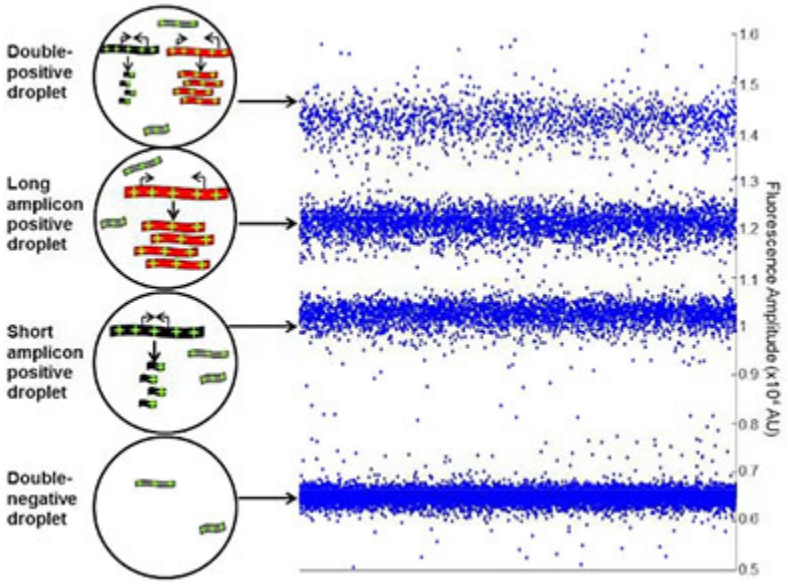
Docket #: S13-343

Customizable Genotyping with Digital PCR Platform

Researchers in Prof. Hanlee Ji's laboratory have developed a robust, highly customizable assay for quantifying genetic aberrations on a digital PCR platform. This technology could be used for a variety of genotyping applications, such as mutation detection, copy number analysis, and studies of fragmented DNA (such as with formalin-fixed paraffin embedded tissues). For example, with single nucleotide variants this technology employs a set of two primers with non-complementary “tail” regions that vary in length depending on which allele they are designed to bind (e.g. wild type or mutant). After PCR, these primers produce amplicons that can be quantified independently using a non-specific fluorescent DNA binding dye (such as EvaGreen).

The use of tails allows for direct control of the amplicon size and therefore the fluorescent amplitude of the positive partitions. This eliminates the need for an optimized TaqMan probe and enables detection of smaller amplicons than is allowed with traditional TaqMan detection schemes. With this new system, the region of interest and the reference gene are both amplified in the same well to eliminate the need for standards and to minimize sampling error.



Schematic of Droplet-digital PCR with Non-Specific Dye. *Droplets are formed pre-PCR by randomly sequestering fragmented template DNA into equal volume partitions. The first population of droplets, corresponding with the lowest fluorescent amplitude, has only the unamplified background template DNA (gray). The second population represents the droplets containing only the short amplicon template (black). The third population represents droplets with only the long amplicon template (red). The population with the highest amplitude represents droplets containing both amplified targets.*

Stage of Research

The inventors have demonstrated that this method can detect mutations that are present in less than 1% in an otherwise wild type sample, and identify copy number change in a sample of 10% tumor admixture with normal DNA. The researchers have also demonstrated wide applicability in tracking oncogenic mutations at single molecule resolution in circulating DNA.

Applications

- **Genetic aberration quantification** on droplet-digital PCR platform, for detecting:
  - single nucleotide polymorphisms
  - copy number variation
  - genomic DNA integrity
  - splice variant quantification

    - gene expression with cDNA
    - small RNA quantification

Advantages

- **Highly customizable:**
    - no need to optimize TaqMan probe oligonucleotide
    - interrogate a wide range of targets regardless of neighboring nucleotide context not limited by spectral overlap, multiplexing is dependent on length of tail
- **Robust:**
    - retains accuracy found in TaqMan-based assays
    - minimal sampling error because both the region of interest and reference gene are measured from the same template in the same well
    - eliminates need for standards
- **Sensitive** - can detect and quantify genetic aberrations at very low concentrations
- **Scalable:**
    - enables multiplexing with tail length and does not require dyes of different wavelengths
    - cost effective when scaling to a large number of genes
    - simple oligonucleotide synthesis
    - transferable to any assay previously optimized for general bulk PCR
    - optimize only two primers for new assay (compared to three in TaqMan chemistry)
- **Small template-specific target region:**
    - increases PCR efficiency
    - shorter amplicons preferable for detecting degraded DNA (for example, in formalin-fixed paraffin embedded samples)

Publications

- Christina Wood-Bouwens, Billy T. Lau, Christine M. Handy, HoJoon Lee, Hanlee P. Ji, Single color digital PCR provides high-performance detection of cancer mutations from circulating DNA, Journal of Molecular Diagnostics, Sept. 2017, Vol. 19, Issue 5; http://dx.doi.org/10.1016/j.jmoldx.2017.05.003.

- Published PCT Patent Application
- Miotke, LK; Lau, BT; Rumma, RT; Ji, HP, High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR, *Anal Chem.*, published online Jan. 31, 2014.

Patents

- Published Application: WO2015095225
- Published Application: 20160304936
- Published Application: 20200017902
- Issued: 10,465,238 (USA)

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