PRINTERIA

Notebook

Part adaptation

THURSDAY 7/6

We made a liquid culture of the BBa_P10500 to purify it the following day.

FRIDAY 8/6

Miniprep results for BBa_P10500:

IG	Name	260/280	DNA Conc. ng/μL	DNA amount μg
12	BBa_P10500	1,98	143,827	7,19135

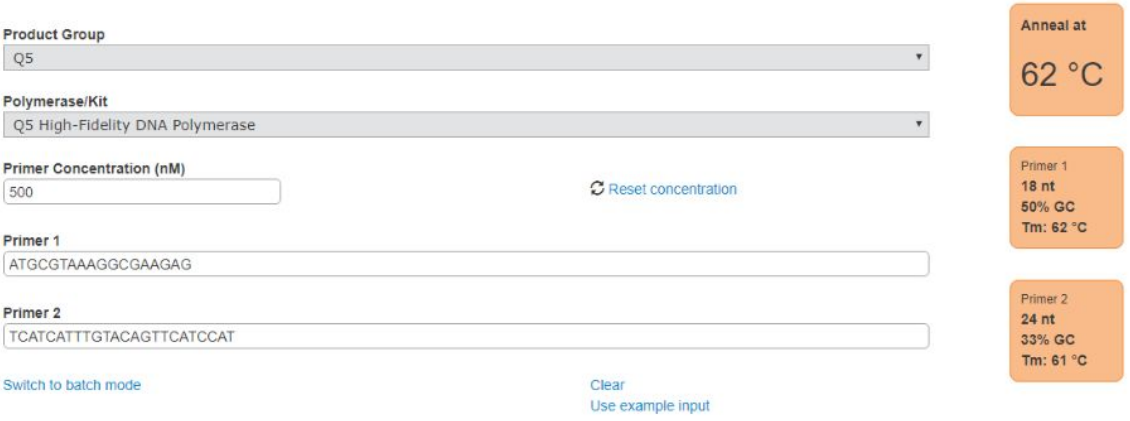
Primer design for GFP Superfolder (BBa_K2656013)

Oligo forward: GCGCCGTCTCGCTCGAATGCGTAAAGGCGAAGAG

Oligo reverse: GCGCCGTCTCGCTCAAAGCTCATCATTTGTACAGTTCATCCAT

Sequence from BBa_I746908 , insert 720bp.

In all the designed primers we added the proper prefixes and suffixes to make the parts Golden Gate compatible.

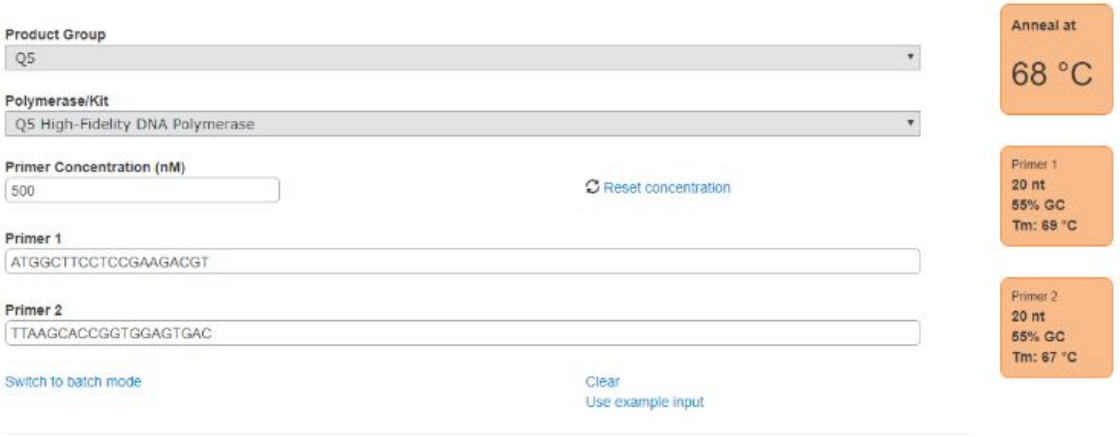


Primer design for mRFP (BBa_K2656014)

Oligo forward: GCGCCGTCTCGCTCGAATGGCTTCCTCCGAAGACGT

Oligo reverse: GCGCCGTCTCGCTCAAAGCTTAAGCACCGGTGGAGTGAC

Plasmid pYB010tc , insert: 675bp



WEDNESDAY 13/6

In the lab we had CDSs for the mRFP and the sfGFP, so we used primers to get them.

Data for the PCR of sfGFP y mRFP:

Tm 2	Anneal temp	Template	PCR Product	Extension Time Calculated
61	62	pSB1C3-I746908 sfGFP	755	45 seg
67	68	pYB010tc mRFP	713	45 seg

Thermocycler program for sfGFP

PCR	**1**		
Step	**Temperature**	**Time**	
Hot Start	98°C		
Initial denaturation	98°C	30 secs	
35 Cycles			
Denatuation	98°C	30 secs	
Anneal	62°C	30 secs	(62,1)
Extension	72°C	45 secs	30sec/kb
Final extension	72°C	5 minutes	
Hold	4-10°C		

Program for mRFP

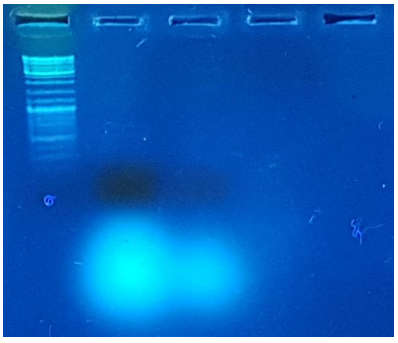
PCR	**2**		
Step	**Temperature**	**Time**	
Hot Start	98°C		
Initial denaturation	98°C	30 secs	
35 Cycles			
Denatuation	98°C	30 secs	
Anneal	68 °C	30 secs	(67,9)
Extension	72°C	45 secs	30sec/kb
Final extension	72°C	5 minutes	
Hold	4-10°C		

Td=65,8, G=5,5

- We have added a 5'UTR region to the RBS in benchling between the RBS and the ATG in order to improve translations.

THURSDAY 14/6

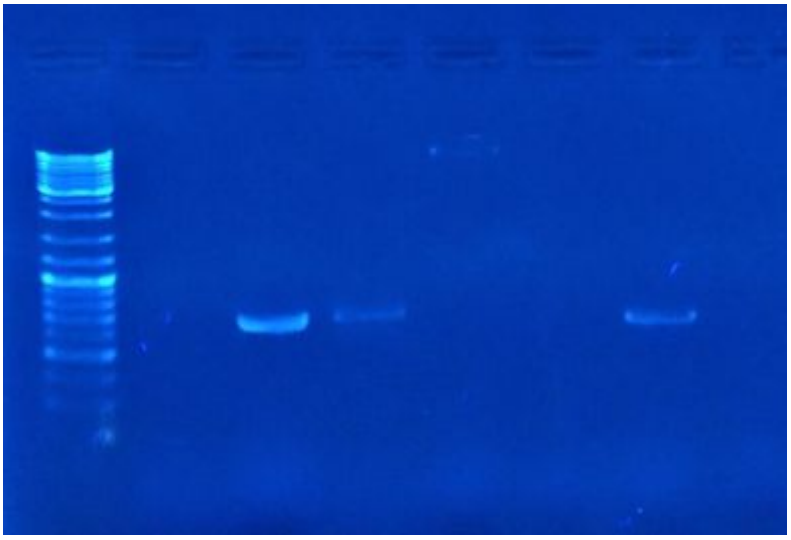
Electrophoresis results for the PCR: empty



We repeat the pcr:

For the repetition we will use other templates and other reaction conditions

Templates used to amplify:Gel: Ladder, control 1, 1.1 (I746903), 1.2 pSB1C3-I746908 (previous PCR), pGreen alpha 2, control 2, 2.1 (pCB9ta-mRFP) 2.2(PCB9ta E/S-mRFP)



Golden Gate reaction to assembly sfGFP and mRFP into BBa_P10500

FRIDAY 15/6

- We have changed the sequence of BBa_K592101 in order to avoid a restriction site for BsaI

- Electroporation: C -, 2 C+, BBa_P10500 with sfGFP, BBa_P10500 with mRFP, and plate culture

MONDAY 18/6

Minipreps of K2656013 (sfGFP) and K2656014(mRFP). DNA concentration for pUPD with mRFP and sfGFP after Miniprep.

Name	260/280	ng/µL	µg
K2656014 3	1,945	60.89	3,0445
K2656014 3	2,001	70.253	3,51265
K2656014 3	1,956	50.475	2,52375
K2656013 1	2,091	39.958	1,9979
K2656013 2	2,011	61.882	3,0941
K2656013 3	1,956	60.089	3,00445

Colony PCR

TUESDAY 19/6

- Electrophoresis sfGFP PCR

It did not work; there are no bands (only the ladder)

Electrophoresis mRFP PCR

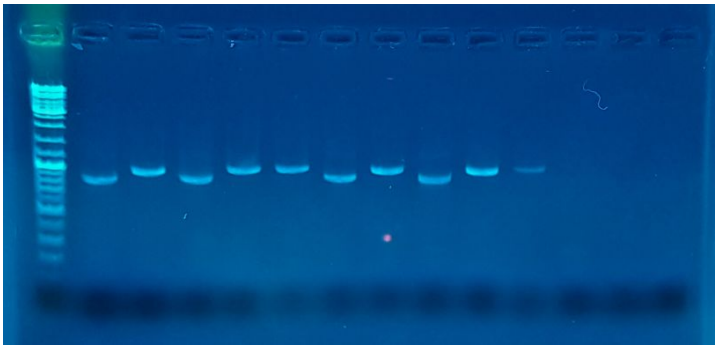
Did not work.

They both need to be repeated.

WEDNESDAY 20/6

- Electrophoresis sfGFP:

1: Ladder, 2-8: 1-7 sfGFP colony PCR, 9-11: sfGFP plasmid PCR, 12: C+ BBa_P10500 , 13: C+ Ale's plasmid idk xdd, 14: C-

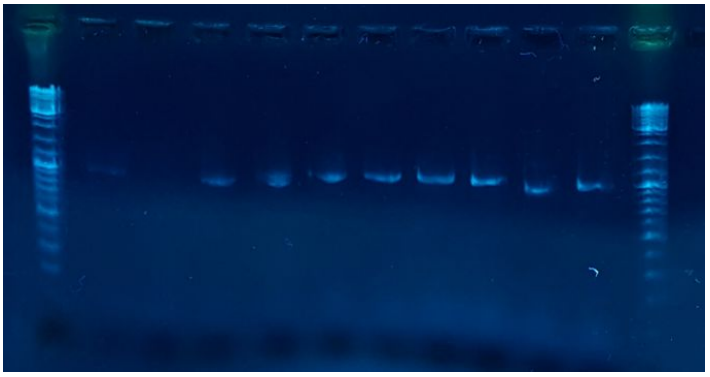


We find sfGFP in colonies number 1, 3 and 6.

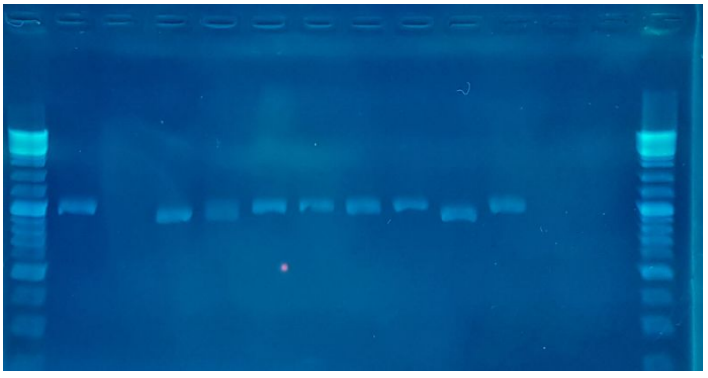
We choose to use **number 1 from plasmid and number 3 from colony**

Electrophoresis mRFP:

1: Ladder, 2-8: 1-7 sfGFP colony PCR, 9-11: sfGFP plasmid PCR, 12: ladder



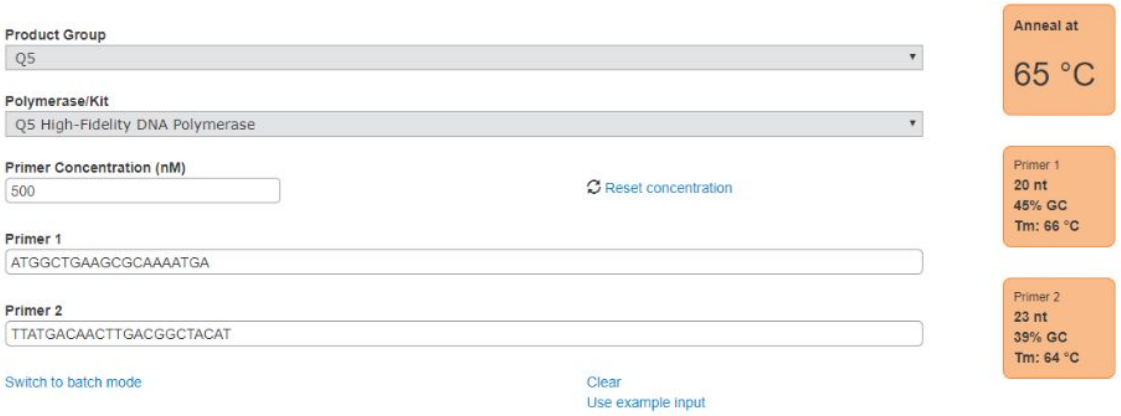
We are repeating this electrophoresis with an agarose gel with more agarose concentration (1.5%)



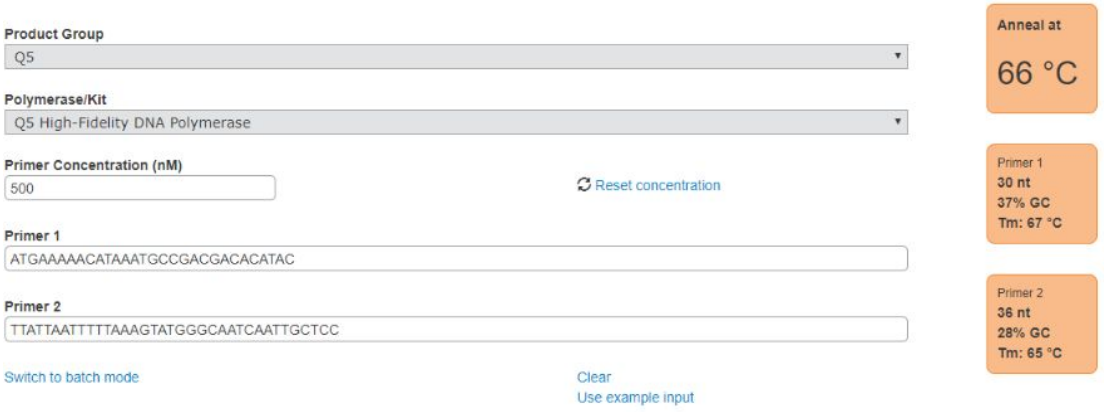
We choose to use **number 2 from plasmid** and **number 3 from colony.**

-Stock has been made for GFP1 and mRFP2. GFP3 and mRFP3 has been inoculated in liquid medium.

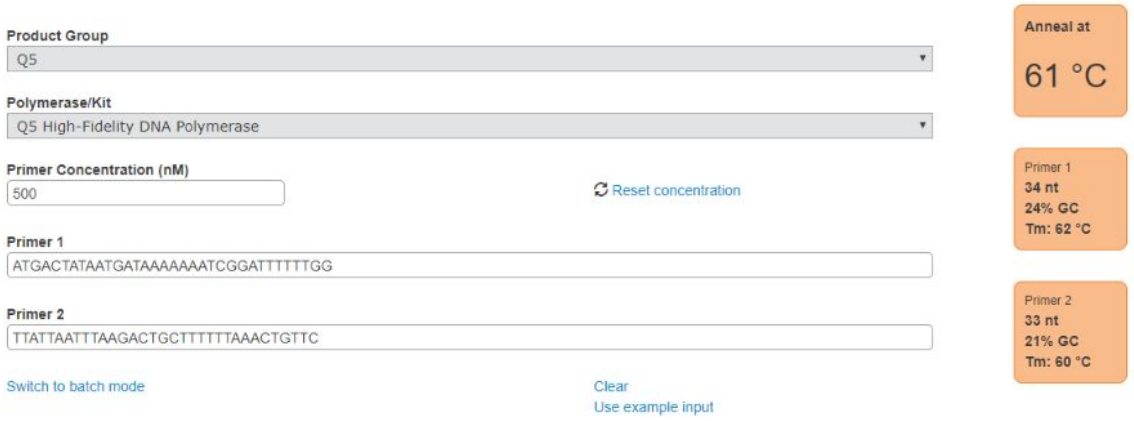
Primer design for BBa_K2442103, from pSB1C3-I746908 (sfGFP)



Primer design for BBa_C0062 from pCB2tc (luxR)



Primer design for BBa_C0161 from pYB06k30 (luxI)



Purification of plasmids from the colonies used in the colony pcr (sfGFP and mRFP):

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	**ng/µL**
sfGFP	0,09	0,069	0,043	0,035	0,018	1,939	**34,681**

mRFP	0,07	0,056	0,038	0,02	0,01	2,051	**19,944**

MONDAY25/6

Golden Gate reactions for cloning the gBlocks into BBa_P10500 vectors these parts:

J23106	**J61100**	**J23101**	**J61101**	**I179005**	**B0034**

Miniprep for BBa_P10500:

IG	Name	260/280	DNA Conc. ng/µL	**DNA amount µg**
12	BBa_P10500	1,98	143.827	**7,19**

TUESDAY 26/6

PCR for amplifying araC, Lux R and Lux I
Transformation of the Golden Gate results from the past day with electroporation.

WEDNESDAY 27/6

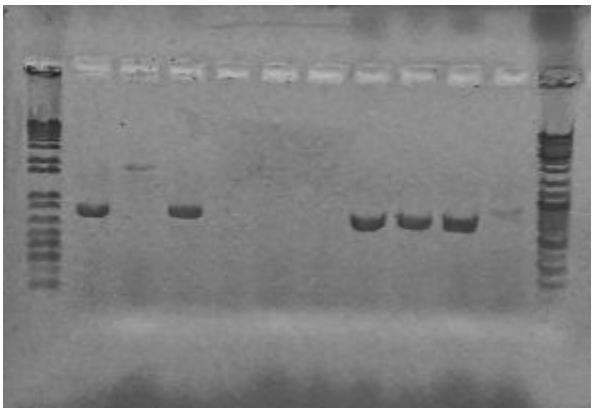
None of the PCRs worked. We repeat them

PCR cycles:

PCR	**2**		
Step	**Temperature**	**Time**	
Hot Start	98°C		
Initial denaturation	98°C	30 seconds	
35 Cycles			
Denatuation	98°C	10 seconds	
Anneal	68 °C	30 seconds	Td=64.3
Extension	72°C	1 min	1seg/kb
Final extension	72°C	5 minutes	
Hold	4-10°C		

GRADIENT:
Desired temperature 64.3°C
Grad: 4.5°C
Arac=1 LuxI=2 LuxR=3
Columns: 5, 2 and 8 respectively
64.7°C 61°C 65.9°C

Results: 1:ladder; 2: araC Normal; 3: araC DSMO; 4: araC Enhancer; 5: luxI Normal; 6: luxI DSMO; 7: luxI Enhancer; 8: luxR Normal; 9: luxR DSMO; 10: luxR Enhancer; 11: C-



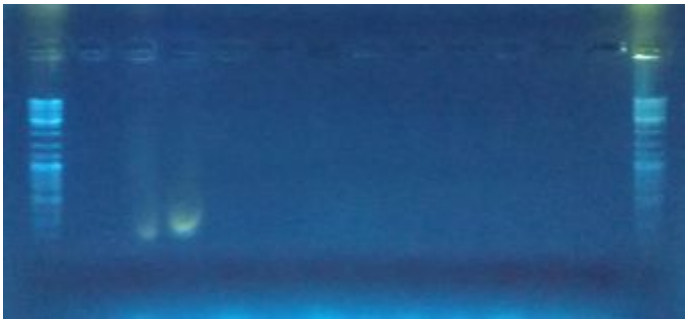
Purification before using in Golden Gate reaction:

Name	260/280	ng/µL	ug
AraC	1,921	39.982	**1.19946**
AraC GC enhanced	1,898	30.465	**0.91395**
LuxR DSMO	1,771	23.285	**0.69855**
LuxR GC enhanced	1,879	33.091	**0.99273**

We will use AraC and LuxR GC enhanced
We will repeat the LuxI PCR

Transformation results from the previous day:
Only B0034 has positive results. The others need to be transformed again. Colony PCR for corroborating this positive result:

Colony PCR
Electrophoresis gel:



We grow in liquid medium colonies 2 and 3

Assembly of gBlocks and PCR products into BBa_P10500

THURSDAY 28/6

Transofrmation of: J23106, J61100, J23101, J61101, I79005, BBa_K592101, BBa_K592009, BBa_K338001, BBa_R0061, BBa_R0062, ACY07122.1, B0015, B0032, B0030, AraC, LuxR adapted into BBa_P10500

Miniprep and DNA concentration for previous colonies 2 and 3 of B0034 adapted into BBa_P10500

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
Colony 2	0,102	0,075	0,043	0,044	0,022	1,936	**43,549**
Colony 3	0,106	0,075	0,04	0,051	0,026	1,979	**51,107**

Repetition of the LuxI PCR with DMSO and other templates

FRIDAY 29/6

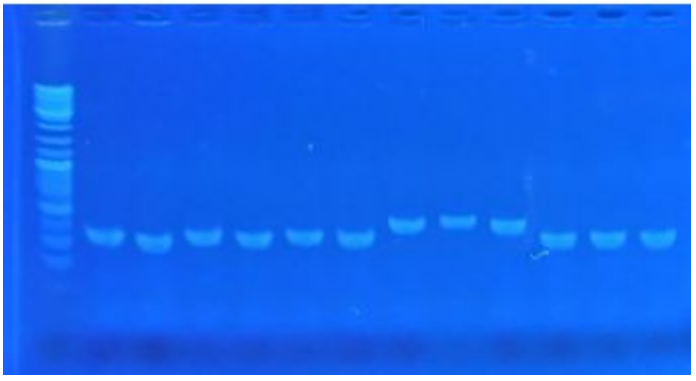
Transformation results:
LuxR, AraC, I79005, B0032; K338001, J61101, K592101, ACY07122.1 adapted into BBa_P10500 are correctly transformed

The other need to be repeated:
Colony PCR:

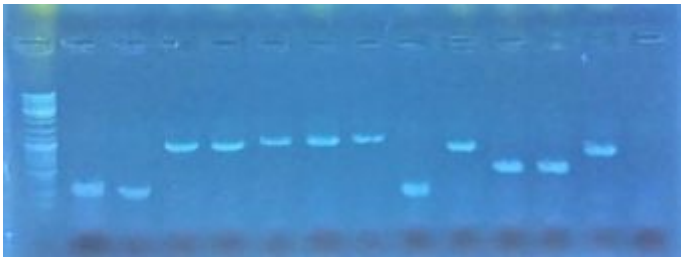
LuxR	AraC	**I79005**	**B0032**	**K338001**	**J61101**	K592101
3	3	3	3	3	3	3

ACY07122.1	B0015	C+ from blue colony	C-	Blue colony from J61101 50ul plate	C+ from BBa_P10500
3	1	1	1	1	1

Results:
1: ladder
2-4: I79005
5-7: B0032
8-10: K338001
11-13: J61101



1: ladder, 2:B0015, 3-5: ACY07122.1,6-8:LuxR, 9-11:AraC, 12:c+, 13: c-



SATURDAY 30/6

Purification and stock of:

	BBa_K2656016	BBa_K2656017	BBa_K2656000	pACA4	BBa_K2656001	pACA6	pACA7	BBa_K2656016	BBa_K2656026
	LuxR	AraC	I79005	B0032	K338001	J61101	K592101	ACY07122.1	B0015
colonies:	2 and 3	1 and 2	2 and 3	1 and 2	1 and 3	1 and 2	2 and 3	1 and 2	1

-Colony PCR of plates B0034 (in this case, PCR using the domesticated BBa_P10500), R0015 and R0061.

-Transformation and growth in solid medium of BBa_P10500 K592009, R0062 and B0030

SUNDAY 1/7

Plates re-plated from the previous GB reaction grew well.

We picked 3 colonies from each for Colony PCR with the following numbering:

K592009 is #2 ~(1050bp)
R0062 is # 5 (~370bp)
B0030 is #9 (~360bp)

3 colonies from each plus one negative control

We will colony pcr only R0062 and B0030, they have the same length. (25sec of extension time, the rest of the parameters the same). Colonies from yesterday (R0061dom and B0015dom) grew well, they are in the fridge to do stock and miniprep on Monday.

MONDAY 2/7

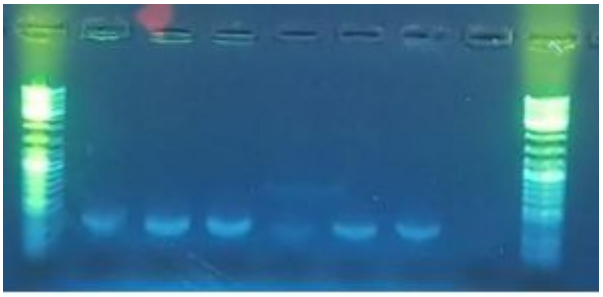
Plasmids purifications from Saturday:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656000.1	0,128	0,096	0,051	0,065	0,039	1,681	**65,384**
BBa_K2656000.2	0,114	0,092	0,071	0,031	0,015	2,107	**31,312**
pACA4.1	0,156	0,114	0,059	0,086	0,049	1,749	**85,66**
pACA4.2	0,12	0,085	0,048	0,06	0,03	1,987	**60,115**
BBa_K2656001.1	0,1	0,086	0,07	0,018	0,009	2,092	**18,113**

BBa_K2656001.2	0,101	0,072	0,041	0,048	0,025	1,94	**48,18**
pACA6.1	0,114	0,08	0,04	0,062	0,033	1,906	**62,326**
pACA6.2	0,112	0,089	0,055	0,045	0,027	1,674	**45,248**
pACA7.1	0,188	0,126	0,048	0,126	0,07	1,793	**126,353**
pACA7.2	0,103	0,074	0,041	0,049	0,027	1,859	**49,261**
BBa_K2656016.1	0,138	0,096	0,052	0,073	0,036	2,014	**72,718**
BBa_K2656016.2	0,085	0,067	0,046	0,027	0,015	1,827	**26,905**
BBa_K2656026	0,101	0,075	0,042	0,046	0,026	1,769	**45,793**
BBa_K2656016.1	0,081	0,065	0,044	0,027	0,013	2,008	**26,899**
BBa_K2656016.2	0,111	0,083	0,045	0,054	0,03	1,806	**54,4**
BBa_K2656017.1	0,153	0,102	0,042	0,1	0,052	1,921	**99,821**
BBa_K2656017.2	0,127	0,092	0,05	0,065	0,035	1,877	**64,959**
BBa_P10500	0,086	0,067	0,046	0,029	0,014	2,086	**29,061**

Electrophoresis gel for: R0062 (5) and B0030 (9)

1: ladder; 2-4: R0062; 5-7: B0030; 10: C-



We choose colonies 2 and 3 for R0062 and 2 and 3 for B0030

DNA conc. for BBa_K2656026 and BBa_K2656002:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656026.2	0,185	0,118	0,044	0,128	0,066	1,931	**127,626**
BBa_K2656002.1	0,176	0,113	0,042	0,123	0,064	1,925	**122,657**

Golden Gate reaction for the assembly of these fragments into BBa_P10500:

J23106	J61100	J23101	BBa_R0061	J23102	pBadminimal

Transformation of:

J23106	J61100	J23101	J23102	pBadminimal	BBa_R0061

TUESDAY 3/7

All transformations went well.

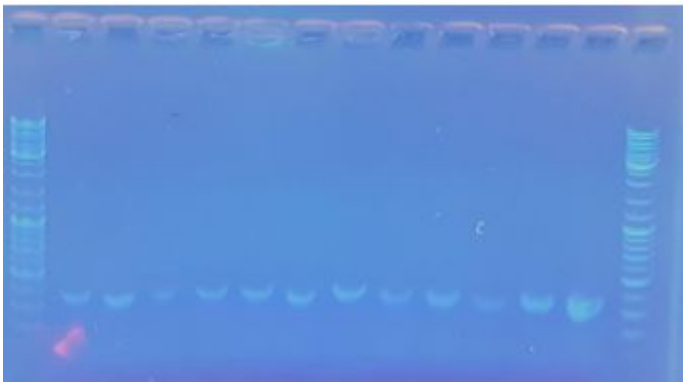
Colony PCR

2 reactions because of the inserts length
1st PCR:

	l (bp)
J23106	387
J23102	390
J61100	373
J23101	390

ext time=25 secs

Electrophoresis gel for the 1st PCR:
Ladder, 1.1, 2.1, 3.1, 5.1, 1.2, 2.2, 3.2, 5.2, 1.3, 2.3, 3.3, 5.3, ladder



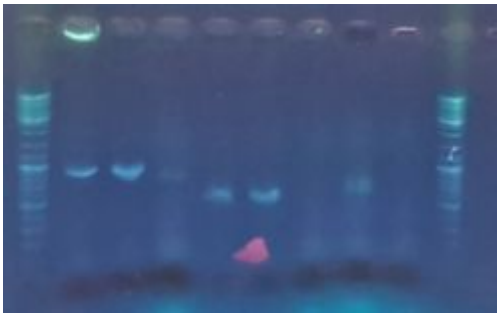
We choose colonies:
1.2 and 1.3; 2.1 and 2.2; 3.2 and 3.3; 5.1 and 5.3

2nd PCR:

	l (bp)
K592009	1050
pBadminal	660

extension time= 1 min

Results:
Ladder, 4.1, 4.2, 4.3, 6.1, 6.2, 6.3, C+, C-,ladder



We choose colonies 4.1, 4.2, 6.1, 6.2 to do miniprep

Purification of BBa_P10500 using 2 different silica columns:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
blue	0,189	0,115	0,044	0,134	0,064	2,088	**134,265**
yellow	0,134	0,088	0,039	0,085	0,043	1,986	**85,162**

Miniprep for BBa_K2656003 and pACA15. DNA conc:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656003.1	0,175	0,11	0,042	0,124	0,061	2,015	**123,613**
BBa_K2656003.2	0,142	0,091	0,04	0,093	0,046	2,04	**93,189**
pACA15.1	0,125	0,084	0,041	0,074	0,036	2,031	**74,105**
pACA15.2	0,158	0,098	0,038	0,11	0,054	2,028	**110,42**

WEDNESDAY 4/7

- Stock made of BBa_K2656003 and pACA15

MONDAY 9/7

Making working disolutions of all the parts in BBa_P10500 (50 ng/uL)

TUESDAY 10/7

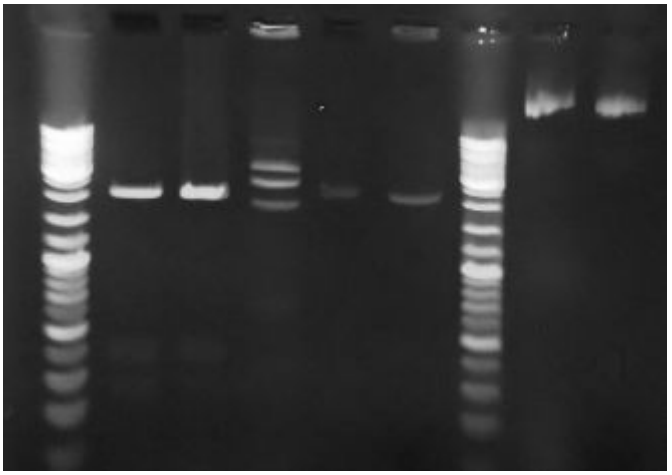
Repeating the minipreps of the parts:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656013	0,157	0,114	0,059	0,08	0,042	1,903	**79,865**
BBa_K2656014	0,197	0,125	0,05	0,13	0,063	2,065	**129,637**
BBa_K2656000	0,23	0,148	0,055	0,156	0,08	1,958	**156,318**
BBa_K2656001	0,175	0,114	0,049	0,109	0,052	2,072	**108,778**
BBa_K2656016	0,209	0,134	0,052	0,139	0,069	2,003	**138,999**
BBa_K2656016	0,11	0,08	0,045	0,048	0,023	2,038	**47,515**
pACA13	0,123	0,096	0,061	0,044	0,023	1,93	**43,861**
BBa_K2656008	0,178	0,114	0,043	0,117	0,059	1,992	**116,709**

Results of sequences 1-15
pACA 1, pACA 2 and pACA 7 are wrong.
pACA 14 and pACA 15 were sent with the opposite names. Check and fix it.

Comprobation restriction analysis with E-P of pACA 1, pACA 2, pACA 7, pACA 14 and pACA 15
Digestion 20min @ 37ºC:
Final Conc 25 ng/uL and final volume 30 uL

Gel for the digestion of BBa_K2656013, BBa_K2656014, pACA7, BBa_K2656003, pACA15



Bands are not ok.

Analysis of sequencing results: pACA18, BBa_K2656006, BBa_K2656007 and pACA21
pACA18 and BBa_K2656006 are ok. pACA 1, pACA 2, pACA 7, pACA 14, pACA 15, pACA 16, pACA 17,BBa_K2656007 and pACA 21 will be resequenced.

WEDNESDAY 11/7

Assembly of BBa_K2656019(LuxI):

Minipreps of BBa_K2656016 and pACA 13:

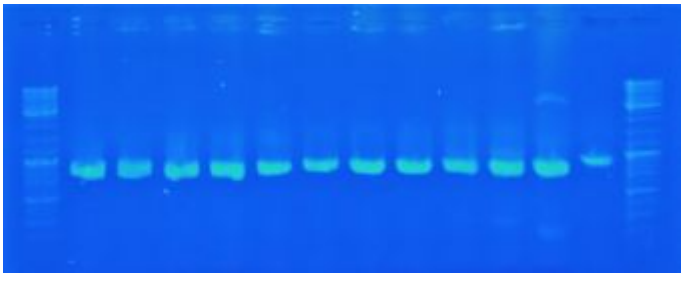
Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656016	0,224	0,135	0,042	0,171	0,087	1,969	**171,027**
pACA13	0,145	0,094	0,040	0,095	0,048	1,988	**95,233**

THURSDAY 12/7

Transformation results:

cPCR BBa_K2656019 (luxI):

Electrophoresis:
Expected size: 924 pb
Ladder, colonies 1 to 10, control + (BBa_P10500), control -, ladder



We will miniprep colonies 1 and 2.

FRIDAY 13/7

Miniprep the tubes that are in the incubator when they are grown appropriately. Stock made.
DNA conc:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656019.1	0,205	0,123	0,042	0,153	0,075	2,046	**153,141**
BBa_K2656019.2	0,17	0,106	0,039	0,122	0,061	1,988	**121,809**

MONDAY 16/7

PCR for mrfp and sfGFP:

Templates

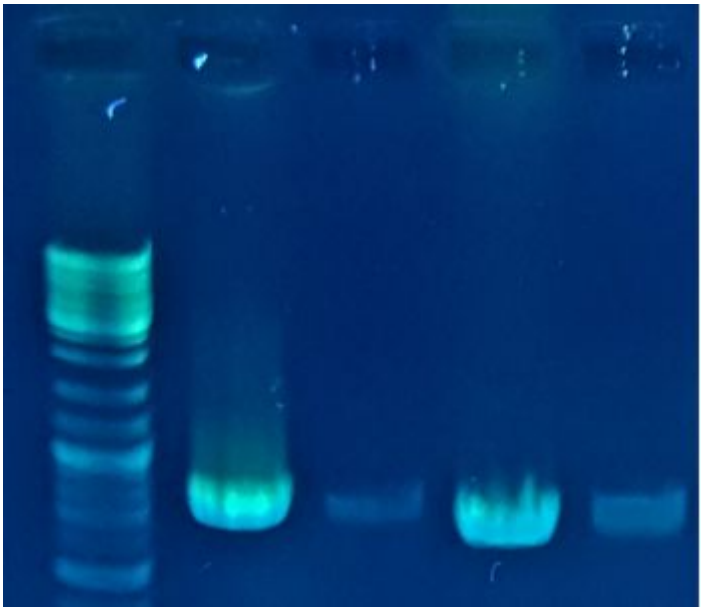
sfGFP	mRFP1
I746903	pCB9ta

Reactions:

number	ID
1	sfGFP
2	C- sfGFP
3	mRFP
4	C- mRFP

Thermocycler: **Td=65,8, G=5,5** Extension time: 45 secs

Results:
1: ladder 2: sfGFP GB domesticated 3:C- sfGFP 4: mRFP GB domesticated 5: C- mRFP



TUESDAY 17/7

Minipreps:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656005.1	0,176	0,113	0,044	0,121	0,062	1,948	**121,383**
BBa_K2656008.1	0,226	0,137	0,041	0,175	0,09	1,95	**175,209**
pACA21.1	0,227	0,138	0,043	0,173	0,089	1,945	**172,533**

sfGFP and mRFP PCR clean up:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
sfGFP	0,172	0,113	0,045	0,116	0,062	1,869	**116,231**
mRFP	0,138	0,094	0,045	0,083	0,044	1,908	**83,067**

GG reaction for BBa_K2656013, BBa_K2656014 (sfGFP and mRFP)

WEDNESDAY 18/7

- Transformation of BBa_K2656013 (sfGFP) and BBa_K2656014 (mRFP) via electroporation (10G electrocompetent strain used).

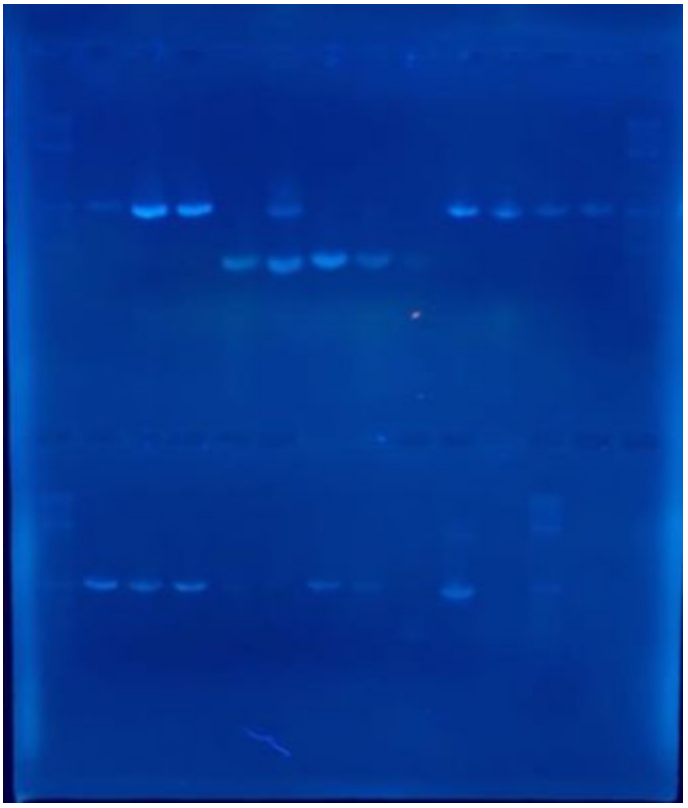
Minipreps of the actual pACA 14, pACA 15 BBa_K2656005 BBa_K2656008 and pACA 1421

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
14.1	0,126	0,085	0,043	0,076	0,039	1,964	**76,164**
14.2	0,123	0,084	0,043	0,072	0,037	1,965	**72,235**
15.1	0,204	0,124	0,039	0,157	0,081	1,948	**156,961**
15.2	0,273	0,173	0,062	0,201	0,105	1,914	**201,305**
17.2	0,203	0,123	0,04	0,154	0,079	1,961	**154,16**
20.2	0,117	0,083	0,043	0,065	0,035	1,872	**65,196**
21.2	0,229	0,139	0,041	0,18	0,094	1,915	**179,523**

THURSDAY 19/7

-cPCR BBa_K2656013 and BBa_K2656014: 10 colonies for each pACA

Electophoresis gel:
1: ladder, 2 to 12: colonies sfGFP 1-10 and mRFP 1 to 2, 13: ladder
1: ladder, 2 to 9: colonies 3 to 10 mRFP, 10: C+ (BBa_P10500), 11: C-, 12: ladder



We choose colonies 1 and 3 (BBa_K2656013) and 3 and 5 (BBa_K2656001) to miniprep

NOTE: NOW BBa_K2656005, BBa_K2656008 and BBa_K26560141 ARE CORRECTLY LABELLED AND READY TO USE

FRIDAY 20/7

Minipreps

Name	260	280	260/280	ng/µL
sfGFP colony 1	0,089	0,044	2,002	**88,882**
sfGFP colony 3	0,053	0,026	2,074	**53,351**
mRFP colony 3	0,169	0,083	2,027	**168,578**
mRFP colony 5	0,131	0,064	2,031	**130,573**

MONDAY 23/7

GG reaction for the Gblocks:

J61100.2
B0030.2
B0032.2
B0034.2
J61101.2
K592101_LVA
K592101
E0040
K082003
mRFP_LVA

Transformation of 10G electrocmpetent cells with 5 uL GB reaction. C+: BBa_P10500. Plate in Cm + Chromomax. 300 uL and 50 uL.

TUESDAY 24/7

C+: blue colonies. C-: there are colonies. Plates for each adapted parr: There are white colonies in all plates, but these plates were not ok because of the incorrect antibiotic spread on the surface the day before and because there was a lawn in each plate (even plating 50 uL). We replated with 30 uL of the transformed cells.

WEDNESDAY 25/7

There are colonies in each plate. We are going to pick them.

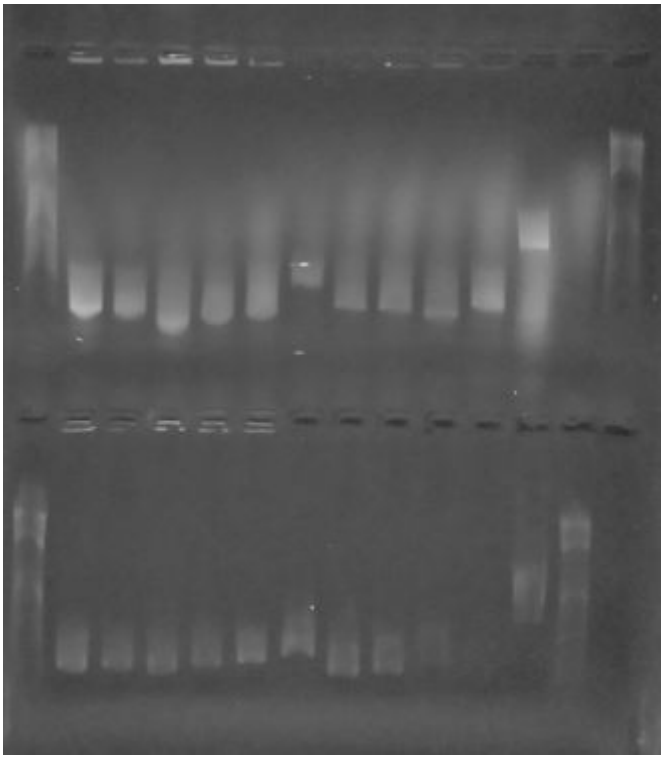
Gblocks domesticated with the GB, transformed and plated:

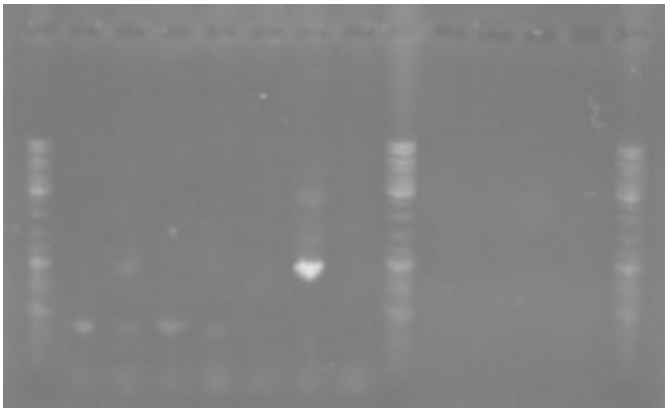
J61100.2
B0030.2
B0032.2
B0034.2
J61101.2
K592101_LVA
K592101
E0040
K082003
mRFP_LVA

1st cPCR (number 1 to 5. RBS). We picked 5 colonies of each gblock.
5*5= 25 + Positive control + negative control
Ext time 30 secs
C+: BBa_P10500. 2 ng pipetting 0.5 uL.

2nd cPCR. 5 colonies of each pACA.
C+: BBa_P10500. 2 ng pippeting 0.5 uL.
Extension time 1 min

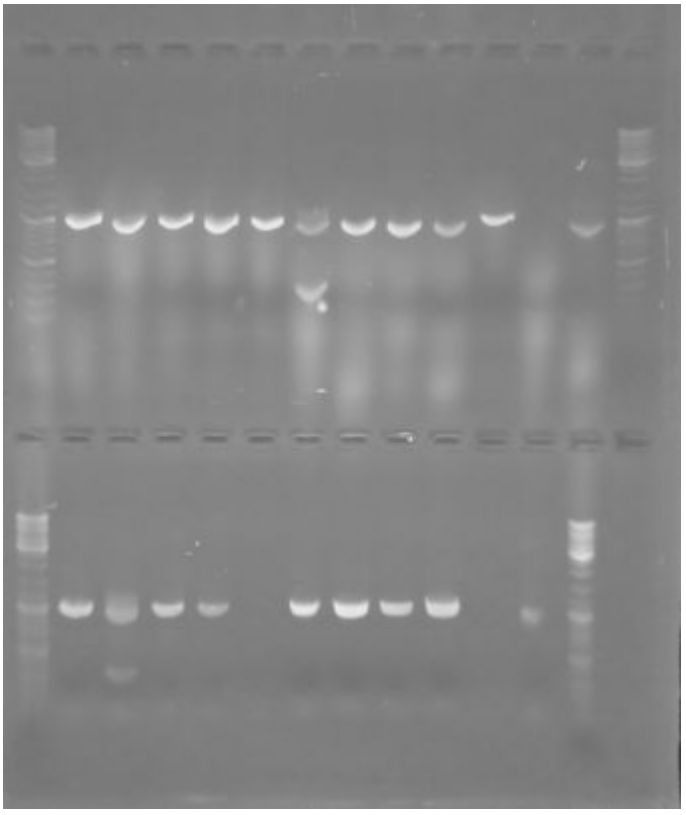
Electrophoresis gel for 1st PCR:
Ladder, J61100.2 (5 colonies), B0030.2 (5 colonies), c+, c-, ladder.
Ladder, B0032.2 (5 colonies), B0034.2 (5 colonies), c+, ladder.
Ladder, J61101.2 (5 colonies), c+, c-, ladder.

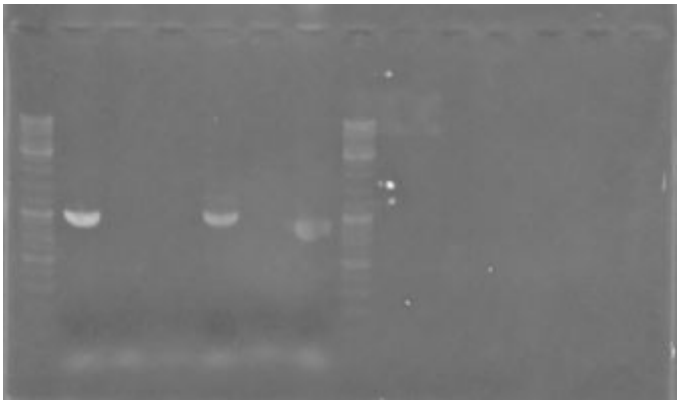




For first PCR: We choose colonies 1 and 2; 2 and 3; 1 and 3; 1 and 2; 1 and 3

Electrophoresis gel for 2nd PCR:
Ladder, K592101_LVA (5 colonies), K592101 (5 colonies), c-, c+, ladder.
Ladder, E0040 (5 colonies), K082003 (5 colonies), c+, ladder.
Ladder, mRFP_LVA (5 colonies), c+, ladder.





For the 2nd PCR: colonies 1 and 3; 5; 1 and 3; 2 and 4; 1 and 4

Minipreps of BBa_K2656008, BBa_K2656009, BBa_K2656010, BBa_K2656011, BBa_K2656012:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656008.1	0,158	0,1	0,041	0,105	0,053	1,998	**105.279**
BBa_K2656008.2	0,18	0,114	0,046	0,122	0,061	1,99	**121.681**
BBa_K2656009.1	0,11	0,076	0,04	0,058	0,029	2,007	**58.037**
BBa_K2656009.2	0,168	0,106	0,044	0,11	0,054	2,035	**110.441**
BBa_K2656010.1	0,149	0,095	0,041	0,095	0,046	2,043	**94.944**
BBa_K2656010.2	0,169	0,104	0,039	0,117	0,057	2,038	**117.135**

BBa_K2656011.1	0,131	0,09	0,046	0,072	0,036	1,975	**72.068**
BBa_K2656011.2	0,156	0,108	0,055	0,088	0,045	1,95	**88.218**
BBa_K2656012.1	0,174	0,112	0,047	0,113	0,057	1,979	**112.97**
BBa_K2656012.2	0,165	0,102	0,039	0,112	0,056	2,016	**112.414**

FRIDAY 27/7

Minipreps: BBa_K2656020 , BBa_K2656021, pACA 30, BBa_K2656023,pACA 32

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656020.1	0,187	0,118	0,047	0,132	0,067	1,977	**132,279**
BBa_K2656020.2	0,123	0,085	0,043	0,07	0,036	1,94	**69,956**
BBa_K2656021	0,113	0,076	0,039	0,066	0,033	1,997	**65,716**
BBa_K2656022.1	0,164	0,105	0,043	0,111	0,056	1,984	**111,43**
BBa_K2656022.2	0,164	0,102	0,038	0,116	0,059	1,978	**116,342**
BBa_K2656023.1	0,16	0,099	0,039	0,11	0,055	2,022	**110,21**
BBa_K2656023.2	0,139	0,091	0,041	0,088	0,045	1,982	**88,469**
BBa_K2656024.1	0,2	0,136	0,071	0,118	0,059	2,015	**118,317**
BBa_K2656024.2	0,128	0,089	0,048	0,07	0,036	1,956	**69,671**

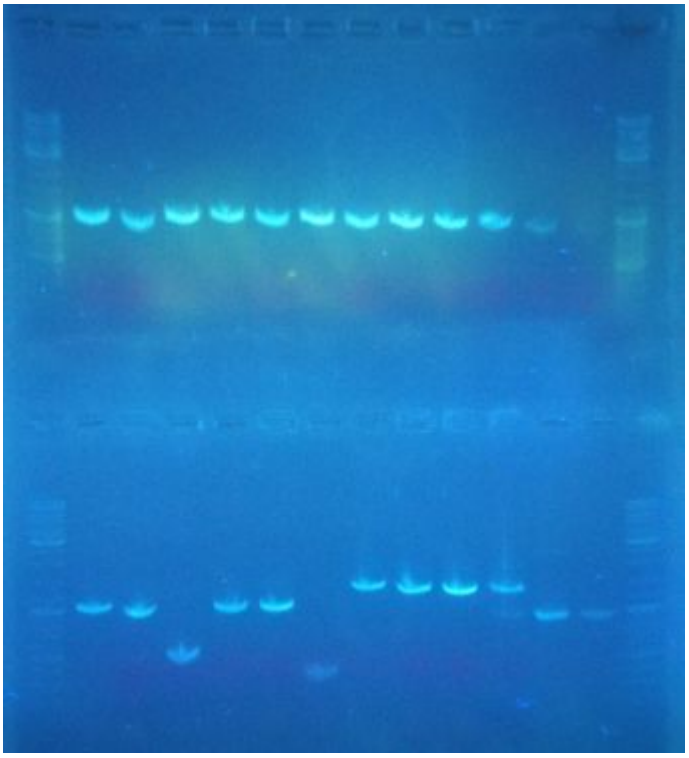
MONDAY 30/7

Sequences of BBa_K2656021, BBa_K2656022, BBa_K2656023, BBa_K2656024 were not correct. Repeat assembly of BBa_K2656022,BBa_K2656023,BBa_K2656024 the mint odor gblock (it wil be BBa_K2656025) into BBa_P10500. (We forgot to do the BBa_K2656021 again here, but we did it later).

WEDNESDAY 1/8

Colony PCR of BBa_K2656022, BBa_K2656023, BBa_K2656024 and BBa_K2656025. We pick 5 colonies for each adapted part.
Ext. time = 1min

Electrophoresis gel:
ladder; colonies 1-5 of BBa_K2656023; colonies 1-5 of BBa_K2656022; C+; C-; ladder
ladder; colonies 1-5 of BBa_K2656024; colonies 1-5 of BBa_K2656025, C+, C-, ladder



To do miniprep:
BBa_K2656023: colonies 3 and 4
BBa_K2656022: colonies 1 and 2
BBa_K2656024: colonies 4 and 5
BBa_K2656025: colonies 2 and 3

THURSDAY 2/8

GB reaction to domesticate K592101 (BBa_K2656021) into BBa_P10500:

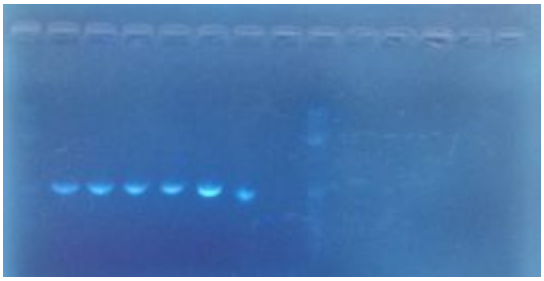
Minipreps for BBa_K2656022, BBa_K2656023, BBa_K2656024, BBa_K2656025

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	**ng/µL**
BBa_K2656022.**1**	0,191	0,117	0,043	0,133	0,065	2,027	**132,531**
BBa_K2656022.**2**	0,193	0,116	0,039	0,14	0,069	2,044	**140,257**
BBa_K2656023.**1**	0,204	0,124	0,044	0,144	0,071	2,022	**143,712**
BBa_K2656023.**2**	0,185	0,116	0,042	0,129	0,065	1,979	**128,832**
BBa_K2656024.**1**	0,226	0,13	0,039	0,171	0,082	2,084	**171,3**
BBa_K2656024.**2**	0,353	0,197	0,04	0,297	0,148	2,014	**297,266**

BBa_K2656025.**1**	0,09	0,066	0,04	0,036	0,018	2,008	**35,99**
BBa_K2656025.**2**	0,103	0,07	0,04	0,049	0,022	2,252	**48,76**

FRIDAY 3/8

Colony PCR of BBa_K2656021:
ladder, colonies 1 to 5, BBa_P10500 (C+), C-, ladder



We choose colonies 1 and 2 to do the Miniprep.

SATURDAY 4/8

Miniprep BBa_K2656021.

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656021.1	0,09	0,073	0,053	0,024	0,012	1,925	**24,029**
BBa_K2656021.2	0,071	0,058	0,041	0,017	0,009	1,861	**16,703**

Very low conc. It may be because of the new buffers used. Grow it again on Monday and Miniprep.

TUESDAY 7/8

Miniprep of BBa_K2656021.1 was done using all the new home-made buffers.
Miniprep of BBa_K2656021.2 was done using the same buffers and protocol but washing with the AQ buffer instead of PB and PE.

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656021.1	0,121	0,086	0,045	0,065	0,034	1,913	**65,002**
BBa_K2656021.2	0,192	0,122	0,043	0,139	0,073	1,905	**138,512**

SATURDAY 11/8

Purification BBa_K2656026:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656026.3	0,078	0,063	0,044	0,025	0,013	1,902	**25,287**

TUESDAY 14/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656026.1	0,166	0,111	0,048	0,099	0,052	1,909	**99,287**
BBa_K2656026.2	0,15	0,1	0,044	0,087	0,045	1,947	**87,156**
BBa_K2656026.3	0,137	0,095	0,046	0,071	0,037	1,928	**71,485**

WEDNESDAY 15/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656014	0,107	0,08	0,046	0,044	0,024	1,816	**43,896**
BBa_K2656000	0,133	0,091	0,042	0,074	0,038	1,92	**73,893**
BBa_K2656004	0,116	0,084	0,045	0,053	0,028	1,877	**52,897**
BBa_K2656022	1,142	0,551	0,049	1,07	0,489	2,187	**1070,362**

THURSDAY 23/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656013.1	0,143	0,104	0,059	0,064	0,033	1,916	**64,074**
BBa_K2656014.1	0,124	0,09	0,052	0,05	0,025	2	**50,139**
BBa_K2656016.1	0,109	0,081	0,048	0,041	0,021	1,925	**40,686**
BBa_K2656026.1	0,131	0,094	0,048	0,062	0,034	1,841	**61,697**

BBa_K2656017.1	0,099	0,075	0,051	0,028	0,012	2,322	**27,966**
BBa_K2656002.1	0,086	0,067	0,043	0,022	0,011	1,991	**22,457**
BBa_K2656003.1	0,131	0,09	0,042	0,068	0,036	1,895	**68,191**
BBa_K2656009.2	0,087	0,066	0,041	0,026	0,012	2,15	**25,545**
BBa_K2656020.1	0,123	0,086	0,041	0,061	0,031	1,922	**60,52**
BBa_K2656022.2	0,102	0,073	0,04	0,041	0,021	1,981	**40,985**
BBa_K2656004.1	0,124	0,085	0,041	0,062	0,031	2,016	**61,531**
BBa_K2656019.2	0,113	0,082	0,047	0,044	0,022	2,009	**44,383**

FRIDAY 24/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656022	0,094	0,073	0,047	0,026	0,013	1,996	**25,614**
BBa_K2656002.1	0,128	0,091	0,045	0,062	0,032	1,9	**61,677**
BBa_K2656000.1	0,106	0,083	0,054	0,031	0,017	1,896	**31,392**
BBa_K2656017.1	0,109	0,079	0,044	0,044	0,022	2,011	**44,074**
BBa_K2656009.1	0,113	0,081	0,043	0,049	0,025	1,958	**48,559**

TUESDAY 28/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
pACA18.1	0,164	0,11	0,045	0,098	0,052	1,871	**97,503**
pACA18.2	0,167	0,11	0,043	0,104	0,054	1,906	**103,61**
BBa_K2656025.1	0,134	0,096	0,048	0,064	0,035	1,86	**64,168**
BBa_K2656025.2	0,157	0,104	0,044	0,092	0,048	1,927	**92,058**

THURSDAY 30/8

Miniprep:

Name	260 Raw	280 Raw	320 Raw	260	280	260/280	**ng/µL**
AraC	0,185	0,121	0,049	0,114	0,057	1,997	**114**
B0032	0,126	0,089	0,046	0,059	0,029	2,027	**59,216**
J61101	0,126	0,087	0,043	0,062	0,03	2,077	**61,733**
B0030	0,17	0,111	0,044	0,105	0,053	1,992	**105,268**
B0034	0,099	0,073	0,041	0,036	0,018	2,073	**36,436**
J61100	0,129	0,087	0,04	0,067	0,032	2,067	**67,094**

WEDNESDAY 5/9

Miniprep:

Name	Location	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
AraC c.2	B3	0,092	0,068	0,042	0,037	0,019	2,022	37,495

MONDAY 10/9

Miniprep:

Name	Location	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µL
BBa_K2656010	E2	0,19	0,119	0,043	0,131	0,067	1,952	130,941
BBa_K2656026	E3	0,168	0,113	0,054	0,098	0,05	1,94	97,668