Preparation of EmfI cell stocks

1. Sacrifice the pregnant mouse when embryos are 14 dpc. Moisten the belly with 70% ethanol and dissect out the uterus.
2. Transfer the uterus to a 100mm Petri dish containing PBS, and dissect the embryos (~10) away from the uterus and all the membranes. Transfer embryos into a 50ml Falcon tube containing 20-30ml of PBS. Wash the embryos with PBS by inverting the Falcon tube up and down. Repeat this procedure 2-3times.
3. Transfer embryos into a new Petri dish in a minimal volume of PBS. Remove heads with a scalpel; remove all internal organs (liver, heart, kidney, lungs and intestine) with sharp edged forceps.
4. Transfer embryo carcasses into a 50ml Falcon tube and wash 3 times with the PBS solution (~30ml). Transfer embryo carcasses to a new Petri dish.
5. Mince all carcasses (~10) in the minimal volume (about 1ml) of trypsin/EDTA using 2 scalpels. Put 7-10 small glass beads and a small stir bar into a 50ml Falcon tube. With a 5ml pipette, transfer finely minced embryo carcasses into a 50ml falcon tube containing glass beads and a stir bar. Wash the plate with 5ml of trypsin to get the tissue remains from the Petri dish and add to the Falcon tube containing minced embryo carcasses, glass beads and a stir bar. Add another 5ml of trypsin into that same Falcon tube to end up with a total of 10ml of trypsin. Add 100μl of DNase (~0.2-0.5mg/ml).
6. Incubate for 30minutes at 37^0C with constant stirring. Use a hotplate placed into a 37^0C incubator with a magnetic stir on. Place the tube in a rack attached to a hotplate. Make sure that the tube content is being uniformly stirred.
7. Observe the tube content. The solution will look cloudy, which is normal. If you see a lot of undigested DNA, add another 100μl of DNase. Add another 10ml of the trypsin/EDTA solution and repeat step 6. Can repeat step 7 once more if needed.
8. Collect the cell suspension into a new 50ml Falcon tube and add 3ml of FBS to stop the trypsin activity. Rinse glass beads twice with 3ml of the feeder

medium and add this wash solution containing EmfIs to the rest of EmfI suspension.

9. Spin cells at 270g for 8 minutes.
10. Resuspend the cell pellet in a needed volume of the feeder medium and plate onto a 150mm TC dishes (~1embryo/plate).
11. Observe cells in about 3hours. Virtually all feeder cells will attach to the plate by that time. Small round cells will be floating in suspension; these are the blood cells that are not going to attach to the plate.
12. Change medium the next day. By the end of that day plates will be about 60%confluent. Observe the cell morphology. Some granulation around the cell nuclei will be apparent which is considered to be normal.
13. Passage 1plate to 5plates of the same size on the day when plates look confluent (2-3 days).
14. Freeze the rest of the plates when they reach confluence (3-4 days). All cells from one plate should be frozen in one vial in 1ml. Store cells at -70^0C for one day. Transfer vials to liquid nitrogen.

Mitomycin treatment of EmfI cells

1. On Tuesday, 4pm, thaw one vial of non-Mitomycin-C treated feeders onto four 15 cm plates (25 ml medium into each plate).
2. Friday, pass each plate onto four 15 cm plates.
3. Monday, Mitomycin-C treatment for 2 hours and freeze one plate per vial.

Feeder cell plate preparation

Remove freezing medium and resuspend cells in 24 ml of feeder medium. Plate cells in gelatinized plates (150 μl/well of 96 well plate; 12 ml/10 cm; 4 ml/6cm; 2 ml/well of 6 well; 0.5 ml/well of 4 well or 24 well).

EmfI medium:

10% FBS, 1xPen/Strep, 1x non Essential AA, 1x NaP, 1x L-Glutamine