

# Cell Centered Database

University of California, San Diego

[maryann@ncmir.ucsd.edu](mailto:maryann@ncmir.ucsd.edu)

Microscopy Product #:4064 112006e

For the most updated information, please visit

<http://ccdb.ucsd.edu/CCDBWebSite/main?event=displaySum&mpid=4064>

| Image2D   | Reconstruction | Segmentation |
|---|----------------|--------------|
|  |                |              |

## Project Information:

|                     |  |
|---------------------|--|
| PROJECT_ID          | P1723  |
| PROJECT_NAME        | Localization of Metabotropic Glutamate Receptors in Alpha Synuclein Overexpressing Mouse             |
| PROJECT_DESCRIPTION | Characterization of staining for mGluR5 glutamate receptor in animal model of Parkinsonian disorders |
| LEADER              | <a href="#">Diana Price</a>  |
| FUNDING_AGENCY      | Branfman Family Foundation   |
| PROJECT_START_DATE  |  |
| PROJECT_END_DATE    |  |
| COLLABORATORS       | Edward Rockenstein, Eliezer Masliah, <a href="#">Mark Ellisman</a>                                   |
| PUBLICATION1        |  |
| PUBLICATION2        |  |
| PUBLICATION3        |  |

| Experiment Information - |  |
|--------------------------|--|
| PURPOSE                  | To determine the relationship between mGluR5 and alpha synuclein staining in different lines of alpha synuclein overexpressing mouse |
| TITLE                    | Comparison of mGluR5 and synuclein staining  |
| EXPERIMENTER             | Diana Price  |
| EXPERIMENT_NAME          |  |
| EXPERIMENT_DATE          |  |

| Subject Information - |                      |
|-----------------------|----------------------|
| GROUP_BY              | Genetic Modification |
| SUBJECT_NAME          | Thy-1/asyn           |
| FIXATION_METHOD_ID    |                      |
| SCIENTIFIC_NAME       | mus musculus         |
| SPECIES               | mouse                |
| STRAIN                | C57BL/6-DBA/2        |
| AGE                   | days                 |
| AGECLASS              | adult                |
| ANIMAL_NAME           |                      |
| LITTER_ID             |                      |
| SEX                   | male                 |
| VENDOR                | Eliezer Masliah      |
| WEIGHT                | grams                |

| Tissue -            |              |
|---------------------|--------------|
| ANATOMIC_LOCATION   |              |
| MICROTOME           | vibratome    |
| ORIENTATION         | coronal      |
| THICKNESS           | 80 um        |
| TISSUE_PROD_STORAGE |              |
| EXTERNAL_FILE_NAME  |              |
| TISSUE_GROUP_TYPE   | triple label |

| Microscopy Product Information - |  |
|----------------------------------|--|
| MICROSCOPY_PRODUCT_ID            | 4064   |
| IMAGE_BASENAME                   | 112006e  |
| CREATE_DATE                      | 2006-11-20 00:00:00.0  |
| INSTRUMENT                       | Olympus Fluoview 1000  |
| MICROSCOPE_TYPE                  | LASER SCANNING CONFOCAL                                      |
| PLANE_COUNT                      | 1  |
| PRODUCT_TYPE                     | SURVEY   |
| PURL                             |  |
| SESSION_NAME                     |  |
| TELESCIENCE_SRB                  | P1723/Experiment_3482/Subject_256/Tissue_369/Microscopy_4064 |
| X_RESOLUTION                     | .207 um/pixels   |
| Y_RESOLUTION                     | .207 um/pixels   |
| XSIZE                            | 1024   |
| YSIZE                            | 1024   |

## Protocol:

For more information on transgenic animals, see: Rockenstein et al 2002 E. Rockenstein, M. Mallory, M. Hashimoto, D. Song, C.W. Shults, I. Lang and E. Masliah, Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters, J Neurosci Res 68 (2002), pp. 568-578

Specimen processing: Tissue section acquisition from transgenic animals

Animals were deeply anesthetized with Nembutal<sup>®</sup> (pentobarbital) and perfused via intracardiac catheterization. Perfusion with oxygenated Ringer's solution containing 250U/ml heparin, 0.2 mg/ml xylocaine and 1% dextrose was followed by 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS) (both at 37 degrees Celsius). The brains were carefully removed from the skull and postfixed for 1 hour in the same fixative used in the perfusion. The brain was blocked and cut into 2 mm thick sections using an acrylic brain matrix (David Kopf; Tujunga, CA) to facilitate reproducibility of sections. These thick sections were then sectioned into 80 micron thick coronal sections using a Vibratome (VT1000E, Leica Microsystems, Wetzlar, Germany).

Specimen processing: Immunocytochemistry

Tissue sections were incubated with monoclonal anti- $\alpha$ -syn (1:250; BD Transduction Laboratories, San Diego, CA, Catalog #AB610787) and rabbit anti-mGluR5 (1:250; Chemicon, Temecula, CA, Catalog #AB5675) followed by incubation with donkey anti-mouse Alexa Fluor 488 (1:100, Molecular Probes, Carlsbad, CA) and donkey anti-rabbit RRX (1:100, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) overnight at 4°C. The immunolabeling procedure consisted of the following steps: (1) 6x5 min rinses in 0.1 M PBS; (2) 1 hr blocking step in PBS containing 3% normal donkey (NDS), 0.1% Triton X-100, 1% fish gelatin, and 1% BSA; (3) 48 hr incubation in primary antibodies diluted in working buffer (PBS, 1% NDS) at 20 degrees C; (4) 6 x 5 minute rinses in working buffer; (5) 24 hr incubation in working buffer containing donkey anti-mouse Alexa Fluor 488 (Molecular Probes, Carlsbad, CA) and donkey anti-rabbit RRX (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). (6) 6 x 10 min rinses in working buffer; (7) 3 x 10 min rinses in PBS; (8) the sections were free floated onto slides and coverslipped using ProLong mounting media (Invitrogen Molecular Probes, Carlsbad, CA) with DAPI nuclear stain. Controls for the mGluR5 antibody experiments included both preabsorption with the control peptide (Chemicon, Catalog #AG374), as well as primary omission studies, which both revealed a lack of non-specific staining. Controls for other antibodies used were performed via omission of primary antibodies, and revealed no non-specific staining. All steps were conducted at 4 degrees C, on wet ice and with ice-cold solutions

# Specimen Preparation Information:

| Specimen Preparation Information - |   |
|------------------------------------|---|
| PROTOCOL_ID                        | 15692   |
| PROTOCOL_NAME                      | Immunolabeling P1723  |
| PROTOCOL_DESCRIPTION               | Double labeling immunolabeling of alpha synuclein and mGIR5   |
| Protocol Steps:                    | 1)Molecular Localization(15740)<br>2)Molecular Localization(15749)<br>3)Stain(15765)<br>4)Chemical(15690)<br>5)Microtomy(15691) |

## Molecular Localization (15740)

### Molecular Target

MOLECULAR TARGET ID: 15741  
MOLECULAR LOCALIZATION ID: 15740  
MOLECULE: synuclein  
ISO FROM: alpha  
MOLECULAR CLASS: protein  
ABBREVIATION: Snca  
ENTREZ\_ID: 20617

### Probe used

PROBE ID: 15742  
CONTROLS: omitted primary antibody

Antibody ID: 15743  
Clonality: monoclonal  
Raised in animal: mouse  
Antibody type: IgG

### Reagent (15696)

|              |                               |
|--------------|-------------------------------|
| Reagent name | anti alpha synuclein antibody |
|--------------|-------------------------------|

|             |  |
|-------------|--|
| Temperature |  |
|-------------|--|

#### Chemical

|  |
|--|
| Chemical ID: 15695<br>Chemical name: anti alpha synuclein antibody<br>Vendor: BD Transduction Laboratories<br>Concentration: .25 %<br>Catalog number: AB610787 |
|--|

|  |
|--|
| Chemical ID: 15704<br>Chemical name: normal donkey serum<br>Concentration: 1 % |
|--|

|  |
|--|
| Chemical ID: 24<br>Chemical name: phosphate buffer<br>Concentration: .1 M<br>pH: 7.4 |
|--|

|  |
|--|
| Chemical ID: 31<br>Chemical name: saline<br>Concentration: .9 %<br>Chemical notes: normal saline |
|--|

### Detection method

Molecule reagent ID: 15709  
Molecular type: antibody  
Chromagen :Alexa 488

## Molecular Localization (15749)

### Molecular Target

MOLECULAR TARGET ID: 15750  
MOLECULAR LOCALIZATION ID: 15749  
MOLECULE: metabotropic glutamate receptor  
ISO FROM: 5  
MOLECULAR CLASS: protein  
ABBREVIATION: GRM5  
ENTREZ\_ID: 108071

### Probe used

PROBE ID: 15751  
CONTROLS: omitted primary antibody

Antibody ID: 15752  
Clonality: polyclonal  
Raised in animal: rabbit  
Antibody type: IgG

### Reagent (15714)

|              |                      |
|--------------|----------------------|
| Reagent name | anti mGluR5 antibody |
|--------------|----------------------|

|             |  |
|-------------|--|
| Temperature |  |
|-------------|--|

|          |   |
|----------|---|
| Chemical | Chemical ID: 15719<br>Chemical name: anti mGluR5 antibody<br>Vendor: Chemicon<br>Concentration: .25 %<br>Catalog number: AB5675 |
|----------|---|

### Detection method

Molecule reagent ID: 15721  
Molecular type: antibody  
Chromagen :Rhodamine Red X

|  |   |   |
|--|---|---|
| <b>Stain (15765)</b>   |   |   |
| Stain ID   | 15765   |   |
| Prepared by  | Diana Price   |   |
| Temperature  |   |   |
| Stain notes  | DAPI is dissolved in ProLong Mounting medium and applied at time of coverslipping |   |
| Reagent  | <b>Reagent (15760)</b>  |   |
|  | Reagent name  | DAPI in ProLong   |
|  | Temperature   |   |
|  | Chemical  | Chemical ID: 15758<br>Chemical name: DAPI<br>Concentration: |
| Chemical ID: 15759<br>Chemical name: ProLong mounting medium<br>Vendor: Molecular Probes<br>Concentration: |   |   |

|                                  |           |
|----------------------------------|-----------|
| <b>Chemical Fixation (15690)</b> |           |
| Time of fixation                 |           |
| Temperature                      | 37 C      |
| Fixative volume                  |           |
| Fixation method                  | perfusion |

|                          |           |
|--------------------------|-----------|
| <b>Microtomy (15691)</b> |           |
| Microtome                | 0         |
| Thickness                | 80 um     |
| Temperature              |           |
| Embedding agent          | 0         |
| Microtomy notes          | Vibratome |

| Specimen Description - |                        |
|------------------------|------------------------|
| ANATOMICAL_DETAIL      | 17004                  |
| ATLAS_COORD            | , ,                    |
| ORGAN                  | brain                  |
| REGION                 | dentate gyrus          |
| SYSTEM                 | central nervous system |

## Imaging Parameters:

| Image Type -                    |  |
|---------------------------------|--|
| OPTICAL_SECTION_SERIES          | 17003  |
| OPTICAL_SECTION_SERIES_D<br>ESC | Only a single optical section was acquired for each image. |

| Light Microscopy Product - |                                       |
|----------------------------|---------------------------------------|
| LMPRODUCT_ID               | 17005                                 |
| IMMERSION_MEDIUM           | oil                                   |
| LENS                       | Olympus PlanApo 60X oil               |
| LENS_MAGNIFICATION         | 60 X                                  |
| MOUNTING_MEDIUM            | Prolong (Molecular Probes)            |
| NUMERICAL_APERTURE         | 1.42                                  |
| LM_NOTES                   | DAPI was added to the mounting medium |



### Confocal channel (17008)

|                   |       |
|-------------------|-------|
| Confocal image ID | 17008 |
|-------------------|-------|

|            |           |
|------------|-----------|
| Fluorophor | Alexa 488 |
|------------|-----------|

|       |       |
|-------|-------|
| Color | Green |
|-------|-------|

|                       |        |
|-----------------------|--------|
| Excitation wavelength | 488 nm |
|-----------------------|--------|

|                     |        |
|---------------------|--------|
| Emission wavelength | 520 nm |
|---------------------|--------|

### Molecular Localization (15740)

|                  |  |
|------------------|--|
| Molecular Target | MOLECULAR TARGET ID: 15741<br>MOLECULAR LOCALIZATION ID: 15740<br>MOLECULE: synuclein<br>ISO FROM: alpha<br>MOLECULAR CLASS: protein<br>ABBREVIATION: Snca<br>ENTREZ_ID: 20617 |
|------------------|--|

### Confocal channel (17014)

|                   |       |
|-------------------|-------|
| Confocal image ID | 17014 |
|-------------------|-------|

|            |                 |
|------------|-----------------|
| Fluorophor | Rhodamine Red X |
|------------|-----------------|

|       |     |
|-------|-----|
| Color | Red |
|-------|-----|

|                       |        |
|-----------------------|--------|
| Excitation wavelength | 543 nm |
|-----------------------|--------|

|                     |        |
|---------------------|--------|
| Emission wavelength | 591 nm |
|---------------------|--------|

### Molecular Localization (15749)

|                  |   |
|------------------|---|
| Molecular Target | MOLECULAR TARGET ID: 15750<br>MOLECULAR LOCALIZATION ID: 15749<br>MOLECULE: metabotropic glutamate receptor<br>ISO FROM: 5<br>MOLECULAR CLASS: protein<br>ABBREVIATION: GRM5<br>ENTREZ_ID: 108071 |
|------------------|---|

**Confocal channel (17022)**

Confocal image ID 17022

Fluorophor DAPI

Color Blue

Excitation wavelength 405 nm

Emission wavelength 461 nm

**Stain (15765)**

Stain ID 15765

Stain reagent ID 15760

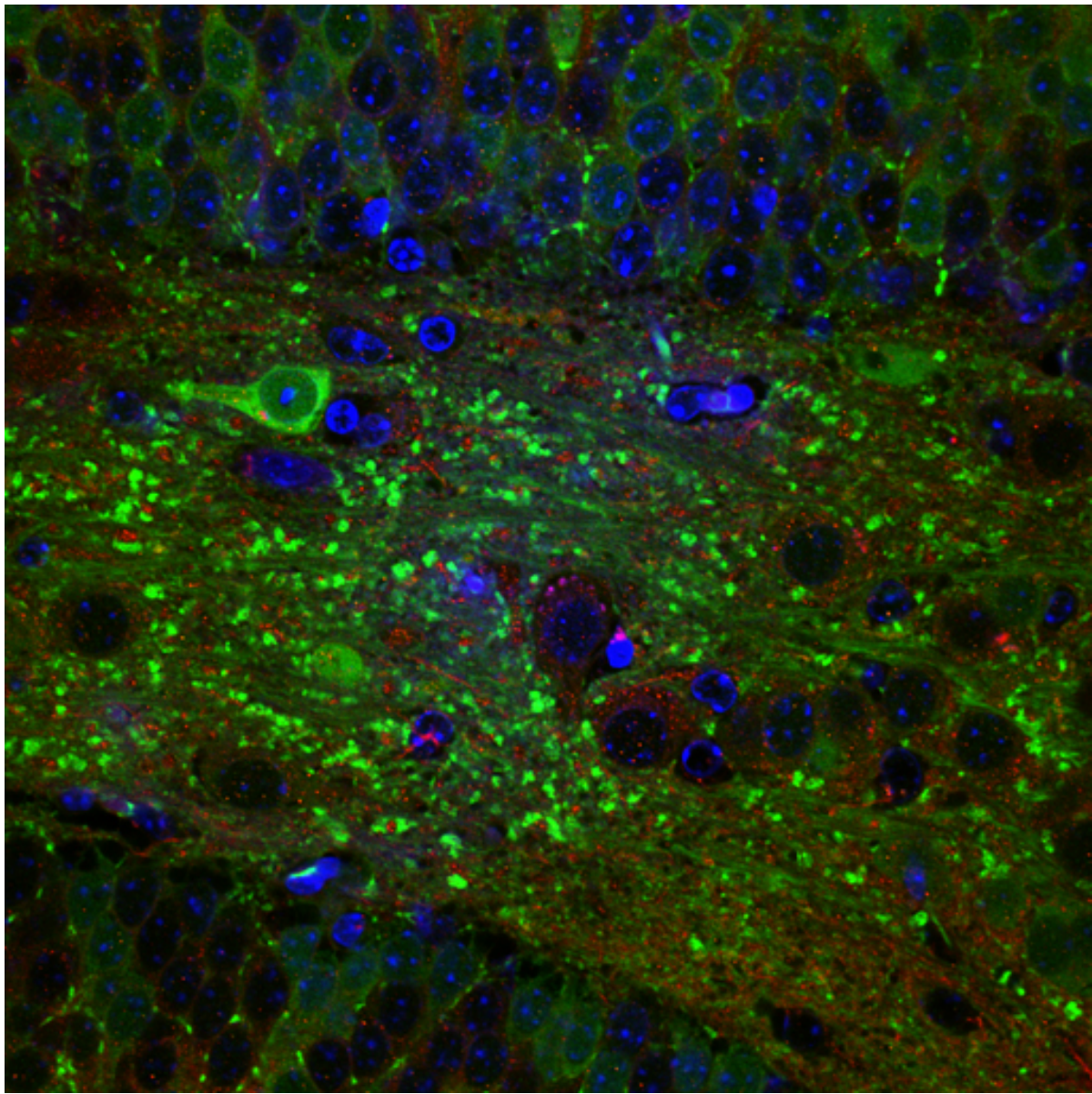
Prepared by Diana Price

Temperature

Stain notes DAPI is dissolved in ProLong Mounting medium and applied at time of coverslipping

# Raw 2D Image

Raw Low Resolution 2D Image -



| Raw 2D Image -    |   |
|-------------------|---|
| IMAGE2D_ID        | 17030   |
| IMAGE_DATE        | 2006-11-20 00:00:00.0   |
| IMAGE_DESC        | Zip archive containing the 3 channel image file in tiff format (112006e_RGB.tif). Also included is the .oif header file generated by the Olympus Fluoview, which gives additional detail on microscope settings.  |
| IMAGE_FILE_FORMAT | tiff  |
| IMAGE_FILE_NAME   | /usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006e_img.jpg   |
| RAW_DATA_FILE     | /telescience/home/CCDB_DATA_USER.portal/P1723/Experiment_3482/Subject_256/Tissue_369/Microscopy_4064/112006e_img.zip  |
| THUMBNAIL_DESC    | Triple labeled confocal image of the dentate gyrus of a transgenic mouse engineered to overexpress alpha synuclein under the Thy-1 promotor, immunolabeled for mGluR5 (red), alpha synuclein (green) and counterstained with DAPI (blue) to reveal cell nuclei. |
| THUMBNAIL_FILE    | /usr/local/tomcat/webapps/FileUploadTool/temp_file_upload/112006e_img_thmb.jpg  |
| X_RESOLUTION      | .207 um/pixel   |
| Y_RESOLUTION      | .207 um/pixel   |
| X_SIZE            | 1024 pixels   |
| Y_SIZE            | 1024 pixels   |

## **USER AGREEMENT**

Data Sharing and Citation Policy: The mission of the CCDB is to promote data sharing among scientists interested in cellular and subcellular anatomy and in developing computer algorithms for 3D reconstruction and modeling of such data. Data sets may be viewed or shared at the discretion of the author of the data. In some cases, the data may be freely viewed and downloaded without contacting the original author while in other cases, permission of the author may have to be obtained prior to downloading the data. In either case, failure to cite or give proper credit to the original authors who collected these data in subsequent published articles or presentations is a material breach of this User Agreement. CCDB requires all researchers re-analyzing these published data via the CCDB access to reference the original published article and the CCDB. An example of an appropriate acknowledgement is provided on the CCDB web site. CCDB is not in a position to police every intended use of these data. The scientific community will self-police the compliance of this contractual obligation.

## **DISCLAIMER**

THE DATA PROVIDED BY THE CCDB ARE FREELY DISTRIBUTED AND WITHOUT CHARGE. THESE DATA ARE PROVIDED BY THE CCDB "AS IS" AND WITHOUT ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT, TO ANY THIRD PARTY RIGHTS. IN NO EVENT SHALL THE CCDB BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THESE DATA, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.

## **USER NOTIFICATION**

For large size image data, it will take several minutes to download, please be patient. Thanks!

## **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 acknowledgement 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