8th June, 2015

- The following biobrick parts were revived from the **iGEM 2015 distribution plates** using the iGEM protocol Standard protocol.
- Transformation was carried with Ultra-competent cells for **DH5α** strain of ***E.coli*** (with 200µL of LB).
- 200µL of the revived product was plated on LA+antibiotic.
- The plates were kept at 37℃ overnight (12-16 hours).

Part Number	**Location in Kit**	**Backbone**
J23110 (Constitutive Promoter)	Plate 4, Well 19D	Ampicillin
K592025 (RBS+amilCP)	Plate 1, Well 19C	Chloramphenicol
J04450 (RFP)	Plate 4, Well 2H	Ampicillin

Negative Control: For both antibiotics (Amp and Cam)
Positive Control: RFP

9th June, 2015

Part Number	**Number of colonies**
J23110 (Promoter)	Many colonies
K592025 (RBS+amilCP)	Many single colonies
J04450 (RFP)	Lawn growth
Negative Control	No colonies

- Next, the colonies were inoculated in 5mL LB+ 0.1% antibiotic.
- They were kept in an incubator at 37℃ overnight (12-16 hours) at around 180-200 rpm.

10th June, 2015

- Growth was observed in K592025, but no growth in J23110.
- Again a colony was picked from the plate with J23110 and inoculated in 5mL LB+ 0.1% antibiotic.
- They were kept in an incubator at 37℃ overnight (12-16 hours) at around 180-200 rpm.

11th June, 2015

- Again no growth was observed in J23110 (even after inoculating twice).
- Transformation was done again for J23110
- Different promoter was also transformed.

Part Number	Location in Kit	Backbone
J23119 (Constitutive Promoter)	Plate 3, Well 17O	Chloramphenicol

13th June, 2015

- The two promoters were tranformed with 200µL SOC media which was prepared using the iGEM protocol (earlier transformations with LB).
- 200µL of the revived product was plated on LA+antibiotic.
- The plates were kept at 37℃ overnight (12-16 hours).

14th June, 2015

Part Number	Number of colonies
J23110 (Promoter 1)	30
K592025 (RBS+amilCP)	3
J23119 (Promoter 2)	47
Negative Control	No colonies

- The colonies obtained were inoculated in 5mL LB+ 0.1% antibiotic.
- They were kept in an incubator at 37℃ overnight (12-16 hours) at around 180-200 rpm.

18th June, 2015

Plasmid isolation was carried out using the **Alkaline Lysis Protocol** from Sambrook and Maniatis.
Resuspended in TE Buffer.

Results of Nanodrop:

Sample	Vial Number	A260/A280	Concentration (ng/µL)
K592025	1	1.99	6691.9
	2	2.11	4749.5
	3	2.12	4158.3
J23119	1	2.10	4865.3
	2	2.09	2757.4
	3	2.10	4902.3
J23110	1	2.09	5121.8
	2	2.15	8148.0
	3	2.13	3223.8

- The alkaline lysis buffer I wasn't added with RNase.
- The plasmid isolation was repeated with RNase added to Buffer I.

27th June, 2015

- The colonies were again inoculated in 5mL LB+ 0.1% antibiotic.
- They were kept in an incubator at 37℃ overnight (12-16 hours) at around 180-200 rpm.

28th June, 2015

- RNase (10mg/mL stock) was added to Alkaline Lysis Buffer I.
- Resuspension in milliQ water.

Results of Nanodrop:

Sample	Vial Number	A260/A280	Concentration (ng/µL)
K592025	1	2.13	56.9
	2	1.39	67.4
	3	2.06	58.5
J23119	1	2.16	34.1

	2	2.15	32.3
	3	1.39	116.1
J23110	1	2.08	158.4
	2	2.02	3222.1
	3	1.95	580.4

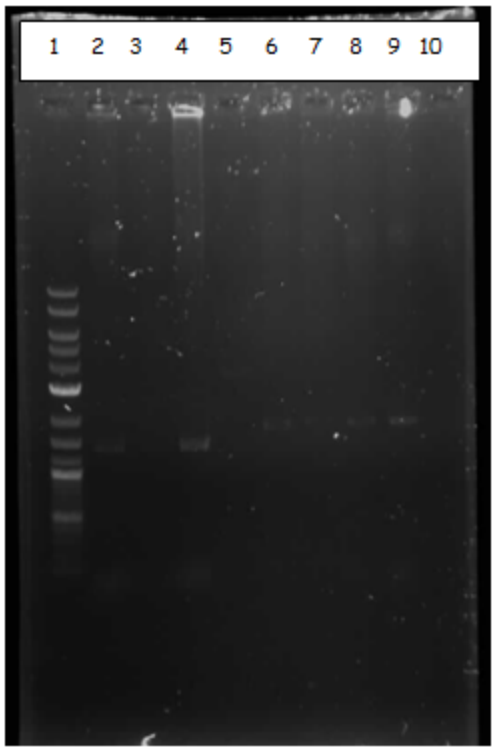
29th June, 2015

- The colonies were again inoculated in 5mL LB+ 0.1% antibiotic.
- They were kept in an incubator at 37℃ overnight (12-16 hours) at around 180-200 rpm.

30th June-10th July, 2015
Again, alkaline lysis was done.

Results of Nanodrop:

Date	**Sample**	**Vial Number**	**A260/A280**	**Concentration (ng/µL)**
30/6	J23119	1	1.94	2376.8
30/6	J23119	2	1.95	2301.2
30/6	J23119	3	1.88	1662.9
8/7	J23119	1	1.99	1616.5
8/7	J23110	2	1.98	618.9
10/7	K592025	1	1.96	1641.1
10/7	K592025	2	1.97	1457.0
10/7	K592025	3	1.99	1404.6



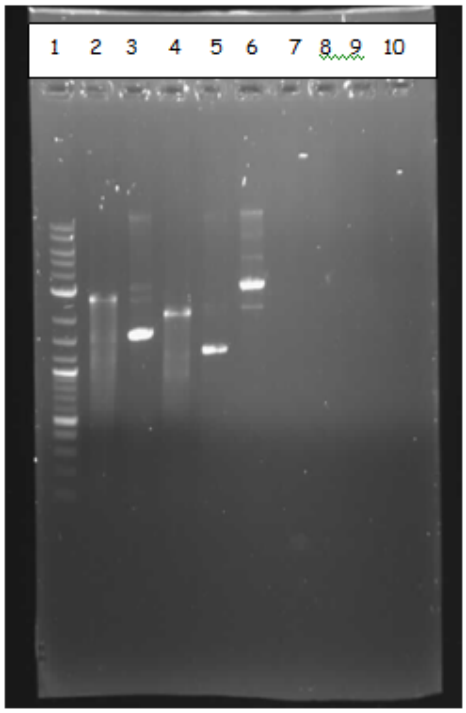
Legend:

1. 2log ladder
2. 3,17O (<2.1kb)-Promoter
3. 3,17O (<2.1kb)-Promoter
4. 3,17O (<2.1kb)-Promoter
5. 3,17O (<2.1kb)-Promoter
6. 4,19D (<2.1kb)-Promoter
7. 1,19C (<2.8kb)-amilCP
8. 1,19C (<2.8kb)-amilCP
9. 1,19C (<2.8kb)-amilCP
10. Blank

26th August and 3rd September

Results for Nanodrop by Qiagen spin mini kit

Sample	A260/A280	Concentration (ng/μL)
J23119	2.04	121.1
J23119	1.89	329.3
K592029	1.96	158
K592029	1.88	407.3



Legend:

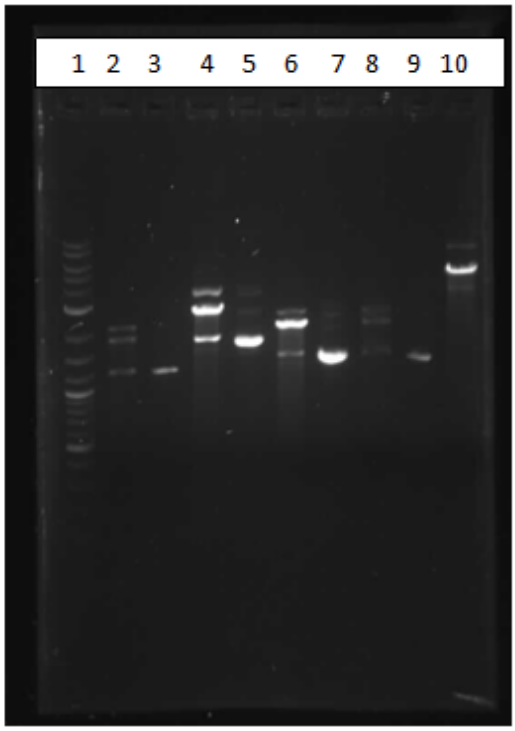
1) ladder

2)1,19C (chromoprotein)-cut (~2.9kb)

3)1,19C (chromoprotein)-uncut (<2.9kb)

4)3,17O (promoter)-cut(~2.1kb)

5)3,17O (promoter)-uncut(<2.1kb)



Legend:

1) 2log DNA ladder
2) Other
3) Other
4) 1,19C (chromoprotein)-cut (~2.9kb)
5) 1,19C (chromoprotein)-uncut (<2.9kb)
6) 3,17O (promoter)-cut(~2.1kb)
7) 3,17O (promoter)-uncut(<2.1kb)

26th August

Single digestion reaction

(in µL)	K592025	J23119
EcoRI	0.3	0.3
DNA	2	2
10XEcoRI Buffer	1	1
100X BSA	0.1	0.1
Distilled water	6.6	6.6
Total Volume	10	10

27th August

3A assembly double digestion

(in µL)	Promoter(J23119)	amilCP(K592025)	Linear backbone
DNA	2	2	10
10X Buffer2	2.5	2.5	2.5
100X BSA	0.5	0.5	0.5
EcoRI	0.5	-	0.5
SpeI	0.5	-	-
PstI	-	0.5	0.5
XbaI	-	0.5	-
DpnI	-	-	0.5
Distlled water	14	14	5.5
Total Volume	20	20	20

Kept at 37°C for 4 hours, heat-kill at 80°C for 20min.

Colonies obtained (~10), were inoculated and miniprep was done with qiagen kit.

Table3: Double digestion for 2A assembly

2A assembly Double digestion:

(in µL)	Promoter(J23119)	amilCP(K592025)
DNA	7.8	7
10X Buffer2	1	1
100X BSA	0.2	0.2
SpeI	0.5	0.5
PstI	0.5	-
XbaI	-	0.5
Distilled water	0	0.8
Total Volume	10	10

9th September