BROAD CLINICAL LABS

PRODUCT DATASHEET

Total RNA Sequencing

Incorporating globin and ribosomal depletion

Product Overview

Total RNA sequencing is an invaluable research tool that provides a more holistic view of the transcriptome. This can include insight into both coding and non-coding regions with roles in regulatory mechanisms and the detection of alternative splicing events and post-transcriptional modifications that provide a deeper understanding of gene regulation and isoform diversity.

Broad Clinical Labs (BCL) has performed a thorough evaluation of available technologies and processing conditions to provide the highest quality total RNA (with a combined globin depletion / rRNA depletion step) by working closely with scientists within and outside the Broad Institute. The BCL generates cDNA libraries that are depleted of globin and rRNA regions, and possess a superior quality with increased complexity. This increased complexity enables us to achieve a high sequencing depth of up to 100 million reads aligned in pairs, while keeping the duplication rates low. The enhanced complexity of these libraries facilitates the identification of a greater number of transcripts and protein coding genes compared to our conventional mRNA workflow (Fig 1).

BCL is able to offer a highly scalable total RNA product with rRNA and globin depletion by leveraging core competencies in process design, molecular biology, laboratory automation, and integrated LIMS and analysis tools. RNA samples are processed using a globin/rRNA depletion and stranded cDNA library construction sample preparation kit modified to include custom UMI adapters and primers for improved performance, multiplexing, and integration into our automated platform. RNA library quantity is verified by both PicoGreen™ QC and qPCR prior to sequencing.

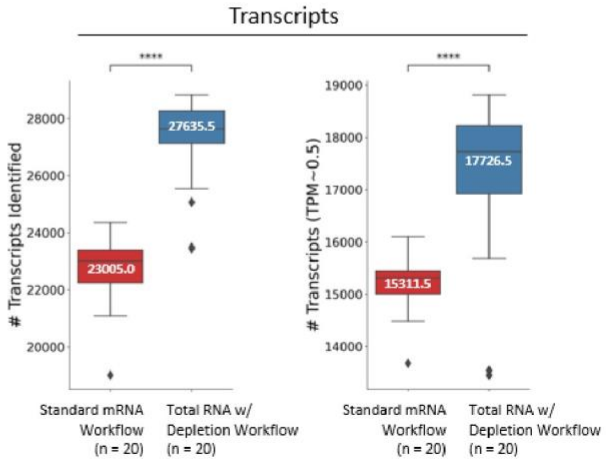

Figure 1. Comparison of the number of transcripts detected in 20 blood samples processed with both mRNA and Total RNA workflows.

What's Included

- Sample receipt
- Combined globin RNA and rRNA depletion, stranded cDNA synthesis and library construction with UMIs
- Illumina Sequencing (2x 151bp Reads) on NovaSeqX

Input Requirements

- 2 µg (volume > 50 µL ; 40 ng/µL) of purified total RNA derived from blood
- Minimum 24 samples / batch
- Recommended RQS value ≥ 7.0
- Minimum sample data including: collaborator participant or sample ID, sample tube barcode, sample type, RQS

Data Deliverable

- 80 - 100 Million reads aligned in pairs.
- De-Multiplexed, aggregated DRAGEN CRAM file aligned to human genome assembly (hg38)