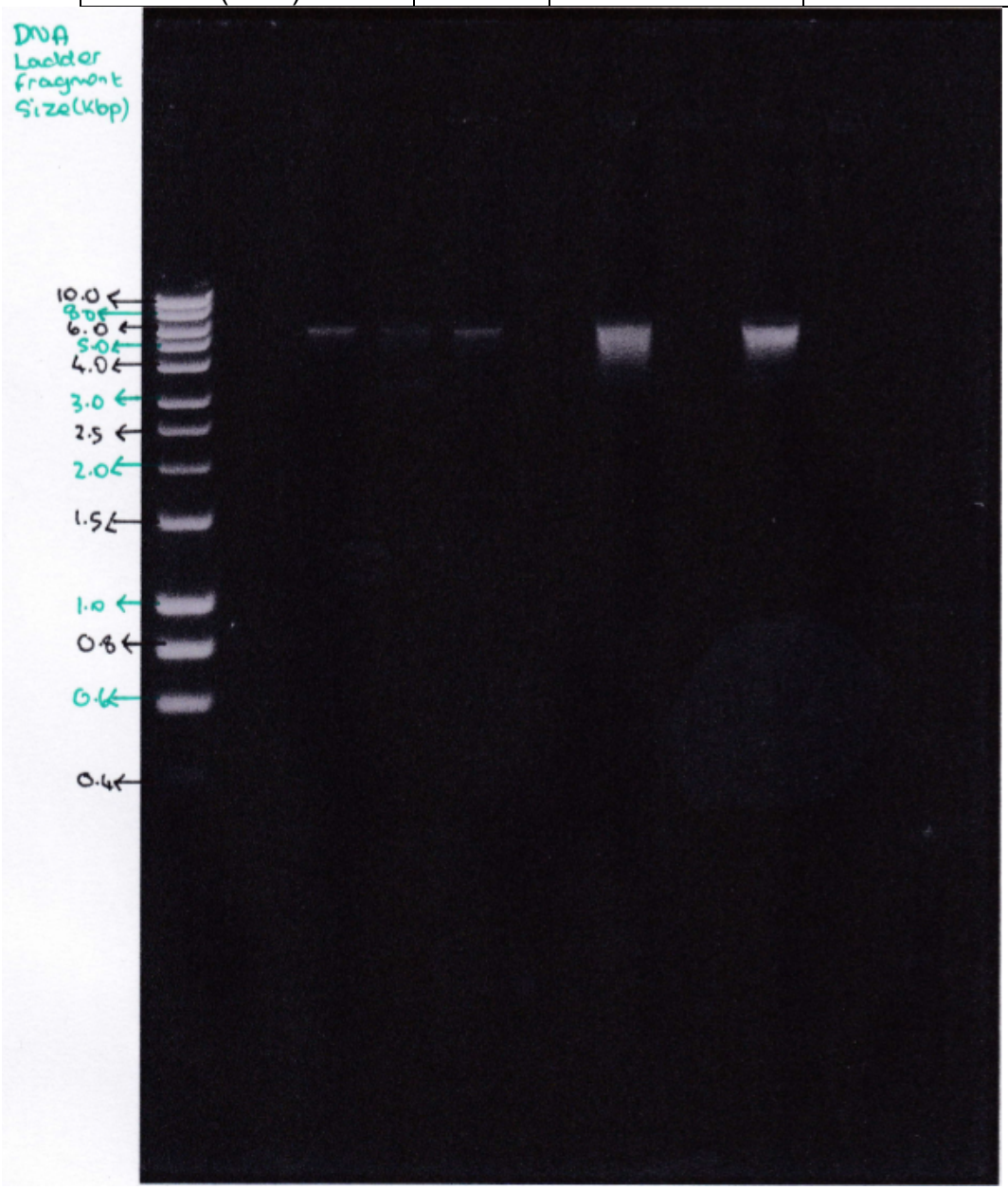
Monday 9th September

Gel of plasmid digest

Eppendorf	DNA	Dye	TAE buffer
	Volume of component (µl)		
1 (Ladder)	3	3.5	13.5
2 (None)	3	3.5	13.5
3 (EcoR1)	3	3.5	13.5
4 (Pst1)	3	3.5	13.5
5 (Both)	15	3.5	1.5
6 (Both)	15	3.5	1.5



Lane 1: ladder, Lane 2:empty, Lane 3: none, Lane 4: Ecor1, Lane 5 : Pst1, Lane 6: empty, Lane 7: both, Lane 8: empty, Lane 9: both, lanes 10 and 11 empty.

Note: No cuts Ecor1 and Pst1 occurred during digests.

norV and NrfA digests

- NrfA - 23µl, 2.0µl of both Xba1 and Pst1, 3.0µl buffer H
- norV

Eppendorf	Restriction enzyme	Buffer H	Distilled water	norV DNA
	Volume of component (µl)			
1 (None)	0.0	0.8	4.2	3
2 (Ecor1)	0.2	0.8	4.0	3
3 (Pst1)	0.2	0.8	4.0	3
4 (Both)	2.0 of each	3.0	0	23

- Both digests can be carried out for 2.5 hours at 37ºC.

LB agar plates (400ml)

Tryptone 4.0g
NaCl 4.0g
Yeast extract 2.0g
Agar 6.0g
Distilled water 400ml

1. Produce stock solution into large glass measuring jar. Seal and autoclave. When autoclaving agar stock solution, you must return for the end time of the autoclave cycle.
2. Prepare the required number of agar plates, within close area to Bunsen burner.
3. Label all agar plates with Cm (abbreviation for the antibiotic chloramphenicol).
4. Addition of chloramphenicol 0.4 ml (34 mg dissolved within 1ml of ethanol, has to be added in ratio of 1:1000 to stock solution) to agar stock solution, when at about 50°C. You must not add before this temperature, or the stock antibiotic will be denatured.
5. Pour around 20ml of agar liquid into each plate, taking care that the solution doesn't begin to solidify.
6. Flame the agar using the Bunsen burner, if bubbles are present.
7. Allow agar to set, then turn plates upside down and place in oven for around 20 minutes.
8. Seal plates together and place in plate bag bag, store in cold room. Ensure that they are upside down.

Solubilising of 3 BioBrick's

1. Ring part 1A on plate 1, part 4B on plate 3, part 1O on plate 4, using a marker pen.
2. Puncture foil of located parts, using pipette tip.
3. Resuspend dried DNA with 10µl of HPLC grade water.
4. Remove contents of well using pipette and store at -20°C, for future use.

Digest of colony 2 plasmid

- Digests carried out for two different miniprep samples from colony 2 plasmid.

Eppendorf	Restriction enzyme	Buffer H	Distilled water	Colony 2 plasmid DNA
	Volume of component (µl)			
1(None)	0	0.8	4.2	3
2 (EcoR1)	0.2	0.8	4.0	3
3 (Pst1)	0.2	0.8	4.0	3
4 (Both)	2.0 of each	3.0	0	23.0

- Digests were carried out for 2.5 hours at 37ºC

Colony 2 overnight

- Resuspension of a single colony of colony 2 in 10ml of LB media.
- Grow overnight at 37ºC in the shaker.

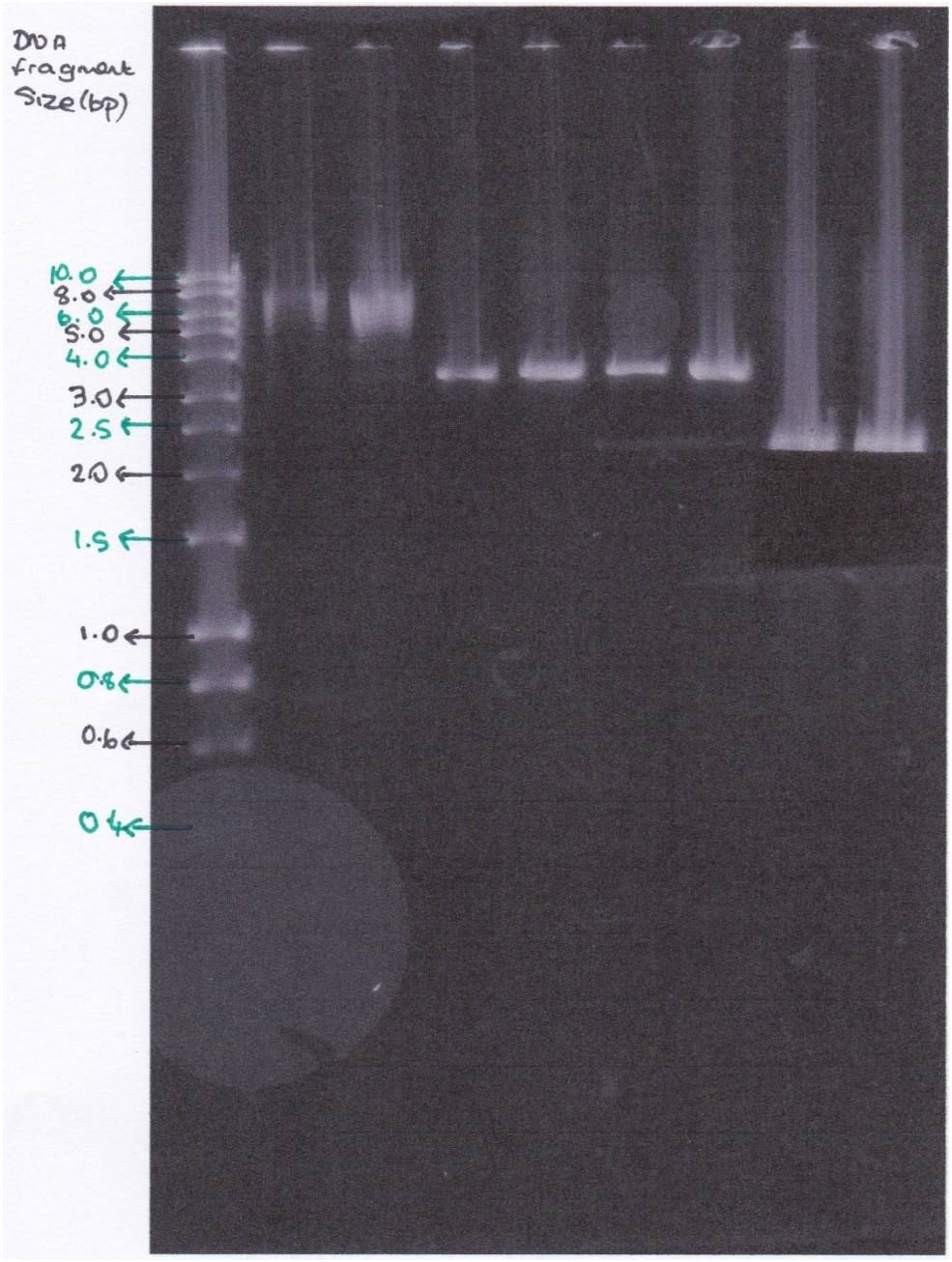
Tuesday 10th September

Gel of plasmid digest

1. Preparation of gel as previous.
2. Quantities of each component put into gel.

Eppendorf	DNA	Dye	TAE buffer
	Volume of component (µl)		
1 (Ladder)	3	3.5	13.5
2 (None) (1)	3	3.5	13.5
3 (None) (2)	3	3.5	13.5
4 (Ecor1) (1)	3	3.5	13.5
5 (Ecor1) (2)	3	3.5	13.5
6 (Pst1) (1)	3	3.5	13.5
7 (Pst1) (2)	3	3.5	13.5
8 (Both) (1)	15	3.5	1.5
9(Both) (2)	15	3.5	1.5

Note: (1) and (2) correspond to the sample number of the colony 2 miniprep.



Lane 1: empty, Lane 2: ladder, Lane 3: none (1), Lane 4: none (2), Lane 5: Ecor1(1), Lane 6: Ecor1 (2), Lane 7: Pst1 (1), Lane 8: Pst1 (2), Lane 9: Both (1), lane 10: Both (2), lane 11: empty.

Plasmid and insert purified from gel

1. Cut out required bands. Use scalpel on UV light box.
Note: UV light is on for a short as possible, due to it damaging DNA. Also, cut as close to band as possible, so that there is as little amount of gel as possible.
2. Put samples into pre-weighed Eppendorf's and reweigh.

	Mass (g)		
	Eppendorf	Eppendorf and gel	gel
Sample 1	0.9864	1.0877	0.1013

Sample 2	0.9864	1.1178	0.1314
Sample 3	0.9907	1.0474	0.0567
Sample 4	0.9864	1.0315	0.0451

3. Addition of binding buffer in 1:1 volume to gel.
4. Incubation at 55ºC for 10 minutes, inverting every 2-3 minutes to aid process of gel dissolving.
5. Transfer of samples to GeneJET purification column, centrifuge for 1 minute at 12,000 rpm for 60 seconds. Discard flow through, then placed column back into collection tube.
6. Addition of 700µl of wash buffer too each sample, centrifuge for 1 minute at 12,000 rpm for 60 seconds.
7. Centrifuge empty column, again.
8. Put column into an Eppendorf tube, add 50µl of elution buffer. Centrifuge for 1 minute at 12,000 rpm.
9. Store DNA at -20ºC.

Digest norV and NrfA

23µl of norV/NrfA purification from PCR,
2µl of Ecor1
2µl of Pst1
3µl of 10x buffer H (Roche)
- Above quantities into eppendorf and incubate at 37ºC for 2.5 hours.

Transformations of BioBricks

1. Competent cells on ice. Label 2.0ml centrifuge tubes with different ligation ratios.

2. 2µl of BioBricks 1 1:A, 3 4:B, 4 1:O, should be pipetted into its corresponding tube. A different pipette tip should be used for each.

3. 50µl of competent cells should then be pipetted into each tube. Once this is added, flick each tube, to ensure that they are mixed.

4. Incubate on ice for 30 minutes.

5. Place cells into a 42°C water back, for 1 minute, to heat-shock the cells. To allow the cells to recover, transfer them back to ice and leave them for 5 minutes.

6. Add 200µl of SOC media per tube, and incubate at 37°C for 2 hours. Label agar plates in with each ratio and volume of cells to be added.

7. In turn pipet 70µl from each tube onto the appropriate plates, and spread the mixture evenly across the plate. When doing this make sure that the spreading rod is constantly sterile, using ethanol and Bunsen flame. Cool rod on the agar, before spreading cells across plate. Once complete, tape plates in stacks and place in 37°C oven for incubation overnight.

Note: all transformations were successful, due to colonies being present on all plates.

Miniprep of colony 2 overnight

1. Centrifuge cells for 6 minutes at 4000 rpm. Discard supernatant.
2. Resuspend pelleted cells in 250µl of Resuspension Solution, vortex to ensure cells are resuspended completely. Transfer to Eppendorf tube.
3. Addition of 250µl of Lysis Solution to each Eppendorf. Mix by inverting tube 6 times.
4. Add 350µl of Neutralisation Buffer to each Eppendorf, mix by inverting 6 times.
5. Centrifuge for 5 minutes at 12,000 rpm.
6. Transfer supernatant to GeneJET spin column.
7. Centrifuge for 1 minute at 12,000 rpm and discard flow-through.
8. Add 500µl of Wash Solution and centrifuge for 60 seconds at 12,000 rpm. Discard flow-through.
9. Repeat step 8.
10. Discard flow-through and centrifuge for another 60 seconds at 12,000 rpm.
11. Transfer GeneJET spin column to Eppendorf tube. Add 50µl of Elution Buffer, leave to stand for 2 minutes. Then centrifuge for 2 minutes at 12,000 rpm.
12. Store colony 2 plasmid x2, collected from miniprep at -20ºC

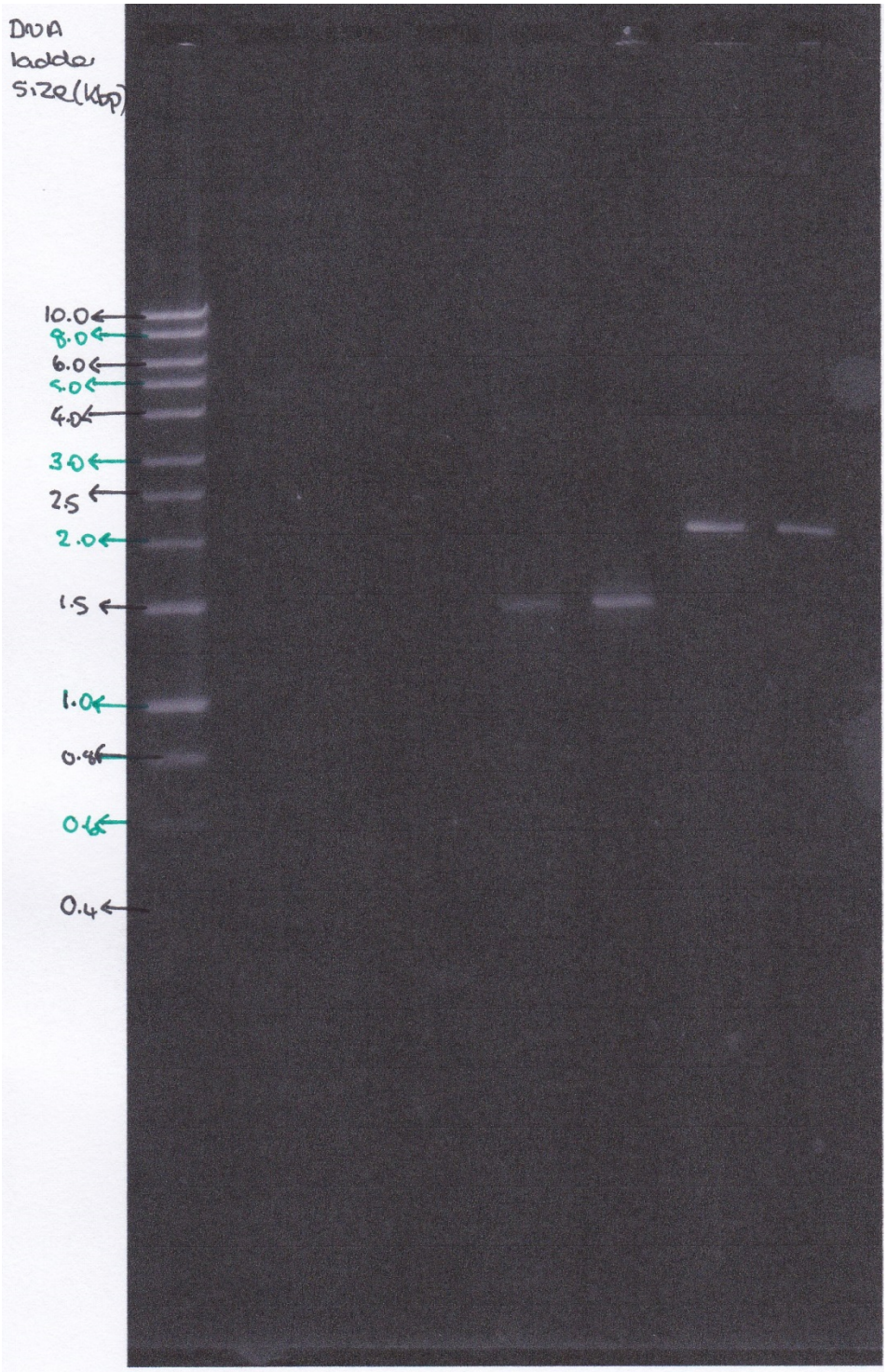
Wednesday 11th September

Gel of digested norV, NrfA and plasmid

1. Preparation of gel as previous.
2. Quantities of each component put into gel.

Eppendorf	DNA	Dye	TAE buffer
	Volume of component (µl)		
1 (Ladder)	3	3.5	13.5
2 (norV) (1)	3	3.5	13.5
3 (norV) (2)	3	3.5	13.5
4(NrfA) (1)	3	3.5	13.5
5 (NrfA) (2)	3	3.5	13.5
6 (Plasmid) (1)	3	3.5	13.5
7 (Plasmid) (2)	3	3.5	13.5

Note:
- norV 1 cut with Ecor1 and Spe1
- norV 2 cut with Ecor1 and Pst1
- NrfA 1 cut with Xba1 and Pst1
- NrfA 2 cut with Ecor1 and Pst1
- Plasmids from originally different miniprep samples from colony 2 (samples 1 and 2).



Lane 1: empty, Lane 2: ladder, Lane 3: empty, Lane 4: norV (1), Lane 5: norV(2), Lane 6: NrfA (1), Lane 7: NrfA (2), Lane 8: Plasmid (1), Lane 9: Plasmid (2), lanes 10 and 11: empty.

Note: gel was used to work out the concentration of components, in order to use them in a ligation.
Nor V: 3.066ngµl^{-1}
NrfA: 2.3ngµl^{-1}
Plasmid: 4ngµl^{-1}

Ligations of norV, NrfA and plasmid

- Ligation carried out using plasmid from colony 2, norV and NrfA from colony PCR.

	Component volume added (µl)				
Ligation ratio (P:I)	norV	Buffer	Enzyme	Water	Plasmid
1:5	3.5	1.0	1.0	-	5.0
1:3	1.0	1.0	1.0	2.0	5.0
1:1	0.3	1.0	1.0	2.7	5.0
1:0	-	1.0	1.0	5.0	5.0

	Component volume added (µl)				
Ligation ratio (P:I)	NrfA	Buffer	Enzyme	Water	Plasmid
1:3 (1 & 2)	8.15	2.0	2.0	6.6	1.25
1:1	2.7	1.0	1.0	2.0	1.25
1:0	0	1.0	1.0	6.75	1.25

	Component volume added (µl)					
Ligation ratio (P:I)	norV	NrfA	Buffer	Enzyme	Water	Plasmid
1:3	0.25	8.15	2.0	2.0	5.6	1.25
1:1	0.16	2.7	1.0	1.0	3.89	1.25
1:0	0	0	1.0	1.0	6.75	1.25

- Ligation carried out at 4ºC overnight, using T4 ligase and buffer.