

A Systems Biology Approach to Accelerate Biomarker Discovery

Gene expression analysis has become an invaluable tool for preclinical and clinical studies to investigate transcriptional profiles and gene signatures associated with treatment response and resistance to drugs. The HTG EdgeSeq™ Oncology Biomarker Panel (OBP) is thoughtfully designed to include targets that enable insights for applications such as general systems biology, tumor biology and immunology (*Figure 1*).

Panel Overview

The HTG EdgeSeq Oncology Biomarker Panel contains probes to 2,549 genes and is compatible with solid and liquid biopsy sample types (*Table 1*). The panel was designed to provide comprehensive coverage in a single assay, unlike other platforms where you may need to run multiple panels to obtain equivalent gene coverage. The HTG EdgeSeq Oncology Biomarker Panel (*Figure 1*) generates more output than competing targeted panels.

Table 1: HTG EdgeSeq Oncology Biomarker Panel Overview

Number of Targets	2,549
Sample Types and Recommended Input Amount	FFPE: 8-9 mm ² PAXgene: 500 µL Extracted RNA: 35 ng Cells: ≥3,000 cells
Time to Results for 96 Samples	36 hours
Data Analysis	HTG EdgeSeq Reveal Software



- AMPK Pathway
- Angiogenesis
- Apoptosis
- Cardio Toxicity
- Cell Cycle
- Cluster of Differentiation
- DMPK
- DNA Repair
- EGF / PDGF Pathway
- EGFR / HER Pathway
- FGFR Pathway
- Hedgehog Pathway
- Hypoxia
- Immuno Oncology
- JAK / STAT Pathway
- MAP Kinase Pathway
- NFkB Pathway
- Other Genes of Interest
- PI3K / AKT Pathway
- Receptors
- Stem Cells
- Stress Toxicity
- Tissue Specific
- WNT Pathway

Figure 1: The HTG EdgeSeq Oncology Biomarker Panel includes targets for each of the pathways listed, making it applicable for a wide range of system biology and oncology applications.

HTG EdgeSeq Oncology Biomarker Panel

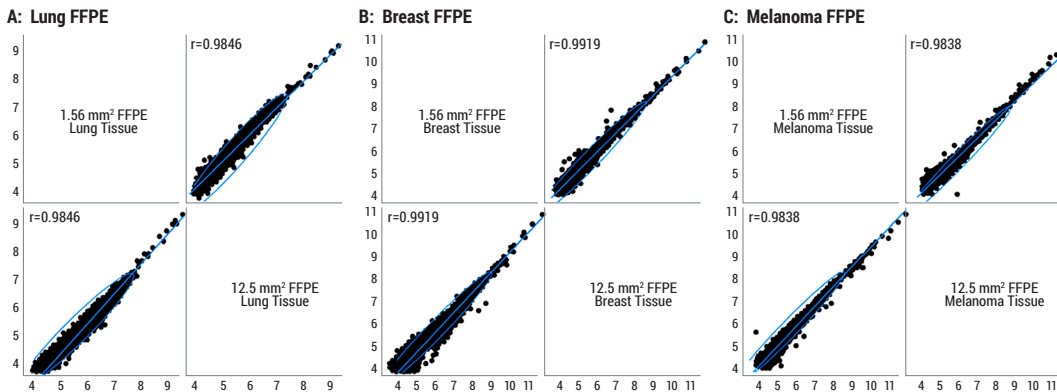


Figure 2: Three (3) FFPE tissues (A. lung, B. breast, and C. melanoma) were lysed and processed with the HTG EdgeSeq Oncology Biomarker Panel at two input amounts, 12.5 mm² and 1.56 mm², per sample. Turner *et al.*, 2019

Small Sample Input

Clinical and research studies are often delayed or halted by lack of quality and quantity of patient samples, while the demand for additional molecular information continues to increase. While RNA-Seq may be considered the gold standard for measuring gene expression, it requires a substantial number of high-quality FFPE sections to generate high quality data. On the other hand, the HTG EdgeSeq extraction-free chemistry is highly versatile, using relatively small sample input amounts to generate high quality data. A high correlation is shown (Figure 2) between sample input of 1.56 mm² and 12.5 mm² per well. Previous studies (Turner *et al.*, 2019) have successfully developed molecular subtype classification using only one section of FFPE tissue, highlighting the low sample input requirement.

Reproducibility with Small and Archival Samples

The overwhelming majority of archival clinical oncology samples are formalin-fixed, paraffin-embedded (FFPE) tissue. Many archival FFPE samples, especially pre-treatment biopsy specimens, are too small and/or too old to reliably generate sufficient RNA for gene expression analysis applications using standard RNA extraction methodologies. This can lead to sample exclusion from a cohort, therefore reducing the overall statistical power of the data set. The extraction-free HTG EdgeSeq chemistry, which is compatible with many solid and liquid biopsy types, enables high sample pass rate and precise measurements of gene expression, even from small, archival samples over 10 years old. In one example, Srour *et al.*, 2021 performed gene expression studies for breast cancer using the HTG EdgeSeq OBP on archival sentinel lymph node biopsies as old as 14 years, with a 100% sample pass rate. Lipchik *et al.*, 2016 evaluated the reproducibility of sample age and size by comparing FFPE sections between 3.125 mm² and 71.1 mm² from FFPE samples that were 5 to 12 years old (Figure 3). Using both breast and colorectal samples, they showed correlation coefficients > 0.98 between technical

replicates and concluded that the HTG EdgeSeq OBP provides a solid platform to obtain gene expression data from FFPE samples far too small for reliable RNA extraction. Lastly, O'Rourke *et al.*, 2020 compared HTG EdgeSeq technology with RNA-Seq across various sample types and found HTG EdgeSeq technology produced fewer QC failures compared to whole transcriptome RNA-Seq while using significantly less tissue input.

Panel Performance

Sensitivity and Extraction Bias

Sensitivity of a gene expression assay can be significantly reduced by multiple factors, including RNA extraction, PCR amplification and ligation, none of which are part of the HTG EdgeSeq workflow (Brown *et al.*, 2018). The NGS based HTG EdgeSeq workflow and extraction-free chemistry offers

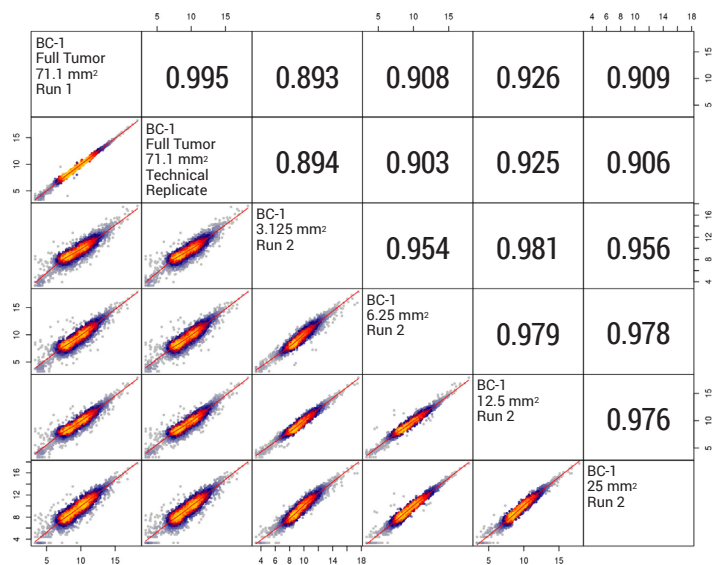


Figure 3: The HTG EdgeSeq Oncology Biomarker Panel produces high intra-sample reproducibility of samples between 3.125 and 71.1 mm² and 5-10 years in age. Lipchik *et al.*, 2016

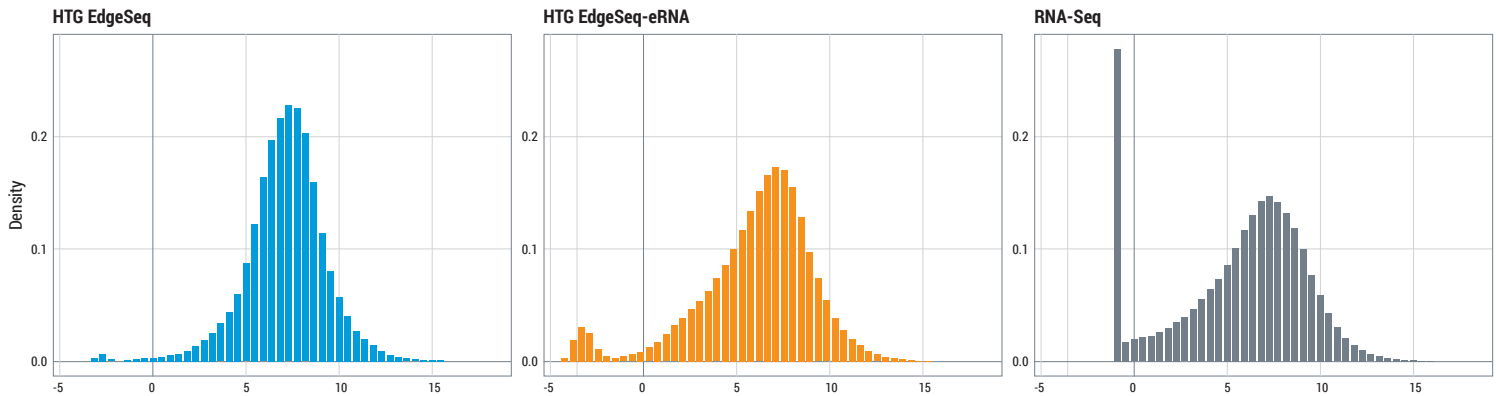


Figure 4: Gene expression comparison between HTG EdgeSeq extraction-free sample input (left), HTG EdgeSeq with extracted RNA (middle) and RNA-Seq with extracted RNA (right). Data generated by HTG Molecular Diagnostics, Inc.

high correlation to RNA-Seq (Ran *et al.*, 2020), while reducing extraction bias associated with RNA purification. The comparison above illustrates this bias, showing the increase in zero count genes for samples using RNA extraction (Figure 4, right and middle) compared to samples using the HTG EdgeSeq extraction-free chemistry (Figure 4, left). This increase in genes with zero counts in samples using RNA extraction highlights the potential bias caused by RNA extraction, which can result in the loss of signal for a significant number of genes.

Accuracy

Reproducibility is the ability to generate the same answer over and over while accuracy is the assurance that the answer is correct. Zhang *et al.*, 2021 evaluated classification accuracy using a 5-fold cross validation between prostate cancer patients with and without neoadjuvant therapy and area under the curve (AUC) for HTG EdgeSeq OBP and nanoString nCounter PanCancer Immune Profiling Panel (data not shown). In addition to showing a high degree of correlation between the two platforms, they showed that the HTG EdgeSeq platform had the highest accuracy and AUC, when compared to the nanoString nCounter platform (Table 2). The authors conclude that the HTG EdgeSeq platform is uniquely suited for analyzing gene expression in clinically derived FFPE tissue samples and has the potential to provide gene-expression data that can advance the development of clinical biomarkers.

Table 2. Accuracy and Area Under the Curve

	Accuracy		Area Under the Curve	
	HTG OBP	nS Immune	HTG OBP	nS Immune
Fold 1	88%	63%	100%	100%
Fold 2	75%	63%	92%	67%
Fold 3	78%	33%	78%	83%
Fold 4	86%	86%	100%	100%
Fold 5	50%	38%	60%	73%
Average	75%	56%	86%	85%

Zhang *et al.*, 2021

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Panel Products

Kit Configurations and Catalogue Numbers

Kit Name	Configuration	Illumina Sequencers	Thermo Fisher Scientific Ion Torrent Sequencers
HTG EdgeSeq Oncology Biomarker Panel	2 x 8	916-002-208	916-002-308
HTG EdgeSeq Oncology Biomarker Panel	4 x 8	916-002-008	916-002-108
HTG EdgeSeq Oncology Biomarker Panel	1 x 24	916-002-224	916-002-324
HTG EdgeSeq Oncology Biomarker Panel	4 x 24	916-002-024	916-002-124
HTG EdgeSeq Oncology Biomarker Panel	1 x 96	916-002-096	916-002-196

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