

Cell Type Bacterial, gram positive

Molecules Electroporated DNA: plasmids, various sizes

Species Used *Staphylococcus aureus*, RN4220**Before the Pulse**

Cell Growth Medium Trypticase soy broth (Difco)

Growth Phase at Harvest O.D. (600) = 0.3 to 0.8

Pre-pulse Incubation 1 minute

Wash Solution 500 mM sucrose

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 0°C (ice)

Electroporation Medium 500 mM sucrose

Cuvette Gap 0.2 cm

Cell Density 1 X 10 (10) cells / ml

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.001 to 1.0 µg

DNA Resuspension Buffer TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

Capacitor 25 µF

Volume of DNA 1 to 2 µl

Resistor (Pulse Controller) 100 Ω

After the Pulse

Time Constant 2.5 msec

Outgrowth Medium SMMP (see comments)

Relevant Publications and/or Comments**Note:** exponential values designated in parentheses.

SMMP: equal volumes of 2X SMM and 4X PAB.

SMM (pH 6.5): 1M sucrose, 0.4 M maleic acid, 0.4 M MgCl₂.

PAB: Antibiotic Medium 3 (Difco).

Outgrowth Temperature 0 °C

Length of Incubation 15 minutes

Selection Method or Assay Used Various antibiotics: Tc, Em, Km, Pc.

Electroporation Efficiency Varies from 10 to 3.0 X 10 (5) transformants / µg

Per Cent Survival 5 to 10%

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Survey Number

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