



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Streptococcus sanguis*, FW213

Molecules Electroported DNA: plasmid, 6 to 12 kB.

Before the Pulse

Cell growth medium	Brain Heart Infusion (BHI) +0.2% glucose	Growth phase at harvest	O.D. (600) = early log phase
Wash solution	10 mM Tris-Cl (pH 6.0), 0.5 M sucrose	Pre-pulse incubation	10 mM Tris-Cl (pH 4.0), 0.5 M sucrose

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	10 mM Tris-Cl (pH 4.0), 0.5 M sucrose	Cuvette Gap	0.2 cm
Cell Density	10 (9) cells / ml	Voltage	2.5 kV
Volume of Cells	50 µl	Field Strength	12.5 kV/cm
DNA Concentration	50 µg / ml	Capacitor	25 µF
DNA Resuspension Buffer	1x TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) 200 Ω
Volume of DNA	1 to 2 µl	Time Constant	4.0 to 4.3 msec

After the Pulse

Outgrowth Medium	THB (Difco) + 0.625 M sucrose
Outgrowth Temperature	37 °C
Length of Incubation	2 to 3 hours
Selection Method or Assay Used	antibiotic selection
Electroporation Efficiency	10 (4) transfectants / µg, using supercoiled plasmid DNA
Per Cent Survival	30 to 40 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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Date Submitted 3/4/91

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