Automated extraction of genomic DNA for Next-Generation Sequencing using the Xceler8™ Nucleic Acid Extraction Platform

Introduction

Today's complex genomics questions demand a depth of information beyond the capacity of traditional DNA sequencing technologies. Next-Generation Sequencing (NGS) offers ultra-high throughput, scalability, and speed and is an essential tool used in academic, translational, and clinical research settings. NGS workflows based on hybridization capture enable the interrogation of all mutation classes (point mutations, short indels, gene fusions, and copy number variants) in specific regions of interest. These protocols typically require the preparation of a genomic DNA (gDNA) fragment library from which regions of interest are captured with pre-defined probe panels. Mid- to high-nanogram quantities of input gDNA (of sufficient quality to support efficient pre-capture amplification of 150 to >350 bp library fragments) is required for successful sequencing.

In this application note we provide comparative performance data on gDNA extracted using the Xceler8™ (X8) Platform (a highly flexible and ***beyond-simple*** automated system) and widely used- magnetic bead-based automated system (Auto Q1), spin column-based automated system (Auto Q2) and a spin column-based manual DNA extraction kit (Manual Q). The study demonstrates that the automated Xceler8 Platform is able to effectively isolate gDNA from various cultured human cell lines without compromising its integrity, and the extracted gDNA is suitable for sensitive downstream applications such as NGS.

Materials and Methods

Samples

Three human cell lines were selected for this study- MIA PaCa-2, PLC/PRF/5 and A549. Approximately 1 x 10^6 cells were resuspended in 200 µL of 1X PBS and were used for each extraction run.

DNA extraction

gDNA was extracted from MIA PaCa-2, PLC/PRF/5 and A549 human cell lines using four different extraction methods. 1. X8™ Genomic DNA Cartridge Kit (Cat# X8-GD-001-24) together with X8™ OneTouch Instrument (Cat# X8-OT-101-IN), 2. A magnetic bead-based automated system (Auto Q1), 3. A spin column-based automated system (Auto Q2) and 4. A spin column-based manual DNA extraction kit (Manual Q). All samples were normalized to equal final elution volume for fair comparison.

Spectrophotometry

Yield of the extracted gDNA was assessed using NanoDrop™ 2000 Spectrophotometer (ThermoFisher Scientific). UV absorbance readings were taken at 230 nm, 260 nm and 280 nm, and absorbance ratios (A260/A280 and A260/A230) were determined.

Whole Exome Sequencing (WES)

The gDNA extracted using the various methods was used as the template to run the POLARIS® Clinical Exome Test, a laboratory developed

whole exome sequencing test that provides sequencing data using a custom NGS-based workflow. The 39 Mbp target region includes coding sequences of 19,396 human genes. The POLARIS Clinical Exome Test was developed and validated in accordance with the College of American Pathologists (CAP) and Ministry of Health (MOH), Singapore, guidelines, within the CAP-accredited and PHMC licensed POLARIS Laboratory at Genome Institute of Singapore (POLARIS@GIS). The libraries were sequenced using MiSeq™ System (Illumina).

Results and Discussion

In this study, gDNA was extracted from three different human cell lines using the Xceler8 (X8) Platform, as well as two other automated platforms (Auto Q1 and Auto Q2) and a widely used column-based manual DNA extraction kit (Manual Q) as reference methods. The gDNA was extracted from each cell line in triplicates and used to prepare NGS libraries and run the POLARIS Clinical Exome Test. The gDNA purified using the various extraction methods from the three different human cell lines were then assessed by comparing library construction, sequencing analysis metrics and results from whole exome sequencing. Pre-capture library concentration, on-target rate and coverage are important library preparation and primary sequencing analysis metrics in NGS. Those metrics were assessed to evaluate the quality of the extracted gDNA. Results are summarized in Table 1 and Figure 1.

Table 1. Comparative NGS library Quality Control (QC) metrics for gDNA extracted from human cell lines. *SEM- Standard Error of the Mean.

DNA EXTRACTION METHOD	PRE-CAPTURE LIBRARY CONCENTRATION		PERCENTAGE TARGETED BASES WITH ≥30x COVERAGE	
	MEAN CONC. (ng/ µL)	SEM*	MEAN CONC. (ng/ µL)	SEM*
X8	23.38	1.04	82.20	2.24
Auto Q1	28.17	1.90	77.94	0.45
Auto Q2	27.71	0.91	81.62	1.55
Manual Q	25.76	4.62	81.19	2.23

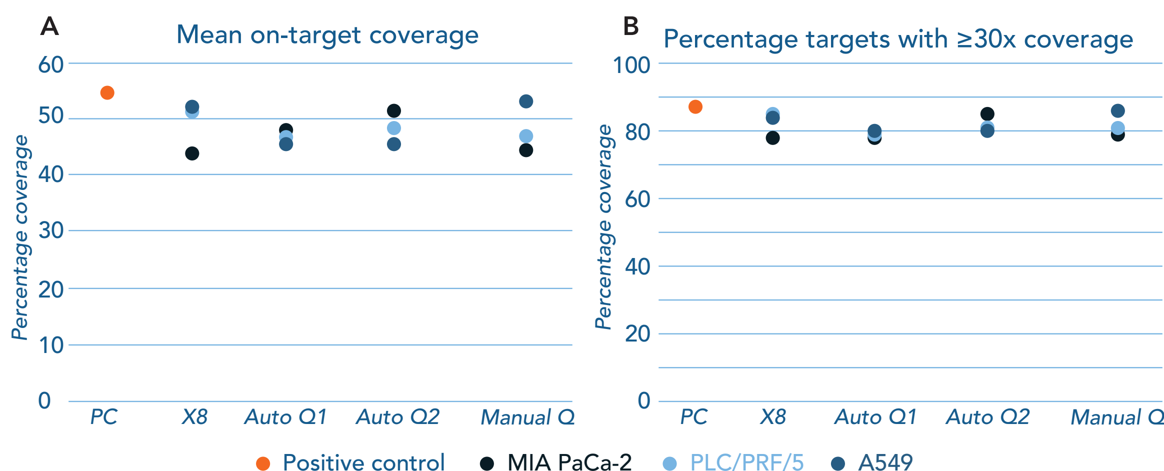

Figure 1. Comparison of mean on-target coverage (A) and percentage targets with ≥30x coverage (B). QC metrics obtained by sequencing the indicated samples were similar between the X8 Platform, other automated systems (Auto Q1 and Auto Q2), and the manual workflow (Manual Q). PC - Positive Control.

Beside the QC metrics, data generated by the POLARIS Clinical Exome Test workflow were also analyzed. The test provides a comprehensive coverage of the human exome with more than 95% of the target bases covered at ≥30x and an average sequencing depth of ≥100x. It can detect single nucleotide polymorphisms (SNPs), small insertions and deletions (Indels). Results for A549 cell line samples are shown in Table 2 and as an example.

Table 2. Number of variants detected from gDNA extracted from A549 cell line using various extraction methods.

DNA EXTRACTION METHOD	PASSED FILTER	SNPs	INSERTIONS	DELETIONS	INDELS
X8	29642	27480	1003	1134	6
Auto Q1	29746	27494	1032	1192	6
Auto Q2	29812	27575	1027	1188	6
Manual Q	29736	27552	1021	1136	8

Number of SNPs, insertions and deletions detected in A549 cell line were very similar for samples extracted by the different methods. Similar results were obtained for MIA PaCa-2 and PLC/PRF/5 cell lines.

A Whole Exome Sequencing (WES) analysis of the NGS data showed that the number of rare variants (with allele frequency <1%) in gDNA samples extracted using the Xceler8 Platform were comparable for all three cell lines to those obtained using the other automated and manual reference methods (Figure 2). This demonstrates that the Xceler8 Platform can efficiently isolate high-quality gDNA suitable for comprehensive downstream applications.

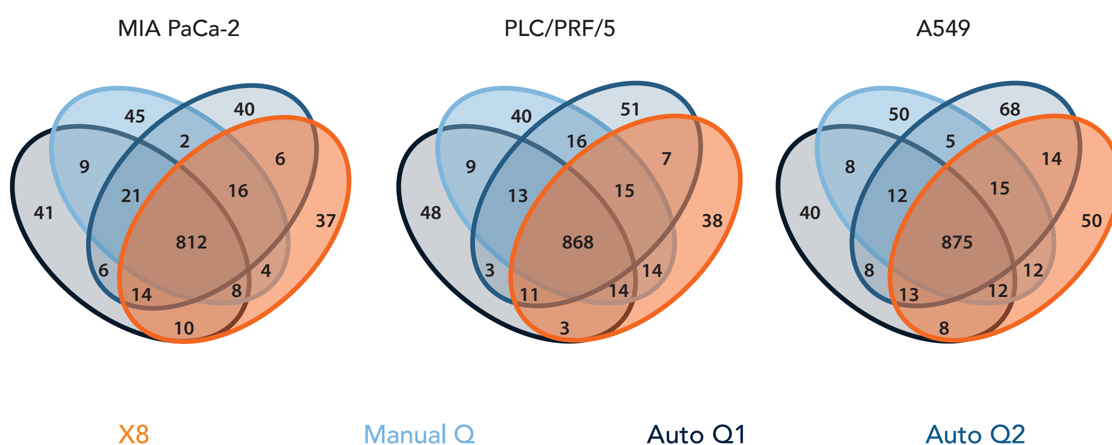

Figure 2. Number of rare variants (with allele frequency <1%) identified using Whole Exome Sequencing (WES) analysis in gDNA extracted from MIA PaCa-2, PLC/PRF/5 and A549 human cell lines. gDNA was extracted using the X8 Platform, one manual extraction method (Manual Q), and two other widely used automated platforms (Auto Q1 and Auto Q2).

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Conclusions

In this study we demonstrated the Xceler8 Platform ensures consistent and reproducible extraction of gDNA from human cell line samples across different users. Overall, results demonstrated the gDNA isolated using the Xceler8 Platform shows equivalency when compared to the other automated and manual reference methods and that the extracted gDNA can be used for sensitive downstream applications such as Next Generation Sequencing.

The comparative NGS data generated using the gDNA extracted using the Xceler8 Platform and the other reference methods (Auto Q1, Auto Q2 and Manual Q), showed equivalency in performance. However, in overall user experience the Xceler8 Platform shines across the other reference methods. One BioMed's novel chemistry-based approach simplifies the product architecture, thus making the load-and-go system ideal for labs looking for automation with high flexibility and low maintenance. The fully integrated Xceler8 Platform provides users with a seamless and ***truly walk-away*** experience. To conclude, all of these benefits combined with the reliable technical performance makes the Xceler8 Platform perfect for labs that are tired of conventional manual or automated nucleic acid extraction methods.

Acknowledgements

The data for this Application Note was generated as part of an independent benchmarking study done at the Centre for Genome Diagnostics at Genome Institute of Singapore (GIS).

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SAM-0015-EN *Rev A*

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