BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Corynebacterium glutamicum* ATCC 13032, *Brevibacterium flavum* ATCC 21475

Molecules Electroporated DNA: plasmid pCW1 (a shuttle vector between *E. coli* and *Coryneform* bacterium

Before the Pulse

Cell growth medium ABG + 0.5% Tween 80 + 2.5% glycine

Growth phase at harvest O.D. (600) =0.25

Pre-pulse incubation ice

Wash solution 15% glycerol

The Pulse

Electroporation Temperature 4 °C

Electroporation Medium 15% cold glycerol

Cell Density 5 x 10 (10) / ml

Volume of Cells 50 to 60 µl

DNA Concentration 0.4 µg / µl

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

Volume of DNA 1 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 200 Ω (Pulse Controller)

Time Constant 4.6 to 4.9 msec

After the Pulse

Outgrowth Medium SMMC buffer

Outgrowth Temperature 30 °C

Length of Incubation 12 hr

Selection Method or Assay Used Neomycin, 10 µg / ml

Electroporation Efficiency 10 (5) transformants / µg DNA

Per Cent Survival 15 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

Institution Address

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Date Submitted 12/4/90

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