Technical Note

Benchmark Study of HTG Transcriptome Panel and NanoString nCounter PanCancer Immune Profiling and Custom Panels

Purpose

The purpose of this technical note is to showcase the results of a cross-platform comparison of the gene expression profiles of the same formalin-fixed paraffin-embedded (FFPE) tissue samples run with the HTG EdgeSeq™ platform using HTG Transcriptome Panel (HTP) and NanoString (NS) nCounter® platform using nCounter PanCancer Immune Profiling and Custom Panels.

Introduction

Gene expression profiling is a powerful tool that can be used to understand disease processes and to identify biomarkers that can be used for diagnosis, disease prognosis, and treatment efficacy. HTG Molecular Diagnostics recently developed HTP, a proprietary extraction-free human transcriptome panel that provides accurate and precise quantitative gene expression data for over 19,000 human genes[1]. Other commonly used technologies for the analysis of gene expression include RNA Sequencing (RNA-Seq) and the NS nCounter panels. While the advantages of the HTG EdgeSeq™ platforms over RNA-Seq have previously been established[1-4], this tech note compares the HTP and two NS panels, and highlights the unique advantages of the HTP.

Both the HTG EdgeSeq and the NS nCounter Platforms are commonly used for targeted gene expression profiling. The key features of the HTP and NS panels are shown in Table 1. The NS nCounter panels use fluorescent probes targeted against genes of interest that can be individually counted without amplification using an automated workflow. However, they can only measure the expression of up to 800 genes, which is not favorable for biomarker discovery, and they require extracted RNA (eRNA). The HTP, on the other hand, can measure the expression of up to 19,398 human genes using targeted nuclease protection probes (NPP). In addition to the 24-fold higher number of transcripts that can be investigated, one of the main advantages of the HTP is that it employs a proprietary extraction-free method of sample preparation, which bypasses the risk of RNA extraction bias[5] caused by the removal of small or partially degraded RNA species during the extraction process. The extraction-free process also ensures RNA is not lost due to the low efficiency of the extraction resulting in less sample input requirement to generate equivalent amounts of addressable RNA.

Table 1. Comparison of HTP and NanoString Panels		
	HTP	**NanoString nCounter**
Probe design	Gene-specific nuclease protection probes	Fluorescent reporter and capture probes
Sample Preparation	Extraction-free sample preparation avoids RNA extraction bias, and less sample is required	RNA extraction
Sample Amount	70 ng extracted RNA 11-22 mm^2 FFPE tissue 400 µL PAXgene	100 ng extracted RNA
Number of genes or transcripts detected	Up to 19,398	Up to 800-limited biomarker discovery capabilities
Processing Time	3 days/96 samples	2 days*/12 samples

* Does not include time for RNA extraction

Cross-platform comparisons of NS and HTG platforms have previously been reported in the literature[6,7]. Zhang et al. performed a cross-platform comparison of immune-related gene expression of eRNA from 52 patients to assess intratumor immune responses following cancer immunotherapy using the HTG EdgeSeq Oncology Biomarker and Precision Immuno-Oncology Panels and the NS nCounter PanCancer Immune Response Panel and found a high level of consistency across platforms[6]. In a separate study, Godoy et al. compared the HTG miRNA Whole Transcriptome Assay (WTA) to the NS nCounter platform, concluding that HTG EdgeSeq miRNA WTA is the most reproducible and has the lowest detection bias[7]. Both studies showcase the robust performance of HTG EdgeSeq panels in comparison to NS technology.

This technical note is the first to highlight the results of a cross-platform comparison of the new HTG Transcriptome Panel and two NS panels, including the NS nCounter PanCancer Immune Profiling Panel and a NS Custom Panel using the same FFPE samples. The data presented in this tech note show that there is a high level of cross-platform concordance between HTP and the NS panels.

Methods and Statistical Analyses

A customer cohort of 45 FFPE diffuse large B-cell lymphoma (DLBCL) tissues were run at HTG on the HTP as part of the HTP Early Adopter Program[†] and at NanoString using the NS nCounter PanCancer Immune Profiling Panel and a NS Custom Panel. Briefly, the HTP is a research use only (RUO) panel designed to measure the expression of 19,398 human mRNA transcripts in FFPE, PAXgene and eRNA samples. For more information about the performance of the HTP, refer to the HTP Product Sheet (www.htgmolecular.com/resources/category/product-sheets). The NS

[†]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 Professor Wolfram Klapper at the University Hospital Schleswig-Holstein Kiel, Germany. The samples were processed at HTG's commercial laboratory (VERI/O ™).

nCounter PanCancer Immune Profiling and NS Custom Panels are also RUO panels. The NS PanCancer Immune Profiling Panel can measure the gene expression of 770 genes implicated in cancer immunotherapy. The NS custom panel used in this analysis measures the expression of 117 genes known to play critical roles in lymphoma biology.

For the HTP, one 5µm FFPE tissue section per sample was lysed using HTG's proprietary Lysis Buffer. The sample lysates were placed on an HTG EdgeSeq processor where gene specific NPPs were added and allowed to hybridize to their target RNAs. S1 nuclease was added to remove unhybridized NPPs and RNAs, leaving behind only NPPs hybridized to their target RNAs, resulting in a 1:1 ratio of probes and mRNA targets. Subsequently, sequencing adapters and molecular barcode tags were added by PCR. The tagged samples were cleaned, quantified, pooled, and sequenced using Illumina's NextSeq 500/550 system. Data from the sequencer were processed and reported by the HTG EdgeSeq Parser software.

For the NS panels, RNA was extracted from the samples and approximately 100 ng of eRNA per sample was mixed with gene-specific fluorescent probes and allowed to hybridize to the target mRNA overnight. Samples were then placed in a NS nCounter Prep Station where excess probes were removed, and probe/RNA complexes were retained in an nCounter cartridge. Sample cartridges were then placed in a NS nCounter Digital Analyzer for data collection.

Matched samples across platforms were compared and expressions from the matched probes were analyzed. Samples that failed the HTP and NS post-sequencing quality control (QC) metrics were not included in this analysis. Table 2 lists the comparisons and captures the number of matching probes and samples for each comparison.

Table 2. Panel and Sample Information

Comparison	Number of Matched Samples	Number of Matched Probes
HTP vs NS PanCancer Immune Profiling Panel	13	580
HTP vs NS Custom Panel	24	79

Results

The gene expression profiles of 13 samples that were run on the HTP and NS PanCancer Immune Profiling Panel, as well as 24 samples that were run on the HTP and the NS Custom Panel were used to compare the dynamic range, sample-wise, and gene-wise correlations between platforms (Figure 1). Figure 1A demonstrates that the dynamic range, or range of expression, for the HTP and NS panels is similar. Figure 1B shows the median sample-wise pairwise correlation, measured using different correlation coefficients for the different comparisons[8]. The purpose of using these three different coefficients is that they can provide different nuances of the data under investigation: the Pearson coefficient captures the familiar linear relationship (either between genes or within samples), while the Spearman coefficient describes the monotonic relationship (either linear or not), and ultimately, the Tabatabai coefficient is a robust measure

which shows the linear relationship but is less sensitive to influential observations. The correlation coefficients ranged between 0.81 to 0.89, suggesting that there is strong agreement between the HTP and NS gene expression results for these samples, especially considering that these two platforms use different sample input types (extraction-free lysate with HTP versus eRNA with NS). Lastly, Figure 1C shows the gene-wise correlations between panels‡. Fifty percent of the genes have a Spearman correlation higher than 0.50 between the HTP and NS PanCancer Panel and greater than 0.75 between the HTP and NS Custom Panel. The correlation coefficients for the gene-wise analysis will be impacted by the range of gene expression, among other factors. If the expression variability is small, for example, the correlation coefficient will be low, but this does not indicate a lack of alignment between the two technologies.

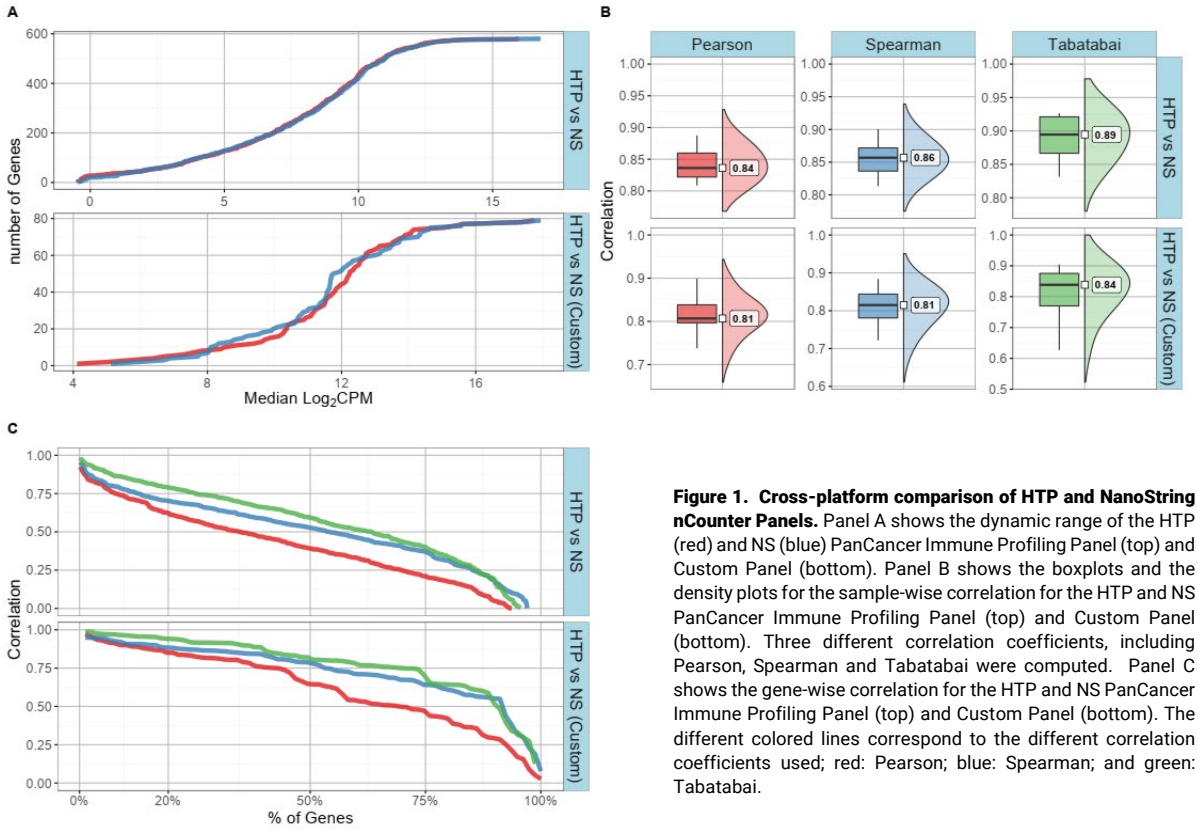

Figure 1. Cross-platform comparison of HTP and NanoString nCounter Panels. Panel A shows the dynamic range of the HTP (red) and NS (blue) PanCancer Immune Profiling Panel (top) and Custom Panel (bottom). Panel B shows the boxplots and the density plots for the sample-wise correlation for the HTP and NS PanCancer Immune Profiling Panel (top) and Custom Panel (bottom). Three different correlation coefficients, including Pearson, Spearman and Tabatabai were computed. Panel C shows the gene-wise correlation for the HTP and NS PanCancer Immune Profiling Panel (top) and Custom Panel (bottom). The different colored lines correspond to the different correlation coefficients used; red: Pearson; blue: Spearman; and green: Tabatabai.

‡ The Pearson correlation coefficient was computed on the log_2CPM-transformed counts using all the probes for the HTP vs. NS (Custom) comparison. For the HTP vs. NS comparison – as a way to avoid comparing non-expressed genes – only probes with a value larger than 4 in both assays were selected. In addition to this – in order to take into account, the artifact induced by some influential samples – a robust linear correlation[7] was added to the comparisons in addition to the more familiar Pearson correlation coefficient.

Conclusions

Collectively, the results indicate that there is a high level of cross-platform concordance between the HTP and the NS nCounter PanCancer Immune Profiling and Custom Panels. The panels had similar dynamic ranges and the correlations between the GEP of the same FFPE samples run on the HTP versus NS panels were above 0.8, suggesting that the agreement between the HTP and NS gene expression results is strong. While the analysis suggests that the performance of the platforms is similar, the HTP has some unique advantages. The HTP, for example, can measure the expression of nearly 20,000 human genes versus up to 800 with NS, without having to extract RNA from samples. These features make HTP better suited for biomarker discovery and for use with small, older, or degraded FFPE samples from which eRNA is difficult to obtain.

References

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