Main specifications for the DS3000

■ Main unit specifications

Item	Details
Number of capillaries	4
Capillary length	36 cm
Sample format	8-tube strip × 4
Device control	Touch panel PC
Number of Dyes	6
Application	Sequencing analysis / Fragment analysis
Size	400 (W) × 600 (D) × 600 (H) mm
Weight	45 kg
Performance guarantee temperature	15 – 30˚C
Performance guarantee humidity	20 – 80% RH (no condensation)
Power input	100 – 240 ±10% VAC, 50/60 Hz
Rated power	260 VA
Supported secondary analysis software	• Mutation Surveyor (SoftGenetics, LLC, sold separately) • GeneMarker (SoftGenetics, LLC, sold separately) • GeneMarker HID (SoftGenetics, LLC, sold separately)

■ Run module specifications

Run Module	Application	Polymer type	Contiguous Read Length*1 (bp, QV20 CRL)	Average run time (minutes)
Fast_Sequence36_Polymer7	Sequencing analysis	Polymer7	≥600	≤32
Standard_Sequence36_Polymer7	Sequencing analysis	Polymer7	≥700	≤60
BDx_Fast_Sequence36_Polymer7	BDx sequencing analysis	Polymer7	≥600	≤32
BDx_Standard_Sequence36_Polymer7	BDx sequencing analysis	Polymer7	≥700	≤60

Run Module	Application	Polymer type	Average run time (minutes)	Sizing precision*2 (bp, 50 – 400 bp)
Fragment_Analysis36_Polymer7	Fragment analysis	Polymer7	≤35	NA
Fragment_Analysis36_Polymer4	Fragment analysis	Polymer4	≤44	<0.16

*1 Contiguous Read Length (bp, QV20 CRL) is measured with BigDye® Terminator v3.1. Sequencing Standard Kit (Thermo Fisher Scientific, sold separately)

*2 Sizing precision (bp, 50 - 400 bp) is measured with PowerPlex™ ESI17 Fast Allelic Ladder and WEN ILS 500 ESS. (Promega®, sold separately)

· BigDye is a registered trademark of Thermo Fisher Scientific Inc.

· Promega is a registered trademark of Promega Corporation.

■ Consumables specifications

Product name	Part number	Details	Remarks
Capillary Cartridge 36 cm	613-0330	1 pcs	Storage temperature: 15 – 30˚C
Buffer	613-0252	Anode Buffer × 2 cartridges Cathode Buffer × 2 cartridges	Storage temperature: 2 – 10˚C
Polymer7	613-0251	4 cartridges	Storage temperature: 2 – 10˚C
Polymer4	613-0250	4 cartridges	Storage temperature: 2 – 10˚C
Septa for Cathode Buffer Cartridge	613-7231	10 pcs	
Retainer for Cathode Buffer Cartridge	613-7233	4 pcs	
Septa for 8 well tubes	613-7230	24 pcs	
Base and Retainer for 8 well tubes	613-7232	4 pcs	
Anode Electrode Assembly	613-7263	1 pcs	

Specifications in this catalog are subject to change with or without notice,
as Hitachi High-Tech Corporation continues to develop the latest technologies and product for its customers.

CAUTION:For correct operation, follow the instruction manual when using the instrument.

NOTICE:The system is For Research Use Only, and is not intended for any animal or human therapeutic or diagnostic use.
Hitachi High-Tech does not guarantee the performance on the document with every possible sample under every possible condition.

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HTB-DS-E003 2021.08

Technical Report 3

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Microsatellite Instability Analysis using DS3000 Compact CE Sequencer

Abstract

Microsatellite instability (MSI), an accumulation of somatic mutations in microsatellites or short tandem repeat sequences, is a consequence of DNA mismatch repair (MMR) system deficiency, and thus considered as a biomarker for multiple types of cancers. In MSI analysis, DNA fragments were distributed in single-base resolution and compared with the reference data on a capillary electrophoresis (CE).
Therefore, CE system is required to establish both the accurate and reproducible sizing of the fragments. Using DS3000 Compact CE Sequencer (hereafter called DS3000), we introduce an end-to-end workflow of MSI analysis from DNA extraction to data analysis.

DS3000
Compact CE Sequencer

Materials and Methods

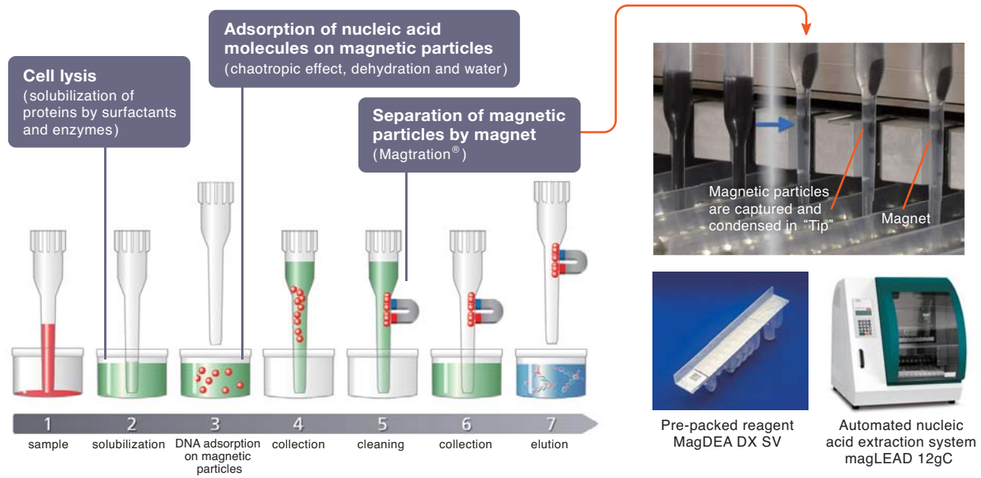
【Starting materials】

Starting materials were Microsatellite instable (MSI) FFPE DNA Reference Standard (HD830, Horizon Discovery Ltd) and its normal counterpart, microsatellite stable (MSS) FFPE DNA Reference Standard (HD831). Both materials were derived from the same human cell line and provided in curled FFPE sections.

【 DNA Extraction 】

Genomic DNA was extracted using an automated nucleic acid extraction system, magLEAD 12gC (Precision System Science. Figure 1). Approximately 10 μg of genome DNA were obtained with sufficient quality for the following analysis (A260/A280 ≥ 1.95).

■ Overview of DNA extraction by the magLEAD system



■ Yield and quality of the extracted genomic DNA

Sample	Conc.(ng/μl)	Quantity(μg)	A260/A280	A260/A230
MSS	54.8	11.0	1.96	1.80
MSI	59.4	11.8	1.95	1.85

Figure 1:Overview of genome extraction

【 PCR and CE 】

Five MSI markers (mononucleotide repeats) and two control markers (pentanucleotide repeats) were co-amplified and fluorescently-labeled from 2 ng of the genomic DNA using the MSI Analysis System, Version 1.2 (Promega®). The PCR samples were mixed with the Internal Lane Standard 600 (ILS600) size standard (Promega®) and run on the DS3000 CE Sequencer. The run protocol was briefly summarized in Table 1.

Table 1：Performance of Polymer 7

Polymer	Run time	Run voltage
Polymer 7	～35 min	13 kV

【 Data Analysis 】

Peak calling and sizing were performed on GeneMarker ver. 3.0.1 (SoftGenetics) following to the installation of MSI panel and ILS600 size standard definition file. MSI specific peaks were identified by comparing the sizing data with its normal counterpart.

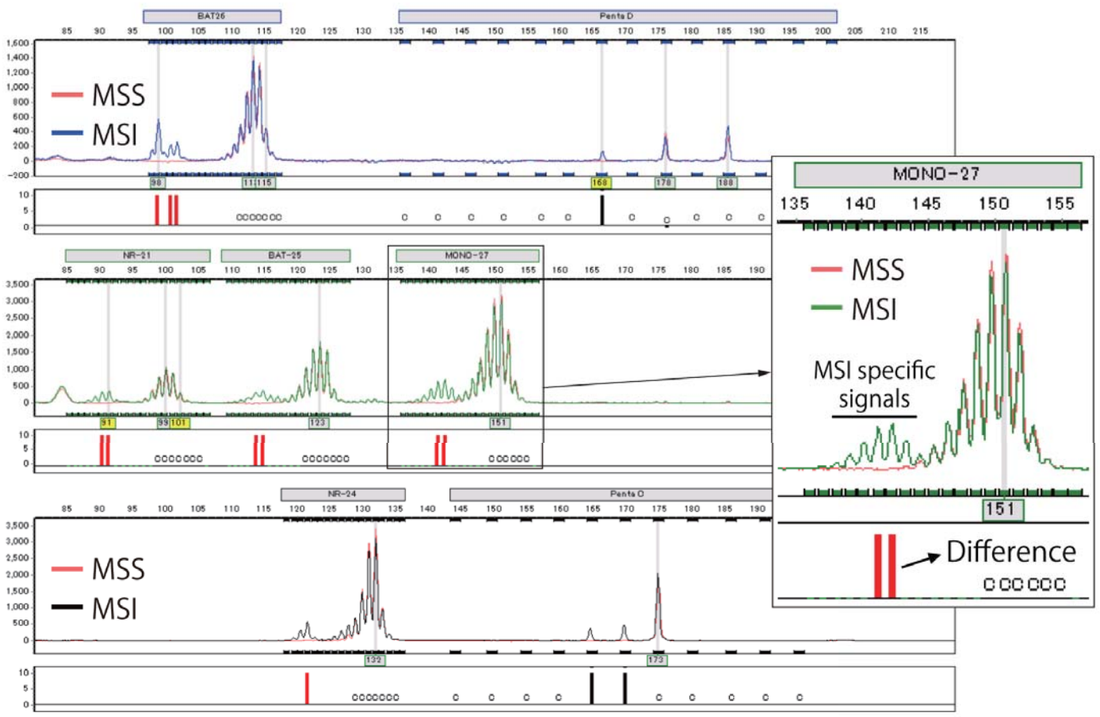


Figure 2：Overlay View of MSI analysis

The electropherogram of MSS (pink line) and MSI (blue, green, and black lines) were overlaid.
The red vertical bars designate the MSI-specific peaks where the peak-to-peak difference between MSI and MSS is significant.
Each of the "C" indicates the predefined reference position where an allele peak of MSS appears.
Based on a peak-to-peak comparison between MSI and MSS, the MSI-specific peaks were successfully identified at all five markers.
In contrast, peaks in common with MSS were all overlapped within +/-0.1 bp difference.

Technical Report 3

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【 Workflow from DNA extraction to MSI analysis starting with FFPE samples 】

The time for each step represents the estimated walk-away time and does not include hands-on time.

Conclusion

MSI analysis was performed on DS3000 using a commercially available paraffin-embedded sections. The run files were analyzed on GeneMarker v3.0.1, and MSI-specific peaks on all five markers were identified successfully. In addition, peaks in common with MSS overlapped within 0.1 bp difference. These results indicate the reliable performance of DS3000.

Caution

- DS3000 Compact CE Sequencer is for Research Use Only and not intended for diagnostic procedures.
- This document shows an example of verification under limited samples and environments.
- It does not guarantee that data equivalent to those in this document can be obtained with every sample under every environment.