AN INTEGRATED SYSTEM FOR TARGETED NEXT-GENERATION SEQUENCING THAT ENABLES SIMULTANEOUS ANALYSIS OF DNA MUTATIONS, RNA FUSIONS AND GENE EXPRESSION IN RESIDUAL CLINICAL FFPE, FNA, AND LIQUID BIOPSIES

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SUMMARY
QuantideX® NGS technology can interrogate both DNA and RNA targets from total nucleic acid (TNA) using a streamlined and unified workflow and customizable content.

• An evaluation of two prototype QuantideX NGS panels, a 146-marker panel for thyroid cancer and a 187-marker panel for non-small cell lung cancer, demonstrated detection of DNA mutations, gene fusions and quantitative measures of mRNA targets.

• This NGS approach can unveil multi-omic data from a single TNA specimen in pursuit of improved diagnostic yields and more informed assessments of mutation-negative samples.

INTRODUCTION
The capability of NGS to interrogate a broad range of DNA mutations in a single assay has precipitated a paradigm shift in precision medicine from single-target assays to highly multiplexed NGS panels. Current NGS panels have increased the breadth of content but do not support the analysis of RNA and DNA markers in a unified assay. We present QuantideX NGS, a comprehensive approach for targeted clinical NGS that enables simultaneous quantification of DNA and RNA through a streamlined workflow compatible with low-input total nucleic acid (TNA) derived from the most challenging clinical specimens including FFPE, FNA, and liquid biopsies.

METHODS
FFPE, FF, plasma and serum specimens used in this study were obtained through collaborations (NSCLC FFPEs provided by MD Anderson Cancer Center) or acquired through tissue banks. Thyroid FNAs were provided through collaborations or were residual clinical specimens from Asuragen’s CLIA lab. Whole transcriptions (WT) RNA-Seq was performed on 68 thyroid FFPEs to discover novel mRNA and fusion diagnostic markers. Targeted NGS Thyroid and NSCLC panels were developed using Asuragen’s QuantideX NGS technology. Targeted NGS QC was performed with a novel PCR assay that quantifies functional DNA and RNA from TNA. PCR-based target enrichment was conducted using QuantideX targeted NGS reagents and sequenced on a MiSeq® (Illumina). Library sequences were analyzed using QuantideX NGS Reporter, a bioinformatic analysis suite that directly incorporates pre-analytical QC information to improve the accuracy of variant calling, fusion detection and RNA quantification.

RESULTS
Current NGS panels have increased the breadth of content but do not support the analysis of RNA and DNA markers in a unified assay. We present QuantideX NGS, a comprehensive approach for targeted clinical NGS that enables simultaneous quantification of DNA and RNA through a streamlined workflow compatible with low-input total nucleic acid (TNA) derived from the most challenging clinical specimens including FFPE, FNA, and liquid biopsies.

• The described NGS technologies are versatile, offer high analytical sensitivity, and can be applied to liquid biopsies to reveal low-abundance mutations in circulating tumor DNA.

• A 187-marker DNA/RNA NGS panel for NSCLC reported mutations, RNA fusions, and mRNA transcripts from 97 lung cancer specimens with results consistent with TCGA reference data.

• The described NGS technologies are versatile, offer high analytical sensitivity, and can be applied to liquid biopsies to reveal low-abundance mutations in circulating tumor DNA.

CONCLUSIONS
• The interrogation of both DNA and RNA markers in thyroid cancer using QuantideX NGS technology enabled 95% sensitivity for malignant thyroid FNA biopsies and improved the interpretation of mutation-negative FNA specimens.

• A 187-marker DNA/RNA NGS panel for NSCLC reported mutations, RNA fusions, and mRNA transcripts from 97 lung cancer specimens with results consistent with TCGA reference data.

• The described NGS technologies are versatile, offer high analytical sensitivity, and can be applied to liquid biopsies to reveal low-abundance mutations in circulating tumor DNA.

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