

illustra plasmidPrep Midi Flow Kit

Introduction

The illustra™ plasmidPrep Midi Flow Kit is designed for the purification of high yields of transfection-grade plasmid DNA. The high capacity of the medium included in the kit allows for the purification of large yields of plasmid DNA that are proportional to the input sample volume (up to ~ 250 µg of plasmid from 50 ml of culture for high copy number plasmids or 500 ml of culture for low copy number plasmids).

We performed a comparative evaluation of the illustra plasmidPrep Midi Flow and Qiagen™ Plasmid Midi kits. The increased capacity provided by the purification medium in the illustra plasmidPrep Midi Flow Kit allowed for the isolation of significantly greater yields of both high and low copy number plasmids compared with the Qiagen Plasmid Midi Kit.

illustra plasmidPrep Midi Flow Kit delivers:

- **High yields:** Upwards of of 300 µg with excellent reproducibility.
- **Dependable flow rates:** High selectivity for plasmid DNA by using high-capacity Fast Flow columns which contain a strong anion exchange purification medium that has excellent flow characteristics, exceptional chemical and pH stability.
- **High purity:** Yields plasmid DNA with up to 7-fold less endotoxin compared with Qiagen's Plasmid Midi Kit.
- **Simpler purification:** Columns are pre-equilibrated for immediate use; color-coded caps and bottles with matching protocol steps minimize the chance for error; quick reference protocol card provides instructions at a glance for experienced users.

Method overview

The illustra plasmidPrep Midi Flow Kit uses a three-step plasmid purification process (Fig 1) consisting of a modified alkaline lysis procedure coupled with centrifugal clarification; an anion-exchange purification by gravity-flow using the Fast Flow Plasmid medium provided in a disposable, pre-equilibrated column format; and finally, concentration and desalting of the purified plasmid DNA by isopropanol precipitation and centrifugation.

We compare the yield, purity, and quality of plasmid DNA prepared with the illustra plasmidPrep Midi Flow Kit with that of the Qiagen Plasmid Midi Kit.

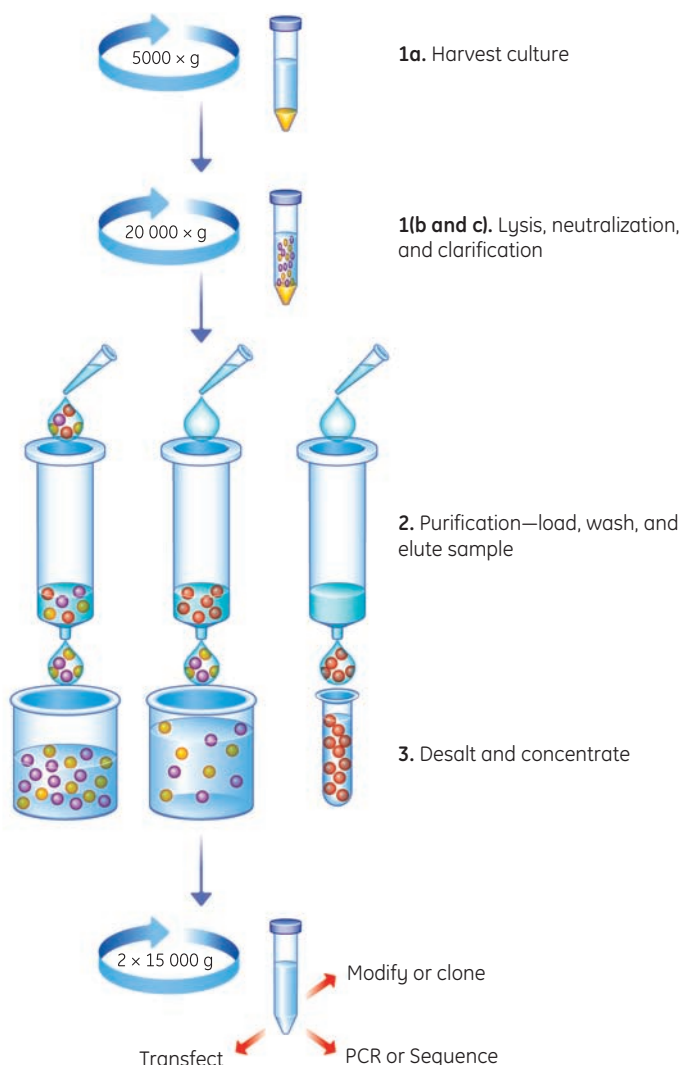


Fig 1. Schematic representation of the illustra PlasmidPrep Midi Flow Kit protocol.



Yield and purity

The superior capacity of the Fast Flow Plasmid purification medium used in the illustra plasmidPrep Midi Flow kit permits the processing of larger culture volumes than the Qiagen Plasmid Midi Kit and provided significantly greater overall yields of plasmid DNA from midi-scale procedures with both high copy and low copy number vectors (Table 1 and Fig 2).

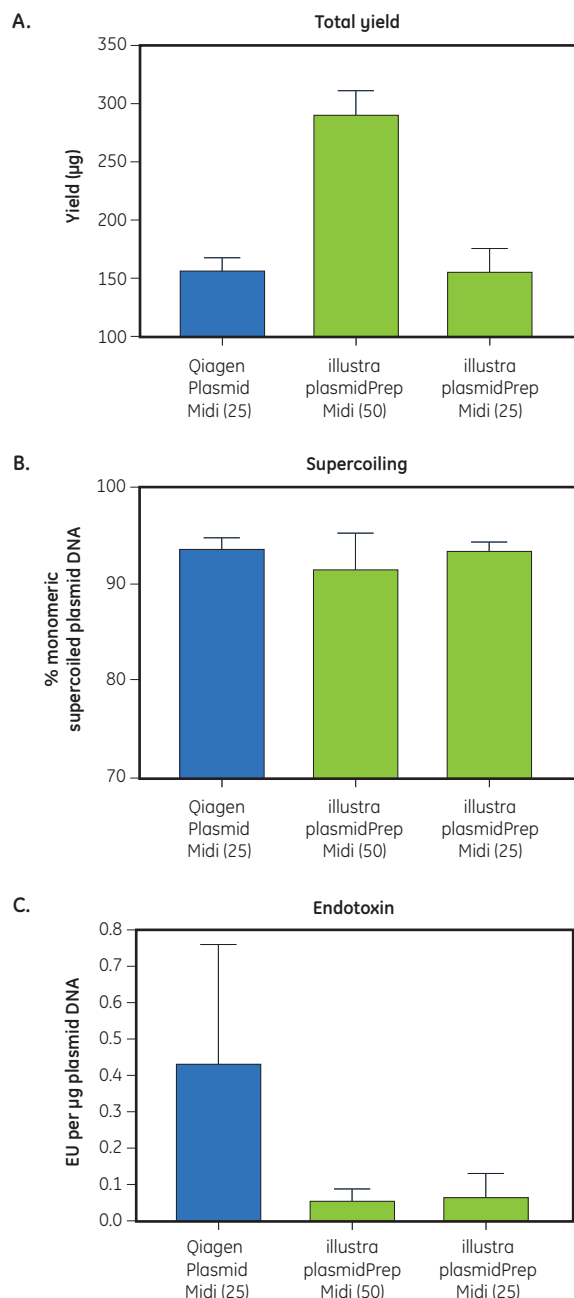


Fig 2. Yield and purity values for high copy number plasmid DNA (pCORON/EGFP) prepared with the illustra plasmidPrep Midi Flow and Qiagen Plasmid Midi kits according to manufacturer's instructions. Processed culture volume in parentheses; n = 12 with 3 researchers.

Plasmid DNA samples prepared with both the illustra plasmidPrep Midi Flow and the Qiagen Plasmid Midi kits provided UV spectrophotometric data that was equivalent and within traditionally accepted ranges of 1.8 to 2.0 for $A_{260}:A_{280}$ ratios, and 2.2 to 2.4 for $A_{260}:A_{230}$ ratios (Table 1); indicating that the presence of salt, RNA, and protein contaminants were negligible. Agarose gel electrophoresis was used to confirm the absence of fast and slow migrating contaminating nucleic acids such as RNA and genomic DNA (Fig 3) and subsequent densitometric analysis showed that very high levels (> 90%) of supercoiled monomeric plasmid DNA were isolated using both kits (Figs 2B and 3).

Endotoxin contamination was significantly lower for high and low copy number plasmid DNA samples prepared with the illustra plasmidPrep Midi Flow Kit compared to the Qiagen Plasmid Midi Kit (Table 1 and Fig 2C). The low level of endotoxin contamination is a reflection of the highly selective nature of the Fast Flow Plasmid purification medium for plasmid DNA, and the optimized anion-exchange system used in the illustra plasmidPrep Midi Flow Kit.

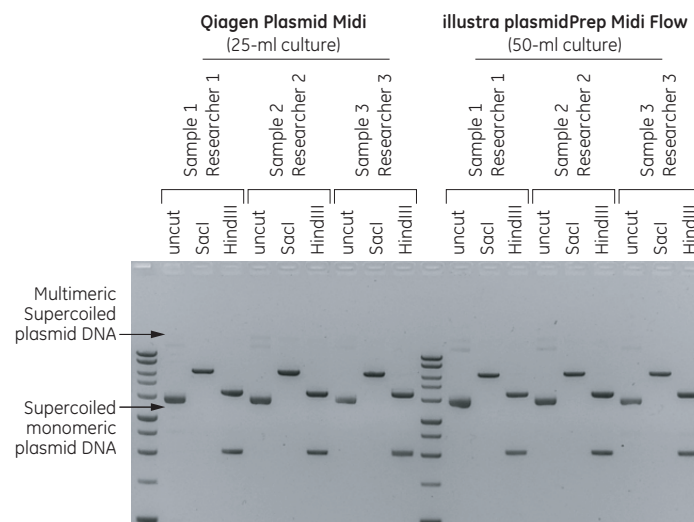


Fig 3. DNA agarose gel showing uncut samples with predominantly supercoiled and monomeric forms of plasmid DNA and the absence of endonuclease activity. The gel also shows complete restriction enzyme digestion of plasmid DNA samples prepared with both the Qiagen Plasmid Midi and illustra plasmidPrep Midi Flow kits according to manufacturers' instructions. Plasmid DNA was purified from *E. coli* cells (25 and 50 ml) harboring a 6.3-kb high copy number plasmid.

Performance of purified plasmid DNA in downstream applications

Plasmid DNA samples isolated using the Qiagen Plasmid Midi and illustra plasmidPrep Midi Flow kits could be digested to completion with a range of restriction enzymes including SacI and HindIII (Fig 3). The plasmid DNA was also suitable for PCR and autosequencing applications, with equivalent mean autosequence read length values (based on Phred20 quality scores) for both the illustra plasmidPrep Midi Flow and Qiagen Plasmid Midi kits (Fig 4). Plasmid DNA purified with the illustra plasmidPrep Midi Flow Kit was successfully transfected into a range of mammalian cells including COS7, HeLa, and SH-SY5Y cells.

Table 1. Yield and purity values for high copy and low copy number plasmid DNA prepared with the illustra plasmidPrep Midi Flow and the Qiagen Plasmid Midi kits

Kit	Plasmid	Copy number	Culture volume (ml)	Overall plasmid DNA yield (µg)		Endotoxin (EU/µg plasmid DNA)		A ₂₆₀ :A ₂₈₀ ratio		A ₂₆₀ :A ₂₃₀ ratio	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
Qiagen Plasmid Midi	pCORON/EGFP	High	25	154.6	11.1	0.43	0.33	1.90	0.03	2.30	0.04
illustra plasmidPrep Midi Flow	pCORON/EGFP	High	25	154.3	18.8	0.06	0.06	1.87	0.03	2.35	0.01
illustra plasmidPrep Midi Flow	pCORON/EGFP	High	50	289.6	20.9	0.05	0.03	1.90	0.01	2.34	0.08
Qiagen Plasmid Midi	pBHG	Low	100	107.4	3.0	5.47	1.30	1.92	0.01	2.29	0.03
illustra plasmidPrep Midi Flow	pBHG	Low	150	191.37	7.7	0.13	0.02	1.93	0.01	2.29	0.00

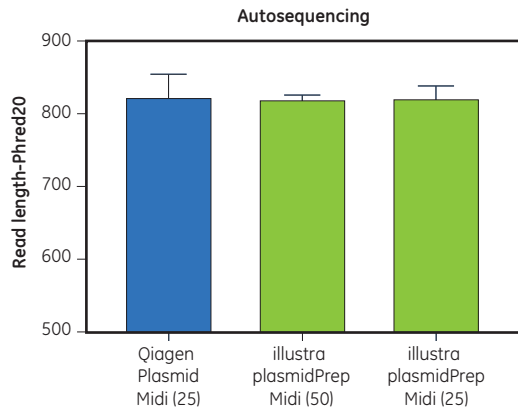


Fig 4. Mean autosequence read length using Phred20 quality measurement for high copy number plasmid DNA samples (n = 12; 3 researchers) prepared with the Qiagen Plasmid Midi Prep and illustra plasmidPrep Midi Flow kits according to manufacturers' instructions. Processed culture volume is shown in parentheses.

Summary

The illustra plasmidPrep Midi Flow Kit consistently produces high yields of pure plasmid DNA—with low levels of endotoxin contamination—for use in a range of demanding cellular and molecular biology applications including: transfection, enzymatic modification, amplification, and automated sequencing.

Ordering information

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