



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactobacillus reuteri*, 100-63(3)
 a gastrointestinal isolate from fowl crop

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)
Wash solution	3.5X SMEB; Luchansky <i>et.al.</i> (1988) Bio-Rad Bulletin 1350 :1-3	Pre-pulse incubation	0°C for 1 min

The Pulse

Electroporation Temperature	0 °C	Instruments Used	Gene Pulser® apparatus
Electroporation Medium	3.5 x SMEB (1x = 272 mM sucrose, 1 mM EDTA)	Cuvette Gap	0.4 cm
Cell Density	10 (9) cells / ml	Voltage	2.5 kV
Volume of Cells	800 µl	Field Strength	6.25 kV/cm
DNA Concentration	10 µg	Capacitor	25 µF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) Not used**
Volume of DNA	5 µl	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	1.6 x 10 ⁽¹⁾ transformant / µg DNA (avg); 1x10 ⁽¹⁾ to 1.2x10 ⁽⁴⁾ range
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 Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 12/20/90
Survey Number 071