

Isolation of high molecular weight DNA suitable for long-read sequencing platforms

Long-read sequencing technologies like those offered by Pacific Biosciences (PacBio) and Oxford Nanopore Technologies have enabled research in complex genome assembly and revolutionized genomics applications. To produce longer, more contiguous reads, researchers leveraging long-read sequencing rely on intact and high-quality high molecular weight (HMW) DNA. DNA that is low in purity and yield or fragmented in length may adversely affect long-read sequencing metrics such as read length, read depth and coverage, accuracy, and structural variant detection.

Here, we isolated HMW DNA from whole blood using nucleic acid isolation kits from various suppliers and evaluated DNA integrity, yield, and profile with regard to standard HMW criteria. Table 1 summarizes the isolation kits evaluated in this study along with their referenced workflows.

High purity and yield of large DNA fragments were observed using the Applied Biosystems™ MagMAX™ HMW DNA Kit, indicating its likelihood to perform well with downstream long-read sequencing technologies.

Table 1. Summary of HMW DNA extraction kits utilized in this experiment.

Supplier	Kit	Cat. No.	User manual	Workflow*	Input volume (μL)	Elution volume (μL)
Thermo Fisher Scientific	MagMAX HMW DNA Kit	A36626	MAN1001478, Revision A	Whole blood	200	100
New England Biolabs	Monarch™ HMW DNA Extraction Kit for Cells & Blood	T3050	Monarch HMW DNA Extraction Kit for Cells & Blood, Version 3.0_03/24	Whole blood, low input	200	100
PacBio	Nanobind™ HT 1 mL blood kit	102-762-800	102-573-500, Rev: 03	Whole blood	200	100
Promega	Wizard™ HMW DNA Extraction Kit	A2920	Instructions for Use of Product A2920, 7/22 revision	Whole blood	300	100
Qiagen	MagAttract™ HMW DNA Kit	67563	MagAttract HMW DNA Handbook, March 2020	Whole blood	200	100
RevoluGen	Fire Monkey™/Fire Flower™ (10) Kit	N/A**	Fire Monkey/Fire Flower (10) Kit Instructions for Use, 2022-10	Whole blood, elution of Fraction A	200	100
				Whole blood, elution of Fraction B	200	80
Zymo Research	Quick-DNA™ HMW MagBead Kit	D6060	Quick-DNA HMW MagBead Kit Instruction Manual	Whole blood	200	50

* Indicates the sample workflow followed in the user manual.

** Associated Cat. No. not available. When ordering online, the product is specified as "Fire Monkey - 10 columns kit".



HMW DNA purity

A_{260}/A_{280} and A_{260}/A_{230} ratios were averaged from isolated HMW DNA using a Thermo Scientific™ NanoDrop™ spectrophotometer (Table 2). Kits from multiple suppliers all resulted in A_{260}/A_{280} ratios around the typically accepted range of 1.8–2.0, indicating minimal presence of protein and impurities, and average A_{260}/A_{230} ratios around the typically accepted range of 2.0–2.2, indicating minimal presence of organic compounds, salts, and carbohydrates. Averages were determined using HMW DNA from three whole blood samples.

HMW DNA isolation yields

Total yield (µg) was obtained from isolated HMW DNA utilizing the Invitrogen™ Qubit™ 1X dsDNA Broad Range (BR) Assay Kit on the Invitrogen™ Qubit™ Flex Fluorometer (Figure 1). Yields were averaged by isolation technology from multiple suppliers across all three donors. The highest yield observed was obtained using the MagMAX HMW DNA Kit from Thermo Fisher Scientific.

Size distribution of HMW DNA

The Femto Pulse™ system from Agilent Biosciences was utilized to analyze the distribution of genomic DNA by the total percent of isolated material (Table 3). While typical long-read sequencing platforms utilize >40 kb fragments of HMW DNA, optimal performance may be achieved with fragments longer than 100 kb. Highest mass percentages (for >40 kb and >100 kb fragments) were observed with kits from Thermo Fisher Scientific and PacBio.

Table 2. Spectrophotometry results.

Supplier	A_{260}/A_{280}		A_{260}/A_{230}	
	Average	Standard deviation	Average	Standard deviation
Thermo Fisher Scientific	1.84	0.02	2.59	0.08
New England Biolabs	2.10	1.03	1.11	0.69
PacBio	1.80	0.05	1.84	0.11
Promega	1.70	0.20	1.66	0.56
Qiagen	1.80	0.27	0.89	0.28
RevoluGen: Fraction A	1.74	0.11	−9.64	13.24
RevoluGen: Fraction B	1.45	0.44	−0.52	6.56
Zymo Research	1.84	0.02	2.00	0.15

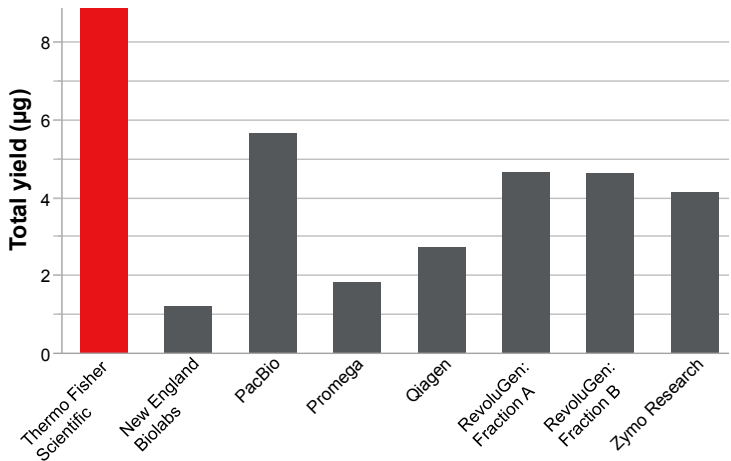


Figure 1. Total yields of HMW DNA.

Table 3. Percent size distribution of total genomic recovery.

Supplier	<10 kb	>40 kb	>100 kb
Thermo Fisher Scientific	4.17%	80.70%	76.73%
New England Biolabs*	NA		
PacBio	5.39%	86.60%	81.38%
Promega**	7.39%	70.11%	36.51%
Qiagen†	12.02%	69.54%	58.21%
RevoluGen: Fraction A	14.23%	24.73%	4.90%
RevoluGen: Fraction B	5.50%	38.00%	7.00%
Zymo Research	2.65%	78.71%	24.30%

* Nucleic acid isolated was not observable on the Femto Pulse system.
** Two samples were removed from analysis because they were outside the concentration range.
† Isolated nucleic acid only indicated one of the three donors to have HMW DNA greater than 100 kb, which shifted the average percentage.

HMW DNA for long-read success

The MagMAX HMW DNA Kit is suitable for obtaining DNA fragments >100 kb from fresh and frozen blood, cultured cells, and tissues using manual or automated protocols (Figure 2). Consisting of the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit with an added booster solution, the kit maintains the expected quality and consistency of MagMAX products while expanding the capabilities of DNA size recovery.

When integrating the MagMAX HMW DNA Kit into an automated workflow on a Thermo Scientific™ KingFisher™ system, HMW DNA purification can be completed with a total hands-on time of approximately 20 minutes and a total turnaround time of <2 hours. The MagMAX HMW DNA Kit can be ordered in kitted or bulk format, allowing for scale-up and customization needs.

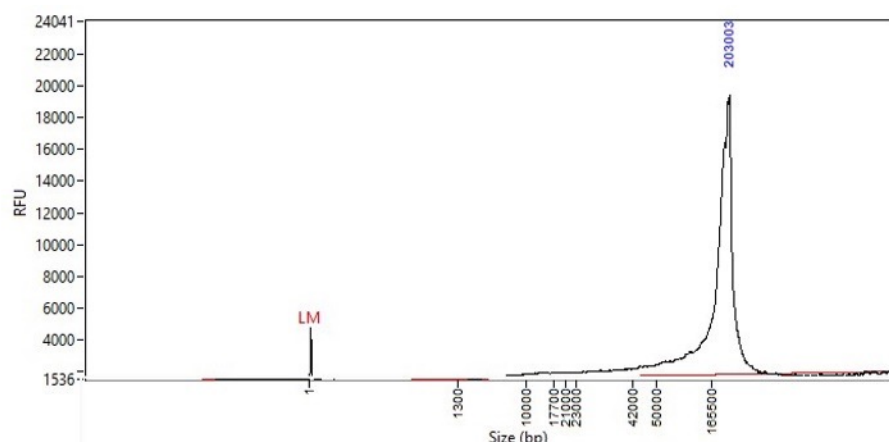


Figure 2. Isolation of HMW DNA fragments. The trace profile depicted is a representation of isolated HMW DNA from whole blood using the MagMAX HMW DNA Kit. The trace was obtained using the Femto Pulse system and analysis kit. LM: ladder marker; RFU: relative fluorescence unit

Ordering information

Product	Quantity	Cat. No.
MagMAX HMW DNA Kit	100 preps	A36626
MagMAX HMW DNA Kit with Cell and Tissue Extraction Buffer	100 preps	A36627
MagMAX DNA Multi-Sample Ultra 2.0 Kit	1 kit	A36570
MagMAX HMW DNA Booster, 100 preps	150 µL	A36624
MagMAX HMW DNA Booster, 1,000 preps	1 mL	A36625
MagMAX DNA Multi-Sample Ultra 2.0 Proteinase K	45 mL	A36578
MagMAX DNA Multi-Sample Ultra 2.0 Binding Beads	45 mL	A36579
MagMAX DNA Multi-Sample Ultra 2.0 Wash I Solution	1.1 L	A36580
MagMAX DNA Multi-Sample Ultra 2.0 Lysis/Binding Solution	450 mL	A36581
MagMAX DNA Multi-Sample Ultra 2.0 Elution Solution	120 mL	A36582
MagMAX DNA Multi-Sample Ultra 2.0 Enhancer Solution	45 mL	A36583
MagMAX Cell and Tissue DNA Extraction Buffer, 100 preps	60 mL	A45469
MagMAX Cell and Tissue DNA Extraction Buffer, 1,000 preps	600 mL	A45470

Learn more at thermofisher.com/hmwdna

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