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Electrotransformation of Agrobacterium Tumefaciens with PSP AP-VF2 and pCambia 1301 constructs / Tan Kong Hooi.

Lihat sebelah

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

By

Tan Kong Hooi

Research Report submitted in partial fulfillment of the
requirements for the degree of
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PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:
Electrotransformation of *Agrobacterium tumefaciens* with PSP'AP-VF2 and pCAMBIA 1301 constructs oleh Tan Kong Hooi, No Matrik UK7870 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi) Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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°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_2O	-	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 µg/µL and 1.338 µg/µL 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 seterusnys 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.