



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Legionella pneumophila*

Molecules Electroported DNA: plasmid, pLAW330, 14 kB, supercoiled

Before the Pulse

Cell growth medium AYE medium (see notes)

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

Pre-pulse incubation None

Wash solution 10 % glycerol

The Pulse

Electroporation Temperature 4 °C

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Medium 10% glycerol

Cell Density 10 (11) cells/ ml

Cuvette Gap 0.2 cm

Volume of Cells 40 µl

Voltage 2.3 kV

DNA Concentration 1 µg/ µl

Field Strength 11.5 kV/cm

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

Capacitor 25 µF

Volume of DNA 1 to 2 µl

Resistor (Pulse Controller) 100 Ω

Time Constant 2.4 msec

After the Pulse

Outgrowth Medium AYE medium (see notes)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

AYE medium: (per liter)

10 g N-(2-acetamido)-2-aminoethanesulfonic acid

10 g Yeast Extract

0.4 g L-cysteine

0.25 g Fe NO₃

pH to 6.9 with KOH

Outgrowth Temperature 37 °C

Length of Incubation 5 hours

Selection Method or Assay Used Kanamycin, chloramphenicol

Electroporation Efficiency 10 (5) transformants / µg DNA

Per Cent Survival 90 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 9/12/92

Survey Number 203

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