MOUSE EMBRYONIC FIBROBLASTS

1. Preparation of primary mouse embryonic fibroblasts

MEF cells are isolated from mouse embryos at day 14 of gestation as described by Robertson (1987):

At animal facility:

- Isolate embryos and wash once in PBS; dissect individual embryos to remove the head and soft tissues (liver, heart, and other viscera) and wash the carcasses twice in PBS.

- Mince the embryo carcasses into fine pieces in a small volume of trypsin-EDTA (just cover all the embryos) and keep this on ice untill all embryos are sampled.

From here on perform all handlings in the normal tissue culture room and <u>not</u> in the ES tissue culture room!!

- Add more trypsin/EDTA solution (2ml trypsin solution in total for 10 embryos), mix well with the embryo tissues and incubate the mixture at 37 °C for 30 min. Dissociate the tissues by vigorous pipeting with a glass Pasteur pipet and a grey pipeting balloon.

- Add 10 ml of complete medium and transfer digested tissues into a 50 ml tube and dissociate again until no big pieces are seen any more.

- Allow the large pieces of tissue debris to settle down and transfer the supernatant into a clean tube.

- Plate the cell suspension onto 162 cm^2 flasks (about 1 embryo per flask); culture the cells for 24-36 hrs, and freeze the cells in two vials per embryo. Culture medium is complete medium + β-mercaptoethanol, -LIF, +penicillin, + streptomycin. Freezing medium is complete medium + 10% DMSO.

- <u>NOTE</u>: Test the cells for mycoplasm contamination.
  Cells cannot be used before they are tested!

2. Preparation of mitotically inactive mouse embryo fibroblasts

- Thaw 1 vial of primary MEF and start culture in a 162 cm^2 flask using 30 ml of complete medium + β -mercaptoethanol, -LIF, +penicillin/streptomycin. Gaz the flasks with 5% CO_2 and keep the lids closed.

- Culture the cells till confluency and passage 1: 6 by washing the cells once

with PBS, trypsinizing the cells with trypsin/EDTA, 4ml per flask, 5 min 37 °C (shorter is even better, certainly not too long), neutralizing with medium and dividing into 6 new flasks.

- Culture the cells again till confluency and passage 1: 6.
  The cells will tend to grow somewhat slower and some times have difficulty to grow to confluency. It should take approximately 5-7 days to grow till confluency in this phase.

- Trypsinize the cells as described above. Collect the neutralized batches and spin down for 5 minutes at 1200 rpm. Remove the supernatant but leave a little bit of fluid and resuspend the cells in this small volume. Add 1 ml of medium and resuspend the cells again and than add the final volume (see below).
  The cells are usually frozen down in portions of 80 cm^2 and 40 cm^2.

- Irradiation of the MEFs. Take the cells on ice to H6. Place them in the cartridge filled with ice, and place the sample into the irradiation chamber. Irradiate the cells with 2500 rads. Meanwhile, prepare complete medium containing 20% DMSO and label the cryovials. Bring the cells back to H4.

- Resuspend the cells and add an equal volume of complete medium + 20% DMSO. Distribute the cells over the vials, put them on ice and freeze them down centrally.

The whole procedure for 30 flasks takes about 2 h for 2 persons.

Example: 30 flasks of 162 cm^2 = 4800 cm^2
=> 60 vials of 40 cm^2
=> 30 vials of 80 cm^2
Cells in 30 ml complete medium --> irradiate
Add 30 ml of complete medium + 20% DMSO giving 60 ml
- 60 x 0.5 ml => 40 cm^2 vials
- 30 x 1.0 ml => 80 cm^2 vials