Presented at AACR 2017

Poster #5354

Evaluation of Quantity, Quality, and Performance with the TruSight® Tumor 170 Solid Tumor Profiling Assay of Nucleic Acids Extracted from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Sections

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INTRODUCTION

Solid tumor profiling assays need to deliver accurate and consistent results in the face of decreased quality and quantity of nucleic acids extracted from FFPE samples. Understanding the performance of a particular solid tumor profiling assay with FFPE tissue is critical, but with limited and non-renewable samples available to most assay-developers, the sample number used to understand this performance can be small. TruSight Tumor 170 (TST170) is an Illumina developed comprehensive solid tumor profiling panel targeting 170 genes using DNA and RNA from FFPE samples. In order to confirm the robustness of the assay with FFPE tissue, 2310 FFPE samples were brought in-house and evaluated. Quantity of both DNA and RNA extraction were determined by various methods, including AccuClear™, Qubit™ and Quantifluor® fluourometric assays. Overall, >95% of the samples achieved the minimum concentrations required for the TruSight Tumor 170 assay. As a surrogate for DNA quality, we measured the amplification potential of the nucleic acid by assessing a ΔCq value using quantitative PCR after normalization to a fixed input mass. To assess RNA quality, we used the DV200 metric, which measures the percentage of RNA fragments >200 nucleotides in length. We examined ΔCq and DV200 values across different tissues and didn't find a significant difference between tissues. Finally, we assessed the ability of samples to pass the sample quality control (QC) metrics in the TruSight Tumor 170 assay. These QC metrics ensure accurate variant calling, with a sensitivity and specificity of ≥95%. We found that samples that had a ΔCq value of ≤5 and a DV200 value of ≥20 achieved a QC success rate above 95%. This data highlights the need for further investigation into the methods for extraction, quantification and quality assessment of nucleic acids for solid tumor profiling and underscores the robustness of TruSight Tumor 170 with FFPE samples.

MATERIALS AND METHODS

DNA and RNA were extracted from formalin-fixed paraffin-embedded (FFPE) samples using the Qiagen AllPrep kit. Following extraction DNA samples were quantified using Qubit (n = 245) or AccuClear (n = 3146) and RNA samples were quantified using BioAnalyzer (n = 349), Fragment Analyzer (n = 1707) or Quantifluor fluorometric assay (n = 2249). Following quantitiation, samples were assessed for quality. DNA samples were assessed by qPCR using the Illumina FFPE QC Kit (WG-321-1001) along with a control cell line sample with a known input mass. Delta Cq (ΔCq) values, a measure of amplifiabilty, were then assigned to each sample. For optimal TST170 performance, Illumina recommends that samples have a ΔCq ≤ 5. RNA samples were assessed for quality by determining the DV200 value, using the size distribution traces from the Bioanalyzer or Fragment Analyzer traces. The DV200 value indicates the percent of the sample that is 200 nucleotides in length or longer, an indicator of the level of sample degradation. For optimal TST170 performance, Illumina recommends that samples have a DV200 value of ≥ 20. Following quantitation and quality assessment, samples that met the minimum input threshold (3.3 ng/µl for DNA, 4.7 ng/µl for RNA), regardless of quality, were processed through the TruSight Tumor 170 assay. Briefly, DNA samples were sheared to prepare for library preparation and RNA samples were converted to cDNA. Subsequently, both sample types were run in parallel through library preparation followed by a hybrid capture enrichment targeting 170 key cancer genes. Samples were evaluated for performance based on quality control (QC) metrics established during the development of the TruSight Tumor 170 assay.

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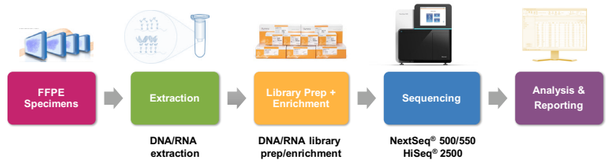
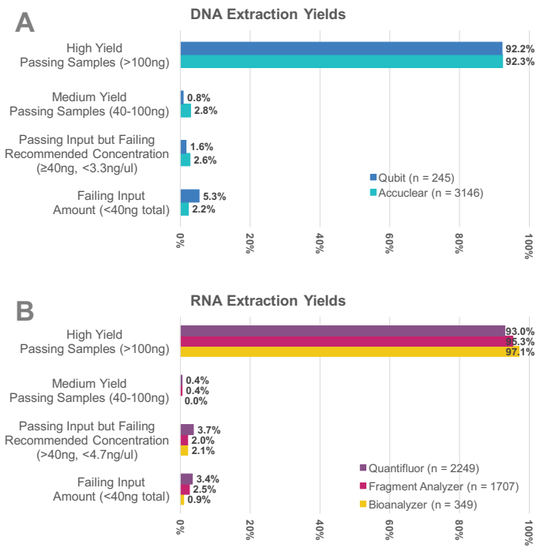
WORKFLOW



RESULTS

Figure 1 - Extraction of FFPE samples yield sufficient material for the TST170 Assay

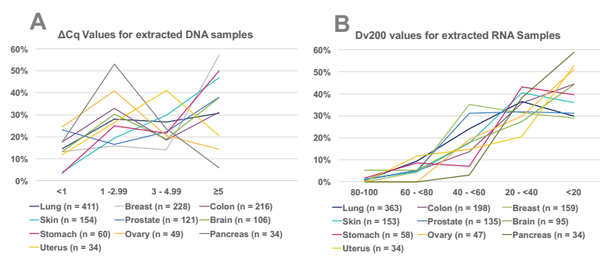


RESULTS

A) Yields from DNA sample extractions were binned by total yield (<40ng, >40ng but below the recommended input concertation, 40-100ng and >100ng) and the percent of samples in each bin were calculated. Greater than 95% of samples gave sufficient material for subsequent testing.

B) Yields from RNA sample extractions were binned by total yield (<40ng, >40ng but below the recommended input concertation, 40-100ng and >100ng) and the percent of samples in each bin were calculated.

Figure 2 – Sample Qualification Metrics

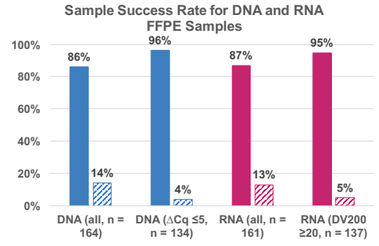


A) The percent of DNA samples that fall into ΔCq bins (<1, 1 - 2.99, 3 - 4.99, ≥ 5) were plotted based on tissue type. Differences in quality of samples were observed based on tissue type, with breast tissue yielding the most samples with ΔCq values ≥ 5 and Pancreas tissue yielding the fewest samples with ΔCq values ≥ 5.

B) The percent of RNA samples that fall into DV200 bins (80 - 100, 60 - <80, 40 - <60, 20 - <40, <20) were plotted based on tissue type. Sample quality was typically in the DV200 range of 20 – 60 with some tissues, such as pancreas, uterus, and ovary having more than 50% of samples with DV200 values of <20.

RESULTS

Figure 3 – Sample Performance in the TruSight Tumor 170 Assay



DNA (blue, 62 unique samples) and RNA (pink, 62 unique samples) were processed through the TruSight Tumor 170 Assay using the standard 40 ng input, regardless of sample quality and then assessed for performance against sample QC criteria. When sample quality is not taken into account the success rate for the TST170 assay was >85% for both DNA and RNA samples. Further analysis of this data set, with samples that fall outside the recommended quality cutoffs removed, showed that for DNA (42 unique) and RNA (52 unique) at least 95% of samples for both DNA and RNA pass sample QC criteria.

CONCLUSION

Standard extractions from FFPE embedded samples provide sufficient material (40ng) in >95% of samples that were extracted by Illumina. Further analysis showed that in the samples extracted at Illumina, the quality of the samples varied by tissue type. The quality of samples, in particular pancreas, ovary, and uterus, tended towards lower quality; however, these samples were mostly procured from commercial vendors and had often been stored for several years prior to being received and extracted. Finally, this data shows that the TruSight Tumor 170 panel is a robust assay that generates passing sample QC data in >85% of samples with varying quality, and in >95% of samples that have quality metrics that fall within the recommendations for the kit.

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