



Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative	Molecules Electroported	DNA: cosmids, plasmids up to 50 kB
Species Used	<i>E. coli</i> , DH5 α , HB101; <i>Salmonella typhimurium</i> , <i>Salmonella senftenberg</i>		

Before the Pulse

Cell growth medium	LB	Growth phase at harvest	O.D.(660) = 0.3
Wash solution	10% glycerol	Pre-pulse incubation	Minimal

The Pulse

Electroporation Temperature	+/- 2°C	Instruments Used	Gene Pulser® apparatus & Pulse Controller
Electroporation Medium	10% glycerol		
Cell Density	10 (11) cfu / ml	Cuvette Gap	0.1 cm
Volume of Cells	50 μ l	Voltage	1.6 kV
DNA Concentration	0.5 to 2 μ g / μ l	Field Strength	16 kV/cm
DNA Resuspension Buffer	Not given	Capacitor	25 μ F
Volume of DNA	2 μ l	Resistor	(Pulse Controller) 400 Ω
		Time Constant	4 to 8 msec

After the Pulse

Outgrowth Medium	SOC	Relevant Publications and/or Comments
Outgrowth Temperature	35°C	Note: exponential values designated in parentheses.
Length of Incubation	1 hour	SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl ₂ , 10 mM MgSO ₄ , 20 mM glucose.
Selection Method or Assay Used	Antibiotic selection: tetracycline, kanamycin, ampicillin	
Electroporation Efficiency	Not done (very poor for <i>S. senftenberg</i>)	
Per Cent Survival	approximately 50 %	

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 9/11/92

Survey Number 200

© Bio-Rad Laboratories, 1993