



TECHNICAL MANUAL

MSI Analysis System, Version 1.2

Instructions for Use of Product
MD1641

For Research Use Only. Not for use in diagnostic procedures

MSI Analysis System, Version 1.2

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Manual.
 E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

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1. Description

1.A. The MSI Analysis System

Microsatellites are short, tandem repeated DNA sequences 1–6 base pairs in length. These repeats are distributed throughout the genome and often vary in length from one individual to another due to differences in the number of tandem repeats at each locus. Microsatellite markers can be used to detect a form of genomic instability called microsatellite instability (MSI). MSI is a change in length of a microsatellite allele due to insertion or deletion of repeat units during DNA replication and failure of the DNA mismatch repair system to correct these errors.

The MSI Analysis System, Version 1.2, is a fluorescent PCR-based assay to detect MSI in human cells. Typically, MSI analysis involves comparing allelic profiles of microsatellite markers generated by DNA amplification from matching normal and test samples, which may be mismatch-repair (MMR) deficient. Alleles that are present in the test sample but not in corresponding normal samples indicate MSI. The MSI Analysis System includes fluorescently labeled primers for co-amplification of seven markers including 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; Figure 1 and Table 1). The mononucleotide markers are used for MSI determination, and the pentanucleotide markers are used to detect potential sample mixups or contamination. An internal lane size standard (Figure 2) is added to the amplified samples to assure accurate sizing of alleles and adjust for run-to-run variation. The PCR products are separated by capillary electrophoresis (CE), and the output data may be analyzed with fragment analysis software, such as GeneMapper® software (Applied Biosystems), to determine MSI status of test samples. To simplify data analysis, there are available panels and bins text files that automatically assign genotypes using GeneMapper® software. See Section 7 for additional information on data analysis.

Note: If you have questions or need support with capillary electrophoresis instruments or configurations, contact Promega Technical Services at: genetic@promega.com

Table 1. The MSI Analysis System Locus Information.

Marker Name	Major Repeat Sequence	Size Range (bp) ¹	2800M Alleles (bp) ²	Primer Dye ³
NR-21	(A) ₂₁	80–105	102	JOE
BAT-26	(A) ₂₆	80–118	115	FL
BAT-25	(A) ₂₅	107–129	124	JOE
NR-24	(A) ₂₄	100–135	132	TMR
MONO-27	(A) ₂₇	131–165	151	JOE
Penta C	(AAAAG) _{3–15}	137–225	175, 180	TMR
Penta D	(AAAAG) _{2–17}	120–250	182, 187	FL

¹Rare alleles outside of these size ranges may exist. Allele sizes may vary when using different polymers or instrument configurations.

²Allele sizes for 2800M Control DNA were determined using the Applied Biosystems® 3500 Genetic Analyzer with POP-7® polymer and a 50cm capillary.

³TMR = carboxy-tetramethylrhodamine; FL = fluorescein; JOE = 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

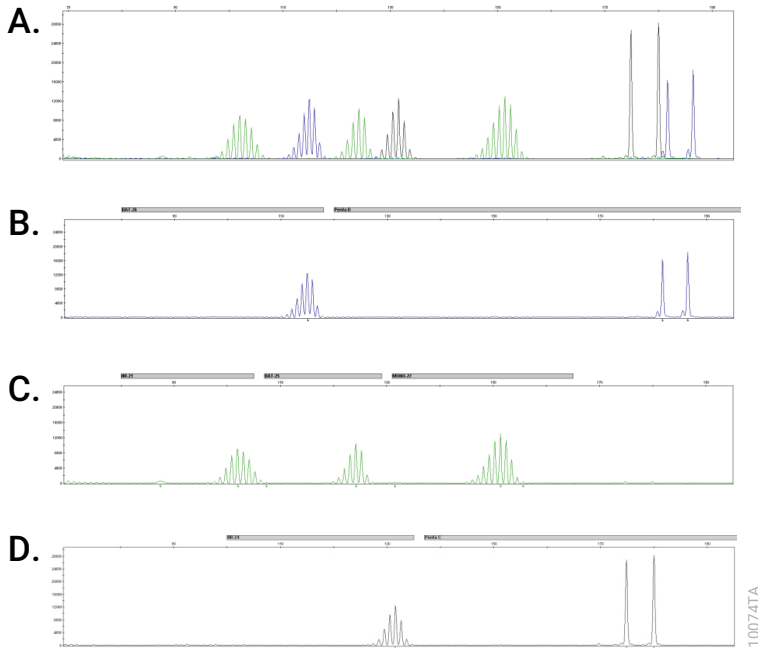


Figure 1. The MSI Analysis System, Version 1.2. A single normal genomic DNA template (2800M Control DNA; 1–2ng) was amplified using the MSI Analysis System, and the PCR products were analyzed using an Applied Biosystems® 3500 Genetic Analyzer with POP-7® polymer and a 50cm capillary array. **Panel A.** An electropherogram showing the five mononucleotide loci and two pentanucleotide loci. **Panel B.** An electropherogram showing peaks of the fluorescein-labeled loci: BAT-26 and Penta D. **Panel C.** An electropherogram showing the peaks of JOE-labeled loci: NR-21, BAT-25 and MONO-27. **Panel D.** An electropherogram showing the peaks of the TMR-labeled loci: NR-24 and Penta C.

The mononucleotide repeat markers included in the MSI Analysis System were selected for high sensitivity and specificity to alterations in samples containing mismatch repair defects. These mononucleotide repeat markers are quasi-monomorphic; that is, almost all individuals are homozygous for the same common allele for a given marker (see Section 9.D for allele frequencies). Using monomorphic markers simplifies data interpretation. The pentanucleotide repeat markers were selected for their high level of polymorphism and low degree of MSI to uniquely identify samples, helping to confirm that the test sample and the paired normal samples are from the same individual. The pentanucleotides should **not** be used for determining microsatellite instability.

1.B. The Internal Lane Standard 600

The Internal Lane Standard 600 (ILS 600) contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length (Figure 2). Each fragment is labeled with carboxy-X-rhodamine (CXR) and is detected separately (as a fourth color) in the presence of MSI Analysis System-amplified material. The ILS 600 is used in each CE injection to increase precision in analysis when using the MSI Analysis System.

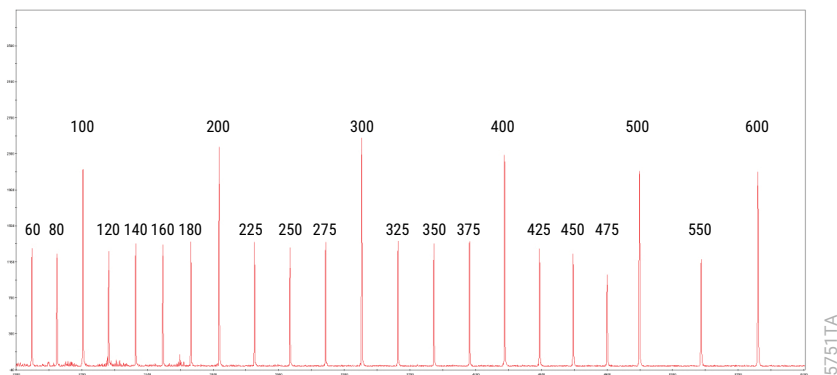


Figure 2. Internal Lane Standard 600.



2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
MSI Analysis System, Version 1.2	100 reactions (50 reaction pairs)	MD1641

For Research Use Only. Not For Use In Diagnostic Procedures. Each system contains sufficient reagents to perform 100 reactions (50 reaction pairs). Includes:

Pre-amplification Components Box

- 1 × 100µl MSI 10X Primer Pair Mix
- 1 × 300µl Gold ST★R 10X Buffer
- 1 × 1.25ml Nuclease-Free Water
- 1 × 25µl 2800M Control DNA (10ng/µl)

Post-amplification Components Box

- 1 × 150µl Internal Lane Standard 600

Storage Conditions: Store all components at –30°C to –10°C. The MSI 10X Primer Pair Mix and Internal Lane Standard 600 are light-sensitive and must be stored in the dark.

Available Separately

PRODUCT	SIZE	CAT. #
PowerPlex® 4C Matrix Standard	5 preps	DG4800

Not for Medical Diagnostic Use.

3. DNA Extraction Methods

Obtaining sufficient high-quality DNA from formalin-fixed, paraffin-embedded (FFPE) tissues can be problematic. DNA is often degraded due to prolonged or unsuitable fixation of the tissue sample before embedding in paraffin. Promega offers automated and manual genomic DNA extraction systems for purifying high-quality DNA from FFPE tissue sections. The Maxwell® RSC DNA FFPE Kit (Cat.# AS1450) provides an easy, automated method for purifying genomic DNA from FFPE tissue samples without the use of hazardous organic solvents, such as xylene. The ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2352) provides a flexible, all-inclusive manual method for purifying genomic DNA from FFPE tissue without hazardous solvents or overnight digestion. Please contact your local Promega Branch Office or Distributor (contact information available at: www.promega.com) or e-mail: genetic@promega.com for more information.

4. DNA Amplification Using the MSI Analysis System

Materials to Be Supplied by the User

- thermal cycler (see specific information on instruments and thermal cycling conditions in Section 9.A)
- microcentrifuge
- 0.2ml (thin-walled) microcentrifuge tubes, MicroAmp® reaction tube strips or MicroAmp® optical 96-well reaction plates (Applied Biosystems)
- 1.5ml microcentrifuge tubes
- aerosol-resistant pipette tips
- GoTaq® MDx Hot Start Polymerase Cat.#D6001 [or similar hot-start Taq DNA Polymerase at $\geq 5\text{u}/\mu\text{l}$ concentration such as AmpliTaq Gold® DNA polymerase (Life Technologies Cat.# N8080242)]

The MSI Analysis System is optimized to amplify 1–2ng of genomic DNA in a 10 μl reaction volume using the protocols detailed below. However, input DNA amounts should be optimized to adjust for variations in DNA yield and quality due to differences in samples, DNA isolation methods or both. Using excessive amounts of DNA template may result in peak heights exceeding the linear detection range of the CE instruments. Using insufficient DNA template can result in low PCR yields, and peak heights may fall below detection limits (50–175RFU). Accurately quantitating template DNA is important. We recommend quantitating the FFPE template DNA using the Promega QuantiFluor® ONE dsDNA System (Cat.# E4870, E4871) or QuantiFluor® dsDNA System (Cat.# E2670). Testing at Promega has shown that DNA quantitation using the QuantiFluor® ONE dsDNA System with the Quantus™ Fluorometer provided results more similar to those of qPCR from DNA samples extracted from three FFPE tissue types as compared to the NanoDrop® 2000 instrument (1).

4.A. Amplification Setup

Note: We strongly recommend using gloves and aerosol-resistant pipette tips to prevent cross contamination. We recommend keeping all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup. We recommend that every time a microcentrifuge tube is vortexed, briefly centrifuge the tubes at maximum speed. The protocol provided below is an example that uses 2 μl of sample with 8 μl of amplification mix. The reaction can accommodate more DNA template input if needed. To add a larger DNA template volume, the water and amplification mix volumes need to be adjusted accordingly (e.g., 4 μl of sample with 6 μl of amplification mix contains 3.85 μl of water per reaction).

1. Thaw the Gold ST★R 10X Buffer, MSI 10X Primer Pair Mix and Nuclease-Free Water.
2. Mix these reagents by vortexing for 5–10 seconds before each use. A precipitate may form in the Gold ST★R 10X Buffer. If this occurs, warm the buffer briefly at 37°C, then vortex until the precipitate is in solution.
3. To prepare the Amplification Mix, determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for losses during pipetting.
4. Place one 0.2ml microcentrifuge tube for each reaction into a rack, and label appropriately. Alternatively, use MicroAmp® optical 96-well reaction plates.

- Table 2 shows the component volumes per sample when using a DNA template volume of 2µl in a 10µl reaction volume. If a different template volume is required, the water volume should be adjusted accordingly.

Table 2. Amplification Mix for the MSI Analysis System

Component	Volume Per Reaction
Nuclease-Free Water	5.85µl
Gold ST★R 10X Buffer	1.00µl
MSI 10X Primer Pair Mix	1.00µl
Hot-start Taq DNA polymerase (5u/µl)	0.15µl
Total reaction volume	8.00µl

Note: Pipetting volumes smaller than 1µl is inaccurate. We recommend preparing enough Amplification Mix to avoid pipetting such small volumes.

- Combine the final volumes of Nuclease-Free Water, Gold ST★R 10X Buffer, MSI 10X Primer Pair Mix and hot-start DNA polymerase in a sterile 1.5ml tube. Mix gently.
- Transfer 8µl of Amplification Mix to the bottom of each reaction tube or well.
- Pipet 2µl of template DNA (1–2ng) for each sample into the bottom of the appropriate tube or well containing Amplification Mix.

Notes:

- The MSI Analysis System was optimized using 1–2ng of DNA template. Further optimization of template input quantity may be required to accommodate your laboratory’s samples and chosen quantification method. Highly crosslinked or degraded samples may require higher template quantities. Template DNA should be mixed well before transferring to the tube or well containing Amplification Mix.
 - Store DNA templates in nuclease-free water or TE⁻⁴ buffer [10mM Tris HCl (pH 8.0), 0.1mM EDTA]. If the template DNA is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, DNA sample volume should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations, depending on the source of template DNA and the extraction procedure used.
- For the positive amplification control, dilute the 2800M Control DNA 0.5–1ng/µl in Nuclease-Free Water. Pipet 2µl of the diluted DNA into the bottom of the tube or well containing Amplification Mix. Mix by pipetting several times.
 - For the negative amplification control, pipet Nuclease-Free Water (instead of template DNA) into a microcentrifuge reaction tube or well containing Amplification Mix. Mix by pipetting several times. We recommend briefly centrifuging reaction tubes or plates following reaction set up to ensure liquids are at the bottom of the wells prior to amplification.

Note: Use the same volume of positive or negative control as template DNA sample, if possible.

4.B. Amplification Thermal Cycling

Thermal cycling with the MSI Analysis System, Version 1.2, requires modified ramp rates for optimal amplicon balance. Additional examples of cycling protocols can be found in Section 9.A. Using other thermal cyclers may require optimizing ramp times or ramp speeds.

For faster cycling without optimization, a complete 5X GoTaq® Multiplex Master Mix can be used with the MSI Analysis System, Version 1.2, and is available separately for purchase. Contact your local Promega representative or contact genetic@promega.com for more information.

1. Place the tubes or MicroAmp® optical 96-well reaction plates in a thermal cycler.
2. Select and run the recommended protocol. See Section 9.A for example thermal cycler conditions.
3. After the thermal cycling protocol is complete, store samples at 4°C overnight or -20°C for longer, protected from light.

Notes:

- a. Long-term storage of amplified samples at 4°C or higher can produce artifacts.
- b. DNA quantity and quality affect PCR yield. Use the recommended DNA purification methods and accurate quantitation to minimize problems. Cycle number may be modified to adjust for variations in DNA amount but should not exceed 32 cycles.

5. Capillary Electrophoresis

5.A. Spectral Calibration

The PowerPlex® 4C Matrix Standard (Cat.# DG4800) is required for spectral calibration prior to use of the MSI Analysis System, Version 1.2, on all CE instruments. This Matrix Standard uses the F DyeSet configuration on Applied Biosystems® instruments or Promega 4-dye configuration on the Spectrum Compact CE System. For protocols and additional information about spectral calibration, see the *PowerPlex® 4C Matrix Standard Technical Manual #TMD048*, available online at:

www.promega.com/protocols/

Proper spectral calibration is critical to evaluate multicolor fragment analysis systems. For answers to questions about spectral calibration or additional protocols for other instruments, contact Promega Technical Services at:

genetic@promega.com

5.B. Sample Preparation

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- aerosol-resistant pipette tips
- crushed ice or an ice-water bath
- 96-well plate or strip tube and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)



The quality of formamide is critical. Use only the recommended formamide. Freeze formamide in aliquots at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.



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.

Note: The Internal Lane Standard 600 is included in each well to standardize analysis of amplified samples and uses a fourth color.

1. Prepare a loading cocktail by combining and mixing the Internal Lane Standard 600 (ILS 600) and formamide as follows:
$$[(0.5\mu\text{l ILS 600}) + (9.5\mu\text{l formamide})] \times (\# \text{ wells})$$

Note: Add 1–2 additional wells into the calculation above to account for pipetting errors when preparing the loading cocktail. The volume of internal lane standard (ILS) used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. However, this ILS volume adjustment is not usually necessary when adequate injection times are used.
2. Mix for 10–15 seconds using a vortex mixer.
3. Pipet 10µl of formamide/ILS mix prepared in Step 1 into individual wells for each amplified sample or control.
4. Add 1µl of amplified sample to the wells containing formamide/ILS. Each well to be included in the injection must contain formamide or other manufacturer-recommended liquid, regardless of if whether it will be used to collect data. This ensures the injection occurs properly. Cover wells with appropriate septa.
5. Centrifuge plate briefly to remove air bubbles from the wells if necessary.
6. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument

6. CE Instrument Setup

6.A. Detecting Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers

Refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide* for the instrument maintenance schedule and instructions on how to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide*. Below is an abbreviated description of set up on this instrumentation.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch. Set the oven temperature to 60°C, and then select **Start Pre-Heat**. Once the Oven Temperature and Detection Cell Temperature values turn green, proceed with the first injection.
2. Prior to the first analysis using the MSI Analysis System Version 1.2, you must create an Instrument Protocol, Size Standard, Sizing Protocol and Assay.
 - 2.a. To create a new Instrument Protocol, navigate to the Library, select **Instrument Protocols**, and create a new Instrument Protocol. Select Application Type as **Fragment**, then select the Capillary Length and Polymer consistent with the desired configuration. Select the DyeSet created in Section 5.A. In Run Module, select the module that is consistent with the instrument configuration used (e.g., if running POP-7® on a 50cm array, select **FragmentAnalysis50_POP7**). Name the protocol a descriptive name and then save.

Note: Sensitivities of instruments may vary. Default run conditions are a good starting point but the injection time and voltage may be adjusted in the Instrument Protocol. Signal should be within the dynamic range of the 3500 Series Genetic Analyzers. Saturation on this instrument series occurs at greater than ~25,000RFU. A target of 5,000–10,000RFU for the mononucleotide markers with control DNA is a reasonable target; however, the user may need to optimize conditions to minimize artifacts and maximize signal.

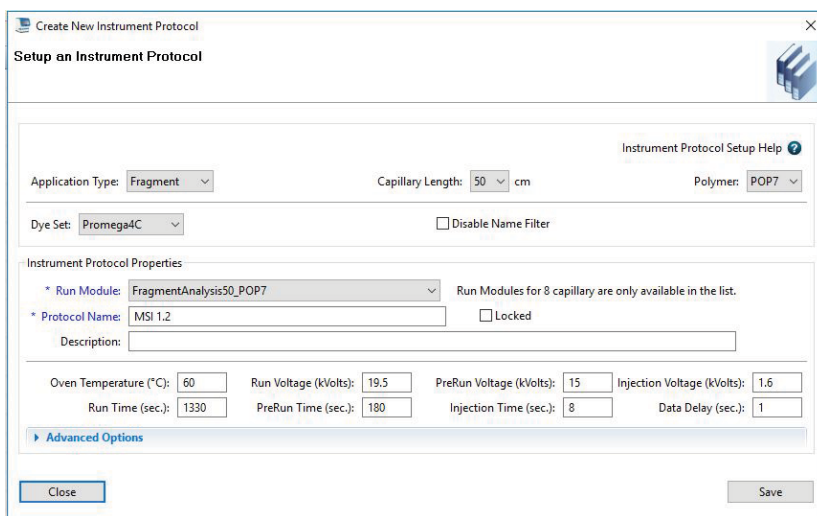


Figure 3 shows the 'Create New Instrument Protocol' dialog box. The 'Setup an Instrument Protocol' section includes: Application Type: Fragment, Capillary Length: 50 cm, Polymer: POP7, Dye Set: Promega4C, and a 'Disable Name Filter' checkbox. The 'Instrument Protocol Properties' section includes: Run Module: FragmentAnalysis50_POP7, Protocol Name: MSI 1.2, and a 'Locked' checkbox. The 'Description' field is empty. The 'Parameters' section includes: Oven Temperature (°C): 60, Run Voltage (kVolts): 19.5, PreRun Voltage (kVolts): 15, Injection Voltage (kVolts): 1.6, Run Time (sec.): 1330, PreRun Time (sec.): 180, Injection Time (sec.): 8, and Data Delay (sec.): 1. There is an 'Advanced Options' section and 'Close' and 'Save' buttons.

Figure 3. Default instrument protocol for the Applied Biosystems® 3500xL Genetic Analyzer using POP-7® polymer and a 50cm capillary array.

6.A. Detecting Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzers (continued)

- 2.b. To create a new Size Standard for the Sizecalling protocol, navigate to the Library. Select **Size Standards**, and then select **Create**.

Assign the Size Standard a descriptive name such as "ILS 600". Choose **Red** as the Dye Color. The fragments in the size standard are 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases.

- 2.c. To create a new Sizecalling Protocol, navigate to the Library. Select **Sizecalling Protocols**, and then select **Create**.

Assign a descriptive protocol name. Select the size standard created in Step 2.b. Leave all other settings as default or optimize if desired.

- 2.d. To create a new Assay, navigate to the Library. Select **Assays**, and then select **Create**.

In the 'Create New Assay' window, select the Instrument Protocol created in Step 2.a and the Sizecalling Protocol created in Step 2.c. Assign a descriptive assay name. Select the application type **Fragment**. An Assay is required for all named samples on a plate.

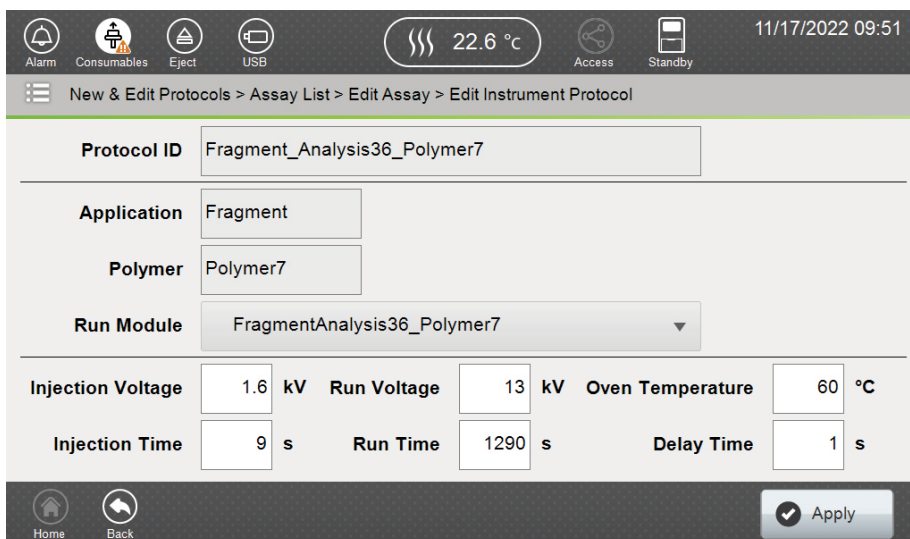
3. To create a New Plate, navigate to the Library, and from the Manage menu, select **Plates** and then **Create**.
4. Assign a descriptive plate name. Select the plate type as **Fragment** from the drop-down menu and select the Capillary Length and Polymer configuration.
5. Select **Assign Plate Contents**.
6. Assign names to wells including samples or controls.
7. In the lower left portion of the screen, under Assays, use the 'Add from Library' option to select the Assay created in Step 2.d. Choose the **Add to Plate** button, and close the window.
8. Highlight the named wells including samples and controls, and then select the Assay to assign it to the wells. Alternatively, select **Table View** and then select the Assay from the Assay drop down field.
9. Select **Link Plate for Run**.
10. The 'Load Plate' window will appear. Select **Yes**.
11. In the 'Run Information' window, assign a Run Name. Select **Start Run**.

6.B. Detecting Amplified Fragments Using the Promega Spectrum Compact CE System

Refer to the instrument user manual for instructions on required consumables, cleaning, installing the capillary array, performing a spatial calibration and adding polymer. Analyze the samples as described in the *Spectrum Compact CE System Operating Manual #TMD058*. Below is an abbreviated description of set up on this instrumentation.

1. Preheat the instrument to 60°C for at least 30 minutes prior to sample run.
2. Prior to the first analysis using the MSI Analysis System Version 1.2, you must create an Instrument Protocol, Size Standard, Sizecalling Protocol and Assay.
 - 2.a. Create an Instrument Protocol by selecting **Instrument Protocols** in the 'New & Edit Protocols' screen. Select **Create** in the footer. In the 'New Instrument Protocol' screen, enter a unique Protocol ID, select **Fragment** as the application then define the Polymer type and array length. In the Run Module drop-down menu, select the module that is consistent with the instrument configuration used (e.g., if running Polymer 7 and a 36cm array, select **Fragment_Analysis36_Polymer7**). An example of the default conditions for Polymer 7 and 36cm array are shown below in Figure 4.

Note: Sensitivities of instruments may vary. Default run conditions are a good starting point but the injection time and voltage may be adjusted in the Instrument Protocol. Signal should be within the dynamic range of the Spectrum Compact CE Instrument. Saturation on this instrument series occurs at greater than 25,000RFU. A target of 5,000–10,000RFU for the mononucleotide markers with control DNA is reasonable target; however, the user may need to optimize conditions to minimize artifacts and maximize signal.



The screenshot shows the 'Edit Instrument Protocol' screen with the following settings:

- Protocol ID:** Fragment_Analysis36_Polymer7
- Application:** Fragment
- Polymer:** Polymer7
- Run Module:** FragmentAnalysis36_Polymer7
- Injection Voltage:** 1.6 kV
- Run Voltage:** 13 kV
- Oven Temperature:** 60 °C
- Injection Time:** 9 s
- Run Time:** 1290 s
- Delay Time:** 1 s

The interface includes a top status bar with icons for Alarm, Consumables, Eject, USB, a temperature display of 22.6 °C, Access, and Standby, and a date/time display of 11/17/2022 09:51. A bottom navigation bar contains Home, Back, and an Apply button.

Figure 4. Default Instrument Protocol Settings for the Spectrum Compact CE System with Polymer 7 and a 36cm array.

6.B. Detecting Amplified Fragments Using the Promega Spectrum Compact CE System (continued)

- 2.b. Select **Sizecalling Protocols** in the 'New & Edit Protocols' screen. Select **Create** in the footer. Add a unique Protocol ID. In the 'Size Standard' tab, select **ILS600** from the drop-down menu. Other settings can be left as default or optimized if desired.
- 2.c. Select **Assay** in the 'New & Edit Protocols' screen. Select **Create** in the footer. Add a unique Protocol ID, select **Fragment** as the Application. Select the Polymer type in the drop-down menu. Select the Dye Set created/used for spectral calibration in Section 5.A. Then select the Instrument Protocol and Analysis Protocol created in Steps 2.a and 2.b from the respective drop-down menus.

Note: Edits made to the Instrument or Analysis Protocol from the 'Edit Assay' screen do not change the parameters of those protocols stored in the library. Changes are only stored with the specific assay.
3. Select **Fragment Analysis** from the 'Main Menu' screen. Enter a unique run ID, if desired. Follow on screen prompts to assign sample details to a new strip. When editing the strip information, select the Assay, Dye Set, Instrument Protocol and Analysis Protocol created in Step 2.

6.C. Detecting Amplified Fragments Using the Applied Biosystems® 3130 or 3130x/ Genetic Analyzer

Refer to the instrument user manual for instructions on required consumables, cleaning, installing the capillary array, performing a spatial calibration and adding polymer. Analyze the samples as described in the user manual for the Applied Biosystems® 3130 or 3130x/ Genetic Analyzer. Below is an abbreviated description of set up on this instrumentation.

1. In the Module Manager, select **New**. Select **Regular** in the Type drop-down list, and select the fragment analysis module default for the configuration used (e.g., **FragmentAnalysis50_POP7** or **FragmentAnalysis36_POP4**) in the Template drop-down list. Give a descriptive name to your run module, and select **OK**.

Notes:

- a. If using 36cm, POP-4® configuration, lengthen the run time to 2,000 seconds.
 - b. Sensitivities of instruments may vary. Default run conditions are a good starting point but the injection time and voltage may be adjusted in the Module Manager. Signal should be within the dynamic range of the Applied Biosystems® 3130 Series Genetic Analyzers. Saturation on this instrument series occurs at greater than 7,000RFU. A target of 1,000–3,000RFU for the mononucleotide markers with control DNA is reasonable target; however, the user may need to optimize conditions to minimize artifacts and maximize signal.
2. In the Protocol Manager, select **New**. Type a name for your protocol. Select **Regular** in the Type drop-down list, and select the run module you created in the previous step in the Run Module drop-down list. Lastly, choose **F** in the Dye-Set drop-down list. Select **OK**.
 3. In the Plate Manager, create a new plate record as described in the instrument user manual. In the dialog box that appears, select **GeneMapper—Generic** in the Application drop-down list, and select the appropriate plate type (96-well). Add entries in the owner and operator windows, and select **OK**.
Note: If autoanalysis of sample data is desired, refer to the instrument user manual for instructions.
 4. In the GeneMapper® plate record, enter sample names in the appropriate cells. Scroll to the right. In the Results Group 1 column, select the desired results group. In the Instrument Protocol 1 column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select **OK**.
Note: To create a new results group, select **New** in the drop-down menu in the Results Group column. Select the 'General' tab, and enter a name. Select the 'Analysis' tab, and select **GeneMapper—Generic** in the Analysis type drop-down list.
 5. Place plate containing samples in the instrument and close the instrument doors.
 6. In the spectral viewer, confirm that dye set F is active, and set the correct active calibration for dye set F.
 7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.
 8. Once the plate record is highlighted, select the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.
 9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green **Run Instrument** arrow becomes enabled.
 10. Select the green **Run Instrument** arrow on the toolbar to start the sample run.

7. Data Analysis

7.A. MSI Analysis Overview

Detecting MSI is based on comparing allelic profiles generated by amplifying matching normal and test sample DNA. The appearance of novel alleles in the test sample indicates microsatellite instability (Figure 5). The MSI Analysis System co-amplifies and detects a panel of microsatellite markers that have been shown to be sensitive and specific for detecting MSI-H samples with mismatch repair deficiencies. Specific PCR product sizes and the respective fluorescent dyes used for labeling of each microsatellite marker are described in Table 1.

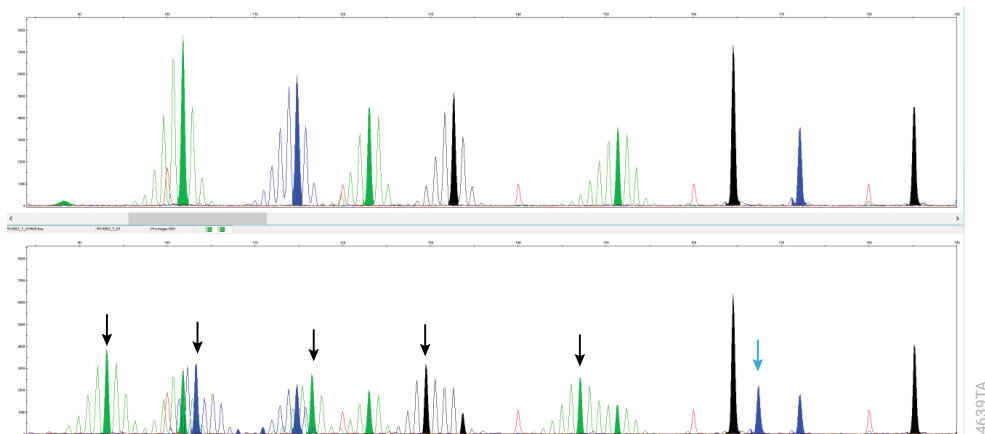


Figure 5. Microsatellite instability assays using the MSI Analysis System. Matching normal sample (top panel) and MSI-H test sample (bottom panel) were analyzed using the MSI Analysis System. Two nanograms of genomic DNA was amplified and analyzed using an Applied Biosystems® 3500 Genetic Analyzer with POP-7® polymer and a 50cm capillary array. The presence of new alleles in the test sample (indicated by the black arrows) that were not present in the normal sample indicates MSI. See Figure 1 for additional information on expected data from the MSI Analysis System, Version 1.2.

Commonly, when using a five mononucleotide panel, samples in which greater than 40% of microsatellite markers are altered (≥ 2 altered markers out of 5) are classified as MSI-H. Samples with less than 40% mononucleotide repeat markers altered may be classified as microsatellite stable (MSS). While the occurrence of only one altered mononucleotide marker in mismatch repair-deficient cells is uncommon in colorectal samples, it may be more common in other sample types. Repeating the MSI assay or performing additional methods of analysis may be necessary to accurately classify these samples. Pentanucleotide markers are not intended for use in MSI classification.

Amplifying microsatellite markers will yield one or two major allele peaks, depending on whether the individual is homozygous (one peak) or heterozygous (two peaks) for that marker. In addition, amplifying microsatellite markers often generates artifact peaks due to stutter. Electropherograms of mononucleotide markers show a number of less intense stutter peaks at 1bp intervals from the most prominent or true allele peak (Figure 6). Instability for a marker is recognized as alterations in the lengths of the microsatellite due to deleting or inserting a repeating unit, which produces a novel length allele in test DNA compared to matching normal DNA. The mononucleotide markers included in the MSI Analysis System are nearly monomorphic so that most individuals are homozygous for the same common allele for a given marker (see Section 9.D for allele frequencies).

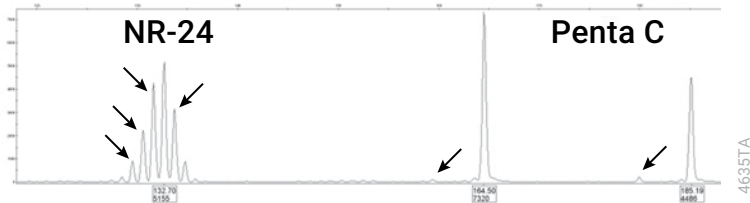


Figure 6. Stutter peaks. Stutter peaks (indicated by arrows) generated by polymerase slippage during PCR amplification of short tandem repeats are clearly visible at the mononucleotide marker NR-24 (left) at 1bp intervals from the true or tallest allele peak. Stutter artifacts decrease as microsatellite repeat length increases, so much smaller stutter peaks also can be seen at the Penta C marker, 5bp from true allele peaks.

Two polymorphic pentanucleotide repeat markers, Penta C and Penta D, are included in the MSI Analysis System to help confirm that the test sample and matching normal samples are from the same individual. Alleles found in the normal sample also should be present in the test sample if both samples are from the same individual. MSI has been observed in 14% of MSI-H samples in Penta C and 35% in Penta D, so additional pentanucleotide alleles also may be present in some MSI-H samples (as seen in Figure 5 denoted with a blue arrow); however, this is not specific or sensitive for mismatch repair deficiency. A matching probability between 1:10 and 1:16 was calculated for Penta C, and between 1:18 and 1:33 for Penta D based upon allele frequency data. Matching probability is the average number of randomly selected people you would have to survey before you find two with the same allelic pattern. Using both pentanucleotide markers should allow detection of sample mixups over 99% of the time.

7.B. Importing Panels and Bins Text Files for GeneMapper® Software, Version 4.0 and Higher

To facilitate analysis of data generated with the MSI Analysis System, Version 1.2, we have created panels and bins text files to automatically assign genotypes using GeneMapper® software, versions 4.0 and higher are available. Panels and bins text files for the MSI Analysis System, Version 1.2, can be downloaded from the Promega web site at:

www.promega.com/resources/software-firmware/fragment-analysis/msi-analysis-system-v1-2-panels-and-bins-request/

1. Open the GeneMapper® software.
2. Select **Tools**, then **Panel Manager**.
3. Highlight the **Panel Manager** icon in the upper left pane.
4. From the menu, select **File**, then **Import Panels**.
5. Navigate to the file location where the panels and bins text files were saved on your computer. Select the panel file, then **Import**.
6. In the navigation pane, highlight the Promega MSI folder that you just imported.
7. Select **File** then **Import Bin Set**.
8. Navigate to the file location where the panels and bins text files have been saved on your computer. Select the bin file, then **Import**.
9. At the bottom of the Panel Manager window, select **OK**. The Panel Manager window will close automatically.

7.C. Creating an Analysis Method Using GeneMapper® Software

1. Select **Tools** then **GeneMapper Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **New** and a new analysis method dialog box will open.
4. Select **Microsatellite**. Select **OK**.
5. Enter a descriptive name for the analysis method, such as "Promega MSI".
6. Select the 'Allele' tab (Figure 7).
7. Select the bin set corresponding to the Promega MSI System, **Promega MSI**.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.
9. Enter the values shown in Figure 7 for proper filtering of peaks when using the MSI Analysis System. These filter settings will allow the user to display plotted data with the highest peak labeled in each set of amplified fragments within each mononucleotide marker range. These settings can be changed if an alternative labeling strategy is desired. For an explanation of the proper usage and effect of these settings, refer to your GeneMapper® software documentation.

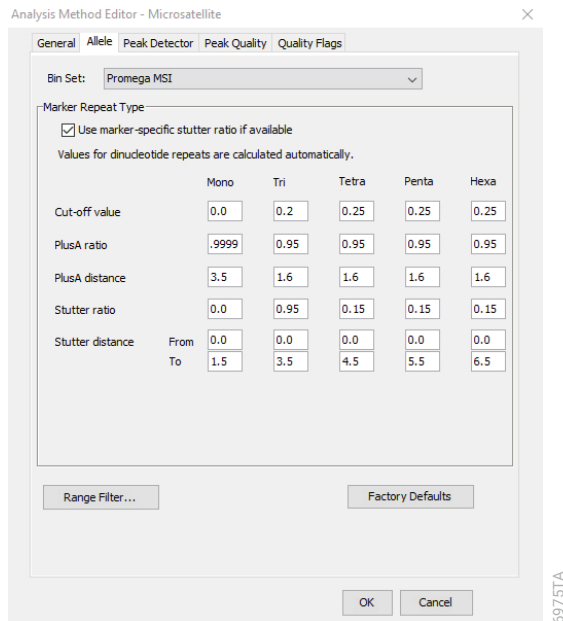
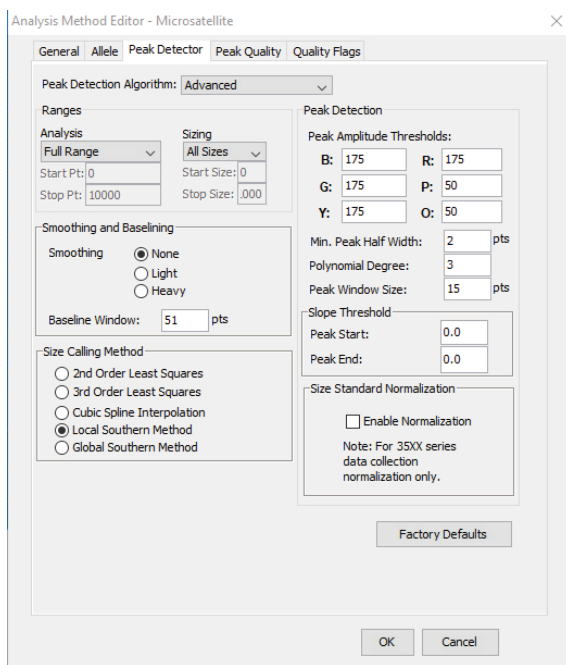


Figure 7. The Analysis Method Editor. The PlusA ratio setting for Mono repeats is 0.9999.

10. Select the 'Peak Detector' tab. We recommend the settings shown in Figure 8. Peak amplitude thresholds should be determined by the individual laboratory based on desired sample peak intensity range, dynamic range of the instrument, and noise or background signal that is observed in analyzed data. Peak amplitude thresholds between 50RFU and 200RFU are commonly used. For Applied Biosystems® 3130 series Genetic Analyzers, a threshold of 50RFU is a good starting point. For newer instruments such as the Applied Biosystems® 3500 Genetic Analyzers, 175RFU may be more appropriate.
11. Select **OK** to save your settings.



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Figure 8. The recommended settings for the 'Peak Detector' tab.

7.D. Creating a Size Standard

1. Select **Tools**, then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **New**.
4. Select **Basic or Advanced** (Figure 8). The type of analysis method selected must match the peak detection algorithm selected on the 'Peak Detector' tab for the analysis method. Select **OK**.
5. Enter a detailed name, such as "ILS 600", in the Size Standard Editor. Make sure that red is selected as the color for the size standard dye.
6. Enter the sizes of the internal lane standard fragments into the table (60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases).
7. Select **OK**.

7.E. Processing Data

1. In the File menu, select **New Project**, then **Microsatellite**.
2. Import sample files into a new project using the “Add Samples to Project” selection under the File menu.
3. In the Sample Type column, use the drop-down menu to select **Sample**, **Positive Control** or **Negative Control** as appropriate.
4. In the Analysis Method column, select the analysis method created in Section 7.C.
5. In the Panel column, select **Promega MSI** from the Promega MSI folder. This is the panels file that was imported in Section 7.B.
6. In the Size Standard column, select the size standard that was created in Section 7.D.
7. Select **Analyze** (green arrow button) to start data analysis.

7.F. Reviewing the Size Standard

When correctly labeled, the ILS 600 should give a Sizing Quality score near 1.0. Problems with ILS analysis (extra peaks, mislabeling, spectral pull up, etc.) will generate a Sizing Quality score of between 0 and 1.0 and may result in sample analysis failure. For more information refer to your GeneMapper® software documentation.

1. Select all samples in the ‘Samples’ tab by selecting **Edit**, then **Select All**.
2. Open the Size Match Editor by selecting **Analysis**, then **Size Match Editor**.
3. View the ‘Size Matches’ tab to review the Size Quality score, size standard peaks and size standard peak labels for each sample.
4. Determine if all peaks in the size standard are present and labeled correctly for the ILS 600 as shown in Figure 2.

7.G. Reviewing Analyzed Sample Data

For data analysis, the GeneMapper® analysis software, or a similar fragment analysis software such as GeneMarker® Software, is required. Fragment analysis software transforms raw data into electropherograms with accompanying data tables displaying PCR fragment lengths (in base pairs) and peak heights (in RFU). Results should be reviewed to eliminate artifact peaks caused by bleedthrough (Figure 9), stutter or CE spikes. Additional information on known artifacts is summarized in Section 7.H.

Note: For more information or assistance using other fragment analysis software, contact Promega Technical Service at: genetic@promega.com or refer to the software user manual for detailed instructions on fragment analysis and genotyping.

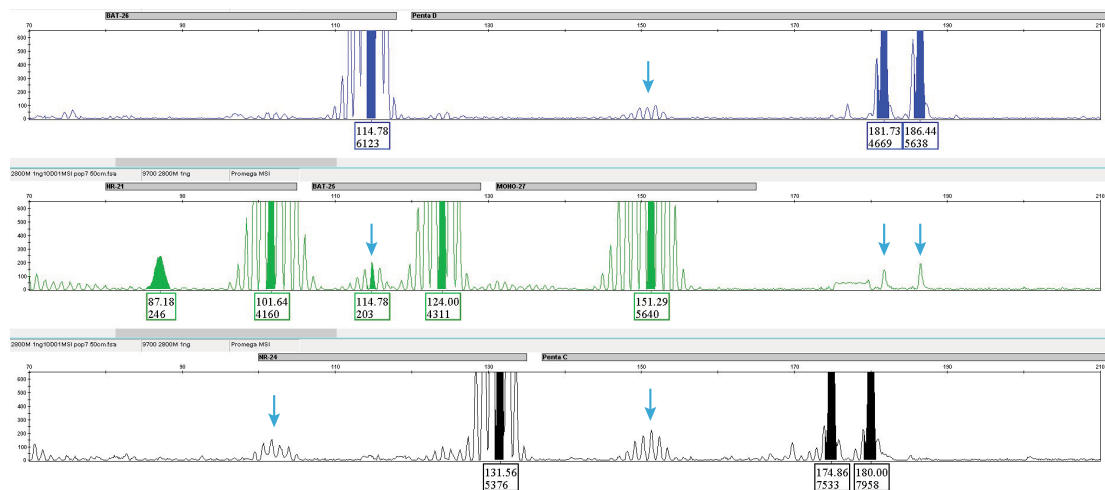


Figure 9. Bleedthrough or pull-up peaks. Small peaks (indicated by arrows) that correspond to much higher peaks in another color channel are called bleedthrough or pull-up peaks. In this example bleedthrough peak heights were ~150–200RFU compared to the ~4,000–7,000RFU peak heights of the true alleles present. Bleedthrough can occur when peak heights are excessive or if a poor or incorrect matrix was applied to the samples.

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7.H. Known Artifacts

A known broad peak artifact in the JOE (green) channel has been observed inside the NR-21 panel around ~80–93bp on an Applied Biosystems® 3500 Genetic Analyzer. An example is pictured below (Figure 10). This may be called as an allele by GeneMapper® Software but should be excluded when performing analysis.

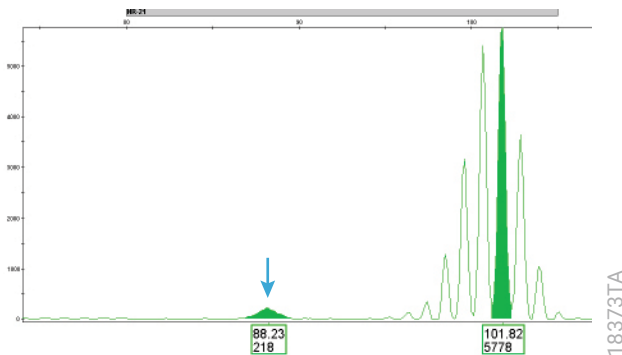


Figure 10. Example of broad peak artifact. Electropherogram of the JOE (green) channel at NR-21 with a blue arrow that indicates the known broad peak artifact. In this example, the broad peak artifact was called as an allele by the GeneMapper® software.

Other amplification artifacts may occur when too much DNA, or low-quality DNA, is used as input. The baseline signal in the fluorescein channel may become elevated and resemble stutter peaks at 1bp intervals (Figure 11). This elevated baseline is DNA dependent and not observed in every sample. Adopting the suggested analysis parameters for GeneMapper® analysis in Section 6 will help filter out these artifacts when identifying and calling alleles for these markers.

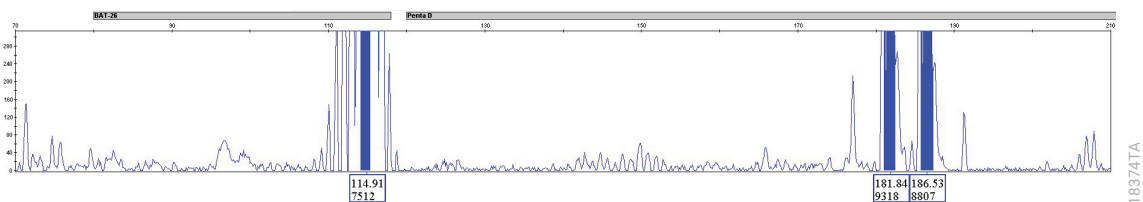


Figure 11. Example of baseline artifact. Electropherogram of the FL (blue) channel showing baseline “chatter”.

Adjustment of the Peak Amplitude Threshold in the analysis parameters may be necessary to reduce calling of baseline artifacts as alleles, an example is shown with a blue arrow in Figure 12. Optimization of the PlusA distance or Peak Amplitude Threshold in the Analysis Method may help reduce the number of incorrectly identified alleles on the edge of the stutter filter settings by the GeneMapper® algorithm. However manual identification and removal of erroneously called peaks may still be necessary (Figure 12).

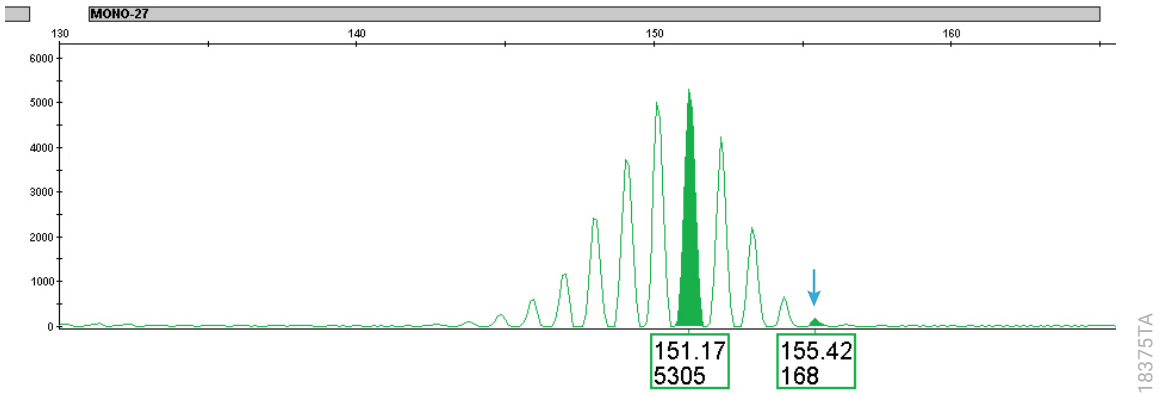


Figure 12. Example of an artifact allele. Electropherogram of the JOE (green) channel showing MONO-27 with the main allele called by GeneMapper® Software analysis method at 151bp. An artifact at 155bp is called due to the distance from the main allele such that stutter filtering is not effective for the last stutter peak.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: genetic@promega.com

For troubleshooting GeneMapper® software, refer to *GeneMapper® Software Reference and Troubleshooting Guide*.

Symptoms

Weak fluorescent signal for allele peaks

Causes and Comments

Insufficient template DNA. Make sure DNA is accurately quantitated, and use the recommended amount of template DNA.

Reinject with increased injection time.

Impure DNA template. Impurities in DNA preparation may inhibit PCR. Clean up DNA, or prepare new genomic DNA.

Poor-quality DNA. Improper or prolonged fixation of paraffin-embedded samples can result in low DNA yields and poor-quality DNA. Repeat amplification with additional DNA input (e.g., 4–10ng).

Poor capillary electrophoresis injection (ILS 600 peaks also affected). Reinject sample. Check the syringe pump system for leakage. Check the laser power.

Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.

High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover from DNA sample of K⁺, Na⁺, Mg²⁺ or EDTA can negatively affect PCR. Changes in pH also may affect PCR.

Thermal cycler or tube problems. Confirm PCR program is correct. Use recommended thermal cycler and tubes. Heat block calibration may be required.

Samples were not properly denatured before loading. Heat-denature samples for the recommended time and cool on crushed ice or in an ice-water bath immediately prior to loading the capillary.

Amplification reaction components were not added to the bottom of the tube or well. Gently tap the tube or plate to move the liquid to the bottom, or centrifuge briefly.

Symptoms

Causes and Comments

Excessive fluorescent signal for allele peaks

Too much template DNA.

- Make sure DNA was accurately quantitated, and use 1–2ng in PCR.
- Reinject with reduced injection time.
- Dilute PCR product 1:5 to 1:10 in sterile deionized water or 1X Gold ST★R Buffer prior to preparing loading solution.

Extra peaks visible in one or all dye colors

Microsatellite artifacts (e.g., stutter). Amplifying microsatellite loci generated artifacts that appear as smaller peaks 1bp above or below the prominent mononucleotide repeat allele or 5bp below a pentanucleotide repeat allele. Stutter product peak heights will be higher if too much template DNA was used.

Excess amount of DNA. We recommend 1–2ng of DNA. Amplifying >2ng of DNA template may result in a higher number of stutter products. Use less DNA template, or reduce the number of cycles in the amplification program by 2 or more cycles.

Pull-up or bleedthrough. Pull-up can occur when peak heights are excessive or if a poor or incorrect matrix was applied to the samples. Increase the peak amplitude threshold (i.e., 150–200RFU) in the analysis method and reanalyze. If problems persist, generate a new matrix or perform a new spectral calibration, and apply the new matrix to samples.

Samples were not properly denatured prior to loading. Heat-denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to loading the capillary.

Contamination with another template DNA or previously amplified DNA. Cross contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.

8. Troubleshooting (continued)

Symptoms

Preferential amplification of smaller loci

Causes and Comments

DNA was cross-linked in sample. DNA prepared from paraffin-embedded samples often is cross-linked with other DNA or protein molecules, preventing amplification of longer DNA fragments.

Degraded DNA. DNA template was degraded into smaller fragments with the larger loci showing diminished yield.

Insufficient template DNA. Use the recommended amount of template DNA.

Excess amounts of DNA. We recommend 1–2ng of DNA. Amplifying >2ng DNA template may result in an imbalance in yields, with the smaller loci showing more amplification product than the larger loci. Use less DNA template, or reduce the number of cycles in the amplification program by 2 or more cycles.

9. Appendix

9.A. Examples of Thermal Cycling Conditions for the MSI Analysis System, Version 1.2

Note: Thermal cyclers other than those listed below may be used, but will require optimization of cycling conditions, and may require additional validation in your lab.

Protocol for the GeneAmp® PCR System 9600 Thermal Cycler

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

94°C for 30 seconds

ramp 68 seconds to 58°C, hold for 30 seconds

ramp 50 seconds to 70°C, hold for 1 minute

for 10 cycles, then:

90°C for 30 seconds

ramp 60 seconds to 58°C, hold for 30 seconds

ramp 50 seconds to 70°C, hold for 1 minute

for 20 cycles, then:

60°C for 30 minutes

4°C soak

Protocol for the GeneAmp® PCR System 9700 Thermal Cycler

Note: GeneAmp® PCR System 9700 thermal cycling conditions are in 9600 emulation mode and use the silver block.

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds,
ramp 29% to 58°C for 30 seconds,
ramp 23% to 70°C for 1 minute
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds,
ramp 29% to 58°C for 30 seconds,
ramp 23% to 70°C for 1 minute
for 20 cycles, then:

60°C for 30 minutes

4°C soak

Protocol for the Applied Biosystems Veriti® Thermal Cyclers in 9600 Emulation Mode

In the main menu screen, touch **Tools Menu**. In the 'Tools Menu' screen, touch **Convert a Method** to open the Convert Method Wizard. Touch the check box next to "9600 Emulation Mode". Touch the **next arrow**. Then enter the cycling conditions below, select options to alter ramp rates. For more information on running in emulation mode, consult the Veriti® Thermal Cycler technical manual.

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds,
ramp 29% to 58°C for 30 seconds,
ramp 23% to 70°C for 1 minute
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds,
ramp 29% to 58°C for 30 seconds,
ramp 23% to 70°C for 1 minute
for 20 cycles, then:

60°C for 30 minutes

4°C soak



9.A. Examples of Thermal Cycling Conditions for the MSI Analysis System, Version 1.2 (continued)

Protocol for the Applied Biosystems ProFlex® Thermal Cyclers

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 1.5°C/sec to 94°C for 30 seconds

ramp 1.2°C/sec to 58°C for 30 seconds

ramp 0.4°C/sec to 70°C for 60 seconds
for 10 cycles, then:

ramp 1.5°C/sec to 90°C for 30 seconds

ramp 1.2°C/sec to 58°C for 30 seconds

ramp 0.4°C/sec to 70°C for 60 seconds
for 20 cycles, then:

60°C for 30 minutes

4°C soak

Protocol for the Eppendorf MasterCycler® X50 and Nexus Thermal Cyclers

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 0.7°/sec to 94°C for 30 seconds

ramp 0.5°/sec to 58°C for 30 seconds

ramp 0.2°/sec to 70°C for 1 minute
for 10 cycles, then:

ramp 0.7°/sec to 90°C for 30 seconds

ramp 0.5°/sec to 58°C for 30 seconds

ramp 0.2°/sec to 70°C for 1 minute
for 20 cycles, then:

60°C for 30 minutes

4°C soak

Protocol for the Bio-Rad C1000™ Thermal Cycler

Thermal Cycling Protocol

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 0.6°/sec to 94°C for 30 seconds

ramp 0.8°/sec to 58°C for 30 seconds

ramp 0.3°/sec to 70°C for 1 minute

for 10 cycles, then:

ramp 0.6°/sec to 90°C for 30 seconds

ramp 0.8°/sec to 58°C for 30 seconds

ramp 0.3°/sec to 70°C for 1 minute

for 20 cycles, then:

60°C for 30 minutes

4°C soak

9.B. Reference

1. *Quantitating DNA from FFPE samples using the QuantiFluor® ONE dsDNA System and the Quantus™ Fluorometer*
Application Note AN268, Promega Corporation.

9.C. Related Products

Product	Size	Cat. #
PowerPlex® 4C Matrix Standard	5 preps	DG4800
Spectrum Compact CE System	each	CE1304
Maxwell® RSC Instrument	each	AS4500
Maxwell® RSC DNA FFPE Kit	48 preps	AS1450
ReliaPrep™ FFPE gDNA Miniprep System	10 reactions	A2351
	100 reactions	A2352
QuantiFluor® ONE dsDNA System	100 reactions	E4871
	500 reactions	E4870
QuantiFluor® dsDNA System	1ml	E2670



9.D. Allele Frequencies for Mononucleotide Repeat Loci

Allele sizes were determined using the ABI PRISM® 3100 Genetic Analyzer with POP-4® polymer and a 36cm capillary. Note that allele sizes may vary when using different polymer or instrument configurations.

BAT-25			
Size (bp)	Caucasian-American	African-American	Asian-American
113	1	1	0
114	0	1	0
115	0	12	0
116	0	7	0
117	0	1	0
118	0	8	0
119	0	14	0
120	7	111	4
121	108	103	93
122	58	12	30
123	8	5	0
124	0	0	0
n =	182	274	127

BAT-26			
Size (bp)	Caucasian-American	African-American	Asian-American
103	0	23	0
104	0	0	0
105	0	0	0
106	0	2	1
107	1	0	0
108	0	0	0
109	0	0	0
110	0	0	0
111	0	4	0
112	14	38	3
113	140	131	115
114	22	57	8
115	0	0	0
n =	177	255	127

NR-24			
Size (bp)	Caucasian-American	African-American	Asian-American
130	60	197	49
131	113	34	77
132	0	0	0
133	0	2	2
n =	173	233	128

NR-21			
Size (bp)	Caucasian-American	African-American	Asian-American
94	1	2	8
95	1	0	0
96	0	3	0
97	0	60	0
98	27	100	14
99	134	62	89
100	14	9	23
101	1	0	1
n =	178	236	135

MONO-27			
Size (bp)	Caucasian-American	African-American	Asian-American
142	0	1	0
143	0	0	0
144	0	0	0
145	0	0	0
146	0	0	0
147	0	0	0
148	0	3	0
149	59	88	42
150	107	141	83
151	5	4	1
152	0	1	0
153	0	0	0
154	0	1	0
155	0	0	1
n =	172	239	127

9.E. Summary of Changes

The following changes were made to the 2/23 revision of this document:

1. Removed instructions for ABI PRISM® 310 Genetic Analyzer.
2. Updated Sections 1, 3, 4, 5, 7 and 9.
3. Provided instrument instructions in Section 6, including the Spectrum Compact CE Instrument.
4. Added additional thermal cycling protocols (Section 9.A).
5. Moved to a new template.



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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.