Lung extraction for histology

Introduction

Materials

- 1 big scissor, 1small scissor for the brain, 1 tweezers with teeth, 1 tweezers with curved ends, 1 thicker tweezers.
- 1ml syringes (1 syringe /animal + 1 for avertin)
- 20G catheter (BD Cat # 381703 or SURFLO® Teflon I.V. Catheter 20G x 1 1/4" Pink sc-360098 from Santa Cruz)
- 15ml Falcon tubes, 2/animal, one with PBS to do the "lavage" and wash the catheter in the end, and an empty one to put the collected "lavage".
- 50ml tube with 45ml of 4%PFA or 10% Formalin
- 50ml tube with PBS/5mM EDTA
- 10ml syringe with 27G needle (only one)
- Suture wire (Cat #20090710)
- Butterfly tube/needle
- Eppendorf tubes, 2/animal, one for the cytokines and the other for the tail.
- 20ml syringe to inflate the lung (can be re-used), 1/experiment (only needed in special cases)
- Surgery scissor – It is in the dissecting drawer, in the TC room, inside a small box. Make sure you only take it out of the box to cut the trachea and put it back in!

Procedure

Perfusion - Cytokines and immune cell isolation

1. Put the mouse to sleep with 0.37ml (female) or 0.47ml (male) of avertin (concentration is 12.5mg Tribromoethanol/ml).
2. Lay down the animal to the styrofoame with the needles, fix the 4 limbs with 18G needles
3. Spray it with 70% EtOH
4. Open the animal in a V-shaped manner from the belly until both sides of the ribs.

5. Carefully open the thoracic membrane (DO NOT damage the lungs).
6. Fill a 10ml syringe with PBS/5mM EDTA.
7. Make a cut in the liver so the animal can bleed out.
8. CRITICAL Insert the needle in the right ventricle, push the plunger slowly and continuously. (if you miss the right ventricle you just have to wait longer until you see the lungs becoming white!)
9. Pinch the animal's lip with a needle so its head stays steady.
10. Open the skin in the neck.
11. Carefully rip the membrane covering the trachea using the tweezers with teeth. (need a picture)
12. Pass a suture wire around the trachea using the curved tweezers.
13. With the surgery scissor perpendicular to the trachea tube make a nick in the tube wide enough for inserting the catheter (DO NOT cut the entire trachea).
14. Insert the catheter and make a knot with the suture around the catheter/membrane (not to hard or else you will obstruct the liquid passage)
15. Hold the catheter in place with two needles (one on each side) to prevent it to go forward (when you press the plunger). (picture)
16. Using a new syringe for each animal, gently insert 0.8ml of PBS/5mM EDTA and remove the liquid to an eppendorf tube. Separate the first wash into a eppendorf tube (This is generally called "Lavage")
17. Repeat the last step 4x more. Each time add fresh PBS for the wash. Combine all the washes from the 2^{nd} time to 5^{th} time to a 15ml tube and put them on ice until you finish.
18. Take the catheter away and rinse it with PBS.
19. Centrifuge the both fractions at 600g for 10mins and recover the supernatant of the first fraction into a new tube and label it as "Cytokines". Discard the supernatant of the other fractions.
20. Resuspend the both cell pellet fraction in 1ml of freezing buffer (10% DMSO, 10% FBS 80% DMEM) and label it as "Lavage".
21. Freeze it at -80C, and transfer the cells to -150ºC freezer the next day.

Lung procedure

22. Insert the needle in the left ventricle and pump 30ml of PFA or Formalin.

23. Carefully insert the “formalin labeled” catheter and inject 2ml of 4%PFA or 10%Formalin to inflate the lungs, let the lung sit for 10min (so that when you remove the lung, it’s already fixed. Even the lung collapse when you take out the lung, it’s already preserved). (make sure there is no leaking in the trachea).

24. Put the lung still connected with the heart in 10% formalin overnight.

Embedding in Paraffin

Day1: (4.5hrs)

25. 10% Buffered Formalin 24 hours

26. PBS 3 x 15 min

27. 70%EtOH at 4°C until ready for next step (1hr < t < 1wk)

Day2:

28. If necessary, trim at angle desired for sectioning (flat side will be face down when embedding), remove the heart and suture.

29. 80% EtOH 2 x 30min

30. 90% EtOH 2 x 30 min

31. 95% EtOH 30 min

32. 95% EtOH Overnight at 4°C

33. MELT WAX (100mL of wax / 20ml vial is plenty), note that wax comes in pellets, so solid volume will be roughly 2x liquid volume.

Day3 (6.5 hours)

34. 100%EtOH 2 x 30 min

35. 50% EtOH/ 50% Histoclear 30 min

36. Pipet Histoclear with glass pipets

37. 100% Histoclear 30min

38. 50% Histoclear/ 50% Paraffin Wax 30min

39. In the incubator to keep wax melted

40. 100% Paraffin 3 x 1 hour

41. Fill mold with wax (keep in incubator/heated 56C to 62C). For embedding lungs, use Peel-A-Way Embedding Mold Square-S22.

42. Place organ with the flat, sliced side face down (the posterior side face down)

Sectioning Protocol for Trimming Lungs

43. Size of sample is about 0.5cm
44. Collect about 5-10 representative positions throughout the block
45. 100µm trim between each position
46. 100µm used for general samples (13 positions)
47. 1-2 slides per collection
48. Total: 13-26 slides (depending on slides taken per position)
49. Use H&E staining slides for all
50. CRITICAL 400µm used for samples containing significant tumors in the middle of the lung, at a distance which will yield the largest surface area of the lung
51. 6 slides per collection (do at 2 separate positions), Total: 12 slides
52. Use 2 H&E slides (1 per position), 10 SuperFrost slides (5 per position)
53. 15 H&E slides (13 from general sample, 2 from samples with significant tumors) + 10 SuperFrost slides (from samples with significant tumors) = 25 slides
54. GOAL: To have 15-10 representative, consistent, equidistant sections throughout the entire lung to be stained by H&E, and to have an additional 10 slides from the middle of the lung, which best represent the tumor burden phenotype of the lung, for use in other staining such as for IHC.