



# Gene Pulser® Electroprotocols

\* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

|                  |                                  |                       |  |
|------------------|----------------------------------|-----------------------|--|
| <b>Cell Type</b> | Mammalian, adherent              | <b>Molecules</b>      | DNA: varies, fibronectin $\beta$ -gal, |
| <b>Species</b>   | Human, fibroblast; Human Hep3b2, | <b>Electroporated</b> | genomic DNA, CMUB, etc.                |
| <b>Used</b>      | hepatocyte; Mouse, L-cells.      |                       |  |

## Before the Pulse

|                           |   |                                |           |
|---------------------------|---|--------------------------------|-----------|
| <b>Cell growth medium</b> | MEM + 10% Fetal Calf Serum (GIBCO/BRL, Sigma) | <b>Growth phase at harvest</b> | Log       |
| <b>Wash solution</b>      | Phosphate Buffered Saline and Trypsin         | <b>Pre-pulse incubation</b>    | 5 minutes |

## The Pulse

|                                    |   |                         |   |
|------------------------------------|---|-------------------------|---|
| <b>Electroporation Temperature</b> | 25 °C                                   | <b>Instruments Used</b> | Gene Pulser® apparatus & Capacitance Extender |
| <b>Electroporation Medium*</b>     | MEM                                     | <b>Cuvette Gap</b>      | 0.4 cm  |
| <b>Cell Density</b>                | 1 to 10 x 10 <sup>6</sup> cells / pulse | <b>Voltage</b>          | 0.320 kV                                      |
| <b>Volume of Cells</b>             | 500 $\mu$ l                             | <b>Field Strength</b>   | 0.8 kV/cm                                     |
| <b>DNA Concentration</b>           | 40 to 100 $\mu$ g DNA per pulse         | <b>Capacitor</b>        | 500 $\mu$ F                                   |
| <b>DNA Resuspension Buffer</b>     | TE (10 mM Tris, 1 mM EDTA, pH 8.0)      | <b>Resistor</b>         | (Pulse Controller) $\Omega$ none              |
| <b>Volume of DNA</b>               | 20 to 40 $\mu$ l                        | <b>Time Constant</b>    | Not given                                     |

## After the Pulse

|                                       |                     |  |   |
|---------------------------------------|---------------------|--|---|
| <b>Outgrowth Medium</b>               | MEM                 | <b>Relevant Publications and/or Comments</b> |   |
| <b>Outgrowth Temperature</b>          | 37 °C               | <b>Note:</b>                                 | exponential values designated in parentheses. |
| <b>Length of Incubation</b>           | 48 hours to 1 month |  |   |
| <b>Selection Method or Assay Used</b> | GPT and Neomycin    |  |   |
| <b>Electroporation Efficiency</b>     | 60%                 |  |   |
| <b>Per Cent Survival</b>              | 50 %                |  |   |

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