



Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast
Species Used *Saccharomyces cerevisiae*, SEY 6210

Molecules Electroporated DNA: YCp50 plasmid DNA (yeast centrameric shuttle vector)

Before the Pulse

Cell growth medium YPDA: 10% yeast extract, 20% bacto peptone, 20% glucose, 2 µg / ml adenine

Growth phase at harvest 2.0 x 10⁽⁷⁾ cells / ml

Pre-pulse incubation E-buffer (see notes) on ice for at least 5 minutes

Wash solution E-buffer (see notes)

The Pulse

Electroporation Temperature Room temperature
Electroporation Medium E-buffer (see notes)

Instruments Used Gene Pulser® apparatus

Cell Density 2.0 x 10⁽⁹⁾ cells / ml

Cuvette Gap 0.2 cm

Volume of Cells 50 µl

Voltage 0.54 kV

DNA Concentration 10 to 100 µg

Field Strength 2.7 kV/cm

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

Capacitor 25 µF

Volume of DNA 2 µl

Resistor (Pulse Controller) none Ω

Time Constant 10 to 20 msec

After the Pulse

Outgrowth Medium YPDA

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

E. Meilhoc *et. al.* Biotechnology **8**: 223-227, 1990.

Outgrowth Temperature 30 °C

Length of Incubation 2 hours

Selection Method or Assay Used YMM - ura (4 days) [yeast introgen base without amino acids, Difco]

Wash solution:

1. 10 mM Tris pH 8.0, 25 mM DTT in YPDA at 30° C for 10 min.

2. E-buffer: 10 mM Tris HCl pH 7.5, 270 mM Sucrose, 1mM MgCl₂

Electroporation Efficiency 2.5 x 10⁽⁵⁾ transfectants / µg DNA

Per Cent Survival 50 %

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 6/30/91

Survey Number 180

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