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 as well as other tumors at variable frequency. Studies have confirmed that MSI tumors have a better prognosis than microsatellite stable (MSS) colorectal cancers, and MSI cancers do not have the same response to the chemotherapeutic strategies used to treat microsatellite stable tumors.

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 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.

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.

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:

Bio-Rad MSI ddPCR Test Configuration

The Bio-Rad MSI ddPCR Workflow

1. Generate droplets
2. Perform PCR
3. Read and analyze results

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 other external standards or replicates, making it an ideal technology for clinical implementation.

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

4. Adapted from Gruber SB, Kohlmann W. The genetics of hereditary nonpolyposis colorectal cancer. J Natl Comp Cancer Net. 2002; 1:137-44

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.”