



Multiplex PCR-SSCP: A highly effective and efficient method of mutation detection and analysis

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Abstract

AIM: The method of PCR-SSCP we used previously for P53 mutation detection could only analyze one exon at a time. In addition, DNA extraction from microdissection samples is tedious and produces small amounts of DNA for analysis. To increase the efficiency of mutation detection and analysis while conserving the amount of DNA used, multiplex PCR, to amplify four exons simultaneously, and applied to human esophageal cancer samples for P53 mutation detection in this study.

METHODS: Multiplex PCR involves three successive PCR amplifications: (1) two sequence-specific primers are used, one containing a universal tail at its 5'-end, (2) the universal tail from the first round and another nested sequence-specific primers containing another universal tail at its 5'-end are used, (3) the two

distinct universal tails from the first and second rounds are used. We analyzed exons 5 to 8 of the *p53* gene in 8 paraffin-embed dedhuman esophageal squamous cell carcinoma specimens from Linzhou (Linxian, originally), Henan, China. Several different running and labeling conditions were tried in the multiplex PCR-SSCP to determine the most efficient and the highest mutation-detecting conditions. Direct sequencing was followed to get sequence of shift bands in four exons.

RESULTS: After numerous trials, it was demonstrated that internal labeling of primers and a running condition of 50 watts for 4-5 h produced the most reliable and reproducible results. From the 8 samples analyzed, 4 out of 8 (50%) possessed shift bands, with 3 out of these 4 (75%) having multiple shift bands, amounting to a total of 9 shift bands overall. This mutation rate of 50% is consistent with published results. Sequencing analysis revealed 7 confirmed mutations, while 2 bands have been confirmed as garbage bands. The multiple P53 mutations at different exons detected in a single sample demonstrate the benefits of multiplex PCR-SSCP.

CONCLUSION: Our result suggest that multiplex PCR-SSCP is a highly effective and efficient method of mutation detection and analysis and that internal labeling of primers and a running condition of 50 watts for 4.5 h produced the most reliable and reproducible results.

Key words: Esophageal neoplasms; *p53* gene; Mutation; DNA; Polymerase chain reaction

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