

ESTABLISHMENT OF *AGROBACTERIUM*
TUMEFACIENS WITH P_{35S} GUS⁺ AND
P_{35S} GUS⁻ CONSTRUCTS

SHAH HONG HOON

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
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Electrotransformation of Agrobacterium Tumefaciens with PSP
AP-VF2 and pCambia 1301 constructs / Tan Kong Hooi.



PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

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ELECTROTRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS* WITH
PSP'AP-VF2 AND PCAMBIA 1301 CONSTRUCTS

By

Tan Kong Hooi

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Faculty of Science and Technology
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FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

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Disahkan oleh:

.....
Penyelia Utama

DR. CHA THYE SAN'
Pensyarah

Nama:

Jabatan Sains Biologi
Fakulti Sains dan Teknologi

Cop Rasmi:

Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 7/5/2006

.....
Penyelia Kedua

DR. RAZIZ BIN AHMAD (Ph.D)
LECTURER

Nama:

Dept of Biological Sciences
Faculty of Science and Technology
University College of Science
and Technology Malaysia
21030 Kuala Terengganu.

Cop Rasmi:

Tarikh: 4/5/2006

.....
Ketua Jabatan Sains Biologi

Nama:

PROF. MADYA DR. NAKISAH BT. MAT AMIN
Ketua

Cop Rasmi:

Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 7/5/06

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LIST OF ABBREVIATIONS

°C	-	degree celcius
kb	-	kilo base pair
bp	-	base pair
mM	-	milimolar
mL	-	milliliter
μL	-	microliter
rpm	-	round per minute
M	-	molar
OD	-	optical density
ddH ₂ O	-	double distilled water
ng	-	nanogram
μg	-	microgram

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ABSTRACT

Agrobacterium-mediated transformation is an effective plant genetic transformation method. The application of such method on microalgae transformation is extremely rare. In this study, the PSP'AP-VF2 construct which carries the gene encoding for antisense cDNA palmitoyl ACP-thioesterase and pCAMBIA 1301 construct which carries the reporter GUS gene were transferred into *Agrobacterium tumefaciens* strain LBA4404. The PSP'AP-VF2 and pCAMBIA 1301 constructs were successfully extracted from *E. coli* stock with the purity of 2.00 and 1.80 respectively and the concentration was 1.308 $\mu\text{g}/\mu\text{L}$ and 1.338 $\mu\text{g}/\mu\text{L}$ respectively. The competent *A. tumefaciens* was successfully prepared and were electroporated with both constructs at 2.2 kV. Both transformed *A. tumefaciens* samples showed five distinct bands in the plasmid extraction. The PSP'AP-VF2 transformed *A. tumefaciens* was further screened with PCR and the primers used were PTE-VF1 and PTE-VR2. Three out of five PCR product samples showed the distinct bands. The results obtained from this study indicated that both constructs were successfully transferred into *A. tumefaciens*. The transformed *A. tumefaciens* will be used to transform *Chlorella* sp. in the future study.

Elektrotransformasi *Agrobacterium tumefaciens* dengan konstruk PSP'AP-VF2 dan pCAMBIA 1301

ABSTRAK

Transformasi berpanduan *Agrobacterium* merupakan salah satu kaedah transformasi genetic tumbuhan yang berkesan. Aplikasi kaedah transformasi tersebut ke atas mikroalga masih jarang. Dalam kajian ini, konstruk PSP'AP-VF2 yang mempunyai gen antisen cDNA palmitoyl ACP-thioesterase dan pCAMBIA 1301 yang mempunyai GUS gen dimasukkan ke dalam *A. tumefaciens* dengan kaedah elektroporasi. Kedua-dua konstruk PSP'AP-VF2 dan pCAMBIA 1301 telah berjaya diekstrak daripada *E. coli* dengan ketulenan 1.80 dan 2.00 masing-masing, dan kepekatannya ialah 1.308 µg/µL dan 1.338 µg/µL masing-masing. Sel elektrokompeten *A. tumefaciens* telah disediakan dengan berjaya dan seterusnya dielektroporasikan pada 2.2 kV dengan konstruk-konstruk tersebut. Dalam pengekstrakan plasmid, sampel-sampel daripada kedua-dua *A. tumefaciens* yang telah dielektroporasikan menunjukkan lima band yang jelas. Kehadiran konstruk PSP'AP-VF2 dalam *A. tumefaciens* telah dikesan dengan PCR, dan primer PTE-VF1 dan PTE-VR2 telah digunakan. Tiga daripada lima sampel PCR product menunjukkan bands yang jelas. Keputusan yang diperoleh menunjukkan kedua-dua konstruk telah dimasukkan ke dalam *A. tumefaciens*. *A. tumefaciens* yang telah ditransformasi akan digunakan untuk transformasi *Chlorella* sp. dalam kajian masa depan.