



Gene Pulser® Electroprotocols

Cell Type Plant, protoplasts
Species Used *Hedyotis corymbosa*

Molecules Electroported DNA: pROA93, 16.2 kB, circular.

Before the Pulse

| | | | |
|---------------------------|--|--------------------------------|---------------|
| Cell growth medium | Murashige & Skoog's medium + 2% sucrose + 2 mg /l, 2,4-D, 1 g/l casein hydrolysate (GIBCO/BRL) | Growth phase at harvest | Not given |
| Wash solution | Not given | Pre-pulse incubation | 10 min on ice |

The Pulse

| | | | |
|------------------------------------|-----------------------------------|-------------------------|---|
| Electroporation Temperature | 20 °C | Instruments Used | Gene Pulser® apparatus & Capacitance Extender |
| Electroporation Medium | Phosphate HEPES buffered saline | Cuvette Gap | 0.4 cm |
| Cell Density | 1 x 10 ⁽⁶⁾ protoplasts | Voltage | 0.2 to 0.4 kV |
| Volume of Cells | 1 µl | Field Strength | 0.250 to 1.125 kV/cm |
| DNA Concentration | 40 µg/ml | Capacitor | 10 to 960 µF |
| DNA Resuspension Buffer | Not given | Resistor | (Pulse Controller) none Ω |
| Volume of DNA | 5 to 10 µl | Time Constant | Not given |

After the Pulse

| | | | |
|---------------------------------------|---|--|--|
| Outgrowth Medium | MS medium + 6% glucose + 2 µg/l 2,4-D + 0.1% casein hydrolysate | Relevant Publications and/or Comments | Note: exponential values designated in parentheses. |
| Outgrowth Temperature | 25 °C | | |
| Length of Incubation | 2 days | | |
| Selection Method or Assay Used | CAT | | |
| Electroporation Efficiency | Good | | |
| Per Cent Survival | Not given | | |

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 2/22/91

Survey Number 182

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