BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *E. coli; Legionella pneumophila,* strain Nottingham N -7

Molecules Electroporated DNA: pUC 19, about 2.7 kB; pLP116 (a pUC 19 derivative), about 2.8 kB

Before the Pulse

Cell growth medium Buffered charcoal yeast extract (BCYE- α) agar supplemented with L-cysteine

Wash solution Phosphate buffered saline (PBS)

Growth phase at harvest O.D. (600) =18 hr. growth on BCYE-α agar

Pre-pulse incubation on ice for 30 min.

The Pulse

Electroporation Temperature 0 °C

Electroporation Medium Electroporation buffer described by Dower: see notes

Cell Density 10 (9) cells / ml

Volume of Cells 800 µl

DNA Concentration 5µg/ml

DNA Resuspension Buffer TE buffer (40 mM Tris, 2 mM EDTA (disodium), pH 7.9

Volume of DNA 2 to 10 µl

Instruments Used Gene Pulser® apparatus

Cuvette Gap 0.4 cm

Voltage 2.5 kV

Field Strength 6.25 kV/cm

Capacitor 25 µF

Resistor Pulse Controller not used**. NOT RECOMMENDED (see notes).

Time Constant 4.5 to 4.8 msec

After the Pulse

Outgrowth Medium BCYE - α, supplemented with L- cysteine agar

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method or Assay Used 50 µg / ml ampicillin

Electroporation Efficiency 10 (6) transformants / µg DNA

Per Cent Survival 60 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses. Electroporation medium is buffer described by Dower: 270 mM sucrose, 1 mM MgCl2, 7 mM NaPO4, pH 7.4, filter-sterilized.

Ref: American Society for Microbiology Annual Meeting, Dallas, Texas, 1991, Abstract H-9.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Ref: Dower,W.J., Miller,J.F. and Ragsdale,C. 1988. *NAR* **16**(13):6127-6145.

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