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Development of direct monitoring to analyze molecular marker related to genetic diseases

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Background and Aims: Nowadays, with increasing molecular biology application in medical science, variable methods have been developed to diagnose wide range of genetic diseases. One of these methods are PCR based DNA markers that have a common step for staining, detection and analyzing. Commonly used dye for staining steps is ethidium bromide that is time consuming, carcinogenic and dangerous pollution for human and environment respectively. Therefore, introduction of new techniques with high speed and safety seems essential. This method will provide possibility of detecting DNA fragment by comparing the standard DNA size ladder. This study investigates a new DNA staining and monitoring method through attachment a visible dye reporter component to DNA standard ladder and DNA amplified fragments. The first step to achieve this goal, is setting up PCR based molecular DNA ladder
Methods: Specific PCR primers were designed according to pUC57 vector sequences using software oligo ver5. With preparing related constructs, target DNA fragments were amplified based on Multiplex PCR and were analyzed on TBE agarose gel.
Results: we have described how to provide the primary genetic materials, design primers and optimize a PCR that we would be able to produce DNA ladder. The results show the first series of DNA fragments ,located between 100-500 bp have been successfully obtained and the length of DNA fragments were estimated by comparing the known DNA size ladder (Fermentas).
Conclusions: Introduction of a new method for monitoring and analyzing DNA fragment profiles on agarose gel electrophoresis via labeling DNA fragment ends with specific reporter dye will enable us to develop new PCR based detection kits for specific genetic disorders. This technique is safe, rapid and sensitive to diagnose genetic disorders that can be widely used in molecular medicine laboratories.

Keywords: DNA ladder; PCR; Primer; Genetic disease