Multiplexed, high-throughput SNP analysis by free-solution conjugate microchannel electrophoresis

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Technologies that allow for high-throughput, economical, and accurate single nucleotide polymorphism (SNP) genotyping are becoming crucial for modern genomic analyses. Here, we present a method for multiplexed single-base extension (SBE) genotyping that takes advantage of the unique separation modalities made possible via end-labeled free-solution electrophoresis (ELFSE). A series of monodisperse "drag-tags" was created using both chemical and biological synthesis and used to achieve the high-resolution separation of genotyping reaction products by microchannel electrophoresis without a polymeric sieving matrix. A highly multiplexed SBE reaction was performed in which 16 unique drag-tagged primers simultaneously probe 16 p53 gene loci, with an abbreviated thermal cycling protocol of only 9 minutes. The drag-tagged SBE products were rapidly separated by ELFSE in both capillaries and microfluidic chips with genotyping accuracy in excess of 96%. The separation requires less than 70 seconds in a glass microfluidic chip, or about 20 minutes in a commercial capillary array sequencing instrument.