iGEM
Team USP-Brazil
Lab Notebook

Protocols:
The circuit construction was done following these protocols:

Transformation protocol:

1. Thaw the cells on ice for 10 minutes
2. Add 1-5uL of plasmid to 50uL of competent cells. Mix gently
3. Incubate the cells on ice for 20-30 minutes
4. Spread 20uL of antibiotics for each plate
5. Water bath the cells at 42°C for 1 minute
6. Put back on ice for 2 minutes
7. Add 950uL of LB to the tube. Incubate at 37°C for 30-60 minutes
8. Centrifuge and ressuspend the cells with 100uL LB. Plate and incubate the cells overnight at 37°C

PCR protocol:

Ligation protocol:

Vector	Insert	10x Buffer	Ligase	H2O
1,5uL	10uL	2uL	1uL	5,5uL

Ligate 16°C for 30 minutes and heat kill 80°C for 20 minutes.

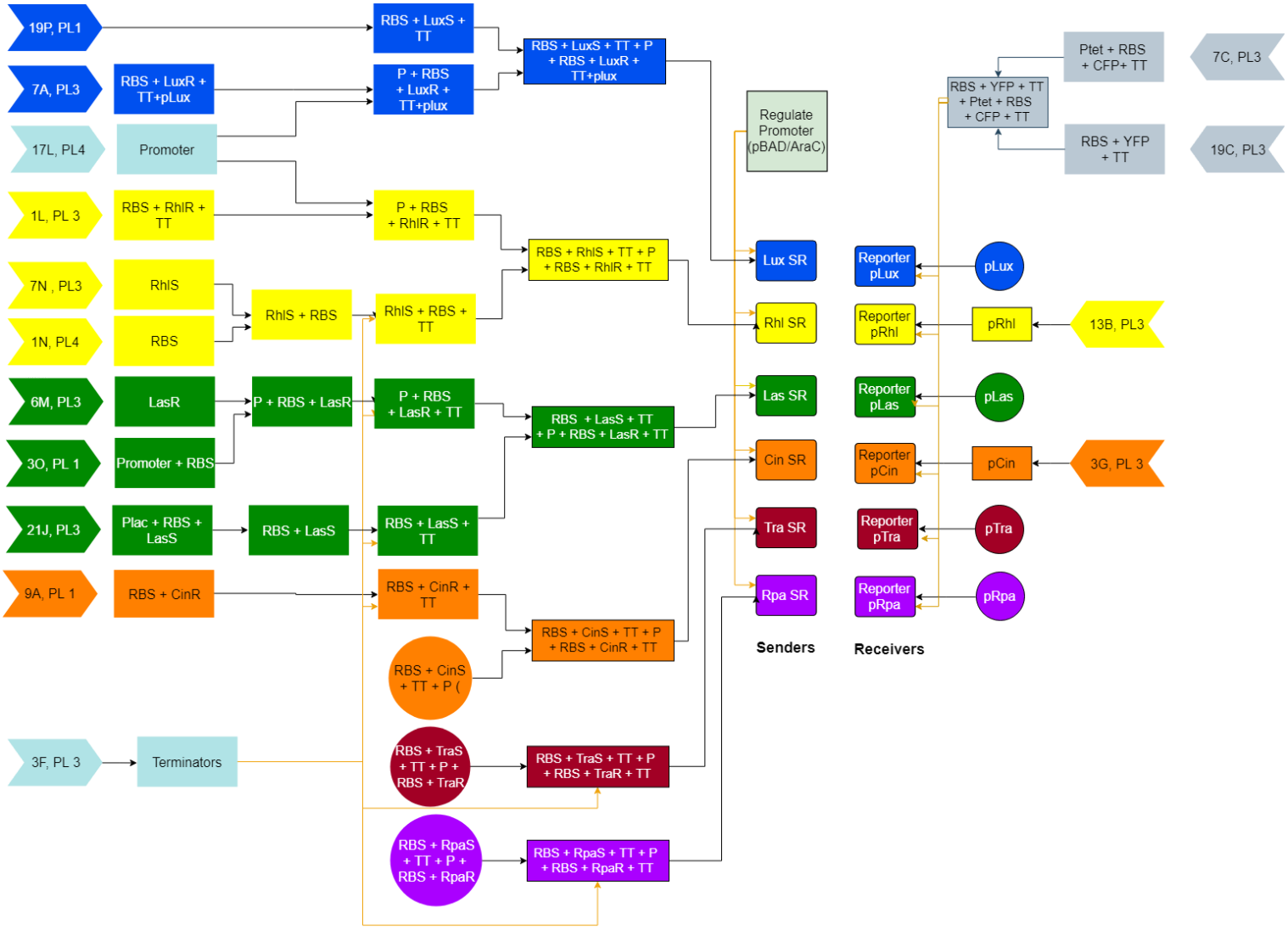
Digestion protocol:

We used EcoRI, SpeI, NotI, XbaI or PstI for digestion of biobricks.

Plasmid	2.1 Buffer 10x	Enzymes	H2O
20uL	3uL	1uL	To 30uL

Incubate the restriction digest at 37°C for 30 minutes and then 80°C for 20 minutes

Construction:
To build up all the 12 plasmids, we mostly used biobricks from the 2017 distribution kit. The flowchart below shows all the cloning procedures we have done.



Date: May 28th, 2018 (Felipe)
Experiment: Chemocompetent DH5a pre inoculum
Results/Discussion
50uL frozen DH5a to LB
Overnight at 37°C

----------x----------

Date: May 29th, 2018 (Felipe, Vinicius, Catharina, Fábio)
Experiment: Distribution kit sequences transformation
2017 iGEM distribution kit
Sequences:
Plate 1:

3O (plate 1): BBa_R0078 (Promoter cinR and HSL regulated)

Plate 2:

19P (plate 2): BBa_K876060

Plate 3:

13B BBa_I14017 [P(Rhl): rhlR and C4HSL regulated promoter
7C:BBa_I13602 (Tet operator with CFP reporter (with LVA tag)
3F:BBa_B0015 (Double terminator B0010-B0012)
3G:BBa_Roo78 (Promoter CinR and HSL regulated)
21J: BBa_K0084007 (Lac repressible lasI generator (no LVA)
19K:BBa_E0430 EYFP (RBS+LVA-TERM)
1L:BBa_I466 (RhlR protein generator)
6M: BBa_C0179 (LasR activator for *P. aeruginosa*)
7N: BBa_C0070 (Autoinducer synthetase for N-butyryl-HSL (BHL) and HHL
17L:BBa_J23104 (Constitutive promoter family member)
1N: BBa_0034 (RBS)
7P:BBa_I0462 (LuxR protein generator)

Result (05/30):

Plate 2 mistakenly resuspended, it was supposed to be plate 1: remake
All plates were successfully transformed
Exception: 7P (P4) with wrong antibiotic (chloramphenicol)

----------x----------

Date: May 30th, 2018 (Felipe)
Experiment: Bacteria inoculum at LB and antibiotic
Results/Discussion
5mL LB in 15mL falcon
5uL antibiotic

----------x----------

Date: June 1st, 2018 (Felipe, Vinicius, Andressa); Chuck
Experiment: Miniprep of 30/05 bacteria
Results/Discussion:
Cells freezed at -20°C
DNA did not fully precipitate (at miniprep first step)

Plate	Plasmid	Concentration(ng/uL)	Results
Plate 4	1N (RBS)	9,7	x
	17L (Promoter)	7,5	x
	7P (LuxR)	34,1	ok
PLate 3	21J (Plac+RBS+LasS)	28,5	ok

	7N (RhlS)	14,1	ok/x
	6M (LasR)	26,7	ok
	1L (RBS+RhlR+TT)	13	ok/x
	19K (RBS+LVA-TERM)	15,3	ok/x
	7C (Ptet+RBS+CFP+TT)	40	ok
	3G (CinR promoter)	11,5	x
	13B (Rhl promoter)	10,8	x
	3F (Terminators)	16,7	ok/x

----------x----------

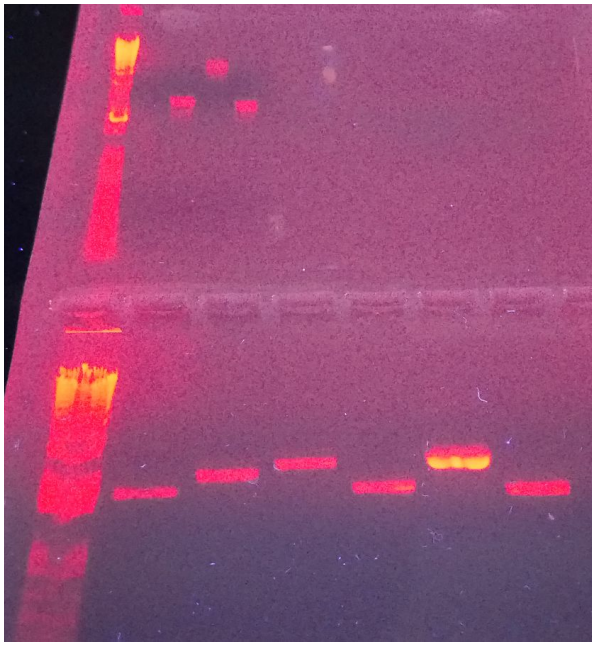
Date: June 4th, 2018 (Felipe)
Experiment: Pre inoculum of failed plasmids (x and ok/x)
Plate 4: 1N , 17L
Plate 3: 7N, 1L, 19K, 3G, 13B, 3F

----------x----------

Date:June 4th, 2018 (Felipe)
Experiment: Miniprep gel (01/06)
Results/Discussion:
Plate 4: 7P
Plate 3: 7C, 21J, 6M, 7N, 1L, 3F, 19K

----------x----------

Date: June 5th, 2018
Experiment: 04/06 Miniprep (Felipe)
Results/Discussion
17L, Pl4, didn't grow



top	L	7P (pl 4)	7C (pl 3)	21J (pl 3)	6M (pl 3)
		x	ok	ok	ok

bottom	L	3G	7N	19K	3F	1L	13B	1N
	ok	ok	ok	ok	ok	ok	ok	x

----------x----------

Date: June 6th, 2018 (Henrique)
Experiment: Transformation of plasmids from distribution kit and Miniprep of plasmíd 17L (plate 4)

Transformed cells:
30 (placa 1); 9A (placa 1); (19P (placa 1); 1N (RBS); 7P (placa 4)

----------x----------

Date: June 6th, 2018 (Felipe)
Experiment: Digestão overnight de plasmídeos

Partes e enzimas:

Plasmid	3F, plate 3	7C, plate 3	21J, plate 3	7N, plate 3	6M, plate 3	19K, plate 3	

Enzyme	E+X	E+S

Reaction::

	x1
Plasmíd	20uL
2.1 Buffer 10x	3uL
Enzymes	1uL cada
H2O	5uL

Overnight at 37°C for later heat inactivation at 80°

----------x----------

Date: June 7th, 2018 (Henrique)
Experiment: Purificação das digestões e, 06/06
2 géis

----------x----------

Date: June 7th ,2018
Experiment: Ligation of 06/06 digestion
Ligações: 3F+21J; 3F+7N; 3F+6M; 3F +7C+19K
Reação:

	x1
Vetor	1,5uL
Inserto	10uL
Buffer	2uL
Ligase	1uL
H2O	5,5uL

----------x----------

Date: Junho 07, 2018
Experiment: Transformation of 19P (placa 1); 7p (Plate 4) and ligations 19P, placa 1); (7P, placa 4); (19K.+7C); (7N+3F); 21J +3F); (6M + 3F)

----------x----------

Date: June 11th, 2018. (Felipe)
Experiment: Colony PCR of 06/06

	1x
DNA	1 colônia
Forward	0,5uL
Reverse	0,5uL
dNTP	0,5uL
Taq	0,25uL
Buffer	2,5uL
H2O	21uL
	25,25

Colonies: 6M+3F; 7N+3F; 21J+3F; 19K+7C
Results/Discussion: No positives results. Lack of a negative control.

----------x----------

Date: Junho 13, 2018
Experiment: Repetition of previous PCR reaction

	1x
DNA	1 colônia
Forward	0,5uL
Reverse	0,5uL
1,25M dNTPs	4uL
Taq	0,125uL
10x Buff	2,5uL
H2O	17uL

----------x----------

Date: June 13th , 2018.
Experiment: Bacteria plating
Everything on plate 3

Plate 1: 6/6 e 10/06
Plate 4: 06/06 e 10/06

Results/Discussion
13B (plate 3) did not grow. Remake.

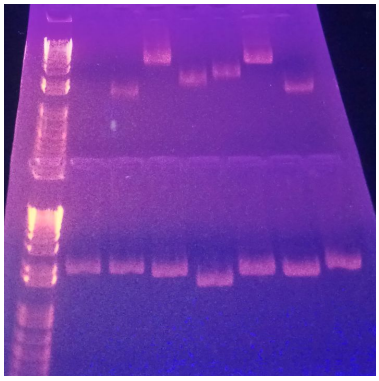
----------x----------

Date: Junho 14, 2018.
Experiment: Inoculum of 06/13 bacteria (Felipe)
14 tubes with 2mL of LB medium

Next Steps:

----------x----------

Date: June 15th, 2018
Experiment: Miniprep of 06/13
Gel:
Ladder - 7P - 1N (RBS) - 17L - 9A - 30 - 14P - 3F
Ladder - 1L - 19K - 6M - 3G - 21J - 7N - 7C
Stored in glycerol at 80 degrees.
Results/Discussion: 7P not appearing, rest seems to be ok.



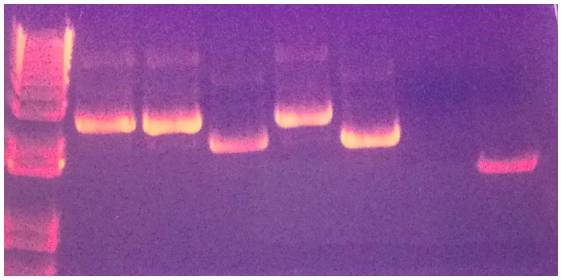
----------x----------

Date: June 19th, 2018.
Experiment: Inoculum of plates 13B and 19K+7C ligation
2mL of LB medium

----------x----------

Date: June 20th, 2018
Experiment: Miniprep of 13B plates, 19K+7C ligation (06/19) (Felipe)
Results/Discussion
Gel: Ladder - Colony 1-Colony2-Colony 3-Colony 4-Colony 5-Colony 6-Colony13B

Ok	ok	X	Ok	X	XXX	Ok



----------x----------

Date: June 20yh, 2018
Experiment: Ligation inoculum (3F + 6M and 7N + 21J)
2mL of LB medium

----------x----------

Date: Plasmid digestion

Parts	19K + 7C	17L, plate 4	13B, plate 3	3G, plate 3	1L, plate 3
Enzymes	X+P	S + P			X+P

Reaction:

Plasmid	2.1 Buffer 10x	Enzymes	H2O
20uL	3uL	1uL	5uL

Overnight at 37°C for heat-inactivation (80°C) the following day

----------x----------

Date: June 21th, 2018
Experiment: Heat inactivation of 3g and 13B digestions (Felipe)
80°C for 20 minutes

----------x----------

Date: 21th, June 2018.
Experiment: Colony PCR of 06/20 inoculum (with IGP60) (Felipe)
Reaction

Inoculum	Forward Primer	Reverse Primer	10mM dNTPs	Taq	10x Buffer	50mM MgCl2	H2O
1uL	0,5uL	0,5uL	0,5uL	0,125uL	2,5uL	0,75uL	19,125uL

Gel:

Ladder	Negative control	Positive control	1 (colony 1	2 (colony 5	3 (colony 6	4 (colony 2)	5 (colony 4)	6 (colony 3)	7	8
	-	3O, plate 2	6M + 3F						7N + 3F	

Ladder	9	10	11	12	13	14	15	16	17	18
	7N + 3F				21J + 3F					

Results/Discussion: No results

----------x----------

Date: June 26th, 2018.
Experiment: Colony PCR of 06/20 inoculum (21th June repetition) (Felipe)
PCR:

Inoculum	Forward Primer	Reverse Primer	10mM dNTPs	Taq	10x Buffer	50mM MgCl2	H2O
1uL	0,5uL	0,5uL	0,5uL	0,125uL	2,5uL	0,75uL	19,125uL

Gel:

	Colonies								
Ladder	1	3	4	1	2	4	1	2	3
	6M			7N			21J + 3F		

Results/Discussion: all ok
Next Steps:

----------x----------

Date: June 27th, 2018
Experiment: Inoculum of positive ligations at 06/20 (Felipe)
Ligation 6M+3F: colony 3
Ligation 7N+3F: colony 2
Ligation 21J + 3F: colony 2

2mL of LB medium

----------x----------

Date: June 27th, 2018
Experiment: Plasmid digestion (Felipe)
Plasmids:

3O, plate 1	1N, plate 4 (RBS)	6M + 3F	7N + 3F	9A, plate 1	3F, plate 3
S+P		X+P		E+S	E+X

Reaction:

Plasmid	2.1 Buffer 10x	Enzymes	H2O
20uL	3uL	1uL	5uL

----------x----------

Date: June 29th, 2018
Experiment: Ligation of 27th digestion (Felipe and Lucas)
Purification gel:

Ladder	6M + 3F	7N+3F

Ladder	4A, plate 1	4A, plate 1	4A, plate 1	4A, plate 1

Ligation reaction:

Vector	Insert	10x Buffer	Ligase	H2O
1,5uL	10uL	2uL	1uL	5,5uL

Transformation:
7P, plate 4 - Carbenicillin
(LuxR)

3O+ (6M+3F)- Clorofenicol
(Promoter + RBS + LasR+ terminator)

1N + (7N+3F) - Carbenicillin
(RhlS+RBS+terminator)

9A+3F - Clorofenicol
(RBS+CinR+ terminator)

----------x----------

Date: July 3rd, 2018
Experiment: Inoculum of 1-6 colonies from 06/29 transformation (Felipe)

----------x----------

Date: July 3rd, 2018
Experiment: Transformation of 7A (plate 3) (Felipe)
Substitution of 7P (plate 4)= LuxR

7A (plate 3)= RBS+LuxR+terminator+plux

Electroporation
Results/Discussion: No growth

----------x----------

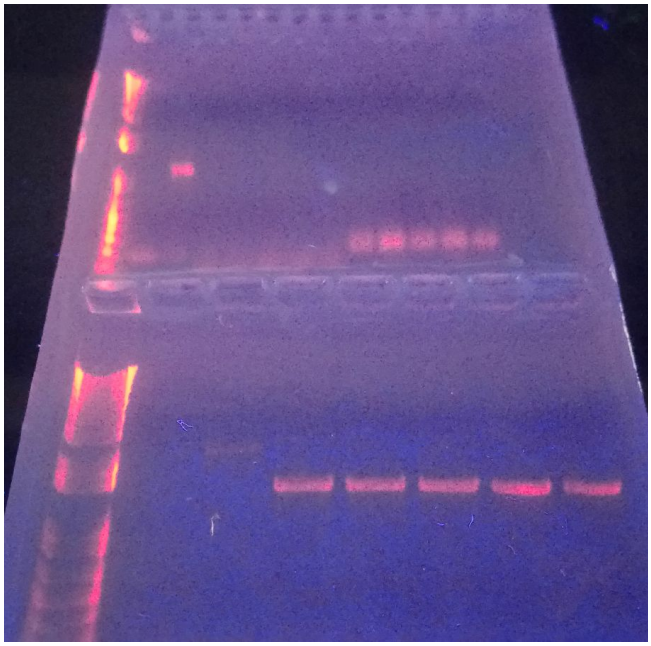
Date: July 4th, 2018.
Experiment: PCR of 07/03 Inoculum (Felipe and Lucas)
Reaction:

DNA	Forward	Reverse	dNTPs	10x Buffer	MgCl2 50mM	Taq plat.	H2O
1uL	0,5uL	0,5uL	0,5uL	2,5uL	0,75uL	0,1uL	19,15uL

Gel:

Ladder	Neg. Control	Positiv. Control	2	3	4	5	6	7	8	9	10	11
	-		1N + (7N + 3F)					9A + 3F (closed plasmid)				

Ladder	12	13	14	15	16	17	18
	9A + 3F	3O + (6M+ 3F)					



Results/Discussion: 3O was the wrong part. Rest just didn't work

----------x----------

Date: Jult 4th, 2018.
Experiment: Transformation of 7A, plate 3 (Felipe and Lucas)
Heat shock

----------x----------

Date: July 4th, 2018.
Experiment: Miniprep of 07/03 inoculum. (?)

----------x----------

Date: July 10th, 2018.
Experiment: Plasmid digestion (Felipe)
Plasmids and enzymes:

3O	17L	7A (after purification)
S+P		X+P

Reaction:

Plasmid	2.1 Buff	Enzymes	H2O
20uL	3uL	1uL each	5uL

----------x----------

Date: July 10th, 2018.

Experiment: Inoculum of 13B, 3G and 17L (Felipe, Vinicius's plate)
2mL of LB medium

----------x----------

Date: July 10th, 2018.
Experiment: Ligations 3O + (6M+3F) and 17L + 7A
Reaction:

Insert	Vector	Buffer	Ligase	H2O
10uL	1,5uL	2uL	1uL	5,5uL

Transformation of ligations above and 19P from plate 1 of 2017.

----------x----------

Date: July 10th, 2018.
Experiment: Inoculum of colonies from 06/28
1N+ (7N+3F) and 9A + 3F
Colonies 7-12

----------x----------

Date: July 10th, 2018.
Experiment: Miniprep of 23B and 3G plasmids

----------x----------

Date: 12th July, 2018.
Experiment: PCR of previous inoculum (07/10)
9A + 3F and 1N +(7N+3F)
Reaction:

DNA	Forward	Reverse	dNTPs	Buffer	MgCl2	Taq	H2O
1uL	0,5uL	0,5uL	0,5uL	2,5uL	0,75uL	0,1uL	19uL

Results/Discussion: No results

----------x----------

Date: 12th July, 2018.
Experiment: Inoculum of transformations 17L+7A and 3O + (6M+3F)

----------x----------

Date: July 12th, 2018.
Experiment: Plasmid digestion
Digestion:

1N	7N+3F	9A	3F
S+P	X+P	E+S	E+X

Left at 37°C overnight

----------x----------

Date: July 13th, 2018.
Experiment: PCR of 07/12 inoculum (Felipe)
Reaction:

DNA	Forward	Reverse	dNTPs	Buffer	MgCl2	Taq	H2O
1uL	0,5uL	0,5uL	0,5uL	2,5uL	0,75uL	0,1uL	19uL

Gel:

Ladder	Neg. Control	1	2	3	4	5	6
		17L+7A					

Ladder	Positiv control	7	8	9	10	11	12
		3O+(6N+3F)					

----------x----------

Date: July 13th, 2018.
Experiment: Ligation and transformation of 9A + 3F and 1N+ (7N+3F)

Vector	Insert	Ligase	H2O	Buffer
1,5uL	10uL	1uL	5,5uL	2uL

----------x----------

Date: ?
Experiment: ?
Results/Discussion
Next Steps:

----------x----------

Date: July 17th, 2018

Experient: Colony PCR and Inoculum

Results/Discussion:

Sample ID/name	Expected Size	Size on gel
19P, PL1 (RBS+LuxS+TT)	~800	**800 - O.K.**
1N+(7N+3F) (RBS+RhIS+TT)	~800	~900 (Inconclusive)
9A+3F (RBS+CinR+TT)	~950	~950 from colony 2 (Inconclusive)
3O+(6M+3F) (P+RBS+LasR+TT)	~900	~900 (Inconclusive)
Negative control	None	**~900 (Contamination)**

Next Steps:

- Make another primer solution
- Miniprep 19P, 1N+(7N+3F), 9A+3F and 3O+(6M+3F)
- Digest 1N+(7N+3F), 9A+3F and 3O+(6M+3F) with NotI.

Date: July 18th, 2018

Experiment: Miniprep and Digestion (NotI)

Results/Discussion:
19P, PL1 (RBS+LuxS+TT): - Confirmed!
9A+3F (RBS+CinR+TT): ~200kb (Terminator only, repeat ligation)
1N+(7N+3F) (RBS+RhIS+TT): No plasmid (repeat).
3O+(6M+3F) (P+RBS+LasR+TT): - only 2000kb band (repeat).

Next Steps: Repeat ligations 1N+(7N+3F), 9A+3F and 3O+(6M+3F).
Date: July 19th
Experiment: Digestion 19P, 7A+17L, pLas and pRSFDuet
Results/Discussion:

Digestions were successful. All bands could be visualized in the gel with the expected size.
Problem: pRSFDuet had low concentration. Miniprep another culture.

Next Steps:

Miniprep: pRSFDuet
Digest: psb1K3
Ligation and transformation: (19P+7A+17L+psB1K3) and (pLAS+19K,7C+pRSFDuet)

----------x----------

Date: August 2nd, 2018.
Experiment: Miniprep of 07/31 inoculum
Digestion and purification:

DNA	Buffer	Enzymes	H2O
20uL	3uL	1uL each	5uL

9A	6M+3F	7N+3F
E+S	X+P	

----------x----------

Date: August 2nd, 2018
Experiment: Ligation and transformation 9A+3F (Felipe)
----------x----------

Date: August 2nd, 2018

Experiment: Digestion pLux, (13B, PL3), pTra, pRpa and (3G, PL3) with EcoRI and SpeI. Ligation and Transformation
Results/Discussion: pLux, pTra, pRpa and (3G, PL3) were successfully digested. There was no plasmid on 13B, PL3 sample.
Next Steps: Repeat digestion of another 13B, PL3 Sample with EcoRI and SpeI.
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Date: August 7th, 2018.
Experiment: PCR of gblocks CinSR; TraSR; RpaSR and (21J+3F) (Felipe and Caio)
Results/Discussion:

Just positive control and 21J worked. One minute of extension.

----------x----------

Date: August 7th, 2018.
Experiment: Transformation of 3A promoters assembly (Guilherme and Caio)
Parts: pLux, pRhI, pCin, pTra and pRpa

----------x----------

Date: August 8th, 2018.
Experiment: Inoculum of transformed colonies at 08/07 (Caio)
Three colonies each

----------x----------

Date: August 8th, 2018.
Experiment: 3A assembly of (Las+LasR), (RhlS + RhlR) (Guilherme and Felipe)

----------x----------

Date: 09th August, 2018.
Experiment: PCR of 09/08 inoculum (Caio)
pCin and pTra successful
Results/Discussion: pCin and pTra successful

----------x----------

Date: 9th August, 2018.
Experiment: 3A assembly (Guilherme)
Parts:CinSR+(9A+3F); TrasR+pBAD);RpaSR+pBAD

----------x----------

Date: 9th August, 2018.
Experiment: PCR of 08/07 transformations (promoters) (Caio)
Only pCin and pTra heve worked. More colonies from other promoter transformations to be re-strained.

----------x----------

Date: 10th August, 2018.
Experiment: PCR of yesterday's re-strains (Guilherme)
Only pCin and pTra heve worked. More colonies from other promoter transformations to be re-strained.

----------x----------

Date: August 13th, 2018.
Experiment: Digestion (XbaI ans PstI) of 19K+7C to try and ligate promoters again.
Posterior 3A assembly of pRhI+(19K+7C)+pRSF, RhlS+RhlR+pSB1K3

----------x----------

Date: August 15th, 2018.
Experiment: Colony PCR of gBlock transformations TraSR and RpaSR and pRhI
Experiment: Miniprep and analytical digestion of LasSR, RhlSR, pLux and pRpa
Experiment: Co-transformation of LuxSR, LasSR, pSB1A3 w/ pLux (Amp+Kan)

----------x----------

Date: August 28th, 2018.

Experiment: Plate reader test with control parts (EYFP/ECFP). Left growing overnight in 2mL LB (50mg/mL chloramphenicol). Tested with and without IPTG and measured 100uL in a black, clear bottom greiner 96-well plate. Excitation/Emission wavelengths used were 439-476 and 514-527

	OD600	CFP	YFP(e^4)
19K	3,7	377,41	5,3
14J, pl3	3,9	901,36	7
7C	4,1	448,2	7,3
19K+7C	3,5	443,44	6,1
19K (IPTG)	-	455,78	2,9
14J, pl3 (IPTG)	-	1350,4	9,3
7C (IPTG)	-	741,53	3,3
19K+7C (IPTG)	-	700,51	6,2

Expected results for CFP, as only 19K should not present constitutive CFP expression. YFP results too variable, but some difference perceptible in inducing 14J for YFP expression.

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Date: August 29th, 2018.
Experiment: Co-transformation plates slathered with arabinose to check for naked-eye fluorescence.

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Date: September 12th, 2018.
Experiment: Haven't been documenting our lab work, but already started doing assays with all promoters (except for pRhl). Assay here with 2mL LB in 15mL tubes, 10mM arabinose, measuring fluorescence after 12h. Excitation/Emission wavelengths used were 439-476 and 514-527
Results: Inconsistent values of YFP/CFP. Cannot discern controls from tests, despite the clear difference when seeing fluorescence with naked eye.

----------x----------

Date: September 22th, 2018.
Experiment: Assay with Lux Sender and all promoters with 2mL LB in 15mL tubes, 10mM arabinose, measuring fluorescence every 3h then overnight. Excitation/Emission wavelengths used were 439-476 and 514-527

Results: Fluorescence just clearly visibly different from LB after 24h. Inconsistent values of YFP/CFP at the end, as we cannot discern controls from tests, despite the clear difference when seeing fluorescence with naked eye.

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Date: September 25th, 2018.

Experiment: Tested the same as 09/22, this time in a different plate reader, and with different dilutions to check if we can induce at a state where cells would be more visible.

Results: Fluorescence still just clearly visibly different from LB after 24h. Inconsistent values of YFP/CFP at the end, as we cannot discern controls from tests, despite the clear difference when seeing fluorescence with naked eye. We'll try getting rid of LB in further assays.

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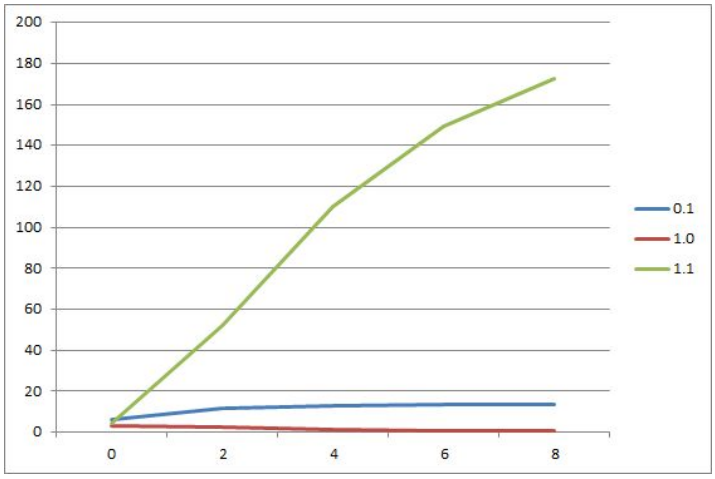
Date: September 26th, 2018.

Experiment: Assay with Lux Sender and Lux promoter with 2mL LB in 15mL tubes, 2,5mM arabinose, measuring fluorescence every 2h. Excitation/Emission wavelengths used were 430-480 and 500-530, same as litarature, and with different dilutions to check if we can induce at a state where cells would be more visible. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.

Results: Much better results. Small wavelength separation was really messing our measurements, and CFP control is now much more discernable from blank autofluorescence. Control measurements were also constant and reasonable. Clear induction by arabinose seeable, except for when inducing at high OD. Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver

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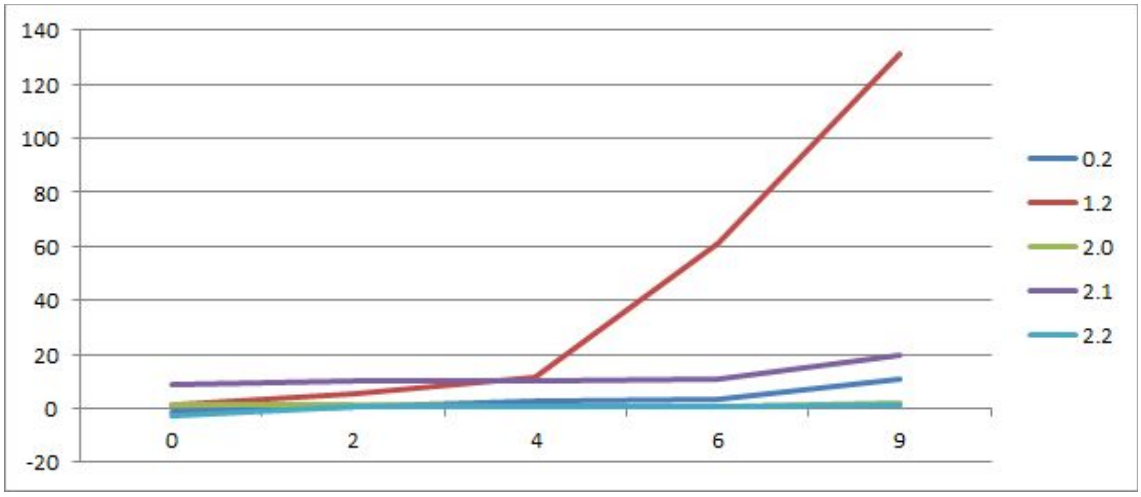
Date: September 27th, 2018.

Experiment: Assay with Lux and Las Senders and Las promoter with 2mL LB in 15mL tubes, 2,5mM arabinose, measuring fluorescence every 2h. Excitation/Emission

wavelengths used were 430-480 and 500-530. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.
Results: Good induction for Las promoter. Couldn't activate it with Las Sender though, something must be wrong with that construct. Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Sender/Receiver

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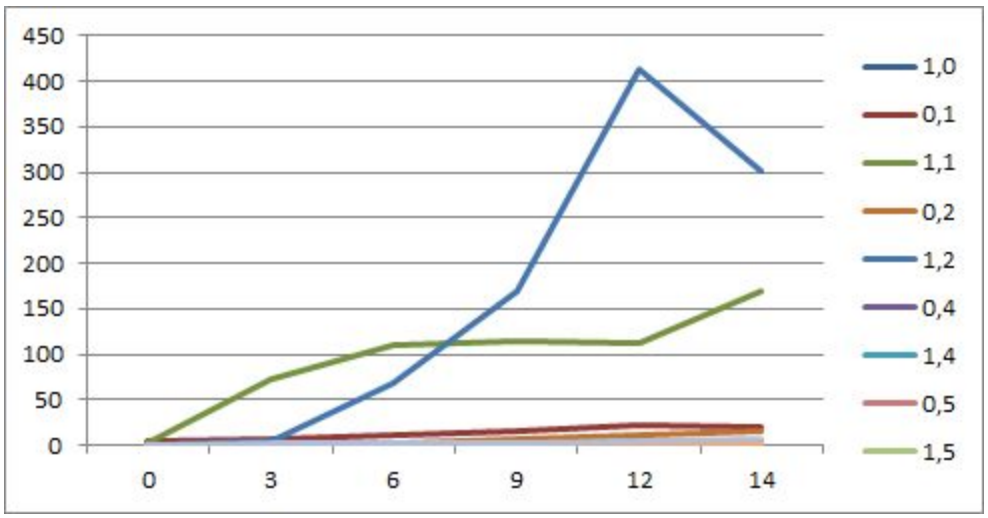
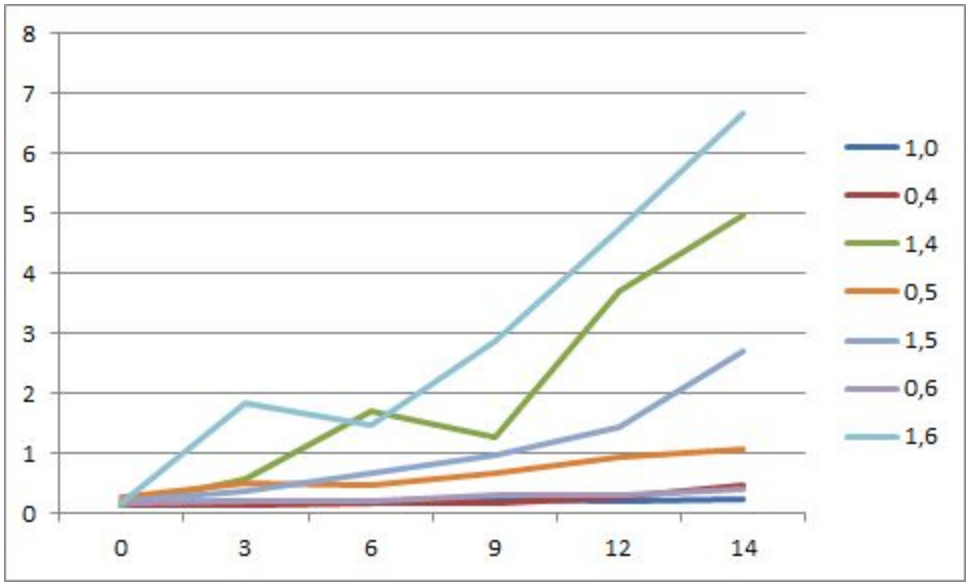
Date: September 30th, 2018.
Experiment: Assay with Lux Sender and every promoter but pRhl with 2mL LB in 24-well plate, 2,5mM arabinose, measuring fluorescence every 3h. Excitation/Emission wavelengths used were 430-480 and 500-530. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.
Results: Good induction with pLux and pLas, as expected, and weak expression from the others at later stages of the experiment. Graph for YFP/CFP ratio:





Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Receiver, 4: Cin Receiver, 5: Tra Receiver, 6: Rpa Receiver

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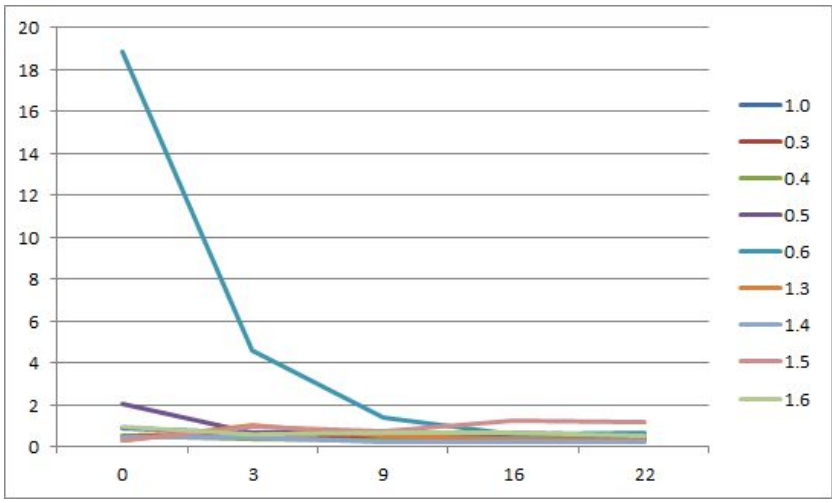
Date: September 30th, 2018.

Experiment: Assay with Lux Sender and every promoter but pLux and pLas with 2mL LB in 15mL tubes, 2,5mM arabinose, measuring fluorescence every 3h. Excitation/Emission wavelengths used were 430-480 and 500-530. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.

Results: No activity at all. Must be because of aeration condition differences between 24-well plates and 15mL tubes. 0.6 had big fluorescence in the beggining, but we attribute this to wells not being clean, as this doesn't even have a receptor protein.

Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Receiver, 3: Rhl Receiver, 4: Cin Receiver, 5: Tra Receiver, 6: Rpa Receiver

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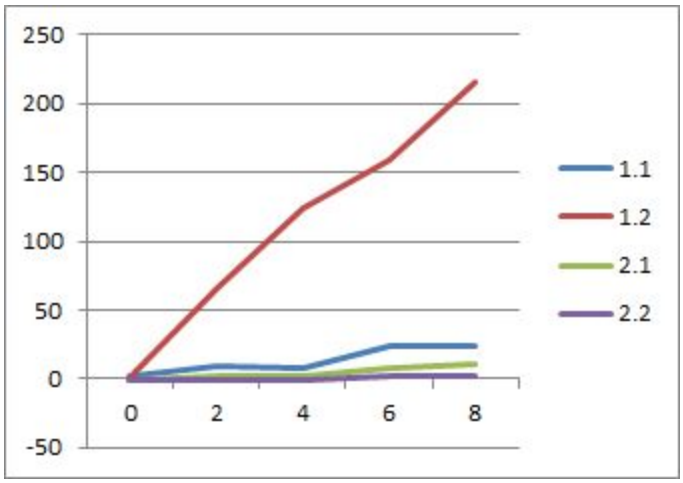
Date: October 10th, 2018.

Experiment: As the dusseldorf collaboration arrived, we are testing their synthetic 3OC6 HSL. Assay with Lux Sender and Lux, Las promoters with 2mL LB, 10^{-4} mM 3OC6 HSL, measuring fluorescence every 2h. Excitation/Emission wavelengths used were 430-480 and 500-530. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.

Results: Weaker induction of pLux and pLas. We should use this alongside with arabinose induction to be able to compare results. Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Receiver, 4: Cin Receiver, 5: Tra Receiver, 6: Rpa Receiver

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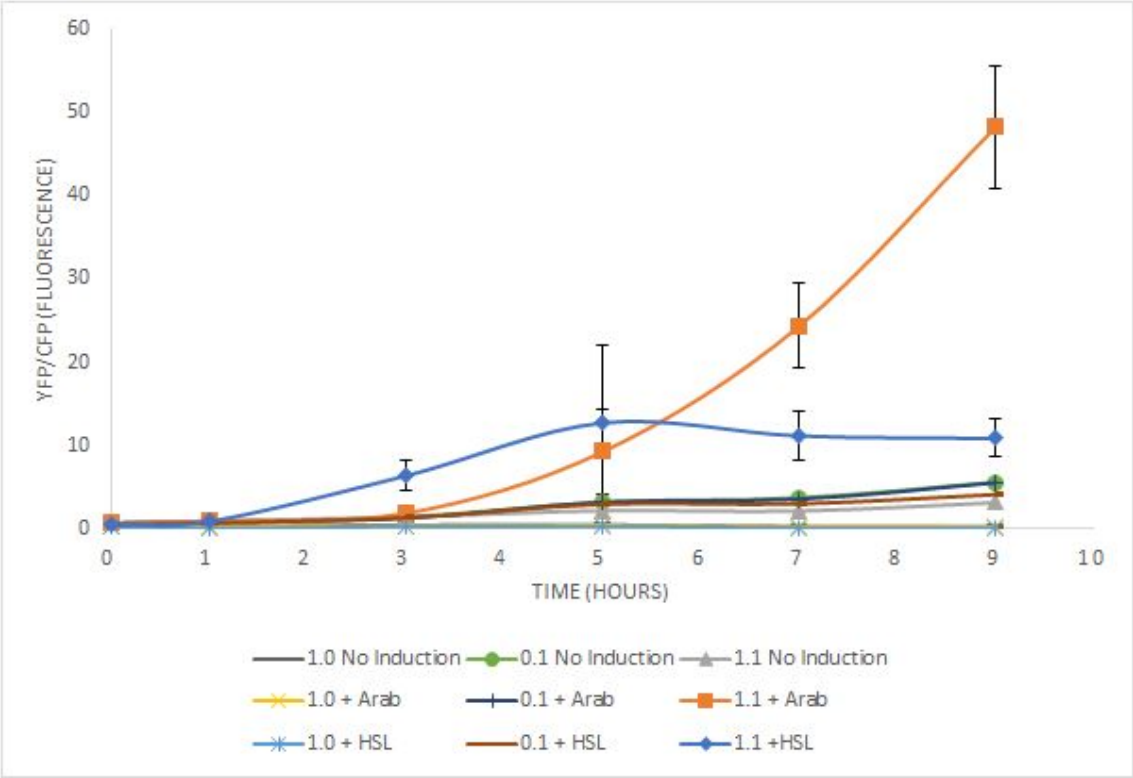
Date: October 10th, 2018.

Experiment: Assay with Lux Sender and Lux promoter with 2mL M9, 2,5mM arabinose or 10^{-4} mM 3OC6 HSL, measuring fluorescence every 2h. Excitation/Emission wavelengths used were 430-480 and 500-530. Cells grown in M9 media to test for blank autofluorescence and possibility of a kinetic experiment with shorter measurement intervals.

Results: Weaker and slower induction of pLux, and with the synthase-induced response stronger than only using synthetic HSL. Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Receiver, 4: Cin Receiver, 5: Tra Receiver, 6: Rpa Receiver

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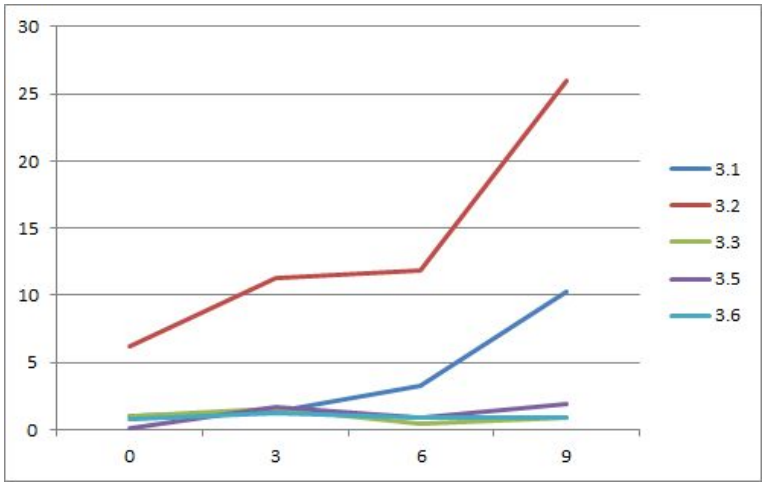
Date: October 10th, 2018.

Experiment: Assay with Rhl Sender and all promoters except of pCin (didn't grow) with 2mL LB, 2,5mM arabinose, measuring fluorescence every 2h. Excitation/Emission wavelengths used were 430-480 and 500-530. Also centrifuged cells after sampling and resuspended them in H2O, to clear LB autofluorescence.

Results: Good pLux and pLas activity, weaker than with Lux Sender. Graph for YFP/CFP ratio:



Being 0: empty Sender/Receiver plasmid, 1: Lux Sender/Receiver, 2: Las Receiver, 3: Rhl Sender/Receiver 4: Cin Receiver, 5: Tra Receiver, 6: Rpa Receiver

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