

Harvesting Mouse Embryonic Fibroblasts from Murine Embryos:

Thomson Lab 2004

1. Inject approximately 0.5 ml avertin IP into a 13 or 14 day pregnant mouse*.
When mouse is anesthetized, perform a cervical dislocation.
2. Saturate abdomen with 70% Ethanol and pull back the skin to expose the peritoneum.
With sterile tools, cut open the peritoneal wall to expose the uterine horns.
Remove the uterine horns and place them in a 10 cm dish. Wash three times with 10 ml PBS w/o CaMg.
3. Cut open each embryonic sac with a scissors and release the embryos into the dish.
4. Using two pairs of watchmakers forceps, remove the placenta and membranes from the embryo. Once they have been removed, dissect out the visceral tissue (ie. anything that is dark in color). Place the embryos in a clean petri dish and wash three times with 10 ml PBS.
5. With a curved iris scissors, FINELY mince the tissue. When your hand is too tired to mince any more, add 2 ml Trypsin/EDTA and continue to mince. Add an additional 5 ml of Trypsin/EDTA and incubate at 37°C (for about 20 minutes). At this time return to step 1 and start another mouse.
6. Perform steps 1-4 and return to the embryos in Trypsin/EDTA.
7. Pipet the embryos in Trypsin/EDTA vigorously, until few chunks remain. Return plate to the incubator for an additional 10 minutes.
8. Neutralize the Trypsin/EDTA with about 20 ml culture medium**, and transfer the contents of the dish to a 50 ml conical tube.
9. Mix the contents of the tube well, and evenly add to T75 culture flasks containing 20 ml culture medium. There should be approximately 3 embryos per T75.
10. Place these flasks in a 37°C incubator overnight.
11. Return to the embryos sitting in PBS, and begin process at step 5 again.
12. The next day, change the medium to get rid of debris and toxic cell death products.
13. When flasks are becoming about 80-90% confluent and still in the log growth phase, it is a good time to freeze them. In general, this happens about the second day after preparing the embryos. It may happen sooner or later, so keep watch over your flasks.

Notes:

* We have been using CF-1 mice for fibroblast preparations

** Culture medium is:

88%	DMEM
10%	FBS
1%	NEAA
1%	PenStrep

*** As with any new cell in the lab, a representative sample should be tested for mycoplasma