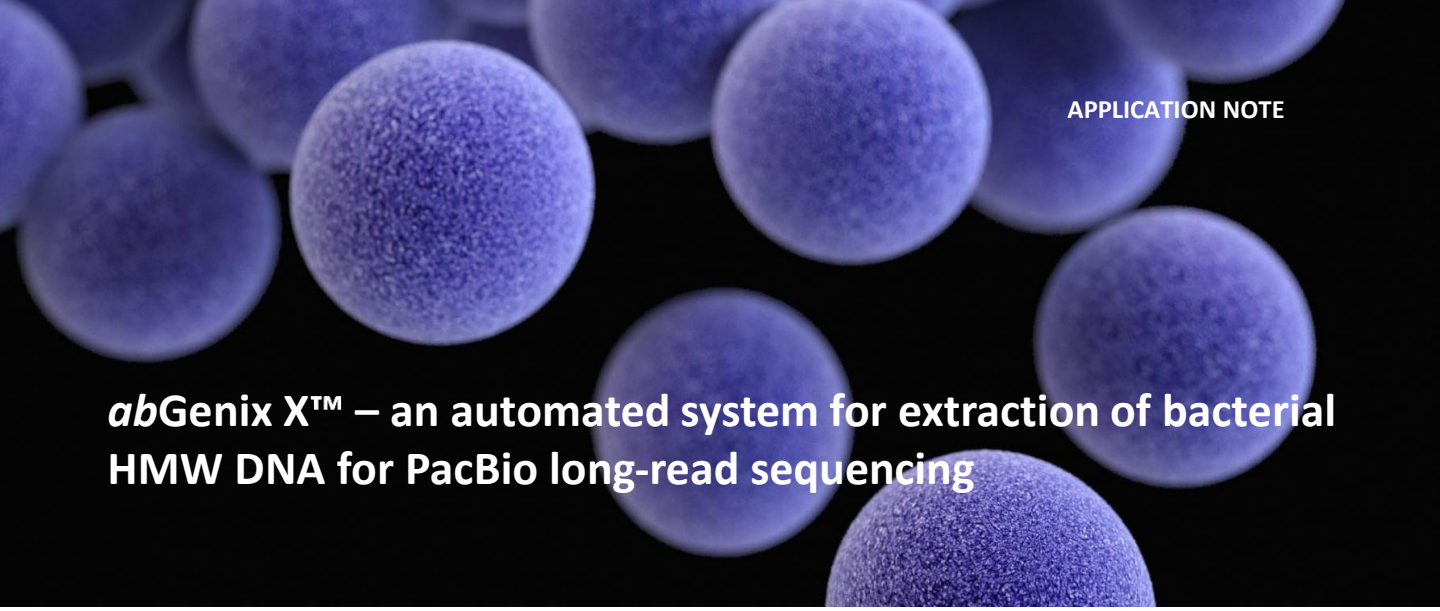

APPLICATION NOTE

*ab*Genix X™ – an automated system for extraction of bacterial HMW DNA for PacBio long-read sequencing

Introduction

High-throughput sequencing of metagenomes offers unprecedented insights into the diversity and gene pool of naturally occurring microbes and viruses that occupy soils, marine habitats and host-associated environments.

Assembling of short reads generated by short-read sequencing is inherently challenging and often leads to highly fragmented assemblies. This increases the likelihood of generating composite genomes that include contigs from multiple distinct populations. By circumventing the problems associated with short-read assembly, long-read sequencing offers a compelling solution to the ideal of reconstructing complete genomes from metagenomes.

The efficacy of long-read sequencing heavily depends on the structural integrity of the input DNA. This makes extraction of high-quality High Molecular Weight (HMW) DNA a crucial upstream procedure for long-read sequencing.

In this study we evaluated quality of DNA extracted by our new *ab*Genix X™ HMW DNA Cartridge Kit for long-read sequencing on PacBio sequencing system.

Materials and Methods

Samples

Genomic DNA was extracted from Gram-positive (*Streptococcus agalactiae*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica*). 1 x 10^9 or 2 x 10^9 bacterial particles resuspended in 200 µL of PBS were taken for each extraction run.

DNA extraction

DNA was extracted from bacteria using the *ab*Genix X™ HMW DNA Cartridge Kit in conjunction with the *ab*Genix X™ Instrument.

Quality Control

Yield, integrity and purity of the extracted DNA were assessed using the Femto Pulse System, Qubit 4 fluorometer (in conjunction with Qubit 1X dsDNA HS Assay Kit) and NanoDrop™ 2000 spectrophotometer.

Long read sequencing

Long read sequencing was conducted using the SMRTbell® prep kit 3.0 on PacBio Sequel IIe system at Next Level Genomics Pte Ltd.

Results and Discussion

Yield and purity of the extracted HMW DNA were assessed using the NanoDrop™ 2000 spectrophotometer and Qubit 4 fluorometer (**Table 1**) and integrity by the Femto Pulse System (**Table 2**). The results demonstrate that DNA extracted using the *ab*Genix X™ system meeting quality requirements for long-read sequencing.

The extracted DNA was used as a template for library preparation and consecutive sequencing. Bacterial samples were multiplexed, percentage of the individual libraries vary from 3 to 5%.

The polymerase N50 shows that the purified DNA was of high quality to allow the enzyme to read the insert multiple times. After demultiplexing, microbial genomes were assembled using SMRT® Link software. The reads were aligned and mapped on the reference sequences (**Figure 1**). The key mapping metrics are summarized in **Table 3**.

Distribution of majority of read length was between 2000 bp and 25,000 bp for *S. agalactiae* and between 3000 bp and 13,000 bp for *E. coli, P. aeruginosa* and *S. enterica* (**Figure 2**).

The mapping and assembling results demonstrate even distribution of reads across the reference sequences that shows non-selective and uniform extraction of bacterial DNA when using the *ab*Genix X™ system.

Table 1. Yield and purity (ratio of absorbance at 260 and 280 nm) of HMW DNA extracted from bacterial samples using the automated *ab*Genix X™ HMW DNA Cartridge Kit measured by Qubit 4 and NanoDrop™ 2000, respectively.

Sample	Sample input	Total DNA yield (µg)	DNA purity (A 260/280)
S. agalactiae	1 x 10^9	20.90	1.85
E. coli	2 x 10^9	7.53	1.55
P. aeruginosa	2 x 10^9	18.45	1.64
S. enterica	1 x 10^9	3.78	1.80

Table 2. Quantitative fragment analysis of DNA samples extracted by the *ab*Genix X™ HMW DNA Cartridge Kit conducted using the Femto Pulse System.

Sample type	% DNA >20 kb	% DNA >50 kb	% DNA >100 kb	% DNA >150 kb
S. agalactiae	93	72	67	63
E. coli	97	63	29	26
P. aeruginosa	92	67	41	31
S. enterica	96	71	47	38

Table 3. Alignment mapping metrics.

Metric	*S. agalactiae* sample 1	*S. agalactiae* sample 2	*E. coli*	*P. aeruginosa*	*S. enterica*
Percentage of the library	**5%**	**5%**	**3%**	**4%**	**3%**
Mean Concordance (mapped)	99.76%	99.77%	99.83%	99.85%	99.85%
Number of CCS* Reads (total)	68,837	45,643	134,487	73,820	88,894
Number of CCS Reads (mapped)	68,681	45,546	134,261	73,733	88,736
Number of CCS Bases (mapped)	851,593,036	619,325,243	1,135,292,460	571,755,638	723,565,192
CCS Read Length Mean (mapped)	12,212	13,447	8,412	7,739	8,111
CCS Read Length N50 (mapped)	13,618	14,980	8,919	8,140	8,636
CCS Read Length 95% (mapped)	19,794	21,808	12,973	11,835	12,585
CCS Read Length Max (mapped)	33,013	40,316	44,020	16,769	18,780
Polished Contigs	5	3	2	1	2
Maximum Contig Length	1,446,693	2,326,586	4,581,832	6,275,431	4,873,909
NS0 Contig Length	1,446,693	2,326,586	4,581,832	6,275,431	4,873,909
Sum of Contig Length	2,404,027	2,373,564	4,675,439	6,275,431	4,967,741

NOTE: *CCS – Circular consensus sequencing.

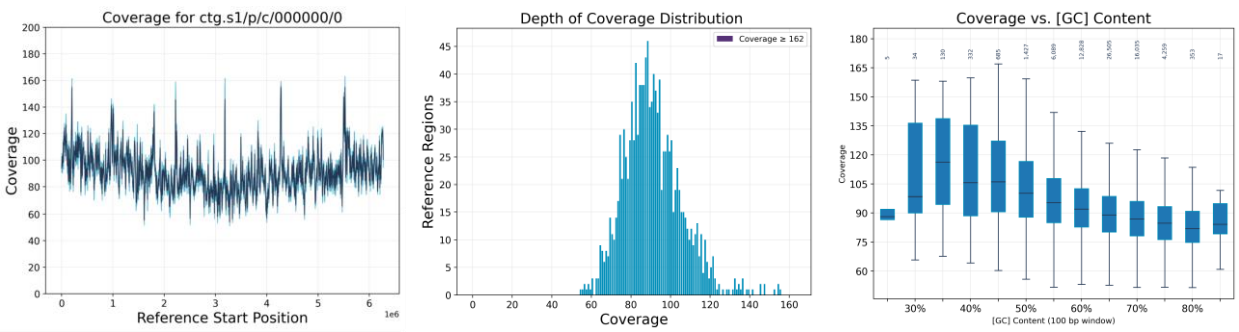

Figure 1. An example of mapping analysis for *P. aeruginosa*.

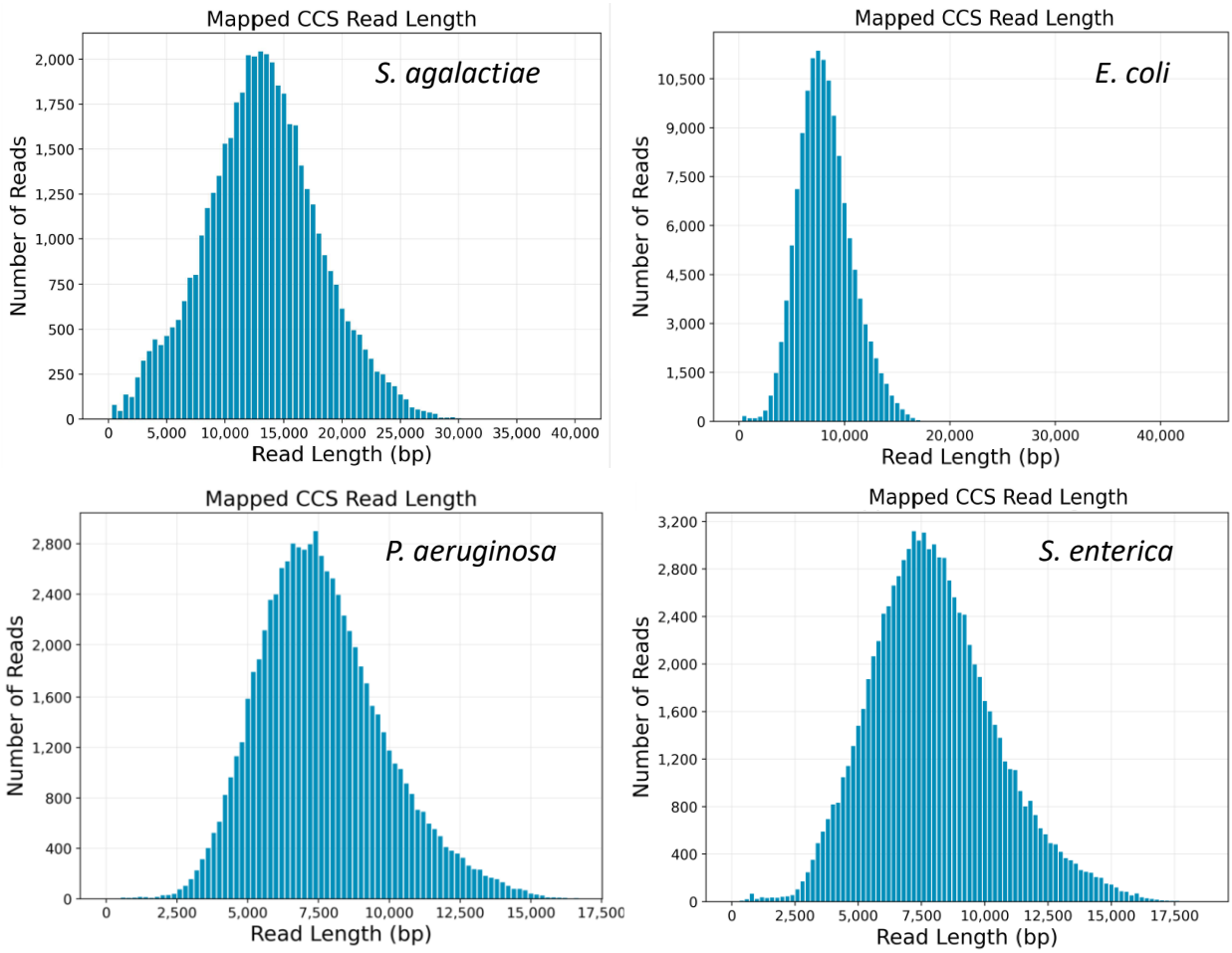

Figure 2. Distribution of read lengths for bacterial samples extracted using the *ab*Genix X™ Nucleic Acid Purification System for different bacteria species.

Conclusions

Results of this study demonstrate that the *ab*Genix X™ system enables extract high-quality HMW DNA from various bacteria species. The extracted DNA is suitable for PacBio's sequencing systems, and the quality of reads is comparable to samples processed by other DNA extraction methods.

Beside extracting high-quality HMW DNA, the automated *ab*Genix X™ system providing users with a seamless and walk-away experience that makes it ideal for labs that currently cope with manual nucleic acid extraction methods.

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