

Primary Mouse Embryonic Fibroblasts or Feeders

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Materials:

Female mice with timed pregnancies.

See the “Guide to Better Mouse Rooms, Timed Pregnancies/Plugging”.

Mouse dissection tools (at least a good surgical scissors, and a variety of forceps, sterile)

Sterile PBS, room temp

0.25% trypsin

0.1% gelatin in water or 0.2% gelatin in PBS

DMEM with 10% FCS, Pen/Strep/Glu, non-essential amino acids

DNase (100mg/mL, which is 1000x final concentration)

10cm sterile dishes

For Pooled embryos

one 16 or 18G needle, 20mL syringe

For Individual embryos

24-well plate

ependorf tubes

a 16 or 18G needle and a 5mL syringe for each embryo

1. Dissect mouse embryos 12.5-14.5 p.c. (I prefer 12.5-13.5, you can go as long as 16.5, but the embryo is less fibroblast and more cartilage and other organs by then). Remove each uterine horn as a long “sausage tube” and place in a petri dish of sterile PBS. Remove the individual yolk sacs and place them in a separate dish of PBS. Remove embryos from yolk sac and place in PBS in an individual 24-well plate if you need to keep embryos separate for genotyping, or place them all in a single dish of PBS if they have same genotype. The rest of this protocol is for pooled embryos, but can be scaled down for individual embryos.
2. Remove head and internal organs from each embryo. Place “trunk” of embryos into 10-15 ml of **0.25%** trypsin (1 mL trypsin per embryo) in a 50 mL conical tube. If you need to genotype, use the head in the eppendorf tube to extract DNA as you would from a piece of mouse tail.
3. Pull the embryos and trypsin through a 16 or 18 gauge needle into a 20 ml syringe to disaggregate the embryos. Squirt back into the tube. Save the needle and syringe carefully by dropping it into a new 50 mL conical. (Do not re-cap the needle! This is dangerous!)
4. Rock cells/trypsin gently at 37°C for 20-30 minutes. If you don't have a warm rocker, you can also use waterbath and then invert the tube every 3-5 minutes. During this time, prepare the 10cm dishes that your cells will be plated onto by coating them in a 1% or 2% gelatin. If I am dissecting E14.5 embryos, I use one 10cm plate per harvested embryo. If I have E12.5, I use about 2 dishes for every

- 3 embryos. If you are using small, individual embryos you can use a 6cm dish or 2 wells of a 6-well plate for each embryo. The cells are accustomed to being packed together in the embryo, so they are happiest very confluent at first.
5. Pull everything through the syringe 1 more time, discard syringe in sharps bin.
 6. Add 20 ml media and add DNase to final concentration of 100 micrograms/ml. Pipet up and down aggressively. You want to try to break up the genomic DNA – it can interfere with the procedure. Some people like to make the mix “foamy” to help – it won’t hurt the cells.
 7. You may need to “suck off” a floating gelatinous glob of junk sometime during this procedure - be careful, because you could also suck off your cells. This floating stuff is usually no more than 10% of the volume.
 8. Incubate in this trypsin/media/DNase solution for 15 minutes.
 9. Spin down your tube or tubes of cells – 1500+ rpm for 5 minutes.
 10. Wash with media once to get rid of trypsin and DNase.
 11. Remove the gelatin and plate cells on to the 10 cm dishes. These cells are passage 0 (p0). Change media the next day. Cells should be growing nicely, and may be ready to split already.

The following are the steps to then make your MEFs into Feeders for ES cells:

12. When confluent, split each dish onto 2x 15 cm dishes. This is a 1:6 split.
13. When confluent, freeze down about half of the cells at 3×10^6 cells/vial. I call this passage 2 (passage 0 on the 10 cm dish, passage 1 on the 15 cm, and now passage 2 in the vials. Each vial can be thawed onto one 15cm dish as P2). Take the other half of your batch and split them 1:6 again. They are happy, actively growing cells now, but they will stop growing at passage 4 or 5 if they are simply primary fibroblasts. We have isolated MEFs from TPA-/- mice that will continue to divide and be happy for 6-7 passages.
14. To make MEFs into Feeders, take the vials of frozen cells, place with some dry ice in a beaker, and expose to 6000 RADs in the gamma cell irradiator. Now, each vial of 3×10^6 cells will cover one 10cm plate (or 55cm^2) when you thaw it. Alternately, some people like to create irradiated stocks with 5×10^6 cells, thawing one vial onto 2 10cm plates (covering 110cm^2)
15. The End – good luck!