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Optimization of Pyrosequencing Method to Detect IVS1-NT5 β-Globin Gene Mutation in β-Thalassemia

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Abstract : β-thalassemia incidence in Indonesia is reaching 2.500 cases per year, so that it is still a threat for Indonesian's society due to the high therapeutic cost. Prenatal diagnosis for detecting β-globin gene mutation is important to be developed in order to decrease β-thalassemia incidence. Pyrosequencing, a new DNA sequencing method, owns the ability to detect DNA mutation quickly for prenatal diagnosis importance. A specific β-globin gene mutation for Indonesian population is needed to make the mutation detection process become more effective, which is the mutation in the intervening sequence 1 nucleotide 5 (IVS1-NT5) region. Hence, the objective of this study is to know the optimal pyrosequencing condition to detect the IVS1-NT5 β-globin gene mutation in β-thalassemia. The sample used was the stored DNA material which the mutation had been detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for pyrosequencing optimization. There were two steps of optimization, both in the amplification step using polymerase chain reaction (PCR), and pyrosequencing step. The obtained result in amplification step was 55^{o}C for primer annealing temperature. In pyrosequencing step, the enzyme and substrate storage condition, cartridge's needle performance, and quality of sequencing primer were observed as factors which influence peak formation in the pyrograms, Nevertheless, due to the absence of the proper peaks on the pyrograms of this study, the pyrosequencing method to detect IVS1-NT5 is not yet optimized.
Keywords : β-globin, β-thalassemia, IVS1-NT5, Pyrosequencing.

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