cells protocol

Composition Feeder medium (for 1L)

- 2x 500 ml H_2O (Sigma or Gibco; embryo, cell or tissue culture tested)
- 13,5 g DMEM powder (Gibco; cat. 12800-116)
- 120 ml FBS
- 26 ml non-essential amino acids (Gibco)
- 24 ml glutamine (Gibco)
- 8,6 µl mercapto-ethanol (Sigma)
- 3,79 g $NaHCO_3$(Gibco)
- 24 ml Penicillin/Streptomycin (optional)
- Filter sterilize with a 0,22 µm filter

1.Plating of embryonic feeders

Method A:

1. Take a cryovial from the liquid nitrogen tank and place it immediately in a hot water bath of 37°C(for 2 to 3 minutes).
2. As soon as the cryovial is thawed, rinse the outside of the cryovial with 70% ethanol before placing it into the flow cabinet.
3. Pipette the MEF cells up and down in the cryovial with a 1 ml pipette.
4. Transfer the contents of the cryovial (all MEF cells) to a 15 cm dish, which already contains 25 ml of feeder medium.
5. Shake the dish smoothly in a circular and 8-shape movement for an optimal distribution of MEF cells.
6. Place the dish in the incubator.
7. After 30 minutes, shake the dish again.
8. After 1 hr, shake the dish again.
9. After 2 to 3 hrs, tap the dish and if the cells are well attached to the dish, refresh the feeder medium (not essential).

OR

Method B:

1. Take a cryovial from the liquid nitrogen tank and place it immediately in a hot water bath of 37°C(Take it back out of the water bath before the last clump of ice are melted in the cryovial).
2. As soon as the cryovial is thawed, rinse the outside of the cryovial with 70% ethanol before placing it into the flow cabinet.
3. Aspirate first 10 ml of feeder medium in a 10 ml pipette, followed by the thawed cell suspension. By sucking up very slowly a few air bubbels, one by one, the air bubbles that rise in your pipette will mix the thawed cell suspension (which still contains freezing

medium) with the feeder medium.

4. To get rid of the freezing medium, transfer the cell suspension into a Falcon tube and centrifuge for 5 minutes at 1000 rpm.
5. Aspirate the supernatant and resuspend the cell pellet gently in feeder medium.
6. Transfer the contents to a 15 cm dish and add feeder medium till your reach a total volume of 25ml.
7. Shake the dish smoothly in a circular and 8-shape movement for an optimal distribution of MEF cells.
8. Place the dish in the incubator.
9. After 30 minutes, shake the dish again.
10. After 1 hr, shake the dish again.