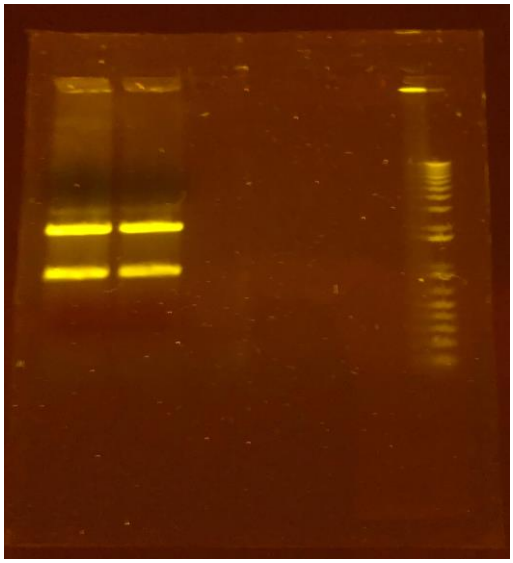
5/29 - 31/5: Wet Lab Training Session 1
3/6 - 5/6: Wet Lab Training Session 2

Week 1

10th June:

Experiment Title	Backbone Digestion + Electrophoresis
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	Gel are solidified for around 10 min. Electrophoresis run for 25 minutes under 130V.
Results	From picture of gel electrophoresis result, the band is quite clear, and the enzymes seem to work well. For the ladder, only right hand side of it ran, maybe is caused by the gel's default.
Comments	For gel to solidified, could wait for longer time.
Photos	 Sample1 Sample 2 Ladder

Details:

- 600ng pSB1C3-K608002-I13401 for each sample

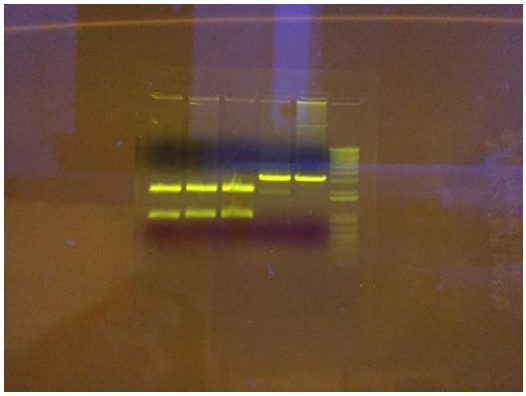
Backbone Con. (µl/ml)	ddH2O (µl)	DNA(µl)	CutSmart Buffer (µl)	PstI (µl)	EcoRI(µl)
246.6	10.8	2.4	1.8	1.5	1.5
114.5	8.0	5.2	1.8	1.5	1.5

Experiment Title	Gel purification

Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	N/A
Results	Result are 2.3μl/ml and 3.3μl/ml respectively.
Comments	The results of purification are extremely low, tried again the gel purification test again tomorrow to see whether the problem is only for this case.
Photos	N/A

Experiment Title	Transformation of E1010, I13401, K68002, J61002
Protocol	Transformation protocol from iGEM https://parts.igem.org/Help:Protocols/Transformation
Changes in Protocol	Using LB instead of SOC media
Results	Nothing grew out overnight.
Comments	Maybe the amount of DNA is too little. Also, the time of cold shock could be longer. Make sure all the experiments are done on the ice.
Photos	N/A

11th June:

Experiment Title	Backbone Digestion + Electrophoresis
Protocol	Refer to Appendix Section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	The bands are cleanly cut. Indicating that the enzymes are working well.
Comments	Please cut the inserts with E & P as well
Photos	 Sample1 Sample 2 Sample 3 Control E Control P Ladder

Details:

- 600ng pSB1C3-K608002-I13401 for each sample

Backbone Conc. (μg/ml)	ddH_2O (μl)	DNA (μl)	CutSmart Buffer (μl)	PstI (μl)	EcoRI (μl)
246.6	10.8	2.4	1.8	1.5	1.5
211.5	10.4	2.8	1.8	1.5	1.5
114.5	8	5.2	1.8	1.5	1.5

- For control

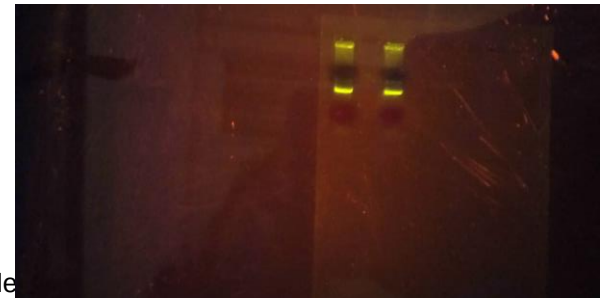
Backbone Conc. (μg/ml)	ddH_2O (μl)	DNA (μl)	CutSmart Buffer (μl)	PstI (μl)	EcoRI (μl)
114.5	9.5	5.2	1.8	---	1.5
114.5	9.5	5.2	1.8	1.5	---

Experiment Title	Gel Purification
Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	N/A
Results	Concentrations: 5.4μg/ml, 3.8μg/ml, 4.2μg/ml respectively
Comments	The gel purification efficiency is too low. Need to optimize this protocol.
Photos	N/A

Experiment Title	Transformation of E1010, I13401, K68002, J61002
Protocol	Transformation protocol from iGEM kit plate http://parts.igem.org/Help:Protocols/Transformation
Changes in Protocol	Use LB instead of SOC media Use 2 ng of DNA instead of 1ng
Results	Nothing grew on the plates after a day.
Comments	Troubleshoot: Maybe the kit plate is unreliable. One can try to transform other parts. Remember to use the amp agar plate when transforming J61002.
Photos	N/A

12th June:

Experiment Title	Testing for gel purification efficiency using new wash buffer
Protocol	Refer to Appendix Section 1 protocols, 1.3

Changes in Protocol	2 samples of pSB1C3 with old and new buffer DNA- 300ng Elution Buffer- 25µL(10+15)
Results	Sample1- 29.6% efficiency Sample 2- 30.2% efficiency
Comments	Not high enough. Repeated with the commercial kit
Photos	Sample 

Experiment Title	Gel purification with the commercial kit
Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	2 samples of pSB1C3 with the commercial kit DNA- 300ng Elution Buffer- 25µL(10+15)
Results	Sample1- 140% efficiency Sample 2- 150% efficiency
Comments	Abnormal results probably due to contamination
Photos	N/A

Experiment Title	Backbone Digestion
Protocol	Refer to Appendix section 1 protocols, 1.1
Changes in Protocol	N/A
Results	N/A
Comments	Incubated at 37 for one hour
Photos	N/A

Details:

- 500ng pSB1C3

Backbone Con. (µl/ml)	ddH2O (µl)	DNA(µl)	CutSmart Buffer (µl)	PstI (µl)	EcoRI(µl)
246.6	10.8	2.4	1.8	1.5	1.5

Experiment Title	Testing cell competency
Protocol	iGEM cell competency testing kit protocol https://parts.igem.org/Help:2017_Competent_Cell_Test_Kit
Changes in Protocol	N/A
Results	Nothing grew on the plates.
Comments	The cells have lost their competency
Photos	N/A

13th June:

Experiment Title	Testing the commercial kit for gel purification again
Protocol	Refer to Appendix section 1 protocols, 1.2 and 1.3
Changes in Protocol	2 samples of pSB1C3 with new buffer and commercial kit DNA- 500ng Elution Buffer- 30µL(15+15)
Results	Efficiency: Sample1-105%, Sample2-160%
Comments	Abnormally high yield observed again
Photos	

Experiment Title	Mini-prep GFP K608002-I13401
Protocol	Refer to Appendix Section 1 protocols, 1.8
Changes in Protocol	N/A
Results	

Sample	Conc. (µg/ml)	A260/A230	A260/A280

	<table><tr><td>1</td><td>184.1</td><td>2.22</td><td>1.86</td></tr><tr><td>2</td><td>114.7</td><td>2.136</td><td>1.89</td></tr><tr><td>3</td><td>120.9</td><td>2.125</td><td>1.865</td></tr></table>
Comments	High yield → very nice
Photos	N/A

Experiment Title	Testing the viability of cells
Protocol	Spreading the competent cells in the fridge on to an antibiotic-free plate.
Changes in Protocol	N/A
Results	The cells are alive.
Comments	N/A

Experiment Title	Making LB and plates for making competent cells
Protocol	Adding 10g Tryptone, 5g of yeast extract, 10g NaCl, 15g of agar into a bottle and fill the water to 1 liter. Autoclave. (pick up around 1 pm) Making plates under sterile conditions.
Changes in Protocol	Half of them are for plates without antibiotics; half of them are with Ampicillin
Results	Plates
Comments	N/A

Experiment Title	Streak out bacteria for Ultra-competent cells
Protocol	Spreading the competent cells getting from KC’s lab on to an antibiotic-free plate.
Changes in Protocol	N/A
Results	The cells grew overnight
Comments	Cells are prepared for Monday’s Ultra-competent cells

14th June:

Experiment Title	Transformation of BBa_J23100 and BBa_J23104 with KC lab competent cells

Protocol	Refer to Appendix Section 1 protocols, 1.4
Changes in Protocol	Heat shock for 70 seconds, incubate in room temperature over the weekends.
Results	The cells grew overnight
Comments	N/A

Experiment Title	Making LB for Ultra-Competent Cells
Protocol	Three 1L LB broth, one 250mL broth Autoclave. (pick up around 1 pm) Making plates under sterile conditions.
Changes in Protocol	N/A
Results	Left on bench
Comments	N/A

Week 2

17th June:

Experiment Title	Making Lysate Buffer
Protocol	10ml Tris-base (pH 8.2 adjusted with acetic acid) 14ml Mg glutamate 60ml K glutamate Top up to 998ml ddH2O
Changes in Protocol	N/A
Results	N/A
Comments	Add 2ml of DTT when ready to use

Experiment Title	Making Ultra-Competent Cells
Protocol	Refer to Appendix Section 1 protocols, 1.11
Changes in Protocol	In sample 1, added 10ml into flask; sample 2, added 4ml; sample 3, added 2ml.
Results	Put in the 18^{o}C incubator at 16:40 and took out at 9:10 in the next day. 9:20 18/06/2019

Sample	1	2	3
OD_{600}	0.190	0.056	0.021

Comments	N/A

18th June:

Experiment Title	Making Ultra-Competent Cells (Cont)
Protocol	Refer to Appendix Section 1 protocols, 1.11
Changes in Protocol	Add more starter culture into the flasks next time.
Results	At 09:20 Sample: 1 | 2 | 3 OD_{600}: 0.190 | 0.056 | 0.021 At 15:00: Sample: 1 | 2 | 3 OD_{600}: 0.370 | 0.120 | 0.035 At 15:45: Sample: 1 | 2 | 3 OD_{600}: 0.422 | 0.130 | 0.043 At 16:55: Sample: 1 | 2 | 3 OD_{600}: 0.495 | N/A | N/A
Comments	Ultra-competent cells kept under -80°C

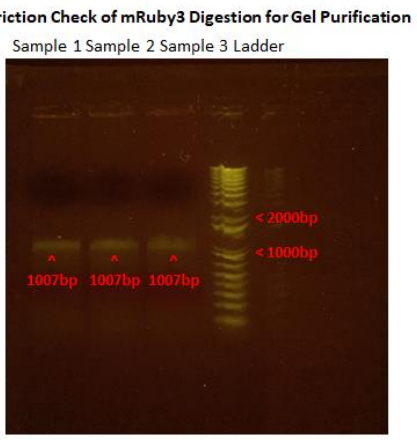
Experiment Title	1
Protocol	Refer to Appendix section 1 protocols, 1.1
Changes in Protocol	N/A
Results	N/A
Comments	Incubated at 37°C for one hour
Photos	N/A

Details:

- 100ng mRuby3

Backbone Con. (µg/µl)	ddH2O (µl)	DNA(µl)	CutSmart Buffer (µl)	PstI (µl)	EcoRI(µl)

10	4.8	10	1.8	0.7	0.7
10	4.8	10	1.8	0.7	0.7
10	4.8	10	1.8	0.7	0.7

Experiment Title	Electrophoresis + mRuby3 Gel Purification
Protocol	Refer to Appendix section 1 protocols, 1.2 and 1.3
Changes in Protocol	3 samples of mRuby3 DNA- 100ng per eppendorf Elution Buffer- 30µL(15+15)
Results	Concentrations: 2.616µg/ml, 2.502µg/ml, 4.000µg/ml
Comments	Abnormally high yield observed again
Photos	

Experiment Title	Ligation of mRuby3 insert to PSB1C3 backbone
Protocol	Refer to Appendix section 1.1
Changes in Protocol	N/A
Results	Not Checked
Comments	N/A
Photos	N/A

19th June:

Experiment Title	Mini-Prep J23104-J61002

<table>
<tr><td>Protocol</td><td>Refer to Appendix Section 1 protocols, 1.8</td></tr>
<tr><td>Changes in Protocol</td><td>N/A</td></tr>
<tr><td>Results</td><td><table>
<tr><td>Sample</td><td>Conc. (μg/ml)</td><td>A260/A230</td><td>A260/A280</td></tr>
<tr><td>1</td><td>75.96</td><td>2.717</td><td>1.901</td></tr>
</table>
In total 50 μL</td></tr>
<tr><td>Comments</td><td>Reasonable result</td></tr>
<tr><td>Photos</td><td>N/A</td></tr>
</table>

Experiment Title	mClover3 sequence digestion + Electrophoresis (#1)
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	Electrophoresis ran at 130V for 30 min
Results	By looking at the result of electrophoresis, there are only bands close to the well, which might indicate digestion isn't successfully done
Comments	It might be the problem of not getting the enzyme into the sample properly, the enzyme could be stick to the eppendorfs' wall and not well mixed with samples
Photos	N/A

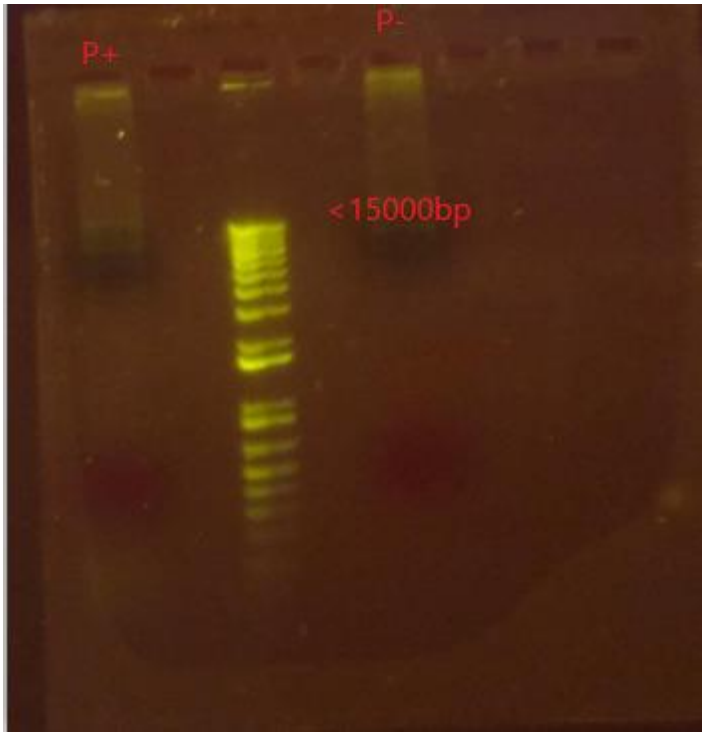
Experiment Title	mClover3 sequence digestion + Electrophoresis (#2)
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	Electrophoresis ran at 130V for 25 min
Results	Dim bands at targeted region.
Comments	The reason why the band look so dim in the picture could be the amount we took for each sample is too low.
Photos	

Experiment Title	mClover3 Gel Purification
Protocol	Refer to Appendix section 1 protocols, 1.3
Changes in Protocol	2 samples of mClover3 DNA- 100ng per eppendorf

	Elution Buffer- 30µL(15+15)
Results	
Comments	The result is fine.
Photos	N/A

Experiment Title	PCR of J23104_J61002 (RFP)
Protocol	Refer to Appendix section 1 protocol 1.12
Changes in Protocol	2 samples- primer and control (without primers) Reaction volume- 20uL RNA forward and reverse primers conc- 10uM Mistakes: the amount of DNA we put in is incorrect. We put in 150 ng, which is 10x of the normal concentration
Results	Failed P+: actual pcr products (no clear band of target size of 850 bp) Ladder: invitrogen 1kb+ P-: no primer control (same band smear as P+)
Comments	1 hour Using old tube of Q5

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 72°C 72°C	10 seconds 30 seconds 30 seconds
Final Extension	72°C	2 minutes

Photos	

Experiment Title	Transformation of mRuby3
Protocol	Refer to Appendix section 1 protocols, 1.4
Changes in Protocol	N/A
Results	No colonies on the plate.
Comments	Could be the problem of transformation or the problem of new batch of competent cells. Needed troubleshooting (testing the competent cells with kits and plasmid).
Photos	N/A

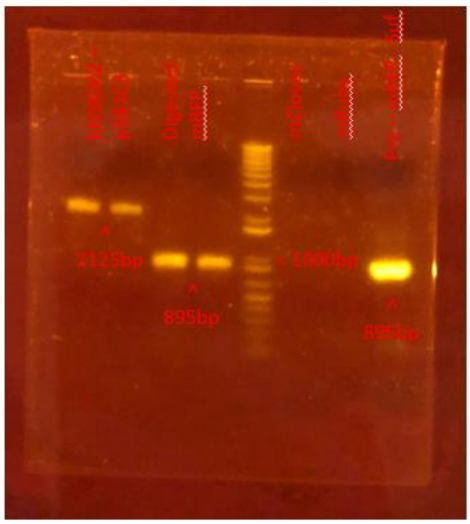
20th June:

Experiment Title	Ligation of mClover3 insert to PSB1C3 backbone
Protocol	Refer to Appendix section 1.1
Changes in Protocol	N/A
Results	Not Checked
Comments	N/A
Photos	N/A

Experiment Title	Transformation of BBa_K608002
Protocol	Transformation protocol from iGEM https://parts.igem.org/Help:Protocols/Transformation
Changes in Protocol	Using LB instead of SOC media; use 2 ng of DNA instead of 1ng
Results	Three visible colonies grew on the plate. (later usage for colony PCR)
Comments	N/A
Photos	N/A

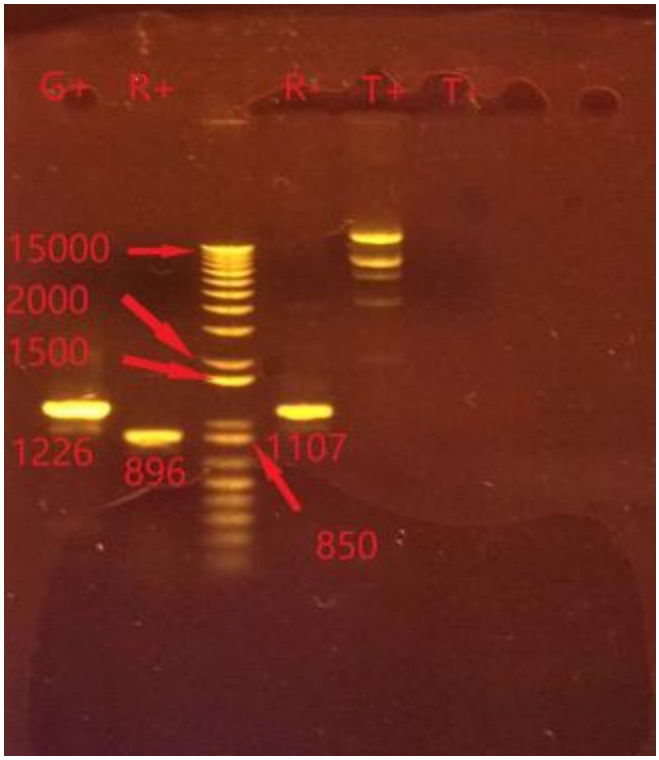
Experiment Title	Testing competency of ultra competent cells and competency cell kit
Protocol	Transformation protocol from iGEM competent cell kit http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit
Changes in Protocol	Using LB instead of SOC media; use 0.1 ng and 0.01 ng from BBAJ04450 (RFP from the testing kit DNA) and 75.96ng and 7.596ng of J23104-J61002 (RFP from our stock)
Results	For all plates spread have colonies on, which indicate the competent cells are working well.
Comments	The team ran out of 1.5ml eppendorf tubes, so we used 0.5ml tubes for the two J23104-J61002 plates during transformation. We also spread 40ul of ampicillin on our agar plates before spreading the transformed cells to ensure that bacterial selection occurs efficiently
Photos	N/A

Experiment Title	Ligation verification of mClover and mRuby
Protocol	PCR with Q5 polymerase (NEB)
Changes in Protocol	N/A
Results	Since there are no visible bands, we inferred that the ligation of mClover and mRuby failed.
Comments	

Photos	

Experiment Title	PCR of J23104-J61002 with overhang primers
Protocol	Refer to https://international.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491
Changes in Protocol	Reaction volume- 25uL RNA forward and reverse primers conc- 10uM Amount of template added: 15ng per reaction
Results	G+: GFP plasmid (VF2/VR)--positive control. R+: RFP plasmid (overhang primers fwd/bwd)--wt we actually need. Ladder R-: RFP plasmid (VF2/VR)--control to overhang primers. T+: RFP plasmid only. T-: No template control
Comments	The R+ showed target band at around 900bp, which is correct. This means PCR succeeded However, the T+ (RFP template) only showed multiple bands, larger than expected sizes.

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 72°C 72°C	10 seconds 30 seconds 30 seconds
Final Extension	72°C	5 minutes

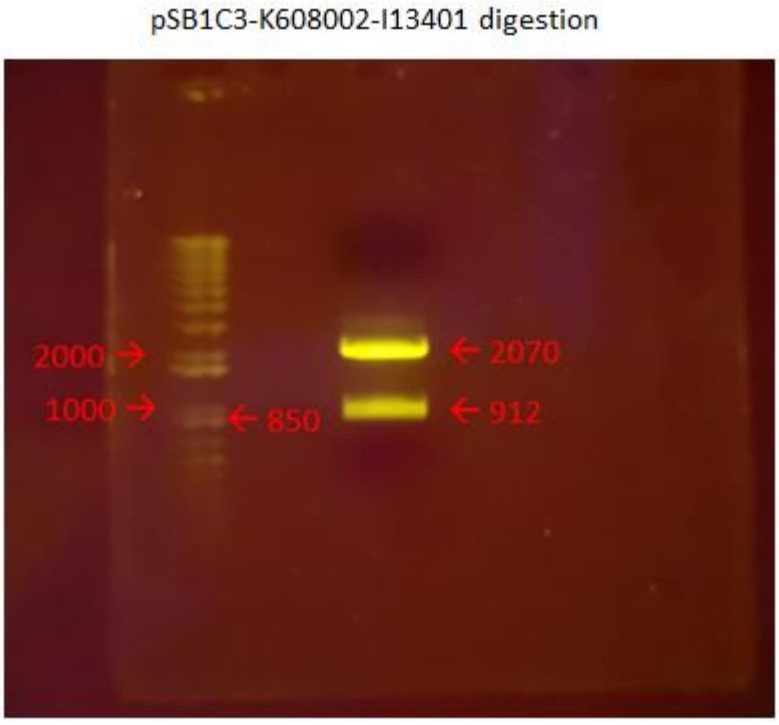
photos	

21st June:

Experiment Title	Gel Purification of RFP
Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	N/A
Results	Concentrations: 3.3µg/ml, 3.5µg/ml respectively
Comments	N/A
Photos	N/A

Experiment Title	Colony Streaking of BBa_K608002
Protocol	Refer to Appendix Section 1 protocols, 1.5
Changes in Protocol	N/A
Results	3 plates streaked in 37°C incubator
Comments	N/A
Photos	N/A

Experiment Title	Colony PCR of BBa_K608002
Protocol	Refer to Appendix Section 1 protocols, 1.
Changes in Protocol	N/A
Results	Colony plates 2 and 3 can be used later
Comments	Red band appeared in colony plate 1
Photos	N/A

Experiment Title	Backbone Digestion + Electrophoresis (for next week's ligation troubleshooting)
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	Gel are solidified for around 15 min. Electrophoresis run for 25 minutes under 130V.
Results	From picture of gel electrophoresis result, the band is quite clear, can clearly distinguish backbone's band and insert's.
Comments	N/A
Photos	

Details:

- 1000ng pSB1C3-K608002-I13401 for each sample

Backbone Con. (µl/ml)	ddH2O (µl)	DNA(µl)	CutSmart Buffer (µl)	PstI (µl)	EcoRI(µl)
114.5	4.5	8.7	1.8	1.5	1.5

Experiment Title	Gel purification
Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	N/A
Results	<table><tr><td>Sample</td><td>Conc. (µg/ml)</td><td>A260/A230</td><td>A260/A280</td></tr><tr><td>1</td><td>11.47</td><td>0.089</td><td>2.097</td></tr><tr><td>2</td><td>7.119</td><td>0.054</td><td>2.282</td></tr></table>
Comments	Need to find out the reason why ratio of A260/A230 and A260/A280 are abnormal, it could be contaminated by other factors.
Photos	N/A

Week 3

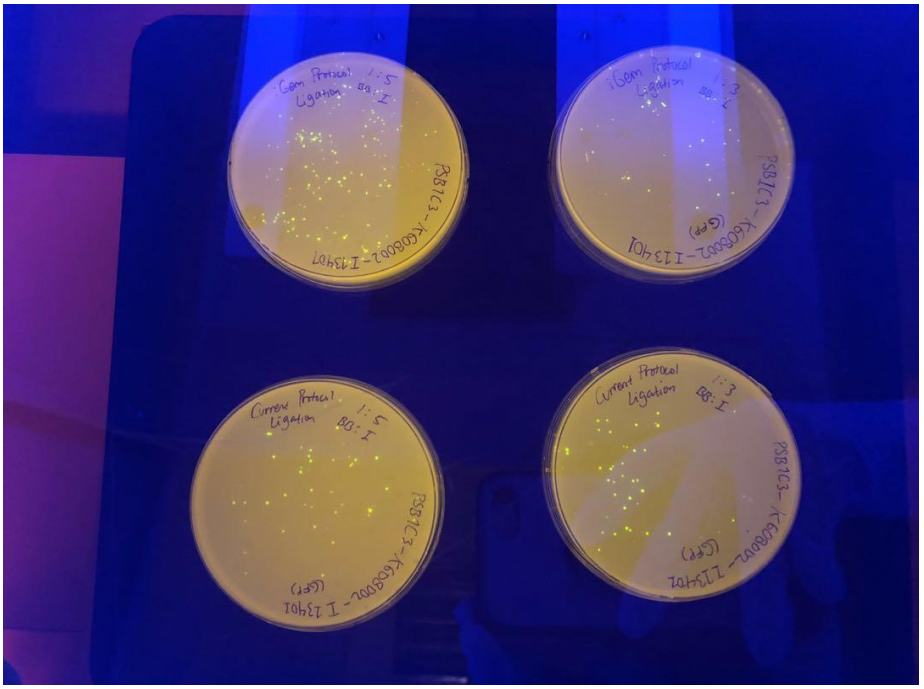
24th June:

Experiment Title	Digestion of RFP_PCR(X,P) product and K618002_pSB1C3(S,P)
Protocol	Refer to protocol 1.1
Changes in Protocol	No changes
Results	
Comments	Gel extraction yield is very low
Photos	N/A

Experiment Title	Ligation optimisation using IGEM and lab protocol (GFP)
Protocol	Refer to protocol 1.1
Changes in Protocol	Total vol- 15ul Insert (K608002-I13401) conc- 7.12ug/ml Vector (pSB1C3) conc- 11.47ug/ml <table><tr><td></td><td>Current protocol</td><td>IGem protocol</td></tr></table>

	<table><tr><td>V/I ratio</td><td>1:3</td><td>1:5</td><td>1:3</td><td>1:5</td></tr><tr><td>Vector</td><td>10ng- 0.87ul</td><td>10ng- 0.87ul</td><td>25ng- 2.18ul</td><td>25ng- 2.18</td></tr><tr><td>Insert</td><td>13.3ng- 1.86ul</td><td>22.1ng- 3.10ul</td><td>33.1ng- 4.65ul</td><td>55.1ng- 7.74ul</td></tr><tr><td>T4 buffer</td><td>1.5ul</td><td>1.5ul</td><td>1.5ul</td><td>1.5ul</td></tr><tr><td>T4 ligase</td><td>0.75ul</td><td>0.75ul</td><td>0.75ul</td><td>0.75ul</td></tr><tr><td>H2O</td><td>10.02ul</td><td>8.78ul</td><td>5.92ul</td><td>2.83ul</td></tr></table>
Results	See transformation
Comments	In this experiment, there is no control, which is important though. And in this case, for the ligation, we did 50 min 25°C and 10 min 37°C, while the su temperature for 2 hours.
Photos	N/A

Experiment Title	Transformation of ligated GFP product (K608002-I13401)
Protocol	Refer to protocol 1.4
Changes in Protocol	Used cell competency test kit transformation protocol due to successful results obta 1ul DNA added
Results	From the photo, the iGEM protocol 1:5 gives the best result.From the photo, the iGE
Comments	Can adjust current protocol to iGEM protocol 1:5, which gives the best result.

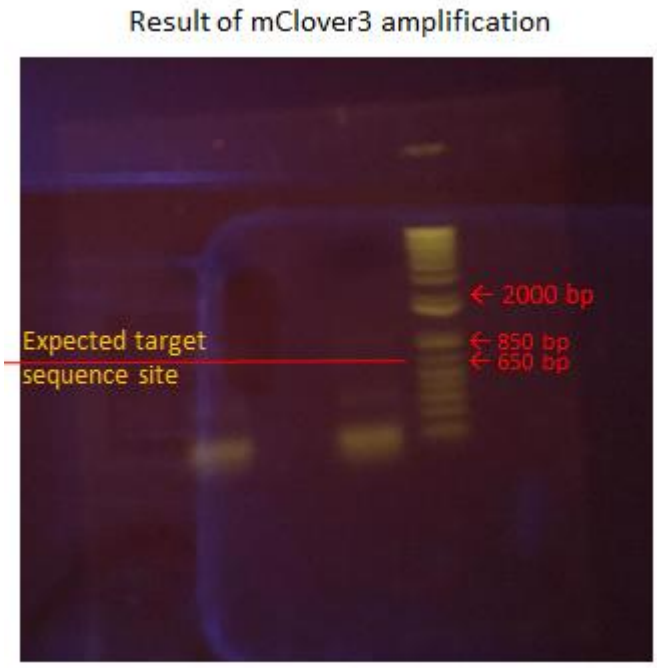
Photos	

Experiment Title	Ligation of RFP_PCR and K608002_pSB1C3
Protocol	Protocol 1.1 (highly modified)
Changes in Protocol	Due to the extremely low concentration of digested DNA, the volume we 25ng K608001_pSB1C3 (15ul) 31.6ng RFP_PCR (18ul) 1.5ul T4 ligase 3.45u; T4 buffer
Results	
Comments	
Photos	

25th June:

Experiment Title	mClover3 PCR amplification
Protocol	Q5 PCR protocol from NEB (https://international.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491)
Changes in Protocol	Since the protocol mention the template DNA amount to be variable - put in 100ng of DNA
Results	N/A - see “Amplified mClover3 Electrophoresis + gel purification”
Comments	Wrong DNA amount (too much), but either no clear band at the correct site

	(which is around 700 bp), need troubleshooting for amplification again.
Photos	N/A

Experiment Title	Amplified mClover3 Electrophoresis + gel purification
Protocol	Refer to protocol 1.2, 1.3
Changes in Protocol	Electrophoresis run for 25 minutes under 130V.
Results	The final conc. is 1.834 ug/ml in 30ul
Comments	The amplification failed. Before PCR, there were 100ng of mClover3. After amplification,the amount became less. (Result due to originally wrong amount of DNA during amplification)
Photos	

Experiment Title	mRuby ligation to pSB1C3
Protocol	Refer to protocol 1.1
Changes in Protocol	Total Volume- 20ul mRuby conc- 4.00ul/ml BB conc- 3.62ul/ml V/I ratio- 1:3 BB amount- 25ng

Vector	6.9ul
Insert	9.4ul

	<table><tr><td>T4 buffer</td><td>2ul</td></tr><tr><td>T4 ligase</td><td>1ul</td></tr><tr><td>H2O</td><td>0.7ul</td></tr></table>
Results	Used for transformation
Comments	N/A
Photos	N/A

Experiment Title	Transformation of ligated mRuby product and control pSB1C3
Protocol	Refer to protocol 1.4
Changes in Protocol	Used cell competency test kit transformation protocol due to successful results obtained 1ul DNA added
Results	No colonies on the plate
Comments	After one more day for it in the incubator, there are colonies growing out. But the colonie gonna comparing the colonies with one on June 26th's mRuby plate.
Photos	N/A

26th June:

Experiment Title	mRuby3 PCR amplification
Protocol	Q5 PCR protocol from NEB (https://international.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491)
Changes in Protocol	<table><tr><td>Step</td><td>Temperature</td><td>Time</td></tr><tr><td>Initial Denaturation</td><td>98°C</td><td>30 seconds</td></tr><tr><td>35 Cycles</td><td>98°C 72°C 72°C</td><td>10 seconds 30 seconds 30 seconds</td></tr><tr><td>Final Extension</td><td>72°C</td><td>2 minutes</td></tr></table>
Results	N/A - see "Amplified mRuby3 Electrophoresis + gel purification"
Comments	N/A

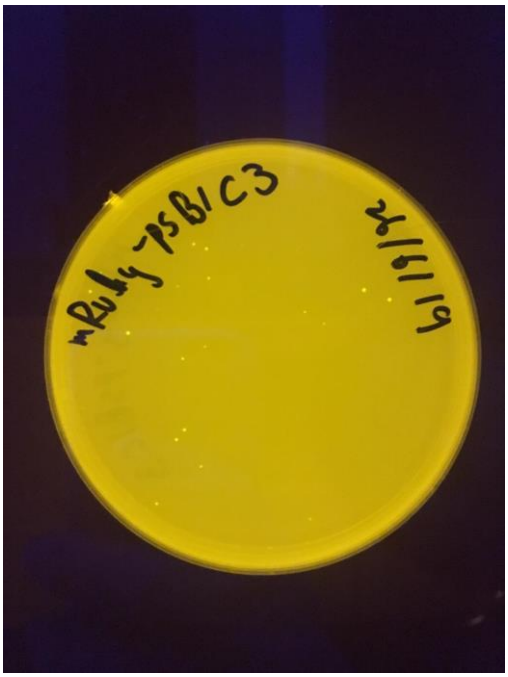
Photos	N/A

Experiment Title	Amplified mRuby3 Electrophoresis + gel purification
Protocol	Refer to protocol 1.2, 1.3
Changes in Protocol	Electrophoresis run for 25 minutes under 130V.
Results	The final conc. is 15.9 ug/ml in 30ul
Comments	The amplification is success.
Photos	N/A

Experiment Title	Repeating mRuby ligation to pSB1C3
Protocol	Refer to protocol 1.1
Changes in Protocol	Total Volume- 20ul mRuby conc- 4.00ul/ml BB conc- 3.62ul/ml V/I ratio- 1:3 BB amount- 25ng
Results	Used for transformation
Comments	N/A
Photos	N/A

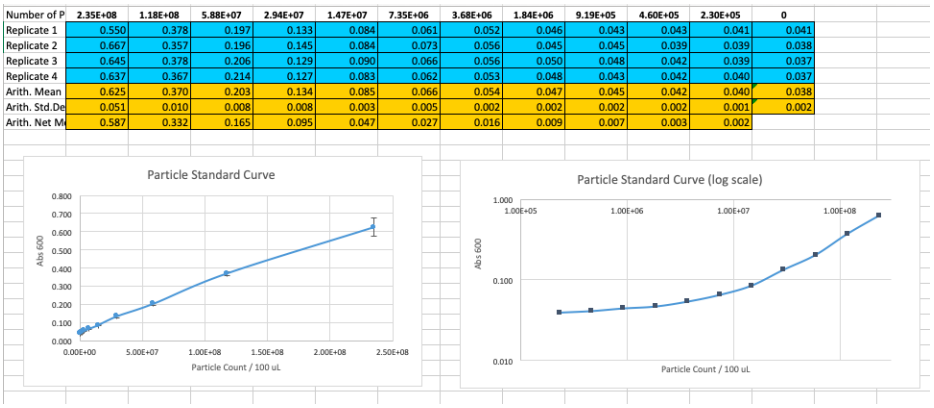
Vector	6.9ul
Insert	9.4ul
T4 buffer	2ul
T4 ligase	1ul
H2O	0.7ul

Experiment Title	Transformation of ligated mRuby product and control pSB1C3
Protocol	Refer to protocol 1.4
Changes in Protocol	Used cell competency test kit transformation protocol due to successful results obtaine 1ul DNA added
Results	Green colonies obtained- abnormal
Comments	Take one of the colonies to plate reader testing.

Photos	

27th June:

Experiment Title	Particle calibration on plate reader
Protocol	Reference to 2018 iGEM interlab protocol calibration 2
Changes in Protocol	Plate reader setting WellScan pattern: Fill (9 points) absorbance: 600nm Shake: Once before reading
Results	Excel file with reduced data and calibration standard curve https://drive.google.com/file/d/1gkTnE5N0YVnx2WrwpmzqCRxIy84q04Fo/view?usp=sha
Comments	Values should form a linear 1:1 on both linear and log scale. But due to lack of sensitivity wells of lower concentrations could not be distinguished well.

Photos	

Number of P	2.35E+08	1.18E+08	5.88E+07	2.94E+07	1.47E+07	7.35E+06	3.68E+06	1.84E+06	9.19E+05	4.60E+05	2.30E+05	0
Replicate 1	0.550	0.378	0.197	0.133	0.084	0.061	0.052	0.046	0.043	0.043	0.041	0.041
Replicate 2	0.667	0.357	0.196	0.145	0.084	0.073	0.056	0.045	0.045	0.039	0.039	0.038
Replicate 3	0.645	0.378	0.206	0.129	0.090	0.066	0.056	0.050	0.048	0.042	0.039	0.037
Replicate 4	0.637	0.367	0.214	0.127	0.083	0.062	0.053	0.048	0.043	0.042	0.040	0.037
Arith. Mean	0.625	0.370	0.203	0.134	0.085	0.066	0.054	0.047	0.045	0.042	0.040	0.038
Arith. Std.De	0.051	0.010	0.008	0.008	0.003	0.005	0.002	0.002	0.002	0.002	0.001	0.002
Arith. Net M	0.587	0.332	0.165	0.095	0.047	0.027	0.016	0.009	0.007	0.003	0.002	

Experiment Title	Inoculate colonies grew on 26/6 and 25/6's mRuby ligation plates
Protocol	Refer to protocol 1.7
Changes in Protocol	Used ampicillin
Results	Used in 28th plate readers' verifying
Comments	N/A
Photos	N/A

28th June:

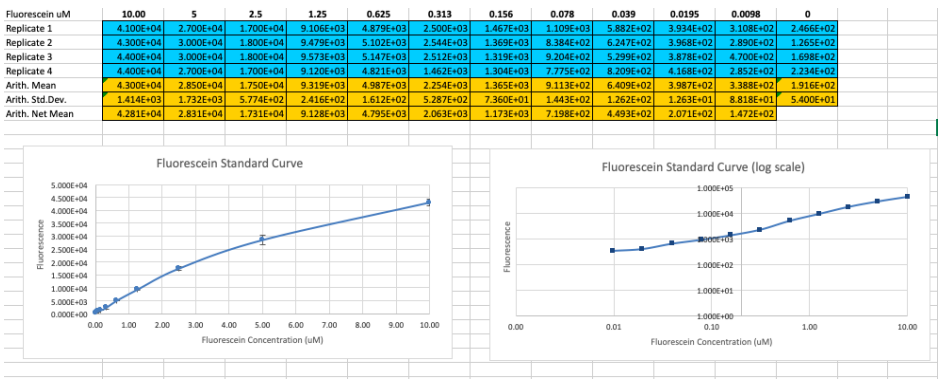
Experiment Title	Verifying unknown colonies
Protocol	Plate wells 1st run: Ex:490nm, Em:525nm (GFP wavelengths) 2ns run: Ex: 558nm, Em:592nm (mRuby3 wavelengths) 3rd run: Ex: 505nm, Em:515nm (mClover3 wavelengths)
Changes in Protocol	N/A
Results	1st run (GFP):

	1	2	3	4
F	Glowing colonies (plated on 26/6)			
G	Non-glowing colonies (plated on 25/6)			

	1	2	3	4	avg
F	1.2e4	1.2e4	1.1e4	1.2e4	1.2e4

<table>
<tr><td></td><td>
<table>
<tr><td>G</td><td>656.17</td><td>586.12</td><td>582.11</td><td>590.91</td><td>603.83</td></tr>
</table>
2nd run (mRuby3):
<table>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>avg</td></tr>
<tr><td>F</td><td>251.38</td><td>231.88</td><td>230.72</td><td>237.75</td><td>237.93</td></tr>
<tr><td>G</td><td>261.06</td><td>237.81</td><td>223.82</td><td>226.07</td><td>237.19</td></tr>
</table>
3rd run (mClover3):
<table>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>avg</td></tr>
<tr><td>F</td><td>7.1e4</td><td>6.7e4</td><td>6.3e4</td><td>5.9e4</td><td>6.5e4</td></tr>
<tr><td>G</td><td>6.3e4</td><td>6.2e4</td><td>6.1e4</td><td>6.0e4</td><td>6.2e4</td></tr>
</table>
</td></tr>
<tr><td>Comments</td><td>Possible interpretations:
<table>
<tr><td>1st run</td><td>F >>> G: F contains GFP, G doesn’t contain GFP</td></tr>
<tr><td>2nd run</td><td>F ~ G (low levels): both F and G don’t contain mRuby3</td></tr>
<tr><td>3rd run</td><td>F ~ G (high levels): both F and G contain mClover3</td></tr>
</table>
</td></tr>
<tr><td>Photos</td><td></td></tr>
</table>

<table>
<tr><td>Experiment Title</td><td>Fluorescence calibration on plate reader</td></tr>
<tr><td>Protocol</td><td>Reference to 2018 iGEM interlab protocol calibration 3</td></tr>
<tr><td>Changes in Protocol</td><td>Plate reader settings:
<table>
<tr><td>WellScan pattern</td><td>Fill (9 points)</td></tr>
<tr><td>Excitation</td><td>485nm</td></tr>
<tr><td>Emission</td><td>525nm</td></tr>
</table>
</td></tr>
<tr><td>Results</td><td>https://drive.google.com/file/d/1Gs_nbwrPgn25gMrB00lvpMouZym60_8y/view?usp=shari</td></tr>
<tr><td>Comments</td><td>Okay curve</td></tr>
</table>

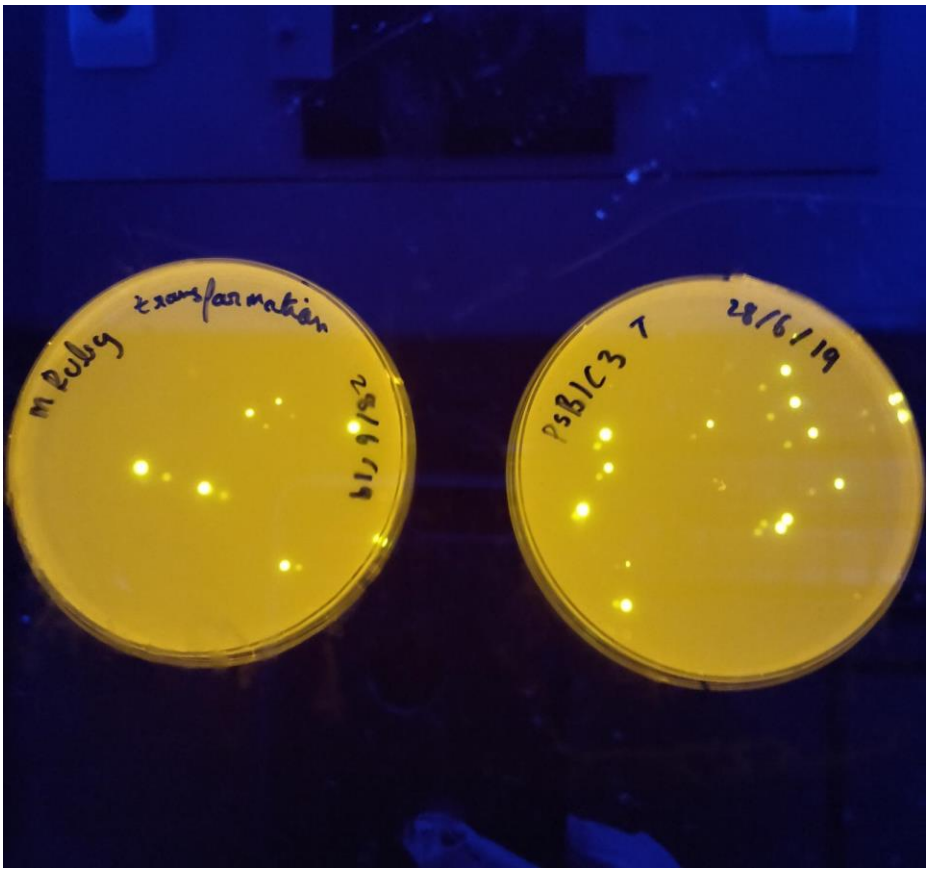
Photos	

Fluorescein uM	10.00	5	2.5	1.25	0.625	0.313	0.156	0.078	0.039	0.0195	0.0098	0
Replicate 1	4.100E+04	2.700E+04	1.700E+04	9.106E+03	4.879E+03	2.500E+03	1.467E+03	1.109E+03	5.882E+02	3.934E+02	3.108E+02	2.466E+02
Replicate 2	4.300E+04	3.000E+04	1.800E+04	9.479E+03	5.102E+03	2.544E+03	1.369E+03	8.384E+02	6.247E+02	3.968E+02	2.890E+02	1.265E+02
Replicate 3	4.400E+04	3.000E+04	1.800E+04	9.573E+03	5.147E+03	2.512E+03	1.319E+03	9.204E+02	5.299E+02	3.878E+02	4.700E+02	1.698E+02
Replicate 4	4.400E+04	2.700E+04	1.700E+04	9.120E+03	4.821E+03	1.462E+03	1.304E+03	7.775E+02	8.209E+02	4.168E+02	2.852E+02	2.234E+02
Arith. Mean	4.300E+04	2.850E+04	1.750E+04	9.319E+03	4.987E+03	2.254E+03	1.365E+03	9.113E+02	6.409E+02	3.987E+02	3.388E+02	1.916E+02
Arith. Std.Dev.	1.414E+03	1.732E+03	5.774E+02	2.416E+02	1.612E+02	5.287E+02	7.360E+01	1.443E+02	1.262E+02	1.263E+01	8.818E+01	5.400E+01
Arith. Net Mean	4.281E+04	2.831E+04	1.731E+04	9.128E+03	4.795E+03	2.063E+03	1.173E+03	7.198E+02	4.493E+02	2.071E+02	1.472E+02	

Experiment Title	Repeating amplified mRuby ligation to pSB1C3
Protocol	Refer to protocol 1.1
Changes in Protocol	Total Volume- 20ul mRuby conc- 4.00ul/ml BB conc- 3.62ul/ml V/I ratio- 1:3 BB amount- 25ng
Results	Used for transformation
Comments	N/A
Photos	N/A

Vector	6.9ul
Insert	9.4ul
T4 buffer	2ul
T4 ligase	1ul
H2O	0.7ul

Experiment Title	Transformation of ligated mRuby product and control pSB1C3
Protocol	Refer to protocol 1.4
Changes in Protocol	2ul DNA added
Results	Green colonies obtained for mruby and control- abnormal
Comments	N/A

Photos	

Week 4

July 2nd:

Experiment Title	mClover3 PCR amplification
Protocol	Q5 PCR protocol from NEB (https://international.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491)
Changes in Protocol	

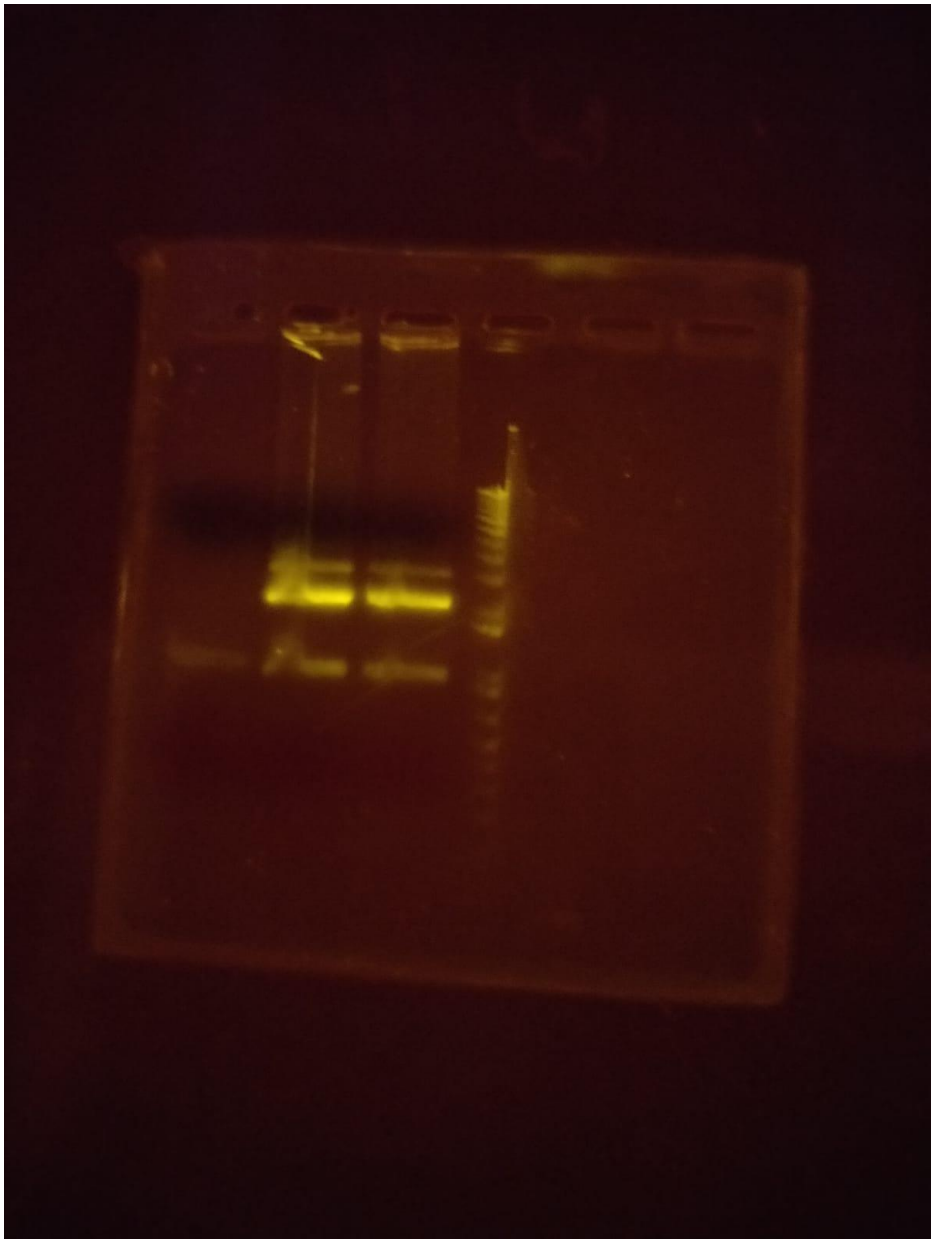
Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C 72°C 72°C	10 seconds 30 seconds 30 seconds
Final Extension	72°C	2 minutes

Results	N/A - see “Amplified mClover3 Electrophoresis + gel purification”
Comments	N/A
Photos	N/A

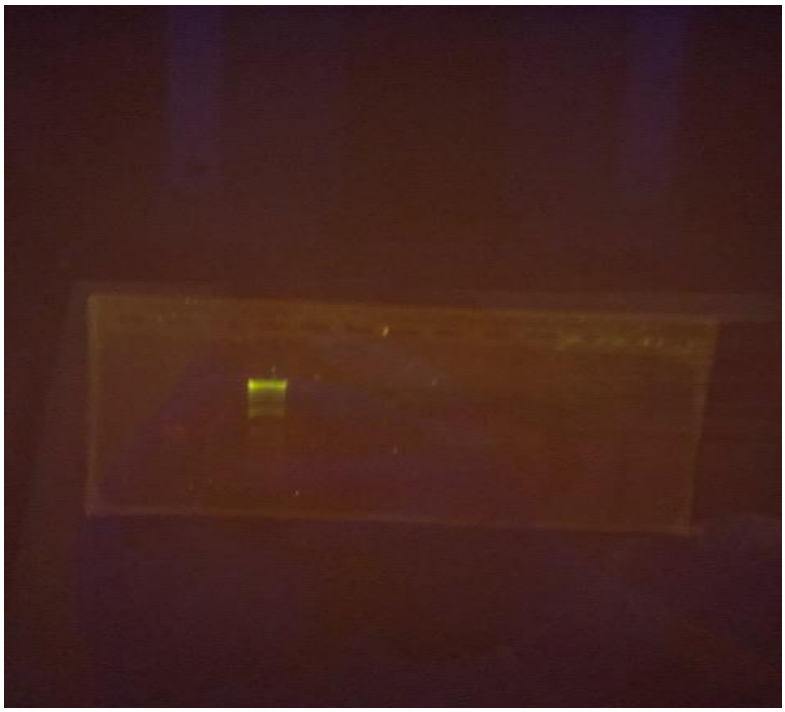
Experiment Title	Amplified mClover3 Electrophoresis + gel purification
Protocol	Refer to protocol 1.2, 1.3
Changes in Protocol	Electrophoresis run for 25 minutes under 130V.
Results	The final conc. is 23ug/ml in 30ul
Comments	The amplification is success.
Photos	N/A

Experiment Title	Digestion of mClover and backbone
Protocol	Refer to protocol 1.1
Changes in Protocol	No changes
Results	N/A
Comments	N/A
Photos	N/A

Experiment Title	Gel purification
Protocol	Refer to Appendix Section 1 protocols, 1.3
Changes in Protocol	N/A
Results	N/A
Comments	N/A

Photos	

Experiment Title	Digestion of CasX fragment1&2, dCasX fragment 2 + Electrophoresis
Protocol	Refer to protocol 1.1，1.2
Changes in Protocol	No changes
Results	Nothing for CasX2 and dCasX2, extremely dim bands for CasX1
Comments	N/A

Photos	

Details:

- 100ng for each sample

concentration (μg/μl)	ddH2O (μl)	DNA(μl)	CutSmart Buffer (μl)	PstI (μl)	EcoRI(μl)
10	5.2	10	1.8	0.5	0.5

Experiment Title	Digestion of CasX fragment1 + Electrophoresis
Protocol	Refer to protocol 1.1，1.2
Changes in Protocol	No changes
Results	Dim bands
Comments	Should do pcr before digestion
Photos	

Details:

- 100ng for each sample

concentration (μg/μl)	ddH2O (μl)	DNA(μl)	CutSmart Buffer (μl)	PstI (μl)	EcoRI(μl)
10	5.2	10	1.8	0.5	0.5

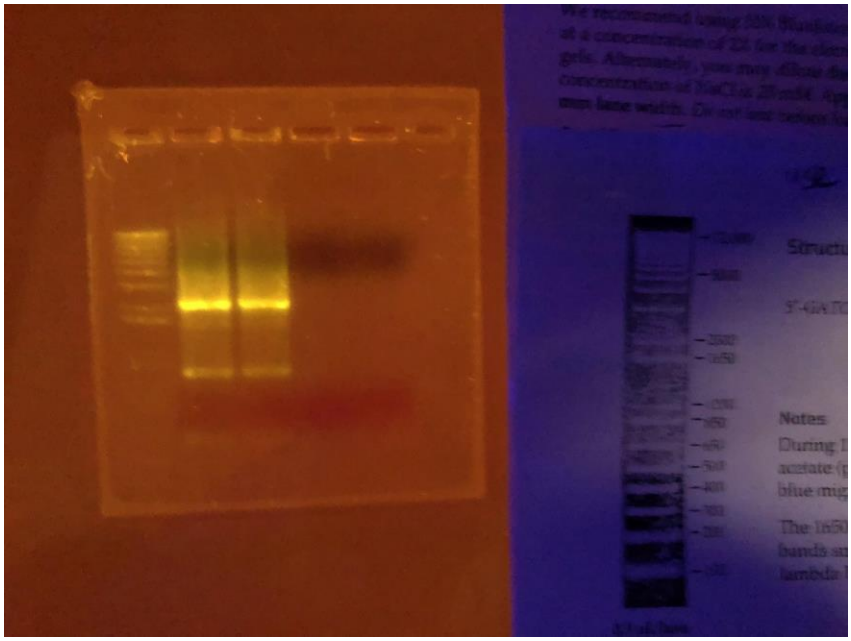
July 3rd:

Experiment Title	Digested mClover ligate to pSB1C3
Protocol	Refer to protocol 1.1
Changes in Protocol	
Results	Used for transformation
Comments	N/A
Photos	N/A

Experiment Title	Transformation of ligated mClover product and control pSB1C3 and negative control of ne plates (made on 28/6)
Protocol	Refer to protocol 1.4
Changes in Protocol	5ul DNA added
Results	Green colonies obtained for mClover and control(abnormal)
Comments	The negative control on new plates showed nothing, which indicates the new plates are wo fine. However, for the control plate, the green colonies could indicates the contamination in backbone vector, so whether the green colonies are mClover should still be checked.
Photos	N/A

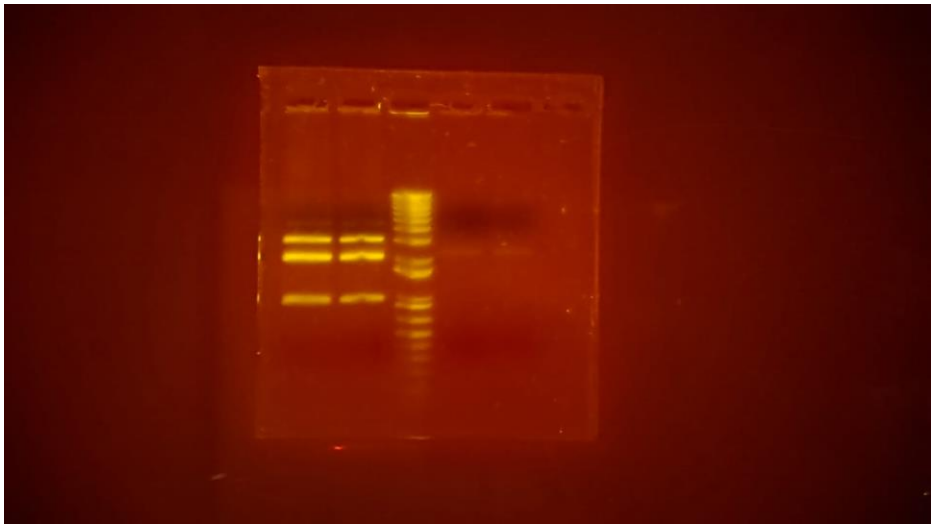
July 4th:

Experiment Title	CasX fragment 1(pcr) & dCasX fragment 2(digestion) Electrophoresis + gel purification
Protocol	Refer to protocol 1.2, 1.3
Changes in Protocol	N/A
Results	Nothing for dCasX frg2(right), clear bands for CasX frg1(left).
Comments	Definitely need to do pcr first

Photos	

July 5th:

Experiment Title	Digestion(E,P) of casx f1 pcr product and psb1c3
Protocol	Refer to protocol
Changes in Protocol	Use 50ul reaction instead of 18ul
Results	Nothing for dCasX frg2(right), clear bands for CasX frg1(left).
Comments	Definitely need to do pcr first

Photos	

Week 6

July 15th:

Experiment Title	Transformation of Ho Yi Mak dCas9 plasmid
Protocol	Refer to protocol 1.4
Changes in Protocol	Since the plasmid is very large (12kbp), we tried to heat shock them with 45, 60, 75, and 90 secs respectively.
Results	All the plates have colonies and they grow very well.
Comments	We need to miniprep the dCas9 transformed cells.
Photos	N/A

July 16th:

Experiment Title	Transformation of K1323002 plasmid (from 2017) #1
Protocol	Refer to protocol 1.4
Changes in Protocol	2ul DNA added, heat shock for 45 sec.
Results	No colony grew
Comments	N/A
Photos	N/A

Experiment Title	Inoculation and plate streaking for Ho Yi Mak dCas9 transformed cells
Protocol	Refer to protocol 1.7
Changes in Protocol	N/A
Results	The inoculation went well.
Comments	The inoculation is for the miniprep tomorrow.
Photos	N/A

July 17th:

Experiment Title	Transformation of K1323002 plasmid (from 2017) #2
Protocol	Refer to protocol 1.4
Changes in Protocol	2ul DNA added, heat shock for 45 sec.
Results	No colony grew
Comments	The DNA amount for plasmid from 2017 kit plate wasn't enough
Photos	N/A

Experiment Title	Miniprep for Ho Yi Mak dCas9 cells
Protocol	Refer to protocol 1.8
Changes in Protocol	N/A
Results	
Comments	The cell pellets weren't resuspended properly, so the high DNA yield may be due to contamination.
Photos	N/A

Experiment Title	Digestion verification for dCas9 miniprep product
Protocol	Refer to protocol 1.1, 1.2
Changes in Protocol	N/A
Results	

Comments	The band size is correct. Therefore the transformation should be successful.
Photos	N/A

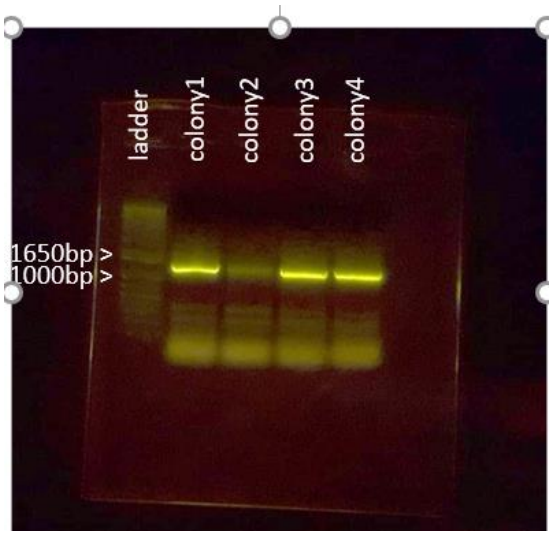
July 18th:

Experiment Title	Transformation of K1323002 plasmid #3
Protocol	Refer to protocol 1.4
Changes in Protocol	1ul DNA added (one plate for 2017 kit plate, 2 plates from 2016 kit plate), heat shock for 60 sec.
Results	One plate labeled 2016 kit plate grew colonies (control group - GFP plasmid grew)
Comments	Taking colonies to colony PCR
Photos	N/A

July 19th:

Experiment Title	Transformation of K1323002 plasmid #4
Protocol	Refer to protocol 1.4
Changes in Protocol	For two plates, 1ul DNA is added respectively from the 2016 kit plate. For the other one, 3 ul DNA is added from the 2017 kit plate. All three plates are heat shocked for 60 sec.
Results	None of the plates have colonies
Comments	The kitplates of not very reliable
Photos	N/A

Experiment Title	Colony PCR for dCas9 plasmid
Protocol	NEB Taq polymerase protocol
Changes in Protocol	NA

Results	
Comments	The primer site of this plasmid is flipped comparing to other PSB1C3 plasmids. Therefore, instead of getting the 5700bp insert, we got 1200bp bands, which are PCRed backbone fragments.
Photos	As shown above

Experiment Title	Inoculation of dCas9 transformed cells #1
Protocol	Refer to protocol 1.7
Changes in Protocol	N/A
Results	Since we forgot to add antibiotics, we need to redo this step again
Comments	The inoculation is for the miniprep and digestion verification.
Photos	N/A

Week 7

July 22nd:

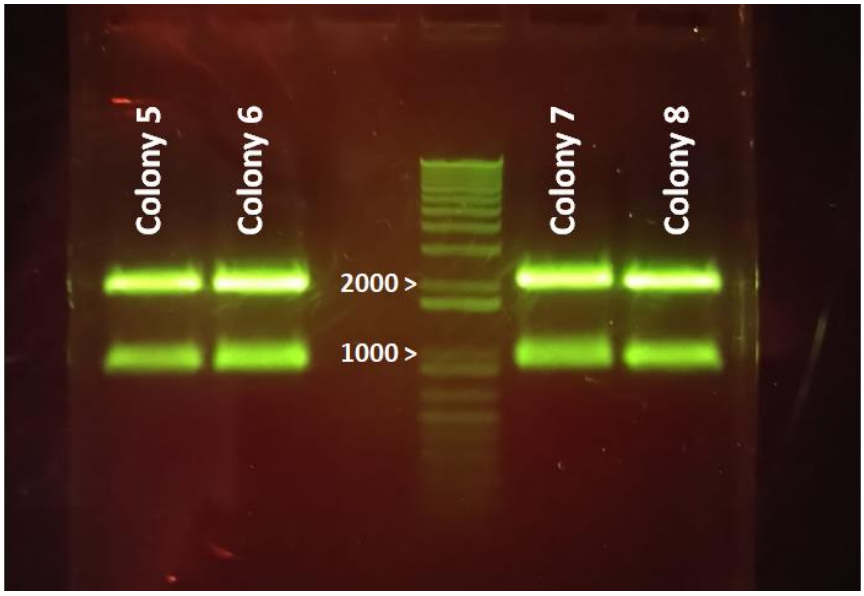
Experiment Title	Inoculation of dCas9 transformed cells #2
Protocol	Refer to protocol 1.7
Changes in Protocol	N/A
Results	
Comments	The inoculation is for the miniprep and digestion verification tomorrow.
Photos	N/A

July 23rd:

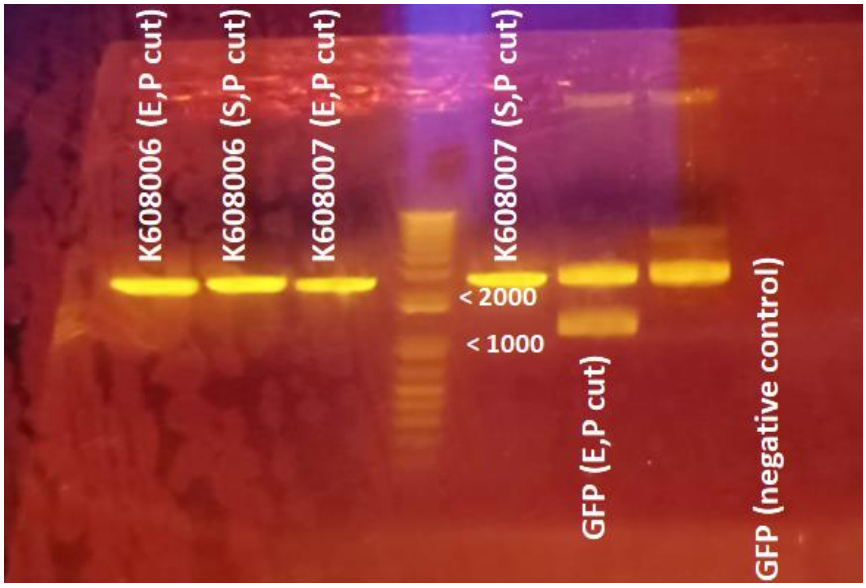
Experiment Title	Digestion of dCas9 + Electrophoresis#1
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	The bands of the gel aren't clear.
Comments	Try to do an overnight digestion under 16°C. For electrophoresis, we should try with lower voltage and longer time.
Photos	

July 24th:

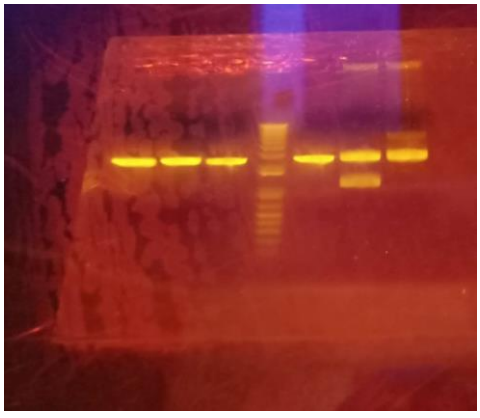
Experiment Title	Digestion of dCas9 + Electrophoresis#2
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	Incubate overnight under 16°C. Ran the gel with 100V for 40 min.
Results	The band size matches with that of digested GFP rather than dCas9.

Comments	Since the colonies glow green and the digestion result also matches with GFP, we suspected that this plate of dCas9 cells is contaminated by GFP. We autoclaved and cleaned most of our utensils and equipment that afternoon.
Photos	

Experiment Title	Digestion of K608006 and K608007 + Electrophoresis #1
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	Although the positive control sample has two bands with the correct size, all the other samples have only one band.
Comments	The insert of the plasmid is only 55 bp so we doubted that whether the DNA ran out of the gel. We decided to use GeneRuler 50bp DNA ladder to check the existence of the insert for next digestion.

Photos	

July 25th:

Experiment Title	Digestion of K608006 and K608007 + Electrophoresis #1
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	Although the positive control sample has two bands with the correct size, all the other samples have only one band.
Comments	The insert of the plasmid is only 55 bp so we doubted that whether the DNA ran out of the gel. We decided to use GeneRuler 50bp DNA ladder to check the existence of the insert for next digestion.
Photos	

July 26th:

Experiment Title	Digestion of K608006 and K608007 + Electrophoresis #2
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	The electricity power might be switched off not long after we started running the gel, so the gel electrophoresis failed.
Comments	
Photos	N/A

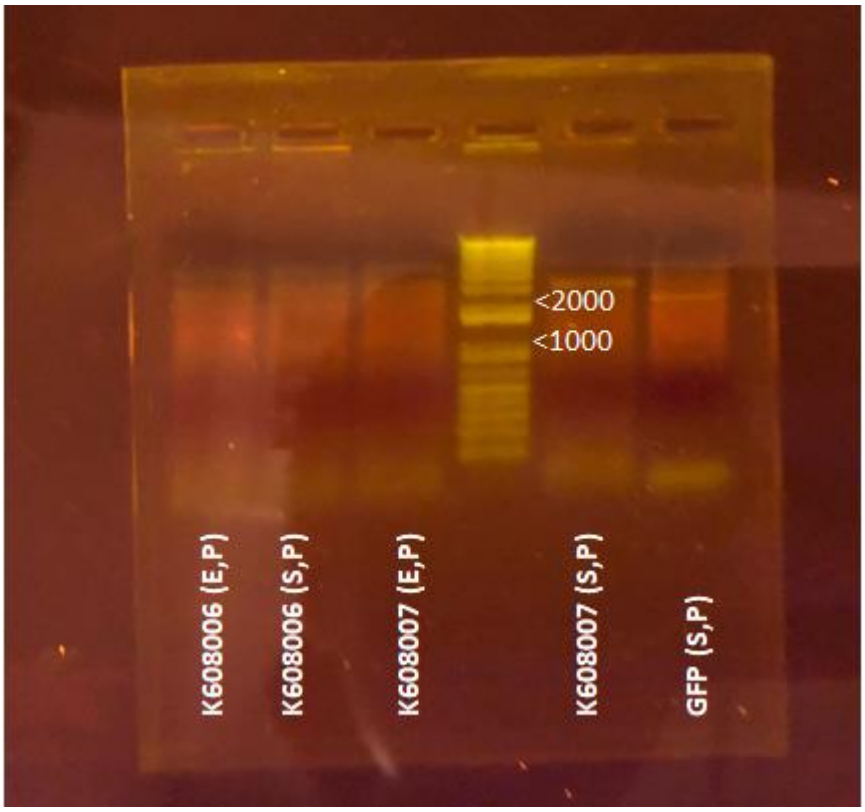
Experiment Title	Digestion of K608006 and K608007 + Electrophoresis #3
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	
Comments	
Photos	N/A

Experiment Title	Inoculation of dCas9 transformed cells
Protocol	Refer to protocol 1.7
Changes in Protocol	N/A
Results	
Comments	(The inoculation was stop at the end of the day, and was put in the fridge. Inoculation will continue on Monday.)
Photos	N/A

Week 8

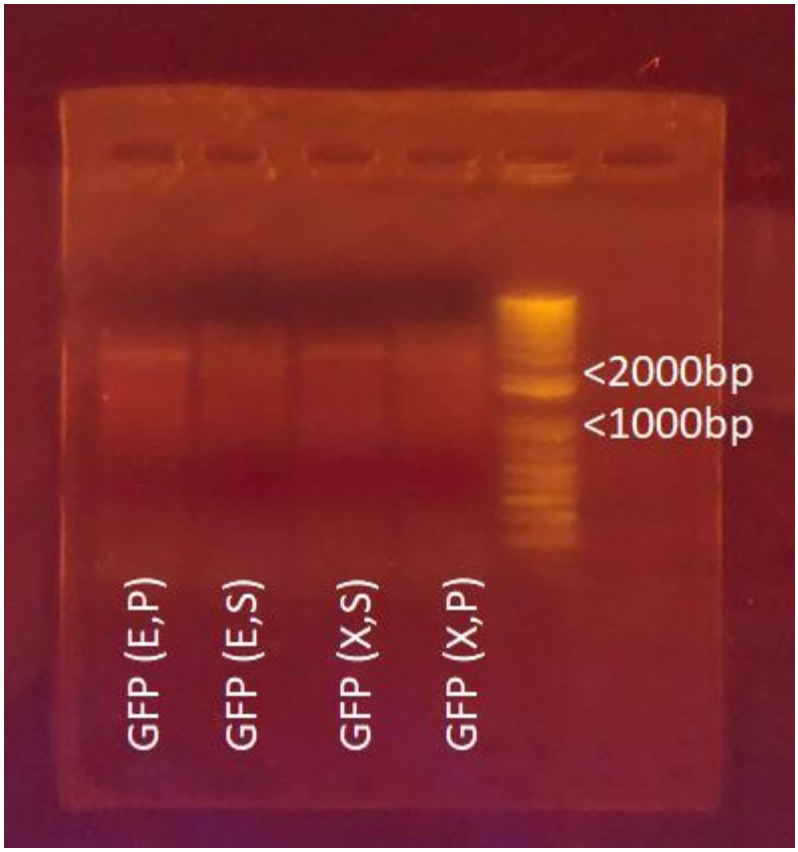
July 29th:

Experiment Title	Digestion of promoter & RBS (K608006 & K608007)

Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	There are smears for all the samples and no clear band show.
Comments	Maybe the enzyme are not working well
Photos	

Experiment Title	Inoculation of K608006 and K608007 plasmid
Protocol	Refer to protocol 1.7
Changes in Protocol	N/A
Results	
Comments	The inoculation is for the miniprep and digestion verification tomorrow.
Photos	N/A

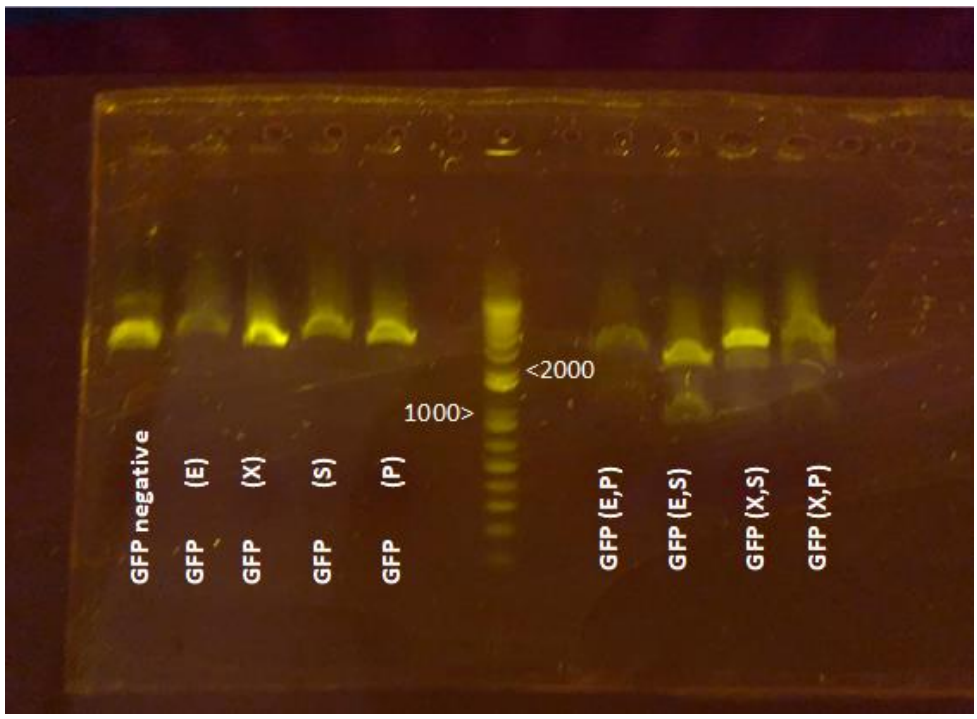
Experiment Title	Testing SpeI and XbaI enzymes: GFP digestion#1
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2

Changes in Protocol	N/A
Results	There are no bands on the gel.
Comments	We need to re do the digestion again.
Photos	

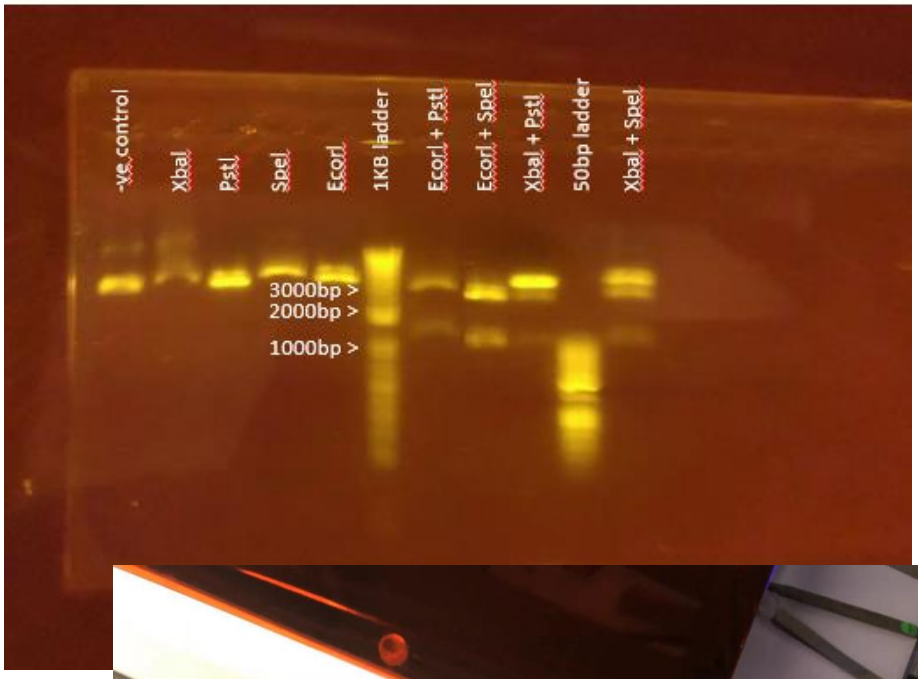
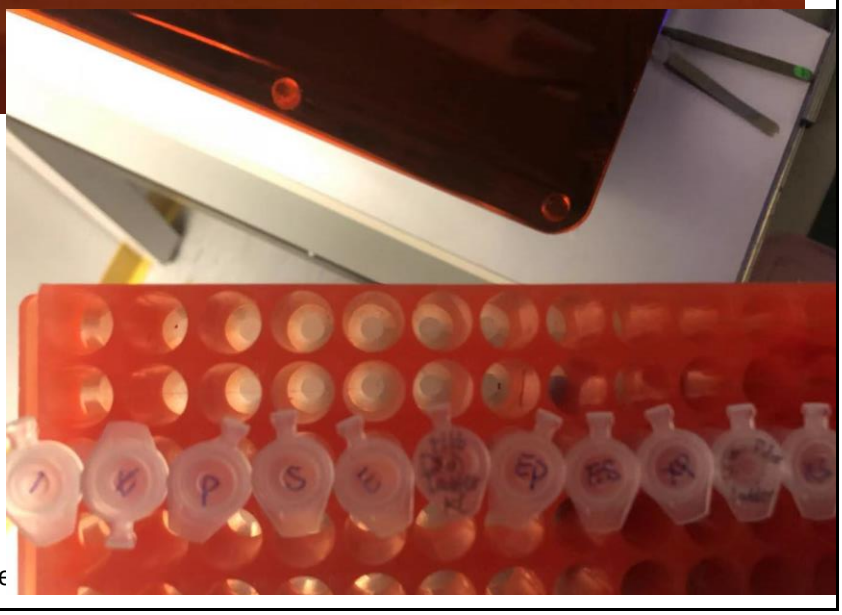
Experiment Title	Testing SpeI and XbaI enzymes: GFP digestion#2
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	
Comments	
Photos	N/A

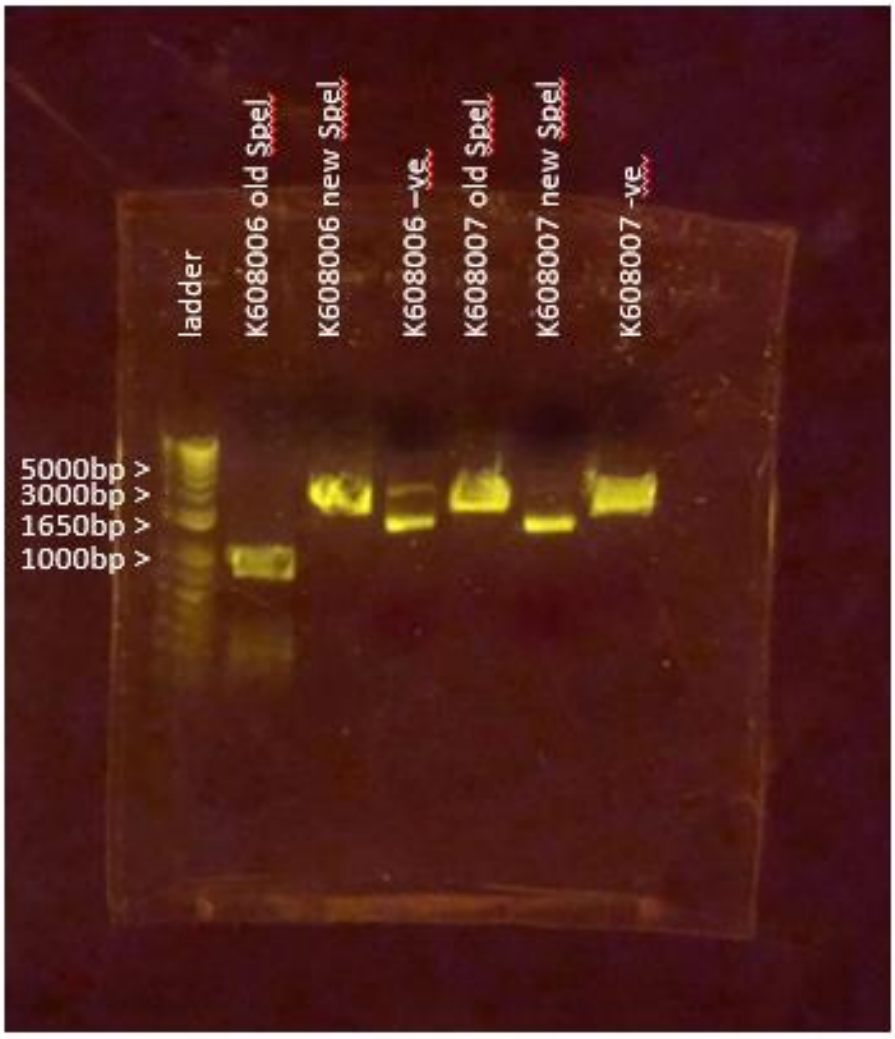
July 30th:

Experiment Title	Digestion of promoter & RBS (K608006 & K608007)
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	
Comments	The uncut plasmids run faster than the linearized ones
Photos	N/A

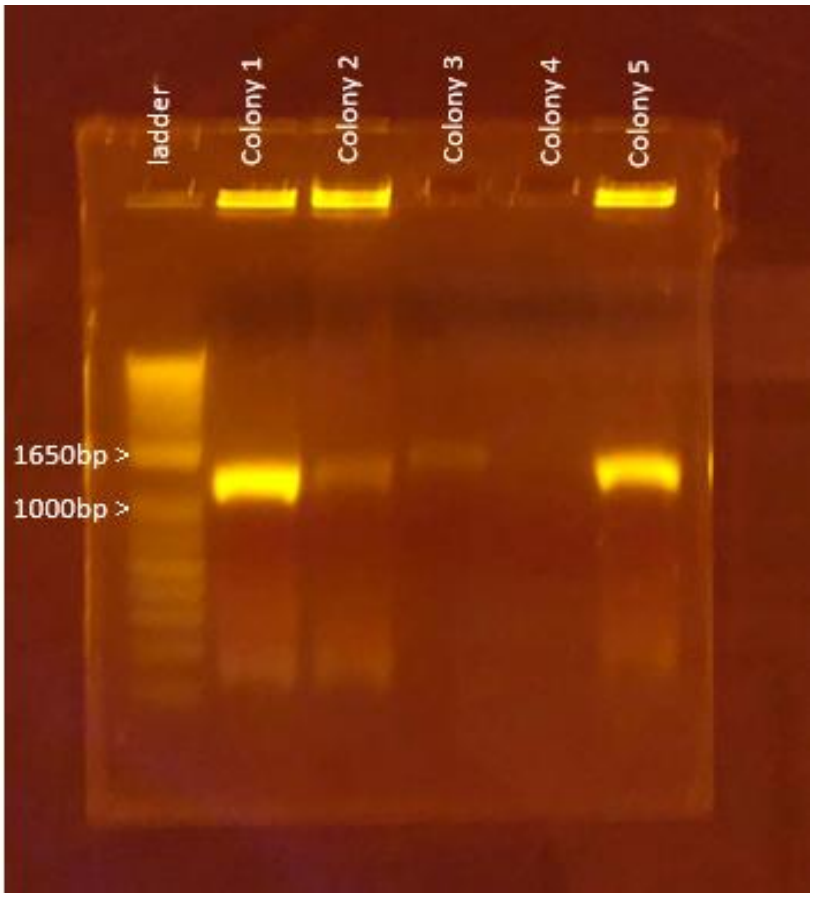
Experiment Title	Testing E,X,S,P (digestion and gel electrolysis)
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	For single enzyme's cut result, due to the reverse band shape we couldn't tell the actual size of bands.
Comments	All the bands are extremely strange, plan to check the TAE buffer
Photos	

July 31th:

Experiment Title	Electrophoresis (for the restriction enzyme test again)
Protocol	Refer to protocol
Changes in Protocol	N/A
Results	
Comments	
Photos	  -The orde

Experiment Title	Digestion and gel electrolysis of promoter & RBS (K608006 & K608007)
Protocol	Refer to protocols,
Changes in Protocol	N/A
Results	
Comments	All the bands are extremely strange, plan to check the TAE buffer
Photos	

Experiment Title	Colony PCR for K1323002 dCas9 with VF2 and VR
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2

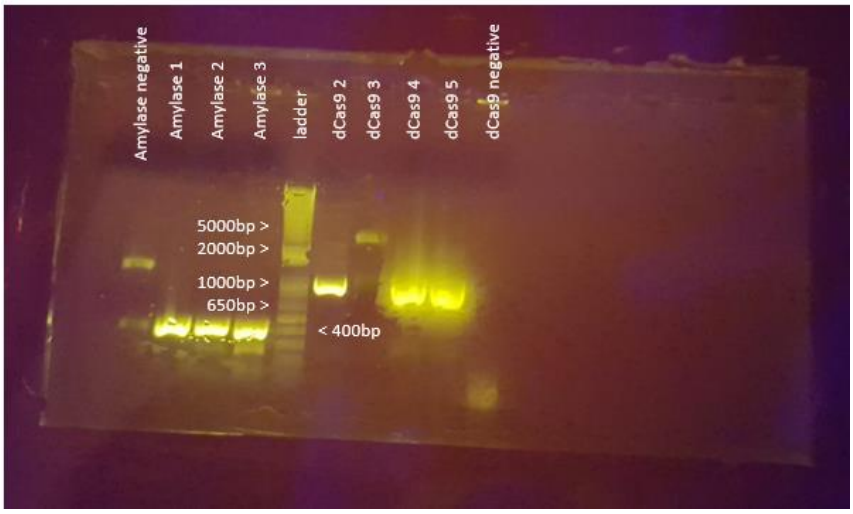
Changes in Protocol	N/A
Results	Several colonies show bands between 1000bp and 1650 bp, which matches with our prediction
Comments	N/A
Photos	

Experiment Title	Transformation of minipreped K1323002
Protocol	1.4
Changes in Protocol	N/A
Results	All the plates have substantial colonies
Comments	But doing transformation with the minipreped DNA is unreliable
Photos	N/A

August 1st:

Experiment Title	Testing SpeI and XbaI enzymes: GFP digestion#2
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	
Comments	N/A
Photos	N/A

August 2nd:

Experiment Title	Colony PCR for K1323002 with prefix and suffix
Protocol	Refer to Appendix section 1 protocols, 1.1 and 1.2
Changes in Protocol	N/A
Results	dCas9 3 has a band that's close to 5000, which could be the correct colony
Comments	
Photos	

Experiment Title	Gel purification for K608006 and K608007
Protocol	1.3
Changes in Protocol	N/A
Results	K608006: 3.641

	K608007: 6.961
Comments	N/A
Photos	N/A

Week 9

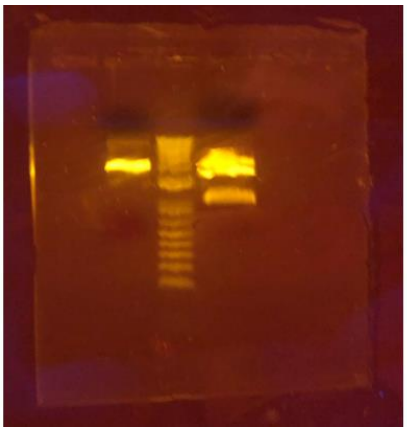
August 5th:

Experiment Title	Inoculated K1323002 colony 3
Protocol	Refer to Appendix section 1 protocols, 1.7
Changes in Protocol	Added C antibiotics
Results	
Comments	The inoculation is for the miniprep and digestion verification tomorrow.
Photos	N/A

Experiment Title	Transformation of 2017 K1723000 #2
Protocol	protocol 1.4
Changes in Protocol	Heat shock for 1 minute
Results	Nothing grew on the plates
Comments	
Photos	N/A

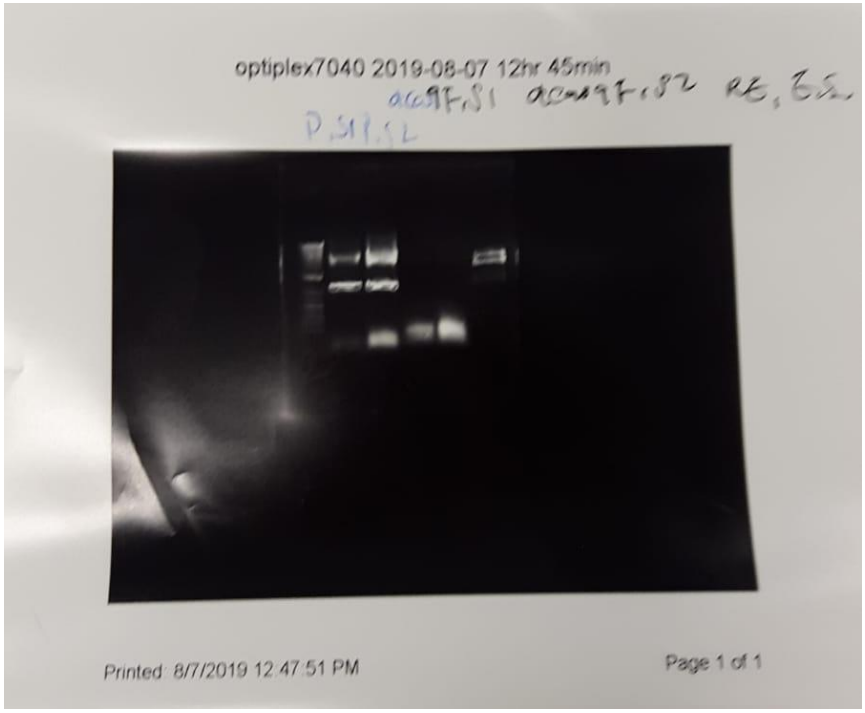
August 6th:

Experiment Title	Miniprep of part K1323002, taken from Colony 3
Protocol	Standard Protocol of the Miniprep kit (GeneJET miniprep kit)
Changes in Protocol	-
Results	Concentration: 101.1µg/ml A260/A230: 1.68 A260/A280: 1.90
Comments	Sample to be used for PCR and Restriction digestion check.
Photos	N/A

Experiment Title	RE check for K1323002
Protocol	Protocol 1.1,1.2
Changes in Protocol	N/A
Results	The bands are close to 5000
Comments	
Photos	 

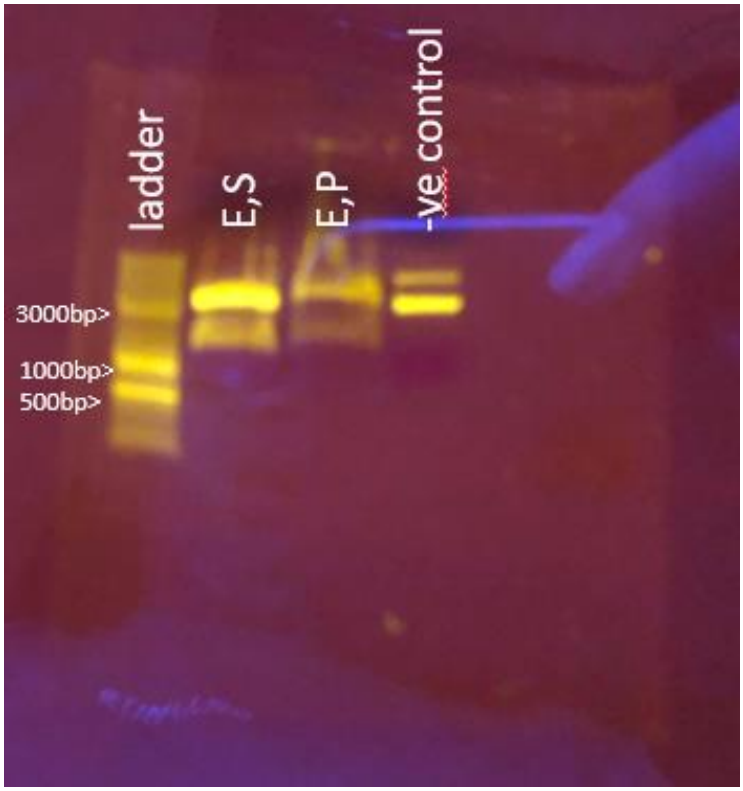
August 7th:

Experiment Title	Gel electrophoresis of PCR product of K1323002 and the restriction digestion
Protocol	1.2 Gel Electrophoresis
Changes in Protocol	For forward primer, 2 different primers were used: - dCas9 F, a primer made for the dCas9 part

	- Prefix F, a primer made for the biobrick prefix For both scenarios, the reverse primer used was the suffix reverse primer made for the biobrick suffix
Results	N/A
Comments	Order of the samples loaded on the gel is, from left to right: ladder / P,S1 / P,S2 / F,S1 / F,S2 / RE digestion(E,S)
Photos	

Experiment Title	Transformation of 2018 kitplate K1723000#1
Protocol	protocol 1.4
Changes in Protocol	Heat shock for 1 minute
Results	Nothing grew on the plates
Comments	
Photos	N/A

Experiment Title	RE check for K132002
Protocol	protocol 1.1, 1.2
Changes in Protocol	N/A

Results	The band that is around 2000bp should be the backbone, and the band that is more than 3000bp is the insert. The band sizes look resonable.
Comments	
Photos	

August 8th:

Experiment Title	Stocking IDT product
Protocol	
Changes in Protocol	Heat shock for 1 minute
Results	Nothing grew on the plates
Comments	
Photos	N/A

August 9th:

Experiment Title	Hifi assembly

<table>
<tr><td>Protocol</td><td>NEB HiFi kit protocol + HiFi transformation protocol (https://international.neb.com/-/media/catalog/datacards-or-manuals/e2621_man.pdf)
<table>
<tr><td>component</td><td>Amount (ng)</td><td>Concentration (ng/uL)</td><td>Volume (uL)</td></tr>
<tr><td>iGEM provided Linearized backbone pSB1C3</td><td>63.36</td><td>105.6</td><td>0.6</td></tr>
<tr><td>Insert 1 (GFPsgGFP)</td><td>126.72</td><td>10</td><td>12.67</td></tr>
<tr><td>Insert 2 (asGFP)</td><td>126.72</td><td>10</td><td>12.67</td></tr>
<tr><td>Master Mix</td><td>/</td><td>/</td><td>10</td></tr>
<tr><td>H2O</td><td>/</td><td>/</td><td>0</td></tr>
</table></td></tr>
<tr><td>Changes in Protocol</td><td>Total reaction volume: 20uL changed to 35uL
(DNA insert concentrations are low)</td></tr>
<tr><td>Results</td><td>Failed. No colony on plates after transformation</td></tr>
<tr><td>Comments</td><td>Linearized backbone may not include suffix XbaI site and prefix SpeI. therefore overlap may not bind well. To proceed, we make our own linearized backbone by ourselves to restore more bp within overlap</td></tr>
<tr><td>Photos</td><td>N/A</td></tr>
</table>

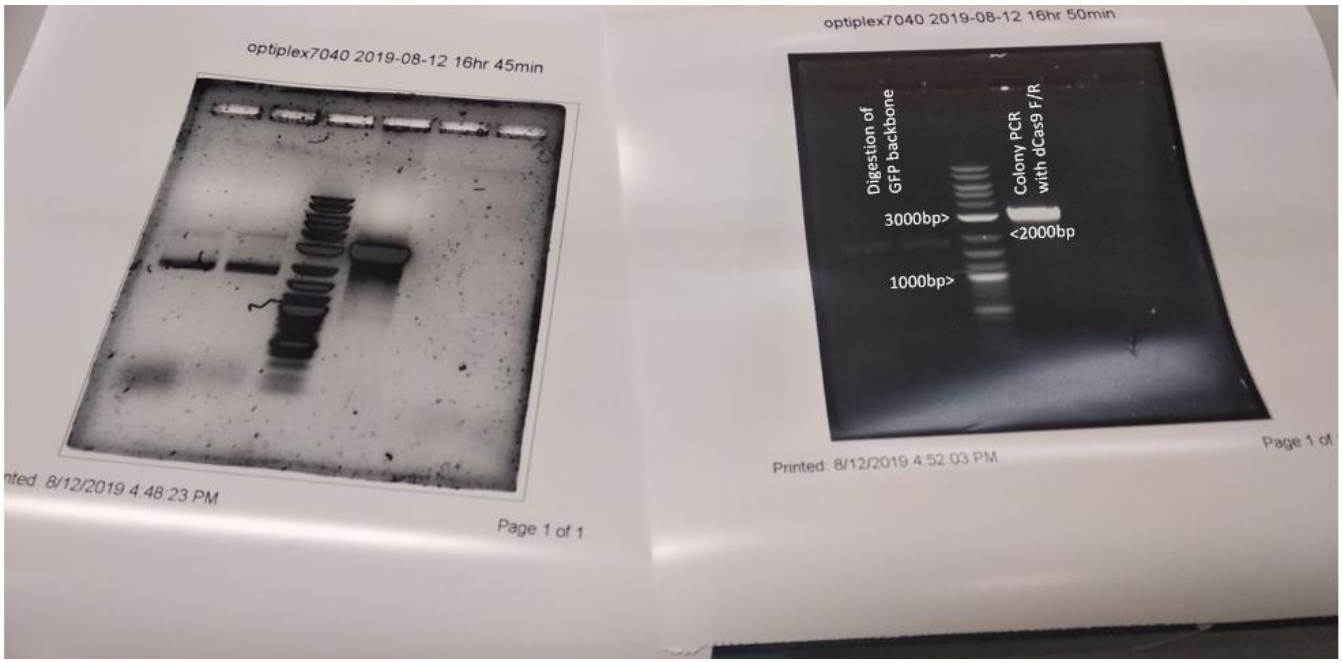
Week 10

August 12th:

Experiment Title	Colony PCR of K1323002
Protocol	NEB Taq PCR protocol
Changes in Protocol	
Results	Nothing grew on the plates
Comments	
Photos	N/A

Experiment Title	Digestion of GFP-PSB1C3 backbone
Protocol	protocol 1.1, 1.2
Changes in Protocol	N/A

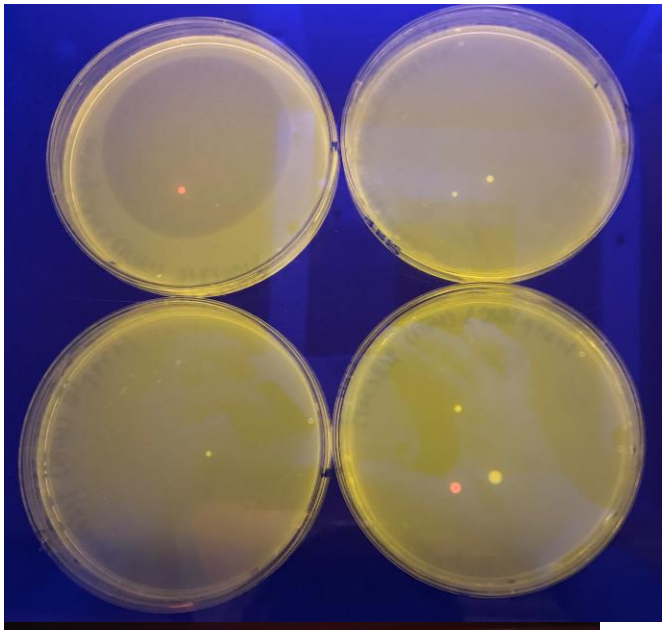
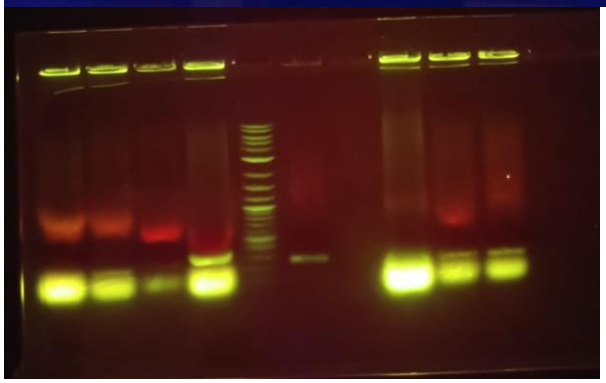
Results	
Comments	
Photos	N/A



Experiment Title	Transformation of J04450 (PSB1C3 backbone)
Protocol	protocol 1.4
Changes in Protocol	N/A
Results	
Comments	
Photos	N/A

August 13th:

Experiment Title	Colony PCR for possible HiFi-ligated transformants
Protocol	NEB Taq PCR protocol
Changes in Protocol	
Results	None of the colonies is of the correct clone Correct band size should be ~2.5kb
Comments	Modify linearizing backbone method and try HiFi with new linearize backbone again

Photos	 

Experiment Title	Linearizing pSB1C3 with XS cut
Protocol	
Changes in Protocol	
Results	After gel purification, the DNA concentration is higher than the initial undigested DNA concentration and impurities level is very high. Did not use the digested product as linearized backbone for HiFi.
Comments	Try PCR backbones with PrefixR and SuffixF primers to linearize
Photos	

Experiment Title	Transform remaining HiFi products from 9/8

Protocol	NEB HiFi transformation protocol
Changes in Protocol	2uL -> 3uL HiFi product into competent cell (our total volume of HiFi reaction is expanded from 20uL to 35uL)
Results	Colony grown. RFP colonies present. GFP colonies absent.
Comments	Try PCR backbones with PrefixR and SuffixF primers to linearize
Photos	

Experiment Title	Inoculation of RFP in pSB1C3
Protocol	NEB HiFi transformation protocol
Changes in Protocol	2uL -> 3uL HiFi product into competent cell (our total volume of HiFi reaction is expanded from 20uL to 35uL)
Results	Colony grown. RFP colonies present. GFP colonies absent.
Comments	Try PCR backbones with PrefixR and SuffixF primers to linearize
Photos	

August 14th:

Experiment Title	Miniprep pSB1C3 RFP and hym dCas9
Protocol	
Changes in Protocol	
Results	pSB1C3 RFP: 130.1ng/uL Hym dCas9: 730.4ng/uL
Comments	
Photos	

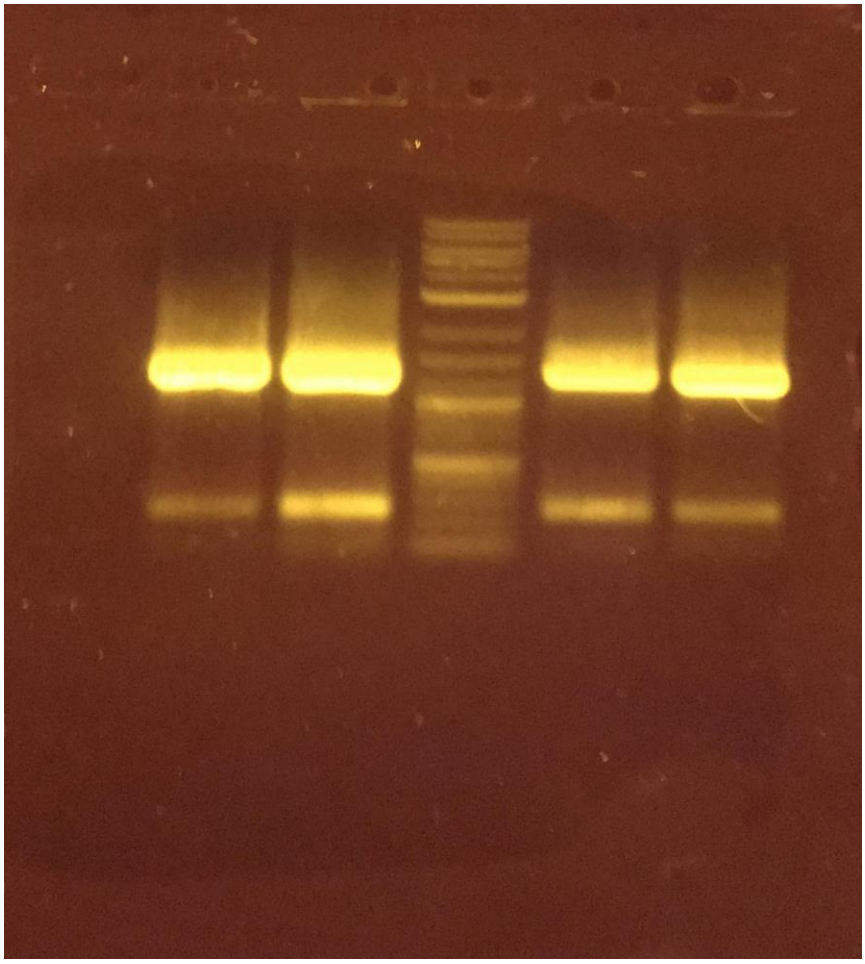
Experiment Title	HiFi linearized pSB1C3 with insert 1,2
Protocol	NEB HiFi kit protocol (https://international.neb.com/-/media/catalog/datacards-or-manuals/e2621_man.pdf)
Changes in Protocol	
Results	
Comments	

Photos	

August 15th:

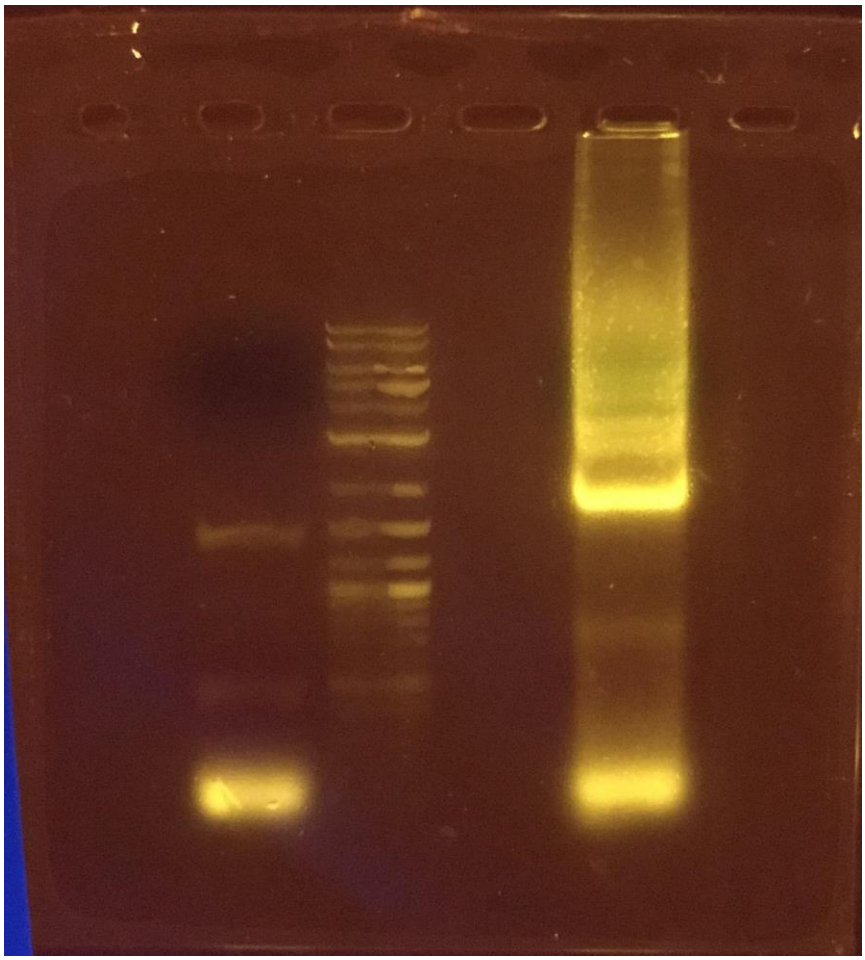
Experiment Title	Transform HiFi products from 14/8
Protocol	HiFi transformation protocol
Changes in Protocol	
Results	Colonies are red instead of green. HiFi not successful
Comments	Backbones have very low concentration
Photos	

Experiment Title	PCR HiFi products with VF2 VR primers
Protocol	
Changes in Protocol	
Results	No band at the right size. HiFi failed.
Comments	Backbones have very low concentration

Photos	

August 16th:

Experiment Title	PCR linearize pSB1C3 and gel purification
Protocol	Thermo Scientific GeneJET Gel Extraction Kit https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012661_GeneJET_Gel_Extraction_UG.pdf
Changes in Protocol	Elution volume 20uL
Results	PCR successful Gel purification concentration: 15.12ng/uL
Comments	

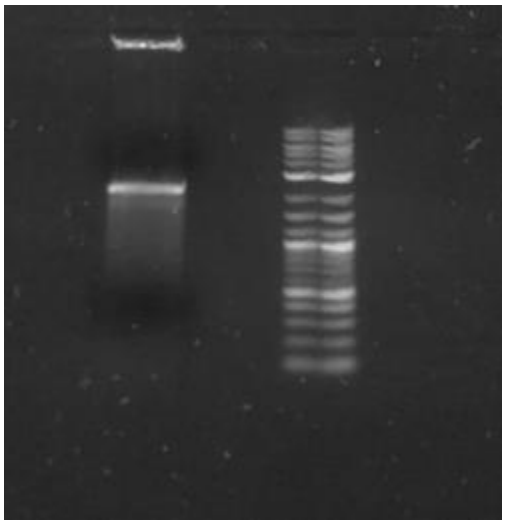
Photos	

Week 11

August 21st:

Experiment Title	Resuspension of IDT product dCas9 control
Protocol	IDT spec sheet
Changes in Protocol	Added 20uL MQ in 1000ng DNA, i.e. 50ng/uL
Results	
Comments	

Experiment Title	Digest dCas9 control insert with ES + gel purification
Protocol	Gel purification using GeneJET kit.

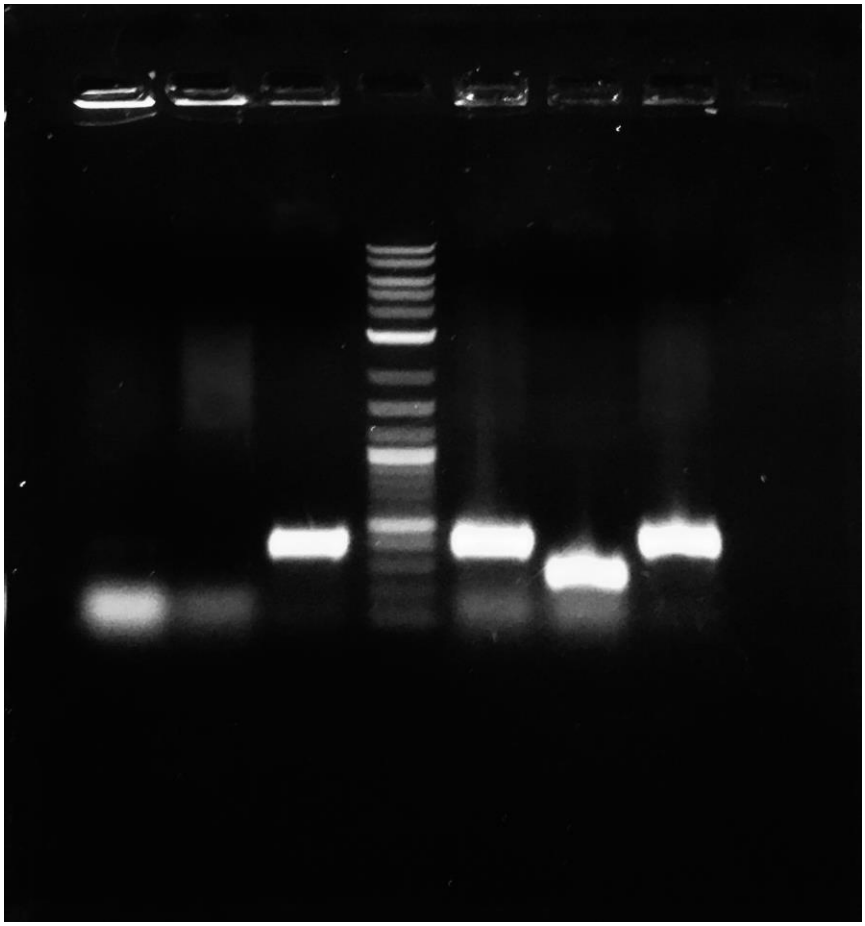
Changes in Protocol	Elution volume 20uL
Results	Concentration is very very low (~0ng/uL)
Comments	No need to run gel after digestion as undigested and digested bands cannot be distinguished. Use heat inactivation instead.
Photos	

Experiment Title	Ligation of dCas9 control into B0015-pSB1C3 (EX cut) +transform
Protocol	
Changes in Protocol	Estimated amount of dCas9 control insert in gel purification product is 11ng. Backbone added to ligation: 3ng
Results	Colonies present on plates
Comments	
Photos	

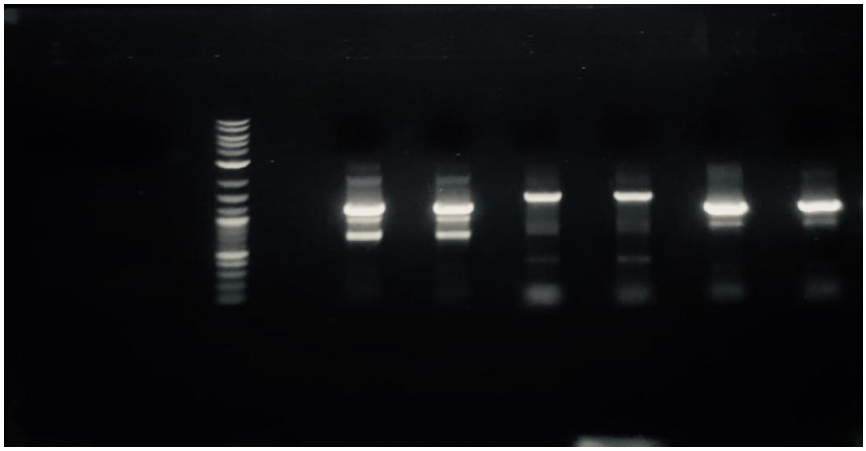
August 22nd:

Experiment Title	Amplify dCas9 control IDT product + PCR cleanup
Protocol	NEB Q5 PCR, prefix suffix primers PCR cleanup: GeneJET gel purification kit
Changes in Protocol	
Results	PCR cleanup very low resulting concentration: ~2ng/uL
Comments	

Photos	

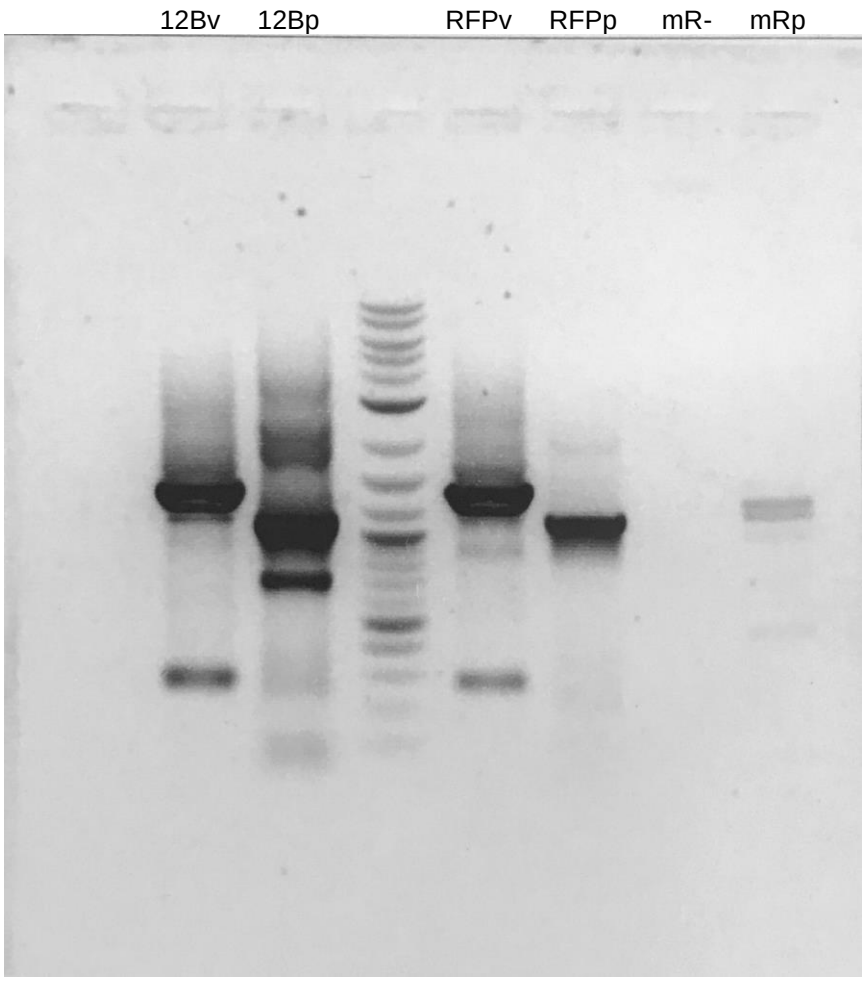
Experiment Title	Colony PCR + inoculation of dCas9 control - B0015-pSB1C3
Protocol	6 colonies Taq PCR. VF2 VR
Changes in Protocol	
Results	
Comments	VF2 VR without insert ~400bp. Ligation failed

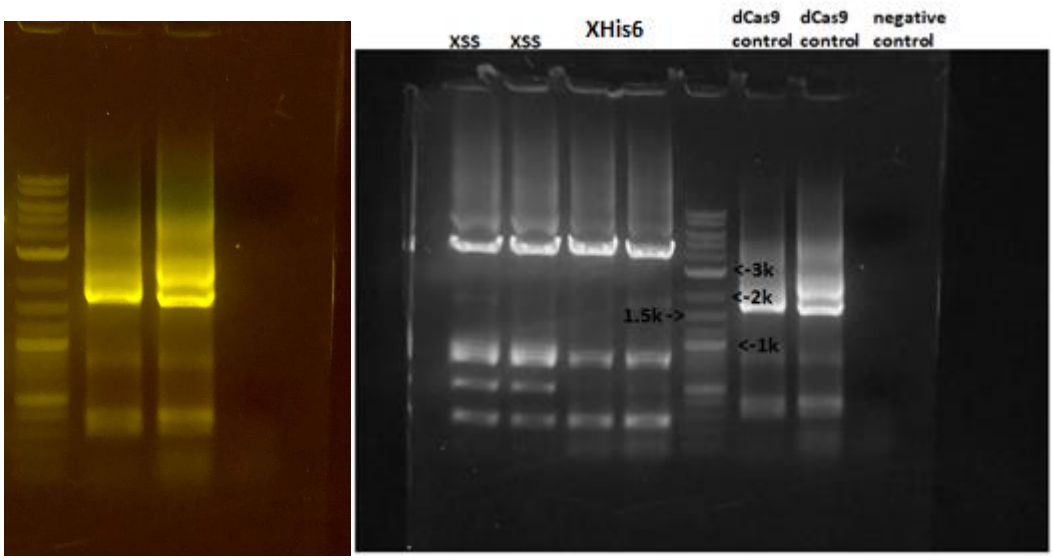
Experiment Title	Amplify dCas9 control IDT product, insert 1,2,3 + gel purification
Protocol	NEB Q5 PCR, Homemade gel purification buffers, GeneJET
Changes in protocol	Elution volume: 2x15uL; 20uL
Results	GG- asG- RG- GG1 GG2 asG1 asG2 RG1 RG2

<table>
<tr><td></td><td>  </td></tr>
<tr><td>Comments</td><td>All band at right size
Gel purification resulting concentration ~5ng/uL</td></tr>
</table>

<table>
<tr><td>Experiment Title</td><td>HiFi assembly of direct PCR product</td></tr>
<tr><td>Protocol</td><td>
<table>
<tr><td></td><td>inserts1+2+pSB1C3</td><td>Positive control</td></tr>
<tr><td>Insert 1 PCR product (320ng/uL)</td><td>0.6uL</td><td></td></tr>
<tr><td>Insert 2 PCR product (480ng/uL)</td><td>0.5uL</td><td></td></tr>
<tr><td>Linearized pSB1C3 (15.12ng/uL)</td><td>6uL</td><td></td></tr>
<tr><td>pUC19 (positive plasmid control)</td><td></td><td>3.33uL</td></tr>
<tr><td>Positive insert control</td><td></td><td>6.66uL</td></tr>
<tr><td>HiFi Master Mix</td><td>10uL</td><td>10uL</td></tr>
<tr><td>MQ</td><td>2.9uL</td><td>0uL</td></tr>
<tr><td>Total volume</td><td>20uL</td><td>20uL</td></tr>
</table>
</td></tr>
<tr><td>Changes in Protocol</td><td>See above</td></tr>
<tr><td>Results</td><td>See next experiment</td></tr>
<tr><td>Comments</td><td></td></tr>
</table>

August 23rd:

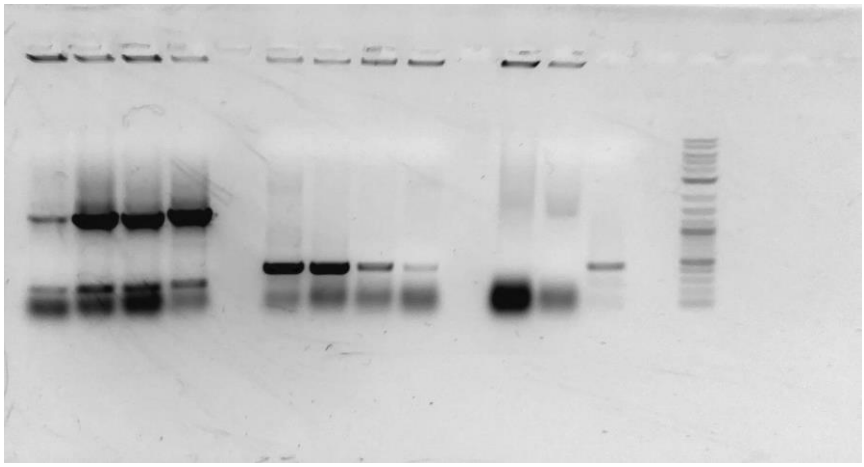
Experiment Title	PCR check for HiFi products
Protocol	Taq PCR, VF2 VR, Prefix Suffix primers
Changes in Protocol	
Results	 12Bv: insert 1 - insert 2 - pSB1C3 (VF2,VR) 12Bp: insert 1 - insert 2 - pSB1C3 (prefix, suffix primers) RFPv: RFP-pSB1C3 (VF2,VR) RFPp: RFP-pSB1C3 (prefix, suffix primers) mR-: mRuby digested (no primers) mRp: mRuby digested (prefix, suffix primers)
Comments	Insert 1+2 expected size: 2521 Correct faint band from prefix suffix primers Possible explanations for bright bands: 1) Original inserts unligated 2) Taq polymerase cannot extend

Experiment Title	PCR amplification of dCas9 control + gel purification
Protocol	GeneJET gel purification kit
Changes in Protocol	Pre warm elution buffer to 52C before elution Sit column for 3 minutes before spin Elution volume 15uL
Results	
Comments	Concentration increased significantly: 26.44ng/uL and 19.xxng/uL
Photos	dCas9 control 

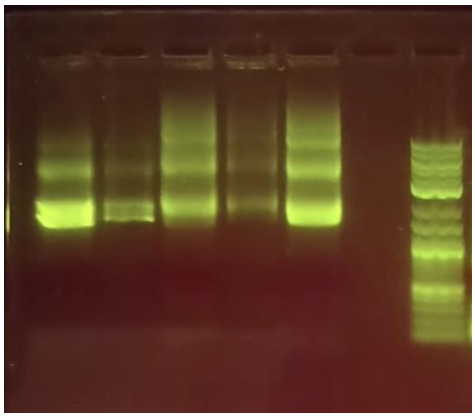
Week 12

August 27th:

Experiment Title	Colony PCR for HiFi/ ligation products products
Protocol	
Changes in Protocol	
Results	Hifi insert1+2+pSB1C3 dCas9 cut and ligate -ve -ve +ve

	
Comments	Not correct size
Photos	

Experiment Title	Q5 PCR linearize pSB1C3
Protocol	NEB Q5 PCR, prefix reverse suffix forward primers From RFP-pSB1C3
Changes in Protocol	
Results	
Comments	
Photos	

Experiment Title	Q5 PCR amplify dCas9 control IDT products + gel purification
Protocol	NEB Q5 PCR, prefix suffix primers
Changes in Protocol	
Results	Right size, resulting concentration: 3.67ng/uL

Comments	
Photos	

August 28th:

Experiment Title	HiFi (insert 1+2+ pSB1C3) + transform
Protocol	<table><tr><td>Insert 1 (GG)</td><td>0.6</td></tr><tr><td>Insert 2 (asG)</td><td>0.6</td></tr><tr><td>Linearized pSB1C3</td><td>0.3</td></tr><tr><td>2X Master Mix</td><td>5</td></tr><tr><td>MQ</td><td>3.5</td></tr></table>
Changes in Protocol	
Results	Some green colonies -> streak
Comments	
Photos	

August 29th:

Experiment Title	Digestion of dCas9 control (ES cut)
Protocol	<table><tr><td>EcoR1</td><td>0.5</td></tr><tr><td>Spe1</td><td>0.5</td></tr><tr><td>dCas9 control</td><td>14</td></tr><tr><td>Cut Smart buffer</td><td>2.5</td></tr><tr><td>MQ</td><td>7.5</td></tr></table>
Changes in Protocol	
Results	
Comments	
Photos	

Experiment Title	Digestion of inserts 2, 3
Protocol	

EcoR1	0.4
Spe1	0.4
Insert 2 (asG)	8.98
Insert 3 (RG)	5.29
Cut Smart buffer	1.8
MQ	asG: 6.82 RG: 10.51

Changes in Protocol	
Results	
Comments	
Photos	

Experiment Title	Ligations
Protocol	

	dCas9 ctrl - pSB1C3	Multistep RGG	One step GGG-pSB1C3
dCas9 control insert	12		
Insert 1 (GG)			11.5
Insert 2 (asG)		8.5	11.5
Insert 3 (RG)		8.5	
B0015-pSB1C3	2.2		2.2
T4 ligase	1	1	1.5
buffer	2	2	3
MQ	2.8	0	0.3

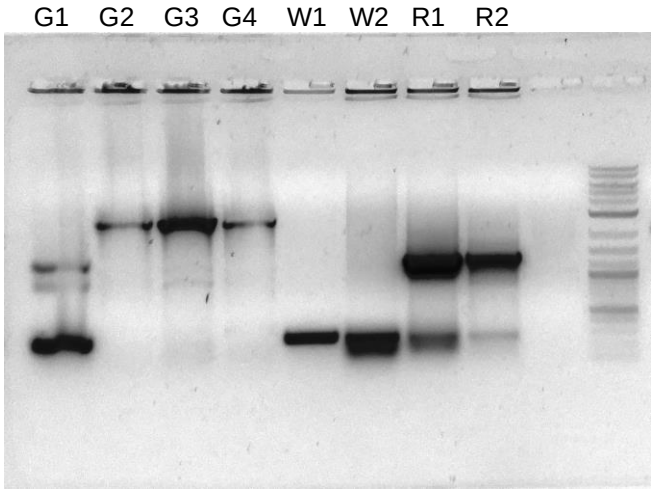
Changes in Protocol	
Results	

Comments	
Photos	

August 30th:

Experiment Title	Colony PCR for green, white and red colonies of HiFi plates + inoculate + streak
Protocol	
Changes in Protocol	
Results	
Comments	G2-4 are the right size (~2500+400bp)
Photos	

	colonies	+ve control (RFP-pSB1C3)
DNA	/	0.2
VF2	0.5	0.5
VR	0.5	0.5
dNTP	0.5	0.5
Taq	0.6	0.6
Thermal pol	2.5	2.5
MQ	20.4	20.2



G1-4: green colonies
W1-2: white colonies
R1-2: red colonies

Experiment Title	Linearize backbone from I
Protocol	

	pSB1C3	B0015-Sal1	B0015-Sal1	-ve	+ve
K608002 - pSB1C3					0.2
I13401 - pSB1C3	0.5			10	
B0015		0.2	0.2		
Prefix R	1.25				1.25
Suffix F	1.25				1.25
TT R		2	1		
Prefix F			1		
Master Mix	12.5	12.5	12.5	12.5	12.5
MQ	9.5	10.3	10.3	2.5	9.8

Changes in Protocol	
Results	
Comments	
Photos	

Week 13

September 2nd:

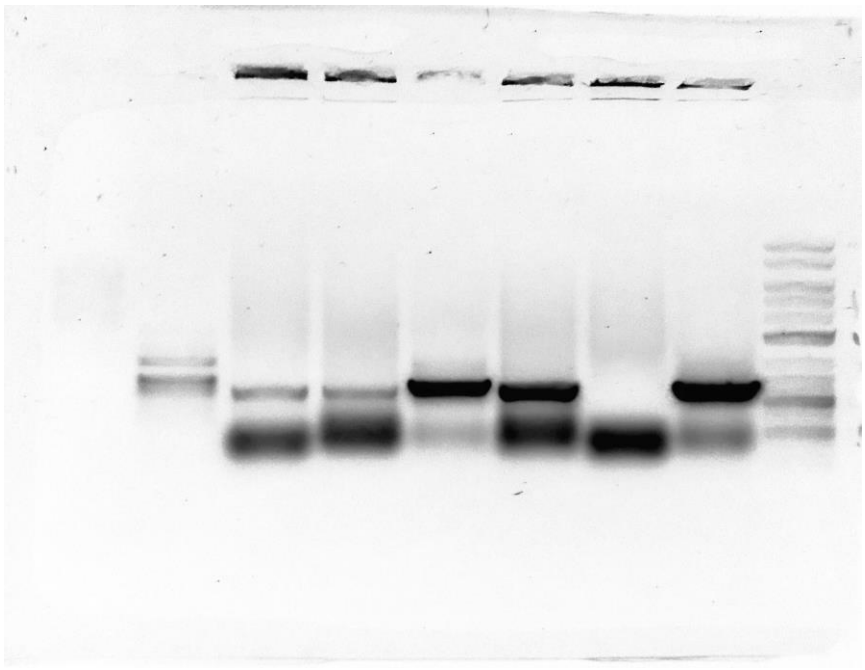
Experiment Title	HiFi for (inserts 2+3+pSB1C3)
Protocol	
Changes in Protocol	

Insert 2 (asG)	0.6
Insert 3 (RG)	0.6
Linearized pSB1C3	0.3
2X Master Mix	5
MQ	3.5

Results	failed
Comments	

Photos	

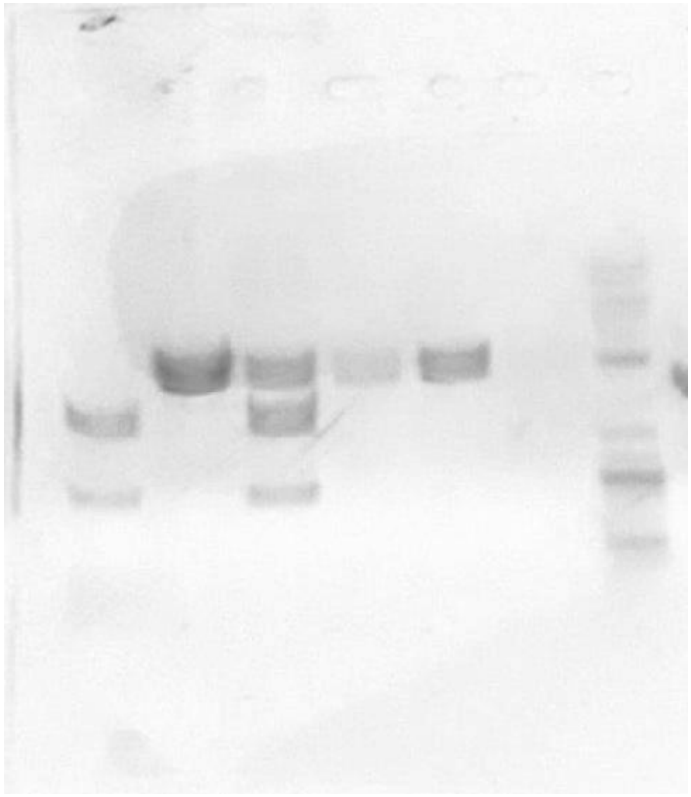
September 3rd:

Experiment Title	colony PCR dCas9 control - B0015
Protocol	taq PCR, prefix forward suffix reverse primers
Changes in Protocol	
Results	
Comments	Expected band size: 2.7kb
Photos	

Experiment Title	Digestion of GFP/ RFP for promoter assay

Protocol

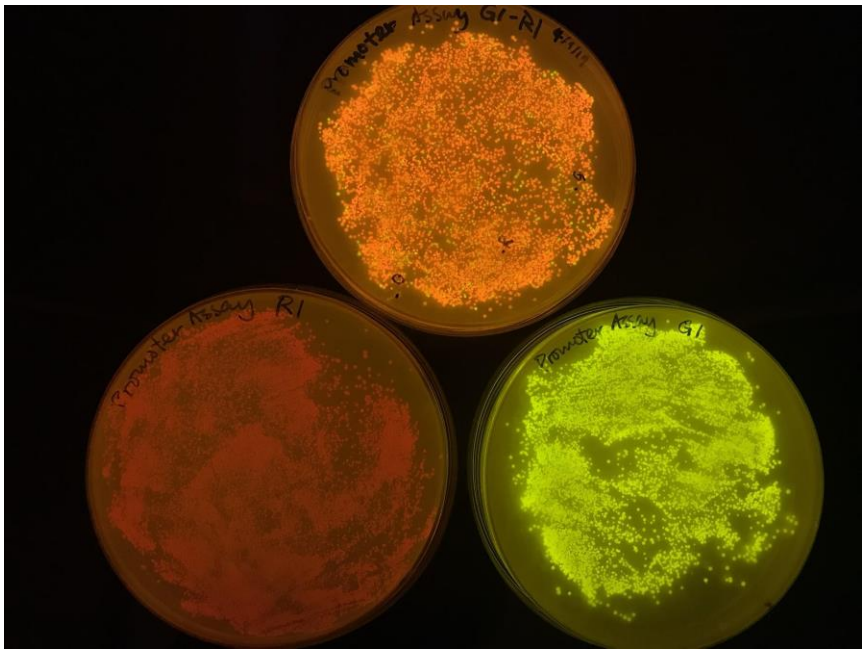
	R1	R2	R3	G1	G2	G3	+ve
	K608002-RFP-pSB1C3	J23104-J61002-RFP	K608002-RFP	pSB1C3-K608002_I13401 Kelly	pSB1C3-K608002-I13401	K608002-J13401-pSB1C3	RFP=pSB1C3
EcoR1	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Spe1	0.3	0.3	0.3				0.3
Xba1				0.3	0.3	0.3	

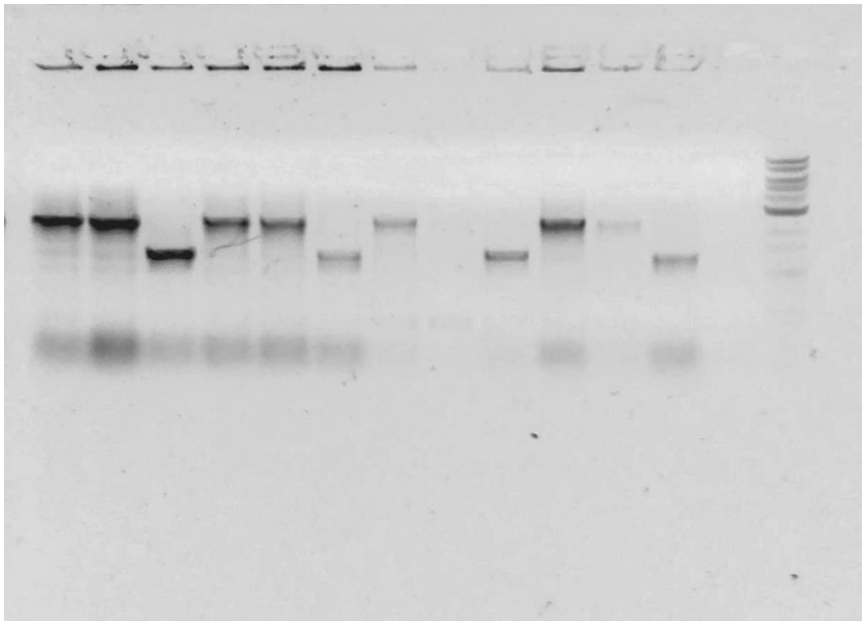
	DNA	3	15.6	15.6	15.6	3	15.6	3
	CS	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	MQ	12.6	0	0	0	12.6	0	12.6
Changes in Protocol	R1 R2 R3 G1 G2 G3 							
Results								
Comments	Expected band size: R1,R2: 900kb; G1,G3: 3kb							
Photos								

Experiment Title	Ligation for promoter assay				
Protocol		G1pSB1C3-R1	G1pSB1C3-R3	G2pSB1C3-R1	G2pSB1C3-R3
	G1	0.84 (10ng)	0.84 (10ng)		
	G2			0.28 (16ng)	0.28 (16ng)
	R1	3.33 (10ng)		3.33 (16ng)	

	<table><tr><td>R3</td><td></td><td>8.97 (10ng)</td><td></td><td>8.97 (16ng)</td></tr><tr><td>Buffer</td><td>2</td><td>2</td><td>2</td><td>2</td></tr><tr><td>T4 ligase</td><td>1</td><td>1</td><td>1</td><td>1</td></tr><tr><td>MQ</td><td>12.83</td><td>7.19</td><td>13.39</td><td>7.75</td></tr></table>
Changes in Protocol	
Results	
Comments	
Photos	

September 4th:

Experiment Title	Transform promoter assay ligation
Protocol	8 plates: G1, R1, R3, -ve G1-R1, G1-R3, G2-R1, G2-R3
Changes in Protocol	
Results	
Comments	
Photos	

Experiment Title	Colony PCR for promoter assay transformants
Protocol	
Changes in Protocol	
Results	O O G O O G O O G O O G -ve 
Comments	Expected band size: ~2.5kb
Photos	

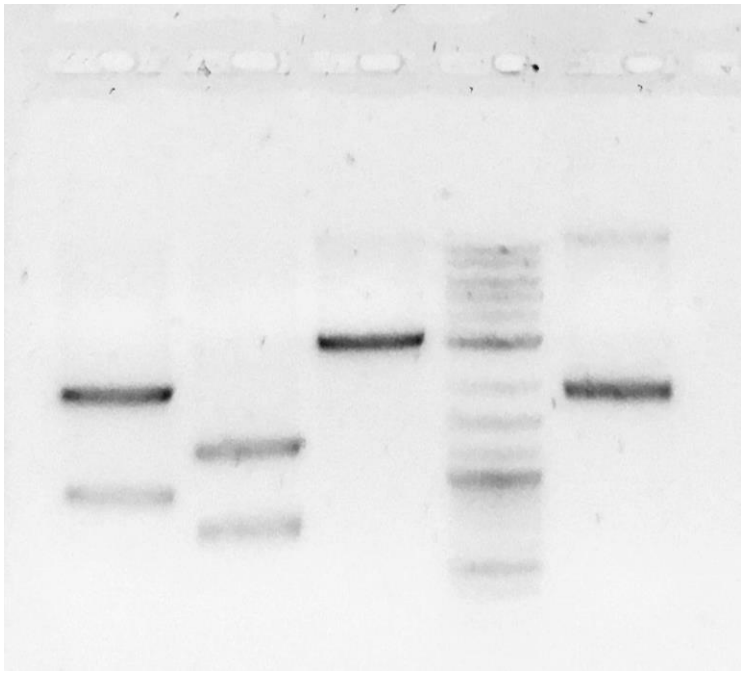
Experiment Title	Colony PCR dCas9 control-B0015-pSB1C3
Protocol	
Changes in Protocol	
Results	
Comments	
Photos	

September 5th:

Experiment Title	RE check dCas9 control-B0015-pSB1C3
Protocol	

		EP	EP NdeI	E	-ve
	DNA	5	5	5	5
	Enzyme	1	1.5	0.5	0
	Buffer	1.8	1.8	1.8	1.8
	MQ	10.2	9.7	10.7	11.2
Changes in Protocol					
Results					
Comments	Expected sizes: EP: 2070, 2330 EP Ned1: 1666, 645, 2070 E: 4252				

EP EP Nde1 E -ve

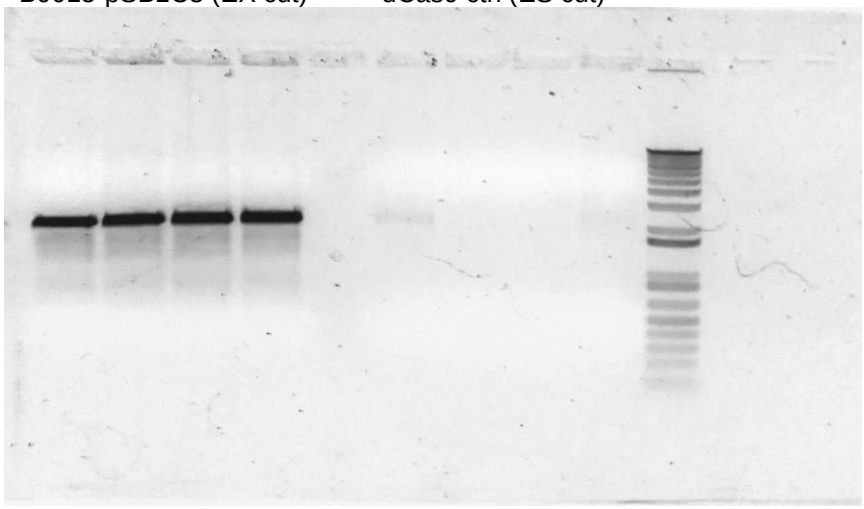


Photos	

Experiment Title	Digestion of dCas9 control and B0015-pSB1C3
Protocol	
Changes in Protocol	

	dCas9 ctrl tube1	dCas9 ctrl tube2	B0015-pSB1C3
DNA	8	15.2	5
E	0.5	0.5	0.5
X			0.5
S	0.5	0.5	
Buffer	1.8	1.8	1.8
MQ	7.2	0	10.2

Cut and purified B0015-pSB1C3

Results	B0015-pSB1C3 (EX cut) dCas9 ctrl (ES cut) 
Comments	
Photos	

September 6th:

Experiment Title	
Protocol	

Changes in Protocol	
Results	
Comments	
Photos	