

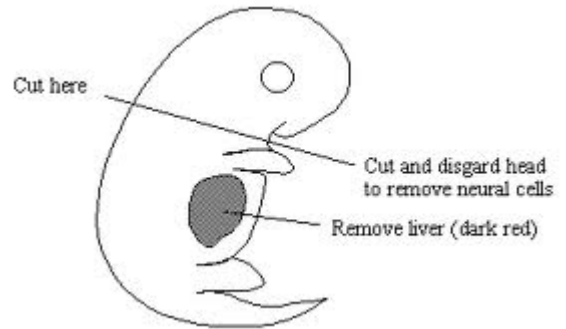
Isolation of Mouse Embryonic Fibroblasts

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Procedure:

- 1) Collect embryos at E13.5 or E14.5
 - a. Wash embryos in phosphate buffered saline (PBS)
- 2) Using forceps, remove placenta and membranes from embryo. Dissect out liver using forceps and cut off head using scalpel (see image). Place embryos in a clean petri dish.



- 3) Finely mince tissue using forceps. Add 10 ml of 0.05% Trypsin-EDTA (Gibco, 25300-054) and continue to mince tissue by pipetting tissue up and down. Incubate tissue at 37 °C for 20 minutes.
- 4) Resuspend tissue and re-incubate at 37 °C for an additional 15 minutes.
- 5) Neutralize the Trypsin-EDTA digest solution by adding 20 ml of culture medium (DMEM + 10 % FBS + antibiotics) and transfer contents through 70 μ m strainer into 50 ml conical tube.
- 6) Pellet cells via centrifugation at 1 kRPM for 5 min
- 7) Aspirate media and add 20 ml of culture medium. Plate onto a T25 flask (1 embryo/flask). Place flask in 37 °C incubator overnight.
- 8) Change medium to get rid of dead cells and cell debris.
- 9) Can freeze cells when at 80-90 % confluency or can use cells for experiments.