UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF ALLELE SPECIFIC – POLYMERASE CHAIN REACTION FOR DETECTING GENETIC POLYMORPHISM OF APOLIPOPROTEIN E

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TABLE OF CONTENTS

ABSTRACT

Alzheimer is one of the diseases that destroy brain cells, causing problems with thinking, memory and behavior. Patients' quality of life is compromised because the disease can affect work, social life and lifestyle. So, it is vital to determine and identify this disease at early stage so that later modification can me made as it also can be caused by environment factor besides genetic factor. Apolipoprotein E-ε4 allele was found to be the major genetic risk for Alzheimer's disease. Therefore, this study is aimed to develop Allele specific – polymerase chain reaction (AS-PCR) method, validate the method and later use it for detecting of the types and frequencies of the genetic variants of APOE. After DNA extraction, the DNA was used as samples in PCR reaction to determine the APOE polymorphism by using allele-specific primers. We have found the most suitable primers (F1wt, F1mt, R1cm, F2wt, F2mt, and R2cm) for multiplex PCR to detect the polymorphism of APOE genotype. Temperature of 54 °C was found to be the most suitable for these allele-specific primers to anneal to their specific complementary sequences in the template DNA. Touchdown PCR was shown to increase specificity of PCR reaction in this study. Addition of 5% DMSO also improved the specificity and PCR yield. Optimisation of PCR was shown to be successful either by increasing the sensitivity or the specificity of PCR reaction. Increase PCR sensitivity means that increase detection of true target sequence while improving the specificity of reaction result in amplification of the correct sequence.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Polymerase chain reaction (PCR) is a technique commonly used in molecular biology to amplify a piece of deoxyribonucleic acid (DNA) by *in vitro* enzymatic replication. By using PCR method, we can amplify the DNA exponentially from a piece of DNA as a template for replication. PCR can be performed with no restrictions on the form of DNA, besides we can perform a wide array of genetic manipulations by using this method.

In this study, allele specific polymerase chain reaction (AS-PCR) would be developed to genotype DNA samples for Apolipoprotein E (APOE) polymorphism. A multiplex AS-PCR approach with combinations of two or more pairs of oligonucleotide primers for PCR will be designed to detect first and second mutation site of APOE gene concurrently. First mutation site of APOE gene can be transcribed to 112 amino acids, while second mutation site of APOE can be transcribed to 158 amino acids. The specific primers designed allow amplification only if the nucleotide at the 3' end of the PCR