TECHNICAL MANUAL

OncoMate™ MSI Dx Analysis System

Instructions for Use of Product
MD3140

CE

INSTRUCTIONS FOR
USE OF PRODUCT
MD3140

IVD

Rev 0
TM595

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OncoMate™ MSI Dx Analysis System

1. Introduction

1.1 Overview of the OncoMate™ MSI Dx Analysis System

This guide describes the OncoMate™ MSI Dx Analysis System and is the primary source for information about its intended use, components, limitations, protocol, troubleshooting and more. The OncoMate™ MSI Dx Analysis System is a CE-marked IVD medical device that uses a fluorescent PCR-based assay as part of a broader laboratory workflow (Figure 1) to analyze formalin-fixed, paraffin-embedded (FFPE) tissue samples for microsatellite instability (MSI). The OncoMate™ 5C Matrix Standard (Cat.# MD3850) is required for the analysis of OncoMate™ MSI Dx Analysis System amplified products by capillary electrophoresis. Instructions for the use of the OncoMate™ 5C Matrix Standard are provided in the *OncoMate™ 5C Matrix Standard Technical Manual* #TM597.

The complete workflow surrounding use of the OncoMate™ MSI Dx Analysis System involves DNA extraction from FFPE tissue sample, quantification of DNA, amplification of specific microsatellite markers via multiplex PCR, analysis of amplified DNA fragments by capillary electrophoresis and analysis of capillary electrophoresis fragment data using a fragment analysis software. Users of the OncoMate™ MSI Dx Analysis System must select suitable workflow components based on the requirements provided in Section 3. Consult the technical literature for the selected DNA extraction method, DNA quantification method, thermal cycler, capillary electrophoresis instrument and fragment analysis software for additional information.

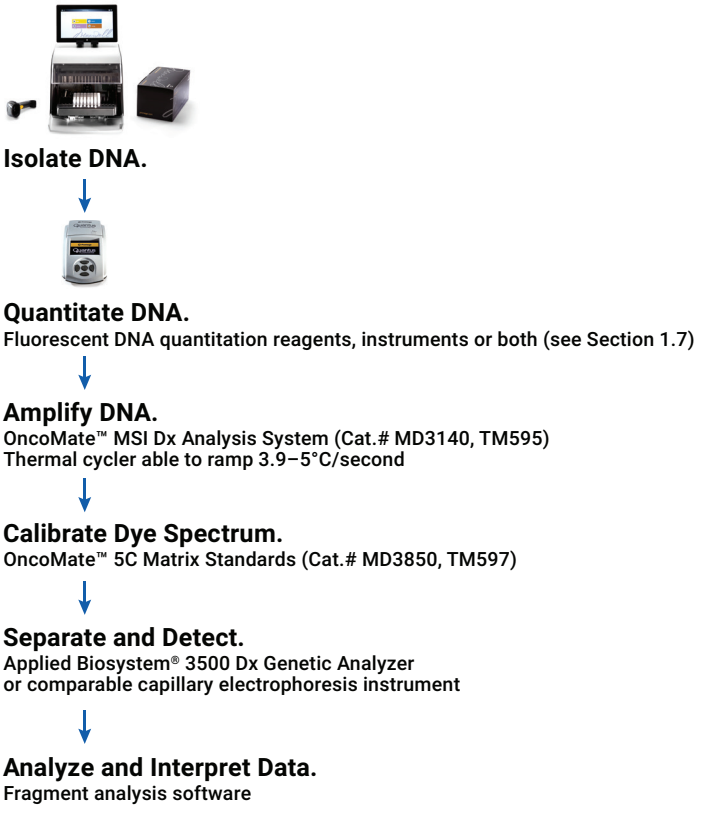


Figure 1. Laboratory workflow associated with the OncoMate™ MSI Dx Analysis System.

1.2 Product Name

OncoMate™ MSI Dx Analysis System
Cat.# MD3140, 100 reactions Σ 100

1.3 Abbreviations and Definitions

bp, base pair

CRC, colorectal cancer

DCS, data collection software

dMMR, deficient-mismatch-repair

dsDNA, double-stranded DNA

ESMO, European Society for Medical Oncology

FFPE, formalin-fixed, paraffin-embedded

HNPCC, hereditary nonpolyposis colorectal cancer

ICI, immune checkpoint inhibitor

LOH, loss of heterozygosity

MMR, mismatch repair

MSI, microsatellite instability

MSI-H, microsatellite instability high: sample interpretive result when two or more markers are unstable

MSS, microsatellite stable: sample interpretive result when fewer than two markers are unstable

PCR, polymerase chain reaction

RFU, relative fluorescence units

QC, quality control

NA, not applicable

Allele: one of two or more forms (e.g., length) of a DNA marker

Bleedthrough: capillary electrophoresis artifact where peaks from one dye channel are detected in a second dye channel at approximately the same size (bp); also known as “spectral pull-up”

DNA marker: a sequence of interest within the genome

Heterozygous: the alleles for a DNA marker have different forms

Homozygous: both alleles for a DNA marker have the same form

Mononucleotide repeat: DNA structure where the same nucleotide base is repeated

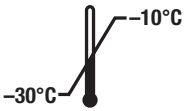
Quasi-monomorphic: a DNA marker for which most individuals are homozygous for the same common allele

Stable: allele size change indicative of mismatch repair deficiency is absent

Stutter peak: a PCR artifact resulting from slippage of the polymerase during in vitro DNA replication. Stutter peaks are one or more repeat units shorter or longer than the true allele peak

Unstable: allele size change indicative of mismatch repair deficiency is present

1.4 Key to Symbols Used

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at –30°C to –10°C		Manufacturer
	Conformité Européenne		Irritant
	Catalog number		Lot number
	Do not reuse		Contains sufficient for <n> tests
	Use by		Protect from light
	Consult instructions for use		

1.5 Intended Use

The OncoMate™ MSI Dx Analysis System is a PCR-based fragment-sizing test used to determine microsatellite instability (MSI) status in DNA purified from human formalin-fixed paraffin-embedded (FFPE) tissue samples derived from solid tumors.

The OncoMate™ MSI Dx Analysis System generates allelic profiles from tumor and non-tumor FFPE tissue samples from the same patient through polymerase chain reaction (PCR) amplification of DNA microsatellite markers, followed by size separation of the amplified markers using capillary electrophoresis. MSI status is determined by comparing the allelic profiles. An expansion or reduction in the length of repetitive DNA sequences in the tumor cell DNA when compared to the normal cell DNA from the same patient indicates MSI. Normal and tumor tissue from the same patient must be tested at the same time and data from both samples must be available for comparison for results to be valid.

The OncoMate™ MSI Dx Analysis System is not intended to diagnose a specific disease. It is intended for use with patients already diagnosed with cancer who may benefit from additional genetic testing. Test results obtained using the product must be interpreted by healthcare professionals in conjunction with other clinical findings, family history and laboratory data. This product is intended for professional use only.

1.6 Background Information

Microsatellites are short, repetitive DNA sequences consisting of repeat units of one to six nucleotides [e.g., $(A)_n$, $(CA)_n$, $(AAAAG)_n$]. Microsatellite sequences are distributed throughout the human genome and are prone to insertion and deletion errors during DNA replication. Normally, errors are repaired by the cellular DNA mismatch-repair (MMR) system. MSI is observed when MMR function is deficient and DNA replication errors are not repaired, resulting in different lengths of microsatellite alleles in MMR-deficient tissue versus normal tissue samples (Bacher, 2004; Boland, 2010; Ionov, 1993; Umar, 2004; Wang, 2017). Among microsatellites, mononucleotide repeats are the most likely to show instability (Bacher, 2004; Ionov, 1993). Microsatellite instability may result from several underlying mechanisms, including somatic and germline mutation of MMR genes and alterations to MMR gene promoter regions. In individuals with MMR defects, the accumulation of mutations may lead to cellular dysfunction and, eventually, cancer (Boland, 2010; Le, 2015; Timmermann, 2010).

Microsatellite instability is highly correlated with Lynch Syndrome. Lynch Syndrome is an autosomal dominant disorder resulting from germline mutations in any of the four major MMR genes (MLH1, MSH2, MSH6, PMS2) and is a predisposition to cancer. Identification of Lynch Syndrome individuals enables early cancer detection and intervention at a point in the disease where survival can be more easily influenced. At the genome level, a failure of an MMR gene leads to a loss of expression or function of the corresponding MMR protein. Microsatellite instability results from MMR deficiency and therefore informs, along with clinical and family history, an underlying genetic susceptibility. Common Lynch Syndrome-associated cancers include colorectal and endometrial cancers; however, Lynch Syndrome has been identified in virtually all solid tumor types (Cortes-Ciriano, 2018; Hause, 2016; Latham, 2018). Multiple clinical oncology associations endorse universal screening of colorectal and endometrial cancers for MSI to identify candidates for further genetic testing for Lynch Syndrome, including the European Society for Medical Oncology (ESMO; Stjepanovic, 2019), the National Comprehensive Cancer Network (CRC, EC), and the American Society of Clinical Oncology (Sepulveda, 2017; Stoffel, 2015).

More recently, microsatellite instability has been associated with response to immune checkpoint inhibitor (ICI) therapeutics (Le, 2015; Overman, 2017). ICI therapies target immune suppression receptors or their ligands and, with treatment, an individual's immune cells are more effective at combating the cancer. Due to defective DNA MMR function, MSI-high (MSI-H) tumors are hypermutated and produce more neoantigens or novel epitopes, some of which are thought to be immunogenic and trigger an immune response against the cancer (Le, 2017). As a result, MSI-H tumors are often antigenic and show more lymphocyte infiltration, a scenario in which the patient is likely to respond to an ICI. Identification of patients who could benefit from these treatments provides additional options and information for health care professionals. MSI has been observed in many tumor types (Cortes-Ciriano, 2018; Hause, 2016; Latham, 2018), and response to ICI has been demonstrated across MSI-H tumors from multiple sites of origin (Le, 2015). The European Society for Medical Oncology (ESMO) has recognized the importance of MSI and mismatch repair deficiency testing to support patient eligibility for ICI therapies and has summarized their recommendations surrounding MSI testing for this purpose (Luchini, 2019, Stjepanovic, 2019). The ESMO Translational Research and Precision Medicine Working Group recommends MSI testing on the spectrum of Lynch Syndrome tumors because of the potential usefulness of ICI in these cancers and the identification of Lynch Syndrome can benefit extended family members. MSI by PCR and dMMR analysis by IHC are recommended to be used together because of past reports of high false positive rates with local laboratory developed tests. Finally, the committee recommends panels of five mononucleotide repeat markers because of their higher sensitivity and specificity over panels including dinucleotide repeat markers (Luchini, 2019).

1.7 Test Principle

The OncoMate™ MSI Dx Analysis System is a fluorescent, multiplex PCR-based test to detect DNA sequence length changes in microsatellite regions of tumor cell DNA relative to the same regions from the patient's normal cells. The OncoMate™ MSI Dx Analysis System targets seven microsatellite markers: five mononucleotide-repeat markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) and two pentanucleotide-repeat markers (Penta C and Penta D; Table 1, Figure 2). These mononucleotide-repeat markers are analyzed to determine MSI status and were selected for high sensitivity and specificity to alterations in repeat lengths in samples containing mismatch repair defects. For each of the five mononucleotide-repeat markers, allele size(s) (in base pair, bp) in the tumor sample are compared to those in the normal sample to determine if a new allele representing MSI is present (Figure 3). A marker is interpreted as unstable when a new allele is detected that has a size difference of at least 2.75bp (3 bases minus 0.25 base pair to accommodate sizing precision of capillary electrophoresis) compared to the reference allele in the normal sample. A tumor sample is considered MSI-H when two or more markers are interpreted as unstable. A tumor sample is considered MSS when fewer than two markers are interpreted as unstable (Hampel, 2008; Boland, 1998). While these markers are quasi-monomorphic (i.e., most individuals are homozygous for the same common alleles) and normal tissue will typically display a single distribution of peaks at each mononucleotide marker, low frequency heterozygosity or variation in normal-tissue allele length between individuals has been observed. Because of the possibility for heterozygosity, data from paired normal and CRC tumor samples must be interpreted together to determine tumor MSI status. Two pentanucleotide-repeat markers are analyzed as an identity confirmation between the normal and tumor DNA samples and were selected for their high level of polymorphism and lower degree of MSI (Bacher, 2004). When all alleles detected in the normal sample are also present in the tumor sample, the sample identity is supported.

Table 1. Expected Amplified Size Ranges and Detection Channels for the Markers Included in the OncoMate™ MSI Dx Analysis System Amplification Kit. Ranges correspond to expected sizes for data generated using POP-7® polymer and a 50cm capillary array.

Mononucleotide Markers	Repeat Structure	Detection Channel	Amplified Size Range
BAT-26	$A_{(26)}$	Blue	83 to 121bp
NR-21	$A_{(21)}$	Green	83 to 108bp
BAT-25	$A_{(25)}$	Green	110 to 132bp
MONO-27	$A_{(27)}$	Green	134 to 168bp
NR-24	$A_{(24)}$	Yellow (displayed black)	103 to 138bp
Pentanucleotide Markers	**Repeat Structure**	**Detection Channel**	**Amplified Size Range**
Penta D	$AAAGA_{(2–17)}$	Blue	123 to 253bp
Penta C	$AAAAC_{(4–17)}$	Yellow (displayed black)	140 to 228bp

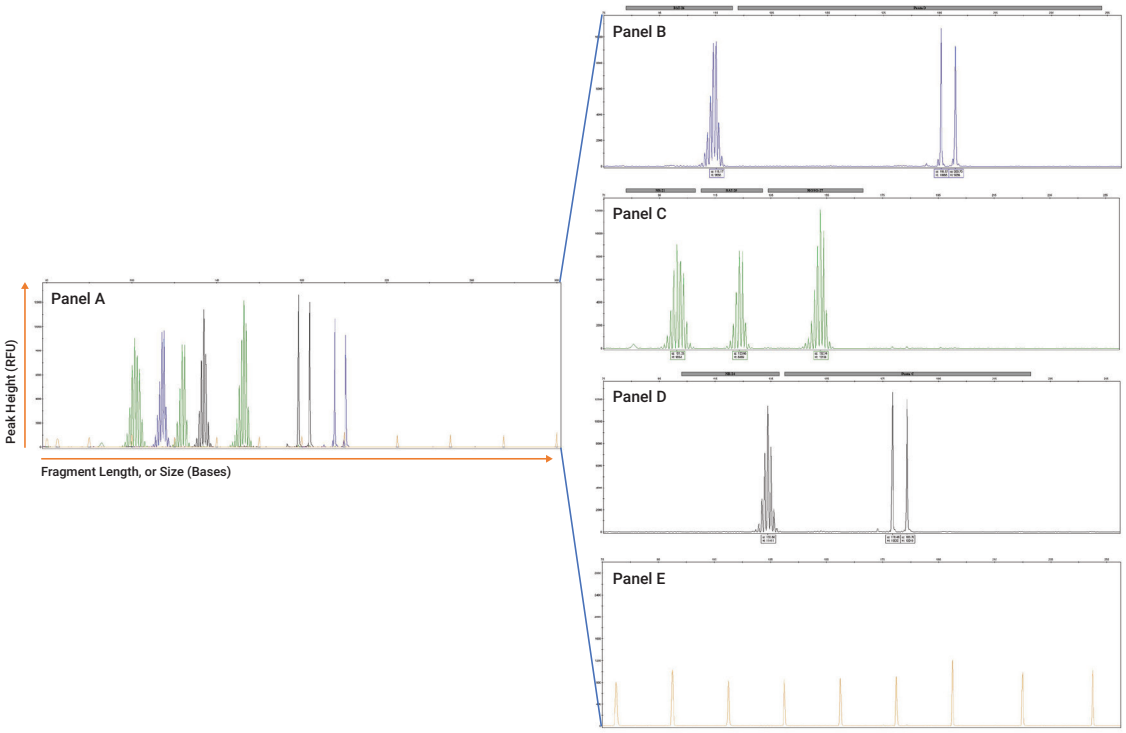


Figure 2. OncoMate™ MSI Dx Analysis System data example. A single genomic DNA template (1ng) was amplified using the OncoMate™ MSI Dx Analysis System, and the PCR products were analyzed using the Applied Biosystems® 3500 Dx Genetic Analyzer with POP-7® polymer and 50cm capillary array. **Panel A.** An electropherogram showing the simultaneous detection of all fluorescently labeled DNA fragments. **Panels B–E.** Microsatellite data displayed by detection channel, allowing easier interpretation. Panel E illustrates Size Standard 500 fragment peaks.

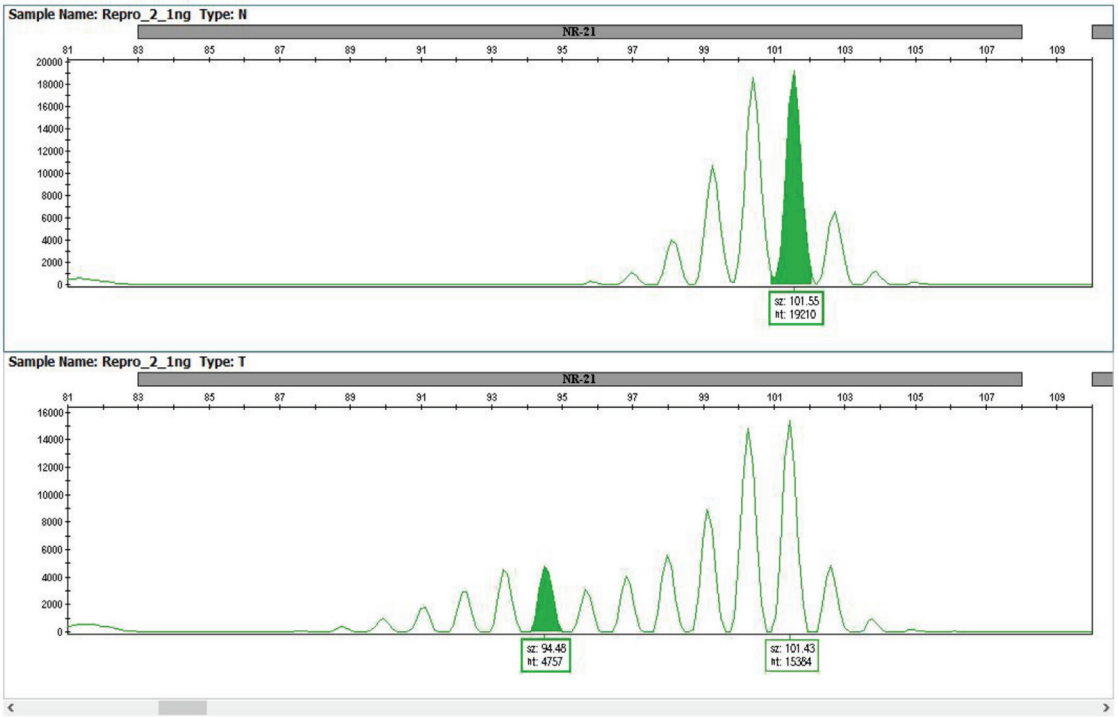


Figure 3. Stability assessment of mononuclotide markers. In the example above, the microsatellite alleles of interest are highlighted. For a mononucleotide marker, if the size difference between the new allele in the tumor sample and the reference allele in the normal sample is at least 2.75bp, the marker is interpreted as unstable. In this example, the reference allele in the normal sample (top electropherogram) is 101.55bp, while the new allele in the tumor sample (bottom electropherogram) is 94.48bp. The size difference between these two alleles is 7.07bp; this difference is ≥2.75bp, therefore the NR-21 marker is interpreted as unstable.

Using multiplex PCR with fluorescent-dye-labeled primers, all seven microsatellite regions are amplified within the same reaction to generate dye-labeled PCR products. PCR products and a fluorescently labeled size standard, Size Standard 500, are then added to formamide and heat-denatured. The resulting single-stranded DNA is electrokinetically injected into a capillary electrophoresis instrument, where the DNA fragments are separated based on size and detected through the incorporated fluorescent label. Analysis of the size standard alongside the microsatellite DNA fragments permits accurate sizing and direct size comparisons between samples. Following capillary electrophoresis, the resulting DNA fragment data (.fsa) files generated by the instrument can be analyzed using a fragment analysis software to determine fragment size and identify alleles.

2. Product Components and Storage Conditions

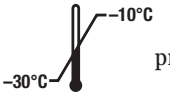
2.1 Materials Provided with the OncoMate™ MSI Dx Analysis System (Cat.# MD3140)

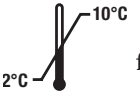
Σ 100 This product contains sufficient reagents to perform 100 reactions (50 paired reactions).

The following materials are included:

COMPONENT	SIZE	PART#
OncoMate™ MSI 5X Primer Mix	**200µl**	**MD705A**

Includes: Fluorophore-labeled and unlabeled primers for BAT-26, Penta D, NR-21, BAT-25, MONO-27, NR-24 and Penta C in a buffered solution.

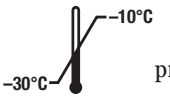
Storage Conditions: Pre-amplification area; 

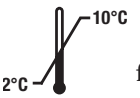
prior to use; 

following first use. Protect from light.

COMPONENT	SIZE	PART#
OncoMate™ MSI 5X Master Mix	**200µl**	**MD280A**

Includes: GoTaq® MDx Hot Start DNA Polymerase, dNTPs, magnesium chloride and salts in a buffered solution with stabilizers.

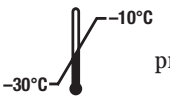
Storage Conditions: Pre-amplification area; 

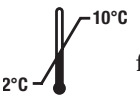
prior to use; 

following first use.

COMPONENT	SIZE	PART#
2800M Control DNA, 10ng/µl	**25µl**	**MD810A**

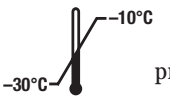
Includes: Cell-line derived male genomic DNA standard in a buffered solution.

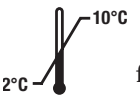
Storage Conditions: Pre-amplification area; 

prior to use; 

following first use.

COMPONENT	SIZE	PART#
Water, Amplification Grade	**1.25ml**	**MD193A**

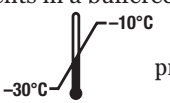
Storage Conditions: Pre-amplification area; 

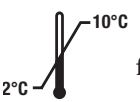
prior to use; 

following first use.

COMPONENT	SIZE	PART#
Size Standard 500	**100µl**	**MD500A**

Includes: Fluorophore-labeled DNA fragments in a buffered solution.

Storage Conditions: Post-amplification area; 

prior to use; 

following first use. Protect from light.

2.2 Storage and Handling of the OncoMate™ MSI Dx Analysis System

Upon receipt, store all components at –30°C to –10°C in a nonfrost-free freezer. Before first use, store the 2800M Control DNA at 2°C to 10°C for at least 8 hours. After the first use, store the OncoMate™ MSI Dx Analysis System at 2°C to 10°C for up to 3 months. The OncoMate™ MSI 5X Primer Mix and Size Standard 500 are light-sensitive and must be stored in the dark. Do not refreeze. Store pre-amplification and post-amplification reagents in separate rooms and use with dedicated pipettes, tube racks, etc. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect results.

2.3 Materials Not Provided

Laboratory Reagents

- DNA extraction system (See Sections 3.3 and 3.4)
- OncoMate™ 5C Matrix Standard (Promega Cat.# MD3850)
- Hi-Di™ Formamide (e.g., Applied Biosystems Cat.# 4404307)
- Fluorescent-dye-based dsDNA quantification reagents (see Section 3.4)
- Nuclease-Free Water (Promega Cat.# MC1191)

Note: It is critical to use high-quality Hi-Di™ Formamide. Freeze Hi-Di™ Formamide in aliquots at –30°C to –10°C. Multiple freeze-thaw cycles or long-term storage at 2–10°C may cause formamide breakdown. Poor-quality formamide may contain ions that compete with DNA during injection, resulting in lower peak heights and reduced sensitivity.

Laboratory Supplies

Note: The following laboratory equipment is required in two distinct areas of the laboratory: one for pre-amplification procedures and one for post-amplification procedures.

- Set of calibrated precision pipettes capable of delivering 1µl to 1,000µl
- Aerosol-resistant pipette tips (10µl to 1,000µl)
- 1.5ml microcentrifuge tubes
- MicroAmp® Optical 96-Well Reaction Plate with Barcode (Thermo Fisher Scientific Cat.# 4306737)
- MicroAmp® 8-Cap Strip, clear (Thermo Fisher Scientific Cat.# N8010535) (pre-amplification only)
- Personal microcentrifuge (“mini centrifuge”)
- Centrifuge compatible with 96-well plates (e.g., mini plate centrifuge)
- Microcentrifuge tube racks
- Vortex mixer
- Nonfrost-free freezer at –30°C to –10°C
- Refrigerator at 2°C to 10°C
- Crushed ice (post-amplification only)
- DNA extraction equipment (e.g., heat blocks, centrifuges), based on selected method (pre-amplification only)

Instruments and Accessories

- DNA extraction instrument, if method is automated
- DNA quantification instrument and associated supplies
- Capillary electrophoresis instrument and associated supplies
- Thermal cycler compatible with 96-well plates

Software

- Fragment analysis software (i.e., software capable of analyzing .fsa files from a capillary electrophoresis instrument)

3. Before You Begin

3.1 Warnings and Precautions

Chemical Safety Warning: Some reagents used with fragment analysis are potentially hazardous. Handle and dispose of hazardous materials according to the guidelines established by your institution. Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide. Consult the safety data sheet for formamide, provided by the manufacturer, prior to use.

Safety Data Sheet Statement: Important information regarding the safe handling, transport and disposal of this product is contained in the Safety Data Sheet (SDS). SDSs for all reagents provided in the kits are available online at: **www.promega.com/resources/msds/** or upon request from Promega Technical Services at: **genetic@promega.com**

Biosafety Precautions: The OncoMate™ MSI Dx Analysis System is intended for use with formalin-fixed paraffin-embedded tissues. Follow the guidelines established by your institution for the handling and disposal of these tissues.

PCR Precautions: The quality of purified DNA, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. Therefore, the OncoMate™ MSI Dx Analysis System requires strict adherence to the recommended procedures for amplification and fluorescence detection described in this manual.

Minimizing Contamination: PCR-based microsatellite analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling kit components, assembling amplification reactions and analyzing amplification products. Store and use reagents and materials used prior to amplification (OncoMate™ MSI 5X Master Mix, OncoMate™ MSI 5X Primer Mix, 2800M Control DNA and Water, Amplification Grade) in a separate room from those used following amplification (Size Standard 500). Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup. Always include a negative control reaction (i.e., no template) to detect reagent contamination and a positive control reaction to verify reagent performance. Wear gloves and use aerosol-resistant pipette tips to prevent cross-contamination.

3.2 Specimen Requirements

Tissue sections suitable for use with the assay are prepared using 10% neutral-buffered formalin following standard pathology practices and contain sufficient nucleated cells and a tumor content of ≥20% tumor cells, based on a pathology review. Performance evaluation of the OncoMate™ MSI Dx Analysis System was conducted using DNA extracted with the Maxwell® CSC Instrument and Maxwell® CSC DNA FFPE Kit from colorectal cancer FFPE tissue samples meeting these criteria.

3.3 DNA Extraction Methods and DNA Quality Requirements

Obtaining sufficient high-quality DNA from formalin-fixed, paraffin-embedded (FFPE) tissues can be problematic. DNA may be degraded due to prolonged or unsuitable fixation of the tissue sample before embedding in paraffin, and amplification inhibitors from the FFPE sample or the extraction method may carry through the extraction process. It is the user's responsibility to verify the compatibility of the selected DNA purification method with this assay. PCR assay controls may be used to identify the presence of amplification inhibitors in purified DNA extracts.

Performance of the OncoMate™ MSI Dx Analysis System was evaluated using DNA extracted from colorectal cancer FFPE tissue samples using the Maxwell® CSC DNA FFPE Kit (Cat.# AS1350) in combination with the Maxwell® CSC Instrument (Cat.# AS6000). The presence of up to 5% ethanol or up to 50µM guanidine hydrochloride in the extracted DNA was demonstrated not to interfere with OncoMate™ MSI Dx Analysis System performance.

3.4 DNA Quantity Requirements

The OncoMate™ MSI Dx Analysis System amplification kit is intended for use with 1ng of DNA. DNA must be quantified prior to use. UV-absorbance measurements are unreliable for quantifying dsDNA purified from FFPE tissue samples. The use of a fluorescent, dsDNA-binding dyes or quantitative PCR is recommended to estimate the DNA concentration. Both of these methods provide more reliable quantification of DNA purified from FFPE tissue samples, particularly when DNA concentrations are low or when DNA eluates contain common contaminants (e.g., RNA, free nucleotides, solvents, detergents, protein or salts).

The OncoMate™ MSI Dx Analysis System was evaluated with CRC DNA samples quantified using dsDNA-binding dyes capable of accurately measuring dsDNA concentrations as low as 0.05ng/µl. During performance evaluation, DNA was quantified using the QuantiFluor® dsDNA System (Cat.# E2670) which is intended for general laboratory use.

3.5 Thermal Cycler Requirements

Performance of the OncoMate™ MSI Dx Analysis System was evaluated using the Thermo Fisher Scientific Veriti®, Eppendorf MasterCycler® and Bio-Rad C1000 Touch™ thermal cyclers, which share the following minimum specifications:

Maximum Block Ramp Rate: 3.9°C/second to 5°C/second

Temperature Accuracy: ±0.25°C (at ≥90°C)

Temperature Uniformity: <0.5°C (at ≥90°C)

Heated lid capable of reaching 103°C to 105°C

The performance of this assay may be negatively affected by the use of thermal cyclers with specifications outside of the indicated ranges.

3.6 Capillary Electrophoresis Instrument Requirements

OncoMate™ MSI Dx Analysis System amplification products are separated by fragment length (size) using capillary electrophoresis. Except for selecting a dye set based on the AnyDye method for spectral calibration, the default Fragment Analysis method provided by the data collection software for fragment analysis was used, and injection time was varied to achieve the appropriate fragment peak heights. For 2800M Control DNA, a peak height of approximately 8,000 relative fluorescence units (RFU) was targeted for the seven OncoMate™ MSI Dx Analysis System markers. Performance of the OncoMate™ MSI Dx Analysis System was evaluated using the Applied Biosystems® 3500 Dx Genetic Analyzer configured with POP-7® polymer and a 50cm capillary array. Run conditions used during performance evaluation are listed in Appendix B (Section 11.2). Capillary electrophoresis instruments compatible for use with the OncoMate™ MSI Dx Analysis System will share the following specifications:

Number of dyes detected: ≥5

Capillary array length: array lengths suitable for single-base resolution, including 50cm

Separation matrix: POP-7® or equivalent

Excitation wavelength (approximate): 480nm to 520nm

Detection optics: Promega dyes require emission capture from approximately 500nm to 630nm

Resolution range: 1bp resolution from 60bp to ≥300bp

Sizing precision (repeatability, expressed as standard deviation): ≤0.15bp across a range from 60bp to ≥300bp

It is the user's responsibility to verify the compatibility of the selected capillary electrophoresis instrument and analysis method with OncoMate™ MSI Dx Analysis System amplified products.

3.7 Spectral Calibration Requirements for the Capillary Electrophoresis Instrument

Prior to first use, perform a spectral calibration of the capillary electrophoresis instrument using the OncoMate™ 5C Matrix Standard (Cat.# MD3850). Spectral calibration is performed following *OncoMate™ 5C Matrix Standard Technical Manual* #TM597 with reference to the user guide for the capillary electrophoresis instrument. This procedure provides the data necessary for the capillary electrophoresis instrument to resolve the overlapping fluorescent signals from the multiple OncoMate™ MSI Dx Analysis System dye-labeled DNA fragments. Calibration settings used during performance evaluation are listed in Appendix A (Section 11.1). Perform a new spectral calibration in accordance with the capillary electrophoresis instrument manufacturer's recommendations. For example, a new spectral calibration is required after any major maintenance on a capillary electrophoresis system, such as changing the excitation source (e.g., laser), calibrating or replacing the optics, or changing the polymer type or capillary array.

3.8 Data Analysis Software Requirements

Fragment analysis software is required to analyze OncoMate™ MSI Dx Analysis System capillary electrophoresis data. Suitable fragment analysis software will accept fragment data (.fsa) files, calculate fragment size using the Local Southern method, attribute fragments to a marker by size range and fluorescent label (i.e., color), tabulate and display data from a mixture of fragment sizes (≥65bp to 300bp) and colors (≥5), and print and export analyzed data according to calculated allele size and marker. Suitable software also will display data quality information (e.g., off-scale peaks) captured during capillary electrophoresis and calculate basic QC parameters (e.g., identification of broad peaks) based on user-defined settings.

Data generated during the performance evaluation of the OncoMate™ MSI Dx Analysis System was analyzed using GeneMapper® Software version 5.0 and the analysis method settings listed in Appendix C (Section 11.3).

3.9 Analytical Threshold Requirements

The user must determine the analytical (i.e., detection) threshold of the assay prior to data analysis. The analytical threshold defines the RFU value above which sample signals can be distinguished from instrumentation noise, allowing the user to confidently analyze fragment data. Establishing an analytical threshold is critical to ensure the quality of patient data (see Section 7.5). Suitable procedures for determining the analytical threshold are available in technical literature (e.g., Bregu, 2013). During performance evaluation of the OncoMate™ MSI Dx Analysis System on the Applied Biosystems® 3500 Dx Genetic Analyzer, we used an analytical threshold of 175RFU across all dye channels, determined using the upper limit approach, which is based on linear regression of peak intensities generated from samples containing different DNA inputs (Bregu, 2013).

4. Assay Protocol

Keep pre-amplification and post-amplification reagents in separate rooms. Prepare DNA template dilutions and amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup. Wear gloves and use aerosol-resistant pipette tips to prevent DNA cross-contamination. Use a fresh pipette tip when adding each DNA sample, the 2800M Control DNA and Water, Amplification Grade (for negative controls) to amplification reactions.

4.1 Dilution of FFPE Tissue DNA Extracts

The OncoMate™ MSI Dx Analysis System requires a dsDNA input of 1.0ng per reaction, delivered in a volume of 1µl to 6µl. We recommend diluting DNA to a constant concentration across samples so that 1ng DNA is added to each reaction in a constant volume. During performance evaluation of the OncoMate™ MSI Dx Analysis System, all DNA templates were diluted to 0.5ng/µl and 2µl of diluted template DNA was added to each reaction.

Complete the following steps for DNA samples that require dilution prior to amplification:

1. Vortex DNA sample three times for 5 seconds each at maximum speed, and centrifuge briefly (1 to 2 seconds), if necessary, in a mini centrifuge to collect the sample at the bottom of the tube.

   Note: Failure to adequately mix DNA samples and dilutions may result in quantification errors and poor performance of the OncoMate™ MSI Dx Analysis System.

2. Dilute DNA sample in Nuclease-Free Water (Cat.# MC1191) to an appropriate concentration to allow addition of 1.0ng of dsDNA to each amplification reaction in the desired template volume (see Section 4.3). To maximize accuracy, pipet volumes of ≥1µl when diluting sample DNA.

   Note: Use Nuclease-Free Water if sample dilution is required. PCR amplification efficiency can be greatly altered by changes in pH (due to added Tris-HCl) or available magnesium concentration (due to chelation by EDTA) in the amplification reaction when Tris- or Tris-EDTA-based diluents are used. Failure to adequately mix DNA samples and dilutions may result in quantification errors and poor performance of the OncoMate™ MSI Dx Analysis System. Prepare fresh sample DNA dilutions for each experiment. Storing diluted sample DNA for future use may result in poor amplification and assay failure.

4.2 2800M Control DNA Dilution

The 2800M Control DNA serves as a positive amplification control (Section 7.3), not an MSI control. The OncoMate™ MSI Dx Analysis System is designed for use with a 2800M Control DNA input of 1.0ng, delivered in a volume of 1.0µl to 6.0µl. Prior to use, store the 2800M Control DNA, 10ng/µl, at 2°C to 10°C for a minimum of 8 hours. We recommend diluting the control DNA to the same concentration as the test samples. During performance evaluation of the OncoMate™ MSI Dx System, 2800M Control DNA was diluted to 0.5ng/µl and 2µl of diluted control DNA was added to each reaction.

1. Vortex the 2800M Control DNA three times for 10 seconds each at maximum speed.
2. Dilute 2800M Control DNA in Nuclease-Free Water (Cat.# MC1191) or Water, Amplification Grade, so that 1.0ng is added to the positive control reaction in the desired volume (1–6µl). See Table 2 for example dilutions. To ensure accuracy, pipet volumes ≥1µl when preparing 2800M Control DNA dilutions.

Table 2. Diluting the 2800M Control DNA.

Volume of DNA Template Per Reaction	Volume of 2800M Control DNA (10ng/µl)	Volume of Water[1]
1.0µl	2.0µl	18µl
2.0µl	2.0µl	38µl
3.0µl	2.0µl	58µl
4.0µl	2.0µl	78µl
5.0µl	2.0µl	98µl
6.0µl	2.0µl	118µl

[1]Nuclease-Free Water or Water, Amplification Grade

Note: Prepare a fresh 2800M Control DNA dilution for each experiment. Storing diluted 2800M Control DNA for future use may result in poor amplification of the positive control and assay failure.

4.3 Preparation of OncoMate™ MSI Dx Analysis System Amplification Reactions

1. At the first use, thaw the OncoMate™ MSI 5X Primer Mix, OncoMate™ MSI 5X Master Mix and Water, Amplification Grade, completely. After the first use, store reagents at 2°C to 10°C for up to 3 months.
2. Centrifuge tubes briefly (1–2 seconds) in a mini centrifuge to bring contents to the bottom, and vortex reagents three times for 3 seconds each at maximum speed. Do not centrifuge after vortexing, as this may cause the reagents to form a concentration gradient in the tube.
3. Label a new MicroAmp® Optical 96-Well Reaction Plate with Barcode.
4. Determine the number of reactions to be assembled. This must include at least one positive amplification control and one negative amplification control reaction for each plate processed. Add additional reactions to compensate for pipetting error. While this approach consumes a small amount of each reagent, it ensures that sufficient PCR amplification mix is available for all samples.
5. Assemble the PCR amplification mix as described in Table 3. Add the final volume of Water, Amplification Grade, OncoMate™ 5X Master Mix and OncoMate™ 5X Primer Mix to a clean, 1.5ml tube. The template DNA will be added to each reaction well individually at Step 7.

   Note: Table 3 assumes that the DNA template is delivered to the reaction in Step 7 in a constant volume across all samples.

Table 3. Assembly of PCR Amplification Mix.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	to a final volume of 10µl	×		=	
OncoMate™ MSI 5X Master Mix[2]	2µl	×		=	
OncoMate™ MSI 5X Primer Mix	2µl	×		=	
Template DNA (1.0ng)	up to 6µl				
Total Reaction Volume	10µl				

[1]Combine Water, Amplification Grade, OncoMate™ 5X Master Mix and OncoMate™ 5X Primer Mix in a new 1.5ml tube. The template DNA will be added to each reaction well individually at Step 7.

[2]A precipitate may form in the OncoMate™ MSI 5X Master Mix. Presence of the precipitate will not affect DNA amplification using the OncoMate™ MSI Dx Analysis System.

6. Vortex the PCR amplification mix three times for 3 seconds each at maximum speed, and then pipet one reaction's worth of PCR amplification mix into each well of the reaction plate(s) used for samples and controls.

   Note: Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or marker-to-marker imbalance. Add PCR amplification mix to the wells of the reaction plate as soon as the mix is prepared. Proceed promptly with Steps 7 through 10, followed immediately by thermal cycling.
7. Vortex each diluted FFPE template DNA (prepared in Section 4.1) three times for 5 seconds each at maximum speed, and centrifuge briefly (1–2 seconds) in a mini centrifuge to collect the liquid at the bottom of the tube. Pipet 1.0ng of the sample into the designated well on the plate containing PCR amplification mix. Mix by pipetting several times.

8. Vortex the diluted 2800M Control DNA (prepared in Section 4.2) three times for 10 seconds each at maximum speed, and centrifuge briefly (1–2 seconds) in a mini centrifuge to collect the liquid at the bottom of the tube. Pipet 1.0ng of the 2800M Control DNA dilution into the well(s) reserved for the positive control reaction(s). Mix by pipetting several times.
9. For the negative amplification control, pipet a volume of Water, Amplification Grade that is equivalent to the template DNA volume into the well(s) reserved for the negative control reaction(s). Do not add template DNA to the negative control reactions. Mix by pipetting several times.

   Note: Failure to amplify and analyze negative and positive control reactions may introduce uncertainty during data analysis of results and complicate troubleshooting. Positive and negative control reactions must be included on every plate.
10. Cap the wells with MicroAmp® 8-Cap Strips, and centrifuge briefly in a mini plate centrifuge to bring contents to the bottom of the wells and remove air bubbles.

4.4 Thermal Cycling

1. Ensure that the heated lid has reached the programmed temperature (103–105°C), and place the reaction plate in a thermal cycler. Close the thermal cycler lid.
2. Program the thermal cycler following the manufacturer's instructions to run the protocol specified in Figure 4. Ensure that the reaction volume is set to 10µl. The total cycling time, including ramping, is approximately 1 hour and 15 minutes.

Thermal Cycling Protocol[1]
96°C for 1 minute, then:
96°C for 10 seconds
58°C for 1 minute
72°C for 30 seconds
for 29 cycles, then:
60°C for 10 minutes, then:
4°C hold

[1]Reaction volume: 10µl; Heated lid: 103° to 105°C

Figure 4. Thermal cycling protocol for the OncoMate™ MSI Dx Analysis System.

3. After completion of the thermal cycling protocol, proceed with fragment analysis by capillary electrophoresis, or store amplification products protected from light overnight at 2°C to 10°C or long-term at –30°C to –10°C.

Note: Long-term storage of amplified samples at temperatures greater than –10°C may produce artifacts that interfere with data analysis.

4.5 Preparation of the Capillary Electrophoresis Instrument

See Section 3.6 for capillary electrophoresis instrument requirements. Follow the manufacturer's instructions for operation and maintenance of the selected capillary electrophoresis instrument and use of the associated polymer and capillary arrays.

1. Ensure that instrument polymer and buffers are not expired and that a sufficient number of samples or injections are available to complete the planned analysis.
2. If applicable, preheat the capillary electrophoresis oven according to the manufacturer's instructions for at least 30 minutes before starting a run.

4.6 Preparation of OncoMate™ MSI Dx Analysis System Amplified Fragments for Capillary Electrophoresis

1. If amplified samples were stored at −30°C to −10°C, thaw them completely before proceeding. Vortex for 5 seconds, and centrifuge the plate for 5 to 10 seconds in a mini plate centrifuge to collect contents at the bottom of wells.
2. Determine the number of wells required to analyze all amplified samples, including the positive and negative control reactions. Add to this number any unused wells from which an injection will be initiated plus additional wells to compensate for pipetting error.
3. Vortex the Size Standard 500 three times for 3 seconds each at maximum speed, and prepare the capillary electrophoresis loading cocktail as directed in Table 4.

Note: Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Table 4. Capillary Electrophoresis Loading Cocktail Preparation.

Loading Cocktail	Volume Per Well	×	Number of Wells	=	Final Volume
Hi-Di™ Formamide	9.5µl	×		=	
Size Standard 500	0.5µl	×		=	
Total Volume	10µl				

4. Vortex the loading cocktail three times for 3 seconds each at maximum speed, and centrifuge briefly (1–2 seconds) in a mini centrifuge to collect the reagents at the bottom of the tube.
5. Pipet 10µl of loading cocktail into each required well of a MicroAmp® Optical 96-Well Reaction Plate with the selected capillary electrophoresis instrument.

   Note: Loading cocktail or Hi-Di™ formamide must be added to every well from which an injection is initiated, whether amplified products also are added to the well or not. Failure to add loading cocktail or Hi-Di™ formamide to a well that is injected may result in damage to the capillary array and run failure.
6. Add 1µl of amplified sample or control reaction to each designated well.
7. Cover wells with septa according to the instrument manufacturer's instructions.

8. Centrifuge the plate for 5 to 10 seconds to bring the formamide-sample mixture to the bottom of each well and remove air bubbles.
9. Denature samples at 95°C for 3 minutes in a thermal cycler, and then immediately chill the reaction plate on crushed ice for at least 3 minutes. Denature samples just prior to loading the plate onto the capillary electrophoresis instrument.

   Note: Do not close the heated lid of the thermal cycler, as this may melt the plate septa.
10. Load the plate onto the capillary electrophoresis instrument according to the manufacturer's instructions. If applicable, ensure that the oven has reached the required temperature prior to loading.

4.7 Detection of Amplified Fragments by Capillary Electrophoresis with Fluorescence Detection

Consult the technical manual for the selected capillary electrophoresis instrument for information on completing a fragment analysis run, including how to configure the spectral calibration and fragment analysis methods, adding information for samples, loading denatured samples onto the instrument, initiating a run, and retrieving data for a completed run. When programming the instrument, start with the default fragment analysis method for the specific instrument configuration. Optimization of injection time may be required to meet individual laboratory needs. It is the user's responsibility to ensure that the conditions selected for capillary electrophoresis are compatible with the assay. Contact your local Promega branch office or distributor, or e-mail: **genetic@promega.com** for more information.

5. Data Analysis

5.1 Introduction

Capillary electrophoresis of OncoMate™ MSI Dx Analysis System amplification products results in data (.fsa) files that require downstream analysis using fragment analysis software (Figure 5). The objectives of data analysis are to provide basic information about data quality, size fragment peaks across the defined analysis range, assist in filtering "stutter" peaks from allele peaks present in each marker, and summarize allele size(s) by marker for downstream results interpretation. Perform the following steps to analyze capillary electrophoresis data using fragment analysis software:

Complete once, prior to first analysis; see Appendix C (Section 11.3) for instructions:

- Define the known sizes for the size standard fragments (i.e., create or import the assay Size Standard)
- Assign the analysis size range for each marker (i.e., create or import the assay Panels)
- Set the analysis parameters (i.e., create or import the Analysis Method)
- Optional: Manage data viewing (i.e., create or import Table, Plot and Report Settings)

Complete for each batch of samples analyzed; see Section 5.2 for instructions:

- Import the data files
- Select the analysis inputs (i.e., assay Size Standard, assay Panels and Analysis Method)
- Perform the data analysis
- Summarize the allele size by marker

GeneMapper® Software was used to evaluate the performance of the OncoMate™ MSI Dx Analysis System. Final data analysis settings used during performance evaluation of the assay are provided for reference in Appendix C (Section 11.3). These settings were optimized to identify the smallest (in bp) distinct allele in each mononucleotide-repeat marker and to distinguish it from stutter fragment peaks for downstream analysis. Under specific circumstances, distinct alleles present in a sample may be filtered when using the recommended analysis settings. These filtered alleles are not used for determining marker stability (see Section 6.4), as they are not the smallest distinct allele for that marker. The workflow to analyze fragment data is illustrated in Figure 5 and described below in Section 5.2 using GeneMapper® Software as an example. For additional instructions on analyzing fragment data using GeneMapper® Software, refer to the Thermo Fisher Scientific *DNA Fragment Analysis by Capillary Electrophoresis User Guide*.

Notes:

1. Analysis Method and Panels settings were determined for the OncoMate™ MSI Dx Analysis System using data generated with an Applied Biosystems® 3500 Dx Genetic Analyzer with POP-7® polymer and a 50cm array. The analysis of data acquired using different capillary electrophoresis instrument configurations may require modified panel and analysis method files. For assistance, contact your local Promega branch office or distributor, or e-mail: **genetic@promega.com**
2. The Analysis Method settings shown in Appendix C may serve as a model to develop analysis settings for your capillary electrophoresis system. However, the analytical thresholds ("Peak Amplitude Thresholds" in the "Peak Detector" tab of the GeneMapper® 'Analysis Method Editor') must be determined by the individual laboratory based on the targeted sample peak intensity, dynamic range of the instrument, and noise or background signal observed in analyzed data (refer to Section 7.5). An analytical threshold of 175RFU for each color channel (B, G, Y, O) was employed during performance evaluation of the OncoMate™ MSI Dx Analysis System. Analytical thresholds for suitable capillary electrophoresis instrumentation are typically between 50RFU and 200RFU. For an explanation of the proper usage and effect of the various analysis settings, refer to the Thermo Fisher Scientific *DNA Fragment Analysis by Capillary Electrophoresis User Guide*.

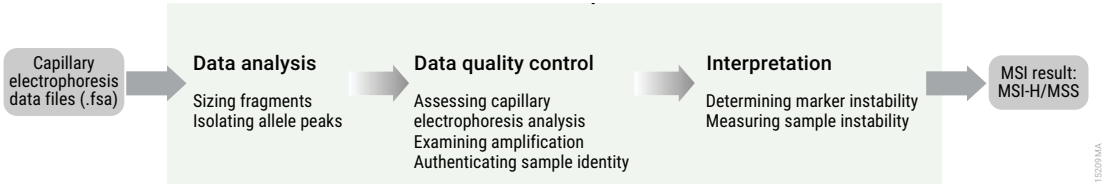


Figure 5. Analysis workflow for OncoMate™ MSI Dx Analysis System capillary electrophoresis data.

5.2 Analyzing Data

Instructions for data analysis using GeneMapper® Software are provided here for your convenience. These instructions assume that you have imported the available GeneMapper® settings files described in Appendix C (Section 11.3) or created similar setting files. When using other software for data analysis, refer to the corresponding user guide for information and instructions.

1. Open the GeneMapper® Software, version 5.0 or 6.0.
2. Select the "New Project" icon ( ) from the tool bar, and choose **Microsatellite** as the project type.
3. Import data by selecting the "Add Samples To Project" icon ( ) from the tool bar.
4. In the "Table Setting" drop-down menu in the tool bar, select **OncoMate_MSI_Dx_Table_v1.0**.
5. If the "Sample Type" field is not populated, choose **Sample**, **Positive Control** or **Negative Control** to describe each samples.
6. For all control and patient samples, select **OncoMate_MSI_Dx_Analysis_v1.0** for the "Analysis Method", **OncoMate_MSI_Dx_Panels_v1.0** for the "Panel" and **OncoMate_MSI_Dx_Size_v1.0** for the "Size Standard".
7. Select the "Analyze" icon ( ) from the tool bar to analyze all samples. When prompted, provide a descriptive name for the project in the "Save Project" pop-up window and select **OK**.

   Note: Following analysis, the samples may be sorted using the **Sort…** option under the **Edit** menu (e.g., by **Sample Type** and **Sample Name**). Reordering the samples so that each normal and tumor sample pair are adjacent in the sample list will aid in the display and review of the samples when using the provided "Plot Settings" file (see Step 8). Sorting also may be used to ensure that electropherograms for the normal and tumor sample pair are printed on the same page (see Step 9c).
8. Review control and patient samples for quality, as described in Section 6.3. Select rows containing the control or patient samples and select the "Display Plots" icon ( ) from the tool bar to view electropherograms and tabulated allele data. In the "Plot Setting" drop-down menu in the tool bar, select **OncoMate_MSI_Dx_Plot_v1.0**.

   Note: Review of sample electropherograms is necessary for assessing data quality. We recommend reviewing the controls first, followed by the patient samples. Control samples selected alongside patient samples will be continuously displayed in the "Samples Plot" window and cannot be minimized, crowding the screen.
9. Review valid patient samples for MSI status, as described in Sections 6.4 and 6.5. Display sample data as described in Step 8. To facilitate data interpretation, sizing data may be exported for downstream analysis several ways, including those described here.

   Note: These data export options are provided for information only. It is the responsibility of the laboratory executing the test to determine the most suitable data format to be used for downstream MSI status determination. For assistance in developing a reporting method for your laboratory, contact your local Promega branch office or distributor, or e-mail: **genetic@promega.com**

a. A text file including filtered allele sizes and other sample data may be exported as a "Genotypes Table" as follows:

i. From the main screen, select one or more patient and control samples and select the "Display Plots" icon ( ) from the toolbar to open the "Samples Plot" window.

ii. If the "Genotypes Table" is not displayed below the electropherogram(s), select the "Genotypes Table" icon ( ) from the toolbar.

iii. Select **Export Table** from the **File** menu, and save the file for downstream analysis.

b. A text file including filtered allele sizes and other sample data may be exported as a "Report" as follows:

i. From the main screen, select one or more patient and control samples and select the "Report Manager" icon ( ) from the toolbar to open the "Report Manager" window.

ii. In the "Report Setting" drop-down menu in the tool bar, select **OncoMate_MSI_Dx_Report_v1.0**.

iii. Select **Export…** from the **File** menu, name the file and select **Export**.

Note: Different data fields are available for export as a "Genotypes Table" versus a "Report." Fields may be added to meet the specific needs of your laboratory by editing the provided OncoMate™ "Table Settings" or "Report Settings" files in the GeneMapper® Manager.

c. A portable document format (.pdf) data summary displaying electropherograms and tabulated allele sizes may be saved as follows:

i. From the main screen, select one or more patient and control samples and select the "Display Plots" icon ( )to open the "Samples Plot" window.

ii. Select **Print…** from the **File** menu. Choose **Microsoft Print to PDF** (or equivalent) as the "Print Service" and **Landscape** as the orientation. Select **Print** and provide a sample name for the resulting .pdf file.

Note: Multiple .pdf files may be generated during this process, requiring different file names. For this reason, labs may choose to save a separate .pdf for each sample pair.

6. Review and Interpretation of Results

This section reviews the considerations for assessing data quality and interpreting MSI status for samples analyzed using the OncoMate™ MSI Dx Analysis System. Fragment analysis software may provide multiple options for data display and review. For example, GeneMapper® Software provides a variety of options for displaying and summarizing sample data. For additional information on reviewing data, refer to Thermo Fisher Scientific's *DNA Fragment Analysis by Capillary Electrophoresis User Guide*.

6.1 Understanding OncoMate™ MSI Dx Analysis System Data

The analysis of OncoMate™ MSI Dx Analysis System PCR products results in DNA fragment length data for each of the microsatellite regions amplified. Data from a matched normal and tumor sample pair are interpreted by evaluating the five mononucleotide-repeat markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) and two pentanucleotide-repeat markers (Penta C and Penta D) amplified by the OncoMate™ MSI Dx Analysis System.

Mononucleotide-repeat markers are analyzed to determine patient MSI status. The amplification of mononucleotide-repeat markers by PCR produces a main allele peak and an approximately bell-shaped distribution of flanking "stutter" peaks, each separated by 1bp (Bacher, 2004). Within any individual stutter peak distribution, the peak with the highest fluorescence value (RFU) is the allele peak (Figure 6). More than one stutter peak distribution and allele may be present per marker for either normal (when heterozygous) and tumor (when heterozygous or unstable) tissue samples. Fragment analysis software, such as GeneMapper® Software, uses peak-filtering algorithms to isolate alleles present in each marker from the "stutter" peaks. The size of these alleles is displayed on an electropherogram and tabulated for downstream MSI interpretation.

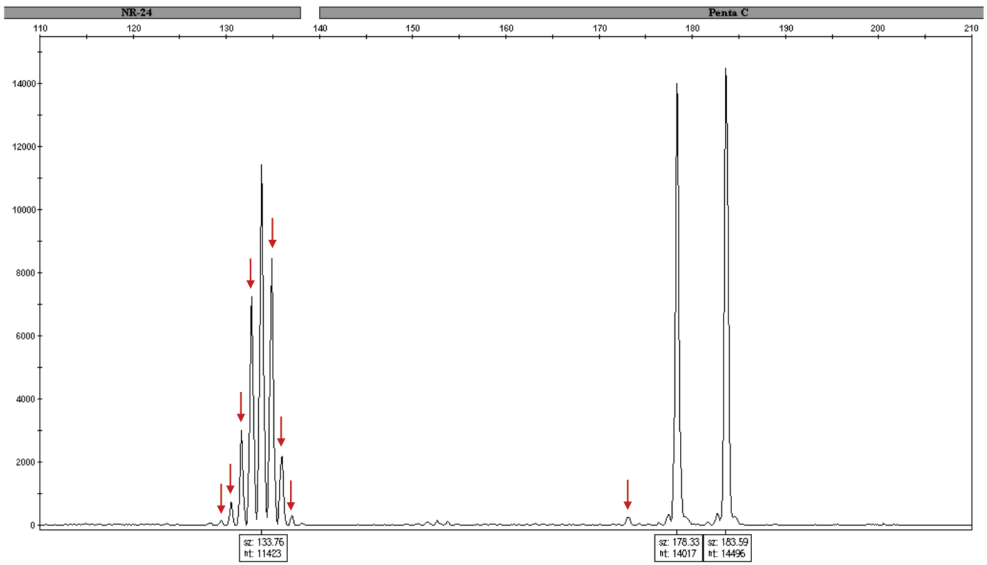


Figure 6. Stutter peaks (indicated by arrows) generated by polymerase slippage during PCR amplification of short tandem repeats are clearly visible at mononucleotide markers at 1bp intervals from the genuine, or tallest, allele peak, represented here using the NR-24 marker (left). Much smaller stutter peaks also may be seen at pentanucleotide makers 5bp from genuine allele peaks, represented here using the Penta C marker.

When a marker in a tumor sample is unstable, one or more novel alleles that are distinct from the normal allele(s) will be detected (Figure 7). A difference in allele size is detected by comparing the data from the tumor sample to its matched normal sample. Tumor samples are classified as MSI-H when ≥40% of the mononucleotide-repeat markers exhibit new alleles (i.e., when ≥2 of 5 markers show "altered allelic profiles"). Tumor samples with fewer than two altered mononucleotide-repeat markers may be classified as MSS (Umar, 2004; Boland, 1998).

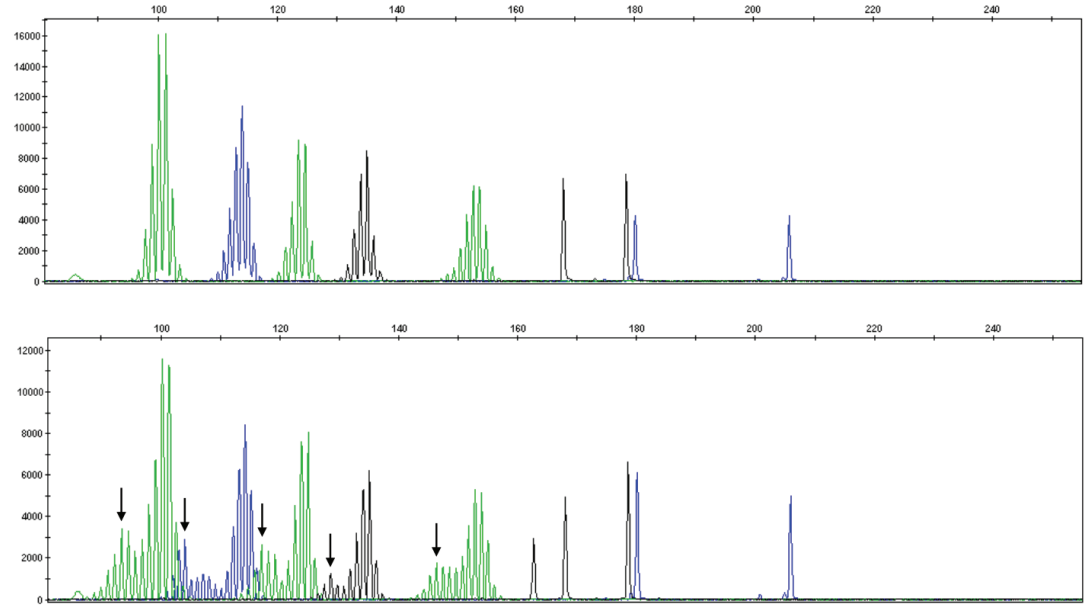


Figure 7. Matching normal (top panel) and tumor (bottom panel) patient samples were analyzed using the OncoMate™ MSI Dx Analysis System. One nanogram of DNA was amplified and analyzed using an Applied Biosystems® 3500 Dx Genetic Analyzer with POP-7® polymer and a 50cm capillary array. The presence of new alleles in the tumor sample (indicated by arrows) that were absent from the normal reference sample indicates marker instability. The sample shown is MSI-H, as ≥2 mononucleotide-repeat markers show instability.

6.2 Summary of Known Amplification Artifacts and Capillary Electrophoresis Anomalies

When using POP-7® polymer and a 50cm capillary array for capillary electrophoresis, a known amplification artifact is observed within the NR-21 marker as a single broad peak in the size range of 83bp to approximately 90bp (Figure 8). This peak should not be considered as an allele when determining the stability of the NR-21 marker. The presence of low-intensity, n−1bp peaks is common for pentanucleotide-repeat markers, Penta D and Penta C.

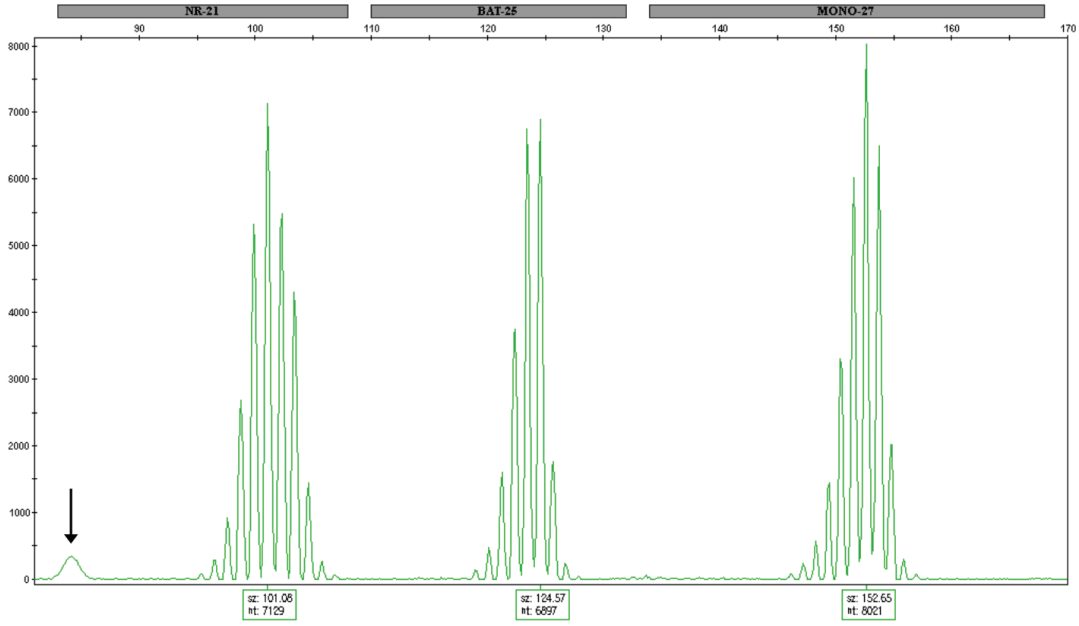


Figure 8. An amplification artifact (indicated with an arrow) may be observed within the NR-21 marker as a single broad peak in the size range of 83bp to approximately 90bp. This peak should not be considered as an allele when determining the stability of the NR-21 marker.

Other amplification artifacts may occur when too much DNA is used as input to the OncoMate™ MSI Dx Analysis System. The baseline signal in the BAT-26 marker (blue channel) may become elevated and jagged. Except for extreme inputs (e.g., ≥4 ng 2800M Control DNA), adopting the suggested peak-filtering parameters for GeneMapper® analysis (see Appendix C) will filter these artifacts out when identifying (i.e., calling) alleles for the BAT-26 marker.

Other known, but random, anomalies may be observed during capillary electrophoresis. Three such rare anomalies predominate: failed injections, broad peaks and signal spikes.

a. When an injection fails, little or no sample DNA is injected into capillary array. In these cases, a Sizing Quality QC failure (see Table 5) will be observed due to the lack (or poor quality) of Size Standard 500 peaks. Sizing Quality is a feature of GeneMapper® Software in which a score is calculated based on peak shape, size matching and the size-calling curve. The setting is found in the analysis method.

b. A peak may be detected during capillary electrophoresis that exhibits a broad (i.e., not sharp) morphology. A broad peak may originate from crystals or other aberrant material migrating through the capillary array.

c. A signal "spike" may be observed during capillary electrophoresis in the form of a near-zero width peak that spans all color channels. Such spikes are detected and ignored by most fragment analysis software when identifying alleles.

6.3 Quality Control Requirements for Data Interpretation

Understanding the quality of OncoMate™ MSI Dx Analysis System data is critical for proper interpretation of patient MSI status. A set of assay controls (Positive and Negative Controls) must be in place to ensure confidence in the data acquired with this assay and used for MSI interpretation (Section 7). Before the MSI status of a sample is determined, a series of data quality (QC) checks are performed to establish user confidence in data quality (Table 5). Most of the QC checks described below require visual inspection of the sample electropherograms; an evaluation of only the allele table from a given sample batch is insufficient to ascertain data quality.

The sample types evaluated for each QC check and the impact of a failed QC check are provided in Table 5. Following a data QC evaluation, each patient sample will have a status of either 'Invalid,' meaning that MSI interpretation is not possible due to a critical data QC failure, or 'Valid,' meaning that no critical QC failure was observed or that, despite a QC failure, interpretation of patient MSI status may still be possible based on the guidance provided in Section 6.5.3. When a control sample fails certain data QC checks, the quality of all associated patient samples may be compromised, and the 'Invalid' result applies to all samples in the batch. When positive and negative controls have passed all QC tests, a patient sample may pass or fail the data QC tests on a sample-by-sample basis. The patient sample status associated with a QC failure based on whether the failure is observed for a positive or negative amplification control or for the patient sample. Samples that fail QC requirements can be addressed according to instructions provided in the Troubleshooting section of this technical manual. A recommended progression of data quality check is as follows:

1. Evaluate sizing quality of all control and patient samples

2. Evaluate batch controls (i.e., the positive and negative amplification controls).

3. Evaluate patient samples

Table 5. Data QC Tests to be Completed Before MSI Interpretation to Understand the Quality of OncoMate™ MSI Dx Analysis System Data.

				Patient sample status when either a control sample or a patient sample fails the corresponding QC test	
Section	**QC Test**	**Description of QC test**	**Samples evaluated[1]**	**Control sample fails QC[2]**	**Patient sample fails QC[3]**
6.3.1	Sizing quality QC	A quality score is calculated based on peak shape, size matching and the size-calling curve.	+, –, N, T	All samples invalid	Sample invalid
6.3.2	Negative Control QC	For the negative amplification control, there must be no peaks detected above the analytical threshold.	–	All samples invalid	Patient samples not evaluated
6.3.3	Positive Control Identity QC	For the positive amplification control, the alleles present in the pentanucleotide markers must match the expected alleles for the 2800M Control DNA within 1.5 base pairs.	+	All samples invalid	Patient samples not evaluated
6.3.4	Off-Scale Peak QC	The intensity (RFU) of peaks in a given sample must not exceed the maximum detectable range of the capillary electrophoresis instrument.	+, N, T	All samples invalid	Sample invalid
6.3.5	Spectral Pull-Up QC	Peaks that are aligned by length in separate dye channels are evaluated for spectral pull-up (i.e., signal bleedthrough between dye channels).	+, N, T	All samples invalid	Sample invalid
6.3.6	Broad Peak QC	The width of peaks must not exceed the value assigned for MSI analysis.	+, N, T	See footnote 4	See footnote 4
6.3.7	Allele Amplification QC	At least one allele above the analytical threshold must be present within each marker.	+, N, T	All samples invalid	Marker invalid; sample may be interpreted
6.3.8	DNA Contamination QC	For each pentanucleotide marker, there can be no more than two alleles present in the normal sample.	+, N	All samples invalid	Sample invalid
6.3.9	Patient Sample Identity QC	For each pentanucleotide marker, the alleles identified in the normal sample must be present in the tumor sample (within 1.5 base pair).	T	Controls not evaluated	See footnote 5
6.3.10	Tumor Signal QC	For mononucleotide markers that have been interpreted as stable, allele peak height(s) in the tumor sample must be sufficient to ensure assay sensitivity. The required peak intensity is based on the analytical threshold and the sample tumor content.	T	Controls not evaluated	Marker invalid; sample may be interpreted

[1] N, normal sample; T, tumor sample; +, positive control; –, negative control

[2] MSI interpretive result for all patient samples in the batch when a control sample exhibits the associated QC issue.

[3] Column indicates whether patient samples are invalid or may still be interpreted when affected by the associated QC issue despite failure for a particular marker. The direction provided requires control samples to have passed all QC tests. See interpretation guidelines in Section 6.5.

[4] Acknowledge the known broad-peak artifact between 83bp and 90bp in NR-21 during data QC evaluation as unrelated to instability. All samples should be interpreted as invalid if a control sample exhibits a broad peak other than the known NR-21 artifact, and the affected marker(s) should be interpreted as invalid if a broad peak other than the known NR-21 artifact is observed in a patient sample.

[5] When the Patient Sample Identity QC fails due to absence of an allele, the sample may be interpretable based on the guidance provided in Section 6.5.3. Otherwise, the sample is invalid.

6.3.1 Evaluate All Samples for Sizing Quality (Sizing Quality QC)

Sizing quality metrics may be provided by capillary electrophoresis instruments and/or displayed in fragment analysis software. Sizing quality issues are commonly associated with a failure of the capillary electrophoresis analysis and occur on a single-capillary basis (i.e., not for every capillary of an injection). Samples exhibiting Sizing Quality issues will display no (or few) fragment peaks or fragment peaks that broaden (i.e., lose resolution) with increasing size (Figure 9). Evaluate sizing quality for every sample and control analyzed as the sizing information is critical to the test result.

To assess sizing quality, ensure that the Size Standard 500 (orange) dye channel is displayed in the fragment analysis software and demonstrates the expected peak profiles for the 60bp through 300bp size standard fragments. A patient or control sample result is invalid if either of the following attributes indicating poor sizing quality are identified:

a. A sizing quality flag was returned by the capillary electrophoresis data collection or fragment analysis software.

   Note: Only the 60bp through 300bp Size Standard 500 fragments are used for sizing MSI markers. Apparent quality issues for Size Standard 500 peaks greater than 300bp do not affect fragment sizing.

b. One or more of the 13 Size Standard 500 fragments from 60bp through 300bp are not labeled or are labeled incorrectly (60bp, 65bp, 80bp, 100bp, 120bp, 140bp, 160bp, 180bp, 200bp, 225bp, 250bp, 275bp and 300bp). This may occur due to severe peak-to-peak sloping or peak broadening within the size standard. Occasionally, aberrant peaks may be detected during capillary electrophoresis that could lead to size standard peak misidentification.

   Note: Capillary electrophoresis “spikes” (i.e., zero-width peaks) may be present but will not interfere with fragment sizing unless they are mistakenly labeled as size standard peaks by the fragment analysis software.

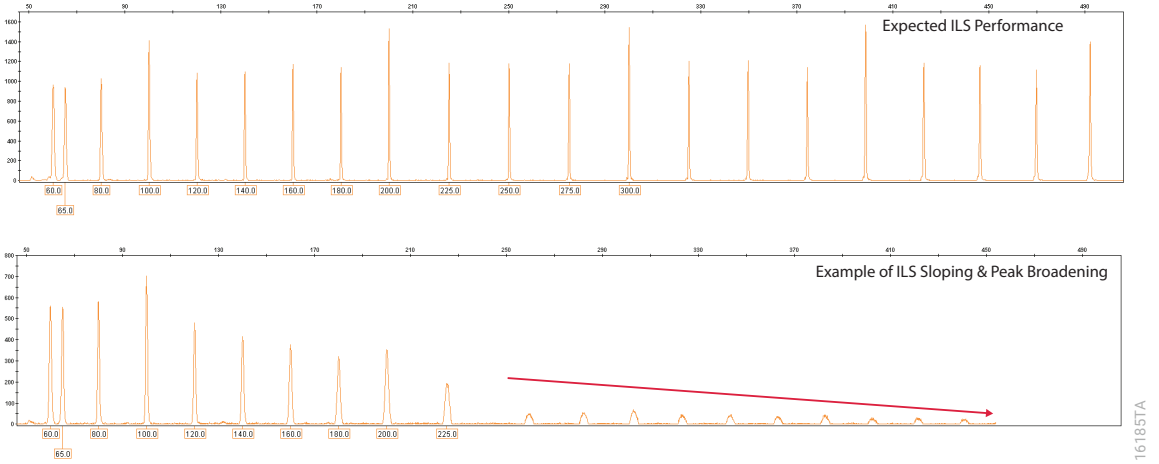


Figure 9. The upper panel shows the expected result for the Size Standard 500. In the lower panel, extreme sloping and peak broadening were observed, indicative of a Sizing Quality issue.

6.3.2 Evaluate the Negative Control Reaction for Amplification (Negative Control QC)

A negative amplification control must be analyzed alongside patient samples. Fragment peak(s) detected above the analytical threshold in the sample dye channels (blue, green and yellow/black) for the negative amplification control indicate that contamination may have occurred. A contaminated negative control would indicate potential data quality issues for the entire batch of samples. Therefore, all patient samples in a batch with a failed negative control sample should be considered invalid.

6.3.3 Evaluate the Pentanucleotide-Repeat Markers for the Positive Control Reaction for Identity (Positive Control Identity QC)

A positive amplification control sample, prepared with 2800M Control DNA, must be analyzed alongside patient samples to provide evidence for assay integrity (i.e., that the expected results were observed). The Positive Control Identity QC is evaluated using the Penta D and Penta C markers. The positive amplification control sample exhibits two alleles at each pentanucleotide-repeat marker, which are detected within the following size ranges (bp) based on our performance evaluation data (conditions listed in Appendices B and C):

a. Penta D: 193.5–196.5 and 199–202

b. Penta C: 176.5–179.5 and 182–185

A failure of the positive control to meet these criteria indicates potential data quality issues for the entire batch of samples. Therefore, all patient samples analyzed in a batch with a failed positive control sample should be considered invalid.

6.3.4 Evaluate Peak Intensities for Off-scale Results (Off-Scale Peak QC)

Capillary electrophoresis instruments have a limited range to detect fluorescent signals from dye-labeled DNA fragments. In some situations, the fluorescent signal from OncoMate™ MSI Dx Analysis System amplification products may exceed the detector's upper limit. When this happens, an Off-Scale Peak QC flag will be returned by the instrument and displayed in the fragment analysis software. Off-scale peak intensities may result in signal bleedthrough into other dye channels (Figure 10; Section 6.3.5). Since the bleedthrough peak patterns will resemble the original signal pattern, this bleedthough may be mistaken for an allele in a normal or tumor sample, complicating MSI interpretation. A patient or control sample should be considered invalid when off-scale results are observed. Off-scale results observed for 2800M positive amplification controls indicate a systemic issue when preparing or amplifying OncoMate™ MSI Dx Analysis System reactions and potential data quality issues for the entire batch of samples being tested. In batches where a positive control fails the Off-Scale Peak QC, all samples in the batch must be interpreted as invalid and addressed according to guidance provided in Section 9. Evaluate data for off-scale results in every sample analyzed and the 2800M amplification control.

6.3.5 Evaluate All Markers for Spectral Pull-Up (Spectral Pull-Up QC)

When off-scale results are observed or when spectral calibration of the capillary electrophoresis instrument is inadequate (e.g., because of adjustments to the instrument optics during a service call), fluorescent signals from dye-labeled fragments may be detected outside of the intended color channel at approximately the same size (bp) as the true signal. This situation is referred to as spectral pull-up or bleedthrough. When bleedthrough is detected in other dye channels, a QC flag may be displayed by the fragment analysis software.

Refer to the technical manual for the selected fragment analysis software for setting parameters to detect spectral pull-up. Parameters matching those used during evaluation of the OncoMate™ MSI Dx Analysis System are provided in Appendix C.

Since the peak patterns will resemble the original signal pattern, this bleedthough may be mistaken for an allele in a normal or tumor sample, complicating MSI interpretation. Bleedthrough signals present in 2800M positive amplification controls would indicate potential data quality issues for the entire batch of samples in the assay. For these reasons, affected patient or control samples should be considered invalid if bleedthrough is observed above the analytical threshold. Evaluate data for bleedthrough in every patient sample analyzed and in the positive amplification control. To assess samples for bleedthrough peaks, display all dye channels displayed on the same plot (Figure 10). Look for peaks of the same size that are present in two or more dye channels. Genuine peaks in a dye channel will be of significantly higher intensity than bleedthrough peaks in the other dye channel(s). For OncoMate™ MSI Dx Analysis System fragments, bleedthrough may be observed in the following patterns:

a. BAT-26 peaks may be observed in the NR-21 or BAT-25 panels

b. Penta D peaks may be observed in the BAT-25 or MONO-27 panels

c. NR-21 peaks may be observed in the BAT-26 or NR-24 panels

d. BAT-25 peaks may be observed in the BAT-26, Penta D or NR-24 panels

e. MONO-27 peaks may be observed in the Penta D, NR-24 or Penta C panels

f. NR-24 peaks may be observed in the NR-21, BAT-25 or MONO-27 panels

g. Penta C peaks may be observed in the MONO-27 panel

In some situations, genuine fragment peaks may be aligned across dye channels and confused for bleedthrough. On these occasions, repeat spectral calibration of the capillary electrophoresis instrument and repeat the assay to provide confidence in the data and the resulting MSI interpretation.

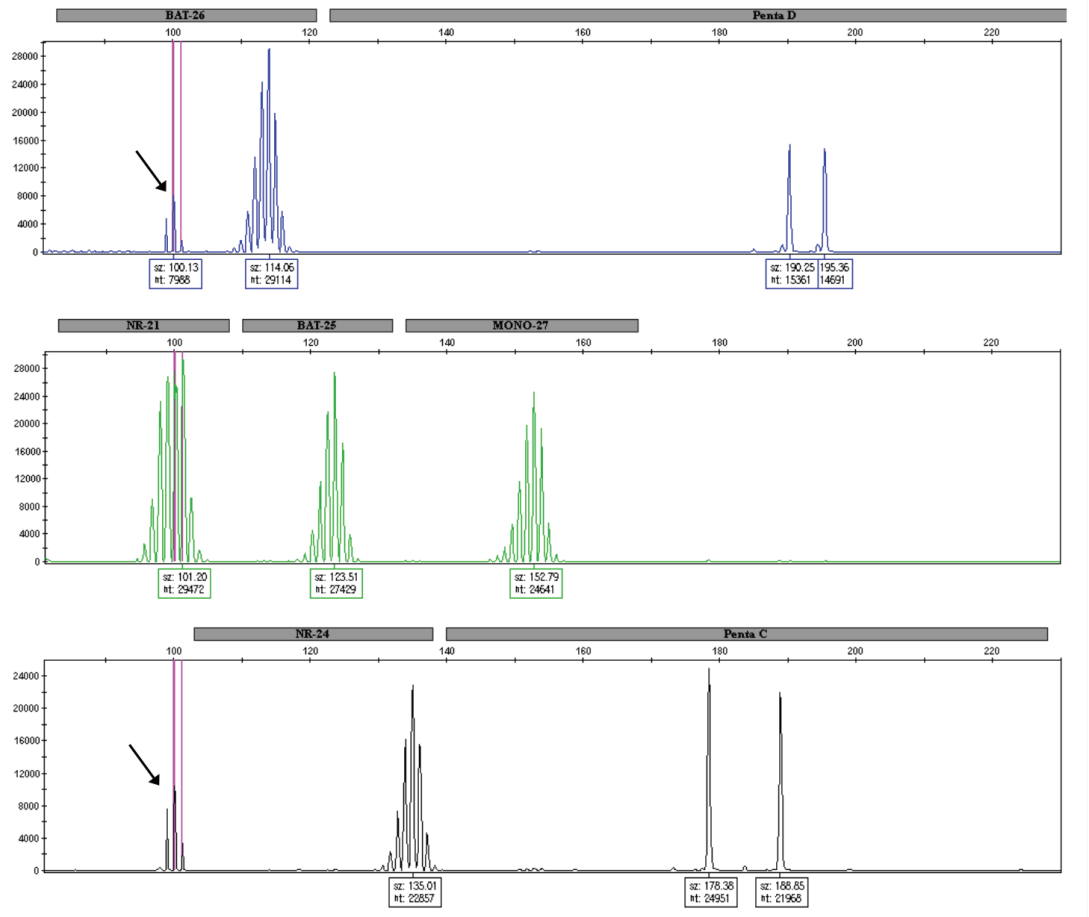


Figure 10. Off-scale normal tissue sample, with prominent signal bleedthrough between dye channels. Peaks in NR-21 exceeded the detection limit of the capillary electrophoresis instrument, resulting in an off-scale flag and bleedthrough peak of a similar pattern at other markers. **Panel A.** Markers in the blue dye channel, highlighting a cluster of signal bleedthrough peaks from NR-21 into BAT-26. **Panel B.** Markers in the green dye channel, highlighting off-scale fragment peaks for NR-21. **Panel C.** Markers in the yellow/black dye channel; the bleedthrough peaks from NR-21 into this dye channel fall outside of the marker panels.

6.3.6 Evaluate All Markers for Broad Peaks (Broad Peak QC)

Broad fragment peaks may be observed and called as alleles in OncoMate™ MSI Dx Analysis System data. These peaks exhibit a gentle rise and fall pattern that spans several base pairs and are easily distinguished from genuine fragment peaks, which rise and fall sharply across a 1bp to 2bp span. Broad peaks may be observed sporadically as a result of abnormal PCR amplification or capillary electrophoresis analysis. A known broad-peak amplification artifact is often observed between 83bp and 90bp in the OncoMate™ MSI Dx Analysis System NR-21 marker (see Section 6.2 for more details). When broad peaks are observed, a QC flag may be displayed by the fragment analysis software.

Refer to the technical manual of the selected fragment analysis software to set parameters to detect broad peaks. Parameters matching those used during performance evaluation of the OncoMate™ MSI Dx Analysis System are provided in Appendix C (Section 11.3).

Inspect patient and control samples at each marker for broad peaks that were called as alleles. A broad peak at NR-21 between 83bp and 90bp is unrelated to microsatellite instability and its detection does not indicate a Broad Peak QC failure. When a broad peak is detected for one or more markers, the quality of the data is diminished. Data interpretation may still be possible for unaffected markers based on guidance provided in Section 6.5. Broad peaks in control sample(s) may indicate potential data quality issues for the entire batch of samples. In batches where a control sample fails the Broad Peak QC, all samples in the batch must be interpreted as invalid and addressed according to guidance provided in Section 9.

6.3.7 Evaluate All Markers for Allele Amplification (Allele Amplification QC)

Inspect patient samples and 2800M positive controls at each marker to assess whether a genuine fragment peak is present above the analytical threshold (see Sections 3.9 and 7.5). When no allele is detected for one or more markers, the quality of the sample data is diminished. Document markers for which Allele Amplification QC fails. Data interpretation may still be possible for unaffected markers based on guidance provided in Section 6.3. **Note:** At least one allele must be detected in both the normal and tumor sample for a given mononucleotide-repeat marker to be interpreted. Missing alleles in 2800M positive amplification control indicate potential data quality issues for the entire batch of samples. In batches where a positive control sample fails the Allele Amplification QC, all samples in the batch must be interpreted as invalid and addressed according to guidance provided in Section 9.

6.3.8 Evaluate the Positive Amplification Control and Normal Patient Samples for DNA Contamination (DNA Contamination QC)

Analysis of the number of alleles detected in pentanucleotide-repeat markers can sometimes provide information on sample contamination from outside sources of human DNA. Count the number of alleles identified for each pentanucleotide-repeat marker in the positive amplification control and normal patient samples. The DNA Contamination QC fails when three or more alleles are detected for Penta C or Penta D. Genomic instability associated with certain tumors may result in more than two alleles being present in the tumor sample. Therefore, evaluation of patient tumor samples by the DNA Contamination QC is uninformative. Failure observed for the 2800M positive amplification control sample would indicate potential data quality issues for the entire batch of samples. In batches where a positive control sample fails the DNA Contamination QC, all samples in the batch must be interpreted as invalid and addressed according to guidance provided in Section 9. Patient normal samples that fail the DNA Contamination QC should be considered invalid and addressed according to guidance provided in Section 9.

6.3.9 Evaluate Pentanucleotide-Repeat Markers in Patient Samples for Identity (Patient Sample Identity QC)

Compare alleles in each pentanucleotide-repeat marker to provide confidence that normal and tumor samples were derived from the same patient. The Patient Sample Identity QC is passed when all alleles identified in the normal sample are also present in the tumor sample. Genomic instability associated with certain tumors may result in more or fewer alleles being present in the tumor sample relative to the normal sample. The presence of additional alleles in the tumor sample has been observed and does not result in Patient Sample Identity QC failure. Additional alleles in the tumor sample at the Penta markers does not indicate MSI. On the other hand, a missing allele in the tumor sample may indicate a sample switch or loss of heterozygosity (LOH) at the marker (see Section 6.5.3). Sample pairs that fail the Patient Sample Identity QC due to a missing allele in the tumor sample may still be interpretable based on the guidelines provided in Section 6.5.3. Sample pairs that fail the Patient Sample Identity QC for other reasons should be considered invalid and addressed according to guidance provided in Section 9. Positive and negative controls are not evaluated for the Patient Sample Identity QC.

6.3.10 Evaluate Mononucleotide-Repeat Markers in Tumor Samples for Signal (Tumor Signal QC)

For each mononucleotide-repeat marker, when no new alleles are identified in the tumor sample relative to the normal sample, the marker is interpreted as stable. However, the normal ("reference") allele in the tumor sample must be present at a sufficient intensity, based on sample tumor content, to ensure that new alleles are detected above the analytical threshold. Therefore, the peak intensity at each apparently stable mononucleotide-repeat marker must be inspected to determine whether the allele intensity is sufficient to ensure the assay's sensitivity to detect new alleles.

For performance evaluation of the OncoMate™ MSI Dx Analysis System, we determined the analytical threshold to be 175RFU with a minimum tumor content of 20%, corresponding to a tumor fraction of 0.2. To ensure assay sensitivity, we used the following formula to calculate a minimum required normal-allele peak intensity of 700RFU in apparently stable mononucleotide-repeat markers in tumor samples:

$$\text{Tumor Signal Intensity (RFU)} \geq \text{Analytical Threshold} \times \frac{1}{\text{Minimum Tumor Fraction}} \times (1 - \text{Minimum Tumor Fraction})$$

At a minimum peak intensity of 700RFU, a new allele present at 20% (a tumor fraction of 0.2) of the total sample would have an expected peak height of 175RFU (i.e., 175RFU is 20% of the total signal of 875RFU, while 700RFU is 80% of the total signal of 875RFU). Thus, ensuring that the signal intensity is sufficient to be confident in a stable call for a given marker in samples with ≥ 20% tumor content. Document markers that fail Tumor Signal QC. Data interpretation may still be possible for unaffected markers based on guidance provided in Section 6.5. Positive and negative controls are not evaluated for the Tumor Signal QC.

6.4 Determination of Mononucleotide-Repeat Marker Stability

Analysis of mononucleotide-repeat markers in paired normal and tumor samples determines marker stability, which informs tumor MSI status. Stability is evaluated on a marker-by-marker basis, and instability is identified by the presence of one or more new microsatellite alleles in the tumor sample relative to the normal sample from the same patient. In some tumors, subtle allele alterations may be present and distinct new alleles may not be identified by existing fragment analysis software; for such samples, visual comparison of electropherograms for differences may identify instability. In other tumors, such as those from MSI-High CRC patients, alterations to microsatellite profiles are pronounced, and distinct new alleles with flanking stutter peaks of decreasing intensity are present. Repeat analysis of a sample with increased tumor content and/or with different capillary electrophoresis injection time(s) may be informative when marker stability is equivocal or indeterminate (Guedes, 2017; Wang, 2017; Boyle, 2014). The process for determining marker stability is summarized in Figure 11. Table 6 summarizes the possible results when determining the stability of mononucleotide-repeat markers. Marker stability cannot be determined for patient samples identified as invalid due to a critical data QC failure (Section 6.3).

During performance evaluation of the OncoMate™ MSI Dx Analysis System, we employed a simple algorithm for determining marker stability: For each of the five mononucleotide-repeat markers in the patient's normal and tumor sample, the smallest allele identified was considered the allele of interest for subsequent comparisons. The size difference between the alleles of interest in the normal and tumor samples was calculated to determine marker stability. When this difference was ≥3bp (implemented in practice as ≥2.75bp to account for the sizing precision of the capillary electrophoresis instrument), the marker was interpreted as unstable.

Table 6. Mononucleotide-Repeat Marker Stability Calls Inferred by Comparing Alleles Present in the Normal and Tumor Samples.

Marker Stability Call	
The stability call assigned to each marker for the sample	
Value	**Description**
Stable	Microsatellite instability was not detected for the mononucleotide-repeat marker. No new alleles were detected in the tumor sample, or the size difference between a new tumor allele and the normal reference allele was insignificant (<2.75bp).
Unstable	Microsatellite instability was detected for the mononucleotide-repeat marker. A new allele was detected in the tumor sample, and the size difference between this allele and the normal reference allele was significant (≥2.75bp).
Invalid	Microsatellite instability could not be assessed for the mononucleotide-repeat marker. Sample data quality was unacceptable due to a critical QC failure (see Section 6.3).

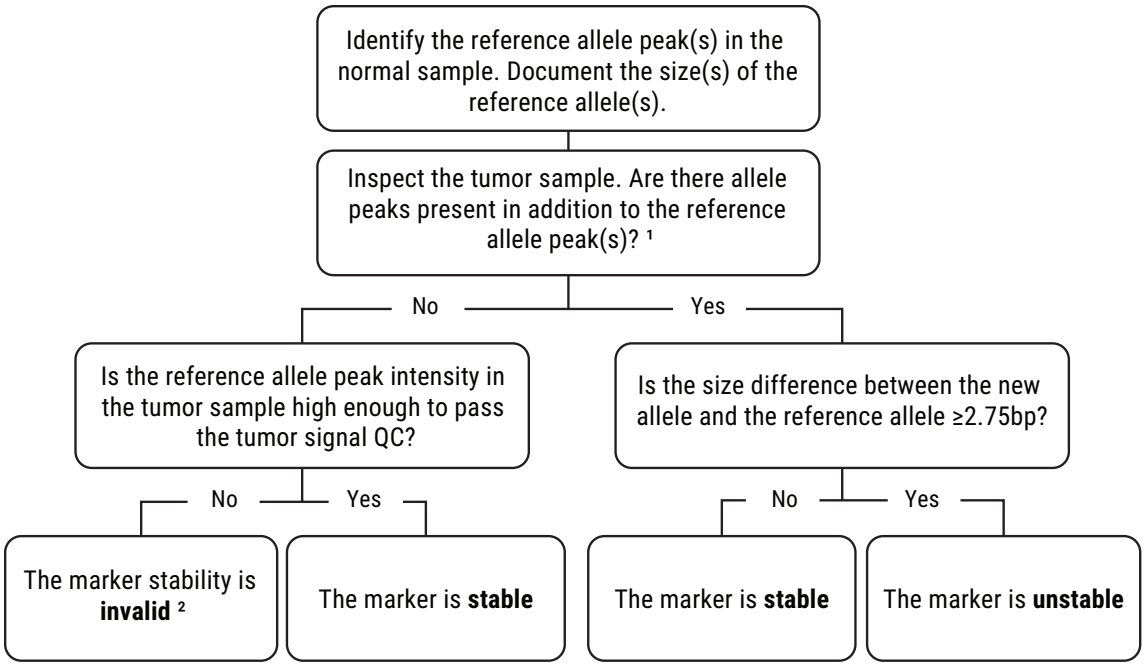


Figure 11. General guidance for assessing mononucleotide-repeat marker stability for patient samples. Follow the flow chart for each mononucleotide-repeat marker that met the data QC requirements specified in Section 6.3. Document the stability of each marker, and then proceed to Section 6.5, Interpretation of Tumor MSI Status. **Note:** For a given mononucleotide-repeat marker to be interpreted, all QC checks must pass for that marker in both the normal and tumor samples.

6.5 Interpretation of Tumor MSI Status

6.5.1 Summary

The process for interpreting sample MSI status based on the stability calls determined for OncoMate™ MSI Dx Analysis System markers is summarized in Figure 12. Table 7 summarizes end points for tumor sample MSI status interpretation. This method follows consensus rules for determining sample MSI status established at the US National Cancer Institute Workshop on Microsatellite Instability (Boland, 1998). When no critical QC failures are observed, sample MSI status is interpreted as follows:

A tumor sample is interpreted as MSI-H when two or more mononucleotide-repeat markers are unstable. A tumor sample is interpreted as MSS when fewer than two mononucleotide-repeat markers are interpreted as unstable (Boland, 1998; Hampel, 2008; Umar, 2004).

An MSI determination is still possible when invalid results are obtained for a subset of markers (see Section 6.5.2) or when a Patient Sample Identity QC failure is observed (see Section 6.5.3). Guidance for interpreting such samples is provided below. Samples exhibiting fewer than two valid mononucleotide-repeat markers cannot be interpreted and must be addressed according to guidance provided in Section 9.

Table 7. Tumor MSI Status Inferred from the Sum of Marker Stability Results.

Value	Description
MSS	MSS (microsatellite stable) indicates that fewer than two mononucleotide-repeat markers were identified as unstable.
MSI-H	MSI-H (MSI high) indicates that two or more mononucleotide-repeat markers were identified as unstable.
Invalid	Invalid indicates that sample data quality is unacceptable due to a critical data QC failure (see Table 5). No MSI interpretation can be made from these data. When a batch has failed data QC due to an issue with either the positive or negative amplification control, all samples within that batch must be interpreted as invalid. See the troubleshooting section of this manual for guidance on resolving QC failures that lead to invalid results.

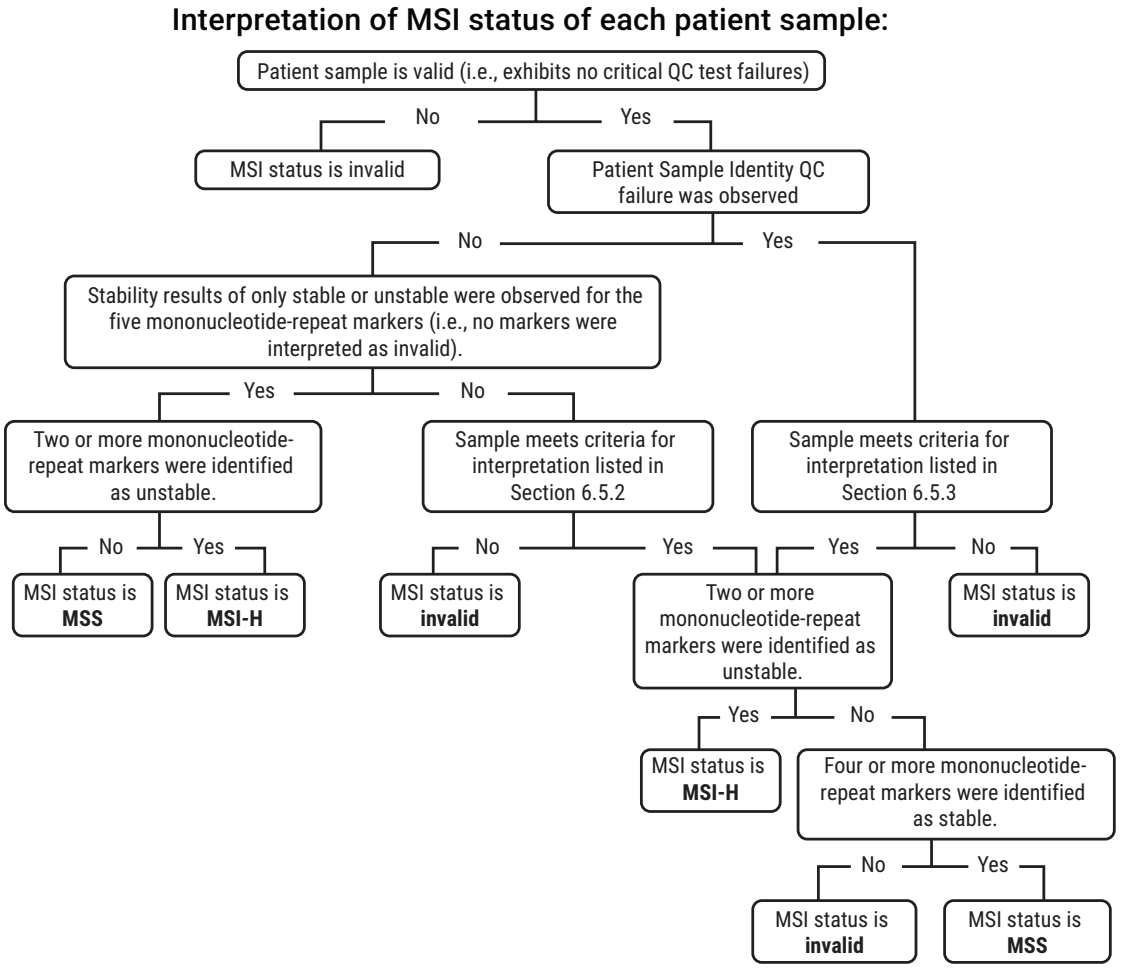


Figure 12. General guidance to determine the MSI status of a tumor sample. Follow the flow chart for each sample passing data QC.

6.5.2 MSI Interpretation When a Subset of Markers Exhibit Invalid Stability Results

When allele peak intensities for mononucleotide-repeat markers are too low for data evaluation, samples will exhibit Tumor Signal QC or Allele Amplification QC failures and a marker stability status of invalid. When a broad peak interferes with allele peak sizing, a sample will exhibit a Broad Peak QC failure and a marker stability status of invalid.

An MSI interpretation may be possible for a patient sample exhibiting invalid stability results at a subset of mononucleotide-repeat markers if the following conditions are true:

1. Low marker peak heights could not be rectified by a) increasing the DNA input to the OncoMate™ MSI Dx Analysis System amplification reaction or b) re-analyzing a different FFPE tissue section.
2. No QC failures are observed for either pentanucleotide-repeat marker.
3. The only data QC failures are the Tumor Signal QC, Allele Amplification QC or Broad Peak QC.
4. A stable or unstable call could be made for two or more mononucleotide-repeat markers.

If these conditions are met, the sample may be interpreted with the remaining, valid stability data using the following guidelines (Boland, 1998):

1. If two or more mononucleotide-repeat markers are unstable, interpret the tumor sample as MSI-H.
2. If four or more mononucleotide-repeat markers are stable, interpret the tumor sample as MSS.
3. Otherwise, the sample cannot be interpreted. The sample MSI status will be invalid.

6.5.3 MSI Interpretation When Patient Sample Identity QC Failure is Observed

Within tumor samples the pentanucleotide markers may lose or display additional alleles that complicate interpretation of identity with normal samples. Common genetic events observed with tumor tissue include LOH and generalized genomic instability, which may interfere with sample authentication (i.e., confirmation of identity between matched normal and tumor samples). Pentanucleotide-repeat markers may display a different number of alleles in a tumor sample relative to the matched normal sample. The presence of additional alleles in a tumor sample does not prompt a data QC failure.

On the other hand, cases of pronounced allelic imbalance (e.g., loss of heterozygosity, LOH) may result in a pentanucleotide allele that is present in normal sample but is not called an allele in a tumor sample. Severe allelic imbalance is observed when one of the normal pentanucleotide alleles is lost (i.e., greatly diminished in peak height and not identified as an allele) in the tumor. This state can lead to an apparent Patient Sample Identity QC failure. To confirm if a "lost" normal allele is present above the analytical threshold and was simply filtered by the software allele-calling algorithm, open the sample file in the fragment analysis software and hover the cursor over the "lost" peak in the electropherogram for the tumor sample. When hovering over a potential "lost" peak in the GeneMapper® software, you will see a vertical black line appear to indicate that the peak was above the analytical threshold but was filtered and thus not identified as an allele (Figure 13). Alternatively, you can zoom in on a potential "lost" peak to ensure that the lost peak is above the analytical threshold. If the selected fragment analysis software does not support this feature, verify that the peak height of the lost allele is above the analytical threshold.

An MSI interpretation may be possible for a patient sample exhibiting an apparent Patient Sample Identity QC failure if the following conditions are true:

1. The QC failure was observed for a single pentanucleotide-repeat marker (i.e., Penta C or Penta D, not both).
2. The "lost" normal allele is present in the tumor sample above the analytical threshold but was filtered as stutter by the fragment analysis software's allele-calling algorithm because of the much greater height of another allele in the same panel.
3. No QC failures are observed for the mononucleotide-repeat markers.

If these conditions are met, data can be interpreted using the following guidelines:

1. If two or more mononucleotide-repeat markers are unstable, interpret the tumor sample as MSI-H.
2. If four or more mononucleotide-repeat markers are stable, interpret the tumor sample as MSS.

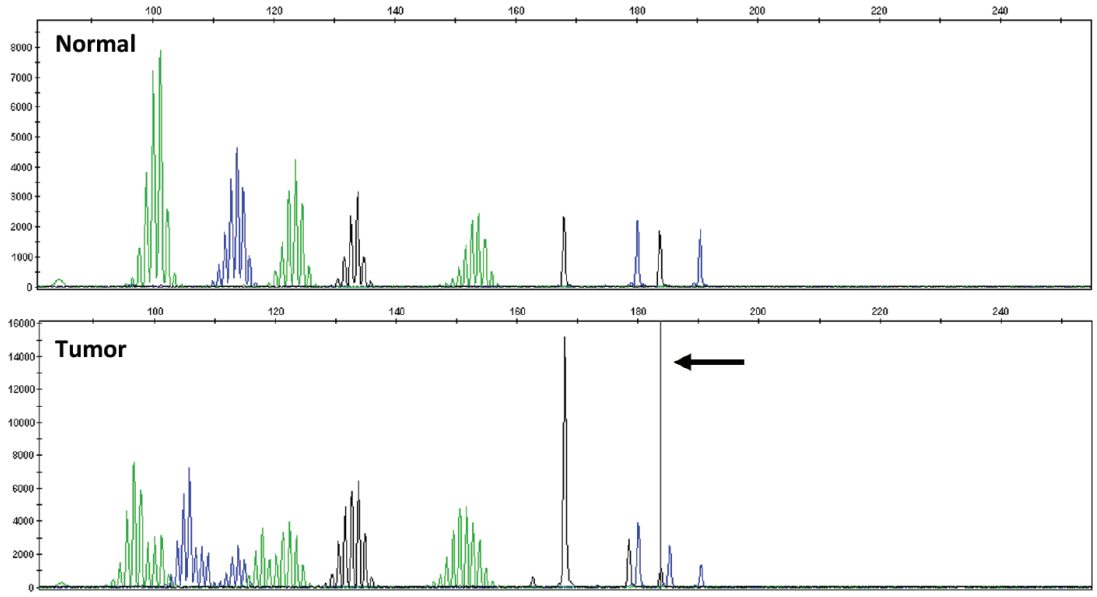


Figure 13. An example of pronounced allele imbalance. In cases of pronounced allelic imbalance in pentanucleotide-repeat markers, a vertical black line will be displayed when the cursor is hovered over a "lost" normal peak in the tumor sample electropherogram if this peak is present and above the analytical threshold but was filtered as stutter due to the much greater peak height of the 168bp allele in the same panel.

7. Assay Quality Controls

7.1 Spectral Calibration

During capillary electrophoresis, dye-labeled OncoMate™ MSI Dx Analysis System amplification products are separated by size and detected. Prior to analysis, the selected capillary electrophoresis instrument is calibrated with matrix standards to distinguish the specific dyes used in the assay. The OncoMate™ 5C Matrix Standard (Promega Cat.# MD3580) consists of DNA fragments labeled with five different fluorescent dyes (fluorescein, JOE, TMR-ET, CXR-ET and WEN) in one tube. Perform a spectral calibration according to general parameters provided in Appendix A (Section 11.1). Once generated, the spectral calibration file is applied automatically during sample detection to account for the spectral overlap among the dyes and to separate the raw fluorescent signals into individual dye signals.

7.2 Matched Normal Tissue Sample

Mononucleotide-repeat markers can show heterozygosity or variation in normal-tissue allele length between individuals (Bacher, 2004). To account for such variations in normal alleles, a matched normal tissue sample must be processed in parallel with every tumor sample. Additionally, review of the normal tissue sample can be important when assessing the tumor sample for subtle alterations in allele length. Tumor samples commonly comprise a mixture of normal and tumor cells. Novel tumor allele(s) may overlap stutter peaks from the normal cell component within the tumor sample, and the direct comparison to the normal tissue may be necessary to interpret that data. If analysis of a tumor or normal sample must be repeated for any reason, the paired tumor or normal sample must be rerun as well to account for possible run-to-run variations.

7.3 Positive and Negative Controls

Positive and negative (no-template) control amplification reactions using 2800M Control DNA and Water, Amplification Grade, respectively, must be analyzed concurrently with patient samples to verify assay performance. At least one 2800M Control DNA amplification reaction and one negative control amplification reaction must be included in each plate of patient samples. The negative control reaction is analyzed to ensure that no unexpected amplification occurred in no-template reactions. Unexpected amplification in a negative control reaction indicates DNA contamination leading to an invalid assay result. Negative controls should not have amplified peaks above the analytical threshold. The positive control reaction is analyzed to demonstrate that the amplification chemistry performed as expected. Unexpected results in a positive control may indicate a suboptimal or failed amplification. See Table 8 for expected 2800M Control DNA results.

Table 8. Expected Results Using 1ng of 2800M Control DNA.

Marker Name	2800M Alleles (bp)[1,2]
NR-21	101
BAT-26	115
BAT-25	124.5
NR-24	134
MONO-27	152.5
Penta C	178.5, 184
Penta D	195.5, 200.5

[1]Allele sizes were determined using the Applied Biosystems® 3500 Dx Genetic Analyzer with POP-7® polymer and a 50cm capillary. Sizes determined on other instruments or with different consumable configurations may vary slightly.

[2]Instrument-to-instrument and day-to-day variability in the performance of capillary electrophoresis instruments may result in an approximately ±1bp difference in the expected allele sizes for 2800M Control DNA.

7.4 Capillary Electrophoresis Standards

All analyzed samples and controls must contain Size Standard 500. Size Standard 500 contains a series of 21 DNA fragments of known lengths (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 500bp). Each fragment is labeled with WEN dye and is detected separately (as a fourth color, orange) in the presence of OncoMate™ MSI Dx Analysis System amplified products during capillary electrophoresis. The Size Standard 500 is used to assign sizes to the amplified products for each sample and control using the Local Southern method (Southern, 1979). The size standard controls for capillary-to-capillary variations in sizing precision during capillary electrophoresis and allows direct comparison of samples across the capillary electrophoresis run. Only the 60bp to 300bp fragments are analyzed using the selected fragment analysis software.

7.5 Analytical Threshold

An analytical threshold must be established and applied during data analysis with fragment analysis software to ensure confidence in MSI interpretation. Several methods to establish a suitable analytical threshold are available, and these may be found in published literature (e.g., Bregu, 2013). During performance evaluation of the OncoMate™ MSI Dx Analysis System, we determined the analytical threshold to be 175RFU using the upper limit approach, which is based on linear regression of peak intensities generated from samples containing different DNA inputs (Bregu, 2013).

8. Specific Limitations

Tumor content of tumor samples may affect the ability of fragment sizing software to identify an allele. Tumor content must be ≥20% and nucleated-cell abundance must be sufficient, based on standard pathological characterization. Tumor samples that do not meet these criteria are not suitable for use with the OncoMate™ MSI Dx Analysis System.

The OncoMate™ MSI Dx Analysis System was evaluated using input DNA purified from FFPE colon tissue samples using the Maxwell® CSC Instrument and Maxwell® CSC FFPE DNA Kit, followed by analysis of the amplification products using the Applied Biosystems® 3500 Dx Genetic Analyzer with GeneMapper® Software version 5.0. Before using other DNA extraction methods, capillary electrophoresis instruments, fragment analysis software or analysis methods, the user must determine that those methods are suitable for use with the OncoMate™ MSI Dx Analysis System.

Fragment sizing software has varying abilities to identify an altered tumor allele or microsatellite marker pattern. Compare the tumor sample to a normal tissue sample from the same patient for accurate interpretation.

The microsatellite loci included in the system are quasimonomorphic. In rare cases heterozygous allele patterns have been observed. Compare the tumor sample to a normal tissue sample from the same patient for accurate interpretation.

Instruments and instrument accessories used with the OncoMate™ MSI Dx Analysis System must be maintained according to the manufacturer recommendations and meet the criteria defined for use with this product.

9. Troubleshooting

For questions not addressed here, consult the user guide for the applicable instrument or accessory or contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com** or e-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Size Standard 500 fragments showed low resolution, fewer fragments than expected or low peak intensities, resulting in a Sizing Quality QC failure (amplicons also may be affected)	Bubbles were present in the instrument fluidics. Follow the instrument manufacturer's procedure for clearing the bubbles in the instrument fluidics, and then repeat capillary electrophoresis analysis for the affected samples, including positive and negative controls.
	Contaminants or crystal deposits were present in the polymer. When replenishing the polymer, ensure it is brought to room temperature as directed by the manufacturer. Repeat capillary electrophoresis analysis for the affected samples, including positive and negative controls.
	One or more capillaries were blocked. Refill the capillary array, and repeat capillary electrophoresis analysis for the affected samples, including positive and negative controls. You may need to install a new capillary array.
	Size Standard 500 was not added to the capillary electrophoresis loading cocktail. Prepare fresh capillary electrophoresis loading cocktail including Size Standard 500, and repeat capillary electrophoresis analysis for all samples and controls.
	The capillary tips were not in contact with loading cocktail. Ensure that 11µl of loading cocktail-sample mixture was added to each required well of the 96-well plate and that the plate was centrifuged briefly prior to starting the capillary electrophoresis run. Repeat capillary electrophoresis analysis for the affected samples, including positive and negative controls. Review the electropherogram to troubleshoot the root cause of the Sizing Quality QC flag.

Symptoms	Causes and Comments
Capillary electrophoresis run failed to start when prompted	Bubbles were present in the capillary electrophoresis instrument fluidics. Remove bubbles following the manufacturer's instructions. Restart the capillary electrophoresis run.
	An error occurred on the system computer. Reboot the capillary electrophoresis instrument and the instrument's computer following the manufacturer's instructions, and then restart the capillary electrophoresis run.
	One or more of the instrument consumables was expired or has reached the sample limit. Replace the expired or exhausted instrument consumable(s), and then restart the capillary electrophoresis run.
Unexpected peaks were detected in the Size Standard 500 dye channel, resulting in a Sizing Quality QC failure	Capillary electrophoresis-related artifacts. Aberrant peaks may be observed during capillary electrophoresis. When these affect the orange dye channel, the Size Standard 500 peaks may be obscured or the sizes assigned incorrectly by the fragment analysis software. Repeat capillary electrophoresis analysis for the affected samples, including positive and negative controls. Change capillary electrophoresis consumables if problem persists. Review the electropherogram to troubleshoot the root cause of the Sizing Quality QC flag.
Poor amplification or marker-to-marker imbalance in allele intensities was observed for the 2800M Control DNA amplification reaction (samples also may be affected)	The 2800M Control DNA stock solution or 2800M Control DNA dilution was not vortexed sufficiently. Low or excessive peak intensities may be observed, including excessive amplification of Penta D alleles. Repeat amplification reactions and capillary electrophoresis analysis, including positive and negative controls.
	The PCR amplification mixture was not vortexed sufficiently. Repeat amplification reactions and capillary electrophoresis analysis, including positive and negative controls.
	The 2800M Control DNA dilution was prepared previously and reused. Prepare a fresh dilution of 2800M Control DNA, and repeat amplification reactions and capillary electrophoresis analysis, including a freshly prepared positive control sample and a negative control.

Symptoms	Causes and Comments
Low peak heights for the normal or tumor samples. Tumor samples may have a Tumor Signal QC failure.	Insufficient template DNA. Low peaks heights can interfere with data interpretation. Allele peak intensities in the tumor samples must be high enough to ensure that new alleles are detected above the analytical threshold (see Section 6.3.10). Allele intensities for the normal sample must be ≥ the analytical threshold. Make sure DNA was accurately quantified and diluted, and repeat amplification using 1.0ng of template DNA if a quantification error is discovered. Otherwise, repeat the amplification with more DNA. If the problem persists, repeat the assay with DNA prepared from a different tissue section.
	Impure DNA template. Impurities in DNA samples may inhibit PCR. Consult the manufacturer's instructions for the selected DNA extraction method for guidance. You may need to change to a different DNA extraction method if sufficient DNA quality cannot be obtained. Performance evaluation of the OncoMate™ MSI Dx Analysis System was done using DNA purified from CRC FFPE tissue samples using the Maxwell® CSC Instrument and Maxwell® CSC FFPE DNA Kit. Other methods may not provide the same quality of DNA.
	Poor capillary electrophoresis injection (Size Standard 500 peaks also are affected). Repeat capillary electrophoresis analysis for affected samples and positive and negative controls.
	Poor-quality formamide was used. Use only Hi-Di™ 3500 Dx Series formamide.
	High salt concentration or altered pH. Do not dilute the FFPE DNA template in TE buffers. Use only nuclease-free water to dilute template DNA.
	Thermal cycler or reaction plate problems (positive control reaction also was affected). Confirm that the PCR program is correct and that the selected thermal cycler meets the requirements for the OncoMate™ MSI Dx Analysis System (see Section 3.5). Calibration of heat block may be required. Use only MicroAmp® plates.
	Samples were not properly denatured before capillary electrophoresis. Heat-denature samples for 3 minutes and cool on crushed ice or in an ice-water bath for at least 3 minutes immediately prior to CE.

Symptoms	Causes and Comments
Low peak heights for the normal or tumor samples. Tumor samples may have a Tumor Signal QC failure. (continued)	Poor-quality or degraded DNA. Improper or prolonged fixation of paraffin-embedded samples can result in low DNA yields and poor-quality DNA. Improper storage of the DNA following successful DNA extraction may also result in DNA degradation. Repeat the amplification with more DNA, or repeat DNA preparation using a different tissue section.
	Amplification reaction components were not added to the bottom of the well during PCR setup. Prior to thermal cycling, centrifuge the plate briefly in a mini plate centrifuge to bring contents to the bottom of the wells and remove air bubbles.
Fluorescent signal for allele peaks exceeded the dynamic range of the selected capillary electrophoresis instrument	Too much template DNA. Make sure DNA is accurately quantified and diluted. Repeat amplification using 1.0ng of template DNA if a quantification error is discovered. Otherwise, take one of the following approaches: 1) repeat the amplification reaction with less DNA, or 2) dilute amplification products 1:8 in loading cocktail (e.g., 1µl of amplification products plus 7µl of loading cocktail; see Table 4 for loading cocktail composition) and repeat capillary electrophoresis analysis using 1µl of the diluted amplification products. Ensure the DNA extraction method is suitable for FFPE tissue samples and the DNA has been stored appropriately for the method used.
	FFPE sample DNA was degraded or cross-linked. Degraded or cross-linked FFPE DNA templates may exhibit preferential amplification of smaller markers, particularly NR-21. Verify correct preparation conditions for FFPE samples and storage conditions for FFPE DNA extracts. One of the following approaches may be taken to address off-scale samples: 1) repeat the amplification reaction with less DNA, or 2) dilute amplification products 1:8 in loading cocktail (e.g., 1µl of amplification products plus 7µl of loading cocktail; see Table 4 for loading cocktail composition) and repeat capillary electrophoresis analysis using 1µl of the diluted amplification products. If problem persists, repeat the amplification with less DNA. You may need to repeat DNA preparation and analysis using a different tissue section.

Symptoms	Causes and Comments
Unexpected peaks were visible in one or more dye colors	Stutter peaks were mistaken for microsatellite alleles. Amplification of microsatellite markers generates artifacts that appear as smaller peaks 1bp above or below the prominent mononucleotide-repeat allele(s) or 1bp or 5bp above or below a pentanucleotide-repeat allele(s). Stutter product peak heights will be higher if too much template DNA was used.
	Excess amount of DNA. Use 1.0ng of DNA template. Amplification of >4ng of DNA template may result in elevated baseline artifacts in BAT-26 and a higher number of stutter peaks.
	Pull-up or bleedthrough. Pull-up, also known as bleedthrough, can occur when peak heights are excessive or if the quality of the spectral calibration was poor. Repeat spectral calibration of selected capillary electrophoresis instrument. Make sure DNA is accurately quantified and diluted. Repeat amplification using 1.0ng of template DNA. If problem persists, repeat the amplification with less DNA.
	Samples were not properly denatured prior to loading. Heat-denature samples for 3 minutes, and cool on crushed ice or in an ice-water bath for at least 3 minutes immediately prior to capillary electrophoresis.
	Capillary electrophoresis-related artifacts. Aberrant peaks may be observed during capillary electrophoresis. Ensure that polymer is warmed to room temperature before installation and use. Repeat injections. Repeat amplification and capillary electrophoresis if problem persists.
	Contamination of control or patient sample with another template DNA or amplified product. Cross-contamination can be a problem. Use aerosol-resistant pipette tips and change gloves regularly.
	An unstable tumor sample was mistakenly analyzed as a normal reference sample. Verify that samples were identified and labeled correctly. Repeat amplification and capillary electrophoresis analysis of affected samples to confirm suspected root cause.

Symptoms	Causes and Comments
Preferential amplification of smaller markers	DNA was cross-linked. DNA prepared from formalin-fixed, paraffin-embedded samples often is cross-linked with other DNA or protein molecules, preventing amplification of longer DNA fragments. Repeat amplification using more DNA. If saturation of smaller markers is observed with more DNA, repeat DNA extraction and sample analysis using a different tissue section. If problem persists, FFPE tissue sample quality may be too low for successful analysis.
	Degraded DNA. DNA template was degraded into smaller fragments, with the larger markers showing diminished yield. Verify correct storage conditions for 2800M Control DNA and FFPE DNA samples. Repeat amplification reaction using more DNA. If saturation of smaller markers is observed with more DNA, repeat DNA extraction and sample analysis using a different tissue section. If problem persists, FFPE tissue sample quality may be too low for successful analysis.
	Insufficient template DNA. Make sure DNA is accurately quantified and diluted, and repeat amplification using 1.0ng of template DNA if a quantification error is detected. If problem persists, repeat the amplification reaction with more DNA, or repeat DNA preparation using a different tissue section.
One or more pentanucleotide alleles present in the normal sample were absent from the tumor sample	Normal and tumor DNA samples from different individuals were analyzed as a sample pair. Ensure that normal or tumor samples were paired correctly on the PCR plate and during capillary electrophoresis analysis. Ensure samples were labeled correctly. Repeat capillary electrophoresis analysis of affected samples with proper sample pairing, including positive and negative control reactions. If problem persists, repeat amplification reactions.
	Tumor sample exhibits loss of heterozygosity. See Section 6.5.3 and Figure 13 for more information about interpreting data from these tumor samples.

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11. Additional Information

Contact information available at: **www.promega.com**; e-mail: **genetic@promega.com**

11.1 Appendix A

Spectral calibration settings used during performance evaluation of the OncoMate™ MSI Dx Analysis System with the Applied Biosystems® 3500 Dx Genetic Analyzer are shown below.

The following settings are suitable for completing the spectral calibration using Applied Biosystems® 3500 Series Genetic Analyzers:

Dye set template: AnyDye (3500 Series)

Dye order: 5, blue; 4, green; 3, yellow; 2, red; 1, orange (purple deselected)

Matrix Condition Number Upper Limit: 8.0

Locate Start Point After Scan: 300

Locate Start Point Before Scan: 5000

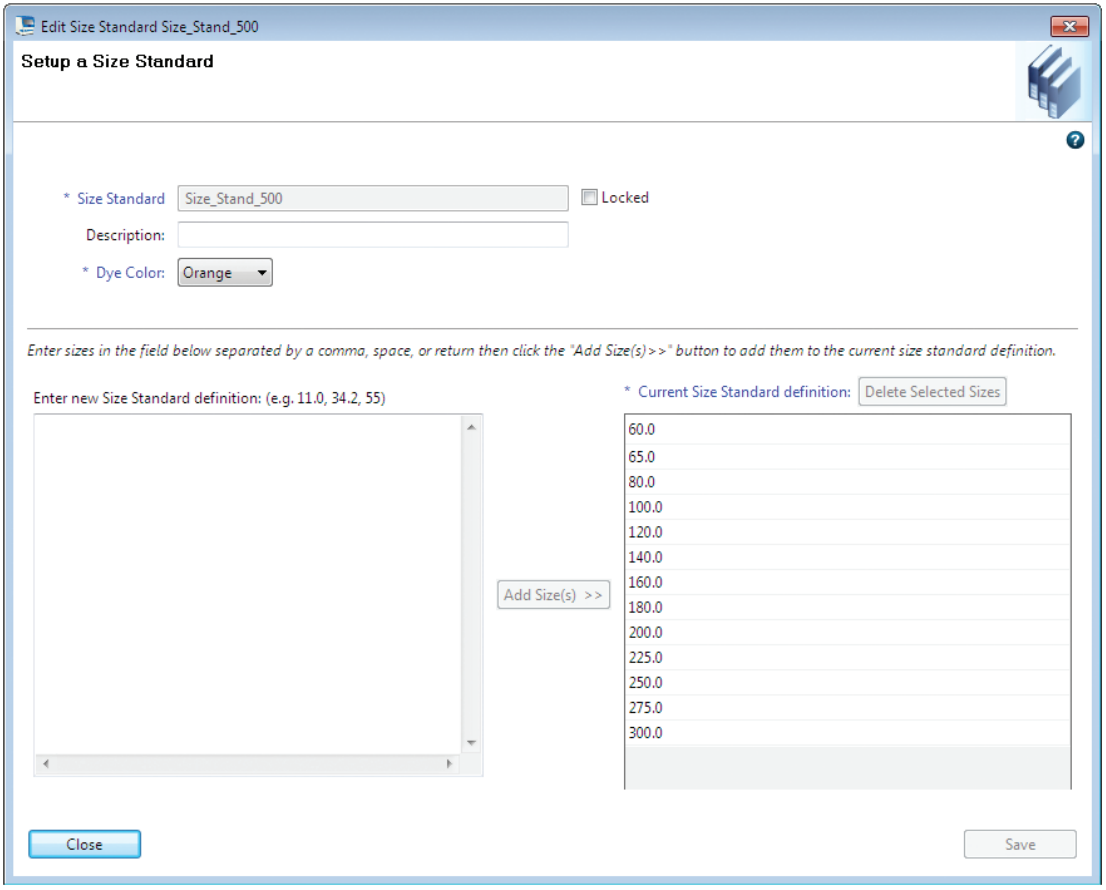
Limit Scans To: 6500

Sensitivity: 0.4

Minimum Quality Score: 0.95

11.2 Appendix B

Fragment analysis settings used during performance evaluation of the OncoMate™ MSI Dx Analysis System with the Applied Biosystems® 3500 Dx Genetic Analyzer are shown below.



16190TA

Figure 14. Capillary electrophoresis instrument "Size Standard" definition.

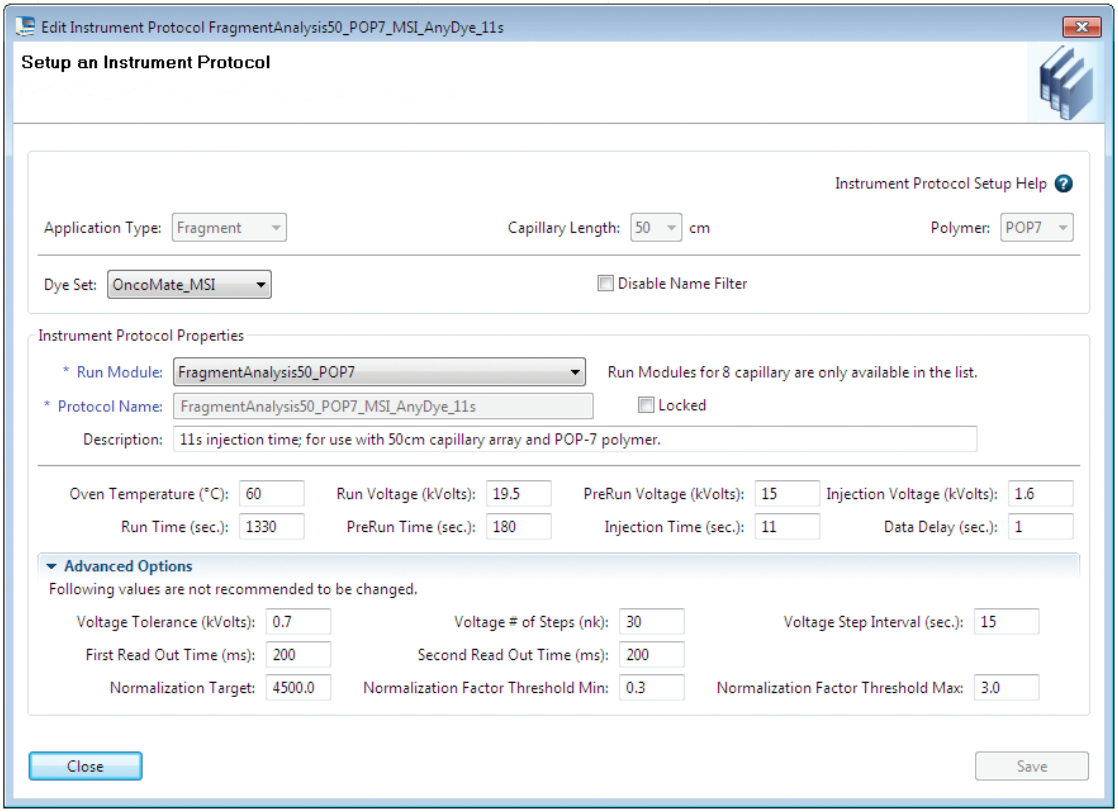


Figure 15. Capillary electrophoresis instrument "Instrument Protocol" settings.

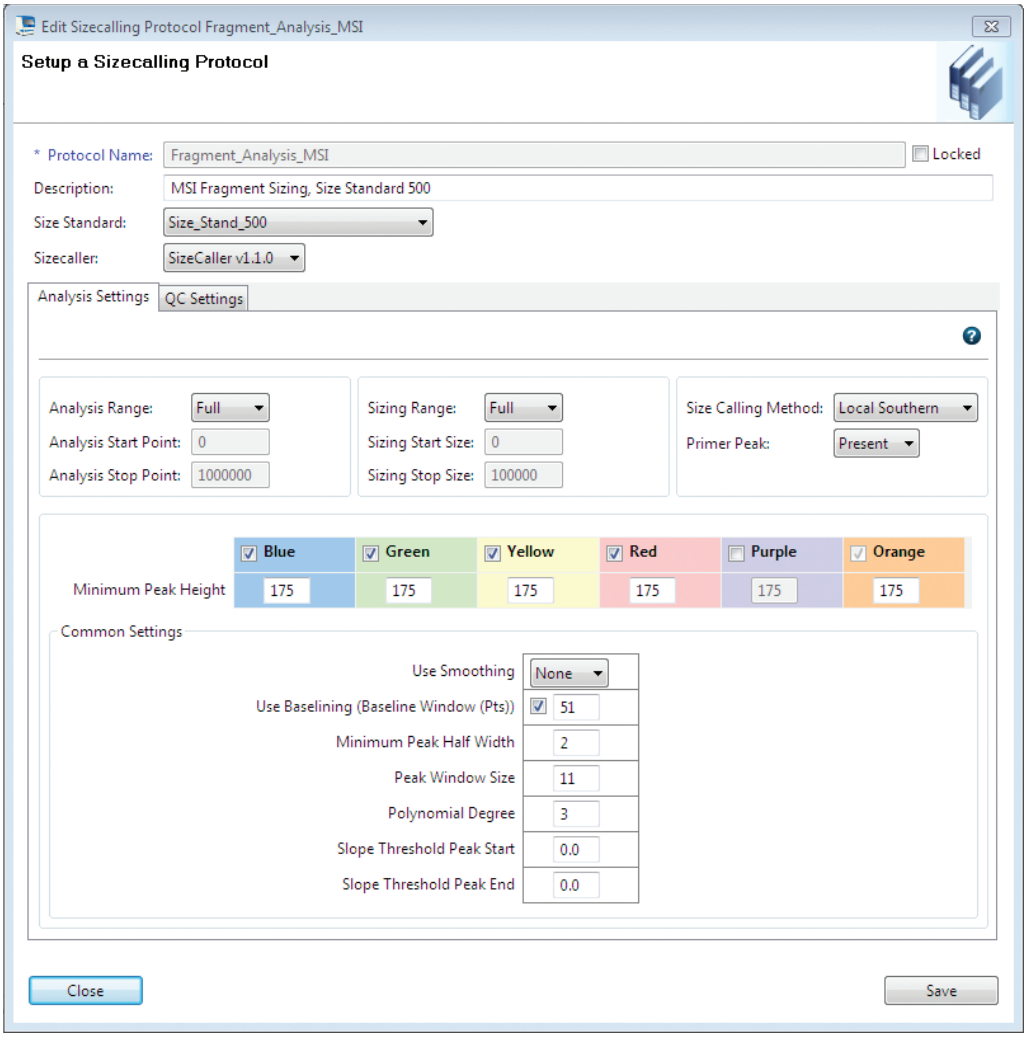


Figure 16. Capillary electrophoresis instrument "Sizecalling Protocol" settings.

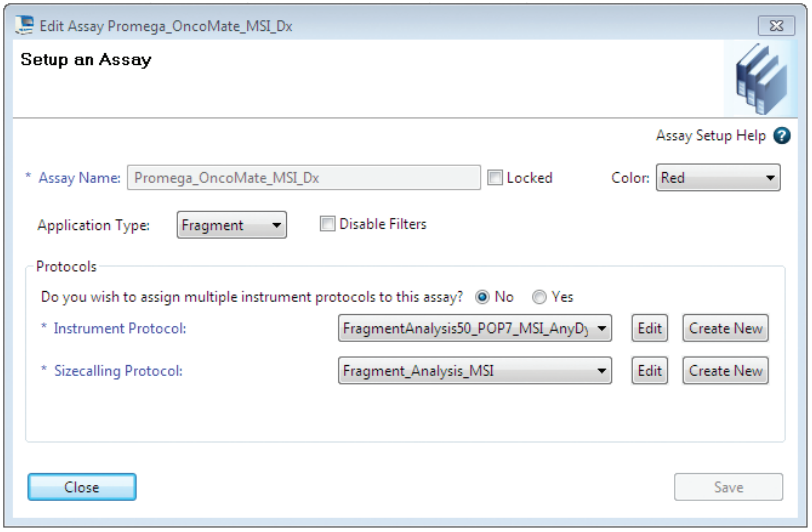


Figure 17. Capillary electrophoresis instrument "Assay" settings.

11.3 Appendix C

Data Analysis Settings for GeneMapper® 5.0 Used During Performance Evaluation of the OncoMate™ MSI Dx Analysis System.

Note: GeneMapper® setting files and data analysis instructions are provided for convenience and apply specifically to capillary electrophoresis data generated using a 50cm array and POP-7® polymer. Users analyzing OncoMate™ MSI Dx Analysis System amplified products with other capillary electrophoresis instrument configurations must verify the suitability of data analysis instructions for their specific instrument configuration. User-created analysis methods may be more suitable for a given instrument configuration.

Importing Settings Files for GeneMapper

To facilitate the analysis of data generated with the OncoMate™ MSI Dx Analysis System, we have created files (Panels, Analysis Method, Size Standard, Plot Settings, Table Settings and Report Settings) for import into GeneMapper® Software versions 5.0 and 6.0. These can be obtained from the Promega web site at: **www.promega.com/OncomatePanels**. Panels, Size Standard and Analysis Method definitions are also provided below (Tables 9 and 10, Figure 19). Refer to literature associated with GeneMapper® Software versions 5.0 and 6.0 for additional information.

1. Open the GeneMapper® Software, version 5.0 or 6.0.
2. Select **Tools** and then **Panel Manager**.
3. Select **Panel Manager** in the upper left pane (Figure 18).
4. From the menu, select **File**, then **Import Panels**. The Panel Manager icon must be selected (highlighted blue) for the **Import Panels** option to be available for selection.
5. Navigate to the file location where the panels text file was saved on your computer. Select the file **OncoMate_ MSI_Dx_Panels_v1.0**, then **Import**.
6. At the bottom of the Panel Manager window, select **Apply** then **OK**.
7. Select **Tools** and then **GeneMapper Manager**.
8. Select the "Size Standards" tab, and then select **Import** at the bottom of the GeneMapper Manager window.
9. Navigate to the file location where the Size Standard file was saved on your computer. Select the file **OncoMate_ MSI_Dx_Size_v1.0**, then **Import**.
10. With the GeneMapper Manager still open, repeat the process described in Steps 8 and 9 to import the remaining settings files (Analysis Method, Plot Settings, Table Settings and Report Settings).
11. When all settings files are imported, select **Done** at the bottom of the GeneMapper Manager window.

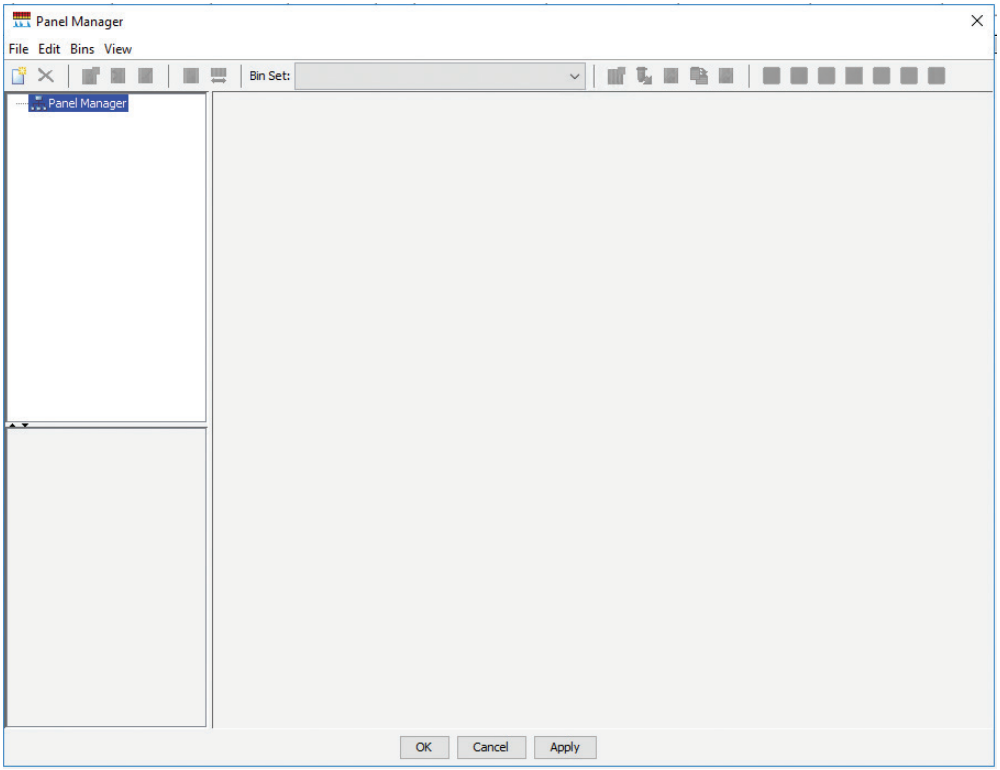


Figure 18. The GeneMapper® Panel Manager window, with the Panel Manager icon selected. The "Import Panels" option (Step 4) is accessible from the "File" menu when the Panel Manager icon is selected (highlighted in blue).

Table 9. Marker Panel definition.

Marker	Dye Channel	Panel Range (bp)[1]		Repeat Type[2]
		Start	End	
BAT-26	blue	83	121	1
Penta D	blue	123	253	5
NR-21	green	83	108	1
BAT-25	green	110	132	1
MONO-27	green	134	168	1
NR-24	yellow	103	138	1
Penta C	yellow	140	228	5

[1]The expected size range for amplicons in each marker must be defined in the selected fragment analysis software.

[2]The microsatellite type is defined in this table as 1 (for mononucleotide-repeat markers) and 5 (for pentanucleotide-repeat markers).

Table 10. Size Standard definition. The Size Standard 500 contains fragment peaks from 60bp to 500bp, although analysis of the 60bp through 300bp fragment peaks is sufficient for sizing of OncoMate™ MSI Dx Analysis System fragments.

Size Standard Fragment #	Fragment Size (bp)
1	60
2	65
3	80
4	100
5	120
6	140
7	160
8	180
9	200
10	225
11	250
12	275
13	300

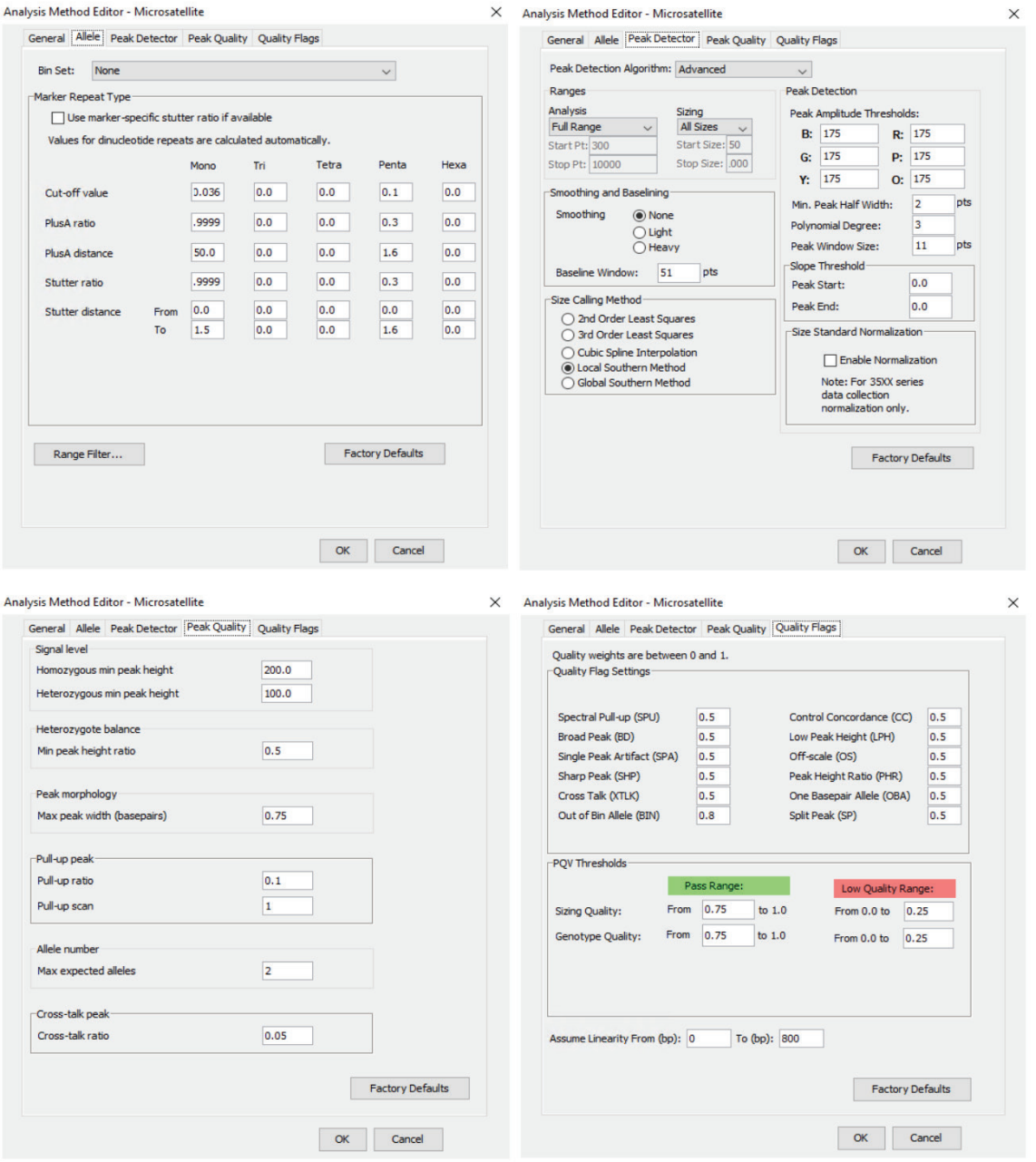


Figure 19. Analysis settings used for performance evaluation of the OncoMate™ MSI Dx Analysis System.

[a] U.S. Pat. No. 9,139,868, European Pat. No. 2972229 and other patents pending.

[b] TMR-ET, CXR-ET and WEN dyes are proprietary.