Technical Information

Next-generation plasmid DNA purification – a comparison of current kits

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Since QIAGEN launched the world's first kit for purification of plasmid DNA, technologies have been developed to make plasmid DNA preparation even more convenient and reduce endotoxin levels in line with scientists' needs in the laboratory. A lot of well-known companies have developed kits, and the choice of solutions available has never been greater. We compared popular advanced-technology kits to see their relative performance in speed, convenience of handling, yield and endotoxin levels.

QIAGEN® Plasmid *Plus* Kits are convenient to use, yielding reliable, high-quality results for the generation of transfection-grade DNA. We compared them with various other current plasmid kits, all of which generate transfection-grade DNA. The comparison was based on handling, speed and the formats of the different kits. Yields and, where appropriate, endotoxin levels (an endotoxin removal step is not included in some kits tested), were also compared.

Materials and methods

The kits compared were chosen for their equivalence of declared yield and endotoxin levels within the range of kits provided by the same supplier:

- QIAGEN Plasmid *Plus* Maxi Kit
- MACHEREY-NAGEL Nucleobond® Xtra Midi
- Invitrogen™ PureLink™ Fast Low-Endotoxin Maxi Plasmid Purification Kit
- ZymoPURE™ II Plasmid Maxiprep Kit* using the optional endotoxin removal step
- Promega PureYield™ Plasmid Maxiprep System

Bacterial strain and plasmid

E. coli DH5α harboring pCMVβ plasmid (7164 bp) was used for all comparisons.

Bacterial culture

E. coli DH5α was plated on agar plates with the appropriate antibiotics and incubated overnight at 37°C. Starter cultures were prepared with the appropriate antibiotic using a single colony. These cultures were grown overnight in flasks containing 5 ml culture media LB (1% peptone, 0.5% yeast extract, 1% NaCl). They were then used to inoculate several main cultures in 500 ml flasks. ▷

* At the time of the study, the kit was called ZymoPURE-EndoZero Plasmid Maxiprep Kit.

Main cultures were incubated overnight at 37°C and 85 rpm. To generate identical samples, we pooled all cultures. *E. coli* biomass production (cell density) was measured by OD600. Cells from a 100 ml culture were harvested by centrifugation in a 50 ml falcon tube and stored at –20°C for plasmid DNA purification. Plasmid DNA was isolated in quadruplicate.

Endotoxin levels

Endotoxin levels were measured using the Limulus amoebocyte lysate (LAL) test following the manufacturer's instructions (Lonza). For each kit, a 1/100 dilution of 2 samples were tested in triplicate. A standard curve of the test ranged between 0.005 EU/ml and 50 EU/ml.

Handling

Based on the information and protocols provided by the suppliers, we assigned points for convenience – from one point (low convenience) to three points (high convenience). Parameters assessed included: the type of protocol (with vs. without precipitation and vacuum vs. gravity flow), the duration of processing the protocol, the convenience of handling (hurdles in the protocol), the possibility of up- or down-scaling due to different formats.

Yield and visualization

Plasmid DNA yield and quality (A_{260}/A_{280} ratios, A_{260}/A_{230} ratio) were determined using the QIAxpert® system and the A_{260} dsDNA App (2.4.0.1). Plasmid DNA was visualized by agarose gel electrophoresis running ~500 ng DNA per lane on a 0.8% agarose gel at 100 V for ~60 minutes, and 60 V for 60 minutes. For size comparison, standard DNA was used (left: GelPilot High Range Ladder [6 µl]; right: GelPilot 1 kb Ladder [6 µl]).

Results

Handling and convenience

Comparison of handling, speed, protocol complexity and formats (with and without endotoxin removal) and the points given show differences in the convenience of use of the kits tested (Table 1). The points for each category were added together to generate a convenience level. QIAGEN Plasmid *Plus* Kit rates highest with a convenience level of 9. This level was achieved due to quick and efficient handling, and a simple protocol that fits many formats.

Table 1. Handling, speed, protocol and formats

Kit	Comments	Protocol	Formats available	Convenience level
QIAGEN Plasmid *Plus*	Very simple and convenient protocol	• Without precipitation • Vacuum	★★★ ● ● ● ●	9
M&N NucleoBond Xtra Midi	Recommended culture volume depends on OD. After measuring OD, the volume of culture input is determined for the Midi or Maxi protocol – time-consuming.	• With precipitation • Gravity flow	★ ● ●	3
Invitrogen PureLink Low-endotoxin Maxi	Very simple and convenient protocol	• Without precipitation • Vacuum	★ ● ●	7
ZymoPURE II Plasmid Maxiprep Kit	Endotoxin removal optional after plasmid elution by centrifugation of the eluate through an additional spin column	• Without precipitation • Vacuum	★★ ● ● ●	7
Promega PureYield Plasmid Maxiprep	Least convenient protocol in terms of handling and speed, compared to all other vacuum protocols	• Without precipitation • Vacuum	★ ● ●	5

●: Midi ●: Maxi ●: Mega ●: Giga

Endotoxin levels

Low endotoxin levels mean reproducible results with transfection experiments. Plasmid DNA samples purified using QIAGEN Plasmid *Plus* Kits had lower endotoxin levels than samples prepared with the other kits (Figure 1). The endotoxin-removal step in the ZymoPURE Kit protocol using the EndoZero spin did not lead to significantly lower levels of endotoxin (Figure 1).

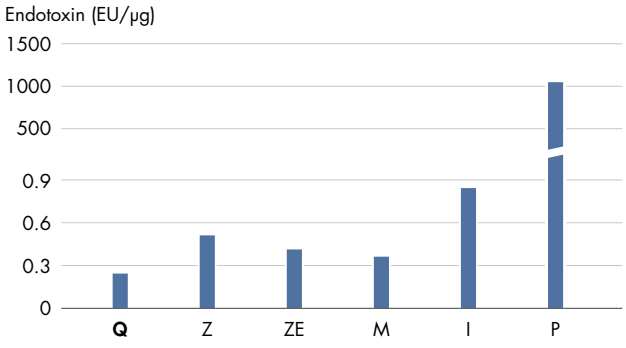

Figure 1. Lower endotoxin levels. Comparison of five plasmid DNA kits shows wide differences in levels of endotoxins. Endotoxins were measured as described in materials and methods in plasmid DNA samples prepared using the kits indicated. Purification with QIAGEN Plasmid *Plus* Kits resulted in plasmid DNA samples with the lowest endotoxin levels. **Q:** QIAGEN Plasmid *Plus* Kit; **Z:** ZymoPURE II without EndoZero step (no ET wash); **ZE:** ZymoPURE II with EndoZero step; **M:** Macherey-Nagel Nucleobind Xtra; **I:** IVGN PureLink Fast Low ET;**P:** Promega PureYield.

Processing time

Processing times were calculated using the protocols supplied. The time needed for steps were determined empirically and applied to all kits using a standard number of 12 samples. The QIAGEN Plasmid *Plus* Kit protocol is the fastest with a range of times for the other kits (Figure 2).

Yield and analysis of plasmid DNA

Yield and quality of plasmid DNA as assessed by agarose gel electrophoresis were comparable for all kits tested (Figure 3).

A QIAxpert Instrument (Application: A_{260} dsDNA [2.4.0.1]) was used to determine DNA yields: the A_{260}/A_{280} and the A_{260}/A_{230} ratio. A_{260}/A_{230} was >2.0 and A_{260}/A_{280} ratio was between 1.8 and 2.0. Quality and yield were comparable over all samples (data not shown).

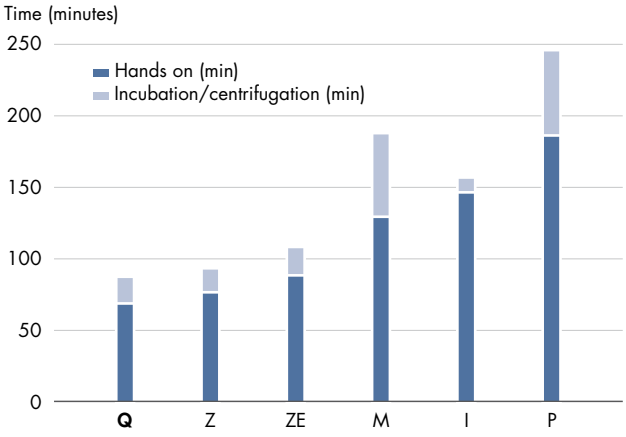

Figure 2. Faster protocols. Processing time for 12 samples specified by handling and centrifugation/incubation time. QIAGEN Plasmid *Plus* Kits purify plasmid DNA from 12 samples quicker than other kits. **Q:** QIAGEN Plasmid *Plus* Kit; **Z:** ZymoPURE II without EndoZero step (no ET wash); **ZE:** ZymoPURE II with EndoZero step; **M:** Macherey-Nagel Nucleobind Xtra; **I:** IVGN PureLink Fast Low ET; **P:** Promega PureYield.

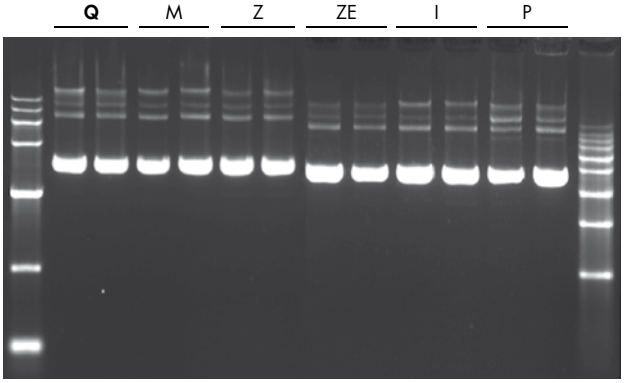

Figure 3. Visual analysis of plasmid DNA. Plasmid DNA analyzed by agarose gel electrophoresis shows similar results from the different kits tested. **Q:** QIAGEN Plasmid *Plus* Kit; **M:** Macherey-Nagel Nucleobind Xtra; **Z:** ZymoPURE II without EndoZero step (no ET wash); **ZE:** ZymoPURE II with EndoZero step; **I:** IVGN PureLink Fast Low ET; **P:** Promega PureYield.

Conclusions

When all parameters are taken into consideration, all kits performed well, especially in yield. There are significant differences in handling and protocol time. QIAGEN Plasmid *Plus* Kits perform best in handling, protocol time and endotoxin removal. The combination of its features makes the QIAGEN Plasmid *Plus* Kit the first choice.

Good endotoxin levels were achieved with M&N MACHEREY-NAGEL Nucleobond® Xtra Midi, the protocol time is very long. We saw no difference using the ZymoPURE II Plasmid Maxiprep Kit with or without the optional EndoZero step. The Promega PureYield™ Plasmid Maxiprep System includes an endotoxin removal buffer, but yield higher endotoxing levels that are comparable with those obtained using silica slurry.

QIAGEN kits can be easily used in different formats (96-well to Giga) for extra convenience and more reproducible results. Reliability is key to the use of the same quality from transfection screening to large-volume experiments.

The low level of endotoxin that is achieved with QIAGEN Plasmid *Plus* Kits guarantee reproducible results of transfection experiments on different levels: only the effect of the transfected protein is measured and no effects due to the presence of endotoxins. Different cell lines can be compared, because the same DNA can be used to transfect them.

QIAGEN Plasmid *Plus* Kits are the solution of choice, as QIAGEN Plasmid *Plus* DNA can be used for nearly every experiment. They combine better endotoxin removal with more convenient handling and faster protocols, without compromising on plasmid DNA yield or quality. For experiments that demand endotoxin levels below 0.1 EU/µg DNA, QIAGEN EndoFree® Kits will deliver this level.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

To try QIAGEN Plasmid *Plus* Kits, visit **www.qiagen.com/ngplasmidpure**.