Electroporation of targeting vectors into ES cells

DNA Prep.

- Prepare CsCl-purified DNA for electroporation by linearizing the DNA, extracting once with phenol, then twice with chloroform followed by an ethanol precipitation. Wash the ethanol precipitate with 70% ethanol, then fill the tube containing DNA with fresh 70% ethanol to sterilize the contents for at least 2 hours.

- In a tissue culture hood, pour off the ethanol. Spin the DNA briefly, and in the hood, remove the last traces of ethanol. Allow the DNA to air dry by leaving the tube open in the tissue culture hood for 5-10 minutes. Dissolve the DNA in sterile water and add an equal volume of sterile 2X ZAP buffer for a final concentration of 1 µg/ml DNA.

Electroporation

- Trypsinize ES cells from 1 T75 flask (expect ~1-3X10^7), spin cells out and wash once in 1X ZAP buffer, resuspend in 0.8ml 1X ZAP

- Add 60 µg linearized DNA to ES cells in ZAP buffer and transfer the mixture to a Biorad or comparable electroporation cuvette (0.4 cm gap). Electroporate at 800 volts, 3µFarads.

- Let cells recover in the cuvette for 10 min. at room temperature.

- Mix electroporated cells with ES medium (no G418 or gancyclovir) and plate onto a 10 cm dish of feeders at a density of 2X10^6 cells per feeder dish. Do not use tissue culture flasks. Note, the area of a 10 cm dish is approximately that of a T75.

- The next day and on subsequent days, feed cells with drug-containing medium. During the 2-3 days after drug addition, there will be a lot of cell death. When changing the medium, wash the monolayer 1-2 times with HBSS to remove dead cells before adding new medium.

- 5-7 days after electroporation, colonies will be ready for picking into 24 well plates.