**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. To meet this demand, PerkinElmer has released the new BioQule NGS System: a fully automated, walkaway sample preparation system for preparing up to 8 libraries simultaneously. Through the incorporation of an integrated thermal cycler, an on-board bead wash system, easy to use user interface and integrated optical device, the BioQule NGS System can automate and quantify an endless library of NGS methods.

**Methods**

56 libraries were generated using the NEXTFLEX Rapid DNA-Seq Kit 2.0 for Illumina® Platforms reagent kit, automated on the PerkinElmer BioQule NGS System from 20 ng 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. A LabChip® GX Touch™ nucleic acid analyzer 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.

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**Manual Workflow: NEXTFLEX Rapid DNA-Seq Kit 2.0**

- Thaw ERA Reagents
- Thermocycle ERA
- Thermocycle Ligation
- Bead Wash #1
- Thermocycle PCR
- Bead Wash #1
- Run BioQule NGS System

**BioQule Workflow: NEXTFLEX Rapid DNA-Seq Kit 2.0**

- Thaw Plate
- Run BioQule NGS System

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For research use only. Not for use in diagnostic procedures.
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 a LabChip GX Touch nucleic acid analyzer and through a fluorescent concentration measurement device, such as a 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 concentrations were compared between the BioQule NGS System and Fisher Scientific Qubit Flex Fluorimeter (Figure 3).

Library Results:
The automated protocols generated NGS libraries with higher yields than the libraries constructed manually. With an input of 20 ng of DNA and 9 PCR cycles, 33 µl of 35-65 ng/µL library DNA was generated. When manual and automated libraries were measured for sizing with the LabChip GX Touch nucleic acid analyzer, 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 (Figure 5). Manual and automated preparations of the E. coli libraries featured comparable values for relevant sequencing metrics including GC percentage and Alignment rate (Figure 6).
Figure 4. Library traces measured for (A) manual and (B) automated E. coli inputs on the LabChip® GX Touch™ nucleic acid analyzer.

Figure 5. The libraries prepared with the BioQule NGS System showed comparable values for sequencing metrics to libraries prepared manually.
Conclusion

The NEXTFLEX Rapid DNA-Seq Kit 2.0 automated on the BioQule NGS System allows for the robust generation and quantification of high-quality sequencing libraries from 20ng of E. coli gDNA with a 98% 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 an increase in hands on time.

Figure 6. Display of sequencing quality metrics. GC bias and Alignment are all comparable between E. coli libraries prepared manually and on the BioQule NGS System.