BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli*, MC1061, modified to contain F' (host for M13)

Molecules Electroporated DNA: M13 recombinant RF (double stranded) and single-stranded DNA

Before the Pulse

Cell growth medium LB

Growth phase at harvest O.D. (600) =0.4 - 0.6

Pre-pulse incubation none; done as fast as possible

Wash solution 10% glycerol, 4 washes at 4°C

The Pulse

Electroporation Temperature 0 to 4 °C

Electroporation Medium 10% glycerol

Cell Density As high as possible: just vortexed drained pellet.

Volume of Cells 40 µl

DNA Concentration 7.5 µg/µl

DNA Resuspension Buffer 10% glycerol

Volume of DNA 5 µl + 40µl cells

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.450 kV

Field Strength 12.2 kV/cm

Capacitor 25 µF

Resistor 400 Ω (Pulse Controller)

Time Constant 9.1 to 9.6 msec

After the Pulse

Outgrowth Medium SOC

Outgrowth Temperature 25 °C

Length of Incubation 1 to10 min., plated immediately

Selection Method or Assay Used M13 plaques, no drugs

Electroporation Efficiency 1-5x10(10) (RF); 1-50x10(8) (single-stranded)transformants / µg DNA

Per Cent Survival Not measured

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Transformation efficiency for M13 is about 3x as high as pUC plasmid.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor

Institution Address

Telephone Number

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Date Submitted 10/16/90

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