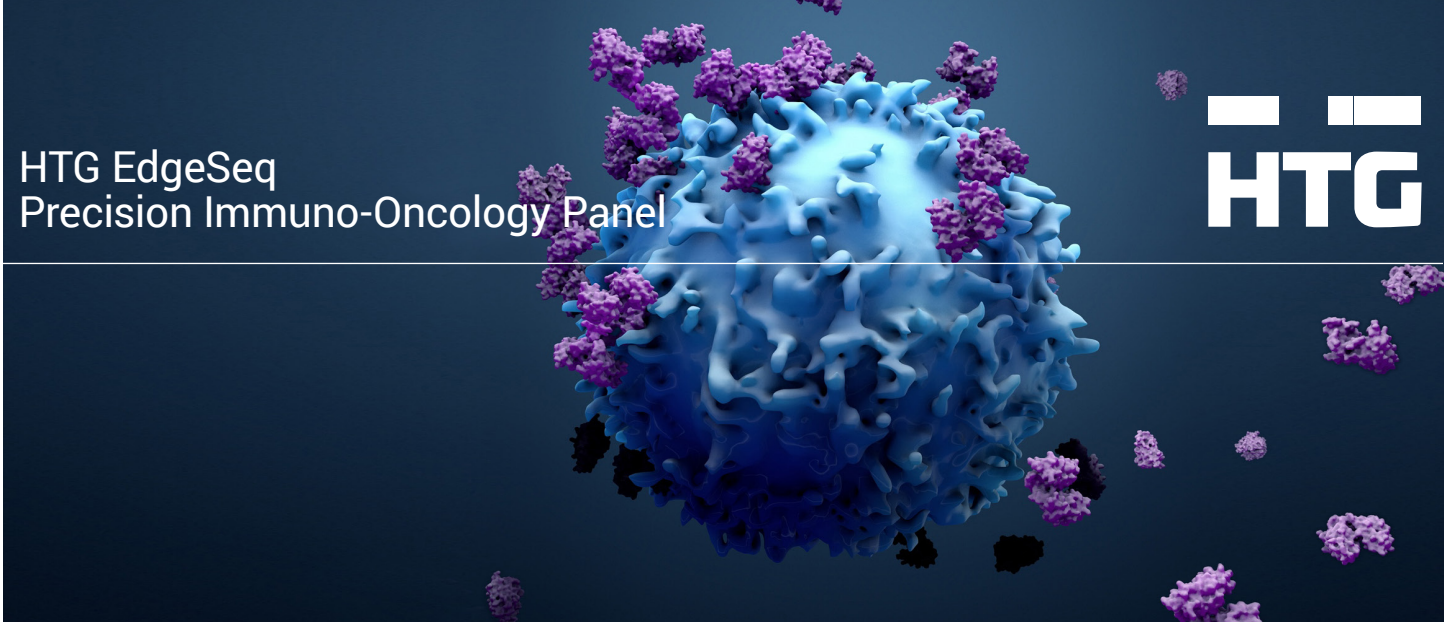

Enabling you to Decipher Complexities of the Tumor Microenvironment

Gene expression analysis has become an invaluable tool for preclinical and clinical studies to investigate transcriptional profiles and gene signatures associated with disease state, treatment response, and disease outcome. The HTG EdgeSeq™ Precision Immuno-Oncology Panel (PIP) is carefully designed to include targets that enable evaluation of gene expression associated with oncology and the immune response, both inside the tumor and in the surrounding microenvironment *(Figure 1)*.

Panel Overview

The HTG EdgeSeq Precision Immuno-Oncology Panel includes probes that provide insights for identifying relevant immune resistance mechanisms in the tumor microenvironment. This panel can simultaneously interrogate 1,392 targets, using common solid and liquid sample input types, in less than three days *(Table 1)*. Sample inputs are low across all sample types allowing researchers to get reliable results from small biopsies. 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 Precision Immuno-Oncology Panel *(Figure 1)* generates more output than competing targeted panels.

Table 1: *HTG EdgeSeq Precision Immuno-Oncology Panel Overview*

Number of Targets	1,392
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

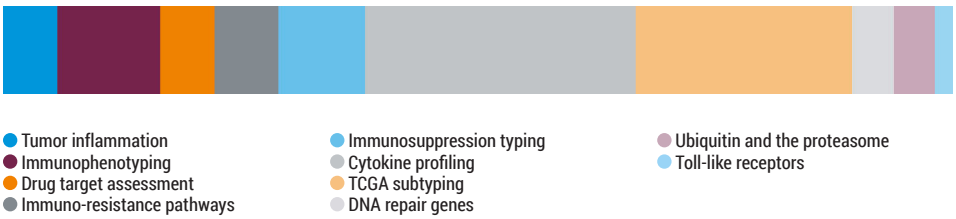

Figure 1: *The HTG EdgeSeq Precision Immuno-Oncology Panel includes targets for each of the processes listed, making it applicable for a wide range of immuno-oncology applications.*

HTG EdgeSeq
Precision Immuno-Oncology Panel

Small Sample Input

Clinical and research studies are often delayed by lack of sufficient patient samples, while the demand for additional molecular information continues to increase. While many consider RNA-Seq to be the gold standard for measuring gene expression, it requires substantial quantities of high-quality extracted RNA, that can be difficult to generate with small or archival samples. On the other hand, the HTG EdgeSeq technology's extraction-free chemistry is highly versatile, using relatively small sample input amounts to generate high quality data. A high correlation (0.98-0.99) is shown between sample replicates across a range of different sample input amounts ranging from 0.75 mm^2 up to 12 mm^2 per well *(Figure 2)*. These data illustrate the high performance of the HTG EdgeSeq Precision Immuno-Oncology Panel across a wide range of sample inputs, including FFPE sample inputs of less than 1.0 mm^2 per well.

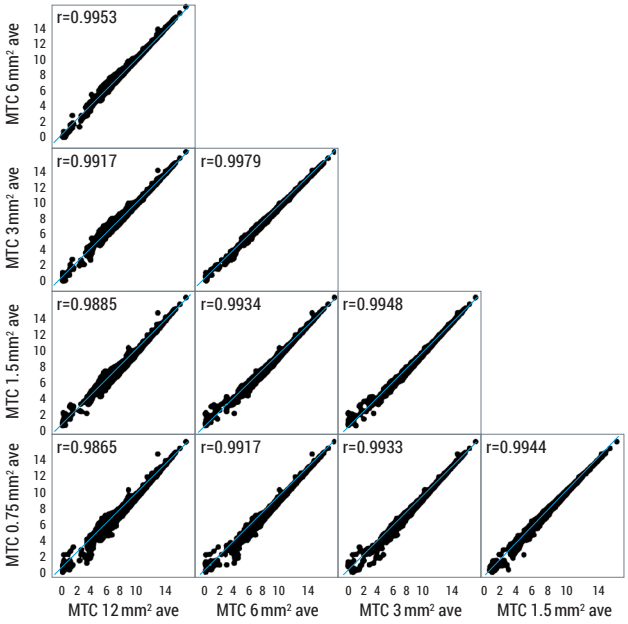

Figure 2: *Multi-tissue FFPE tissues were lysed and processed with the HTG EdgeSeq PIP at sample input concentrations between 0.75 mm^2 and 12 mm^2. Data generated by HTG Molecular Diagnostics, Inc.*

Reproducibility with Small and Archival Samples

The most difficult archival samples are formalin-fixed, paraffin-embedded (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. The proprietary HTG EdgeSeq extraction-free sample preparation chemistry enables researchers to generate quality data with a high degree of reproducibility from samples that would otherwise not meet sample input requirements. This is done by preserving partially degraded and small RNAs which might be lost during standard RNA extraction. For example, Bell *et al.*, 2020, were successful in completing gene expression studies for sinonasal cancer using archival tissue as old as 15 years, with a 93% sample pass rate.

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 high correlation to RNA-Seq (Ran *et al.,* 2020), while reducing extraction bias associated with RNA purification. This bias is illustrated as the increase in zero count genes for samples using RNA extraction *(Figure 3, right and middle)* compared to samples using the HTG EdgeSeq extraction-free chemistry *(Figure 3, 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.

Reproducibility

In addition to being sensitive, an assay must be both reproducible and accurate. As mentioned above, the introduction of bias can greatly affect reproducibility especially when working with limited quantity of precious samples that may potentially contain compromised RNA material. When using liquid biopsy samples, the HTG EdgeSeq Precision Immuno-Oncology Panel produces highly reproducible data, even when using liquid sample types like PAXgene *(Figure 4)*. Together, these results show the high degree of reproducibility generated by varying input amounts and sample types in the HTG EdgeSeq Precision Immuno-Oncology Panel.

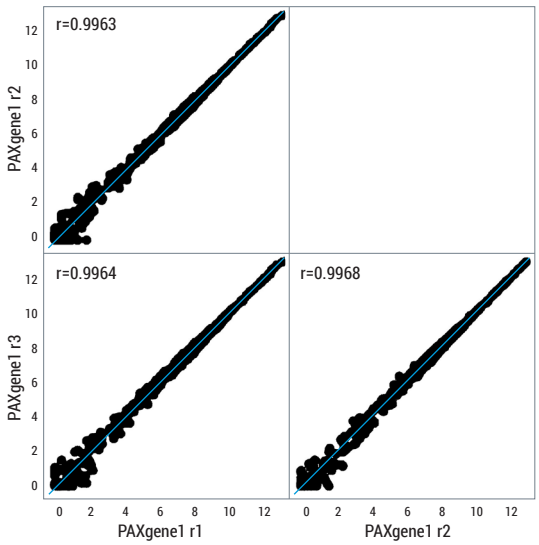

Figure 4: *Replicate analysis of PAXgene samples processed with the HTG EdgeSeq PIP. Data generated by HTG Molecular Diagnostics, Inc.*

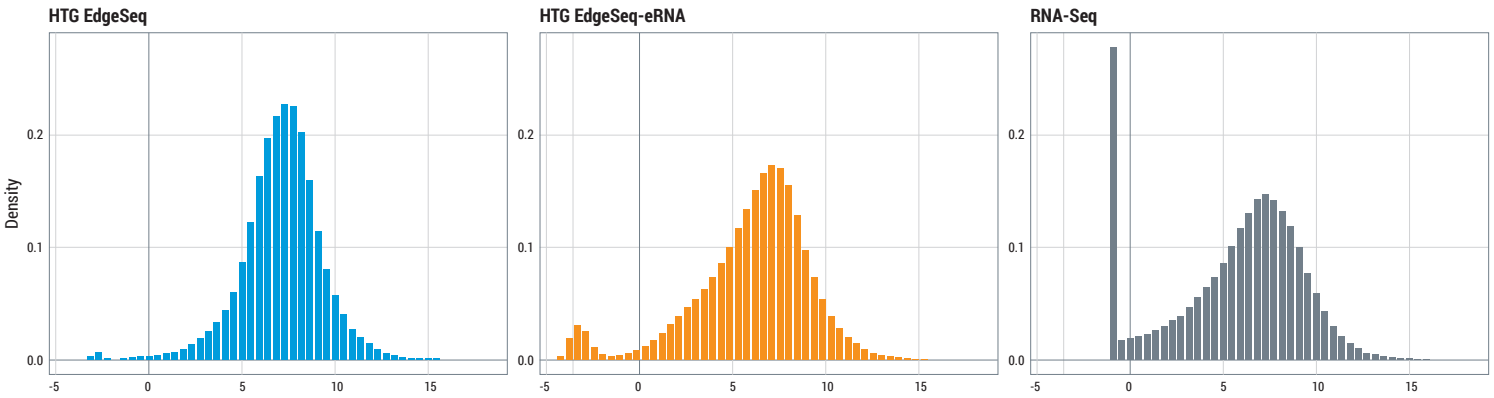

Figure 3: *Gene expression comparison between HTG EdgeSeq extraction-free sample input (left), HTG EdgeSeq with extracted RNA (middle) and RNA-Seq with extracted RNA (right). Data generated by HTG Molecular Diagnostics, Inc.*

Accuracy

Reproducibility is the ability to generate the same answer while accuracy is the assurance that the answer is correct. Zhang *et al.,* 2021 evaluated classification accuracy using a 5-fold crossed validation between prostate cancer patients with and without neoadjuvant therapy and area under the curve (AUC) for the HTG EdgeSeq Precision Immuno-oncology Panel and the nanoString nCounter PanCancer Immune Profiling Panel (data not shown). In addition to showing a high degree of correlation between the two platforms, the authors showed that the HTG EdgeSeq platform had the highest accuracy and AUC, when compared to nanoString's nCounter platform *(Table 2).* This led the authors to 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 PIP	nS Immune	HTG PIP	nS Immune
Fold 1	100%	63%	100%	100%
Fold 2	88%	63%	100%	67%
Fold 3	89%	33%	94%	83%
Fold 4	86%	86%	100%	100%
Fold 5	63%	38%	73%	73%
Average	**85%**	**56%**	**94%**	**85%**

Zhang et al., 2021

Lastly, Zhang *et al.,* 2021 found that while many common genes across the two platforms were significantly correlated, the HTG EdgeSeq Precision Immuno-Oncology Panel was able to detect a group of genes that were not detected by the nanoString platform. These genes were highly reproducible and were involved in tumor progression either through mediating tumorigenesis, proliferation, metastasis, avoiding the immune system, or conferring resistance *(Table 3).*

Table 3. *Consistency Comparison between HTG EdgeSeq Precision Immuno-Oncology and nanoString Immune Panel*

Genes Matched (% Matched Genes)	617	22.7%
Genes Significantly Correlated ($p < 0.05$)	560	90.8%
Highly Correlated Genes ($r > 0.5$)	475	77.0%

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Panel Products

Kit Configurations and Catalogue Numbers

Kit Name	Configuration	Illumina Sequencers	Thermo Fisher Scientific Ion Torrent Sequencers
HTG EdgeSeq Precision Immuno-Oncology Panel	2 x 8	916-011-208	916-011-308
HTG EdgeSeq Precision Immuno-Oncology Panel	4 x 8	916-011-008	916-011-108
HTG EdgeSeq Precision Immuno-Oncology Panel	1 x 24	916-011-224	916-011-324
HTG EdgeSeq Precision Immuno-Oncology Panel	4 x 24	916-011-024	916-011-124
HTG EdgeSeq Precision Immuno-Oncology Panel	1 x 96	916-011-096	916-011-196

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.

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