JAX: expression - RNA_Seq,

Molecular Methods Description:

• Tissues preserved in RNAlater were homogenized in TRIzol (ThermoFisher Scientific) using a gentleMACS dissociator (Miltenyi Biotec Inc). Total RNA was isolated using the miRNeasy Mini kit (Qiagen) according to manufacturer's protocols, including the optional DNase digest step. RNA quality and concentration were assessed using the RNA 6000 Nano LabChip assay on the 2100 Bioanalyzer instrument and Nanodrop 2000 spectrophotometer (Thermo Scientific). * Non-stranded libraries were constructed using TruSeq RNA Library Prep Kit v2 (Illumina). PolyA containing mRNA was isolated using oligo-dT magnetic beads, followed by RNA fragmentation, first and second strand cDNA synthesis, ligation of Illumina-specific adapters containing a unique barcode sequence for each library, and PCR amplification. Libraries were checked for quality and concentration using the DNA 1000 assay (Agilent Technologies) and quantitative PCR (KAPA Biosystems), according to the manufacturers' instructions. * Libraries were pooled and sequenced 75 bp paired-end on the NextSeq 500 (Illumina) using NextSeq High Output Kit v2 reagents (Illumina), or 100 bp paired-end on the HiSeq2500 (Illumina) using TruSeq SBS v3 reagents (Illumina).

Analysis Description:

RNAseq data were processed with Xenome to extract human sequences. The human sequences were aligned to the hg38 transcriptome with Bowtie2, with expression levels estimated by RSEM. RSEM estimated counts were upper quantile normalized.