

## **Broad Stranded RNA-Seq Method**

### **cDNA Library Construction**

Total RNA was quantified using the Quant-iT™ RiboGreen® RNA Assay Kit and normalized to 5ng/ul. Following plating, 2 uL of ERCC controls (using a 1:1000 dilution) were spiked into each sample. An aliquot of 200ng for each sample was transferred into library preparation which was an automated variant of the Illumina TruSeq™ Stranded mRNA Sample Preparation Kit. This method preserves strand orientation of the RNA transcript. It uses oligo dT beads to select mRNA from the total RNA sample. It is followed by heat fragmentation and cDNA synthesis from the RNA template. The resultant 500bp cDNA then goes through library preparation (end repair, base 'A' addition, adapter ligation, and enrichment) using Broad designed indexed adapters substituted in for multiplexing. After enrichment the libraries were quantified using Quant-iT PicoGreen (1:200 dilution). After normalizing samples to 5 ng/uL, the set was pooled and quantified using the KAPA Library Quantification Kit for Illumina Sequencing Platforms. The entire process is in 96-well format and all pipetting is done by either Agilent Bravo or Hamilton Starlet.

### **Illumina Sequencing**

Pooled libraries were normalized to 2nM and denatured using 0.1 N NaOH prior to sequencing. Flowcell cluster amplification and sequencing were performed according to the manufacturer's protocols using either the HiSeq 2000 or HiSeq 2500. Each run was a 101bp paired-end with an eight-base

index barcode read. Data was analyzed using the Broad Picard Pipeline which includes de-multiplexing and data aggregation.