

# Myriad Genomic Instability Status Assay Technical Specifications

Effective Date: May 2017

## Description of Analysis

Myriad Genomic Instability Status Assay is an *in vitro* diagnostic test utilizing Illumina HiSeq next generation sequencing to assess tumor genomic instability in primary or metastatic ovarian cancer and breast cancer. This analysis is performed on genomic DNA isolated from formalin fixed, paraffin embedded (FFPE) tumor tissue.

## Description of Method

Acceptable sample types are FFPE tumor tissue from blocks or slides of primary or metastatic ovarian cancer and breast cancer. The portion of the tumor should measure at least 5x5mm and contain 20% or greater tumor cellularity determined using the adjacent hematoxylin and eosin stained (H&E) section. In cases where blocks are not available, optimal Genomic Instability Status Assay testing requires 5-11 unstained slides sectioned in the following order: one 2-5  $\mu\text{m}$  tissue section mounted on a charged slide for H&E staining followed by 4-10 consecutive 10 $\mu\text{m}$  unstained sections mounted on uncharged slides for macrodissection of tumor. Patient DNA is extracted and purified from the tumor specimen, assigned a unique bar-code for robotic-assisted continuous sample tracking, and submitted for molecular testing.

## *Genomic Instability Status analysis*

The Genomic Instability Status assay is a custom hybridization capture panel that targets SNPs distributed across the genome. Whole genome next generation sequencing libraries are hybridized to this panel to enrich for sequence spanning these SNP locations. The enriched library is then sequenced using a HiSeq 2500 next generation sequencer (Illumina). The sequencing reads are used to generate allele specific copy number profiles, and these profiles are used for calculation of the Genomic Instability score. This score is calculated based upon an aggregate analysis of loss of heterozygosity, (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). A validated cutoff is applied to the Genomic Instability score that provides a positive or negative result for the patient. For more information please refer to Timms et al *Breast Cancer Research* 2014.

## Performance Characteristics

### *Analytical concordance:*

For analytical concordance of the Genomic Instability Assay, 206 individual anonymized breast and ovarian resection tumor DNA samples were tested in two laboratories. Concordance was demonstrated to be 98.06%.

### *Test reproducibility:*

Analytical validation studies included reproducibility study for NGS. A total of 18 unique formalin-fixed, paraffin-embedded (FFPE) breast and ovarian tumor specimens were tested in at least 12 replicates over 6 independent runs consisting of different combinations of instruments, reagent lots, operators and data reviewers. The design of this study enabled the evaluation of both inter-run reproducibility, which assessed the precision of analytical calls of sample replicates across assay batches, and intra-run repeatability which assessed the precision of analytical calls of sample replicates within assay batches. Genomic Instability scores were also assessed for inter- and intra-run reproducibility across these samples. The samples demonstrated a minimum standard deviation of differences in Genomic Instability scores between 0.54 (min) and 1.57 (max). In a separate study, 4 samples (two breast and two ovarian primary re-section samples) were analyzed by the Genomic Instability Status assay across three batches. Each batch contained at least three replicates per sample. The overall standard deviation of Genomic Instability Scores was 1.34.

## Interpretive Criteria:

### *“Genomic Instability Status: Positive”*

The test results demonstrate homologous recombination deficiency.

### *“Genomic Instability Status: Negative”*

The test results demonstrate homologous recombination proficiency.