

Automated nucleic acid purification with the chemagic™ Body Fluid 1k kit on the BioQule™ NGS system

Introduction

The increase of decentralization in diagnostic efforts is demanding an easy to use and reliable automated sample preparation systems in a low-throughput settings. Revvity's BioQule™ NGS System, a fully automated, walkaway sample preparation system, can automate the nucleic acid (NA) Purification for up to 16 samples in a single workflow. Here, the BioQule™ NGS System used chemagic™ Body Fluid 1k Kit (CMG-1049-BQ) to purify high-quality DNA and RNA from input of 40 µL Human Whole Blood. The purified samples are downstream processed for real-time polymerase chain reaction (qPCR) and next-generation sequencing (NGS). For complete automated workflow of NA purification from whole blood to a sequencing ready library preparation on the BioQule™ NGS System, please refer to the application note "Bleed to Read - From Blood Samples to Ready-To-Use Library For NGS."

For research use only.
Not for use in diagnostic procedures.

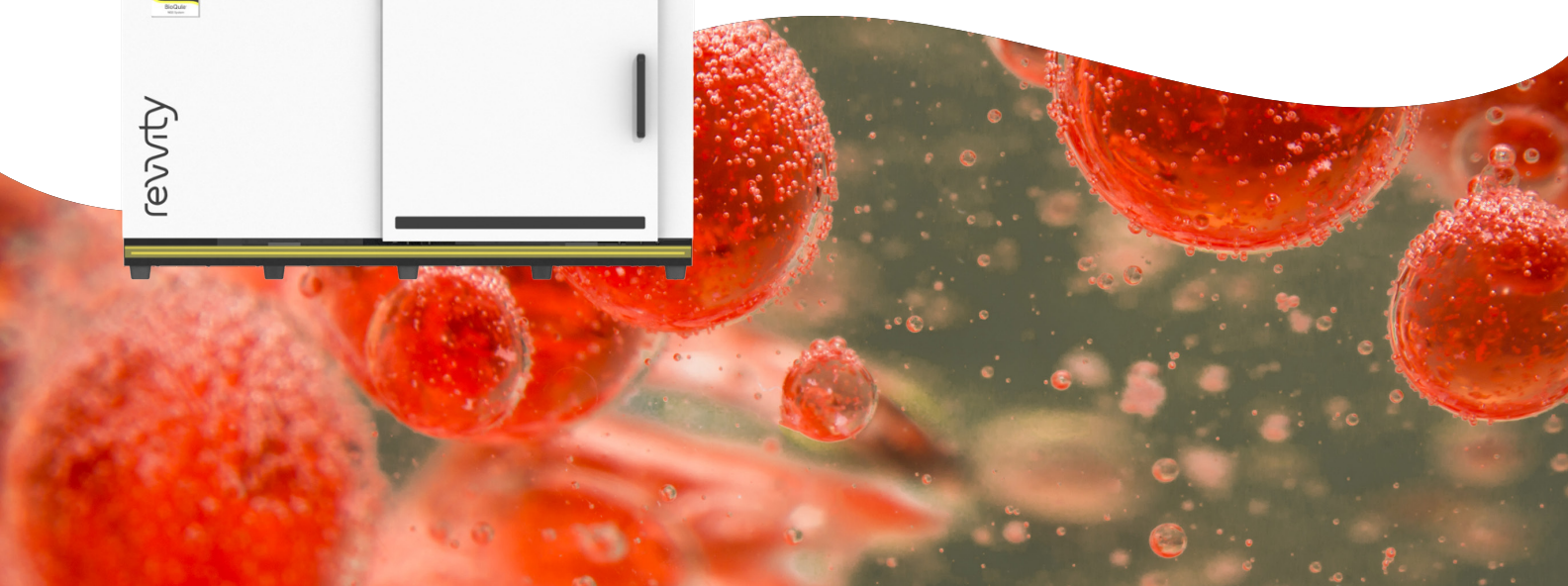
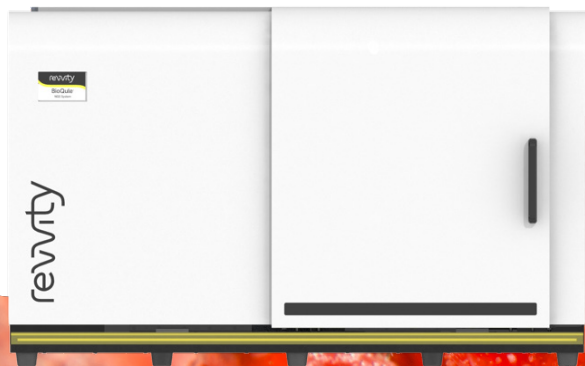
Manual Workflow



Automated Workflow



Figure 1. Manual and Automated Workflow comparisons of chemagic™ Body Fluid 1k Kit (CMG-1049-BQ) for Human Whole Blood



Methods

Total of 32 Human Whole Blood samples in Sodium Citrate anticoagulants were used at 40 µL sample volume for the purification protocol in BioQule™. NA Purification was tested at two different sample storage conditions - Fresh (stored at 4° C, analyzed 1 - 7 days after collection) and Frozen (stored at -20° C for at least 48 hours after collection). The blood samples were collected from a donor with White Blood Cell count of $\frac{7.5 \times 10^9}{\mu\text{L}}$. The purified NA was eluted in 30 µL of elution buffer using chemagic™ Body Fluid 1k Kit (CMG-1049-BQ)*.

The general workflow comparison is visualized in Figure 1. An Agilent BioTek Gen5™ Microplate Reader and Imager software was used to assess the quality (A_{260}/A_{280}) of the output, whilst Fisher® Scientific Qubit® Flex Fluorometer was used to assess the quantity (ng/µL) of the DNA.

The purified NA were further downstream processed for library preparation using NEXTFLEX® Rapid XP V2 DNA-Seq kit (for Illumina® Platforms) at 50 ng total DNA input and 4 PCR cycles. A LabChip® GX Touch™ nucleic acid analyzer was used to assess the size range of the library outputs. Additionally, a standard qPCR protocol targeting Homo sapiens gene Albumin (ALB) was performed with 5 µL of eluates. An Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System was used here.

* Note chemagic™ Body Fluid 1k Kit (CMG-1049) can isolate NAs from body fluids such as blood, serum, plasma, urine, stool suspensions, nasal-, vaginal- and buccal swabs, sputum, cerebrospinal fluid, puncture, cell suspensions, amniotic fluid, and others.

Results

Nucleic Acid Purification

The BioQule™ NGS System was able to yield comparable concentrations of purified double-stranded DNA (dsDNA) from Whole Blood. With an input of 40 µL of Fresh Human Whole Blood, a 30 µL eluate of 20.6 - 37.0 ng/µL dsDNA was extracted, while for Frozen Human Whole Blood samples 17.3 - 38.8 ng/µL of dsDNA was extracted.

High quality DNA is required for further downstream processes where the absorbance measured at 260 nm wavelength are commonly used to quantify DNA. A_{260}/A_{280}

ratios of 1.8 - 2.0 often indicates comparatively pure DNA. Extracts from the BioQule™ NGS System exhibited an average ratio of 1.98 across each storage condition.

qPCR amplification and quantification

The qPCR protocol targeted the ALB gene from the DNA extracts. The extracts had a Quantification Cycle (Cq) that was in the expected range (Table 1).

Table 1. Average quantity and quality metrics of the extracts from Human Whole Blood using the BioQule NGS system.

Storage conditions	dsDNA yield per mL of sample input (µg/mL)	A_{260}/A_{280}	Cq
Fresh	25.0	1.98	24.5
Frozen	25.1	1.98	24.9

Library Preparation

The purified DNA extracts from Human Whole Blood using the BioQule NGS system yielded consistent sequencing-ready libraries using NEXTFLEX® Rapid XP V2 DNA-Seq kit (For Illumina® Platforms) at normalized 50 ng of DNA input and 4 PCR cycles. (Figure 2).

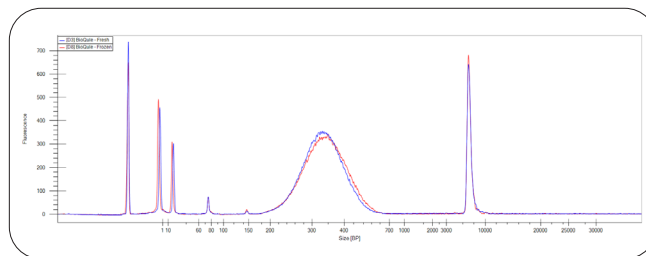


Figure 2. Comparison of library traces generated from gDNA purified using the BioQule NGS system.

Conclusion

The chemagic™ Body Fluid 1k Kit (CMG-1049-BQ) chemistry automated on the BioQule NGS System allows for a robust purification of genomic DNA from 40 µL Human Whole Blood for downstream processing of qPCR quantification and NGS library preparation.



Learn more about BioQule™ NGS System



Learn more about automated nucleic acid purification with chemagic™ technology



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