General instructions for single cuvette electroporation with DNA targeting vectors

1. Vector preparation
   Digest 50 μg vector DNA with an enzyme which linearizes the vector in the appropriate position.
   Take a small aliquot ($1/100^{th}$ is plenty) to assess completion of digest on a gel and ethanol precipitate the remainder (15 minutes on ice, 15 minutes in micro-centrifuge is more than sufficient to recover everything).
   Rinse pellet twice with 70% ethanol to remove excess salt, leaving the second 70% wash in the microfuge tube.
   Transfer tube of DNA to a functioning TC hood for all remaining processing (wipe outside of tube down with etOH first).
   Remove 70% ethanol from DNA pellet in the TC hood, and leave tube open to dry in hood (usually 25-40 minutes).
   Resuspend dry pellet in 100 μl sterile PBS, close tube and leave overnight in hood to resuspend.

2. Cell preparation
   Pass ES cells from a confluent T25 flask to a T150 48 hours prior to electroporation.
   Feed the following day.
   Feed the cells again the next day, 4 hours prior to electroporation.

3. Electroporation
   Trypsinize cells (10 min for feeder-free JM8, 15 min for feeder dependent) and dilute with normal growth medium.
   Spin cells down (3 minutes, 1.3K) and resuspend in 10 ml PBS.
   Count cells in haemocytometer and respin. Resuspend cell pellet thoroughly (usually about 2-5 x 10^7 cells) in 100 μl room temperature PBS.
   Pipette cell suspension into 1.7mL microfuge tube containing DNA, pipette up and down once to mix cells and DNA, and quickly transfer to .4mm electroporation cuvette (Biorad). Cover cuvette and *immediately* electroporate cells using BioRad Gene Pulser II (800V, 3.0μF, time constant should be approx. 0.04ms).
   Allow cells to recover 20 minutes at room temperature in the TC hood. Dilute cells in medium and plate on 10 cm tissue culture dishes (gelatinized or with feeders) in medium without selection drug. (Seed at least 2 replicate plates at the optimal seeding density; but seeding density will depend on the particular experiment and targeting efficiency so if you are unsure test a range).
   Change medium the following day to medium containing the selection drug of choice. Maintain cells under selection for about 10 days, changing the medium daily for at least the first 5-7 days, or until reasonable size colonies are visible.