Immunohistochemistry: Perilipin (by Frank Ko)  

Tissue Preparation:

1. Fix the bone samples in 4% PFA for 24 hrs at 4°C  
2. Decalcify the samples with 20% EDTA at 4°C for 7 days (change the EDTA daily)  
3. Paraffin processing (histocore)

Before starting:

- Make 1L 1X TBS  
  - 100 mL 10X TBS  
    - 10X TBS (1L): 60.5 g Tris (Fisher BP152)  
      87.6g NaCl (Fisher BP358)  
      pH to 7.5  
      Bring to 1L w/ ~800 mL H₂O  
  - 900 mL H₂O  
- Prepare humid chambers (w/ Whatman paper)  
- Make 4L 1X TNT  
  - 400 mL 10x TBS  
  - 3600 mL dH₂O  
  - 2 mL Tween-20 (Sigma P2287): add while stirring

Staining process: (use TSA kit, Perkin Elmer #NEL700A)

1. Incubate slides at 60°C on slide warmer for at least 1 hr

2. Deparaffinize and dehydrate (use clean solutions!)  
   a. Xylene, 3x 3 min  
   b. 100% EtOH, 2x 2min  
   c. 95% EtOH 2 min

3. Rehydrate in 1X TBS, 5 min RT

4. 3% H₂O₂/MeOH 10 min RT  
   a. 25 ml 30% H₂O₂ + 225 mL MeOH

5. dH₂O, 5 min, RT

6. Equilibrate sections by rinsing with 1X TBS
7. Circle sections with Pap-pen

8. Incubate with TNB at RT in moist chamber for 30 min

9. 1° antibody diluted in TNB, RT in moist chamber for O/N at 4°C
   a. Perilipin (Abcam ab3526) 1:50 dilution in TNB

10. Wash: 1X TNT, 3x 5 mins at RT

11. Biotinylated 2° Ab diluted in TNB, in moist chamber for 30 min
    a. antiRabbit IgG, 1/200 (Vector BA1000)

12. Wash: 1X TNT, 3x 5 min at RT

13. SA-HRP (from kit) 1:100 dilution in TNB, 30 mins at RT

14. Wash: 1X TNT, 3x 5 min at RT

15. Biotynil Tyramid (kit) 1:50 dilution in amplification diluent, 5 min RT

16. Wash: 1X TNT, 3x 5 min at RT

17. SA-HRP (from kit) 1:100 dilution in TNB, 30 min RT

18. Wash: 1X TNT, 3x 5 min at RT

19. For HRP detection, use DAB (VECTOR SK-4100)
   In 5ml dH2O:
     Add 2 drops buffer; mix well
     Add 4 drops DAB; mix well
     Add 2 drops H2O2; mix well
     Add 2 drops NiCl (optional)

20. Incubate until color develops (2-10 mins)

21. Wash 5 mins in dH2O

22. Counterstain (optional)

23. 95% EtOH 2 min

24. 100% EtOH 2x 3 min

25. Xylene 2x 5 min