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Rapid and optimized protocol for efficient PCR-SSCP genotyping for wide ranges of species

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Abstract

Single-strand conformation polymorphism (SSCP) is a reproducible and sensitive method for the detection of genetic polymorphisms and mutations in a wide range of polymerase chain reaction (PCR) products. However, the applications of this technique are largely confined to a set of cumbersome

optimizations. Herein, a non-time-consuming method for PCR-SSCP that can be conducted with minimum efforts and less technical expertise is presented. The main concept of this simplified technique was based on many optimizations that were conducted to ensure the highest possible sensitivity to detect genetic polymorphism without incorporating sophisticated equipment and tedious efforts. The optimum gel concentration, temperature, and time requirements were strictly adjusted so as not to be further modified before applying this method for genotyping purposes. Furthermore, minimized silver staining steps were combined with this method to further minimize time and effort. It was confirmed that the performed adjustments were not reduced the overall sensitivity of the technique. Therefore, the suggested method can be utilized to genotype a wide range of PCR products (mainly from 200 to 600 bp) without the need for further optimizations and modifications. This study proposes a rapid SSCP protocol for genotyping PCR products using simple, low-cost, and friendly to perform recipes.

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Contributions

MAB; conducted most of the experiments. MBSA; designed, supervised the work and wrote the manuscript. TRA; participated in the experiments and analyzed the data. TMA; co-supervised the work. HHD, THH, ATA, DA, MKAA, IAF, AHA; participated in the experiments. HOH and AMM; analyzed the data.

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Ethics declarations

Disclosure statement

The authors declare that they have no competing interests.

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