ELECTROPORATION OF *Clostridium sp.* WITH
POWER-LOW CIRCULAR CONSTRUCT

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2007

ELECTROPORATION OF *Chlorella* sp. WITH pCAMBIA 1304 CIRCULAR CONSTRUCT

By

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Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
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UNIVERSITI MALAYSIA TERENGGANU
2007

This project should be cited as:

Yeong, Y.Y. 2007. Electroporation of *Chlorella* sp. with pCAMBIA 1304 circular construct. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Universiti MalaysiaTerengganu. 43p.

UNIVERSITI MALAYSIA TERENGGANU

JABATAN SAINS BIOLOGI
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ACKNOWLEDGEMANTS

Firstly, I would like to thank my supervisor, Dr. Cha Thye San for his guidance and encouragement during the research. I thank him for giving me opportunities to be one of his undergrad student. His scientific advices and comments have always been inspirational to me for completing this project. I am indeed very grateful for working and learning his amazing wealth knowledge with him on the field of molecular biology.

Secondly, I thank to all members of the team: Tan Choy Won, Banreet Kaur A/P Bud Singh, Oh Hong Yew, Lua Chung Liang and Norbaiti Bt. Mohd Isa for all discussion and stuff we shared that made my time during research became very enjoyable.

Thirdly, I would like to express my appreciation to all lab assistants especially Mr. Mazrul for their help in handling of materials and equipments in UMT's Biotechnology Lab.

Finally, I would like to thank my family: my parents and sisters for their kind support and encouragement. Without their love and support, I would not have been here writing my thesis. I would also like to thank my friends who have always been helpful when help is needed. I am very thankful to my friends: Saw Ying Yu and Chong Hoi Fen for their comment on manuscript.

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LIST OF ABBREVIATIONS

ACP	Acyl Carrier Protein
AP	Antisense Palmitoyl-ACP Thioesterase
BBM	Bold Basal Medium
bp	Base pair
CoA	Coenzyme A
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleic Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
g	Gram
L	Liter
LB	Lurie Bertani
M	Molar
$MgCl_2$	Magnesium Chloride
mL	Mililiter
Msec	Millisecond
µg	Microgram
µL	Microliter
OD	Optical Density
TAE	Tris-Acetate-EDTA

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ABSTRACT

An efficient method for electrically introducing pCAMBIA 1304 circular plasmid into *Chlorella* cells was developed. This method is useful for molecular genetic studies and commercial applications where the genes of interest responsible for the production of a valuable nutrient are electrically introduced into the recombinant *Chlorella* sp. The pCAMBIA 1304 circular plasmid carrying the hygromycin phosphotransferase (*hpt*) selectable marker, green fluorescent protein (*gfp*) and β-glucuronidase (*gus*) reporter genes regulated by CaMV 35S promoter was used to transform the *Chlorella* cells. The pCAMBIA 1304 circular plasmid was successfully extracted from *E. coli* and verified by PCR technique with three sets of primers combination. The primers combination 35S-F/35S-R amplify the CaMV 35S promoter which produced a band of 326bp while primers combination GG-F/GG-R managed to amplify *gfp*: *gus* fusion genes with size of 676bp and primers combination 35S-F/GG-R for amplification both CaMV 35S promoter and *gfp*: *gus* fusion genes with size of 1462bp. The effect of pCAMBIA 1304 circular plasmid was examined on six MicroPulser programme (Bio-Rad) with electrical voltages from 1.5 to 3.0kV. Programme Ec3 delivered a voltage of 3.0kV was found in higher transformation efficiency under 10μg/mL hygromycin selection. The putative recombinant *Chlorella* colonies can be further confirmed the present of the pCAMBIA 1304 plasmid into it by using PCR technique.

ELECTROPORASI *Chlorella* sp. DENGAN KONSTRUK BULAT pCAMBIA 1304

ABSTRAK

Satu kaedah yang efisyen direkakan untuk memasukkan plasmid bulat pCAMBIA 1304 secara elektrik ke dalam sel-sel *Chlorella*. Kaedah ini adalah digunakan untuk mengkaji genetik molekul dan kegunaan komersial di mana gen-gen yang diminati bertanggungjawab untuk menghasilkan nutrisi yang bernilai telah memasukkan ke dalam *Chlorella* sp. rekombinan secara elektrik. Plasmid bulat pCAMBIA 1304 yang membawa gen hygromycin phosphotransferase (*hpt*) sebagai penanda memilih, green fluorescent protein (*gfp*) dan β-glucuronidase (*gus*) sebagai gen-gen pengesan yang dikawal oleh CaMV 35S promoter telah digunakan untuk transformasi sel-sel *Chlorella*. Plasmid bulat pCAMBIA 1304 telah berjaya diekstrak dari *E. coli* dan disahkan oleh PCR teknik dengan mengunakan tiga kombinasi pencetus. Kombinasi pencetus 35S-F/35S-R mengamplifikasi CaMV 35S promoter yang menghasilkan jalur bersize 326bp sementara kombinasi pencetus GG-F/GG-R berupaya untuk amplifikasi gen-gen *gfp*: *gus* yang bercantum dengan jalur bersize 676bp dan kombinasi pencetus 35S-F/GG-R untuk tujuan amplifikasi kedua-dua CaMV 35S promoter dan gen-gen *gfp*: *gus* yang bercantum dengan jalur bersize 1462bp. Kesan yang dibawa oleh plasmid bulat pCAMBIA 1304 telah dikajikan dengan enam program MicroPulser (Bio-Rad) yang menghasilkan eletrik volta dari 1.5 kepada 3.0kV. Program Ec3 yang menghantarkan elektrik volta 30kV didapati bahawa mempunyai frekuensi transformasi yang tertinggi dibawah pemilihan 10μg/mL hygromycin. Koloni-koloni *Chlorella* rekombinan yang putatif ini boleh diteruskan untuk mengesahkan kehadiran pCAMBIA 1304 plasmid ke dalamnya melalui PCR teknik.