Plasmid purification

Effortless maxi-scale plasmid purification with the KingFisher PlasmidPro Maxi Processor

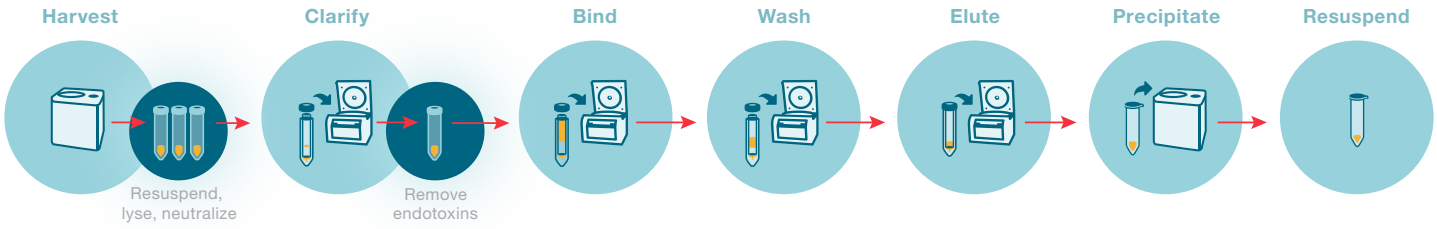
- **Centrifugation-free**—no centrifugation or vacuum filtration needed for plasmid DNA (pDNA) purification
- **Innovative technology**—utilizes an all-in-one prefilled reagent cartridge that enables setup in less than 5 minutes
- **Fully automated**—simply pour in the culture and press Start. pDNA is eluted in less than 75 minutes with no manual processing steps
- **Endotoxin-free**—less than 0.1 endotoxin unit (EU) per microgram of isolated pDNA

Introduction

Plasmid DNA purification has a vital role in molecular biology research [1]. pDNA isolated at maxi scale is utilized in a variety of downstream applications, including mRNA synthesis, vaccine development, antibody production, cell and gene therapy, and viral vector production [2]. With the growing demand for high-quality pDNA for molecular biology applications, reliable and automated pDNA purification processes have become increasingly important. pDNA can be purified effectively using a traditional column-based method. However, column-based purification is labor-intensive and time-consuming, as multiple centrifugation and vacuum filtration steps may be needed to isolate pDNA. Column-based purification is thus a bottleneck for researchers seeking to obtain high-quality pDNA quickly.

The fully automated Thermo Scientific™ KingFisher™ PlasmidPro Maxi Processor can streamline the pDNA purification workflow by reducing processing bottlenecks (Figure 1). The KingFisher PlasmidPro Maxi Processor is an advanced plasmid purification system that uses an all-in-one prefilled cartridge with the same chemistry as the Invitrogen™ PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit. With innovative technology and a user-friendly interface, this system is a powerful tool for efficient and reliable maxi-scale pDNA purification. The simple automated workflow saves valuable time, and fewer resources are needed to obtain high-quality pDNA for demanding downstream applications. Here we provide a comprehensive overview of the performance of the KingFisher PlasmidPro Maxi Processor for maxi-scale purification of four plasmids ranging from ~2.7 kb to ~11.4 kb in size. We assessed its capabilities in terms of pDNA yield, purity, supercoiled pDNA quality, and transfection efficiency.

Column-based manual plasmid workflow



KingFisher PlasmidPro Maxi Processor plasmid purification system

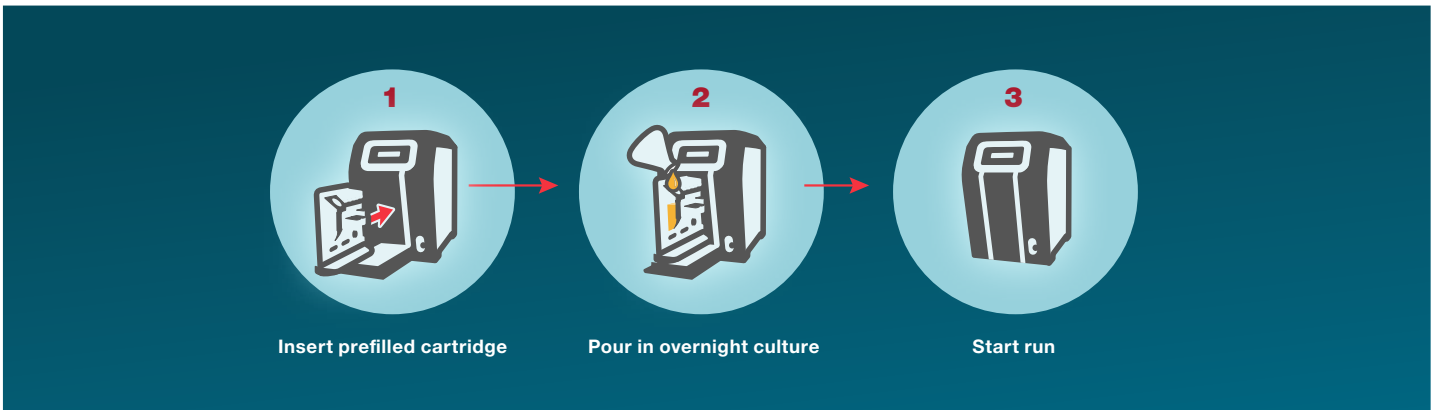


Figure 1. Comparison of automated and manual plasmid purification workflows. The KingFisher PlasmidPro Maxi Processor is simple to use and enables fully hands-free plasmid purification in less than 75 minutes. The completely hands-on workflow for column-based plasmid purification can be effective, but it is time-consuming and labor-intensive.

Materials and methods

Bacterial culture

Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* were transformed with plasmid A (~2.7 kb), plasmid B (~5.1 kb), or plasmid D (~11.4 kb), and Invitrogen™ MAX Efficiency™ DH5α Competent Cells (*E. coli*) were transformed with plasmid C (~6.6 kb). A single colony from each plasmid transformation was grown overnight to prepare a glycerol stock. The glycerol stocks were stored at –80°C, then used to prepare bacterial cultures for pDNA isolation. The cultures were grown overnight in Gibco™ LB Broth (1X) with continuous shaking at 225 rpm in a 37°C incubator. The medium was supplemented with 100 µg/mL carbenicillin to select for cells transformed with plasmid B, 100 µg/mL ampicillin to select for cells transformed with plasmid A or plasmid C, or 50 µg/mL kanamycin to select for cells transformed with plasmid D. The optical densities of the cultures were measured on a Thermo Scientific™ NanoDrop™ spectrophotometer after 14–16 hours of growth.

pDNA isolation

pDNA was purified using the KingFisher PlasmidPro Maxi Processor equipped with the Thermo Scientific™ KingFisher™ PlasmidPro Maxi Processor Endotoxin-Free Cartridge. The bacterial cultures for plasmids A, B, C, and D were grown overnight, and 150 mL of each culture was processed. Data were analyzed from 74 extractions performed on nine different KingFisher PlasmidPro Maxi Processors (plasmid A, n = 19; plasmid B, n =13; plasmid C, n = 21; plasmid D, n = 21). The performance of the pDNA purified using the automated KingFisher PlasmidPro system was compared to pDNA isolated manually from the same cultures using the column-based PureLink Expi Endotoxin-Free Maxi Plasmid Purification Kit according to the user guide.

pDNA analysis

The eluted samples were analyzed using the Thermo Scientific™ NanoDrop™ Eight UV-Vis Spectrophotometer to determine pDNA yields and A_{260}/A_{280} and A_{260}/A_{230} ratios. A subset of each plasmid type was run on a 1% agarose gel for 75 minutes at a constant voltage of 75 V to visually assess the amounts of supercoiled pDNA, genomic DNA, and RNA contamination in the samples. The supercoiled pDNA was quantified by densitometry using images acquired on the Invitrogen™ iBright™ FL1500 Imaging System, and the data were analyzed using JMP™ software 16.0.

Endotoxin measurements

Twenty-six representative samples across all four plasmids were analyzed for the presence of endotoxins using the Endosafe™ nexgen-PTS™ endotoxin testing system. The results were compared to the endotoxin levels in pDNA samples obtained by the manual column-based plasmid purification process.

Residual *E. coli* genomic DNA (gDNA) in purified pDNA

Residual *E. coli* gDNA in each sample was measured to assess the purity of the plasmid preparations. The Applied Biosystems™ TaqMan™ Ba04646242_s1 ECs4157 qPCR assay, which targets a single-copy gene in the *E. coli* genome, and Applied Biosystems™ TaqMan™ Fast Advanced Master Mix were used for the analysis. Serial dilutions of gDNA from the control TOP10 or DH5α strains of *E. coli* were used to construct standard curves of C_t values against concentration to estimate the percentage of gDNA in the plasmid preparations. The purified pDNA samples were diluted 1:100, and 2 µL aliquots from the samples were tested in triplicate reactions. The data were analyzed using JMP software 16.0.

pDNA transfection efficiency

The efficiency of transfection with pDNA purified on the KingFisher PlasmidPro Maxi Processor was compared to the efficiency of transfection with pDNA purified using the manual process. Transfection of HuH-7 cells maintained in DMEM with low glucose and 10% FBS was performed using 100 ng of purified plasmid B or C diluted with Invitrogen™ Lipofectamine™ 3000 Transfection Reagent and Gibco™ Opti-MEM™ Reduced Serum Medium with 10,000 cells per well, with six replicates. The plate was incubated at 37°C with 5% CO_2 for 20–24 hours. For plasmid B, which contained a secreted alkaline phosphatase (SEAP) reporter sequence, 25 µL of the culture medium was assayed for gene expression using the Invitrogen™ Phospha-Light™ SEAP Reporter Gene Assay System, following standard procedures. Luminescence generated by the assay was measured using a FLUOstar™ Omega microplate reader. The Invitrogen™ Luc Screen™ Extended-Glow Luciferase Reporter Gene Assay System was used to assess the efficiency of transfection with plasmid C, which contained a luciferase sequence. Luciferase bioluminescence was measured in the culture plate after incubating the cells for 10 minutes at room temperature. The data were analyzed using JMP software 16.0.

Results

The optical densities (OD_{600}) of the cultures transformed with plasmids A, B, C, and D, after 14–16 hours of culturing, were 2.3, 2.8, 3.1, and 2.8, respectively. The total pDNA yields obtained after purification on the KingFisher PlasmidPro Maxi Processor are shown in Figure 2A. Even with large culture volumes, the total plasmid A yields isolated with the automated purification process were similar to what would be expected with a manual purification process. The average A_{260}/A_{280} and A_{260}/A_{230} ratios for all purified samples were >1.8 and >2.0, respectively, demonstrating comparable results that would satisfy purity requirements for all downstream applications (Figure 2B).

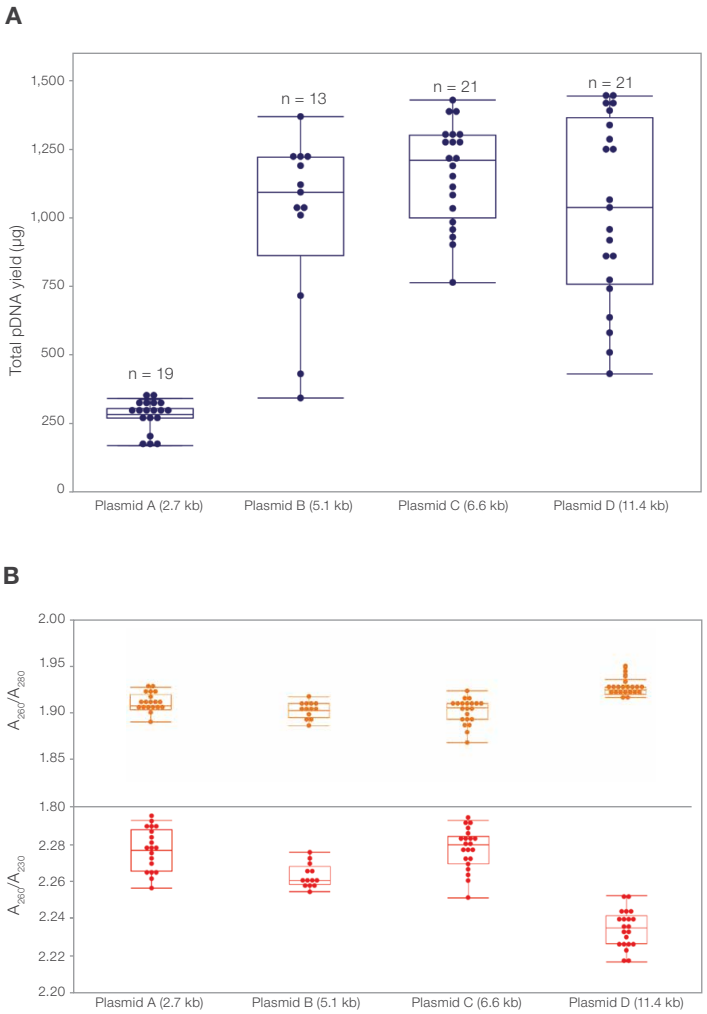


Figure 2. Yields and purities of four plasmids purified on the KingFisher PlasmidPro Maxi Processor. (A) Total pDNA yields. The box plots show the distributions of the data, with each dot representing an individual purification. **(B)** A_{260}/A_{280} and A_{260}/A_{230} pDNA purity ratios.

pDNA isoforms

Highly supercoiled pDNA is required for a variety of downstream applications [3]. The purified plasmids were analyzed by agarose gel electrophoresis, which showed predominantly supercoiled pDNA with no visible traces of RNA or gDNA contamination across samples (Figure 3A). Over 80% of the pDNA purified on the KingFisher PlasmidPro Maxi Processor was of the supercoiled isoform (Figure 3B), which was comparable to the amount of supercoiled isoform obtained with the manual process. These results demonstrate that fully automated, maxi-scale plasmid purification on the KingFisher PlasmidPro Maxi Processor can provide pDNA of superior quality to meet the demands of biotherapeutics workflows.

Residual *E. coli* gDNA content

E. coli gDNA must be removed during the plasmid purification process because residual host cell DNA has the potential to interfere with downstream applications. qPCR was used to determine the residual *E. coli* gDNA content in 37 representative purified samples of plasmids B, C, and D. The amount of residual gDNA in samples purified on the KingFisher PlasmidPro Maxi Processor was within 1% of that left over after manual purification (Figure 4). The data highlight the efficiency of the KingFisher PlasmidPro Maxi Processor at removing bacterial gDNA.

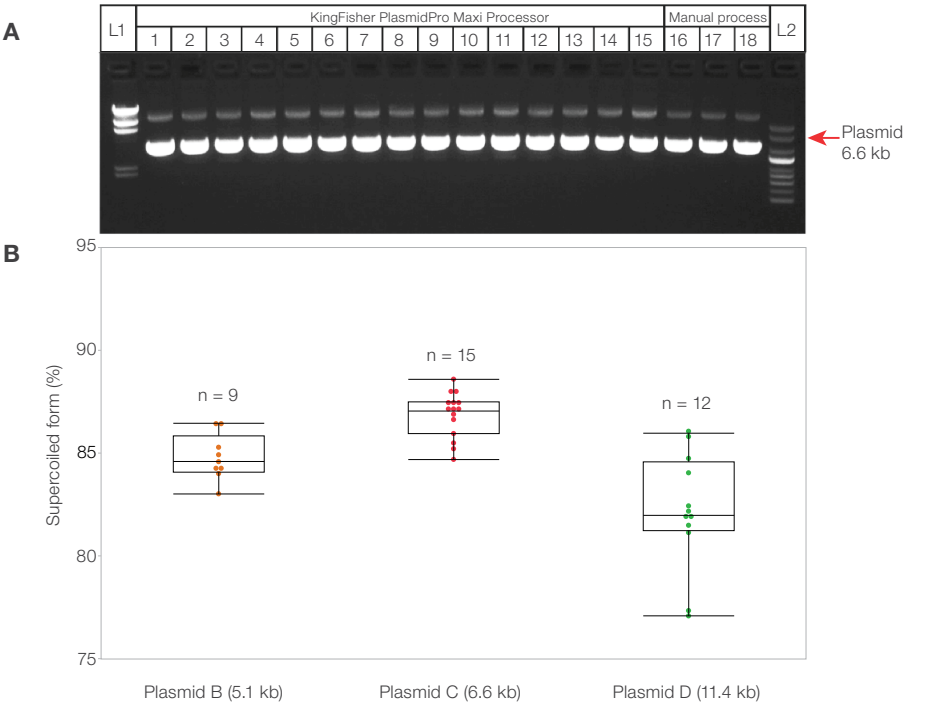


Figure 3. Results of gel electrophoresis and densitometric analysis of pDNA isoforms. (A) Representative samples of plasmid C (6.6 kb, lanes 1–15) purified using the KingFisher PlasmidPro Maxi Processor are compared to pDNA purified manually with a column-based kit (lanes 16–18), in a 1% agarose gel. L1: linearized dsDNA ladder. L2: supercoiled pDNA ladder. Supercoiled pDNA was the predominant form at 6.6 kb. **(B)** Percentages of supercoiled pDNA in a subset of samples purified using the KingFisher PlasmidPro Maxi Processor. The box plots show the distributions of data, with each dot representing an individual purification. For all three plasmids, the average percentage of supercoiled pDNA exceeded 80% and was comparable to the average percentage of supercoiled pDNA in samples purified using the manual process (88%).

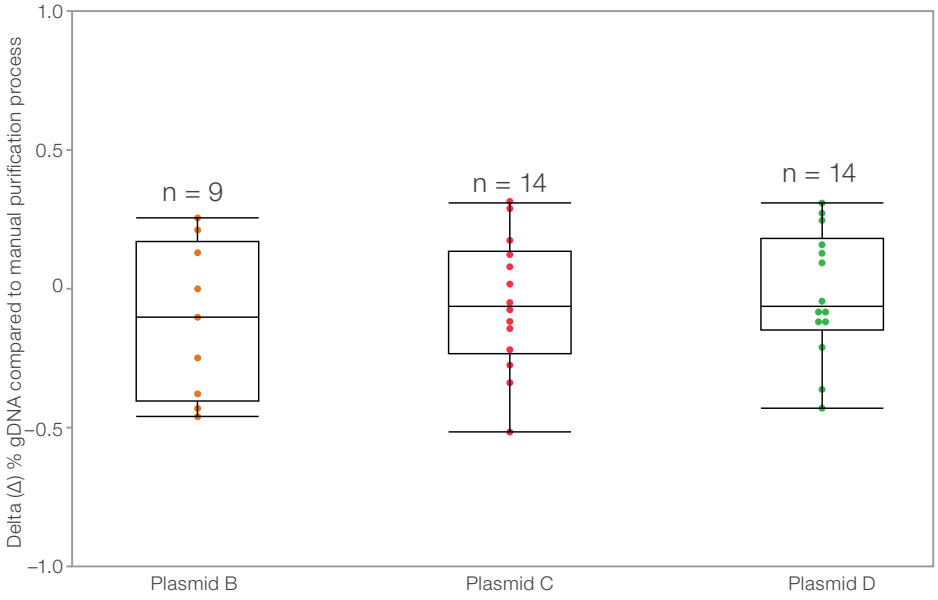


Figure 4. Relative percentages of bacterial gDNA remaining in purified plasmid preparations. Representative data showing the amounts of residual gDNA in pDNA samples purified on the KingFisher PlasmidPro Maxi Processor. The percent change for each plasmid is relative to the amount of gDNA left over after manual purification of the same plasmid. The box plots show the distributions of the data, with each dot representing an individual purification.

Endotoxin content

Endotoxins are lipopolysaccharide components in the cell walls of gram-negative bacteria. They are released into solution during cell lysis and get carried along with pDNA through purification [4]. Although endotoxin levels vary with bacterial strain, plasmid type, and culture conditions, pDNA that contains less than 0.1 endotoxin unit (EU) per µg is considered endotoxin-free (advanced transfection grade) and suitable for biotherapeutics workflows in drug discovery research. The prefilled cartridge for the KingFisher PlasmidPro Maxi Processor includes an endotoxin removal buffer that neutralizes endotoxins. The endotoxin content was measured in 26 representative pDNA samples that included all four plasmids. Samples S1–S26 were purified using the KingFisher PlasmidPro Maxi Processor, and the endotoxin levels in the samples were compared to endotoxin levels in samples from the same cultures after manual purification with the PureLink Expi Endotoxin-Free Maxi Plasmid Purification Kit (Table 1). The endotoxin levels in all samples purified on the KingFisher PlasmidPro Maxi Processor were below 0.1 EU/µg and comparable to those measured in pDNA samples isolated using the manual purification process.

pDNA transfection efficiency

The downstream application of purified pDNA to express reporter genes is important in gene therapy, functional genomics, and protein production. Reporter gene expression in mammalian HuH-7 cells transfected with plasmid B or plasmid C purified on the KingFisher PlasmidPro Maxi Processor was compared to expression of the same genes in HuH-7 cells transfected with plasmid B or plasmid C purified manually. Expression of the *SEAP* reporter gene from plasmid B and the luciferase gene from plasmid C was equivalent after pDNA purification with the KingFisher PlasmidPro Maxi Processor and the manual process (Figure 5). These results indicate effective pDNA delivery and reporter gene expression, demonstrating that pDNA purified on the KingFisher PlasmidPro Maxi Processor is highly stable and suitable for mammalian cell transfection.

Table 1. Endotoxin content in pDNA samples purified using the KingFisher PlasmidPro Maxi Processor.

Plasmid and bacterial strain	Sample ID	Endotoxin assay results (EU/µg)
Plasmid A (2.7 kb) TOP10	S1	<0.009
	S2	<0.045
	S3	<0.043
	S4	<0.043
	S5	<0.039
	Manual process	<0.016
Plasmid B (5.1 kb) TOP10	S6	0.021
	S7	<0.013
	S8	<0.012
	S9	<0.034
	Manual process	0.023
Plasmid C (6.6 kb) DH5α	S10	<0.011
	S11	<0.017
	S12	<0.010
	S13	<0.010
	S14	<0.015
	S15	<0.014
	S16	<0.010
	S17	<0.023
	S18	<0.012
	S19	<0.009
	S20	<0.012
	S21	<0.018
	Manual process	<0.011
Plasmid D (11.4 kb) TOP10	S22	<0.010
	S23	<0.010
	S24	<0.027
	S25	<0.025
	S26	<0.009
	Manual process	0.081

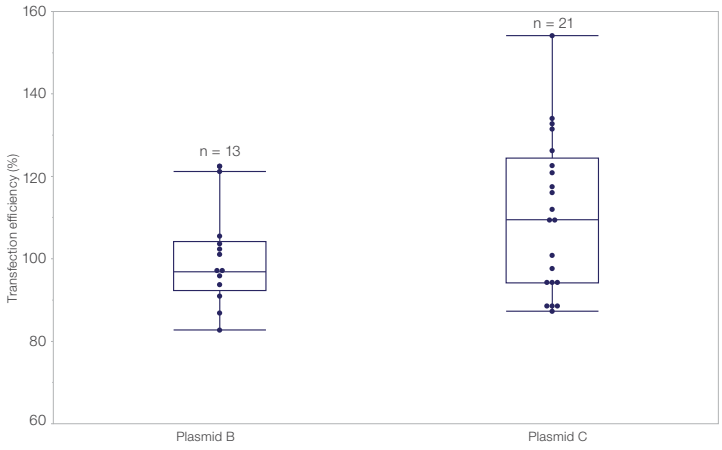


Figure 5. Efficiency of transfection with plasmids purified on the KingFisher PlasmidPro Maxi Processor relative to manually purified plasmids. Plasmids B and C were used to transfect HuH-7 mammalian cells. The *y*-axis values indicate transfection efficiency relative to the pDNA isolated using the manual purification process (set at 100% for both plasmids). The data represent reporter gene expression after transfection with 100 ng of pDNA isolated using the KingFisher PlasmidPro Maxi Processor. *SEAP* expression from plasmid B and luciferase expression from plasmid C were compared to those of pDNA purified from the same overnight bacterial cultures using the column-based manual purification process. The box plots show the distributions of the data, with each dot representing an individual purification.

Conclusion

In this study, we evaluated the performance of the KingFisher PlasmidPro Maxi Processor by purifying four plasmids ranging in size from ~2.7 kb to ~11.4 kb. The purified pDNA was assessed in terms of yield, isoform, transfection efficiency, and purity, including endotoxin and genomic DNA content. The performance and quality of pDNA isolated on the KingFisher PlasmidPro Maxi Processor were comparable to those achieved with a manual column-based purification process. All pDNA samples processed using the automated workflow contained predominantly supercoiled pDNA with no visible traces of RNA or genomic DNA, and their endotoxin content was well below 0.1 EU/µg each. These results show that plasmid DNA isolated on the KingFisher PlasmidPro Maxi Processer would be suitable for protein production, mRNA synthesis, vaccine development research, and cell and gene therapy.

References

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Ordering information

Product	Unit size	Cat. No.
KingFisher PlasmidPro Maxi Processor	1 instrument	PPMX1000
KingFisher PlasmidPro Maxi Processor Endotoxin-Free Cartridge	Pack of 4	A54072
KingFisher PlasmidPro Maxi Processor, extended warranty package	1 instrument	A66427

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