illustra plasmidPrep Mini Spin Kit

Introduction

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 molecular biology applications.

We examined the performance of the illustra plasmidPrep Mini Spin Kit vis-à-vis the QIAprep™ Spin Miniprep Kit from Qiagen™ and found that the entire plasmid DNA purification process can be completed with the illustra plasmidPrep Mini Spin Kit in ~ 9 min (without compromising plasmid DNA purity or quality) whereas the same procedure took ~ 19 min with the QIAprep Spin Miniprep Kit.

illustra plasmidPrep Mini Spin Kit delivers:

- **Fast results:** Isolates plasmid DNA in less than 10 min. This represents a time saving of > 50% compared with QIAprep Spin Miniprep Kit.
- **High purity:** Plasmid DNA samples that are free of RNA and genomic DNA contamination, that lack any residual nuclease activity, and exist in a predominantly supercoiled configuration.
- **High quality:** Excellent performance in several downstream applications including—restriction enzyme analysis, ligation, cloning, DNA sequencing, and PCR.
- **Simpler purification:** Increased ease of use provided by minimal changes in pipetting volume from one part of the protocol to the next, less centrifugation steps, color-coded kit components, and a lack of organic solvents.

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 the illustra plasmidPrep Mini Spin Kit was more than 50% faster than a similar extraction using the QIAprep Spin Mini Kit (Fig 1).

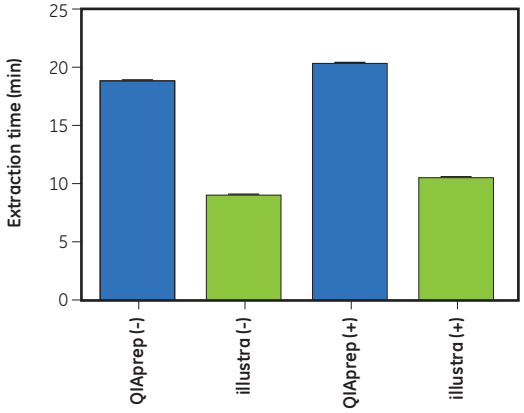


Fig 1. The extraction time from a single miniprep using either the illustra plasmidPrep Mini spin or the QIAprep Spin Miniprep kits. For statistical analysis and relevance, we employed 3 different researchers to isolate 9 different plasmid DNA samples. Plus (+) refers to the inclusion of an optional 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 4 cultures were in a supercoiled configuration (Figs 2 and 3).

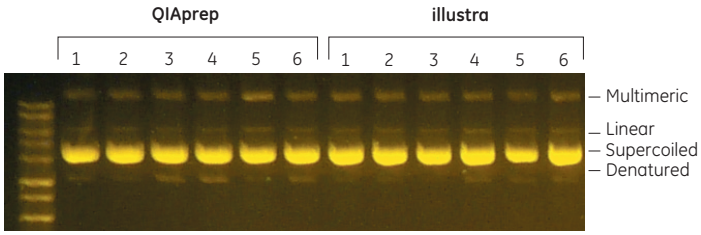


Fig 2. A representative image of undigested plasmid DNA (400 ng) extracted from culture 1. Plasmid minipreps were performed using the illustra and QIAprep kits.

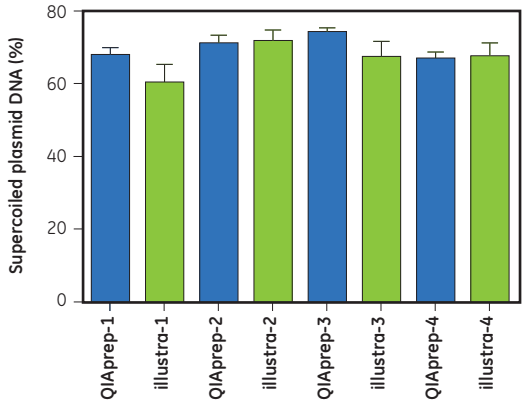


Fig 3. Amount of supercoiled plasmid DNA expressed as a percentage of total plasmid DNA. No significant differences were apparent (ANOVA, p-value > 0.05) between the amounts of supercoiled plasmid DNA in extracts derived from either the illustra or QIAprep miniprep kits. The numbers denote the particular culture used for plasmid DNA extraction. The results were generated using the Kodak™ 1D Image Analysis software.

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 the 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 (Fig 4).

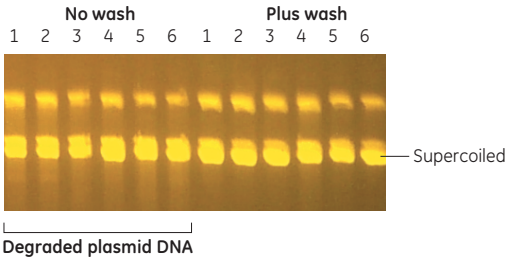
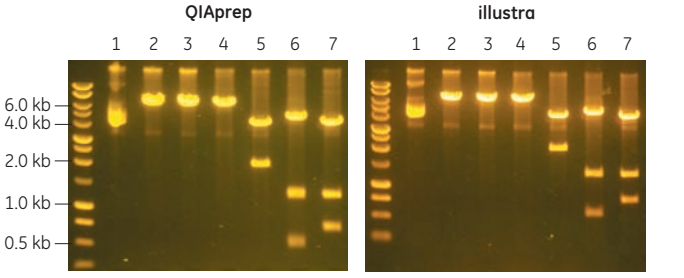


Fig 4. Plasmid DNA samples after incubation for potential nuclease activity. A representative image is shown of plasmid DNA samples extracted using the illustra plasmidPrep Mini Spin Kit from *E. coli* strain HB101 (*EndA*⁺) containing pCORON1002/EGFP-C1 plasmid.

Compatibility with downstream applications

The plasmid DNA samples isolated with either the QIAprep or illustra kits were digested to completion in all the restriction digests performed in this study (Fig 5)—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 that plasmid DNA samples isolated with both kits were digested to completion with HindIII (Fig 6), suggesting that both illustra and QIAprep kits produced plasmid DNA samples with negligible salt content.



1—Undigested plasmid DNA
2—NheI (6313 bp)
3—SacI (6313 bp)
4—BamHI (6313 bp)
5—HindIII (4273 and 2040 bp)
6—PsiI (4497, 1252 and 564 bp)
7—HindIII/NheI (4273, 1278, and 762 bp)

Fig 5. 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.

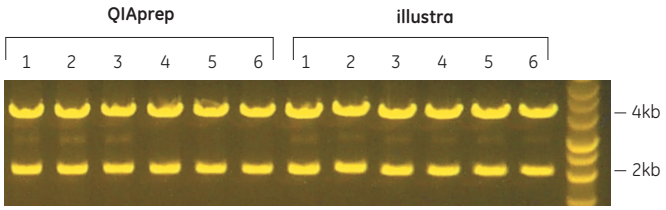


Fig 6. HindIII restriction digests (1 unit, 37°C for 1 h) of plasmid DNA samples. A representative image is shown of plasmid DNA samples extracted from culture 1.

Endpoint PCR

All the miniprep products were of sufficient quality (irrespective of the purification kit used) to facilitate the amplification of a 1187-bp product (Fig 7). 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 PCR products using either nonproofreading (*Taq* DNA polymerase) or the proofreading polymerases (*PfuTurbo*™ and Vent™ DNA polymerases).

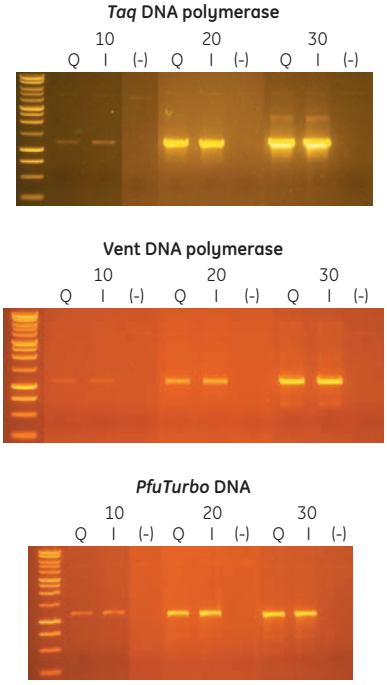


Fig 7. Endpoint PCR analysis. Lanes Q and I represent amplification products from QIAprep and illustra derived plasmid DNAs, respectively; (-) represents no template control reactions. The numbers 10, 20, and 30 denote the number of PCR cycles performed. PCR analysis was performed on several plasmid DNA samples. Representative data is shown for single plasmid DNA isolations using either the QIAprep or illustra kits.

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 (Fig 8). 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).

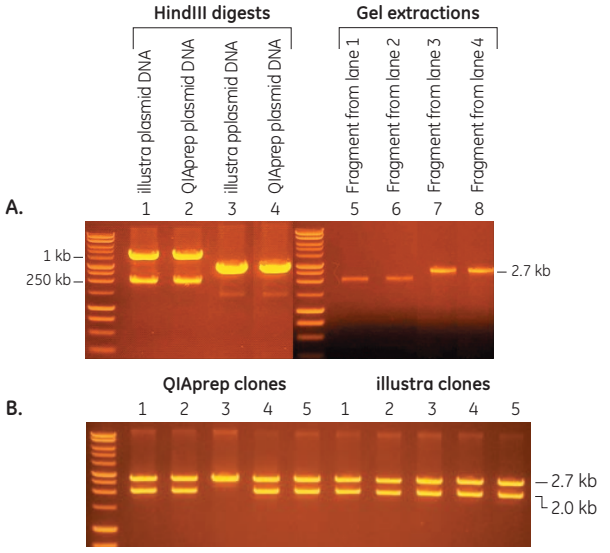


Fig 8. Ligation and cloning results. (A) Lanes 1 and 2 (pCORON1002/EGFP-C1); 3 and 4 (pUC18) are HindIII digests (1 and 3 are plasmid DNA samples isolated with the illustra plasmidPrep Mini Spin Kit whereas lanes 2 and 4 depict plasmid DNA purified with the QIAprep Spin Miniprep Kit. The pCORON1002/EGFP-C1 digest products are ~ 2 and ~ 4 kb in size, that of pUC18 is ~ 2.7 kb. Lanes 5, 6, 7, and 8 are the gel-extracted fragments. (B) Five randomly selected recombinant plasmid DNAs (derived from QIAprep and illustra kits) were digested with HindIII. The correct religation pattern is ~ 2.0 and 2.7 kb.

Summary

The illustra plasmidPrep Mini Spin Kit is a versatile plasmid DNA purification system that achieves a considerable reduction in process time compared to Qiagen's QIAprep Spin Miniprep Kit without compromising plasmid DNA yield, purity or quality.

References

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Ordering information

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