BIO-RAD

Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative	**Molecules Electroporated**	DNA: plasmid, pUC13 & subclones; pCDNA-1 (CDM8 varient)
Species Used	*E. coli*, DH5α		

Before the Pulse

Cell growth medium	LB; made w/1% BACTO-tryptone, 0.5% Bacto-yeast; no NaCl added, no pH adjustment.	**Growth phase at harvest**	O.D. (600) =0.4 to 0.6
		Pre-pulse incubation	None
Wash solution	water; best available; 4°C		

The Pulse

Electroporation Temperature	25 °C	**Instruments Used**	Gene Pulser® apparatus and Pulse Controller
Electroporation Medium	water		
Cell Density	2 x 10 (10) cells / ml * (see notes)	**Cuvette Gap**	0.2 cm
		Voltage	1.75 kV
Volume of Cells	0.06 to 0.1 ml	**Field Strength**	8.75 kV/cm
DNA Concentration	1pg DNA	**Capacitor**	25 μF
DNA Resuspension Buffer	SOC	**Resistor**	200 Ω (Pulse Controller)
Volume of DNA	1 μl	**Time Constant**	4.5 to 5.0 msec

After the Pulse

Outgrowth Medium	SOC (1 ml added immediately)
Outgrowth Temperature	37 °C
Length of Incubation	1 hour
Selection Method or Assay Used	Ampicillin
Electroporation Efficiency	1 x 10 (9) transformants / μg DNA
Per Cent Survival	about 25%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
* prepare by resuspending pellet in 2 volumes of deionized water.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4,
20 mM glucose.

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 3/11/91

Survey Number 019