

The Complete Solution for Microsatellite Instability (MSI-H) Analysis

Systems to purify high-quality DNA from formalin-fixed paraffin-embedded tissue samples and to amplify seven informative genetic loci in a single multiplex reaction. Performance and convenience from Promega!

Microsatellite Instability (MSI) Analysis System

The MSI Analysis System, Version 1.1^(a-e) is a fluorescent multiplex PCR-based^(f) assay for detection of microsatellite instability (MSI), a form of genomic instability. This instability is due to either insertion or deletion of repeating units during DNA replication, and failure of the DNA mismatch repair system (MMR) to correct these errors. MSI analysis typically involves comparison of allelic profiles of microsatellite markers generated by amplification from matching normal tissue and tumor samples. New alleles in the tumor sample not found in the corresponding normal sample indicate the presence of MSI.

The MSI Analysis System, Version 1.1, includes fluorescently labeled primers (marker panel) for co-amplification of seven markers for analysis of the MSI-high (MSI-H) phenotype, including five nearly monomorphic mononucleotide repeat markers and two highly polymorphic pentanucleotide repeat markers. Amplified fragments are detected using either the ABI PRISM[®] 310 or 3100 Genetic Analyzer after spectral calibration.

Mononucleotide	Pentanucleotide
BAT-25	Penta C
BAT-26	Penta D
MONO-27	
NR-21	
NR-24	

Mononucleotide markers are highly sensitive and specific markers for MSI-H samples, while the pentanucleotide markers provide controls for sample mix-ups and/or contamination, providing the choice of direct analysis of tumor samples, or traditional analysis of tumor and normal tissue pairs.

The MSI Analysis Macro software facilitates data analysis by providing automated assignment of genotypes using ABI Genotyper[®] software.

This configuration meets the recommendations proposed at the 2002 NCI Workshop for MSI Testing (1,2) and overcomes the misclassification, lack of sensitivity and stutter-artifact problems inherent in the five-marker Bethesda Panel.

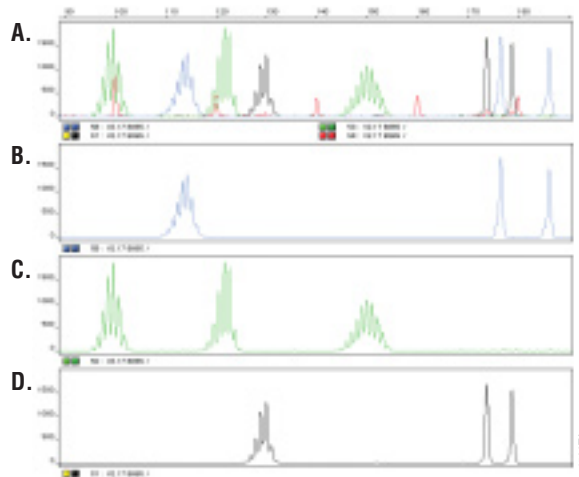


Figure 1. Analysis of MSI Phenotype. One nanogram of purified normal human DNA from a single individual was amplified and analyzed on an ABI PRISM[®] 3100 Genetic Analyzer. **Panel A.** Multiplex amplification with the MSI Analysis System, Version 1.1. Three-color amplification products range in size from 98 to 209 bases. **Panel B.** Amplification of BAT-26 (103–115base) and Penta D (135–201 base) fragments. **Panel C.** Amplification of NR-21 (94–101 bases), BAT-25 (114–124 base) and MONO-27 (142–154 base) fragments. **Panel D.** Amplification of NR-24 (130–133 bases) and Penta C (143–194 base) fragments.

Benefits

- **Understand Complete MSI Phenotype:** A single multiplex PCR amplifies five informative mononucleotide repeats for MSI-H determination.
- **Confidence in Sample Identification:** Co-amplification of highly polymorphic pentanucleotide repeat markers provides internal sample tracking.
- **Consistent Data Analysis:** Available MSI Analysis Macro software simplifies and standardizes allele size determination.

DNA Purification from Fixed Tissue Samples

The MagneSil® Genomic, Fixed Tissue System provides an easy method to purify high-quality genomic DNA from formalin-fixed paraffin-embedded tissue samples for PCR analysis. Samples are treated with Proteinase K overnight prior to purification. The system allows purification of up to 12 samples in less than 1 hour without the use of organic solvents.

The MagneSil Genomic, Fixed Tissue System purifies ultra-clean genomic DNA free of PCR inhibitors, including very small DNA fragments. This allows consistent amplification of PCR products as large as 1,800bp.

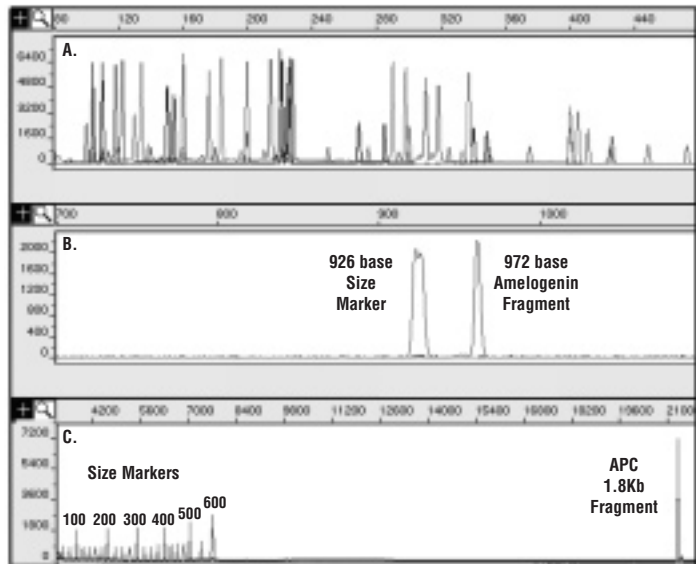


Figure 2. Analysis of DNA purified from paraffin-embedded, formalin-fixed 10µm thin sections using the MagneSil Genomic, Fixed Tissue System. Purified DNA was amplified and then analyzed on an ABI PRISM® 3100 Genetic Analyzer. **Panel A.** Amplification with a set of 16 fluorescently labeled primers. Amplification products range in size from 104 to 420 bases. **Panel B.** A 972-base fragment amplified using an amelogenin primer set. **Panel C.** A 1.8kb fragment amplified from the APC gene. Results will vary depending on the degree of cross-linking due to formalin fixation.

Benefits

- **Ease of Use:** Simple protocol eliminates organic solvent extraction of samples.
- **Consistent Amplification:** System provides highly pure DNA free of PCR inhibitors, including very small fragments of DNA.
- **Superior Performance:** Demonstrated amplification of fragments as large as 1,800bp.
- **Confidence:** Optimized for use with MSI Analysis System.

Ordering Information

Product	Size	Cat.#
MSI Analysis System, Version 1.1(a-e)*	50 reactions	MD1641
MagneSil® Genomic, Fixed Tissue System**	100 samples	MD1490
MSI Analysis Macro*	1 each	MD1650

*For Research Use Only. Not for use in diagnostic procedures.

**For Laboratory Use.

Additional Information

Protocols	Part#
MSI Analysis System, Version 1.1, Technical Manual http://www.promega.com/tbs/tm255/tm255.html	TM255
MagneSil® Genomic, Fixed Tissue System, Technical Bulletin http://www.promega.com/tbs/tb319/tb319.html	TB319
MSI Analysis Macro, Technical Bulletin http://www.promega.com/tbs/tb338/tb338.html	TB338

References

1. Umar, A., *et al.* (2004) Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl Cancer Inst.* **96**, 261–268.
2. Bacher, J.W., *et al.* (2004) Development of a fluorescent multiplex assay for detection of MSI-High tumors. *Disease Markers* **19** (in press).

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