Baylor/Rice SATC Protocol

Title: SATC Tissue Processing, Imaging, and Analysis
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Submitted: 10/4/21

Tissue Preparation for Imaging

1) Remove the organ or tissue sample from the euthanized mouse and remove extraneous material.

2) If necessary, remove a small section, and freeze immediately in liquid nitrogen for molecular analysis.

3) Place organ in 10 ml of freshly prepared 4% paraformaldehyde (in PBS) in a 20 ml vial.

4) Incubate for 20 - 24 hours at 4˚C with gentle agitation.

5) Remove paraformaldehyde solution and replace with 30% sucrose in PBS.

6) Incubate for 20 - 24 hours at 4˚C with gentle agitation.

7) Embed tissues in OCT in trays, marking orientation for sectioning.

8) Freeze on dry ice, and store at -80˚C.

Imaging

1) Section frozen tissue blocks at 14 microns, and place three non-consecutive sections on one slide.

2) Mount with DAPI stain.

3) Image slides on Zeiss Axio Scan.Z1 scanner, using the 20X objective and Cy5 (for tdTomato) and DAPI fluorescent filters.

Image processing

1) Open all .czi files from a tissue in Zen Lite (Blue)

2) Under the Graphics menu, add Scale Bar for each image. Adjust size and placement as necessary.

3) At the Channels line under the Dimensions tab at the bottom of the screen, change the color of the Cy5 channel from Red to the Gold LUT.

4) Under the Display tab at the bottom of the screen, hit the Min/Max button to adjust the range of the histogram. Highlight the gold Cy5 channel and note the White value. Repeat for all the images.

5) Manually adjust the White value from the gold Cy5 channel in all images until it matches the highest values in the tissue set.

6) Save the processed image as .czi in a separate “Processed images” folder

7) To export the image, switch tabs on the left to Processing. Choose single image, and under Method, choose Image Export. Set the file type to PNG. Re-size the image to 20%. Ensure Burn-in Graphics is checked. Click the target button next to the Zoom setting to re-size the scale bar after the image was re-sized. If necessary, choose Define Subset, and choose a desired scene. Next to Export to, choose the destination folder. Click the Apply button to export the image. For subsequent images, only clicking the target button should be necessary.

Analysis

1) Examine images using ZEN software. Note:
   a) Presence or absence of tdTomato (or other relevant fluorophore) signal
   b) Structural integrity of organ, based on DAPI staining
   c) Attempt to identify type of cell expressing tdTomato
   d) Attempt to identify a pattern of tdTomato expression

2) Quantify extent of tdTomato fluorescence using ImageJ
   a) Count nuclei using Threshold/Binarize/Watershed algorithms, or manually
   b) Count tdTomato positive cells, either manually, or using Threshold/Binarize/Watershed algorithms
   c) Report data as percentage of positive cells per nuclei

Recipes

4% Paraformaldehyde

In fume hood:

1. Heat 70 ml of dH_2O to 60° C. **DO NOT OVERHEAT**!
2. Add 4 g. Paraformaldehyde and stir bar, cover and stir at 60° C.
3. Add 1 drop of 2N NaOH.
4. Keep stirring until solution clears with a few particles. Should be no more than 30 min.
5. Remove from heat. Add 10ml 10xPBS.
6. Bring pH of solution to 7.2 with 1% HCl (about 1 ml).
7. Add dH_2O to final volume (100 ml).

8. Filter (40 micron) and cool to 4° C on ice. Store at 4° C wrapped in foil.

Good for 2 weeks. Monitor for microorganism growth

30% sucrose

1. 5ml 10xPBS
2. 25ml dH_2O
3. 15g sucrose
4. dH_2O up to 50 ml
5. Filter (20 micron). Store at 4° C wrapped in foil.

Good indefinitely. Monitor for microorganism growth