To detect microsatellite instability (MSI), NGS is used to analyze 29 homopolymer loci within 28 amplicons, including BAT-25 and BAT-26, by sequencing tumor-only DNA on an Illumina MiSeq Sequencer. The output NGS data is analyzed at each locus by computational tools to determine MSI status of tumor samples comparing the number of peaks and average indel lengths to a normal reference population. **Scoring of MSI by NGS:** Results are scored as "Unstable (MSI-H)" or "Stable". Unstable colorectal carcinomas, endometrial carcinomas, and some other types of neoplasms may be indicative of hereditary Lynch syndrome, or may only identify microsatellite instability in the neoplasm which is not indicative of a hereditary condition. This assay detects the majority, but not all unstable neoplasms with a sensitivity and specificity of 96% and 100%, respectively. Although a stable result may be attributed to the absence of instability, the possibility of a very small neoplastic cell population that is unstable but below the limit of detection cannot be excluded. Assuming a diploid population of cells, the limit of detection is 10% unstable cells in a background of stable cells.

To measure CD8 IHC expression pattern of tumor infiltrating lymphocytes (TILs), the Dako Autostainer and standard IHC (M7103, Dako) techniques are used to obtain the characteristics and infiltration pattern of CD8+ T-cells. The reviewed IHC sections are required to contain at least 100 neoplastic cells to ensure the tumor is evaluable by IHC. **Interpretation of CD8 TILs by IHC:** A "strongly infiltrating" pattern refers to CD8 TILs staining that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and in >50% of the tumor examined. A "moderately infiltrating" pattern refers to CD8 TILs staining that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and in 10 - ≤50% of the tumor examined. A "minimally infiltrating" pattern refers to CD8 TILs staining that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and in 5 - ≤10% of the tumor examined. An "excluded" pattern represents restriction of >95% of all CD8 TILs in a tumor to the periphery or interstitial stromal areas and not actively invading nest or groups of neoplastic cells. A "non-infiltrating" pattern refers to CD8 TILs staining that infiltrate nests of neoplastic cells in a non-overlapping fashion and with <5% of the tumor showing an infiltrating pattern.

Amplicon-based targeted next generation sequencing (NGS) for digital gene expression detection **(RNA-Seq)** is used to interrogate 43 **T-cell receptor signaling (TCRS)** genes and **8 tumor infiltrating lymphocytes (TILs) genes including CD8,** that have functions across the cycle of immunity. **Interpretation of TCRS and TILs gene expression by RNA-Seq:** Each gene is compared to a reference population derived from 167 unique tumors, normalized to a value between 1 and 100, and scored as the percentile (relative) rank (% Rank). TCRS gene expression is interpreted as "Very High" for genes ranked 95-100, "High" for genes ranked 85-94, "Moderate" for genes ranked 50-84, and "Low" for genes ranked 20-49, and "Very Low" for genes ranked 0-19. TILs gene expression is interpreted as "High" for genes ranked 75-100, "Moderate" for genes ranked 25-74, and "Low" for genes ranked 0-24. CD8 TILs gene expression is also used to characterize tumors as hot or cold, and is interpreted as "Highly Inflamed" for genes ranked 75-100, "Moderately Inflamed" for genes ranked 25-74, and "Non-inflamed" for genes ranked 0-24.

OmniSeq Advance comprehensive genomic profiling uses NGS **DNA-Seq** to detect mutations (single nucleotide variants, insertions, deletions and indels), and copy number variants in 118 oncogenes and 26 tumor suppressor genes. DNA-Seq detects gain-of-function mutations in oncogenes using a hotspot coverage strategy, while copy number analysis uses complete exon analysis to detect high level amplification. DNA-Seq also detects loss of function mutations in tumor suppressor genes using a complete coding sequence coverage strategy, while copy number analysis detects homozygous deletions. NGS RNA-Seq is performed for oncogene fusion analysis. For single nucleotide variants (SNVs), the assay has a sensitivity and PPV of 97.0% and 97.9%, respectively. For insertions, deletions and indels, the assay has a sensitivity and positive predictive value (PPV) of 82.0% and 96.7%, respectively. SNVs, insertions, deletions and indels in samples with a minimum of 20% tumor nuclei are reliably detected with 95% sensitivity at a minimum VAF of 14.6% and an analytical sensitivity of 79.8% at a VAF of 5%. Copy number variants are reliably detected in samples with a minimum of 50% tumor nuclei, with an assay sensitivity and PPV of 93% and 90%, respectively. For fusions, the assay has no minimum neoplastic cell requirements due to RNA-based method of detection, with both sensitivity and PPV of 100%. Knowledge of partners is required for fusion detection. The assay reports coding DNA and predicted protein changes using Standard Human Genome Variation Society (HGVS) nomenclature (http://www.hgvs.org/varnomen) for detected variants. When analysis does not meet criteria for 95% confidence in a negative result call for a specific variant position, the result for that variant is reported as indeterminate. Detected variants that do not meet FDA variant classification guidelines for actionability, are non-synonymous, and are not reported if present in the 1,000 Genomes database at a prevalence of 1% or greater, or for tumor suppressor genes, are also deleterious in at least one protein modeling database (SIFT or PolyPhen) and reported as variants of unknown therapeutic significance.

OmniSeq Advance Therapy Considerations for checkpoint inhibition and targeted therapy are reported following FDA biomarker evidence classification guidelines as outlined in *Approach to Tumor Profiling Next Generation Sequencing Tests (FDA CDRH, 2017)* using the OmniSeq Knowledgebase. **OmniSeq Advance** also reports negative results for therapeutic associations with FDA Level 1 and Level 2 evidence for selection for checkpoint inhibition or targeted therapy. The OmniSeq Knowledgebase is proprietarily curated by OmniSeq for final clinical and genomic content. While OmniSeq reviews this information to help ensure accuracy, decisions about patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration the patient's condition, family history, physical examinations, information from other diagnostic and laboratory tests, and patient preferences, in accordance with standard of care practice. There is no guarantee that markers reported in this test will result in therapeutic efficacy or lack of therapeutic efficacy for any drug known to target markers in this test. It is possible that therapeutic implications associated with markers identified by this test are not suitable for a specific patient.

OmniSeq Advance was developed and its performance characteristics determined by OmniSeq, Inc., Buffalo, NY. The U.S. Food and Drug Administration (FDA) has not approved or cleared the RNA-Seq, DNA-Seq, MSI, or IHC TILs expression pattern of CD8 test components, however, FDA approval or clearance is not currently required for the clinical use of these tests. The FDA has approved the PD-L1 IHC components of the test for in vitro diagnostic use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. This test should not be regarded as investigational or for research use. OmniSeq, Inc. is authorized under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and by the New York State Clinical Laboratory Evaluation Program (NYSCLEP) to perform high-complexity testing.