

ELECTROPORATION OF *Chlamydomonas* WITH
95 S- β GALACTOSIDASE CIRCULAR CONSTRUCT

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ELECTROPORATION OF *Chlorella* sp. WITH
35S-AP CIRCULAR CONSTRUCT

By

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Research Report submitted in partial fulfillment of
the requirements for the degree of
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RESEARCH REPORT VERIFICATION**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ELECTROPORATION OF *Chlorella* sp. with 35S-AP CIRCULAR CONSTRUCT oleh BANREET KAUR A/P BUD SINGH no. matrik:UK9968 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
DHA	Docosahexanoic Acid
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleic Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
EPA	Eicosapentanoic Acid
g	Gram
L	Liter
LB	Lurie Bertani
M	Molar
MgCl ₂	Magnesium Chloride
mL	Mililiter
μg	Microgram
μL	Microliter

OD	Optical Density
PUFA	Polyunsaturated fatty acid
TAE	Tris-Acetate-EDTA
YEM	Yeast-extract Mannitol

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ABSTRACT

Electroporation has been given much attention in recent years due to its easy ability to act as a tool of genetic manipulation to generate transgenic plants. The availability of essential polyunsaturated fatty acids in *Chlorella* sp. makes it a marketable source of omega-3 and omega-6. The 35S-AP plasmid DNA was extracted from *Escherichia coli*. The purity of the extracted plasmid was 1.78 while the concentration was 0.67 $\mu\text{g/mL}$. This suggests that the plasmid obtained is of high quality. The 35S-AP plasmid was amplified with PCR technique using three primer combinations. The primer combinations 35S-F/35S-R produced a band of 326 bp. The primer combinations PTE-VF1/PTE-VR2 produced a band of 617 bp. The final primer combinations 35S-F/PTE-VF1 produced a distinct band of 943 bp. A series of voltage was tested to determine the most suitable voltage by electroporation of 35S-AP circular plasmid using the Bio-Rad Micropulser electroporation apparatus. The program Ec3 with a voltage of 3.0kV was selected to electroporate *Chlorella* sp. with 35S-AP circular plasmid. The electroporated cells were cultured on BBM with 10 $\mu\text{g/mL}$ hygromycin for selection of putative recombinant *Chlorella* sp. Sixteen putative colonies were randomly selected and transferred to BBM grid plates with 10 $\mu\text{g/mL}$ and 15 $\mu\text{g/mL}$ hygromycin respectively. The putative transformed colonies grew on 10 $\mu\text{g/mL}$ hygromycin but no growth was observed on 15 $\mu\text{g/mL}$ hygromycin. Electroporation condition needs to be optimized to successfully transform *Chlorella* sp.

Elektropolorasi *Chlorella* sp. dengan Plasmid Bulat 35S-AP

ABSTRAK

Elektroporasi telah diberi perhatian lebih sejak kebelakangan ini kerana kebolehnya sebagai alat manipulasi genetik untuk menjana tumbuhan transgenik. Kandungan asid lemak poli tak tepu yang terdapat dalam *Chlorella* sp. menjadikannya satu sumber pendapatan dengan adanya omega-3 dan omega-6. Plasmid DNA 35S-AP telah diekstrak daripada kultur *Escherichia coli* dengan ketulenan sebanyak 1.78 sementara kepekatannya adalah 0.67 µg/mL. Ini menunjukkan bahawa plasmid yang diperoleh berkualiti tinggi. Plasmid DNA 35S-AP telah diamplifikasikan mengguna teknik PCR 3 kombinasi-kombinasi primer 35S-F/35S-R menunjukkan jalur sebanyak 326 bp, manakala kombinasi primer PTE-VF1/PTE-VR2 dan 35S-F/PTE-VF1 menunjukkan jalur sebanyak 617 bp dan 943 bp masing-masing. Satu siri voltan diuji dengan menggunakan plasmid bulat 35S-AP untuk menentukan voltan yang paling sesuai menggunakan alat elektropolorasi MicroPulsert Bio-Rad. Program Ec3 dengan voltan sebanyak 3.0kV dipilih untuk elektropolorasi plasmid bulat 35S-AP. Sel yang dielektropolorasikan dikultur pada BBM 10 µg/mL hygromycin untuk pemilihan. Enam belas koloni dipilih secara rambang dan dikultur di atas piring bergrid BBM yang masing-masing mengandungi hygromycin sebanyak 10 µg/mL dan 15 µg/mL hygromycin. Beberapa koloni rekombinasi putatif dapat dilihat pada BBM 10 µg/mL hygromycin manakala tiada koloni yang dapat dilihat pada BBM 15 µg/mL. Keadaan elektropolarasi untuk *Chlorella* sp. perlu dioptimumkan untuk metransformasi *Chlorella* sp. dengan jayanya.