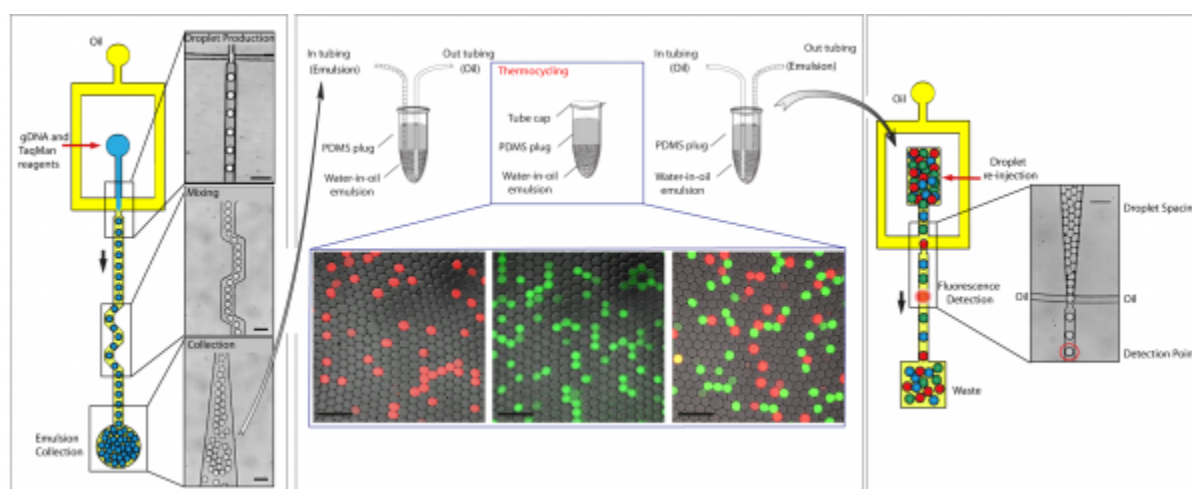


Droplet-based digital PCR microfluidic procedures for genetic and epigenetic alteration(s) detection.

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Genetic and epigenetic alterations within tumor DNA can be used as highly specific biomarkers to distinguish cancer cells from their normal counterparts. These biomarkers are potentially useful for the diagnosis, prognosis, treatment and follow-up of patients. In order to have the required sensitivity and specificity to detect rare tumoral DNA in stool, blood, lymph and other patient samples, a simple, sensitive and quantitative procedure to measure the ratio of mutant to wild-type genes is required. We have developed a procedure allowing the highly sensitive detection of mutated DNA in a quantitative manner within complex mixtures of DNA. In the original work, by using a droplet-based microfluidic system to perform digital PCR in millions of picoliter droplets, we demonstrated the possibility to have an accurate and sensitive quantification of mutated *KRAS* oncogene in gDNA. This technique enabled the determination of mutant allelic specific imbalance (MASI) in several cancer cell-lines and the precise quantification of mutated *KRAS* gene in the presence of a 200 000-fold excess of unmutated *KRAS* genes. The sensitivity is only limited by the number of droplets analyzed. Furthermore, it is also possible to perform multiplex analysis allowing the detection of several different mutations within a single biomarker, mutations in different biomarkers, or even to perform whole targeted pathway analysis. We have now developed droplet-based digital procedures for the highly sensitive detection of a wide range of mutations, copy number variations, DNA hypermethylation and for the quantitative analysis of miRNA and mRNA expression. Future aims of the project are the optimization and validation of this method for its widespread use in clinical oncology.



Overview of the droplet-based procedure developed for the highly sensitive detection of rare sequences within biological samples. (a) An aqueous phase containing the gDNA, PCR reagents and TaqMan® probes specific for the wild-type and mutant genes is emulsified using a microfluidic device. (b) The emulsion is collected in a PDMS-sealed and thermocycled. During DNA amplification, the TaqMan® probes are cleaved and the corresponding fluorophores are released. Light micrographs of drop production, mixing, collection and

re-injection are shown (scale bars 60 μ m). **Middle Panel: Fluorescence confocal microscopy analysis of thermocycled droplets.** gDNA extracted from homozygous cell lines bearing wild-type KRAS alleles (1) and a heterozygous cell-line bearing both mutant and wild-type KRAS alleles (2) were analyzed. Red-fluorescent droplets contain wild-type DNA, green-fluorescent droplets contain mutant DNA, yellow-fluorescent droplets contain both mutant and wild-type DNA, and non-fluorescent droplets do not contain target DNA (scale bar 100m) (from Pekin et al., 2011).

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