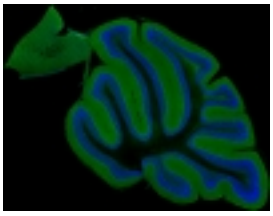
Cell Centered Database

University of California, San Diego

Maryann Martone

Microscopy Product #:33 102003a

For the most updated information, please visit
http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=33

Image2D	Reconstruction	Segmentation
		

Project Information:

PROJECT_ID	P1187
PROJECT_NAME	Correlated Imaging Approaches and Multiscale Databases for Research in Parkinson's Disease
PROJECT_DESCRIPTION	characterization of a mouse model of human alpha synuclein overexpressor
LEADER	Diana Price
FUNDING_AGENCY	The Branfman Family Foundation
PROJECT_START_DATE	2002-09-01 00:00:00.0
PROJECT_END_DATE	2003-06-30 00:00:00.0
COLLABORATORS	M.H. Ellisman; M. Martone; G.A. Johnson; E. Masliah
PUBLICATION1	Price DL;Martone ME; Masliah MH; Ellisman MH (2003) High-resolution Large-Scale 3-D Mapping Studies of Alpha-Synuclein Immunoreactivity in Transgenic Mice Overexpressing Human Alpha-Synuclein. Society for Neuroscience Abstract.
PUBLICATION2	Price DL, Chow SK, MacLean NAB, Hakozaki H, Peltier S, Martone ME, Ellisman MH (2006) High-Resolution Large-Scale Mosaic Imaging using Multiphoton Microscopy to Characterize Transgenic Mouse Models of Human Neurological Disorders. Neuroinformatics. 2006;4(1):65-80.
PUBLICATION3	

Experiment Information -	
PURPOSE	to determine the distribution of alpha-synuclein immunolabeling in control and transgenic mouse tissue
TITLE	Alpha-synuclein immunolabeling for large-scale mapping study
EXPERIMENTER	Diana Price
EXPERIMENT_NAME	
EXPERIMENT_DATE	2003-07-28 00:00:00.0

Subject Information -	
GROUP_BY	genetic manipulation
SUBJECT_NAME	transgenic
FIXATION_METHOD_ID	2
SCIENTIFIC_NAME	mus musculus
SPECIES	mouse
STRAIN	Unspecified
AGE	290 days
AGECLASS	adult
ANIMAL_NAME	
LITTER_ID	
SEX	female
VENDOR	
WEIGHT	32 grams

Tissue -	
ANATOMIC_LOCATION	large scale at level of cerebellum
MICROTOME	vibratome
ORIENTATION	sagittal
THICKNESS	80 um
TISSUE_PROD_STORAGE	p1187 #2
EXTERNAL_FILE_NAME	
TISSUE_GROUP_TYPE	anterior/posterior region

Microscopy Product Information -	
MICROSCOPY_PRODUCT_ID	33
IMAGE_BASENAME	102003a
CREATE_DATE	2003-10-20 00:00:00.0
INSTRUMENT	BioRad RTS 2000MP Multiphoton
MICROSCOPE_TYPE	multiphoton
PLANE_COUNT	
PRODUCT_TYPE	optical section series/mosaic
PURL	NA
SESSION_NAME	
TELESCIENCE_SRB	P1187/Experiment_21/Subject_21/Tissue_26/Microscopy_33
X_RESOLUTION	.237 pixels/um
Y_RESOLUTION	.237 pixels/um
XSIZE	52
YSIZE	37

Protocol:

P1187 Exp. 10A 2-photon Study: Branfman Project 7/28/03

Description: alpha-synuclein + nuclear stain for large scale mapping study in striatal region

Protocol

1. Perfusion

Nembutal; 4% paraformaldehyde
2 hr. postfix in 4% para

2. Sectioned on Vibratome

Thickness = 80 microns
3 blocks at 2 mm each from anterior (A, B, C) + cerebellum

3. sections stored in cryoprotectant at -20 (7/30/03)

4. Wash 6x with PBS 1X (on ice)

5. Make up blocking buffer

PBS w/o NaCl = buffer used
Total amount needed = 33 mls x 3

For each 33 ml:

Ingredient	Amount
0.8 PBS	6.6 ml 5X PBS + 24.2 ml 2x distilled H20
3% NDS (24 , 4)	0.96 ml
1% fish gel	0.33 ml
0.1% Triton X-100	0.0332 ml
1% BSA	0.33 g

6. Block slices (1 hr) in blocking buffer

7. Make up working buffer

Use blocking buffer to dilute to working buffer

Ingredient	200ml	150ml	100ml	50ml
Blocking buffer	20 ml	15 ml	10 ml	5 ml
0.1% Triton	0.2 ml	0.15 ml	0.1 ml	0.05 ml
1X PBS	180 ml	135 ml	90 ml	45 ml

8. Wash 1X5 minutes with working buffer

9. Dilute 1o Abs in working buffer

anti-alpha-synuclein; Host = Rabbit; 1:500 = 4 microliters in 2 ml WB

Vial	Contents/Tx	Total Volume	Amt Ab added/vial
1.	a-synuclein	2 ml	4 + 2 ?l
2.	Control	2 ml	Omitted

4 microliters x 2 vials= 8 x 3 animal = 24 microliters total alpha-synuclein

10. Place on shaker in cold room labeled & covered with aluminum foil overnight

11. Wash 6x with working buffer

12. Prepare 2o Abs (for confocal immunolabeling)

donkey a.....rabbit AF488 @ 1:100 (MBIRN Box 5)

13. Let sit on cold room shaker covered with foil for 2-24 hrs

14. Prepare nuclear stain

a. Final solution = equal parts 2xPBS + 1:100 Hoescht 33342 in ddH2O
b. Prepare each separately.
c. Once added together, you should not observe any precipitation.
d. If precipitation is observed?. Do not use the solution!
e. 2 ml x total number of vials = total ml solution needed

15. 30 min staining with nuclear stain

16. Wash 6x with 1X PBS 0.8

Image Type -	
OPTICAL_SECTION_SERIES	25
OPTICAL_Z_RESOLUTION	2.5 um

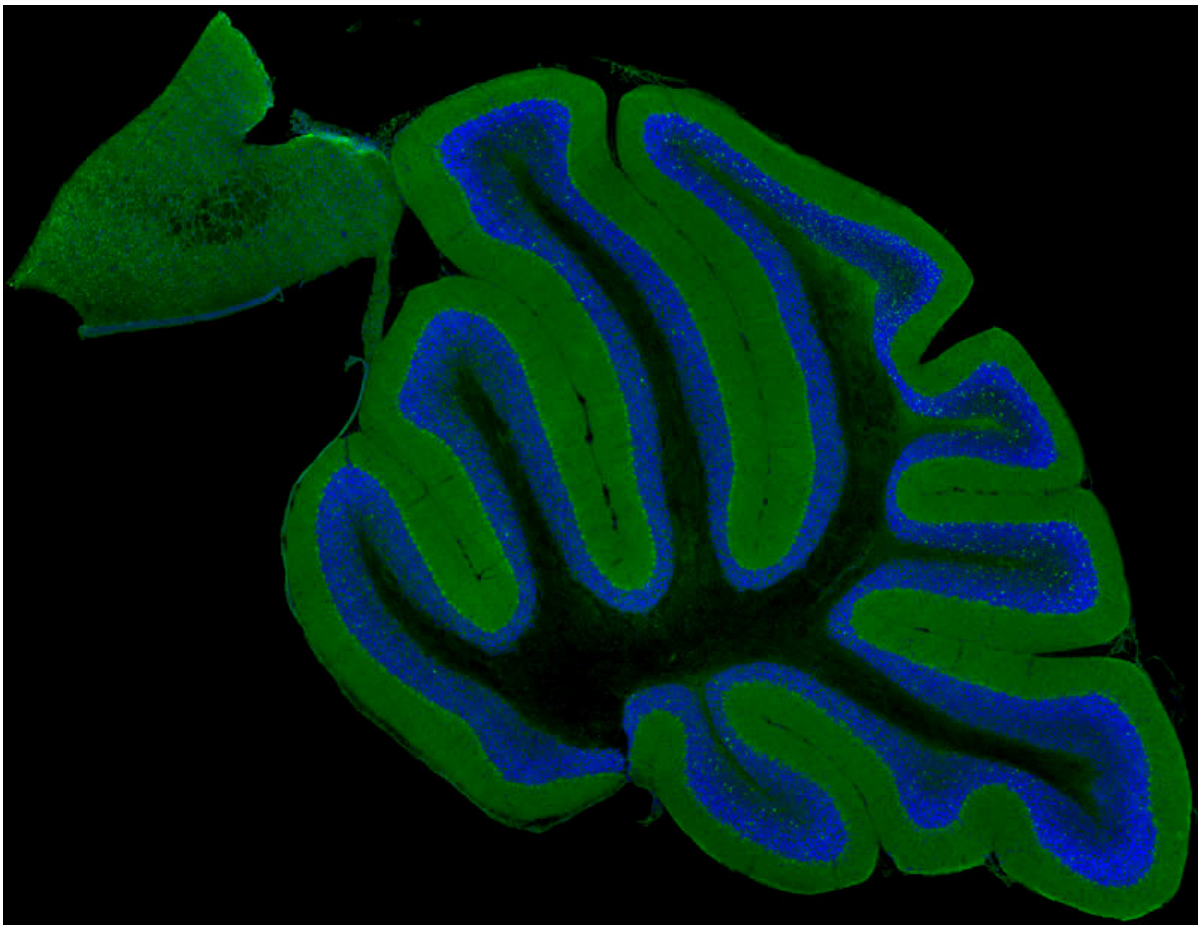
Specimen Description -	
ANATOMICAL_DETAIL	33
ATLAS	Paxinos and Franklin #105
ATLAS_COORD	.48, ,
CELL_TYPE	unspecified
MAP_LOCATION	Feb2004Diana/P1187/Experiment_10/663_tg/cerebellum/102003a_atlas.jpg
ORGAN	brain
REGION	cerebellum
SYSTEM	central nervous system

Light Microscopy Product -	
LMPRODUCT_ID	26
COVER_SLIP_THICKNESS	1 um
IMMERSION_MEDIUM	oil
LENS	Nikon Plan Fluor
LENS_MAGNIFICATION	60 x

Light Microscopy Product -	
MOUNTING_MEDIUM	vectashield with slow fade
NUMERICAL_APERTURE	1.4
REFRACTIVE_INDEX	1

Reconstruction

Reconstruction Image -



Reconstruction -	
RECONSTRUCTION3D_ID	33
ALIGNMENT_METHOD	automatic
ALIGNMENT_PROGRAM	IMOD
BASENAME_ORIGFILE	NA
CROPPING_COORDINATE1	,
CROPPING_COORDINATE2	,
RECON_DESC	Manual Alignment
RECON_PROGRAM	IMOD
RECON_TYPE	optical section series/mosaic
THUMBNAIL	P1187/102003a_vt.jpg
VOLUME_DIMENSION	, ,
VOLUME_NAME	Feb2004Diana/P1187/Experiment_10/663_tg/cerebellum/102003ARGBcombined.tiff
VOXEL_SCALE	, ,
RECONSTRUCTION_IMAGES_ID	33
NEUROINFORMATICA_URL	http://ccdb-aims.ucsd.edu:8880/showMe.jsp?instGUID=C26B5F3A-80D3-0D9F-EADD-663E71BF30D4
RECON_IMAGE_DESC	Image mosaic of a section through mouse cerebellum showing the distribution of alpha synuclein (green) in transgenic mouse overexpressing alpha synuclein. Section was counterstained with a nuclear stain (blue) to reveal the locations of cell somata
RECON_FILE_NAME	Feb2004Diana/102003a.jpg
VOLUME_THUMBNAIL	P1187/102003a_vt.jpg

USER AGREEMENT

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USER NOTIFICATION

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ACKNOWLEDGEMENT

Data used from the CCDB should be appropriately referenced, including both the author of the data and the CCDB. If the data were from a published study, the reference is included in the database record. The following reference should be cited for the CCDB:

Martone, M. E., Gupta, A., Wong, M., Qian, X., Sosinsky, G., Ludaescher, B., and Ellisman, M. H. A cell centered database for electron tomographic data. J. Struct. Biology 138: 145-155, 2002.

In addition, the support for the Cell Centered Database should be included in the acknolwedgement section of any publication:

The Cell Centered Database is supported by NIH grants from NCRR RR04050, RR RR08605 and the Human Brain Project DA016602 from the National Institute on Drug Abuse, the National Institute of Biomedical Imaging and Bioengineering and the National Institute of Mental Health, and NSF grants supporting the National Partnership for Advanced Computational Infrastructure NSF-ASC 97-5249 and MCB-9728338.

Maryann Martone