BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Lactobacillus plantarum,* strain MGD 286

Molecules Electroporated DNA: pGK12, covalently closed circular form, 4.3 kB; pNZ12, covalently closed circular form, 4.3 kB

Before the Pulse

Cell growth medium MRS (Difco) + 1% D,L - threonine

Growth phase at harvest O.D. (600) =0.5 to 1.0 (average = 0.7)

Pre-pulse incubation None

Wash solution Distilled deionized water, room temperature

The Pulse

Electroporation Temperature 23°C (room temperature)

Electroporation Medium 30% PEG 1000 (Dow, Sigma, filter sterilized)

Cell Density 10 (9) cells / ml

Volume of Cells 100 μl

DNA Concentration ?

DNA Resuspension Buffer TE (10 mM Tris, 1 mM EDTA, pH 8.0)

Volume of DNA 1.5 μl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 1.5 kV

Field Strength 7.5 kV/cm

Capacitor 25 μF

Resistor (Pulse Controller) 400 Ω

Time Constant 3.8 to 4.2 msec

After the Pulse

Outgrowth Medium MRS + 1% D,L - threonine

Outgrowth Temperature 37 °C

Length of Incubation 1 hour

Selection Method or Assay Used pGK12: 2 μg/ml erythromycin + lincomycin, then chloram.(10 μg/ml)
pNZ12: chloramphenicol (10 μg/ml)

Electroporation Efficiency pGK12: 2x10 (3) / μg DNA
pNZ12: 6.5x10 (2) / μg DNA

Per Cent Survival 25 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Strain MGD286: Hill, H.A. & Hill,J.E. 1986. *Current Microbiol.* **13**:91-94.

Method a modification of: Josson K. *et al.* 1989.*Plasmid* **21**:9-20.

Plasmid pGK12: Kok, J., *et al.* 1984. *Appl. Environ. Microbiol.* **48**: 726-731.

Plasmid pNZ12: De Vos,W. 1986. Biomolecular Engineering in the European Community (Magnien, E., ed.) pp.465-471. Martinus Nijoff, Dordrecht.

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