Exp 1: Plasmid Isolation

Experimental Aim : To isolate Plasmid (pET28a and m-Cherry) from DH5 Alpha Cells.

Method:

Innoculate the following cultures with appropriate concentration of antibiotics.

1. DH5Alpha with pET28a in LB+ kan
2. Normal DH5Alpha in LB+ kan [negative control]
3. Normal DH5Alpha in LB [control]
4. DH5Alpha with mCherry Plasmid in LB+Amp

NANODROP RESULT :

	ng/uL	**A260/A280**	**A260/A230**
pET28a	13.6	1.84	1.34
Negative control	26.4	1.83	0.90
mCherry plasmid	34.8	1.93	1.18

02/06/21

Exp 2: Restriction Digestion & Gel Run

Experimental Aim: To confirm the functionality of the restriction enzymes by digesting the isolated plasmids.

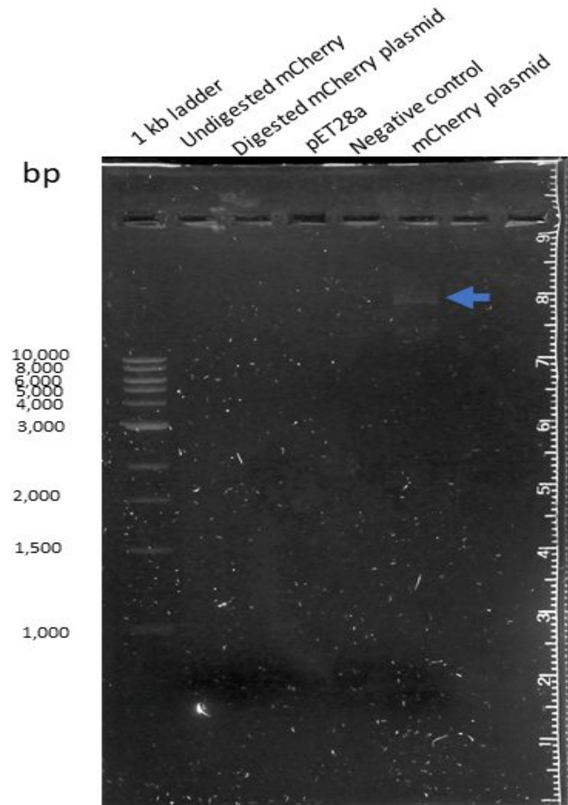
Method:
The isolated mCherry and pET28a plasmid was digested using two restriction enzyme :

1. EcoRI (NEB)
2. BamH1 (NEB)

Master Mix:

- 40.7- RNAse free water
- 5uL- 10x NEB buffer
- 1uL EcoRI + 1uL BamHI
- 2.3 uL plasmid DNA

Agarose Gel electrophoresis



OBSERVATION :

1. No bands were visible for restriction digested products.
2. pET28a band was also not visible on the gel.
3. 2 bands were visible for mCherry plasmid (size:~15kb) sample which could be the plasmid as the band was above 10 kb ladder.

mCherry plasmid size : 15kb
mCherry fragment size : 2.5 kb

Exp 1: Plasmid Isolation

Experimental Aim: To isolate pET28a and mCherry from DH5Alpha Cells.

Method: Inoculate the following cultures overnight at 37 C in a shaker incubator set at 225 RPM.

	LB	**Antibiotic (Stock conc.)**	**Antibiotic (Working Conc.)**	**Observations after 13 hrs**
pET28a	6mL	100 mg/mL Kan	50 ug/mL	turbid
Negative control I	6mL	100mg/mL Kan	50 ug/mL	No turbidity
mCherry	6mL	100 mg/mL Amp	100 ug/mL	turbid
Negative control II	6mL	100 mg/mL Kan	100 ug/mL	No turbidity

04/06/21

NANODROP RESULT :

	ng/uL	A260/A280	A260/A230
pET28a	1150.7	1.98	2.02
mCherry plasmid	1013.1	1.96	2.09

Exp 2: Restriction Digestion & Gel Run

Experimental Aim: To confirm the functionality of the restriction enzymes by digesting the isolated plasmids.

Method:
The isolated mCherry and pET28a plasmid was digested using the following restriction enzymes :

- EcoRI (NEB)
- BamH1 (NEB)
- HindIII (H.F) (NEB)

Restriction Digestion Master Mix

	mCherry	**pET28a**
Template	1uL	1uL
Buffer (20X)	2uL	2uL
Enzyme 1	1uL (BamHI)	1uL (BamHI)
Enzyme 2	1uL (EcoRI)	1uL (HindIII)
Mili Q	15uL	15uL

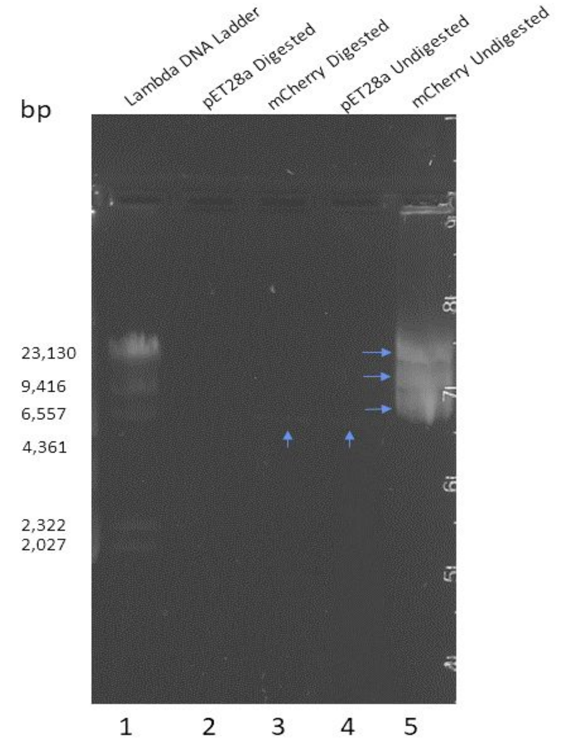
Agarose Gel electrophoresis

- mCherry digested with BamHI and EcoRI
- pET28a digested with BamHI and HindIII H.F

Observation and conclusion:

1.Faint bands observed near 6kb ladder for pET28a and mCherry digested products (unexpected).

2. Undigested mCherry has 3 bands between 6kb and 23kb. Three bands could probably be different conformations of the plasmid.



Transformation of DH5-Alpha

Experimental Aim: To transform the isolated plasmid into competent DH5-Alpha and test the competency of the cells.

Method: The cells were transformed according to the Transformation Protocol.

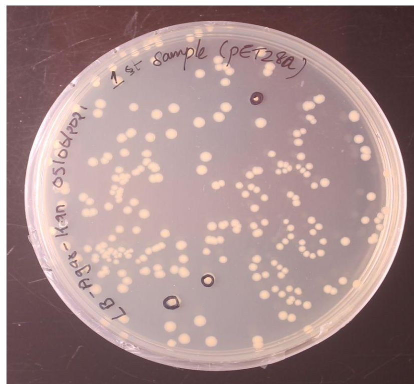
Sample Name	Concentration(ng/uL)	Isolation on
pET28a	1150.7	04/06/21
pET28a	271.5	27/05/21
mCherry	1013.1	04/06/21
mCherry	1472.8	31/05/21

05/06/21

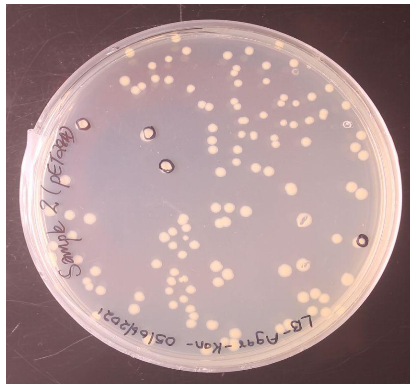
Plating Transformed Cells

1. Sample1: LB+kan
2. Sample 2: LB+kan
3. Sample 3: LB+Amp
4. Sample 4: LB+Amp

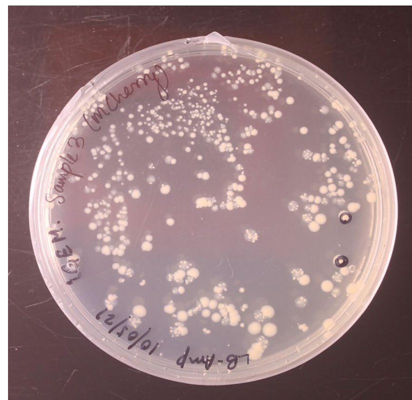
Result: All four samples had colonies after 24 hours



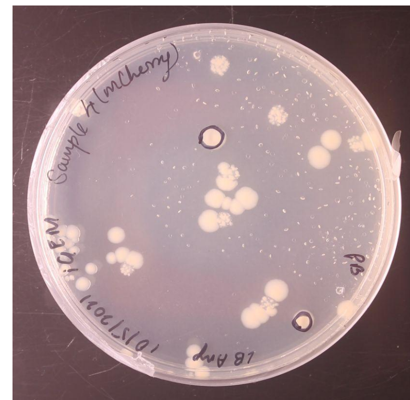
pET28a (Sample 1)
LB+kan (50 ug/mL)



pET28a (Sample 2)
LB+ kan (50 ug/mL)



mCherry (Sample 3)
LB+ amp (100 ug/mL)



mCherry (Sample 4)
Lb+amp (100 ug/mL)