



Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive
Species Used *Mycobacterium smegmatis*

Molecules Electroported DNA: plasmids, various sizes

Before the Pulse

Cell growth medium	Middlebrook 7H9 Broth (Difco) [MB]	Growth phase at harvest	O.D. (600) = mid- log
Wash solution	10% glycerol	Pre-pulse incubation	None

The Pulse

Electroporation Temperature	Room temperature	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	10% glycerol	Cuvette Gap	0.2 cm
Cell Density	Not given	Voltage	1.25 kV
Volume of Cells	100 µl	Field Strength	6.25 kV/cm
DNA Concentration	varies	Capacitor	25 µF
DNA Resuspension Buffer	400 to 600 µl MB	Resistor	(Pulse Controller) 800 Ω
Volume of DNA	Not given	Time Constant	9 msec

After the Pulse

Outgrowth Medium	MB	Relevant Publications and/or Comments Note: exponential values designated in parentheses. Ref: Lee, M.H., Pascopella, L., Jacobs, Jr., W.R. and Hatfull, G.F., <i>PNAS</i> 88 :3111-5.1991. See also: Snapper, <i>et.al.</i> <i>PNAS</i> 85 :6987-6991.1988.
Outgrowth Temperature	37 °C	
Length of Incubation	2 hours	
Selection Method or Assay Used	MB / usually kanamycin sensitive	
Electroporation Efficiency	Usually very good	
Per Cent Survival	Not given	

Name of Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 3/8/91
Survey Number 079