BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Lactobacillus fermentum*, RF 14, a gastrointestinal isolate from pig intestine

Molecules Electroporated DNA: pGT633, covalently closed circular form, a native 9.8kB erythromycin resistant *Lactobacillus* plasmid.

Before the Pulse

Cell growth medium Lactobacilli MRS broth (Difco)

Growth phase at harvest O.D. (600) =0.8 (log phase cells)

Pre-pulse incubation 0°C for 1 min

Wash solution 3.5X SMEB; Luchansky *et.al.* (1988) Bio-Rad Bulletin **1350**:1-3

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium 3.5 x SMEB (1x = 272 mM sucrose, 1 mM MgCl2)

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 10 µg

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

Volume of DNA 5 µl

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used Erythromycin 25 µg / ml; requires a minimum expression time of 3 hr. to recover transformants

Electroporation Efficiency 5 x 10 (3) transformants / µg DNA, avg. Range: 4 X10 (2) to 4 X 10 (5)

Per Cent Survival 17 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. **Ref:** H.J. Connell, "Investigation of Methods for the Transformation of Gastrointestinal Strains of Lactobacilli with Plasmid pGT633" (1990). Ph.D. thesis, University of Otago, Dunedin, NEW ZEALAND (2) This work was carried out under the supervision of Dr. G. Tannock, Dept. of Micro., University of Otago, Box 56, Dunedin, NEW ZEALAND. PH: +64-3-4797713, Fax 64-3-4741607. Questions regarding the availability of strains and the pGT633 should be directed to him.**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submittor

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Date Submitted 12/20/90

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