NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES

Optimizing NEXTFLEX Rapid XP library preparation on the Revvity BioQule system for viral genomic sequencing

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Optimization of NEXTFLEX library preparation parameters is necessary for specific specimen types

BACKGROUND

Many laboratories employ automated liquid handling systems to streamline sample preparation for next-generation sequencing (NGS). Systems range from large, expensive platforms with multiple liquid-dispensing arms and integrated accessories (e.g., shakers, heating blocks, plate readers and thermocyclers) to small, low-cost systems containing a single liquid-dispensing arm and no accessory equipment. Each system has advantages and disadvantages with regard to cost, ease of operation, dispensing accuracy and throughput. The Revvity BioQule system (Figure 1), which employs a simplified reagent and cartridge preparation loading strategy, allows easy automation of library construction for low throughput runs. The system has automated thermal cycling, an on-board magnet, and optical quantification capabilities, which allows library preparation, clean-up and quantitation of up to eight libraries in a single run. In this study, we investigated the performance of library preparation using the Revvity NEXTFLEX Rapid XP V2 DNA-Seq kit on the BioQule system.

Figure 1. General overview of the Revvity BioQule system

METHODS

Random cDNA from 39 poliovirus environmental isolates, and tiled amplicons from six nasopharyngeal swab specimens positive for SARS-CoV-2 (SC2) and eight stool specimens positive for norovirus were used as input for NEXTFLEX library preparation. After preparation on the BioQule, library concentration and fragment lengths were determined using the Invitrogen QuantIT system and Agilent Tapestation D1000 assay, respectively. NEXTFLEX library preparation on the BioQule and subsequent sequencing on the Illumina MiSeq platform were preformed using the parameters/kits shown in Table 1.

RESULTS

Final Library Concentration and Average Fragment Size

- Final library concentration estimates based on NEXTFLEX optical quantification were higher for 50 out of 53 libraries compared to quantification using the Invitrogen Quant-iT system (Figure 2)
  - Median 2.7-fold difference
  - Pearson correlation coefficient (r) value of 0.71
- Average fragment size after library preparation was 544 base pairs (bp) for poliovirus libraries, 373 bp for SC2 libraries, and 462 bp for norovirus libraries (Figure 3)
  - Observed differences in library fragment size may be due to the nature of the starting material (i.e., random cDNA vs amplicon)

Illumina Sequencing Run Metrics

- Increasing the number of library preparation PCR cycles to 13 improved the clustering density for sequencing runs with poliovirus samples
  - 171 K/mm^2 with 6 cycles of PCR compared to 604 K/mm^2 with 13 cycles of PCR (Table 1)
- Target clustering density and quality scores were obtained for norovirus amplicons with 10 minutes of fragmentation time and 10 cycles of PCR
  - 1096 K/mm^2 clustering density (target range 1000-1200 K/mm^2) and 88% of bases higher than Q30 (target > 80%) (Table 1)

CONCLUSIONS

Our results indicate library preparation parameters such as fragmentation time and PCR cycles need to be optimized depending on the library input material (i.e., random cDNA vs amplicon). Future BioQule experiments will focus on continued optimization of run parameters for NEXTFLEX library preparation and testing of the Illumina DNA Library Preparation protocol.

FIGURES/TABLES

Table 1. NEXTFLEX Rapid XP V2 DNA-Seq library preparation parameters and Illumina sequencing kits/settings and run metrics.

		Sequencing Run				
		Run 1	Run 2	Run 3	Run 4	Run 5
Library Preparation/ BioQule Parameters	**Input Amount (ng)**	40	7-22	40		
	Target Virus (# of samples)	Polio (6) and SC2 (6)	Polio (7)	Polio (6)	Polio (20)	Norovirus (8)
	Input Type†	Random cDNA and amplicon	Random cDNA	Random cDNA	Random cDNA	Amplicon
	Fragmentation Time	10 minutes				
	PCR Cycles	10	13	13	6	10
Sequencing Parameters and Run Metrics	**Loading Concentration**	15 pM		12.5 pM	15 pM	
	Read Length (bp)	2 X 250			2 X 150	
	Kit Type	Std	Micro	Nano	Std	Nano
	Clustering Density (K/mm²)	110	NA*	604	171	1096
	Clusters Passing Filter (%)	47	NA*	89	86	83
	%≥Q30	74	NA*	81	86	88

*Sequencing run did not complete, but samples were still used for library concentration and fragment length comparisons
†Amplicon sizes average 550 bp for SC2 tiled amplicons and ~7.5kb for norovirus amplicons.

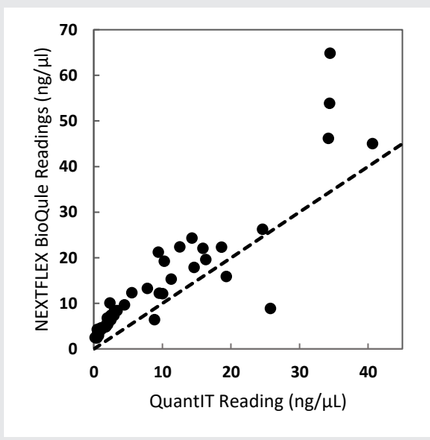

Figure 2. Estimated library concentrations based on NEXTFLEX BioQule readings (y-axis) vs Invitrogen QuantIT (x-axis). Each point represents a single library, with the dotted line denoting equal concentration readings between the two methods; points falling above the line denote higher concentration reading based on BioQule quantitation and points falling below the line denote higher concentrations readings based on QuantIT.

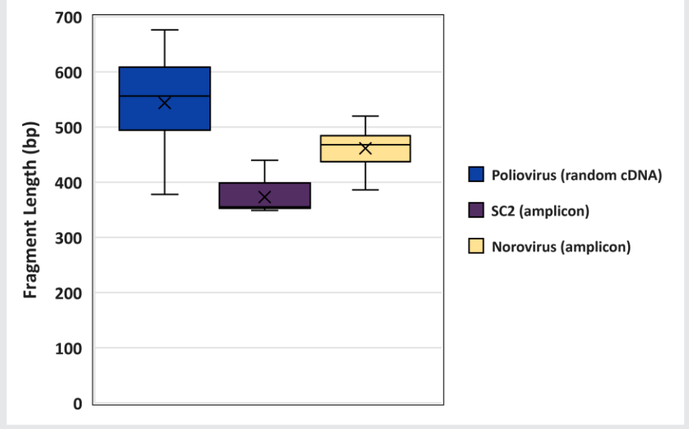

Figure 3. Average fragment length for poliovirus (blue), SC2 (purple) and norovirus (yellow) libraries.

SCAN HERE FOR MORE INFORMATION

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