Multi-Site Reproducibility and Analytic Accuracy of the HTG EdgeSeq Immuno-Oncology Assay

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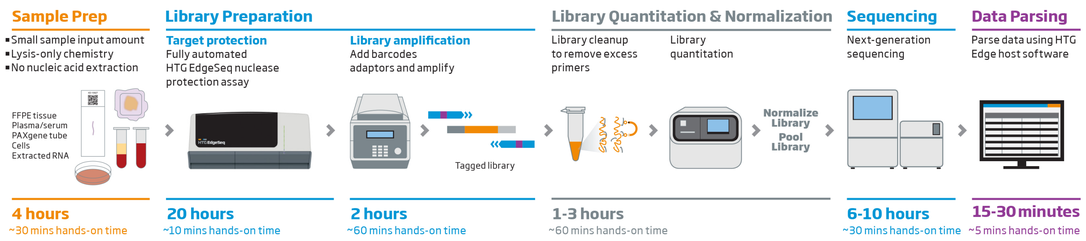
Introduction

HTG EdgeSeq assays employ automated quantitative nuclease protection assay (qNPA) coupled to next-gen sequencing to enable multiplex mRNA expression analysis. This study utilizes data that were generated under a standardized protocol that is used for every HTG EdgeSeq system installation. Data from seven sites (a mixture of central laboratory and academic genomic labs) have been summarized to demonstrate assay reproducibility and analytic accuracy of the HTG EdgeSeq Immuno-Oncology Assay. The HTG EdgeSeq Immuno-Oncology Assay is comprised of probes targeting 549 genes implicated in the host immune response to tumors. The assay can be used to measure expression levels using a single section of formalin-fixed, paraffin-embedded (FFPE) tissue. All sequencing was performed with the Illumina MiSeq per manufacturer's specifications.

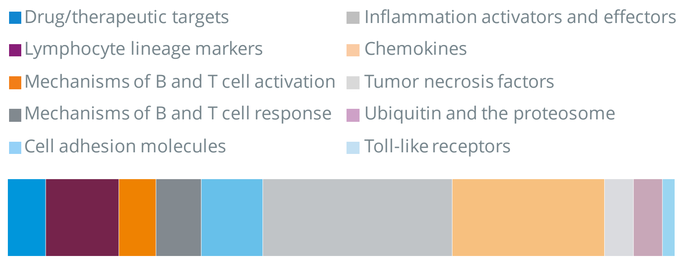
HTG EdgeSeq Immuno-Oncology Assay

HTG EdgeSeq Workflow



HTG EdgeSeq Immuno-Oncology Assay

The HTG EdgeSeq system requires **as little as one 5 µm section** of a core needle biopsy — providing more answers from less sample.



Methods

Eight lung FFPE samples (seven non-small cell lung cancer [NSCLC] and one metastatic melanoma, per Table 1) were lysed at 10 mm^2/35 µL in triplicate. The lysates were randomized to 24 wells of a 96-well plate and processed using the HTG EdgeSeq system and sequenced on an Illumina MiSeq sequencer.

Baseline data were generated at HTG Molecular Diagnostics, Tucson, AZ. These baseline data were then compared to data generated at seven sites after HTG EdgeSeq system installation and calibration to demonstrate instrument performance. The baseline data included processing the study samples on three different HTG EdgeSeq processors. Samples run at external sites were performed on single instruments.

Table 1. Lung FFPE Samples.

FFPE 1	Lung, Squamous
FFPE 2	Lung, Adeno
FFPE 3	Lung, Melanoma
FFPE 4	Lung, Squamous
FFPE 5	Lung, Adeno
FFPE 6	Lung, Adeno
FFPE 7	Lung, Adeno
FFPE 8	Lung, Squamous

Performance Metrics:

1. Reproducibility of expression across FFPE samples on five genes (PD-L1 (CD274), PD-1 (PDCD1), PD-L2 (PDCD1LG2), CTLA4, JAK4 (TYK2)). Reproducibility between baseline and site probe-level expression was qualitatively assessed using Tukey mean-difference (a.k.a. Bland-Altman) plots.
2. Distribution of mean-variance relationship of expression over the set of 549 genes, as well as summary statistics of triplicate standard deviation (SD) and coefficient of variation (CV).
3. Agreement between the triplicate samples over all genes. Agreement was measured through Pearson correlation coefficient (r) and concordance correlation coefficient (CCC).

Results: Baseline Performance

Distribution of Expression (Figure 1)

Data from subsequent field validation studies will be compared to data from Processor 31.

Success: It is expected that outside of very low expressing probes that sequenced data should demonstrate similar mean-variance patterns of expression between processors.

Correlation between replicates (Figure 2)

Success: Replicate samples show similar probe level expression.

Variation (Figure 3)

The HTG estimates of inter-quartile range (IQR) and SD for replicates averaged across processors will be used as a baseline to compare with subsequent sites.

Success: qualitatively similar values for FFPE samples.

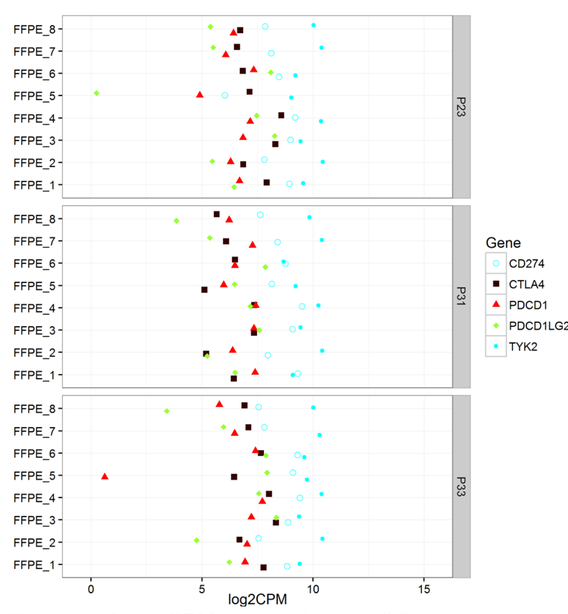


Figure 1. Log_2 (CPM) expression for all five genes.

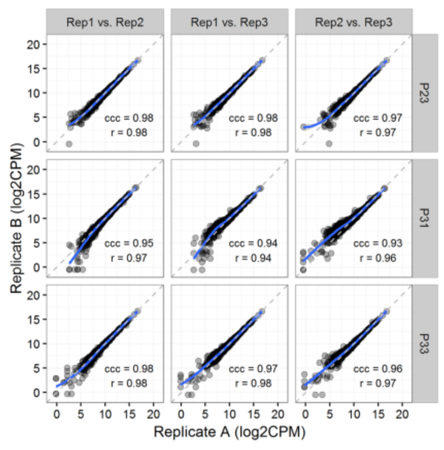


Figure 2. Representative regression plots for first FFPE sample.

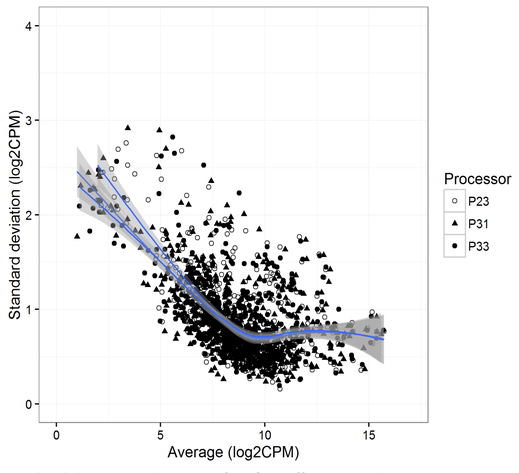


Figure 3. Mean-variance plot for all genes by processor.

Results: Seven Sites

Agreement to Baseline Expression (Figure 4)

Expression of specific probes from field validation studies are compared to data from Processor 31. Most probes show a difference very close to zero (y-axis) over all expression levels (x-axis).

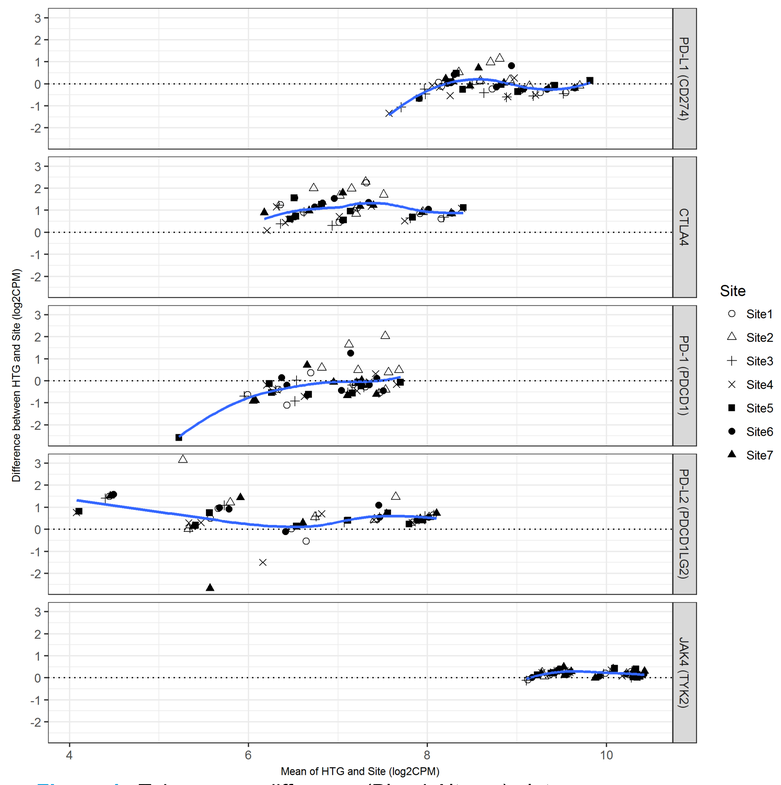


Figure 4. Tukey mean-difference (Bland-Altman) plots.

Mean-Variance Relationship (Figure 5)

The between-FFPE variance, measured by SD and coefficient of variation, CV, (on the y-axis) by average expression (on the x-axis) is similar for each site (in red) compared to the variance by average expression seen in data generated at HTG. Variability is higher for lower levels of expression, but for most samples the SD, even at the lowest levels of expression, is below 2 SD. These plots demonstrate a level of analytic accuracy as seen with low variability even at low levels of expression.

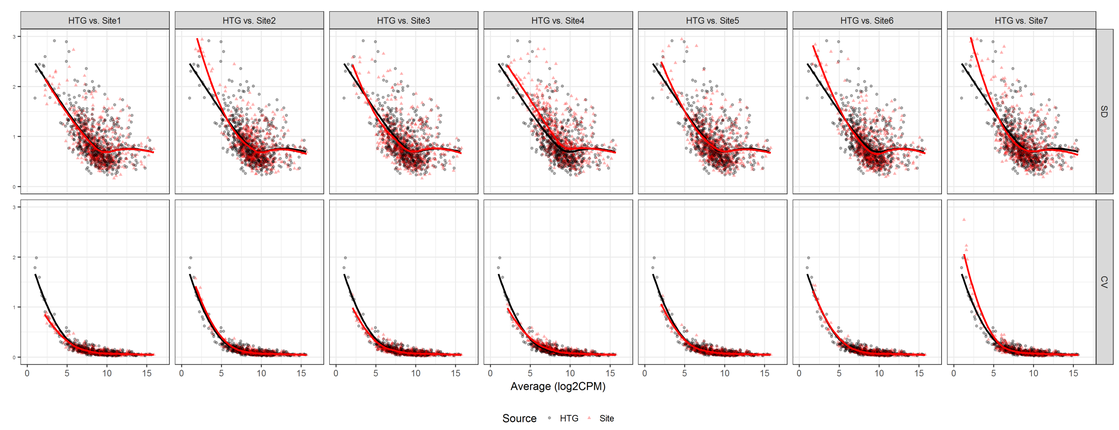


Figure 5. Mean-variance (SD) and mean-coefficient of variance (CV) plots; y-axis is SD or CV and x-axis is the mean of expression of all FFPE samples by each probe ranked from low to high expression.

Correlation between sites and HTG (Figure 6)

Correlation between the data generated by HTG and each of the sites shows a very high degree of correlation. These plots are based on averaging expression values over all eight FFPE samples. Most of the expression values are between log2 (CPM) of 5 and 10, approximately 32 and 1,024 counts, after adjustment by total aligned counts.

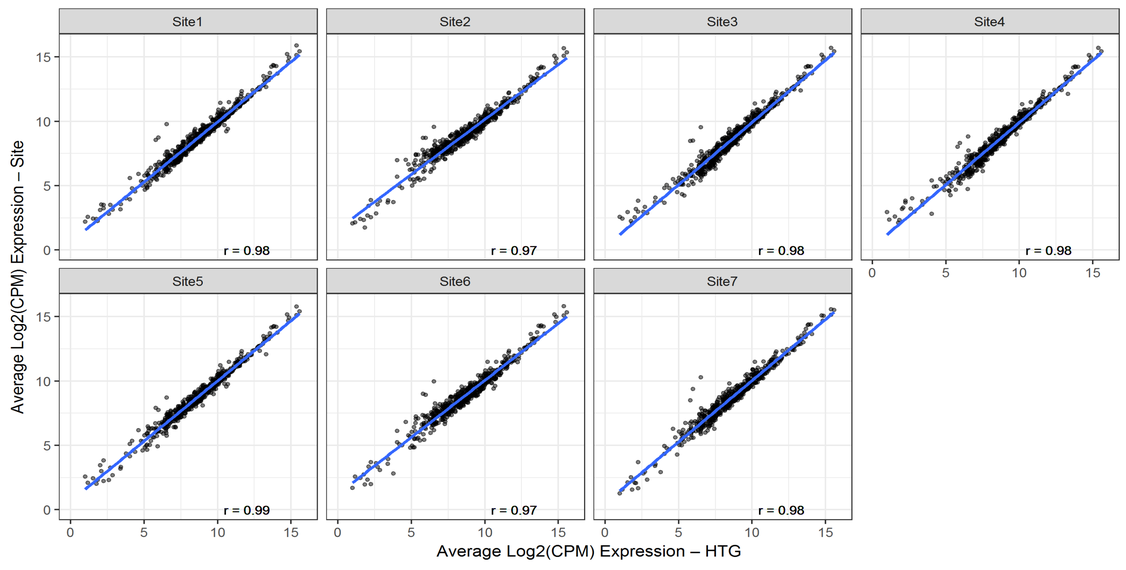


Figure 6. Regression and Pearson correlation coefficient between baseline (HTG) and site runs; expression is averaged across eight FFPE samples.

HTG EdgeSeq Immuno-Oncology Assay

The HTG EdgeSeq Immuno-Oncology Assay and the HTG EdgeSeq system provide reliable and accurate gene expression as demonstrated across a total of seven independent sites.

The baseline data show that variability between HTG EdgeSeq processors is low, there is good correlation between replicate measurements within samples, and most genes have less than 1SD difference between non-small cell lung cancer samples.

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