# Electroporation of ES Cells

**Materials & Reagents required:**

- Electroporator and 0.4 cm cuvette
- EmbryoMax ES Cell Qualified Electroporation Buffer (Cat. No. ES-003-D)
- ES Cell Medium:
    - DMEM (Cat. No. SLM-220-B)
    - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
    - 1% Nucleosides,100x (Cat. No. ES-008-D)
    - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
    - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
    - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
    - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
    - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Ice
- Incubator, 37 °C/5% $CO_2$
- 25–40 µg Linearized construct DNA, ethanol precipitated and dried
- PMEF Feeder cell coated plates
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)

**Procedure:**

1. The evening before the electroporation is to be performed, prepare 4 plates with PMEF cells.
2. The morning that the electroporation is to be performed feed the ES cells fresh ES Cell Medium.
3. Later that afternoon, harvest the ES cells as described previously, and determine the cell count. $1x10^7$ ES cells is the minimum number of ES cells required for electroporation. If there is excess, freeze the cells down as previously described.
4. Centrifuge the cells required for electroporation at 300 xg for 10 minutes, and then aspirate the medium.
5. Resuspend the ES cell pellet in 600 µL of Electroporation Buffer.

6. 25–40 µg of knockout construct DNA (purified) should already be linearized, ethanol precipitated and dried as a pellet. In a sterile hood, dissolve the DNA pellet in 30 µL of Electroporation Buffer, and then add the solution to the ES cells. Mix well and leave for 5 minutes at room temperature.
7. Place the ES cells in a 0.4 cm electroporation cuvette. Electroporate the suspension at 500 µFD, 0.24 kV. The time constant produced should be between 6.9 and 7.9 milliseconds (optimal 7.2). Following electroporation, place the cuvette on ice for 10 minutes.
8. Transfer the electroporated ES cells to 40 mL of ES Cell Medium and mix gently using a Pasteur pipette.
9. Plate the ES cell suspension (10 mL per feeder plate, total of 4 plates). Ensure that the PMEF Feeder Cell Medium is removed prior to the addition of cells.
10. Incubate for approximately 36 hours at 37 °C and 5% $CO_2$ prior to antibiotic selection.