Exp 1: Plasmid Isolation

Experimental Aim: To isolate the pET28a plasmid from transformed DH5-Alpha cell culture.

Observations: The amount of pellet formed was less considering it was a 10 mL culture.

Result: Plasmid was isolated from the culture and resuspended in 125 uL of 0.1X TE Buffer and stored at -20 C.

Conclusion: The Plasmid yield may be low due to the the following reason(s)

- Insufficient time for proper culture growth.(can be taken after 2 days also)
- Buffers not prepared well.
- Did not add kanamycin to the LB broth (no selection pressure).

10/05/21

Exp 2: Streaking DH5-Alpha cells (ampicillin resistant) on LB Agar Plates with Ampicillin

Experimental Aim: To learn the basic lab protocols for preparation of LB agar plates with ampicillin antibiotic.

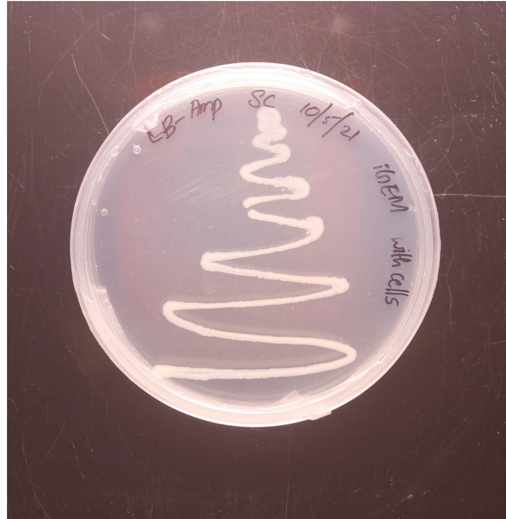
Method: LB agar plates with ampicillin were prepared. Ampicillin resistant DH5-Alpha cells were streaked onto the plates and incubated for 12 hours at 37 C

Observation: Colonies were observed on the plates.

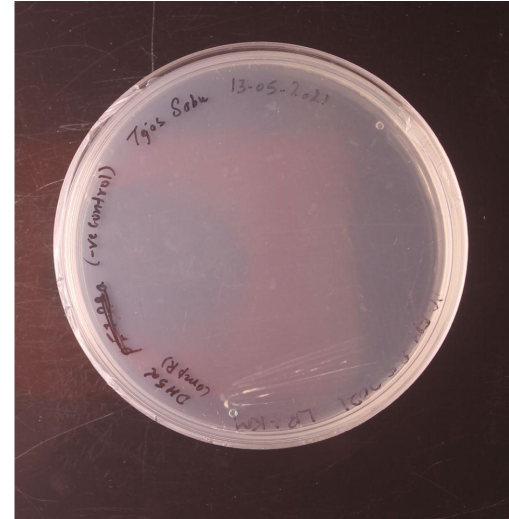
Results: DH5-Alpha bacterial colonies were observed on the plates after 12 hours of incubation.

Conclusion: DH5-Alpha cells could grow on Lb agar plates with ampicillin because of the ampicillin resistant plasmid within them.

Growth of Ampicillin Resistant DH5-Alpha cells



Ampicillin resistant DH5-Alpha cells on LB agar plates with ampicillin antibiotic



Ampicillin resistant DH5-Alpha cells on LB plates with kanamycin (Negative control)

Exp 1: Streaking DH5-Alpha cells on LB Agar plates

Experimental Aim: To prepare a fresh stock of DH5-Alpha cells for future use.

Method: Single colony of DH5-Alpha cells from previously prepared plate was streaked onto fresh LB agar plates.

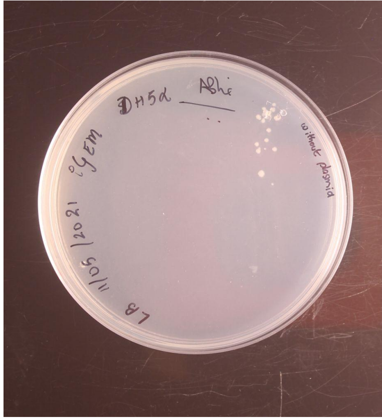
Results:
DH5-Alpha colonies were observed on 3 plates having Lb agar only.

No colonies were observed on the plates with Lb agar and ampicillin.

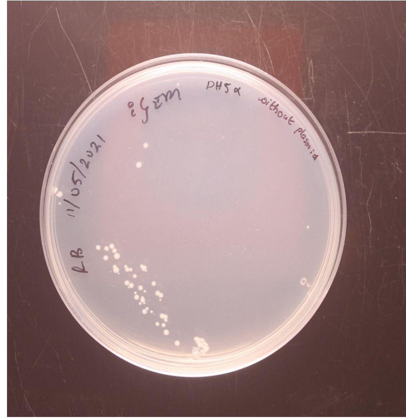
Conclusion: The DH5-Alpha cells did not have any plasmid with ampicillin resistant gene which supports the above observations.

11/05/21

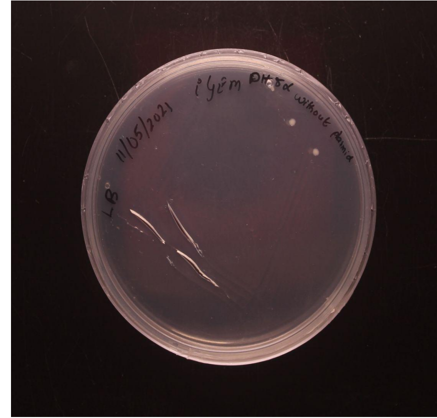
Growth of DH5-Alpha cells on LB agar plates



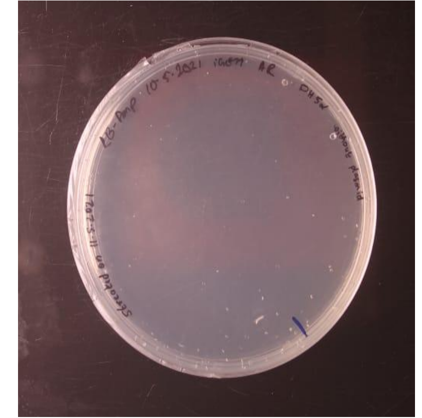
DH5a on LB plate



DH5a on LB plate



DH5a on LB plate



Negative control (DH5-Alpha on LB+amp plate)

Conclusions :

The reason why only few colonies were visible in the Lb media could be:

- Improper streaking method.
- Colonies were not picked properly.

Agarose Gel Electrophoresis

Experimental Aim: To confirm the presence and purity of plasmid(pET-28a) isolated on 10-05-2021

Method: The isolated plasmid DNA was made to run on a 1% Agarose Gel.

Observations:

No bands observed near the 5 kb ladder (plasmid size: 5369bp)

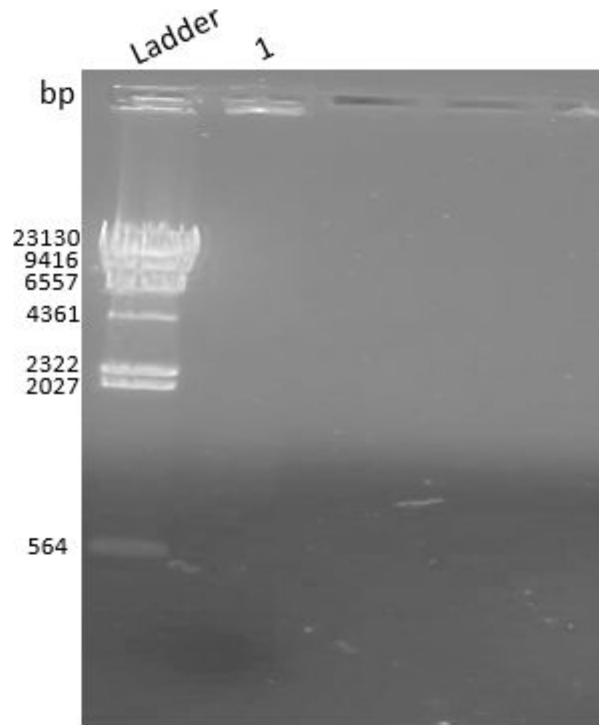
The Ladder DNA concentration was high hence the bands were not proper.

Result: The isolated plasmid DNA(5369bp) did not provide any bands near the 5 kb ladder DNA

Conclusion: The absence of bands could have been due to following reasons

- Improper plasmid isolation method.
- The plasmid yield was very less than expected. Digested plasmid should have also been used for better conclusion.

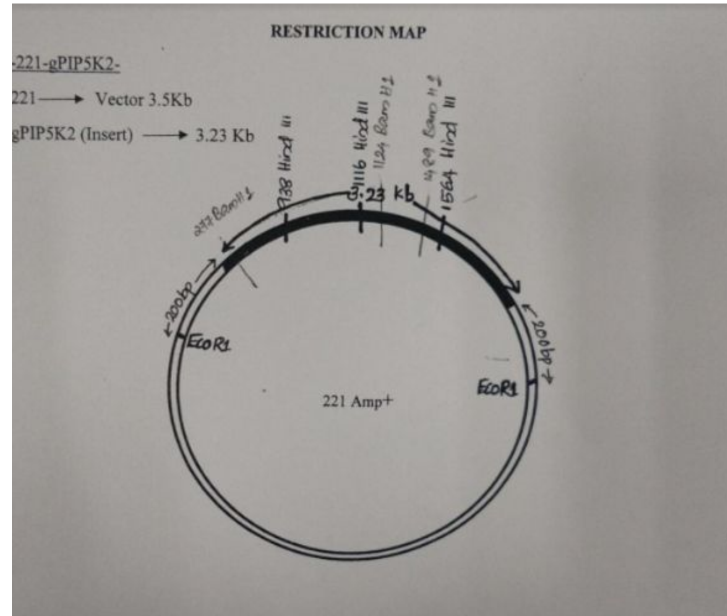
Agarose Gel Electrophoresis



Annotations:

Ladder: Lambda DNA digested with HindIII
1: Isolated Plasmid Dna (50uL)

Exp 1: Restriction digestion of pGEM-Teasy221 (with gPIPK52 insert) using EcoRI and HindIII



Restriction Map of pGEM-Teasy221 Plamsid with gPIP5K2 insert

12/05/21

Experimental Aim: To digest the plasmid DNA using EcoRI and HindIII and run the digested fragments on a 1% agarose gel.

Observations: Obtained 2 prominent bands between 5-6 kb in both digestions. Ladder DNA bands were not clear.

Results : The bands that were to be obtained:

With HindIII (3 fragments) : 6000 bp, 178 bp, 448 bp

With EcoRI (2 fragments) : 2997 bp, 3630 bp

The bands that were obtained during the experiment :

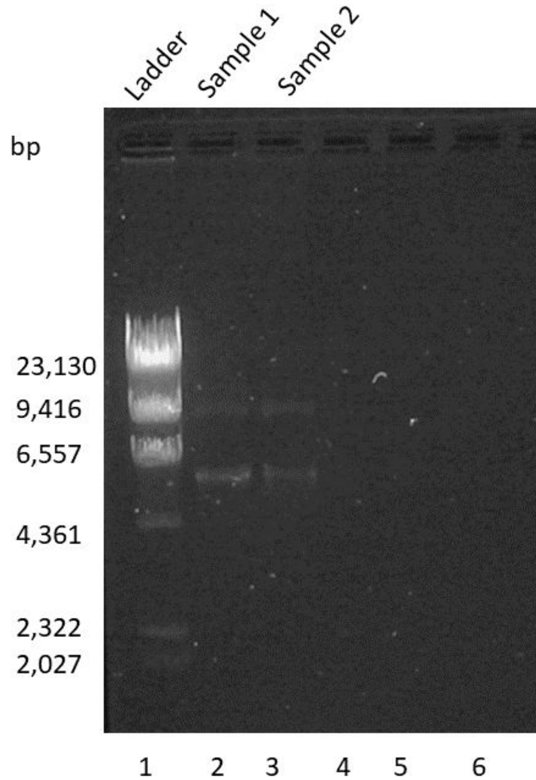
With HindIII: 2 bands between 5-6 kb

With EcoRI : 2 bands between 5-6 kb

Conclusions:

- EcoRI digestion wasn’t successful.
- Obtained a band at 6 kb for HindIII digestion but other bands were not seen properly.
- The ladder DNA was did not run properly.(high concentration)
- Did not have any positive (plasmid digested with restriction enzyme known to work) or negative control (undigested plasmid)

Agarose Gel Electrophoresis of Plasmid DNA



Annotations:

Sample 1- pGEM-Teasy221 digested with HindIII

Sample 2 - pGEM-Teasy221 digested with EcoRI

Sample 1: pGEM-Teasy221 digested with Hind III
Sample 2: pGEM-Teasy221 digested with EcoRI

Inoculation of DH5-Alpha cells for preparation of competent cells

Experimental Aim:

To prepare primary inoculate of :

- DH5-ALpha cells with pET28-a for plasmid isolation and
- DH5-Alpha without plasmid for preparation of competent cells.

Prepared 10 mL of culture for each.

Observation: Turbidity in the cultures medium after 12 hours of incubation.

12-05-2021

Sample	LB	Antibiotic (Stock Conc.)	Antibiotic (Working Conc.)	Observations after 13 hrs
pET28a (NL)	5 mL	100 mg/mL of Kan	50 ug/mL of Kan	Turbid
pET28a (NL)	5 mL	100 mg/mL of Kan	50 ug/mL of Kan	Turbid
pET28a (NL)	5 mL	100 mg/mL of Kan	50 ug/mL of Kan	Turbid

13-05-2021

Exp 1: Plasmid(pET-28a) isolation from DH5-ALpha cells and Agarose gel electrophoresis

Experimental Aim:

- To isolate the pET-28a plasmid from DH5-Alpha cells [study group]
- Similar procedure to be performed for DH5-Alpha cell culture without any plasmid [Negative control]
- To run the digested plasmid on 1% agarose gel

Observation: glassy white pellet obtained in both control as well as study group.

Conclusion: Reason for obtaining pellet in negative control could be due to following reasons:

- Genomic DNA contamination (could use more amount of isopropanol to precipitate more Genomic DNA)
- RNA contamination (did not use RNAse)

13-05-2021

Estimating concentration of plasmid isolated using Nanodrop

Method: 1.5 uL of Plasmid DNA (suspended in 125uL 0.1X TE buffer) was placed on a nanodrop and values were noted.

Samples	Concentration of ds DNA	A (260/280)	A (260/230)
Negative control (Normal DH5-Alpha cells)	2323.5 ng/uL	2.03 (RNA contamination)	2.25
Isolated Plasmid(pET28a) from DH5-Alpha Sample 1	3982.6 ng/uL	2.13 (RNA contamination)	2.20
Isolated Plasmid(pET28a) from DH5-Alpha Sample 2	3569 ng/uL	2.14 (RNA contamination)	2.24

Optimum Absorbance values for Nanodrop assay

	DNA	**RNA**
A (260/280) [RNA/protein contamination]	1.8→2 Optimum is 1.8	1.8→ 2 Optimum is 2
A (260/230) [Salt and other contamination]	2 → 2.2	2→ 2.2

Agarose Gel Electrophoresis

Experimental Aim: To run the isolated plasmid DNA and the negative control.

Observation:

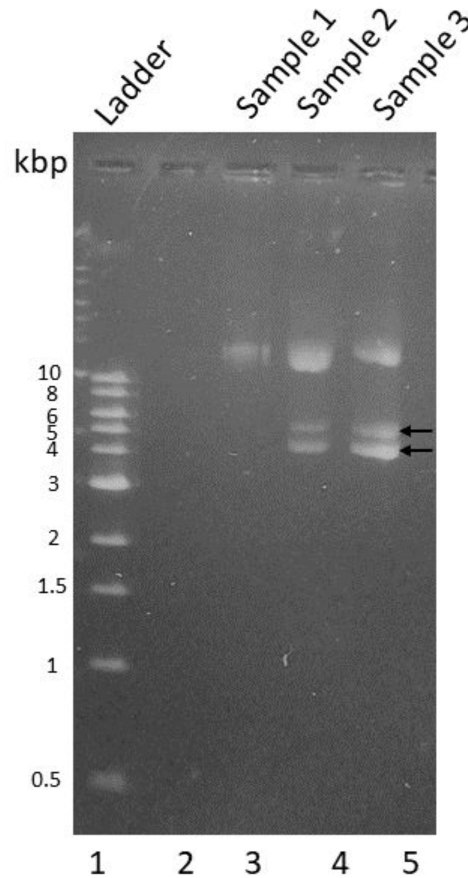
Obtained 2 bands with for the isolated plasmid DNA (pET-28a)

only a single band was observed for the negative control.

Result: Obtained 2 bands for the isolated plasmid. One of band is between 5 Kb and 6 Kb which is in correspondence to the actual plasmid length of 5369 bp.

The gel was stored at 4 C.

Agarose Gel Electrophoresis



Sample 1: Negative Control
Sample 2: pET28a
Sample 3: pEt28a

Conclusion:

- The results support that the plasmid isolation was successful. This can be confirmed only after transforming normal DH5-Alpha cells with the plasmid isolated today.
- The reason for presence of two bands in the sample could be due to the different conformations of plasmid DNA.
- The top band would possible correspond to genomic DNA contamination as it was in line with band from the negative control.

Experiment 2: Competent cell preparation

Experimental Aim: Preparation of DH5a competent cells.

Observation: Measured absorbance at 600nm.

Time	OD
14:01	0.1
14:45	0.1
15:16	0.24
15:47	0.4

Result: Aliquoted 50uL of competent cells into ~70 (0.5mL) tubes. Stored the tubes at -80 degree celsius.

Exp 3: Transforming DH5-Alpha cells with pET-28a

Experimental Aim:

To transform competent DH5-Alpha cells with the isolated plasmid pET-28a.

Streak the transformed cells on LB+kanamycin plate and compare the result with a

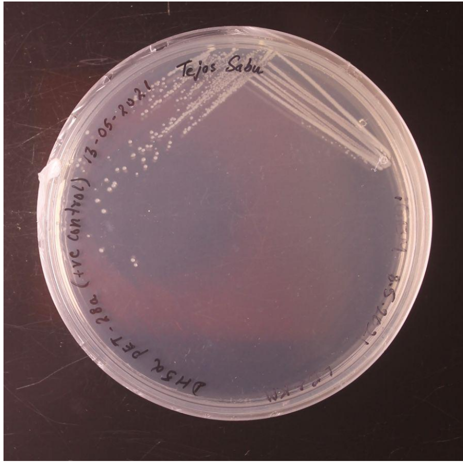
positive control group (Streaking Kanamycin Resistant DH5-Alpha cells on LB+kan plate[30ug/mL])

and negative control group (Streaking Kanamycin resistant Dh5-Alpha cells on LB+amp plate[100 ug/mL])

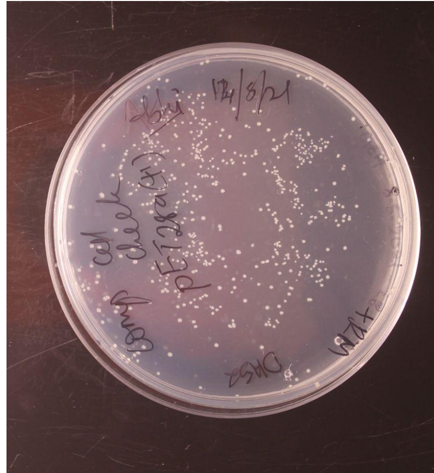
Observation:

- Negative control: No colonies were formed. (bubbles were seen on the plate)
- Positive control: Colonies were observed on the plate.
- Study group : Colonies were observed.

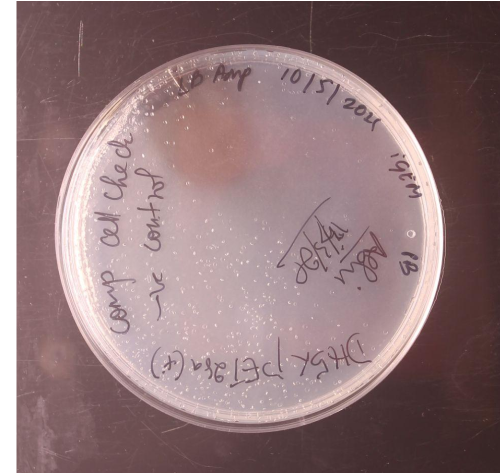
Competent DH5-Alpha cells streaked on LB plates



DH5-Alpha with pET-28a streaked on LB+kan plate
(Positive control)



Transformed DH5-Alpha cells streaked on LB+kan plate
(Study group)



DH5-Alpha with pET-28a streaked on LB+ampicillin plate
(Negative control)

Result and Interpretations

Sample Space	**Conclusions**
Study Group	Colonies observed. Competency achieved.
Positive control	Colonies observed. Plasmid containing DH5-Alpha cells show phenotypic change
Negative control	No colonies observed. No contamination

Exp 1: Plasmid Extraction from Agarose Gel

Experimental Aim: To extract pET-28a from agarose gel concentration of purified plasmid was estimated using a nanodrop.

Nanodrop result:

DNA concentration	**A (260/280)**	**A (260/230)**
8 ng/uL	3.32	0.01 (salts and other contaminants present)

Note: below 20 ng/uL concentration of DNA the A (260/280) values are not reliable.

14-05-2021

Exp 2: Transformation of DH5-Alpha cells with plasmid pSB1C3 from iGEM Kit plate

Experimental Aim:

To transform the competent DH5-Alpha cells with pSB1C3 vector containing RFP construct and streak the cells on to LB+CAM (5ug/mL) plates to test transformation success.
Compare the results with a negative control plate (Streaking DH5-Alpha on LB+CAM Plate)

Observation: No growth was observed in both plates

Conclusions: Transformation wasn't successful

Experiment 1: Plasmid(pET28a) Isolation from transformed DH5-Alpha cells

Aim: To isolate plasmid DNA(pET-28a) from transformed DH5-Alpha cells and visualise the plasmid on 1% agarose gel.

Method:

- 5mL of inoculate was transferred from each of the 4 falcon tubes.

where 1,2,3 were the study groups and 4 was the negative control

Observations:

- No pellets in samples 1,2 and 4.
- Glassy white pellet was observed in sample 3.

Results: The pellet may be the plasmid pET28a along with genomic DNA contamination but cannot ensure until we run it on a gel.

15-05-2021

Experiment 2: Restriction digestion of plasmid (pET-28a)

Experimental Aim: To perform restriction digestion of pET-28a (isolated on 14-05-2021) using BamHI and HindIII and run the digested plasmid on 1% agarose gel.

Observations: a band was obtained around 5kb.

Conclusion: Not certain whether the plasmid is digested since the difference in the base pairs between the BamHI and HindIII is 20bp (which is not visible).

Agarose Gel Electrophoresis

Aim: To run the following samples on the 1% agarose gel and visualise the bands.

- Isolated Plasmid (pET-28a) [3 study samples and 1 negative control]
- pSB1C3 from iGEM KIT.
- Restriction digestion of pET28a with BamHI and HindIII

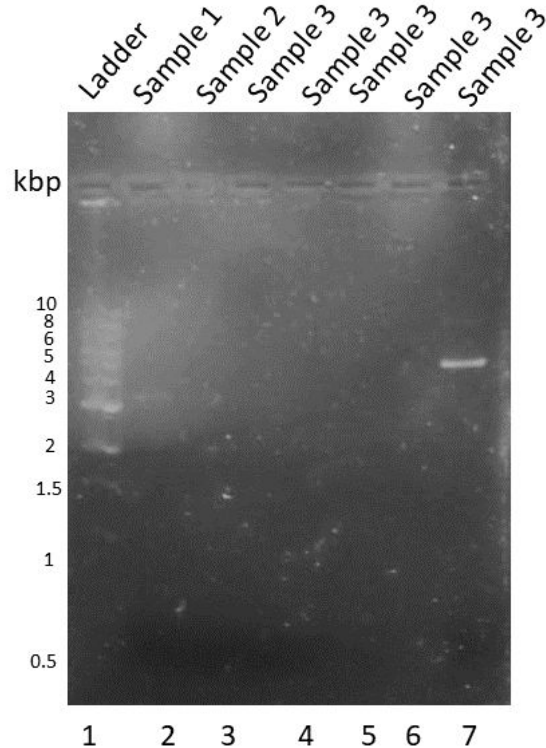
Observations:

2 bands observed in lane 2 and 3 corresponding to 3kb

A single band around 5kb was observed in lane 8

No bands were observed in other lanes.

Agarose Gel Electrophoresis



Sample 1: 1 Kb ladder DNA
Sample 2 : pET28a sample 1
Sample 3: pET28a sample 2
Sample 4: pET28a sample 3
Sample 5: Negative control.
Sample 6: GFP plasmid from iGEM kit
Sample 7: pET28a digested with HindIII and BamHI

Conclusions::

- The pellet that we got in sample 3 is not the plasmid DNA since we did not get a band around 5kb.
- Unexpected bands present at 3kb which could be some conformational form of the plasmid.
- The digested pET28a vector which has a size btw 5.3 kb but the band is btw 4 and 5 kb.(The ladder might not have run properly)

Exp 3: Transformation of competent DH5-Alpha cells

Experimental Aim:
To transform competent DH5-Alpha cells with GFP, RFP constructs (in pSB1C3) as well as pET28a. The transformed cells are to be streaked on LB+Cam [5ug/mL] plate (for GFP and RFP constructs) and LB+Kan [50ug/mL] plate (for pET-28a)

Observation :

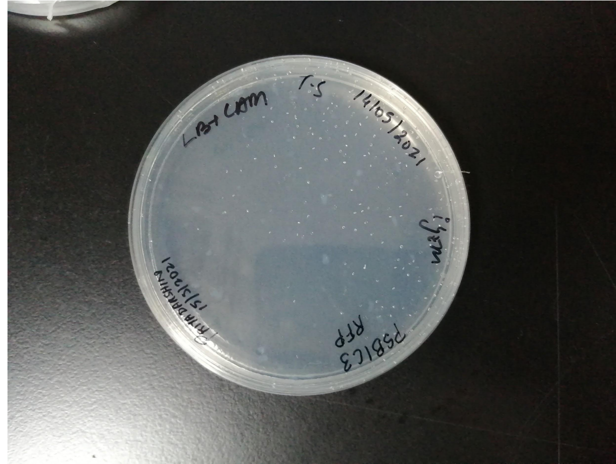
- Two potential colonies in the plate with GFP transformation.
- No colonies seen in the plate with RFP transformation.
- No colonies seen in the plate with pET28a(+) vector.

Results :

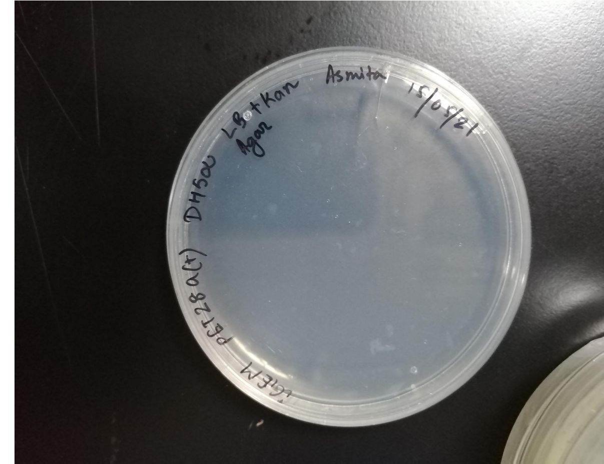
Observed growth on pET28a and GFP containing plasmids whereas RFP containing plasmid did not show any growth.

Growth of transformed DH5-Alpha cells on prepared plates

GFP transformation (chloramphenicol resistant bacteria)



RFP transformation (chloramphenicol resistant bacteria)



pET28a(+) vector transformation (kanamycin resistant bacteria)