illustra tissue & cells genomicPrep Midi Flow Kit

Introduction

The illustra™ tissue & cells genomicPrep Midi Flow Kit is designed for high-yield extraction and purification of genomic DNA (gDNA) from tissue samples ranging in size from 50 to 200 mg. With the kit, gDNA can be obtained in less than 3 h using columns that have been designed to accommodate both gravity- and spin-based methods. Resultant gDNA is of high molecular weight, yield, and purity. The purified gDNA is suitable for use in downstream applications such as cloning, PCR, restriction enzyme digestion, and DNA sequencing.

illustra tissue & cells genomicPrep Midi Flow Kit delivers:

- **High yields:** Isolates up to 20% more gDNA than Qiagen™ Genomic-tip 100/G (in comparative tests with identical sample inputs) and accepts up to twice as much input material enabling yields exceeding 250 µg.
- **High quality:** Optimized protocol produces intact gDNA that is > 48 kb in size (Fig 1) for use in a wide range of applications, including long PCR.
- **Dependable flow rates:** Fast Flow purification medium minimizes column clogging for higher sample throughput.
- **Simpler purification:** Columns are pre-equilibrated for immediate 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.
- **Fast results:** Optimized lysis procedure and final desalting without precipitation saves hours from prep time; centrifugation protocol provides even faster results.

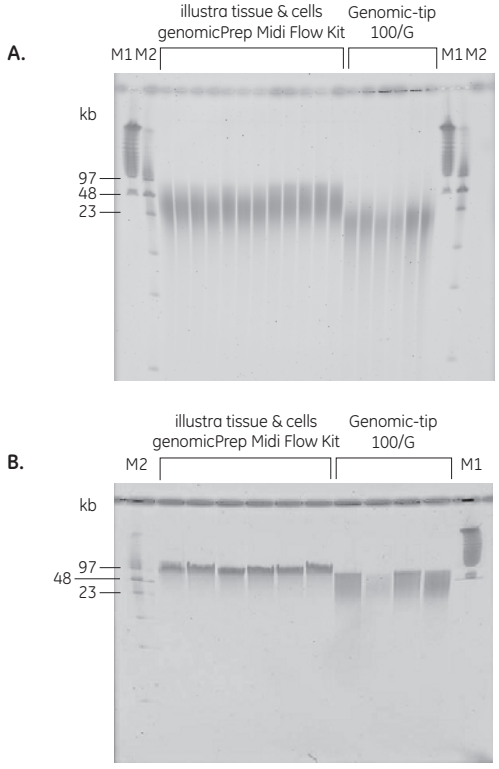


Fig 1. (A). Sizing by pulsed-field gel electrophoresis (PFGE) of gDNA purified from rat liver tissue. Genomic DNA was isolated from 80 mg rat liver using either the illustra tissue & cells genomicPrep Midi Flow Kit or the Genomic-tip 100/G from Qiagen. (B) Sizing by PFGE of gDNA purified from Chinese hamster K1 cells. Genomic DNA was isolated from using either the illustra tissue & cells genomicPrep Midi Flow Kit or the Genomic-tip 100/G from Qiagen. M1 = lamda PFGE marker; M2 = Low range molecular weight PFGE marker. Genomic DNA was isolated according to manufacturers' instructions. Genomic DNA (250 ng) was loaded for all samples.

Method overview

The illustra tissue & cells genomicPrep Midi Flow Kit uses a simple and efficient three-stage process that employs an optimized lysis procedure, gDNA purification by anion exchange chromatography, and a trouble-free desalting process.

The lysis procedure is performed in a single step and is completed in about two hours. RNase A may be used to remove RNA contamination following lysis.

Genomic DNA purification is achieved using Fast Flow Genomic 250 columns, which contain a Fast Flow anion exchange chromatography medium with excellent flow characteristics, exceptional chemical and pH stability, and a high capacity for biomolecules. Genomic DNA binds to the medium in high-salt concentrations that prevent the binding of potential impurities. The DNA can subsequently be eluted in buffers containing higher salt concentrations.

The Fast Flow Genomic 250 columns are pre-equilibrated and designed for both gravity flow and centrifugation processes. The buffer used for pre-equilibration has been optimized to prevent the majority of impurities from binding to the medium, leading to higher purity and yield of gDNA. Pre-equilibrated columns circumvent the need for an equilibration step, which in conjunction with the excellent flow characteristics, facilitate purification, resulting in reduced hands-on and total processing time.

Desalting is performed using Sephadex™ G-25 DNA grade medium. The Sephadex G-25 column provides a simple and rapid desalting process that removes > 99% of the salt from the purified gDNA solution. This process is highly reproducible and can be completed in approximately 15 min. This method alleviates the need to perform isopropanol precipitation, which requires substantially longer time (3 to 4 h) and yields results that can be highly variable.

The method is summarized in Figure 2 with specifications shown in Table 1.

Table 1. Specifications for illustra tissue & cells genomicPrep Midi Flow Kit

Feature	Specification	
Sample type	Animal tissue	Cultured mammalian cells
Sample input size	< 200 mg of animal tissue	< 2.0×10^7 cultured cells
Binding capacity	> 250 µg	> 250 µg
Yield	100 to 135 µg[1]	80 to 100 µg[2]
Purity (A_{260}/A_{280})	> 1.7	> 1.7
Time/prep (sample to desalting)	< 3 h	< 4 h
Product size	> 20 kb	> 20 kb
Elution volume	3.5 ml	3.5 ml

[1] Values shown derived from 80 mg rat liver samples; actual yields will vary depending on tissue type used.

[2] Values shown derived from 2.0×10^7 MRC5 cells; actual yields will vary depending on cell type used.

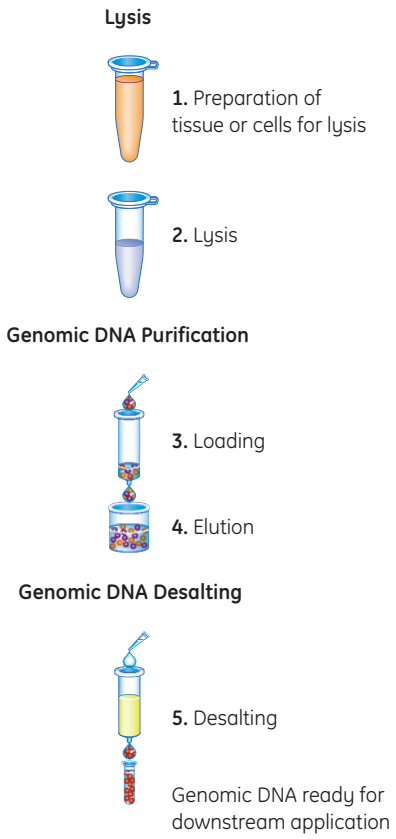


Fig 2. Schematic representation of the three-stage (lysis, purification, desalting) gDNA isolation protocol employed by the illustra tissue & cells genomicPrep Midi Flow Kit.

High yields with high quality

The high capacity of the Fast Flow Genomic 250 column combined with the optimized protocol enable the illustra tissue & cells genomicPrep Midi Flow Kit to produce high yields of gDNA from both tissues and cells. In comparative tests using 80 mg rat liver tissue, the illustra tissue & cells genomicPrep Midi Flow Kit produced an average yield of 121.3 µg gDNA compared to an average yield of 88.8 µg gDNA using Genomic-tip 100/G from Qiagen. For cells, yields from a sample input of 2×10^7 cells ranged from 80 to 100 µg for MRC5 and HK293 cells to 40 to 60 µg for CHO K1 cells. Table 2 summarizes the comparative yield and purity data obtained for the illustra tissue & cells genomicPrep Midi Flow Kit and Genomic-tip 100/G.

The illustra tissue & cells genomicPrep Midi Flow Kit yields high-quality gDNA from tissues. The purified gDNA was largely intact with sizes larger than 48 kb. In a comparison with Genomic-tip 100/G from Qiagen, the purified gDNA obtained using illustra blood genomicPrep Midi Flow Kit was of larger size with less shearing (Fig 1A).

The illustra tissue & cells genomicPrep Midi Flow Kit also produces intact, well-sized gDNA from cultured cells (Fig 1B). The purified gDNA exhibited sizes over 48 kb with practically no shearing compared with gDNA obtained with the Genomic-tip 100/G.

The purity of the gDNA isolated using illustra tissue & cells genomicPrep Midi Flow Kit was also high. For rat liver tissue, the kit delivered a purity of 1.76; the Genomic-tip 100/G produced gDNA with a purity of 1.79. Purity for gDNA derived from various cell lines, including CHO K1, MRC5, and 293K cells, ranged from 1.7 to 1.85. A purity ratio of 1.7 to 1.9 indicates that the gDNA is pure for all standard molecular biology applications.

Table 2. Comparative yields and purities for gDNA obtained with the illustra tissue & cells genomicPrep Midi Flow Kit and the Genomic-tip 100/G from Qiagen[1]

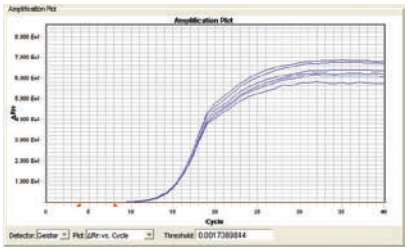
Kit	Yield (µg) ± sd	Purity (A_{260}/A_{280}) ± sd
Illustra tissue & cells genomicPrep Midi Flow Kit	121.3 ± 14.0	1.76 ± 0.02
Genomic-tip 100/G	88.8 ± 8.0	179 ± 0.06

[1] Comparison was performed using 80 mg rat liver according to manufacturers' instructions. n = 12 for illustra tissue & cells genomicPrep Midi Flow Kit; n = 6 for Genomic-tip 100/G.

Compatibility with downstream applications

Real-time PCR

Several gDNA samples isolated from rat liver tissue were evaluated in real-time PCR experiments (Fig 3). All samples displayed very similar amplification curves, demonstrating the reproducible quality of the isolated gDNA and its compatibility for use in real-time PCR applications.



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Genomic-tip 100/G

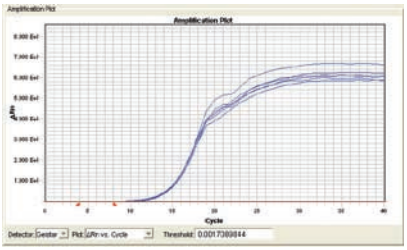

Fig 3. Real-time PCR amplification from gDNA extracted from 80 mg rat liver using illustra tissue & cells genomicPrep Blood Midi Flow Kit and Genomic-tip 100/G from Qiagen. Genomic DNA was isolated according to manufacturers' instructions. Genomic DNA (100 ng) was used as template. Primers for glyceraldehyde-3-phosphate dehydrogenase were used.

Restriction enzyme digestion

The purity and concentration of gDNA isolated from tissues using illustra tissue & cells genomicPrep Midi Flow Kit enables its direct use in restriction enzyme digestions. Tests with 10 restriction enzymes (AseI, BamHI, BglII, EcoRI. HindIII, HincII, NcoI, PstI, SacI, and XbaI) demonstrated that the purified DNA was free from restriction enzyme inhibitors. Results for EcoRI are shown in Figure 4.

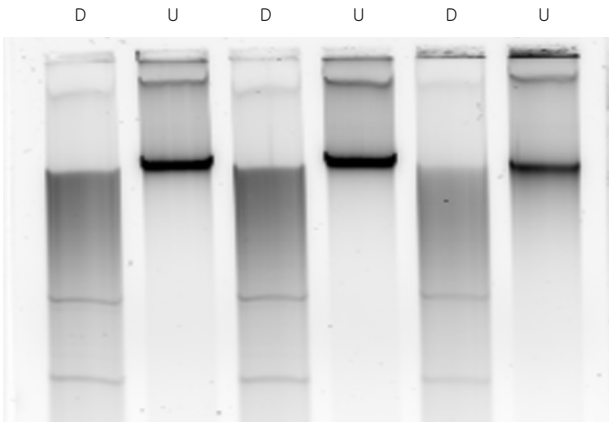

Fig 4. EcoRI digest of gDNA obtained from rat liver with the illustra tissue & cells genomicPrep Midi Flow Kit. U = uncut DNA (no enzyme); D = gDNA digested with EcoRI.

Summary

The illustra tissue & cells genomicPrep Midi Flow Kit uses a convenient and efficient protocol to routinely deliver high yields (100 to 135 µg) of high-quality intact gDNA. The gentle lysis conditions yield gDNA that is > 48 kb in size and largely intact. The kit features a simplified protocol that saves time at the lysis and desalting stages. The purified gDNA can be used directly in downstream applications including restriction digestion and real-time PCR.

Ordering information

illustra tissue & cells genomicPrep Midi Flow Kit (25 preps)	28-9042-73

Asia Pacific: Tel: +65 6275 1830 Fax: +65 6275 1829 **Australasia** Tel: + 61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01/57606-1619 Fax: 01/57606-1627 **Belgium** Tel: 0800 73 888 Fax: 02 416 82 06 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, & South East Europe** Tel +43 1 972720 Fax: +43 1 97272 2750 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Eire** Tel: 01494 544000 Fax: 0044 1494 542010 **Finland & Baltics** Tel: +358-(0)9-512 39 40 Fax: +358 (0)9 512 39 439 **France** Tel: 01 6935 6700 Fax: 01 6941 9677 **Germany** Tel: (089) 96281 660 Fax: (089) 96281 620 **Greater China** Tel: +852 2100 6300 Fax: +852 2100 6338 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: +81 3 5331 9336 Fax: +81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7304 **Middle East & Africa** Tel: +30 210 9600 687 Fax: +30 210 9600 693 **Netherlands** Tel: 0800 82 82 82 1 Fax: 0800 82 82 82 4 **Norway** Tel: 815 65 555 Fax: 815 65 666 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S** Tel: +7 (495) 956 5177 Fax: +7 (495) 956 5176 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 1990 Fax: 018 612 1910 **Switzerland** Tel: 0848 8028 12 Fax: 0848 8028 13 **UK** Tel: 0800 616928 Fax: 0800 616927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

www.gelifesciences.com/illustra

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

imagination at work

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala
Sweden

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan