Monday 22nd July

1. Inoculate 50µl strain into 10ml of LB solution, grow overnight.
2. Preparation of tips and conical flasks in autoclave, for use the following day.

Tuesday 23rd July

Preparation of ampicillin agar plates

- 250ml of distilled water
- 2.5g of tryptone
- 2.5g NaCl
-1.25g of yeast extract
- 3.75g of agar

1. Produce stock solution into large glass measuring jar. Seal and autoclave.
2. Prepare the required number of agar plates, within close area to Bunsen burner.
3. Label all agar plates with ampicillin.
4. Addition of ampicillin to agar stock solution, when at about 50°C. You must not add before this temperature, or the stock antibiotic will be denatured. Must be added as at a ratio of 1:1000, ampicillin to total volume of agar stock solution. 0.25ml of ampicillin at a concentration of 50µg/ml, should be added to 250ml stock solution. This should be prepared by adding 50µg of ampicillin to 1ml of water and to syringe filter.
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 leave for 20 minutes.
8. Seal plates together in stacks, put in cold room overnight.

Preperation of SOC media

- 2.0g of tryptone
- 0.5g of yeast extract
- 19mg of KCl
- 50mg Nacl
- 100ml of distilled water
- 95.2mg of $MgCl_2$
- 24.0mg of $MgSO_4$

1. Weight out masses of solids.
2. Addition of solids to an appropriately sized glass jar.
3. Addition of distilled water.
4. Seal and autoclave.

Competent cell production

1. Inoculate 5ml (1:20) of Top10 e.coli cells into 100ml of LB stock solution. Leave to grow at 37°C. Keep checking the OD600, stop growing up cells when between 0.4 and 0.45.

Time (minutes)	OD600
0	0.167
20	0.176

35	0.279
55	0.451

2. Place cells in the cold room for 10mins.

3. Prechill centrifuge by running a cycle for 10 minutes, to reduce its temperature. Centrifuge bottles can be prechilled on ice, along with $CaCl_2$ and $CaCl_2$ glycerol, on ice.

4.Harvest cells at 3000rpm, 4^{o}C for 8 mins.

5. Pour away supernatant, then gently resuspend pellet in chilled 100mM $CaCl_2$, 25ml. (Do not use a pipette to re-suspend, swirl flask!)

6. Incubate on ice for 10mins.

7. Harvest cells at 3000rpm, 4oC for 8 mins.

8. Resuspend pellet in 2ml of 100mM CaCl2, 30% (v/v) glycerol and divide into 100ul aliquots (eppendorf tubes). This could be done in an eppendorf tube container, which has been in a -20^{o}C freezer.

9. Store at -80^{o}C

Colony PCR for norV and NrfA

1. Dilution of primers to produce 100pmol/µl

Primer	Volume of sterile water added (µl)
norV_F	114
norV_R	104
NrfA_F	79
NrfA_R	229

2. Suspension of 1 colony of top10 *E.coli* cells in 50µl of sterile water.
3.

Components added to each PCR tube	Volume added to each tube (µl)
$MgCl_2$ Buffer	5
dNTP's	5
F_primer	1
R_primer	1
DNA template	2
Taq polymerase	1
Water	35
Total	50

Section of program	Time (minutes)	Temperature (°C)
Initial	15	105
Main cycle 30x		
Initial denaturation	3	94
Annealing	0.75	55
Extension	3	72
Final extension	5	72

Wednesday 24th July

Agarose gel electrophoresis of PCR product

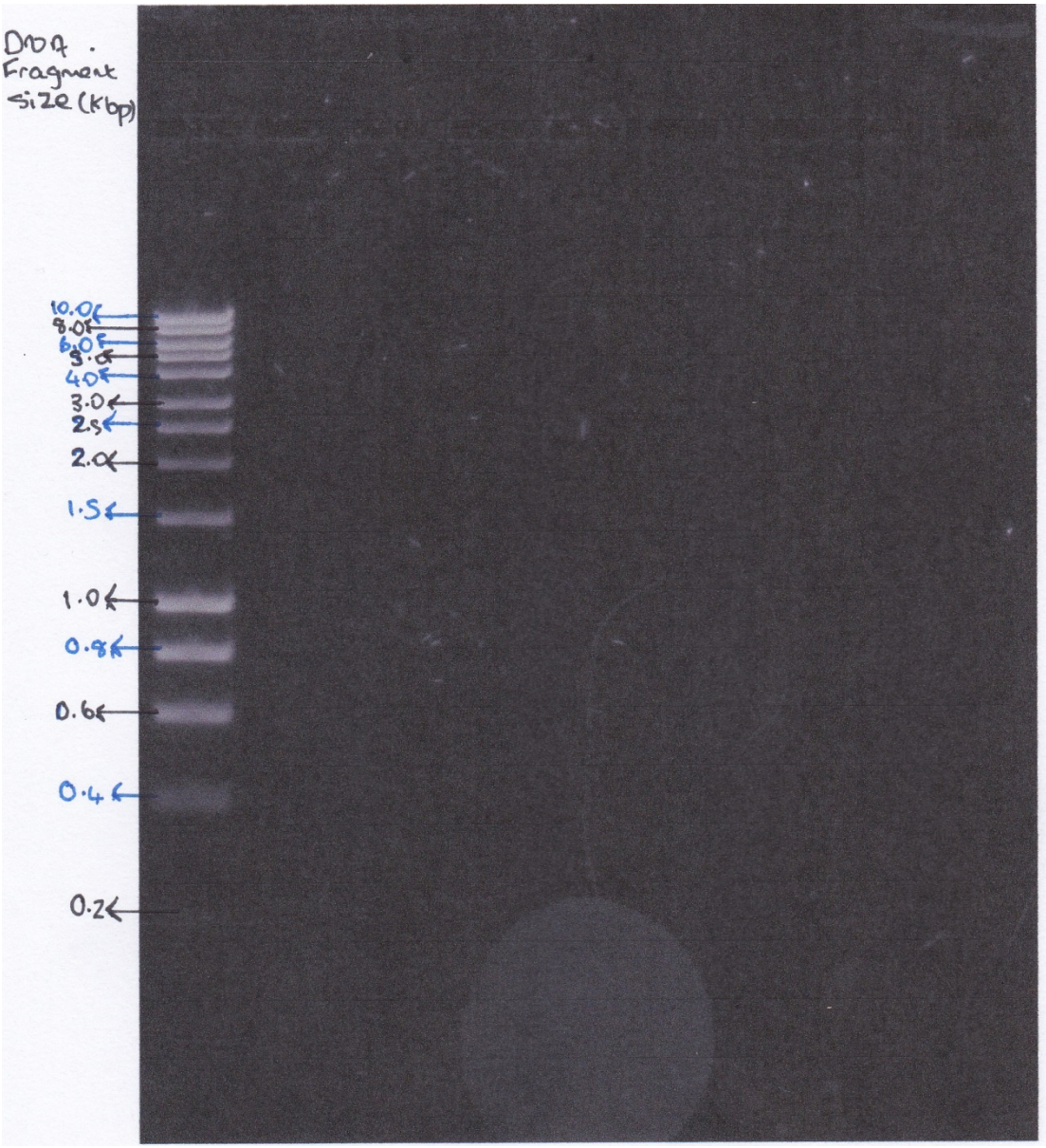
1. Preparation of gel:
- 0.75g of agarose (1.5 g/100ml concentration)
- 50ml 1x TAE buffer
- Dissolve agarose into buffer, using microwave.
- Allow to cool
- Addition of 1μl of cyber safe stain

2. Poor agarose solution into gel tank and leave to set.
3. Addition of 1x TAE buffer to gel tank, make sure to cover sides, allow only a small volume to cover the top of the gel.
4. Make up the required contents of each gel.

Component	Volume of component added to each eppendorf		
	1	2	3
DNA	-	5	5
Dye	3.5	3.5	3.5
Buffer	11.5	11.5	11.5
DNA ladder	5	-	-

Note: tube 1- DNA ladder, tube 2- PCR product norV, tube 3-PCR product NrfA

5. Pipette the 20µl from each eppendorf tube into corresponding well.
6. Run gel at 100 (volts/mv), until the gel front is within 1-2 cm of end of the gel.
7. Once gel is done, image using scanner.



Note: PCR was unsuccessful, no bands on the gel for promoter or gene.

Solubilising of GFP

1. Ring part 14K on plate 5, using a marker pen.
2. Puncture foil of 14k, 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.

Transformation of GFP within plasmid into Top10 *E.coli* cells

1. Competent cells on ice. Label 2.0ml centrifuge tubes with GFP 1 and 2.

2. Iµl of solubilised GFP plasmid 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 200ul of SOC media per tube, and incubate at 37°C for 2 hours. Label agar plates in triplicate, with the concentration of DNA which shall be added to each.

7. In turn pipet 70ul 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.

Thursday 25th July

Preparation of GFP *E.coli* overnight

1. Preparation of ampicillin, 50µg within 1 ml of HPLC water.
2. Lifting of individual colonies and resuspend within 5ml of LB buffer.
3. Incubate the two samples at 37°C, overnight.

Friday 26th July

Miniprep of GFP *E.coli* colonies

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 GFP collected from miniprep at -20ºC

Restriction digests of miniprep product

1. Following volumes of each component are to be added to corresponding eppendorf tubes. A repeat from each should be carried out, using 2 miniprep products.

Digestion type	DNA	Restriction enzyme	Buffer	Distilled water
	Volume of component (µl)			
None	3	-	0.8	4.4
Single	3	0.2	0.8	4.2
Double	3	0.2 (each)	40.8	4.0

2. Incubate for 2 hours at 37°C
3. Prepare agarose gel.
4. Run digests on gel, to see if successful.