Culturing ES Cells (E14Tg2A line)

I. Prepare gelatin-coated flasks for ES cell passage:

(1) Add enough 0.1% gelatin in PBS to the flask to cover its base. Swirl until gelatin / PBS mixture coats entire base of flask. Let sit for at least 10 minutes.
(2) Aspirate off excess gelatin / PBS. The flask is now ready for ES cell passaging. (Note that if a small amount of gelatin / PBS liquid remains in the flask, it will not be harmful to ES cells.)

II. Passage ES cells:

(3) Aspirate media from ES cells. Rinse cells in pre-warmed PBS, using enough PBS to completely cover the cells.
(4) Add pre-warmed trypsin / EDTA to ES cells. For a 25 cm^2 flask, 0.5 ml trypsin is sufficient. Place flask in incubator for 2-5 minutes, until cells become dissociated.
(5) Add pre-warmed ES cell media to trypsinized cells: 5 mls ES cell media should be sufficient to inactivate the 0.5 mls trypsin. Pipette up and down until colonies become dissociated to single cells.
(6) Add 1 ml of the trypsinized ES cells to the gelatinized flask (this amounts to a 1:5 split). Also add 4 mls of ES cell media to the new flask.

III. General notes about E14Tg2A ES cells:

ES cells are routinely passaged every 2-3 days. On the in-between days when ES cells are not passaged, it is best to change their media. Thus, ES cells require daily attention. The feeder-independent ES cell line E14Tg2A grows very quickly and rapidly acidifies the media, turning it yellow in color. When the medium becomes acidified (either by not changing the media every day, or by passaging the cells at too low of a density) the cells may undergo crisis. Crisis can lead to excess differentiation and cell death, which greatly compromises their totipotency. This will cause a significant reduction in their future ability to undergo germline transmission, even if they appear to recover and look healthy at the time of injection.

To prevent cells from differentiating, they should be dissociated down to single cells during their passage. If they are not thoroughly dissociated, they are likely to form large clumps after passage, and the cells within these clumps will then differentiate or die. Similarly, cells should not be plated at very low density, as that may cause them to form large clumps which contain differentiated cells prior to reaching confluence.

Media & Reagents:

0.1% Gelatin in PBS

Place 2% gelatin stock bottle in 37 °C water bath until gelatin is melted. Add 25 mls liquified gelatin to 500 mls PBS. (There is no need to filter this mixture so long as the individual components have been maintained under sterile conditions.)

ES Cell Media

500 mls 1X MEM Media
5 mls 100 mM Sodium Pyruvate
5 mls 100X nonessential amino acids
50 mls BioWhittaker or GIBCO FBS (don't use CellGro FBS for ES cell media)
5 mls 100X Penicillin/Streptomycin
1.75 µl 14.3 M B-mercaptoethanol
µl LIF (use homemade LIF at a final [] of 1000 units/ml.
This is 250 µl of the LIF stock made on 6/01)

-filter media if you use homemade LIF, since it is not sterile.

***Note --** if you wish to add the drug neomycin (G418/Geneticin) to your ES cell media for selection, the recommended final concentration is 200 µg/ml. Our Geneticin stock from SIGMA is at a concentration of 50 µg/µl, and it is stored in the tissue culture fridge.