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

Imaging

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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 by the use of 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