



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactococcus lactis*, subspecies *cremoris* & *lactis*, LM0230, H2, HP

Molecules Electroporated DNA: pMU1328, pSA3, pGB301, pJDC9, pAMB1; 3 to 30kB, primarily covalently closed circular form.

Before the Pulse

Cell growth medium	M17 glucose broth (ATCC)	Growth phase at harvest	O.D. (600) = 0.3 to 0.7
Wash solution	water	Pre-pulse incubation	ice

The Pulse

Electroporation Temperature	0 °C (ice)	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	water	Cuvette Gap	0.2 cm
Cell Density	10 (10) x 10 (11) cells / ml	Voltage	2 to 2.5 kV
Volume of Cells	40 µl	Field Strength	10 to 12.5 kV/cm
DNA Concentration	100 to 500 ng in 1 to 10 µl	Capacitor	25 µF
DNA Resuspension Buffer	water or TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) 200 Ω
Volume of DNA	1 to 10 µl	Time Constant	3.9 to 4.7 msec

After the Pulse

Outgrowth Medium	M17 glucose+0.5 M sucrose broth
Outgrowth Temperature	30°C
Length of Incubation	1 hour
Selection Method or Assay Used	antibiotic marker
Electroporation Efficiency	10 (4) transformants / µg DNA
Per Cent Survival	Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
 Powell *et al.*, 1988. *Appl. Environ. Microbiol.* **54**:655-660.

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Survey Number 075

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