



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactococcus lactis*, subspecies *lactis*, LM0230

Molecules Electroported DNA: pGB301, 9.8 kB.

Before the Pulse

Cell growth medium	M-17 glucose (ATCC)	Growth phase at harvest	O.D. (600) = (stationary)
Wash solution	Distilled water	Pre-pulse incubation	1 min. on ice

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller BTX Transfactor® 100
Electroporation Medium	Distilled water	Cuvette Gap	0.2 / 0.1 cm
Cell Density	5 X 10 ⁽¹⁰⁾ cells / ml	Voltage	2.5 / 1.6 kV
Volume of Cells	Not given	Field Strength	12.5 / 16 kV/cm
DNA Concentration	1 µg	Capacitor	25 µF
DNA Resuspension Buffer	10 mM Tris, 1 mM EDTA, pH 8.0	Resistor	(Pulse Controller) 200 Ω
Volume of DNA	3 to 5 µl	Time Constant	3.0 to 3.5 msec

After the Pulse

Outgrowth Medium	M-17-Glucose (no antibiotic selection)	Relevant Publications and/or Comments
Outgrowth Temperature	32 °C	Note: exponential values designated in parentheses.
Length of Incubation	2 hours	McIntyre, D.A. & Harlander, S.K. 1989. <i>Appl. Envir. Microbiol.</i> 55 : 604.
Selection Method or Assay Used	Erythromycin resistance	McIntyre, D.A. & Harlander, S.K. 1989. <i>Appl. Envir. Microbiol.</i> 55 : 2621.
Electroporation Efficiency	10 (3) to 10 (5) transformants / µg	McIntyre, D.A. & Harlander, S.K. 1990. 'Genetic Manipulation Techniques for Lactic Cultures'. <i>In: Proc. XXIII Int. Dairy Cong.</i> 2 : 1578(1990).
Per Cent Survival	10 %	

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Date Submitted 9/10/90

Survey Number 076

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