BIO-RAD

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid, pUC13 & subclones; pCDNA-1 (CDM8 varient)

Before the Pulse

Cell Growth Medium LB; made w/1% BACTO-tryptone, 0.5% Bacto-yeast; no NaCl added, no pH adjustment.

Growth Phase at Harvest O.D. (600) =0.4 to 0.6

Pre-pulse Incubation None

Wash Solution water; best available; 4°C

The Pulse

Instruments Used Gene Pulser® apparatus and Pulse Controller

Electroporation Temperature 25 °C

Electroporation Medium water

Cell Density 2 x 10 (10) cells / ml * (see notes)

Volume of Cells 0.06 to 0.1 ml

DNA Concentration 1 pg DNA

DNA Resuspension Buffer SOC

Volume of DNA 1 µl

Cuvette Gap 0.2 cm

Voltage 1.75 kV

Field Strength 8.75 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.5 to 5.0 msec

After the Pulse

Outgrowth Medium SOC (1 ml added immediately)

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicillin

Electroporation Efficiency 1 x 10 (9) transformants / µg DNA

Per Cent Survival about 25%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
* prepare by resuspending pellet in 2 volumes of deionized water.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.

Name of Submittor John E. Mapoles, Ph.D.

Institution Address Univ of Colorado HSC; GI; B-158
4200 E. 9th Ave
Denver, CO 80262

Survey Number

019