QUANTIDEX® TARGETED RNA-SEQ ENABLES SENSITIVE AND ACCURATE DETECTION OF GENE FUSIONS, *MET* EXON 14 SKIPPING AND EXPRESSION PROFILING OF FFPE LUNG CANCER SPECIMENS

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SUMMARY

- The QuantideX® NGS RNA Lung Cancer Kit is a research tool for analyzing recurrent gene fusions, *MET* exon 14 skipping and gene expression in lung cancer specimens.
- The QuantideX® RNA QC assay predicts false-negative risk.
- Fusions and *MET* e14 skipping events are detected to <5% positive in a background of wild-type cells.
- Evaluation of over 300 FFPE specimens spanning 3 clinical cohorts further demonstrates kit performance.

INTRODUCTION

RNA fusions and splice variants, such as *MET* exon 14 skipping, are recognized as important therapeutic targets in non-small cell lung cancer (NSCLC) and a growing number of other solid tumors. Despite the emerging importance of these targets to cancer research, NGS assays that analyze RNA markers currently lag behind DNA sequencing efforts in workflow efficiency and in the rigor of analytical performance evaluations.

METHODS

NSCLC cell lines and FFPE specimens from three cohorts were analyzed by the QuantideX® NGS RNA Lung Cancer Kit. Two cohorts were collected at MD Anderson Cancer Center and a third cohort was provided by Asuragen. Positive fusions and *MET* exon 14 skipping events were confirmed by PCR and capillary electrophoresis, or digital PCR. Admixtures and input titrations of cell-line and select FFPE specimens were evaluated to determine assay sensitivity and robustness to low-input, low-quality nucleic acids.

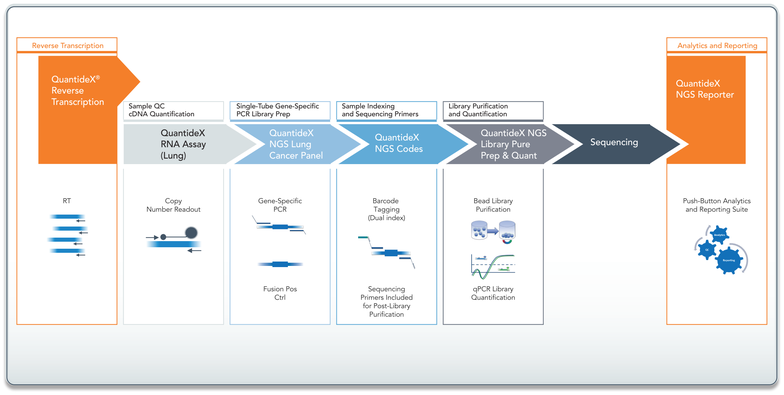

Figure 1. Overview of QuantideX NGS RNA Lung Cancer Kit from wet to dry bench analytics.

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 AGBT 2016

RESULTS

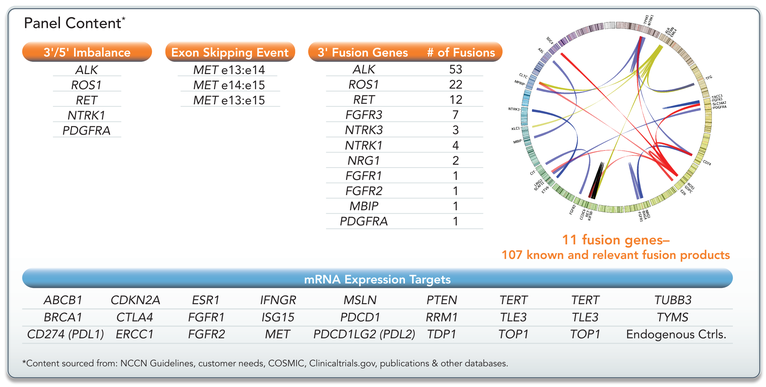

Figure 2. QuantideX NGS RNA Lung Cancer Kit content. Covers 107 recurrent gene fusions including *ALK*, *RET* and *ROS1*, *MET* ex14 skipping and 23 mRNA markers of prognostic and theranostic value.

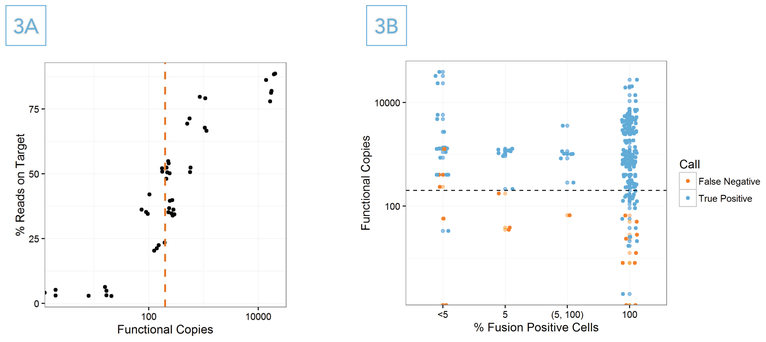

Figure 3. QuantideX RNA QC predicts library quality and false-negative risk. A) Fraction of library reads mapping to intended targets is predicted by library input copies. **B)** Fusion positive libraries prepared with <200 functional copies are at risk for false-negative calls. Dashed line in both plots indicates minimum recommended input of 200 functional copies.

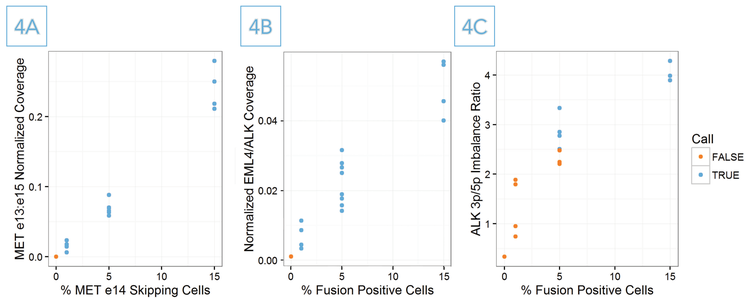

Figure 4. QuantideX NGS Assay detects fusions and splice variants down to 1:100 cells. A) Admixture of a *MET* Δe14 positive cell-line in the background of wild-type cells. **B)** Fusion and **C)** 3′/5′ imbalance status for an admixture of *EML4-ALK* positive FFPE in the background of a negative FFPE across multiple technical replicates.

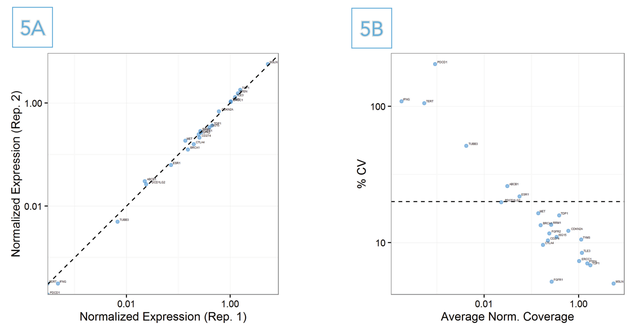

Figure 5. RNA expression markers accurately and reproducibly quantified by kit in multi-operator, multi-day study. A) Representative inter-operator, concordance of mRNA expression between FFPE technical replicates **B)** %CV over 12 FFPE replicates. Line indicates 20% CV.

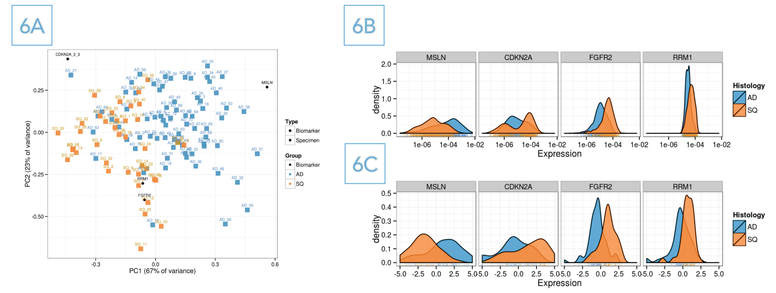

Figure 6. Select mRNA expression markers distinguish squamous (SQ) from adenocarcinomas (AD). A) PCA analysis of 4 mRNA markers over MDACC Cohort 1. **B)** TCGA and **C)** MDACC Cohort 1 show similar SQ and AD distributions for select mRNAs.

	Cohort QC Status				Specimen	QC	Fusion	Imbalance	*MET* Δe14
	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
Asuragen Tumor Bank	112	78	24	10	LC104	At Risk	*EML4-ALK*	None	N
					LC107	Pass	*EML4-ALK*	ALK	N
					CL138	Pass	*EML4-ALK*	ALK	N
					LC143	Pass	*EML4-ALK*	ALK	N
					LC159	Pass	*KIF5B-RET*	RET	N
					LC163	At Risk	*SLC34A2-ROS1*	None	N
					LC170	Pass	*EML4-ALK*	ALK	N
					LC191	Pass	*EML4-ALK*	ALK	N
					LC192	Pass	*EML4-ALK*	ALK	N
					LC209	Pass	*EML4-ALK*	ALK	N
					LC220	Pass	*EML4-ALK*	ALK	N
					LC227	At Risk	*EML4-ALK*	None	N
					LC77	Pass	*KIF5B-RET*	None	N
					LC93	Pass	*EML4-ALK*	ALK	N
					LC95	Pass	*EML4-ALK*	ALK	N
					LC98	Pass	*EML4-ALK*	ALK	N

Table 1. Summary of 3 FFPE NSCLC clinical cohorts: MDACC Cohort 1 (CNBs), MDACC Cohort 2 and Asuragen Tumor Bank (surgical resections). QuantideX QC categorization is shown per cohort. All specimens with detected fusion or *MET* ex14 skipping events are shown with 26/317 (8.2%) of the evaluable specimens testing positive. Only 5% of FFPE specimens failed QC metrics.

CONCLUSIONS

- The QuantideX NGS RNA Lung Cancer Kit is an efficient and accurate tool for profiling low-input NSCLC specimens with sensitivity to <5% positive.
- Sample-Aware™ bioinformatics flags libraries at risk of false-negative calls, enabling confident evaluation of poor-quality specimens.
- The QuantideX® NGS system is a modular and extensible framework upon which to develop NGS assays for precision medicine applications.

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