mAb3D Immunohistochemistry Data Production

Overview

Monoclonal antibodies (mAbs) were screened via immunohistochemistry on 100µm-thick sagittal mouse brain sections. Tissue sections were processed and prepared using conditions mimicking those for whole-mount staining and imaging. Results provide indication of antibody's suitability for use in whole-mount. Tissue preparation and immunohistochemistry were performed manually, and pipeline protocols have been refined to balance both robustness and simplicity for optimal results based on our observations and experience.

Antibody Selection

Monoclonal antibodies were obtained against a broad range of endogenous proteins present in the brain, including transcription factors, interneuron subtype markers, and non-neuronal markers. Thank you to our collaborators at JHU/CDI Labs Protein Capture Reagent Program and at NeuroMab for providing mAbs. Additional mAbs were obtained from the Developmental Studies Hybridoma Bank and from various commercial vendors.

Sample Collection

Screening was performed on standard PFA-fixed brain tissues from adult wild type mice (CD1 and C57/B6, male and female) following approved institutional protocols. On Day 1, mice were transferred from housing facility to procedure room; care was taken to minimize animal stress during the move. Mice were allowed to acclimatize to new environment for 1-3 hours prior to procedure commencement. Mice were anesthetized using isoflurane delivered via VetEquip RC2 anesthesia system and transcardially perfused using Gilson Minipuls 3 peristaltic pump at 9mL/min. The cardiovascular system was perfused first with 10mL of 1x PBS with 10µg/mL heparin, then 60mL of 4% paraformaldehyde with 2µg/mL heparin, then finally with 30mL of 1x PBS with 10µg/mL heparin.

Brains were carefully dissected out of the skulls and fixed overnight in 4% PFA while nutating at 4°C. On protocol Day 2, brains were washed back into 1xPBS/0.02%NaN_3 while nutating at room temperature, for 3 washes total.

Blocking

Either immediately preceding or following the delipidation step, brains were washed in B1n solution for 1-2 days (3 washes/day) for blocking and antigen retrieval.

Delipidation

Brains were delipidated by washing up and down a methanol gradient. Specifically, brains were cut into hemispheres and washed at room temperature with nutation for a half hour each in 100% B1n, 20%/40%/60%/80% MeOH/B1n, 100% MeOH, 100% dichloromethane, 100% MeOH, 80%/60%/40%/20% MeOH/B1n, 100% B1n. Hemispheres were then washed to 1xPBSw/NaN_3 for long-term storage.

Sectioning

Hemispheres were embedded in 2% Agarose/1xPBS and sectioned to 100µm on a Leica VT1000 S vibratome. Hemispheres were cut from medial side to lateral side, yielding approximately 36 total sagittal sections per hemisphere (with section #1 the most medial section and section #36 the most lateral section). Sections were placed in well-plates with 1xPBS/0.2% NaN_3 for long-term storage.

Immunolabeling

Tissue sections for immunohistochemistry were individually selected to optimize assessment of each protein target. The expected target distribution for each mAb was estimated based on the corresponding gene expression profile provided in the Allen Brain Atlas In-Situ Hybridization database. The anatomical sagittal range possessing high expression for that protein target was determined; a single sagittal section within that range was assigned to the corresponding mAb.

Staining was performed according to either the Sequential Method or the 1:1/1:2 Preconjugation Methods described in “mAb3D-Protocols_ThickSectionBasedAntibodyScreening”, with 1µg mAb per 800µL PTxwH staining buffer. For mAbs originally produced in mouse, IgG subclass-specific secondary antibodies were used. Secondary antibodies used were pGt-Ms-IgG1-A568 (ThermoFisher, A21124, Lot#1964384), pGt-Ms-IgG1-A594 (Jackson, 115-585-205, Lot#137745), FAB-Gt-Ms-IgG1-A647 (Jackson, 115-607-185, Lot#149375), pGt-Ms-IgG2a-A594 (Jackson, 115-585-206, Lot#136566), FAB-Gt-Ms-IgG2a-A647 (Jackson, 115-607-186, Lot#14834), pGt-Ms-IgG2b-A568 (ThermoFisher, A21144, Lot#2045340), pGt-Ms-IgG2b-A594 (Jackson, 115-585-207, Lot#140267), FAB-Gt-Ms-IgG2b-A647 (Jackson, 115-607-187, Lot#149378), and FAB-Gt-Rb-A647 (Jackson, 111-607-008, Lot#146584).

Following staining, sections were mounted on slides using ACB mounting media. Slides were sealed the next day and imaging commenced the day following.

Imaging

Wide-field fluorescence imaging was performed on a Keyence BZ-X810 using Chroma ET-mCh/TR and ET-CY5 NX filter cubes. Images were stitched using Keyence BZ-X810 Analyzer software and compressed and adjusted using ImageJ.

Assessment

During screening process, images were reviewed manually by three independent reviewers to evaluate presence of specific immunolabeling signal. Additional quantitative measurements will follow.

In Progress

1. Cross-validation: with the accumulated list of positive mAbs, we will perform cross validation with multiple mAb clones against the same protein target (or same cell population) to evaluate their consistency.
2. Multiplex capability: we will incorporate available multiplex labeling techniques to provide a compatible reference list over brain targets.
3. Whole mount: whole brain (or hemisphere) profiling will be done for all validated mAbs for complete 3D pattern reference.

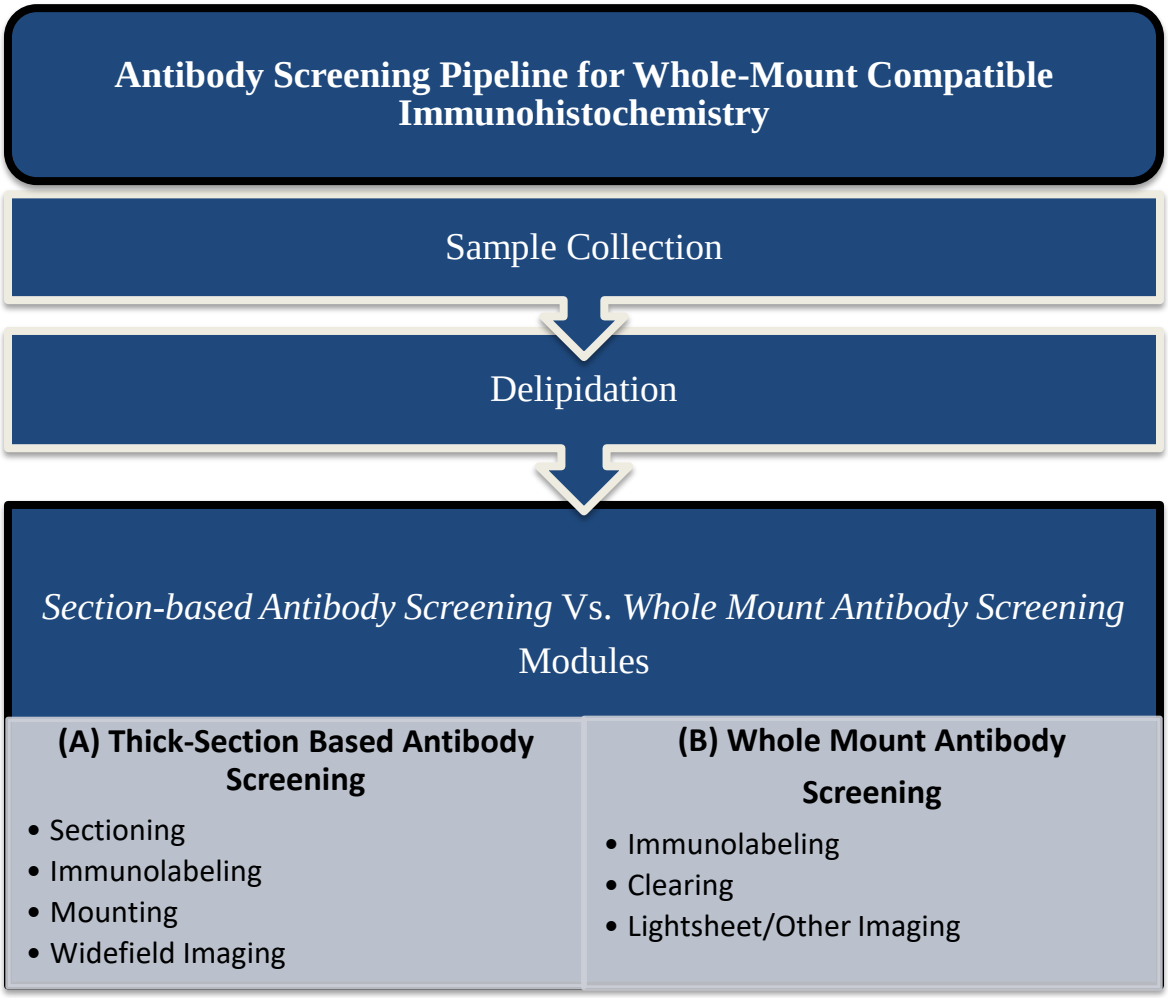

Diagram 1. *Antibody validation pipeline workflow*