incubation → XhoI/BamHi
pSB1C3 + RFP → XbaI/SpeI



The firs lane indicates that the MaSp1 + pJP22 was adequately cut for *Chlamydomonas* transformation.
The second lane indicates that pSB1C3 has, indeed, a XhoI site, being inadequate for procedures that use this enzyme.
A3 and C3 were mistakenly cut, but the size was compatible with a non-digested plasmid.
The last lane indicates that Fábio's PCR of gb2 worked.

----------10/07----------

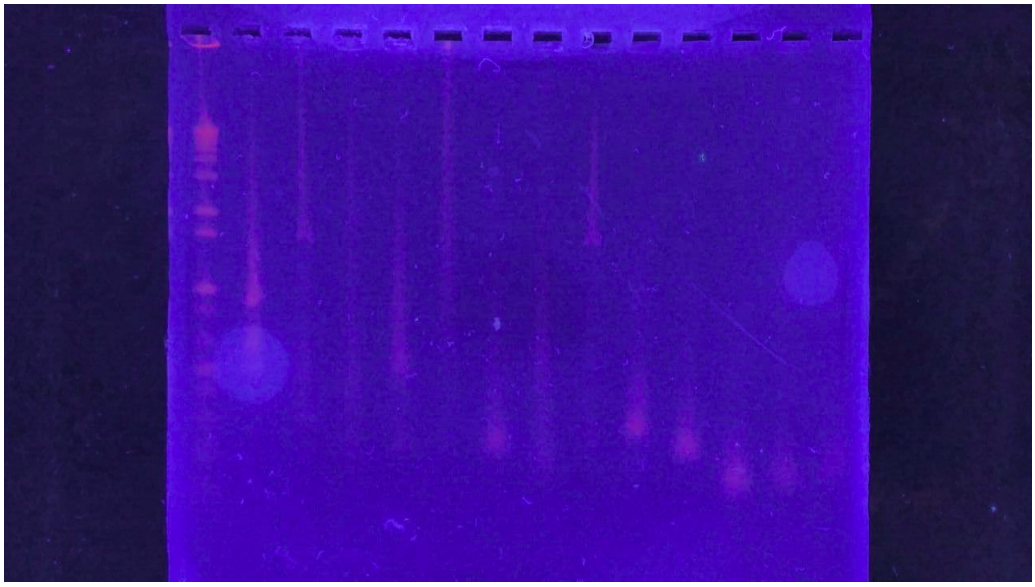
Miniprep of plasmid

PCR with Q5 and X7 buffers
Fabio

Samples amplified: LysK (56), MV-L (57), β-Gal (59), Lip Thela (60), Ea Masp (62), Type 2 (63), Silwa 1 (64), Silwa 2 (65), gBlock 2 (68), USER (75).
Samples were amplified in duplicates with Q5 and X7 buffers. Not all samples were run in electrophoresis due to gel size limitations.

	Final [ ]	Volume		Final [ ]	Volume
Q5 Buffer	X	12,5µL	GC Buffer	X	2,5µL
Pf (0060)	0,5µM	1,25µL	dNTPs	0,5µM	0,625µL
Pr (0061)	0,5µM	1,25µL	Pf (0060)	0,5µM	0,625µL
Template	-	1µL	Pr (0061)	0,5µM	0,625µL
H_2O	-	9µL	Betaine	1M	2,5µL
Total	-	25µL	Polymerase 7X	-	1µL
			Template	-	1µL
			H_2O	-	3,625µL
			Total	-	12,5µL

Sample location in gel													
	Q5								X7				
Ldd	(-)	LysK	MV-L	EaMasp	Type 2	Silwa 1	Silwa 2	gBlock 2	Type 2	Silwa 1	Silwa 2	gBlock 2	(-)



gb1 + pSB1C3 clone in JM110 cells - digestion
Tiago

The gb1 plsmid was cloned in competent JM110 cells, due to ClaI *dam* methylation sensitivity. A purified miniprep tube (29 microliters) was then subjected to a full digestion with:
-ClaI
-PstI-HF
-CutSmart Buffer

At 37ºC overnight

Preparation of sequencing reaction
Brayan
Standard protocol from Chemistry Institute was used.

Miniprep
Viviane

PCR purification
Viviane

Gel purification
Viviane

Digestion
Viviane

PSB1C3 and PSB1A3 transformations
Fabio

NIne electroporation transformations were made with competent cells prepared earlier the same day. The plasmids used were PSB1C3 + Promoter, PSB1C3 + Terminal, PSB1C3 + Resistance, PSB1C3 + gBlock 2, PSB1A3 + Masp 2, PSB1C3 Negative Control, PSB1A3 + RFP (from iGEM kit plate 4, position 2H), ????