



Gene Pulser® Electroprotocols

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

Molecules Electroported DNA: plasmids, various sizes

Before the Pulse

Cell growth medium	Trypticase soy broth (Difco)	Growth phase at harvest	O.D. (600) = 0.3 to 0.8
Wash solution	500 mM sucrose	Pre-pulse incubation	1 minute

The Pulse

Electroporation Temperature	0°C (ice)	Instruments Used	Gene Pulser® apparatus Pulse Controller
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	Capacitor	25 µF
DNA Resuspension Buffer	TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) 100 Ω
Volume of DNA	1 to 2 µl	Time Constant	2.5 msec

After the Pulse

Outgrowth Medium	SMMP (see comments)
Outgrowth Temperature	0°C
Length of Incubation	15 minutes
Selection Method or Assay Used	Various antibiotics: Tc, Em, Em, Km, Pc.
Electroporation Efficiency	Varies from 10 ⁽⁵⁾ to 3.0 X 10 ⁽⁵⁾ transformants / µg
Per Cent Survival	5 to 10 %

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).

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 3/7/91

Survey Number 082

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