A MODULAR NEXT-GENERATION SEQUENCING TECHNOLOGY THAT COMBINES TARGETED ENRICHMENT AND BIOINFORMATICS ANALYSES TO REVEAL RNA EXPRESSION AND DNA MUTATIONS IN LUNG CANCER

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SUMMARY

- The QuantideX® NGS System* enables broad, sensitive and accurate profiling of RNA and DNA from challenging clinical research specimens.
- Analysis of 273 NSCLC specimens from 3 clinical cohorts was performed using the QuantideX® NGS RNA Lung Cancer Kit* and a prototype DNA lung panel.
- Molecular features identified by QuantideX NGS were consistent with disease subtype and analytically concordant with an independent NGS method.

INTRODUCTION

RNA fusions and aberrant splicing events, such as *MET* exon 14 skipping, are important therapeutic targets in non-small cell lung cancer (NSCLC) and other solid tumors. Quantification of RNA variants can complement DNA analyses to capture the dynamic molecular state of tumor cell populations and inform treatment strategies. We describe a novel targeted next-generation sequencing (NGS) technology that offers both modularity and systems-level integration to achieve accurate RNA and DNA profiling from challenging clinical specimens.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded (FFPE) or fine-needle aspiration (FNA) specimens from three cohorts, including the BATTLE-2 clinical trial, were collected at MD Anderson Cancer Center. BATTLE-2 tissues were also sequenced using FoundationOne® (Foundation Medicine). Pre-analytical QC was performed using novel qPCR assays that quantify distinct populations of amplifiable RNA and DNA from total nucleic acid. RNA or DNA was enriched by PCR using the QuantideX NGS RNA Lung Cancer Kit, or a prototype DNA panel with common non-primer-based reagents, and sequenced on a MiSeq® System (Illumina). Data analysis was achieved using QuantideX® NGS Reporter, an analysis suite that directly incorporates pre-analytical QC data into the variant calling algorithm to improve the identification of DNA mutations and RNA fusions.

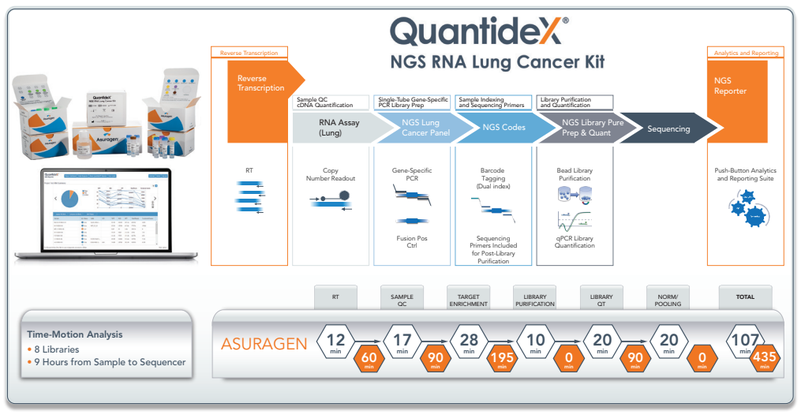

Figure 1. Overview of the QuantideX NGS workflow from nucleic acid quantification to variant analysis. Workflow for the RNA Lung Cancer Kit is shown. The prototype targeted DNA-Seq workflow follows an identical process but excludes the reverse transcription step and enlists a DNA-specific bioinformatic analysis module.

*For Research Use Only – Not For Use In Diagnostic Procedures
Preliminary research data. The performance characteristics of this assay have not yet been established.
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RESULTS

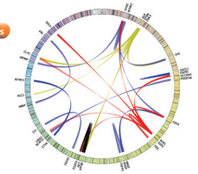
2A

QuantideX NGS RNA Lung Cancer Kit**

3'/5' Imbalance
ALK
ROS1
RET
NTRK1
PDGFRA

Exon Skipping Event
MET e13:e14
MET e14:e15
MET e13:e15

3' Fusion Genes	# of Fusions
ALK	53
ROS1	22
RET	12
FGFR3	7
NTRK3	3
NTRK1	4
NRG1	2
FGFR1	1
FGFR2	1
MBIP	1
PDGFRA	1



11 fusion genes–
107 known and relevant fusion products

mRNA Expression Targets							
ABCB1	*CDKN2A*	*ESR1*	*IFNGR*	*MSLN*	*PTEN*	*TERT*	*TUBB3*
BRCA1	*CTLA4*	*FGFR1*	*ISG15*	*PDCD1*	*RRM1*	*TLE3*	*TYMS*
CD274 (PDL1)	*ERCC1*	*FGFR2*	*MET*	*PDCD1LG2 (PDL2)*	*TDP1*	*TOP1*	Endogenous Ctrls.

**Content sourced from: NCCN Guidelines, customer needs, COSMIC, Clinicaltrials.gov, publications & other databases.

2B

QuantideX NGS DNA Lung Prototype Panel

DNA Targets	
AKT1	*APC*
ALK	*GNAS*
BRAF	*IDH1*
EGFR	*SMAD4*
HRAS	*TP53*
KRAS	*TSHR*
MET	*CDKN2A*
NRAS	*EIF1AX*
RET	*CTNNB1*
PIK3CA	*PTEN*

Figure 2. QuantideX NSCLC NGS Panels. A) The QuantideX NGS RNA Lung Cancer Kit covers 107 recurrent gene fusions involving *ALK, RET* and *ROS1, MET* e14 skipping and 23 mRNA markers of clinical research value. **B)** The prototype NSCLC DNA-Seq panel covers 55 hotspot regions in 20 genes selected from NCCN guidelines, COSMIC prevalence and NSCLC literature.

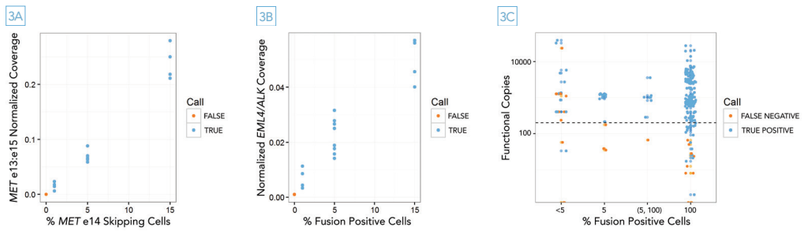


Figure 3. QuantideX NGS RNA Lung Cancer Kit Analytical Performance. Admixture of **A)** *MET* exon 14 skipped cell line or **B)** *EML4-ALK* positive FFPE in the background of wild-type cells with positive detection down to 1 in 100 cells. **C)** Libraries prepared with <200 functional copies are at risk for false-negative calls. The dashed line indicates the minimum recommended input of 200 functional copies.

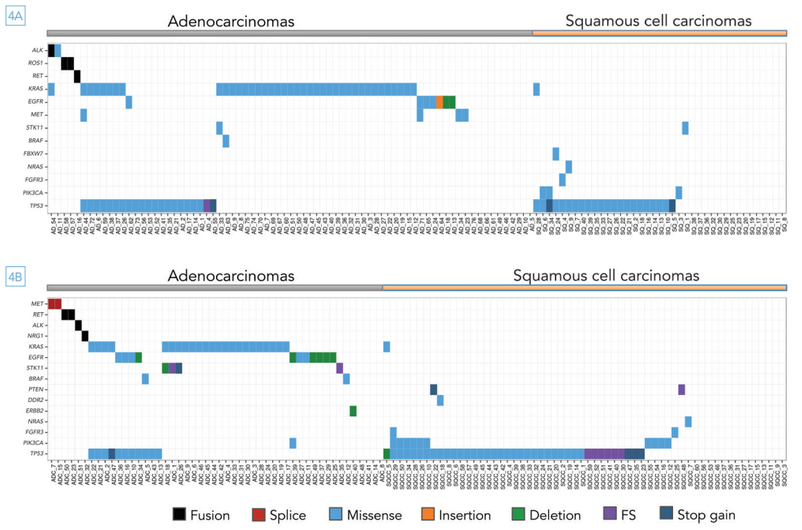


Figure 4. Multi-omic characterization of MDACC cohorts using the QuantideX NGS RNA Lung Cancer Kit and the DNA Lung Cancer Prototype Panel. A) MDACC Cohort 1 (CNBs). **B)** MDACC Cohort 2 (surgical resections). Mutations identified in both cohorts were consistent with the underlying histopathology. For example, DNA mutations in *KRAS, EGFR* and RNA fusions involving *ALK, RET* and *ROS1* were restricted to adenocarcinomas. Note that SQ_9 (*NRAS* mutant) in Cohort 1 was characterized as a poorly differentiated NSCLC with squamous cell carcinoma features, whereas SQ_28 (*KRAS* mutant) was identified as a poorly differentiated NSCLC with adenocarcinoma features (TTF1+, p40-).

	Cohort QC Status				Specimen	QC	Fusion	Imbalance	*MET* e13:15
	Total	Pass	At Risk	Fail					
MDACC Cohort 1	113	78	27	8	AD16	At Risk	*KIF5B-RET*	None	N
					AD54	Pass	*EML4-ALK*	*ALK*	N
					AD57	Pass	*EZR-ROS1*	None	N
					AD58	Pass	*CD74-ROS1*	None	N
MDACC Cohort 2	110	109	1	0	ADC7	Pass	None	None	Y
					ADC15	Pass	None	None	Y
					ADC23	Pass	*CCDC6-RET*	*RET*	N
					ADC32	Pass	*CD74-NRG1*	None	N
					ADC50	Pass	*KIF5B-RET*	*RET*	N
					ADC51	Pass	*EML4-ALK*	*ALK*	N

Table 1. Summary of targeted RNA-Seq results in two FFPE NSCLC clinical cohorts. MDACC Cohort 1 (CNBs) and MDACC Cohort 2 (surgical resections). All specimens with detected fusion or *MET* e14 skipping events are shown with 10/215 (4.7%) of the evaluable specimens testing positive and only 3.6% of specimens failing QC.

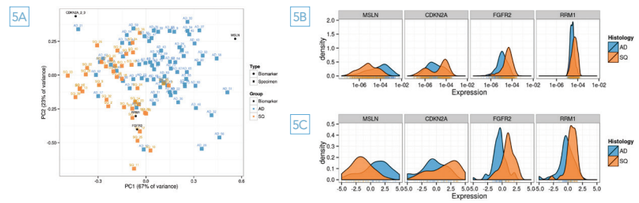


Figure 5. Select mRNA expression markers revealed by targeted RNA-Seq distinguish squamous cell carcinomas (SQ) from adenocarcinomas (AD). A) PCA analysis of 4 mRNA markers over MDACC Cohort 1. **B)** TCGA and **C)** MDACC Cohort 1 shows similar SQ and AD distributions for select mRNAs.

BATTLE-2 ID	Mutation	%Variant	FMI Call
B2_002	*TP53* p.Y163H	**63.3**	+
B2_023	*BRAF* p.G469A	**14.6**	**N/A**
B2_026	*KRAS* p.A59E	**34.7**	+
B2_026	*TP53* 346*	**29.9**	+
B2_027	*TP53* p.S241fs*6	**14.6**	**N/A**
B2_044	*TP53* p.G245C	**87.6**	+
B2_060	*TP53* p.P190L	**24.5**	+
B2_060	*EGFR* p.E746_A750delELREA	**54.0**	+
B2_076	*TP53* p.G245C	**95.2**	+
B2_078	*TP53* p.R273P	**41.9**	+
B2_084	*TP53* p.L348F	**51.9**	+
B2_091	*TP53* p.R273H	**18.9**	+
B2_103	*EGFR* p.L858R	**72.0**	+
B2_103	*EGFR* p.T790M	**39.4**	+
B2_103	*TP53* p.T126H	**25.4**	+

BATTLE-2 ID	Mutation	%Variant	FMI Call
B2_108	*KRAS* p.G12C	**33.3**	+
B2_108	*TP53* p.E294D	**49.6**	+
B2_108	*TP53* p.E294fs*12	**49.7**	+
B2_117	*KRAS* p.G12C	**50.9**	+
B2_120	*KRAS* p.G12V	**21.9**	+
B2_120	*TP53* p.R248Q	**21.0**	+
B2_121	*KRAS* p.G12C	**86.1**	+
B2_121	*TP53* p.G154V	**71.0**	+
B2_143	*EGFR* p.T790M	**21.8**	**N/A**
B2_143	*TP53* p.Y234C	**21.8**	**N/A**
B2_143	*EGFR* p.L747_E749delLRE	**23.9**	**N/A**
B2_145	*TP53* p.H214R	**63.7**	**N/A**
B2_147	*CTNNB1* p.S37F	**17.8**	+
B2_147	*MET* p.T1010I	**59.0**	-
B2_324	*PIK3CA* p.E545K	**11.3**	**N/A**

Table 2. BATTLE-2 DNA variants from FoundationOne NGS analysis of tissue specimens were in 97% agreement with Asuragen targeted DNA-Seq results from FNA smears. Smears contained only ~100 to a few hundred cells with 24/50 yielding sufficient material for sequencing. By comparison, "FMI Call" refers to variants identified by FoundationOne using tissue of higher quality/quantity and less challenging to sequence. Samples without FMI call data are indicated as "N/A".

CONCLUSIONS

- The QuantideX NGS System is a highly sensitive and accurate technology for the comprehensive characterization of clinical research specimens.
- *Sample-Aware*™ bioinformatics flags libraries at risk of false-negative calls, enabling confident evaluation of low-input, poor-quality specimens.
- The QuantideX NGS System is a modular and extensible framework to develop targeted RNA and DNA assays for precision medicine applications.

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