Procedure to generate passage zero Swiss-Webster mouse embryonic fibroblasts (MEFs).

- Kill timed-pregnant Swiss-Webster mice when embryos would be at day 14, by cervical dislocation. Swab the abdomen with 70% alcohol.

- Using a scissors cut open the abdomen cavity and remove the uteri (2/mouse) and place in a 50ml conical tube containing sterile PBS.

- Wash embryos by transferring into another 50ml tube containing sterile PBS until no blood remains (1-2X).

- Transfer the embryos to a 100mm Petri dish containing 1X PBS. Cut apart the uterus by cutting between the embryos.

- Transfer embryos to another 100mm Petri dish with PBS and remove amniotic sac and placenta from the embryo.

- Place the embryos in a 100mm dish containing DMEM+10%FCS with P/S. Cut off head of the embryo. Remove and discard all internal red organs (spleen, liver, heart lungs...all internal organs).

- Mince embryos using a razor blade/scalpel into as small as possible pieces. Spin down pieces in a 50ml conical tube and resuspend in 10-12 ml of 0.2% collagenase containing 20% serum plus 10 ul/ml of 1mg/ml DNase stock solution.

- Vortex and incubate cells for 1-2 hours at 37°C. Vortex cells every 30mins.

- At the end of the incubation period syringe the mixture 4-6X through a 20-gauge needle. Bring the volume of the cells up to 50ml in DMEM+10 % GemCell fetal calf serum (FCS).

- Spin at 1400 rpm for 5 minutes.

- Resuspend cells. Make up the volume to 10ml using DMEM+ 15% GemCell FCS.

- Count cells and plate at 3X10^7 cells/100mm dish. This is about 1 embryo equivalent per 100mm dish.

- Incubate at 37°C overnight, and then change media.

- Cells will be confluent the next day freeze @ 2X10^6 cells/vial or passage a dish 1:10.