Background
Therapeutic antibodies targeting immune checkpoint molecules have been approved by the FDA for the treatment of several types of cancer. Currently, evaluation of the tumor checkpoint blockade is limited to FDA-approved IHC assays measuring PD-L1 ligand status which is subjective and not analytically robust. As the number of antibodies targeting immune checkpoints expands, assays that can evaluate additional biomarkers in tumor specimens are needed to accurately predict patient response to these drugs. To address these issues, custom immune response NGS assay was developed to measure the transcript level of 54 genes involving T-cell receptor signaling (TCRS) and tumor infiltrating lymphocytes (TILs) in solid tumors varying in heterogeneity, disease, biopsy type and age. As part of the study, we evaluated the impact these specimen attributes and the complex tumor microenvironment have on the immune gene expression signature and their role as possible assay interferents.

Methods
Studies were designed to evaluate the analytical performance of a targeted RNA-seq assay for FFPE samples from NSCLC, melanoma, RCC, HNSCC, kidney and bladder cancer (Figure 1). To assess degree of assay tolerance to the wide range of specimen attributes that are inherent in tumors, samples and mixed samples of various histopathologic characteristics were included.

- 167 FFPE specimens: NSCLC, melanoma, RCC, HNSCC, kidney and bladder cancer
- RNA (10ng)
- OncomineTM Immune Response Research Assay (ThermoFisher)
- Ion Chef and S5XL (ThermoFisher)
- Torrent Suite and immuneResponseRNA plugin (ThermoFisher)

Results
Principal Component Analysis (PCA) performed on gene expression of a reference population of 167 samples revealed three distinct immune response signatures (Figure 2).

Immune Response Gene Signature

Further correlation and over-representation analysis was performed to determine impact of several specimen related quantitative and qualitative meta-variables on these three immune signatures (Figure 3, Tables 1-4):

- Disease (Bladder, HNSCC, Kidney, NSCLC, Melanoma)
- T-Path (GI, GU, Head & Neck, Hematopoietic, Musculoskeletal, Nervous System, Skin, Thorax)
- PMR (Primary, Metastatic, Recurrent)
- Specimen Year (2002 – 2013)
- Specimen type (excisional bx, incisional bx, resection, FNA, NCB, punch bx)
- Architecture (disaggregated, disrupted, intact, partially intact)
- TILs (infiltrating apparent, infiltrating not apparent, lymphoid structures, not apparent, strongly infiltrating)
- Stroma quality (fibrotic, mixed, NE)

Conclusion
Tumor samples harbor a mixture of potential immune assay interferents, including variable benign, neoplastic and immune cells populations with both naive and reactive stroma contributing to a complex tumor microenvironment that is difficult to catalogue prior to testing. Using a Immune Response assay, we evaluated the gene expression of 167 solid tumors and demonstrated that the immune signatures in the samples tested were maintained for the majority of characteristics studied within a specified range. As expected, strongly infiltrating TIL status was significantly associated with the high expression group and under represented in the low expression cluster. High expressing tumors favored metastatic lesions over primary, and demonstrated a lower prevalence of thorax and incisional biopsies. Other attributes including architecture, neoplastic content, T-Path, PMR, specimen type, tissue amount and specimen age were not over-represented in any immune signature.

Our study demonstrates that the host immune signature present in the tumor microenvironment is sufficiently strong to withstand a wide range of tumor heterogeneity, thereby reducing the need of extensive tissue macrodissection and the exclusion of samples previously thought to be non-evaluable.