Accurate Detection of Microsatellite Instability (MSI) in Clinical Samples Using the Pillar oncoReveal MSI Panel, a Single-Tube Multiplex PCR-based NGS Assay and Analysis Software Solution

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ABSTRACT

Introduction MSI is caused by defects in MMR genes and results in increased insertion and deletion mutations within simple repeat regions in the genome. Inheritance of MMR mutations is associated with Lynch syndrome and 15% of colorectal cancers exhibit MSI. Diagnosis of MSI can inform patient prognosis and clinical decision-making. Accurate detection tools are therefore required to facilitate timely diagnosis of MSI. We report the performance of an integrated NGS assay comprised of a single-tube multiplex PCR-based panel and Pillar's PiVAT alignment software with MSIsensor -based detection.

Methods Analyses were conducted on 56 pairs of tumor and matched normal FFPE samples. All had accompanying clinical MSI status information (blinded until after the Pillar data was generated) verified by another MSI detection method. For analysis of MS site variation in normal samples, 69 samples representing individuals from multiple ethnicities were examined. The samples were assayed with the oncoReveal MSI Panel. Sequencing was performed on Illumina sequencers using a PE2x150 sequencing protocol. Data was analyzed by PiVAT, and MSIsensor was run on either the primary BWA alignment output or PiVAT filtered, locally re-aligned, and paired-end assembled BAM files with coverage normalization and FDR threshold set to 1.

Results MSI was assessed using MSIsensor, which compares the distribution of MS site lengths in tumors versus matched normal samples. Preliminary analyses revealed a greater separation of MSI scores between MSI-positive and -negative tumors when MSIsensor was run on PiVAT BAM files versus primary BWA BAM files. ROC analysis indicated that at a threshold of 45% of altered MS sites, sensitivity and specificity were optimized at 97% and 100%, respectively. MS sites exhibiting low variability between normal samples of different ethnicities might represent a more stable class of targets and be more predictive of MSI if altered in tumors. We therefore performed hierarchical clustering on MS site difference scores between 69 samples of different ethnicities and the GIAB TV2 standard. MS sites clustered into 3 distinct groups of high, medium, and low variability. Importantly, the subset of low variability sites exhibited high difference scores between MSI-positive and -negative tumors. When limited to this group, MSIsensor results were concordant with analyses run on the full set, indicating the utility of this subset.

Conclusions We developed a robust assay for MSI detection in multiple tumor types that encompasses library generation, data analysis, and diagnosis with high sensitivity and specificity. We also identified a subset of MS sites within the panel that were highly predictive of MSI status and exhibited low variability between normal tissue samples.

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ASSAY SENSITIVITY AND SPECIFICITY

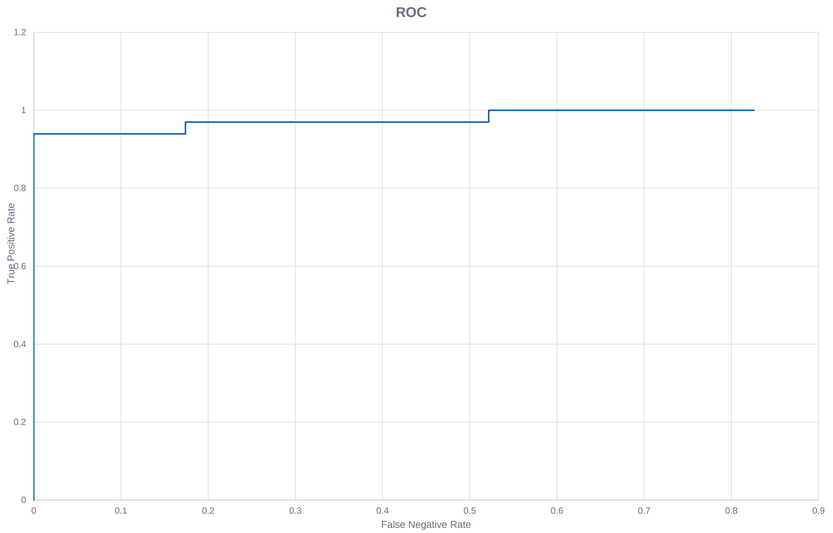

Figure 1 – Accurate detection of MSI with the oncoReveal panel in 56 clinical samples representing matched tumor/normal pairs. Samples' clinical status was determined at the collection site using a gold standard MSI detection kit.

MICROSATELLITE VARIABILITY IN NON-TUMOR SAMPLES

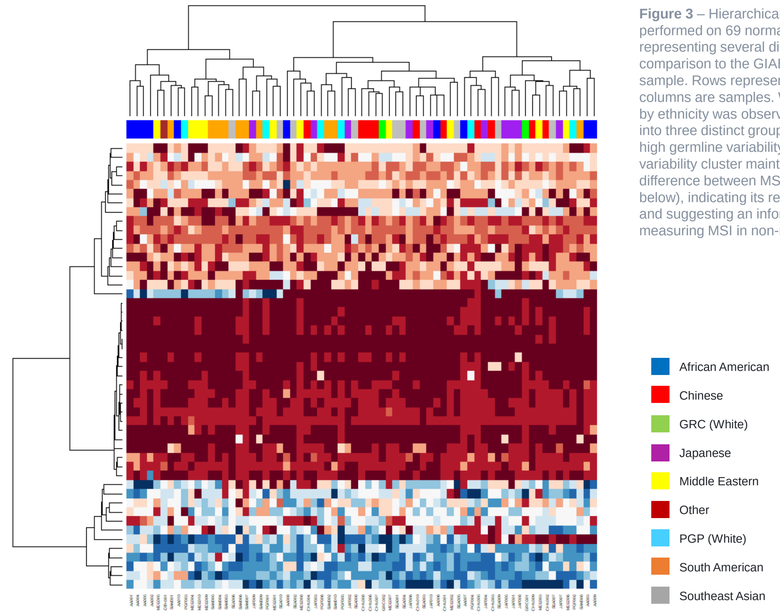

Figure 3 – Hierarchical clustering was performed on 69 normal tissue samples representing several different ethnicities, in comparison to the GIAB TV2 standard reference sample. Rows represent individual MS sites and columns are samples. While no clear clustering by ethnicity was observed, MS sites clustered into three distinct groups of low, medium, and high germline variability. The low-germline-variability cluster maintained a high degree of difference between MSS and MSI tumors (see below), indicating its relevance to diagnostics and suggesting an informative capability in measuring MSI in non-matched contexts.

MICROSATELLITE SITE TARGETS

Target_Name	Bethesda Panel	Promega Panel	17 high informative
D17S250(gt)20-(N)x-(ta)7	+		
D2S123(ac)21p5	+		
D5S346(TG)20p5	+		
BAT25(T)25	+	+	+
BAT26(A)27	+	+	+
MONO27(T)28		+	+
NR21(A)21		+	+
NR24(T)23		+	+
38 Additional Targets			12 selected

Table 1 – The oncoReveal MSI panel consists of 46 PCR amplicons targeting 53 MS sites in the human genome, including the sites comprising 1st and 2nd generation gold standard MSI testing kits and sites included in several high-impact studies from a literature review. A 17-target subset with high informative value was determined by examining MS site variability between normal tissue samples of various ethnicities (see below).

PiVAT DATA PROCESSING

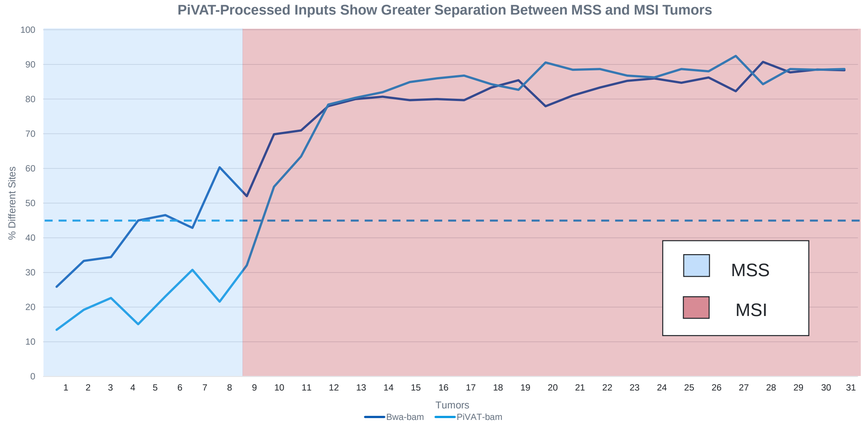

Figure 2 – PiVAT data processing improves MSI detection by applying read-quality filtration, paired-end assembly, and local realignment, resulting in greater difference scores between MSS and MSI tumors in a subset of the clinical dataset. Standard BWA alignment files are used as a comparison. The 45% threshold used to differentiate MSS and MSI is shown with a dotted line.

INFORMATIVE TARGET SELECTION

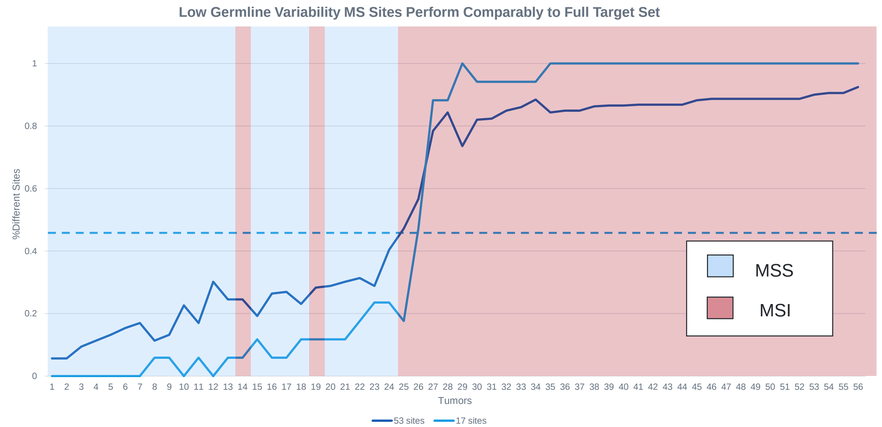

Figure 4 – MSI detection in 56 clinical tumor samples. Two false negatives result in a sensitivity of 97%, while maintaining 100% specificity. Limiting the targets to the 17 low-germline-variability set (above) produced comparable results with apparently greater separation between scores in MSS vs MSI tumor samples, albeit with one additional false negative call for a sample near the threshold.