



Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative	Molecules Electroporated	DNA: pRK290, about 20 kB, covalently closed, circular (see notes). Plasmid contains kanamycin resistance cartridge.
Species Used	<i>Legionella pneumophila (philadelphia)</i> , <i>Legionella longbeachae</i>		

Before the Pulse

Cell growth medium	Harvested from BCYE agar	Growth phase at harvest	O.D. (600) = not applicable, harvested directly from plate
Wash solution	Distilled water	Pre-pulse incubation	5 to 10 minutes at 4°C in distilled water

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	Distilled water	Cuvette Gap	0.2 cm
Cell Density	10 (6) to 10 (7) cells / ml	Voltage	2.5 kV
Volume of Cells	40 µl	Field Strength	12.5 kV/cm
DNA Concentration	100 ng	Capacitor	25 µF
DNA Resuspension Buffer	TE buffer	Resistor	200 Ω (Pulse Controller)
Volume of DNA	2 µl	Time Constant	4.8 msec

After the Pulse

Outgrowth Medium	Liquid BCYE - α medium	Relevant Publications and/or Comments	Note: exponential values designated in parentheses. pRK290 described in: <i>PNAS</i> 77 : 7347-7351 (1980).
Outgrowth Temperature	37 °C		
Length of Incubation	6 hours		
Selection Method or Assay Used	kanamycin resistance (25 µg / ml)		
Electroporation Efficiency	7 x 10 (3) / ng DNA (<i>L. pneumophila</i>); 10/ng DNA (<i>L. longbeachae</i>)		
Per Cent Survival	not calculated		

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 10/15/90

Survey Number 051

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