



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactobacillus reuteri*, 100-23,
 a gastrointestinal isolate from rat stomach

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)

Pre-pulse incubation 0°C for 1 min

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

The Pulse

Electroporation Temperature 0°C

Instruments Used Gene Pulser® apparatus

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

Cell Density 10 (9) cells / ml

Cuvette Gap 0.4 cm

Volume of Cells 800 µl

Voltage 12.5 kV

DNA Concentration 10 µg

Field Strength 6.25 kV/cm

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

Capacitor 25 µF

Volume of DNA 5 µl

Resistor (Pulse Controller) Not used**

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10 ml

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.

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 2.6 x 10⁽¹⁾ transformants / µg DNA, avg. Range: 5 to 8.6 X 10⁽²⁾

Per Cent Survival 17%

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 12/20/90

Survey Number 068

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