BROAD CLINICAL LABS

PRODUCT DATASHEET

Whole Transcriptome Sequencing

Product Overview

Whole transcriptome sequencing is an invaluable research tool for 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 Clinical Labs has performed a thorough evaluation of available technologies and processing conditions including read depth, fragment insert size, and sample cleanup to provide the highest quality whole transcriptome sequencing product.

Leveraging core competencies in process design, molecular biology, laboratory automation and integrated LIMS and analysis tools, we are able to process >500 whole transcriptome samples per week (>25,000 annually). We have performed more than 80,000 whole transcriptomes to date and have served as the sequencing center for the Genotype Tissue Expression (GTEx) project[1].

RNA samples are processed using a standard strand-specific mRNA sample preparation kit, modified for improved performance, multiplexing, and integration into our automated platform. 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 variation. RNA library quality and insert size is assessed by RQS value (equivalent to RIN), and RNA library quantity is verified by both PicoGreen™ QC and qPCR prior to sequencing.

What's Included

- Sample receipt and ERCC RNA control addition
- Poly(A) selection and Stranded cDNA Synthesis

What's Included (cont.)

- Illumina Sequencing (2x 151bp Reads)
- Sample Fidelity QC (96 SNP fingerprinting) available with Sample Qualification of matching DNA

Input Requirements

- 250ng of purified total RNA; or fresh/frozen tissue, blood, or cell pellets that yield >250ng purified RNA
- RQS value ≥ 5.5
- Minimum Sample data including collaborator participant ID, collaborator sample ID, and participant biological sex

% Stranded	% mRNA Bases	% PF Bases Aligned	Insert Size	Deliverable
99.5	73.0	93.8 ± 0.4	500bp	50M or 100M paired reads

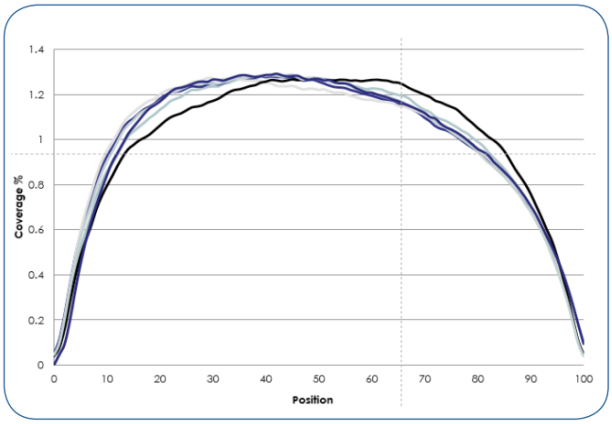

Figure 1. Top: expected performance of Broad Clinical Labs' Whole Transcriptome Sequencing product. Bottom: typical gene body coverage percentile (relative coverage) 5' to 3' across the transcript.

Data Deliverable

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

[1] https://gtexportal.org/home/