

Complement IHC Studies with the HTG EdgeSeq PATH Assay

The HTG EdgeSeq PATH Assay was designed to complement immunohistochemistry (IHC)-based studies with mRNA gene expression data from 470 genes. Using a single formalin-fixed, paraffin-embedded (FFPE) tissue section, researchers may collect data similar to IHC results typically obtained from multiple sections of FFPE tissue. To examine the relationship between gene expression as detected by mRNA and protein (IHC), a well-understood model system, invasive breast cancer, was chosen for comparison.

Methods: Serial sections of 12 cases of invasive breast carcinoma were stained using standard IHC methods. All assessments were made by two qualified pathologists, with re-reads performed in cases where discordant results were obtained. Scoring was performed using standard guidelines. Samples scored positive by these guidelines are plotted in orange, negative in grey.

A single FFPE section from each case was processed using the HTG EdgeSeq PATH Assay following the instructions in the package insert and then sequenced on an Illumina MiSeq sequencer. Data were transformed into \log_2 counts per million (CPM) prior to analysis.

Measurement of hormone receptor in breast cancer

The assessment of breast cancers for hormone receptor positivity is used to subtype and classify breast cancer based upon the suspected oncogenic drivers.

Hormone receptor assessment is typically performed via immunohistochemistry; measurement of the mRNA from the *ESR1* and *PGR* genes (estrogen receptor 1 and progesterone receptor), was performed to determine how well it agreed with the traditional method.

As seen in Figures 1 and 2, clear differences in signal are obtained from the HTG EdgeSeq PATH Assay data between tumors with cells staining above and below the typical 1% cut-off used to determine positivity of both receptors. Overall, correlative results were obtained, demonstrating similarity in measuring hormone receptors via mRNA profiling and IHC.

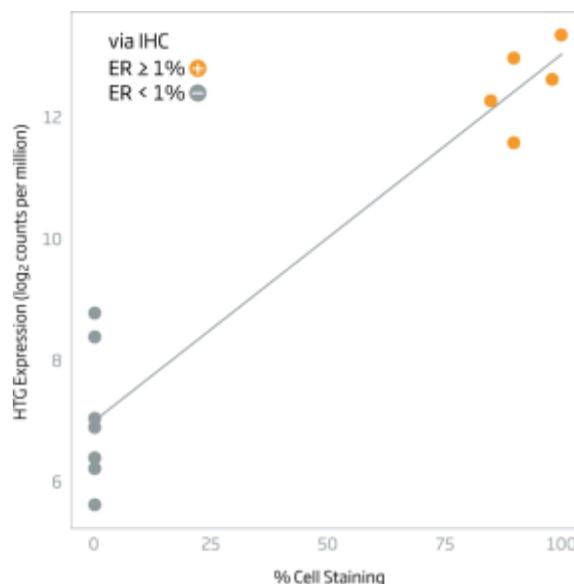


Figure 1: Agreement between the HTG EdgeSeq PATH Assay and IHC in the measurement of estrogen receptor (ER). The percentage of cells staining positive via IHC was assessed by a pathologist using standard guidelines.

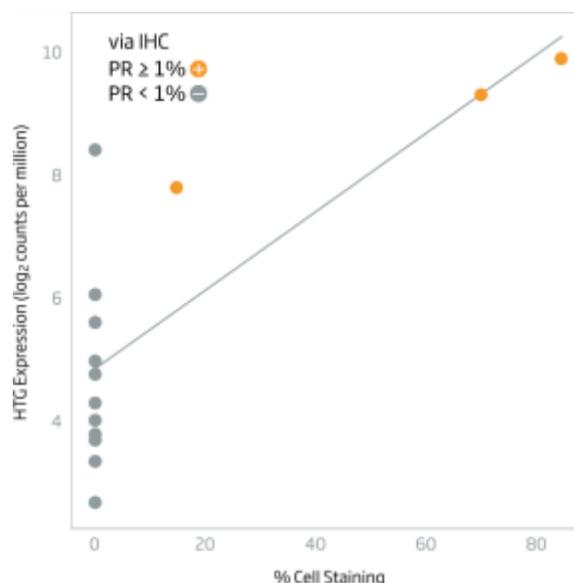


Figure 2: Agreement between the HTG EdgeSeq PATH Assay and IHC in the measurement of progesterone receptor (PR). The percentage of cells staining positive were assessed by a pathologist using standard guidelines.

Measuring HER2/ERBB2

The HER2 (ERBB2) gene is frequently amplified in the genomes of invasive breast cancer. While HER2 gene amplification may be assessed by various methodologies, including *in situ* hybridization, most labs view protein expression levels via IHC as the gold-standard.

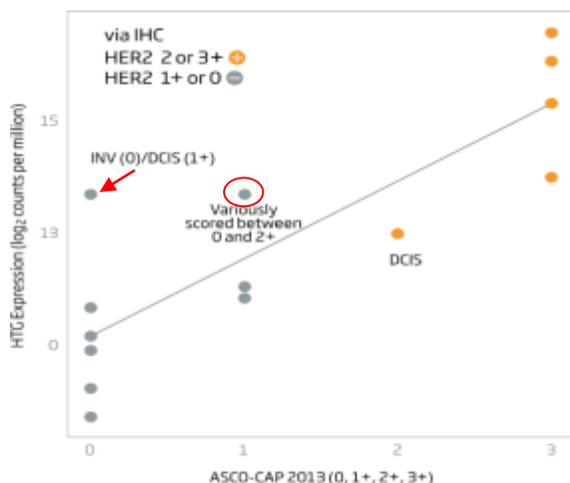


Figure 3: Agreement between the HTG EdgeSeq PATH Assay and IHC in the measurement of the HER2 / ERBB2 receptor. The stained sections were assessed by a pathologist using standard ASCO guidelines.

Comparison of the numeric output from the HTG EdgeSeq PATH Assay to the standard IHC scoring methods for invasive cancer shows general agreement between the data sets.

Assessing the Ki67 cell proliferation marker

Expression of the Ki67 marker is closely associated with cell proliferation, with higher levels of the marker indicating faster growing, more aggressive tumors.

Comparison of the gene expression levels obtained by the HTG EdgeSeq PATH Assay and

positive staining cells from IHC show a close relationship, with a clear separation of tumors staining above and below the 16% cut-off typically used to determine positivity in IHC.

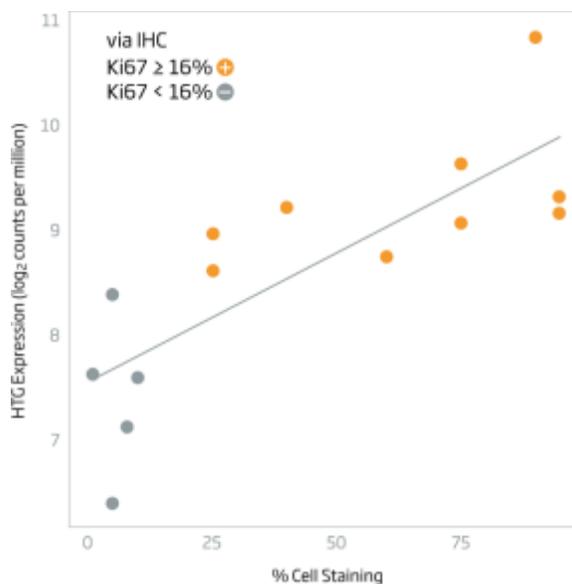


Figure 4: Agreement between the HTG EdgeSeq PATH Assay and IHC in the measurement of the Ki67 marker. The percentage of cells staining positive were assessed by a pathologist using standard guidelines.

Summary

Using a single section of FFPE tissue, the HTG EdgeSeq PATH Assay produces data for 470 genes commonly measured by IHC.

The data generated are generally comparable to those obtained with IHC, which requires one section of tissue per marker.

The HTG EdgeSeq PATH Assay has the potential to consolidate hundreds of IHC tests into a single workflow, allowing the researcher to obtain more data while conserving valuable tissue.