Sera-Xtracta Genomic DNA Kit

HIGH YIELD EXTRACTION AND PURIFICATION OF GENOMIC DNA FROM WHOLE BLOOD

The Sera-Xtracta™ Genomic DNA Kit provides an effective solution for the extraction and purification of genomic DNA from whole blood. It addresses the growing demand to increase the throughput of nucleic acid purification in the lab by using faster, easy to handle, automated procedures that deliver reliable results. Sera-Xtracta Genomic DNA Kit (Fig 1) facilitates the transition from column purification to magnetic bead-based purification and provides high-purity extraction that enables low limits of detection. The kit yields genomic DNA with purity and quality that is compatible with most molecular biology techniques, including cloning, restriction enzyme digestion, polymerase chain reaction (PCR) amplification, genotyping applications and next-generation sequencing (NGS).

The Sera-Xtracta Genomic DNA Kit protocols minimize shearing, resulting in high quality intact genomic DNA. The procedure can be completed in less than 90 minutes and generates DNA yields of 4–8 µg from 200 µL sample input volume with a purity ratio (A_{260}/A_{280}) greater than 1.7 and minimal carryover of RNA. The kit is optimized for processing 50–200 µL of whole blood.

Fig 1. The Sera-Xtracta Genomic DNA Kit for the extraction and purification of genomic DNA from whole blood includes silica-coated magnetic beads, proteinase K, lysis buffer, binding buffer, two wash buffers, and gDNA elution buffer.

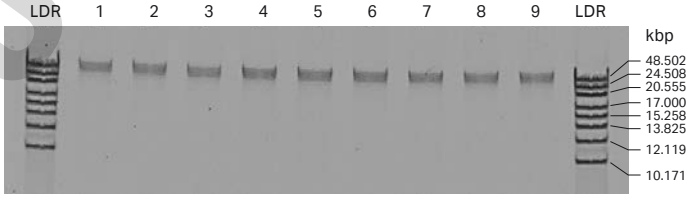

Fig 2. Gel electrophoresis of purified genomic DNA (0.8% agarose gel in 1X TAE buffer). LDR is the marker.

Typical results from whole blood

Genomic DNA was purified from 200 µL whole blood using the Sera-Xtracta Genomic DNA Kit. Samples were collected in EDTA collection tubes. Multiple isolations were carried out to determine reproducibility of DNA quality, yield and purity.

All DNA samples isolated using the Sera-Xtracta Genomic DNA Kit were of high quality, containing single prominent DNA bands ≥ 48 kb. Extracts were free from visible RNA contamination and smearing beneath the major genomic DNA band, demonstrating minimal fragmentation during the extraction process (Fig 2).

Yields of genomic DNA isolated from 200 µL whole blood, stabilized with EDTA, were very reproducible, with a mean of 4.5 µg dsDNA per isolation (Qubit™ dsDNA BR assay), as shown in Figure 3. $A_{260/280}$ nm of 1.76 and $A_{260/230}$ nm of 1.96 (NanoDrop™ 2000) readings indicate the high purity of the eluted DNA, as shown in Figure 4.

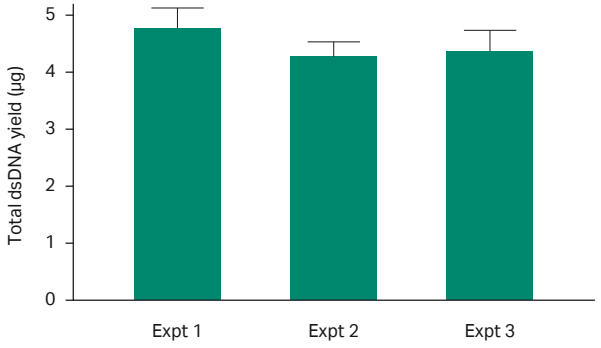

Fig 3. Yield of genomic DNA from replicate 200 µL inputs of whole blood. Graph shows one reading taken from three replicates (n = 3) for each experiment; error bars are +SD.

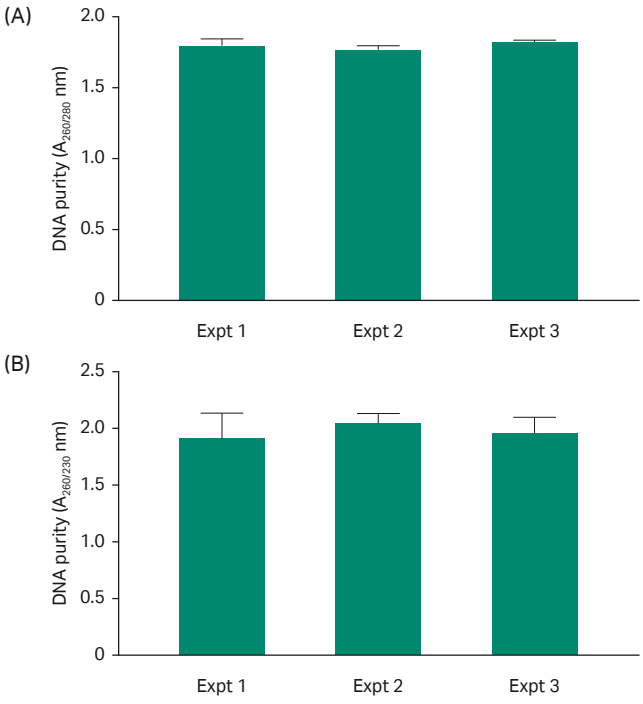

Fig 4. Purity of genomic DNA from replicate 200 µL inputs of whole blood. Graphs show 3 readings taken from three replicates (n = 9) for each experiment, error bars are +SD. (A) DNA purity $A_{260/280}$ nm; (B) DNA purity $A_{260/230}$ nm.

Scalability

The Sera-Xtracta Genomic DNA Kit has been optimized to accommodate different sample input volumes, ranging from 50 µL to 200 µL, to provide flexibility to accommodate sample availability and yield requirements.

The Sera-Xtracta Genomic DNA Kit provides scalable DNA extraction from different volumes of whole blood. Results demonstrate a linear correlation between the input volume and DNA yield. Average dsDNA yields: 200 µL, 6.85 µg; 100 µL; 3.40 µg. 50 µL, 1.66 µg (Fig 5).

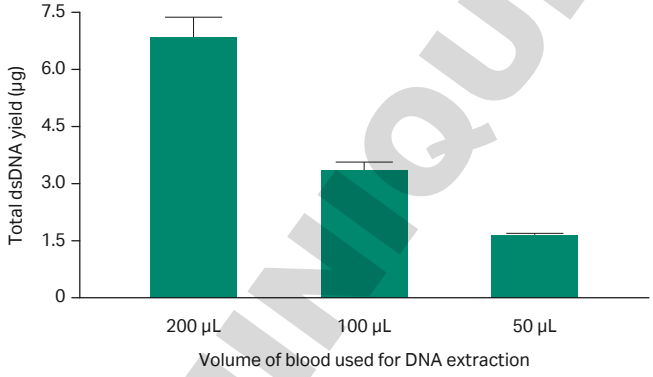

Fig 5. Yield of genomic DNA from 50 µL, 100 µL, and 200 µL inputs of whole blood. Graph shows one reading taken from four replicates (n = 4); error bars are +SD.

Comparison: automated DNA extraction process compared to MagMAX DNA Multi-Sample Ultra 2.0 Kit using the KingFisher Duo Prime system

The ability to automate the Sera-Xtracta Genomic DNA Kit was demonstrated on the KingFisher™ Duo Prime system, a magnetic bead-based automation platform (Thermo Fisher Scientific).

An automation script* was developed for the Sera-Xtracta Genomic DNA Kit based on the manual protocol. This was tested alongside the supplier's recommended protocol for the MagMAX™ DNA Multi-Sample Ultra Kit. The resulting purifications were analyzed for yield and purity.

*An automation script for KingFisher Duo Prime is available on request from Scientific Support (cytiva.com/support/scientific-support)

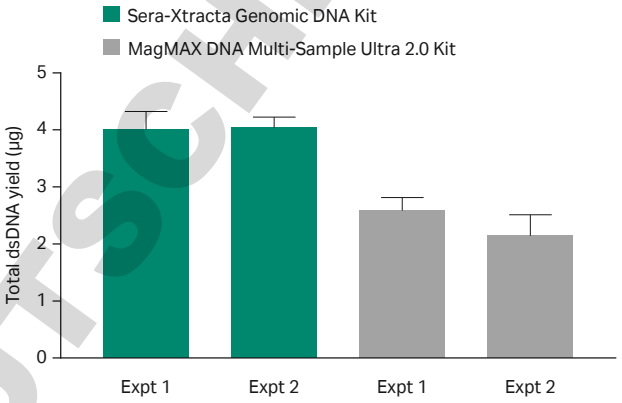

Fig 6. Yield of genomic DNA from replicate 200 µL inputs of whole blood, processed on the KingFisher Duo Prime system. Graph shows one reading taken from twelve replicates (n = 12) for each experiment; error bars are +SD.

DNA yields from the same blood sample, processed on the KingFisher Duo Prime system, were consistently higher when using Sera-Xtracta Genomic DNA Kit when compared with MagMAX DNA Multi-Sample Ultra 2.0 Kit (when run using their respective automation scripts).

Purity of DNA samples isolated from the same blood sample, processed on the KingFisher Duo Prime system using the Sera-Xtracta Genomic DNA Kit, were comparable to those extracted using the MagMAX DNA Multi-Sample Ultra 2.0 Kit when run using their respective automation scripts (Fig 7).

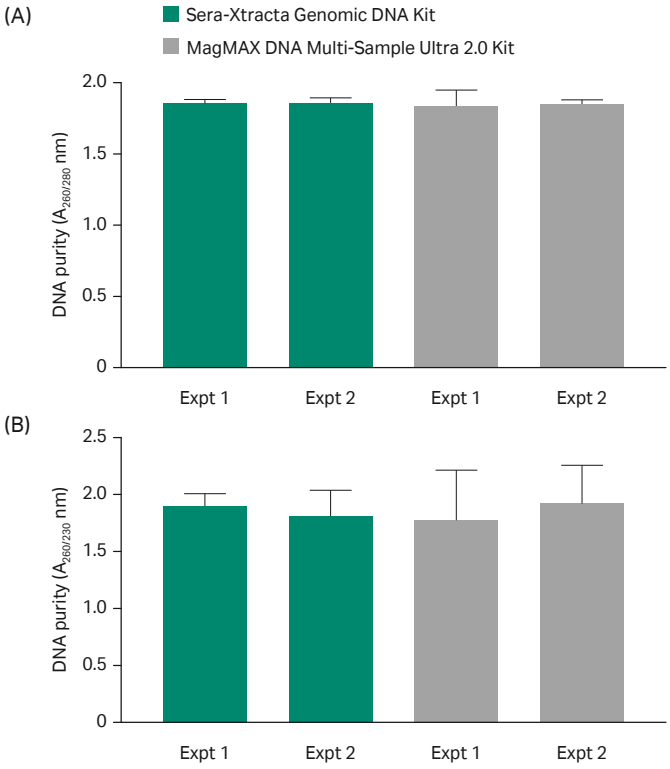

Fig 7. Purity of genomic DNA from replicate 200 µL inputs of whole blood, processed on the KingFisher Duo Prime system. Graphs show one reading taken from twelve replicates (n = 12) for each experiment, error bars are +SD. (A) DNA purity $A_{260/280}$ nm; (B) DNA purity $A_{260/230}$ nm.

This data is based on a minimum of three independent experiments and/or 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.

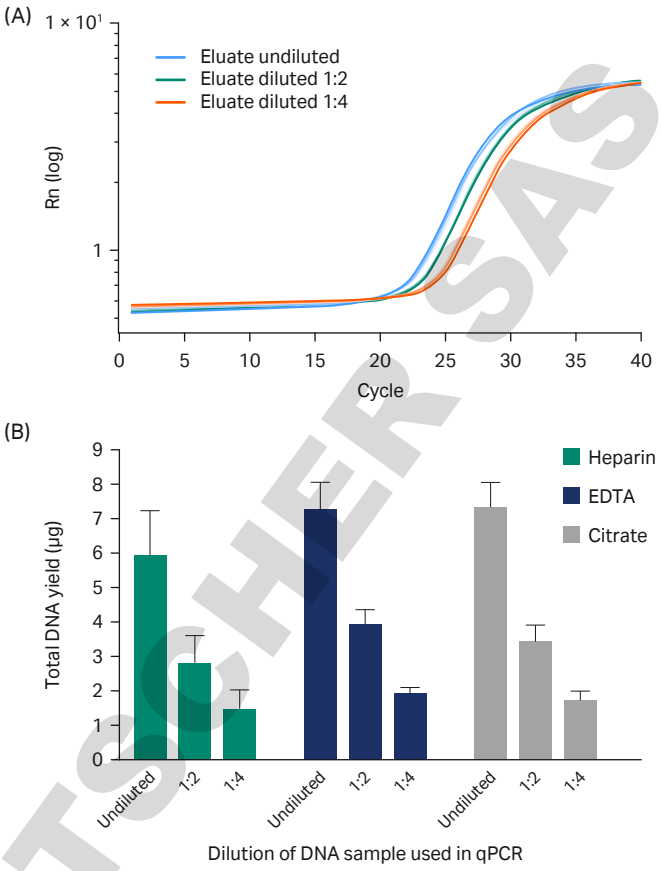

Fig 8. Real-time qPCR amplification of DNA isolated from whole blood stabilized with EDTA, heparin, and citrate. (A) Three sample input volumes were used in qPCR reactions. Amplification curves demonstrate a linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors. (B) Graph shows one reading taken from six replicates (n = 6) for each sample, error bars are +SD.

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.

Whole blood from a single donor was collected into EDTA, heparin and citrate blood collection tubes. DNA was isolated from 200 µL aliquots using the standard Sera-Xtracta Genomic 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).

No inhibition was observed in DNA isolated from anticoagulated whole blood collected in all three collection tubes, as shown in Figure 8A.

No inhibition was observed in samples purified from anticoagulated blood using the Sera-Xtracta Genomic DNA Kit. Isolated DNA was diluted, as indicated in Figure 8B, to enable different volumes of eluate to be used in qPCR reactions. Results demonstrate a highly linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors.

There were no significant differences ($p > 0.05$, one-way ANOVA) in Ct values obtained from undiluted eluates, diluted eluates (1:2 and 1:4 dilutions) and No Template Controls (qPCR reactions containing no DNA eluate) for the internal PCR control (IPC) assay (data not shown).

Comparison versus MagAttract HMW DNA Kit, MagaZorb DNA Mini-Prep Kit, and MagMAX DNA Multi-Sample Ultra Kit

All DNA purifications were carried out using 200 µL whole human blood in accordance with kit manufacturer's instructions. Yield and purity of genomic DNA was analyzed using the NanoDrop 2000. Yield of double stranded DNA was determined using the Qubit dsDNA BR assay (Fig 9 and Fig 10).

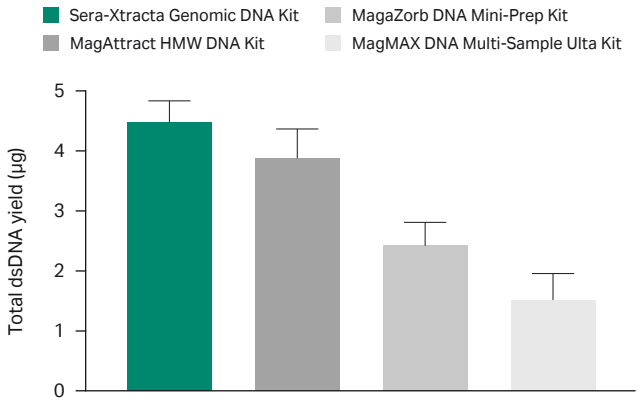

Fig 9. Double stranded DNA yield from three separate experiments. Graph shows one reading taken from nine replicates (n = 3 per experiment), error bars are +SD. Yield of double stranded genomic DNA from the same blood samples was higher using the Sera-Xtracta Genomic DNA Kit when compared to the MagAttract™ HMW DNA Kit (Qiagen), MagaZorb™ DNA Mini-Prep Kit (Promega), and MagMAX DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific).

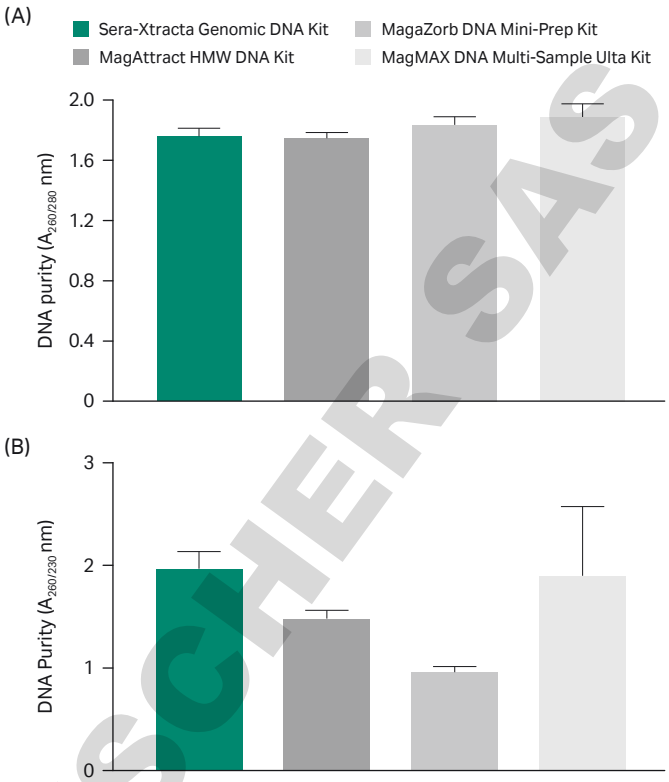

Fig 10. DNA purity by $A_{260/280}$ nm and $A_{260/230}$ nm ratios from three separate experiments. Graphs show three readings taken from nine replicates (n = 27), error bars are +SD. (A) Purity ($A_{260/280}$ nm ratio) of genomic DNA from the same blood samples is consistently higher than 1.70 using the Sera-Xtracta Genomic DNA Kit. (B) Purity ($A_{260/230}$ nm ratio) of genomic DNA from the same blood samples is consistently higher when using the Sera-Xtracta Genomic DNA Kit when compared to MagAttract HMW DNA Kit and MagaZorb DNA Mini-Prep Kit.

The high-purity DNA extracted using the Sera-Xtracta Genomic DNA Kit is suitable for use in downstream applications, including real-time quantitative PCR (Fig 8, A and B) and next-generation sequencing (Table 1).

This data is based on a minimum of three independent experiments and/or 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.

Performance of isolated genomic DNA in next-generation sequencing

For any NGS platform, success depends to a large extent on using DNA of high quality. Yield of double-stranded DNA (dsDNA) and its integrity status are important quality metrics.

DNA samples isolated using the Sera-Xtracta Genomic DNA Kit contained single prominent DNA bands ≥ 48 kb, while RNA and fragmented DNA were undetectable (Fig 1), thus demonstrating that the high-quality DNA obtained with the Sera-Xtracta Genomic DNA Kit is suitable for use in NGS experiments.

DNA was isolated from 200 µL aliquots of whole blood using the standard Sera-Xtracta Genomic DNA Kit protocol. DNA libraries were then generated using the Nextera™ DNA Flex library Prep Kit (illumina™ 20018704) andNGS was performed on the NextSeq™ 550 Sequencing System according to the manufacturer's instructions.

Table 1. Next-generation sequencing of genomic DNA isolated with the Sera-Xtracta Genomic DNA Kit

Attribute	Specifications
Total PF reads	101 595 298
Percent Q30 bases	87.9%
Percent duplicate paired reads	2.4%

Read level statistics

Read	Total aligned reads	Percent aligned reads
1	48 845 992	96.2%
2	48 551 075	95.6%

Base level statistics

Read	Total aligned bases	Percent aligned bases	Mismatch rate
1	6 670 676 694	89.1%	1.00%
2	6 531 989 114	87.2%	1.17%

Genomic DNA isolated with Sera-Xtracta Genomic DNA Kit couples seamlessly with downstream library preparation protocols for NGS, allowing the generation of high-quality sequencing reads.

Ordering information

Product	Pack size	Product code
Sera-Xtracta Genomic 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	29437807
Sera-Mag™ Select	5 mL	29343045
	60 mL	29343052
	450 mL	29343057
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
Ready-To-Go GenomiPhi 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
MagRack Maxi	15 mL/50 mL tubes	28986441
MagRack 6	1.5 mL/2.0 mL microtubes	28948964

cytiva.com/Sera-Xtracta-gDNA

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