BIO-RAD

Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram negative
Species Used	*E. coli*, DH5α
Molecules Electroporated	DNA: plasmid from 2kb to 30 kb from a ligation mix, sometimes supercoiled.

Before the Pulse

Cell growth medium	LB
Growth phase at harvest	O.D. (600) = between 0.4 and 0.6
Pre-pulse incubation	30 seconds to 1 minute
Wash solution	double distilled water (ice cold) and (0°C, ice cold) glycerol

The Pulse

Electroporation Temperature	0 °C
Electroporation Medium	10% glycerol/water
Cell Density	Not given
Volume of Cells	40 μl
DNA Concentration	See notes
DNA Resuspension Buffer	Not given
Volume of DNA	from 0.5 μl to 2 μl
Instruments Used	Gene Pulser® apparatus and 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	Not given

After the Pulse

Outgrowth Medium	SOC
Outgrowth Temperature	37 °C
Length of Incubation	20 min. to 1 hour
Selection Method or Assay Used	Have used carpenicillin, kanamycin, tetracycline, Xgal / IPTG (depending on plasmid)
Electroporation Efficiency	10 (9) transformants / μg DNA
Per Cent Survival	Not given

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

DNA concentration: Depends on ligation-usually straight out of gel slice (low melt) ligation.
SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl2, 10 mM MgSO4,
20 mM glucose.
LB: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

Name of Submittor

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Date Submitted 3/4/91

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