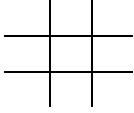


Mouse Primary Fibroblast Tissue Culture (Ariel/Eric)

- Prepare DMEM w/ 10% FCS and pen strep
- “Scratch” the bottom of the wells of a 24 well plate with a 19 gauge needle to produce a checkerboard pattern

i.e.



1. Sac mice and take tissue from organ of interest
 - store organ on ice in supplemented DMEM
2. In a flow hood chop up the tissue finely
3. Add a small piece of tissue to the intersection of two scratches so that cells can adhere and migrate from the sample onto the tissue culture dish.
4. Allow tissue piece to sit for 1-5min. on the dish w/o media
5. Add 1 drop of media to the tissue piece
 - incubate 30min. in tissue culture incubator
6. Gently add appropriate volume of media to the tissue culture dish to submerge tissue
7. Replenish media as needed

Adherent fibroblasts will be observed ~2-3 days after plating and confluent wells are observed ~3-4 weeks after plating.