Examining neuronal cell type specificity using FOXP2

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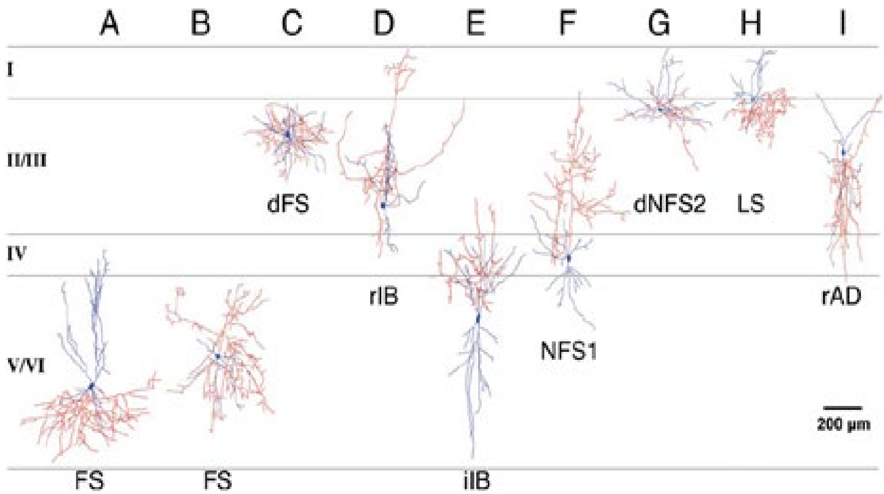
Who is Nazeli Acosta?

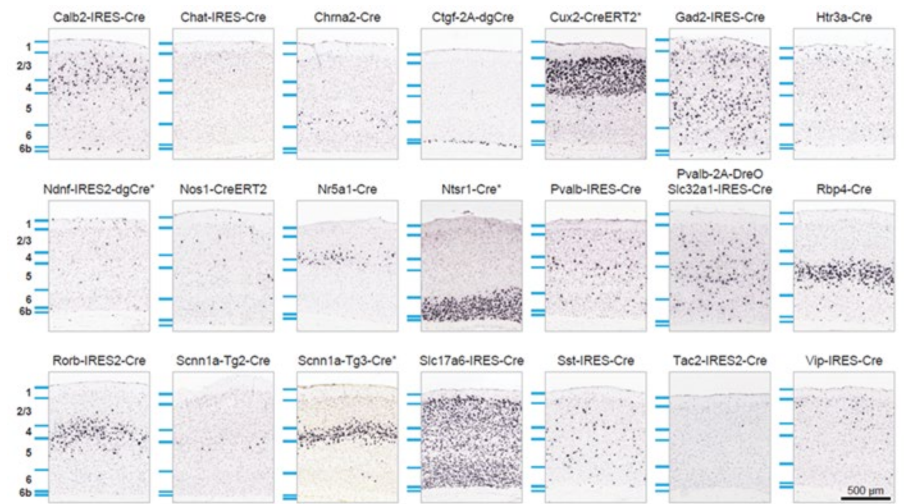
Rising Sophomore at Providence College studying Biomedical Engineering

Interested in tutoring, intellectual debates, finding sustainable solutions to problems in the developing world etc.

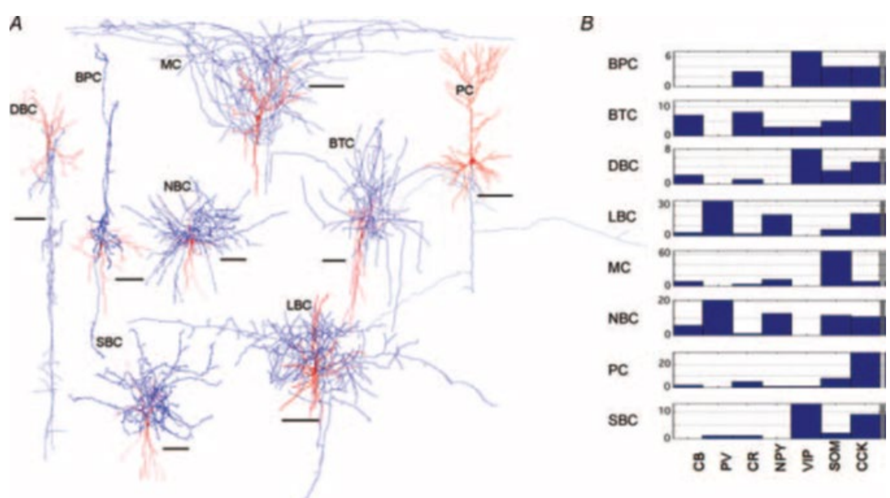
Attempts to classify cellular diversity in mouse cortex



Miyoshi et al. J. Neurosci. 2007



Tasic, Menon…Zeng (2016) Nature Neuroscience



Toledo-Rodriguez *et al.* J Physiol 2005

New approaches are needed to study brain structure, function and disease in human

Animal models of Alzheimer disease: historical pitfalls and a path forward

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PNAS

Genomic responses in mouse models poorly mimic human inflammatory diseases

Junhee Seok[a,1], H. Shaw Warren[b,1], Alex G. Cuenca[c,1], Michael N. Mindrinos[a], Henry V. Baker[c], Weihong Xu[a], Daniel R. Richards[d], Grace P. McDonald-Smith[e], Hong Gao[a], Laura Hennessy[f], Celeste C. Finnerty[g], Cecilia M. López[c], Shari Honari[f], Ernest E. Moore[h], Joseph P. Minei[i], Joseph Cuschieri[j], Paul E. Bankey[k], Jeffrey L. Johnson[h], Jason Sperry[l], Avery B. Nathens[m], Timothy R. Billiar[l], Michael A. West[n], Marc G. Jeschke[o], Matthew B. Klein[j], Richard L. Gamelli[p], Nicole S. Gibran[j], Bernard H. Brownstein[q], Carol Miller-Graziano[k], Steve E. Calvano[r], Philip H. Mason[e], J. Perren Cobb[s], Laurence G. Rahme[t], Stephen F. Lowry[r,2], Ronald V. Maier[j], Lyle L. Moldawer[c], David N. Herndon[g], Ronald W. Davis[a,3], Wenzhong Xiao[a,t,3], Ronald G. Tompkins[t,3], and the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program[4]

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Review Article

Lost in translation: animal models and clinical trials in cancer treatment

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Abstract: Due to practical and ethical concerns associated with human experimentation, animal models have been essential in cancer research. However, the average rate of successful translation from animal models to clinical cancer trials is less than 8%. Animal models are limited in their ability to mimic the extremely complex process of human carcinogenesis, physiology and progression. Therefore the safety and efficacy identified in animal studies is generally not translated to human trials. Animal models can serve as an important source of *in vivo* information, but alternative translational approaches have emerged that may eventually replace the link between *in vitro* studies and clinical applications. This review summarizes the current state of animal model translation to clinical practice, and offers some explanations for the general lack of success in this process. In addition, some alternative strategies to the classic *in vivo* approach are discussed.

Lost in translation: Treatment trials in the SOD1 mouse and in human ALS

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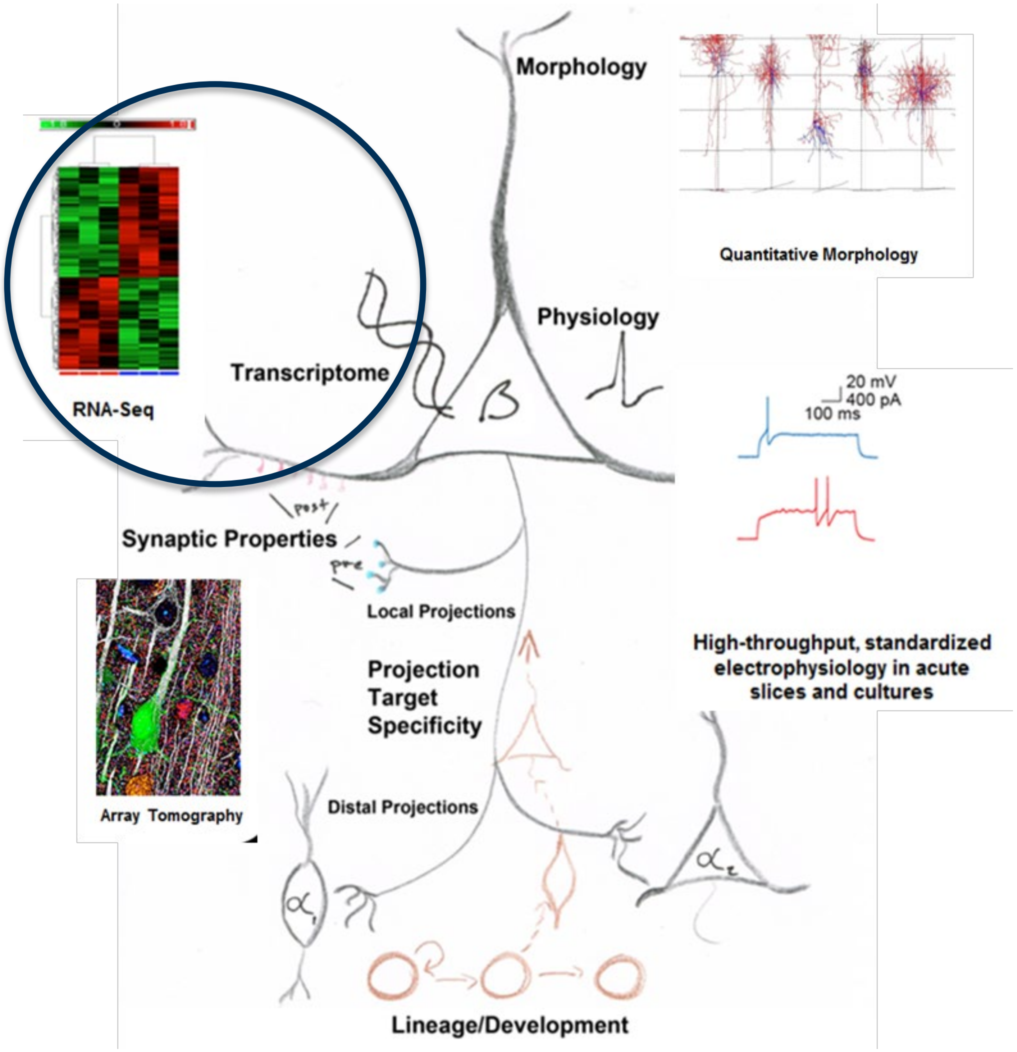
Available online 3 January 2007

Human Cell Types Program: Using a systematic approach to study cell types in human neocortex



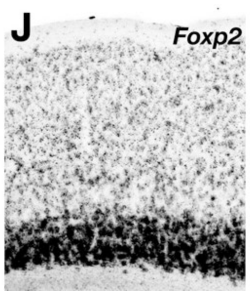
FOXP2 is associated with language and defines specific cortical cell types

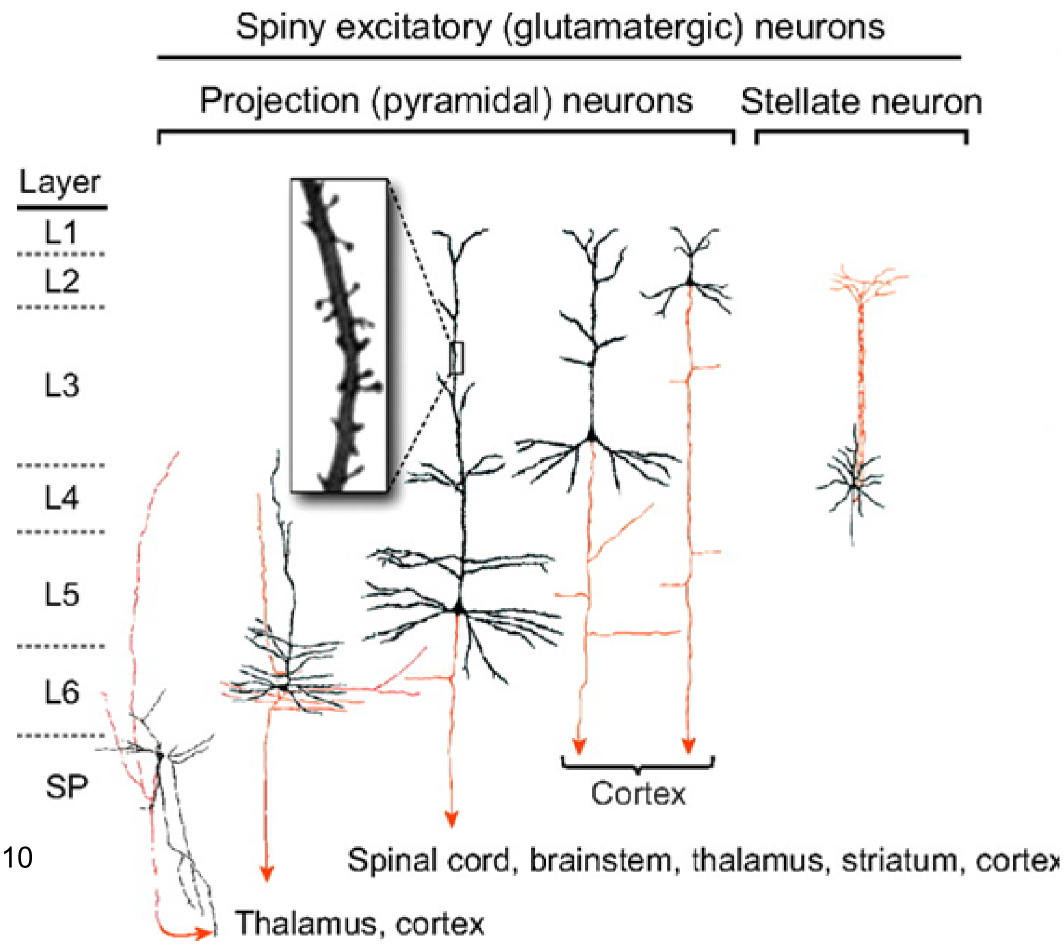
It is a marker of layer 6 neurons in the cortex

This suggests **FOXP2** is involved in the feedback loop between the thalamus and the cortex

L5

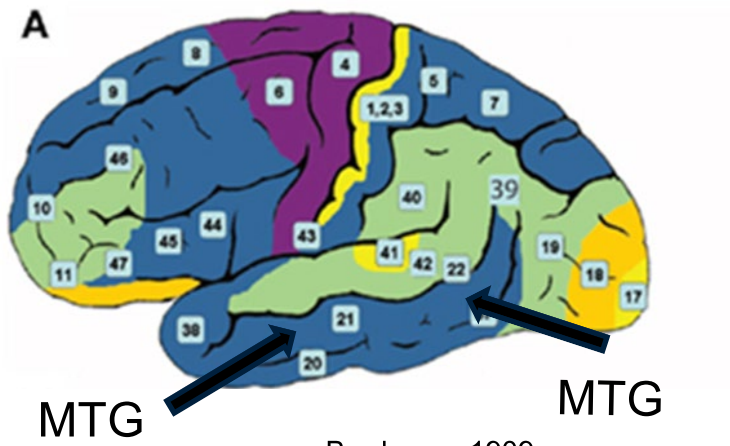
L6



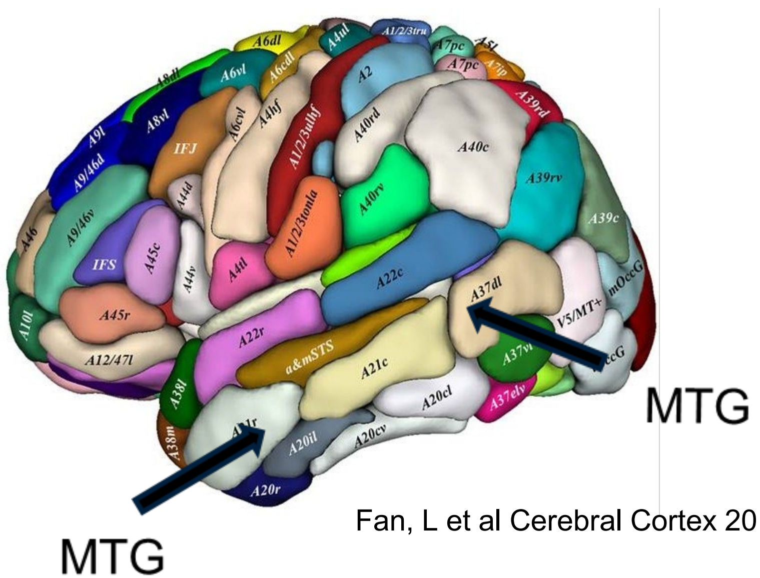


Rowell et al. Journal of Comparative Neurology, 2010

Middle Temporal Gyrus

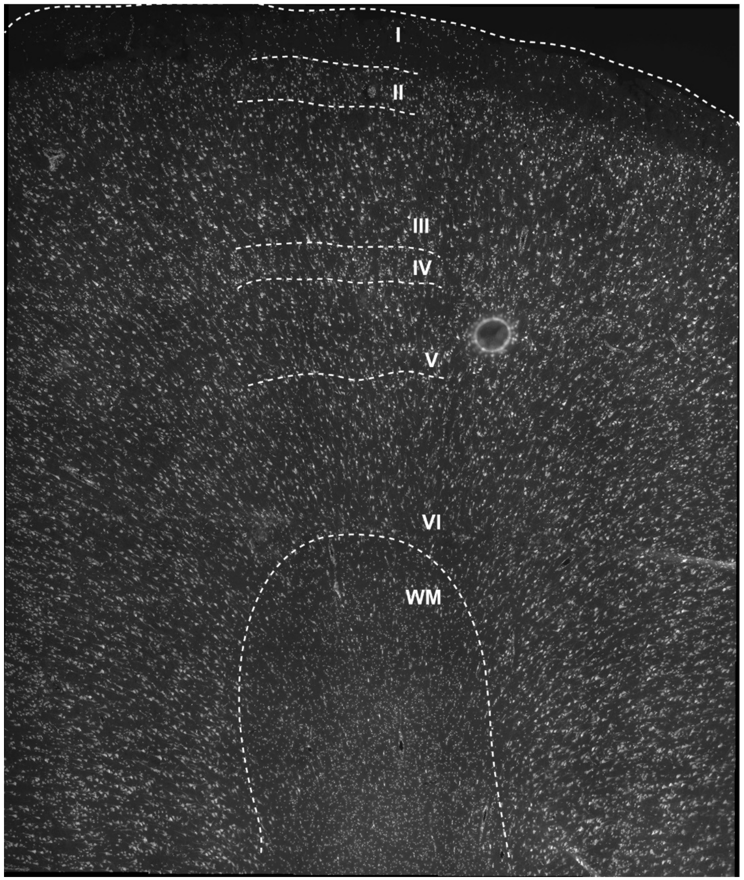


Brodmann 1909

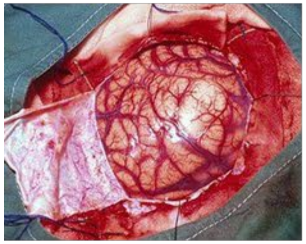


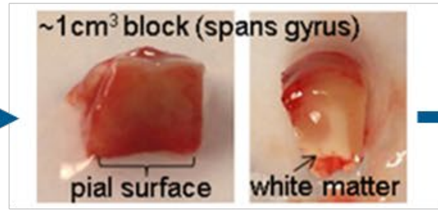
Fan, L et al Cerebral Cortex 2016

NeuN staining of the mid temporal gyrus

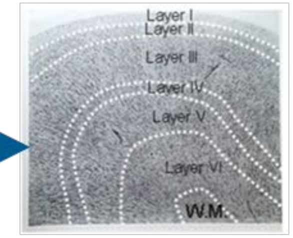


Processing of neurosurgical tissue





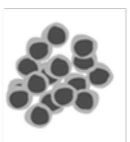
Vibratome section at 350 um



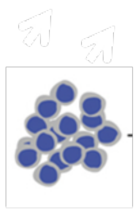
Dissect layer of interest

Pool dissected samples and proceed to cell dissociation

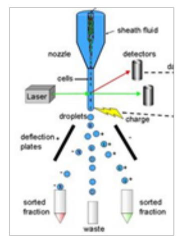
Isolation and sorting of single cells via FRISCR



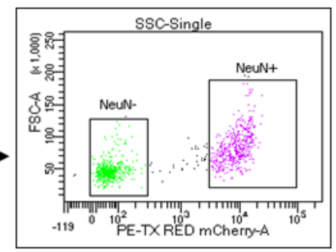
Dissociate and fix



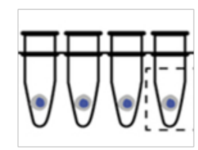
Permeabilize and stain with FOXP2



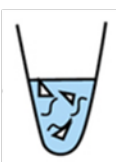
FACS



Select FOXP2+/FOXP2- events

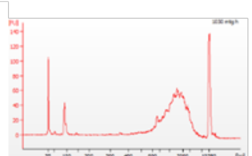


Sort single cells

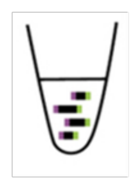


Lyse nuclei or cells and reverse cross-link

Purify and elute mRNA



cDNA library preparation



RNAseq

Thomsen et al. Nature 2016

Procedures

Test out FOXP2 via Immunohistochemistry on fixed human tissue slice

↓

Neurosurgical live tissue preparation

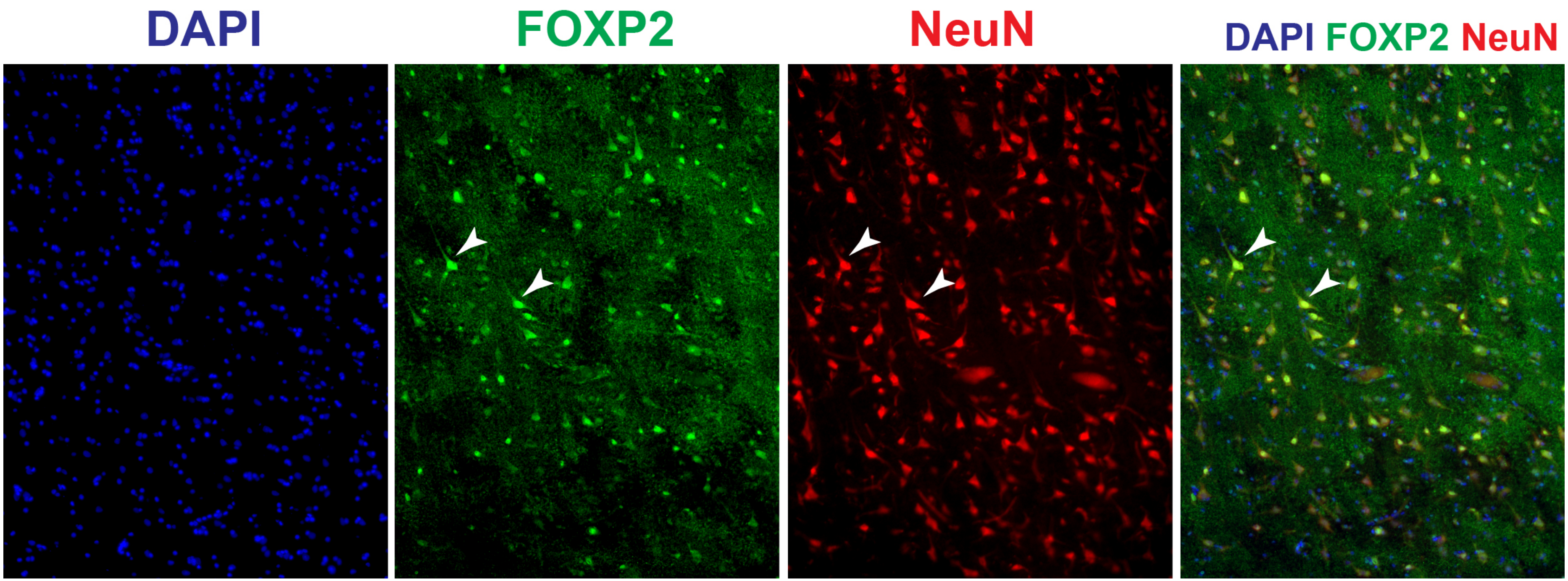
↓

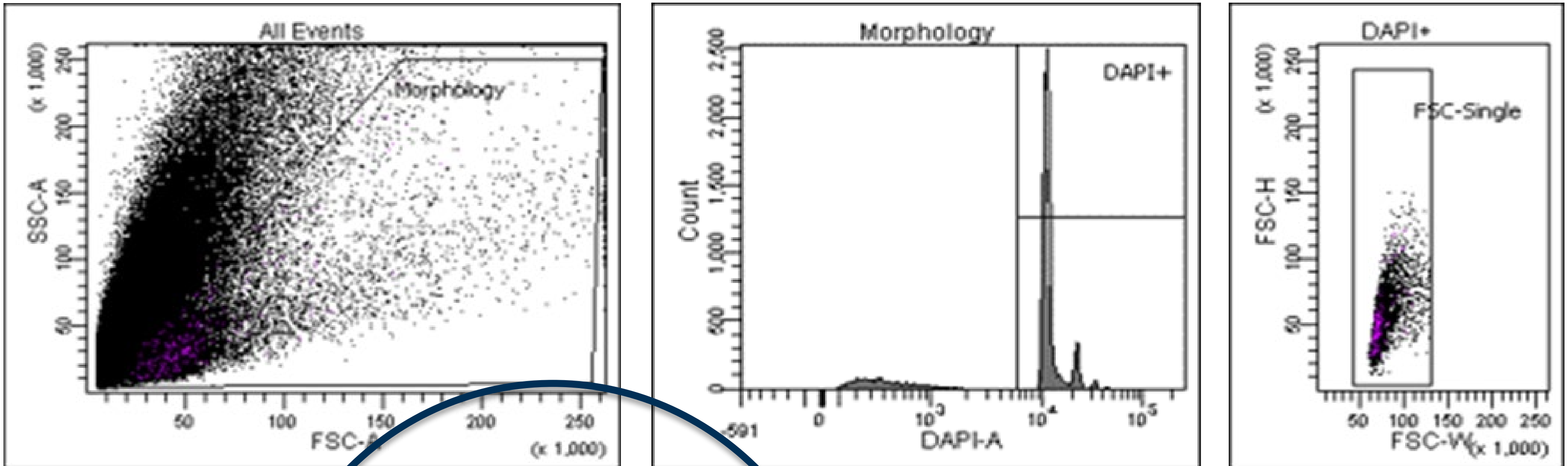
Test out FOXP2 via FACS (Flow cytometry) on dissociated whole cells

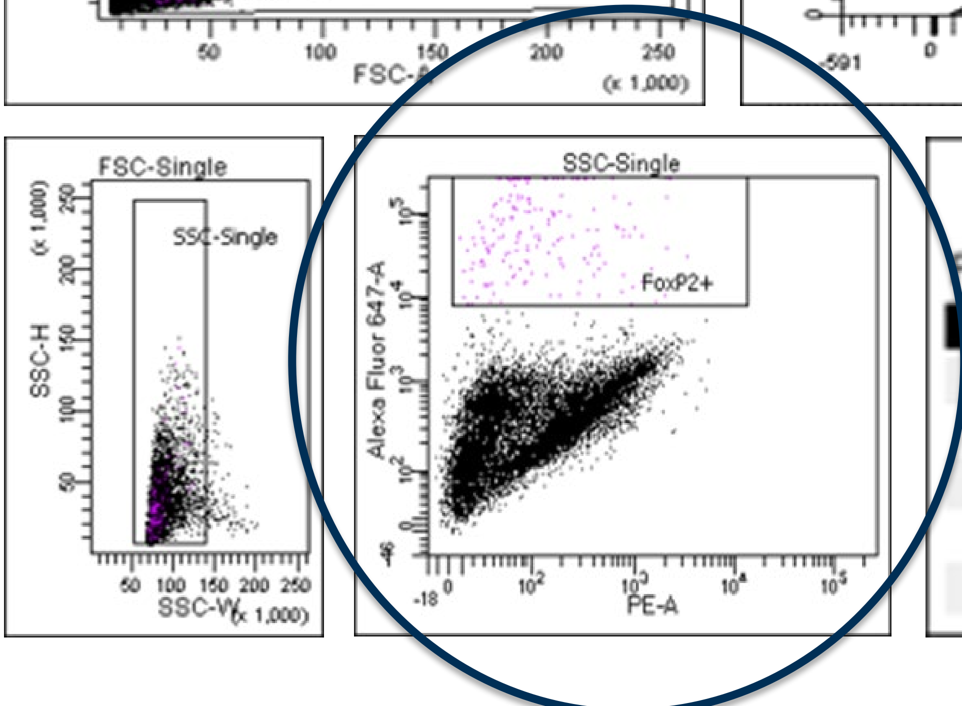
↓

Sort labeled FOXP2 positive single cells and proceed to remainder of FRISCR method

IHC and FACS staining results



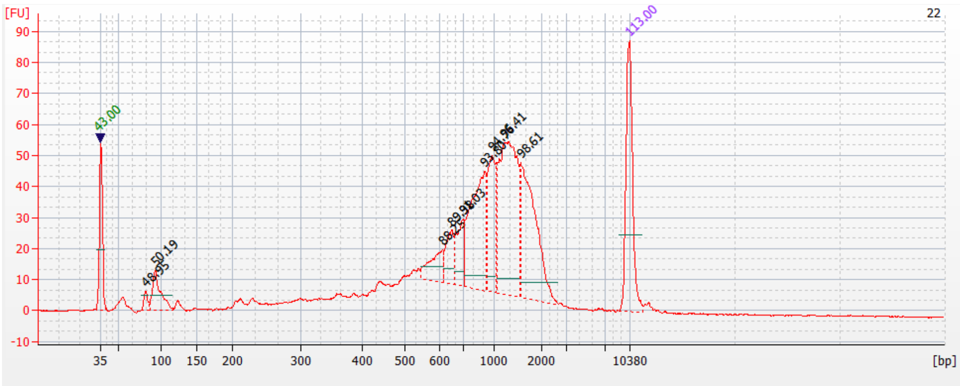




Tube: NeuN_PE FoxP2 1_1000 AF647

Population	#Events	%Parent	%Total
All Events	218,125	####	100.0
Morphology	16,340	7.5	7.5
DAPI+	12,269	75.1	5.6
FSC-Single	11,826	96.4	5.4
SSC-Single	11,525	97.5	5.3
FoxP2+	179	1.6	0.1

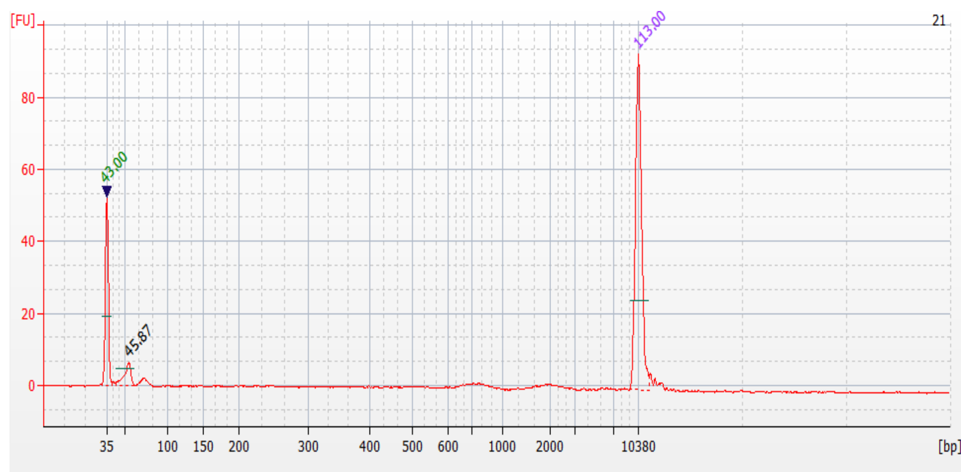
Bioanalyzer Results



		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
▶	◀	35	125.00	5,411.3	Lower Marker
2		82	13.81	254.6	
3		93	60.99	989.8	
4		612	27.85	69.0	
5		648	26.91	62.9	
6		700	33.18	71.8	
7		906	107.17	179.3	
8		977	54.99	85.2	

Results | Peak Table | Region Table | Legend

High quality cDNA from FOXP2 cell



		Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]	Observations
▶	◀	35	125.00	5,411.3	Lower Marker
2		55	28.10	780.4	
3	▶	10,380	75.00	10.9	Upper Marker

Poor quality cDNA from FOXP2 cell

Acknowledgements

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Christine MacDonald
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