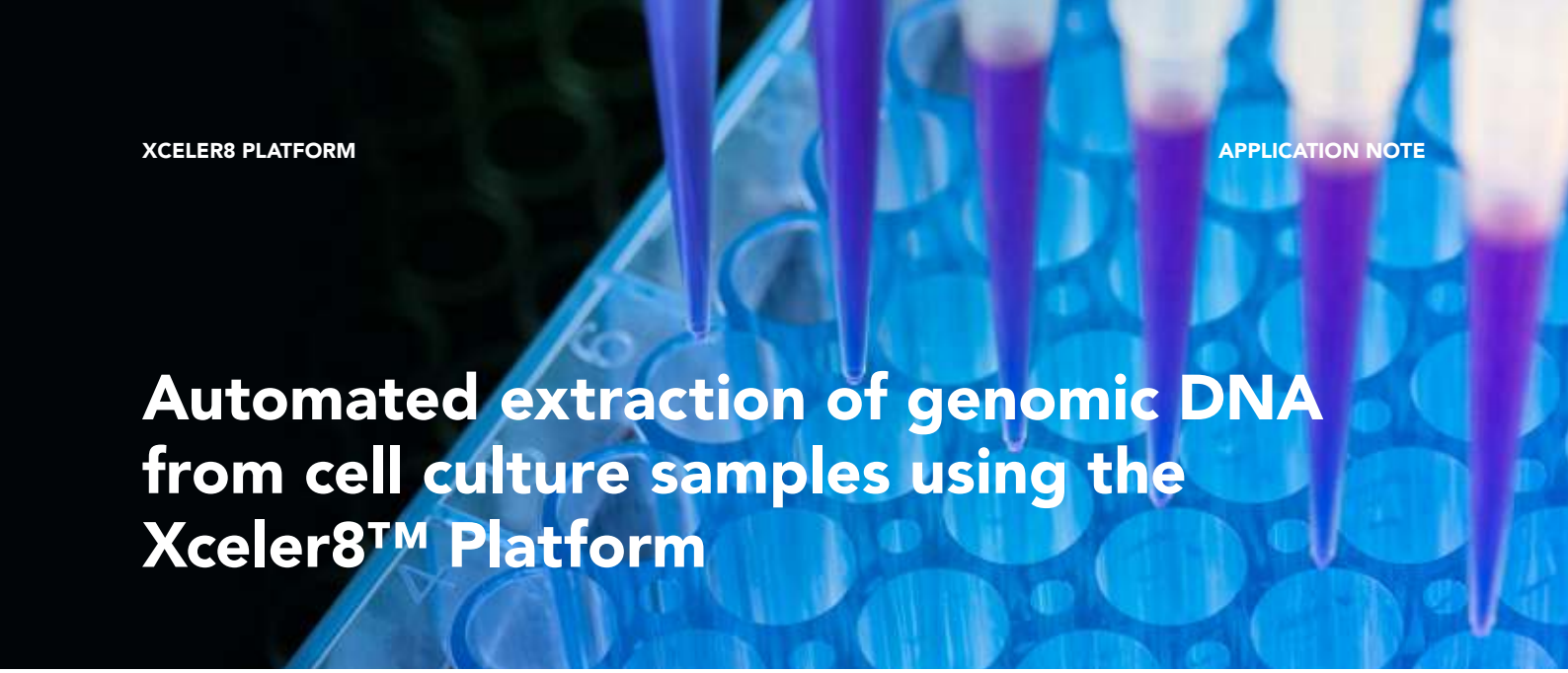

Automated extraction of genomic DNA from cell culture samples using the Xceler8™ Platform

Introduction

In many molecular laboratories today, nucleic acid is extracted and purified using both manual and automated extraction protocols. Starting with good quality and quantity of genomic DNA (gDNA) typically results in better application performance. Therefore, it is important the extracted gDNA meet the criteria of high quality, purity and yield.

Most laboratories with low to medium sample throughput choose the manual extraction workflows due to the high cost and complexity of automation. However, manual nucleic acid extraction methods are generally labor intensive and can introduce the risk of contamination, errors, and user variability.

In this application note we provide comparative performance data on gDNA extracted using the Xceler8™ Platform (a highly flexible and ***beyond-simple*** automated system) and two widely used column-based manual DNA extraction kits. The study demonstrates that the automated Xceler8 Platform is able to effectively isolate gDNA from cell culture samples, without compromising its integrity, and the extracted gDNA is suitable for sensitive downstream applications such as Real-Time PCR.

Materials and Methods

Samples

gDNA was extracted from HeLa and NCI-H1975 human cell lines. ~1 x 10^6 cells were resuspended in 200 µL of 1X PBS for each extraction run. Several batches of samples were prepared and run at various time points by different operators, using multiple lots of reagents.

DNA extraction

gDNA was extracted from HeLa and NCI-H1975 human cell lines using the X8™ Genomic DNA Cartridge Kit (Cat# X8-GD-001-24) together with X8™ OneTouch Instrument (Cat# X8-OT-101-IN) and two widely used column-based manual DNA extraction kits as reference methods. All samples were normalized to equal final elution volume for fair comparison.

Spectrophotometry

Yield of the extracted gDNA was assessed using the Invitrogen Qubit 4 Fluorometer in conjunction with Qubit 1X dsDNA HS Assay Kit (ThermoFisher Scientific).

Real-Time PCR

1 µL of isolated gDNA was added to 20 µL of Real-Time PCR reaction mixture containing 10 µL of 2X SYBR Select PCR Master Mix (ThermoFisher Scientific) and primers specific to human *GAPDH* gene. The extracted gDNA were amplified under the following cycling conditions: 95 °C for 2 min for an initial denaturation, 45 cycles of 95 °C for 3 sec for denaturation, 58 °C for 10 sec for annealing and extension and the resulting PCR products were then run on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

Results and Discussion

In this study, gDNA was extracted from HeLa and NCI-H1975 human cell lines using the X8 Genomic DNA Cartridge Kit in conjunction with X8 OneTouch Instrument and two column-based manual DNA extraction kits as reference. The gDNA yield was assessed using the highly sensitive Qubit 1X dsDNA HS Assay Kit. Overall, the concentration of gDNA extracted using the Xceler8 Platform was either higher or comparable to the gDNA extracted using the two reference methods (Figure 1).

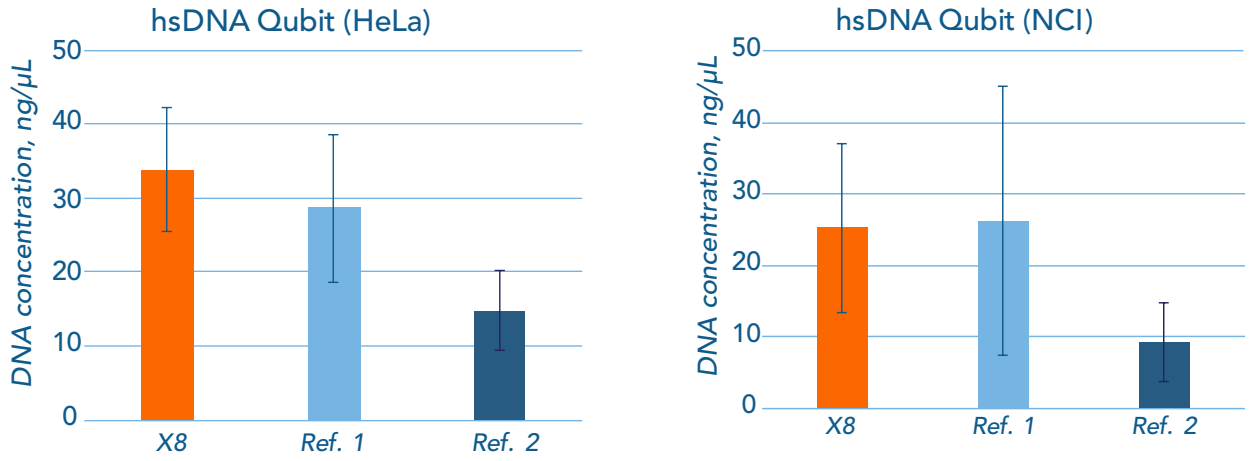

Figure 1. gDNA was isolated from HeLa (L) and NCI-H1975 cells (R) using the automated X8 Genomic DNA Cartridge Kit and two column-based manual DNA extraction kits (Ref. 1 and 2). The gDNA yield was then assessed using the highly sensitive Qubit 1X dsDNA HS Assay Kit.

Variability of gDNA yield was observed across the samples, since they were prepared in several batches in order to obtain representative set of data. This phenomenon was related to the samples batches, not performance of the kits used in this study. Also, quality of gDNA was tested by Real-Time PCR with human *GAPDH* gene specific assay on CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The Real-Time PCR results demonstrated either equivalency or superiority of the automated Xceler8 Platform compared to the two manual column-based kits used as reference (Tables 1 and 2, Figure 2).

Table 1. Real-Time PCR results for HeLa cells (~1 x 10^6 cells per sample).

DNA EXTRACTION PROTOCOL	NO. OF SAMPLES	AVE Ct Value	STANDARD DEVIATION
X8™ Genomic DNA Cartridge Kit	10	25.78	0.73
Manual method Ref. 1	6	26.46	0.73
Manual method Ref. 2	6	29.88	0.55

Table 2. Real-Time PCR results for NCI-H1975 cells (~1 x 10^6 cells per sample).

DNA EXTRACTION PROTOCOL	NO. OF SAMPLES	AVE Ct Value	STANDARD DEVIATION
X8™ Genomic DNA Cartridge Kit	9	27.52	1.38
Manual method Ref. 1	3	27.61	0.40
Manual method Ref. 2	2	32.56	1.87

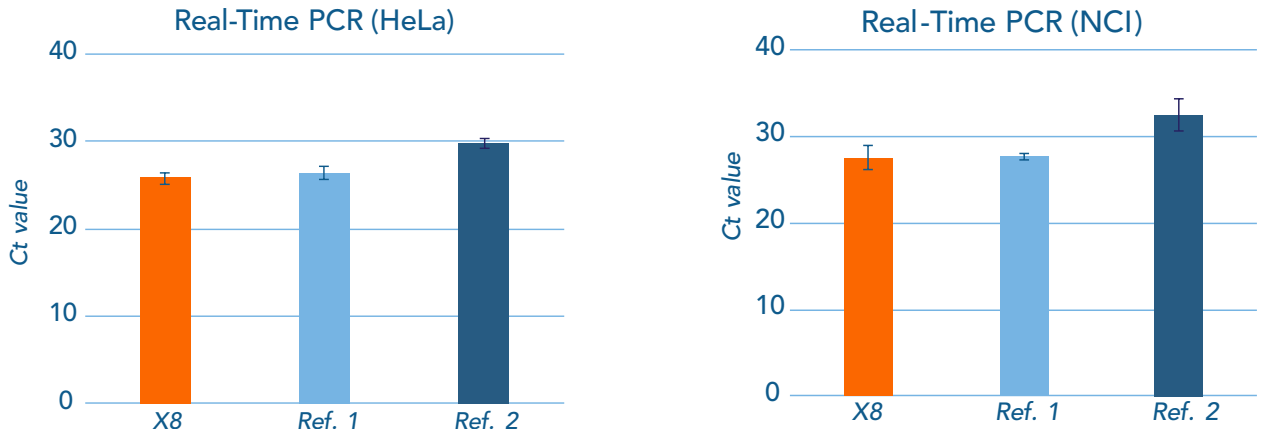

Figure 2. gDNA was isolated from HeLa (L) and NCI-H1975 cells (R) using the automated X8 Genomic DNA Cartridge Kit and two column-based manual DNA extraction kits (Ref. 1 and 2). Comparative Ct values* for *GAPDH* gene were quantified in a bar graph. *Ct values are inversely proportional to the amount of nucleic acid in the samples.

Conclusions

In this study we demonstrated the Xceler8 Platform ensures consistent and reproducible extraction of gDNA from cell culture samples across different users and that the extracted gDNA can be used for sensitive downstream applications such as Real Time-PCR. Overall results demonstrated the gDNA isolated using the Xceler8 Platform shows either equivalency or superiority when compared to the two manual column-based kits used as reference.

One BioMed's novel chemistry based technology simplifies the product architecture, thus making the load-and-go system ideal for labs looking for high flexibility at an affordable price. The fully integrated platform also eliminates the need for ancillary equipment and user-supplied reagents, thus providing users with a seamless and ***truly walk-away*** experience. To conclude, all of these features combined with the reliable technical performance makes the Xceler8 Nucleic Acid Extraction Platform ideal for labs that currently cope with manual nucleic acid extraction methods.

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