A COMPREHENSIVE MULTI-SITE EVALUATION OF THE QUANTIDEX® NGS PAN CANCER KIT* YIELDS REPEATABLE AND REPRODUCIBLE NGS ANALYSIS OF LOW-QUANTITY FFPE TUMOR BIOPSIES

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SUMMARY

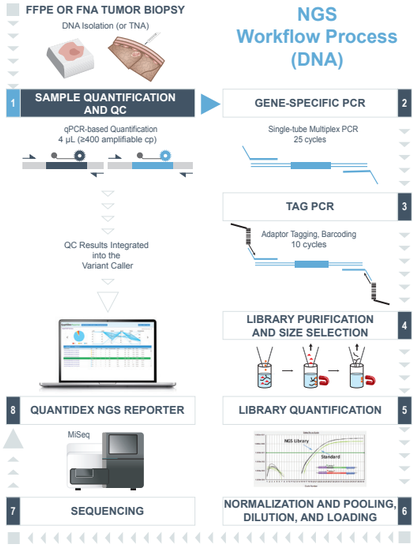
- A comprehensive NGS methodology, the QuantideX NGS Pan Cancer Kit, was evaluated at UCLA, Greenwood Genetic Center, and Asuragen, Inc. through a structured assessment of training, proficiency, accuracy and repeatability studies.
- The multi-site study yielded repeatable and concordant variant calls using Asuragen tumor bank samples, kit controls and an independent FFPE cohort from Georgia Regents University.
- The QuantideX NGS Pan Cancer Kit and QuantideX NGS Reporter software offers single-sourced integrated chemistry, simplified workflow and analytics solution that performs with high accuracy and reproducibility in challenging sample-types with low DNA input.

INTRODUCTION

Complex NGS workflows and generic data analysis pipelines impact the ability to establish inter-laboratory accuracy and reproducibility. Here we present outcomes from a multi-site evaluation of a novel comprehensive NGS technology that combines single-source reagents with a variant caller that is informed by pre-analytical QC data using low-quantity inputs from residual FFPE tumor biopsies, FFPE analytical mixtures, intact cell line DNA and mixtures of cell line tumor DNA.

METHODS

All sites utilized the QuantideX NGS Pan Cancer Kit, a next-generation sequencing (NGS) oncology panel that offers a comprehensive workflow solution integrating reagents, controls, and a novel bioinformatics suite for the sequencing of 21 genes relevant to a diverse set of human cancers (Figure 1). The kit and reagents comprise pre-analytical sample QC, 2-step PCR-based targeted library enrichment, purification, library quantitation and custom sequencing primers for use with the MiSeq® (Illumina) system. Variant calls across 21 cancer genes using the QuantideX NGS Reporter data analysis and reporting tool were linked to pre-analytic sample QC results (functional copy numbers). A total of seven unique MiSeq runs were performed by both external sites (Figure 2). Study samples included analytical FFPE titration series, cancer cell lines, FFPE and FNA residual tumor biopsies analyzed at Asuragen and distributed to evaluation sites for distinct sample set category analysis - Training, Proficiency, Accuracy and Repeatability. Each NGS library preparation included an NTC, residual clinical FFPE DNA control comprised of a 5% BRAF p.V600E mutation, a Multi-Variant synthetic control and five clinical residual specimens.



QUANTIDEX NGS PAN CANCER KIT			
Gene	Codon Range	Gene	Codon Range
ABL1	249-258	HRAS	9-20
	303-319		59-76
AKT1	16-27		113-121
AKT2	16-26	IDH1	122-134
ALK1	1174-1196	IDH2	122-134
	1274-1278		163-174
BRAF	465-474	JAK2	607-620
	591-612	KIT	557-579
EGFR	486-493		815-826
	709-722	KRAS	9-20
	737-761		55-65
	767-798		104-118
	849-861		137-148
ERBB2	755-769	MET	1245-1256
	774-788	NRAS	9-20
	839-847		55-67
	877-883		110-119
FGFR1	123-136		144-150
	250-262	PDGFRA	560-572
FGFR3	247-260		840-852
	363-374	PIK3CA	540-551
	638-653		1038-1049
FLT3	829-840	RET	916-926

Figure 1. The QuantideX NGS Pan Cancer Kit overcomes the complexity of NGS using a simplified workflow and integrated sample analysis and reporting software. A 46 amplicon panel of current and emerging targets of clinical significance in human cancer, represents >1,600 known COSMIC variants reported in 21 genes.

*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 AMP 2015

RESULTS

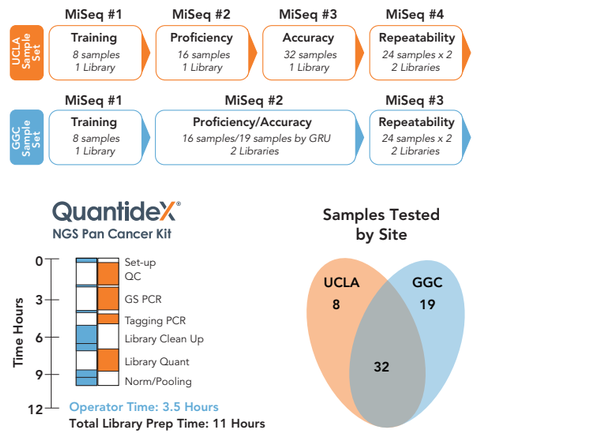


Figure 2. The multi-site study design, sample set and workflow accommodated the rapid and thorough assessment of the NGS kit. A total of 91 distinct sample runs were performed (40 UCLA, 51 GGC). UCLA and GGC tested 32 common samples; UCLA has 8 unique and GRU provided 19 tumor specimens. Evaluation sites were fully trained with the integrated workflow and capable of MiSeq instrument loading within 2-3 days.

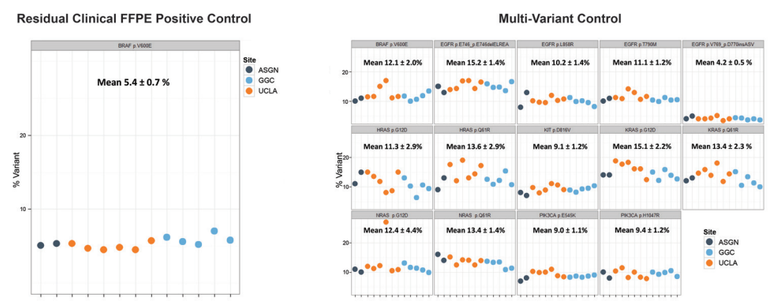


Figure 3. Kit controls reliably reported targeted variants in the FFPE Positive Control and Multi-Variant Control across sites and runs. The kit controls yielded a BRAF p.V600E variant at 5.4% in the FFPE Control and positive detection of 14 variants with a mean of 12% in the Multi-Variant Control.

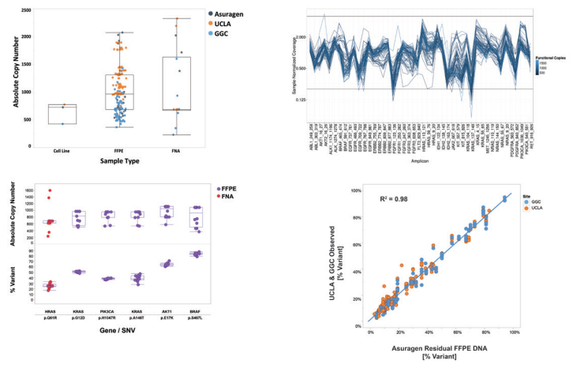


Figure 4. Multi-site variant detection in residual clinical FFPE tumor and intact DNA by NGS is highly correlated to reference results across 3 sites. Inter-laboratory repeatability for qualification of amplifiable DNA was within 2-fold and generated reliable variant calls using low-quantity amplifiable inputs from residual FFPE or FNA (spanning 230 to 2,325 copies). Amplicon normalized coverage was within a 5-fold range across NGS runs and sites for 99% of samples. Variant call percentages showed excellent agreement across 3 sites.

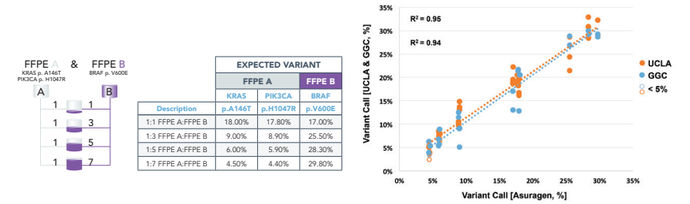


Figure 5. Titration of FFPE tumor DNA revealed dose-dependent detection of mutations to 5% of total NGS reads. Linearity from 5% – 32% mutation was observed for KRAS, PIK3CA and BRAF hotspots, in agreement with expected results in maintenance of sensitivity and positive predicted value (PPV). Two KRAS p.A146T (C>T) calls were detected below 5%.

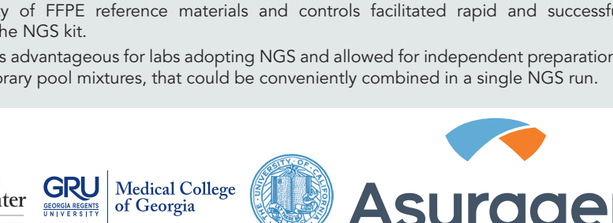


Figure 6. Independent sample preparations and multiplex library pools from repeatability studies yielded reproducible variant calls and uniform coverage. For each site, multiplex library pools were prepared independently by replicate, then combined and loaded onto a single MiSeq run.

Gene	Variant Call	Variant (Count)
AKT1	E17K	1
BRAF	S467L	1
BRAF	V600E	11
ERBB2	D769Y	1
ERBB2/KRAS	I767M/G12D	1
HRAS	Q61R	1
IDH1/NRAS	R132C/Q61L	1
IDH1/NRAS	R132C/Q61R	1
KRAS	G12D	5
KRAS	G13D	2
KRAS	G12C	2
KRAS	A146V	1
KRAS	G12V	6
NRAS	Q61R	2
NRAS	G12S	2
NRAS	Q61K	1
PIK3CA	H1047R	2
PIK3CA	E545K	1
PIK3CA/KRAS	H1047R/A146T	1
None	None	5
Total		48

AGREEMENT MATRIX FOR 48 UNIQUE RESIDUAL CLINICAL SAMPLES ASURAGEN (N=30) AND GRU (N=18)		Reference	
		POS	Wt
Evaluation Sites QuantideX Pan Cancer DNA	POS	43*	0
	Wt	1†	4

Figure 7. Variant detection of 48 unique residual clinical specimens by the QuantideX NGS Pan Cancer Kit were concordant between sites and testing methods. Variant calls were observed ranging from 4% to 93% across 8 different genes. *A positive KRAS sample was reported p.G12D using qPCR and p.G12V using NGS. One tested sample was omitted for orthogonal comparison because the variant was outside the NGS primer design and Sanger sequencing using exon spanning primers failed. †An EGFR deletion (p.E746_E749delELRE) was detected at 2.5% below the 5% threshold.

CONCLUSIONS

- Evaluation studies of the QuantideX NGS Pan Cancer Kit provided a standardized and simplified workflow to rapidly characterize performance across sites, samples and NGS runs.
- Technologists at two external clinical laboratories were fully trained with the integrated workflow and capable of MiSeq instrument loading within 3 days.
- The availability of FFPE reference materials and controls facilitated rapid and successful evaluation of the NGS kit.
- The workflow is advantageous for labs adopting NGS and allowed for independent preparations of multiplex library pool mixtures, that could be conveniently combined in a single NGS run.

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