Making embryonic fibroblast feeder cells

- Set up matings between mice of any strain or genotype. If $G418^R$ feeders are needed, choose animals that will ensure passage of a *Neo* allele to all progeny. $G418^R$ feeders are not needed for routine culture of ES cells, but are needed for culturing ES cells after electroporation.

- Next day (day 0.5) check females for plugs

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- On day 13.5, sacrifice females, remove and decapitate embryos, dissect the embryos to remove internal organs and wash away blood with HBSS, mince embryo carcass in Trypsin-EDTA solution as finely as possible with scalpels.

- Incubate at 37°C for 20-30 minutes, add 10% FCS DMEM medium, transfer tissue suspension into 50 ml tubes, pipette vigorously up and down 20 times but avoid foaming.

- Allow the tube to sit for 5 min. undisturbed to let any large pieces of tissue settle. Remove the supernatant to a fresh tube and spin at 150Xg for 5 min. Plate cells in tissue culture flasks using one T175 flask per embryo and 25 ml feeder medium per flask.

- Change medium the next day, and let cells grow to confluence feeding every third day if that length of culturing is needed.

- To freeze cells, wash the monolayer with 10 ml HBSS, add 5 ml warmed trypsin and incubate at room temperature for 3 minutes or until cells are released. Add 2ml 10% FCS DMEM and transfer pooled cells to a 50 ml tube. Spin cells 150Xg for 10 minutes. Resuspend cells in 10% FCS DMEM (0.5ml per T175) and add 0.5ml to a freezing vial containing 0.5ml 2X freezing solution. Freeze cells at -70°C overnight then store in liquid N_2.

- Gamma-irradiation of EF cells: thaw a vial of EF cells to 5 T175 flasks and expand to confluence. Trypsinize cells, Resuspend in 5 ml 10% FCS DMEM in a 15 ml tubes. Gamma-irradiate with 3000 rads. Spin cells 150Xg 5 minutes, Resuspend in fresh buffer and freeze for later use, or plate onto gelatinized plates for use during the next 6-48 hours as feeders for ES cells. It is useful to freeze feeders in different sized aliquots so that when they are thawed, they will provide a confluent monolayer for one T25 (~10^6 cells), one T75 (~$3X10^6$ cells) or five T75s (~$1.5X10^7$ cells).