Tuesday 3rd September

LB agar plates (400ml solution)

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 0.5 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 leave for 20 minutes.
8. Seal plates together in the plate bag, store in cold room. Ensure that they are upside down.

LB media production (200ml)

Tryptone	2g
Yeast extract	1.0g
Sodium Chloride	2g
Distilled water	200ml

- Above quantities of components into a glass jar, seal and autoclave.

Transformations of ligated norV into linearised plasmid

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

2. 2µl 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 70ul 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: no colonies grew, therefore meaning that all ligations were unsuccessful.

Thursday 5th September

Digest of colony 1 and 2 plasmid and norV

Eppendorf	Restriction enzyme 1	Restriction enzyme 2	Buffer H	DNA
	Volume of component (µl)			
1 (Plasmid colony 1)	2	2	3	23
2 (Plasmid colony 2)	2	2	3	23
3 (norV)	2	2	3	23

- Plasmid restriction enzymes: 1 Ecor1, 2 Pst1
- norV restriction enzymes: 1 Ecor1, 2 Spe1 (product for all in one ligation)
- 37ºC for 2.5 hours.
- Store in freezer.

PCR for norV and NrfA

1. Preparation of primer working stock solution - 2µl of primer and 18µl of water.
2. Suspension of 2 separate colonies into 50µl of water, to act as template.

Eppendorf	Buffer	dNTP's	Primer-F	Primer-R	DNA template	Taq polymerase	Water
	Volume of component in each eppendorf (µl)						
1(norV 1)	5	5	1.5	1.5	0.1	0.5	36.4
2(norV 1)	5	5	1.5	1.5	1	0.5	35.5
3(NrfA 1)	5	5	1.5	1.5	0.1	0.5	36.4
4(NrfA 1)	5	5	1.5	1.5	1	0.5	35.5
5(norV 2)	5	5	1.5	1.5	0.1	0.5	36.4
6(norV 2)	5	5	1.5	1.5	1	0.5	35.5
7(NrfA 2)	5	5	1.5	1.5	0.1	0.5	36.4
8(NrfA 2)	5	5	1.5	1.5	1	0.5	35.5

Section of program	Time (minutes)	Temperature (°C)
Initial	15	95
Main cycle 39x		

Initial denaturation	0.5	94
Annealing	0.5	50
Extension	3.5	72
Final extension	20	72

Note: store in fridge (4ºC) overnight.

Buffer production for competent cells

100mM $CaCl_2$
$CaCl_2$ 1.471g
Distilled water 100ml

100mM $CaCl_2$ + 30% v/v glycerol
$CaCl_2$ 1.471g
Glycerol 30ml
Distilled water 70ml

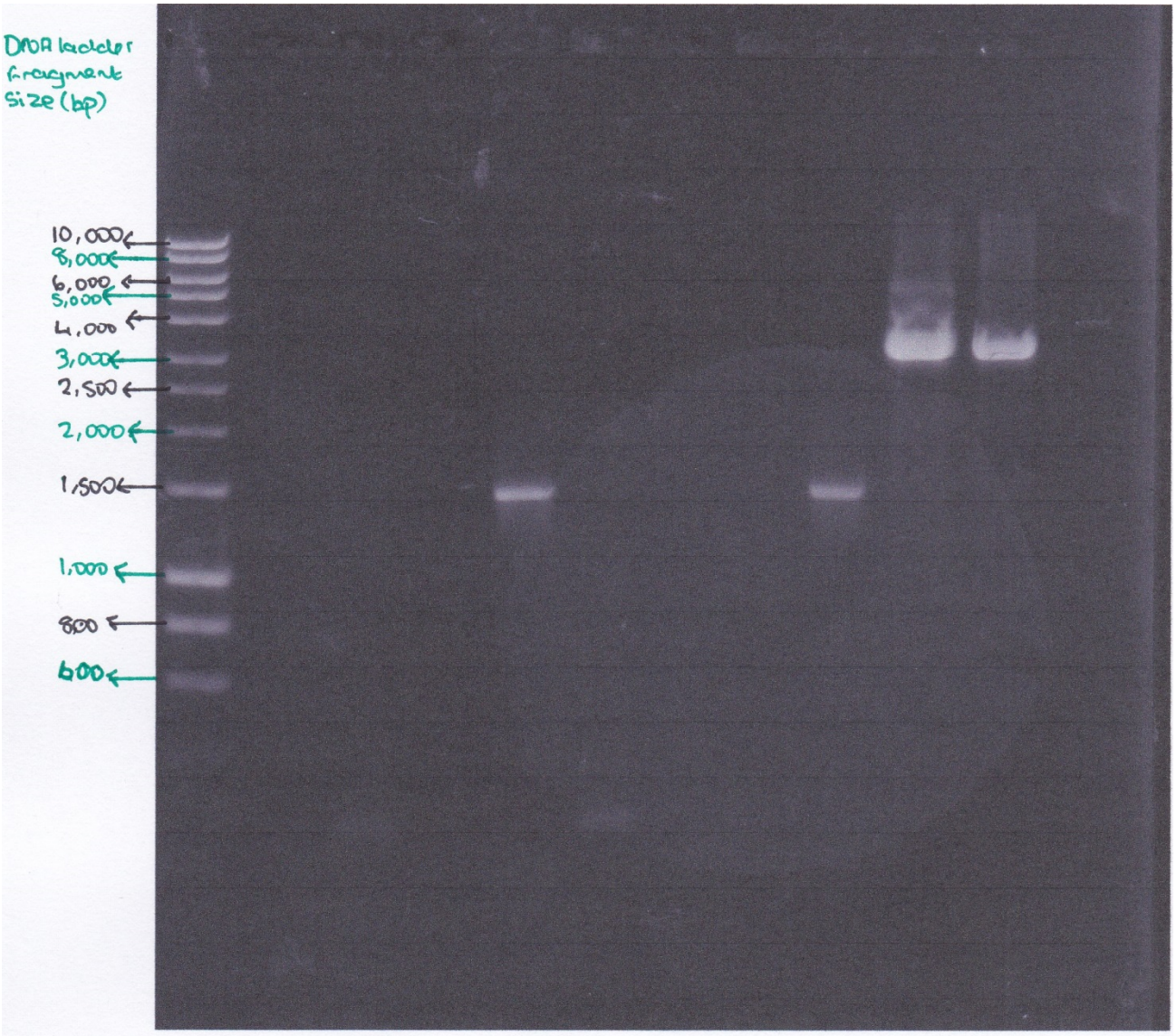
1. Inoculate 50µl strain into 10ml of LB solution, grow overnight at 37ºC

Friday 6th September

Gel of digested plasmid and PCR product

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 (NrfA 0.1)	3	3.5	13.5
4 (NrfA 1)	3	3.5	13.5
5 (norV 0.1)	3	3.5	13.5
6 (norV 1)	3	3.5	13.5
7 (NrfA 0.1)	3	3.5	13.5
8 (NrfA 1)	3	3.5	13.5
9(norV 0.1)	3	3.5	13.5
10(Colony 1 digest)	15	3.5	1.5
11(Colony 2 plasmid)	15	3.5	1.5



Lane 1: ladder, Lane 2:Colony 1 norV, Lane 3: Colony 1 norV, Lane 4: Colony 1 NrfA, Lane 5 : Colony 1 NrfA, Lane 6: Colony 2 norV, Lane 7: Colony 2 norV, Lane 8: Colony 2 NrfA, Lane 9: Colony 2 NrfA, lane 10: Colony 1 plasmid digest, lane 11:Colony 11 plasmid digest

Note: PCR in lanes 2, 4,5 and 8 were successful. Digests from both colonies were not.

Production of component cells

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

Time (minutes)	OD600
0	0.207
15	0.328
45	0.487

Note: growth of cells was stopped slightly too late.

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 cells at -80^{o}C

Purification of PCR products 2, 4, 5 and 8

1. Add 225µl of Buffer PB to PCR product.
2. Bind DNA by adding sample to QIAquick column. Centrifuge for 60 seconds at 13,000 rpm and discard flow through.
3. Wash by adding 0.75ml of Buffer PE and centrifuge for 60 seconds at 13,000 rpm, discard flow through.
4. Centrifuge column for a further 60 seconds at 13,000 rpm.
5. Place QIAquick columns into Eppendorf.
6. Elute by adding 50µl of Buffer EB to centre of the membrane. Leave stand for 1 minute, then centrifuge the column for 60 seconds and 13,000 rpm.
7. Store both norV and NrfA DNA at -20ºC.

Gel of digested plasmid (Ecor1 and Pst1)

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)	15	3.5	1.5
2 (digest 1)	15	3.5	1.5
3 (digest 1)	15	3.5	1.5
4 (digest 1)	15	3.5	1.5
5(digest 2)	15	3.5	1.5
6 (digest 2)	15	3.5	1.5
7(digest 2)	15	3.5	1.5

Lane 1 and 2: empty, lane 3: ladder, lane 4: empty, lane 5, 6 and 7: digest 1, lane 8:empty, lane 9, 10, and 11: digest 2.

Note: Neither digest was successful.

Restriction digests of 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 left on the bench over the weekend.