A novel suite of enzyme mixes enable robust hybrid capture sequencing from low quality FFPE DNA

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In cancer genomics, a common source of DNA is formalin-fixed, paraffin-embedded (FFPE) tissue from patient surgical samples. FFPE DNA poses many notable challenges for preparing NGS libraries, including low input amounts and highly variable damage from fixation, storage, and extraction methods. Due to the high cost of sequencing and variability of coverage, regions of interest are often specifically enriched using hybrid capture-based approaches, but these methods require a high input of diverse, uniform DNA library to achieve the coverage required for somatic mutation identification in tumor samples.

We developed a new DNA repair enzyme mix, enzymatic fragmentation mix, and library amplification PCR master mix, optimizing the activities of these mixes using FFPE samples ranging from DIN 1.8 to 6.8 to maximize yield, WGS library quality, and target enrichment library performance. Combining DNA damage repair and a novel enzymatic fragmentation mix upstream of library preparation reduced the false positive rate in somatic variant detection by repairing damage-derived mutations, and also improved the library yield, quality metrics, complexity, coverage uniformity, and hybrid capture library quality metrics. The new PCR master mix boosts the library yield without compromising library quality in FFPE-derived samples, allowing flexibility in the PCR cycles used to accommodate high-throughput processing of FFPE samples of highly varied quality. This library prep workflow was evaluated with multiple sequencing platforms including Illumina, MGI, and Element Biosciences.

This new suite of enzyme mixes allows even highly damaged FFPE samples to achieve high-quality libraries with sufficient input for hybrid capture. Increasing the useable reads and coverage enables robust detection of somatic variants as demonstrated using both reference standard DNA and FFPE samples. Finally, the use of enzymatic fragmentation and a flexible PCR master mix make this FFPE library prep workflow compatible with high-throughput and automation-based workflows.