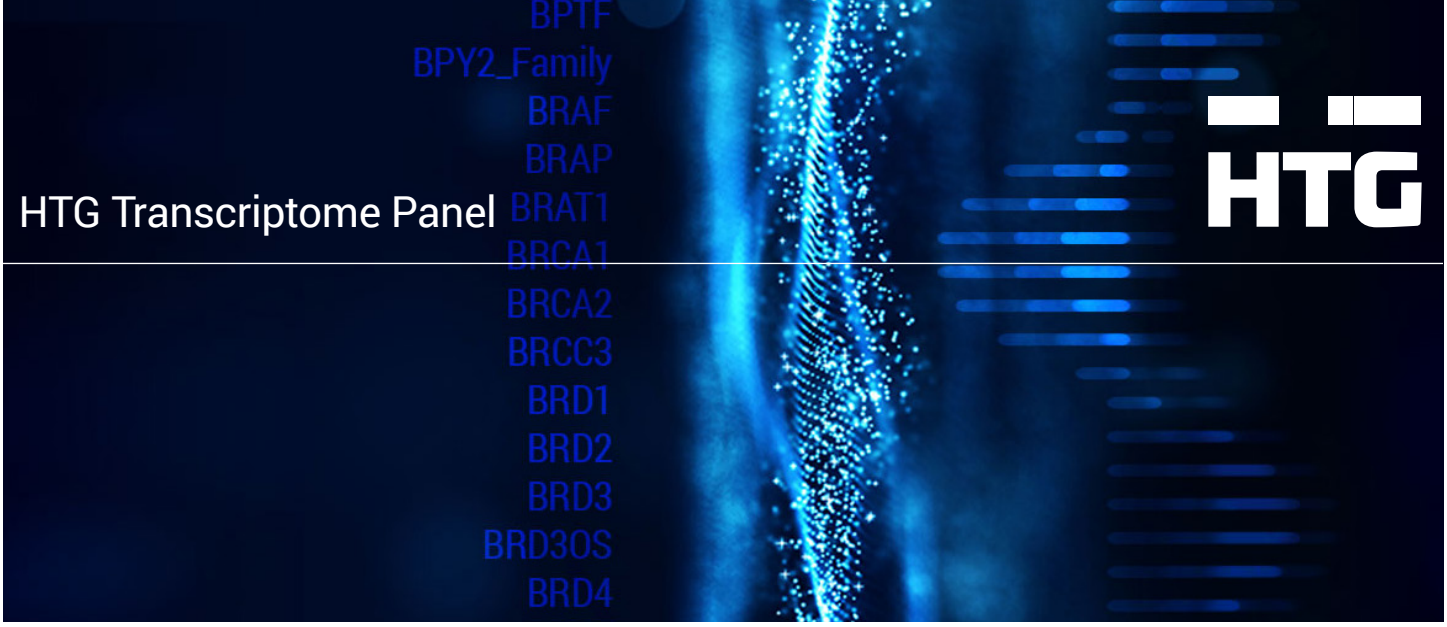

Comprehensive Profiling of the Human mRNA Transcriptome

Gene expression analysis has become an invaluable tool for preclinical and clinical studies to investigate biomarker profiles and gene signatures associated with disease state, treatment response, and outcome. The HTG Transcriptome Panel (HTP) is expertly designed to provide extensive coverage of most human mRNA transcripts including isoforms in a single panel *(Figure 1)*.

Panel Overview

The HTG Transcriptome Panel includes probes to most known human mRNA transcripts, providing insight into the gene expression of a sample. The panel has the ability to interrogate expression in FFPE samples using 19,616 probes, generating data for up to 96 samples in less than three days. The panel contains 19,398 probes specific to human mRNA transcripts, 4 positive control probes, 100 negative control probes, 22 genomic DNA probes, and 92 external RNA control consortium (ERCC) probes. The panel allows researchers to generate reliable results using limited sample amount with only 11.0 mm^2 FFPE tissue from a 5 µm FFPE section. HTG EdgeSeq™ Reveal software provides an integrated statistical analysis solution that is web-based and simple-to-use. Researchers can quickly produce data files formatted for many of the common bioinformatics pipelines in addition to generating publication quality figures.

Table 1: *HTG Transcriptome Panel Overview*

Number of Probes	19,616
Sample Type and Recommended Input Amount	FFPE: ≥11 mm^2
Time to Results for 96 Samples	<3 days
Integrated Data Analysis	HTG EdgeSeq Reveal Software (4.0.0)

The probes in the HTP were selected against the NCBI human genomic database and screened against the NCBI RefSeq RNA database for primary accession of the gene of interest.

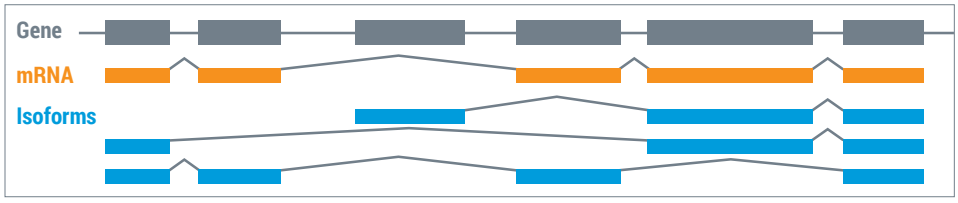

Figure 1: *The HTP targets known mRNA transcripts including many isoforms of the human transcriptome, making it applicable for a wide range of discovery and clinical applications.*

Low Sample Input

Critical studies can often be thwarted by lack of sufficient samples, while the demand for comprehensive molecular information continues to increase. RNA-Seq is considered to be the gold standard for evaluating transcriptome data, however, it requires substantial quantities of high-quality extracted RNA to meet sample input requirements. This can be difficult to generate with small and/or archival samples. On the other hand, HTG's extraction-free chemistry is highly versatile, using relatively small sample input amounts to generate high quality human mRNA transcriptome data. In a head to head comparison, HTP was able to generate high quality transcriptome data using only 1 or 2 slides whereas RNA-Seq required 4-8 slides to extract sufficient RNA for similar analysis *(Table 2)*.

Table 2. *Comparison of HTG and RNA-Seq platforms.*

	HTP	RNA-Seq
Number of FFPE Slides Used	1-2*	4-8
Sample Type Used	Extraction-free FFPE	Extracted RNA
Overall Pass Rate	100% (24/24)	75% (18/24)**
Pass Rate for Samples Older than 10 Years	100% (13/13)	63% (7/11)

** Only a single sample required two sections.*
*** Samples failed to generate sufficient extracted RNA to process using RNA-Seq.*

Archival Samples

The most difficult archival samples are FFPE tissues that have been stored at room temperature for a decade or more. Many of these FFPE samples are also small, making it difficult to reliably extract sufficient RNA for gene expression analysis. Our proprietary extraction-free sample preparation chemistry enables researchers to generate quality data with a high degree of precision from samples that would otherwise not meet sample input requirements for RNA-Seq *(Table 2)*. This is done by preserving partially degraded and small RNAs which might be lost during standard RNA extraction. As depicted in *Figure 2*, top row, HTP demonstrates a high degree of repeatability with samples sectioned from blocks less than five years old and blocks greater than ten years old *(Figure 2,* bottom row). This data shows that HTP demonstrates excellent repeatability and offer some advantages to RNA-Seq when analyzing archival samples.

Comparison of HTG Transcriptome Panel to RNA-Seq

The ability of the HTP to identify differentially expressed genes was compared to RNA-Seq across multiple cancer indications. Log fold-changes in gene expression showed strong correlation between gene expression data from RNA-Seq and HTP using FFPE samples. HTP shows equivalent results using a fraction of the sample input and is able to generate higher sample pass rate when compared to RNA-Seq *(Figure 3, Table 2).*

Precision

An assay that can profile approximately 20,000 mRNA target must be both precise and accurate. Measurement of precision is especially important when working with limited quantity of precious samples that may potentially contain compromised RNA material. The precision of HTP was evaluated using FFPE samples across multiple plates, operators, processors and lots. Precision was high across all conditions demonstrating high precision *(Table 3)*.

Table 3. *Precision of HTG Transcriptome Panel.*

	Mean	Median
Overall Precision	0.940	0.952

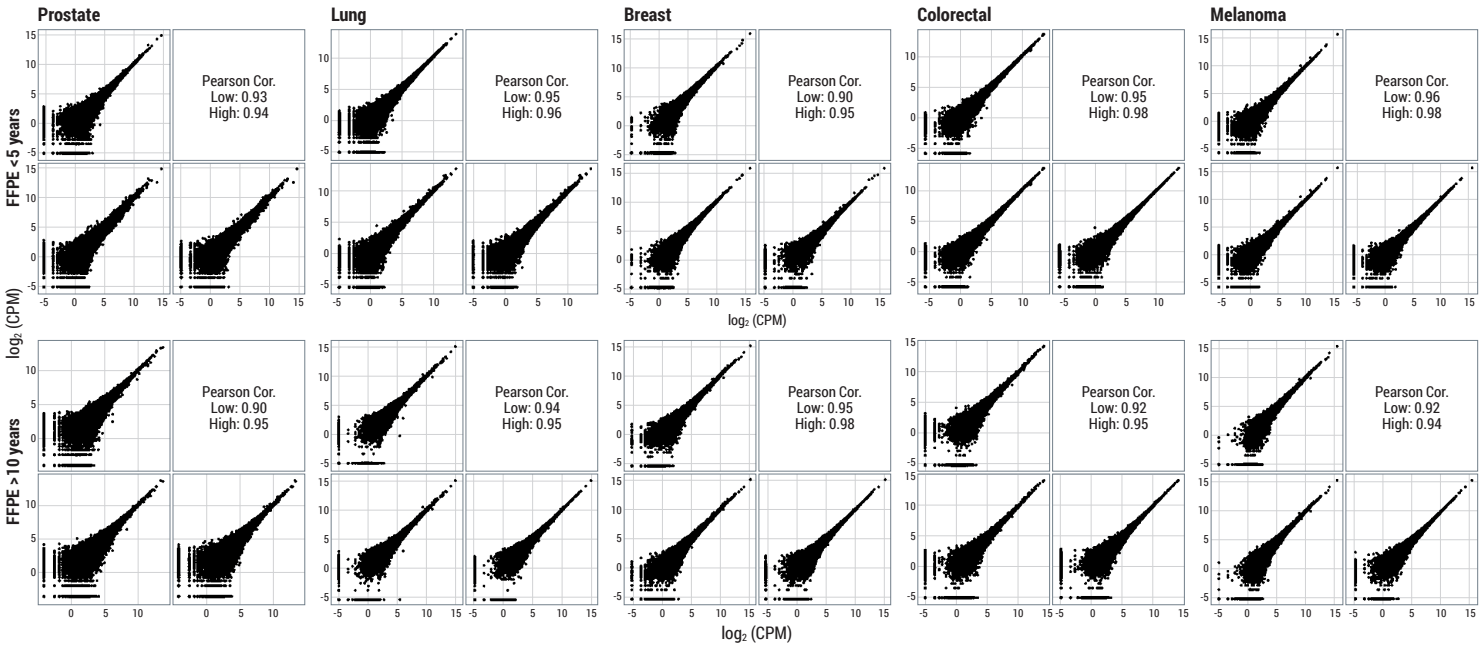

Figure 2. *Multi-tissue FFPE samples were lysed and processed with the HTG Transcriptome Panel from blocks less than 5 years old (top row) and blocks greater than 10 years old (bottom row).*

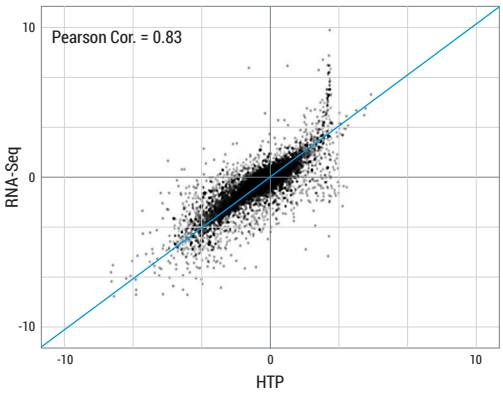

Figure 3: *Differential gene expression analysis comparison between HTG Transcriptome Panel and RNA-Seq.*

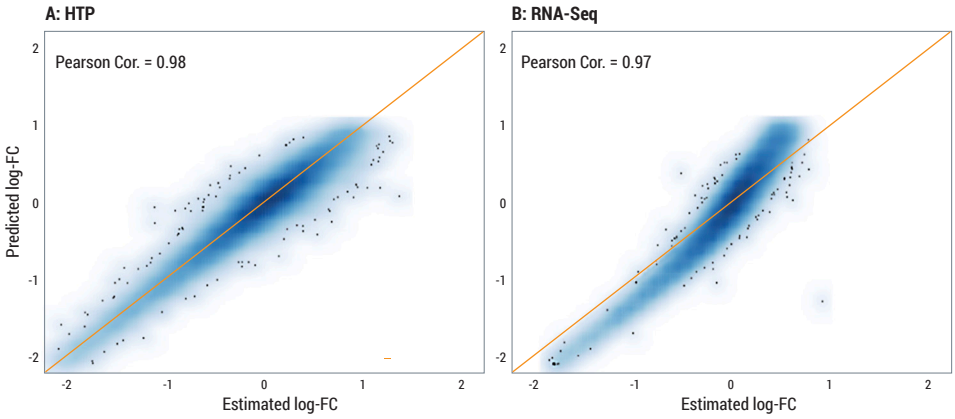

Figure 4: *Accuracy of differential expression analysis of HTG Transcriptome Panel and RNA-Seq. Pearson Cor. between the predicted and observed log fold-changes (log-FC) of HTP (A) and RNA-Seq (B).*

Accuracy and Dynamic Range

Precision is the ability to generate the same answer consistently while accuracy is the assurance that the answer is correct. The accuracy of the HTP was determined using a mixture of defined ratios of previously characterized tissue samples. Accuracy of gene expression was assessed through differential expression by comparing expected and observed log fold-changes of the tissue mixtures. *Figure 4* shows correlation between the observed and predicted log-fold changes were 0.98 for the HTP (A) and 0.97 for RNA-Seq (B) as measured by the Pearson correlation coefficient (Pearson Cor.).

The HTP shows strong linearity across a wide dynamic range. The differential expression was measured using the ERCC exogenous RNA controls. The ERCCs were spiked in at various known ratios across multiple FFPE samples from different cancer indications. The observed linear response of the panel spans the 10^6-fold concentration range covered by the ERCC controls, and indicates consistent assay performance over a broad dynamic range.

Integrated Data Analysis

The HTG EdgeSeq Reveal software is a powerful, simple-to-use solution to interrogate and generate novel insights without a complicated analysis pipeline. Reveal can help assess low expressing genes and help researchers address common NGS and assay quality metrics in addition to quickly create publication quality figures for visualizing data generated from the transcriptome analysis. Reveal provides features that can be utilized when analyzing the data from the HTP including:

- User selected probe annotation for use with all visualizations
- Paired sample analysis
- Additional output file formats (R, Tab) for easy integration into common bioinformatics pipelines.

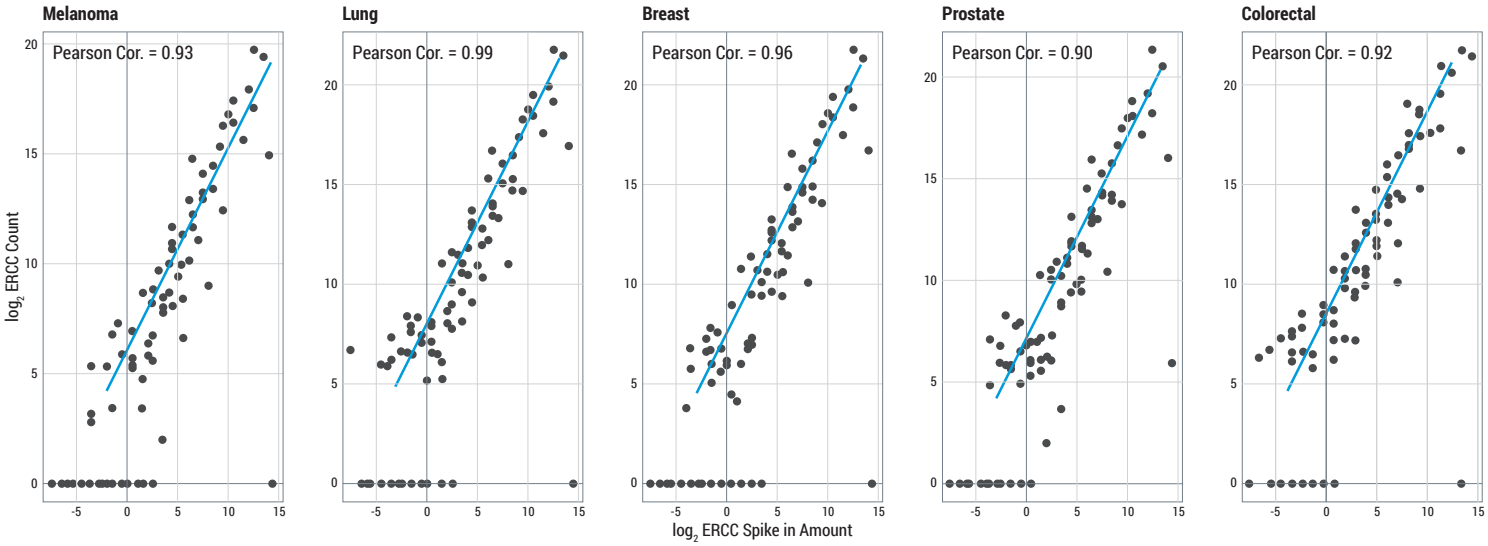

Figure 5. *Assessment of assay linearity and dynamic range using spiked-in ERCC transcripts. The predicted log_2 transformed amounts of ERCC spike-in (X-axis) were correlated with the actual log_2-transformed amounts of ERCC (Y-axis).*

VERI/O Laboratory Services

Trusted Partner for Gene Expression Analysis

Accelerate your research or drug development programs for precision medicine. Our high-quality and high-throughput molecular facility provides a comprehensive gene expression solution using our proprietary HTG EdgeSeq platform. VERI/O™ laboratory's highly experienced team is an extension of your organization to meet your study needs with high accuracy and reproducibility. Find out why VERI/O laboratory is the right partner for your program.

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- Comprehensive program management to support research and clinical studies
- Flexible and scalable solution with a track record of successful projects
- Rapid turnaround time to meet your program timelines
- Comprehensive bioanalytical study reports

Panel Products

Kit Configurations for Illumina Sequencers

Kit Name	Configuration	Catalogue Number
HTG Transcriptome Panel	4 x 8	HTG-001-008
HTG Transcriptome Panel	2 x 8	HTG-001-208
HTG Transcriptome Panel	4 x 24	HTG-001-024
HTG Transcriptome Panel	1 x 24	HTG-001-224
HTG Transcriptome Panel	1 x 96	HTG-001-096

About HTG

Our mission is to illuminate the transcriptome with targeted gene expression profiling and empower actionable insights for clinical research. HTG's comprehensive gene expression solutions have enabled generation of biomarker signatures, link expression profiling to map novel pathways and decode complexities of diseases.

Learn more at htgmolecular.com

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Rev 3: 10-AUG-2021