BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, DH5α, XL1- Blue, NM522, JM109, MV1190

Molecules Electroporated DNA: cDNA library in pBS-SKII+ (3 to7 kB), Bluescript™ vectors (3 to 7 kB), (single stranded M13 phage DNA).

Before the Pulse

Cell growth medium LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Growth phase at harvest O.D. (600) =0.9

Pre-pulse incubation 10% glycerol

Wash solution glass distilled water

The Pulse

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cell Density 1 liter cells @ 0.9 O.D. (600) to 2 ml cell slurry

Volume of Cells 40 µl

DNA Concentration Not given

DNA Resuspension Buffer glass distilled water

Volume of DNA 0.5 to 2 µl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 4.8 msec

After the Pulse

Outgrowth Medium SOB

Outgrowth Temperature 37 °C

Length of Incubation 1 hr. 225 rpm

Selection Method or Assay Used LB-carbampicillin 100 µg / µl

Electroporation Efficiency Strains without F':10 (9) to 5 x 10 (10), with F': 10 (8) to 10 (9) tranformants / µg

Per Cent Survival not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. I've found that I can increase my transformation efficiency by 10 - 50 fold by being ultra careful to use the purest reagents and water available, autoclaving everything that will come into contact with cells (and I mean everything - including pipet tips and microfuge tubes) in glass distilled water prior to sterilization, and by using glassware that has never been washed with soap or bleach. Also, I've found that it is important to work quickly while preparing cells and to keep them cold.
SOB: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 20 mM glucose.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 8/21/90

Survey Number 035