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Universiti Malaysia Terengganu

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Electroporation of Chlorella sp. with linearized psp AP-VF2 construct / Lua Chung Liang.

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Electroporation of *Chlorella* sp. with linearized PSP'AP-VF2 construct

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RESEARCH REPORT VERIFICATION

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Electroporation of *Chlorella* sp. With linearized pSP'AP-VF2 plasmid oleh Lua Chung Liang, no. matrik: UK 10570 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,Universiti Malaysia Terengganu.

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LIST OF ABBREVIATIONS

ACP	Acyl Carrier Protein
AP	Antisense Palmitoyl-ACP Thioesterase
bp	Base pair
cDNA	Complementary Deoxyribonucleic Acid
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
µg	Microgram
µL	Microliter
OD	Optical Density
TAE	Tris-Acetate-EDTA
BBM	Bold's Basal Medium

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ABSTRACT

Electroporation is an important genetic transformation tool that has been used in plant genetic engineering to generate a wide variety of fertile transgenic plants. The PSP'AP-VF2 construct carries antisense palmitoyl-ACP thioesterase cDNA driven by Sesquiterpene synthase promoter. The AP2 construct was isolated from *E.coli*. The purity of the DNA obtained was 1.81 and its concentration was 0.15 µg/µL. The PSP'AP-VF2 plasmid was verified by PCR technique with primer combinations of PTE-VF1/PTE-VR2 and PTE-VF1/Pro-VF2. The size of amplified bands were 617 bp and 1047 bp respectively. The desired plasmid was successfully linearized by using *Eco*R1 restriction enzymes and verified by 1.0% agarose gel electrophoresis. Purified linearized product shows the purity of 1.91 and its concentration was 0.40 µg/µL. Different parameters were used to determine the suitable voltage for transformation using p35S-AP. Agr mode (2.2kV) shows the growth of *Chlorella* sp. colonies on the plates and was selected to be used in the electroporation of *Chlorella* sp with PSP'AP-VF2. The PSP'AP-VF2 construct was successfully electroporated into wild type *Chlorella* sp. at 2.2 kV in 0.1 cm cuvette. The putative transgenic *Chlorella* sp. was spreaded on BBM primary agar plates containing 10 µg/mL of hygromycin. Colonies on primary agar plates was randomly selected and transferred to grid plate containing 10µg/mL hygromycin.

Tajuk: Elektroporasi *Chlorella* sp. dengan konstruk linear PSP'AP-VF2

ABSTRAK

Electroforasi merupakan suatu alat transformasi genetik yang amat penting dan dipraktik secara meluas dalam kajian genetik untuk menghasilkan tumbuhan transgenik yang subur. Konstrak PSP'AP-VF2 mengandungi antisen palmitol-ACP thioesterase cDNA yang dipandu oleh promoter Sesquiterpene synthase. Konstrak AP2 telah diekstrak dari *E.coli*. Ketulenan yang diperoleh ialah 1.81 dan kepekatannya ialah 0.15μg/μL. Plasmid PSP'AP-VF2 disahkan dengan menggunakan kaedah PCR dengan kombinasi primer PTE-VF1/PTE-VR2 dan PTE-VF1/Pro-VF2. Saiz jalur yang diperolehi ialah 617 bp dan 1047 bp. Plasmid yang dikehendaki berjaya dilerai menjadi lurus dengan menggunakan enzim pembatasan *Eco*R1 dan seterusnya dikenalpasti ketulennya dengan menggunakan kaedah elektroforesis gel agaros 1.0%. Ketulenan produk tersebut ialah 1.91 dan kepekatannya ialah 0.40 μg/μL. Parameter yang berlainan telah digunakan untuk menentukan voltan yang plaing sesuai untuk transformasi *Chlorella* dengan menggunakan p35S-AP. Fungsi Agr (2.2 kV) menunjukkan pertumbuhan koloni *Chlorella* di dalam piring dan dipilih untuk digunakan dalam elektroporasi dengan menggunakan PSP'AP-VF2. Konstrak PSP'AP-VF2 telah berjaya dielektroporasikan kepada *Chlorella* liar pada 2.2 kV di dalam kuvet 0.1 cm. *Chlorella* tersebut dipindah ke dalam piring BBM mengandungi 10μg/mL hygromycin. Koloni yang tumbuh di atas piring tersebut dipindah ke dalam piring bergrid mengandungi 10μg/mL hygromycin.