ThermoFisher SCIENTIFIC

DNA extraction

Performance comparison of maxi-scale plasmid DNA isolation kits

Summary

- The GeneJET Endo-Free Plasmid Maxiprep Kit provides yields that are comparable to those of similar kits from Qiagen and Macherey-Nagel.
- The GeneJET Endo-Free Plasmid Maxiprep Kit has a centrifuge option, and an even quicker vacuum manifold option, that extract high-purity plasmid DNA with minimal gDNA contamination.
- The GeneJet Endo-Free Plasmid Maxiprep Kit provides endotoxin-free, transfection-grade pDNA in half the time of comparable maxi-scale kits from Qiagen and Macherey-Nagel.

Introduction

Plasmid DNA (pDNA) extraction has recently garnered attention due to increased research on, and production of, mRNA vaccines. Vaccine development is a sensitive application that requires transfection of high-quality pDNA, free of endotoxin contaminants and extraction buffers, as the quantification of plasmid concentrations can be compromised if endotoxins remain.

Maxi-scale preps lie in the middle of the spectrum between micro-scale and giga-scale preps. They are reliably used for screenings to ensure high quality, high yield, and consistent reproducibility for downstream applications such as transfection and PCR. Here the performance of the Thermo Scientific™ GeneJET™ Endo-Free Plasmid Maxiprep Kit is compared to two other suppliers' kits, the Qiagen™ EndoFree™ Plasmid Maxi Kit and the Macherey-Nagel™ NucleoBond™ Xtra Maxi EF kit.

Materials and methods

A 3 L batch of Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* with a high–copy number positive control vector expressing secreted alkaline phosphatase (pSEAP2, TakaraBio) was grown in Gibco™ LB Broth with carbenicillin until an OD_{600} of 3.6 was reached. A 5 L batch of Thermo Scientific™ DH5α Competent Cells with low–copy number Invitrogen™ pBR322 was grown in the same type of medium until an OD_{600} of 3.8 was reached.

The cultures were spun down at 4,000 x *g* for 15 minutes to form 50 mL pellets. The remaining medium was decanted, and the pellets stored at –20°C. All extractions were performed in triplicate using the recommended high-copy or low-copy input volume for each kit in this study. For the high-copy plasmid, the input volumes for the GeneJet kit, the Qiagen kit, and the Macherey-Nagel kit were 150 mL, 100 mL, and 300 mL of culture per replicate, respectively. For the low-copy plasmid, the input volumes for the GeneJet kit, the Qiagen kit, and the Macherey-Nagel kit were 150 mL, 250 mL, and 600 mL of culture per replicate, respectively.

At the time of use, the cell pellets were thawed in a room temperature water bath for 15 minutes, and then resuspended in the kits' respective resuspension buffers. pDNA was isolated following each kit's standard protocol, and then common downstream analyses were performed. The GeneJET kit provides two options for its workflow—using a centrifuge or a quicker vacuum manifold. The vacuum manifold option was used for this study. The Thermo Scientific™ NanoDrop™ 8000 Spectrometer was used to determine concentration and purity, and gel electrophoresis was used to visually assess the quantity of gDNA and supercoiled pDNA. 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 5,000 cells per well in six replicates. The plate was incubated at 37°C with 5% CO_2 for 18–24 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). A 1% agarose gel was run for 10 minutes to see an RNA band running ahead of the supercoiled pDNA band, and continued for an additional 65 minutes to see the separation of gDNA and supercoiled pDNA bands. Endotoxin levels were tested using the Endosafe™ Nexgen-PTS™ instrument (Charles River Laboratories) with the limulus amebocyte lysate (LAL) assay.

invitrogen

The yields of high-copy pDNA obtained using the GeneJET kit, Qiagen kit, and Macherey-Nagel kit are shown in Figure 1. The yields were comparable between the GeneJET and Macherey-Nagel kits, while the Qiagen kit yielded approximately half of what the other two kits did. The sample purity ratios obtained using the NanoDrop 8000 Spectrophotometer are shown in Figure 2. Gel electrophoresis showed no RNA contamination across all samples (Figure 3A), and samples obtained using the GeneJET kit appeared to have the lowest amount of other pDNA isoforms, compared to the samples obtained with the Qiagen kit, which had more nicked and linear isoforms, and with the Macherey-Nagel kit, which had the linear isoform (Figure 3B). All of the high-copy plasmid samples had endotoxin levels <0.1 EU/µg (Table 1), the criterion for being classified as endotoxin-free and suitable for transfection. Luminescence from transfection is comparable across all three samples, which indicates that the functional amount of plasmid is similar across all three samples (Figure 4).

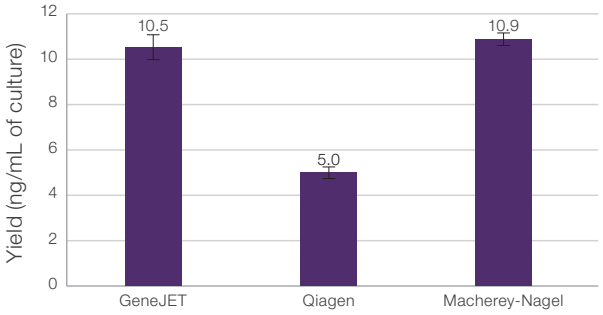

Figure 1. Average yields of high-copy pDNA purified using three different kits. Yields represent averages of triplicate extractions.

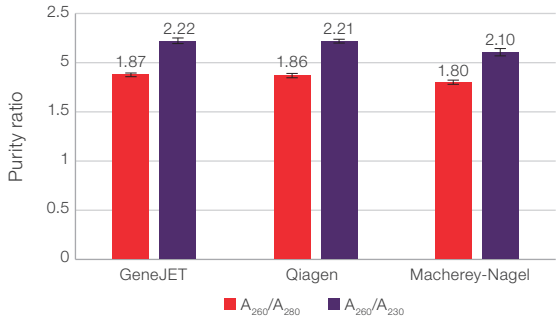

Figure 2. Purity ratios of high-copy pDNA isolated using three different kits. Absorbance was measured with the NanoDrop 8000 Spectrophotometer.

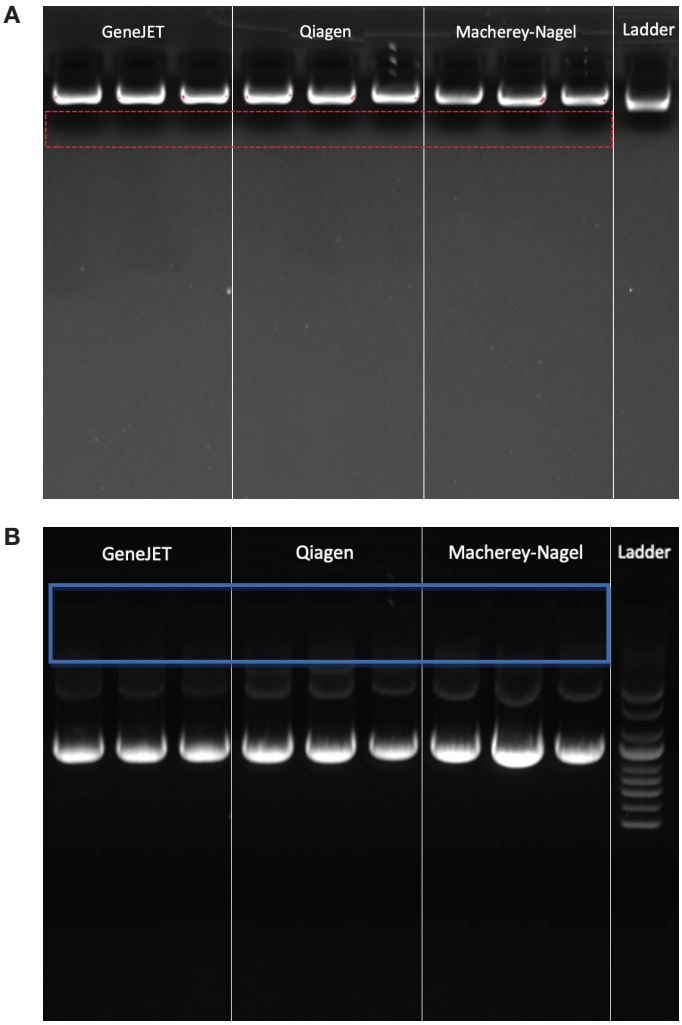

Figure 3. High-copy pDNA isolated using three different kits. (A) A gel was run for 10 minutes to visualize RNA contamination. The red box indicates where the band would be if there was RNA contamination. **(B)** The gel was run for an additional 65 minutes to separate the gDNA and supercoiled pDNA bands. The blue box indicates where the gDNA would be present.

Table 1. LAL assay results for high-copy and low-copy plasmid samples obtained using three different kits. The endotoxin levels in all samples were <0.1 EU/µg, which is the upper limit to be classified endotoxin-free.

	Endotoxin level (EU/µg)	
	High copy	Low copy
GeneJET kit	0.004	0.011
Qiagen kit	<0.005	<0.047
Macherey-Nagel kit	<0.001	0.036

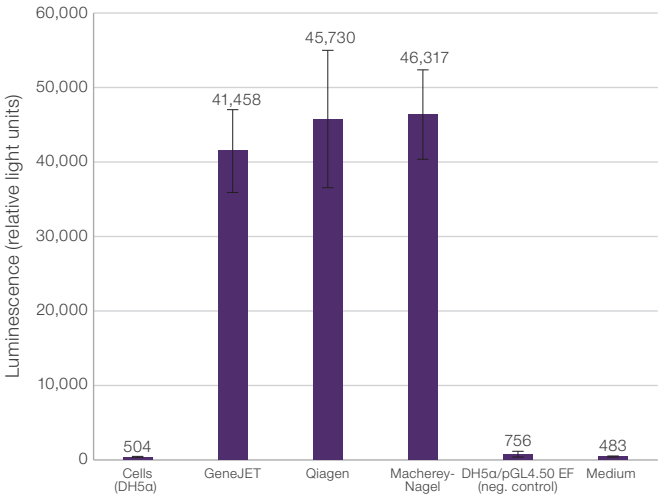

Figure 4. SEAP luminescence after transfection of pDNA into HuH-7 cells. pDNA was isolated using three separate kits. Luminescence was measured using a FLUOstar Omega instrument.

The yields of low-copy pDNA obtained using the GeneJET kit, Qiagen kit, and Macherey-Nagel kit are shown in Figure 5. The yields were comparable between the GeneJET and Macherey-Nagel kits, while the Qiagen kit yielded approximately 20–25% of what the other two kits did. The low-copy plasmids had comparable A_{260}/A_{280} and A_{260}/A_{230} ratios across all samples, but the ratios were slightly lower than those of the high-copy plasmid samples, which is expected (Figure 6). Gel electrophoresis showed no RNA contamination across all samples (Figure 7A), and the samples extracted using the GeneJET kit had the least gDNA contamination (Figure 7B). It is possible the sample obtained using the Macherey-Nagel kit had resin carried over from the column into the eluate, as indicated by the bright pDNA band seen at the bottom of the loading well (Figure 7B). All of the low-copy plasmid samples also met the criterion for being endotoxin-free (Table 1), with endotoxin levels <0.1 EU/µg.

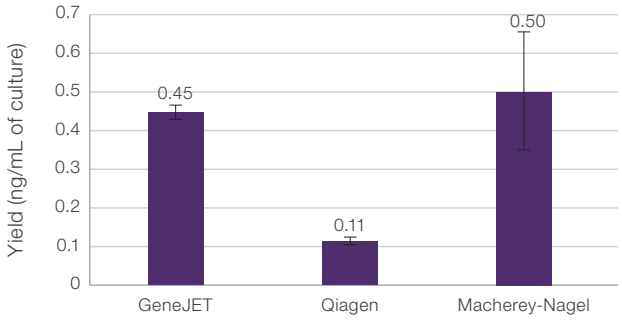

Figure 5. Average yields of low-copy pDNA purified using three different kits. Yields represent averages of triplicate extractions.

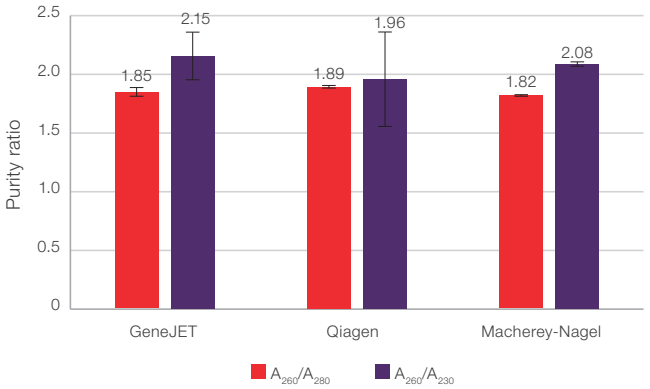

Figure 6. Purity of pDNA isolated from low-copy samples obtained using three different kits. Absorbance was measured using the NanoDrop 8000 Spectrophotometer.

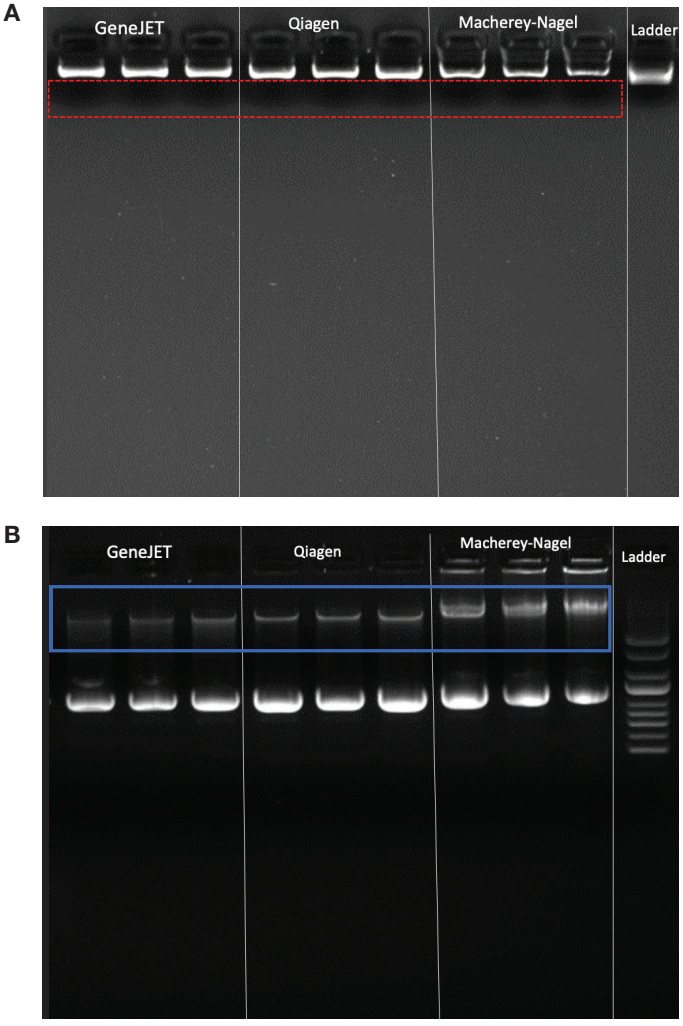

Figure 7. High-copy pDNA isolated using three different kits. (A) A gel was run for 10 minutes to visualize RNA contamination. The red box indicates where the band would be if there was RNA contamination. **(B)** The gel was run for an additional 65 minutes to separate the gDNA and supercoiled pDNA bands. The blue box indicates the gDNA.

The vacuum manifold option in the GeneJET Endo-Free Plasmid Maxiprep Kit does not require pelleting and resuspension of pDNA with isopropanol. The clarified lysate has a high salt concentration, which promotes binding to the silica column, and pDNA is eluted directly from the column, saving the user at least 30 minutes of time [1]. The Qiagen EndoFree Plasmid Maxi Kit and Macherey-Nagel NucleoBond Xtra Maxi EF kit are gravity-flow kits, which take about twice as long as the GeneJET Endo-Free Plasmid Maxiprep Kit. The Qiagen EndoFree Plasmid Maxi Kit uses a syringe in the lysate clarification step, which makes the process fast but creates potential issues for a novice who may not have experience with syringes and not be familiar with the amount of pressure that is required; there is also a possibility of accidentally spilling some of the clarified lysate as the syringe is pushed [2]. The Macherey-Nagel NucleoBond Xtra Maxi EF kit is a gravity-flow kit with a workflow similar to that of the Qiagen kit; however, it requires a much larger culture input volume than the Qiagen kit. This is challenging, as it requires growing large amounts of culture for pelleting, and freezer space for storage. The input for the Macherey-Nagel NucleoBond Xtra Maxi EF kit is closer to that of a mega-scale kit, so the kit is more aptly compared to other mega-scale kits. The GeneJET Endo-Free Plasmid Maxiprep Kit provides yields comparable to those of the Macherey-Nagel kit, but has a much easier and faster workflow. For the low-copy samples, the standard deviation of the sample quantities isolated using the Macherey-Nagel kit was higher compared to the samples isolated with the other kits; one replicate had a significantly higher concentration, possibly due to the relatively high input volume of resuspended cells. The GeneJET kit avoids these issues with a much lower culture volume input—a quarter of the volume required by the Macherey-Nagel kit—while delivering a comparable yield.

Conclusions

In this study, three maxi-scale pDNA isolation kits—the GeneJET Endo-Free Plasmid Maxiprep Kit, Qiagen EndoFree Plasmid Maxi Kit, and Macherey-Nagel NucleoBond Xtra Maxi EF kit—were compared for their performance and ease of use. All three kits provided high-purity pDNA with comparable high yields. However, the GeneJET Endo-Free Plasmid Maxiprep Kit has an easier workflow that saves time while maintaining high yield and purity.

References

1. Thermo Scientific GeneJET Endo-Free Plasmid Maxiprep Kit (2014). Retrieved October 21, 2022, from https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012672_GeneJET_Endofree_Plasmid_Maxiprep_UG.pdf
2. Endofree plasmid kits. QIAGEN. Retrieved October 23, 2022, from https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/plasmid-dna/endofree-plasmid-kits/

Ordering information

Product	Cat. No.
GeneJET Endo-Free Plasmid Maxiprep Kit	K0861
One Shot TOP10 Chemically Competent *E. coli*	C404006
DH5α Competent Cells	EC0112
Plasmid pBR322	15367014
Carbenicillin Antibiotic	10177012
NanoDrop Eight Spectrophotometer (replaces the discontinued NanoDrop 8000 Spectrophotometer)	NDE-GL
Dulbecco's Modified Eagle Medium (DMEM), low glucose, pyruvate	11885084
Fetal Bovine Serum, qualified, One Shot format, United States	A3160502
Lipofectamine 3000 Transfection Reagent	L3000001
Opti-MEM I Reduced Serum Medium	31985062
Phospha-Light SEAP Reporter Gene Assay System	T1015

Learn more at **thermofisher.com/plasmid**