

Bacteria genomicPrep Mini Spin Kit

GENOMIC DNA PURIFICATION

Bacteria genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of genomic DNA (gDNA) from gram-negative (G-) and gram-positive (G+) bacteria. The procedure for G- bacteria can be completed in about 40 minutes (sample to gDNA). Purified gDNA is suitable for molecular biology applications including cloning, restriction enzyme digestion, PCR, and genotyping.

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 fast results.
- **Optimized kit:** Dedicated kit is 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:** High yield reproducibility minimizes the need to repeat experiments.

Method overview

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

The 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, G+ bacteria must be pre-lysed with lysozyme. An optional RNase step can be performed to yield RNA-free gDNA.

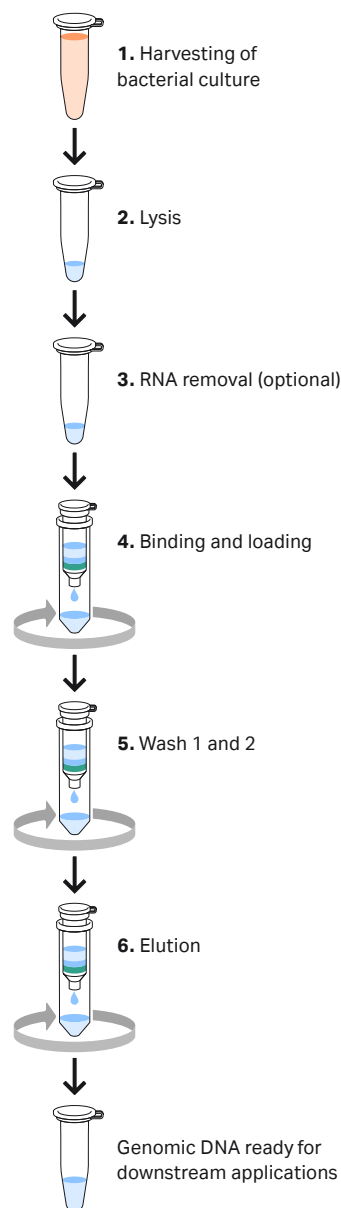


Fig 1. Schematic representation of the gDNA isolation protocol employed by the Bacteria genomicPrep Mini Spin Kit. The method can be completed in about 40 minutes, excluding the optional RNA removal step, for G- bacteria and in about 60 minutes for G+ bacteria.

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 μ L 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* DH5 α , TOP10, JM109, and G+ bacteria such as *Bacillus*. Typical yields are 4 to 12 μ g 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 ($A_{260}/A_{280} = \sim 1.8$). Specifications for Bacteria genomicPrep Mini Spin Kit are shown in Table 1.

Table 1. Specifications for Bacteria genomicPrep Mini Spin Kit

Feature	Specification	
	Gram-negative bacteria	Gram-positive bacteria
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 μ L	200 μ L
Typical yield ¹	4 to 12 μ g gDNA	5 to 10 μ g gDNA
Purity (A_{260}/A_{280})	> 1.7	> 1.7
Time per prep ²	~ 40 minutes	~ 60 minutes
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 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).

When the optional RNase step is included, the genomicPrep protocol yields RNA-free gDNA (Fig 3).

Reproducible yields

The Bacteria genomicPrep Mini Spin Kit delivers consistent, reproducible yields of gDNA with the coefficient of variation for genomicPrep yields at 25.5% (Table 2).

Yields of gDNA purified using 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 2. Yield and purity using Bacteria genomicPrep Mini Spin Kit¹

Kit	Yield (μ g) \pm sd	Purity (A_{260}/A_{280}) ²
Bacteria genomicPrep Mini Spin Kit	5.1 \pm 1.3	1.86 \pm 0.05

¹ Purification was performed using 2×10^9 cells of *E. coli* (DH5 α) according to manufacturers' instructions. The data is an average of 36 samples. For yield, $p = 0.036$; for purity, $p = 0.000$.

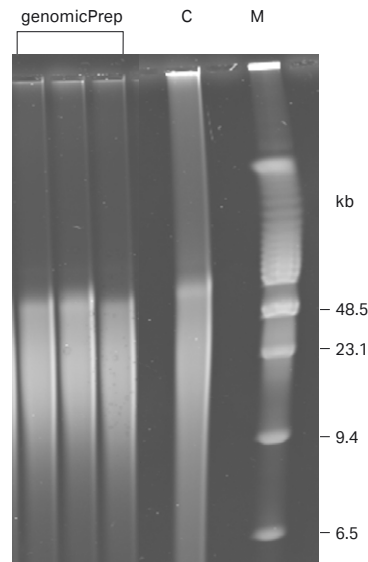


Fig 2. Sizing of gDNA from *E. coli* DH5 α by pulsed-field gel electrophoresis (PFGE). Genomic DNA was isolated from 2×10^9 cells using the Bacteria genomicPrep Mini Spin Kit. Samples contained 250 ng of purified gDNA. All samples were run on the same gel. M = low-range PFGE marker. C = commercially available bacterial gDNA.

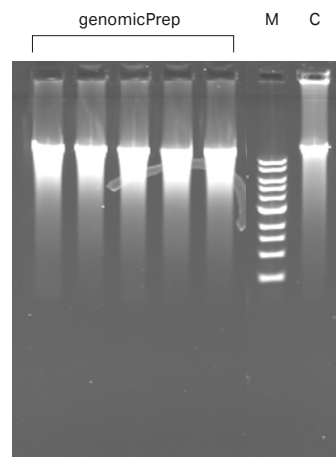


Fig 3. Detection of RNA contamination in samples of purified gDNA. Gel was loaded with 20 μ L of eluted gDNA solution from Bacteria genomicPrep Mini Spin Kit. Genomic DNA from *E. coli* DH5 α was isolated according to manufacturers' instructions, including an RNase treatment step. C = commercially available bacterial gDNA. M = 1 kb molecular weight marker.

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

Organism ¹	Yield (μ g \pm sd) ²	Purity (A_{260}/A_{280}) ²
<i>E. coli</i> JM109	5.0 \pm 0.7	1.91 \pm 0.02
<i>E. coli</i> TOP10	6.0 \pm 0.1	1.91 \pm 0.01
<i>Bacillus subtilis</i>	10.7 \pm 0.6	1.98 \pm 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 Bacteria genomicPrep Mini Spin Kit performs effectively in quantitative real-time PCR. The performance of gDNA purified using the Bacteria genomicPrep Mini Spin Kit was assessed 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) (Fig 4).

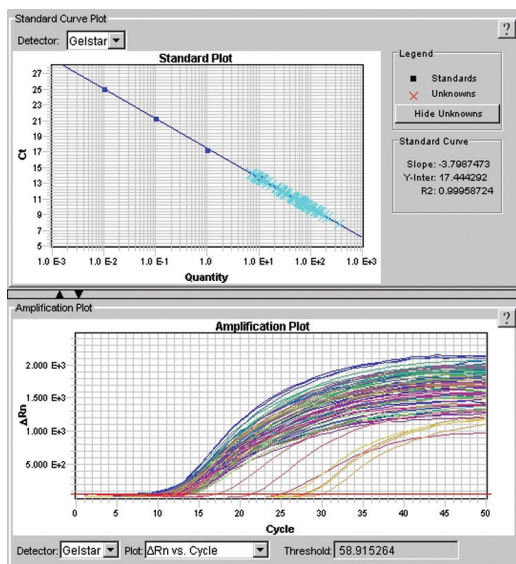


Fig 4. Real-time PCR amplification *E. coli* DH5α gDNA purified with Bacteria genomicPrep Mini Spin Kit. Real-time PCR assays were performed using PuReTaq™ Ready-To-Go™ PCR Beads and the primer sets for the *E. coli* 16S rRNA gene. One microliter of the eluted gDNA solution, corresponding to 10 to 30 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.

Long PCR

The large size and high amount of intact gDNA produced using 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.

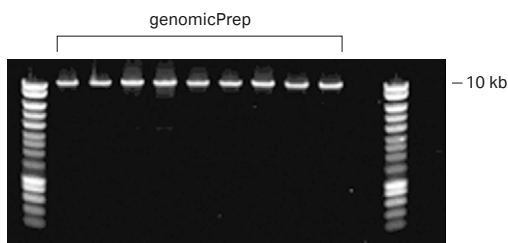


Fig 5. Amplification of an 11-kb amplicon from purified bacterial gDNA. Five microliters of the eluted gDNA solution, corresponding to 50 to 150 ng of the gDNA was used per reaction. Following PCR, equal volumes from each reaction were resolved on 0.8% agarose gel stained with ethidium bromide. M = 1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

Restriction enzyme digestion

The purity and concentration of gDNA isolated using 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).

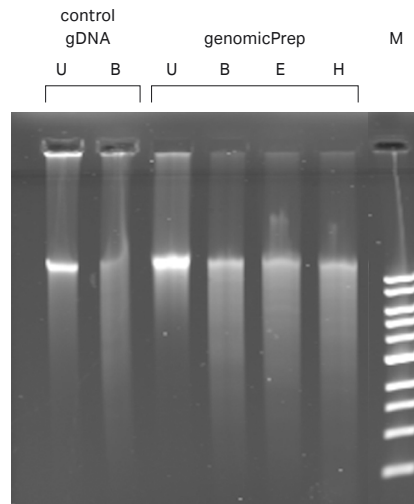


Fig 6. Restriction enzyme digestion of gDNA purified using Bacteria genomicPrep Mini Spin Kit. Purified samples containing 250 ng of gDNA were used for digestion reactions with 20 to 80 units of enzyme at either 37°C for 1 hour or 37°C for 18 hours. The results were visualized on 0.8% agarose gel. U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

Summary

The 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 minutes from G- bacteria or in about 60 minutes for G+ bacteria. The optimized 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 × 10⁹ cells. The purified gDNA can be used directly in downstream applications such as real-time PCR and long PCR, as well as restriction enzyme digestions, cloning, and genotyping.

Ordering information

Product	Quantity	Product code
Bacteria genomicPrep	50 preps	28904258
Mini Spin Kit	250 preps	28904259

[cytiva.com/genomicPrep](https://www.cytiva.com/genomicPrep)

For local office contact information, visit [cytiva.com/contact](https://www.cytiva.com/contact)

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