

Cell Type Bacterial, gram positive

Molecules DNA: plasmid, pC194, 2.9 kB,
Electroporated double-stranded, supercoiledSpecies *Staphylococcus aureus* RN4220
Used**Before the Pulse**

Cell Growth Medium Tryptic soy broth

Growth Phase at Harvest O.D. (600) = 0.4 [early log]

Pre-pulse Incubation 1 minute, room temperature

Wash Solution 500 mM sucrose

The Pulse

Instruments Used Gene Pulser® apparatus & Pulse Controller

Electroporation Temperature 25 °C

Electroporation Medium 500 mM sucrose

Cuvette Gap 0.2 cm

Cell Density 10(10) cells/ ml

Voltage 2.5 kV

Volume of Cells 40 µl

Field Strength 12.5 kV/cm

DNA Concentration 0.5 µg/µl

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

Capacitor 25 µF

Volume of DNA 1 µl

Resistor (Pulse Controller) 100 Ω

After the Pulse

Time Constant 2.4 to 2.5 msec

Outgrowth Medium SMMI (Staphylococcus Medium, ATCC 454)

Relevant Publications and/or Comments**Note:** exponential values designated in parentheses.
Ref: Kraemer and Landolo, *Curr. Microbiol.* 21:373 (1990).
Efficiencies of $\geq 1 \times 10^6$ transformants/µg is rare.

Outgrowth Temperature 37 °C

Length of Incubation 1.5 to 2 hours

Selection Method or Assay Used Antibiotic resistance-chloramphenicol

Electroporation Efficiency up to 10(6); often 10(4) to 10(5) transformants / µg

Per Cent Survival Unknown

Name of Submitter Allen Gies

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Survey Number

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