



Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast
Species Used *Saccharomyces cerevisiae*, strain S288C; a, α and a/ α

Molecules Electroporated DNA: YEp351/352, pRS 303 - 316, YEp50, usually supercoiled, 6-9 kB.

Before the Pulse

Cell growth medium YEPD (ATCC#1202/1245) or synthetic

Growth phase at harvest Log phase 80 to 100 Klett units

Pre-pulse incubation 10 mM Tris-HCl, pH 7.5, 1 M Sorbitol

Wash solution Water

The Pulse

Electroporation Temperature Room temperature

Instruments Used Gene Pulser® apparatus & Capacitance Extender, Pulse Controller

Electroporation Medium YPD, 1 M Sorbitol

Cuvette Gap 0.1 cm

Cell Density Concentrated 100 x

Voltage 0.55 kV

Volume of Cells 60 to 100 μ l

Field Strength 5.5 kV/cm

DNA Concentration 1 μ g / μ l

Capacitor 25 μ F

DNA Resuspension Buffer YEPD or SOC - yeast

Resistor (Pulse Controller) 600 Ω

Volume of DNA 7 to 10 μ l

Time Constant 5 to 6 msec

After the Pulse

Outgrowth Medium synthetic plates, - URA, -LEU, -HIS; No soft agar.

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 30 ° C, sometimes 24° C

Length of Incubation 48 hours

Selection Method or Assay Used -URA, -LEU, -HIS, or combinations of them.

Electroporation Efficiency 1000 to 3000 transfectants / μ g DNA

We are not interested in electroporation as such, but it is a very convenient method to introduce DNA into yeast cells. Electroporation is much less time consuming than the other methods available and also easier to perform. Room temperature is used because otherwise the time constant becomes too high and you get fewer transformants. This may also be used with frozen yeast cells but then the efficiency drops a lot.

Per Cent Survival Not known

Name of Submitter
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Date Submitted 10/16/90

Survey Number 177

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