

## Introduction

### Abstract

Our lab is using comparative genomics protocols to explore the evolution of the insulin-like signaling pathway in *Drosophila* species spanning 40M years of evolution. This project aims to annotate an ortholog of the *Drosophila melanogaster* gene *microtubule star (mts)* in the incompletely characterized *Drosophila ananassae* genome. Genome annotation is the process of creating gene models, or labelling areas of interest in a species' genome for further investigation, using genome comparisons and large data sets characterizing gene expression, which is the production of RNA from select regions of genomic DNA. We are using comparative genomics and bioinformatics protocols to identify and build a detailed gene model of the putative *mts* gene in *D. ananassae*. We will annotate all unique protein-coding isoforms of this gene in *D. ananassae*. Our research tools include a custom mirror of the UCSC Genome Browser, NCBI BLAST, the model organism database FlyBase, and custom bioinformatics tools developed by the Genomics Education Partnership. Annotation of a large number of insulin-like signaling pathway genes will enable further investigation of the evolution of these genes and their regulatory regions in the context of their action within the insulin-like signaling pathway.

### Experimental Questions

Does the *mts* gene exist in *D. ananassae*? Is synteny preserved between *D. melanogaster* and *D. ananassae* in a genomic region of interest? What is the gene model of the *D. ananassae mts* gene?

### Background

Orthologs are homologous genes that arose in a common ancestor and are present in related species. The utilization of a relatively closely related species as a reference species enables the search for orthologs in the unannotated genomes of related species. Our research examines the *mts* or *microtubule star* gene, a component of the insulin-like signaling pathway in the reference species *Drosophila melanogaster*. We searched for *mts* in the unannotated species *Drosophila ananassae*. Differences between orthologs could provide insight into pathway modifications and constraints. In the future, additional insulin-like signaling pathway genes will be annotated for analysis.

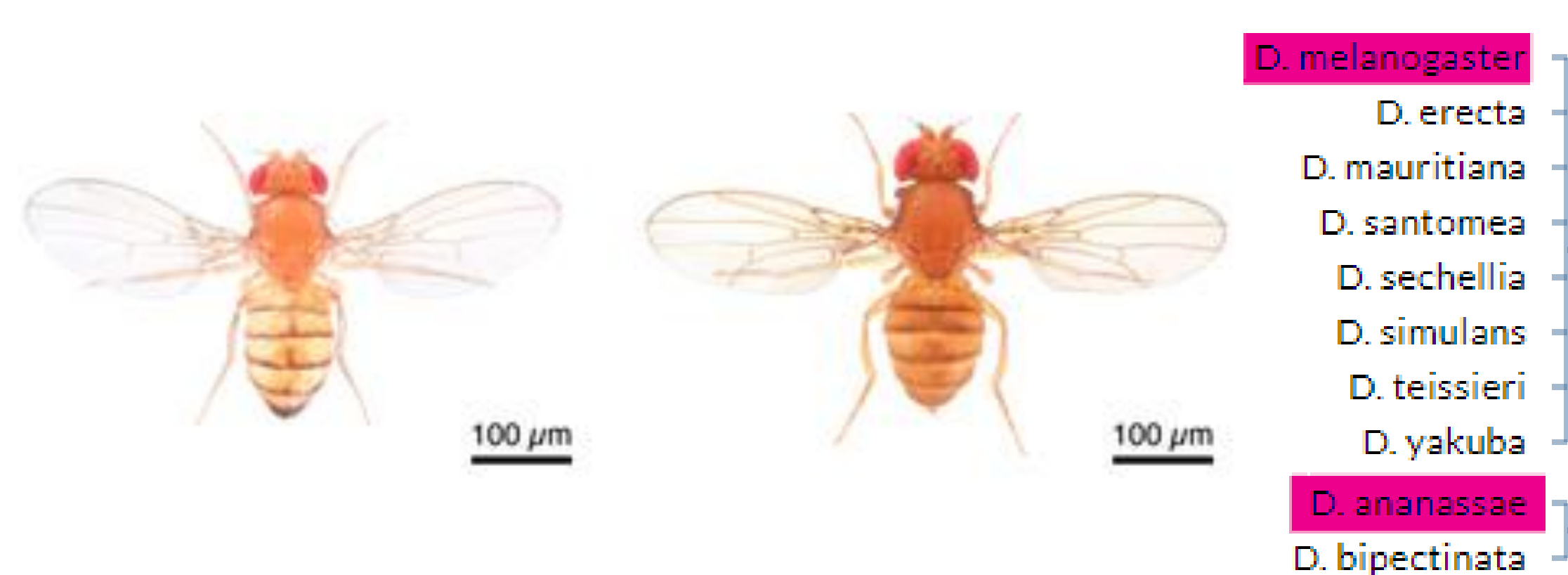


Figure 1: *D. melanogaster* (left), *D. ananassae* (middle), and a *Drosophila* phylogeny. From [UCSC Genome Browser Gateway](#).

## Methods

### Synteny

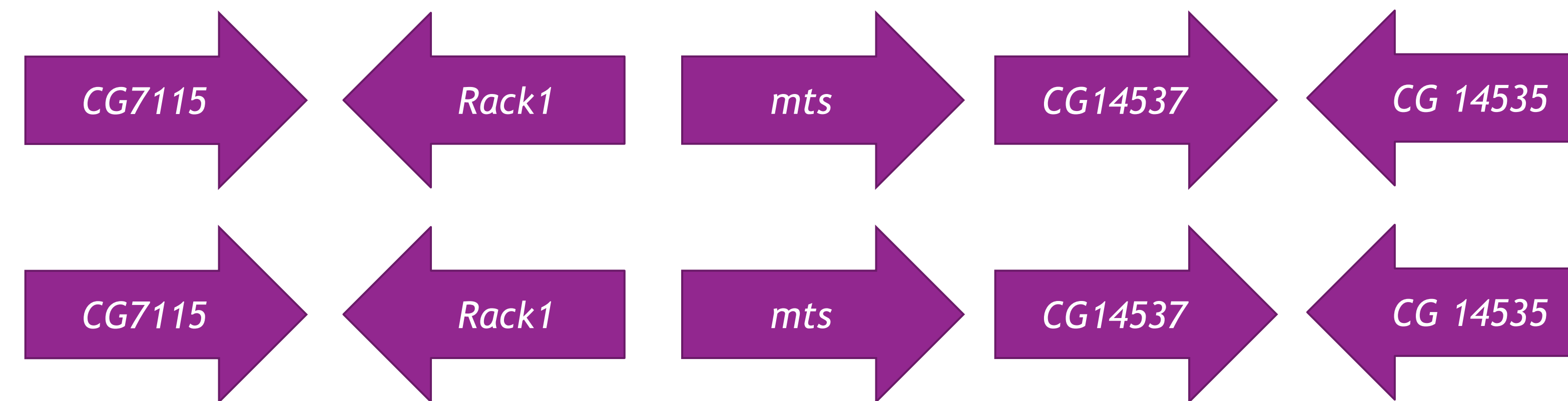


Figure 2: *D. melanogaster* (chr2L scaffold) (top) and *D. ananassae* (NC\_057930 scaffold) (bottom). The preservation of the order and gene direction between organisms demonstrates local synteny.

### Gene Annotation Workflow

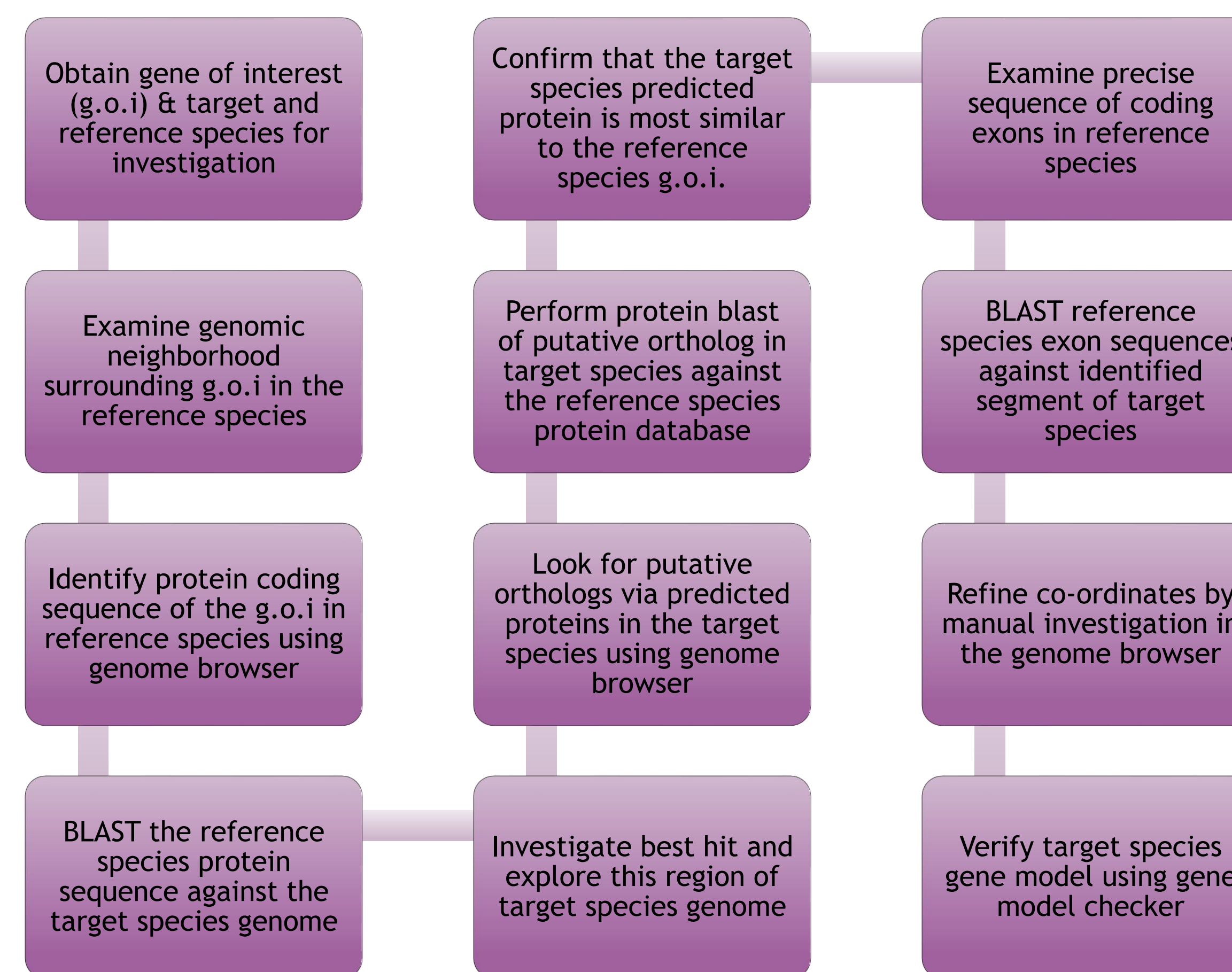


Figure 3: Gene annotation workflow used to carry out the annotation of the *mts* gene in *D. ananassae*. Adapted from [The Genomics Education Partnership](#).

## Results

### Custom Gene Model Generated for *mts* in *D. ananassae*

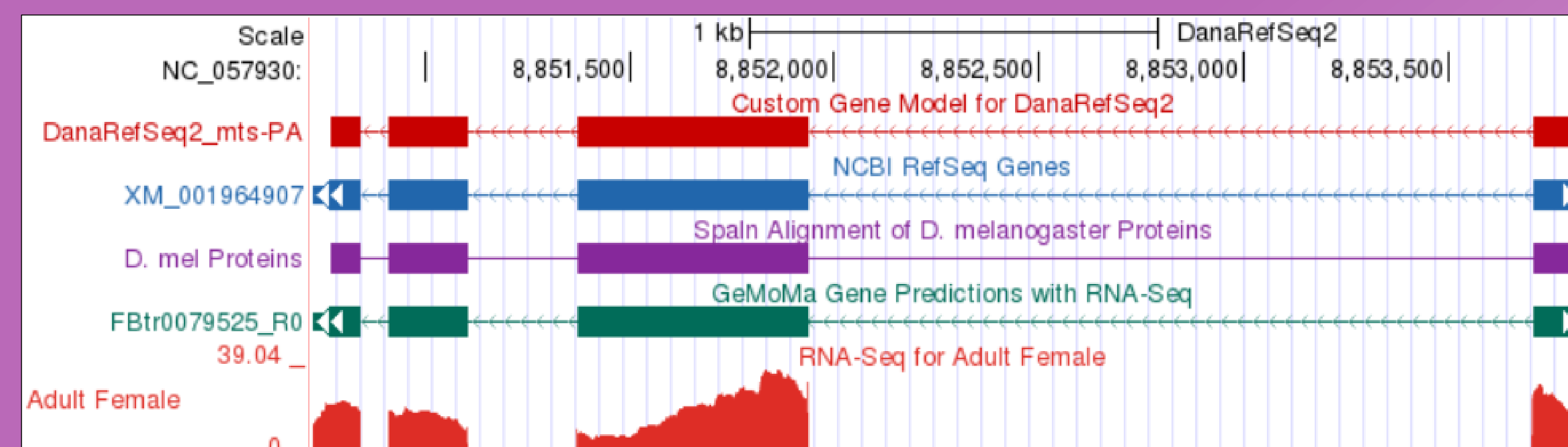


Figure 4: Snapshot of a mirror of the [UCSC Genome Browser](#) containing the custom gene model (annotated *mts* gene) in *D. ananassae* (ASM1763931v2). Our *mts* gene model is well-supported by multiple lines of evidence.

## Protein Alignment

Dme1_mts-PA	1	MEDKATTKDLQNIQNECQLTETQVRTLCDKAKEITLSKESNVQEVKCPVTYCGDVHC	60
DanaRefSeq2_mts-PA	1	MEDKATTKDLQNIQNECQLTETQVRTLCDKAKEITLSKESNVQEVKCPVTYCGDVHC	60
Dme1_mts-PA	61	QFHDLMELFRIGGKSPDNYLFGDYVDRGYYSVETVLLVALKVRYRERITILRGNHES	120
DanaRefSeq2_mts-PA	61	QFHDLMELFRIGGKSPDNYLFGDYVDRGYYSVETVLLVALKVRYRERITILRGNHES	120
Dme1_mts-PA	121	RQLTQVYGFYDECLRKYGNANVWYFTDLFDYLPALTALVDGQIFCLHGGLSPSIDSLDHI	180
DanaRefSeq2_mts-PA	121	RQLTQVYGFYDECLRKYGNANVWYFTDLFDYLPALTALVDGQIFCLHGGLSPSIDSLDHI	180
Dme1_mts-PA	181	RALDRLQEVPHGPMCDLLWSDDRRGGWGISPRGAGYTFGQDISETFNNTNGLTLVSR	240
DanaRefSeq2_mts-PA	181	RALDRLQEVPHGPMCDLLWSDDRRGGWGISPRGAGYTFGQDISETFNNTNGLTLVSR	240
Dme1_mts-PA	241	HQLVMEGYNWCNDRNVVTIFSAFNVCYRCGNQALMELDDSLKFSFLQDPAPRRGEPHY	300
DanaRefSeq2_mts-PA	241	HQLVMEGYNWCNDRNVVTIFSAFNVCYRCGNQALMELDDSLKFSFLQDPAPRRGEPHY	300
Dme1_mts-PA	301	TRRTPDYFL	309
DanaRefSeq2_mts-PA	301	TRRTPDYFL	309

Figure 5: Protein alignment of *mts*-PA in *D. ananassae* against *mts*-PA in *D. melanogaster*. From [Gene Model Checker](#).

## Gene Dot Plot

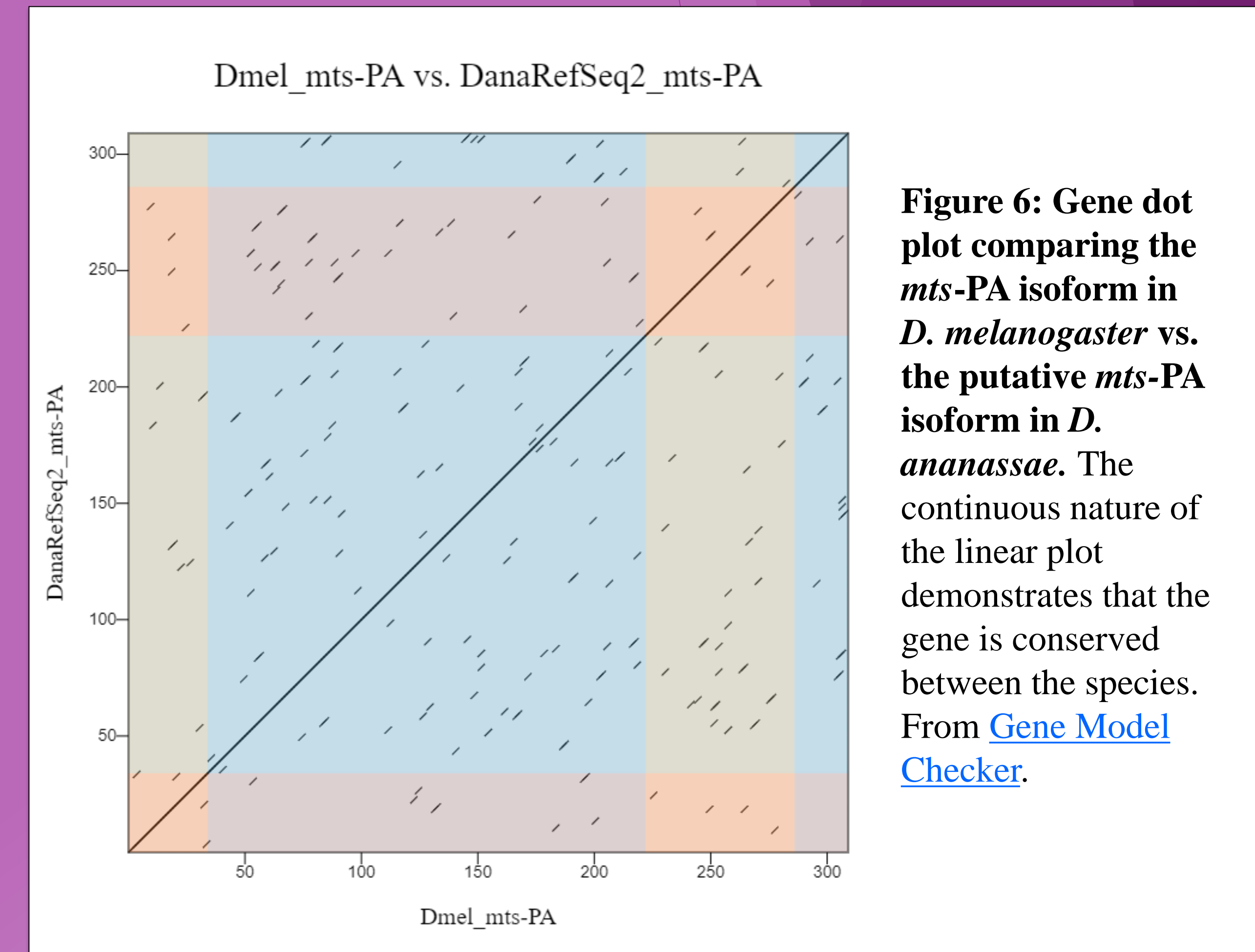


Figure 6: Gene dot plot comparing the *mts*-PA isoform in *D. melanogaster* vs. the putative *mts*-PA isoform in *D. ananassae*. The continuous nature of the linear plot demonstrates that the gene is conserved between the species. From [Gene Model Checker](#).

## Discussion

Our work indicates that the ortholog of *D. melanogaster mts* is present in *D. ananassae*. This genomic region is syntenic and we have generated a *D. ananassae mts* gene model. Our next step is to draft a manuscript and submit this custom gene model for publication. Future investigation could encompass the annotation of additional pathway genes and the analysis of gene and regulatory region evolution of the insulin-like signaling pathway through comparative genomics.

### Acknowledgements:

- Jaeda Doctolero and John Over
- [The Genomics Education Partnership](#) (GEP) & [The Reed Lab](#)