Legend:

aiiA: Autoinducer inactivation enzyme from Bacillus; hydrolyzes acetyl homoserine lactone (BBa_C0060)
yhjH: yhjH Gene From E.coli str. K12 (BBa_K861090)
epsE: EpsE Molecular Clutch Gene of B. subtilis (BBa_K143032)
P+RBS: Promoter T7 and RBS (BBa_K525998)
RBS: RBS (Elowitz 1999) -- defines RBS efficiency (BBa_B0034)
T: T1 from E. coli rrnB (BBa_B0010)
DT: double terminator (B0010-B0012)

May

May/29/2017
Protocol: Sterilization and antibiotic stock
Lab materials were sterilized at 121°C for 15 minutes.

- 10 µL tips box (3)
- 200 µL tips box (3)
- 1 mL tips box (3)
- 1.5 mL Eppendorf tubes bottle (2)
- L shaped glass spreader (2)
- Pipettes (5)

First, calculations were done using as a reference the webpage openwetware.com. Each antibiotic was weighed and added to a 1.5 mL amber eppendorf tube, then, 1.5 mL of absolute alcohol was added and mixed until homogeneous. Stored at -20°C.
Chloramphenicol: 0.051g, labeled as “Cl”.
Ampicillin: 0.075g, labeled as “Am”.

May/30/2017
Protocol: Antibiotic stock and competent cells
First, calculations were done in order to get a concentration of 100 µg/mL. 15g of ampicillin was weighed and added to a 1.5 mL amber eppendorf tube, then, 1.5 mL of absolute alcohol was added and mixed until homogeneous. Stored at -20°C.
TOP10 competent cells

May/31/2017
Protocol: Parts resuspension
From the distribution kit, transformed *E. coli* competent cells and plated on CAM and AMP plates.

DNA	Plate	Well	Antibiotic
P+RBS	1 (2015)	3M	CAM
aiiA	2	4H	CAM
T1	4 (2015)	16G	AMP
DT	3	3F	CAM
RFP	4	4B	CAM

June

June/01/2017
Protocol: 100 mM and 50 mM $CaCl_2$ preparation and overnight cultures
$CaCl_2$ (5.59 g) added to 500 mL and 1 L of distilled water.
LB medium (10 mL) inoculated with TOP 10 (200 µL). 37°C, no agitation.

June/02/2017
Protocol: Competent cells
TOP 10 competent cells.

June/03/2017
Protocol: Transformation
Transformation P+RBS (CAM), aiiA (CAM), RFP (CAM), T (AMP) and RFP (AMP) into TOP 10 competent cells.

June/04/2017
Results: Inoculated plates after 22 incubation hours showed:
Negative Control (NC) - LB (w/o antibiotic) → Countless extended growth.
NC - LB (CAM) → Extended growth with 8 punctuated colonies, translucent and yellowish.
P+RBS (CAM) → Two colonies, medium and big size, both translucent and yellowish; entire edges and slightly elevated.
aiiA (CAM) → Two colonies, one punctuated colony and the other one small and round, both translucent and yellowish; entire edges and slightly elevated.
RFP (CAM) → No growth.
T (AMP) → One whitish, opaque, slightly dry, big size colony.
RFP (AMP) → Same morphology as T (AMP), one whitish, opaque, slightly dry, big size colony.
Protocol: Overnight cultures

8 LB medium tubes (10 mL) inoculated by duplicate with: P+RBS, aiiA, T and RFP. It was taken only half colony from those plates with just one colony, also, an extra tube was inoculated with a colony from the NC - LB (CAM) in order to see why this occurred.

June/05/2017
Results: From the overnight cultures, both aiiA and T, showed turbidity. There was no growth at all in NC - LB (CAM) tube which leads to conclude that contamination occurred during plate extension.
Protocol: Miniprep, Nanodrop and Electrophoresis
Plasmid extraction of: aiiA, P+RBS and T.
Nanodrop

Part	First Measurement	Second Measurement	Third Measurement	Average
P+RBS	36.3/1.98	36.6/1.94	35.9/1.93	36.26/1.95
aiiA	116.7/2.03	119/2.02	118.7/2.02	118.13/2.02
T	35.3/1.82	34.1/1.88	33.9/1.89	34.43/1.86

Measurements: Concentration / 260/280

June/06/2017

Protocol: Electrophoresis, psB1A3 resuspension, Nanodrop and competent cells

Plasmid resuspension

DNA	Plate	Well	Antibiotic
psB1A3	4	2H	AMP

Nanodrop

Part	First Measurement	Second Measurement	Average
psB1A3	2.6/1.63	1.5/1.74	2.05/1.68

Measurements: Concentration / 260/280

TOP 10 competent cells.

June/07/2017
Protocol: DNA resuspension and transformation
Parts taken from the 2017 kit:

DNA	Plate	Well
aiiA	2	4H
yhjH	1	13D
P+RBS	1	3M
GFP	3	6I
RFP	3	6G
CFP	3	19C

Transformation of yhjH, aiiA, P+RBS, RFP, CFP and GFP into TOP 10 competent cells.

June/08/2017
Results: From RFP, CFP and GFP plates, only RFP and CFP showed fluorescence.
Protocol: Overnight cultures
LB (CAM) plates inoculated with aiiA, yhjH, P+RBS, RFP, GFP and CFP due to some irregularities seen on the first plates.

June/09/2017
Results: RFP and CFP showed fluorescence. LB medium (CAM) tubes were inoculated with RFP and CFP (1μL/mL), 37°C for 24 hours.
Protocol: Overnight cultures
LB medium inoculated with DH5α, 37°C and 225 rpm agitation.

June/10/2017
Protocol: Antibiotic stock and transformation
1.5 mL of CAM prepared. Transformation of yhjH, aiiA, P+RBS and RFP into DH5α competent cells.

June/11/2017
Protocol: Overnight cultures
10 mL LB medium tube inoculated with TOP 10 cells. Incubated for 18 hours, 37°C and 225 rpm agitation.

June/12/2017
Protocol: Alkaline Lysis Miniprep solutions preparation and overnight cultures
Solution 1 (100 mL): Sucrose (15 g), Tris (0.6057 g), EDTA (1.8612 g) and distilled water (80 mL). pH adjusted to 7.9 and sterilized for 15 min.
Solution 2 (100 mL): NaOH (8 g), SDS (1 g) and distilled water (80 mL).
Solution 3 (100 mL): Potassium acetate (29.442 g), Glacial acetic acid (11.5 mL) and distilled water (88.5 mL).
40 mL LB medium tube inoculated with TOP 10 cells. 37°C and 225 rpm agitation.

June/13/2017
Protocol: Competent cells
TOP 10 competent cells.

June/14/2017
Protocol: Transformation
Transformation of yhjH, aiiA, P+RBS, RFP and NC into TOP 10 competent cells.

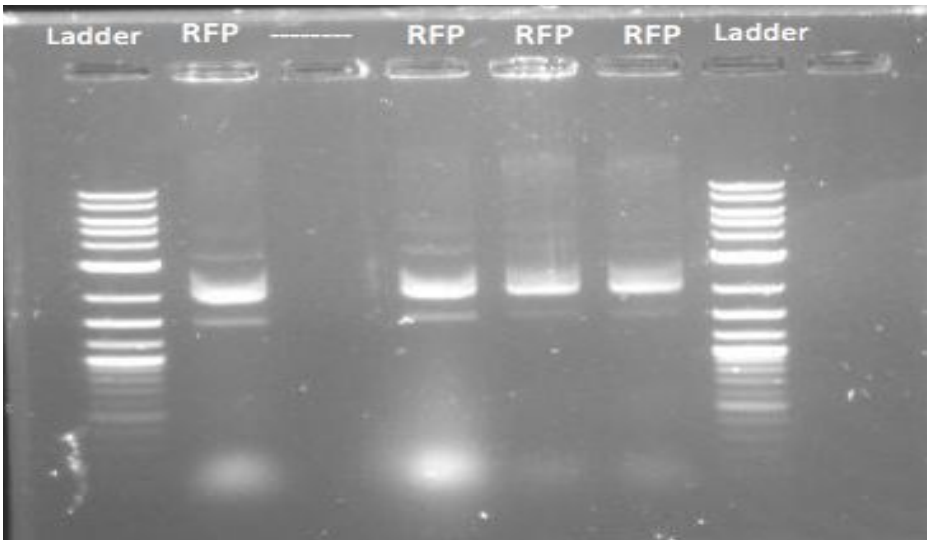
June/15/2017
Results: RFP, yhjH and aiiA showed growth of a single colony on each one of them, RFP's colony with a reddish color.
Protocol: Overnight cultures and sodium acetate solution preparation
3 LB medium tubes (10 mL) inoculated with 300 µL of: RFP (2) and CFP. 37°C and 225 rpm agitation.
Solution 3M (50 mL): Sodium acetate (12.3045 g) and distilled water (30 mL).

June/16/2017
Protocol: Miniprep
Alkaline lysis plasmid extraction by duplicate of RFP1 and RFP2.

June/17/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Third Measurement	Average
RFP1	2592.3/2.01	2594.8/1.97	2454.5/1.97	2547.2/2
RFP2	3750.5/2.07	3746.1/2.07	3572/2.08	3689.5/2.07

Measurements: Concentration / 260/280



Results: The weight expected was of 3139 pb, the sixth strip has a weight around 3000 pb, slightly below than expected but contaminated by proteins.

June/18/2017
Protocol: Overnight cultures
6 LB+CAM medium (60 mL) tubes inoculated with 300 µL of aiiA (2), yhjH (2), RFP and NC.
37°C and 225 rpm agitation.

June/19/2017
Protocol: Competent cells and transformation
TOP 10
Transformation of P+RBS, epsE, RFP1, RFP2, RFP3 and NC into TOP 10 competent cells.

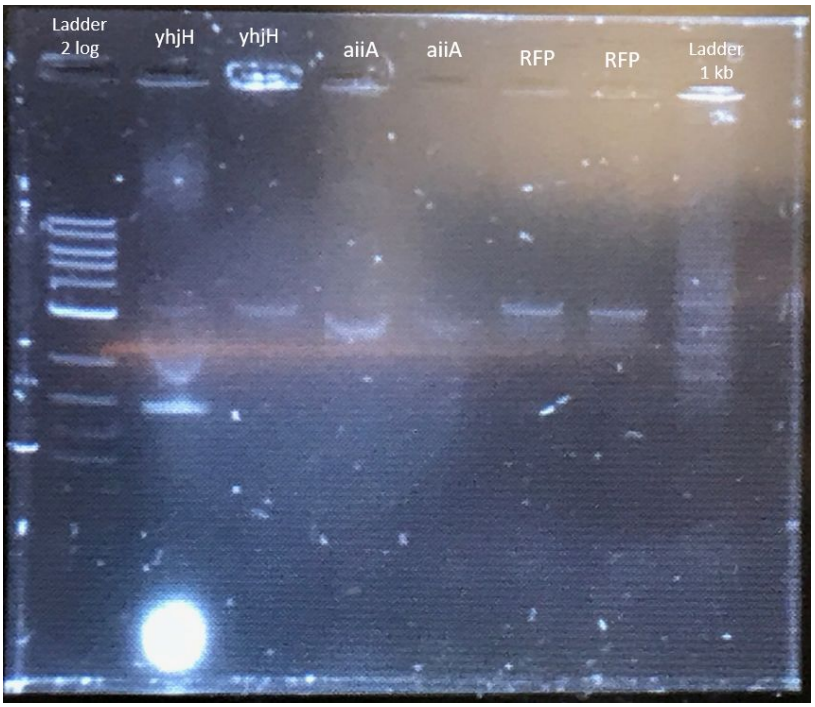
June/20/2017
Results: From the transformation, RFP1, RFP2, RFP3 and P+RBS showed growth, it was not the case for epsE because it was cultured in CAM instead of AMP.
Protocol: Miniprep and Nanodrop
Plasmid extraction (alkaline lysis) by duplicated of aiiA, yhjH and RFP.
Nanodrop

Part	First Measurement	Second Measurement	Average
yhjH1	5362/2.05/2.36	5383/2.05/2.35	5372.5/2.05/2.355
aiiA1	2932.8/1.98/2	3026.7/1.98/2	2979.75/1.98/2
RFP1	992.1/1.95/2.21	1156.5/1.91/1.92	1074.3/1.93/2.06
yhjH2	288.4/1.83/1.81	286/1.84/1.82	287.2/1.835/1.815
aiiA2	1903.8/2.03/2.28	1936.6/2.01/2.29	1920.2/2.02/2.285

RFP2	179/1.93/2.27	185.5/1.92/2.28	182.25/1.925/2.275

Measurements: Concentration / 260/280 / 260/230

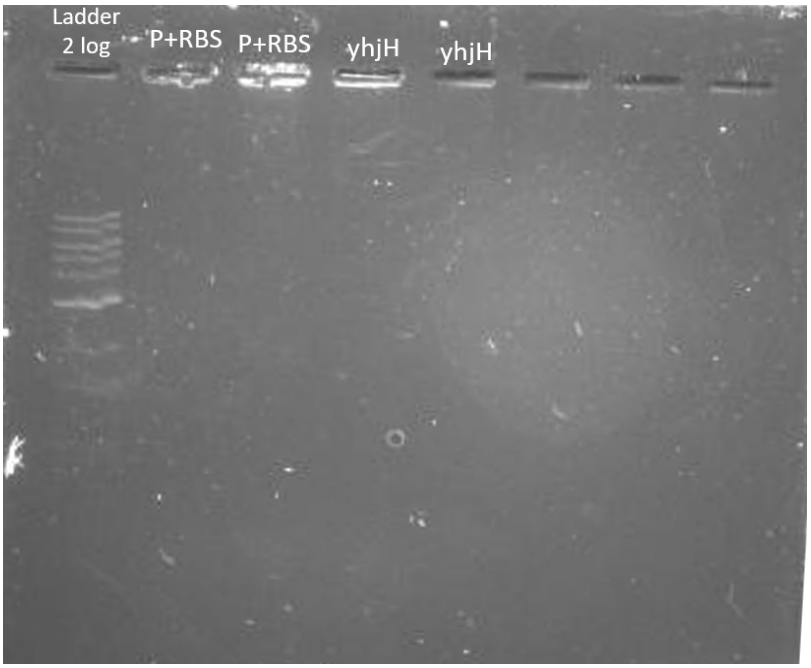
June/21/2017
Protocol: Miniprep, overnight cultures and electrophoresis
Plasmid extraction (alkaline lysis) by duplicated of P+RBS.
2 plates inoculated with 200 µL of epsE and NC each one, and one last plate as a NC. 37°C and 225 rpm agitation.



June/22/2017
Protocol: Antibiotic stock, Miniprep, Nanodrop and electrophoresis
1.5 mL of AMP prepared.
Plasmid extraction (alkaline lysis) by duplicated of P+RBS.
Nanodrop

Part	First Measurement	Second Measurement	Third Measurement	Average
P+RBS1	38.3/1.5/1.04	47.3/1.47/0.99	50.9/1.52/1.03	45.5/1.496/1.02
P+RBS2	1385.4/1.68/1.2	733.5/1.63/1.28	303.4/1.7/1.26	807.4/1.67/1.24

Measurements: Concentration / 260/280 / 260/230



June/23/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Third Measurement	Average
P+RBS1	240.6/1.98/2.24	244.5/1.96/2.23	251.8/1.94/2.18	245.6/1.96/2.21
P+RBS2	30.3/1.69/1.89	16.2/1.59/1.78	15.4/1.7/1.92	19.76/1.78/1.86

Measurements: Concentration / 260/280 / 260/230

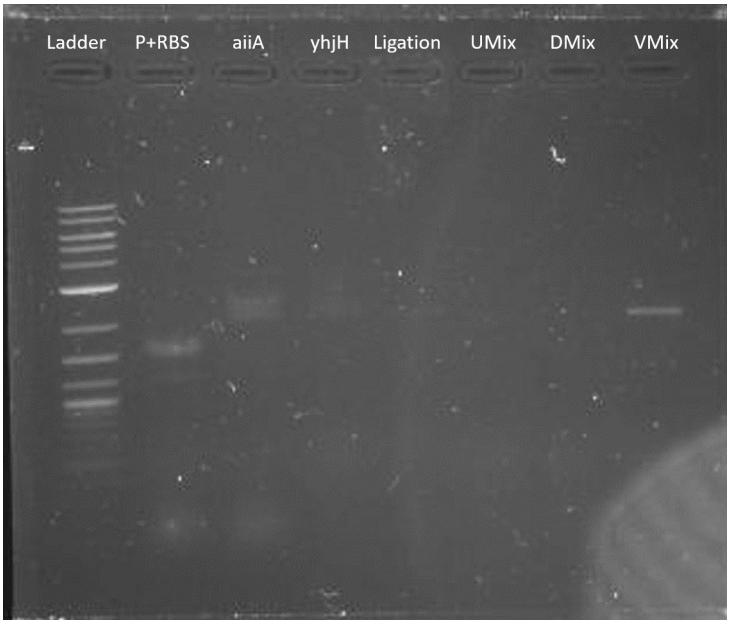
June/24/2017
Protocol: Overnight cultures
Erwinia Amylovora cultured on a nutrient agar plate by the quadrant streak plate method, 28°C. epsE and NC cultured on LB agar plates added with AMP, 37°C.

June/25/2017
Protocol: Digestion and Ligation
aiiA digestion (Upstream Mix: 2.0358 µL of P+RBS, 1 µL of EcoRI, 1 µL of SpeI, 5 µL of 10x NE Buffer and 40.9642 µL of nuclease free water. Downstream Mix: 2.0833 µL of aiiA, 1 µL of XbaI, 1 µL of PstI, 5 µL of 10x NE Buffer and 40.9167 µL of nuclease free water. Vector Mix: 30.39 µL of psB1A3, 1 µL of EcoRI, 1 µL of PstI, 5 µL of 10x NE Buffer and 12.61 µL of nuclease free water).

June/26/2017
Protocol: TAE 1x preparation, overnight cultures ,Nanodrop and electrophoresis
TAE 1x (500 mL): 10 mL of TAE 50x and 490 mL of distilled water, sterilized (121.5 °C) for 15 min.
Cultures of P+RBS, aiiA, yhjH and *Erwinia Amylovora.*
Nanodrop

Part	First Measurement	Second Measurement	Average
Downstream Mix	194.3/1.88/1.22	220/1.88/1.23	207.15/1.88/1.225
Upstream Mix	107.3/1.42/0.49	103.9/1.44/0.52	105.6/1.43/0.505
Vector Mix	38.9/1.03/0.26	74.3/1.45/0.66	56.6/1.24/0.46
Ligation	728.⅓.95/2.12	702.9/4.31/2.6	71505/4.13/2.36

Measurements: Concentration / 260/280 / 260/230

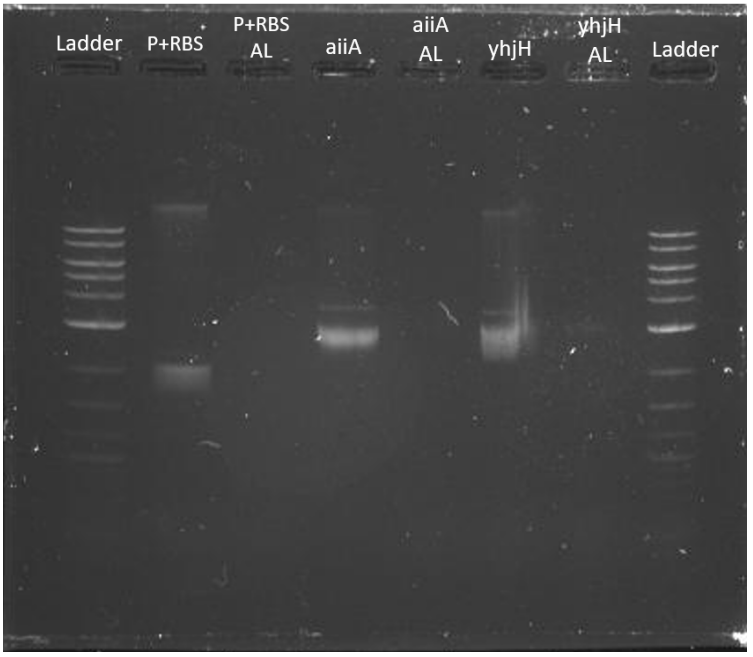


June/27/2017
Protocol: Miniprep, Buffer TE preparation and overnight cultures
Plasmid extraction of: P+RBS, aiiA and yhjH.
Plasmid extraction by alkaline lysis of: P+RBS, aiiA and yhjH.
Buffer TE: 0.0605 g of Tris and 0.0186 g of EDTA, pH 7.9 and 40 mL of distilled water.
3 LB medium (15 mL) tubes inoculated with 200 µL of aiiA, P+RBS and yhjH. 37°C and 225 rpm agitation.

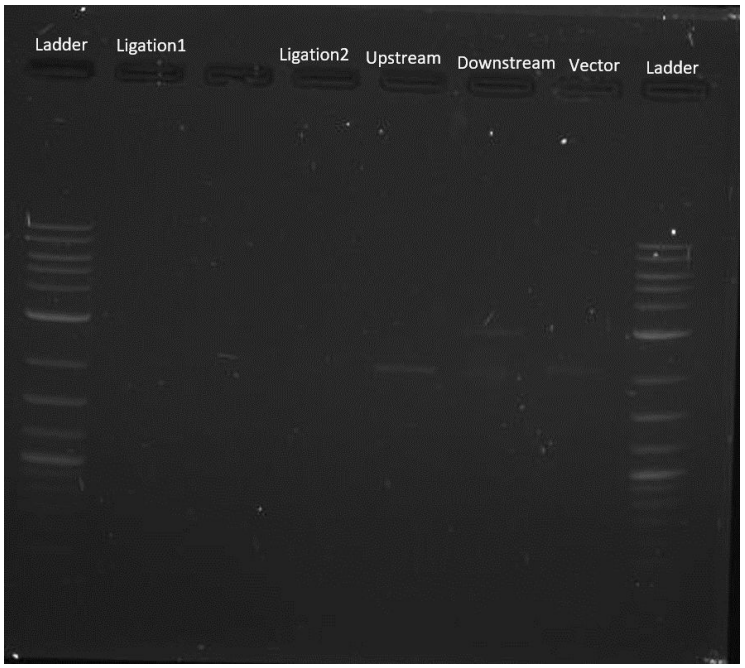
June/28/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Average
P+RBS	5.5/1.62/4.67	6.9/1.69/3.17	6.2/1.655/3.92
yhjH	18.5/1.84/2.86	13.2/1.67/3	15.85/1.755/2.43
aiiA	10.4/1.62/6.05	7.5/1.6/3.74	8.95/1.61/4.895

Measurements: Concentration / 260/280 / 260/230



June/29/2017
Protocol: Digestion, Ligation and electrophoresis
aiiA digestion (Upstream Mix: 10.266 µL of P+RBS, 1.5 µL of EcoRI, 1.5 µL of SpeI, 5 µL of Cutsmart and 31.734 µL of nuclease free water. Downstream Mix: 6.311 µL of aiiA, 1.5 µL of XbaI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 35.686 µL of nuclease free water. Vector Mix: 30.39 µL of psB1A3, 1.5 µL of EcoRI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 11.6048 µL of nuclease free water.)
Ligation Mix: 2 µL of Upstream Mix, 2 µL of Downstream Mix, 2 µL of Vector Mix, 2 µL of Ligase Buffer, 1.5 µL of Ligase and 10.5 µL of nuclease free water.



June/30/2017
Protocol: Digestion, Ligation and overnight cultures
aiiA digestion (Upstream Mix: 10.266 µL of P+RBS, 1.5 µL of EcoRI, 1.5 µL of SpeI, 5 µL of Cutsmart and 31.734 µL of nuclease free water. Downstream Mix: 6.311 µL of aiiA, 1.5 µL of XbaI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 35.7 µL of nuclease free water. Vector Mix: 30.39 µL of psB1A3, 1.5 µL of EcoRI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 11.6048 µL of nuclease free water.)
Ligation Mix: 2 µL of Upstream Mix, 2 µL of Downstream Mix, 2 µL of Vector Mix, 2 µL of Ligase Buffer, 1.5 µL of Ligase enzyme and 10.5 µL of nuclease free water.
Nutrient broth (10 mL) tube inoculated with TOP 10, 37°C.

July

July/01/2017
Protocol: Competent cells and transformation
TOP10 competent cells
Transformation of epsE, DT and RFP into TOP 10 competent cells.

July/02/2017
Protocol: Overnight cultures
Two nutrient broth tubes (50 mL) inoculated with TOP 10 cells.

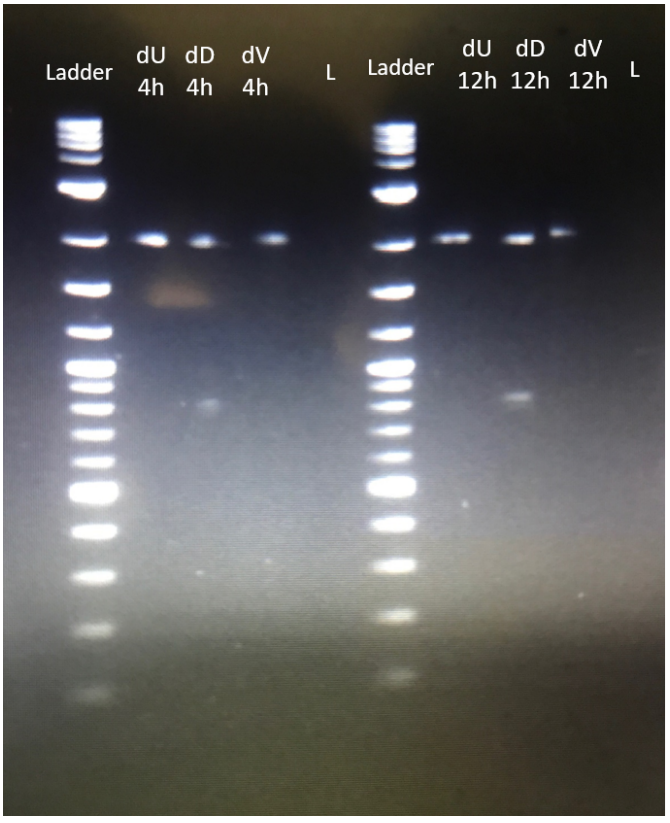
July/03/2017
Protocol: Competent cells and transformation
TOP10 competent cells
Transformation of epsE, DT, RFP and NC into TOP 10 competent cells.

July/04/2017
Protocol: Overnight cultures
P+RBS, yhjH and aiiA cultured in nutrient broth 2x, 37°C and 225 rpm agitation.

July/05/2017
Protocol: Digestion, Ligation and competent cells
This protocol was done twice, a four hours aiiA digestion and twelve hours aiiA digestion (Upstream Mix: 10.266 µL of P+RBS, 1.5 µL of EcoRI, 1.5 µL of SpeI, 5 µL of Cutsmart and 31.734 µL of nuclease free water. Downstream Mix: 6.311 µL of aiiA, 1.5 µL of XbaI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 35.686 µL of nuclease free water. Vector Mix: 30.39 µL of psB1A3, 1.5 µL of EcoRI, 1.5 µL of PstI, 5 µL of Buffer 3.1 and 11.6048 µL of nuclease free water.)
Ligation Mix: 2 µL of Upstream Mix, 2 µL of Downstream Mix, 2 µL of Vector Mix, 2 µL of Ligase Buffer, 1.5 µL of Ligase enzyme and 10.5 µL of nuclease free water.
TOP 10 cells.

July/06/2017
Protocol: Transformation
Transformation of epsE, DT, RFP and NC into TOP 10 competent cells.

July/07/2017
Protocol: Electrophoresis



July/10/2017
Protocol: Competent cells
TOP 10 competent cells.

July/11/2017
Protocol: Competent cells, transformation
TOP 10 competent cells.
Transformation of epsE, DT and RFP into TOP 10 competent cells.

July/12/2017
Protocol: Overnight cultures
Four nutrient broth tubes (10 mL) inoculated with aiiA, yhjH and P+RBS, 37°C and 225 rpm agitation.

July/13/2017
Protocol: Overnight cultures
Four LB broth tubes (10 mL) inoculated with epsE, DT and NC, 37°C and 225 rpm agitation.

July/14/2017
Protocol: Miniprep
Plasmid extraction by duplicate (alkaline lysis) of DT, epsE and NC.

July/15/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Average
epsE1	1897.5/1.77/1.49	1234.5/1.97/1.93	1566/1.87/1.71
epsE2	970.9/2.02/2.16	1024.3/1.97/1.93	997.7/1.995/2.045
DT1	1160.4/1.96/1.83	1164.7/1.97/1.85	1162.55/1.965/1.84
DT2	836.6/1.98/1.91	836.3/1.98/1.91	836.45/1.98/1.91
NC1	771.2/1.98/2.03	805.1/1.96/1.93	788.15/1.97/1.98
NC2	539.1/1.98/2.16	571.3/1.99/2.21	555.2/1.985/2.185

Measurements: Concentration / 260/280 / 260/230

July/16/2017
Protocol: Miniprep
Alkaline lysis plasmid extraction by duplicate of DT, epsE and NC.

July/17/2017
Protocol: Nanodrop
Nanodrop

Part	First Measurement	Second Measurement	Average
epsE1	977.9/1.87/1.2	948.1/1.84/1.19	963/1.855/1.195
epsE2	7773.8/1.93/1.49	6721.3/1.92/1.47	7247.55/1.925/1.48
DT1	1704.3/1.97/1.82	1704.6/1.97/1.82	1704.45/1.97/1.82
DT2	5590.7/1.99/1.9	5548.9/1.99/1.9	5569.8/1.99/1.9
NC1	1991/2.01/1.89	1981.3/2.01/1.89	1986.15/2.01/1.89
NC2	6203.6/2.01/1.87	6150.8/2.01/1.88	6177.2/2.01/1.875

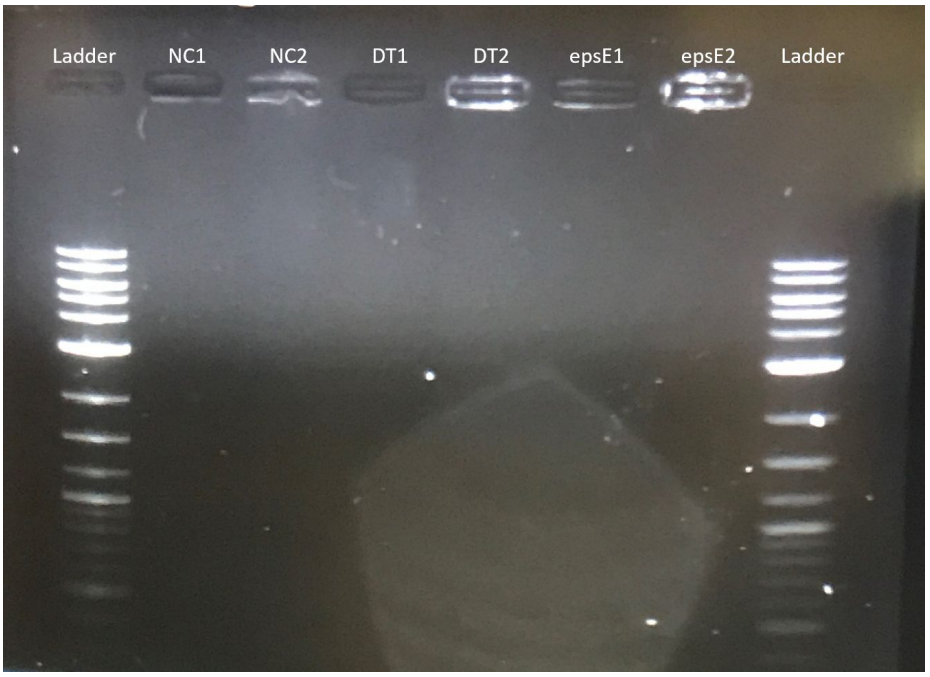
Measurements: Concentration / 260/280 / 260/230

July/18/2017
Protocol: Miniprep
Alkaline lysis plasmid extraction by duplicate of DT, epsE and NC.

July/19/2017
Protocol: Overnight cultures, Nanodrop and electrophoresis
Nutrient agar added with sucrose inoculated with *Erwinia Amylovora*. Two nutrient broth tubes (10 mL) inoculated with TOP10 cells.
Nanodrop

Part	First Measurement	Second Measurement	Average
epsE1	134.6/1.93/2.06	137/1.92/2.05	135.8/1.925/2.055
epsE2	89.4/1.76/1.02	99.7/1.73/0.95	94.55/1.745/0.985
DT1	594.2/1.95/2.16	581.3/1.95/2.17	587.75/1.95/2.165
DT2	1082.3/1.98/2	1080.5/1.97/2.09	1081.4/1.975/2.045
NC1	297.6/1.95/2.14	346.7/1.97/2.17	322.15/1.96/2.155
NC2	1172.8/1.99/2.17	1194.5/1.98/2.21	1183.65/1.985/2.19

Measurements: Concentration / 260/280 / 260/230



July/20/2017
Protocol: Competent cells, transformation
TOP10 competent cells
Transformation of NC, epsE, DT, Ligation 4h and Ligation 12h into TOP10 competent cells.

July/21/2017
Protocol: Overnight cultures
LB and nutrient broth added with sucrose, inoculated with DT, epsE, Ligation 4h and Ligation 12h.

July/22/2017
Protocol: Miniprep
Alkaline lysis plasmid extraction by duplicate of epsE, Ligation 4h and Ligation 12h.

July/23/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Average
epsE1	1591.4/2.05/2.03	1569.2/2.06/2.07	1580.3/2.055/2.05
epsE2	1285.9/2.09/2.36	1278.2/2.1/2.37	1282.05/2.095/2.365
L4h1	1780.5/2.1/2.34	1736.9/2.11/2.34	1758.7/2.105/2.34

L4h2	116.5/2.02/2.11	143.1/2.02/2.18	129.8/2.02/2.145
L12h1	2449.3/2.12/2.29	2325.2/2.11/2.29	2387.25/2.115/2.29
L12h2	1603.7/2.1/2.29	1604.7/2.08/2.28	1604.2/2.09/2.285

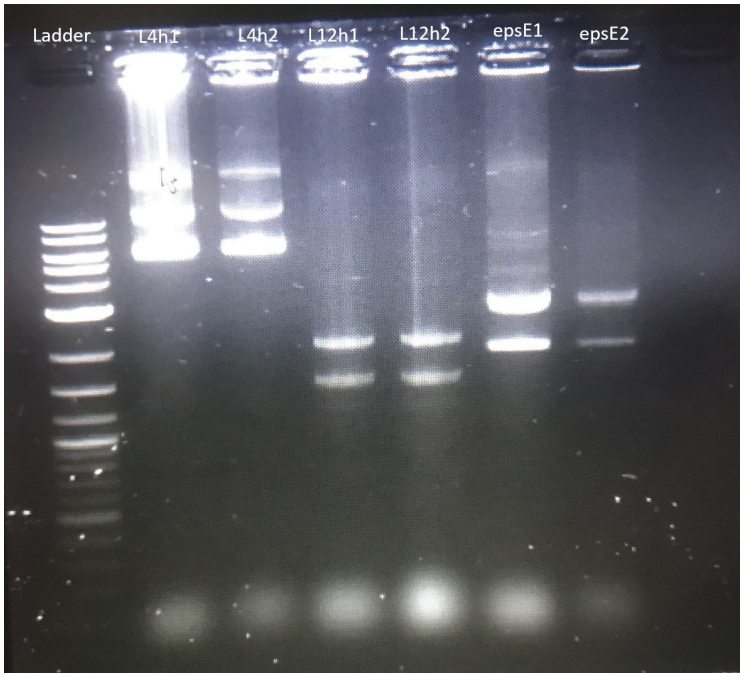
Measurements: Concentration / 260/280 / 260/230

July/24/2017
Protocol: Miniprep
Plasmid extraction by alkaline lysis of epsE, Ligation 4h and Ligation 12h.

July/25/2017
Protocol: Nanodrop and electrophoresis
Nanodrop

Part	First Measurement	Second Measurement	Average
epsE1	3355.5/2.12/2.28	3307.2/2.11/2.3	3331.35/2.115/2.29
epsE2	1483.9/2.03/1.91	1504.5/2.03/1.93	1494.2/2.03/1.92
L4h1	2655.7/2.11/2.23	2615.1/2.11/2.25	2635.4/2.11/2.24
L4h2	1359.9/2.1/2.36	1391.6/2.11/2.38	1375.75/2.105/2.37
L12h1	3515.6/2.14/2.25	3585.2/2.14/2.24	3550.4/2.14/2.245
L12h2	3857.3/2.14/2.23	3839.9/2.15/2.24	3845.6/2.145/2.235

Measurements: Concentration / 260/280 / 260/230



July/27/2017
Protocol: Miniprep
Plasmid extraction by alkaline lysis of epsE, Ligation 4h (colony 1,2,3) and Ligation 12h (colony 1,2,3).

July/28/2017
Protocol: Nanodrop, parts resuspension, competent cells, transformation and digestion
Nanodrop

Part	First Measurement	Second Measurement	Average
L4hC1	604.2/2.06/2.29	591.8/2.06/2.32	598/2.06/2.305
L4hC2	1973.9/2.13/2.23	1973.5/2.13/2.23	1973.7/2.13/2.23
L4hC3	2482.9/2.13/2.23	2496.8/2.13/2.23	2489.85/2.13/2.23
L12hC1	744.1/2.08/2.47	710.5/2.08/2.46	727.3/2.08/2.465
L12hC2	780.3/2.07/2.32	769/2.1/2.29	774.65/2.085/2.305
L12hC3	994.5/2.09/2.41	767.5/2.1/2.42	881/2.095/2.415
epsE	1880.7/2.12/2.21	1916.8/2.13/2.22	1898.75/2.125/2.214

Measurements: Concentration / 260/280 / 260/230
Parts resuspension

Part	Plate	Well
(T) BBa_B0011	4 (2016)	1B
(DT) BBa_B0015	3	3F

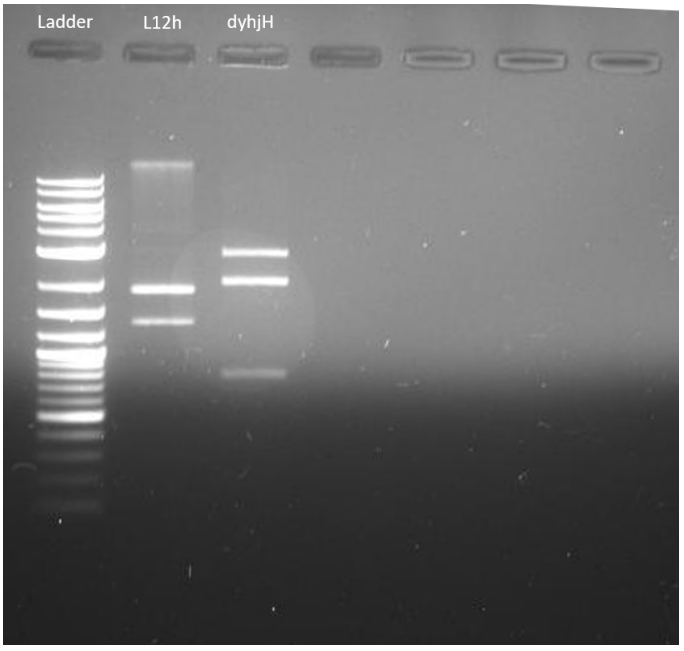
TOP10 competent cells
Transformation of T and DT into TOP10 competent cells.
yhjH digestion (Digestion Mix: 6.2 µL of yhjH, 1.5 µL of PstI, 1.5 µL of XbaI, 5 µL of 3.1 NE buffer and 35.8 µL of nuclease free water).

July/29/2017
Protocol: Miniprep, Nanodrop, ligation and electrophoresis
Plasmid extraction of P+RBS+ aiiA.
Nanodrop

Part	First Measurement	Second Measurement	Third Measurement	Average
P+RBS+aiiA	24.1/1.84/2.21	24.4/1.74/1.77	24/1.83/1.21	24.16/1.8/1.73

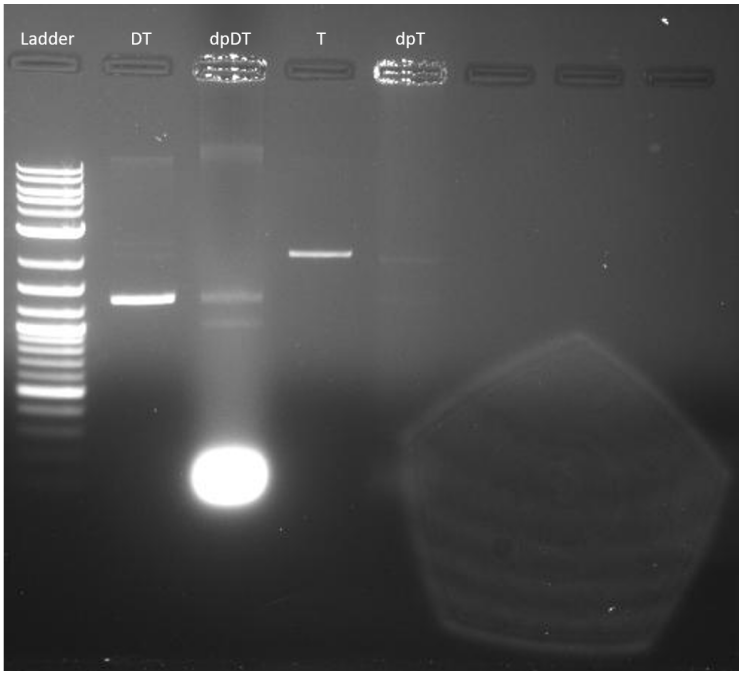
Measurements: Concentration / 260/280 / 260/230

yhjH ligation (Ligation Mix: 10.5 µL of nuclease free water, 2 µL of T4 ligase buffer, 2 µL of digested Vector Mix, 2 µL of Upstream Mix (P+RBS), 2 µL of Downstream Mix and 1.5 µL of T4 ligase).



August

August/01/2017
Protocol: Miniprep
Plasmid extraction of DT.
Plasmid extraction by alkaline lysis of yhjH+RBS.



August/02/2017

Protocol: Partial digestion, Miniprep, digestion, Nanodrop and electrophoresis

Partial digestion of T (Mix: 5 µL of DNA, 2.5 µL Buffer cutsmart. 0.5 µL XbaI enzyme and 17 µL of nuclease free water) and double terminator (Mix: 10 µL of DNA, 2.5 µL of buffer cutsmart, 0.5 µL of XbaI enzyme and 12 µL of nuclease free water).

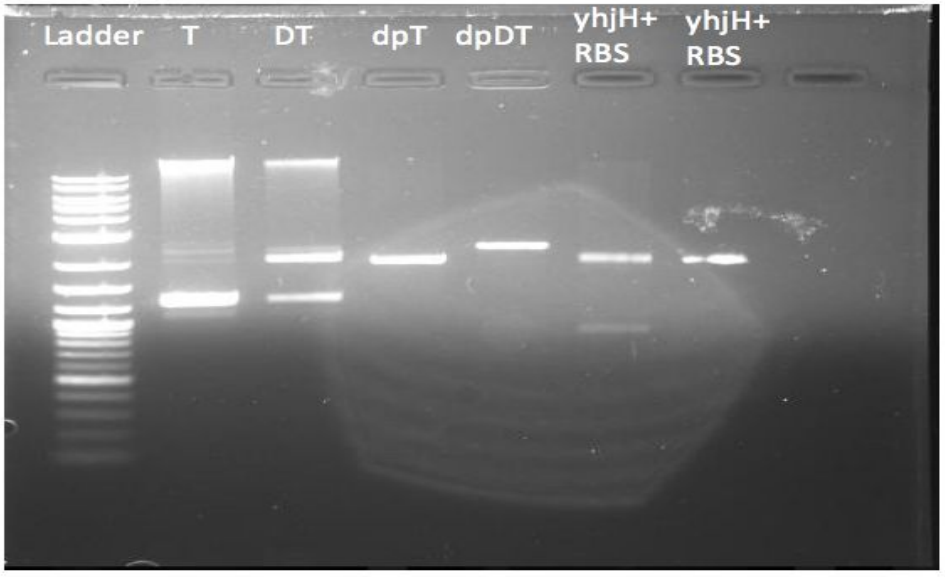
Plasmid extraction of T.

Nanodrop

Part	First Measurement	Second Measurement	Average
yhjH+RBSC1	3822.2/2.14	3855.3/2.14	3838.75/2.14
yhjH+RBSC2	1124,3/2.25	1598/2.11	1361.15/2.18
yhjH+RBSC3	3776.9/2.12	3718.4/2.01	3747.65/2.065

Measurements: Concentration / 260 / 280

12-hour digestion of T (Mix:1.5 µL XbaI enzyme,1.5 µL PstI enzyme, 5 µL buffer, 33.3 µL nuclease free water and 8.7 µL of DNA) and CAM (PSB1C3) vector (Mix:1.5 µL EcoRI enzyme,1.5 µL PstI enzyme, 5 µL buffer, 21.7 µL nuclease free water and 20.3 µL of the vector)



August/03/2017

Protocol: Miniprep, partial digestion, Nanodrop and Ligation

Plasmid extraction of P+RBS+yhjH

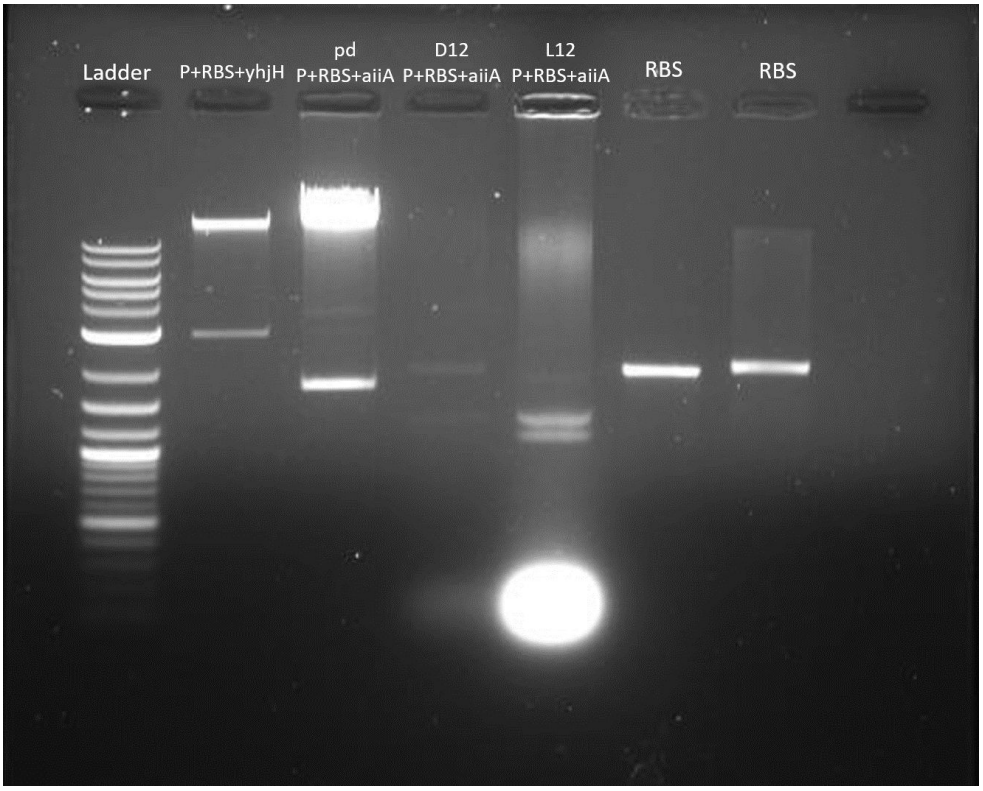
Partial digestion P+RBS+yhjH (Mix: 5 µL of DNA, 2.5 µL Buffer cutsmart. 0.5 µL XbaI enzyme and 17 µL of nuclease free water) and RBS (Mix: 5 µL of DNA, 2.5 µL Buffer cutsmart 0.5 µL XbaI enzyme and 17 µL of nuclease free water), by an hour.

Nanodrop

Part	First Measurement	Second Measurement	Average
RBSC1	3066.1/2.1	3071.7/2.09	3068.5/2.95
RBSC2	86.1/1.91	86.6/1.94	86.35/1.925

Measurements: Concentration / 260/280

12-hour ligation for biobrick of aiiA (Mix: 2 µL (P+RBS+aiiA) + 2 µL T+ 2 µL CAM vector + 2 µL Ligation buffer + 2 µL ligase enzyme and 10.5 µL nuclease free water).



August/04/2017

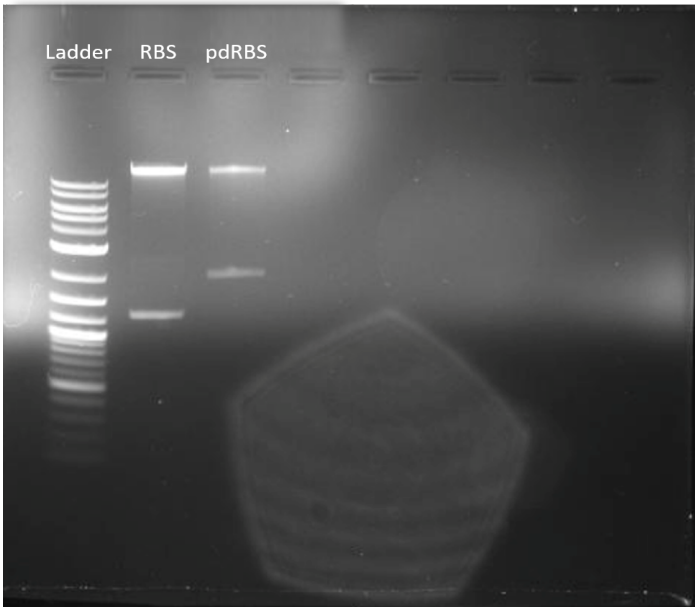
Protocol: Miniprep, partial digestion, competents cells and transformation

Plasmid extraction of RBS

Partial digestion of RBS (Mix: 5 10 µL of RBS, 2.5 µL Buffer cutsmart. 0.5 µL XbaI enzyme and 12 µL of nuclease free water),

TOP10 competent cells

Transformation of aiiA final biobrick into TOP10 competent cells.



August/08/2017
Protocol: Ligation
12-hour ligation of the yhjH final biobrick (Mix:2 µL of P+RBS+yhjH + 2 µL T+ 2 µL CAM backbone + 2 µL Ligation buffer + ligase enzyme + 10.5 µL nuclease free water).

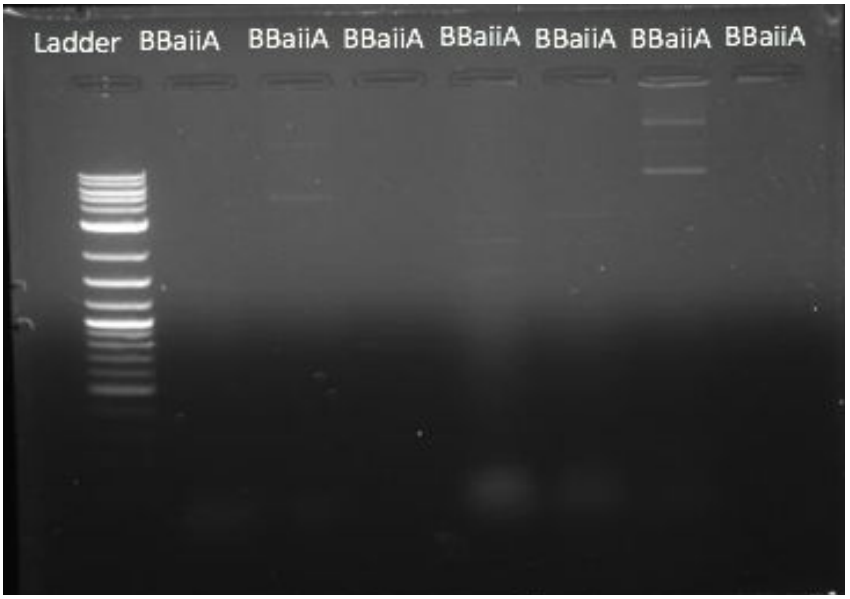
August/09/2017
Protocol: Antibiotic stock, chemically competent cells, transformation and miniprep
TetR stock with a concentration of 35mg/mL in a volume of 1.5mL
Plasmid extraction of aiiA biobrick (6 colonies).
BL21(DE3) Chemically competent cells.
Transformation of P+RBS+epsE (TetR), biobrick of aiiA (CAM) and yhjH biobrick (CAM).

August/10/2017
Protocol: Overnight cultures, Nanodrop, partial digestion, miniprep and electrophoresis
5 LB broth tubes inoculated with 5 colonies of yhjH biobrick, 37 °C, 225 r.p.m.
Nanodrop

Part	First Measurement	Second Measurement	Average
Biobrick yhjH C1	2559.8/2.09	2559.3/2.08	2559.55/2.085
Biobrick yhjH C2	1611.2/2.08	1336/2.08	1473.6/2.08
Biobrick yhjH C3	26.8/1.91	27.4/1.88	27.1/1.895
Biobrick yhjH C4	4284/2.07	4326.9/2.09	4305.45/2.08
Biobrick yhjH C5	1681.9/2.13	1775.1/ 2.14	1728.5/2.135

Measurements: Concentration / 260/280

Plasmid extraction by alkaline lysis of yhjH+RBS
Partial digestion of the aiiA biobrick (5 colonies), all colonies followed this mix: (5 µL of DNA, 2.5 µL Buffer cutsmart. 0.5 µL XbaI enzyme and 17 µL of nuclease free water).
Partial digestion of aiiA biobrick at the standard conditions (5 colonies).



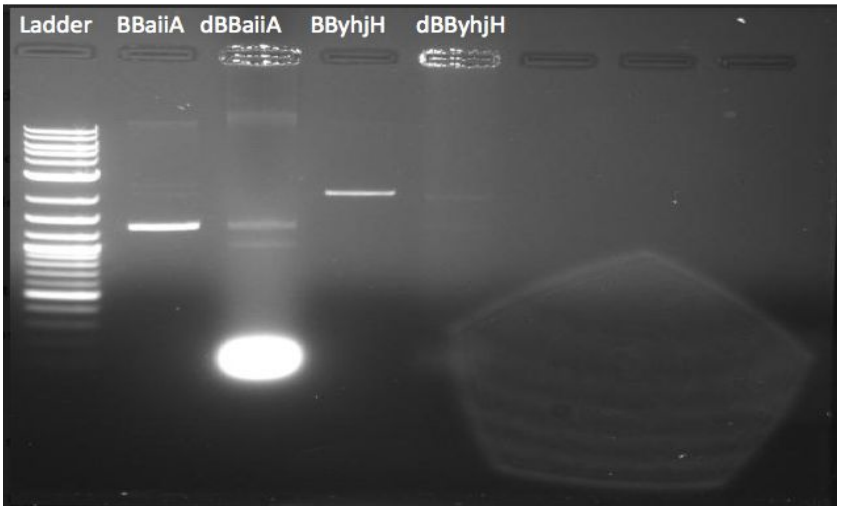
August/11/2017
Protocol: Digestion, Ligation, overnight cultures and miniprep
12-hour digestion of yhjH+RBS (Mix:5.74 µL of (P+RBS+yhjH) + 5 µL Cutsmart buffer + 1.5 µL SpeI + 1.5 µL EcoRI and 10.5 µL nuclease free water) and 12-hour digestion of RBS (Mix: 5.74 µL of RBS + 5 µL Cutsmart buffer + 1.5 µL SpeI + 1.5 µL EcoRI and 10.5 µL nuclease free water). 12-hour ligation of epsE+T (Mix: 2 µL of epsE + 2 µL T+ 2 µL CAM backbone + 2 µL Ligation buffer T4 + 10.5 µL nuclease free water + T4 Ligase enzyme).
LB broth tubes inoculated with aiiA biobrick.
Plasmid extraction by alkaline lysis of 5 colonies of the yhjH biobrick.

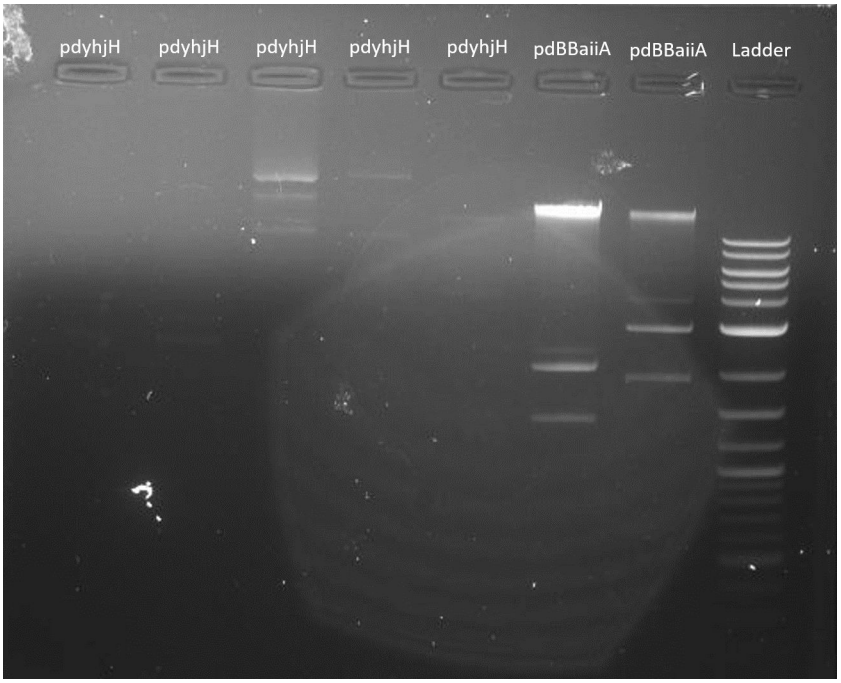
August/12/2017
Protocol: Miniprep, 12-hour digestion, partial digestion, Nanodrop and electrophoresis
Plasmid extraction by alkaline lysis of yhjH.
Plasmid extractions of yhjH biobrick and aiiA biobrick (2 colonies).
12-hour digestion of kanR backbone (7.2 µL vector, 5 µL of buffer 3.1, 1.5 µL PstI, 1.5 µL EcoRI, 35 µL of nuclease free water).
Partial digestion of yhjH (5 colonies), aiiA biobrick (2 colonies).

Nanodrop

Part	First Measurement	Second Measurement	Average
yhjH C1	781.2/2.08	699.2/1.94	740.2/2.01
yhjH C2	1807.1/ 2.09	1773.8/2.09	1790.45/2.09
yhjH C3	1472.1 / 2.10	1339/2.08	1405.55/2.09
yhjH C4	1946.8/2.12	1420.4/2.08	1683.6/2.1
kanR Backbone	10.7/2.3	11.9/ 2.27	11.3/2.285

Measurements: Concentration / 260/280



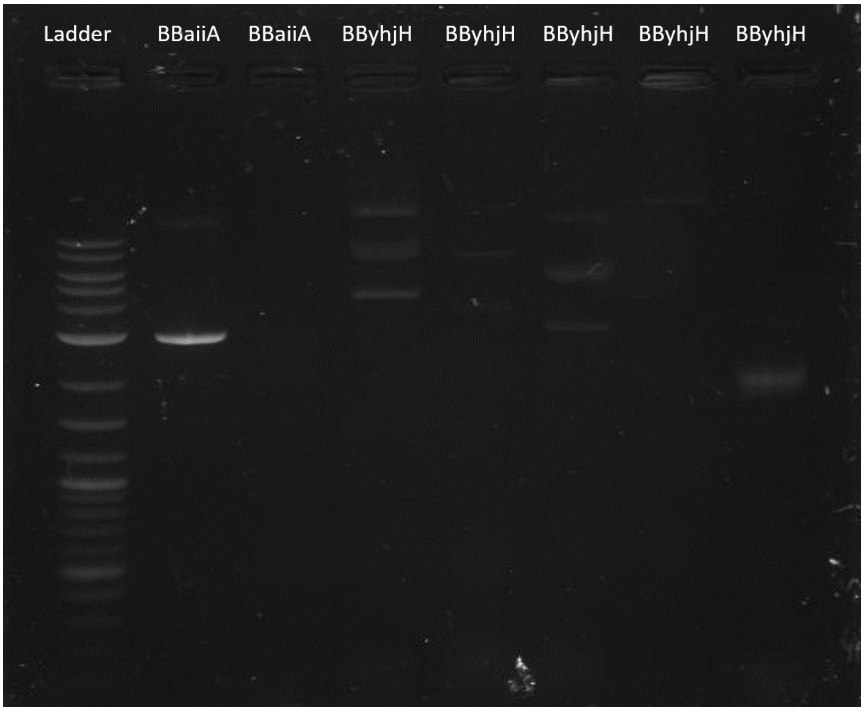


August/13/2017
Protocol: Ligation and overnight cultures
12-hour ligation of yhjH in kanR vector.
LB broth tubes inoculated with 4 colonies of the yhjH biobrick.

August/14/2017
Protocol: Miniprep, competent cells, transformation and overnight cultures
Plasmid extraction of aiiA biobrick.
TOP10 competent cells
Transformation of RFP, RBS + yhjH, epsE+T and RBS+epsE into TOP10 competent cells.
4 LB agar plates inoculated with epsE+T (CAM), RFP (CAM), RBS+yhjH (TetR) and RBS+epsE (TetR).

August/15/2017
Protocol: Overnight cultures, partial digestion, miniprep, chemically competent cells and transformation
LB agar plate supplemented with sucrose inoculated with Erwinia Amylovora.
Partial digestion by alkaline lysis of yhjH biobrick (5 colonies).
Plasmid extraction of aiiA biobrick.
TOP10 competent cells
Transformations of epsE+T (CAM), RBS+yhjH (TetR), RBS+epsE (TetR) and RFP (TetR).

August/16/2017
Protocol: Overnight cultures and electrophoresis
LB agar plate (CAM) inoculated with RBS + epsE.



August/17/2017
Protocol: Overnight cultures and calibration curve of *Erwinia Amylovora*
12 LB agar plates inoculated with yhjH (CAM), epsE+T (CAM), RBS+epsE (5 colonies) (CAM) and RBS + yhjH (2 colonies) (TetR).
Calibration curve of *Erwinia Amylovora* was prepared to predict the growth rate, to know the exact time when *Erwinia* reaches its optimal density to begin with the competent cells protocol.

August/18/2017
Protocol: Miniprep
Plasmid extraction of yhjH biobrick.
Plasmid extraction by alkaline lysis of epsE+T, yhjH + RBS (2 colonies) and epsE+RBS (5 colonies).

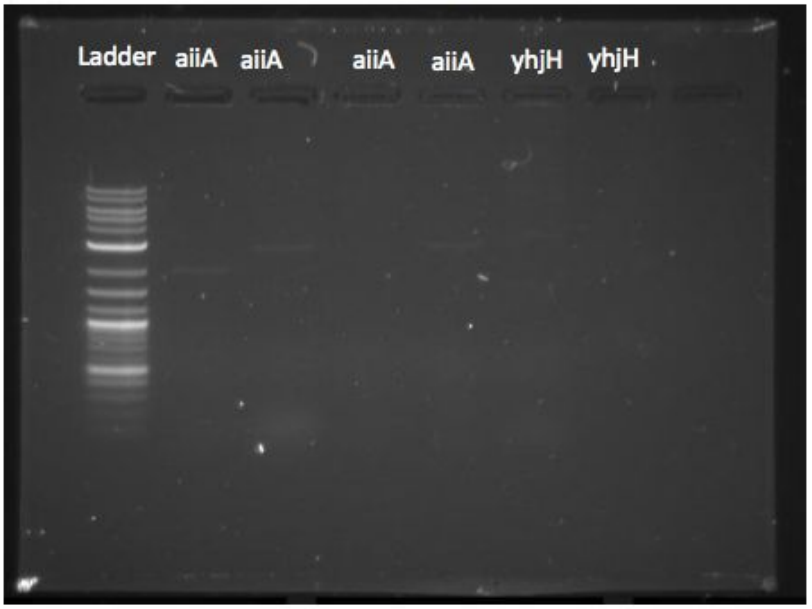
August/19/2017
Protocol: Transformation
Transformation of *Erwinia Amylovora* with aiiA into BL21(DE3) electrocompetent cells.

August/20/2017
Protocol: Chemically competent cells and overnight cultures
BL21(DE3) Chemically competent cells.
LB agar (CAM) inoculated with P+RBS+epsE, LB agar (TetR) inoculated with RBS+epsE, RBS+yhjH, epsE+T and RFP.

August/25/2017
Protocol: Competent cells and transformation
TOP10 competent cells.
Transformation of yhjH biobrick and RBS+yhjH.

August/26/2017
Protocol: Overnight cultures, competent cells and transformation
5 LB broths supplemented with sucrose tubes (CAM) inoculated with *Erwinia Amylovora* previously transformed with biobrick of aiiA. 3 LB broth tubes (CAM) inoculated with yhjH biobrick. LB broth tube (TetR) inoculated with yhjH+RBS, 28 °C, 255 rpm.
TOP10 competent cells.
Transformations of RBS+yhjH and P+RBS+epsE (TetR).

August/27/2017
Protocol: Overnight cultures, miniprep and electrophoresis
LB agar supplemented with sucrose inoculated with *Erwinia Amylovora*.
LB broth tubes inoculated with yhjH biobrick and RBS+yhjH.
Plasmid extraction by alkaline lysis of yhjH, aiiA and RBS+epsE.



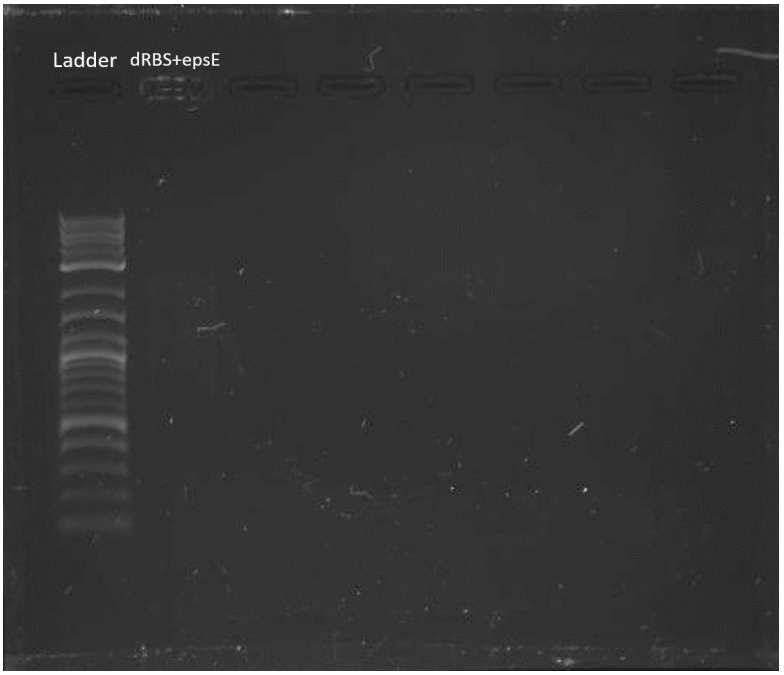
August/28/2017
Protocol: Miniprep, partial digestion and electrophoresis
Plasmid extraction by alkaline lysis of biobrick yhjH (3 extractions) and RBS+yhjH.
Partial digestion of the extraction at the standard conditions.

August/29/2017
Protocol: Partial digestion
Partial digestion of yhjH biobrick (3 colonies) and RBS + yhjH.

August/30/2017
Protocol: Partial digestion, miniprep and Nanodrop
Partial digestion of the plasmid extractions of yhjH biobrick and RBS+ yhjH both of them with the standard mix of partial digestion at the standard conditions.
Plasmid extraction of yhjH biobrick and RBS+epsE.
Nanodrop

Part	Concentration	260/280	260/230
RBS+epsE_1	36.6	1.92	6.25
RBS+epsE_2	38.5	1.93	5.09
yhjHBL21_1	85.3	1.87	2.63
yhjHBL21_2	84.1	1.82	2.33

August/31/2017
Protocol: Ligation and electrophoresis
12-hour ligation of RBS+yhjH) in TetR vector and (P+RBS+epsE) in TetR vector.



September

September/02/2017
Protocol: Miniprep
Plasmid extraction of biobrick aiiA and biobrick of yhjH.

September/03/2017
Protocol: Miniprep
Plasmid extraction by alkaline lysis of RBS+yhjH (5 colonies) and P+RBS+epsE (5 colonies).

September/05/2017
Protocol: Overnight cultures
8 LB broth tubes (5 mL) 4 tubes added with CAM and the other 4 tubes added with TetR, inoculated with *Erwinia Amylovora*, RBS+yhjH, P+RBS+epsE (5 colonies) and RBS+aiiA.

September/06/2017
Protocol: Miniprep and characterization
Plasmid extraction by alkaline lysis of *Erwinia* wild and transformed aiiA *Erwinia* (4 colonies).
Trials of characterization: Inoculation of *Erwinia Amylovora* in apple slices, in a total of 6 plates, Erwinia was inoculated in different ways: plates 1 through 4 containing transformed Erwinia with different aiiA colonies, plate 5 containing *Erwinia* wild and plate 6 containing an apple without inoculation used as a negative control. Each plate divided in two, with each part inoculated by different techniques.

September/07/2017
Protocol: Miniprep and overnight cultures
Plasmid extraction by alkaline lysis of P+RBS+epsE and *Erwinia Amylovora*.
LB broth (TetR) tubes inoculated with RBS+yhjH and RBS+epsE (CAM).

September/08/2017
Protocol: Partial digestion and miniprep.
Standard mix in standard conditions to perform partial digestion of aiiA *Erwinia*, *Erwinia* wild and P+RBS+epsE.
Plasmid extraction of RBS+yhjH and RBS+epsE.

September/09/2017
Protocol: Nanodrop, Miniprep, partial digestion, overnight cultures and 12-hour digestion
Plasmid extraction of RBS+yhjH and RBS+epsE.
Partial digestion of RBS+yhjH and RBS+epsE.
LB broth tubes inoculated with RBS.
12-hour digestion of epsE+RBS.
Nanodrop

Part	First Measurement	Second Measurement
P+RBS+epsE	43.4/1.82	44.5/1.81
P+RBS+yhjH	64.4/1.81	68.4/1.82

Measurements: Concentration / 260/280

September/10/2017
Protocol: Miniprep, partial digestion, transformation and Ligation.
Plasmid extraction of (Two colonies)
Partial digestion of P+RBS+epsE.
Transformation of *Erwinia Amylovora* into electrocompetent aiiA biobrick and yhjH biobrick.
12-hour ligation of of RBS+epsE+T in TetR vector and RBS + yhjH in TetR vector.

September/11/2017
Protocol: Partial digestion, digestion, chemically competent cells and transformation and overnight cultures
Partial digestion with standard mix of RBS+epsE and RBS+yhjH.
12-hour digestion of P+RBS+epsE (Mix: 7.3 µL DNA, 1.5 µL XbaI enzyme, 1.5 µL PstI enzyme, buffer 5 µL and 34.7 µL nuclease free water).
LB agar plate(TetR) inoculated with ligated transformations.

September/12/2017
Protocol: 12-hour ligation and overnight cultures
12-hour ligation of epsE biobrick (P+RBS+epsE+T)

LB broth tubes inoculated with transformations, RBS+epsE+T and RBS+yhjH inoculated in LB agar plates.

September/13/2017:
Protocol: Overnight cultures, miniprep, transformation
Erwinia Amylovora and BL21(DE3) inoculated in LB broth tubes supplemented with sucrose.
Plasmid extraction by alkaline lysis of RBS+epsE+T and RBS+yhjH.

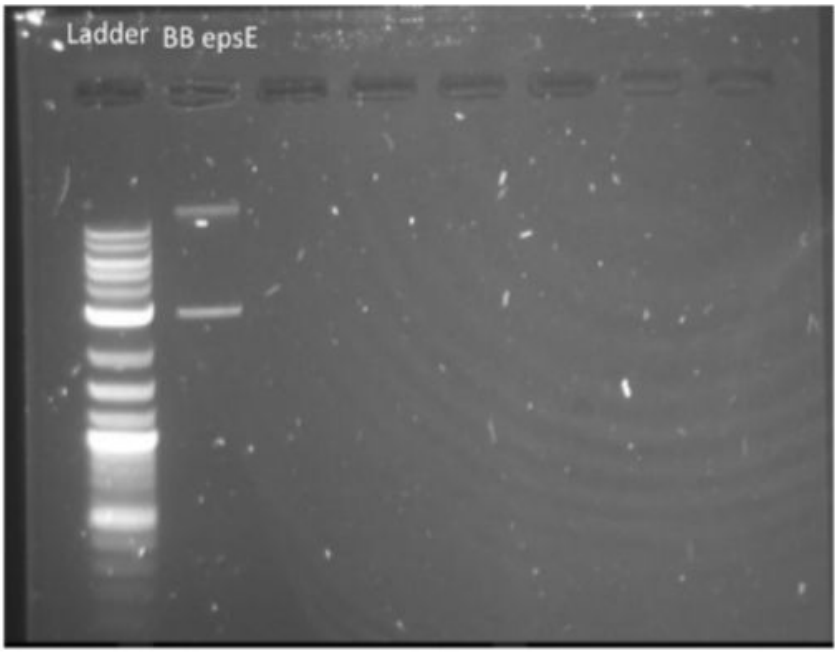
September/14/2017
Protocol: Solutions preparation for colorimetry tube test, digestion and overnight cultures
Solutions preparation for the colorimetry microtiter-plate test.
12-hour digestion of RBS.
LB broth tubes inoculated with BL21(DE3) and transformed epsE biobrick.

September/15/2017
Protocol: Miniprep, digestion, overnight cultures
Plasmid extraction by alkaline lysis of BL21(DE3) and *Erwinia*
12-hour digestion of RBS+yhjH.
LB broth tubes inoculated with RBS.
SDS polyacrylamide gel electrophoresis with SDS page of aiiA *Erwinia Amylovora*.

September/16/2017
Protocol: Miniprep, partial digestion, digestion and Ligation
Plasmid extraction of RBS and RBS+yhjH (two colonies)
Plasmid extraction by alkaline lysis of epsE biobrick (5 colonies) and RBS+epsE+T (3 colonies).
Partial digestion of RBS and RBS+yhjH.
12-hour digestion and ligation of RBS+ yhjH in TetR vector.

September/17/2017
Protocol: Overnight cultures and Ligation
LB broth tubes inoculated with plasmid extractions.
12-hour ligation of RBS+yhjH in TetR vector, RBS+epsE in CAM vector and RBS+epsE+T in TetR vector.

September/18/2017
Protocol: Miniprep and Partial digestion
Plasmid extraction of epsE biobrick.
Partial digestion of epsE biobrick.



September/19/2017
Protocol: Transformation
Transformation of epsE biobrick into BL21(DE3).

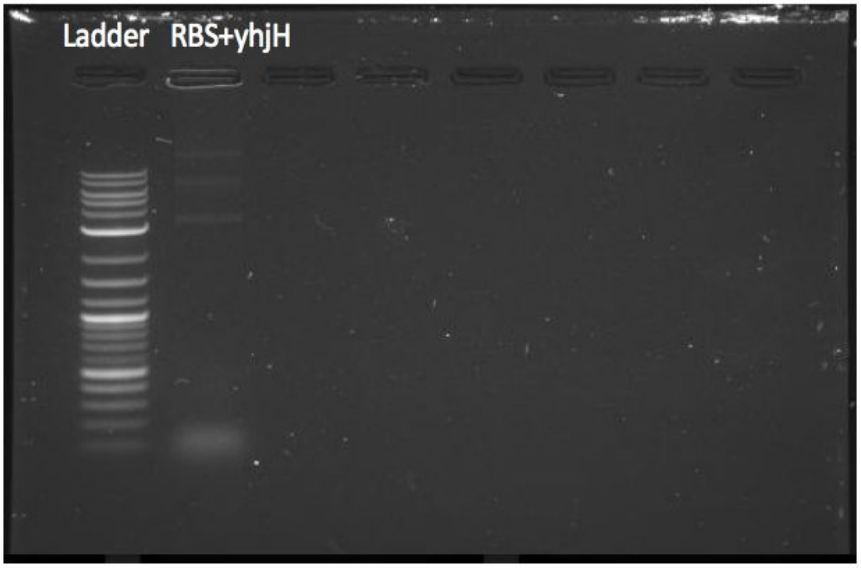
September/20/2017
Protocol: Electroporation
Electroporation of *Erwinia Amylovora* with epsE biobrick.

September/21/2017
Protocol: Overnight cultures
LB broth tubes inoculated with epsE biobrick transformations.

September/22/2017
Protocol: Miniprep
Plasmid extraction by alkaline lysis of RBS+yhjH (two colonies) and epsE biobrick.

September/23/2017
Protocol: Overnight cultures
LB agar plates supplemented with sucrose inoculated with *Erwinia Amylovora* and LB broth tubes inoculated with RBS+yhjH.

September/24/2017
Protocol: Miniprep and electrophoresis
Plasmid extraction by alkaline lysis of RBS+yhjH.



October

October/01/2017
Protocol: Overnight cultures
LB broth tubes supplemented with sucrose inoculated with *Erwinia Amylovora* and LB broth tubes inoculated with aiiA and yhjH biobricks.

October/02/2017
Protocol: Overnight cultures and miniprep
LB broth tubes inoculated with TOP10
Plasmid extraction by alkaline lysis of the biobricks (15 colonies).

October/03/2017
Protocol: Partial digestion
Partial digestion of the 15 plasmid extractions(biobricks and *Erwinia Amylovora*).

October/04/2017
Protocol: Transformation
Transformation by electroporation of epsE into *Erwinia Amylovora*.

October/05/2017
Protocol: Overnight cultures
Inoculation of 24-hour Elisa plates, BL21(DE3) with epsE biobrick transformation.

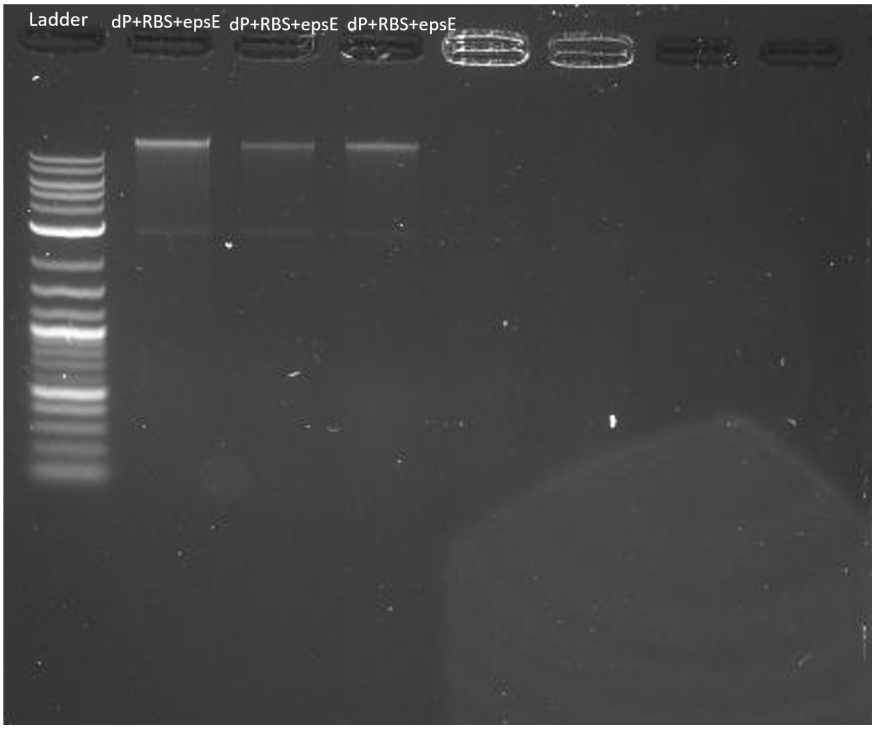
October/06/2017
Protocol: Miniprep, partial digestion and colorimetry
Plasmid extraction by alkaline lysis of epsE biobrick.
Partial digestion of epsE biobrick.
Biofilm quantification using 24-hour tubes with safranine and microtiter plate test.

October/09/2017
Protocol: DNA resuspension
Resuspension of IDT aiiA and IDT epsE.

October/10/2017
Protocol: Overnight cultures
LB agar plates inoculated with BL21(DE3). Two plates inoculated with transformations of IDT aiiA (AMP) and IDT epsE (AMP). Another plate inoculated with epsE (CAM).

October/11/2017
Protocol: Digestion and overnight cultures
Digestion of IDT aiiA and IDT epsE.
Inoculation of electroporations and BL21(DE3).

October/12/2017
Protocol: Miniprep, partial digestion, overnight cultures and electrophoresis
Plasmid extraction of epsE biobrick in *Erwinia Amylovora*.
Partial digestion of epsE biobrick in *Erwinia Amylovora.*
Inoculation of *Erwinia Amylovora* and transformations of yhjH, aiiA and epsE.



October/13/2017
Protocol: Chemically competent cells, transformation and characterization
BL21(DE3) Chemically competent cells.
Transformation of IDT aiiA and IDT epsE parts.
Inoculation on the apple leaves for characterization trials.

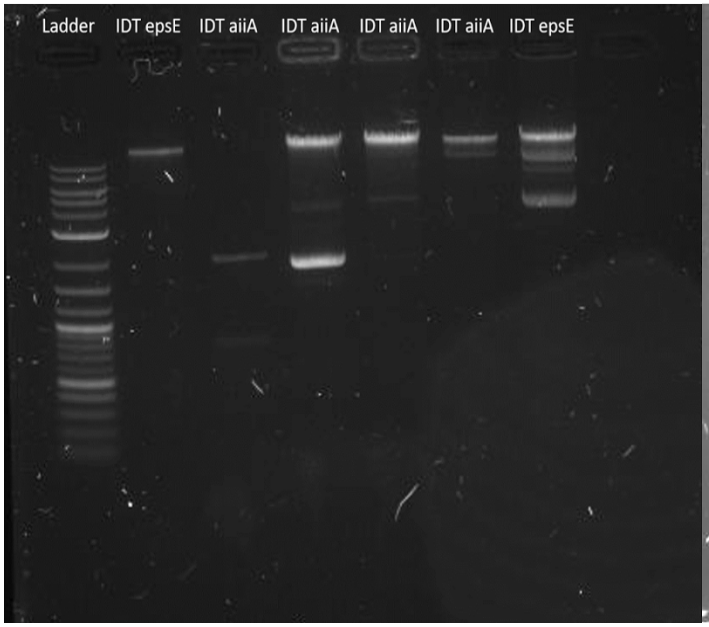
October/14/2017
Protocol: Transformation, digestion and overnight cultures
Transformation of IDT sequences (aiiA and epsE).
12-hour digestion of IDT sequences (aiiA and epsE).
LB agar plates inoculated with RFP and BL21(DE3). Inoculation of *Erwinia Amylovora* for the collaboration with TEC CEM team.

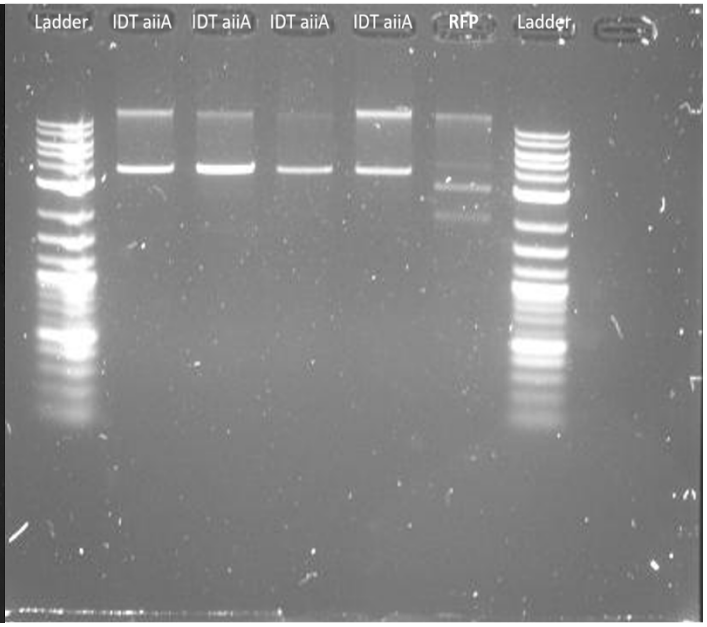
October/15/2017
Protocol: Miniprep, digestion, overnight cultures and electroporation
Plasmid extraction of RFP.
12-hour digestion of RFP.
Inoculation of BL21(DE3) and *Erwinia Amylovora*.
Electroporation of IDT aiiA.

October/16/2017
Protocol: Chemically competent cells, overnight cultures and transformation
BL21(DE3) Chemically competent cells.
LB agar plates supplemented with sucrose inoculated with *Erwinia Amylovora*.
Transformation of IDT dilutions sets of 48 hours *Erwinia* and 48 hours of BL21(DE3).

October/17/2017
Protocol: Overnight cultures and colorimetry solutions preparation
Inoculation of aiiA and epsE transformations and aiiA *Erwinia Amylovora*.
Solutions preparation for the colorimetry microtiter-plate test for quantification of biofilm formation.

October/19/2017
Protocol: Overnight cultures, digestion and electrophoresis
Inoculation of yhjH, epsE and pseudomonas as a positive control in *Erwinia Amylovora*.
Separation of Elisa plates.
12-hour digestion of IDT aiiA and IDT epsE.





October/20/2017
Protocol: Miniprep
Plasmid extraction of IDT parts *Erwinia Amylovora*.

October/21/2017
Protocol: Digestion, transformation and characterization
12-hour digestion of RFP.
Transformation by electroporation of epsE IDT.
Inoculation in apple slices as a part of the characterization trials.

ERWINIOONS