Detection of Microsatellite Instability (MSI) Using oncoReveal™ Core LBx, a Multiplexed, Single Tube, Liquid Biopsy Assay

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ABSTRACT

Introduction. Inactivating mismatch repair genes can lead to microsatellite instability (MSI), resulting in increased insertions and deletions (indels) in simple repeat regions called microsatellites (MS) and the consequent introduction of alleles of varying MS length within the tumor genome (MSI-High or MSI-H). This phenotype is observed in several cancer types, including 15% of all colorectal cancers, and is prevalent in Lynch Syndrome. Accurate and timely detection of MSI using a non-invasive, liquid biopsy test is important for predicting the efficacy of immunotherapies that take advantage of increased expression of neoantigens in MSI-H tumors. MSI detection in tumors without matched normal tissue is confounded by natural diversity in MS length polymorphisms, necessitating a standard normal reference sample. To address this challenge, we developed a research-use-only (RUO) liquid-biopsy based multiplexed, single tube, targeted NGS-based panel for detecting MSI status in matched or unmatched tumors, in addition to SNV/indel and CNV detection of important cancer genes in cfDNA samples. Here, we demonstrate its performance in a diverse set of MSI-stable (MSS) and MSI-H samples.

Methods. We identified 27 informative MS sites from previous analyses of 198 clinical samples with known MSI status. Primers were designed using Pillar's VersaTile® software. The product size range was 55-130bp. Due to lack of commercially available cfDNA samples with known MSI status, we validated our designed primers on 17 pairs of matched tumor-normal FFPE samples. The resulting libraries were sequenced on Illumina's NextSeq or MiSeq machines and the data was analyzed using Pillar's secondary analysis platform, PiVAT® (Pillar Biosciences Variant Analysis Toolkit). MSIsensor (Niu et al, 2014) was used to call MSI status in both matched- and unmatched-calling mode. Unmatched calling was performed using a pooled normal sample of multiple ethnic backgrounds.

Results. PCR amplification of products was verified from commercially available cfDNA samples. In FFPE samples, we calculated the PiVAT MSI score, or the % of MS sites with >0.1 χ2-score between tumor and normal MS allele length distributions. PiVAT MSI score ≤45% indicated MSS status, >45% indicated MSI-H status. We estimated a minimum requirement of ~5,000 reads per amplicon to consistently and accurately call MSI status. Using this method, we were able to correctly call MSI-H vs MSS in all tumor samples with matched calling. For unmatched calling using a pooled reference, increasing the PiVAT MSI score cutoffs to 60% of sites with >0.2 χ2-score achieved identical 100% sensitivity and specificity.

Conclusion. We developed an (RUO) cfDNA kitted assay oncoReveal LBx for MSI detection in addition to SNV/indel and CNV detection. We demonstrate high performance with matched and unmatched normals, supporting the feasibility of such panels for liquid biopsy testing.

MICROSATELLITE SITE TARGETS

Target_Name	Historically Used Sites	Commercially Used Sites	Unmatched tumor calling subset
BAT25(T)25	+	+	+
BAT26(A)27	+	+	+
MONO27(T)28		+	+
NR21(A)21		+	+
23 Additional Targets			9 selected

Table 1 – The oncoReveal Core LBx panel consists of 27 PCR amplicons targeting 28 MS sites in the human genome, including a subset of sites comprising 1st and 2nd generation gold standard MSI testing kits and sites included in several high-impact studies from a literature review. A 13-target subset with high informative value was determined by examining MS site variability between normal tissue samples of various ethnicities (data not shown).

RESULTS

MSI Detection in Tumors with Matched Normal Comparator

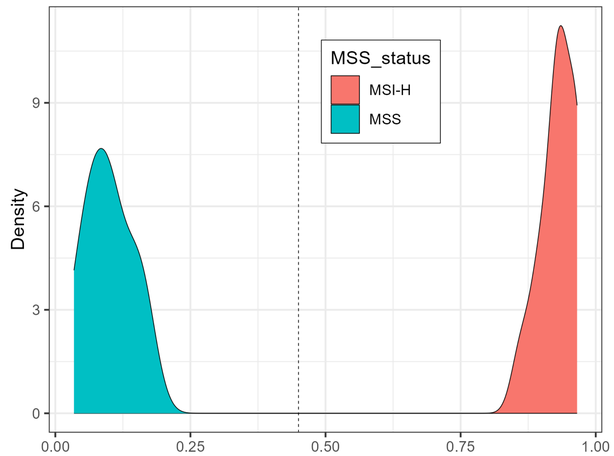

Figure 1. Matched MSI Calling: Using a per-site cutoff of 0.1 χ2 difference score and basing MSI-H positive call on >=45% of MSI sits meeting this criteria, matched tumor/normal samples were well differentiated using the oncoReveal Core LBx analysis pipeline. Maximum MSI scores for MSS samples were <= 0.25 and minimum MSI score for MSI-H samples was >= 0.75, resulting in a very high differentiating capability.

MSI Detection in Unmatched Tumors Using Synthetic Pooled Comparator

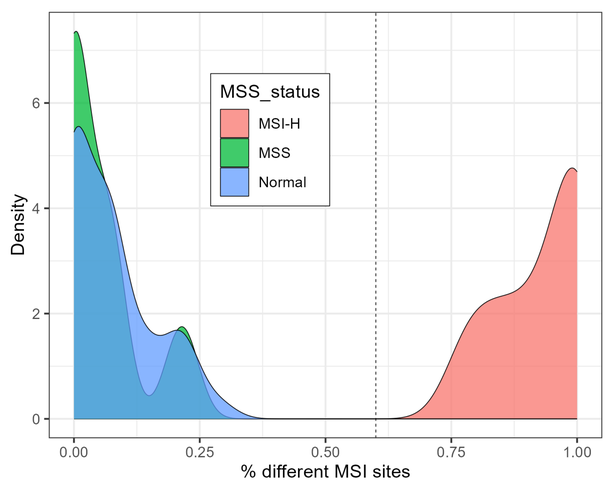

Figure 2. Unmatched MSI Calling: Using a per-site cutoff of 0.2 χ2 difference score and basing MSI-H positive call on >=60% of MSI sits meeting this criteria, unmatched tumor samples were well differentiated using the oncoReveal Core LBx analysis pipeline. Maximum MSI scores for MSS samples were <= 0.30 and minimum MSI score for MSI-H samples was >= 0.65. Cutoffs were set to minimize the likelihood of false positive calls in tumors without a direct normal comparator, where MSI site length polymorphisms based on normal population heterogeneity is more likely to occur.

RESULTS

MSI Detection in Diluted MSI-H Sample Libraries

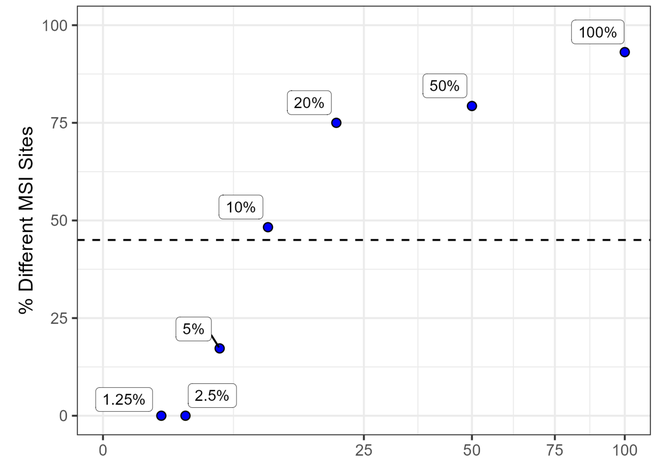

Figure 3 Dilution Series of MSI-H Signal: A dilution series of the well characterized MSI-H cell line DLD-1 in a normal cf LBX sample was performed in order to simulate decreasing tumor content and the limit of detection of MSI signal. Accurate positive MSI calls were still obtained down to the 10% dilution level.

Performance Summary

Definition	Characterized in	Validation results
NPA TN * 100 / (FP + TN)	Clinically characterized samples Human normal†	100%
PPA TP * 100 / (TP+FN)	Clinically characterized samples	100%

Table 2. Summary of performance metrics from secondary analysis pipeline. The table summarizes negative percent agreement (NPA) and positive percent agreement (PPA) for the normal and positive FFPE samples tested in this study. The panel has very high sensitivity and specificity of 100%

CONCLUSIONS

- We present here oncoReveal™ Core LBx, a research-use-only (RUO) liquid biopsy panel that includes MSI detection capability.
- We demonstrate successful amplification of targets in cfDNA samples and high sensitivity and specificity of our panel in FFPE samples, even when tumor/normal signal was reduced to 10%.
- Limited availability of commercial cfDNA standard samples limits a comprehensive assessment of the panel.
- Detection of MSI positive samples represents an important feature forward in cancer research and the molecular profiling of liquid biopsy samples.
- Larger studies would be needed to establish the clinical utility of this panel.