BIO-RAD

**Gene Pulser® Electroprotocol**

**Cell Type** Bacterial, gram positive

**Species Used** *Lactobacillus gasseri,* 100-5, a gastrointestinal isolate from pig intestine

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

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**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**

**Instruments Used** Gene Pulser® apparatus

**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, pH8.0)

**Volume of DNA** 5 µl

**Cuvette Gap** 0.4cm

**Voltage** 12.5 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** 2.6 x 10 (1) transformants / µg DNA, avg. Range: 5 to 8.6 X 10 (2)

**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 ofLactobacilli 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.

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**Survey Number**

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