Spatial multiomic assay for studying interneuron heterogeneity in the brain

Chengxin Zhou[1], Yifan Wang[1], Debia Wakhloo[1], Anushka Dikshit[1], Li-chong Wang[1], Maithreyan Srinivasan[1]

1. Advanced Cell Diagnostics, a Bio-techne brand, Newark, CA, USA

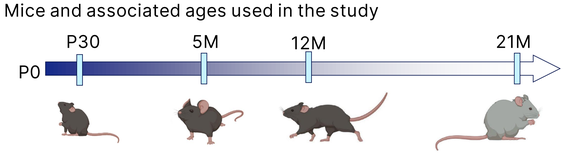
INTRODUCTION

We have a next-generation, protease-free RNAscope spatial multiomics workflow. The newly developed novel protease-free RNAscope workflow is optimized for same-slide detection of both protein and RNA biomarkers with unparalleled sensitivity and tissue morphology. Here, we demonstrated the simultaneous detection of 3 neuron-specific RNA markers and various neuronal and non-neuronal protein markers in FFPE mouse brain sections. The current assay revealed the spatial distributions of interneuron subtypes in the mouse brains and their changes over the 2 years life span. The robust and specific NeuN IF staining in aging mouse brains also provided a metric for the total neuron change in the juvenile and aged brains. We believe the new protease-free pretreatment method developed by ACD can provide a useful platform for multiomic detections of RNA, DNA and protein targets in the same tissue sections and therefore provides a better correlation between the different biomolecules in the biological cascades.

METHODS

Mouse FFPE brain sections were collected from C57BL/8j mice from 4 different postnatal age groups: 1 month, 5 months, 12months, and 21 months. The brain sections were baked and dewaxed, cleaned in ethanol for 30min, and boiled with RNAscope target retrieval buffer for 30min at 100 Celsius. The slides were subsequently treated with ACD manual PretreatPro reagent for 30min at 40 Celsius (Fig.1). After the pretreatment, the slides were hybridized with ISH probes targeting mouse *Pvalb*, *Sst*, and *Vip* mRNAs for 2 hours at 40 Celsius. The fluorescent ISH signals were then developed using our RNAscope Multiplex v2 detection kit. Immediately following the ISH detection, the slides were incubated with various primary antibodies against Cd31 (1:200), Cd8 (1:200), Cd3e (1:200), and NeuN (1:1000), diluted in PBS+0.1%BSA respectively, for overnight at 4 Celsius. Slides were washed in PBS to remove free antibodies then stained with secondary antibodies conjugated with AlexFluoro750 fluorophore. The slides were mounted with ProlongGold mounting media and imaged under a 20X epifluorescent scanner (Phenoimager HT) with multispectral capacity. For image quantifications, QuPath software suite was used to analyze the percentages of *Vip, Sst,* or *Pvalb* positive neurons in the different brain cortex.

Mice and associated ages used in the study



RNAscope manual Multiplex v2 Protease-free workflow schematic

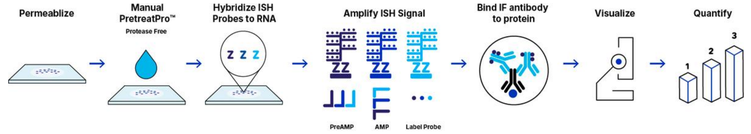


Figure 1. A newly developed protease-free pretreatment workflow that enables simultaneous RNAscope ISH staining for RNAs and conventional IHC/IF/ICC staining for proteins on the same tissue slides.

RESULTS

New protease-free Multiplex v2 assay can be used with frozen tissues

Fixed frozen mouse hindbrain

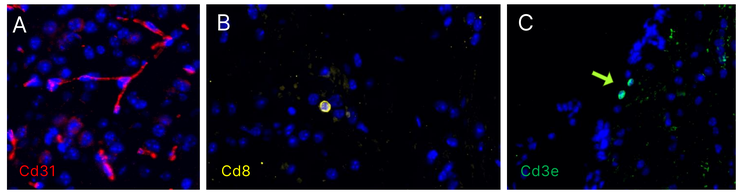


Figure 2. Sequential IF staining in fixed frozen mouse hindbrain and meninges following RNAscope protease-free Multiplex v2 pretreatment. PretreatPro treated tissues showed robust immunopositivity against the two protease-sensitive epitopes Cd31 (A) and Cd8 (B) in the tissues as well as the protease-resistant epitope Cd3e (C), suggesting our new pretreatment method supports both ISH and IHC staining.

New protease-free workflow rescues NeuN antibody signal in FFPE tissue

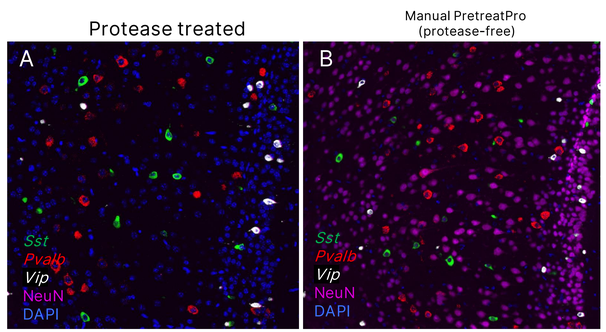


Figure 3. Spatial multiomic staining with RNAscope Multiplex (3 mRNAs) + sequential IF (NeuN) in FFPE mouse brain cortex. Although our RNAscope protease pretreatment boosted highly sensitive and specific ISH detections in the mouse brain (A), NeuN epitopes were degradated by the protease and led to false immuno-negativity in the same slide IF staining. In contrast, the new PretreatPro pretreatment allowed RNAscope Multiplex staining and a sensitive subsequent IF staining for NeuN protein (B).

RNAscope demonstrates spatial changes in transcriptional expressions of *Pvalb* and *Vip* in aged mouse cortical interneurons

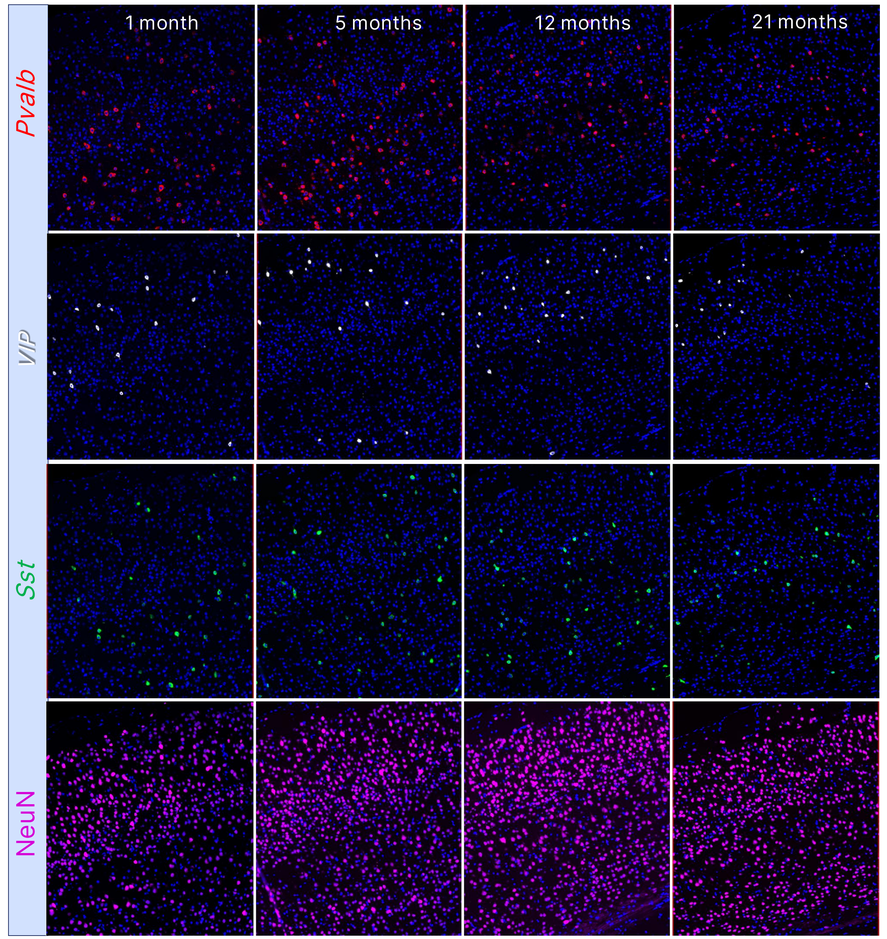


Figure 4. Representative images of protease-free Multiplex v2 ISH staining and sequential NeuN IF staining in mouse brain cortexes of different postnatal age groups. First 3 rows: RNAscope Multiplex v2 FISH signals (Row1=Pvalb, Row2=Vip, Row3=Sst). Bottom row: NeuN sequential immunofluorescent staining on the same slides with mouse anti-NeuN monoclonal antibody.

CONCLUSIONS

- RNAscope Multiplex v2 manual assay provides detection of RNA and protein targets simultaneously with high signal intensity provided by TSA amplification.
- The new PretreatPro reagent allows for a protease-free solution to successfully detect target proteins without compromising on signal quality.
- This assay was successfully used to analyze interneuron marker changes with aging in FFPE mouse brain tissues.