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Development and evaluation of cell line-derived FFPE reference material for MSI assay validation

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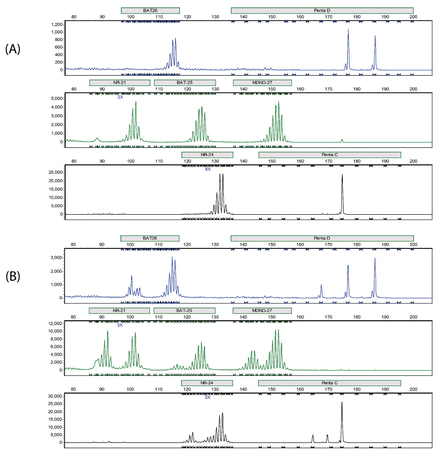
Abstract

Microsatellite Instability (MSI) is defined by variance in the repeat count of microsatellite motifs and occurs in cells that are deficient in one or more mismatch repair proteins. MSI is present in varying cancer types, but is most commonly found in colorectal, gastric and endometrial cancer. Patients with early stage colorectal cancers that display MSI have a better prognosis and show a better response to chemotherapy compared to those with microsatellite stable tumours. MSI, alongside additional markers such as tumour mutational burden, is also a positive predictive biomarker for immune checkpoint inhibition.

Identification of MSI tumours in the diagnostic laboratory is traditionally performed by fluorescent multiplex PCR, evaluating the microsatellite length of two mononucleotide repeats and three dinucleotide repeats (Bethesda Panel) or a commercially available kit, consisting of five mononucleotide markers (recommended NCI Panel) alongside IHC staining for the four MMR proteins MSH2, MSH6, MLH1, and PMS2. With the increase in MSI testing related to the recent FDA approval of Keytruda®, several companies and laboratories are now developing newer and better PCR-based and next-generation sequencing assays to assess MSI.

To control for error, we have developed a pair of cell line-derived MSI/MSS reference samples covering the most commonly used MSI biomarkers. MSI/MSS cell lines were mixed at biologically-relevant ratios and processed into FFPE to serve as a whole-process control. Our data support the suitability of this material on a variety of different platforms and with a high degree of consistency throughout various FFPE batches. In conclusion, our cell line-derived reference samples represent a commutable control to support MSI assay development and validation.

MSI control showed MSI-H results using Promega MSI Analysis System

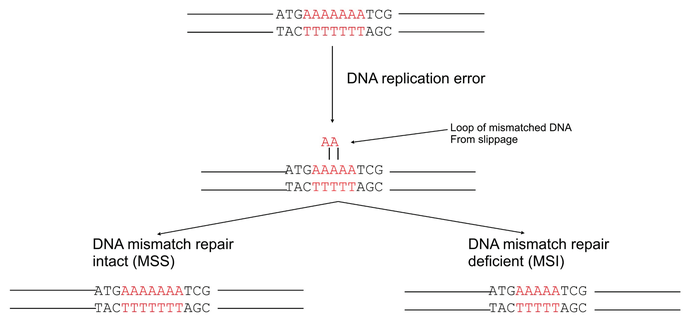


DNA was extracted from each FFPE material and analysed with Promega MSI Analysis kit. Electropherograms from MSS control (A) and MSI control (B) are shown. MSS control showed a single peak at each microsatellite marker. In contrast, MSI control showed an extra peak at each microsatellite, suggesting the sample was MSI-H. The data has been summarised in Table 1.

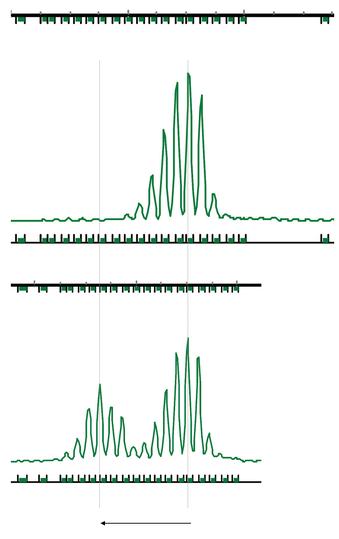
Data were kindly provided by Newcastle upon Tyne Hospital, Northern Molecular Genetics Service.

Introduction

Microsatellite instability (MSI) occurs in cells in which one or more mismatch repair genes are deficient.



Identification of MSI is performed using fluorescent multiplex PCR to evaluate the microsatellite length in order to compare normal tissues and tumour tissues. Representative images are shown below. The tumour sample gained an extra peak following the PCR amplification and this is defined as MSI.



MSI control showed MSI at various commonly tested microsatellite markers

Table 1. Summary of results from Promega MSI Analysis System

Sample	NR-21	NR-24	BAT-25	BAT-26	MONO-27
MSS	S	S	S	S	S
MSI	I	I	I	I	I

Table 2. Summary of results from Diatech Titano MSI

Sample	BAT-25	BAT-26	NR-21	NR-24	D2S123	D17S250	TGFβ	BAT-40	D18S58	D5S346
MSS	S	S	S	S	S	S	S	S	S	S
MSI	I	I	I	I	I	inconclusive	I	I	I	I

Table 3. Summary of results from Biocartis Idylla MSI Assay

Sample	ACVR2A	BTBD7	DIDO1	MRE11	RYR3	SEC31A	SULF2
MSS	S	S	S	S	S	S	S
MSI	I	I	I	I	I	I	I

DNA were extracted from each FFPE material and analysed with three commercially available PCR based MSI analysis kits, Promega's MSI Analysis System (Table 1), Diatech's Titano MSI kit (Table 2), and Biocartis' Idylla MSI Assay (Table 3).

Our materials showed MSI at a range of microsatellite locus including Bethesda panel and NCI recommended panel. Therefore these materials are suitable as reference standards for MSI/MSS analysis.

Methods

MSS and MSI cell lines were fixed with formalin and embedded into paraffin to produce FFPE blocks. gDNA were extracted from 15µm curls and their microsatellite status was analysed with commercially available fluorescent multiplex PCR based MSI analysis kit. Commonly analysed microsatellite markers are summarised in a table.

	Microsatellite markers
Bethesda panel	BAT-25, BAT-26, D2S123, D17S250, D5S346
NCI recommended panel	NR-21, NR-24, BAT-25, BAT-26, MONO-27
Additional markers commonly tested for	NR-22, NR-27, BAT-40, D18S58, CAT-25

Conclusions

Our prototype materials showed MSI at commonly analysed microsatellite markers, including Bethesda panel and NCI recommended panel.

This indicated our cell line-derived reference samples represent a commutable control to support MSI assay development and validation.