SuraSeq® Targeted NGS Assays: Integrated, Cross-Platform Reagents That Enable Accurate Detection of Cancer Gene Mutations in Residual Clinical FFPE, FNA, and Biofluid Biopsies

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SUMMARY

- Next-generation sequencing (NGS) is an enabling technology for precision medicine applications, but clinical implementation is challenged by the lack of process integration from specimen-to-report.
- We developed a comprehensive, cross-platform NGS workflow for FFPE, FNA and biofluid specimens, known as SuraSeq® targeted NGS, that links functional DNA and RNA quantification (Quantidex™ DNA and RNA assays) with bioinformatic analyses.
- We illustrate the value of this integrated approach using three targeted NGS assays: a 21-gene pan-cancer panel, a TP53 exon panel, and a 104-gene panel that combines both DNA and RNA sequencing of markers of thyroid cancer.
- Analyses across a total of 312 FFPE, FNA, and biofluid specimens revealed accurate calls down to 1-5% mutations from as few as 200 copies of amplifiable DNA, and improvements in clinical sensitivity by combining both DNA and RNA variants in thyroid cancer.

INTRODUCTION

The capabilities of NGS to interrogate somatic mutations in cancer gene panels have spurred the rapid adoption of this technology for precision medicine. However, NGS workflows that integrate analytically-defined controls, pre-analytical and in-process QC assays, and standardized bioinformatics and reporting are sorely needed. We describe a comprehensive sequencing approach that supports both exon- and hotspot-targeted NGS using low DNA or RNA inputs on either Illumina MiSeq or Ion Torrent PGM instruments.

MATERIALS AND METHODS

DNA functional quality was determined using Quantidex™ DNA or RNA assays (adapted from Sah et al., 2013), and PCR-based target enrichment was conducted using SuraSeq® NGS reagents (modified from Hadd et al., 2013). Controls included cancer cell-line DNA mixtures and novel designs for synthetic gBlock constructs (IDT), as well as residual clinical specimens obtained under IRB approval. Sequencing procedures for MiSeq (Illumina) and PGM (Thermo Fisher) followed manufacturer's instructions. Bioinformatic methods (SuraSight® software) for DNA and RNA analyses were developed in-house.

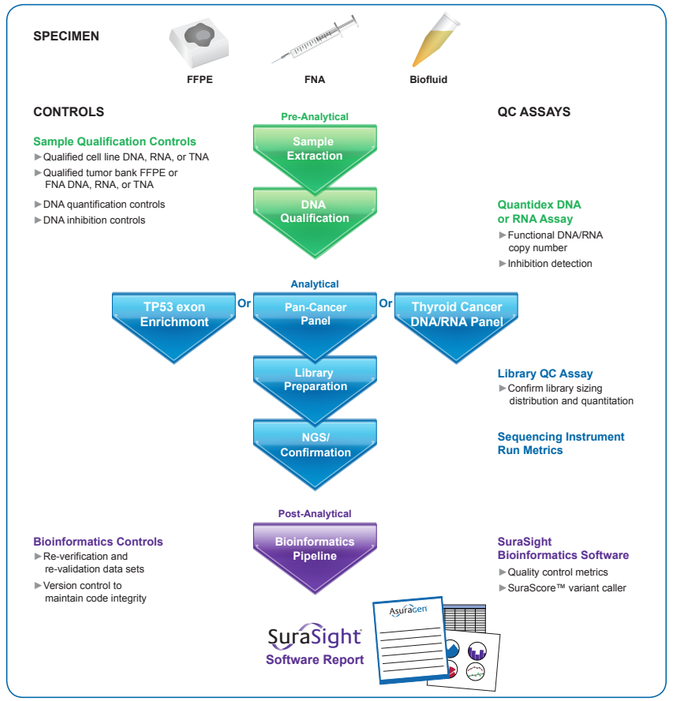


Figure 1. Workflow for SuraSeq® targeted NGS assays featuring three distinct NGS panels.

Presented at AMP 2014

RESULTS

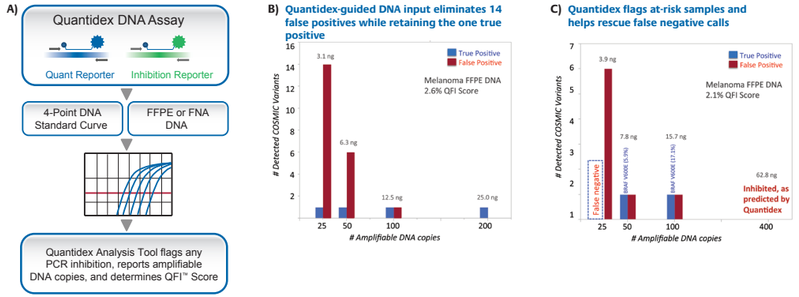
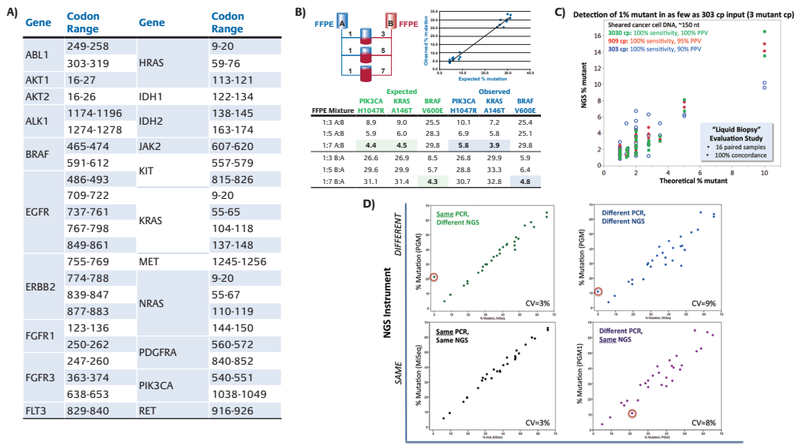


Figure 2. Quantification of amplifiable DNA by the Quantidex™ DNA assay guides input into the NGS enrichment step to help assure the accuracy of variant calling. A) Quantidex assay workflow, **B)** Rescue of NGS false positives in melanoma FFPE DNA, **C)** Rescue of NGS false positives and a false negative, and confirmed inhibition from a high-input melanoma FFPE DNA.



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	138-145
	1274-1278		163-174
BRAF	465-474	JAK2	607-620
	591-612	KIT	557-579
	486-493		815-826
EGFR	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	PIK3CA	840-852
	363-374		540-551
	638-653		1038-1049
FLT3	829-840	RET	916-926

Figure 3. Accurate detection of low-abundance mutations in FFPE and biofluid DNA using a SuraSeq™ pan-cancer panel, and comparisons of MiSeq and PGM instruments. A) Hot spot coverage across 21 cancer genes, **B)** Quantification to <5% variant in FFPE mixtures, **C)** Detection of low-level mutations in cell-line DNA, and concordance study results in 16 biofluid samples, **D)** Variations in mutation quantification across 40 FFPE samples, comparing distinct PCRs and NGS instruments (MiSeq and PGM).

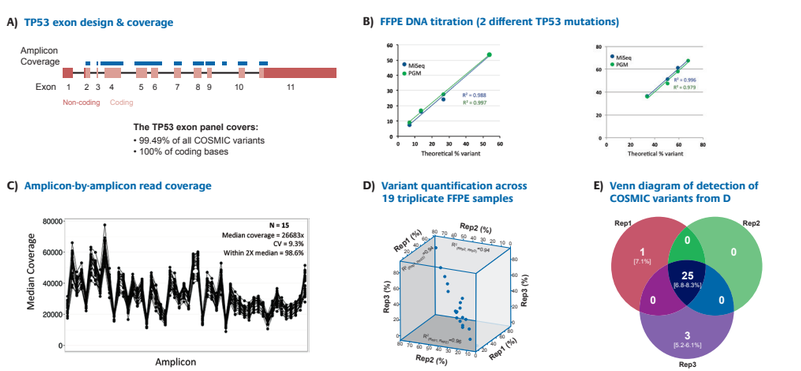
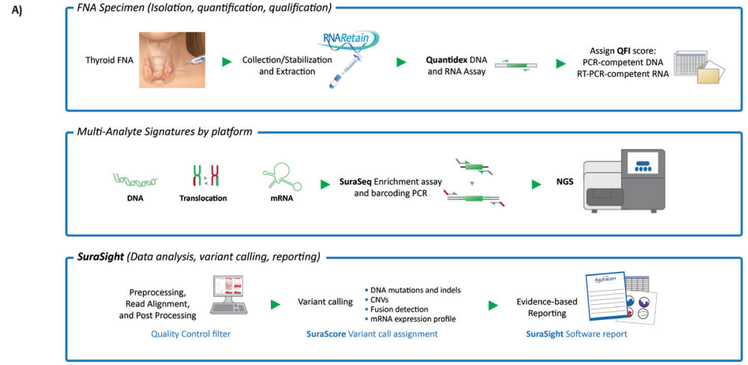


Figure 4. Design and evaluation of a TP53 exon sequencing assay. A) Panel design, **B)** Titration of two FFPE DNA samples to 7-9% variant, **C)** Coverage uniformity, **D)** Correlation of variant quantification across triplicate FFPE samples, **E)** Sample-level concordance from Fig. 4D. Note that all discordant variants shown in Fig. 4E are near the LOD of 5%, and are exclusively derived from 2 samples with low QFI scores (3.7 and 6.1%).



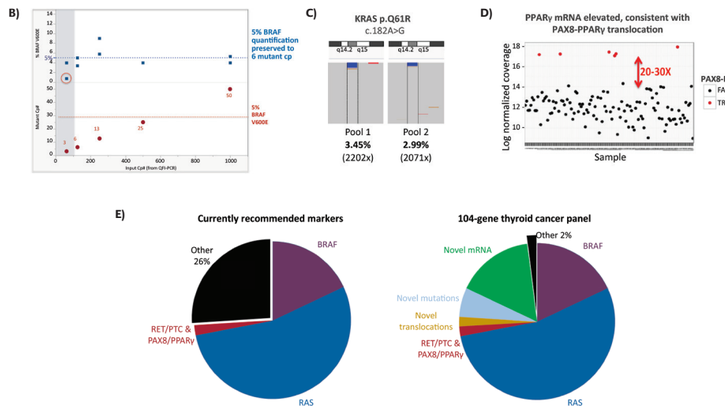


Figure 5. A 104-gene panel workflow integrates total nucleic acid (TNA) inputs with unified DNA and RNA enrichment, "built-in" confirmation, and sample-specific bioinformatics to improve the detection of thyroid cancer. A) Comprehensive NGS system approach, **B)** Optimized DNA variant detection from FNA TNA, **C)** Confirmation of a low-level KRAS mutation in FNA through orthogonal panel design, **D)** Intra-assay confirmation of a PAX8-PPARγ translocation, **E)** Superior sensitivity for thyroid cancer detection in 50 cytologically indeterminate FNAs using the 104-gene panel compared to currently recommended clinical markers.

CONCLUSIONS

- Quantification of functional DNA and RNA using Quantidex™ Assays guides DNA, RNA, and TNA inputs, flags PCR inhibition, and rescues NGS false positives and negatives.
- SuraSeq targeted NGS utilizes a systems approach to accurately quantify variants in hot spot and exon sequencing panels across both MiSeq and PGM instruments.
- Repeatability studies and cross-platform comparisons demonstrate a high level of agreement in variant calling while also illuminating sources of variation.
- A 104-gene thyroid cancer panel can improve the detection of malignant FNA biopsies from cytologically indeterminate specimens by optimizing TNA inputs, providing "built-in" confirmation of DNA and RNA variants, and compounding diagnostic value across multiple sentinels of dys-regulated disease pathways.

Research use only – not for use in diagnostic procedures.
Preliminary research data. The performance characteristics of this assay have not yet been established.