Sunday 5/12/13

RBS Vector Prep

Digested the B0034 part with EcoRI and XbaI

Treated with CIP

PCR Purified

T7 Insert Prep

PCR the I712074 part

Ran a gel to isolate the 594 bp sequence

Extracted DNA from gel

Tuesday 5/14/13:

T7 Insert Prep Continued

Digested with EcoRI and SpeI

Cell cultures:

1) 3 mL SOB Media + 3 uL Amp + K137007 (fimE)

2) 3 mL SOB Media + 3 uL Amp + 1A3 (hbiF)

Transformation:

hbiF into CCs

Plated onto 1 amp resistance plate, in incubator

RBS B0034 Digest

With SpeI and PstI

Wednesday 5/15/13

Miniprep K137007 (fimE)

Miniprep HbiF 1A3

Transform HbiF **Note:** Possible Contamination

Monday 5/20/13

Gel extraction of HbiF

Ran gel of FimE, seemed to be only cut by one enzyme.

Wednesday 5/22/13

CIP treated colonies 3, 4, 5 of M1

Ligated M3 (XP) and B0034 (SP), left reaction going in fridge overnight

Ligated M1 (SP) and HbiF (XP), left reaction going in fridge overnight

Thursday 052313:

Ligated M1 (SP) and HbiF (XP), left reaction going in fridge overnight (colonies 3,4 and **5**)

Thursday 5/30/13

Digested M2 (col 1,2,5) and M5 (col 1,2,3) with XP

Digested M4 3 (col 1-3) with EcorI and AgeI

Tuesday 6/4/13

Orange: Ran out B0034 (SP) and M1 col 3 CIP treated (XP) vectors to gel extract, however little DNA was present. Re-innoculated from master plate for new miniprep cultures

Green: Inoculated M1-3, M2-1,2,5 and M3 in Amp tubes

Wednesday 6/5/13

Orange: Miniprepped B0034 and M1 col. 3 and digested M1 with S/P

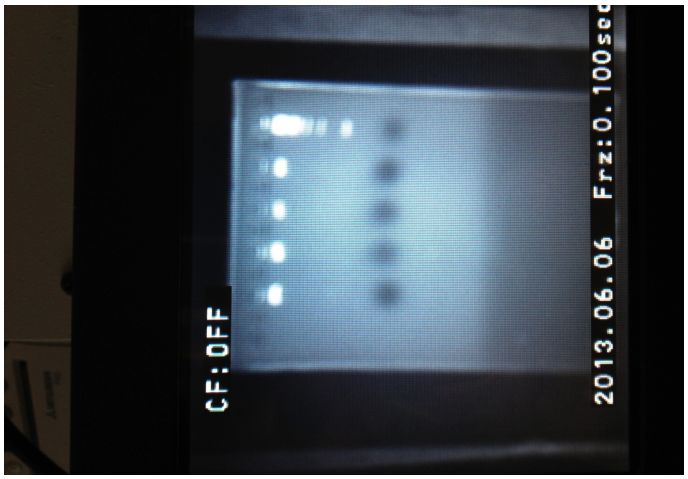
Green: Miniprepped M1-3, M2-1,2,5 and M3 (50uL final volume), digested B0034 with SP

Thursday 6/6/13

Green: Ran ladder, M2-col 1 M2-col 2 M2 col 5 and Hbif

Miniprepped HbiF, ran B0034 on gel and extracted

The compact flash drive did not work, so i took a picture with my phone, that's why the quality is not so great. However, I also captured the picture on the machine and it's still on in case you need a better picture.



I don't know if you could see it from this either?

https://mail-attachment.googleusercontent.com/attachment/u/0/?ui=2&ik=d720431768&view=att&th=13f1a7da8ac28b0d&attid=0.1&disp=inline&safe=1&zw&saduie=AG9B_P8xe-NDEJ55utXV-k972s3J&sadet=1370539062324&sads=EQ-W-_SPRaBjNZylJcPGN3phAps

Orange: Ran out and gel extracted M1 col.3 (SP) digest

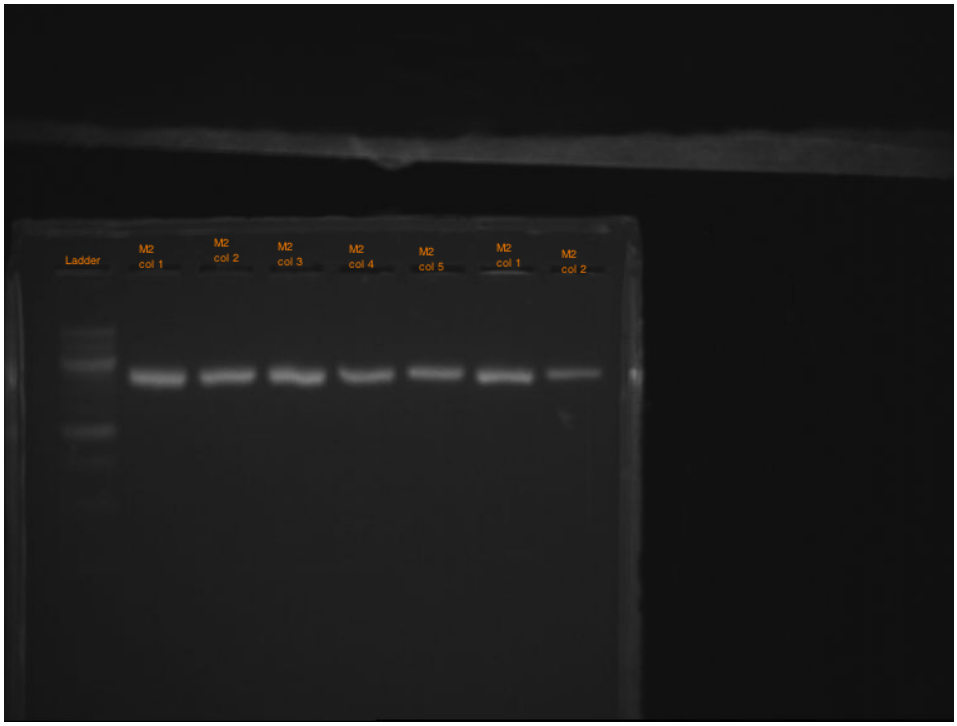
Yellow: colony PCR on M5 and M4, colonies A-J

Friday 6/7/2013

Yellow: Ran colony PCR of M5 and M4 colonies, no bands were found in any lanes

Blue: Ran some old M2 colony PCR's (1st 5 lanes) and Lilly's PCR of the first 2 colonies (last 2

lanes). All the colonies appeared to be the correct size (expected fragment size is ~818). Colonies 1,2, and 5 were sent for sequencing on this day. Results should be back on Tuesday.

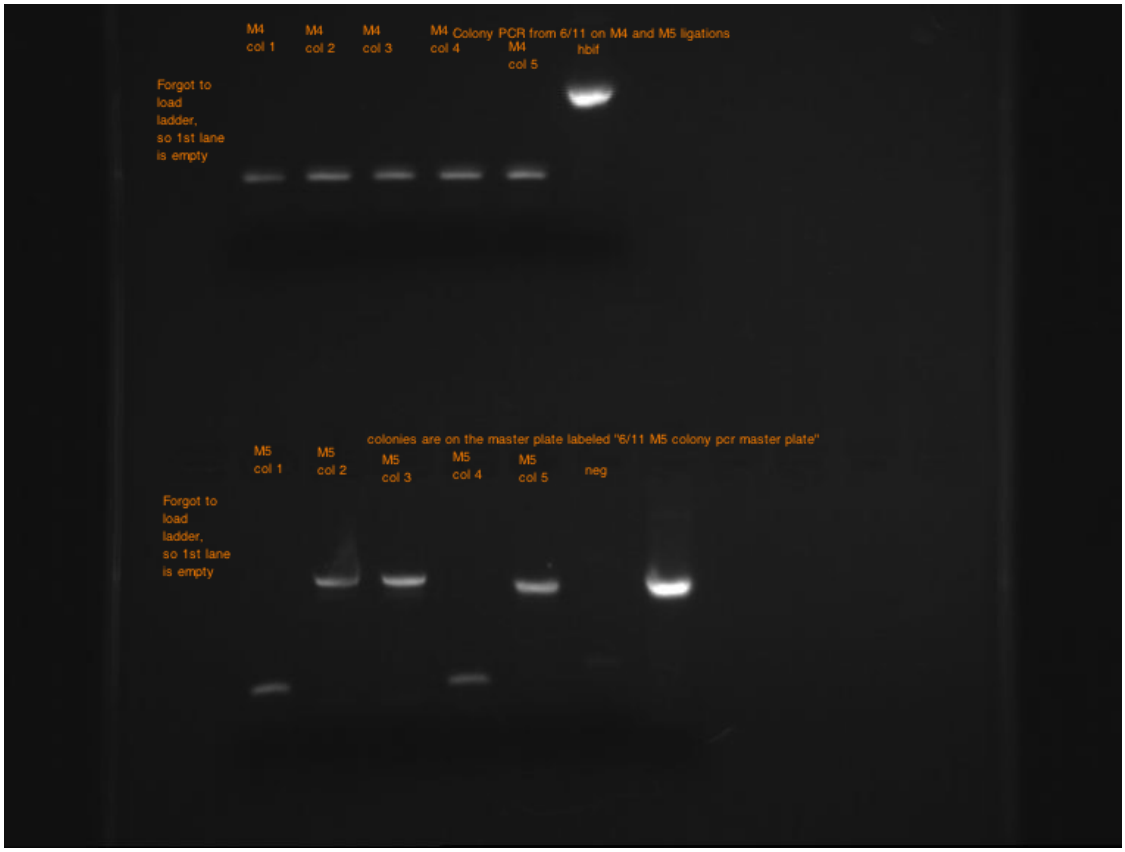


Saturday 6/8/13

Completed Gel extraction for B0034 (40ul and 20ul sample)
Ran gel of M1, cut out band. still needs to be gel extracted (in blue rack)

Monday 6/10/13
Green: Ligated B0034 with M3
Orange: Gel purified, CIP treated and Ligated M1 col. 3 (SP) with hbiF (XP) O/N @ 4 C
Corey: Digested hbiF with XP for 8 hours.

Tuesday 6/11/13
Green: Ran colony PCR of old M4 and M5 ligations; Transformed M5 ligation



Orange: Transformed M4 Ligation

Blue: Ran gel of HbiF XP digest (cut out fragment, was the right size, ~600bp)
Gel extracted, ligated with M1

Ran gel of old M4 and M5 ligations:

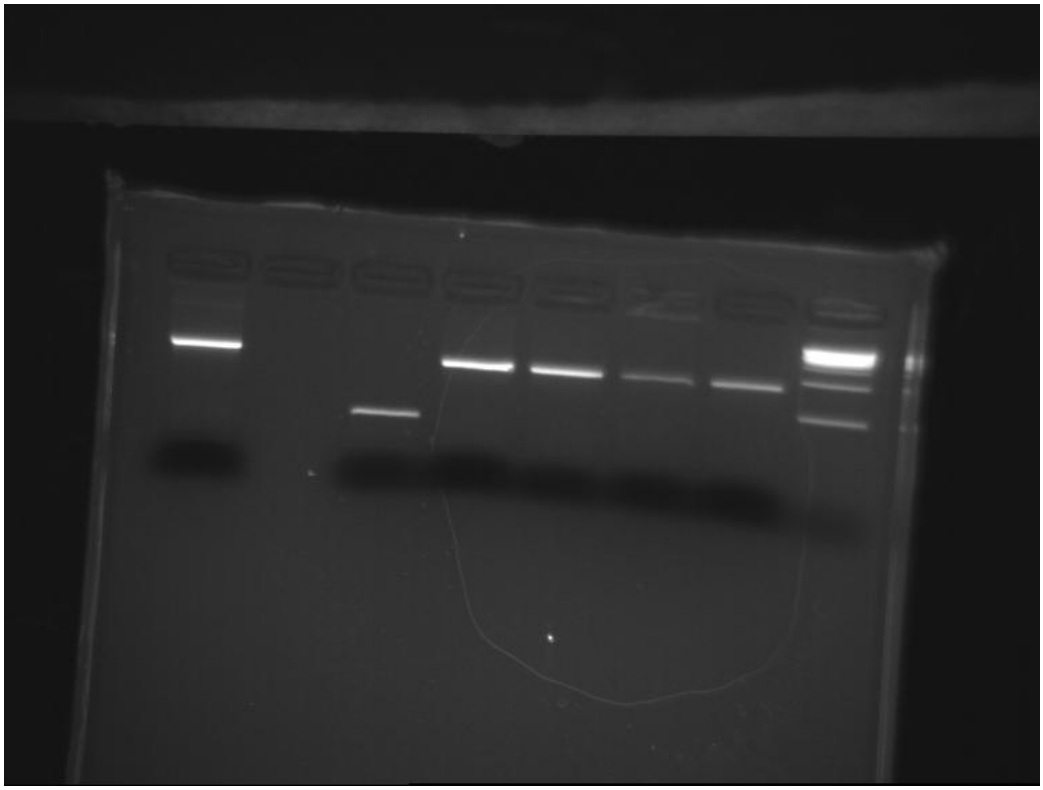
Wednesday 6/12/13

Green: Ran colony PCR of M5 transformation; transformed I719005 from wells

Orange: Colony PCR of M4 transformation colonies 1-5

Thursday 6/12/13

Orange: Ran out the M4 possibles on a 2% gel, not sure how you guys are doing the fancy labelling, but left to right we have HbiF control, blank, col 5, 4, 3, 2, 1, 1kb ladder. It looks to me as if colony 5 is not a successful ligation, however the other 4 appear to have the hbiF gene there as well. Innocualted O/N cultures of 1-4 from master plate to miniprep and sequence



Green: Ran gel of M5 colony PCR, no bands other than ladder (even positive control)

6/14/13
Orange: Miniprepped M4 Col 1.,2. and set up tubes for sequencing. These need to be taken to the core, but it is already closed today. I will leave them in the freezer in back in the orange team rack. If any of you are able to take them down to the sequencing core on monday it would be great, if not I may be able to do it myself. Thanks in advance

6/16/13

6/18/13
Green: Digested M2 with X and P, extracted and transformed A340620

6/19/13
Green: Gel extracted M2 digest, ran vent/colony PCR of A340620

6/20/13
Orange: Digested B0034 with X/S and fimE with X/P. Treated B0034 with CIP and gel extracted both insert and vector from gel.

6/21/13
Orange: Gel extracted fimE (X/P) and B0034 (X/S) and set up a ligation of these two parts

Set up a ligation of M1 and fimE, however for both samples the fimE DNA was very low concentration. I set up one set of standard vector:insert ratio and another with a surplus of the fimE with hope of getting luckier with a ligation.
Green: Digested M2 (XP) and A340620 (SP), ran gel to extract M2

6/24/13
Green: Finished gel extract of M2, ligated M2 with A340620 (no backbone!), removed pSB1C3, pSB1A3, and pSB1AT3 from wells and transformed all

6/25/13
Green: Ligated M9 with backbone and transformed, pulled I13500 and pSB2K3 out of wells and transformed, inoculated 7 colonies of pSB1C3, and 2 colonies of pSB1A3 and pSB1AT3.

6/26/13
Green: Inoculated pSB2K3, digested many parts with many things

Thursday 6/27/13
Green: No ligations were done today. M12 (tet inverter), psB1C3, and M2 (EA) were gel extracted. M9 did not show up on gel at all. We also VentPCRed M9. Everything is in rack.

Orange: PCR of hbiF, B0034 (x5), fimE (x5), M7, M5, M2, F2622 (x3), M12 (x5)
Green: Ligated M2, his tag, and chlor backbone, miniprepped kan backbone

Friday 6/28/13
Orange: Mass digests of parts:

	HbiF	B0034	fimE	M7	M5	M2	F2622	M12	A340620	Natural	Co

											nservative
ES		ES	ES	ES			ES	ES	ES	ES	ES
XP		Xp	XP		XP	XP		XP	XP		
EA						EA					
EP										EP	EP

ES: x 9
XP: x 7
EA: 1
EP: x3

Example of the digests in the incubator

ES	B0034	fimE	M7	F2622	M12	A340620	Natural	Conserved	pSB1C3
XP	B0034	fimE	M5	M2	M12	A340620			
EP	Natural	Conserved	pSB2k3	pSB2K3					
EA	M2	M2							

Saturday June 29 2013

Mike- Gel extracted digests, Notes:

Bands look uncut: one of M2(EA), M5(XP), fimE(XP), M2(XP)
Uncut, slightly half cut, and faintly cut: M12(XP)
Definitely cut: one of M2(EA), 1c33rdfrombottom(ES), 1c32ndfrombottom(ES), fimE(ES), A340620(ES), M12(ES)
Unsure if cut; bands very faint but definitely there: consfimSON(ES), NatfimSOFF(EP),consfimSON(EP), NatfimSOFF(ES)
half cut or cut: M7(ES)

Re-do the following digests:

XP: M5, fimE[2], M2(only 10uL), M12, B0034[2,5]
ES: consfimSON, NatfimSOFF, M7, B0034[2,5], F2622[2,3]
EP: consfimSON, NatfimSOFF, pSB1C3(rSAP),pSB1A3(rSAP),pSB1AT3(rSAP)

The F2622 PCR failed. Needs to be re-done or other F2622's digested.

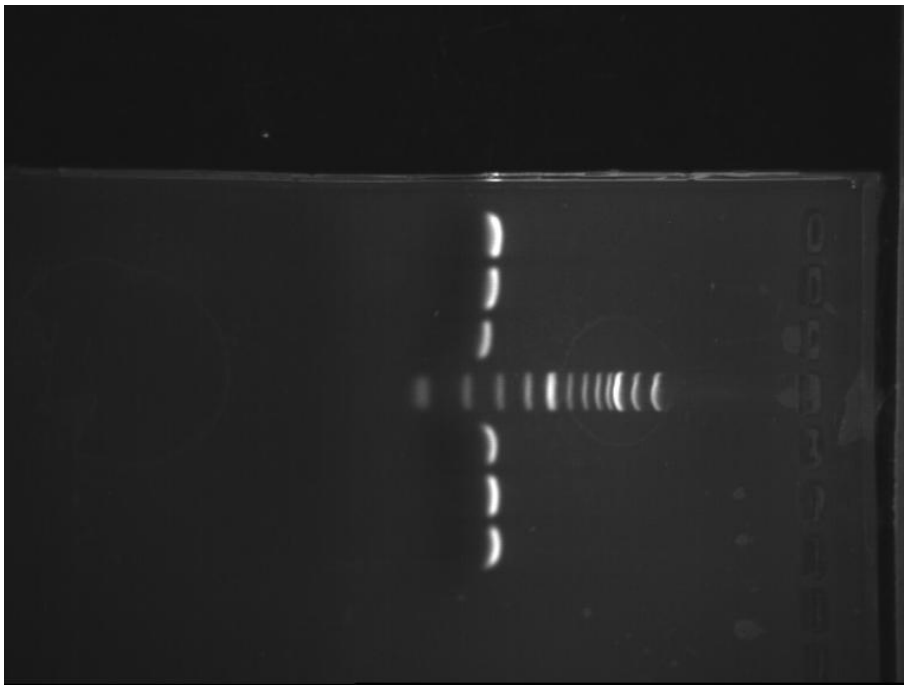
pSB1C3, 1A3, 1AT3 should be digested with EP and the 2nd and 3rd from bottom bands should be extracted and used in ligations to determine which band is the correct one to cut out.

Monday Jul 1 2013

completed the following 3A ligations: M6, M8, M10, M9, M13, M4, Natural fimS off, Cons FimE on, His + M2

7/2/13
Green: Colony PCR of M12/RBS ligation

7/3/13
Green: All M12+RBS colonies appear to have the wrong band size (should be about 850)



Green: The transformation of the Chlor backbone, M2 EA, and Histag did not work. The backbone and M2 was for sure not the problem. Neither was the plate. Maybe His tag? I don't know, we'll see. Mike is doing that same transformation to see if it works or not.

7/9/13
Green: Digested Switch(ES), M12(XP), and Kan backbone (EP), ligated them, and transformed

7/10/13

Green:

- PCRed M1 and “Switch tet”
- Gel extraction of fimE.
- Digested:
    - 2 M12 samples with ES and XP (one of the XP samples is mislabeled as ES)
    - A340620 with ES and XP
    - M9L2 with ES and XP
    - M9L3 with ES and XP
    - FimE (K137007) with ES and XP
    - Hbif (K880000) with XP
    - M1 and “Switch tet” PCR with ES
- inoculated reverse B0030-RFP (K199021), an RFC25 part (K157006), RBS-GFP (I13500), and the old switch double reporter (MI2F)

7/12/13

Green: Added rSAP to Kan backbone, attempted to religate, retransform switch+tet

7/17/13

Green: Religated (again) switch + tet + amp backbone this time.

7/18/13

Green: Transformed switch/tet ligation (used 150 uL comp cells, used 100 uL pre-centrifugation and 100 uL resuspended after centrifugation)

7/19/13

Green: Ran vent colony PCR of 3 colonies from each plate of switch/tet ligation

RNA secondary structure shielding the ends can inhibit ligation. Addition of DMSO to 10% (v/v) can increase ligation in these cases.

Might be worth looking into adding 10% DMSO to our ligations? I highly doubt this is the issue but we can give it a shot.

* Too much ligation mixture was added to the cells. Add between 1-5 µl to 50 µl competent cells.

Another possibility

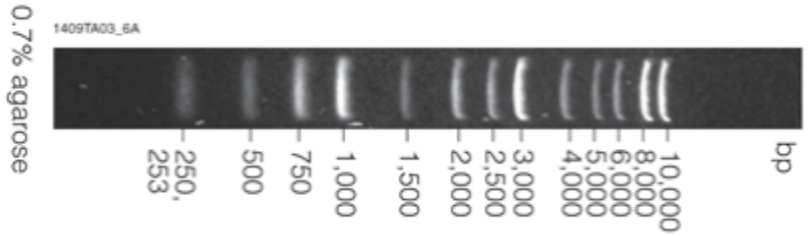
As a test, digest some vector with EcoRI, gel isolate (but don’t CIP treat). Ligate it to itself. You should get a lawn of colonies when you transform. If not, soemthing (probably your agarose) is eating the ends

Possible something (single strand nuclease?) removing sticky ends from the digest

products

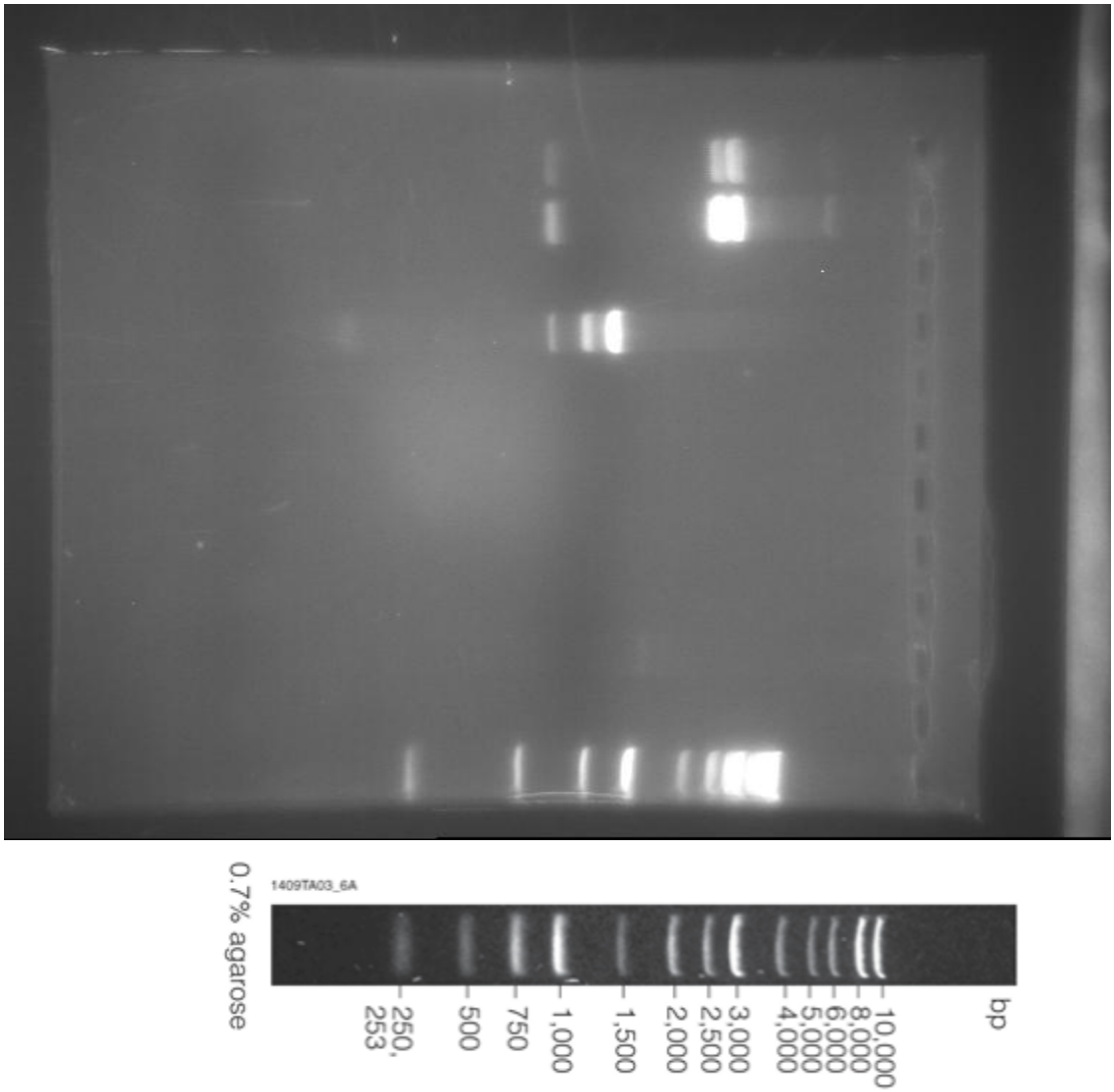
7/20/13
Green: Ran gel (see gel folder for more exposures)

Lane	Gel 1	Lengths	Gel 2	Lengths
1	1kb Ladder promega	see below	Hbif XP	561
2	M1 ES	58	M2 XP	579
3	nat FimS OFF ES	421	M2 EA	579
4	cons FimS ON ES	421	M9L1 SP	999
5	cons FimS ON ES	421	M9L2 ES	999
6	switch tet PCR ES	1006	M9L2 XP	999
7	M12 XP	620	M9L3 ES	999
8	M12 ES	620	M9L3 XP	999
9	M12 ES	620	A340 ES	999
10	M12 ES	620	A340 XP	999
11	FimE XP	558	A340 XP	999
12	FimE ES	558	1kb Ladder promega	see below

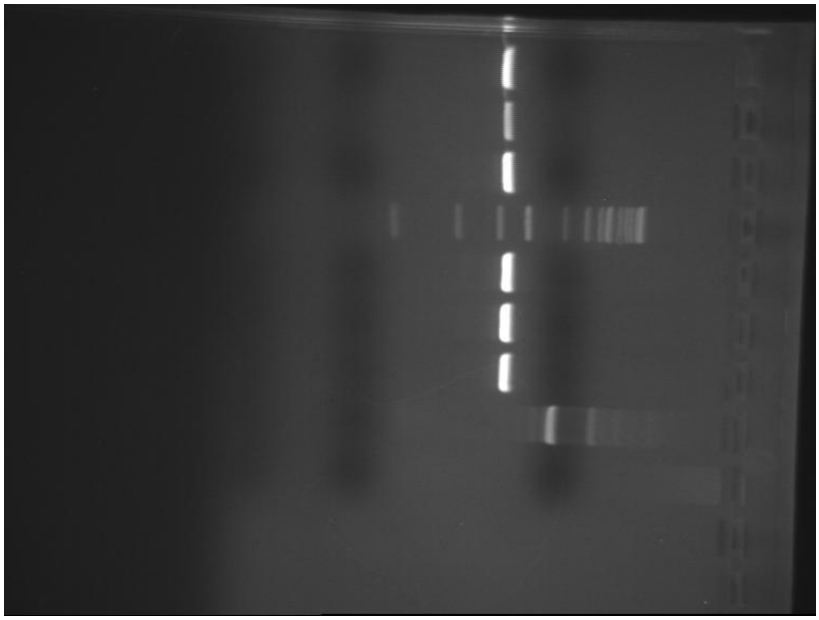


Gel 1: exposure 0.4s (UVP00294-00296)

Gel 2: exposure 0.4s (UVP00297-00301)



Ran gel of switch/tet ligation colony PCRs, not sure if correct band size (1kb ladder)



7/23/13
Green: Digested NatOFF (col 2) and NatON with ES, PCR amplified NatON and NatOFF

7/24/13
Green: Ligated both NatOFF and NatON with M12 and C backbone, and transformed
Blue: Performed Mutagenic PCR to add xhoI and bamhi sites to fime and hbif. Sequencing will have to be repeated as the sample was too concentrated. The results were sloppy, but it did seem like the pcr had worked. fime especially looked good
Transformed:
psb1t3
pet28b
1c3 positive control

Transformations failed. The positive control was terrible as well, only a few colonies. The transformation used 50ul of comp cells, so one with 200ul will be used.

Various backbones were digested with EP and SAP treated. A340620 was pcr amplified.

7/25/13
Magic Green: M28 and M29 transformations failed (all red colonies), treated Chlor backbone with rSAP and religated to switches+tet

7/26/13

Magic Green:
8 ligations
• Ligation 1: M2 xp + a340620
• Ligation 2: M2 xp extract + a340620 (inadequate DNA)
• Ligation 3: M2 xp + M9L2
• Ligation 4: M2 xp extract + M9L2 (inadequate DNA)
Each with the following protocols:
#1: http://parts.igem.org/Help:Protocols/Ligation
#2: http://openwetware.org/wiki/DNA_Ligation
• using 1uL of vector and 3uL of each insert
• used LIGATIONJJ thermocycler program

7/30
Magic Green:
Miniprep PCR: 12X mastermix
- 444 uL H2O
- 60uL buffer
- 30 uL primer 1
- 30 uL primer 2
- 12 uL dNTP

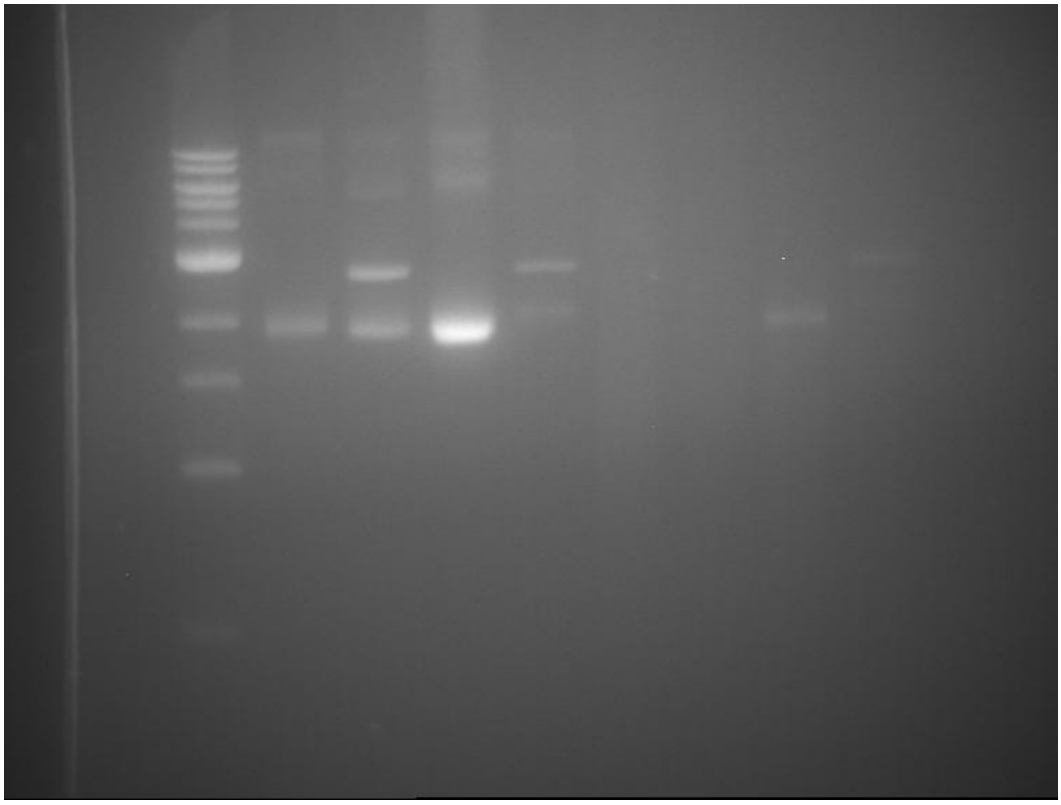
Vent PCR of K199021, psb4C5, FimE, K157006, B0034, K137080, K137070, K176001, MI8, and Psb1AT3. Also, all of these are Amp resistant. The control was F2622.

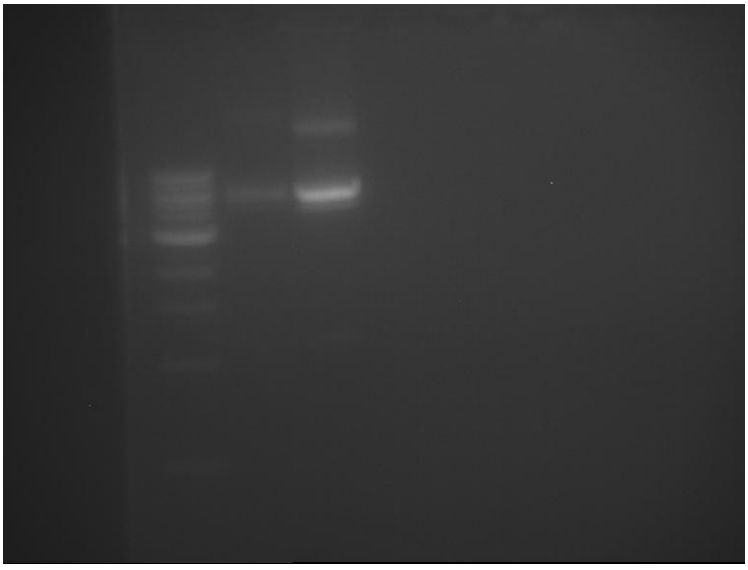
7/31/13
Narly Yellow: tried gel extracting the B0034 and FimE PRC and miniprep products. It turns out we didn't have enough DNA. From left to right: FimE miniprep product, FimE PRC product, B0034 PCR product, B0034 miniprep product. I think we will have to re-digest FimE and B0034.

8/1/13
New Orange:
PCR amplification of K137007 (FimE)
Master plates in fridge on orange shelf

8/2/13
New Orange:
PCR purified half of the products from yesterday
Digested both with XP (leftover PCR product in freezer)
Waiting to receive S03948 (edit: received!)

Yellow: Digested A340620 with ES, M2 with XP, and pSB2K3 with EP and treated backbone with SAP
Ran a Gel with 1kb ladder, M2 uncut, M2 XP, M2 uncut, M2 XP, A340620 PCR purified uncut, A340620 PCR purified ES, A340620 ES uncut, A340620 ES



Ran a separate gel with 1kb ladder, pSB2K3 EP, pSB2K3 EP (Jonah's) then cut out the backbone at 4425 bp 

Green: innoculated and plated MSBT 2012 frozen stocks
- K137007 (FimE) frozen stock
- B0034 1A3 frozen stock
- pSB1K3 frozen stock

- M2 1A2 frozen stock
- M2 1A3 frozen stock
- B0034 other frozen stock
- K137080 frozen stock

8/3/13

Orange:

- PCR amplification (2 of each):
  - S03948
  - FimE colony from orange rack
  - Q04121 (from well)
  - M10 (from Josh)
  - Cons-FimE
  - Cons-Hbif
- PCR purified one of each.
- Digested (both purified and not purified PCR products):
  - S03948 (ES)
  - FimE (XP)
  - Q04121 (ES)
  - M10 (EP)
- Streaked a plate of S03948 and inoculated S03948 and M10 1A3 col 1.
- Mini-prepped 12 tubes for the green team.

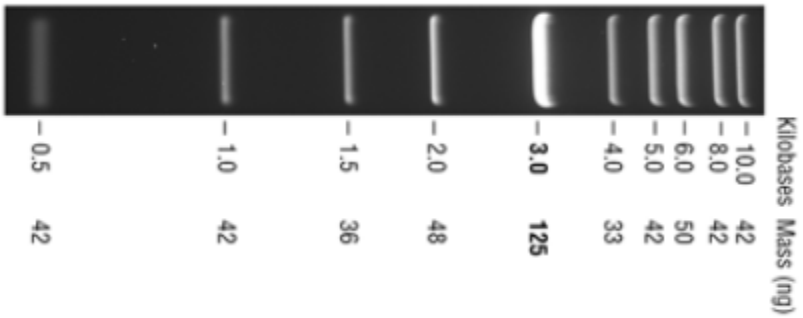
8/4/13

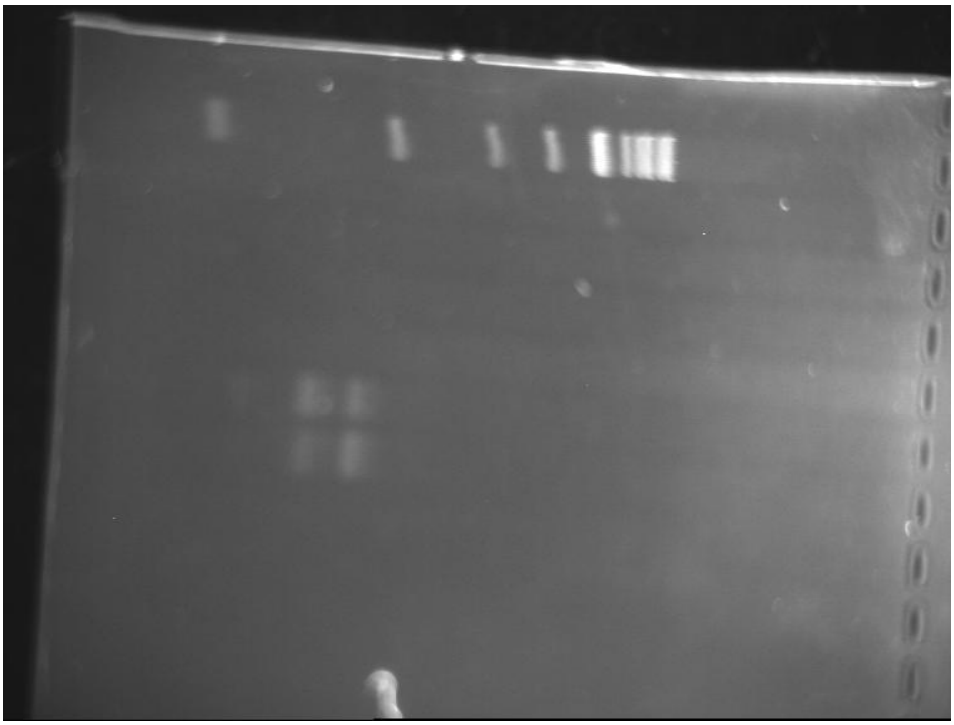
Orange:

- Mini-prepped S03948 and M10 inoculations
- Ran gel with Corey of parts digested yesterday
- May not be able to perform ligations/transformations due to lack of T4 ligase and LB+Kan plates

Yellow:

- Gel of parts that Jonah digested



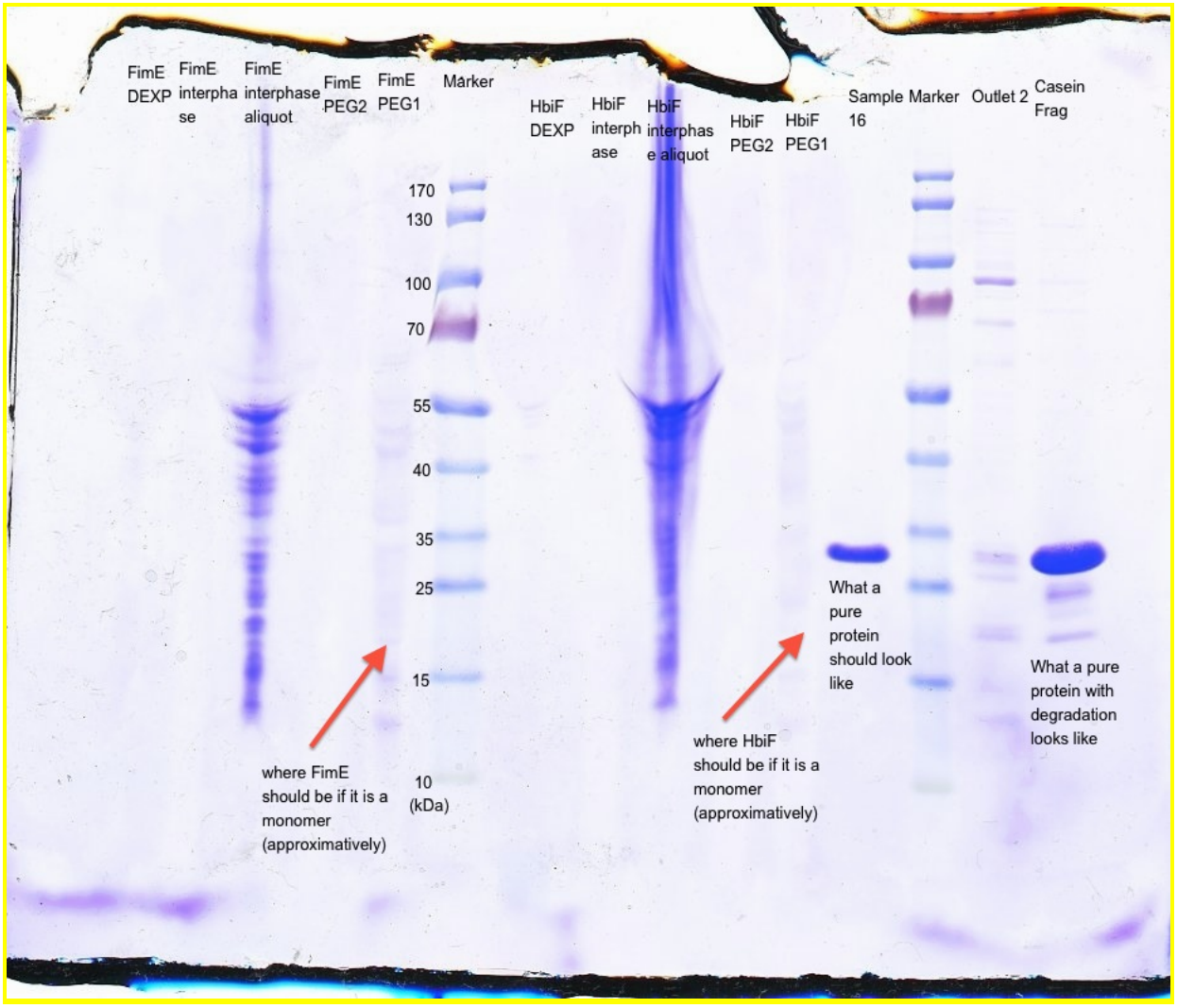


- ran 12 M46 ligations (M2 + Lac Inverter + pSB2K3) and plated
1, 5, 9 m2 1 Q 1 backbone
2, 6 ,10m2 1 Q2 backbone
3 , 7 , 11 m2 2 q1 backbone
4 , 8 12 m2 2 q2 backbone

1-4 (1, 1, 1, 1 ligase, 21 water) 16 C 45 mins
5-8(3, 3, 1, 1 buff, .5 ligase) 16c 45 mins
9-12 (2 , 2 , .5 backbone, 1 buff, .5 ligase, 2.5 water) 16c 45 mins

8/6/13
Narly Yellow:
Protein purification gel.

Correction: doesn't necessarily need to be a monomer when I said it should appear because I boiled the proteins anyway.



8/7

Green:

M10 photometer measurements

- M10 1A3 col1 mini: .015 ug/uL
- M10 1A3 col1 mini 2: .025 ug/uL
- M10 1A3 PCR: .086 ug/uL
- M10 1A3 purified PCR: .006 ug/uL
- pSB1C3: .031 ug/uL

Digests of each with EcoRI and PstI

Orange: Mass Vent colony PCR (26 samples)

- M44 ligation colonies (20 total, 2x colonies 1-10), psb1AT3 transformation positive control template (1), psb1AT3 red colony from fridge (1), cons FimE for Josh (2), 14-1 & 15-4 for mike (2 of each)

8/8
Green: Test K12 MG1655 pKD4 frozen stock
- inoculated frozen stock at 9:30am
- at noon
    - plated on Kan and no antibiotic
    - inoculated new tube and added Kan to the old one

Orange:
- Made tet, gen, kan, and plain LB plates
- inoculate M44 ligations, integration plasmid (pAH153), constitutive Hbif, and MG1655 pKD46 frozen stock
- miniprepped cons FimE, 14-1, 15-4, pAH153
- streaked pAH153

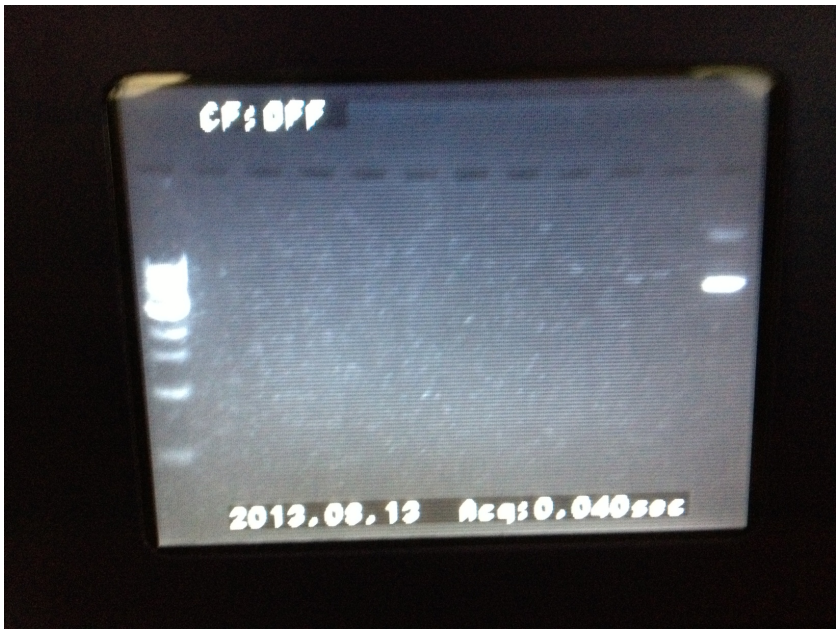
8/9
Green:
16:30: moved grown inoculations
19:30: streaked MG1655 pKD46 from antibiotic-free inoculation
20:30: reinoculated M44 ligations, constitutive Hbif, and MG1655 pKD46 frozen stock with old amp

8/10
Green:
15:30: moved grown plates and inoculations

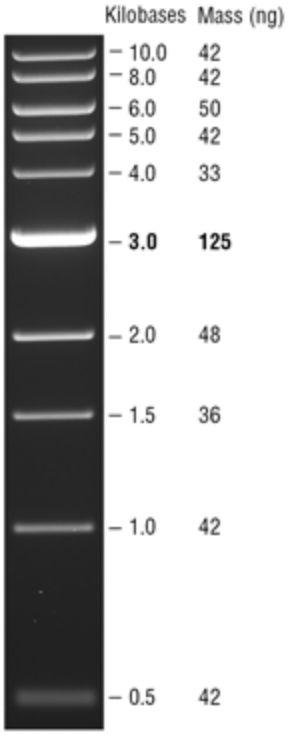
8/11
Green:
1:00: inoculate tet and amp antibiotic test using 1AT3 and 2K3
- 1 red stripe on tube = old amp stock
- 2 red stripes on tube = new amp stock
- blue stripe on tube = tet
- no stripe on tube = plain LB
2:30: calibrate microplate reader and incubate diluted calibration dilutions with the plate
17:30: put incubations in fridge and inoculated M10 1k3 col 4
- pcr purification

- cons-FimE miniprep PCR
- cons-Hbif miniprep PCR

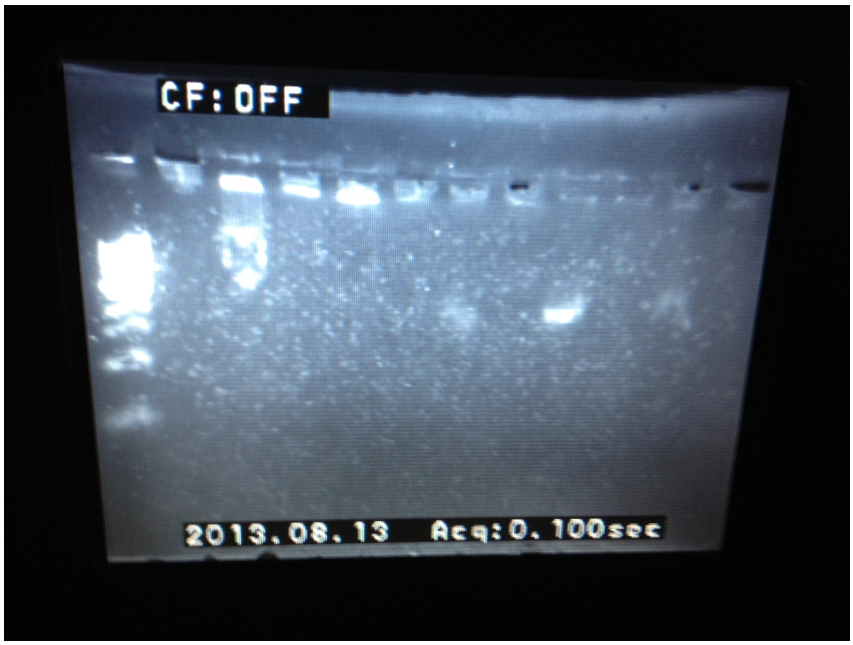
- digest pcrs and their purifications
- gel of 8/7 and today's digests (pictures below) [Help from Blue and Orange team]



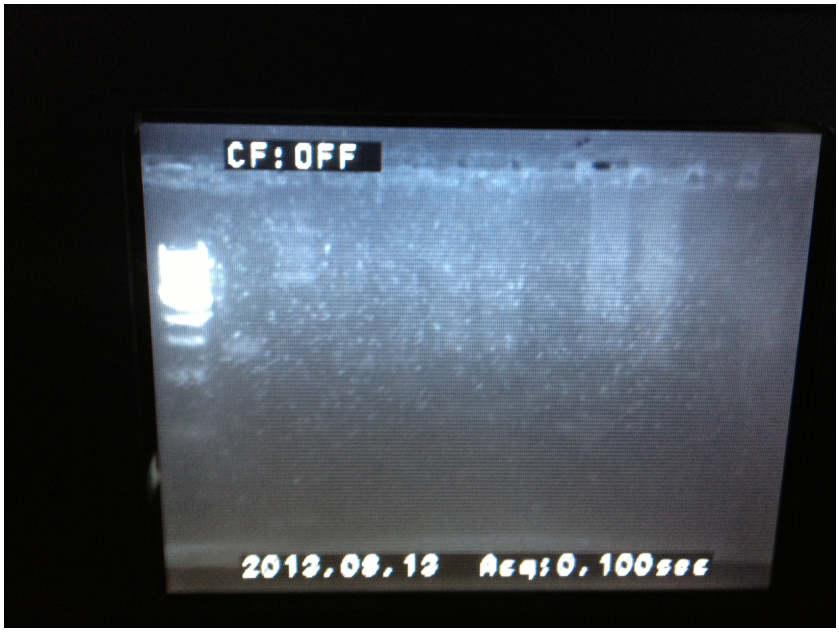
Left to right: 1kb ladder, M44 col 10 (lane 2) - 1 (lane 11) EP PCR pur, pSB2K3 EP SAP mini



Left to right: 1kb ladder (1), pSB1K3 EP SAP mini (2), pSB1C3 EP SAP mini (3), cons HbiF EP (4), cons FimE EP (5), 1C3 EP (6), M10 PCR EP (7), M10 PCR pur EP (8), M10 mini 1 EP (9), M10 mini 2 EP (10), negative control (11), Mike's part (12)



Bad gel, but...
Left to right: 1kb ladder (1), pAH153 EP SAP mini (2), pSB2K3 EP mini (3), pSB1K3 EP mini (4), pSB1C3 EP mini (5), pAH153 EP mini (6), cons FimE EP col PCR (7), cons HbiF EP mini PCR (8), cons FimE EP mini PCR (9), cons HbiF EP mini PCR pur (10), cons FimE EP mini PCR pur (11), 1AT3 EP ? col PCR (12)



Left to right: 1kb ladder, 1AT3+ EP col PCR, M44 10 (lane 3) - 1 (lane 10) EP col PCR

Orange:
PCR purification of M44 ligation colonies
Mass digests with green team of M44 PCRs, their purifications, and plasmid backbones
Ran two massive gels of M44 colony PCRs, backbones, and M10 parts

- Appears that the M44 ligations were unsuccessful (ligation will be attempted again using pSB2K3 instead of pSB1AT3; will try with linearized gel extracted backbone and backbone only treated with SAP, not gel extracted)

Made chlor, amp, tet, and gen plates

8/12
Green:
- switch orientation taq PCR assay (54.1 +/- 4.4C annealing gradient)
    - mg1655 Kan Resitant stock
    - mg1655 Amp Resistant stock
    - bl21 DE3
    - neb10beta

3:30: index Mg1655 plates at 43C and put growth curve experiment in fridge
13:00: ran incubated calibration plate in reader
15:30: replaced calibration plate with growth experiment and moved nonantibiotic plates
17:00: transfomations
- transformed M10 colonies in Bl21 and DH5alpha
- transformed circuit controls (K137018 and K137019)

0:00: fridged growth experiment

8/13
Green:
12:00: started new run of growth experiment
13:00: new run crashed
13:20: continued run

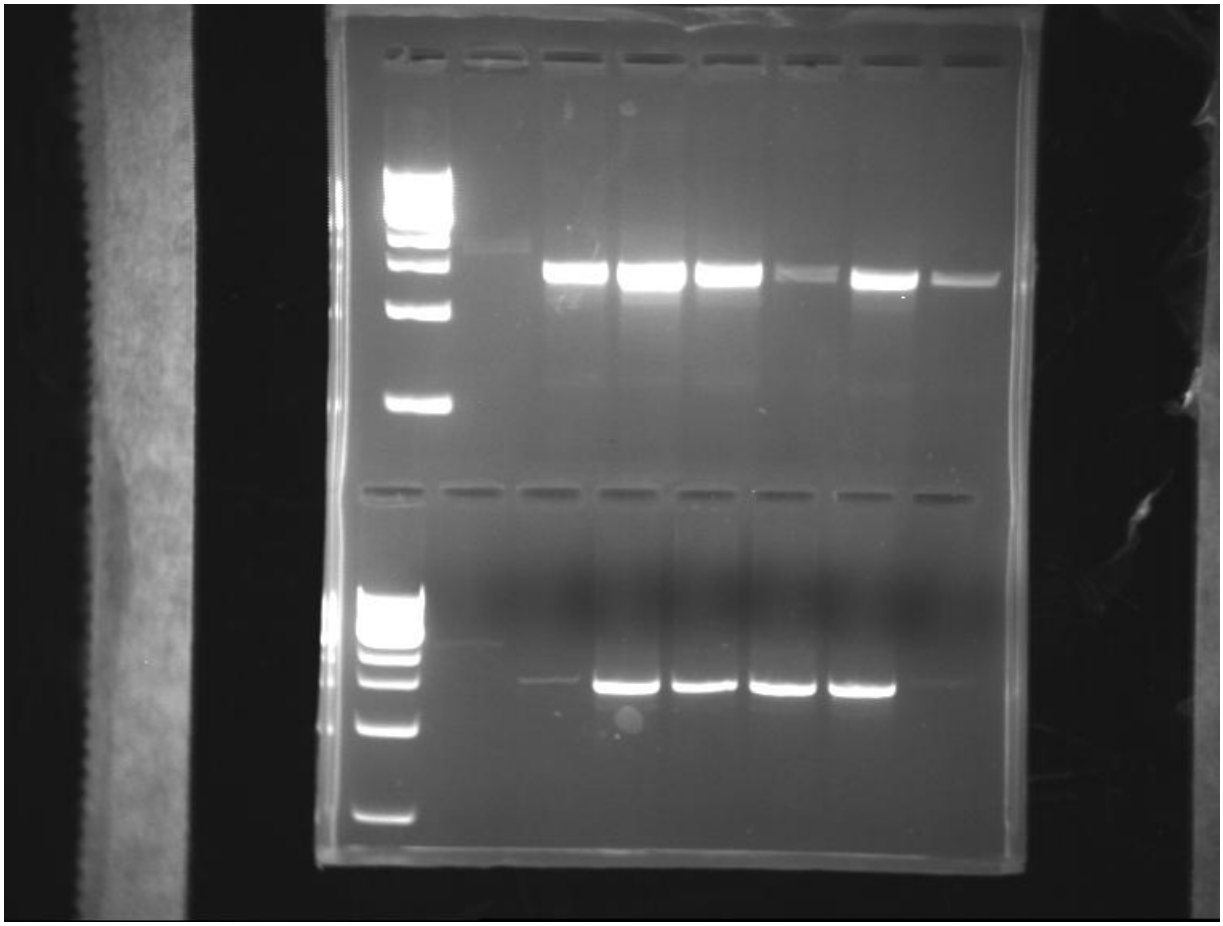
Orange:
Re-tried M44 ligations, transformed, grew overnight in incubator

8/14
Orange:
- Two of the four ligation transformations grew colonies. Only the ligations using non gel extracted backbone produced colonies. +control 2K3 grew colonies (only slightly red though), -control 1A3 did not grow colonies (good), and the two ligations that did grow have both red and white colonies.
    - Vent PCR of the colonies, index plate made, inoculated

Green:
22:15: inocculated ‘cured’ MG1655 stock for comp cells
22:45: indexed M10 in Bl21 and DH5alpha
23:45: inoculated registry orders

8/15
Green:
00:30: inoculated switch controls, T9002, and backbones
- made ‘cured’ MG1655 comp cells

Orange:
-Gel



- Minipreps eluted with EB

8/16
Green:
3:30: inoculate Drew's minipreps, last failed inoculations, and 'cured' MG1655
16:00: diluted MG1655 'cured' colonies in old amp culture
23:30: moved grown inoculations

8/17
Green: purified SAP treated backbones

8/18
Green: used backbones varieties in ligations according to construct tracking document

8/20
Orange: Transformation of 16 ligations for green and orange team.

8/23

Orange: Met to discuss a plan.

Week of 8/25

Recovered the synthesized switches by PCR using the custom biobrick primers and finally got ligations to amilcp and gfp to work! Co transformed the switches with our inducible recombinase generator. Saw green and white colonies when expected!