CULTURING PRIMARY FIBROBLASTS FROM MOUSE TISSUE BIOPSIES

Remove 1-2mm piece of ear or tail from rodent (younger is better, but we have successfully used 6-month old mice) and rinse tissue well with Wescodyne (iodine-based antimicrobial solution) followed by 70% ethanol. Then rinse the tissue piece 2X in either PBS containing 100ug/ml kanamycin or in PBS containing standard tissue culture (TC) amounts of penicillin + streptomycin.

Working in a 6cm TC dish under a laminar flow hood, mince each single ear or tail biopsy very well with sterile razor blade/scalpel into a 0.25ml mixture of collagenase and dispase neutral protease (4mg/ml each in DMEM). Alternatively, you may mince in standard TC trypsin (though this works less well). After mincing finely, add another 0.25 ml collagenase mixture (or trypsin) to the plate, and incubate the minced tissue for 30 minutes at 37°C.

Add 6mls of MEF media (DMEM +10%FBS + pen/strep) to the 6cm plate and incubate overnight at 37°C, 5% CO_2. The following day, aid the dissociation of any very large pieces of tissue by titerating with a sterile Pasteur pipette. Maintain sterility. Continue incubating the tissue for an additional 3 days at 37°C, 5% CO_2 to allow the cells to adhere and spread away from the tissue chunks.

After 3-4 days, the adherent cells are usually sparse, and a few of the larger tissue chunks may still remain. If desired, you may remove the tissue chunks to a new 6cm plate. Feed cells in the original plate with fresh MEF media, and add media to the newer plate with the chunks. Continue incubating cells.

Cells will begin to undergo rapid proliferation over the next several days. This expansion can be assisted by using a 5%O_2- 3%CO_2 incubator (if available), but the cells will also grow in a standard 37°C, 5%CO_2 incubator. When nearly confluent, cells may be passed or frozen.

This protocol was modified from Jaenisch Lab protocol (Lengner @wi.mit.edu) by Kathy Hoover of the Jones Lab (kathleen.hoover@umassmed.edu) and has been successfully used for tail tissue biopsies from mice and rats.