

## How do I update functional annotation in Apollo?

1. Log into VEuPathDB
2. From the gene record page select “View and update community annotations in Apollo”
3. With a right click on the gene in the Annotated Transcript track open the drop-down menu and select “Create new annotation > gene”
4. Right click on the gene in the User-created Annotations, select “Open Annotation”
5. The annotation panel is on the right-hand side. You can now add functional annotation, i.e. Symbol, description.
6. Select the tab Gene Product to add a product description with metadata. There are additional tabs to add Gene Ontology terms (the tab GO) and the tab DbXref to add publications.
7. Once you’ve added the functional annotation select from the Status drop-down menu “Finished”.

**ToxoDB**  
Release 52  
20 May 2021  
Toxoplasma Informatics Resources

**TGME49\_247520** hypothetical protein

Type: protein coding gene  
Chromosome: XII  
Location: TGME49\_chrXII:3,390,518..3,392,745(-)  
Species: *Toxoplasma gondii*  
Strain: ME49  
Status: Reference Strain

View 1 user comment, or add a comment  
View and update community annotations in Apollo

**Annotations** Tracks Ref Sequence Organism Users Groups Admin

Show All Show Visible Only

868deed5-89df-47c9-bc7f ☒ ID All Types GO GP Prov

Reference Sequence All Users All Statuses

Rows 25 1-1 of 1

Name	Seq	Type	Length	Updated
TGME49_247520-126_1	TGME49_chrXII gene	2,228	May 26, 2021	
TGME49_247520-126_1-00001	mRNA	2,228	May 26, 2021	

gene: TGME49\_247520-126\_1 Link to annotation Close(s)

**Details** GO Gene Product Provenance DbXref Comment Attributes

☒ Sync name with transcript ☐ Obsolete

Type gene Status No status selected

Name TGME49\_247520-126\_1

Symbol WIP

Aliases ("," separated)

Description

Location 3390518 - 3392745 strand(-) Partial: ☐ 3' ☐ 5'

Ref Sequence TGME49\_chrXII

Owner ucb.40063473

Created May 26, 2021 04:07 PM

Updated May 26, 2021 04:07 PM

**Add new Gene Product to TGME49\_247520-126\_1**

GO Annotation Guidance

Product Host wave complex interacting pr ☐ Alternate Evidence ECO:0000314 ☐ All ECO Evidence

IDA (ECO:0000314): direct assay evidence used in manual assertion Evidence Code Info

With Prefix ID + Add

Reference PMID 31600500 + Add

Note + Add

Save Cancel

**Details** GO Gene Product Provenance DbXref Comment Attributes

☒ Sync name with transcript ☐ Obsolete

Type gene Status ☒ No status selected ☐ Not Finished ☐ Finished ☐ Requires Curator

Name TGME49\_247520-126\_1

Symbol WIP

## How do I update structural annotation in Apollo?

1. Log into VEuPathDB
2. From the gene record page select “View and update community annotations in Apollo”
3. With a right click on the gene in the Annotated Transcript track open the drop-down menu and select “Create new annotation > gene”
4. Right click on the gene in the User-created Annotations, select “Open Annotation”
5. The annotation panel is on the right-hand side. Select the tab Tracks.
6. Select evidence tracks from Draggable Annotation
7. Drag and drop the evidence into the User-created Annotations area
8. With a right-click on the gene in the User-created Annotations area open the drop-down menu. There are options to merge exons, split and duplicate genes. Choose the modification as appropriate.
9. Once you’ve finished modifying the gene structure, choose the Annotations tab and select the status finished.

The screenshot displays the ToxoDB web interface for *Toxoplasma gondii* ME4. The main panel shows a genomic track with various annotations. A sidebar on the left contains navigation and filtering options. Numbered callouts (1-9) indicate the following steps:

- 1**: Select the gene track in the main panel.
- 2**: Click the 'Add' button in the sidebar to add a new annotation.
- 3**: Click 'Create new annotation' in the 'gene' dropdown menu.
- 4**: Click 'Open Annotation (alt-click)' to open the annotation editor.
- 5**: Click the 'Annotations' tab in the sidebar.
- 6**: Check the 'RNA-Seq Evidence for Introns Novel with Strong Evidence' checkbox in the 'Comparative Genomics' section.
- 7**: Click the 'Annotations' tab in the sidebar.
- 8**: Click 'Change annotation type' and select 'Merge' in the dropdown menu.
- 9**: Click 'Save' in the 'Details' panel to save the annotation.

## How do I know if my new gene structure is correct in Apollo?

1. Check if your gene has a Start and Stop site. You can do this by zooming in. Alternatively, right click on the gene to open the menu and select Get sequence. With this option you can recheck if there is a correct Start. You only need to check for correct start and stop sites if your gene does not have annotated UTRs.
2. Check splice sites. In most Eukaryotes the majority of splice sites at the exon/intron boundaries appear as 5'-...exon]GT/AG[exon...-3'. An exclamation mark in Apollo shows non-canonical splice sites.
3. Check if you can annotate UTRs, for example using RNA-Seq data. A useful track that could help with this is the "Combined RNA-Seq plot". This track combines all RNA-Seq plots available for your genome of interest. You can find this track by searching for "combine".
4. Check sequence similarity by running blast. Right click on the gene in the User-created Annotations area, open the drop down menu and select Get sequence. Copy the sequence to the clipboard and run blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The screenshot displays the Apollo genome browser interface with several key components:

- Top Panel:** Shows the reference sequence and user-created annotations for the gene *TGME49*. A right-click context menu is open over a gene model, showing options: "Get Sequence (meta-click)", "Get GFF3 (alt-meta-click)", and "Zoom to Base Level".
- Gene Model:** A track showing the gene structure with exons as boxes and introns as lines. A right-click context menu is also open over the gene model, showing the same three options.
- Sequence Window:** A window titled "Sequence" showing the nucleotide sequence of the gene. The sequence is:
 

```
>24a0283c-cf6e-4adc-b66f-4de6928f4e89 [sequence:exon] 245
residues [TGME49_chrX:3440383-3442160] [peptide]
MGNACKKNTAKTPTRKEADLAERKERQEREAKAEAEKARAEAEKNAADKAEAEERRAA
EAREERESARKEAEAEAEAEAEAEERLRKEAEKKKAEAEKRAEEQRAAEAEAEQ
RAREEAERRKAEAEAEAEAEAEAEERERQAEALQEEMSPREKYDKLASPEDSASETTMATQPKV
AEHSSAAVTDRTSVVGYTVTPCDMASIDETAKYLSKRCGCDLGDQHDENECPICRHIDLS
APLLN
```
- Annotations Panel:** A panel on the right showing various annotation tracks. The "combine" track is selected, and the "Transcriptomics" track is checked, showing the "TgondilME49 combined RNAseq plot".

## Which evidence code should be used when adding GO terms or gene products in Apollo?

1. IDA is used if the GO term/product has been experimentally verified
2. ISS: GO term/product is inferred from similarity. For GO annotations add GO\_REF:0000024 as reference. GO references describe the methods and procedures used in creating such annotations.
3. ISM: GO term/product is inferred from InterPro/Pfam domain. Add GO\_REF:000011 in the reference field for GO annotations.
4. IMP: GO term/product is inferred from mutant phenotype

Additional information on evidence codes can be found here:

<http://geneontology.org/docs/guide-go-evidence-codes>

A list of GO references can be found here:

<http://current.geneontology.org/metadata/gorefs/index.html>

The figure displays four screenshots of the Apollo GO Annotation Guidance interface, each illustrating a different evidence code and its associated fields. The interface is divided into sections for Aspect, Go Term, Relationship between Gene Product and GO Term, Evidence, With, Reference, and Note.

**1. IDA (ECO:0000314):** This screenshot shows the 'cellular component' aspect with the GO term 'apicoplast (GO:0020011)'. The relationship is 'part of'. The evidence code is 'ECO:0000314', which is highlighted. The 'With' section shows 'Prefix' and 'ID' fields. The 'Reference' section shows 'PMID' with the value '17449654'.

**2. ISS (ECO:0000250):** This screenshot shows the 'biological process' aspect with the GO term 'histone methylation (GO:0016571)'. The relationship is 'involved in'. The evidence code is 'ECO:0000250', which is highlighted. The 'With' section shows 'UniProtKB' and 'Q86X55'. The 'Reference' section shows 'GO\_REF' with the value '0000024'.

**3. ISM (ECO:0000255):** This screenshot shows the 'molecular function' aspect with the GO term 'nucleic acid binding (GO:0003676)'. The relationship is 'enables'. The evidence code is 'ECO:0000255', which is highlighted. The 'With' section shows 'InterPro' and 'IPR003029'. The 'Reference' section shows 'GO\_REF' with the value '0000011'.

**4. IMP (ECO:0000315):** This screenshot shows the 'biological process' aspect with the GO term 'response to drug (GO:0042493)'. The relationship is 'involved in'. The evidence code is 'ECO:0000315', which is highlighted. The 'With' section shows 'Prefix' and 'ID' fields. The 'Reference' section shows 'PMID' with the value '24352242'.