APPLICATION NOTE

Bleed to read - from blood samples to ready-to-use library for NGS.

Introduction

Next-Generation Sequencing (NGS) is becoming readily accessible, however, combining an automated nucleic acid (NA) purification from biological samples with automated preparation of sequencing ready library still stands as bottleneck for ease use in NGS protocols. Part of this is due to the lack of a reliable and user-friendly complete automation system for low throughput NA purification and library preparation. Revvity's BioQule™ NGS System can fully automate both the NA purification and library preparation for up to 8 samples in a single workflow, reducing the risk of human error and minimizing hands-on time to only 20 minutes. This fully walk-away sample preparation and quantification benchtop instrument comes at low-cost for automating library preparation from Human Whole Blood. The results in this application note are focused on utilizing the BioQule Bleed to Read kit on the BioQule NGS System for Whole Genome Sequencing (WGS) with enzymatic fragmentation.

BioQule™ NGS System

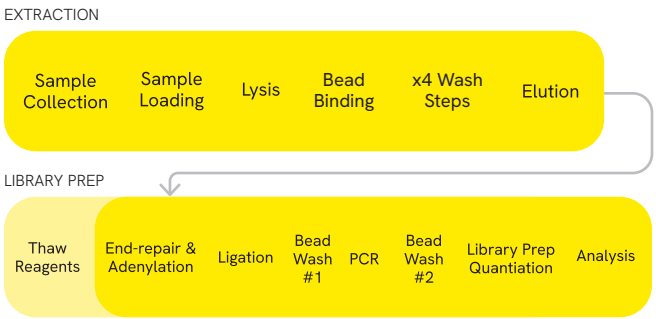
Methods

A total of 24 samples of Human Whole Blood were collected with both Sodium Citrate and EDTA anticoagulants for downstream purification and library preparation. The BioQule Bleed to Read kit (P/N 900-000021) was used for nucleic acid extraction and library preparation from 40 µL of sample input. Both the sample types were tested at two different storage conditions - Fresh (stored at 4° C, processed 1 - 7 days after collection) and Frozen (stored at -20° C for at least 48 hours after collection).

The NEXTFLEX™ Unique Dual Index Barcodes (NOVA-514150) at 4 PCR cycles were used for library preparation. A LabChip™ GX Touch™ nucleic acid analyzer was used to assess the size range and yield of the library outputs.

All the results of Automation workflow (BioQule NGS system) and Manual workflow were compared in this application note (Figure 1). The MiniSeq® platform at 2x150 (Illumina®, San Diego, CA) was used to sequence all samples.

Manual Workflow



Automated Workflow

Figure 1: Comparison of Manual and Automated Workflow for nucleic acid purification from Human Whole Blood and library preparation for NGS.

Results

The final volume of generated library was 35 µL where both the automated workflow and manual workflow exhibited similar yield and molecular weight range with no adapter dimers across all conditions (Sodium citrate and EDTA anticoagulants at 4° C and -20° C) (Figure 2).

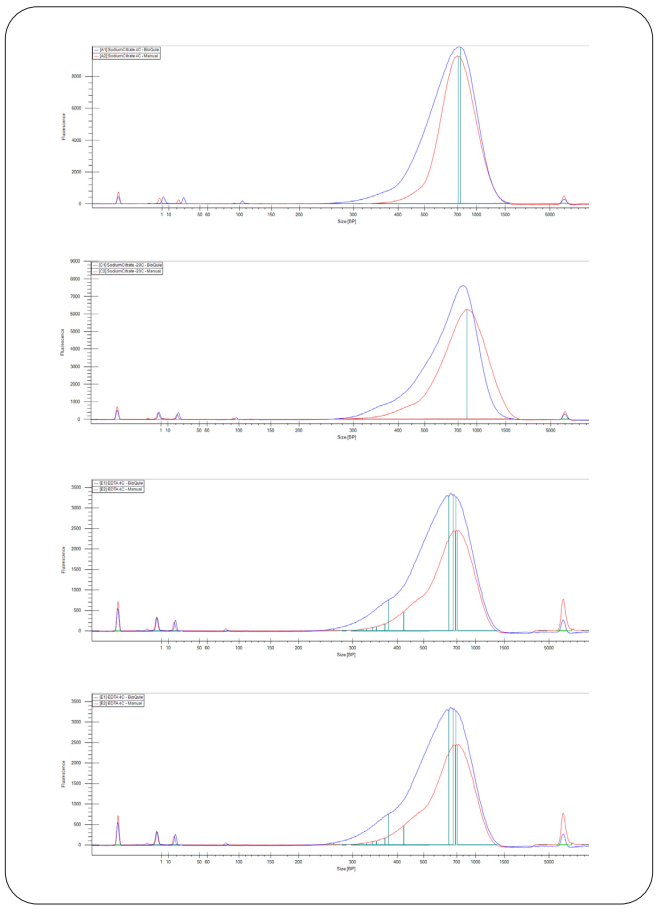


Figure 2: Comparison of electropherogram traces from Automated workflow (blue) and Manual workflow (red) for sample preparation at conditions: (A) Sodium Citrate sample kept at 4° C, (B) Sodium Citrate sample kept at -20° C, (C) EDTA sample kept at 4° C, and (D) EDTA sample kept at 4° C.

The sequencing quality of the libraries are evaluated by the insert sizes across each condition as well as analysis of the GC bias. The results show comparable insert sizes with limited variance (Figure 3) and exhibit minimal variability in normalized coverage across the 20 – 60 % GC windows (Figure 4).

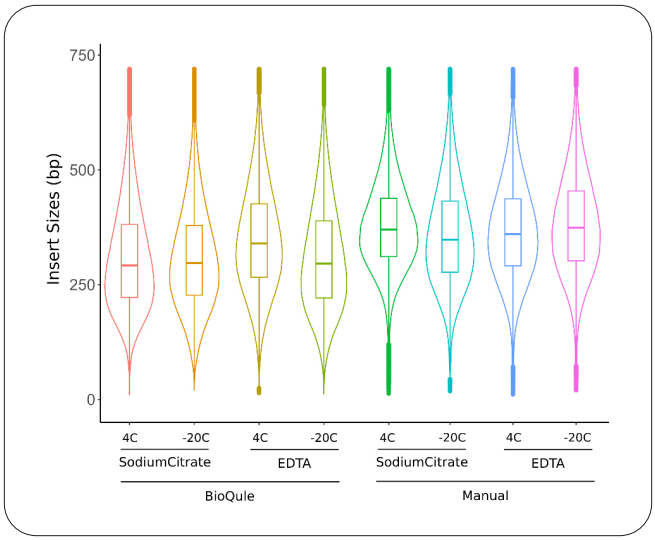


Figure 3: Comparison of insert sizes for sequencing in libraries prepared from BioQule NGS system and Manual workflow at each anticoagulant and storage conditions (Sodium Citrate and EDTA anticoagulant samples at Fresh and Frozen storage conditions)

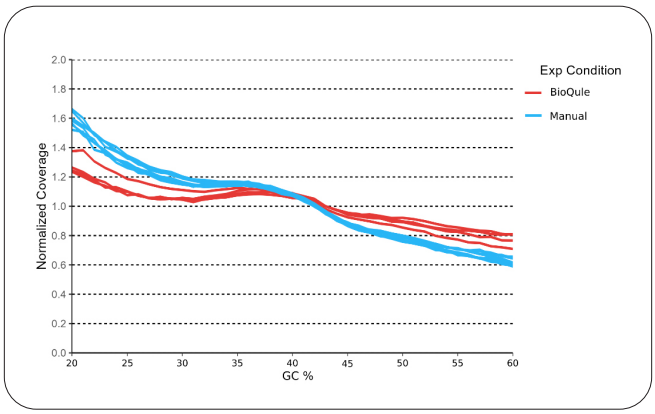


Figure 4: Analysis of GC bias grouped in BioQule NGS system and Manual workflow. Minimal variability in normalized coverage is seen across the ~95 % of the human genome represented by the windows of 20 – 60 % GC content.

Conclusion

The BioQule NGS System can fully automate the preparation of consistent sequencing-ready libraries from Human Whole Blood samples, whether with Sodium Citrate or EDTA anticoagulants at Fresh (stored at 4° C) or Frozen (stored at -20° C) storage conditions. The BioQule Bleed to Read kit (P/N 900-000021) is adopted on the BioQule NGS system to produce up to 8 libraries in a single run with expected size range and yield. The automated workflow requires only 20 minutes of user hands-on time, making the full workflow from blood sample to NGS sequencing ready libraries accessible to labs of all sizes and technical expertise.

Learn more about Bioqule NGS System

Learn more about automated nucleic acid purification with chemagic™ technology