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 the performance can be small. TruSight Tumor 170 (TST170) is an Illumina developed comprehensive solid tumor profiling panel targeting 170 genes and was designed to robustly interrogate 70% of the tumor genome using DNA and RNA from FFPE samples. In order to confirm the robustness of the assay with limited and non-renewable samples available to most assay-developers, the sample extraction method was optimized using DNA and RNA from FFPE samples. In order to confirm the robustness of the assay with FFPE samples, 2310 FFPE samples were brought in-house and evaluated. Quantity of DNA and RNA extraction were determined by various methods, including AssayClean® and Quantifluor®. 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 the TruSight Tumor 170 assay.

Results
A) Yields from DNA sample extractions were binned by total yield (>40ng, >40ng but before the recommended input concentration, 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 before the recommended input concentration, 40-100ng and >100ng) and the percent of samples in each bin were calculated.

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