UNIVERSITI TEKNOLOGI MARA

AMPLIFICATION OF HUMAN *PREGNANE X RECEPTOR* GENE BY USING POLYMERASE CHAIN REACTION

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

November 2008

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my project supervisor, Dr. Rosmadi b.Yusoff for always has been there for me to offer his time, knowledge, guidance and advices which are important and supportive.

A special thank to Prof.Dr.Zaki Mohd Salleh and Assoc. Prof. Dr. The Lay Kek for their detailed and constructive comments which has been great value throughout completing this thesis.

Acknowledgements are also made to the staff of pharmacogenomics laboratory and the postgraduate students for their excellent guidance and assistance during the laboratory work.

Last but not least, my warmest gratitude goes to my parents and friends for their loving support and patience.

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ABSTRACT

Polymerase chain reaction (PCR) is a fast and inexpensive technique used to amplify, or make many copies of, small segments of DNA. In the mean time, cloning is another method to amplify a specific gene that involves transferring a DNA fragment of interest from one organism to a self-replicating genetic element such as a bacterial plasmid (cloning vector). This followed by propagation of DNA of interest in a foreign host cell. In this study, the objective of this study is to amplify Human *Pregnane X Receptor* (h*PXR*) Gene using Polymerase Chain Reaction for use in cloning and expression. Initially, both forward and reverse primers for h*PXR* gene are designed followed by reconstitution of the primers to prepare working stock for the polymerase chain reaction (PCR). The composition for PCR experiment was determined and a suitable PCR condition was designed to amplify the primers. Gel electrophoresis then was performed and the bands appeared were visualized under UV transilluminator. The result showed that the product of h*PXR* gene was obtained by using 55 ^{0}C as annealing temperature. As a conclusion, in this study the h*PXR* gene was successfully amplified. This product could be used further for the expression of the protein.

CHAPTER 1

INTRODUCTION

1.1 Polymerase Chain Reaction (PCR)

PCR (polymerase chain reaction) is one of the most popular methods in biological and biomedical bench work today. The principle of polymerase chain reaction (PCR) was design by American chemist, Kary Mullis. By the mid 1980s, the techniques were used for the first time to diagnose a disease, when researchers identified the gene for sickle cell anemia. At about the same time, the method was introduced to forensic medicines. Differed from gene cloning, PCR is carried out in a single test tube by mixing DNA with a set of reagents and placing the tube in thermal cycler. Thermo cycler is equipment that enables the mixtures to be incubated at a series of temperatures that varied in a preprogrammed manner. The basic steps in PCR experiments are as follows:

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

ii) Lowering the temperature to 55°C causes the primers to bind to the DNA, a process known as hybridization or annealing. The resulting bonds are stable only if the