BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram positive

Species Used *Enterococcus faecalis* JH2-2 and UV202

Molecules Electroporated DNA: pLAR33 (an 18kB ds DNA plasmid), pAM401 (an 10.4 kB ds DNA plasmid)

Before the Pulse

Cell growth medium Brain Heart Infusion (BHI) broth (Difco)

Growth phase at harvest O.D. (600) =1.0 (exponential)

Pre-pulse incubation Buffer, cuvettes, and cells placed on ice

Wash solution 1.5x EP (see notes)

The Pulse

Electroporation Temperature 25 °C

Electroporation Medium 1.5x EP

Cell Density Cells concentrated 140x

Volume of Cells 50 µl

DNA Concentration 1 ng DNA

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

Volume of DNA 2 µl

Instruments Used Gene Pulser® apparatus
Pulse Controller

Cuvette Gap 0.2 cm

Voltage 2.5 kV

Field Strength 12.5 kV/cm

Capacitor 25 µF

Resistor 200 Ω (Pulse Controller)

Time Constant 5.6 to 5.8 msec

After the Pulse

Outgrowth Medium BHI -Difco

Outgrowth Temperature 37 °C

Length of Incubation 3 hours

Selection Method or Assay Used BHI agar plates with 10 µg / ml erythromycin

Electroporation Efficiency 2 x 10 (3) transformants / ng DNA

Per Cent Survival Not determined

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Wash solution: EP=1mM HEPES (pH7.4),1mM MgCl2, 0.5M sucrose

Name of Submittor

Institution Address

Telephone Number

Fax Number

Date Submitted 8/21/90

Survey Number 060