Overcoming Limitations of RNA-Seq Library Construction from FFPE Samples Using a Novel Workflow

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RNA expression studies in oncology are critical for diagnosis, therapy decisions and prognosis. Availability of fresh tumor specimens for high quality analysis is rare, especially, for long-term ouIn this poster, we report an effective RNA-Seq method to interrogate small RNAs and long RNAs from archived breast cancer FFPE tissues. We demonstrate the efficiency of using a novel library preparation method using engineered retrotransposon enzyme called SEQzyme. This enzyme's unique modus operandi combines cDNA synthesis and adapter ligation in one continuous synthesis reaction and drastically reducing workflow times.

In our study, we compared two different extraction methods for FFPE samples. Our FFPE data was benchmarked against matching fresh frozen samples. The total number of genes detected was similar across the workflows and between FFPE and fresh frozen samples. We utilized a custom data analysis pipeline designed for use with our workflow enabling detection of RNAs greater than 20 bp. We showcase detection of different RNA biotypes - mRNAs, lncRNAs, and smRNAs. Significantly greater number of miRNAs were captured with our method. We demonstrate enhanced recovery of smaller fragments from degraded FFPE sample and enable a more complete transcriptome analysis. By unlocking more information from FFPE samples using RNA-Seq we demonstrate the gene expression in breast cancer samples. This study can be extended to other sample types to impact translational disease research