

Plasmid isolation

Experimental Aim:

To isolate pET28a from DH5-Alpha.

Method:

Inoculate the following cultures (5mL culture in 50mL falcon tubes) and incubate in a shaker incubator at 37°C at 225 RPM.

- Dh5alpha with pET28a in LB+kan [50ug/mL] (Study Group)
- Dh5alpha without pET28a in Lb+kan [50ug/mL] (Negative Control)
- Dh5alpha without pET28a in Lb (Control)

Observations: pellets observed in all three test tubes after first centrifugation → antibiotic may not be active or contamination

| Sample | LB | Antibiotic (Stock Conc.) | Antibiotic (Working Conc.) | Observations after 13 hrs |
|--|-----------|---------------------------------|-----------------------------------|----------------------------------|
| Dh5alpha with pET28a | 5 mL | 100 mg/mL of Kan | 30 ug/mL of Kan | Turbid |
| Dh5alpha without pET28a | 5 mL | 100 mg/mL of Kan | 30 ug/mL of Kan | Turbid |
| Dh5alpha without pET28a (Control) | 5 mL | None | 50 ug/mL of Kan | Turbid |

Nanodrop Result

| Sample | ng/uL | A(260/280) | A(260/230) |
|--------------------------------------|--------------|-------------------|-------------------|
| Plasmid sample 1 (pET28a) | 75.8 | 1.98 | 1.97 |
| Plasmid sample 2 (pET28a) | 271.5 | 2.06 | 2.02 |
| Negative control | 23 | 2.21 | 0.51 |
| Negative control | 29.7 | 1.97 | 1.69 |

Agarose Gel electrophoresis

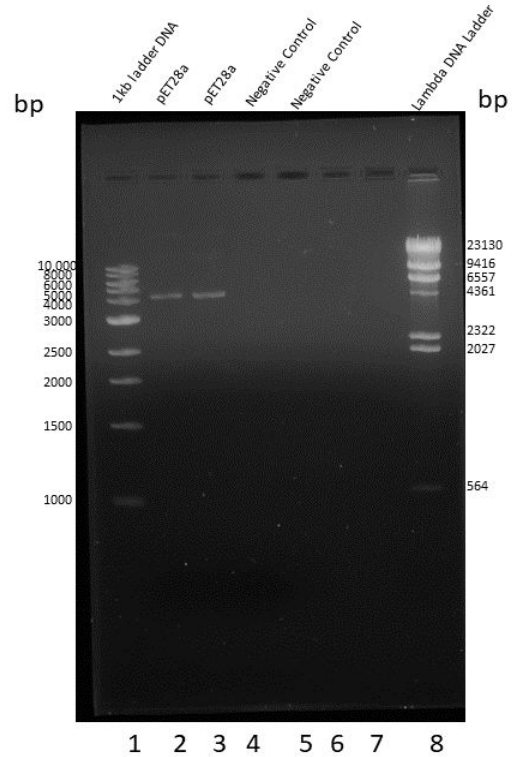
1% agarose gel was prepared and the following samples were loaded :

- Plasmid sample 1 : pET28a isolated from DH5Alpha (2mL)
- Plasmid sample 2: pET28a isolated from DH5Alpha (2mL+1mL)
- Negative Control : DH5Alpha in LB+kan
- Negative Control : DH5Alpha in LB

Results and Conclusions:

- Observed band between 4000 and 5000 bp → Could be pET28a plasmid
- Plasmid pET28a to be confirmed after transformation and plating.

Agarose Gel Electrophoresis



Inoculation for Plasmid Isolation

Set I

- DH5Alpha with pET28a in LB+kan
- DH5Alpha in LB +kan (negative control)
- DH5Alpha in LB (negative control)

| Sample | LB | Antibiotic (Stock Conc.) | Antibiotic (Working Conc.) | Observations after 13 hrs |
|--|-----------|-------------------------------------|---------------------------------------|--------------------------------------|
| 1.DH5Alpha with pET28a | 5 mL | 100 mg/mL of Kan | 50 ug/mL of Kan | Turbid |
| 2.DH5Alpha without pET28a | 5 mL | 100 mg/mL of Kan | 50 ug/mL of Kan | No turbidity |
| 3. Dh5Alpha without | 5 mL | None | 50 ug/mL of Kan | No turbidity |

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Inoculation for Plating

Set II

1. DH5Alpha with gfp plasmid in LB+ Cam
2. DH5Alpha with rfp plasmid in LB +Cam

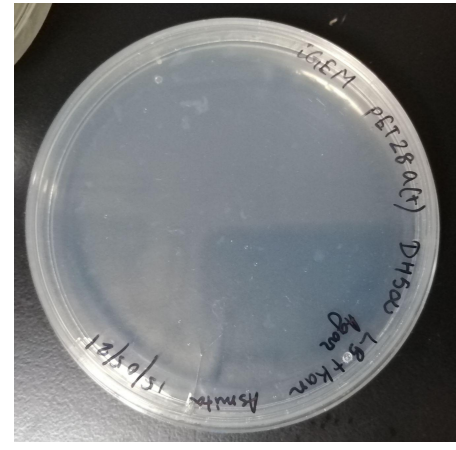
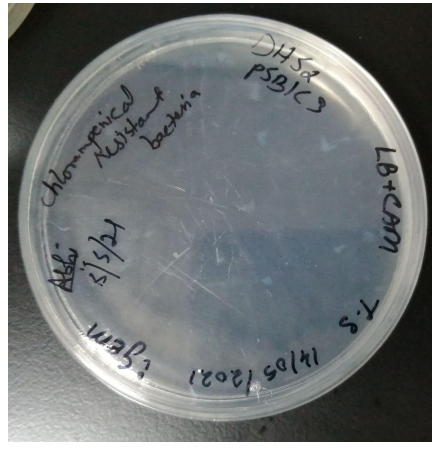
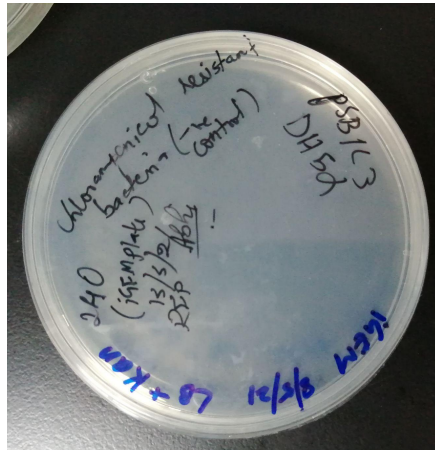
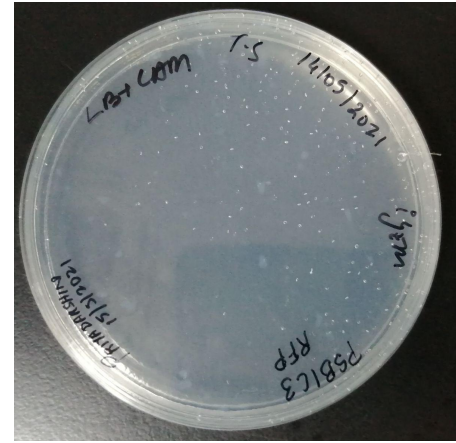
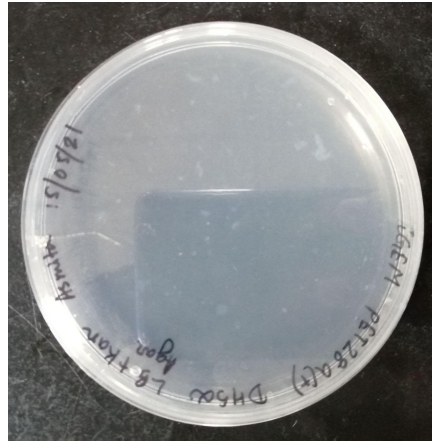
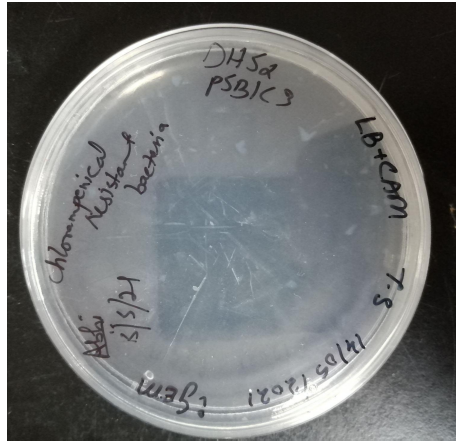
Cam stock: 5mg/mL ; Working concentration: 10 ug/mL

Set III

1. DH5Alpha with gfp plasmid in LB+ Cam
2. DH5Alpha with rfp plasmid in LB +Cam

Cam stock: 5mg/mL ; Working concentration: 20 ug/mL

Streaked Plates



Plasmid isolation for Set I

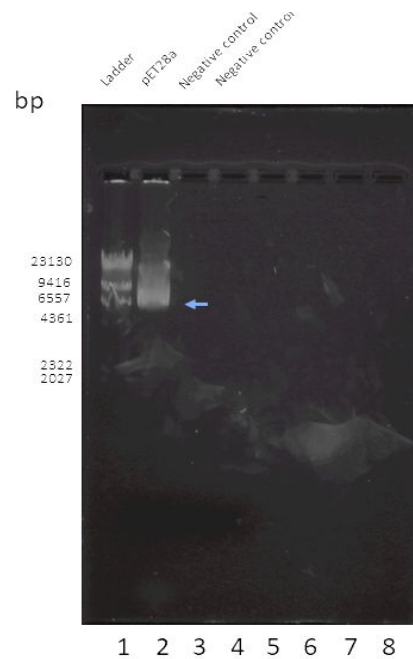
Observation :

- Pellet observed for DH5Alpha (kan+) in LB+Kan
- No Pellet observed for DH5 ALpha (kan-)in LB + Kan
- No Pellet observed for DH5 ALpha (kan-)in LB.

Nanodrop Result for Set I

| Samples | ng/uL | A(260/280) | A(260/230) |
|------------------|--------|------------|------------|
| pET28a | 2928.5 | 1.99 | 2.25 |
| Negative Control | 7.6 | 2.23 | 0.25 |
| Negative Control | 5.4 | 2.32 | 0.28 |

Agarose Gel Electrophoresis for plasmid isolated from Set I



Results and Conclusions:

- Observed band between 4000 and 5000 bp → Could be pET28a plasmid
- Plasmid pET28a to be confirmed after transformation and plating.

Plasmid Isolation

Experimental Aim : Isolate pET28a from DH5Alpha Cells.

Method:

Inoculation :

DH5Alpha with mCherry plasmid in LB (5mL)+ Ampicillin (working: 100 ug/mL)

Observation :

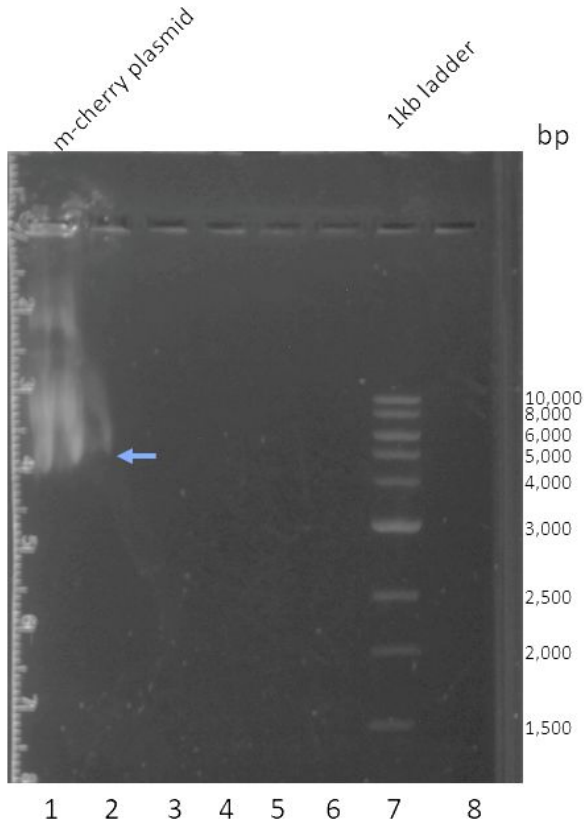
The solution was turbid after 12 hours

White Pellet obtained at the end of plasmid isolation.

Nanodrop result

| Sample | ng/uL | A(260/280) | A(260/230) |
|-------------------------|--------------|-------------------|-------------------|
| m-Cherry Plasmid | 447.3 | 1.65 | 0.92 |

Agarose Gel run



Observation and conclusion

- Obtained a smear instead of bands indicating that it could be genomic DNA contamination.
- Did not obtain any bands above 10kb ladder (m-Cherry plasmid has a size of 15 kb)