

UNIVERSITI TEKNOLOGI MARA

**AMPLIFICATION OF HUMAN *UGT1A1*
USING PCR FOR USE IN CLONING AND
EXPRESSION**

AHMAD BIN SHAFIE

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ABSTRACT

UDP-glucuronosyltransferase (*UGT1A1*) is one of the families of enzymes which catalyze the transfer of glucuronic acid to a range of endogenous compounds and xenobiotic during Phase II drug metabolism. It facilitates the elimination of compounds in either urine or bile. The cloning of this enzyme in vitro thus allows further studies including drug-drug, drug-herb interaction studies to be performed. In this project, we aim to amplify *UGT1A1* gene from human liver DNA bank which can be used for subsequent analysis. Specific primers flanking the complete *UGT1A1* coding region was designed. The specificity of the primers were evaluated using Oligo Explorer 1.2 software. A specific PCR protocol was developed to amplify the gene from the human liver DNA which was used as the template. The amplicon targeted composes of 1602 base pairs of nucleotides (533 amino acids). The gene was amplified using PCR protocol that was optimized in house. Gel electrophoresis was performed to confirm the success of amplification. The band of interest which was 1602 base pairs in size was observed under UV transilluminator after gel electrophoresis. *UGT1A1* was successfully amplified from human liver DNA using PCR. The amplicon can then be used for cloning and expression of *UGT1A1* enzyme.

CHAPTER 1

INTRODUCTION

1.1 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is an important method to amplify specific DNA sequences in vitro. This principle was designed by American chemist, Kary Mullis. The process itself is an extremely straightforward extension of the properties of DNA replication. The impact of the polymerase chain reaction (PCR) on molecular biology, forensic science and the diagnosis of human genetic diseases have been immense (Saiki *et al.*, 1985; Mullis and Faloona, 1987). At its limits, PCR is able to amplify rapidly a specific region of single DNA molecule in vitro to yield sufficient quantities that later use for further studies including drug-drug and drug-herb interaction studies (Mullis, 1990). The basic principle of PCR involves denaturation, annealing and elongation (extension) process.

- i) The reaction solution containing DNA molecules (to be copied), polymerases (which copy the DNA), primers (which serve as starting DNA) and nucleotides, dNTPs (which are attached to the primers) is heating at 95 °C. This cause the two complementary strands to separate, a process known as denaturing or melting.