

Cell Type Bacterial, gram positive

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

Species Used *Legionella pneumophila***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

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cuvette Gap 0.2 cm

Cell Density 10 (11) cells/ ml

Voltage 2.3 kV

Volume of Cells 40 µl

Field Strength 11.5 kV/cm

DNA Concentration 1 µg/ µl

Capacitor 25 µF

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

Resistor (Pulse Controller) 100 Ω

Volume of DNA 1 to 2 µl

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-acetanido)-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%

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Survey Number

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