



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactobacillus* sp., strain 100-33

Molecules Electroported DNA: plasmid pLAR33, 18kB, supercoiled.

Before the Pulse

Cell growth medium	MRS broth (DIFCO)	Growth phase at harvest	O.D. (600) = 0.6 to 1.0 (log)
		Pre-pulse incubation	none
Wash solution	2% glycerol in 10 mM NaPO ₄ (pH 6.0)		

The Pulse

Electroporation Temperature	0 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	2% glycerol in 10 mM NaPO ₄ (pH 6.0)		
Cell Density	100x growth when harvested	Cuvette Gap	0.2 cm
Volume of Cells	50 µl	Voltage	2.5 kV
DNA Concentration	1 µg	Field Strength	12.5 kV/cm
DNA Resuspension Buffer	Not given	Capacitor	25 µF
Volume of DNA	1 to 5 µl	Resistor	(Pulse Controller) 200 Ω
		Time Constant	1 to 3 msec

After the Pulse

Outgrowth Medium	MRS broth
Outgrowth Temperature	37 °C
Length of Incubation	1 to 3 hrs, depends on selection.
Selection Method or Assay Used	Antibiotic resistance (erythromycin)
Electroporation Efficiency	2 X 10 ⁽⁵⁾ transformants / µg DNA
Per Cent Survival	1%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We have found that the electroporation of plasmids into our intestinal *Lactobacilli* isolates is highly strain-dependent. Our efficiencies range from 0 to 2 X 10⁽⁵⁾.

Name of Submitter
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Date Submitted 8/21/90

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