September

September 2nd

Experiment: Transformation
Responsables: María José
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from August 22th (Lin_S.aureus and MCS 1) and August 29th (MCS 2).
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_S.aureus + bbC RFP	C	✓	✓
MCS_Lacto + bbA RFP (1)	A	✗	✓
MCS_Lacto + bbA RFP (2)	A	✗	✗
C+ (bbA RFP)	A	✓	✗
C-	A	✗	✗
C-	C	✗	✗

September 2nd

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 August 31st indexed plate were cultured.
Results: No results needed.

September 2nd

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications: Double amount of enzymes was used and it was incubated for 2 hours at 37°C.
Results:

Part	Enzymes	Result
Lin_C.diff	E+S	✓
P3_GFP_Caract	X+P	✓
P3_TetR	X+P	✓

September 2nd

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 in September. 1 µL of ligase (400.000 U/ml) was used per 20uL of rxn. Incubation was incubated overnight 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_C.diff	8.65	P3_GFP_Caract	8.65	bbT RFP	0.5
Lin_C.diff	8.65	P3_TetR	8.65	bbT RFP	0.5
Lin_C.diff	8.65	P3_ARNas_GFP	8.65	bbT RFP	0.5
Lin_C.diff	8.65	P3_ARNas_Lis	8.65	bbT RFP	0.5

- Should be in Kanamycin resistance

Results: No results needed.

September 2nd

Experiment: Bacterial culture for plasmid isolation

Responsables: Pablo Delgado and María Durán

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

Protocol modifications or specifications:

Name	Resistance	To do
Lin_Sin2 + P3_TetR (1)	T	Preserve
Lin_Sin2 + P3_TetR (2)	T	Preserve
AgrC_AgrD_Wt -> pellet was red	A	Preserve
Lin_Sin2	A	Preserve
P3_GFP_Caract	A	Preserve and Miniprep
P3_TetR	A	Preserve and Miniprep
P3_ARNas_GFP	C	Preserve and Miniprep
P3_ARNas_Lis	C	Preserve and Miniprep
Ter_BBa_B0015	A	Preserve and Miniprep
AgrB_AgrD_Wt	A	Preserve and Miniprep
AgrC_AgrD_Wt + Prom_C.diff	T	Preserve and Miniprep
Lin_Sin2 + P3_ARNas_GFP (1)	T	Preserve and Miniprep
Lin_Sin2 + P3_ARNas_GFP (2)	T	Preserve and Miniprep
Lin_Sin2 + P3_ARNas_Lis	T	Preserve and Miniprep

Results: No results needed.

September 3rd

Experiment: PCR

Responsables: Paula

Protocol code: PCR

Protocol modifications or specifications: Minipreps from September 2nd were amplified. Each reaction was supplemented with 1uL of $MgCl_2$ and they were amplified using V2F and VR primers. 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 1.2% agarose gel at 70V for 1 hour.

Results: No amplicons were observed.

September 3rd

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
P3_TetR	A	137.5	1.82
P3_GFP_Caract	A	411.7	1.85
P3_ARNas_GFP (1)	C	190.2	1.84
P3_ARNas_Lis	C	58.8	1.86
Lin_Sin2 + P3_ARNas_GFP (2)	A	65.5	1.85
Lin_Sin2 + P3_ARNas_GFP (3)	A	216.5	1.87
Lin_Sin2 + P3_ARNas_Lis (6)	T	50.5	1.87
AgrD_AgrB_E.coli	T	64.6	1.81
Ter_B0015	T	148.7	1.87

September 3rd

Experiment: Transformation

Responsables: María José

Protocol code: Trans_Ec

Protocol modifications or specifications: Samples transformed are the ligations from August 22th (Lin_S.aureus and MCS 1) and August 29th (MCS 2).

Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff + P3_GFP_Caract	T	✓	✗
Lin_C.diff + P3_TetR	T	✓	✗
Lin_C.diff + P3_ARNas_GFP	T	✓	✗
Lin_C.diff + P3_ARNas_Lis	T	✓	✗
bbT RFP (C+)	T	✓	✗
C -	T	✗	✗
AgrC_AgrD + Prom_C.diff	T	✓	✓

******Note:This ligations should not be in Tetra

September 3rd

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from August 22th (Lin_S.aureus and MCS 1) and August 29th (MCS 2).
Results:

Name	Resistance	Result	
		Red colonies	White colonies
AgrC_AgrA_WT + Prom_C.diff	T	✓	**1**
C+ (bbT RFP)	T	✓	✗
C-	T	✗	✗

- ligation should not be in Tetra

September 3rd

Experiment: Index Plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: White colonies from the Lin_S.aureus transformation of September 2nd were stroke.
Results: No results needed.

September 4th

Experiment: Index Plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: White colony from the AgrC_AgrA_WT + Prom_C.diff transformation of September 3rd were stroke.

- This plate was discarded because ligation should be in another backbone.

Results: No results needed.

September 4th

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

Ter_908	A	503	1.85
Ter_667	A	639.2	1.86
Ter_NisA	A	572	1.87
SinTer	A	490.6	1.84

September 4th

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:

Name	Resistance	To do
Lin_C.diff	A	Miniprep
Lin_S.aureus (1)	C	Miniprep
Lin_S.aureus (2)	C	Miniprep
P3_TetR	A	Miniprep
P3_GFP_Caract	A	Miniprep
P3_ARNas_GFP	C	Miniprep
P3_ARNas_Lis	C	Miniprep

Results: No results needed.

September 4th

Experiment: Restriction

Responsables: Pablo Delgado

Protocol code: Rest

Protocol modifications or specifications: None

Results:

Ligation	Resistant	Result
Lin_Sin2 + P3_GFP_Caract	C	✓
Lin_Sin2 + P3_TetR (1)	T	✗
Lin_Sin2 + P3_TetR (2)	T	✗
Lin_Sin2 + ARNas_GFP (2)	T	✗
Lin_Sin2 + ARNas_GFP (3)	T	✗
Lin_Sin2 + ARNas_Lis	T	✗

*To prove that the ligations are assembled, a digestion with BamHI and SalI can be made. The results expected must show two bands in the assembled samples (the two enzymes cut the sequence), if not, just one, corresponding to the linearized plasmid. The miniprep of the piece should be in the same agarose gel and also, the sample separately digested with both enzymes, to use them as reference.

September 4th

Experiment: Bacterial culture for AIP Extraction

Responsables: Pablo Delgado
Protocol code: standard lab procedure
Protocol modifications or specifications: 20 mL of AgrD_AgrB_E.coli was precultured overnight.
Results: Non result needed

September 5th

Experiment: Transformation
Responsables: María José
Protocol code: Trans_Ec
Protocol modifications or specifications: Sample transformed is the ligation from September 4th.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
AgrC_AgrA_WT	A	✗	✓
bbA + RFP (C+)	A	✓	✗
C-	A	✗	✗

September 5th

Experiment: Plasmid Isolation
Responsables: Pablo Delgado and María José
Protocol code: ThermoScientific Kit, K0503
Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 40µL of nuclease free water.
Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
Lin_C.diff	C	153.4	1.87
Lin_S.aureus (1)	C	147	1.86
Lin_S.aureus (2)	C	189	1.9
Lin_Sin2	A	372.6	1.85
P3_GFP_Caract	A	438.3	1.86
P3_TetR	A	201.5	1.85
P3_ARNas_GFP	C	177.8	1.85
P3_ARNas_Lis	C	187	1.86

September 5th

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications:
Results:

Part	Resistant	Result

Lin_C.diff	C	Not Checked
Lin_S.aureus (1)	C	Not Checked
Lin_S.aureus (2)	C	Not Checked
Lin_Sin2	A	Not Checked
P3_GFP_Caract	A	Not Checked
P3_TetR	A	Not Checked
P3_ARNas_GFP	C	Not Checked
P3_ARNas_Lis	C	Not Checked

September 5th

Experiment: Ligation

Responsables: Paula Thiel and Pablo Delgado

Protocol code: Lig

Protocol modifications or specifications: 1 µL of ligase (400.000 U/ml) was used in a 20uL reaction. Incubation was carried overnight at 16ºC. Ligation was prepared as shown in the next table:

Upstream part	Insert part Volume (µL)	Downstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_C.diff	8.25	P3_GFP_Caract	8.25	bbK*	0.5
Lin_C.diff	8.25	P3_TetR	8.25	bbK*	0.5
Lin_C.diff	8.25	P3_ARNas_GFP	8.25	bbK*	0.5
Lin_C.diff	8.25	P3_ARNas_Lis	8.25	bbK*	0.5
Lin_S.aureus	8.25	P3_GFP_Caract	8.25	bbK*	0.5
Lin_S.aureus	8.25	P3_TetR	8.25	bbK*	0.5
Lin_S.aureus	8.25	P3_ARNas_GFP	8.25	bbK*	0.5
Lin_S.aureus	8.25	P3_ARNas_Lis	8.25	bbK*	0.5
Lin_Sin2	8.25	P3_GFP_Caract	8.25	bbK*	0.5
Lin_Sin2	8.25	P3_TetR	8.25	bbK*	0.5
Lin_Sin2	8.25	P3_ARNas_GFP	8.25	bbK*	0.5
Lin_Sin2	8.25	P3_ARNas_Lis	8.25	bbK*	0.5
AgrC_AgrA_Wt*	8.25	Prom_C.diff*	8.25	bbK*	0.5

*Digested before this day

Results: No results needed.

September 5th

Experiment: AIP induction

Responsables: María José

Protocol code: InEc
Protocol modifications or specifications: Two treatments with 10 mL of preculture and 10 mL of LB (each one) were prepared. At the induction step, 1 mM of IPTG was added to the cultures and then, both of them were incubated for 1 hour at 37ºC. The first one was filtered with a 0.2 um filter and the second one was centrifuged.
Results: SDS-PAGE from September 6th.

September 5th

Experiment: AIP extraction
Responsables: María José
Protocol code: ExEc
Protocol modifications or specifications: Pellet from AIP induction was resuspend in 5 mL of Lysis Buffer.
Results: SDS-PAGE from September 6th.

September 6th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from September 5th.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 + P3_GFP_caract	K	✓	✓
Lin_Sin2 + P3_TetR	K	✓	✓
Lin_Sin2 + P3_ARNas_GFP	K	✓	✓
Lin_Sin2 + P3_ARNas_Lis	K	✓	✓
Lin_S.aureus + P3_GFP_caract	K	✓	✓
Lin_S.aureus + P3_TetR	K	✓	✓
Lin_S.aureus + P3_ARNas_GFP	K	✓	✓
Lin_S.aureus + P3_ARNas_Lis	K	✓	✓
Lin_C.diff + P3_GFP_caract	K	✓	✓
Lin_C.diff + P3_TetR	K	✓	✓
Lin_C.diff + P3_ARNas_GFP	K	✓	✓
Lin_C.diff + P3_ARNas_Lis	K	✓	✓
AgrC_AgrA_WT + Prom_C.diff	K	✓	✓
bbK + RFP (C+)	K	✓	✓
C-	K	✗	✓

September 6th

Experiment: Index Plate
Responsables: María José
Protocol code: standard lab procedure

Protocol modifications or specifications: Two colonies from the AgrC_AgrA_WT transformation of September 5th and August 30th were stroke.

September 6th

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

September 6th

Experiment: SDS-PAGE
Responsables: María José
Protocol code: SDSPAGE
Protocol modifications or specifications: SDS-PAGE was run with the samples of the AIP induction and extraction from September 5th. The gel was run for 30 min at 90 V and 1 hour and a half at 120 V.
Results: The pellet from AIP extraction was the only one with protein bands in this SDS-PAGE.

September 7th

Experiment: AIP induction
Responsables: María José
Protocol code: InEc
Protocol modifications or specifications: The culture was prepared with 25 mL of preculture, 25 mL of LB medium and 500 uL of ampicilin. At the induction step, 1 mM of IPTG was added and then, it was incubated overnight.
Results: The culture didn't show any fluorescence (GFP).

September 9th

Experiment: Restriction
Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications: Double amount of enzymes was used and it was incubated for 1 hour at 37°C.
Results:

Part	Enzymes	Result
Lin_S.aureus*	E+P	**Not Checked**
Lin_Sin2	E+P	**Not Checked**
AgrC_AgrA_Wt*	E+P	**Not Checked**

P3_GFP_Caract	E+P	**Not Checked**
P3_TetR	E+P	**Not Checked**

*From IDT original gBlocks

September 9th

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 in September. 1 µL of ligase (400.000 U/ml) was used in a 20uL reaction. Incubation was incubated overnight at 16ºC. Ligation was made as shown in the next table:

Upstream part	**Insert part Volume (µL)**	**Backbone**	**Backbone Volume (µL)**
Lin_C.diff	16,5	bbC RFP	0.5
Lin_S.aureus	16,5	bbC RFP	0.5
Lin_Sin2	16,5	bbC RFP	0.5
AgrC_AgrA_Wt	16,5	bbC RFP	0.5
P3_GFP_Caract	16,5	bbC RFP	0.5
P3_TetR	16,5	bbC RFP	0.5

Results: No results needed.

September 9th

Experiment: Bacterial culture for AIP Plate Induction
Responsables: María José
Protocol code: a transformant with AIP was cultured in 5mL of LB with ampicillin.
Protocol modifications or specifications: None
Results: No results needed.

September 10th

Experiment: AIP plate induction
Responsables: María José
Protocol code: InEc_plate
Protocol modifications or specifications: 100 uL preculture was inoculated in two plates with 1mM of IPTG, 10 mL of LB medium and 100 uL of ampicillin.
Results: Colonies didn't show any fluorescence (GFP).

September 11th

Experiment: Transformation
Responsables: Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from September 5th and September 9th.
Results: Kanamycin plates got contaminated.

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 + P3_GFP_Caract	K	✓	✓
Lin_Sin2 + P3_TetR	K	✓	✓
Lin_Sin2 + P3_ARNas_GFP	K	✓	✓
Lin_Sin2 + P3_ARNas_Lis	K	✓	✓
bbK + RFP (C+)	K	✓	✓
C-	K	✗	✓
Lin_C.diff	C	✓	✗
Lin_S.aureus	C	✓	✗
Lin_Sin2	C	✓	✓
AgrC_AgrA_Wt	C	✓	✗
P3_GFP_Caract	C	✓	✓
P3_TetR	C	✓	✓
bbC + RFP (C+)	C	✓	✗
C-	C	✗	✗

September 11th

Experiment: AIP plate induction
Responsables: Pablo Delgado
Protocol code: InEc_plate
Protocol modifications or specifications: 100 uL preculture was inoculated in two plates with 1mM of IPTG, 10 mL of LB medium and 100 uL of ampicillin.
Results: Colonies didn't show any fluorescence (GFP).

September 11th

Experiment: AIP induction
Responsables: María José
Protocol code: InEc
Protocol modifications or specifications: The culture was prepared with 5 mL of preculture, 50 mL of LB medium and 500 uL of ampicillin. At the induction step, 1 mM of IPTG was added and then, it was incubated overnight.
Results: The culture didn't show any fluorescence (GFP).

September 12th

Experiment: Index Plate

Responsables: Paula Thiel
Protocol code: standard lab procedure
Protocol modifications or specifications: White colonies from the transformation of September 11th were stroke.
Results:

Name	Number in index plate	Resu
Lin_Sin2	1	White
P3_TetR	1-12	Red colonies: 6 and 10 White colonies: 1-5, 7-9 an
P3_GFP_caract	1-8	White colonies:1-8

September 12th

Experiment: Streaking
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: One colony of BL21 *E. coli* was stroke.
Results: No results needed.

September 15th

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: Colonies number 2 and 3 from P3_GFP_Caract and P3_TetR index plates, and number 1 from Lin_Sin2 index plate were cultured.
Results: No results needed.

September 15th

Experiment: Transformation
Responsables: María José
Protocol code: Trans_Ec
Protocol modifications or specifications: AgrD_AgrB_E.coli miniprep from September 3rd was transformed in a BL21 strain.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
AgrD_AgrB_E.coli	A	✗	✓
bbA + RFP (C+)	A	✓	✗
C-	A	✗	✗

September 16th

Experiment: Plasmid Isolation

Responsables: Paula Thiel

Protocol code: ThermoScientific Kit, K0503

Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 40µL of nuclease free water.

Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
Lin_Sin2	C	276.6	1.85
P3_GFP_Caract (1)	C	411.6	1.84
P3_GFP_Caract (2)	C	350.5	1.85
P3_GFP_Caract (3)	C	261.0	1.84
P3_TetR (2)	C	595.7	1.85

September 16th

Experiment: Transformation

Responsables: Pablo Delgado

Protocol code: Trans_Ec

Protocol modifications or specifications: Samples transformed were all the ligations in stock. Transformations were done with 3uL of ligation.

Results:

Name	Resistance	Result	
		Red colonies	White colonies
Lin_C.diff	C	✓	✗
Lin_C.diff	C	✓	✗
Lin_C.diff	C	✓	✗
Lin_S.aureus	C	✓	✓
Lin_S.aureus	C	✓	✓
Lin_S.aureus	C	✓	✗
Lin_S.aureus	C	✓	✗
Lin_S.aureus	C	✓	✗
AgrC_AgrA_Wt	C	✓	✓
AgrC_AgrA_Wt	C	✓	✓
AgrC_AgrA_Wt	C	✓	✓
AgrC_AgrA_Wt	C	✓	✗
AgrC_AgrA_Wt	C	✓	✗
bbC + RFP (C+)	C	✓	✗
C-	C	✗	✗

September 16th

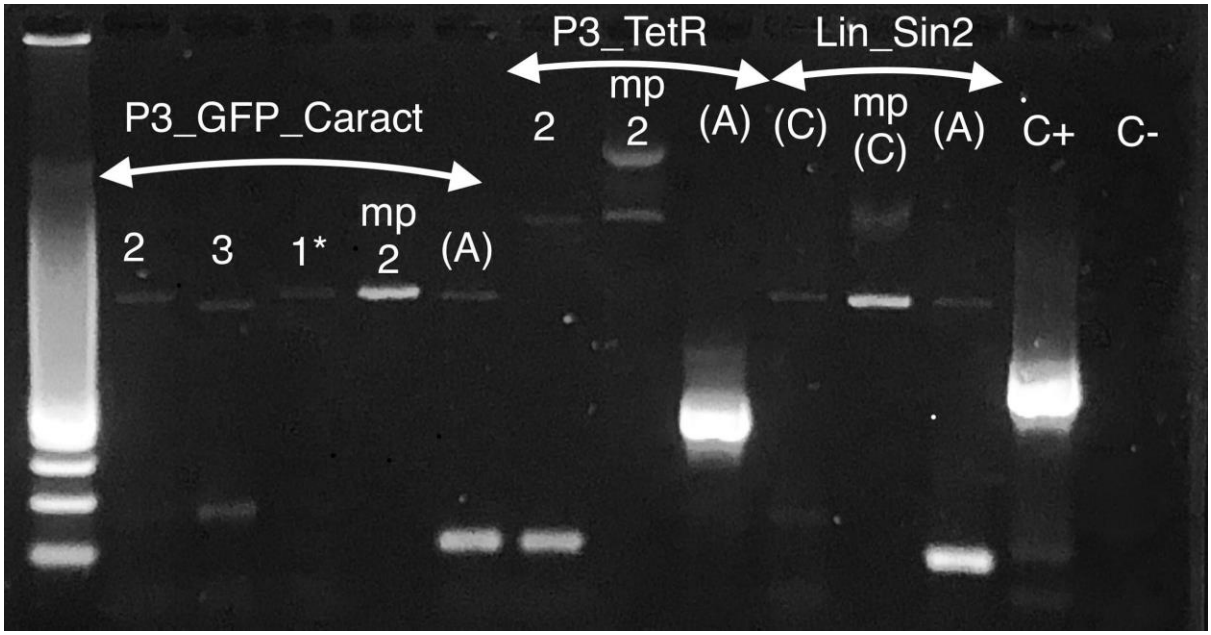
Experiment: PCR

Responsables: Paula and Pablo

Protocol code: PCR

Protocol modifications or specifications: Minipreps from September 16th and September 5th were amplified. Each reaction was supplemented with 1uL of $MgCl_2$ and they were amplified using V2F and VR primers. The thermocycler program was set with 30 sec of alignment at 56°C and 2:45 min of extension. Products were run in a 1.0% agarose gel at 80V for 1 hour.

Results:



September 16th

Experiment: AIP plate induction

Responsables: Paula Thiel

Protocol code: InEc_plate

Protocol modifications or specifications: 100 uL preculture was inoculated in a plate with 1mM of IPTG, 10 mL of LB medium and 100 uL of ampicillin.

Results: Non green colonies were present.

September 17th

Experiment: AIP induction

Responsables: María José

Protocol code: InEc

Protocol modifications or specifications: The culture was prepared with 10 mL of preculture, 90 mL of LB medium and 1000 uL of ampicillin. At the induction step, 1 mM of IPTG was added and then, it was incubated for 1 hour. No positive or negative culture was made.

Results: The culture didn't show any fluorescence (GFP).

September 17th

Experiment: AIP extraction

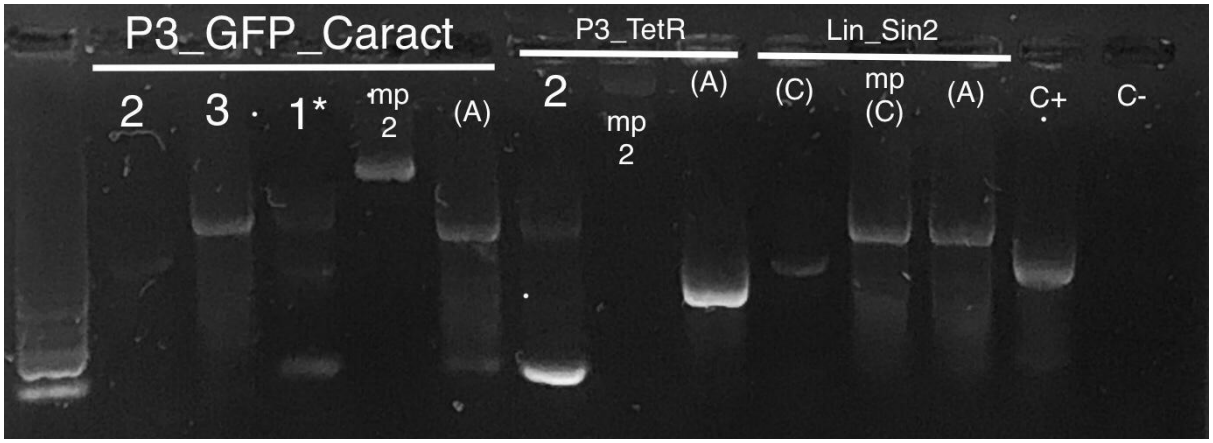
Responsables: María José

Protocol code: ExEc

Protocol modifications or specifications: Pellets from AIP induction were resuspended in 10 mL of Lysis Buffer. Before centrifugation, all resuspended pellets were boiled for 30 min.
Results: SDS-PAGE from September 21th.

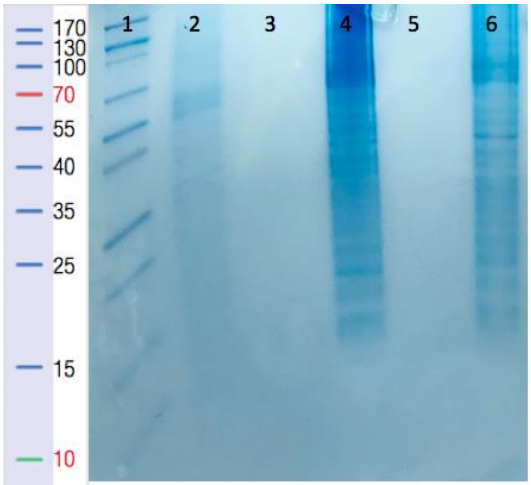
September 17th

Experiment: PCR
Responsables: Paula and María José
Protocol code: PCR
Protocol modifications or specifications: Minipreps from September 16th and September 5th were amplified. Each reaction was supplemented with 1uL of $MgCl_2$ and they were amplified using V2F and VR primers. The thermocycler program was set with 30 sec of alignment at 56°C and 2:45 min of extension. Products were run in a 1.0% agarose gel at 80V for 1 hour.
Results:



September 18th

Experiment: SDS-PAGE
Responsables: María José
Protocol code: SDSPAGE
Protocol modifications or specifications: A SDS-PAGE was run with the samples of the AIP induction and extraction from September 17th. The gel was run for 20 min at 90 V and 80 min at 120 V.
Results:



(1) Molecular weight marker. (2) AIP culture after induction (3) AIP extracell proteins. (4) AIP intracell proteins. (5) AIP soluble intracell proteins after extraction. (6) AIP insoluble intracellular proteins after extraction.

September 18th

Experiment: Index Plate
Responsables: María José
Protocol code: standard lab procedure
Protocol modifications or specifications: One white colony from the transformation of AgrD_AgrB_E.coli (Ampi) in BL21 strain was stroke.
Results: no results needed

September 18th

Experiment: Index Plate
Responsables: Paula Thiel
Protocol code: standard lab procedure
Protocol modifications or specifications: White colonies from the transformation of September 11th were stroke.
Results:

Name	Number in index plate	Results
Lin_C.diff	Non	Part lost
Lin_S.aureus	1-2	White colony: 2 Red colony: 1
AgrC_AgrA_Wt	1-5	White colony: 4 Red colonies: 1-3,5

September 18th

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: Samples transformed are the ligations from september 9th.
Results:

Name	Resistance	Result	
		Red colonies	**White colonies**
Lin_Sin2 (C)	C	✗	2
bbC + RFP (C+)	C	✓	✗
C-	C	✗	✗

September 18th

Experiment: Ligation
Responsables: Pablo Delgado

Protocol code: Lig

Protocol modifications or specifications: 1 µL of ligase (400.000 U/ml) was used in 20uL of reaction. Incubation was incubated overnight 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 (A)	8.25	P3_GFP_Caract (A)	8.25	bbC	0.5

Results: no results needed

September 18th

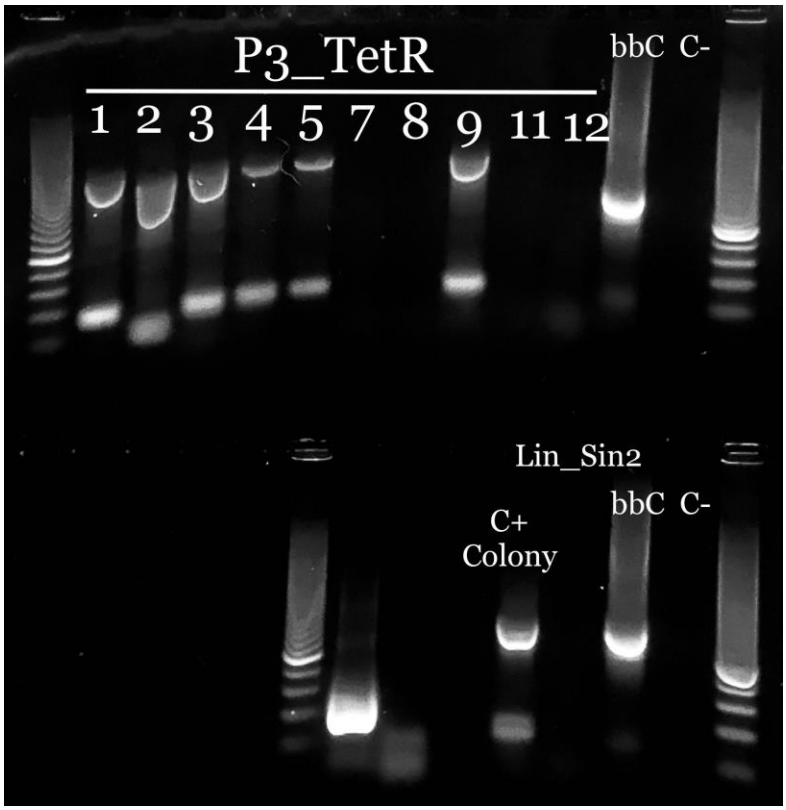
Experiment: Colony PCR

Responsables: Pablo Delgado

Protocol code: Col_PCR

Protocol modifications or specifications: Index plate from September 12th was amplified. Thermo Master Mix 2X was use with V2F and VR primers, 2 uL of supernatant of cold-hot treatment of the colonies in 25uL of NFW was used. The thermocycler program was set with 30 sec in denaturing, 30 sec of alignment at 56°C and 2:45 min of extension. Products were run in a 1.0% agarose gel at 80V for 2 hours.

Results:



September 19th

Experiment: Transformation

Responsables: Pablo Delgado

Protocol code: Trans_Ec

Protocol modifications or specifications: Samples transformed are the ligations from September 18th.

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 (A) + P3_GFP_Caract (A)	C	✓	
bbC + RFP (C+)	C	✓	✗
C-	C	✗	✗

September 19th

Experiment: Plasmid Isolation

Responsables: María José Durán

Protocol code: ThermoScientific Kit, K0503

Protocol modifications or specifications: At the final step, plasmidial DNA was eluted using 40µL of nuclease free water.

Results:

Name	Resistance	Concentration (ng/uL)	Absorbance
AgrD_AgrB_BL21	A	251.4	1.85

September 19th

Experiment: AIP induction

Responsables: María José

Protocol code: InEc

Protocol modifications or specifications: The culture was prepared with 10 mL of preculture, 90 mL of LB medium and 1000 uL of ampicillin. At the induction step, 1 mM of IPTG was added and then incubated for 1 hour. The positive and negative control was prepared with 1 mL of preculture, 9 mL of LB and 100 uL of ampicillin.

Results: The culture didn't show any fluorescence (GFP). Another protein induction with a C+ and a C- with same volume needs to tested to have a better comparison.

September 19th

Experiment: Colony PCR

Responsables: Paula Thiel and Pablo Delgado

Protocol code: Col_PCR

Protocol modifications or specifications: White colonies from index plate (September 18th) were amplified. Thermo Master Mix 2X was used with V2F and VR primers. 2 uL of supernatant of cold-hot treated colonies (resuspended in 25 uL of NFW) was added in the respective reaction. Also, a PCR of the miniprep from September 19th was done. The thermocycler program was set with 30 sec in denaturing, 30 sec of alignment at 56°C and 2:45 min of extension. Products were run in a 1.0% agarose gel at 80V for 1 hour.

Results: no expected gel bands were observed.

September 19th

Experiment: Index Plate
Responsables: Paula Thiel
Protocol code: standard lab procedure
Protocol modifications or specifications: White colonies from the transformation of September 18th were stroke.
Results:

Name	Number in index plate	Results
Lin_Sin2	1-3	White colony: 2 Red colonies: 1 and 3

September 19th

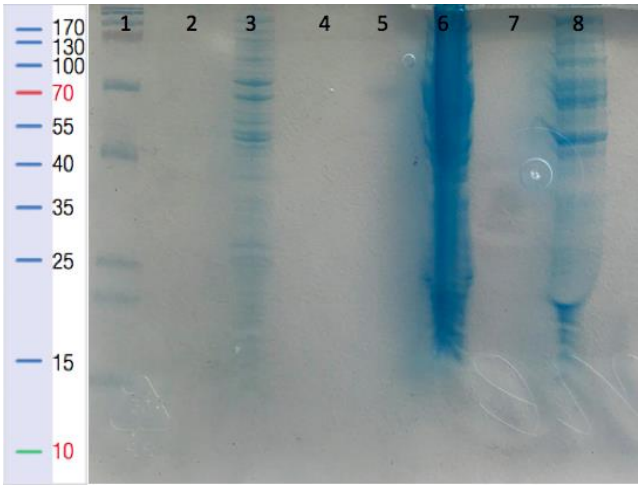
Experiment: Bacterial culture for plasmid isolation
Responsables: Paula Thiel
Protocol code: 5mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with one transformed colony.
Protocol modifications or specifications: Colony number 2 of Lin_S.aureus, number 4 of AgrC_AgrA_WT and number 2 Lin_Sin2 were cultured.
Results: No results needed.

September 20th

Experiment: AIP extraction
Responsables: María José
Protocol code: ExEc
Protocol modifications or specifications: Pellet from AIP induction was resuspend in 5 mL of Lysis Buffer.
Results: SDS-PAGE from September 21th.

September 21st

Experiment: SDS-PAGE
Responsables: María José
Protocol code: SDSPAGE
Protocol modifications or specifications: A SDS-PAGE was run with the samples of the AIP induction and extraction from September 20th. The gel was run for 30 min at 90 V and 90 min at 120 V.
Results:



(1) Molecular weight marker. (2) C+ soluble intracellular proteins after extraction. (3) C+ insoluble intracellular proteins after extraction. (4) AIP centrifuged supernatant after induction. (5) AIP soluble intracellular proteins after extraction. (6) AIP insoluble intracellular proteins after extraction. (7) AIP insoluble intracellular proteins after extraction. (8) C- soluble intracellular proteins after extraction. (9) C- insoluble intracellular proteins after extraction.

September 23rd

Experiment: Restriction

Responsables: Pablo Delgado

Protocol code: Rest

Protocol modifications or specifications: Double amount of enzymes was used and it was incubated for 30 min at 37°C.

Results:

Part	Enzymes	Result
Lin_Sin2 (C)	E+S	✓
P3_ARNas_GFP (C)	X+P	✓
P3_ARNas_Lis (C)	X+P	✓

September 23rd

Experiment: Ligation

Responsables: Pablo Delgado

Protocol code: Lig

Protocol modifications or specifications: Enzyme T4 used was a new one bought from NEB in September. 1 µL of ligase (400.000 U/ml) was used in 20uL of reaction. Incubation was incubated overnight 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 (C)	8.25	P3_ARNas_GFP (C)	8.25	bbA	0.5
Lin_Sin2 (C)	8.25	P3_ARNas_Lis (C)	8.25	bbA	0.5

Results: No results needed.

September 24th

Experiment: Ligation Restriction for confirmation

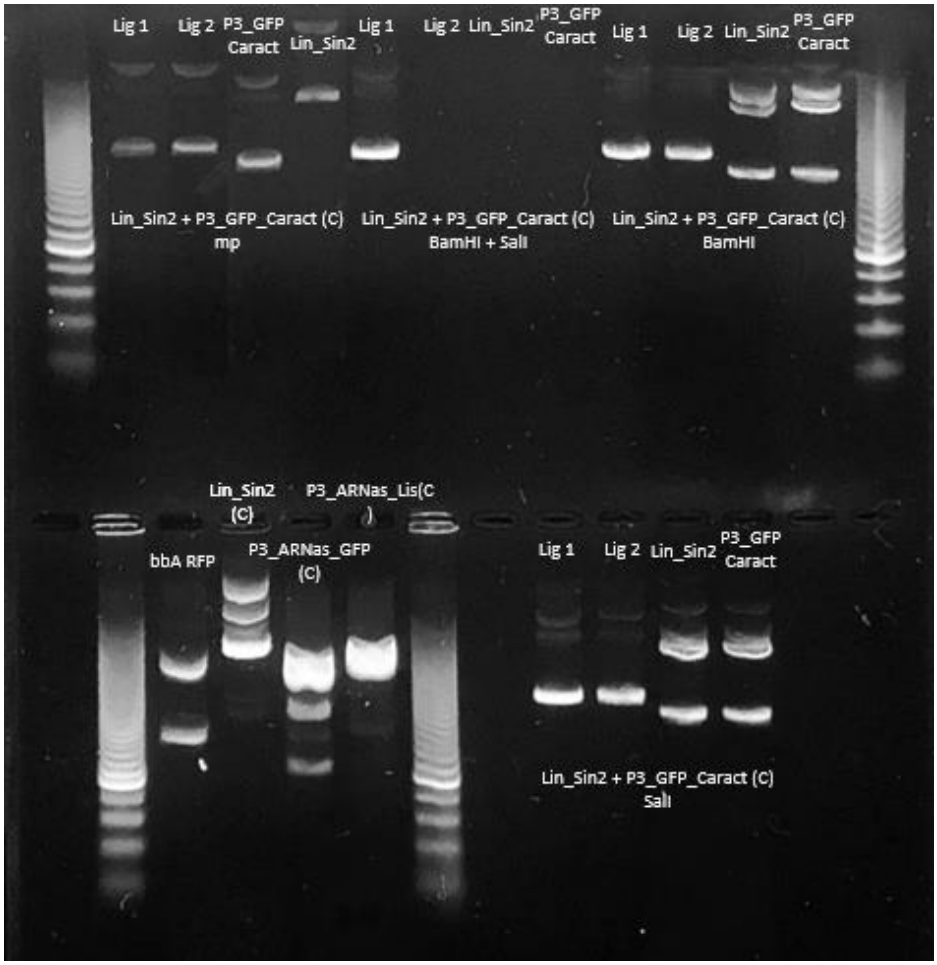
Responsables: Pablo Delgado

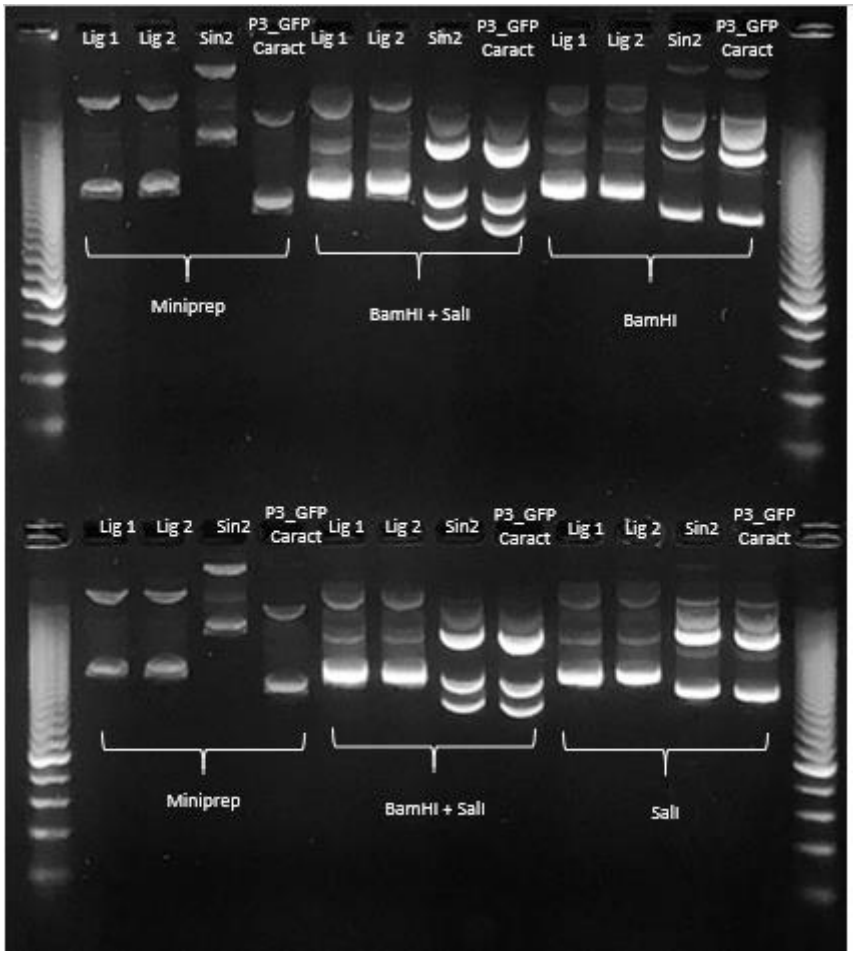
Protocol code: Lig

Protocol modifications or specifications: Reaction was incubated at 37°C for 30min.

Results:

Part	Enzymes	Number of Bands
mp 1 - Lin_Sin2 + P3_GFP_Caract (C)	Non	**2**
mp 2 - Lin_Sin2 + P3_GFP_Caract (C)	Non	**2**
mp Lin_Sin2 (A)	Non	**2**
mp P3_GFP_Caract (A)	Non	**2**
1 - Lin_Sin2 + P3_GFP_Caract (C)	BamHI + SalI	**2**
2 - Lin_Sin2 + P3_GFP_Caract (C)	BamHI + SalI	**0**
Lin_Sin2 (A)	BamHI + SalI	**0**
P3_GFP_Caract (A)	BamHI + SalI	**0**
1 - Lin_Sin2 + P3_GFP_Caract (C)	BamHI	**1**
2 - Lin_Sin2 + P3_GFP_Caract (C)	BamHI	**1**
Lin_Sin2 (A)	BamHI	**3**
P3_GFP_Caract (A)	BamHI	**3**
1 - Lin_Sin2 + P3_GFP_Caract (C)	SalI	**3**
2 - Lin_Sin2 + P3_GFP_Caract (C)	SalI	**3**
Lin_Sin2 (A)	SalI	**3**
P3_GFP_Caract (A)	SalI	**3**


Lig 1
Lig 2
P3_GFP Caract
Lin_Sin2
Lig 1
Lig 2
Lin_Sin2
P3_GFP Caract
Lig 1
Lig 2
Lin_Sin2
P3_GFP Caract
Lin_Sin2 + P3_GFP_Caract (C) mp
Lin_Sin2 + P3_GFP_Caract (C) BamHI + SalI
Lin_Sin2 + P3_GFP_Caract (C) BamHI
Lin_Sin2 (C)
P3_ARNas_Lis(C)
bbA RFP
P3_ARNas_GFP (C)
Lig 1
Lig 2
Lin_Sin2
P3_GFP Caract
Lin_Sin2 + P3_GFP_Caract (C) SalI



September 24th

Experiment: PCR

Responsables: Pablo Delgado

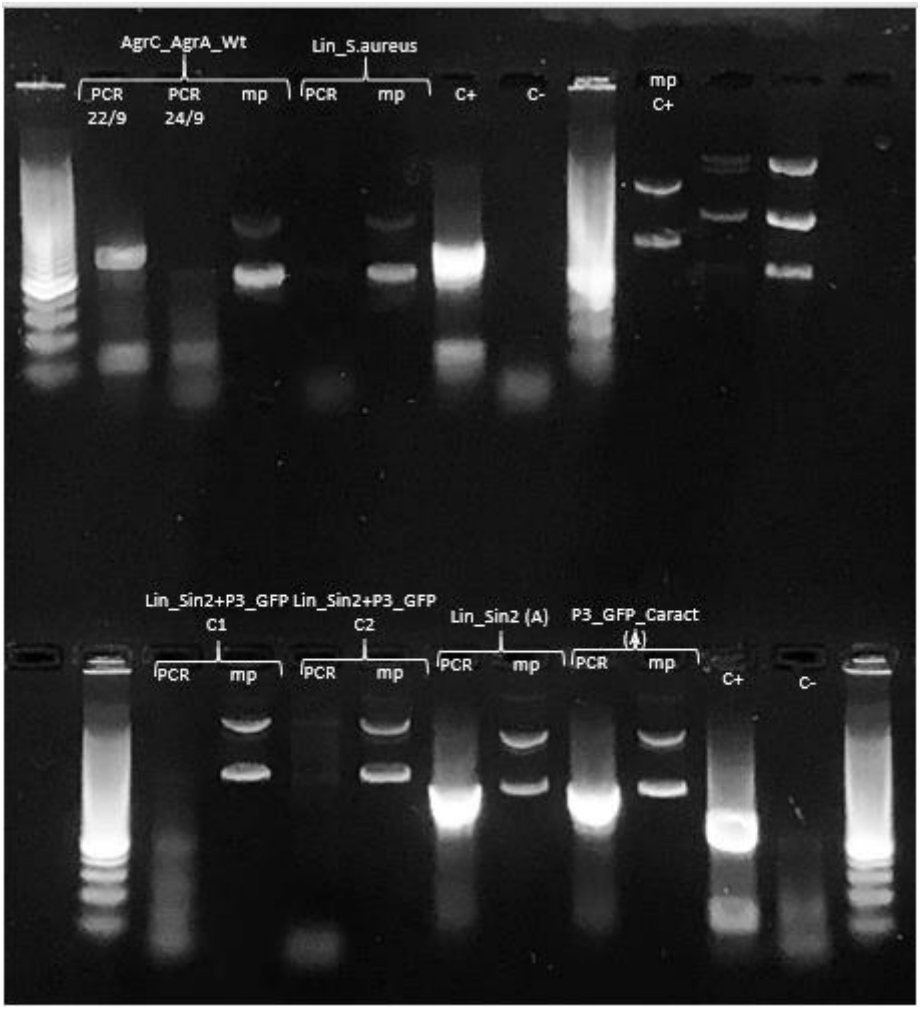
Protocol code: PCR

Protocol modifications or specifications: Two reactions were established, first one amplifying AgrC_AgrA_Wt (C) from September 22th and Lin_S.aureus (C) from today's miniprep, with its respective controls. The thermocycler program was set with 30 sec in denaturing, 30 sec of alignment at 56°C and 2:45 min of extension.

In the second reaction the two ligation from september 22th Lin_Sin2 + P3_GFP_Caract (C) were amplified, with its respective controls. 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% agarose gel at 80V for 1 hour.

Results:



September 24th

Experiment: Transformation

Responsables: Pablo Delgado

Protocol code: Trans_Ec

Protocol modifications or specifications: Samples transformed are the ligations from September 18th.

Name	Resistance	Result	
		Red colonies	White colonies
Lin_Sin2 (A) + P3_GFP_Caract (A)	C	✓	✓
bbC + RFP (C+)	C	✓	✗
C-	C	✗	✗
Lin_Sin2 (C) + P3_ARNas_GFP (C)	A	✓	✓
Lin_Sin2 (C) + P3_ARNas_Lis (C)	A	✓	✓
bbA + RFP (C+)	A	✓	✗
C-	A	✗	✗

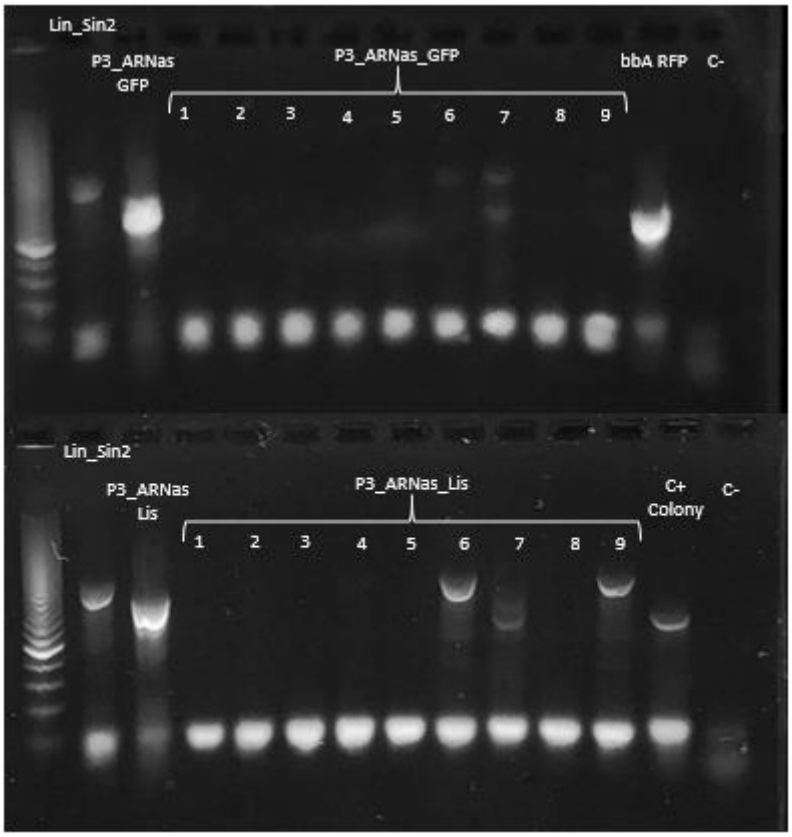
September 25th

Experiment: Colony PCR

Responsables: Pablo Delgado

Protocol code: Col_PCR

Protocol modifications or specifications: Ten white colonies from Lin_Sin2 (C) + P3_ARNas_GFP (C) and ten from Lin_Sin2 (C) + P3_ARNas_Lis (C) were resuspended in 25 uL of NFW, heated 20 min at ~90°C, 20min at -20°C and heated again 20min at ~90°C,. Then, the debris was formed by centrifuging 2 min at 7000 g and the supernatant was used as DNA template for the PCR. The thermocycler program was set with 1 hour at 25°C, 1 min of denaturalization, 2 min of alignment at 56°C and 3:45 min of extension.
Products were run in a 1% agarose gel at 80V for 2 hours.
Results: 3 colonies look like they have the insert



September 25th

Experiment: Bacterial culture for plasmid isolation
Responsables: Pablo Delgado
Protocol code: 10 mL of Luria Bertani (LB) culture media supplemented with the respective antibiotic were inoculated with samples Lin_Sin2+P3_ARNas_GFP (A) (colony 7) Lin_Sin2+P3_ARNas_Lis (A) (colonies 6 and 9), according with today's Colony PCR.
Protocol modifications or specifications: None.
Results: No results needed.

September 25th

Experiment: AIP plate induction
Responsables: María José
Protocol code: InEc_plate
Protocol modifications or specifications: 100 uL preculture was inoculated in a plate with 1mM of IPTG, 10 mL of LB medium and 100 uL of ampicillin. Positive control was ILC's transformants in a plate

with LB and chloramphenicol and the negative control, AgrA_AgrC_WT's transformants in a plate with IPTG, LB and ampicillin.
Results: No induction was observed.

September 25th

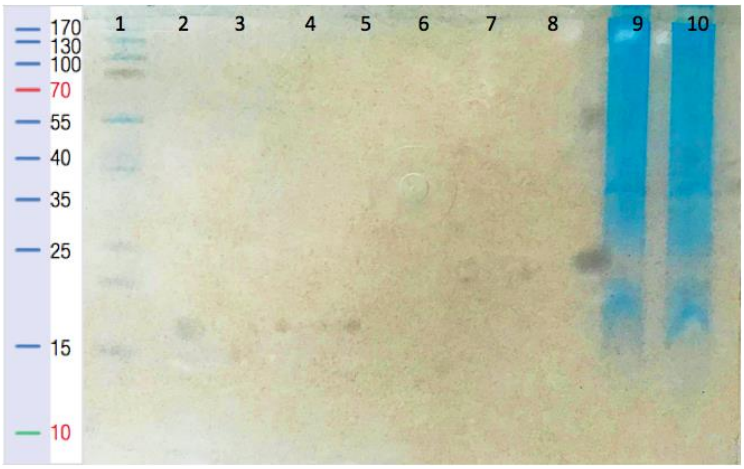
Experiment: AIP induction
Responsables: María José
Protocol code: InEc
Protocol modifications or specifications: The culture was prepared with 10 mL of preculture, 90 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM of IPTG was added and then, it was incubated 6 hours. The positive control (ILC2) and negative control (AIP not induced) were prepared with 10 mL of preculture and 90 mL of LB and 100 mg/mL of ampicillin. After incubation, 30 mL of AIP induction were filtered with a 0.22 um filter and the others 70 mL were centrifuged.
Results: no results needed.

September 25th

Experiment: AIP extraction
Responsables: María José
Protocol code: ExEc
Protocol modifications or specifications: Pellets from AIP induction (AIP, C+, C-) were resuspended in 10 mL of Lysis Buffer. Before centrifugation, all resuspended pellets were boiled for 30 min.
Results: SDS-PAGE from September 26th.

September 26th

Experiment: SDS-PAGE
Responsables: María José
Protocol code: SDSPAGE
Protocol modifications or specifications: A SDS-PAGE was run with the samples of the AIP induction and extraction from September 25th. The gel was run for 30 min at 90 V and 90 min at 120 V.
Results:



(1) Molecular weight marker. (2) AIP filtered supernatant after induction. (3) C- filtered supernatant after induction. (4) AIP centrifuged supernatant after induction. (5) C- centrifuged supernatant after induction. (6) AIP soluble intracellular proteins after extraction. (7) C+ soluble intracellular proteins

after extraction. (8) C- soluble intracellular proteins after extraction. (9) AIP insoluble intracellular proteins after extraction. (10) C+ insoluble intracellular proteins after extraction.