BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, NM522

Molecules Electroporated DNA: plasmid DNA with inserts; pUC and pUC derivatives; relaxed circle

Before the Pulse

Cell growth medium conventional growth medium (NaCl Tryptone); LB broth; yeast extract

Wash solution Distilled water

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

Pre-pulse incubation 1 min. on ice

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol (distilled water)

Cell Density 1/500 initial volume

Volume of Cells 50 µl

DNA Concentration 1 to 10 ng / µl

DNA Resuspension Buffer Distilled water

Volume of DNA 10 µl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.1 cm

Voltage 2.0 kV

Field Strength 20 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.2 msec

After the Pulse

Outgrowth Medium LB growth medium

Outgrowth Temperature 37 °C

Length of Incubation 30 min.

Selection Method or Assay Used Ampicillin, 40 µg / µl

Electroporation Efficiency > 10 (8) transformants / µg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 10/31/90

Survey Number 042