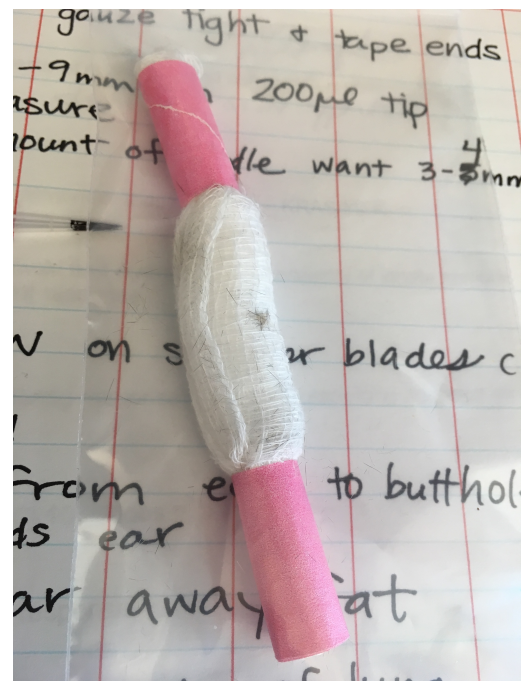


Mouse Orthotopic Lung Injections

1. Make pillow – roll gauze pad up tight and tape ends
2. Make guide – 8-9mm of 200uL tip, use marker to mark complete guide piece (see bottom picture)
 - After cut, remeasure guide
 - Measure amount of needle exposed – should be 3-4mm
 - Tape guide to syringe/needle
3. Lay anesthetized mouse on its right side on top of pillow – pillow should be placed near shoulder blades close to neck – pillow should not be directly under injection site
 - Make sure mouse is laying completely flat and is not rotated in one direction or the other
4. Shave area around incision site – remove excess hair with moistened gauze pad
5. Swab site with iodine swabs
6. Bisect mouse along the midline from ear to butthole, move over ~1/2" to the left towards the head, that will be incision site
7. Make cut/incision wide enough for you to work
 - Make sure to use scissors perpendicular with animal, failure will create jagged incision site edges – do not angle it
8. Readjust mouse if necessary – rib cage should be flat but laying at a 45° angle compared to surface
9. Remove and cut away all fat from incision site
10. Find the edge of the lung in the rib cage – should see lung moving horizontally
11. Find the rib nearest to the edge of the lung and count 3 ribs over to the left towards the head of the mouse
12. Take pre-loaded 30-gauge needle in right hand (hold it in such a way that you are depressing the plunger with your index finger) – use left hand as a guide to steady yourself and inject in between the 2nd and 3rd ribs
 - Push needle into tissue completely perpendicular to mouse/lung – any angle could make needle miss lung
13. Once the guide piece reaches the tissue, wait a second or two to prevent leakage, and dispense total volume of cells into lung
14. Squirt a generous amount of lidocaine on incision site
15. Staple wound closed with appropriate number of staples (1-3)
16. Ear tag mouse
17. Inject 100uL of carprofen via IP into mouse
18. Weigh mouse – record ear tag number, cell line, and body weight
19. Place mouse in box on warming pad – visually check to see if mice are ok



Needle Guide (black)



Gauze Pillow