<u>Quick Histology Protocol for Spleens and Tumors</u>

Total time: 2 days for **Fixation, Dehydration, Embedding, Cutting, and Slide Prep**

Steven Tran 6/16/2017

*Read through the entire protocol before starting it.

**This is a simplified version of the original protocol. For a more detailed guide, please refer to Edward Yang's "Epic Histology Protocol".

***If the entire histology process is started at a reasonable time on harvest day, then you will be able to view the slides under the microscope in two days. For example, if you start Monday morning, you can start viewing on Wednesday morning.

<u>Fixation and Dehydration</u> (1 day + overnight)

Materials needed - have all of this ready before harvesting tissues

- Plates with RP5/TCM/PBS for transporting tissues from mouse room to lab
- Empty plates for soaking tissues in PFA/Sucrose
- 2% PFA (diluted from 16% PFA in **1X PBS**)
- 10% Sucrose (diluted from 10% Sucrose in **1X PBS**)
- 30% Sucrose (stock solution in **1X PBS**)

1. Sacrifice mouse and harvest spleen/tumor and place into labeled wells on the plate in media/PBS.
2. **20min to 1h**: Transfer tissues into another plate filled with **2% PFA.** Cover with **aluminum foil**, and shake at **4 C** for either **20 min for tissues with reporter** or **up to 1 h for tissues without reporter**.
    a. If you fix a tissue with YFP/GFP for more than 20 min, you will lose the reporter signal.
3. **6h**: Aspirate 2% PFA from the wells and replace with **10% Sucrose.** Cover with **aluminum foil**, and shake at **4 C** for **6h**.
4. **Overnight**: Aspirate 10% Sucrose from the wells and replace with **30% Sucrose**. Cover with **aluminum foil**, and shake at 4 C **overnight**.
    a. If in a rush, when the tissue sinks to the bottom of the well, you can move on to the embedding step.

Embedding (1 hr)

Materials needed - prepare all of this while waiting for the shaking steps

- 2-Methylbutane
- Dry Ice
- Cryomolds (plastic molds for storing tissues long term)
- OCT
- 2 Shallow Glass Containers
- Ice bucket
- Plates with tissues in 30% sucrose

1. Label as many **cryomolds** with desired description for as many tissues.
2. Place **dry ice** in **ice bucket** such that there is a flat surface, and then place the **shallow glass containers** directly on the flat dry ice surface.
3. Pour a small amount of **2-Methylbutane** into the **shallow glass containers** just enough to cover the bottom of the container.
4. Wait about **5 minutes** for the 2-Methylbutane to cool down to the the temperature of the dry ice.
5. **For each tissue sample**:
    a. Fill the cryomold halfway full with OCT.
    b. Place cryomold into shallow glass container containing cold 2-Methylbutane.
    c. As the OCT starts to freeze and turn white, place the tissue into the cryomold.
    d. **Immediately** fill the cryomold to the top with OCT.
    e. Wait for all the OCT to freeze completely; if part of tissue still exposed, add OCT on top of the exposed area.
    f. Place cryomolded sample onto the dry ice on the side to maintain frozen state.
    g. Repeat for all samples.
6. **Store at -80 C. This is an indefinite stopping step.**

Staining (half a day with lots of waiting time)

The slides should always be covered/in the dark unless you are moving them around to wash or adding stain. Otherwise, photobleaching occurs.

1. Rinse slides in 1X PBS for **10 minutes**.
2. While the slide is rinsing, thaw enough **universal blocking buffer** for 100uL per sample.
    a. For each sample, 50 uL is used for the blocking step and 50 uL is used to dilute antibodies in.
3. While the slide is still rinsing, make your stain and dilute in **universal blocking buffer**. You can use up to four colors without DAPI or three colors including DAPI, so choose your stain wisely. AF647 and AF488/FITC are bright, while AF594 is very dim. Below are the recommended concentrations based on titrations done with spleens/B16 tumors:
    a. AF647 **CD4, CD8, B220 - 1:100**
    b. AF647 **45.1, 45.2 - 1:75**
    c. AF594 **CD4, CD8 - 1:75**
    d. AF488/FITC **45.1, 45.2, CD4, CD8, B220 - 1:100**
    e. **These will vary according to tissue. But in general, less stain is better to prevent nonspecific staining.
4. Dry slides on paper towels for **5 minutes** or **until almost dry**.
5. Draw dime sized circle of **hydrophobic pap pen** around tissue sample. Draw over the circle **3-4** times to ensure that there is a “wall” of pap pen to prevent the stain from escaping.
6. Dry pap pen for **5 minutes** or **until almost dry**.
7. Add 50uL of **universal blocking buffer** to the dime sized area and incubate for **1 hour.**
8. Rinse slides in 1X PBS for **10 minutes**.
9. Dry slides on paper towels for **5 minutes** or **until almost dry**.
10. Add 50uL of **FC block** to the dime sized area and incubate for **30 minutes**.
11. Dry slides on paper towels for **5 minutes** or **until almost dry**.
12. Add 50uL of **stain** to the dime sized area and incubate for **1 to 2 hours.**
13. Wash 3x in 1X PBS for **5 minutes** each for a total of **15 minutes**. Replace PBS after each wash.
14. Dry slides on paper towels for **5 minutes** or **until almost dry**.
15. Add 50uL of 1:1000 mM DAPI for **5 minutes**.
16. Wash 3x in 1X PBS for **5 minutes** each for a total of **15 minutes**. Replace PBS after each wash.
17. Dry slides on paper towels for **5 minutes** or **until almost dry**. Wipe remaining pap pen residue with a kimwipe without touching the tissue (washing should have gotten most of it off though).
18. Add 1 drop or recommended volume of antifade mounting medium to the sample, cover with a glass coverslip without producing bubbles, and seal completely with nail polish to prevent the antifade from leaking and ruining the sample.
19. Dry slides for **2 hours** or **overnight** before viewing.