



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *Acetobacter xylinum*, ATCC 23769

Molecules Electroported DNA: cosmid pRK311, 22 kB, CsCl gradient purified DNA

Before the Pulse

Cell growth medium Schramm & Hestrin medium:
glucose 20 g/l; peptone 5g/l; yeast extract 5g/l; Na₂HPO₄ 2.7g/l; citric acid 1.15g/l; pH 6.2
Wash solution Cold water, then 10% glycerol

Growth phase at harvest O.D.(660) = 0.47

Pre-pulse incubation 1 min.

The Pulse

Electroporation Temperature 4°C
Electroporation Medium 10% glycerol
Cell Density 4x10⁽⁹⁾ cells / ml
Volume of Cells 40 µl
DNA Concentration 125 µg / ml
DNA Resuspension Buffer 10 mM Tris, pH 8.0, 1mM EDTA
Volume of DNA 2 µl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.1 cm

Voltage 1.8 kV

Field Strength 18 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.0 msec

After the Pulse

Outgrowth Medium Schramm and Hestrin medium

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 28°C

Length of Incubation 3 hr.

Selection Method or Assay Used tetracycline resistance

Electroporation Efficiency 1600 transfectants / µg DNA

Per Cent Survival 100%

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 3/6/91

Survey Number 001

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