Game Changing Plasmid Purification Isolation:

Thermo Fisher presents a High Throughput, Centrifugation Free method for isolating Transfection Grade Plasmid DNA

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Abstract

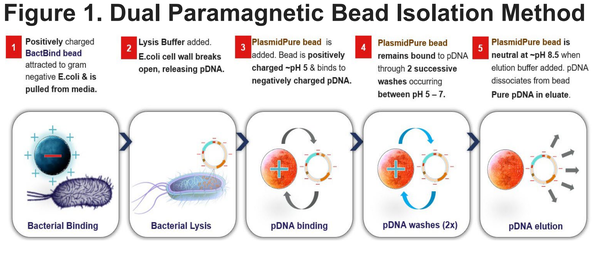
Plasmid purification has recently moved into the spotlight with the development of mRNA vaccines and widely accepted gene therapies using viral vectors. With heightened demand to shorten development times of biotherapeutics, Pharma and Biotechnology industries are seeking solutions to expedite the pace of research by removing bottlenecks and inefficiencies stunting the development of future therapeutics. Bacterial cell pelleting has been identified as a historical bottleneck when isolating pDNA.
In response to industry demands, Thermo Fisher Scientific has developed the MagMAX™ Pro HT NoSpin™ Plasmid Isolation technology for use in high throughput workflows focused on development of novel biotherapeutics. Unlike other extraction methods, this isolation technique utilizes dual paramagnetic bead-based separation, eliminating pre-processing inefficiencies (i.e., centrifugation, vacuum filtration) entirely.

Here, we demonstrate pDNA isolation using the MagMAX™ Pro HT NoSpin™ chemistry in conjunction with KingFisher Flex, Presto, and Apex Sample Preparation Purification Systems.
Our method results in consistently high yields (18-21µg) of transfection grade pDNA, in a fraction of the time, compared to traditional plasmid miniprep purification techniques (96 samples processing capacity in ~35 min). Isolated pDNA is suitable for immediate use in a variety of downstream applications such as endonuclease digestion and mammalian transfection. Additionally, our plasmid purification method offers flexible adaptation for various workflows: standalone benchtop KingFisher purification systems, and liquid handling robotic systems.

Introduction

MagMAX™ Pro HT NoSpin™ Plasmid Miniprep kit chemistry is ideal for high throughput workflows requiring high quality pDNA. This dual, paramagnetic bead isolation technique provides rapid and easily automatable method for (1) isolating *E.coli* cells in culture, through exploitation of the electrochemical charge differential inherent to the bacterial cell wall structure using our BactBind Beads and (2) then pDNA isolation after an alkaline lysis using our Plasmid Pure Beads.

Figure 1. Dual Paramagnetic Bead Isolation Method



Introduction (continued)

Figure 2. KingFisher Flex (benchtop)

Figure 3. KingFisher Apex (benchtop)

Figure 4. KingFisher Presto (Integrated with Liquid Handler)

Materials and Methods

MagMAX™ Pro HT NoSpin™ Plasmid Miniprep Kit Sample Preparation

E. coli strain TOP10/pSEAP2 is grown overnight shaking at 225 RPM in a 37◦C incubator. 1mL of culture was put into individual wells of a KingFisher 96 deep-well plate, and the plate processed on the KingFisher™ Apex System. The elution plate, containing purified pDNA, was utilized to obtain NanoDrop™ results and for downstream assays to detect endotoxin.

Test Method(s)

Samples analyzed utilizing NanoDrop™ Eight Spectrophotometer to obtain pDNA yield, A260/280 ratio, A260/230 ratio, and % E. coli bound to paramagnetic bead. Samples were run on 1% agarose gel to examine DNA quality. Samples were tested for endotoxin utilizing the Charles River Laboratory Endosafe® nexgen-PTS™ system and the Quant-iT™ Endotoxin Detection Assay Kit.

Transfection efficiency of the pDNA from the high-copy One Shot TOP10/pSEAP culture was measured using HuH-7 cells maintained in DMEM with low glucose and 10% FBS. Transfection was performed with 100 ng of pDNA diluted in Invitrogen™ Lipofectamine™ 3000 Transfection Reagent and Gibco™ Opti-MEM™ medium, with 10,000 cells per well in six replicates. The plate was incubated at 37C with 5% CO2 for 20-24 hours. Following incubation, 25 µL of the culture medium was assayed for the SEAP reporter protein using the Invitrogen™ Phospha-Light™ SEAP Reporter Gene Assay System following standard procedures. The luminescence generated by SEAP acting on the substrate was measured using a FLUOstar™ Omega instrument (BMG Labtech).

Data Analysis

Data was analyzed utilizing JMP 17.

Results

Yields and Purity

Figure 5. KingFisher Flex and Apex pDNA Yield and Purity: Representative purity ratios from samples grown in both LB and TB media with a large, 11.4kb plasmid. Data taken from a representative run with n= 96 processed in a KingFisher 96 deep-well plate on a KingFisher ™ Flex (Left) and Apex (Right) instrument.

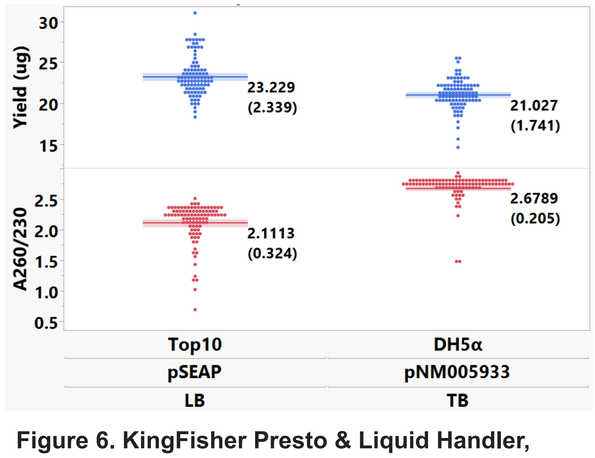


Figure 6. KingFisher Presto & Liquid Handler, pDNA Yield: Representative purity ratios from samples grown in both LB media with a medium sized, 5.4kb plasmid. Data taken from a representative run with n= 96 processed in 4 KingFisher 96 deep-well plates on an integrated KingFisher Presto/Liquid Handler.

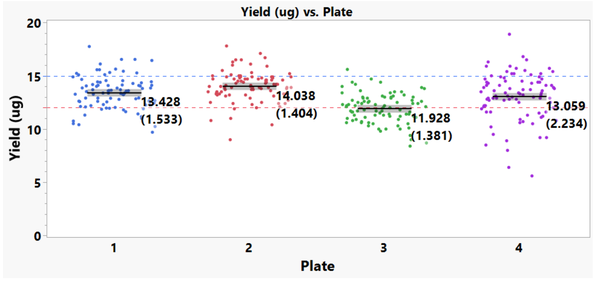


Figure 7: Endotoxin Analysis; pDNA extracted from six randomized samples from the KingFisher Presto Extraction with TOP10/pSEAP2 in LB culture.

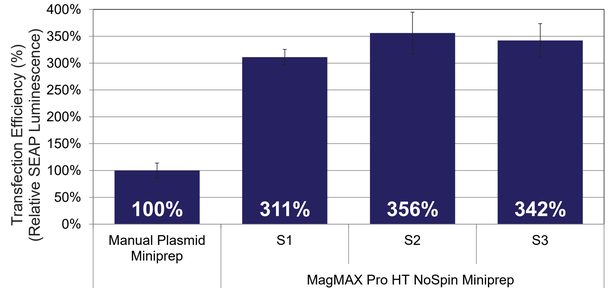
Sample ID	Conc. (ng/µL)	Diln.	value	EU/mL	EU/µg	Grade
4-A1	121.4	1000	=	0.672	**0.445**	Transfection Grade
4-A10	119.6		=	0.695	**0.504**	
4-A19	128.1		<	0.661	**0.390**	
4-A28	134		<	0.698	**0.373**	Low Endo
4-A37	108.5		<	0.836	**0.460**	
4-A46	125.4		<	0.835	**0.398**	

Results (continued)

Transfection Efficiency

Figure 8: SEAP luminescence after transfection of pDNA into HuH-7 cells

pDNA was isolated using MagMAX™ Pro HT NoSpin Miniprep Kit and GeneJET™ Plasmid Miniprep Kit. Luminescence was measured using a FLUOstar™ Omega instrument.



Conclusions

- Process 96 plasmid culture samples, start to finish, on KingFisher Sample Prep Purification Systems: 35 minutes or less
- Our method provides consistently, high yields (18 -21 µg).
- Our method obtains low endotoxin levels, (< 1 EU/µg) that enable direct downstream use in transfection and other biotherapeutic applications.
- Easily automatable with liquid handling robotic systems to extract four, ninety-six well plates per batch as a walkaway solution

References

1. Stadler J, Lemmens R, Nyhammar T. 2004. Plasmid DNA purification. Journal of Gene Medicine; 6(Suppl 1):S54eS66.

2. Schneier M, Razdan S, Miller AM, Briceno ME, Barua S. 2020. Current technologies to endotoxin detection and removal for biopharmaceutical purification. Biotechnology and Bioengineering 117:2588–2609.

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