Quantitative detection of circulating tumor DNA by droplet-based digital PCR.


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Circulating tumor DNA (ctDNA) is present in plasma of individuals with advanced cancers. It 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 (sorafenib, panitumumab). 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 wild-type DNA can be as low as 1/100,000.

The study describes the use of droplet-based digital PCR for detection and quantitation of frequent KRAS mutations in ctDNA from plasma of patients with advanced colorectal cancer. Furthermore, we demonstrate that multiple digital PCR*1 enables testing samples for different mutations simultaneously.

By segregating individual target DNA molecules into millions of aqueous nanoliter droplets, our procedure allows for extremely precise, sensitive, and fast quantification of mutated genes. The sensitivity of the procedure was confirmed by measuring 1/200,000 dilution of mutant DNA over five decades of concentration to 95% confidence intervals (represented by the orange shading).

**Figure 3:** Sensitivity of the method. Performance of droplet-based digital PCR was compared to a qPCR method identifying a single sample of 100 ng DNA with a mutation frequency of 1%. The fluorescent signal of each droplet is analyzed. Droplets can also be analyzed with confocal microscopy. The TaqMan® probes recognizing wild-type alleles carried a red-fluorescent fluorophore and TaqMan® probes specific for the mutant sequences carried a green fluorophore.

**Figure 4:** Detection of KRAS codon 12 mutations in DNA isolated from plasma of a patient with metastatic colon cancer. The DNA sample was diluted in a range of 100 ng to 1 pg and analyzed with droplet-based digital PCR. The detection limit was 100 copies of the mutation per 200,000 copies of wild-type DNA. The method is thus, both sensitive and quantitative, the sensitivity being limited only by the number of droplets analyzed.

**Figure 5:** Performing multiplex reactions in droplets. Multiplexing enables development of assays for biomarker panels at reduced cost and sample consumption.

**Figure 6:** Quantitative detection of circulating tumor DNA in plasma of patients with metastatic colorectal cancer. The DNA sample was diluted in a range of 100 ng to 1 pg and analyzed with droplet-based digital PCR. The detection limit was 100 copies of the mutation per 200,000 copies of wild-type DNA. The method is thus, both sensitive and quantitative, the sensitivity being limited only by the number of droplets analyzed.

**Figure 7:** Detection of KRAS codon 12 mutation in DNA isolated from plasma of a patient with metastatic colorectal cancer. The DNA sample was diluted in a range of 100 ng to 1 pg and analyzed with droplet-based digital PCR. The detection limit was 100 copies of the mutation per 200,000 copies of wild-type DNA. The method is thus, both sensitive and quantitative, the sensitivity being limited only by the number of droplets analyzed.

Detecting and quantifying circulating tumor DNA in plasma of patients with metastatic CRC has high sensitivity and specificity. We anticipate that the method will be employed in multiple applications in the clinic, including diagnosis, cancer recurrence monitoring, and treatment management.

**References**