Electroporation of ES Cells

(Based on Bradley Lab Protocol Collection Electroporation)

DNA Preparation:

1. The DNA to be used for electroporation should be high-quality and clean DNA (Qiagen-Endo free Maxi-prep kit). For applications where linerized DNA is needed (targeting vectors), do a large-scale, overnight digest on your DNA.

2. Extract the large-scale digest once with an equal volume of phenol/chloroform and once with an equal volume of chloroform. Precipitate the DNA with ethanol, dry down, and resuspend in TE or water. It's handy if the DNA is at 1 µg/µl, but not necessary. Some people think that this step is optional, so if you're in a big hurry, just inactivate your enzyme and proceed.

Cell Preparation:

1. Embryonic stem cells (80% confluent for best results) should be fed 2-4 hours before harvesting.

2. Trypsinize cells and resuspend in 10ml ES cell medium. Take a 200µl aliquot to count, and spin the rest down for 7' at 1000rpm in the table-top centrifuge. The normal yield of cells is 3.0×10^7 cells/10 cm plate.

3. Resuspend the pellet in PBS, to a density of 1.1×10^7 cells/ml. Count a 20µl sample to confirm that you are within a reasonable amount of this number.

Electroporation:

1. Mix appropriate amounts of DNA and cells together in a 15 ml tube (25 µg of DNA and 0.9 ml of cells for each electroporation). Leave the tube at room temperature for 5' (this is optional).

2. Aliquot the mixture into electroporation cuvettes (4 mm gap). Try to avoid touching the metal plates. Place the cuvette in the electroporation holder with the electrodes in contact with the metal holding clips.

3. Set the BTX 830 electroporator to Low voltage mode, 250 V (Voltage), 1 ms (Pulse Length) and 1 pulse. (For the Biorad GenePulser: 230V, 500 µF (requires the capacitance extender) and press the two red buttons to electroporate. The machine will flash "Ch 9" and will beep when electroporation is complete, at which time you should release the buttons. The time constant should be between 5.6 and 8.0.)

4. Leave the cuvette at room temperature for 5 minutes and then plate the cells. If you will be doing drug selection, you can plate the entire reaction. If you are doing Cre electroporation to remove a selection cassette, and will not have any drug selection marker to use, then plate out 1200 and 600 cells on separate plates. Each cuvette to 2-4 10 cm plates.

5. Approximately 2/3 of the cells will die, leaving you with a manageable number for picking later.

6. Drug selection can begin 24 hours after electroporation. First two day, use 200 µg/ml of G418 (0.4 ml of 50 mg/ml active form G418 in 100 ml ES medium), and then 150 µg/ml G418 (0.3 ml G418 per 100 ml medium) and 10 mM Glanciclovir (10 µl to 100 ml of ES cell medium).

7. Colonies may be picked as early as 8 days, are best around 10-12 days, but may be recovered up to 18-21 days after the electroporation.