Cell Isolation Protocol:

Based on lung primary cell isolation protocol (using mouse lungs) from Barker lab and Owens lab protocols with substitutions as necessary.

Supplies needed:

- Eppendorf tube with 0.5 mL HBSS (w/ Ca2+ Mg2+) and 10 uL Pen-Strep
- Cooler and ice
- Sterile scalpels
- Sterile 50 mL conical tube
- Sterile 70 uM filter
- 2 mm conical tube, sterile
- Sterile 1 mL syringe (for plunger)
- Round petri dish (to use as cutting surface)
- Digestion solution
    - Collagenase I @ 100 u/mL (100 uL of 1000u/mL stock)
    - DNAase @ 50 units/mL: 25 uL of 2000 u/mL aliquot (New England Biolabs RNase-free)
        - *Note*: May need to adjust if mixing new stock solution from Roche lyophilized DNase (in refrigerator). Consider use of up to 500 u/mL.
    - HBSS w/ Ca2+ Mg2+ (875 uL, or enough to make 1 mL total volume based on above volumes)
    - Final volume 1 mL (plenty for small biopsy)
- Culture medium (Lonza Fibroblast Medium), 1 mL
- Telfa (optional)
- T25 flask

Steps:

1. Prepare flasks: add 5 mL complete growth media to T25 flask. Place flasks in 37degC incubator and allow media to pre-equilibrate to temperature for at least 30 minutes prior to adding cells.
2. Fill one Eppendorf tube with 0.5 mL HBSS or PBS. Weigh this and record weight. Place in refrigerator to chill.
3. Bring Eppendorf tube to OR in cooler with ice pack and tube rack (don't put tube directly on ice, it will freeze). Collect tissue into Eppendorf tube. Place back in cooler and return to lab immediately.
4. Weigh Eppendorf tube/tissue and calculate mass of tissue.
5. Wash tissue 2-3x with HBSS or PBS. Washing onto Telfa works well. Try to rinse away as much blood as possible.
6. Place tissue on cap of petri-dish (do not use culturing surface of petri dish, to avoid tissue/cell adherence). Mince tissue into small chunks (no more than 2 mm/side) using scalpel.
7. Transfer to appropriate volume of digestion solution. Total 1 mL.
8. Place tube in tube rack on gentle orbital shaker in in incubator, at 37 C for 4-6 hours, until tissue appears almost completely dissolved.

9. Place a 70 uM filter onto a 50 mL tube and pour digestion solution and tissue unto the filter. Rinse 2 mL centrifuge tube with PBS or HBSS and pour onto filter to ensure no tissue or cells left behind.
10. Use a sterile syringe plunger to gently work any chunks of tissue through the filter. Can rinse filter with a few mLs of PBS or HBSS to help rinse through any additional cells.
11. Centrifuge cell suspension at 300 xg for 10 minutes.
12. Aspirate supernatant completely
13. Re-suspend pellet with 1 mL media, gently pipetting up and down a few times to ensure an even single cell suspension. Transfer to prepared T25 flask.
14. Incubate at 37 degC with 5% CO_2.
15. Re-eval for feeding at 24-36 hours.