302c Fast, Sensitive P53 Mutation Detection by Microchip Electrophoresis-Based Tandem Sscp/Heteroduplex Analysis

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Genetic mutation detection promises to revolutionize the diagnosis and treatment of cancer by enabling the correlation of prognosis with specific sequence alterations. Mutations in the p53 gene, in particular, are known to be important in the pathogenesis of a variety of human cancers. Single-strand conformational polymorphism (SSCP) and heteroduplex analysis (HA) are two excellent and complementary electrophoretic methods for genetic mutation detection, because of their simplicity, breadth of application, and low cost. In SSCP, mutant DNA is mixed with wild-type DNA, denatured, and snap-cooled so that in dilute solutions, single-stranded DNA conformers form. Changes in DNA sequence lead to different conformations, which can then be separated by electrophoresis in entangled polymer media. In HA, mutant DNA is mixed with wild-type DNA, denatured, and slowly cooled, allowing some mutant and wild-type single strands of DNA to anneal to each other. Sequence non-complementarities in the heteroduplexes between the hybridized mutant and wild-type DNA can lead to conformational differences relative to wild-type and mutant homoduplexes, and in some cases are separable by electrophoresis. In order to make a clinically feasible mutation detection system, we have been working to optimize tandem-SSCP/HA on a microchip electrophoresis platform by investigating the importance of variables such as polymer matrix properties, electric field strength, DNA sample stability and purification, etc. We show that single-base mutations in the p53 gene exons 5-9 can be detected by chip electrophoresis with separation times of less than 10 minutes. This research has also been extended to patient tumor samples to create a more clinically applicable system for mutation detection.