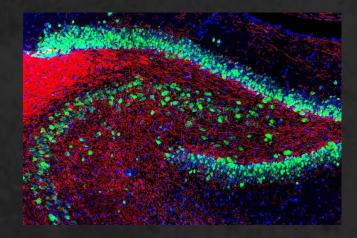
Multiplexed Imaging For Brain Tissue

Mohamed Masoud

TReNDS Reading-Group Oct 2022

Motivation

Staining of brain cell types is critical not only to identify brain cell structure but to identify specific cells in the brain.



Why Multiplex?

Multiplex techniques enable neuroscientists to measure multiple molecular targets, simultaneously, in a single experiment.

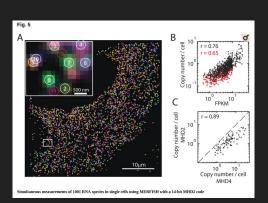
This technique facilitates molecular profiling of individual cells in the context of a tissue, thereby providing a snapshot of cellular function.

Antibody-based detection of proteins can reveal the localization of a particular protein, suggesting a particular function. For example, in neurons, <u>NeuN</u> is localized to the nucleus.

Using multiplexing techniques, researchers can examine the protein's co-localization of their favorite protein. For example, colocalization of their protein of interest with a postsynaptic protein like PSD95 can suggest a postsynaptic function.

Multispectral Image Support

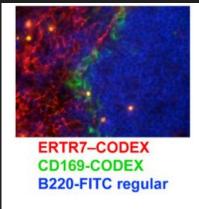
-				The fact of the	# of Samples	Constitution of the contract o
Assay Name	Biological Enti	Assay Type	Bulk or Single	File Format(s)	planned	Median File Size
H&E	histology	imaging	bulk	.svs (aperio)ir	230	50 MB/svs
CODEX	protein	imaging	single cell	.tiff	30	3Gb/tiff 10-300 files per sample
Ex-SEQ	RNA	imaging	single cell	.tiff	30	5 gb/tif, 20 tiffs per sample
MERFISH	RNA	imaging	single cell	tiff, txt	30	Should be similar to CODEX
MIBI	protein	imaging	single cell	.tiff, msdf (raw)	30	< 50mb/tiff. <500mb/msdf
Spatial transcri	RNA	NGS+imaging	near-single cell	.tif, .fastq, .tsv	30	14 Gb (raw imaging), 200 Mb (processed imaging)



Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging $\,\star\,$

Goltsev Y, Samusik N, Kennedy-Darling J, Bhate S, Hale M, Vazquez G, Black S, Nolan GP

Cell, 2018 – Journal Article ---



RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells *

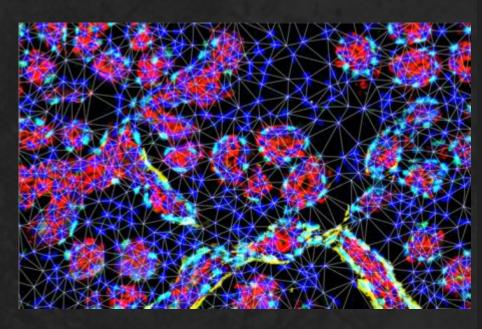
Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X

Science, 2015 - Journal Article ...

Source: Gutman

Spatial Proteomics

Imaging-based spatial proteomics methods enable quantitative and spatial analysis of over 40 protein markers across a whole tissue section at single-cell resolution.



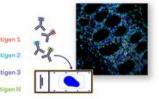
Main Pipeline

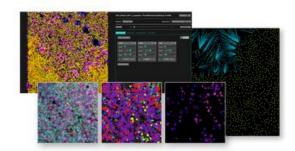












Source: IONPATH



ARTICLE



Check for update

OPEN https://doi.org/10.1038/s41467-021-21735-x

Whole-brain tissue mapping toolkit using largescale highly multiplexed immunofluorescence imaging and deep neural networks

Dragan Maric

1,3,4

Aditi Singh

2, Aryan Mobiny

Aryan

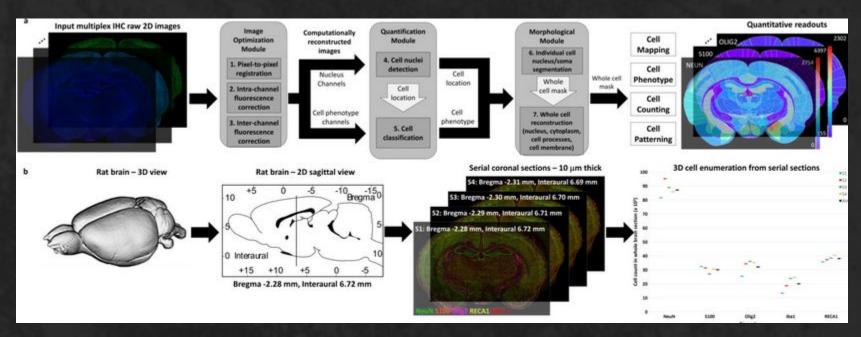
Aryan Mobiny

Aryan

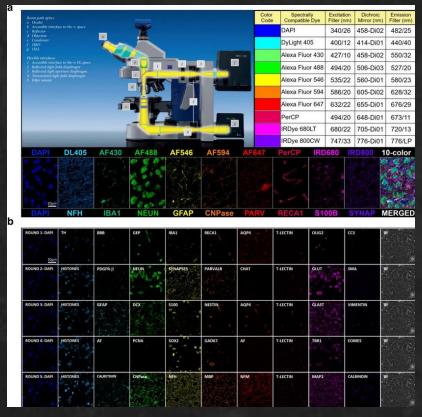
Aryan Mobiny

Aryan M Hien Van Nguyen², Andrea Sedlock¹, Kedar Grama ² & Badrinath Roysam ^{2,4⊠}

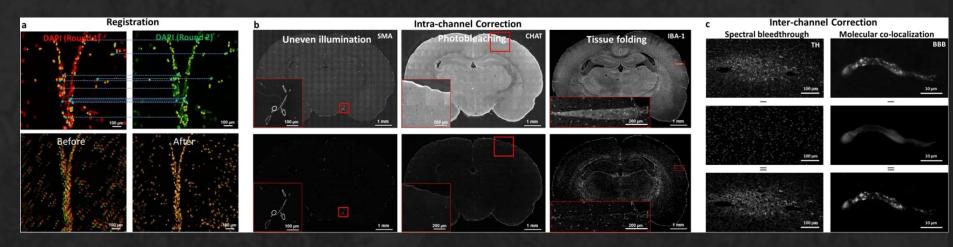
Overview of the whole brain tissue phenotyping pipeline



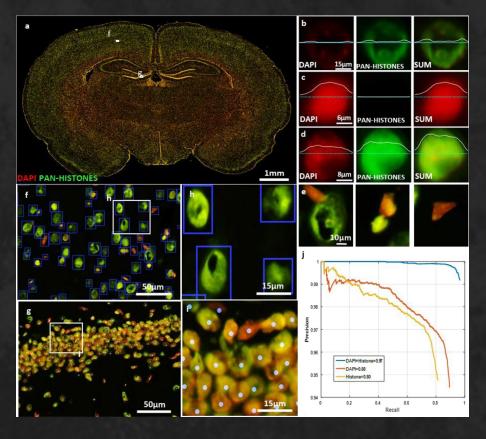
Overview of the multiplex IHC staining and multispectral epifluorescence imaging platform.

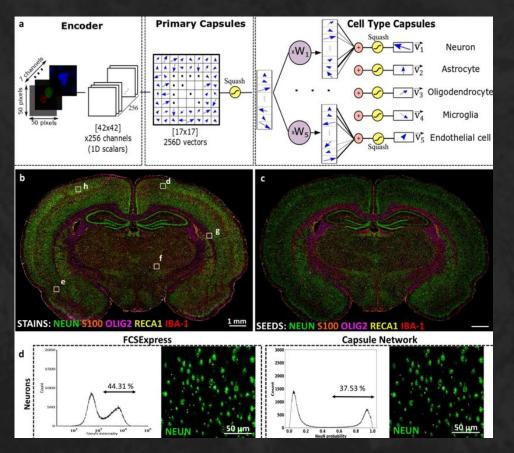


Overview of the post-acquisition image optimization module



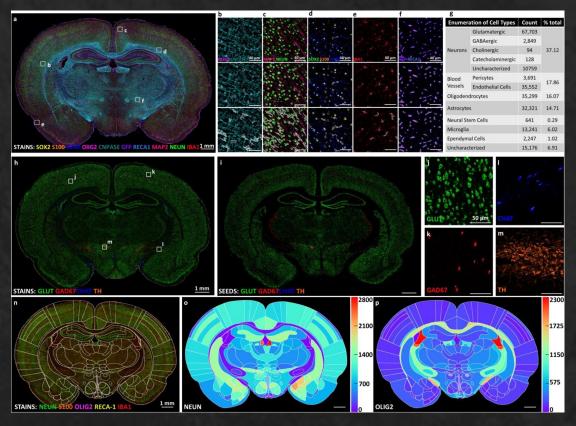
Improved nuclei detection in whole brain images using a multiplexed approach





Source: Maric et al.

Summary of quantification and morphological modules





Panel 1 - Neuroinflammator		lv. t	In 1	DOID II	I	al	la · .	I	. P. C
Antibody	Target/Function	Vendor	Product #	RRID #		Clone	Conjugate	Reactivity	Application
CD3	All T cells	Thermo Fisher Sci	MA1-7630	AB_2073347	Mouse IgG3	RIV9	Unconjugated	Hu, Ms, Rt	IHC-FoFr
CD45R/B220	All B cells	Thermo Fisher Sci	14-0460-82	AB_467275	Mouse IgG2b	HIS24	Unconjugated	Rt	IHC-FoFr
MHC Class II	Dendritic Cells	Thermo Fisher Sci	14-0920-82	AB_467384	Mouse IgG1	HIS19	Unconjugated	Rt	IHC-FoFr
BA1	All Microglia, Macrophages	Wako Chemicals	019-19741	AB_839504	Rabbit IgG	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
BA1	All Microglia, Macrophages	Synaptic Systems	234006	AB_2619949	Chicken IgY	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
IBA1	All Microglia, Macrophages	Synaptic Systems	234004	AB_2493179	Guinea Pig IgG	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
CD68	Activated Macrophages	Abcam	ab125212	AB_10975465	Rabbit IgG	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-FoFr
TMEM 119	Resident Brain Microglia	Synaptic Systems	400211	AB_2814935	Mouse IgG2a	97G1C1	Unconjugated	Rt	IHC-P, IHC-FoF
Panel 2 - Neural Stem Cells	and Astroglial Cell Lineage Phenotyping:		70						7
Antibody	Target/Function	Vendor	Product #	RRID #	Host Ig class	Clone	Conjugate	Reactivity	Application
Human/Mouse/Rat Sox2	Neural Stem Cells, Neural Progenitors	BD Biosciences	561469	AB_10694256	Mouse IgG1	030-678	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Human/Mouse/Rat Sox2	Neural Stem Cells, Neural Progenitors	Thermo Fisher Sci	14-9811-82	AB_11219471	Rat IgG2a	Btjce	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Mouse/Rat Nestin	Neural Stem Cells, Neural Progenitors	Millipore Sigma	MAB353	AB_94911	Mouse IgG1	Rat 401	Unconjugated	Ms, Rt	IHC-FoFr
Vimentin	Astrocytes-Differentiating Stage	Synaptic Systems	172004	AB_2832221	Guinea Pig IgG	Polyclonal	Unconjugated	Ms, Rt	IHC-FoFr
Vimentin	Astrocytes-Differentiating Stage	Synaptic Systems	172006	AB_2800525	Chicken IgY	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Vimentin	Astrocytes-Differentiating Stage	Millipore Sigma	AB5773	AB_11212377	Chicken IgY	Polyclonal	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
5100β	Astrocytes-Fully Mature Stage	Millipore Sigma	MAB079-1	AB_571112	Mouse IgG2a	15E2E2	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
GFAP	Astrocytes-Reactive Stage	Agilent/Dako	556330	AB 396368	Mouse IgG2b	Cocktail	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
GFAP	Astrocytes-Reactive Stage	Thermo Fisher Sci	13-0300	AB_2532994	Rat IgG2a	2.2B10	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
GFAP	Astrocytes-Reactive Stage	Thermo Fisher Sci	PA1-10004	AB_1074620	Chicken IgY	Polyclonal	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
GFAP	Astrocytes-Reactive Stage	Agilent/Dako	Z0334	AB 10013382	Rabbit IgG	Polyclonal	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
GLAST	Astrocytes-Glutamate Transport	Synaptic Systems	250114	AB_2619957	Guinea Pig IgG	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
GLAST	Astrocytes-Glutamate Transport	Miltenyi Biotec	130-095-822	AB 10829302	Mouse IgG2a	ACSA-1	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
AQP4	Astrocytes-BBB Integrity	Abcam	ab9512	AB 307299	Mouse IgG3	[4/18]	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Panel 3 - Oligodendroglial C							, ,		
Antibody	Target/Function	Vendor	Product #	RRID #	Host Ig class	Clone	Conjugate	Reactivity	Application
Olig2	Oligodendrocytes-Gene Transcription	Millipore Sigma	MABN50	AB 10807410	Mouse IgG2a	211F1.1	Unconjugated	Hu, Ms, Rt	IHC-P. IHC-FoF
CNPase	Oligodendrocytes-Soma, Processes	Millipore Sigma	MAB326	AB_2082608	Mouse IgG1	11-5B	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
CNPase	Oligodendrocytes-Soma, Processes	Novus Biologicals	MA5-35002	AB 2848907		CL2887	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Myelin-MAG	Oligodendrocytes-Myelinating	BioLegend	851702	AB 2749880	Mouse IgG2b	B11F7	Unconjugated	Hu, Ms, Rt	IHC-P. IHC-FoF
Myelin-PLP	Oligodendrocytes-Myelinating	Millipore Sigma	MAB388	AB 177623	Mouse IgG2a	PLPC1	Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-FoF
Myelin-MBP	Oligodendrocytes-Myelinating	BioLegend	808402	AB 2564742	Mouse IgG2b	SMI99	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Myelin-MBP	Oligodendrocytes-Myelinating	Millipore Sigma	MAB386	AB_94975	Rat IgG2a	aa 82-87	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-FoF
Panel 4 - Neuronal Cytoarch		1	1	1	1		,-0	1	,
Antibody	Target/Function	Vendor	Product #	RRID#	Host Ig class	Clone	Conjugate	Reactivity	Application
VeuN	Neurons-Fully Mature	Millipore Sigma	MAB377	AB 2298772	Mouse IgG1	A60	Unconjugated	Hu, Ms, Rt	IHC-P. IHC-For
NeuN	Neurons-Fully Mature	Millipore Sigma	ABN78	AB 10807945	Rabbit IgG	Polyclonal	Unconjugated	Hu, Ms, Rt	IHC-P, IHC-For
VeuN	Neurons-Fully Mature	Millipore Sigma	ABN91	AB 11205760		Polyclonal	Unconjugated	Ms, Rt	IHC-P, IHC-Fol
NeuN	Neurons-Fully Mature	Millipore Sigma	ABN90P	AB 2341095	Guinea Pig IgG		Unconjugated	Hu, Mk, Ms, Rt	IHC-P, IHC-For
ACUIA	iveurons runy macule	Millipore Sigma	T8660	AB 477590		SDL.3D10	Onconjugateu	TIU, IVIK, IVIS, IX	mic-r, mic-ror

Supplementary Table 2. Library of spectrally compatible secondary antibodies for 10-plex fluorescence IHC staining

Secondary Antibody	DL405°	DY395XL ^c	AF430°	AF488°	AF546°	AF594°	AF647°	PerCP ^e	IRD680LT ^d	IRD800CW ^d
Goat Anti-Mouse IgG1	115-475-205 ^b	Custom	Custom ^b	A21121°	A21123ª	A21125°	A21240°	115-125-205 ^b	926-68050 ^d	926-32350 ^d
Goat Anti-Mouse IgG2a	115-475-206 ^b	Custom ^d	N/A	A21131°	A21133°	A21135°	A21241°	115-125-206 ^b	926-68051 ^d	926-32351 ^d
Goat Anti-Mouse IgG2b	115-475-207 ^b	Custom ^d	N/A	A21141°	A21143°	A21145°	A21242ª	115-125-207 ^b	926-68052 ^d	926-32352 ^d
Goat Anti-Mouse IgG3	115-475-209 ^b	Custom	N/A	A21151°	N/A	A21155°	115-605-209 ^b	115-125-209 ^b	Custom ^d	Custom ^d
Goat Anti-Mouse IgM	115-475-075 ^b	Custom	N/A	A21042 ^a	A21045°	A21044 ^a	A21238°	115-126-075 ^b	926-68080 ^d	926-32280 ^d
Goat Anti-Rat IgG (min x Ms)*	112-475-167 ^b	Custom ^d	N/A	A11006°	A11081°	A11007°	A21247°	112-126-071 ^b	926-68029 ^d	926-32219 ^d
Goat/Donkey Anti-Rabbit IgG	711-475-152 ^b	Custom	A11064°	A11034°	A11035a	A11037 ^a	A21245°	711-126-152 ^b	926-68023 ^d	926-32213 ^d
Goat/Donkey Anti-Chicken IgG	703-475-155 ^b	Custom	N/A	A11039°	A11040a	A11042 ^a	A21449°	703-126-155 ^b	926-68028 ^d	926-32218 ^d
Goat/Donkey Anti-Guinea Pig IgG	706-475-148 ^b	Custom ^d	N/A	A11073°	A11074a	A11076°	A21450°	706-126-148 ^b	926-68030 ^d	926-32411 ^d
Donkey F(ab') ₂ Anti-Sheep IgG	713-475-147	N/A	N/A	A11015°	A21098a	A11016°	A21448°	713-126-147	N/A	N/A

AF: Alexa Fluor; DL: DyLight; DY: Dyomics; IRD: Infra Red Dye; PerCP: peridinin-chlorophyll-protein; XL:Extra Long.

^aThermo Fisher Scientific; ^bJackson ImmunoResearch; ^cDyomics; ^dLI-COR Biosciences; ^eBD Biosciences.

*Min x Ms: Highly adsorbed for minimal crossreactivity with mouse IgG/IgM antibodies.

Tabulated entries represent vendor product/catologue numbers.

Custom: Custom conjugated by vendor.

Supplementary Table 3. Standard filter selection for 10-color epifluorescence imaging with matrix crosstalk analysis performed by AVR Optics using Semrock SearchLight Spectra Viewer

Standard 10-color	Filter Setup 1	Filte	r Set Specificat	tions ^a	Signal from Primary Set ^b	1970 mg	- Laborator		F	ilter Signa	Crosstalk	(% Transmi:	ssion)°	A.0-2-11		Company of the Compan
Fluorophore	Standard Filter Set	Exciter	Dichroic	Emitter	Fluorescence Signal (mW)	Fluorophore	DAPI	DL405	AF430	AF488	AF546	AF594	AF647	PerCP	IRD680LT	IRD800CW
DAPI	DAPI-5060C	FF01-377/50	FF409-Di03	FF02-447/60	7.03E-07	DAPI	100.00%	201.99%	0.47%	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
DyLight 405	LED-DAPI-B	FF01-378/52	FF409-Di03	FF02-447/60	1.42E-06	DL405	47.68%	100.00%	0.24%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Alexa Fluor 430	LuciferYellow-C	FF01-438/24	FF482-Di01	FF01-538/40	2.81E-07	AF430	0.21%	0.00%	100.00%	31.10%	2.23%	0.00%	0.00%	0.38%	0.05%	0.00%
Alexa Fluor 488	FITC-3540C	FF01-482/35	FF506-Di03	FF01-536/40	6.08E-07	AF488	0.00%	0.00%	15.07%	100.00%	1.63%	0.00%	0.00%	0.17%	0.00%	0.00%
Alexa Fluor 546	TRITC-B	FF01-543/22	FF562-Di01	FF01-593/40	8.61E-07	AF546	0.00%	0.00%	0.17%	0.36%	100.00%	53.54%	0.00%	0.07%	0.00%	0.00%
Alexa Fluor 594	SpRed-B	FF01-586/20	FF605-Di02	FF01-628/32	1.65E-06	AF594	0.00%	0.00%	0.01%	0.01%	5.94%	100.00%	1.84%	0.03%	0.00%	0.00%
Alexa Fluor 647	Cy5-4040-C	FF02-628/40	FF660-Di02	FF01-692/40	6.38E-07	AF647	0.00%	0.00%	0.00%	0.00%	0.07%	9.84%	100.00%	4.67%	29.94%	0.00%
PerCP	N/A*	FF01-488/50	FF640-FDi02	FF01-676/29	7.61E-07	PerCP	0.00%	0.00%	1.41%	0.39%	0.89%	4.30%	0.68%	100.00%	0.55%	0.00%
IRDye 680LT	Cy5.5-C	FF02-655/40	FF685-Di02	FF01-716/40	5.37E-07	IRDye 680LT	0.00%	0.00%	0.00%	0.00%	0.01%	0.45%	44.88%	2.50%	100.00%	0.00%
IRDye 800CW	Су7-В	FF01-708/75	FF757-Di01	FF02-809/81	8.68E-07	IRDye 800CW	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.09%	0.10%	14.06%	100.00%

Standard 10-color	Filter Setup 2	Filte	r Set Specificat	tions"	Signal from Primary Set ^b	984 84 8	X 11 1 1 1 1 1 1	o or a second con-	F	ilter Signal	Crosstalk	(% Transmi	ssion)°	141970000		
Fluorophore	Standard Filter Set	Exciter	Dichroic	Emitter	Fluorescence Signal (mW)	Fluorophore	DAPI	DL405	DY395XL	AF488	AF546	AF594	AF647	PerCP	IRD680LT	IRD800CW
DAPI	DAPI-5060C	FF01-377/50	FF409-Di03	FF02-447/60	7.03E-07	DAPI	100.00%	201.99%	1.37%	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
DyLight 405	LED-DAPI-B	FF01-378/52	FF409-Di03	FF02-447/60	1.42E-06	DL405	47.68%	100.00%	0.68%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Dyomics 395XL	LF405/LP-B	FF01-390/40	Di02-R405	BLP01-405R	6.70E-06	DY395XL	10.78%	17.46%	100.00%	1.75%	3.19%	9.13%	0.52%	7.51%	3.04%	0.00%
Alexa Fluor 488	FITC-3540C	FF01-482/35	FF506-Di03	FF01-536/40	6.08E-07	AF488	0.00%	0.00%	7.80%	100.00%	1.63%	0.00%	0.00%	0.17%	0.00%	0.00%
Alexa Fluor 546	TRITC-B	FF01-543/22	FF562-Di01	FF01-593/40	8.61E-07	AF546	0.00%	0.00%	0.76%	0.36%	100.00%	53.54%	0.00%	0.07%	0.00%	0.00%
Alexa Fluor 594	SpRed-B	FF01-586/20	FF605-Di02	FF01-628/32	1.65E-06	AF594	0.00%	0.00%	0.15%	0.01%	5.94%	100.00%	1.84%	0.03%	0.00%	0.00%
Alexa Fluor 647	Cy5-4040-C	FF02-628/40	FF660-Di02	FF01-692/40	6.38E-07	AF647	0.00%	0.00%	0.03%	0.00%	0.07%	9.84%	100.00%	4.67%	29.94%	0.00%
PerCP	N/A*	FF01-488/50	FF640-FDi02	FF01-676/29	7.61E-07	PerCP	0.00%	0.00%	1.59%	0.39%	0.89%	4.30%	0.68%	100.00%	0.55%	0.00%
IRDye 680LT	Cy5.5-C	FF02-655/40	FF685-Di02	FF01-716/40	5.37E-07	IRDye 680LT	0.00%	0.00%	0.00%	0.00%	0.01%	0.45%	44.88%	2.50%	100.00%	0.00%
IRDye 800CW	Су7-В	FF01-708/75	FF757-Di01	FF02-809/81	8.68E-07	IRDye 800CW	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.09%	0.10%	14.06%	100,00%

[&]quot;Filter set specifications are based on the selection of standard off-the-shelf filter sets that most closely matched the spectral properties of the selected fluorophores, as determined using Semrock SearchLight Spectra Viewer. Standard filter setup 1 was selected for a panel of 10 commonly used spectrally compatible non-tandem flurophores. Standard filter setup 2 was selected to include a custom DY-395XL, a mega-Stokes shift dye, in place of AF430, to improve spectral compatibility with AF488.

Details of each standard filter set specifications are available in SearchLight here: https://searchlight.semrock.com/?sid=f46f2125-6930-4622-86c0-a9d02c5a6f84.

Abbreviations: AF: Alexa Fluor; DAPI: 4',6-diamidino-2-phenylindole; DL: DyLight; DY: Dyomics; FITC: Fluorescein isothiocyanate; IRD: Infra Red Dye; PerCP: Peridinin-chlorophyll-protein; TRITC: Tetramethylrhodamine-isothiocyanate;

bEstimated fluorescence signal in mW of the primary filter set. This parameter was used as a reference in Semrock SearchLight Spectral Viewer to make sure the filter set would produce sufficient signal in comparison with single fluorophore optimized sets.

Filter crosstalk was computed by AVR Optics using Semrock SearchLight Spectra Viewer for the fluorophores and filter specifications listed above using the following microscope setup: Zeiss Axiolmager. Z2 epifluorescence microscope, Excelitas X-Cite 200DC mercury vapor short arc broad spectrum lamp, and Hamamatsu Orca-Flash 4.0 V2 sCMOS camera. Cross-talk values exceeding 10% signal transmission required for optimal 10-color fluorescence imaging are highlighted in yellow.

^{*}N/A: Not Available; Standard PerCP filter set for epifluorescence imaging is not currently available from Smrock. Instead, filters were selected based on a good signal and signal-to-noise without regard to other fluorophores in the 10-color panel.

Supplementary Table 4. Custom filter selection for 10-color epifluorescence imaging with matrix crosstalk analysis performed by AVR Optics using Semrock SearchLight Spectra Viewer

Custom 10-color Filter Setup 1	111	Filter Set Speci	fications ^a	Signal from Primary Set ^b					Filter Signal Cr	osstalk (% Trans	mission) ^c			2 17.77	1,11,13
Fluorophore	Exciter	Dichroic	Emitter	Fluorescence Signal (mW)	Fluorophore	DAPI	DL405	AF430	AF488	AF546	AF594	AF647	PerCP	IRD680LT	IRD800CW
DAPI	FF01-340/26	FF458-Di02	FF01-482/25	6.58E-08	DAPI	100.00%	0.00%	1.69%	2.28%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
DyLight 405	FF01-400/12	FF414-Di01	FF01-440/40	3.53E-07	DL405	5.95%	100.00%	0.14%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Alexa Fluor 430	FF01-427/10	FF458-Di02	FF01-550/32	9.61E-08	AF430	0.33%	0.00%	100.00%	12.28%	7.39%	0.00%	0.00%	0.29%	0.00%	0.00%
Alexa Fluor 488	FF01-494/20	FF506-Di03	FF01-527/20	3.38E-07	AF488	0.00%	0.00%	4.53%	100.00%	0.10%	0.00%	0.00%	0.10%	0.00%	0.00%
Alexa Fluor 546	FF01-535/22	FF560-Di01	FF01-580/23	4.28E-07	AF546	0.00%	0.00%	0.22%	1.12%	100.00%	9.00%	0.00%	0.07%	0.00%	0.00%
Alexa Fluor 594	FF01-586/20	FF605-Di02	FF01-628/32	1.59E-06	AF594	0.00%	0.00%	0.01%	0.01%	5.90%	100.00%	1.89%	0.03%	0.00%	0.00%
Alexa Fluor 647	FF02-632/22	FF655-Di01	FF01-676/29	4.48E-07	AF647	0.00%	0.00%	0.00%	0.00%	0.08%	3.93%	100.00%	4.08%	1.90%	0.00%
PerCP	FF01-494/20	FF648-Di01	FF01-673/11	2.02E-07	PerCP	0.00%	0.00%	0.50%	0.41%	0.62%	3.18%	0.61%	100.00%	0.00%	0.00%
IRDye 680LT	FF01-680/22	FF705-Di01	FF01-720/13	1.87E-07	IRDye 680LT	0.00%	0.00%	0.00%	0.00%	0.00%	0.04%	7.70%	1.79%	100.00%	0.00%
IRDye 800CW	FF01-747/33	FF776-Di01	FF01-776/LP	5.96E-07	IRDye 800CW	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.08%	100.00%

Custom 10-color Filter Setup 2		Filter Set Speci	fications*	Signal from Primary Set ^b					Filter Signal Cro	osstalk (% Trans	mission) ^c				.11.11
Fluorophore	Exciter	Dichroic	Emitter	Fluorescence Signal (mW)	Fluorophore	DAPI	DL405	DY395XL	AF488	AF546	AF594	AF647	PerCP	IRD680LT	IRD800CW
DAPI	FF01-340/26	FF458-Di02	FF01-482/25	6.58E-08	DAPI	100.00%	0.00%	6.87%	2.28%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
DyLight 405	FF01-400/12	FF414-Di01	FF01-440/40	3.53E-07	DL405	5.95%	100.00%	0.16%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Dyomics 395XL	FF01-409/32	FF552-Di02	FF01-572/15	1.06E-06	DY395XL	0.48%	0.00%	100.00%	0.63%	5.00%	0.20%	0.00%	0.03%	0.00%	0.00%
Alexa Fluor 488	FF01-494/20	FF506-Di03	FF01-527/20	3.38E-07	AF488	0.00%	0.00%	1.35%	100.00%	0.10%	0.00%	0.00%	0.10%	0.00%	0.00%
Alexa Fluor 546	FF01-535/22	FF560-Di01	FF01-580/23	4.28E-07	AF546	0.00%	0.00%	0.80%	1.12%	100.00%	9.00%	0.00%	0.07%	0.00%	0.00%
Alexa Fluor 594	FF01-586/20	FF605-Di02	FF01-628/32	1.59E-06	AF594	0.00%	0.00%	0.15%	0.01%	5.90%	100.00%	1.89%	0.03%	0.00%	0.00%
Alexa Fluor 647	FF02-632/22	FF655-Di01	FF01-676/29	4.48E-07	AF647	0.00%	0.00%	0.03%	0.00%	0.08%	3.93%	100.00%	4.08%	1.90%	0.00%
PerCP	FF01-494/20	FF648-Di01	FF01-673/11	2.02E-07	PerCP	0.00%	0.00%	0.39%	0.41%	0.62%	3.18%	0.61%	100.00%	0.00%	0.00%
IRDye 680LT	FF01-680/22	FF705-Di01	FF01-720/13	1.87E-07	IRDye 680LT	0.00%	0.00%	0.00%	0.00%	0.00%	0.04%	7.70%	1.79%	100.00%	0.00%
IRDye 800CW	FF01-747/33	FF776-Di01	FF01-776/LP	5.96E-07	IRDye 800CW	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.08%	100.00%

"Filter set specifications were custom selected from off-the-shelf exciter, dichroic and emitter filters available from Semrock using the following criteria based on calculations performed within Semrock SearchLight Spectral Viewer; (1) for excitation filters, we searched for sufficient overlap between the fluorophore excitation spectrum and the excitation source, and selected filters so as to minimize cross-excitation among the 10 fluorophores of interest without detrimentally compromising the excitation of the specific fluorophore for which the excitation filter was selected. This was an iterative process as the criteria for determining sufficient excitation and minimal crosstalk were the 'primary' fluorescence signal as determined in SearchLight's optimization calculator compared to the 'secondary' signals of the other fluorophores in the panel. As a benchmark, we determined that for a given primary fluorophore, signals of "10E-07 mW were good while signals of "10E-08 mW were acceptable with increased integration times and verified viability experimentally; (2) the dichroic mirror was selected based on proximity to the emission filter, ideally achieving a state of transmission just before the passband of the emission filter, to give an additional level of blocking for unwanted excitation light; (3) emission filters were selected based on overlap with the fluorophore emission spectra and an average optical density (OD) blocking of OD6 across the excitation bandwidth, with as narrow bandpass properties as possible to minimize cross-talk among the 10 fluorophores of interest without detrimentally compromising specific emission signal of the specific fluorophore for which the emission filter was selected, which was also an iterative process in conjunction with the excitation filter; and (4) to improve signal to noise in cases where the excitation and emission filters are very close, where possible based on the available choices, at least a "1% separation in wavelength of the falling edge of the excitation filter

*Estimated fluorescence signal in mW of the primary filter set. This parameter was used as a reference in Semrock SearchLight Spectral Viewer to make sure the filter set would produce sufficient signal in comparison with single fluorophore optimized sets.

Filter crosstalk was computed by AVR Optics using Semrock SearchLight Spectra Viewer for the fluorophores and filter specifications listed above using the following microscope setup: Zeiss Axiolmager.Z2 epifluorescence microscope, Excelitas X-Cite 200DC mercury vapor short arc broad spectrum lamp, and Hamamatsu Orca-Flash 4.0 V2 sCMOS camera. Crosstalk values exceeding 10% signal transmission required for optimal 10-colorfluorescence imaging are highlighted in yellow.

Details of each custom filter set specifications are available in SearchLight here: https://searchlight.semrock.com/?sid=a9cbb659-0301-4866-b249-e6a04d9033c2.

Abbreviations: AF: Alexa Fluor; DAPI: 4',6-diamidino-2-phenylindole; DL: DyLight; DY: Dyomics; IRD: Infra Red Dye; LP: Long Pass; PerCP: Peridinin-chlorophyll-protein; XL:Extra Long.

Supplementary Table 5. List of primary and secondary antibodies for 10-plex IHC staining protocol in Fig 2b

Fluorophore	Channel#	Biomarker	Target Cell/Function	1st step reagent*	2nd step reagent*
DAPI	C1	4',6-diamidino-2-phenylindole (DAPI)	All Nucleated Cells	N/A	DAPI (TFS, D1306, AB_2629482)
DL405	C2	Neurofilament Heavy Chain (NFH)	Neurons-Axonal Processes	Chicken IgY anti-NFH (MS, AB5539, AB_11212161)	Donkey anti-Chicken IgY-DL405 (JIR, 703-475-155, AB_2340373)
AF430	C3	Ionized calcium binding adaptor molecule 1 (IBA1)	Microglia, Macrophages	Rabbit anti-IBA1 (Wako, 019-19741, AB_839504)	Goat anti-Rabbit IgG-AF430 (TFS, A11064, AB_2534111)
AF488	C4	Neuronal Nuclei (NeuN)	Neurons-Nucleus, Soma	Guinea Pig IgG anti-NeuN (MS, ABN90P, AB_2341095)	Goat anti-Guinea Pig IgG-AF488 (TFS, A11073, AB_2534117)
AF546	CS	Glial fibrillary acidic protein (GFAP)	Reactive Astrocytes	Rat IgG2a anti-GFAP (TFS, 13-0300, AB_2532994)	Goat anti-Rat IgG-AF546 (TFS, A11081, AB_2534125)
AF594	C6	2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase)	Oligodendrocytes	Mouse IgG2b anti-CNPase (NB, NBP2-46617, N/A)	Goat anti-Mouse IgG2b-AF594 (TFS, A21145, AB_2535781)
AF647	C7	Parvalbumin	Interneurons-Subset	Sheep IgG anti-Parvalbumin (RDS, AF5058, AB_2173907)	Donkey anti-Sheep IgG-AF647 (TFS, A21448, AB_2535865)
PerCP	C8	Rat Endothelial Cell Antigen 1 (RECA1)	Endothelial Cells	Mouse IgG1 anti-RECA1 (TFS, 14-0360-82, AB_467212)	Goat anti-Mouse IgG1-PerCP (JIR, 115-125-205, AB_2338631)
IDRye 680LT	C9	S100 calcium-binding protein β (S100β)	Pan-Astrocytes	Mouse IgG2a anti-S100β (MS, MAB079-1, AB_571112)	Goat anti-mouse IgG2a-IRDye 680LT (LCB, 926-68051, AB_2783643)
IRDye 800CW	C10	Synaptophysin	Neurons-Synapses	Mouse IgM anti-Synaptophysin (MS, MAB329, AB_94786)	Goat anti-mouse IgM-IRDye 800CW (LCB, 926-32280, AB_2814919)

^{*}Each primary antibody/ligand/dye and secondary antibody was used at 1 µg/ml final concentration (i.e., 1:1,000 dilution of 1 mg/ml stock concentration) with sufficient volume to cover the tissue section (typically 250-500 µL/per section).

Vendor Abbreviations: ABC: Abcam; BL: BioLegend; JIR: Jackson ImmunoResearch; LCB: Li-Cor Biosciences; MS: Millipore Sigma; NB: Novus Biologicals; RDS: R&D Systems; TFS: Thermo Fisher Scientific.

Reagent information in parenthesis (by order listed): Vendor, Product#, RRID#.

RRID#: Research Resource Identifier.

	Round#Channel#	nd secondary antibodies for 50-plex IHC staining protoco	Target/Function	1st step reagent*	2nd step reagent*
DAPI	R1C1	4',6-diamidino-2-phenylindole (DAPI)	All Nucleated Cells	N/A	DAPI (TFS, D1306, AB 2629482)
L405	R1C2	Tyrosine Hydroxylase (TH)	Neurons-Catecholaminergic	Chicken IgY anti-TH (ABC, ab76442, AB_1524535)	Donkey anti-Chicken IgY-DL405 (JIR, 703-475-155, AB_2340373)
/395XL	R1C3	Blood Brain Barrier (BBB)	Endothelial Cells	Mouse IgM anti-BBB (BL, 836803, AB_2687236)	Goat anti-Mouse IgM-DY395XL (LCB, custom, RRID: N/A)
/A	R1C4	Green Fluorescent Protein (GFP)	Endothelial Cells	N/A (endogenously expressed GFP)	N/A (endogenously expressed GFP)
F546	R1C5	lonized calcium binding adaptor molecule 1 (IBA1)	Microglia	Guinea Pig IgG anti-IBA1 (SS, 234005, AB_2493179)	Goat anti-Gunea Pig IgG-AF546 (TFS, A11074, AB_2534118)
F594	R1C6	Rat Endothelial Cell Antigen 1 (RECA1)	Endothelial Cells	Mouse IgG1 anti-RECA1 (TFS, 14-0360-82, AB_467212)	Goat anti-Mouse IgG1-AF594 (TFS, A21125, AB_2535767)
F647	R1C7	Aquaporin-4 (AQP4)	Astrocyte Terminals	Mouse IgG3 anti-AQP4 (Abcam, ab9512, AB 307299)	Goat anti-mouse IgG3-AF647 (JIR, 115-605-209, AB 2338920)
erCP	R1C8	Tomato Lectin (TL)	Endothelial Cells, Microglia	Tomato Lectin-Biotin (VL, B-1175, AB 2315475)	Streptavidin-PerCP (JIR, 016-120-084, AB 2337241)
RDve 680LT	R1C9	Oligodendrocyte Transciption Factor 2 (Olig2)	Oligodendrocytes	Mouse IgG2a anti-Olig2 (MS, MABN50, AB 10807410)	Goat anti-Mouse IgG2a-IRDye 680LT (LCB, 926-68051)
RDve 800CW	R1C10	Cleaved Caspase-3 (CC3)	Apoptotic Cells	Rabbit IgG anti-CC3 (MS, AB3623, AB 91556)	Donkey anti-Rabbit IgG-IRDye 800CW (LCB, 926-32213)
right Field	R1C11	N/A	Cell Morphology	N/A	N/A
	Round#Channel#	Biomarker	Target/Function	1st step reagent	2nd step reagent
API	R2C1	DAPI	All Nucleated Cells	N/A	DAPI (TFS, D1306, AB 2629482)
01.405	R2C2	Pan-Histones H1, H2A, H2B, H3, H4 (HIST)	All Nucleated Cells	Mouse IgG2b anti-HIST cocktail mix (MBLI)**	Goat anti-mouse leG2b-DL405 (JIR. 115-475-207, AB 2338801)
Y395XL	R2C3		Pericytes-Subset	Mouse IgG3 anti-PDGFRβ (SCB, sc-365464, AB_10847085)	
F488	R2C4	Neuronal Nuclei (NeuN)	Neurons-Pan Specific	Chicken IgY anti-NeuN (MS, ABN91, AB_11205760)	Goat anti-Chicken IgY-AF488 (TFS, A11039, AB_2534096)
F546	R2C5	Synaptohysin (SYN)	Neurons-Pan Specific	Mouse IgM anti-SYN (MS, MAB329, AB_94786)	Goat anti-Mouse IgM-AF546 (TFS, A21045, AB_2535714)
F594	R2C6	Parvalbumin (PARV)	Interneurons-Subset	Guinea Pig IgG anti-PARV (SS, 195004, AB_2156476)	Goat anti-Gunea Pig IgG-AF594 (TFS, A11076, AB_2534120)
F647	R2C7	Choline Acetyltransferase (ChAT)	Neurons-Cholinergic	Mouse IgG1 anti-ChAT (MS, MAB5270, AB_2079753)	Goat anti-Mouse IgG1-AF647 (TFS, A21240, AB_2535809)
erCP	R2C8	Tomato Lectin (TL)	Endothelial Cells, Microglia	Tomato Lectin-Biotin (VL, B-1175, AB_2315475)	Streptavidin-PerCP (JIR, 016-120-084, AB_2337241)
RDye 680LT	R2C9	Glutaminase GLS2 (GLUT)	Neurons-Glutamatergic	Rabbit IgG anti-GLUT (Abcam, ab113509, AB_10866157)	Donkey anti-Rabbit IgG-IRDye 680LT (LCB, 926-68023, AB_1070616
RDve 800CW	R2C10	Smooth Muscle Actin (SMA)	Pericytes-Subset	Mouse IgG2a anti-SMA (TFS, 14-9760-82, AB 2572996)	Goat anti-Mouse IgG2a-IRDye 800CW (LCB, 926-32351, AB 278299
right Field	R2C11	N/A	Cell Morphology	N/A	N/A
	Round#Channel#	Biomarker	Target/Function	1st step reagent	2nd step reagent
DAPI	R3C1	DAPI	All Nucleated Cells	N/A	DAPI (TFS, D1306, AB 2629482)
01405	R2C2	Pan-Histones H1, H2A, H2B, H3, H4 (HIST)	All Nucleated Cells	Mouse IgG2b anti-HIST cocktail mix (MBLI)**	Goat anti-mouse (gG2b-DL405 (JIR, 115-475-207, AB_2338801)
0Y395XL	R3C3				
		Glial Fibrillary Acidic Protein (GFAP)	Astrocytes-Reactive	Rat IgG anti-GFAP (TFS, 13-0300, AB_2532994)	Goat anti-Rat IgG-DY395XL (LCB, custom, RRID: N/A)
AF488	R3C4	Doublecortin (DCX)	Neurons-Newly Born	Rabbit IgG anti-DCX (Abcam, ab18723, AB_732011)	Goat anti-Rabbit IgG-AF488 (TFS, A11034, AB_2576217)
AF546	R3C5	S100 calcium-binding protein β (S100β)	Astrocytes-Pan Specific	Mouse IgG2a anti-S100β (MS, MAB079-1, AB_571112)	Goat anti-Mouse IgG2a-AF546 (TFS, A21133, AB_2535772)
AF594	R3C6	Nestin (NST)	Immature Neural and Endothelial Cells	Mouse IgG1 anti-NST (MS, MAB353, AB_94911)	Goat anti-Mouse IgG1-AF594 (TFS, A21125, AB_2535767)
AF647	R3C7	Aquaporin-4 (AQP4)	Astrocyte Terminals	Mouse IgG3 anti-AQP4 (Abcam, ab9512, AB_307299)	Goat anti-mouse IgG3-AF647 (JIR, 115-605-209, AB_2338920)
PerCP	R1C8	Tomato Lectin (TL)	Endothelial Cells, Microglia	Tomato Lectin-Biotin (VL, B-1175, AB_2315475)	Streptavidin-PerCP (JIR, 016-120-084, AB_2337241)
RDve 680LT	R3C9	EAAT1 (GLAST)	Astrocytes-Pan Specific	Guinea Pig IgG anti-GLAST (SS, 250114, AB 2619957)	Donkey anti-Guinea Pig IgG-IRDye 680LT (LCB, 926-68030, AB 1070
RDve 800CW	R3C10	Vimentin (VIM)	Immature Neural and Endothelial	Chicken IgY anti-VIM (MS, AB5733, AB 11212377)	Donkey anti-Chicken IgY-IRDve 800CW (LCB, 926-32218, AB 18500)
Bright Field	R3C11	N/A	Cell Morphology	N/A	N/A
stains in Round #4	Round#Channel#	Biomarker	Target/Function	1st step reagent	2nd step reagent
DAPI	R4C1	DAPI	All Nucleated Cells	N/A	DAPI (TFS, D1306, AB 2629482)
	R2C2	Pan-Histones H1, H2A, H2B, H3, H4 (HIST)	All Nucleated Cells	Mouse IgG2b anti-HIST cocktail mix (MBLI)**	Goat anti-mouse IgG2b-DL405 (JIR, 115-475-207, AB 2338801)
		Pan-Histories H1, HZA, HZB, H3, H4 (HIST)		N/A	
DL405					N/A
0Y395XL	R4C3	Autofluorescence (AF)	N/A		
DY395XL AF488	R4C4	Proliferating Cell Nuclear Antigen (PCNA)	All Actively Proliferating Cells	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993)	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784)
0Y395XL AF488 AF546	R4C4 R4C5	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (Sox2)	All Actively Proliferating Cells Neural Precursors, Astrocytes	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256)	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB_2535765)
DY395XL AF488 AF546 AF594	R4C4 R4C5 R4C6	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (Sox2) Gutamic Acid Decarboxylase 67 (GAD67)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256) Mouse IgG2a anti-GAD67 (MS, MAB5406, AB_2278725)	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB_2535765) Goat anti-Mouse IgG2a-AF594 (TFS, A21135,AB_2535774)
DY395XL AF488 AF546 AF594 AF647	R4C4 R4C5 R4C6 R4C7	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (Sox2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256) Mouse IgG2a anti-GAD67 (MS, MABS406, AB_2278725) N/A	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB_2535765) Goat anti-Mouse IgG2a-AF594 (TFS, A21135,AB_2535774) N/A
)Y395XL \F488 \F546 \F594 \F647	R4C4 R4C5 R4C6	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (Sox2) Gutamic Acid Decarboxylase 67 (GAD67)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256) Mouse IgG2a anti-GAD67 (MS, MAB5406, AB_2278725)	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB_2535765) Goat anti-Mouse IgG2a-AF594 (TFS, A21135,AB_2535774)
07395XL 4F488 4F546 4F594 4F647 DerCP	R4C4 R4C5 R4C6 R4C7	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (Sox2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256) Mouse IgG2a anti-GAD67 (MS, MABS406, AB_2278725) N/A	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB_2535765) Goat anti-Mouse IgG2a-AF594 (TFS, A21135,AB_2535774) N/A
DY395XL AF488 AF546 AF594 AF647 DerCP RDye 680LT	R4C4 R4C5 R4C6 R4C7 R1C8	Proliferating Cell Nuclear Antigen (PCNA) SRY (sex determining region Y)-box 2 (5ox2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tbr1	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB_423993) Mouse IgG1 anti-Sox2 (BD, 561469, AB_10694256) Mouse IgG2a anti-GAD67 (MS, MAB5406, AB_2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB_2315475)	Goat anti-Mouse IgG3-AF488 (TFS, A21151, AB, 2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21123, AB, 2535765) Goat anti-Mouse IgG2-AF594 (TFS, A21125, AB, 2535774) N/A Streptawidin-PerCP (JIR, 016-120-084, AB, 2337241)
DY395XL AF488 AF546 AF594 AF647 PerCP RDye 680LT IRDye 800CW	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10	Proliferating Cell Nuclear Antigen (PCNA) SRY (zex determining region Y)-box 2 (Sox2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tbr1 Ecomes (Tbr2)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Immature	Mouse IgG3 anti-PCNA (GTX, GTXM2237, AB 22393) Mouse IgG2 anti-Sot2 (BD, 551469, AB 10694256) Mouse IgG2 anti-G4D67 M/S, MABS406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr I (Abcam, ab31390, AB 2200219) Rat IgG2 anti-Tbr I (TS, 144.875-82, AB, 11042577)	Goat anti-Mouse IgG-1AF548 (TFS, A21151, AB, 2353784) Goat anti-Mouse IgG-1AF546 (TFS, A21123, AB, 2353785) Goat anti-Mouse IgG2-AF554 (TFS, A21135, AB, 2355772) N/A Streptavidin-PerCP (JIR, 016-120-084, AB, 2337241) Donkey anti-Rabbi IgG-IRDyc 880XT (JCB, 395-68023, AB, 1070616 Goat anti-Rat IgG-RiVye 800XV (IGC, 926-32219, AB, 1850025)
OY395XL AF488 AF546 AF547 OerCP ROye 680LT RDye 800CW Bright Field	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11	Proliferating Cell Nuclear Antigen (PCNA) System Control of Contr	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology	Mouse IgG3 anti-PCNA (GTX, GTX40237, AB, 423993) Mouse IgG1 anti-Sov2 (BD, S51649, AB, 16942459) Mouse IgG2 anti-G4D67 (MS, MAB5406, AB, 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB, 2315475) Rab IgG2 anti-Tbr1 (Abcam, ab31540, AB, 2200219) Rat IgG2 anti-Tbr2 (TFS, 14-4875-82, AB, 11042577) N/A	Goat anti-mouse IgG3-AF488 (TFS, A21151, AB_2535784) Goat anti-Mouse IgG1-AF546 (TFS, A21125, AB_2535765) Goat anti-Mouse IgG2-AF546 (TFS, A21125, AB_2535765) Goat anti-Mouse IgG2-AF549 (TFS, A21135, AB_2535774) N/A Streptawdin-PerCP (JIR, 016-120-084, AB_2337241) Donkey anti-Rabbit IgG-IRDye 580LT (JCB, 926-68023, AB_1070616 Goat anti-Rat IgG-IRDye 580LT (JCB, 926-32219, AB_1850025) N/A
Y395XL F488 F546 F594 F647 erCP RDye 680LT RDye 800CW right Field tains in Round #5	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11 Round#Channel#	Proliferating Cell Nuclear Antigen (PCNA) SY (see determine region Y)-box (Sex2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) That Eomes (Tbr2) N/A Bloomarker	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Target Function	Mouse IgG3 anti-PCNA (GTX, GTXM0237, RA 273993) Mouse IgG1 anti-Soz (BD, 551469, AB 10694256) Mouse IgG2 anti-GAD67 M/S, MA65406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31540, AB 2200219) Rat IgG2 anti-Tbr2 (TS, 14-4875-82, AB 11042577) N/A List step reagent	Goat anti-Mouse IgG-1AF548 (TFS, A21151, AB, 2353784) Goat anti-Mouse IgG-1AF546 (TFS, A21152, AB, 2353784) Goat anti-Mouse IgG2-AF594 (TFS, A21155,AB, 2555776) N/A Streptawdin-PerCP (JIR, 016-120-084, AB, 2337241) Denkey anti-Raibbi IgG-IRIQP 680LT (J.CB, 926-58023, AB, 1070516 Goat anti-Rat IgG-IRIQP 800CW (J.CB, 926-32219, AB, 1850025) N/A Zond step reagent
Y395XL F488 F546 F594 F647 erCP RDye 680LT Dye 800CW right Field tains in Round #5	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11 Round#Channel# R5C1	Proliferating Cell Nuclear Antigen (PCNA) SPY (sex determining region Y)-box 2 (Sox2) Gutamir And Detarbonylase 67 (GAD67) Autofluorescence (AF) Torato Lectin (TL) Tor1 Leomes (Tor2) N/A Blomarker DAP)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABEAGE N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Target/Function All Nucleated Cells	Mouse IgG3 anti-PCNA (CTX, GTXM0237, A8 23393) Mouse IgG1 anti-Son2 (BD, S51695, A8 10694256) Mouse IgG2 anti-Son2 (BD, S51695, A8 10694256) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31540, A8 2200219) Rat IgG2 anti-Tbr2 (TFS, 14-4875-82, A8 11042577) N/A Ist step reagent N/A	Goat anti-mouse IgG3-AF488 (TF3, A21151, AB _2535784) Goat anti-Mouse IgG1-AF546 (TF3, A21151, AB _2535755) Goat anti-Mouse IgG2-AF594 (TF3, A21135, AB _25357574) N/A Streptawdin-PerCP (JIR, 016-120-084, AB _2337241) Donkey anti-Raibbit IgG-IROye 580LT (I.CR, 926-58023, AB _1070618 Goat anti-Rait IgG-IROye 580LT (I.CR, 926-58023, AB _1070618 Goat anti-Rait IgG-IROye 800CW (I.CR, 926-32219, AB _1850025) N/A Znd step reagent DAIP (TFS, 01306, AB _2629482)
Y395XL F488 F594 F594 F647 erCP ROye 680LT ROye 800CW right Field tains in Round #5 API L405	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11 R4C11 R5C1 R2C2	Proliferating Cell Nuclear Anigen (PCNA) SY feas determining region Y-box (Sex2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tor1 Ecmes (Tbr2) N/A Biomarker DAPI PAP-Histones H1, H2A, H2B, H3, H4 (HIST)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Targer, Function All Nucleated Cells All Nucleated Cells	Mouse IgG3 anti-PCNA (GTX, GTXA0237, RA 22393) Mouse IgG1 anti-Soz (BD, 55168, R3 16694255) Mouse IgG2 anti-GAD67 (MS, MA85406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr I (Abcam, ab31540, AB 2200219) Rabbit IgG anti-Tbr I (Abcam, ab31540, AB 2200219) N/A List tep reagent N/A Mouse IgG2 anti-HiST cocktail mix (MBU)**	Goat anti-Mouse (GC1-AF548 (TFS, A21151, A8, 2353784) Goat anti-Mouse (GC1-AF546 (TFS, A21152, A8, 2553755) Goat anti-Mouse (GC1-AF546 (TFS, A21153,A8, 255575) Goat anti-Mouse (GC1-AF594 (TFS, A21153,A8, 2555774) N/A Streptavidin-PerCP (JIR, 016-120-084, A8, 2337241) Donnkey anti-Raibbi (gG-Ribye 800LT (LC6, 926-68023, A8, 1070516 Goat anti-Rat (gG-Ribye 800LY (LC6, 926-32219, A8, 1850025) N/A Zoot step reagent DAPI (TFS, D1306, A8, 2653482) DAPI (TFS, D1306, A8, 2653482)
Y395XL F488 F5846 F594 F647 erCP tDye 680LT tDye 80CW right Field tains in Round #5 API L405 F430	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11 Round#Channel# R5C1 R2C2 R2C3	Proliferating Cell Nuclear Antigen (PCNA) SYY (sex determining region Y)-box 2 (Sox2) Gutamic And Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tor1 Eomes (Tibr2) N/A Biomarker DAP! Pan-Histones H1, H2A, H2B, H3, H4 (HIST) Carletinin (CRT)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABergic N/A Endothelal Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Imature Cell Morphology Target/Function All Nucleated Cells All Nucleated Cells Interneurons-Subset	Mouse IgG3 anti-PCIA (CTX, GTXM0237, AB 23393) Mouse IgG1 anti-So2 (BD, 561469, AB 10694256) Mouse IgG2a anti-GAD67 [MS, MAB5406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31940, AB 2200219) Rab IgG2a anti-Tbr2 (TFS, 14-4875-82, AB 11042577) N/A Ist step reagent N/A Mouse IgG2b anti-HiST cocktail mix (MBLI)** Mouse IgG2b anti-HIST cocktail mix (MBLI)**	Goat anti-mouse (§G3-AF488 (TFS, A21151, A8, 2353784) Goat anti-Mouse (§G1-AF546 (TFS, A21123, A8, 2535786) Goat anti-Mouse (§G2-AF534 (TFS, A21123, A8, 2535776) N/A Streptavidin-PerCP (JIR, 016-120-084, A8, 2337241) Donkey anti-Rabbit (§G-RDyc 880LT (LCR, 926-68023, A8, 1070516 Goat anti-Rabbit (§G-RDyc 800CW (LCR, 926-32219, A8, 1850025) N/A Zood step reagent DAP (TFS, D1306, A8, 2629482) Goat anti-mouse (§G2-D-DL055 (JIR, 115-475-207, A8, 2338801) Goat anti-Rabbit (§G-AF430 (TFS, A11064, A8, 2534111))
Y395XL FA88 FF546 FF594 FF647 FF647 FF647 FF04P ROPE 880LT ROPE 880LT ROPE 800LW right Field tains in Round #5 API LL405 FA30 FF430	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C11 R4C11 R5C1 R2C2 R2C2 R2C3 R2C4	Proliferating Cell Nuclear Anigen (PCNA) SY feas determining region Y-box (Sex2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tori Eemes (Tbr2) N/A Blomarker DAPI Par-Histones H1, H2A, H2B, H3, H4 (HIST) Calretinin (RT) 2,3 'Cyclic roucleotide 3'-phosphodiesterase (CNPase)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GABAergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Targer Ffunction All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific	Mouse IgG3 anti-PCNA (GTX, GTXM0237, RA 22393) Mouse IgG1 anti-Soz (BD, 55168, R4 10594255) Mouse IgG2 anti-GAD67 (MS, MA85406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr I (Abcam, ab31340, AB 2200219) Rabbit IgG anti-Tbr I (Abcam, ab31340, AB 2200219) N/A Ist IgG2 anti-Tbr I (TFS, 144875-82, AB 11042577) N/A Macus IgG2 anti-HiST cocktail mix (MBU)** Rabbit IgG anti-CRI (MS, A85054, AB 2068506)	Goat anti-Mouse (GC)-AF488 (TFS, A21151, AB, 2353794) Goat anti-Mouse (GC)-AF586 (TFS, A21152, AB, 2535756) Goat anti-Mouse (GC)-AF596 (TFS, A21155,AB, 2535774) N/A Streptavidin-PerCP (JIR, 016-120-084, AB, 23337241) Donkey anti-Rabbi (gG-RR)ve 800 T (JCB, 926-68023, AB, 1070616 Goat anti-Rat (gG-RR)ve 800CW (JCB, 926-32219, AB, 1850025) N/A Zond step reagent DAPI (TFS, D1306, AB, 2629482) [R, 115-475-207, AB, 2338801] Goat anti-Rabbit (gG-PC)-MOS (JR, 115-475-207, AB, 2338801) Goat anti-Rabbit (gG-PC)-MOS (JR, 115-475-207, AB, 2338801) Goat anti-Mouse (gG-PC)-MOS (JR, 115-475-207, AB, 2338501) Goat anti-Mouse (gG-PC)-MOS (JR, 115-475-207, AB, 2338501) Goat anti-Mouse (gG-PC)-MOS (JR, 21110, AB, 2333154)
Y395XL F488 F594 F647 F647 P647 P0ye 880LT Dye 800CW right Field L405 F430 F4488 F546	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C10 R4C11 R2C2 R2C3 R2C3 R2C4 R2C5	Proliferating Cell Nuclear Antigen (PCNA) SYY (sex determining region Y)-box 2 (Sex2) Gutamic And Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) That Eemes (Tbr2) N/A Blomarker DAPI Pan-Histones HL, H2A, H2B, H3, H4 (HIST) Carletinin (CRI) 2,3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) Neurofilament Haay Chain (NFH)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GaBergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Imature Cell Morphology Target/Function All Nucleated Cells All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific Neurons-Evanal Innervation	Mouse IgG3 anti-PCIA (CTX, GTXM0237, AB 23393) Mouse IgG1 anti-So2 (BD, 561469, AB 10694256) Mouse IgG2 anti-GAD67 [MS, MABS406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31940, AB 2200219) Rabbit IgG anti-Tbr2 (TFS, 14-4875-82, AB _11042577) N/A Ist step reagent N/A Mouse IgG2b anti-HIST cocktail mix (MBILI)** Rabbit IgG anti-Tbr1 (MS, AB5054, AB 2068506) Mouse IgG1 anti-CNPase (MS, MAB336, AB 2008208) Mouse IgG1 anti-CNPase (MS, MAB336, AB 2008208)	Goat anti-Mouse IgG-3.4F488 (TFS, A21151, AB, 2353784) Goat anti-Mouse IgG-1.4F546 (TFS, A21152, AB, 2353784) Goat anti-Mouse IgG-1.4F546 (TFS, A21123, AB, 2353774) N/A Streptavidin-PerCP (JIR, 016-120-084, AB, 2337241) Donkey anti-Raibbit IgG-1RDyc 880UT (ICB, 936-68023, AB, 1070616 Goat anti-Raib IgG-1RDyc 880UT (ICB, 936-68023, AB, 1070616 Goat anti-Raibbit IgG-187048 (DIR, 115-187-207, AB, 1850025) N/A DAPI (TFS, 01305, AB, 2629482) Goat anti-Raibbit IgG-3.4F340 (TFS, A11064, AB, 2533111) Goat anti-Mouse IgG-3.4F340 (TFS, A11064, AB, 2533764) Goat anti-Mouse IgG-3.4F340 (TFS, A21121, AB, 2533764) Goat anti-Mouse IgG-3.4F340 (TFS, A21121, AB, 2533764) Goat anti-Mouse IgG-3.4F340 (TFS, A21121, AB, 2533764) Goat anti-Mouse IgG-3.4F346 (TFS, A21121, AB, 2533764)
V395XL F488 F546 F594 F647 F647 F647 F647 F647 F647 F647 F64	R4C4 RAC5 RAC6 R4C7 R1C8 R4C9 R4C10 R4C10 R4C10 R4C10 R4C10 R4C10 R5C1 R5C1 R5C2 R7C3 R7C4 R7C5 R7C6 R7C6	Proliferating Cell Nuclear Antigen (PCNA) SYF (see determining region Y-box (Sex2) Gutamic Acid Decarbonylase 67 (GAD67) Autofluorescence (AF) Tonato Lectin (TI) Tor. Eomes (Tbr2) N/A Biomarker DAPI Par-Histones H1, H2A, H2B, H3, H4 (HIST) Calretinin (ERT) 2;3 'Cyclic - nucleotide 3'-phosphodiesterase (CNPase) Neurofilament Heavy Chain (NFH) Neylin Basic Potein (MSP)	All Actively Proliferating Cells Neurons-GABAergic Neurons-GABAergic Neurons-GABAergic Neurons-Pyramidal Mature Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Target-Fruction All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific Neurons-Axonal Innervation Oligodendrocytes-Pan Specific Neurons-Axonal Innervation Oligodendrocytes-Myelinating	Mouse IgG3 anti-PCINA (GTX, GTXM0237, RA 273993) Mouse IgG anti-Soz (BO, 55166, R3 10594255) Mouse IgG anti-GAD67 (MS, MA85406, A8 2278725) N/A Nouse IgG2a anti-GAD67 (MS, MA85406, A8 2278725) N/A Rabbit IgG anti-Tbr I (Abcum, ab31340, A8 2200219) Rabbit IgG anti-Tbr I (Abcum, ab31340, A8 2200219) N/A Ist tepreagent N/A Mouse IgG2b anti-HIST cocktail mix (MBU)** Rabbit IgG anti-CRT (MS, A85054, A8 2068506) Chicken IgG anti-CRT (MS, A85054, A8 2068506) Chicken IgY anti-NFH (MS, A85539, A8 211212161) Rat IgG2a MBC, MS, MA8386, A8 49475)	Goat anti-Mouse (gG-3A-FA88 (TFS, A21151, A8, 2535784) Goat anti-Mouse (gG-3A-FS918, A21152, A8, 2535755) Goat anti-Mouse (gG2-AFS914 (TFS, A21135,A8, 2535774) Norseptavidin-PerCP (JIR, 016-120-084, A8, 23337741) Donkey anti-Rabbit (gG-RD)e 6800T (LCS, 926-68023, A8, 1070616 Goat anti-Rat (gG-RD)e 6800T (LCS, 926-68023, A8, 1070616 Goat anti-Rat (gG-RD)e 800CW (LCS, 926-32219, A8, 1850025) N/A Zood step reagent OAPI (TFS, D1306, A8, 2629482) Goat anti-Rabbit (gG-AF480) (TFS, A11064, A8, 2534111) Goat anti-Mouse (gGZ-D-0405 (JIR, 115-475-207, A8, 2338801) Goat anti-Mouse (gGZ-PG-0405 (JIR, 115-475-207, A8, 2338801) Goat anti-Chicken (gY-AF480 (TFS, A11004, A8, 2534027)
V395XL F488 F546 F594 F647 F647 F647 F647 F647 F647 F647 F64	R4C4 R4C5 R4C6 R4C7 R1C8 R4C9 R4C10 R4C10 R4C11 R2C2 R2C3 R2C3 R2C4 R2C5	Proliferating Cell Nuclear Antigen (PCNA) SYY (sex determining region Y)-box 2 (Sex2) Gutamic And Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) That Eemes (Tbr2) N/A Blomarker DAPI Pan-Histones HL, H2A, H2B, H3, H4 (HIST) Carletinin (CRI) 2,3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) Neurofilament Haay Chain (NFH)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GaBergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Imature Cell Morphology Target/Function All Nucleated Cells All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific Neurons-Evanal Innervation	Mouse IgG3 anti-PCIA (CTX, GTXM0237, AB 23393) Mouse IgG1 anti-So2 (BD, 561469, AB 10694256) Mouse IgG2 anti-GAD67 [MS, MABS406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31940, AB 2200219) Rabbit IgG anti-Tbr2 (TFS, 14-4875-82, AB _11042577) N/A Ist step reagent N/A Mouse IgG2b anti-HIST cocktail mix (MBILI)** Rabbit IgG anti-Tbr1 (MS, AB5054, AB 2068506) Mouse IgG1 anti-CNPase (MS, MAB336, AB 2008208) Mouse IgG1 anti-CNPase (MS, MAB336, AB 2008208)	Goat anti-Mouse IgG3-AF548 (TFS, A21151, AB, 2353784) Goat anti-Mouse IgG2-AF546 (TFS, A21152, AB, 2353784) Goat anti-Mouse IgG2-AF554 (TFS, A21123, AB, 2535776) N/A Streptavidin-PerCP (JIR, 016-120-084, AB, 2337241) Donkey anti-Rabbit IgG-RPDy 680/LT (LCB, 976-680/23, AB, 1070616 Coat anti-Rat IgG-RPDy 880/CW (LCB, 926-32219, AB, 1850025) N/A Zond step reagent DAPI (TFS, D130S, AB, 2639482) Goat anti-Rabbit IgG-AF430 (TFS, A11064, AB, 2538111) Goat anti-Mouse IgG2-D4065 (JIR, 115-475-207, AB, 2338801) Goat anti-Mouse IgG3-P488 (TFS, A21121, AB, 2535764) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2535764) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2535764) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2535764)
W395XL F488 F546 F594 F594 F647 erCP DUpe 880LT TUpe 80CW right Field API LL05 F430 F438 F546 F594	R4C4 RAC5 RAC6 R4C7 R1C8 R4C9 R4C10 R4C10 R4C10 R4C10 R4C10 R4C10 R5C1 R5C1 R5C2 R7C3 R7C4 R7C5 R7C6 R7C6	Proliferating Cell Nuclear Antigen (PCNA) SYF (see determining region Y-box (Sex2) Gutamic Acid Decarbonylase 67 (GAD67) Autofluorescence (AF) Tonato Lectin (TI) Tor. Eomes (Tbr2) N/A Biomarker DAPI Par-Histones H1, H2A, H2B, H3, H4 (HIST) Calretinin (ERT) 2;3 'Cyclic - nucleotide 3'-phosphodiesterase (CNPase) Neurofilament Heavy Chain (NFH) Neylin Basic Potein (MSP)	All Actively Proliferating Cells Neurons-GABAergic Neurons-GABAergic Neurons-GABAergic Neurons-Pyramidal Mature Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Target-Fruction All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific Neurons-Axonal Innervation Oligodendrocytes-Pan Specific Neurons-Axonal Innervation Oligodendrocytes-Myelinating	Mouse IgG3 anti-PCINA (GTX, GTXM0237, RA 273993) Mouse IgG anti-Soz (BO, 55166, R3 10594255) Mouse IgG anti-GAD67 (MS, MA85406, A8 2278725) N/A Nouse IgG2a anti-GAD67 (MS, MA85406, A8 2278725) N/A Rabbit IgG anti-Tbr I (Abcum, ab31340, A8 2200219) Rabbit IgG anti-Tbr I (Abcum, ab31340, A8 2200219) N/A Ist tepreagent N/A Mouse IgG2b anti-HIST cocktail mix (MBU)** Rabbit IgG anti-CRT (MS, A85054, A8 2068506) Chicken IgG anti-CRT (MS, A85054, A8 2068506) Chicken IgY anti-NFH (MS, A85539, A8 211212161) Rat IgG2a MBC, MS, MA8386, A8 49475)	Goat anti-Mouse (gG-3A-FA88 (TFS, A21151, A8, 2535784) Goat anti-Mouse (gG-3A-FS918, A21152, A8, 2535755) Goat anti-Mouse (gG2-AFS914 (TFS, A21135,A8, 2535774) Norseptavidin-PerCP (JIR, 016-120-084, A8, 23337741) Donkey anti-Rabbit (gG-RD)e 6800T (LCS, 926-68023, A8, 1070616 Goat anti-Rat (gG-RD)e 6800T (LCS, 926-68023, A8, 1070616 Goat anti-Rat (gG-RD)e 800CW (LCS, 926-32219, A8, 1850025) N/A Zood step reagent OAPI (TFS, D1306, A8, 2629482) Goat anti-Rabbit (gG-AF480) (TFS, A11064, A8, 2534111) Goat anti-Mouse (gGZ-D-0405 (JIR, 115-475-207, A8, 2338801) Goat anti-Mouse (gGZ-PG-0405 (JIR, 115-475-207, A8, 2338801) Goat anti-Chicken (gY-AF480 (TFS, A11004, A8, 2534027)
W395XL F488 F546 F594 F647 erCP BODye 800CW right Field tains in Round #5 tAPI L405 F430 F438 F546 F594 F647 erCP	RAC4 RAC5 RAC6 RAC7 RIC8 RAC9 RAC10 RAC10 RAC11 ROund#Channel# RSC1 RC2 RC2 RC3 RC4 RC5 RC6 RC6 RC6 RC7	Proliferating Cell Nuclear Antigen (PCNA) SY feas determining region Y-box (Sex2) Gutamic Acid Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TU) Tor. Tomato Lectin (TRT) 2,3" Cyclic - uncelectide 3"-phosphodiesterase (CMPase) Neurofiament Heavy Chain (NFH) Neurofiament Medium Chain (NFH) Neurofiament Medium Chain (NFM) Tomato Lectin (TU)	All Actively Proliferating Cells Neurons-GABAergic Neurons-GABAergic Neurons-GABAergic Neurons-Pyramidal Mature Neurons-Pyramidal Mature Neurons-Pyramidal Immature Cell Morphology Target Fruction All Nucleated Cells Interneurons-Subset Oligodendrocytes-Pan Specific Neurons-Axonal Innervation Oligodendrocytes-Ayelinating Neurons-Axonal Innervation Oligodendrocytes-Ayelinating Neurons-Axonal Innervation Oligodendrocytes-Myelinating Neurons-Axonal Innervation Endothelial Cells, Microglia	Moure IgG3 anti-PCNA (GTX, GTXM0237, RA 22393) Moure IgG anti-Soz (B) 55166, R3 10594255) Moure IgG2a anti-GAD67 (MS, MA85406, AB 2278725) Moure IgG2a anti-GAD67 (MS, MA85406, AB 2278725) N/A Babbit IgG anti-Tor1 (Abcam, ab31340, AB 22000219) Rabbit IgG anti-Tor1 (Abcam, ab31340, AB 22000219) N/A Ist tepreagent N/A Moure IgG2b anti-HIST cocktail mix (MBU)** Rabbit IgG anti-CRT (MS, AB5054, AB 2068206) Chicken IgV anti-NFH (MS, AB5053, AB 2068206) Chicken IgV anti-NFH (MS, AB5539, AB 11212161) Rat IgG2a MS (MS, MA8386, AB 39475) Moure IgG2a anti-NFH (MS, AB5539, AB 2151276) Moure IgG2a anti-NFH (MS, AB5539, AB 2151276) Moure IgG2a anti-NFH (MS, AB5539, AB 2151276) Moure IgG2a anti-NFH (MS, MA8386, AB 39475)	Goat anti-Mouse (gG2-AFA88 (TFS, A21151, A8, 2535784) Goat anti-Mouse (gG2-AFS918 (A21123, A8, 2535765) Goat anti-Mouse (gG2-AFS94 (TFS, A21135,A8, 2535774) ASSESSED (GOAT (GOAT) (G
Y395XL IF488 IF546 IF594 IF647 PerCP ROye 680LT ROye 800CW Iright Field	RAC4	Proliferating Cell Nuclear Antigen (PCNA) SYf (sex determining region Y)-Box 2 (Sex2) Gutamic And Decarboxylase 67 (GAD67) Autofluorescence (AF) Tomato Lectin (TL) Tor1 Eomes (Tbr2) N/A Biomarker OAP! 2-3"-Histones HL, H2A, H2B, H3, H4 (HIST) Calretinin (CRT) 2-3"-Vicin-nucleotide 3"-phosphodiesterase (CMPase) Neurofilament Heavy Chain (NFH) Myelin Sasic Protein (MBP)	All Actively Proliferating Cells Neural Precursors, Astrocytes Neurons-GaBhergic N/A Endothelial Cells, Microglia Neurons-Pyramidal Mature Neurons-Pyramidal Imature Cell Morphology Target/Function All Nucleated Cells All Nucleated Cells Interneurons-Subset Digodendrocytes-Pan Specific Neurons-Avandi Innervation Oligodendrocytes-Myelinating Neurons-Avandi Innervation	Mouse IgG3 anti-PCIA (GTX, GTXM0237, AB 23393) Mouse IgG1 anti-So2 (BD, 561469, AB 10694256) Mouse IgG2 anti-GD67 [MS, MA6S406, AB 2278725) N/A Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Tomato Lectin-Biotin (VL, B-1175, AB 2315475) Rabbit IgG anti-Tbr1 (Abcam, ab31340, AB 2200219) Rabbit IgG anti-Tbr1 (Abcam, ab31340, AB 2200219) N/A 11st step reagent N/A Mouse IgG2b anti-HST cocktail mix (MBIJ)** Rabbit IgG anti-CTX (MS, A85054, AB 2068506) Mouse IgG2 anti-HST cocktail mix (MBIJ)** Rabbit IgG anti-CTX (MS, A85054, AB 2068506) Mouse IgG1 anti-CWase (MS, MA6326, AB 2062608) Chicken Igf anti-HST (MS, A8538, AB 11212161) Rat IgG2a-MBP (MS, MA6386, AB 94975) Mouse IgG2a-MBP (MS, MA6386, AB 94975)	Goat anti-Mouse IgG3-AF548 (TFS, A21151, AB, 2353784) Goat anti-Mouse IgG3-AF548 (TFS, A21152, AB, 2353784) Goat anti-Mouse IgG2-AF534 (TFS, A21123, AB, 2353774) N/A Streptavidin-PerCP (JIR, 016-120-084, AB, 2337241) Donkey anti-Rabbit IgG-RB/by 680UT (JCB, 936-68023, AB, 1070616 Goat anti-Rat IgG-RB/by 800CW (JCB, 926-32219, AB, 1850025) N/A Zood step reagent DAPI (TFS, D1305, AB, 262-3482) Goat anti-Rabbit IgG-AF430 (TFS, A11064, AB, 2534714) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2353764) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2353764) Goat anti-Mouse IgG1-AF488 (TFS, A21121, AB, 2353764) Goat anti-Rabbit IgG-AF430 (TFS, A11007, AB, 10561522) Goat anti-Rabbit IgG-AF450 (TFS, A11007, AB, 10561522) Goat anti-Rabbit IgG-AF546 (TFS, A11007, AB, 10561522) Goat anti-Rabit IgG-AF546 (TFS, A11007, AB, 2555510)

Commission	T-1-1- 7	Cell phenotyping results

Juppleine	entary 1a	bie 7. Cei	pnenoty	ping resu	iits																											
Cell ID	centroid_x	centroid_y	xmin	ymin	xmax	ymax	NeuN	8100	Olig2	lba1	RECA1	Cleaved Caspase-3	Tyrosine Hydroxylase	Blood Brain Barrier	GFP	PDGFR beta	Parvalbumin	Choline Acetyltransferas	GFAP	Smooth Muscle Actin	Glutaminase	Doublecortin	Sox2	PCNA	Vimentin	GAD67	Tbr1	Eomes	Calretinin	Nestin	Aquaporin-4	Calbindin
1	38157	29149	38142	29135	38173	29164	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2	10900	28364	10880	28356	10921	28372	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	0
3	10939	28358	10923	28352	10955	28365	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	1	0	0	1	1	0
4	10833	28350	10813	28339	10853	28362	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	0
5	10790	28348	10771	28338	10809	28358	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	0
6	10903	28352	10885	28346	10921	28358	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	0
7	10968	28351	10954	28346	10982	28357	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0	0
8	10899	28341	10882	28330	10917	28353	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
9	10962	28340	10948	28333	10976	28348	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0
10	10869	28339	10857	28331	10882	28347	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
11	10993	28338	10977	28330	11009	28347	0	0	1	0	0	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0
12	10674	28331	10656	28320	10692	28343	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	0
13	10720	28329	10703	28318	10737	28340	0	0	1	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0
14	10698	28330	10688	28321	10708	28340	0	0	1	0	0	0	1	1	1	1	0	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0
15	11032	28325	11019	28317	11046	28334	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0
16	10674	28316	10660	28308	10688	28324	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0
17	10779	28309	10760	28297	10799	28321	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
18	11064	28310	11043	28300	11085	28321	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0
19	10628	28308	10611	28298	10646	28319	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0
20	11117	28300	11097	28288	11137	28313	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0
21	10600	28304	10585	28297	10615	28312	0	0	1	0	0	0	0	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0
22	10817	28302	10810	28294	10825	28310	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
23	11393	28293	11370	28279	11416	28307	0	0	1	0	0	0	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	0	0	1	1	0
24	10553	28295	10540	28288	10566	28303	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	1	0	0	1	1	0
25	10580	28287	10564	28278	10597	28296	0	0	1	0	0	0	1	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0
26	10532	28288	10522	28282	10542	28295	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	1	0
27	11142	28283	11126	28271	11158	28295	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	1	0