Making 1^0 Fibroblasts from Mouse Embryos

1. Use females that are 12.5 to 14.5 days pregnant.

2. Working with one at a time, anesthetize and sacrifice each mouse. Remove the uterus, trimming excess tissue, and place in 10 cm dish of sterile PBS.

3. Using a different set of sterile scissors, forceps and gloves, and working on a different (sterile) diaper, remove each embryo from the uterus. You may want to transfer the embryos to a fresh dish of PBS if first dish is too messy.

4. For each embryo remove the head with sterile blade and place in 1.5 ml tube (see step 7). Put the rest of the body into a sterile 1 cc syringe with 18 gauge needle attached. Place the syringe into a 15 ml tube.

5. Add 1 ml trypsin to syringe, use plunger to push embryo and trypsin through needle. Break up tissue by pulling back on plunger (thus suctioning tissue mixture back into syringe) and pushing through again. Repeat for a total of 3 squishies. The multiple squishies are not required when using d12.5 embryos.

6. Cap 15 ml tubes and incubate at 37^{o}C in tissue culture CO_2 incubator for approximately 5-10 minutes.

7. DNA: To the 1.5 ml tubes containing heads add tail cutting buffer plus Proteinase K and continue with your favorite DNA prep protocol.

8. 1° Fibroblasts: Prepare M15 media (but without BME) and put 20 ml in each sterile plate (one plate per embryo; do not use STO feeder plate nor gelatin). Label each plate with embryo number and note that this is passage 0 ("p0").

9. To each tube of embryo tissue: use transfer pipette to add 1ml to 2ml media to tube, break up tissue further by pulling mixture up into pipette and squirting out again approximately 4 times. Use 10 ml pipette to add enough media to bring volume to 5 ml and transfer all to plate.

10. Put plates in incubator. Change the media 24 hours later. At 48 hours later typsinize the cells and plate onto 2 fresh untreated 10cm plates (now "p1"). Two or three days later the cells will be sufficiently confluent to freeze down. Alternatively, if faced with many clones, trypsinize each plate into freeze down media and freeze cells (each plate = 1 vial) at 48 hours post plating. These cells remain p0. When cells are needed for an experiment, thaw 1 vial per plate and pass each plate 1:2 at 24 hours (now cells are p1). Can freeze back one of the plates if not needed as a duplicate point in the proposed study.

You will need:
pregnant females, syringe, needle, avertin
two sets of scissors & forceps; scalpel or razor blades
beaker of 70% EtOH
diapers, gloves
for each embryo: 15 ml tube, 1 cc syringe, 18 gauge needle
typsin, PBS, 10 cm dishes
M15 without BME: M15= DMEM buffered with Na-bicarb., and supplemented with 15% fetal calf serum + glutamine and pen/strep.