ES CELL ELECTROPORATION

April 2015 Revision

Standard protocol is routinely used for most ES cells (including JM8, JM8.F6, JM8.N4, JM8A3, JM8A3.N1, JM8A1.N3, E14, R1 and Bruce 4 cells.)

For **feeder-independent** ES cells such as JM8N4, JM8A, etc; prepare 2-4 x 10 cm dishes by pre-gelatinizing and adding 10 ml ES medium to each, place in 37^{o}C incubator until they are required.

For **feeder-dependent** ES cells such as R1, JM8, JM8.F6, etc; plate G418 resistant feeders 1-2 days prior to electroporation. When plates are needed for the electroporation, replace feeder medium with 10 ml ES medium, and place in a 37^{o}C incubator until they are required.

Media formulations and suppliers of reagents for culturing KOMP distributed ES cell lines can be found on the Protocols page for JM8 cells and sublines on the KOMP website.

Electroporation

1. On the day of the electroporation, make sure the ES cell cultures look undifferentiated; plates should be 80% confluent for best results.
2. Change the medium on the ES cells 2-4 hours prior to electroporation. Feeding will increase the likelihood of obtaining homologous recombinants. This is because homologous recombination occurs at a higher rate in cells that are actively undergoing mitosis (i.e., still in their log phase of growth).
3. Wash ES cell dishes with PBS, trypsinize, add ES medium to inactivate the trypsin and pipette up and down to make a single cell suspension.
4. Gently centrifuge the cells at ~1000 rpm for 5 min.
5. Remove the supernatant and resuspend cells in ~1-2 ml cold PBS.
6. Determine the cell density using a haemocytometer and dilute with CA/Mg free PBS to 1 x 10^{7} cells in 0.9 ml PBS, the density necessary for a single electroporation (a single 10 cm dish of ES cells at approximately 80% confluency should contain 2 - 3 x 10^{7} cells.
7. For each electroporation, mix together the 0.9 ml of 1 x 10^{7} ES cell suspension and ~50 ul of DNA (the concentration can be variable; currently we're using ~20 ug per each electroporation.)
8. Transfer to a 0.4 cm electroporation cuvette and immediately electroporate as follows.
9. Place the cuvette in the electroporation holder and pulse the cells. For the BioRad Gene Pulser II (Richmond, CA), our settings are: 230 mVolts x 500 uF capacitance. The time constant will usually be in the range of 6.0 – 7.5.

10. After the electroporation, place the cuvette at room temperature for 15 – 20 minutes. Transfer the contents from one cuvette into one or two of the pre-warmed medium-containing dishes according to the number of colonies anticipated. Control 10 cm dishes or 6 well dishes can also be set up for no G418 treatment or no DNA included in the electroporation.
11. After approximately 24 hours, gently remove the medium in the cell dishes, and re-feed with selection medium or control medium as appropriate. Continue changing the medium daily for 7-10 days. In the case of neo selection, the medium is supplemented with ~150-200 ug/ml of G418 (we're currently using Geneticin, Invitrogen, #10131-035, 50 mg/ml at 200 ug/ml for selection.) It may be necessary to perform a "Kill Curve" to determine the exact drug concentration for each cell type and possibly each lot of G418.

    In the case of puromycin selection, we are currently using Invitrogen puromycin, in liquid form, A11138-02, 20 mg/ml which we aliquot and store frozen. For JM8N4 cells, e.g., we find that selection with 0.6 - 0.8 ug/ml of puromycin is sufficient and we usually begin selection on day 2 or day 3, after observing the outgrowth of the cells. This agent seems to kill the cells more rapidly than G418 and 5 – 7 days or less under selection may be sufficient. Again, it may be necessary to carry out a time course determination with each lot of puromycin or each type of cells. In particular, R1 cells seem to be more resistant to puromycin than JM8 cells and as much as 2 ug/ml may be required.

    For cells requiring feeders, it will be necessary to use feeders which are resistant to the selection agent, e.g., DR4 feeders may be used for puromycin and G418 selection experiments.
12. Continue the selection until colonies become apparent and grow to a size that is amenable to picking (usually takes 6-10 days.) Colonies may then be picked, trypsinized into individual 96 wells and allowed to grow until about 80% confluent before splitting for freezing, genotyping, etc. We routinely prepare at least 2 backup frozen plates in addition to a plate for genotyping; one plate will be used to retrieve and expand the positive clones and one is frozen as backup. Although they are expensive, Thermo Scientific #3725 Matrix 96 well plates with tube inserts (vials) are useful for the 2 backup plates since they allow for thawing single samples. For the 2 plates which will be frozen, cells are diluted in 2x freezing media and capped with sterile mineral oil. The 96 well plates containing 96 vials are then labelled and frozen in Styrofoam containers at -80°C for several days. The 96 well plates are then moved to vapor phase liquid nitrogen tanks for long term storage.