**Gene Pulser® Electroprotocol**

**Cell Type**  
Fungal / Yeast

**Species Used**  
Pichia pastoris GTS115

**Molecules Electroporated**  
DNA: plasmid, pHILD2 & D4, linearized, 8 to 10 kB.

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### Before the Pulse

**Cell Growth Medium**  
Yeast Extract Potato Dextrose, YEPD, (DIFCO)

**Growth Phase at Harvest**  
O.D. (600) = 1.3

**Pre-pulse Incubation**  
5 minutes, 4 °C

**Wash Solution**  
Cold water two times; then 1 M sorbitol, one time

### The Pulse

**Electroporation Temperature**  
25 °C but sample & cuvette at 4 °C

**Electroporation Medium**  
1 M sorbitol

**Cell Density**  
300x concentration from harvest density

**Volume of Cells**  
50 μl

**DNA Concentration**  
0.5 to 2 μg / pulse

**DNA Resuspension Buffer**  
1 M sorbitol

**Volume of DNA**  
1 to 5 μl

**Cuvette Gap**  
0.2 cm

**Voltage**  
1.5 kV

**Field Strength**  
7.5 kV/cm

**Capacitor**  
25 μF

**Resistor**  
(Pulse Controller) 400 Ω

**Time Constant**  
approximately 8.0 msec

**Outgrowth Temperature**  
30 °C

**Length of Incubation**  
3 to 5 days

**Selection Method or Assay Used**  
Complimentation of histidine auxotrophy

**Electroporation Efficiency**  
approx. 1000 transformants / μg DNA

**Per Cent Survival**  
Not tested

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205