abGenix X™ - First cartridge-based Automated platform for extraction of Ultra-High Molecular Weight DNA from whole blood for ONT long-read sequencing

Introduction

DNA extracted from blood is a subject of study in various fields such as genetic and genomic research, medical diagnostics, personalized medicine, forensic science, pharmacogenomics, etc.

Ultra-High Molecular Weight (uHMW) (>300 kb) DNA is critically important in Oxford Nanopore Technologies' (ONT) long read sequencing technology, since the technology is nearly unlimited by the DNA length and uHMW DNA resulting in much longer sequencing reads. uHMW DNA tends to have lower error rates compared to shorter fragments, long reads can span repetitive elements, which are difficult to resolve with short reads alone. Also, use of uHMW DNA allows for better detection of structural variants (e.g., deletions, insertions, and inversions) as well as chromosomal aberrations.

We evaluated quality of DNA extracted from whole blood by our automated *ab*Genix X™ system and a newly developed *ab*Genix X™ HMW DNA Cartridge Kit and its compatibility with the ONT's library preparation and long-read sequencing chemistry. We used the Monarch® HMW DNA Extraction Kit for Tissue (New England Biolabs) as reference method.

Materials and Methods

Sample

A single donor human blood from a commercial source was used in this study. 2 mL of whole blood was taken per each extraction.

DNA extraction

Total DNA was extracted using the *ab*Genix X™ HMW DNA Cartridge Kit in conjunction with the *ab*Genix X™ Instrument and Monarch® HMW DNA Extraction Kit for Tissue (NEB).

DNA quality and yield

Yield and integrity of the extracted DNA were assessed by Agilent's Femto Pulse System and ProSize fragment analysis software.

Long read sequencing

About 42 µg of DNA was taken for each library preparation as recommended by the manufacturer. DNA libraries were prepared using ONT's Ultra-Long DNA Sequencing Kit V14. Sequencing was conducted on the PromethION 24 A Series platform (using R10.4.1 Flow Cells).

Results and Discussion

DNA yield, purity and integrity

DNA was extracted from a single donor whole blood using the *ab*Genix X™ HMW DNA Cartridge Kit and Monarch® HMW DNA Extraction Kit for Tissue simultaneously in duplicates. Capillary electrophoresis showed DNA length peaks between 103 and 190 Kb for both methods (**Figure 1**). Comparative analysis of DNA integrity by the ProSize fragment analysis software demonstrated an equivalency between gDNA extracted by the both methods at lower size threshold (**Table 1**). However, increasing size threshold to 200 kb revealed the slight increment of uHMW DNA in samples extracted with the *ab*Genix X™ HMW DNA Cartridge Kit compared to the reference method.

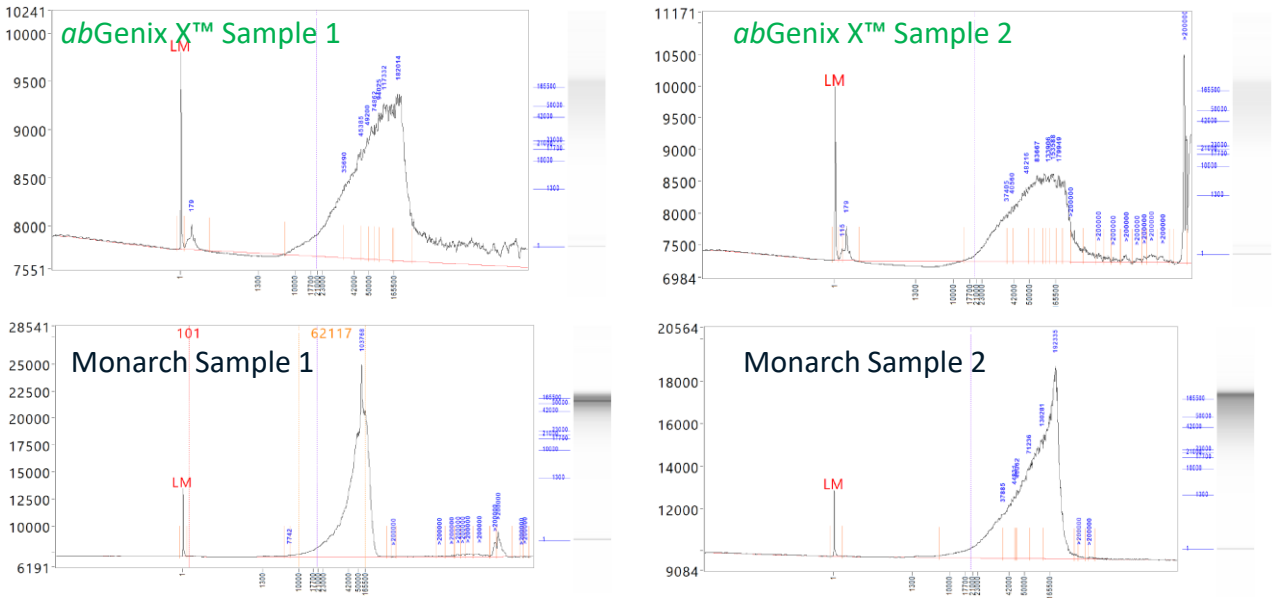

Figure 1. Quantitative fragment analysis of DNA samples extracted by the *ab*Genix X™ HMW DNA Cartridge Kit (top panel) and Monarch® HMW DNA Extraction Kit for Tissue (bottom panel) using the Femto Pulse System.

Table 1. Fragment size analysis of DNA extracted by the both kits using the ProSize software. Genomic Quality Number (portion of DNA) at different size thresholds.

Sample	Genomic Quality Number (GQN)* size threshold					
	10 kb	20 kb	50 kb	100 kb	150 kb	200 kb
*ab*Genix X™ Sample 1	9.7	9.4	5.8	4.4	2.9	1.3
*ab*Genix X™ Sample 2	9.6	9.5	6.1	4.9	3.6	2.5
Monarch Sample 1	9.8	9.5	4.9	3.7	2.6	1.6
Monarch Sample 2	9.9	9.6	6.4	4.9	3.2	0.9

***NOTE.** The genomic quality number (GQN) shows the fraction of the total measured concentration that lies above the size threshold specified by the user. The GQN scores the sample on a scale of 0 to 10, with 0 indicating none of the sample exceeds the threshold and 10 indicating 100 % of the sample lies above the threshold value [1].

Long-read sequencing

DNA libraries were prepared using the Ultra-Long DNA Sequencing Kit V14 in accordance with the manufacturer's protocol. Long-read sequencing was conducted on the PromethION 24 A Series platform using R10.4.1 Flow Cells. Major sequencing outputs of the runs are summarized in **Table 2.** Distribution of read lengths was similar for samples are extracted by the test and reference methods (**Figure 2**).

Table 2. Data output for DNA extracted from whole blood using the *ab*Genix X™ HMW DNA Cartridge Kit and Monarch® HMW DNA Extraction Kit for Tissue.

Kit		*ab*Genix X™ HMW DNA Cartridge Kit (AITbiotech)		Monarch® HMW DNA Extraction Kit for Tissue (NEB)	
Sample		**1**	**2**	**1**	**2**
Total Bases		40.33 Gb	45.67 Gb	58.56 Gb	48.54 Gb
Median Read Quality		18.4	18.3	17.0	17.2
Number of Reads		2.01 M	2.34 M	1.73 M	1.51 M
Read Length N50	Total	58,012	57,587	77,153	62,339
	Q15	61,900	62,210	78,450	62,750
	Q18	63,540	64,000	78,800	63,120
	Q20	66,650	67,160	75,700	61,510
	Q30	**75,880**	**74,900**	72,030	57,690
Number, % and gigabases of reads above quality cutoffs	>Q10	1.71 M (84.9%) 36.91 Gb	1.97 M (84.2%) 41.64 Gb	1.43 M (82.7%) 49.80 Gb	1.25 M (83.0%) 41.36 Gb
	>Q15	1.45 M (72.0%) 33.25 Gb	1.67 M (71.4%) 37.49 Gb	1.13 M (65.3%) 39.80 Gb	1.01 M (67.0%) 33.67 Gb
	>Q18	1.07 M (53.2%) 28.18 GB	1.24 M (53.0%) 31.83 Gb	0.69 M (40.0%) 24.01 Gb	0.63 M (41.7%) 21.40 Gb
	>Q20	0.68 M (33.8%) **21.97 Gb**	0.79 M (33.8%) **25.15 Gb**	0.26 M (15.0%) 7.37 Gb	0.23 M (15.2%) 7.38 Gb
	>Q30	693 (0.034%) **26.20 Mb**	977 (0.042%) **44.44 Mb**	447 (0.026%) 9.64 Mb	277 (0.018%) 8.05 Mb

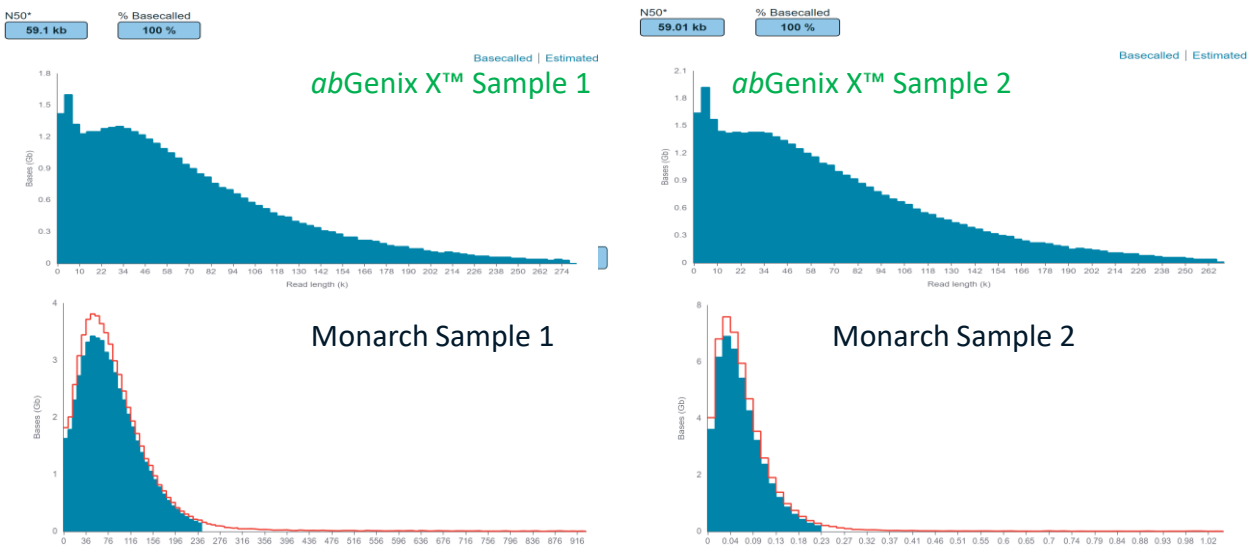

Figure 2. Read length distribution for the DNA extracted from whole blood by the *ab*Genix X™ HMW DNA Cartridge and Monarch® HMW DNA Extraction Kits.

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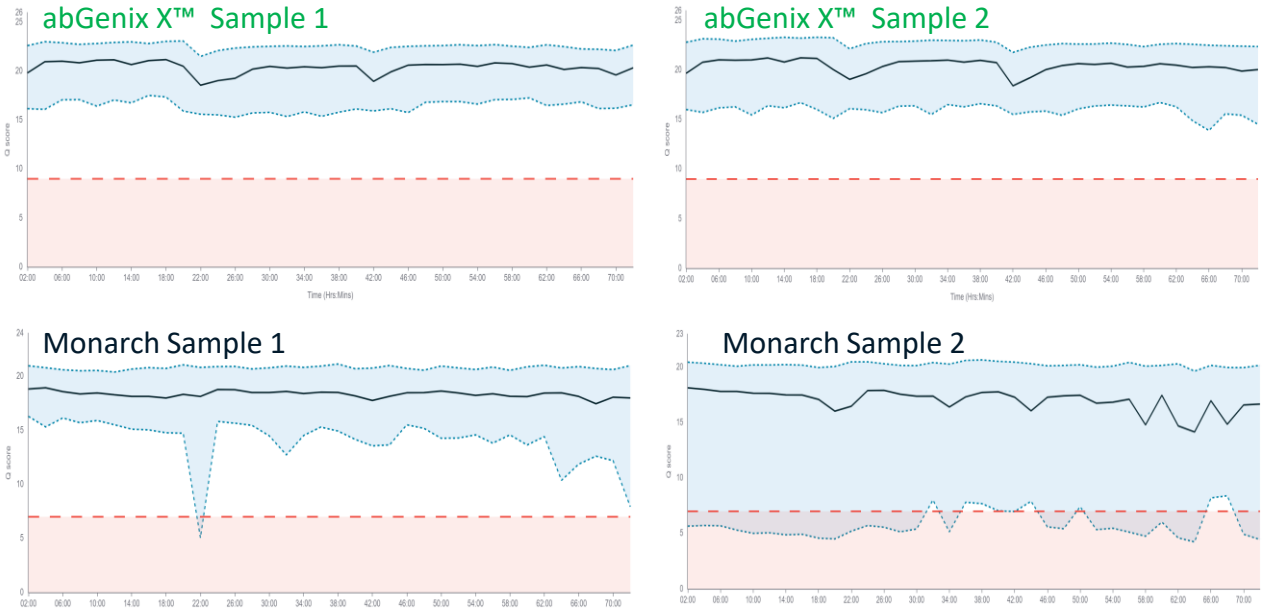

Figure 3. Q score (Quality score) for the DNA samples extracted from whole blood.

In spite of the total number of bases generated was slightly higher for the samples extracted with the Monarch® kit (48 – 58 Gb) compared to the *ab*Genix X™ samples (40 – 45 Gb), DNA extracted using the system generated higher number of high-quality (Q30) long reads (Read Length N50) *ab*Genix X™ as well as higher number of DNA reads at >Q20 and >Q30 (**Table 2**). Qualitative analysis of the reads showed the overall higher quality of the reads for samples extracted using the *ab*Genix X™ HMW DNA Cartridge Kit (**Figure 3**). Analysis of the longest read lengths with cut-off at Q20 demonstrated a higher number of long reads for samples extracted by the *ab*Genix X™ system compared to the reference method (**Figure 4**). The sequencing data strongly corelate with the capillary electrophoresis and ProSize fragment analysis data (**Table 2**). This results clearly indicate that the *ab*Genix X™ system is able to extract ultra-long intact DNA molecules.

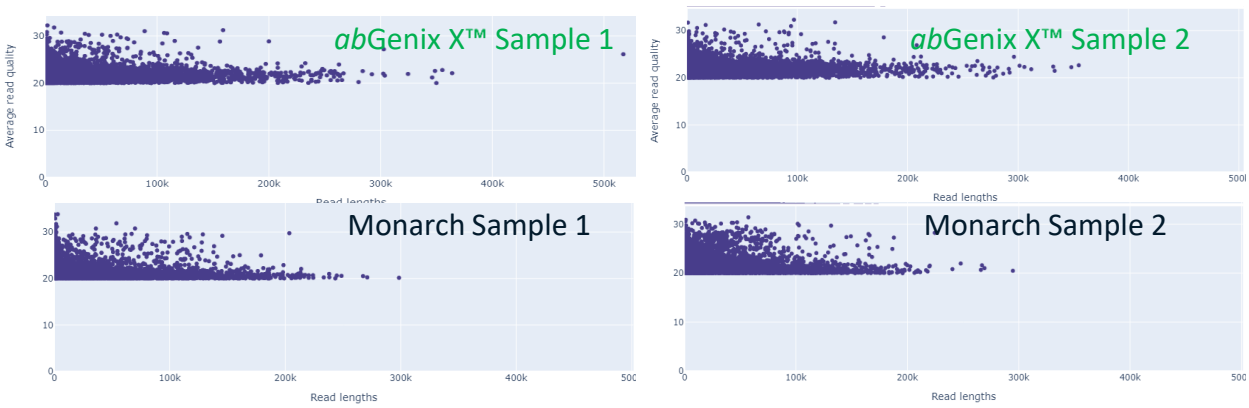

Figure 4. Dot plots Q20 / Read Lengths vs Average read quality.

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Conclusions

Overall results show either equivalency or superiority of the *ab*Genix X™ Platform compared to the reference manual kit. The *ab*Genix X™ Platform thus appears as an efficient and reliable solution for extraction of uHMW DNA from whole blood and the quality of extracted DNA is meet the requirements for the nanopore library preparation and long-read sequencing technology.

Results demonstrate that AITbiotech's solid phase technology makes extraction of high-quality uHMW simple, and automation of the extraction procedure holds great promise for accelerating research and clinical diagnostics.

References

1. Pocernich C., Uthe J., Siembieda S. Genomic DNA Extractions Compared with the Agilent Femto Pulse System. *Agilent Technologies Application Note*, publication number 5994-0754EN, 2019. simple

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