BIO-RAD

Gene Pulser® Electroprotocols

Cell Type	Bacterial, gram positive
Species Used	*Lactobacillus reuteri,* DSM 20016
Molecules Electroporated	DNA: *L. reuteri* erythromycin resistaance plasmid, pLUL631 (10.2kB, covalently closed circular form) and derivatives.

Before the Pulse

Cell growth medium	*Lactobacillus* Carrying Medium (LCM) + 40 mM glucose
Wash solution	Distilled water
Growth phase at harvest	O.D. (600) = 0.5 to 1.0 (mid-log)
Pre-pulse incubation	Ice, 1 to 3 min

The Pulse

Electroporation Temperature	0 °C
Electroporation Medium	30% PEG 1500 /distilled water
Cell Density	Concentrated culture ~100X: 10 (10) to10 (11) cells / ml
Volume of Cells	100 μl
DNA Concentration	0.001 to 5 μg
DNA Resuspension Buffer	TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) or water
Volume of DNA	< 10μl
Instruments Used	Gene Pulser® apparatus
Cuvette Gap	0.2 cm
Voltage	2.0 to 2.5 kV
Field Strength	10.0 to 12.5 kV/cm
Capacitor	25 μF
Resistor	(Pulse Controller) 200 Ω
Time Constant	0.5 msec

After the Pulse

Outgrowth Medium	LCM + 40 mM glucose
Outgrowth Temperature	37 °C
Length of Incubation	1.5 hrs
Selection Method or Assay Used	erythromycin or chloramphenicol resistance, 10 μg / ml
Electroporation Efficiency	10 (7) to 10 (8) transformants / μg DNA
Per Cent Survival	Not known

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

pLUL631: Axelsson, L.T., *et al.* 1988. *Plasmid* , **20**:171-174.

Electroporation: Axelsson,L.T. & Ahrne, S.E.I. 1990. Transformation of *Lactobacillus reuterii* with electroporation:studies on the erthromycin resistance plasmid pLUL631. *In*: Axelsson,L. *Lactobacillus reuteri*, a member of the gut bacterial flora. Dissertation. Report 44. Dept. Microbiology, Swedish Univ., Uppsala, Sweden

Ahrne,S.,& Axelsson,L. 1990. FEMS Microbiology. Rev. Abstract-A13, **87**: 12.

Name of Submittor

Institution Address

Telephone Number

Fax Number

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