MOLECULAR CLONING OF THE T7 RNA POLYMERASE *T7RNAP* GENE AND CHLORAMPHENICOL ACETYLTRANSFERASE *CAT* GENE

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By

LOO DE JING

A project report submitted to the department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biotechnology

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ABSTRACT

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Mitochondria are organelles found in eukaryotes that are responsible for energy production. To date, mitochondrial gene expression is not well understood. Some mitochondrial biological processes involve regulation by nuclear-encoded factors that are targeted to the mitochondria by mitochondrial targeting sequence (MTS). Translational fusion of MTS and foreign gene permits the delivery of the foreign protein into mitochondrial matrix. Previous study showed that nuclear-encoded MTS-targeted T7 RNA polymerase (T7RNAP) is able to transcribe foreign DNA in the mitochondria. A previous study showed that the *CAT* gene is a potential selectable marker for yeast mitochondrial transformation. However, for expressing *CAT* gene in the mitochondria, the gene sequence needs to be recoded according to the mitochondrial genetic code. Therefore, it may be possible to express a mitochondrial recoded *CAT* (*mCAT*) gene under the control of a promoter, in a cell that expresses *T7RNAP*. As a part of the effort to develop this mitochondrial *mCAT* expression system, this study aims to clone three gene constructs, namely the *T7RNAP* chimeric gene, the *T7RNAP* gene, and the *mCAT*

gene. All these gene constructs will be cloned into the cloning vector pENTR/D-TOPO and subsequently, subcloned into yeast expression vectors. In this study, the *T7RNAP* and *mCAT* genes were amplified by chain reaction (PCR) and the *T7RNAP* gene was constructed by overlap extension PCR. All three constructs were cloned into the cloning vector pENTR/D-TOPO. DNA sequencing showed that the orientation and sequences of *T7RNAP* and *mCAT* inserts were correct. This sequence deletion probably occurred during the overlap extension PCR process. The *T7RNAP* and *mCAT* genes were successfully subcloned into the yeast expression vectors pDEST52 and pMOD, respectively. The orientation and sequence of both genes in the expression vectors were verified by DNA sequencing.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and sincere appreciation to my supervisor Associate Professor Dr. Wong Hann Ling for his indispensable guidance, invaluable support, encouragement and motivation throughout the course of my project. His passionate, kind, helpful and patient character drove me to face the challenges and problems encountered in this journey.

Besides, I would also like to extend my appreciation the postgraduate students, Mr. Toh Wai Keat and Mr. Ng Wen Guang for their enlightening guidance and compassionate support throughout the period of my final year project.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Loo De Jing

APPROVAL SHEET

This project report entitled "**MOLECULAR CLONING OF THE T7 RNAP *T7RNAP* GENE AND CHLORAMPHENICOL ACETYLTRANSFERASE *CAT* GENE**" was prepared by LOO DE JING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biotechnology at Universiti Tunku Abdul Rahman.

Approved by:

_____

(DR. WONG HANN LING) Date:……...

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Date: _____

PERMISSION SHEET

It is hereby certified that **LOO DE JING** (ID No: **11ADB04449**) has completed this final year project entitled "MOLECULAR CLONING OF THE T7 RNAP *T7RNAP* GENE AND CHLORAMPHENICOL ACETYLTRANSFERASE *CAT* GENE" under the supervision of Dr. Wong Hann Ling (Supervisor) from the Department of Biological Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(LOO DE JING)