MOLECULAR CLONING OF
NANOLUCIFERASE FOR
BIOLUMINESCENCE RESONANCE ENERGY
TRANSFER

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MOLECULAR CLONING OF NANOLUCIFERASE FOR BIOLUMINESENCE RESONANCE ENERGY TRANSFER

By

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ABSTRACT

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KANG SIANG YU

Nanoluciferase (Nluc) is an enzyme that emits bioluminescence by catalyzing the oxidation of furimazine into furimamide without the use of ATP. It is small as it is only made up of 171 amino acids. It fuses with green fluorescent protein (GFP) to generate bioluminescence resonance energy transfer (BRET) construct to identify the interaction between proteins. Under the presence of furimazine, energy was transferred from Nluc to GFP causing it to emit fluorescence which is detected as BRET signal. This BRET is dependent on the distance and orientation of interaction between energy donor and acceptor, as well as, the overlapping of donor emission spectrum and acceptor excitation spectrum. The objectives of this project are to clone and express Nluc, as well as, generate BRET constructs by overlap extension PCR for fusing Nluc and GFP variants. The Nluc was amplified from extracted plasmid pNL1.1 by polymerase chain reaction (PCR) and was inserted into pBAD-TOPO® expression vector by TA cloning. After that, electroporation was carried out to transform electrocompetent Escherichia coli TOP10 with the pBAD-TOPO® vector. The colonies formed were subjected to colony PCR to screen for the success tansformants. Lastly, the expression of Nluc
was detected by addition of Nano-Glo® Luciferase Assay Substrate and the emitted luminescence was detected by ChemiDoc™ MP Imaging System. Apart from this, 6 different BRET constructs were generated by overlap extension PCR between Nluc and GFP variants. As the result, 6 out of 15 randomly picked colonies were successfully expressed and synthesized Nluc with high signal-to-noise ratio. The non-luminescent colonies either carried non-functional Nluc, where the Nluc may be inserted in opposite orientation or the Nluc insert was absent. On the other hand, BRET constructs with different GFP variants were generated. However, transformation of BRET constructs was not success due to arcing occurred while performing electroporation.
ACKNOWLEDGEMENT

First of all, I owe my utmost gratitude to my supervisor, Dr Wong Hann Ling for his knowledge, caring and enthusiasm in guiding me along my final year project and providing useful suggestions in solving problems faced in research and thesis writing. I am very grateful to have an excellent supervisor like Dr Wong. Besides, I would like to deeply thank postgraduate students Mr Toh Wai Keat and Mr Ng Wen Guang for providing the information and materials needed in FYP, as well as, lending hand whenever I faced problems in FYP and presentation. Furthermore, I would like to thank my labmates Ms Lim Min Zi, Ms Joanne Lam, Ms Ong Wei Chi, Ms Christina Chin and Mr Bryan Sonylah, as well as, my family and friends for providing me moral support.
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.

______________________
KANG SIANG YU
This project report entitled "MOLECULAR CLONING OF NANOLUCIFERASE FOR BIOLUMINESCENCE RESONANCE ENERGY TRANSFER" was prepared by KANG SIANG YU and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biotechnology at Universiti Tunku Abdul Rahman.

Approved by:

___________________________
(Assoc. Prof. Dr Wong Hann Ling) Date:......................

Supervisor

Department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman
It is hereby certified that **KANG SIANG YU** (ID No: **12ADB04925**) has completed this final year project entitled “MOLECULAR CLONING OF NANOLUCIFERASE FOR BIOLUMINESCENCE RESONANCE ENERGY TRANSFER” 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,

_____________________

(KANG SIANG YU)
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List of chemicals and materials used and their respective manufacturers.

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