



Gene Pulser® Electroprotocols

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

Molecules Electroported DNA: plasmids, pC194, pE194, pTV32; phage DNA, 80α.

Before the Pulse

Cell growth medium	Tryptic Soy Broth (TSB - Difco)	Growth phase at harvest	O.D. (540) = 0.55 to 0.6
Wash solution	10% glycerol	Pre-pulse incubation	30 min. ice with DNA

The Pulse

Electroporation Temperature	0 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	Not given	Cuvette Gap	0.2 cm
Cell Density	1 X 10 ⁽¹¹⁾ cells / ml	Voltage	2.5 kV
Volume of Cells	50 µl	Field Strength	12.5 kV/cm
DNA Concentration	0.1 to 0.2 µg / ml	Capacitor	25 µF
DNA Resuspension Buffer	5 parts 4X SOC, 4 parts SMM, 0.5 parts 10% BSA + DNA	Resistor	(Pulse Controller) 100 Ω
Volume of DNA	10% volume, ≤ 5 µl	Time Constant	2.4 msec

After the Pulse

Outgrowth Medium	4X SOC, SMM, 10%
Outgrowth Temperature	37 °C (30°C for sensitive plasmids)
Length of Incubation	90 min.
Selection Method or Assay Used	BM agar + antibiotics
Electroporation Efficiency	10 (5) transformants / µg
Per Cent Survival	80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

SMM: 1M sucrose, 0.04 M maleic, 0.04 MgCl₂.

SMM + 4X SOC: 5.5 parts SMM 4 parts 4X SOC, 0.5 parts 10 % BSA, filter sterilized.

BM: 1.0% peptone, 0.5% yeast extract 0.1 % glucose, 0.5% NaCl, 0.1% K₂HPO₄, 1.2 % agar.

4X SOC: 8.0% tryptone, 2.0% yeast extract, 40mM NaCl, 10 mM MgCl₂, 40 mM MgSO₄, 80mM glucose. To the freshly autoclaved base containing tryptone and yeast extract, add the appropriate amount of individually prepared sterile stocks (2M for glucose, 1M for all inorganic salts). Filter sterilize and aseptically tube.

Name of Submitter
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Date Submitted 8/24/90

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