

Gene model for the ortholog of *Ptp61F* in *Drosophila ananassae*

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Abstract

Gene model for the ortholog of *Protein tyrosine phosphatase 61F* (*Ptp61F*) in the *Drosophila ananassae* May 2011 (Agencourt dana_caf1/DanaCAF1) Genome Assembly (GenBank Accession: GCA_000005115.1). This ortholog was characterized as part of a developing dataset to study the evolution of the Insulin/insulin-like growth factor signaling pathway (IIS) across the genus *Drosophila* using the Genomics Education Partnership gene annotation protocol for Course-based Undergraduate Research Experiences.

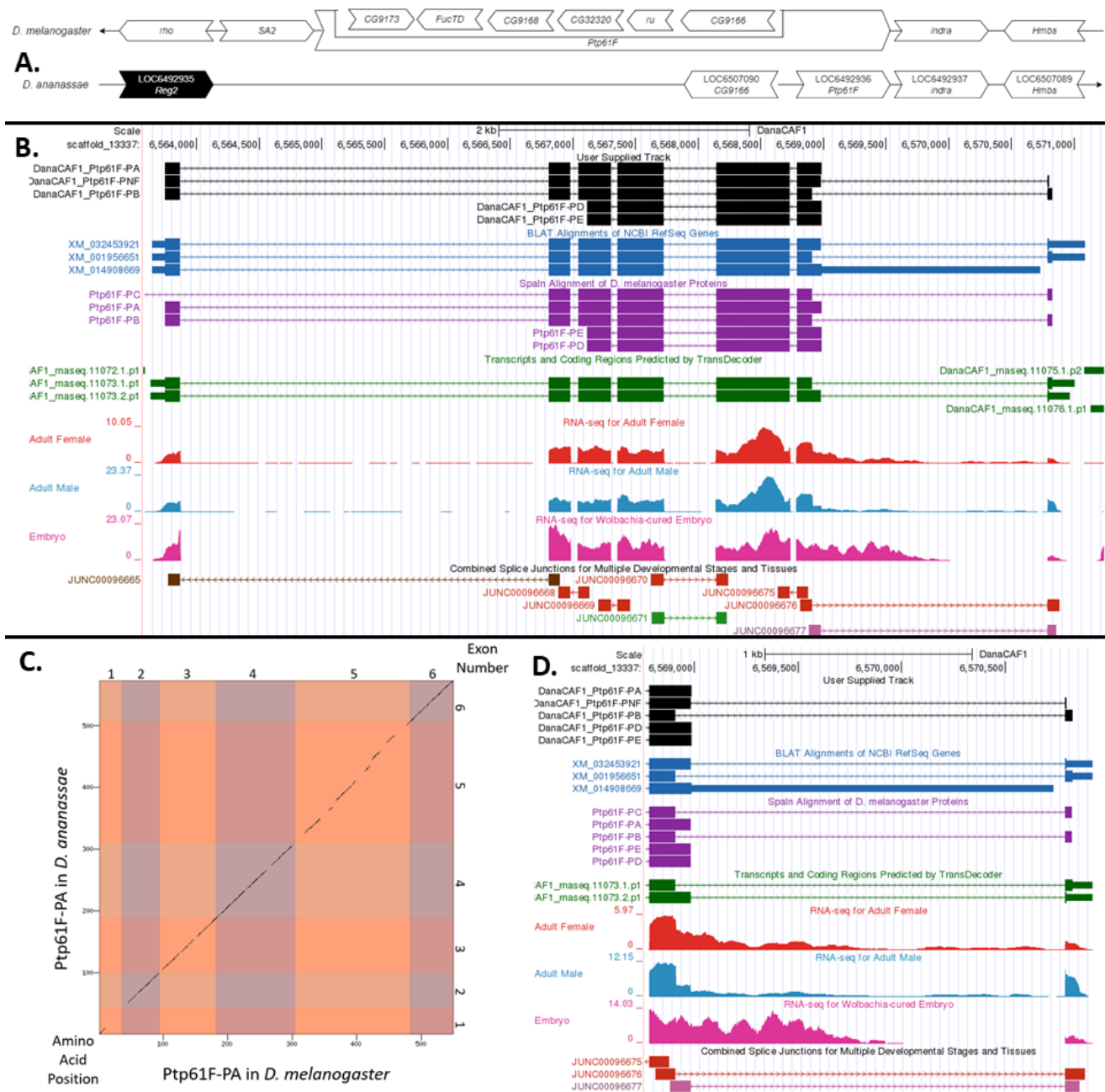


Figure 1. Genomic neighborhoods for *Ptp61F* in *Drosophila melanogaster* and *Drosophila ananassae*:

(A) Synteny comparison of the genomic neighborhoods for *Ptp61F* in *Drosophila melanogaster* and *Drosophila ananassae*. Thin underlying arrows indicate the DNA strand within which the gene-*Ptp61F*-is located in *D. melanogaster* (top) and *D. ananassae* (bottom). The thin arrow pointing to the right indicates that *Ptp61F* is on the positive (+) strand in *Drosophila ananassae*, and the thin arrow pointing to the left indicates that *Ptp61F* is on the negative (-) strand in *D. melanogaster*. The wide gene arrows pointing in the same direction as *Ptp61F* are on the same strand relative to the thin underlying arrows, while wide gene arrows pointing in the opposite direction of *Ptp61F* are on the opposite strand relative to the thin underlying arrows. White gene arrows in *Drosophila ananassae* indicate orthology to the corresponding gene in *D. melanogaster*, while black gene arrows indicate non-orthology. Gene symbols given in the *Drosophila ananassae* gene arrows indicate the orthologous gene in *D. melanogaster*, while the locus identifiers are specific to *Drosophila ananassae*.

(B) Gene Model in GEP UCSC Track Data Hub (Raney et al., 2014). The coding-regions of *Ptp61F* in *Drosophila ananassae* are displayed in the User Supplied Track (black); coding CDSs are depicted by thick rectangles and introns by thin lines with arrows indicating the direction of transcription. Subsequent evidence tracks include BLAT Alignments of NCBI RefSeq Genes (dark blue, alignment of Ref-Seq genes for *Drosophila ananassae*), Spaln of *D. melanogaster* Proteins (purple, alignment of Ref-Seq proteins from *D. melanogaster*), Transcripts and Coding Regions Predicted by TransDecoder (dark green), RNA-Seq from Adult Females, Adult Males, and *Wolbachia*-cured Embryos (red, light blue, and pink, respectively; alignment of Illumina RNA-Seq reads from *Drosophila ananassae*), and Splice Junctions Predicted by regtools using *Drosophila ananassae* RNA-Seq (SRP006203, SRP007906, PRJNA257286, PRJNA388952). Splice

junctions shown have a minimum read-depth of 10 with 50-99, 100-499, 500-999, >1000 supporting reads in green, pink, brown, and red, respectively. **(C) Dot Plot of Ptp61F-PA in *D. melanogaster* (x-axis) vs. the orthologous peptide in *Drosophila ananassae* (y-axis).** Amino acid number is indicated along the left and bottom; coding-CDS number is indicated along the top and right, and CDSs are also highlighted with alternating colors. Line breaks in the dot plot indicate mismatching amino acids at the specified location between species. **(D) Gene Model in GEP UCSC Track Data Hub** (Raney et al., 2014). The same evidence tracks as Figure 1B are shown in this image. We hypothesize that the isoform Ptp61F-PC does not exist in *D. ananassae*. In addition, we hypothesize that there is a novel isoform, Ptp61F-PNF.

Description

This article reports a predicted gene model generated by undergraduate work using a structured gene model annotation protocol defined by the Genomics Education Partnership (GEP; thegep.org) for Course-based Undergraduate Research Experience (CURE). The following information in this box may be repeated in other articles submitted by participants using the same GEP CURE protocol for annotating *Drosophila* species orthologs of *Drosophila melanogaster* genes in the insulin signaling pathway.

"In this GEP CURE protocol students use web-based tools to manually annotate genes in non-model *Drosophila* species based on orthology to genes in the well-annotated model organism fruitfly *Drosophila melanogaster*. The GEP uses web-based tools to allow undergraduates to participate in course-based research by generating manual annotations of genes in non-model species (Rele et al., 2023). Computational-based gene predictions in any organism are often improved by careful manual annotation and curation, allowing for more accurate analyses of gene and genome evolution (Mudge and Harrow 2016; Tello-Ruiz et al., 2019). These models of orthologous genes across species, such as the one presented here, then provide a reliable basis for further evolutionary genomic analyses when made available to the scientific community." (Myers et al., 2024).

"The particular gene ortholog described here was characterized as part of a developing dataset to study the evolution of the Insulin/insulin-like growth factor signaling pathway (IIS) across the genus *Drosophila*. The Insulin/insulin-like growth factor signaling pathway (IIS) is a highly conserved signaling pathway in animals and is central to mediating organismal responses to nutrients (Hietakangas and Cohen 2009; Grewal 2009)." (Myers et al., 2024).

"*D. ananassae* (NCBI:txid7217) is part of the *melanogaster* species group within the subgenus *Sophophora* of the genus *Drosophila* (Sturtevant 1939; Bock and Wheeler 1972). It was first described by Doleschall (1858). *D. ananassae* is circumtropical (Markow and O'Grady 2005; <https://www.taxodros.uzh.ch>, accessed 1 Feb 2023), and often associated with human settlement (Singh 2010). It has been extensively studied as a model for its cytogenetic and genetic characteristics, and in experimental evolution (Kikkawa 1938; Singh and Yadav 2015)." (Lawson et al., 2024).

We propose a gene model for the *Drosophila ananassae* ortholog of the *D. melanogaster* Protein tyrosine phosphatase 61F (*Ptp61F*) gene. The genomic region of the ortholog corresponds to the uncharacterized protein [XP_014764155.1](#) (Locus ID [LOC6492936](#)) in the May 2011 (Agencourt dana_caf1/DanaCAF1) Genome Assembly of *Drosophila ananassae* ([GCA_000005115.1](#), *Drosophila* 12 Genomes Consortium et al., 2007). This model is based on RNA-Seq data from *Drosophila ananassae* ([SRP006203](#), [SRP007906](#), [PRJNA257286](#), [PRJNA388952](#); Graveley et al., 2011) and *Ptp61F* in *D. melanogaster* using FlyBase release FB2023_03 ([GCA_000001215.4](#)) (Larkin et al., 2021; Gramates et al., 2022; Jenkins et al., 2022).

The protein product of *Ptp61F* (PTP61) negatively regulates the Insulin/TOR pathway by dephosphorylating the insulin receptor (InR) (Wu et al., 2011; Buszard et al., 2013). PTP61Fm-mediated dephosphorylation of InR requires an interaction with the SH2/SH3 adaptor protein Dock ([dreadlocks](#)) while PTP61Fn dephosphorylates InR in a Dock-independent manner (Clemens et al., 1996; Buszard et al., 2013; Willoughby et al., 2017). The *Ptp61F* gene in *Drosophila melanogaster* was first isolated using rat PTP61F cDNA in a low stringency hybridization screening method (McLaughlin and Dixon 1993). *Ptp61F* is differentially spliced where the longer isoform (PTP61Fm) is targeted to the ER by the hydrophobic C-terminal domain, and the shorter isoform (PTP61Fn) is targeted to the nucleus by a nuclear localization signal (NLS) (McLaughlin and Dixon 1993). PTP61F plays a role in the regulation of a variety of signaling pathways through negative regulation of tyrosine kinases including Janus kinase/signal transducers and activators of transcription (JAK/STAT), Mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (EGFR), and platelet-derived growth factor/vascular endothelial growth factor receptor (PVR) (Baeg et al., 2005; Buszard et al., 2013; Tchankou-Nguetcheu et al., 2014; Willoughby et al., 2017).

Synteny

The reference gene, *Ptp61F*, occurs on chromosome 3L in *D. melanogaster* and is flanked upstream by *rhomboid* (*rho*) and *Stromalin 2* (*SA2*) and downstream by *indra* and *Hydroxymethylbilane synthase* (*Hmbs*). There are also six genes nested within *Ptp61F* in *D. melanogaster*: [CG9173](#), [FucTD](#), [CG9168](#), [CG32320](#), [ru](#), and [CG9166](#). The *tblastn* search of *D. melanogaster* Ptp61F-PA (query) against the *Drosophila ananassae* (GenBank Accession: [GCA_000005115.1](#))

Genome Assembly (database) placed the putative ortholog of *Ptp61F* within scaffold_13337 at locus [LOC6492936](#) ([XP_014764155.1](#))— with an E-value of 7e-28 and a percent identity of 84.48%. Furthermore, the putative ortholog is flanked upstream by [LOC6492935](#) ([XP_001956685.1](#)) and [LOC6507090](#) ([XP_001956686.1](#)), which correspond to *Reg-2* and *CG9166* in *D. melanogaster* (E-value: 0.0 and 2e-140; identity: 89.62% and 83.55%, respectively, as determined by *blastp*) (Figure 1A; Altschul et al., 1990). The putative ortholog of *Ptp61F* is flanked downstream by [LOC6492937](#) ([XP_001956688.1](#)) and [LOC6507089](#) ([XP_001956689.1](#)), which correspond to *indra* and *Hmbs* in *D. melanogaster* (E-value: 2e-96 and 0.0; identity: 32.20% and 86.85%, respectively, as determined by *blastp*). The putative ortholog assignment for *Ptp61F* in *D. ananassae* is supported by the following evidence: The *blastp* and *tblastn* results support the presence of a *Ptp61F* ortholog in this location in *D. ananassae* although synteny is not completely conserved. In *D. melanogaster*, the Ptp61F-PC isoform has 6 genes nested within it, but this characteristic is not present in *D. ananassae*, so we hypothesize that the Ptp61F-PC isoform does not exist in this species. *CG9166* is present in both genomic neighborhoods, but in slightly different locations (i.e., not nested within *Ptp61F*), and the first and second downstream genes (*indra* and *Hmbs*) are orthologous. Therefore, we conclude that [LOC6492936](#) is the correct ortholog of *Ptp61F* in *D. ananassae* (Figure 1A).

Protein Model

Ptp61F in *D. melanogaster* has mRNA isoforms: Ptp61F-RA, Ptp61F-RB, Ptp61F-RC, Ptp61F-RD, and Ptp61F-RE. Ptp61F-RE and Ptp61F-RD have identical coding sequences. mRNA isoform Ptp61F-RA contains six CDSs, Ptp61F-RB and Ptp61F-RC have seven CDSs, and Ptp61F-RD and Ptp61F-RE have four CDSs. In *D. ananassae*, the isoform count is not conserved (see Special characteristics of the protein model), and we predict five total isoforms (Ptp61F-PA, Ptp61F-PB, Ptp61F-PD, Ptp61F-PE, and Ptp61F-PNF). The sequence of Ptp61F-PA in *Drosophila ananassae* has 76.87% identity (E-value: 0.0) with the protein-coding isoform Ptp61F-PA in *D. melanogaster*, as determined by *blastp* (Figure 1C). Given that *D. ananassae* and *D. melanogaster* belong to distinct species groups, the observed degree protein divergence is well explained and consistent with that seen in other ortholog comparisons between these two species (*Drosophila* 12 Genomes Consortium et al., 2007). Coordinates of this curated gene model are stored by NCBI at GenBank/BankIt (accessions [BK064622](#), [BK064623](#), [BK064624](#), [BK064625](#), and [BK064626](#)). These data are also archived in the CaltechDATA repository (see “Extended Data” section below).

Special characteristics of the protein model

We hypothesize that the isoform Ptp61F-PC does not exist in *D. ananassae*. In addition, we hypothesize that there is a new isoform, Ptp61F-PNF, due to alternative splicing. Ptp61F-PC in *D. melanogaster* has a long first intron that has six genes nested within it, but this does not appear in *D. ananassae* (Figure 1A). A *tblastn* search of the amino acid sequence of the first CDS in Ptp61F-PC in *D. melanogaster* against the *D. ananassae* genome did not return any results, and there are no predicted splice junctions consistent with a long first intron for Ptp61F-PC in this species. This leads us to conclude that the Ptp61F-PC isoform is not present in *D. ananassae*. Finally, we determined that [XM_032453921](#) ([LOC6492936](#)) is a novel isoform, as there is not an orthologous isoform in *D. melanogaster* that has the same gene structure. It has seven CDSs, and its sixth CDS is longer than the corresponding CDS in isoform Ptp61F-PB (Figure 1D). The seventh CDS of the proposed novel isoform is only eight base pairs long (including the stop codon), as compared to 37 base pairs long for the seventh CDS of isoform Ptp61F-RB. The sixth and seventh CDSs of this novel isoform are supported by a splice junction with a score of 457 (Figure 1D, shown in pink). These features of [XM_032453921](#) ([LOC6492936](#)) lead us to believe that new alternative splicing has occurred, resulting in a novel isoform. This novel isoform has 75.86% protein identity (E-value: 0.0) to the *D. melanogaster* Ptp61F-PB isoform. We propose that this isoform be named Ptp61F-PNF.

Methods

Detailed methods including algorithms, database versions, and citations for the complete annotation process can be found in Rele et al. (2023). Briefly, students use the GEP instance of the UCSC Genome Browser v.435 (<https://gander.wustl.edu>; Kent WJ et al., 2002; Navarro Gonzalez et al., 2021) to examine the genomic neighborhood of their reference IIS gene in the *D. melanogaster* genome assembly (Aug. 2014; BDGP Release 6 + ISO1 MT/dm6). Students then retrieve the protein sequence for the *D. melanogaster* reference gene for a given isoform and run it using *tblastn* against their target *Drosophila* species genome assembly on the NCBI BLAST server (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Altschul et al., 1990) to identify potential orthologs. To validate the potential ortholog, students compare the local genomic neighborhood of their potential ortholog with the genomic neighborhood of their reference gene in *D. melanogaster*. This local synteny analysis includes at minimum the two upstream and downstream genes relative to their putative ortholog. They also explore other sets of genomic evidence using multiple alignment tracks in the Genome Browser, including BLAT alignments of RefSeq Genes, Spaln alignment of *D. melanogaster* proteins, multiple gene prediction tracks (e.g., GeMoMa, Geneid, Augustus), and modENCODE RNA-Seq from the target species. Detailed explanation of how these lines of genomic evidence are leveraged by students in gene model development are described in Rele et al. (2023). Genomic structure information (e.g., CDSs, intron-exon number and boundaries, number of isoforms) for the *D. melanogaster* reference gene is retrieved through the Gene Record Finder (<https://gander.wustl.edu/~wilson/dmelgenerecord/index.html>; Rele et al., 2023). Approximate splice sites within the target gene are determined using *tblastn* using the CDSs from the *D. melanogaster* reference gene. Coordinates of CDSs

are then refined by examining aligned modENCODE RNA-Seq data, and by applying paradigms of molecular biology such as identifying canonical splice site sequences and ensuring the maintenance of an open reading frame across hypothesized splice sites. Students then confirm the biological validity of their target gene model using the Gene Model Checker (<https://gander.wustl.edu/~wilson/genechecker/index.html>; Rele et al., 2023), which compares the structure and translated sequence from their hypothesized target gene model against the *D. melanogaster* reference gene model. At least two independent models for a gene are generated by students under mentorship of their faculty course instructors. Those models are then reconciled by a third independent researcher mentored by the project leaders to produce the final model. Note: comparison of 5' and 3' UTR sequence information is not included in this GEP CURE protocol (Gruys et al., 2025).

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Extended Data

Description: Zip file containing FASTA, PEP, and GFF. Resource Type: Model. File: [DanaCAF1_Ptp62E.zip](#). DOI: [10.22002/7tpn6-87t55](https://doi.org/10.22002/7tpn6-87t55)

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