



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Staphylococcus aureus*, RN4220

Molecules Electroporated DNA: pC194 (2.9 kB), pE194 (2.9 kB), pI258 (2.9 kB), pMH109 (7.4 kB), pMH120 (7.8 kB), supercoiled.

Before the Pulse

Cell growth medium B2 broth (see notes)

Growth phase at harvest Mid-log; 260 Klett units, #66 red filter.

Pre-pulse incubation None

Wash solution See notes

The Pulse

Electroporation Temperature Room temperature, 20 °C
Electroporation Medium 10% glycerol

Instruments Used Gene Pulser® apparatus & Pulse Controller

Cell Density 3 x 10⁽¹⁰⁾ cells / ml

Cuvette Gap 0.1 cm

Volume of Cells 60 µl

Voltage 2.3 kV

DNA Concentration 17 µg DNA / µl

Field Strength 23 kV/cm

DNA Resuspension Buffer B2 broth

Capacitor 25 µF

Volume of DNA 1 to 6 µl

Resistor (Pulse Controller) 100 Ω

Time Constant 2.5 msec

After the Pulse

Outgrowth Medium NYE broth: 1% casein hydrolyzate (Sigma), 0.5% yeast extract, 0.5% NaCl

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
B2 broth = 1% casein hydrolyzate (Sigma), 2.5% yeast extract (Difco), 0.5% glucose, 0.1% KHPO₄, 0.5% NaCl pH 7.5, overnight culture diluted 1/25 in 25 ml B2 broth in 300 ml Klett flask.

Outgrowth Temperature 37 °C

Length of Incubation 2 hours

Selection Method or Assay Used NYE agar and appropriate selective agent

Wash Solution: 3 washes deionized water; 1/5 wash 10% glycerol; 1/10 wash 10% glycerol (Wash volume refers to volume of growth medium).

Electroporation Efficiency 2 x 10⁽⁸⁾ CFU per µg DNA

Per Cent Survival 2 %

Name of Submitter
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Date Submitted 12/23/91

Survey Number 083

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