MOL231: Functional investigation of neurite guidance receptors *Dscam* and *Neogenin* in *Nematostella vectensis* by identifying knockout animals and transgene reporter carriers

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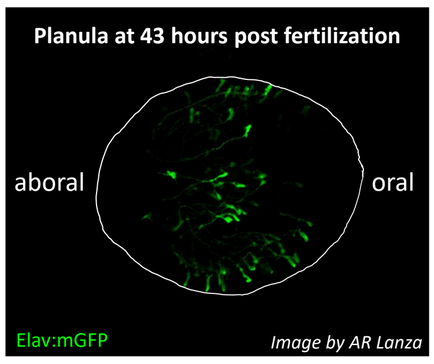
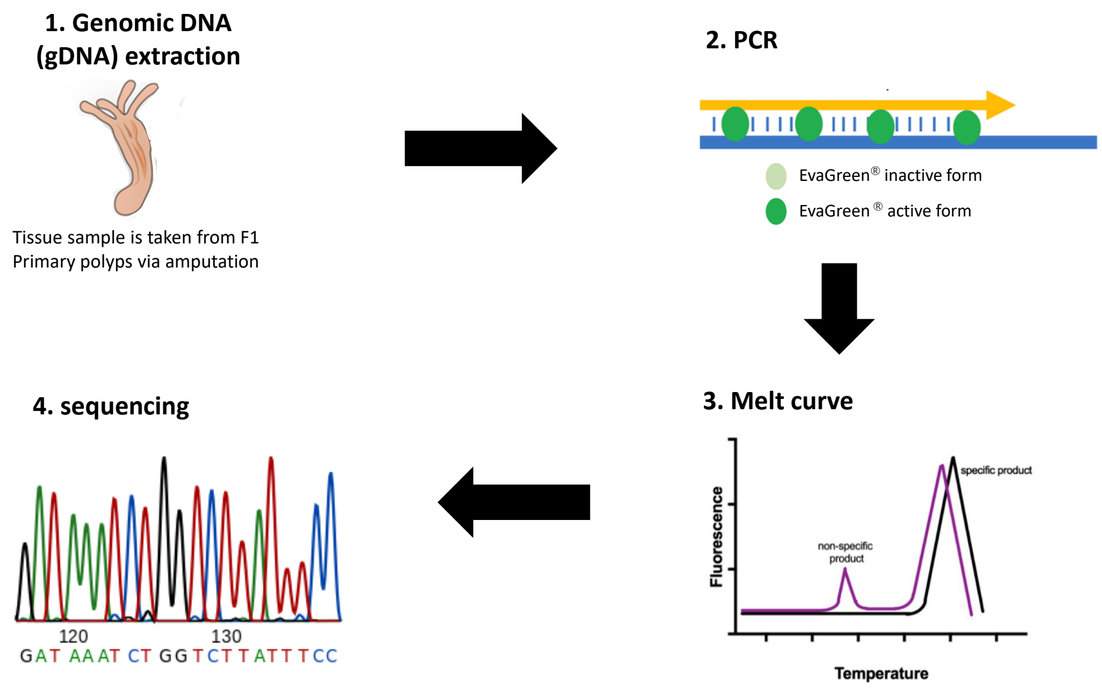
Background

Establishing connections between neurons is essential for forming a nervous system. These connections are formed by axonal outgrowths which connect neurons together. The receptors Dscam and Neogenin function in axon guidance in centralized nervous systems, but it is yet unknown whether they play a role in the formation of simple nerve nets, like that of the sea anemone, *Nematostella vectensis*. During early *Nematostella* development, neurite outgrowth occurs primarily in an aboral direction (away from the mouth). By inhibiting the function of these receptors, we hope to determine their role in guiding early neurite outgrowth. Cas9 protein and guide RNAs targeting *Dscam* and *Neogenin* were injected into *Nematostella* embryos to introduce mutations in the genes. Furthermore, a transgenic reporter line was created to help visualize where the Dscam receptor is expressed in the *Nematostella* nervous system.



Early neurites grow in an aboral direction

Methods



AIM: To identify CRISPR/Cas9 mutants by genotyping individual animals and to identify carriers of *Dscam::memGFP* transgene

Results

Dscam mutant melt curves

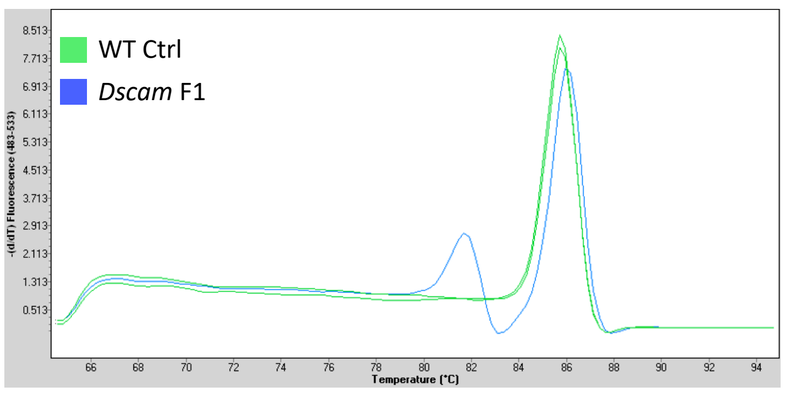


Figure 1.1 – Melt curve analyses were conducted using gDNA extracted from the F1 offspring of (parent) animals previously injected with an sgRNA targeting *Dscam*. Mutant melt curves indicate that there is variation in the nucleotide sequence when compared to control. Mutant melt curves were detected for F1 offspring from this particular F0 parent.

Sequence mutation

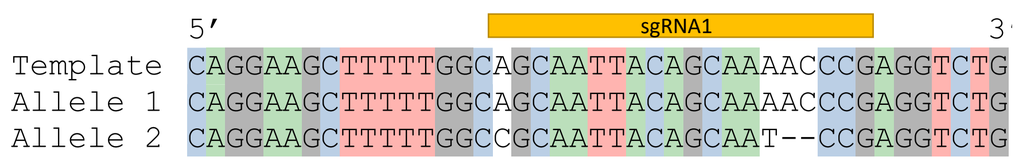


Figure 1.2 – Individuals who produced a mutant melt curve were selected for further sequence analysis. Our results show that the CRISPR/Cas9 sgRNA which targeted the *Dscam* gene resulted in 2 nucleotide substitutions as well as a 2 bp deletion.

Amino acid sequence

WT LFGSNYSKTRGLTLETPHSVHARTPASSGISRVPESAFGSRYEIGRRSAAS

Mutant LFGRNYSNPRSDPRDATLGARSDPCIGDIPSTRVCVRI✱VRDRPTECRV

Figure 1.3 – The mutant nucleotide sequence was translated into its amino acid/protein sequence. We saw that the nucleotide substitutions and deletions together produced a premature stop codon (red asterisk) in the protein sequence.

Neogenin mutant melt curves

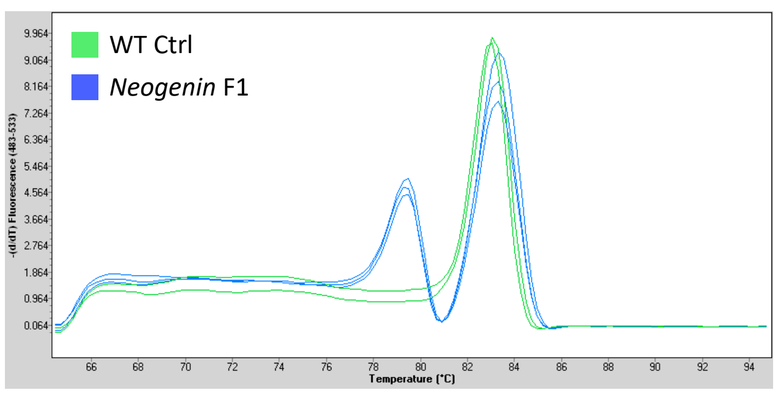


Figure 2.1 – Melt curve analyses were conducted using gDNA extracted from the F1 offspring of (parent) animals previously injected with an sgRNA targeting *Neogenin*. Mutant melt curves indicate that there is variation in the nucleotide sequence when compared to control. Mutant melt curves were detected for F1 offspring from this particular F0 parent.

Sequence mutation

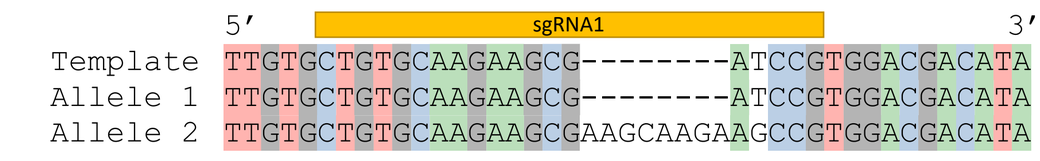


Figure 2.2 – Individuals who produced a mutant melt curve were selected for further sequence analysis. Our results show that the CRISPR/Cas9 sgRNA which targeted the *Neogenin* gene resulted in an 8 bp insertion and 1 nucleotide substitution.

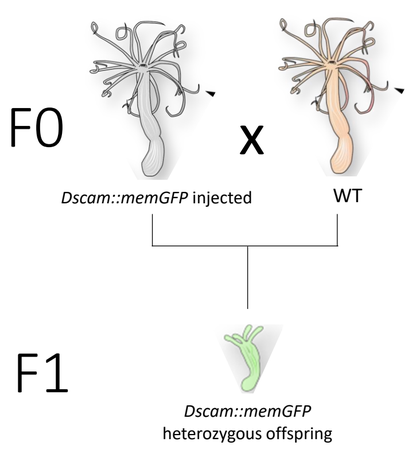
Amino acid sequence

WT LCKKRSVDDIQMPRRPPPTYKSAIQSNGSAKKKKEEKPPDLWINHTENMEMKPLTS

Mutant LCKKRSKNPWTTYKCLEGRRQLINRPSSQMGPQRKRKKKNLPIFGLTTRRTWK✱NL

Figure 2.3 – The mutant nucleotide sequence was translated into its amino acid/protein sequence. We saw that the nucleotide substitutions and insertion together produced a premature stop codon (red asterisk) in the protein sequence.

Identification of F0 carrier of the *Dscam::memGFP* transgene



SCAN ME

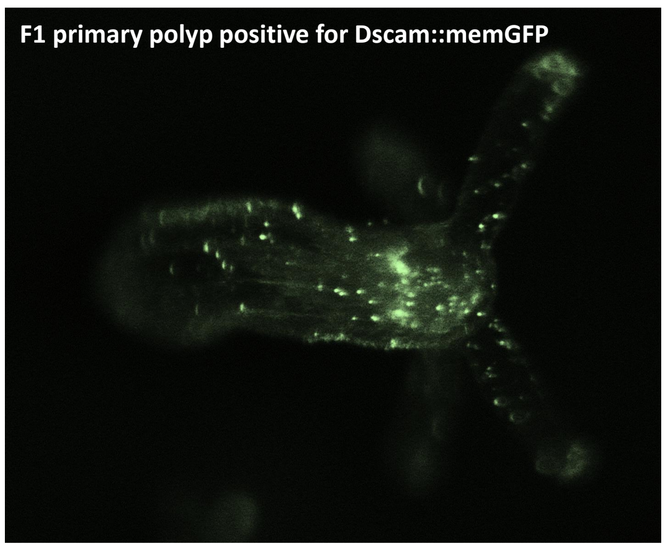


Figure 3 – F0 animals injected with the *Dscam::memGFP* construct were raised to adulthood and crossed with WT in order to produce heterozygous F1 offspring. We identified four F1 individuals that were positive for GFP transgene.

Summary and conclusion

1. Individual F1 offspring carrying CRISPR/Cas9 induced mutations were positively identified for both the *Dscam* and *Neogenin* receptor genes indicating our Cas9 protein and guide RNAs work.
2. These nucleotide mutations resulted in a premature stop codon in the amino acid sequence of both *Dscam* and *Neogenin* and may inhibit the function of the protein.
3. Identified an F0 parent animal carrying the *Dscam:memGFP* transgene as well as four positive F1 offspring confirming germline transmission.
4. Altogether, these tools will aid in assessing the role of Dscam and Neogenin in neurite guidance