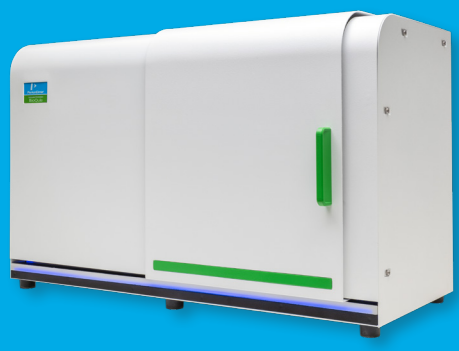
APPLICATION NOTE

NEXTFLEX® Rapid XP DNA-Seq Kit Automated on the BioQule™ NGS System

BioQule™ NGS System



Introduction

The proliferation of next-generation sequencing (NGS) requires the development of easy to use, robust and fully automated workflows to enable the decentralization of NGS library formation. Integrated enzymatic fragmentation, such as in the NEXTFLEX Rapid XP DNA-Seq kit, is a simpler more streamlined workflow for NGS library preparation. To meet this demand, PerkinElmer has automated the NEXTFLEX Rapid XP DNA-Seq kit, on the new BioQule NGS System: a fully automated, walkaway sample preparation system for preparing up to 8 libraries simultaneously. The BioQule NGS System can automate an endless library of NGS methods through the incorporation of an integrated thermal cycler, an on-board bead wash system, easy to use user interface and an integrated optical device.

Methods

70 libraries were generated using the NEXTFLEX Rapid XP DNA-seq kit for Illumina® Platforms reagent kit, automated on the PerkinElmer BioQule NGS System from 10ng of Covaris-shared E. coli gDNA. A visual comparison of the manual vs automated workflow can be seen in Figure 1. NEXTFLEX® barcodes were also used. 9 cycles of PCR amplification were performed. An Agilent® Bioanalyzer® System was used to assess the molecular weight range of the library outputs, a Fisher® Scientific Qubit® Flex Fluorometer was used to assess the quantity of the library outputs. The times involved in each step of the automated workflow can be seen in Figure 2.

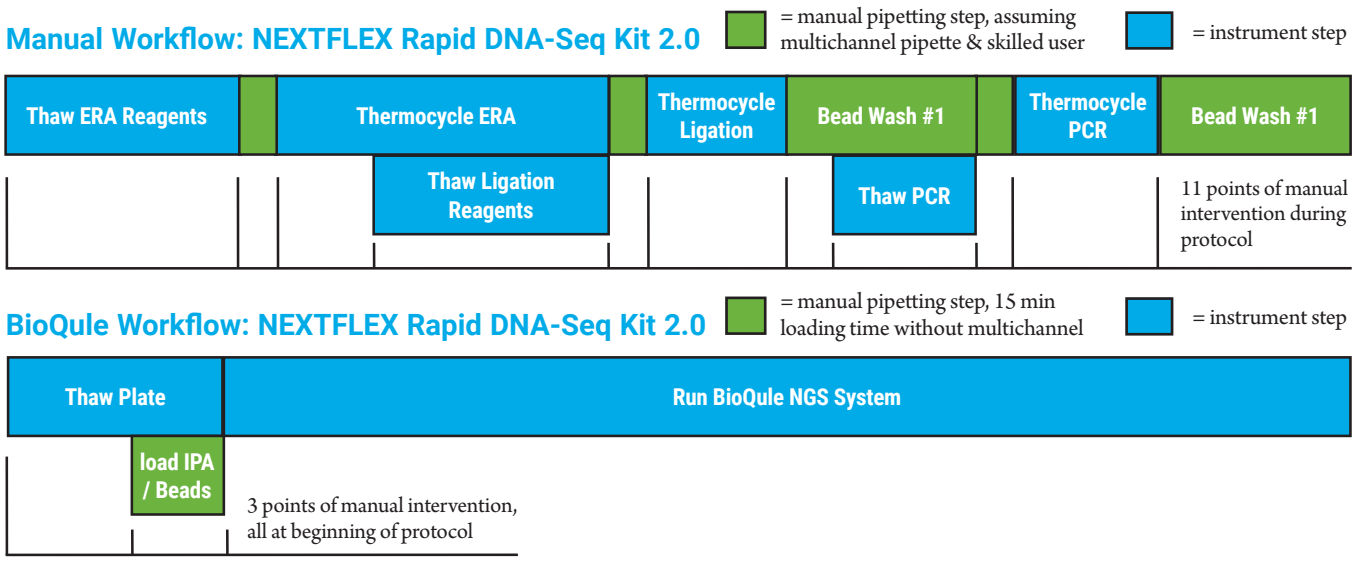


Figure 1. *Manual Workflow vs BioQule NGS Workflow*

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Figure 2. *NEXTFLEX Rapid XP DNA-Seq Workflow on the BioQule NGS System*

Quality Control Results:

Quality control is an important aspect of the NGS workflow, both to verify that libraries were made correctly and to normalize library yields before pooling for sequencing. When performing the manual version of these assays, quality control is performed on an Agilent® Bioanalyzer® System and through a fluorescent concentration measurement device, such as Fisher® Scientific Qubit® Flex Fluorimeter.

On the BioQule NGS System, fluorescent quantification of the library outputs is integrated into the automated workflow, avoiding the need for manual quantification of libraries. To demonstrate the accuracy of the BioQule quantification, calculated outputs were compared between the BioQule NGS System and Fisher® Scientific Qubit® Flex Fluorimeter (Figure 3).

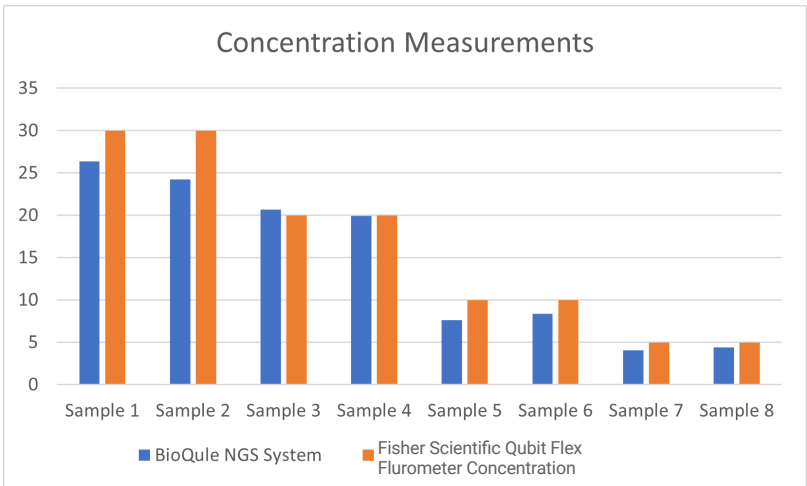
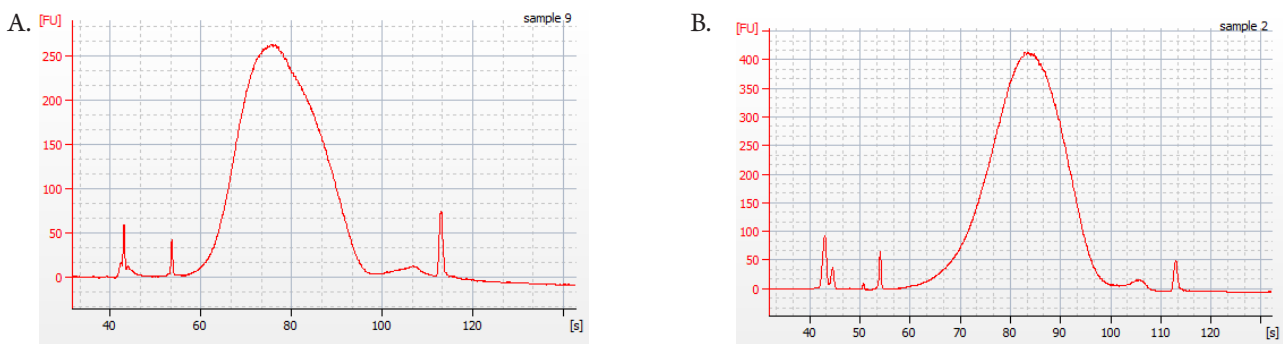


Figure 3. *The BioQule NGS System concentration measurements compared to the Fisher® Scientific Qubit Flex Fluorimeter concentration measurements of a known standard curve. The BioQule NGS System measures concentrations equivalent to the gold standard.*

Library Results:



Manual Library Prepared with the NEXTFLEX Rapid XP DNA-Seq Kit

Library Prepared by the BioQule NGS System Using the NEXTFLEX Rapid XP DNA-Seq Kit

Figure 4. *Library traces measured for (A) manual and (B) automated 10 ng E. coli inputs on the Agilent® Bioanalyzer® System.*

The automated protocols generated NGS libraries with higher yields than the libraries constructed manually. With an input of 10 ng of DNA and 9 PCR cycles, 33 µL of 13-20 ng/µL library DNA was generated. When manual and automated libraries were measured for sizing with the Agilent® Bioanalyzer® System, similar yield and molecular weight range were observed (Figure 4).

Following the quantitation and sizing of the final libraries, samples were sequenced on an Illumina® MiSeq® sequencer. Libraries generated using the BioQule platform generated commensurate performance to those generated by an experienced lab technician. Manual and automated preparations of the E. coli libraries featured comparable values for relevant sequencing metrics including GC percentage, depth of coverage and average insert size (Figure 5).

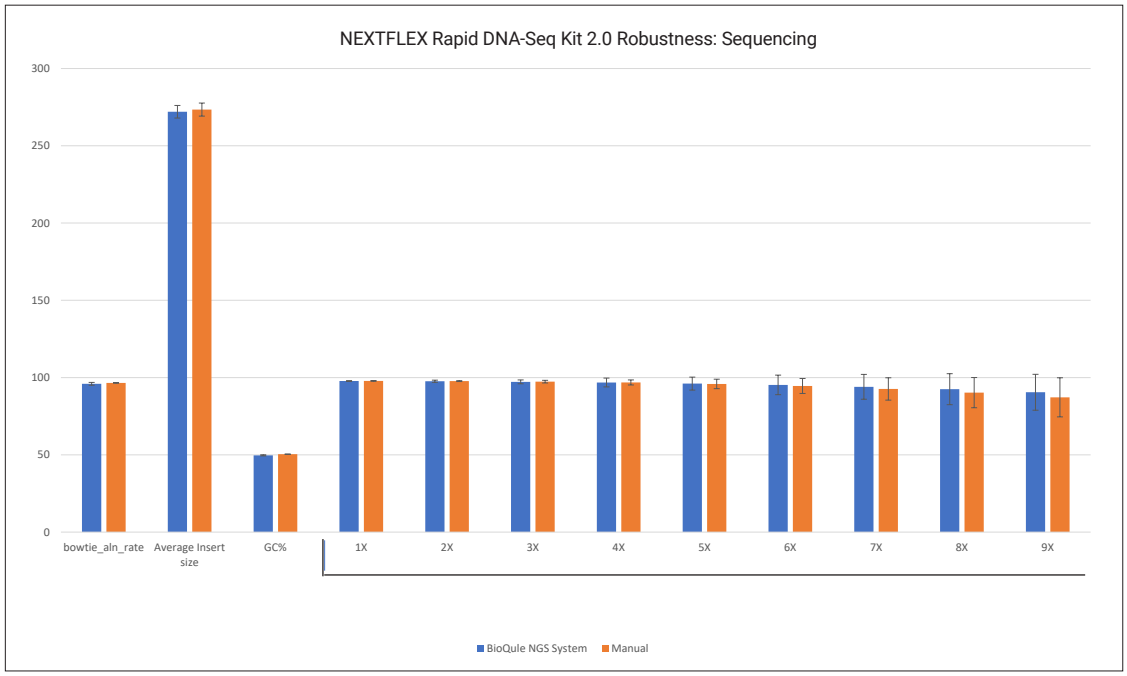


Figure 5. *Depth of Coverage of Sequencing Data*

Conclusion

The NEXTFLEX® Rapid XP DNA-Seq Kit automated on the BioQule NGS System allows for the robust generation and quantification of high-quality sequencing libraries from 10ng of E. coli gDNA with a 95% success rate. This allows the creation of up to 8 libraries with only 15 minutes of hands-on user time. Fluorescent quantification of library outputs, such as the fluorescent quantification on the BioQule NGS system, allows for easy normalization of libraries without the purchase of an additional instrument or increase in hands on time.

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