

Evaluation of Microsatellite Instability (MSI) using Ion Torrent Sequencing technology

ThermoFisher
SCIENTIFIC

Sameh El-Difrawy¹, Anelia Kraltcheva², Asha Kamat¹, Alice Zheng¹, Janice Au-Young¹ and Simon Cawley¹

¹Thermo Fisher Scientific, South San Francisco, CA.; ²Thermo Fisher Scientific, Carlsbad, CA.

INTRODUCTION

Cancer-associated instabilities at microsatellite locations throughout the genome have been shown to be predictive of response to immunotherapy treatment. A Microsatellite Instability High (MSI-H) status can result when the DNA Mismatch Repair (MMR) system fails to work properly. In 1997 NCI recommended utilizing a panel of five MSI markers for detecting Colorectal cancer (CRC). The traditional approach uses CE and utilizes the difference in marker profile among a tumor/normal tissue pair to determine the MSI Status of that tumor.

Recently, there has been a growing demand to develop more sensitive solutions to MSI detection with a larger number of markers. NGS provides a natural solution for that demand with the ability to process multiple samples and a large number of markers. MSI markers are mostly very long homopolymers, di- and tri-nuc short tandem repeats, the type of motifs that are not easily amplified or sequenced accurately due to the existence of different artifacts including stutter.

Here, we describe an NGS-based method to assess Microsatellite Instability (MSI) status in tumor-only and tumor-normal samples utilizing Ion AmpliSeq™ or Ion AmpliSeq™ HD technologies and an Ion GeneStudio™ S5 next-generation sequencer.

MATERIALS AND METHODS

There has recently been a significant increase in literature surveying the landscape of microsatellite Instability using data from the Cancer Genome Atlas (TCGA) and other programs[1-4]. These publications were used to identify an initial set of loci shown to be sensitive to MSI in different types of cancers. Primers for those markers were designed and ordered for both CE, and Ion AmpliSeq/AmpliSeq™ HD chemistries and sequenced on an Ion GeneStudio S5™ Series sequencer and 3500xL CE sequencer.

Optimal sequencing conditions were identified to accurately characterize a diverse marker set that includes monomers that vary in length between 10 BP and 40 BP in addition to di- and tri-nucleotide STR markers.

A bioinformatics pipeline, in the form of a Torrent suit plugin, MSICall, was developed to process the aligned bam files generated by Ion GeneStudio S5. Reads mapped to the locations of each marker were grouped. For each read a homopolymer signal is measured and statistics of the signal across a large number of reads is evaluated against an expected metric to estimate a per marker/direction MSI score. A total MSI score is calculated by adding the individual marker scores after some simple noise filtering.

We evaluated performance of the assay and algorithm with a set of 32 clinical research samples including CRC, Endometrial and Gastric carcinoma tumors in both MSI-High and MSS status. Tissue samples used for evaluation were purchased from Folio Biosciences. The resulting scores were in concordance with results from capillary electrophoresis studies.

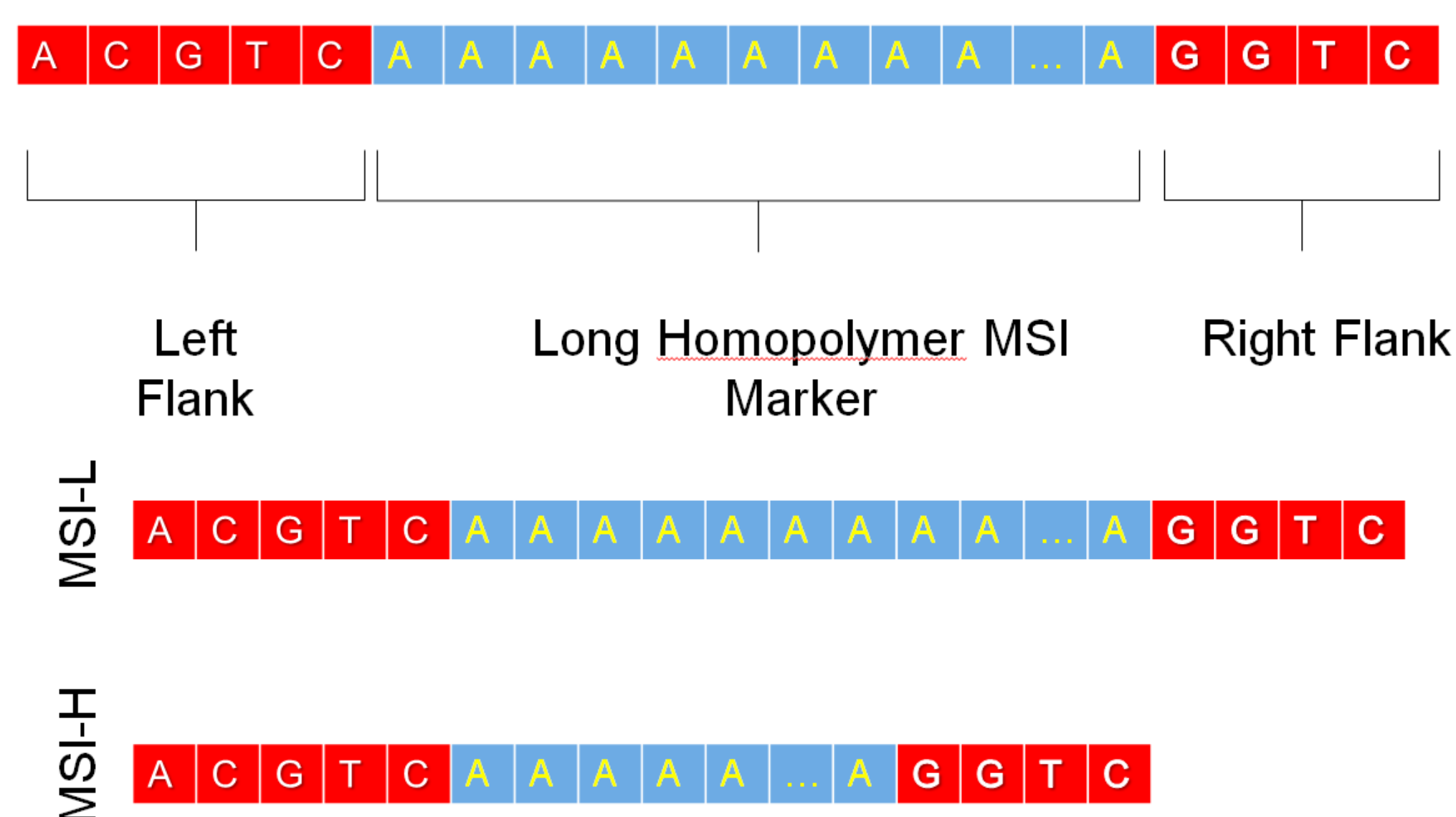


Figure 1: Identification of unstable MSI markers.

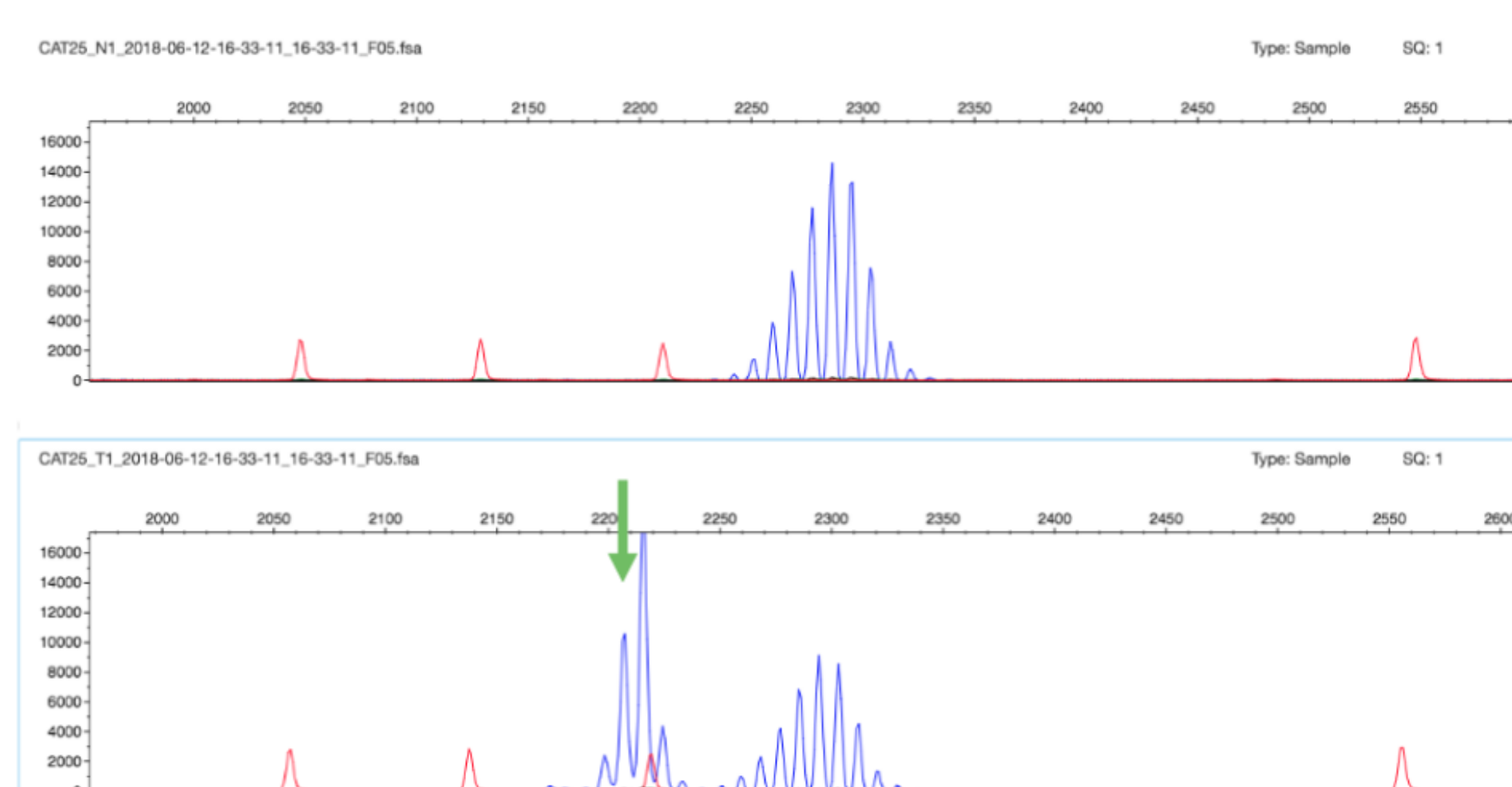


Figure 2: MSI-H homopolymer marker behavior observed in CE. Tumor (bottom) and matching normal (top)

RESULTS

MSI Score	CRC Tumor	Endo Tumor	Gastric Tumor	Endo Normal	CRC Normal	Endo Normal	Gastric Normal
BAT25	1.88	1.52	0	0	0.41	0	0
BAT26	1.15	1.64	1.51	0.86	0.01	0	0
NR21	3.29	2.11	1.37	0.19	0.04	0.05	0.12
NR24	2.74	2.15	0.79	0	0.22	0.09	0.33
NR_22	2.11	2.87	1.22	0.1	0.36	0.28	0.01
MSIM_1	1.15	0.92	2.41	0	0.18	0.15	0.12
:	:	:	:	:	:	:	:
LIMCH1	0.48	0.11	0.58	0.08	0.08	0.04	0.04
PRMD2	0.45	0.78	0.23	0	0.02	0.12	0.07
RNF43	1.94	0.14	0.11	0.09	0.08	0.2	0.07
MSI Score	125.33	97.92	128.53	0.86	6.37	0	1.26

Table 1: Classification of MSI status using multiple markers.

Tumor Type	MSI-H	Normal
CRC	161.76	8.08
CRC	103.09	8.48
Gastric	71.6	22.61
Endometrial	48.98	2.45

Table 2. Identification of MSI-High and Normal Samples: MSI score is assigned to each sample across multiple markers. Higher score is indicative of MSI-H status.

CE

	GC-2	GC-5	EC-2	EC-4	EC-5
NR21	+		+		
BAT26	+				+
BAT25	+		+		+
NR24	+		+		
MONO27	+		+		
MSI	High	Stable	High	Stable	High

NGS

Sample/Donor ID	NGS Result	MSI score
Gastric Cancer Tumor-GC2	MSI-H	81.56
Gastric Cancer Tumor-GC5	Normal	5.38
Endometrial Cancer Tumor-EC2	MSI-H	85.71
Endometrial Cancer Tumor-EC4	Normal	16.09
Endometrial Cancer Tumor-EC5	MSI-L	27.83

Table 3: Concordance of results for CRC and non-CRC tumor samples: CE (Top) and NGS (Bottom).

CONCLUSION

A next-generation sequencing based assay using 76 markers and associated Bioinformatics pipeline were developed to assign MSI status to tumor samples with great precision. The accuracy of the system was validated using an orthogonal test. MSI status can be assigned using tumor-only or tumor-normal samples.

REFERENCES

- [1] R Bonneville, MA Krook et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO Precis Oncol. 2017
- [2] J Hempelmann, C Lockwood et al. Microsatellite instability in prostate cancer by PCR or next-generation sequencing, Journal for ImmunoTherapy of Cancer 20186:29
- [3] Y Maruvka, K Mouw et al. Analysis of somatic microsatellite indels identifies driver events in human tumors, Nature Biotechnology 35, 951-959
- [4] I Cortes-Ciriano, S Lee et al. A molecular portrait of microsatellite instability across multiple cancers, Nature Communications 8, 15180

ACKNOWLEDGMENTS

Xinzhan Peng; Guobin Luo; Sihong Chen; Kevin Heinemann; Kyusung Park; Cristina Van Loy; Jennifer Kilzer; Bo Ding; Yutao Fu; Eugene Ingerman; Cheng Zong Bai Daniel Mazur; Mark Andersen; Rob Bennett

For Research Use Only. Not for use in diagnostics procedures