

## Microsatellite Instability Analysis with GeneMarker®

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### Introduction

Microsatellites are stretches of DNA where a 1-5 base pair sequence is repeated several times. The most common microsatellite in the humans is a dinucleotide repeat of CA which occurs tens of thousands of times across the genome. Microsatellite instability (MSI) is a condition where repeat units are gained or lost within a locus resulting in length polymorphism. Certain repeat regions are known to be highly polymorphic and heritable. In the forensics community, these sites are useful when identifying markers for DNA fingerprinting. Conversely, microsatellite instability within and around certain genes can have devastating effects due to the possibility of frameshift mutations.

MSI is a key factor in several cancers including colorectal, endometrial, ovarian and gastric cancers (1, 2, 3). Colorectal cancer studies have demonstrated two mechanisms for MSI occurrence. The first is in hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch Syndrome, where an inherited mutation in a mismatch-repair gene causes a microsatellite repeat replication error to go unfixed. The replication error results in a frameshift mutation that inactivates or alters major tumor suppressor genes - key genes in the regulation of the cell cycle and, ultimately, the prevention of cancer. The second mechanism whereby MSI causes colorectal cancer is an epigenetic change which silences an essential mismatch-repair gene. In both cases, microsatellite insertions and deletions within tumor suppressor gene coding regions result in uncontrolled cell division and tumor growth (4).

Five markers have been recommended by the National Cancer Institute to screen for MSI in HNPCC tumors (often called Bethesda markers) (5). Generally, MSI detection in two of the markers is considered a positive result or high probability of MSI (MSI-H). Recently, Promega® Corporation released a microsatellite instability assay called MSI Analysis System\*. The kit includes five mononucleotide markers which were chosen for their sensitivity and specificity (6). Included in GeneMarker is the MSI Analysis System panel for quick, accurate MSI analysis.

### Procedure

#### GeneMarker MSI Analysis Procedure

1. Import data files (fsa, abi, ab1, scf)
2. Select the **Run** icon to launch the Run Wizard
3. Recommended Settings:

#### Template Selection

- a. Panel – *Promega MSI*
- b. Size Standard – User-defined
- c. Standard Color – User-defined
- d. Analysis Type – *Fragment (Animal)*

#### Data Process

- e. Select *Load Default*

#### Additional Settings

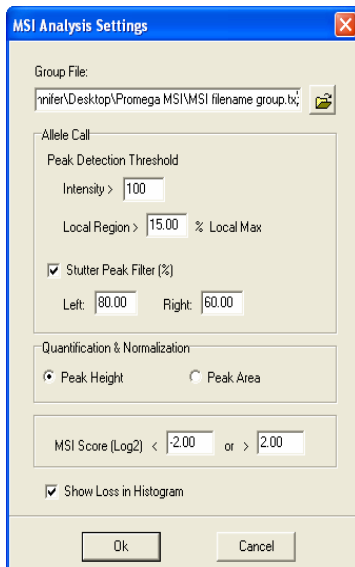
- f. Peak Score – *Reject < 1 Check 7 < Pass*

4. After the data is processed, select **Applications > MSI Analysis**

5. In the **MSI Analysis Settings** box, upload a tab-delimited text file that identifies Reference samples (first column) and Tumor samples (second column) to the *Group File* field

- a. Use the **File Name Group Tool** in the **Tools** menu to create a group file using GeneMarker.

6. Adjust settings as desired and click **OK**
7. The main MSI Analysis window appears
8. Click the **Print** icon to print *MSI Analysis Report*

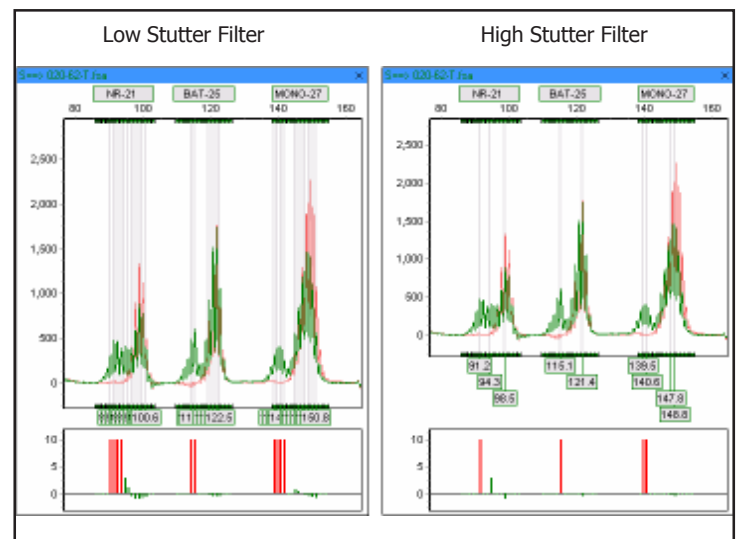


### Results

In GeneMarker's MSI Analysis module, tumor samples are compared to normal samples based on peak-to-peak comparison. Differences between the two traces are displayed in a Trace Comparison Histogram below each electropherogram. Within the electropherogram, the tumor sample trace is overlain on a light red reference trace. In this way, the clinician can easily visualize where the areas of instability exist.

There are a few options for MSI display which will vary by personal preference. In the MSI Analysis Settings box there are several options for filtering the allele call. The peak detection thresholds, Intensity and Local Region are designed to filter out noise near the baseline. The Stutter Filter right and left thresholds are applied after the peak-to-peak comparison with the reference trace and are designed so that the user can minimize the number of bars in the Peak Comparison Histogram.

**Figure 1** shows a low stutter filter setting (Left: 60, Right: 40) compared to the same trace with a higher stutter filter setting applied (Left: 99, Right: 99).



**Figure 1.** A low stutter filter shows a high number of identified "instability" peaks. As the stutter filter setting is increased, fewer "instability" peaks are identified.

A second tool to assist the analyst in displaying only the most relevant peaks in a microsatellite instable marker is through the MSI Score setting. MSI Score is calculated using a log<sub>2</sub> ratio plot of tumor sample versus reference. In this way, the normalized intensities of the reference sample can be accurately compared to the tumor sample thereby presenting a more meaningful analysis. In **Figure 2**, a low MSI Score setting (-2, +2) is compared to a high MSI Score setting (-2, +4) for the same sample.



**Figure 2.** When the MSI Score threshold is lowered, more peaks are identified as instable (red histogram bars). As the MSI Score threshold is increased, only the most relevant peaks are identified as instable.

## Discussion

With GeneMarker's new MSI Analysis feature tumor samples can be quickly and accurately screened for microsatellite instabilities. Stutter filtering of mononucleotide repeat markers and a unique MSI Scoring algorithm take the commonly used "three-second rule" down to less than a second! GeneMarker automates the analysis process and creates an easy-to-read report. The MSI Analysis Report includes electropherograms and histograms for each dye color and a MSI Positive/Negative determination table for each marker. With these features, analysts can quickly and accurately assess MSI-High or Low for each patient sample.

## Acknowledgements

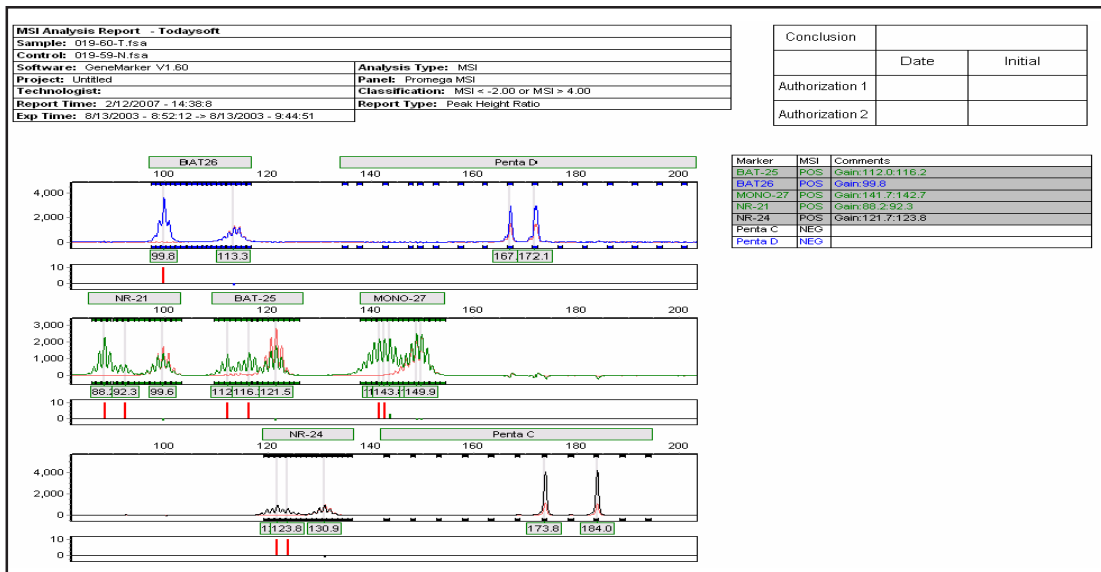
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**Figure 3.** GeneMarker's MSI Analysis Report

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