June

June 3rd

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 May 31st were cultured: B0015, R0040, E0840, R0010, I13504, E0240, bbC RFP and bbA RFP.
Results: No results needed.

June 3rd

Experiment: Streaking
Responsables: Paula Thiel
Protocol code: One colony was stroke in a LB plate with the respective antibiotic.
Protocol modifications or specifications: None.
Results:

Name	Resistance	Result
J23100	A	✓
J23101	A	✓
J23110	A	✓

June 4th

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
B0015	C	100.5	1.84
R0040	C	122.2	1.86
E0840	C	193.0	1.86
R0010	C	193.7	1.78
I13504	C	157.0	1.81
E0240	C	154.9	1.83
pCloranfenicol	C	203.9	1.84
pAmpicilina	A	235.9	1.85

June 4th

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

Name	Resistance	Result
E0040	A	✓
J23108	A	✗
J23114	A	✓
C+	A	✓
C-	A	✗

June 5th

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 June 4th were cultured: J23114 and E0040. As well as the following colonies from June 3rd plate streaking: J23100, J23101 and J23110.
Results: No results needed.

June 6th

Experiment: Plasmid Isolation
Responsables: Paula Flores
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
E0040	A	277.3	1.86
J23100	A	439.1	1.86
J23101	A	344.7	1.86
J23110	A	388.9	1.86
J23114	A	383.7	1.87

June 6th

Experiment: *L. casei* ATCC 0334 growth kinetics
Responsables: Anthony Mora
Protocol code: GC_Lacto
Protocol modifications or specifications: None
Results: It need to be repeated because the initial OD was too high.

June 7th

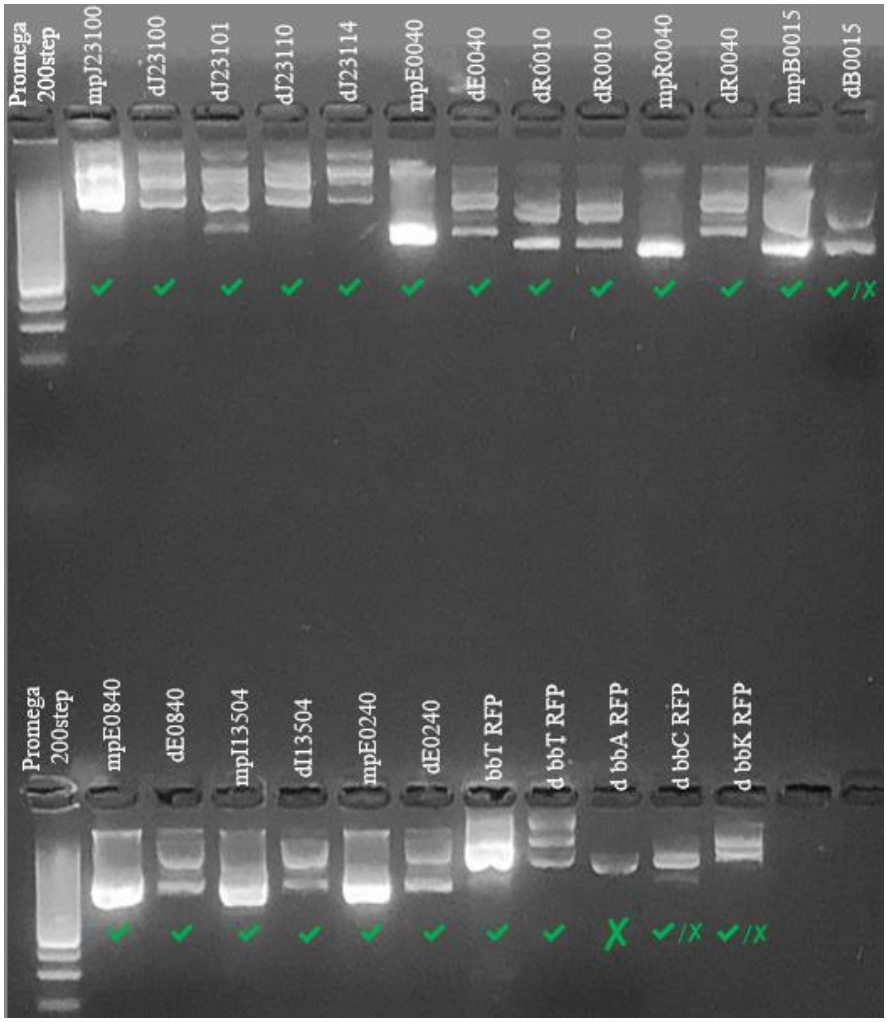
Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications: None
Results:

Name	Resistance	Result
J23101	A	✗
J23108	A	✗
C+	A	✓
C-	A	✗

June 7th

Experiment: Restriction
Responsables: Pablo Delgado and Paula Thiel
Protocol code: Rest
Protocol modifications or specifications: Samples were run in 1.5% agarose gel for 40 minutes at 80V.
Results:

Part	Enzymes	Result
E0040	E+S	✓
B0015	X+P	Uncertain
I13504	X+P	✓
E0240	X+P	✓
E0840	X+P	✓
J23100	E+S	✓
J23101	E+S	✓
J23110	E+S	✓
J23114	E+S	✓
R0010	E+S	✓
R0040	E+S	✓
bbA RFP	E+P	✗
bbC RFP	E+P	Uncertain
bbT RFP	E+P	✓
bbK RFP	E+P	Uncertain



June 7th

Experiment: Ligation

Responsables: Pablo Delgado and Paula Thiel

Protocol code: Lig

Protocol modifications or specifications: Ligation was made as shown in the next table:

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
E0040	3	**B0015**	1	**bbT RFP**	3
J23100	1	**I13504**	3.5	**bbT RFP**	3
J23100	1	**E0840**	3.5	**bbT RFP**	3
J23100	1	**E0240**	3.5	**bbT RFP**	3
J23101	1	**I13504**	3.5	**bbT RFP**	3
J23101	1	**E0840**	3.5	**bbT RFP**	3
J23101	1	**E0240**	3.5	**bbT RFP**	3
J23110	1	**I13504**	3.5	**bbT RFP**	3
J23110	1	**E0840**	3.5	**bbT RFP**	3
J23110	1	**E0240**	3.5	**bbT RFP**	3
J23114	1	**I13504**	3.5	**bbT RFP**	3

J23114	1	**E0840**	3.5	**bbT RFP**	3
J23114	1	**E0240**	3.5	**bbT RFP**	3
R0010	1	**I13504**	3.5	**bbT RFP**	3
R0010	1	**E0840**	3.5	**bbT RFP**	3
R0010	1	**E0240**	3.5	**bbT RFP**	3
R0040	1	**I13504**	3.5	**bbT RFP**	3
R0040	1	**E0840**	3.5	**bbT RFP**	3
R0040	1	**E0240**	3.5	**bbT RFP**	3

Results: No results needed.

June 7th

Experiment: Twist Clonal Genes resuspension
Responsables: Pablo Delgado
Protocol code: As indicated by the manufacturer
Protocol modifications or specifications: First 4 terminators came from Twist Bioscience, those were resuspended in Tris-EDTA Buffer pH8.0 up to around 200 ng/µL.
Results: No results needed.

Twist code	Laboratory name	Resistance	Twist mass sended (ng)	Volume added (uL)	Concentration (ng/uL)
Terminador_908	**Ter_908**	A	3971	19.9	199.54
Terminador_Control	**sinTer**	A	6512	32.5	200.37
Terminador_NisA	**Ter_NisA**	A	4344	21.7	200.18
Terminador_667	**Ter_667**	A	5238	26.2	199.92

June 7th

Experiment: Transformation
Responsables: Pablo Delgado and Paula Thiel
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations and Clonal Genes resuspension were transformed, 2uL were added to the cells.
Results:

Name	Resistance	Result
E0040 + B0015	T	✗
J23100 + I13504	T	✗
J23100 + E0840	T	✗
J23100 + E0240	T	✗
J23101 + I13504	T	✗
J23101 + E0840	T	✗
J23101 + E0240	T	✗
J23110 + I13504	T	✗
J23110 + E0840	T	✗

J23110 + E0240	T	✗
J23114 + I13504	T	✗
J23114 + E0840	T	✗
J23114 + E0240	T	✗
R0010 + I13504	T	✗
R0010 + E0840	T	✗
R0010 + E0240	T	✗
R0040 + I13504	T	✗
R0040 + E0840	T	✗
R0040 + E0240	T	✗
C +	T	✓
C -	T	✗
Ter_908	A	✓
sinTer	A	✓
Ter_NisA	A	✓
Ter_667	A	✓
C +	A	✓
C -	A	✗

June 10th

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

Name	Resistance	Concentration (ng/uL)	Absorbance
Ter_908	A	533.7	1.89
sinTer	A	557.4	1.88
Ter_NisA	A	460.0	1.87
Ter_667	A	260.0	1.89

June 17th

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

Name	Resistance	Result
K358006	C	✓
C+	C	✓
C-	C	✓ (few colonies grew)

June 18th

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 June 17th were cultured: K358006 and bbC RFP.

Results: No results needed.

June 20th

Experiment: Plasmid isolation

Responsables: Pablo Delgado

Protocol code: ThermoScientific Kit, K0503

Protocol modifications or specifications: Cultures were harvest on June 19th and the pellet was conserved at 4°C. At the final step, plasmidial DNA was recovered using 30µL of nuclease free water.

Results: Shown below

Name	Resistance	Concentration (ng/uL)	Absorbance
bbC RFP	C	452.9	1.87
K358006 - 1	C	338.9	1.87
K358006 - 2	C	396.6	1.88
K358006 - 3	C	394.9	1.87

June 20th

Experiment: Restriction

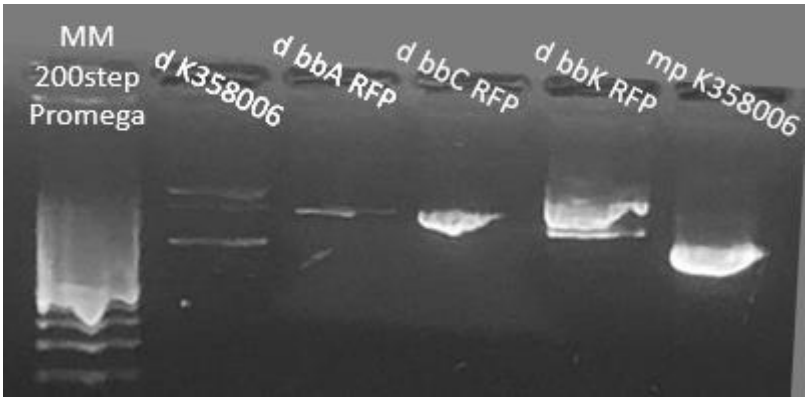
Responsables: Paula Thiel and María José

Protocol code: Rest

Protocol modifications or specifications: Samples were run in 1.5% agarose gel for 30 minutes at 90V.

Results:

Part	Enzymes	Result
K358006	X+P	✓
bbA RFP	E+P	Uncertain
bbC RFP	E+P	Uncertain
bbK RFP	E+P	✓



June 20th

Experiment: Ligation

Responsables: Pablo Delgado and Paula Thiel

Protocol code: Lig

Protocol modifications or specifications: Ligation was made as shown in the next table:

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
R0010	7.5	**K358006**	7.5	**bbA RFP**	1.5
R0040	7.5	**K358006**	7.5	**bbA RFP**	1.5
J23100	7.5	**I13504**	7.5	**bbT RFP**	1.5
J23100	6	**I13504**	6	**bbT RFP**	2

Results: No results needed.

June 20th

Experiment: Transformation

Responsables: Pablo Delgado

Protocol code: Trans_Ec

Protocol modifications or specifications: Incubation took 16 hours at 37°C and plates were placed under day light at room temperature for a better visualization of RFP negative ligation.

Results:

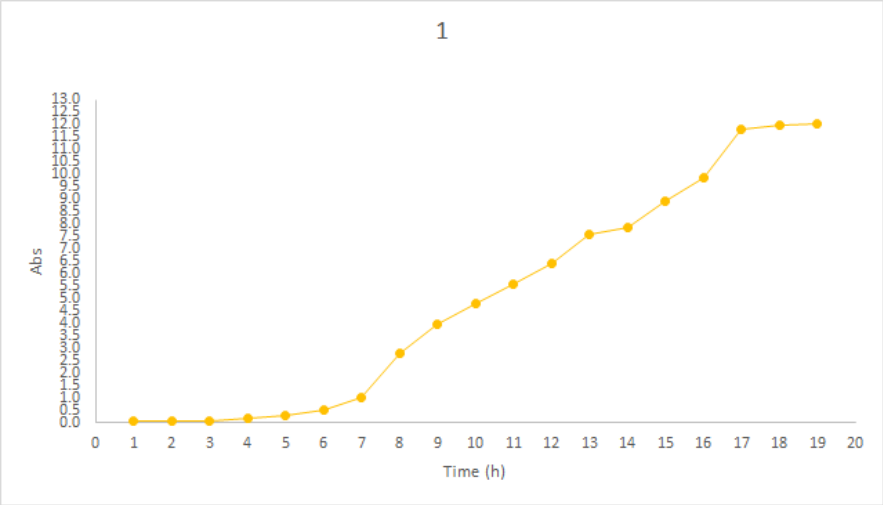
Name	Resistance	Result	
		Red colonies	White colonies
R0010 + K358006	A	✓	✓
R0040 + K358006	A	✓	✓
C+	A	✓	✗
C-	A	✗	✓ (Two little white colonies grew)
J23100 + I13504	T	✓	✗
J23100 + I13504	T	✓	✗
C+	T	✓	✓
C-	T	✗	✗

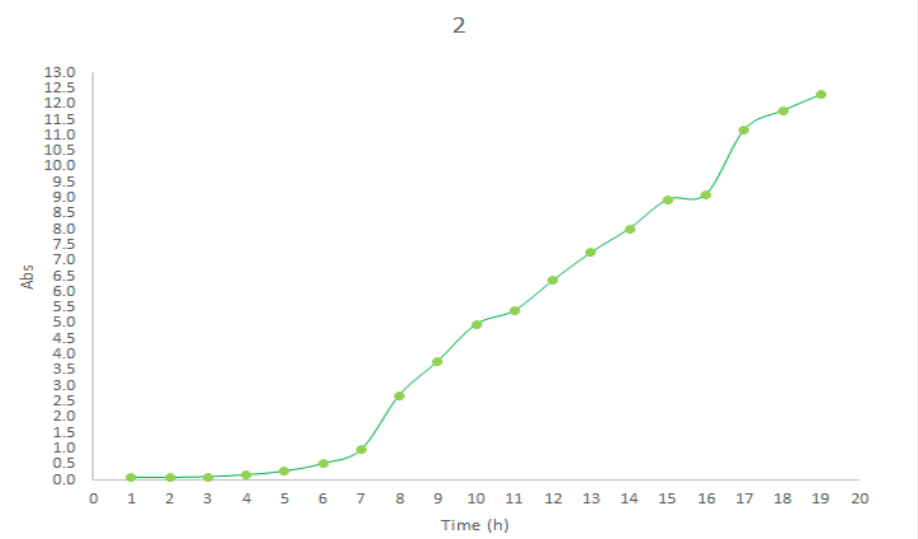
June 21st

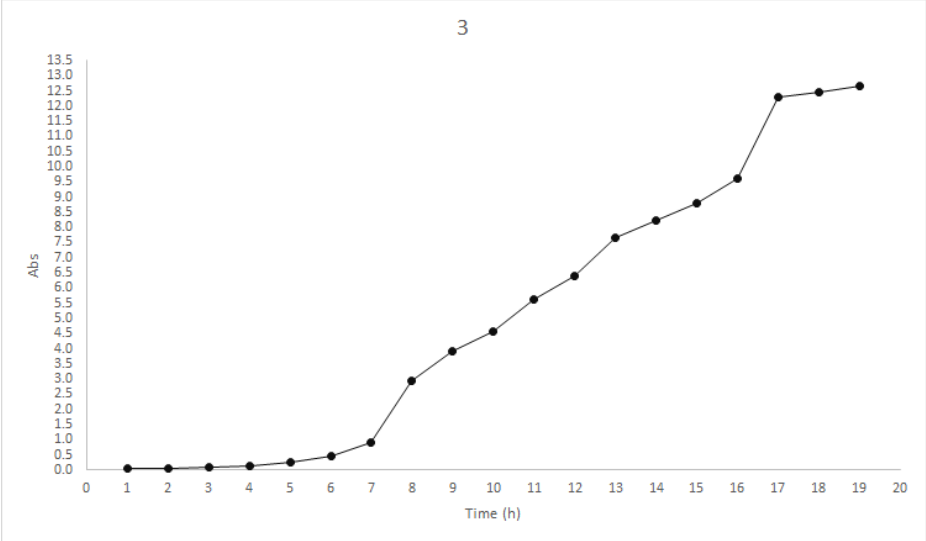
Experiment: *L. casei* ATCC 0334 growth kinetics
Responsables: Anthony Mora
Protocol code: GC_Lacto
Protocol modifications or specifications: Incubation took 19 hours at 37°C, 120 rpm and OD was measured by spectrophotometer.
Results:

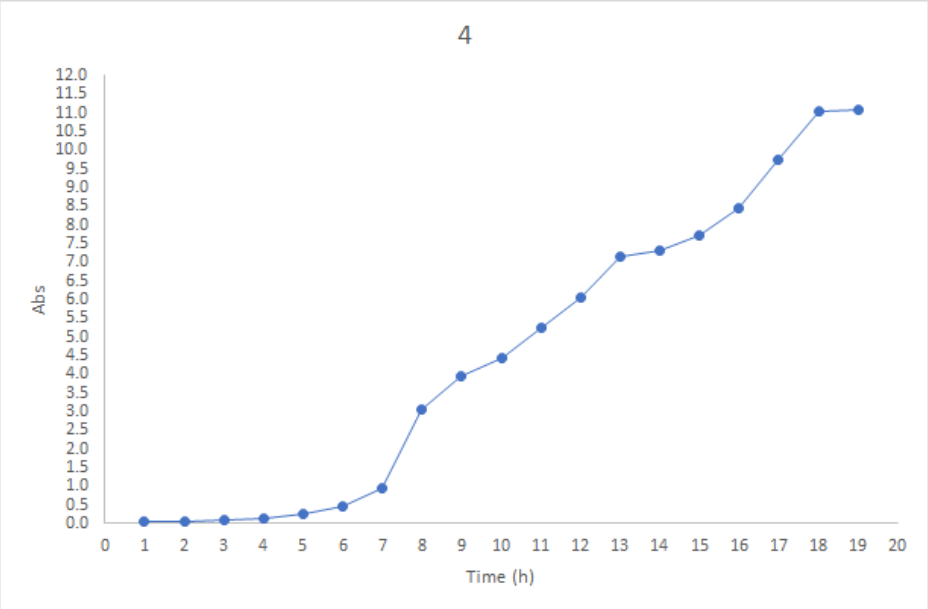
Repeat	1	2	3	4
Time (h)	Abs (OD_{600})			
1	0.071	0.064	0.063	0.060
2	0.079	0.067	0.066	0.063
3	0.096	0.090	0.089	0.087
4	0.160	0.149	0.147	0.152
5	0.288	0.267	0.251	0.257
6	0.543	0.512	0.472	0.458
7	0.992	0.956	0.918	0.930
8	2.780	2.700	2.950	3.060
9	3.990	3.780	3.930	3.960
10	4.810	4.960	4.550	4.420
11	5.600	5.400	5.630	5.230
12	6.400	6.360	6.400	6.060
13	7.580	7.260	7.660	7.150

14	7.870	8.020	8.210	7.330
15	8.900	8.950	8.780	7.710
16	9.850	9.120	9.620	8.450
17	11.820	11.190	12.300	9.750
18	11.970	11.790	12.450	11.040
19	12.030	12.300	12.660	11.100

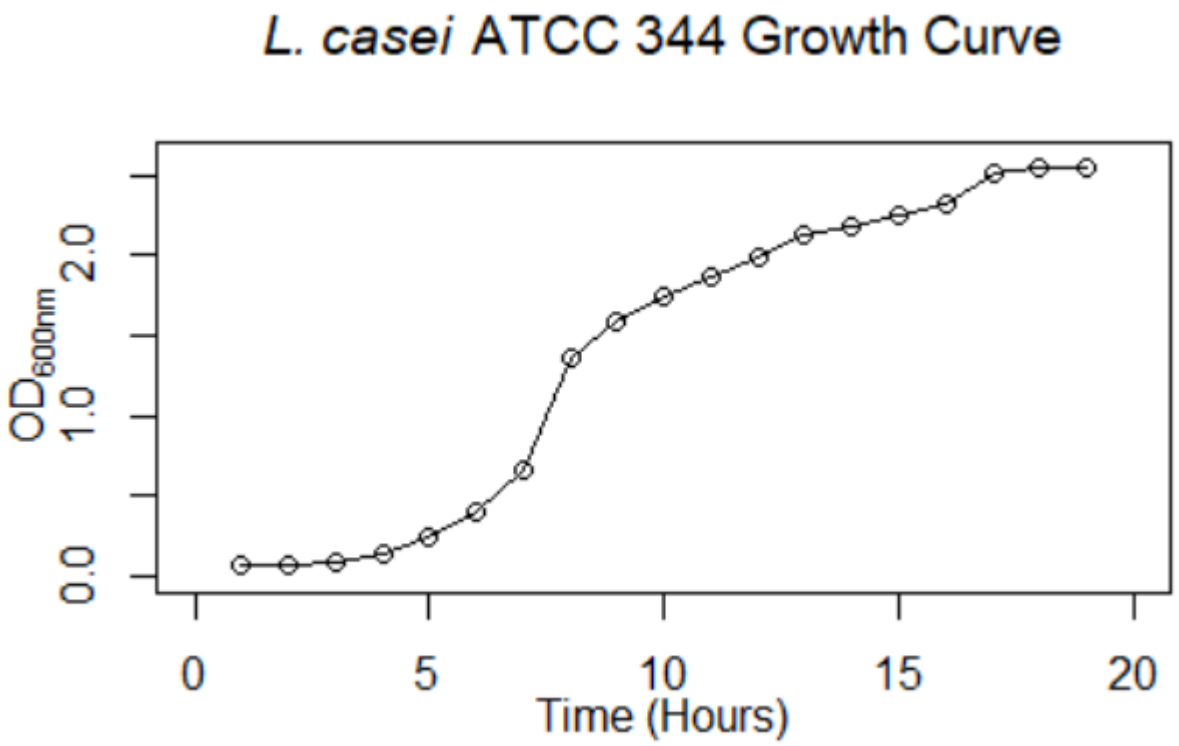








Normalized Curve:



Code in R

```
#Llamado de los datos
datos <- read.csv2("C:/Users/jp299/Desktop/diffEASY/Curvas de
Lacto/Junio/Cinética_Lactobacillus_21-6-19.csv", stringsAsFactors=F)
datos
names (datos)[1] = "Hora"
names (datos)[7] = "ODnorm"
datos

#Grafico de lineas #OD por separado
par(mar=c(3,3,3,1))
par("mar")

plot(datos$Hora, datos$ODnorm, type="o", pch=1, col="black",lty=1, xlab="", ylab="", lwd=0,
    main= substitute (paste(italic('L. casei'), " ATCC 344 Growth Curve")), ylim=c(0,2.6), xlim=c(0,20))

par(new=TRUE)
ylab.name = expression("OD" ["600nm"])
mtext(ylab.name, side=2,line=1.75)
mtext("Time (Hours)",side=1,line=1.75)
```

June 24th

Experiment: Colony PCR
Responsables: Pablo Delgado
Protocol code: Col_PCR

Protocol modifications or specifications: 1 white colony from R0010 + K358006 and 2 white colonies from R0040 + K358006 were checked by PCR with the V2F and VR standard BioBricks primers.
Results: No expected bands were obtained.

June 24th

Experiment: IDT gBlocks resuspention
Responsables: Pablo Delgado
Protocol code: As indicated by the manufacturer
Protocol modifications or specifications: All gene parts from IDT were resuspended in 100uL of nuclease free water up to around 10ng/µL, as recommended by the supplier.
Results: No results needed.

IDT code	Laboratory name	Resistance	IDT mass sended (ng)	Volume added (uL)	Concentration (ng/uL)
AgrC_AgrA_Linker_C.diff	**Lin_C.diff**	Fragment	1000	100	10
AgrC_AgrA_Linker_S.aureus	**Lin_S.aureus**	Fragment	1000	100	10
AgrC_AgrA_Linker_Sin2	**Lin_Sin2**	Fragment	1000	100	10
AgrC_AgrA_WildType_C.diff	**AgrC_AgrA_WT**	Fragment	1000	100	10
P3_GFP_Caracterizar	**P3_GFP_Caract**	Fragment	1000	100	10
P3_TetR	**P3_TetR**	Fragment	1000	100	10
P3_ARNas_GFP	**P3_ARNas_GFP**	Fragment	1000	100	10
P3_ARNas_Lisina	**P3_ARNas_Lisina**	Fragment	1000	100	10
Promotor_C.diff	**Prom_C.diff**	Fragment	1000	100	10
MCS_Lacto	**MCS_Lacto**	Fragment	1000	100	10

June 24th

Experiment: Restriction
Responsables: Pablo Delgado and Paula Thiel
Protocol code: Rest
Protocol modifications or specifications: gBlocks fragments from IDT were cut with EcoRI + PstI to insert them in the iGEM backbones. 21.5uL of resuspended gBlocks were used, which gave a final concentration of 8.6ng/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

June 24th

Experiment: Ligation
Responsables: Pablo Delgado and Paula Thiel
Protocol code: Lig
Protocol modifications or specifications: Ligation was made as shown in the next table:

Upstream part	Insert part Volume (µL)	Backbone	Backbone Volume (µL)
Lin_C.diff	14	**bbA RFP**	3
Lin_S.aureus	14	**bbA RFP**	3
Lin_Sin2	14	**bbA RFP**	3
AgrC_AgrA_WT	14	**bbA RFP**	3
P3_GFP_Caract	14	**bbA RFP**	3
P3_TetR	14	**bbA RFP**	3
P3_ARNas_GFP	14	**bbA RFP**	3
P3_ARNas_Lisina	14	**bbA RFP**	3
Prom_C.diff	14	**bbA RFP**	3
MCS_Lacto	14	**bbA RFP**	3

June 24th

Experiment: Transformation
Responsables: Pablo Delgado
Protocol code: Trans_Ec
Protocol modifications or specifications:
Results:

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

Results were changed on june 26th because after two days of incubation colonies started to turn red

June 25th

Experiment: Transformation
Responsables: Pablo Delgado and María José
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations used were the ones prepared on June 20th.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
R0010 + K358006	T	✗	✗
R0040 + K358006	T	✗	✗
R0010 + K358006	T	✗	✗
R0040 + K358006	T	✗	✗
C+ (bbT RFP)	T	✓	✗
C-	T	✗	✗

June 25th

Experiment: Colony PCR
Responsables: Pablo Delgado and María José
Protocol code: Col_PCR
Protocol modifications or specifications: 5 white colonies from every IDT transformation were checked by PCR with the V2F and VR standard BioBricks primers.

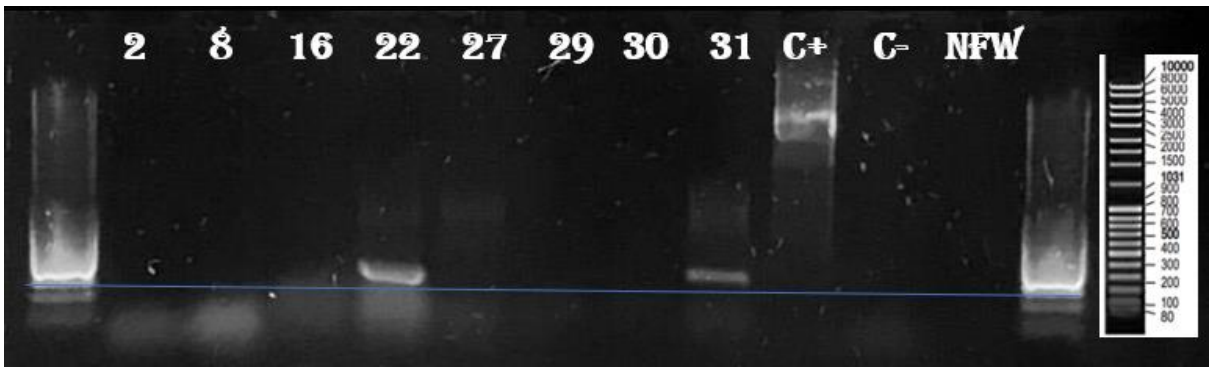
Results: All colonies of the indexed plate grew red. Also, the agarose gel showed contamination and no control amplification.

June 26th

Experiment: Colony PCR
Responsables: Pablo Delgado
Protocol code: Col_PCR
Protocol modifications or specifications: white colonies from the transformation of the IDT gblocks from June 24th were selected and indexed. Also, one sample of each gBlock was amplified with primers V2F and VR, with 56°C of Tm and 2:45 min of extension, just to see if there was a preview of the desired genes. It is necessary to repeat the Colony PCR with all white colonies.

Name	Number in index plate	Siz
Lin_C.diff + bbA RFP	-	2
Lin_S.aureus + bbA RFP	1-6	2
Lin_Sin2 + bbA RFP	7-14	2
AgrC_AgrA_WT + bbA RFP	15-20	2
P3_GFP_Caract + bbA RFP	21-23	1
P3_TetR + bbA RFP	-	2
P3_ARNas_GFP + bbA RFP	24-28	1
P3_ARNas_Lisina + bbA RFP	-	1
Prom_C.diff + bbA RFP	-	1
MCS_Lacto + bbA RFP	29-30	1
C+ (bbA RFP from colonie)	31	

Results: Agarose gel at 0.7%, ran for 50min at 70V

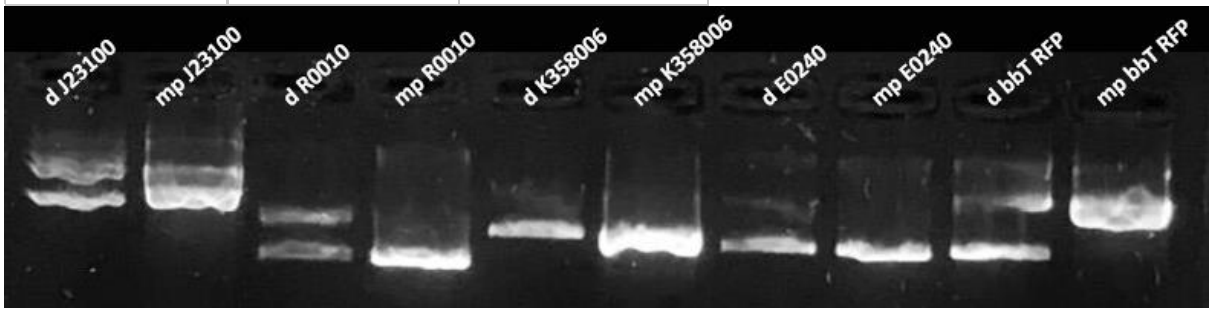


June 26th

Experiment: Restriction

Responsables: Pablo Delgado
Protocol code: Rest
Protocol modifications or specifications: Samples were run in 0.7% agarose gel for 50 minutes at 60V.

Part	Enzymes	Result
R0010	E+S	✓
J23100	E+S	✓
K358006	X+P	✓ / ✗
E0240	X+P	✓ / ✗
bbt RFP	E+P	✓



June 26th

Experiment: Ligation (Optimization)
Responsables: Pablo Delgado
Protocol code: Lig
Protocol modifications or specifications: samples were incubated 16 hours at 16°C and denatured for 20 min at 65°C. Digestions used were prepared the same day (June 26th). On June 27th the same reagents must be used to verify if the ligation gives better results with an incubation time of 16 hours (overnight) at 16°C or just 15 min at room temperature.
Ligation was made as shown in the next table:

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
R0010	7.5	**K358006**	7.5	**bbT RFP**	1.5
J23100	7.5	**E0240**	7.5	**bbT RFP**	1.5

Results: No results needed.

June 27th

Experiment: Ligation (Optimization)
Responsables: María José Durán
Protocol code: Lig

Protocol modifications or specifications: samples were incubated 15 min at room temperature and denatured for 20 min at 65°C. Digestions used were prepared the day before (June 26th).
Ligation was made as shown in the next table:

Upstream part	Upstream part Volume (µL)	Downstream part	Downstream part Volume (µL)	Backbone	Backbone Volume (µL)
R0010	7.5	**K358006**	7.5	**bbT RFP**	1.5
J23100	7.5	**E0240**	7.5	**bbT RFP**	1.5

Results: No results needed.

June 27th

Experiment: Transformation
Responsables: Paula Thiel and María José
Protocol code: Trans_Ec
Protocol modifications or specifications: Ligations used were the ones prepared on June 26th and 27th.
Results:

Name	Resistance	Result	
		Red colonies	White colonies
R0010 + K358006_Overnight	T	✓	✗
R0010 + K358006_15min	T	✓	✗
J23100 + E0240_Overnight	T	✓	✗
J23100 + E0240_15min	T	✓	✗
C+ (bbT RFP)	T	✓	✗
C-	T	✗	✗

June 27th

Experiment: Plasmid isolation
Responsables: María José Durán
Protocol code: ThermoScientific Kit, K0503
Protocol modifications or specifications: Eluted in 50 uL of water instead of TE Buffer.
Results: Shown below

Name	Resistance	Concentration (ng/uL)	Absorbance (260/280)
pAmpicilina	A	132.7	1.81
pTetraciclina	T	64.6	1.84
pCloranfenicol	C	100.1	1.81
pKanamicina	K	363.8	1.86

June 27th

Experiment: Colony PCR
Responsables: Paula Thiel and María José

Protocol code: Col_PCR

Protocol modifications or specifications: Samples used correspond to the white colonies selected in June 25th. 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 70V for 90 minutes.

Results: