[redacted] OF *AGROBACTERIUM TUMEFACIENS* WITH [redacted]

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Electrotransformation of Agrobacterium Tumefaciens with PSP'AP-VF1 and Pcambia 1302 contructs / Willy Yee.

Lihat sebelah

ELECTROTRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS* WITH PSP'AP-VF1 AND PCAMBIA1302 CONSTRUCTS

By

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PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: TRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS* WITH PSP'AP-VF1 AND PCAMBIA1302 CONSTRUCTS oleh Willy Yee, no. matrik: uk 8020 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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LIST OF ABBREVIATIONS

Bp	Basepair
cDNA	Complimentary DNA
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphates
E. coli	*Escherichia coli*
EDTA	Ethylene Diamine Tetraacetic Acid
Kb	Kilobase
Kv	Kilo Volts
LB	Luria-Bertanni
$MgCl_2$	Magnesium Chloride
$NaCl_2$	Sodium Chloride
OD	Optical Density
TAE	Tris Acetate EDTA
GFP	Green Fluorescent Protein
rpm	Revolution per minute
PCR	Polymerase Chain Reaction

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ABSTRACT

Electroporation is an efficient transformation method for transformation of bacteria. Recombinant *Agrobacterium* in which the T-DNA has been replaced with genes of interest are the most efficient vehicles used for the genetic transformation of plants. The PSP'AP-VF1 construct harboring the antisense cDNA of palmitoyl-ACP thioesterase gene driven by a sesquiterpene promoter fragment and pCAMBIA1302 construct harboring the green fluorescent protein gene were successfully electroporated into *Agrobacterium tumefaciens* strain LBA4404. PCR amplification of the antisense palmitoyl-ACP thioestease gene from the extracted plasmid of transformed *A. tumefaciens* confirmed the presence of PSP'AP-VF1 construct in all five recombinant *Agrobacterium* colonies. The presence of pCAMBIA1302 in recombinant colonies was confirmed by the presence of plasmid. Four out of five recombinant colonies that were screened for pCAMBIA1302 were positive. Recombinant colonies confirmed by PCR and plasmid extraction were selected and inoculated in YEM Kanamycin (50μg/mL) media for further use. This study could be carried out further by digesting the pCAMBIA1302 plasmid with appropriate restriction enzymes or by PCR amplification with gene specific primers to further confirm the presence of the plasmid in the recombinant *Agrobacterium.*

ELEKTROTRANSFORMASI *Agrobacterium tumefaciens* DENGAN KONSTRUK PSP'AP-VF1 DAN pCAMBIA1302

ABSTRAK

Elektroporasi adalah kaedah yang amat efisyen dalam transformasi bakteria. *Agrobacterium* rekombinan di mana T-DNAnya telah diganti dengan gen-gen yang diminati merupakan vektor yang paling efisyen dalam transformasi genetik tumbuhan. Konstruk PSP'AP-VF1 yang mengandungi cDNA antisens gen palmitoyl-ACP thioesterase dan serpihan promoter sesquiterpena dan konstruk pCAMBIA1302 yang mengandungi gen GFP berjaya dielektroporasikan ke dalam *Agrobacterium tumefaciens* stren LBA4404. Amplifikasi gen antisense palmitoyl-ACP thioestease daripada plasmid yang dipencil menunjukkan kehadiran konstruk PSP'AP-VF1 dalam kesemua lima koloni rekombinan *Agrobacterium*. Kehadiran pCAMBIA1302 dalam koloni rekombinan dikesan melalui kehadiran plasmid. Empat daripada lima koloni rekombinan yang ditransformasi dengan pCAMBIA1302 menunjukkan keputusan positif. Koloni rekombinan yang telah disahkan dengan kaedah PCR dan ekstraksi plasmid dipilih dan dikultur dalam YEM Kanamycin (50µg/mL) untuk pengunaan selanjutnya. Kajian ini boleh diteruskan dengan menghadamkan pCAMBIA1302 dengan enzim penyekat yang sesuai atau dengan amplifikasi PCR dengan pencetus-pencetus spesifik-gen untuk mengesahkan kehadiran pCAMBIA1302 dengan lebih lanjut.