Analytical Performance of a Novel Microsatellite Instability (MSI) Droplet Digital PCR Test

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Introduction: Microsatellite Instability

Microsatellites are tandem repeats of 1-6bp that are spread throughout the genome. Microsatellite length variation is observed in tumor cells with impaired mismatch repair (MMR). The variable microsatellite length phenotype is called Microsatellite Instability and is considered a phenotypic marker of mismatch repair deficiency, with prognostic and therapeutic implications. Microsatellite instability (MSI) is seen in gastrointestinal, endometrial, and colorectal tumors[1] as well as other tumors at variable frequency[2]. Studies have confirmed that MSI tumors have a better prognosis than microsatellite stable (MSS) colorectal cancer, and MSI cancers do not have the same response to the chemotherapeutic strategies used to treat microsatellite stable tumors[3].

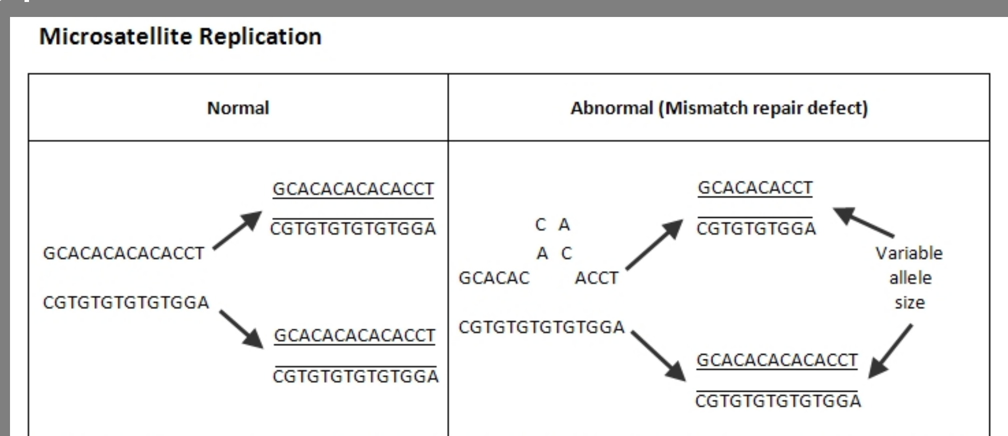

Figure 1[4]. Correct and erroneous replication of a microsatellite region. Functional MMR would correct errors such as the one shown in the right panel, but when MMR is deficient such errors accumulate.

We developed a highly sensitive Droplet Digital PCR (ddPCR) test for determining MSI status by testing 5 common microsatellite markers. The test can be used on either tumor tissue (fixed or fresh) DNA or plasma cell-free (cf) DNA and it does not require testing of matched normal samples for analysis.

Bio-Rad ddPCR Workflow

Figure 2. Universal Droplet Digital PCR setup procedure on Bio-Rad QX200 Instrument System

The Bio-Rad MSI ddPCR test follows the efficient, universal workflow of Droplet Digital PCR setup that tolerates variation between operators, days, instruments, or laboratories and requires no external standards or replicates, making it an ideal technology for clinical implementation.

Bio-Rad MSI ddPCR Assay Design

This novel MSI ddPCR test utilizes a competitive-probe drop-off assay design, with two probes competing for the same target sequence. Depending on mutation level/microsatellite length, one or the other probe is out-competed; indicating microsatellite stability or instability.

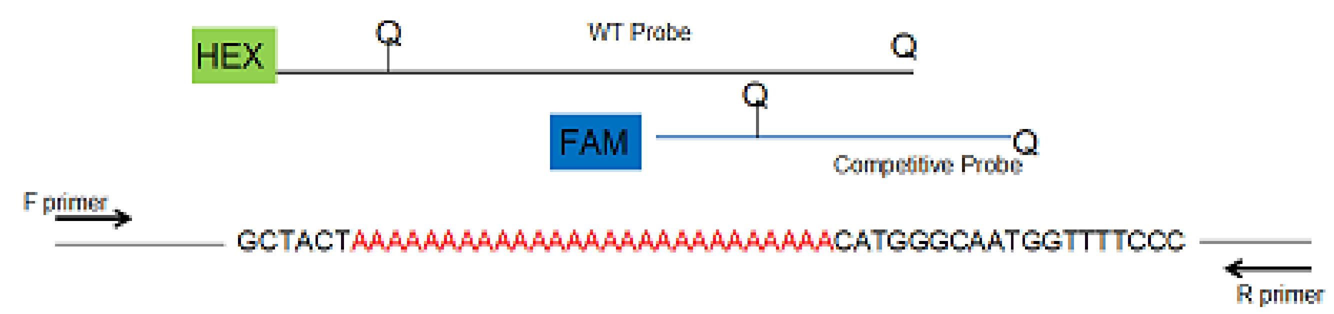

Figure 4. Schematic representing sample microsatellite site, with two competitive complementary probes

With wildtype target molecules, both probes bind and get hydrolyzed at an even rate, resulting in a "double-positive" droplet. With mutant (repeat shortened) target molecules, the full-length probe will be out-competed by the competitive probe, resulting in a single-positive droplet.

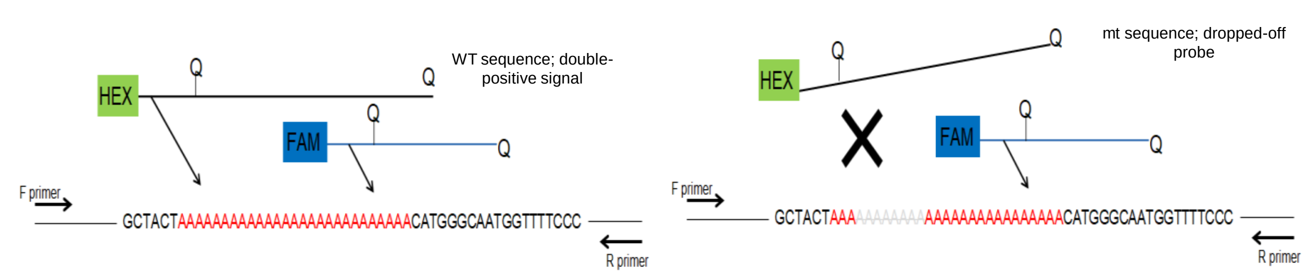

Figure 5. Probe competition on a WT (left) and mt (shortened, right) microsatellite target.

Bio-Rad MSI ddPCR Test Configuration

The Bio-Rad MSI ddPCR test analyzes 5 common microsatellite markers in a total of 3 reaction wells, featuring assay duplexing to minimize sample volume requirements:

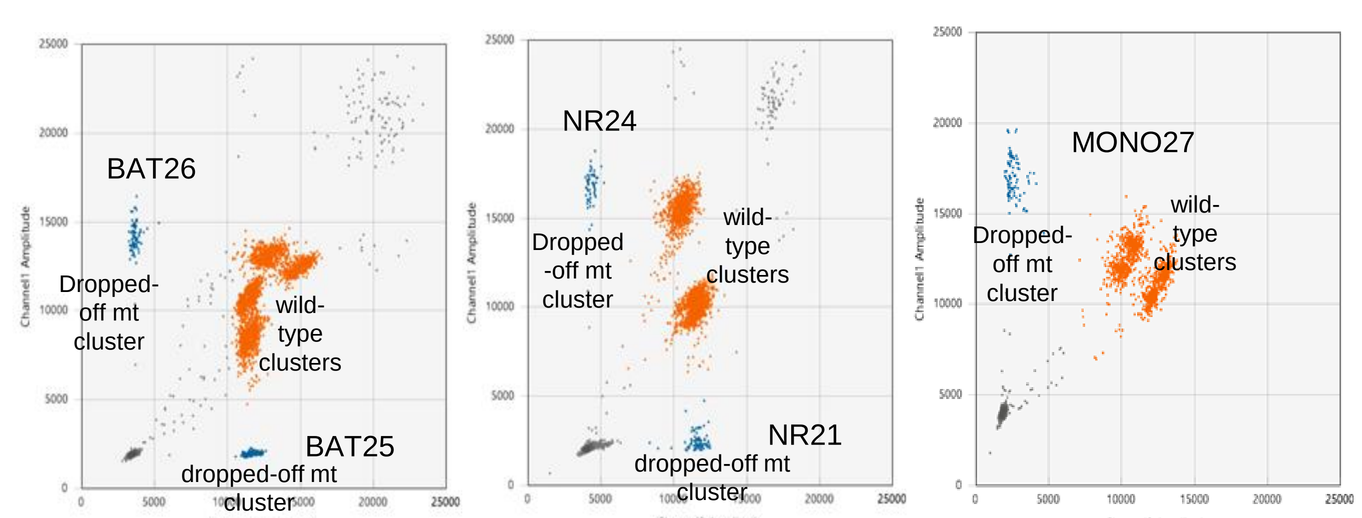

Figure 3. Two-dimensional ddPCR plots of the 3 MSI reactions testing a real MSI-H patient cfDNA sample. Wild-type molecules are in the orange clusters, and the mutant molecules (corresponding to microsatellite instable targets) are in the blue clusters. Individual targets are labeled on each plot.

MSI ddPCR Analytical Sensitivity and Performance

The sensitivity of the MSI assay to variable length deletions was determined using contrived samples consisting of mixtures of DNA from MSS and MSI-H cell lines, as well of synthetic ultramers of defined repeat lengths. The assay can distinguish mutant (shortened) from wild-type alleles if the length difference is at least 2 base pairs in the microsatellite repeat region.

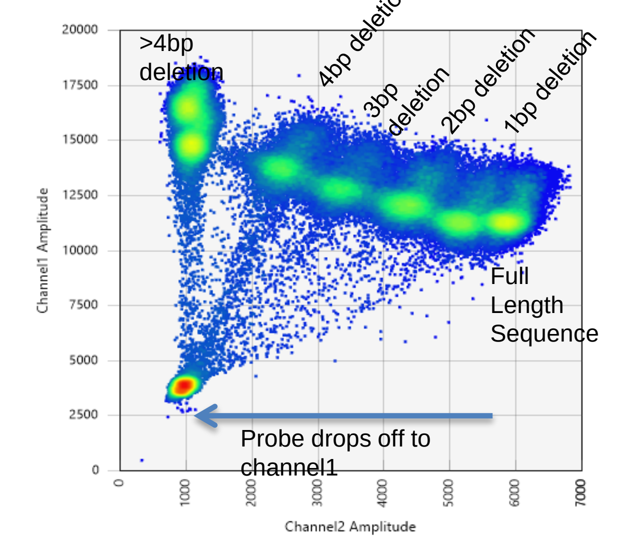

Figure 6. Overlaid two-dimensional ddPCR plot (in heatmap) showing drop off clusters at different deletion bp lengths for a singleplex MSI assay (mt in FAM channel1)

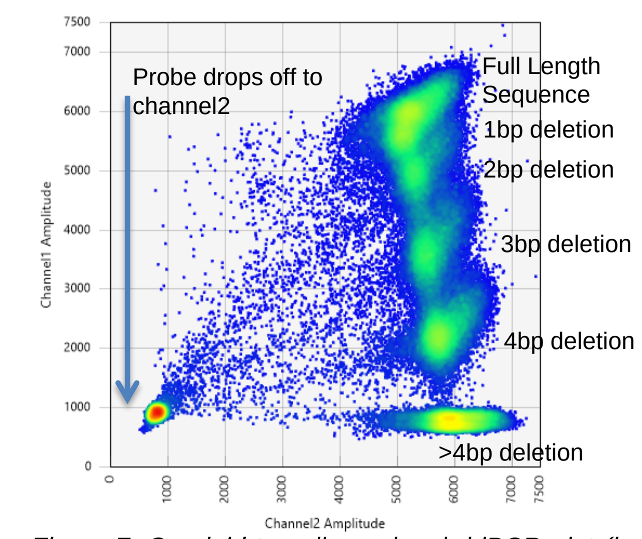

Figure 7. Overlaid two-dimensional ddPCR plot (in heatmap) showing drop off clusters at different deletion bp lengths for a singleplex MSI assay (mt in HEX channel2)

Serial titration of MSI-H cell line DNA into MSS cell line DNA resulted in an analytical sensitivity of at least 0.125%, with all contrived samples detected as positive down to the lowest dilution tested. Limit of blank (LOB) for each of the 5 markers was either 0 or 1 mutant copy/marker

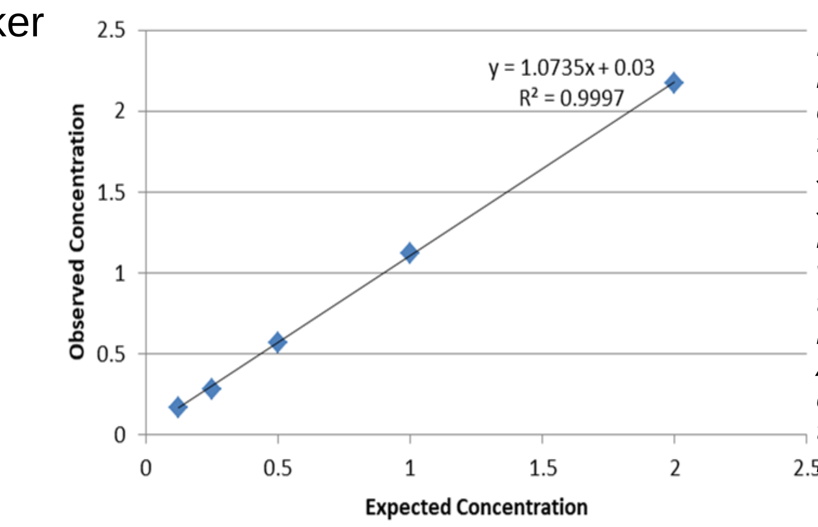

Figure 8. Graph of linearity for Bio-Rad ddPCR MSI test. A 2-fold serial dilution series of contrived sample made from cell line DNA was tested with the 3 MSI assays, two wells per assay; 13 replicates per dilution. All replicates were detected positive down to 0.125% MSI fraction input

Conclusion

The Bio-Rad ddPCR MSI test offers an analytical sensitivity of 0.125% or below and a LOB of 0-1 mutant copy/test. It can distinguish MT from WT with as little as 2bp difference without requiring a paired normal sample for analysis, comparison, making this test well suited for MSI detection in various tissue sample types or in liquid biopsy.

References

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See corresponding poster # ST083/Abstract # 16976 "Clinical Validation of the Bio-Rad ddPCR MSI Test for the Detection of Microsatellite Instability in Tumor and Plasma cfDNA Samples"

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