

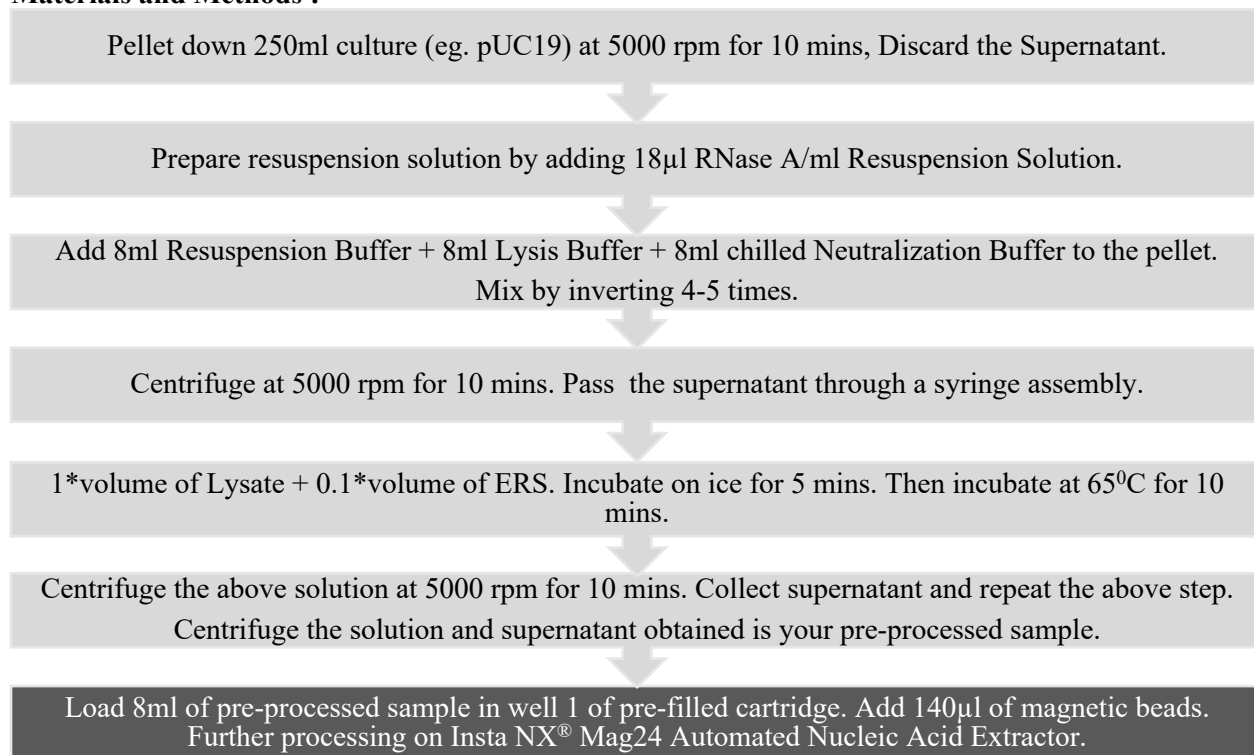
Rapid purification of Sequencing grade Endotoxin-free Plasmid Maxiprep DNA using HiMedia's Mag24 Platform



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Extraction of nucleic acids is the starting point in any molecular biology study and hence is considered as a crucial process. Plasmids are considered a major driving force of prokaryote evolution, as they can migrate between populations, making them potent agents of lateral DNA transfer and microbial warfare. The importance of plasmids goes beyond microbial evolution, as they are widely used as vectors for genetic engineering in basic research (e.g., random mutagenesis) as well as applications in biotechnology (e.g., insulin production), synthetic biology, agriculture (e.g., genetic engineering of crops) and medicine (e.g., biopharmaceuticals). The demand for efficient production methods of plasmid DNA (pDNA) has increased vastly in response to rapid advances in the use of pDNA in gene therapy and in vaccines since the advantageous safety concerns associated with non-viral over viral vectors. Plasmid DNA purified from bacterial cells can be contaminated with endotoxin to different extents, depending on the purification method. Reports indicate that endotoxin can decrease transfection efficiency in many eukaryotic cell lines. HiMedia's HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification Kit provides an endotoxin-free, high yield plasmid DNA and hassle-free automated solution for extraction.

Materials and Methods :-



Results

I. Extraction performance evaluation:

Plasmid DNA (high-copy plasmid pUC19 and low-copy plasmids pEG202, YEp13 and YCp50) from bacterial cultures were isolated using HiMedia ‘HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification Kit [MB515PC-24]’ and its performance was compared with commercially available competitor Endotoxin-free Plasmid Maxi kit. HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification [MB515PC-24] kit gave **better total yield (up to 700µg)** and good spectrophotometric ratios (A_{260}/A_{280} ratio ≥ 1.8) with **intact DNA band** (supercoiled) in **lesser time (60 mins in machine)** when compared with competitor kit. **Repeatability** of the performance (yield) of the MB515PC-24 kit was confirmed by replicate testing and was found to be satisfactory. Dilutions (1:10, 1:100) of all the plasmids showed proportionate decrease in DNA yield indicating that the **concentration** and **yield** of undiluted eluate is **accurate**.

Plasmid	Sample	Protocol	Yield (ng/µL)	Total Yield (µg/mL)	Purity (A_{260}/A_{280})	Purity (A_{260}/A_{230})
pEG202	Replicate 1	HM	800.40	720.36	1.88	2.32
		OTH	109.40	98.46	1.89	2.40
	Replicate 2	HM	783.70	705.33	1.88	2.31
		OTH	110.40	99.36	1.90	2.38
	Replicate 1 (1:10)	HM	80.25	72.23	1.89	2.29
		OTH	10.40	9.36	1.75	1.81
	Replicate 1 (1:100)	HM	6.20	5.58	2.14	1.48
		OTH	0.65	0.59	---	-3.25
YEp13	Replicate 1	HM	593.45	534.11	1.86	2.19
		OTH	22.60	20.34	1.85	2.06
	Replicate 2	HM	563.65	507.29	1.85	2.16
		OTH	22.55	20.30	1.84	2.23
	Replicate 1 (1:10)	HM	59.45	53.51	1.85	2.16
		OTH	2.35	2.12	1.74	1.42
	Replicate 1 (1:100)	HM	5.60	5.04	1.90	1.81
		OTH	0.20	0.18	0.67	-0.57
YCp50	Replicate 1	HM	637.20	573.48	1.91	2.26
		OTH	364.15	327.74	1.91	2.40
	Replicate 2	HM	625.70	563.13	1.91	2.24
		OTH	368.45	331.61	1.91	2.39
	Replicate 1 (1:10)	HM	67.05	60.35	1.86	2.24
		OTH	39.65	35.69	1.90	2.35
	Replicate 1 (1:100)	HM	7.40	6.66	1.70	1.37
		OTH	4.40	3.96	2.15	1.54
pUC19	Replicate 1	HM	639.45	575.51	1.93	2.51
		OTH	483.35	435.02	1.92	2.34
	Replicate 2	HM	656.35	590.72	1.93	2.51
		OTH	492.10	442.89	1.92	2.35
	Replicate 1 (1:10)	HM	67.15	60.44	1.89	2.52
		OTH	48.15	43.34	1.91	2.27
	Replicate 1 (1:100)	HM	7.30	6.57	1.85	1.45
		OTH	4.15	3.74	2.02	1.93

Table 1: Total yield and purity measurements of plasmid DNA isolated from kits **HiMedia (HM)** and **Competitor (OTH)**.

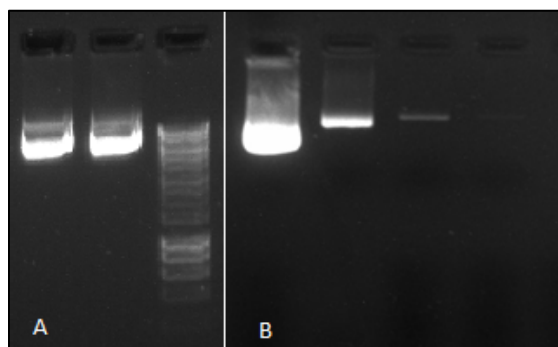


Figure 1: High Quality of Plasmid DNA (gel image).

A: 2µl of Plasmid DNA (pEG202) extracted by MB515PC-24 kit loaded along with 1kb molecular ladder run on 1% agarose gel

B: 5µl of dilutions of extracted plasmid DNA (pEG202) run as Neat, 1:10, 1:100 and 1:1000 run on 1% agarose gel

II. Restriction Digest of Plasmid DNA:

Plasmid DNA isolated using HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification [MB515PC-24] is of high quality and suitable for downstream application such as **restriction digestion**. Plasmid DNA (low-copy plasmid YEp13) from bacterial culture was isolated using MB515PC-24 and subjected to restriction digestion with various restriction enzymes. The purified plasmid is compatible for restriction digestion and the restriction map was verified.

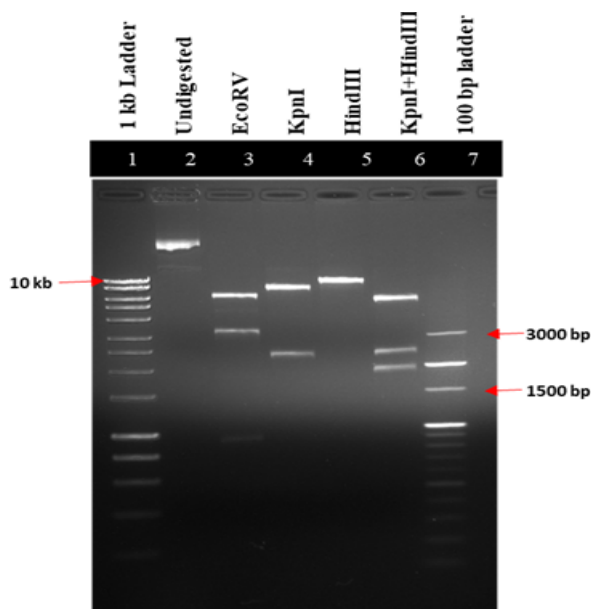


Figure 2: Restriction digestion pattern of YEp13 vector.

III. Sequencing data of plasmid DNA purified using the HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification Kit [MB515PC-24]:

Plasmid DNA isolated using HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification [MB515PC-24] is of high quality and suitable for downstream application such as **sequencing**. Plasmid DNA (high-copy plasmid pUC19) from bacterial culture was isolated using MB515PC-24 and subjected to sanger sequencing using M13 primers. The result showed blast identity of **>99%** over a **958 bp** contiguous sequence when aligned with complete sequence of Cloning vector pUC19. This indicates that the purified Plasmid DNA is of high quality compatible with sequencing application.

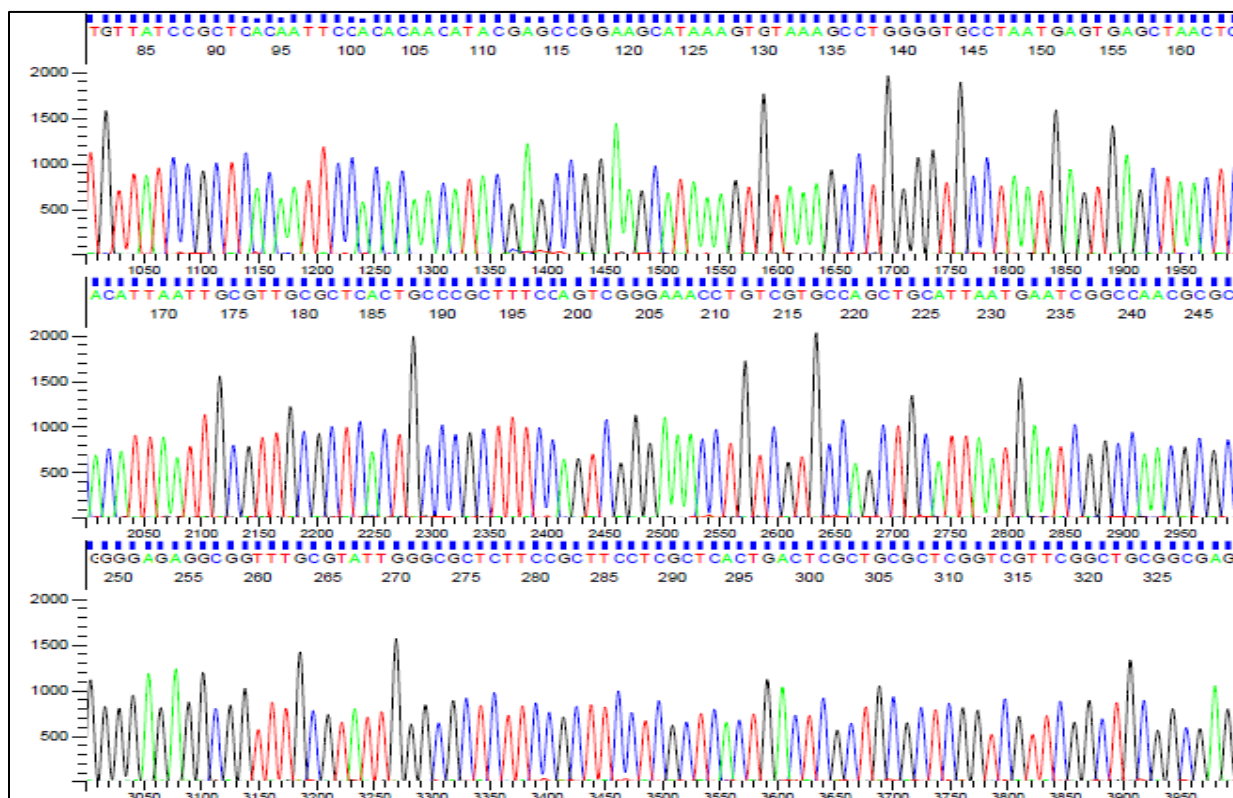


Figure 3: Partial electropherogram sequence of Plasmid DNA of pUC19

Conclusion :-

HiPurA® Pre-filled Cartridges for Endotoxin-free Plasmid Maxiprep DNA Purification Kit offers a reliable hassle-free automated solution of isolating up to 700 µg of endotoxin-free good quality plasmid DNA.



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