August

August 1st

Experiment: PCR
Responsables: Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: All minipreps from July 30th and july 31th of the IDT gblocks was used as template for a PCR to confirmed its presence. Prefix and Suffix iGEM primers was used, with an annealing temperature of 56°C and a extension of 2:45 min.
Results:
No amplification was observed, just the plasmid.

August 1st

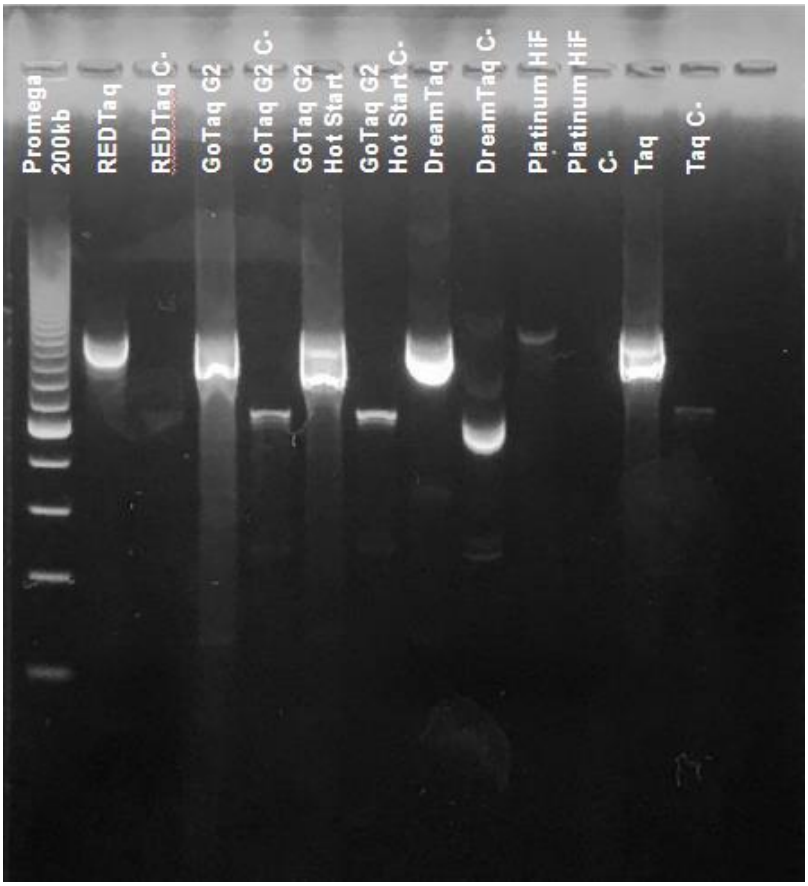
Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: None
Results:

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

August 2nd

Experiment: PCR
Responsables: Paula Thiel
Protocol code: PCR
Protocol modifications or specifications: Different polymerases were tested by the amplification of miniprep K358006 (June, 20th). Polymerases tested are the followings: REDTaq® Sigma, Go TaqG2® Promega, Go TaqG2 Hot Start® Promega, DreamTaq® Thermo Fisher Scientific, Platinum HiF® Invitrogen, Taq Promega. All reactions were prepared as described by the manufacturer. Amplicons were run in a 1.5% agarose gel at 80V for 2 hours.
Results:



August 5th

Experiment: PCR
Responsables: Paula Thiel and Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: Samples amplified are the followings: IDT devices, minipreps from July 27th, July 30th and July 31th, K358006 and RFP. The reaction was prepared using REDTaq polymerase. Products were run in 1.5% agarose gel for 2 hours.
Results: No expected bands were obtained.

August 5th

Experiment: Transformation
Responsables: María José
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	✗	✗
MCS_Lacto + pSB1A3 linearized plasmid*	A	✗	✗
C+ (bbA RFP)	A	✗	✗
C-	A	✗	✗

*Should be red

August 6th

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

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

*Should be red

August 6th

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications: gBlock assembly was verified. 1000ng of plasmid were used in 25uL of reaction. bbT-RFP vector plasmido was the digested on June 22th

Part	Enzymes	Result
Lin_Sin2	E+S	
P3_GFP_Caract	X+P	
P3_TetR	X+P	
ILC1	E+P	

August 6th

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. Ligation was made as shown in the next table:

Upstream part	Insert part Volume (µL)	Downstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_Sin2	8.5	**P3_GFP_Caract**	8.5	**bbT RFP**	0,5
Lin_Sin2	8.5	**P3_TetR**	8.5	**bbT RFP**	0,5
MCS_Lacto	~9			**ILC1**	0,5

Results: No result needed.

August 7th

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: None
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 + P3_GFP_Caract	T	✓	✗
Lin_Sin2 + P3_TetR	T	✓	✓
MCS_Lacto +ILC1*	C	✗	✗
C+ (bbT RFP)	T	✓	✗
C-	T	✗	✗

August 8th

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: bbA -RFP and bbT-RFP were cultured
Results: bbT - RFP cultures did not show growing

August 8th

Experiment: Index plate
Responsables: Pablo Delgado
Protocol code: standard lab procedures

Protocol modifications or specifications: Colonies from August 7th were strooke in a new plate to confirm their colour. The 4 white colonies of the Lin_Sin2 + P3_TetR part and 3 of the MCS were selected.

August 8th

Experiment: Restriction
Responsables: Paula Thiel
Protocol code: Rest
Protocol modifications or specifications: IDT gBlocks were digested with EcoRI and PstI. Final concentration of DNA was 7.5ng/µL. Also, double amount of enzymes was used, and it was incubated for 4 hours at 37°C.
Results: No results needed.

August 8th

Experiment: Ligation
Responsables: Pablo Delgado
Protocol code: Lig
Protocol modifications or specifications: Incubation took 20 hours at 16ºC. Ligation was made as shown in the next table:

Upstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_C.diff	17.3	**bbC RFP**	0.5
Lin_S.aureus	17.3	**bbC RFP**	0.5
AgrC_AgrA_WT	17.3	**bbC RFP**	0.5
P3_ARNas_GFP	17.3	**bbC RFP**	0.5
P3_ARNas_Lisina	17.3	**bbC RFP**	0.5
MCS_Lacto	17.3	**ILC1**	0.5

Results: No results needed.

August 9th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from August 10th and August 6th were transformed.
Results:

Name	Resistance	Result	
		Red colonies	White colonies

Lin_Sin2 + P3_GFP_Caract	T	X	X
Lin_Sin2 + P3_TetR	T	X	X
Lin_C.diff	C	X	X
Lin_S.aureus + bbC	C	X	X
P3_ARNas_GFP	C	X	X
P3_ARNas_Lisina	C	X	X
Prom_C.diff	C	X	X
MCS_Lacto	C	X	X
RFP_bbC (C+)	C	X	X
C-	C	X	X
RFP_bbT (C+)	T	X	X
C-	T	X	X

August 9th

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
RFP_bbA	A	295.4	1.83
RFP_bbA	A	275.7	1.84

August 9th

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: Transformed colonies from of Lin_Sin2 + P3_TetR from August 7th were cultured.
Results: No results needed.

August 10th

Experiment: Streaking
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: One colony of *E. coli* DH5a was stroke in a LB plate.
Results: No results needed.

August 10th

Experiment: Plasmid Isolation
Responsables: María José
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
Lin_Sin2 + P3_TetR (1)	T	341.4	1.86
Lin_Sin2 + P3_TetR (2)	T	172.3	1.87
Lin_Sin2 + P3_TetR (3)	T	185.4	1.91

August 10th

Experiment: PCR
Responsables: María José
Protocol code: PCR
Protocol modifications or specifications: Samples amplified were the minipreps Lin_Sin2 + P3_TetR from the same day, Lin_Sin2 from July 31th and P3_TetR from July 30th. The reaction was prepared using REDTaq polymerase.
Results: PCR was made with less extension time of the corresponded.

August 12th

Experiment: Plasmid Isolation
Responsables: María José
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
RFP_bbT	T	120.4	1.82

August 12th

Experiment: PCR
Responsables: Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: Samples amplified were the minipreps Lin_Sin2 + P3_TetR from the August 12th, Lin_Sin2 from July 31th and P3_TetR from July 30th. The reaction was prepared using REDTaq polymerase.

Reactive	Volumen (uL)
Nuclease Free water	18.25
RedTaqBuffer 10X (Promega Buffer)	2.5
dNTP's	0.5
V2F Primer	0.25
VR Primer	0.25
RedTaq Polimerase	1.25
ADN (5ng/uL)	2

Results: No gene was amplified

August 13th

Experiment: PCR
Responsables: Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: Samples amplified were the minipreps Lin_Sin2 + P3_TetR from the August 12th, Lin_Sin2 from July 31th and P3_TetR from July 30th. The reaction was prepared using REDTaq polymerase.

Reactive	Volumen (uL)
Nuclease Free water	15.25
RedTaqBuffer 10X (Promega Buffer)	2.5
dNTP's	2
V2F Primer	1
VR Primer	1
RedTaq Polimerase	1.25
ADN (5ng/uL)	2

Results: No band was observed

August 13th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from August 10th were transformed.

Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff	C	✓	✓
Lin_S.aureus + bbC	C	✓	✗
P3_ARNas_GFP	C	✓	✓
P3_ARNas_Lisina	C	✓	✓
Prom_C.diff	C	✓	✓
MCS_Lacto	C	✓	✗
RFP_bbC (C+)	C	✓	✗
C-	C	✗	✗

August 16th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from August 10th were transformed.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff	C	✓	✓
Lin_S.aureus + bbC	C	✓	✓
P3_ARNas_GFP	C	✓	✓
P3_ARNas_Lisina	C	✓	✓
Prom_C.diff	C	✓	✓
MCS_Lacto	C	✓	✗
RFP_bbC (C+)	C	✓	✗
C-	C	✗	✗

August 16th

Experiment: Ligation
Responsables: Paula Thiel
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. Ligation was made as shown in the next table:

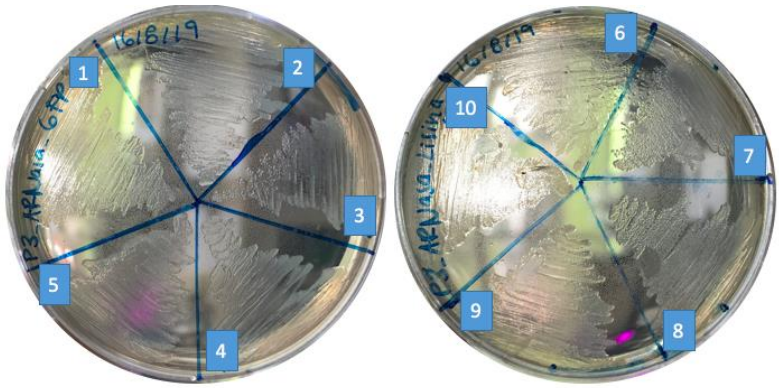
Upstream part	Insert part Volume (µL)	Downstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_Sin2	6.5	**P3_GFP_Caract**	6.5	**bbT RFP**	0.5

Results: No results needed.

August 17th

Experiment: Index plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: None
Results:

Name	Date	Number in index plate	
P3_ARNas_GFP	August 14th	1-5	PCR
P3_ARNas_Lis	August 14th	6-10	Colonies PCR



August 17th

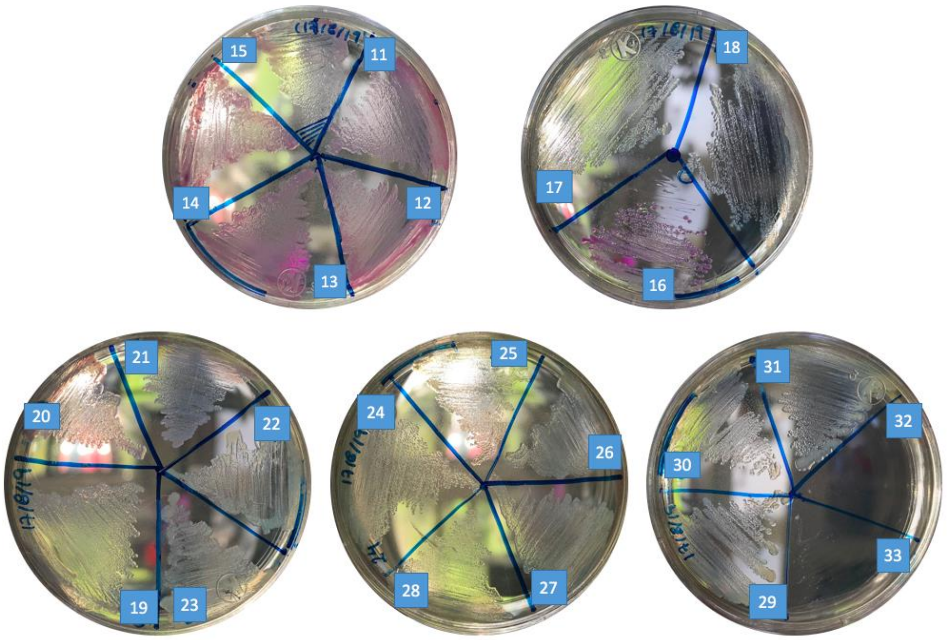
Experiment: Transformation
Responsables: María José
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligation from August 16th was transformed.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 + P3_GFP_Caract	C	✓	X
RFP_bbC (C+)	C	✓	X
C-	C	X	X

August 17th

Experiment: Index plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: None
Results:

Name	Date	Number in index pl
Lin_C.diff	August 13th	11
Prom_C.diff	August 13th	12-15
Lin_C.diff	August 16th	16-18
Lin_S.aureus	August 16th	19-21
Prom_C.diff	August 16th	22-28
MCS_Lacto	August 16th	29-31
Lin_Sin2 + P3_GFP_Caract	August 16th	32-33

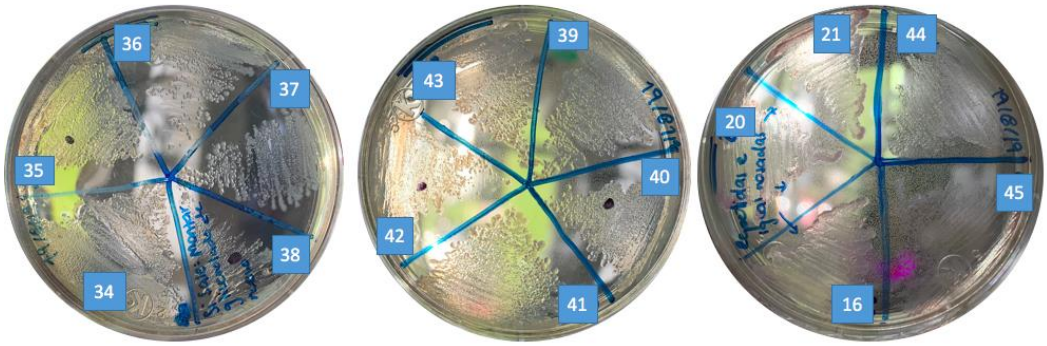


August 19th

Experiment: Index plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: The colonies 16, 20 and 21 are the same as the index plate of August 17th.
Results:

Name	Date	Number in index plate	
Lin_Sin2 + P3_GFP_Caract	August 17th	34-44	Only th PCR

Lin_C.diff	August 16th	16 and 45	Red col
Lin_S.aureus	August 16th	20 and 21	Repeat same r



August 19th

Experiment: Plasmid Isolation
Responsables: María José
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
P3_ARNas_GFP (1)	Cl	100	1.82
P3_ARNas_GFP (3)	Cl	90	1.63
P3_ARNas_GFP (5) -> Desechado	Cl	51.8	1.73
P3_ARNas_Lis (6) -> Desechado	Cl	42.6	1.70
P3_ARNas_Lis (8) -> Desechado	Cl	50.2	1.82
P3_ARNas_Lis (10) -> Desechado	Cl	60.7	1.84

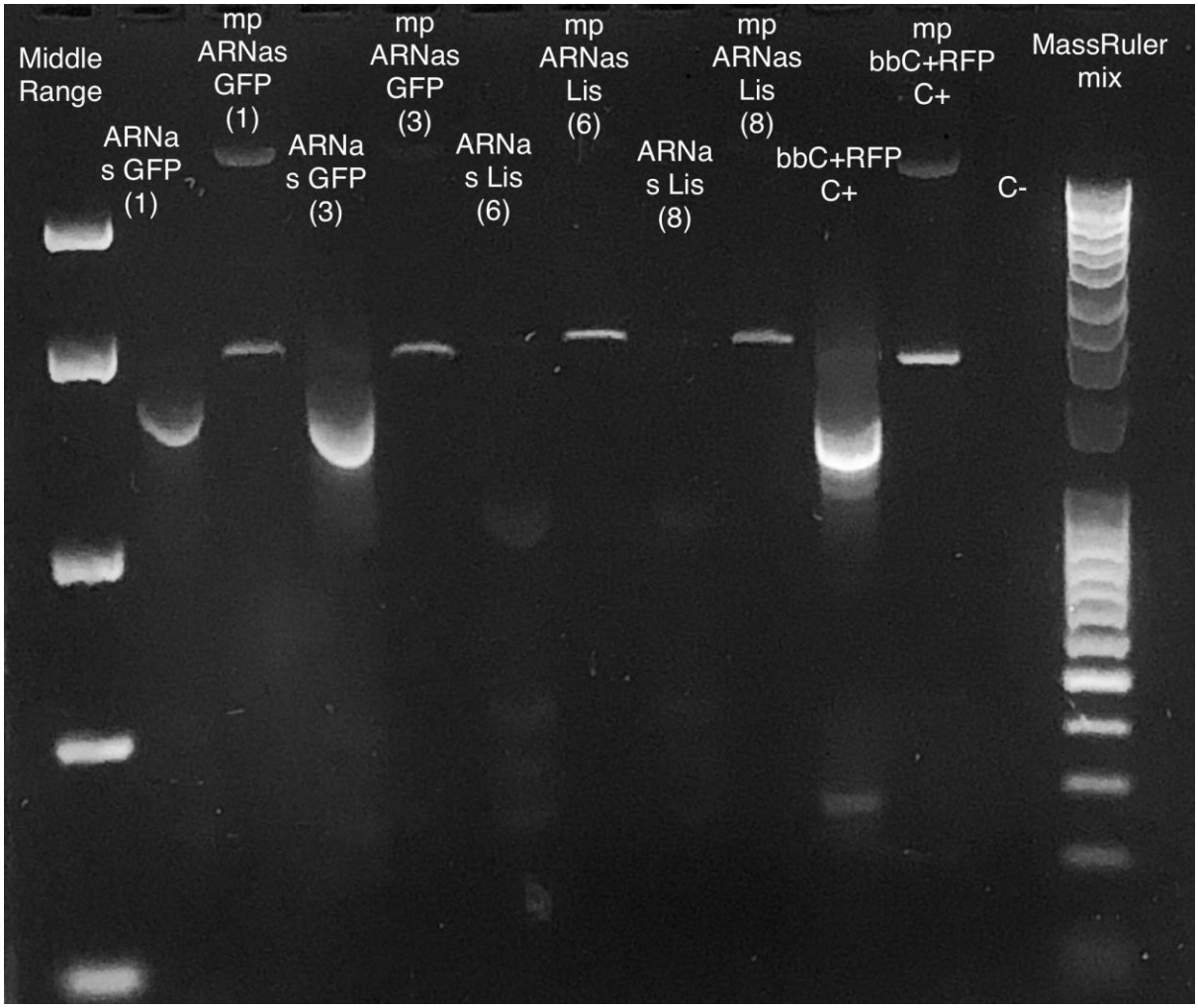
August 19th

Experiment: PCR
Responsables: María José
Protocol code: PCR
Protocol modifications or specifications: Samples amplified were the minipreps P3_ARN_GFP and P3_ARNas_Lis from the same day. The reaction was prepared using REDTaq polymerase.

Reactive	Volumen (uL)
Nuclease Free water	18.25
RedTaqBuffer 10X (Promega Buffer)	2.5

dNTP's	0.5
V2F Primer	0.25
VR Primer	0.25
RedTaq Polimerase	1.25
ADN (5ng/uL)	2

Results:



August 20th

Experiment: Plasmid Isolation

Responsables: María José

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
Lin_C.diff (17)	Cl	69.9	1.84
Lin_C.diff (18)	Cl	77.3	1.86
Prom_C.diff (22)	Cl	97.2	1.69
Prom_C.diff (25)	Cl	72.8	1.91
Prom_C.diff (28)	Cl	64.3	1.83

August 20th

Experiment: PCR
Responsables: Paula Thiel
Protocol code: PCR
Protocol modifications or specifications: PCR made with the Lin_C.diff and Prom_C.diff variants was amplified using V2F and VR conditions and a extension of 1:30min.
Results: No amplification was observed

August 20th

Experiment: Bacterial culture for plasmid isolation
Responsables: María José
Protocol code: 5mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with one transformed colony.
Protocol modifications or specifications: White colonies from of August 19th indexed plate were cultured.
Results: No results needed.

August 21th

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

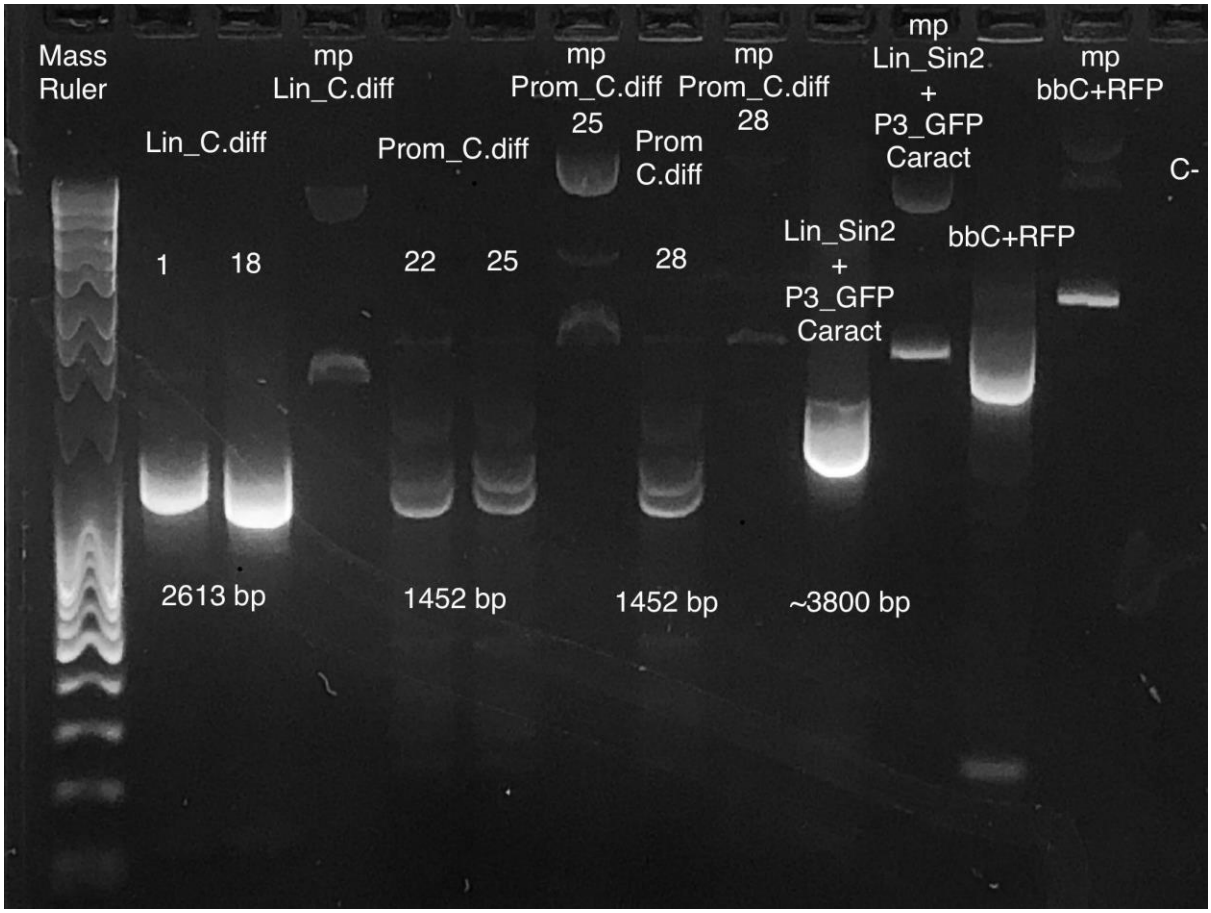
Name	Resistance	Concentration (ng/uL)	Absorbance
Lin_Sin2 + P3_GFP_Caract	Cl	535.7	1.86

August 21st

Experiment: PCR
Responsables: María José
Protocol code: PCR

Protocol modifications or specifications: PCR was made with the Lin_Sin2 + P3_GFP_Caract miniprep from the same day and the samples amplified the day before (Lin_C.diff and Prom_C.diff). It was amplified using V2F and VR conditions and a extension of 3:45min.

Results:



August 21st

Experiment: Transformation

Responsables: María José

Protocol code: Trans_Ec

Protocol modifications or specifications: Ligation from August 16th was transformed.

Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff	C	✗	✓
Lin_S.aureus	C	✗	✗
P3_ARNas_Lis	C	✗	✓
Prom_C.diff	C	✗	✓
MCS_Lacto	C	✗	✗
C+	C	✓	✗
C-	C	✗	✗

August 22nd

Experiment: Index plate
Responsables: Paula Thiel
Protocol code: stand lab procedure
Protocol modifications or specifications: White colonies from the transformation of August 19th were stroke.
Results:

Name	Number in index plate	Res
Prom_Cdiff	1-3	All w
P3_ARNas_Lis	4	Wh
Lin_C.diff	5	Re

August 22nd

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications: IDT gBlocks were digested with EcoRI and PstI. Parts used were: MCS_lacto, Prom_Cdiff, P3_ARNas_Lisina, Lin_S.aureus, Lin_Cdiff. Final concentration of DNA was 7.5ng/µL. Also, double amount of enzymes was used, and it was incubated for 4 hours at 37°C.
Results: No results needed.

August 22nd

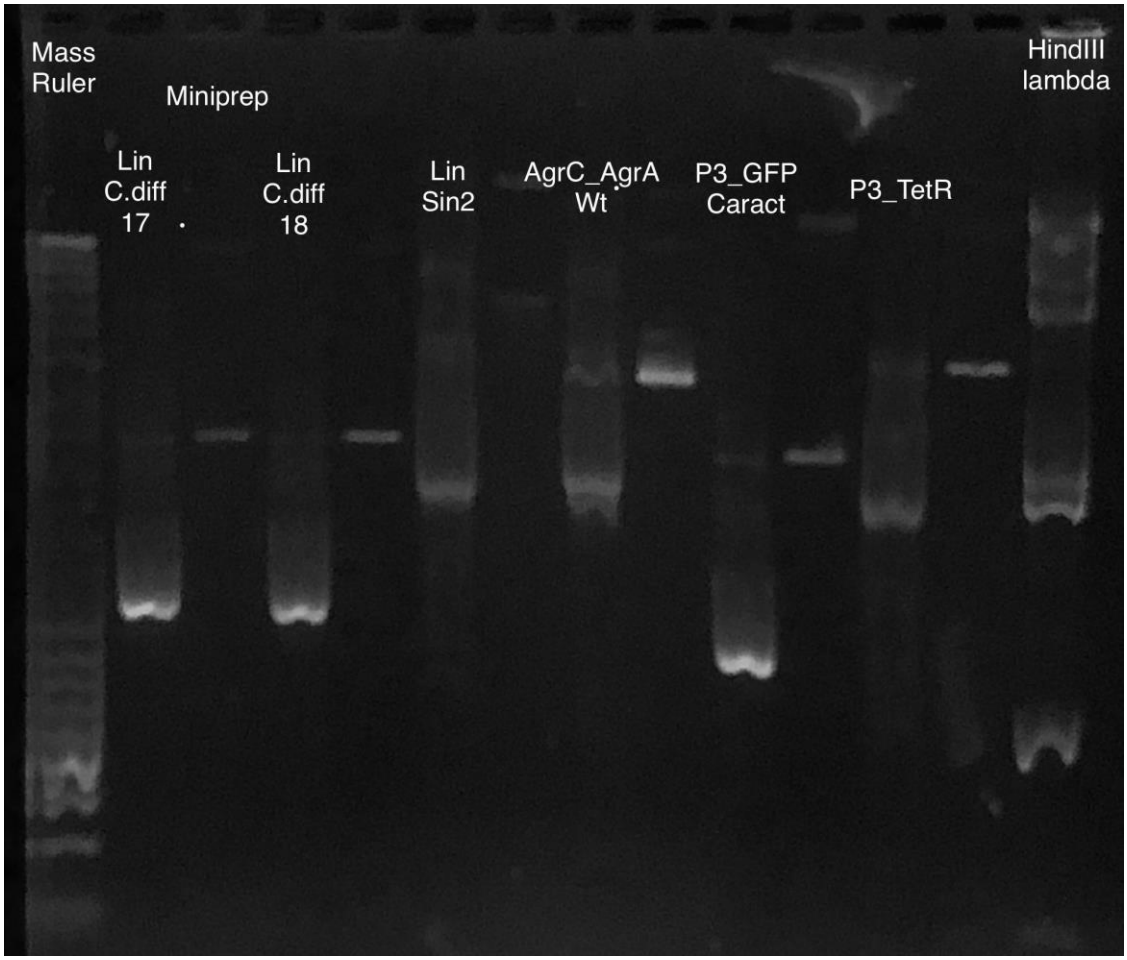
Experiment: Ligation
Responsables: Paula Thiel
Protocol code: Lig
Protocol modifications or specifications: Enzyme T4 used was a new one bought from NEB. 0.2µL of ligase (2.000.000 U/ml) was used. Incubation took 16 hours at 16ºC. Ligation was made as shown in the next table:

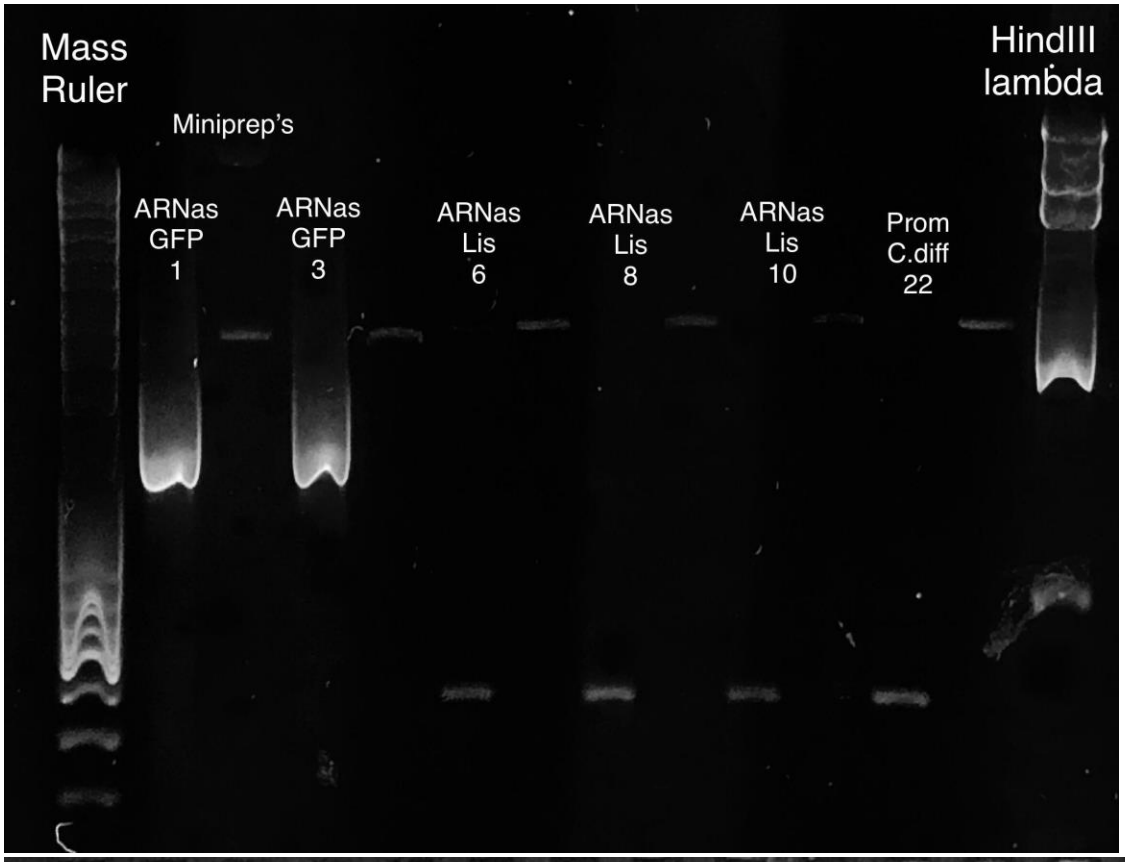
Insert	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
MCS_lacto	17.3	**pRBA**	0.5
Prom_Cdiff	17.3	**bbC RFP**	0.5
P3_ARNas_Lisina	17.3	**bbC RFP**	0.5
Lin_Saureus	17.3	**bbC RFP**	0.5
Lin_Cdiff	17.3	**bbC RFP**	0.5

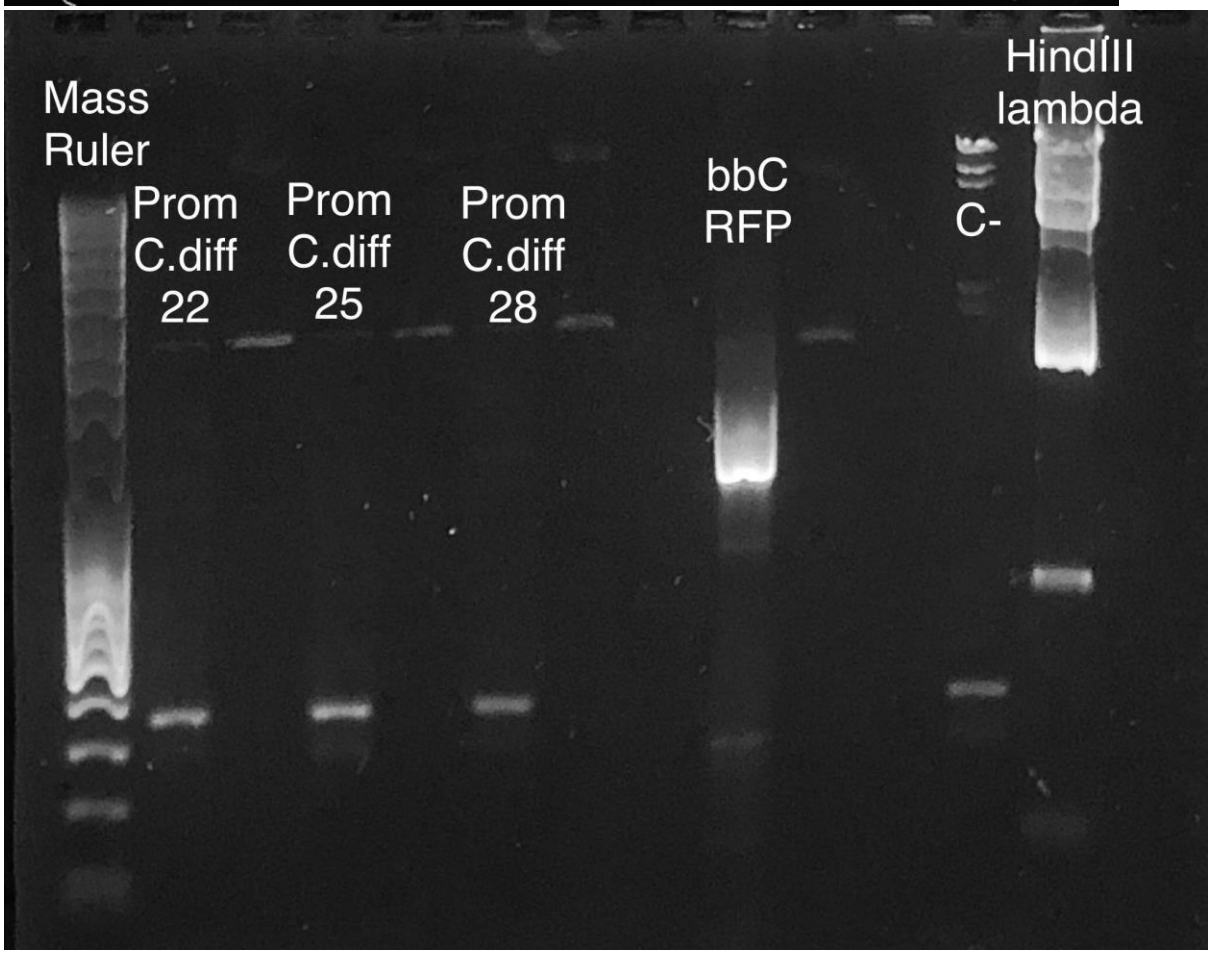
Results: No results needed.

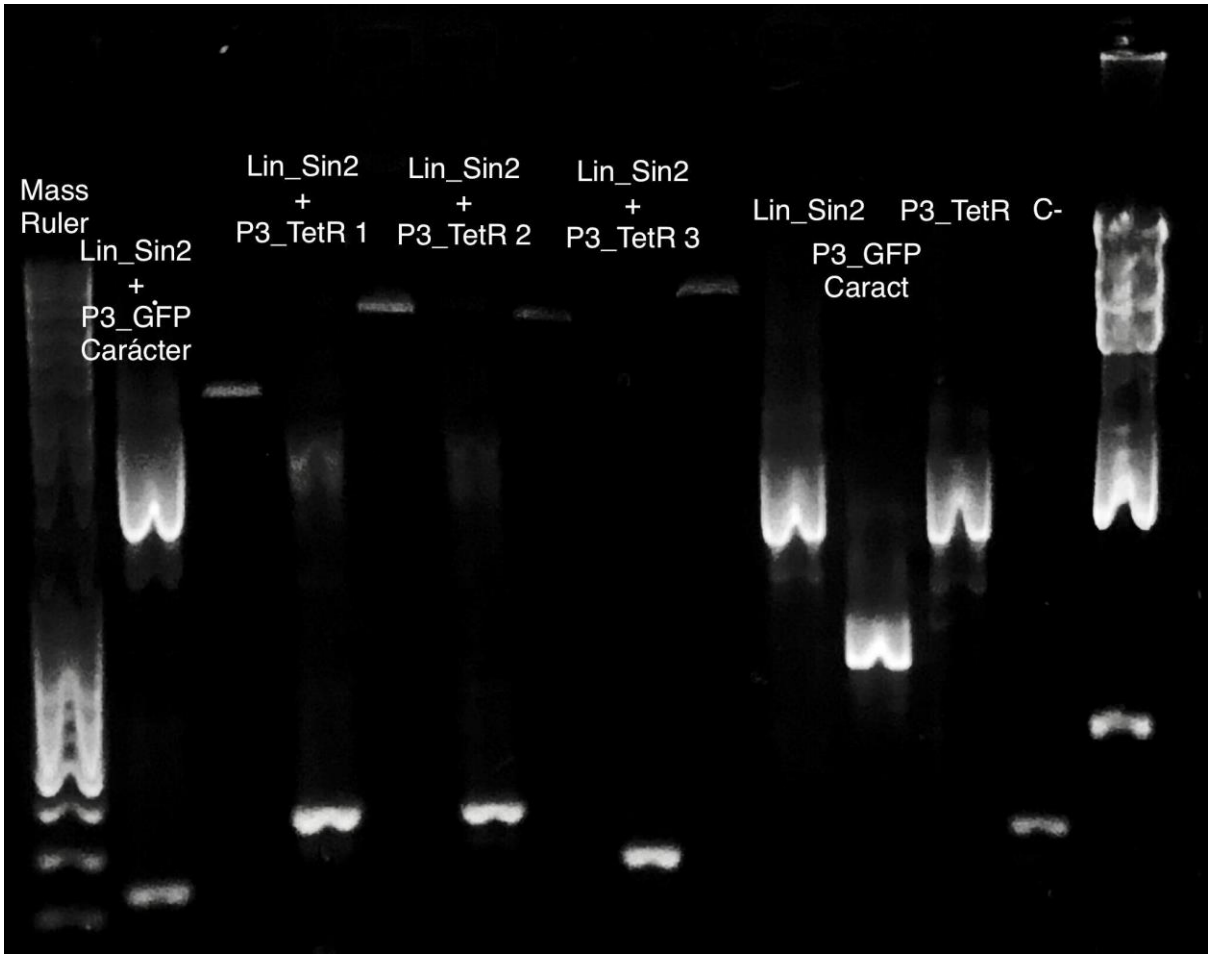
August 22nd

Experiment: PCR
Responsables: Pablo Delgado
Protocol code: PCR
Protocol modifications or specifications: PCR of all parts present in miniprep was performed. It was amplified using V2F and VR (56°C) conditions and with an extension of 4:00 min.
Results:




Mass
Ruler
Miniprep's
ARNas
GFP
1
ARNas
GFP
3
ARNas
Lis
6
ARNas
Lis
8
ARNas
Lis
10
Prom
C.diff
22
HindIII
lambda


Mass
Ruler
Prom
C.diff
22
Prom
C.diff
25
Prom
C.diff
28
bbC
RFP
C-
HindIII
lambda



Part	Status in Gel	Status in Sequencing	Part	Status in Gel	Status in Sequencing
Lin_C.diff (17)	✗	-	P3_ARNas_Lis (8)	✗	-
Lin_C.diff (18)	✗	-	P3_ARNas_Lis (10)	✗	-
Lin_Sin2	✓	-	Prom_C.diff (22)	✗	-
AgrA_AgrC_WT	✓	-	Prom_C.diff (25)	✗	-
P3_Caract	✓	-	Prom_C.diff (28)	✗	-
P3_TetR	✓	-	Lin_Sin2 + P3_GFP_Caract	✓/✗	-
P3_ARNas_GFP (1)	✓	-	Lin_Sin2 + TetR (1)	✓/✗	-
P3_ARNas_GFP (3)	✓	-	Lin_Sin2 + TetR (2)	✓/✗	-
P3_ARNas_Lis (6)	✗	-	Lin_Sin2 + TetR (3)	✗	-

August 23rd

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from August 22th were transformed.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff + bbC RFP	C	✓	✓
Lin_S.aureus + bbC RFP	C	✓	✓
P3_ARNas_Lis + bbC RFP	C	✓	✓
Prom_C.diff + bbC RFP	C	✓	✓
MCS_Lacto + pRB-A	A	✗	✓
C+ (bbC RFP)	C	✓	✗
C-	C	✗	✗

August 23rd

Experiment: Bacterial culture for plasmid isolation
Responsables: María José
Protocol code: 5mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with one transformed colony.
Protocol modifications or specifications: White colonies from of august 22th indexed plate were cultured.
Results: No results needed.

August 24th

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
Prom C. diff (1) -> Desechado	C	256.7	1.85
Prom C.diff (2) -> Desechado	C	163.1	1.79
Prom c.diff (3) -> Desechado	C	214.9	1.84
P3 ARN lis (4)	C	280.4	1.83

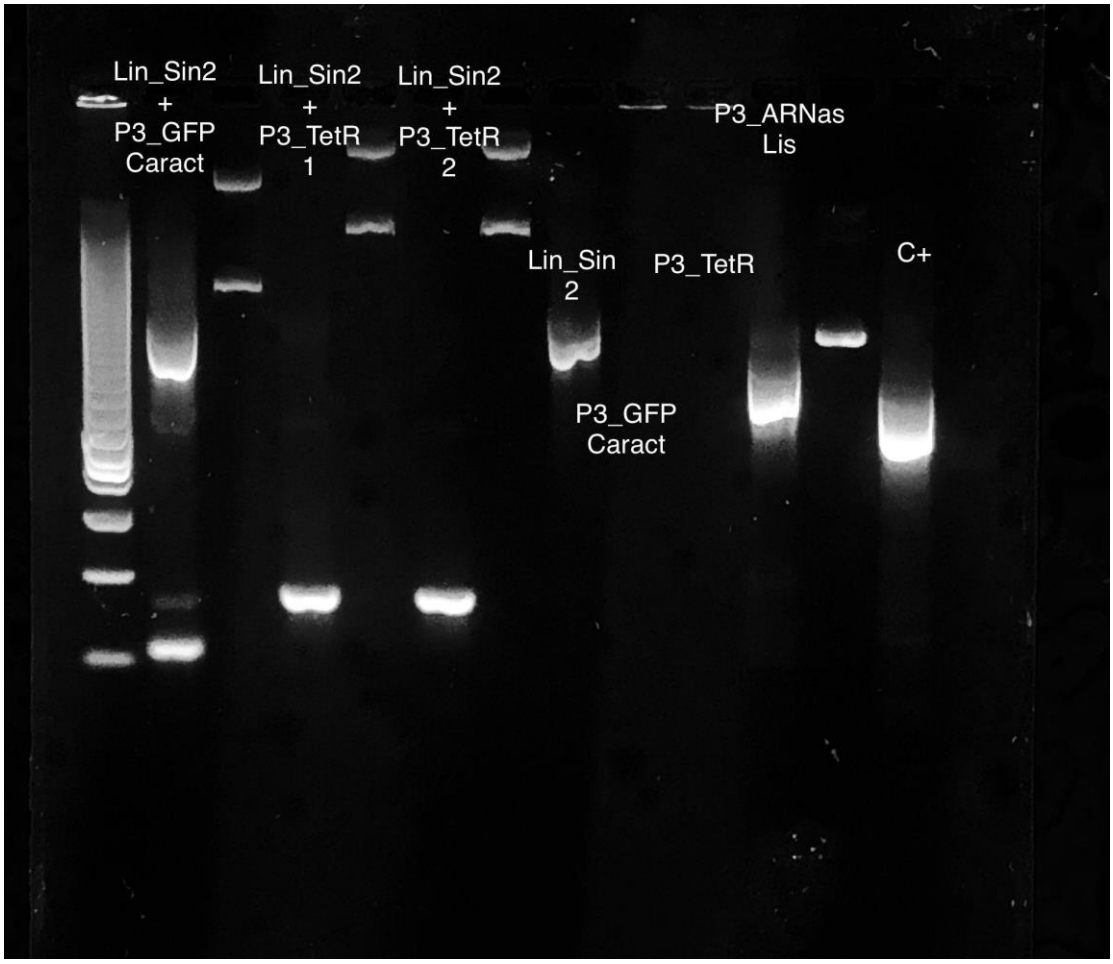
August 24th

Experiment: Index plate
Responsables: Paula Thiel
Protocol code: standard lab procedures
Protocol modifications or specifications: White colonies from the transformation of August 23rd were stroke.

Name	Number in index plate	Results
Prom_Cdiff	1-2-3	White
P3_ARNas_Lis	1	White
Lin_S.aureus	1-2	White
Lin_C.diff	-	Red

August 24th

Experiment: PCR
Responsables: Paula
Protocol code: PCR
Protocol modifications or specifications: The following samples were amplified: PCR products from August 22nd of ligations Lin_Sin2 + P3_GFP_Caract and Lin_Sin2_ + P3_TetR, minipreps from August 24th and minipreps of Prom C.diff (colonies 22, 25 and 28). It was amplified using V2F and VR conditions and a extension of 4min. Products were run in a 1.6% agarose gel at 70V for 2 hours.
Results: Any of the Prom_C.diff part presents an amplification.



August 27th

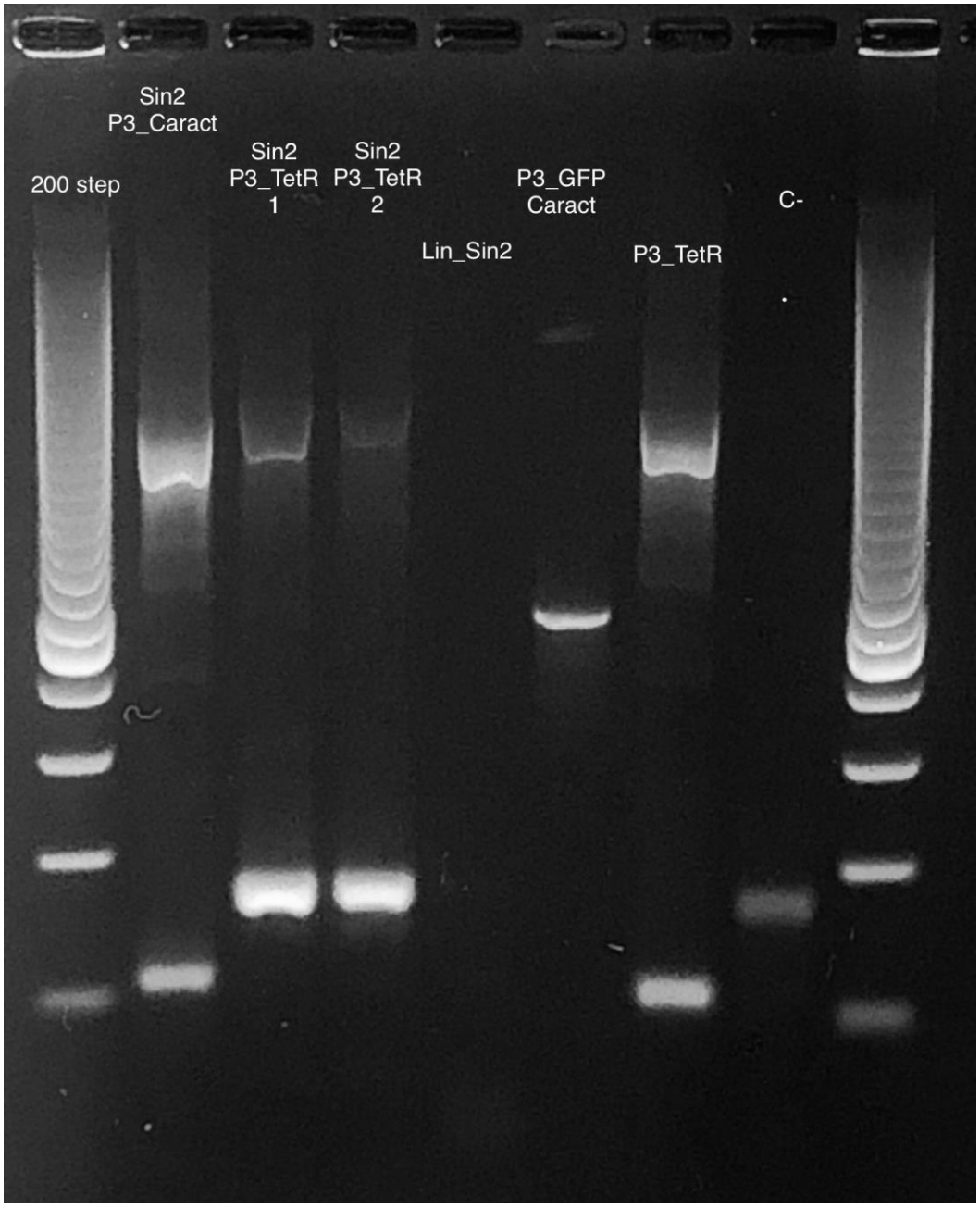
Experiment: PCR

Responsables: Paula and María José

Protocol code: PCR

Protocol modifications or specifications: The following samples were amplified: minipreps of Lin_Sin2 + P3_GFP_Caract, Lin_Sin2_TetR (1), Lin_Sin2_TetR (2), Lin_Sin2, P3_GFP_Caract and P3_TetR. Each reaction was supplemented with 1uL of $MgCl_2$ and they were amplified using V2F and VR primer. Reactions were incubated for 2 hours at room temperature and the thermocycler program was set with 1 min of denaturalization, 2 min of alignment at 56°C and 3:45 min of extension. Products were run in a 1.6% agarose gel at 70V for 2 hours.

Results:



August 27th

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations from August 22th were transformed.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff + bbC RFP	C	✓	2
Lin_S.aureus + bbC RFP	C	✓	4

MCS_Lacto + pRB-A	A	✗	6
C+ (bbC RFP)	C	✓	✗
C-	C	✗	✗

August 27th

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: White colonies from August 24th indexed plate were cultured.

Results: No results needed.

August 28th

Experiment: Plasmid Isolation

Responsables: Pablo Delgado

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
Prom C. diff (1)	C	150.3	1.85
Prom C.diff (2)	C	105	1.85
Prom c.diff (3)	C	110	1.86
Lin_S.aureus (1)	C	70.7	1.88
Lin_S.aureus (2)	C	61.5	1.86

August 28th

Experiment: PCR

Responsables: Pablo Delgado

Protocol code: PCR

Protocol modifications or specifications: The following samples were amplified: minipreps of Prom_C.diff (1:3), Lin_S.aureus (1:2). Each reaction was supplemented with 1uL of $MgCl_2$ and they were amplified using V2F and VR primer. Reactions were incubated for 1 hour at room temperature and the thermocycler program was set with 1 min of denaturalization, 1 min of alignment at 56°C and 3:00 min of extension. Products were run in a 1% agarose gel at 70V for 2 hours.

Results: No positive results were observed.

August 28th

Experiment: Index plate

Responsables: Pablo Delgado

Protocol code: standard lab procedure

Protocol modifications or specifications: White colonies from the transformation of August 27th were stroke.

Results:

Name	Number in index plate	Results
Prom_C.diff	1-3	white colonies: index #2
Lin_S.aureus	1-4	white colonies: index #4

August 29th

Experiment: Twist Clonal Genes resuspension

Responsables: Pablo Delgado

Protocol code: as indicated by the manufacturer

Protocol modifications or specifications: terminator Ter_908 from Twist Bioscience and the AIP part (AgrB_AgrD_E.coli) were resuspended in NFW up to around 100 ng/µL.

Twist code	Laboratory name	Resistance	Concentration (ng/uL)
Terminador_BBa_B0015	Ter_908	A	100
AgrB_AgrD_E.coli	AIP	A	100

Results: No results needed.

August 29th

Experiment: Restriction

Responsables: Pablo Delgado

Protocol code: Rest

Protocol modifications or specifications: IDT gBlocks were digested with EcoRI and PstI. Parts used were: MCS_lacto and Lin_Cdiff. Also, double amount of enzymes was used. It was incubated by 1 hour at 37°C.

Results: No results needed.

August 29th

Experiment: Restriction

Responsables: Pablo Delgado

Protocol code: Rest

Protocol modifications or specifications: Double amount of enzymes was used and it was incubated for 15 min at 37°C.

Results:

Part	Enzymes	Result
Lin_Sin2	E+S	✓
P3_ARNas_GFP	X+P	✓

P3_ARNas_Lisina	X+P	✓
AgrC_AgrA_Wt	E+S	**Not Checked**
Prom_C.diff	X+P	**Not Checked**
bbA	E+P	✓
bbK	E+P	✓

August 29th

Experiment: Ligation
Responsables: Paula Thiel and 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. Ligation was made as shown in the next table:

Upstream part	Insert part Volume (µL)	Downstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_Sin2	8.65	**P3_ARNas_GFP**	8.65	**bbT RFP**	0.5
Lin_Sin2	8.65	**P3_ARNas_Lis**	8.65	**bbT RFP**	0.5
AgrC_AgrA_WT		**Prom_C.diff (1)**		**bbT RFP**	0.5
Lin_C.diff		**-**	-	**bbA RFP**	
MCS_lacto		**-**	-	**bbA RFP**	

August 30th

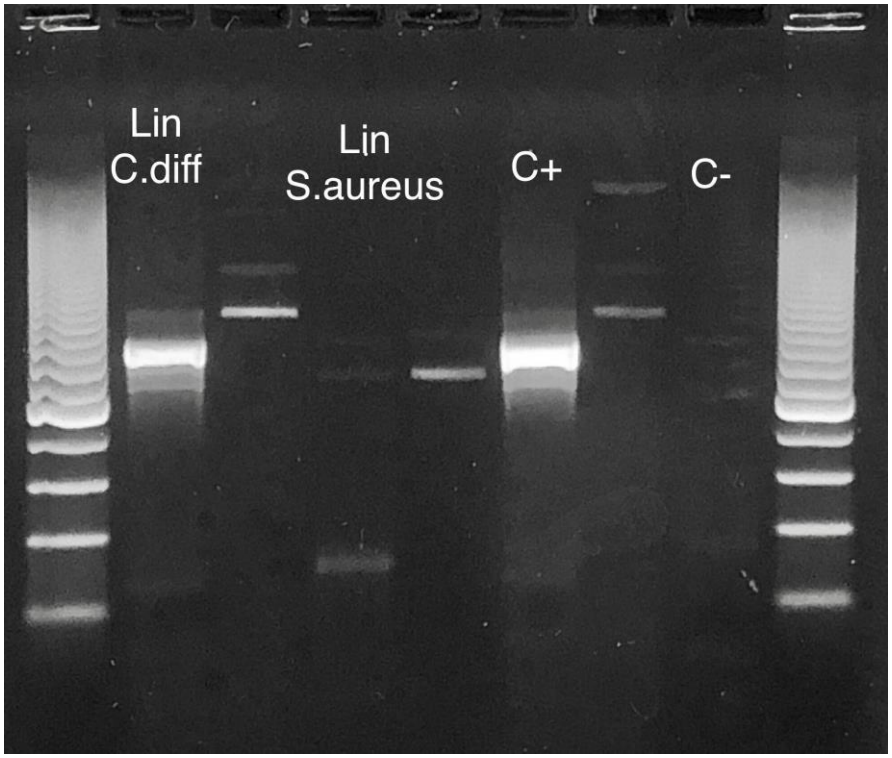
Experiment: Plasmid Isolation
Responsables: Paula Thiel
Protocol code: ThermoScientific Kit, K0503
Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 30µL of nuclease free water.
Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
Lin_C.diff (2)	C	207.0	1.86
Lin_S.aureus (4) -> Descartado	C	161.1	1.85

August 30th

Experiment: PCR

Responsables: Paula and Pablo
Protocol code: PCR
Protocol modifications or specifications: Minipreps from August 30th were amplified using V2F and VR conditions and an extension time of 2:45 min. Products were run in a 1.2% agarose gel at 70V for 1 hour.
Results:



August 30th

Experiment: Index plate
Responsables: Paula Thiel
Protocol code: standard lab procedure
Protocol modifications or specifications: White colonies from the transformation of August 29th were stroke.
Results:

Name	Number in index plate	Results
AgrB_AgrD_E.coli	1-2	✓
B0015	1-2	✓

August 30th

Experiment: Transformation
Responsables: Pablo Delgado and Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from August 29th and the minipreps are specified in the following table.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 + P3_ARNas_GFP	T	✓	25
Lin_Sin2 + P3_ARNas_Lis	T	✓	1
AgrC_AgrA_WT + Prom_C.diff	T	✓	2
Lin_C.diff + bbA_RFP	A	✓	0
MCS_lacto + pRBA	A	✗	✗
C+ (bbT_RFP)	T	✓	✗
C-	T	✗	✗
C-	A	✗	✗

August 31st

Experiment: Index plate

Responsables: María José

Protocol code: standard lab procedures

Protocol modifications or specifications: White colonies from the transformation of August 30th were stroke.

Results:

Name	Number in index plate	Results
Lin_Sin2 + P3_ARNas_GFP	1-5	✓
Lin_Sin2 + P3_ARNas_Lis	6	✓
AgrC_AgrA_WT + Prom_C.diff	7-8	✗