

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

YEONG YUN YEE

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
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ELECTROPORATION OF *Chlorella* sp. WITH pCAMBIA 1304 CIRCULAR
CONSTRUCT

By

Yeong Yun Yee

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Disahkan oleh: / Verified by:

.....
[Signature]
Penyelia Utama / Main Supervisor

Nama: **DR. CHA THYE SAN**
Pensyarah
Cop Rasmi: Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 29/4/07

.....
[Signature]
Ketua Jabatan Sains Biologi / Head, Department of Biological Sciences

Nama: **DR. AZIZ BIN AHMAD**
Ketua
Cop Rasmi: Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 30/4/07

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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 10 μ g/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.

ABSTRAK

Satu kaedah yang efisien 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 dimasukkan 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 menggunakan 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 elektrik volta dari 1.5 kepada 3.0kV. Program Ec3 yang menghantarkan elektrik volta 30kV didapati bahawa mempunyai frekuensi transformasi yang tertinggi dibawah pemilihan 10 μ g/mL hygromycin. Koloni-koloni *Chlorella* rekombinan yang putatif ini boleh diteruskan untuk mengesahkan kehadiran pCAMBIA 1304 plasmid ke dalamnya melalui PCR teknik.