BROAD CLINICAL LABS

PRODUCT DATASHEET

Stranded mRNA Sequencing

Product Overview

Stranded mRNA 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 stranded mRNA sequencing product.

Leveraging core competencies in process design, molecular biology, laboratory automation and integrated LIMS and analysis tools, we are able to process >500 stranded mRNA samples per week (>25,000 annually). We have performed more than 80,000 stranded mRNA samples 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 sample quality and insert size is assessed by RIN, and the quantity of libraries generated from RNA is verified by both PicoGreenTM 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)

Input Requirements

- Total RNA: ≥800ng total; 50-300µL volume; 10-110ng/µL concentration preferred, 6.5ng/µL minimum acceptable concentration; RIN > 5.5 required, RIN >7 preferred
- PAXgene preserved whole blood, fresh frozen tissue, and cell pellet also accepted
- Minimum Sample data including collaborator participant ID, collaborator sample ID, and participant biological sex

Data Deliverable

- 50 Million, 75 Million, or 100 Million reads aligned pairs
- CRAM File

Expected Performance Specification of Stranded mRNA				
% Stranded	**%mRNA Bases**	**% PF Bases Aligned**	**Insert Size**	**Deliverable**
99.5	73.0	93.8± 0.4	500bp	50M, 75M, or 100M paired reads

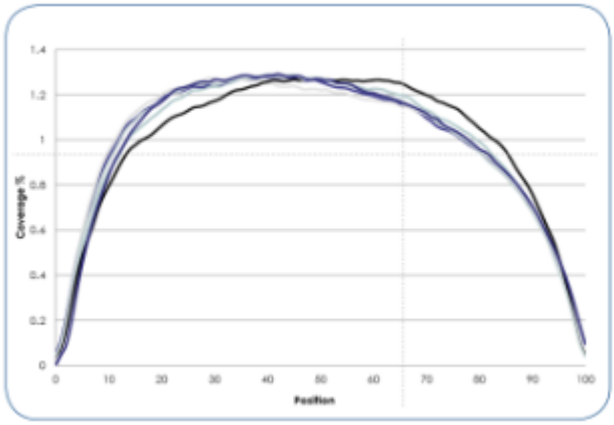

Figure 1.Typical gene body coverage percentile (relative coverage) 5' to 3' across the transcript.

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