Automated extraction of genomic DNA from gram-positive bacteria using the Xceler8™ Nucleic Acid Extraction Platform

Introduction

Bacterial cells are surrounded by cell surface structures that allow them to thrive in extreme environments. Though cell walls of both gram-positive and gram-negative bacteria have peptidoglycan, the quantity, thickness, length distribution, and the degree of cross-linking is more extensive in gram-positive bacteria than in gram-negative bacteria. Therefore, isolating high quality genomic DNA (gDNA) from gram-positive bacteria requires additional steps and special lysis methods to overcome the strong peptidoglycan barrier and then disrupt the cell walls. Due to these added steps in the sample preparation workflow many laboratories prefer manual extraction methods. 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 a widely used column-based manual DNA extraction kit. The study demonstrates that the automated Xceler8 Platform is able to effectively isolate gDNA from gram-positive bacterial samples, without compromising its integrity, and the extracted gDNA is suitable for sensitive downstream applications such as Real-Time PCR.

Materials and Methods

Samples

Approximately 2 x 10^9 *Streptococcus agalactiae* bacterial cells were resuspended in 200 µL of 1X PBS and were used 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 *S. agalactiae* using the X8™ Genomic DNA Cartridge Kit (Cat# X8-GD-001-24) together with X8™ OneTouch Instrument (Cat# X8-OT-101-IN) as well as a column-based manual DNA extraction kit as a reference method. All samples were normalized to equal final elution volume for fair comparison.

Lysozyme treatment

To enhance lysis 3 mg of lysozyme was added to each sample. For the gDNA extracted using the Xceler8 Platform we compared two different lysozyme incubation workflows.

1) On-Board incubation. The sample with the lysozyme was transferred into the sample well of the X8 Genomic DNA Cartridge and the protocol for Gram Positive bacteria- Lysozyme was selected. This allowed for the lysozyme treatment to happen On-Board the X8 OneTouch Instrument. On-Board incubation simplifies workflow as user can add sample and lysozyme to the X8 Cartridge and select the appropriate automated protocol. However as lysozyme treatment takes additional time, it increases the

instrument run time per sample.

2) External incubation. The sample with lysozyme was incubated for 30 min at 37 °C on a heat block and then transferred to the sample well of the X8 Genomic DNA Cartridge and the Gram Positive bacteria protocol was selected. Since this workflow has external lysozyme treatment on a heat block, it restricts the instrument run time to only the automated extraction protocol, and is be a more efficient workflow for users processing multiple samples and are looking for shorter instrument run times. For the gDNA extracted using column-based manual DNA extraction kit the lysozyme treatment was done externally on the heat block.

Spectrophotometry

Yield of the extracted gDNA was assessed using NanoDrop™ 2000 Spectrophotometer (ThermoFisher Scientific) and the Invitrogen Qubit 4 Fluorometer in conjunction with Qubit 1X dsDNA HS Assay Kit (ThermoFisher Scientific).

Agarose gel electrophoresis

Integrity of the extracted gDNA was assessed by electrophoresis using 0.8% agarose gel. Pictures were taken by the GelDoc Go Imaging system (Bio-Rad). 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 target specific primer mix. Cycling conditions: 95 °C for 5 min for an initial denaturation, 40 cycles of 95 °C for 2 sec for denaturation, 60 °C for 10 sec for annealing and extension. The PCR was run on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

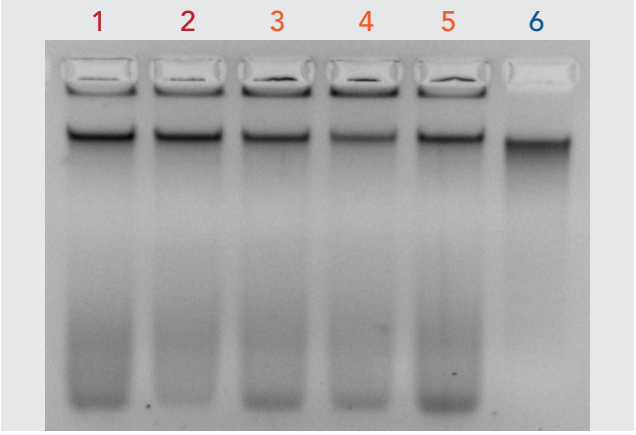

Figure 1. gDNA was isolated from gram-positive bacteria *S. agalactiae* using the Xceler8 Platform (lanes 1-2: X8 External incubation, lanes 3-5: X8 On-Board incubation) and a reference manual column-based DNA extraction kit (lane 6).

Results and Discussion

In this study, gDNA was extracted from *Streptococcus agalactiae* cells using the X8 Genomic DNA Cartridge Kit in conjunction with X8 OneTouch Instrument and one column-based manual DNA extraction kits as reference. 20 µL of each 200 µL eluate was then run on 1X TAE

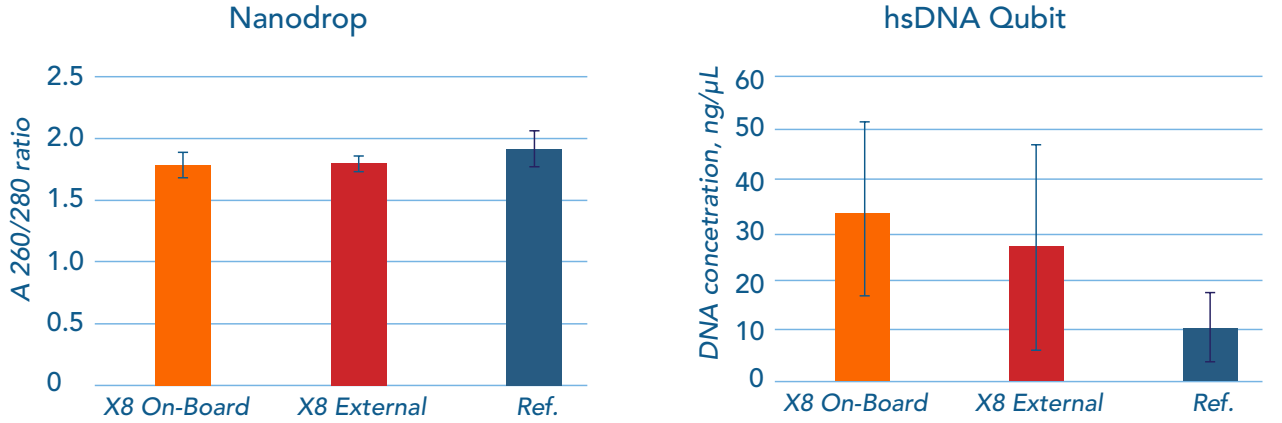

Figure 2. gDNA was isolated from *S. agalactiae* using two different workflows on the automated Xceler8 Platform and a column-based manual DNA extraction kit (Ref.). The extracted gDNA was characterized with respect to purity and yield using the NanoDrop™ 2000 Spectrophotometer (L) and the highly sensitive Qubit 1X dsDNA HS Assay Kit (R) respectively.

Table 1. Comparative spectrophotometric analyses of gDNA extracted from *S. agalactiae*.

DNA EXTRACTION PROTOCOL	SAMPLES	ABSORBANCE RATIO (260/280) (NANODROP)			DNA CONCENTRATION (ng/ µL QUBIT)	
		RANGE	AVE	SD	AVE	SD
X8 On-Board	51	1.50 – 1.94	1.78	0.09	33.31	17.68
X8 External	6	1.73 – 1.86	1.80	0.05	26.61	18.79
Column-based manual method	8	1.64 – 2.07	1.92	0.14	10.67	6.77

0.8% agarose gel to visually inspect the extracted gDNA (Figure 1). Extracted gDNA showed high integrity that is comparable to the reference method. To assess the purity and yield of the extracted gDNA, eluates were measured by two spectrophotometric methods- NanoDrop™ 2000 and Qubit 4 Fluorometer (Figure 2). Purity of the extracted gDNA was assessed by measuring ratio of absorbance at 260 nm and 280 nm by NanoDrop™ 2000. Column-based manual method and X8 External protocols showed comparable results, while there were several outliers when the X8 On-Board protocol was used (Table 1).

The gDNA yield was measured by highly sensitive Qubit 1X dsDNA HS Assay Kit. In most cases concentration of DNA extracted using the Xceler8 Nucleic Acid Purification System was either higher or comparable to those obtained using the reference method. However, both methods demonstrated a wide range of sample-to-sample variability in gDNA concentrations: from 5.61 to 59.00 ng/µL for the Xceler8 system and from 4.44 to 24.80 ng/µL for the reference method. This can be explained by variability between several batches of samples prepared and run at different time.

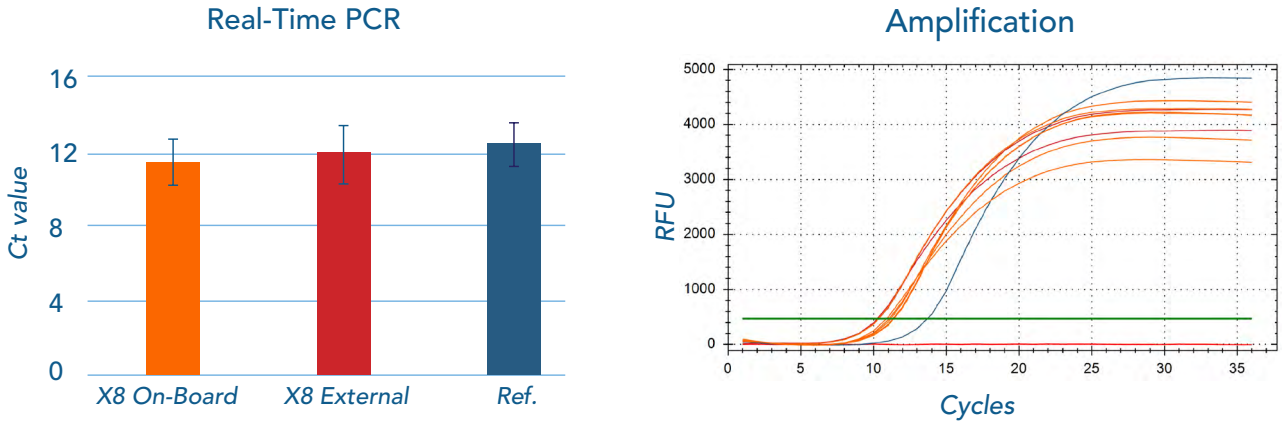

Figure 3. gDNA was isolated from *S. agalactiae* using two different protocols on the automated Xceler8 Platform (On Board and External incubation) and a column-based manual DNA extraction kit (Ref.). Comparative Ct values* were quantified in a bar graph (L). Real-Time PCR plot (R) Blue curve – gDNA template from the sample extracted by reference method, orange and red curves – DNA templates from the samples extracted by the X8-On Board and External incubation respectively. The flat green line is the Negative Control. *Ct values are inversely proportional to the amount of nucleic acid in the samples.

Table 2. Real-Time PCR results for *S. agalactiae* (~2 x 10^9 cells per sample).

DNA EXTRACTION PROTOCOL	NO. OF SAMPLES	AVE CT VALUE	STANDARD DEVIATION
X8 On-Board	51	11.57	1.26
X8 External	6	12.11	1.48
Column-based manual method	8	12.53	1.27

Additionally, quality of gDNA was assessed by Real-Time PCR with *S.agalactiae* specific assay on CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The Real-Time PCR results demonstrated the slight superiority of the automated Xceler8 Platform over the manual column-based kit (Figure 3). The average Ct values were 0.9 and 0.4 earlier for X8 On-Board and External incubation protocols respectively (Table 2). For most samples results obtained using the Xceler8 Platform were either superior or comparable to the manual column-based reference method.

Conclusions

In this study we demonstrated the Xceler8 Platform ensures consistent and reproducible extraction of gDNA from gram-positive bacteria (e.g., *S. agalactiae*) samples across different users. For the lysozyme treatment, the Xceler8 Platform supports two different workflows: 1) On-Board the X8 OneTouch Instrument (increased instrument run time). 2) External- (requiring the sample to be transferred to the X8 Genomic DNA Cartridge after 30 min). gDNA isolated using the X8 On-Board method showed slightly better performance than the X8 External method. Overall, results demonstrated the gDNA isolated using the Xceler8 Platform shows either equivalency or superiority when compared to the widely used manual column-based kit used as reference and that the extracted gDNA can be used for sensitive downstream applications such as Real Time-PCR.

One BioMed's novel chemistry based approach 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 provides 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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