Highly multiplexed spatial mapping of diverse gene signatures and cell type specific markers across mouse brain using the RNAscope™ HiPlexUp in situ hybridization assay

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Introduction

Transcriptomic studies have ushered into an era of single cell technologies that are crucial for both classifying and characterizing known and novel cell populations of complex heterogenous tissues. However, such techniques are limited using dissociated cells that result in the loss of spatial organization of these cell populations, thus requiring a highly multiplexed approach that can interrogate gene expression at a single cell resolution while retaining the morphological context.

We sought to utilize the RNAscope HiPlex and HiPlexUp assay and reagents to spatially map diverse gene signatures identified by single cell RNA sequencing (scRNAseq) and known neuronal cell-type specific markers. With the previous HiPlex-12 reagent workflow, we spatially mapped the novel medium spiny neuronal (MSN) D1 and D2 subtypes identified by scRNAseq (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016). Our new HiPlexUp reagent workflow enables for simultaneous detection of up to 48 targets on a single tissue section. This iterative target detection process allows for a highly sensitive and specific mRNA visualization without compromising the structural integrity of the tissue morphology. In addition to visualizing the previously confirmed major and minor D1 and D2 MSN subtypes (*Drd1, Htr7, Pcdh8, Th, Synpr, Crym, Wfs1, Calb1, Drd1, Cnr1, and Foxp1*) we also visualized neuronal markers *(Fam84b, Lhx6, Crh, Vip, Tac1, Moxd1, Slc6a1, Sst, Chrna2, Gad2, Slc32a1, Gria1, Grin1, Cx3cr1, Chrm1, Chrm3, Oprd1, Chrnb2, Gabr2, Vglut1, Vglut2, Gad2, Calb2, and Pvalb*) and ubiquitously expressed genes (*Polr2a, Ppib, Ubc, Hprt, ActB, Tubb3, Bin1, Ldha, Gapdh, Pgk1, Bhlhe22, and Cplx2*) of the mouse brain. The markers were expressed across various region of interests such as the olfactory bulb, caudate putamen, hypothalamus and cerebral cortex. These diverse expression patterns serve as an invaluable tool is understanding the region-specific functional significance of these neuronal genes.

Lastly, we demonstrated the utility of our image registration software resolving this 48-plex data by zoning into our targets of interest. In conclusion, Single-cell transcriptomics combined with spatial mapping by the RNAscope technology is well suited for resolving heterogeneous tissues at cellular resolution and providing insights into cellular organization and function of diverse cell types in healthy and disease states.

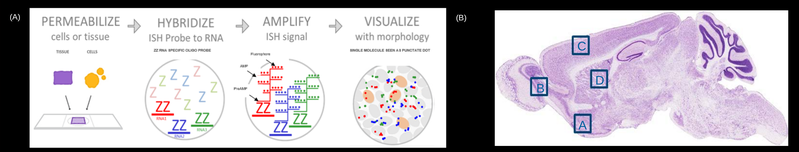
RNAscope Technology and Experimental Design

Tissue preparation: Sagittal sections (10 µm thick) of fresh frozen brain tissue from 6 weeks old C57/BL6 male mice were purchased from Acepix.

RNAscope™ in situ hybridization: The RNAscope HiPlex Assay (HiPlex 12 reagent kit) and HiPlexUp Ancillary upgrade kit from Advanced Cell Diagnostics were used for gene expression analysis in the brain

Imaging and quantification: Images were acquired using the Vectra Polaris Scanner and microscope. Composite images were generated using RNAscope HiPlex image Registration Software.

Figure 1. (A)The RNAscope Assay workflow and (B) Regions of Interest analyzed with the HiPlexUp assay



Results

RNAscope HiPlex and HiPlexUp Assay

Figure 2. *In situ* hybridization workflow using the RNAscope HiPlexUp assay and the RNAscope HiPlex image registration software.

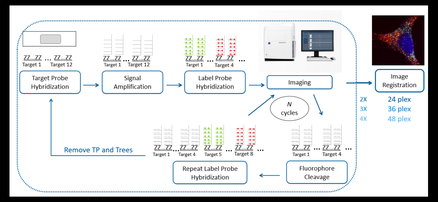


Figure 3. Selected markers to demonstrate the utility of the RNAscope HiPlexUp Assay in simultaneously detecting 48 markers comprising of commonly used neuroscience related probes, positive control targets and markers previous identified by scRNAseq

R1 Plex1-12	Channel	Label Probe	Positive
Cycle 1	T1	488	Mm-Polr2A-T1
	T2	550	Mm-Ppib-T2
	T3	647N	Mm-Ubc-T3
	T4	750	Mm-Hprt1-T4
Cycle 2	T5	488	Mm-Actb-T5
	T6	550	Mm-Tubb3-T6
	T7	647N	Mm-Bin1-T7
	T8	750	Mm-Ldha-T8
Cycle 3	T9	488	Mm-Gapdh-T9
	T10	550	Mm-Pgk1-T10
	T11	647N	Mm-Bhlhe22-T11
	T12	750	Mm-Cplx2-T12

R2 Plex13-24	Channel	Label Probe	Positive
Cycle 1	T1	488	Mm-Htr7-T1
	T2	550	Mm-Pcdh8-T2
	T3	647N	Mm-Slc32a1-T3
	T4	750	Mm-Th-T4
Cycle 2	T5	488	Mm-Synpr-T5
	T6	550	Mm-Crym-T6
	T7	647N	Mm-Wfs1-T7
	T8	750	Mm-Calb1-T8
Cycle 3	T9	488	Mm-Drd1a-T9
	T10	550	Mm-Drd2-T10
	T11	647N	Mm-Cnr1-T11
	T12	750	Mm-Foxp1-T12

R3 Plex24-36	Channel	Label Probe	Positive
Cycle 1	T1	488	Mm-Fam84b-T1
	T2	550	Mm-Lhx6-T2
	T3	647N	Mm-Crh-T3
	T4	750	Mm-Vip-T4
Cycle 2	T5	488	Mm-Tac1-T5
	T6	550	Mm-Moxd1-T6
	T7	647N	Mm-Grin2C-T7
	T8	750	Mm-Slc6a1-T8
Cycle 3	T9	488	Mm-Calb2-T9
	T10	550	Mm-Sst-T10
	T11	647N	Mm-Chrna2-T11
	T12	750	Mm-Gad2-T12

R4 Plex37-48	Channel	Label Probe	Positive
Cycle 1	T1	488	Mm-Gria1-T1
	T2	550	Mm-Grin1-T2
	T3	647N	Mm-Cx3cr1-T3
	T4	750	Mm-Chrm1-T4
Cycle 2	T5	488	Mm-Chrm3-T5
	T6	550	Mm-Oprd1-T6
	T7	647N	Mm-Chrnb2-T7
	T8	750	Mm-Gabra2-T8
Cycle 3	T9	488	Mm-Vglut1-T9
	T10	550	Mm-Vglut2-T10
	T11	647N	Mm-Gad1-T11
	T12	750	Mm-Pvalb-T12

Figure 4. Spatial mapping of all the *Drd1a/Drd2* striatal sub-populations in mouse brain. 12 target probes were used to simultaneously detect *Drd1a*, *Drd2*, *Foxp1*, *Pcdh8*, *Synpr*, *Htr7*, *Meis2*, *Calb1*, *Crym*, *Cnr1*, *Wfs1*, and *Th* using the HiPlex 12 Ancillary reagent kit

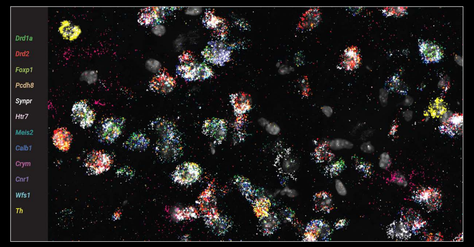


Figure 5. Detection of the major and minor D1 and D2 subtypes simultaneously in the mouse brain. (A) Visualization of the D1 (Red) and D2 (Green) major and minor populations on the same sagittal section. (B) Detection of cells co-expressing *Drd1a* and *Drd2* (marked by yellow circles), which could be done in the same section/field of view as in (A) using the HiPlex Image registration software.

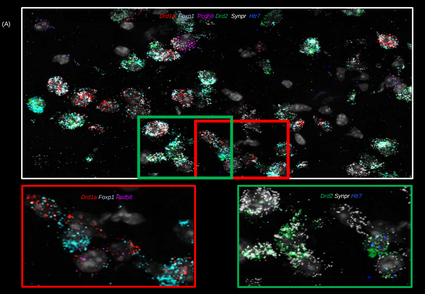
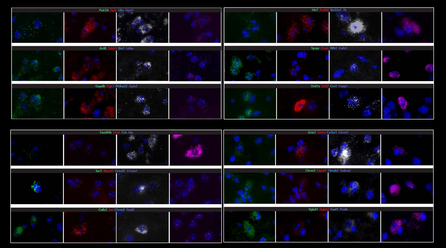


Figure 6. Spatial mapping of all neuroscience related probes in mouse brain. Simultaneous detection of 48 neuroscience related targets visualized using the RNAscope HiPlexUp assay at a single cell level and sorted individually using the HiPlex image registration software



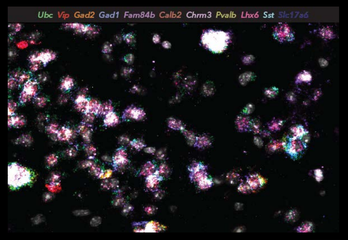
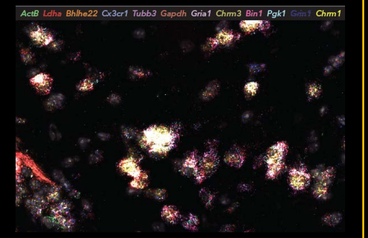


Figure 7. Neuroscience related markers in mouse brain visualized in the Hypothalamus . RNAscope HiPlexUp assay visualized several neuronal markers across multiple regions of interest in the sagittal section of mouse brain. *Ubc, Vip, Gad2, Gad1, Fam84b, Calb2, Chrm3, Pvalb, Lhx6, Sst* and *Slc17a6* were identified in the Hypothalamus region

Figure 8. Neuroscience related markers in mouse brain visualized in the Olfactory Bulb . RNAscope HiPlexUp assay visualized several neuronal markers across multiple regions of interest in the sagittal section of mouse brain. *ActB, Ldha, Bhlhe22, Cx3cr1, Tubb3, Gapdh, Gria1 Chrm3, Bin1, Pgk1, Grin1* and *Chrm1* were identified in the Olfactory Bulb region



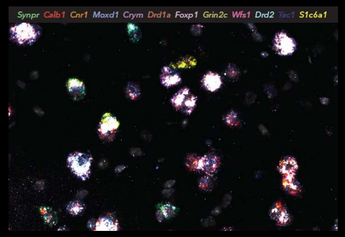
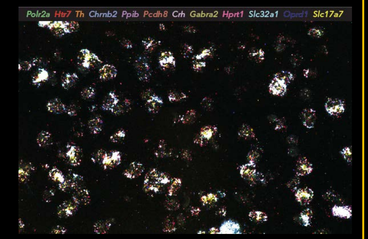


Figure 9. Neuroscience related markers in mouse brain visualized in the Cerebral Cortex. RNAscope HiPlexUp assay visualized several neuronal markers across multiple regions of interest in the sagittal section of mouse brain. *Synpr, Calb1, Cnr1, Moxd1, Crym Drd1a, Foxp1, Grin2c, Wfs1, Drd2, Tac1* and *Slc6a1* were identified in the Cerebral cortex region

Figure 10. Neuroscience related markers in mouse brain visualized in the Caudate and Putamen. RNAscope HiPlexUp assay visualized several neuronal markers across multiple regions of interest in the sagittal section of mouse brain. *Polr2a, Htr7, Th, Chrnb2, Ppib, Pcdh8, Crh, Gabra2, Hprt1, Slc32a1, Oprd1* and *Slc17a7* were identified in the Caudate and Putamen region



Conclusion

- scRNA-seq is a powerful approach to cell type discovery and classification, however, the resulting cell types and markers require confirmation and mapping with spatial context.
- Multiplexed RNAscope ISH complements scRNA-seq experiments by putting scRNA-seq findings in tissue context at single-cell resolution and single-molecule sensitivity.
- The new RNAscope HiPlex technology enables simultaneous visualization of up to 48 targets and multiple cell types and subtypes on the same tissue section, providing a powerful new tool for neuroscience and beyond.

References

- Gokce O, Stanley GM, Treutlein B, Neff NF, Camp JG, Malenka RC, Rothwell PE, Fuccillo MV, Sudhof TC, Quake SR. Cellular Taxonomy of the Mouse Striatum as Revealed by Single-Cell RNA-Seq. *Cell Rep*. 2016;16(4):1126-1137.
- Allen Mouse Brain Atlas, 2004.