Sera-Xtracta™ HMW DNA Kit

HIGH YIELD EXTRACTION AND PURIFICATION OF DNA FROM BLOOD, BUFFY COAT, SALIVA, CULTURED CELLS AND TISSUE SAMPLES

The Sera-Xtracta™ High-Molecular-Weight (HMW) DNA Kit is designed for reproducible extraction of genomic DNA (gDNA) from a wide range of sample types (Table 1), including:

- Whole blood (treated with citrate, heparin or Ethylenediaminetetraacetic acid (EDTA)
- Buffy coat
- Saliva
- Cultured mammalian cells
- Solid tissue samples

The kit is powered by SeraSil-Mag™ silica-coated magnetic beads and also includes optimized buffers and Proteinase K for efficient genomic DNA extraction from different samples, following a simple lyse, bind, wash and elute protocol. The extraction protocols are designed to efficiently remove contaminants and PCR inhibitors while minimizing shearing, resulting in high quality high-molecular-weight genomic DNA with minimal RNA carryover. The kit is optimized for processing 50 µL to 2 mL input volumes to yield high-molecular-weight genomic DNA with a high purity and quality that is compatible with molecular biology techniques, including cloning, restriction enzyme digestion, PCR amplification, genotyping applications and next-generation sequencing (NGS).

Fig 1. The Sera-Xtracta™ HMW DNA Kit for the extraction and purification of genomic DNA includes silica-coated magnetic beads, Proteinase K, lysis buffer, binding buffer, two wash buffers, and gDNA elution buffer.

The Sera-Xtracta™ HMW DNA Kit (Product code: **29429140**) contains enough reagents for 96 purifications when processing sample volumes up to 200 µL. The kit allows for scaling up the sample input volume to 2 mL*.

*Scaling up sample volumes will affect the number of reactions per kit.

Table 1. Recommended sample input and expected yields from different sample types when using a 200 µL sample input protocol†. Average yield for all samples (excluding tissue samples) was measured using Qubit™ dsDNA BR assay (Invitrogen). Tissue samples were measured using the NanoPhotometer™ NP80 Touch (Implen)

Sample	Recommended sample input amount	Average yield (µg)
Whole blood	200 µL	4.5
Saliva		
• Frozen saliva	200 µL	4.1
• Saliva stored in Oragene™ collection kit[1] (DNA Genotek)	200 µL	2.7
Buffy coat[2]	Up to 6.6 × 10^6 cells	34.7
Cultured mammalian cells[3]		
• U-2 OS (human osteosarcoma) cells	Up to 1 × 10^6 cells	12.4
• A451 (human squamous carcinoma) cells	Up to 1 × 10^6 cells	17.7
Solid tissue		
• Fresh mouse kidney	17–25 mg	65.0
• Frozen mouse kidney	17–25 mg	54.6
• Fresh mouse lung	17–25 mg	38.3
• Frozen mouse lung	17–25 mg	31.5
Elution volume[4]	100 µL	–
Typical purity (A_{260}/A_{280} and A_{260}/A_{230})	> 1.7	–
Typical product size[5]	> 60 kb	–

† Refer to Sera-Xtracta™ HMW DNA Kit user guide for details of 1 mL and 2 mL sample input protocols.

[1] Saliva collected and stored in the Oragene™ saliva self-collection collection kit (DNA Genotek, product code OG-500)

[2] Recommended input quantity for 200 µL scale. Note: input volume will depend upon cell count of the starting material. It is possible to process larger volumes of buffy coat using the 1 mL or 2 mL sample input protocols* if cell concentrations are low.

[3] Recommended input quantity for 200 µL scale. If genomic DNA content is greater than 6 pg per cell (as for some immortalised cell lines), reduce input cell number accordingly. Note: yields may vary according to cell type and growth state.

[4] Recommended elution volume: the end user has the option to vary this according to downstream application requirements.

[5] Data generated on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent) which only enables gDNA sizing up to 60 kb. Pulse field gel electrophoresis (PFGE) data is available for gDNA isolated from blood (page 5), demonstrating high-molecular-weight DNA sizes > 200 kb when using Sera-Xtracta™ HMW DNA Kit.

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Typical results from whole blood

Genomic DNA was purified from 200 µL of whole blood collected in EDTA blood collection tubes using the Sera-Xtracta™ HMW DNA Kit. Results are shown in Figures 2 and 3.

With multiple isolations carried out to determine reproducibility of DNA yield, quality and purity, the data presented here shows that all DNA samples isolated using the Sera-Xtracta™ HMW DNA Kit were of high quality and integrity, containing single high-molecular-weight DNA bands > 60 kb* (Fig 2A) and DIN values > 9.7. Extracts were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process.

* Refer to page 5 (Figs 8A and 8B) for pulse field gel electrophoresis data, demonstrating the Sera-Xtracta™ HMW DNA Kit is capable of isolating high-molecular-weight DNA > 200 kb.

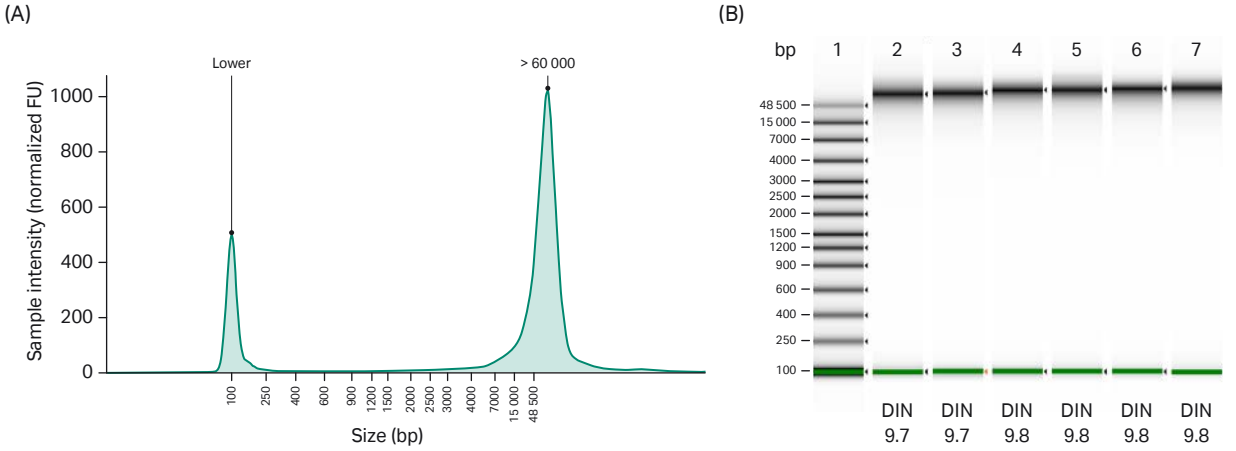

Fig 2. Analysis of genomic DNA purified from replicate 200 µL inputs of whole human blood and analyzed on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent). The samples can be visualized as (A) electropherogram and (B) gel image. Sample details: (1) Ladder (size in bp), (2 to 7) six gDNA samples extracted from replicate 200 µL inputs of whole blood. The DNA Integrity Number (DIN) is displayed under the individual gel lane in Fig 2B with a high DIN indicating highly intact gDNA, and a low DIN indicating strongly degraded gDNA.

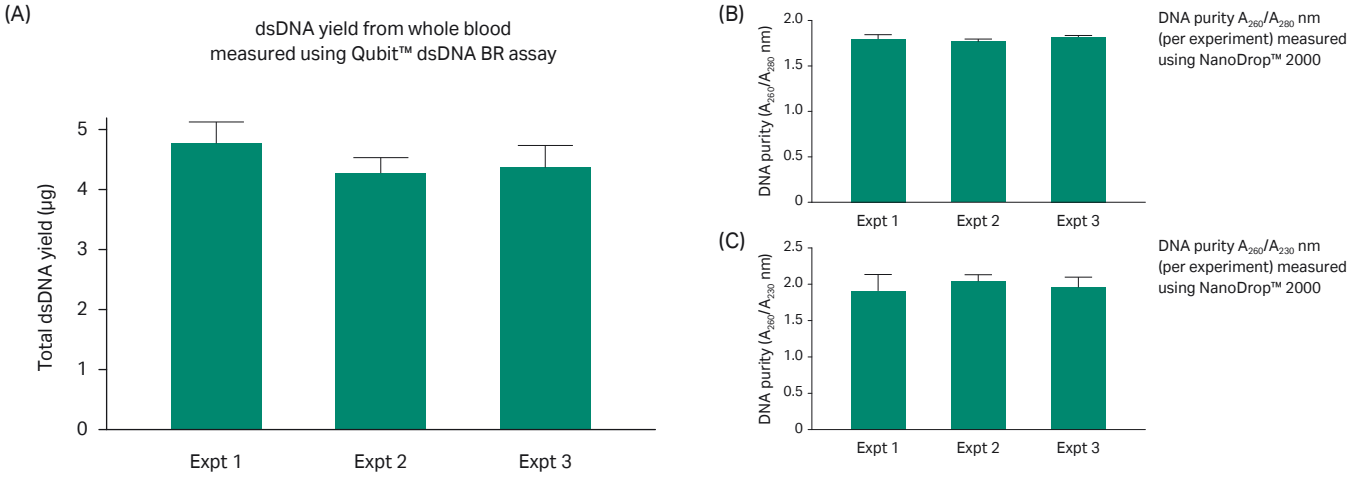

Fig 3. Yield and purity of genomic DNA isolated from replicate 200 µL inputs of whole blood stabilized with EDTA. (A) Yields were reproducible, with a mean of 4.5 µg dsDNA per isolation (Qubit™ dsDNA BR assay, Invitrogen). (B, C) Average A_{260}/A_{280} nm and A_{260}/A_{230} nm purity readings were 1.76 and 1.96 respectively (NanoDrop™ 2000, ThermoFisher Scientific), indicating high purity of eluted DNA. Graphs show three readings taken from three replicates (n = 3) for each experiment, error bars are +SD.

Flexible sample input volume

The Sera-Xtracta™ HMW DNA Kit provides robust chemistry for extracting DNA from different sample input volumes up to 2 mL to provide efficient scale-up and flexibility around sample availability and yield requirements. To demonstrate this, two experiments were performed: standard to low volume (200 µL – 50 µL) and standard to high volume (200 µL to 2 mL) to isolate genomic DNA from whole blood stabilized with EDTA.

Data demonstrates a linear correlation between the input volume and DNA yield, indicating that Sera-Xtracta™ HMW DNA Kit provides scalable DNA extraction from different volumes of whole blood.

Note: if the volume of the input sample is less than the required 200 µL, it can be made up to the required volume for extraction with a physiological buffer such as phosphate-buffered saline (PBS). However, this is not critical. For example, if the starting sample volume is lower than 200 µL, satisfactory results can be achieved using the 200 µL protocol without making the volume up to 200 µL with buffer.

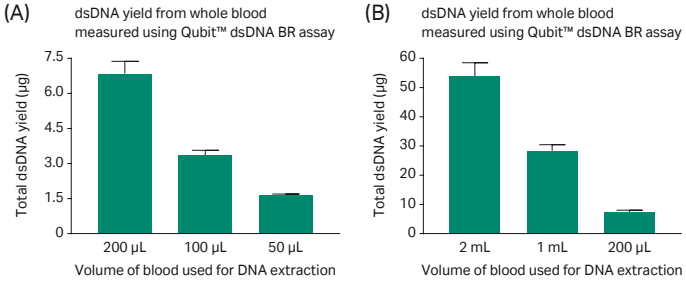

Fig 4. Yield of genomic DNA extracted from different input volumes of whole blood. (A) Samples processed using the Sera-Xtracta™ HMW DNA Kit standard 200 µL protocol, (B) Standard 200 µL, 1 mL and 2 mL protocol. Graphs show one reading taken from four replicates (n = 4), error bars are +SD. Average dsDNA yields in (A): 6.85 µg, 3.40 µg and 1.66 µg for 200 µL, 100 µL and 50 µL input respectively. Average dsDNA yields in (B): 7.33 µg, 28.33 µg and 53.87 µg for 200 µL, 1 mL and 2 mL input respectively (Qubit™ dsDNA BR assay, Invitrogen).

Comparison of automated DNA extraction process compared to MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher Scientific) using the KingFisher™ Duo Prime system (Thermo Fisher Scientific)

Sera-Xtracta™ HMW DNA Kit provides ease of use for both manual and automated setup. To demonstrate suitability of the protocol for an automated extraction, we developed (using the manual protocol) a Sera-Xtracta™ HMW DNA Kit script* for the KingFisher™ Duo Prime system, a magnetic bead-based automation platform (Thermo Fisher Scientific).

We compared the performance of the automated extraction from blood sample with the supplier's recommended protocol for the MagMAX™ DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific). The resulting purifications were analyzed for yield and purity.

DNA yields from the same blood sample processed on the KingFisher™ Duo Prime system (Thermo Fisher Scientific) were consistently higher using Sera-Xtracta™ HMW DNA Kit when compared with MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher Scientific). Purity of DNA samples were comparable for both kits (when run using their respective automation scripts).

*Automation scripts for processing samples on the KingFisher™ Duo Prime (Thermo Fisher Scientific) are available on request from Scientific Support (cytiva.com/support/contact-us).

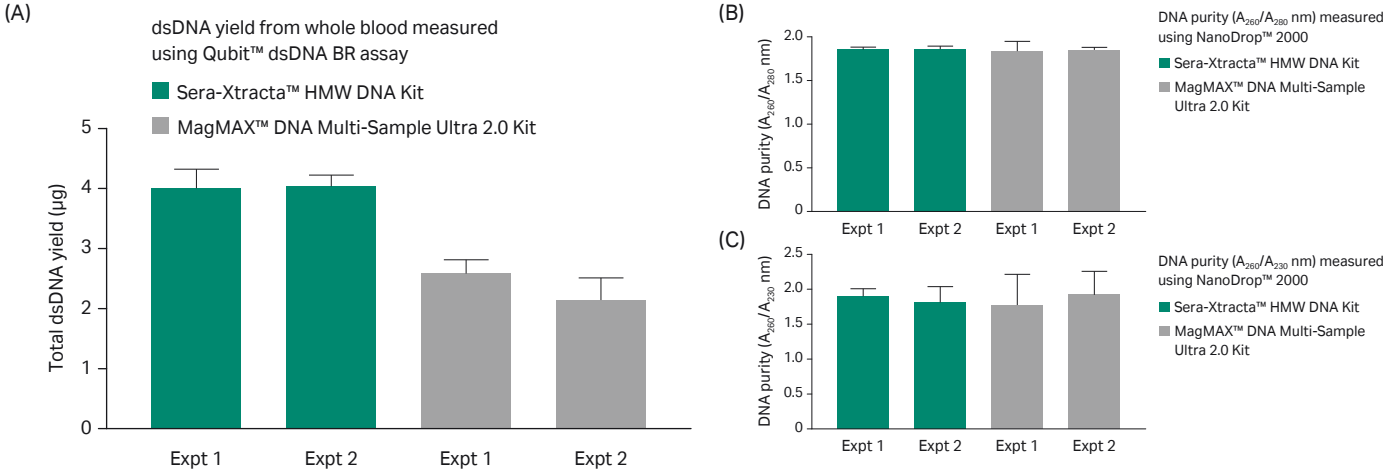

Fig 5. Yield and purity of genomic DNA from replicate 200 µL inputs of whole blood processed on the KingFisher™ Duo Prime system. Graph shows one reading taken from twelve replicates (n = 12) for each experiment, error bars are +SD. (A) dsDNA yield from whole blood measured using Qubit™ dsDNA BR assay. (B, C) DNA purity $A_{260/280}$ and $A_{260/230}$ were greater than 1.8 respectively.

Removal of PCR inhibitors from whole blood collected in EDTA, Heparin and Citrate tubes

Downstream applications such as real-time quantitative PCR (qPCR) and next-generation sequencing (NGS) are highly sensitive to the presence of inhibitors such as heme, anticoagulants, enzymes and divalent cations. To demonstrate the removal of such inhibitors, whole blood from a single donor was collected in EDTA, Heparin and Citrate blood collection tubes. We isolated DNA from 200 µL aliquots using the standard Sera-Xtracta™ HMW DNA Kit protocol. The purified DNA was serially diluted and subjected to real-time qPCR amplification using a kit containing a pre-formulated Internal PCR Control (IPC), designed to identify samples that contain PCR inhibitors (Quantifiler™ human DNA Quantification Kit, Thermo Fisher Scientific).

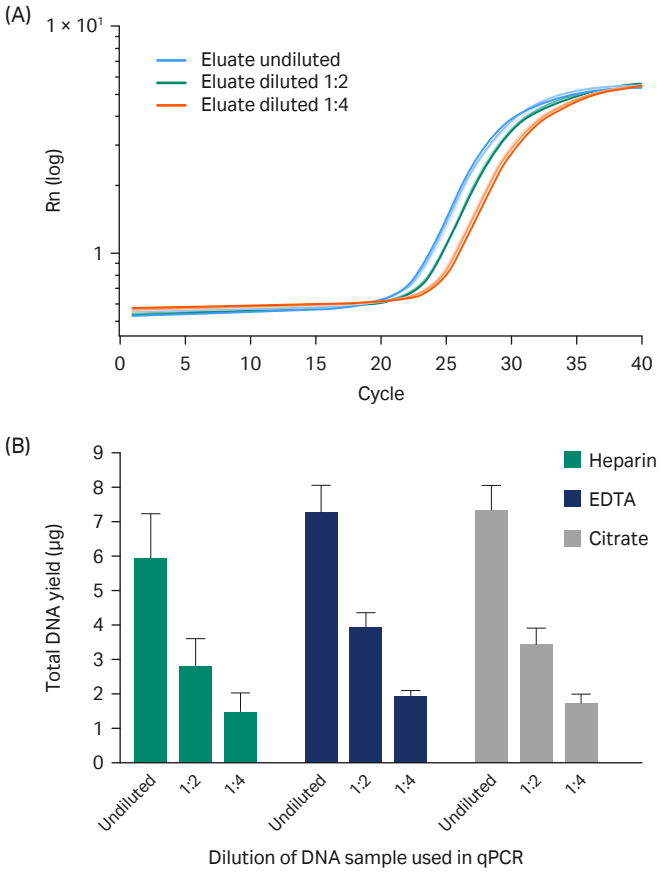

Fig 6. Real-time qPCR amplification of DNA isolated from whole blood stabilized with EDTA, heparin, and citrate. (A) Three sample input volumes were used in qPCR reactions. Amplification curves demonstrate a linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors. (B) Graph shows one reading taken from six replicates (n = 6) for each sample, error bars are +SD.

Isolated DNA was diluted, as indicated in Figure 6B, to enable different volumes of eluate to be used in qPCR reactions. There were no significant differences ($p>0.05$, one-way ANOVA) in Ct values obtained from undiluted eluates, diluted eluates (i.e. 1:2 and 1:4 dilutions) and no template controls (i.e., qPCR reactions containing no DNA eluate) for the Internal PCR Control (IPC) assay (data not shown).

Data shown here demonstrate a highly linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors in DNA isolated from whole blood collected in all three blood collection tubes.

Comparison versus MagAttract™ HMW DNA Kit (Qiagen), MagaZorb™ DNA Mini-Prep Kit (Promega) and MagMAX™ DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific)

We carried out all DNA purifications using 200 µL whole human blood in accordance with kit manufacturer's instructions. We analyzed yield and purity of genomic DNA using the NanoDrop™ 2000 (Thermo Fisher Scientific). Yield of double-stranded DNA was determined using the Qubit™ dsDNA BR assay (Invitrogen).

Fig 7A shows yield of double-stranded genomic DNA isolated from blood using the Sera-Xtracta™ HMW DNA Kit was higher when compared to the MagAttract™ HMW DNA Kit (Qiagen), MagaZorb™ DNA Mini-Prep Kit (Promega) and MagMAX™ DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific). Fig 7B shows purity (A_{260}/A_{230} nm ratios) of genomic DNA were consistently higher using the Sera-Xtracta™ HMW DNA Kit when compared to the MagAttract™ HMW DNA Kit (Qiagen) and the MagaZorb™ DNA Mini-Prep Kit (Promega). Fig 7C shows the A_{260}/A_{280} nm ratios were consistently higher than 1.70.

The highly pure DNA extracted using the Sera-Xtracta™ HMW DNA Kit is suitable for use in downstream applications, including quantitative PCR (Figs 6A and 6B) and next-generation sequencing (Figs 9A–9D and 10).

This data is based on a minimum of three independent experiments / replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol/recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during October and November 2019 and is held at this location.

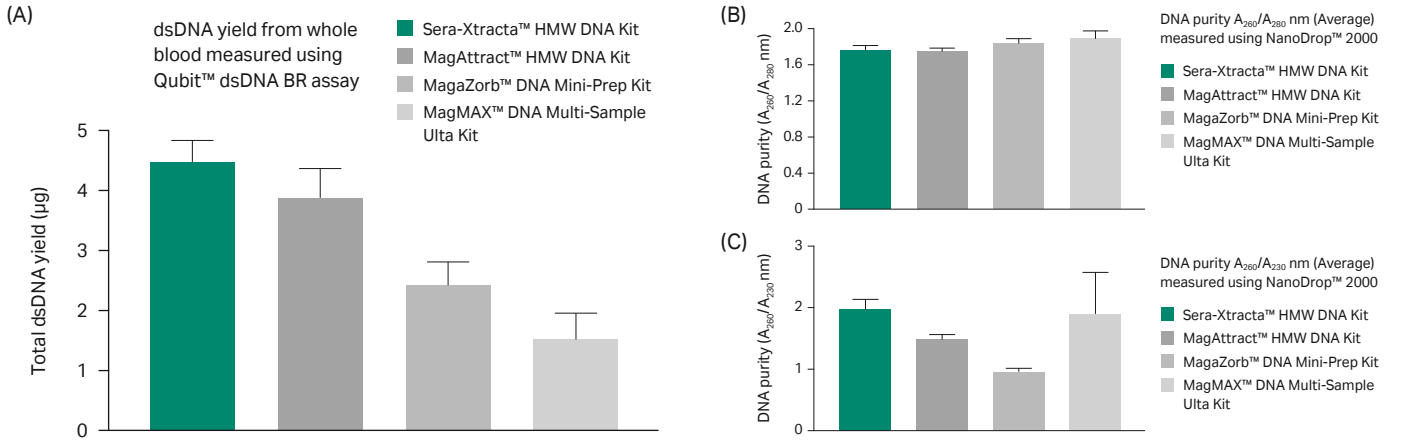

Fig 7. Double-stranded DNA yield and purity from three separate experiments. Graphs show one reading taken from nine replicates (n = 3 per experiment), error bars are +SD.

Pulse field gel electrophoresis

We determined the integrity of genomic DNA isolated from human whole blood (stabilized with EDTA) using the Sera-Xtracta™ HMW DNA Kit by pulse field gel electrophoresis (PFGE) (Fig 8A). The Sera-Xtracta™ HMW DNA Kit provides successful isolation of high quality and high-molecular-weight (> 200 kb) genomic DNA that would be suitable for third-generation sequencing (3GS). Recent advances in third-generation sequencing, including longer read lengths, higher throughputs and lower costs provide great benefits to genomics. Long reads contain more information compared to short reads, enabling genome assembly, detection of rare variants, transcript reconstruction, metagenomics and other emerging areas of modern biology and medicine.

The Sera-Xtracta™ HMW DNA Kit outperformed products from other suppliers and provided greater yields of high-molecular-weight DNA (size ranges 100 to 200 and > 200 kb) (Fig 8B). We quantified DNA size based on the MidRange PFG Marker (New England Biolabs, N0342S) run on the edges of the gel. We split the samples into four groups and measured the percent of total signal in each group. The groups were < 50 kb, 50 to 100 kb, 100 to 200 kb and > 200 kb. Data for < 100 kb size ranges are not shown.

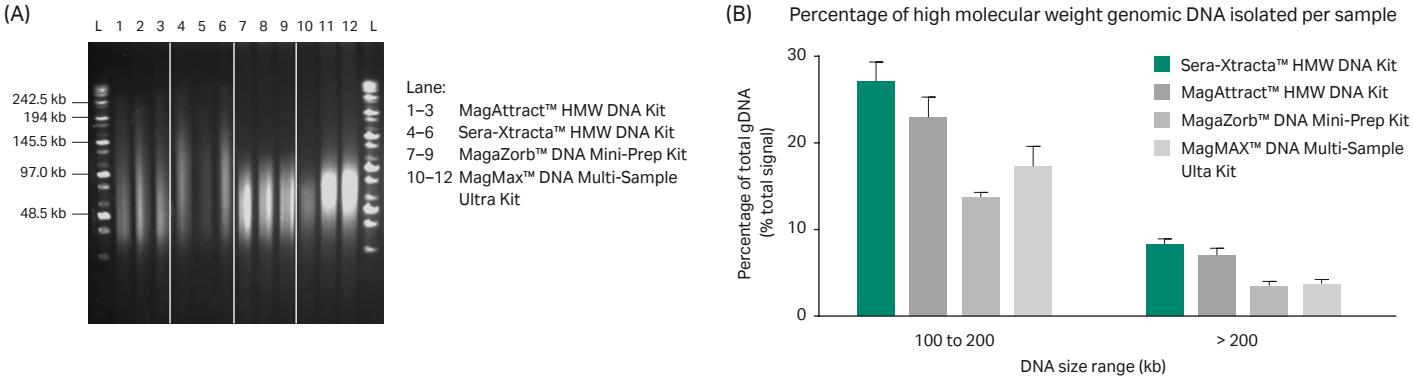

Fig 8. Pulse field gel electrophoresis (PFGE) of genomic DNA isolated from 200 µL whole human blood stabilized with EDTA. Purifications were carried out in accordance with kit manufacturer's instructions. (A) PFGE data showing isolation of high-molecular-weight DNA from three technical replicates. Sample details: L, MidRange PFG Marker (New England Biolabs, N0342S). Lanes 1 to 3, MagAttract™ HMW DNA Kit (Qiagen). Lanes 4 to 6, Sera-Xtracta™ HMW DNA Kit (Cytiva). Lanes 7 to 9, MagaZorb™ DNA Mini-Prep Kit (Promega). Lanes 10 to 12, MagMAX™ DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific). (B) Graph showing the percentage of high-molecular-weight genomic DNA isolated per sample (within size ranges: 100 to 200 kb and > 200 kb). Graph shows one reading taken from three replicates (n = 3) for each experiment; error bars are +SD.

Next-generation sequencing: Quality of sequencing reads from DNA libraries prepared from genomic DNA isolated through manual and automated extraction protocols

Yield of dsDNA and its integrity status are important quality metrics in any sequencing run. DNA samples isolated using the Sera-Xtracta™ HMW DNA Kit contained prominent DNA bands > 100 kb, while RNA and fragmented DNA were undetectable (Fig 8A). This demonstrates that the high-quality DNA obtained with the Sera-Xtracta™ HMW DNA Kit is suitable for use in NGS experiments.

DNA was isolated from 200 µL aliquots of whole blood using both the manual and automated Sera-Xtracta™ HMW DNA Kit protocols. Automated extraction was performed using the KingFisher™ Duo Prime System (Thermo Fisher Scientific). DNA libraries were then generated using the Nextera™ DNA Flex Library Prep Kit (illumina) and NGS was performed on the NextSeq™550 Sequencing System (illumina) according to the manufacturer's instructions.

Data from Figures 9 and 10 show that DNA extracted using both manual and automated protocols of the Sera-Xtracta™ HMW DNA Kit generated high quality libraries of similar complexity, with sequencing reads of comparable coverage.

Both sets of libraries produced reads with an average of 110.7 ± 8.2 million reads per library (Fig 9A). Reads were properly paired and mapped to unique positions on the human reference genome (Fig 10) with a low percentage of duplicates and an average of 87.1 ± 1.5% of the bases displaying a phred-score > Q30.

Reads were properly distributed among chromosomes, indicating good sequencing coverage. The coverage is almost identical among all the sequencing libraries (Fig 10). Overall, these results indicate that with both the manual and automated extraction protocols, the Sera-Xtracta™ HMW DNA Kit is robust enough to deliver high quality DNA which is suitable for sensitive applications such as NGS.

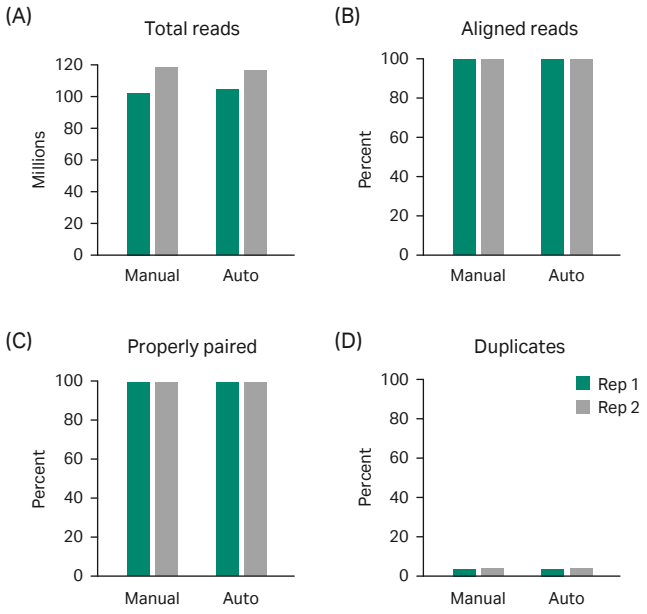

Fig 9. Comparison of alignment performance for libraries generated using the Nextera™ Flex Library Preparation kit (illumina) and input DNA isolated using manual and automated Sera-Xtracta™ HMW DNA Kit protocols. (A) Reads per library (B, C) High percentage of reads that were properly aligned reads to reference genome (D) Low percentage of duplicate reads.

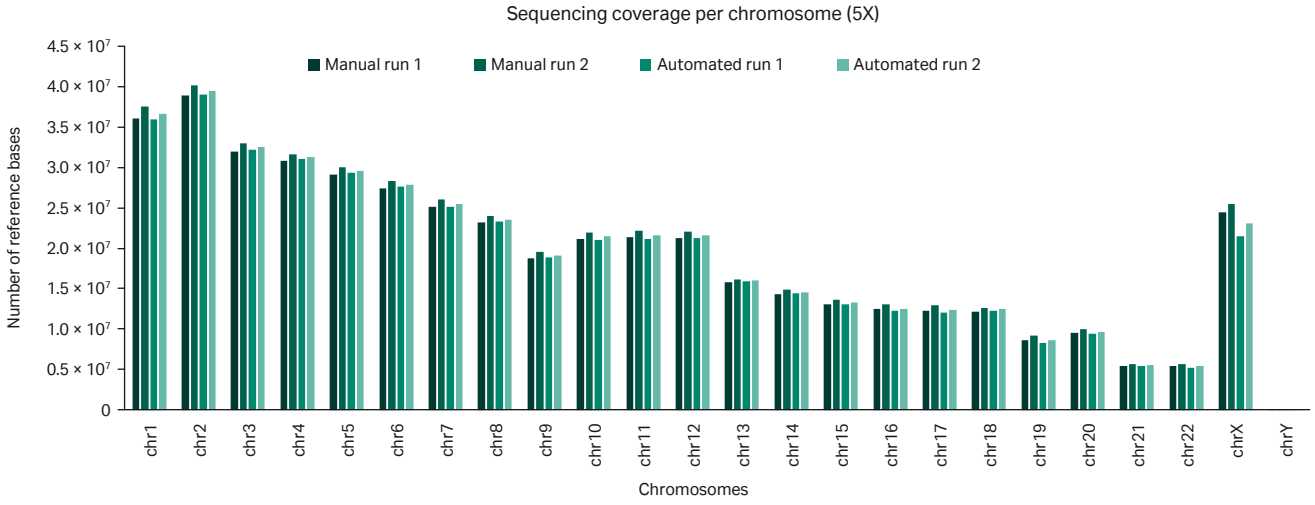

Fig 10. Sequencing coverage at individual chromosomes for libraries derived from input DNA isolated using manual and automated Sera-Xtracta™ HMW DNA Kit protocols.

Extraction of genomic DNA from buffy coat

To determine suitability of Sera-Xtracta™ HMW DNA Kit to extract DNA from buffy coat, we isolated genomic DNA from 200 µL aliquots of buffy coat (processed from fresh whole blood collected in EDTA collection tubes) using the standard 200 µL sample input protocol in the Sera-Xtracta™ HMW DNA Kit user guide. Prior to extraction, we diluted the buffy coat to provide a range of cell concentrations from 0.75 × 10^6 to 6.6 × 10^6 cells, and we carried out multiple isolations to determine reproducibility of DNA quality, yield and purity.

All DNA samples isolated using the Sera-Xtracta™ HMW DNA Kit were of high quality and integrity, containing single high-molecular-weight DNA bands and DIN values > 9.0. The gel image indicates that all extracted gDNA samples were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process. Yields of genomic DNA isolated from buffy coat samples were reproducible and demonstrated an approximate linear correlation between cell quantity and DNA yield. The average yield across all samples was 5.45 µg dsDNA per 1 × 10^6 cells (SD = 0.321) for input quantities ranging from 0.75 to 6.6 × 10^6 cells per extraction (Qubit™ dsDNA BR assay, Thermo Fisher Scientific). Eluted DNA was highly pure with an average A_{260}/A_{280} nm of 1.88 (SD = 0.021) and A_{260}/A_{230} nm of 2.05 (SD = 0.309) (NanoDrop™ 2000, Thermo Fisher Scientific).

Note: maximum bead capacity was exceeded when number of cells used per extraction increased above 6.6 × 10^6 (~35 µg). For processing higher input quantities of cells, isolate DNA using a 1 mL sample input protocol (refer to Sera-Xtracta™ HMW DNA Kit user guide for details).

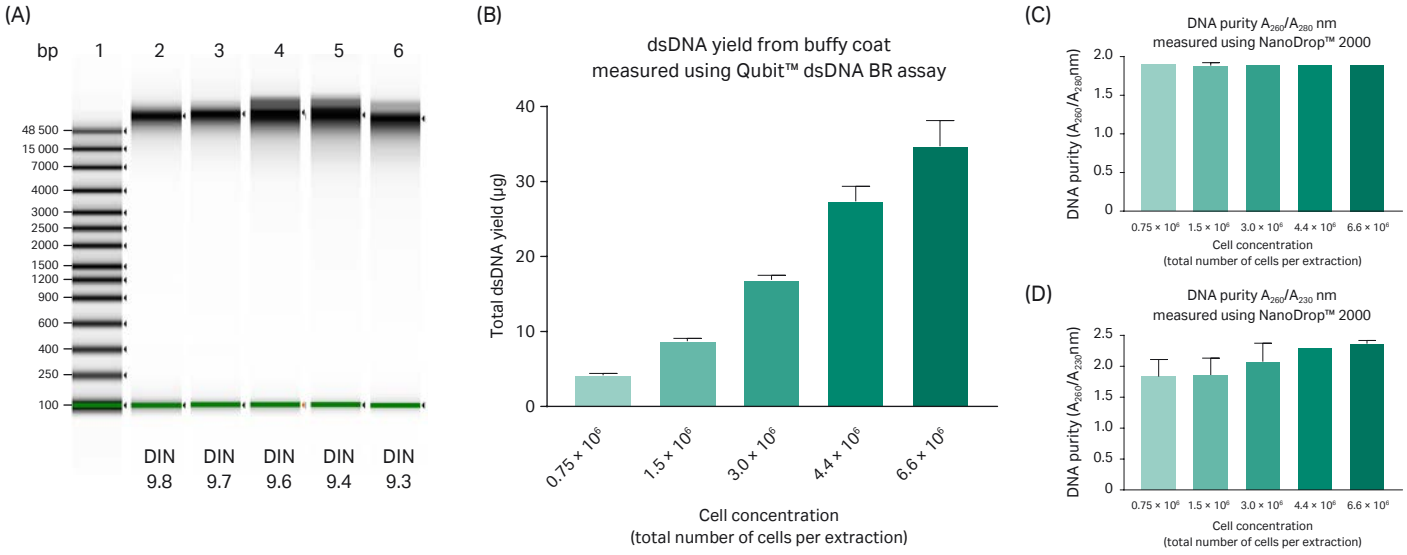

Fig 11. gDNA samples purified from five different concentrations of buffy coat samples. (A) Gel images of samples analyzed on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent). Sample details: (1) Ladder (size in bp), (2) 0.75 × 10^6 cells per extraction, (3) 1.5 × 10^6 cells, (4) 3.0 × 10^6 cells, (5) 6.6 × 10^6 cells and (6) 4.4 × 10^6 cells. The DIN is displayed under the individual gel lane. A DIN range is between 1–10, with a high DIN indicating highly intact gDNA, and a low DIN a strongly degraded gDNA. (B) dsDNA yield from buffy coat, measured using Qubit™ dsDNA BR assay. (C) DNA purity A_{260}/A_{280} nm measured using NanoDrop™ 2000. (D) DNA purity A_{260}/A_{230} nm measured using NanoDrop™ 2000. (B, C, D) Graphs show three readings taken from three technical replicates (n = 9) for each experiment; error bars are +SD.

Extraction of genomic DNA from saliva

We extracted genomic DNA from 200 µL and 2 mL saliva samples using the standard 200 µL or 2 mL sample input protocols in the Sera-Xtracta™ HMW DNA Kit user guide. Saliva was collected from healthy adult donors, pooled and stored as follows prior to gDNA isolation: (1) frozen saliva: 2 mL aliquots of saliva were immediately frozen at -80°C. When ready to use, aliquots were thawed on ice. (2) Oragene™ saliva self-collection kit (DNA Genotek): saliva samples were collected according to the manufacturer's instructions and incubated overnight at 25°C prior to DNA isolation.

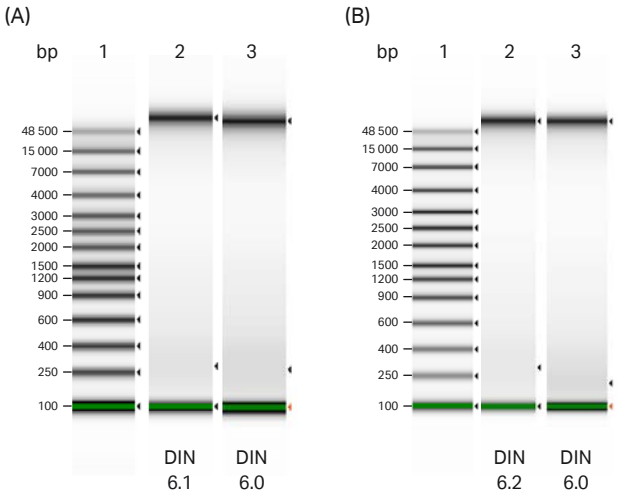

Fig 12. Genomic DNA purified from 200 µL and 2 mL input volumes of frozen saliva and analyzed on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent). The DIN is displayed under the individual gel lanes. (A) Sample details: (1) Ladder (size in bp), lanes 2, 3: 2 mL frozen saliva (B) Sample details: (1) Ladder (size in bp), lanes 2, 3: 200 µL frozen saliva. DIN range is between 1–10, with a high DIN indicating highly intact gDNA, and a low DIN a strongly degraded gDNA.

Data represented in Figure 12 and Table 2 shows DNA samples isolated from two different donor pools (processed using the standard 200 µL or 2 mL sample input protocols in the Sera-Xtracta™ HMW DNA Kit user guide) were of good quality and integrity, with up to 80% of DNA longer than 60 kb and DIN values ≥ 6.0. Extracts were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process.

Table 2. Data derived from three technical replicates for two experimental repeats (n = 6). Average yield and purity were determined using Qubit™ dsDNA BR assay (Invitrogen) and a NanoDrop™ Lite (Thermo Fisher Scientific) respectively

Sample type	Donor ID	Sample input volume*	Average dsDNA Yield (µg)	average A_{260}/A_{280} nm
Frozen saliva	Donor 1	200 µL	4.1	> 1.80
Frozen saliva	Donor 1	2 mL	22.7	> 1.80
Oragene™ Saliva Self-Collection Kit (DNA Genotek)	Donor 2	200 µL	2.7	> 1.70
Oragene™ Saliva Self-Collection Kit (DNA Genotek)	Donor 2	2 mL	17.3	> 1.70

*Refer to Sera-Xtracta™ HMW DNA Kit user guide for details of 1 mL and 2 mL sample input protocols.

Extraction of genomic DNA from solid tissues

We purified genomic DNA from 17–25 mg of fresh and frozen mouse kidney and lung tissue using the tissue protocol described in the Sera-Xtracta™ HMW DNA Kit user guide. For each experiment, we used three pieces of tissue from the same organ, and the experiment was repeated on two separate occasions (n=6) to determine reproducibility of DNA quality, yield and purity. Error bars are +SD.

All DNA samples isolated using the Sera-Xtracta™ HMW DNA Kit were of high quality and integrity, containing single high-molecular-weight DNA bands > 60 kb and DIN values > 8.8 (Fig 13A). Extracts were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process. Average DNA yield and DNA purity is indicated in Table 3, showing data derived from six biological replicates per tissue type, two experimental repeats (n = 6). DNA yield is without RNase treatment.

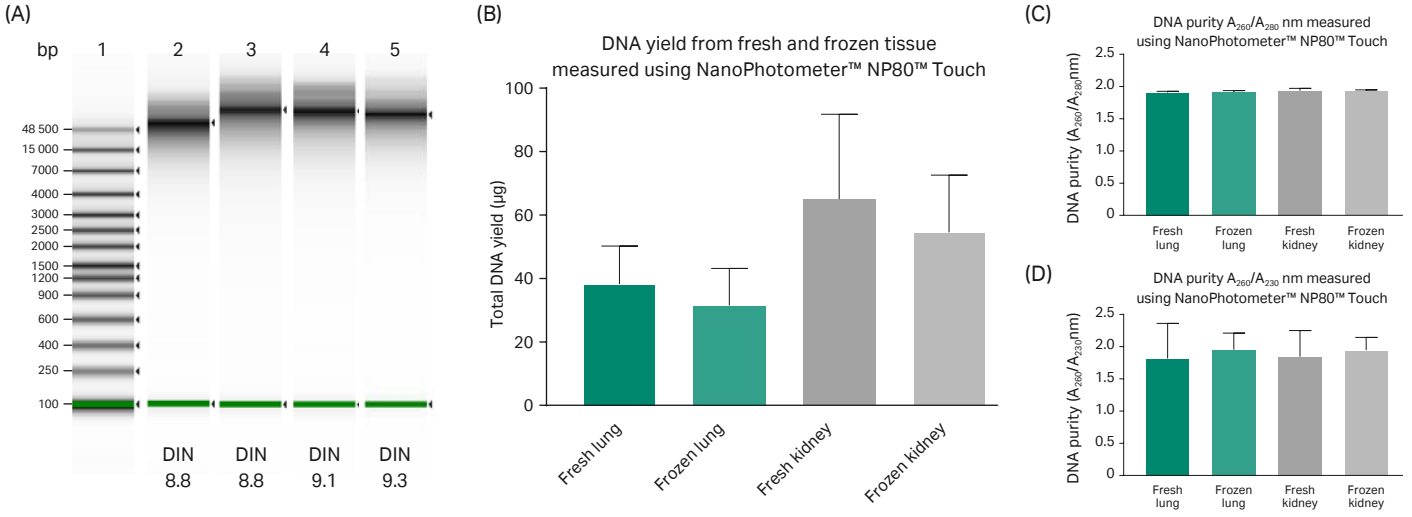

Fig 13. Examples of four gDNA samples extracted from fresh and frozen mouse tissue and analyzed on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent). Graphs show data from two separate experiments combined. Readings were taken from three biological replicates for each experiment (n = 6), error bars are +SD. Average yield and purity were determined using NanoPhotometer™ NP80 Touch (Implen™). (A) Gel image, sample details: (1) Ladder (size in bp), (2) frozen kidney, (3) fresh kidney, (4) frozen lung, (5) fresh lung. A high DIN indicates highly intact gDNA, and a low DIN a strongly degraded gDNA. (B) DNA yield from fresh and frozen tissue. (C) Genomic DNA purity (A_{260}/A_{280}) from fresh and frozen mouse lung and kidney tissue. (D) Genomic DNA purity (A_{260}/A_{230}) from fresh and frozen mouse lung and kidney tissue.

Table 3. Average DNA yield from fresh and frozen mouse lung and kidney tissue samples*

Tissue type	DNA yield (µg)	Average A_{260}/A_{280} nm	Average A_{260}/A_{230} nm
Fresh mouse lung	38	1.9	> 1.8
Frozen mouse lung	32	1.9	> 1.9
Fresh mouse kidney	65	1.9	> 1.8
Frozen mouse kidney	55	1.9	> 1.9

*Recommended input tissue quantity: 17–25 mg per extraction

Extraction of genomic DNA from cultured mammalian cells

We purified genomic DNA from 1 × 10^6 and 5 × 10^6 U-2 OS (human osteosarcoma) cells using the cultured cell protocol described in the Sera-Xtracta™ HMW DNA Kit user guide. Cultured cells were harvested and diluted to the desired concentration for isolation. We then extracted genomic DNA using the standard 200 µL and 2 mL sample input protocols (suitable for processing up to 1 × 10^6 cells or 5 × 10^6 cells per extraction* respectively).

* Recommended input quantity. If genomic DNA content is greater than 6 pg per cell (as for some immortalized cell lines), Reduce input cell number accordingly.

All DNA samples isolated using the Sera-Xtracta™ HMW DNA Kit were of high quality and integrity, containing single high-molecular-weight DNA bands with DIN values > 9.0. Extracts were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process. Yields of genomic DNA isolated from two concentrations of U-2 OS cells demonstrated an approximate linear correlation between cell number and dsDNA yield, with average A_{260}/A_{280} nm and A_{260}/A_{230} nm ratios above 1.9, indicating high purity of eluted DNA, as shown in Table 4 Data was derived from four technical replicates (n = 4). Average yield and purity were determined using Qubit™ dsDNA BR assay (Invitrogen) and NanoDrop™ 2000 (Thermo Fisher Scientific) respectively. Cells were processed using the standard 200 µL and 2 mL sample input protocols† in the Sera-Xtracta™ HMW DNA Kit user guide.

† Note: U-2 OS cells are chromosomally highly altered, with chromosome counts in the hypertriploid range (containing three sets of chromosomes), hence greater quantity of gDNA extracted from 1 × 10^6 cells than the expected 6 µg (assuming 6 pg gDNA per cell).

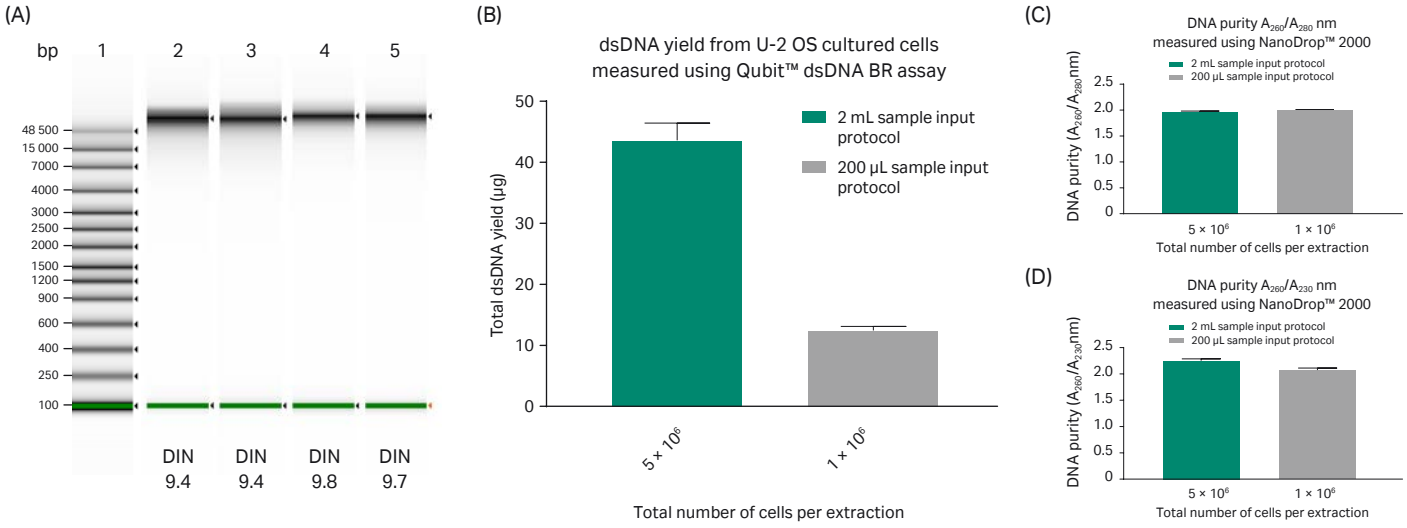

Fig 14. Replicate gDNA samples extracted from two different input quantities of U-2 OS (human osteosarcoma) cells and analyzed on the 4150 TapeStation™ system (Agilent) with the Genomic DNA ScreenTape™ assay (Agilent). Graphs show readings from four technical replicates (n = 4) for each experiment; error bars are +SD. (A) Gel image, sample details: (1) Ladder (size in bp), lanes 2, 3: 1 × 10^6 cells per extraction (processed using 200 µL sample input protocol), lanes 4, 5: 5 × 10^6 cells per extraction (processed using 2 mL sample input protocol). DIN is displayed under the individual gel lane. A high DIN indicates highly intact gDNA, and a low DIN indicates a strongly degraded gDNA. (B) dsDNA yield from U-2 OS cultured cells measured using Qubit™ dsDNA BR assay. (C, D) Purity of genomic DNA from U-2 OS (human osteosarcoma) cells measured using NanoDrop™ 2000.

Table 4. Average yield and purity of dsDNA extracted from U-2 OS (human osteosarcoma) cells

Sample input volume‡	Number of cells per extraction§	dsDNA yield (µg)	Average A_{260}/A_{280} nm	Average A_{260}/A_{230} nm
200 µL	1 × 10^6	12.45	> 1.9	> 1.9
2 mL	5 × 10^6	43.60	> 1.9	> 1.9

‡ Scaling up sample volumes will affect the number of reactions per kit. Refer to Sera-Xtracta™ HMW DNA Kit user guide for details of 1 mL and 2 mL sample input protocols.
§ Note: U-2 OS cells are chromosomally highly altered, with chromosome counts in the hypertriploid range (containing three sets of chromosomes), hence greater quantity of gDNA extracted from 1 × 10^6 cells than the expected 6 µg (assuming 6 pg gDNA per cell).

Conclusions

The Sera-Xtracta™ HMW DNA Kit is designed for efficient isolation of high-molecular-weight DNA from a wide range of sample types including whole blood, buffy coat, saliva, cultured mammalian cells and solid tissue. The kit uses magnetic bead-based technology. The protocol is flexible enough to allow purification of DNA from these sample types in less than 90 minutes, giving high yield and pure HMW DNA suitable for a range of molecular biology techniques including long-read sequencing. Recent advances in third-generation sequencing, including longer read lengths, higher throughputs and lower costs provide great benefits to researchers addressing challenges in genomics and other emerging areas of modern biology and medicine. The Sera-Xtracta™ HMW DNA Kit protocols are optimized to address the challenges of obtaining DNA of sufficient size, quantity and quality that is essential to long-read sequencing.

Ordering information

Product	Pack size	Product code
Sera-Xtracta™ HMW DNA Kit	96 purifications*	29429140

*Based on 200 µL sample input of blood

Related products	Pack size	Product code
Sera-Xtracta™ Cell-Free DNA Kit	96 purifications (2 mL input)	29437807
Sera-Mag™ Select	5 mL	29343045
	60 mL	29343052
	450 mL	29343057
RT-qPCR Kit	100 reactions	29639678
AIC Mix	100 reactions	29639678
Direct RT-qPCR Kit	100 reactions	29656615
Sera-Xtracta™ Virus/Pathogen Kit	96 extractions	29506009
	1000 extractions	29514201
PuReTaq™ Ready-To-Go™ PCR Beads	Multiwell plate, 96 reactions	27955701
	Multiwell plate, 5 × 96 reactions	27955702
	0.5 mL tubes, 100 reactions	27955801
	0.2 mL hinged tube with cap, 96 reactions	27955901
GenomiPhi™ V2 DNA amplification kit	100 reactions	25660031
	500 reactions	25660032
GenomiPhi™ Ready-To-Go™ V3 DNA amplification kit	96 reactions	25660196
	480 reactions	25660197
GFX™ PCR DNA and Gel Band Purification Kit	10 purifications	28903466
	100 purifications	28903470
	250 purifications	28903471
GFX™ 96 PCR Purification Kit	96 purifications	28903445
Blood genomicPrep Mini Spin Kit	10 purifications	28904263
	50 purifications	28904264
	250 purifications	28904265
Tissue and cells genomicPrep Mini Spin Kit	50 purifications	28904275
	250 purifications	28904276

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