BROAD INSTITUTE

Human Whole Transcriptome Sequencing

GENOMIC SERVICES

Product Data Sheet

Highlights

- **Experienced provider as the sequencing center for the Genotype Tissue Expression (GTEx) project**
- **Performance at scale - Over 24,000 whole transcriptomes have been sequenced at Broad to-date.**

Strand-Specific RNA Sequencing

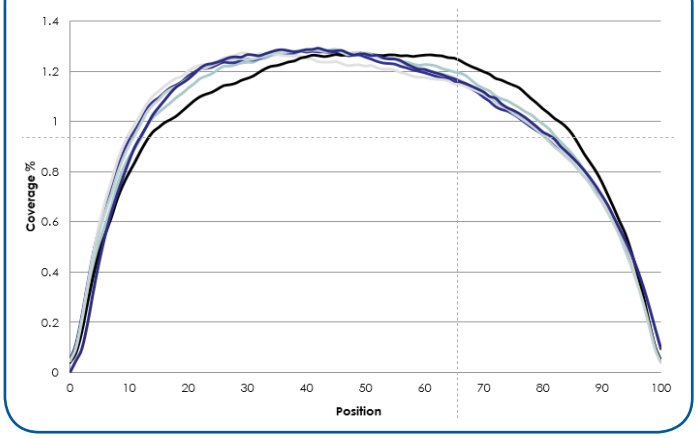
Whole Transcriptome Sequencing has become an invaluable tool for performing fundamental research surrounding transcript discovery and expression analyses including isoform detection, fusion transcript discovery, differential expression profiling, allele specific mutation validation, and transcriptome assembly. Working closely with scientists within and outside the Broad Institute, Broad Genomic Services has performed a thorough evaluation of available technologies and optimized processing conditions including read depth, fragment insert-size, and sample cleanup to provide the highest quality whole transcriptome sequencing products possible.

Leveraging core competencies in process design, molecular biology, laboratory automation and integrated LIMS and analysis tools we have developed products capable of producing hundreds of high-quality whole transcriptome libraries per week.

Specifications

% Stranded	% mRNA Bases	% PF Bases Aligned	Insert Size	Deliverable
99.5	73.0	93.8 ± 0.4	500bp	50M paired reads

Coverage Across Transcripts



Product Details:

RNA samples are prepared using the Illumina TruSeq Stranded mRNA sample preparation kit, modified for improved performance, multiplexing and integration into our automated platform. Ther-moFisher ERCC RNA controls are added prior to Poly(A) selection providing additional control for variability, including: quality of the starting material, level of cellularity, RNA yield, and batch to batch variability. RNA quality and insert-size is assessed by Caliper LabchipGXII producing a RQS value (equivalent to RIN). RNA quantity is determined by RiboGreen. Samples are then sequenced on the HiSeq4000.

What's Included:

- Sample Receipt & Plating
- ERCC RNA Control Addition
- Poly(A) selection
- Stranded cDNA Synthesis
- Illumina HiSeq 4000 Sequencing (2x 101 Bp Reads)
- Sample Fidelity QC (96 SNP fingerprinting) Available with Sample Qualification of matching DNA

Data Deliverable:

- 100 Million reads aligned in pairs
- STAR aligned to human genome assembly (hg19)
- De-Multiplexed, aggregated Picard BAM file with, insert size and alignment summary
- Data accessed via secure online digital transfer

Product Inputs:

- 150ng of purified total RNA
- Fresh/frozen tissue, blood, or cell pellets that preferably yield >250ng of RNA (separate charge will be applied for extractions)
- Minimum Sample data including - Collaborator Participant ID, Collaborator Sample ID, Gender