A Simple and Versatile Next-Generation Sequencing Technology for Co-Detection of RNA Structural Variants and DNA Mutations in Lung Cancer

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Introduction

Mutational categories associated with NSCLC initiation and progression include single-nucleotide variants (SNVs), RNA fusions and deletions (INDELs), and copy number variations (CNVs), as well as RNA-based fusion events. To address these challenges, various methods have been developed, including FISH, immunohistochemistry (IHC), and, more recently, next-generation sequencing (NGS). FISH and IHC are laborious, expensive, and time-consuming, creating additional material and labor costs, and erecting barriers to broader adoption. To address these issues, we evaluated a simple, easy-to-use, next-generation sequencing (NGS) technology. The QuantideX® NGS RNA Lung Cancer and DNA Hotspot 21 Kits from Asuragen* are exons 14 skipping events (e13:e15), 23 mRNA targets, and three endogenous control transcripts.

Materials and Methods

Study 1 assessed weighted input: Twenty DNA Hotspot 21 libraries and twenty-two RNA Lung Cancer libraries were analyzed along with controls. RNA fusions were called with 82 to 11,965 paired-end reads passing instrument filter for RNA Lung Cancer libraries vs DNA Hotspot 21. DNA mutations were detected down to 5% allele frequency. No call was made for the four negative RNA samples or the three negative DNA samples. The high library concentration allowed for accurate results when no more than 24 samples were tested. To generate accurate results when no more than 24 samples were tested, the resultant libraries were combined following the library pooling strategies below. The final concentration value for both RNA- and DNA-based pools and then combined at a 3:1 ratio.

Table 1. QuantideX® NGS Panels. *All the QuantideX® NGS Lung Cancer Kit segments include exon-skipping variants, exon deletion variants, and expression targets. **The QuantideX® NGS DNA Hotspot 21 Kit segments include exon-skipping variants, exon deletion variants, and expression targets.

Table 2. Paired-End Reads Passing Instrument Filter for RNA Lung Panel Libraries vs DNA Hotspot 21. *The resultant libraries were combined following the library pooling strategies below. **Data analysis was performed using QuantideX® NGS Report, an integrative bioinformatics platform that enables seamless integration of data from all of the variant calling algorithms.***

Conclusions

• QuantideX® NGS Lung Cancer and DNA Hotspot 21 libraries could be processed simultaneously and pooled sample-to-sample to generate accurate results when no more than 24 samples were tested. To support up to 48 libraries per flow cell, libraries from the two panels could be formulated in separate RNA and DNA-based panels and then combined as a 3:1 ratio.

• The results demonstrate that QuantideX® NGS Kits can be used in a high-throughput manner to co-detect multiple categories of RNA and DNA variants in less than three days.

• The simplicity and speed of the approach, coupled with the standardized workflow, has the potential to increase the accessibility of an NGS-based approach to analyze numerous tissue samples from NSCLC molecular results.

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