

Cell Type Bacterial, gram negative

Molecules DNA: cosmid pRK311, 22 kB, CsCl
Electroporated gradient purified DNASpecies Used *Acetobacter xylinum*, ATCC 23769**Before the Pulse**Cell Growth Medium Schramm & Hestrin medium:
glucose 20 g/l; peptone 5g/l; yeast
extract 5g/l; Na₂HPO₄ 2.7g/l; citric
acid 1.15g/l; pH 6.2

Growth Phase at Harvest O.D.(660) = 0.47

Pre-pulse Incubation 1 min.

Wash Solution Cold water, then 10% glycerol

The Pulse

Instruments Used Gene Pulser® apparatus

Electroporation Temperature 4 °C

Electroporation Medium 10% glycerol

Cuvette Gap 0.1 cm

Cell Density 4 x 10⁹ cells / ml

Voltage 1.8 kV

Volume of Cells 40 µl

Field Strength 18 kV/cm

DNA Concentration 125 µg / ml

DNA Resuspension Buffer 10 mM Tris, pH 8.0, 1mM EDTA

Capacitor 25µF

Volume of DNA 2 µl

Resistor 200 Ω (Pulse Controller)

After the Pulse

Time Constant 4.0 msec

Outgrowth Medium Schramm and Hestrin medium

Relevant Publications and/or Comments**Note:** exponential values designated in parentheses.

Outgrowth Temperature 28 °C

Length of Incubation 3 hr.

Selection Method or Assay Used tetracycline resistance

Electroporation Efficiency 1600 transfectants / µg DNA

Per Cent Survival 100%

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Survey Number

001