PMLB: mutation - Illumina HiSeq 2000, WES

Molecular Methods Description:

DNA was isolated using a gSYNC■ DNA Extraction Kit (FroggaBio Cat# GS100) following user guide directions. Flash frozen tumor up to 25 mg of fragments were dissociated with 200 _l of GST Buffer and 20 _l of Proteinase K then vortex thoroughly and incubated at 60■C overnight. This extraction method was based on using a DNA spin column with buffers and centrifugation to remove impurities, and finally eluting the purified DNA. The exome capture was performed using the Agilent SureSelect Human All Exon 50Mb kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer■s instructions. The captured DNA of PDX models and their matched normal were sequenced on the Illumina HiSeq 2000 platform (Illumina, San Diego,CA), and paired-end sequences (2 x 101 bp) were generated for each sample.

Analysis Description:

Xenome(v1.0.1) was used to filter out mouse stromal reads by aligning the reads to DNA of the NOD-SCID mouse., the remaining reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner (v0.7.12). Quality control, local realignment of indel, base quality score recalibration (BQSR), duplicate reads marking and further processing of the mapped reads were performed using the standard Genome Analysis Toolkit (GATK) pipeline (v3.4), samtools (v1.2) and Picard (v1.140) (https://broadinstitute.github.io/picard/).The pipeline generated a single Binary Alignment Map (BAM) file for each sample (either PDX or matched normal) that includes reads, calibrated quantities, and alignments to the genome.