APPLICATION NOTE

Automating NGS library preparation with the NEXTFLEX rapid XP V2 DNA-Seq kit.

Introduction

The Sciclone® and Zephyr® family of NGS workstations are ideal for rapid and reliable NGS library construction. With the extensive library of pre-developed, vendorqualified protocols, users can quickly automate their NGS workflows. Automating library construction workflows reduces hands-on time and variability. This solution of the liquid handling workstations and NEXTFLEX® Rapid XP V2 DNA-Seq kit, combined with NEXTFLEX proprietary normalization beads produces consistent amount of DNA for all samples in a library pool. Thus, shortening the time needed for quantification and pooling preparation for sequencing by up to 3 hours/96 sample. The bead-based normalization protocol binds a specific amount of DNA to the beads and after elution the libraries have approximately the same concentration even when differing inputs are used. An automated workflow for each workstation has been demonstrated in Figure 1.

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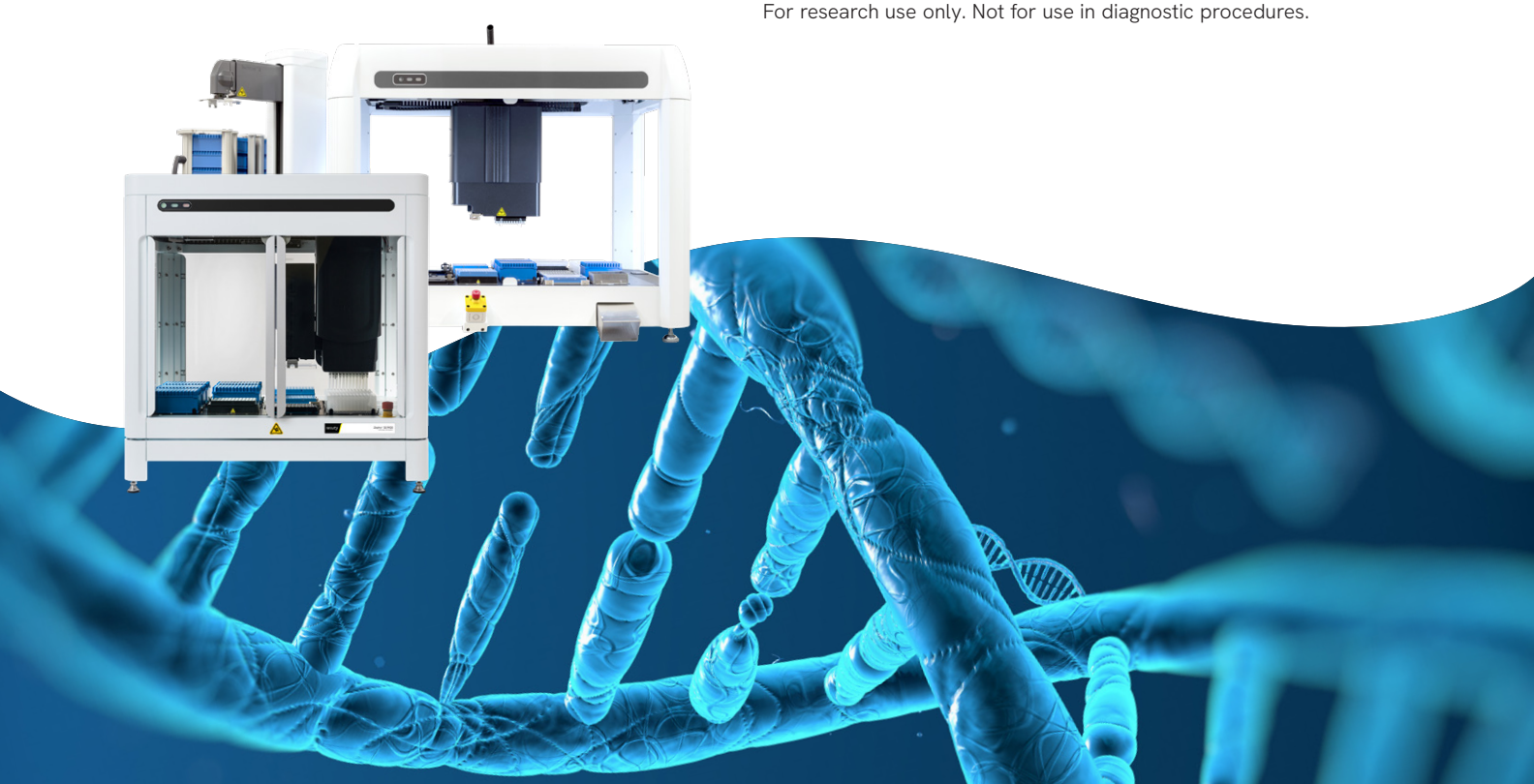

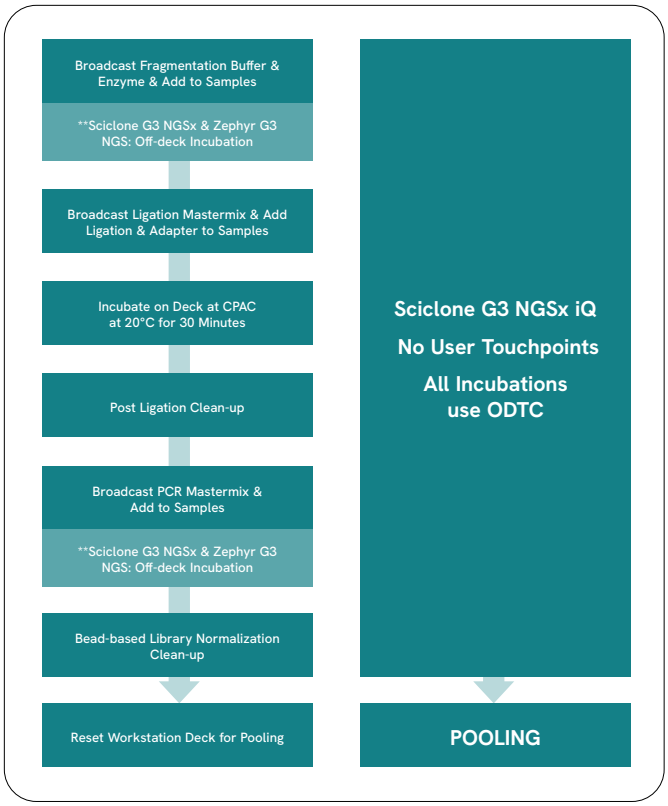

Figure 1. Automated workflow of NEXTFLEX Rapid XP V2 DNA-Seq on the Zephyr, Sciclone G3 NGSx, and Sciclone NGSx iQ Workstations. The Sciclone G3 NGSx iQ workstation utilizes an ODTC (On-Deck Thermal Cycler) and does not require user interactions during automated workflow. Note: **User touchpoint if running samples on Zephyr NGS or Sciclone G3 NGSx workstations.

Experimental setup

To demonstrate the automation of the NEXTFLEX Rapid XP V2 DNA-Seq kit on the workstations, a sample plate was prepared for each system with 24 Human Promega DNA. Each well contained a total of 10 ng input. Samples were dispensed to the first and last row of the 96 well plate (A and H).

Application method

To start the application, the user will open a library prep workbook, indicate the number of columns to run and then aliquot the reagents based on the volumes indicated (figure 2). The user will then proceed to start the application (figure 3) and the software will prompt the user to enter the number of columns being processed, starting column for the adapter plate (figure 4) and then the software will display images guiding the user through deck set up (figure 5). Once the deck is set up correctly, the user will click Finish to start the method.

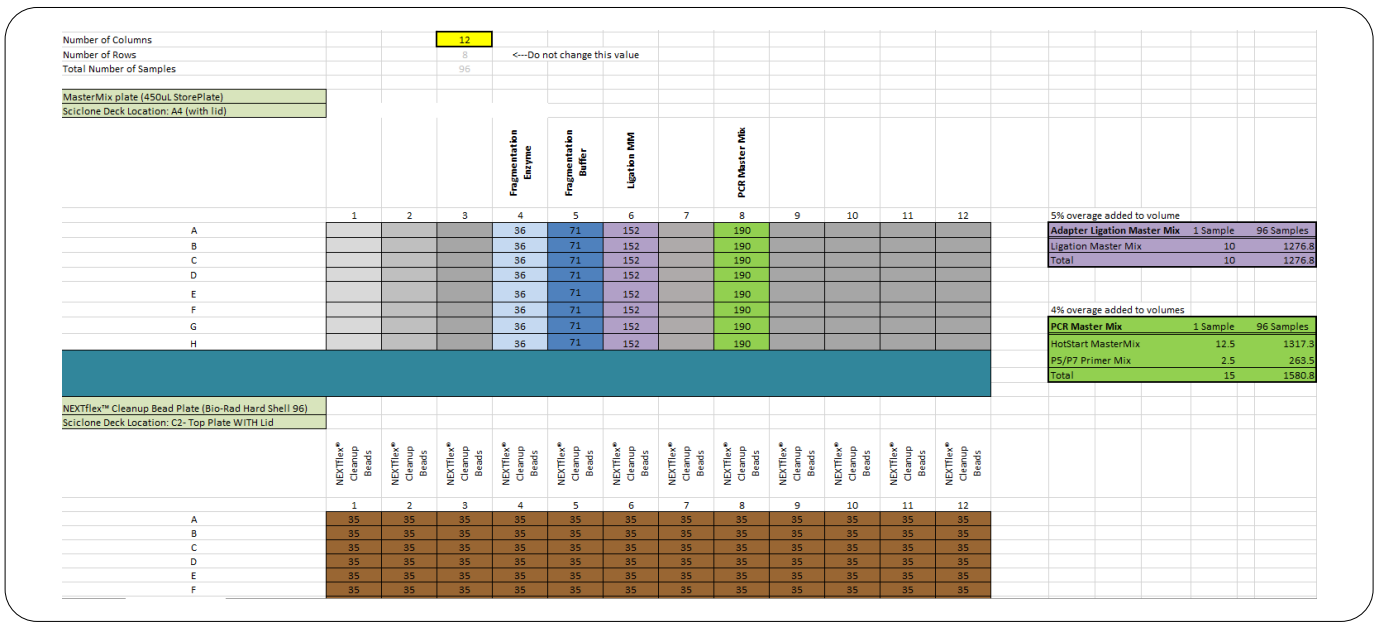

Figure 2. NEXTFLEX Rapid XP V2 DNA-Seq Workbook. The workbook details plate type, layout, deck location and reagent volumes calculated from user input.

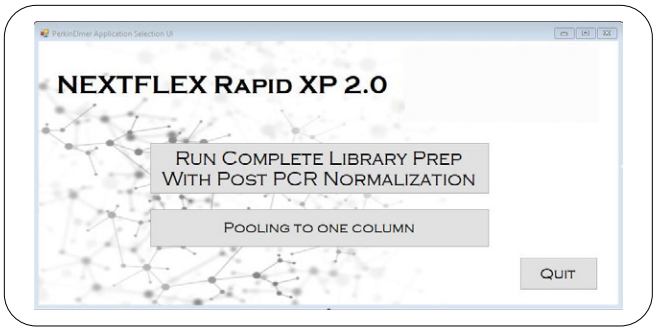

Figure 3. NEXTFLEX Rapid XP V2 DNA-Seq start of software user interface.

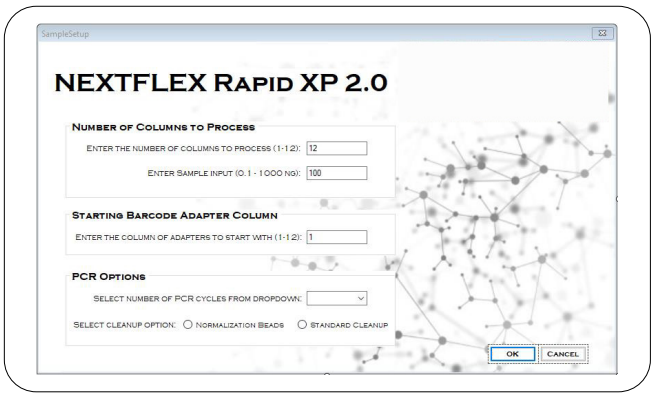

Figure 4. NEXTFLEX Rapid XP V2 DNA-Seq user interface for Sciclone G3 NGSx iQ workstation.

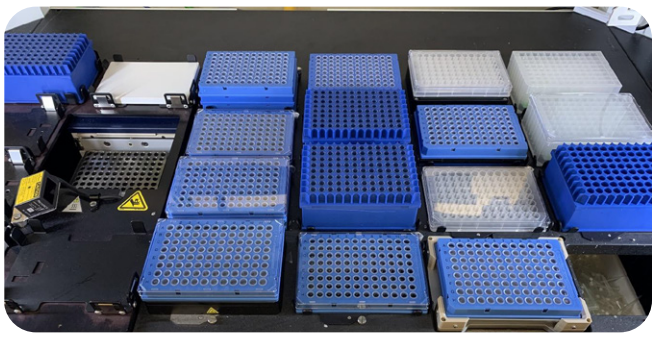

Figure 5. Final deck layout of NEXTFLEX Rapid XP V2 kit on Sciclone G3 NGSx iQ.

The workstation will start the liquid handling by broadcasting the fragmentation buffer and enzyme to 96 well plates. The sample is then aspirated, added to the fragmentation buffer and finally added to the fragmentation enzyme. Depending on the workstation, this will either trigger (i) a software prompt for the user to remove the plate for off deck incubation (Zephyr and Sciclone G3 NGSx workflow) or (ii) the integrated gripper will move the plate into the on-deck thermal cycler (ODTC) on Sciclone G3 NGSx iQ instrument. While the samples are incubating, the liquid handler will broadcast the adapter ligation master mix to a clean plate.

Post incubation, the sample plate is placed back onto the on-deck shaker (Zephyr manually or Sciclone automatically) and the adapter ligation master mix and barcode adapters are added to the samples. The samples are then incubated at 20°C for 30 minutes. Once the sample finish incubating, the adapter ligated DNA goes through a bead purification with NEXTFLEX beads and washed twice with 80% ethanol. The samples are eluted in water, then added to the PCR master-mix. After the samples are mixed, the software will i) on Zephyr and Sciclone G3 NGSx instruments prompt the user to remove and thermal cycle the plate or ii) on Sciclone G3 NGSx iQ instrument the integrated gripper will move the plate to the ODTC and in both cases, the software will thermal cycle at the following conditions:

- 98°C for 45 seconds
- 7 cycles* of PCR at 98°C for 15 seconds, 60°C for 30 seconds and lastly 72°C for 30 seconds
- 72°C for 1 minute

**** Number of PCR Cycle depends on the input DNA. This information is available on the kit user manual***

Once the PCR protocol is finished in the thermal cycler, the samples are returned on deck, normalization beads are added to plate and incubated for 8 minutes. The samples will go through a total of two ethanol washes and eluted in elution buffer. Each sample is quantified using Thermo Fisher Scientific's Qubit® dsDNA HS Assay kit to check for recovery and examined using the LabChip® GX Touch™ nucleic acid analyzer for proper

Results

A set of 24 sample with 10 ng input was run on Sciclone G3 NGSx iQ, Sciclone G3 NGSx, and Zephyr G3 NGS workstations. NEXTFLEX Rapid XP V2 DNA-seq Kit utilizes normalization beads that helps in bypassing the quantification step. In our study we used the qubit to compare the average library yield between three workstations. All three workstations produced libraries within the expected range. The Zephyr G3 NGS workstation produced libraries with average yields of 2.42 ng/µL, Sciclone G3 NGSx workstation 3.36 ng/µL and Sciclone G3 NGSx iQ workstation 3.83 ng/µL. The expected yield after the full library prep workflow is >1 ng/ µL. The GX Touch instrument with NGS 3K Assay and X-Mark Chip was used to determine the size of the final libraries. The libraries aligned with the expected range with target peak ~425 bp (Figure 6). Sequencing metrics for the Sciclone NGSx iQ libraries are shown in Figures 7-9.

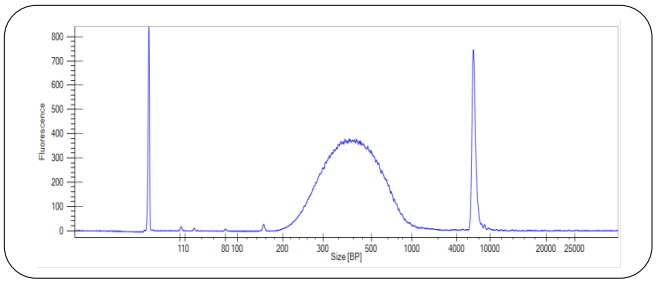

Figure 6. LabChip GX Touch trace of final library using the NGS 3K Assay and X-Mark LabChip.

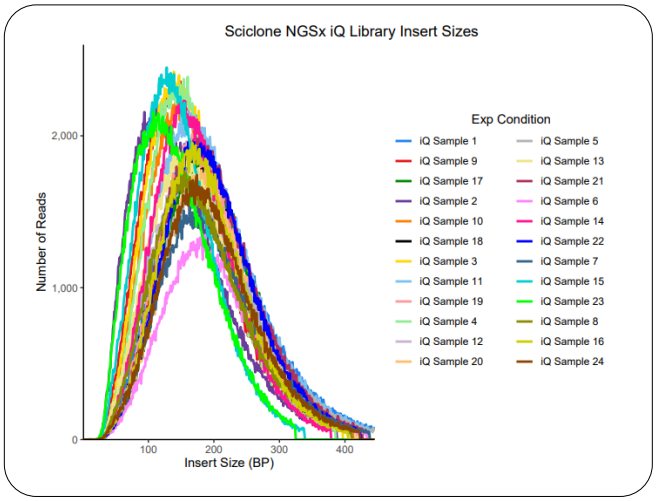

Figure 7. Insert size for 24 libraries prepared on the Sciclone NGSx iQ workstation.

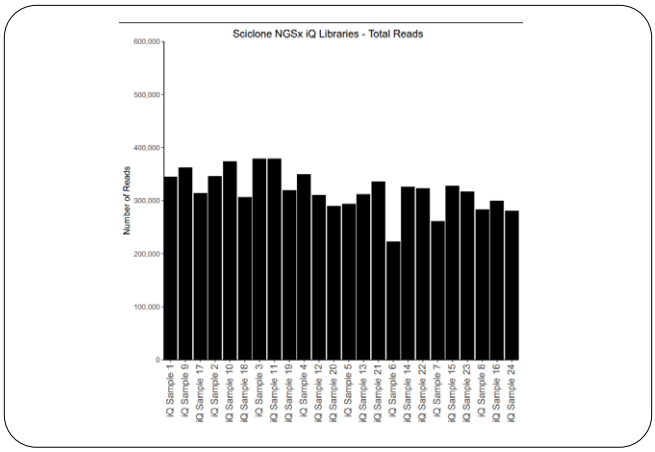

Figure 8. Total reads for 24 sequenced libraries prepared on the Sciclone NGSx iQ workstation.

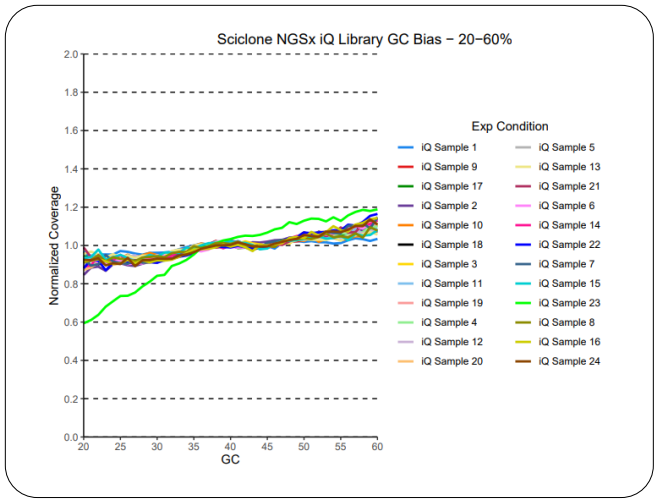

Figure 9. GC Bias for 24 sequenced libraries that were prepared on the Sciclone NGSx iQ workstation.

Conclusions

NEXTFLEX Rapid XP V2 DNA-Seq kit automation on the Zephyr and Sciclone liquid handling workstations, combined with NEXTFLEX proprietary normalization beads produced consistent libraries ready to be sequenced on an Illumina® sequencing platform. The use of normalization beads reduced the cost and saved time for the user and required only limited access to the Thermo Fisher® Scientific Qubit® and/or Agilent® fragment analyzer. The normalized libraries were prepared in as little as 6 hours with only 2X user intervention on Zephyr and Sciclone NGSx workstations and no intervention on Sciclone NGSx iQ workstation (with complete hands-off walkaway). The yield and size of libraries prepared on these workstations were within the expected range. The automated workflow with software guided deck set-up, allows users to eliminate user errors, reduce labor cost while increasing throughput andimproving sample tracking.

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