

Cell Type Bacterial, gram negative

Molecules Electroporated DNA: pRK290 , about 20 kB, covalently closed, circular (see notes). Plasmid contains kanamycin resistance cartridge.

Species Used *Legionella pneumophila (philadelphia)*, *Legionella longbeachae***Before the Pulse**

Cell Growth Medium Harvested from BCYE agar

Growth Phase at Harvest O.D. (600) = not applicable, harvested directly from plate

Pre-pulse Incubation 5 to 10 minutes at 4°C in distilled water

Wash Solution Distilled water

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

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

DNA Resuspension Buffer TE buffer

Capacitor 25 µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.8 msec

Outgrowth Medium Liquid BCYE - a medium

Relevant Publications and/or Comments**Note:** exponential values designated in parentheses.pRK290 described in: *PNAS* 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 (*L. pneumophila*); 10/ng DNA (*L. longbeachae*)

Per Cent Survival not calculated

Name of Submitter Dr. Michael W. Heuzenroeder

Institution Address Institute of Medical and Veterinary Science
Clinical Microbiology
P. O. Box 14 Rundle Mall,
Adelaide 5000, South Australia

Survey Number

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