Plasmid purification

High-throughput, centrifugation-free plasmid DNA extraction using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit

Summary

- **Centrifugation-free**—eliminates the need for traditional centrifugation steps for plasmid DNA purification
- **Innovative technology**—uses novel magnetic bead–based technology to capture bacterial cells from culture and bind and elute plasmid DNA
- **Automated**—high-throughput dual paramagnetic bead-based plasmid DNA isolation on Thermo Scientific™ KingFisher™ systems
- **High yield**—purifies up to 20 µg of high-copy plasmid DNA from 1 mL of bacterial culture
- **Superb quality**—transfection-grade plasmid DNA isolation with an enhanced endotoxin removal buffer

Introduction

The purification of plasmid DNA (pDNA) plays a vital role in molecular biology research [1]. Isolation of pDNA from 1–2 mL of overnight bacterial culture is generally called a miniprep. pDNA is utilized in various downstream applications, including cloning, sequencing, vaccine development, antibody production, cell and gene therapy research, and viral vector production, to name a few [2]. Continuing developments in pDNA isolation technology have provided researchers with various options for efficiently isolating and purifying pDNA for specific applications. However, these advancements have been limited by the multiple centrifugation steps needed to pellet the bacterial cells and isolate pDNA. These are rate-limiting steps in the process of isolating high-quality pDNA in a shorter time frame with fewer hands-on steps. The high-throughput pDNA purification method of the Applied Biosystems™ MagMAX™ Pro HT NoSpin Plasmid Miniprep Kit reduces these processing bottlenecks by eliminating centrifugation steps, improving the workflow for biotherapeutic drug development.

The MagMAX Pro HT NoSpin Plasmid Miniprep Kit utilizes advanced dual paramagnetic bead-based technology to enable automation of various steps involved in pDNA purification using the benchtop Thermo Scientific™ KingFisher™ Flex or Apex Purification System. Briefly, these steps include binding of bacterial cells from an overnight culture to magnetic beads, cell lysis, removal of cellular debris, binding of pDNA to magnetic

beads, washing away of impurities, and elution of purified pDNA. The resulting pDNA is high in supercoiled isoforms and devoid of contaminating bacterial genomic DNA and RNA, and the enhanced endotoxin removal buffer yields transfection-grade pDNA that is suitable for various downstream applications. The MagMAX Pro HT NoSpin Plasmid Miniprep Kit is fully automation-ready with liquid handling robotic systems. Furthermore, the reagent volumes provided with the kit are designed for high-throughput workflows. The integration of the Thermo Scientific™ KingFisher™ Presto™ Purification System with qualified liquid handlers enables rapid extraction, higher processing efficiency, and consistently high-quality pDNA.

Materials and methods

Bacterial culture

Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* was transformed in order to grow two different plasmids: pNM005933 (11.4 kb) and pSEAP2 (5.1 kb). A single colony from each plasmid transformation was cultured overnight to prepare glycerol stocks that were then used to grow bacterial cultures for pDNA isolation using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit. Briefly, TOP10 cells carrying pNM005933 were grown overnight in Gibco™ LB broth (1X) supplemented with 50 µg/mL kanamycin, while TOP10 cells carrying pSEAP2 were grown overnight in LB broth supplemented with 100 µg/mL carbenicillin. Both cultures were subjected to continuous shaking at 225 rpm in a 37°C incubator. The optical density (OD_{600}) of the TOP10 cells carrying pNM005933 and pSEAP2 reached 2.6 and 2.4, respectively, after 14–16 hours, as measured by a Thermo Scientific™ NanoDrop™ Spectrophotometer.

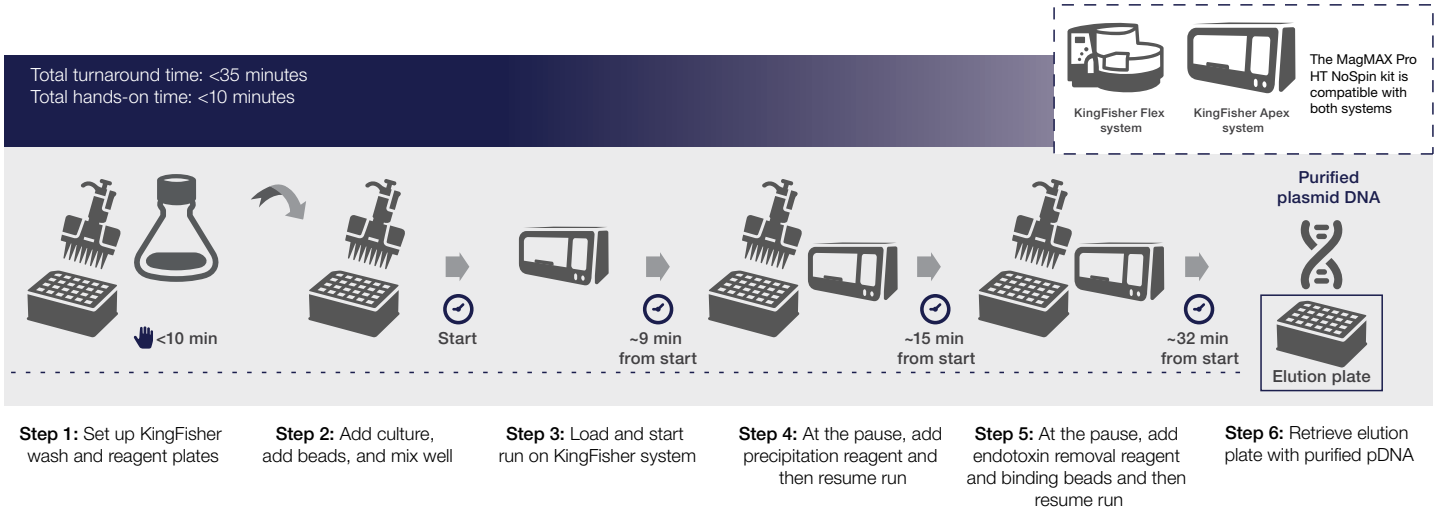

Figure 1. Benchtop extraction workflow for pDNA purification using a KingFisher system and the MagMAX Pro HT NoSpin Plasmid Miniprep Kit.

pDNA isolation on KingFisher systems

Four 96 deep-well extractions were used for each plasmid type (n = 384). Briefly, 1 mL aliquots of culture were transferred to the wells of a KingFisher 96 deep-well plate (4 plates per plasmid type). The plates were then processed on the KingFisher Flex or Apex Purification System using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit protocol of the user guide and following the extraction workflow shown in Figure 1. The benchtop extraction workflow with the KingFisher system enables isolation of pDNA from 96 samples in less than 35 minutes, with less than 10 minutes of hands-on time. The elution plate containing 100 µL per well of purified pDNA can then be used for downstream analysis.

pDNA analysis

Eluted samples were analyzed utilizing the Thermo Scientific™ NanoDrop™ Eight Spectrophotometer to obtain pDNA yield, A_{260}/A_{280} ratio, and A_{260}/A_{230} ratio measurements. pDNA was then run on a 1% agarose gel to visually assess the quality of pDNA isoforms and contamination with genomic DNA and RNA. A subset of four randomized eluted samples for each plasmid was further analyzed by restriction enzyme digestion. After digestion with BamHI, the samples were run in duplicate on a 1% agarose gel to identify and distinguish individual pDNA size and quality. The Invitrogen™ iBright™ FL1500 Imaging System was used to image and analyze the gel for pDNA isoforms.

Endotoxin measurements

Six representative pSEAP2 pDNA samples, purified on the KingFisher Apex system, were selected to analyze for the presence of endotoxins using the Endosafe™ nexgen-PTS™ system (Charles River Laboratories).

pDNA transfection efficiency

Transfection efficiency of the high-copy pSEAP2 plasmid 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 37°C with 5% CO_2 for 20–24 hours. Following incubation, 25 µL of the culture medium was assayed for the secreted alkaline phosphatase (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).

Results

pDNA yield and purity

The total yields of pDNA obtained from two high-copy plasmids produced by TOP10 cells in LB medium and purified using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit from four 96 deep-well plates for each plasmid are shown in Figure 2. The average total yield for pNM005933 using the KingFisher Flex system was 16.2 µg with a standard deviation (SD) of 2.9. The average total yield for pSEAP2 using the KingFisher Apex system was 18.8 µg with a SD of 4.0. The average A_{260}/A_{280} and A_{260}/A_{230} ratios for sample purity were >1.8 and >2.0, respectively, which meet the purity standards required for downstream analysis [3] (Figure 2).

pDNA quality

Highly supercoiled pDNA is required for various downstream applications [4]. Here, gel electrophoresis showed predominantly supercoiled pDNA from both plasmids, with no traces of visible RNA or genomic DNA contamination across all samples (S1–S4, Figure 3A, 3B). The MagMAX Pro HT NoSpin Plasmid Miniprep Kit isolation yields abundant supercoiled pDNA and low amounts of other topological forms, demonstrating the quality of pDNA desired for biotherapeutics application workflows. Restriction enzyme digestion was used to assess the size and identity of the plasmid (Figure 3A, 3B). BamHI digests of the 5.1 kb pSEAP2 had a correct banding pattern of 3,300 bp and 1,800 bp. Similarly, digests of the 11.4 kb pNM005933 showed the correct banding pattern of 7,000 bp and 4,000 bp.

Endotoxin measurements

Endotoxins, also known as lipopolysaccharides, are cell wall components of gram-negative bacteria that are released into solution upon cell lysis and co-purify with pDNA during isolation [5]. The MagMAX Pro HT NoSpin Plasmid Miniprep Kit is supplied with an endotoxin removal buffer to neutralize the endotoxins and provide transfection-ready pDNA. Table 1 shows endotoxin measurements for six representative pSEAP2 samples isolated from TOP10 cells that were grown in LB. The endotoxin levels did vary between the samples, but they all were at or below 1.0 EU/µg. Hence, pDNA isolated with the MagMAX Pro HT NoSpin Plasmid Miniprep Kit is classified as transfection-grade, suitable for biotherapeutics workflows in early drug discovery research. However, the endotoxin levels may vary depending on the bacterial strain, plasmid type, and culture conditions used.

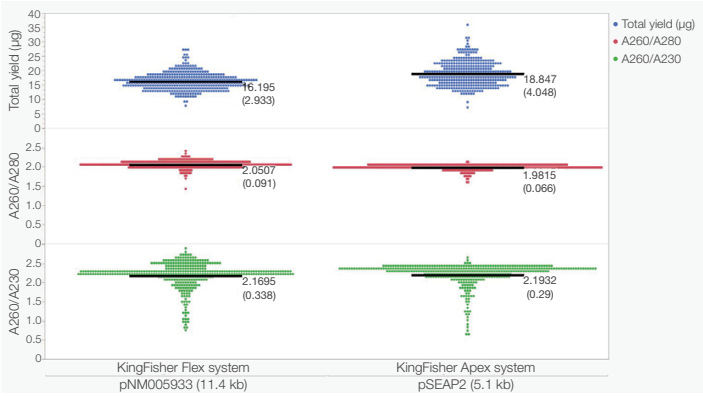

Figure 2. pDNA yield and purity following purification on the KingFisher Flex and Apex systems. TOP10 cells carrying either pNM005933 or pSEAP2 were grown in LB medium and processed on the KingFisher Flex and KingFisher Apex systems, respectively. Total yield and purity ratios (A_{260}/A_{280} and A_{260}/A_{230}) were determined for each purification. Data represent four 96 deep-well plate runs (n = 384) for each plasmid, with mean (± SD). Data were analyzed using JMP™ software 16.0 (SAS Institute Inc.).

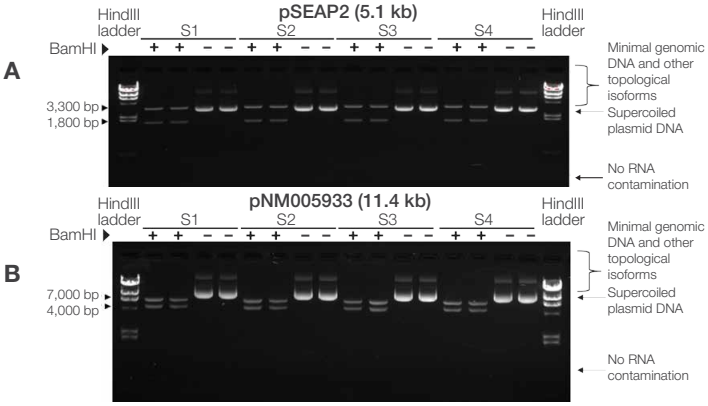

Figure 3. Agarose gel electrophoresis to assess the presence of DNA isoforms and quality of pDNA. (A) pSEAP2 and **(B)** pNM005933 samples extracted using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit contained minimal genomic DNA, nicked pDNA, and single-stranded pDNA, the majority being supercoiled pDNA. Restriction digestion with BamHI generated the correct banding pattern for both plasmids.

Table 1. Endotoxin measurements* for pDNA isolated using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit.

Sample	Endotoxin (EU/µg)
S1	<0.4
S2	<0.7
S3	0.3
S4	<0.3
S5	1.0
S6	<0.3

* Measurements were taken from six randomized samples from two different KingFisher Apex system extractions (S1–S3 from one run and S4–S6 from the second run). pDNA was extracted from TOP10 cells carrying pSEAP2 plasmid that were grown in LB medium.

pDNA transfection efficiency

SEAP protein expression from pDNA transfected into Huh-7 cells is high, which further indicates the suitability of pDNA for mammalian cell transfection (Figure 4). Variation in transfection efficiency among the six samples could be attributed to differences in the percentage of supercoiled pDNA. These results also provide insight into the intra-run variability for a benchtop workflow with two different KingFisher instruments and two different operators, where the first three samples (S1–S3) were from the same run while the other three samples (S4–S6) were from an additional set of runs from the same overnight culture. Overall, the supercoiled pDNA isolated using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit is very stable and successfully enters the mammalian cell, resulting in the desired protein expression (Figure 4).

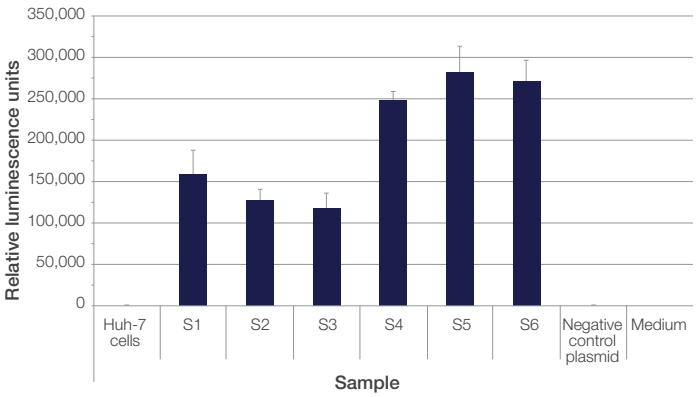

Figure 4. SEAP-based luminescence assay of Huh-7 cells transfected with purified DNA. A culture of TOP10 cells carrying pSEAP2 was grown, and the plasmid DNA was isolated using the MagMAX Pro HT NoSpin Plasmid Miniprep Kit with the KingFisher Apex purification system. The isolated pDNA was used to transfect Huh-7 cells, and the SEAP expression of six randomized samples (S1–S6) was measured using the luminescence-based Phospha-Light SEAP Reporter Gene Assay System.

Conclusion

The MagMAX Pro HT NoSpin Plasmid Miniprep Kit has a simple workflow that saves time while providing supercoiled pDNA of high yield and purity. High-quality pDNA enables numerous applications, such as antibody production, vaccine development, and cell and gene therapy research. Furthermore, the kit is easily integrated into a liquid handling system for high-throughput workflows.

Authors

Huntley Polanshek, Ron Abruzzese, Rasika Sunnadeniya, Karessa Garza, and Jay Bhandari, Thermo Fisher Scientific, Austin, TX.

Ordering information

Description	Quantity	Cat. No.
MagMAX Pro HT NoSpin Plasmid Miniprep Kit	96 reactions	A58309
	384 reactions	A58310
	1,152 reactions	A58311
	2,304 reactions	A58312

References

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4. Higgins NP, Vologodskii AV (2015) Topological behavior of plasmid DNA. *Microbiol Spectr* 3(2). doi: 10.1128/microbiolspec.plas-0036-2014
5. Schneier M, Razdan S, Miller AM et al. (2020) Current technologies to endotoxin detection and removal for biopharmaceutical purification. *Biotechnol Bioeng* 117(8):2588–2609. doi: 10.1002/bit.27362

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