

JAX: mutation - Actionseq20,

Molecular Methods Description:

- Macro dissection-enriched FFPE tissue sections were used for DNA extraction. Target exons and introns were enriched by hybrid-capture (Roche Nimblegen). * Libraries were prepared incorporating barcodes as unique molecular identifiers. * Libraries were pooled and sequenced 150?bp paired-end on the NextSeq 500 (Illumina). Forward and reverse reads are, respectively, R1 and R3. Fastq file R2 contains the index reads.

Analysis Description:

Mutational analysis was performed using the JAX ActionSeq Genome Analytics (AGA) pipeline, which includes fastp to preprocess the reads. Reads were mapped to the reference human genome GRCh38 using the BWA-MEM alignment tool with ALT-Aware mapping. SAM/BAM files were sorted and duplicates removed using Picard, then variants were called with UnifiedGenotyper, Mutect2, and Pindel, then merged, filtered and annotated.