Cloning and Misc Labbook

Date **October 4, 2013**
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Contents

8/13 James - Transformation practice

Transformed biobricks K808026, K936000, K936013, K808025 into competent E.coli (alpha-select Bioline) cells on 25ug/mg chloraphenicol plates. Failure, no cells grew.

Problems to solve: faulty plates, too strong antibiotic, faulty DNA from registry. Eventually some cells grew, low transformation efficiency

Will retransform. pUC19 (standard plasmid/amp resistant) transform, place half on plates we made and half on plates made at the centre. Also do 90 minute stage on flat horizontal instead of in vertical mixer.

4/8/13 Second transformation

K808026, K936000, K936013, K808025 into competent E.coli (alpha-select Bioline) cells on 25ug/mg chloraphenicol plates. One or two colonies.

pUC19 +ve onto Amp plate (no colonies)

Overall failure. No cells on positive control. Plated 1/5th transformation reaction onto plates from CSynBI. Will check this afternoon.

5/8/13 Solving transformation issues.

Cutinase, LC-Cutinase, FSc colonies picked and streaked onto fresh Chloramphenicol plate. incubating O/N.

6/8/13 MK + James setting up overnight cultures

from colonies streaked and those grown from CSynBI plates. to miniprep tmr and send for sequencing.

colonies picked into 3mL LB and 1.5 uL Chlo (25ug/ml) tubes (in shaking incubator on 6th floor)

-LC-cut1 (BBa_K936000 colony number 1)

-LC-cut2 (BBa_K936000 colony number 2)

-PelB LC-Cut (BBa_K936013)

-FSc 3 (BBa_K808025 colony number 3)

-Fsc RIK (BBa_K808025 colony number)

-Est13 (BBa_ K808026)

6/8/13 Sisi and Jemma transformation

K808026, K936000, K936013, K808025 into gold competent E.coli (alpha-select Bioline) cells on 25ug/mg chloraphenicol plates.

Control: pUC19 +ve onto Amp plate

40 sec heat shock, 90 minute stage on flat horizontal mixer. Culture observed after one night.

Overall very low efficiency, a few cells grow. Could because of inefficient heat transfer in heat shock machine/our lab techniques/amount of DNA used. Will use water bath for heat shock next time.

6/8/13: J: Preparation for sequencing:

>1.8 = good

>100ng/uL = good

DNA=260, protein=280

	LC1	LC2	PelB-LC	Fsc3	Fsc Rich	Est13
OD260-280	1.85	1.77	1.74	1.81	1.81	1.92
ng/uL	83.9	75.2	54.5	89.8	69.0	46.8

Want 500ng @ 100ng/uL

Results: LC1,LC2 and PelB-LC were correct but the others were not. Therefore we binned Fsc FscRich and Est13..

7/8/13

Prepared agar

2 LB+ agar

Both at 300mL (7.5g LB premix, 8.4g agar)

8/8 /13

poured plates from 300 mL, with Chlo (25 uM)

8/8/13: J+S

We transformed:

- PelB-LC (Part:BBa_K936013) **(from our miniprep)**
- LC1 (BBa_K936000), **(from our miniprep)**

- LC2 (BBa_K936000), **(from our miniprep)**
- promoterJ23104 + RBSb0034 (BBa_K608002), **(from parts distribution)**
- amilCP Blue (BBa_K592009), **(from parts distribution)**
- stress response (BBa_K639003) **(from parts distribution)**

into Chloraphenicol LB plates where we streaked them O/N

9/8/13 PCR to linearise backbone (MK)

template: pSB1C3 - LC-Cut

P1: G1002

P2: G1003

program: 3x(95.20,55.20,72.40)

measured: C=938.1 ng/ul A(260/280)=1.54

10/8/13 Transform PUT enzymes, stress sensor and P+RBS into MG1655 ()

2ul DNA, 50ul MG1655 cells. 30min on ice, 30sec 42oC heatshock, 3min ice, 250ul LB, 90min horizontal incubation 37oC, 200ul transform mix onto Chloraphenicol LB plates (36ug/ml antibiotic conc) O/N rest in fridge.

DNA:

- PelB-LC (Part:BBa_K936013) **(from our miniprep)**
- LC1 (BBa_K936000), **(from our miniprep)**
- promoterJ23104 + RBSb0034 (BBa_K608002), **(from parts distribution)**
- stress response (BBa_K639003) **(from parts distribution)**

12/8/13 pSB1C3 backbone cont. from 9/8/13 ()

PCR reaction PCR purified with bioline kit and resuspended in 30ul ddH20. DNA spec 50.8ng/ul

Send to sequencing pSB1C3 2070bp (5ul at 20.7ng/ul). seq id: pSB1C3

- 2.5ul DNA + 2.5ul ddH20
- So 1ul of 10uM G1002 primer and 2ul dH20 for every 2 sequencing reactions.

Digest pSB1C3 PCR product, Total 30ul digests

Make linearised vector for cloning	Make vector which can reanneal and circularise for empty vector control
10x Fast Digest green buffer 3ul	10x Fast Digest buffer 3ul
EcoRI 1ul	XbaI 1ul
PstI 1ul	SpeI 1ul
DNA (1ug) 20ul	DNA (254ng) 5ul
H2O 5ul	H20 20ul

Hyper ladder I 7ul, pSB1C3 (EP digest), blank lane, pSB1C3 (XS), amilCP PCR

Gel dodgy need to make fresh TAE or TB.

observed results from stress sensor. it looks leaky BBa_K639003

12/8/13 overnight cultures (MK, JP)

set up from these, with 25 uM Chlo

- J23104+RBSB0034 (in MG1655)
- LC Cutinase (in MG1655)
- PelB LC Cutinase (in MG1655)
- osmY (BBa_K892008) in the cells they sent it to us
- ABC transporter (K258008) in the cells they sent it to us
- stress sensor (K639006) in the cells they sent it to us

12/8/13 streak, James

- LARD1 on amp plate

12/8/13 transformation of amilCP (BBa_K592009), Jemma

into Top10 alpha cells, 30uL cells, 5uL DNA. lots of colonies grew. the next day. the problems with transformaition solved. :) -> alphaselect cells were baaad, we are using top10 from now on.

12/8/13 PCR and purification

template: amilCP from distribution

primers: VF2 and VR

measured ABS after: 69.2 ug/ml

purified and only 9.5 ng/ml remained. :(something was not woing there.

1-7

5ml 0.05

13/8 Jemma and Margarita

- Day culture - amil CP blue **K592009**
- Glycerol stocks - osmy **K892008**, stress **K639006**, promoter/RBS J23104+RBSB0034 **K68008**, ABC transporter **K639006,** pel B LC Cut **K936013**, LT cutinase **K936000**
- Miniprep

	Abs (ug/ul)	A 260/280
LC 1	41.8	1.79
LC 2	50.2	1.91
PLC 1	83.4	1.90
PLC 2	154.7	1.88
OsmY 1	73.9	1.88
OsmY 2	131.2	1.87
ABC 1	34.7	1.88
ABC 2	26.8	1.82
P + RBS 1	94.4	1.89
P + RBS 2	80.7	1.87
Stress	145.9	1.85

all these were sent for sequencing (results: 17/08)

13/8 PCR: adding his tag to LC-Cut and pelB-LC-Cut (MK)

template: LC1 (06/08 miniprep) , PLC (06/08 miniprep)

P1: Fw_his_suf

P2: Rv_his_cut

mix: 50 uL reactions

program:

aneal: 55, 20

elongation time: 72, 15

run on gel, cut it (made new buffer, problems with gels solved.)

the band cut out was around 600BP. That is not good. The elongation time was too short as well. I am repeating this tmr with longer elongation time.

13/8 miniprep day culture amilCP (MK)very low concentration DNA, so made new O/N culture in 4mL

	concentration (ng/ul)	A (260/280)
amilCP1	10	1.86
amilCP2	9.6	2

sequenced. The results were not so good.

13/8/13 setup WASTE condiioned media and L-Lactic acid experiment

Three O/N cultures (from colonies A-C).

14/8/13 WASTE condiioned media and L-Lactic acid experiment

100ul culture into

- 4ml LB
- 4ml WASTE conditioned media
- 4ml of LB + 4ul L-Lactic Acid

Put 200ul into 96 well plate in the order listed on drive folder experimental data 14-8-13

Cells didn't grow well / or maybe it was different plate reader? (14-8-13)

14/8/13 WASTE EXP second attempt

Picked colonies E+F from stress sensor BBa_K639003 and grew O/N in 4ml LB+ Chlaram-phenicol (1:2000 dilution 25ug/ml)

14/8/13 miniprep (James and Matt)

LARD1	BBa_K258001	9.4 ng6ul
phaA	BBa_K338003	19.7 ng/ul
amilCP blue/3	BBa_K592009	14 ng/ul

amilCP blue/3 was sequenced. see 17/08 sequencing results for details

14/8/13 gel purification (Jemma)

14/8/13 PCR to put His tag onto LC-CUT and pelB-LC-CUT (Mara)

template: LC1, PLC

P1: Fw_his_suf

P2: Rv_his_cut

mix:

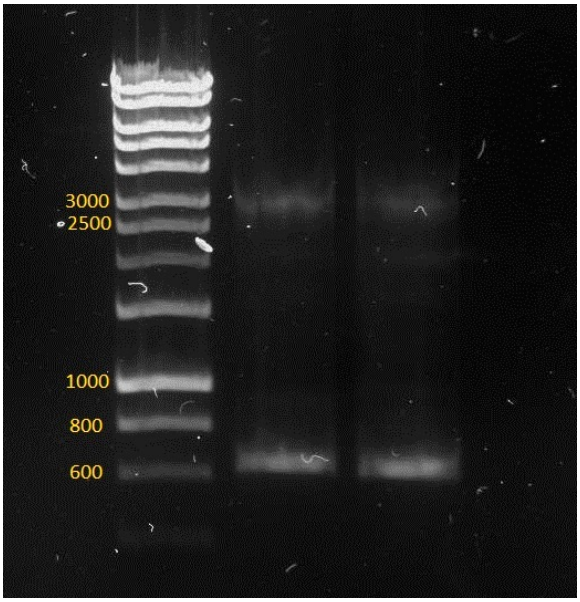
2x50 mL reactions

program:

aneal: 55, 20

elongation: 72, 50

run 16ul reaction (from 50 ul individual reaction volumes)



There is some unspecific amplification product at around 600BP. However, there is also a product around 300BP, which is the size we are looking for.

We run the whole reaction and cut out the bands at 3000.

They were purified from the gel.

	concentration (ug/ul)	purity (A 260/280)
pel B LC cut, his tag	41.1	3.09
LC cut, his tag	16.5	1.87

14/8/13 gradient PCR to determine optimal anealing T for the his-PCR reaction

template: LC1 (6/08 miniprep) only

P1: Fw_his_suf

P2: Rv_his_cut

mix:

12x25 ul reactions.

program:

anealing 50-60 degrees range., 20

elongation: 72,50

We did not see any substantial increase in specificity.

16/08/13 Digest with SpeI, PstI (MK)

P+RBS1 plasmid (from 13/8) 2 ul = 700 ng

P+RBS2 plasmid (from 13/8) 8.7 ul= 700 ng

16/08/13 Digest with Xba, PstI (MK)

amilCP1 PCR product (see 15/8 for details) 2.1 ul= 500 ng

LC1 plasmid (from 07/08) 6 ul = 500 ng

PLC plasmid (from 07/08) 9.2 ul = 500 ng

unfortunately, I lost these samples when I tried to purify them from the gel. :(I am not sure why this happened, it could be because I heated the samples up to 60 degrees instead of 50 (in protocol) because the gel melted very slowly. Therefore, the next gel will be 0.8 and not 1% so that it's easier to melt.

Also, only 2 gel purification columns were available so, I only did two. Here are the results:

	concentration (ug/ul)	purity (A 260/280)
amilCP	3.3	3.58
PLC	4.6	4.92

Also, I used up most of the LC1 and PLC plasmids so I had to use different DNA when I repeated the experiment the next day.

16/08/13 Digest with Xba, PstI (MK)

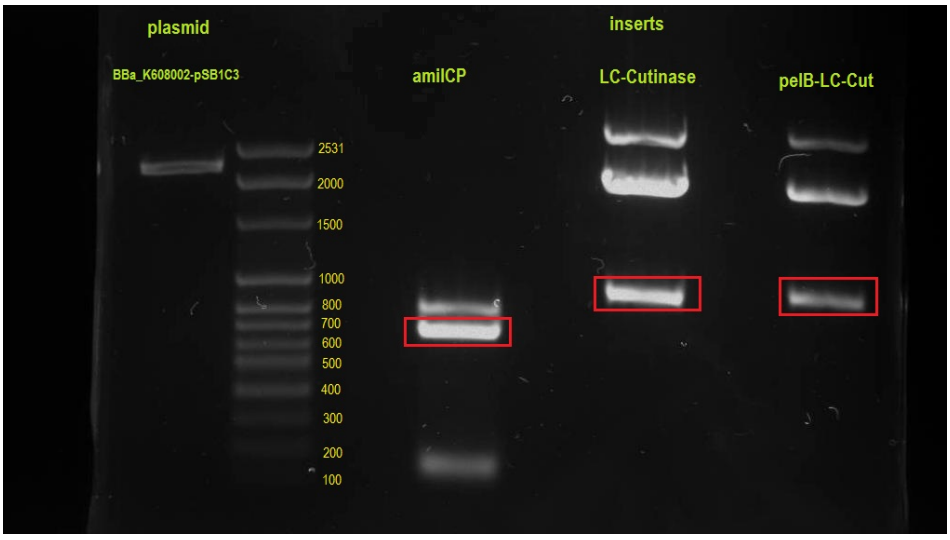
amilCP1 PCR product (see 15/8 for details) 3 ul= 650 ng

LC2 plasmid (from 07/08) 6.7 ul = 500 ng

PLC2 plasmid (from 13/08) 3.3 ul = 500 ng

16/08/13 Run on gel and cut out (MK)

I run the above digest (whole 20 ul reaction) on a 0.8% gel. I also run 2ul of P+RBS plasmid on the side. The ladder is 6ul of HL 100+. We cut out the bands shown with red rectangle.



19/08/13Digest with Xba, PstI (IB)

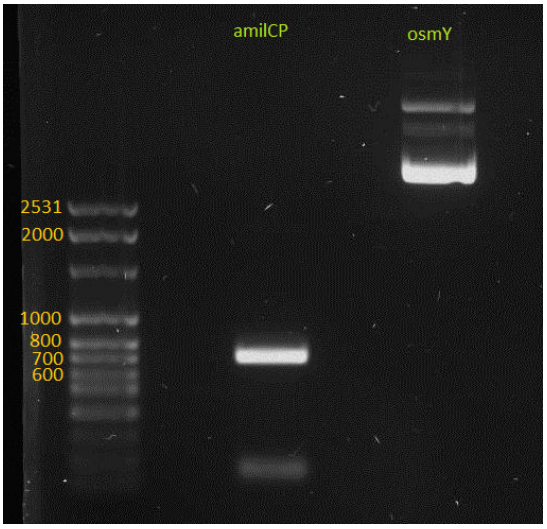
amilCP3 PCR product (see 15/8 for details) 5.2 ul= 500 ng

OsmY2 Plasmid (from 13/08) 3.8 ul = 500 ng

Incubated at 37 degrees for 40 mins.

AmilCP, OsmY2 digest run on gel

0.8% gel



19/08/13Digest with Xba, PstI (MK)

repeated osmY2 and also addad osmY1

Sisi + James

picked 3 colonies D E F from stress plate, prepared O/N culture

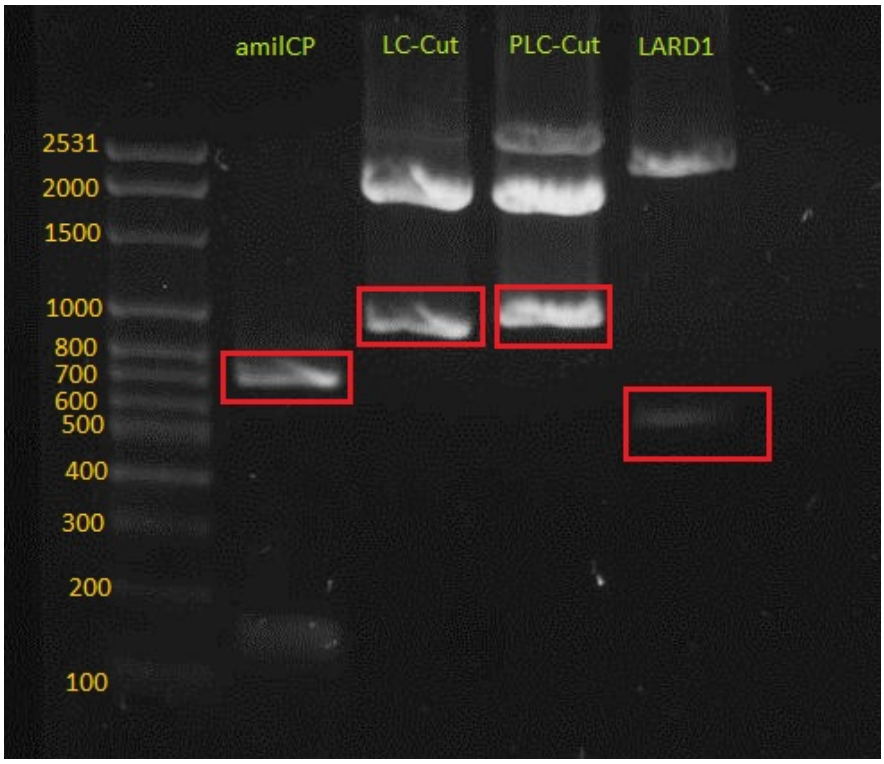
20/08/13Digest with Xba, PstI (MK)

amilCP3 (13/08)

LC2 (7/08)

PLC (13/08)

LARD1 (15/08)



Gel purification

	amilCP	LC	PLC	LARD1
C (ng/ul)	6.5	6.9	7.2	3.3
A (260/280)	2.59	2.49	2.26	4.6

Dephosphorylation of backbone.

P+RBS1 (15/08) all of the reaction.

Ligation

10ul reactions. (1buffer, 1enzyme, 1ATP)

	amilCP	LC	PLC	LARD1
insert	2.7	2.7	2.7	6
vector	0.5	0.5	0.5	0.5

21/08 send stuff for sequencing (MK):

- **pSb1C3 (12/8/13)** (5ul at 20.7ng/ul) with Primer G1003
- phaABC4 (16/08/2013) 31.8ng/ul with Primer VR

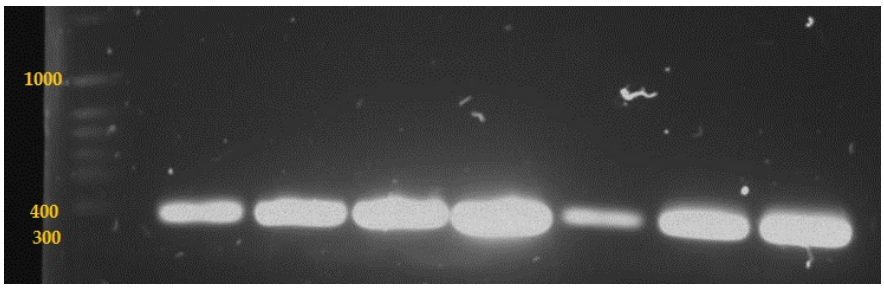
21/8/13 4x pSB1C3 backbone

Stress Biosensor (1+2) and PJ23104+RBSB0034 (3+4) were the two templates. Numbers eleate to reaction numbers. In total 4 reactions.

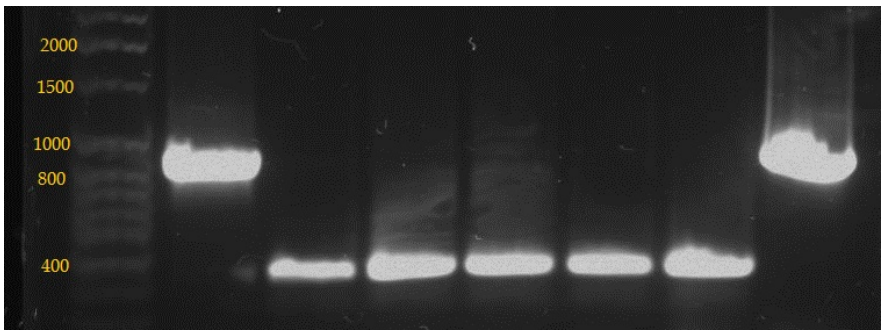
Programme stored on eppendorf nexus.

22/8/13 colony PCR from ligation (MK)

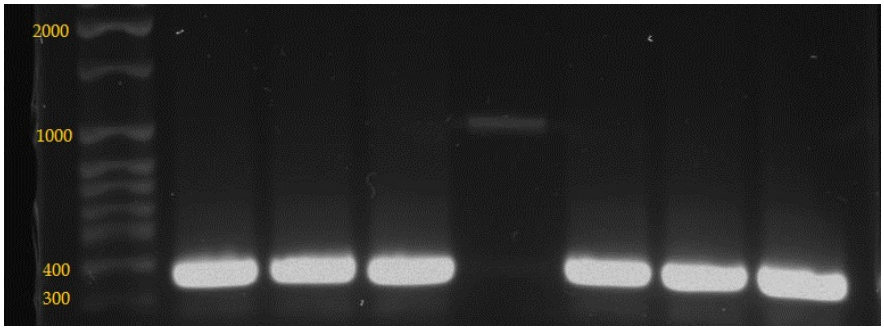
amilCP: no good. empty vector only :((also, the colonies were all white)



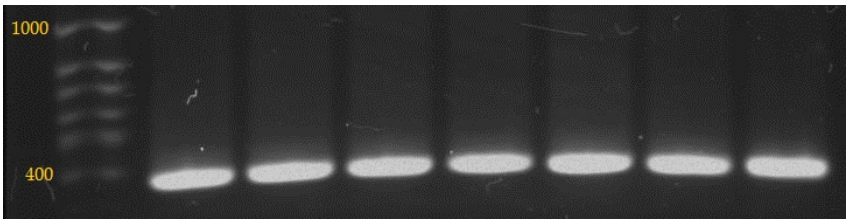
LC-Cut: 1 and 7 look good. O/N culture setup



PelB-LC-Cut: 4 looks promising. O/N culture is setup from it.



LARD1: no good. :(



I could actually see blue colonies on the amilCP ligation plate after a few days. So the ligation was not that bad afterall, I was just unlucky with picking colonies.

22/8/13 ligation of amilCP again (MK)

insert - amilCP(20/08) - 6.7ul

vector - p+RBS1 (20/08) - 0.4ul

22/8/13 streaked LC1, LC7, PLC4 colonies from ligation onto polyDEGA plates.

23/08/13 miniprep PLC4, LC7

23/08/13 - Transformation

ligation of milCP-P+RBS1 (22/8) reaction (all 10ul by mistake...) into NEB10 cells

regeneration of cells in SOC for 1.5h at 37 in greece

23/08/13- PCR of PLC4 (23/08) and LC7 (23/08)

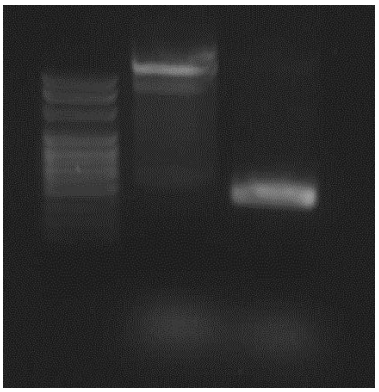
For 25microlitres of reaction

0.5microlitres of template added(PLC4 109.2ng/microl, LC7 130.8ng/microl)

Annealing temp of primers at 55degrees, VF2 and VR

run the PCR - 1:

LC good, 2:PLC not good



26/8/13 Transformation of the first seven synthesised biobricks into NEB 5

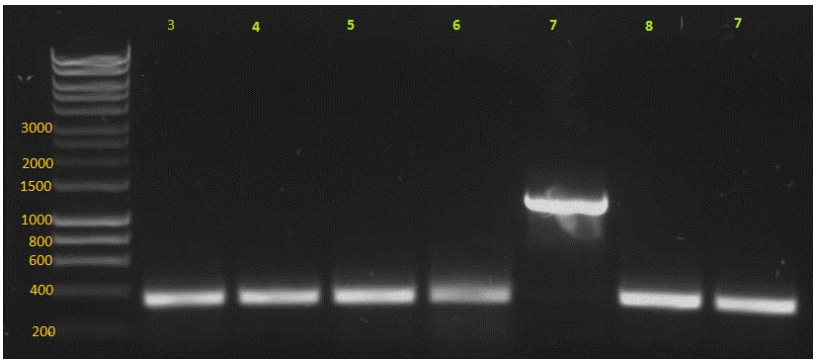
BBa_K1149002	PUR ESTCS2
BBa_K1149003	PueA
BBa_K1149004	PueB

BBa_K1149006	PulA
BBa_K1149007	Proteinase K
BBa_K1149008	PelB-Proteinase K

BBa_K1149011	Phaz2

27/8/13 colony PCR

P+RBS+PLC transformation, O/N culture setup from 7 and 11



27/8/13 Picked colonies for O/N culture ()

BBa_K1149002	PUR ESTCS2
BBa_K1149003	PueA
BBa_K1149004	PueB

BBa_K1149006	PulA
BBa_K1149007	Proteinase K
BBa_K1149008	PelB-Proteinase K

BBa_K1149011	Phaz2

these were successfully transformed from Invitrogen suppllied synthesized DNA on 26/8/13.

28/08 miniprep

amilCP A	27/08/2013	25.3	miniprep	1.87
amilCP B	27/08/2013	20.5	miniprep	1.88
LARD1 2	27/08/2013	54.3	miniprep	1.85
LARD1 5	27/08/2013	55.7	miniprep	1.87

29/08/2013 send stuff for sequencing:

VF2 primer:

- amilCP A (27/8 MK)
- amilCP B (27/8 MK)
- LARD1 2 (27/8 MK)
- LARD1 5 (27/8 MK)
- LC7 (23/08 MK)
- PLC 7 (28/08 SF)
- PCL 11 (28/08 SF)

VF2 and VR primers:

- phaBC (28/08 SF)
- stress (28/08 SF)

28/8/13 O/N cultures of first seven potential biobricks were glycerol stocked and minipreped

BBa_K1149002	PUR ESTCS2 131.9ng/ul
BBa_K1149003	PueA 142.9ng/ul
BBa_K1149004	PueB 145ng/ul

BBa_K1149006	PulA 142.8ng/ul
BBa_K1149007	Proteinase K 145.1ng/ul
BBa_K1149008	PelB-Proteinase K 165.9ng/ul

BBa_K1149011	Phaz2 134.3ng/ul

28/8/13 4 new biobricks transformed into TOP 10.

BBa_K1149001	Secretion Toolkit

BBa_K1149005	PudA

BBa_K1149009	CLE
BBa_K1149010	Phaz1

28/8/13 transformation

Sisi transformed amilCP A(27-08), LC7(23-08) and PLC7(28-08) into E. coli MG1655.

Results: LC7 plate has many pink colonies, therefore the cells are actually MCherry cells instead of LC7

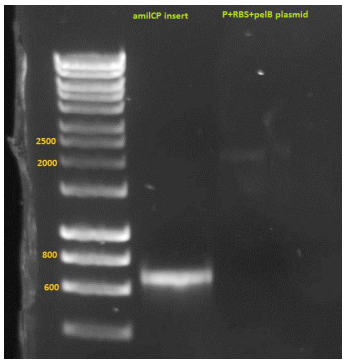
28/8/13 INFUSION REACTION (MK)

PCR with PfuUltra, 50uL reactions:

1., template: PLC7 (28/08) P1: G1002 P2: pelB reverse

2., template: amilCP3 (14/08) P1: pelB-amilCP P2:amilCP-sufix

run 5uL (by 5ul ladder)



PCR purify:

amilCP: 81.7 ng/ul 1.88

P+RBS+pelB: 30.2 ng/ul 1.8

Infusion reaction

2 ul 5* Infusion Enzyme Mix

5.1 ul H2O

1.6 ul P+RBS+pelB (linearised vector)= 48 ng

1.2 ul amilCP (insert)=98 ng

transformation

into 50 ul NEB10 cells

5uL of the reaction

30/8/13 O/N culture (for glycerol stock)

amilCP A colony 1.

PLC7 colony A

30/8/13 Digest of pSB1C3 Backbone

Enzyme Master Mix for Linearised pSB1C3 Plasmid Backbone (20ul total)

2 ul 10x FAST Buffer

1ul EcoRI

1ul PstI

8ul linearized DNA (25ng/ul 200ng total)

8 ul dH20 (total 20ul)

Digest Plasmid Backbone (10ng/ul DNA)

Digest 37C/60 min, heat kill 80C/20 min

Add 2.2ul Antarctic Phosphatase buffer (final 1X)

Add 1□L Antarctic Phosphatase

Incubate 60 mins at 37°C.

Heat-inactivate for 5 mins at 65°C.

Then move to ligation see 31/8/13

30/8/13 sent stuff for sequencing:

VF2 and VR primers:

- phaBC (28/08)
- PLC7 (28/08)

VF2 primer>

- pelB PCR product (29/08) (the one we used for infusion)

The sequencing results from PLC11 came back without a promoter+RBS.

The results from PLC7 had the P+RBS but did not have the LC-Cut. :(not good, not good…

The pelB PCR product was something totally different. (BLAST shows some bit of some genomic DNA.) I think maybe I managed to amplify a bit of the genome and Infused that to amilCP. No surprise it's not being expressed. :(

31/8/13 Progress on Cloning Synthesis Biobricks

pSB1C3 ready for ligation (8rxns)

3:1 ratio.

	DNA post gel extraction	Insert (ng=ul)	Vector (25ng)	10X ligase buffer	T4 ligase	dH20 (up to 20ul)
PelB PK	12.5ng/ul	**77ng / 6.2ul**	2.9	2ul	1ul	**.9ul**
PK	11.7ng/ul	**77ng / 6.6ul**	2.9	2ul	1ul	**.5ul**
Phaz2	19.1ng/ul	**84ng / 4.4ul**	2.9	2ul	1ul	**2.7ul**
9002	12.5ng/ul	**99ng / 7.9ul**	2.9	2ul	1ul	**0ul**
9003	12.2ng/ul	**81ng / 6.6ul**	2.9	2ul	1ul	**.5ul**
9004	18.8ng/ul	**75ng / 4ul**	2.9	2ul	1ul	**3.1ul**
9006	26.5ng/ul	**60ng / 2.3ul**	2.9	2ul	1ul	**4.8ul**
MM		-	**20.3ul**	**14ul**	**7ul**	-

Add 5.9ul MM to insert + h20 (red means adjusted to be 10ul max)

BBa_K1149001	Secretion Toolkit	GeneArt transformed into NEB5
BBa_K1149002	PUR ESTCS2	Potentially Biobrick in NEB10
BBa_K1149003	PueA	Ligation may have failed, retry ligation
BBa_K1149004	PueB	Potentially Biobrick in NEB10
BBa_K1149005	PudA	GeneArt transformed into NEB5

BBa_K1149006	PulA	Potentially Biobrick in NEB10
BBa_K1149007	Proteinase K	Potentially Biobrick in NEB10
BBa_K1149008	Proteinase K	Potentially Biobrick in NEB10
BBa_K1149009	CLE	GeneArt transformed into NEB5
BBa_K1149010	Phaz1	GeneArt transformed into NEB5
BBa_K1149011	Phaz2	Potentially Biobrick in NEB10

2/9/13 MASS-SPEC sample preparation

Label properly and take to Lisa first thing in the morning. First, dissolve **Stock** in a falcon/eppendorf tube and dilute that, to 0.001 g/mL: **with ddH2O and LB** in an eppendorf tube.

EG: 1uL of 1.11g/mL stock

3HB: 80 uL of 100mM stock (

for GC-MS:

- 1mg of 3HB in ddH2O
- 1mg 3HB in LB
- 1mg Lactic acid in ddH2O
- 1mg Lactic acid in LB
- 1mg EG in ddH2O
- 1mg EG in LB

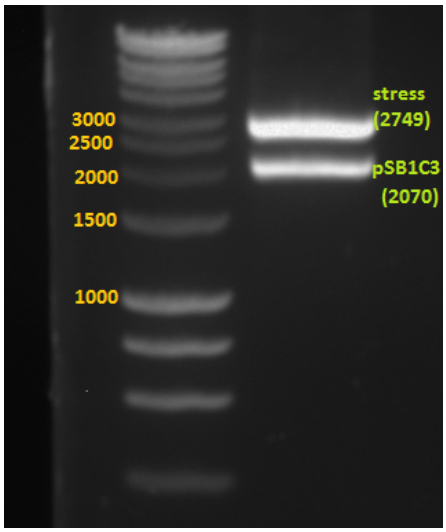
for MOLDI:

- 1mg PHB in ddH2O

2/9/13 digest for backbone (MK)

stress (13/8) with EcoRI and PstI

cut out band



2/9/13 Cloning planning

BBa_K1149001	Secretion Toolkit	GeneArt transformed into NEB5
BBa_K1149002	PUR ESTCS2	Potentially Biobrick in NEB10
BBa_K1149003	PueA	Ligation may have failed, retry ligation
BBa_K1149004	PueB	Potentially Biobrick in NEB10
BBa_K1149005	PudA	GeneArt transformed into NEB5
BBa_K1149006	PulA	Potentially Biobrick in NEB10
BBa_K1149007	Proteinase K	Potentially Biobrick in NEB10
BBa_K1149008	Proteinase K	Potentially Biobrick in NEB10
BBa_K1149009	CLE	GeneArt transformed into NEB5
BBa_K1149010	Phaz1	GeneArt transformed into NEB5
BBa_K1149011	Phaz2	Potentially Biobrick in NEB10

Streaked 2 colonies onto new chloramphenicol plate. 6hr day growth to 6pm.

BBa_K1149002

BBa_K1149004

BBa_K1149006

BBa_K1149007

BBa_K1149008

BBa_K1149011

- Need to setup O/N cultures of above, if they are OK, miniprep, send for seq and transform tomorrow.

Second Biobrick wave

- Need to setup O/N culture (Kanomycin?) and miniprep next day, do EP digest and purify insert. I recommend you run 9003 second EP digest (from other dayon gel with the other 4 Biobricks.

BBa_K1149001

BBa_K1149005

BBa_K1149009

BBa_K1149010

3/9/13 Biobrick cloning

Construct	Backbone	ng/ul
PK	pSB1C3	32.2
PelB PK	pSB1C3	38.3
Phaz2	pSB1C3	88.3
9002	pSB1C3	122
9004	pSB1C3	33.8
9006	pSB1C3	73.6
Secretion Toolkit	geneart backbone	168.9
Phaz1	geneart backbone	123.6
CLE	geneart backbone	143.2
9005	geneart backbone	113.8

All potential biobricks sent for sequencing with VF2 primers.

4/9/13 Biobrick update

Biobrick Number	Description	Status	Gene Art Backbone ng/ul	pSB1C3 ng/ul
BBa_K1149001*	Secretion Toolkit	Have Gene Art plasmid	168.9	
BBa_K1149002	PUR ESTCS2	Biobrick trans-formed MG1655 (Picking several more backup colonies)	131.9	122 A: 57.5 B: 53.7
BBa_K1149003*	PueA	Have Gene Art plasmid	142.9	
BBa_K1149004	PueB	Have Gene Art plasmid	145	
BBa_K1149005	PudA	Have Gene Art plasmid	113.8	
BBa_K1149006	PulA	Biobrick trans-formed MG1655 (Picking several more backup colonies)	142.8	73.6 A: 32.4 B: 31.4
BBa_K1149007*	Proteinase K	Have Gene Art plasmid	145.1	
BBa_K1149008*	PelB-Proteinase K	Have Gene Art plasmid	165.9	
BBa_K1149009	CLE	Have Gene Art plasmid	143.2	
BBa_K1149010	Phaz1	Have Gene Art plasmid	123.6	

BBa_K1149011* Phaz2	Have Gene Art plasmid	134.3
BBa_K1149012 Bdh1	Not synthesised yet	
BBa_K1149013 Bdh2	GeneArt Backbone-transformed Picked two colonies to extract Gene Art plasmid	A: 134.3 B: 134.5

5/9/13 Miniprepped DNA

Bdh2 colonies A+B in geneart plasmid were miniprepped. See table above for colonies A+B dna ammount.

9002+9006 BB colonies A+B to have extra woing colonies of these Biobricks (BB). Also sent for sequencing.

5/9/13 prepared 5 constructs for Biobrick ligation

	DNA post gel extraction	Insert (ng=ul)	Vector (25ng)	10X ligase buffer	T4 ligase	ATPdH20 (up to 20ul)	
bdh2A	46.5	*1.5*	2.9	2ul	1ul	**2**	*10.6*
bdh2B	2.9	*5.1*	2.9	2ul	1ul	**2**	*7*
CLE	2.5	*5.1*	2.9	2ul	1ul	**2**	*7*
Phaz1	16.1	*4*	2.9	2ul	1ul	**2**	*8.1*
9004	35.1	*5.1*	2.9	2ul	1ul	**2**	*7*
9005	3.1	*5.1*	2.9	2ul	1ul	**2**	*7*
MM	-	-	**17.4**	**12**	**6**	12	-

7.9ul of MM to each

ligating O/N 16oC

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	Have Gene Art plasmid
BBa_K1149002	PUR ESTCS2	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149003*	PueA	Have Gene Art plasmid
BBa_K1149004	PueB	ligation into pSB1C3
BBa_K1149005	PudA	ligation into pSB1C3
BBa_K1149006	PulA	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149007*	Proteinase K	Have Gene Art plasmid
BBa_K1149008*	PelB-Proteinase K	Have Gene Art plasmid
BBa_K1149009	CLE	ligation into pSB1C3
BBa_K1149010	Phaz1	ligation into pSB1C3
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	Not synthesised yet
BBa_K1149013	Bdh2	ligation into pSB1C3

All digests will be EcoRI+PstI

Digest planning: Secretion signals

3 fragments generated.

1: 2,243 bp - From EcoRI[370] To PstI[2613] INSERT

2: 1,604 bp - From PstI[2613] To PstI[4217]

3: 680 bp - From PstI[4217] To EcoRI[370]

Digest planning: 9002

3 fragments generated.

1: 2,740 bp - From EcoRI[384] To PstI[3124] INSERT

2: 1,619 bp - From PstI[3124] To PstI[4743]

3: 694 bp - From PstI[4743] To EcoRI[384]

Digest planning: 9003

3 fragments generated. EP

1: 2,228 bp - From EcoRI[384] To PstI[2612] INSERT

2: 1,619 bp - From PstI[2612] To PstI[4231]

3: 694 bp - From PstI[4231] To EcoRI[384]

Digest planning: 9004

1: 2,075 bp - From EcoRI[384] To PstI[2459] INSERT

2: 1,619 bp - From PstI[2459] To PstI[4078]

3: 694 bp - From PstI[4078] To EcoRI[384]

Digest planning: 9005

3 fragments generated.

1: 1,930 bp - From EcoRI[384] To PstI[2314] INSERT

2: 1,619 bp - From PstI[2314] To PstI[3933]

3: 694 bp - From PstI[3933] To EcoRI[384]

Digest planning: 9006

3 fragments generated. Need to triple digest.

1: 1,642 bp - From EcoRI[384] To PstI[2026] INSERT

2: 1,619 bp - From PstI[2026] To PstI[3645]

3: 694 bp - From PstI[3645] To EcoRI[384]

Digest planning: PK

1: 2,131 bp - From EcoRI[370] To PstI[2501] INSERT

2: 1,604 bp - From PstI[2501] To PstI[4105]

3: 680 bp - From PstI[4105] To EcoRI[370]

Digest planning: PelB PK

1: 2,140 bp - From EcoRI[370] To PstI[2510] INSERT

2: 1,604 bp - From PstI[2510] To PstI[4114]

3: 680 bp - From PstI[4114] To EcoRI[370]

Digest CLE

3 fragments generated.

1: 1,838 bp - From EcoRI[384] To PstI[2222] INSERT

2: 1,619 bp - From PstI[2222] To PstI[3841]

3: 694 bp - From PstI[3841] To EcoRI[384]

Phaz1:

need to triple digest

1: 1,619 bp - From EcoRI[377] To PstI[1996] INSERT

2: 1,612 bp - From PstI[1996] To PstI[3608]

3: 687 bp - From PstI[3608] To EcoRI[377]

Digest planning: Phaz2 Need to triple digest.

2 fragments generated.

1: 2,369 bp - From PstI[2716] To EcoRI[384]

2: 2,332 bp - From EcoRI[384] To PstI[2716] INSERT

BDH2

3 fragments generated.

1: 1,804 bp - From EcoRI[384] To PstI[2188] INSERT

2: 1,619 bp - From PstI[2188] To PstI[3807]

3: 694 bp - From PstI[3807] To EcoRI[384]

6/9/13 Dephosphorylate

2.2 buffer, 1AP enzyme

6/9/13 Cloning update, 9002+9006 sequencing

9002A: perfect match at start then sequencing signal declines, also some mutations in middle of enzyme that shift alignment they do fluoresce

9002B: perfect match at start then sequencing signal declines, also some mutations in middle of enzyme that shift alignment they do fluoresce

9006A: perfect match at the start, then some mutations that shift alignment

9006B: perfect match at the start, then some mutations that shift alignment

6/9/13 digest

Secretion signals

9003

PK

PelB PK

6/9/13 O/N culture

4mL LB 2uL chlo,

- BBa_9006 colony 3, 4
- BBa_9002 colony 3, 4

7/9/13 ligation plan

	DNA post gel ex-traction	Insert (ng=ul)fff	Vector (25ng)fff	10X ligase buffer fff	T4 ligase	ATP	dH20 (up to 20ul) fff
PK	7.7ng/ul	10	2.9	2ul	1ul	**2**	2.1
PelB PK	21.1	3.8	2.9	2ul	1ul	**2**	8.3
9003	17.7	4.5	2.9	2ul	1ul	**2**	7.6
Secretion Toolkit	16.3	4.9	2.9	2ul	1ul	**2**	7.2
MM	-	-	11.6	8	4	8	-

7.9ul of MM to each

7/9/13 Update on cloning Biobricks

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	ligation into pSB1C3
BBa_K1149002	PUR ESTCS2	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149003*	PueA	ligation into pSB1C3
BBa_K1149004	PueB	O/N GA then digest
BBa_K1149005	PudA	digest
BBa_K1149006	PulA	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149007*	Proteinase K	ligation into pSB1C3
BBa_K1149008*	PelB-Proteinase K	ligation into pSB1C3
BBa_K1149009	CLE	digest
BBa_K1149010	Phaz1	Have Gene Art plasmid
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	Not synthesised yet
BBa_K1149013	Bdh2	digesting

need GA DNA 9003/PelB-PK/9004/

8/9/13 Update on cloning Biobricks

O/N cultures from glycerol stocks of geneart plasimid constructs

9003 49.3ng/ul, **9004** 58.2ng/ul, **9005** 93.7ng/ul, **pelB PK** 62.3ng/ul

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	transforming NEB10
BBa_K1149002	PUR ESTCS2	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149003*	PueA	transforming NEB10
BBa_K1149004	PueB	ligating
BBa_K1149005	PudA	ligating
BBa_K1149006	PulA	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149007*	Proteinase K	transforming NEB10
BBa_K1149008*	PelB-Proteinase K	transforming NEB10
BBa_K1149009	CLE	ligating
BBa_K1149010	Phaz1	Have Gene Art plasmid
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	Synthesised
BBa_K1149013	Bdh2	ligating

8/9/13 ligation plan

	DNA post gel ex-traction	Insert (ng=ul)	Vector (25ng)	10X ligase buffer	T4 ligase	ATPdH20 (up to 20ul)	
CLE		5.1	2.9	2ul	1ul	2	7
9004		5.1	2.9	2ul	1ul	2	7
9005		5.1	2.9	2ul	1ul	2	7
bdh2		5.1	2.9	2ul	1ul	2	7
MM	-	-	11.6	8	4	8	-

7.9ul of MM to each

9/9/13 Cloning update

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	miniprepped and glycerol stock(NEB10)
BBa_K1149002	PUR ESTCS2	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149003*	PueA	miniprepped and glycerol stock (NEB10)
BBa_K1149004	PueB	transformed MG1655
BBa_K1149005	PudA	transformed MG1655
BBa_K1149006	PulA	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149007*	Proteinase K	miniprepped and glycerol stock(NEB10)
BBa_K1149008*	PelB-Proteinase K	miniprepped and glycerol stock(NEB10)
BBa_K1149009	CLE	transformed MG1655
BBa_K1149010	Phaz1	Have Gene Art plasmid
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	Synthesised
BBa_K1149013	Bdh2	transformed MG1655
BBa_K1149014	Putative Permease	Synthesised

- O/N of phaz2 + PK/PelBPK/9003/SecToolKit
- Need to aliquot pSB1C3 digested backbone into 25ng/ul
- miniprep day cultures

10/9/13 Cloning Update

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	miniprepped and glycerol stocked(NEB10) sending for seq = 89.3ng/ul
BBa_K1149002	PUR ESTCS2	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149003*	PueA	miniprepped and glycerol stocked(NEB10) sending for seq = 50.5ng/ul

BBa_K1149004	PueB	Highly likely to be Biobrick in MG1655
BBa_K1149005	PudA	Highly likely to be Biobrick in MG1655
BBa_K1149006	PulA	Biobrick transformed MG1655 (Picking several more backup colonies)
BBa_K1149007*	Proteinase K	miniprepped and glycerol stocked(NEB10) sending for seq = 112.9ng/ul
BBa_K1149008*	PelB-Proteinase K	miniprepped and glycerol stocked(NEB10) sending for seq = 82.1 ng/ul
BBa_K1149009	CLE	Highly likely to be Biobrick in MG1655
BBa_K1149010	Phaz1	Have Gene Art plasmid
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	Synthesised
BBa_K1149013	Bdh2	Highly likely to be Biobrick in MG1655
BBa_K1149014	Putative Permease	Synthesised

- Send for sequencing PK, PelB-PK, 9003, 9001, PelBLCutinase, PhaBC DONE
- O/N cultures, miniprep next day and send for sequencing: 9004,9005, CLE, bdh2 DONE
- 9002 and 9006 need to pick more colonies to check so 1 O/N culture each MG1655.

11/9/13

- O/N of phaz2 (Gene Art).

10/9/13 (MK)

- PLC A (from BBa_K936020 in MG 1655) to glycerol stock, miniprep and sent for sequencing (83.1 ng/ul) and it□s good. put it into a woing box.
- phaBC41 (from glycerol stock) glycerol stocked coz the previous one melted (sorry I binned that) and minipreped, sent for sequencing (157.6 ng6ul)

other minipreps:

	conc	A 260/280
9006	21.2	2.2
9002	27.7	1.92
PK A	14.2	1.83
PK B	11.1	1.72
PPK A	16.7	1.92
PPK B	9.7	2.05
SecA	15.5	2.05
Sec B	21.2	2.06

9003 A	12.2	2.35
9003 B	13.3	2.34

10/9/13 Transformation (MK)

- bdh1 from synthesis (1ul of 1ug/ul)
- EV ligation 5ul
- PK lig 5ul (Proteinase K)
- PPK lig 5ul (pelB proteinaseK)

please sometime/9/13 send biobricks to registry

shipment 01867

Tube	Part	Plasmid	Resistance	Notes
1	BBa_K1149020	pSB1C3	C	amilCP_A (27/08/13) 25.3 ng/ul
2	BBa_K1149021	pSB1C3	C	LARD1_5 (27/09/13) 55.7 ng/ul

11/9/13 Cloning update

Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	no seq results., resending and picking more colonies
BBa_K1149002	PUR ESTCS2	Picked additonal colony, sent for seq
BBa_K1149003*	PueA	mostly aligns, but multiple mutations
BBa_K1149004	PueB	Highly likely to be Biobrick in MG1655, 2x colonies sent for seq.
BBa_K1149005	PudA	Highly likely to be Biobrick in MG1655, 2x colonies sent for seq.
BBa_K1149006	PulA	Picked additional colony, sent for seq
BBa_K1149007*	Proteinase K	mostly aligns, but multiple mutations
BBa_K1149008*	PelB-Proteinase K	no seq results., resending and picking more colonies
BBa_K1149009	CLE	Highly likely to be Biobrick in MG1655, 2x colonies sent for seq.
BBa_K1149010	Phaz1	Have Gene Art plasmid

BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	GeneArt transformed to MG1655
BBa_K1149013	Bdh2	Highly likely to be Biobrick in MG1655, 2x colonies sent for seq.
BBa_K1149014	Putative Permease	Synthesised

miniprep results

- CLE A 144.6ng/ul
- CLE B 168.2ng/ul
- bdh2 A 168.5ng/ul
- bdh2 B 163.6ng/ul
- 9004 A 213.2ng/ul
- 9004 B 205.9ng/ul
- 9005 A 37.4ng/ul
- 9005 B 47.9ng/ul
- 9002 I 200.4ng/ul
- 9006 I 216.8ng/ul

All of these sent for seq as described above.

12/9/13 Tasks to do and cloning update

Resend for sequencing (since they failed see above table)

- Secretion toolkit
- PelB-PK

Pick 2x colonies

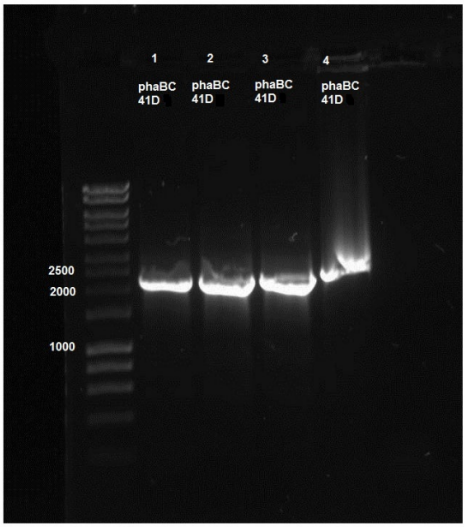
- Secretion toolkit
- PelB-PK
- PK
- 9003

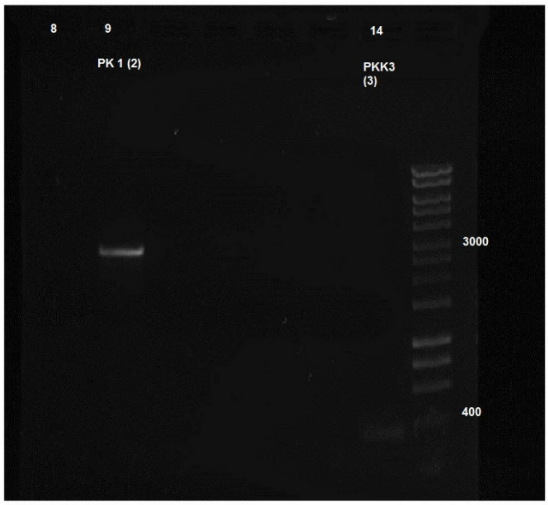
Biobrick Number	Description	Status
BBa_K1149001*	Secretion Toolkit	no seq results., resending and picking more colonies
BBa_K1149002	PUR ESTCS2	Biobrick (most recent one)
BBa_K1149003*	PueA	mostly aligns, but multiple mutations, picked more colonies
BBa_K1149004	PueB	9004A: Biobrick 9004B: Biobrick (has slightly better coverage too)
BBa_K1149005	PudA	Not biobrick, re-pick colonies for seq, consider re-diesting out insert

BBa_K1149006	PulA	Biobrick (most recent one)
BBa_K1149007*	Proteinase K	mostly aligns, but multiple mutations, picked more colonies
BBa_K1149008*	PelB-Proteinase K	no seq results., resending and picking more colonies
BBa_K1149009	CLE	CLEA: Biobrick CLEB: mutated Base deletion - G1024
BBa_K1149010	Phaz1	Have Gene Art plasmid
BBa_K1149011*	Phaz2	Have Gene Art plasmid
BBa_K1149012	Bdh1	GeneArt transformed to MG1655
BBa_K1149013	Bdh2	Bdh2A: biobrick (This one best). bdh2B: mutated biobrick (last two bases little weird?).
BBa_K1149014	Putative Permease	Synthesised

12/9/13 Colony PCR (MK)

colony PCR from the transformations and from previous phaBC41D colonies (re-streaked to single cells)





Biobrick Number	Description	Status	Glycerol stock in MG1655?
BBa_K1149001*	Secretion Toolkit	no seq results., resending and picking more colonies	
BBa_K1149002	PUR ESTCS2	Biobrick Res send for seq 11/9/13 I Then bin rest if OK	plate/DNA/ glycerol stock-11/9/13 9002 I
BBa_K1149003*	PueA	**BINED DNA, finding more colonies**	
BBa_K1149004	PueB	9004A: Biobrick 9004B: Biobrick (has slightly better coverage too).	plate/DNA/ glycerol stock-9004A+B 9/9/13
BBa_K1149005	PudA	Not biobrick, re-pick colonies for seq, consider re-diesting out insert, **binned A+B 11/9/13, +9005 ligation+ transformed ligation plate**	
BBa_K1149006	PulA	Biobrick Res send for seq 11/9/13 I Then bin rest if OK	plate/DNA/ glycerol stock-11/9/13 9002 I
BBa_K1149007*	Proteinase K	mostly aligns, but multiple mutations, picked more colonies, **binned 10/9/13 DNA**	
BBa_K1149008*	PelB-Proteinase K	no seq results., resending and picking more colonies **binned 10/9/13 DNA**	
BBa_K1149009	CLE	CLEA: Biobrick **CLEB: mutated Base deletion - G1024 bined**	plate/DNA/ glycerol stock-CLEA 9/9/13

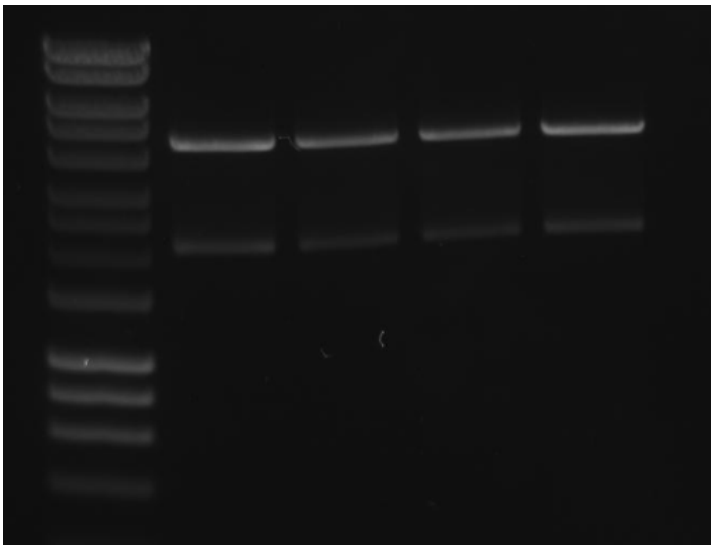
BBa_K1149010	Phaz1	Have Gene Art plasmid	
BBa_K1149011*	Phaz2	Have Gene Art plasmid	
BBa_K1149012	Bdh1	GeneArt transformed to MG1655	
BBa_K1149013	Bdh2	Bdh2A: biobrick (This one best). **bdh2B: mutated biobrick (last two bases little weird?). binned**	plate/DNA/ glycerol stock- Bdh2A 9/9/13
BBa_K1149014	Putative Permease	Synthesised	

12/9/13 Digest (MK)

phaABC4, phaBC41D, phaBC1 (41D), phaBC2 (41D)

they are all the same thus we have no phaBC in theres. :(

fail.



13/9/13 bdh2 PCR

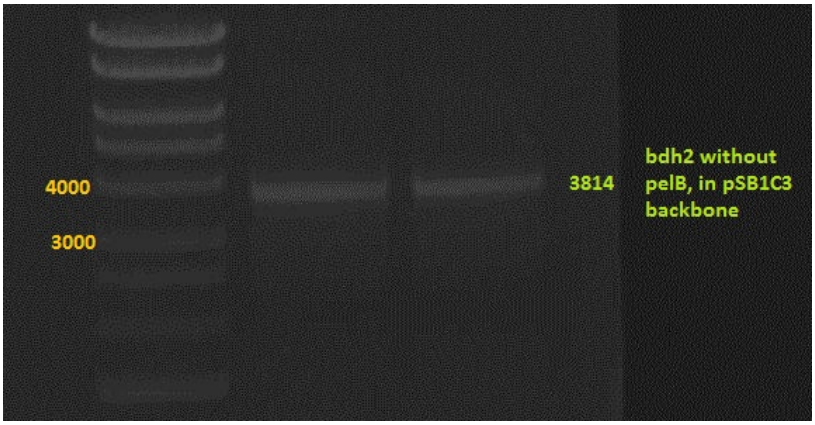
template: bdh2_A (verified by sequencing) in pSB1C3

P1: bdh2_Fw

P2:RBS_Rv

Pfu polimerase

program: 95:20, 55:20, 72:30



I have cut out and purified the PCR product.

13/9/13 cloning update

Biobrick Number	Description	Status	Glycerol stock in MG1655?
BBa_K1149001*	Secretion Toolkit	no seq results., resending and picking more colonies	
BBa_K1149002	PUR ESTCS2	Biobrick: plate/DNA/ glycerol stock-11/9/13 9002 I Biobrick Resend for seq with BBa_G1004	Yes and plate
BBa_K1149003*	PueA	**BINED DNA, finding more colonies**	
BBa_K1149004	PueB	Biobrick: plate/DNA/ glycerol stock-9004A+B 9/9/13 B slightly better coverage so send that one	Yes and plate

BBa_K1149005	PudA	Have Gene Art plasmid	
BBa_K1149006	PulA	Biobrick: plate/DNA/ glycerol stock-11/9/13 9006 I Biobrick Resend for seq with BBa_G1004	Yes and plate
BBa_K1149007*	Proteinase K	mostly aligns, but multiple mutations, picked more colonies, **binned 10/9/13 DNA**	
BBa_K1149008*	PelB-Proteinase K	no seq results., resending and picking more colonies **binned 10/9/13 DNA**	
BBa_K1149009	CLE	Biobrick: plate/DNA/ glycerol stock-CLEA 9/9/13	Yes and Plate
BBa_K1149010	Phaz1	Have Gene Art plasmid	
BBa_K1149011*	Phaz2	Have Gene Art plasmid	
BBa_K1149012	Bdh1	GeneArt transformed to MG1655	
BBa_K1149013	Bdh2	Biobrick: plate/DNA/ glycerol stock-Bdh2A 9/9/13	Yes and Plate
BBa_K1149014	Putative Permease	Synthesised	

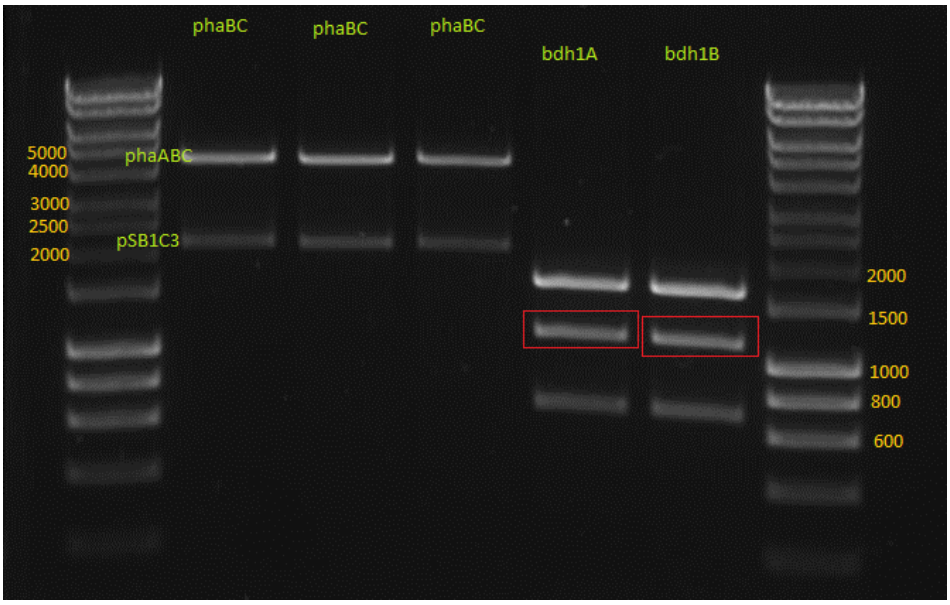
- Sequence 9002+9006 I with G1004 (prefix binding)

  Sequence with VF2
- Secretion toolkit x2
- PelB-PK x2
- PK x2
- 9003 x2

14/09/13 Digest with Pst1 and Xba (MK)

1: phaBC 41D, the plasmid from the glycerol stock-culture that was also sent for sequencing

2: phaBC41D1 and 3: phaBC41D2 - restreaked from original colony that the glycerol stock was from, ON, miniprep.



The phaBC is clearly not pha BC but phaABC in fact. This means that all the experimental data that we collected for phaCB (phaBC) is invalid. :(

phaBC expected 2952

phaABC expected 4201

pSB1C3 is 2070

bdh1A, bdh1B - the bdh1 contstruct that from geneart. miniprep from MG1655. I am gel purifying this and ligating it into pSB1C3 backbone.

14/09/13 AP treatment

pBS1C3 MM sample with 3.3 ul AP, 2 ul buffer

10 min 37 degrees

2min 75 degrees

14/09/13 Dpn1 digest (MK)

the pcr products from inverse PCR in order to remove original plasmid.

- bdh2 gel purified (13/9/13) (9ng/ul)
- phaBC 41 gel purified (see PHB lab book)
- phaBC 42 gel purified (see PHB lab book)
- phaBC 42 ligation (was transformed previously but did not make colonies, well, we'll give it one more try)

14/09/13 Ligations (MK)

in 20 ul reactions

	bdh2	phaBC41	phaBC42	bdh1A	bdh1B
insert	(10ul) ~100 ng	(10ul) ~100 ng	(10ul) ~100 ng	(6ul) 75ng	(12ul) 75ng
vector	-	-	-	(0.5ul) 25ng	(0.5ul) 25ng

15/09/13 Transformation (MK)

5ul of each into 30ul MG1655 cells.

- phaBC42 old
- phaBC41
- phaBC42
- bdh2
- bdh1A
- bdh1B

only bdh2 grew. :(so I repeated the thing with NEB5 cells.

16/09/13 Transformation (MK)

into 50ul NEB5 cells.

- phaBC42 old
- phaBC41
- phaBC42
- bdh1A
- bdh1B

16/09/13 O/N (MK)

4 cultures picked from bdh2. miniprep and send for seq tmr.

16/09/13 Streak (MK)

- 9002, estCS2 ara
- 9003, pul A xylose
- 9004, pueB Xylose
- 9006, pulA ara

onto Impranil-light plates. (with appropriate induction)

Biobrick update 16/9/13

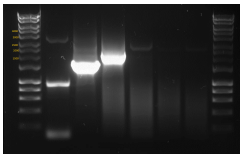
Biobrick Number	Description	Status	Glycerol stock in MG1655?
BBa_K1149001*	Secretion Toolkit	Biobrick Sec 2	No NEB10
BBa_K1149002	PUR ESTCS2	Biobrick: plate/DNA/ glycerol stock-11/9/13 9002 I	Yes and plate
BBa_K1149003*	PueA	Biobrick 9003 1 and 2	No NEB10
BBa_K1149004	PueB	Biobrick: plate/DNA/ glycerol stock-9004A+B 9/9/13 B slightly better coverage so send that one	Yes and plate
BBa_K1149005	PudA	Have Gene Art plasmid	
BBa_K1149006	PulA	Biobrick: plate/DNA/ glycerol stock-11/9/13 9006 I	Yes and plate
BBa_K1149007*	Proteinase K	Biobrick PK1	No NEB10
BBa_K1149008*	PelB-Proteinase K	BioBrick PelBPK2	No NEB10
BBa_K1149009	CLE	Biobrick: plate/DNA/ glycerol stock-CLE-A 9/9/13	Yes and Plate
BBa_K1149010	Phaz1	Have Gene Art plasmid	
BBa_K1149011*	Phaz2	Have Gene Art plasmid	
BBa_K1149012	Bdh1	GeneArt transformed to MG1655, being digested	
BBa_K1149013	Bdh2	Biobrick: plate/DNA/ glycerol stock-Bdh2-A 9/9/13	Yes and Plate

BBa_K1149014	Putative Permease	Synthesised

16/09/13 miniprep bdh2 (MK)

	A	A 260/280	seq
1	147		4bp missing (including ATG)
2	115		lots missing
3	129		lots missing
4	98		lots missing
5	34.7	1.83	lots missing
6	31.3	1.83	scar and prefix missing

19/09/13 Contamination check , because of contamination on Western



We conclude that the DNA samples we used are not contaminated and the reason for the extra band on the western is something else.

20/09/13 YebF PCR, with PfU

- template:secretion toolkit, geneart
- P1: fus-Yebf
- P2: pref-yebF

program: 95:20, 60:20, 72:15, 35X

it didin□t wo. >(Should do a gradient PCR in order to figure out the best ™ but due to lack of time, I□m leaving this for now.

19/09/13 Ligations

purified DphaABC, digested it with Pst1, Xba.

20.7 ng/ul (1.82)

purified P+RBS1, digested, AP treated (from 20/8)

26.5 ng/uL

purified from pooled phaBC1 and phaBC2. (14.9) and cut out the right size band from a gel.

	insent	vector
1 bdh2	-	bdh2 (100ng, repeat)
2 phaCB	-	phaCB (100ng)
3 Dpha1	DphaCAB (75ng)	P+RBS1 (25ng)
4 Dpha2	DphaCAB (75ng)	pSB1C3 (25ng)
5 Phaz1	Phaz1 (75ng)	pSB1C3 (25ng)
6 bdh1	bdh1A (75ng)	pBS1C3 (the other, 25ng)

The MM (it's not MM, it is the pSB1C3M. run out now….)

16degrees ON.

Going to heat inactivate the ligase before I am transforming.

I used a new NEB ligase and ligase buffer from Biochemistry because the previous ligations did not wo and I think that the ATP or the enzyme might have been the reason. I have purified the DNA samples for the same reason, as they were only heat inactivated before…

20/09/13 Transformation

5ul of all the above ligation into NEB5 cells.

We got colonies the next day. :)

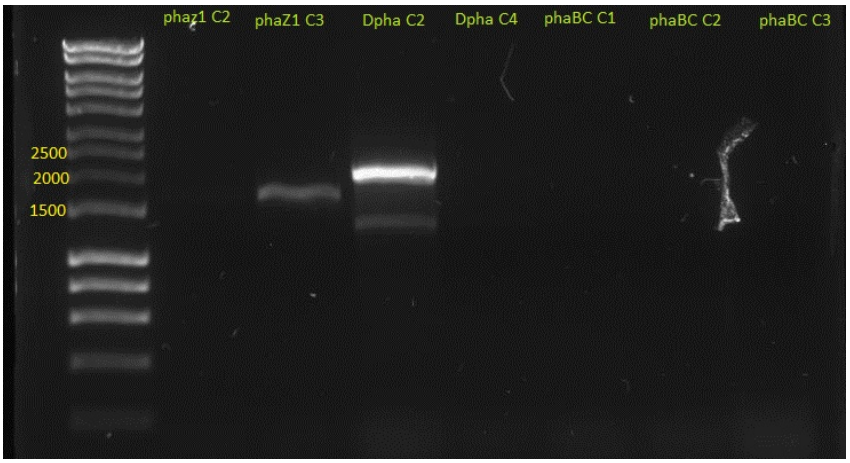
21/09/13 Colony PCR

Picked 7 colonies from each plate (each ligation.) The PCR Did not wo and the band sizes are not corresponding to anything we would expect. I put the samples directly into the PCR reaction, which was maybe not the best thing to do.

22/09/13 Colony PCR , again with some colonies

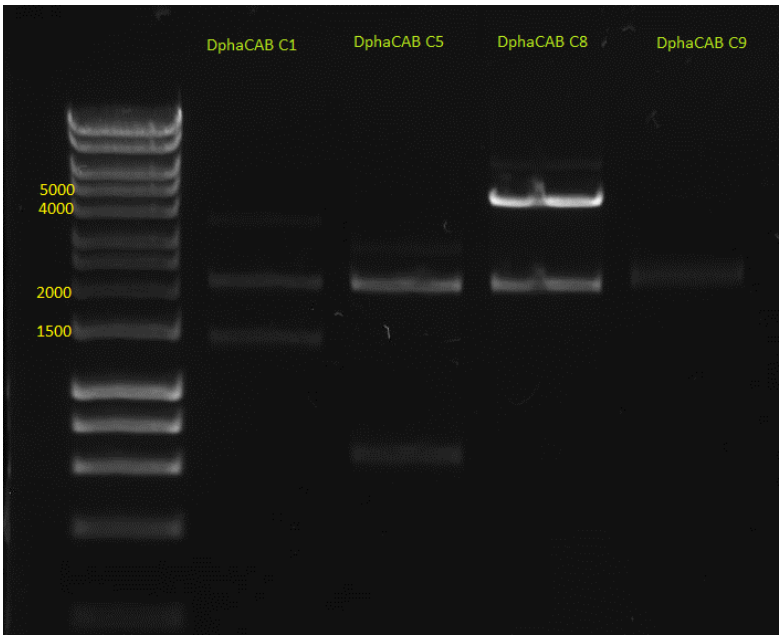
I prepared the templates where I diluted the bacteria picked from the plate in 20uL H2O. This gave nicer results in some cases but no results most of the time. So, there probably was not enough template.

What James suggests is to boil the bacteria in H2O, centrifuge them and use a bit of the supernatant as template. We'll see if this wos next time I do colony PCR.



I set up O/N from some Dpha1 ligations so that I can have a look at the plasmids by digest and not do any more colony PCR-

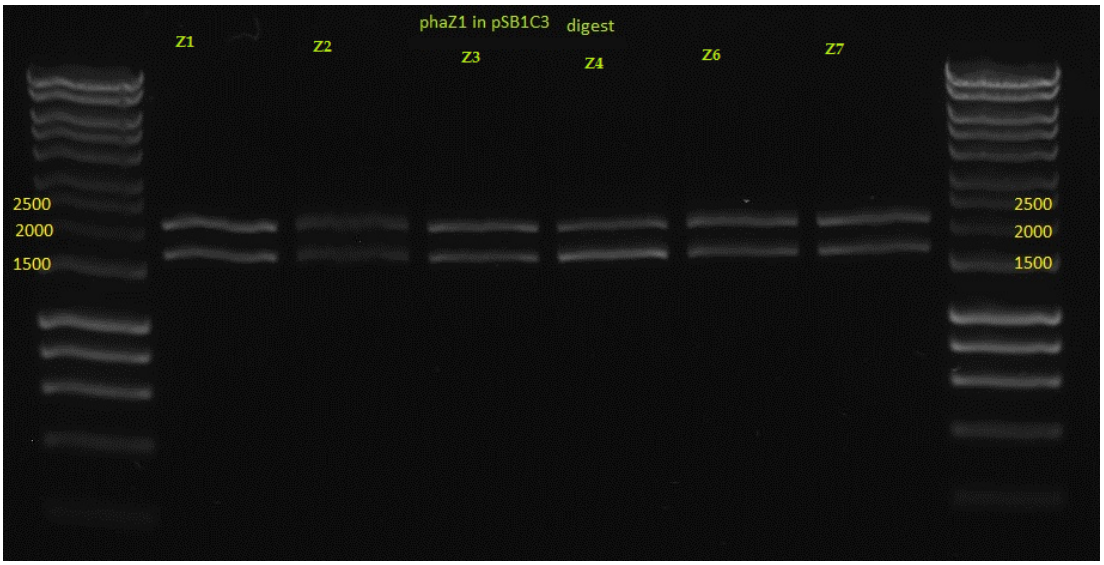
23/09/13 miniprep and digest phaCAB with new Promoter and RBS



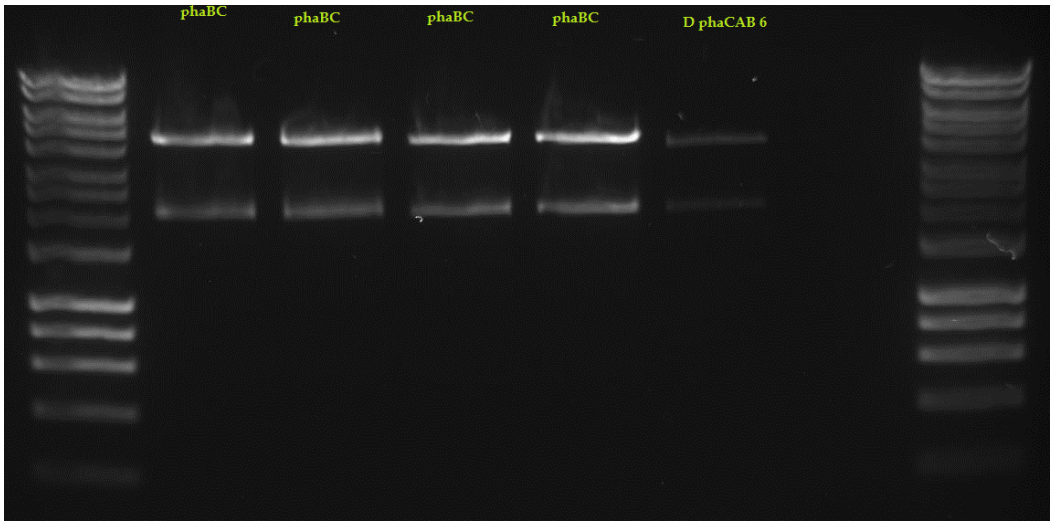
sent phaCAB colonyC8 for sequencing.

Turns out that it has the E.coli P+RBS (BBa_K608002) But also the native promoter and RBS as well. We have submitted this as a biobrick and decided to miniprep more plasmids from different colonies in order to find one that has the new PRBS only and not the native one as well.

24/9/13 Miniprep and digest phaZ1



They are all looking fine and we have sent all of them for sequencing. However, unfortunately, the BioBrick submission deadline is tomorrow. And we decided not to sent any DNA that we have not sequenced.



The phaBC are all too large size and probably have phaCAB instead of just BC. Therefore I made more O/N to screen more plasmids.

The phaCAB6 looks good and it was sent for sequencing. (I also did a new O/N for miniprep because the DNA concentration was low in the sample. Plus I added one more DphaCAB (#7).

24/9/13 Transformations into MG1655

- DphaCAB 8
- DphaCAB 6
- phaZ1 4
- phaZ1 6
- bdh2 4 (the pelB has been succesfully removed from the gene)

Biobrick update 24/9/13

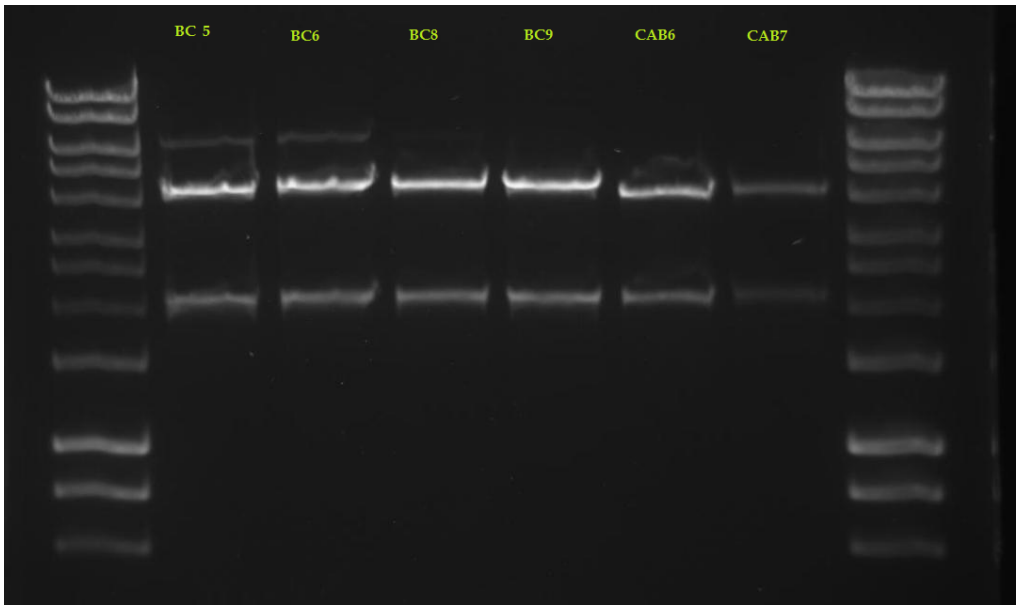
Biobrick Number	Description	Status	Glycerol stock in MG1655?
BBa_K1149001*	Secretion Toolkit	Biobrick Sec 2	not relevant
BBa_K1149002	PUR ESTCS2	Biobrick: plate/DNA/ glycerol stock-11/9/13 9002 I	Yes and plate
BBa_K1149003*	PueA	Biobrick 9003 1 and 2	Yes and plate
BBa_K1149004	PueB	Biobrick: plate/DNA/ glycerol stock-9004A+B 9/9/13 B slightly better coverage so send that one	Yes and plate
BBa_K1149005	PudA	Have Gene Art plasmid	
BBa_K1149006	PulA	Biobrick: plate/DNA/ glycerol stock-11/9/13 9006 I	Yes and plate
BBa_K1149007*	Proteinase K	Biobrick PK1	Yes and plate
BBa_K1149008*	PelB-Proteinase K	BioBrick PelBPK2	Yes and plate
BBa_K1149009	CLE	Biobrick: plate/DNA/ glycerol stock-CLE-A 9/9/13	Yes and Plate
BBa_K1149010	Phaz1	potential biobrick	maybe
BBa_K1149011*	Phaz2	Sent for sequencing.	

BBa_K1149012	Bdh1	GeneArt transformed to MG1655, being digested	
BBa_K1149013	Bdh2	Biobrick: plate/DNA/ glycerol stock-Bdh2-A 9/9/13	Yes and Plate
BBa_K1149014	Putative Permease	Synthesised	

25/09/13 miniprep and digest

find concentrations of plasmids on database

(200ng of each)



We have sent all of these for sequencing.

25/09/13 Streak

I streaked EV, native phaCAB and new phaCAB (#8) onto Nile Red plate in a continuous lawn so that we can image under UV and see if the new phaCAB produces more bioplastic.