



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Lactobacillus* sp., strain ES1

Molecules Electroported DNA: pLAR 33 ,18 kB plasmid, double stranded DNA .

Before the Pulse

Cell growth medium MRS broth (DIFCO)

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

Pre-pulse incubation Buffer, cuvettes, cells placed on ice

Wash solution 2% glycerol in 7 mM NaPO₄ (pH 6.0)

The Pulse

Electroporation Temperature 25 °C

Instruments Used Gene Pulser® apparatus
Pulse Controller

Electroporation Medium 2% glycerol in 10 mM NaPO₄ (pH 6.0)

Cell Density Cells concentrated 80X

Cuvette Gap 0.2 cm

Volume of Cells 50 µl

Voltage 2.0 kV

Field Strength 10 kV/cm

DNA Concentration 1 µg

Capacitor 25 µF

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

Resistor (Pulse Controller) 200 Ω

Volume of DNA 2 µl

Time Constant 2.2 msec

After the Pulse

Outgrowth Medium MRS broth

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

This transfer is briefly discussed in *Plasmid* **23**:119-125 (1990) and was taken from *FEMS Microbiol. Lett.* **44**:173-177 (1987).

Length of Incubation 6 hrs ,depends on selection.

Selection Method or Assay Used Antibiotic resistance (10 µg / ml erythromycin) on MRS plates

Electroporation Efficiency 2 X 10⁽⁵⁾ transformants / µg DNA

Per Cent Survival 0.03 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 8/21/90

Survey Number 074

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