Undergraduate Research Symposium
ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT
PROJECT 47

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## *Modeling Autism Spectrum Disorder in Caenorhabditis elegans*

Neurologin (NLG) and Neurexin (NRX) are proteins located on the pre and postsynaptic terminals of neurons. They are responsible for the formation and maintenance of these synapses. Mutations found in the NLG and NRX genes have been linked to Autism Spectrum Disorder (ASD).

The goal of my research has been to use the nematode, *Caenorhabditis elegans*, to create a model organism displaying ASD like behaviors. In humans, there are multiple NLG and NRX genes. However, in *C. elegans* there is only one neuroligin gene and one neurexin gene. The single gene provides a model to better understand their human counterparts.

The first aspect of my research was to sequence existing strains of *C. elegans* which have alterations in their neurexin gene. These mutant strains were expected to demonstrate behavioral deficits, however they did not, and we wanted to further characterize the mutations. To accomplish this I utilized PCR to amplify the region where the mutations are located. The fragments were then sequenced. We found that the expected mutations were indeed present. In one strain, VC1416, I discovered that there is an, in frame, 54 amino acid deletion. In a different strain, SG1, I found that there is a 1498 bp deletion, with the deletion of 135 amino acids deleted out of frame.

The second aspect of my work is to create a complete knockout of the NLG gene using CRISPR/Cas9 technology. This process included cloning the 5' and 3' flanking regions of the *C. elegans* NLG-1 gene. These fragments were assembled along with selection markers to generate the new knockout plasmid. Synthetic DNA encoding the 5' and 3' guide RNA sequences were cloned individually into two additional plasmids. The accuracy of the cloning was then verified through Sanger sequencing.

The third aspect of my research is to introduce the human NLG-3 gene into the *C. elegan* genome. This process required me to add the human NLG-3 cDNA and three artificial *C. elegans* introns. Construction was completed and sequence analysis of the completed plasmid is ongoing.

The fourth aspect of my research will be to introduce the human NLG-3 mutations, R451C and G221R associated with ASD. This aspect of my project is currently in the initial stages and is where my future work will be heading. All these plasmid constructs will be used to generate transgenic *C. elegans*.

# Modeling Autism Spectrum Disorder in *Caenorhabditis elegans*

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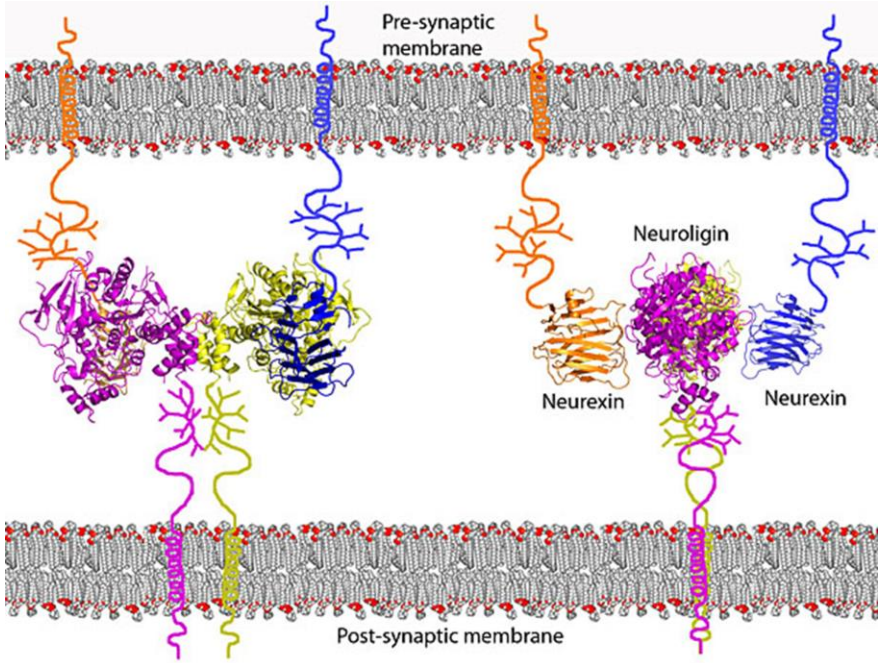
## Introduction

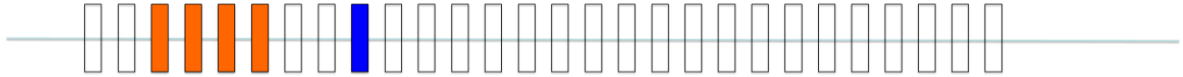
- Neuroligin (NLG) and Neurexin (NRX) pre and postsynaptic proteins
  - Responsible for the formation and maintenance of synapses
  - Mutations found in the NLG and NRX linked to Autism Spectrum Disorder (ASD). (Cao et al. 2017).

## Research Objective

- Characterize the known Neurexin Mutations.
- Create mutant strains of the nematode *Caenorhabditis elegans*
  - Complete knockout of Neuroligin
  - Humanized version of Neuroligin

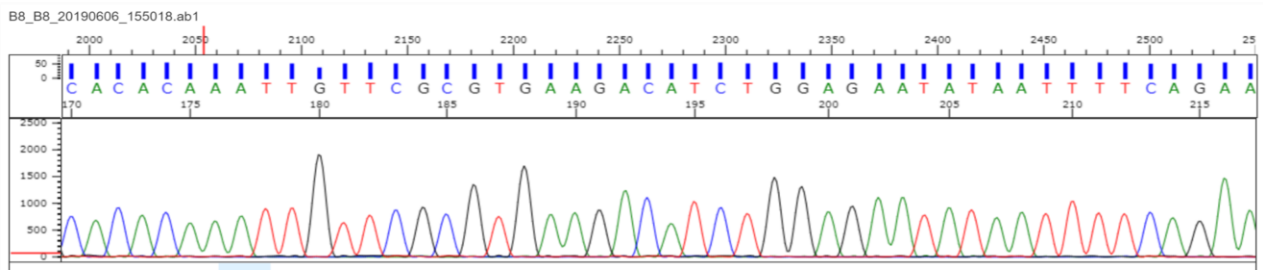



## Characterization of Strains VC1416 and SG1



**SG1** **VC1416**

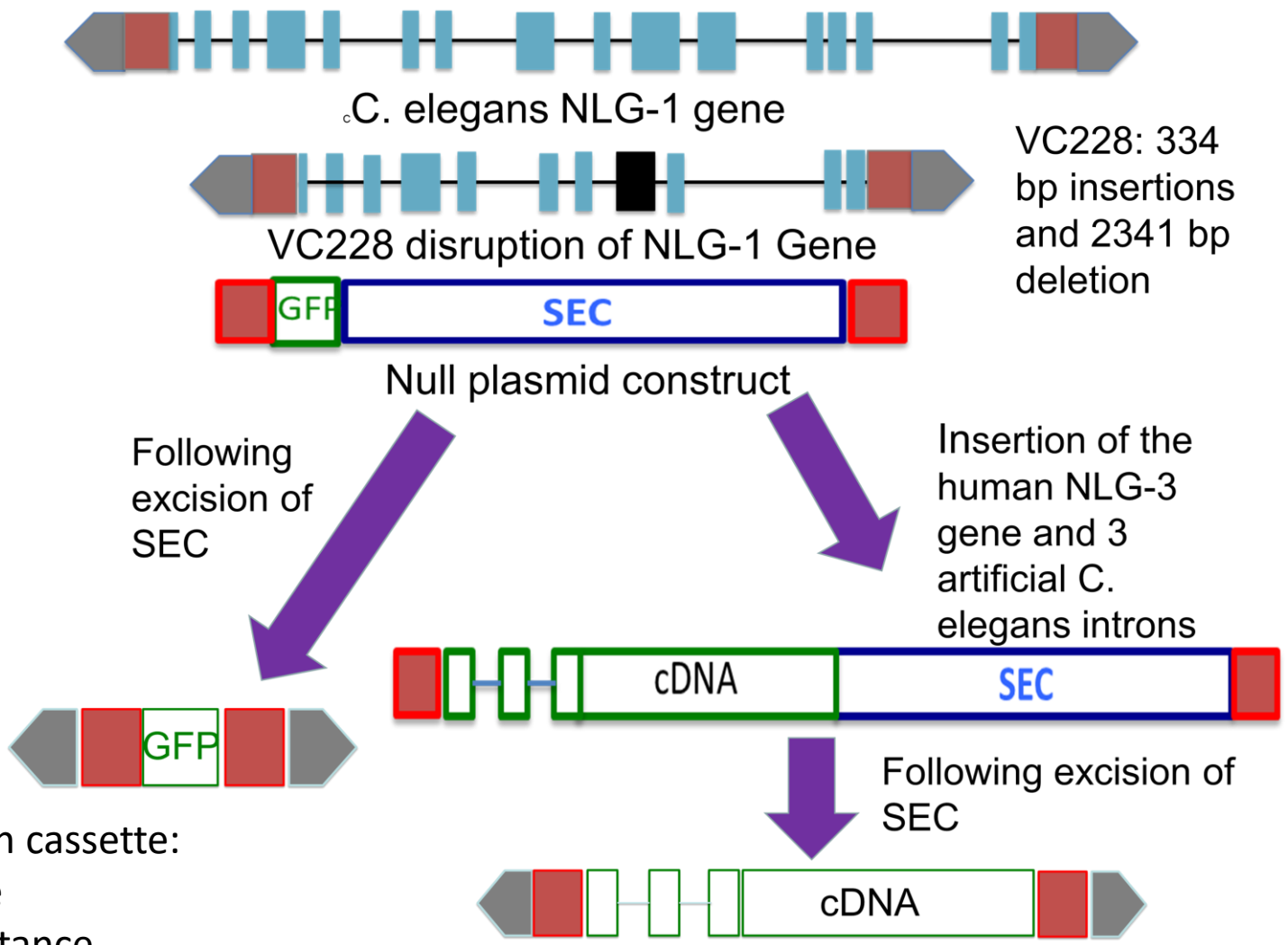
N2 (wt): 28 exons, 1716 amino acids
**VC1416:** 861 bp deletion, exon 9 deleted, 54 amino acids
**SG1:** 1498 bp deletion, exons 3-6 deleted, 136 amino acids




*C. elegans* NLG-1 gene

VC228 disruption of NLG-1 Gene

# Plasmid constructs for novel NLG-1 strains of *C. elegans*




SEC Self-excising selection cassette:

- Roller phenotype
- Hygromycin resistance
- Temperature Induced Cre

## Results

- VC1416 has an 861 bp deletion with exon 9 deleted (54 amino acids).
- SG1 has a 1498 bp deletion with exons 3-6 deleted (136 amino acids)
- Both the null and the human replacement plasmids have been created
- DNA sequencing shows multiple substitutions.

## Discussion

- Why don't the SG1 mutants show more behavioral deficits.
- The substitutions were most likely introduced during the multiple steps of PCR in the Gibson assembly protocol.
- We are currently creating a new construct with minimal PCR steps.
- The lab is working on creating the NRX-1 nulls and human replacement constructs.


## Acknowledgments

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## Future Work

- Fix substitutions with limited PCR
- Microinject the null constructs
- Introducing the R451C and G221R mutation to the humanized construct.
  - Mutations are associated with ASD in humans.

## References


Cao X, Tabuchi K. 2017. Functions of synapse adhesion molecules neurexin/neuroligins and neurodevelopmental disorders. Neurosci Res. 116:3–9. [accessed 2020 Mar 12]