BIO-RAD

Gene Pulser® Electroprotocol

Cell Type Bacterial, gram negative

Species Used *E. coli*, DH5α

Molecules Electroporated DNA: plasmid ,(pBluescript, pSVCAT, pBR322)

Before the Pulse

Cell Growth Medium 2 x YT

Growth Phase at Harvest O.D. (600) =1.0

Pre-pulse Incubation on ice for 20 minutes

Wash Solution ice-cold water

The Pulse

Instruments Used Gene Pulser® apparatus, Pulse Controller,

Electroporation Temperature 0 °C

Electroporation Medium 10% glycerol

Cell Density 5 x 10 (7) cfu / µl

Volume of Cells 20 µl

DNA Concentration 1 µg / ml

DNA Resuspension Buffer TE buffer (pH 8.0)

Volume of DNA 1 µl

Cuvette Gap 0.1 cm

Voltage 1.5 kV

Field Strength 15kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used Ampicilin

Electroporation Efficiency 0.5 to 1.0 x 10 (9) cfu / µg DNA

Per Cent Survival 38 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Reference: T. Tsuji, *et al*. (1990)PNAS.**87**:8835-8839.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
2xYT: 1.6% Bacto tryptone,1.0% Bacto yeast extract, 0.5% NaCl.

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Survey Number

021