Plasmid Isolation

Experimental Aim: To isolate pET28a from DH5-Alpha Cells.

Method : 20 mL LB was inoculated with DH5-Alpha cells containing pET28a. Appropriate antibiotic was added to the culture and incubated for 13 hours at 225 RPM in 37°C.

Sample	**LB**	**Antibiotic (Stock Conc.)**	**Antibiotic (Working Conc.)**	**Observations after 13 hrs**
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid

21/06/21

Nanodrop Results

Sample	ng/ul	A(260/A280)	A(260/230)
pET28a (NL)	404.8	1.91	2.26

Results:
The isolated Plasmid were stored at -20°C freezer for further use.

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Sample	**LB**	**Antibiotic (Stock Conc.)**	**Antibiotic (Working Conc.)**	**Observations after 13 hrs**
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid

22/06/21

Nanodrop Result

Sample	ng/ul	A(260/A280)	A(260/230)
pET28a (NL)	86.2	1.85	1.77

Results:
The isolated Plasmid were stored at -20°C freezer for further use.

Exp 1: Plasmid Isolation

Experimental Aim: To isolate pET28a from DH5-Alpha Cells.

Method : 40 mL LB was inoculated with DH5-Alpha cells containing pET28a. Appropriate antibiotic was added to the culture and incubated for 13 hours at 225 RPM in 37°C.

Sample	LB	Antibiotic (Stock Conc.)	Antibiotic (Working Conc.)	Observations after 13 hrs
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid

23/06/21

Nanodrop Result

Sample	ng/ul	A(260/A280)	A(260/230)
pET28a (NL)	4001.7	2.07	2.22
pET28a (NL)	17847.8	2.02	2.44

Results:
The isolated Plasmid were stored at -20°C freezer for further use.

Exp 2: Plasmid Isolation

Experimental Aim: To isolate pET28a from DH5-Alpha Cells.

Method : 40 mL LB was inoculated with DH5-Alpha cells containing pET28a. Appropriate antibiotic was added to the culture and incubated for 13 hours at 225 RPM in 37°C.

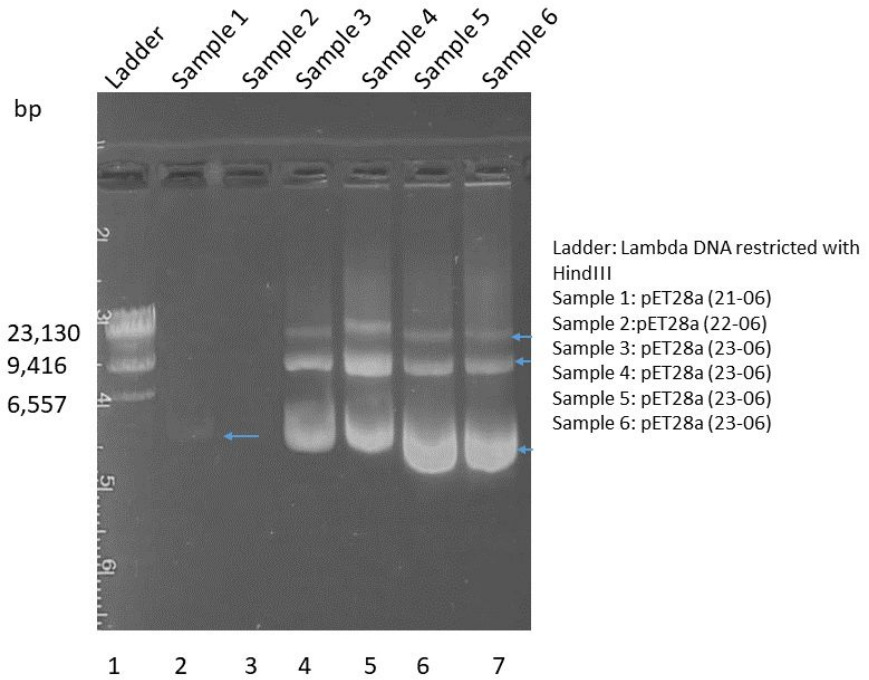
Sample	LB	Antibiotic (Stock Conc.)	Antibiotic (Working Conc.)	Observations after 13 hrs
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid
pET28a (NL)	20 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid

Nanodrop Result

Sample	ng/ul	A(260/A280)	A(260/230)
pET28a (NL)	26542.5	2.01	2.36
pET28a (NL)	15859.2	2	2.27

Results:
The isolated Plasmid were stored at -20°C freezer for further use.

Agarose Gel Electrophoresis



Results and Conclusions:

- The ladder did not run properly. Some bands were very faint
- Sample1,3,4 showed desired size of 5.3 kb.
- Sample 5 and 6 showed bands a bit lower in size than sample 1 and 2.
- A lot of smear was observed during gel run which could have been due to genomic DNA contamination.

25/06/21

PLASMID ISOLATION

Experimental Aim: To isolate pET28a from DH5-Alpha Cells.

Method : 30 mL LB was inoculated with DH5-Alpha cells containing pET28a. Appropriate antibiotic was added to the culture and incubated for 13 hours at 225 RPM in 37°C.

Sample	LB	Antibiotic (Stock Conc.)	Antibiotic (Working Conc.)	Observations after 13 hrs
pET28a (NL)	10 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid
pET28a (NL)	10 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid
pET28a (NL)	10 mL	100 mg/mL of Kan	30 ug/mL of Kan	Turbid

26/06/21

Nanodrop Result

Sample	Conc.(ng/uL)	A260/A280	A260/A230
pET28a(NL)	47.2	1.8	1.65
pET28a(NL)	57.6	1.82	1.88
pET28a(NL)	52.8	1.8	1.64

Results:
The isolated Plasmid were stored at -20°C freezer for further use.