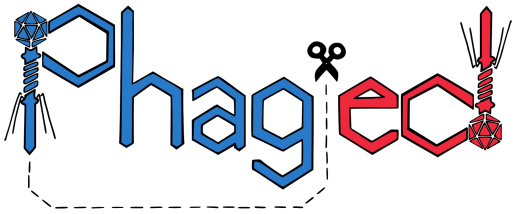
## Week 1 1/5/17 - 5/5/17

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!

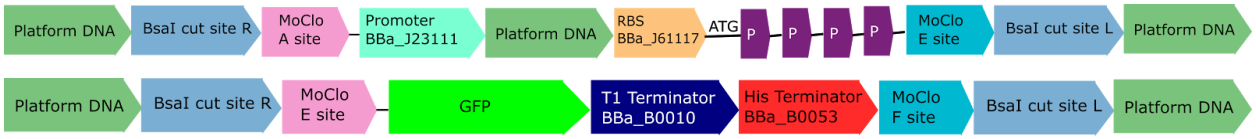
## Week 2 8/5/17 - 12/5/17

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.




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

1. Experimental planning: we decided which phages everyone will work with (T7 for me!), and also what genes we'll be targeting. 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.




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

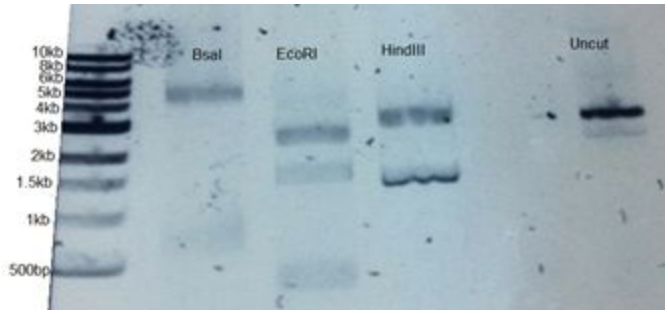
1. Worked with Lydia Mapstone to make competent BL21 DE3
2. We were visited by students from Leiden University - we gave a presentation about our project and surveyed them to gauge their opinions about it.

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

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

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

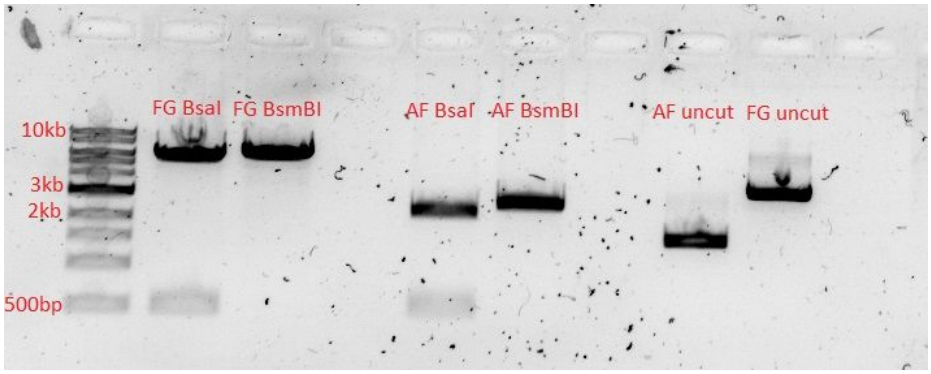
1. Worked with Lydia Mapstone to check DVK_AF plasmid: digestion with BsaI, EcoRI and HindIII followed by gel electrophoresis. Results suggested that this wasn't the correct plasmid, as the sizes of the bands were not as expected.




2. Worked with Lydia Mapstone to make KAN + X-Gal + IPTG agar plates.
3. Ordered T7 homology flank constructs from IDT.

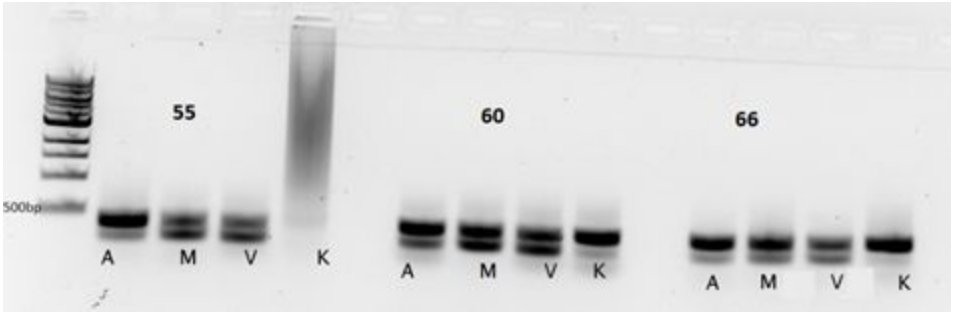

## Week 7 12/6/16 - 16/6/17

1. Worked with Lydia Mapstone 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 Lydia Mapstone to resuspend and PCR amplify our gene target constructs that were synthesised by IDT. PCR was carried out at various

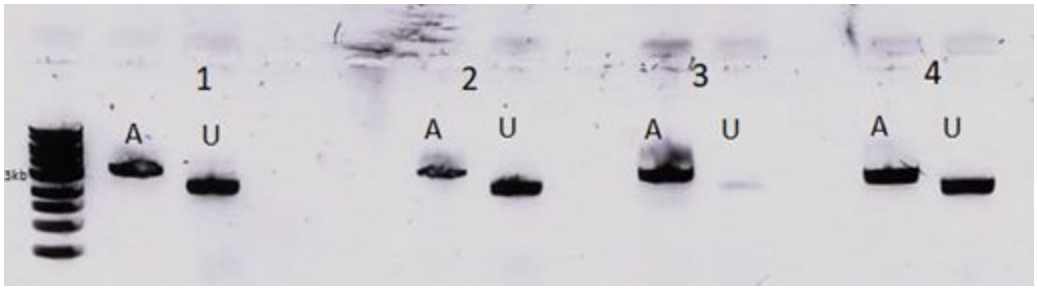
annealing temperatures and PCR products were checked by gel electrophoresis.




3. Worked with Lydia Mapstone to carry out MoClo of DVK_AF + GFP + KPC, and subsequent transformation of *E. coli* TOP10 with the MoClo plasmid.

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

1. Worked with Lydia Mapstone to carry out MoClo of DVK_AF + GFP + ampC/vanA, and subsequent transformation of *E. coli* TOP10 with the MoClo plasmid.
2. Worked with Lydia Mapstone 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.

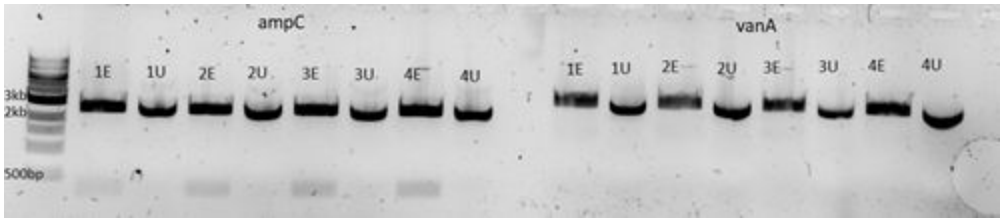



3. Worked with Lydia Mapstone to check the success of ampC MoClo via extraction of the plasmid using miniprep, digestion with AvrII, EcoRI and EcoRI+PstI, and gel electrophoresis. Band sizes were not as expected, suggesting an incorrect insertion into the MoClo plasmid.
4. Worked with Lydia Mapstone to repeat DVK_AF + GFP + vanA MoClo and TOP10 transformation, as no white colonies were produced by the previous attempt.

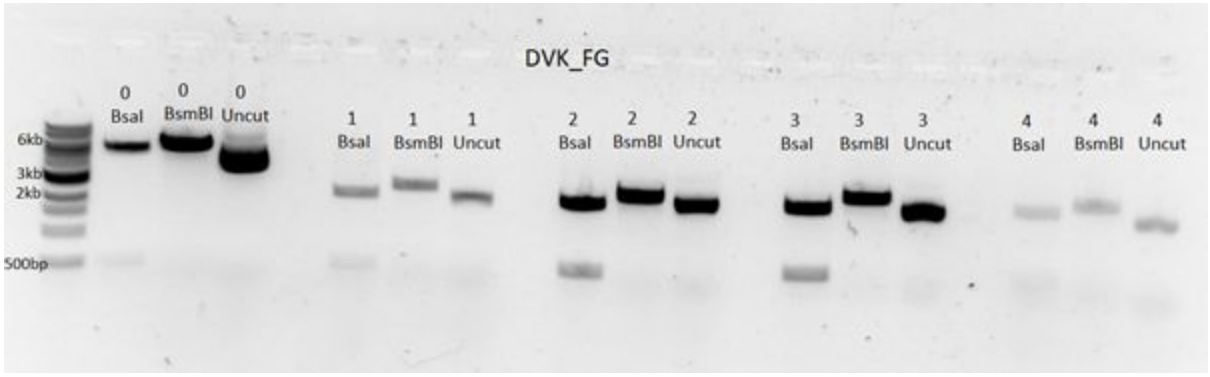
## Week 9 26/6/17 - 30/6/17

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 Lydia Mapstone 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.

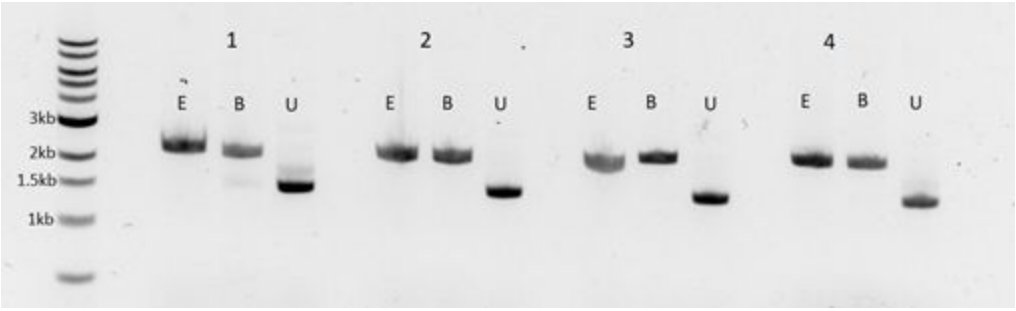



3. Resuspended T7 homology flank DNA synthesised by IDT.
4. Took more minipreps of DVK_FG from the DH5alpha, and digested these with BsaI and BsmBI before checking with gel electrophoresis. All samples except the original yielded the correct band sizes, suggesting that the original colony may have been contaminated. The correct minipreps were used for further DVK_FG work.




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

1. Performed MoClo to combine DVK_FG + T7 homology flanks + KPC, and transformed into *E. coli* TOP10. Success was checked via miniprep, digestion with AvrII and BbsI, and gel electrophoresis. Band sizes were as expected, so a glycerol stock of the successfully transformed TOP10 was made up.
2. Performed MoClo to combine DVK_FG + T7 homology flanks + vanA, and transformed into *E. coli* TOP10. Success was checked via miniprep, digestion with EcoRI and BbsI, and gel electrophoresis. Band sizes were not as expected, so the MoClo was deemed to have failed.

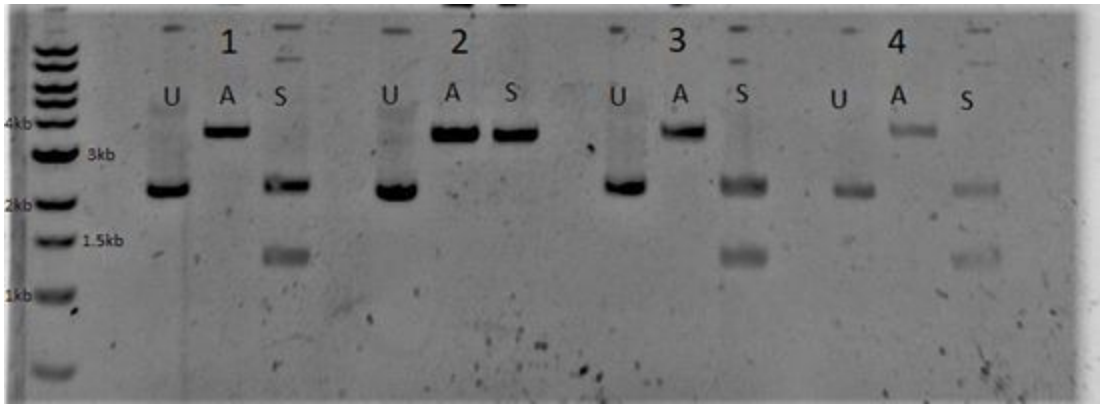



## Week 11 10/7/17 - 14/7/17

1. Prepared the pCas9 plasmid via digestion with BsaI, gel electrophoresis and gel extraction.
2. Resuspended T7 spacer oligos synthesised by Sigma, and prepared them via phosphorylation and annealing.
3. Attempted ligation of T7 spacers into pCas9 and transformation into BL21 DE3. No colonies grew on chloramphenicol plates, suggesting that the ligation was unsuccessful.

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

1. PCR to replace medium-strength Anderson promoter in the target construct with a stronger version, to see if this would produce visible GFP.
2. Performed MoClo of DVK_AF + GFP + KPC_new_Anderson and transformed into *E. coli* TOP10. Success was checked via miniprep, digestion with AvrII and SpeI and gel electrophoresis. Bands of the expected sizes were produced by 3 of the 4 samples tested.




3. Repeated MoClo of DVK_FG + T7 homology flanks + vanA, and checked success via digestion with EcoRI, BbsI and SpeI. Band sizes were again not as expected, so the MoClo was deemed to have failed.

4. Re-attempted ligation of T7 spacers into pCas9, and transformation into BL21 DE3. Again, no colonies were produced.

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

1. Re-attempted ligation of T7 spacers into pCas9, and transformation into BL21 DE3. Again, no colonies were produced.
2. Worked with Lydia Mapstone and Yuri Matsueda to examine testing platform *E. coli* using fluorescence microscopy. Elise Darmon provided the microscope and advised us on its use. The GFP did not seem to be being expressed.

## Week 14 31/7/17 - 4/8/17

1. Worked with Filippo Abbondanza to prepare samples for sequencing at Edinburgh genomics.