

Reveal



Immune, Stroma and TME signatures — all in the HTG EdgeSeq Reveal software.

The Tumor Microenvironment (TME) is a complex and dynamic environment which plays a role in the body’s response to growth and metastasis of tumors (*Galon et al., 2006; Hanahan et al., 2012*). The HTG EdgeSeq™ Reveal Oncology Signatures, built upon the HTG EdgeSeq Precision Immuno-Oncology Panel (PIP) were developed to enable researchers to better understand the tumor microenvironment, including tumor inflammation and stromal response, to a tumor. As part of HTG’s EdgeSeq Reveal software, each of these signatures can be applied within the software for rapid gene expression visualization.

- **Verification utilized RNA-Seq and HTG EdgeSeq data** from over 1,000 formalin-fixed, paraffin-embedded (FFPE) tissue samples
- **xCell algorithm** (*Aran et al., 2017*) used to generate reference immune, stroma and TME scores
- **HTG EdgeSeq Reveal Immune scores** orthogonally compared to multiple commonly used inflammation IHC markers in an independent cohort of nearly 75 FFPE samples
- **Tissue-agnostic scores verified** with multiple different tumor types
- **Can be applied to existing and new data** using proprietary HTG EdgeSeq Reveal software

HTG EdgeSeq Immune, Stroma and TME Primary and Orthogonal Signature Verification Workflow Overview

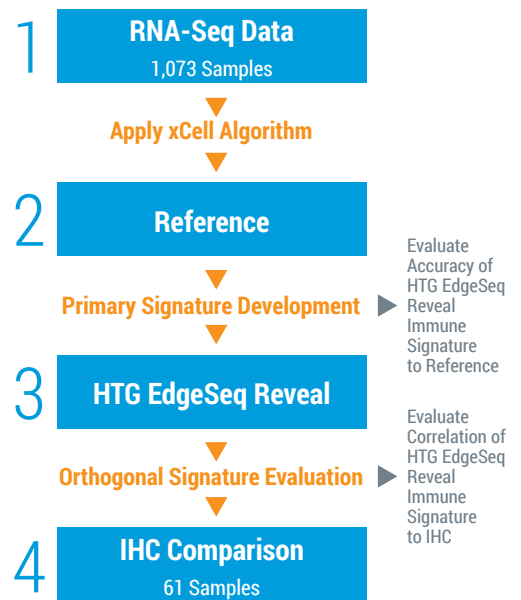


Figure 1: Over 1,000 FFPE samples run on both RNA-Seq (xCell) and HTG EdgeSeq platforms for primary signature development and nearly 75 FFPE samples of an independent cohort were compared between HTG EdgeSeq Reveal Immune Signature and IHC to support primary verification studies.

Signature Verification and Guardbanding

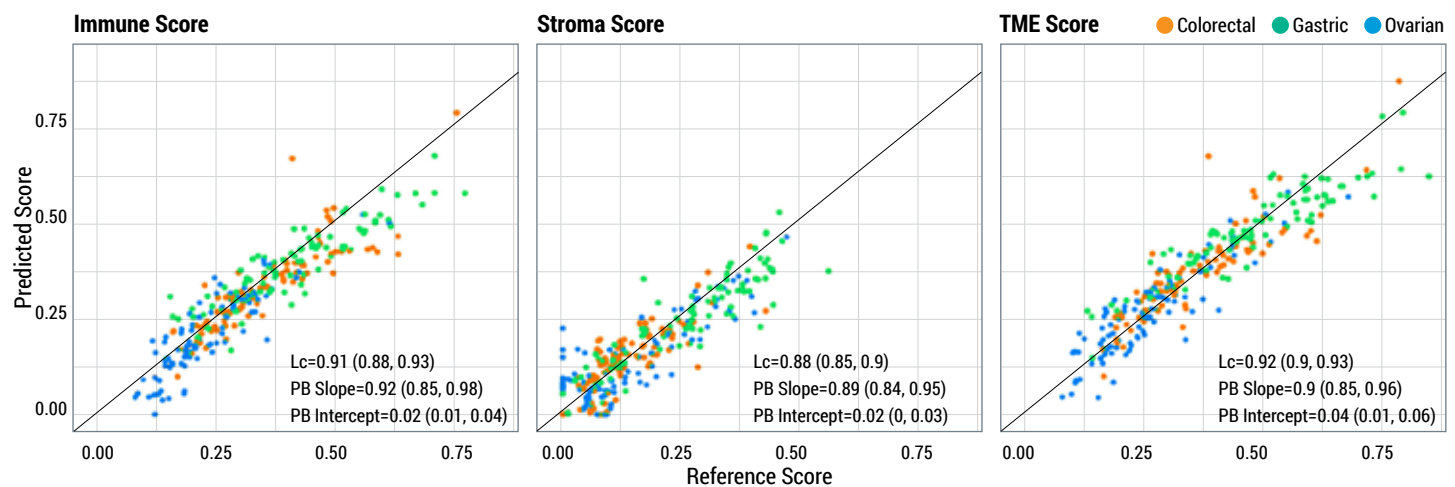
Well-known GEP immune signatures (CIBERSORT, xCell, MCP, EPIC, TIMER and quanTIseq) typically utilize a primary and orthogonal signature verification strategy to ensure correct data correlation analysis and biological relevance.

- **Primary verification** showed Lin's concordance correlation coefficients of 0.91, 0.88 and 0.92 when comparing the reference xCell score to the HTG EdgeSeq Reveal Immune, Stroma and TME scores.
- **Orthogonal verification** between IHC markers for CD3, CD4, CD68, CD8, and PD-1 antibody staining to HTG EdgeSeq Reveal Immune score showed good correlation.

Robust performance of GEP inflammation and stroma signatures to fluctuations in sample input and tumor macrodissection ensure sample score does not change based on amount of tissue or macrodissection method used.

- **Robust signatures** to sample inputs as small as a single FFPE section of a needle biopsy.
- **Robust signatures** irrespective of macrodissection method utilized.

Predicted Accuracy for Immune, Stroma and TME Scores



HTG EdgeSeq Reveal

HTG EdgeSeq Reveal software streamlines analysis of biomarker data, allows visualization of complex gene expression profiles, and facilitates application of informative gene signatures.

- Data quality control, and correlation analysis
- Normalization and PCA (Principal Component Analysis) tools
- Interrogate immune-related, inflammation and key cancer associated signaling pathways
- Generate and visualize gene expression patterns utilizing volcano plots and heat maps

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Figure 2: The y-axis shows predicted score from HTG EdgeSeq Reveal Signatures and the x-axis shows the xCell reference scores. The Lin's concordance correlation coefficients (Lc) and Passing-Bablok (PB) are shown for each signature.