TECHNICAL NOTE

Challenges working with FFPE

Considerations when working with FFPE

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

FFPE OVERVIEW

The Broad Institute Genomics Platform has made several improvements to standardize workflows, optimize success rates, decrease artifacts and bring FFPE samples to high coverage. Since 2015, the Platform has sequenced close to 5,000 FFPE samples allowing us to determine several difficulties when working with degraded materials. Lower yields in library preparation, libraries with lower molecular weight and highly duplicated with similar percentage selections as in non-FFPE samples, (Figure 1), along with less predictability in the relationship between Gb & target coverage are factors that need to be accounted for. 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).

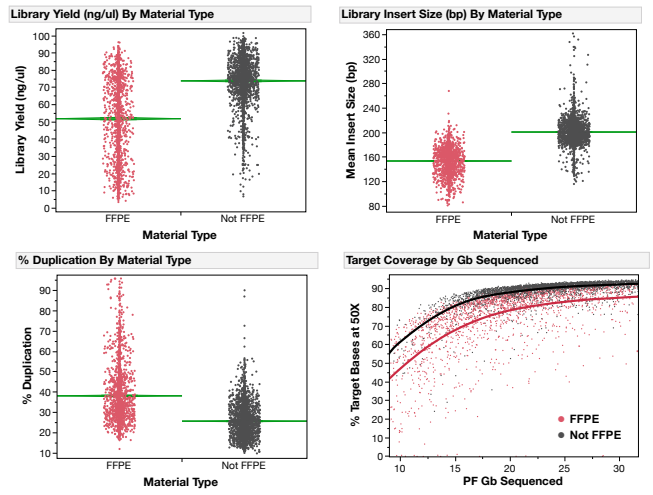

Figure 1: comparisons between FFPE & non-FFPE samples in library yield (ng/ul), size (bp), % duplication, and & selected by sample type

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

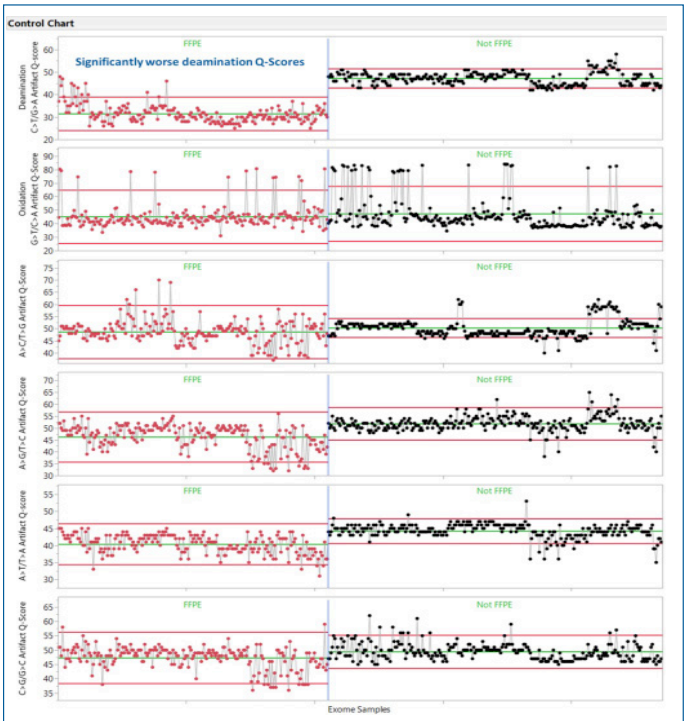

Figure 2: Comparisons in Q-scores for all possible base changes between FFPE & non-FFPE samples

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

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