<u>Culturing Embryonic Fibroblasts</u>

1. Establish a timed pregnancy to produce a litter at approximately E13-14.

2. Euthanize the pregnant mother and swab the belly in 70% EtOH.

3. Remove the uterus containing the embryos into a sterile dish. Remove to the TC hood and add filtered phosphate buffered saline (PBS). Rinse embryos to remove blood and remove to another dish with PBS. Keep in PBS until mincing.

4. In the hood, dissect the embryo from the uterus and discard the liver, spleen and the head.

5. Mince embryo ~5 min in a 10cm dish with sterile scalpel blades, then add 3 ml of 0.25% Trypsin-EDTA. Mince further, then add another 2 mL of 0.25% Trypsin-EDTA, triturate and transfer to a 15 mL conical tube.
   -Soak dissection tools in 70% EtOH between embryos to prevent contamination.

6. Incubate 10 minutes at 37ºC, with occasional agitation. Begin at step 4 with another embryo at this point.

7. Triturate the sample to break apart tissue, then allow larger unbroken tissue to settle. Remove cell suspension into a fresh 15 ml tube and add up to 12 ml with growth media.

8. Count cells and plate 1.5 x 10^6 cells per 10cm plate. To count cells, add 15 µL of cell suspension to the hemocytometer and count cells within the 4x4 counting brackets (take average of the four counting grids). The average is the number of cells per 0.1 µL of the suspension.

9. 24 hrs after plating, change the media to remove debris. Grow to 90% confluence, splitting to 25% confluence when necessary (keep cells within this range to promote maximal growth). After the first few passages, only fibroblast-like cells remain. Growth rate should progressively decline in successive passages until transformation occurs, at approximately 1-3 months in culture. Cell doubling time should fall between 24-72 hrs.

Fibroblast Media: DMEM + 10% FBS + 100µg/mL L-glutamine + 100µg/mL penicillin + 100µg/mL streptomycin.