ELECTROPORATION OF *Chlorella sp.* WITH
PSP-AP 2 CIRCULAR CONSTRUCT

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ELECTROPORATION OF *CHLORELLA* sp. WITH PSP-AP2 CIRCULAR CONSTRUCT

By

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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

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ABSTRACT

The microalgae *Chlorella* sp. is used as a nutrient-dense foods and sources of fine chemicals such as lipid, protein, chlorophyll, vitamins and mineral that improve health. The genetic transformation was conducted to enhance the polyunsaturated fatty acids which contribute for human health. *Chlorella* sp. was cultured successfully and used to prepare electrocompetent cells.The objectives of this study were to transform the *Chlorella* sp. with PSP-AP2 circular construct and select the putative recombinant *Chlorella* cells. The plasmid DNA of PSP-AP2 construct was extracted from *E.coli*. the presence of the PSP-AP2 construct was detected with two primer combinations. The primers PTEVF1/PTEVR2 amplified a band of 617 bp while the primers PTEVF1/ProVF2 amplified a band of 1047 bp. The 35S-AP circular plasmid was used to determine a suitable voltage by using 6 different voltages. The programme "EC3" with a voltage of 3.0kV was selected to electroporate the PSP-AP2 circular plasmid. The transformed *Chlorella* cells were cultured on BBM solid medium with 10µg/mL hygromycin to select the putative recombinant *Chlorella* cells. From the primary plate, sixteen putative recombinant *Chlorella* cells were randomly selected and cultured on the BBM grid plates with 10µg/mL. Colonies was obtained on BBM grid plate with 10µg/mL hygromycin. The *Chlorella* cells show transient expression whereby high efficiency cannot be obtained. Further study should be carried out to verify the presence of PSP-AP2 construct in genomic DNA of putative recombinant *Chlorella* cells with PCR techniques.

Elektropolorasi *Chlorella* sp. dengan Plasmid Bulat PSP-AP2

ABSTRAK

Chlorella sp. ialah sejenis mikroalga yang mengandungi pelbagai nutrient seperti lipid, protein, pigmen klorofil, vitamin, dan mineral yang meningkatkan kesihatan manusia. *Chlorella* sp. telah dikultur dan digunakan sebagai sel elektrokompeten bagi kegunaan elektroporasi. Plasmid DNA telah diekstrak daripada *E.coli* dan pengesahan kewujudan konstruk bulat PSP-AP2 dijalankan dengan menggunakan 2 jenis kombinasi primer (PTEVF1/PTEVR2 dan PTEVF1/ProVF2) dan memperolehi 2 jalur yang bersaiz 617bp (PTEVF1/PTEVR2) dan 1047bp (PTEVF1/ProVF2). Plasmid bulat 35S-AP digunakan dalam elektroporasi sel *Chlorella* dengan 6 voltan yang berbeza. Program "EC3"dengan voltan 3.0kV dipilih untuk elektropolorasi yang sesuai untuk plasmid bulat. Elektroporasi dijalankan sekali lagi dengan menggunakan plasmid bulat konstruk PSP-AP2. Sel *Chlorella* yang telah dielektroporasi dikultur ke atas agar BBM yang mengandungi hygromycin (10μg/mL) dan diperhati selepas satu minggu. Sel *Chlorella* yang putatif didapati bertumbuh. Enam belas koloni putatif dipilih secara rawak dan kultur pada piring petri bergrid BBM dengan 10μg/mL hygromycin. Selepas diperhatikan satu minggu, koloni didapati bertumbuh. Kajian ini boleh diteruskan dengan mengekstrak genomik DNA sel *Chlorella* dan menjalankan pengesahan kewujudan konstruk PSP-AP2 di dalam sel *Chlorella* dengan teknik PCR.