INDEPENDENT EVALUATION OF A FULLY INTEGRATED NEXT-GENERATION SEQUENCING TECHNOLOGY FOR THE ACCURATE DETECTION OF ONCOGENIC RNA FUSIONS AND ABERRANT SPLICING EVENTS IN LUNG CANCER

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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 kit demonstrated concordant results across two laboratories using 30 total nucleic acid (TNA) isolates derived from clinically-relevant specimens such as FFPE tumor biopsies.
- Agreement was also achieved for 8 residual clinical FFPE samples comparing the QuantideX NGS RNA Lung Cancer Kit* and FISH.
- Results comparing the QuantideX NGS RNA Lung Cancer Kit* and the nCounter® Vantage™ Lung Gene Fusion Panel were in agreement for both the original cohort and residual FFPE samples.

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. We present a new targeted RNA-Seq Lung Cancer assay that can report 107 known oncogenic gene fusions, *MET* exon 14 skipping, 26 expression markers, and 5 imbalance markers for novel fusion detection using a streamlined sample-to-answer workflow.

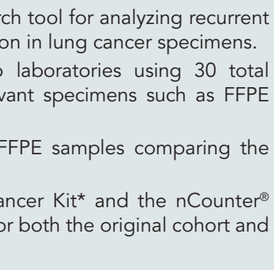
MATERIALS AND METHODS

The QuantideX NGS RNA Lung Cancer Kit* was evaluated at Asuragen (Austin, TX) and Jewish General Hospital (Montreal, QC, Canada). A set of 30 samples derived from banked tumors and cell lines plus two controls were analyzed at Asuragen using the MiSeq® System (Illumina) and used over three study phases. An additional set of FFPE-derived residual clinical research samples that were previously annotated by FISH were evaluated in the final study phase. A subset of samples were also evaluated using the nCounter Vantage Lung Gene Fusion Panel (NanoString Technologies; The Journal of Molecular Diagnostics (2014) 16(2):229-43).

Panel Content*

3'/5' Imbalance	Exon Skipping Event
ALK	*MET* e13:e14
ROS1	*MET* e14:e15
RET	*MET* e13:e15
NTRK1	
PDGFRA	

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.

Figure 1. QuantideX NGS RNA Lung Cancer Kit* content. The panel covers 107 recurrent gene fusions including *ALK, RET* and *ROS1, MET ex14* skipping, 23 mRNA markers of prognostic and theranostic value and 3 internal control mRNA markers.

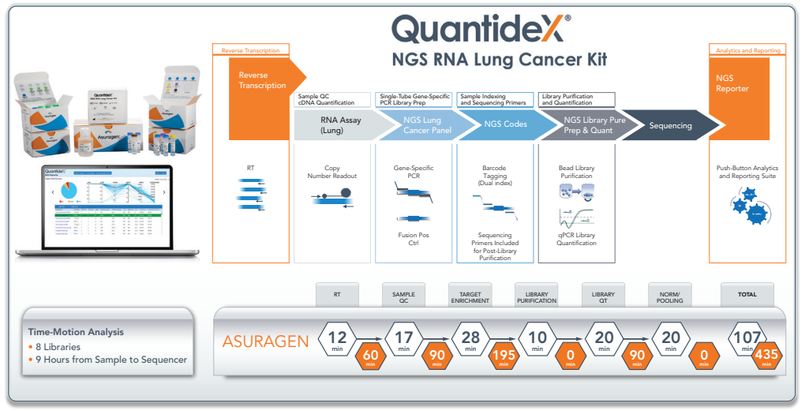


Figure 2. Overview of QuantideX NGS RNA Lung Cancer Kit* from wet lab to dry bench analytics. The workflow is designed to minimize the number of steps, operator hands-on-time, and overall turn-around-time. Time-Motion Analysis shows both operator hands-on-time and instrument time. The bundled QuantideX® Reporter software package runs locally on a standard computer.

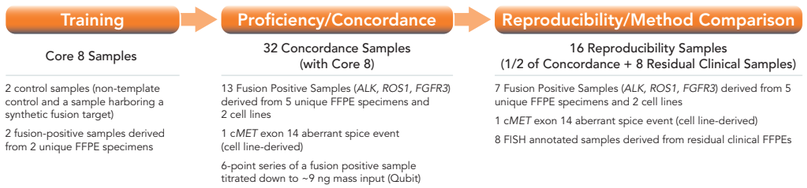


Figure 3. Summary of the site evaluation study design. The study was designed with three phases for NGS evaluation: Training (8 libraries), Proficiency & Concordance (32 libraries), and Reproducibility & Method Concordance (16 libraries in duplicate plus additional evaluator libraries). Specifically, Jewish General Hospital was trained within two days, and they achieved a successful proficiency run in the second phase (Table 1). A subset of samples were evaluated by NanoString's Vantage Lung Gene Fusion Panel. Eight additional samples that were previously annotated by FISH were evaluated using both systems (Table 2). Analysis of mRNA expression was not part of this evaluation.

RESULTS

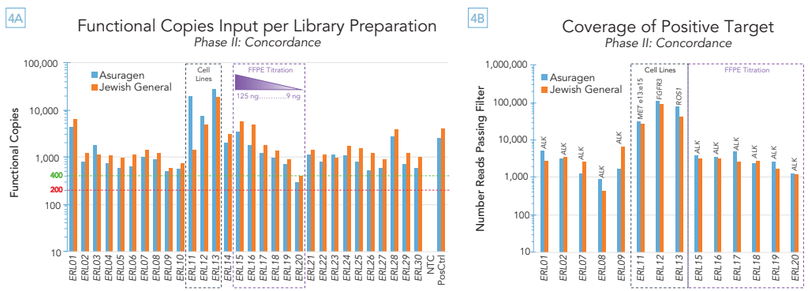


Figure 4. Quantification of both the RNA functional copy number and the on-target coverage for fusions and splice events was consistent between sites. A) Comparison of the functional copy input measured using the QuantideX RNA Assay (Lung). Results were consistent between sites (1.4-fold mean difference with a standard deviation of 0.5-fold). **B)** Comparison of the on-target coverage for each of the positive samples. Results were consistent between sites (1.1-fold mean difference with a standard deviation of 0.9-fold). Even coverage across titration series libraries demonstrated successful normalization and pooling.

	Asuragen Annotations			Concordance (32)			Reproducibility Set 1 (16)			Reproducibility Set 2 (16)		
Sample ID	Source	Mutation Status	Imbalance Status	Expected Fusion	Expected Imbalance	*Met* e13:e15	Expected Fusion	Expected Imbalance	*Met* e13:e15	Expected Fusion	Expected Imbalance	*Met* e13:e15
NTC	Control	Neg	Neg	none	none	none	none	none	none	none	none	none
Pos	Control	Synthetic *ALK*	Neg	✓	none	none	✓	none	none	✓	none	none
ERL1	FFPE	*EML4:ALK*	*ALK*	✓	✓	none	✓	✓	none	✓†	✓	none
ERL2	FFPE	*EML4:ALK*	*ALK*	✓	✓	none	✓	✓	none	Failed	Failed	Failed
ERL3	FFPE	Neg	Neg	none	none	none	none	none	none	none	none	none
ERL4	FFPE	Neg	Neg	none	none	none	none	none	none	none	none	none
ERL5	FFPE	Neg	Neg	none	none	none	none	none	none	none	none	none
ERL6	FFPE	Neg	Neg	none	none	none	none	none	none	none	none	none
ERL7	FFPE	*EML4:ALK*	*ALK*	✓	✓	none	✓	✓	none	✓†	✓	none
ERL8	FFPE	*EML4:ALK*	*ALK*	✓	✓	none	✓	✓	none	✓†	✓	none
ERL9	FFPE	*EML4:ALK*	*ALK*	✓	✓	none	✓	✓	none	✓†	✓	none
ERL10	FFPE	Neg	Neg	none	none	none	none	none	none	none	none	none
ERL11	Cell	*MET* e13:e15	Neg	none	none	✓	none	none	✓	none	none	✓
ERL12	Cell	*FGFR3:TACC3*	Neg	✓	none	none	✓	none	none	✓	none	none
ERL13	Cell	*SLC32A2:ROS1*	*ROS1*	✓	✓	none	✓	✓	none	✓	✓	none
ERL14	FFPE	Neg	Neg	none	none	none						
ERL15	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL16	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL17	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL18	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL19	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL20	FFPE	*EML4:ALK*	*ALK*	✓	✓	none						
ERL21	FFPE	Neg	Neg	none	none	none						
ERL22	FFPE	Neg	Neg	none	none	none						
ERL23	FFPE	Neg	Neg	none	none	none						
ERL24	FFPE	Neg	Neg	none	none	none						
ERL25	FFPE	Neg	Neg	none	none	none						
ERL26	FFPE	Neg	Neg	none	none	none						
ERL27	FFPE	Neg	Neg	none	none	none						
ERL28	FFPE	Neg	Neg	none	none	none						
ERL29	FFPE	Neg	Neg	none	none	none						
ERL30	FFPE	Neg	Neg	none	none	none						

Expected fusions, imbalance and splice variant calls were accurately called in 63 of 64 libraries

One library, *ERL2*, failed both QC and NGS for Reproducibility Set 2

†Samples had low coverage false positive contaminants that were resolved based on plate position

Table 1. Jewish General Hospital NGS results compared to Asuragen sample annotations. Out of 64 libraries performed at Jewish General Hospital, 63 yielded the expected calls both for mutation and imbalance status (after resolving contamination events in reproducibility set 2). One sample failed analysis, which was identified at the QC step (not shown).

Concordance with Orthogonal Test Methods						
Sample ID	FISH	NanoString Vantage Calls	Asuragen QuantideX Fusion Calls	Fusion Concordance	Asuragen QuantideX Imbalance Calls	Imbalance Concordance
ERL2	Neg	*EML4(13):ALK(20)*	*EML4(13):ALK(20)*	✓	ALK	✓
ERL7	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL10	Neg	Neg	Neg	-	Neg	-
ERL13	Neg	*SLC34A2(4):ROS1(32)*	*SLC34A2(4):ROS1(32)*	✓	*ROS1*	✓
ERL14	Neg	Neg	Neg	-	Neg	-
ERL15	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL16	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL17	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL18	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL19	Neg	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	ALK	✓
ERL20	Neg	unknown *ALK*-fusion	*EML4(6):ALK(20)*	✓	ALK	✓
ERL21	Neg	Neg	Neg	-	Neg	-
ERL22	Neg	Neg	Neg	-	Neg	-
ERL23	Neg	Neg	Neg	-	Neg	-
ERL24	Neg	Neg	Neg	-	Neg	-
JGH1	Neg	Neg	Neg	-	Neg	-
JGH2	Neg	Neg†	Neg	-	Neg†	-
JGH3	*ROS1* split	*SLC34A2(4):ROS1(32)*	*SLC34A2(4):ROS1(32)*	✓	Neg*	X
JGH4	Neg	Neg	Neg	-	Neg	-
JGH5	Neg	Neg	Neg	-	Neg	-
JGH6	Neg	Neg	Neg	-	Neg	-
JGH7	*ROS1* split	*EZR(10)-ROS1(34)*	*EZR(10)-ROS1(34)*	✓	*ROS1*	✓
JGH8	*ALK* split	*EML4(6):ALK(20)*	*EML4(6):ALK(20)*	✓	Neg*	X

Table 2. Summary of QuantideX NGS RNA Lung Cancer Kit* FFPE sample agreement with orthogonal methods. Samples identified as fusion positive by either FISH (8 residual clinical samples) or NanoString (8 residual clinical samples + 15 Asuragen subset samples) demonstrated 100% agreement for fusion-positive calls. Imbalances for two samples (*) were not called but were near the positive threshold. One sample (†) was near threshold for an *ALK* imbalance for both NanoString and NGS technologies.

CONCLUSIONS

- An independent laboratory was trained to proficiency in two days using the QuantideX NGS RNA Lung Cancer Kit*.
- The kit workflow, from sample to sequencer, can be completed in about 9 hours.
- High inter-operator, and intra- and inter-run reproducibility, was achieved at two different sites with FFPE samples using inputs down to <10 ng.
- NGS fusion results were in 100% agreement with *ALK* and *ROS1* translocation calls from orthogonal assays performed with 23 FFPE samples.

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*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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