



Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative	Molecules Electroporated	DNA: various plasmids
Species Used	<i>Bradyrhizobium japonicum</i> ; <i>E. coli</i> , species unspecified		

Before the Pulse

Cell growth medium	For <i>B. japonicum</i> , see reprint; for <i>E. coli</i> , standard protocol, see Pulse Controller or <i>E. coli</i> Pulser™ Manual.	Growth phase at harvest	O.D. (600) =varies - usually exponentially growing
Wash solution	Sterile distilled water	Pre-pulse incubation	5 min on ice

The Pulse

Electroporation Temperature	See notes	Instruments Used	Gene Pulser® apparatus Pulse Controller
Electroporation Medium	10 % glycerol	Cuvette Gap	0.2 cm
Cell Density	10 ⁽⁹⁾ to 10 ⁽¹⁰⁾ cfu / ml	Voltage	2.5 kV
Volume of Cells	40 µl	Field Strength	12.5 kV/cm
DNA Concentration	Varies: 12 ng / ml to 4 µg / ml	Capacitor	Not given
DNA Resuspension Buffer	Distilled water	Resistor	200 Ω (Pulse Controller)
Volume of DNA	2 µl	Time Constant	5 msec

After the Pulse

Outgrowth Medium	For <i>B. japonicum</i> , see reprint: YEGG	Relevant Publications and/or Comments	Note: exponential values designated in parentheses.
Outgrowth Temperature	30 °C		Electroporation temperature: cells and cuvette are on ice - then pulsed
Length of Incubation	20 hr		Publications: Gerinot, M.L., Morisseau, B.A. & T. Klapatch. 1990. Electroporation of <i>Bradyrhizobium japonicum</i> <i>Mol. Gen. Genet.</i> 221 :287
Selection Method or Assay Used	Various drug resistances		The info on this sheet is for <i>B. japonicum</i> . See reprint. For <i>E. coli</i> we just use standard conditions (see Pulse Controller or <i>E. coli</i> Pulser™ Manual).
Electroporation Efficiency	1.8 x 10 ⁽⁵⁾ (<i>B. japonicum</i>) transformants / µg DNA		
Per Cent Survival	20 to 95 %		

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 3/21/91

Survey Number 048

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