Development of an expanded microsatellite instability panel with automated data analysis

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Introduction

Microsatellites are genetic motifs consisting of 1-6 base pair repeats. These sequences are susceptible to replication errors that can result in deletions and insertions. Normally, these errors are corrected by the DNA mismatch repair (MMR) system, however, when deficiencies in the DNA MMR system are present, microsatellite replicate errors accumulate in the genome. This phenomenon is commonly referred to as microsatellite instability (MSI). The evaluation of MSI is increasingly being used by clinical researchers for two main purposes: 1) to inform the diagnosis of a type of neoplastic inherited syndrome termed Lynch Syndrome[1] and 2) to assess the effectiveness of oncology immunotherapy treatment options.

Microsatellite instability is generally associated with better clinical outcomes after immunotherapy treatment[2] and is most frequent in uterine corpus endometrial carcinomas, colorectal adenocarcinomas, and stomach adenocarcinomas[3].

PD-1 blockade in tumors with mismatch-repair deficiency

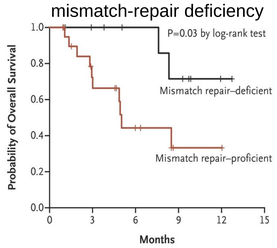


Figure 2B, Le et al. 2015, *NEJM*

Microsatellite instability frequency across multiple cancer types

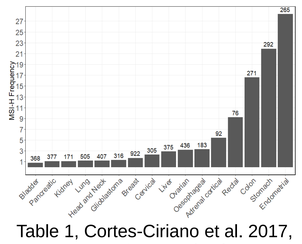
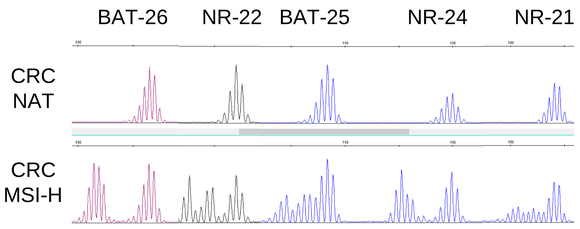


Table 1, Cortes-Ciriano et al. 2017, *Nature Communications*

In 2017, FDA's approval of KEYTRUDA® (pembrolizumab) for any patients with solid tumors harboring MSI or mismatch repair deficiency marked a paradigm shift in biomarker-guided therapy research. This has led to increased research utilizing MSI as a predictive biomarker for the effectiveness of immune-checkpoint inhibition. However, clinical researchers have indicated that current solutions to detect MSI are few and have limitations, including insufficient markers for applications across multiple tumor types and cumbersome data analysis.

Bethesda Panel, Gold Standard for MSI detection in CRC



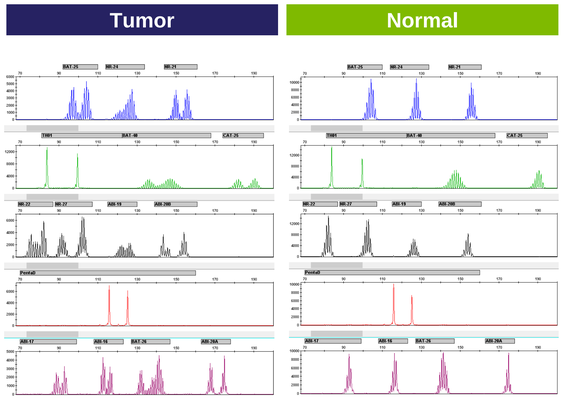
Thermo Fisher Scientific has improved upon the Bethesda panel and standard workflow by developing a MSI assay that has:

- A fast, simple workflow
- Low sample input (2 ng FFPE DNA)
- Expanded content from the Bethesda panel
- Automated analysis and interpretable results
- Tumor-only analysis

Methods

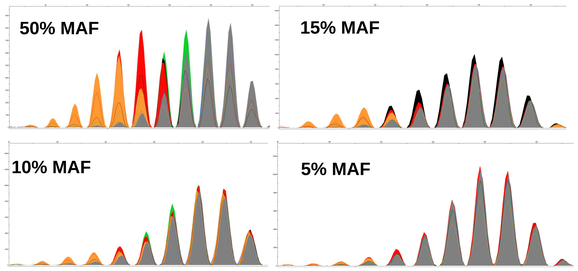
Fast workflow: 3.5 hours from DNA to answer

15 markers multiplexed across 5 dye channels



- The ABI MSI assay contains 13 mononucleotide MSI markers: 8 markers were derived from the literature and National Cancer Institute guidelines. Five additional markers internally identified for monomorphism and high sensitivity in multiple cancer types were included as well
- The assay also contains two sample identification markers to determine sample mixup or contamination
- Additionally, the new ABI MSI markers are less heterozygous than the Bethesda set indicating that they will have superior performance in tumor-only analysis (data not shown)

GeneArt synthetic constructs reveal detection complexities

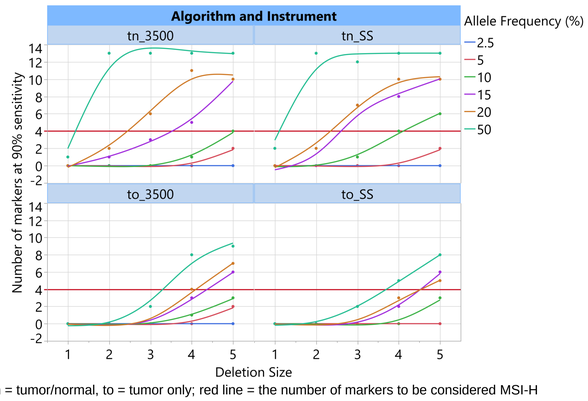


Deletion size: = WT, = 1 bp, = 2 bp, = 3 bp, = 4 bp, and = 5 bp

- Detection of instability is a complex interplay between deletion size and mutant allele fraction present in a sample
- We generated synthetic constructs to: 1) understand the peak morphology of difficult to assess MSI samples and 2) train the algorithm at various allele frequencies and with variable deletion sizes for each homopolymer

Results

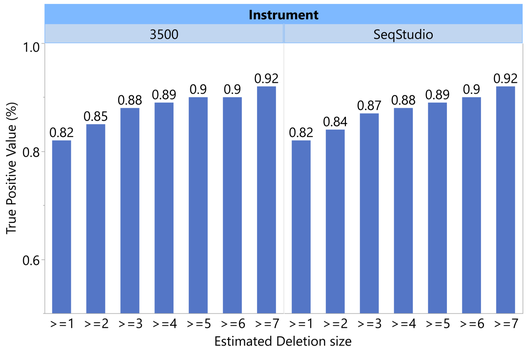
Algorithm performance on synthetic constructs



tn = tumor/normal, to = tumor only; red line = the number of markers to be considered MSI-H

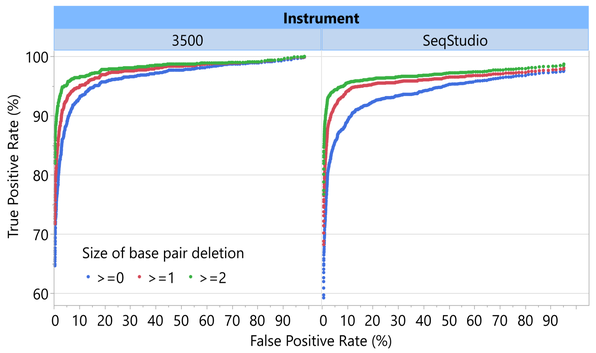
- High sensitivity is achieved in samples with 3-5 base pair deletions at 20% allele frequency

Tumor-Only Analysis on clinical samples, >98% specificity



- Tumor-only analysis in cancer types with large deletions like colon and gastric cancer will see high sensitivity

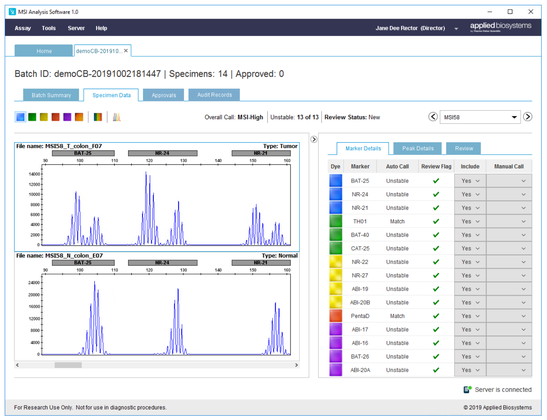
Tumor-Normal Analysis on clinical samples

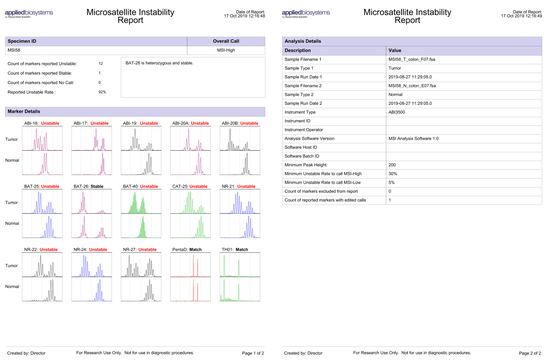


- Tunable algorithm parameters allow us to maximize sensitivity and specificity on both the Applied Biosystems™ 3500 and SeqStudio™ Genetic Analyzer Systems

ABI MSI software illustration: automatic calls and reports

- Automated genotyping solution for streamlined analysis and reporting, saving customers time and effort required by current manual analysis





Conclusions

- The ABI MSI Assay achieves robust identification of microsatellite instability in multiple cancer types with low sample input
- In addition, we developed MSI Analysis Software that has fast analysis with automated calling at sensitivity and specificity
- This easy-to-use MSI detection solution is normal control optional, cutting the cost per sample in half

References

1 Vaksman, Zalman, and Harold R. Garner. "Somatic microsatellite variability as a predictive marker for colorectal cancer and liver cancer progression." *Oncotarget* 6.8 (2015): 5760.

2 Le, Dung T., et al. "PD-1 blockade in tumors with mismatch-repair deficiency." *New England Journal of Medicine* 372.26 (2015): 2509-2520.

3 Cortes-Ciriano, Isidro, et al. "A molecular portrait of microsatellite instability across multiple cancers." *Nature communications* 8 (2017): 15180.

Acknowledgements

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