BIO-RAD

Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative	**Molecules Electroporated**	DNA,various supercoiled plasmids - CsCl pure; Qigen column, after microdialysis
Species Used	*E. coli*, N99, DH 5α		

Before the Pulse

Cell growth medium	LB	**Growth phase at harvest**	O.D. (600) =whatever
		Pre-pulse incubation	Short as possible; however long it takes to set up.
Wash solution	Twice in cold water; twice in 10% glycerol		

The Pulse

Electroporation Temperature	Not given	**Instruments Used**	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	10% glycerol		
Cell Density	Not given	**Cuvette Gap**	0.2 cm
		Voltage	2.5 kV
Volume of Cells	Not given	**Field Strength**	12.5 kV/cm
DNA Concentration	Not given	**Capacitor**	25 μF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA,pH 8.0)	**Resistor**	200 Ω (Pulse Controller)
Volume of DNA	1 to 10 μl	**Time Constant**	4.0 to 5.0 msec

After the Pulse

Outgrowth Medium	SOC (Add glucose after autoclaving and cooling)
Outgrowth Temperature	30 °C (N99) 37 °C (DH5α)
Length of Incubation	4hr (N99); 2hr (DH5α)
Selection Method or Assay Used	Ampicillin, kanamycin resistance
Electroporation Efficiency	2 to 4 x 10 (8) transformants / μg DNA
Per Cent Survival	Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
I have electroporated temperature-sensitive mu-lysogens with no problems. I was concerned, because even a slight increase in temperature will result in cell death. As long as I keep things on ice and work quickly, there seems to be no problem with temperature induction by the electroporation.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

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Date Submitted 8/27/90

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