# *Modeling Human Diseases in the Nematode Caenorhabditis elegans*

*Marvin L. Bayne, Ph.D.*
*Fellow*
*Charles A Dana Research Institute for Scientist Emeriti*
*Drew University*

# *Education and Training*

- AB Biology

  Western Maryland College

  (now McDaniel College)


- MS and PhD Biochemistry and Molecular Biology

  Northwestern University


- Post-Doctoral Training

  Johns Hopkins University

# Work Experience

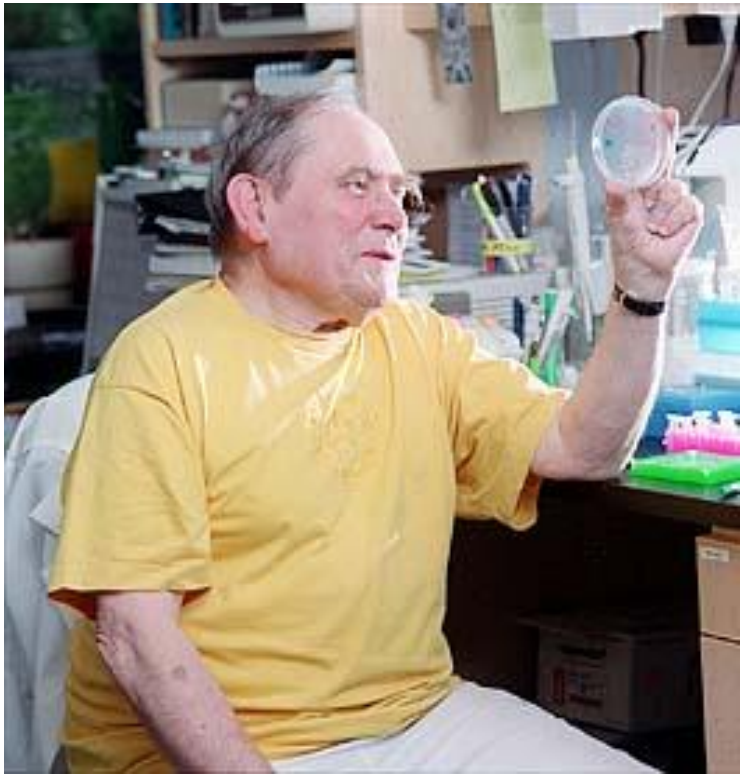
- Unigene Laboratories — 2.5 years
- Merck Research Laboratories — 11 years
- Schering-Plough Research Institute — 14 years
- Merck Research Laboratories — 1 year

- Charles A Dana Research Institute for Scientists Emeriti — 2016-

# *Research Topics*

- Mechanism and Enzymology of DNA Replication
- Gene Synthesis, Recombinant Protein Expression, and Protein Engineering
- G Protein-Coupled Receptor Cloning and Drug Development Programs
- Genomics, Biomarkers, Translational Research
- **Molecular Neurobiology of *Caenorhabditis elegans***

# *Why Study Worms?*



Sydney Brenner

“Thus we want a multicellular organism which has a short life cycle, can be easily cultivated, and is small enough to be handled in large numbers, like a micro-organism. It should have relatively few cells, so that exhaustive studies of lineage and patterns can be made, and should be amenable to genetic analysis.” **--Excerpts from Proposal to the Medical Research Council, 1963**

# *C. elegans as a Model System*

- **Easy to Cultivate**
  - Small: ~1mm in length
  - Grown on agar plates of *E. coli* bacteria, can be scaled up in liquid culture
  - Large brood size: ~300; short generation time: ~3 days
- **Genetic Analysis Tools**
  - Forward Genetics:
    - EMS mutagenesis, transposons mutagenesis
    - Self fertilizing hermaphrodites allows easy clonal expansion
  - Reverse Genetics:
    - Gene Knockouts/Replacements: CRISPR/Cas-9
    - Gene Knockdowns thru RNAi bacterial delivery system
- **Transparent:**
  - Allows use of **Green Fluorescent Protein** tagged promoter fusions and proteins to follow expression in vivo

- **Can be used to Model Human Diseases**
  - ~ 60-80% of *C. elegans* genes have human counterparts
  - ~42% of human disease genes have *C. elegans* counterparts
  - Can generate “humanized” worms; replace worm gene with human counterpart

# *C. Elegans Milestones*

- 1963: Brenner proposal
- 1998: First multicellular organism fully sequenced
- 2002: Cell Lineage Nobel Prize to Brenner, Horvitz and Saulston
- 2006: RNAi discovery Nobel Prize to Fire and Mello
- 2008: Nobel Prize to Chalfie for GFP in *C. elegans*
- 2011: First connectome completed

# *Resources Available*

- C. *elegans* Genetic Stock Center, U of Minnesota
  - Mutant stocks, knockouts and point mutations
  - GFP fusions
  - Wild type strains from different ecosystems
  - Related, sequenced species eg *C. briggsea*
- C. *elegans* scientific community
  - WormBook
  - WormBase
  - Worm Breeder's Gazette
  - New York Area Worm Discussion Group

# *Role of C. elegans Research in Drug Discovery*

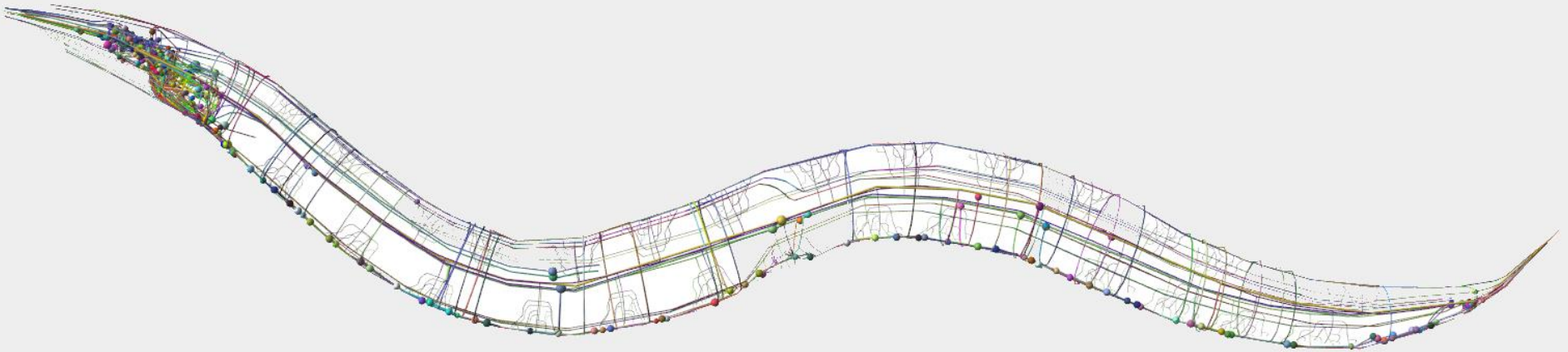
- Target identification
- Mechanism of action studies
  - Traditional Chinese Medicines
- Identification of off-target effects
  - Clozapine
- Phenotypic screening
- “Humanized” worms for lead identification

# *C. elegans Models of Human Disease*

- **Neurobiology**
  - Alzheimer's Disease
  - **Parkinson's Disease**
  - Huntington's Disease
  - Nicotine addiction
  - ALS
  - **Autism Spectrum Disorders**
- Metabolic Disease
  - Insulin signalling and resistance
  - Fat accumulation
- Aging
- Cancer pathways

# *Nervous System of C. elegans*

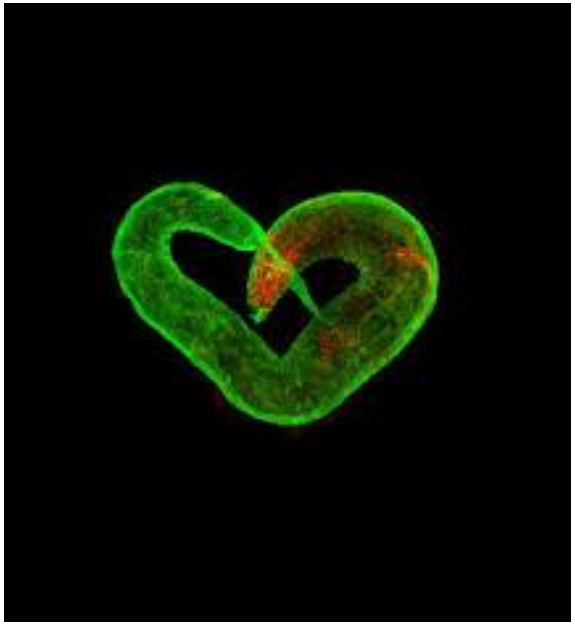
- 302 neurons out of a total of 959 cells
  - 32 chemosensory neurons
  - 8 dopamine neurons
- Complete “connectome” determined
  - 6393 chemical synapses
  - 1410 neuromuscular junctions
  - 890 gap junctions



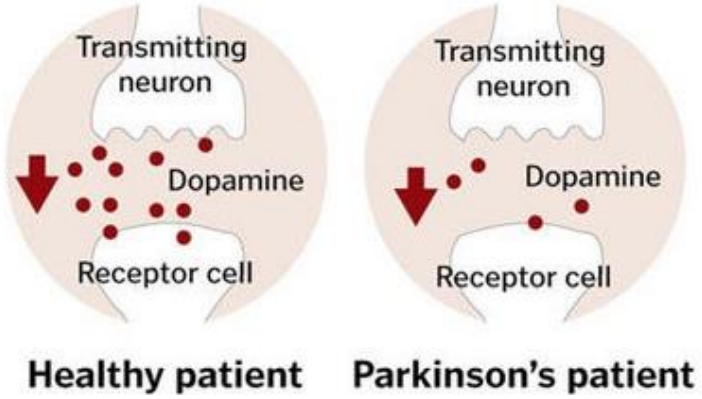
# *Bayne Lab 2019*

***Stefanie DeFronzo, Lexi Holroyd,, Erin Heller, Shivani Mody, Karishma Patel, Stephanie Wang, Mehek Agrawal, Krishna Patel***

- Current Projects
  - Parkinson's Disease
  - Autism Spectrum Disorder



# *Parkinson's Disease*




Parkinson's disease is a degenerative neurological disease affecting dopamine producing neurons

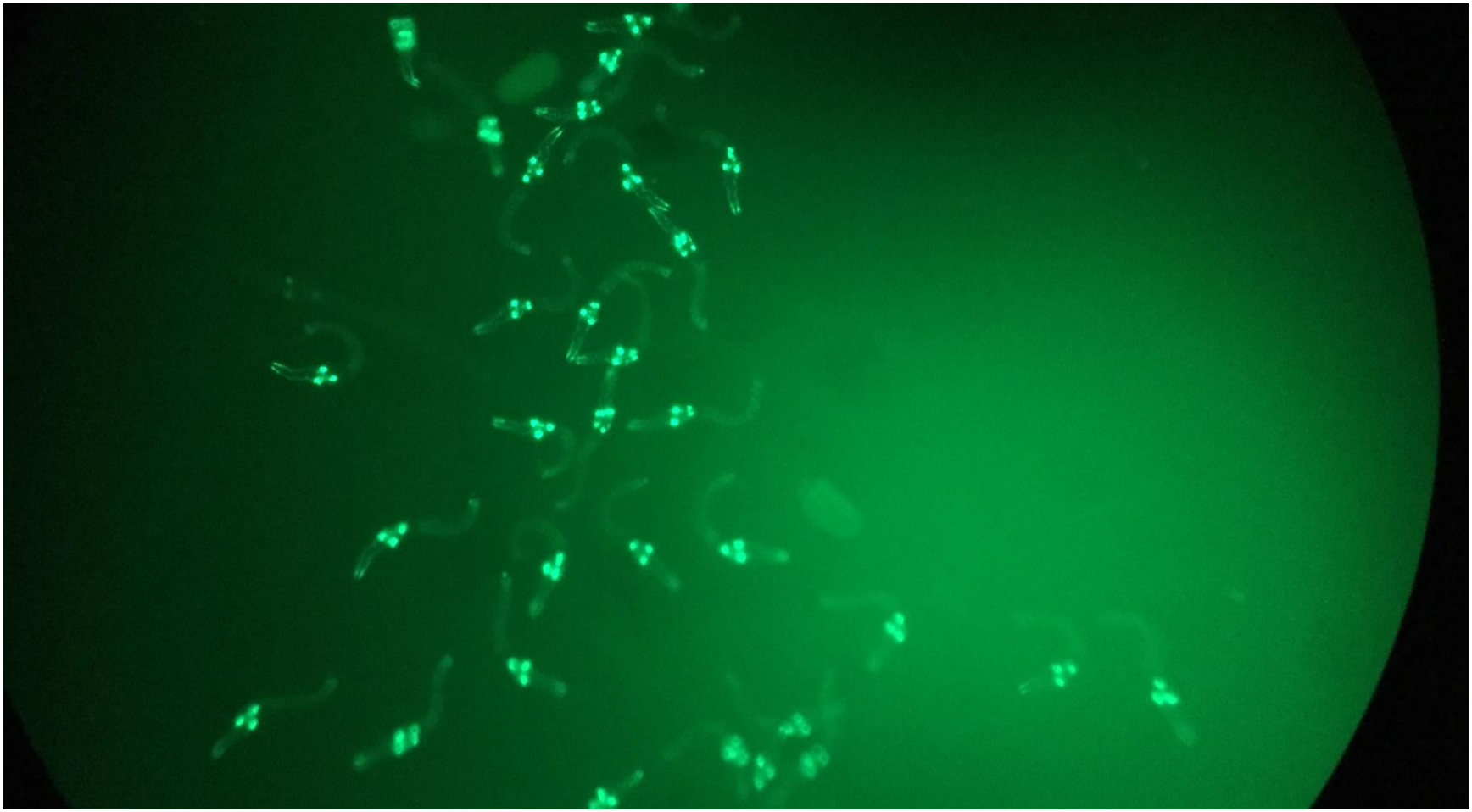
Damage to dopaminergic neurons can be caused by genetic defects, environmental factors such as exposure to neurotoxins like pesticides, or traumatic brain injury.

Symptoms include tremors, slowness of  movements, gait problems

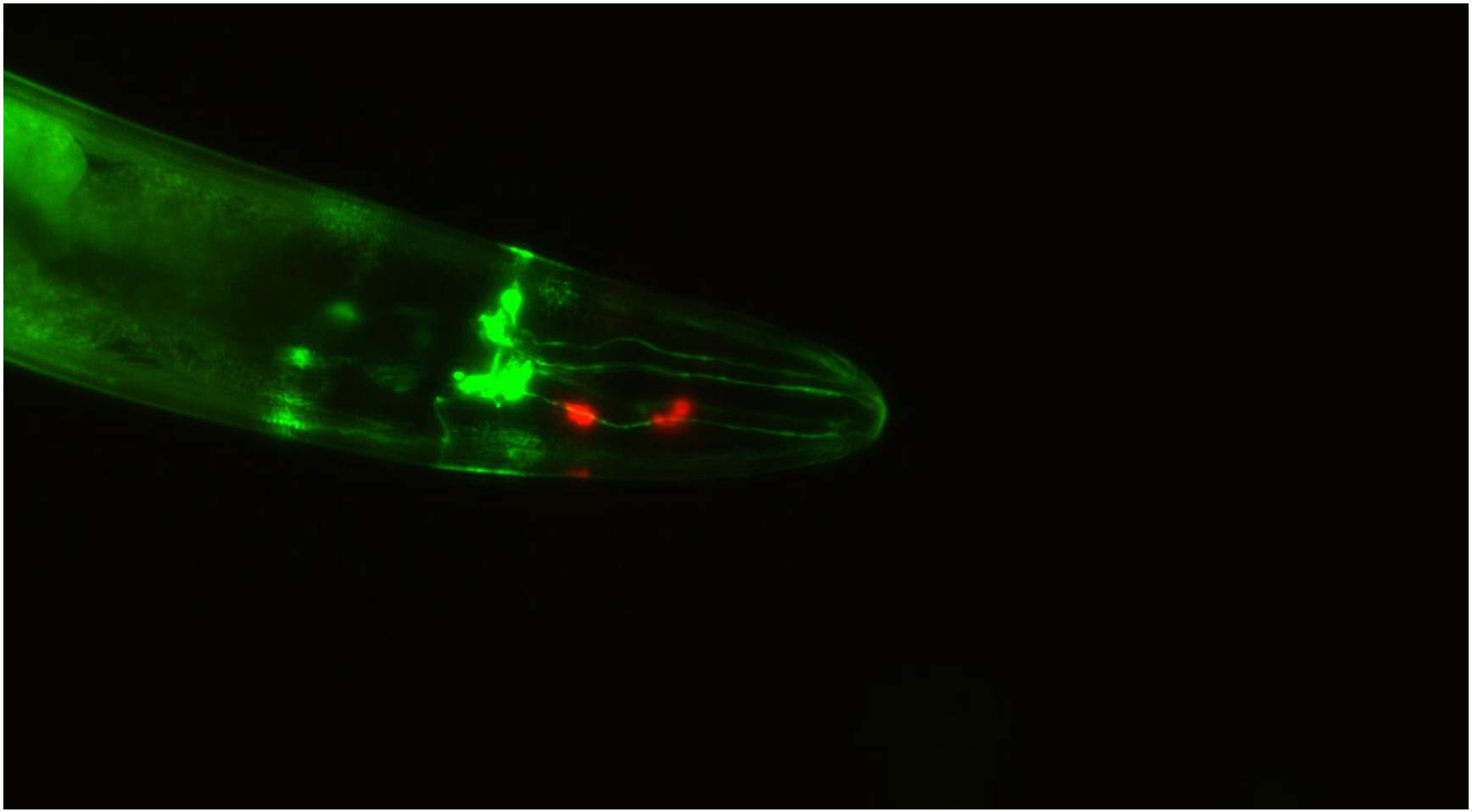
# *C. elegans Models of Parkinson's Disease*

- **Genetic**: *C. elegans* strains expressing human PD related genes resulting in age-dependent degeneration of dopaminergic neurons
  - Alpha-synuclein: **A53T** mutation
  - LRRK2: **G2019S** mutation
- **Neurotoxins**: chemical degeneration of dopamine neurons
  - MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
  - 6-OHDA (6-hydroxyl dopamine)
- Degeneration of dopamine neurons can be monitored using worms expressing **Green Fluorescent Protein** specifically in the 8 dopaminergic neurons
- Parkinson's model worms develop movement abnormalities mimicking movement disorders in Parkinson's disease patients
  - Swim to crawl paralysis, swimming induced paralysis
- We are using these models to identify drugs and/or genes that protect dopamine neurons

# *Dopamine Neurons Expressing GFP*

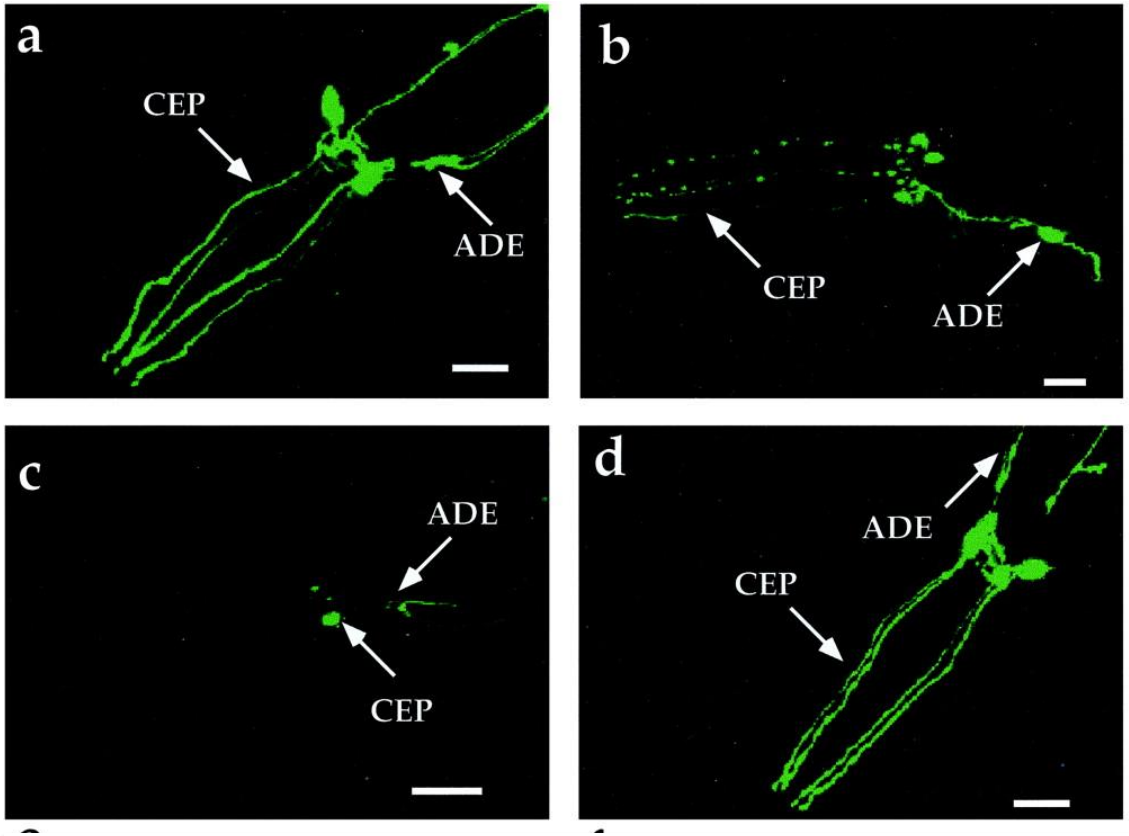


# *C. elegans MGM7*
# *Melissa Medina C'17*



# *6-OHDA Damage to C. elegans Dopamine Neurons inhibited by Dopamine Transporter Antagonists*

Nass R, Hall DH, Miller DM, Blakely RD (2002) Neurotoxin-induced degeneration of dopamine neurons in Caenorhabditis elegans. Proc Natl Acad Sci U S A 99,3264-3269.



# *Worm Models of Parkinson's Disease*

- **VM6365 pdat-1::GFP, pdat-1::ICE**
  - Reported loss of GFP (DA neurons) in late L1 stage
  - No GFP staining in adults
- **JVR203 pdat-1::GFP, pdat-1::α-synuclein (A53T)**
  - Age dependent loss of dopamine neurons
- **JVR168 pdat-1::GFP, pdat-1::LRRK2 (G2019S)**
  - Age dependent loss of dopamine neurons
- **Dat-1**
  - **loss of activity mutation in dopamine transporter**
- **Cat-2**
  - **mutation in biosynthesis of dopamine**
- **Dop-3**
  - **mutation in dopamine receptor**

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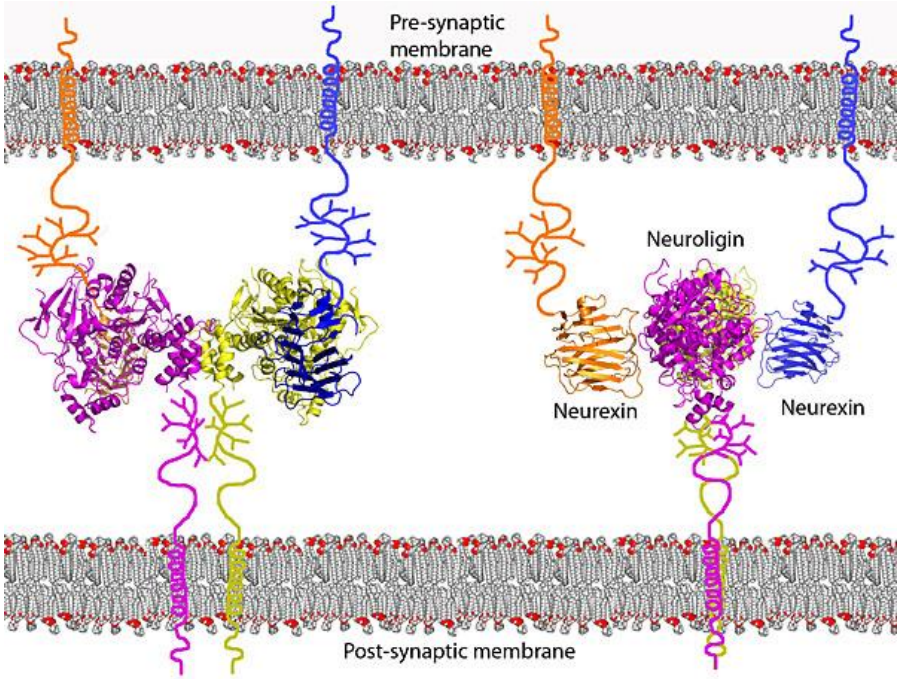
# *Goals*

- Correlate loss of dopamine neurons (through loss of GFP signal) to behavioral assays
- Test known LRRK2 inhibitors
- Establish screen for new LRRK2 inhibitors

# Autism Spectrum Disorder




Autism Spectrum Disorder (ASD) , refers to a range of conditions characterized by challenges with social skills, repetitive behaviors, speech and nonverbal communication, as well as by unique strengths and differences.

Neuroligins and neurexins are involved in establishing and maintaining synaptic connections

Mutations in genes for neuroligins and neurexins are associated with ASD

# *C. elegans Models of Autism Spectrum Disorder*

- Disruption of the *C. elegans* neuroligin-1 gene and the neurexin-1 genes result in sensory deficits.
  - Increased sensitivity to mercury toxicity (Thiomersal)
  - Lack of chemotaxis response to 1-octanol
  - Lack of osmotic avoidance
  - Insensitivity to thermal gradients
  - Behaviors mediated through the ASH neurons
- *C. elegans* neuroligin deletions can be rescued by microinjection of human wild type neuroligin genes but not by genes carrying mutations associated with ASD.

# *Goals of ASD Project*

- Characterize Neuroligin and Neurexin mutants available from C. elegans Genetics Center
- Generate null mutants of NLG-1 and NRX-1 by CRISPR/Cas9 technology
- Confirm/Identify behavioral deficits
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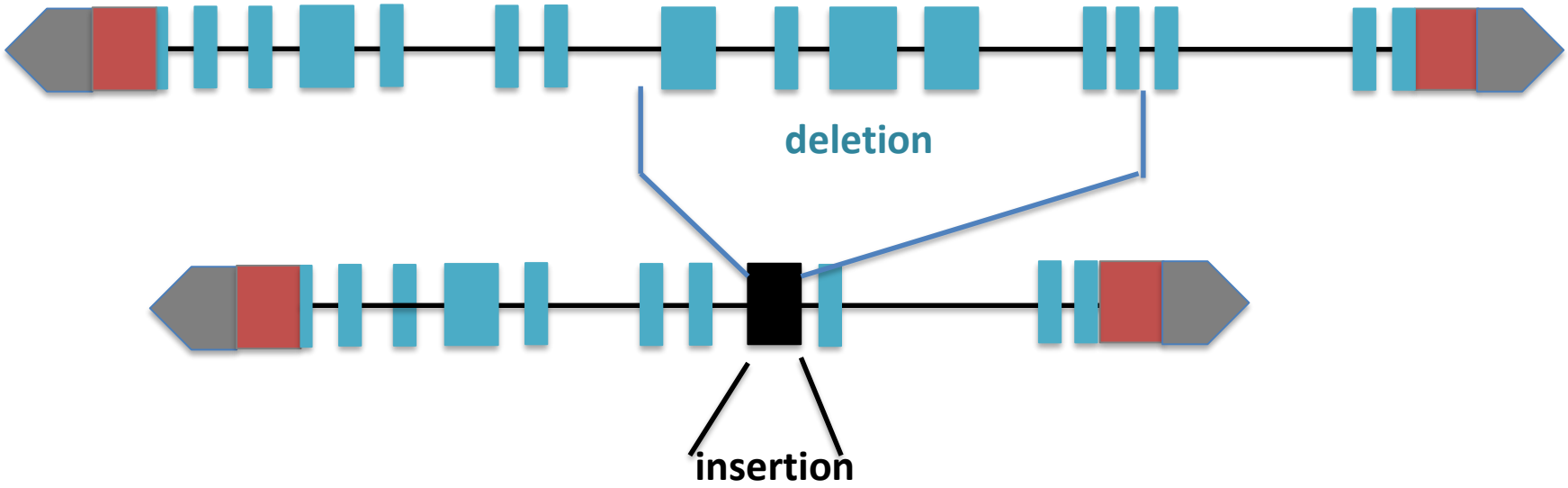
# *Neuroligin and Neurexin Mutants from CGC*

- Neuroligin
  - N2 (wt): 16 exons, 845 amino acids
  - VC228: 2341 bp deletion, 334 bp insertion, loss of exons 8-13

- Neurexin
  - N2 (wt): 28 exons, 1716 amino acids
  - VC1416: 861 bp deletion, exon 9 deleted, 54 amino acids
  - SG1: 1498 bp deletion, exons 3-6 deleted, 136 amino acids

# VC228 (ok259)

**334 bp insertion, 2341 bp deletion**



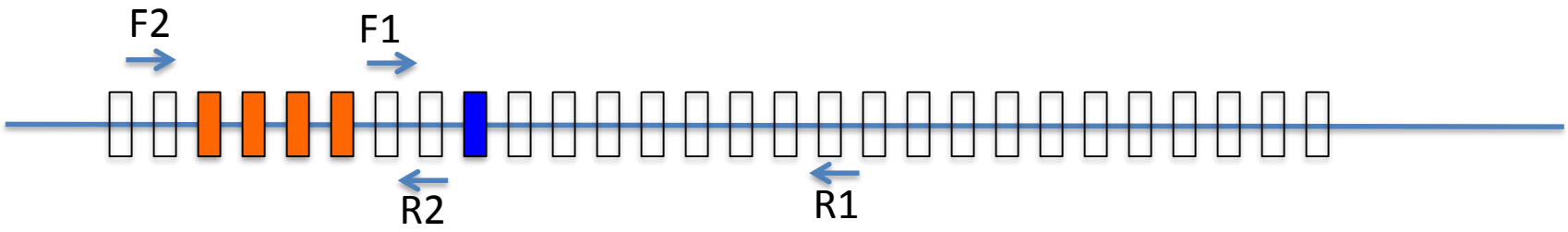

Why isn't VC228 more defective?
Why are only ASH neuron functions affected?

Generate a complete deletion of the NLG-1 gene by CRISPR/Cas9

# *Neurexin Mutants from CGC*

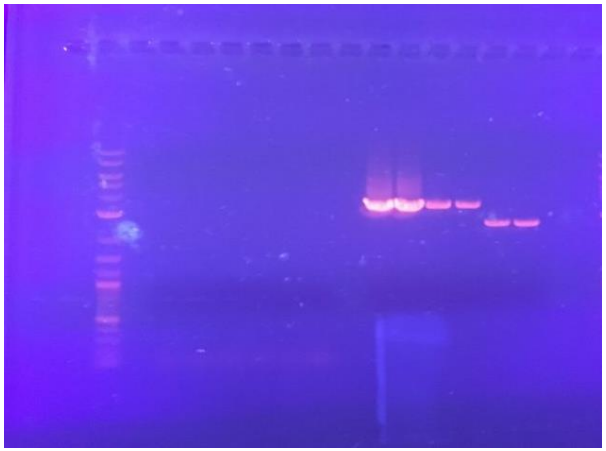
- N2 (wild type): 28 exons, 1716 amino acids
- VC1416: 861 bp deletion
- SG1: ~1500 bp deletion

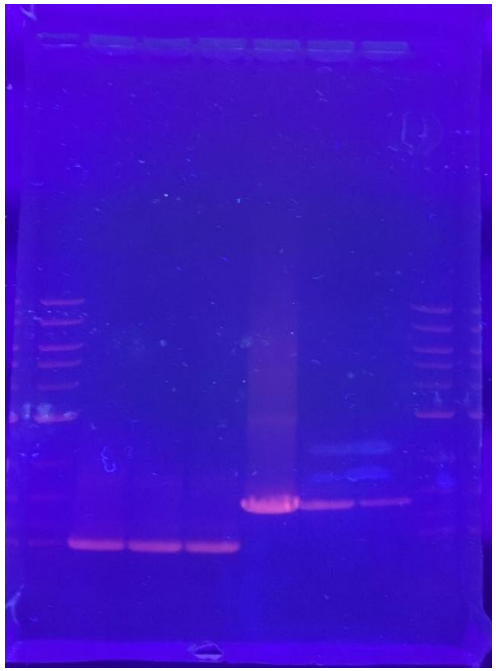
- Exact location and number of amino acids lost was unknown. Genomic DNA from N2, SG1 and VC1416 was isolated an used as template for PCR to amplify the deleted regions of NRX-1. The PCR products were then sequenced using the Sanger chain termination procedure




Locate region of deletion by PCR from N2, VC1416 and SG1 genomic DNA
Sequence PCR products showing deletions
Blast sequence of VC1416 and SG1 deletions against N2 genomic DNA
Identify deletion and determine exons and amino acids deleted

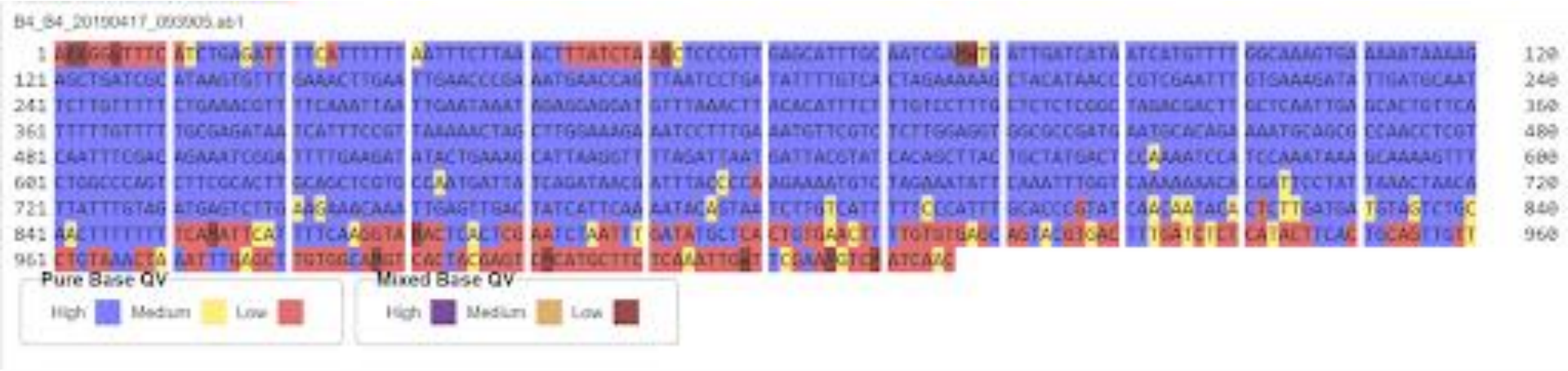
F1/R1




F2/R2

# 1416 Deletion Sequence

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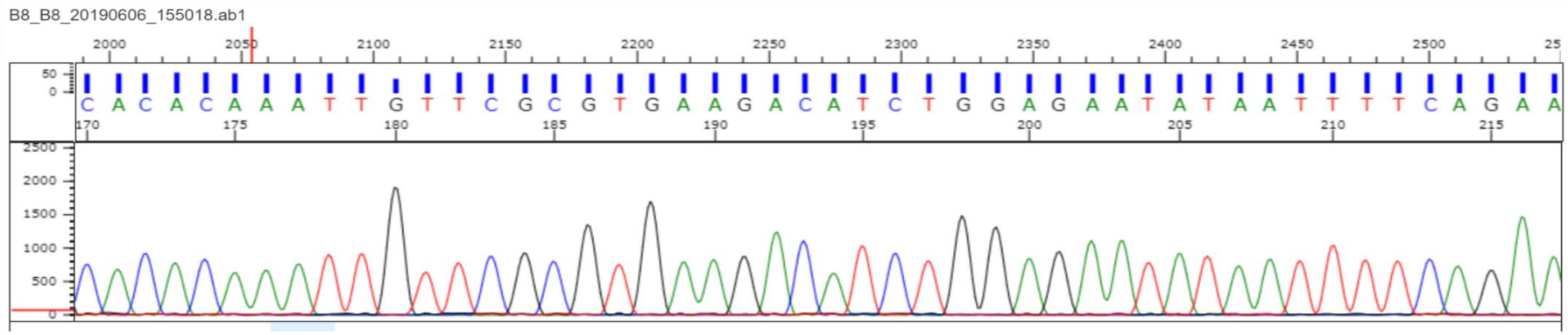
# SG1 Deletion Sequence

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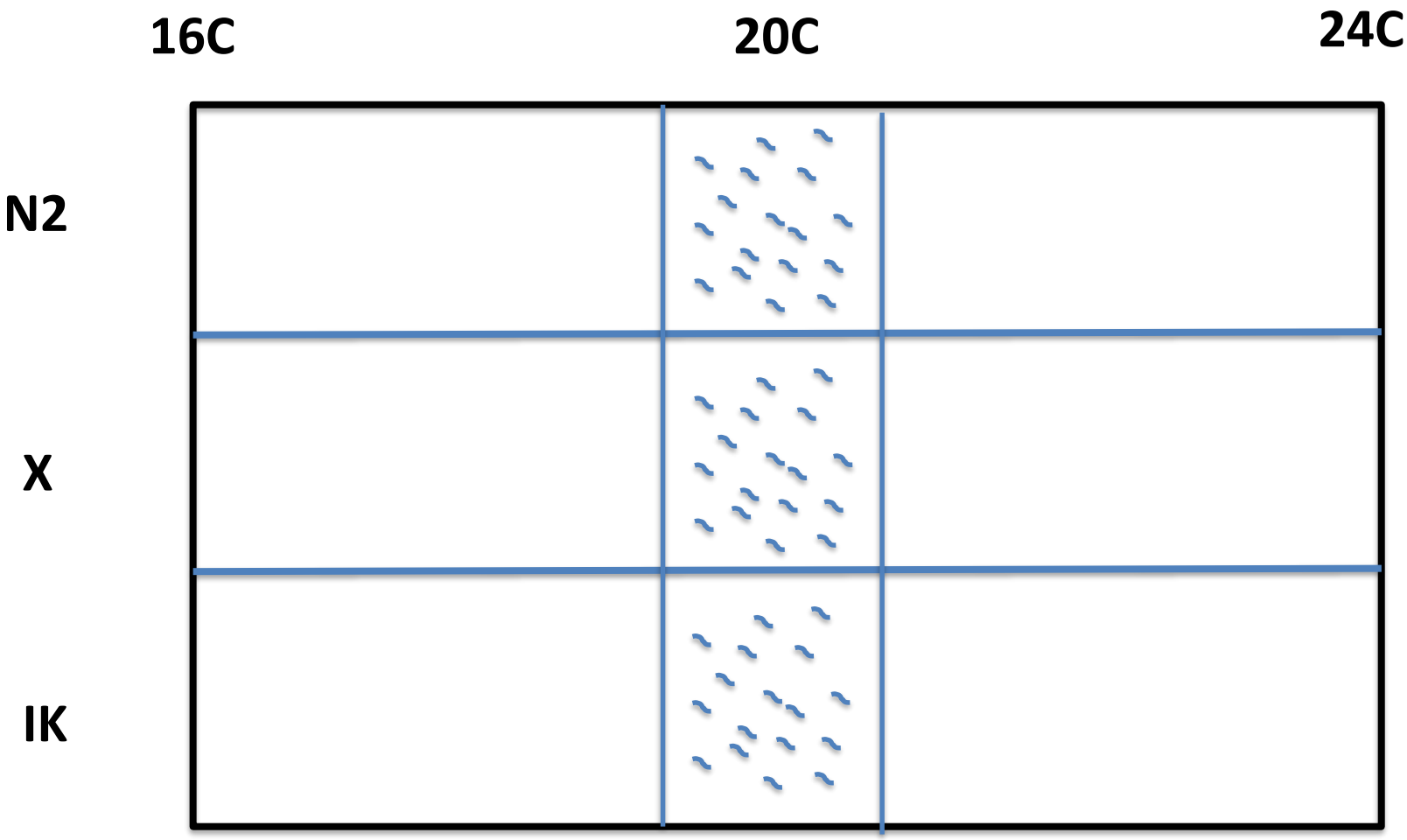
gcgcgggatagtgacgaaattgaaagagaacacaagcggaagcaaagaaaccaaaga

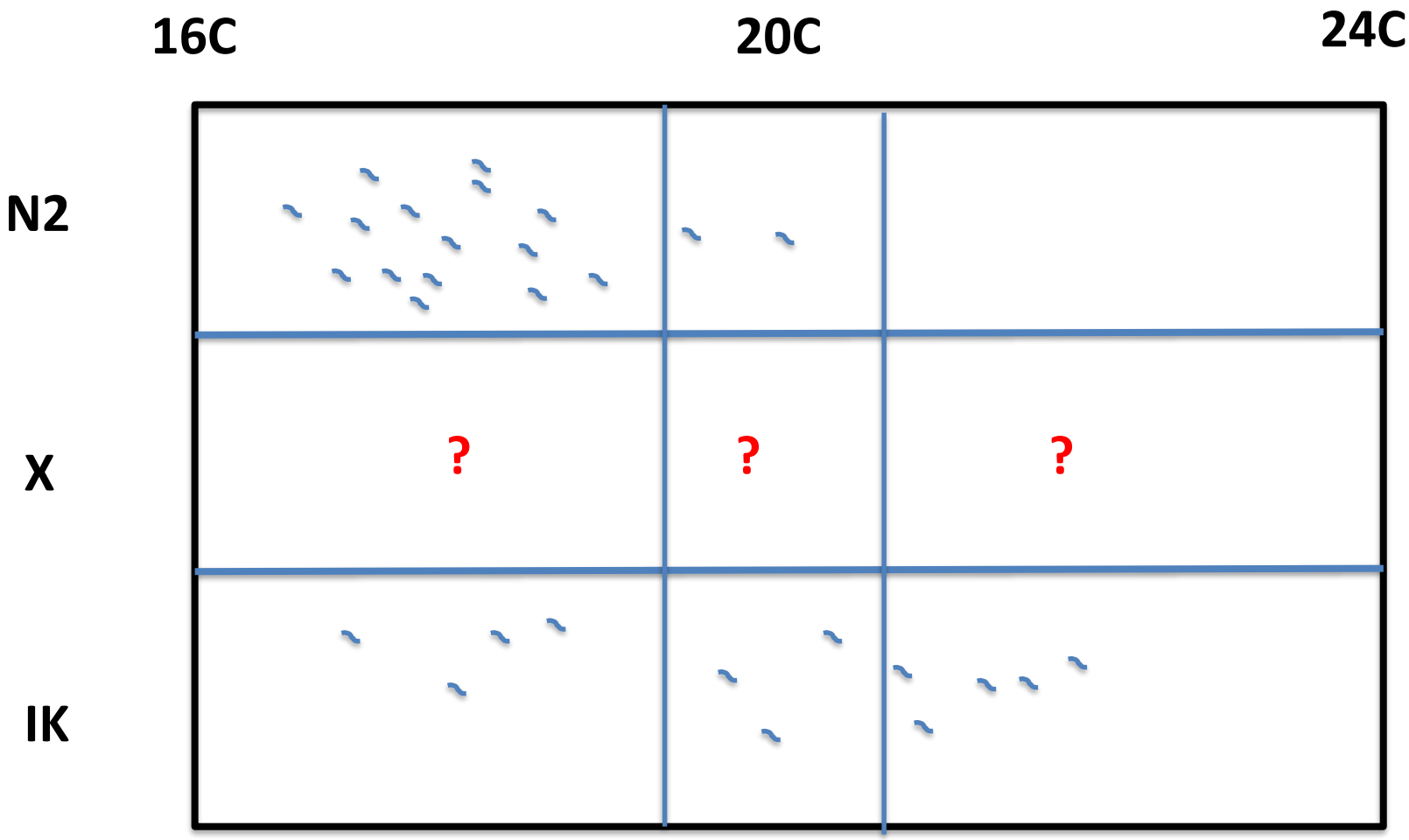
# *Behavioral Assays for Neuroligin and Neurexin Mutants*

- Octanol insensitivity
- Increased sensitivity to Thiomersal
- Insensitivity to thermal gradients

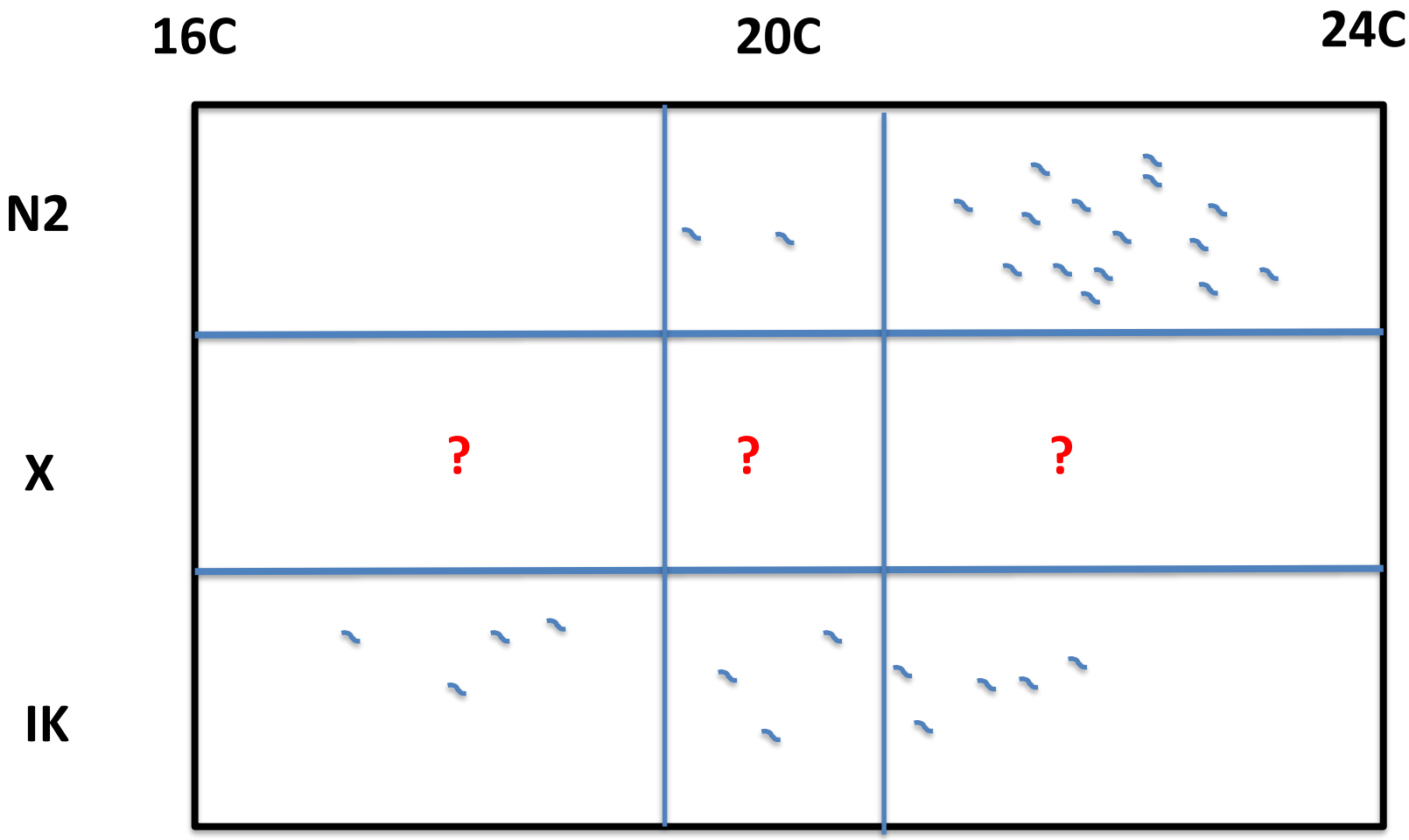
# *Thermotaxis Assay*



# *Thermotaxis Assay Tc=16*



# *Thermotaxis Assay Tc=24*

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# *Behavioral Assays for Neuroligin and Neurexin Mutants*

- Octanol insensitivity
- Increased sensitivity to Thiomersal
- Insensitivity to thermal gradients
- Initial experiments failed to replicate literature data
- To better understand the roles of neuroligin and neurexin in synapse creation and function in *C. elegans* we want to completely remove the coding regions of these genes using CRISPR/Cas9.

# *Goals of ASD Project*

- Characterize Neuroligin and Neurexin mutants available from C. elegans Genetics Center
- **Generate null mutants of NLG-1 and NRX-1 by CRISPR/Cas9 technology**
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# *CRISPR/Cas9 Deletion of C. elegans NLG-1*



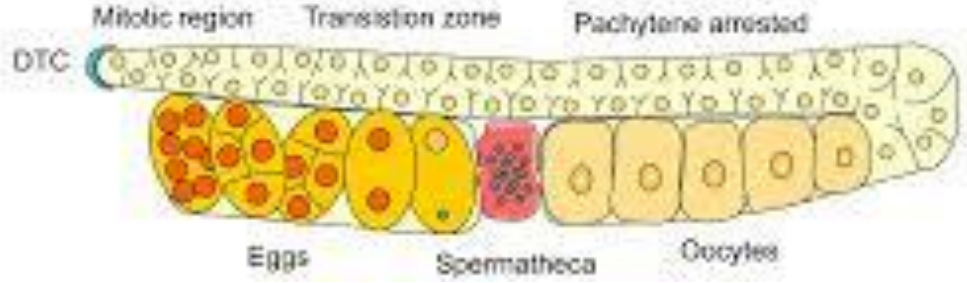
**GFP** SEC Self-excising selection cassette
Roller phenotype
Hygromycin resistance
Temperature Induced Cre

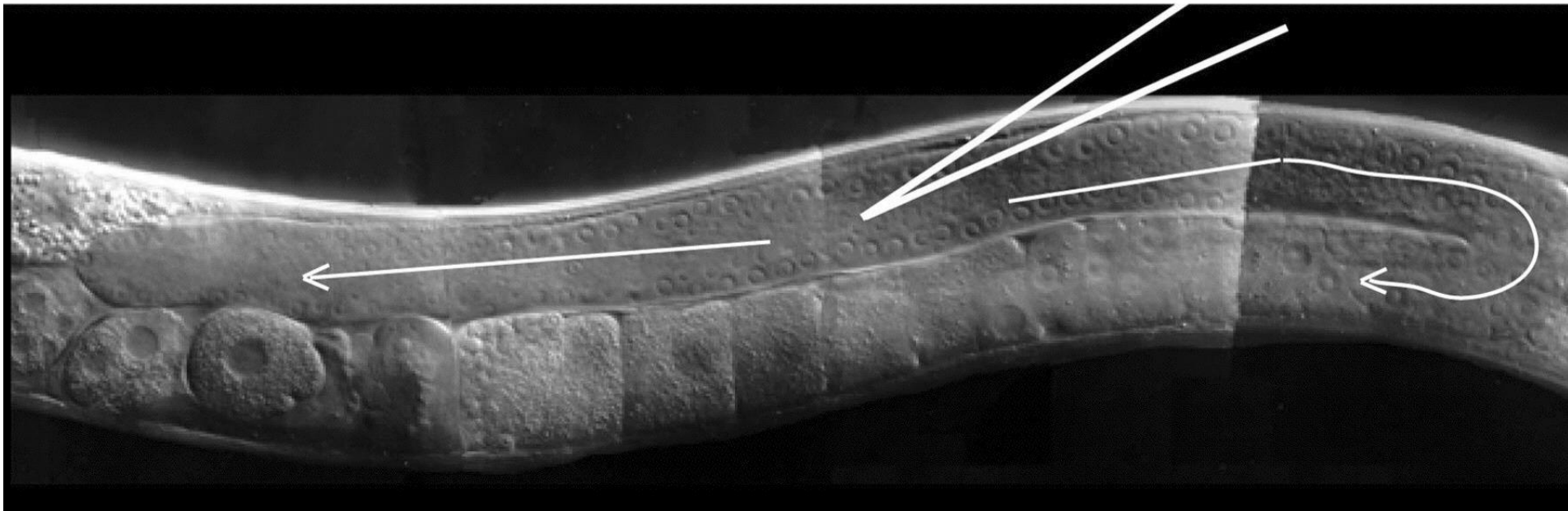

Null Repair Template

Post selection
Excision of SEC

# *Microinjection*

- Prepare microinjection plasmid cocktail
  - Repair template (5'and 3'Flanks, SEC and insert)
  - Guide RNA expression plasmids
  - Cas9 expression plasmid
- Inject DNA into the syncytial gonad of young adult hermaphrodites
- After injection, transfer worms to new plate
- After 3 days add hygromycin to plate
- After 6-7 days select viable "rollers" to new plate
- Heat shock L1 larvae at 34C for 4 hours to excise SEC


Mitotic region
Transistion zone
Pachytene arrested
DTC
Eggs
Spermatheca
Oocytes



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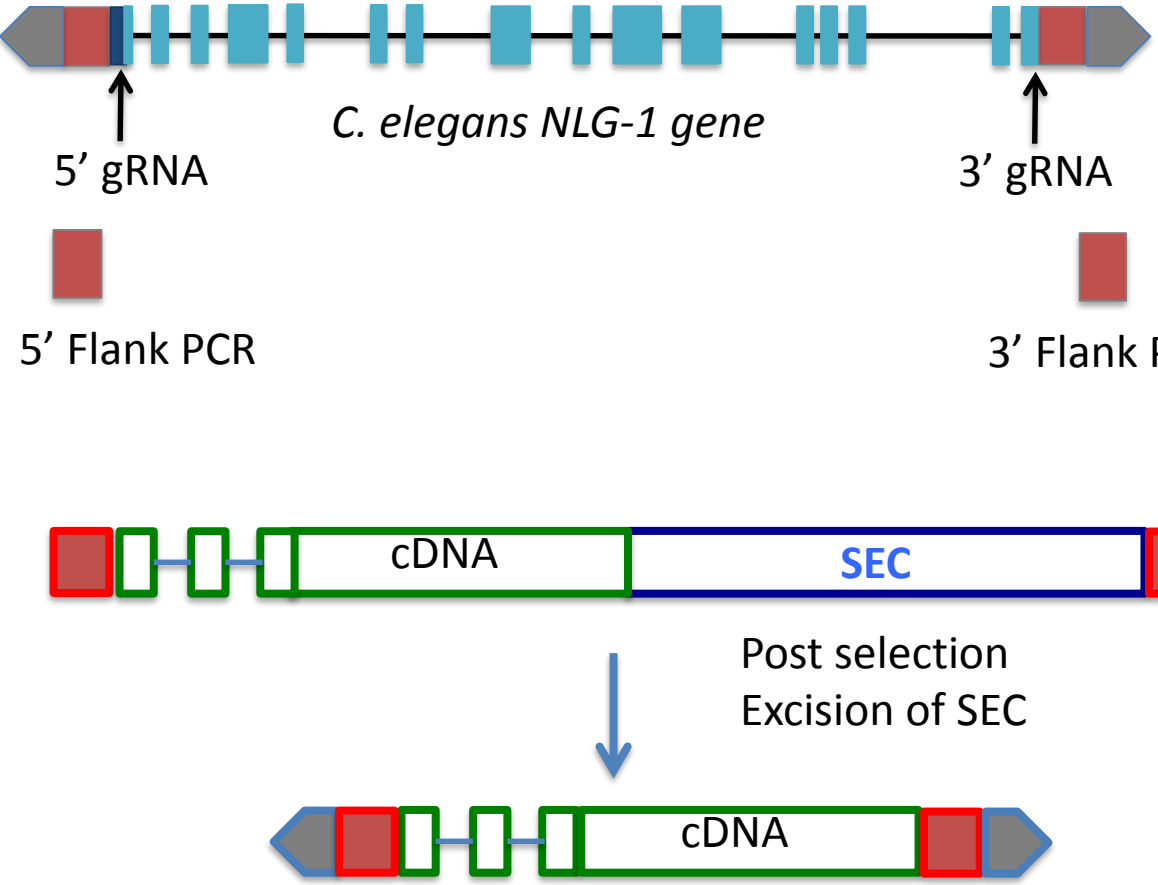
- Generate null mutants of NLG-1 and NRX-1 by CRISPR/Cas9 technology
- Confirm/Identify behavioral deficits
- **Replace worm NLG-1 and NRX-1 genes with human genes**
- Replace worm NLG-1 and NRX-1 genes with human variants associated with ASD
- Develop GRASP (GFP Reconstitution Across Synaptic Partners) assay to screen for restoration of normal function

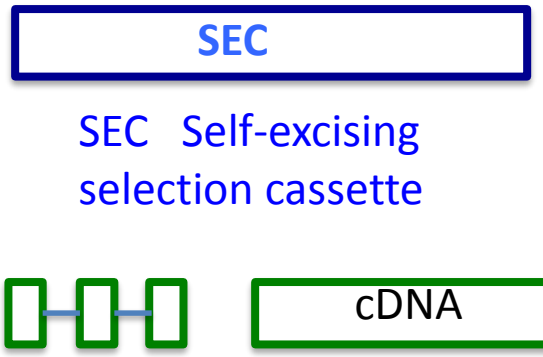
# *New C. elegans Models of ASD: Humanized Worms*

- We plan to clone the human neuroligin-3 gene, introduce the **R451C** and **G221R** mutations and generate humanized *C. elegans* strains using CRISPR/Cas9; then look for genes or compounds to reverse the effects of the mutations.

- We also plan to clone the human neurexin-2 gene, introduce the **L81Q** mutation and generate humanized *C. elegans* strains using CRISPR/Cas9; then look for genes or compounds to reverse the effects of the mutations.

# CRISPR/Cas9 Replacement of C. elegans NLG-1 with Human NLG-3



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# Site Directed Mutagenesis: R451C

```
G     K       D       T      L       R      E      T       I      K       F
    M
NLG-3   GGT AAG GAC ACC CTG CGA GAG ACC ATC AAG TTC ATG

                        TTC CTG TGG GAC ACA CTC TGG TAG TTC
        GGT AAG GAC ACC CTG TGT GAG ACC ATC AAG TTC ATG

                                              PCR with mismatch primer

           G     K       D     T       L      C
E       T      I      K       F    M
R451C   GGT AAG GAC ACC CTG TGT GAG ACC ATC AAG TTC ATG
```

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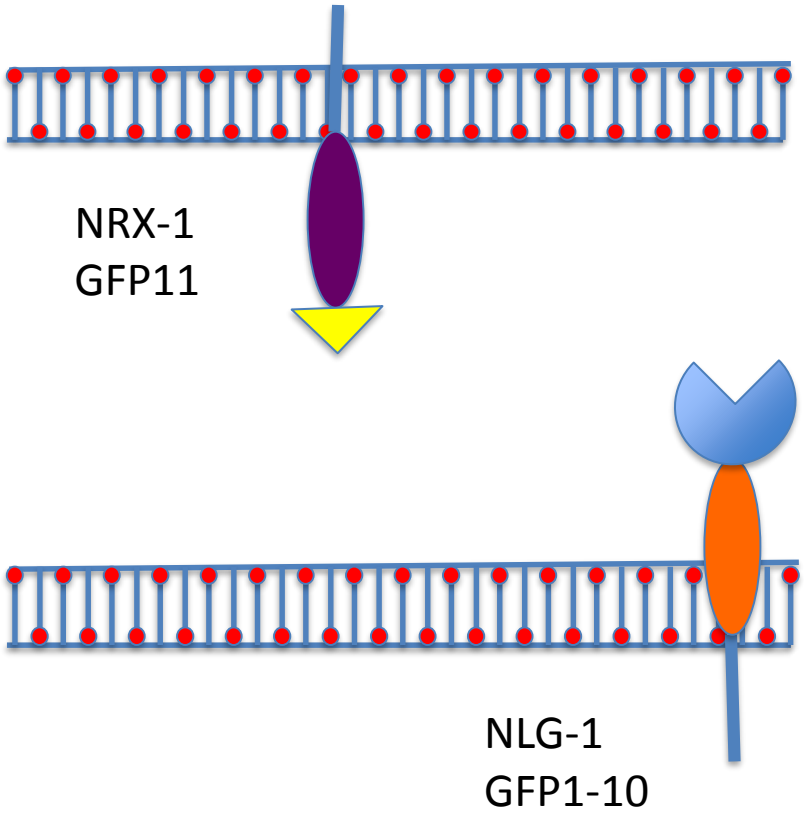
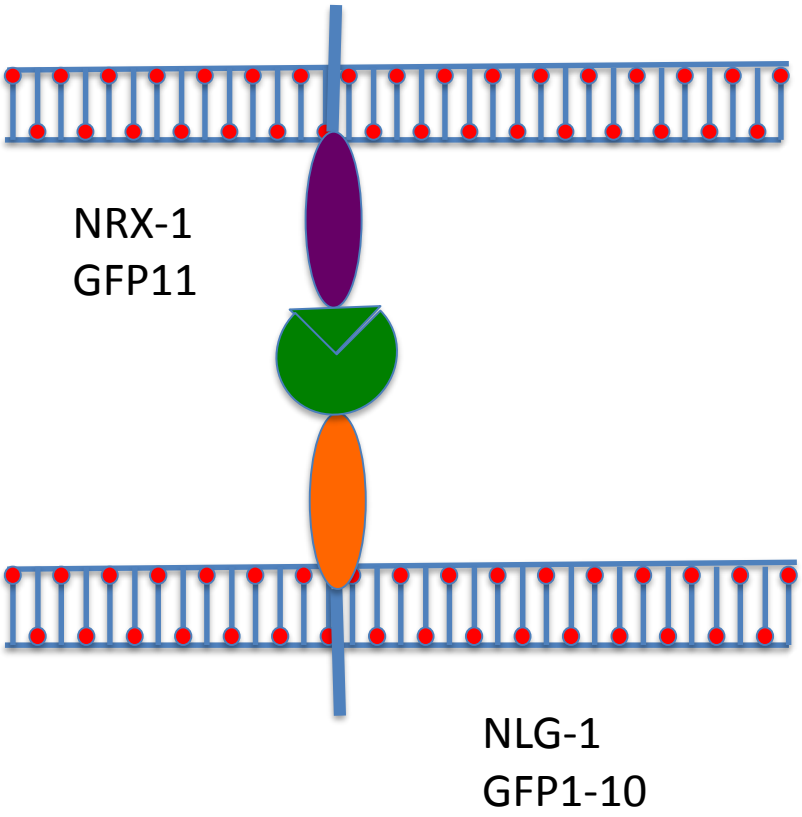
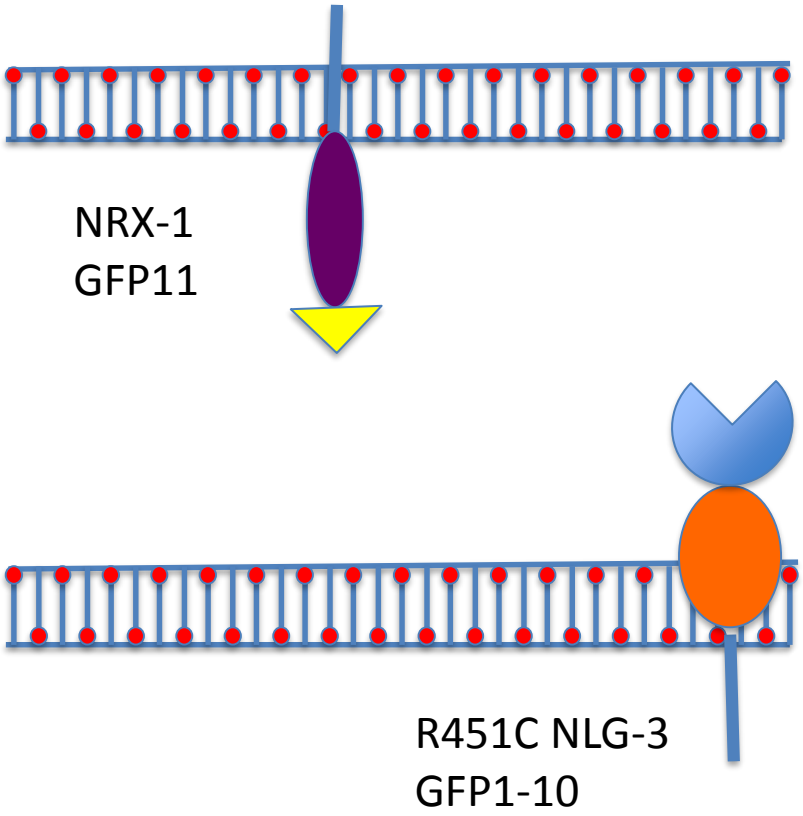
# GRASP: GFP Reconstitution Across Synaptic Partners



# *GRASP: GFP Reconstitution Across Synaptic Partners*



# *GRASP Screen for Re-Activators of Mutant NLG-3*



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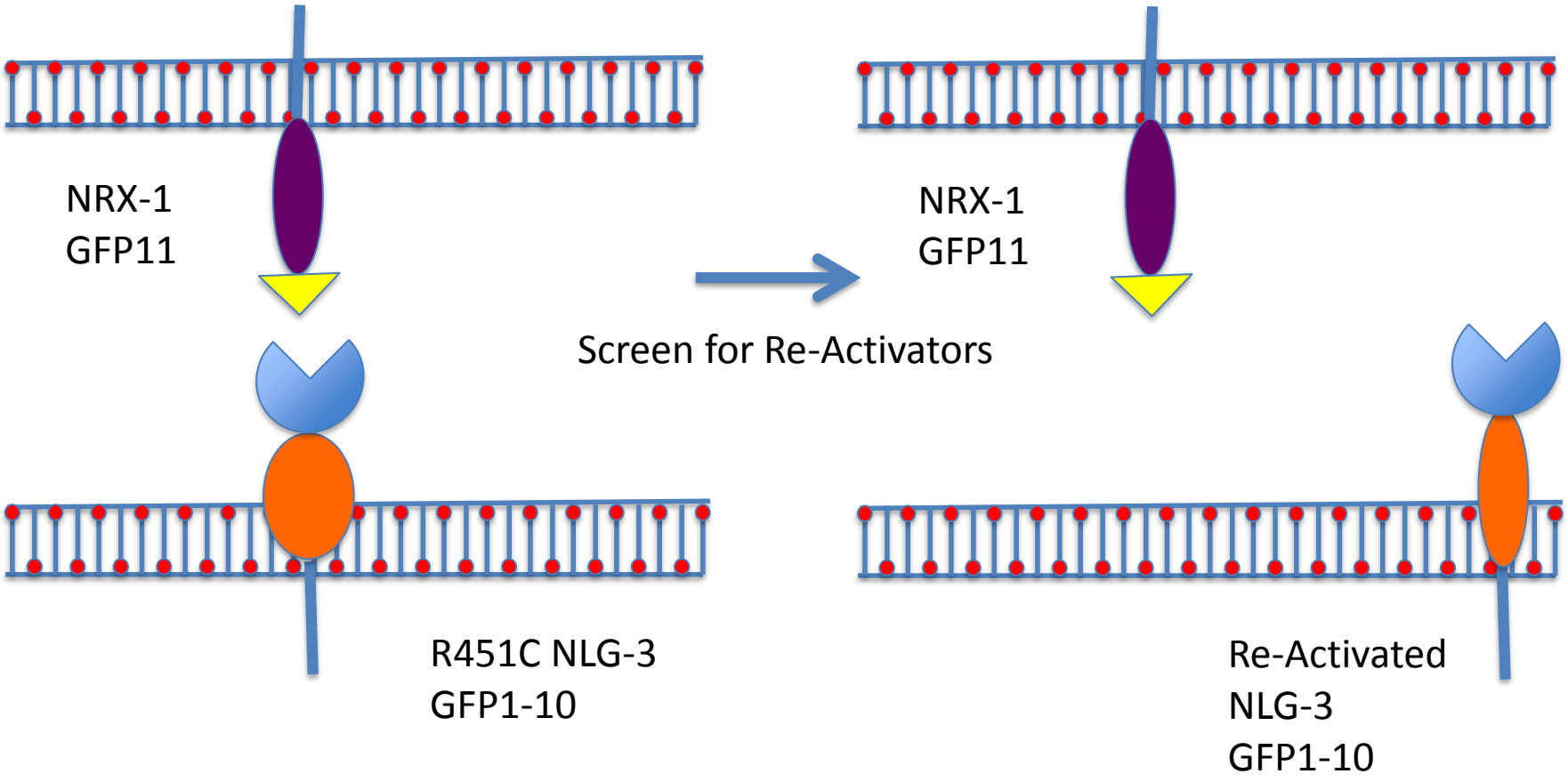

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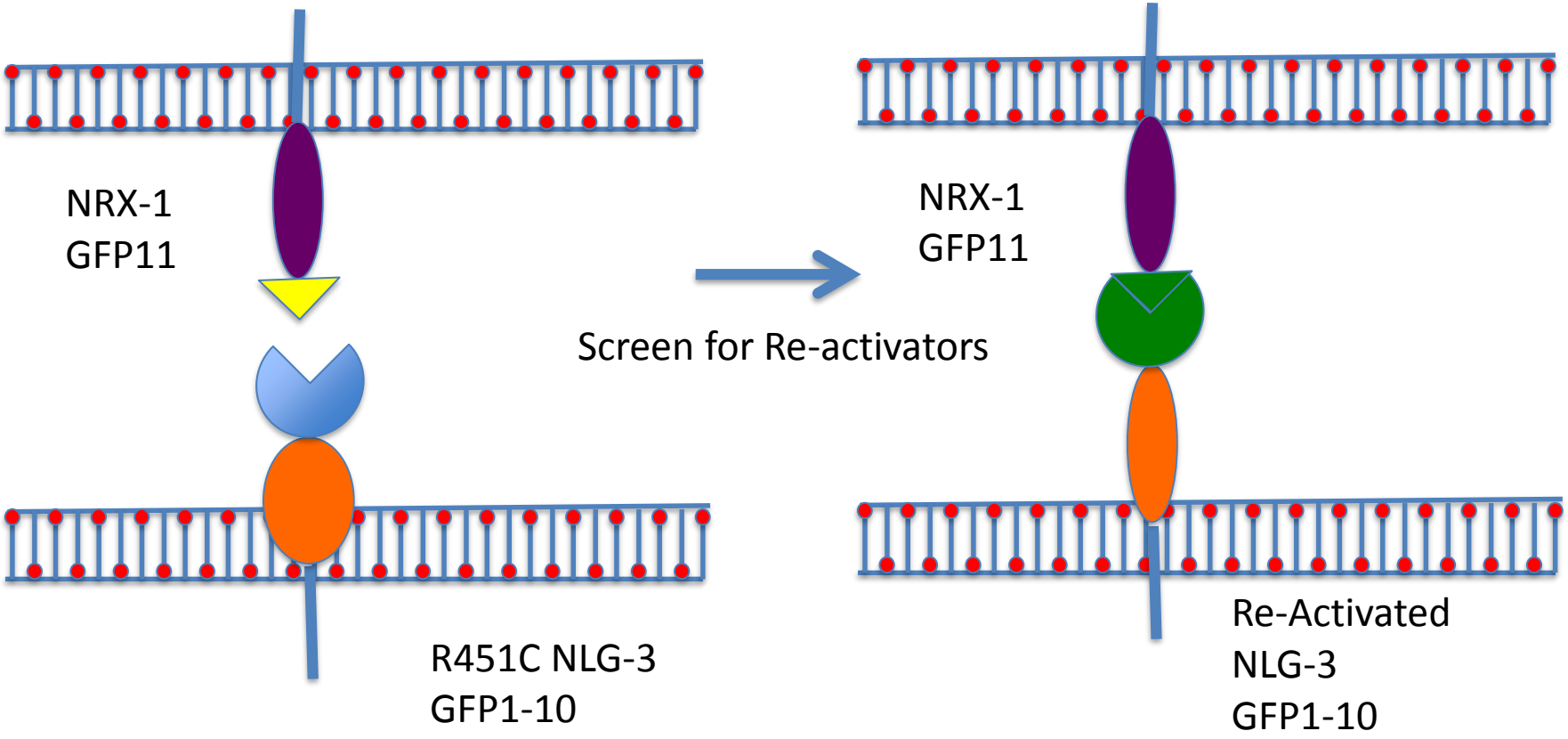

# *GRASP Screen*

- Tag NLG-1 with GFP1-10
- Tag NRX-1 with GFP11
- Generate transgenic worms via CRISPR/Cas9
- Mate to generate hybrids NLG-1 chrX, NRX-1 chrV
  - Alternative: Multiplex CRISPR/Cas9
- Identify synapses
- Tag humanized NLG and NRX genes
- Tag mutant humanized genes
- Screen for compounds to allow formation of GFP with mutant NLG or NRX genes

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