



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Mycobacterium*, unspecified strain

Molecules Electroporated DNA: plasmid constructs
 (*Mycobacteria/E.coli* constructs)

Before the Pulse

Cell growth medium Tryptic soy broth (Difco)

Growth phase at harvest O.D. (600) = mid to late log phase

Pre-pulse incubation 10% sucrose, 75mM phosphate buffer

Wash solution Distilled water

The Pulse

Electroporation Temperature 0 °C
Electroporation Medium 10% sucrose, 75mM phosphate buffer

Instruments Used Gene Pulser® apparatus
 Pulse Controller

Cell Density 10⁽¹⁰⁾ to 10⁽¹²⁾ cells / ml

Cuvette Gap 0.2 cm

Volume of Cells 100 to 200 µl

Voltage 2.4 kV

DNA Concentration 1 to 5 µg DNA

Field Strength 12 kV/cm

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

Capacitor 25 µF

Volume of DNA ?

Resistor (Pulse Controller) 600 Ω

Time Constant 5 to 10 msec

After the Pulse

Outgrowth Medium Tryptic Soy Broth or Middlebrook 7H9 broth (Difco)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 35 °C

Length of Incubation overnight with shaking

Selection Method or Assay Used antibiotic selection

Electroporation Efficiency 1 x 10⁽³⁾ to 10⁽⁶⁾

Per Cent Survival Not given

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 6/6/91

Survey Number 077

© Bio-Rad Laboratories, 1993