BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Mycobacterium smegmatis*, ATCC 607

Molecules Electroporated DNA: plasmid, pAL 5000

Before the Pulse

Cell growth medium Mycobacteria, 7H11 (Difco)

Growth phase at harvest O.D. (600) = 0.5 to 1.0

Pre-pulse incubation None

Wash solution 272 mM sucrose,7 mM potassium phosphate,1 mM $MgCl_2$, pH 7.0

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium 272 mM sucrose,7 mM potassium phosphate,1 mM $MgCl_2$, pH 7.0

Cell Density 1 X 10 (9) cells / ml

Volume of Cells 400 μl

DNA Concentration 10 μg / ml

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

Volume of DNA 10 to 20 μl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) 200 Ω

Time Constant 4.5 to 5.0 msec

After the Pulse

Outgrowth Medium Mycobacteria, 7H11 (Difco)

Outgrowth Temperature 37 °C

Length of Incubation 15 to 21 days

Selection Method or Assay Used Not given

Electroporation Efficiency Not given

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

Institution Address

Telephone Number

Fax Number 58-2-861-1258

Date Submitted 3/19/91

Survey Number 080