BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Lactobacillus acidophilus* ADH; gastrointestinal isolate from human faeces

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) =log phase cells, 0.8

Pre-pulse incubation 0°C for 1 min.

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

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium 3.5X SMEB (1x = 272 mM sucrose, 1 mM $MgCl_2$)

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)

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. **See comments

Time Constant 10 to 15 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (Difco) 10ml

Outgrowth Temperature 37 °C

Length of Incubation 3 hrs.

Selection Method or Assay Used Erythromycin, 25 µg/ml; the Em(R) gene of pGTG33 requires a min.expression time of 3hr.to recover

Electroporation Efficiency Average 8.6 x 10 (1) transformants / µg DNA

Per Cent Survival 17 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. **It is NOT RECOMMENDED to use high voltage with out the Pulse Controller. **Ref:** **(1)** H. J. Connell, "Investigation of Methods for the Transformation of Gastointestinal Strains of Lactobacilli with Plasmid pGT633." Ph.D. Thesis, University of Otago, Dunedin, NEW ZEALAND (1990) **(2)** This work was carried out under the supervision of Dr. G. Tannock , Dept. of Microbiol., Univ. of Otago, P. O. Box 56, Dunedin, NEW ZEALAND; PH:+64-3-4797713; FAX: 64-3-4741607. Questions regarding the availability of strains and the plasmid pGT633 should be directed to him.

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