



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *E. coli*, DH5 α

Molecules Electroported DNA: plasmid ,(pBluescript, pSVCAT, pBR322)

Before the Pulse

Cell growth medium 2 x YT

Growth phase at harvest O.D. (600) =1.0

Pre-pulse incubation on ice for 20 minutes

Wash solution ice-cold water

The Pulse

Electroporation Temperature 0 °C
Electroporation Medium 10% glycerol

Instruments Used Gene Pulser® apparatus, Pulse Controller, Capacitance Extender

Cell Density 5 x 10⁽⁷⁾ cfu / μ l

Cuvette Gap 0.1 cm

Volume of Cells 20 μ l

Voltage 1.5 kV

DNA Concentration 1 μ g / ml

Field Strength 15kV/cm

DNA Resuspension Buffer TE buffer (pH 8.0)

Capacitor 25 μ F

Volume of DNA 1 μ l

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Reference: T. Tsuji, *et al.*

(1990)PNAS. **87**:8835-8839.

SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose.

2xYT: 1.6% Bacto tryptone, 1.0% Bacto yeast extract, 0.5% NaCl.

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicilin

Electroporation Efficiency 0.5 to 1.0 x 10⁽⁹⁾ cfu / μ g DNA

Per Cent Survival 38 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 4/11/91

Survey Number 021

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