AMP 2023 Abstract #1575857

Microsatellite Instability (MSI) detection with TrueMark™ MSI Assay

Jieying Chu, Faqiang Wu, Shiloh Sprankles, Fernando J. Lopez-Diaz, Steven P Rivera, Vincent Funari

NeoGenomics Laboratories, Aliso Viejo, CA 92656

Introduction: MSI is detected as a result of a change in the lengths of microsatellites caused by deletions or insertions of short repeating nucleotide sequences in tumor DNA. Comparison to the germline DNA from the same individual or to a panel of normal samples is required. MSI status has clinical use for identifying patients with HNPCC/Lynch Syndrome and has for prognostic and therapeutic decision-making purposes. The TrueMark™ MSI Assay detects MSI through multiplex PCR and fragment analysis of 13 microsatellite markers. Additionally, two highly variable short tandem repeat (STR) sequences are included to confirm sample identity. Data was analyzed with the TrueMark™ MSI Analysis Software which can make automated MSI status calling for each marker with or without matching normal and offer a faster and easier analysis.

Methods: FFPE-derived DNA from 82 clinical samples (42 known MSI-H and 40 MSS samples; 43 colon, 39 non-colon including endometrium, uterus, cervix, pancreas, stomach, esophagus, liver, prostate, lymph node, and breast) were collected. The lowest native tumor content was 20% for colon and 40% for non-colon samples. To test the assay's performance at 20%, 30% and 40%, matched tumor and normal samples were mixed. All samples were run with TrueMark™ multiplex PCR and fragment analysis with 1 ng of input, and were analyzed with and without matched normal with the TrueMark™ MSI Analysis Software. Unflagged marker calls generated by the software were accepted, while flagged marker calls were reviewed and either accepted outright or called manually by comparing to matching normal markers if available. The cutoff for making an MSI-H determination is 30 % of markers or greater being unstable, an MSS call is between 0% and 30 %. The final calls of MSI status were compared to the Promega MSI PCR assay results whose calls were made manually. Over 460 clinical samples have been run with the assay so far and individual marker's performance have been investigated.

Results: At native tumor purity with matching normal (colon 20-80%, and non-colon 40-90%), the assay was 100% sensitive and specific. Without matching normal, the sensitivity was 94% sensitive and 100 % specific. The sensitivity for colon diluted to 20% tumor content with matching normal were 100% and 88% without. The sensitivity for non-colon diluted to 40% tumor with matching normal was 100% and 75% without. At 30% non-colon tumor with normal was 100% sensitive and 69% without. Specificity was 100% in all cases.

Conclusions: At 1ng DNA input (minimum input 0.25ng), TrueMark™ MSI Assay showed good performance. For all native samples (colon tumor content 20-80%, non-colon tumor content 40-90%), the assay showed 100% and 94% sensitivity with and without matching normal, with specificity being 100% in all cases.

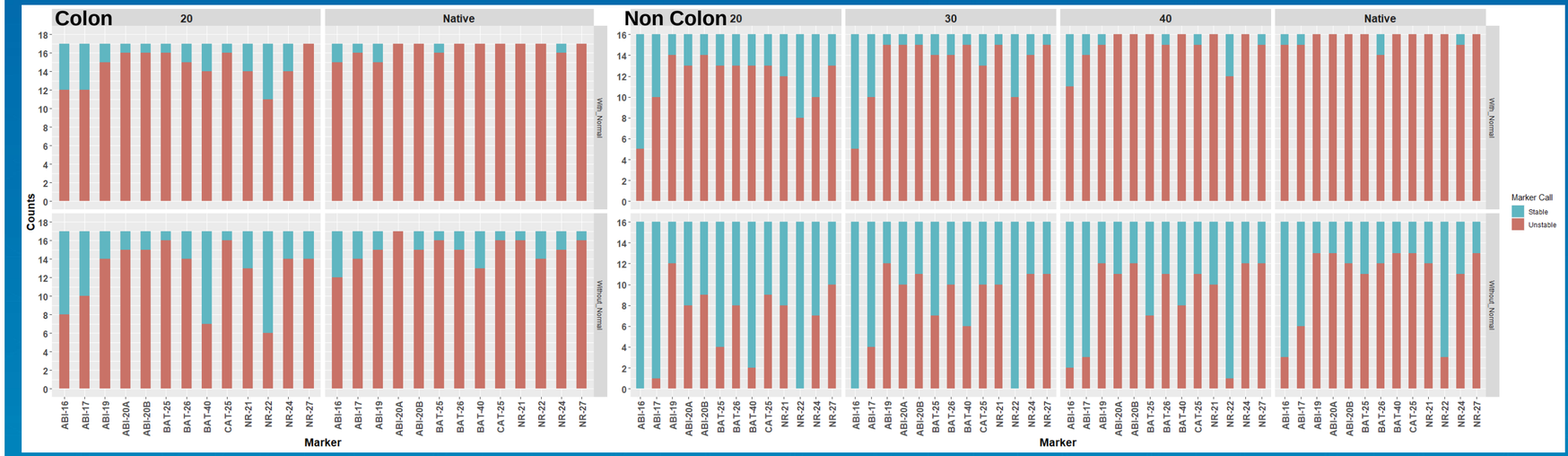
Jieying.Chu@neogenomics.com

Good performance of the TrueMark™ MSI Assay

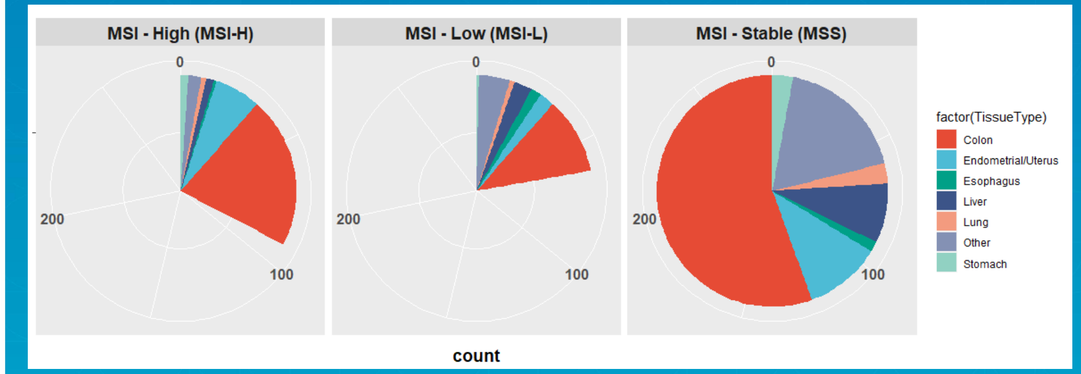
Tumor content		Colon (20-80%)	non-colon (40-90%)	all tissues
Native (20-90%)	w/ normal	100	100	100
	w/o normal	94	94	94
Dil. 20%	w/ normal	100	94	97
	w/o normal	88	63	76

Tumor content		Colon (20-80%)	non-colon (40-90%)	all tissues
Undiluted (20-90%)	w/ normal	100	100	100
	w/o normal	100	100	100
Dil. 20%	w/ normal	100	100	100
	w/o normal	100	100	100

ABI markers showed higher instability under different circumstances



One month clinical data monitor



Conclusions

- Assay showed 100% and 94% sensitivity with and without matching normal with 1ng DNA input.
- Some MSI markers (ABI & NR-22) were more prone to lose instability than others when normal wasn't available or tumor content decreased.
- Colon samples were more feasible to call MSI status reliably without matching normal, with tumor content as low as 20%
- Colon samples were 60% of clinical MSI samples, followed by endometrial/uterus, esophagus, and liver/lung

Reference

Cicek MS, Lindor NM, Gallinger S, et al. Quality assessment and correlation of microsatellite instability and immunohistochemical markers among population- and clinic-based colorectal tumors results from the Colon Cancer Family Registry. J Mol Diagn. May 2011;13(3):271-81. doi:10.1016/j.jmoldx.2010.12.004

Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors. *Clin Cancer Res*. Jul 1 2019;25(13):3753-3758. doi:10.1158/1078-0432.CCR-18-4070