JULY

Date: Wednesday, 7/4

Objective(s):

PCR amplification of Fragments 1 - 7.

Day Summary:

PCR amplification of Fragment 5 did not produce any bands on the agarose gel after gel electrophoresis. PCR amplification for Fragment 5 needs to be repeated.

Procedure:

1. 7 PCR tubes were labelled for Fragments 1 - 7.

2. Reagents to the 7 PCR tubes for the 7 fragments were added as per the tables below.

   PCR tube 1 for Fragment 1:

Reagent	*Amount (µL)*
PrimeSTAR Max Premix	25.0
20 µM 3GT - p15a F Primer	0.75
20 µM ANS - JYaraC R Primer	0.75
A8C EL222 (< 200 ng)	4.0
ddH_2O	19.5
Total	50.0

   PCR tube 2 for Fragment 2:

Reagent	*Amount (µL)*
PrimeSTAR Max Premix	25.0
20µM LasR - KanR F Primer	0.75
20µM DFR - ColE R Primer	0.75
AHL ColE backbone (108 ng/µL)	1.8
ddH_2O	21.7
Total	50.0

   PCR tube 3 for Fragment 3:

Reagent	*Amount* (µL)
PrimeSTAR Max Premix	25.0
20µM F3H - LasR F Primer	0.75
20µM KanR - LasR R Primer	0.75
AHL ColE backbone (108 ng/µL)	1.8
ddH_2O	21.7
Total	50.0

PCR tube 4 for Fragment 4:

Reagent	*Amount* (µL)
PrimeSTAR Max Premix	25.0
RSF - plac F Primer	0.75
OsPKS - plac R Primer	0.75
EbK IPTG	3.5
ddH_2O	20.0
Total	50.0

PCR tube 5 for Fragment 5:

Reagent	*Amount* (µL)
PrimeSTAR Max Premix	25.0
AmpR - RSF F Primer	0.75
plac - RSF R Primer	0.75
pRSF (392 ng/µL)	0.5
ddH_2O	23.0
Total	50.0

PCR tube 6 for Fragment 6:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
4CL - AmpR F	0.75
RSF - AmpR R	0.75
MCS	1.60
ddH_2O	21.9
Total	50.0

PCR tube 7 for Fragment 7:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
OsPKS - MCS F Primer	0.75
con - MCS R Primer	0.75
MCS (120 ng/µL)	1.6
ddH_2O	21.9
Total	50.0

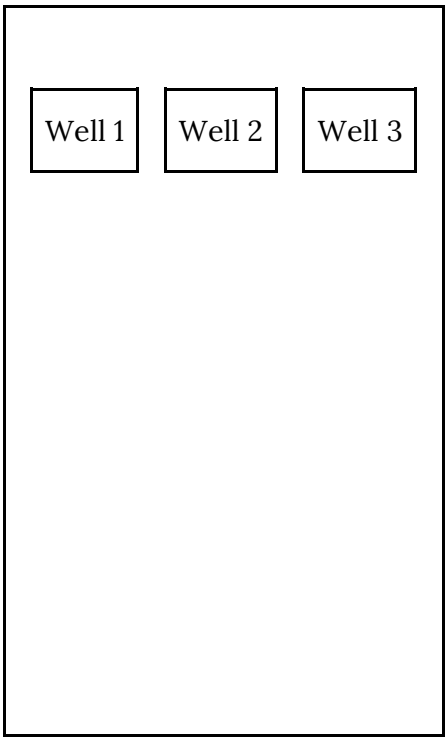
3. PCR tubes 1 - 7 were spun down for 5 secs to mix the reagents thoroughly before running PCR. Settings:

T (°C)	Time		
98	2 min		
98	10 sec		
55	5 sec	}	X 35 Cycles
72	35 sec		
72	2 min		

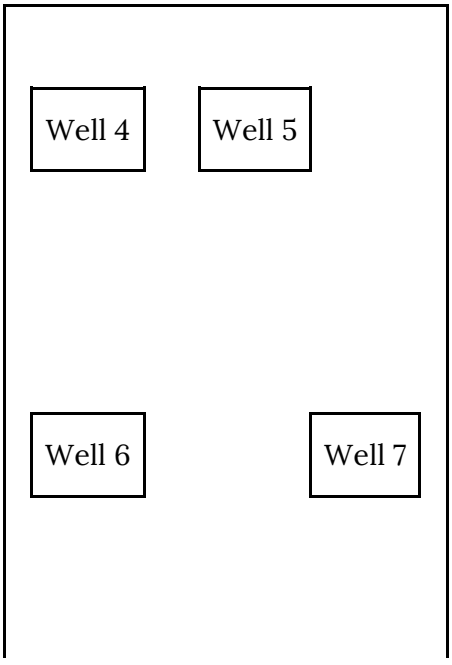
12	Hold

4. 2 pieces of 1% agarose gel were casted. EtBr was added as a nucleic acid stain (intercalating agent) for subsequent UV visualisation. The gels were casted in the configuration below because the bands were expected to be able to separate sufficiently after running through half the gel.

Agarose Gel A



Agarose Gel B



5. After PCR amplification was completed, the 7 samples were resuspended with 5X loading dye (1 part dye to 4 parts sample) before being loaded in the agarose wells.

   Amount of 5X loading dye to add to sample = 50 µL/4 = 12.5 µL
   Total volume to add to each cell = 50 µL + 12.5 µL = 62.5 µL

6. Gel electrophoresis was run for 30 min at 120 V for Gel A and 22 min at 120 V for Gel B.

7. 7 Eppendorf tubes 1 - 7 were labelled for Fragments 1 - 7 respectively. The masses of the 7 different Eppendorf tubes were weighed (Table 1, before).

8. After gel electrophoresis, the gels were visualised under UV light. The bands that were fluorescent under UV light were cut out from the gels and transferred into their respective Eppendorf tubes. Each tube was weighed again (Table 1, after). The mass of the cut gel was calculated by subtracting the original mass of the Eppendorf tube from the mass of the Eppendorf tube after transferring the cut gel into the tube. The amount of Buffer QG to add to each eppendorf carrying the cut gel was then estimated by multiplying the mass of the cut gel by 3.

9. The DNA from the cut gel was extracted as described in QIAgarose Gel Extraction Kit Protocol.

Table 1: Gel electrophoresis gel extraction preparation.

Tube	1	2	3	4	5	6	7
Before	1.1087	1.1028	1.1025	1.1086	No band	1.1002	1.1021
After	1.7003	1.7874	1.8254	1.7357	No band	1.8687	1.7807
Mass of Gel extracted	0.5916	1.6846	0.7229	0.6351	No band	0.76856	0.678
Amount of Buffer QC to add	1.7748	2.0538	2.1687	1.8753	No band	2.3055	2.0

10. The DNA concentration of each sample was then measured and reflected in Table 2 (below).

Table 2: DNA concentration of each fragment after PCR amplification.

Fragment Number	Fragment	Plasmid(s)	Concentration (ng/µL)
1	JYP15a - CMR - araC	2	115.0
2	KanR - ColE1	3,4,5	129.0
3	Las	3,5	132.0

4	lacI	1	125.0
5	RSF	1	-
6	AmpR	1	126.0
7	MCS	1	133.0

Additional comments: PCR amplification was successful for Fragments 1-4, 6 & 7. Failure of PCR amplification of Fragment 5 could be due to poor annealing of primers to target gene.

Date: Monday, 7/9
Objective(s):
PCR amplification of Fragments 5, 8 - 11.

Day Summary:
Successful PCR amplifications of all fragments.

Procedure:

1. 5 PCR tubes were labelled for Fragments 5, 8 - 11.

2. Reagents to the 5 PCR tubes for the 5 fragments were added as per the tables below.

   PCR tube 5 for Fragment 5:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
AmpR - RSF F Primer	0.75
plac - RSF R Primer	0.75
pRSF (392 ng/µL)	0.5
ddH_2O	23.0
Total	50.0

   PCR tube 8 for Fragment 8:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
COIE-DFR For	0.75
LasR-F3H Rev	0.75

F3H-DFR gBlock	0.5
ddH_2O	23
Total	50.0

PCR tube 9 for Fragment 9:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
Plac-Ospks F	0.75
MCS - Ospks R	0.75
Ospks mini prep	1.2
ddH_2O	22.3
Total	50.0

PCR tube 10 for Fragment 10:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
MCS - Con F	0.75
4CL - PAL R	0.75
PEaaat S mini prep	1.5
ddH_2O	22
Total	50.0

PCR tube 11 for Fragment 11:

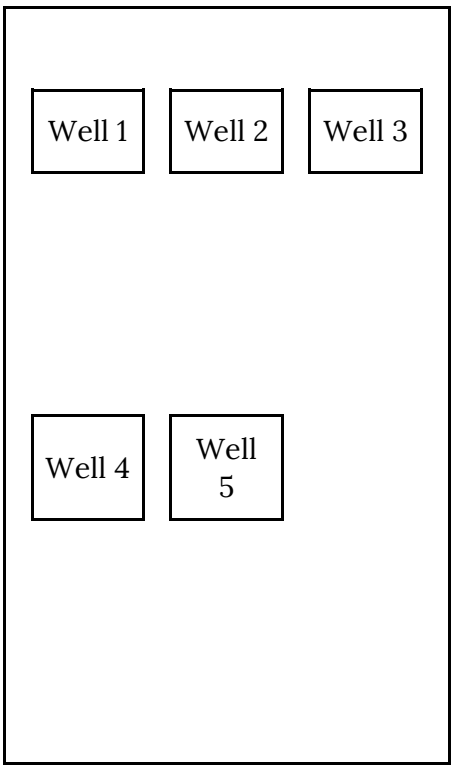
Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
4CL - PAL F	0.75
AmpR - 4Cl R	0.75
PEaaat S mini prep	1.5

ddH_2O	22
Total	50.0

3. PCR tubes 5, 8 - 11 were spun down for 5 secs to mix the reagents thoroughly before running PCR. Settings:

T (°C)	Time	
98	2 min	
98	10 sec	
55	5 sec	} X 35 Cycles
72	30 sec	
72	2 min	
12	Hold	

4. A 1% agarose gel was casted. EtBr was added as a nucleic acid stain (intercalating agent) for subsequent UV visualisation. The gels were casted in the configuration below because the bands were expected to separate sufficiently after running through half the length gel.



5. After PCR amplification was completed, the 5 samples were resuspended with 5X loading dye (1 part dye to 4 parts sample) before being loaded in the agarose wells.

   Amount of 5X loading dye to add to sample = 50 µL/4 = 12.5 µL
   Total volume to add to each cell = 50 µL + 12.5 µL = 62.5 µL

6. Gel electrophoresis was run for 22 min at 120 V.

7. 5 Eppendorf tubes 5, 8 - 11 were labelled for Fragments 5, 8 - 11 respectively. The masses of the 5 different Eppendorf tubes were weighed (Table 1, before).

8. After gel electrophoresis, the gel was visualised under UV light. The bands that were fluorescent under UV light were cut out from the gel and transferred into their respective Eppendorf tubes. Each tube was weighed again (Table 1, after). The mass of the cut gel was calculated by subtracting the original mass of the Eppendorf tube from the mass of the Eppendorf tube after transferring the cut gel into the tube. The amount of Buffer QG to add to each Eppendorf carrying the cut gel was then estimated by multiplying the mass of the cut gel by 3.

9. The DNA from the cut gel was extracted as described in QIAgarose Gel Extraction Kit Protocol.

10. The DNA concentration of each sample was then measured and reflected in Table 2 (below).

Table 2: DNA concentration of each fragment after PCR amplification.

Fragment Number	Fragment	Plasmid(s)	Concentration (ng/µL)
5	RSF	1	121.0
8	F3H - DFR	3,5	61.3
9	OsPKS	1	66.9
10	PAL	1	108.0
11	4CL	1	74.9

Date: Tuesday, 7/10

Objective(s):

Gibson assembly of Plasmids 1 and 3. Transformation of Plasmids 1 and 3 into DH5α competent cells before plating them onto LB + Amp and LB + Kan plates respectively.

Day Summary:

Gibson assembly was completed for Plasmids 1 and 3 and plated onto LB + Amp and LB + Kan plates respectively to form colonies overnight.

Procedure:

1. 2 Eppendorf tubes were labelled for Plasmids 1 and 3 respectively.

2. Reagents for the Gibson assembly for each of the plasmids were added to the 2 Eppendorf tubes as per the tables below.

   Eppendorf tube 1 for Gibson assembly for Plasmid 1 (PAL - 4CL - MCS - OsPKS):

Reagent	Amount (µL)
2X Gibson Assembly Master Mix	5.0
Lac I	0.3
OsPKS	0.5
MCS	0.3
PAL	0.6
4CL	0.6
AmpR	0.2
RSF	0.2
ddH2O	2.3

   Eppendorf tube 3 for Gibson assembly for Plasmid 3 (F3H - DFR):

Reagent	Amount (µL)
2X Gibson Assembly Master Mix	5.0
KanR - colE	0.4
F3H - DFR	1.0
Las	0.2
ddH2O	3.4

3. Spin down Eppendorf tubes 1 & 3 for 10 minutes to mix reagents thoroughly. Short spin only.

4. Carry out Gibson assembly for Eppendorf tubes 1 & 3. Settings: 50C for 1 hour and 12C infinite

5. 2 electroporation cuvettes and 2 Eppendorf tubes 1a & 3a were labelled for Plasmids 1 and 3 respectively.

6. 2 µL of Plasmid 1 and Plasmid 3 was pipetted into electroporation cuvettes 1 & 3 respectively. Keep on ice.

7. 50 µL of DH5α competent cells was pipetted into each of electroporation cuvettes 1 & 3. Wipe down the sides of the electroporation cuvettes. Electroporate.

8. The bunsen burner was turned on to keep the surrounding environment sterile prior to opening the LB bottle. 500µL of LB was added to each of electroporation cuvettes 1 & 3. Resuspend before transferring all contents to fresh Eppendorf tubes 1a & 3a respectively.

9. DH5α competent cells in Eppendorf tubes 1a & 3a were allowed to recover for 1 h at 37°C and 220 rpm.

10. One plate of LB + Kan agar was prepared and labelled Plate 3. One plate of LB + Amp was prepared and labelled Plate 1.

11. 100 µL of cells transformed with Plasmid 1 was plated onto the LB + Amp plate (Plate 1) and 100 µL of cells transformed with Plasmid 3 was plated on LB + Kan plate (Plate 3). Plates were incubated overnight at 37°C.

Date: Wednesday, 7/11

Objective(s):
Choice of colonies for overnight cultures in Plates 1 & 3.

Day Summary:
One colony was observed to grow on Plate 1. 2 colonies were chosen from Plate 3 (picture below) for overnight culture.

Overnight culture 1 for colony from Plate 1 was unsuccessful. Overnight culture for Plate 3 was successful.

Procedure:

1. 2 culture tubes were labelled F3H - DFR A & B.

2. 2 colonies from Plate 3 and 1 colony from Plate 1 were chosen to cultivate for overnight culture. They were scraped off and added to X µL of LB broth in culture tubes F3H - DFR A & B and PAL - 4CL - MCS - OsPKS respectively for overnight incubation at 37°C, 220 rpm.

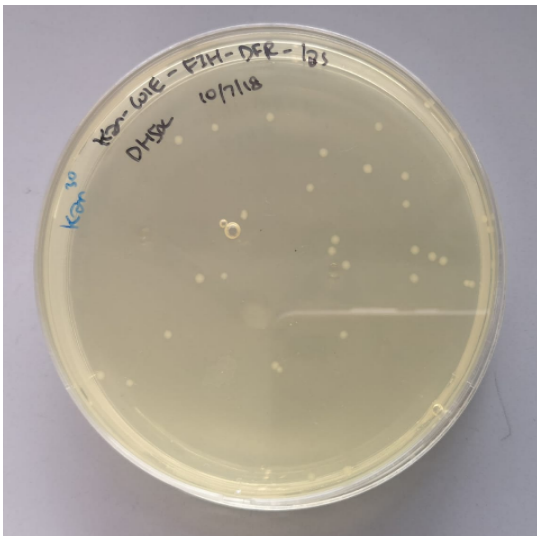


Image 1: Plate 3. Colonies that grew on Plate 3 (LB + Kan agar) are assumed to carry Plasmid 3, which confers kanamycin resistance.

Additional comments: Failure of overnight culture may be due to failure in Gibson assembly of Plasmid 1, or in transformation of Plasmid 1 to DH5α competent cells.

Date: Thursday, 7/12

Objective(s):

(a) DNA purification of Plasmid 3 for DNA sequencing.
(b) Repetition of transformation of Plasmid 1 into DH5α competent cells and plating onto LB + Amp agar plate to grow colonies.

Day Summary:

(a) DNA purification of Plasmid 3 in F3H - DFR A & B yielded insufficient (Less than 60ng/microl) concentration for DNA sequencing.
(b) One colony was observed to have grown on the Plate 1. YP verified

Overnight culture 1 for colony from Plate 1 was unsuccessful. Overnight culture for Plate 3 was successful.

Procedure:

1. DNA purification of Plasmid 3 from culture tubes F3H - DFR A & B was carried out as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol.

2. Plasmid concentrations of samples F3H - DFR A & B were measured and recorded in the table below.

Sample	Concentration (ng/µL)
F3H - DFR A	33.64
F3H - DFR B	50.02

3. 1 electroporation cuvette and 1 Eppendorf tube were labelled for Plasmids 1.

4. 2 µL of Plasmid 1 was pipetted into new Electroporation Cuvette 1. Keep on ice.

5. 50 µL of DH5α competent cells was pipetted into Electroporation Cuvette 1. Wipe down the sides of the electroporation cuvette. Electroporate.

6. The Bunsen burner was turned on to keep the surrounding environment sterile prior to opening the LB bottle. 500µL of LB was added to Electroporation Cuvette 1. Resuspend before transferring all contents to new Eppendorf Tube 1 respectively.

7. DH5α competent cells were in Eppendorf tubes 1 were allowed to recover for 1 h at 37°C and 220 rpm.

8. One plate of LB + Amp was prepared and labelled Plate 1.

9. 100 µL of cells transformed with Plasmid 1 was plated onto the LB + Amp plate (Plate 1) and incubated overnight at 37°C.

Date: Friday, 7/13

Objective(s):

(a) DNA purification of Plasmid 3 for DNA sequencing.
(b) An overnight culture of the single colony found on Plate 1 was grown overnight at 37°C, 220 rpm.

Day Summary:

(a) DNA purification of Plasmid 3 still yielded insufficient concentration for DNA sequencing. Another 2 colonies from Plate 3 were selected to be grown in an overnight culture at 37°C, 220 rpm.
(b) Overnight culture of single colony was successful.

Procedure:

1. DNA purification of Plasmid 3 from culture tubes F3H - DFR A & B was carried out as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol.
2. Plasmid concentrations of samples F3H - DFR A & B were measured and recorded in the table below.

Sample	Concentration (ng/µL)
F3H - DFR A	27.4
F3H - DFR B	19.5

3. 2 colonies were chosen from Plate 3 to cultivate for overnight culture. They were scraped off and added to X µL of LB broth in culture tubes F3H - DFR A & B for overnight incubation at 37°C, 220 rpm.

4. The single colony was scraped from Plate 1 and added to 6 mL of LB broth in new culture tube PAL - 4CL - MCS - OsPKS for overnight incubation at 37°C, 220 rpm.

Date: Saturday, 7/14
Objective(s):
DNA purification of Plasmids 1 and 3.

Day Summary:
DNA concentration for both plasmids were sufficiently high to be sent for sequencing.

Procedure:
1. DNA purification of Plasmid 3 from culture tubes F3H - DFR A & B and Plasmid 1 from culture tube PAL - 4CL - MCS - OsPKS was carried out as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol.
2. Plasmid concentrations of samples F3H - DFR A & B and PAL - 4CL - MCS - OsPKS were measured and recorded in the table below.

Sample	Concentration (ng/µL)
F3H - DFR A	91.3
F3H - DFR B	91.9
PAL - 4CL - MCS - OsPKS	102

Date: Tuesday, 7/17
Objective(s):
Analysis of sequencing results for Plasmid 3 samples F3H-DFR A & B and Plasmid 1 (de novo) sample PAL - 4CL - MCS - OsPKS.

Day Summary:
DNA plasmids were submitted for sequencing on 180716 and sequencing results returned on 180717. Sequencing for deNoVo was successful for this round. Sequencing for F3H was unsuccessful for this round.

Results:
F3H-DFR A
LasR-F3H_R: 2771-3760: Correct
F3H 216_R: Failed; genes were missing

F3H-DFR B
LasR-F3H_R: 2601-3748: Correct
F3H 216_R: Failed; genes were missing

De novo
plac- OsPKS _ F: 1588 - 2584: Correct
Possible solution: Design primer and sequence it downwards, after 2584.

OsPKS - 215E: 573 - 1748: Correct
DNA sequencing for deNoVo has been successful for this round.

Possible solution for F3H-DFR: Clone F3H - DFR A & B fragment, insert missing F3H 216_R using Gibson Assembly and resend for sequencing again.

Date: Thursday, 7/19
Objective(s):
PCR amplification of Fragments 12 & 13.

Day Summary:
PCR was successful. Despite low concentration of Fragment 13 after PCR, concentration is still successful for Gibson Assembly.

Procedure:

1. 2 PCR tubes were labelled for Fragments 12 & 13.

2. Reagents to the 2 PCR tubes for the 2 fragments were added as per the tables below.

   PCR tube 12 for Fragment 12:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
New JyaraC - ANS For	0.75
New 3GT - ANS Rev	0.75
New ANS gBlock	0.5
ddH_2O	23.0
Total	50.0

   PCR tube 13 for Fragment 13:

Reagent	Amount (µL)
PrimeSTAR Max Premix	25.0
New ANS - 3GT For	0.75
New P15a - 3GT Rev	0.75
New 3GT gBlock	0.5
ddH_2O	23

Total	50.0

3. PCR tubes 12 & 13 were spun down for 5 secs to mix the reagents thoroughly before running PCR. Settings:

T (°C)	Time	
98	2 min	
98	10 sec	
55	5 sec	} X 35 Cycles
72	20 sec	
72	2 min	
12	Hold	

4. 1 piece of 1% agarose gel was casted according to the configuration below. EtBr was added as a nucleic acid stain (intercalating agent) for subsequent UV visualisation.

5. After PCR amplification was completed, the 2 samples were resuspended with 5X loading dye (1 part dye to 4 parts sample) before being loaded in the agarose wells.

   Amount of 5X loading dye to add to sample = 50 µL/4 = 12.5 µL
   Total volume to add to each cell = 50 µL + 12.5 µL = 62.5 µL

6. Gel electrophoresis was run for 30 min at 120 V.

7. 2 Eppendorf tubes 1 & 2 were labelled for Fragments 12 & 13. The masses of the 2 different Eppendorf tubes were weighed.

8. After gel electrophoresis, the gels were visualised under UV light. The bands that were fluorescent under UV light were cut out from the gels and transferred into their respective Eppendorf tubes. Each tube was weighed again. The mass of the cut gel was calculated by subtracting the original mass of the Eppendorf tube from the mass of the Eppendorf tube after transferring the cut gel into the tube. The amount of Buffer QG to add to each Eppendorf carrying the cut gel was then estimated by multiplying the mass of the cut gel by 3.

9. 9. The DNA from the cut gel was extracted as described in QIAgarose Gel Extraction Kit Protocol.

10. 10. The DNA concentration of each sample was then measured and reflected in Table 1 (below).

Table 1: DNA concentration of each fragment after PCR amplification.

Fragment Number	Fragment	Plasmid(s)	Concentration (ng/μL)
12	ANS	2	116.30
13	3GT	2	18.36

Additional comments: The band for 3GT was very faint. May be due to low starting concentration. However, the final concentration is still sufficient for Gibson assembly.

Date: Friday, 7/20

Objective(s):

Gibson assembly of Plasmids 2 and 3. Transformation of Plasmid 2 & 3 into DH5α competent cells before plating them onto a LB + CmR plate and LB + KanR plate respectively.

Day Summary:

Gibson assembly was completed for Plasmids 2 and 3 and plated onto LB + CmR and LB + Kan plates respectively to form colonies overnight.

Procedure:

1. 2 Eppendorf tube was labelled for Plasmid 2 & 3.

2. Reagents for the Gibson assembly for Plasmid 2 & 3 were added to the Eppendorf tubes 2 & 3 as per the tables below respectively.

   Eppendorf tube 2 for Gibson assembly for Plasmid 2:

Reagent	Amount (μL)
2X Gibson Assembly Master Mix	5.0
New JYP15a - CmR - araC PCR	0.6
ANS PCR	0.3
3GT PCR	2.5
ddH2O	0.6

   Eppendorf tube 3 for Gibson assembly for Plasmid 3:

Reagent	Amount (μL)
2X Gibson Assembly Master Mix	5.0

F3H Partial Fragment	0.4
Las - KanR - ColE - DFR	1.2
ddH2O	3.4

3. Spin down Eppendorf tubes 2 & 3 for 10 secs to mix reagents thoroughly. Short spin only.

4. Carry out Gibson assembly for Eppendorf tube 2 & 3. Settings: 50C for 1 hour and 12°C for infinite hold.

5. 2 electroporation cuvettes and 2 Eppendorf tubes 2a & 3a were labelled for Plasmid 2 & 3 respectively.

6. 2 µL of Plasmid 2 & 3 each were pipetted into 2 Eppendorf tubes containing 50µL of DH5α competent cells respectively. Keep on ice.

7. The mixture in both Eppendorf tubes were resuspended. The entire contents of the 2 tubes were then pipetted into their respective electroporation cuvettes 2 & 3. Wipe down the sides of the electroporation cuvettes. Electroporate.

8. The Bunsen burner was turned on to keep the surrounding environment sterile prior to opening the LB bottle. 500µL of LB was added to each of electroporation cuvettes 2 & 3. Resuspend before transferring all contents to fresh Eppendorf tubes 2a & 3a respectively.

9. DH5α competent cells in Eppendorf tube 2a & 3a were allowed to recover for 45 min at 37°C and 220 rpm.

10. One plate of LB + CmR agar was prepared and labelled Plate 2. One plate of LB + KanR agar was prepared and labelled Plate 3.

11. 100 µL of cells transformed with Plasmid 2 was plated onto the LB + CmR plate (Plate 2). 100 µL of cells transformed with Plasmid 3 was plated onto the LB + KanR plate (Plate 3) The plates were incubated overnight at 37°C.

Date: Friday, 7/20

Day Summary:

dsDNA concentrations for Plasmid 2 (ANS 1 & ANS 2) were low after plasmid purification (<60 ng/µL) but were still sent for sequencing. Plasmid 3 (DFR 1 & DFR 2) were sent for sequencing. Naringenin was added to acidic, neutral and basic conditions to observe variations in its colour at these different conditions.

Procedure:

DNA Purification for Plasmids 2 and 3

1. DNA purification was carried out for the 4 plasmids (DFR 1, DFR 2, ANS 1 & ANS 2) as described in 'QIAprep Plasmid DNA Purification Using QIAprep Spin Miniprep Kit and a Microcentrifuge' protocol.

2. Plasmid concentrations of samples DFR 1, DFR 2, ANS 1 and ANS 2 were measured and recorded in the table below.

Sample	Concentration (ng/µL)
DFR 1	52.3
DFR 2	70.1
ANS 1	44
ANS 2	42.2

Characterisation of Naringenin

1. Prepare 2 50 mL glass bottles for the preparation of (i) 40 mL Milli-Q, (ii) pH 2 and (iii) pH 12.

2. Insert a stirring magnet into bottles (ii) and (iii) to facilitate stirring when the acid and base are added in respectively.

3. Measure the pH of pure Milli-Q using a pH probe. The pH of Milli-Q is measured to be pH 6.

4. To prepare (ii) pH 2 (acidic), drops of 6N HCl were added into the bottle until pH 1.6 was reached.

5. To prepare (iii) pH 12 (basic), drops of 1M NaOH were added into the bottle until pH 12 was reached.

6. pH of the naringenin stock that is dissolved in DMSO (100 mM) was measured to be ~pH 6 (about the same pH as the Milli-Q solution).

7. 5 µL of 100 mM naringenin dissolved in DMSO was set as the standard volume of naringenin used across all tests. Testing was carried out as per follows:

Test	pH of solution added	pH of final mixture	Colour	Comments
1	1.6	3	Clear	-
2	6	4	Clear	-
3	12	8	Clear	-
4	14 (1M NaOH)	10	Orange	Addition of 5, 10, 15 µL 1M NaOH all produced the same orange.

5	1 (1M HCl)	1	Clear	-

After the first round of testing, it was clear that the addition of a highly concentrated base caused the conversion of naringenin from a clear (or very pale yellow) liquid to a deep orange. Further testings are as follows:

Test	1st	2nd	3rd	Final pH	Colour
1	40 µL 1M NaOH	15 µL 1M HCl	-	1	Yellow but turns orange eventually.
2	5 µL 1M NaOH	20 µL 1M HCl	-	1	Yellow
3	10 µL 1M NaOH	20 µL 1M HCl	-	1	Yellow
4	15 µL 1M NaOH	20 µL 1M HCl	-		Yellow
5	15 µL 1M HCl	40 µL 1M NaOH	-		Orange
6	5 µL 1M NaOH	20 µL 1M HCl	40 µL 1M NaOH		Orange

Date: Tuesday, 7/24

Objective(s):
Running HPLC of naringenin standard

Day Summary:
Overnight running of HPLC of different concentrations of pure naringenin

Procedure:

1. 100 mM concentration of pure naringenin was thawed.

2. The 100 mM concentration was then diluted to the following concentrations; 1 mM, 0.75 mM, 0.5 mM and 0.1 mM, using DMSO as naringenin is hydrophobic. Once the dilution was done, the tubes were placed in a centrifuge for a short spin.

3. Four HPLC vials were prepared and 100µL of each dilution were transferred into their respective vials. The vials were then carefully inspected for bubbles.
4. The buffer system used was 100 mM Ammonium acetate and 100% acetone. The pumps were then purged with their respective buffer.

5. The column was connected to the system and the samples were keyed in.

6. The column was attached to the HPLC machine using the arrow on the column as reference. It was ensured that there was no leakage and that the pressure was maintained below 100 bar.

7. HPLC cycle was conducted in the following manner:

Action	*Time (mins)*	*Volume (µL)*	*Remarks*
Flush	15	1	Methanol
Equilibrate	15	1	Methanol
Blank	46	10	-
Equilibrate	15	1	Methanol
100µM of naringenin	46	10	-
Equilibrate	15	1	Methanol
500µM of naringenin	46	10	-
Equilibrate	15	1	Methanol
750µM of naringenin	46	10	-
Equilibrate	15	1	Methanol
1mM of naringenin	46	10	-
Equilibrate	15	1	Methanol
Flush	15	1	Methanol

Date: Wednesday, 7/25

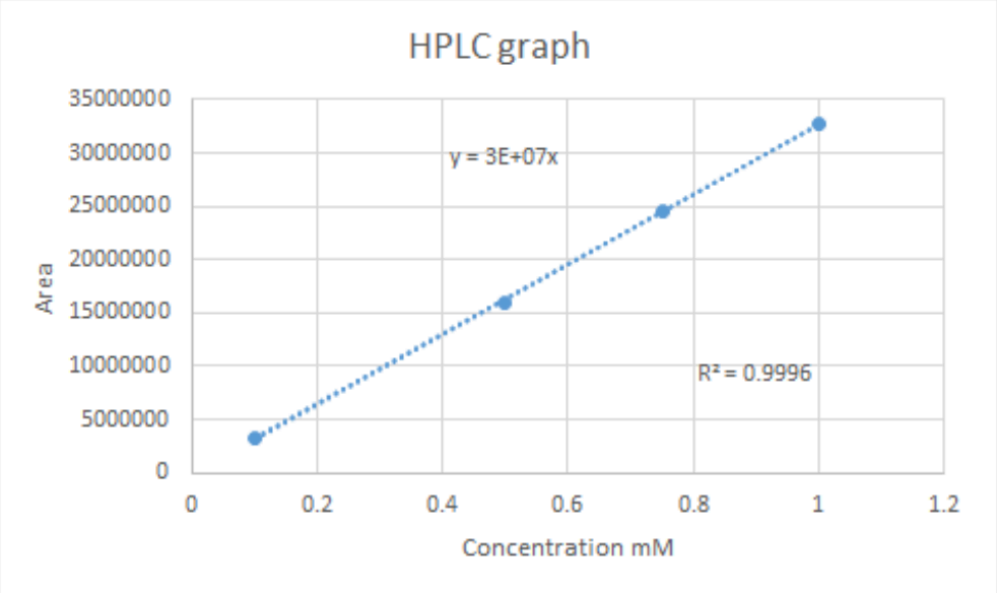
Objective(s):

Analyse the HPLC results of standard naringenin

Day Summary:

(a) The absorbance vs time graph has definite peaks.
(b) A linear relation was obtained between area and concentration.

Results:



Date: Tuesday, 7/31

Day Summary:

Induction of Plasmids 2 & 3 at 30/37°C for 2 days after OD has reached 0.6. Negative controls were set up (uninduced).

Procedure:

Samples

No.	Sample	Temperature (°C)	Duration (Days)
1	F3H-DFR Control	30	2
2	F3H-DFR Induced	30	2
3	F3'H-FNS Control	30	2
4	F3'H-FNS Induced	30	2
5	F3H-DFR Control	37	2
6	F3H-DFR Induced	37	2
7	F3'H-FNS Control	37	2
8	F3'H-FNS Induced	37	2

Samples 2 and 6 were induced with 1 mM AHL inducer at for two days. Samples 4 and 8 were induced with 200 nM tetracycline and **kept in the dark** for two days. All samples were left in the shaking incubator at their respective temperatures.