July

July 2nd

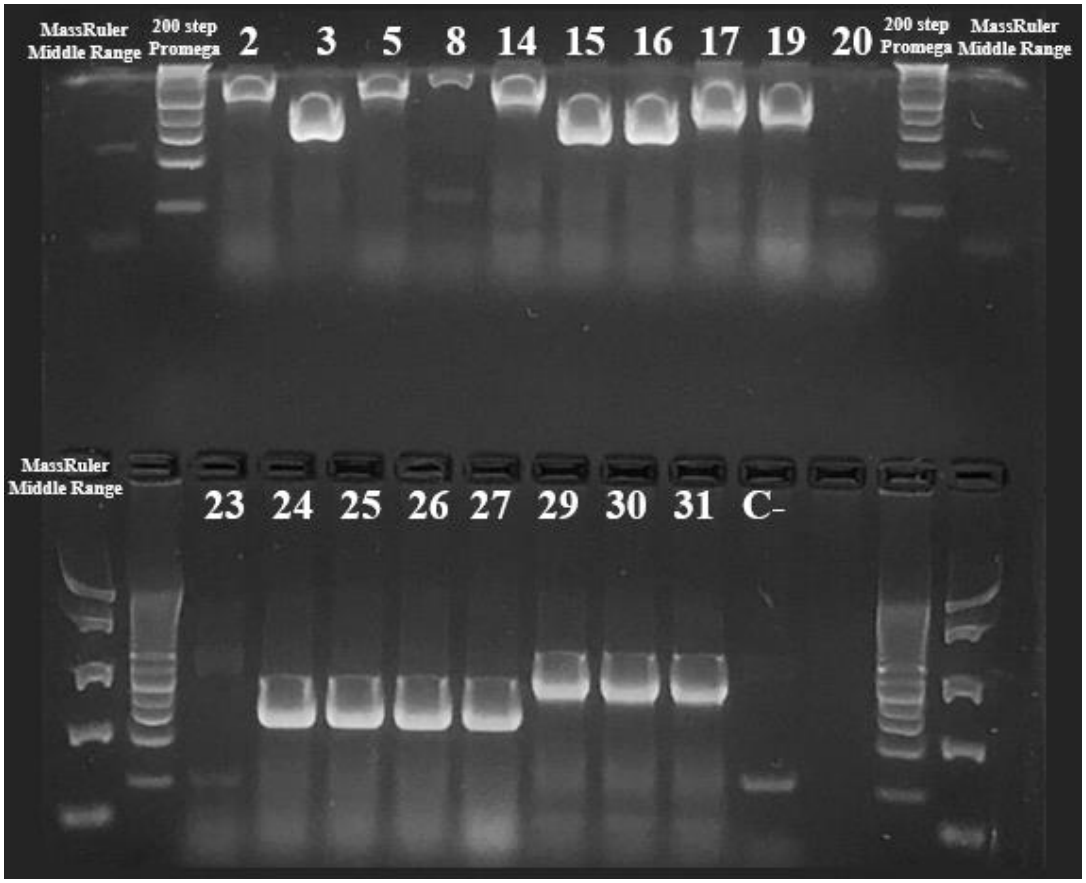
Experiment: *L. casei* ATCC 0334 plate streaking and MRS broth inoculation.
Responsables: Anthony Mora
Protocol code: standard lab procedures
Protocol modifications or specifications: None
Results: No results needed.

July 3rd

Experiment: *L. casei* ATCC 0334 competent cells preparation.
Responsables: Anthony Mora
Protocol code: CC_Lc
Protocol modifications or specifications: None
Results: No results needed

July 4th

Experiment: Electrophoresis gel
Responsables: Pablo Delgado
Protocol code: an 1.5% agarose gel was prepared and run
Protocol modifications or specifications: Samples amplified on June 27th were run again in an 1.5% agarose gel for 120min at 60V.
Results:



July 4th

Experiment: Colony PCR

Responsables: Pablo Delgado

Protocol code: Col_PCR

Protocol modifications or specifications: Samples used correspond to the same colonies selected on June 27th. They were amplified using V2F and VR standard primers. For the thermocycler program, the annealing temperature was set at 55°C and the extension time at 2:45 minutes. PCR products were run in 1.5% agarose gel at 60V for 120 minutes. An AmpliTaq up to 5Kb, from Applied Bioscience was used, to test the Promega Polymerase.

Results: No bands were observed in the gel, the process will be repeated with another enzyme.

July 4th

Experiment: Gel band extraction

Responsables: Paula Thiel

Protocol code: QIAquick Gel Extraction Kit (50)

Protocol modifications or specifications: Digestions were run in 1.5% agarose gel at 60V for 1 hour. Samples run are the followings: R0010, K358006, E0240 and pSB1T3. The final DNA was eluted in 15µL of nuclease free water.

July 4th

Experiment: Ligation

Responsables: Paula Thiel and María José Durán
Protocol code: Lig
Protocol modifications or specifications: Digested parts were obtained from the previous gel band extraction. Samples were incubated for 15 min at room temperature.

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
R0010	5	**K358006**	5	**bbT RFP**	1
R0010	5	**E0240**	5	**bbT RFP**	1

July 4th

Experiment: Transformation
Responsables: María José Durán
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations used were the ones prepared the same day.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
R0010+K358006	T	✗	✗
R0010+E0240	T	✗	✗
C+	T	✗	✓
C-	T	✗	✗

July 5th

Experiment: Colony PCR
Responsables: Pablo Delgado
Protocol code: Col_PCR
Protocol modifications or specifications: Samples used correspond to the same colonies selected on June 27th. They were amplified using V2F and VR standard primers. For the thermocycler program, the annealing temperature was set at 55°C and the extension time at 2:45 minutes. PCR products were run in 1.5% agarose gel at 60V for 120 minutes. A Platinum™ Taq DNA Polymerase High Fidelity up to 20Kb, from ThermoFisher, was used.

Name	Number in index plate	Size (bp)
Lin_C.diff + bbA RFP	-	2613
Lin_S.aureus + bbA RFP	2-3-5	2634
Lin_Sin2 + bbA RFP	8-14	2625
AgrC_AgrA_WT + bbA RFP	15-16-17-19	2634
P3_GFP_Caract + bbA RFP	23	1131

P3_TetR + bbA RFP	-	2092
P3_ARNas_GFP + bbA RFP	24-25-26-27	1431
P3_ARNas_Lisina + bbA RFP	-	1257
Prom_C.diff + bbA RFP	-	1452
MCS_Lacto + bbA RFP	-	1062
C+ (bbA RFP from colonie)	31	

Results: No bands were observed in the gel, the process will be repeated with another enzyme.

July 5th

Experiment: Transformation
Responsables: María José Durán
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations used were the ones prepared on July 4th.
Results:

Name	Resistance	Result	
		Red colonies	**White colonies**
R0010+K358006	T	X	X
R0010+E0240	T	X	X
C+	T	X	X
C-	T	X	X

July 10th

Experiment: Ligation
Responsables: Paula Thiel and María José Durán
Protocol code: Lig
Protocol modifications or specifications: Upstream parts were digested on June 7th. Downstream parts were digested on June 26th. Normal backbone was digested on June 7th. Linearized backbone was digested on July 4th. T4 Ligase - Promega Corporation was used. Samples were incubated for 3 hours at room temperature.

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
J23100	3	**E0240**	3	**bbT RFP**	1
J23100	3	**E0240**	3	**bbT RFP***	1

* Linearized plasmid from gel band extraction

July 10th

Experiment: Transformation
Responsables: María José Durán and Paula Thiel
Protocol code: Trans_Ec

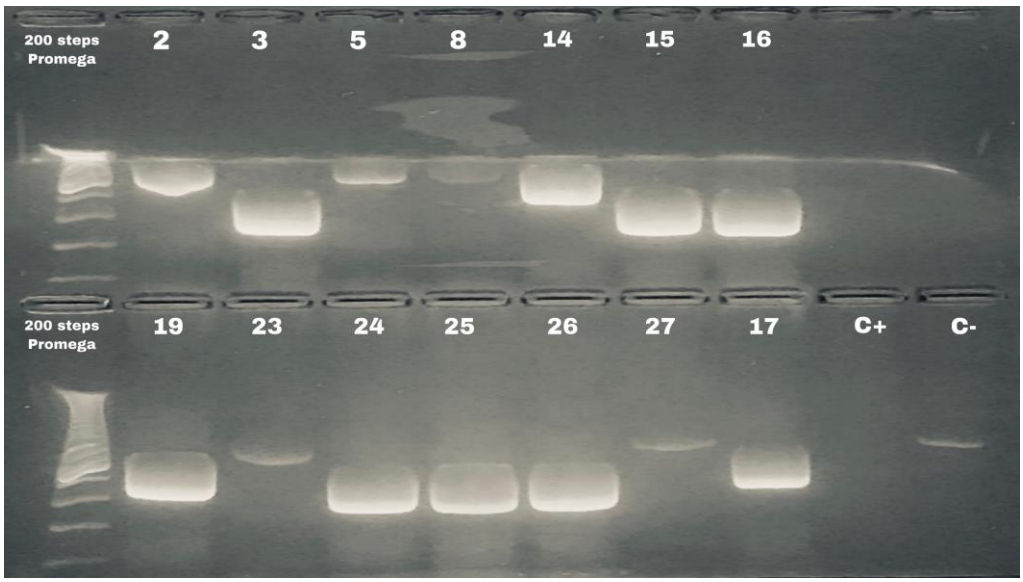
Protocol modifications or specifications: Ligations with the upstream part J23100 were the ones prepared the same day, and Ligations with the upstream part R0010 were prepared on July 4th.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
J23100+E0240	T	✓	✗
J23100+E0240*	T	✗	✗
R0010+K358006	T	✗	✗
R0010+E0240	T	✗	✗
C+	T	✓	✗
C-	T	✗	✗

* Linearized plasmid from gel band extraction

July 10th

Experiment: Colony PCR
Responsables: Paula Thiel and María José Durán
Protocol code: Col_PCR
Protocol modifications or specifications: Samples used correspond to the same colonies selected on July 4th. They were amplified using Prefix and Suffix biobricks standard primers. For the thermocycler program, the annealing temperature was set at 55°C and the extension time at 2:45 minutes. PCR products were run in 1.5% agarose gel at 90V for 60 minutes.
Results:



July 10th

Experiment: Streaking
Responsables: María José Durán
Protocol code: standard lab procedures
Protocol modifications or specifications: One colony of DH5α from National University was stroke in a LB plate.

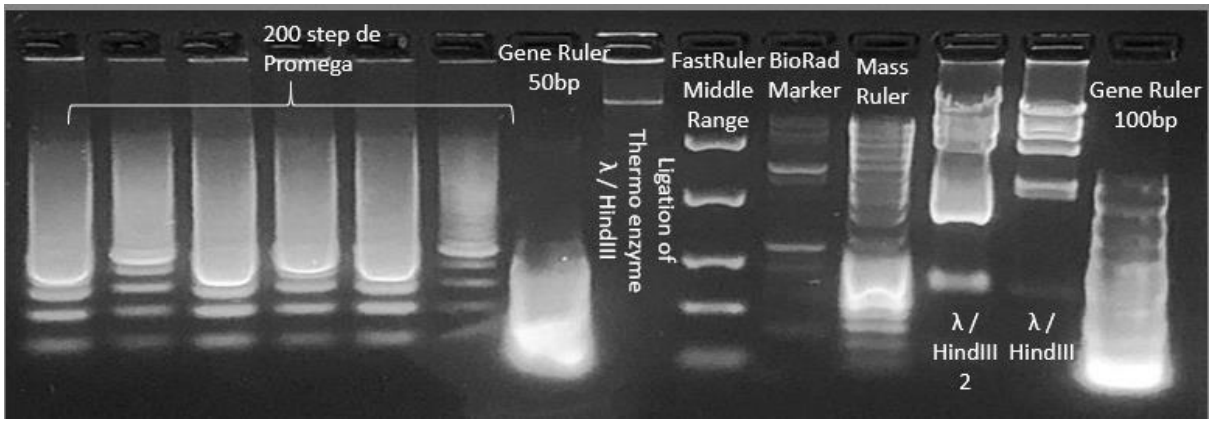
July 11th

Experiment: DNA molecular markers

Responsables: Pablo Delgado

Protocol code: a 1% agarose gel was prepared

Protocol modifications or specifications: Different molecular markers were used to see which ones still work. Those were run at 60V per 60 min in a agarose gel 1%.



July 11th

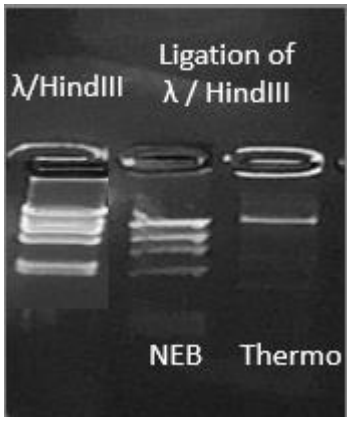
Experiment: Ligase enzyme effectivity assay

Responsables: Pablo Delgado and María José Durán

Protocol code: None

Protocol modifications or specifications: T4 from NEB and Thermo were used to see if they can ligate the lambda-hindIII marker, the mixture was established with 1uL of the enzyme and 3uL of the marker, results were run in a agarose gel at 1% for one hour at 60V.

Results: We concluded that NEB ligase present in the lab do not work property, instead Thermo enzyme do works.



July 11th

Experiment: Ligation

Responsables: Pablo Delgado and María José Durán

Protocol code: Lig

Protocol modifications or specifications: After ligase effectivity assay, Thermoscientific kit will be used for a while. J23100 used was prepared on June 7th, E0240 on June 26th and plasmid bbT + RFP digestion on 26th. Linearized plasmid used was prepared on June 4th from a band extraction.

Master Mix

Reactive	1X (uL)
Nuclease Free Water	4
Vector part	1
Upstream part	5
Downstream part	5
Ligase Buffer 5X	4
Ligase	1
TOTAL	**20**

Reaction was incubated for 15 min at 22°C and inactivated by 10 min at 65°C.

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
R0010	5	**K358006**	5	**bbT RFP**	1
J23100	5	**E0240**	5	**bbT RFP**	1
J23100	5	**E0240**	5	**bbT RFP***	1

* Linearized plasmid from gel band extraction

July 11th

Experiment: Transformation

Responsables: Pablo Delgado and María José Durán

Protocol code: Trans_Ec

Protocol modifications or specifications: Ligations were prepared the same day. Ligation identified as Linearized was obtained from the gel band extraction (07/04).

Results:

Name	Resistance	Result	
		Red colonies	White colonies
R0010+K358006	T	✓	✓
J23100+E0240	T	✓	✗
J23100+E0240*	T	✗	✗
C+	T	✓	✗
C-	T	✗	✗

* Linearized plasmid from gel band extraction

July 12th

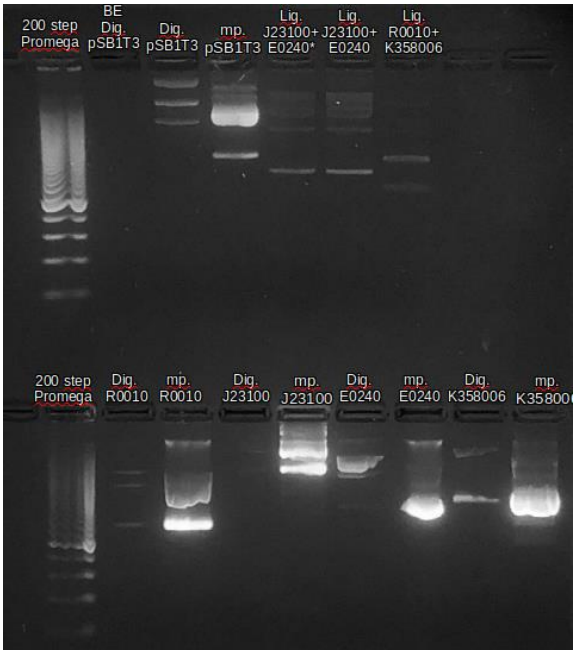
Experiment: Electrophoresis

Responsables: Paula Thiel

Protocol code: an agarose gel was prepared

Protocol modifications or specifications: Several samples were run in 1.5% agarose gel at 60V for 120min. Samples used are the followings: band extraction of digested pSB1T3 from July 4th (BE Dig.pSBT3), digested plasmid from June 7th (Dig. pSB1T3), miniprep of pSB1T3 from June 27th (mp. pSB1T3), Ligations from July 11th (Lig. J23100+E0240*, Lig. J23100+E0240 and Lig. R0010+K358006), digested K358006 from June 20th, digestions from June 7th (Dig. R0010, Dig. J23100, Dig. E0240), minipreps from June 4th (mp. R0010 and mp. E0240), K358006 miniprep from June 20th and J23100 miniprep from June 6th.

Results:



July 12th

Experiment: Colony PCR

Responsables: Pablo Delgado

Protocol code: Col_PCR

Protocol modifications or specifications: Samples used correspond to 5 white colonies from the transformation of June 11th, especifically, R0010+K358006, witch has a PCR extension of about 1856bp. Samples were amplified with V2F and VR standard primers, with 2uL of the DNA hot extraction, visualization was shows in an agarose gel 1.5% with 60 min at 80V.

Results: No expected bands were observed.

July 12th

Experiment: Ligation

Responsables: Paula Thiel

Protocol code: Lig

Protocol modifications or specifications: Thermo Scientific T4 DNA Ligase kit was used. Inserts used were prepared on June 24th and digested plasmid pSB1T3+RFP on June 26th. Samples were incubated at 22°C for 15 minutes and inactivated at 65°C for 10 minutes.

Insert	Insert (µL)	Backbone	Backbone Volume (µL)
Lin_C.diff	7	**bbT RFP**	1
Lin_S.aureus	7	**bbT RFP**	1
Lin_Sin2	7	**bbT RFP**	1
AgrC_AgrA_WT	7	**bbT RFP**	1
P3_GFP_Caract	7	**bbT RFP**	1
P3_TetR	7	**bbT RFP**	1
P3_ARNas_GFP	7	**bbT RFP**	1
P3_ARNas_Lisina	7	**bbT RFP**	1
Prom_C.diff	7	**bbT RFP**	1
MCS_Lacto	7	**bbT RFP**	1

July 12th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from the same day were transformed in *E.coli*.
Results:

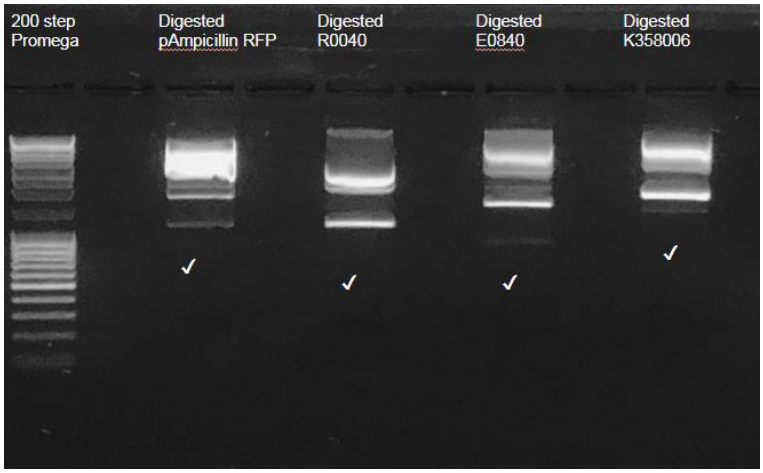
Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff	T	✓	✗
Lin_S.aureus	T	✓	✗
Lin_Sin2	T	✓	✗
AgrC_AgrA_WT	T	✓	✗
P3_GFP_Caract	T	✓	✗
P3_TetR	T	✓	✗
P3_ARNas_GFP	T	✓	✗
P3_ARNas_Lisina	T	✓	✗
Prom_C.diff	T	✓	✗
MCS_Lacto	T	✓	✗
C+ (pSB1T3+RFP)	T	✓	✗
C-	T	✗	✗

July 15th

Experiment: Restriction
Responsables: Paula Thiel and Pablo Delgado
Protocol code: Res
Protocol modifications or specifications: Samples were run in 1.5% agarose gel for 60 minutes at 90V.
Results:

Part	Enzymes	Result
E0840	X+P	✓
R0010	E+S	✓

K358006	X+P	✓
bbA RFP	E+P	✓



July 15th

Experiment: Gel band extraction

Responsables: Paula Thiel

Protocol code: QIAquick® Gel Extraction Kit (50)

Protocol modifications or specifications: Plasmid digestion prepared the same day was used. As there were two possible bands that could correspond to the linearized plasmid, both were extracted separately and named as “up” and “down”, respectively. The final DNA was eluted in 15µL of nuclease free water.

Results: no results needed

July 15th

Experiment: Transformation

Responsables: Pablo Delgado

Protocol code: Trans_Ec

Protocol modifications or specifications: Minipreps used for the transformation are the followings: R0010 and ILC1.

Results:

Name	Resistance	Result	
		Green colonies	White colonies
R0010	C	✗	✓
ILC1	C	✓	✓
C-	C	✗	✗

July 16th

Experiment: Restriction

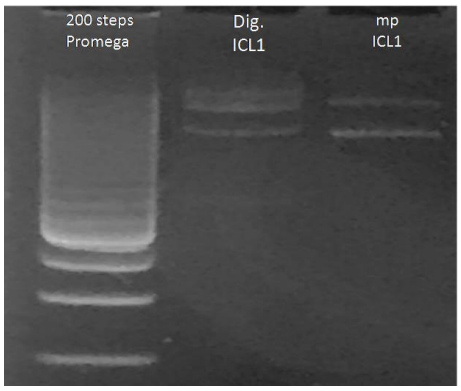
Responsables: Pablo Delgado and María José Durán

Protocol code: Res

Protocol modifications or specifications: Samples were run in 1.8% agarose gel for 60 minutes at 90V.

Results:

Part	Enzymes	Result
ILC1	E+P	✓



July 16th

Experiment: Bacterial culture for plasmid isolation

Responsables: Paula Thiel

Protocol code: 5mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with one transformed colony.

Protocol modifications or specifications: The following transformants from July 16th and July 11th were cultured: R0010, ILC1 and bbT RFP.

Results: No results needed.

July 16th

Experiment: Ligation

Responsables: Pablo Delgado

Protocol code: Lig

Protocol modifications or specifications: Thermo Scientific T4 DNA Ligase kit was used. Insert used was prepared on July 16th and digested plasmid bbA+RFP on July 15th. Two different treatments were tested: incubation at 22°C for 15 minutes and incubation at 22°C for 1 hour. Samples were inactivated at 65°C for 10 minutes.

Insert	Insert (µL)	Backbone	Backbone Volume (µL)	Treatment
ILC1	5	**bbA RFP "up" band**	1	15 minutes
ILC1	5	**bbA RFP "down" band**	1	15 minutes
ILC1	5	**bbA RFP "up" band**	1	1 hour
ILC1	5	**bbA RFP "down" band**	1	1 hour

Results: No results needed.

July 16th

Experiment: Transformation

Responsables: Pablo Delgado and María José Durán

Protocol code: Trans_Ec

Protocol modifications or specifications: Ligations from the same day were transformed. Two different treatments were tested: transforming with 1µL of ligation and transforming with 5µL of ligation.

Results:

Name	Volume of ligation used (uL)	Incubation	Resistance	Red colonies	Green colonies	White colonies
Upper band	1	15min	C	0	9	2
Upper band	5	15min	C	0	9	7
Upper band	1	1 hour	C	0	58	22
Upper band	5	1 hour	C	0	86	5
Upper band	1	15min	A	1	0	0
Upper band	5	15min	A	1	1	0
Upper band	1	1 hour	A	4	3	3
Upper band	5	1 hour	A	14	1	0
Down band	1	15min	C	0	6	1
Down band	5	15min	C	2	4	3
Down band	1	1 hour	C	17	21	4
Down band	5	1 hour	C	0	31	14
Down band	1	15min	A	0	0	1
Down band	5	15min	A	0	0	0
Down band	1	1 hour	A	1	0	3

Down band	**5**	**1 hour**	**A**	**0**	**0**	**0**
C+ bbC-RFP	**-**	**-**	**C**	**✓**	**0**	**0**
C+ bbA-RFP	**-**	**-**	**A**	**✓**	**0**	**0**

July 17th

Experiment: Plasmid Isolation

Responsables: Paula Thiel and María José Durán

Protocol code: ThermoScientific Kit, K0503

Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 30µL of nuclease free water.

Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
bbT	T	502.0	1.85
R0010	C	214.6	1.85
ILC1	C	390.5	1.86

July 22nd

Experiment: Restriction

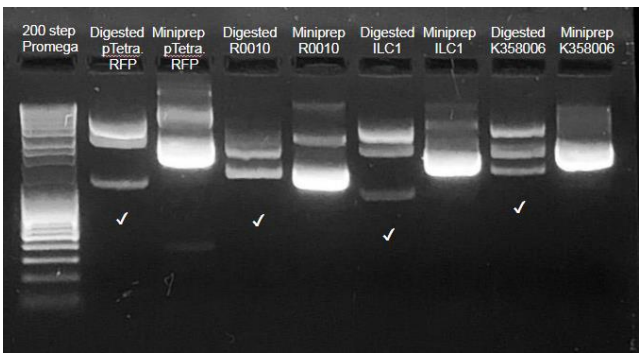
Responsables: María José Durán

Protocol code: Res

Protocol modifications or specifications: Samples were run in 1.5% agarose gel for 60 minutes at 90V.

Results:

Part	Enzymes	Result
ILC1	E+P	✓
R0010	E+S	✓
K358006	X+P	✓
bbT	E+S	✓



July 22nd

Experiment: Ligation

Responsables: María José Durán

Protocol code: Lig

Protocol modifications or specifications: NEB T4 DNA Ligase kit was used. All digested parts were prepared the same day. Two different treatments were tested: incubation at room temperature for 15 minutes and incubation at 16°C for 1 hour.

Insert	Insert (µL)	Backbone	Backbone Volume (µL)	Treatment
ILC1	5	**bbT RFP**	1	15 minutes
ILC1	5	**bbT RFP**	1	1 hour

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)	Treatment
R0010	5	**K358006**	5	**bbT RFP**	1	15 minutes
R0010	5	**K358006**	5	**bbT RFP**	1	1 hour

July 22nd

Experiment: Transformation

Responsables: Paula Thiel

Protocol code: Trans_Ec

Protocol modifications or specifications: Ligations from the same day were transformed. Two different treatments were tested: transforming with 1µL of ligation and transforming with 5µL of ligation.

Results:

Name	Resistance	Result		
		Green colonies	Red colonies	White colonies
ILC1 (T) 15 min 1 µL	T	X	X	X
ILC1 (T) 15 min 5 µL	T	X	X	X
ILC1 (T) 1 hour 1 µL	T	X	X	X
ILC1 (T) 1 hour 5 µL	T	X	X	X
R0010+K358006 (T) 15 min 1 µL	T	X	X	X
R0010+K358006 (T) 15 min 5 µL	T	X	X	X
R0010+K358006 (T) 1 hour 1 µL	T	X	X	X
R0010+K358006 (T) 1 hour 5 µL	T	X	X	X
C+	T	X	X	X
C-	T	X	X	X

July 23rd

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from July 22nd were transformed. Two different treatments were tested: transforming with 1µL of ligation and transformed with 5µL of ligation.
Results:

Name	Resistance	Result		
		Green colonies	Red colonies	White colonies
ILC1 (T) 15 min 1 µL	T	2	✓	X
ILC1 (T) 15 min 5 µL	T	10	✓	X
ILC1 (T) 1 hour 1 µL	T	0	✓	X
ILC1 (T) 1 hour 5 µL	T	12	✓	X
R0010+K358006 (T) 15 min 1 µL	T	0	✓	1
R0010+K358006 (T) 15 min 5 µL	T	0	✓	4
R0010+K358006 (T) 1 hour 1 µL	T	0	✓	4
R0010+K358006 (T) 1 hour 5 µL	T	0	✓	5
C+	T	0	✓	X
C-	T	X	X	X

July 24th

Experiment: Index plate
Responsables: Pablo Delgado
Protocol code: standard lab procedures
Protocol modifications or specifications: All white colonies of R0010+K358006 in bbT plasmid from the transformation from July 23th were subcultured in fresh medium with the respective antibiotic. The objective was to confirm the white color of the colonies.
Results: No results needed.

July 24th

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Res
Protocol modifications or specifications: gBlocks fragments from IDT were cut with EcoRI + PstI to insert them in the iGEM backbones. 21uL of the gBlick resuspension were used, which gave a final concentration of 8.4ng/uL in the digestion tubes.

Part	Enzymes	Result
Lin_C.diff	E+P	Can not be checked

Lin_S.aureus	E+P	Can not be checked
Lin_Sin2	E+P	Can not be checked
AgrC_AgrA_WT	E+P	Can not be checked
P3_GFP_Caract	E+P	Can not be checked
P3_TetR	E+P	Can not be checked
P3_ARNas_GFP	E+P	Can not be checked
P3_ARNas_Lisina	E+P	Can not be checked
Prom_C.diff	E+P	Can not be checked
MCS_Lacto	E+P	Can not be checked
bbA RFP	E+P	Not checked
pSB1A3 linearized plasmid	E+P	Can not be checked

Results: No results needed.

July 24th

Experiment: Ligation
Responsables: Pablo Delgado
Protocol code: Lig
Protocol modifications or specifications: Enzyme T4 used was a new one bought from NEB. 0.2µL of ligase (2.000.000 U/ml) was used. Incubation took 16 hours at 16ºC and then inactivated at 65ºC for 10 min. Ligation was made as shown in the next table:

Upstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_C.diff	16.8	**bbA RFP**	1
Lin_S.aureus	16.8	**bbA RFP**	1
Lin_Sin2	16.8	**bbA RFP**	1
AgrC_AgrA_WT	16.8	**bbA RFP**	1
P3_GFP_Caract	16.8	**bbA RFP**	1
P3_TetR	16.8	**bbA RFP**	1
P3_ARNas_GFP	16.8	**bbA RFP**	1
P3_ARNas_Lisina	16.8	**bbA RFP**	1
Prom_C.diff	16.8	**bbA RFP**	1

MCS_Lacto	16.8	**pSB1A3 linearized plasmid**	1

Results: No results needed.

July 25th

Experiment: Transformation
Responsables: Paula Thiel and María José Durán
Protocol code: Trans_Ec
Protocol modifications or specifications: None
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff + bbA RFP	A	✓	1
Lin_S.aureus + bbA RFP	A	✓	11
Lin_Sin2 + bbA RFP	A	✓	8
AgrC_AgrA_WT + bbA RFP	A	✓	4
P3_GFP_Caract + bbA RFP	A	✓	7
P3_TetR + bbA RFP	A	✓	3
P3_ARNas_GFP + bbA RFP	A	✓	✗
P3_ARNas_Lisina + bbA RFP	A	✓	✗
Prom_C.diff + bbA RFP	A	✓	7
MCS_Lacto + pSB1A3 linearized plasmid*	A	✗	✗
C+ (bbA RFP)	A	✓	✗
C-	A	✗	✗

*Should be red

July 26th

Experiment: Index plate
Responsables: Pablo Delgado
Protocol code: standard lab procedures
Protocol modifications or specifications: All white colonies of the transformation from July 25th were subcultured in fresh medium with the respective antibiotic. The objective was to confirm the white color of the colonies.

Name	Number in index plate	Size (bp)

P3_GFP_Caract + bbA RFP	From 1 to 7	1131
P3_TetR + bbA RFP	From 8 to 10	2092
Prom_C.diff + bbA RFP	From 11 to 17	1452
Lin_C.diff + bbA RFP	18	2613
Lin_S.aureus + bbA RFP	From 19 to 29	2634
Lin_Sin2 + bbA RFP	From 30 to 37	2625
AgrC_AgrA_WT + bbA RFP	From 38 to 41	2634

Results: No results needed.

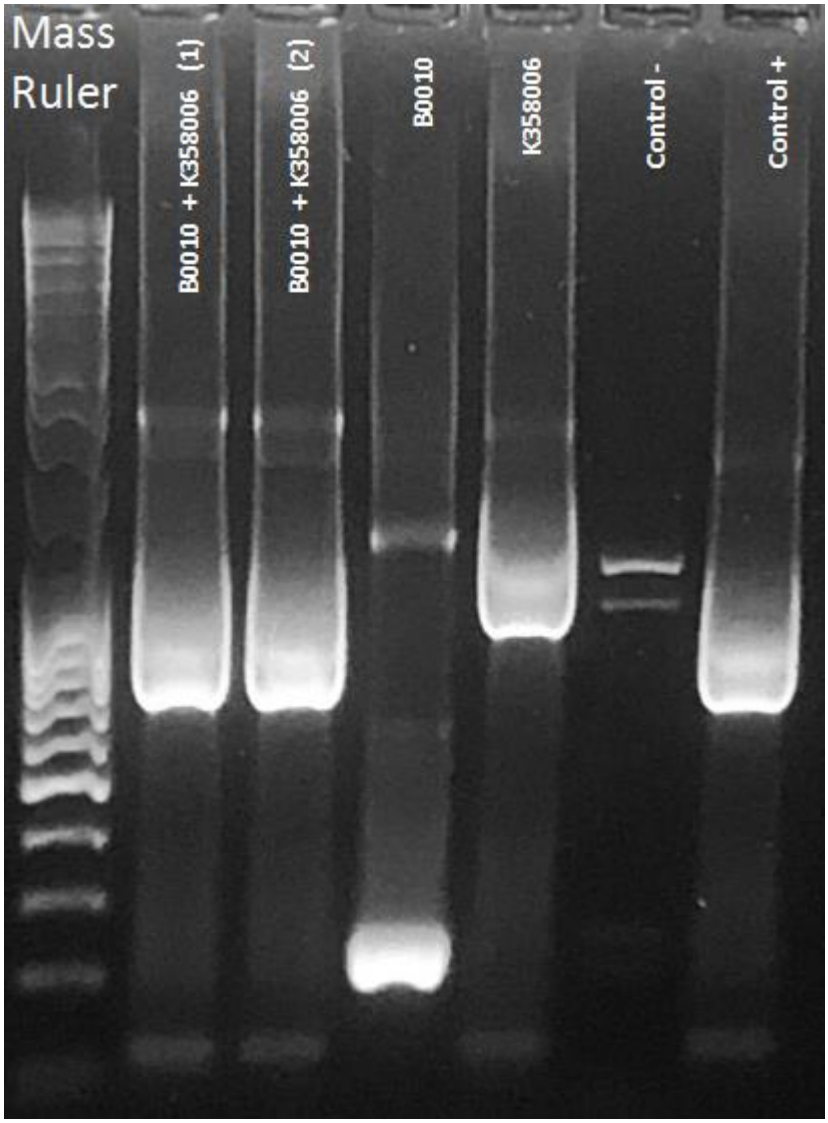
July 27th

Experiment: Plasmid Isolation
Responsables: Paula Thiel
Protocol code: ThermoScientific Kit, K0503
Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 30µL of nuclease free water.
Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
R0010+K358006 (1)	T	269.4	1.88
R0010+K358006 (2)	T	385.8	1.88

July 29th

Experiment: PCR
Responsables: Paula Thiel and Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: Samples used correspond to the isolated plasmid from July 27th. For the thermocycler program, the annealing temperature was set at 56°C and the extension time at 2:00 minutes. PCR products were run in 1.6% agarose gel at 80V for 60 minutes. Prefix and Sufix iGEM primers were used.
Results:



July 29th

Experiment: Preparation of *Clostridium difficile* RNA extraction solutions.
Responsables: Noé Chaves.
Protocol code: RNA_Cd
Protocol modifications or specifications: The solutions were made in greater volumes due to possible repetitions that would be needed in further days.
Results: No results needed.

July 29th

Experiment: Bacterial culture for plasmid isolation
Responsables: Pablo Delgado
Protocol code: 5mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with one transformed colony.
Protocol modifications or specifications: The following colonies of the indexed plate from July 26th were cultured: 1, 6, 10, 13, 18, 19, 24, 32, 38
Results: 18 culture was red.

July 30th

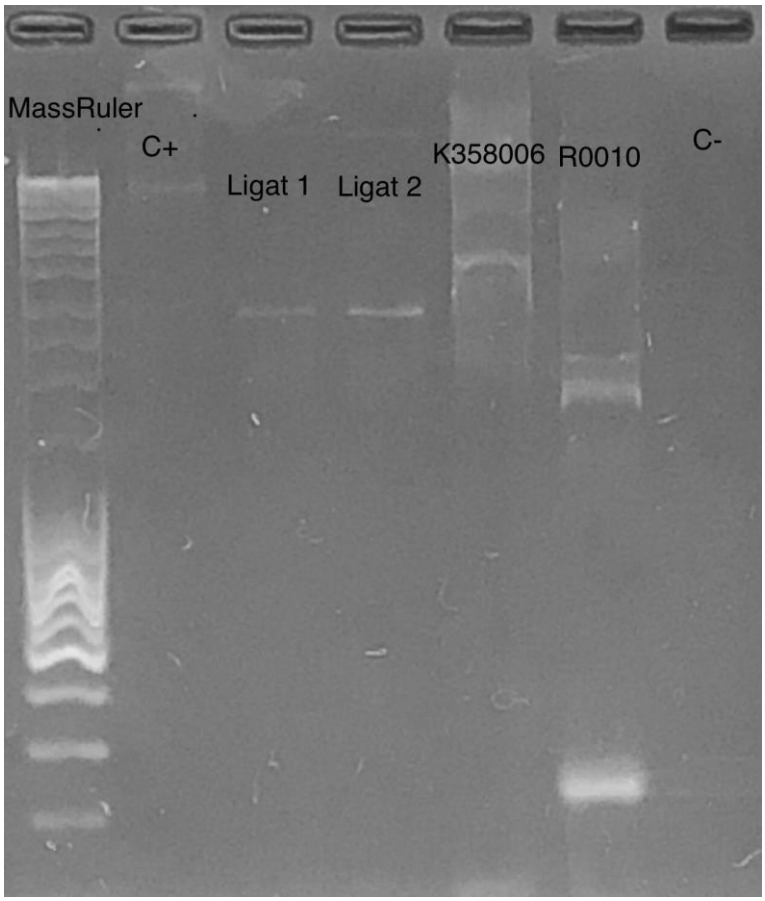
Experiment: PCR

Responsables: Pablo Delgado and María José Durán

Protocol code: PCR

Protocol modifications or specifications: Samples used correspond to the isolated plasmid from July 27th. It is a replicate of the PCR from yesterday, with less miniprep of the lysine ligated (1:10 dilution). For the thermocycler program, the annealing temperature was set at 56°C (Prefix and Sufix iGEM primers) and the extension time at 2:45 minutes. PCR products were run in 1.6% agarose gel at 70V for 90 minutes.

Results:



July 30th

Experiment: Fast miniprep PCR

Responsables: Pablo Delgado and María José Durán

Protocol code: PCR

Protocol modifications or specifications: PCR with prefix and suffix iGEM primers of the cultures from yesterday with the IDT genes were performed with the extraction protocol of 15min at 95ºC and 15min at 10ºC, in order to disrupt the cell wall. Annealing temperature of 56ºC and a extension of 2:45min were used.

Results: No bands were observed in the gel

July 30th

Experiment: Plasmid Isolation

Responsables: Pablo Delgado

Protocol code: ThermoScientific Kit, K0503

Protocol modifications or specifications: Thermo kit was used to extract plasmid from the cells 6 and 10 of the indexed plate from the culture of the day before.

Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
P3_GFP_Caracter (6)	A	220	1.82
P3_TetR (10)	A	508	1.84

July 30th

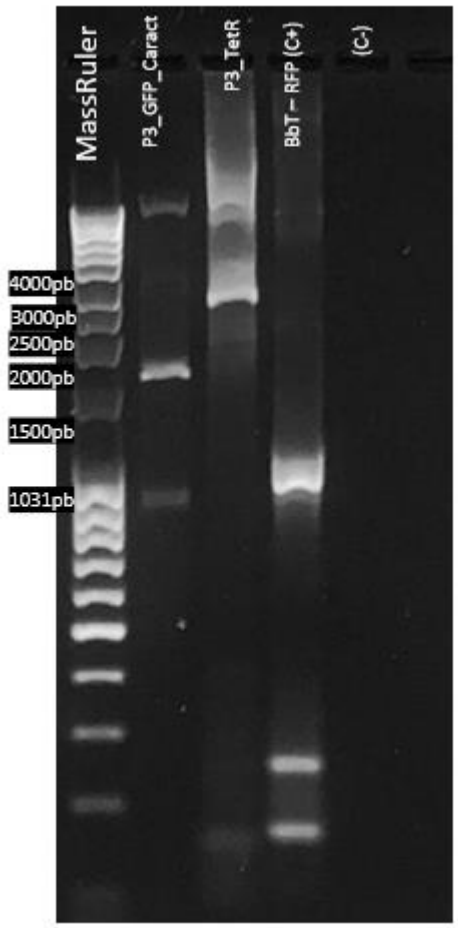
Experiment: PCR

Responsables: Pablo Delgado

Protocol code: PCR

Protocol modifications or specifications: P3_GFP_Caract and P3_TetR miniprep was used as template for a PCR. Prefix and Suffix iGEM primers were used, with an annealing temperature of 56ºC and a extension of 2:00min.

Results:



July 31st

Experiment: Plasmid Isolation
Responsables: Pablo Delgado
Protocol code: ThermoScientific Kit, K0503
Protocol modifications or specifications: Thermo kit was used to extract plasmid from the cells 6 and 10 of the indexed plate from the culture of the day before.
Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
Prom_C.diff + bbA RFP (13)	A	426	1.85
Lin_S.aureus + bbA RFP (19)	A	103	1.86
Lin_S.aureus + bbA RFP (24)	A	376	1.86
Lin_Sin2 + bbA RFP (32)	A	409	1.84
AgrC_AgrA_WT + bbA RFP (38)	A	229	1.86

July 31st

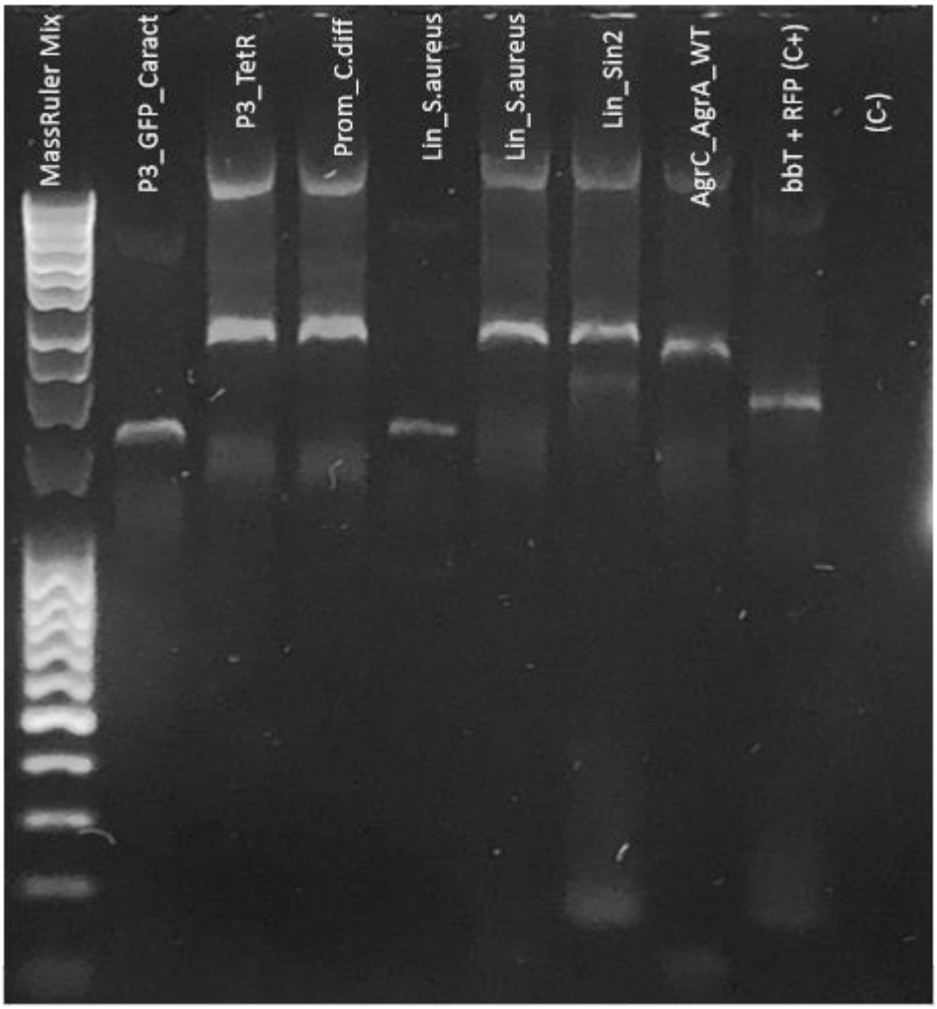
Experiment: PCR
Responsables: Pablo Delgado and María José Durán
Protocol code: PCR
Protocol modifications or specifications: All minipreps from July 30th and July 31st of the IDT gblocks were used as template for a PCR. Prefix and Suffix iGEM primers were used, with an annealing temperature of 56ºC and a extension of 2:45min.

Codes for parts from IDT:

IDT name	Laboratory name	Length (bp)	Plasmid resistance insertion
AgrC_AgrA_Linker_C.diff	**Lin_C.diff**	**2613**	**A**
AgrC_AgrA_Linker_S.aureus	**Lin_S.aureus**	**2634**	**A**
AgrC_AgrA_Linker_Sin2	**Lin_Sin2**	**2625**	**A**
AgrC_AgrA_WildType_C.diff	**AgrC_AgrA_WT**	**2643**	**A**
P3_GFP_Caracterizar	**P3_GFP_Caract**	**1131**	**A**

P3_TetR	**P3_TetR**	**2092**	**A**
P3_ARNas_GFP	**P3_ARNas_GFP**	**1431**	**A**
P3_ARNas_Lisina	**P3_ARNas_Lisina**	**1257**	**A**
Promotor_C.diff	**Prom_C.diff**	**1452**	**A**
MCS_Lacto	**MCS_Lacto**	**1062**	**A**

Results:



July 31st

Experiment: Transformation
Responsables: María José Durán
Protocol code: Trans_Ec
Protocol modifications or specifications: None

Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff + bbA RFP	A	X	X
P3_ARNas_GFP + bbA RFP	A	X	X
P3_ARNas_Lisina + bbA RFP	A	X	X
MCS_Lacto + pSB1A3 linearized plasmid*	A	X	X
C+ (bbA RFP)	A	X	X
C-	A	X	X

*Should be red