SCALABLE, MODULAR COMPONENTS AND RIGOROUS QUALITY CONTROL FOR NEXT GENERATION SEQUENCING IN THE CLINICAL LABORATORY

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SUMMARY

- The QuantideX® NGS workflow is flexible to accommodate low to high-throughput sample requirements, incorporating pre-analytical, in-process and post-analytical quality controls.
- The kit includes pre-analytical sample qualification, 2-step PCR-based targeted library enrichment, library purification, library quantification and custom sequencing primers for use with the MiSeq® system.
- The Pan Cancer panel targets hotspot next generation sequencing of clinically relevant genes found throughout multiple tumor types.

INTRODUCTION

Targeted assays using next-generation sequencing (NGS) are driving advances in cancer and genetic disease diagnosis. To ensure the accuracy of variant calls and relevance to precision medicine, NGS-based assays require additional layers of quality control and safeguards. A streamlined NGS assay enables rapid turnaround time from sample prep to patient reporting. With this goal in mind, we developed a modular and scalable reagent kit for NGS analysis of FFPE-extracted DNA that integrates rigorous pre-analytical, analytical and post-analytical quality controls and provides a fast and streamlined workflow.

METHODS

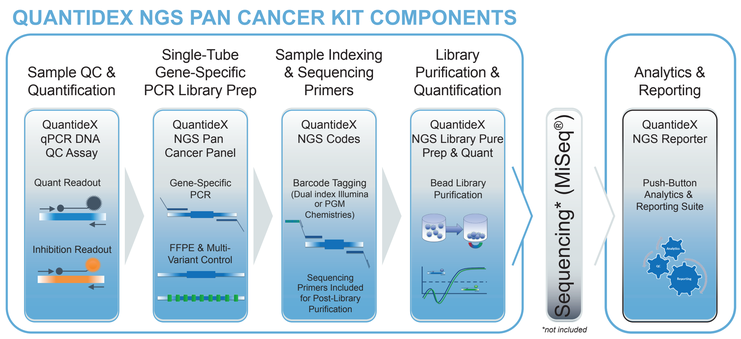


Figure 1. The QuantideX NGS Pan Cancer Kit provides an all-in-one NGS solution. A comprehensive workflow integrating reagents, controls, and a novel bioinformatics suite for the sequencing of an oncology panel relevant to a diverse set of human cancers.

QuantideX DNA Assay

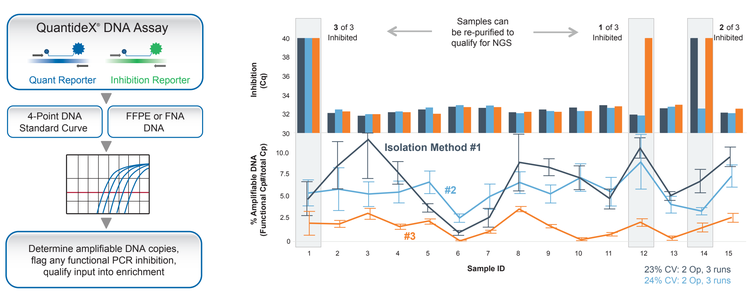


Figure 2. The QuantideX DNA Assay can identify variations in sample quality and effect of inhibitors. Functional DNA is assessed using a qPCR-based method to assess functional copy number, which is integrated into downstream analysis. 15 Melanoma FFPE tumor biopsies were isolated with three different methods showing variation in yield (lower axis) and inhibition (upper axis). Percent amplifiable DNA is plotted with error bars for standard deviation of 3 independent runs.

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 SLAS 2016

QuantideX NGS Pan Cancer Panel

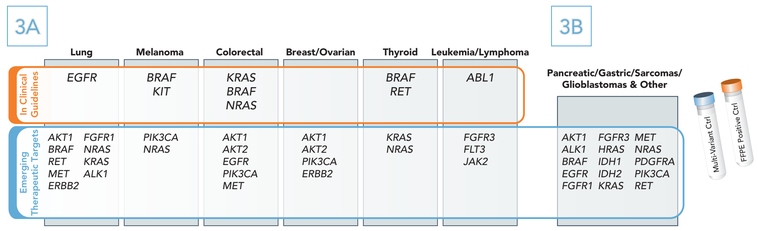
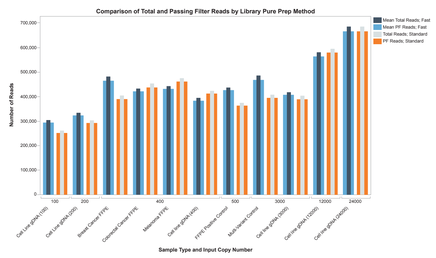


Figure 3. The QuantideX NGS Pan Cancer Panel uses a multiplexed primer pool and process controls to ensure the accuracy of library preparation and analysis. (A) The panel targets 21 genes across 46 regions of current and emerging targets of clinical significance in human cancer. **(B)** Each batch of NGS library preparation includes true FFPE positive control comprised of a 5% BRAF p.V600E mutation and a Multi-Variant process control containing 14 COSMIC variants of 3 different types (Indel, Substitution, Deletion) that are targeted by the panel.

QuantideX Codes

Figure 4. Dual-index codes with mastermix-free preparation ensures sample integrity. Index codes are formulated to include platform-specific adaptor sequences for targeted PCR products with custom adapters. Codes are provided in individual tubes, racked in an SBS format plate. Each sample during the indexing reaction is given a unique pairwise "set" of index codes.

QuantideX Library Pure Prep



Method	Standard	Fast
Bind off magnet?	Yes	Yes
Bind incubation	4 min	--
Bind magnet time	4 min	20 sec
Wash off magnet?	Yes	No
Resuspend beads during wash?	Yes	No
Wash incubation	2 min	--
Wash magnet time	2 min	20 sec
Dry incubation time	2 min	1 min
Elute off magnet?	Yes	Yes
Elute incubation	4 min	1 min
Elute magnet time	4 min	20 sec
Total plate moves (on/off magnet)	7	3
Total incubation and magnet time	26 min	3.33 min

Figure 5. QuantideX Library Pure Prep's bead purification methodology is simple, fast and robust to DNA copy number input. Total reads and reads passing filter is similar when procedure is varied between "standard" and "fast" in replicate analysis of FFPE samples and kit controls over broad range of input copies (100 to 24,000).

QuantideX Library Quant

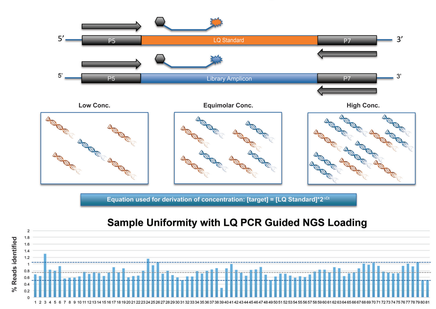


Figure 6. Library Quant guides loading of the MiSeq using an internal single-point reference for the quantification of targeted NGS libraries using qPCR. Differences in cycle threshold (ΔCt) are used to quantify the sample library and guide the normalization of sample pooling.

QuantideX Sequencing Reagents

	PhiX Alignment	
Samples/Run	Expected	Observed
8	7.40%	7.50%
81	7.40%	8.20%
192	7.40%	6.10%

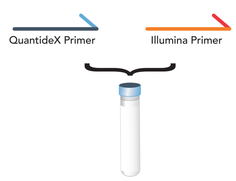


Figure 7. Premixed primers for QuantideX and standard Illumina libraries for efficient, controlled sample loading. Custom sequencing primer mixes and a diluent are provided in the kit for detection and loading of pooled QuantideX libraries and an Illumina PhiX sequencing control. The alignment of the PhiX sequencing control, mixed with QuantideX libraries, is consistent with expected results. Total reads are affected by total samples, number of amplicons, yield and sequencing quality.

QuantideX NGS Reporter

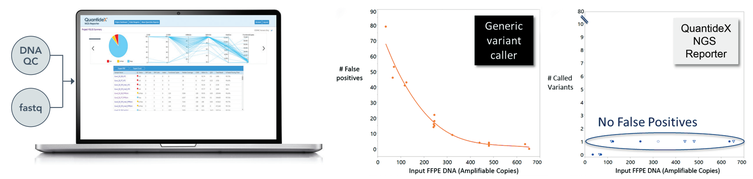


Figure 8. QuantideX NGS Reporter software is a locally installed NGS analysis solution. The QuantideX variant caller incorporates sample-specific pre-analytical QC data, thereby increasing the likelihood that precious samples can be processed by NGS. The number of false-positive calls in low-quality 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, as compared to a caller without pre-analytical QC.

RESULTS

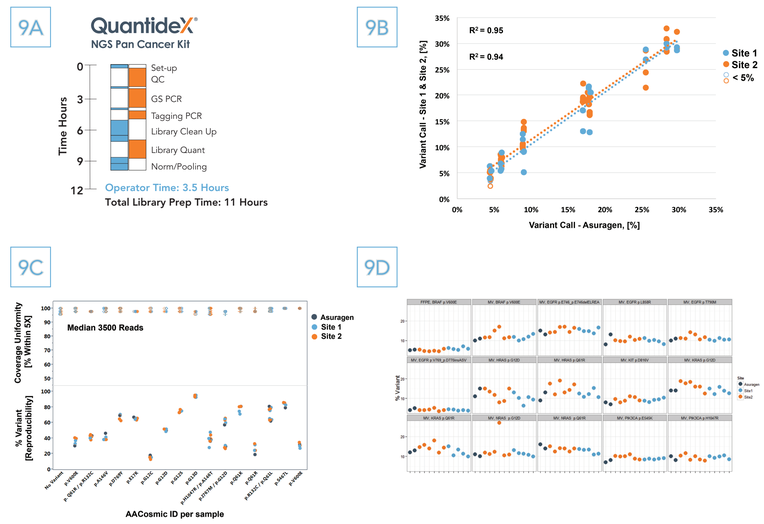


Figure 9. Reproducible and accurate variant call detection in controls and clinical samples is achieved using an efficient workflow. (A) Manual library preparation using the QuantideX NGS Pan Cancer Kit for 20 samples reduced hands-on operator time to 3.5 hours. **(B)** Titration of FFPE tumor DNA revealed dose-dependent detection of mutations to 5% of total NGS reads in multiple laboratories, maintaining sensitivity and positive predicted value (PPV). **(C)** Independent sample preparations and multiplex library pools from repeatability studies yielded reproducible variant calls and uniform coverage. **(D)** Controls included in the kit reliably reported targeted variants in the FFPE Positive Control and Multi-Variant Control across sites and runs.

CONCLUSIONS

- Our focus on controls and scalability addresses important considerations for the adoption and expeditious validation of NGS methods within the clinical laboratory.
- Due to the modular workflow approach, alternative panels may be substituted to the same workflow for greater applicability to emerging targets of clinical utility.

- The modular components and simple workflow may be easily translated to automated processes.

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