



## Gene Pulser® Electroprotocols

**Cell Type** Fungal / Yeast  
**Species Used** *Saccharomyces cerevisiae* - lines derived from S288C

**Molecules Electroporated** DNA: pUC19 and Bluescript™ -based plasmids.

### Before the Pulse

**Cell growth medium** YEPD (ATCC#1202/1245)

**Growth phase at harvest** 0.5 to 1.0 at O.D.600; ~100 mls log phase culture concentrated 200x, in

**Pre-pulse incubation** None required

**Wash solution** Water

### The Pulse

**Electroporation Temperature** Room temperature

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

**Electroporation Medium** Water

**Cell Density**  $6 \times 10^{10}$  cells / ml

**Cuvette Gap** 0.2 cm

**Volume of Cells** 40 to 50  $\mu$ l

**Voltage** 0.6 kV

**DNA Concentration** Total DNA = 0.2 to 1.0  $\mu$ g

**Field Strength** 3.0 kV/cm

**DNA Resuspension Buffer** Not given

**Capacitor** 25  $\mu$ F

**Volume of DNA**  $\leq 5 \mu$ l

**Resistor** (Pulse Controller) 200  $\Omega$

**Time Constant** Not given

### After the Pulse

**Outgrowth Medium** Add water to final vol. 150-200  $\mu$ l. Plate on selective synthetic media (SD)

#### Relevant Publications and/or Comments

**Note:** exponential values designated in parentheses. Washed and concentrated cells can be stored by adding 80% glycerol to a final concentration of 20% and freezing at -80°C. To electroporate thawed cells: pellet cells, remove glycerol containing supernatant, wash cells in 0.5-1.0 ml water, and resuspend cells in water to original volume. Efficiency is not affected significantly by freezing but you must remove glycerol.

**Outgrowth Temperature** 30 °C

**Length of Incubation** 2 days

**Selection Method or Assay Used** Amino acid or nucleotide dropout

**Electroporation Efficiency** 2 to 10,000 transfectants /  $\mu$ g DNA

**Comment:** removing glycerol after thawing cells may help by simply removing lysed cell contents that would alter media conductivity - this could impact efficiency (alters the time constant, t).

**Per Cent Survival** Not given

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

**Telephone Number**

**Fax Number**

**Date Submitted** 9/14/90

**Survey Number** 179

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