



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactobacillus acidophilus*

Molecules Electroporated DNA: pACMI (or pACM2), pAMG10, pCK98, pBSKTAU, pGK12, pUB110. See Comments.

Before the Pulse

Cell growth medium Lactobacilli MRS broth (DIFCO Laboratories)

Growth phase at harvest O.D. (600) = 1.0

Pre-pulse incubation No incubation

Wash solution See Comments

The Pulse

Electroporation Temperature 4 °C
Electroporation Medium pZB (1x)

Instruments Used Gene Pulser® apparatus
 Pulse Controller

Cell Density 2 x 10⁽⁸⁾ to 8 x 10⁽⁸⁾ cells / ml

Cuvette Gap 0.4 cm

Volume of Cells 0.8 ml

Voltage 2.5 kV

DNA Concentration 200 µg / ml

Field Strength 6.25 kV/cm

DNA Resuspension Buffer TE buffer (pH 8.0) (10mM Tris, 1mM EDTA)

Capacitor 25 µF

Volume of DNA 5 µl

Resistor 1000 Ω (Pulse Controller)

Time Constant 3.0 to 4.8 msec

After the Pulse

Outgrowth Medium Lactobacilli MRS broth (DIFCO)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Wash solution: pZB(1X): 7 mM potassium phosphate (pH 7.4) containing 1 mM MgCl₂ and 272mM sucrose.

Outgrowth Temperature 37 °C

Length of Incubation 3 hour

Selection Method or Assay Used See Comments

Selection Method or Assay Used: Antibiotic resistance method, pACMI (TcR), pAM610 (TcR) pCK98(KmR), pBSKTAU (KmR), pGK12(EmR), pUB110(KmR)

Electroporation Efficiency 3.1 x 10⁽³⁾ to 2.0 x 10⁽³⁾ transformants / µg DNA (average)

Ref: *Biotechnology Letters* (1990). Vol. 12. No. 12. 919-924.

Per Cent Survival 12 %

Name of Submitter
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Date Submitted 1/4/91

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