

Rapid purification of high-quality plasmid DNA using illustra plasmidPrep Mini Spin Kit

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The illustra™ plasmidPrep Mini Spin Kit can be used to rapidly purify high-quality plasmid DNA from small culture volumes (1 to 3 ml) of transformed *E. coli*. The system is fast, efficient, and routinely generates highly pure plasmid DNA yields of up to 15 µg. The yield and quality of the purified plasmid DNA ensures exceptional performance in downstream applications such as restriction enzyme analysis, ligation, cloning, DNA sequencing, PCR and other applications in molecular biology.

Method overview

The protocol consists of three phases: a modified alkaline lysis method is used to disrupt the bacteria (1-3); a centrifugation step to pellet bacterial cellular debris; and the loading of the cleared supernatant onto a purification column that contains a novel silica membrane. The novel membrane facilitates the removal of denatured contaminants using a single wash and drying step prior to plasmid DNA elution. The illustra plasmidPrep Mini Spin Kit employs chaotropic salts to denature potential contaminants and promote the selective binding of plasmid DNA to the silica membrane (4, 5).

Extraction time

The time taken to perform a single plasmid DNA extraction using illustra plasmidPrep Mini Spin Kit was more than 50% faster than a similar extraction using QIAprep™ Spin Mini Kit (Fig 1).

¹ Details of the comparative studies are found in Data File 28-9075-92 AA. The comparative studies were performed at GE Healthcare's laboratories. QIAprep and Qiagen are trademarks of the Qiagen group.

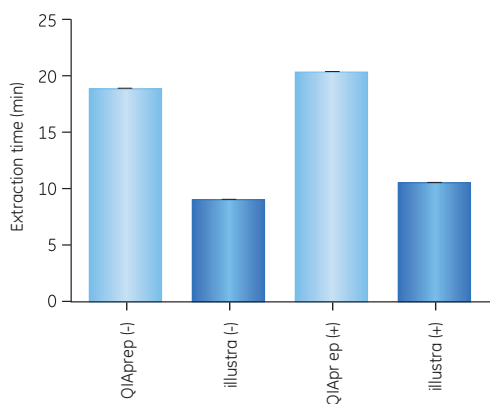


Fig 1. The extraction time from a single miniprep using either the illustra plasmidPrep Mini Spin Kit or the QIAprep Spin Miniprep Kit. For statistical analysis and relevance, we employed three different researchers to isolate nine plasmid DNA samples. Plus (+) refers to the inclusion of an optional nuclease removal step and (-) denotes its omission.

Amount of supercoiled plasmid DNA

The amount of supercoiled plasmid DNA present in a sample can be used to assess the extent of physical damage suffered in the course of the miniprep extraction procedure. The data indicates that 60 to 70% of the plasmid DNA isolated from all four cultures were in a supercoiled configuration (data not shown).

Nuclease activity in plasmid DNA samples derived from the *EndA*⁺ strain HB101

Residual nuclease activity was investigated in plasmid DNA samples purified from *E. coli* HB101 with illustra plasmidPrep Mini Spin Kit. The presence of a partially degraded plasmid DNA smear suggests that residual nuclease activity was present in samples that were not subjected to the nuclease removal wash. Equivalent plasmid DNA samples that had undergone the additional wash did not show any plasmid DNA degradation indicating the removal of nuclease activity (data not shown).

Compatibility with downstream applications

The plasmid DNA samples isolated with either the QIAprep or illustra kit were digested to completion in all the restriction digests performed in this study (Fig 2)—including digests involving low concentrations of the restriction enzyme HindIII (1 unit at 37°C for 1 h). HindIII activity is diminished in the presence of elevated salt concentrations; therefore, HindIII digestion can be utilized to indicate the presence of prohibitively high-salt contamination in the purified plasmid DNA. We observed

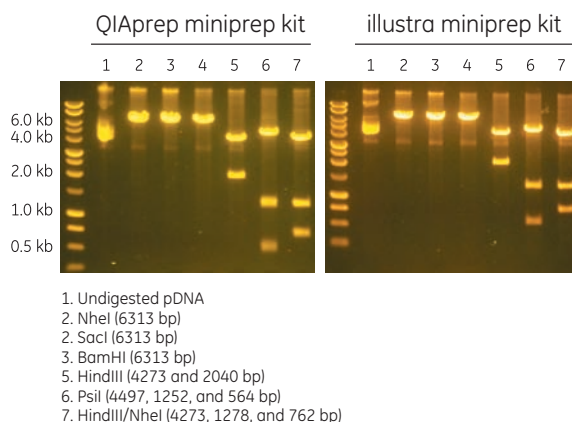


Fig 2. Restriction enzyme digestion of plasmid DNA samples (400 ng, 5 units, 37°C for 1 h). A representative image is shown of single plasmid DNA samples extracted from culture 1.

that plasmid DNA samples isolated with both kits were digested to completion with HindIII (Fig 3), suggesting that both illustra and QIAprep kits produced plasmid DNA samples with negligible salt content.

Endpoint PCR

All the isolated plasmid DNA samples were of sufficient quality (irrespective of the purification kit used) to facilitate the amplification of a 1187-bp product (Fig 4). Comparable band intensities were observed for each individual DNA polymerase when we compared QIAprep to illustra plasmid DNA templates. In addition, the number of PCR cycles did not affect the amplification result thus indicating similar amplification efficiencies for all plasmid DNA templates. The enzymes were chosen to highlight the efficacy of illustra derived plasmid DNA as reliable templates for the amplification of DNA products using either nonproofreading (*Taq* DNA polymerase) or the proofreading polymerases (*PfuTurbo*TM and *Vent*TM DNA polymerases).

Ligation and cloning

Plasmid DNA isolated using the illustra plasmidPrep Mini Spin Kit was shown to be a suitable template for T4 DNA ligase-mediated cloning experiments (data not shown). The ligation, cloning, and transformation efficiencies were comparable for plasmid DNA samples purified with either the illustra or QIAprep miniprep kits (> 300 ampicillin-resistant colonies). Negative control reactions (absence of ligase) produced < 50 colonies.

Conclusions

illustra plasmidPrep Mini Spin Kit is a versatile plasmid DNA purification system saving as much as 10 min in process time compared with the QIAprep Spin Miniprep Kit, without compromising plasmid DNA yield, purity, or quality. Plasmid DNA samples purified with illustra plasmidPrep Mini Spin Kit are of high quality, ensuring excellent performance in several downstream molecular biological techniques including restriction enzyme analysis, PCR, ligation, and cloning.

References

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5. Marko, M.A., *et al.* A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. *Anal. Biochem.* **121**, 382 (1982).

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Ordering information

Product	Code number
illustra plasmidPrep Mini Spin (50 preps)	28-9042-69
illustra plasmidPrep Mini Spin (250 preps)	28-9042-70



Fig 3. HindIII restriction digests (1 unit, 37°C for 1 h) of plasmid DNA samples. A representative image is shown of plasmid DNA samples extracted from culture 1.

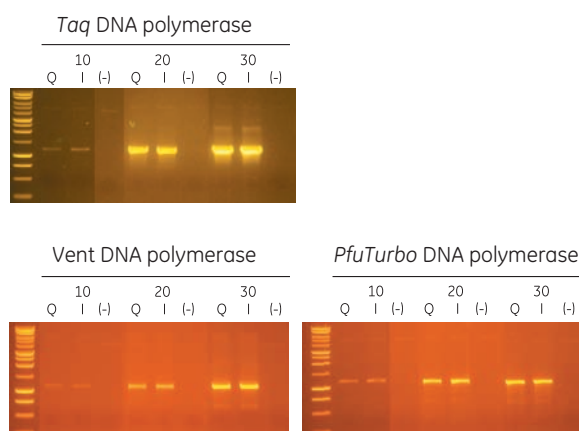


Fig 4. Endpoint PCR analysis. Lanes Q and I represent amplification products from QIAprep spin kit and illustra miniprep kit-derived plasmid DNAs, respectively; (-) represents no template control reactions. The numbers 10, 20, and 30 denote the number of PCR cycles performed. PCR analysis was performed on several plasmid DNA samples. Representative data is shown for single plasmid DNA isolations using either the QIAprep or illustra miniprep kits.