

illustra tissue & cells genomicPrep Mini Spin Kit

Introduction

illustra™ tissue & cells genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from a variety of animal tissues and mammalian cell cultures in just 90 min for tissues and 45 min for cells (sample to elution). DNA produced by the kit supports molecular biology applications including cloning, restriction enzyme digestion, PCR, and genotyping.

illustra tissue & cells genomicPrep Mini Spin Kit delivers:

- **Fast results:** Cuts time from tissue sample to gDNA in half (compared with QIAamp™ DNA Mini Kit), producing high-quality product in just 90 min
- **Simpler purification:** 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 tissue protocol produces intact, RNA-free gDNA that is > 20 kb in size (Fig 1) with a purity of 1.8 (A_{260}/A_{280})
- **High yields:** Consistently high yields from animal tissue, 50% more gDNA than QIAamp DNA Mini Kit

Method overview

The illustra tissue & cells genomicPrep Mini Spin Kit has been optimized to deliver speed with quality. Isolation of gDNA from animal tissues can be completed in about 90 min, and the protocols have been designed to minimize shearing, resulting in high-quality, intact gDNA. The procedure uses a lysis solution in combination with proteinase K and chaotropic agents to release gDNA from various tissue samples and mammalian cells, denature protein components, and promote the selective binding of DNA to a silica-membrane column (1). Contaminants are removed in subsequent wash steps and gDNA is eluted with prewarmed buffer. Buffer chemistry and protocols have been designed to accommodate gDNA extraction from animal tissue and mammalian cell samples.

The kit can be used to extract gDNA from a variety of tissue types, such as liver, kidney, and mouse tails, and mammalian cell lines, including CHO and human lung fibroblasts. Specifications are shown in Table 1.

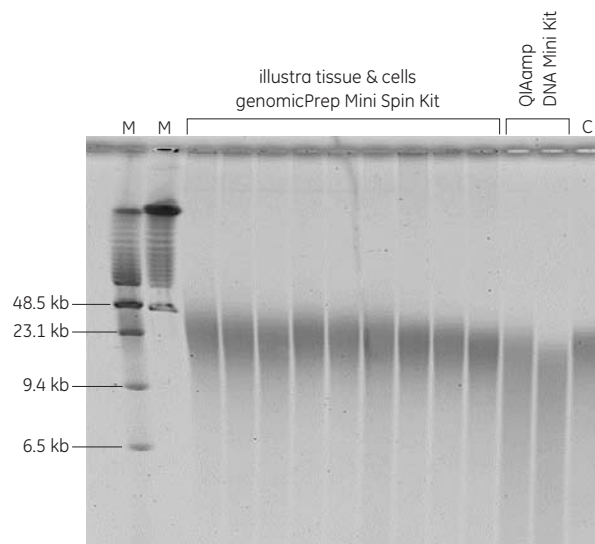


Fig 1. Sizing by pulsed-field gel electrophoresis (PFGE) of gDNA purified from rat liver. Genomic DNA was purified according to manufacturers' instructions. Samples contained 200 ng purified gDNA. C = control rat gDNA. M = molecular weight markers.

Table 1. Specifications for illustra tissue & cells genomicPrep Mini Spin Kit

Feature	Specification	
Sample type	Animal tissue	Cultured cells
Sample input size	5 to 50 mg of animal tissue	Up to 5×10^6 cultured cells
Elution volume	200 μ l	200 μ l
Number of steps	5	5
Binding capacity	> 35 μ g	> 35 μ g
Typical yield	0.5-1.5 μ g DNA/mg of animal tissue ¹	10 to 20 μ g of gDNA (from 5×10^6 cells)
Purity (A_{260}/A_{280})	> 1.75	> 1.75
Time/prep	90 min	45 min
Product size	> 20 kb	> 20 kb

¹ Values shown derived from rat liver samples; actual yields will vary depending on tissue type used.



High quality and purity

The illustra tissue & cells genomicPrep Mini Spin Kit yields high quality gDNA with sizes larger than 20 kb. In a comparison with QIAamp DNA Mini Kit from Qiagen, the purified gDNA obtained from rat liver tissue with the illustra tissue & cells genomicPrep Mini Spin Kit was of larger size with less shearing (Fig 1). The purity of the illustra tissue & cells genomicPrep DNA was also high (Tables 2 and 3). A purity ratio of 1.7 to 1.9 indicates that the gDNA is pure for all standard molecular biology applications.

The illustra tissue & cells genomicPrep Mini Spin Kit also yields intact, well-sized gDNA from cultured mammalian cells. Figure 2 shows pulsed-field gel electrophoresis results for gDNA obtained from human MRC5 cells, which reveals that gDNA was highly intact at nearly 75 kb.

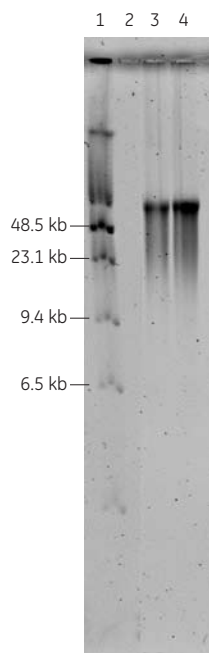


Fig 2. Pulsed-field gel electrophoresis of gDNA purified from MRC5 cells using the illustra tissue & cells genomicPrep Mini Spin Kit. Two separate preparations from different operators are shown (lanes 3 and 4). Lane 1 contains a low-range PFGE marker. Lane 2 was empty.

Consistently high yields

The capacity of the illustra tissue & cells genomicPrep Mini Spin columns is > 35 µg and the columns can handle up to 50 mg of animal tissue. These two factors, together with an optimized protocol result in high yields of gDNA. In comparative tests with QIAamp DNA Mini Kit from Qiagen, illustra tissue & cells genomicPrep Mini Spin Kit delivered over 50% more gDNA from rat liver tissue (Table 2).

Table 2. Comparison of yield between illustra tissue & cells genomicPrep Mini Spin Kit and QIAamp DNA Mini Kit from Qiagen¹

Kit	Yield (µg) ± sd	Purity (A ₂₆₀ /A ₂₈₀) ± sd
illustra tissue & cells genomicPrep Mini Spin Kit	21.2 ± 4	1.84 ± 0.01
QIAamp DNA Mini Kit	13.8 ± 4	1.81 ± 0.03

¹ Comparison was performed using 20 mg of rat liver tissue. Six different rat livers were used for DNA purification. The data is an average of 54 illustra and 18 Qiagen preparations using three different operators.

Table 3 summarizes yields obtained from a variety of animal tissues and cell lines using the illustra tissue & cells genomicPrep Mini Spin Kit. High gDNA yields are obtained from other tissue types such as kidney and mouse tails. Genomic DNA isolation results from cultured lung cells demonstrate the high capacity of the silica resin employed by the kit. Inputs ranging from 1 to 5 million cells give sufficient gDNA for downstream applications.

Table 3. gDNA yield from varying tissues, cells, and input amounts

Tissue type	Sample amount	Yield (µg) ± sd	Purity (A ₂₆₀ /A ₂₈₀) ± sd
Rat liver (n = 54)	20 mg	21.17 ± 4	1.84
Rat kidney (n = 4)	15 mg	19.56 ± 1.7	1.81
Mouse tail (n = 6)	15 mg	11.7 ± 1.7	1.84
CHO cells	1 × 10 ⁵ cells	1.52	1.97
	3 × 10 ⁵ cells	2.57	1.88
	5 × 10 ⁵ cells	3.62	1.88
	1 × 10 ⁶ cells	5.22	1.86
	3 × 10 ⁶ cells	8.79	1.85
	5 × 10 ⁶ cells	13.89	1.84
Kidney HK293 cells (n = 2)	5 × 10 ⁵ cells	16.95 ± 7.3	1.88
Lung MRC5 cells (n = 2)	5 × 10 ⁵ cells	48.36 ± 6.7	1.86

Compatibility with downstream applications

Restriction enzyme digestion

The purity and concentration of gDNA isolated from mammalian tissues using illustra tissue & cells genomicPrep Mini Spin Kit enables its direct use in restriction enzyme digestions. Tests with several enzymes, including HindIII, EcoRI, and BamHI, demonstrated that purified gDNA from animal tissues was free from restriction enzyme inhibitors. Results for HindIII with gDNA isolated from rat liver tissue are shown in Figure 3.

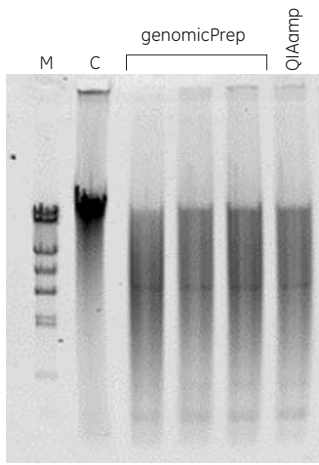


Fig 3. HindIII digest of gDNA. Purified gDNA from the illustra tissue & cells genomicPrep Mini Spin Kit and QIAamp DNA Mini Kit was cut with HindIII. M = Lambda-HindIII molecular weight marker; C = uncut gDNA purified with illustra tissue & cells genomicPrep Mini Spin Kit.

Genomic DNA isolated from cultured cells is also suitable for use in restriction enzyme digestions. In a comparison with gDNA isolated from CHO cells using either the illustra tissue & cells genomicPrep Mini Spin Kit or the QIAamp DNA Mini Kit, gDNA purified from the illustra kit was readily digested, whereas the Qiagen purified DNA inhibited HindIII restriction enzyme activity (Fig 4). The inability to remove RNA (despite RNase treatment) with the Qiagen kit can result in restriction digest failures. RNase A treatment with the illustra kit leaves purified DNA free of such inhibitors that may copurify with RNA.

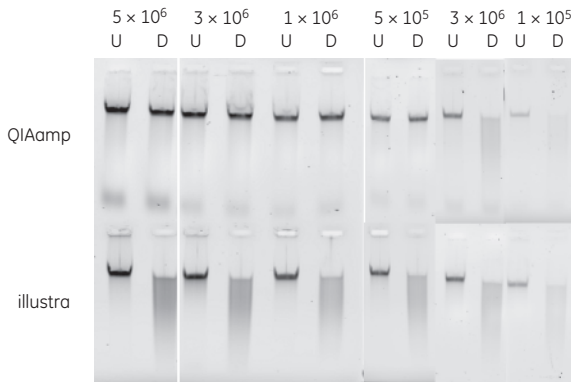


Fig 4. HindIII restriction enzyme digest of gDNA purified from CHO cells using the QIAamp DNA Mini Kit (top gel) and the illustra tissue & cells genomicPrep Mini Spin Kit (bottom gel). Each digestion reaction contained 20 μ l gDNA, and digests were analyzed on 0.8% agarose gel. U = uncut DNA (no enzyme); D = gDNA digested with HindIII. Numbers represent cell amounts from which gDNA was isolated.

Real-time PCR

DNA purified using the illustra tissue & cells genomicPrep Mini Spin Kit performs effectively in quantitative real-time PCR. The sensitivity of gDNA purified using the illustra tissue & cells genomicPrep Mini Spin Kit was compared to gDNA purified using the QIAamp DNA Mini Kit by performing quantitative real-time PCR analysis. The gDNA isolated from rat liver tissue from both kits amplified identical to the standard rat gDNA control, indicating that both samples were free from PCR inhibitors. The sensitivity of gDNA produced by both kits was found to be identical based on Ct values (Fig 5).

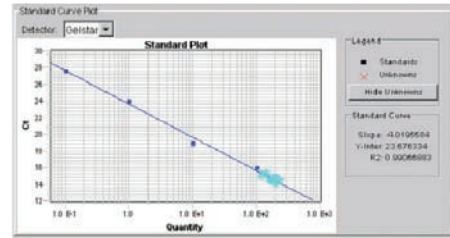


Fig 5. Real-time PCR analysis of gDNA purified from rat liver tissue. Amplification of rat GAPDH gene was performed using 100 ng gDNA purified using the QIAamp DNA Mini Kit (n=6) and the illustra tissue & cells genomicPrep Mini Spin Kit (n=12). The standard curve was generated with 0.1-100 ng of commercially available (Bioline) rat gDNA. The average Ct value for the illustra kit was 14.64 ± 0.38 , and for the QIAGEN kit, it was 14.8 ± 0.49 .

Similar results were found for gDNA purified from cell lines; the Ct values for gDNA from HK293 cells used in PCR was identical for both the illustra tissue & cells genomicPrep Mini Spin Kit and the QIAamp DNA Mini Kit (Fig 6).

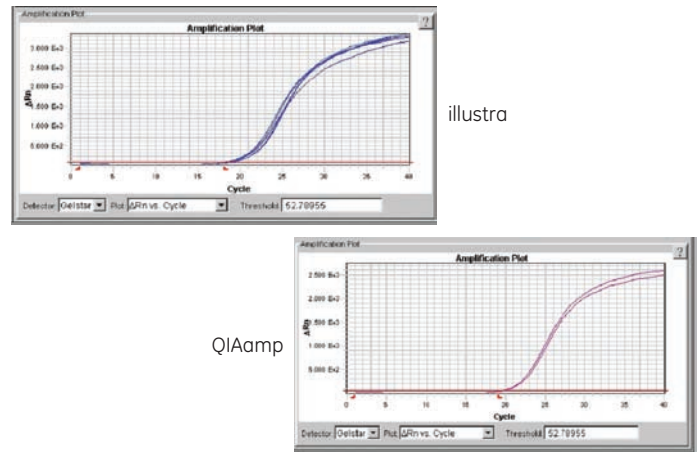


Fig 6. Real-time PCR of gDNA purified from HK293 cells. Amplification reactions used 100 ng gDNA produced by the QIAamp DNA Mini Kit and the illustra tissue & cells genomicPrep Mini Spin Kit.

Summary

The illustra tissue & cells genomicPrep Mini Spin Kit rapidly isolates gDNA from a variety of animal tissues and mammalian cell cultures. For tissues, the total time to extract gDNA is less than 90 min, half the time required with the QIAamp DNA Mini Kit. Genomic DNA purifications performed with the illustra tissue & cells genomicPrep Mini Spin Kit yield consistent results and are highly robust across different sample types. The intact and highly pure gDNA can be used directly in restriction digestion or real-time PCR.

References:

1. Vogelstein, B. and Gillespie, D. Preparative and analytical purification of DNA from agarose, *Proc. Natl. Acad. Sci. USA* **76**, 615-619 (1979).

Ordering information

illustra tissue & cells genomicPrep Mini Spin Kit (50 preps)	28-9042-75
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