



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *Vibrio anguillarum*

Molecules Electroported DNA: plasmid, pKT240, pKT230, pUC19

Before the Pulse

Cell growth medium Marine broth

Growth phase at harvest O.D. (600) = 0.4

Pre-pulse incubation 30°C

Wash solution 272 mM sucrose, 15% glycerol, 7mM NaHPO₄

The Pulse

Electroporation Temperature 0°C

Instruments Used Gene Pulser® apparatus
Pulse Controller

Electroporation Medium 272 mM sucrose, 15% glycerol, 7mM Na HPO₂

Cuvette Gap 0.4, 0.2, 0.1 cm

Cell Density 10 (10) cells / ml

Voltage 0.65 to 2.5 kV

Volume of Cells 40 µl

Field Strength Varied

DNA Concentration 200 ng / µl

Capacitor 25 µF

DNA Resuspension Buffer Not given

Resistor 1000 Ω (Pulse Controller)

Volume of DNA 1 µl

Time Constant 5 to 19 msec

After the Pulse

Outgrowth Medium Marine broth + 1% glucose

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 30 °C

Hamashima, H. *et. al.*, *Microbiol. Immunol.* **34**(f): 703-708 (1990).

Length of Incubation 1 hour

Selection Method or Assay Used Kanamycin, streptomycin, ampicillin

Electroporation Efficiency 4 x 10⁽⁴⁾ transformants / µg DNA

Per Cent Survival 90 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 6/7/91

Survey Number 055

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