

Cell Type Fungal / Yeast

Molecules Electroporated DNA: plasmid, pHILD2 & D4, linearized, 8 to 10 kB.

Species Used *Pichia pastoris* GTS115**Before the Pulse**

Cell Growth Medium Yeast Extract Potato Dextrose, YEPD, (DIFCO)

Growth Phase at Harvest O.D. (600) = 1.3

Pre-pulse Incubation 5 minutes, 4 °C

Wash Solution Cold water two times; then 1 M sorbitol, one time

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 25 °C but sample & cuvette at 4 °C

Electroporation Medium 1 M sorbitol

Cuvette Gap 0.2 cm

Cell Density 300x concentration from harvest density

Voltage 1.5 kV

Volume of Cells 50 µl

Field Strength 7.5 kV/cm

DNA Concentration 0.5 to 2 µg / pulse

DNA Resuspension Buffer 1 M sorbitol

Capacitor 25 µF

Volume of DNA 1 to 5 µl

Resistor (Pulse Controller) 400 Ω

After the Pulse

Time Constant approximately 8.0 msec

Outgrowth Medium Minimal salts plus dextrose (MD)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. This is essentially the method described for *Saccharomyces cerevisiae* by Becker and Guarente, *Methods in Enzymol.*, **194**,182-187(1991).

Outgrowth Temperature 30 °C

Length of Incubation 3 to 5 days

Selection Method or Assay Used Complimentation of histidine auxotrophy

Electroporation Efficiency approx. 1000 transformants / µg DNA

Per Cent Survival Not tested

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Survey Number

205