



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5 α

Molecules Electroported

DNA: plasmid from 2kb to 30 kb from a ligation mix, sometimes supercoiled.

Before the Pulse

Cell growth medium LB

Growth phase at harvest O.D. (600) = between 0.4 and 0.6

Pre-pulse incubation 30 seconds to 1 minute

Wash solution double distilled water (ice cold) and (0°C, ice cold) glycerol

The Pulse

Electroporation Temperature 0°C

Instruments Used Gene Pulser® apparatus and Pulse Controller

Electroporation Medium 10% glycerol/water

Cell Density Not given

Cuvette Gap 0.2 cm

Volume of Cells 40 μ l

Voltage 2.5 kV

Field Strength 12.5 kV/cm

DNA Concentration See notes

Capacitor 25 μ F

DNA Resuspension Buffer Not given

Resistor 200 Ω (Pulse Controller)

Volume of DNA from 0.5 μ l to 2 μ l

Time Constant Not given

After the Pulse

Outgrowth Medium SOC

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37°C

DNA concentration: Depends on ligation-usually straight out of gel slice (low melt) ligation.

Length of Incubation 20 min. to 1 hour

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

Selection Method or Assay Used Have used carpenicillin, kanamycin, tetracycline, Xgal / IPTG (depending on plasmid)

20 mM glucose.

Electroporation Efficiency 10 (9) transformants / μ g DNA

LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Per Cent Survival Not given

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 3/4/91

Survey Number 020

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