Mouse Dermal Fibroblasts Preparation

1. Fill a 150 x 25 mm petri dish with ice. Put newborn mice (2-3 days old) in the petri dish and insert it in an ice bucket. Leave the mice in the ice bucket for 40-60 minutes.

2. Wash dead newborn with water once, then with 70% ethanol twice. Remove ethanol completely.

3. Using sterile techniques under the hood, amputate mice tails and limbs with surgical scissors.

4. Work with one mouse at the time. Cut on the dorsal side and along the length of the body of the mouse with a scalpel such that the skin is cut, but the internal parts of the body are intact. Carefully separate the skin from the rest of the viscera. Put the skin in a petri dish containing 20 ml of PBS.

5. Repeat the procedure for all the skins.

6. Flatten the skins in an empty 150 mm petri dish with the back of a forcep. It's crucial that the skin is perfectly stretched, even at the edges, otherwise peeling is very difficult. The skins should have the dermis facing down.

7. Add approximately 30 ml of trypsin solution (Gibco #15050-040) to each petri dish, and store petri dishes overnight at 4°C. The skins should be floating on the trypsin solution.

8. Put one skin at a time on an empty petri, flatten it again and use forceps to separate the epidermis from the dermis, starting from one corner of the skin.

9. In a flask, per 8 dermis add 12 ml of HBSS and 0.5 ml collagenase solution (10 mg/ml H_2O).

10. Stir the above mix (from step 9) for 30 mins at room temperature.

11. Filter through a sterile gauze.

12. Plate 3-4 ml of the filtered dermis suspension in each 150 mm tissue culture dish containing 20 ml of DMEM + 10% calf serum + antibiotics.

13. Incubate in a 37°C, 5.0 CO_2 incubator.