BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast

Species Used *Dictyostelium discoideum*

Molecules Electroporated DNA: supercoiled and linear

Before the Pulse

Cell growth medium HL5 (peptone, yeast extract, glucose)

(ATCC Media #671)

Wash solution Hepes Buffered Saline

Growth phase at harvest 1 x 10 (6) to 1 x 10 (7) cells / ml

Pre-pulse incubation 5 min.

The Pulse

Electroporation Temperature 4 °C

Electroporation Medium Hepes Buffered Saline

Cell Density 5 x 10 (6) / ml

Volume of Cells 1 ml

DNA Concentration 20 ng to 20 μg

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

Volume of DNA Any volume

Instruments Used Gene Pulser® apparatus & Pulse Controller

Cuvette Gap 0.4 cm

Voltage 1.25 kV

Field Strength 3.125 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) Ω none. NOT RECOMMENED***(see notes)

Time Constant 0 .5 to 0.7 msec

After the Pulse

Outgrowth Medium HL5

Outgrowth Temperature 22°C

Length of Incubation overnight

Selection Method or Assay Used G418

Electroporation Efficiency 10 (-3) transformants/ cell;
6 x 10 (3) transformants / μg DNA.

Per Cent Survival >90 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

HBS: 10mM HEPES, pH 7.2,150 mM NaCl, 5 mM CaCl2

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 11/13/91

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