

PureYield™ Plasmid Miniprep System Comparison: Purification of Large Plasmids

ABSTRACT

Plasmid purification kits are used routinely to obtain plasmid DNA of high quality and purity. PureYield™ Plasmid Systems demonstrate excellent speed, yield, purity and performance compared to several other systems. Here the PureYield™ Plasmid Miniprep System was tested along with three other popular plasmid purification systems for purification of plasmids greater than 10kb. The PureYield™ Plasmid Miniprep System isolated all plasmids tested and performed as well as, if not better than, competitors in terms of yield and purity.

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Introduction

Bacterial plasmids serve as important research tools for a variety of applications including cloning, transfection, cell-free protein expression and DNA sequencing. To isolate the plasmid of interest in most plasmid purification systems, bacterial cells are usually lysed, often using a modified alkaline lysis method(1) , and the plasmid is purified with a DNA-binding column. In recent times, plasmid purification kits have been improved by using the bind-wash-elute procedure to meet the increasing demands for a simple and rapid protocol, resulting in high yields of highly pure plasmid DNA.

PureYield™ plasmid purification systems offer a rapid method for plasmid purification without sacrificing yield or purity(2) . In this article, we compare PureYield™ Plasmid Miniprep System (Cat.# A1222) to NucleoSpin® Plasmid QuickPure (Macherey-Nagel), QuickLyse™ Miniprep Kit (Qiagen) and QIAprep® Spin Miniprep Kit (Qiagen) for extracting large plasmids (>10kb). DNA purity was evaluated by absorbance (A260/A230 and A260/A280). We show that for speed, yield and purity, the PureYield™ Plasmid Miniprep System provides equivalent or superior performance compared to the other miniprep kits.

Comparison of Plasmid Miniprep Systems

The PureYield™ Plasmid Miniprep System provides high-speed purification of high-quality plasmid DNA in 10 minutes or less. Plasmid DNA can be isolated directly from up to 0.6ml of bacterial culture or from pelleted cells from up to 3ml of cell culture as indicated in the protocol. A dye present during the lysis and neutralization steps ensures complete mixing and improves plasmid yields and concentrations. The PureYield™ Plasmid Miniprep System incorporates a unique Endotoxin Removal Wash designed to remove substantial amounts of protein, RNA and endotoxin contaminants from purified plasmid DNA. This feature increases performance in sensitive applications such as transfection and cell-free protein expression reactions.

We compared the performance of the PureYield™ Plasmid Miniprep with the NucleoSpin® Plasmid QuickPure, QuickLyse™ Miniprep Kit and QIAprep® Miniprep when purifying three large plasmids: 10.8kb, 15kb and 18.7kb. DNA yield, purity and quality were evaluated by optical density measurements (A260/A230 and A260/A280). The A260/A280 ratio evaluates the amount of protein present in the purified DNA while the A260/A230 ratio assesses the copurification of chaotropic salts and other contaminants. Table 1 compares the performance specifications of the plasmid purification kits, as supplied in the technical

manual accompanying each kit. The protocols were similar among the kits for the ease of use, although the processing time did vary greatly.

Table 1. Miniprep Plasmid Purification Kit Specifications According to the Manufacturer's Protocol.		
Kit Name	Processing Time	Maximum Sample Capacity
NucleoSpin® Plasmid QuickPure	~11 minutes	1.0–5.0ml
PureYield™ Plasmid Miniprep System	~10 minutes	0.6–3.0ml
QIAprep® Spin Miniprep Kit	<30 minutes	1.5–5.0ml
QuickLyse™ Miniprep Kit	~9 minutes	1.5–3.0ml

Table 1. Miniprep Plasmid Purification Kit Specifications According to the Manufacturer's Protocol.

Of the four kits tested, the PureYield™ Plasmid Miniprep System resulted in the highest yields for 10.8kb and 15kb plasmids (Figure 1). Both the PureYield™ system and Qiaprep® Miniprep resulted in A260/A280 ratios >1.75 for all plasmids tested, while only the QuickLyse™ kit failed to give A260/A230 ratios >1.80. For the QuickLyse™ Miniprep Kit, the fast processing time (Table 1) came at the expense of yield and quality, as plasmid DNA from QuickLyse™ had the lowest yield for 10.8kb and 15kb plasmids and lowest purity for each.

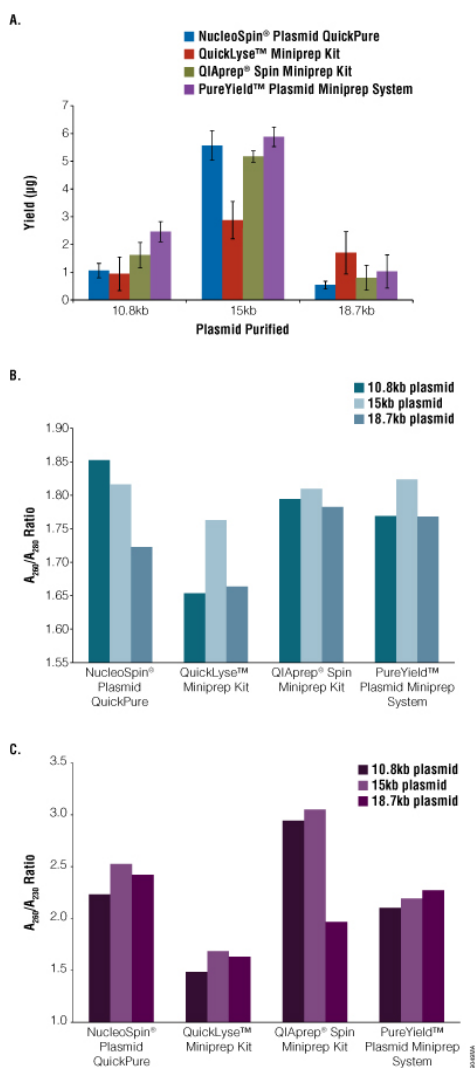


Figure 1. Yield and purity measurements of large plasmids isolated using four popular plasmid miniprep kits. Kit performance was evaluated by comparing the yield and purity of eluted DNA from equivalent culture aliquots. An overnight culture grown from a single colony of a 10.8kb, 15kb or 18.7kb DNA plasmid in KRX cells (Cat.# L3002) was used to inoculate 100ml of LB culture medium containing ampicillin. Optical density measurements were made on the day of plasmid isolations. The culture was split into three sets of four replicates of 1.5ml cultures for each plasmid isolation kit. Data are the average of three sets of four preparations. **Panel A.** Average yield was calculated from absorbance at 260nm. Average purity was calculated by absorbance at 260nm/280nm (**Panel B**) and 260nm/230nm (**Panel C**).

Conclusion

The PureYield™ Plasmid Miniprep System offers rapid purification of plasmid DNA >10kb without sacrificing purity. For speed, yield, and purity, the PureYield™ Plasmid Miniprep System outperforms or is comparable to the competitor kits. The high-quality plasmid DNA is suitable for downstream assays, including demanding applications such as cloning, transfection, cell-free protein expression and DNA sequencing.

REFERENCES

1. Birnboim, H.C. and Doly, J (1979) [A rapid alkaline extraction procedure for screening recombinant plasmid DNA](#). *Nucleic Acids Res.* **7**, 1513–23.
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