4 - Expression Vector/Quick change

FRIDAY, 28/07/2017

Angela Hellyer Did transformations of pBAD33 and pQE60.

SUNDAY, 30/07/2017

2 colonies of pQE-60 were picked and innoculated O/N Zoe Ford Sumaera Rathore

MONDAY, 31/07/2017

Jei Diwakar miniprepped pQE-60 a and pQE-60 b.
Digested with NcoI and BamHI - Buffer 3.1
The vector was dephosphorylated with alkaline phosphatase
Gel was run
Sumaera Rathore extracted the gel.
Zoe Ford did a quick change on C300 3 - Quick-change PCR
Gel for quick change run- (INSERT PIC HERE)
Smear found-need to repeat

TUESDAY, 01/08/2017

Zoe Ford did a quick change on C300 3 - Quick-change PCR . Stored O/N at 4 degrees

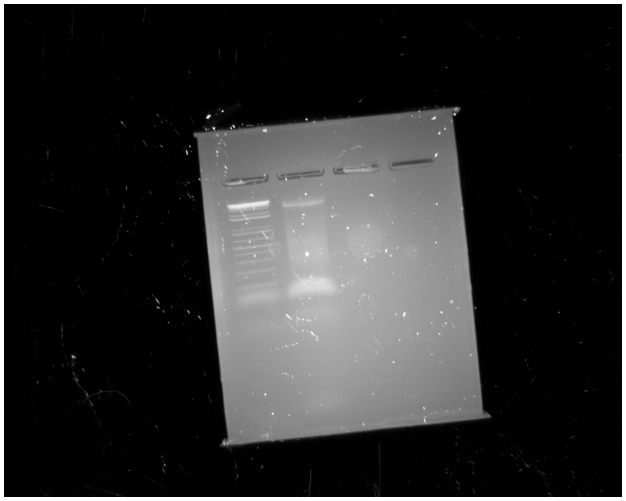
WEDNESDAY, 02/08/2017

Sumaera Rathore inncoulated pBAD33
Transformed and plated Alissa Hummer Sumaera Rathore
Stored overnight.

THURSDAY, 03/08/2017

No colonies found on C300 Quickchange plate - need to PCR again.

02.08 C300 QC.jpg



^correction - four colonies found on conc plate. Leaving to grow up to see if they can be picked. UPDATE they can!

Alissa Hummer and Chun Ngai Au set up a PCR for C100, C200, C250, V100, V150, V200

Sumaera Rathore and Alissa Hummer innoculated the pBAD plate and the C300 plate

FRIDAY, 04/08/2017

Zoe Ford put V300 and V400 in for QC - using qP1F/R.

Jei miniprepped C300 and pBAD

Sumaera Rathore ran C100, C200, C250, V100, V150, V200 on a gel

Jei Diwakar digested pBAD and test digest of C300

QC PCR of V300 and V400 didn't work.

C300 QC nanodrop values :

a - 47.4 (thrown away)

b - 88.3 (sent for sequencing)

c - 73.9

d - 72.9

pBAD nanodrop values:

a - 85.8

b - 72.1

Zoe Ford cut out bands from Sumaera's gel INSERT PIC HERE - NOT V200 though as there was no band. The 5 other parts were gel extracted and left in the freezer as N06 digest (vector). <- but they haven't been digested yet!! ~beware of Zoe's bad labelling~

Reran PCR of V200, left in freezer.

Sumaera Rathore

pBAD - dephosphorylated, gel ran, gel cut out, gel frozen

C300 was sent for sequencing.

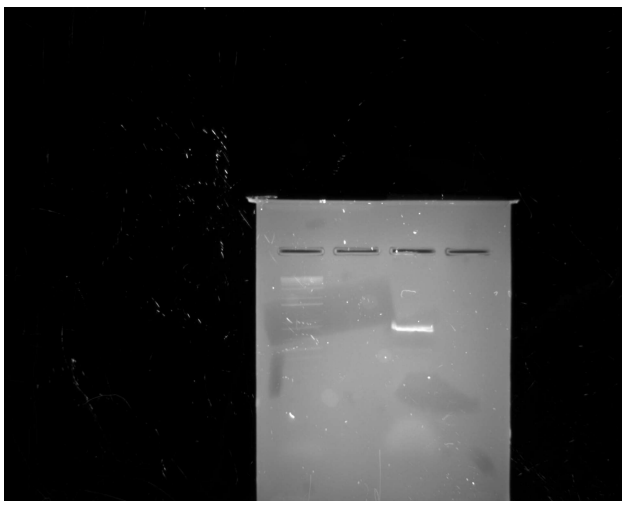
C300 QC was successful!

MONDAY, 07/08/2017

Zoe Ford did another QC PCR on V300 and V400 using qP1F/R, with an annealing temp of 60 and an extension time of 180secs. Ran on gel - couldn't see anything, not even a ladder, going to transform and plate anyway. Digested with DpnI.

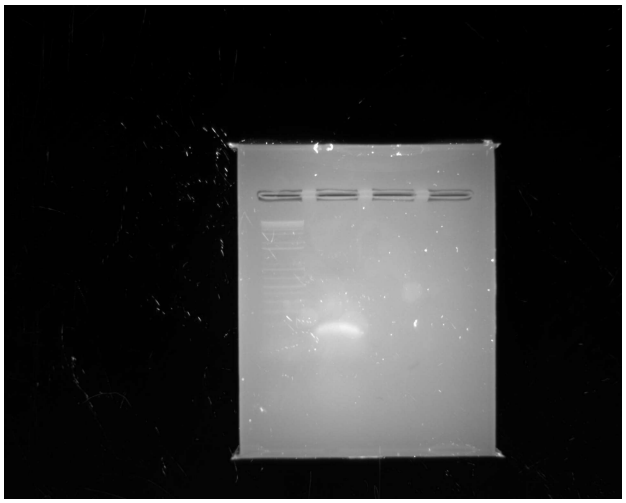
Alissa Hummer PCRed out the part from C307 for going into the expression vector. Ran on gel, cut out band, digested with 1ul of XbaI. Product left in freezer. Will go in pQE-60/C200 and pQE-60/C250 later.

07.08 C300QC-Ex PCR.jpg

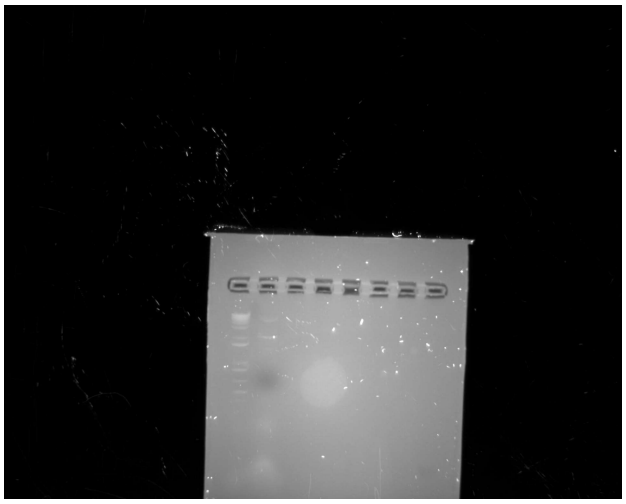


Zoe Catchpole ran PCR product of V200. No band, repeated PCR with a lower annealing temperature (62). Ran gel, cut out band (though there was a mystery larger band) INSERT PIC HERE, gel extracted + frozen.

07.08 V200 PCR.jpg



07.08 V200 PCR-1.jpg

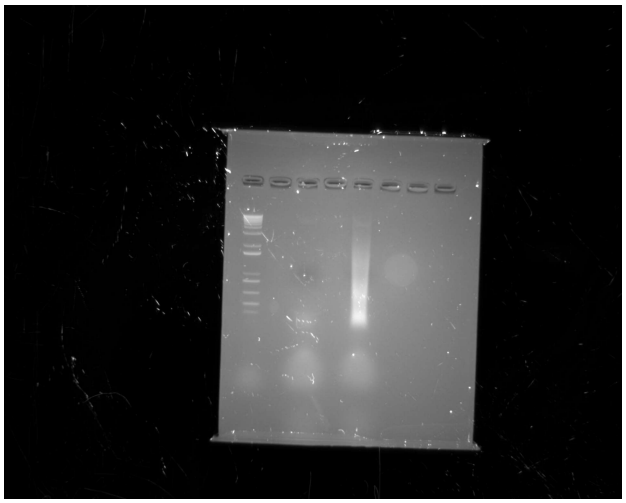


Zoe Ford digested and ligated C100 with pBAD33, and C200, C250, V100, and V150 with pQE-60. Left in PCR machine overnight.

TUESDAY, 08/08/2017

Alissa Hummer repeated the QC PCR for V300 and V400 with annealing temp. 62º (instead of 60), then ran (accidentally all of) the PCR product on a gel ... didn't work

08.08 V300,V400QC.jpg

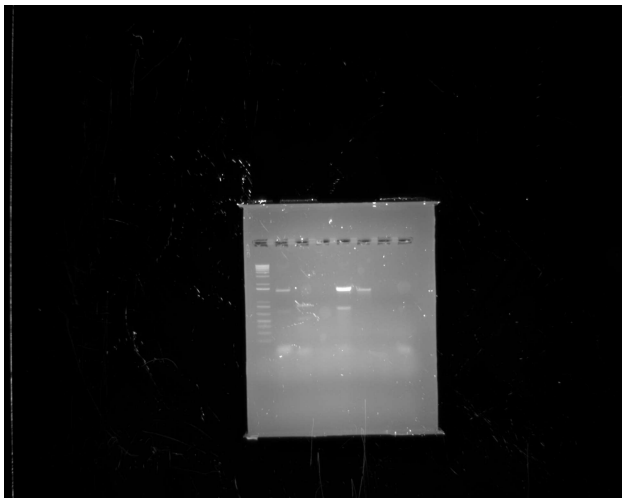


Zoe Catchpole set up a digest of pQE60 with Nco1 and BamH1, heat inactivated, and incubated with alkaline phosphatase.
Alissa Hummer ran this on a gel, Zoe C did gel extraction.
Zoe Catchpole made chloramphenicol and ampicillin plates.
Zoe Ford set up a QC PCR from new 1/15 dilututions for V300 and V400 under FOUR different conditions: annealing temp 62, annealing temp 67, annealing temp 72, and annealing temp 67 using Phusion instead of Q5 (@4 - Phusion PCR)
Zoe Catchpole transformed the C100, C200, C250, V100, V150 parts (finished by Zoe F)
Zoe Ford plated the transformations.

WEDNESDAY, 09/08/2017

No colonies </3
Zoe Catchpole set up another PCR for C100, C200, C250, C300, V100, V150, V200 - annealing temp 67 degrees, 60 sec extension time (as before)

09.08 PCR C1,C2,C25,C3,V1,V15,V2.jpg



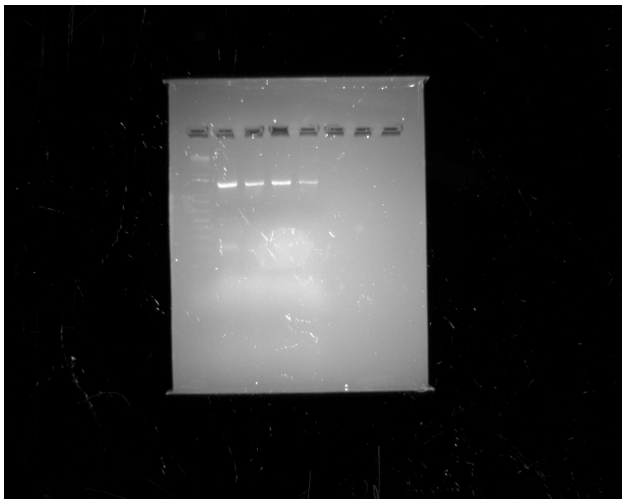
Alissa Hummer digested pQE-60 with Xbal
Gels - Zoe F's QC PCRs didn't work
Zoe C's PCRs got mixed up, rerunning
Alissa's digest worked, Zoe C gel extracted

Zoe F reran the earlier PCR of 7 tubes & ran on a gel - got C100 and V200 (gel in freezer as 09)
Zoe F ligated p60-QE and the digested C300 from Monday

THURSDAY, 10/08/2017

John Myers did a gel extraction on C100 and V200 from yesterday and did a digest on the extracts using XbaI and PstI
Angela Hellyer did a PCR of C200, C250, V100 and V150. CPr conditions 60°C annealing temp. and 30s exension time
Angela Hellyer ran a gel of these and got 4 bands.

10.08 PCR C200,C250,V100,V150.jpg



John Myers did a gel extraction of these bands.
John Myers digested all of these.
Zoe Catchpole made some CM plates.
Angela Hellyer did a transformation of ligated C300 from yesterday and interlab parts, and plated it to grow overnight.
Zoe Catchpole did a ligation of C200, C250, V100, V150, C100 and V200 to leave over night.

FRIDAY, 11/08/2017

Angela Hellyer checkde plates, had colonies for all interlab parts but none for C300 :(
Angela Hellyer did PCR of C300
John Myers gel extracted and digested C300
Zoe Catchpole ligated C300 (overnight)
Angela Hellyer transformed C200, C250, V100, V150, C100 and V200, then plated to grow overnight.
Zoe Catchpole made some amp plates.

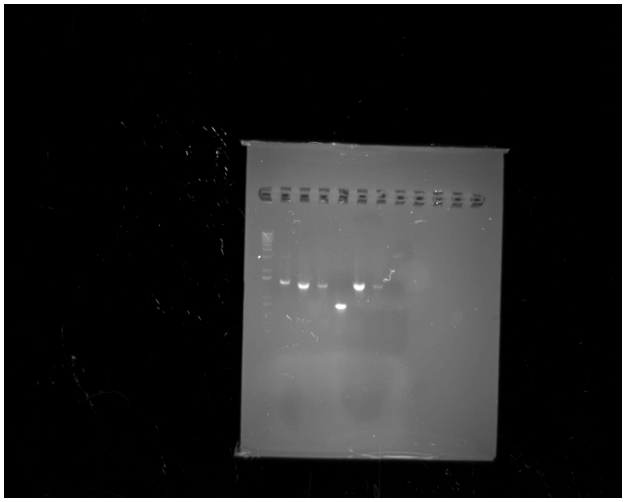
SATURDAY, 12/08/2017

Angela Hellyer took plates out of incubator, no colonies :(
Angela Hellyer put ligations in freezer

MONDAY, 14/08/2017

Zoe Catchpole and Angela Hellyer did a PCR of C100, C200, C250, C300, V100, V150 and V200, then ran on gel

14.08 PCR C1,C2,C25,C3,V1,V15,V2.jpg



John Myers cut out bands and gel extracted and digested

Zoe Catchpole did a PCR of V200 at 62^{o} annealing temp then ran on gel and gel extracted

Zoe Catchpole and John Myers ligated all but c100 and v150. There was not enough stock of BAD (X+P) and pQE60 (N+B).

Zoe Catchpole digested PQE60 with NC01 then ligated using our T4 ligase to troubleshoot.

TUESDAY, 15/08/2017

Zoe Catchpole digested V200

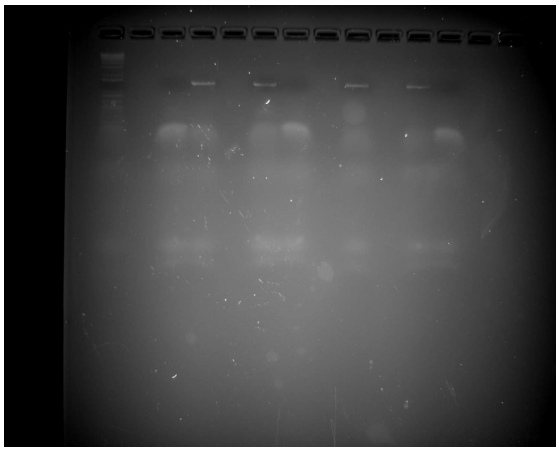
Zoe Catchpole digested pBAD X/P and PQE60 N/B, ran on gel and gel extracted

Zoe Catchpole ligated

WEDNESDAY, 16/08/2017

Zoe Catchpole, Noah Sprent and Alissa Hummer PCRed to Ligated C100-V200., but only C200, C250, V100 and V150 worked. These were left ligating o/n

16.08 PCR from SV.jpg



Gel Lanes - Ladder,Blank,C100,C200,
Blank,C250,C300,Blank,V100,Blank,V150,V200

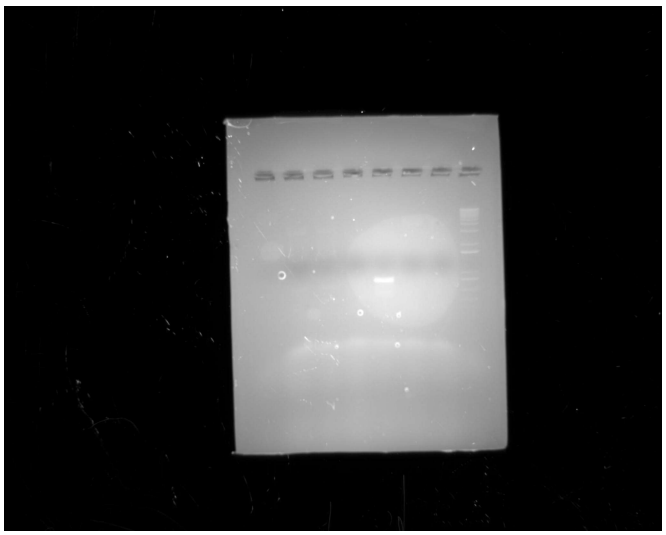
Angela Hellyer repeated the PCR of C100, C300 and V200 then put in freezer with loading dye (in antibiotics box)
Jei Diwakar and Noah Sprent Made a load of AMP plates
Jei Diwakar and Alissa Hummer Transformed C100, V150, and V200

THURSDAY, 17/08/2017

Alissa Hummer ran a gel of the PCR products from 16/08 (2x C100, C300QC, V200). There was only one band for C300QC (and one for C100):

In gel: lanes 1 & 8 = ladder; lanes 2 & 3 = C100, lanes 4 & 5 = C300QC, lanes 6 & 7 = V200

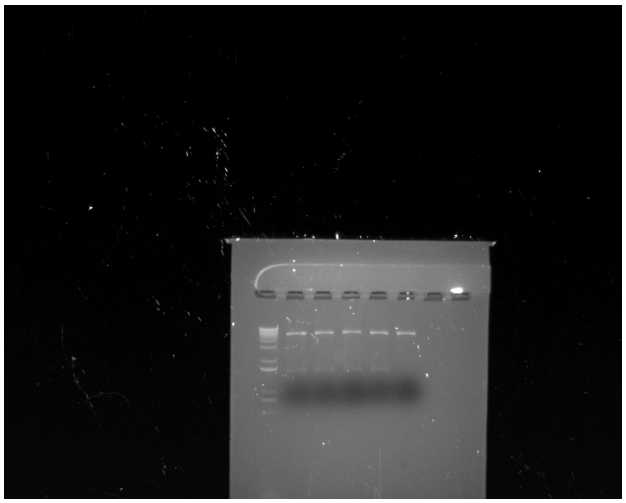
17.08 PCR C100, C300QC, V200.jpg



FRIDAY, 18/08/2017

Jei Diwakar miniprepped parts grown overnight. Only C100 colonies and 1 V200 colony grew. He test-digested and ran them on a gel. Alissa Hummer visualized the gel (see below) and sent C113d for sequencing.

18.08 Miniprep Test Digest C100a-d, V200.jpg



Alissa Hummer transformed and plated C113 and C313, PCR'ed 17/8

SATURDAY, 19/08/2017

Jei Diwakar took out the plates that were plated yesterday. Colonies on C113 but none on C313

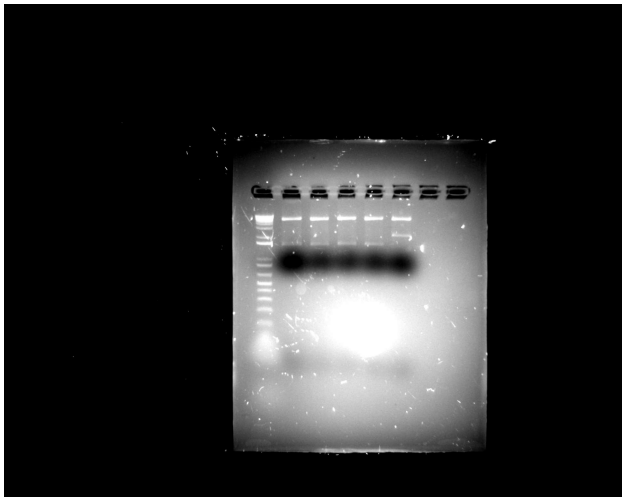
SUNDAY, 20/08/2017

Innoculation

MONDAY, 21/08/2017

Jei Diwakar miniprepped C100 and V200 (only ones that grew O/N). Not correct antibiotic used for all tests.
Test digested
Gel ran.
Will send for sequencing on Wednesday

image.png



TUESDAY, 22/08/2017

Zoe Ford took overlap extension PCR all the way through to ligation yesterday + today for V300 and V400, first PstI site.

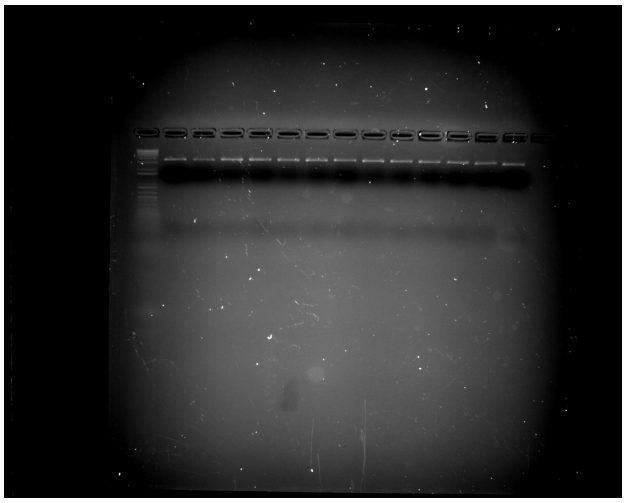
Angela Hellyer did transformations of C312 and C307 and plated.

Kushal Mansatta and John Myers miniprepped C200,C250 x4, V100 x4, V150 x4

Test digested

Gel ran-did not work. No parts found in vectors.

image.png



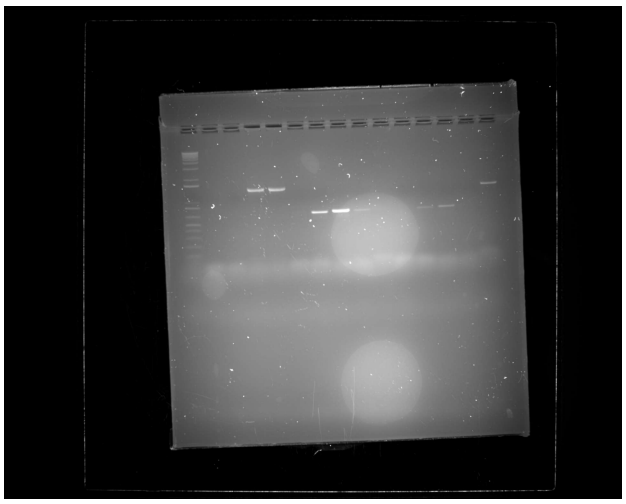
Noah Sprent and Kushal Mansatta ran another PCR of C200,C200WF,C250,C250WF,V100,V100WF,V150,V150WF,V200WF. It was done without DMSO and at 60oc, with 65oc as well for the WF parts

WEDNESDAY, 23/08/2017

Noah Sprent and Jei Diwakar loaded a gel with the PCR from yesterday

Ladder C200 C250 V108 V158 C200WF60 C258WF60 V108WF60 V150WF60 V200WF60 C20065WF C25065WF V108WF65 V158WF65 V200WF65

image.png

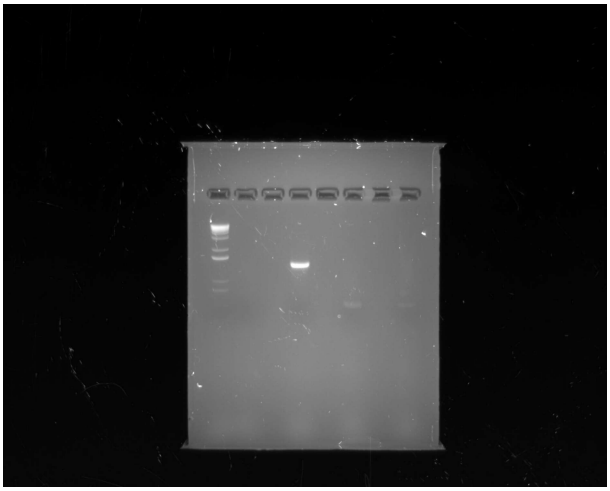


Jei Diwakar and Noah Sprent PCRed C200-V150-V150WF60-V150WF65

The gel was run.

V150 and V150WF worked. C200 didnt work

23.08 - V150 and V150 WF.jpg

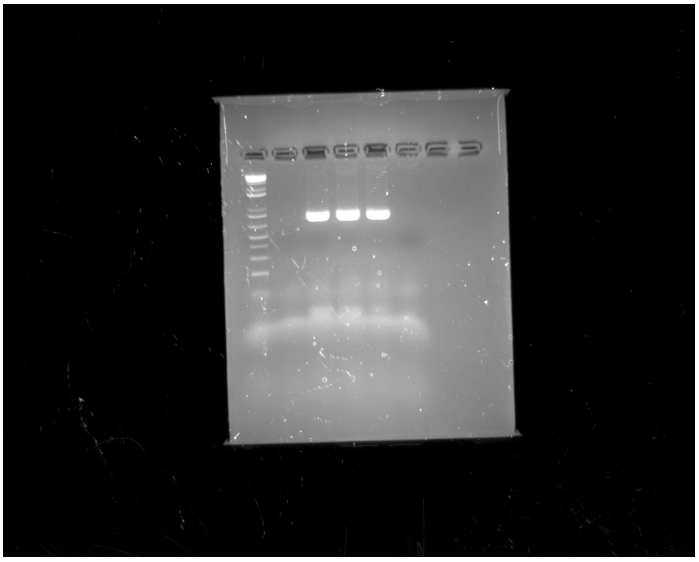


The new primers were resuspended and diluted to be sent off for sequencing

Zoe Catchpole and John Myers did a PCR for:

V100 C100WF62 C100WF65 C100WF67 V200WF
Gel ran
Worked for C100WF only

23.08 - Redo of some parts.jpg



Jei Diwakar and Noah Sprent did a PCR for:
C208-60 C208-62 C208-65
V108-60 V108-62 V108-65
V208WF60 V208WF62 V208WF65

Zoe Catchpole and John Myers Gel extracted:
V160 V160WF C110WF

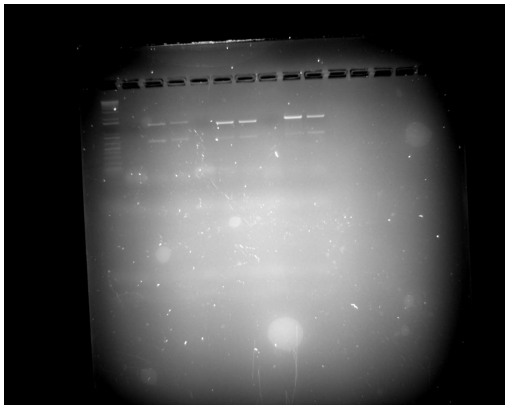
Zoe Catchpole and John Myers transformed and plated:
C212WF
C262
C262WF
V112
V12WF
V162WF
V300 OEP
V400 OEP

Angela Hellyer and Arthur Norman did the Interlab

THURSDAY, 24/08/2017

Zoe Catchpole digested and cleaned up pBAD X/P and pQE60 N/B
Noah Sprent and Jei Diwakar Ran last night's PCR on a gel and cut out the top bands

24.08 C208,V108,V208WF.jpg



Interestingly, the PCRs which worked (at 62 and 65oc) also had 50ul too much liquid in. It is very unclear what was added that was far too much to make it this much, but it worked!
They were then cleaned them up and digested, along with the bits Zoe and John cleaned up last night and test digests of the colonies innoculated last night

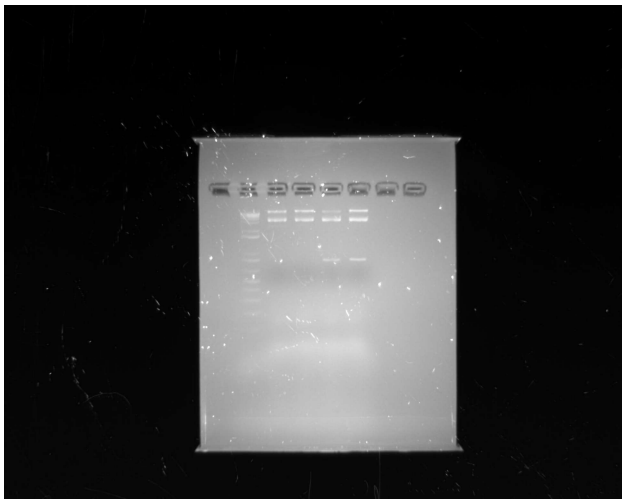
- C208, V108, V160, V160WF, V208WF, C110WF

John Myers cleaned up the digestions and ligated them into the appropriate parts

Sumaera Rathore , Kushal Mansatta and Angela Hellyer did a colony PCR of stuff that was transformed yesterday (V100, V100WF, V150WF, V300oeP, V200WF)

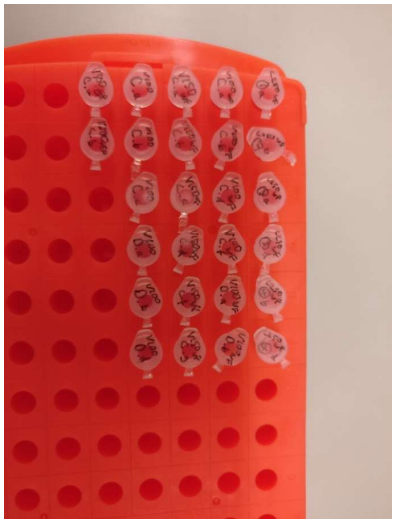
John Myers and Noah Sprent visualised the gels for the test digest, and found two of the 4 colonies had the part in.
Zoe Ford then sent these for sequencing. (C300?)

24_08_17 C300a-d.jpg



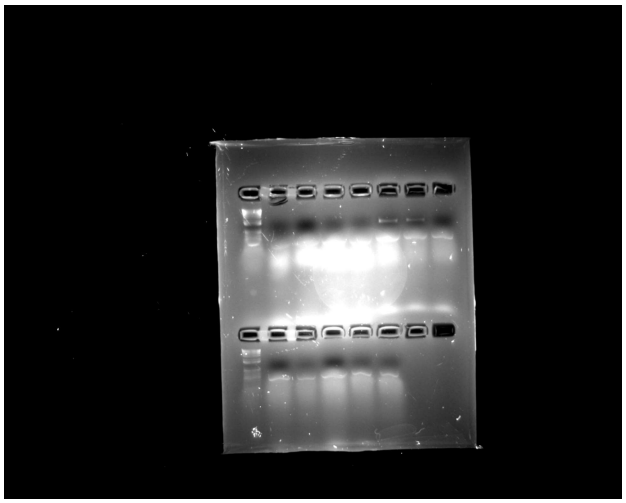
Noah Sprent and John Myers Ran the cPCRs on a gel, and found that only two colonies had the part in (V100c.b and V100c.c). These two were then inoculated by Sumaera Rathore and Kushal Mansatta .

image.png

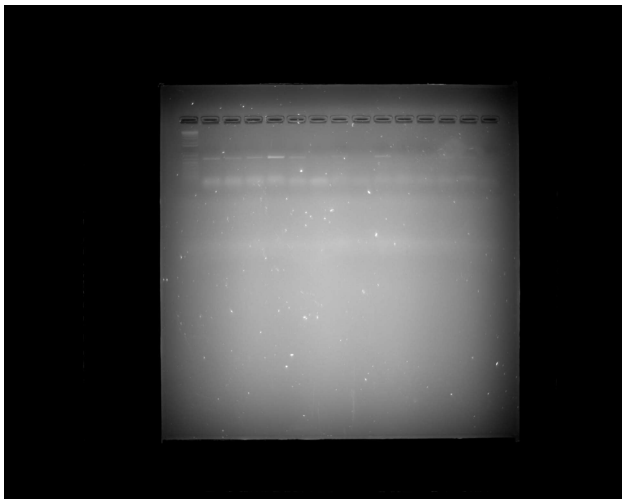


-Order

24_08_17 V150wf V300OEP V100.jpg



24_08_17 C250wf V100wf V150wf.jpg



Kushal Mansatta and Sumaera Rathore Transformed the parts which were ligated, C208, V108, V160, V160WF, V208WF, C110WF

Alissa Hummer , Kushal Mansatta and Sumaera Rathore Ran another PCR

FRIDAY, 25/08/2017

Noah Sprent and Kushal Mansatta miniprepped the V100cb and V100cc parts. V113cb and V113cc were sent for sequencing

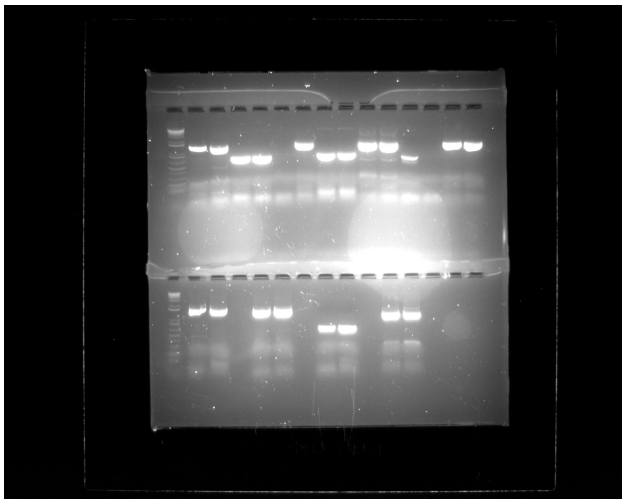
Zoe Catchpole and Angela Hellyer did a colony PCR of plates from yesterday

The PCR's from last night were ran on a gel:

Table1

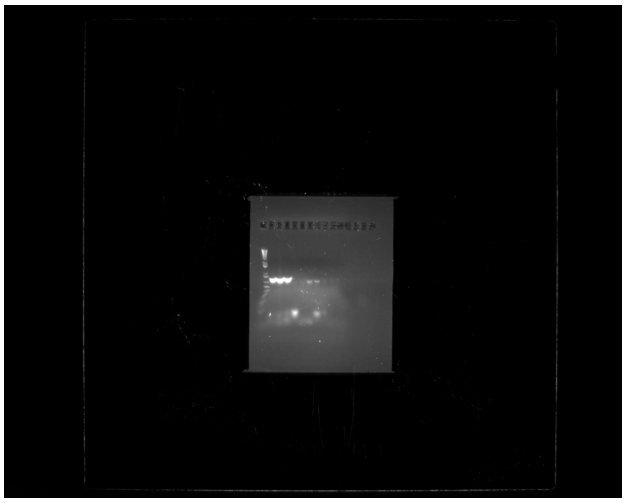
	A	B	C	D	E	F	G
1	Ladder	c200(60)	c200(62)	c200noF(60)	c200noF(62)	c250(60) no band	c250(62)
2	Ladder	v300 OEP (60)	v300 OEP (62)		v400 OEP (60)	V400 OEP (62)	

image.png



Colony PCR: C112 WF a, C112 WF b, C112 WF c, V112 a, V162 a, V162 b, V162 WF a, V212 WF a, V212 WF b, V212 WF c
(1,2 and 7th bands were cut out and extracted to send for sequencing, but we weren't in time :-( )

25.08 Colony PCR.jpg



Pick & inoculate C112 WF a, C112 WF b, C112 WF c, V162 a, V162 b

SATURDAY, 26/08/2017

Zoe Ford miniprepped C112 WF a, C112 WF b, C112 WF c, V162 a, V162 b

SUNDAY, 27/08/2017

Noah Sprent and Angela Hellyer transformed and plated:
C112noF C212 C212 noF C262 V112 V112noF V162 V162noF V212noQ V300EP V400 OEP

TUESDAY, 29/08/2017

Jei Diwakar and Angela Hellyer took out the plates and the only ones with colonies were:
C112noF V212noQ V300EP V400EP- all grew on CM. No AMP colonies found!
The other plates were thrown away.

Noah Sprent Finally sent C112wfb, C112wfd, and V162d colony PCRs for sequencing, as well as C112WFa and V162a from the miniprep. We think there was a mixup with plates, and possible that V162a has nothing in, because should have been b that was sent.

Sumaera Rathore and Arthur Norman set up transformations for C106, V306 and V406 as stocks of concentrated minipreped DNA were running low. Also included PQE60a and pBADa vectors to replenish stocks as there were enough competent cells. Sequencing for these will not be necessary as no ligation steps have been used since they were last sequenced.
Also set up transformations for V213a, C313c and C313d which had previously been transformed but the sequencing DNA may have been contaminated or multiple colonies may have been taken. The colonies for these should be picked, inoculated, minipreped and sent off for sequencing again

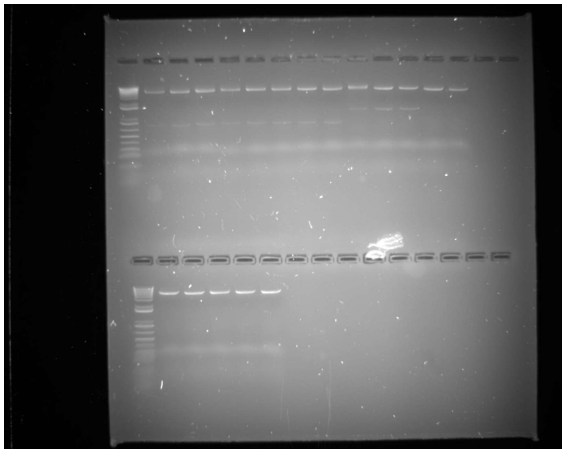
Jei Diwakar and Angela Hellyer did a PCR of C200, C250, C300, V100, V150 and V200. DId 3 of each at 62C. Gel to be run on wednesday morning.

Arthur Norman and Sumaera Rathore inoculated plates from sunday (C112noF, V212noQ, V300oep, V400eop)

WEDNESDAY, 30/08/2017

Noah Sprent and Jei Diwakar Miniprepped and test-digested the colonies inoculated from last night C112noF, V212noQ, V300oep, V400eop. All were done with X+P, therefore no bands were seen for V300 and V400 as they're still in the shipping vector.

30.08 Test Digest.jpg



C113noF dil a-d, C100noF conc a-d, V212noQ a-c,
V300 OEP a-d, V400 OEP a-c.

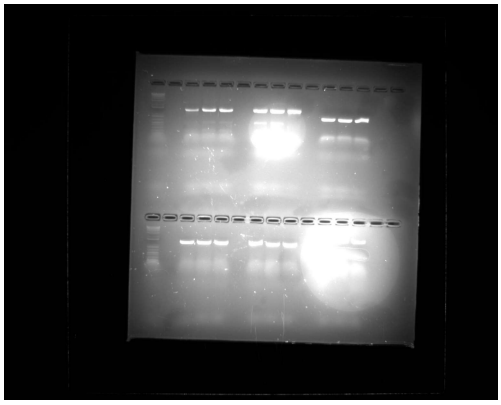
Based on this, C113noFa, V212noQa, V300OEPa and V400OEPa were sent for sequencing
Nanodropped concs:

Table2

	A	B	C	D
1	C113noFa	V212noQa	V300OEPa	V400OEPa
2	31.06	59.40	43.40	41.48

Jei Diwakar
Jei ran last night's PCR on a gel:

30.08 PCR Gel.jpg



C208 C208 C208 C258 C258 C258 C308
C308 C308 V108 V108 V108 V158 V158 V158
V208 V208 V208

Zoe Catchpole and Arthur Norman Then extracted from the gel and cleaned it up, before starting the digestions

John Myers and Angela Hellyer cleaned up the digestions and then put them to ligate overnight.
- C212a, C262a, C312a, V112a, V162a, V212a, and all had the normal amount of everything in (ie. 30ul DNA, 5ul vector)
- C212b, C262b, V112b and V162b had half the amount of everything in so overall volume is half and also were a different ratio so 10ul dna and 2.5ul vector
- C312b and V212b had 20ul dna and 5ul vector and overall volume is normal
C312c and V212c had 10ul dna and 5ul vector and overall volume is normal

Sumaera Rathore and Kushal Mansatta Retransormed the colonies from yesterday that had grown too much to be picked.

C206, C256, V150 dilute, V306, V406, C113b, V213noQa

However, they picked the V212noQa which Jei and Noah had sent for sequencing, rather than the one that had been sent previously.

Noah Sprent Inoculated the three plates that hadn't lawned out last night - C313c, C313d, and PQE60. These were left to grow O/N

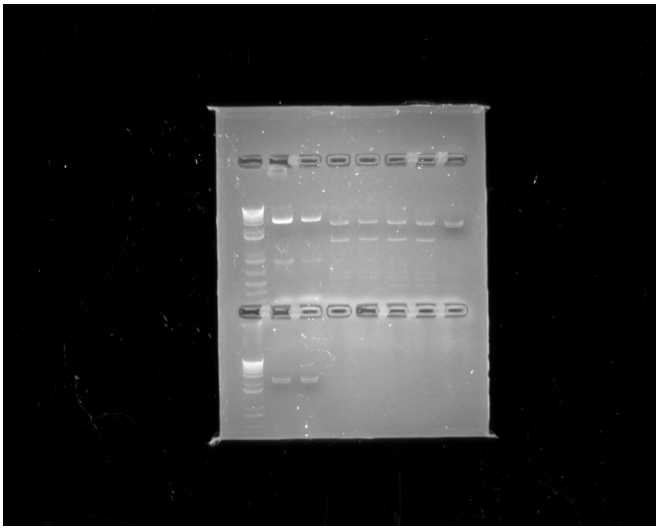
THURSDAY, 31/08/2017

Noah Sprent Took the plates out of the incubator, all had nice, isolated colonies apart from V150
John Myers Inoculated one each of the plates that grew, C206, C256, V306, V406, C113b, V213noQa including C113 that we actually didn't need

John Myers Miniprepped the inoculated colonies from last night, and put pQE-60 in the freezer.

John Myers and Noah Sprent Then test digested C313c and C313d, along with V300 OEP and V400 OEP again with the right enzymes this time

31.08 Test Digest C313+V300OEP+V400OEP.jpg



C313c, C313d, V300OEPa-d, V400a-c

Noah Sprent and John Myers Then proceeded to send lots of weird things for sequencing to work out what's going on If they have a **.5** after their name, then they come from a miniprep that was cleaned up using a purification from enzymatic reactions protocol.

- From the bits miniprepped yesterday
  - C113noFdilb and C113.5noFdilc
  - V213noQb and V313.5noQc (Not a typo, we think tube was mislabelled)
  - V313OEPb and V313.5OEPc
  - V413EOPb and V413OEPc
- From the bits miniprepped today (Which was retransformed from a different lot)
  - C313c
- From the bits miniprepped on Saturday
  - C113.5noFb
  - V163.5noFb
- From the bit transformed last week which we didn't retransform even though we retransformed C313c and d
  - V213.5a
- As a test of our old primers
  - C306

NOTE: V300 and V400 looked like the other way around in the test digest, presumably they got mixed up at some point!
Also, one pBAD part we tested both combinations of R and F for ours and SBS's primers, to see if the primers we've sent them aren't right.

Angela Hellyer and Zoe Catchpole Tranformed all the ligations from yesterday. If they were on AMP then they dropped the concentrations of the antibiotic by a half. They also transformed pBAD-33 and pQE-60 onto these plates as a control.

FRIDAY, 01/09/2017

John Myers Miniprepped the bits that grew overnight, C113b (Just more stock of C100 in vector) @ 143ng/ul, V213noQ @95ng/ul, V306 @174ng/uland V406 @130ng/ul. C206 and C256 didn't grow o/n because they were grown with ampicillin.
V306 and V406 replaced our stock and C113b was added to our stock - All were labelled their names and the date
V213noQ was test-digested

Jei Diwakar Took out the plates from the incubator. The pQE-60 and pBAD-33 controls worked on the plates, but nothing else grew apart from the V213a which had come from a previous miniprep. Seems that the ligations didn't work.

Noah Sprent Retransformed some minipreps that sequencing had previously not worked for:

- From the miniprep on Wednesday
  - C112noF
  - V213noQa
  - V313OEPb
  - V413OEPa
- From last saturday's miniprep - V163noFa
- V213.5a - From a miniprep last week

- These were labelled as N015/N065

Zoe Catchpole Ran a PCR for V100, V150, V200 and V300 (OEP) twice each, but V150 and V300 didn't work

- She then ran those two again, but V300 still didn't work
- The original PCR parts (V100 and V200) were taken through to ligation by John Myers
  - For the digestions the wrong enzymes may have been used, or the wrong enzymes and the DNA, so John switched the labels in the hope that it was the latter
  - After cleanup the concentrations of DNA were: V111 @ 8.55ng/ul and V211 @ 4.02ng/ul, this may actually be below the needed ratio for ligations to be done
- The second PCR was cleaned up and needs digesting before ligation

SUNDAY, 03/09/2017

Kushal Mansatta and Angela Hellyer innoculated a lot of cells

Innoculate parts - Up to 4 colonies of N13/N15 parts and 2 of N06 parts
- C206 and C256 that John inoculated on Thursday but didn't grow
- V213a from plates taken out on Friday
- All the plates from saturday
- competant cells

MONDAY, 04/09/2017

Jei Diwakar , John Myers and Angela Hellyer

- sorted out the numbering system to as follows:
  - expression vector:
    - N13/N63 = expression vector miniprep
    - N14/N64 = retransform from N13
    - N15/N65 = miniprep of N14
  - shipping vector:
    - N06/N56 = shipping vector miniprep
    - N16/N66 = retransfrom from N06
    - N17/N67 = miniprep of N16
- worked out that we needed to reinnoculate the following parts:
  - V213noQ, V216, C114noF, C216, C266
- had to throw away some tubes because there was some confusion with the labelling but have reinnoculated them again
  - ones thrown away were: V214, V215 and C115
- PCRed V100 and V150

- tomorrow need to send V215.5 and V165noFa for sequencing

Sumaera Rathore carried out positive and negative control to check if new batch of cells were competent - they are!

Sumaera Rathore transformed and plated V212 and V112

Sumaera Rathore innoculated C216 and C215 in shipping vector, John Myers and Angela Hellyer innoculated V213noQ, V216, C114noF

Zoe Ford ran first attempt at QC of C200 and C250. No bands. 3 - Quick-change PCR

Zoe Ford ran second attempt at QC at 62 degrees, qcpcr on right hand machine and ran PCR overnight.

John Myers and Arthur Norman miniprepped V167 noF, V213.5 and V300 OEP

TUESDAY, 05/09/2017

Zoe Ford ran 1/5 of PCR products on gel - both bands!!
Digested C200 and C250 QC with DpnI for 1 hour.

Angela Hellyer ran a gell of V110 and V160 from last nights PCR
Then gel extracted these parts.
Then digested them.
Then ligated them.

John Myers miniprepped the parts inoculated the previous day

C217 dil b 128.98 ng/µl
C217 dil a 141.99 ng/µl
C267 dil a 158.10 ng/µl
C267 dil b 220.35 ng/µl
V417 OEP a b 65.59 ng/µl
V417 OEP a a 86.91 ng/µl
C115 noF dil a f 63.21 ng/µl
C115 noF dil a e 80.57 ng/µl
V215 noQ a dil f 68.89 ng/µl
V217 conc e 61.72 ng/µl
V215 noQ a dil g 62.68 ng/µl
V217 conc f 54.39 ng/µl

Sent for sequencing today: V163, V213, V213 noQ, V213, V300oep, V400oep

Zoe Ford set up PCR for C200 and C250, both with and without fluorophore. PCR overnight.

Zoe Catchpole started the transformation of .V100, V200, C200(QC) and C250 (QC) Kushal Mansatta continued and plated with Angela Hellyer .

Arthur Norman innoculated V213noQ for repeat fluorescene experiments.

WEDNESDAY, 06/09/2017

Zoe Catchpole C200 and C250 w/ and w/o fluorophore on gel and gel extracted then Jei Diwakar set up the digestion.

Angela Hellyer ran a PCR of C200 and C250 (3 of each) and ran them on a gel.

John Myers miniprepped V213B noQ, concentration from nanodrop: 84.3 ng/µl

Zoe Catchpole cleaned up c200 and c250 and set up the digestion

Zoe Catchpole digested c300 pQE60 N/B and ran a gel f the vector digest

Jei Diwakar checked sequences- V213 no Q worked. V213 was C100 no F. V163 and V300 OEP and V400 OEP didnt work

Jei Diwakar and Zoe Ford sent V165 noF a dil a for sequencing again with primers of 3x conc (30ul water and 3ul primer stock). A test pQE-60 plasmid was also sent

Zoe Ford PCRed V200 from the shipping vector. Left in small freezer box overnight, labelled V210.

Zoe Ford ligated C200 and C250 parts:

C210b
C260b
C210a + C300 = C512
C260a + C300 =C562
C210noF + C300= C512 no F
C260noF + C300 = C562noF
C212+C211c+C261+C261c were frozen

Zoe Ford plated V112 and V162, left in 37 incubator overnight.

THURSDAY, 07/09/2017

John Myers and Zoe Catchpole miniprepped:

Part	Concentration
V113 vdil b	78.00 ng/µl
C263 conc a	97.49 ng/µl
V213 conc a	164.28 ng/µl
C213 QC conc b	74.18 ng/µl
V113 dil a	71.07 ng/µl
C263 QC dil b	83.61 ng/µl
V213 dil a	15.76 ng/µl
V113 conc a	45.44 ng/µl
C213 QC conc a	104.31 ng/µl
V113 dil c	151.87 ng/µl
V113 dil d	123.22 ng/µl
C263 conc b	158.47 ng/µl
V113 dil a	92.66 ng/µl
V113 dil b	100.51 ng/µl
C213 QC dil b	129.36 ng/µl
C213 QC dil a	147.50 ng/µl

Zoe Ford test digested the minipreps from the morning (some labelling changes may have occurred?)

Gel picture: small gel with two rows of eight wells
Ladder | C213QCa | C213QCb (dil b) | C213QCc (dil a) | C213QCdild | C263QCa (dil b) | C263QCb (conc b)
Ladder | V113a | V113b | V113c | V113 dil c | V113 dil d | C263 conc a | C263 conc b

Gel picture: small gel with four wells
Ladder | V213a (conc a) | V213b | V213c

C213QCb, C213QCc, C263QCa, C263QCb, V213a were the only ones that worked, sent for sequencing.
Suspect all pQE-60 parts had just empty vector

John Myers and HELEN SIYU REN picked colonies from V112 dil & conc, V162 dil & conc, V315 OEPa and V415 OEPa

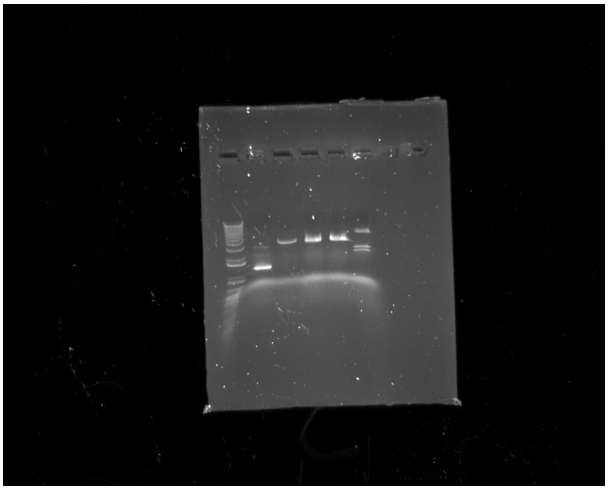
FRIDAY, 08/09/2017

John Myers and Zoe Catchpole miniprepped:

V163 dil b	41.56 ng/µl
V113 conc b	42.90 ng/µl
V113 conc a	140.69 ng/µl
V163 dil c	39.47 ng/µl
V163 conc c	44.05 ng/µl
V163 conc b	63.15 ng/µl
V113 dil b	28.18 ng/µl
V113 dil d	27.40 ng/µl
V163 dil a	82.38 ng/µl
V163 conc d	41.69 ng/µl
V113 conc c	64.51 ng/µl
V315 OEPa dil g	53.36 ng/µl
V415 OEP a conc g	78.66 ng/µl
V315 OEP a dil b	30.14 ng/µl
V415 OEP a conc h	29.25 ng/µl
V113 dil c	98.46 ng/µl
V415 OEP a conc e	45.15 ng/µl
V315 OEP a dil e	29.20 ng/µl
V113 conc d	91.11 ng/µl
V415 OEP a conc f	41.12 ng/µl
V315 OEP a dil f	44.62 ng/µl
V153 dil d	43.56 ng/µl
V113 dild d	43.80 ng/µl

John Myers test digested V113 conc a, V113 dil c, V113 dil d, V163 dil a and V415 OEP a conc g

8_9 V100 V150 V400OEP test digest.jpg



V113 conc a was sent for sequencing

MONDAY, 11/09/2017

John Myers digested C200 QC, C250 QC, V100, V100noF, V150, V150noF, V200 and cleaned them up before ligating them

C261	14.85 ng/µl (Ligated only into PQE60)
V211	11.68 ng/µl
V161	17.90 ng/µl
V111	18.63 ng/µl
V111 No F	15.96 ng/µl
C211	15.77 ng/µl (Ligated only into PQE60)
V161 NoF	16.06 ng/µl

Noah Sprent and Kushal Mansatta miniprepped:

C263 conc a	22.62 ng/µl
C263 conc b	36.92 ng/µl
C263 conc c	65.79 ng/µl
C263 dil a	45.43 ng/µl
C263 dil b	47.84 ng/µl
C263 dil c	42.77 ng/µl
C513 conc a	105.76 ng/µl
C513 conc b	45.42 ng/µl
C513 conc c	96.06 ng/µl
C513 dil a	89.85 ng/µl
C513 dil b	85.46 ng/µl
C213 dil a	78.41 ng/µl
C213 conc a	78.60 ng/µl
C213 conc b	43.96 ng/µl
C213 conc c	41.33 ng/µl
C562 conc a	92.89 ng/µl
C562 conc b	99.63 ng/µl
C562 conc c	75.73 ng/µl
C562 dil a	52.16 ng/µl
C562 dil b	75.75 ng/µl
C562 dil c	87.28 ng/µl
C512 noF conc a	109.84 ng/µl
C512 noF conc b	93.66 ng/µl
C512 noF conc c	82.99 ng/µl
C512 noF dil a	75.46 ng/µl
C512 noF dil b	96.42 ng/µl
C512 noF dil b	96.42 ng/µl
C512 noF dil c	110.85 ng/µl
C562 noF conc a	87.01 ng/µl
C562 noF conc b	82.47 ng/µl
C562 noF conc c	85.86 ng/µl
C562 noF dil a	88.16 ng/µl
C562 noF dil b	135.10 ng/µl

John Myers and Zoe Catchpole digested:

Order left to right:

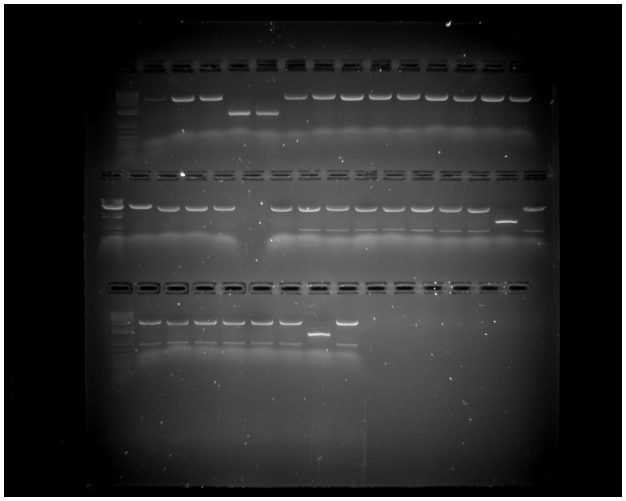
1st row:

C513 dil a N/B C513 dil b N/B C513 conc c N/B C513 conc a N/B C562 noF dil a N/B C562 noF dil b N/B C562 noF conc a N/B C562 noF conc b N/B C562 noF conc a N/B C512 noF dil a N/B C512 noF dil b N/B C562 noF dil c N/B C512 noF dil b N/B C512 noF dil c N/B C562 conc a N/B

2nd row:
C562 conc b N/B C562 dil c N/B C213 conc a N/B C213 dil a N/B C263 dil b N/B C263 conc c N/B C513 dil a X C513 dil b X C513 conc c X C563 conc a X C513 conc a X C513 noF conc a X C563 noF conc c X C563 noF dil b X C513 noF conc c X

3rd row:
C513 noF dil b X C513 noF dil c X C563 conc b X C563 dil c X C563 noF conc b X C563 noF conc a X C513 conc a X C563 noF dil a X

Test Digest 11.09.jpg



No parts were sent for sequencing :'(

John Myers and Zoe Catchpole ran a PCR of C200 and C250 from the shipping vector to form C208, C208 noF, C258 and C258 noF

Noah Sprent Did the first day of the TEV-mCherry from C100 protocol

TUESDAY, 12/09/2017

Zoe Catchpole ran a gel of c208, c208noF, c258 and c258noF.
c200noF and c250 didn't give bands so Zoe Catchpole reran the PCR
John Myers gel extracted C208 and C208 NoF and digested them (The milIQ for the nanodrop is contaminated)

C211	8.57 ng/µl
C261 NoF	10.81 ng/µl

Sumaera Rathore made 8 more Amp plates and Zoe Catchpole made 8 more CM plates
Zoe Catchpole ran a gel of c200 noF, c250 , pQE60 N/B and pqE60300N/B
Zoe Catchpole ligated c212 and c262noF (They are supposed to be labelled C512 and C562 noF)
John Myers ligated C211 NoF and C261

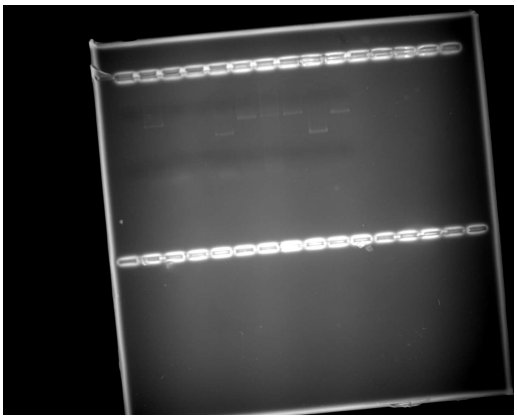
12/09 - Kushal Mansatta Ran a PCR with C207,C257,C207noF,C257noF,C507,C557,V106,V106noF,V206

Noah Sprent Did the second day of the TEV-mCherry from C100 protocol - Inducing with 0.01% arabinose for 3 hours at 30°C

WEDNESDAY, 13/09/2017

12/09 - Noah Sprent Ran the PCR on a gel, visualised with EtBr (no sybr added) and extracted the bands for C208, C258 noF, C508, C558, V100, and V100noF. V208 was actually V200noQ so this wasn't extracted even though there was a band. The ladder cannot be seen probably because it was the last bit left in the tube.

13.09 - PCR.jpg

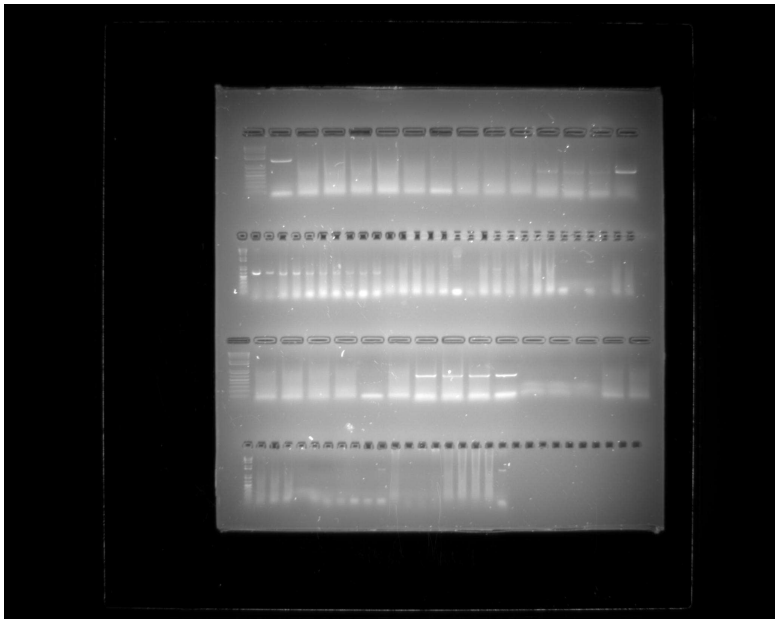


Ladder, C208, C258, C208noF, C258noF, C508, C558, V100, V100noF, V208noQ

12/09 - Arthur Norman Digested them with N and B, along with more pQE60/C300

John Myers and Zoe Catchpole Colony PCRed 77 of the colonies from this morning's plates

13_9 cPCR.jpg



They then inoculated the PCRs from the gel that looked good. (C562 no F conc B, C562 no F conc D, C562 no F conc H, C562 no F conc I, C512 conc F, C262 dil A, C562 no F conc K, C512 dil A, C262 conc E, C562 no F conc L, C512 conc D, V112 conc A, V162 conc C, V162 conc G, C562 no F dil A, C562 no F dil B, C562 no F dil C, C562 no F dil D, C562 no F dil E, C562 no F dil F, C562 no F dil G, C562 no F conc A, C563 no F conc J as well as V112 conc A, V162 conc C and V162 conc G)

13/09 Kushal Mansatta Ran today's PCR, with C207,C257,C207noF,C257noF,C507,C557,V156,V156noF,V206,V500

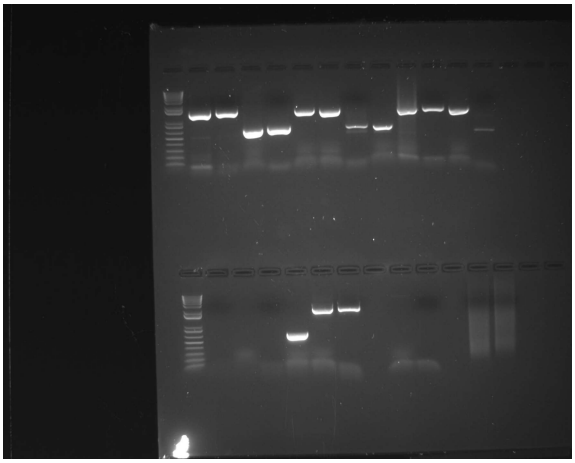
Sumaera Rathore Transformed

Arthur Norman Ran the QC PCR on V400 with a 1 in 10 dilution

THURSDAY, 14/09/2017

13/09 - Noah Sprent Ran yesterday's PCRs on a Gel:

14.09 - 13.09 PCR Gel.jpg



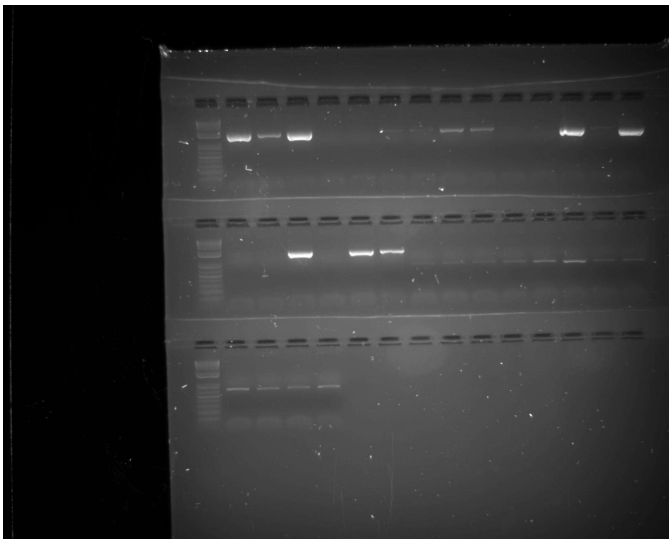
Ladder, C208, C208, C208noF, C208noF, C258, C258,
C258noF, C258noF, C508, C508, C558, C558
Ladder, V158, V158, V158noF, V158noF, V208, V208,
Empty, V500, V500, Empty, QCV4, QCV4

- From this, C208, C208noF, C258, C258noF, and V200 were all doubly-extracted
  - C508 only the second b and was cut, as the first looked suspicious with the streak, and was slightly lower
  - C558 only the first band was extracted
  - V158noF the second band was extracted
  - Some other bands could be seen faintly, including QC, but they didn't merit cutting out
- These were then cleaned up and digested with NcoI-HF and BamHI-HF, along with some PQE60 for ligations

John Myers Then ligated these into the appropriate vectors

Angela Hellyer did a colony pcr of C562 (20 colonies) and C512 noF (12 colonies).

14.09 - cPCR.jpg



order:
ladder,C562a,C562b,C562c,C562d,C562e,C562f,C562g,C562h,C562i,C562j,C562k,C562l,C562m,C562n,
ladder,C562o,C562p,C562q,C562r,C562s,C562t,C512noFa,C512noFb,C512noFc,C512noFd,C512noFe,C512noFf,C512noFg,C512noFh,
ladder,C512noFi,C512noFj,C512noFk,C512noFl

THEN INNOCULATEED (in 8ml LB with amp):
C562a,C562c,C562l,C562q,C562t,
C512noFf,,C512noFi,C512noFj,C512noFk,C512noFl

John Myers miniprepped all the inoculations from yesterday

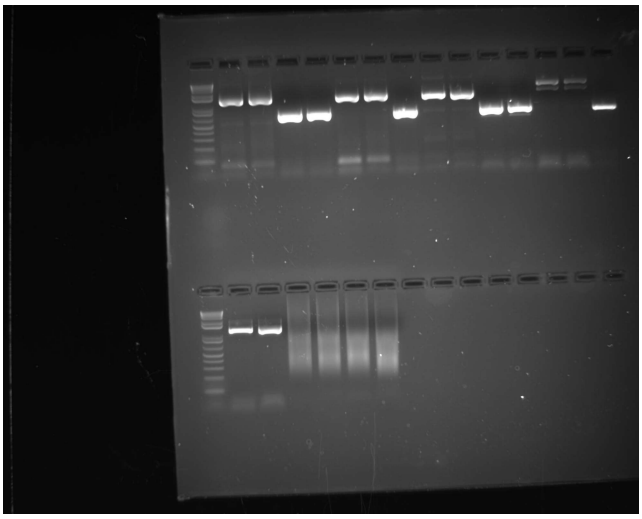
C563 no F conc B	159.06 ng/µl
C563 no F conc D	207.08 ng/µl
C563 no F conc H	214.03 ng/µl
C 563 no F conc I	208.52 ng/µl
C513 conc F	169.70 ng/µl
C262 dil A	161.28 ng/µl
C563 no F conc K	199.39 ng/µl
C513 dil A	172.17 ng/µl
C263 conc E	166.72 ng/µl
C563 no F conc L	149.68 ng/µl
C513 conc D	160.97 ng/µl
V113 conc A	158.60 ng/µl
V163 conc C	128.54 ng/µl
V163 conc G	99.51 ng/µl
C563 no F dil A	186.96 ng/µl
C563 no F dil B	168.01 ng/µl
C563 no F dil C	154.45 ng/µl
C563 no F dil D	156.54 ng/µl
C563 no F dil E	149.49 ng/µl
C563 no F dil F	161.60 ng/µl
C563 no F dil G	190.53 ng/µl
C563 no F conc A	185.52 ng/µl
C563 no F conc J	88.97 ng/µl

Sumaera Rathore transformed and plated C512; V112 noF; V112; V215 noQ; C212; C562, C262 noF

FRIDAY, 15/09/2017

14/09 - Noah Sprent Ran yesterday's PCR on a gel:

15.09 PCR Gel.jpg



Ladder, C208, C208, C208noF, C208noF, C258, C258, C258noF, V108, V108, V108noF, V108noF, V208, V208, C258noF
Ladder, V500, V500, V408.50x, V408.50x, V408.20x, V408.20x

Combined: C208noF, C258noF, V108, V108noF, V208 (Separate bands taken seperately), V500
C208 and C258 - Took one of each and did seperately, one for C208/258 and one for C508/558
Then cleaned up the parts.

14/09 - Zoe Catchpole and John Myers Digested them, along with vector. Then cleaned them up and ligated them.

TEV Purification - Noah Sprent Centrifuged yesterday's cells from the induction but there was no flourescence, so binned them. Worth trying with a better strain.

John Myers and Zoe Catchpole Miniprepped the inoculations from yesterday

Zoe Catchpole Test digested today and yesterday's inoculations, ran them on a massive gel:
- Need to add from stick
C563noF(dilB,concA,**dilA**,conc?,conc?,concK,concD,dilG,**concH**,dilC,dilD,dilF,dilE,concJ)
C563(**Q**,**A**,**c**,**L**)
C513noF(**I**,K,J)
C563T,C513concD,C513dilA,C263concE,V113concG

Only 7 parts worked (**in bold**) and of these, 4 were sent for sequencing (**bold, underlined**)

13/09 - Sumaera Rathore transformed and plated V162 noF; C562; C262 noF; C212 noF; C512; C262; C212

SUNDAY, 17/09/2017

15/09 - Zoe Ford Ran the PCR and QCPCR Kushal had made up on Friday

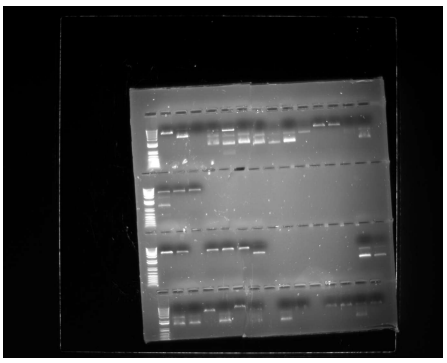
13/09 - Zoe Ford Inoculated the transformations done on Friday

Zoe Ford Also inoculated the ones from the day before

MONDAY, 18/09/2017

13/09 and earlier - John Myers and Jei Diwakar miniprepped the inoculations from Sunday, and test digested them.

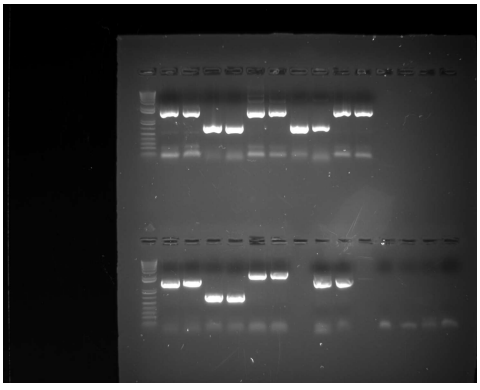
18.09 Test digest 2.jpg



Based on this some were sent for sequencing. Didn't keep a record of which lanes correspond to what other than that the first two lines are C153.

15/09 - Noah Sprent Ran the gel of the PCR

18.09 PCR Gel.jpg



C208,C208,C208noF,C208noF,C258,C258,C258noF,C258noF,C508,C508
V158,V158,V158noF,V158noF,V208,V208,Blank,V508,V508, Blank,V407QC1:20,V407QC1:20,V407QC1:50,V407QC1:50

Extracted and cleaned all the bands except for the QC ones

15/09 - Arthur Norman then digested these, along with vector.

15/09 - Noah Sprent and Jei Diwakar cleaned it all up, but couldn't ligate on account of there being no T4 ligase

14/09 - Sumaera Rathore Transformed the ligations from Friday night

18/09 - Kushal Mansatta Did a new PCR

TUESDAY, 19/09/2017

18/09 - Jei Diwakar Ran last night's PCR on a gel

C208 C208 C208noF C208noF C258 C258 C258noF C258noF C508 C508 C558 C558
V108 V108 V108noF V108noF V208 V208 V508 V508 V608 V608

Noah Sprent and Jei Diwakar gel extracted all of above except C558 because we already have the part.

Noah Sprent and Jei Diwakar ligated C512 X,V512,V212,C212 with old vector digests. All vector digests have now run out. We should have ligated the others on Wednesday but Noah threw them away because he was an idiot.

Kushal Mansatta did a PCR for C208noF, C508, V108noF, V208 and V608 PCR

WEDNESDAY, 20/09/2017

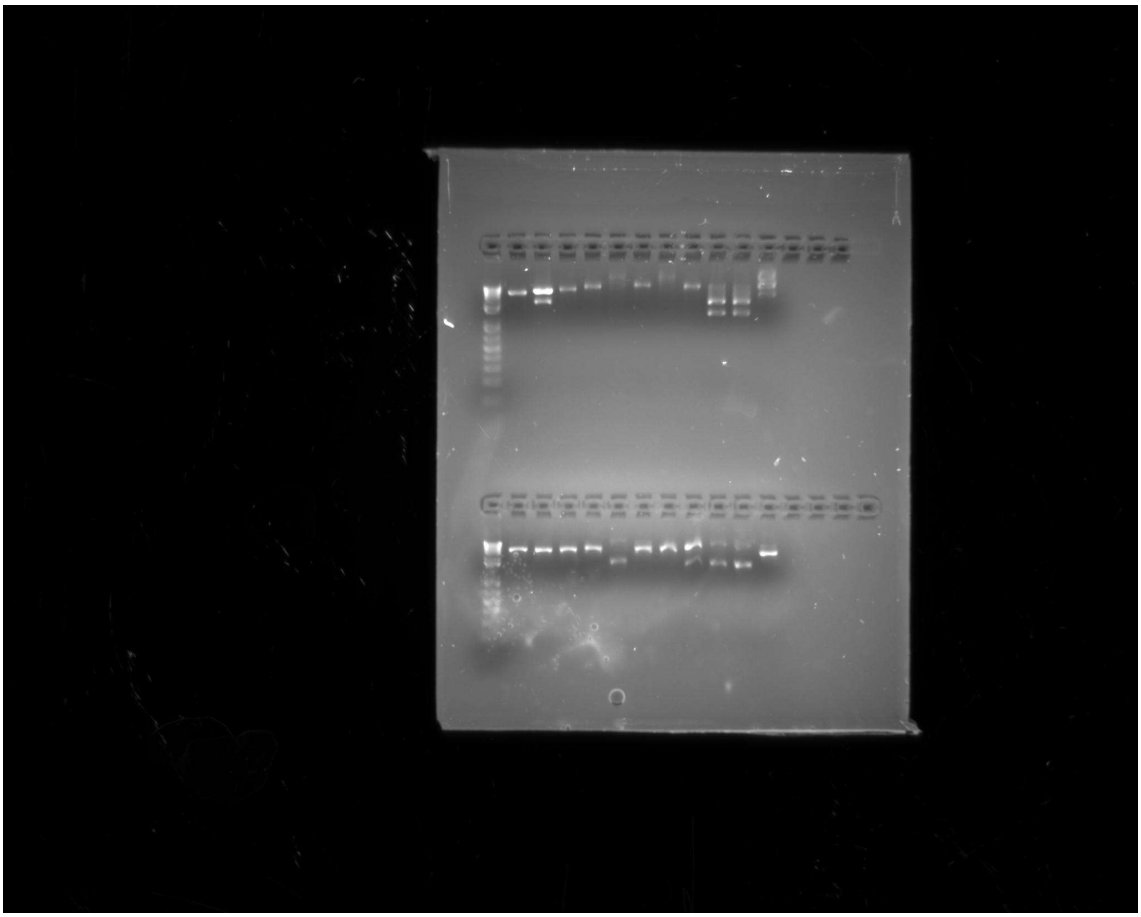
19/09 - Noah Sprent and Zoe Ford Ran only C208noF,C508,V108noF,V208 PCR on a gel and cleaned it up

19/09 - Arthur Norman Digested the parts along with appropriate vector

19/09 - Noah Sprent Cleaned this up and ligated

14/09 - Noah Sprent Miniprepped the inoculations from last night, but only some of them. Didn't nanodrop them all but took a good sample and they were all around 100ng/ul. Then test digested them to give the following gel:

20.09 Test Digest.jpg

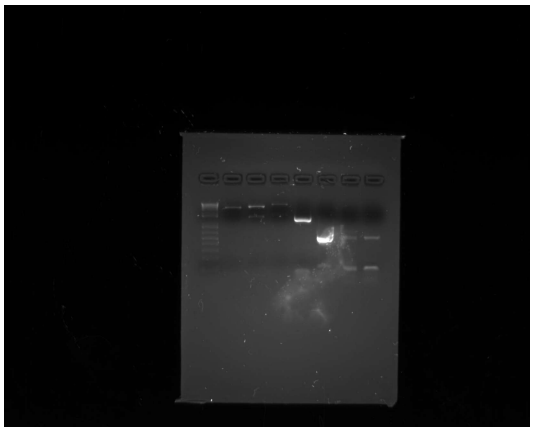


Ladder,
V113noFb,**V213dila**,V213dilb,V213dilc,V213conca,V213concb,V213concc,V513dila,**V513concb,V513concc**,
C313v.d
Ladder,
C213conca,C213concc,C213concd,,C213concf,**C263dila**,C263dilb,C263conce,C563dila,**C513conca**,**C513concb**
,V113noFa

14/09 - The ones in bold looked good, so were sent for sequencing. The ones underlined were controls as they were re-transformations to grow up stock.

Zoe Ford Did a PCR of Monday's sequencing fails to try and see what went wrong, and there could be various explanations for the gel that we got. Interesting is we seem to have amplified some part that is 400bp or so long.

20.09 PCR Check and Vector Digest.jpg



Ladder, pQE60 Vector Digest, C550 Vector Digest, pBAD Vector Digest, C550 Control PCR, C500a, C500b, V150a/b?

18/09 - Sumaera Rathore Made more competent cells and transformed yesterday's ligations along with retransforming the minipreps today that seemed to work from the test digest. Angela Hellyer , Zoe Ford and Jei Diwakar plated them.

15/09 - Zoe Ford Inoculated from yesterday's plates
Jei Diwakar did a C550 assay with ATC and IPTG in LB
Kushal Mansatta did a new PCR for C208noF, C508, V108noF, V208 and V608

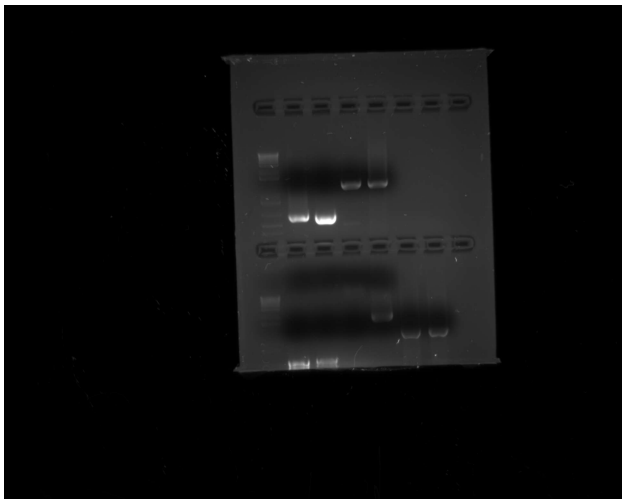
THURSDAY, 21/09/2017

John Myers miniprepped:

C213 conc A	194.15 ng/µl
C213 conc E	226.05 ng/µl
C213 dil A	269.63 ng/µl
C563 conc A	296.46 ng/µl
C563 conc B	216.07 ng/µl
C563 dil A	249.63 ng/µl
V213 conc A	106.99 ng/µl
V213 conc B	107.33 ng/µl
V213 conc C	92.43 ng/µl
V213 conc D	110.68 ng/µl
V213 dil A	89.03 ng/µl
V213 dil B	128.20 ng/µl
V513 conc A	82.84 ng/µl
V513 conc B	102.76 ng/µl

Noah Sprent and Jei Diwakar ran last night's PCR - C208noF, C508, V108noF, V208 and V608 - on a gel and extracted bands for all

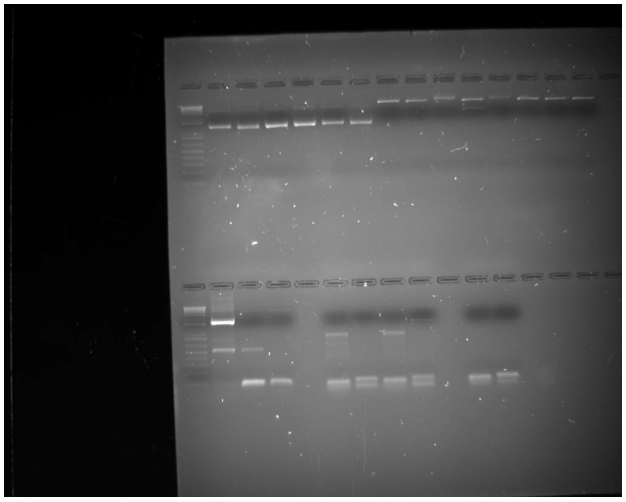
image.png



Kushal Mansatta did a new PCR for C208noF, C508, V108noF, V208 and V608

Kushal Mansatta did a PCR on C263dila (MCS/X control), C513conca (MCS/X), C513concb (MCS/X), V213dila, V513concb, V513concc

image.png



Miniprep test digest and test PCR:

C213 conc A, C213 conc E, C213 dil A, C563 conc A, C563 dilA, V213 concA, V213 conc C, C213 conc D, V213 dil A, V213 dil B, V513 conc A, 513 conc B

C263dila, C513conca, C513concb, V213dila, V513concb, V513concc

FRIDAY, 22/09/2017

Jei Diwakar ran PCR for C208noF, C508, V108noF, V208 and V608 on a gel

Zoe Ford gel extracted. V200 thrown away because we recieved correct sequence.

John Myers miniprepped and test digested:

V113 A conc A	165.93 ng/µl
V113 B conc A	221.79 ng/µl
V113 B conc B	300.08 ng/µl
V113 B conc C	280.81 ng/µl

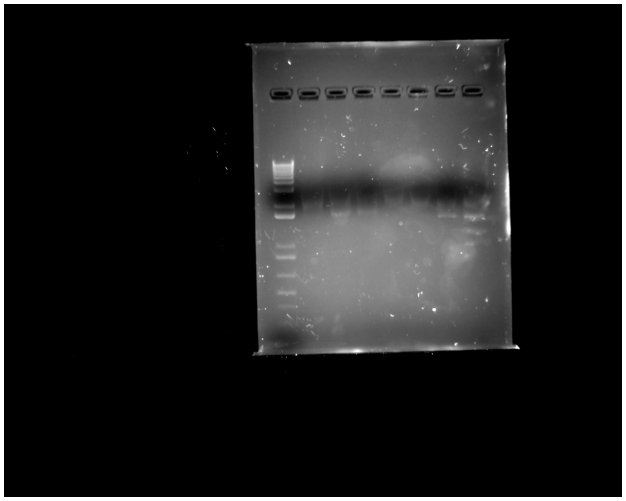
V113 B conc D	201.14 ng/µl
V113 B conc E	216.47 ng/µl
V113 B conc F	316.92 ng/µl
V113 B conc G	292.05 ng/µl
V113 A dil A	183.43 ng/µl
V113 B dil A	214.01 ng/µl
V113 noF conc A	253.33 ng/µl
V113 noF conc B	309.42 ng/µl
V113 noF conc C	296.40 ng/µl
V113 noF conc D	219.34 ng/µl
V113 noF conc E	253.62 ng/µl
V113 noF dil A	321.20 ng/µl
V113 noF dil B	301.78 ng/µl
V107 conc A	401.60 ng/µl
V107 conc B	348.48 ng/µl
V107 conc C	343.36 ng/µl
V107 conc D	286.99 ng/µl
V107 conc E	336.54 ng/µl
V107 conc F	329.64 ng/µl

Noah Sprent digested some vector

Kushal Mansatta did a test PCR of:

C213 conc A, C213 conc A, C213 conc E, C213 conc E, C263 dil A, C263 dil A, C513 conc A, C513 conc A.

image.png



Jei Diwakar repeated C550 assay with M9 media.

Zoe Ford and Noah Sprent diluted the cells required for protein purification.

Also made some inductions for flourescence microscope experiments.

Arthur Norman cleaned up vector digests.

Arthur Norman Set up inductions of V200 (x4), V200noQ (x4), pBAD empty vector (NOT DH5a) for fluorescence microscopy

TUESDAY, 26/09/2017

John Myers and Zoe Catchpole miniprepped stocks of C500:

C513 i	274.95 ng/µl
C513 ii	279.30 ng/µl
C513 iii	287.24 ng/µl

C513 iv	306.15 ng/µl

WEDNESDAY, 27/09/2017

John Myers and Zoe Catchpole miniprepped:

C213 noF conc A	242.26 ng/µl
C513 conc A	274.82 ng/µl
C513 conc B	265.69 ng/µl