A targeted semi-conductor based next-generation sequencing (NGS) test to characterize microsatellite instability in FFPE tumor samples

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INTRODUCTION

Microsatellite instability (MSI) is a form of genetic instability that arises from defects in DNA mismatch repair. MSI is common in colorectal cancer (CRC) and also prevalent in gastric, endometrial, ovarian and other cancers. MSI is important in characterizing prognosis and determining treatment strategies in CRC. In 2017, the FDA approved the PD-1 inhibitor pembrolizumab for patients with advanced MSI-High solid tumors that have progressed following prior treatment.

Traditionally, DNA sequencing by capillary electrophoresis is used to determine MSI status of a matched tumor/normal tissue pair. In 1997 the National Cancer Institute recommended a panel of five MSI markers to detect MSI in CRC.

Given the link between immunotherapy response and MSI, there is a need to develop MSI solutions with a larger number of markers that are applicable to diverse cancer types. Next-generation sequencing (NGS) can broaden the number of markers and process multiple samples simultaneously. However, MSI markers are mainly long homopolymers and di- and tri-nucleotide short tandem repeats (STR), the type of sequence motifs that are not easily amplified or sequenced accurately.

Herein, we describe an NGS-based method to support research of MSI status in tumor-only and matched tumor-normal samples using Ion Ampliseq™ or Ion AmpliSeq™ HD multiplex PCR and the Ion GeneStudio™ S5 next-generation sequencer.

MATERIALS AND METHODS

We developed targets for MSI from internal data and the literature[1-4] and tested those targets using a combination of CE and Ion Ampliseq/AmpliSeq™ HD chemistries and sequencing platforms.

Sequencing conditions were optimized to accurately characterize a diverse set of over 70 markers that included monomers between 10 and 27 base pairs in addition to di- and tri-nucleotide STR markers. An algorithm was developed as a Torrent Suite plug-in (MSICall) to process aligned bam files generated by Ion Torrent sequencing. Reads mapping to the locations of each marker were grouped and a statistical analysis was performed to estimate a directional score on the forward and reverse strands of each marker. A total MSI score for the sample was calculated by summing the individual marker/strand scores that are above noise level. MSI reference materials were purchased from Folio Biosciences. Additionally we tested MSI/MSS custom material manufactured by Horizon Discovery.

We evaluated performance of the assay in a diverse sample set of over 100 CRC, gastric and endometrial cancers having MSI-High and microsatellite stable (MSS) status. The MSI scores derived from the NGS method were in concordance with results determined in parallel using CE.

Figure 1: Templating and Sequencing of the MSI assay using Ion Chef and Ion Gene Studio system.

RESULTS

	CRC Tumor	Endo Tumor	Gastric Tumor	CRC Normal	Endo Normal	Gastric Normal
BAT25	1.88	1.52	0	0.41	0	0
BAT26	1.15	1.64	1.51	0.01	0	0
NR21	3.29	2.11	1.37	0.04	0.05	0.12
NR24	2.74	2.15	0.79	0.22	0.09	0.33
NR_22	2.11	2.87	1.22	0.36	0.28	0.01
MSI_1	1.15	0.92	2.41	0.18	0.15	0.12
	⋮	⋮	⋮	⋮	⋮	⋮
LIMCH1	0.48	0.11	0.58	0.08	0.04	0.04
PRMD2	0.45	0.78	0.23	0.02	0.12	0.07
RNF43	1.94	0.14	0.11	0.08	0.2	0.07
MSI Score	125.3	97.92	128.5	6.37	0	1.26

Table 1: Classification of MSI status using multiple markers in 3 matched tumor/normal pairs. Individual marker scores were derived as the sum of scores on the forward and reverse strands and the sum of the individual marker scores was used to calculate an MSI Score for the sample.

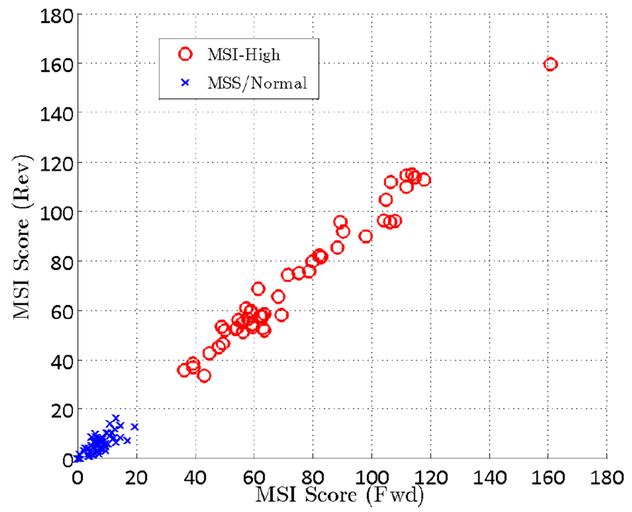

Figure 2: MSI Score distribution in 110 CRC, gastric and endometrial cancer samples. MSI/MSS status was determined by CE. Distribution of calculated MSI Scores for forward (Fwd) and reverse (Rev) strands for MSS/normal and MSI-High samples.

- The data in Figure 2 demonstrated high concordance between the MSI Scores derived from the forward and reverse strands for both MSI-High and MSS samples.
- Samples having MSI-High and MSS status as determined by CE were clearly separated by the NGS method.
- Samples with MSS status had a combined MSI Score of < 40 whereas samples with MSI-High status had MSI Scores > 60.

Data not shown:

- Recommended sample multiplexing levels range from 10 samples per 520 chip, 40 samples per 530 chip to 160 samples per 540 chip.
- The Ion Ampliseq ™ Microsatellite Instability Research panel can be run independently or as a part of a larger panel.

CE

	NR21	BAT26	BAT25	NR24	MONO27	MSI
GC-2	+	+	+	+	+	High
GC-5						Stable
EC-2	+		+	+	+	High
EC-4						Stable
EC-5		+	+			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	MSS	27.83

Table 2: Comparison of results obtained with CE (Top) and NGS (Bottom) for non-CRC tumor samples.

CONCLUSION

- We developed a targeted NGS-based method to assess MSI that leverages Ion AmpliSeq™ or Ion AmpliSeq™ HD multiplex PCR and Ion GeneStudio™ S5 next-generation sequencing. The test is comprised of diverse microsatellite markers including mono- and di-nucleotide repeats that range from 10 to 27 bp.
- A novel analysis algorithm was developed that leverages the unique signal processing properties inherent in semi-conductor sequencing and workflows were developed for tumor only samples as well as paired tumor-normal samples. The test provides results for individual microsatellites and generates a quantitative MSI score for the sample.
- The performance of the assay was verified over a cohort of >100 colorectal, gastric and endometrial cancer samples with MSI status independently assigned by orthogonal on-market tests.
- The MSI panel can be used by itself or integrated into larger targeted sequencing panels.
- MSI tests that leverage the inherent advantages of targeted semiconductor sequencing, including low sample input and fast turn-around time, will support expanded research opportunities into the association of MSI with other targeted alterations and help elucidate the interaction of MSI, DNA repair defects and the response to immune checkpoint inhibition.

The Ion AmpliSeq™ Microsatellite Instability Research Panel is posted on Ampliseq.com and available as a community panel.

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

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