

Plasmid isolation of pET28a using Favor Plasmid Isolation Kit

Note: Plasmid Inoculation failed on 7-06-2021 and 8-06-2021: Could be because of culture plates or LB.

Hence fresh stock of pET28a (from Natesh Lab and Vinesh Lab) and mCherry (from Sadananda Lab) were inoculated in freshly prepared LB on 9-06-2021

Experimental Aim: To isolate plasmid pET28a and mCherry from DH5-Alpha using using Favor Plasmid Isolation Kit

Method: The following cultures were innoculated with respective antibiotic and the the plasmid was isolated next day using the standard protocol provided.

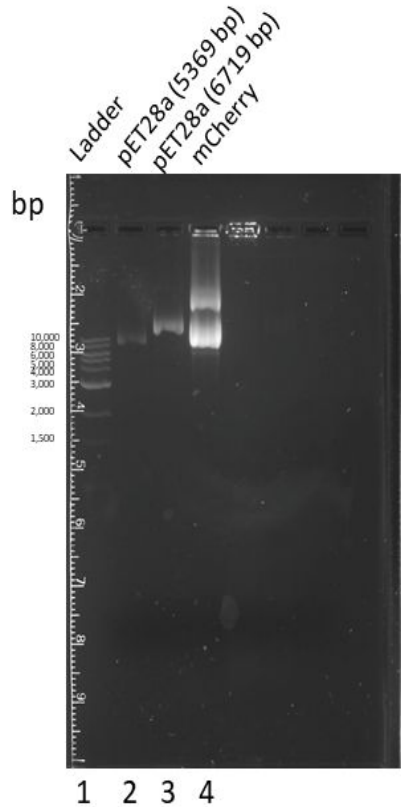
Nanodrop result:

	ng/uL	A(260/280)	A(230/280)
pET28a (NL)	18.5	1.77	1.13
pET28a (VVL)	46.7	1.82	1.52
mCherry	260	1.87	2.13

INOCULATION

DH5-Alpha with	LB	Antibiotic (Stock conc.)	Antibiotic (Working conc.)	Observation after 13 hrs
pET28a (NL)	6mL	100 mg/mL Kan	30ug/mL	turbid
Control 1 (w/o plasmid)	6mL	100 mg/mL Kan	30ug/mL	No turbidity
pET28a with insert (VVL)	6mL	100mg/mL Kan	30ug/mL	turbid
mCherry(SL)	6mL	100mg/mL Amp	100 ug/mL	turbid
Control 2 (w/o plasmid)	6mL	100mg/mL Amp	100 ug/mL	No turbidity
GFP (iGEM kit)	6mL	34 mg/mL Cam	30 ug/mL	No turbidity
Control 3 (w/o plasmid)	6mL	34 mg/mL Cam	30 ug/mL	No turbidity

Agarose Gel Electrophoresis



Size:

Well 1: 1kb Ladder

Well 2: pET28a (5369 bp)

Well 3: pET28a + insert (6719bp)

Well 4: mCherry Plasmid (15,000 bp)

Observations :

- pET28a (NL) and pET28a (VVL) did show bands but were more than 9kb in size. It might be gDNA contamination.
- mCherry sample has 2 bands above the 10kb ladder which might be conformations of the plasmid.

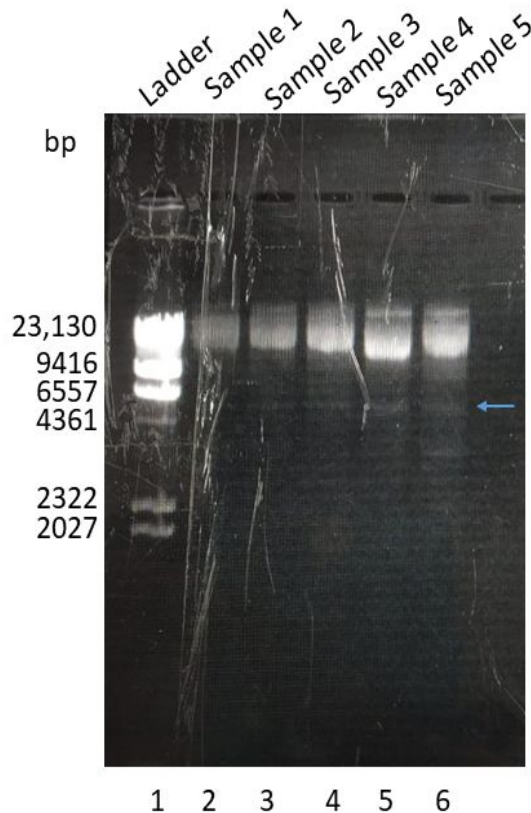
Plasmid isolation of pET28a using MN NucleoSpin Kit

	LB	Antibiotic (Stock conc.)	Antibiotic (Working conc.)	Observation after 13 hrs
pET28a 1	6mL	100 mg/mL Kan	30ug/mL	Turbid
pET28a 2	6mL	100mg/mL Kan	30ug/mL	Turbid
pET28a 3	6mL	100mg/mL Kan	30ug/mL	Turbid
pET28a 4	6mL	100mg/mL Kan	30 ug/mL	Turbid
pET28a 5	6mL	100mg/mL Kan	30 ug/mL	Turbid
Control	6mL	100mg/mL Kan	30 ug/mL	No turbidity

Nanodrop result:

	ng/uL	A(260/280)	A(260/230)
pET28a	37.5	1.76	1.79
pET28a	31.6	1.79	2.06
pET28a	29.8	1.78	2.19
pET28a	37.8	1.83	2.03
pEt28a	40.6	1.71	1.63

Agarose Gel Electrophoresis



Ladder: Lambda DNA
restricted with HindIII
Sample 1,2,3,4,5 :
pET28a plasmid DNA

Observations and conclusions:

- Prominent bands were visible above 10kb ladder → could be gDNA contamination
- Light bands were visible near between 4kb and 6kb for all samples. This could be our required plasmid pET28a.