

Gene Pulser® Electroprotocol

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

Molecules Electroported 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

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cuvette Gap 0.2 cm

Cell Density 3 to 4 x 10¹⁰ / ml

Voltage 2.0 kV

Volume of Cells 50 µl

Field Strength Not given

DNA Concentration 1 to 100 ng

DNA Resuspension Buffer Deionized water

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor (Pulse Controller) 400 Ω

After the Pulse

Time Constant usually 8.2 to 9.2 msec

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, bacterio- phage (top agar) (b-gal+IPTG)

Electroporation Efficiency 5 to 10 x 10⁸ pfu / µg RF DNA

Per Cent Survival 60 to 100 %

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Survey Number

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