QUANTIDEX® NGS REPORTER: A PUSH-BUTTON BIOINFORMATICS ANALYSIS TOOL THAT INTEGRATES PREANALYTIC QC FOR SIMPLE-TO-USE ANALYTICS WITH SUPERIOR VARIANT DETECTION IN RESIDUAL CLINICAL FFPE, FNA, AND LIQUID TUMOR BIOPSIES

Robert Zeigler, Blake Printy, Neal Ormsbee, Dennis Wylie, Jeff Houghton, Sachin Sah, Liangjing Chen, Huiping Zhu, Stephanie Bridger, Julie Krosting, Andrew Hadd, Gary J Latham, and Brian C Haynes
Asuragen, Inc., Austin, TX

SUMMARY

- We present the QuantideX NGS System, a streamlined assay workflow and novel computational approach to variant calling for amplicon-based targeted DNA-Seq that directly incorporates a pre-analytical QC measure of amplifiable template molecules in the sample.
- Data from 474 samples were used to train and validate a "sample-aware" variant caller that can reduce false-positive calls by as much as 80-fold for low-quality samples while retaining high analytical sensitivity.
- The QuantideX NGS Reporter suite is available for use with the QuantideX NGS Pan Cancer Kit*, a fully integrated targeted NGS workflow solution for the detection of clinically relevant variants from a wide range of human cancers.

INTRODUCTION

Clinical research and diagnostics are increasingly reliant on next-generation sequencing (NGS) technologies due to the rich breadth and depth of information they provide. However, complexity of experimentation, heterogeneity of clinical specimens and burden of data analysis pose significant, ongoing challenges that limit the potential of NGS, particularly for oncology applications.

METHODS

Using QuantideX NGS methodology, 474 samples were sequenced on Illumina MiSeq® and/or Ion PGM™ system and functional copies input assessed. Truth was established by confirmation on Luminex (333) and/or replicate sequencing (467). The data were used to train a classifier with and without the QC information under 5-fold cross-validation. Validation of the QuantideX NGS Pan Cancer Kit* and variant caller, was performed with a 98-sample, 3-operator, 3-day validation study, with additional testing on 28 matched FFPE, Plasma, Serum, and/or Fresh Frozen samples.

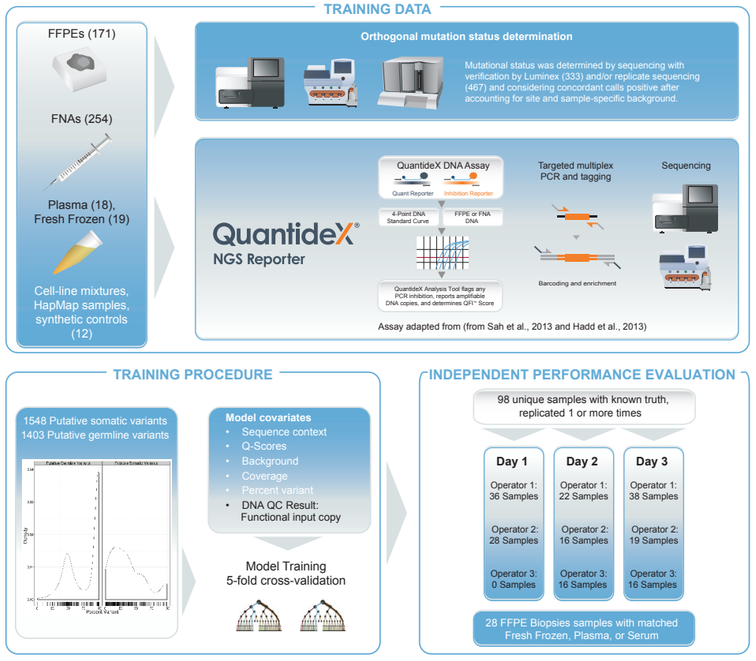

Figure 1. Schematic of data acquisition and classifier training and validation methods.

*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

We present QuantideX NGS Pan Cancer Kit*, a targeted NGS system that utilizes an integrated workflow comprised of optimized QC and library prep reagents, clinically-relevant controls, and novel variant analysis pipeline. Our variant classifier directly incorporates amplifiable DNA template count as a model covariate and was trained on over 400 residual clinical specimens for which truth was independently established. Evaluation on an independent cohort underscores the value of a systems approach to targeted NGS that links pre-analytical, analytical, and post-analytical steps using a streamlined, cross-platform workflow.

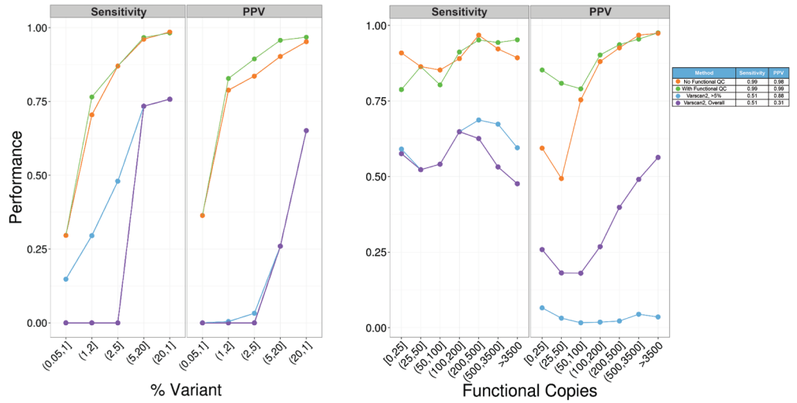

Figure 2. Cross-validation performance of the QuantideX NGS Reporter variant caller. Inclusion of functional QC data results in significant PPV benefit below 200 functional copies, spanning the entire range of % variant. Varscan2 5% is the Varscan2 call set with a 5% minimum variant allele frequency filter applied.

Functional Copies Input	# Variants	Sensitivity	PPV
<= 200	31	0.87	0.93
> 200	340	1.00	1.00

Table 1. Performance of QuantideX NGS Pan Cancer Kit* and Variant Caller in a multi-day, multi-operator study for variants at ≥5% frequency.

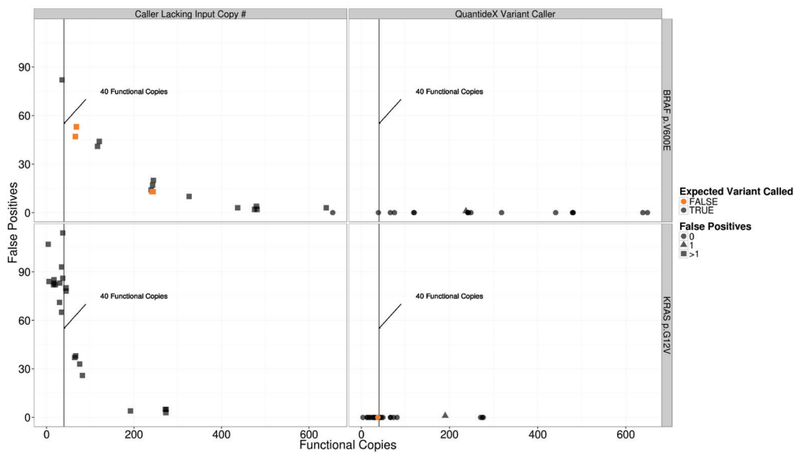

Figure 3. Low functional copies increase false-positive calls in QC-agnostic caller (left column) but not the QuantideX NGS Reporter caller (right column) in BRAF (top row) and KRAS (bottom row) copy-number titration studies. Sample data in black reflect the accurate detection of the target mutation; those in red reflect the absence of this variant (irrespective of whether false-positive calls were present or absent for the same sample). The vertical line is 40 copies of functional input DNA and the triangle shapes represent a single false-positive call.

Tissue	FFPE	Fresh Frozen	Plasma	Serum
Large Intestine	KRAS.G12A (66.7) N=1			None N=2
Large Intestine	KRAS.G12A (68.1) N=1			KRAS.G12A (1.4) N=2
Large Intestine	PIK3CA.E542K (4.3) BRAF.V600E (20.9) N=1	PIK3CA.E542K (9.8) BRAF.V600E (21.6) N=1		None N=2
Large Intestine	PIK3CA.H1047R (24.2) N=1		PIK3CA.E545K (2) PIK3CA.E545K (1.5) N=2	PIK3CA.E545K (1.8) N=2
Large Intestine	KRAS.G12C (13) PIK3CA.E545K (18.4) N=1		None N=2	None N=2
Large Intestine	KRAS.G12D (33.4) N=1	KRAS.G12D (23.8) N=1	None N=2	None N=2
Skin	BRAF.V600E (4.3) BRAF.V600E (4.3) BRAF.V600E (2.3) N=3	BRAF.V600E (5.7) BRAF.V600E (11.8) BRAF.V600E (16.3) N=3	None N=4	None N=2
Soft Tissue	BRAF.V600E (65.5) N=1	BRAF.V600E (46.4) N=1		
Skin	NRAS.Q61K (19.5) N=1	NRAS.Q61K (22.1) N=1	None N=4	None N=2
Lymph Node	BRAF.V600E (55) N=1	BRAF.V600E (53.6) N=1		BRAF.V600E (4.3) BRAF.V600E (4.6) N=2
Lymph Node	BRAF.V600E (88) N=1		BRAF.V600E (18.9) BRAF.V600E (16.1) N=2	BRAF.V600E (9.8) BRAF.V600E (11.9) N=2
Large Intestine	KRAS.Q61L (43) N=1		None N=2	None N=2
Lymph Node	BRAF.V600E (36.4) N=1			None N=2
Lung	KRAS.G12V (33.5) N=1		None N=2	
Lung	PIK3CA.E542K (18.4) N=1	PIK3CA.E542K (18.5) N=1	None N=4	

Table 2. A total of 28 FFPE biopsies with matched fresh frozen, plasma, and serum profiled by the QuantideX NGS Pan Cancer Kit*. Samples were tissue-matched and collected on the same day for each subject to minimize heterogeneity. Inter-biopsy concordant calls are shown in orange, discordant or unconfirmed calls in black, libraries run at <400 functional copies shaded blue, and missing samples shaded grey. 15/28 donors were positive for one or more non-silent mutations with MAF <1% by 1000 genomes.

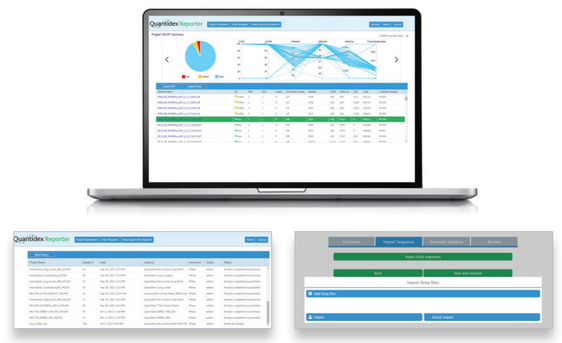

Figure 4. QuantideX NGS Reporter software and interface. The QuantideX variant caller is made available through the QuantideX NGS Reporter analysis software (www.asuragen.com). Shown are screenshots of the software, illustrating the push-button analysis and results.

CONCLUSIONS

- The number of false-positive calls in low-quality samples was dramatically reduced by the QuantideX NGS Reporter variant caller, improving the PPV of variant calling by 51% or more with only an 8% decrease in sensitivity.
- The QuantideX variant caller supports low inputs of residual clinical DNA by incorporating sample-specific pre-analytical QC data, thereby increasing the likelihood that precious samples can be processed by NGS.
- BRAF and KRAS variants in residual clinical FFPE with fewer than 40 functional copies of DNA were identified with the QuantideX variant caller.
- The QuantideX NGS Reporter is available at www.asuragen.com along with the QuantideX NGS Pan Cancer Kit* to enable accurate interrogation of the most challenging clinical specimens.

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