BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Fungal / Yeast

Species Used *Saccharomyces cerevisiae* - lines derived from S288C

Molecules Electroporated DNA: pUC19 and Bluescript™ -based plasmids.

Before the Pulse

Cell growth medium YEPD (ATCC#1202/1245)

Growth phase at harvest 0.5 to 1.0 at O.D.600; ~100 mls log phase culture concentrated 200x, in

Pre-pulse incubation None required

Wash solution Water

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium Water

Cell Density 6 x 10 (10) cells / ml

Volume of Cells 40 to 50 μl

DNA Concentration Total DNA = 0.2 to 1.0 μg

DNA Resuspension Buffer Not given

Volume of DNA ≤5μl

Instruments Used Gene Pulser® apparatus &Pulse Controller

Cuvette Gap 0.2 cm

Voltage 0.6 kV

Field Strength 3.0 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) 200 Ω

Time Constant Not given

After the Pulse

Outgrowth Medium Add water to final vol. 150-200 μl. Plate on selective synthetic media (SD)

Outgrowth Temperature 30 °C

Length of Incubation 2 days

Selection Method or Assay Used Amino acid or nucleotide dropout

Electroporation Efficiency 2 to 10,000 transfectants / μg DNA

Per Cent Survival Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Washed and concentrated cells can be stored by adding 80% glycerol to a final concentration of 20% and freezing at -80°C. To electroporate thawed cells:pellet cells, remove glycerol containing supernatant, wash cells in 0.5-1.0 ml water, and resuspend cells in water to original volume. Efficiency is not affected significantly by freezing but you must remove glycerol.
Comment: removing glycerol after thawing cells may help by simply removing lysed cell contents that would alter media conductivity - this could impact efficiency (alters the time constant, t).

Name of Submittor

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Date Submitted 9/14/90

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