

Plasmid Isolation

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

Method: The following cultures were inoculated and incubated overnight at 37 °C at 225 RPM.

Sample	LB	Antibiotic (Stock conc.)	Antibiotic (Working conc.)	Observatio n after 13 hrs
pET28a (NL)	6mL	100 mg/mL Kan	30ug/mL	Turbid
Control DH5-Alpha w/o plasmid	6mL	100mg/mL Kan	30ug/mL	No Turbidity
Control DH5-Alpha w/o plasmid	6mL	-	-	No turbidity

Nanodrop Result

	ng/uL	A(260/280)	A(230/280)
pET28a (NL)	13221.6	2.06	2.42

The isolated plasmid was stored in -20 °C for later use.

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

Nanodrop Result

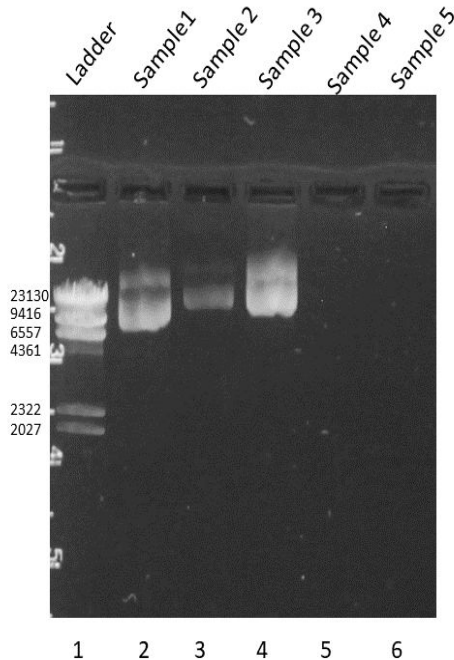
	ng/ul	A(260/A280)	A(260/230)
pET28a (NL)	1146.1	1.93	2.36
pET28a(VVL)	2953.6	1.89	2.36

Result:

The isolated plasmid was stored in -20 C freezer for further use.

Agarose Gel Electrophoresis

- The isolated plasmids were run on 1% Agarose gel.



Ladder: Lambda DNA restricted with HindIII
Sample 1: pET28a (NL)
Sample 2: pET28a (VVL)
Sample 3: pET28a (NL) isolated on 17-06-2021

Results

The desired band size to be obtained were:

Sample 1: 5369 bp

Sample 2: 6719 bp

Sample 3: 5369 bp

We did not obtain desired band sizes..

Restriction Digestion

Experimental Aim: To perform restriction digestion on the isolated plasmid and analyse the result.

Method: The restriction digestion was performed using the standard restriction digestion protocol. The following enzymes were used :

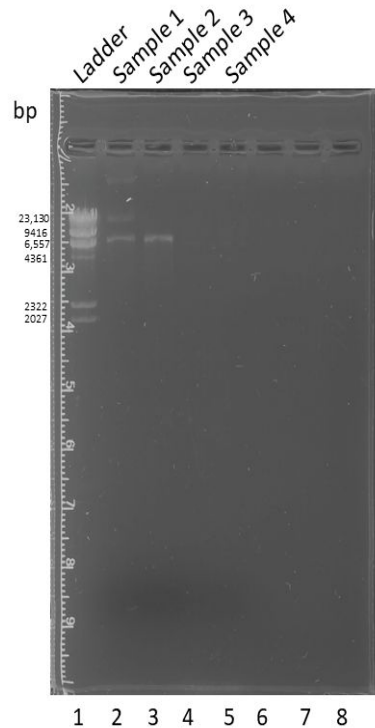
- Single Digestion - EcoR1 with pET28a (NL)
- Double Digestion - EcoR1 , BamH1 with pET28a (VVL)

Results and Observations:

- Sample 1 (undigested) showed 3 bands out of which one band was at the required size of 6.7 kb.
- Sample 2 (digested) which was supposed to show 2 bands one at 5.3 kb and insert of 1.3 kb were not visible. Instead we saw a band near 6.7 kb.
- Sample 3 and 4 did not show any bands.

Agarose Gel Electrophoresis

The digested plasmids were run on a 1% Agarose Gel.



Ladder DNA: Lambda DNA
Digested with HindIII
Sample 1: Undigested pET28 (VV)
Sample 2: Digested pET28 (VV)
Sample 3: Undigested pET28a (NL)
Sample 4: Digested pET28a (NL)