Single molecule droplet-based digital PCR for high sensitivity detection of cancer biomarkers

Taly V.1,2, Pekin D.3, Skhiri Y.4, Baret J.C.5,6, Le Corre D.7, Mazutis L.1, Ben Salem C.8, El Abed A.9, Kotsoopoulos S.6, Hu G.4, Hutchison J. B.4, Link D. R.4, Griffiths A.4, and Laurent-Puig P.10

Introduction

Somatic mutations within tumor DNA are highly specific biomarkers that distinguish cancer cells from normal cells. The biomarkers are likely to be useful for the diagnosis, prognosis, treatment, and follow-up of patients, but use of these biomarkers in clinical oncology requires a highly sensitive strategy that discriminates tumor-specific mutations in a large excess of non-mutated DNA. Digital PCR (dPCR) is an attractive alternative to qPCR due to its superior sensitivity and absolute quantification of target molecules. The RainDance RainStorm® droplet-based microfluidics method utilizes up to 10 million reactions to enable detection of rare mutations in a background of wild-type DNA at levels better than 1 in 200,000.1 The combination of superior sensitivity, unprecedented multiplexing, and flexibility in experiment design provide a powerful genomic analysis platform for new research in cancer including rare variant detection, absolute quantitation of biomarkers, tumor profiling, and the ability to monitor residual disease.

KRAS mutations are found at high rates in several cancers, including colon cancer. The RainDance digital PCR technology is being used to screen colorectal tumor samples from a collection of patients with the goal of correlating the identity and level of one or more KRAS mutation with known clinical outcomes. This system will also be used to validate tumor characterization achieved with next-generation sequencing and Sanger sequencing, and to monitor patient-specific biomarkers for detecting minimal residual disease and disease recurrence.

What is Digital PCR?

Digital PCR is an absolute method of quantifying DNA. Template DNA is diluted to single molecule occupancy per reaction. Using millions of droplets as reaction chambers and end-point amplification, positive PCR reactions are counted as a direct measurement of the number of DNA molecules originally present. Fluorescent probes are commonly used to discriminate PCR(+)/− reactions, and Poisson statistics account for the possibility of multiple molecules occupying the same reaction. Unlike qPCR, standard curves are not necessary.

Superior Sensitivity for Detecting Rare Mutations

Superior sensitivity provides potential for non-invasive diagnostic applications including use of cell-free mutant DNA in blood samples.

Unprecedented Multiplexing Capability With Droplet-Based dPCR

A new method for differentiating targets on the basis of end-point fluorescence intensity was developed by varying the concentration of the fluorescent probes.2 To demonstrate, two panels containing multiple TaqMan® assays for KRAS SNP mutations were assembled. By adjusting concentrations of the probes for each individual assay, 3 or 4 KRAS mutations were measured simultaneously with just VIC and FAM fluorophores. This approach can be expanded to higher plex levels, and it enables detection, identification, and measurement of multiple possible mutations from a single DNA sample.

References

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6. RainDance Technologies, Lexington, Massachusetts, USA

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