Advanced plasmid DNA purification: from transfection-grade to ultraclean endotoxin-free preparations

Over the last three decades, plasmids have played a crucial role as a widely used molecular tool for DNA manipulation and gene expression in various microorganisms and animal cells. Plasmids have many applications in biotechnology and are routinely utilized as cloning vectors in bacteria, and for large-scale protein production (e.g., insulin), gene therapy, mRNA therapy, vaccine development, genetic engineering, and creation of animal-disease models.

One typical workflow involving plasmids is shown in Figure 1, along with key accompanying reagents, kits, and instruments. Robust purification of plasmid DNA, in the amount and purity required for the downstream application of interest, is the key step of the workflow.

During construction of plasmid DNA (cloning, PCR) and subsequent validation (sequencing, enzymatic digestion), small-scale plasmid DNA preparations are generally used. For instance, from 1–5 mL of bacterial culture, one can obtain 5–40 µg of plasmid. Basic molecular biology applications do not require highly pure plasmid, and kits with silica membrane–based columns, such as the Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit, are ideal in this case because of their fast and simple workflows and cost efficiency.

When the downstream application is to transfect mammalian cell lines or inject the plasmids into animal models, large amounts of plasmid DNA (midi-, maxi-, mega-, or giga-scale) are required. For instance, a typical maxiprep uses 100–200 mL of bacterial culture, producing up to 1 mg of plasmid. Another very important requirement for these applications is high purity of the plasmid DNA, which needs to be confirmed by gel analysis, spectrophotometer (A_{260}/A_{280} and A_{260}/A_{230} measurements), and in particular, endotoxin and pyrogen testing using the limulus amebocyte lysate (LAL) or other assays.

Pyrogens are a heterogeneous group of substances of both microbial and nonmicrobial origin. The most potent and widely known among them are the endotoxins, which are cell wall components of gram-negative bacteria. Endotoxins are released into solution upon cell lysis, and they tend to co-purify with the plasmids. Endotoxin contamination in plasmid preparations varies depending on bacterial strain, plasmid and culture conditions, and purification protocol.

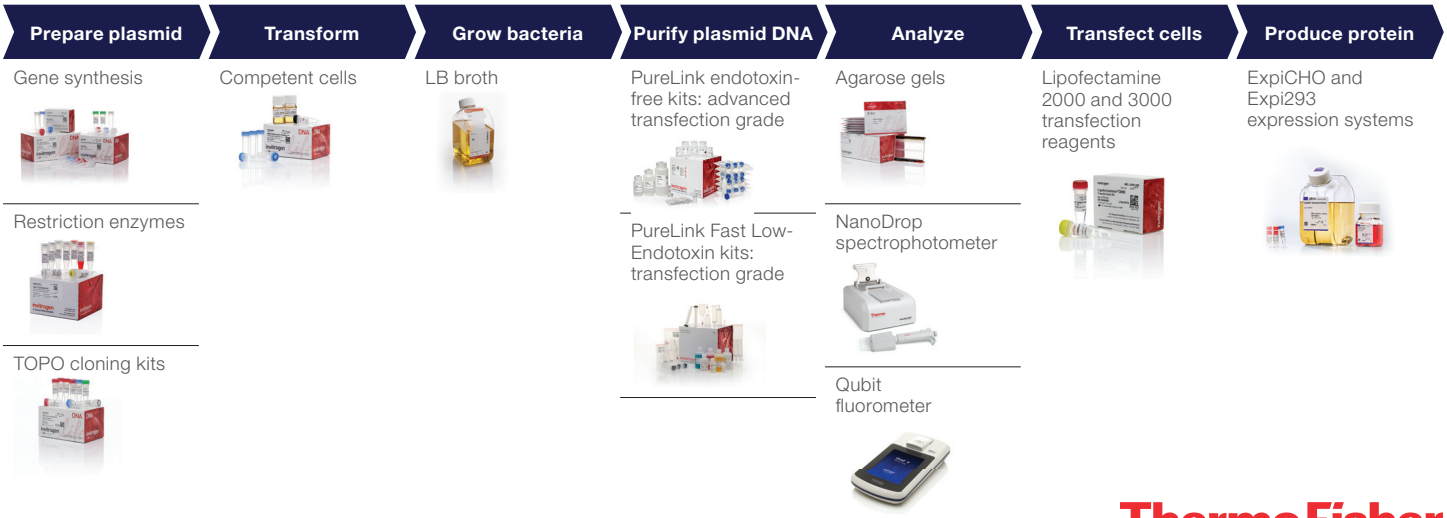

Figure 1. Typical protein expression workflow and key accompanying reagents, kits, and instruments.

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Endotoxins are a source of concern because they can induce nonspecific activation of immune responses in cells and animals, resulting in suboptimal transfection and toxicity in many cell lines, decrease in protein expression in sensitive cells, and life-threatening fever in animal models. To ensure successful *in vitro* and *in vivo* studies, low-endotoxin and endotoxin-free plasmid purification kits are strongly recommended, to produce plasmids with endotoxin contamination guaranteed to be depleted below certain levels. While standard kits produce plasmids with endotoxin levels at 10–100 EU/µg of plasmid, "low-endotoxin" kits generate plasmid DNA with endotoxin levels at <1 EU/µg of plasmid, and "endotoxin-free" kits generate plasmid DNA with endotoxin levels at <0.1 EU/µg of plasmid.

Materials and methods

Single colonies were picked from freshly streaked selective plates and inoculated into 1 mL of Luria-Bertani (LB) medium containing the appropriate selective antibiotic. These starter cultures were incubated at 37°C for 8 hours with vigorous shaking (220–280 rpm). Next, the starter cultures were diluted 1:1,000 in LB medium in a flask with a volume of at least 3 times the volume of the culture, and incubated at 37°C for 12–16 hours with vigorous shaking. The bacterial culture was analyzed using the Thermo Scientific™ NanoDrop™ spectrophotometer to ensure that it reached a cell density of approximately 2–4 × 10^9 cells/mL, or an optical density of 2.0–4.0 at 600 nm (A_{600}). Plasmid DNA was isolated from the freshly harvested bacterial culture using Invitrogen™ PureLink™ kits or other suppliers' products. Downstream analysis used the NanoDrop spectrophotometer to measure yield and purity, agarose gel electrophoresis to visually assess purity, and the Endosafe™-Portable Test System™ (PTS™) test (Charles River) to quantify endotoxin levels.

Results

Invitrogen™ PureLink™ Fast Low-Endotoxin Plasmid Purification Kits use next-generation columns with advanced silica membranes that enable rapid, precipitation-free isolation of transfection-grade (<1 EU/µg) plasmid DNA. The simple precipitation-free protocol yields high-quality plasmids (Figures 2 and 3) and is suitable for standard transfections (Figure 4) and all molecular biology applications such as cloning and sequencing.

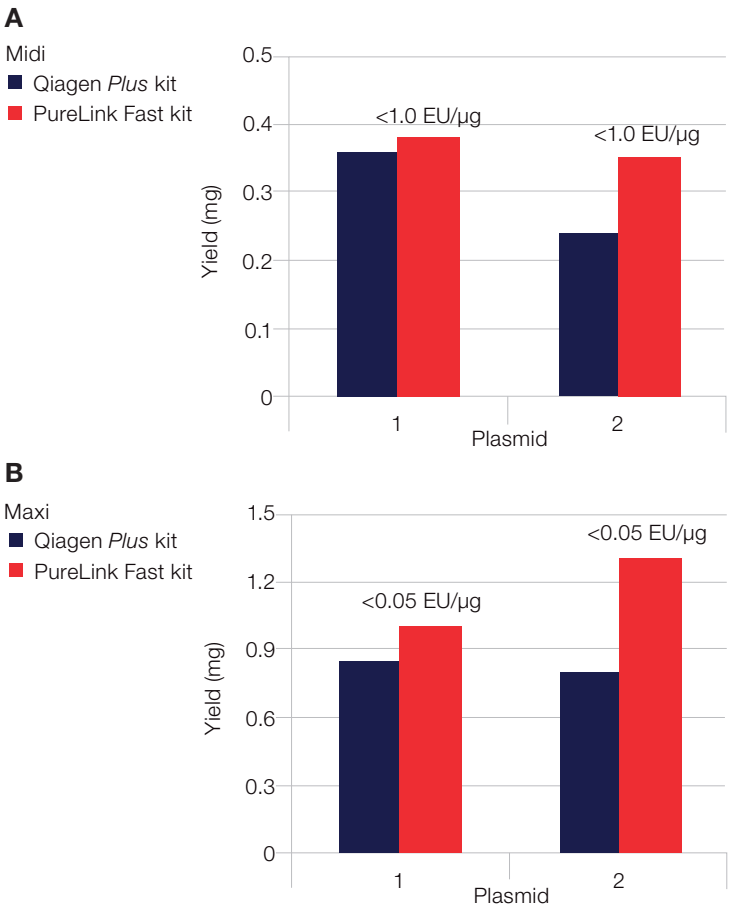

Figure 2. Higher yields of low-endotoxin, transfection-quality plasmid DNA obtained with PureLink Fast purification kits than with kits from another supplier. Two high-copy plasmids with different backbones were purified using PureLink Fast kits and Qiagen Plasmid *Plus* midiprep **(A)** and maxiprep **(B)** kits as described in the product manuals. Endotoxin values (EU/µg) were measured using the Endosafe-PTS test (Charles River) and are provided only for the PureLink Fast preparations.

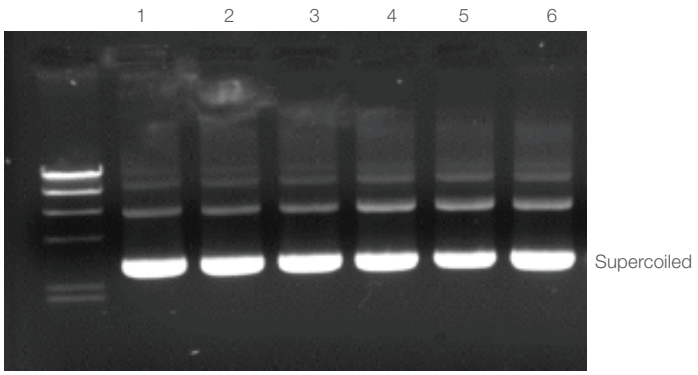

Figure 3. Plasmids purified with PureLink Fast kits are predominantly in the supercoiled form, with no traces of RNA or genomic DNA contamination. Lanes 1–3, PureLink Fast maxipreps; lanes 4–6, Qiagen *Plus* maxipreps; 1% agarose gel analysis, 1 µg plasmid per well.

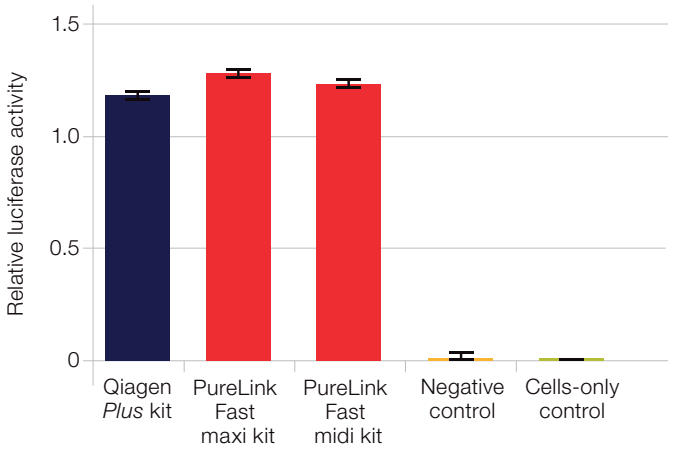

Figure 4. Rapid isolation of transfection-grade plasmid DNA in high yields. pGL4.5-fLuc plasmid DNA was purified with PureLink Fast midi and maxi kits and the Qiagen *Plus* maxi kit, then transfected into HeLa cells with Invitrogen™ Lipofectamine™ 3000 Transfection Reagent. At 24 hours posttransfection, cells were lysed and luciferase protein levels were measured using the Invitrogen™ Dual-Light™ System. Cell viability was not affected, as confirmed with Invitrogen™ PrestoBlue™ reagent.

The rapid protocol of the PureLink Fast Low-Endotoxin Plasmid Purification Kits, based on innovative advanced silica, enables purification of transfection-grade plasmid DNA in as little as 20–30 minutes. The resulting plasmid DNA is of high quality, with endotoxin levels of <1 EU/µg and yields of up to 0.4 mg with the midiprep kit and 1.2 mg with the maxiprep kit. The kits also feature colored buffers for easier identification and visualization of mixing efficiency during lysis and neutralization steps.

Invitrogen™ PureLink™ Expi Endotoxin-Free Plasmid Purification Kits use a novel, proprietary anion-exchange membrane to isolate ultraclean, advanced transfection–grade plasmid DNA in about half the time of current standard protocols. The enhanced membrane used with the vacuum-assisted workflow substantially reduces total purification time, compared to typical resin columns using gravity flow. The simple protocol is designed to provide high yields of high-quality, endotoxin-free plasmid DNA (Figure 5) suitable for advanced applications such as transfection of primary and stem cells, microinjection, gene therapy, and *in vivo* vaccine research.

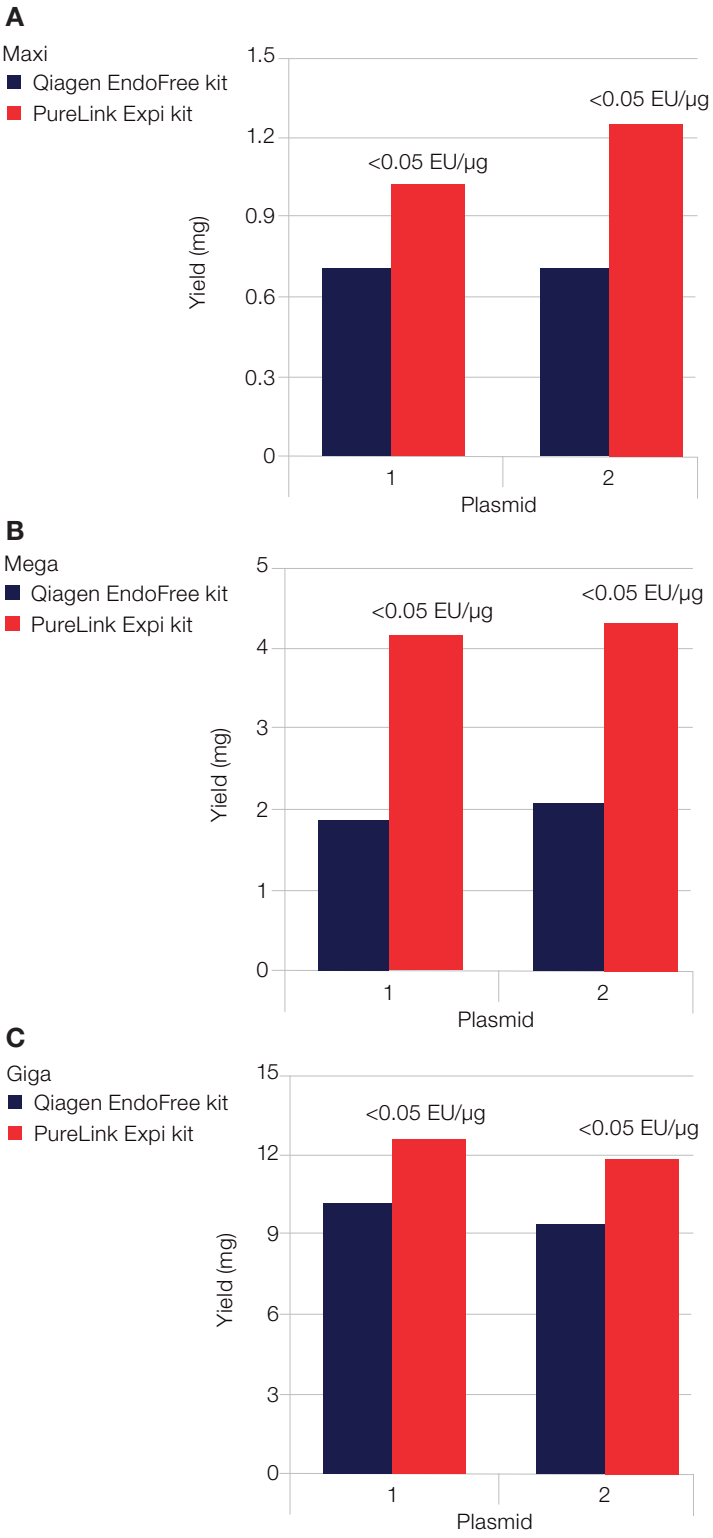

Figure 5. Higher yields of endotoxin-free, advanced transfection–quality plasmid DNA obtained with PureLink Expi purification kits than with kits from another supplier. Two high-copy plasmids with different backbones were purified using PureLink Expi and Qiagen EndoFree maxiprep **(A)**, megaprep **(B)**, and gigaprep **(C)** kits as described in the product manuals. Endotoxin values (EU/µg) were measured using the EndoSafe-PTS test (Charles River) and are provided only for the PureLink Expi preparations.

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The PureLink Expi Endotoxin-Free kits are fully compatible with the Gibco™ ExpiCHO™ and Expi293™ transient mammalian expression systems (Figure 6).

Unlike gravity-flow columns, the PureLink Expi Endotoxin-Free Plasmid Purification Kits provide substantial time savings. One can purify endotoxin-free plasmid DNA in as little as 90 minutes (maxiprep) or 2 hours (megaprep and gigaprep). In addition, high yields of high-quality, endotoxin-free plasmid DNA are achieved—for example, up to 1.5 mg (maxiprep), 5 mg (megaprep), or 15 mg (gigaprep) with <0.1 EU/µg of endotoxin, ideal for transfection of sensitive cell lines or *in vivo* experiments.

Conclusions

The plasmid purification kits described here, along with other reagents, kits, and instruments supporting the entire plasmid workflow highlighted in Figure 1, help ensure successful experiments, including the most challenging intermediate steps and most sensitive *in vitro* and *in vivo* assays. In the last decade, plasmids have evolved from being a tool for fundamental research and manipulations in the research lab to an important tool in the clinical setting, enabling production of therapeutic proteins, gene therapy, and vaccine development. These robust and time-saving solutions would help accelerate discovery and further advance fundamental and applied research involving plasmid DNA.

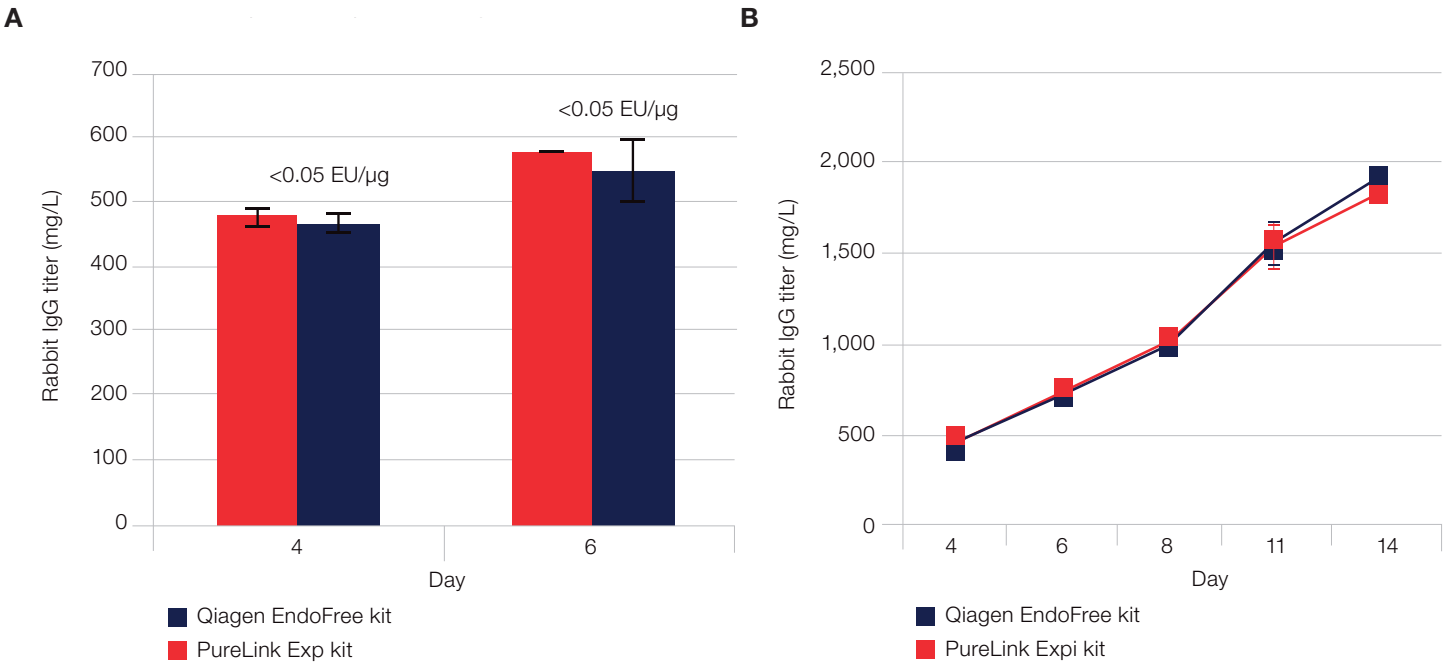

Figure 6. Plasmid DNA purified with PureLink Expi Endotoxin-Free kits is compatible with the Gibco Expi293 and ExpiCHO Expression Systems. Using the **(A)** Expi293 and **(B)** ExpiCHO Expression Systems, cells were transfected with positive controls of rabbit IgG heavy chain and light chain plasmids that were purified using the PureLink Expi megaprep kit or the Qiagen EndoFree megaprep kit. The titer values fall within the expected outputs of the Expi293 and ExpiCHO Expression Systems.

Find out more at **thermofisher.com/plasmidprep**

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