

Notebook

IGEM - CIDEB 2014

General Data

DNA length
(separated)

Gene	Length
NhaS	3,081 bp
L2	3,795 bp
Aroma	3,961 bp
AIDA	2,710 + ? bp

Length of the
genes in
plasmid psB1C3

Gene	Length
pSB1C3	2,070bp
NhaS	1,091bp
L2	1,085bp
Aroma	1,251bp
pUC57	2,710bp
RFP	707bp
AIDA	1,496 bp
irrE	933 bp

Length of the genes in
plasmid pUC57

Gene	Length
NhaS	3,118 bp
Aroma	3,278 bp
Silica	-
IrrE	-

More General Data...

Nomenclature	Meaning
DD/MM (Example, 21/05)	Day/Month
M	Mark
mL	milliliters
uL	microliters
ng	nanogram
Aroma	BSMT1 opt.
Union module	L2 + AIDA

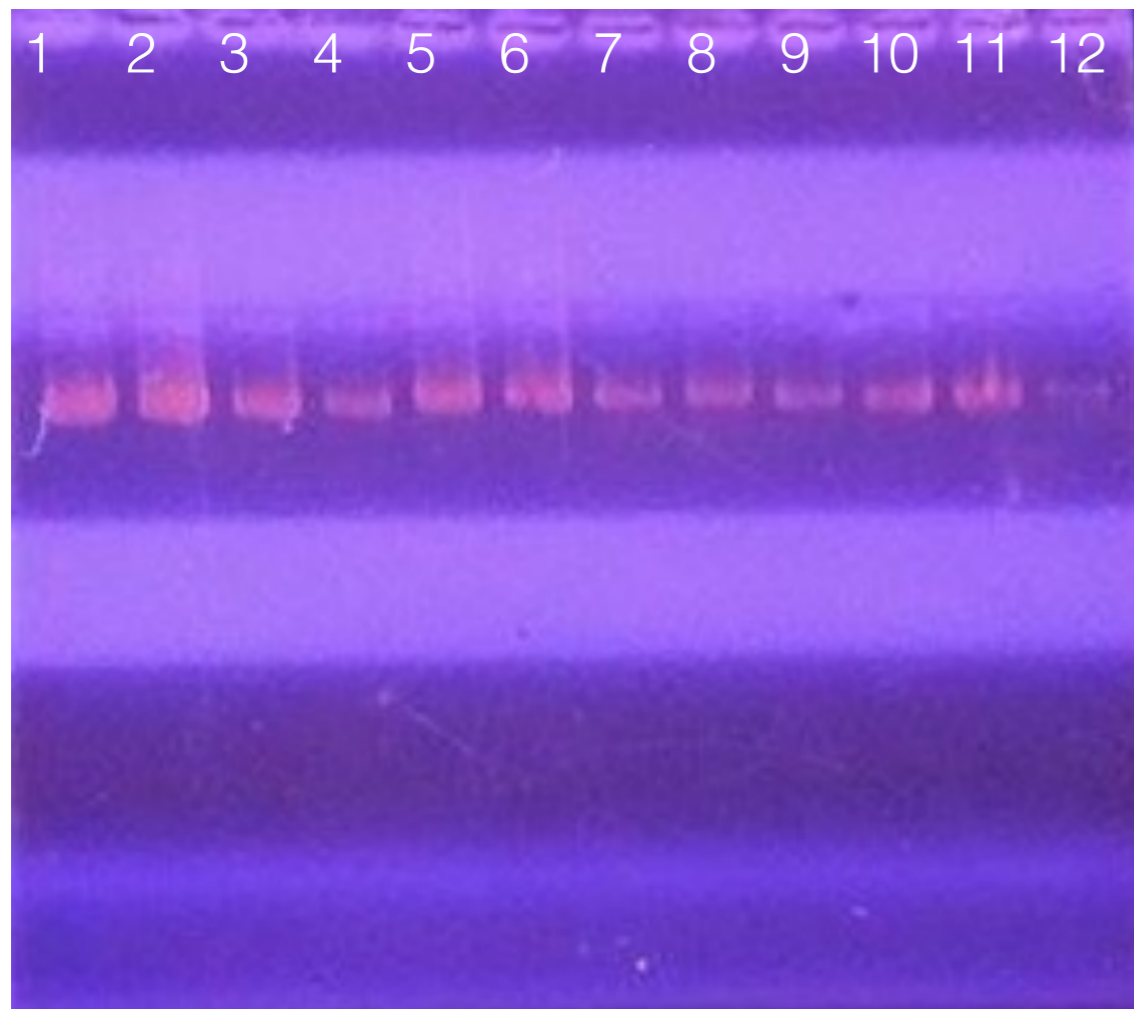
March 13th, 2014

Work in the lab was started and all the needed materials were prepared.

Cloning and DNA extraction of pSB1C3 started

April 19th, 2014

PSB1 plasmid minipreps Minipreps gel



Carri	Gene	Concentration
1	pSB1C3 - 4	50 ng/uL
2	pSB1C3 -3	
3	pSB1C3	
4	pSB1C3	
5	pSB1K3	
6	pSB1K3	
7	pSB1K3	
8	pSB1K3	
9	pSB1A3	
10	pSB1A3	
11	pSB1A3	
12	pSB1A3	

April 22nd, 2014

Synthetic genes were delivered :D

April 23rd, 2014

The synthetic genes were resuspended

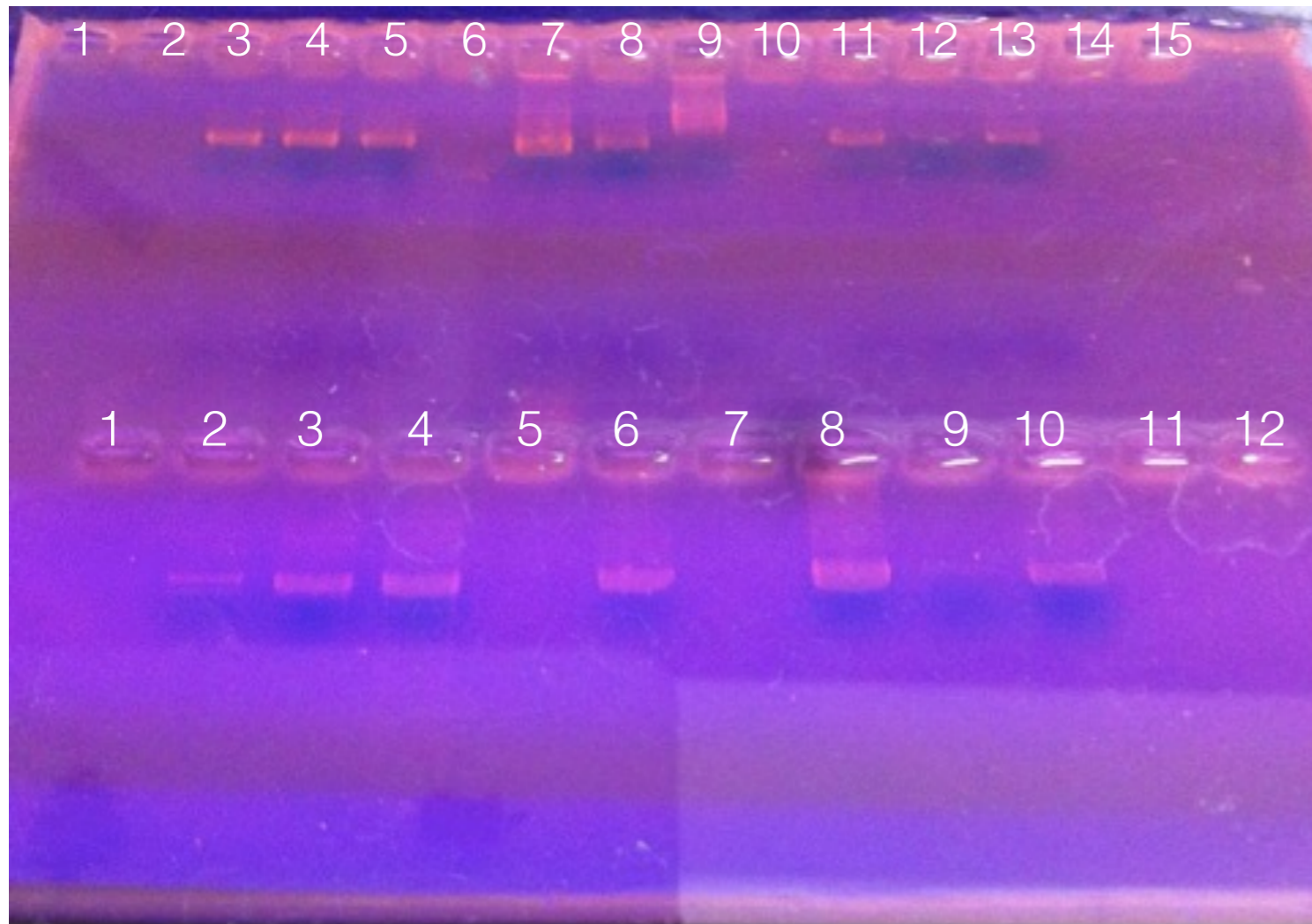
--- April, 2014

- Genes were transformed
- The bacteria were incubated with the genes.
 - Miniprep was made from synthetic genes.

-- April, 2014

Minipreps gel Synthetic genes

L2 1a (L2= the gene) (no.1=the bacteria) (a=can be a,b,c,d o e are the colonies)



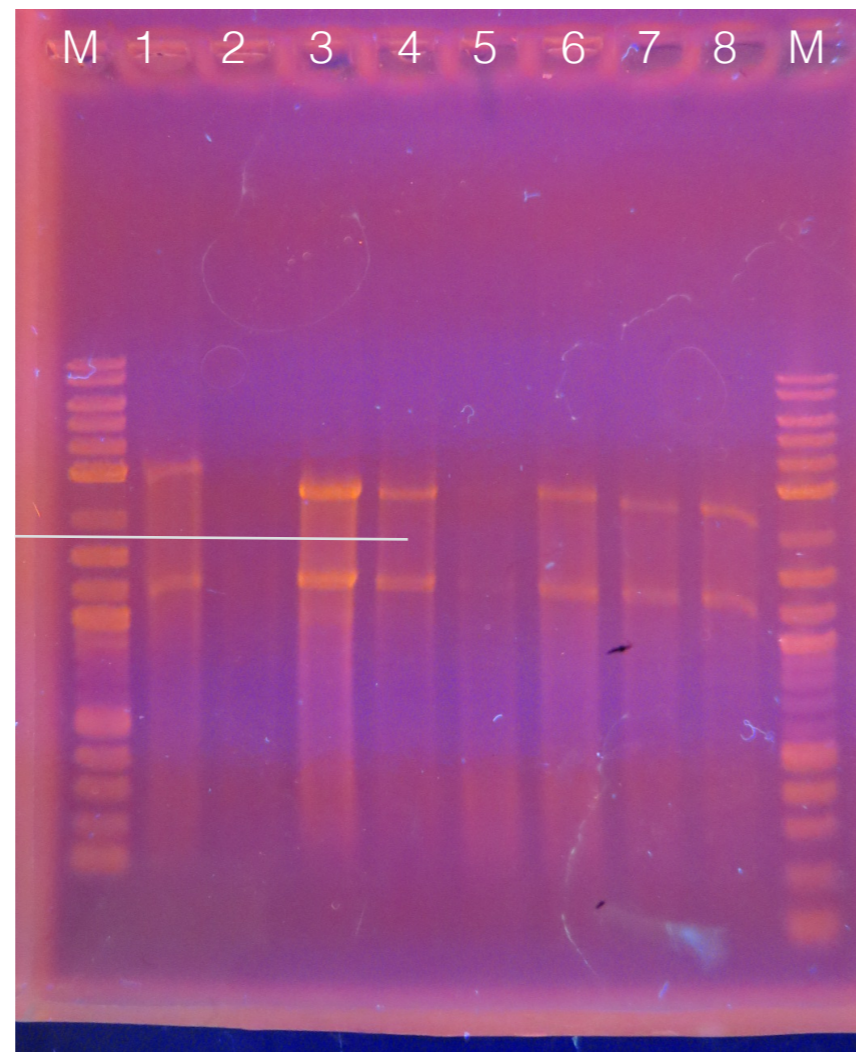
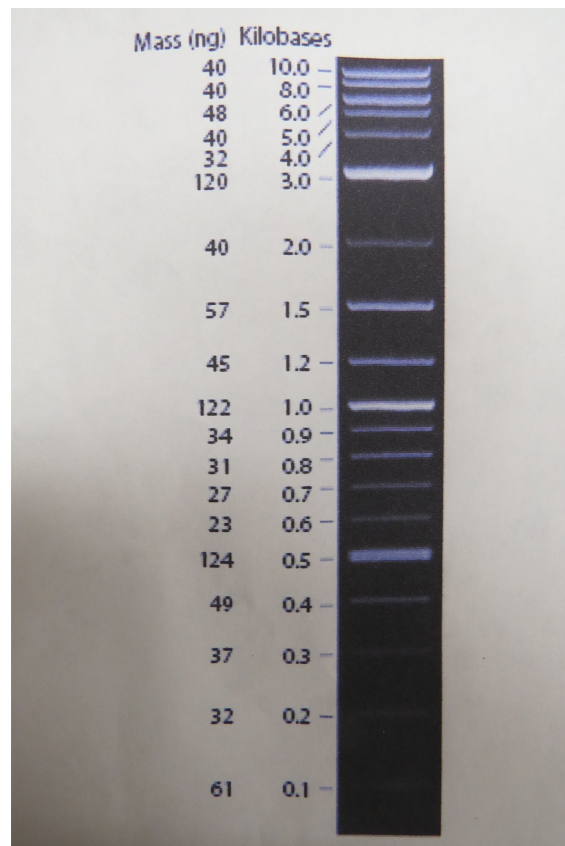
(Carril) Over	Gene	(Carril) below	Gene
1	--	1	--
2	--	2	L2 2a
3	L2 1a	3	L2 2c
4	L2 1b	4	L2 2d
5	L2 1c	5	--
6	--	6	NhaS 2a
7	NhaS 1a	7	--
8	NhaS 1b	8	Aroma 2a
9	NhaS 1c	9	Aroma 2b
10	--	10	Aroma 2c
11	Aroma 1a	11	--
12	Aroma 1b	12	--
13	Aroma 1c		
14	--		
15	--		

May 7th, 2014

Minipreps cutting:
pSB1 plasmids and synthetic genes

Thursday May 8, 2014

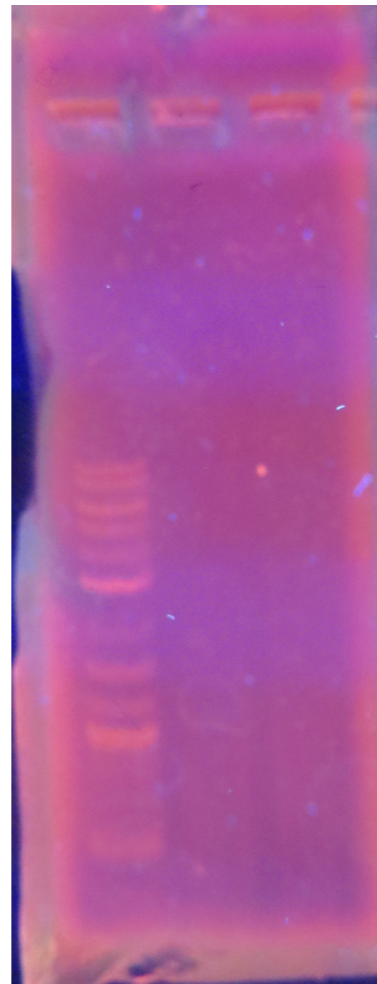
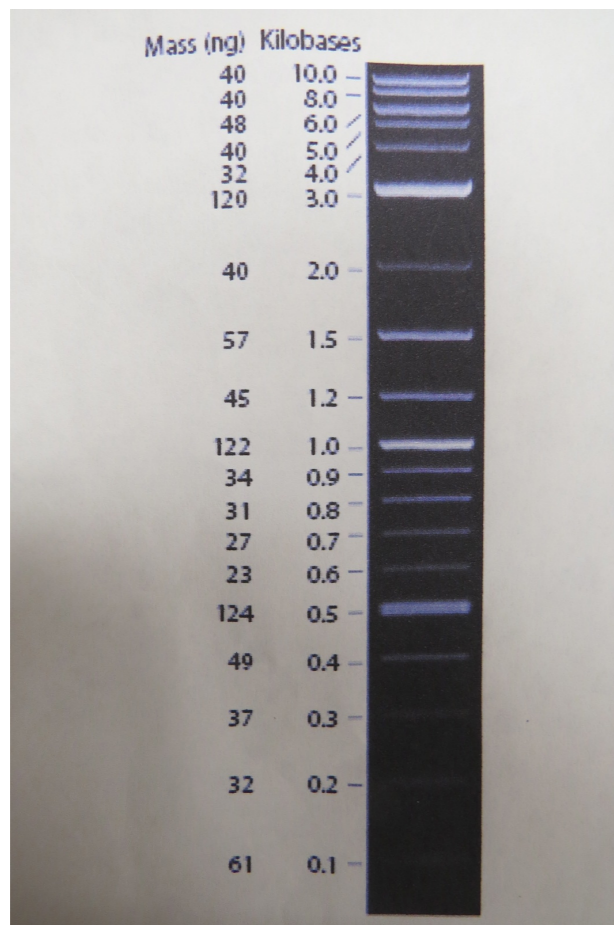
Inoculation tube to tube of AIDA (1 and 2)
Inoculation tube from plate plasmid pSB1 (A3-K3-C3)
Gel test cuts



Gene	Length
pSB1C3	2,070bp
NhaS	1,091bp
L2	1,085bp
Aroma	1,251bp
pUC57	2,710bp
RFP	707bp

Thursday May 8, 2014

Gel test cuts



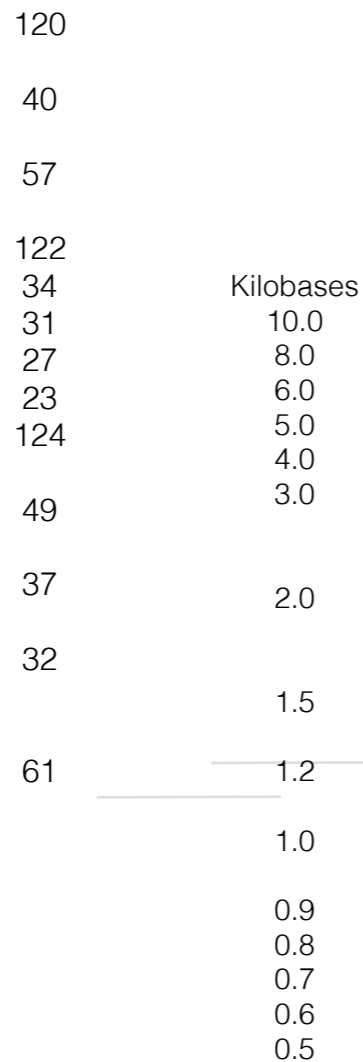
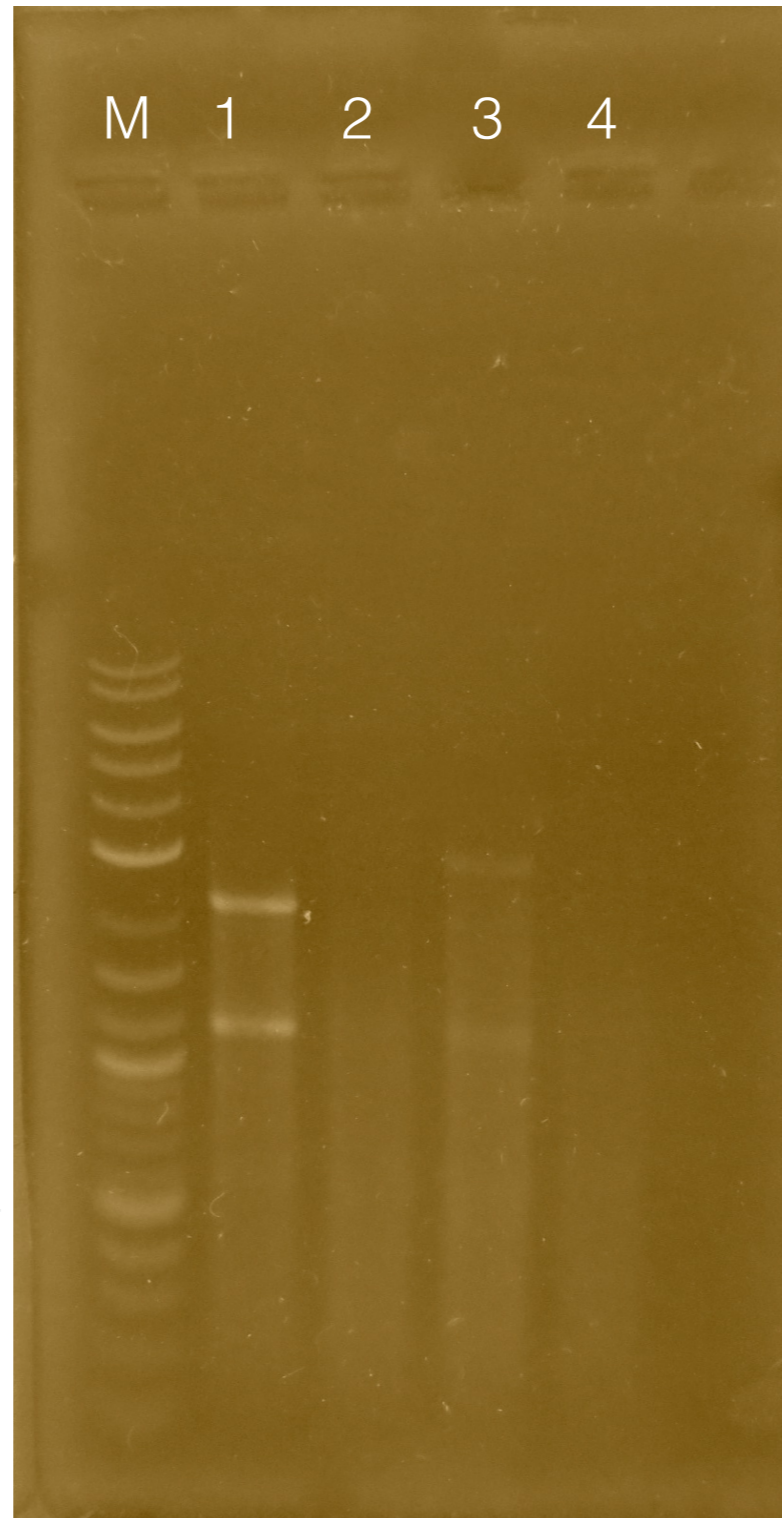
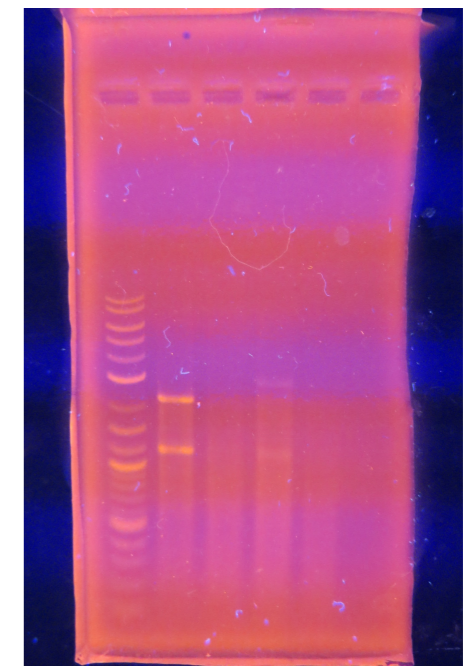
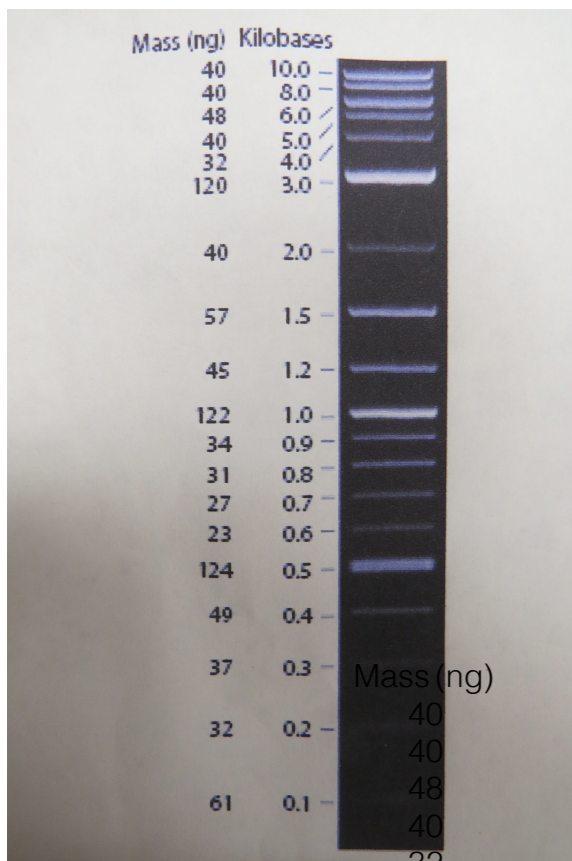
It did not work:c

ArOMA 1,251bp

pUC57 2,710bp

Friday May 9, 2014

Gel slices of synthetic genes



Carril	Gene
1	pSB1C3 (C4)
2	L2 (1a)
3	NhaS (1a)
4	A (1a)

Gene	Lenght
pSB1C3	2,070bp
NhaS	1,091bp
L2	1,085bp
Aroma	1,251bp
pUC57	2,710bp
RFP	707bp

Friday May 9, 2014

Plasmids and AIDA minipreps

Monday May 12, 2014

Synthetic genes inoculation:
-NhaS and AIDA tube to tube
-L2 y Aroma plate to tube

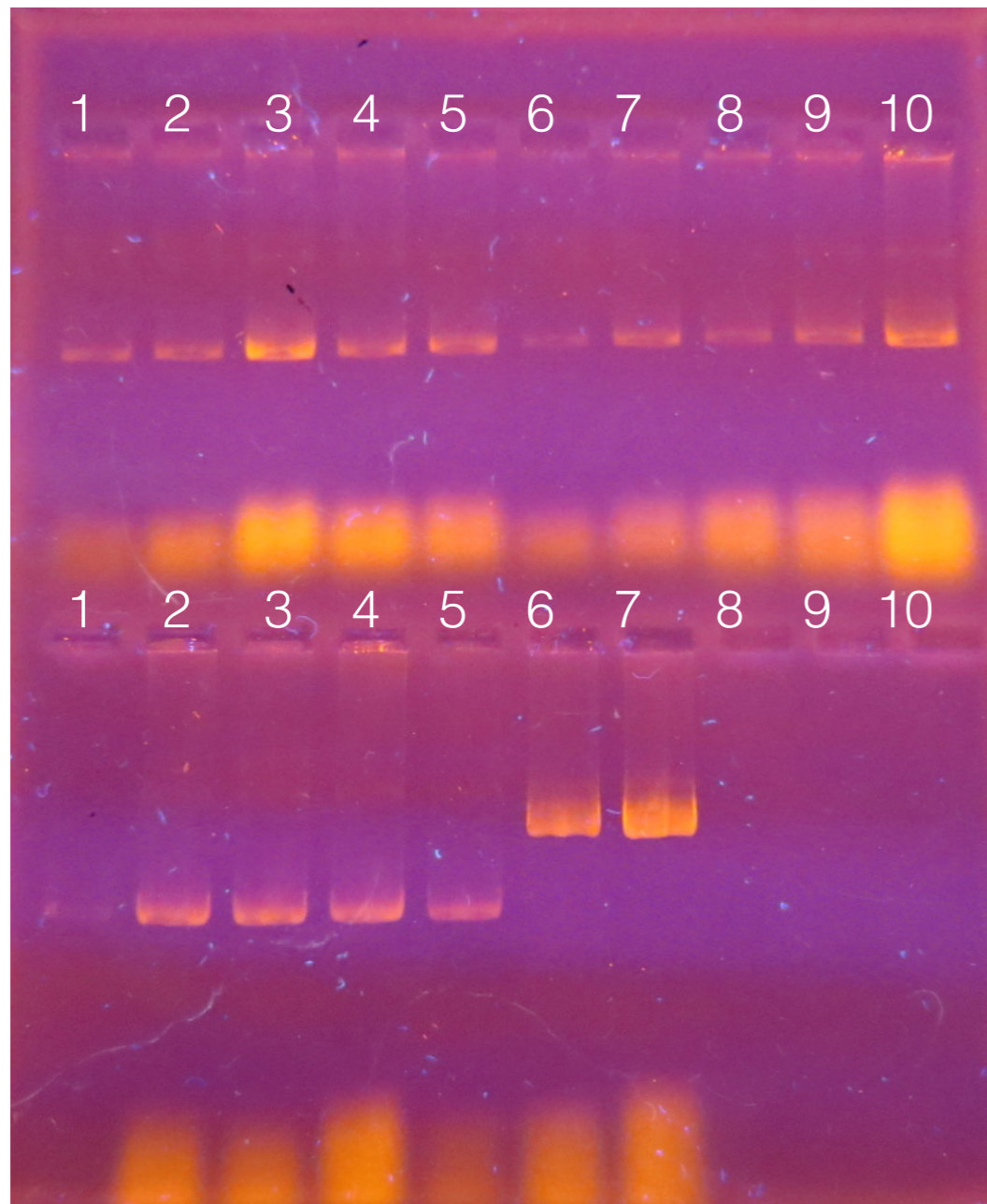
Ligation of the NhaS fragment and pSB1C3 vector

Transformation of the previous ligation

Synthetic genes miniprep

Monday May 12, 2014

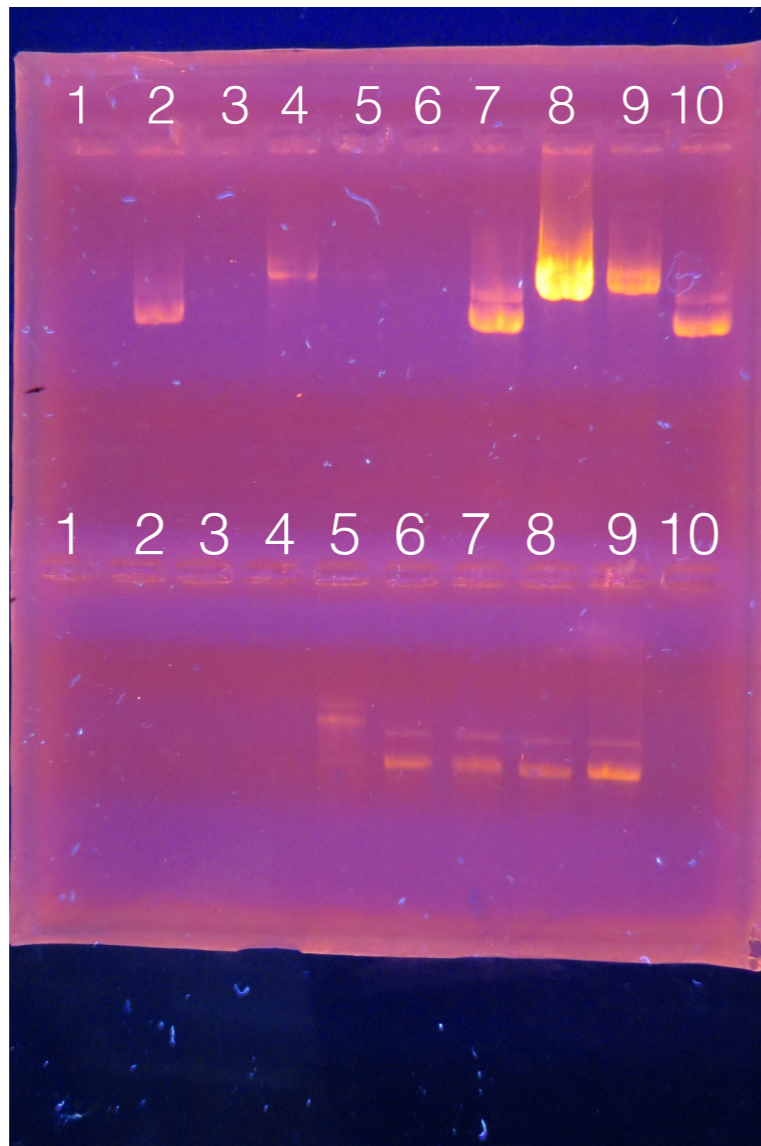
Plasmids and AIDA minipreps gel (9/05)



Carril Arriba	Gene	Concentration			
1	pSB1C3 - 1				
2	pSB1C3 - 2				
3	pSB1C3 - 3	50 ng / uL			
4	pSB1C3 - 4	20 ng/uL			
5	pSB1C3 - 5				
6	pSB1K3 - 1				
7	pSB1K3 - 2				
8	pSB1K3 - 3				
9	pSB1K3 - 4		Carril abajo	Gene	Concentration
10	pSB1K3 - 5		1	pSB1A3 - 1	
			2	pSB1A3 - 2	
			3	pSB1A3 - 3	
			4	pSB1A3 - 4	
			5	pSB1A3 - 5	
			6	AIDA - 1	
			7	AIDA - 2	100 ng/uL

Tuesday May 13, 2014

Gel of Miniprep of genes (previous day)



Carril arriba	Gene
1	L2-5
2	L2-4
3	L2-3
4	L2-2
5	L2-1
6	
7	NhaS-4
8	NhaS-3
9	NhaS-2
10	NhaS-1

Carril abajo	Gene
5	Aroma (Ar) - 5
6	Aroma (Ar) - 4
7	Aroma (Ar) - 3
8	Aroma (Ar) - 2
9	Aroma (Ar) - 1

Wednesday May 14, 2014

Transform ligation PSB1C3 + NhaS Quantitative digestion of pSB1C3 and NhaS

Mix NhaS (1 y2)			
DNA	5uL	x2.2	
Buff H	2uL	x2.2	4.4uL
EcoRI	0.5uL	x2.2	1.1uL
PstI	0.5uL	x2.2	1.1uL
H2O	12uL	x2.2	26.4uL
Total	20uL	x2.2	33uL

Mix pSB1C3 - 3	
DNA	10uL
Buffer H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	7uL
Total	20uL

Quantitative digestion of AIDA, L2, Aroma

Mix Aroma -2	
DNA	5uL
Buff H	1uL
EcoRI	0.5uL
PstI	0.5uL
H2O	3uL
Total	10uL

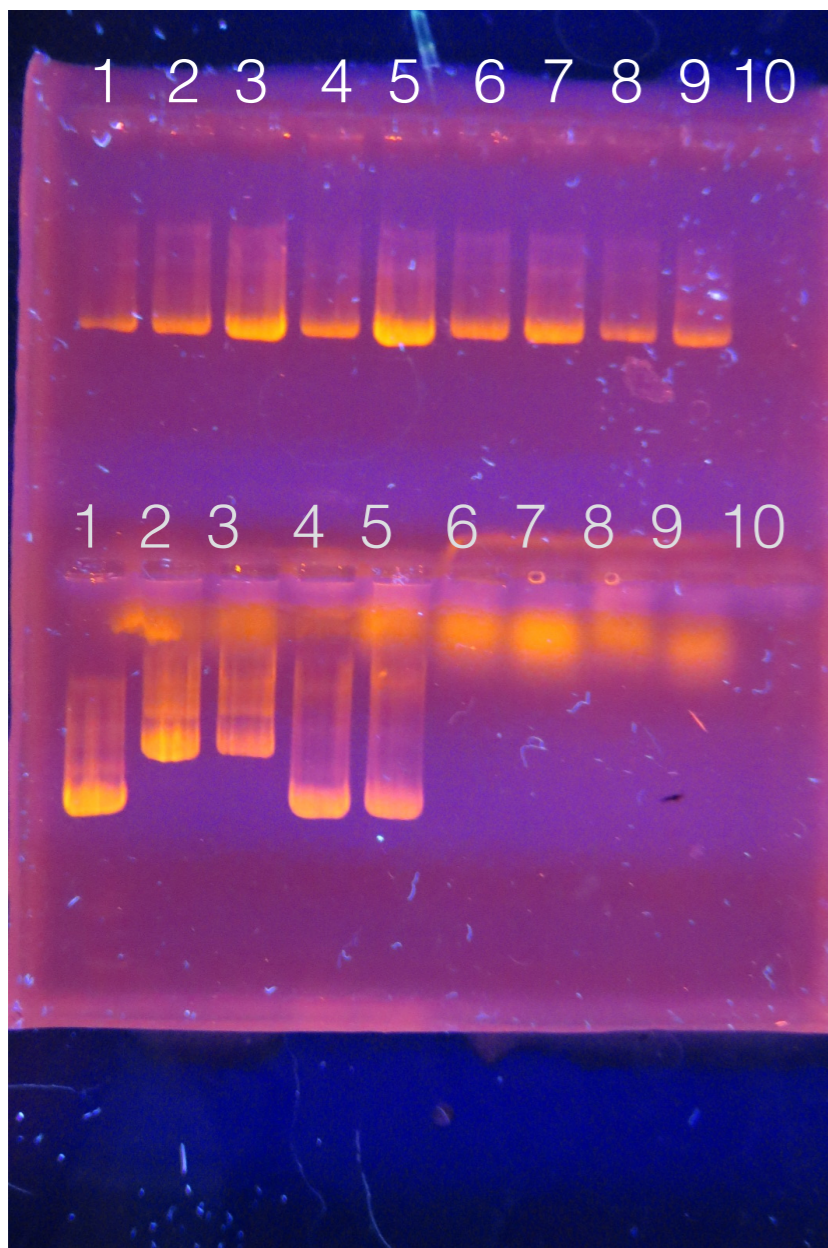
Mix L2 -5	
DNA	5uL
Buff H	1uL
EcoRI	0.5uL
BamHI	0.5uL
H2O	3uL
Total	10uL

Mix AIDA - 2	
DNA	2.5uL
Buff H	1uL
PstI	0.5uL
BglII	0.5uL
H2O	5.5uL
Total	10uL

Tuesday May 13, 2014

Minipreps gel of synthetic genes

Gel of Minipreps



Carril arriba	Gene	Concentration	Carril abajo	Gene	Concentration
1	L2-1		1	NhaS- 1	120 ng / uL
2	L2-2		2	NhaS- 2	100 ng/uL
3	L2- 3		3	NhaS- 3	
4	L2- 4		4	NhaS- 4	
5	L2- 5	50 ng /uL	5	NhaS- 5	
6	Aroma (Ar) - 1				
7	Aroma - 2	50 ng /uL			
8	Aroma - 3				
9	Aroma - 4				
10	Aroma - 5				

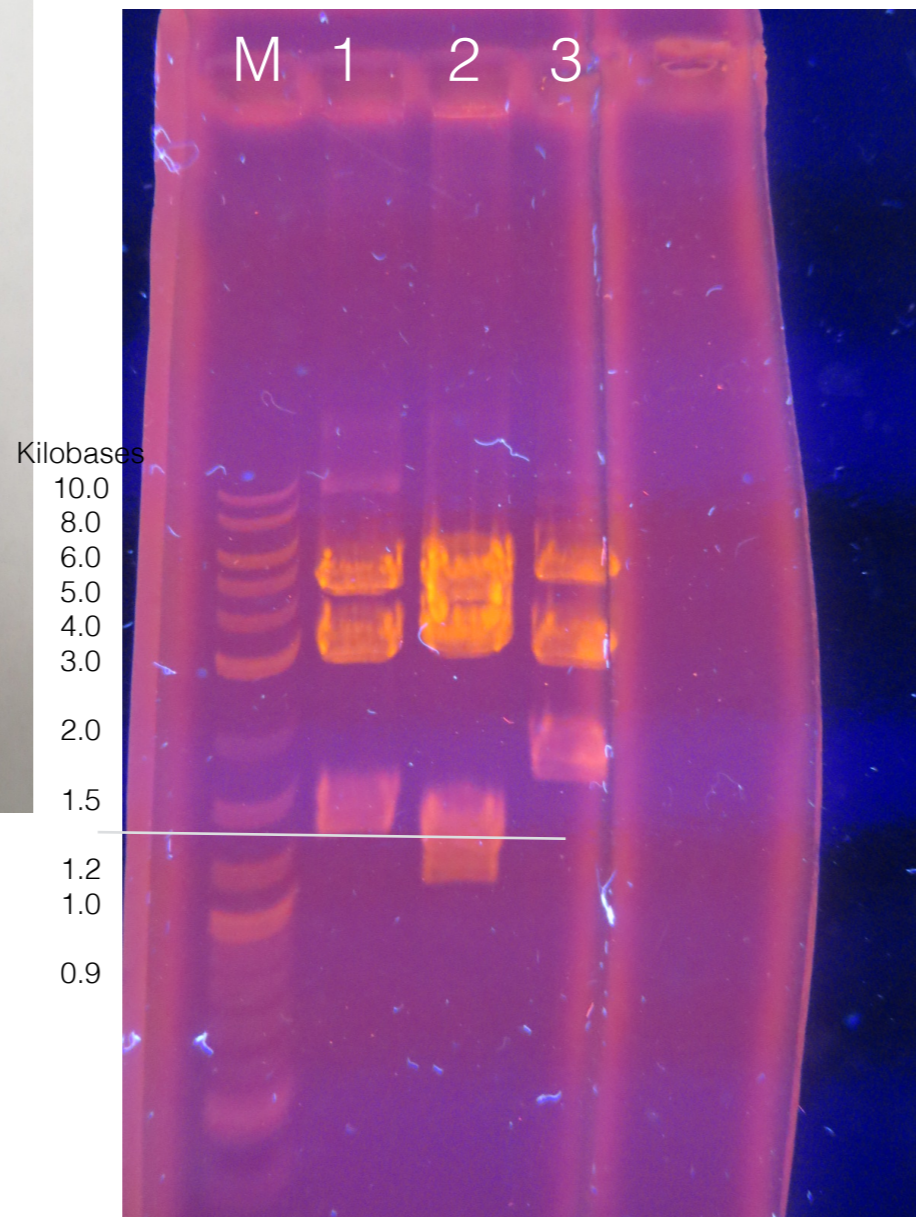
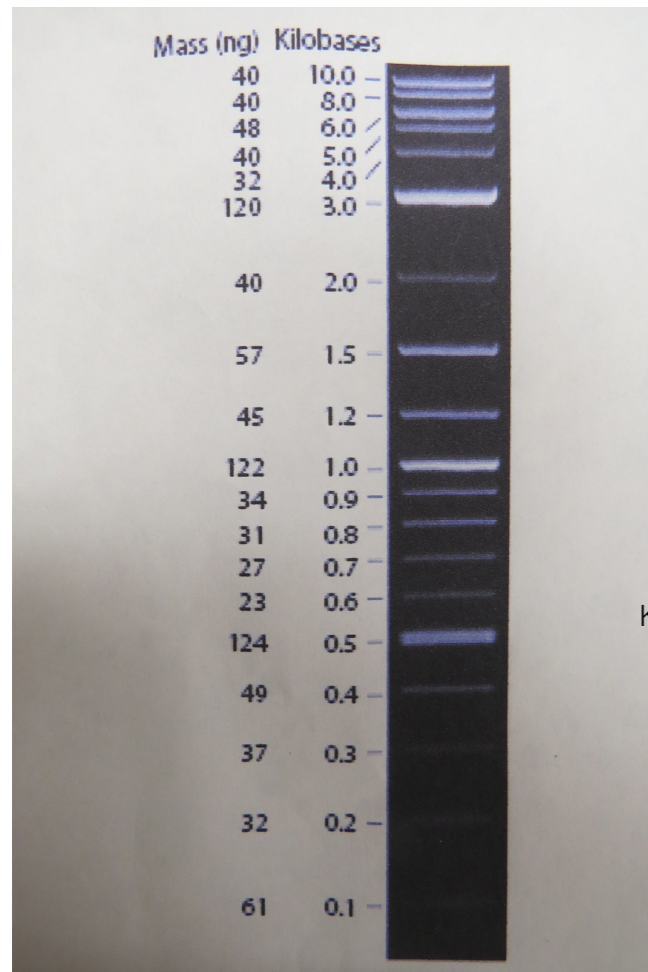
Wednesday May 14, 2014

Transformation of NhaS + pSB1C3

Transformation plating NhaS + pSB1C3 (ligation 12/05)

Wednesday May 14, 2014

Qualitative digestion gel of synthetic genes

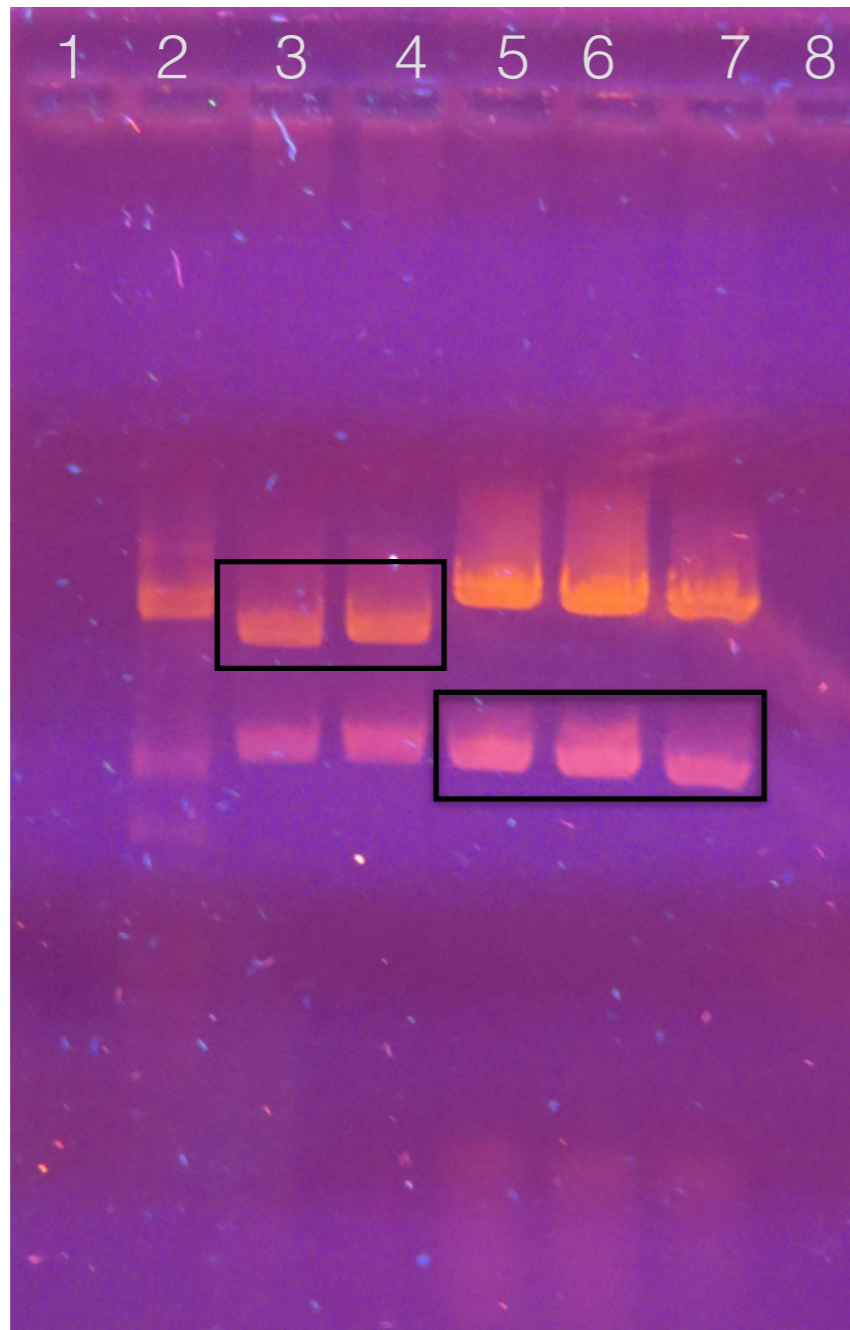


Spot	Gene
1	Aroma 2
2	AIDA-2
3	L2-5

Gene	Length
pSB1C3	2,070bp
NhaS	1,091bp
L2	1,085bp
Aroma	1,251bp
pUC57	2,710bp
RFP	707bp
AIDA	1482bp

Friday May 16, 2014

Quantitative digestion gel (14/05)

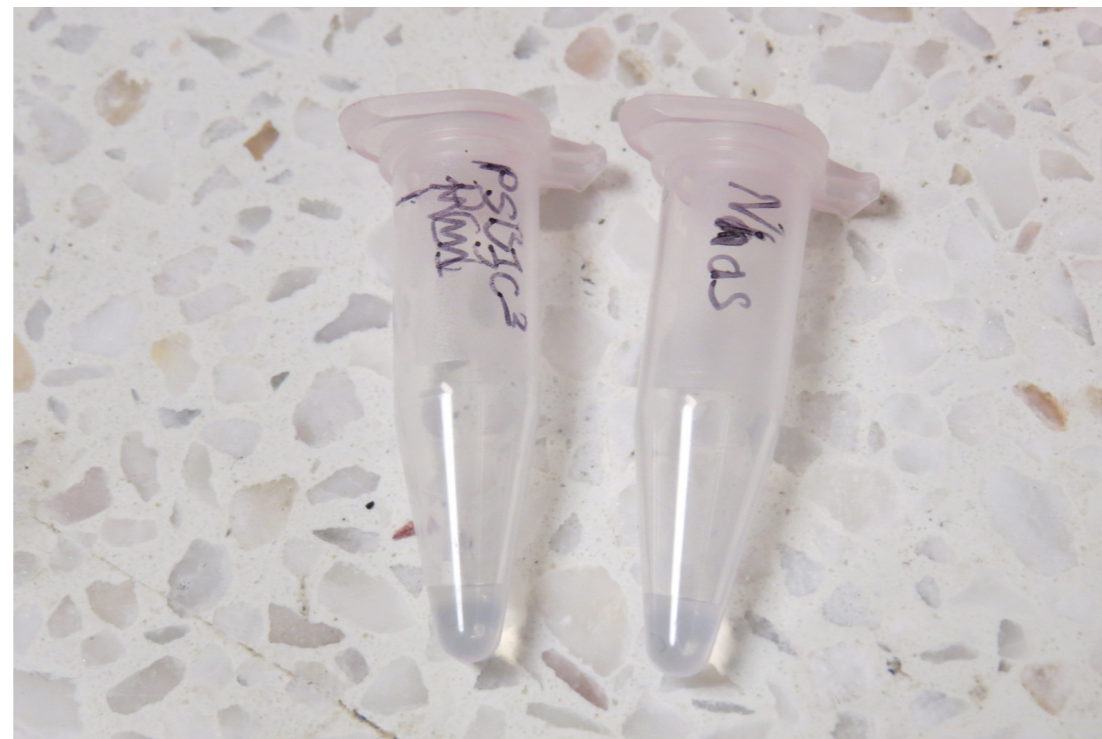


Carril	Gene
1	Loading buffer 10x
2	Marker
3	pSB1C3
4	pSB1C3
5	NhaS
6	NhaS
7	NhaS

Gene	Lenght
pSB1C3	2,070bp
NhaS	1,091bp
RFP	707bp
pUC57	2,710bp

Friday May 16, 2014

Purification of the quantitative digestion (14/05) to ligation



Saturday May 17, 2014

Ligation (purification 16/05)
NhaS + pSB1C3

Ligation and transformation (17/05)

Monday May 19, 2014

Quantitative digestion

Mix L2 -5

DNA	6uL
Buff H	2uL
EcoRI	0.5uL
BamHI	0.5uL
H2O	11uL
Total	20uL

Mix pSB1C3 - 3

DNA	10uL
Buffer H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	7uL
Total	20uL

Mix Aroma -2

DNA	6uL
Buff H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	11uL
Total	10uL

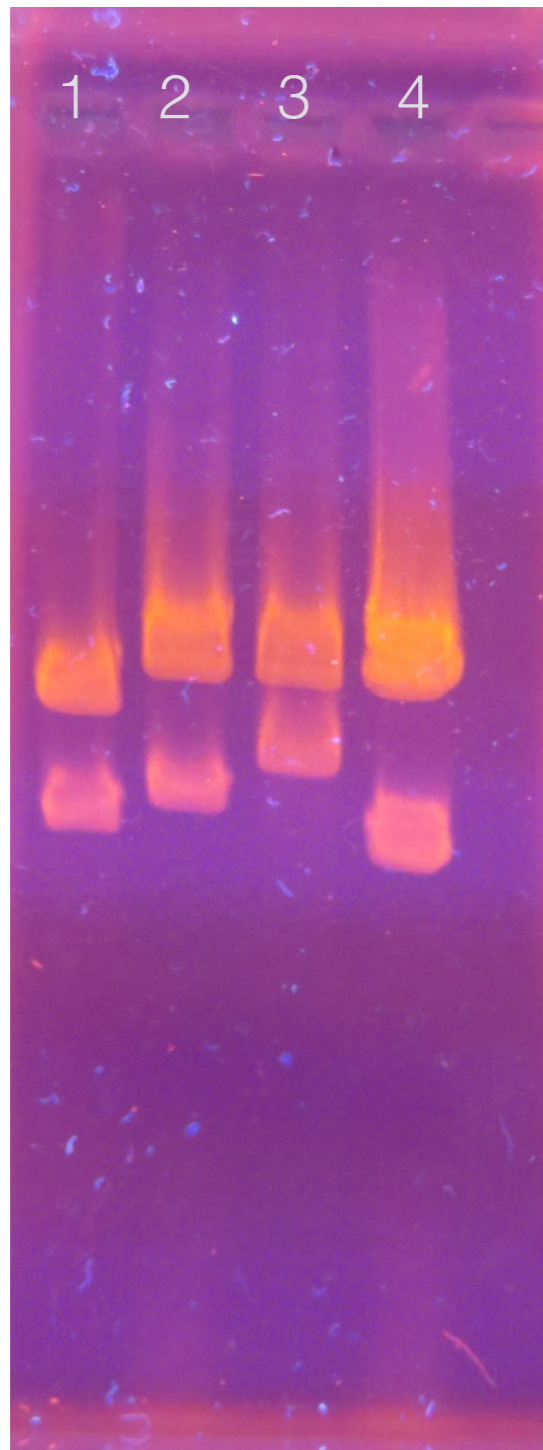
Mix AIDA - 2

DNA	3.5uL
Buff H	2uL
PstI	0.5uL
BglII	0.5uL
H2O	13.5uL
Total	20uL

Monday May 19, 2014

Quantitative digestion gel (19/05)

Purification

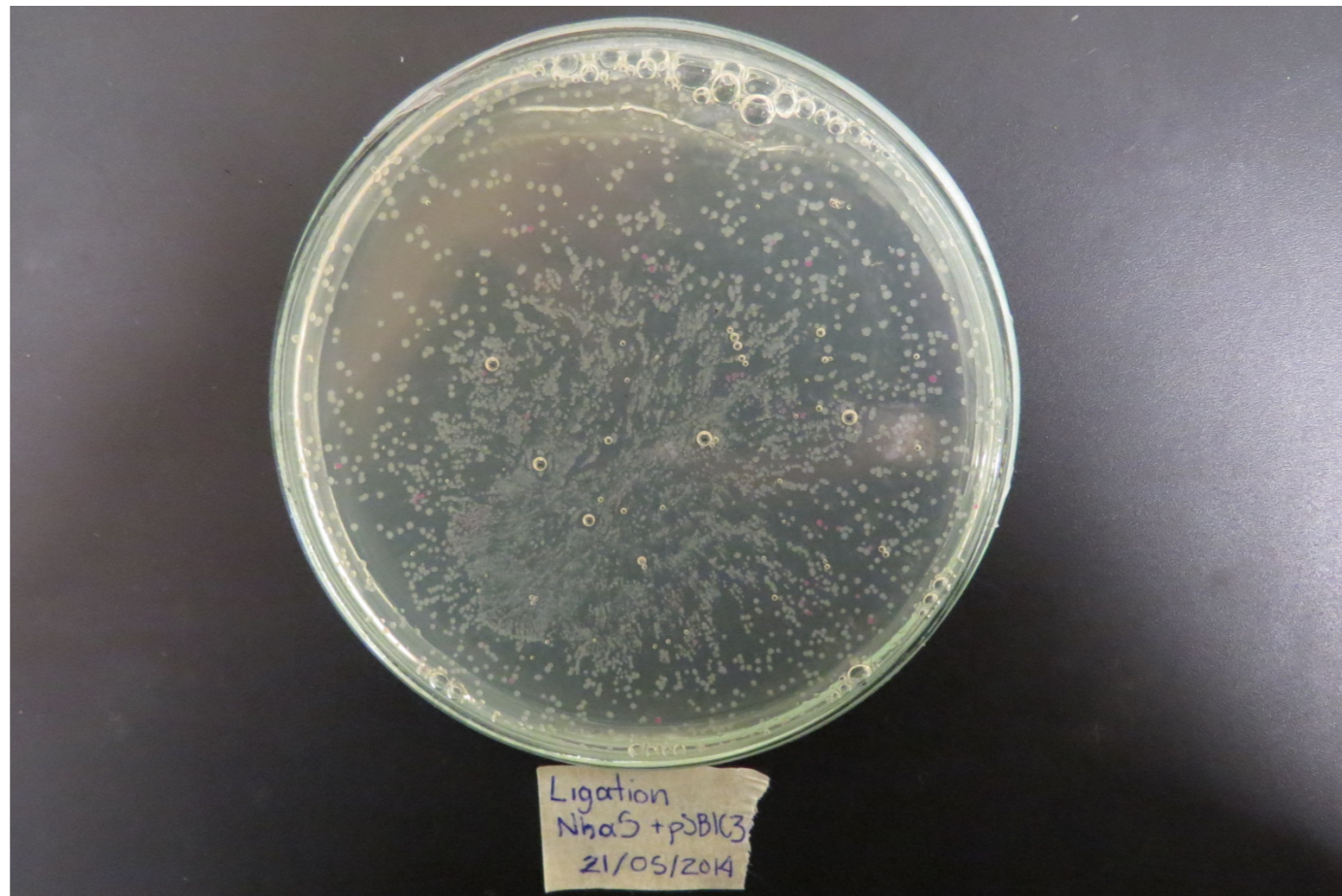


Spot	Gene
1	pSB1C3
2	Aroma
3	AIDA
4	L2

Gene	Length
pSB1C3	2,070bp
NhaS	1,091bp
L2	1,085bp
Aroma	1,251bp
pUC57	2,710bp
RFP	707bp
AIDA	1482bp

Monday May 19, 2014

Transformation NhaS + pSB1C3 (Ligation of 17/05)



Tuesday May 20, 2014

Ligation

Aroma + pSB1C3

Vector	15uL
Inserto	15uL
Buffer	10uL
Enzima	5uL
H2O	5uL
Total	50uL

L2 + AIDA + pSB1C3

Vector	10uL
Inserto	10uL
Inserto	10uL
Buffer	10uL
Enzima	5uL
H2O	5uL
Total	50uL

Inoculation plate - tube

2 tubes of pSB1C3

Wednesday May 21, 2014

Miniprep (inoculation 20/05) pSB1C3

Miniprep gel (previous)



Did not work :(

Wednesday May 21, 2014

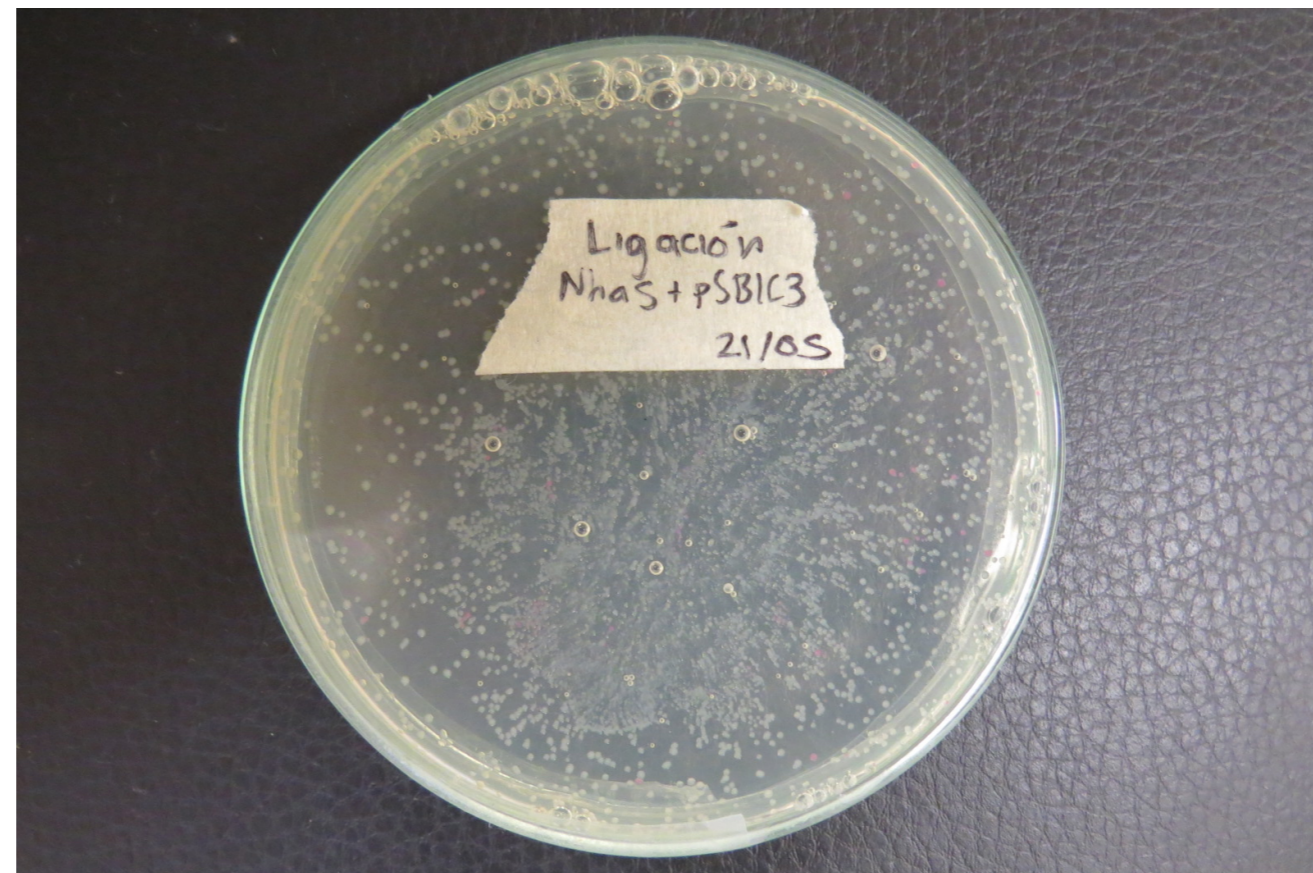
Inoculation plate with beads

Ligation Aroma + pSB1C3

Ligation L2 + AIDA + pSB1C3

Ligation NhaS + pSB1C3

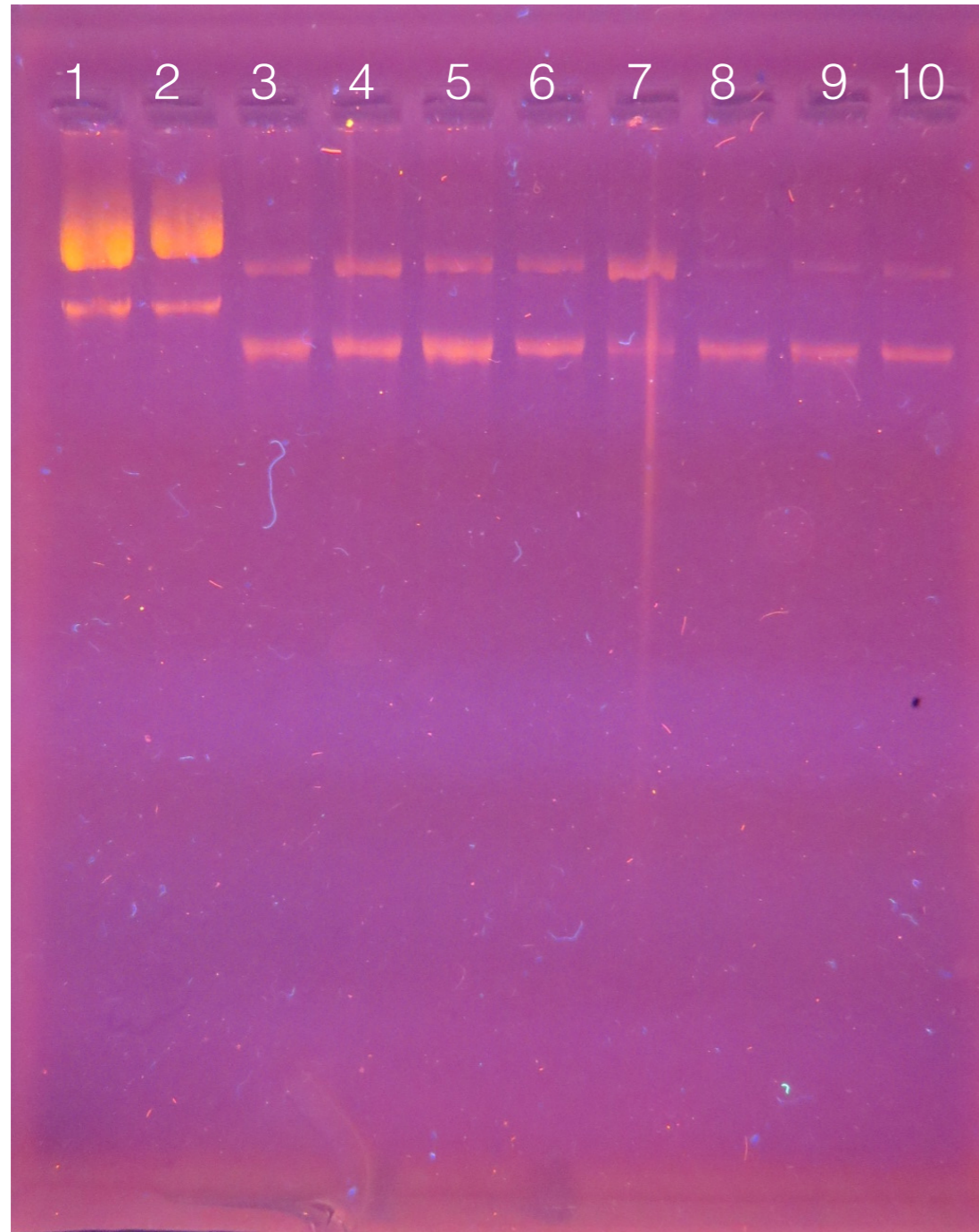
Ligation NhaS + pSB1C3 (New England biolabs - 2 plates)



Wednesday May 21, 2014

Miniprep (inoculation 20/05) pSB1C3

Thursday May 22, 2014



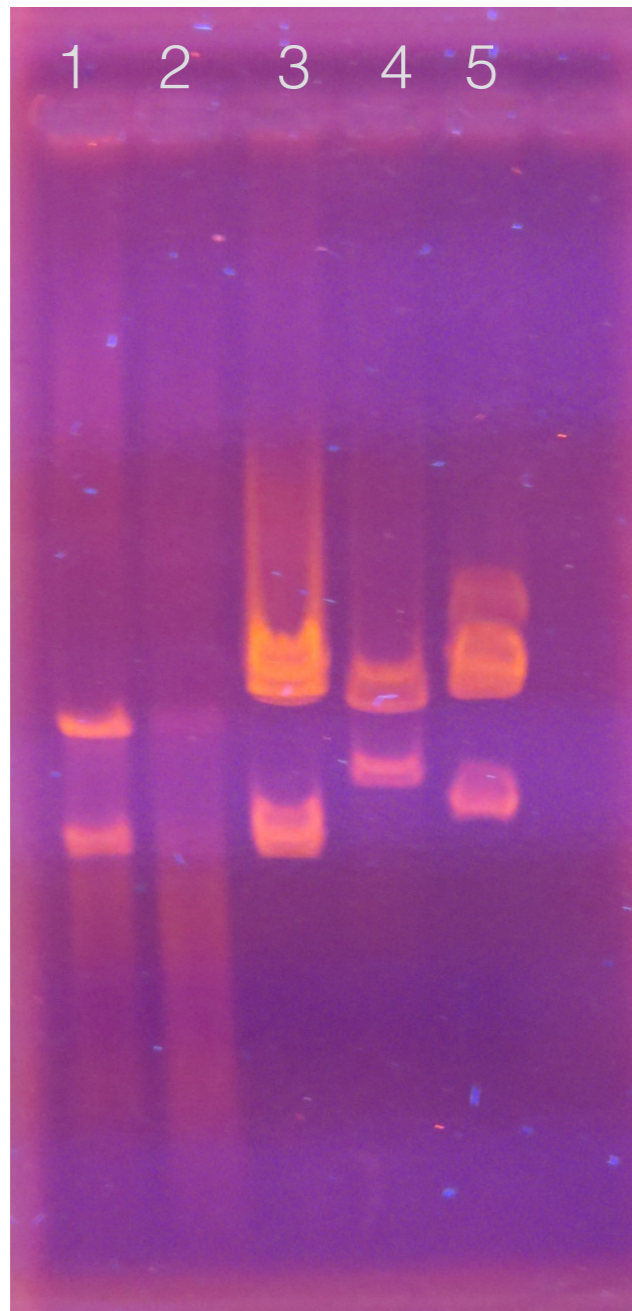
Minipreps
NhaS R (1, 2, 3)
NhaS B (1, 2, 3)
AIDA (1 y 2)
pSB1C3 (1 y 2)

Minipreps gel (previous)

Spot	Gene
1	AIDA-1
2	AIDA-2
3	NhaS - B1
4	NhaS - B2
5	NhaS - B3
6	NhaS - R1
7	NhaS - R2
8	NhaS - R3
9	pSB1C3 - 1
10	pSB1C3 - 2

Thursday May 22, 2014

Gel of the digestion (21/05)

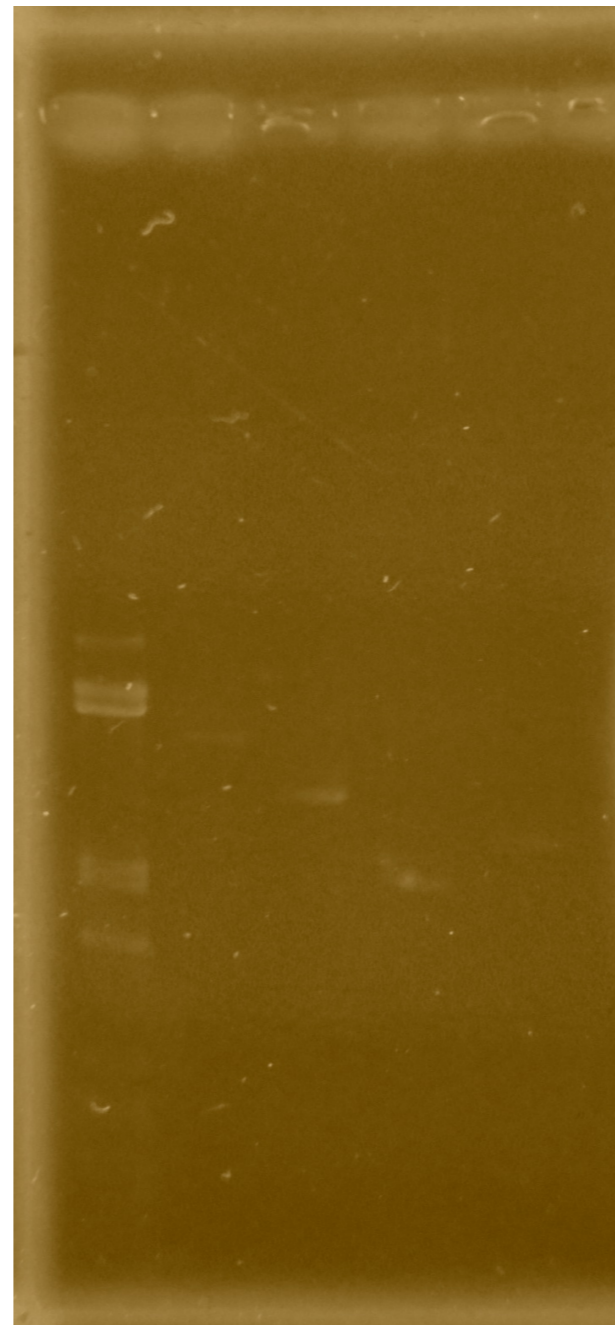
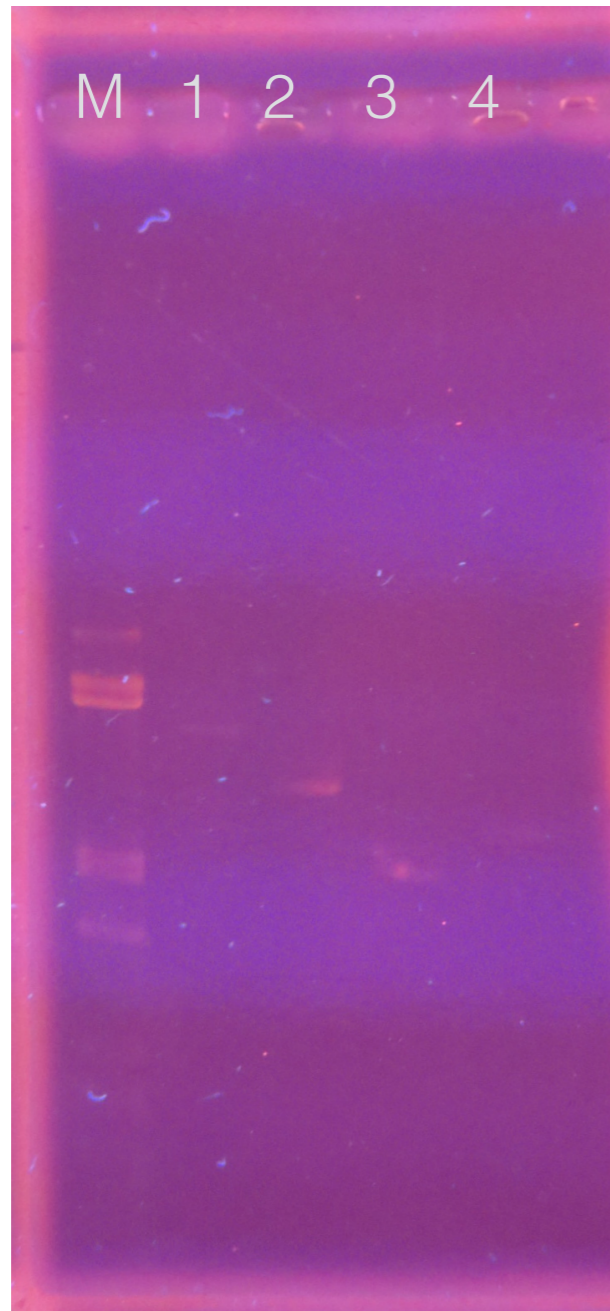


Spot	Gene
1	pSB1C3 - 4
2	pSB1C3 - 5
3	AIDA
4	AIDA
5	Aroma

Gene	Lenght
pSB1C3	2,070bp
L2	1,085bp
Aroma	1,251bp
AIDA	1496bp

Thursday May 22, 2014

Gel of purification from gene segments

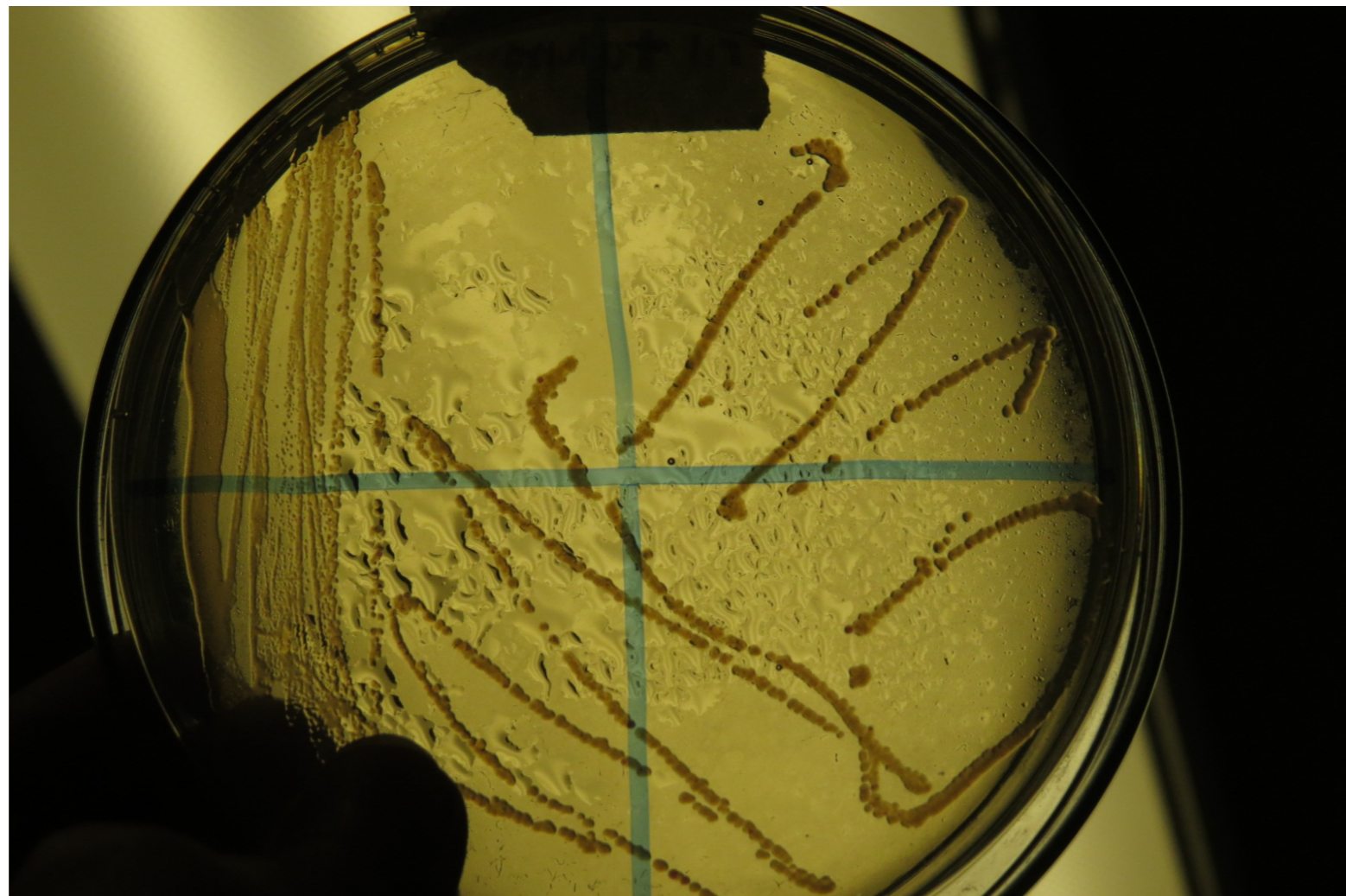


Spot	Gene
1	pSB1C3
2	L2
3	AIDA
4	Aroma

Gene	Lenght
pSB1C3	2,070bp
L2	1,085bp
Aroma	1,251bp
AIDA	1496bp

Friday May 23, 2014

Experimentation with NhaS



Monday May 26, 2014

Inoculation plate - tube (6 tubes of pSB1C3)

Ligation Aroma + pSB1C3

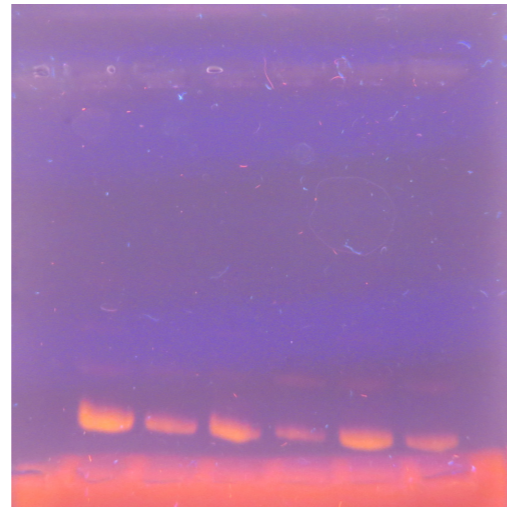
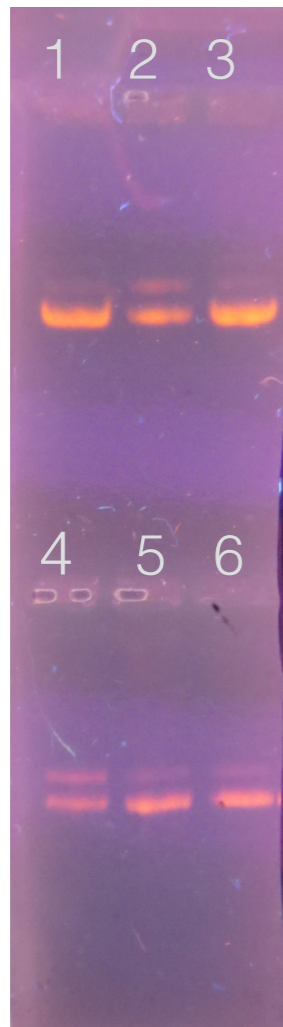
Aroma + pSB1C3

Vector	15uL
Insert	15uL
Buffer	10uL
Enzyme	5uL
H2O	5uL
Total	50uL

Tuesday May 27, 2014

Minipreps pSB1C3 (inoculation 26/05)

Gel from previous minipreps



Spot	Gene
1	pSB1C3 - 1
2	pSB1C3 - 2
3	pSB1C3 - 3
4	pSB1C3 - 4
5	pSB1C3 - 5
6	pSB1C3 - 6

Tuesday May 27, 2014

Quantitative digestion

Mix L2 -2

DNA	9uL
Buff H	2uL
EcoRI	0.5uL
BamHI	0.5uL
H2O	8uL
Total	20uL

Mix Aroma - 3

DNA	9uL
Buffer H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	8uL
Total	20uL

Mix PSB1C3

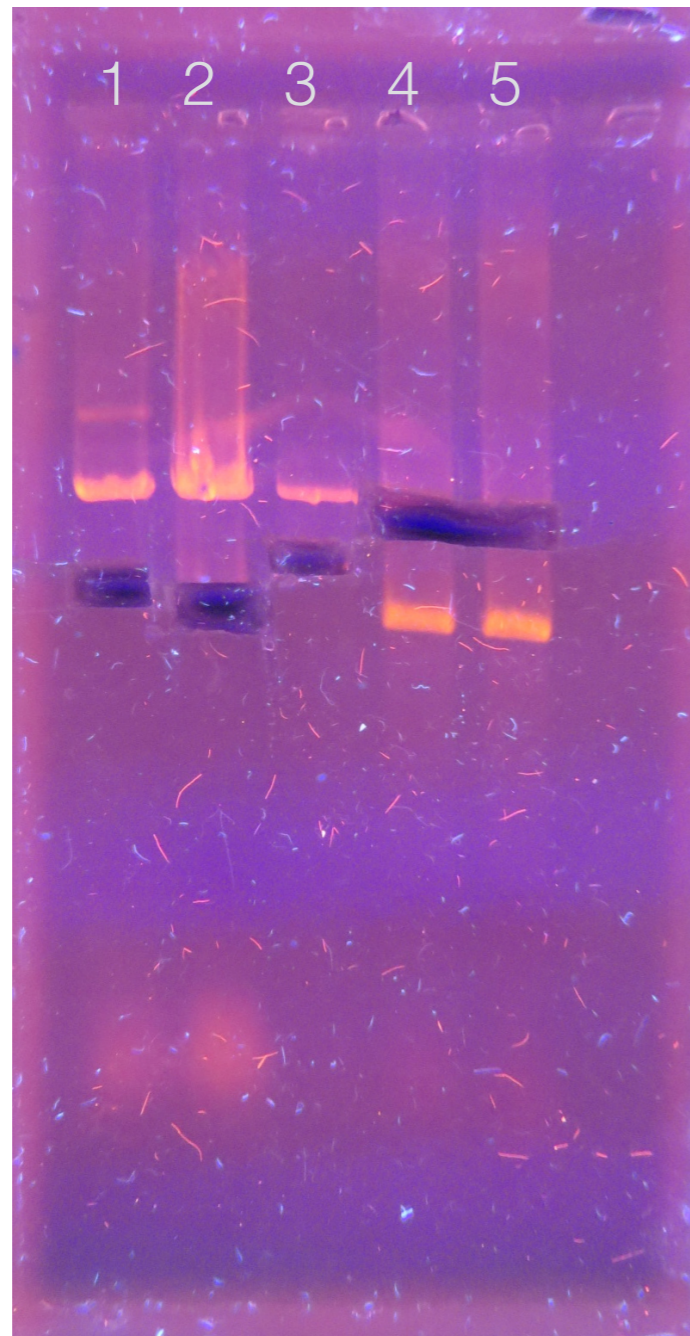
DNA	12uL
Buff H	4uL
EcoRI	0.5uL
PstI	0.5uL
H2O	23uL
Total	40uL

Mix AIDA - 2

DNA	3.5uL
Buff H	2uL
PstI	0.5uL
BglII	0.5uL
H2O	13.5uL
Total	20uL

Tuesday May 27, 2014

Gel digestions (27/05)



Carril	Gene
1	Aroma -3
2	L2 -2
3	AIDA - 2
4	pSB1C3 - 1
5	pSB1C3 - 1

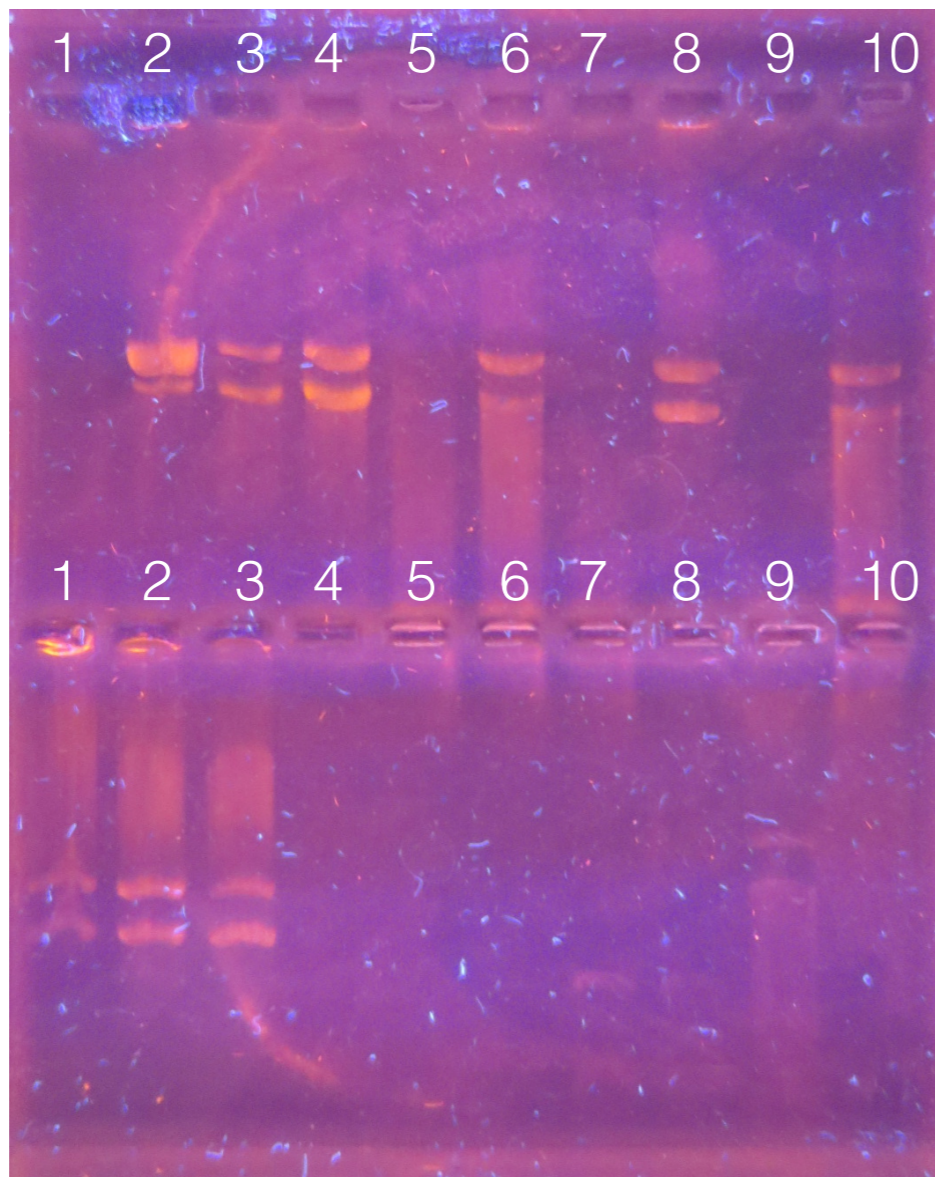
Did not take photo before
cutting :c

Tuesday May 27, 2014

Purification digestion (27/05)

Tuesday May 27, 2014

Gel of old minipreps (before April)

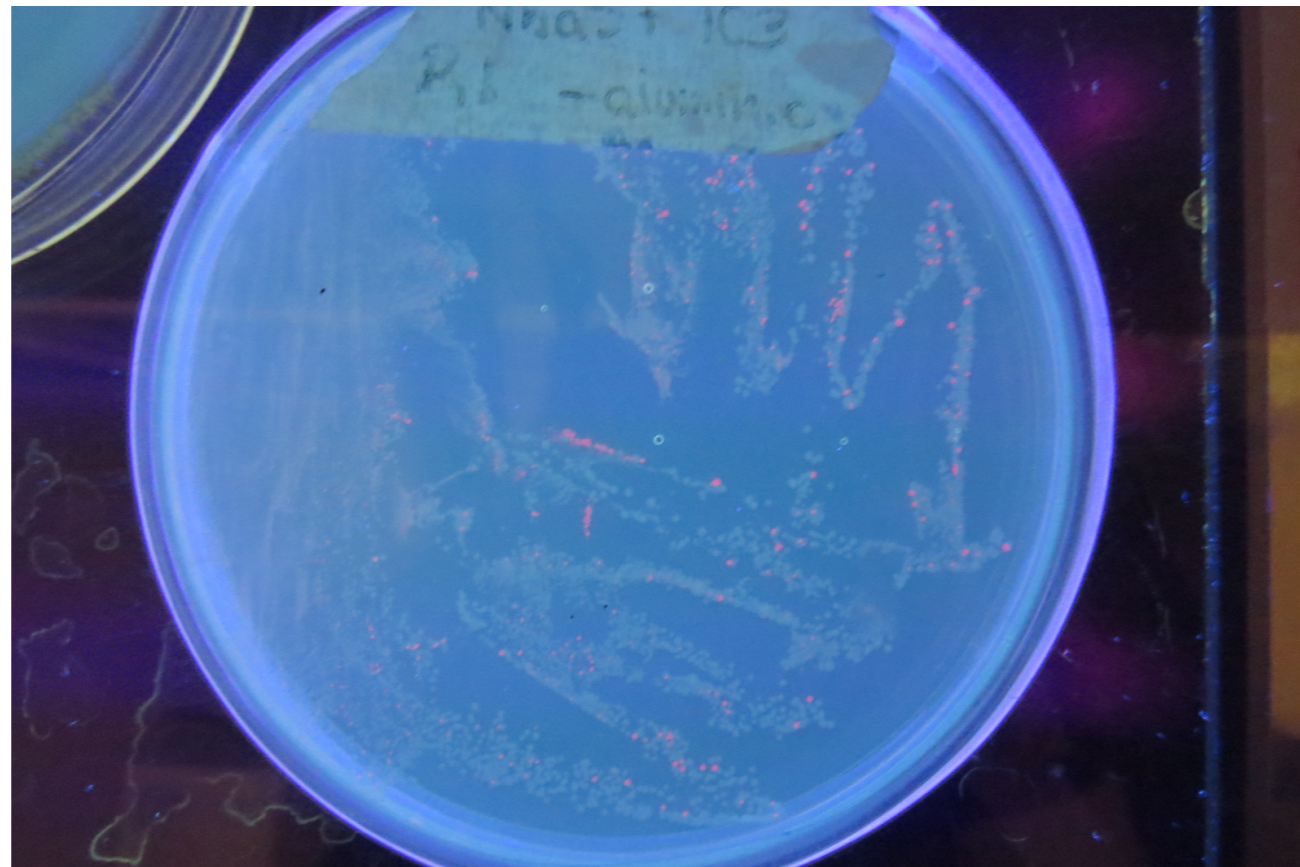


Spot	Gene (pSB1C3)
1	1
2	1
3	2
4	2
5	3
6	3
7	4
8	4
9	5
10	5

Tuesday May 27, 2014

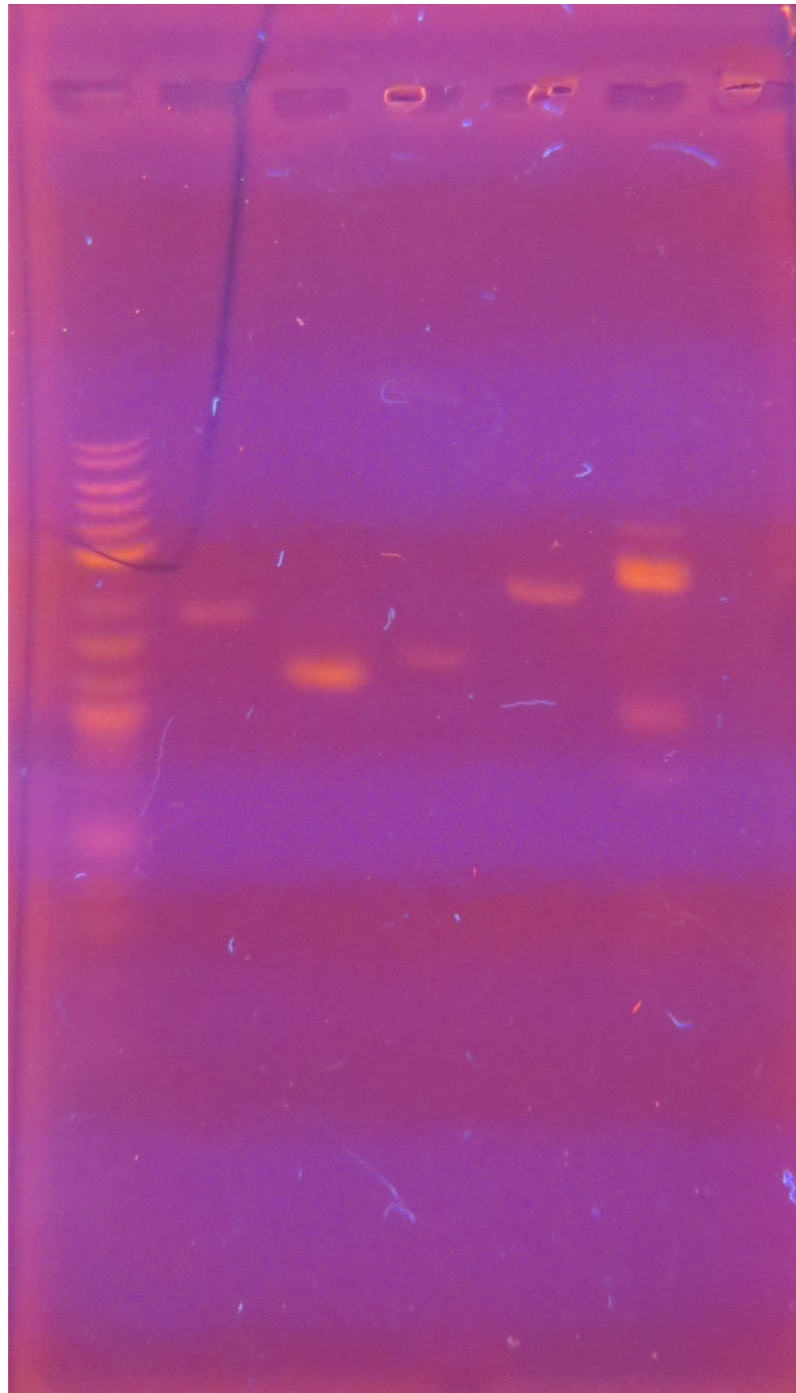
Experimentation of NhaS gene

It was exposed to UV (302nm) in intervals of 10 minutes:
two plates of NhaS in pSB1C3 (See procedure and
results in methods and results tabs)



Wednesday May 28, 2014

Purification gel



Wednesday May 28, 2014

Ligation

AIDA + L2 + pSB1C3

Vector	10 uL
Vector	10 uL
Insert	15 uL
Buffer	10uL
Enzyme	5uL
Total	50uL

Aroma + pSB1C3

Vector	15uL
Insert	15uL
Buffer	10uL
Enzyme	5uL
H2O	5uL
Total	50uL

Wednesday May 28, 2014

Inoculation to plate with beads

Ligation of Aroma 150 uL

NhaS Red 50uL

NhaS Red 50 uL

NhaS White 100 uL

NhaS White 100 uL

Inoculation plate to tube

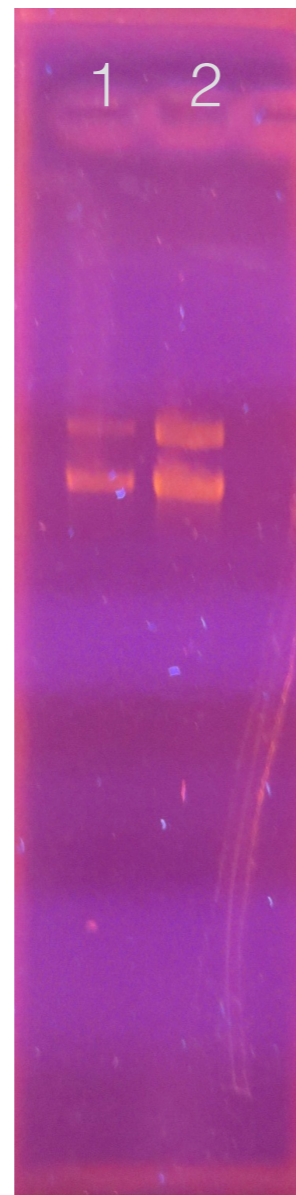
NhaS + 1C3 red

NhaS + 1C3 white

Thursday May 29, 2014

Miniprep of Red and White clones of NhaS for their sequenciation

Miniprep gel (29/06) of NhaS red and white



Spot	Gene
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1	NhaS Red
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2	NhaS white
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Monday June 2, 2014

Inoculation tube - tube (50ml) for midi prep of pSB1C3, Aroma, L2 y AIDA. It was inoculated two tubes of each DNA. Each tube had 25 ml of LB medium.

Monday June 2, 2014

Quantitative digestion for Union module.

Mix L2 -2 (8/05)

DNA	9uL
Buff H	2uL
EcoRI	0.5uL
BamHI	0.5uL
H2O	8uL
Total	20uL

Mix pSB1C3 - 1 (27/05)

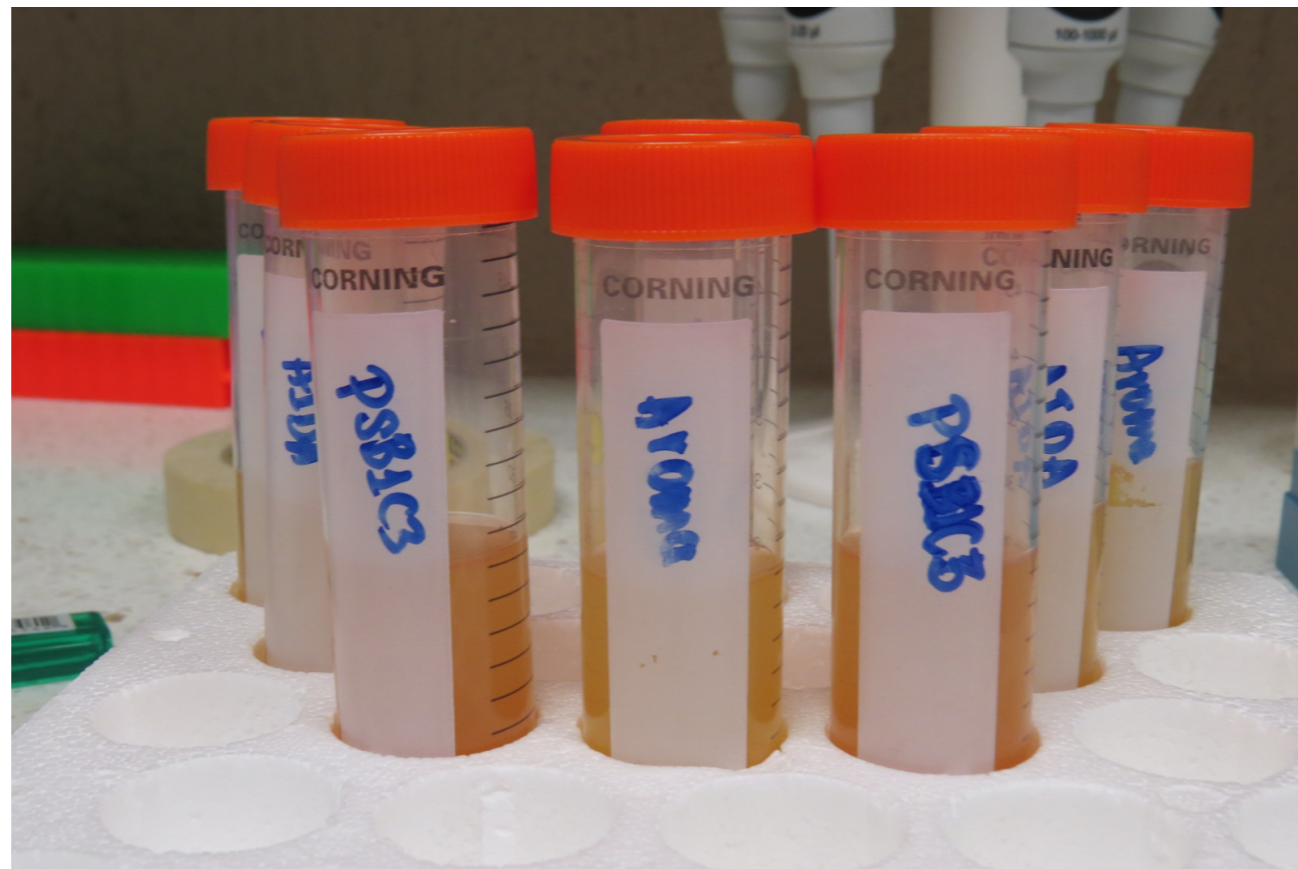
DNA	6uL
Buffer H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	11uL
Total	20uL

Mix AIDA - 2 (12/05)

DNA	3.5uL
Buff H	2uL
PstI	0.5uL
BglII	0.5uL
H2O	13.5uL
Total	20uL

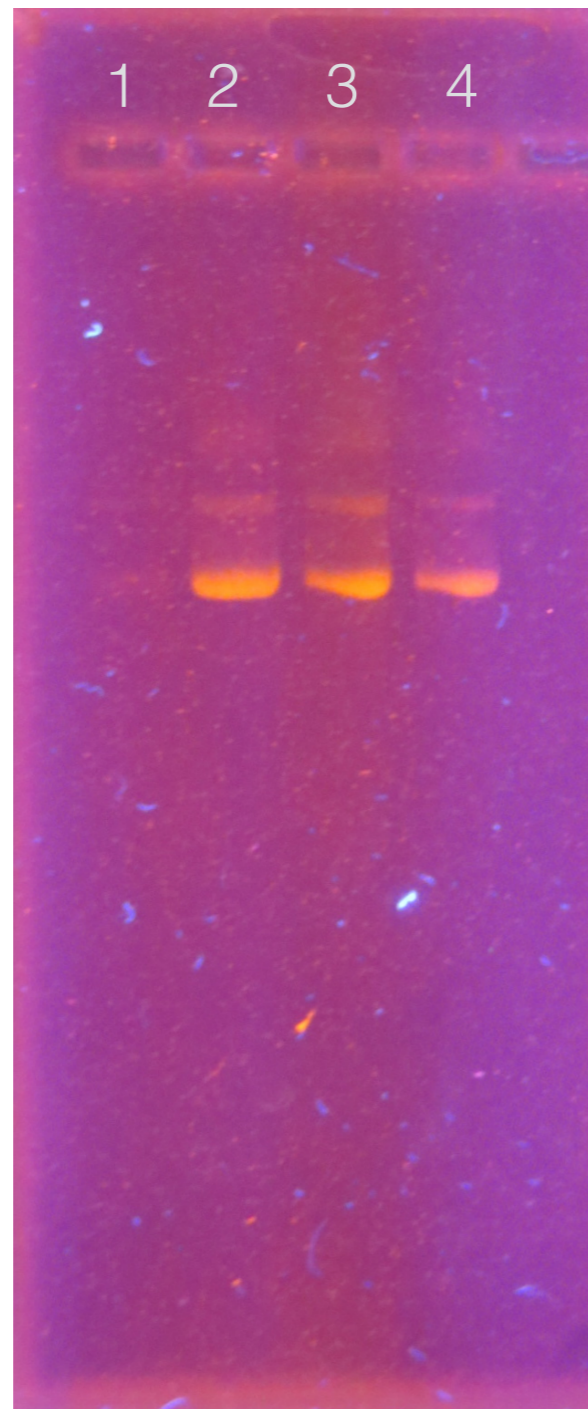
Tuesday June 3, 2014

Midi prep of inoculation 50ml of Aroma, AIDA, L2 and pSB1C3 (2/06)



Tuesday June 3, 2014

Miniprep clones of aroma + 1C3
Miniprep gel of aroma (3/06)



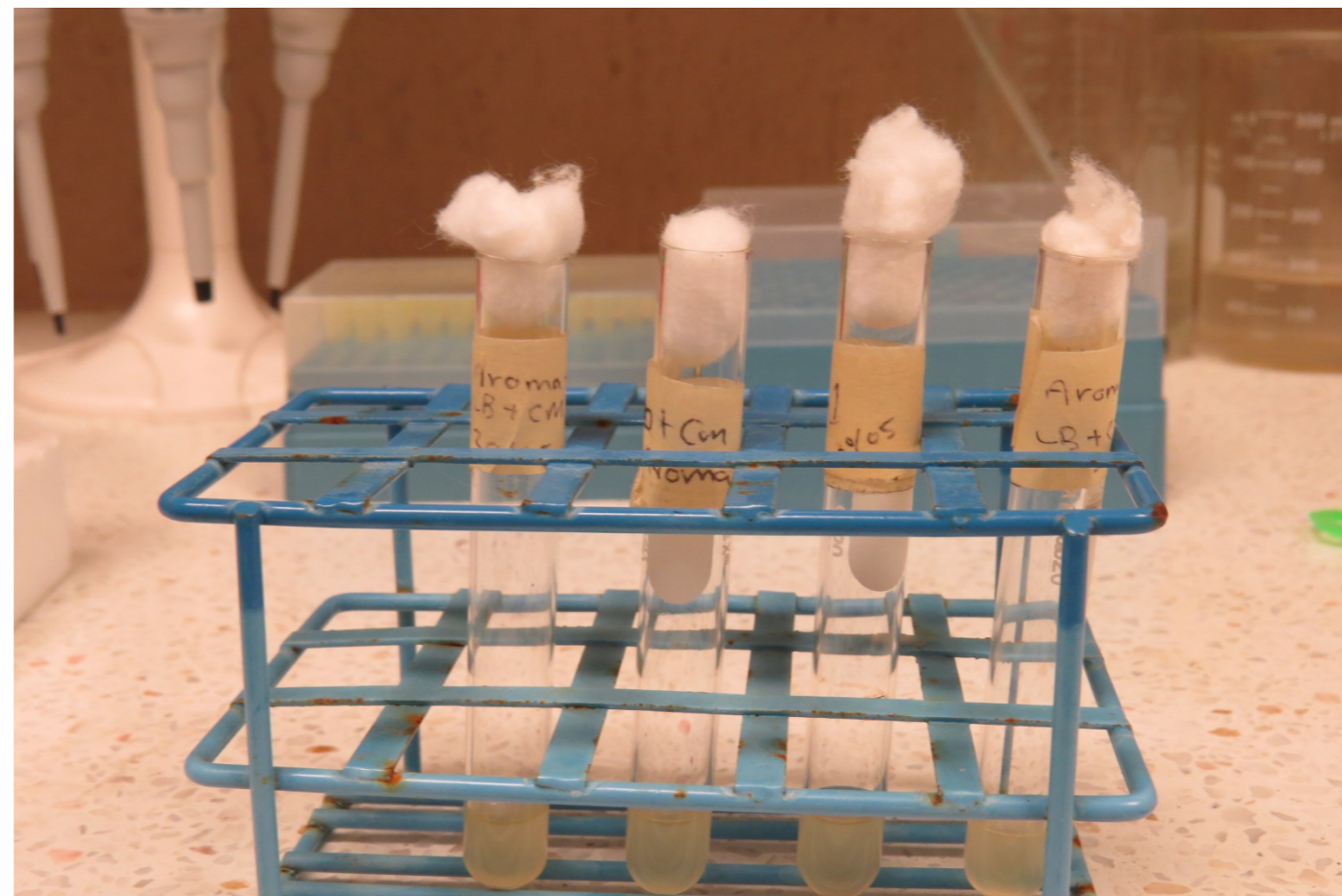
Spot	Gene
1	Aroma - 1
2	Aroma - 2
3	Aroma - 3
4	Aroma - 4

Tuesday June 3, 2014

Inoculation tube to tube of aroma.

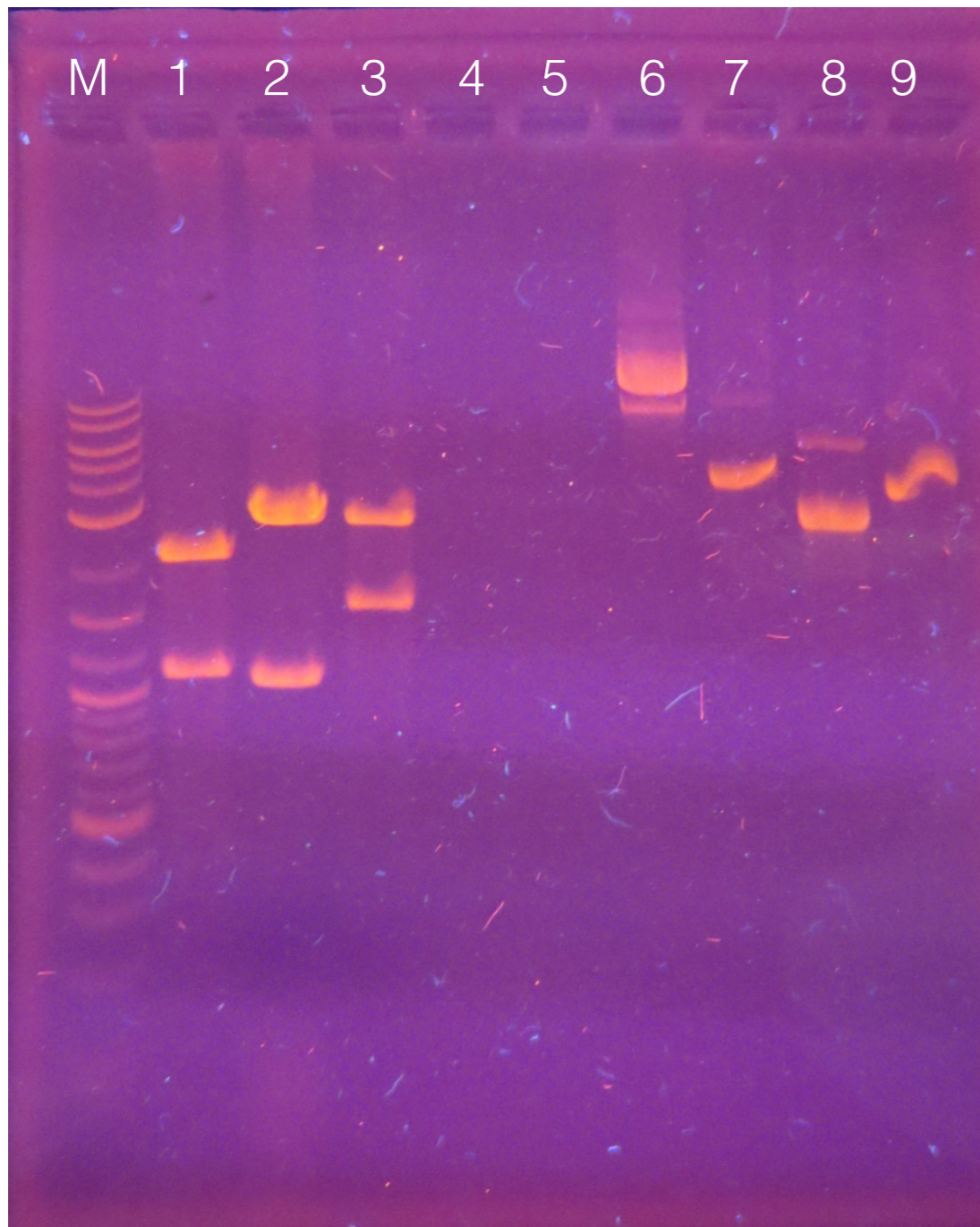
[original tubes (30/05)]

It was inoculated 4 tubes (Aroma 1/2/3/4)



Wednesday June 4, 2014

Quantitative digestion gel (2/06) [right] and of midipreps (3/06) [left]



Spot	Gene
1	pSB1C3
2	AIDA
3	L2
4	---
5	---
6	pSB1C3
7	
8	
9	
10	

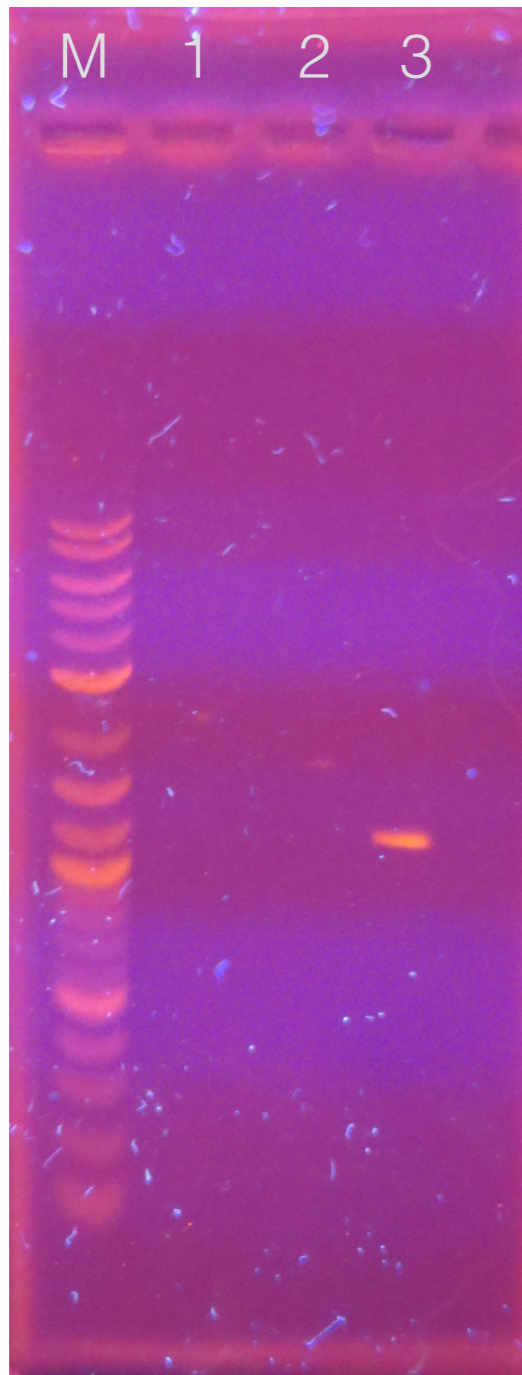
Gene	Length
pSB1C3	2,070bp
L2	1,085bp
AIDA	1482bp

Wednesday June 4, 2014

Digestion purification (2/06)

Thursday June 5, 2014

Purification gel (4/06)



Spot	Gene
1	pSB1C3
2	AIDA
3	L2

Gene	Lenght
pSB1C3	2,070bp
L2	1,085bp
AIDA	1482bp

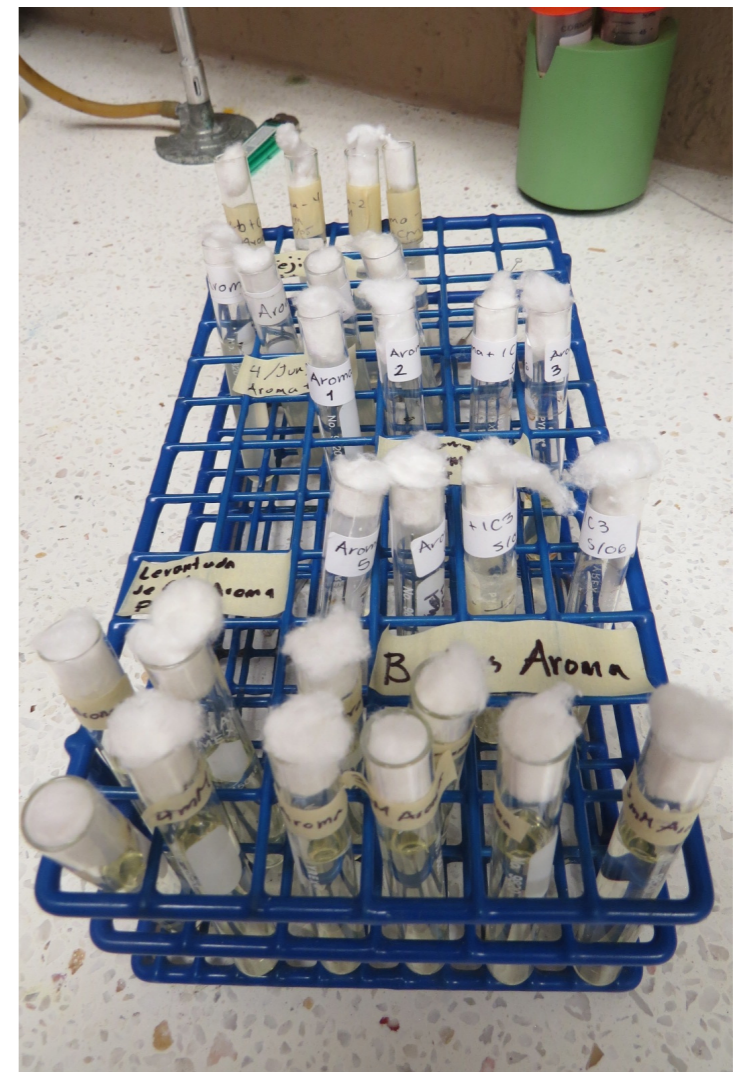
Thursday June 5, 2014

Nhas (Red/White) and Aroma were sent to be sequenced :D

10 plates of Aroma + pSB1C3 with salycilic acid were incoulated

Aroma + 1C3	
2 plates	1 ml of AS to 10 mm
2 plates	2 ml of AS to 10 mm
2 plates	3 ml of AS to 10 mm
2 plates	4 ml of AS to 10 mm
2 plates	5 ml of AS to 10 mm
Total	20 plates

Inoculation of 10 tubes of Aroma +
1C3 + 3ml of 4M of SA



Friday June 6, 2014

Quantitative digestion of AIDA, L2, pSB1C3

Mix L2 -2 (3/06)

DNA	4uL
Buff H	2uL
EcoRI	0.5uL
BamHI	0.5uL
H2O	13uL
Total	20uL

Mix pSB1C3 - 1 (3/06)

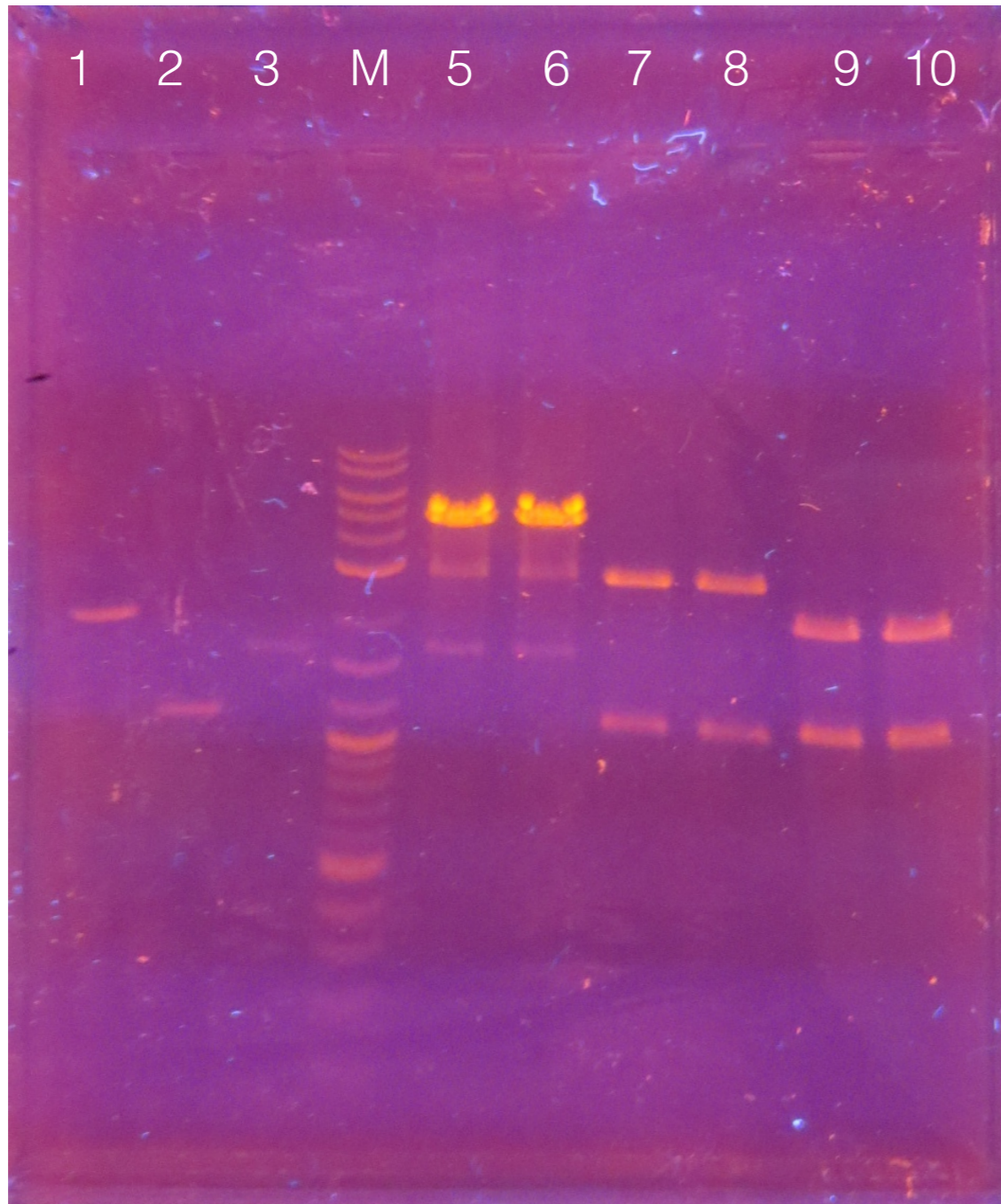
DNA	4uL
Buffer H	2uL
EcoRI	0.5uL
PstI	0.5uL
H2O	13uL
Total	20uL

Mix AIDA - 2 (3/06)

DNA	4uL
Buff H	2uL
PstI	0.5uL
BglII	0.5uL
H2O	13uL
Total	20uL

Monday June 9, 2014

Gel of digestion and purification (4/06)

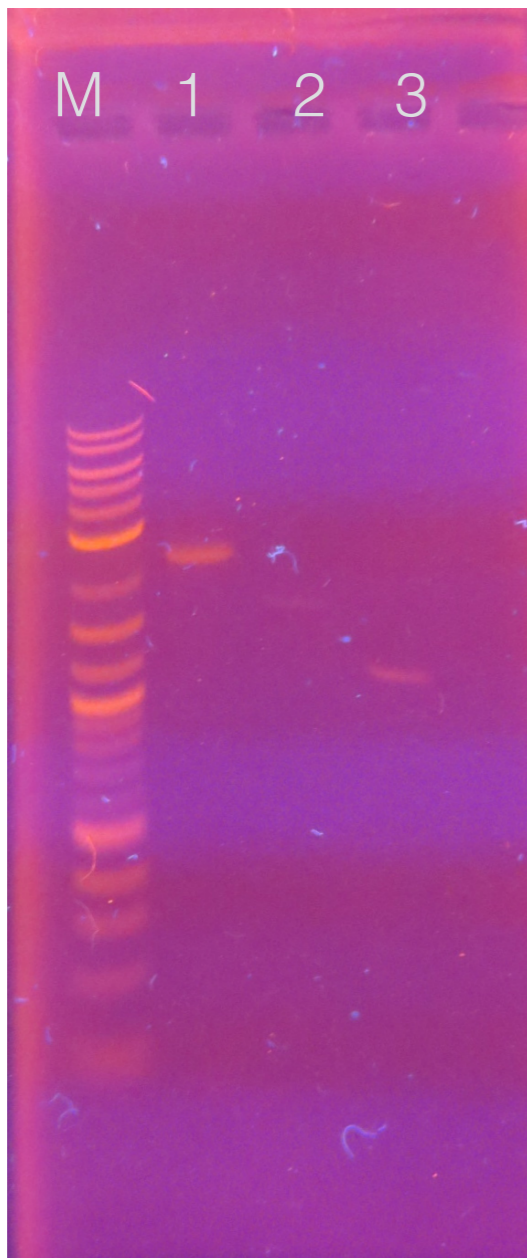


Spot	Gene
1	pSB1C3
2	L2
3	AIDA
4	M
5	pSB1C3
6	pSB1C3
7	L2
8	L2
9	AIDA
10	AIDA

Gene	Length
pSB1C3	2,070bp
L2	1,085bp
AIDA	1482bp

Monday June 9, 2014

Purification of previous digestion (9/06)
Gel of previous purification (9/06)



Spot	Gene
1	pSB1C3
2	AIDA
3	L2

Gene	Lenght
pSB1C3	2,070bp
L2	1,085bp
AIDA	1482bp

Monday June 9, 2014

Inoculation tube - flask of red NhaS, white NhaS and a control bacteria (E.coli without any insert).

(See it in methods and results tabs)

Monday June 9, 2014

Inoculation tube - plate of Aroma to experimentation

Duplicates of each concentration and temperature were
plaqued.

	3ml of AS to 10Mm	3ml of AS to 20 Mm	3ml of AS to 30 Mm	Control
29° C (-32)	2 Petri dishes	2 Petri dishes	2 Petri dishes	1 Petri dish
35° C (+32)	2 Petri dishes	2 Petri dishes	2 Petri dishes	1 Petri dish

(See it in methods and results tabs)

Tuesday June 10th, 2014

NhaS experimentation
Viability in salt

(See it in methods and results tabs)

Wednesday June 11th, 2014

Inoculation of 3 Erlenmeyer flasks to experimentation

1 - NhaS Red

1- NhaS White

1 - Control

Inoculation tube - plaque of NhaS

2 plaques of NhaS Red

2 plaque of NhaS white

Thursday June 12th, 2014

Experimentation of NhaS
Viability in salt

(See it in methods and results tabs)

Friday June 13th, 2014

IrrE arrived from England :D !!!