JAX: expression - hg10st,

Molecular Methods Description:

• Tissue samples were stored in RNAlater (Ambion) per manufacturer's instructions for later homogenization with TRIzol (Life Technologies) using the GentleMacs dissociator (Miltenyi). Total RNA was isolated using the TRIzol Plus Kit (Life Technologies) according to manufacturer's methods including on the column DNase digestion. * Following reverse transcription of RNA with random primers-T7 primers (Affymetrix, Santa Clara, CA), double stranded cDNA was synthesized with the GeneChip WT cDNA Synthesis and Amplification Kit (Affymetrix, Santa Clara, CA). In an in vitro trancription (IVT) reaction with T7 RNA polymerase, the cDNA was linearly amplified to generate cRNA. In the second cycle of cDNA synthesis, random primers were used to generate single stranded DNA in the sense orientation. Incorporation of dUTP in the cDNA synthesis step allowed for the fragmentation of the cDNA strand utilizing uracil DNA glycosylase (UDG) and apurinic/apyrimidinic endonuclease 1 (APE 1) that specifically recognizes the dUTP and allowed for breakage at these residues. Labeling occured by terminal deoxynucleotidyltransferase (TdT) where biotin wasis added by a Affymetrix Labeling Reagent * 2.3µg of biotin-labeled and fragmented cDNA was hybridized onto GeneChip Mouse Gene 1.0 ST Arrays (Affymetrix) for 16 hours at 45 C. Post-hybridization staining and washing was performed according to manufacturer's protocols using the Fludics Station 450 instrument (Affymetrix). * The arrays were scanned with a GeneChipTM Scanner 3000 laser confocal slide scanner.

Analysis Description:

The arrays were processed with the AffyPLM R package, using quantile normalization, no background correction, and fitting to a simple model that treats the log Intensity as a sum of array effect, probe effect, and residual. The array effect is the normalized_expression that is equivalent to the median polished value produced by standard RMA analysis.