Targeted Gene Expression Profiling of Traditional Immunohistochemical Tumor Biomarkers Using Nuclease Protection Coupled with Next-Generation Sequencing

INTRODUCTION

The HTG EdgeSeq PATH Assay enables investigators to measure the expression of 470 human mRNA targets using a proprietary quantitative nuclease protection chemistry coupled with targeted next-generation sequencing (NGS). This panel measures the expression of genes that encode proteins commonly detected by commercially available immunohistochemistry (IHC) antibodies. The HTG EdgeSeq chemistry allows for extraction-free sample preparation and is compatible with limited amounts of tissue from formalin-fixed, paraffin-embedded (FFPE) samples and with cell line controls.

MATERIALS AND METHODS

Sample Input

- Performed 5-point titration curve with breast cancer (BC) FFPE tissue lysates and cell line lysates (data not shown).
- Evaluated expression of genes that encode proteins commonly assessed by IHC.
- Established input range for consistent biomarker expression.

Repeatability

- Intra-run, inter-processor, inter-day repeatability.
  - 20 technical replicates of a single BC FFPE lysate across 5 plates.
- Evaluated through Pearson and Concordance Correlations (Pearson Correlation Coefficient: r, Concordance Correlation Coefficient: CCC)

IHC Agreement

- Compared HTG EdgeSeq PATH Assay (mRNA levels) and IHC (protein levels) results for 14 BC cases.
- Used current ASCO/CAP guidelines for IHC interpretation1,2.
- Assessed four genes that encode proteins commonly assessed by IHC:
  - Estrogen Receptor (ER)
  - Progesterone Receptor (PR)
  - Ki-67
  - Human Epidermal Growth Factor Receptor 2 (HER2)
- Optimized a dichotomized cut-point for the HTG EdgeSeq PATH Assay based on the dichotomous positive/negative status of IHC. Evaluated through Receiver Operating Characteristic (ROC) Curves and Area under the Curve (AUC).

RESULTS

- Similar Probe Expression Across 0.75 mm²/35 µL – 12.0 mm²/35 µL Input Concentrations

- High Intra-run, Inter-processor, and Inter-day Repeatability

CONCLUSIONS

- The HTG EdgeSeq PATH Assay is a reliable tool that measures mRNA levels of targets commonly assessed by IHC using a single 5 µm thick tissue section.
- The recommended FFPE sample input is 6.0 mm²/35 µL.
- The HTG EdgeSeq PATH Assay is repeatable within a run, between processors and between days (median r and CCC > 0.95).
- High agreement between HTG EdgeSeq PATH Assay (mRNA levels) and IHC (protein levels) is observed for selected breast cancer markers.
- The HTG EdgeSeq PATH Assay can serve as a complement to traditional IHC.

REFERENCES


CONTACT

Monica Reinholz, Ph.D.
HTG Molecular Diagnostics
3430 E Global Loop
Tucson, AZ 85719
mreinholz@htgmolecular.com
www.htgmolecular.com
(520) 547-2627