Fact Sheet

Total RNA-Seq Assay

Introduction

Next Generation Sequencing (NGS) is commonly leveraged for transcriptome interrogation and gene expression analysis. The IQVIA Laboratories Total RNA-Seq Assay accepts high- and low-quality samples, including Formalin-Fixed, Paraffin Embedded (FFPE) tissue. The new IQVIA Laboratories Total RNA-Seq Assay offers improved performance and efficiency for RNA sequencing.

Importance of the Assay

The Total RNA-Seq Assay is crucial for accurate and comprehensive analysis of the transcriptome, providing valuable insights into gene and transcript expression and regulation. This assay depletes rRNA and globin mRNA from total RNA to interrogate a wide range of interesting transcripts, including both coding and non-coding RNAs, enabling insights that may be missed with mRNA or RNA exome sequencing.

Key attributes

Suitable for sequencing of biologically interesting RNAs

Improved dynamic range and library complexity over previous methods

Enhanced data quality and accuracy

Assay performance

The assay has demonstrated excellent performance in quality control measures, ensuring reliable and reproducible results.

High data quality

The IQVIA Laboratories total RNA-Seq Assay produces high quality libraries for gene expression applications (Table 1). Benefits include:

- Strong depletion of rRNA and globin mRNAs improves power to detect transcripts of biological interest.
- Improved transcript coverage for better identification of splice junctions.
- High dynamic range for detection of low abundance transcripts.
- Superior accuracy and repeatability of gene quantification.

Table 1. Performance of the IQVIA Laboratories total RNA-Seq Assay on different sample matrices

5 donor samples were processed for each of Fresh Frozen Tissue (FFT), whole blood, and FFPE matrices. Intact Reference Universal Human Reference RNA (UHRR) and Human Brain Reference RNA (HBRR) . 1-3 replicates of each sample (100 ng input) were processed in each of 3 runs. All data was down sampled to 100M clusters/sample. Replicate values were averaged by sample, then samples were averaged by sample matrix.

	INTACT REFERENCE RNA	FRESH FROZEN TISSUE	WHOLE BLOOD	FFPE, DV200 > 55%	FFPE, DV200 < 55%
% rRNA	0.51	1.10	0.74	0.80	0.97
% Globin	0.00	0.00	0.02	0.00	0.00
Library complexity (million fragments)	165.09	119.42	161.14	158.30	94.10
Genes detected	25,038	26,460	23,362	25,375	23,153
% Reads mapped	98.86	98.41	98.93	98.47	97.99

Accurate and reproducible transcriptome profiling

The IQVIA Laboratories Total RNA-Seq Assay has shown accurate and consistent results, highlighting its effectiveness in capturing a comprehensive view of the transcriptome. This assay shows exceptional concordance between technical replicates, as indicated by low coefficients of variation for each gene across runs (Figure 1). In addition, differential expression uncovered by the IQVIA Laboratories Total RNA-Seq Assay shows high correlation (0.92) with mRNA-seq data (Figure 2).

Figure 1. Coefficient of variation of gene expression between runs

Samples were processed in triplicate across each of 3 runs (100 ng input). All data was down sampled to 50M clusters/sample. Genes with low expression (i.e., ≤ 30 raw counts in at least 75% of samples) were excluded and upper quartile normalization was performed. Outliers not shown.

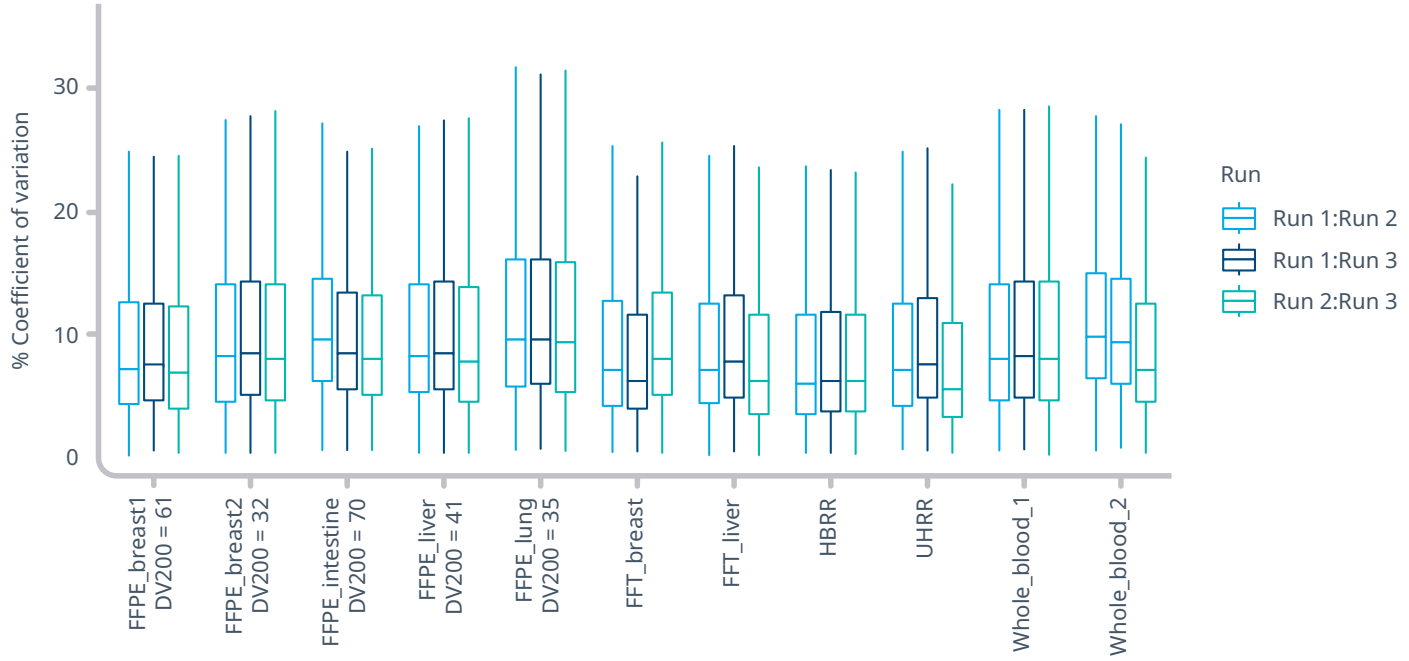


Figure 2. Differential expression between UHRR and HBRR for the IQVIA Laboratories Total RNA-seq assay compared to mRNA-seq data

Relative accuracy was assessed for high quality samples using the correlation of differential expression between UHRR and HBBR. Samples were processed in duplicate or triplicate using the IQVIA Labs Total RNA-seq assay (down sampled to 50M) or using mRNA-seq (down sampled to 30M clusters/sample). Genes with low expression (i.e., ≤ 30 raw counts in at least 75% of samples) were excluded and upper quartile normalization was performed. Log ratios of gene expression were plotted and the Pearson correlation coefficient was determined.

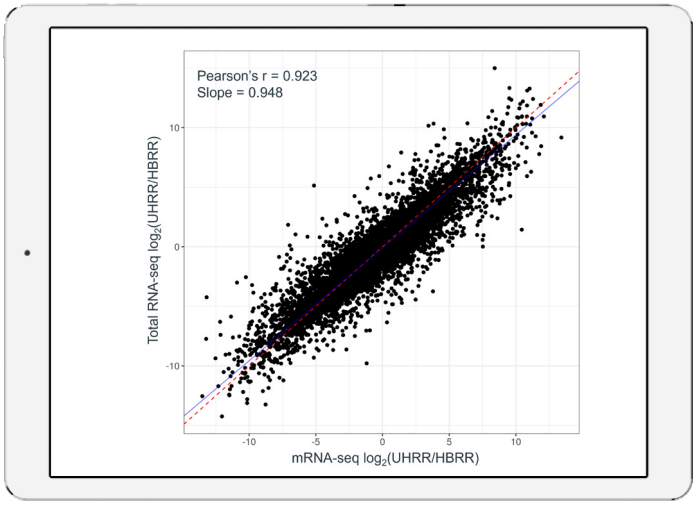


Table 2. Specifications and sample types

The IQVIA Laboratories total RNA-Seq Assay is compatible with various sample types, including RNA samples from FFPE tissues, cells, and blood products.

	FRESH FROZEN TISSUE	BLOOD	FFPE	RNA	CELLS
COLLECTION METHOD	Cryovial	PAXgene Blood Collection Tubes	Paraffin block, curls, or slides	Cryovial	Cryovial
MINIMUM INPUT	100 ng (max 30 mg)	100 ng (2.5 mL)	100 ng (2-4 5-10 µm curls)	100 ng (25 ng/µL in 10 µL nuclease-free water)	100 ng ($1x10^5$ to $4x10^6$ cells)
SHIPPING	Frozen on dry ice (-80°C)	Frozen on dry ice (-80°C)	Cold, with ice packs (4°C)	Frozen on dry ice (-80°C)	Frozen on dry ice (-80°C)
SEQUENCING PLATFORM	NovaSeq 6000 or NovaSeq X Plus				
DELIVERABLES	FASTQs and QC Report, extended analysis available upon request				
REGULATORY	IQVIA Laboratories offers this assay at multiple regulatory levels, including Research Use Only (RUO) and non-CAP/CLIA validated, depending on the intended use of the assay. This test is not intended for patient-specific clinical decision-making. Please reach out to our IQIVA Laboratories Genomics Business Development team to discuss your intended use so we can better guide which regulatory level is most appropriate for your clinical or research program(s).				

Performance improvements

The Total RNA-Seq Assay offers a new and improved solution for transcriptome sequencing, enabling researchers to achieve broader coverage of the transcriptome with improved efficiency and accuracy.

In particular, the new total RNA-seq Assay demonstrates substantial improvements over our previous total RNA-seq assay in two metrics that are key for reliable transcript quantification — insert size and library complexity (Figure 3 and 4).

2-3 replicates of each sample (100 ng input) were processed on each assay. All data was down sampled to 50M clusters/sample. Library complexity was estimated using Picard MarkDuplicates.[1]

Figure 3. Improved insert size

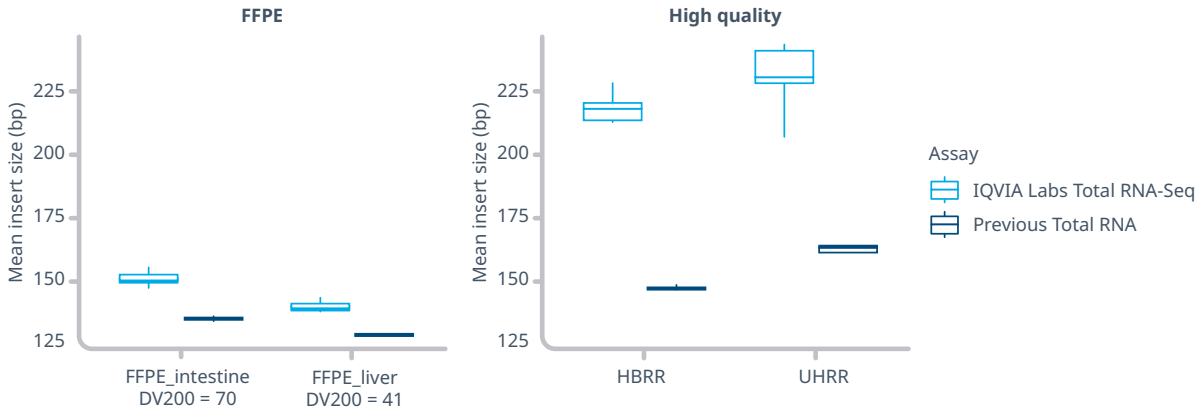
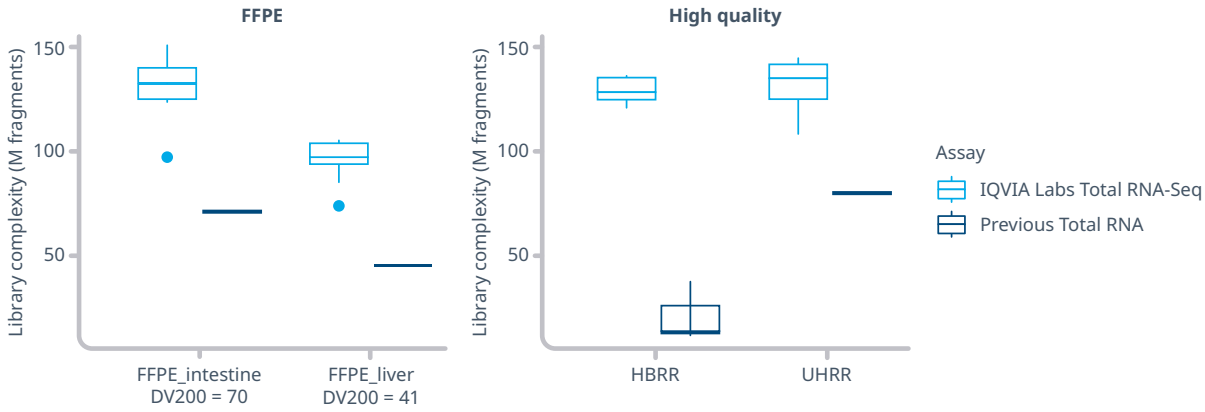


Figure 4. Improved library complexity



References/footnotes

[1]Picard. Broad Institute. http://broadinstitute.github.io/picard.

Global consistency
Harmonized assay testing on **3 continents** for consistent results.

Personalized service
Custom turnaround times for **delivery and analysis.**

Massive data delivery
1.2+ petabytes of genomic data globally in 2024.

Decades of Expertise
Over 20 years in genomics testing.

Industry leader
Leading in genomic testing **since 2001.**

Extensive experience working with the **top 10 pharmaceutical companies.**

IQVIA Genomics has partnered with **> 125 leading/top biotech companies.**

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