BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, covalently closed, pTTQ18, pUC, pBR322, M13

Before the Pulse

Cell growth medium LB or 2X-YT

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

Pre-pulse incubation < 30 sec

Wash solution 10 mM HEPES, pH 7.0,water, water /10 % glycerol

The Pulse

Electroporation Temperature 0°C (ice)

Electroporation Medium water or 10% glycerol/water See comments.

Cell Density >10 (10) cells/ml

Volume of Cells 40 μl

DNA Concentration 2.5 - 250 pg/μl cell suspension

DNA Resuspension Buffer water, 1X TE, 10 mM HEPES

Volume of DNA 1 to 2 μl

Instruments Used Gene Pulser ® apparatus Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 μF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 to 4.8 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37°C

Length of Incubation 1 hour

Selection Method or Assay Used ampicillin

Electroporation Efficiency Not given

Per Cent Survival 10-20%, depends on cell type

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Comments: Necessary to clean up ligations before electroporation. Ligations ethanol precipitated or Gene Clean® purified and resuspended in water or 1mM HEPES, pH 7.0. Also found high efficiencies associated with scrupulously clean apparatus and all equipment and reagents pre-cooled to 0°C. High efficiencies associated with cells grown only to O.D. (600) = 0.5.
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 Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 8/10/90

Survey Number 007