Application of HTG Transcriptome Panel for Targeted Gene Expression Profiling in COVID-19 Cases

Introduction

Coronavirus Disease 2019 (COVID-19), which is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), rapidly became a global pandemic in 2020 resulting in over 6 million deaths and over 470 million cumulative cases worldwide according to the World Health Organization[1] (WHO). In addition to acute respiratory symptoms, persistent symptoms have also been reported with COVID-19 (long COVID) including fatigue, cough, chest tightness, breathlessness, palpitations, myalgia and difficulty to focus[2]. Gene expression profiling (GEP) is being used to help elucidate the mechanisms of disease pathogenesis, genetic factors responsible for inter-individual variability in response to COVID-19, effects on organ tissues and to identify potential therapeutic targets[3-5].

Recently, the HTG EdgeSeq™ Immune Response Panel was used to successfully identify characteristic GEP patterns of immune response in pulmonary draining lymph nodes from COVID-19 cases[6]. In another study, circulating miRNAs were profiled in COVID-19 patients using HTG EdgeSeq miRNA Whole Transcriptome Assay to detect miRNAs that were significantly differentially expressed compared to samples from healthy individuals[7]. This study highlights the transcriptomic profiling of COVID-19 patient samples using the HTG Transcriptome Panel (HTP), could augment discovery, provide insights into the mechanism of disease manifestation, and help determine potential clinical solutions. The recently released HTG Transcriptome Panel (HTP) measures approximately 20,000 human protein-coding genes (mRNAs). This technical note demonstrates the utility value of HTG EdgeSeq proprietary chemistry and application of HTP's comprehensive gene expression profiling technology in GEP of patients infected with COVID-19.

The HTG EdgeSeq library preparation workflow uses Next-Generation Sequencing (NGS) as a readout of the quantitative Nuclease Protection Assay (qNPA). The qNPA is proprietary chemistry, developed by HTG Molecular Diagnostics, that uses targeted probes to protect and ultimately detect and quantify mRNAs in a sample. The HTP is a comprehensive transcriptome panel that employs HTG's extraction-free chemistry and provides accurate and repeatable quantitative gene expression data for 19,398 human mRNA targets. The HTP has been validated for use with a variety of sample types including neoplastic and normal/non-neoplastic formalin-fixed paraffin-embedded (FFPE) tissue samples, extracted RNA from FFPE and fresh frozen tissues, and PAXgene™ blood samples. The HTP was determined to be precise across multiple formulation lots of the HTP probe mix, multiple operators, multiple HTG EdgeSeq processors and multiple sample replicates (Lin's concordance correlation coefficient >0.90, as described in HTP White Paper #3[8]. The HTP was also shown to be accurate and comparable to RNA-Seq (Pearson correlation = 0.83)[8]. The result of this approach is ultra-efficient GEP analysis making optimal use

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of samples including samples of low volume and or quality. In this study, precious samples from COVID-19 cases were evaluated using the HTP.

Methods and Statistical Analyses

This study was conducted as part of the HTP Early Adopter Program[*]. The study cohort included 48 lung non-neoplastic FFPE tissue samples that encompassed 26 COVID-19 cases, 4 influenza pneumonia controls, 9 controls with diffuse alveolar lung damage and 9 normal controls. An HCT-15 cell line (colorectal carcinoma) lysate was used as a plate level control sample. All 48 lung FFPE tissues were lysed at 11 mm^2 / 50 µL in a 5:1 mixture of Lysis Buffer A (LBA) and Proteinase K (PK). The samples were processed following the instructions outlined in the HTP User Manual (P/N 10383264). qNPA reaction was executed with each sample using an HTG EdgeSeq processor and the HTP Assay Reagent pack. The qNPA reaction products were then PCR amplified and tagged using Illumina NGS platform-specific sequence adapters and sample barcodes. The PCR reaction products (libraries) were then cleaned using AMPure® XP Magnetic Beads, quantified, pooled, denatured and sequenced using an Illumina NextSeq 500/550 high output kit. The sequencing FASTQ files were parsed using HTG EdgeSeq™ Parser Software and analyzed using the HTG EdgeSeq™ Reveal statistical analysis package.

Results

The HTP provided accurate and precise data on 10,828 genes with mean counts-per-million above 10 (a typical pre-filter value for differential expression analysis in all 48 samples[9]). Of these expressed genes, 9,386 are not measured by the targeted HTG EdgeSeq Immune Response Panel (IR), suggesting that HTP offers more comprehensive gene expression data. As illustrated below for comparison, there are 1,995 gene targets in common between HTP and Immune Response Panels, 7 gene targets unique to the IR Panel and 17,403 gene targets unique to HTP, opening up avenues for discovery and possible signature development for target identification using differential gene expression analysis. The biological relevance of the HTP data generated in this study is not presented in this Technical Note and is pending publication by the principal investigator of this study[†]. All HCT-15 cell line lysate control samples passed HTP QC metrics with technical replicates having Pearson correlation value ≥0.95.

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[*] The Early Adopter Program (EAP) was introduced as part of the initial launch of the HTP. The EAP allowed a select group of customers access to the HTP for use in their laboratories or through services provided by HTG prior to the commercial launch of the panel. The samples highlighted in this tech note were obtained from Dr. Matthias Matter at the University Hospital Basel, Switzerland. The samples were processed at HTG's commercial laboratory (VERI/O™).

[†] Customer publications in progress

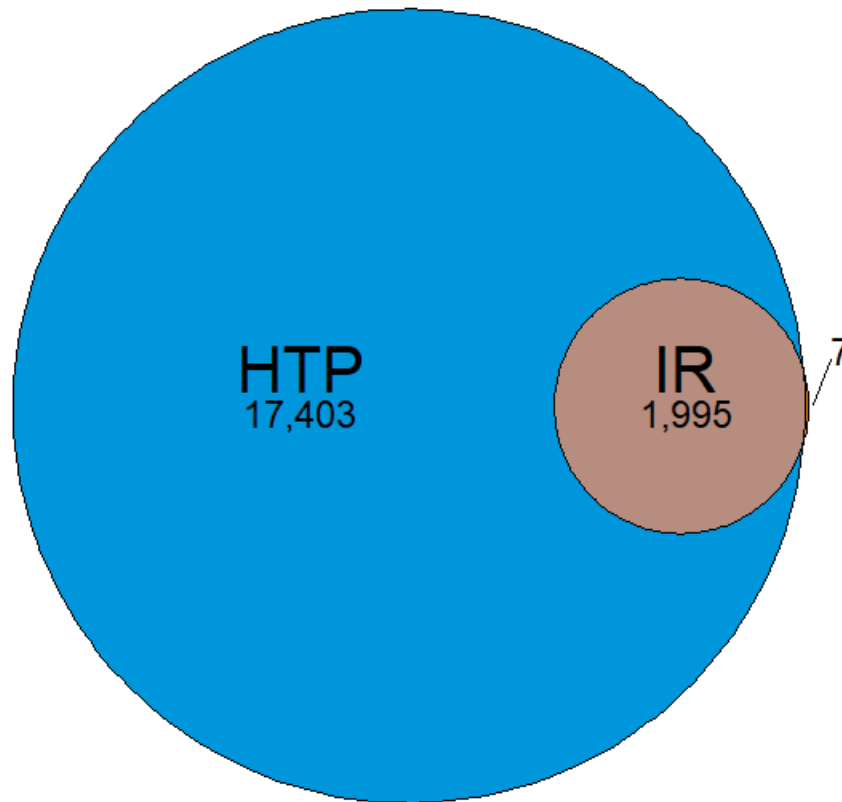## Genes Measured by HTP and IR

Figure 1 – Euler Plot illustrating the number of gene targets unique and overlapping between the HTG Transcriptome Panel (HTP) and the HTG Immune Response Panel (IR). The HTP exhibits 17,403 additional and unique gene targets. IR and HTP panels have 1995 genes in common. IR panel has 7 unique gene targets.

Conclusions

This study demonstrates the power of the HTP technology in targeted profiling of mRNA for use with non-neoplastic FFPE tissue samples derived from COVID-19 patients. This study may provide insights into understanding COVID-19 disease mechanisms and can possibly lead to the discovery of potential therapeutic targets and treatment options for patients.

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