SITE-DIRECTED MUTAGENESIS OF SUPERFOLDER GREEN FLUORESCENT PROTEIN

By

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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

April 2015

ABSTRACT

SITE-DIRECTED MUTAGENESIS OF SUPERFOLDER GREEN FLUORESCENT PROTEIN

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Green Fluorescent Protein (GFP) is a protein made up of 238 amino acid residues that will emit green fluorescence when it is exposed to blue or UV light. It consists of eleven antiparallel beta-strands forming a beta-barrel, with an alpha-helix inside. It has been employed extensively as a reporter gene to visualize the protein-protein interactions (PPI) using GFP in bimolecular fluorescence complementation (BiFC) assays. Numerous researches have been carried out to develop GFP variants, including superfolder GFP (sfGFP), for improving the signal-to-noise (S/N) ratio in the BiFC assays, due to the intrinsic intramolecular interaction of the beta-strands, which causes irreversible assembly of sfGFP in the assays. This limits the use of sfGFP for monitoring dynamic PPI. The main objective of this study is to clone and express variants of sfGFP with mutation at 8th beta sheet and examine if these mutants sfGFP would remain fluoresecent. Mutations in the 8th beta sheet of sfGFP, were separately introduced by polymerase chain reaction (PCR), generating DNA fragments of mutant C-terminal of sfGFP (sfGFP-C).

Subsequently, the N-terminal fragment of sfGFP, was separately fused to the mutant sfGFP-C fragments by overlap extension polymerase chain reaction (OE-PCR). The full-length mutant sfGFP fragments were cloned into the expression plasmid vector pBTOPO® and the reaction mixtures were used to transform competent *Escherichia coli* TOP10. Transformants were screened by blue light irradiation, where putative carrying recombinant plasmid with the sfGFP insert would emit green fluorescence. In summary, all five mutant sfGFP constructs were separately cloned into the pB-TOPO®. However, mutant sfGFP IV was inserted into the expression vector at the opposite orientation, and therefore, was non-functional. Mutant sfGFP exhibited fluorescence loss, while other mutants, produced lower fluorescence than that of the wild-type sfGFP.

ACKNOWLEDGEMENT

Foremost, I owe my utmost gratitude to my supervisor, Dr Wong Hann Ling for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me throughout the lab work and writing of this thesis. I could not have imagined having a better supervisor for my final year project. Besides, I am deeply grateful to postgraduate students Mr Toh Wai Keat and Mr Patrick Liew Jien Pxing for their assistance and helpful comments throughout this project. A special thanks to my fellow labmates, Choh Chin Fei, Goh Hui Ting, Goh Kai Syuen and Loo De Jing for sharing their experiences and tips all the way during FYP. Last but not least, I would like to thank my dearest family and friends for their spiritual and financial support. Without them, I could not have completed this 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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LEE SOCK IM

APPROVAL SHEET

This project report entitled **"<u>SITE-DIRECTED MUTAGENESIS OF SUPERFOLDER GREEN FLUORESCENT PROTEIN</u>"** was prepared by LEE SOCK IM and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biotechnology at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **LEE SOCK IM** (ID No: **12ADB01797**) has completed this final year project entitled **"SITE-DIRECTED MUTAGENESIS OF SUPERFOLDER GREEN FLUOROSCENT PROTEIN"** under the supervision of Assoc. Prof. Dr. Wong Hann Ling 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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(LEE SOCK IM)

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