Lab Notebook

Histidine Kinase

	pages
Deletion in BW25113	**2-16**
Taz	**17-38**
BioBrick Taz	**39-47**
Osmolarity Experiment	**48-53**

Deletion in BW25113

Planning

7/8/2014

- So we've decided not to do the transformations in DH5alphaZ1 since it is problematic for the beta system Tal and Rica are planning.
- We spoke to Lior from Weizmann Institue and we will go there and do the ackA-pta deletion in JW3367-3 which is from the Keio collection and has EnvZ knocked out (Kanamycin instead). The parent strain for the Keio collection is BW25113

7/8/2014

PCR - Amplification of CM resistance from PCR product - take 6

We have two PCR products with concentrations 27.5ng/µl, we will dilute the 27.5ng/µl to get 2ng/µl and do several PCRs

PCR program for amplification of CM resistance from pKD3

stage	**Temp C^0**	**time**
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	70	30 sec
	72	30 sec
Final extension	72	10 min
hold	4	

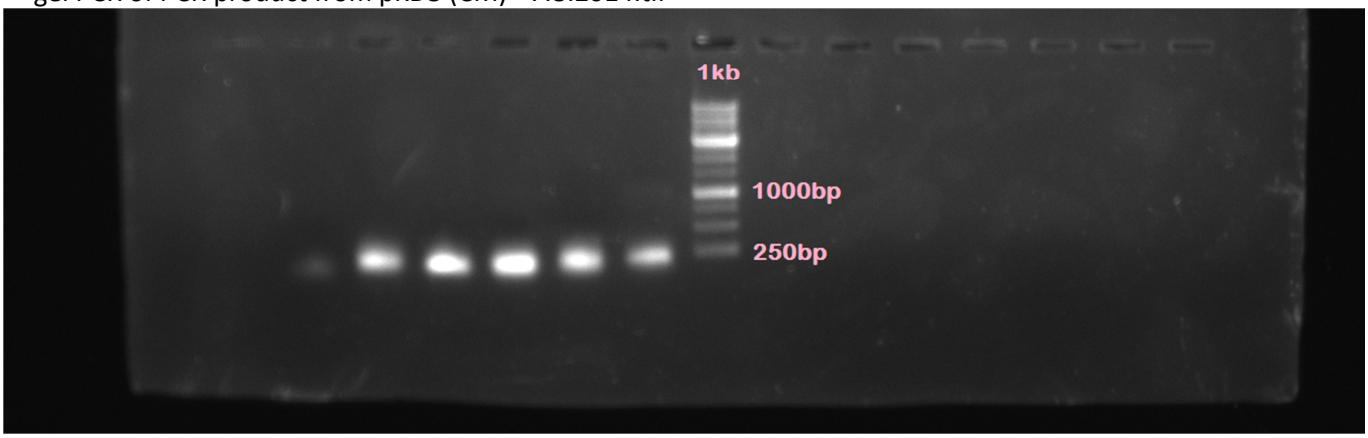
component	**Volume[ml]**	**Volume x4**
UPW	27	108
phusion reaction buffer(x5)	10	40
Template: CMr (2 ng/µl)	5	20
forward primer	2.5	10
reverse primer	2.5	10
DMSO	1.5	6
dNTPs(10 mM)	1	4
Phusion hot start II	0.5	2
tot	50	200

Results from PCR - Amplification of CM resistance from PCR product

7.8.2014

Failed!!!!!

<<gel PCR of PCR product from pKD3 (CM) - 7.8.2014.tif>>



PCR - Amplification of CM resistance from pKD3 - take 7

10/8/2014

PCR program for amplification of CM resistance from pKD3

stage	Temp C^0	time
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	64	30 sec
	72	30 sec
30 cycles	98	10 sec
	70	30 sec
	72	30 sec
Final extension	72	10 min
hold	4	

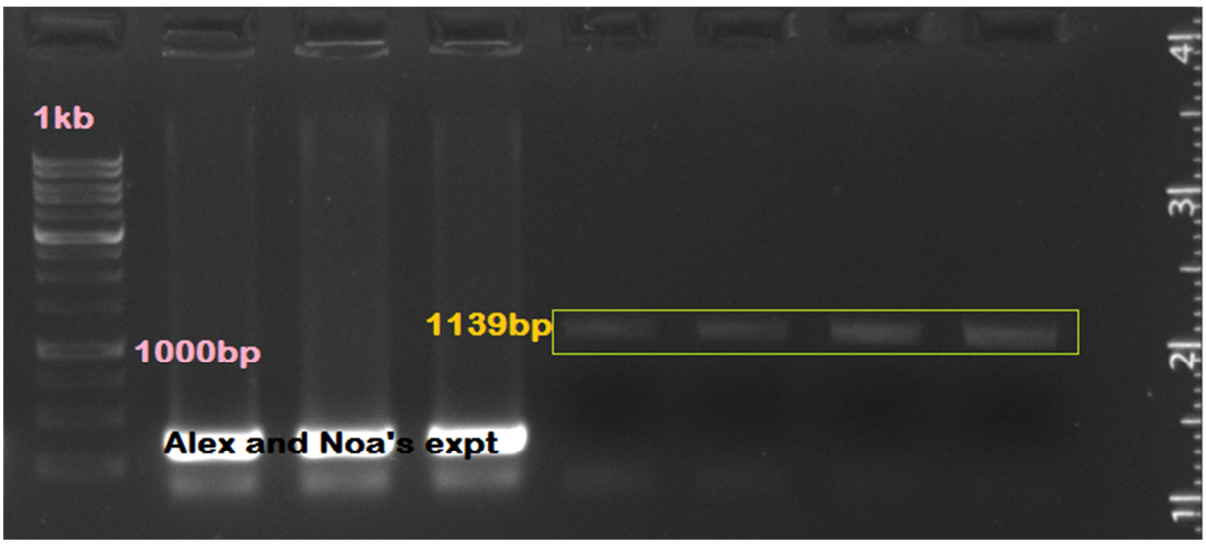
component	Volume[ml]	Volume x4
UPW	27	108
phusion reaction buffer(x5)	10	40
Template: pKD3 (2 ng/µl)	5	20
forward primer	2.5	10
reverse primer	2.5	10
DMSO	1.5	6
dNTPs(10 mM)	1	4
Phusion hot start II	0.5	2
tot	50	200

The concentration of the pKD3 sample is 35.5ng/µl ng/µl. It was diluted to get 2 ng/µl concentration.

Results from PCR - Amplification of CM resistance from pKD3 - take 7

10/8/2014

All 4 succeeded!



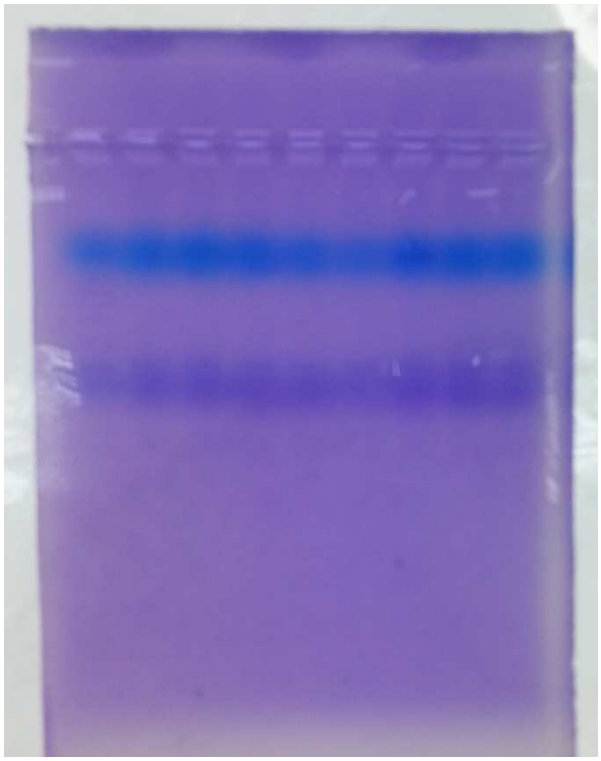
Transforming CM resistance into E.Coli strain JW3367-3

19/8/2014

Karen and Rebecca went to Ron Milo's lab at the Weizmann institute. Elad Hertz, Lior Zelcbuch and Sagit helped us. We ran the products on a gel with crystal violet and cleaned the products from it. We transformed the CM resistance (PCR product) into E. coli strain JW3367-3. We took the plates back to our lab to grow.

Crystal Violet gel

19/8/2014



PCR - verification of ackA-pta deletion - take 1

20/8/2014

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
ackA-pta fw	forward	
ackA-pta rv	reversed	

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x6**
Taq ready mix (x2)	10	60
ackA-pta fw (10 ng/µl)	2	12
ackA-pta rv (10 ng/µl)	2	12
colony	1 colony	
UPW	6	36
total	**20**	**120**

PCR program

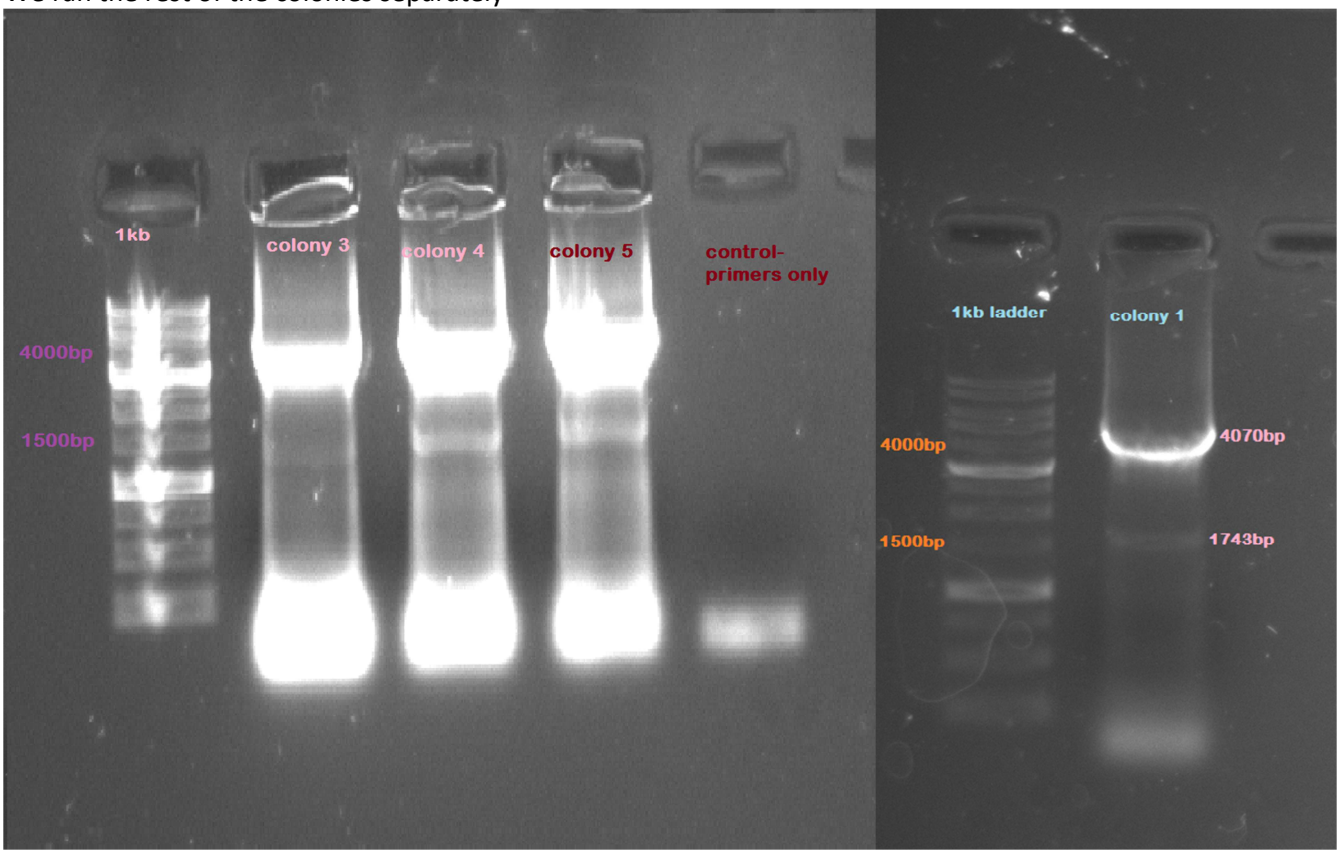
stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	65	30 sec
	72	4 min
Final extension	72	10 min
hold	4	

Expected length of PCR product is 4070bp (deletion didn't work) or 1743bp (deletion did work).

Results from verification PCR - take 1

Rebecca: When I was charging the wells, colony 2 sprayed everywhere so I just ran the gel with the 1KB ladder and colony 1. The result shows the two expected bands) and a lot of products. This might be because the colony 2 sprayed everywhere.

We ran the rest of the colonies separately



PCR - verification of ackA-pta deletion - take 2

We thought that the reason the results from take 2 showed both the expected bands was because the CM resistance might be there as linear DNA so we decided to grow the colonies again for a few more generations.
21/8/2014 Tal did an isolation seeding of the five colonies (each one on its own plate)
22/8/2014 Did PCR verification again - took one colony from each plate again.

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
ackA-pta fw	forward	
ackA-pta rv	reversed	

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x6**
Taq ready mix (x2)	10	60
ackA-pta fw (10 ng/µl)	2	12
ackA-pta rv (10 ng/µl)	2	12
colony	1 colony	
UPW	6	36
total	**20**	**120**

PCR program

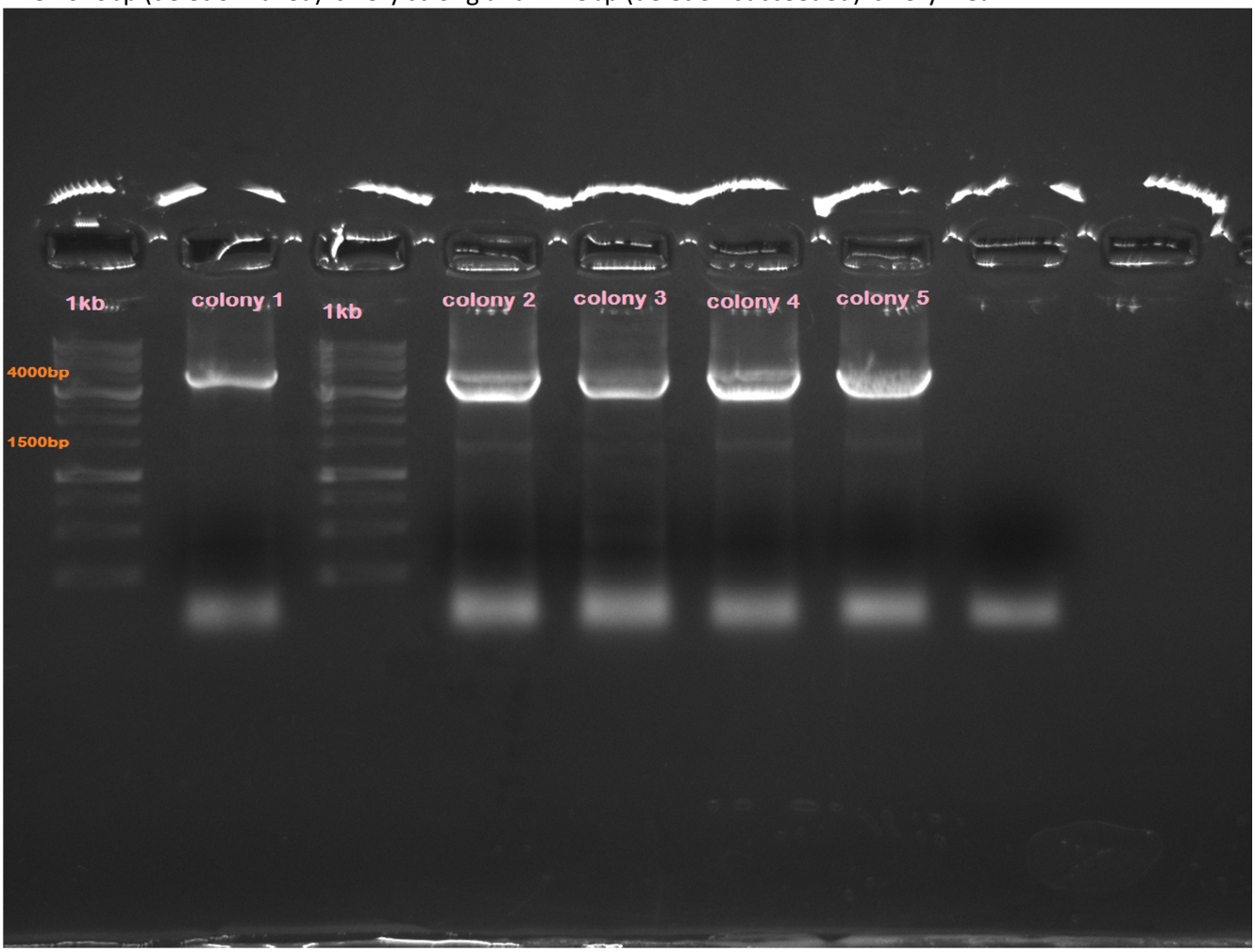
stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	65	30 sec
	72	4 min
Final extension	72	10 min
hold	4	

Expected length of PCR product is 4070bp (deletion didn't work) or 1743bp (deletion did work).

Results from verification PCR - take 2

22/8/2014

Colony 1 splashed a bit so I added another ladder since I was afraid the first one might be messed up.
The 4070bp (deletion failed) is very strong and 1743bp (deletion succeeded) is very weak



PCR - verification of ackA-pta deletion - take 3

24/8/2014 - we are going back to the original plates and doing colony PCR on 10 colonies. We will also run a colony from the wild type BW25113 strain and from the JW3367-3 strain and a tube without anything

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
ackA-pta fw	forward	
ackA-pta rv	reversed	

Reaction mix: make total mix and then divide 20 [µl]

component	Volume[µl]	Volume (ul) x13
Taq ready mix (x2)	10	130
ackA-pta fw (10 ng/µl)	2	26
ackA-pta rv (10 ng/µl)	2	26
colony	1 colony	
UPW	6	78
total	**20**	260

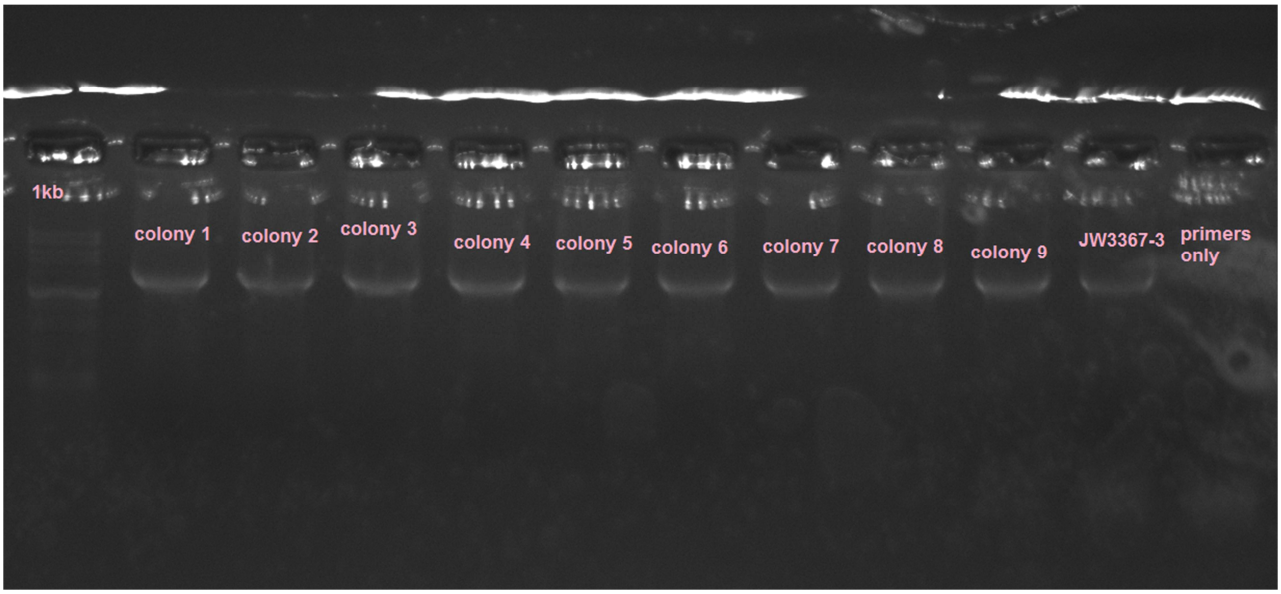
PCR program

stage	Temp C^0	time
Initial denaturation	94	3 min
35 cycles	94	30 sec
	65	30 sec
	72	4 min
Final extension	72	10 min
hold	4	

Expected length of PCR product is 4070bp (deletion didn't work) or 1743bp (deletion did work).

Results from verification PCR - take 3

24/8/2014



Figuring out what happened with the deletion

25/8/2014

Take colony 1,2,3 from 21-22/8 plate and grow starter with cm^r
Take one/two colonies from 24/8 plate and grow starter with cm^r
Take colony of the strain before deletion and grow starter with kan^r

26/8/2014
Did mini-preps for all of them and check with nano-drop if the is a plasmid there - all had plasmids.
Colony 1 - 260λ
Colony 2 - 238λ
Colony 3 - 79λ
Colony 7 - 50.5λ
Colony 8 - 260.5λ

verification of ackA-pta deletion - take 4

31/8/2014 - grew overnight culture of colonies 2, 3, 4, 5 from colony PCR (take 2)

1/9/2014

- Glycerol stock
- Purification of genomic DNA
  - Colony 2: 333.5λ
  - Colony 3: 393.5λ
  - Colony 4: 347.0λ
  - Colony 5: 238.5λ

2/9/2014

- PCR of gDNA
- Run on gel

PCR for verification of ackA-pta deletion (gDNA of colonies 2,3,4,5)

2/9/2014
PCR program

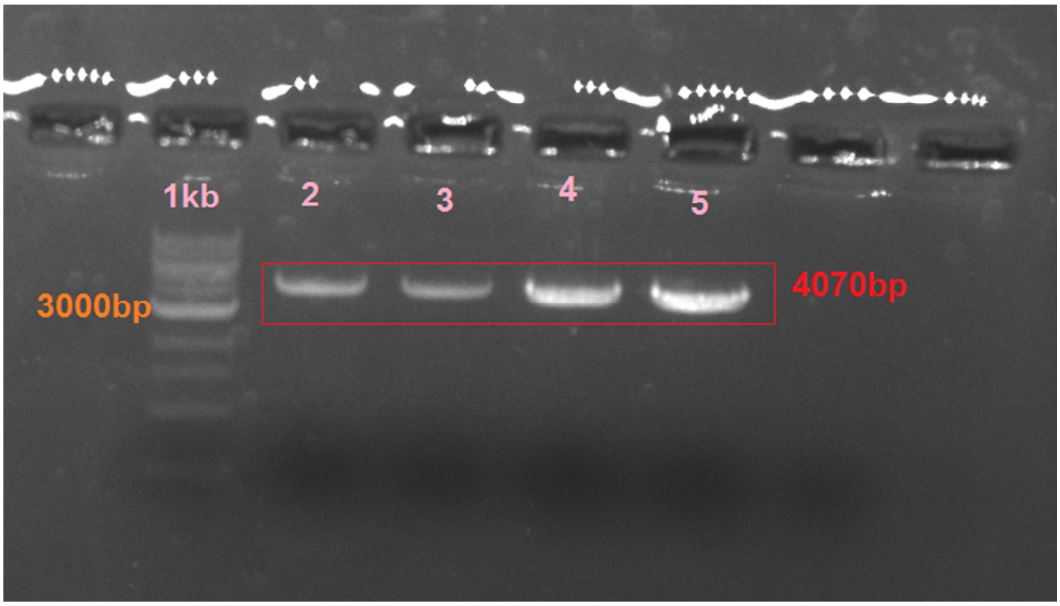
stage	**Temp C^0**	**time**
Initial denaturation	94	00:30
35 cycles	94	00:30
	72	00:30
	72	02:00
Final extension	72	10:00
hold	4	

total time:

component	Volume[ml]
UPW	27
phusion reaction buffer(x5)	10
Genomic DNA (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

PCR for verification PCR (gDNA of colonies 2,3,4,5)

1kb ladder from Roee's lab



pKD46 mini prep

3/9/2014

Did mini-prep of pKD46 from TOP10
(1) 42 ng/microL
(2) 35.5 ng/microL
(graphs were good, but the peak was not so high)
When I did the mini prep I put neutralization solution (200microL) before the lysate solution, than added the lysate and 150microL of the neutralization solution. This might be a problem because the plasmids might have stayed inside the bacteria and not in the clear supernatant.

7/9/2014
Transform pKD46 to JW3367-3

8/9/2014
Grew starter of JW3367-3+pKD46

Deletion of ackA-pta in JW3367-3 - take 2

8/9/2014
Grew starter of JW3367-3 with pKD46 o/n

9/9/2014

DpnI and cleaned CMr PCR product
Made JW3367-3 electrocompetent (and added L-arabinose to activate the pKD46)
Electroporated CMr PCR product
The deletion was done with the following samples:
+L arabinose, +PCR product (CMr) - the deletion should work here
+L arabinose, -PCR product (CMr) - control
-L arabinose, +PCR product (CMr) - control
-L arabinose, -PCR product (CMr) - control
Seeded 8 plates, 2 of each sample.

10/9/2014

Results from transformation - plates:

	+Larab	**-Larab**
+PCR product (CMr)	Lots, lots	One or two colonies
-PCR product (CMr)	One or two colonies	None, none

- Grew starter of 4 colonies from the +,+ plates

11/9/2014

- Did minipreps to all 4 colonies to see if there are plamsids
  - Colony 1: 63.3λ
  - Colony 2: 72.9λ
  - Colony 3: 76.7λ
  - Colony 4: 58.9λ
  - Basically, there were plasmids in all
  - Made a back up plate of these colonies
- Purified genomic DNA from colonies 1 and 4

Colony PCR to verify if deletion of ackA-pta worked

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
ackA-pta fw	forward	
ackA-pta rv	reversed	

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x10**
Taq ready mix (x2)	10	100
UPW	6	60
ackA-pta fw (10 ng/µl)	2	20
ackA-pta rv (10 ng/µl)	2	20
colony	1 colony	
total	**20**	**120**

PCR program

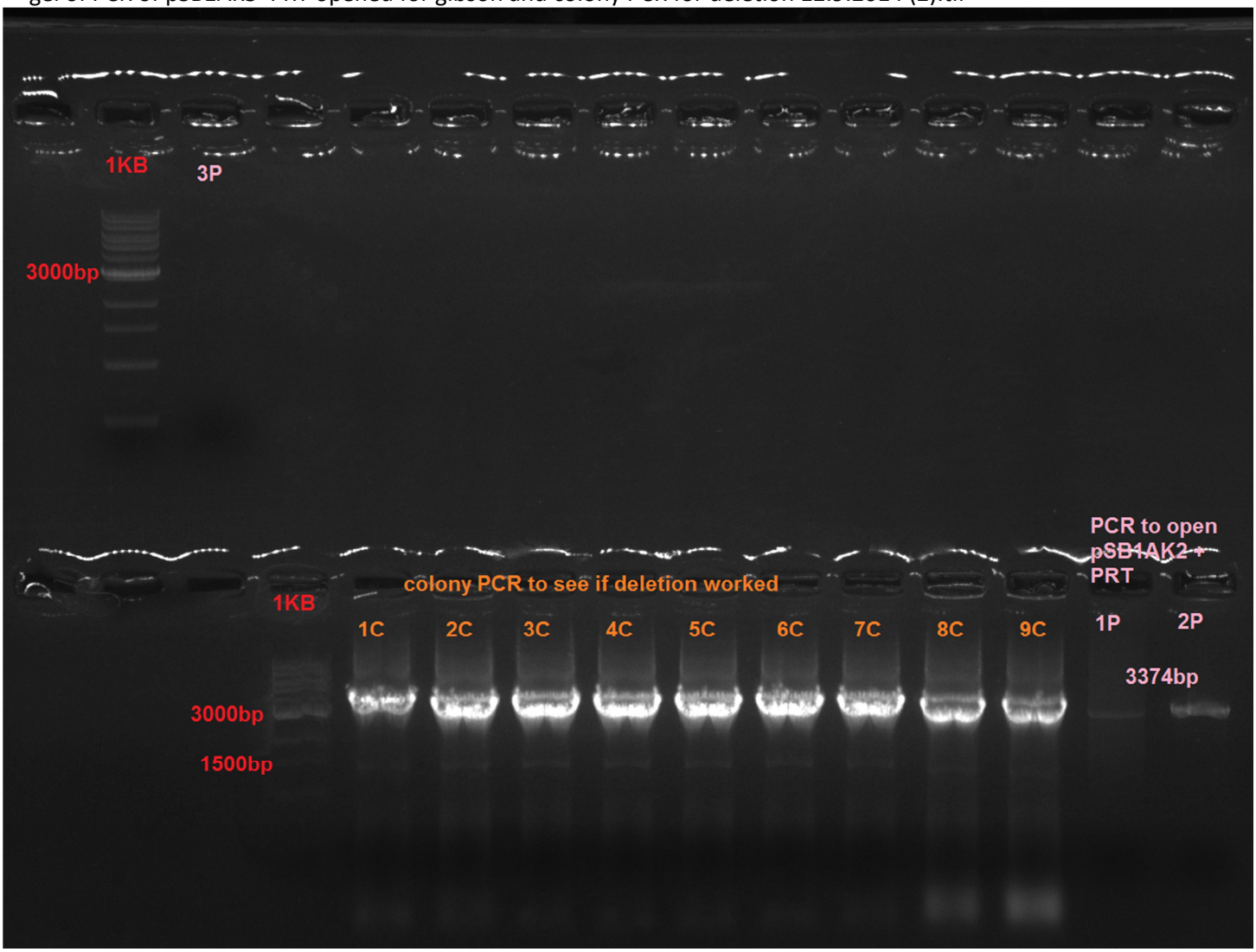
stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	65	30 sec
	72	4 min
Final extension	72	10 min
hold	4	

Expected length of PCR product is 4070bp (deletion didn't work) or 1743bp (deletion did work).

Results of colony PCR

12/9/2014

<<gel of PCR of pSB1AK3+PRT opened for gibson and colony PCR for deletion 12.9.2014 (2).tif>>



Verification of ackA-pta deletion - take 2

14/9/2014

Grew starters overnight of colonies 1-9

15/9/2014

Miniprep to see if there are plasmids:

Colony 1: 109.5λ
Colony 2: 107.5λ
Colony 3: 128.5λ
Colony 4: 195.0λ
Colony 5: 129.0λ
Colony 6: 200.0λ
Colony 7: 274.5λ
Colony 8: 8.5λ
Colony 9: 211.0λ

It looks like Colony 8 has no plasmid. We run on a gel to make sure there is nothing there.
There was nothing there!!!

Grew starter of colony 8

16/9/2014

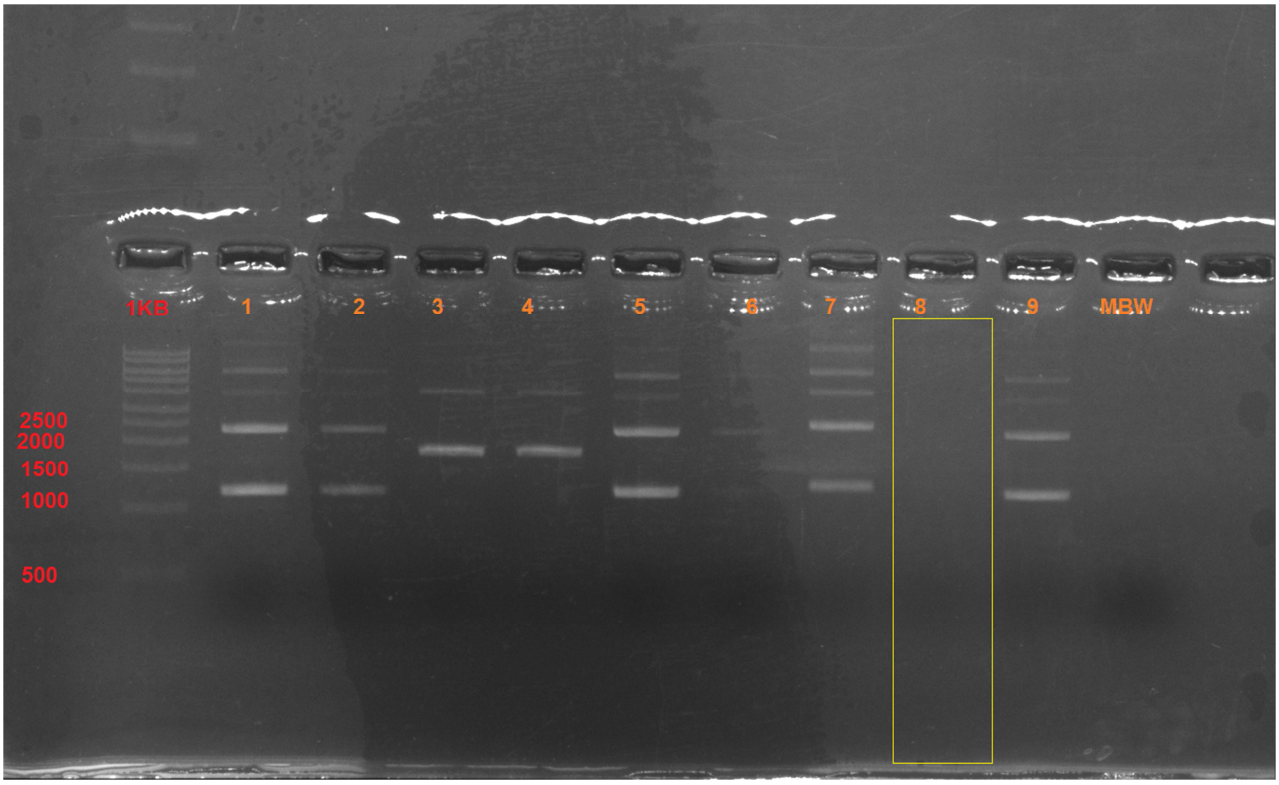
- Glycerol stock of colony 8 in -80 freezer in box of DH5 electrocompetent in spaces 1G, 1H and 1I
- Purified gDNA of colony 8
- PCR of gDNA of colony 8
- Did another miniprep of colony 8 (x2) and got results 100λ, 91.5λ - what does this mean?!?!?!?

17/9/2014

- Run PCR products and minipreps on gel

Gel of minipreps of colonies

15/9/2014



PCR of colony 8

PCR program

stage	**Temp C^0**	**time**
Initial denaturation	94	00:30
35 cycles	94	00:30
	72	00:30
	72	02:00
Final extension	72	10:00
hold	4	

component	Volume[ml]
UPW	27
phusion reaction buffer(x5)	10
Genomic DNA (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

Taz

Building Taz

Received the sequence for Taz1 from the registry (Bba_C0082 on plasmid pSB2K3, in BL1 E. coli bacteria)
29/7/2014 - seed BL1 on Kan plate
30/7/2014 - grow starter of BL1+part - failed
31/7/2014 - grow starter of BL1+part
1/8/2014 - Glycerol stock and Miniprep of BL1+part - concentration: 291.5ng/µl

11/8/2014 - Planning construct of Pcat-RBS-Taz-Ter-Ter
Pcat - Bba_I14033
RBS - Bba_B0034
Ter-Ter - Bba_B0015

We will put the construct into plasmid pSB1AK3 that already contains the double terminator.
Ronen helped us plan the construction.

25/8/2014 -Step 1: reverse PCR of pSB1AK3 containing a double terminator to add Pcat and RBS.
Using the planned primers:
Fw - region containing part of the sequence for Pcat, the sequence for the RBS and part homologous to Ter.
Rev - region containing rest of sequence for Pcat, and part homologous to the Plasmid
26/8/2014 - phosphorylation and ligation (to close the linear plasmid) and transformation into top10 - FAILED!!!
26/8/2014 - transformation in to top10 (take 2)
31/8/2014 - Today we ran the three tubes of PCR product on gel and realized that tube 1 had no product and that is why the phosphorylation/ligation/transformation failed.

- We did phosphorylation and ligation of the PCR product 2

1/9/2014 - Transformed ligated pSB1AK3 into top10
2/9/2014 - We realized we had accidentally plated on CM instead of Kan but the bacteria grew!!! We realized we had the wrong plasmid and that it wasn't pSB1AK3 after all!!!!! So we plated a few bacteria from the glycerol stocks on CM, amp and Kan to figure out which had pSB1AK3 because there had apparently been some miss-labeling
4/9/2014 - Grew starter of strain that had pSB1AK3 (with double terminator)
5/9/2014

- miniprep of pSB1AK3 with double terminator (164.0λ and 132.5λ).
- Reverse PCR

7/9/2014

- Cleaned reverse PCR product and Taz PCR product
- Phosphorylation and ligation

8/9/2014

- we realized that we need to plan new primers to amplify the pSB1AK3+Pcat+RBS+double terminator. Ronen already has the Forward primer so we designed and ordered the reverse primer.
- transformed ligated pSB1AK3+Pcat+RBS+TetR

9/9/2014

- Transformed Bba_M30011 on pSB1C3 (contains RFP under PompC) into Top10. This part came in iGEM 2014 distribution kit plate 2 well 20D.
- Grew starter of top10 with pSB1AK3+Pcat+RBS+TetR

10/9/2014

- Grew starter o/n of Bba_M30011 on pSB1C3 (contains RFP under PompC) inTop10
- Glycerol stock and MP of top10 with pSB1AK3+Pcat+RBS+TetR (iGEM box F9)
  - Concentration of minipreps: 136λ, 238λ

11/9/2014

- Glycerol stock and miniprep of Bba_M30011 on pSB1C3 (contains RFP under PompC) inTop10
  - Concentration of miniprep: 55.7λ

12/9/2014

- Reverse PCR to open pSB1AK3+Pcat+RBS+TetR

14/9/2014

- Clean PCR product (pSB1AK3+Pcat+RBS+TetR), concentrations: (1) 75.5λ, (2) 58.5λ
- Step 2: Gibson to add Taz (used PCR product (1))
- Transformation of Gibson product to top 10 and plate on Kan plates

15/9/2014

- Colony PCR using sequencing primers to see if the Gibson worked
- Run colony PCR on gel - colonies 8 and 9 worked

16/9/2014

- Grew starters of Top10+pSB1AK3 (Taz construct)

17/9/2014

- Miniprep and glycerol stock of Top10+pSB1AK3 (Taz construct)
- Transform Taz construct and Bba_M30011 on pSB1C3 (contains RFP under PompC)

18/9/2014

- Transformation didn't work - Roee said it is because the ORI of the two plasmids are the same

19/9/2014

- Transformed Taz construct (pSB1AK3) into BW25113 and JW3367-3

20/9/2014

- BW25113 transformation worked
- JW3367-3 transformation did not work

22/9/2014

- Colony PCR of BW25113 + Taz construct (pSB1AK3) - worked!

23/9/2014

- Transformation of Taz construct (pSB1AK3) into JW3367-3
- We looked at the sequencing results from the Taz construct on pSB1AK3 and we only saw Taz and no Pcat, RBS or Double Terminator. We think that the problem was that when we did the PCR of the Taz, we didn't do DpnI so the plasmid pSB2K3 (which Taz came on from the registry) was still there and since we grew things on Kan not Amp so we had this original plasmid instead.

24/9/2014

- Transformation didn't work - kan plates

27/9/2014

- Transformation of Taz construct (pSB1AK3) into JW3367-3 (Amp plates)

27/9/2014

- Transformation worked but we think it is a contamination.
- Grew starters of BW25113 and JW3367-3 to make electrocompetent
- Grew starter of BW25113+pSB1AK3 (Taz construct) in Amp
- Grew starter of Top10 + pSB1AK3 with Pcat-RBS-double terminator (I couldn't find the glycerol stock so I took from the old Amp plates I found in the fridge)

28/9/2014

- Starter of BW25113+pSB1AK3 (Taz construct) in Amp did not grow
- Alex and I made BW25113 and JW3367-3 electrocompetent
- Miniprep and glycerol stock (iGEM B box H1) of pSB1AK3 with Pcat-RBS-double terminator
- Reverse PCR to open pSB1AK3 with Pcat-RBS-double terminator between RBS and double-terminator (failed)
- I found the old Taz PCR product and did DnpI (This is what I think our problem was last time)
- I found an old plate in the fridge which had the gibson product and Alex did colony PCR and did back up on Amp+kan. This plate may contain:
  - pSB1AK3 + Pcat-RBS-doubleTerminator that closed on itself (negative result on gel, will grow on Amp+Kan plate)
  - pSB2K3 + Taz (will give positive result on gel, won't grow on Amp+Kan plate)

- pSB1AK3 + Pcat-RBS-TAZ-doubleTerminator that closed on itself (positive result on gel, will grow on Amp+Kan plate)

- I found the glycerol stock of Top10 with pSB1AK3 with Pcat-RBS-double terminator - grew overnight starter on Amp

29/9/2014

- Miniprep of pSB1AK3 with Pcat-RBS-double terminator (concentrations: 153λ (1) and 198λ (2))
  - Sent to sequencing with sequencing primers pSB1C3_Ver_S and pSB1C3_Ver_AS
- PCR of Taz with overhand primers (for Gibson) - Karen did this
- Reverse PCR of pSB1AK3 with Pcat-RBS-double terminator to open for gibson - Shira did this
- Gibson to add Taz to pSB1AK3 with Pcat-RBS-double terminator
- Transform into Top10 - Ittai did this
- Grew starters of pSB1AK3 with Taz construct (from colony 9 from old Gibson plate)

30/9/2014

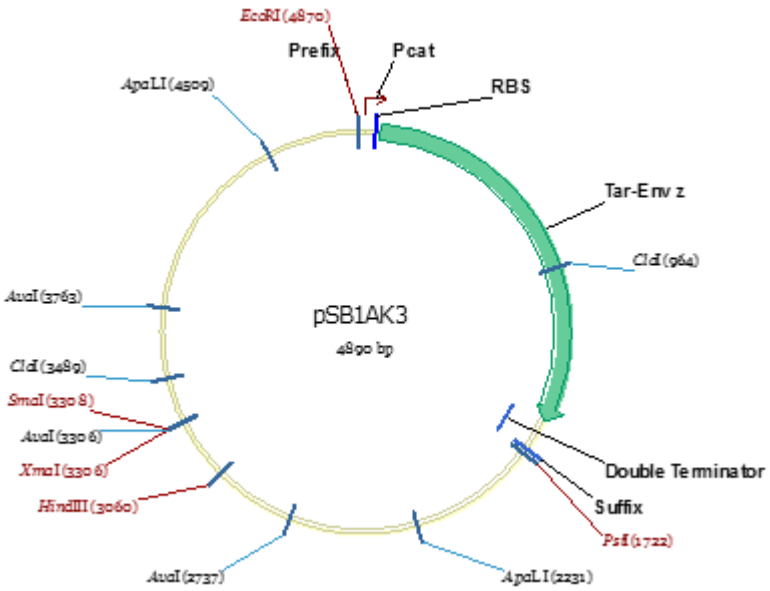
- Minprep of pSB1AK3 with Taz construct (from colony 9 from old Gibson plate)
  - Sent to sequencing with sequencing primers pSB1C3_Ver_S and pSB1C3_Ver_AS
  - Restriction digest with NdeI and BglI (cut in Taz and cut in AmpR)
    - Expected lengths: 3138bp and 1752bp - it worked!!!!!

2/10/2014

- Dual electroporation Transformation of:
  - pSB1AK3+Taz construct
  - pSB1C3+mRFP under PompC (Bba_M30011)

  into
  - BW25113
  - JW3367-3

Taz on pSB1AK3



Reverse PCR to add promoter and RBS to pSB1AK3

25/8/2014

Expected length of PCR product - 3374bp

PCR program

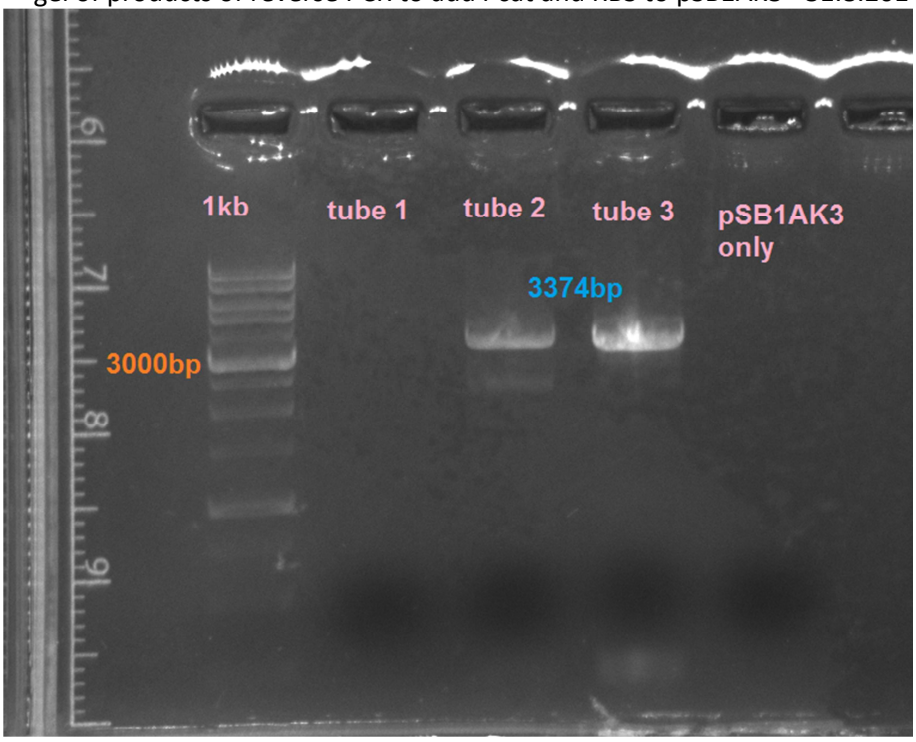
stage	Temp C^0	time
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	61	30 sec
	72	01:45
30 cycles	98	10 sec
	72	30 sec
	72	02:00
Final extension	72	10 min
hold	4	

component	Volume[ml]
phusion reaction buffer(x5)	10
dNTPs(10 mM)	1
forward primer	2.5
reverse primer	2.5
Template: pSB1AK3 (2 ng/µl)	5
Phusion hot start II	0.5
DMSO	1.5
UPW	27
tot	50

Run reverse PCR products on gel

31/8/2014

<<gel of products of reverse PCR to add Pcat and RBS to pSB1AK3 - 31.8.2014.Tif>>



Taz PCR

1/9/14

Taz length: 1516bp

PCR program for amplification Taz

stage	Temp C^0	time
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	63	30 sec
	72	**50 sec**
30 cycles	98	10 sec
	72	30 sec
	72	**50 sec**
Final extension	72	10 min
hold	4	

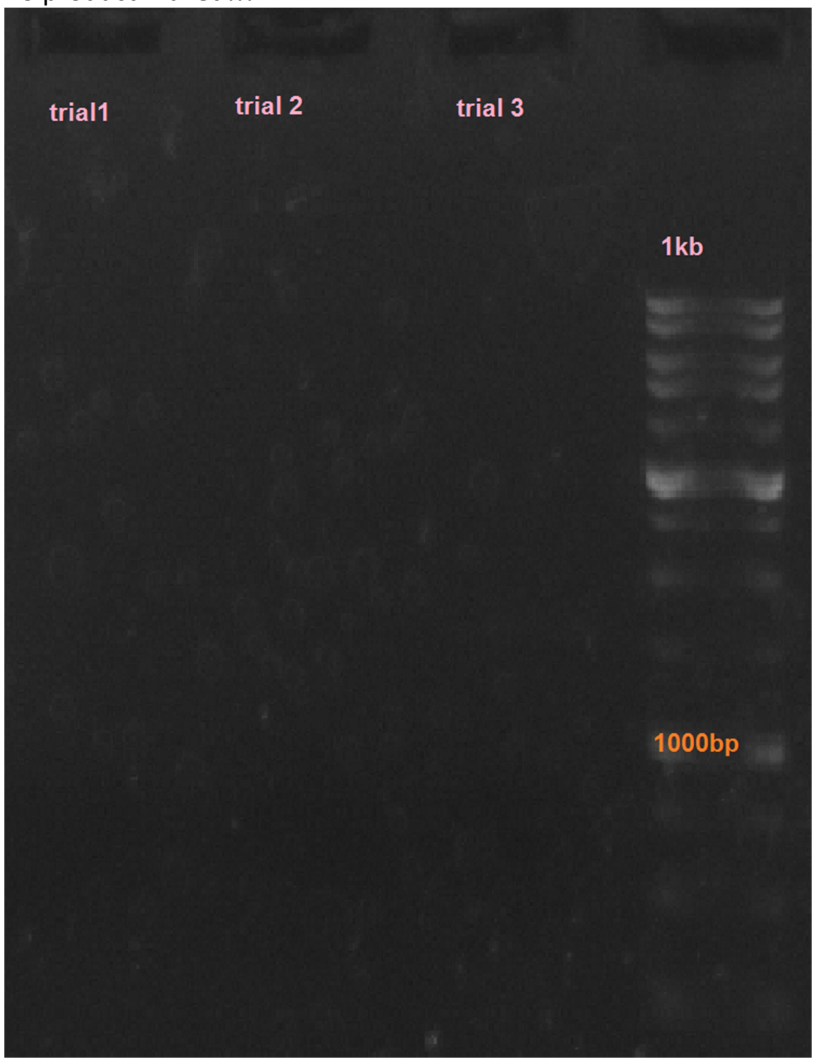
total time:

component	Volume[ml]	Volume x4
UPW	27	108
phusion reaction buffer(x5)	10	40
Template: pSB2K3 (with taz) (2 ng/µl)	5	20
forward primer	2.5	10
reverse primer	2.5	10
DMSO	1.5	6
dNTPs(10 mM)	1	4
Phusion hot start II	0.5	~~2~~
tot	50	200

Results from Taz PCR take 1

1/9/14

No product - failed!!!



Taz PCR (second try)
1/9/14

Taz length: 1500~bp

PCR program for amplification Taz

stage	Temp C^0	Temp C^0	Temp C^0	Temp C^0	time
Initial denaturation	98	98	98	98	30 sec
5 cycles	98	98	98	98	10 sec
	58	**59**	**60**	**61**	30 sec
	72	72	72	72	**50 sec**
30 cycles	98	98	98	98	10 sec
	70	**70**	**70**	**70**	30 sec
	72	72	72	72	**50 sec**
Final extension	72	72	72	72	10 min
hold	4	4	4	4	

total time:

component	Volume[ml]	Volume x4
UPW	27	108
phusion reaction buffer(x5)	10	40
Template: pSB2K3 (with taz) (2 ng/µl)	5	20
forward primer	2.5	10
reverse primer	2.5	10
DMSO	1.5	6
dNTPs(10 mM)	1	4
Phusion hot start II	0.5	~~2~~
tot	50	200

Taz PCR (2) results

Amplification successful

Concentrations:
(58) 183λ
(59) 88.7λ
(60) 217λ
(61) 108λ

Reverse PCR to add promoter and RBS to pSB1AK3

5/9/2014

Expected length of PCR product - 3374bp

PCR program

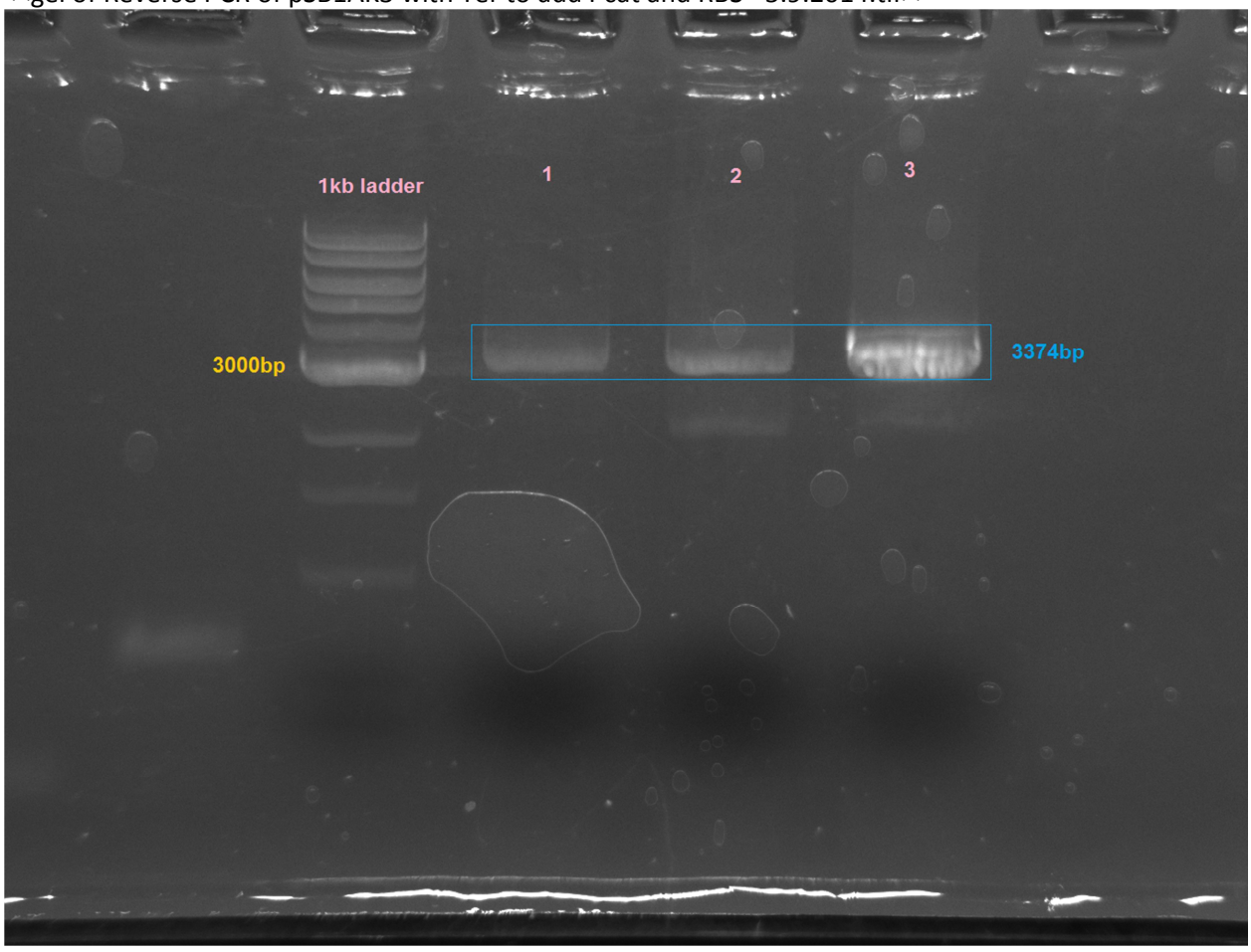
Stage	**Temp C^0**	**time**
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	61	30 sec
	72	01:45
30 cycles	98	10 sec
	72	30 sec
	72	02:00
Final extension	72	10 min
Hold	4	

component	Volume[ul]
phusion reaction buffer(x5)	10
dNTPs(10 mM)	1
forward primer	2.5
reverse primer	2.5
Template: pSB1AK3 (2 ng/µl)	5
Phusion hot start II	0.5
DMSO	1.5
UPW	27
tot	50

Results of reverse PCR products - gel

5/9/2014

<<gel of Reverse PCR of pSB1AK3 with Ter to add Pcat and RBS - 5.9.2014.tif>>



Concentrations:
(1) 116 ng/microL
(2) 121 ng/microL
(3) 147 ng/microL

Reverse PCR to open pSP1AK3 with Pcat+RBS+Ter for gibson - take 1

12/9/2014

PCR program

stage	**Temp C^0**	**time**
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	63	30 sec
	72	01:45
Final extension	72	10 min
hold	4	

component	**Volume[ul]**
UPW	27
phusion reaction buffer(x5)	10
Template: pSB1AK3+PRT (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

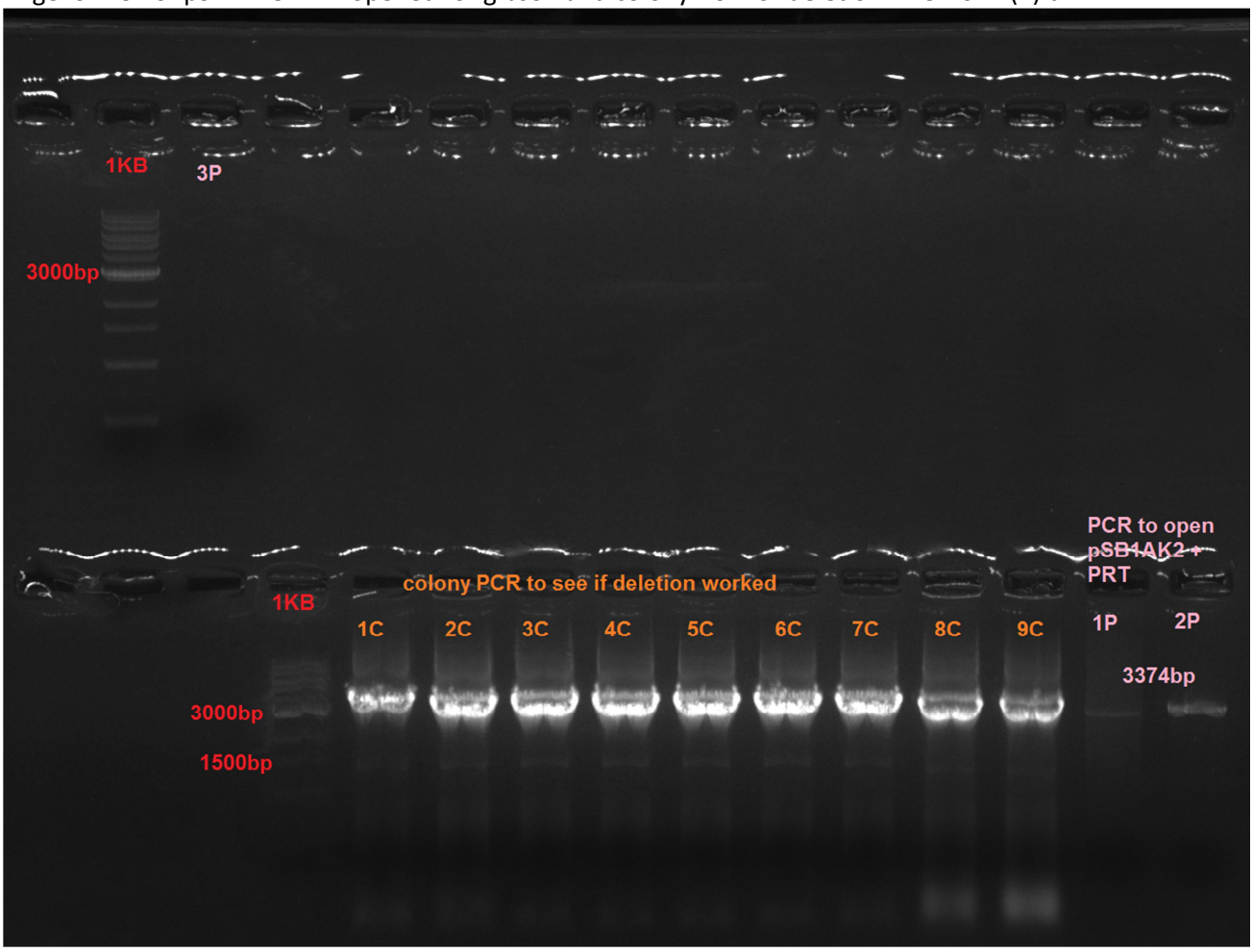
The PCR tubes have the following written on them: **PRT open 1/2/3**

Results of PCR to open pSB1AK3+PRT

12/9/2014

Tubes 1 and 2 worked

<<gel of PCR of pSB1AK3+PRT opened for gibson and colony PCR for deletion 12.9.2014 (2).tif>>



Colony PCR to see if Gibson of Taz construct worked

15/9/2014

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
pSB1C3_Ver_S	forward	57
pSB1C3_Ver_AS	reversed	56.7

Reaction mix: make total mix and then divide 20 [µl]

component	Volume[µl]	Volume (ul) x12
Taq ready mix (x2)	10	120
UPW	6	72
pSB1C3_Ver_S (10 ng/µl)	2	24
pSB1C3_Ver_AS (10 ng/µl)	2	24
colony	1 colony	
total	**20**	

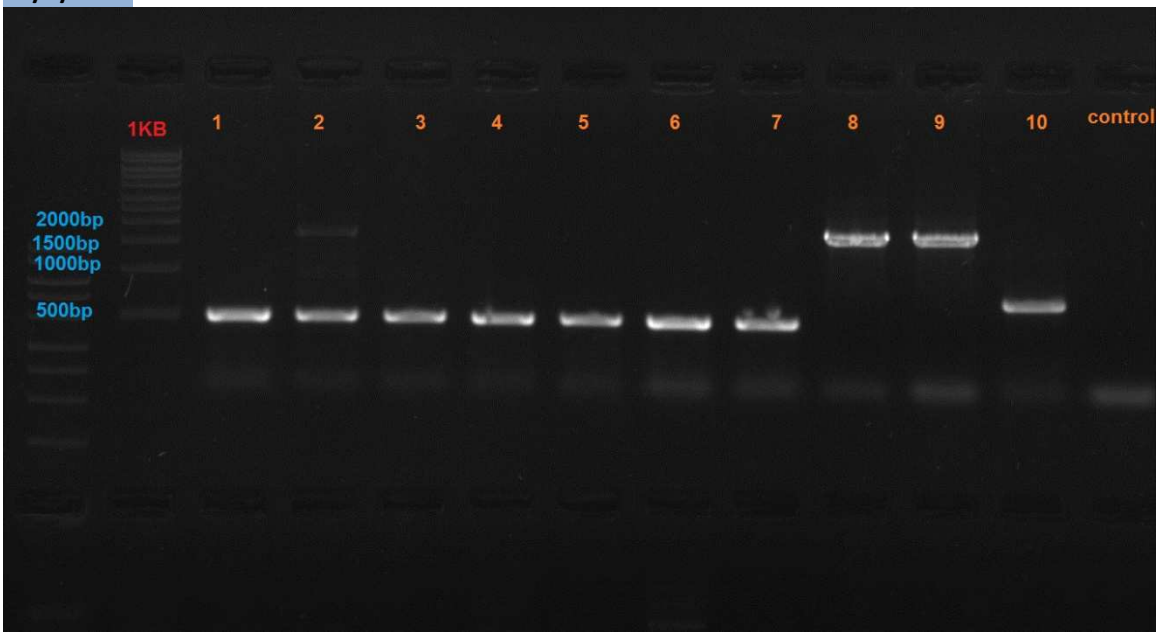
PCR program

stage	Temp C^0	time
Initial denaturation	94	3 min
35 cycles	94	30 sec
	70	30 sec
	72	2 min
Final extension	72	10 min
hold	4	

Expected band: 1974bp - Taz construct

Results of colony PCR

15/9/2014



8 and 9 worked!

Colony PCR plan to see if Taz got into BW25113

22/9/2014

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
pSB1C3_Ver_S	forward	57
pSB1C3_Ver_AS	reversed	56.7

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x12**
Taq ready mix (x2)	10	120
UPW	6	72
pSB1C3_Ver_S (10 ng/µl)	2	24
pSB1C3_Ver_AS (10 ng/µl)	2	24
colony	1 colony	
total	**20**	

PCR program

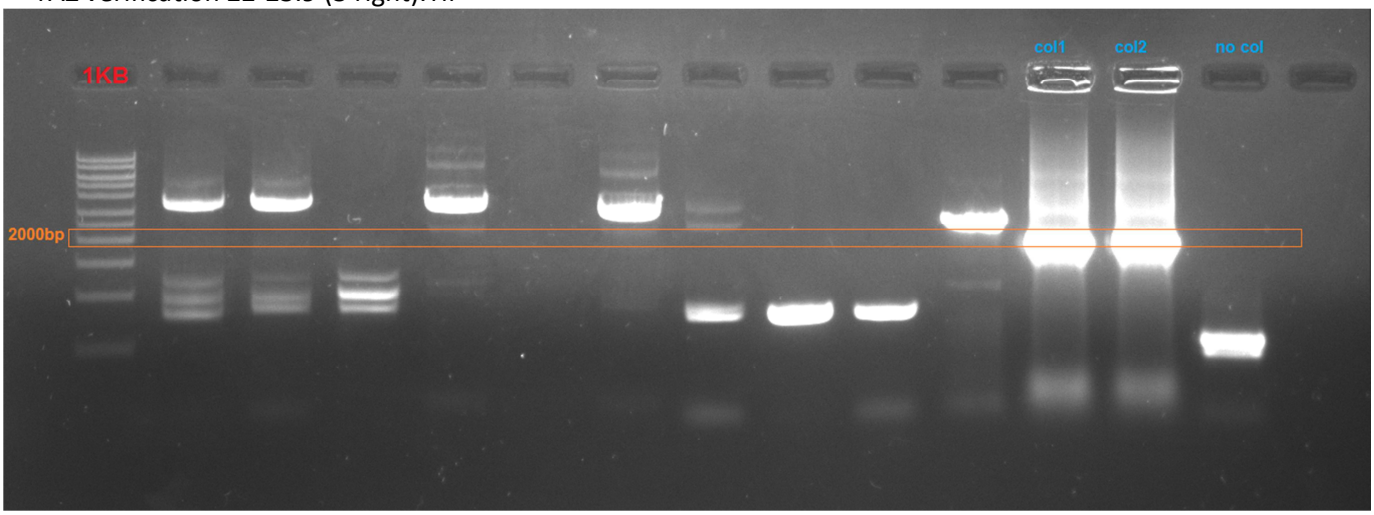
stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	70	30 sec
	72	2 min
Final extension	72	10 min
hold	4	

Expected band: 1974bp - Taz construct

Results of colony PCR

23/9/2014

<<TAZ verification 22-23.9 (3 right).Tif>>



Colony PCR plan to see if Taz got into JW3367-3

22/9/2014

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
pSB1C3_Ver_S	forward	57
pSB1C3_Ver_AS	reversed	56.7

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x14**
Taq ready mix (x2)	10	140
UPW	6	84
pSB1C3_Ver_S (10 ng/µl)	2	28
pSB1C3_Ver_AS (10 ng/µl)	2	28
colony	1 colony	
total	**20**	

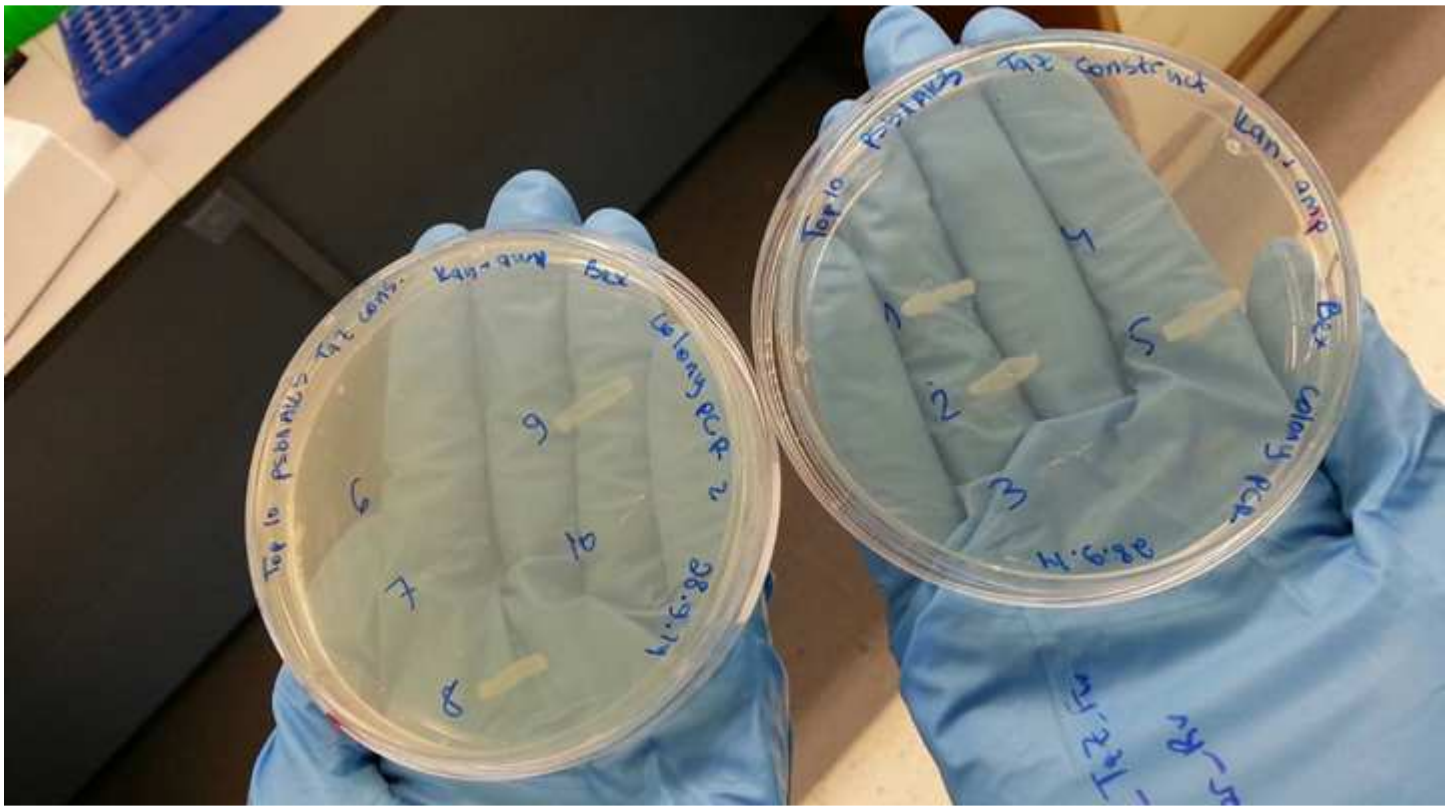
PCR program

stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	60	30 sec
	72	2 min
Final extension	72	10 min
hold	4	

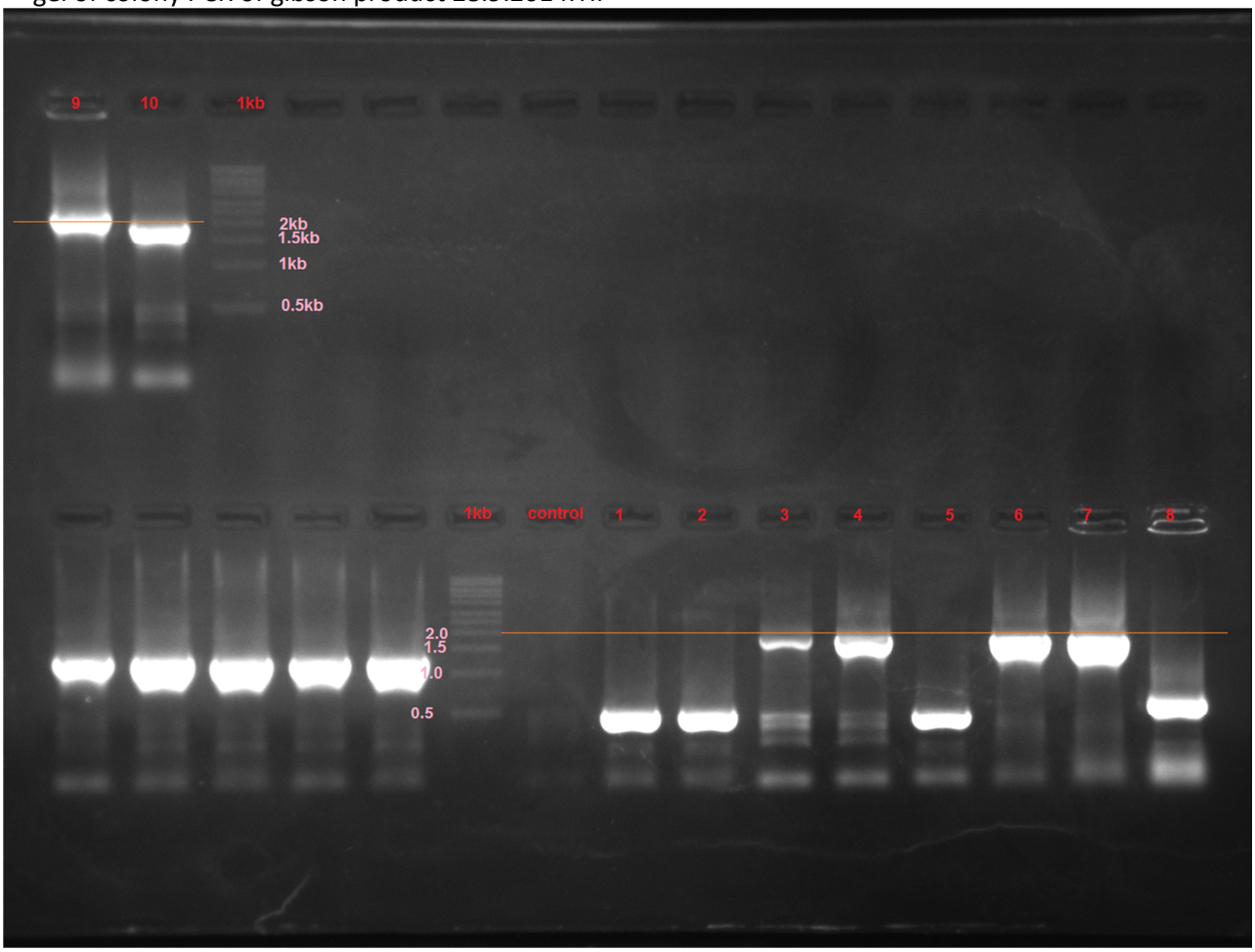
Expected band: 1974bp - Taz construct

Results of colony PCR of gibson product

28/9/2014



<<gel of colony PCR of gibson product 28.9.2014.Tif>>



Reverse PCR to open pSP1AK3 with Pcat+RBS+Ter for gibson - take 2

28/9/2014

	F plasmid Ter
	6g-RBS-Pcat_Rv

PCR program

Stage	**Temp C^0**	**time**
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	63	30 sec
	72	01:45
Final extension	72	10 min
hold	4	

component	Volume[ul]
UPW	27
phusion reaction buffer(x5)	10
Template: pSB1AK3+PRT (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

Results of PCR to open pSB1AK3+PRT

28/9/2014

<<gel of reverse PCR pf pSB1AK3 with PRT and Taz PCR product check 28.9.2014.Tif>>

Taz PCR (take 3)

29/9/2014

Taz length: 1500~bp

PCR program for amplification Taz

stage	Temp C^0	time
Initial denaturation	98	30 sec
5 cycles	98	10 sec
	61	30 sec
	72	**50 sec**
30 cycles	98	10 sec
	70	30 sec
	72	**50 sec**
Final extension	72	10 min
hold	4	

total time:

component	Volume[ml]
UPW	27
phusion reaction buffer(x5)	10
Template: pSB2K3 (with taz) (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

Reverse PCR to open pSP1AK3 with Pcat+RBS+Ter for gibson - take 3

29/9/2014

	F plasmid Ter
	6g-RBS-Pcat_Rv

PCR program

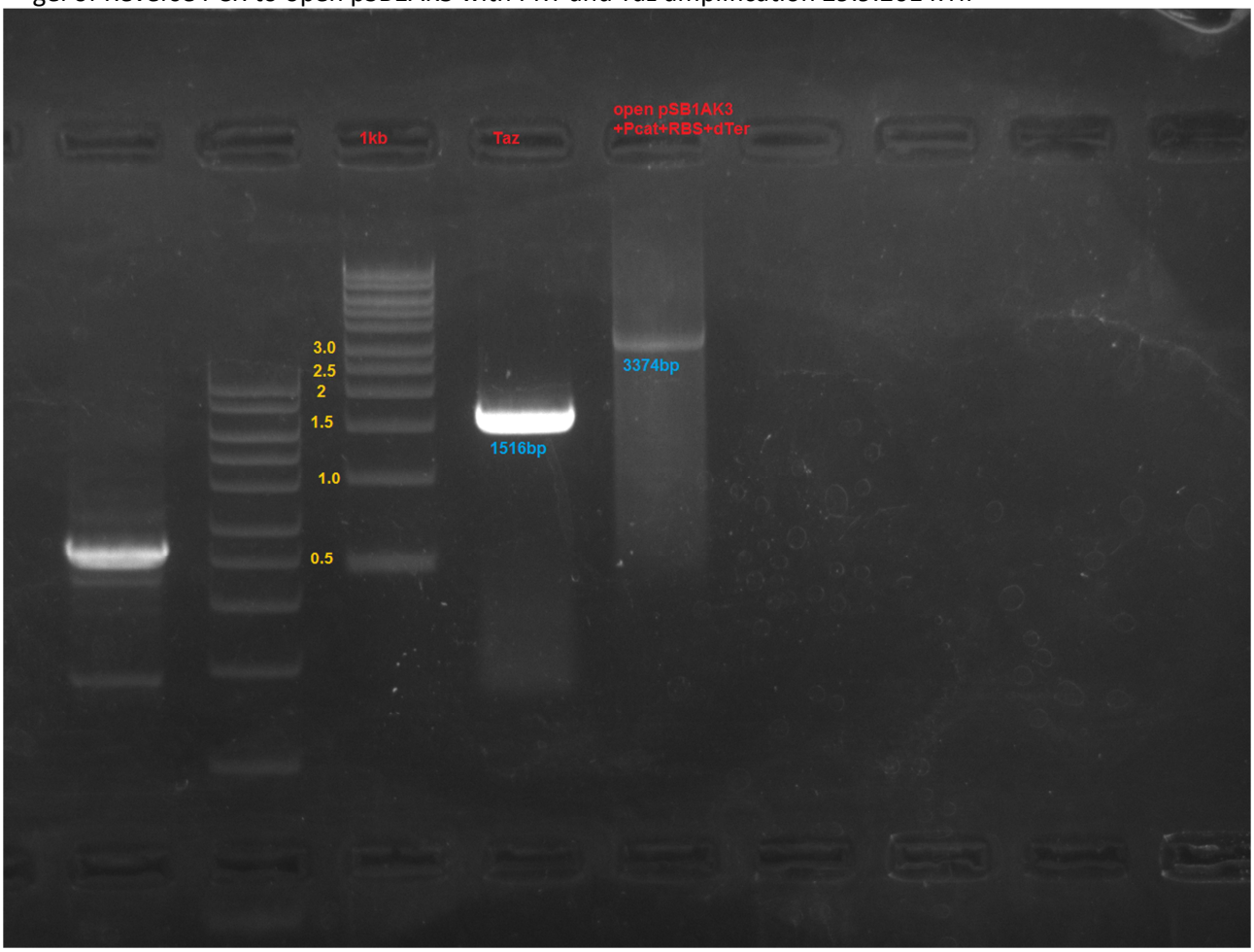
stage	Temp C^0	time
Initial denaturation	98	30 sec
35 cycles	98	10 sec
	63	30 sec
	72	01:45
Final extension	72	10 min
hold	4	

component	Volume[ul]
UPW	27
phusion reaction buffer(x5)	10
Template: pSB1AK3+PRT (2 ng/µl)	5
forward primer	2.5
reverse primer	2.5
DMSO	1.5
dNTPs(10 mM)	1
Phusion hot start II	0.5
tot	50

Results of Revers PCR and Taz PCR

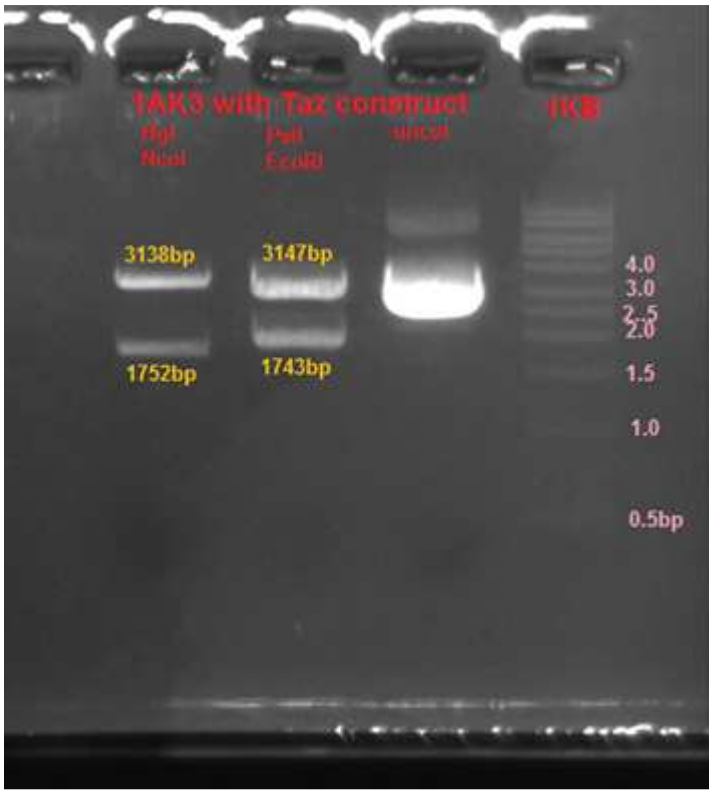
29/9/2014

<<gel of Reverse PCR to open pSB1AK3 with PRT and Taz amplification 29.9.2014.Tif>>



Results of Taz restriction check

We have TAZ!!!!



Colony PCR plan to see if Taz got into BW25113

22/9/2014

Taq readymix PCR

Primer name	**forward/ reversed**	**Tm**
pSB1C3_Ver_S	forward	57
pSB1C3_Ver_AS	reversed	56.7

Reaction mix: make total mix and then divide 20 [µl]

component	**Volume[µl]**	**Volume (ul) x12**
Taq ready mix (x2)	10	120
UPW	6	72
pSB1C3_Ver_S (10 ng/µl)	2	24
pSB1C3_Ver_AS (10 ng/µl)	2	24
colony	1 colony	
total	**20**	

PCR program

stage	**Temp C^0**	**time**
Initial denaturation	94	3 min
35 cycles	94	30 sec
	70	30 sec
	72	2 min
Final extension	72	10 min
hold	4	

Expected band: 1974bp - Taz construct

BioBrick Taz
Preparing Taz for Biobrick

To send Taz as a biobrick we have to put it in pSB1C3

20/9/2014
- Grew starter of Top10 with pSB1C3

21/9/2014
- Miniprep of pSB1C3
- Cut pSB1AK3+Taz and pSB1C3 with EcoRI and PstI restriction enzymes
- Ran on gel
  - got expected band for Taz but unexpected band for rest of plasmid
  - Got nothing for pSB1C3 - Ronen said he has pSB1C3 cut with EcoRI and PstI we can use for ligation

22/9/2014
- Noa did ligation and Transformation

23/9/2014
- Transformation didn't work - contaminated plate (forest!!!)
- Did transformation again

20/9/2014
- Grew starter of Top10 with pSB1C3

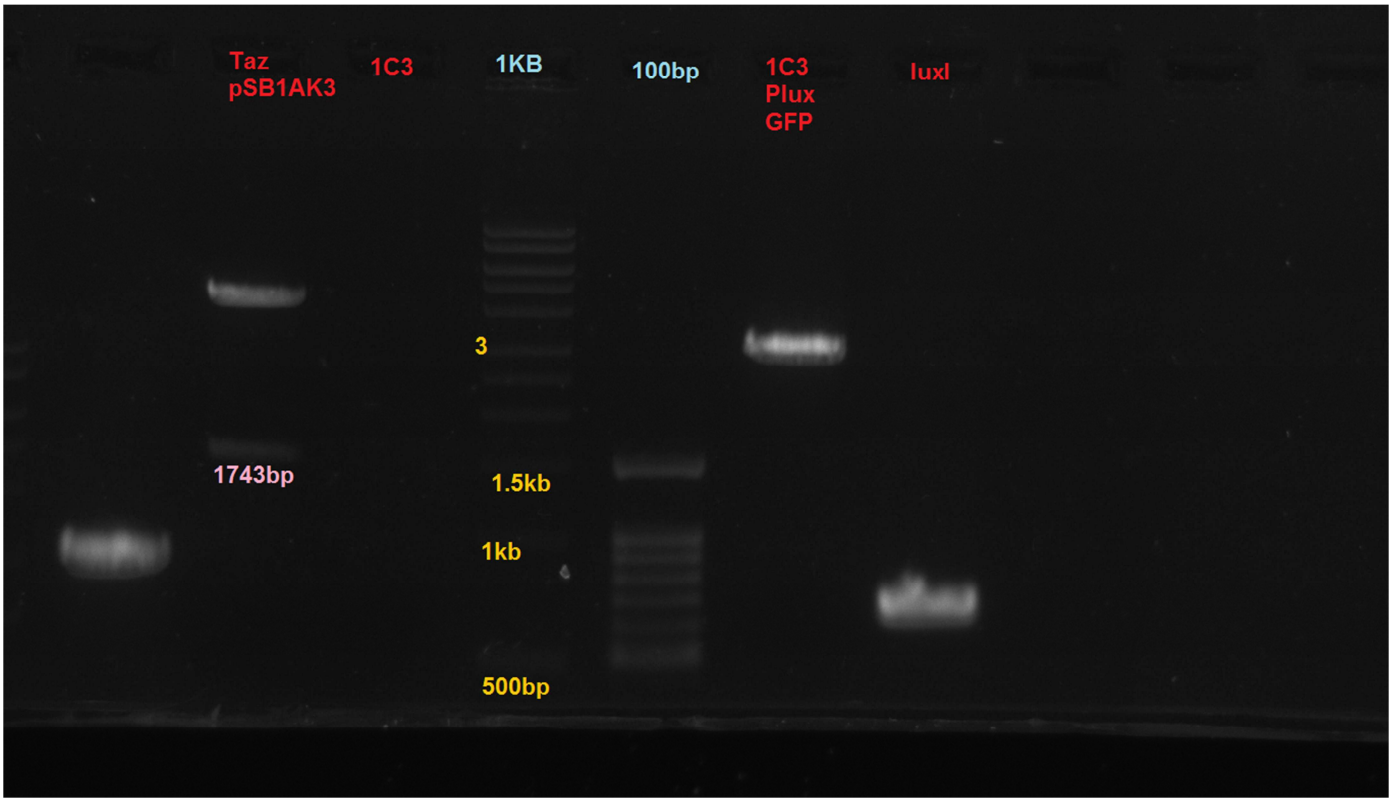
29/9/2014
- Cut pSB1AK3+Taz (the one we found on the old Gibson plate) and pSB1C3 with EcoRI and PstI restriction enzymes
- Ran on gel
  - pSB1AK3 with Taz construct worked, pSB1C3 did not
- Took pSB1C3 from Noa and cut with EcoRI and PstI restriction enzymes
- Ran on gel
  - pSB1C3 worked!!

1/10/2014
1. Clean (from gel) restriction products:
   - Taz construct
   - pSB1C3
2. Ligation of the Taz and pSB1C3 together

2/10/2014
- Transform pSB1C3 + Taz construct (biobrick) into Top10

Results of restriction with PstI and EcoRI - take 1

21/9/2014

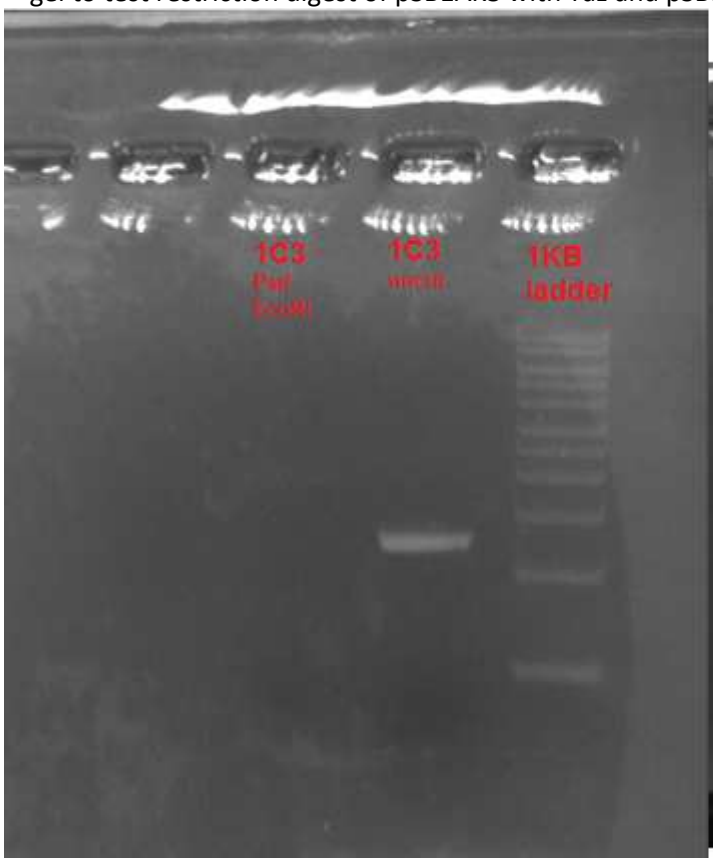


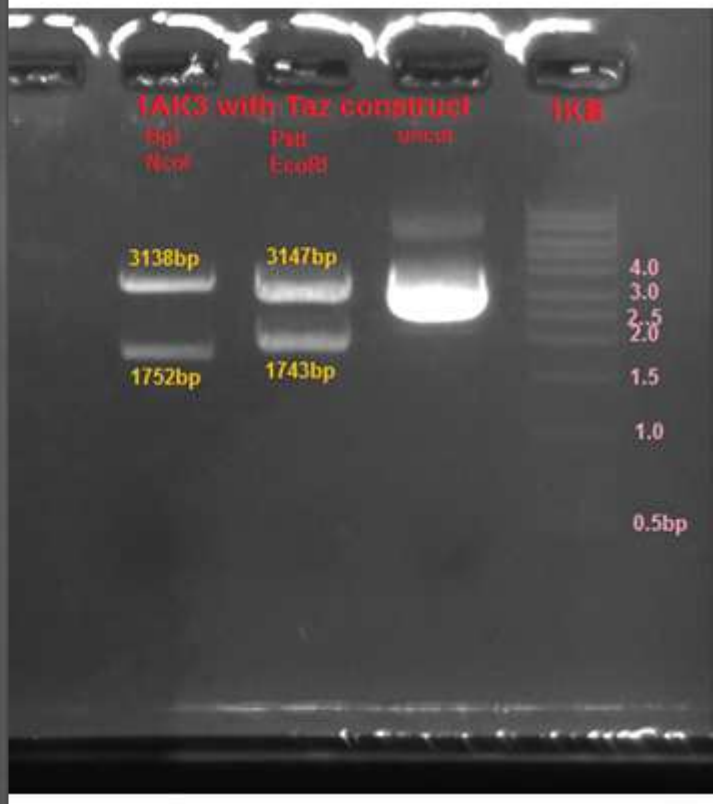
Results of restriction with PstI and EcoRI - take 2

30/9/2014

pSB1AK3 with Taz construct worked, pSB13 did not

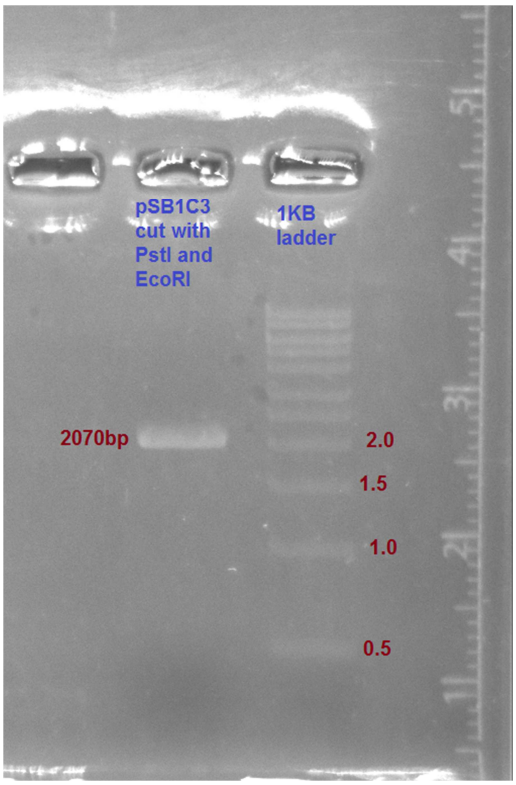
<<gel to test restriction digest of pSB1AK3 with Taz and pSB1C3 30.9.2014.Tif>>





Results of restriction with PstI and EcoRI - take 3

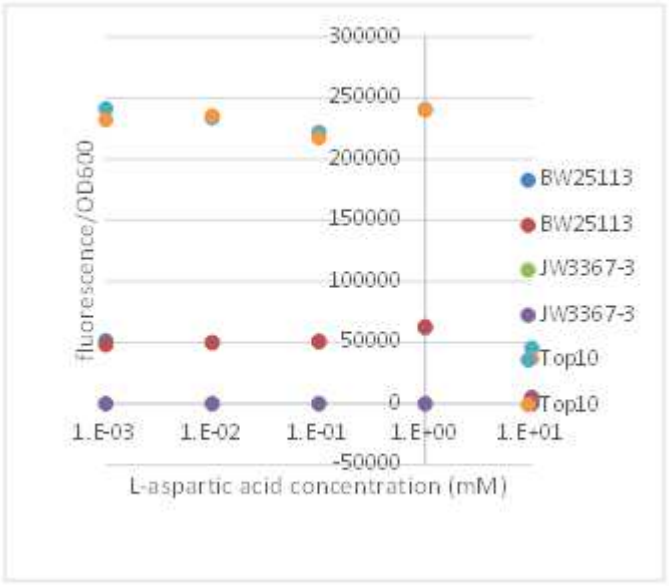
30/9/2014



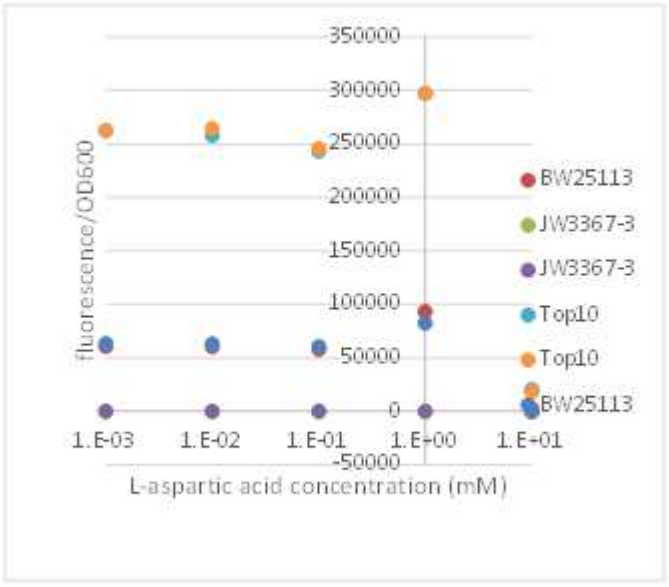
Experiment - take 1

6/10/2014

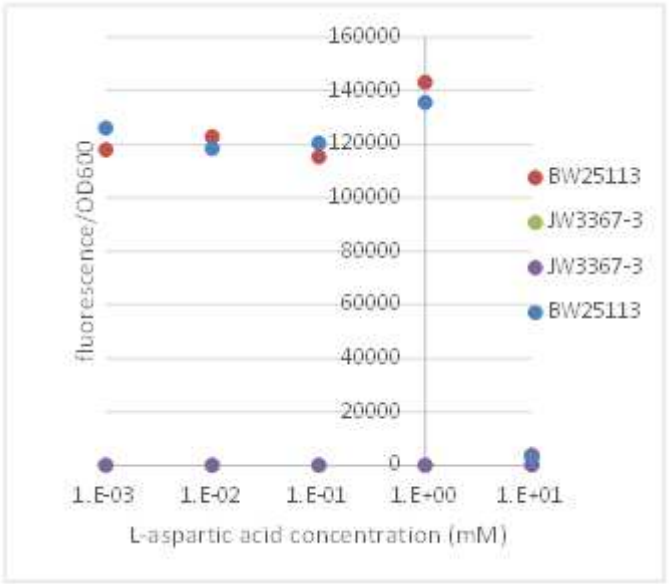
First reading



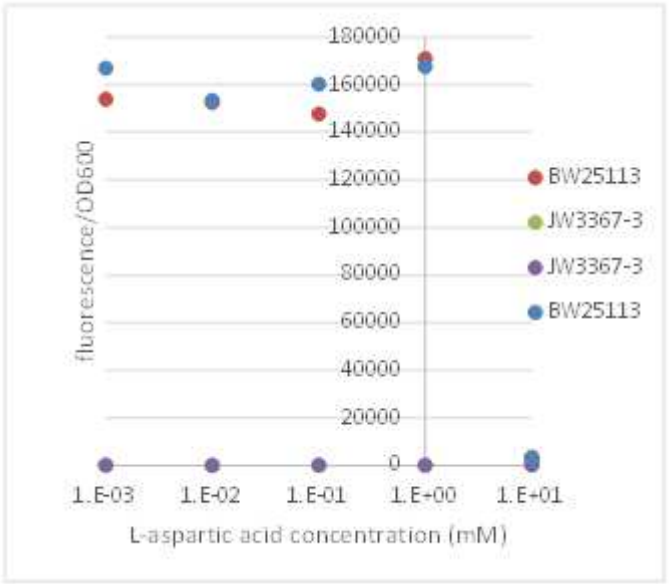
Second reading



Third reading

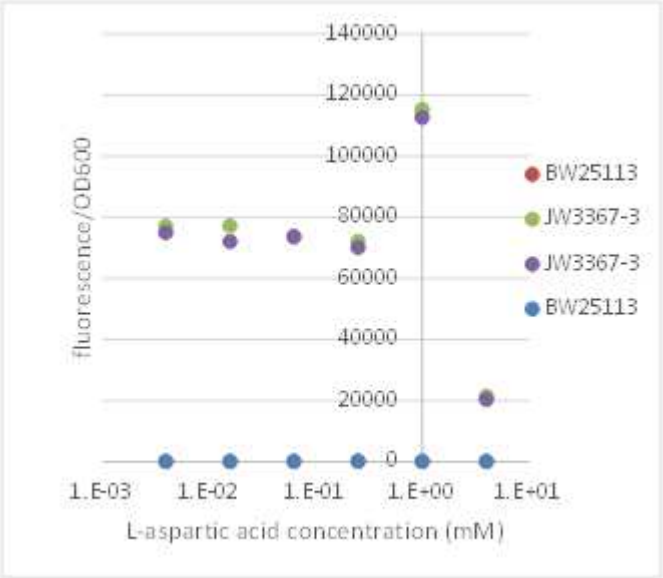


Fourth reading



Experiment take 2

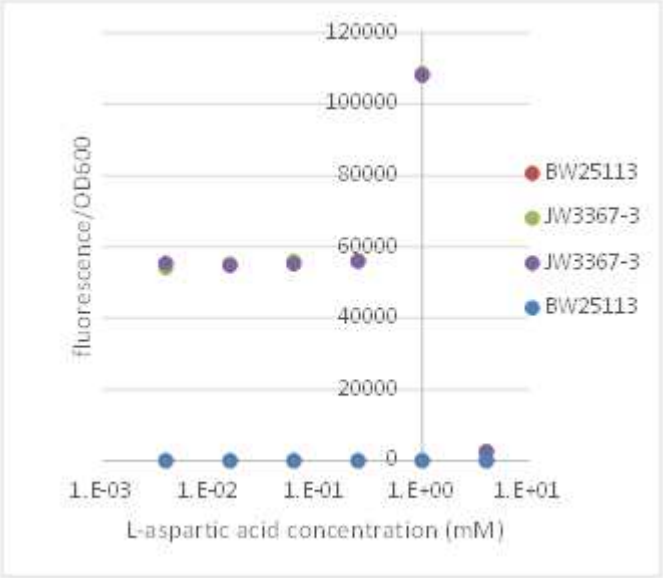
10/10/2014



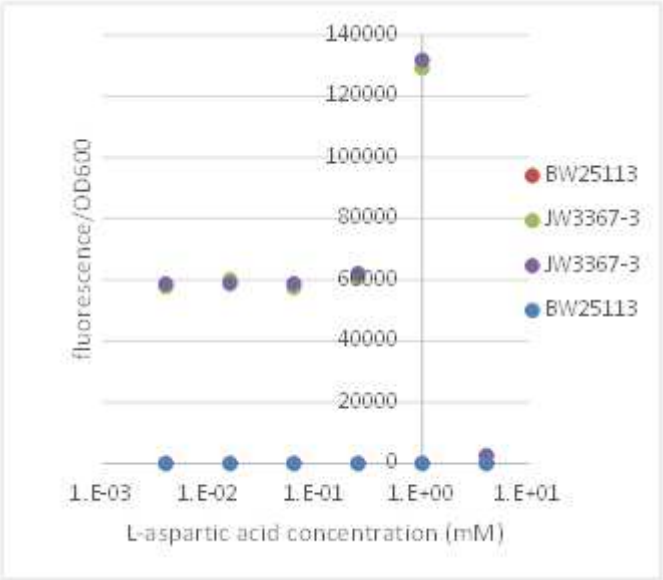
Experiment take 3

11/10/2014

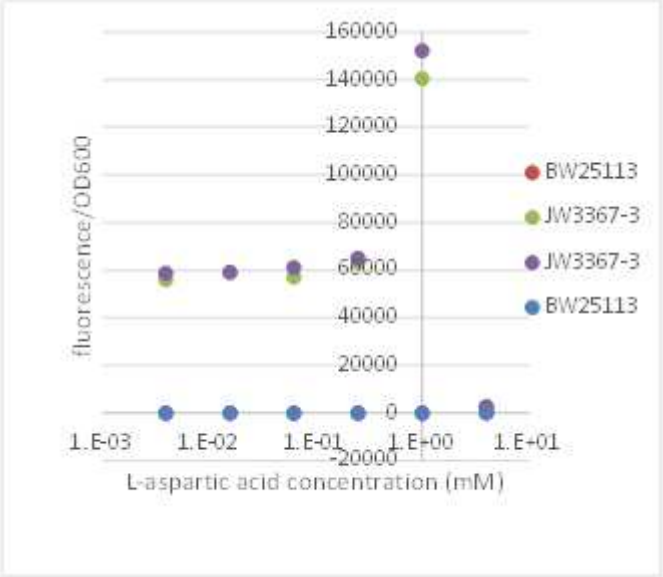
Reading 1



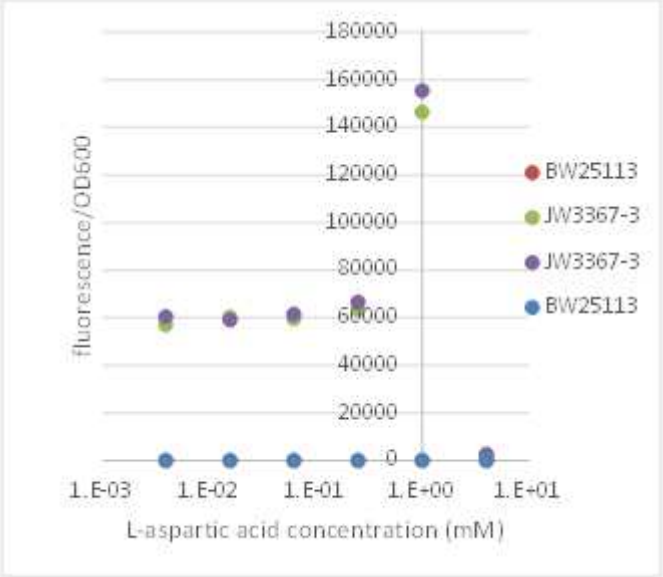
Reading 2



Reading 3



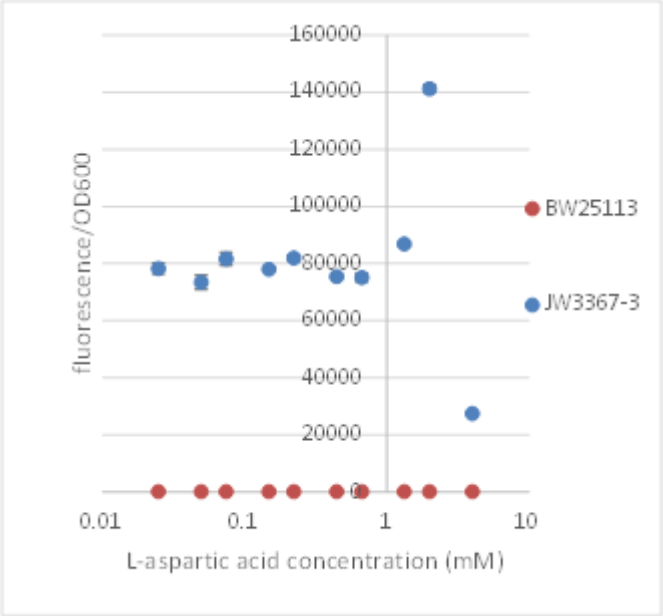
Reading 4



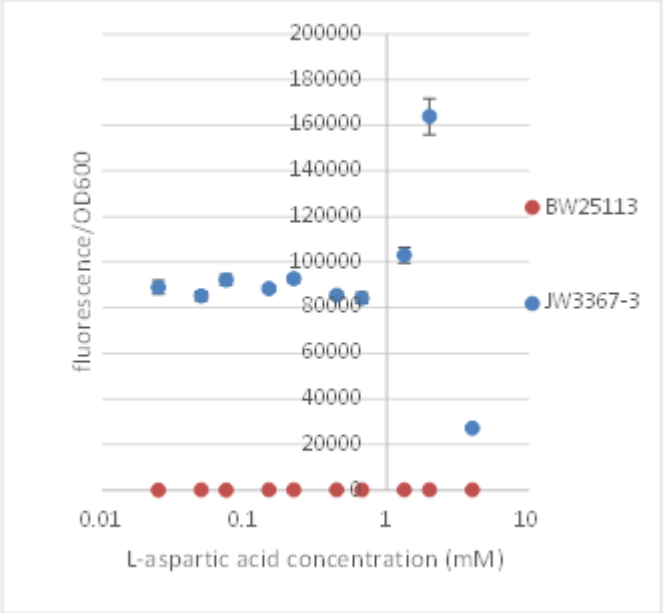
Experiment take 4

14/10/2014

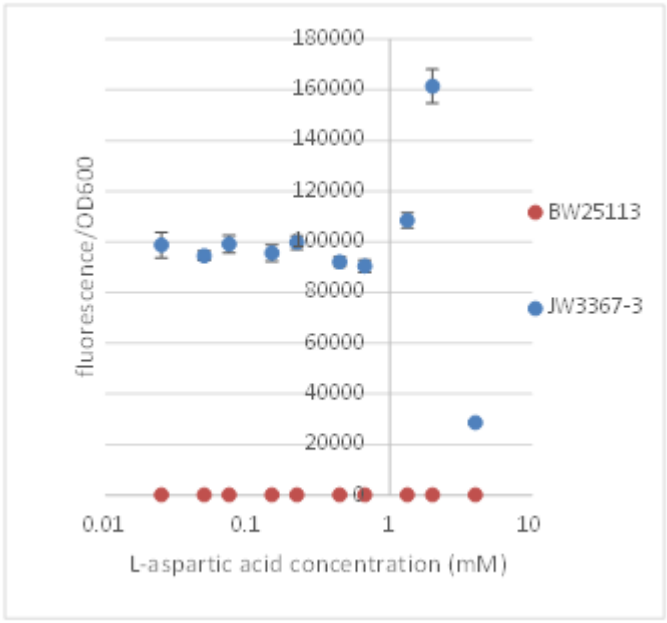
Reading 1



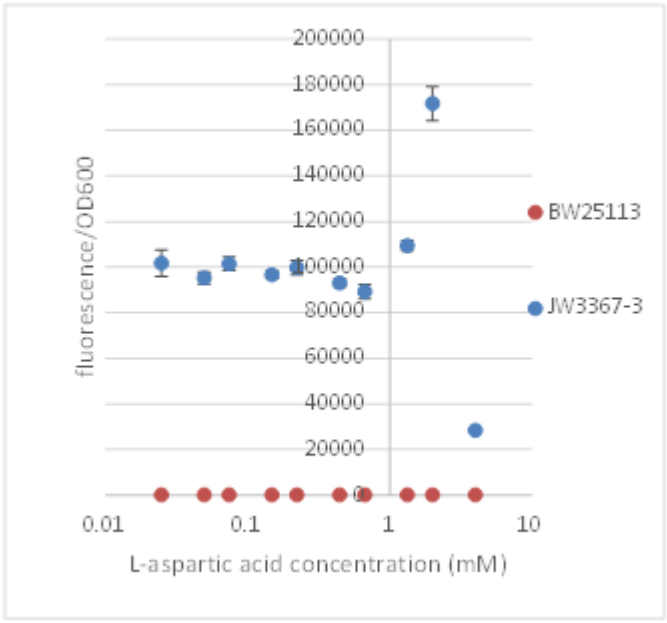
Reading 2



Reading 3



Reading 4



Osmolarity Experiment

Preparing the bacteria

29/9/2014

- Transformed Bba_M30011 on pSB1C3 (contains RFP under PompC) into BW25113 and JW3367-3 (ΔenvZ)

01/10/2014

- Grow starters o/n with CM from colonies from plates
  - BW25113 + pSB1C3+RFP (Bba_M30011)
  - JW3367-3 + pSB1C3+RFP (Bba_M30011)

02/10/2014

- Glycerol stock of
  - BW25113 + pSB1C3+RFP (Bba_M30011)
  - JW3367-3 + pSB1C3+RFP (Bba_M30011)
- Transformation of Mcherry we got from Noa Katz into Top10
  - We need this to experiment on to show that the salt concentration doesn't affect mRFP and only affects the promoter.
  - This failed but we realized we can just use RFP under Plac that we took from the distribution kit in February for the "kids kit"

Experiment take 1

02/10/2014

Today we did the experiment on BW25113 and JW3367-6

JW compared to BW

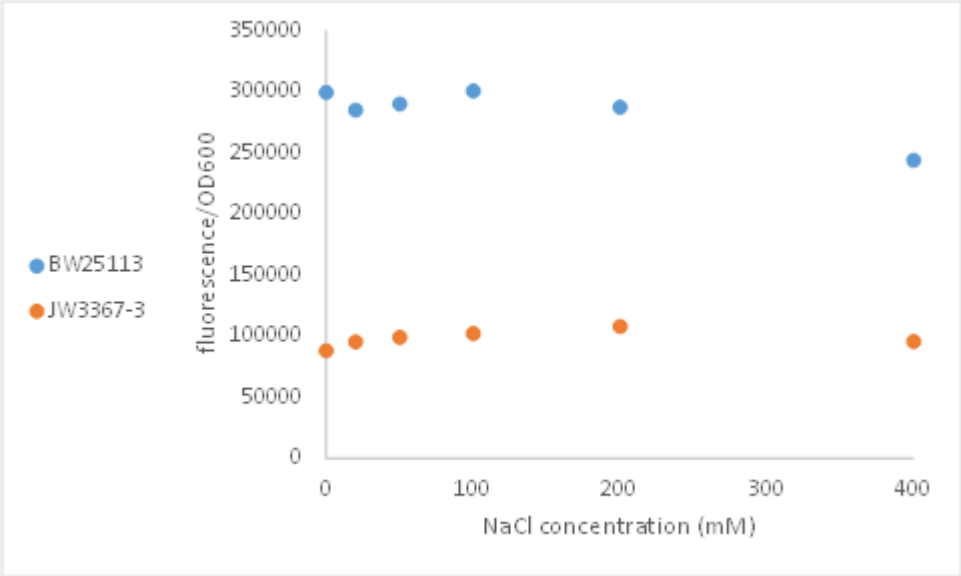
02/10/2014

BW25113 is the wild type E. coli K12 strain
JW3367-3 has the EnvZ deletion (EnvZ is the histidine kinase protein that senses osmolarity and activates the EnvZ/ompR two component signaling system

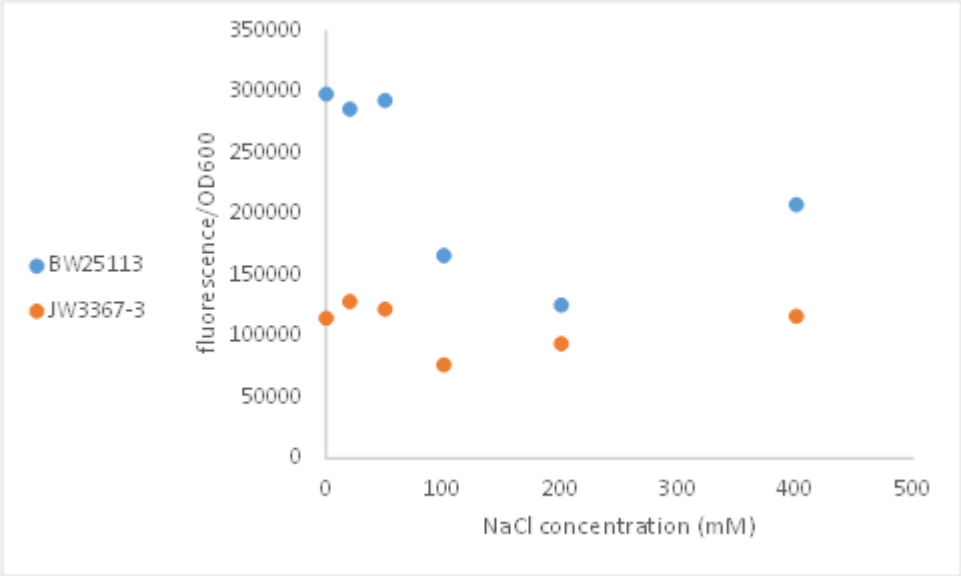
We can see from the results that the fluorescence for the mutant is much lower than that of the wild type which is as was expected

The first reading didn't come out well

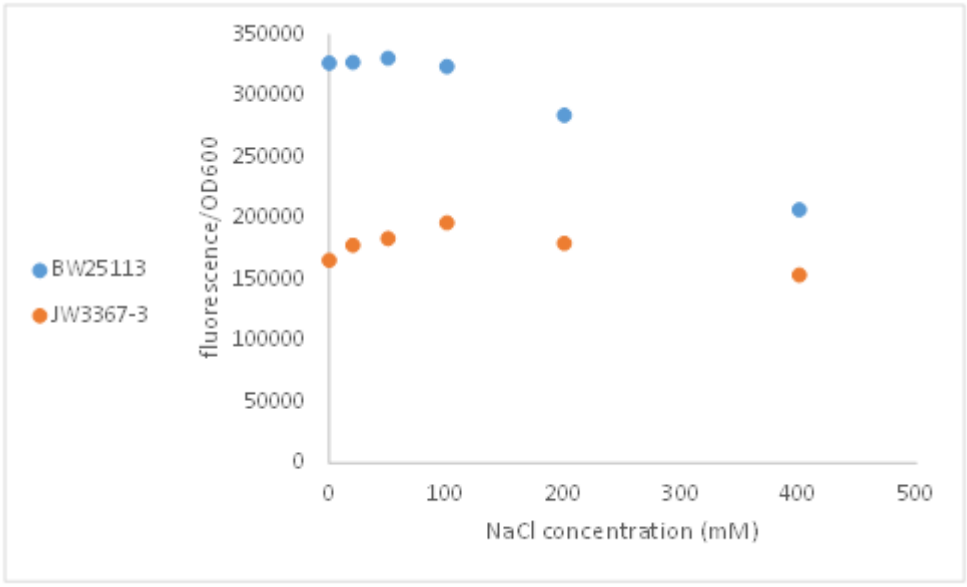
Second reading:



Third reading:



Fourth reading:



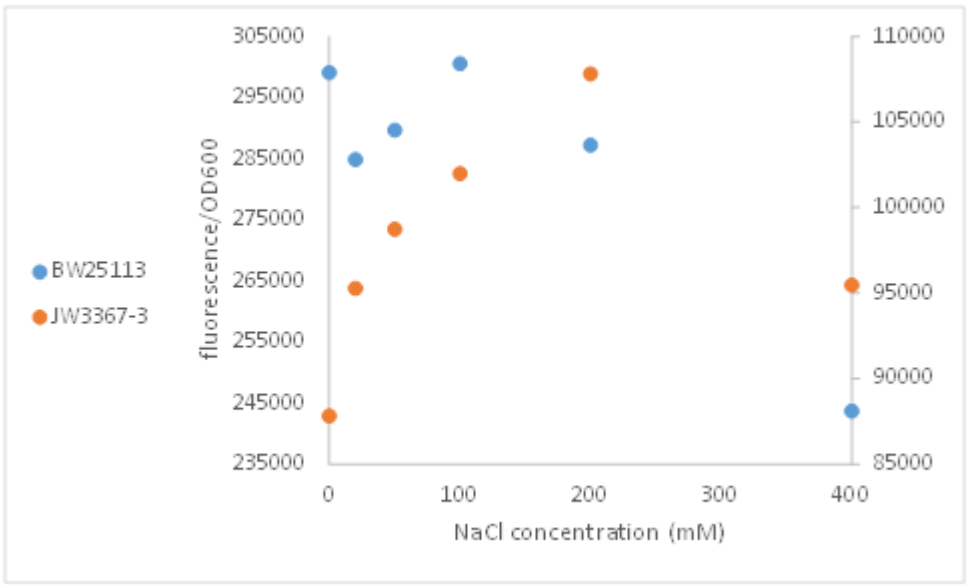
Fluorescence levels at different levels of osmolarity

02/10/2014

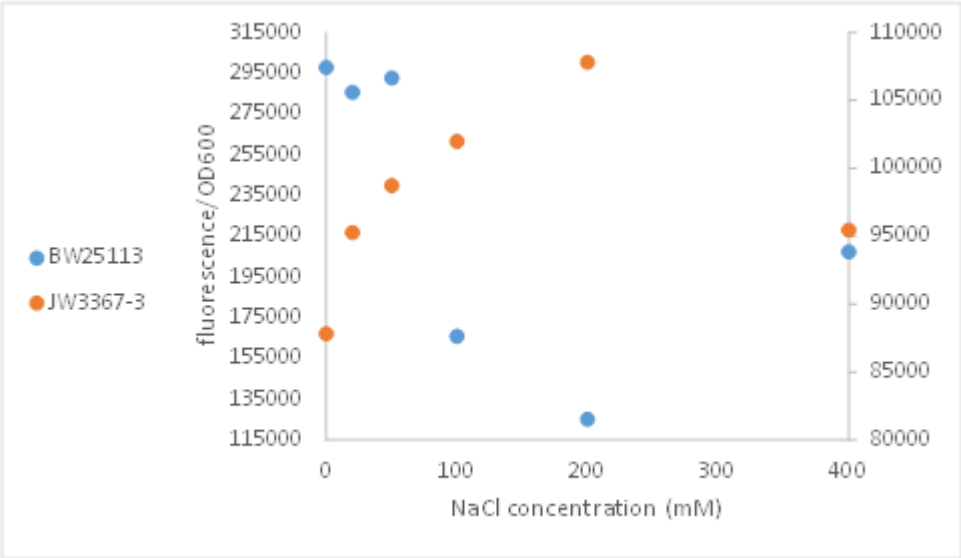
The wild type (BW25113) goes according the left axis
The mutant (JW3367-3) goes according to the right axis

We expected that the wild type would show increasing fluorescence with increasing NaCl concentration but actually we saw a decrease.
We expected that the mutant wouldn't show any increase in fluorescence with increasing NaCl concentration because it doesn't have the EnvZ histidine kinase protein and can't detect osmolarity through that system. However, we saw an increase in fluorescence until 300mM and then a decrease.

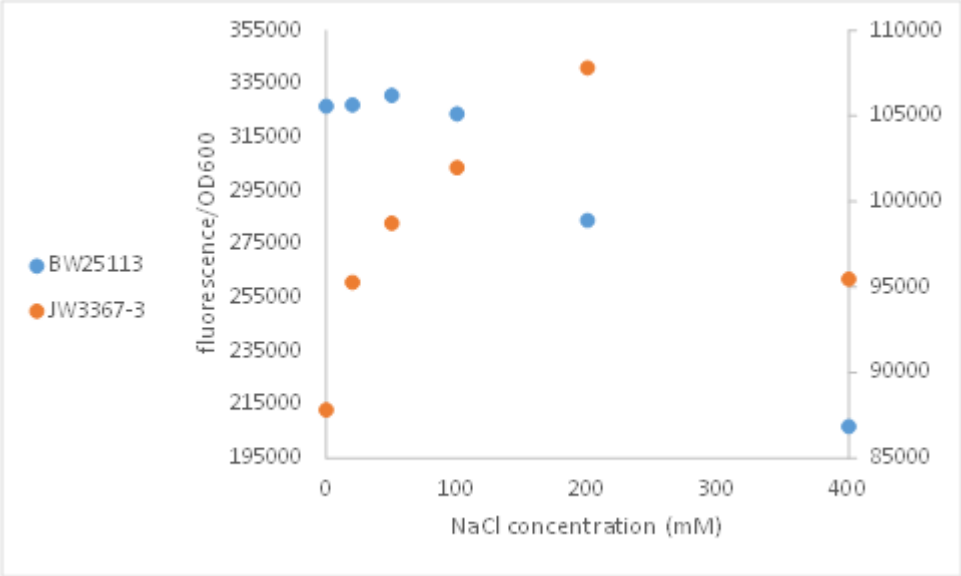
Reading 2:



Reading 3:



Reading 4:



Experiment take 2 - dilutions x3

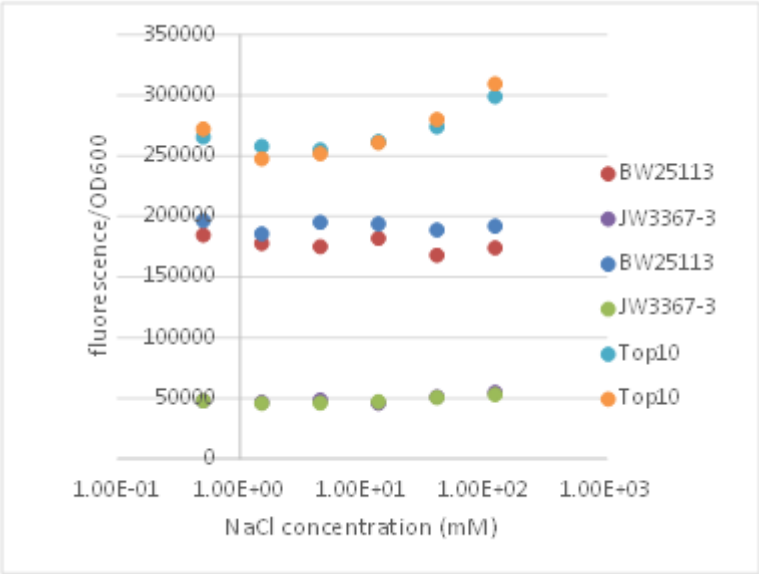
6/10/2014

BW25113 is the wild type E. coli K12 strain
JW3367-3 has the EnvZ deletion (EnvZ is the histidine kinase protein that senses osmolarity and activates the EnvZ/ompR two component signaling system

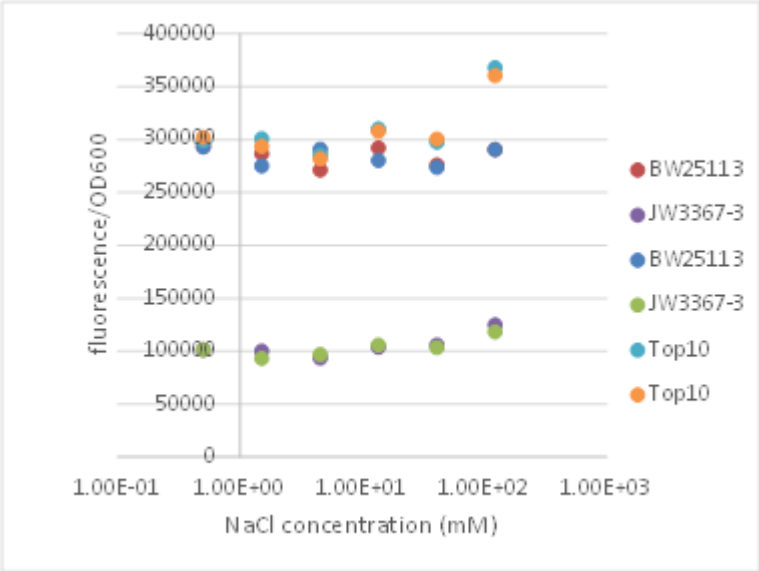
Top10 has RFP under Plac (positive control

We can see from the results that the fluorescence for the mutant is much lower than that of the wild type which is as was expected

First reading:



Second reading:



Experiment take 3 - dilutions x10

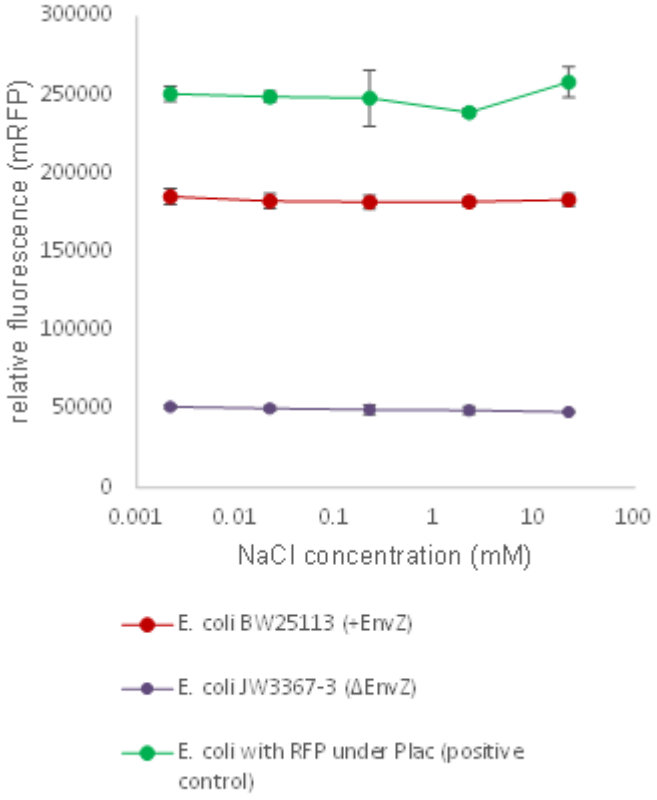
6/10/2014

BW25113 is the wild type E. coli K12 strain
JW3367-3 has the EnvZ deletion (EnvZ is the histidine kinase protein that senses osmolarity and activates the EnvZ/ompR two component signaling system

Top10 has RFP under Plac (positive control

We can see from the results that the fluorescence for the mutant is much lower than that of the wild type which is as was expected

First reading:



Second reading: