

**UNIVERSITI TEKNOLOGI MARA**

**PREPARATION OF A SPECIFIC *CYP2C19*  
TRANSCRIPT FOR *IN VITRO* CLONING**

**SHAFINAZ BINTI AHMAD**

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## ABSTRACT

Cytochrome P450 2C19 is a member of cytochrome P450 that is involved in the metabolism of several important groups of drugs including many proton pump inhibitors and antiepileptics. Genetic polymorphisms of *CYP2C19* are associated with variable ability to metabolize mephenytoin, known as the poor metabolizer and extensive metabolizer phenotypes. The aim of this study is to prepare human *CYP2C19* cDNA for cloning in *E. coli*. Temperature gradient PCR method was used to amplify the *CYP2C*. A set of primers have been designed to produce the target sequence. Few parameters were frequently adjusted to optimize the PCR reaction for higher specificity and reproducibility. *CYP2C19* had to be isolated from the other *CYP2C* genes by using restriction enzyme because of their high similarity in gene sequences. At the end of the study, the result was successful. For the confirmation of the product that had been produced, the sequencing process should be done for further study.

## CHAPTER 1

### INTRODUCTION

#### 1.1 Introduction to the gene and gene cloning

Cytochrome P-450 (CYP) is a hemoprotein enzyme widely distributed from bacteria to mammals. It is involved in catalyzing the oxidation or reduction process of a wide variety of substances including endogenous as well as exogenous compounds. Mammalian CYPs that present in liver microsomes are one of the key enzymatic mechanisms for the metabolism of various drugs, pesticides, environmental pollutants, and carcinogens as well as endobiotics such as steroids, fatty acids and prostaglandins (Zhu *et. al.*, 2002). Some are involved in the activation of procarcinogens while the others may involve in the inactivation of carcinogens. These depend on the types of carcinogens and cancers and the mechanism of carcinogenesis (Shi & Chen, 2004). Mammals possess at least 17 distinct CYP gene families that together code for an estimated 50-60 individual CYP genes in any given species (Zhu *et. al.*, 2002).

In humans, the cytochrome P450 (CYP) 2C subfamily includes at least four functional genes: *CYP2C8*, *-2C9*, *-2C18*, and *-2C19* (Sabine *et. al.*, 2001). The cytochrome P450 2C subfamily accounts for about 10% of all the mammalian P450s that have been recognized to date (Nelson *et. al.*, 1993), and the enzymes comprising this group have been found to be highly heterogeneous in each of the mammalian species that has been extensively characterized (Richardson *et.al.*, 1995). *CYP2C8*, *CYP2C18*, *CYP2C9*, and