



## 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<sub>2</sub>)  
**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 Gastrointestinal 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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**Date Submitted** 12/20/90

**Survey Number** 062

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