RNA-Seq

Library preparation

mRNA libraries

The libraries were prepared using the mRNA-Seq sample preparation kit (Illumina Inc., Cat. # RS-122-2001x2) according to manufacturer's protocol. Briefly, 0.5µg of total RNA were used for poly-A based mRNA enrichment selection using oligo-dT magnetic beads followed by fragmentation by divalent cations at elevated temperature resulting into fragments of 80-250nt, with the major peak at 130nt. First strand cDNA synthesis by random hexamers and reverse transcriptase was followed by the second strand cDNA synthesis. Double stranded cDNA was end repaired 3´adenylated and the 3´-"T" nucleotide at the Illumina adaptor were used for the indexed adaptors ligation. The ligation product was amplified with 15 cycles of PCR.

Sequencing

Each library was sequenced using TruSeq SBS Kit v3-HS, in paired end mode with the read length 2x76bp for the mRNA-Seq experiments. Using the HiSeq2000 instrument (Illumina, Inc) following the manufacturer's protocol. Images from the instrument were processed using the manufacturer's software to generate FASTQ sequence files.