Electroporation of ES cells

ES medium:

75 ml FCS: Hyclone Cat# SH30070.03E
405 ml DMEM: GIBCO 11965-084
5 ml Pen/Strep: GIBCO 15140-122
5 ml NEAA: GIBCO 11140-050
5 ml Sodium Pyruvate: Mediatech Cat# MT25-000-CI
5 ml L-Glutamin: GIBCO 25030-081
0.5 ml LIF: Chemicon ESG1107 (1:10 dilute, aliquot 0.5ml, keep at -20 C)
4ul 2-ME: Sigma M-7522

Electroporation:

1. Trypsinize ES cells. Wash 3x10cm dishes (approximately 2-5 x 10^7 cells) once with PBS and add 0.25% trypsin (2 ml/dish). 37 C 3-5 minutes . Add 2 ml/dish ES medium and pipette up and down vigorously. Aim for a single cell suspension. Spin down cells.
2. Wash pellet with PBS. Spin
3. Wash pellet with PBS. Spin
4. Resuspend pellet in 0.65 mls PBS, pipette up and down in tube containing 100mg DNA in 50ul PBS. Put total volume (0.8 mls) into electroporation cuvette.
5. Electroporate: 200V, 975µF,
6. Let cells sit at room temp for 5 minutes.
7. transfer cells into a 15ml tube with 5ml ES medium. Distribute onto 5x10cm dishes that are pre-seeded with neo-resistant MEF cells.
8. Feed cells everyday. After 24 hours add ES medium supplemented with 300µg/ml G418 (1.5 mls of a 100mg/ml stock for 500 mls medium. To make stock solubilize 1gram in 9.8 mls PBS or DMEM, add 180µl 10N NaOH, filter sterilize, store at -20°C).
9. After 48 hours add gancyclovir to medium. I use a 2 x 10^{-3}M stock (1000X). Store this stock at -20°C.
10. After 7-10 days colonies will be ready to pick.

Picking Colonies:

1. Wash dish with PBS. make a 4x4 grid on the bottom of the plate to help orient the dish under the microscope. Add 10ml PBS/dish.
2. Set P20 at 10µl, pick colony under microscope
3. Place each colony into a separate well of a 96 well dish. Using the grid of a pipette tip box with the grid of the dish makes it easy to keep track.
4. Continue until all 96 wells have a colony in them.
5. Add 50µl 0.25% trypsin-EDTA to each well using a multichannel pipettor.
6. Incubate 5 minutes at 37°C.
7. Add 50 µl ES medium and pipette up and down about 15 times to resuspend the cells as a single cell suspension. Try to minimize bubbles.
8. Transfer the entire cell suspension to a 96 well plate that has been pre-seeded with MEFs. Add 50 µl ES medium for a total volume of 150µl.

9. Feed daily. After 1 or 2 days the wells should be ready to passage. This will have to be a compromise as the cells may not all grow at the same rate.

Splitting cells:

1. Aspirate off medium. Wash each well with 100µl PBS. Add 50µl trypsin-EDTA. Incubate 5 minutes at 37°C.
2. Add 50 µl medium and resuspend the cells. Plate 50 µl onto a new feeder plate and leave the other 50 in the first dish (this will be for DNA). Add 100µl medium to each well. It is CRUCIAL to orient the two plates identically.
3. After one or two days the plate on feeders should be ready to freeze down.

Freezing cells in 96-well plates:

1. Aspirate off medium. Wash each well with 100µl PBS. Add 50µl trypsin-EDTA. Incubate 5 minutes at 37°C.
2. Add 50µl 2x freezing medium and resuspend the cells. 2x freezing medium is 20% DMSO, 60% FCS and 20% ES medium.
3. Add 60µl mineral oil to the top of the wells to prevent evaporation.
4. Wrap the edge of each plate with a piece of parafilm. Place into a well insulated styrofoam box, keep at -80°C.
5. Leave the remaining culture to grow to high density. It is often advisable to split these cells after 2 or 3 days to make a duplicate plate in case you wish to perform more than one Southern blot.

Thawing cells from 96-well plate:

In the late afternoon, place entire 96 well plate onto a floater in 37 C water bath for 3 minutes. Gently pipette lower (aqueous) phase of well into one well of a 24 well plate that has been pre-seeded with MEFs, avoiding oil. Next day , feed cells in early morning . It will take 2-3 days before colonies become apparent and 4-5 days before they will be ready to split.