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THE DEVELOPMENT OF NESTED PCR METHOD FOR DETECTION OF CTLA-4 GENE POLYMORPHISM CT60 (rs3087243)

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ABSTRACT

Cytotoxic T-Lymphocyte Antigen 4 *(CTLA-4)* gene is a protein receptor that causes down regulation in the immune system. Single nucleotide polymorphism (SNP) with rs3087243 in the *CTLA-4* gene has become one of the causes of an autoimmune disease such as type 1 diabetes. Type 1 diabetes develops when there is destruction of pancreatic β cells which leads to absolute insulin deficiency. Nested polymerase chain reaction (PCR) method is used in this study to detect the polymorphism in *CTLA-4* gene. Two set of amplification primers were designed where the first PCR reaction used outer primer to amplify the target sequence. Then, inner primer set was amplified by using first PCR product. Nested PCR method was developed by alteration of the PCR components such as concentration of DNA, primers, number of cycle and annealing temperature in order to get the most specific PCR product. The development of nested PCR was successful as the wild type and mutant allele can be differentiated when the mutant band was disappeared in the agarose gel image at the end of the study. The mutant band managed to disappear as the blood sample was taken from a healthy person. The development in nested PCR method plays an important role in detection of polymorphism that might present in human genes. Thus, allowing an early prevention of the inheritance diseases.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Diabetes is a defect in the body's ability to convert glucose to energy. Glucose is found in food and is an essential nutrient that provides energy for the functioning of the body cells. When food is digested it is changed into fats, protein or carbohydrates. Foods that affect blood sugars are called carbohydrates. Carbohydrates when digested, it will change to glucose. The glucose is then transferred to the blood and is used by the cells for energy. Insulin is needed in order to transfer the glucose from the blood into the cells. (rikama2007, 2012)The blood glucose level is regulated by insulin hormone. Insulin is released into the blood stream by beta-cells found in the islets of Langerhans. In people having diabetes, the process of converting glucose to energy is impaired. (rikama2007, 2012)

In Malaysia, diabetes is known as the silent killer and has given a bad influence to people. There are two main type of diabetes which is type 1diabetes and type 2