Using CRISPR/Cas9 Gene Editing to Test the Hotspot Hypothesis

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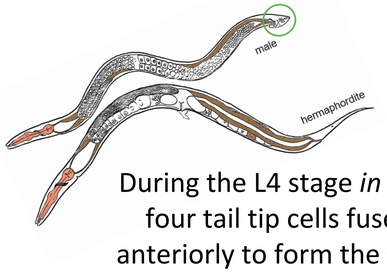
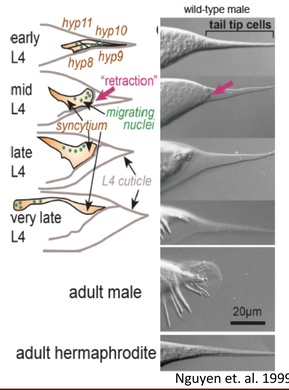
Tail tip morphogenesis (TTM) results in sexually dimorphic tails in *Caenorhabditis elegans*



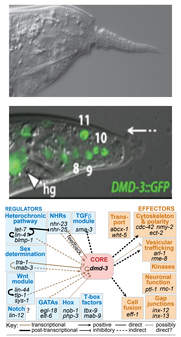
During the L4 stage *in C. elegans*, the four tail tip cells fuse and retract anteriorly to form the short, rounded tip of the adult tail.

TTM happens only in males.



Nguyen et. al. 1999

DMD-3, a DM-Domain transcription factor, is the master regulator of TTM in *C. elegans*



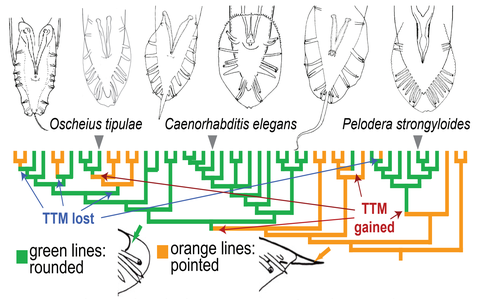
TTM does not occur in DMD-3(-) males

Ectopic expression of DMD-3 in hermaphrodites causes TTM

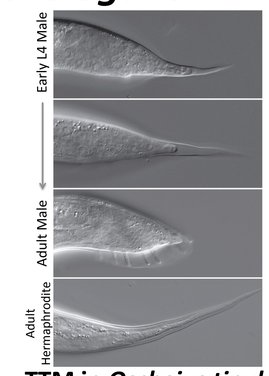
DMD-3 is predicted to be at the center of a bow-tie shaped gene regulatory network

Mason et.al. 2008, Nelson et.al. 2011

TTM evolved multiples times independently in nematode species related to *C. elegans*

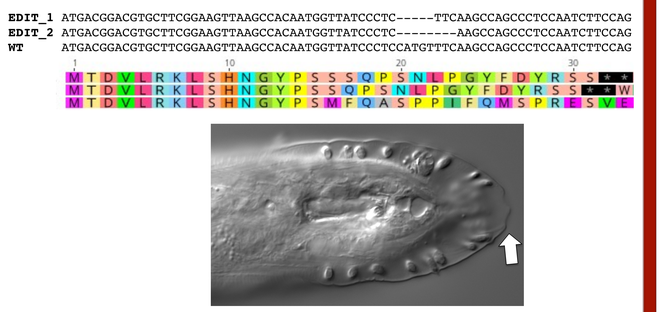


When convergently evolved phenotypes arise due to *de novo* mutations in orthologous loci this locus is referred to as an **evolutionary hotspot** (Martin and Orgogozo, 2013).



TTM in *Oscheius tipulae*

Previously work indicated knockout of the DMD-3 ortholog in *O. tipulae* did not effect TTM. These results did not support the hotspot hypothesis



Tail tip does not extend past the fan in adult DMD-3(-) *O.tipulae* male. Indicating successful TTM

Primer pairs do not amplify desired regions

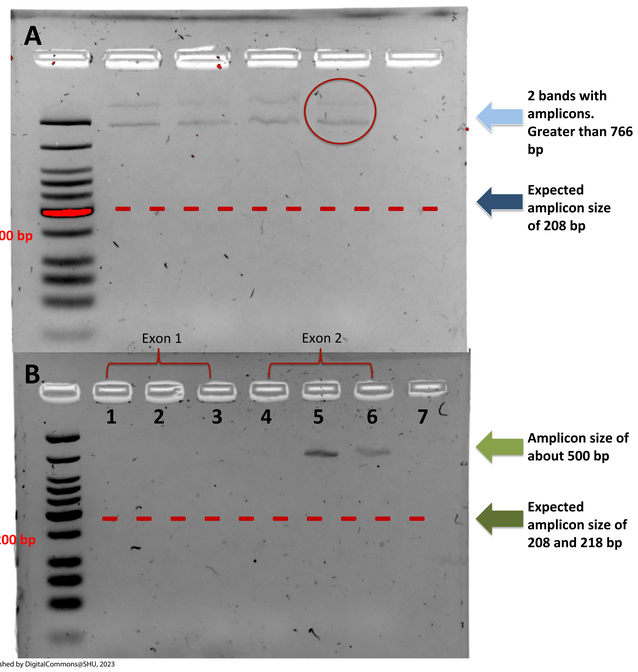


Figure 1. Gel electrophoresis results visualizing DNA amplified by newly designed PCR primers. A) Testing PCR primers to screen for edits made by gRNA #1. The expected amplicon size is 208 base pairs (bp) (dark blue arrow and dashed line). Instead, the amplicon was about 766 bp (light blue arrow). These primers do not amplify the desired region. There is also non-unique binding given the two bands near the top of the gel (red circle). DNA ladder is NEB Low Molecular Weight DNA Ladder**. B)** PCR amplicons from screening primers designed for gRNA #1 and gRNA #2. Lanes 1-3 should contain gRNA #1 amplicons (208 bp) and lanes 4-6 should contain gRNA #2 amplicons (218 bp), dark green arrow and dashed line. gRNA #2 amplicons are about 500 bp and larger than predicted (light green arrow) DNA ladder is NEB Low Molecular Weight DNA Ladder**.**

- A new *O.tipulae* genome was assembled from PacBio sequencing data (Gonzalez et. al. 2021). This new assembly was a higher quality reference genome than the previous Illumina assembly.
- The gene we previously knocked out was not identified as the *dmd-3* ortholog in the new PacBio genome assembly
- We have designed new gRNAs and PCR screening primers to KO newly identified *dmd-3* ortholog
- Currently we are optimizing PCR screening primers

gRNA design strategy :

Target early in the coding region (#1) & target conserved domain (#2)
Screening primers for gRNA #1 should amplify 208bp and span cut site
Screening primers for gRNA #2 should amplify 218bp and span cut site

Future directions

Redesign PCR primers. For primers to be used for CRISPR-Cas9 screening they must amplify only a single and correctly sized genomic region.

References:
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Heteroduplex diagram: https://upload.wikimedia.org/wikipedia/commons/d/de/Formation_of_hetroduplexes_and_homoduplexes.jpg