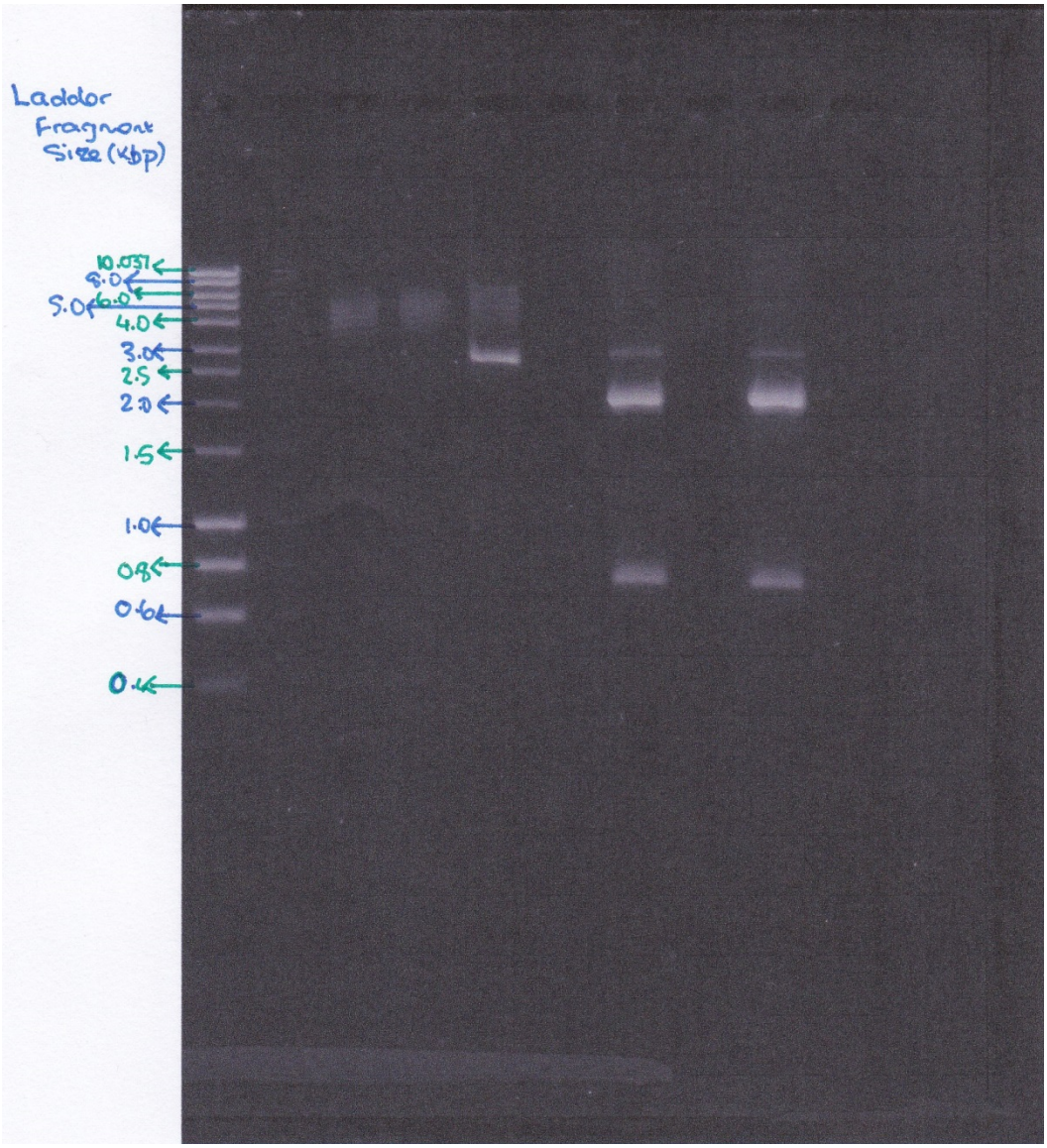
Monday 12th August

Agarose gel of GFP 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 (Xba1)	3	3.5	13.5
4 (Pst1)	3	3.5	13.5
5 (Both)	15	3	0
6 (Both)	15	3	0



Lane 1: empty, Lane 2:ladder, Lane 3: empty Lane 4: none, Lane 5 : Xba1, Lane 6: Pst1, Lane 7: empty, Lane 8: Digest, Lane 9: digest, lanes 10 and 11 empty.

Gel purification of GFP bands

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	0.9994	1.0553	0.0559
Sample 2	0.9903	1.0303	0.040

3. Addition of binding buffer in 1:1 volume to gel. (55.9µl to 1 and 40µl to 2)
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 GFP DNA at -20ºC.

Transformation of ligated product into Top 10 cells

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

2. 10µl of each ligation product 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.

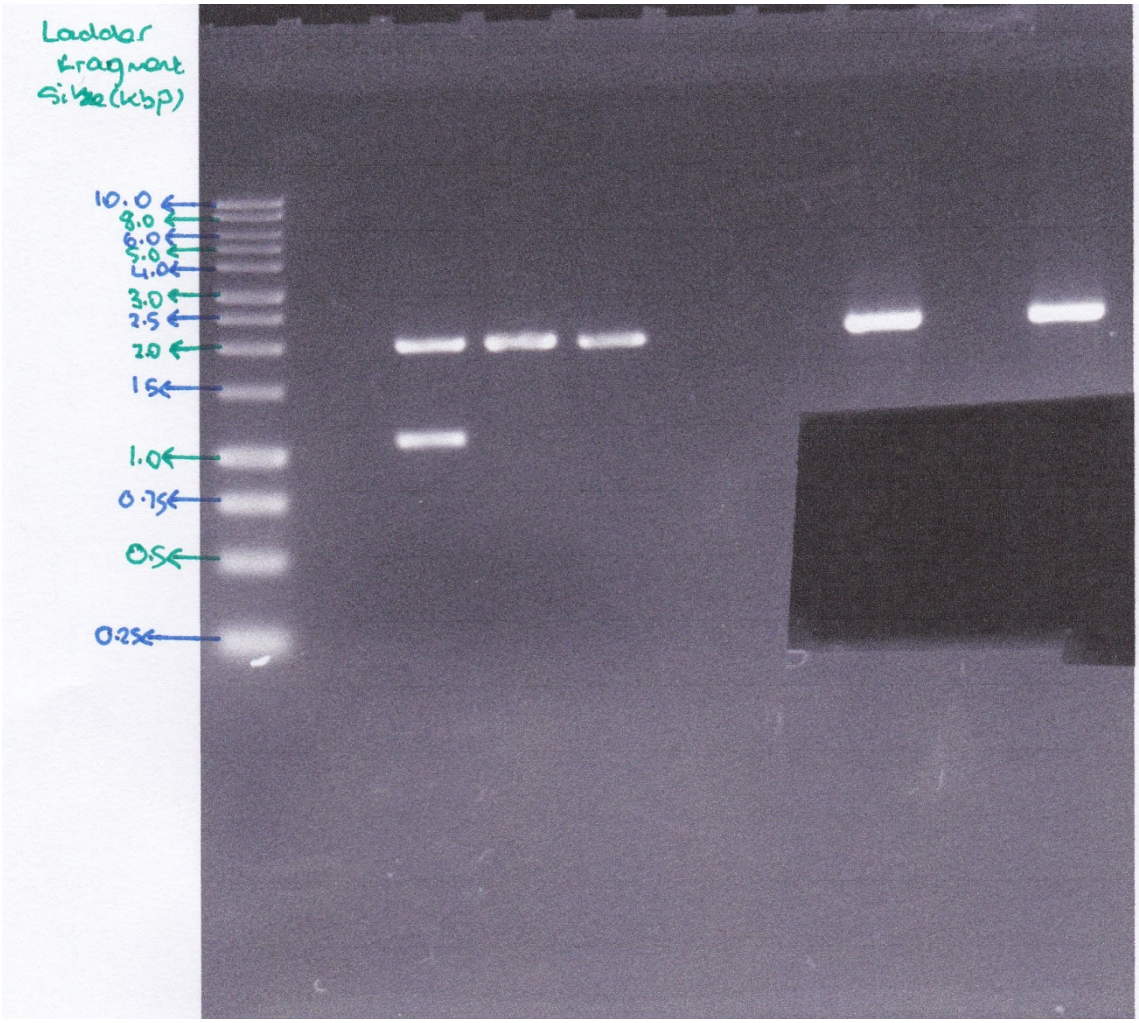
5. Place cells into a 42°C water back, for 1 minute, to heat-shock the cells. To allow the cells to recover, transfer them back to ice and leave them for 5 minutes.

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 200ul and the 50µl from each tube onto the appropriate plate, 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.

Thursday 15th August

Gel of colony digested plasmids 1-4 and digested GFP
- 200 ml of 1x TAE buffer and 3g of agarose.
Note: weigh in beaker before and after put into microwave.
- 1:5 ratio of dye:DNA
- 10µl of digested plasmid from colonies 1-4 loaded onto the gel.
- GFP digest loaded onto gel.



Lane 1: ladder, lane 2: colony 1, lane 3: colony 2, lane 4: colony 3, lane 5: colony 4, lane 6: empty, lane 7: empty, lane 8: GFP, lane 9: empty, lane 10: GFP

Purification of GFP from gel

1. GFP band extract from gel, using razor blade.
2. Weigh gel in Eppendorf tube, then add 3 volumes of buffer QC for 1 mass of gel. Volumes of buffer QC added Eppendorf 1: 976.5µl, Eppendorf 2: 989.1µl, Eppendorf 3: 990µl, Eppendorf 4 928.8µl.
4. Incubate 50ºC for 10 minutes, inverting tubes every 2-3 minutes.

5. Addition of 1 gel volume of isopropanol to gel volume and mix. Volumes of isopropanol added 1: 325.5µl, Eppendorf 2: 329.7µl, Eppendorf 3: 330µl, Eppendorf 4 309.6µl.
5. Sample into QIAquick column, centrifuge for 1 minute at 13,000 rpm. Discard flow through. Apply samples 800µl at a time.
6. Add 0.75ml Buffer PE to column, centrifuge for 1 minute at 13,000 rpm. Discard flow through.
7. Centrifuge for a further 1 minute at 13, 000 rpm. Discard flow through.
8. Place QIAquick column into clean 1.5ml Eppendorf tube.
9. Add 50µl Buffer EB to each tube and centrifuge for 1 minute at 13,000 rpm.
10. Store at -20ºC.