



Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae*, DC5U

Molecules Electroporated DNA: YEp24, plasmid

Before the Pulse

Cell growth medium YEPD (ATCC#1202/1245)

Growth phase at harvest OD(600) = 1.1 to 1.3

Pre-pulse incubation 1 M Sorbitol

Wash solution 2 x Water, 1 M Sorbitol (Becker and Guarante protocol - see notes).

The Pulse

Electroporation Temperature 0 °C (ice)
Electroporation Medium 1 M Sorbitol

Instruments Used Gene Pulser® apparatus & Pulse Controller

Cell Density 3 x 10⁸ / ml

Cuvette Gap 0.2 cm

Volume of Cells 50 to 100 µl

Voltage 1.0 to 1.5 kV

DNA Concentration 1 µg / ml

Field Strength 5.0 to 7.5 kV/cm; optimal: 6.25kV/cm

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Capacitor 25 µF

Volume of DNA 0.5 µl (0.5 µg)

Resistor (Pulse Controller) 200 Ω

Time Constant 4.0 to 5.0 msec

After the Pulse

Outgrowth Medium Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 30 °C

Reference: Becker, D., Guarante, L. *Methods in Enzymol.* **104**:182-187 (1991).

Length of Incubation 3 days

Selection Method or Assay Used Incubate in 1 M Sorbitol for ~ 15 minutes. Place on SD + histidine + leucine + 1 M Sorbitol

Electroporation Efficiency 1.2 x 10⁵ transfectants / µg DNA

Per Cent Survival 40%

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 10/10/90

Survey Number 178

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