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.**

Cell type: Mouse embryonic stem cells

Strain: 129/OLA, subclone E14

Molecules: Linearized and circular DNA

Before the Pulse

Cell growth medium: Complete GMEM consists of 1x GMEM medium (Sigma, #G5154) supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 50 µM -mercaptoethanol, 1x nonessential amino acids, 10% (v/v) fetal bovine serum, and 500–1,000 U/ml of leukocyte inhibitory factor (LIF; Chemicon).

Wash solution: PBS

Growth phase at harvest: 90% confluent

Pre-pulse incubation: None

The Pulse

Electroporation temperature: Ambient

Electroporation medium*: PBS

Cell density: 4×10^6 to 6×10^7 cells

Volume of cells: 0.7 ml

DNA: 5.0–125 µg

DNA resuspension buffer: PBS

Volume of DNA: 100 µl

Instruments used: Gene Pulser Xcell with CE module

Cuvette gap: 0.4 cm

Voltage: 795 V

Field strength: —

Capacitance: 10 µF

Resistance: Infinity

Time constant: 0.2 ms

Relevant comments: electroporation carried out in Time Constant mode using settings of 800 V, 0.2 ms time constant, and 4 mm cuvette.

After the Pulse

Cells are allowed to recover for at least 20 min at ambient temperature, diluted in complete GMEM, and plated out at a density of 2–3 x 10^6 cells per 10 cm dish.

Selection used: G418

Electroporation efficiency: Not determined

Percent survival: About 50%

Protocol Number: 207

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