A158 Forensic Short Tandem Repeat Typing of Single Cells Using Digital Droplet Microfluidics

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After attending this presentation, attendees will learn a single-cell Short Tandem Repeat (STR) typing method for reliable and accurate forensic identification of dilute cellular mixtures.

This presentation will impact the forensic science community by the straightforward analysis of mixtures of single cells, because each cell is typed individually. STR profile interpretation from single cells was feasible even in the presence of background cell-free DNA. This method can be immediately used in common forensic laboratories since no expensive or sophisticated equipment is involved and the final data appears in a familiar conventional format. The improvements in sensitivity and selectivity will lead to more reliable results, especially in cases involving low amounts of cells and mixed cells.

STR forensic identification is a powerful technique that depends upon collecting homogeneous genetic samples from a crime scene for Polymerase Chain Reaction (PCR) amplification; however, real-world samples often present mixtures of cells from different individuals at relatively low concentrations, and thus are difficult to type by conventional methods. Recently, the development of microfluidic technology has enabled digital PCR amplifications in ultralow-volume droplets with single template copy sensitivity. A microfluidic-droplet based approach was developed to perform digital STR typing of single cells with high sensitivity, fidelity, and throughput.

The overall strategy is to isolate individual cells in nanoliter droplets along with primer conjugated beads, release the nuclear DNA for STR amplification, and then analyze the single-cell amplicons linked to each bead. First, a microfluidic droplet generator was constructed with polydimethylsiloxane soft lithography that produced 1.5nL monodisperse agarose microdroplets in fluorinated oil at a high frequency of 444Hz. Second, single cells, along with microbeads functionalized with multiplex primers for STR targets, were dispersed in agarose and compartmentalized within the droplets at a statistically dilute level. The 34µm Sepharose® beads serve as amplicon-binding substrates during PCR to maintain the monoclonality of STR analysis. The unique thermo-responsive sol-gel switching property of agarose enabled the gelled droplets containing the individual cells (and beads) to be processed for various mechanical manipulations and storage while preserving single-genome integrity. Third, the porous structure of the gelled agarose was exploited to introduce a lysis and digestion buffer which released high-molecular-weight genomic DNA from the cell but kept the template DNA entrapped within the droplet. Fourth, the gelled droplets were equilibrated with PCR mixture and redispersed in silicon carrier oil by agitation to form a uniform emulsion of nanoliter PCR reactors. Massively parallel single-cell emulsion PCR was then performed in a standard PCR tube using a conventional thermocycler. This process transferred the STR loci information from the trapped cell onto the co-encapsulated microbead within the droplet. Following amplification, the beads were recovered by removing the oil and disrupting the droplets by melting the agarose. Finally, the STR products immobilized on the beads were detected by reamplifying in PCR plates under appropriate statistically dilute conditions followed by conventional Capillary Electrophoresis (CE) fragment analysis.

The utility of this method for forensic DNA typing was first explored using a 9-plex STR system employing eight core STR loci in the Combined DNA Index System (CODIS) database (D3S1358, D5S818, D7S820, D8S1179, D13S317, D21S11, vWA, and TH01) plus the sex marker Amelogenin. The protocols for the microbead-based multiplex PCR were initially optimized both in bulk solutions and on microdroplets using 9947A female and 9948 male genomic DNA. Sufficient STR fragments were easily produced to enable direct CE analysis after secondary PCR *from single beads*. Using the optimized procedures, complete STR profiles were obtained from individual GM09947 (female) and GM09948 (male) human lymphoid cells.

Based on this success, single-cell identification studies were performed on mixtures of GM09947 and GM09948 cells at different relative concentrations. The cell mixture study exhibited a good linear relationship between the observed and input cell ratios in the range of 0:1 to 10:1, eliminating the complex interpretation of mixed genotypes. STR profiles of single-cells from both pure and mixed cell populations showed that all alleles were correctly called and allelic drop-in/drop-out was not observed. Additionally, the STR profile of target GM09947 cells could be deduced even in the presence of a high-concentration (three copies/droplet) of cell-free 9948 “background DNA.”

Single-Cell STR Analysis, STR Mixture Analysis, Microfluidics

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