



Gene Pulser® Electroprotocols

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|---------------------|---|---------------------------------|---|
| Cell Type | Bacterial, gram positive | Molecules Electroporated | DNA: plasmid pCW1 (a shuttle vector between <i>E. coli</i> and <i>Coryneform</i> bacterium) |
| Species Used | <i>Corynebacterium glutamicum</i> ATCC 13032, <i>Brevibacterium flavum</i> ATCC 21475 | | |

Before the Pulse

| | | | |
|---------------------------|------------------------------------|--------------------------------|-------------------|
| Cell growth medium | ABG + 0.5% Tween 80 + 2.5% glycine | Growth phase at harvest | O.D. (600) = 0.25 |
| Wash solution | 15% glycerol | Pre-pulse incubation | ice |

The Pulse

| | | | |
|------------------------------------|------------------------------------|-------------------------|--|
| Electroporation Temperature | 4°C | Instruments Used | Gene Pulser® apparatus Pulse Controller |
| Electroporation Medium | 15% cold glycerol | Cuvette Gap | 0.2 cm |
| Cell Density | 5 x 10 ⁽¹⁰⁾ / ml | Voltage | 2.5 kV |
| Volume of Cells | 50 to 60 µl | Field Strength | 12.5 kV/cm |
| DNA Concentration | 0.4 µg / µl | Capacitor | 25 µF |
| DNA Resuspension Buffer | TE (10 mM Tris, 1 mM EDTA, pH 8.0) | Resistor | 200 Ω (Pulse Controller) |
| Volume of DNA | 1 µl | Time Constant | 4.6 to 4.9 msec |

After the Pulse

Outgrowth Medium SMMC buffer

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

| | |
|---------------------------------------|-------------------------------|
| Outgrowth Temperature | 30 °C |
| Length of Incubation | 12 hr |
| Selection Method or Assay Used | Neomycin, 10 µg / ml |
| Electroporation Efficiency | 10 (5) transformants / µg DNA |
| Per Cent Survival | 15 % |

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 12/4/90

Survey Number 059

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