Schwer Lab

ES cell targeting using the Bio-Rad Gene Pulser II

Modified from "Introduction of Foreign DNA into Embryonic Stem Cells," Chapter 10, in *Manipulating the Mouse Embryo*, 3rd edition, by Andras Nagy, Marina Gertsenstein, Kristina Vintersten, and Richard Behringer. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2003.

PROCEDURE

1. Begin with an exponentially growing culture of early-passage ES cells. Change the medium ~3 hours (2-4 h) before harvesting the cells.

1-2 days before electroporation: need to prepare sufficient tissue culture dishes, gelatinized (10-cm), containing either G418-resistant iSNLP or MEF feeder cells.

Rinse plated feeders in 10-cm dishes with 10 mL PBS and change to KO-M15 medium about 1 h before electroporation of ES cells.

2. Use 10 mL of pre-warmed PBS (37°C) to rinse ES cells. Trypsinize the cells (2 mL 0.1% Trypsin/Chicken serum for 5 min at 37°C), add 2 mL KO-M15. Pipette cells up and down to produce a single-cell suspension. Add 6 mL KO-M15 (total volume per dish = 10 mL). Combine ES cells from multiple dishes in 50-mL conical, if desired.

3. Centrifuge gently to collect the cells (200 × g for 3 minutes), wash once with 10-20 mL PBS (RT) or electroporation buffer (resuspend very gently), and resuspend as a single-cell suspension.

Count cells (ignore larger feeder cells, count only small ES cells). Adjust to a density of 25 Mio cells/ml in Ca^{++}/Mg^{++}-free PBS or electroporation buffer [use 20 Mio cells in 0.8 mL reaction].

4. For each electroporation, place 0.8 ml (=20 Mio cells) of ES cell suspension in a 0.4-cm-wide sterile cuvette. Avoid air bubbles. Tapping cuvette on bench helps remove air bubbles. Add 20 - 25 µg of linear DNA and mix well by using a P1000 tip. Do not introduce air bubbles. Let the mixture stand at room temperature for 5 minutes. *Not critical to chill the cells before or after electroporation.*

5. Apply a single pulse to the cells. Typical conditions are **230 V, 500 µF** (this requires the capacitance extender).

*Need to **connect shock chamber to lower outlet** (not to Pulse Controller Plus unit). If leaving the pulse controller plus unit connected, must set low range button to "to use high range" and high range button to infinity symbol (see photos at end).*

Perform test electroporation: add 0.8 mL PBS to a Gene Pulser Cuvette (Bio-Rad #165-2088; 0.4-cm electrode gap) and electroporate. Record time constant, actual volt, and sample resistance.

Press both red buttons to electroporate. Machine will flash "Ch 9" and will beep when electroporation is complete. *Hold both buttons until constant beep tone sounds.*

Time constant should read between 5.6 and 8.5 ms if 230 V, 500 µF is used. *See example photos at end of document for settings and outcomes of a typical test electroporation using 0.8 mL PBS.*

Approximately 50% cell death is to be expected with optimal transfection efficiency when PBS is used.

6. After applying the pulse, allow the cells to stand at room temperature in TC hood for 5 minutes (incubation on ice is not necessary). Add KO-M15 to cells in cuvette to gently recover cells. Rinse the cuvette to recover all of the cells.

7. Divide the 0.8-ml sample from the electroporation cuvette equally into two 10-cm tissue culture dishes with feeder cells (can plate on feeders for future selection and on gelatin for non-selective control plate).

8. In all cases, allow the cells to recover for ~24 hours in nonselective medium, i.e., KO-M15 without any added selection agents.

9. After 24 hours, re-feed one tissue culture dish with **nonselective medium**. *This dish serves as a control to assess the number of cells that survived electroporation.*

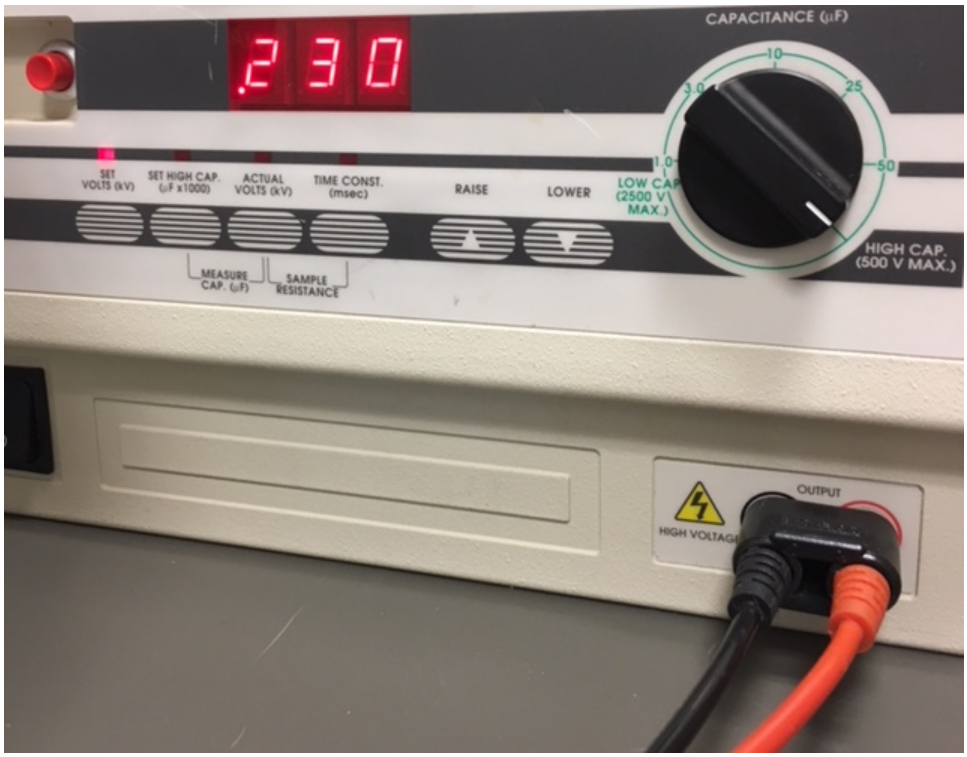
10. Add KO-M15 containing G418 (*200 μg/ml final concentration of the active ingredient based on kill curve test of current Geneticin lot*) to a second tissue culture dish to determine the fraction of cells transformed by integration of the neoR cassette.

Different ES cell lines are not equally sensitive to G418, and different G418 batches are not equally potent. Test the toxicity of the G418 beforehand to find the lowest dose giving 100% killing of non-transfected cells within 5 days.

11. Incubate the cells in culture for ~7-10 days, changing the medium every day.

~50-200 colonies should become visible in selection plate after ~10 days in culture.

Gene Pulser settings examples next pages:



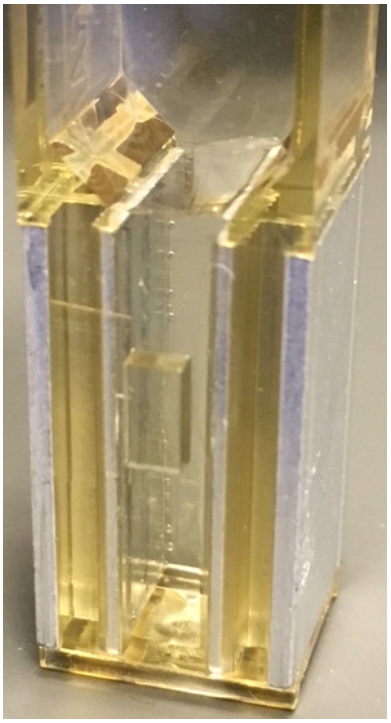
Connect output cables on **bottom output**. Set Capacitance dial to HIGH CAP.
Set Volts (kV) to .230 (=230 V).

Set HIGH CAP (μF x 1000) to .500 (=500 μF)

IF LEAVING THE Pulse Controller PLUS unit connected, MUST SET Low range button to "TO USE HIGH RANGE" and High range button to INFINITY SYMBOL.

Typical readings after test electroporation of 0.8 mL PBS:

actual Volts: 224 V. Time constant: 6.6 ms. Sample resistance: 20 ohms



Air bubbles on electrodes after test electroporation.