BROAD INSTITUTE

Working with FFPE

GENOMIC SERVICES

Technical Note

Considerations when working with FFPE

- **Unique pooling strategies are needed to account for increased variability**
- **Higher rates of C>T/G>A deanimation artifacts require a new set of metrics**

Broad Institute Genomic Services has made several improvements to standardize workflows, optimize success rates, decrease artifacts and bring FFPE samples to high coverage. Since 2015, close to 5,000 FFPE samples have been sequenced, allowing us to determine several difficulties when working with degraded materials. Factors that need to be accounted for include lower yields in library preparation, libraries with lower molecular weight and highly duplicated similar percentage selections as non-FFPE samples (Figure 1), and less predictability in the relationship between Gb and target coverage. FFPE samples also show higher rates of deamination C>T/G>A (Figure 2). These findings resulted in new lab processes, new metrics to define success, and new products designed specifically for FFPE samples (genome & exome). These products now include seperation of pooling from high quality DNA samples and modifications to the PCR protocol which both significantly improve results.

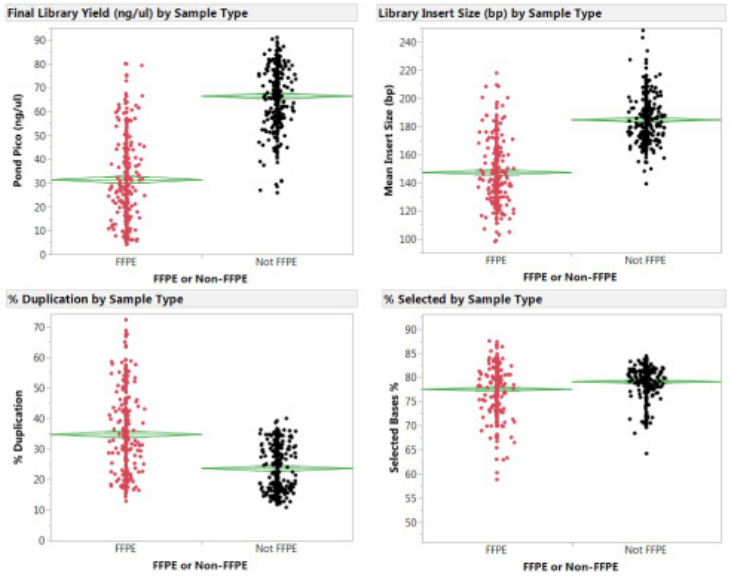

Figure 1: Comparisons between FFPE & non-FFPE samples in library yield (ng/ul), size (bp), % duplication, and selected by sample type

Two statistically significant predictors of successful FFPE sequencing have been identified through performing partition analysis on FFPE exome data sets (Figure 2). The analysis concluded that the main drivers for success were yield after library preparation prior to exome capture and mean insert size as main drivers, with minimum specifications. In addition, FFPE specific QC assays were developed to determine levels of degradation in samples before initiating the sequencing process. Interestingly, age of sample is not a predictor of success, as the technique of the fixation process plays a greater role.

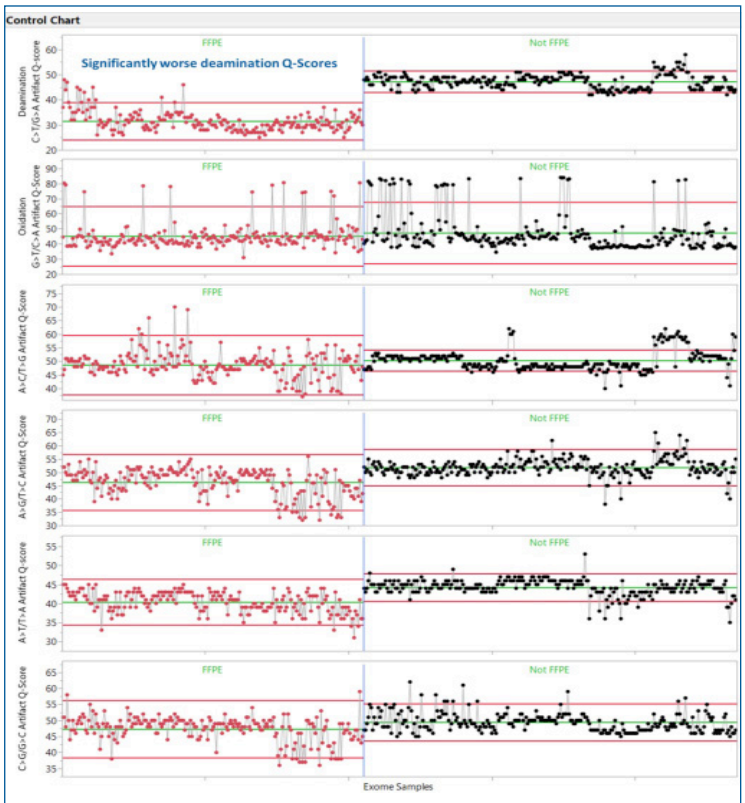

Figure 2: Comparisons in Q-scores for all possible base changes between FFPE & non-FFPE samples

Conclusions

- **Yield after library preparation and insert size are predictors of success**
- **New artifact calculations allow for (G>T/C>A) tracking**
- **Pooling & processing FFPE samples separate from high quality DNA improves performance**
- **Modification of PCR protocol to maintain longer extension times preserves larger fragments**