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DEVELOPMENT OF PCR-BASED METHOD FOR DETECTION OF THE POLYMORPHISM OF N-METHYL-D-ASPARTATE RECEPTOR GENE

FARRAH IDAYU ABDUL MUTALIB

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ABSTRACT

The purpose of this study was to design the allele-specific polymerase chain reaction for detection of the polymorphism of N-Methyl-D-Aspartate receptor gene and establish the frequencies of the selected genetic variants amongst Chinese ethnic in Malaysia. Single nucleotide polymorphism (SNP) of GRIN2B, rs1805247 was selected to be studied. The allele-specific primers were designed by using Oligo Explorer software and the unique sequence of the primers has been sent to First Base Laboratory Sdn. Bhd to be synthesised. The primers were Rcm_2_NMDAR, Fmt_2_NMDAR and Fwt_2_NMDAR. The most suitable annealing temperature for these primers was found to be 67°C where the intended band appeared and matched with DNA sequencing results. In this study, the most common genotype was G/A (55.8%) and A was the predominant allele (62.8%). It can be concluded that the method has been successfully developed because it matched with gold standard validation method. The assay would be useful for detection of the genetic polymorphism of NMDAR rs1805247 because variations that occurred in analysed samples are more than 1%.

CHAPTER 1

INTRODUCTION

1.1.1 Background of study

Polymerase chain reaction (PCR) is the key technique used in molecular genetics for amplifying deoxyribonucleic acid (DNA) sequences as template for replication in vitro by separating the DNA into two strands. By using PCR methods, we can amplify the DNA by more than a million times to produce more copies of the DNA piece. Besides, we can perform a wide array of genetic manipulations by using this method.

In this study, allele specific polymerase chain reaction (AS-PCR) was developed to genotype DNA sample for genetic polymorphism of N-Methyl-D-Aspartate (NMDAR2B) or glutamate receptor ionotropic N-Methyl-D-Aspartate 2B (GRIN2B). Allele specific primers were designed to detect the mutation in selected variant in GRIN2B. In this PCR method, only a single specific primer was used in combination with a common primer to amplify the target sequence. The 3' end of the primer designed should be complementary to the target DNA sequence.