

## **3T3 Transfection using ElectroSquarePorator ECM 830**

### Materials:

1.  $1 \times 10^6$  3T3 cells. (Roughly  $\frac{1}{4}$  of a 80-90% confluent 10cm TC dish of 3T3 cells.)
2. 20ug sterile plasmid DNA per transfection (preferably in nuclease free H<sub>2</sub>O)
3. 4mm gap cuvettes
4. trypsin
5. Growth media (DMEM + 10% FBS + P/S)

### Methods:

#### Done in the Sterile TC Hood:

1. Trypsinize an 80-90% confluent 10cm plate of 3T3 cells. Centrifuge to pellet the cells and remove supernatant. Fully resuspend pellet in 2mL of warm growth media.
2. Add 0.5mL of cell suspension ( $1 \times 10^6$ ) to a 4mm gap cuvette. \*\*\* $1 \times 10^6$  cells was found to be approx.  $\frac{1}{4}$  of a 80-90% confluent 10cm plate. Count cells to be sure\*\*\*
3. Add 20ug of plasmid DNA to the cell suspension in the cuvette. Mix by swirling the pipette tip or carefully pipetting up and down. \*\*\*Pipetting up and down can introduce too many air bubbles into the solution and cause arcing during electroporation. In addition, vigorous pipetting can shear DNA.\*\*\*
4. Cap the cuvettes carefully and carry the samples to the molecular room.

#### Done in the Molecular Room:

5. Turn on the electroporator and set to:

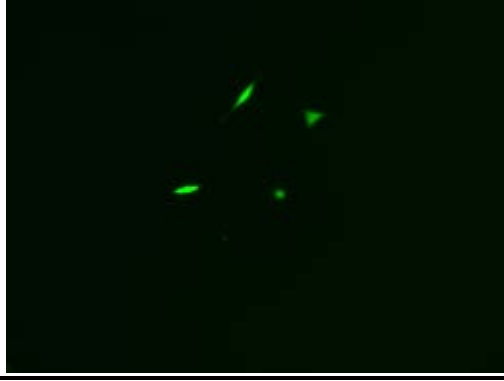
Volts:	600
Pulse length:	500usec
# of pulses:	8
Pulse interval:	500msec
6. Place the cuvette in the safety holder, close the lid, and press the start button.
7. Spray the cuvette and any racks with 70% EtOH before bringing them back to the TC room.

#### Done in the Sterile TC Hood:

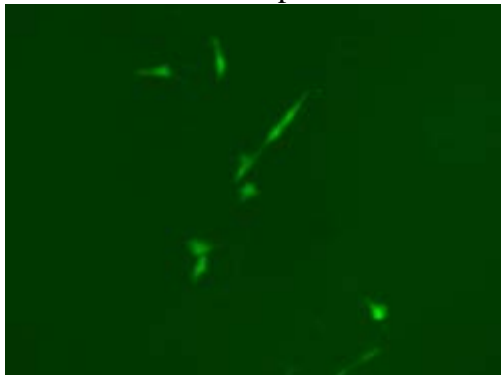
8. Allow the cell suspension to sit undisturbed in the cuvette for 8-10 min.
9. Add 1mL warm growth media to help bring the cells out of the cuvette.
10. Plate the cells from the cuvette on a 10cm (T75 flask) TC dish containing 15mL warm growth media.
11. Plasmid translation will begin in 24 hours, but seems to reach its peak 48-72 hours after transfection. At this point, selective media can be used to isolate stable clones.

All pictures were taken of the same plate at the same magnification (10x).

24 hours after electroporation



48 hours after electroporation



72 hours after electroporation

