



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5 α

Molecules Electroporated DNA: plasmid, various sizes

Before the Pulse

Cell growth medium LB

Growth phase at harvest O.D. (600) = 0.5 ~ 0.6

Pre-pulse incubation Bacteria held at 4 °C prior to pulse.

Wash solution Water

The Pulse

Electroporation Temperature Pulse 25°C (room temperature)

Electroporation Medium Water or 10% glycerol

Cell Density 200 ml culture to 0.5 ml final volume

Volume of Cells 40 to 200 μ l

DNA Concentration 1 pg to 100 ng DNA / pulse

DNA Resuspension Buffer Water or 1/2 x TE

Volume of DNA 1 to 10 μ l

Instruments Used Gene Pulser® apparatus and Capacitance Extender

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 μ F

Resistor 200 Ω (Pulse Controller)

Time Constant 5.0 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Not given

Electroporation Efficiency Max. >10(10) transfectants / μ g pUC DNA

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 5/1/91

Survey Number 025

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