illustra bacteria genomicPrep Mini Spin Kit

Introduction

illustra™ bacteria genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from Gram-negative (G-) and Gram-positive (G+) bacteria. The procedure for G- bacteria can be completed in about 40 min (sample to gDNA) versus 200 min required for the bacteria protocol used with QIAamp™ DNA Mini Kit. Purified gDNA is suitable for molecular biology applications including cloning, restriction enzyme digestion, PCR, and genotyping.

illustra bacteria genomicPrep Mini Spin Kit delivers:

- **Fast results:** convenient, streamlined workflow reduces the number of pipetting volume changes and the overall number of steps to deliver results 80% faster than QIAamp DNA Mini Kit
- **Optimized kit:** dedicated kit optimized for bacterial gDNA with separate protocols for G- and G+ bacteria
- **Ease of 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
- **High quality and purity:** optimized protocol produces intact, RNA-free gDNA that is > 20 kb in size with a purity > 1.8 (A_{260}/A_{280})
- **Reproducible yields:** highly reproducible yields minimizes the need to repeat experiments

Method overview

The illustra bacteria genomicPrep Mini Spin Kit has been optimized to deliver speed with quality. The procedure for G- bacteria can be completed in about 40 min and the protocols have been designed to minimize shearing, resulting in high-quality, intact gDNA. (Fig. 1)

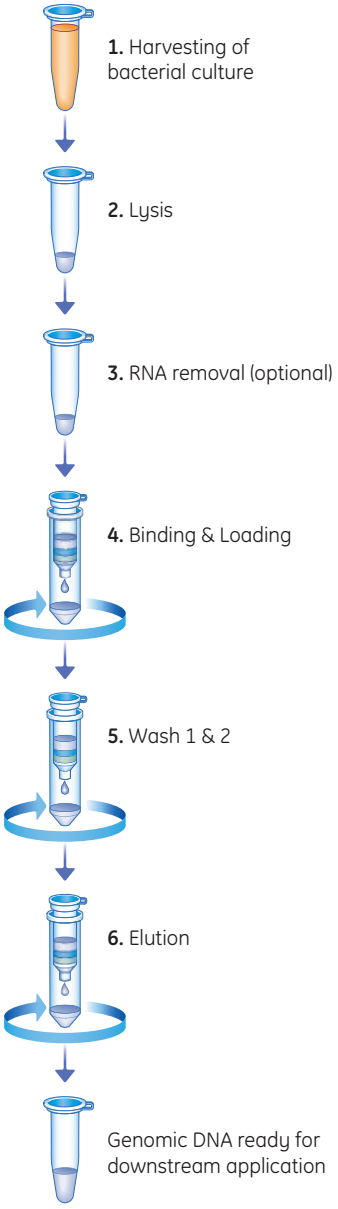

Fig 1. Schematic representation of the gDNA isolation protocol employed by the illustra bacteria genomicPrep Mini Spin Kit. The method can be completed in about 40 min, excluding the optional RNA removal step, for G- bacteria and in about 60 min for G+ bacteria.

The illustra bacteria genomicPrep Mini Spin Kit uses a lysis solution in combination with proteinase K to release gDNA into solution from bacterial cells. Due to the much thicker peptidoglycan layer in G+ bacteria than in G- bacteria, it is necessary to pre-lyse G+ bacteria with lysozyme. An optional RNase step can be performed to yield RNA-free gDNA.

Following lysis, DNA is deproteinated in an extraction solution and gDNA is then bound onto a silica column in the presence of a chaotropic solution. Contaminants are removed using wash steps and DNA is eluted with preheated elution buffer. Purified gDNA can be eluted into 100 or 200 µl of buffer, allowing the preparation of a more concentrated sample when necessary.

The lysis solutions have been optimized to extract gDNA from several strains of G- bacteria such as *E. coli* DH5α, TOP10, JM109, and G+ bacteria such as *Bacillus*. Typical yields are 4 to 12 µg of gDNA per prep. Bacterial numbers ranging from 1 to 4 × 10^9 cells can be used. The kit is designed to give consistent and reproducible recovery of purified gDNA with high purity (A_{260}/A_{280} = ~1.8). Specifications for illustra bacteria genomicPrep Mini Spin Kit are shown in Table 1.

Table 1. Specifications for illustra bacteria genomicPrep Mini Spin Kit

Feature	Specification	
Sample type	Gram-negative bacteria	Gram-positive bacteria
Sample input size	2 × 10^9 cells (A_{600} = 2.0)	2 × 10^9 cells (A_{600} = 2.0)
Elution volume	200 µl	200 µl
Typical yield[1]	4 to 12 µg gDNA	5 to 10 µg gDNA
Purity (A_{260}/A_{280})	> 1.7	> 1.7
Time/prep[2]	~ 40 min	~ 60 min
Product Size	> 20 kb	> 20 kb

[1] Actual yields will vary depending on bacteria strain and growth phase of bacteria.
[2] Time does not include optional RNase treatment.

High quality and purity

The illustra bacteria genomicPrep Mini Spin Kit yields high-quality gDNA with sizes larger than 20 kb. The gDNA is largely intact with minimal shearing (Fig 2). The average purity of the eluted gDNA is higher than that obtained with the QIAamp DNA Mini Kit (Table 2).

When the optional RNase step is included, the genomicPrep protocol yields RNA-free gDNA. Genomic DNA isolated using QIAamp DNA Mini Kit can still contain RNA after RNase treatment (Fig 3).

Table 2. Comparison of yield and purity between illustra bacteria genomicPrep Mini Spin Kit and QIAamp DNA Mini Kit from Qiagen[1]

Kit	Yield (µg) ± sd	Purity (A_{260}/A_{280})
illustra bacteria genomicPrep Mini Spin Kit	5.1 ± 1.3	1.86 ± 0.05
QIAamp DNA Mini Kit	4.2 ± 2.1	1.72 ± 0.12

[1] Comparison was performed using 2 × 10^9 cells of *E. coli* (DH5α) according to manufacturers' instructions. The data is an average of 36 samples for the genomicPrep kit and 35 samples for the QIAamp kit. For yield, p = 0.036; for purity, p = 0.000.

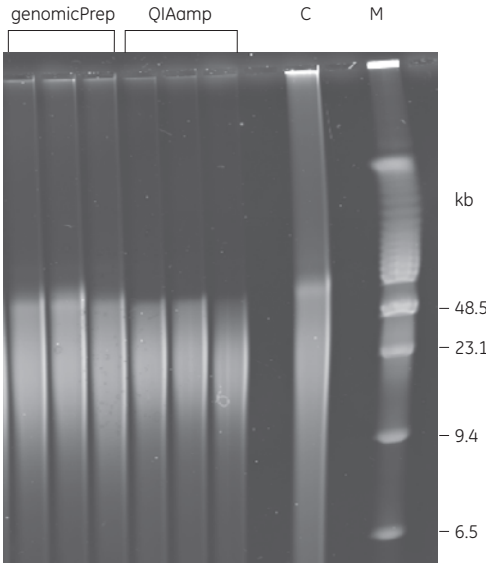

Fig 2. Sizing of gDNA from *E. coli* DH5α by pulsed-field gel electrophoresis (PFGE). Genomic DNA was isolated from 2 × 10^9 cells with either the illustra bacteria genomicPrep Mini Spin Kit or the QIAamp DNA Mini Kit according to manufacturers' instructions. Samples contained 250 ng of purified gDNA. All samples were run on the same gel. M = low-range PFGE marker. C = commercially available bacterial gDNA.

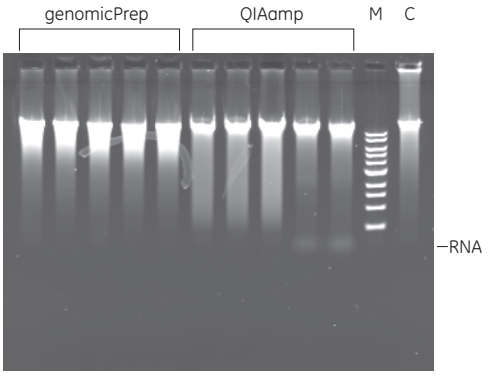

Fig 3. Detection of RNA contamination in samples of purified gDNA. Gel was loaded with 20 µl of eluted gDNA solution from either illustra bacteria genomicPrep Mini Spin Kit or QIAamp DNA Mini Kit. Genomic DNA from *E. coli* DH5α was isolated according to manufacturers' instructions, including an RNase treatment step. C = commercially available bacterial gDNA. M = 1 kb molecular weight marker.

Reproducible yields

The illustra bacteria genomicPrep Mini Spin Kit delivers consistent, reproducible yields of gDNA. In comparative tests with QIAamp DNA Mini Kit, the yield from genomicPrep was 20% more than that from QIAamp (Table 2). Additionally, the coefficient of variation for genomicPrep yields was about half of that for QIAamp (25.5% vs 50%, respectively), which demonstrates that yields from genomicPrep are more consistent than those from QIAamp.

Yields of gDNA purified using illustra bacteria genomicPrep Mini Spin Kit from two other common *E. coli* strains are shown in Table 3. The kit can also be used with G+ bacteria.

Table 3. gDNA yield from different *E. coli* strains and *Bacillus subtilis* (G+ bacterium)

Organism[1]	Yield (µg ± sd)[2]	Purity (A_{260}/A_{280})[2]
E. coli JM109	5.0 ± 0.7	1.91 ± 0.02
E. coli TOP10	6.0 ± 0.1	1.91 ± 0.01
Bacillus subtilis	10.7 ± 0.6	1.98 ± 0.01

[1] gDNA was isolated from 2 × 10^9 cells.
[2] n = 3 for *E. coli* strains; n = 6 for *Bacillus subtilis*.

Compatibility with downstream applications

Real-time PCR

DNA purified using the illustra bacteria genomicPrep Mini Spin Kit performs effectively in quantitative real-time PCR. The performance of gDNA purified using the illustra bacteria genomicPrep Mini Spin Kit was compared to gDNA purified using the QIAamp DNA Mini Kit by performing quantitative real-time PCR analysis. No inhibitory effects were seen in either the change in efficiency of the real-time PCR or the fold amplification (Ct values) between gDNA isolated using either kit (Fig 4). Instead, the significantly higher Ct value for the QIAamp gDNA indicates a lower yield from QIAamp.

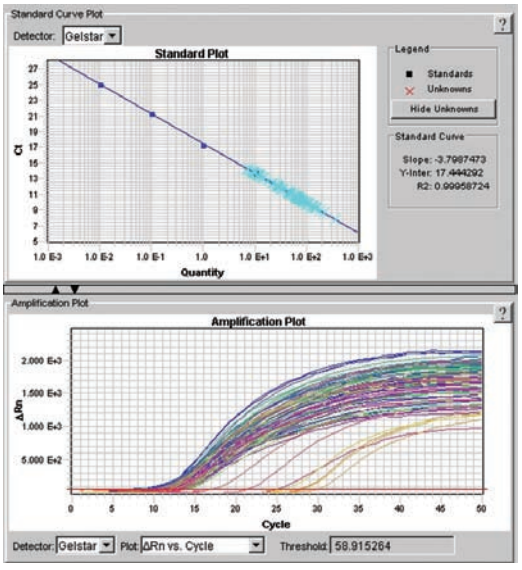

Fig 4. Real-time PCR amplification *E. coli* DH5α gDNA purified with illustra bacteria genomicPrep Mini Kit and QIAamp DNA Mini Kit. Real-time PCR assays were performed using puReTaq™ RTG PCR Beads and the primer sets for the *E. coli* 16S rRNA gene. One microliter of the eluted gDNA solution, corresponding to 10 to 30 ng of gDNA, was used per reaction. A standard curve was obtained using commercially available bacterial gDNA at concentrations ranging from 0.01 to 10 ng. Ct value for genomicPrep was 10.4 ± 1.1 (n = 36) and for QIAamp it was 12.2 ± 1.1 (n = 35). p = 0.000.

Long PCR

The large size and high amount of intact gDNA produced using illustra bacteria genomicPrep Mini Spin Kit makes it suitable for long PCR. In the example shown in Figure 5, 11-kb amplicons were successfully amplified. No inhibitory effects were seen with either genomicPrep or QIAamp purified gDNA.

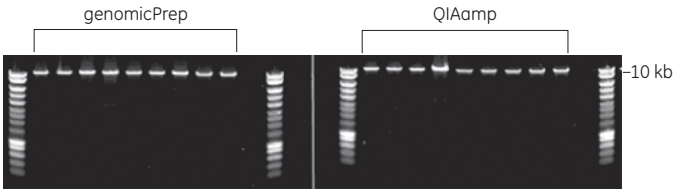

Fig 5. Amplification of an 11-kb amplicon from purified bacterial gDNA. Five microliters of the eluted gDNA solution, corresponding to 50 to 150 ng of the gDNA was used per reaction. Following PCR, equal volumes from each reaction were resolved on 0.8% agarose gel stained with ethidium bromide. M = 1 kb molecular weight marker.

Restriction enzyme digestion

The purity and concentration of gDNA isolated using illustra bacteria genomicPrep Mini Spin Kit enables its direct use in restriction enzyme digestions. Tests with BamHI, EcoRI, and HindIII demonstrated that the purified gDNA was free from inhibitors and that all samples were completely digested (Fig 6).

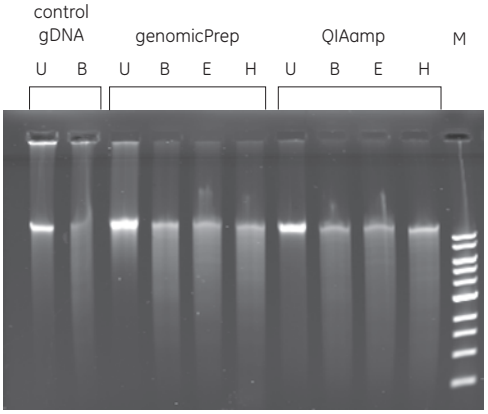

Fig 6. Restriction enzyme digestion of gDNA purified using illustra bacteria genomicPrep Mini Spin Kit and QIAamp DNA Mini Kit. Purified samples containing 250 ng of gDNA were used for digestion reactions with 20 to 80 units of enzyme at either 37°C for 1 hour or 37°C for 18 hours. The results were visualized on 0.8% agarose gel. U = undigested (no enzyme) gDNA; B = BamHI; E = EcoRI; H = HindIII; M = 1 kb molecular weight marker.

Summary

The illustra bacteria genomicPrep Mini Spin Kit rapidly delivers high-quality intact gDNA from G- and G+ bacteria using a convenient and efficient protocol. Genomic DNA can be isolated in about 40 min from G- bacteria or in about 60 min for G+ bacteria. The optimzed lysis conditions yield gDNA that is > 20 kb, intact, and highly pure. Results are reproducible with typical yields of 4 to 12 µg from 2 × 10^9 cells. The purified gDNA can be used directly in downstream applications such as real-time, and long PCR, as well as restriction enzyme digestions, cloning, and genotyping.

Ordering information

illustra bacteria genomicPrep Mini Spin Kit (50 preps)	28-9042-58
illustra bacteria genomicPrep Mini Spin Kit (250 preps)	28-9042-59

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