

Structural annotation in Apollo

Modifying a gene model

In this short tutorial we are showing you step-by-step how to modify an existing gene model in Apollo. Modifying a gene model can include adding additional exons, extending exons, deleting exons, splitting exons and adding UTRs.

1) Accessing Apollo

To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo (1)**. You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo (2)**. Alternatively, go to the **Tools** menu and choose Apollo from the drop-down list (3).

The screenshot shows the ToxoDB gene record page for TGME49_305150. At the top, there's a navigation bar with links for 'My Strategies', 'Searcher', 'Tools' (circled with a red box), 'View', 'Data', 'About', 'Help', and 'Contact Us'. Below the navigation bar, there's a site search bar and a green banner for VEuPathDB. The main content area displays gene details: Type: protein coding gene, Chromosome: IX, Location: TGME49_chrlX:5,245,620..5,257,794(+), Species: *Toxoplasma gondii*, Strain: ME49, Status: Reference Genome. There's a section for 'Add the first user comment' and a link to 'View and update community annotations in Apollo' (circled with a red box). Below this, there's a 'Shortcuts' section with links to Synteny, Alignments, Phenotype, SNPs, Transcriptomics, Protein Features, and Proteomics. A note says 'Also see TGME49_305150 in the Genome Browser or Protein Browser'. Under the 'Gene models' heading (circled with a red box), there's a table showing 13 exons, 1 transcript, and a note about community annotation. At the bottom right, there's a 'Collapse all sections for better performance' button and a 'View in JBrowse genome browser' button. The 'Annotate in Apollo' button is highlighted with a red box (2).

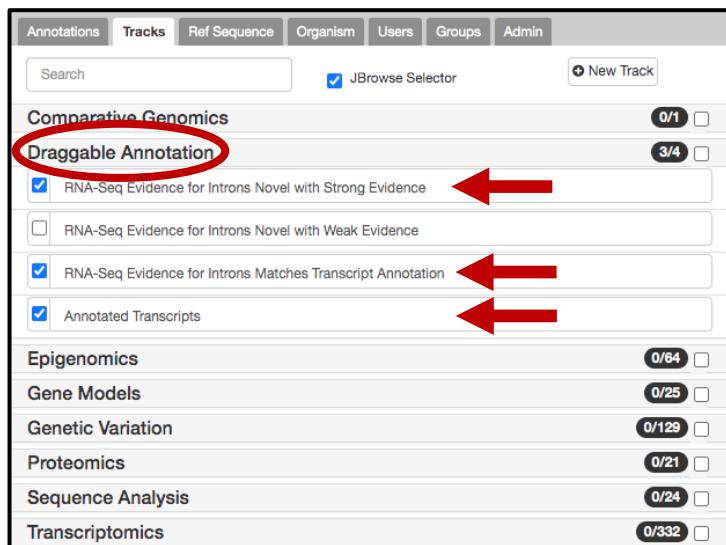
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

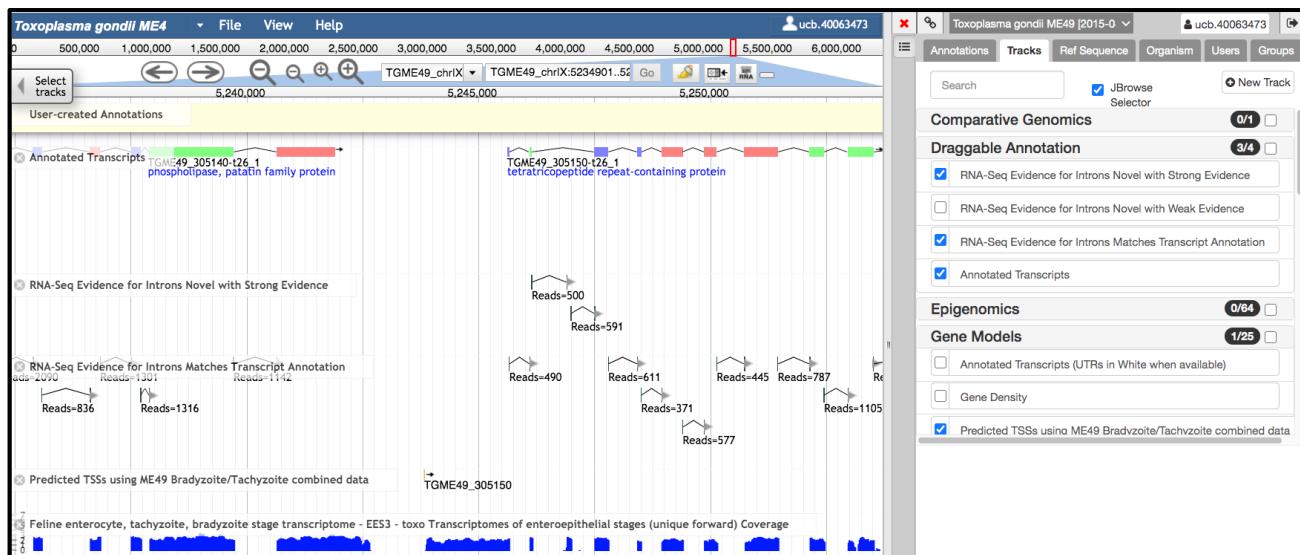
Select on the right-hand side the tab **Tracks**.

The screenshot shows the JBrowse interface for the Toxoplasma gondii ME49 genome. The top navigation bar includes 'File', 'View', 'Help', and a user profile. The main area shows a genomic track for chromosome IX, with a scale from 0 to 6,000,000. Below the track, there are buttons for 'Select tracks', 'Go', and various search functions. On the right, there's a panel with tabs for 'Annotations' (highlighted with a red box) and 'Tracks' (also highlighted with a red box). The 'Annotations' tab shows a table for 'Comparative Genomics' (0/1) and 'Draggable Annotation' (0/4). Other tabs in the panel include 'Ref Sequence', 'Organism', 'Users', 'Groups', and 'Admin'.

Click on the menu item **Draggable Annotation** select **Annotated Transcripts, RNA-Seq Evidence for Introns Novel with Strong Evidence and RNA-Seq Evidence for Introns Matches Transcript Annotation.**

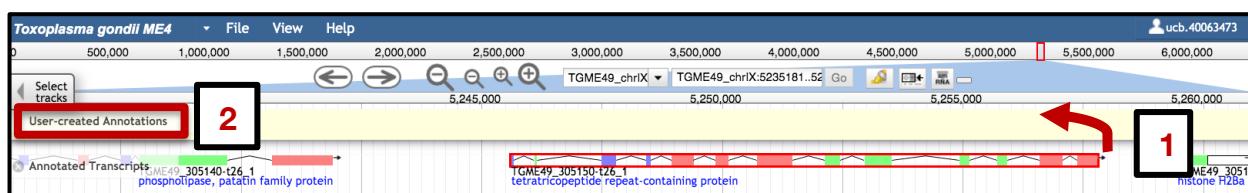


Select additional evidence, i.e. RNAseq plots or predicted TSS (transcription start sites).

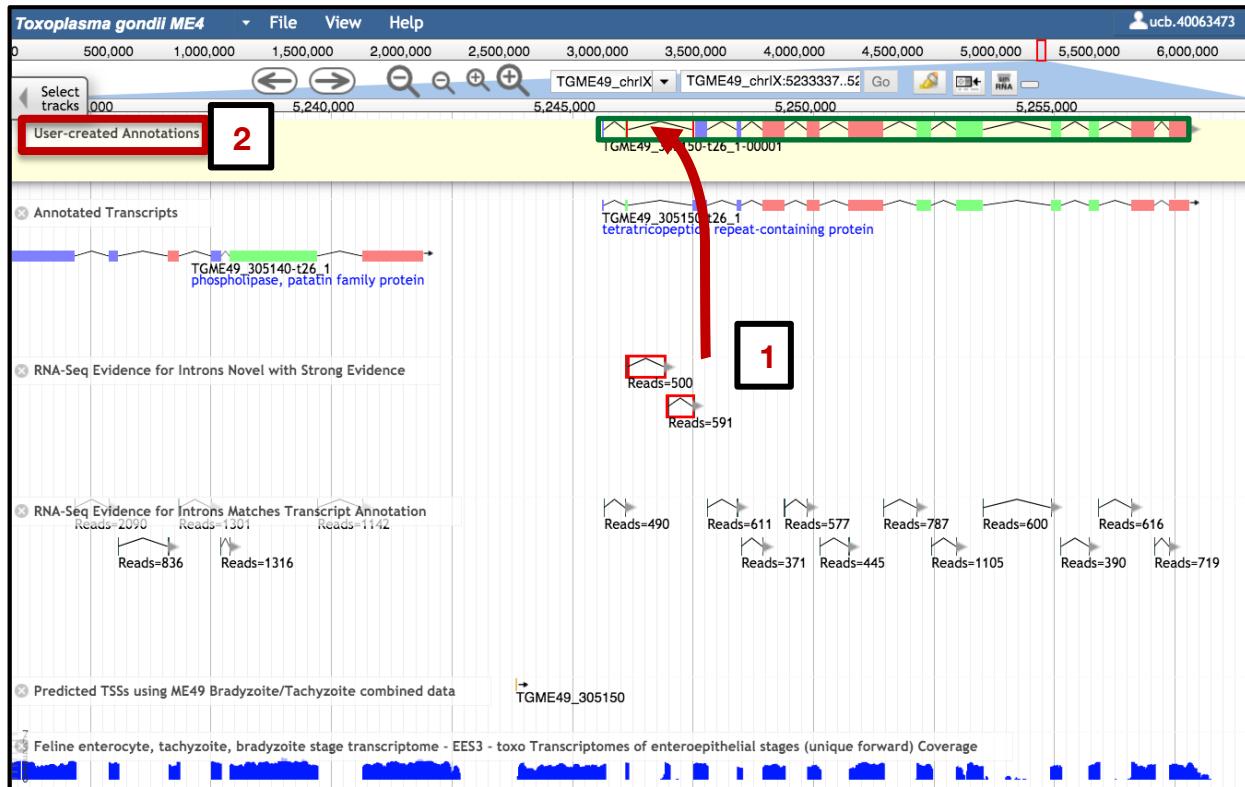


3) Modifying the gene

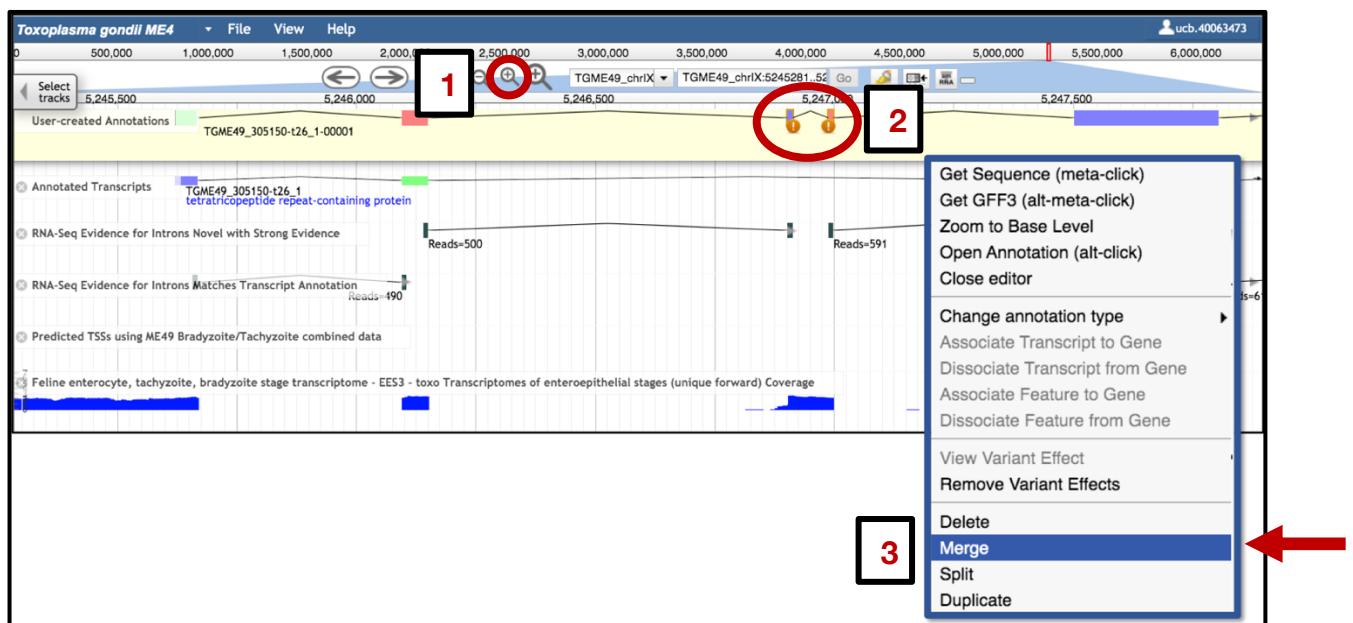
Select the gene model by clicking on one of the introns or by double clicking on the gene model (1). The gene model will show up with red boundaries. Drag and drop the gene into the User-created Annotations track (2). **Please note:** To add one-exon genes into the User-created Annotations area you need to double-click on the gene and then drag it into the user-created annotations area.



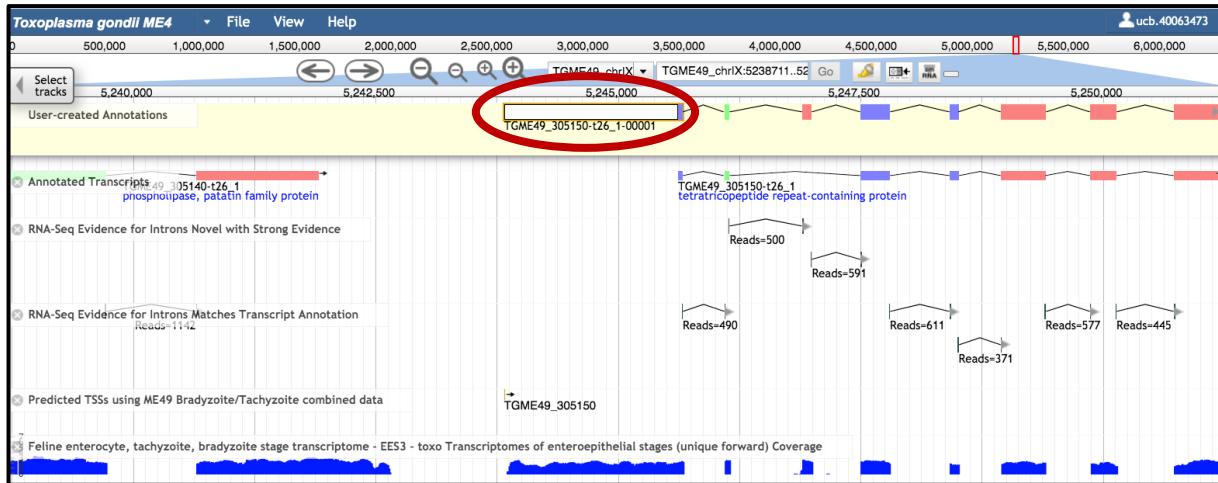
You can either select the intron junctions individually, or hold down the shift key and select both intron junctions with strong evidence (1), drag and drop them into the gene model (2). The gene will get a green box when dragging and dropping the intron evidence.



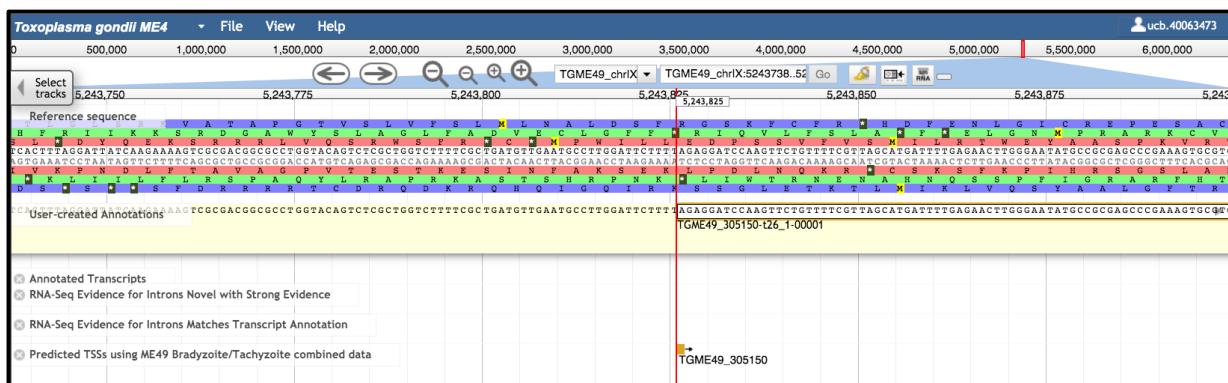
Zoom in by clicking on the + sign on the top (1). Press the Shift key and select the two small exons in the middle (2). With a right-click open the drop-down menu and choose **Merge** (3). Alternatively, select one of the exons you would like to merge, go to the edge of the feature until a little arrow appears and extend the exon until it overlaps with the second exon.



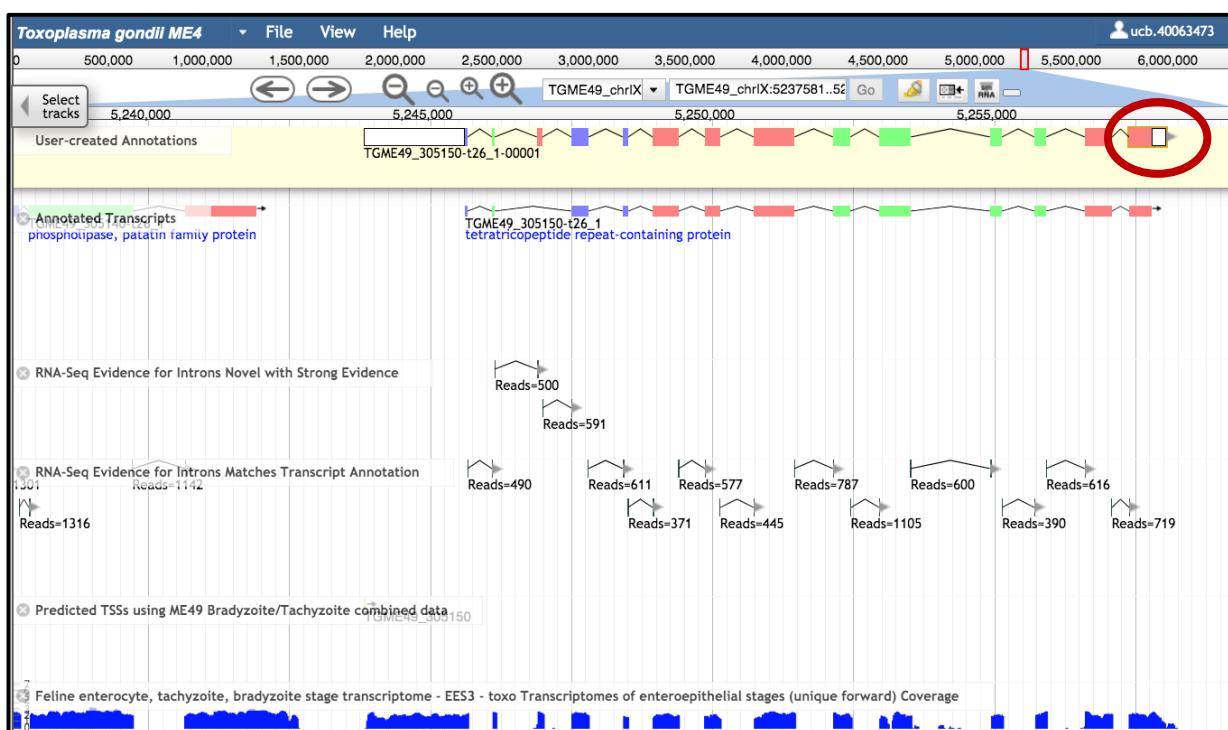
Select the first exon, point your mouse at the edge of the feature, a little arrow will appear, then extend the exon to the transcription start. Apollo will automatically create the 5'UTR!



You can zoom in to recheck the transcription start site.

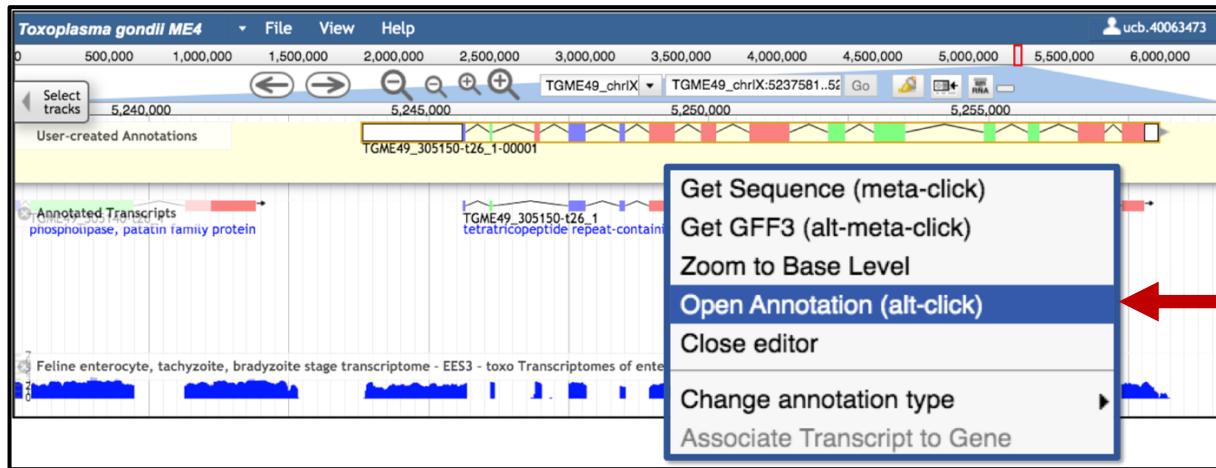


Select the last exon, point your mouse at the edge and extend the exon. Apollo will create the 3'UTR automatically!

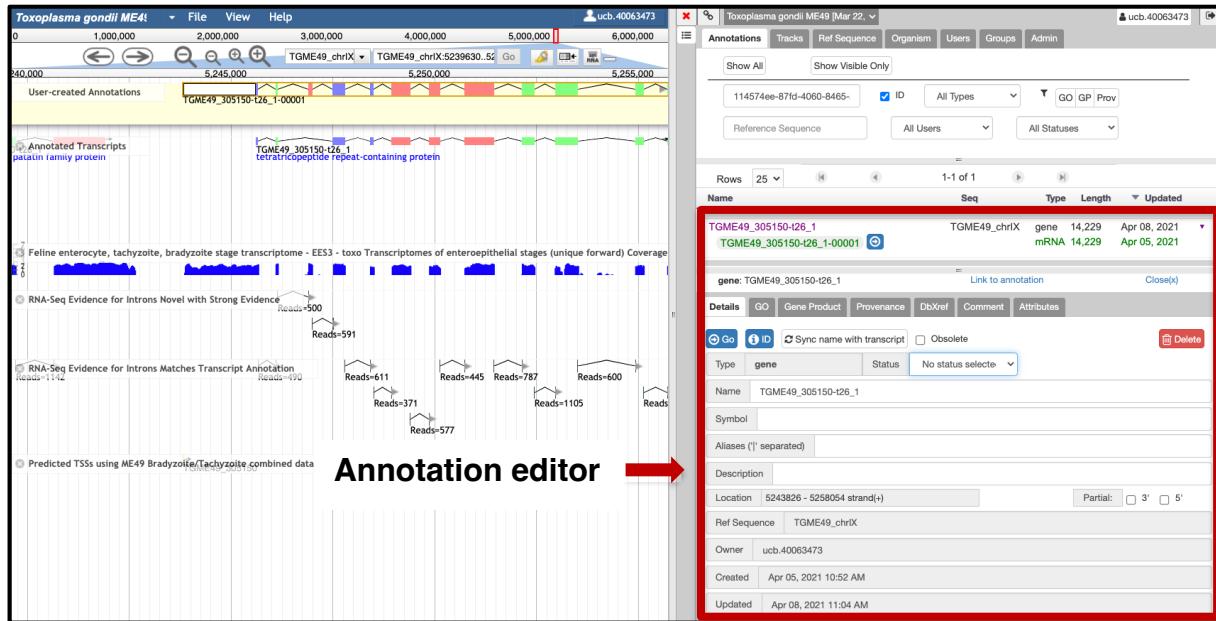


4) Opening of the Annotation editor window

Select the gene in the User-created Annotation track and with a right-click open the drop-down menu and choose **Open Annotation**. Alternatively, you can use the short-cut **alt-click**.

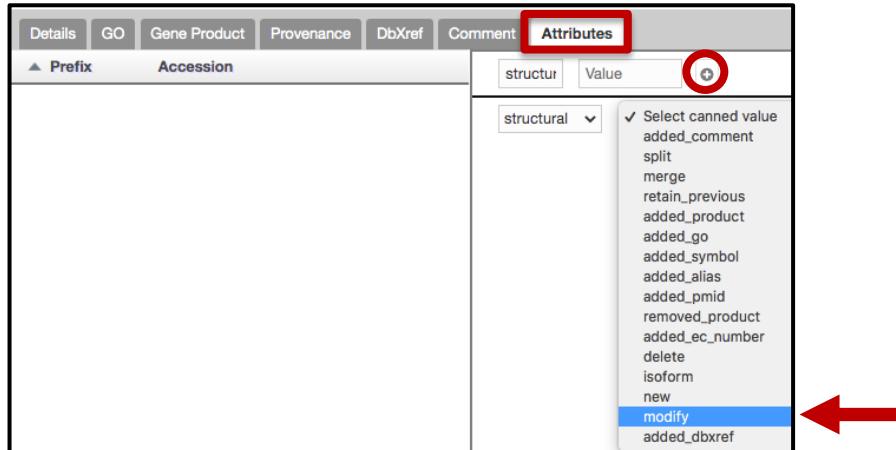


The annotation editor window is now shown on the right-hand side.



5) Finalizing the structural annotation

Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **modify**. Click on the + sign.



If there is no change in the functional annotation choose from the canned tag - **annotation** and from the canned value in the Attributes section **retain previous**. Click on the + sign.

Finally go the Details tab and select the status **Finished** on the gene.

The following day, the corrected gene model is visible on the gene record page in the Community annotations from Apollo track.

Done! For additional questions, please get in touch with the VEuPathDB help desk.

Structural annotation in Apollo Merging/Splitting gene models

In this short tutorial we are showing you step-by-step how to merge/split gene models in Apollo.

1) Accessing Apollo

To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo (1)**. You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo (2)**. Alternatively, go to the **Tools** menu and choose Apollo from the drop-down list (3).

The screenshot shows the ToxoDB gene record page for *TGME49_319312*, a hypothetical protein. The top navigation bar includes links for My Strategies, Searcher, Tools (circled in red), My Works, Data, About, Help, and Contact Us. A guest user is logged in. Below the navigation is a search bar and a green banner for VEuPathDB. The main content area displays gene details: Type: protein coding gene, Chromosome: IV, Location: *TGME49_chrlV:1,041,310..1,050,287(+)*, Species: *Toxoplasma gondii*, Strain: ME49, and Status: Reference Strain. A red circle highlights the "View and update community annotations in Apollo" link. A red box highlights the "Annotate in Apollo" button in the gene models section. A white box highlights the "Tools" menu item in the top navigation bar. The gene models section shows 1 Gene model with 9 exons and 1 transcript. A note indicates the gene is available for community annotation in Apollo.

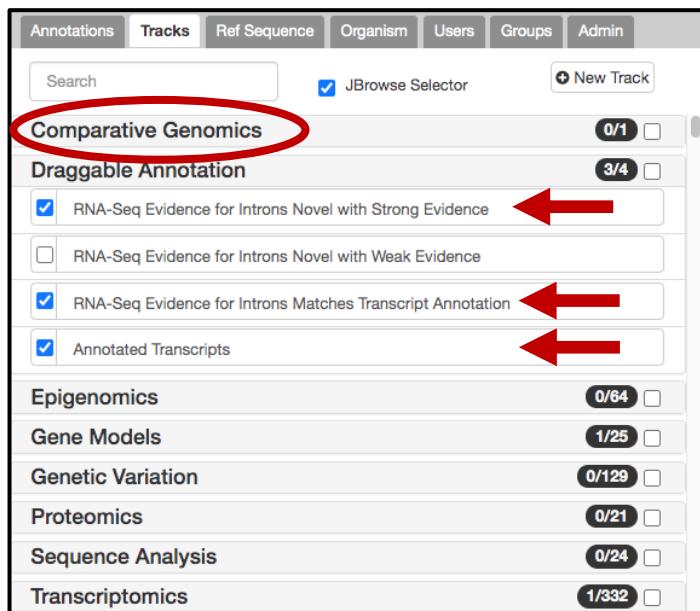
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

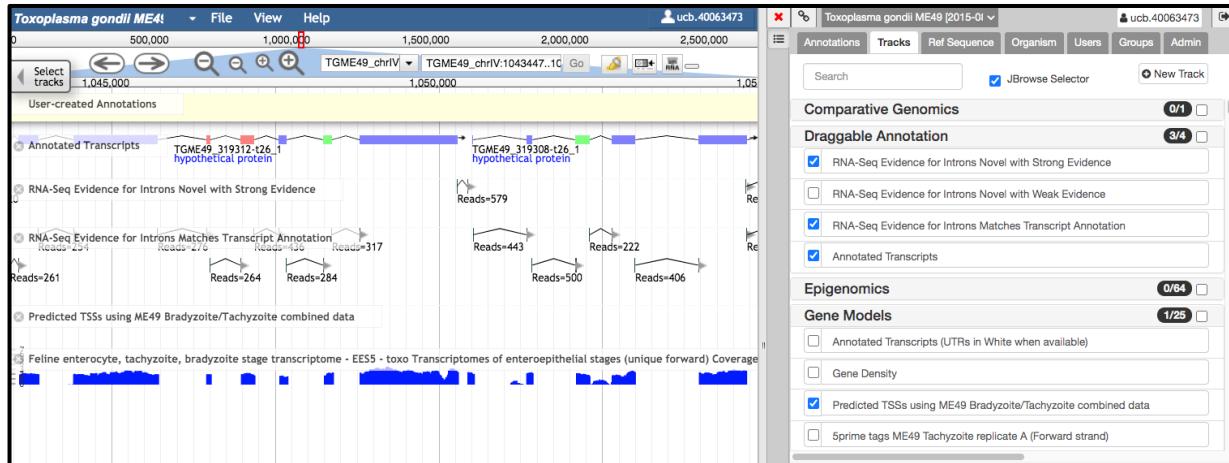
Select on the right-hand side the tab **Tracks**.



Click on the menu item **Draggable Annotation** select **Annotated Transcripts, RNA-Seq Evidence for Introns Novel with Strong Evidence and RNA-Seq Evidence for Introns Matches Transcript Annotation.**



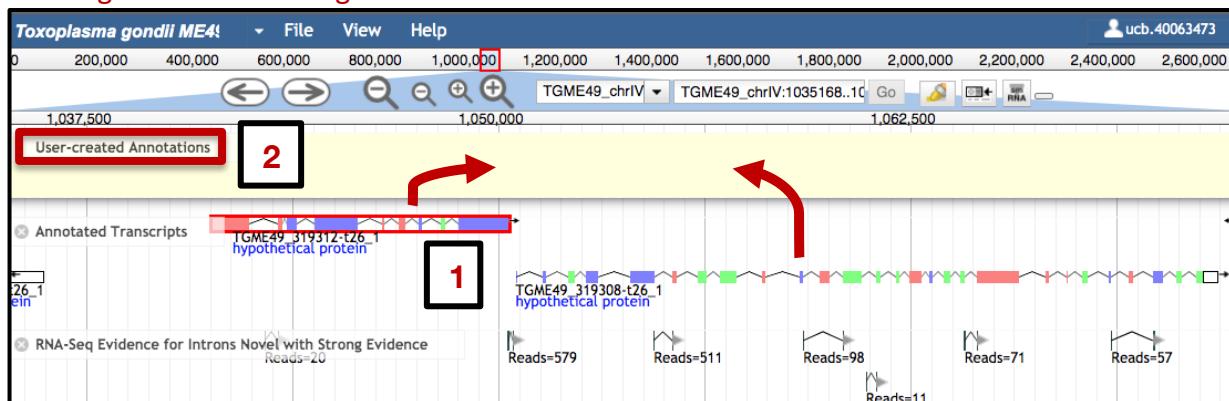
Select additional evidence, i.e. RNAseq plots and predicted TSS (transcription start sites).



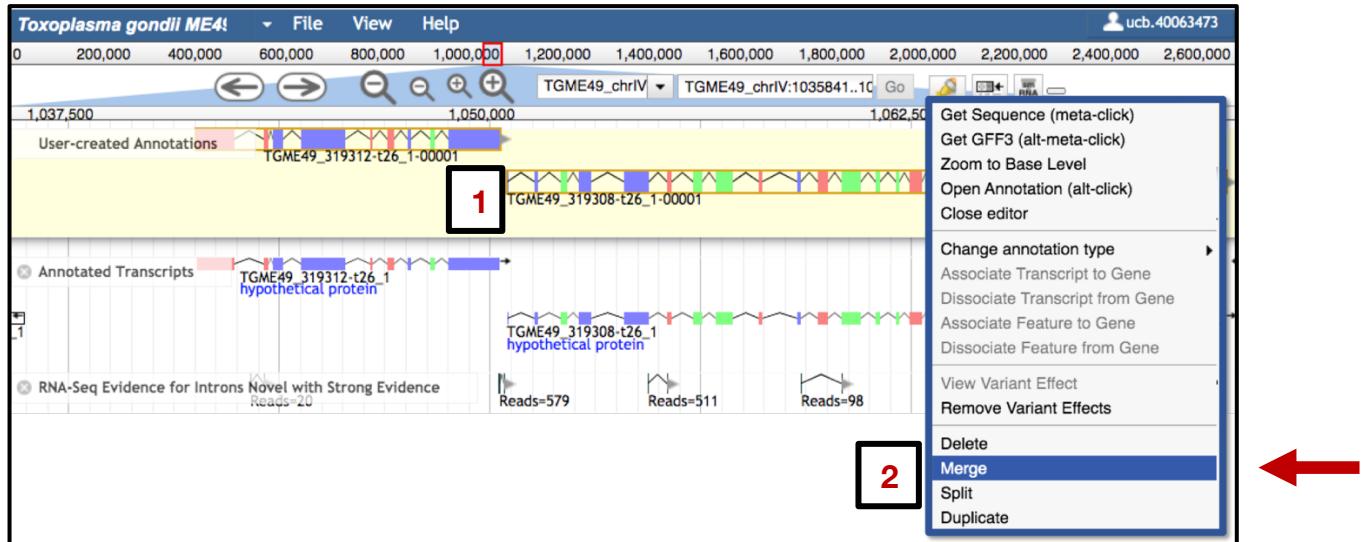
3) Merging genes

Select the gene models that you would like to merge by clicking on one of the introns or by double clicking on the gene model (1). Drag and drop the genes into the User-created Annotations track (2).

Please note: To add one-exon genes into the User-created Annotations area you need to **double-click** on the gene and then drag it into the user-created annotations area.

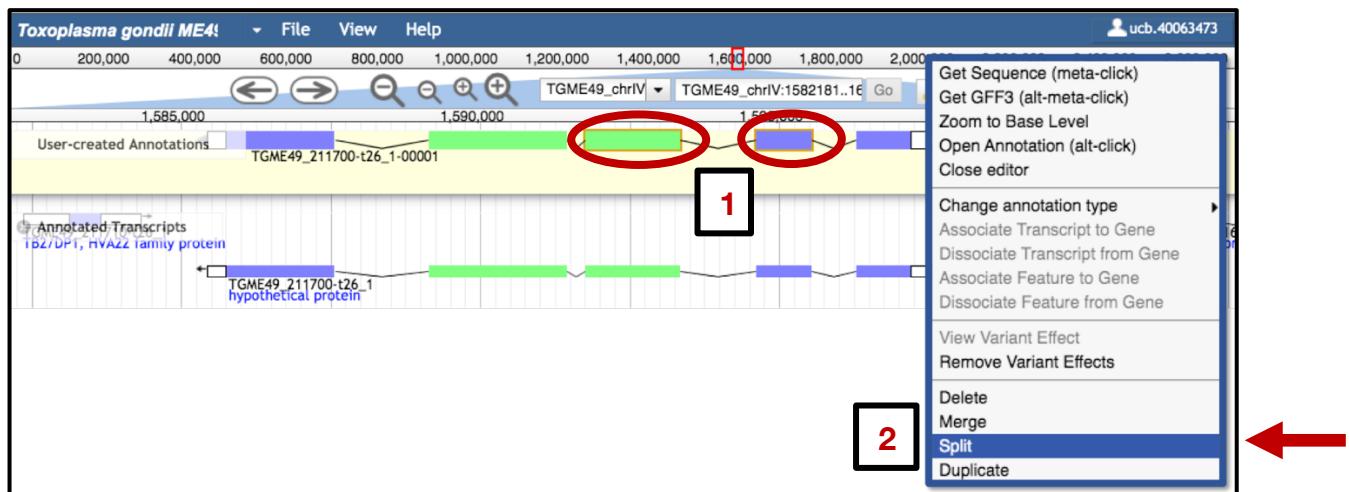


Hold down the shift key and select both gene models in the User-created Annotations track (1). With a right-click open the drop-down menu and choose **Merge** (2).



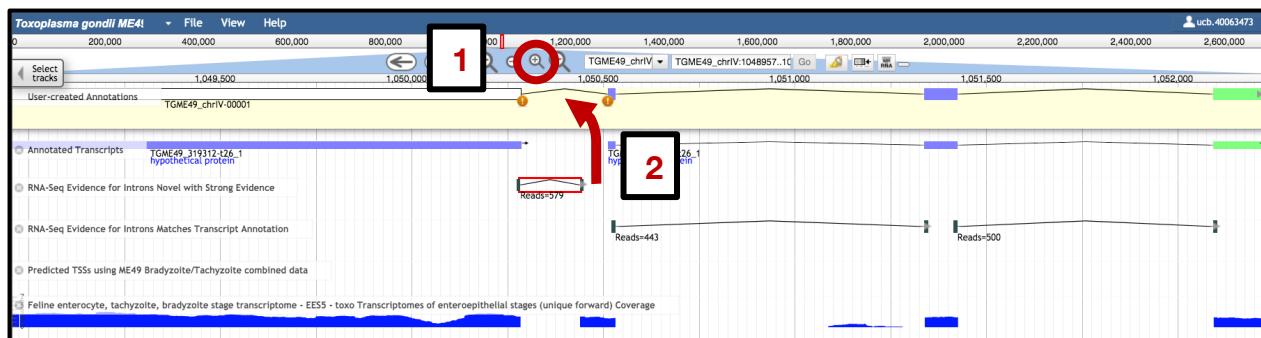
3.1) Splitting gene models

For splitting gene models, select the exons that border the intron that should be split. With a right-click open the annotation drop-down menu and choose split. Once you've split the gene model, recheck if the gene model has the correct start and stop.

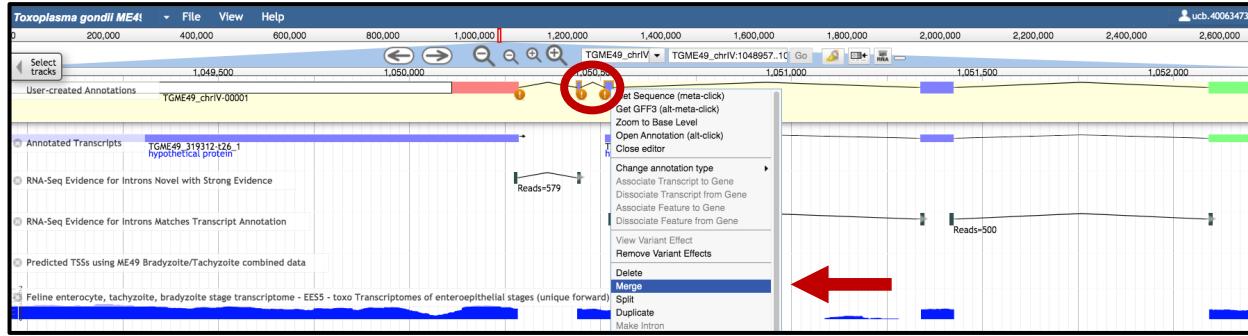


4) Correcting intron-exon boundaries

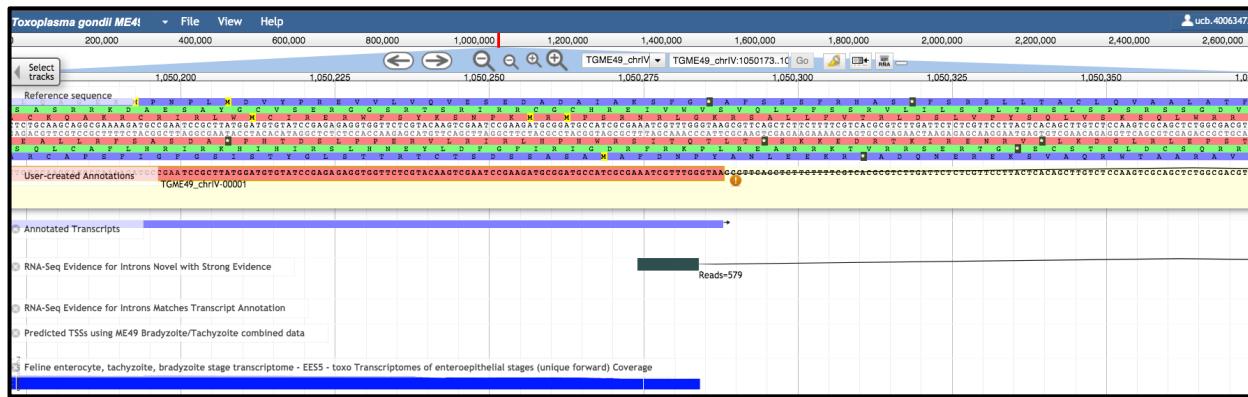
Once you've merged the gene zoom in by clicking on the + sign on the top (1). Select the new splice junction and drag it into the gene model (2).



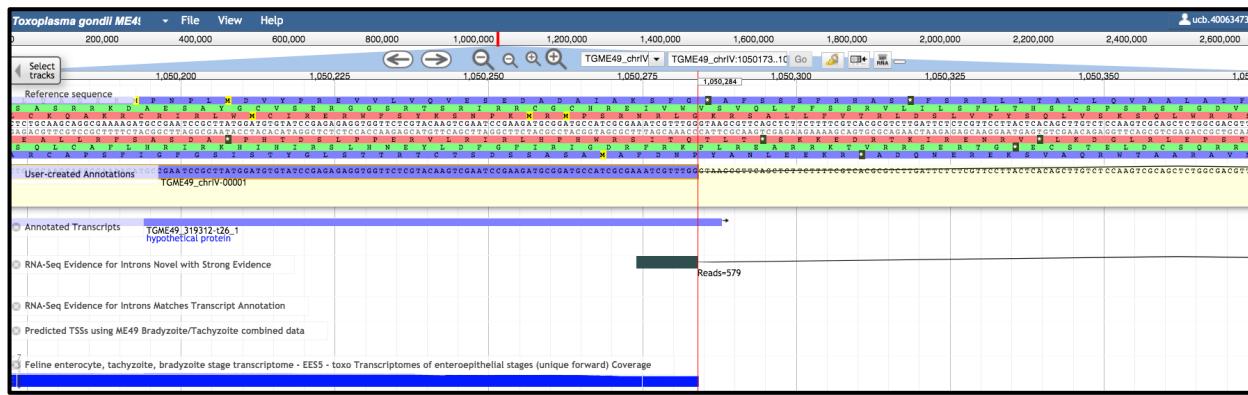
Hold down the shift key and select the two small exons. With a right-click open the drop-down menu and select merge. Hint: The exclamation mark tells you that there are non-canonical splice sites.



Zoom in, select the exon on the left side, point your mouse at the edge of the exon, a little arrow will appear.

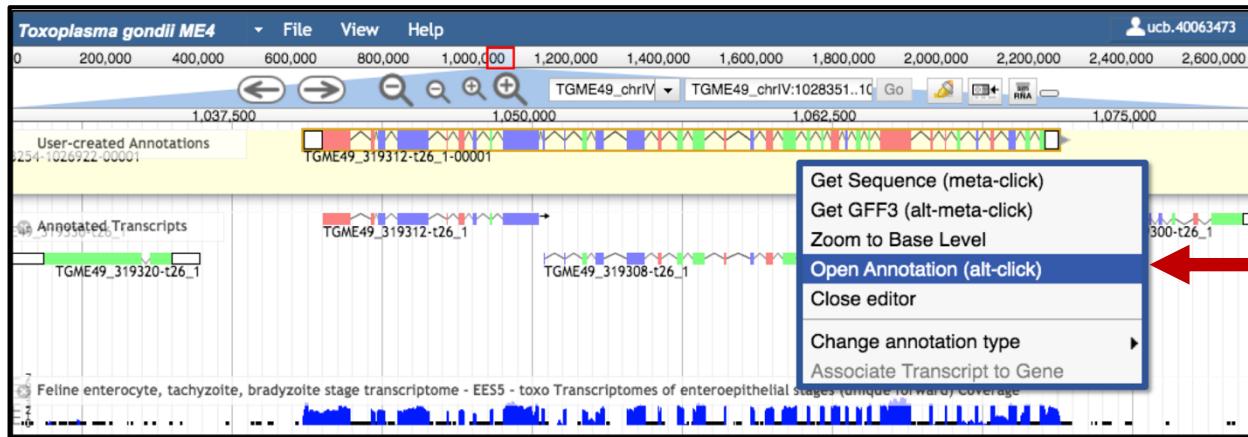


Shorten the exon to the correct splice site.

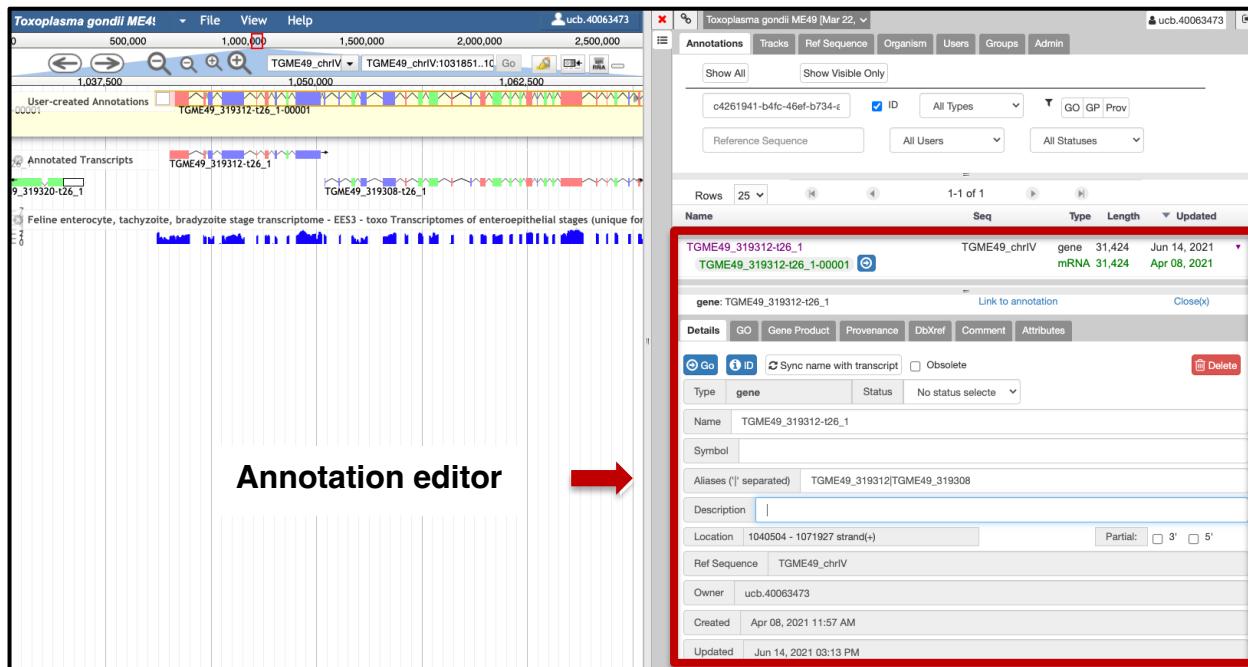


5) Opening of the Annotation editor window

Select the gene in the User-created Annotation track and with a right-click open the drop-down menu and choose **Open Annotation**. Alternatively, you can use the short-cut **alt-click**.

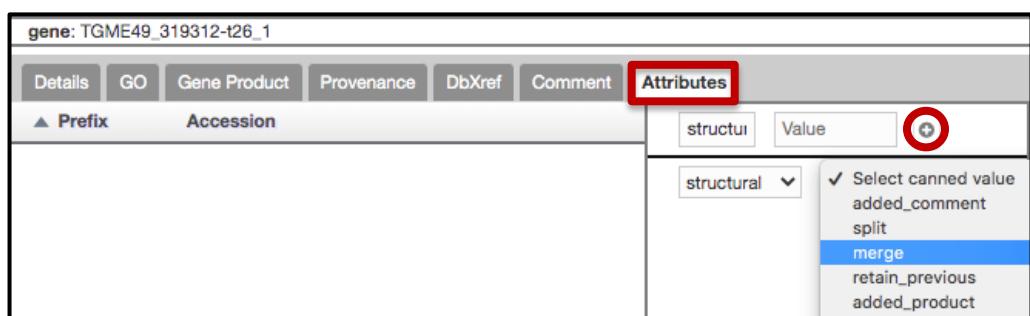


The annotation editor window is now shown on the right-hand side.

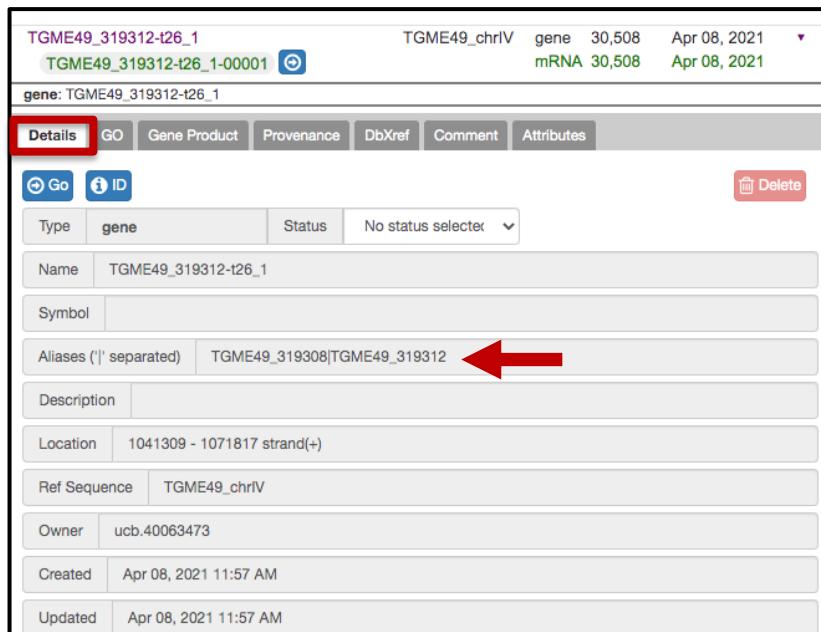


6) Finalizing the structural annotation

Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **merge**. Click on the + sign.



Open the Details section and add the gene IDs that you've merged in the Aliases section. Ideally, also add a Description/Gene Product.



TGME49_319312-t26_1 TGME49_chrlV gene 30,508 Apr 08, 2021
TGME49_319312-t26_1-00001

gene: TGME49_319312-t26_1

Details GO Gene Product Provenance DbXref Comment Attributes

Type gene Status No status selected

Name TGME49_319312-t26_1

Symbol

Aliases ('|' separated) TGME49_319308|TGME49_319312

Description

Location 1041309 - 1071817 strand(+)

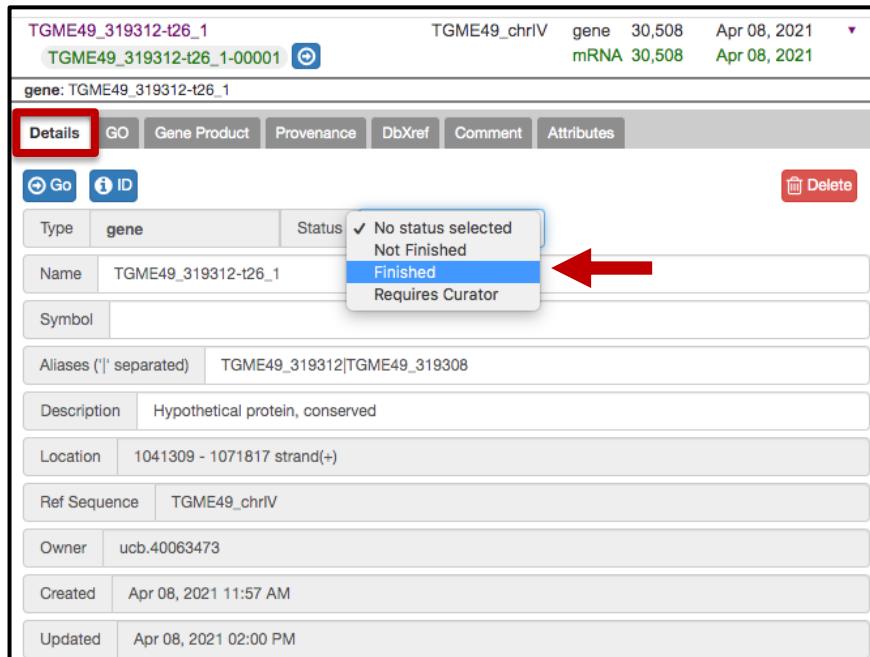
Ref Sequence TGME49_chrlV

Owner ucb.40063473

Created Apr 08, 2021 11:57 AM

Updated Apr 08, 2021 11:57 AM

To finalize the annotation select the status **Finished** on the gene. The following day, the corrected gene model will be visible on the gene record page in the Community annotations from Apollo track.



TGME49_319312-t26_1 TGME49_chrlV gene 30,508 Apr 08, 2021
TGME49_319312-t26_1-00001

gene: TGME49_319312-t26_1

Details GO Gene Product Provenance DbXref Comment Attributes

Type gene Status

No status selected

Not Finished

Finished

Requires Curator

Name TGME49_319312-t26_1

Symbol

Aliases ('|' separated) TGME49_319312|TGME49_319308

Description Hypothetical protein, conserved

Location 1041309 - 1071817 strand(+)

Ref Sequence TGME49_chrlV

Owner ucb.40063473

Created Apr 08, 2021 11:57 AM

Updated Apr 08, 2021 02:00 PM

Done! For additional questions, please get in touch with the VEuPathDB help desk.

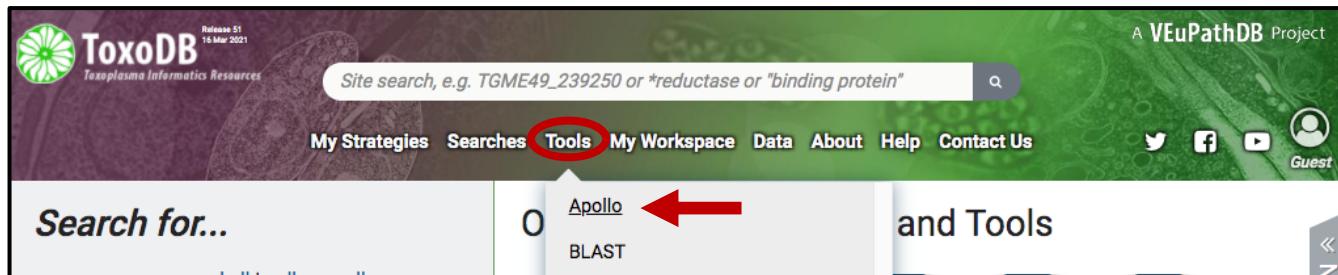
Structural annotation in Apollo

Adding a new gene

In this short tutorial we are showing you step-by-step how to add a new gene in Apollo.

1) Accessing Apollo

To access Apollo select **Tools** from the top menu and choose **Apollo** from the drop-down menu.



Click on the button **Go to Apollo**.

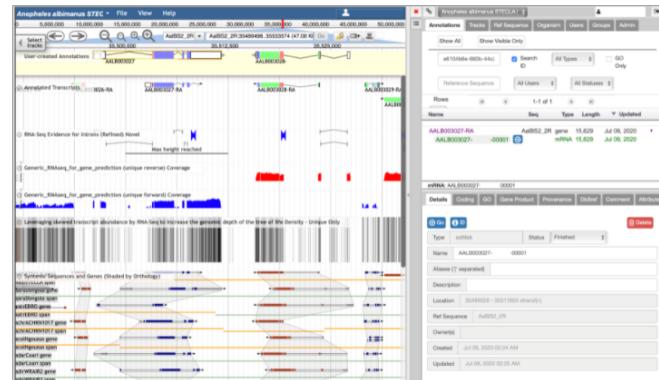
Structural and functional community curation in Apollo

Welcome to the VEuPathDB Apollo service (Dunn et al. 2019), a real time collaborative genome annotation and curation platform.

Use Apollo to integrate new or update current structural and functional data, for gene models in the organisms available in VEuPathDB. Organisms in AmoebaDB, CryptoDB, FungiDB, GiardiaDB, MicrosporidiaDB, PiroplasmaDB, PlasmoDB, ToxoDB, TrichDB, TriTrypDB & VectorBase are available for community curation.

Apollo help and documentation:

- A [sandbox](#) is available for you to get familiar with all Apollo menus, tools, and tracks before you decide to use it for your real gene manual annotations. These changes will not affect any of the organism's official gene set, neither will be preserved.
- Comprehensive webinar to learn [how to use Apollo](#) (57:40 min)
- [Quick commands](#)
- [Functional annotation tutorial](#)
- [About Apollo \(Login required\)](#)
- [User Guide](#)
- [Request feature/Report a bug](#)
- [Powered by JBrowse](#)
- [Web Service API \(Login required\)](#)

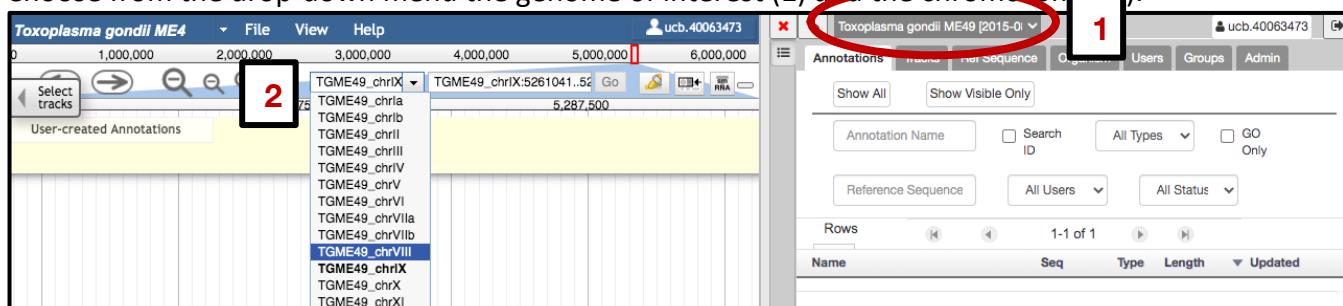


[Go to Apollo](#)

To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Navigate to the genome and chromosome coordinates

Choose from the drop-down menu the genome of interest (1) and the chromosome (2).

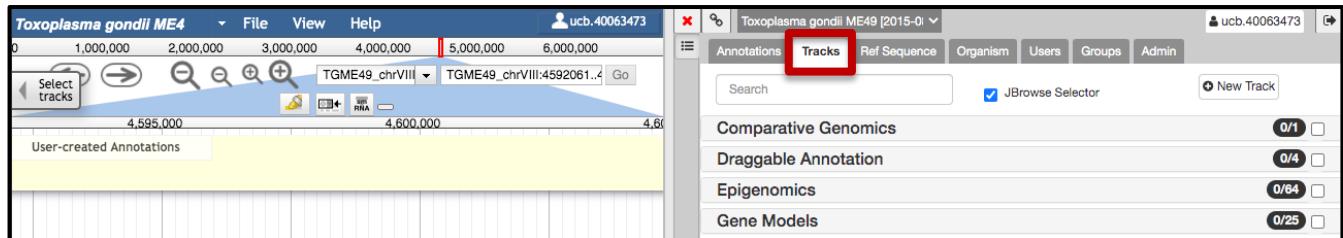


Go to the coordinates with the missing gene. Use the arrows to navigate to the coordinates (1), or type the coordinates in the search box (2).

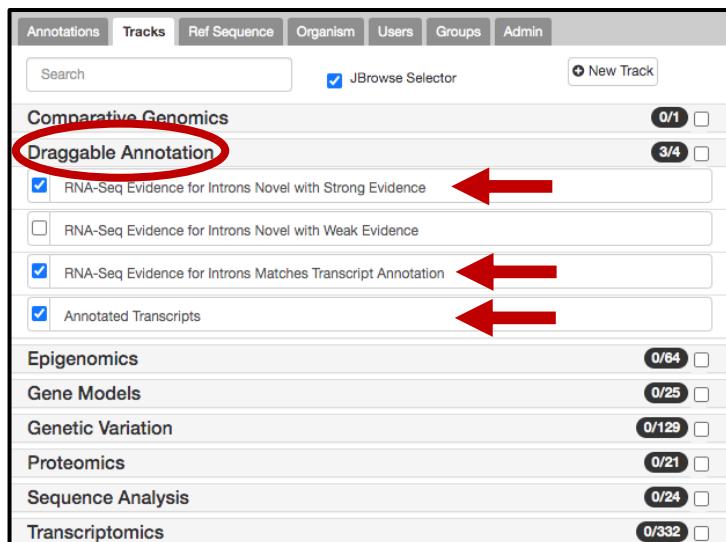


3) Adding draggable annotation and supporting evidence

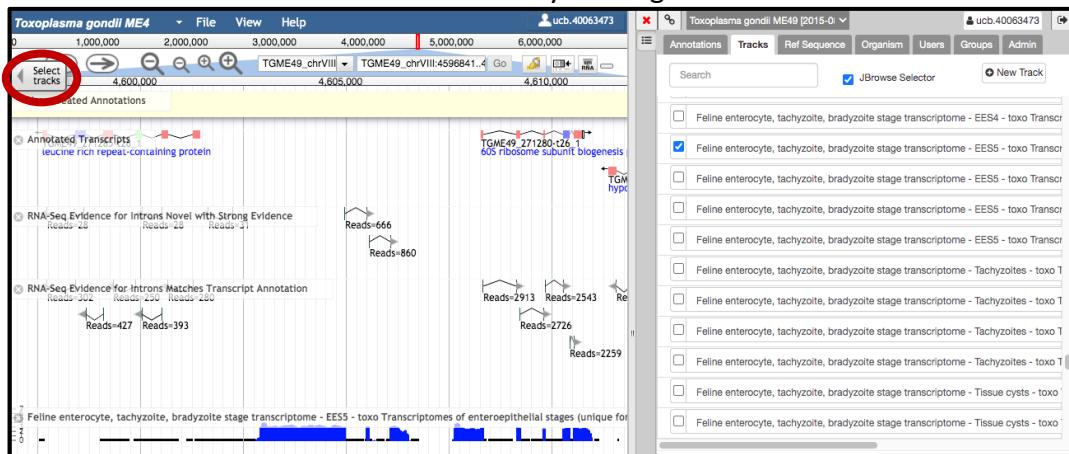
Select on the right-hand side the tab **Tracks**.



Click on the menu item **Draggable Annotation** select **Annotated Transcripts, RNA-Seq Evidence for Introns Novel with Strong Evidence and RNA-Seq Evidence for Introns Matches Transcript Annotation**.

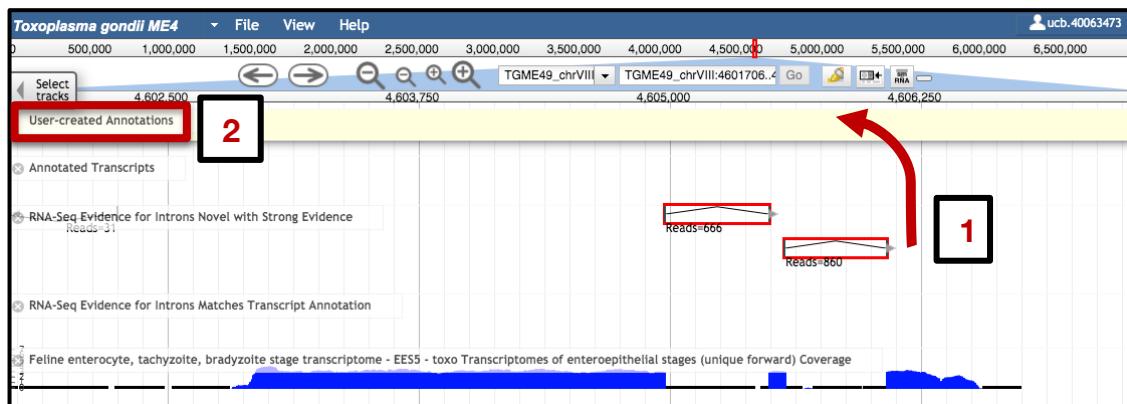


Select additional evidence, i.e. RNAseq plots from the Transcriptomics section. Alternatively, you can select evidence from the JBrowse menu by clicking on **Select tracks**.

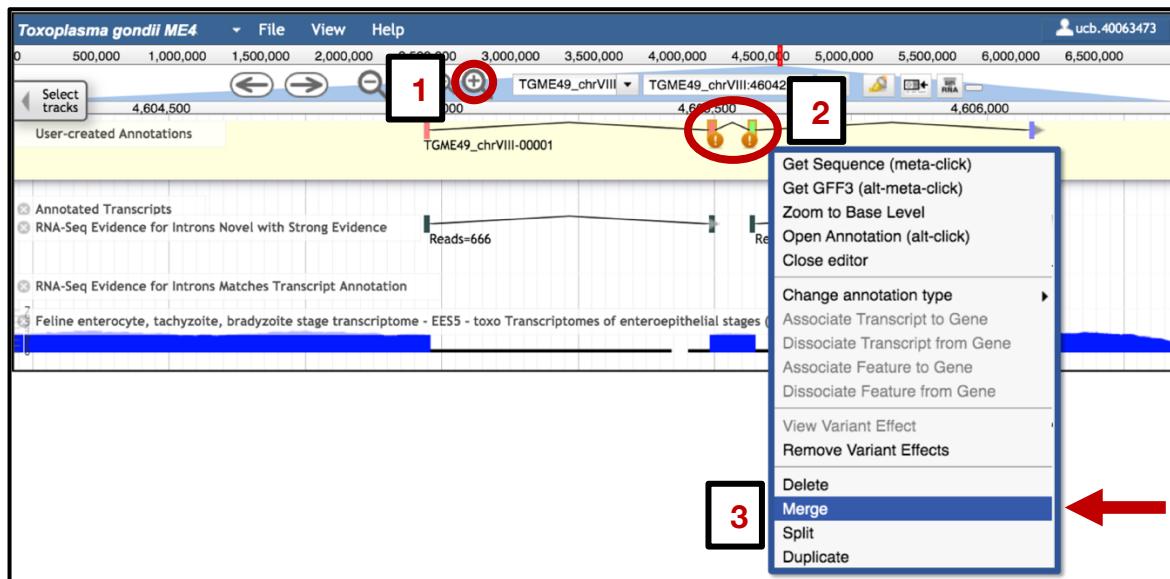


4) Building the new gene

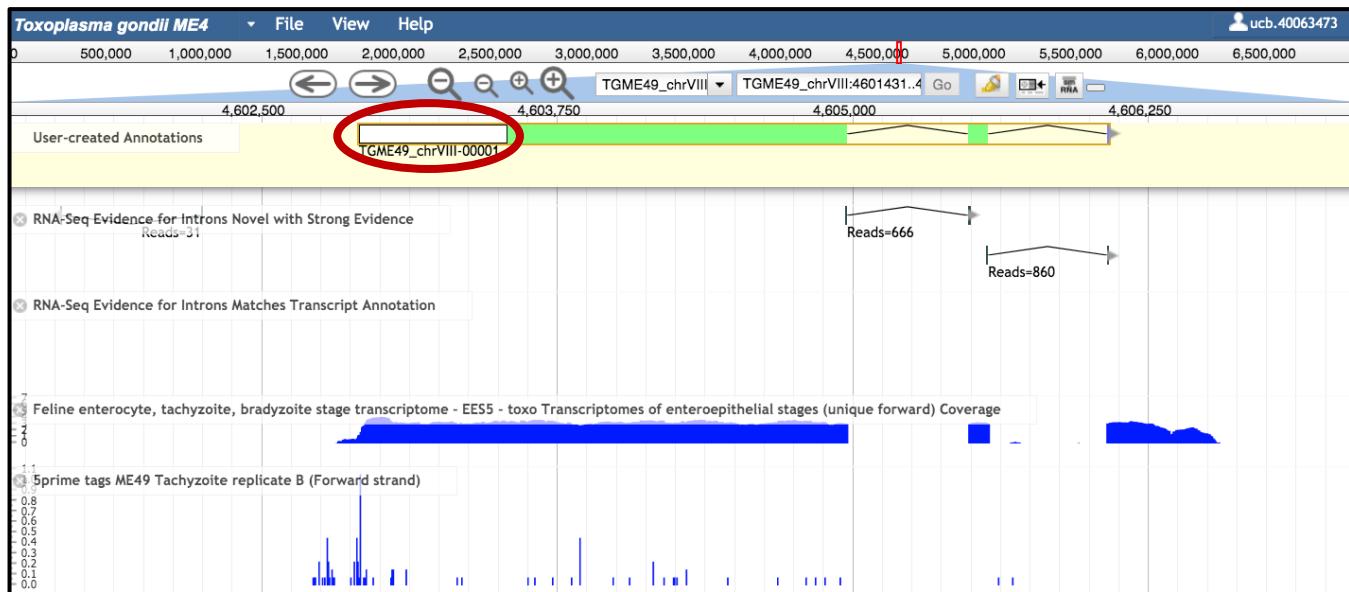
Hold down the shift key and select the introns with strong evidence (1), drag and drop them into the User-created Annotations track (2).



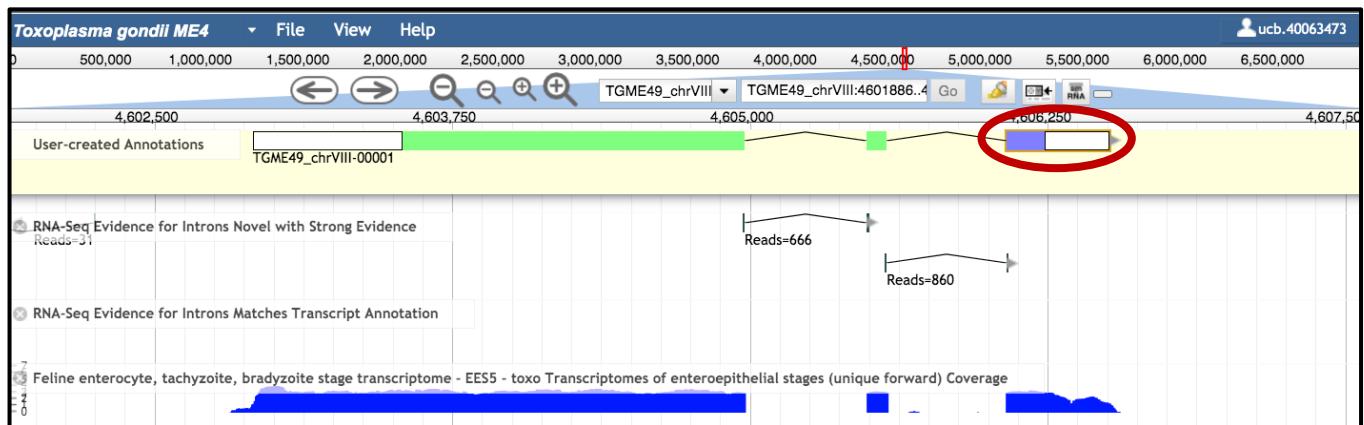
Zoom in by clicking on the + sign on the top (1). Press the Shift key and select the two small exons in the middle (2). With a right-click open the drop-down menu and choose **Merge** (3). Alternatively, select one of the exons you would like to merge, go to the edge of the feature until a little arrow appears and extend the exon until it overlaps with the second exon.



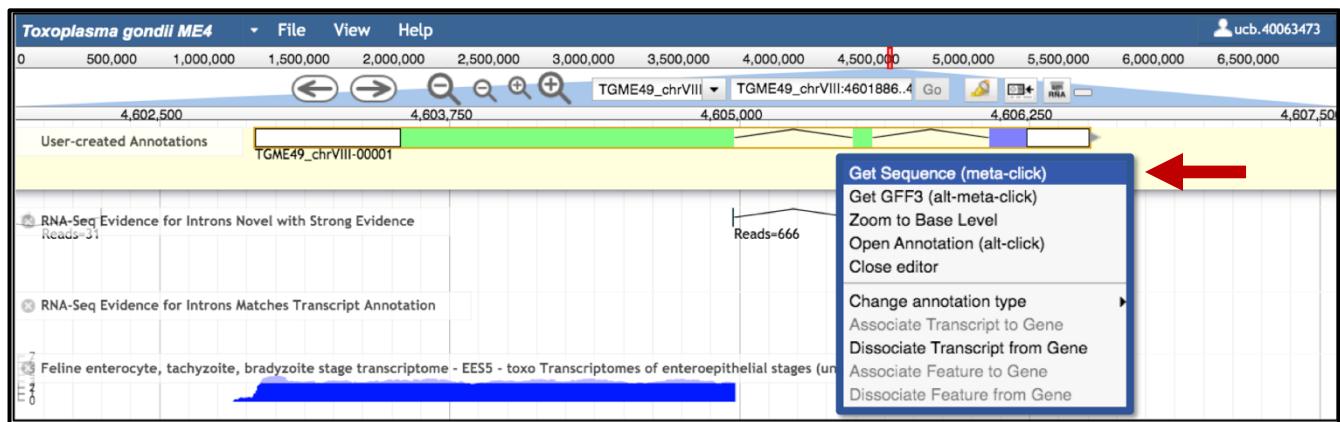
Select the first exon, point your mouse at the edge of the feature until a little arrow appears, then extend the exon to the transcription start. Apollo will automatically create the 5'UTR!



Select the last exon, point your mouse at the edge and extend the exon to the end of the gene model. Apollo will create the 3'UTR automatically!

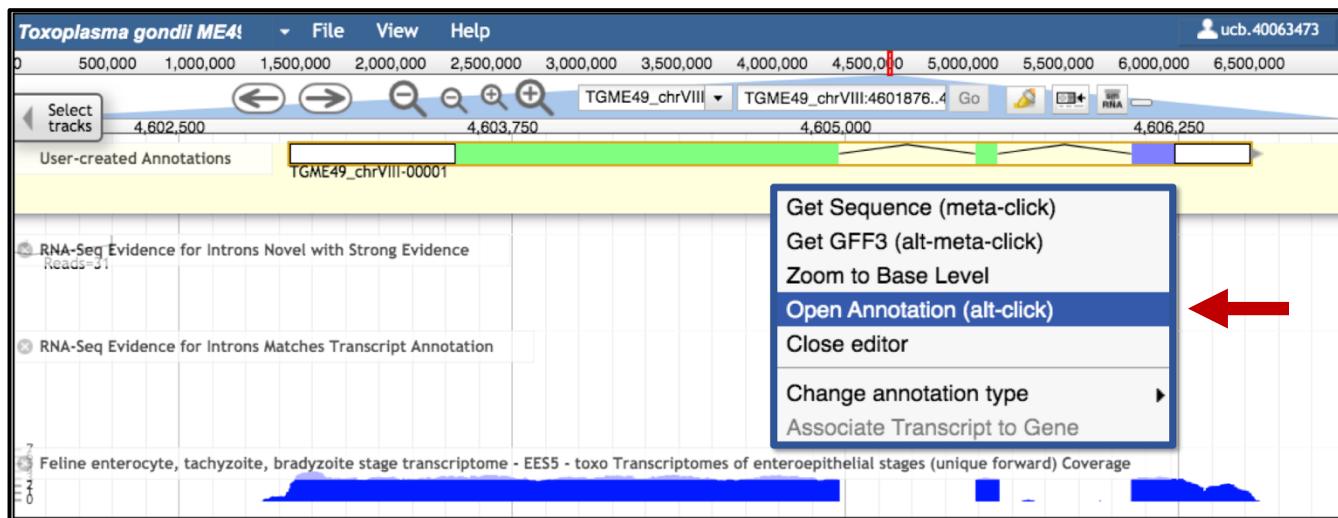


Select the new gene, with a right-click open the annotation drop-down menu and choose **Get Sequence**. Copy the sequence and run blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and InterPro (<https://www.ebi.ac.uk/interpro>) to get additional information about the new gene.

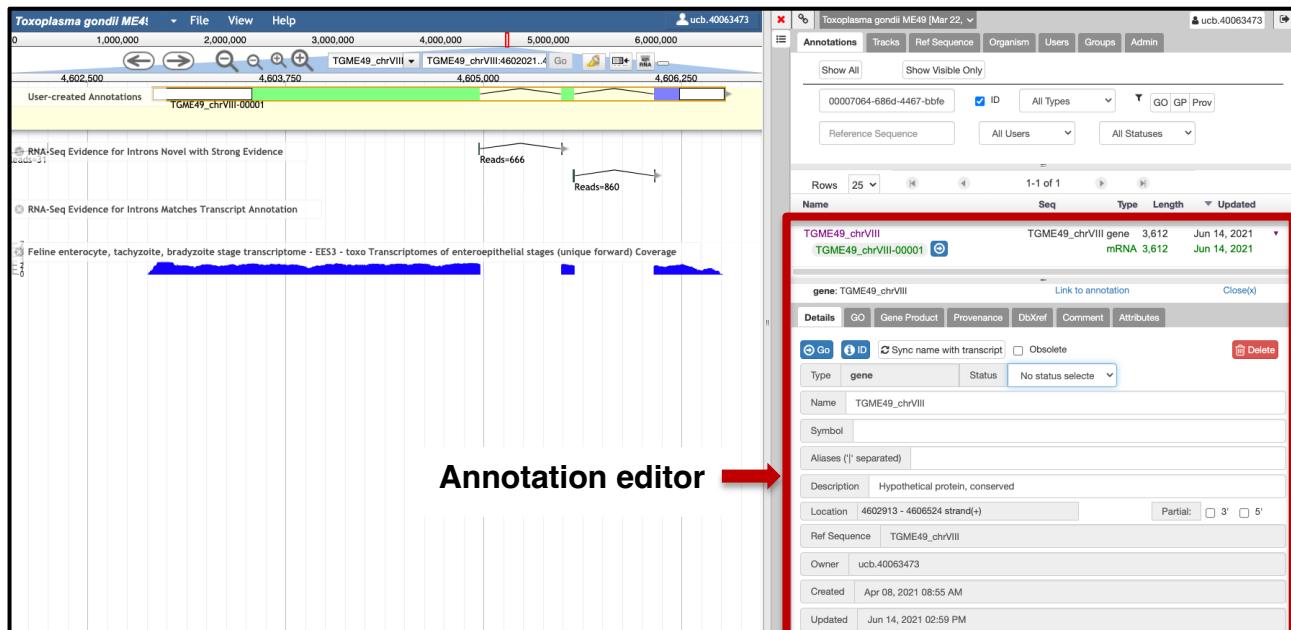


5) Opening of the Annotation editor window

Select the gene in the User-created Annotation track and with a right-click open the drop-down menu and choose **Open Annotation**. Alternatively, you can use the short-cut **alt-click**.



The annotation editor window is now shown on the right-hand side.



6) Finalizing the structural annotation

Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **new**. Click on the + sign.

The screenshot shows the Apollo annotations interface. At the top, the gene record is displayed: TGME49_chrVIII gene 3,603 mRNA 3,603 Apr 04, 2021. Below this, the 'Attributes' tab is selected. In the 'Value' input field, a dropdown menu is open, showing a list of canned values under the 'structural' tag. The 'new' option is highlighted with a blue background and a red arrow points to it from the right side of the screen.

Go to the Details tab, add a description/gene product to your new gene and select the status **Finished** on the gene. The following day, the new gene model will be visible on the gene record page in the Community annotations from Apollo track.

The screenshot shows the Apollo Details tab for the gene TGME49_chrVIII. The 'Status' field is currently set to 'No status selected'. A dropdown menu is open, showing four options: 'No status selected', 'Not Finished', 'Finished', and 'Requires Curator'. The 'Finished' option is highlighted with a blue background and a red arrow points to it from the right side of the screen.

Done! For additional questions, please get in touch with the VEuPathDB help desk.

Structural annotation in Apollo

Alternative transcripts

In this short tutorial we are showing you step-by-step how to create alternative transcripts in Apollo.

1) Accessing Apollo

To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo (1)**. You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo (2)**. Alternatively, go to the **Tools** menu and choose Apollo from the drop-down list (3).

The screenshot shows the Toxodb gene record page for TGME49_315160. At the top, there's a navigation bar with links like 'My Strategies', 'Searcher', 'Tools' (circled in red), 'My', 'Data', 'About', 'Help', and 'Contact Us'. Below the navigation bar, there's a search bar and several data visualization links: Synteny, Alignments, Phenotype, SNPs, Transcriptomics, Protein Features, and Proteomics. The main content area displays gene information: Type: protein coding gene, Chromosome: XI, Location: TGME49_chrXI:4,526,997..4,538,020(-), Species: *Toxoplasma gondii*, Strain: ME49, Status: Reference strain. It also shows 'View 4 user comments, or add a comment' and 'View and update community annotations in Apollo' (circled in red). In the 'Gene models' section, there's a summary: # Exons in Gene 9, # Transcripts 1, and a note: 'This gene is available in Apollo for community annotation. To find out more about Apollo, please visit this help page.' Below this, there are buttons for 'View in JBrowse genome browser' and 'Annotate in Apollo' (circled in red). A red box also highlights the 'Tools' menu item in the top navigation bar.

To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

Select on the right-hand side the tab **Tracks**.

The screenshot shows the Apollo interface. At the top, there's a header with 'Toxoplasma gondii ME4' and a user ID 'ucb.40063473'. Below the header, there's a genome browser view showing a chromosome with coordinates 0 to 6,000,000. On the right, there's a panel with tabs: 'Annotations' (selected), 'Tracks' (circled in red), 'Ref Sequence', 'Organism', 'Users', 'Groups', and 'Admin'. Under the 'Tracks' tab, there are sections for 'Comparative Genomics' (0/1) and 'Draggable Annotation' (0/4). There are also buttons for 'Search', 'JBrowse Selector', and 'New Track'.

Click on the menu item **Draggable Annotation** select **Annotated Transcripts, RNA-Seq Evidence for Introns Novel with Strong Evidence and RNA-Seq Evidence for Introns Matches Transcript Annotation.**

Annotations Tracks Ref Sequence Organism Users Groups Admin

Search JBrowse Selector New Track

Comparative Genomics 0/1

Draggable Annotation 3/4

- RNA-Seq Evidence for Introns Novel with Strong Evidence
- RNA-Seq Evidence for Introns Novel with Weak Evidence
- RNA-Seq Evidence for Introns Matches Transcript Annotation
- Annotated Transcripts

Epigenomics 0/64

Gene Models 0/25

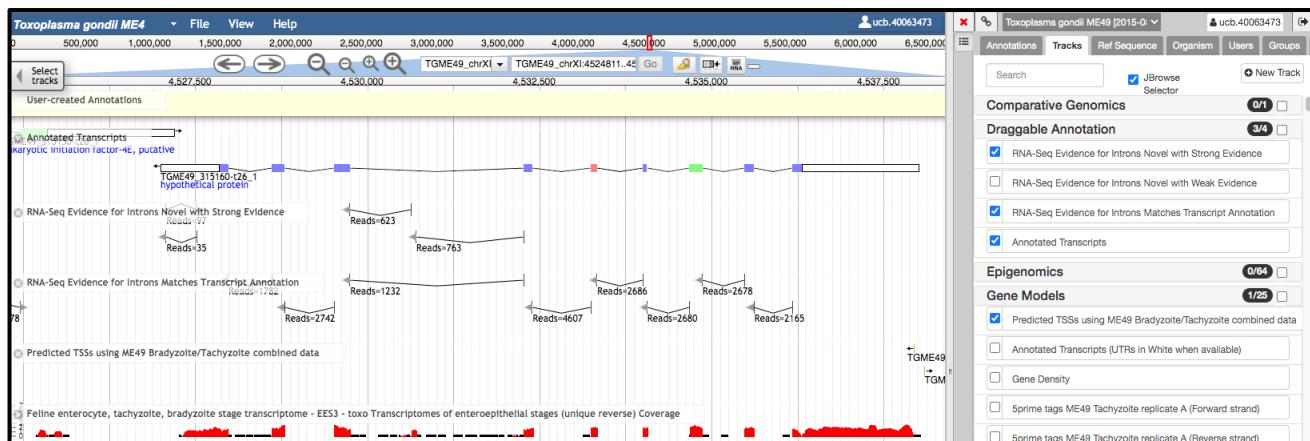
Genetic Variation 0/129

Proteomics 0/21

Sequence Analysis 0/24

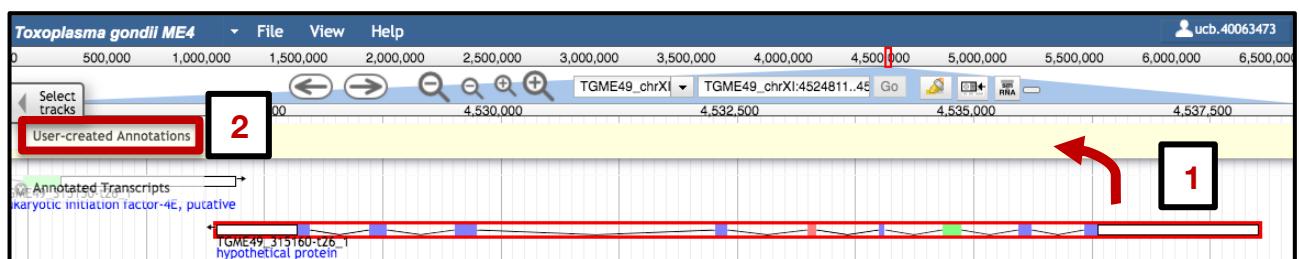
Transcriptomics 0/332

Select additional evidence, i.e. RNAseq plots and predicted TSS (transcription start sites).

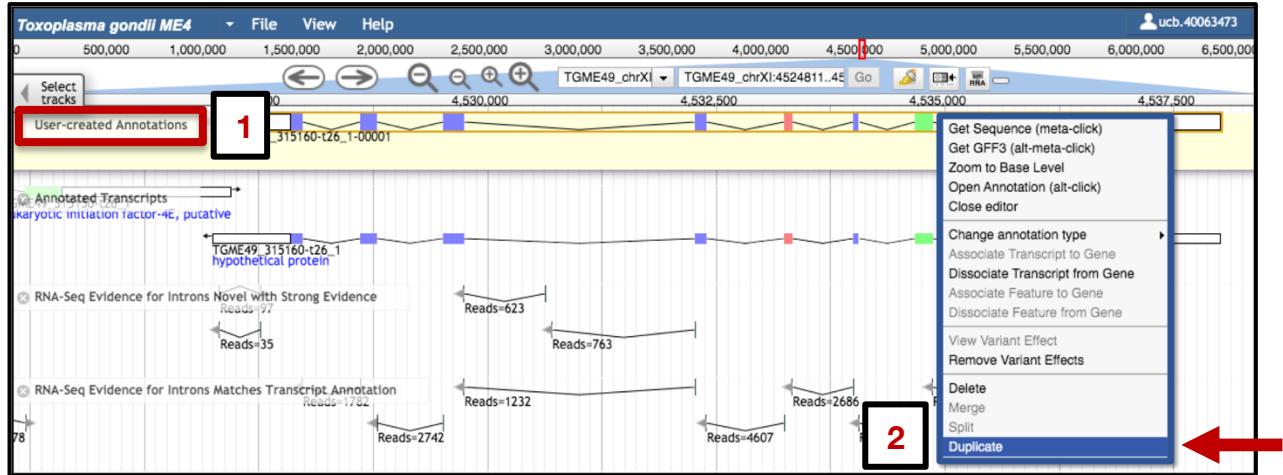


3) Adding alternative transcripts

Select the gene model by clicking on one of the introns or by double clicking on the gene model (1). The gene will show up with red boundaries. Drag and drop the gene into the User-created Annotations track (2). **Please note:** To add one-exon genes into the User-created Annotations area you need to double-click on the gene and then drag it into the user-created annotations area.

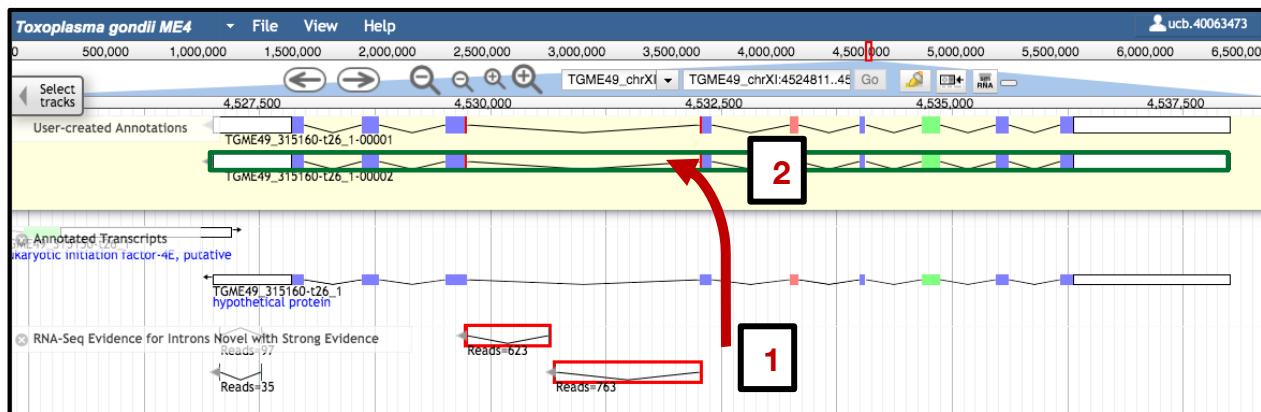


Select the gene in the User-created Annotations area (1). With a right-click open the annotation drop-down menu and choose duplicate (2).

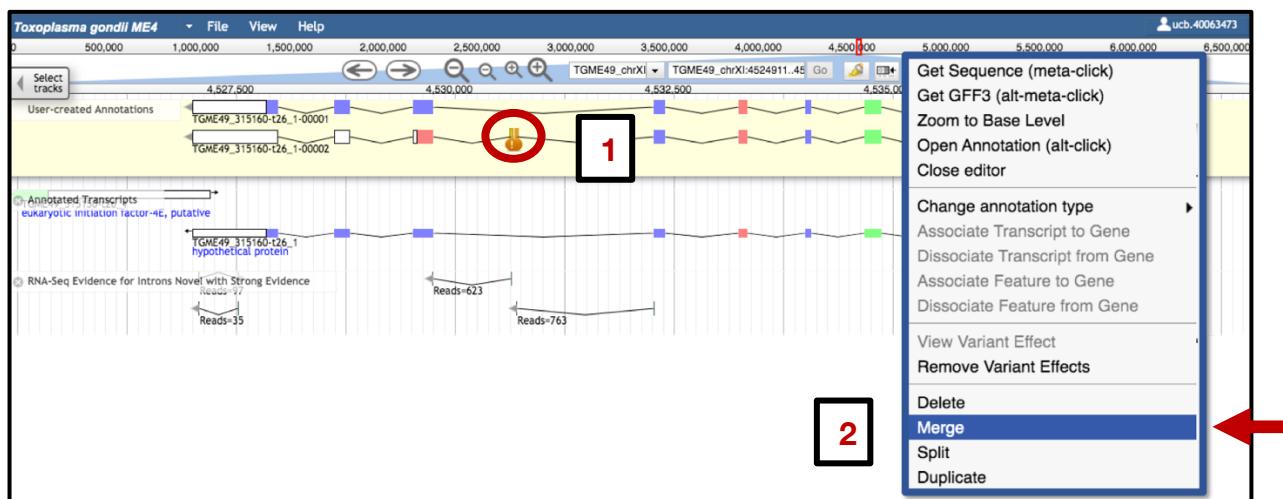


4) Modifying the alternative transcript

Select the intron junctions individually, or hold down the shift key and select both intron junctions with strong evidence (1), drag and drop them into the gene model (2). The gene will get a green box when dragging and dropping the intron evidence.

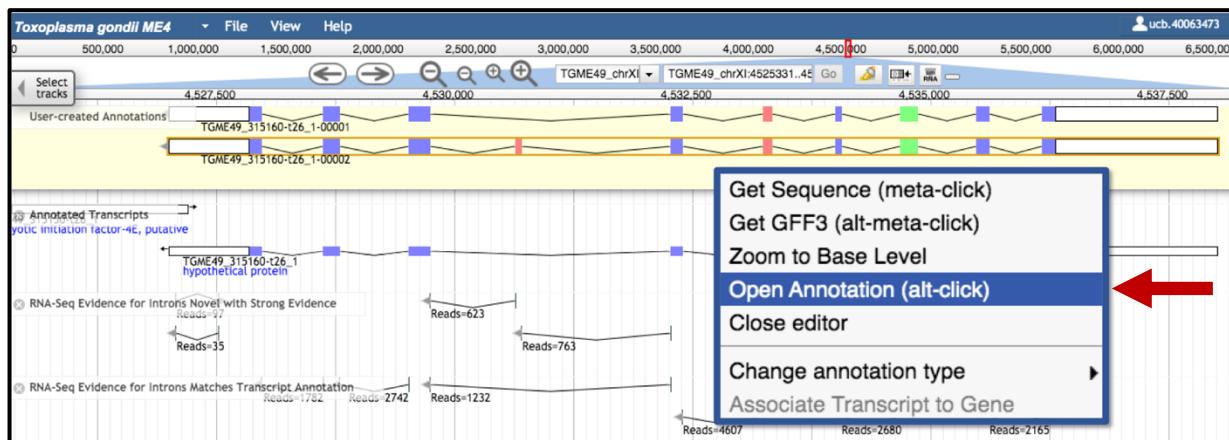


Hold down the shift key and select the two small exons. With a right-click open the drop-down menu and select merge.

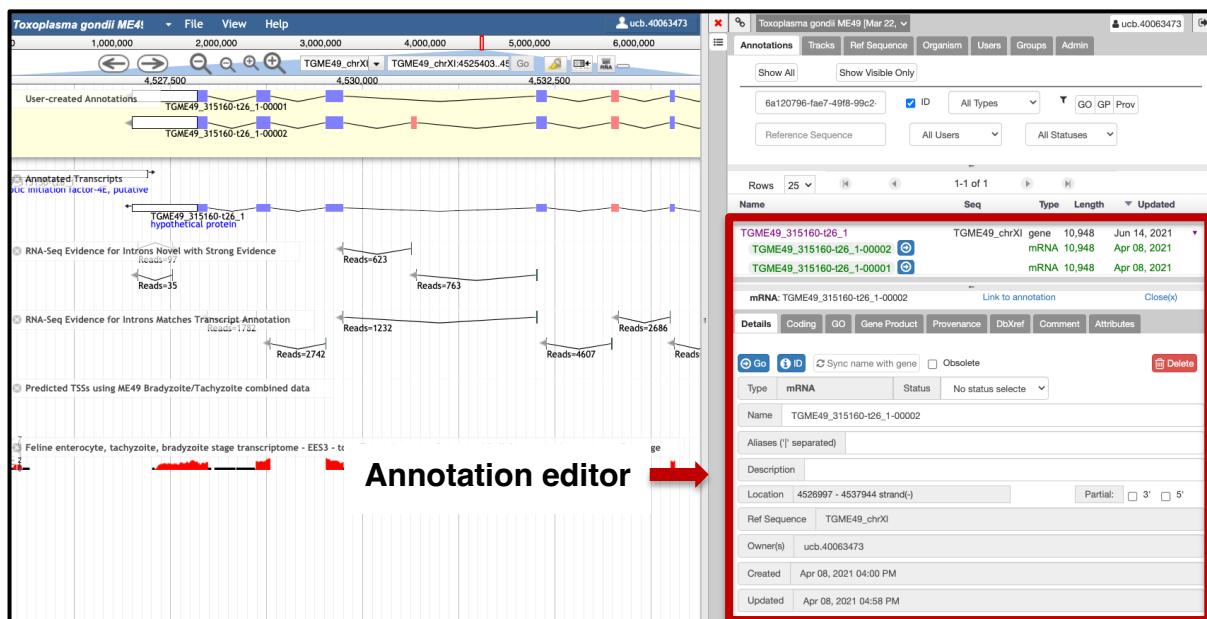


5) Opening of the Annotation editor window

Select one of the transcripts in the User-created Annotation track, with a right-click open the drop-down menu and choose **Open Annotation**. Alternatively, you can use the short-cut **alt-click**.



The annotation editor window is now shown on the right-hand side.



6) Finalizing the structural annotation

Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **isoform**. Click on the + sign.



Go to the Details tab and select the status **Finished**. The following day, the alternative transcript will be visible on the gene record page in the Community annotations from Apollo track.

The screenshot shows the 'Details' tab selected in the top navigation bar. A dropdown menu is open over the 'Status' field, which contains the following options: 'No status selected' (with a checked checkbox), 'Not Finished', 'Finished' (which is highlighted with a blue background and has a red arrow pointing to it), and 'Requires Curator'. The gene record itself includes fields for Type (gene), Name (TGME49_315160-t26_1), Symbol, Aliases, Description, Location (4526996 - 4538020 strand(-)), Ref Sequence (TGME49_chrXI), Owner (ucb.40063473), Created (Apr 08, 2021 03:51 PM), and Updated (Apr 08, 2021 04:00 PM). There are also 'Go' and 'ID' buttons at the top left and a 'Delete' button at the top right.

Done! For additional questions, please get in touch with the VEuPathDB help desk.