UNIVERSITI TEKNOLOGI MARA

CLONING OF HUMAN *GAMMA GLUTAMYL TRANSFERASE (GGT)* GENE

IZZATI BINTI MOHD FAROK

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

Faculty of Pharmacy

October 2008

ACKNOWLEDGEMENTS

Alhamdulillah, thanks to Allah S.W.T for all the blessing and giving me the strength and all the effort to manage and complete this project and also complete my thesis on time in accordance to the guidelines given.

Here, I want to convey my thousand appreciations and special thanks to my thesis supervisor, Assoc. Prof. Dr. Teh Lay Kek, for all her guidance, continuous support and limitless patience in guiding me to complete this thesis. Special thanks to Prof. Dr. Mohd Zaki Salleh and Dr.Rosmadi for their for their support and critics in completing this project.

I also would like to express my special gratitude to Ms. Sharina, Ms. Ainul and Ms.Fazleen who helped me during the practical work. I greatly thank all the other post-graduate students in the Pharmacogenomic Laboratory at Faculty of Pharmacy for all their kindness and willingness to lend a hand when needed.

I also want to thank my family, my friends, my lecturers and also to my coordinator, Dr. Zainul Amiruddin Zakaria for their unlimited support through out this research period. I acknowledge the financial support from Universiti Teknologi Mara (UiTM), Shah Alam, received in the form of laboratory materials and instruments for conducting my thesis.

Finally, thanks to all the people who were directly or indirectly involved in giving me the information during the practical work. I hope this report will fulfill the entire requirement needed to complete this Research Instrumentation (PHM 555) course. May Allah bless us all, InsyaAllah.
Thank you.

TABLE OF CONTENTS

ABSTRACT

The *γ-glutamyl transferase (GGT)* plays an important role in the process of catalyzing the degradation of glutathione. *GGT* is widely used as a marker in preneoplastic lesions in the liver during chemical carcinogenesis. In this study, primers were designed without the restriction sites to investigate the success of cloning. The aim of this study was to clone *GGT* gene in plasmid by means of TA cloning. Methods used in this study were purification of human liver cDNA, PCR for gene amplification, TOPO TA cloning, transformation into *E.coli* and DNA sequencing. PCR was conducted under different annealing temperatures using Takara PCR Thermal Cycler Dice™ Gradient model to determine the optimal annealing temperature. TOPO TA cloning process with high cloning efficiency was completed in 5 minutes. Transformation was done using the One Shot® Chemical Competent *E.coli*. DNA sequencing was also performed using the plasmid samples from transformation process to validate the insert. Plasmid extraction yielded 168 ng/µl of liver cDNA. The PCR based method obtained an optimal annealing temperature for *GGT* amplification, which was at 47°C. Transformation was a success by using *E.coli* as a host where a 10 ml of LB broth was used and incubated at 37°C for 16 hours. Cloning of the *GGT* gene is important as it opens ways for further studies to look into its association with cancer and help in development of medication that prevents the progression of tumors. There is no specific treatment for patients with *GGT* deficiency and thus, researchers may develop protein-based drugs for them. *GGT* cloning can help in the establishment of gene collection in DNA libraries.

CHAPTER 1

INTRODUCTION

The human genome consists of high, medium, and low copy of repetitive DNA and single-copy DNA. The human *gamma glutamyl transferase (GGT)* cDNA was assigned to human chromosome 22 using *in situ* hybridization and at least four copies were identified in man (Collins *et. al.,* 1997). As reported by Sakamuro *et. al.,* (1988), *GGT* consists of 2 peptide chains, heavy and light, each composed of 351 and 189 amino acids, respectively. The peptide chains contain the catalytic site of *GGT*, which are encoded on a single mRNA (Courtay *et. al.,* 1994). *GGT* is a membrane-bound glycoprotein located on the outer surface of the cell membrane.

Breakdown of glutathione is catalyzed by *GGT*, which takes place by transfer of gamma glutamyl moiety of glutathione to a number of acceptors. Suggestion was made that transpeptidase mediates amino acid transport by the reaction of the gamma glutamyl cycle. This cycle is one of the pathways that functions in amino acid transport. Gamma glutamyl amino acids are formed in or on the cell membrane by *GGT* where the gamma glutamyl amino acids are then translocated into the cell before free amino acids are released (Allison & Meister, 1981).