UNIVERSITI TEKNOLOGI MARA

AMPLIFICATION OF HOMO SAPIENS CYP2B6 GENE USING POLYMERASE CHAIN REACTION (PCR) FOR CLONING AND EXPRESSION

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TABLE OF CONTENTS

		Page
TIT	LE PAGE	
APP	PROVAL	
ACKNOWLEGEMENT		
TAE	BLE OF CONTENTS	iii
LIS	T OF TABLES	iv
LIST OF FIGURES		
LIST	Τ OF ABBREVIATIONS	viii
ABS	STRACT	ix
CHA	APTER ONE (INTRODUCTION)	1
CHA	APTER TWO (LITERATURE REVIEW)	
2.1	Cytochrome P450	4
2.2	CYP2B6	5
2.3	Polymerase Chain Reaction (PCR)	6
CHA	APTER THREE MATERIALS AND METHODS	
3.1	Materials	
	3.1.1 Reagents and Chemicals	7
	3.1.2 Instruments	7
3.2	Methods	

ABSTRACT

CYP2B6 is a member of cytochrome P450 group of enzymes. The protein encoded by the CYP2B6 gene is the primary metabolizer of a number of drug substrates, such as buprion, cyclosphosphamide, ifosfamide, pethidine, efavirenz, neverapine, ketamine and propofol. In this study, amplification of CYP2B6 gene was performed by using polymerase chain reaction (PCR). The PCR profile for this study was designed in order to amplify the specific CYP2B6 gene sequence. The primers used in this study were designed and analyzed by using computer software called Oligo Analyzer 1.2® and Oligo Explorer 1.2®. The amplification of CYP2B6 was performed by using designed profile in order to amplify the gene of interest. The PCR product was then been purified by centrifugation using Wizard® SV Gel and PCR Clean-Up System.

CHAPTER ONE

INTRODUCTION

The bulk of responsibility for phase I reaction rests on the cytochrome P450 (CYP450) superfamily enzymes. These hemoproteins are localized in the endoplasmic reticulum and are the final step in an electron transfer chain. CYP450s catalyze the oxidation of therapeutic drugs and other xenobiotics as well as some endogenous compounds. While many tissues in the body contain these enzymes, the principle organs involved are liver and intestines. When a drug is consumed, it may undergo first-pass metabolism in the intestines and liver. Drugs can be absorbed intact, after moderate metabolic activity, or after extensive metabolic activity. A drug then circulates in the blood until it is acquired by another tissue. If the drug reaches the liver again, it may undergo a second-pass metabolism, the most extensive drug metabolism occurs in the liver where CYP450s make up 1-2% of the weight of hepatocytes. A particular metabolic response depends largely on the identity of the drug and an individual's capacity for metabolism of the drug.

CYP2B6 is a member of the Cytochrome P450 group of enzymes. The protein encoded by the CYP2B6 gene is the primary metabolizer of a number of drug substrates, such as bupropion, cyclophosphamide, ifosfamide, pethidine, efavirenz, neverapine, ketamine and propofol. This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of