Lab Journal 1

THURSDAY, 6/18/2020

Overnight cultures of aquired strains

Picked colonies of Shewanella oneidensis, Shewanella putrefaciens and Escherichia coli MFDpir with pipette tips

Dropped pipette tip in empty E-flask

Cultivated in 10 mL LB media overnight at 28, 28 and 37 ^{o}C respectively shaking at 150 RPM

FRIDAY, 6/19/2020

Glycerol stocks of Shewanella Oneidensis and Putrefaciens

500 µL of the overnight culture to 500 µL of 50% glycerol in a 2 mL screw top tube or eppendorf tube

final concentration of glycerol at 25%.

Stored in -80 ^{o}C

OD600 of overnight cultures

	A	B	C	D
1	19/06/2020, Aditi and Julie	Name on eppendorf	OD	
2	E.coli1	Ec1	0.007	No DAP
3	E.coli2	Ec2	0.006	No DAP
4	Shewanella puterfaciens 1	Sp1	0.515	1:10 dilution
5	Shewanella putrefaciens 2	Sp2	0.329	1:10 dilution
6	Shewanella oneidensis 1	So1	0.347	1:10 dilution
7	Shewanella oneidensis 2	So2	0.367	1:10 dilution
8	Shewanella oneidensis 3	So3	0.366	1:10 dilution

No growth was observed and no significant OD600 was recorded in the culture with E coli MFDpir. This is due to the strain requiring supplemented DAP in the media to grow.

THURSDAY, 6/25/2020

Overnight incubation of E. coli DH5α

Picked colonies of E coli containing the DHα conjugation plasmid with pipette tips

Started a 10 mL O/N incubation with 50 ug/ml Kanamycin

FRIDAY, 6/26/2020

Overnight cultures of E. coli DH5α

O/N cultures at a 1:50 dilution had an OD600 of 0.244

With a OD to cell corrolation of $8*10^{8}$ cells/mL/OD, this corresponds to $9.76*10^{9}$ cells/mL

Glycerol Stocks of E. coli DH5α

800 µL of the overnight culture to 200 µL of 85% glycerol in a 2 mL screw top tube or eppendorf tube
final concentration of glycerol at 17%.
Stored in -80 ^{o}C

Extraction of pSRK-Km from E. coli DH5α

Used a QIAgen Miniprep kit to purify the pSRK-Km plasmid from E. coli
4 plasmid prep tubes were made

pSRK-KM

	Tube	Nanodrop (ng/µl)
1	1	79.9
2	2	63.4
3	3	71.8
4	4	76.0

WEDNESDAY, 7/1/2020

Overnight incubation of MFDpir

Picked new colonies of E coli MFDpir
Started a 10 mL O/N incubation with 0.3 mM DAP

THURSDAY, 7/2/2020

Glycerol Stocks of MFDpir

Made 8 glycerol stocks from O/N incubation
Stored in -80 ^{o}C
Threw out old MFDpir glycerol stocks that were incubated without DAP

Chemically Competent MFDpir Cells

Procedure:

1. Take up to 5 mL from the overnight culture and dilute 1:100 with media in E-flask
2. (If needed) add appropriate concentration of growth supplement (0.3 mM for DAP)
3. Incubate at 37^{o}C with shaking until an OD600 of 0.3-0.5
4. Put the flask on ice for 10 minutes
5. Transfer the culture into two 50 mL falcon tubes
6. Centrifuge at 3000 RPM for 7 minutes
7. Pour off the liquid and and resuspend the pellet in 10 mL ice cold $CaCl_2$
8. Centrifuge at 3000 RPM for 5 minutes
9. Pour off the liquid and and resuspend the pellet in 10 mL ice cold $CaCl_2$
10. Keep on ice for 30 minues
11. Centrifuge at 3000 RPM for 5 minutes
12. Resuspend the pellet in 800 uL $CaCl_2$

Made 20 glycerol stocks from chemically competent MFDpir

Used a 100 mL incubation with 0.3 mM DAP from the O/N cultivation
Incubated until a measured OD600 of 0.292 at a 1:2 dilution, meaning the OD600 was approximately 0.6

Stock of chloramphenicol
Dissolved 250 mg chloramphenicol in 10 mL 70% ethanol
Final stock concentration of 25 mg/ml, giving a 1000X solution

FRIDAY, 7/3/2020

Test Transformation (MFDpir and TOP10)
Used biobrick for RFP (BBa_J23100, also for constitutive promoter) and for TraR (BBa_K916000)
No amplification was done prior - 1 uL of the recieved volume was used
Performed a heat shock transformation of both chemically competent MFDpir (prepared the day before) and TOP10

Procedure TOP10:

1. Thaw cells on ice for 15 minutes
2. Add 2 µL KCM (0.5M KCl, 0.15M $CaCl_2$, 0.25 M $MgCl_2$) in a new 1.5 mL tube
3. Add 15-30 ng of plasmid (or 1-8 µL DNA mix)
4. Add dH_2O or ligation mix to a total volume of 10 µL
5. Incubate on ice for 2-5 minutes
6. Add 10 µL competent cells
7. Incubate on ice for 20 minutes
8. Heat shock at 42°C for 1 minute
9. Put on ice for 3 minutes
10. Add 180 µL of prewarmed TSB media
11. Phenotype with shaking for 1 hour at 37°C
12. Plate on petri dish (160 µL on one half, 20 µL on the other half)
13. Incubate overnight at 37°C

Procedure MFDpir:

1. Set heat block to 42°C
2. Thaw a 100 µL vial of chemically-competent cells slowly on ice
3. Add 50 µL cells to the plasmid-containing solution to 1-5 µL DNA mix
4. Incubate sample on ice for 30 minutes
5. Heat shock at 42°C for 45 seconds
6. Incubate on ice for 2 minutes
7. Add SOC media to a total volume of 200 µL
8. Phenotype with shaking at 150 rpm at 37°C for 1 hour
9. Plate on Petri dish
10. Incubate overnight at 37°C

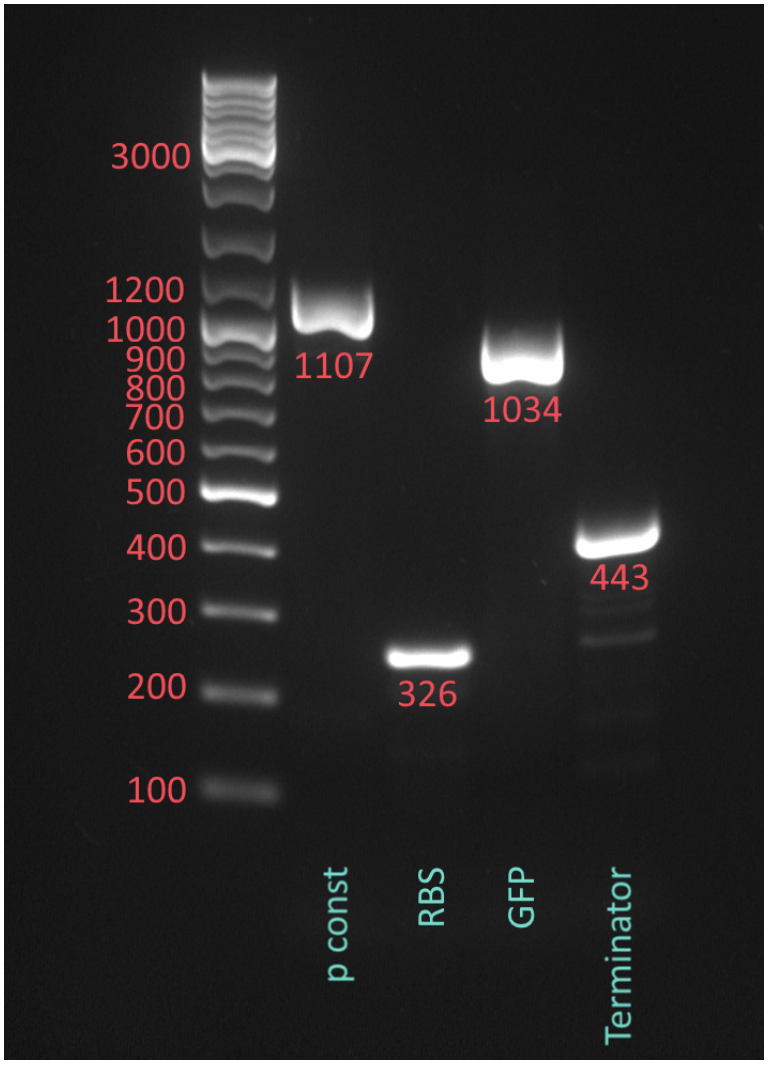
6 samples were prepared. 3 of each cell. 1 RFP, 1 TraR and 1 negative control

Streaked transformants on Cloramphenicol selective plates (with 0.3 mM DAP for MFDpir)
Put in incubator cabinet running P2 (ramp up to 37°C for 24 hours and then ramp down to RT)

BioBrick PCR amplification
BioBricks received from the Uppsala 2020 iGEM team were selected
Ran a PCR amplification of BioBricks containing constitutive promoter (BBa_J23100), RBS (BBa_B0030), GFP (BBa_E0040) and a double terminator (BBa_B0015) with primers VR and VF2

A 1% agarose gel was run at 100 V

PCR_03.07.2020.png



The fragments were of the expected size, suggesting that these BioBricks were correct
The **p const** fragment from BBa_J2300 is not in the standard pSB1C3 backbone, and includes a RBS and RFP downstream the constitutive promoter, giving it its large size

MONDAY, 7/6/2020

Results from Test Transformation

Results from Test Transformation

	A	B	C	D
1		Negative control	RFP	TraR
2	TOP10	no growth	no growth	growth
3	MFDpir	no growth	no growth	no growth

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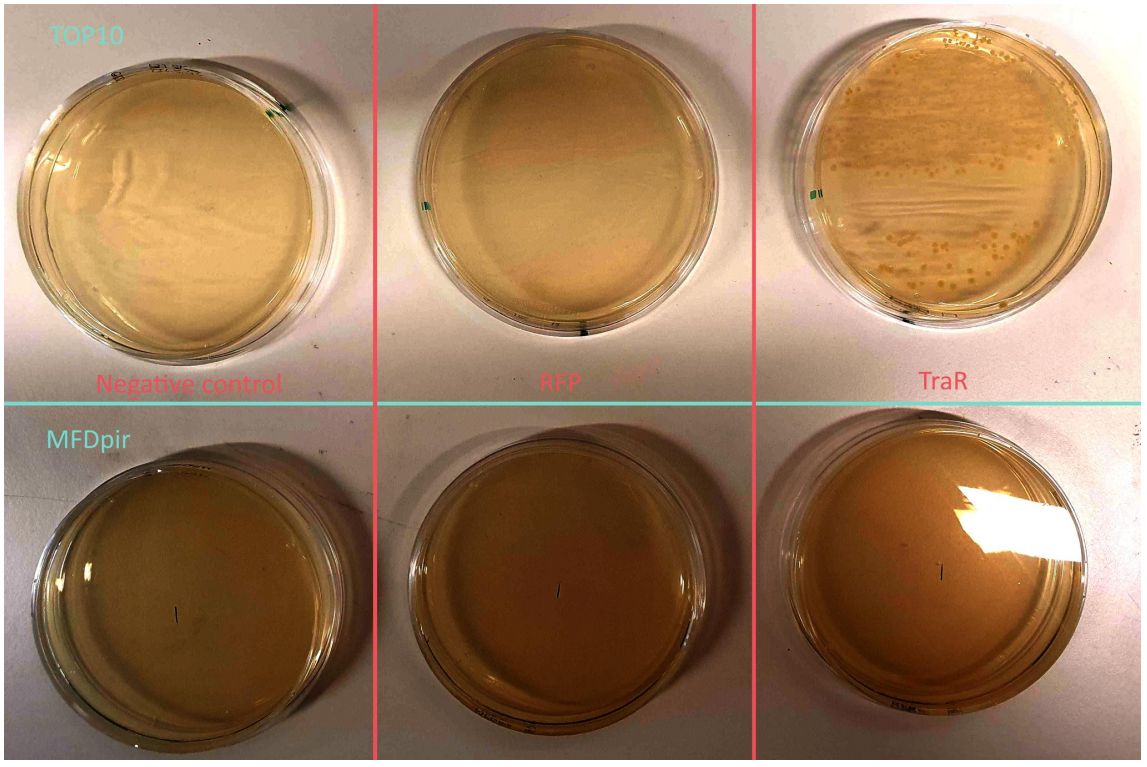


No growth was seen on the negative controls, which is good.
No growth was seen on the RFP transformed cells. This is because we grew the cells on Cloramphenicol plates while the plasmid for BBa_J23100 contains Ampicillin resistance.
Healthy growth occured on the TOP10 plate with TraR plasmid.
Unexpectedly, no growth on the MFDpir TraR plasmid. This may be due to discrepencies in making the cells chemically competent and the follwoing heat shock transformation.

Thus, we decided to do another transformation of MFDpir, this time with the same protocol as found in articles and suggessted by Jonatan martin Rodriguez.

Test Transformation 2
One new 100 uL aliquot of chemically competent MFDpir was taken out, and 40 uL was added to two 1.5 mL tubes
The remaining biobrick for TraR (BBa_K916000) was used (2 uL for each culture)

Procedure MFDpir:

1. Set heat block to 42°C
2. Thaw a 100 µL vial of chemically-competent cells slowly on ice
3. Add 50 µL cells to the plasmid-containing solution to 1-5 µL DNA mix
4. Incubate sample on ice for 30 minutes
5. Heat shock at 42°C for 45 seconds
6. Incubate on ice for 2 minutes
7. Add SOC media to a total volume of 200 µL
8. Phenotype with shaking at 150 rpm at 37°C for 1 hour
9. Plate on Petri dish
10. Incubate overnight at 37°C

Streaked transformants on Cloramphenicol selective plates with 0.3 mM DAP
Put in incubator cabinet running P4 (ramp up to 37^{o}C for 24 hours and then ramp down to 4^{o}C)

Overnight cultures of Shewanella Oneidensis WT and MtrB KO
Picked colonies of Shewanella oneidensis Wildtype and MtrB knockout using pipette tips
Cultivated in 10 mL LB media overnight at 28 ^{o}C with shaking at 150 RPM

Overnight incubation of Chromobacterium Violaceum
Picked colonies of Chromobacterium Violaceum CV026 using pipette tips
Cultivated in 10 mL LB media overnight at 37 ^{o}C with shaking at 150 RPM

TUESDAY, 7/7/2020

Results from Test Transformation 2
Sparse growth on both the plates indicates successful transformation

Glycerol stocks of Shewanella
O/N cultures at a 1:10 dilution had an OD600 of 0.783, which gives an undiluted concentration of 7.83
With a OD to cell corrolation of 8*10^{8} cells/mL/OD, this corresponds to 6.3*10^{9} cells/mL

Made 5 glycerol stocks from each O/N incubation, of which 1 was in a screw top cryotube totaling at 15 tubes (10 MtrB KO, 5 WT)
Stored in -80 ^{o}C

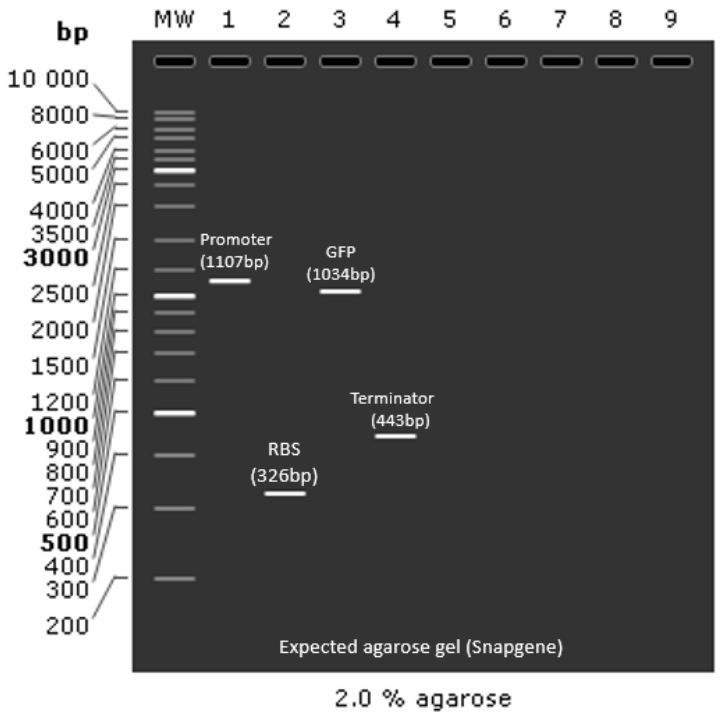
Glycerol Stocks of Chromobacterium
O/N cultures at a 1:10 dilution had an OD600 of 0.713, which gives an undiluted concentration of 7.13
With a OD to cell corrolation of 8*10^{8} cells/mL/OD, this corresponds to 5.7*10^{9} cells/mL

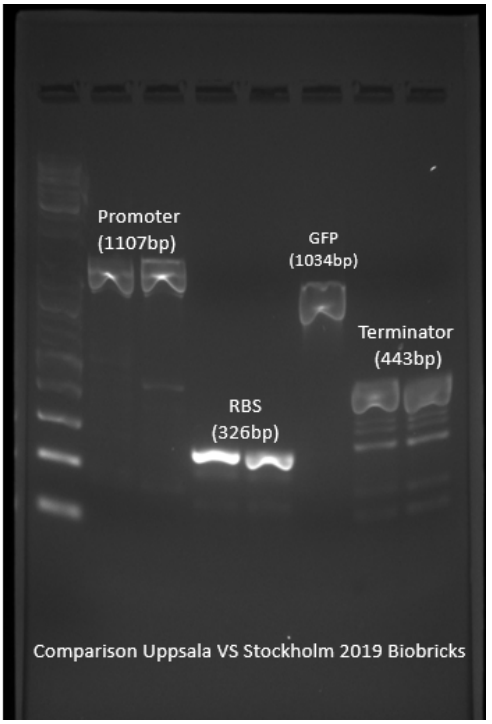
Made 6 glycerol stocks from O/N incubation, of which 1 was in a screw top cryotube
Stored in -80 ^{o}C

BioBrick PCR amplification
BioBricks received from the Stockholm 2019 iGEM team, and the Uppsala 2020 iGEM team were selected
Ran a PCR amplification of BioBricks containing constitutive promoter (BBa_J23100), RBS (BBa_B0030), GFP (BBa_E0040) and a double terminator (BBa_B0015) with primers VR and VF2

A 2% agarose gel was run at 120 V

Gel07_07St_Uppsala_annotated.png





The fragments were of the expected size, suggesting that these BioBricks from both sources were correct

BioBrick in vivo amplification

Started in vivo amplification of 23 biobricks from the Stockholm 2019 kit

BioBricks

	A	B	C	D	E	F
1	Tra R	K916000	2	10I	pSB1C3	1
2	PLux	R0062	2	5H	pSB1C3	2
3	Lux I	C0061	2	3J	pSB1C3	3
4	Lux R (30C6-HSL Receptor)	C0062	6	3O	pSB1A2	4
5	AiiA	C0060	2	3H	pSB1C3	5
6	PRhI	R0071	2	6F	pSB1C3	6
7	Rhl I	C0070	3	8M	pSB1C3	7
8	Rhl R	C0071	3	8O	pSB1C3	8
9	GFP	E0040	6	13K	pSB1A2	9
10	RFP	E1010	3	12M	pSB1C3	10
11	Double Terminator	B0015	3	4E	pSB1C3	11
12	RBS (weak)	B0032	2	1J	pSB1C3	12
13	RBS (strong)	B0030	3	4H	pSB1C3	13
14	RBS (medium)	B0034	6	1M	pSB1A2	14
15	Constitutive Promoter	J23100	6	17C	J61002	15
16	Cin R	C0077	3	10K	pSB1C3	16
17	PCin	R0078	2	4H	pSB1C3	17
18	Las R	C0079	3	10O	pSB1C3	18
19	Las I	C0078	3	10M	pSB1C3	19
20	PLas I	R0079	2	6D	pSB1C3	20
21	pSB1K3		6	6A	J00450	21
22	pSB1C3		4	12P	J00450	22
23	pSB1A3		6	2G	J00450	23
24	m-Cherry	J06504			pSB1C3	

Heat shocked TOP10 E coli to transform the biobricks
Incubated at 37 ^{o}C O/N

THURSDAY, 7/9/2020

BioBrick in vivo amplification

Only 6 of the agar plates showed any growth after 2 days:

- BBa_C0060
- BBa_R0071
- BBa_C0070
- BBa_B0032

- BBa_B0030
- BBa_R0078

5 transformations from previous experiments were also available

- BBa_C0077
- BBa_B0030
- BBa_E0040
- BBa_K916000
- pUC19

Colonies were picked from these and cultured O/N in 15 mL LB with appropriate antibiotic

The remaining BioBricks were transformed and plated
Incubated at 37 ^{o}C O/N

FRIDAY, 7/10/2020

Glycerol stocks of BioBricks
The OD600 of the 11 O/N cultures were taken
Glycerol stocks were prepared and put in -80 ^{o}C

OD600 10/07

	Biobrick	Absorbance
1	R0071	0.336
2	R0030	0.453
3	C0070	0.433
4	K916000	0.501
5	C0077	0.322
6	R0078	0.345
7	E0040	0.320
8	B0032	0.318
9	C0060	0.354

Plasmind prep of BioBricks
Plasmid preps were performed of the O/N cultures to extract the amplified plasmid
Concentration was measured with NanoDrop

Biobricks (1)

	Biobrick	Nanodrop (ng/µL)
1	C0077	95.6
2	C0060	174.8
3	B0032	113.2
4	C0070	83.1
5	B0030	59.4
6	E0040	182.5
7	R0071	70.6
8	R0078	123
9	K91600	157.5
10	J23100	399.7
11	pUC19	552.9

BioBrick in vivo amplification

Colonies were picked from the remaining BioBricks that had grown
These were cultured in 15 mL LB with appropriate antibiotic

Remaining BioBricks that did not work:

- J23100
- pSB1A3
- pSB1C3
- pSB1K3
- C0170
- E1010

Electroporation of BioBricks

The remaining BioBricks are transformed via electroporation rather than heat shocked

SATURDAY, 7/11/2020

Glycerol stocks of BioBricks

The OD600 of the remaining O/N cultures were taken
Glycerol stocks were prepared and put in -80 ^{o}C

OD600 11/07

	Biobrick	Absorbance
1	C0078	0.572
2	C0079	0.480
3	R0062	0.450
4	K1413021	0.462
5	C0062	0.533
6	pSB1C3	0.665
7	C0071	0.433
8	C(R?)0079	0.452
9	J06504	0.576
10	K91600	0.315
11	E1010	0.445
12	C0076	0.455
13	C0061	0.397
14	B0015	0.450

Plasmind prep of BioBricks

Plasmid preps were performed of the remaining O/N cultures to extract the amplified plasmid
Concentration was measured with NanoDrop

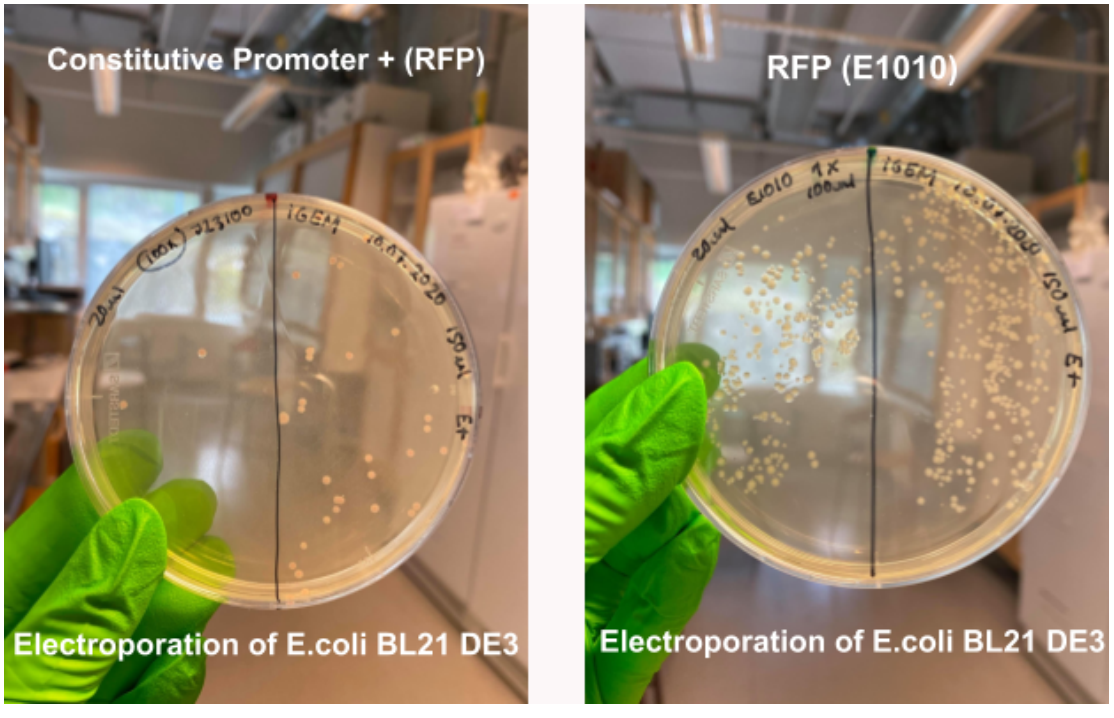
Biobricks (2)

	Biobrick	Nanodrop (ng/µL)
1	K91600	71
2	J06504	142.1
3	B0015	105.7
4	pSB1C3	161.7
5	R0079	122
6	E1010	169
7	C0062	89.2
8	C0061	284.9
9	K1413021	126.8
10	C0071	118.1
11	C0076	141.5
12	R0062	109
13	C0078	133.4
14	C0079	125.1

Electroporation of BioBricks

BBa_J23100 (left) and BBa_E1010 (right) were successfully electroporated

Lab update 16-07.png



They were then incubated O/N to make glycerol stocks and minipreps

OD600 14/07

	Biobrick	Absorbance
1	J23100	0.858
2	pUC19 (control plasmid)	0.699

Biobricks (electroporation)

	Biobrick	Nanodrop (ng/µL)
1	J23100	399.7
2	pUC19 (control plasmid)	552.9

WEDNESDAY, 7/15/2020

Assembly of BioBricks

The following constructs were selected to be assembled, for testing circuits and for building complete constructs

- Constitutive promoter + RBS
- Terminator + Lux promoter
- Terminator + Rhl promoter
- RBS + GFP
- Constitutive promoter in pSRK

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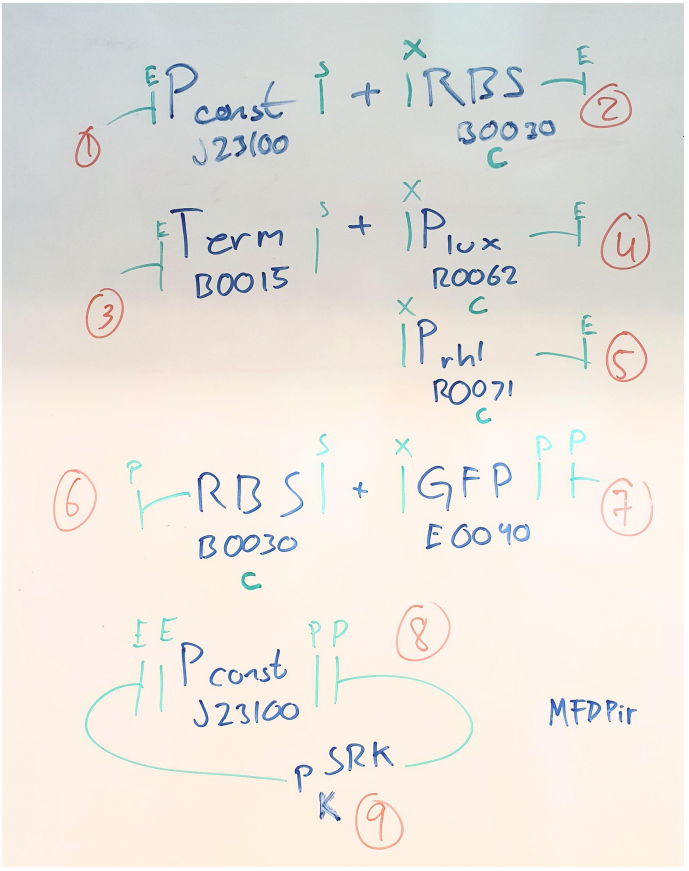

Restriction and digestion

Restriction enzymes used:

- EcoRI
- XbaI
- SpeI
- PstI

Constructs assembled:

- E Constitutive promoter (J23100) S **[1]** + X RBS (strong) (B0030) E **[2]**
- E Double terminator (B0015) S **[3]** + X pLux (R0062) E **[4]**
- E Double terminator (B0015) S **[3]** + X pRhl (R0071) E **[5]**
- P RBS (strong) (B0030) S **[6]** + X GFP (E0040) P **[7]**
- E Constitutive promoter (J23100) P **[8]** + E pSRK P **[9]**

Biobricks (yellow indicates insert DNA)

	Sample	Biobrick No. (BBa_XXXXX)	Length (bp)
1	1	J23100	35
2	2	B0030	15
3	3	B0015	129
4	4	R0062	55
5	5	R0071	53
6	6	B0030	15
7	7	E0040	720
8	8	J23100	35
9	9 (plasmid backbone)	pSRK	5776
10	plasmid backbone	pSB1C3	2070
11	Distance to VF2 primer site		174
12	Distance to VR primer site		140

Procedure restriction digest:

1. Set heat block to 80°C
2. Measure 500 ng of plasmid DNA of each biobrick, the volume is X µL
3. Add (43-X) µL of water
4. Add 5 µL 10X NEB buffer 2.1 each to 1.5 mL tubes
5. Add X µL of DNA
6. Add 1 µL of each restriction enzyme
   (1) p-B1-B2-p
   To open the plasmid before Biobrick 2, use EcoRI and XbaI
   To cut out Biobrick 1 and place before Biobrick 2, use EcoRI and SpeI
   (2) p-B2-B1-p
   To open the plasmid after Biobrick 2, use SpeI and PstI
   To cut out Biobrick 1 and place after Biobrick 2, use XbaI and PstI
7. Incubate at 37°C for 10 minutes
8. For larger DNA like vectors, incubate for 60 minutes to 4 hours (depending on DNA amount)
9. For analytical digest, incubate for 30 minutes
10. (optional) Heat inactivate at 80°C for 20 minutes

Procedure ligation:

Controls:
Biobrick 1 + T4 DNA Ligase (no insert, to check for religation)
Biobrick 2 + T4 DNA Ligase (no insert, to check for religation)
Biobrick 1 + water (no backbone and ligase, to check for undigested plasmid, optional)
Biobrick 2 + water (no insert and ligase, to check for undigested plasmid, optional)

1. Set heat block to 65°C
2. Put empty 1.5 mL tube on ice
3. Thaw and resuspend T4 DNA Ligase Buffer (10X) at room temperature (**NOT** T4 DNA Ligase)
4. Measure 50 ng of Biobrick 2 (plasmid), the volume is X µL (5 µL)
5. Calculate 1:3 molar ratio of Biobrick 2 (plasmid) to Biobrick 1 (insert)
   The volume is Y µL
6. Add (17-X-Y) µL nuclease-free water
7. Add 2 µL T4 DNA Ligase Buffer (10X)
8. Add X µL of Biobrick 2
9. Add Y µL of Biobrick 1
10. Add 1 µL T4 DNA Ligase
11. Gently mix the reaction by pipetting up and down
12. Spin down in tabletop centrifuge briefly
13. Incubate for 30 minutes at room temperature
14. Heat inactivate at 65°C for 10 minutes
15. Chill on ice

The constructs were then transformed into E coli TOP10 via heat shock

THURSDAY, 7/16/2020

Assembly of BioBricks results

E Double terminator (B0015) S **[3]** + X pRhl (R0071) E **[5]**

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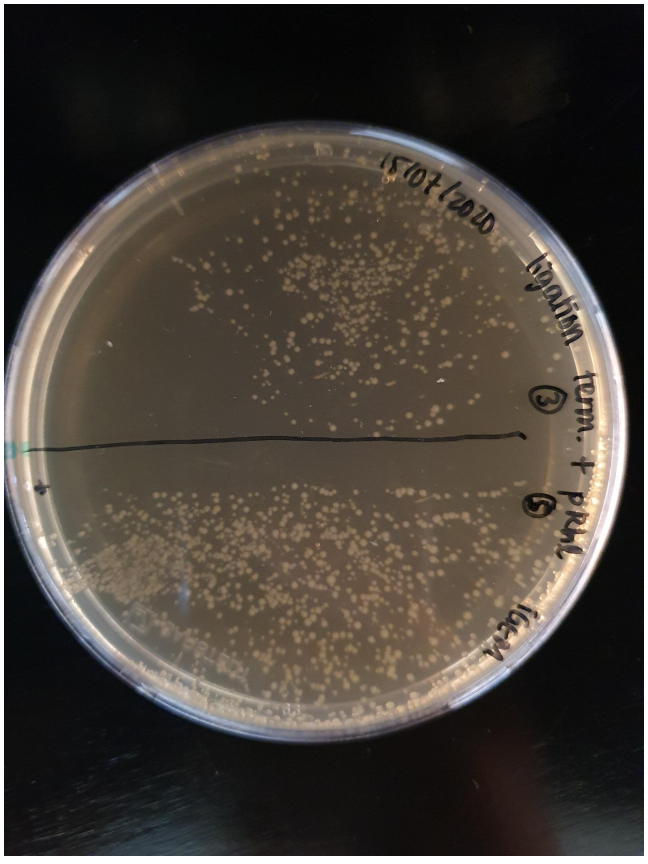

Plenty of growth

Control | X pRhl (R0071) E **[5]**

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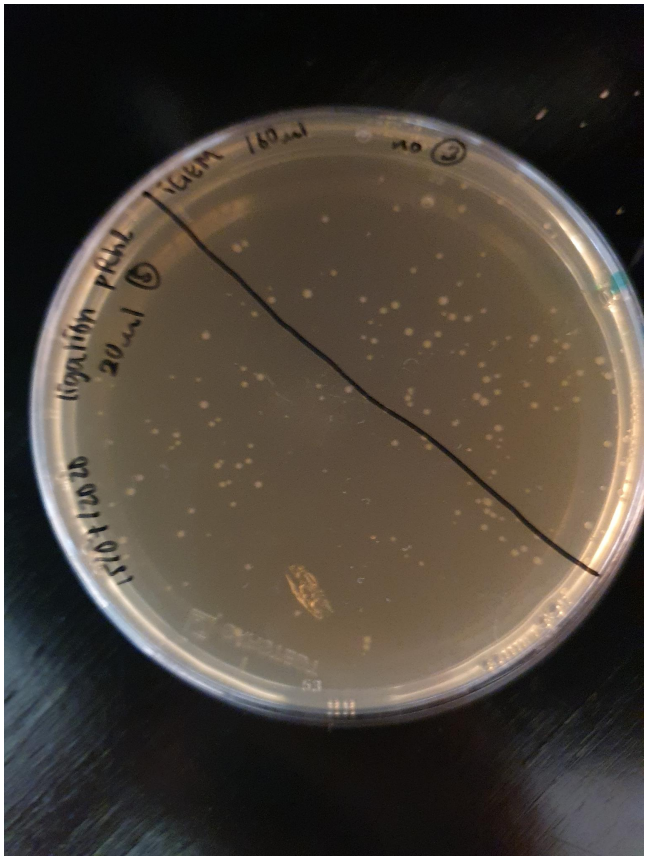

Growth can be seen on the plate, suggesting either incomplete digestion or religation of the plasmid

Control | E Double terminator (B0015) S **[3]**

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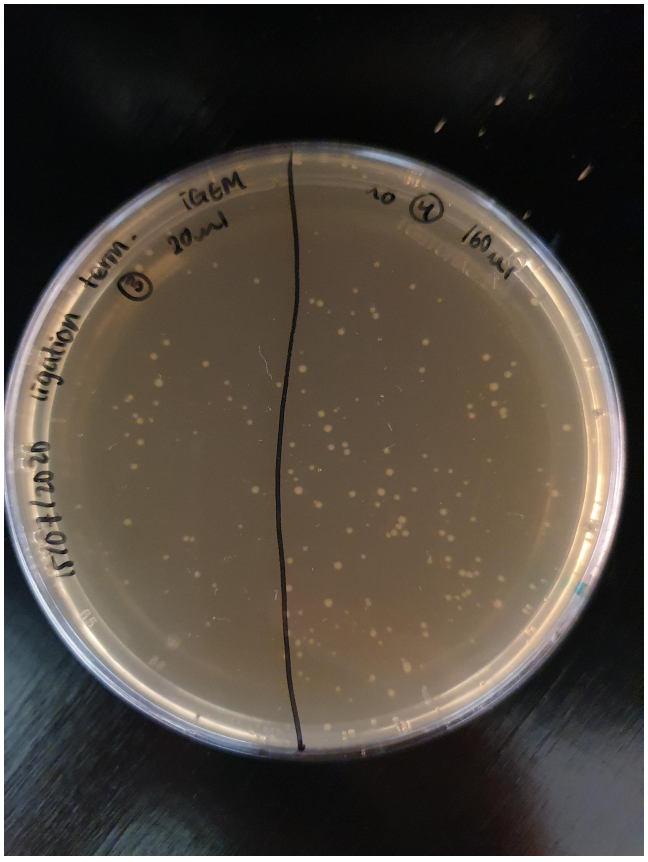

With growth on both background plates, there are a lot of unsuccessful transformants. However, the growth on the control plates do not sum up to the growth on the desired plate, which indicates that there might be colonies with the right insert

E Constitutive promoter (J23100) S **[1]** + X RBS (strong) (B0030) E **[2]**

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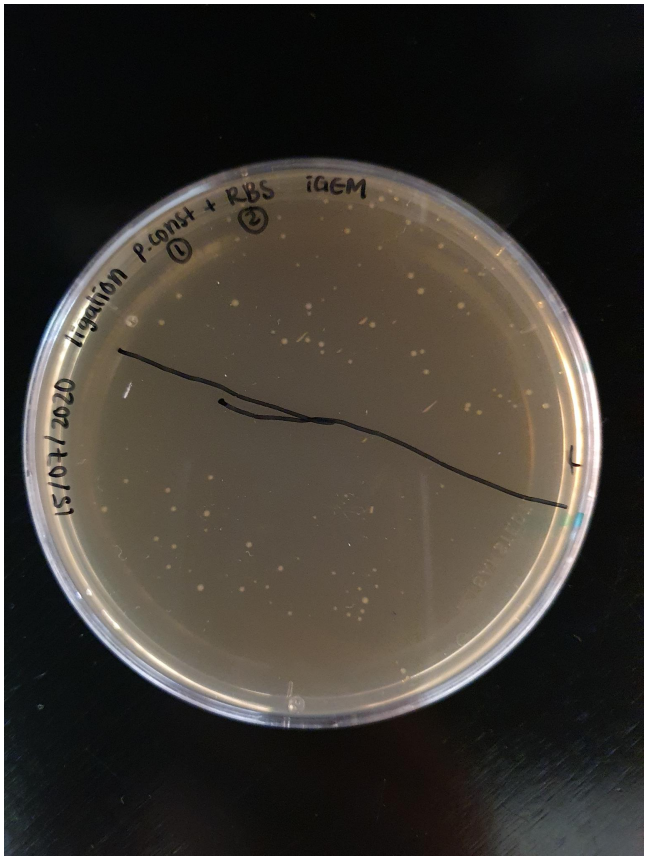

Sparse growth

Control | E Constitutive promoter (J23100) S **[1]**

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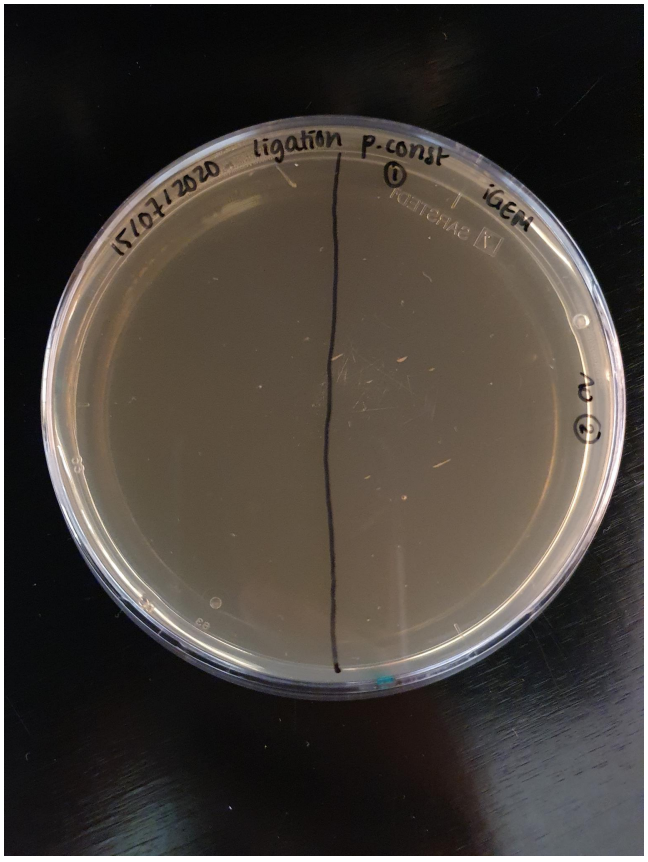

No growth

Control | X RBS (strong) (B0030) E **[2]**

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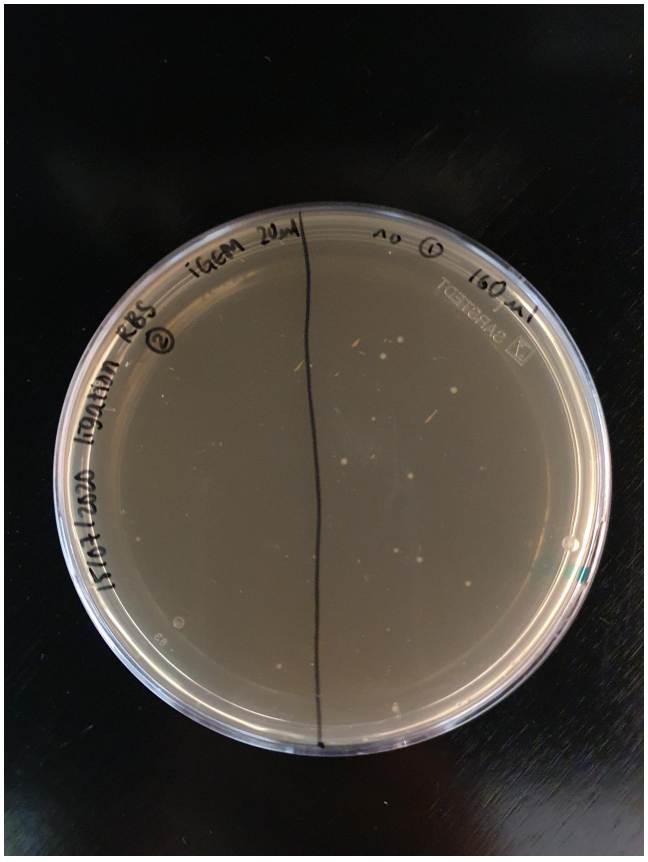

Sparse growth. This is less than that of the desired plate, but chances are small that a correct colony can be found with so few colonies

E Double terminator (B0015) S **[3]** + X pLux (R0062) E **[4]**

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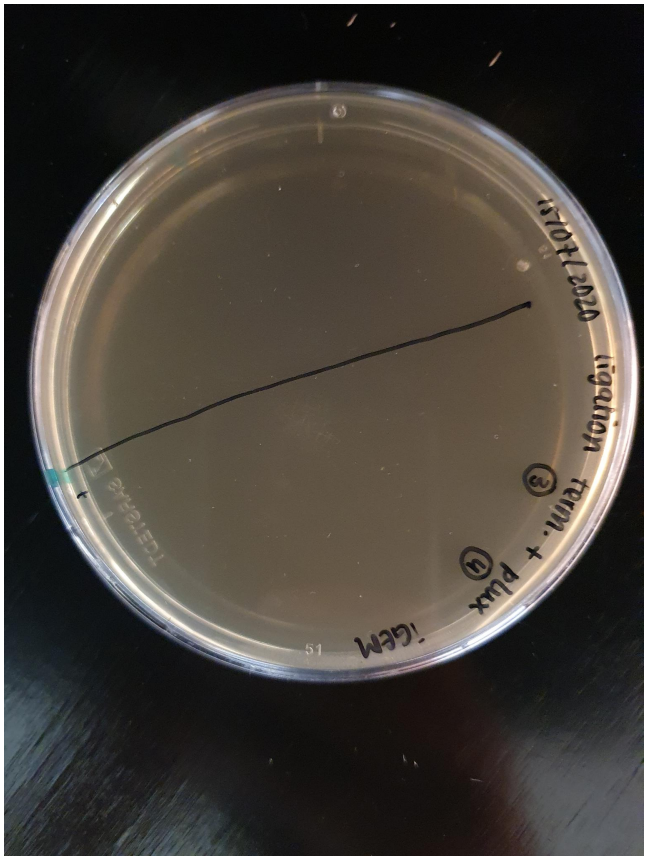
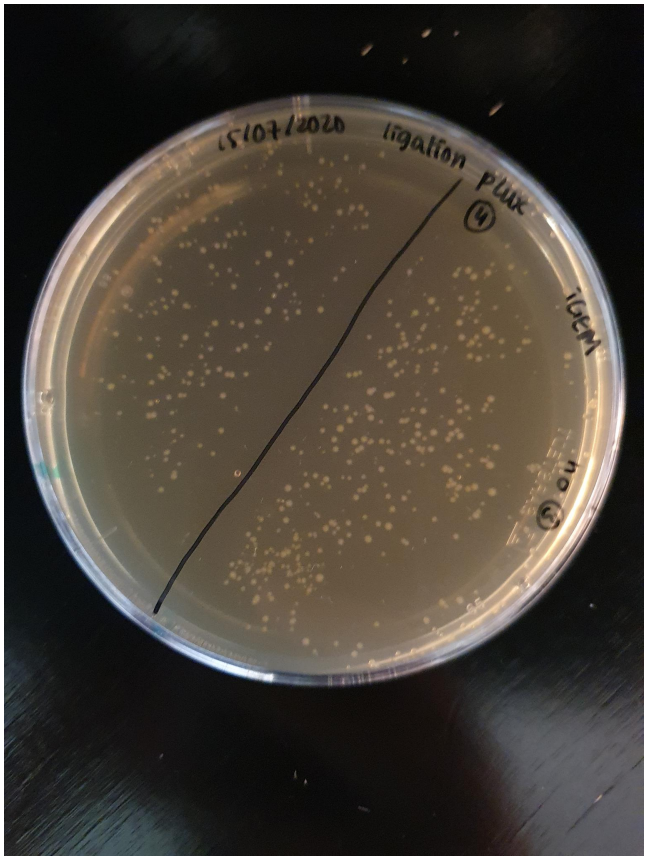


No growth

Control | X pLux (R0062) E **[4]**

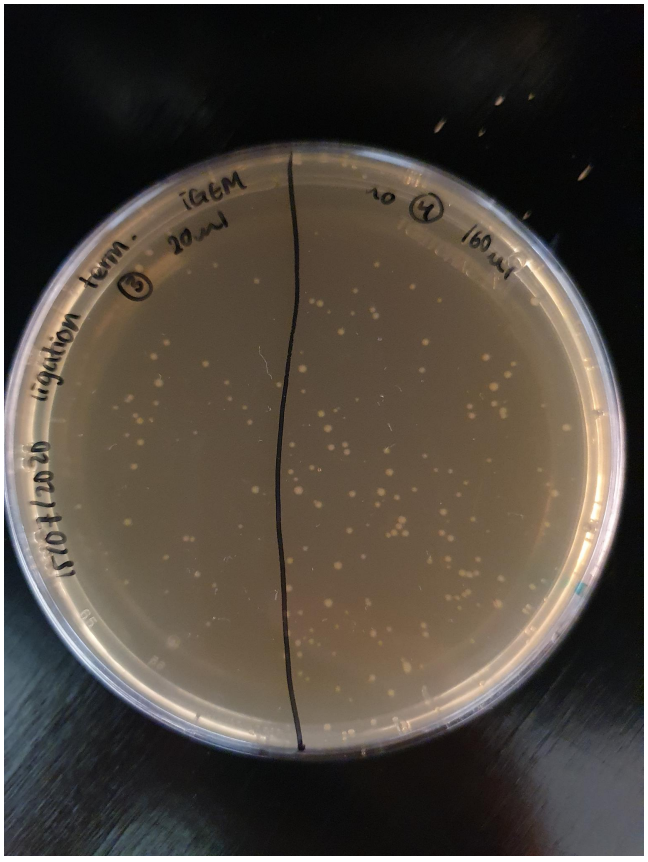
20200717_094955.jpg



Heavy growth

Control | E Double terminator (B0015) S **[3]**

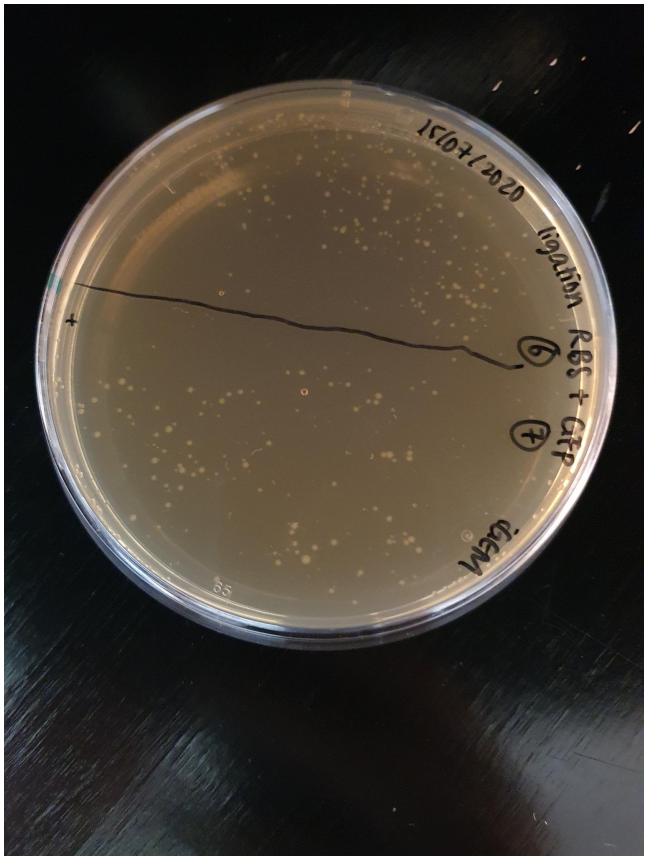
20200717_095011.jpg



Heavy growth. Both the background plates have colonies, while the desired plate does not. The construct may even be detrimental to the cells. Nonetheless, the correct construct cannot be found.

P RBS (strong) (B0030) S **[6]** + X GFP (E0040) P **[7]**

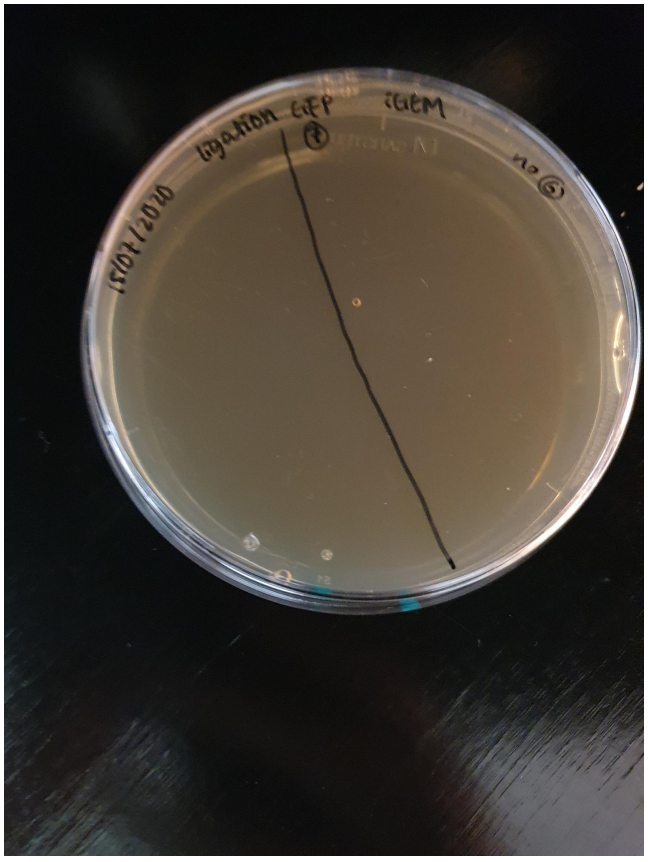
20200717_095042.jpg



Plenty of growth

Control | X GFP (E0040) P **[7]**

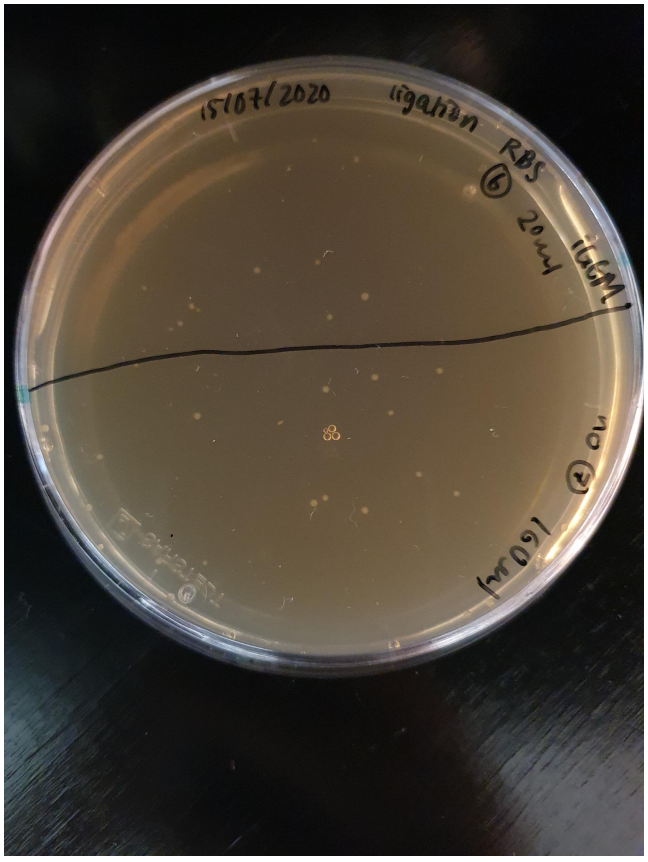
20200717_095058.jpg



No growth

Control | P RBS (strong) (B0030) S **[6]**

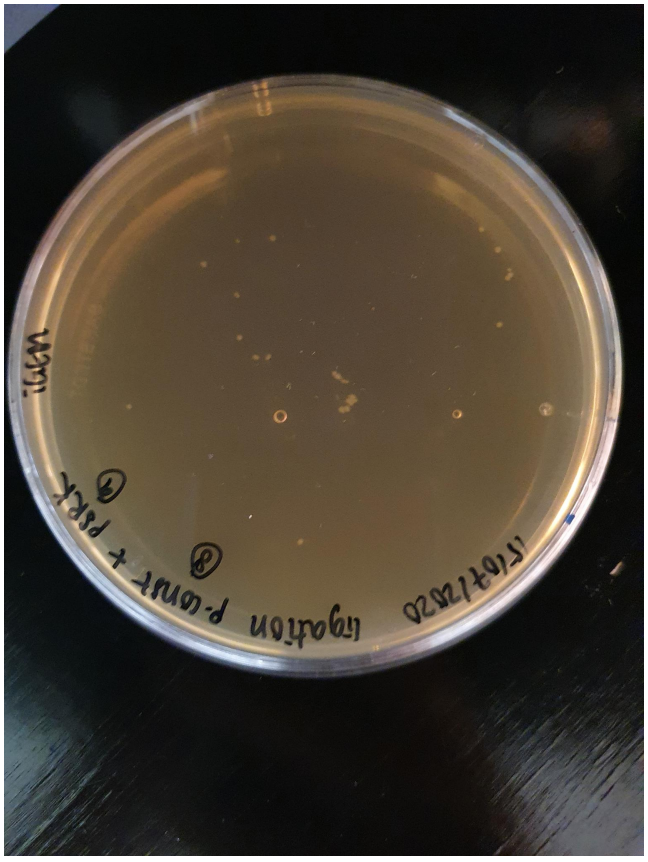
20200717_095113.jpg



Very sparse growth. With little to no growth on the control plates, few colonies should be needed to find the desired construct.

E Constitutive promoter (J23100) P **[8]** + E pSRK P **[9]**

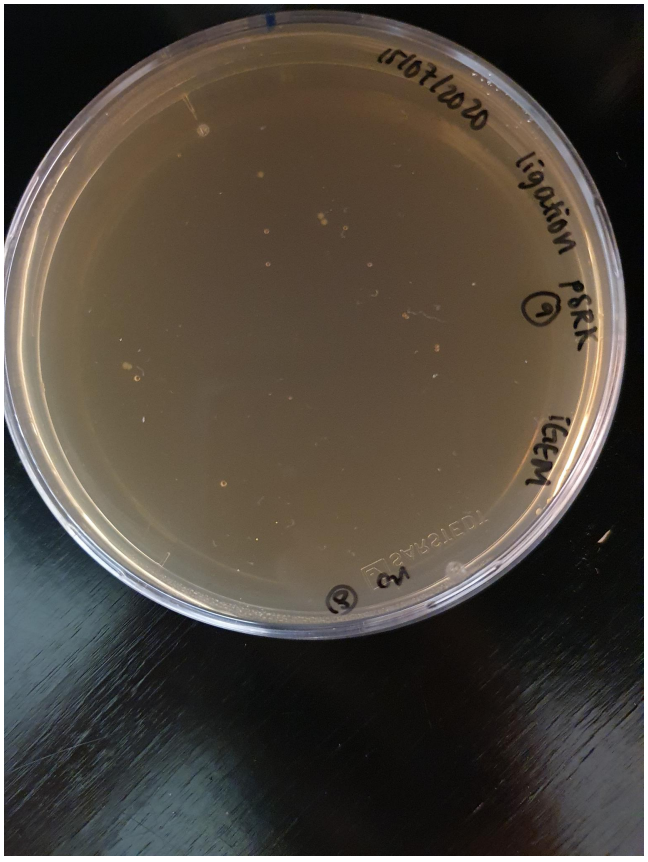
20200717_095126.jpg



Very sparse growth

Control | E pSRK P **[9]**

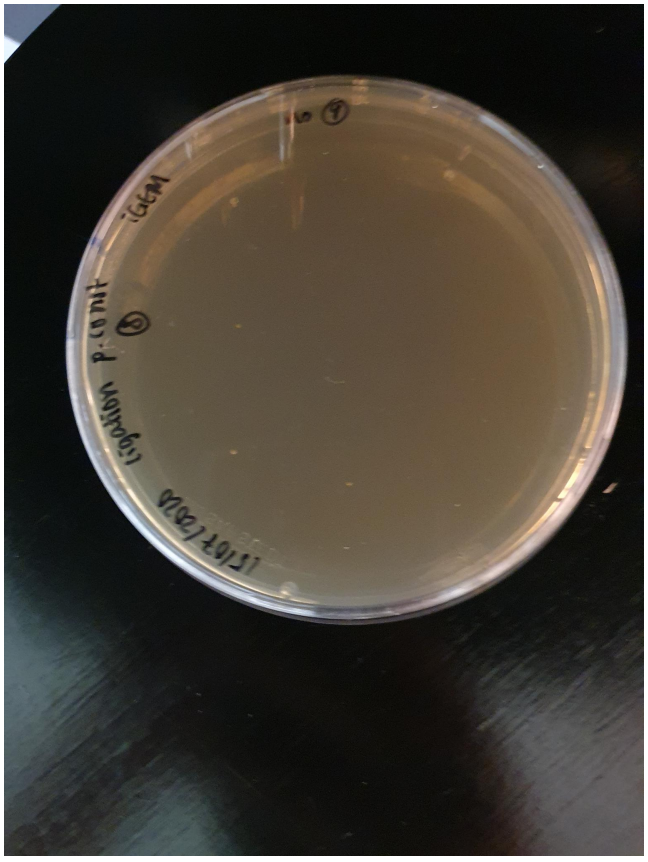
20200717_095139.jpg



Sparse growth

Control | E Constitutive promoter (J23100) P **[8]**

20200717_095151.jpg



No growth. However, with the low amount of growth on the desired plate, chances are slim to find the desired construct.

Colonies were picked and cultivated O/N in appropriate antibiotic

FRIDAY, 7/17/2020

Gel electrophoresis

Gel electrophoresis of the samples showed no successful transformants. While control plates were made to check for ligation, it is unclear whether the digestion even went right. Thus, digestions with controls (undigested, digested with one of the RE, and finally with both) are planned to be carried out

Expected fragment lengths using VF...

	Sample	Expected length (bp)
1	1+2	364
2	1	349
3	2	329
4	3+5	496
5	3	443
6	5	367
7	6+7	1049
8	6	329
9	7	1034

THURSDAY, 7/23/2020

Restriction and digestion

The following restriction digests were repeated:

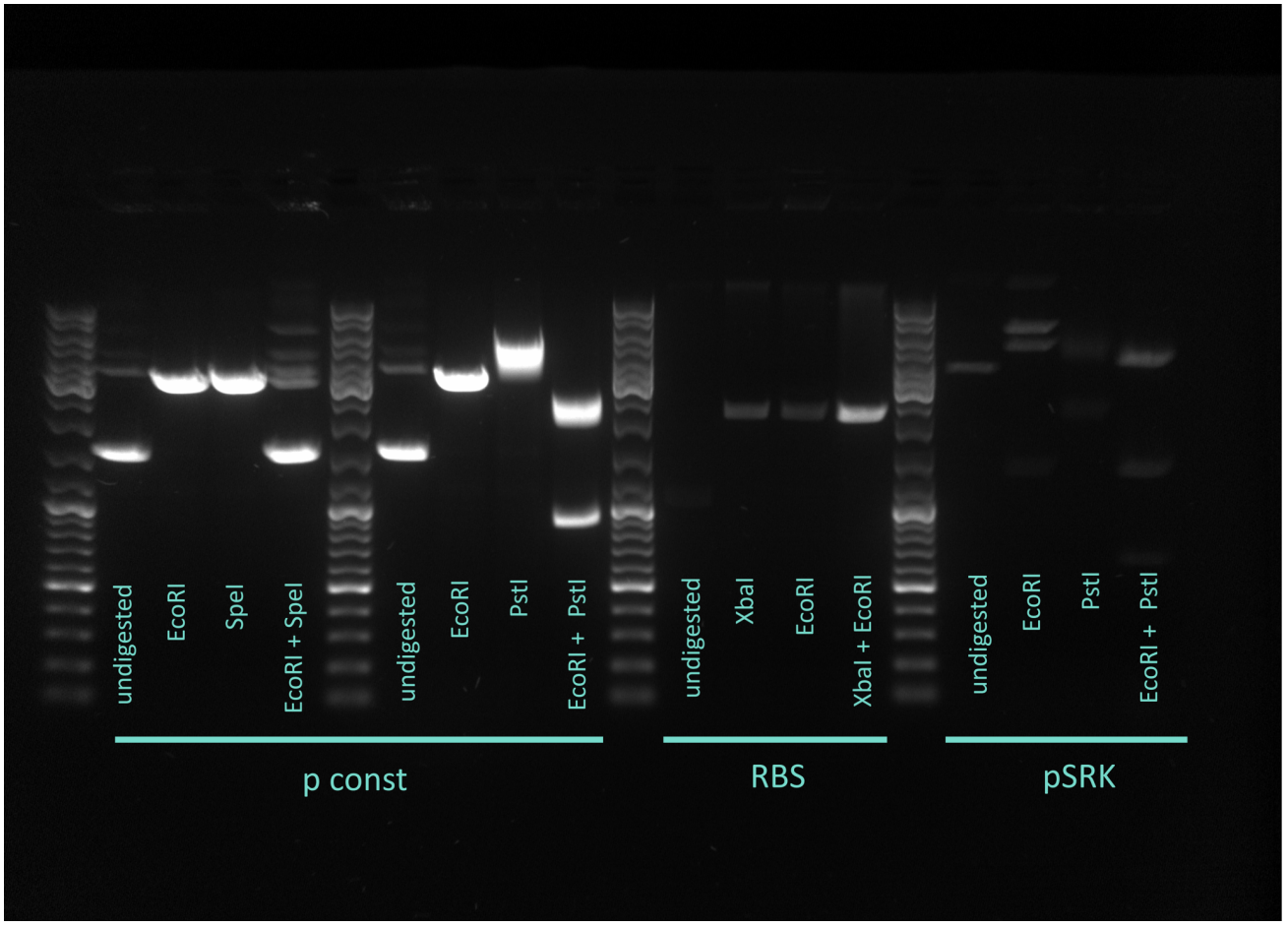
- E Constitutive promoter (J23100) S **[1]** + X RBS (strong) (B0030) E **[2]**
- E Constitutive promoter (J23100) P **[8]** + E pSRK P **[9]**

Digest was run for 10 minutes instead of an hour (in case of star activity of the enzymes)

Gel electrophoresis

With the low transformation efficiency of previous week, a gel electrophoresis was done on the digestion products
90 V 1.5 h

Pconst-ES_Pconst-EP_RBS-XE_pSRK-EP_2020-07-23_14h59m43s.png



The constitutive promoter (J23100) digested with EcoRI and SpeI on their own produce a linear fragment that is of the expected size, the sample digested with both EcoRI and SpeI looks identical to the undigested vector, indicating that digestion was unsuccessful.

The same plasmid digested with EcoRI and PstI seems to be fully digested, with the double digest sample having two fragments - one smaller cut out part, and one larger plasmid. The PstI digested sample and the double digested experience smearing for unknown reasons.

No conclusion can be drawn on the RBS (B0030) as the fragment would be too small to see on the gel.

The digested pSRK experience multiple fragments, in both the single and double digest samples. Further examination of the sequence revelas that there were several valid restriction sites for EcoRI, outside the MCS. Other restriction enzymes would have to be chosen to clone fragments into this vector.

MONDAY, 7/27/2020

pSB1C3 extraction

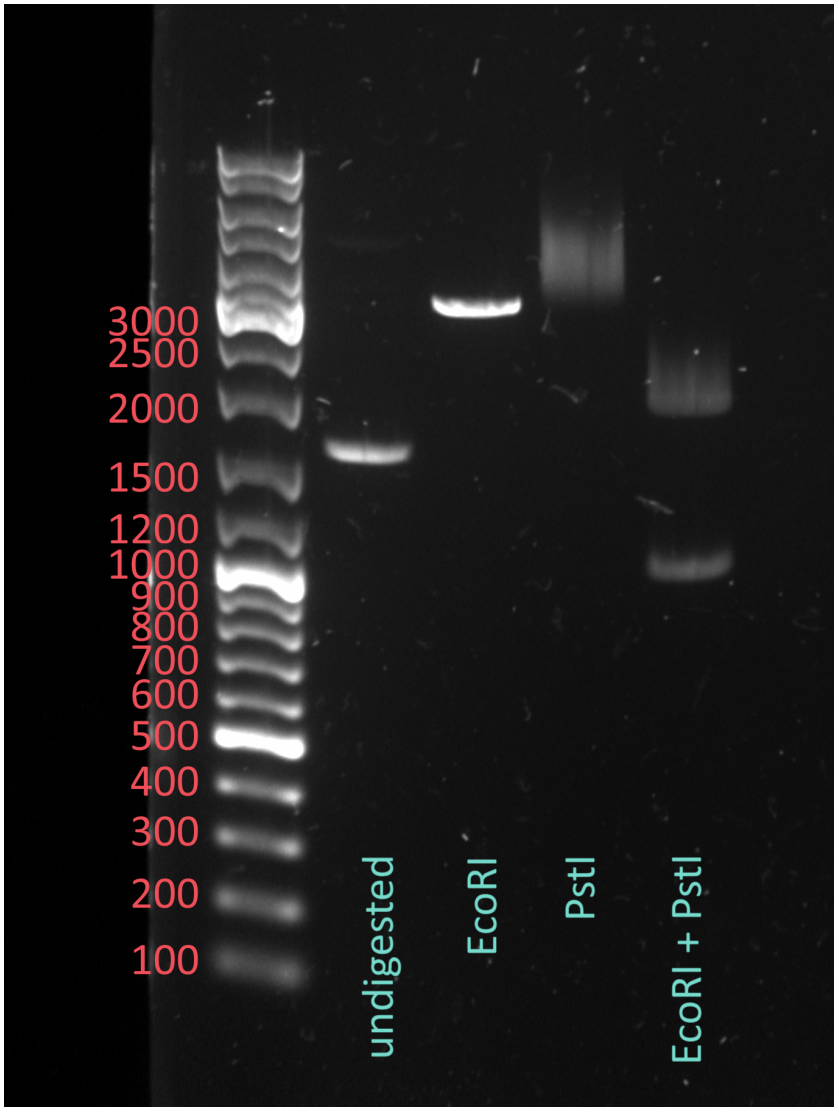
Prepared overnight culture of pSB1C3

TUESDAY, 7/28/2020

pSB1C3 extraction

Restriction digestion of pSB1C3 (from plasmid prep) using EcoRI and PstI
Ran analytical gel electrophoresis of restriction digestion products on thin agarose gel (90 V, 1 h 30 min)

pSB1C3 2020-07-28.png



It was concluded that the smearing may be due to protein in the sample loaded onto the gel. This could be solved by adding SDS, but would not pose a problem to the sample anyways. It was decided to continue with the experiments.

pSB1C3 extraction
An empty plamsind was needed to transform the delivered gBlocks from IDT

Ran gel electrophoresis of EcoRI + PstI product (2 µg) on thick agarose gel
Observed 2 upper bands
Cut 2 upper bands from the gel and extracted plasmid from the gel
Measured plasmid concentration on Nanodrop

pSB1C3 restriction digest with EcoRI ...

	Band position on gel	Nanodrop (ng/µL)
1	top	12.2
2	bottom (product?)	8.7

Isolated plasmid from overnight culture of pSB1C3 with a miniprep as it had run out

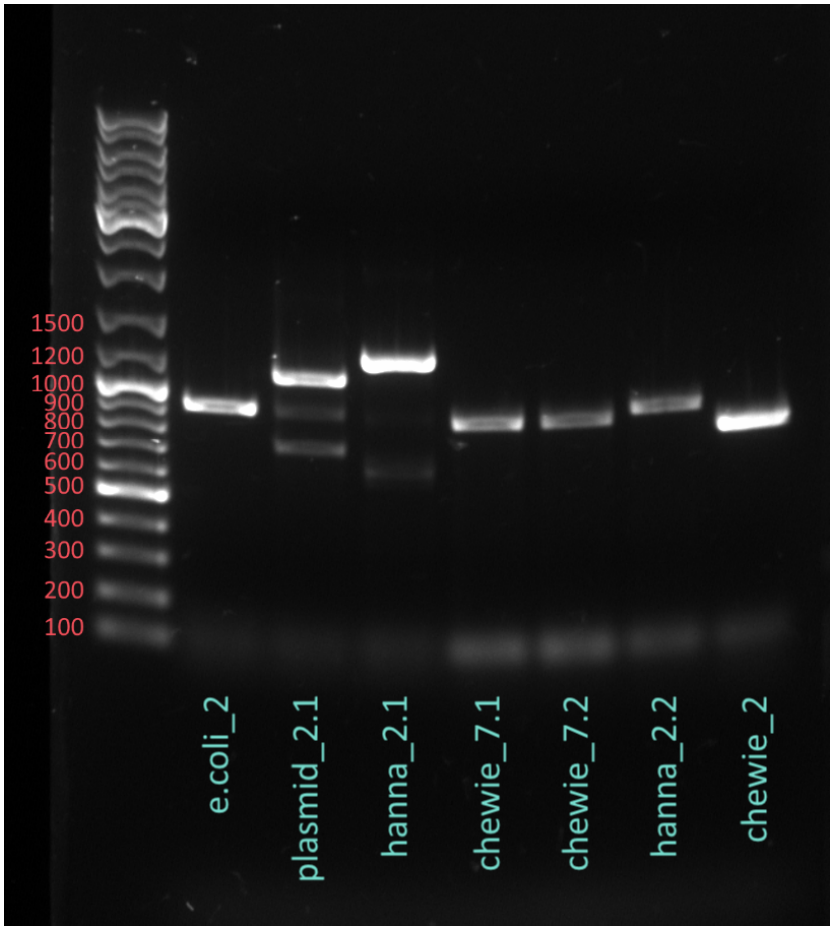
PCR amplification of gBlocks
Delivered gBlocks from IDT were in low quanitity, so it was decided that they would be PCR amplified before transformation

PCR amplified gBlocks:

- e.coli_2
- plasmid_2.1
- hanna_2.1
- hanna_2.2
- chewie_2
- chewie_7.1
- chewie_7.2

Ran gel electrophoresis on PCR products for confirmation of product sizes (90 V, 1 h 30 min)

gblocks 1 200728.png



WEDNESDAY, 7/29/2020

pSB1C3 extraction

Measured plasmid concentration of pSB1C3 on Nanodrop of miniprep

pSB1C3 plasmid prep 27/07

	Set	Nanodrop (ng/µL)
1	1	121.8
2	2	130

PCR amplification of gBlocks

PCR purification of gBlocks:

- e.coli_2
- hanna_2.2
- chewie_2
- chewie_7.1
- chewie_7.2

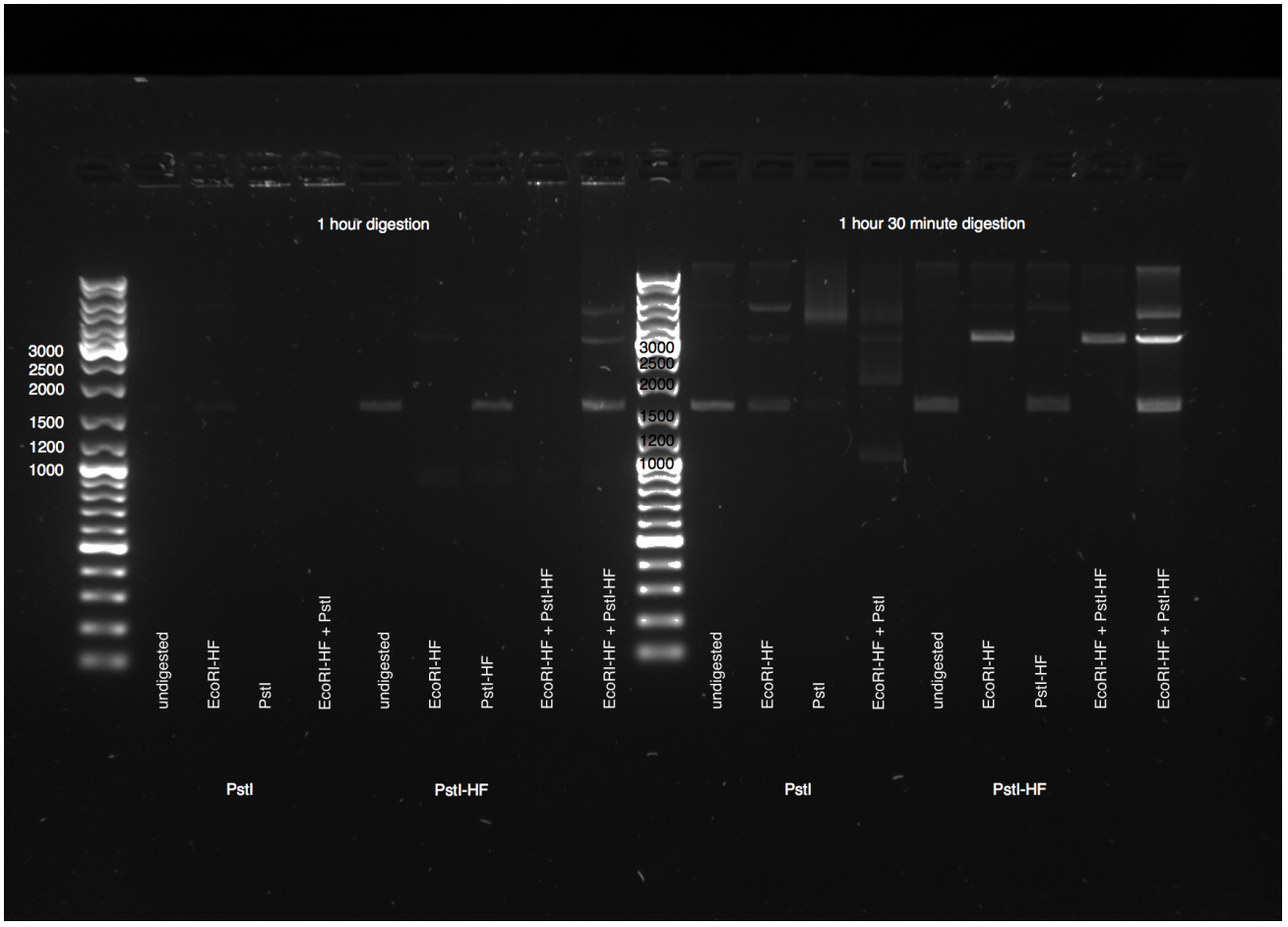
Restriction troubleshooting

Restriction digestion of pSB1C3:

- EcoRI + PstI + NEB buffer 2.1
- EcoRI + PstI-HF + CutSmart buffer

Ran analytical gel electrophoresis after 30 minutes, 1 hour, 1 hour 30 minutes of incubation on thin agarose gel (90 V, 1 h 30 min)

pSB1C3 time 60 & 90 min - 2.png



In vivo amplification of gBlocks

Ligated gBlocks with gel extraction products (28/07)

Incubated at 16^{o}C overnight

THURSDAY, 7/30/2020

In vivo amplification of gBlocks

Transformed TOP10 E.coli with ligation products

FRIDAY, 7/31/2020

In vivo amplification of gBlocks

Transformation plates: observed mostly red colonies, possibly indicating religation or undigested vector of pSB1C3 containing RFP

Resuspended gBlocks
Measured concentration on Nanodrop

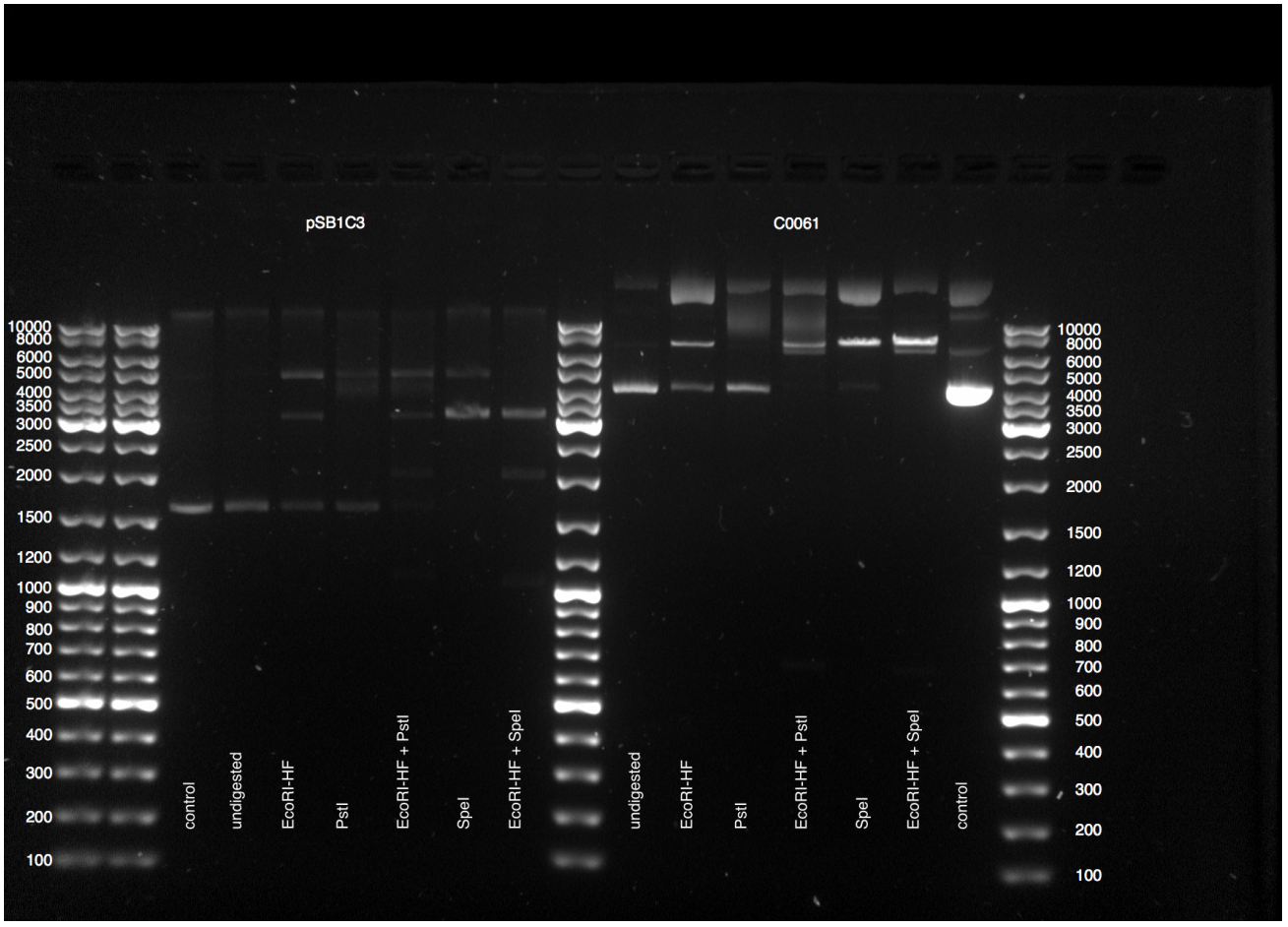
Resuspended gBlocks (2)

	gBlock	Nanodrop (ng/µL)
1	plasmid 1.2	10.1
2	chewie 6.2	10.6
3	chewie 5	10.9
4	chewie 4	11.7
5	plasmid 2.2	10.6
6	heart	12.0

Restriction troubleshooting

Restriction digestion of Biobricks (pSB1C3 and C0061) using a combination of EcoRI + PstI and EcoRI + SpeI
Ran gel electrophoresis of restriction digestion products (90 V, 2 h)

Restriction digest (pSB1C3 + C0061).png



There is no clear amplified band, but rather multiple bands

MONDAY, 8/3/2020

Restriction troubleshooting

Aim: solving the restriction digest problem by varying different parameters on the restriction digest protocol.

Restriction digest 03/08

	Protocol	Antarctic phosphatase	Incubation time	Who does it
1	iGEM protocol	No	30min	Richard
2	iGEM protocol	No	1h	Astha
3	NEB protocol	No	1h	Victoria
4	NEB protocol	No	3h	Julie
5	NEB protocol	Yes	1h	Hanna

NEB protocol:

1. Set heat block to 80°C
2. Measure 500 ng of plasmid DNA of each biobrick, the volume is X µL
3. Add (43-X) µL of water

4. Add 5 µL 10X NEB buffer 2.1 each to 1.5 mL tubes
5. Add X µL of DNA
6. Add 1 µL of each restriction enzyme
   (1) p-B1-B2-p
   To open the plasmid before Biobrick 2, use EcoRI and XbaI
   To cut out Biobrick 1 and place before Biobrick 2, use EcoRI and SpeI
   (2) p-B2-B1-p
   To open the plasmid after Biobrick 2, use SpeI and PstI
   To cut out Biobrick 1 and place after Biobrick 2, use XbaI and PstI
7. Incubate at 37°C for 10 minutes
8. For larger DNA like vectors, incubate for 60 minutes to 4 hours (depending on DNA amount)
9. For analytical digest, incubate for 30 minutes
10. (optional) Heat inactivate at 80°C for 20 minutes

IGEM protocol:

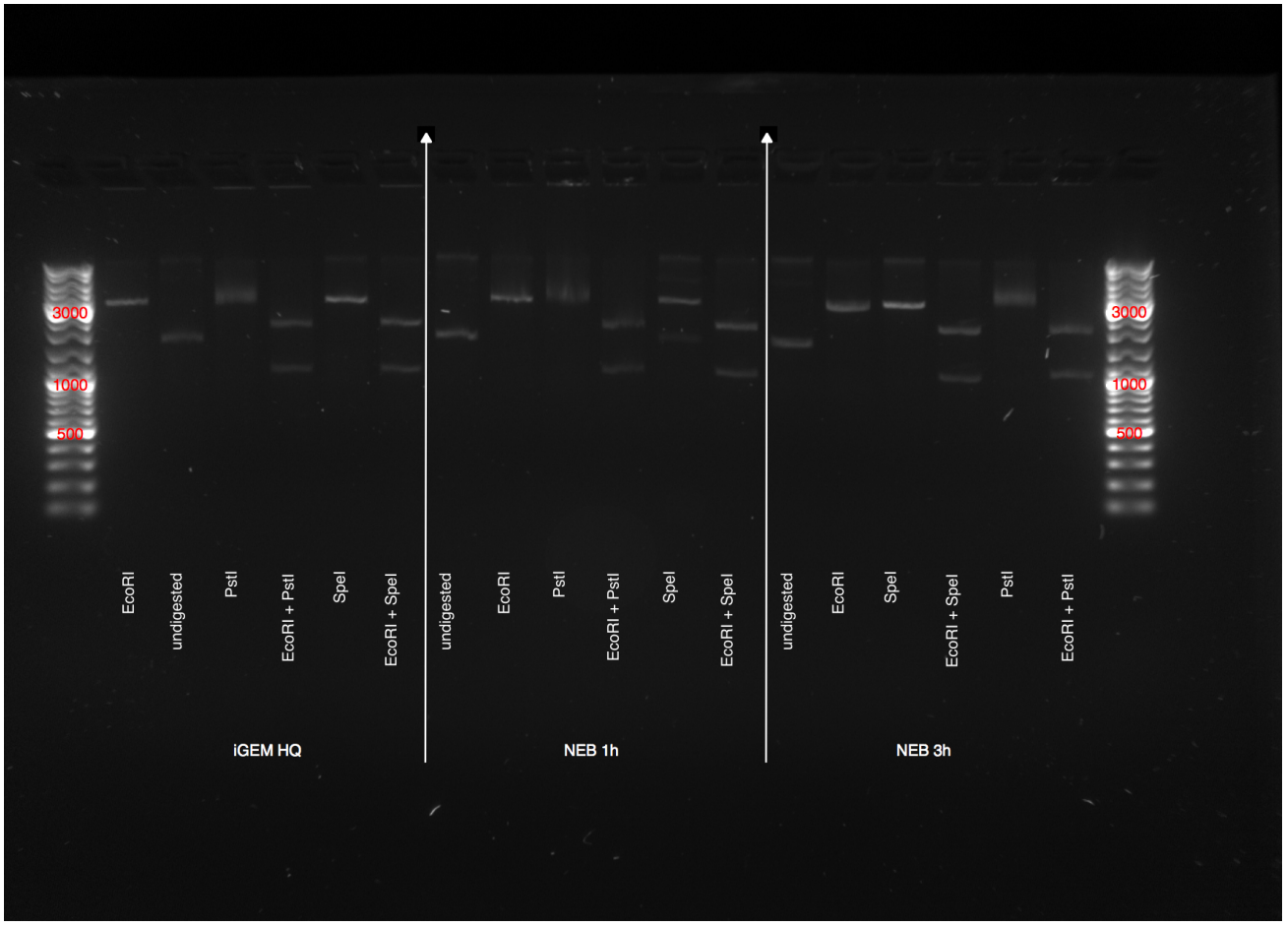
1. Add 250 ng of DNA (volume is X uL) to the PCR tube
2. Add (16-X) uL dH2O
3. Add 2.5 uL NEB buffer 2.1
4. Add 0.5 uL BSA
5. Add 0.5 uL Enzyme 1 (eg, EcoRI)
6. Add 0.5 uL Enzyme 2 (eg, PstI)
7. Mix and spin down breifly
8. Incubate at 37°C for 30 minutes (can be done in thermocycler)
9. Heat inactivate at 80°C for 20 minutes (can be done in thermocycler)
10. Run on analytical gel (approx 8 uL, or 100 ng)

For each of the 4 protocols, digest with these enzymes (6 tubes)

	A	B	C	D	E	F
1	Undigested	EcoRI	SpeI	PstI	EcoRI-PstI	EcoRI-PstI

Insufficient plasmid - protocol (2) and (5) was not completed
Ran gel electrophoresis on restriction digest products (1), (3) and (4) (180 V, 30 min)

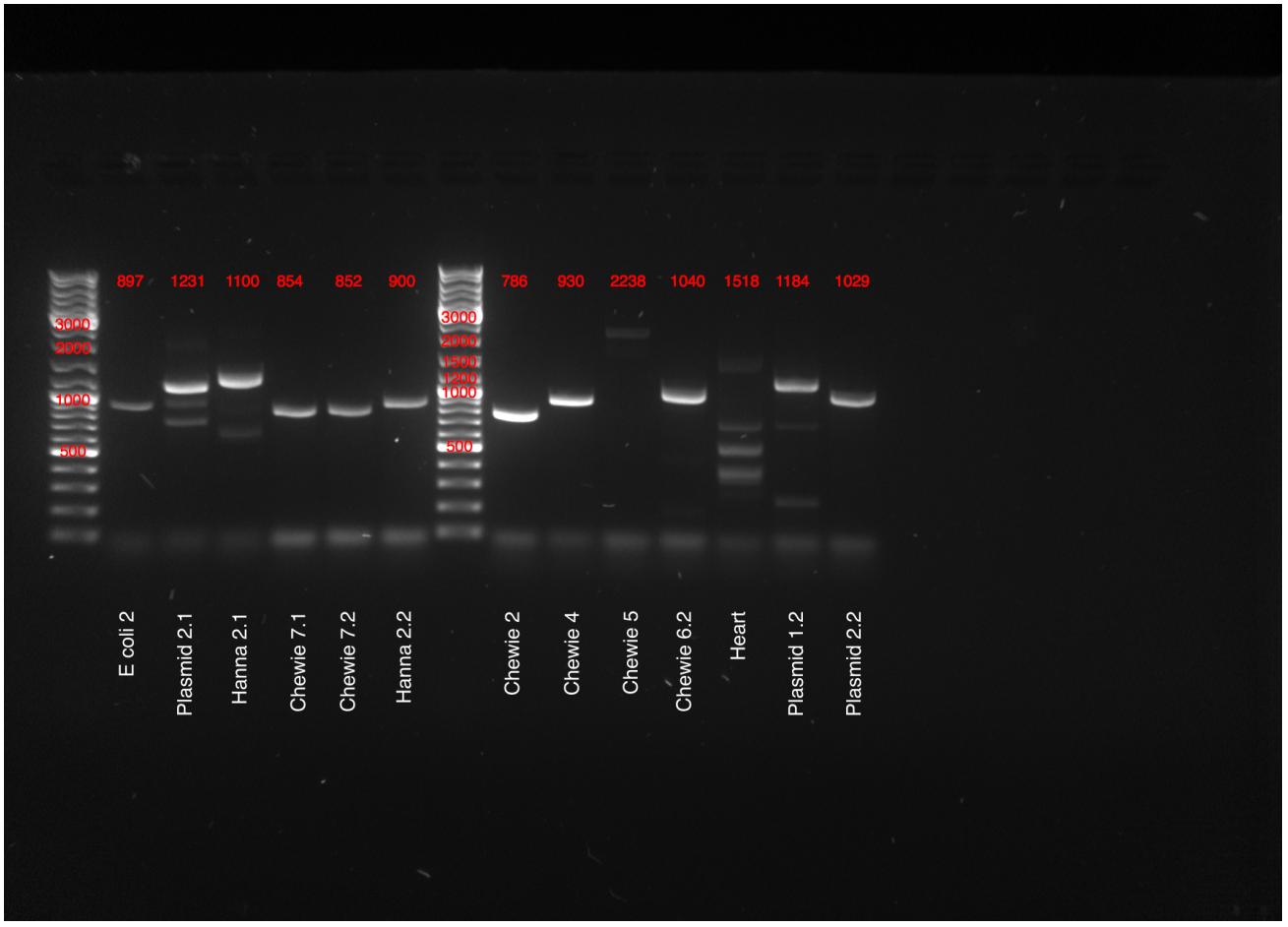
pSB1C3 test digestion 2020-08-03_18h21m24s.png



In vivo amplification of gBlocks

Ran gel electrophoresis on gBlocks PCR products (31/07) (160 V, 30 min)

gblocks 2 2020-08-03_16h44m36s.png



Prepared overnight cultures from glycerol stocks:

- pUC19
- C0060
- C0070
- C0078
- E0040
- pSRK-Km

(although intended, no O/N culture of pSB1C3 w/ RFP coding device was prepared)

TUESDAY, 8/4/2020

pSB1C3 extraction

Prepared O/N culture of pSB1C3

20mL culture and 70mL culture

WEDNESDAY, 8/5/2020

pSB1C3 extraction

Measured OD600 of pSB1C3 O/N cultures

OD600 05/08

	Biobrick	Absorbance	Original OD
1	pSB1C3 (20 mL)	0.794	7.94
2	pSB1C3 (70 mL)	0.723	7.23

Made 4 plasmid preps from O/N cultures
12 tubes were used with 5 mL culture in each. Centrifuged 6 at a time in 4000 RPM in a tabletop centrifuge to pellet.
3 1.5mL tubes were eluted with the same EB, resulting in 4 tubes

pSB1C3 plasmid prep 05/08

	Set	Nanodrop (ng/µL)
1	1	654.8
2	2	651.4
3	3	601.7
4	4	519.2

Started digestion with EcoRI-HF and PstI of 5x4 ug pSB1C3 plasmid

Ran on gel and performed 5x4 ug gel extraction on the double digested pSB1C3 plasmid

PCR amplification of gBlocks
Resuspended gBlocks

gBlocks resuspension (3)

	gBlock	Nanodrop (ng/µL)
1	plasmid 1.1 (N)	7.6
2	chewie 3.2 (O)	10.0

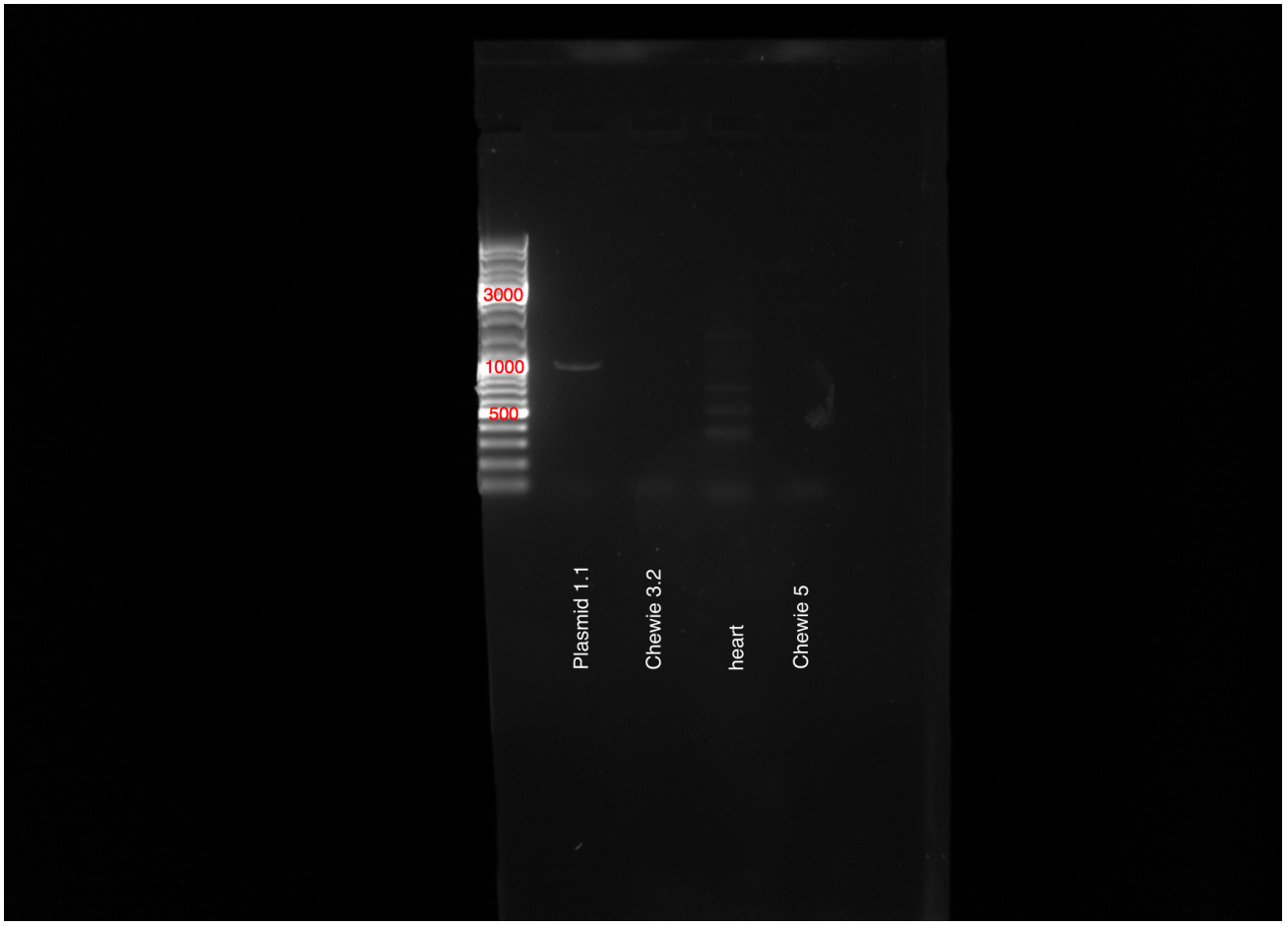
PCR amplified gBlocks: https://tmcalculator.neb.com/#!/main

- plasmid 1.1
- chewie 3.2
- heart
- chewie 5

For heart and chewie 5, used a different PCR program with annealing temperature 65^{o}C and 25 cycles

Ran on gel:

gblocks 3 2020-08-05_19h02m48s.png



Plasmid 1.1 seems to be amplified properly, and the misannealed primer products in heart remains

In vivo amplification of gBlocks
Started restriction digestion of gBlocks A,B2,C1,D,E,F,G,H,J3,L1,M2 with EcoRI-HF and PstI. Used 500ng of DNA for B2 to M2 and 354ng for A (maximum we could get).

Restriction digestion

	Sample	DNA (µL)	Water (µL)	NEB 2.1 Buffer (µL)	EcoRI-HF (µL)	PstI (µL)
1	A	30	13	5	1	1
2	B2	21	22	5	1	1
3	C1	19.2	23.8	5	1	1
4	D	24.8	18.2	5	1	1
5	E	25.8	17.2	5	1	1
6	F	30	13	5	1	1
7	G	16.3	26.7	5	1	1
8	H	22.2	20.8	5	1	1
9	J3	11.6	31.4	5	1	1
10	K1	12	31	5	1	1
11	L1	22.7	20.3	5	1	1
12	M2	21	22	5	1	

Incubated reaction for 30 minutes at 37^{o}C

Started ligation of gBlocks A,B,C,D,E,F,G,H,J,L,M with plasmid pSB1C3 (using the tube with 42ng/µL, corresponding to a volume of 0.8µL) using Hi-T4 DNA ligase
Incubated overnight in the fridge. The ligation was done for vector:insert ratios 1:5, 1:3 and 1:1.

N.B. chewie 5 (I) and heart (K) were not ligated because another PCR was performed on the same day.

THURSDAY, 8/6/2020

In vivo amplification of gBlocks

Ligation reaction

	gBlock	Label	Length (bp)	Nanodrop before RD (ng/µL)	Concentration after RD (ng/µL)	Mass of insert for 1 to 5 (ng)	Volume of insert for 1-5 (µl)	Buffer (µL)	T4 DNA ligase (µL)	Vector (µL)	Water for 1-5 (µL)	Mass of insert for 1 to 3 (ng)	Volume of insert for 1 to 3 (µL)	Buffer (µL)	T4 DNA ligase (µL)	Vector (µL)	Water for 1-3 (µL)	Mass of insert for 1 to 1 (ng)	Volume of insert for 1 to 1 (µL)	Buffer (µL)	T4 DNA ligase (µL)	Vector (µL)	Water for 1-1 (µL)
1	e.coli 2	A	897	11.8	7.08	108.3	15.3	2.0	1.0	0.8	0.9	65	9.2	2.0	1.0	0.8	7.0	21.6	3.1	2.0	1.0	0.8	13.1
2	plasmid 2_1	B	1231	23.7	10	148.7	14.9	2.0	1.0	0.8	1.3	89.2	8.9	2.0	1.0	0.8	7.3	29.7	3.0	2.0	1.0	0.8	13.2
3	hanna 2_1	C	1100	26.1	10	132.9	13.3	2.0	1.0	0.8	2.9	79.7	8.0	2.0	1.0	0.8	8.2	26.5	2.7	2.0	1.0	0.8	13.6
4	chewie 7_1	D	854	20.2	10	103.1	10.3	2.0	1.0	0.8	5.9	61.8	6.2	2.0	1.0	0.8	10.0	20.63	2.1	2.0	1.0	0.8	14.1
5	chewie 7_2	E	852	19.4	10	102.9	10.3	2.0	1.0	0.8	5.9	61.74	6.2	2.0	1.0	0.8	10.0	20.6	2.1	2.0	1.0	0.8	14.1
6	hanna 2_2	F	900	16.3	9.8	108.7	11.1	2.0	1.0	0.8	5.1	65.22	6.7	2.0	1.0	0.8	9.5	21.74	2.2	2.0	1.0	0.8	14.0
7	chewie 2	G	786	30.6	10	94.93	9.5	2.0	1.0	0.8	6.7	56.96	5.7	2.0	1.0	0.8	10.5	19	1.9	2.0	1.0	0.8	14.3
8	chewie 4	H	930	22.5	10	112.3	11.2	2.0	1.0	0.8	5.0	67.3	6.7	2.0	1.0	0.8	9.5	22.4	2.2	2.0	1.0	0.8	14.0
9	chewie 5	I	2238	13	7.8	270.3	34.7	2.0	1.0	0.8	-18.5	162.2	20.8	2.0	1.0	0.8	-4.6	54	6.9	2.0	1.0	0.8	9.3
10	chewie 6_2	J	1040	43.2	10	125.6	12.6	2.0	1.0	0.8	3.6	75.3	7.5	2.0	1.0	0.8	8.7	25.1	2.5	2.0	1.0	0.8	13.7
11	heart	K	1518	41.6	10	183.3	18.3	2.0	1.0	0.8	-2.1	110	11.0	2.0	1.0	0.8	5.2	36.6	3.7	2.0	1.0	0.8	12.5
12	plasmid 1_2	L	1184	22	10	143	14.3	2.0	1.0	0.8	1.9	85.8	8.6	2.0	1.0	0.8	7.6	28.6	2.9	2.0	1.0	0.8	13.3
13	plasmid 2_2	M	1029	23.9	10	124.3	12.4	2.0	1.0	0.8	3.8	74.5	7.5	2.0	1.0	0.8	8.8	24.8	2.5	2.0	1.0	0.8	13.7

Started heat shock transformation of TOP10 E.coli with ligation products (except I and K)
Included controls:
1: E.coli without plasmid (expected results: no growth because bacteria will not have antibiotic resistance)
2: undigested plasmid (expected results: red colonies)
3: digested plasmid without insert (expected results: no growth because plasmid is linearised)
(although intended, ligated plasmid without insert was not included as a control)
Plated on chloramphenicol plates and incubated at 37^{o}C overnight

FRIDAY, 8/7/2020

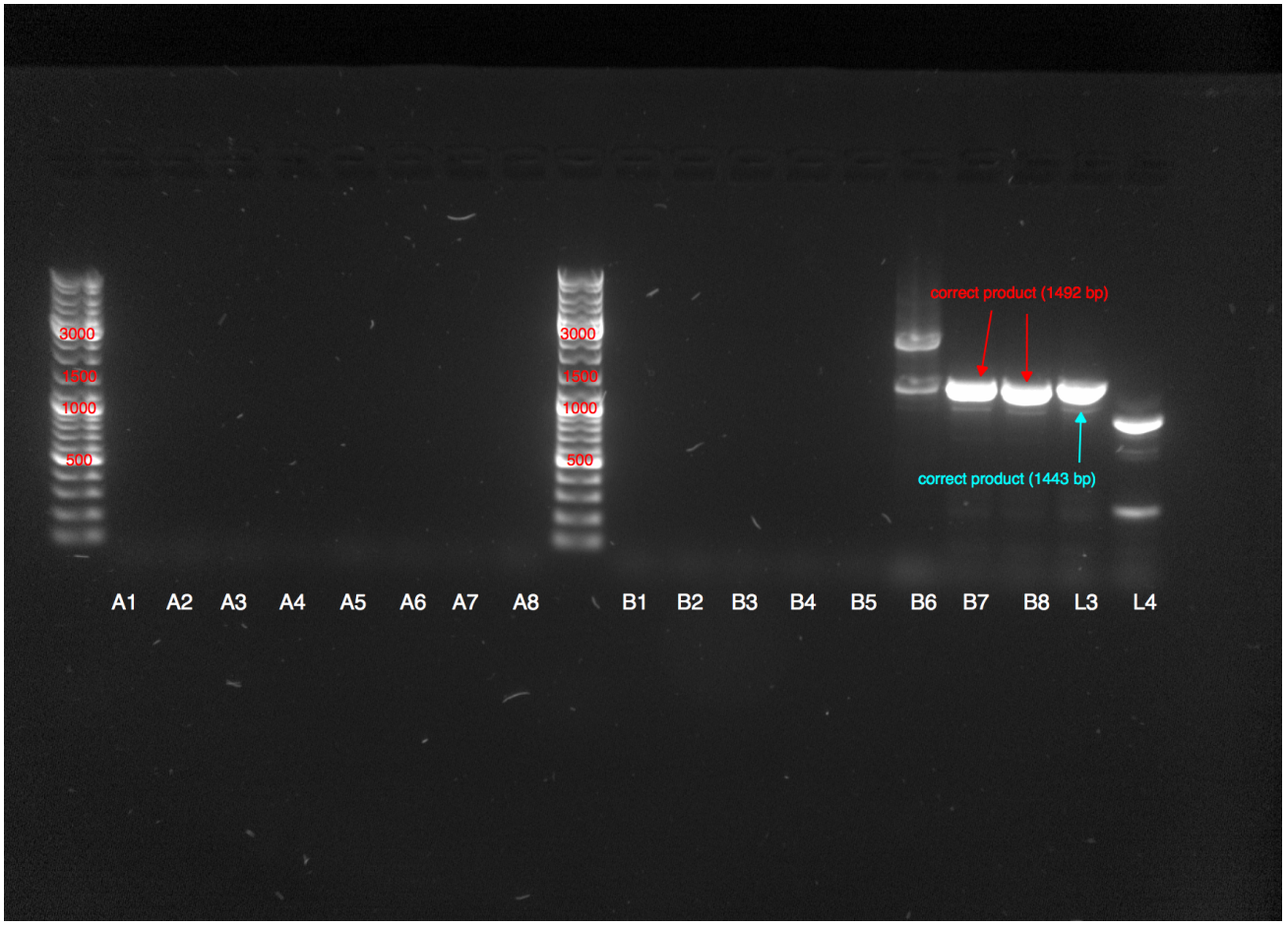
In vivo amplification of gBlocks
Observed white colonies and some red colonies on the transformation plates
No growth on control 1, red colonies on control 2, no growth on control 3
Picked 8 colonies each from 1:5 ligation plates (1:3 for A) and resuspended in 100 µL LB media
Ran colony PCR on the colony suspensions

Colony PCR 07/08

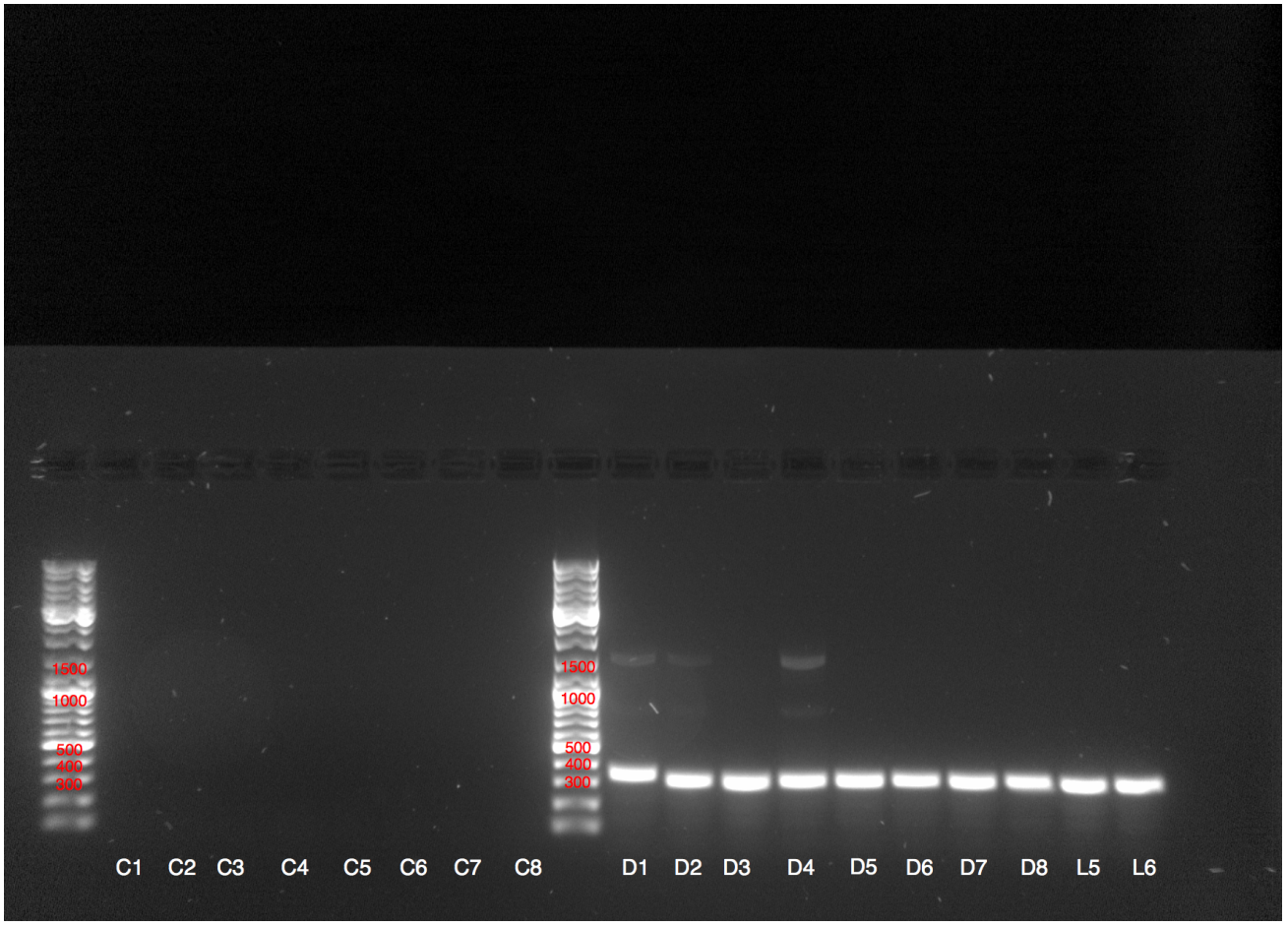
	gBlock	Label	Expected size of colony PCR product with VF2 and VR (bp)
1	e.coli 2	A	1158
2	plasmid 2_1	B	1492
3	hanna 2_1	C	1361
4	chewie 7_1	D	1113
5	chewie 7_2	E	1111
6	hanna 2_2	F	1159
7	chewie 2	G	1045
8	chewie 4	H	1189
9	chewie 6_2	J	1299
10	plasmid 1_2	L	1443
11	plasmid 2_2	M	1288
12	plasmid1_1	N	1226
13	chewie 3_2	O	2107
14	chewie 6_1	P	1328
15	chewie 3_1	Q	2135

Ran gel electrophoresis on colony PCR products (180 V, 30-45 mins)

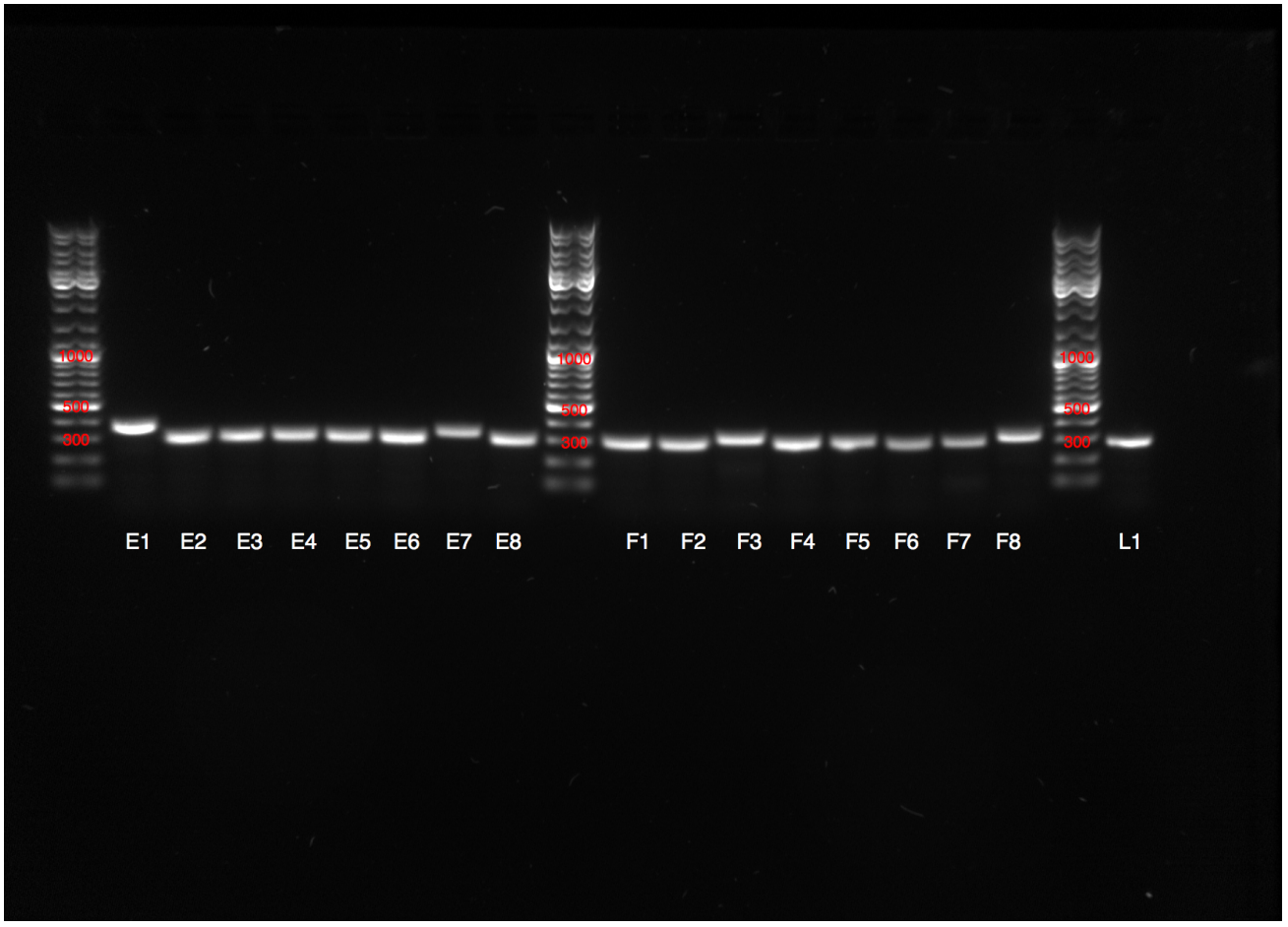
colony PCR gblock ligation A, B, L (3,4) 2020-08-07_19h26m47s.png



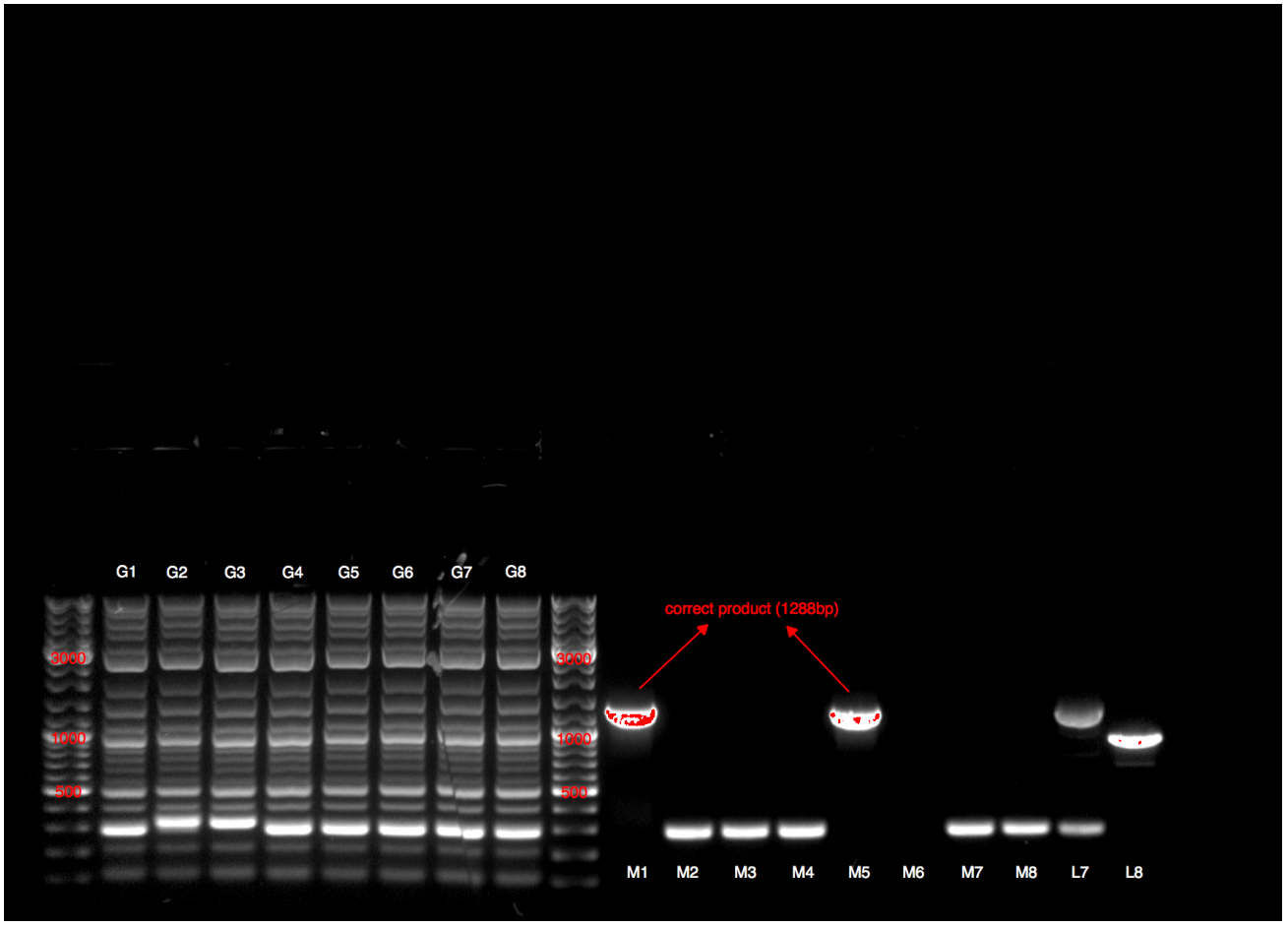
colony PCR gblock ligation C, D, L (5,6) 2020-08-07.png



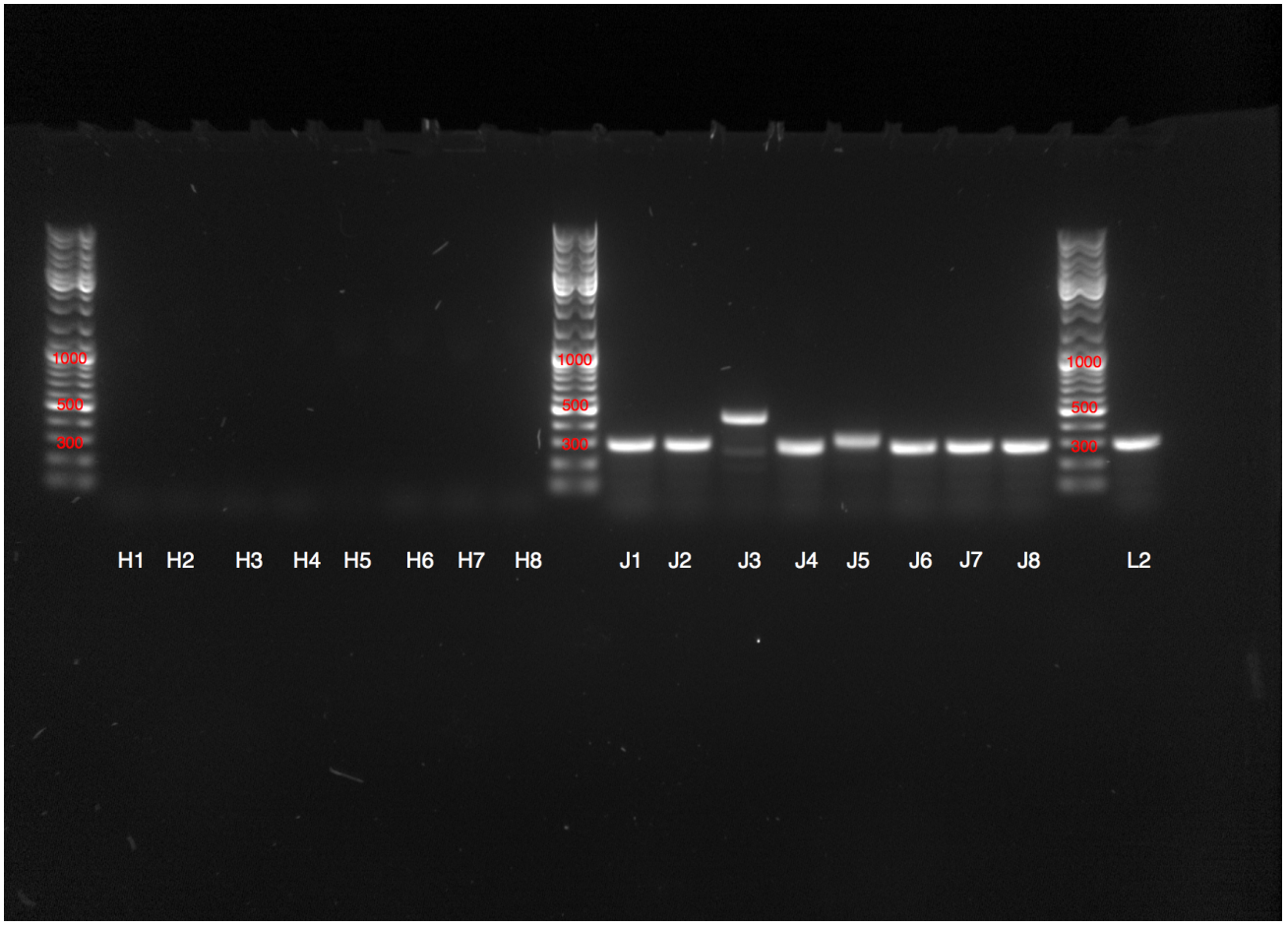
colony PCR gblock ligation E, F, L (1) 2020-08-07_19h34m30s.png



colony pcr gblock ligation G, M, L (7,8) 2020-08-07_18h17m08s.png

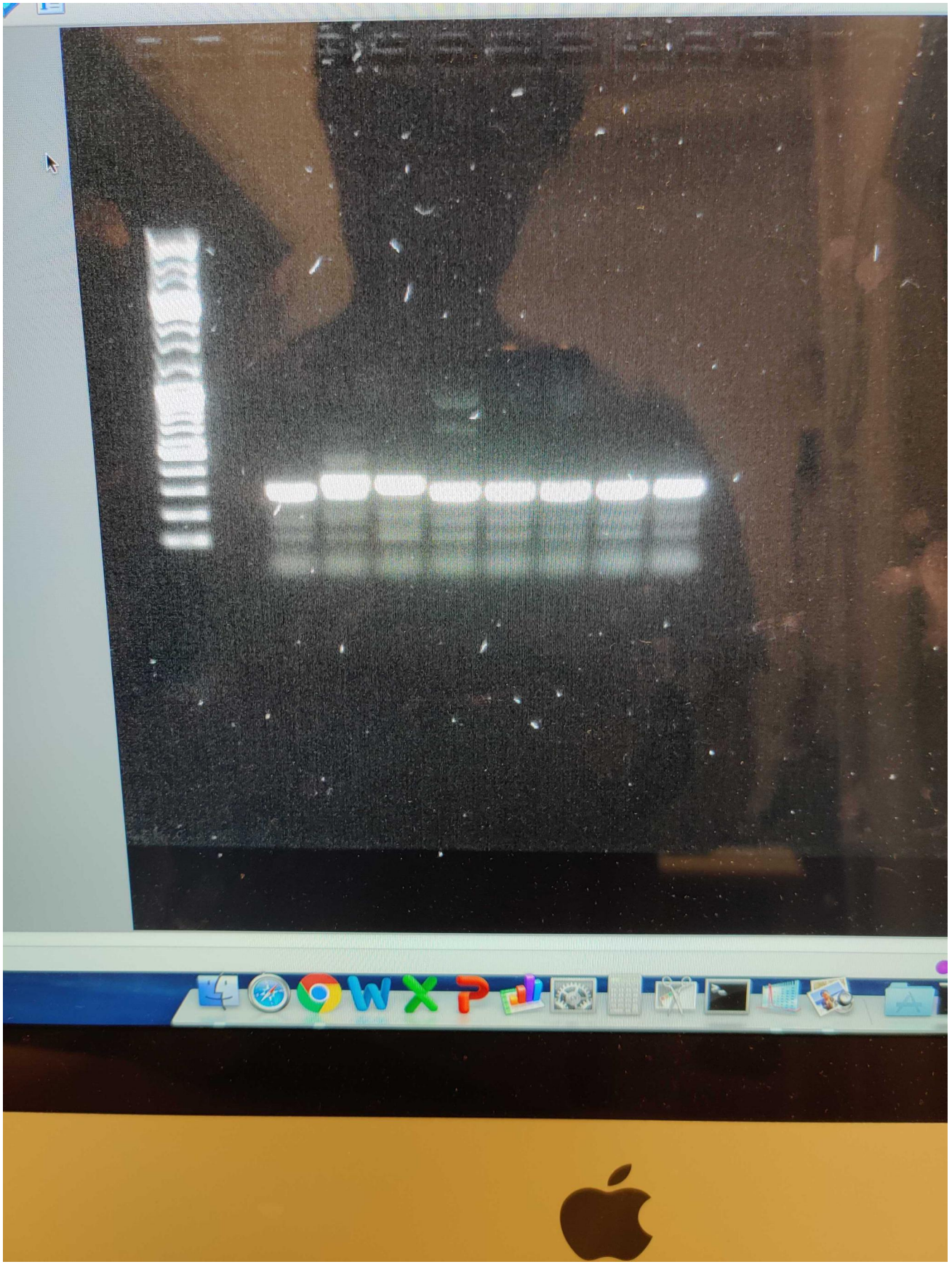


colony PCR gblock ligation H, J, L (2) 2020-08-07_19h37m32s.png



Repeated gel for G to check for the possibility of sample contamination

colony PCR gblock ligation G (repeat)



Weird pattern with low weight remains

Possible explanations:
A, C, H (~100 bp): primer dimers
Loading dye used for 1st gel of sample G was contaminated
<500 bp: religated plasmid without gBlock insert and/or RFP cassette

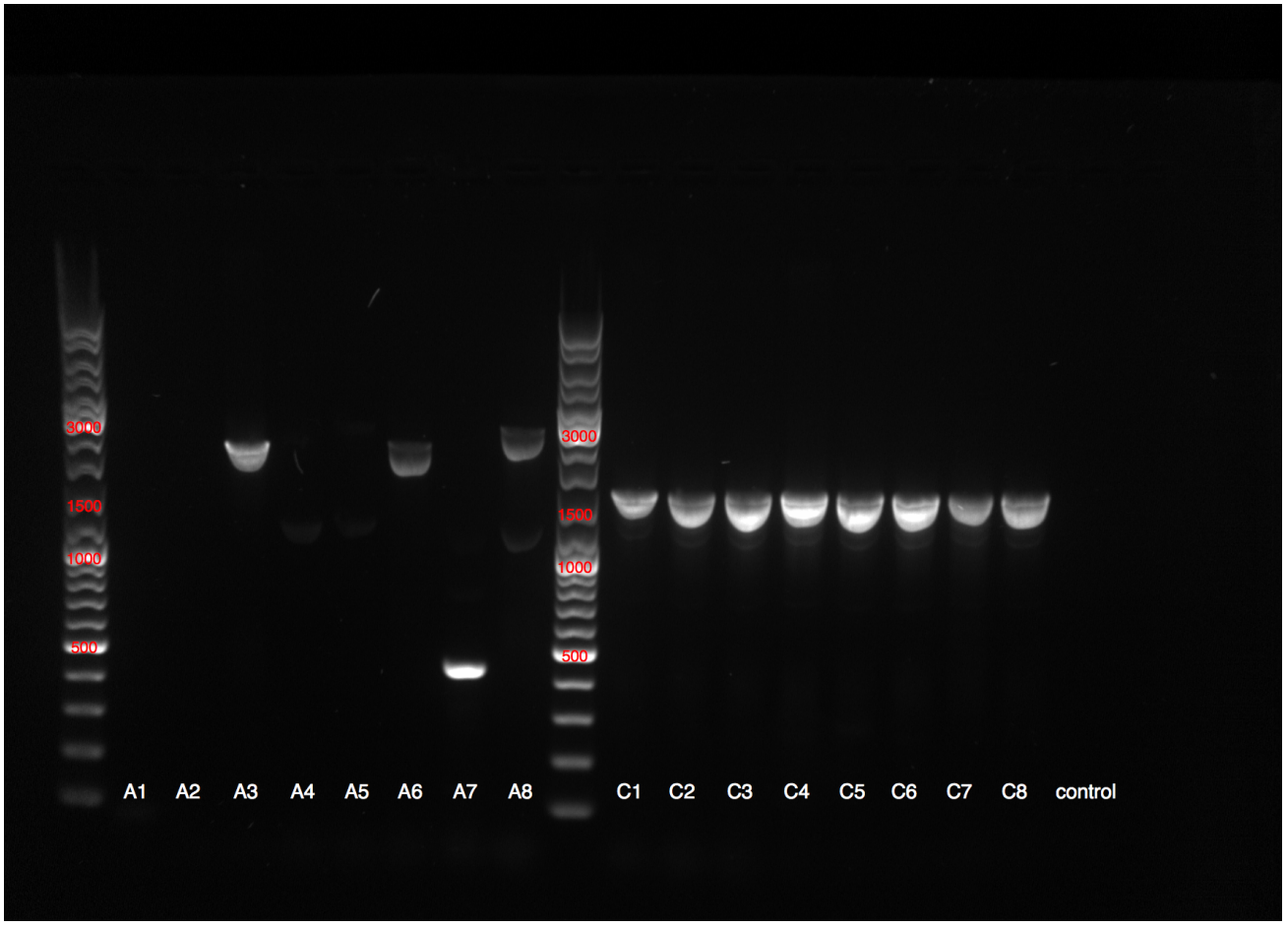
gBlock plasmid prep and glycerol stock
Prepared overnight cultures of B7, B8, M1, M5, L3
Incubated with shaking at 24^{o}C
Stored transformation plates in the fridge
Plate A5 was placed in the incubator at 37^{o}C

MONDAY, 8/10/2020

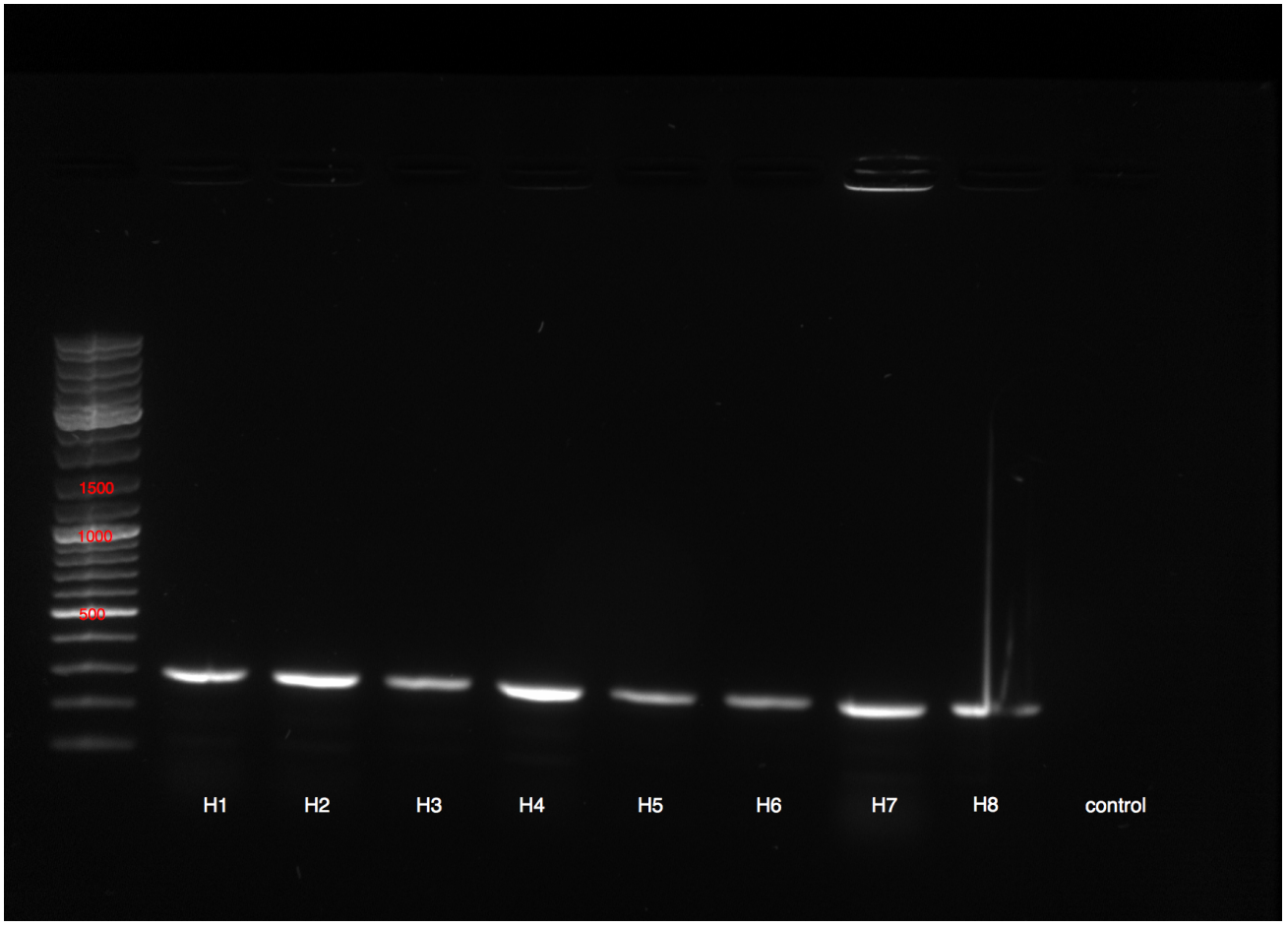
gBlock plasmid prep and glycerol stock
Measured OD600 of B7, B8, M1, M5, L3 o/n cultures
Placed o/n cultures in the fridge

In vivo amplification of gBlocks
Repeated colony PCR of A1-8, C1-8, H1-8 using the same suspension from 07/08
Ran gel of PCR products (180 V, 45 mins)

colony PCR gblock ligation rerun A, C 2020-08-10_16h34m07s.png



colony PCR gblock ligation rerun H 2020-08-10_16h41m54s.png



TUESDAY, 8/11/2020

In vivo amplification of gBlocks

Prepared glycerol stocks of B7, B8, L3, M1, M5

Placed remaining o/n cultures in the fridge

Prepared o/n cultures of Shewanella (wt, ΔmtrB) in LB medium

WEDNESDAY, 8/12/2020

In vivo amplification of gBlocks

Picked 10 new colonies from plates A3, C5, D5, E5, F5, G5, H5, J5

Picked 7 colonies from plate A5 (denoted as A*)

Ran colony PCR using Q5 polymerase protocol in 25 µL reactions

Note:

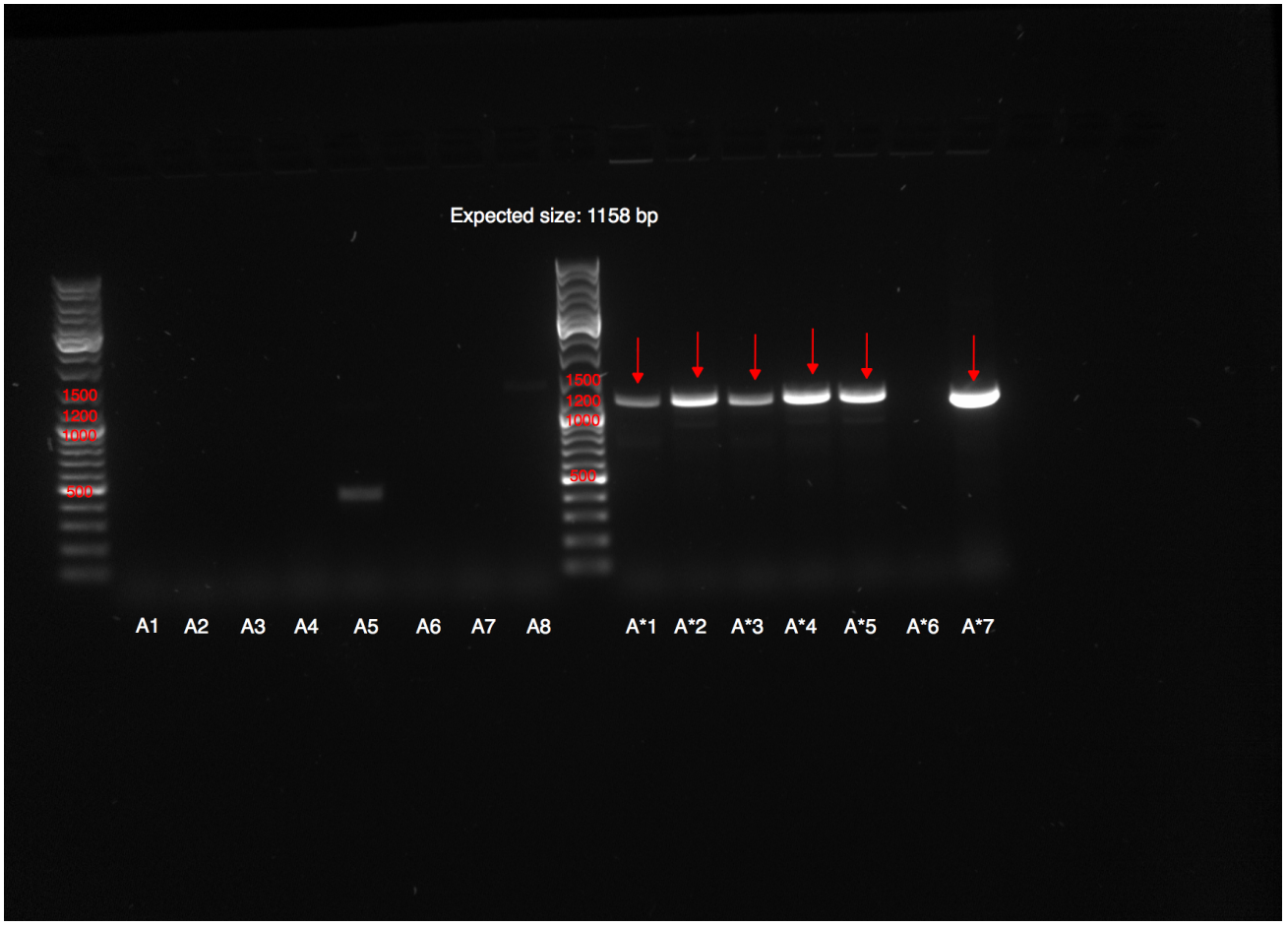
C, F: no red colonies

on gel: well 8 has red colonies (could be RFP)

Ran gel electrophoresis of colony PCR products (180 V, 30 mins)

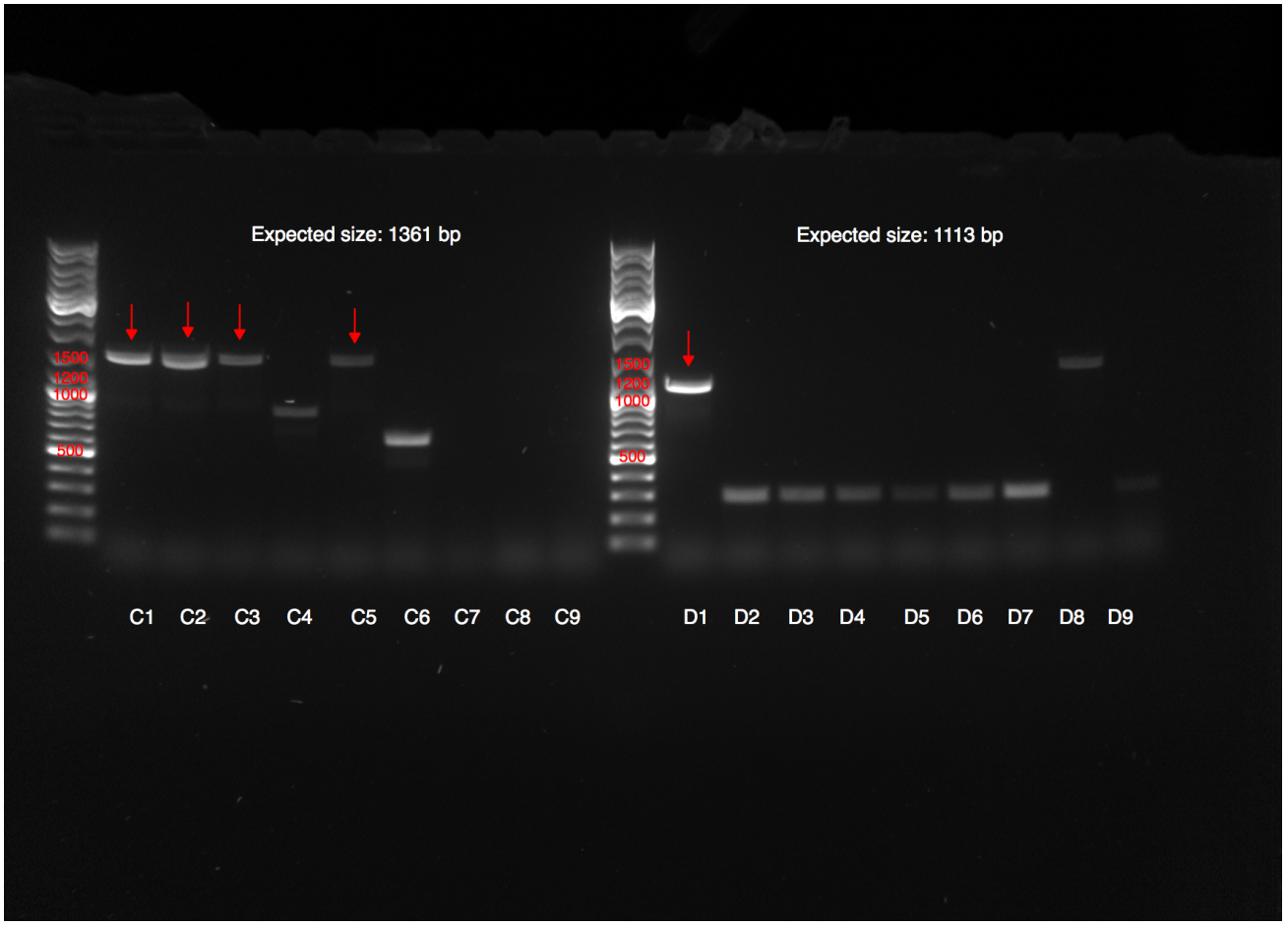
- A and A*

A and A*.png



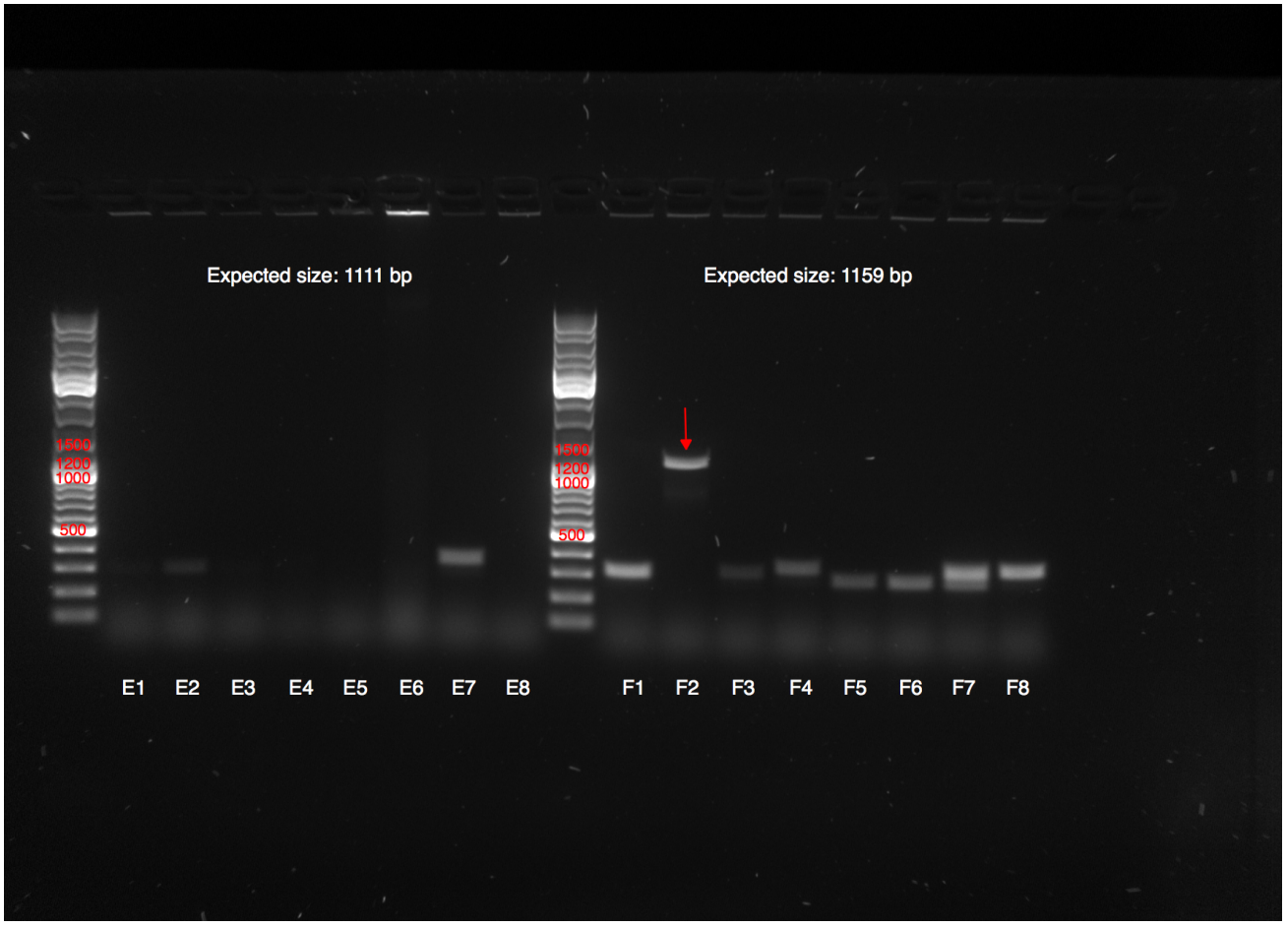
- C and D

C and D.png



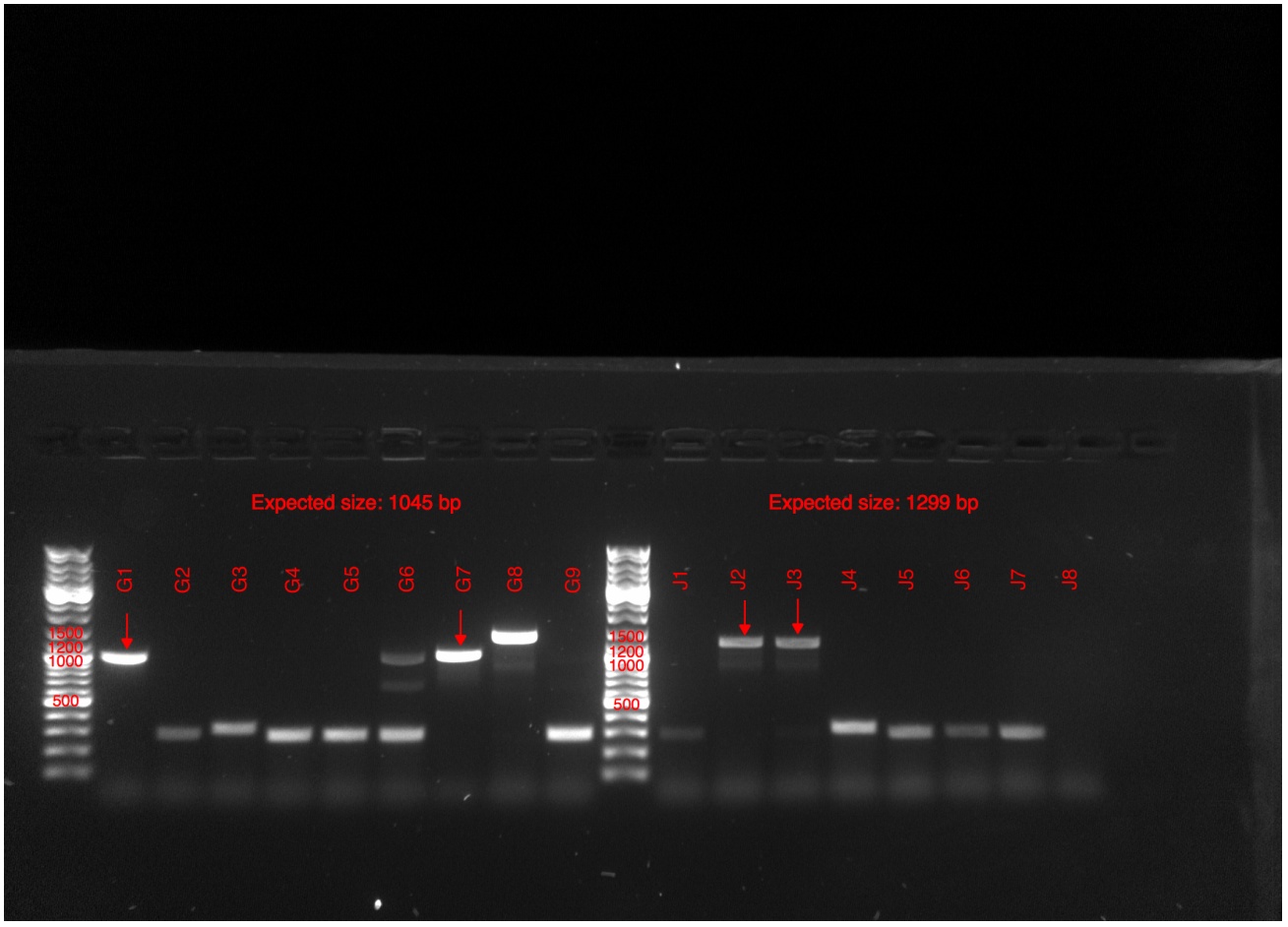
- E and F

E and F.png



- G and J

G and J.png


Expected size: 1045 bp
Expected size: 1299 bp
G1
G2
G3
G4
G5
G6
G7
G8
G9
J1
J2
J3
J4
J5
J6
J7
J8
1500
1200
1000
500
1500
1200
1000
500

- H

H.png

Prepared o/n cultures of A1, A2, A3, A4, A5, A7, C1, C2, C3, C5, D1, F2, G1, G7, J2, J3 in TSB media with chloramphenicol (not enough LB media)

THURSDAY, 8/13/2020

In vivo amplification of gBlocks

Prepared glycerol stocks and plasmid preps of A1, A2, A3, A4, A5, A7, C1, C2, C3, C5, D1, F2, G1, G7, J2, J3
Measured concentration on Nanodrop

Resuspended gBlocks

- chewie 6.1
- chewie 3.1

Measured concentration on Nanodrop

PCR amplification of gBlocks

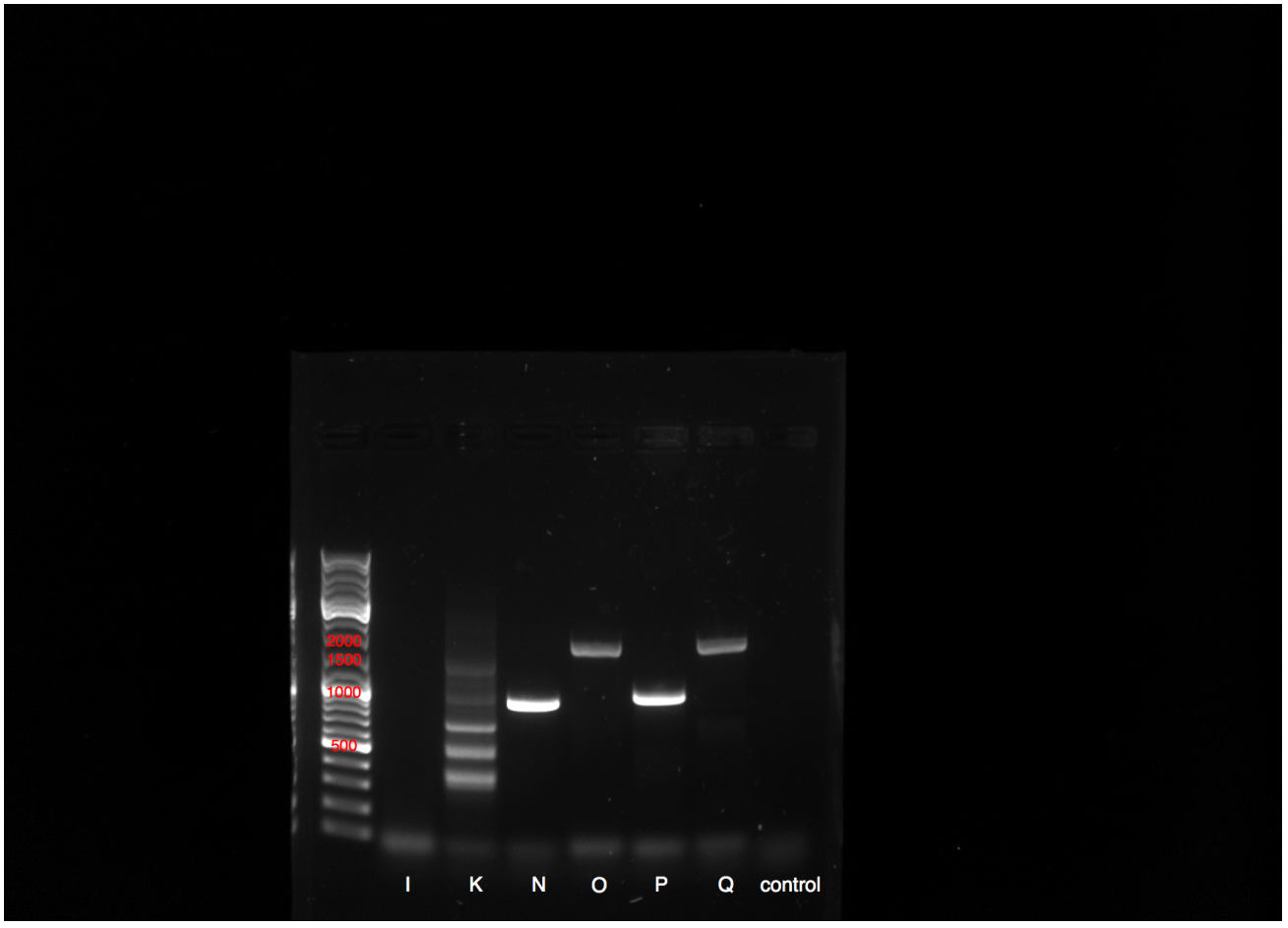
PCR amplified gBlocks using Q5 polymerase:

- chewie 5 (I)
- heart (K)
- plasmid 1.1 (N)
- chewie 3.2 (O)
- chewie 6.1 (P)
- chewie 3.1 (Q)

For chewie 5 and heart, ran Q5 protocol with 25 cycles instead of 12 cycles and annealing temperature 72°C

Ran gel electrophoresis on PCR products (180 V, 30 mins)

gblocks 4 (i, k, n, o, p, q) 2020-08-13_15h03m21s.png



plasmid 1.1, chewie 3.2, chewie 6.1, chewie 3.1 appear to be amplified properly

Colony PCR (Trial 3) - Remaining samples from yesterday

Picked new colonies from E5, F5, H5
E5: Colonies E1-E13 (E12* is a pink colony)
F5: Colonies F1-F12 (no pink colonies grew on the plate)
H5: Colonies H1-H13 (H12* is a pink colony)

Incubated the samples at 37 degrees shaking for circa 45 min

Reaction volumes/Per sample:
25 uL mixture:

- Q5 master mix - 12,5 uL
- VF2 primer - 1,25 uL
- VR primer - 1,25 uL
- Milli Q - 9 uL

- DNA (from incubated colony samples) - 1 uL

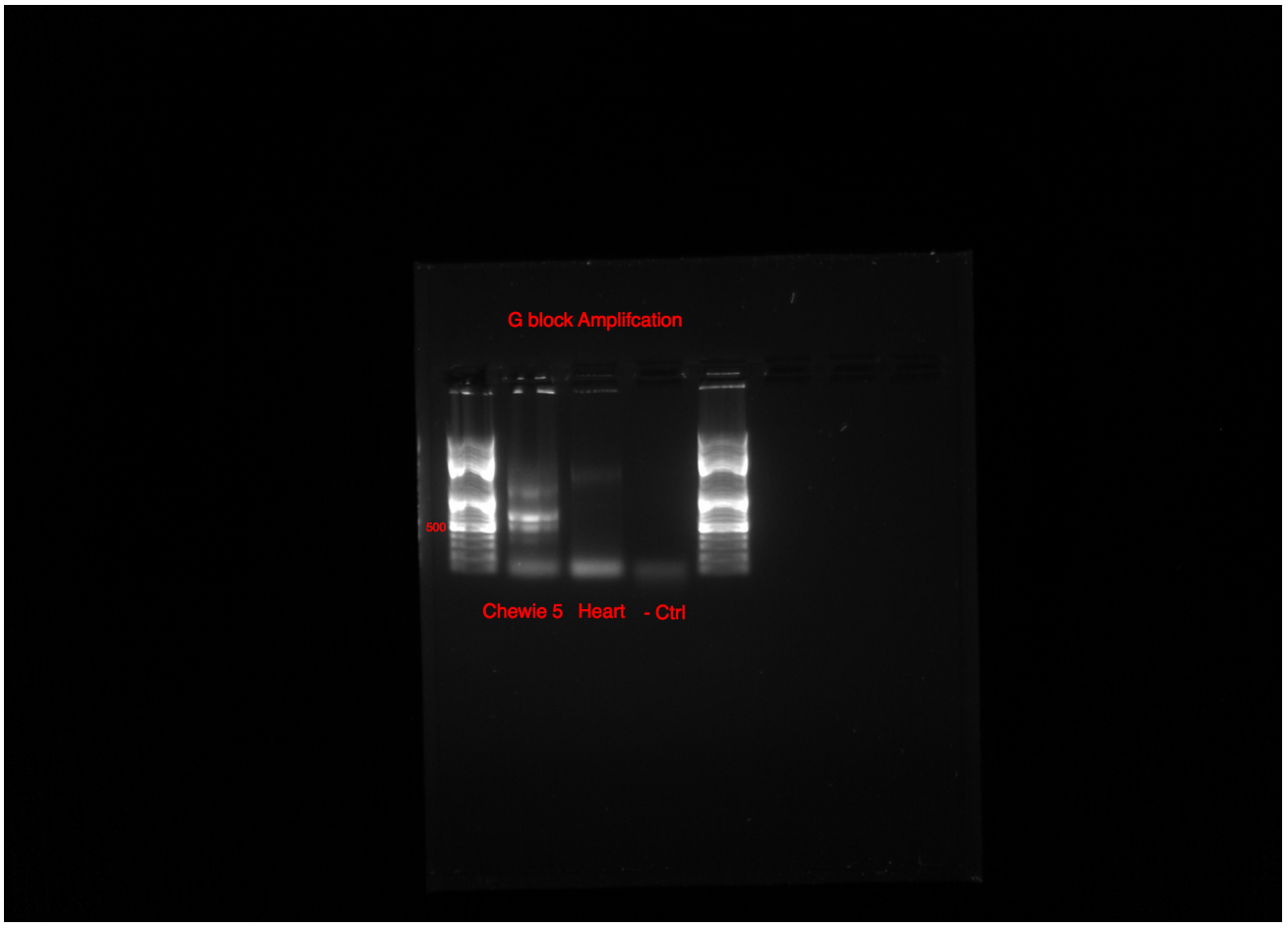
Ran gel of PCR products (180 V, 45 mins)

FRIDAY, 8/14/2020

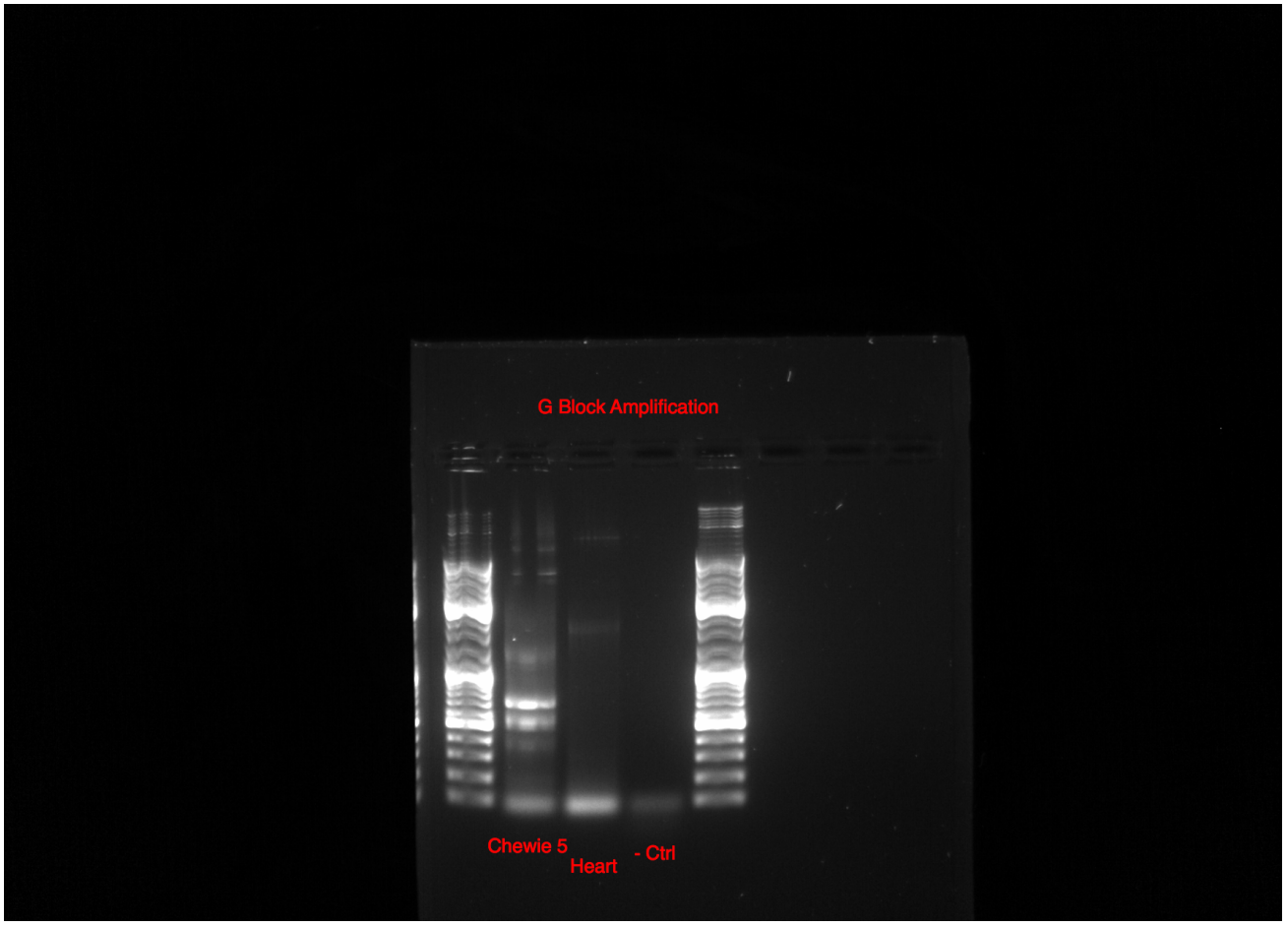
PCR amplification of gBlocks

Reran PCR of chewie 5 (I) and heart (K) using 25 cycles and 65^{o}C annealing temp

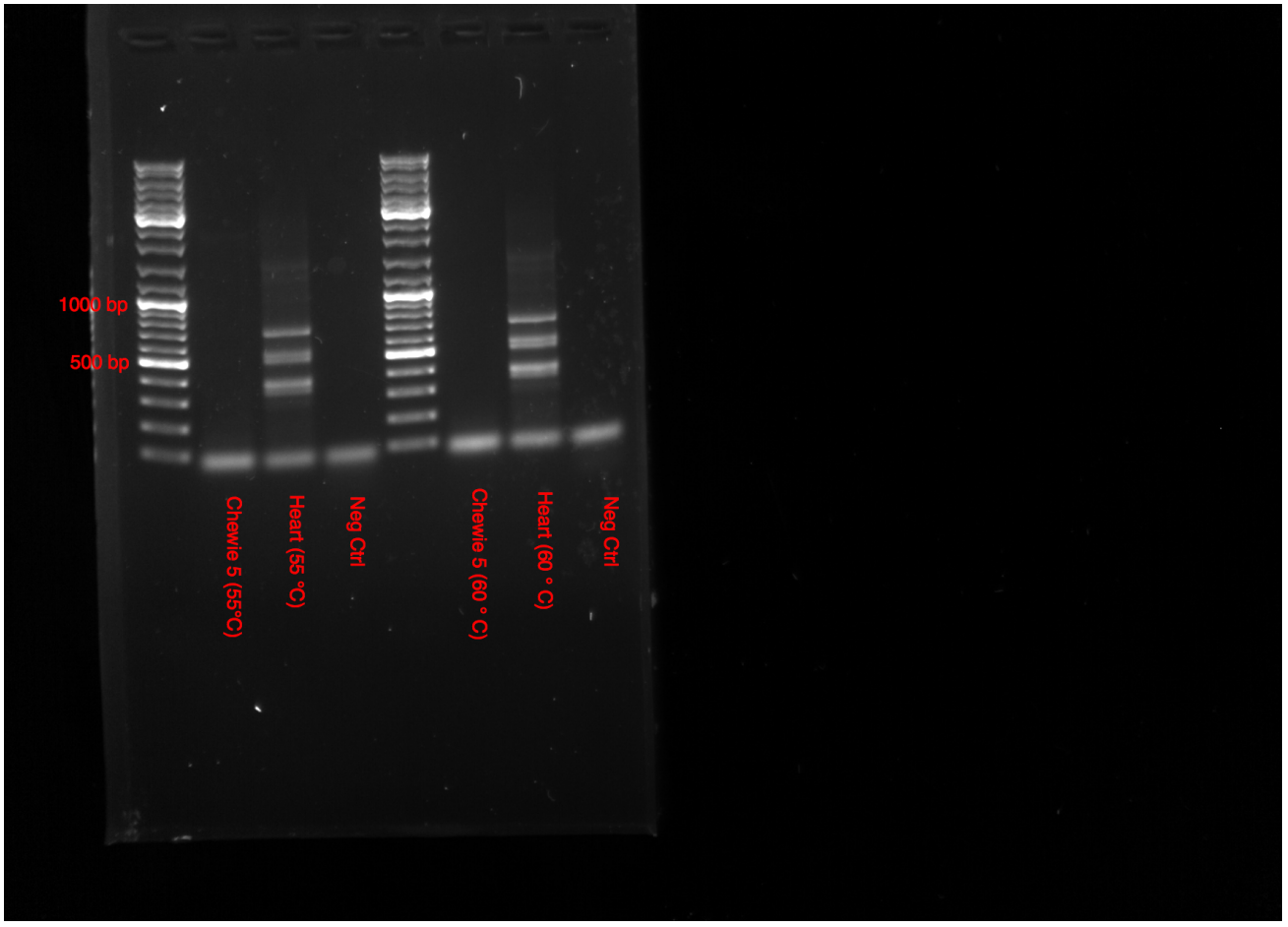
iGEM - G Block PCR (Heart + chewie 5).png



iGEM - G block Amplification - Heart and Chewie (2nd try).png



Heart + Chewie 5 (55° C + 60 ° C annealing).png

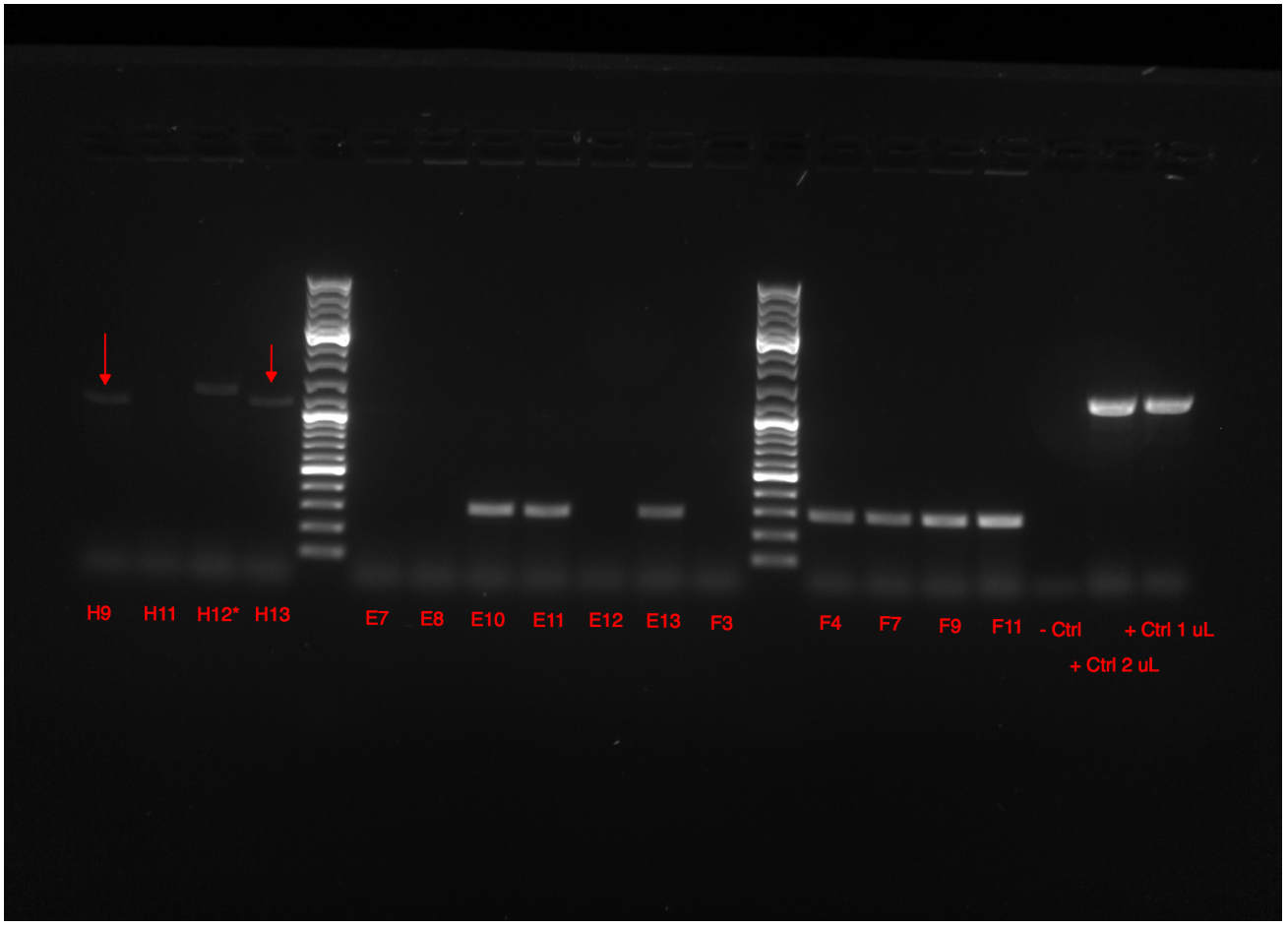


Stored PCR products in fridge/freezer

In vivo amplification of gBlocks

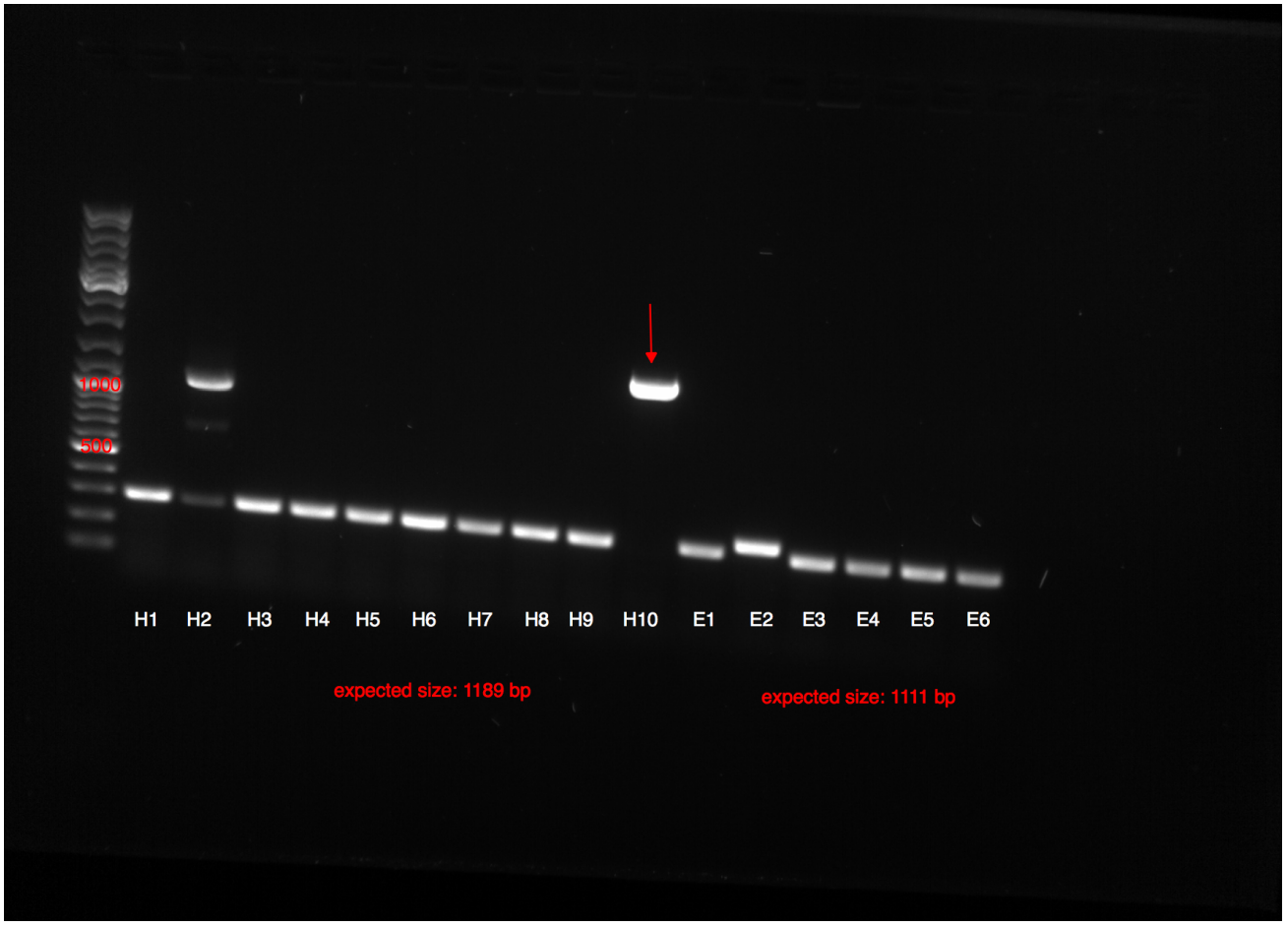
Repeated colony PCR of H9, H11, H12, H13 and 5 of E and 4 of F

iGEM Colony PCR - H, E and F.png

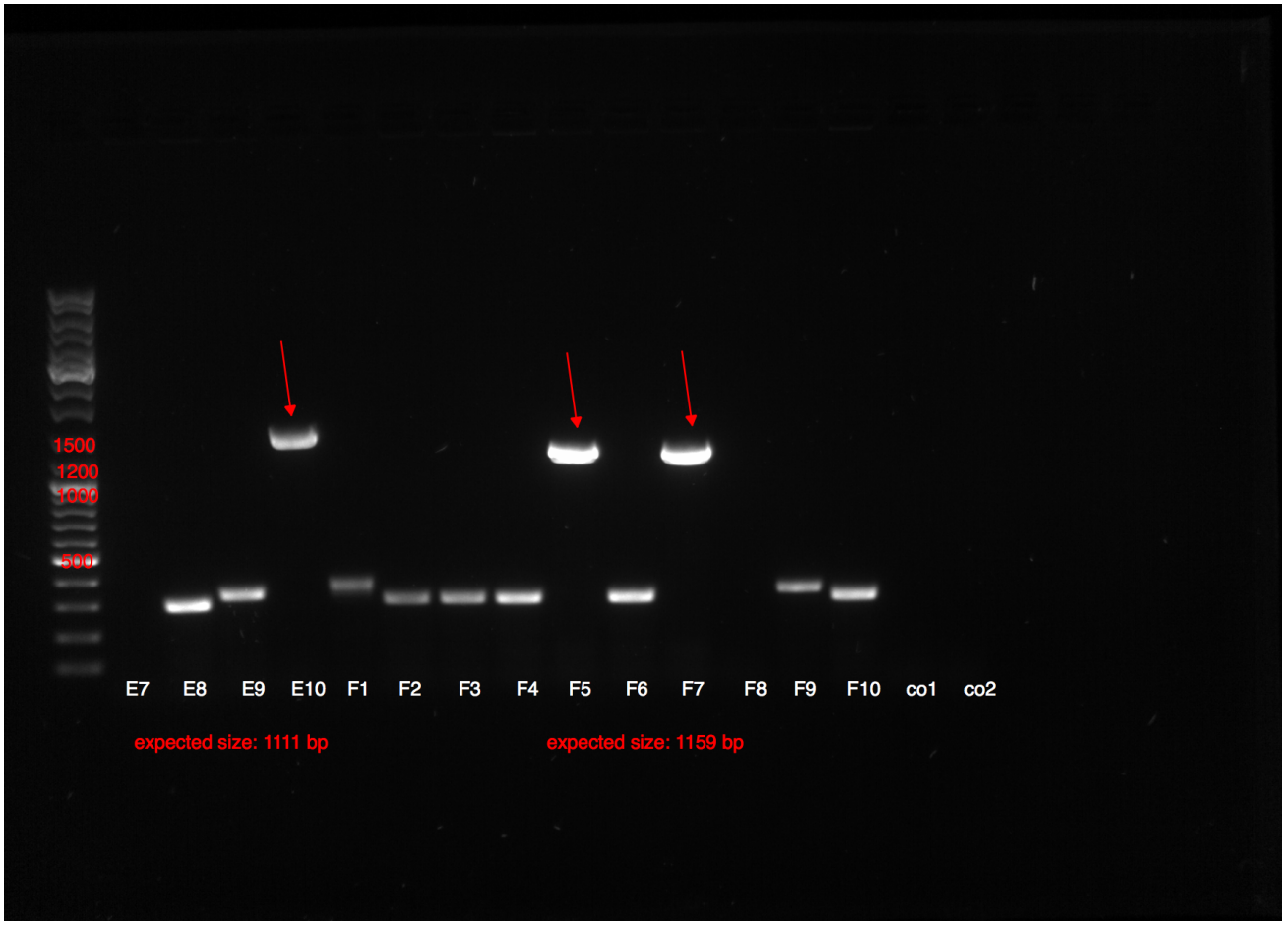


Picked new colonies from E5, F5, H5
Ran colony PCR

h,e aditi 14_08.png



e,f aditi 14_08.png



Prepared weekend cultures of colonies that worked (F5, F7, H2, H9, H13)