

Cell Type Plant

Molecules DNA: pCaMVneo [NPT II]
Electroporated

Species *Lactuca sativa* (aka *chirimen chisha*)
Used

Before the Pulse

Cell Growth Medium MS (Murashige and Skoog)
(GIBCO/BRL)

Growth Phase at Harvest Not given

Pre-pulse Incubation Ice for 10 minutes

Wash Solution Leaf mesophyll protoplasts

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 0 °C

Electroporation Medium 5 mM MES, 70 mM KCl,
0.3 M Mannitol, pH 5.8

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Cell Density 2 x 10 (6) / ml

Volume of Cells 500 µl

Field Strength 0.625 kV/cm

DNA Concentration 100 µg / ml

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA,
pH 8.0)

Capacitor 250 µF

Volume of DNA 50 µg

Resistor (Pulse Controller) none Ω

Time Constant 13.8 msec

After the Pulse

Outgrowth Medium Modified Murashige and Skoog's medium (see
Comments for reference)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: Kazumi Amagasa and Toshiaki Kameya,
J. Japan Soc. Hort. Sci. 57(4): 620-625, 1989.

Outgrowth Temperature 24 °C

Length of Incubation 2 months

Selection Method or Assay Used G418 Geneticin (5 to 20 µg / ml)

Electroporation Efficiency only one

Per Cent Survival 10 to 30 %

Name of Submitter Takashi Ishibashi

Institution Address Nippon Petroleum Refining Co. Ltd.
Petroleum Section, Bio Group
766, Higashi-Toyoi
Kudamatsu-shi, Yamaguchi 744
JAPAN

Survey Number

183