Author: Please complete your labfolder profile.
Entry : 10 June 2015
In Project: Termination

created: 12.09.2015 13:02
updated: 14.09.2015 20:34

The following Biobricks for Termination were resuspended from the Kit Plates and transformed into DH5-alpha competent cells.

Sender Device : luxI : F1610 and K805016 (twins)

Kit locations

1. K805016 : Kit Plate 1, Well 19P, pSB1C3

Receiver Device : luxR : F2620

Kit locations

1. F2620 : Kit Plate 3, Well 4O, pSB1C3

GFP present downstream of Receiver Device : luxR+GFP : T9002

Kit locations

1. T9002 : Kit Plate 3, Well 22J, pSB1C3

The transformed cells were then plated on LB+agar plates with corresponding antibiotic resistance, and incubated for 10-12 hours overnight at 37 deg. Celsius

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 11 June 2015
In Project: Termination

created: 15.09.2015 19:39
updated: 15.09.2015 20:33

11 June 2015 Growth was observed on each of the plates for the transformed biobricks.

Sr. No.	Biobrick Part	Antibiotic Resistance	#No. of colonies
1.0	K805016	Chloramphenicol	3.0
2.0	T9002	Chloramphenicol	30.0
3.0	F2620	Chloramphenicol	12.0
4.0	Negative control	Chloramphenicol	0.0

A single colony was picked from each of the above plates and inoculated in 5ml LB + 5uL antibiotic(Cam).

The cultures were incubated at 37 degrees Celsius overnight, to allow the bacteria to grow till saturation.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 12 June 2015
In Project: Termination

created: 15.09.2015 20:24
updated: 15.09.2015 20:43

12 June 2015

The saturated cultures were stored at 4 degrees Celsius.

2ml of this culture was used to make glycerol stocks (4x0.5ml) which were stored at -80 degrees Celsius.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 14 June 2015
In Project: Termination

created: 15.09.2015 20:39
updated: 15.09.2015 20:42

14 June 2015

The primary culture inoculated on 11 June 2015 was used to make a secondary culture.

For the same, 100uL of the primary culture was added to 10mL LB + 10uL antibiotic (Cam).

This was incubated at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 15 June 2015
In Project: Termination

created: 15.09.2015 20:43
updated: 15.09.2015 22:16

15 June 2015
Plasmid isolation using Alkaline Lysis Protocol.

The DNA was resuspended in TE Buffer.

The DNA concentrations were measured using the Nanodrop.

Biobrick Samples	Sr.No.	Nucleic Acid concentration (ng/uL)	A260/A280
K805016	1.0	43.323	2.02
	2.0	42.416	2.09
	3.0	54.92	2.06
T9002	1.0	31.299	2.12
	2.0	37.811	2.11
	3.0	33.505	2.1
F2620	1.0	25.276	2.14
	2.0	27.866	2.12
	3.0	42.525	2.1

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 16 June 2015
In Project: Termination

created: 15.09.2015 21:07
updated: 18.09.2015 11:01

16 June 2015

The following Biobrick for Termination were resuspended from the Kit Plates and transformed into DH5-alpha competent cells.

Promoter : p(tetR) : R0040

Kit Location:

- Kit Plate 2, Well 6F, pSB1C3

The transformed cells were then plated on LB+agar plates with corresponding antibiotic resistance, and incubated for 10-12 hours overnight at 37 degrees Celsius.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 17 June 2015
In Project: Termination

created: 15.09.2015 21:52
updated: 18.09.2015 11:01

17 June 2015

Growth was observed in the plates.

Sr. No.	Sample	Antibiotic Resistance	#No. of colonies
1.0	R0040	Chloramphenicol	40.0
2.0	Negative Control	Chloramphenicol	0.0

The miniprep samples from 15 June 2015 when loaded on gel,showed a large RNA smear.

The miniprep needed to be repeated.

The following biobrick colonies were inoculated into 2x (5mL LB + 5uL antibiotic (Cam)):

1. K805016
2. R0040
3. F2620
4. T9002

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 18 June 2015
In Project: Termination

created: 15.09.2015 22:01
updated: 18.09.2015 11:02

18 June 2015

Plasmid isolation using Alkaline Lysis protocol.

The DNA obtained was resuspended in 30uL of MilliQ water preheated to 65 degrees Celsius.

Glycerol stocks were made for the promoter, R0040.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 24 June 2015
In Project: Termination
created: 15.09.2015 22:16
updated: 18.09.2015 11:02

24 June 2015

The plasmid DNA isolated on 15 June 2015, resuspended in TE Buffer, needed to be resuspended in hot MilliQ water since the chelating effect of EDTA interferes with restriction digestion. After precipitation and centrifugation, no DNA pellet was obtained. Thus the samples were discarded.

We added RNAse to the plasmid DNA samples isolated on 18 June 2015.

RNAse stock concentration = 10mg/uL

RNAse working concentration = 10ug/uL

1uL of the working concentration RNAse solution was added to the DNA samples, incubated at 37 degrees Celsius for 20 minutes and then stored at -20 degrees Celsius.

The alkaline lysis for all the termination biobricks need to be repeated.

Inoculated the following in 5mL LB + 5uL antibiotic (Cam) :

1. R0040
2. K805016
3. F2620
4. T9002

Incubated the inoculated cultures at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 25 June 2015
In Project: Termination

created: 15.09.2015 22:26
updated: 18.09.2015 11:04

25 June 2015

Plasmid isolation was done using Alkaline Lysis, in which RNAse was added to Solution I.

The DNA concentrations were measured using the Nanodrop.

Biobrick Sample	Sr.No.	Nucleic Acid concentration (ng/uL)	A260/A280
K805016	1.0	1633.3	1.86
	2.0	1488.0	1.83
	3.0	1425.6	1.86
R0040	1.0	1975.3	1.88
	2.0	2193.5	1.87
	3.0	1395.1	1.75
F2620	1.0	1727.1	1.89
	2.0	2273.9	1.89
	3.0		1.9
T9002	1.0	1247.6	1.86
	2.0	1122.3	1.81
	3.0	2175.4	1.92

The isolated plasmids from 18 June 2015 (RNAse added) was run on a gel, to be extracted and purified, to get rid of the RNA fragment contaminations.

Then the DNA concentrations need to be checked on the Nanodrop.

Single digests set up with EcoRI enzyme, in order to run linearized plasmids and check length and concentration in terms of band intensity.

Incubate at 37 degrees Celsius for 2 hours, followed by heat inactivation at 80 degrees Celsius for 10 minutes. Store the digest at -20 degrees Celsius.

Samples	K805016	F2620	T9002	R0040
	(uL)	(uL)	(uL)	(uL)
DNA	0.3	0.3	0.3	0.3
EcoRI	0.3	0.3	0.3	0.3
10x B4	1.0	1.0	1.0	1.0
100x BSA	0.1	0.1	0.1	0.1
Water	8.3	8.3	8.3	8.3
Total	10.0	10.0	10.0	10.0
Mastermix:	10x B4	100x BSA	Water	
	9uL	0.9uL	74.7uL	

A 1% agarose gel was run.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 7 July 2015
In Project: Termination

created: 15.09.2015 23:07
updated: 18.09.2015 11:04

7 July 2015

The following Biobrick for Termination were resuspended from the Kit Plates and transformed into DH5-alpha competent cells.

Promoter : lacI regulated lambda-pL hybrid : R0011

Kit Location:

- Kit Plate 4, Well 5H, pSB1A2

The transformed cells were then plated on LB+agar plates with corresponding antibiotic resistance, and incubated for 10-12 hours overnight at 37 degrees Celsius.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 9 July 2015
In Project: Termination

created: 15.09.2015 23:09
updated: 15.09.2015 23:14

9 July 2015

Growth was seen on the plates.

Inoculated two colonies in 5mL LB + 5uL antibiotic (Amp).

Incubated at 37 degrees Celsius of 12 hours.

Sr. No.	Sample	Antibiotic Resistance	#No. of colonies
1.0	R0011	Ampicillin	30.0
2.0	Negative control	Ampicillin	0.0

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 23 July 2015
In Project: Termination

created: 15.09.2015 23:13
updated: 18.09.2015 11:04

23 July 2015

Inoculated the following biobricks in 5mL LB + 5uL antibiotic :

1. K805016
2. R0011
3. F2620
4. T9002

Incubate at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 24 July 2015
In Project: Termination

created: 15.09.2015 23:16
updated: 16.09.2015 11:38

24 July 2015

Plasmid isolation using Alkaline Lysis.

The DNA was resuspended in 30uL Elution Buffer.

The DNA concentrations were measured using a Nanodrop.

Sample	Sr. No.	Nucleic Acid concentration (ng/uL)	A260/A280
K805016	1.0	2069.1	2.09
	2.0	1922.2	2.05
	3.0	1890.6	2.06
F2620	1.0	1508.5	2.0
	2.0	2267.9	2.06
	3.0	2074.6	2.07
T9002	1.0	2189.8	2.06
	2.0	1652.0	2.07
	3.0	1425.9	2.02
R0011	1.0	2139.9	2.08
	2.0	2189.2	2.04
	3.0	2724.0	2.06

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 27 July 2015
In Project: Termination

created: 16.09.2015 11:31
updated: 18.09.2015 11:04

27 July 2015

Inoculated the following in 3mL LB + 3uL antibiotic for plasmid isolation using QIAGEN Midiprep Kit:

1. K805016
2. R0011
3. T9002
4. F2620

Subcultured the primary culture into 50mL LB + 50uL antibiotic.

Incubated at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 19 August 2015
In Project: Termination
created: 16.09.2015 11:51
updated: 18.09.2015 11:06

19 August 2015

Plasmid isolation using QIAGEN Miniprep Kit.

Resuspended the DNA in 20uL Elution Buffer.

The DNA concentrations were measured on a Nanodrop.

Sample	Sr. No.	Nucleic Acid concentration (ng/uL)	A260/A280
K805016	1.0	84.5	1.93
	2.0	17.2	1.93
F2620	1.0	138.1	1.91
	2.0	18.7	1.91
T9002	1.0	76.6	2.05
	2.0	20.3	2.05
R0011	1.0	77.7	1.92
	2.0	15.6	1.92

Set up a single digest with EcoRI to check plasmid lengths on the gel.

Total reaction volume = 10uL

Incubate at 37 degrees Celsius for 3 hours.

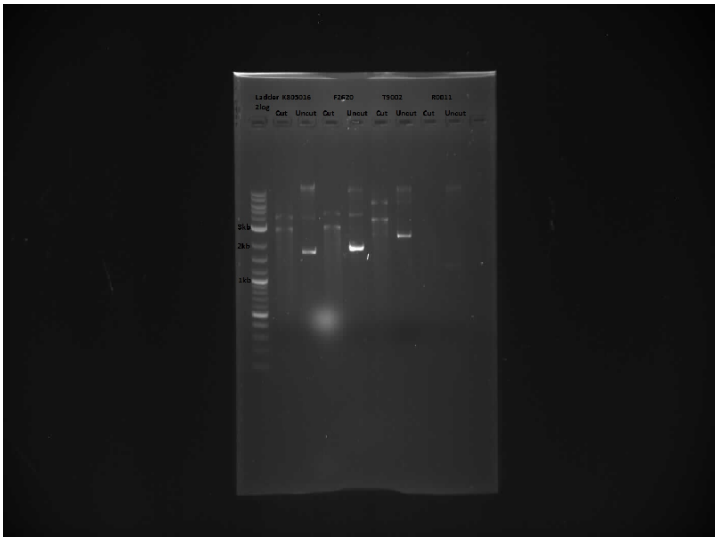
Samples	K805016	T9002	F2620	R0011
	(uL)	(uL)	(uL)	(uL)
DNA	2.0	2.0	1.0	2.0
EcoRI	0.2	0.2	0.2	0.2
Buffer	1.0	1.0	1.0	1.0
100x BSA	0.1	0.1	0.1	0.1
Water	6.7	6.7	7.7	6.7
Mastermix:	Buffer	100x BSA	Water	
	4uL	0.4uL	26.8uL	

A 1 % agarose gel was run, loading cut and uncut plasmids.

Two consecutive bands were seen for the cut DNA. This suggests that the entire DNA got partially digested.

The enzyme probably needed to be incubated for longer.

The bands for the promoter were very faint.



Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 5 September 2015
In Project: Termination

created: 18.09.2015 02:55
updated: 18.09.2015 02:59

5 September 2015

Inoculated the following in 5mL LB + 5uL antibiotic:

- K805016
- F2620
- T9002
- R0011

Incubate at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 6 September 2015
In Project: Termination

created: 18.09.2015 02:59
updated: 18.09.2015 03:05

6 September 2015

Plasmid isolation using QIAGEN Miniprep Kit.

Pre-elution : Heated the miniprep columns + Elution buffer at 37 degrees Celsius for 1.5 hours.

Resuspended the DNA in 20uL of Elution buffer.

The DNA concentration was measured using the Nanodrop.

Sample	Sr. No.	Nucleic Acid concentration (ng/uL)	A260/A280
K805016	1.0	133.3	1.95
F2620	1.0	60.4	1.98
T9002	1.0	205.9	1.89
R0011	1.0	57.2	2.12
	2.0	90.6	2.1

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 8 September 2015
In Project: Termination

created: 18.09.2015 03:05
updated: 18.09.2015 03:18

8 September 2015

Set up a 3A -Assembly to assemble the promoter + Sender construct.

Double Digestion				
	Samples	K805016	R0011	Kan backbone
		(uL)	(uL)	
	DNA	3.0	2.0	Previously digested
	10x B2	2.5	2.5	
	100x BSA	0.5	0.5	
	EcoRI	0.5	-	
	SpeI	0.5	-	
	XbaI	-	0.5	
	PstI	-	0.5	
	Water	13.0	14.0	
	Total	20.0	20.0	
Incubate at 37 degrees Celsius overnight.				
Ligation				
		Control	Sender Device	
		(uL)	(uL)	
	Backbone	2.0	2.0	
	E-S cut (promoter)	-	2.0	
	X-P cut (luxI)	-	2.0	
	Buffer	1.0	1.0	
	T4 Ligase	0.5	0.5	
	Water	6.5	2.5	
	Total	10.0	10.0	
Incubate at 25 degrees Celsius for 7 hours				

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 9 September 2015
In Project: Termination

created: 18.09.2015 03:18
updated: 18.09.2015 03:20

9 September 2015

Transformed 5 uL of the ligated mixture into DH5-alpha competent cells.

Incubated the plates overnight at 37 degrees Celsius.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 10 September 2015
In Project: Termination

created: 18.09.2015 03:20
updated: 18.09.2015 03:22

10 September 2015

No growth seen in any of the plates. Probably the ligation or digestion didn't work.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 11 September 2015
In Project: Termination

created: 18.09.2015 03:21
updated: 18.09.2015 03:23

11 September 2015

Retransformed the leftover 5uL of ligation mixture into DH5-alpha competent cells.

Incubated the plates at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 12 September 2015
In Project: Termination

created: 18.09.2015 03:32
updated: 18.09.2015 06:33

12 September 2015

Inoculated 5 colonies from the ligation transformed plates (Kan backbone).

Set up a 2A-Assembly to get the Promoter-Sender construct in Cam backbone.

The double digest was set up overnight at 37 degrees Celsius.

The ligation was set up at 25 degrees Celsius for 3 hours, and stored at -20 degrees Celsius.

	Ligation 1	Ligation 2
	(uL)	(uL)
Kan backbone	2.0	2.0
E-S cut (R0011)	3.0	2.0
X-P cut (K805016)	3.0	2.0
T4 ligase	0.5	0.5
Buffer	1.0	1.0
Water	0.5	2.5
Total	10.0	10.0

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 14 September 2015
In Project: Termination

created: 18.09.2015 03:38
updated: 18.09.2015 03:40

14 September 2015

Transformed ligations into DH5-alpha competent cells, and incubated the plates at 37 degrees Celsius overnight.

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 15 September 2015
In Project: Termination
created: 18.09.2015 03:40
updated: 18.09.2015 06:36

15 September 2015

No growth seen in the inoculants from 12 September 2015.

About 50 colonies were seen in Ligation 1 transformed plate. Inoculated 5 colonies. About 10 colonies were seen in Ligation 2 transformed plate. Inoculated 3 colonies.

Inoculation was done in 5mL LB + 5uL kanamycin.

Plasmid isolation using QIAGEN Miniprep Kit for the colonies inoculated.

The DNA concentrations were measured using a Nanodrop, after resuspension in 20uL Elution Buffer.

Sample	Sr. No.	Nucleic Acid concentration (ng/uL)	A260/A280
Ligation 1	1.0	91.4	1.95
	2.0	48.9	2.01
	3.0	43.5	2.06
	4.0	45.1	2.05
	5.0	42.5	2.06
Ligation 2	1.0	37.3	2.1
	2.0	34.9	2.09
	3.0	45.1	2.02

Single digest of the miniprep samples to check length of plasmid on a gel.

Samples	L1,1	L1,2	L1,4	L2,1	L2,3
DNA	2.0	4.0	4.0	4.0	4.0
EcoRI	0.3	0.3	0.3	0.3	0.3
Buffer	1.0	1.0	1.0	1.0	1.0
100x BSA	0.1	0.1	0.1	0.1	0.1
Water	6.6	4.6	4.6	4.6	4.6
Total	10.0	10.0	10.0	10.0	10.0

A 1% agarose gel was run, loading the cut and uncut plasmids of the promoter+Sender ligation in pSB1K3 backbone.

Bands were seen at around 3kb, something we expected since the plasmid length should have been around that length.

TOP: Lane 1: 2 log DNA ladder

Lane 2: L1,1 uncut plasmid

Lane 3: L1,1 single digested plasmid (~3kb)

Lane 4: L1,2 uncut plasmid

Lane 5: L1,2 single digested plasmid(~3kb)

Lane 6: L1,4 uncut plasmid

Lane 7: L1,4 single digested plasmid (~3kb)

BOTTOM: Lane 1: 2 log DNA ladder

Lane 2: L2,1 uncut plasmid

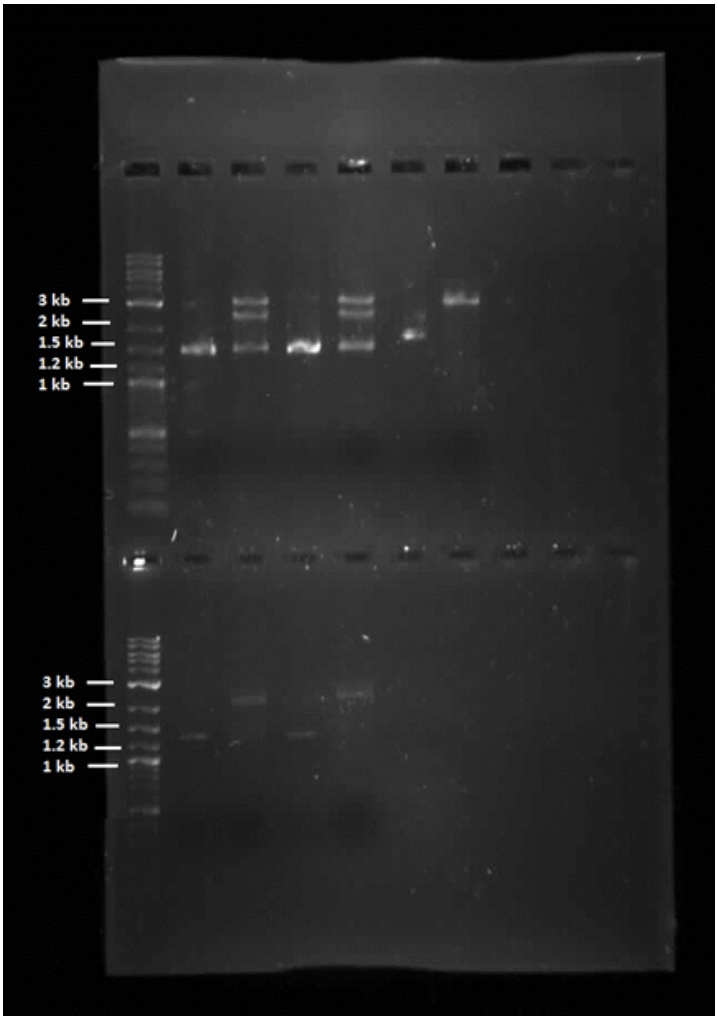
Lane 3: L2,1 single digested plasmid (~3kb)

Lane 4: L2,3 uncut plasmid

Lane 5: L2,3 single digested plasmid (~3kb)

An overnight Double digest was set up to transfer the promoter+sender (LuxI) construct into the pSB1C3 backbone.

The reaction was incubated at 37 degrees Celsius overnight.



Samples	L1,1 (uL)	Cam backbone (uL)
DNA	3.0	10.0
10x B2	2.5	2.5
100x BSA	0.5	0.5
EcoRI	0.5	-
PstI	-	0.5
DpnI	-	0.5
Water	3.0	5.5
Total	10.0	20.0

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 16 September 2015
In Project: Termination

created: 18.09.2015 03:47
updated: 18.09.2015 06:44

A ligation was set up for the double digested construct and E-P digested pSB1C3 backbone. Reaction was carried out at 25 deg. C for 4 hours.

3 ul of the ligation was then transformed into DH5-alpha competent cells. Plates were incubated at 37 deg. C overnight.

	Sender (uL)	Control (uL)
Cam backbone (E-P) cut	2.0	2.0
Kan backbone (E-P) cut	-	2.0
L1,1 (E-P cut)	3.0	-
T4 ligase	0.5	0.5
Buffer	1.0	1.0
Water	3.5	4.5
Total	10.0	10.0

Date:	Signed by:
Date:	Disclosed to and understood by:

Author: Please complete your labfolder profile.
Entry : 17 september 2015
In Project: Termination

created: 18.09.2015 06:41
updated: 18.09.2015 11:00

No colonies were obtained in the plates for sender in Cam backbone transformed into competent cells. The ligation reaction was set up again, followed by transformation in DH5-alpha cells.

A 1:1 co-culture of sender device (BBa_R0011+BBa_K805016) in Kan backbone and receiver (BBa_T9002) in Cam backbone was made in 5 ml LB, incubated at 37 deg. C. This was induced with 1mM IPTG in exponential phase (~2 hours after incubation). This was again incubated for 2 hours at 37 deg C, to check for fluorescence. Control was the same 1:1 co-culture, but uninduced.

Controls: (all cultures in 5ml LB + 5ul antibiotic)

1. Sender device (BBa_R0011+BBa_K805016), uninduced.

2. Sender device (BBa_R0011+BBa_K805016), induced with 1mM IPTG

3. R eceiver device (BBa_T9002), uninduced

4. R eceiver device (BBa_T9002), induced with 1mM IPTG

5. pGFP, induced with 1mM IPTG

6. Positive cntrol - BBa_I20270

7. Negative control - BBa_Roo40

These cultures were then centrifuged at 13000rpm for 2 minutes to obtain bacterial pellet, which was then washed with 1X PBS, washes were repeated 3 times. Finally, pellet was resuspended in 1 ml 1X PBS, out of which 100ul was used for fluorescence measurements in the plate reader.

Slides were prepared for the same samples to observe in a DIC Microscope and check fluorescence.

	Sender
	(uL)
Cam backbone (E-P) cut	2.0
L1,1 (E-P cut)	4.0
T4 ligase	0.5
Buffer	1.0
Water	2.5
Total	10.0

Date:	Signed by:
Date:	Disclosed to and understood by: