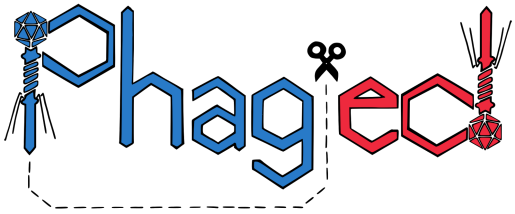
Week 1 1/5/17 - 5/5/17

1. Experimental planning: as a team we discussed the approaches we could take to the project, and came up with an initial design for our constructs.
2. We chose our team name - PhagED!

Week 2 8/5/17 - 12/5/17

1. Experimental planning: the team investigated CRISPR systems and potential target genes
2. We decided on a title for our project and our friend Ellie Powell designed our logo.



3. We carefully designed the logo to have two phages as we are using a two-phage system, CRIPSR-cas symbolism and the Edinburgh university colours. ED stands for Edinburgh.

Week 3 15/5/17 - 19/5/17

1. Experimental planning: we decided which phages everyone will work with (T4 for me!), and also what genes we'll be targeting (choosing important genes in the ESKAPE pathogens).
2. We designed our mock pathogen constructs, Yuri and I choose regions to be used in bla_{KPC} (hereinafter referred to as *KPC*). (Fig.1A)
3. We ordered our target constructs from IDT, after altering them slightly to ensure they didn't contain repetitive regions that would cause difficulties in synthesis.

A

B

C

Overview of the *KPC* construct. A: The allocation of the 51 bp 'chunks' comprising of 17 amino acid residues chosen within *KPC* to be used in the *KPC* construct. The order was scrambled to ensure the KPC protein would be impossible to produce. B: Layout of the *KPC* construct, 'A' indicates the sequence of Moclo fusion site A, as with 'E'. Cyan dashed lines indicate where BsaI will cut. RBS = Ribosomal binding site, L = linker comprising of glycine and serine residues. C: Layout of the *GFP* construct. 'A' indicates the sequence of Moclo fusion site A, as with 'F'. T = terminators, a T1 terminator adjacent to a His terminator.

Week 4 22/5/17 - 26/5/17

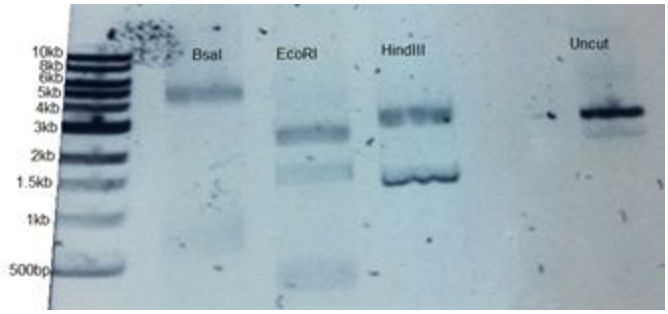
1. Worked with Erin to make competent BL21 DE3.
2. We were visited by students from Leiden University - we gave a presentation about our project and surveyed them to gauge their opinions about it.

Week 5 29/5/17 - 2/6/17

1. We invited teams to our Northern UK Meet-up
2. Worked with Erin to transform *E. coli* TOP10 with DVK_AF plasmid, then extract said plasmid via maxiprep.
3. Worked with Erin to make KAN agar plates.

Week 6 5/6/17 - 9/6/17

1. Worked with Erin to check DVK_AF plasmid: digestion with BsaI, EcoRI and HindIII followed by gel electrophoresis. Results suggested that this wasn't the correct plasmid, as the sizes of the bands were not as expected:

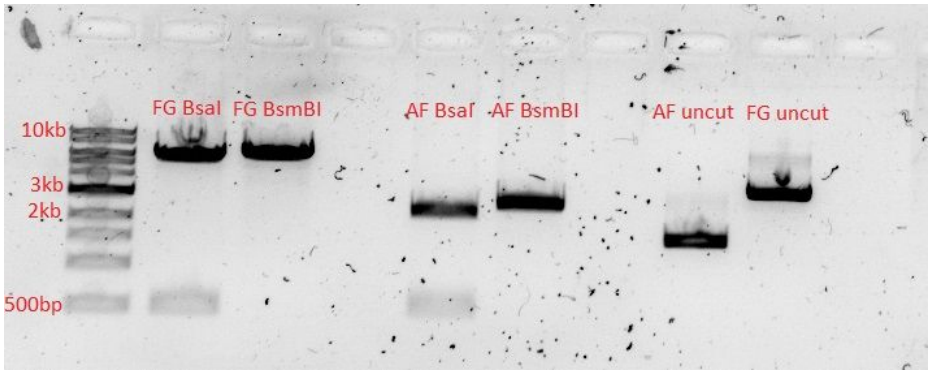


2. Worked with Erin to make KAN + X-Gal + IPTG agar plates.
3. Ordered primers for getting the *vanA* and *KPC* construct to be fused together so they could be placed into T4:

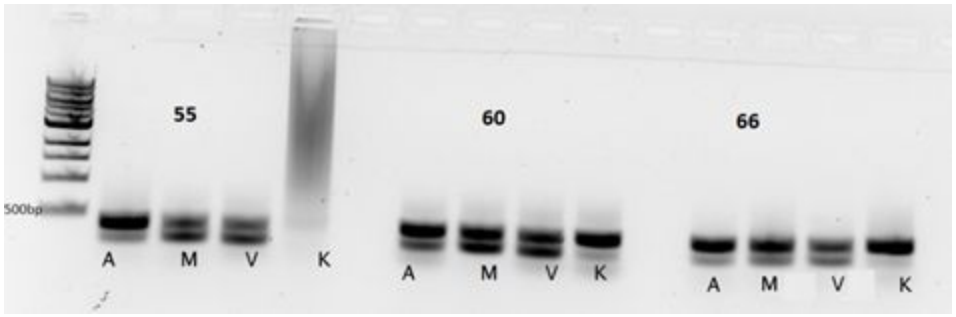
Pair name	Use	Forward Sequence	Reverse Sequence
Construct primers	Amplifying KPC / VanA constructs	GTATCGGGTCTCAGGAG GAT	CGATACGGTCTCTAAGC GCC
Fusion site editors	Editing fusion sites to E and F	GATATGGTCTCAGCTTTA CAGCGAGGCCGTCATC	CTAATGGTCTCTAGCGT ACCGCCATCCTTGTTAG G
Homology + 50bp	Adding 50bp T4 homology to flank	TAAAACTATAGTTGGAAA TTGGAGTCGTTGGGCTA ATAAACTGAGAAAATCCC GGTTTCACGTCATACAGT CG	CAACTGTTGGAGGCCAC GCTTCATGGCCAGCAGT AACACACTTATCATAACA GCGTACCGCCtATCCTT GTTAGGCG
Homology + 100bp	Adding further 50bp homology to flank	CCGACAGATCCTCCTGC TCCAGTAAGAAAAACTCT AGTTAAAAATTATACTAA AACTATAGTTGGAAATTG GAGT	ACGAGAACAGCAATTCC TCCAGTGAATACTTTACT TTGTGTAGCATTCACAA CTGTTGGAGGCCACGCT TCATGG
***SegC* primers**	Bind in T4 homology region	CGACAGATCCTCCTGCT CCAG	GCCTGCTACGAGAACAG CAA
Testing primers	Bind within construct	CTCTGGAGGCGCTTTAC AGC	GCTGTAAAGCGCCTCCA GAG

Week 7 12/6/16 - 16/6/17

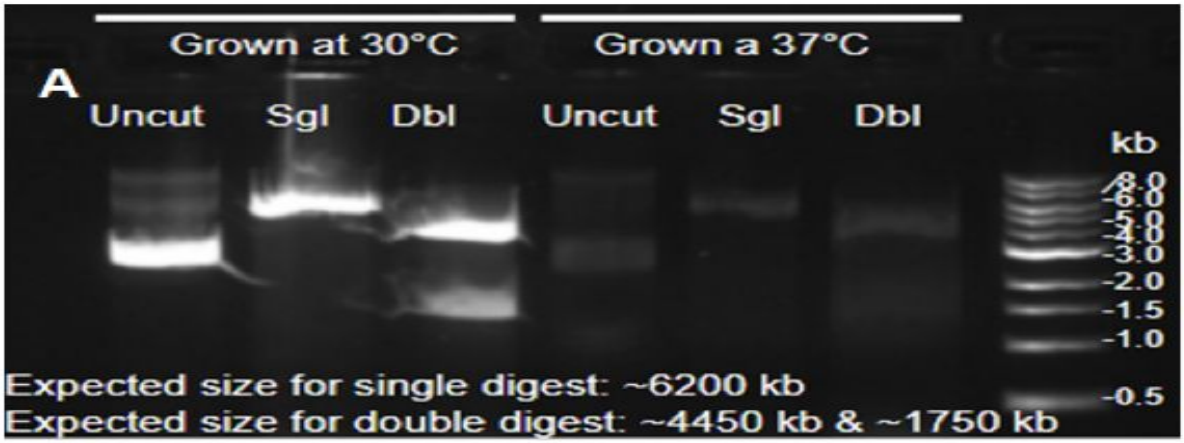
1. Worked with Erin to collect *E. coli* DH5alpha containing DVK_AF and DVK_FG from Miguel Cueva's CIDAR MoClo kit by streaking onto KAN plates. Carried out minipreps, digestion with BsaI and BsmBI and gel electrophoresis. Band sizes were as expected for DVK_AF so this plasmid was used for further work. DVK_FG band sizes were not as expected, so this plasmid was set aside for further investigation.



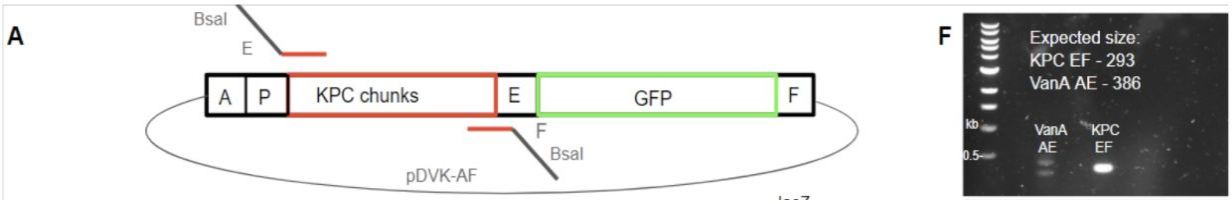
2. Worked with Erin to resuspend and PCR amplify our gene target constructs that were synthesised by IDT. PCR was carried out at various annealing temperatures and PCR products were checked by gel electrophoresis.



3. Worked with Erin to carry out MoClo of DVK_AF + GFP + KPC, and subsequent transformation of *E. coli* TOP10 with the MoClo plasmid.
4. Yale collection order arrived, containing wild type T4 and BL21:DE3:pDK46, which were plated at 30C, Growth curves were conducted of BL21:DE3:pDK46 in SOC and LB at 30C and 37C:

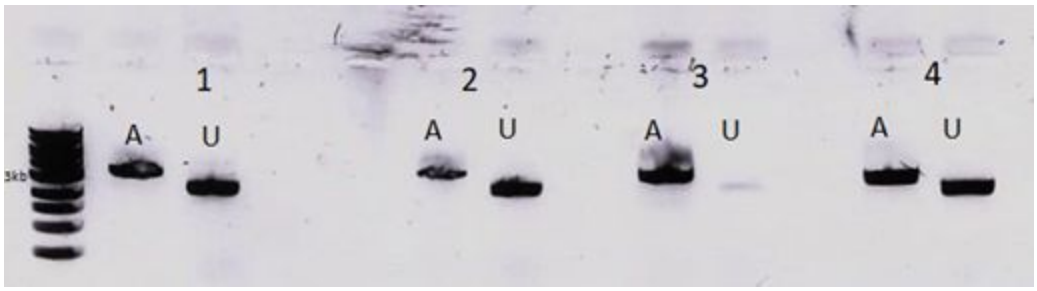


5. PCR of DVKA-F:KPC to create KPC ds construct with E and F fusion sites:

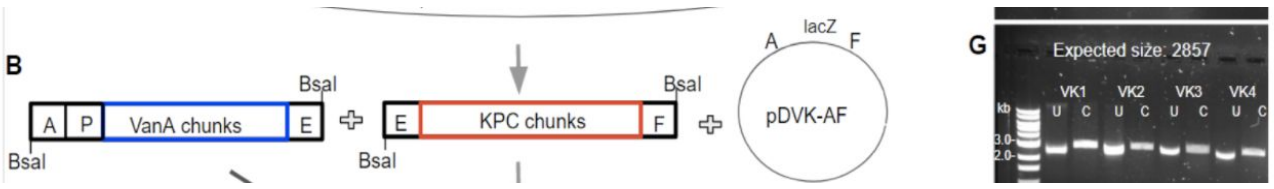


Week 8 19/6/17 - 23/6/17

1. Worked with Erin to carry out MoClo of DVK_AF + GFP + ampC/vanA, and subsequent transformation of *E. coli* TOP10 with the MoClo plasmid.
2. Worked with Erin to check the success of KPC MoClo via extraction of the plasmid using miniprep, digestion with AvrII and gel electrophoresis. The bands were of the expected sizes so the construction of the KPC MoClo construct was deemed to be successful, and a glycerol stock was made.



3. Worked with Erin to check the success of ampC MoClo via extraction of the plasmid using miniprep, digestion with AvrII, EcoRI and EcoRI+PstI, and gel electrophoresis. Band sizes were not as expected, suggesting an incorrect insertion into the MoClo plasmid.
4. Worked with Erin to repeat DVK_AF + GFP + vanA MoClo and TOP10 transformation, as no white colonies were produced by the previous attempt.
5. Visualisation on gel of KPC-EF PRC product,. PCR cleanup of KPC-EF conducted and visualized on gel.
6. Moclo of VanA:A-E, KPC:E-F with pDVKEF conducted and transformed TOP10 cells. pDVK:KPC:VanA plasmid successfully produced in white colonies, and visualised on a gel:

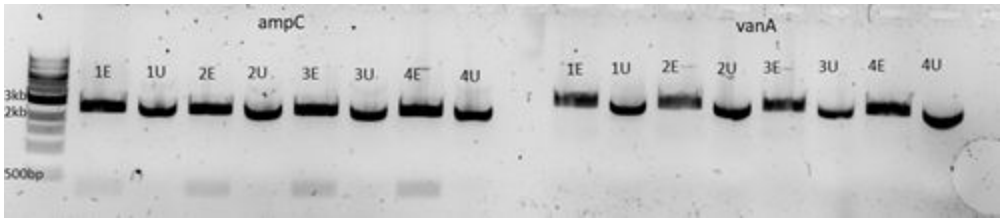


7. New colony of 30C BL21:DE3:pDK46 picked and put in liquid culture at 30C in LB for more pDK46 extraction, RecD strain made chemically competent, miniprep on BL21:DE3:pDK46 and digested to show pDK46 - placed in glycerol stock, transformation of RecD with pDK46.

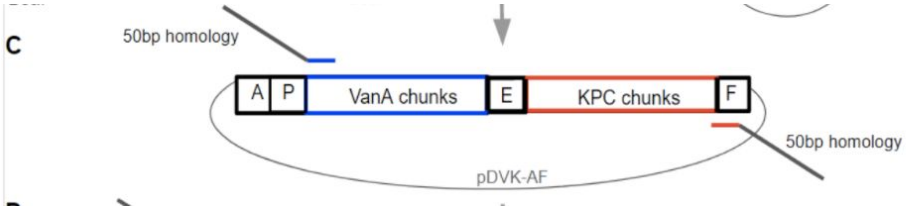
Week 9 26/6/17 - 30/6/17

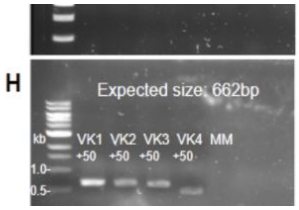
1. Hosted the Northern UK Meet-up - gave visiting teams tours of Edinburgh sites, had pizza for lunch, then each team presented their project ideas.

2. Worked with Erin to check the success of vanA MoClo via extraction of the plasmid using miniprep, digestion with EcoRI, and gel electrophoresis. Band sizes were as expected, suggesting that the construct is correct.

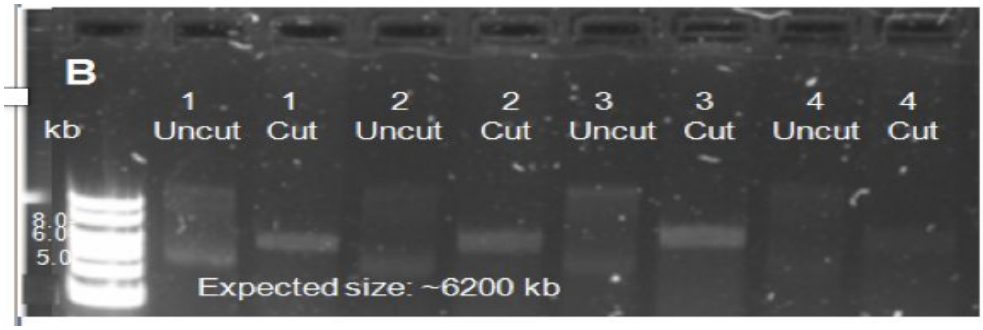


3. PCR on VanA:KPC:DVKAF with primers adding 50bp homology with T4, then visualised:

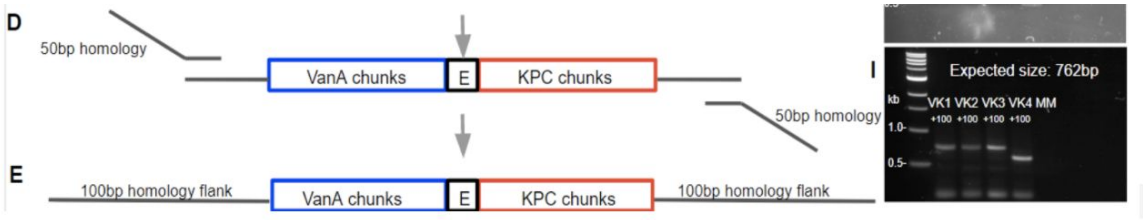




4. Miniprep, digestion and gel electrophoresis of RecD:pDK46:



5. PCR cleanup of VanA:KPC+50bp T4 homology construct and gel electrophoresis of result, PCR of VanA:KPC+50bp with primers adding another 50bp of T4 flanking homology. Gel electrophoresis of final VanA:KPC+100bp T4 homology:
6.



7. Overnight RecD:pDK46 liquid incubation, DH5a, TOP10 and BL21:DE3 plates streaked

8. Gel extraction of VanA:KPC+100bp T4 homology, Tris-Magnesium buffer made.

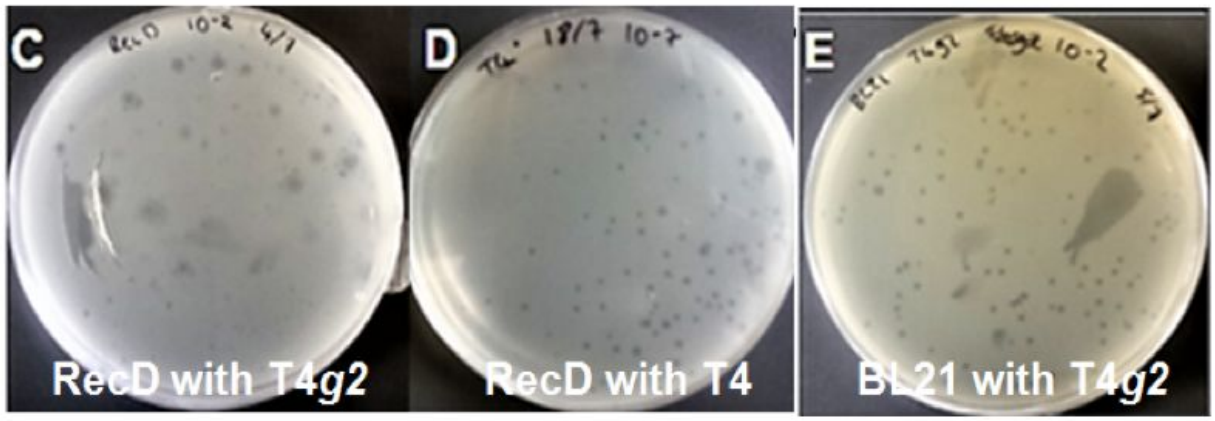
Week 10 3/7/17 - 7/7/17

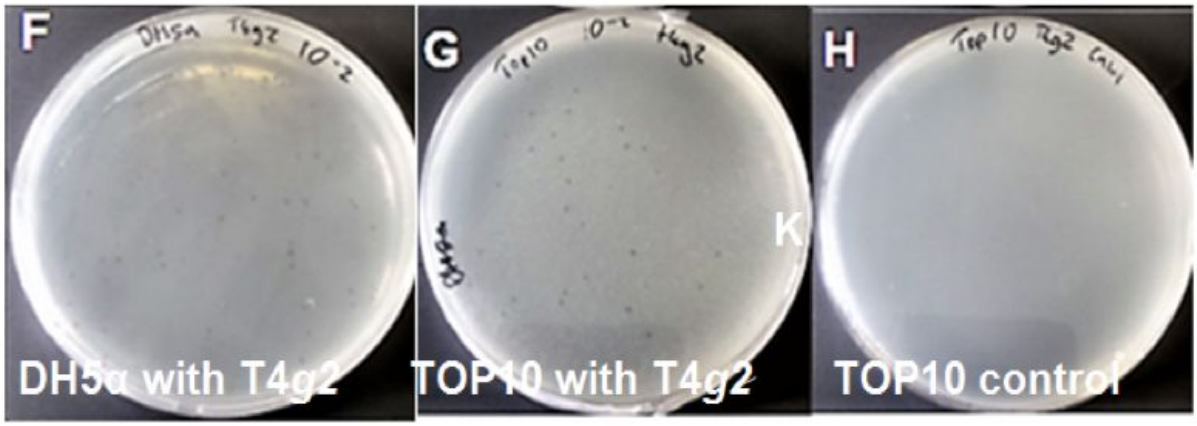
1. Repeat PCR of VanA:KPC+50bp to make stock of final VanA:KPC+100bp ds construct - gel electrophoresis and extraction of final product, MgSO4 buffer produced, overnight liquid culture of DH5a, TOP10, BL21:DE3 and RecD:pDK46.

2. RecD, DH5a, TOP10 and BL21:DE3 grown to OD ~0.6, infected with T4g2 phage dilutions and plated for overnight incubation.

3. Infection with T4g2 failed so repeated phage infection and dilutions with T4g2 on RecD and WT T4 on TOP10.

4. Successful infection on both RecD and TOP10 with T4g2 with WT T4 so repeat infection of DH5a, TOP10 and BL21:DE3 with T4g2 to prove it only works with RecD strain (photo below):





Week 11 10/7/17 - 14/7/17

1. Results of T4g2 on DH5a, TOP10 and BL21:DE3 show it can infect them (photo above).

2. Lysate produced of T4g2 from plaque taken off TOP10, and infected with RecD to make dilutions to 10-6 for calculation of lysate titre.

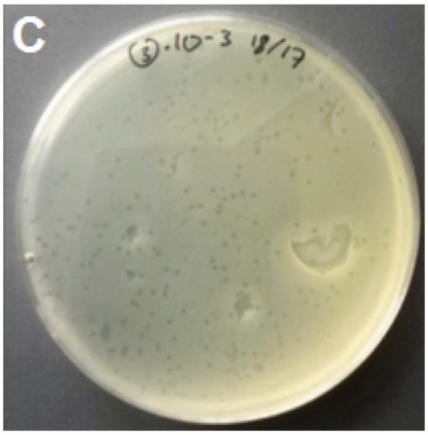
3. Final miniprep and digest of pDK46 RecD to check correct for us in BRED, arabinose produced

4. T4g2 lysate very strong so could not determine titre as all the bacteria were killed (no plaques!). Thus replated with dilutions going to 10-10.

5. Infected RecD cells at OD of 0.6, induced lambda red genes on pDK46 plasmid with 0.1 concentration arabinose, made electrocompetent and then conducted BRED - ds VanA:KPC+100bp T4 homology electroporated into cells to be placed into T4g2, cells were left to lyse after electroporation and 4 lysates produced, 2 of the lysates mixed with fresh RecD cells and serial dilutions produced to make plaques.

6. PCR of big/small plaques found in original T4g2 RecD plate with T4g2 lysate picked from TOP10 plate and compared with WT T4 and P1vir to check all phages are T4, using primers that flank SegC (gene which is replaced by VanA:KPC),

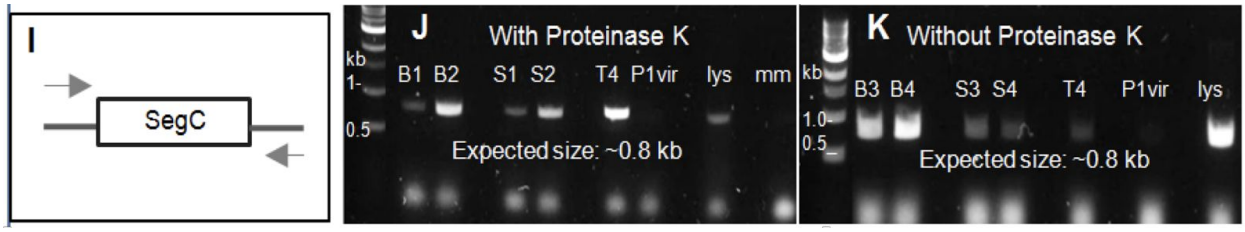
7. 25 plaques from VanA:KPC lysate 2 picked to be ready for PCR screening.



- Photo of T4-gene2 plate containing plaques of recombineered T4-gene2 after BRED

Week 12 17/7/17 - 21/7/17

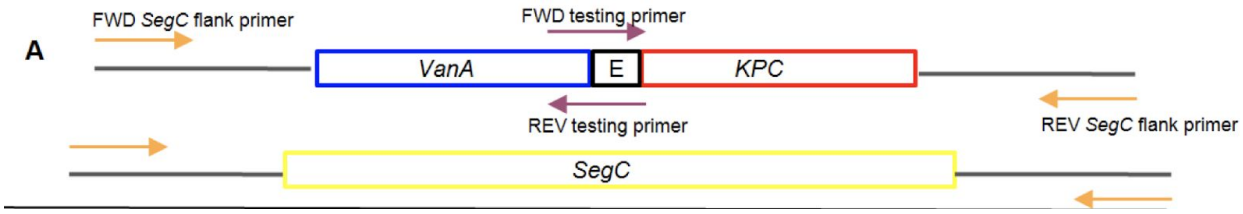
1. Gel electrophoresis of PCR product of different T4 plaques/phages showed all the same phage, which was amplified using *SegC* primers.

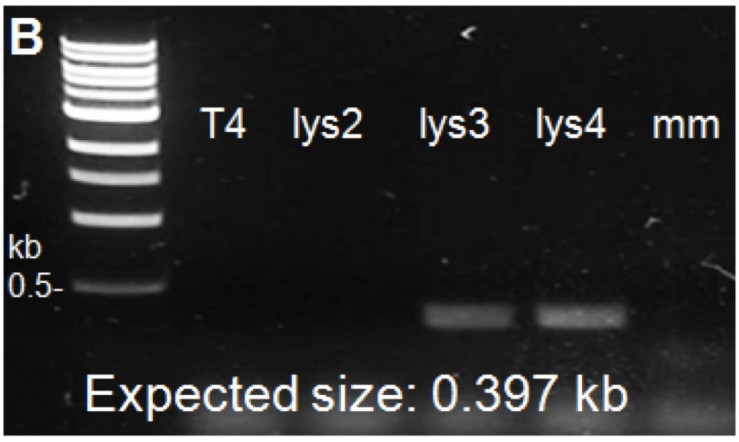


2. Plaques from lysate 2 were screened by PCR and showed by gel electrophoresis to contain no recombinants.

3. Minipep and digestion of BL21:pDK46 and checked correct by gel electrophoresis.

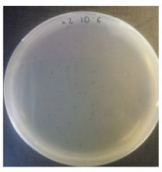
4. PCR screen of VanA:KPC lysate 2, 3, 4 and plaques from lysate 3 with positive control of SegC primers conducted. Lysate 3 and 4 showed evidence of recombinants, no plaques screened showed recombinants:



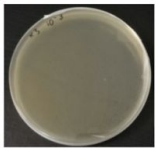


5. 21 further plaques picked from lysate 3 plate and screened, this time with Taq polymerase (all previous PCRs used Q5). Results shown using gel electrophoresis potential recombinants but lots of noise.

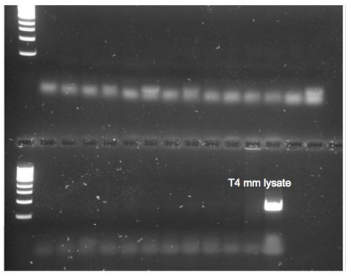
Mon: -Screen of lysate 2

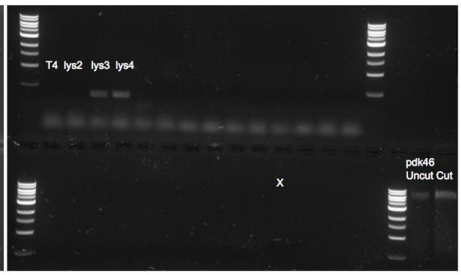


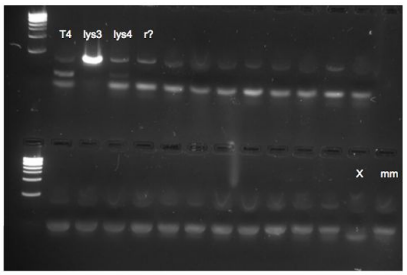
Tues: -Screen of lysate 3
-miniprep + digestion Bl21:pdk46



Weds: -Further screening lysate 3 with taq polymerase
-plated lysate 4 and made new dilutions of lysate 3



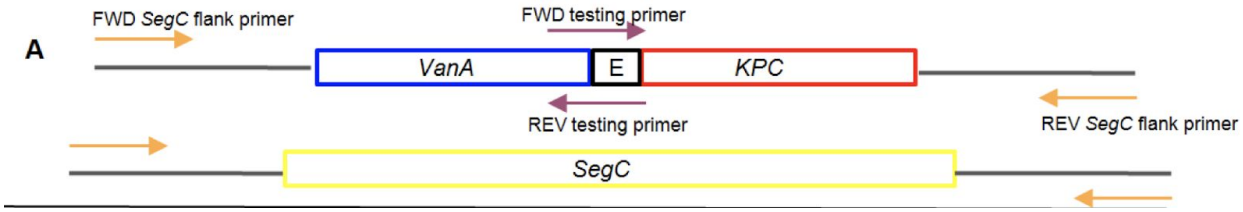




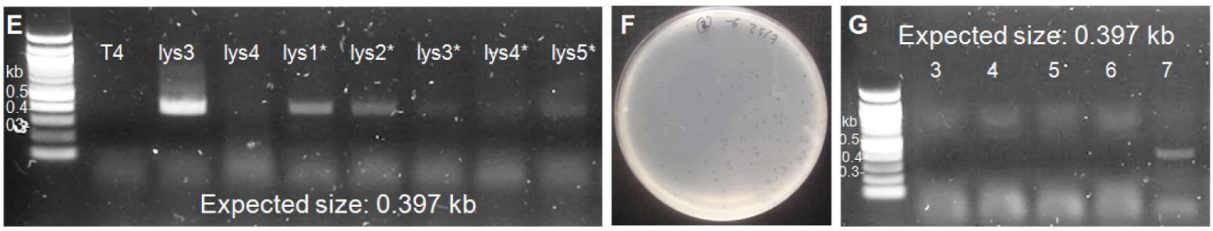
Week 13 24/7/17 - 28/7/17

1. Repeated BRED with wild type T4

2. Continued screening lysate 3 T4-gene2 plaques, and found one with a recombinant phage



3. Screened wild type T4 recombinant lysates and plaques after BRED, and found lysate 1 and 2 had recombinants, and a plaque also showed evidence of a recombinant phage:



The recombineered lytic T4 bacteriophages were therefore discovered on the last day of lab work, so unfortunately could not be purified.