



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative
Species Used *E. coli*, MC1061 or NR9162 (same as MC1061, except *mutS*)

Molecules Electroporated DNA: M13mp2, RF DNA, 7.2 kB, nicked circles double stranded and single stranded DNA.

Before the Pulse

Cell growth medium 2 x YT: 16g Bacto-tryptone, 10 g Bacto-yeast extract, 5 g NaCl per liter

Growth phase at harvest Log phase OD(550) = 0.5 to 0.7

Pre-pulse incubation Variable, on ice

Wash solution deionized water; 10% glycerol

The Pulse

Electroporation Temperature 0 °C
Electroporation Medium 10% glycerol

Instruments Used Gene Pulser® apparatus & Pulse Controller

Cell Density 3 to 4 x 10⁽¹⁰⁾ / ml

Cuvette Gap 0.2 cm

Volume of Cells 50 µl

Voltage 2.0 kV

DNA Concentration 1 to 100 ng

Field Strength Not given

DNA Resuspension Buffer Deionized water

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor (Pulse Controller) 400 Ω

Time Constant usually 8.2 to 9.2 msec

After the Pulse

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

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. We analyze DNA polymerase fidelity *in vitro*. We have observed that when the sample DNA is incubated with less highly purified polymerase preps, the electroporation efficiency decreases dramatically (ca. 2 logs) even after purification of the DNA, whereas efficient transfection by CaCl₂ technique is obtained with the same samples. Electroporation seems to be more sensitive to random nicking of the DNAs by nucleases present in the polymerase preparations. Ref: Thomas, D. *et al.* (1991) *J. Biol. Chem.* **266**: 3744-3751. Eckert, K. *et al.* (1990) *Nucl. Acids Res.* **18**: 3739-3744.

Outgrowth Temperature Room temperature

Length of Incubation 0 to 20 min °C

Selection Method or Assay Used Plaque assay, color screen, bacteriophage (top agar) (β-gal+IPTG)

Electroporation Efficiency 5 to 10 x 10⁽⁸⁾ pfu / µg RF DNA

Per Cent Survival 60 to 100 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 11/15/91

Survey Number 046

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