

#### Preparation of fibroblasts from mouse tail-tips

- (i) Cut the tail from an adult mouse, and wash with PBS.
- (ii) Make a lengthwise incision by an injection needle, peel superficial dermis by hands, and mince the remaining tail into 1-cm pieces with scissors. Place two pieces per well of gelatin-coated 6-well plates, add 2 ml of MF-start medium, and incubate at 37 °C for 5 d, during which time fibroblasts migrate out of the tails.
- (iii) Remove the tissues of tails with sterile forceps and discard. Replace the medium with 2 ml of FP medium, and culture the cells when they reach confluent.
- (iv) Aspirate the medium, wash twice with 2 ml of PBS and add 0.3 ml of 0.05% trypsin/0.53 mM EDTA, and incubate at 37 °C for 10 min.
- (v) Add 2 ml of FP medium, suspend the cells, and transfer and pool to a 15-ml tube. Centrifuge the cells at 160g for 5 min.
- (vi) Discard the supernatant, resuspend the cells with 10 ml of FP medium and plate to 100-mm tissue culture dish (passage 2).
- (vii) When the cells become confluent, remove FP medium, wash once with PBS, and trypsinize with 1 ml of 0.05% trypsin and 0.53 mM EDTA for 5 min. After harvesting, resuspend with 9 ml of FP medium. Passage to new 100-ml dishes at 1:4 dilution (passage 3). These cells usually become confluent within 3–4 d. For the generation of iPS cells, we use tail-tip fibroblasts (TTFs) within three passages to avoid replicative senescence.