Inexpensive, rapid and convenient PCR-minigel SSCP protocol for polymorphisms and mutations analyses of LDL receptor gene

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Abstract

Hypercholesterolemia has been recognized as a major risk factor of atherosclerosis and coronary artery disease. The elevation in plasma low density lipoprotein (LDL) cholesterol is frequently due to genetic alteration at the genetic locus specifying the LDL receptors, leading to defective catabolism of LDL. In order to facilitate the molecular diagnosis of LDL receptor disorder, single strand conformation polymorphism (SSCP) analysis of polymerase chain reaction (PCR) amplified genomic DNA fragments has become a simple and sensitive screening method for identification of DNA polymorphisms and mutations in LDL receptor gene prior to DNA sequencing. In addition, SSCP patterns can be detected by silver staining to avoid hazardous radioactive material or other costly nonradioactive detection techniques. However, the original SSCP protocol is generally large-formatted, which is both time and reagents consuming as well as cumbersome. Minigel SSCP protocols have thus been devised but they involve, although commercially available, costly precast gels. We describe here a nonradioactive PCR-minigel SSCP protocol which is sensitive, inexpensive, rapid, reproducible and manually convenient. The results in this study demonstrate that minigel-SSCP (gel size: 10 cm × 7.3 cm × 0.075 cm) can detect conformation polymorphisms in PCR-fragments with a comparative sensitivity to large gel SSCP (gel size: 30 cm × 40 cm × 0.04 cm) as exemplified by the SSCP analyses of exon 13 of the LDL receptor gene. For minigel SSCP, the reagents for gel components and silver staining are reduced approximately 9 times and 10 times, respectively. For electrophoresis, electrical power is also reduced 10 times. This improved technique can become routinely used for molecular diagnosis of LDL receptor defect as well as for other genetic disorders.

Keywords: Hypercholesterolemia; LDL Receptor Gene; Minigel SSCP; Mutation, PCR; Polymorphism

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