1. At lab bench, remove uterine sac. Transfer to PBS in sterile dish and transfer to laminar flow hood.

2. In laminar flow hood, place small drops of DPBS in sterile dish. With sterile forceps and scissors, dissect limb tissue, removing skin and bone. Mince/fragment limb tissue with forceps. Dissect tail (E17 or older) or head (E16 or younger) and transfer to labeled Eppendorf tube for DNA extraction and genotyping.

3. Transfer muscle tissue to labeled 15ml tube. Add 2 ml of HBSS, 0.25ml of 2% trypsin, 0.25ml of 0.1% DNaseII. Incubate @37°C, with occasional gentle shaking, for 15-20 min.

4. Add 2ml of GM and gently triturate 15X - initially, with pasteur pipette and subsequently, with tapered pasteur pipette (orifice tapered by heating).

5. Centrifuge @ 700 rpm for 5 min; gently resuspend in 5ml GM with pasteur pipette and gently triturate 10-20X with tapered pasteur pipette.

6. Centrifuge @ 700 rpm for 5 min; gently resuspend in 2.5ml GM and filter through cell strainer. Rinse strainer with 2.5ml GM.

7. Transfer filtrate to 6 cm Petri dish and pre-plate @37°C for 15-20 min.


9. Plate 10^6 -2 x 10^6 cells in each of two Matrigel-coated 10 cm dishes. Freeze remainder of cells in 90% FCS and 10% DMSO.

10. The next day, gently treat cells from one dish with trypsin for 45” to 2 min (watch as cells begin to detach), and count cells. Freeze cells from other dish.

11. For cloning, plate 50, 200 or 800 cells / Matrigel-coated 10 cm tissue dish; three dishes at each dilution. Change medium every three days

12. Clones will begin to form at one week, and should be ready to isolate with cloning cylinders at 10-14 days. Expand for freezing; test for muscle differentiation.

**Growth Medium**

DMEM
- 15% fetal calf serum (FCS)
- 2% chick embryo extract (CEE)
- 1 mM glutamine
- 1 mM pyruvate
- 0.1 µg/ml gentamycin
- 20 U/ml γ-interferon

**Differentiation Medium**

DMEM
- 5% horse serum (HS)
- 1 mM glutamine
- 1 mM pyruvate
- 0.1 µg/ml gentamycin