

Digital PCR Liquid Biopsy for KRAS Mutations

This application note describes a novel droplet-based digital PCR method for the non-invasive detection of KRAS in plasma of patients with metastatic colorectal cancer. This highly sensitive and specific method has the potential to be employed in multiple applications in the clinic, including diagnosis, cancer recurrence monitoring, and treatment management.

Circulating Tumor DNA in Cancer

Circulating tumor DNA (ctDNA) is present in plasma of individuals with advanced cancers.¹ ctDNA is a prognostic marker for patients with colorectal cancer (CRC) and it might also be used for predicting the response to targeted therapy. For example, mutations in KRAS indicate which patients will fail to respond to specific therapies (cetuximab, panitumumab).² Although ctDNA is characterized by the presence of a somatic mutation, direct quantitative detection through a simple workflow of such mutant DNA is not feasible by current technologies because the ratio of ctDNA to wildtype DNA can be as low as 1/10,000, often beyond the capabilities of qPCR or dPCR approaches with low reaction numbers.

Multiplexed detection of KRAS mutations in Colorectal Cancer

TaqMan assays for each of the seven most frequent mutations of KRAS were assembled into two multiplex panels covering codons 12 and 13 by mixing mutation-specific VIC and/or FAM TaqMan probes with a single wild-type (VIC) probe and a single pair of PCR primers in each panel. Using control DNA, the heat map histograms reveal that concentrations of probes were tuned to enable discrimination of droplets containing no amplifiable fragments, (PCR negative), wild-type KRAS, or a fragment with a unique KRAS mutation. (Figure 1). The sensitivity of the procedure was confirmed by measuring 1/200,000 dilution of KRAS-mutated cell-line DNA in a background of wild-type DNA.³

DNA was isolated from plasma of a patient with metastatic colorectal cancer. The DNA sample was split and analyzed by a duplex digital PCR assay (Figure 2, top panel) and a multiplex digital PCR assay (bottom panel). The results from the two analyses were similar. In both cases – duplex or multiplex – digital PCR revealed 0.58% and 0.54% of the mutant G12D alleles in the wild type, respectively.

Figure 1
Fluorescence Intensity Scatter Plots

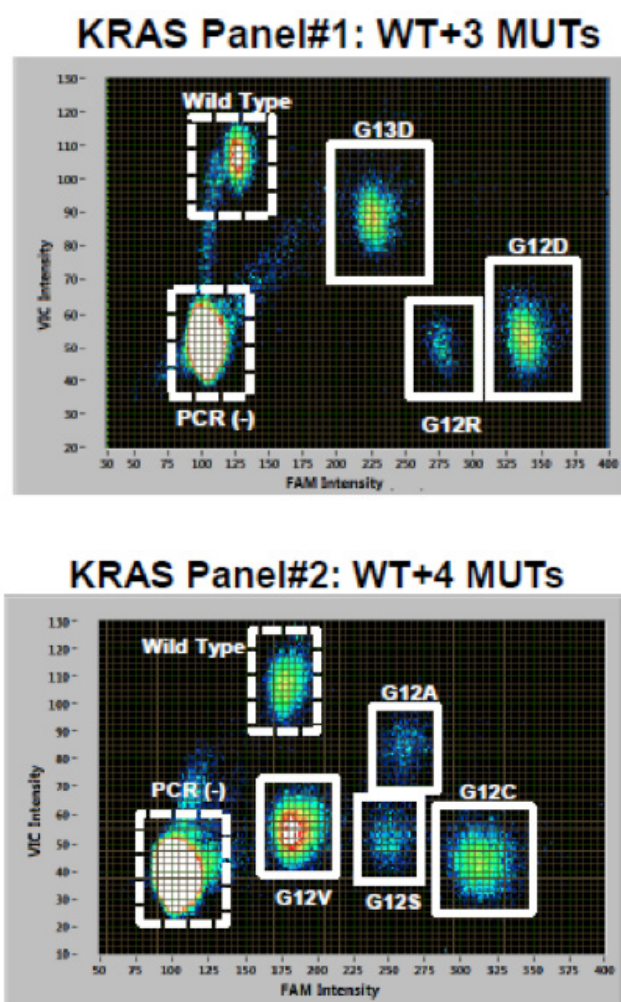
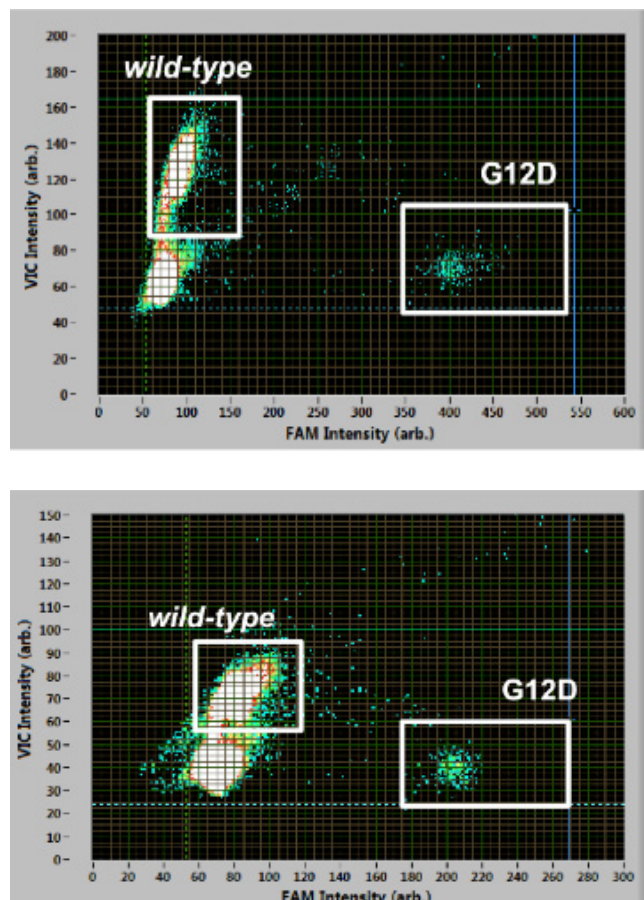


Figure 2



Circulating DNAs isolated from plasma of 54 patients with metastatic colon cancer were analyzed using the KRAS panels.³ DNA concentration in the plasma samples varied by two orders of magnitude and was not correlated with the proportion of mutated DNA, which varied from 42% to 0.1%. 19 samples were expected to be positive for a KRAS mutation based on previous tumor DNA characterization. Results are shown below:

	Tumor KRAS + (19)	Tumor KRAS - (35)
+ For dPCR	16 matched 1 different mutation*	2**
- For dPCR	2	33

*For one KRAS+ tumor, the plasma sample contained a mutation different from the one expected from the tumor characterization.

**Two plasma samples were positive with dPCR for a mutation but no mutation was detected in the tumors. For one of these samples, the initial tumor sample contained less than 15% of tumor cells, and the patient was noted to have a progressive disease at the first evaluation under cetuximab. The other sample was not evaluated for the number of tumour cells due to the small size of the biopsy.

Conclusion

By segregating individual target DNA molecules into millions of droplets, the [RainDrop™ dPCR System](#) allows for sensitive digital detection and quantification of the most frequent KRAS mutations in circulating DNA in plasma. This multiplex “liquid biopsy” approach has applications across a variety of research sample types in the clinic, and a potential role in diagnosis, monitoring, and treatment management decisions in cancer and other progressive diseases.

References

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