Wet lab workflow

SUNDAY, 01/08/2021

Standardization/Calibration of Pipettes:

1. All the pipettes that we plan to work with while performing our wet lab works were collected for the process of calibration.
2. Four members of the wet lab team were involved in performing this work and was divided into two groups each with two members.
3. Eppendorf tubes, distilled water, beakers, weighing machine and pipette tips were taken for performing the process.
4. In Room temperature water has density almost equals to 1gm/ml. So corresponsding weight shows the amount of water pipetted.
5. Procedure followed for standardisation -

- The pipette being calibrated was at first set to a certain value in between the range of the pipette.
- Then a 1.5ml eppendorf tube was taken and tared in a weighing machine.
- Then 1ml of water was filled in the eppendorf using the pipette appropiate number of times.
- Then the eppendorf with water was weighed.
- For standardizing 0.2 to 2 microleter pipette we took 0.01ml of water by setting the pipette volume at 1 microleter.
- We checked for accuracy upto three significant digits.

MONDAY, 02/08/2021

Hood cleaning, fumigation and lab space cleaning :

- All the filters and vents of Laminar air flows were cleaned and the workspace inside the laminar airflow hood were cleaned thoroughly with 70% ethanol and was put on UV for 15mins.
- All the storage facilities (cupboards, almirahs etc.,) were cleaned.
- Fridges (4° C and -20° C) were cleaned and all old unusable reagents and kits were discarded.
- Fumigation process was performed in our lab with formaldehyde and potassium acetate and the labs were sealed post fumigation for 3 days.

THURSDAY, 05/08/2021

1. Cupboards, shelves and almirahs in our working labs were labelled for stocking up of wet lab essentials and easily finding them while working.
2. Pipettes, pipette tip boxes to be used inside the laminar airflow were sepertely labelled from the ones to be not used in the hood.
3. Everything (tissue rolls, spray bottles, aluminium foils, pipette tips, glasswares) were organised accordingly in the shelves.

FRIDAY, 06/08/2021

1. Common Reagents were procured from the common chemistry and biology teaching labs of our Institute in 50ml falcon tubes and was sealed with parafilm
2. The reagents those were procured are -

SATURDAY, 07/08/2021

Received our reagents from Promega Corporation.

MONDAY, 09/08/2021

Before commencement of experiments, medias and agar plates for the same were prepared.

1. LB Broth preparation - (650ml was made)

- 15g of LB premix was mixed with distilled H_2O [1L broth needs 25g of LB premix]
- After dissolving the premix more dH_2O was added to compensate the volume and achieve the total volume of the media being made.
- The conical flask was then made airtight with cotton plug and was sealed with aluminium foil.

2. LB agar preparation - (650ml was made)
- 16.25g of LB premix was mixed with 9.75g of agar in dH_2O. [For 1L of LB agar broth we need 25g of LB premix and 15g agar in dH_2O]
- After dissolving the premix more dH_2O was added to compensate the volume and achieve the total volume of the media being made.
- The conical flask was then made airtight with cotton plug and was sealed with aluminium foil.

The medias were autoclaved at 15 lbs pressure at 121°C for 15 mins. Then the flask with LB Broth was cooled post autoclaving at room temperature before placing stock at 4 °C.
The LB Agar containing flask was cooled a little bit and was then poured in plates inside the hood, each plate was labeled properly before pouring and after some time was kept at 4°C after properly parafilming.

TUESDAY, 10/08/2021

We plan to start with the experiment of biofilm formation as we need to quantify which media produces the maximum amount of biofilm for *Pseudomonas aeruginosa.* So, some more media were made -

1. TSB Media (Tryptic Soy Broth) [100ml was made]-
- 3g of TSB premix was mixed in 100ml of miliQ H_2O. (30g of premix in 1 L)
2. NB Media (Nutrient Broth) [100ml was made] -
- 1.3g of NB premix was mixed in 100ml of miliQ H_2O. (13g of premix in 1L)
3. BH media (Bushnell and Hass media) - (100ml was made)
For 1L BH media preparation we need

BH media Reagents

	Reagents	Amount
1	NaNO3	1g
2	KH2PO4	1g
3	Ammonium Hypophosphate	1g
4	MgSO4.7H20	0.2g
5	FeCl3	0.05g
6	CaCl2.2H2O	0.02g
7	2% Dextrose	20g

- So, accordingly for 100ml of BH media we took 0.1g,0.1g,0.002g,0.005g,0.002g, and 2g of each in order as mentioned in the reagents table.
- Then the pH of the media was adjusted to 7.

All the media containing conical flasks were plugged with cotton and wrapped with aluminum foil and was autoclaved for 15mins at 121 °C. They were cooled at room temperature and then stored at 4 °C.

4. Crystal Violet solution preparation - [100ml was made] (1g in 1L)
- 0.1g of crystal violet was added to 100ml of dH_2O and then magnetic stirring was performed.

WEDNESDAY, 11/08/2021

- We received our ordered products from Tarson

- Tip boxes , beakers, test tubes, eppendorfs (1.5ml and 2ml) were autoclaved and post autoclave were organized as per our needs for the biofilm formation experiment.

THURSDAY, 12/08/2021

We start with our biofilm formation and quantification with different medias for *Pseudomonas aeruginosa.*

1. Primary culture for *Pseudomonas aeruginosa* was done in three different media (NB, LB, TSB) along with controls for each in 15ml falcon tubes and was kept in the incubator at 37°C in shaking condition at 10:30 pm.
2. The protocol for biofilm assay on 96 well plate was chalked out taking references from literatures of previously performed experiments.

FRIDAY, 13/08/2021

At 10:30 am the primary cultures were taken out from the incubator and all controls were found to be non-contaminated.
OD of the primary cultures were measured

Hence we proceeded with the further processes of the Biofilm assay protocol -

- Dilution - 1:100 dilution of all primary cultures were done in respective media and also other two media were plated in 96-well plates, i.e., 990µl of media and 10µl of primary culture.
- Each well was filled with 100µl of the dilution made.
- Three replicates of each were plated along with blanks (pure media); (P) for pseudomonas positive i.e., bacterial primary culture

Well 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	LB (B)	LB (B)	LB (B)	TSB (B)	TSB (B)	TSB (B)	NB (B)	NB (B)	NB (B)			
B	LB x LB (P)	LB x LB (P)	LB x LB (P)	LB x TSB(P)	LB x TSB (P)	LB x TSB (P)	LB x NB (P)	LB x NB (P)	LB x NB(P)			
C	TSB X LB (P)	TSB X LB (P)	TSB X LB (P)	TSB X TSB (P)	TSB X TSB (P)	TSB X TSB (P)	TSB X NB (P)	TSB X NB (P)	TSB X NB (P)			
D	NB X LB (P)	NB X LB (P)	NB X LB (P)	NB X LB (P)	NB X LB (P)	NB X LB (P)	NB X NB (P)	NB X NB (P)	NB X NB (P)			
E												
F												
G												
H												

- The Well plate was incubated in the incubator at 37 °C in non-shaking condition for 24hrs.

SATURDAY, 14/08/2021

OD of the wells were measured at 570nm.

TUESDAY, 17/08/2021

Brainstorming sessions for troubleshooting the biofilm assay protocol. Several new changes were made to the existing protocol to check our hypothesis.
The wet lab team members were subdivided into small groups [Group 1,2,3, and 4]
Changes:

1. We found some of the media to be completely dried up. So we planned to fill the empty wells with water so that it keeps the atmosphere moist.
2. Significant biofilm was not formed so upon doing some more literature survey we found on the 3rd-day biofilm formation shoots up. So incubation time for secondary culture was changed to 72 hours.

3. To get significant result dilution was changed to 1:10 and along with that proportionate dilution was done based on OD.

WEDNESDAY, 18/08/2021

Biofilm assay by Group 1 and Group 4

1. Primary culture of *Pseudomonas aeruginosa* -
- All required glass wares and plastic wares required for the primary culture were UV sterilized for 15 mins.
- 5ml of each media was placed in 50ml falcon tube with positive and controls.
- The tubes were then kept in the incubator for 12 hours at 37°C.

2. Media preparation - LB, NB and TSB medias were prepared each of 300ml and was autoclaved and stored at 4°C.

THURSDAY, 19/08/2021

1. Well plate by Group 4
- OD were checked after the overnight culture.
- Then the further steps of the Biofilm assay protocol were followed -
- This time dilution of 1:10 was done
- The orientation of the plate is as follows -

Well plate of Group 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (B)	LB (B)	LB (B)	TSB (B)	TSB (B)	TSB (B)	NB (B)	NB (B)	NB (B)		Water
C	Water	LB X LB	LB X LB	LB X LB	LB X TSB	LB X TSB	LB X TSB	LB X NB	LB X NB	LB X NB		Water
D	Water	TSB X LB	TSB X LB	TSB X LB	TSB X TSB	TSB X TSB	TSB X TSB	TSB X NB	TSB X NB	TSB X NB		Water
E	Water	NB X LB	NB X LB	NB X LB	NB X TSB	NB X TSB	NB X TSB	NB X NB	NB X NB	NB X NB		Water
F	Water											Water
G	Water											Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

The well plate was then incubated for 72 hours

Well of group 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (B)	LB (B)	LB (B)	TSB (B)	TSB (B)	TSB (B)	NB (B)	NB (B)	NB (B)		Water
C	Water	LB X LB	LB X LB	LB X LB	LB X TSB	LB X TSB	LB X TSB	LB X NB	LB X NB	LB X NB		Water
D	Water	TSB X LB	TSB X LB	TSB X LB	TSB X TSB	TSB X TSB	TSB X TSB	TSB X NB	TSB X NB	TSB X NB		Water
E	Water	NB X LB	NB X LB	NB X LB	NB X TSB	NB X TSB	NB X TSB	NB X NB	NB X NB	NB X NB		Water
F	Water											Water
G	Water											Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

The well plate was then incubated for 72 hour

2. Primary culture of Group 3 - Primary culture of *Pseudomonas aeruginosa* was done in three different medias, and kept in the incubator at 37°C for 12 hrs [kept at 09:30 am]

3. Primary Culture of Group 2 - Primary culture of *Pseudomonas aeruginosa* was done in three different medias, and kept in the incubator at 37°C for 12 hrs [kept at 10:45 am]

4. OD was measured for the primary culture of Group 3 and Group 2 and then the protocol for biofilm assay was followed.

- 1:10 dilution was done, and 200µl was filled in each well.
- The orientation of the plate is as follows -

Well of group 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water											Water
C	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
D	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
E	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
F	Water											Water
G	Water											Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

The well plate was then incubated for 72 hour in shaking condition at 37°C

Well of Group 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water											Water
C	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
D	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
E	Water		NB (P) X NB	LB (P) X LB	TSB (P) X TSB		NB (B)	LB (B)	TSB (B)			Water
F	Water											Water
G	Water											Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

The well plate was then incubated for 72 hour in shaking condition at 37°C

SUNDAY, 22/08/2021

- Protocols prior to plate OD measurement were done as per the biofilm assay protocol.
- OD measured for the plates of Group 1,2,3, and 4.

Analysis:

1. There were discontinuous errors in terms of the measurement. So it was decided to do it for 4-replicates, so even if we get a discontinuous error discarding that we can make a significant statistical average of the 3-data set.

MONDAY, 23/08/2021

1. Primary cultures of *Pseudomonas aeruginosa* were made by Group 1 and 2 and were incubated for 12hrs in incubator at 37°C for 12 hours.
2. Media (LB,NB, and TSB) were prepared.

TUESDAY, 24/08/2021

1. Primary culture of *Pseudomonas aeruginosa* was done by Group 3 and was incubated for 12hrs in incubator at 37°C.
2. OD measurement of the primary culture of Group 1 and 2 was performed and then the next steps as per the protocol of biofilm assay was performed.
3. The orientation of the well plates are as follows -

- Proportionate dilution was done for 0.1 OD and 0.05 OD and 200µl was filled in each well.
- Four replicates for each type was filled in the well plate.

Well group 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB' (C)	LB' (C)	LB' (C)	LB' (C)			LB' (P)	LB' (P)	LB' (P)	LB' (P)	Water
C	Water	NB' (C)	NB' (C)	NB' (C)	NB' (C)			NB' (P)	NB' (P)	NB' (P)	NB' (P)	Water
D	Water	TSB' (C)	TSB' (C)	TSB' (C)	TSB' (C)			TSB' (P)	TSB' (P)	TSB' (P)	TSB' (P)	Water
E	Water	LB" (C)	LB" (C)	LB" (C)	LB" (C)			LB" (P)	LB" (P)	LB" (P)	LB" (P)	Water
F	Water	NB" (C)	NB" (C)	NB" (C)	NB" (C)			NB" (P)	NB" (P)	NB" (P)	NB" (P)	Water
G	Water	TSB" (C)	TSB" (C)	TSB" (C)	TSB" (C)			TSB" (P)	TSB" (P)	TSB" (P)	TSB" (P)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

′ = 0.1 OD ″ = 0.05 OD. The well plate was then incubated for 36 hour in shaking condition at 37°C

Well for Group 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB' (C)	LB' (C)	LB' (C)	LB' (C)			LB' (P)	LB' (P)	LB' (P)	LB' (P)	Water
C	Water	NB' (C)	NB' (C)	NB' (C)	NB' (C)			NB' (P)	NB' (P)	NB' (P)	NB' (P)	Water
D	Water	TSB' (C)	TSB' (C)	TSB' (C)	TSB' (C)			TSB' (P)	TSB' (P)	TSB' (P)	TSB' (P)	Water
E	Water	LB" (C)	LB" (C)	LB" (C)	LB" (C)			LB" (P)	LB" (P)	LB" (P)	LB" (P)	Water
F	Water	NB" (C)	NB" (C)	NB" (C)	NB" (C)			NB" (P)	NB" (P)	NB" (P)	NB" (P)	Water
G	Water	TSB" (C)	TSB" (C)	TSB" (C)	TSB" (C)			TSB" (P)	TSB" (P)	TSB" (P)	TSB" (P)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

′ = 0.1 OD ″ = 0.05 OD. The well plate was then incubated for 72 hour at 37°C

WEDNESDAY, 25/08/2021

1. OD was measured for the primary culture of Group 3 and Group 2 and then the protocol for biofilm assay was followed.

- 1:10 dilution as well as proportionate dilution for 0.01 was done, and 200µl was filled in each well.
- The orientation of the plate is as follows -

well for Group 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (+) X NB	LB(+) X LB	LB(+) X TSB	NB(+) X NB	NB(+) X LB	NB(+) X TSB	TSB(+) X NB	TSB(+) X LB	TSB(+) X TSB	LB (C)	Water
C	Water	LB (+) X NB	LB(+) X LB	LB(+) X TSB	NB(+) X NB	NB(+) X LB	NB(+) X TSB	TSB(+) X NB	TSB(+) X LB	TSB(+) X TSB	LB (C)	Water
D	Water	LB (+) X NB	LB(+) X LB	LB(+) X TSB	NB(+) X NB	NB(+) X LB	NB(+) X TSB	TSB(+) X NB	TSB(+) X LB	TSB(+) X TSB	LB (C)	Water
E	Water	LB (+) X NB	LB(+) X LB	LB(+) X TSB	NB(+) X NB	NB(+) X LB	NB(+) X TSB	TSB(+) X NB	TSB(+) X LB	TSB(+) X TSB	LB (C)	Water
F	Water	NB (C)	NB (C)	TSB (C)	TSB (C)	NB (PD)	NB (PD)	LB (PD)	LB (PD)	TSB (PD)	TSB (PD)	Water
G	Water	NB (C)	NB (C)	TSB (C)	TSB (C)	NB (PD)	NB (PD)	LB (PD)	LB (PD)	TSB (PD)	TSB (PD)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72hour at 37°C

2. Primary culture of *Pseudomonas aeruginosa* was done by Group 4 and was incubated for 12hrs in incubator at 37°C [at 1 am]
3. OD was measured for the primary culture of Group 4 and then the protocol for biofilm assay was followed. [at 1:10pm]
- 1:10 dilution as well as proportionate dilution for 0.01 was done, and 200µl was filled in each well.
- The orientation of the plate is as follows -

Well for group 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (C)	LB (C)	LB (C)	LB (C)	TSB(+) X LB	TSB(+) X LB	TSB(+) X LB	TSB(+) X LB	LB (PD)	LB (PD)	Water
C	Water	TSB (C)	TSB (C)	TSB (C)	TSB (C)	TSB(+) X TSB	TSB(+) X TSB	TSB(+) X TSB	TSB(+) X TSB	LB (PD)	LB (PD)	Water
D	Water	NB (C)	NB (C)	NB (C)	NB (C)	TSB(+) X NB	TSB(+) X NB	TSB(+) X NB	TSB(+) X NB	TSB (PD)	TSB (PD)	Water
E	Water	LB(+) X LB	LB(+) X LB	LB(+) X LB	LB(+) X LB	NB(+) X LB	NB(+) X LB	NB(+) X LB	NB(+) X LB	TSB (PD)	TSB (PD)	Water
F	Water	LB(+) X TSB	LB(+) X TSB	LB(+) X TSB	LB(+) X TSB	NB(+) X TSB	NB(+) X TSB	NB(+) X TSB	NB(+) X TSB	NB (PD)	NB (PD)	Water
G	Water	LB(+) X NB	LB(+) X NB	LB(+) X NB	LB(+) X NB	NB(+) X NB	NB(+) X NB	NB(+) X NB	NB(+) X NB	NB (PD)	NB (PD)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72 hour in still condition at 37°C

FRIDAY, 27/08/2021

1. Plate OD measured for group 1 and group 2

2. Media was prepared

SATURDAY, 28/08/2021

1. OD of plate was measured for group 3 and group 4
2. Primary Culture of of *Pseudomonas aeruginosa* was done by Group 3 and was incubated for 12hrs in incubator at 37°C [at 10:22 pm]

Analysis:

1. Contamination in control made us speculate the fact that there maybe some diffusive problems in the well plate, which was later confirmed by literature. So it was decided to give spaces between wells.

SUNDAY, 29/08/2021

1. Primary Culture of of *Pseudomonas aeruginosa* was done by Group 3 and was incubated for 12hrs in incubator at 37°C [at 7:45am]
2. OD of the primary culture was measured for group 3.
3. The pattern of well plate is - [only proportionate dilution was done]

Well for group 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	NB (PD)		NB (PD)		NB (PD)		NB (PD)				Water
C	Water	LB (PD)		LB (PD)		LB (PD)		LB (PD)				Water
D	Water	TSB (PD)		TSB (PD)		TSB (PD)		TSB (PD)				Water
E	Water	NB (C)		NB (C)		NB (C)		NB (C)				Water
F	Water	LB (C)		LB (C)		LB (C)		LB (C)				Water
G	Water	TSB (C)		TSB (C)		TSB (C)		TSB (C)				Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72 hour in still condition at 37°C

4.

- OD of the primary culture was measured for group 1.
- The pattern of well plate is - [only proportionate dilution was done]

Well FOR GROUP 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (PD)	LB (PD)	LB (PD)	LB (PD)							Water
C	Water	NB (PD)	NB (PD)	NB (PD)	NB (PD)							Water
D	Water	TSB (PD)	TSB (PD)	TSB (PD)	TSB (PD)							Water
E	Water							LB (C)	LB (C)	LB (C)	LB (C)	Water
F	Water							NB (C)	NB (C)	NB (C)	NB (C)	Water
G	Water							TSB (C)	TSB (C)	TSB (C)	TSB (C)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72 hour in still condition at 37°C

5. Primary Culture of of *Pseudomonas aeruginosa* was done by Group 2 and was incubated for 12hrs in incubator at 37°C [7:45 PM]

MONDAY, 30/08/2021

1. OD of the primary culture was measured for group 2.
2. The pattern of well plate is - [only proportionate dilution was done]

Well for group 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	LB (PD)	LB (PD)	LB (PD)	LB (PD)							Water
C	Water	NB (PD)	NB (PD)	NB (PD)	NB (PD)							Water
D	Water	TSB (PD)	TSB (PD)	TSB (PD)	TSB (PD)							Water
E	Water							LB (C)	LB (C)	LB (C)	LB (C)	Water
F	Water							NB (C)	NB (C)	NB (C)	NB (C)	Water
G	Water							TSB (C)	TSB (C)	TSB (C)	TSB (C)	Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72 hour in still condition at 37°C

3. Primary Culture of of *Pseudomonas aeruginosa* was done by Group 3 and was incubated for 12hrs in incubator at 37°C.
4. Competent cells of E.coli DH5α and BL21 were prepared following the protocol and was stored in -80°C.

TUESDAY, 31/08/2021

1. OD of the primary culture was measured for group 3.

2. The pattern of well plate is - [only proportionate dilution was done]

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
B	Water	NB (PD)		NB (PD)		NB (PD)		NB (PD)				Water
C	Water		LB (PD)		LB (PD)		LB (PD)		LB (PD)			Water
D	Water	TSB (PD)		TSB (PD)		TSB (PD)		TSB (PD)				Water
E	Water		NB (C)		NB (C)		NB (C)		NB (C)			Water
F	Water	LB (C)		LB (C)		LB (C)		LB (C)				Water
G	Water		TSB (C)		TSB (C)		TSB (C)		TSB (C)			Water
H	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water

PD = Proportionate Dilution. The well plate was then incubated for 72 hour in still condition at 37°C

3.

- 0.5 M EDTA stock solution (30ml) was made.

1. 5.583 grams of EDTA disodium salt was weighed out
2. It was then dissolved in 25ml of miliQ water and the pH was adjusted with solid NaOH to obtain a pH of 8.0
3. Add 5ml of miliQ water to make the total volume to 30ml
4. Then it was autoclaved.

- 250ml of 50X TAE buffer was made.

1. 60.5g of Tris base was weighed out and dissolved in approximately 200ml of miliQ water
2. Then 14.275ml of 100% glacial acetic acid was added carefully.
3. Then 25ml of of 0.5M EDTA (pH 8) was added
4. pH of buffer should be adjusted around 8.5
5. Then it was stored at room temperature.

- 1X TAE working solution was made

1. 20ml of 50X TAE was mixed with 1000ml of miliQ water.

- 1M NaOH solution was made for pH adjustments

1. 20 ml of 1M NaOH was prepared by adding 0.8g of NaOH pellets in 20ml of dH_2O and then was stored in a falcon tube.

- 50ml of SOB and SOC media was made following the protocol present in the Experiments page.

All buffers and stock solutions were made following the protocols.

WEDNESDAY, 01/09/2021

1. Plate OD was measured for Group 1 at 570nm.
2. 200ml each of LB media (Broth) and Lb agar was prepared and autoclaved at 15 lbs pressure at 121°C for 15 mins. Then the LB agar was poured on Petri plates inside the hood. All were then stored in 4°C
3. Chloramphenicol stock solution of 5ml (25mg/ml) was prepared and kept in 25ml falcon in 4°C.
4. Glucose sterilization was done for the completion of SOC media preparation. Then SOC media was autoclaved and stored in 4°C.

THURSDAY, 02/09/2021

1. Plate OD was measured for Group 2 and Group 3 were measured.
2. 1ml of NFW was aliquoted in 1.5 ml of eppendorfs inside the hood.

3. RFP device was resuspended from kit plate 5 -230.
4. Transformation was performed to check the competency of the E.coli DH5α competent cells made. protocols mentioned in the experiments page were followed.

FRIDAY, 03/09/2021

1. LB agar media was prepared and was autoclaved. Post autoclaved were poured into plates and stored in 4°C.
2. We did not get colonies for the transformation done on 2nd September.

SATURDAY, 04/09/2021

1. Transformation performed again using pUC19 plasmid.

SUNDAY, 05/09/2021

1. Transformation done on 4th September was successful.

MONDAY, 06/09/2021

1. LB Agar (Chloramphenicol) plates made and stored in 4°C.
2. pPlac promoter was resuspended from iGEM kit plate 3 - 3G
3. Transformed into E.coli DH5α cells, and kept for incubation.

TUESDAY, 07/09/2021

Our Mid-semester exams started

WEDNESDAY, 15/09/2021

1. The following were resuspended from iGEM kit plates

- pUC19
- Plac promoter - plate 3 well 3G
- AgrBD - Plate 3 well 8B [iGEM 2018 kit plate]
- AgrAC - Plate 6 well 18L (iGEM 2019 kit plate)
- P2 promoter - Plate 2 well 24H (2018)

2. Transformation was done for all the resuspended biobrick.

THURSDAY, 16/09/2021

1. Transformation was successful for Plac, AgrBD, AgrAC, and P2 promoter.
2. Primary culture was kept at 37°C at 11:30pm

FRIDAY, 17/09/2021

1. Plasmid DNA isolated and kept in -20°C.
2. Gel electrophoresis was performed.

SATURDAY, 18/09/2021

1. Restriction digestion was done for the following according to protocol -
    a. Plac with SpeI and PstI [optimum temperature - 37°C.]
    b. AgrBD with XbaI and PstI [optimum temperature - 37°C.]
    c. AgrCA with XbaI and PstI [optimum temperature - 37°C.]
2. TSAP treatment and heat inactivation were done.
3. As XbaI cannot be heat inactivated, PCR clean up was performed.

SUNDAY, 19/09/2021

1. Gel run and gel extraction were performed.
2. Concentrations of each samples were measured using nanodrop.

MONDAY, 20/09/2021

1. Standardisation of double digestion was done by
    a. The buffer was changed for XbaI and SpeI digestion
    b. Digestion time was increased to 3hrs
2. Gel electrophoresis was performed.

3. Two bands were not so clear, so they were given to PCR for 1 more hour.

TUESDAY, 21/09/2021

1. Some trouble shooting was done for the experiments performed on 18th, 19th and 20th September.
    a. The fragment of Plac and P2 promoter were very small hence they were probably escaping the gel, hence no bands were observed.
    b. Due to this problem we decided to keep the promoter with the vector and cut out the agrCA and agr BD (as they are of larger size)
2. Restriction digestion (double restriction digestion) reactions were set up for Plac and agrBD.
3. Gel electrophoresis was performed.

WEDNESDAY, 22/09/2021

1. Digestion of P2 promoter was performed
2. Phosphatase treatment was done for Plac.
3. Heat inactivation was performed for both agrBD and Plac
4. PCR clean up was performed for AgrBD
5. Gel electrophoresis was performed.

THURSDAY, 23/09/2021

1. Plasmid miniprep was performed for all samples.

FRIDAY, 24/09/2021

1. Tris EDTA Buffer stock (10X) was made following protocol.
2. Plac gel extraction was done.
3. Nanodrop measurement was performed.

SATURDAY, 25/09/2021

1. Restriction digestion was done for agrBD using EcoRI and SpeI
2. Restriction digestion was done for agrCA using EcoRI and SpeI
3. Restriction digestion was done for P2 using EcoRI and XbaI
4. Gel electrophoresis was performed.

SUNDAY, 26/09/2021

1. Sample of agrBD was restriction digested.
2. Then gel electrophoresis was performed and the gel extraction was also done.
3. Concentration was measured using nanodrop,
4. Sample was stored in -20°C.

MONDAY, 27/09/2021

1. Primary culture of pUC19 (Amp+) LB was kept for incubation.

TUESDAY, 28/09/2021

1. pUC19 plasmid isolation was done following the protocols.
2. Plasmid was stored in -20°C.
3. Fresh samples of Plac , agrBD,agrCA and P2 were run in the gel along with digested agrBD and Plac in 0.8% agarose gel.
4. NB agar and NB media were prepared and kept for autoclave along with glassware and equipment.

WEDNESDAY, 29/09/2021

1. DNA concentration measurements of EiCsm6 PCR(done previously), Plac plasmid, Plac digested and AgrBD digested was measured using Nanodrop.
2. PCR cleanup of digested AgrBD and unidirectional vector plasmid was done.
3. Primary culture for biofilm assay was given.

THURSDAY, 30/09/2021

1. Agar LB-chloramphenicol plates were made.

2. Heat inactivation of ligation products (Plac with PSB1C3 + AgrBD) was done.
3. DNA concentration measurements of AgrBD digested PCR and linearised plasmid vector was measured using Nanodrop.
4. Ligation of AgrBD digestion product and Plac promoter was done.
5. Very OD of primary culture was observed, hence a new plate was streaked.

FRIDAY, 01/10/2021

1. Transformation of Plac-AgrBD done and kept in the incubator.
2. Fresh primary culture was put from the above made culture plate.

SATURDAY, 02/10/2021

1. PCR amplification of Dnase I basic, Dnase I composite, and Nisin PV and AgrAC linearised backbone was done.
2. DNase I composite PCR product and linearised backbone was stored.
3. Colony PCR of Plac-AgrBD was done.
4. Gibson unidirectional was started.

SUNDAY, 03/10/2021

1. DNase I, Nisin PV and linearised backbone were again amplified using PCR and the annealing temperature was determined.
2. OD was measured.

MONDAY, 04/10/2021

1. Bidirectional Gibson PCR for P2-GFP, AgrC and AgrA was started.
2. Digestion of DNase I Basic, DNase I Composite, linearised backbone and PUC19 plasmid was done.
3. Ligation of DNase I Basic, DNase I Composite to both the linearised backbone(PSB1C3) and PUC19 and Nisin PV to PSB1C3 was done.
4. New media for biofilm assay was prepared.

TUESDAY, 05/10/2021

1. Transformation of the following was done:
    a. DNase I basic+ PSB1C3
    b. DNase I composite + PSB1C3
    c. DNase I basic + PUC19
    d. DNase I composite + PUC19
    e. Nisin PV + PSB1C3
2. Fresh primary culture was given for biofilm assay.

WEDNESDAY, 06/10/2021

1. Colony PCR of Plac-AgrBD and unidirectional successful
2. Transformation of the above were done into KRX and BL21 strains of E.coli.
3. OD was measured and secondary culture was given.

THURSDAY, 07/10/2021

1. Gel run for P2-GFP, AgrC and AgrA was done followed by PCR cleanup.

FRIDAY, 08/10/2021

1. Colony PCR for DNase I basic and DNase I composite were done.
2. Gel extraction for plasmid backbone (bidirectional) was done.
3. Concentration was measured using Nanodrop

SATURDAY, 09/10/2021

1. Transformation for the bidirectional plasmid was done and the plates were incubated.
2. Post-treatment biofilm assay with DNase I was carried out.

SUNDAY, 10/10/2021

1. Colony PCR was performed for bidirectional Gibson assembly plasmid and corresponding gel was run.

2. Primary culture was given for the viable colonies.
3. Fresh primary culture was put.

MONDAY, 11/10/2021

1. AIP molecules were lyophilised.

WEDNESDAY, 13/10/2021

1. Transformation in BL21 unidirectional and bidirectional.
2. Post-treatment biofilm assay was performed.

THURSDAY, 14/10/2021

1. Primary culture for biofilm assay was given.

FRIDAY, 15/10/2021

1. Primary culture is given in the morning.
2. Secondary culture of 2 unidirectional and 2 bidirectional are given.
3. The production of GFP in the presence and absence of Autoinducer Peptide.
4. Secondary culture with DNase was put for pre-treatment biofilm assay.
5. Plates were kept under incubation for 24 (Plate I) and 72 (Plate II) hours.

SATURDAY, 16/10/2021

1. Similar experiments as previous day were continued.
2. OD of Plate I was measured.

SUNDAY, 17/10/2021

1. Primary culture was given again.

MONDAY, 18/10/2021

1. Secondary culture was given.
2. GFP assay was done from the secondary culture after a proper interval with five different concentration of autoinducer peptide.
3. AIP given for lyophilisation.
4. OD of Plate 2 was measured.

TUESDAY, 19/10/2021

1. AIP was collected and primary culture was given.

WEDNESDAY, 20/10/2021

1. Secondary culture was given.
2. AIP at 5 different concentrations was checked using a fluorometer.