BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast

Species Used *Cryptococcus neoformans*, ma5 mutants

Molecules Electroporated DNA: supercoiled on linear plasmids containing URA5 gene

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Before the Pulse

Cell growth medium YEPD

Growth phase at harvest Logarithmic, O.D.(650) = ~ 1.

Pre-pulse incubation None

Wash solution 270 mM Sucrose,1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5,4 mM DTT

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium 270 mM Sucrose, 10 mM Tris HCl, pH 7.5 (no DTT)

Cell Density Cells concentrated, 100 fold

Volume of Cells 450 μl

DNA Concentration 0.1 to 1.0 μg, in 1 to 10 μl TE

DNA Resuspension Buffer TE Buffer (10 mM Tris, 1 mM EDTA, pH8.0)

Volume of DNA 1 to 10 μl

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.2 cm

Voltage 0.470 kV

Field Strength 2.35 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) none Ω

Time Constant 18 to 22 msec

After the Pulse

Outgrowth Medium None

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used SD media (lacking uracil): 6.7 g yeast nitrogen base per liter without amino acids and 20 g glucose / liter

Electroporation Efficiency 2000 transfectants / μl

Per Cent Survival Not tested

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Note: the protocol described in the paper by Edman and Kwon-Chung, *Mol. & Cell. Biol.*, **10**:4538-4544 (1990) is not the one described above. The one above gives 10-100x greater efficiency.

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