

- T050 -

A SIMPLE SSCP PROTOCOL FOR MUTATIONAL ANALYSIS OF P53 GENE, OPTIMIZED AND EVALUATED IN RESPECT TO DNA SEQUENCING

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The aim of our study was to develop a simple and reliable Single Strand Conformation Polymorphism (SSCP) protocol for mutational analysis of the tumor suppressor gene p53 and to evaluate its efficiency by using previously sequenced DNA samples as controls.

17 ovarian tumor samples, 11 positive and 6 negative for the presence of mutations in exons 5-8 of the p53 gene (verified by DNA Sequencing), a conventional thermostated minilab gel electrophoresis apparatus, non isotopic detection and an inexpensive gel documentation system (including a digital camera, a scanner and a gel analyzer software program) were used. SSCP conditions were optimized in respect to the method's simplicity, reliability and efficiency in mutation detection. Under optimized conditions (14% acrylamide (30:1), room temperature, polyethyleneglycol 0.5%), single stranded DNA conformers are separated in a short time (3.5 to 5h) and 11 out of 12 positive PCR amplicons were clearly detected (SYBR gold fluorescence) while 5 false positives were found in a total number of 49 negative PCR amplicons. The proposed SSCP protocol can be useful for clinical applications as a rapid, inexpensive and reliable screening method for the detection of p53 mutations prior to DNA sequencing.