



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Mycobacterium bovis*, BCG

Molecules Electroporated DNA: plasmid, covalently closed circular form, pYT937, 5.7 kB

Before the Pulse

Cell growth medium Middlebrook 7H9 (Difco)+ Tween 80
Growth phase at harvest O.D. (600) =0.3
Pre-pulse incubation None
Wash solution 10 mM Tris (pH 7.0), 20% sucrose; or 7 mM phosphate buffer, 10% sucrose

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	10 mM Tris (pH 7.0), 20% sucrose	Cuvette Gap	0.2 cm
Cell Density	10 ⁽⁹⁾ cells / ml	Voltage	1.25 kV
Volume of Cells	200 µl	Field Strength	6.25 kV/cm
DNA Concentration	1 to 2 µg	Capacitor	25 µF
DNA Resuspension Buffer	Middlebrook 7H9 + Tween 80	Resistor	(Pulse Controller) 600 Ω
Volume of DNA	10 µl	Time Constant	Not given

After the Pulse

Outgrowth Medium Middlebrook 7H9 + Tween 80

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C
Length of Incubation overnight
Selection Method or Assay Used kanamycin resistance
Electroporation Efficiency 10⁽³⁾ to 10⁽⁴⁾ transformants / µg
Per Cent Survival 50%

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 4/23/91

Survey Number 078

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