BIO-RAD

Gene Pulser® Electroprotocols

Cell Type Bacterial, gram negative

Species Used *Bradyrhizobium japonicum; E. coli,* species unspecified

Molecules Electroporated DNA: various plasmids

Before the Pulse

Cell growth medium For *B. japonicum* , see reprint; for *E. coli* , standard protocol, see Pulse Controller or E. coli Pulser™ Manual.

Wash solution Sterile distilled water

Growth phase at harvest O.D. (600) =varies - usually exponentially growing

Pre-pulse incubation 5 min on ice

The Pulse

Electroporation Temperature See notes

Electroporation Medium 10 % glycerol

Cell Density 10 (9) to 10 (10) cfu / ml

Volume of Cells 40 µl

DNA Concentration Varies: 12 ng / ml to 4 µg / ml

DNA Resuspension Buffer Distilled water

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 Not given

Resistor 200 Ω (Pulse Controller)

Time Constant 5 msec

After the Pulse

Outgrowth Medium For *B. japonicum*, see reprint: YEGG

Outgrowth Temperature 30 °C

Length of Incubation 20 hr

Selection Method or Assay Used Various drug resistances

Electroporation Efficiency 1.8 x 10 (5) (*B. japonicum*) transformants / µg DNA

Per Cent Survival 20 to 95 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Electroporation temperature: cells and cuvette are on ice - then pulsed

Publications: Gerinot, M.L., Morisseau, B.A. & T. Klapatch. 1990. Electroporation of *Bradyrhizobium japonicum Mol. Gen. Genet.* **221**:287

The info on this sheet is for *B. japonicum*. See reprint. For *E. coli* we just use standard conditions (see Pulse Controller or E. coli Pulser™ Manual).

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