Evaluation of the QIAxcel Connect system for NGS library prep QC analysis: experience from a clinical diagnostic laboratory

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Background

Next-generation sequencing (NGS), also known as massive parallel sequencing, has developed rapidly in the past decade. This technology is widely used in molecular diagnostic laboratories and has played a vital role in molecular genetics and in the diagnosis of human diseases. To achieve optimal performance of NGS, quality control (QC) analysis is necessary to provide essential information about library size distribution, peak size, and library concentration for any given library prep kit. Here we evaluated the performance of the QIAxcel Connect system (QIAGEN) as an integral part of NGS library prep QC analysis, including peak size and library concentration, in conjunction with two library prep workflows, Archer (IDT) and Pillar Bioscience.

Materials & Methods

One hundred sixty formalin-fixed paraffin embedded (FFPE) tissue samples from scrolls, 134 FFPE samples from slides, and 126 blood/bone marrow samples were utilized for analysis of the Archer™ FUSIONPlex™ Pan solid tumor v2 panel (Archer FP ST panel), Pillar oncoReveal™ Solid Tumor v2 panel (Pillar ST panel) and Essential MPN panel (Pillar MPN panel), respectively. RNA for the Archer FP ST panel and DNA for the Pillar panels were isolated using standard laboratory procedures. Library prep was performed according to the manufactures' instructions[1-3]. Following library prep, the libraries of each batch were normalized to an "arbitrary" concentration based on the QIAxcel concentration and pooled into one library for each panel. The "true" concentrations of the pooled libraries were measured using Qubit 4 fluorometer (Thermo Fisher Scientific). After dilution, denaturation, and neutralization, sequencing was performed on an Illumina NextSeq 550 Dx instrument and analyzed by the Archer Analysis Unlimited software or the Pillar PiVAT™ Analysis software.

Results

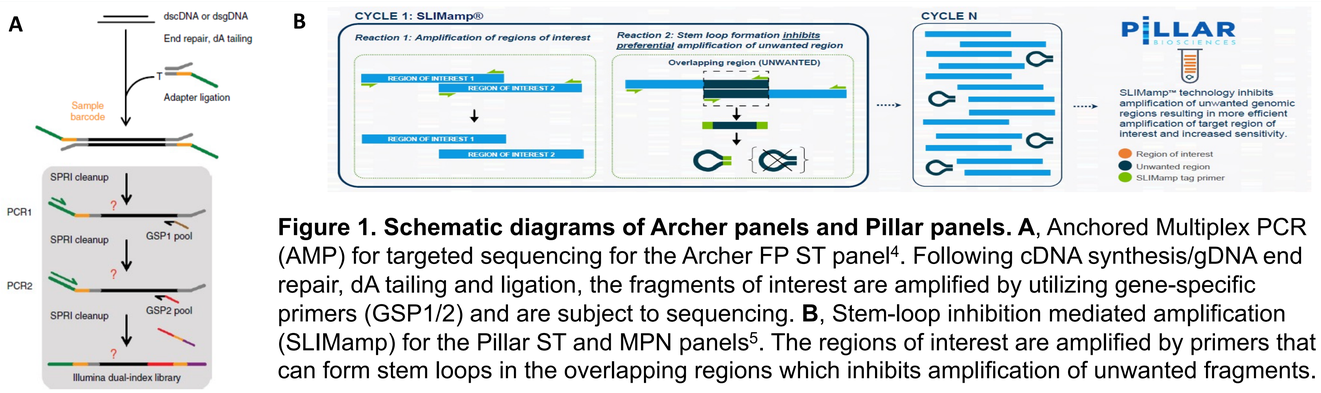
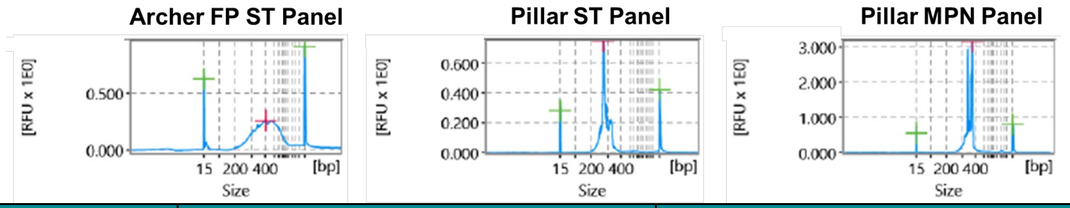


Figure 1. Schematic diagrams of Archer panels and Pillar panels. A, Anchored Multiplex PCR (AMP) for targeted sequencing for the Archer FP ST panel[4]. Following cDNA synthesis/gDNA end repair, dA tailing and ligation, the fragments of interest are amplified by utilizing gene-specific primers (GSP1/2) and are subject to sequencing. **B**, Stem-loop inhibition mediated amplification (SLIMamp) for the Pillar ST and MPN panels[5]. The regions of interest are amplified by primers that can form stem loops in the overlapping regions which inhibits amplification of unwanted fragments.

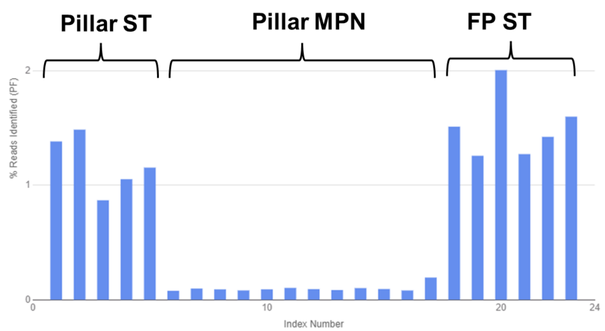
A



B

	Peak Size (bp)			Qubit/QIAxcel (nM/nM)		
	Mean	St. Dev.	%CV	Mean	St. Dev.	%CV
Archer FP ST Panel	304	43	0.14	12.65	4.02	0.32
Pillar ST Panel	277	2	0.01	5.85	1.24	0.21
Pillar MPN Panel	373	11	0.03	5	1.11	0.22

Figure 2. Evaluation of QIAxcel Connect system for NGS library prep QC analysis[6]. A, Examples of electropherograms of the Archer FP ST, Pillar ST, and Pillar MPN panels. **B**, Summary of peak sizes and concentrations from QIAxcel. Peak sizes and concentrations were categorized by each panel. The "arbitrary" concentration from QIAxcel were compared to the "true" concentration from Qubit.



	% Reads Per Sample		
	Mean (%)	St. Dev.	%CV
Archer FP ST Panel	1.6	0.53	0.33
Pillar ST Panel	1.54	0.48	0.31
Pillar MPN Panel	0.1	0.027	0.27

Figure 3. Uniformity of read percentage after normalization using the concentration from QIAxcel. A, Visualization of read percentage in the Sequencing Analysis Viewer software (Illumina). **B**, Summary of reads percentage per sample for each panel.

Discussion & Conclusions

1. We successfully assessed the performance of the QIAxcel Connect system for NGS library prep QC analysis.
2. We created an internal excel table to facilitate the pooling of the panels from different vendors in the same sequencing run, depending on the total reads that were required for each panel.
3. The peak sizes of each panel from the QIAxcel Connect system in this study were reproducible across all the samples and batches.
4. The read percentages of samples from each panel in a batch were uniform, demonstrating that the relative concentration obtained from the QIAxcel Connect system was accurate across the same batch.
5. Employing this system eliminated the need to quantify each individual library. Instead, it allowed us to test one pooled library for each panel, thus reducing reagent cost, labor cost, workflow complexity, and potential human errors.

References

1. Archer™ FusionPlex™ Protocol for Illumina, RA-DOC-047 / REV01
2. Pillar oncoReveal™ Solid Tumor v2 User Manual, UM-0065 version
3. Pillar oncoReveal™ Essential MPN Panel User Manual, MK-0017-6 v1.0
4. Zheng et al., Anchored multiplex PCR for targeted next-generation sequencing, Nat Med. 2014 Dec;20(12):1479-84 (PMID: 25384085)
5. Schenk et al,. Amplification of overlapping DNA amplicons in a single-tube multiplex PCR for targeted next-generation sequencing of BRCA1 and BRCA2, PLoS One. 2017 Jul 12;12(7):e0181062 (PMID: 28704513)
6. QIAxcel Connect System User Manual 08/2023, HB-2890-004