COMPARISONS OF THREE TARGETED NEXT-GENERATION SEQUENCING KITS USING FFPE TUMOR DNA EXPOSES DIFFERENCES IN SPECIMEN COMPATIBILITY, SAMPLE QC, WORKFLOW, AND TURN-AROUND TIME

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SUMMARY

- Commercially available targeted NGS gene panels have found increasing favor for oncology research, drug development, and patient testing for clinically-actionable variants.
- We compared two of the most commonly used cancer hotspot tumor panels, namely AmpliSeq® and TruSight® NGS products, with a new product, the QuantideX® NGS Pan Cancer Kit.*
- Analyses of 60 residual clinical FFPE tumor biopsies supported high levels of variant call accuracy for all three methodologies using the vendors' protocols, but revealed marked differences among the kits in DNA input amount, the fraction of samples passing QC, and hands-on and overall turn-around time.

INTRODUCTION

In oncology, FFPE and FNA tumor biopsies are ubiquitous yet challenging specimens for molecular pathology analyses. Increasingly, targeted NGS gene panels are used to identify clinically-actionable variants in such samples and help guide targeted therapies and individualize patient management. Two of the most commonly used panels are based on AmpliSeq and TruSight chemistries. In this study, we compared and contrasted the analytical performance and time-motion workflows of these two targeted NGS methods using 60 FFPE tumor biopsies. We also compared a new product, the QuantideX NGS Pan Cancer Kit.

METHODS

Residual clinical FFPE biopsies (n=60) and mixtures from 6 different tissues were analyzed. Extracted DNA was quantified using 4 different methods (spectrophotometry, Qubit™ fluorescent dye binding, and two distinct qPCR assays). Functional DNA quality was determined using qPCR and Qubit. DNA samples were qualified for enrichment based upon the suppliers' instructions, and processed using the AmpliSeq Cancer Hotspot Panel v2 (ACHP, ThermoFisher), the TruSight Tumor 26 Panel (TT, Illumina) and the QuantideX NGS Pan Cancer Kit (QPC, Asuragen). NGS data were generated on MiSeq® (TT and QPC) or Ion PGM™ (ACHP) systems. Bioinformatic analyses were performed using each vendor's analysis pipeline. Time-motion analyses were determined using a batch size of 20 samples for ACHP, TT, and QPC.

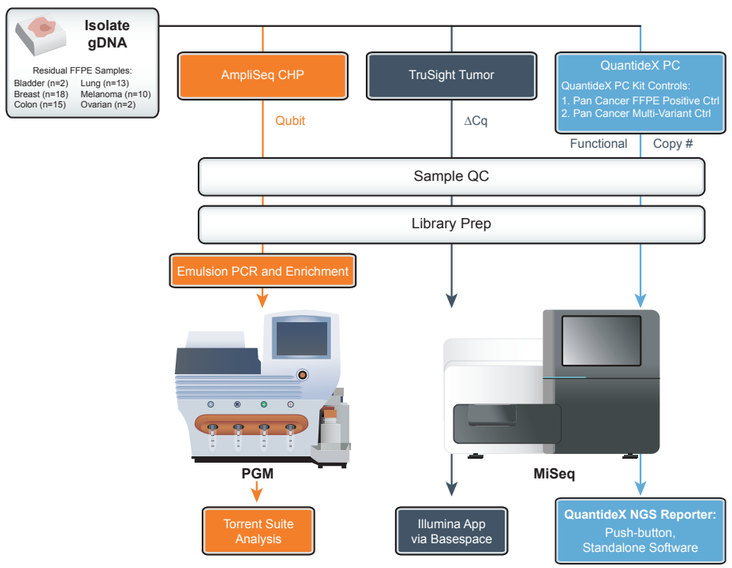

Figure 1. Study design for analytical and workflow comparisons among three targeted NGS kits. All 60 FFPE samples were evaluated with the appropriate QC test for the panel. Passing samples were subjected to library prep and after sequencing were analyzed with each manufacturer's Bioinformatics pipeline.

*Research Use Only – Not For Use In Diagnostic Procedures
Preliminary research data. The performance characteristics of this assay have not yet been established.

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RESULTS

FFPE Sample			AmpliSeq QC Qubit (ng/μL)			TruSight QC qPCR (Δ Cq value)			QuantideX qPCR DNA QC Assay (cp#/μL)		
Bladder-1	Colon-1	Lung-2	0.2	40.7	31.5	16.5	0.6	2.8	3.6	9505.6	4986.6
Bladder-2	Colon-10	Lung-3	2.2	9.8	21.6	4.5	3.6	2.5	839.1	1726.3	3813.1
Breast-1	Colon-11	Lung-4	0.2	33.6	7.5	9.2	1.4	3.7	87.0	6086.6	1137.7
Breast-10	Colon-12	Lung-5	23.8	24.4	8.7	7.1	1.9	5.4	974.0	4606.4	1818.0
Breast-11	Colon-13	Lung-6	24.1	7.0	5.9	4.4	4.8	5.3	3337.9	1140.7	1541.7
Breast-12	Colon-14	Lung-7	18.7	33.1	1.8	3.7	3.3	7.8	2881.8	3024.3	337.0
Breast-13	Colon-15	Lung-8	6.3	25.1	13.6	5.1	1.9	2.1	1103.2	4843.4	2952.8
Breast-14	Colon-2	Lung-9	17.5	3.3	35.4	3.1	5.9	1.6	2167.6	807.4	3497.3
Breast-15	Colon-3	Melanoma-1	12.3	2.5	36.2	3.6	5.0	1.6	1655.7	652.6	7676.7
Breast-16	Colon-4	Melanoma-10	6.0	11.8	56.2	4.7	0.7	0.1	1016.6	3375.5	10090.5
Breast-17	Colon-5	Melanoma-2	8.2	10.8	8.7	4.2	5.0	4.8	1641.8	1659.0	1035.2
Breast-18	Colon-6	Melanoma-3	4.7	13.0	10.4	8.3	5.2	4.8	518.7	1527.5	1946.1
Breast-2	Colon-7	Melanoma-4	0.2	11.3	129.4	9.6	5.3	0.0	53.6	1107.3	17395.3
Breast-3	Colon-8	Melanoma-5	7.1	17.1	3.1	2.6	4.0	6.7	2889.0	2623.8	406.5
Breast-4	Colon-9	Melanoma-6	13.7	46.4	11.6	1.3	3.2	2.7	3284.6	4269.6	2507.5
Breast-5	Lung-1	Melanoma-7	3.9	1.9	23.1	3.8	9.0	0.8	1012.8	262.0	3650.5
Breast-6	Lung-10	Melanoma-8	29.3	23.9	12.2	2.9	1.2	0.9	5647.0	6291.9	3488.9
Breast-7	Lung-11	Melanoma-9	2.0	1.3	4.4	6.1	9.2	6.7	439.5	212.5	533.6
Breast-8	Lung-12	Ovarian-1	35.3	1.2	5.7	1.8	7.3	5.3	6200.5	268.3	952.1
Breast-9	Lung-13	Ovarian-2	9.0	8.5	2.1	12.8	5.6	9.7	58.3	1137.0	125.7
QC Target Value			10 ng of Qubit DNA			Δ Cq of <4.0			>200 functional copies (in 4 uL)		
At-risk or Failed QC			5% (3/60)			52% (31/60)			1.7% (1/60)		
Median DNA input (ng via A260) for samples passing QC			58.4			2100			14.1		
Relative DNA Input Required			4.1			148.9			1		

Table 1. Sample cohort, pre-analytical QC methods, and DNA input for each targeted NGS kit. Acceptable QC criteria for each kit were applied per supplier's instructions included: A minimum of 10 ng Qubit-quantified DNA (12 μL max input volume) (ACHP); ΔCq<4 relative to supplier-provided control (TT); >200 functional DNA copies (>400 copies recommended) (QPC). Failed or at-risk samples for each QC method are indicated in orange. Median values of 62.8 ng/μL (spectrophotometry), 10.6 ng/μL (fluorescent assay), and 1901 amplifiable DNA copies/μL (QuantideX qPCR DNA QC Assay) were obtained across the 60 FFPE tumor DNA samples. The median percent of amplifiable templates was 9.6.

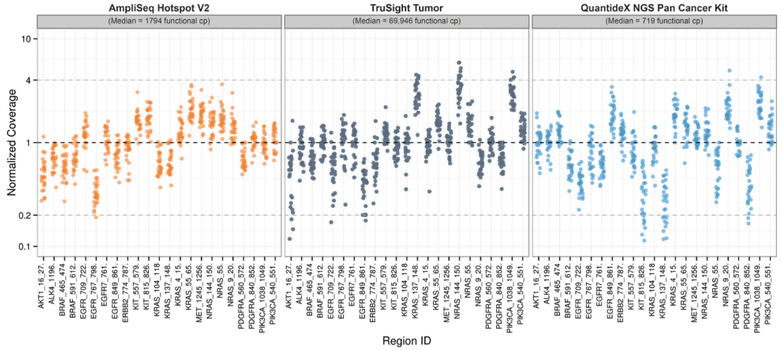

Figure 2. Relative coverage uniformity for common amplicons and FFPE DNA with passing QC across all three targeted NGS kits. Coverage was normalized to the median number of reads for each sample.

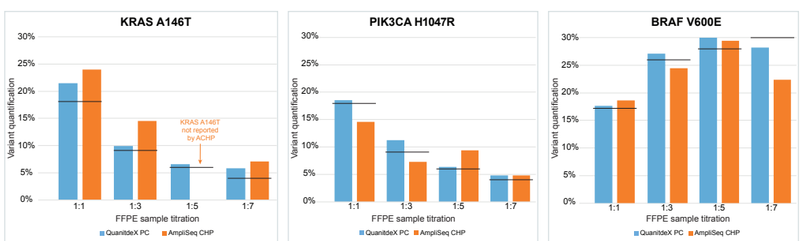

Figure 3. Comparison of analytical sensitivity using a mixture of residual clinical FFPE DNAs. A pair of well-characterized FFPE DNA samples were blended together at ratios of 1:1, 1:3, 1:5, and 1:7 based on functional copy number. These samples were then sequenced from libraries prepared using ACHP and QPC to compare the detection of known mutations at low-copy inputs (400 functional copies). The black hash mark (—) indicates the expected variant frequency based on the mathematical mixing ratio and independent analysis of neat input samples. QPC demonstrated superior qualitative and quantitative mutation detection compared to ACHP.

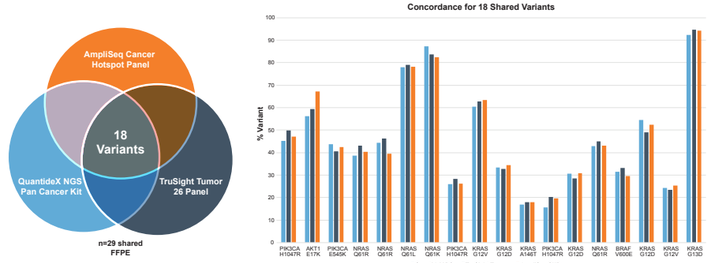

Figure 4. The three targeted NGS panels demonstrated concordant results for 29 FFPE DNA samples that passed each kit's QC criteria. To ensure equity in comparative results, variant call agreement was restricted to gene content that was shared by all three panels. In total, 18 variants were detected, representing 11 distinct mutations in 6 different cancer genes.

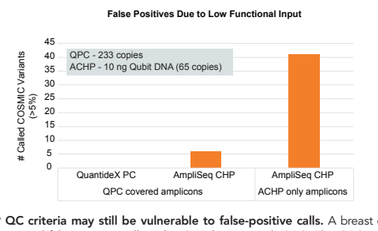

Figure 5. Samples that pass ACHP QC criteria may still be vulnerable to false-positive calls. A breast cancer FFPE that failed TT QC, but passed both ACHP and QPC QC generated false-positive calls with ACHP, but not with QPC. The QPC panel called zero variants overall, yet ACHP called 6 variants (5.3%-16.8%; mean of 8.8%) for shared amplicon regions. Across all ACHP content, the panel called 41 variants.

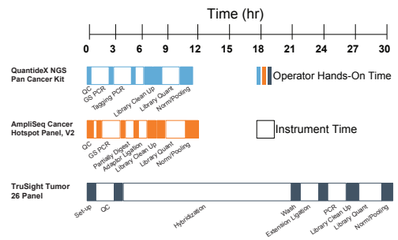

Figure 6. Time-motion analysis of targeted NGS kit workflows. ACHP and QPC demonstrated similar hands-on and total time from DNA-to-sequencer, but TT required more than twice the time. All timepoints were based on actual times recorded during these library preps by the same operator using a common batch size of 20 samples.

CONCLUSIONS

- Using the each manufacturer's QC criteria, the fraction of samples that were unsuitable for targeted NGS varied by 29-fold across the three pan-cancer panels, from 1.7% to 52%.
- The median A260 equivalent input for DNA samples passing pre-analytical QC covered a 150-fold range, from 14.1 ng for the QuantideX NGS Pan Cancer Kit to 58.4 ng for AmpliSeq Cancer Hotspot Panel and 2100 ng for TruSight Tumor.
- Library preparation times from standardized operator runs were (from shortest to longest): QuantideX NGS Pan Cancer Kit (11 hr) <AmpliSeq Cancer Hotspot Panel (12 hr) <TruSight Tumor Panel (31 hr).

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