UNIVERSITI TEKNOLOGI MARA

CLONING AND TRANSFORMATION OF HUMAN MULTIDRUG RESISTANCE GENE 1 (*MDR1*)

HAMIDAH MOKHTARUDDIN

Dissertation submitted in partial fulfilment of requirements of Bachelor of Pharmacy (Hons.)

Faculty of Pharmacy

November 2009

ACKNOWLEDGEMENT

First and foremost, thanks to God and may His blessing be upon our prophet for giving me the strength and determination to successfully complete this research. I would like to express my sincere appreciation to my supervisor Miss Fazleen Haslinda for the continuous support and guidance in completing this research Instrumentation's project. Special thanks to my beloved family for always being there for me, providing me all the encouragement and motivation that I needed. I also love to give special thanks to all postgraduate students especially Kak Sha for their great assistance along this study. Last but not least, my housemates Qairul Azhani Azmin and Shazwani Zulkifli. Thank you very much for your kindness in assisting me with my research.

TABLE OF CONTENTS

		Page
TITLE PAGE		
APPROVAL		i
ACKNOWLEDGEMENTS		ii
TABLE OF CONTENTS		iii
LIST OF TABLES		v
LIST OF FIGURES		vi
LIST OF ABBREVIATIONS		vii
ABSTRACT		viii
CHAPTER ONE (INTRODUCTION)		1
1.1	Introduction	1
1.2	Statement of Problem	2
1.3	Objectives of study	2
1.4	Significance of study	3
1.5	Hypothesis	3
CHAPTER TWO (LITERATURE REVIEW)		
2.1	Multidrug resistance phenotypes	4
2.2	P-glycoprotein	5
2.3	Polymerase Chain Reaction	7
2.4	Cloning of Human *MDR1*	9
2.5	Assay used to determine *MDR1* gene expression	10
2.6	Real Time Polymerase Chain Reaction	10
CHAPTER THREE (MATERIALS AND METHODS)		
3.1	Chemical and Reagents used in PCR	14
3.2	Chemicals and Reagents for Gel Electropheresis and Instruments in PCR	15
3.3	Polymerase Chain Reaction (PCR)	16
3.4	Gel electrophoresis	19
3.5	Purification of PCR Product	21
3.6	Cloning	23
3.7	Transformation	24
	3.7.1 Selective Plate	24
	3.7.2 One Shot Competent Cells	24
3.8	Analysed Transformants	26
	3.8.1 Second PCR	26
	3.8.2 Sequencing Preparation	28
	3.8.2.1 Bacteria Cell Growth	28
	3.8.2.2 Plasmid Extraction	28
	3.8.2.3 Sequencing	30

ABSTRACT

Human *MDR1* encoded for P-glycoprotein (P-gp) formation. P-gp is an ATP dependent efflux transporter which reduces the intracellular concentration of wide range of drugs including the chemotherapy drugs especially when the *MDR1* gene being over expressed. Most of the anticancer agents are the substrates of P-gp thus make the tumour cells are able to survive upon exposure to various anticancer and this will lead to chemotherapy resistance. In order to study about the mechanism of P-gp, human *MDR1* has been cloned and transformed into plasmid of *E.coli* as the reaction vector. Before cloning and transformation take place, amplification of *MDR1* gene was done using Polymerase Chain Reaction (PCR) and followed by gel electrophoresis. The appeared band on gel with 158 bp showed present of desired gene, thus progressed on cloning and transformation methods. As colonies obtained on the selective agar, positive transformants analysis is followed by doing the second PCR and sequencing. These procedures are to confirm the *MDR1* gene is cloned at the correct orientation.

CHAPETER 1

INTRODUCTION

1.1 Introduction

Resistance to chemotherapy is one of the major obstacles to the effective treatment of cancer patients. Such resistance has been associated with rapid drug efflux mediated by Multidrug resistance 1 (*MDR1*) gene (Leith *et al.*, 1999). Human *MDR1* gene encoded for P-glycoprotein (P-gp) formation. P-gp is an ATP dependent efflux transporter. P-gp functions as energy dependent pump and reduces the intracellular concentration of wide range of drugs including the chemotherapy drugs (Bodor *et al.*, 2005; Saito *et al.*, 2003).

The development of chemotherapy resistance is caused by the over expression of P-gp on the membrane of the cancer cells. This membrane glycoprotein is able to confer a *MDR* phenotype by pumping wide spectrum of chemotherapeutics agents like anthracyclines, vinca alkoids, epipodophylline and dactinomycins from the tumor cells (Lau *et al.*, 1998).