Isolation of primary keratinocytes from newborn mice

Note: The isolation of keratinocytes needs an overnight incubation, therefore start in the afternoon. Keratinocytes must be cultured at high cell density. Following first passage, split nor more than 1:2. Following spontaneous immortalization, one can split 1:3. Refer to protocol P71 for culture of mouse keratinocytes. Refer to Lichti, Nature Protocols 3, 799ff, 2008 for more useful background information.

- Use newborn mice, not older than 3 days. Use of prenatal embryos is possible. Procedure detailed below is for newborn or E18.5 dpc embryos.

- Kill by decapitation
- Cool bodies (in a 50ml Falcon) on ice for 1h
- In the meantime prepare under the hood:
    - 50ml falcon with 50% Beta-Iodine/ 50% 1xPBS
    - 50ml falcon with 1xPBS
    - 50ml falcon with 70% EtOH
    - 50ml falcon with 1xPBS
- Then perform the following procedure using one body after the other and keep the rest on ice:
    - Wash and desinfect bodies:
        - 1min 50% Beta-Iodine/ 50% 1xPBS
        - Rinse briefly in 1x PBS
        - 1min 70% EtOH
        - Rinse briefly in 1x PBS
    - Transfer to a sterile 10cm dish (everything must be sterile from here)
    - Cut off tail (used for genotyping) and limbs
    - Make a lengthwise incision along the side of the embryo, from neck to tail
    - Unwrap the mouse body from the back to the front using forceps and the dull part of scissors, take off the skin in one piece.
    - Place the complete skin in 2ml Eppendorf containing 1.5ml (5mg/ml) of dispase for overnight at 4’C.
    - The very next day morning transfer the skin into a petri dish and remove the epidermis from dermis with clean forceps.
    - Place the complete epidermal sheet well-spread in a fresh 3cm petri dish, taking care that the basal layer of the epidermis faces the bottom of the plate (stratum corneum side on top).
    - Put 1ml of trypsin solution (0.025% trypsin in PBS/EDTA 0.02%) below the skin so that complete epidermis floats in trypsin solution.
    - Incubate for 5 mins and then add 2 to 3 ml of the serum containing medium to inactivate trypsin.
    - Now slowly invert the skin and with the help of blunt scalpel softly scratch from one end of the skin to other without tearing the epidermal sheet (this will remove most of the basal cells into the trypsin-serum containing medium).
    - All the viable cells from the basal layer are released in the medium leaving behind transparent stratum corneum. If stratum corneum remains intact as a single sheet, grab with sterile Gilson tip and discard. If in pieces, carefully take up cell suspension with pipette, transfer in Falcon tube and leave stratum corneum pieces behind.
    - Spin down at 1000rpm for 5 to 7mins to collect cells.

- Place cell pellet in growth medium in a 6-10 cm plate (if cell yield is low for biological reasons, use 35 mm dish; high cell density is important).

Note: Plates need to be pre-coated with collagen I for 1hr. Alternatively, cells can be grown on feeder cells (3T3 J1 cells, Mitomycin treated). See corresponding protocols.

Incubate the cells at 32°C, 5% CO_2 in a cell culture incubator.

Note: For the first passage immediately after isolation, grow the cells in complete medium supplemented with 100 µM $CaCl^{2+}$ (final concentration). This will make the cells to adhere well. Following O/N culture, switch back to normal keratinocyte medium containing 50 µM $CaCl^{2}$ (final conc.).

Medium:

We use FAD medium with 50 µM $CaCl^{2+}$ final concentration (FAD low Ca, manufactured for us by Biochrom). The medium contains 10% FCS (FCS-Gold, PAA.), in order to remove calcium ions this has to be pre-treated with Chelex (Bio-Rad; 8g/ 50ml) overnight on a rotating wheel at 4°C, after repeating the procedure, the FCS is sterile filter