UTILITY OF A RAPID TURNAROUND, CLINICALLY-RELEVANT GENE PANEL FOR NEXT GENERATION SEQUENCING (NGS) ANALYSIS OF FFPE SOLID TUMORS

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Disclaimer:
The authors of this abstract have indicated the following conflicts of interest that relate to the content of this abstract: Dr. Hadd is an employee of Asuragen, Inc.

SUMMARY

- The QuantideX® NGS Pan Cancer Kit enables detection of cancer-relevant genes using a simplified workflow and integrated quality controls essential for routine testing.
- This technology integrates pre-analytical QC data with a variant caller to accurately detect SNVs and indels in key cancer genes.
- Assessment of colon and melanoma malignancies were concordant with other methods substantiating this approach for targeted cancer panel analysis.

INTRODUCTION

The widespread adoption of next-generation sequencing (NGS) technologies has redefined the ability to interrogate multiple gene mutations in complex tumor biopsies and to affect precision medicine. To ensure the accuracy of variant calls and relevance to translational medicine, NGS-based assays require additional layers of quality control and safeguards while also meeting the needs of the testing laboratory. We evaluated the QuantideX NGS Pan Cancer Kit to assess capabilities of solid tumor analysis using NGS. The inclusion of controls, streamlined workflow and QuantideX® NGS Reporter, a "sample aware" variant caller, provided rapid turn around and concordant analysis of solid tumor FFPE samples.

DESIGN

Workflow

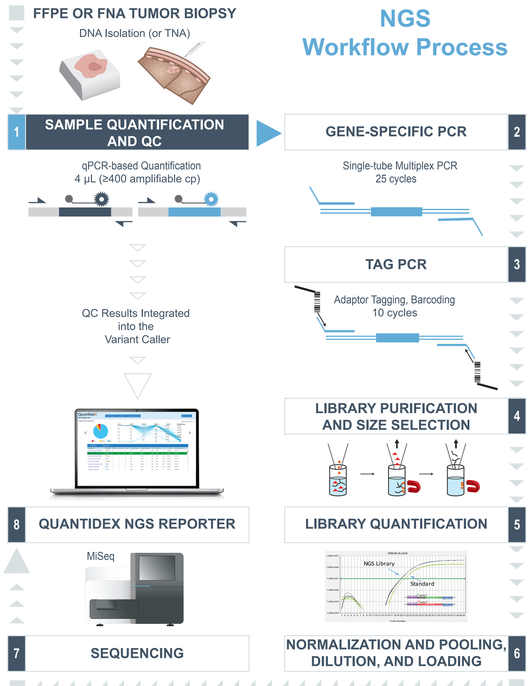


Figure 1.The QuantideX NGS Pan Cancer Kit provides an all-in-one NGS solution. A comprehensive workflow solution integrating reagents, controls, and a novel bioinformatics suite for the sequencing of an oncology panel relevant to a diverse set of human cancers. QC results for DNA samples are included in the variant caller algorithm.

Research Use Only – Not For Use In Diagnostic Procedures
Preliminary research data. The performance characteristics of this assay have not yet been established.
Presented at US CAP 2016

Panel

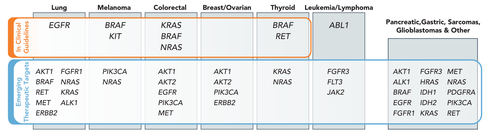
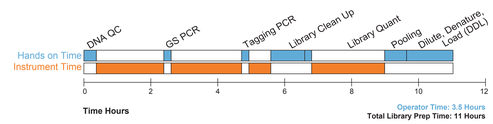


Figure 2. The QuantideX NGS Pan Cancer Kit uses a multiplexed primer pool. The panel targets 21 genes across 46 regions of current and emerging targets of clinical significance in human cancer.

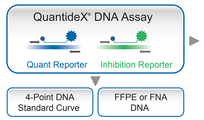
Rapid Turn-Around Time

3A



Quantification of Functional DNA and PCR Readiness

3B



Functional DNA [cp/µL]	PCR Inhibition	Library Prep and NGS Run
<100	No	At Risk
>100	Yes	Dilute or repurify Repeat quantification prior to run
	No	Pass

DNA QC Integrated Variant Caller

3C

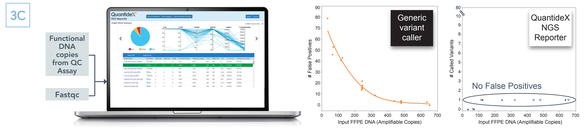


Figure 3. The QuantideX NGS Pan Cancer Kit includes specific design elements that facilitate rapid turn-around time, modular reagents with ease-of-use and an integrated sample-aware variant caller. A) Time-workflow analysis of 20 samples yielded 3.5 hours of hands-on-time within an 11 hour workflow. **B)** The QuantideX DNA Assay informs functional copies of DNA and specific guidance on NGS run criteria. **C)** The QuantideX NGS Reporter software is a locally installed NGS analysis solution that integrates results from pre-analytical QC data to attenuate sample analysis and reduction of false positives at low input.

Positive Controls Reproducibility

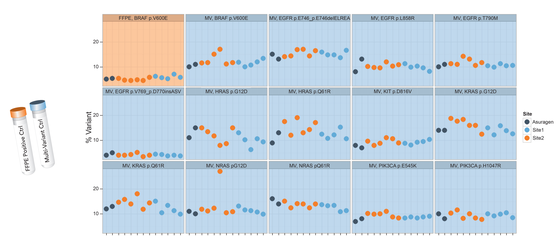
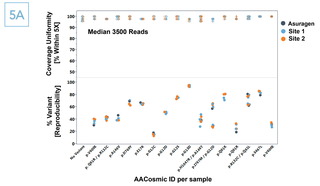


Figure 4. Controls provide analytical QC checks for routine batch processing and proficiency between operators and sequencing runs. The multi-variant control included 12 SNVs and 2 Indels with an average of 12% across different targets. The low input and cutoff variant call of BRAF V600E in an FFPE sourced positive control yielded 5.4% average detection.

RESULTS

FFPE Variant Call Reproducibility

5A



FFPE Variant Call Sensitivity

5B

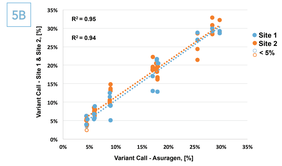


Figure 5. FFPE analysis of multiple sample types and sources yielded highly reproducible variant calls and dose-dependent response. A) Independent sample preparations and multiplex library pools from repeatability studies yielded reproducible variant calls and uniform coverage. **B)** Titration of FFPE tumor DNA revealed dose-dependent detection of mutations to 5% of total NGS reads in multiple laboratories, maintaining sensitivity and positive predicted value (PPV). These results support routine analysis and confirm detection to 5% SNV.

Accuracy

QuantideX NGS Pan Cancer						Single-gene qPCR Result	
Sample Type	Sample ID	Copies	Gene	AA	Percent Variant	Gene	Variant
CRC	GRU001	15370	KRAS	p.G13D	13%	KRAS	G13D
CRC	GRU002	14520	KRAS	p.G12V	13%	KRAS	G12V
CRC	GRU004	8890	KRAS	p.G12V	63%	KRAS	G12V
CRC	GRU005	17890	KRAS	p.G12V	26%	KRAS	G12V
			PIKC3CA	p.Q546K	7%		
CRC	GRU006	20640	KRAS	p.G12V	18%	KRAS	G12V
			PIK3CA	p.H1047R	19%		
CRC	GRU007	5500	KRAS	p.G12V	25%	KRAS	G12D
			PIKC3CA	p.H1047L	8%		
CRC	GRU008	38710	KRAS	p.G12D	49%	KRAS	G12D
CRC	GRU009	23970	KRAS	p.G12D	12%	KRAS	c 12/13
			ERBB2	p.V777L	19%		
CRC	GRU010	39690	KRAS	p.G12D	8%	KRAS	G12D
CRC	GRU011	19560	KRAS	p.G12C	43%	KRAS	c 12/13
			PIK3CA	p.Q546K	7%		
CRC	GRU012	9480	KRAS	p.G12D	12%	KRAS	c 12/13
Mel	GRU013	12540	BRAF	p.V600E	46%	BRAF	V600E
Mel	GRU014	30510	BRAF	p.V600E	19%	BRAF	V600E
Mel	GRU015	1870	BRAF	p.V600E	41%	BRAF	V600E
Mel	GRU016	32430	BRAF	p.V600E	42%	BRAF	V600E
Mel	GRU017	20110	BRAF	p.V600E	42%	BRAF	V600E
Mel	GRU018	42810	BRAF	p.V600E	46%	BRAF	V600E

Table 1. NGS Analysis is 100% concordant to matched variant calls in colorectal (CRC) and melanoma (Mel) tumor biopsies. A total of 4 distinct KRAS codon 12/13 variants in range of 8 to 63%. One sample, GRU007, called p.G12V (>GTT) compared to KRAS p.G12D (>GAT) by previous method. Matched sample variant calls in 6/6 samples in range of 19.5 to 46.2% BRAF p.V600E. Non-matched calls in PIK3CA and ERRB2 were identified in 5 samples supporting advantages of targeted NGS panels over single gene assays.

CONCLUSIONS

- Our results on a small cohort of tumor biopsies show the value of integrating pre-analytical, analytical, and post-analytical steps within a targeted NGS workflow for clinically-relevant gene targets.
- Functional quantification of the input DNA, and incorporation of this sample-specific information into the bioinformatics analysis, can help ensure accurate NGS results in the wake of variable handling and processing of tumor biopsies.
- This simplified approach facilitated the training of new technologists in less than a week, and acquisition of consistent and accurate NGS data in the first run.
- The focus on controls, scalability and expeditious validation addresses important considerations for the adoption of NGS tests and facile incorporation of new test panels.

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