

Preparation of fibroblasts from mouse tail-tips - Kotton Lab

(TTFs= tail tip fibroblasts)

- i. Clean outside of tail with 70% EtOH to sterilize.
- ii. Cut the tail from an adult mouse. (If newborn take entire tail, if adult take 0.25-2cm if alive or entire tail preferably, if sacked; the more tail the more fibroblasts). Place in PBS on ice in 15 cc conical or eppendorf.
- iii. Working in a sterile culture hood with sterile instruments, use sterile forceps to take tail tip out of PBS. Make a lengthwise incision in each tail tip (using an injection needle or scissors). Remove outside skin and hair by peeling back superficial dermis by hand.
- iv. Mince tail tip into 1-cm pieces with scissors, placing each fragment into a waiting well of cold PBS. After washing tail tissue in PBS, place two pieces per well of a gelatin-coated 6-well plate***, add 2 ml of complete media (see below**), and incubate at 37 °C for 5 d, during which time fibroblasts migrate out of the tails.
- v. After 5 days, remove the tissues of tails with sterile forceps and discard. Refeed the remaining adherent fibroblasts in the well by replacing the medium with 2 ml of complete media. Culture until patches of cells are growing nicely (usually 1 day after removal of the tail tissue). Then split 1:1 to spread the patches out into a uniform monolayer (this is passage 0).
- vi. When 90% confluent, passage cells by trypsinizing as usual with 0.05% trypsin/ EDTA for 5 min. Split 1:3 for each passage. Do not passage too early (before 90% confluence) or too late (overgrown) or the cells will senesce. For the generation of iPS cells, we use tail-tip fibroblasts (TTFs) within three passages to avoid replicative senescence. Cells may be frozen in nitrogen (as per standard fibroblast freezing protocol) on passage 1-2, if needed.

** complete media (DMEM; 10% FBS; 1% P/S; +L-Glut)

***preparation of gelatin coated 6 well plate: Add 2 mL of 0.1% sterile gelatin to each well, let sit for at least 5 min, aspirate off, keep plate at room temp until ready to use (the same day).

VERSION HISTORY

2017-04-26	TAL and SJO	TAL/SJO
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