Targeted fluorescence-assisted nuclei sorting from post-mortem human brain

HGEN 396; Winter 2020; Joon Hwan Hong (260832806)

Outline

Project Basis (Rationale)

Targets

Results

Further Directions

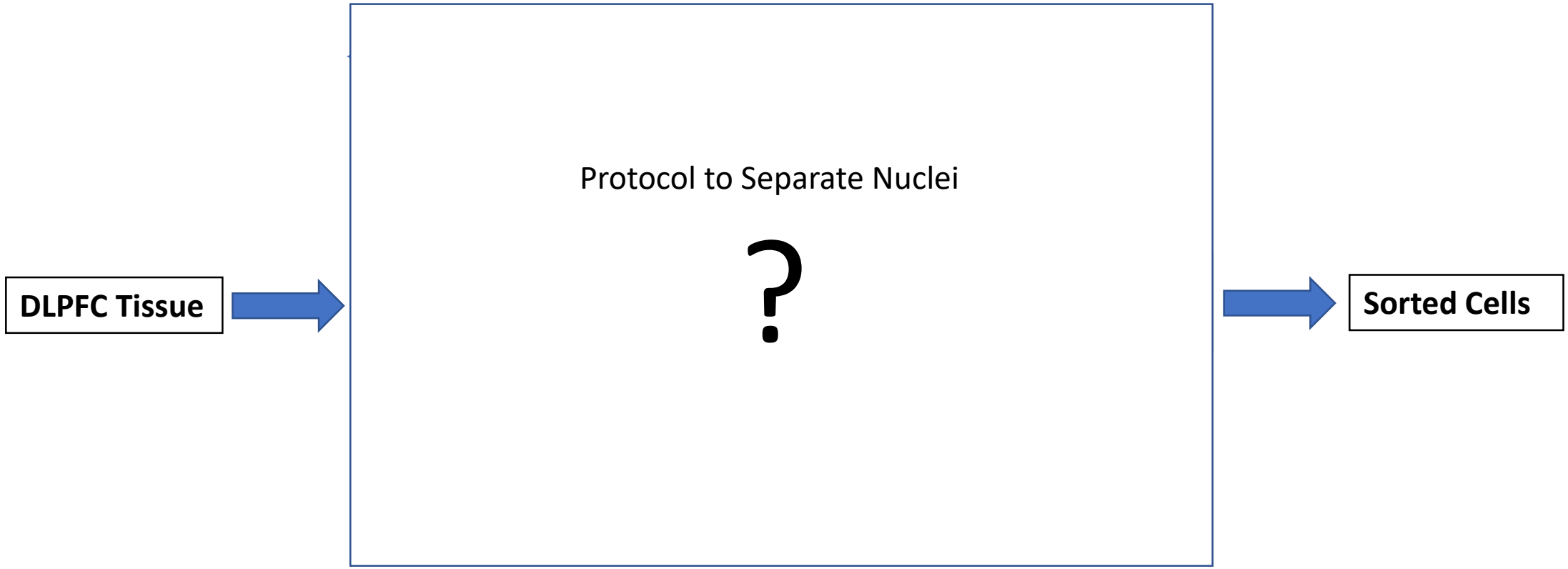
What I Have Learned

Project Basis

- Continuation of previous work done in the lab
  - snRNA-seq identified 26 cell-types
  - DLPFC BA8/9 tissue

- OPC2 and Ex7 cells had largest number of differentially expressed genes between control and MDD
  - Oligodendrocyte precursor cell subtype
  - Deep layer excitatory neuron

Project Basis



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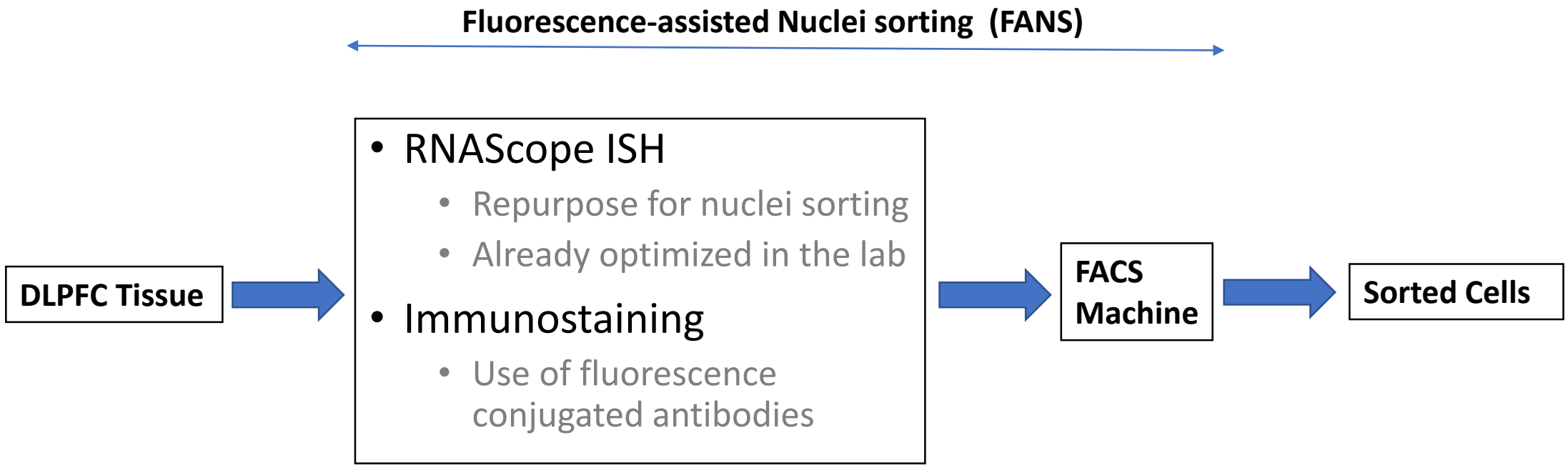
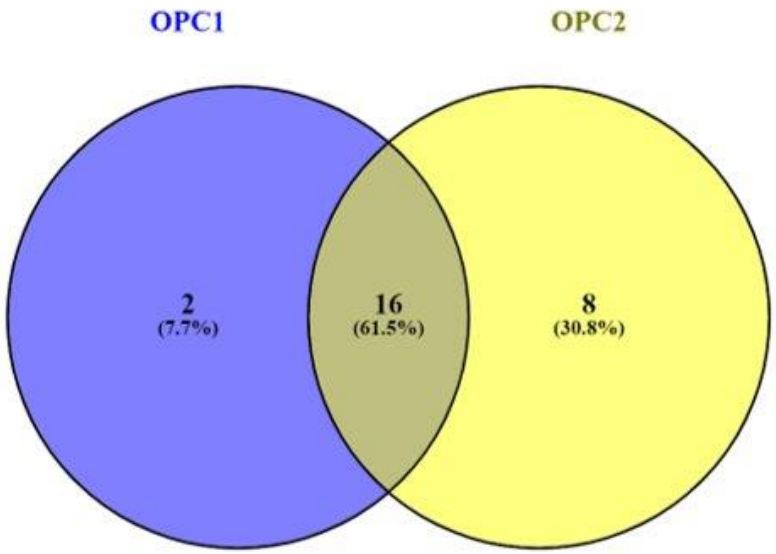
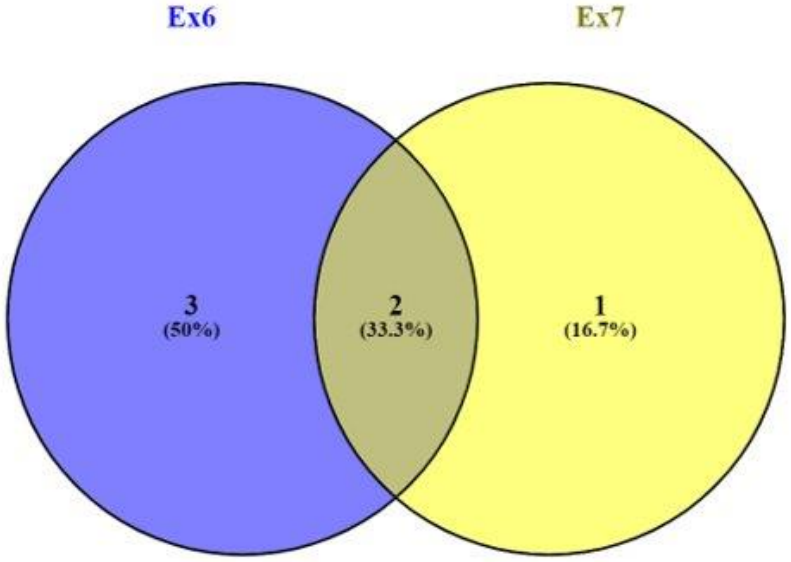


Targets

- OPC2 & OPC1



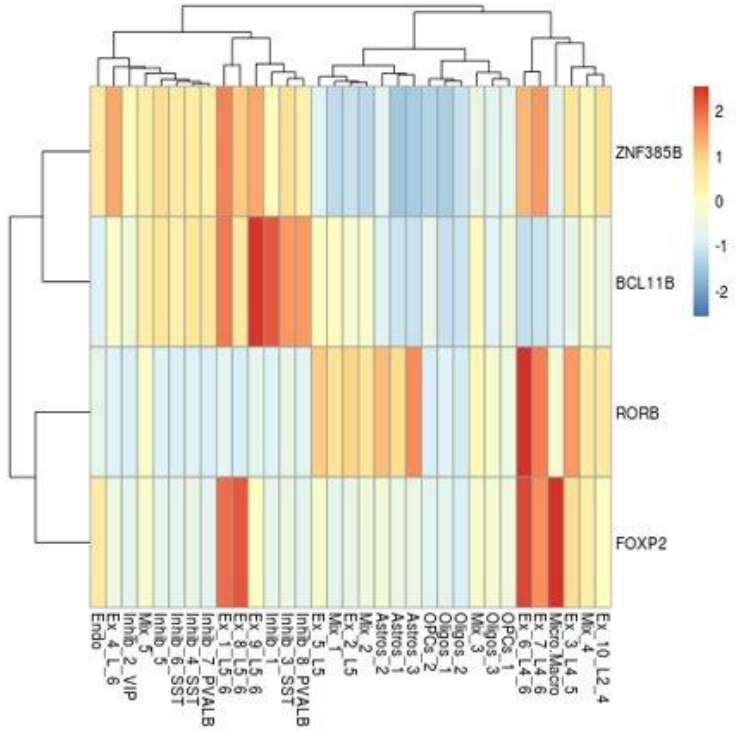
- Ex7 & Ex6



A lot in common for OPC subtypes; Difficult to separate with few marker genes for Ex7 subtype.
→ sorting for both cell types for each

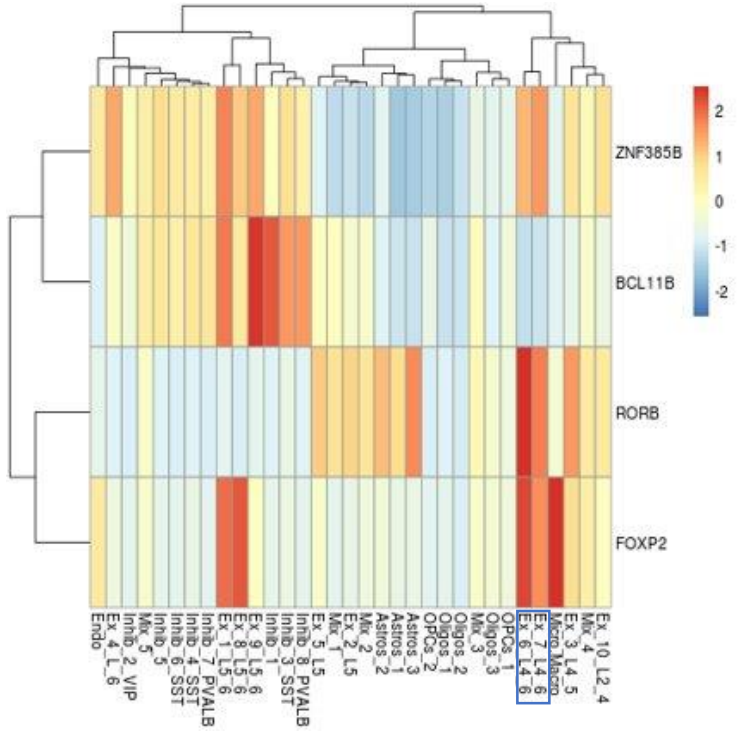
Targets

- Ex6/7
  - **FOXP2**
  - **RORB**



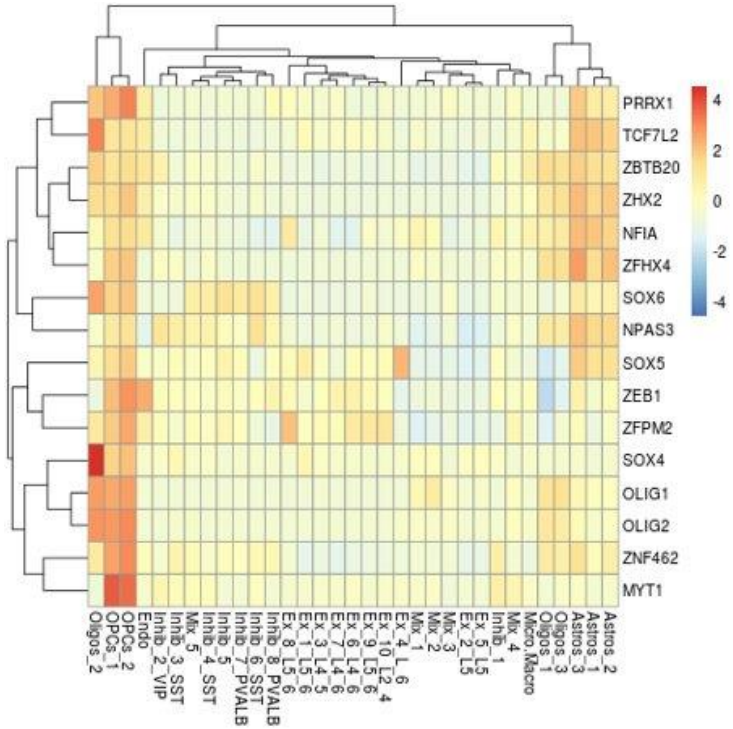
Targets

- Ex6/7
  - **FOXP2**
  - **RORB**
  - NeuN (protocol validation)



Targets

- OPC1/2
  - **MYT1**
  - **PRRX1**
  - **ZFPM2**



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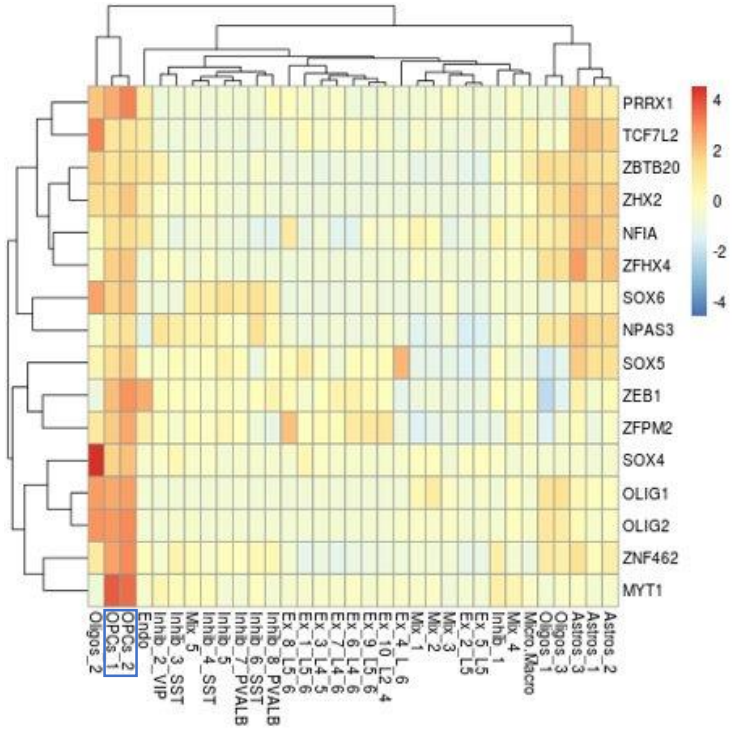
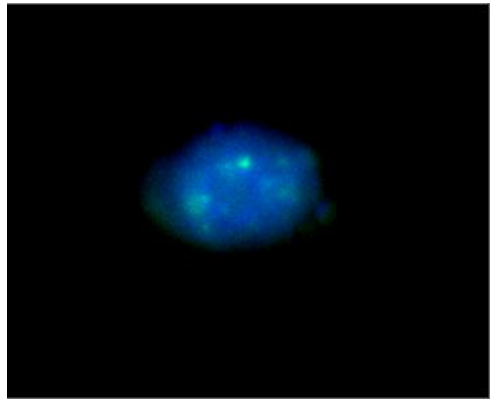
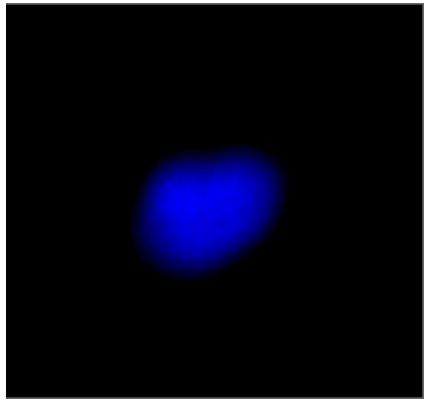
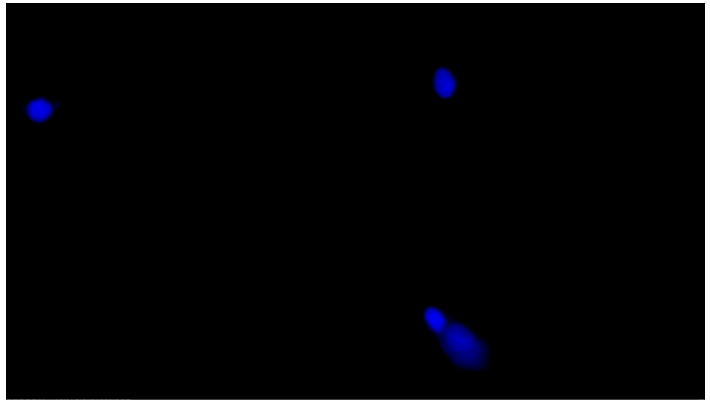


Results

- Jan 20. Nuclei Extraction with RNAScope ISH
  - Loss of nuclei pellets from multiple washes
  - Low nuclei visibility under the microscope
  - No significant signal detected under microscope







Results

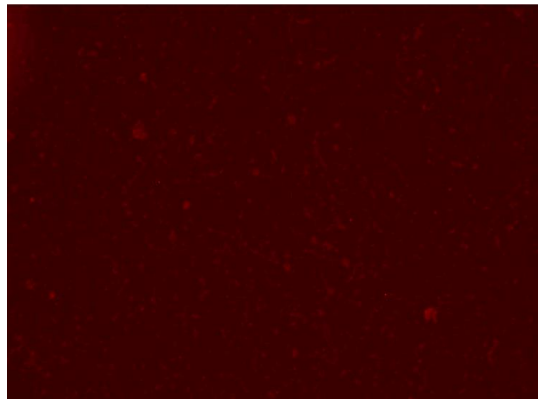
- Jan 24. Nuclei Extraction with AB
  - OPC
    - MYT1
    - PRRX1
    - ZFPM2
  - Ex6/7
    - FOXP2
  - Non-selective

Tube	Animal/ Type	Antibody	Target	Secondary
1	Rabbit Poly	MYT1 (A10824)	OPC	Alexa 488 1/2000 (anti rabbit)
2	Rabbit Poly	PRRX1 (A10237)	OPC	Alexa 488 1/2000 (anti rabbit)
3	Rabbit Poly	ZFPM2 (A9868)	OPC	Alexa 488 1/2000 (anti rabbit)
4	Rabbit Poly	FOXP2 (A5677)	Ex6/7	Alexa 488 1/2000 (anti rabbit)
5	Mouse Mono	FOXP2 (MA-531735)	Ex6/7	Alexa 488 1/2000 (anti mouse)

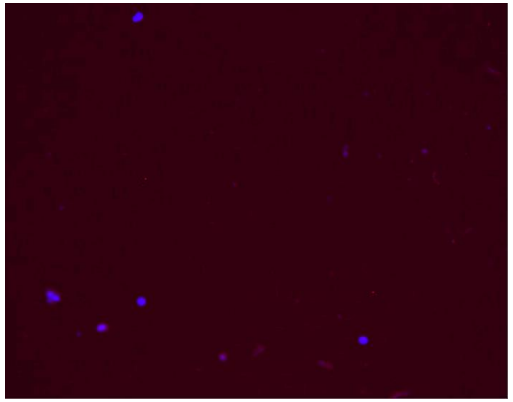
Results

- Jan 31. Nuclei Extraction with RNAScope ISH 2
  - Positive, Negative control, SLC17A7 & GAD1
  - No significant signal detected under microscope; very weak signal

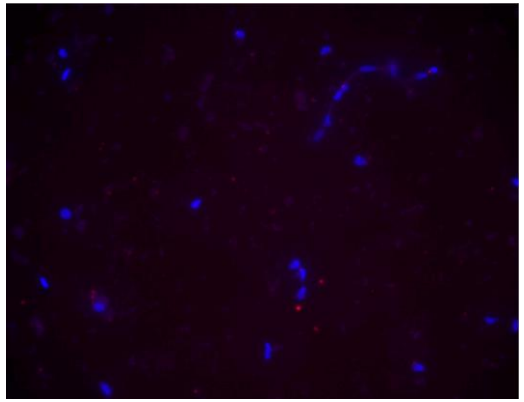
Negative Control



Positive Control



SLC17A7 GAD1



Results

- Feb 7. Nuclei Extraction with AB
  - RORB

Tube	Animal/ Type	Antibody	Target	Secondary
1	Rabbit Poly	RORB(PA5 30152)	Ex 6/7	Alexa 488 1/2000 (anti rabbit)

  - Increased tissue mass for nuclei extraction from 50 mg to 100 mg
    - No significant signal detected under microscope
    - Non-selective binding

Results

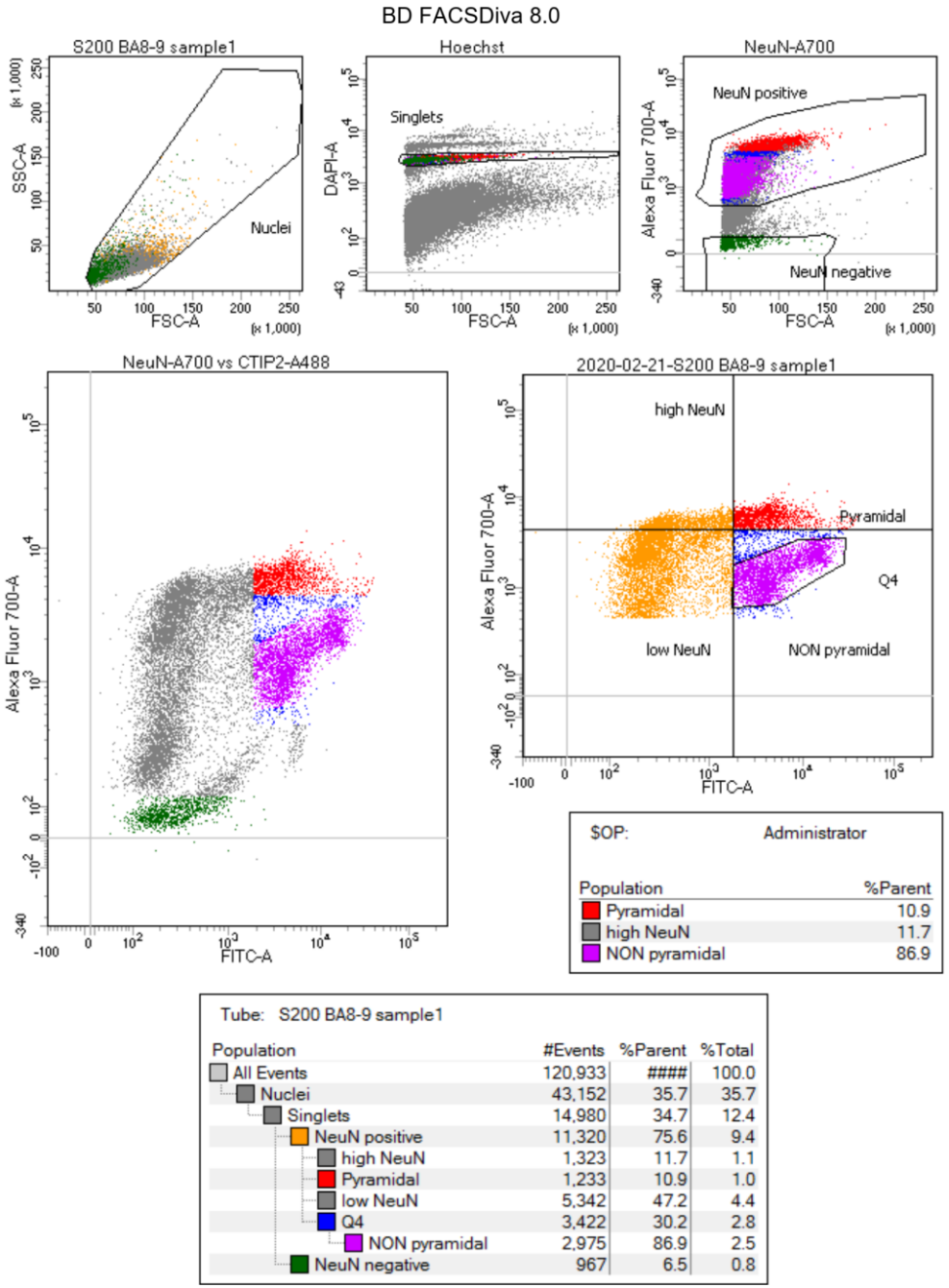
- Feb 14. Nuclei Extraction with AB
  - Tested to see if formalin fixation improved results
    - Tested different concentrations
    - Neither showed significant result under the microscope.

Tube	Animal/ Type	Antibody	Target	Secondary
1	Rabbit Poly	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
2	Rabbit Poly	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
3	Rabbit Poly	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
4	Rabbit Poly	FOXP2 (MA-531735), NeuN-647	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
5 (f)	Rabbit Poly	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
6 (f)	Rabbit Poly	NeuN-647	Ex 6/7	NA
7 (f)	Rabbit Poly	FOXP2 (MA-531735), NeuN-647	Ex 6/7	Alexa 488 1/2000 (anti rabbit)
8 (f)	None	None	Ex 6/7	NA
9 (f)	None	No Hoechst	Ex 6/7	NA

Results

- Feb 21. NeuN Protocol Validation
  - NeuN: Neuronal biomarker
  - Tested if the protocol was the issue

  - Result:
    - Recognized in FACS machine.
    - The protocol worked as expected.



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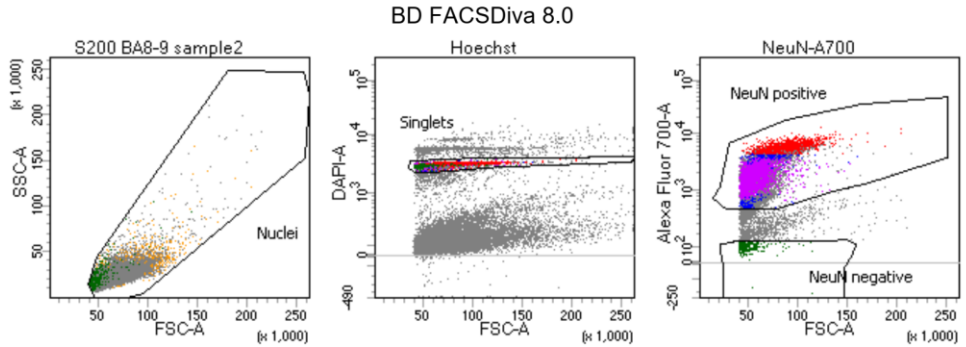
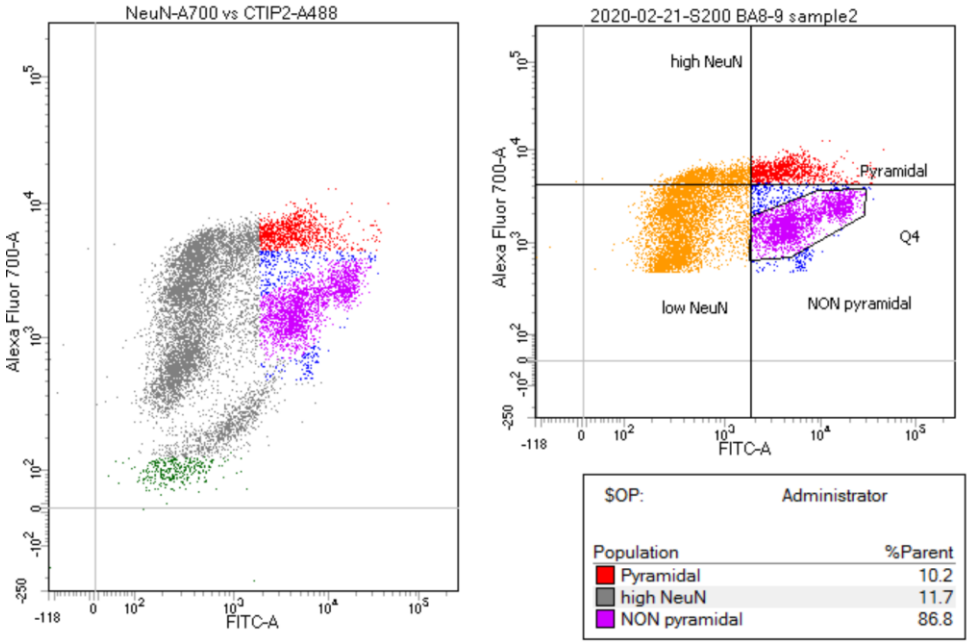




Tube: S200 BA8-9 sample2

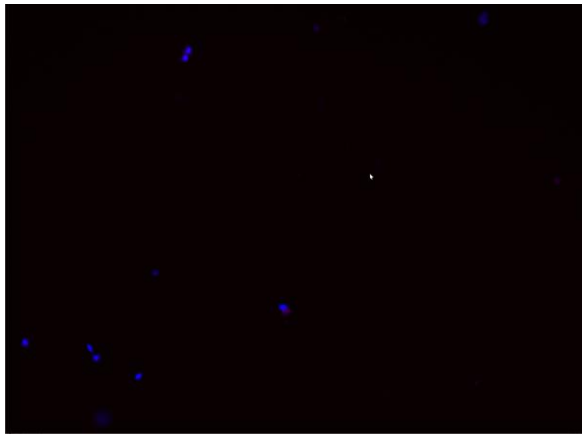
Population	#Events	%Parent	%Total
All Events	93,555	####	100.0
Nuclei	33,076	35.4	35.4
Singlets	11,387	34.4	12.2
NeuN positive	9,569	84.0	10.2
high NeuN	1,124	11.7	1.2
Pyramidal	977	10.2	1.0
low NeuN	4,866	50.9	5.2
Q4	2,602	27.2	2.8
NON pyramidal	2,258	86.8	2.4
NeuN negative	313	2.7	0.3

- FEB 28 Prepare for Modified RNAScope protocol

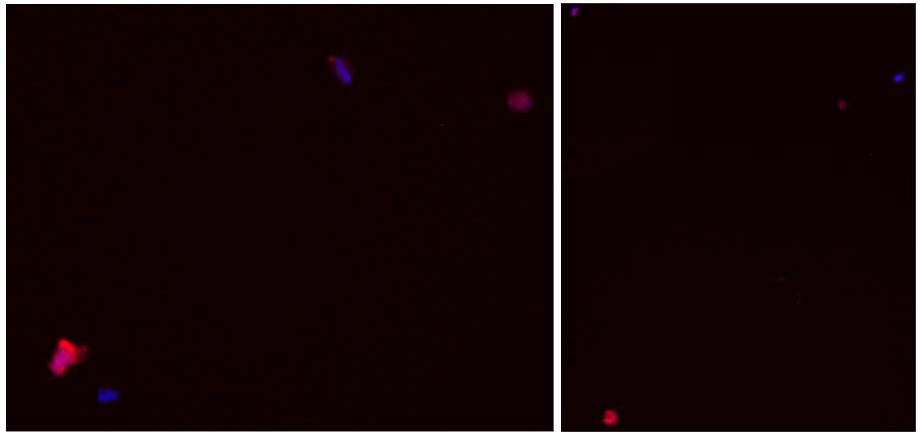
Results

- Mar 2-3. Nuclei Extraction with Modified RNAScope (3)
  - Visually under the microscope: specific?
  - *Could not be detected by FACS machine.*

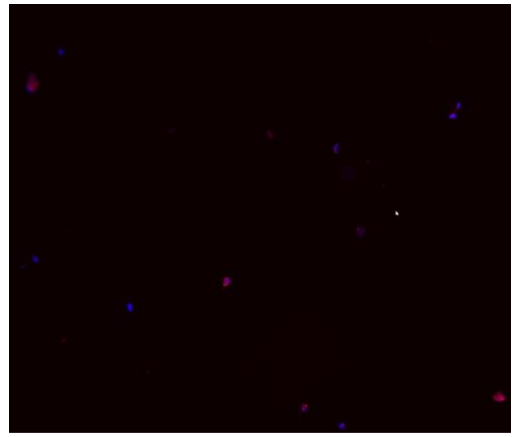
Negative Control



SLC17A7 GAD1



UBC C1

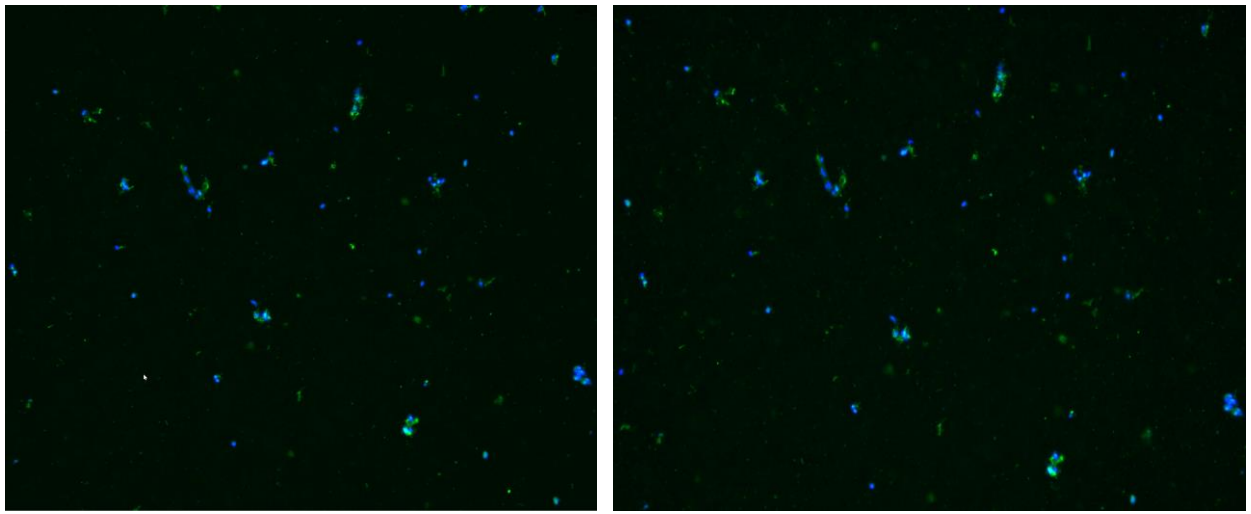


Results

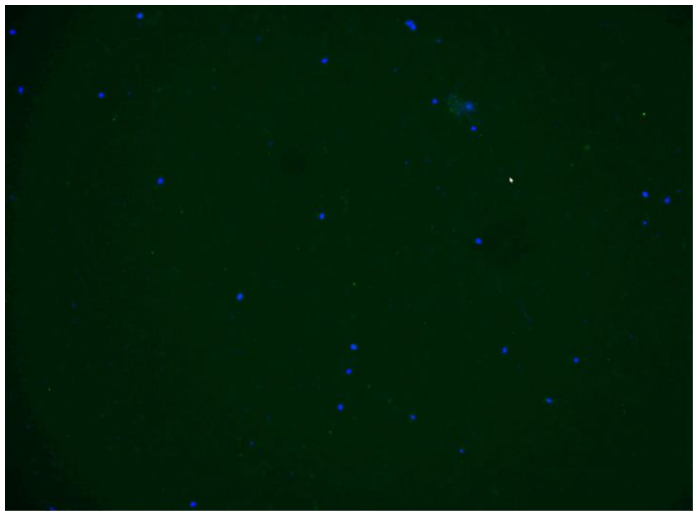
- March 9. Nuclei Extraction with AB
  - FOXP2
  - Retested Mouse-Mono FOXP2 AB (Feb 14th)
    - Likely used Rabbit poly on Feb 14th
    - No significant results under microscope

Tube	Animal/ Type	Antibody	Target	Secondary
1	Goat poly	FOXP2 (PA5-17977)	Ex 6/7	Alexa 488 1/2000 (anti goat)
2 (4C)	Goat poly	FOXP2 (PA5-17977)	Ex 6/7	Alexa 488 1/2000 (anti goat)
3	Mouse Mono	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti mouse)
4 (4C)	Mouse Mono	FOXP2 (MA-531735)	Ex 6/7	Alexa 488 1/2000 (anti mouse)

Goat



Mouse



Probe Synthesis (Probe-Seq v.1.3)

- Mar 13. Probe Design/Synthesis Steps
  - Following procedure from Probe-Seq v.1.3
    - Obtained gene-specific BED files

- Further work stopped due to COVID19

Further Directions

- Test the Probe-seq protocol with the designed probes
- More antibody testing
- FACS Machine usage
- Consistency in nuclei pellets

Overall

Techniques

- Brain sample preparation (cutting)
- Tissue homogenization
- Immunostaining & RNAScope
- Image analysis

Knowledge

- In situ hybridization
- Flow cytometry and cell sorting
- Various __-seq technologies
- Use of transcriptomics for cell specificity
- DNA Methylation in neurons and glia