



## Gene Pulser® Electroprotocols

**Cell Type** Bacterial, gram negative

**Species Used** *E. coli*, MC1061, modified to contain F' (host for M13)

**Molecules Electroported** DNA: M13 recombinant RF (double stranded) and single-stranded DNA

### Before the Pulse

**Cell growth medium** LB

**Growth phase at harvest** O.D. (600) = 0.4 - 0.6

**Pre-pulse incubation** none; done as fast as possible

**Wash solution** 10% glycerol, 4 washes at 4°C

### The Pulse

**Electroporation Temperature** 0 to 4 °C

**Electroporation Medium** 10% glycerol

**Cell Density** As high as possible: just vortexed drained pellet.

**Volume of Cells** 40 µl

**DNA Concentration** 7.5 µg/µl

**DNA Resuspension Buffer** 10% glycerol

**Volume of DNA** 5 µl + 40µl cells

**Instruments Used** Gene Pulser® apparatus  
Pulse Controller

**Cuvette Gap** 0.2 cm

**Voltage** 2.450 kV

**Field Strength** 12.2 kV/cm

**Capacitor** 25 µF

**Resistor** 400 Ω (Pulse Controller)

**Time Constant** 9.1 to 9.6 msec

### After the Pulse

**Outgrowth Medium** SOC

**Outgrowth Temperature** 25 °C

**Length of Incubation** 1 to 10 min., plated immediately

**Selection Method or Assay Used** M13 plaques, no drugs

**Electroporation Efficiency** 1-5x10<sup>(10)</sup> (RF); 1-50x10<sup>(8)</sup> (single-stranded) transformants / µg DNA

**Per Cent Survival** Not measured

#### Relevant Publications and/or Comments

**Note:** exponential values designated in parentheses.

Transformation efficiency for M13 is about 3x as high as pUC plasmid.

**SOC:** 2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>, 20 mM glucose.

**LB:** 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

**Name of Submitter**  
**Institution Address**

**Telephone Number**

**Fax Number**

**Date Submitted** 10/16/90

**Survey Number** 036

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