HEAD-TO-HEAD COMPARISON OF TWO COMMERCIALLY AVAILABLE NEXT-GENERATION SEQUENCING TECHNOLOGIES THAT DETECT GENE FUSIONS IN NON-SMALL CELL LUNG CANCER

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SUMMARY

• The accurate detection of cancer-associated RNA fusions and other variants by next-generation sequencing requires reliable and integrated methods that can support a range of FFPE RNA inputs and quality.
• We compared two kits, the Quantidex® NGS RNA Lung Cancer Kit (QX) (QX) and FusionPlex® ALK, RET, ROS1 v2 Kit (FU) (FP), using 30-288 ng FFPE lung tumor RNA and admixtures down to ≤5% variant.
• Although both kits correctly identified all fusions when QC requirements were met, QX permitted 5-50 fold lower RNA inputs and achieved twice the rate of samples passing QC compared to FP.

INTRODUCTION

The reliable assessment of cancer-associated gene fusions by next-generation sequencing (NGS) is often challenged by low sample input quantity and quality, necessitating rigorous QC assessments to lend confidence to test results. Integration of these QC results with standardized reagents and bioinformatics is critical to assure consistent results from one laboratory to the next. Here we present a head-to-head comparison of two commercially available NGS kits that include reagents and software and are designed to detect non-small cell lung cancer (NSCLC)-related fusions.

MATERIALS AND METHODS

Total nucleic acid was isolated from 20 residual FFPE NSCLC biopsies using FormaPure™ FFPE Extraction Kit (Bedman Couteur), and RNA was quantified using the Qubit® RNA HS Assay Kit (Thermo Fischer Scientific). The isolates were pre-seeded into 24 unique samples, and NGS analysis was performed by an independent laboratory using the Quantidex® NGS RNA Lung Cancer Kit RUO (QX) (Asuragen) and the FusionPlex® ALK, RET, ROS1 v2 kit (RUO) (FP) (using updated FusionPlex chemistry and protocols available Q4 2016, ArcherDX). Libraries were sequenced on the MiSeq® (illumina) and analyzed using each kit’s bioinformatics software suite.

RESULTS

Table 3. Pre- & post-analytical QC results for both QX and FP assays. The QX and FP pre-analytical QC results demonstrated a dose response agreement for sample quality. However, post-analytical QC pass rates were lower for FP than for QX. FP failed samples included samples with sufficiently high mass input and samples with pre-NGS QC scores of less than 28 Cq.

Table 4. Summary of NGS results using the QX and FP assays. The QX NGS results were 100% concordant with the reference results. False-positive and false-negative results are highlighted with ORANGE text. FP NGS results included five false-negative results, four of which occurred in the admixture samples. All missed cases were associated with libraries flagged as failed by post-analytical QC (marked with *). FP NGS also showed weak Evidence flagged as margining for spurious fusions in two samples.

CONCLUSIONS

• Both kits included reagents for library prep, integrated multiple QC metrics to inform interpretations of results, and bioinformatics software for analysis.
• Both kits generated accurate calls when all QC criteria were satisfied.
• Using QX, all 16 sequenced sample libraries exceeded pre- and post-analytical QC requirements, and all fusions were correctly identified.
• For FP only 8/16 samples passed pre-analytical QC to generate reliable calls.
• Our results emphasize the importance of verifying minimum input requirements for each NGS technology and scrutinizing QC checkpoints to ensure reliable results.