

Optional exercise: Genome annotation in Apollo

Apollo is a web interface that uses the JBrowse genome browser and allows you to annotate a genome - including suggesting new gene models, adding UTRs, modifying existing gene models, entering functional information and more.

Learning objectives:

- Accessing Apollo
- Use the menu and navigation bar of Apollo
- Add pre-loaded data tracks
- Changing gene structures in Apollo

1. Accessing Apollo:

Apollo can be accessed from the tools menu.

The screenshot shows the ToxoDB homepage. At the top, there's a navigation bar with links for Site search, My Strategies, Searches, Tools, My Workspace, Data, About, Help, Contact Us, and Guest. A red arrow points to the 'Tools' menu item. Below the navigation bar, there's a section titled 'Overview of Resources' with various links like 'Take a Tour', 'Getting Started', 'Companion', 'CRISPR guide design tool', 'Galaxy', 'Genome browser', 'NCBI Primer3', 'PubMed and Entrez', 'Sequence retrieval', and 'Web services'. On the left, there's a sidebar titled 'Search for...' with categories like Genes, Organisms, Popset Isolate Sequences, RFLP Genotype Isolates, and Genomic Sequences. A green box highlights the 'Getting Started' link under the resources section.

For this optional exercise we will use the VEuPathDB sandbox Apollo instance. The sandbox allows you to practice modifying gene models. The changes you make during your work in the Sandbox will not affect any of the organism's official gene set, neither will they be preserved.

The screenshot shows the Apollo interface for Anopheles gambiae STEC. On the left is a genome browser view with tracks for RNA-seq evidence, gene predictions, and orthology. On the right is a detailed view of a gene model, showing annotations like 'AAU800027-00001' and 'AAU800027-00001'. To the right of the main interface, there are two boxes: 'Experienced Apollo Users' (with a 'Apollo' button) and 'New Users' (with a 'Apollo Sandbox' button). The 'New Users' box is circled with a large red oval.

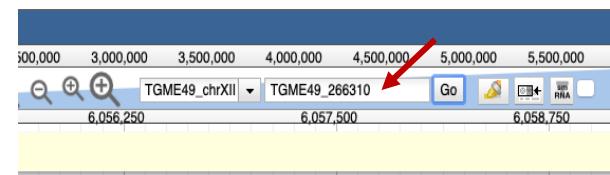
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet, log now into VEuPathDB with your user ID and password.

In the Apollo sandbox, select *T. gondii* ME49 from the genome drop-down menu in the upper left of the page.

The screenshot shows the Apollo browser interface. At the top, there's a navigation bar with 'File', 'View', and 'Help' options. Below it is a genome selection dropdown set to 'Toxoplasma gondii ME49'. To the right of the dropdown is a user profile icon and the identifier 'ulrikeboehme.987772593'. The main area is a genomic track viewer showing a single track labeled 'TGME49_chrV'. On the far right, a sidebar lists various organisms and their last update dates, with 'Toxoplasma gondii ME49 [Jul 01, 2023]' checked.

- Choose a Gene model to correct: Since we have a list of genes from the previous exercise, pick one of those IDs to explore in Apollo. Copy and paste the gene ID in the location box (center of browser page).

Important note: each person should select a different gene ID to prevent editing over each other.



- Add draggable annotation tracks: Draggable annotation tracks allow you to transfer exactly the coordinates of an intron or other feature to the new/corrected gene model that you are creating.

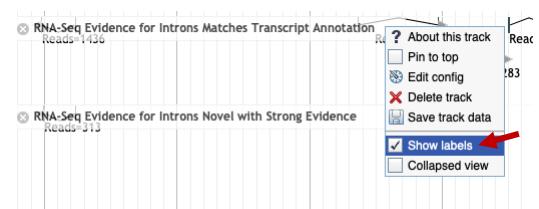
- Select the Tracks tab on the right-hand side and click on the Draggable Annotation menu item.

Select the following tracks:

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

This screenshot shows the Apollo browser with the 'Tracks' tab selected in the sidebar. In the main panel, several tracks are visible, including 'Annotated Transcripts' and 'RNA-Seq Evidence for Introns Novel with Strong Evidence'. A red box highlights the 'Draggable Annotation' section in the sidebar, which contains three selected tracks: 'RNA-Seq Evidence for Introns Novel with Strong Evidence', 'RNA-Seq Evidence for Introns Matches Transcript Annotation', and 'Annotated Transcripts'.

- Label the intron tracks with the number of reads supporting the intron. All tracks have a drop-down menu that is revealed when you hover over the end of the title. Choose **Show labels** from the drop-down menu for the tracks **RNA-Seq evidence for Introns Matches Transcript Annotation** and **RNA-Seq Evidence for Introns Novel with Strong Evidence** to see the number of reads.



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

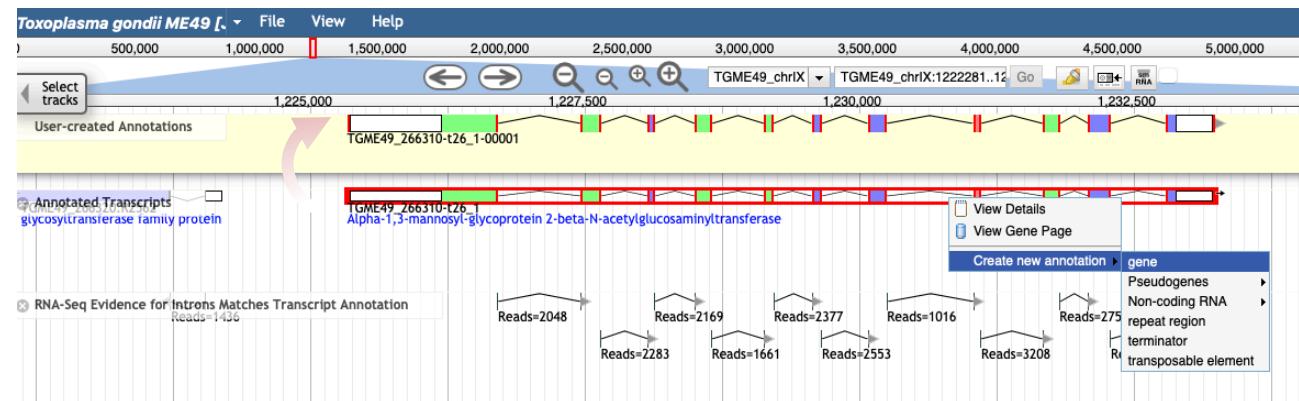
Hint: A track that is useful is the combined RNAseq plot. You can find the track by typing into the Search bar the word **combine**.

The screenshot shows the JBrowse interface with the search bar at the top containing the word "combine". Below the search bar is a list of tracks categorized under "Annotations". One track, "TgondiiME49 combined RNAseq plot", is selected and highlighted with a blue checkmark.

4. Add the gene model into the User-created Annotations area

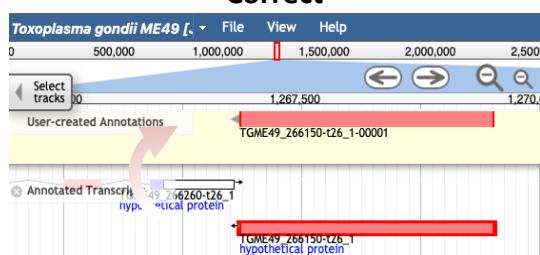
- Select the gene model by clicking on one of the introns or double-click on an exon.
- Once you see a red-border around the gene you can drag and drop the gene model into the pale-yellow User-created Annotations track.
- Instead of dragging the gene model, you can also right click on the gene in the Annotated Transcripts track, select from the drop-down menu **Create new annotation > gene**.

Note: You cannot drag up a single exon. Always select the whole gene and add it into the yellow User-created Annotations area.

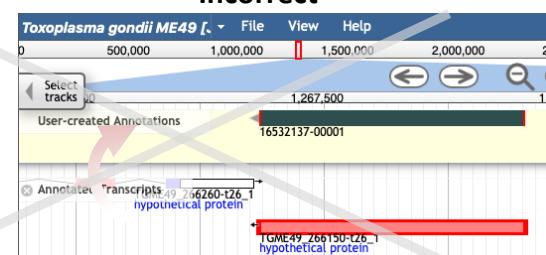


In the event that your gene has a single exon, you need to double-click on the gene. Once you see the red border drag the gene into the pale-yellow User-created Annotations track. The gene model should have a red, green or blue colour indicating the different frames. It should not be a grey box.

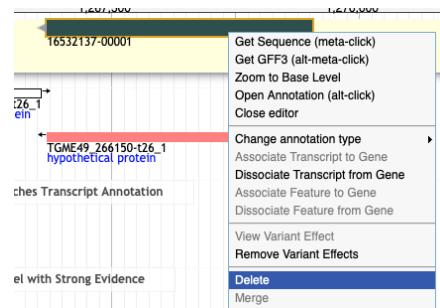
Correct



Incorrect



If you see a grey box when you are trying to drag a single exon gene, you need to delete it and try again. Select the grey box. With a right-click open the menu and choose Delete.

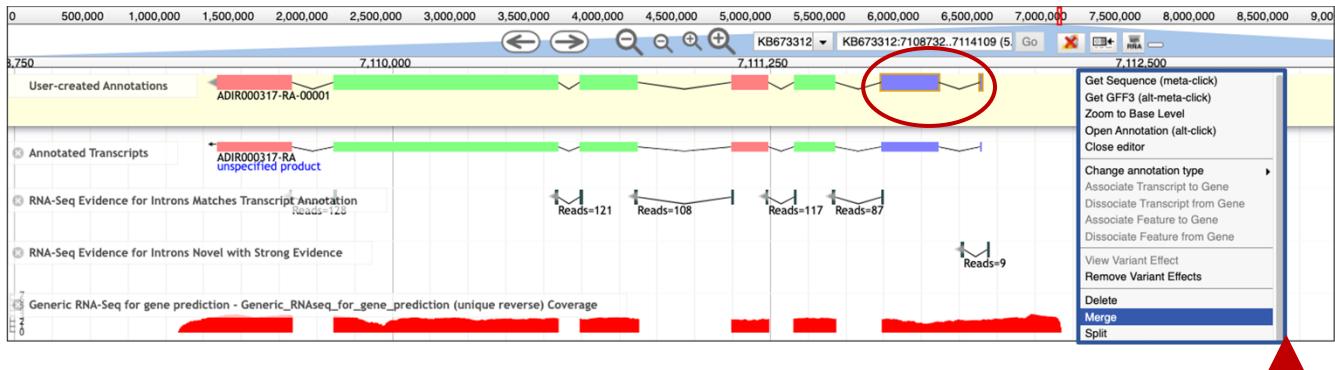


5. **Modify the gene model:** Not every gene model has the same problem. Some may have exon boundaries that do not match the coordinates in the JBrowse tracks. Some may need to be split into two genes, or merged into one gene. Some will have a combination of problems.
- INSPECT YOUR GENE MODEL!** Look at the data tracks such as the intron evidence tracks and the other RNA seq tracks you may have turned on in step 3. What errors do you think need to be corrected?

Please note: Always inspect the **RNA-Seq Evidence for Introns Novel with Strong Evidence** track. This track indicates if your gene of interest has possible missing exons, incorrect exon/intron boundaries or a possible alternative transcript.
 - Below are examples of 10 types of gene problems you may encounter when fixing your genes. Each offers screenshots illustrating how to make edits in Apollo. Choose a hyper link in the list below or scroll down to view the examples and screenshots
 - [Incorrect intron \(see 6.1\)](#)
 - [Incorrect exon/intron boundary \(see 6.2\)](#)
 - [Missing exon \(see 6.3\)](#)
 - [Missing several exons \(see 6.4\)](#)
 - [Genes need to be merged \(see 6.5\)](#)
 - [Genes need to be split \(see 6.6\)](#)
 - [Adding alternative transcripts \(see 6.7\)](#)
 - [Missing gene model \(see 6.8\)](#)
 - [Incorrect start codon \(see 6.9\)](#)
 - [Missing UTRs or incorrect UTR boundaries \(see 6.10\)](#)
 - You can also have a look at the VEuPathDB Apollo YouTube channel. There are short screencasts showing how to modify gene models in Apollo:
<https://www.youtube.com/playlist?list=PLWzQB3i5sYALdtuACxZRowVoghLimhwx>

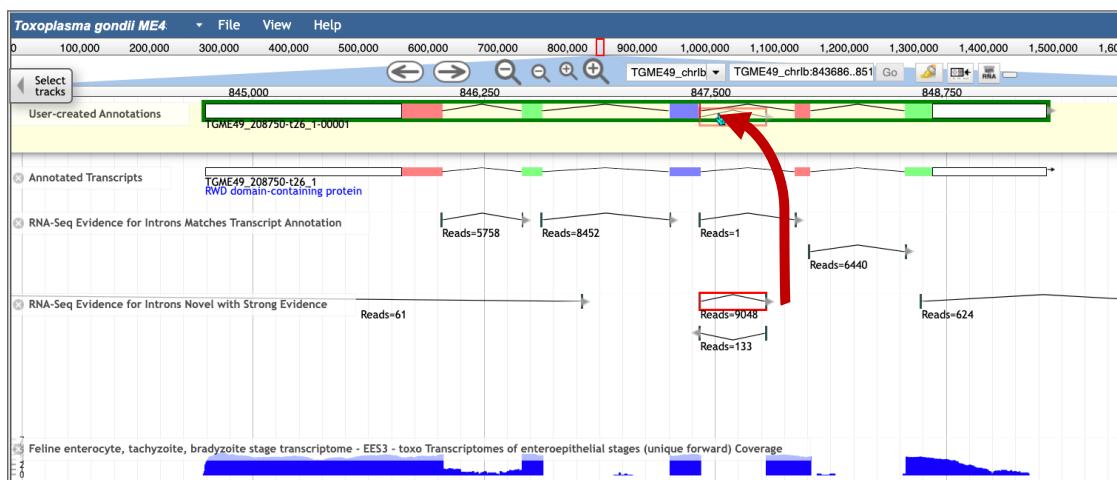
6.1) Incorrect intron

Select the gene model in the pale-yellow User-created Annotations area and with a right-click access the menu. If an intron is not supported select the two exons surrounding the intron by using the shift key and select merge from the menu. Alternatively, select one of the exons, go to the edge of the exon until a little arrow appears and then extend the exon until it overlaps with the second exon.



6.2) Incorrect exon/intron boundary

6.2.a Select the intron evidence, drag it into the user-created Annotations area and drop it onto the gene model. The gene model will get a green border.



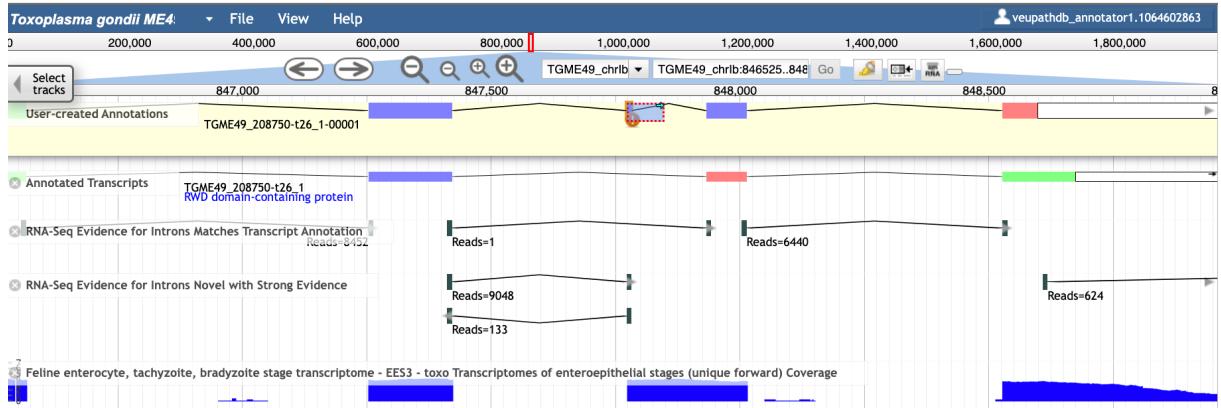
Select OK when you get the message
“Adding features of opposite strand”.

apollo-sandbox.veupathdb.org says
Adding features of opposite strand. Continue?

Cancel

OK

6.2.b Select one of the exons, go to the edge of the new exon until a little arrow appears and extend the exon until it overlaps with the second exon. Alternatively, you can also use the shift key to select both exons, with a right-click open the menu and choose merge.

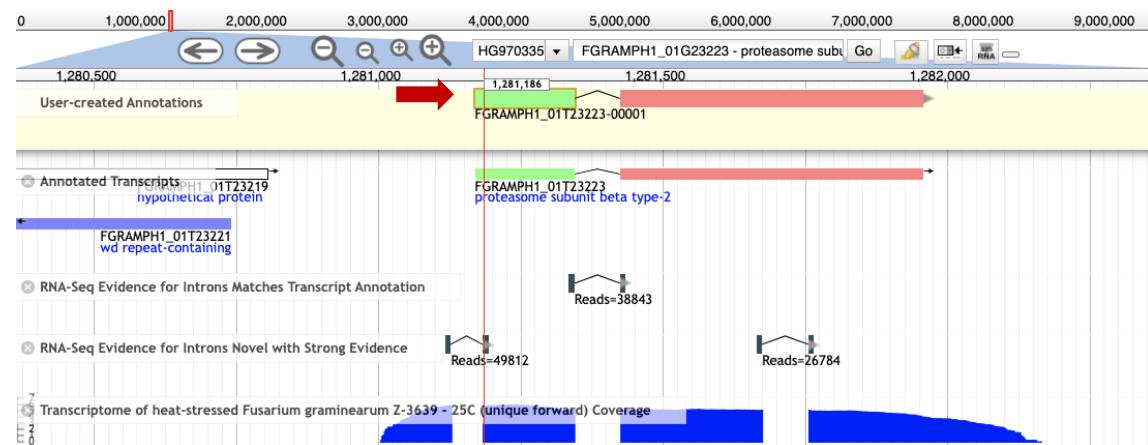


You can recheck if your new gene model is using the correct splice sites by clicking on the intron junctions in the RNA-Seq Evidence for Introns tracks. Red exon borders show up if the splice site corresponds to the evidence.

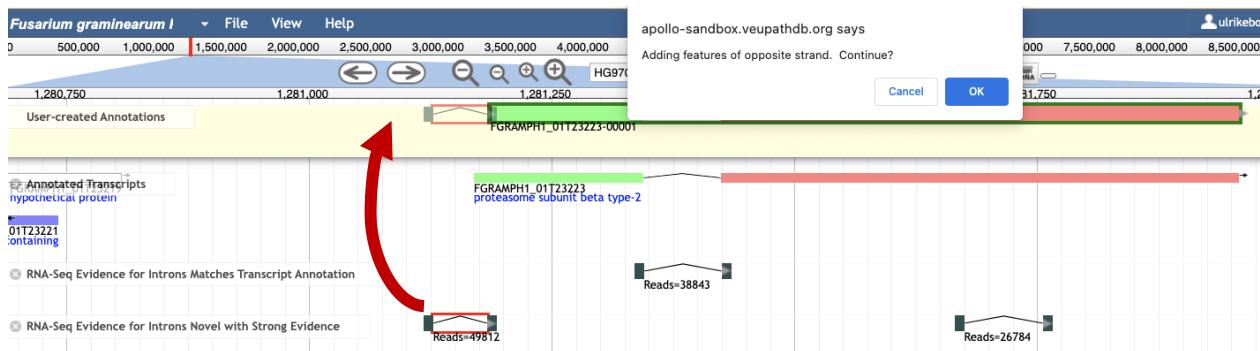


6.3) Adding an exon. In this example, the RNA seq data and the Intron Evidence tracks indicate that there should be another exon. The exon that is annotated is too long and there should be a second exon upstream of that. These instructions outline how to shorten an exon and add a new one.

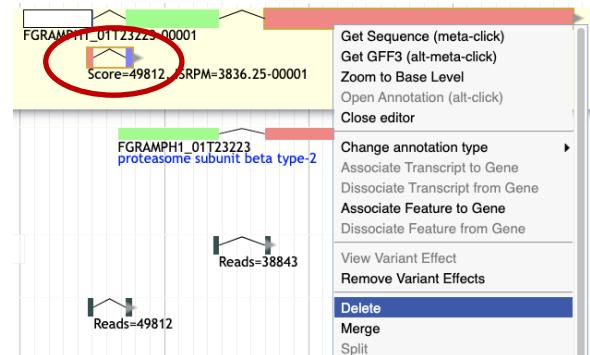
6.3.a Select the existing exon. Point your mouse at the edge of the exon and move the exon boundary so that it fits with the RNA-Seq evidence.



6.3.b Select the intron evidence. Drag it into the user-created Annotations area and drop it onto the gene model. The gene model will get a green border. Select OK when you get the message "Adding features of opposite strand".

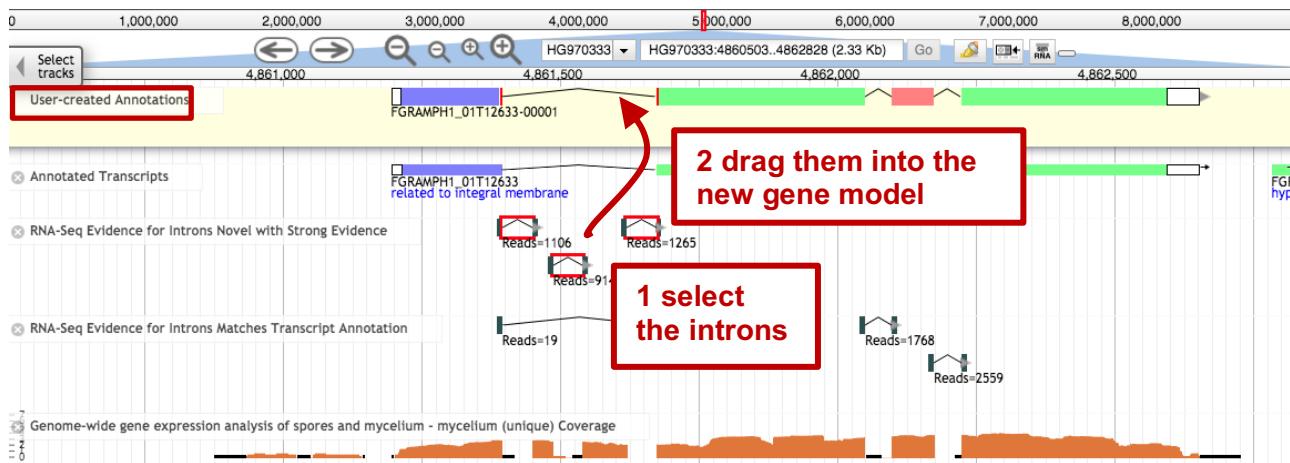


If the intron is being displayed as a separate unit within the User-created Annotation space, right click to delete the intron from the User-created Annotation space and try again. Make sure to drag and drop the intron evidence directly onto the gene. The gene will get a green border.



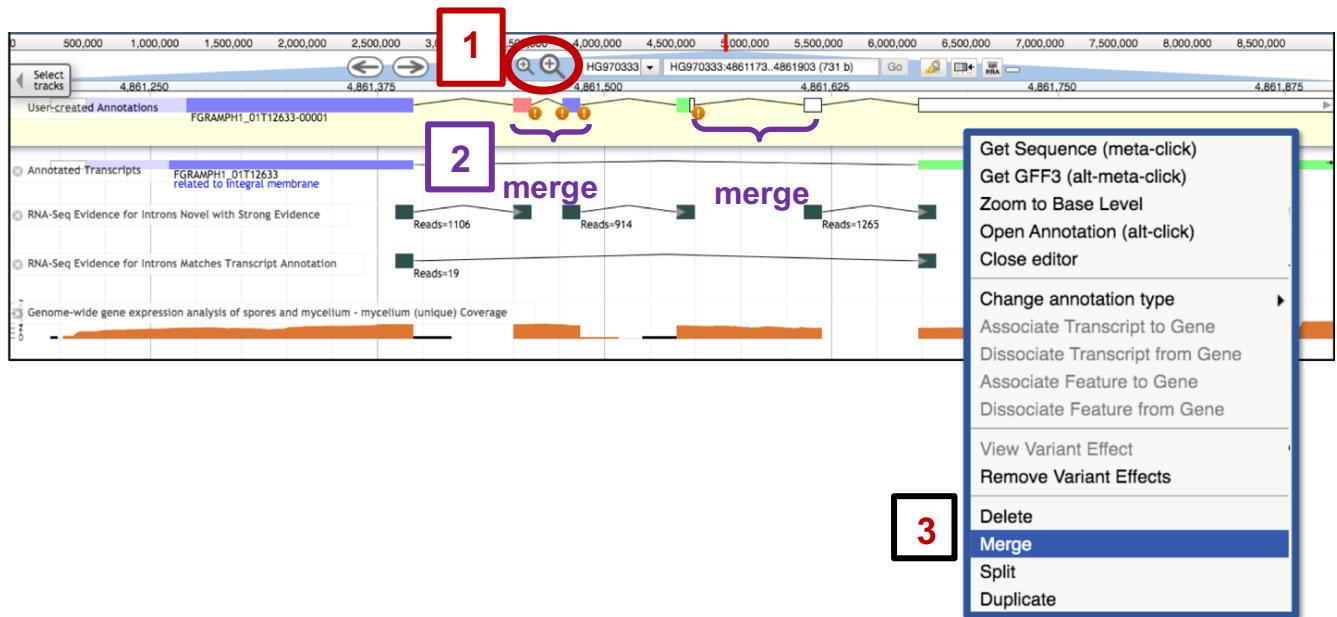
6.4) Adding two or more exons

6.4.a To create new exons drag and drop the intron junctions into the User-created Annotations area. You can either select the intron junctions individually or hold down the shift key and select all intron junctions with strong evidence (1), drag and drop them into the gene model (2). The gene boundaries will show up in green when dragging and dropping.

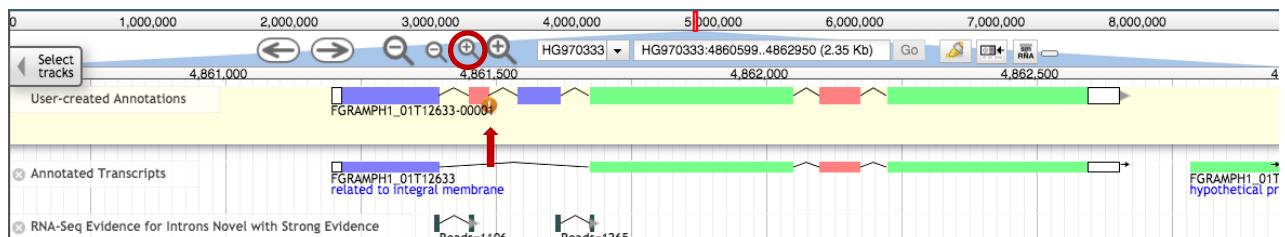


6.4.b Zoom in by clicking on the + sign on the top (1). Press the Shift key and select the exons that should be merged (2). With a right-click open the drop-down menu and choose **Merge** (3).

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.



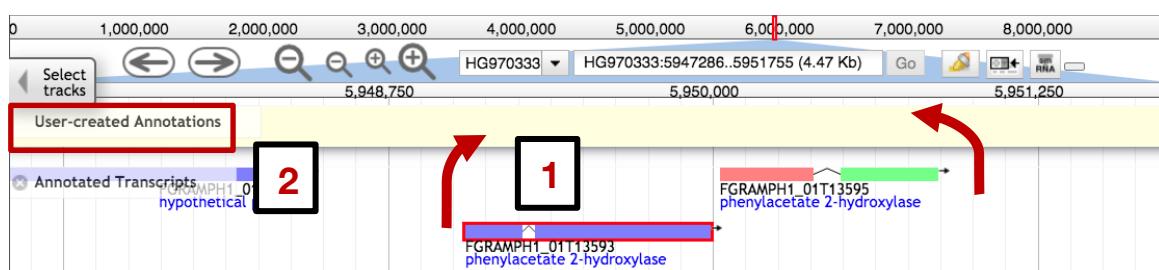
The exclamation marks at the bottom edge of some exons informs about non-canonical splice sites, i.e. GC splice sites. Zoom in by clicking on the + sign and the sequence should become visible. You can check if it is a valid splice site.



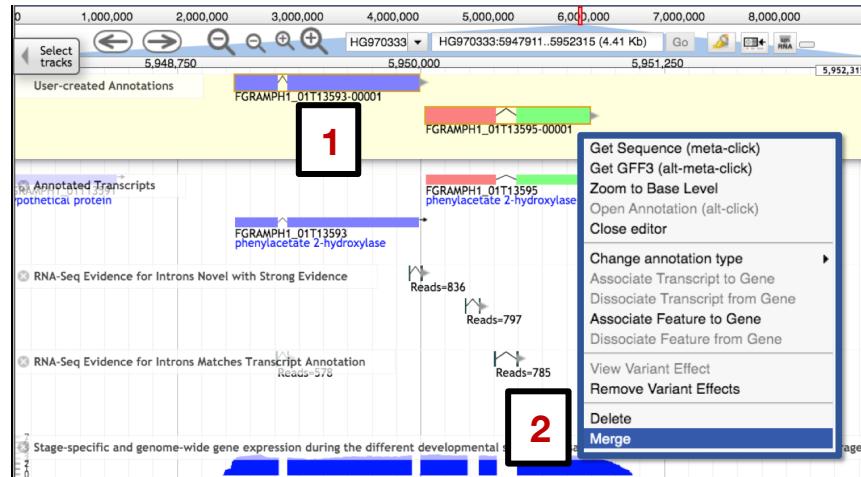
More information on splice sites can be found on this FAQ:
<https://veupathdb.org/veupathdb/app/static-content/faq.html#apollo6>

6.5) Merging two genes

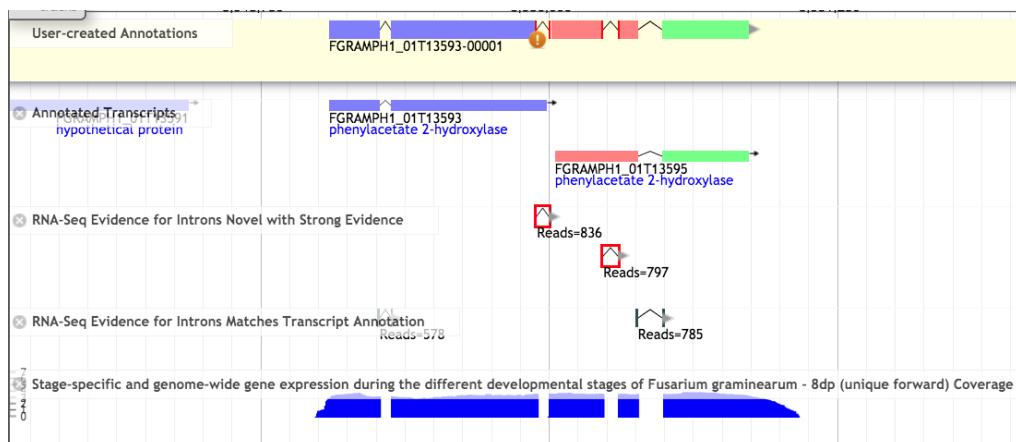
6.5.a Select the gene models that you would like to merge (1). Drag and drop the genes into the User-created Annotations track (2).



6.5.b Now that both genes are in the User-created Annotations track, hold down the shift key and select both gene models (1) in the yellow User-created Annotations area. With a right-click open the drop-down menu and choose **Merge** (2).

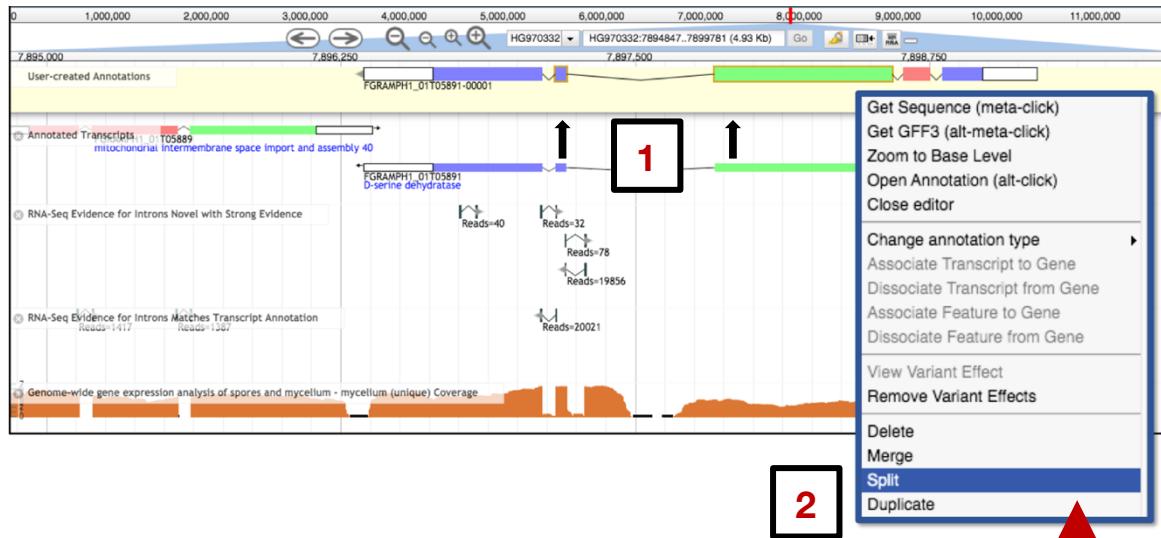


6.5.c Drag and drop the intron junctions into the User-created Annotations area and merge them with the gene. If the exon is longer than the new intron, you need to shorten the exon.

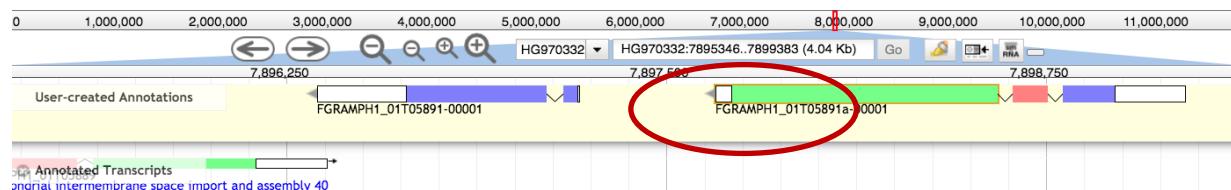


6.6) Splitting genes

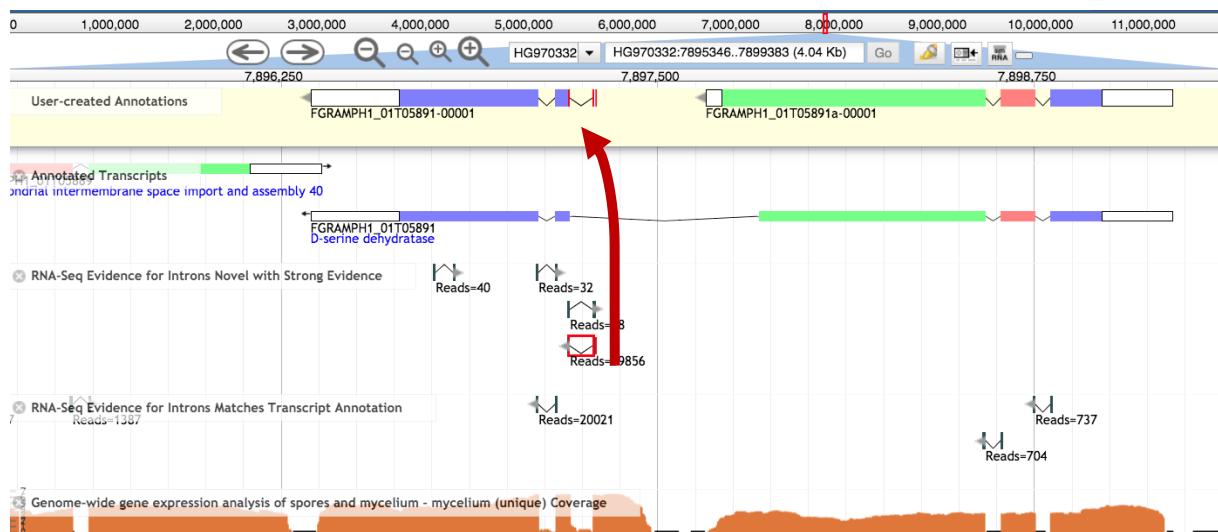
6.6.a To split the gene model, hold down the shift key, mark the two exons that border the intron that should be split (1). With a right-click open the annotation drop-down menu and select **Split** (2).



6.6.b Now that the gene model has been split, the newly created genes need a correct start codon and stop codon. To create the stop, click with your mouse at the end of the gene model and extend it. The 3'UTR will be created automatically!

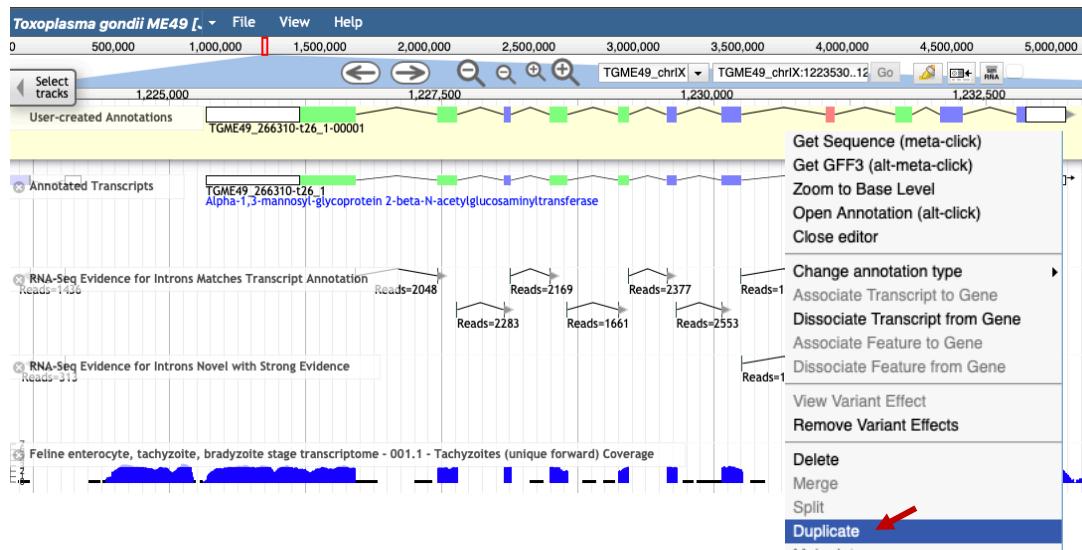


6.6.c The gene model on the left, needs a start codon. Drag and drop the splice junction into the annotation area. Move it up and hover the splice junction over the gene. A green border will show up which indicates that the intron junction has been merged with the gene. Now extend the first exon to include a UTR.

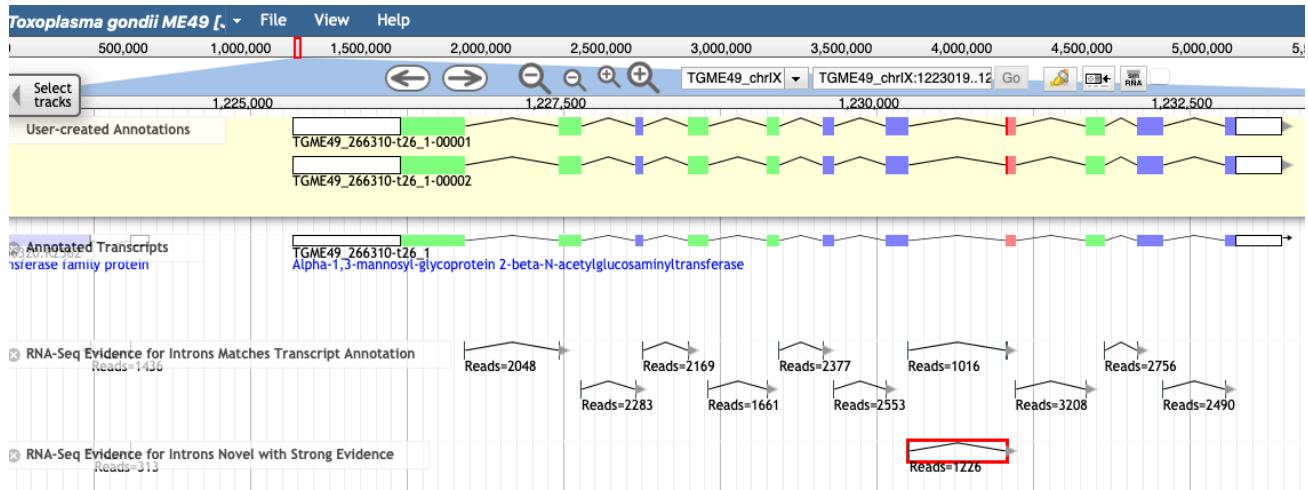


6.7) Adding alternative transcripts

6.7.a Add the gene into the user-created Annotations area. Select the gene in the User-created Annotations area, with a right-click open the drop-down menu and choose duplicate.

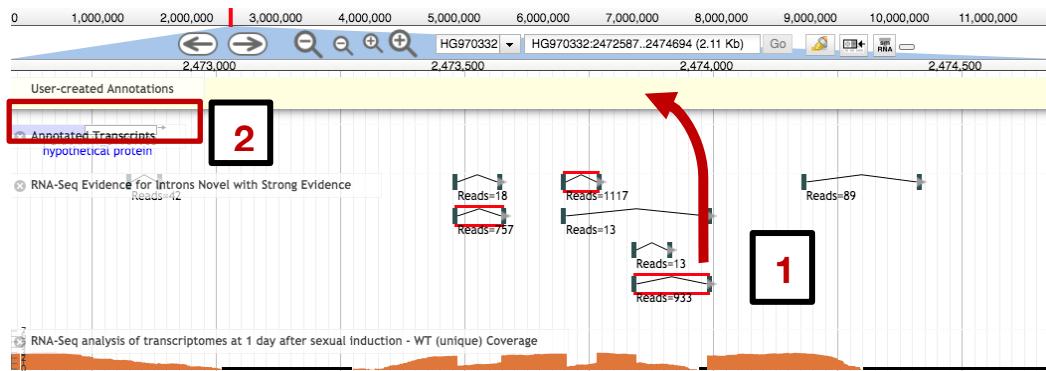


6.7.b There are now two transcripts with different transcript ID extensions: 00001 and 00002. You can now start to modify the alternative transcript.

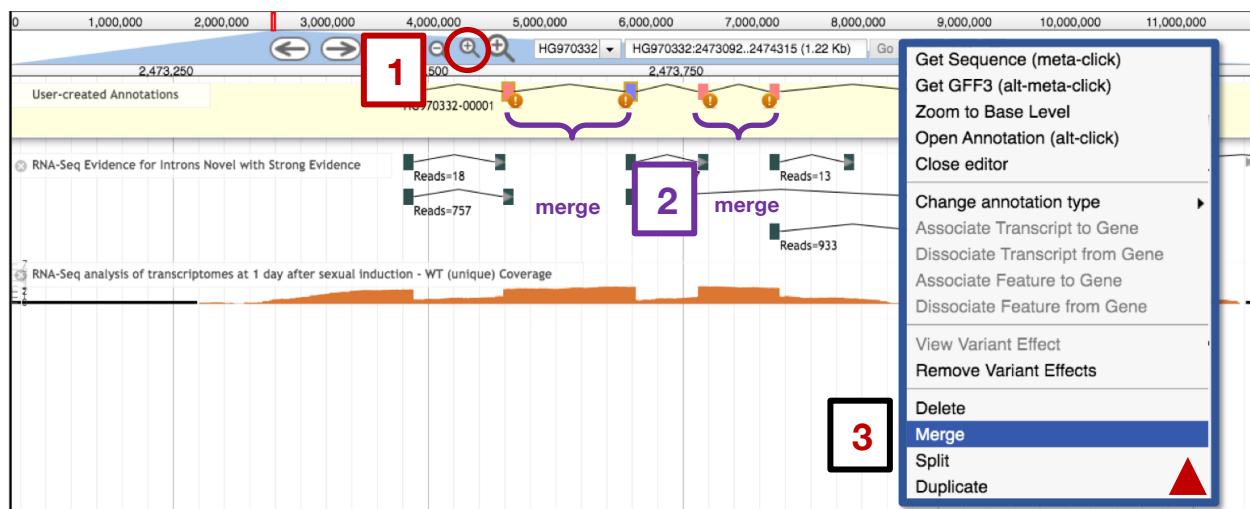


6.8) Creating a new gene

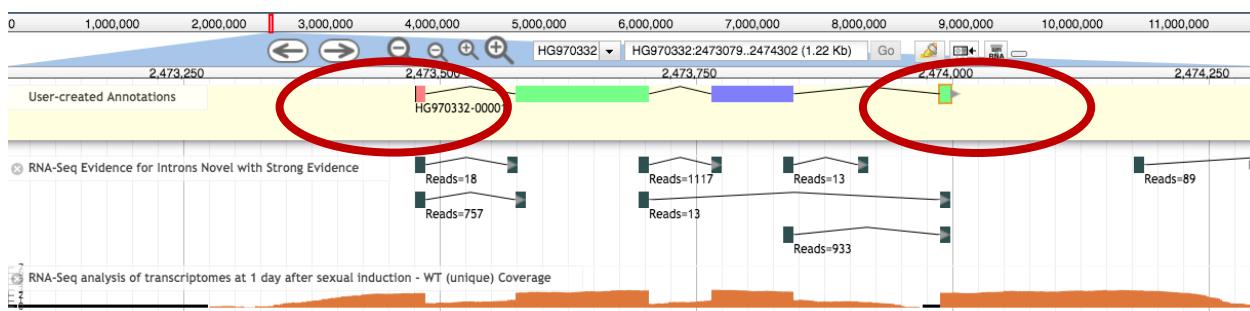
6.8.a Select the supporting evidence, i.e. intron junctions (1). Use the shift key to select more than one intron junction. The selected intron junctions will show up with a red border. Drag and drop them into the User-created Annotations track (2).



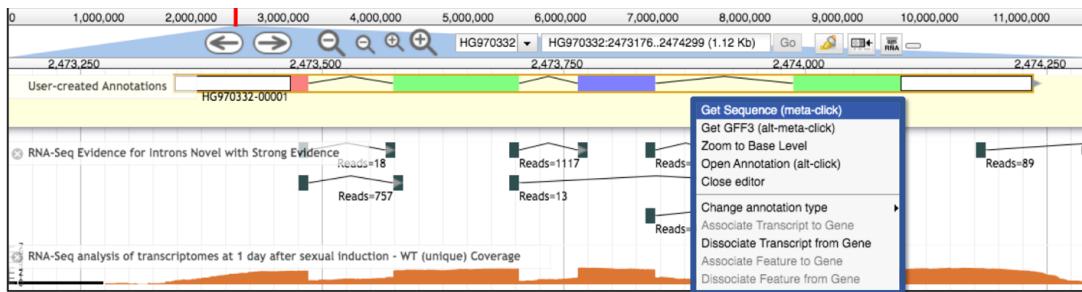
6.8.b Zoom in by clicking on the + sign on the top (1). Press the Shift key and select the exons that should be merged (2). With a right-click open the drop-down menu and choose **Merge** (3). Alternatively, select one of the exons you would like to merge, go to the edge of the feature until a little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



6.8.c Select the first and the last exon, go to the edge of the exon until a little arrow appears and extend it to the start/end. UTRs will be created automatically.

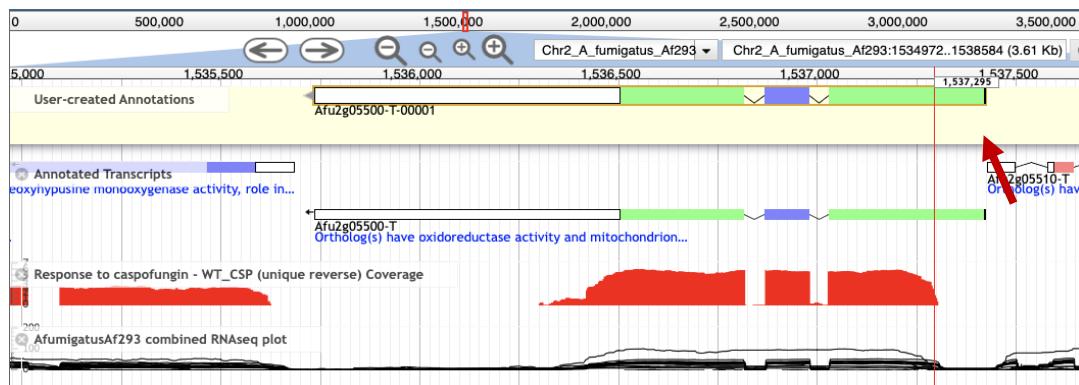


6.8.d Select the new gene model, with a right-click open the annotation drop-down menu and select **Get Sequence**. Copy the sequence, run blast (<https://blast.ncbi.nlm.nih.gov>) and Interpro (<https://www.ebi.ac.uk/interpro>) to get more information about the new gene.

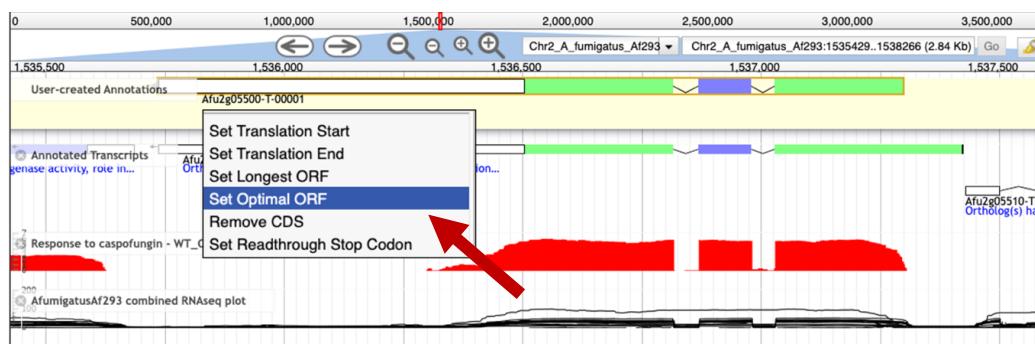


6.9) Incorrect start codon

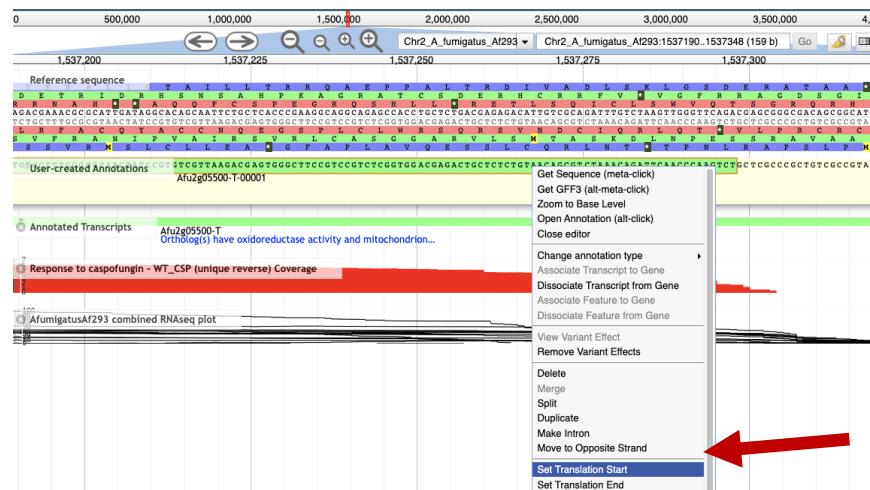
6.9.a Select the gene model in the user-created Annotations area, hover at the end of the first exon and move the exon boundary, so that it fits with the transcript evidence.



6.9.b Open the right-click menu and choose **Set Optimal ORF**. With this option Apollo automatically creates the longest CDS.

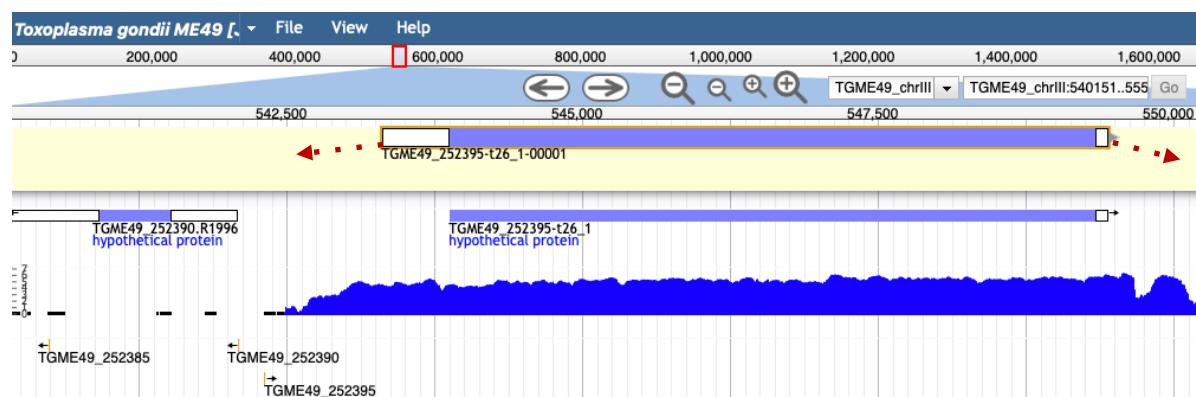


6.9.c Alternatively, you can also zoom in, click on the A of the ATG that you would like as start. Use the option **Set Translation Start** from the right-click menu.

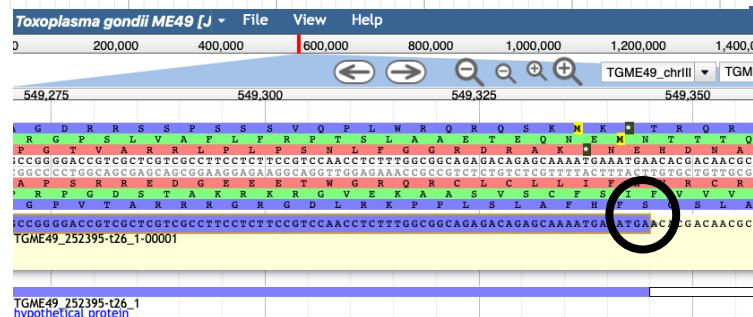


6.10) Adding UTRs

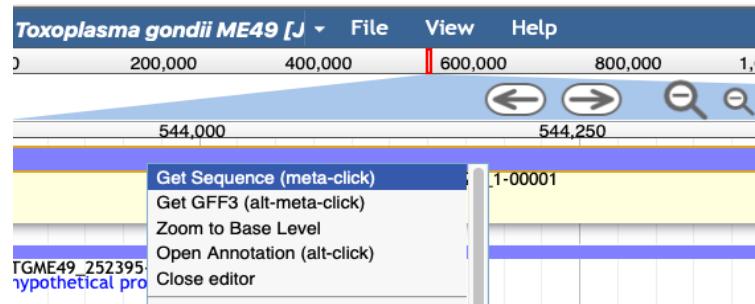
6.10.a Point your mouse at the edge of the feature, a little arrow will appear. Extend the exon to the transcription start/end. Apollo will automatically create UTRs (shown in white).



If you are adding UTRs you don't have to be concerned that your gene model is missing a start and stop codon. If you are not including UTRs always make sure there is a start (ATG) and stop codon (TAA, TAG, TGA). To recheck zoom into the sequence and check.

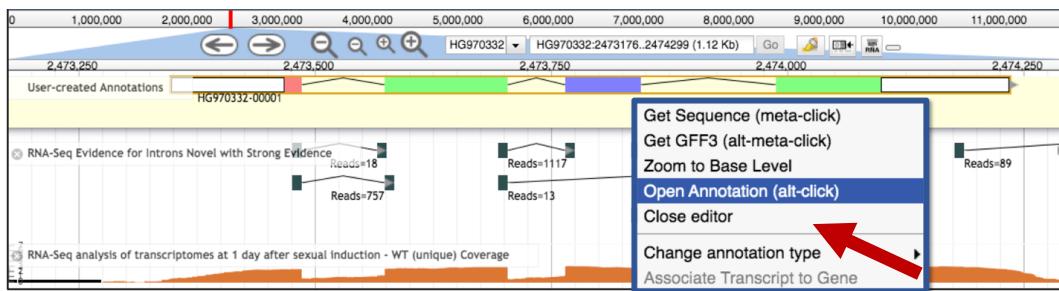


To recheck about the start, you can use the right-click menu and select **GET Sequence** to see if your gene starts with a Methionine.

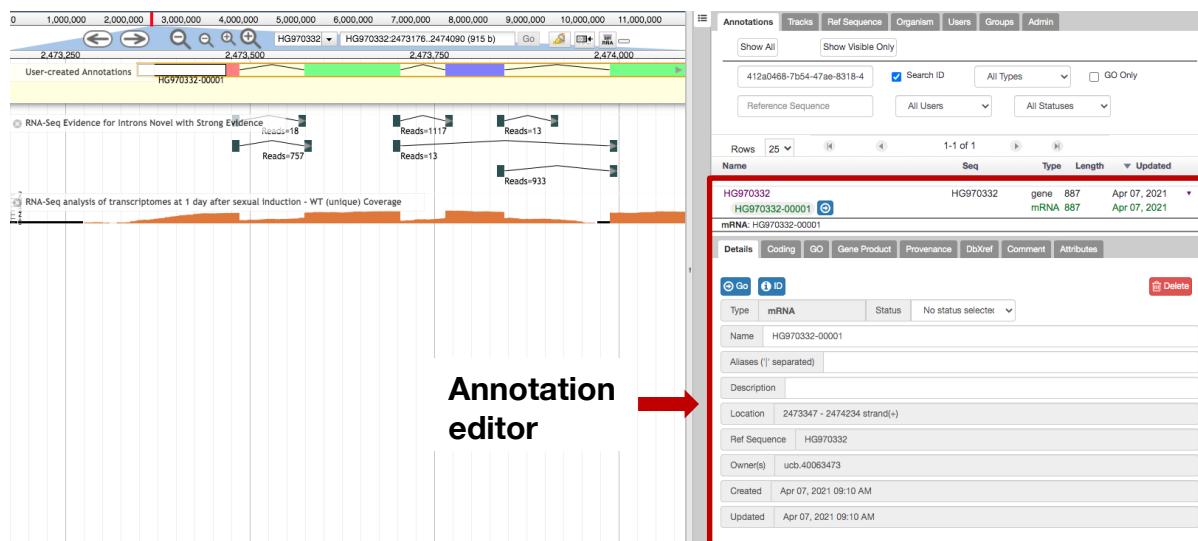


7) Opening of the Annotation editor window

- 7a** 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.



8) Finalizing the structural annotation

8.a Add a product description for new genes, split and merged genes. Finally go to the Details tab and select the status **Finished**.

The screenshot shows a gene detail page for HG970332. At the top, it displays basic information: HG970332, gene 976, Aug 22, 2022, mRNA 976, Aug 22, 2022. Below this is a navigation bar with tabs: Details, GO, Gene Product, Provenance, DbXref, Comment, and Attributes. The Details tab is highlighted with a red box. Under the Details tab, there are fields for Type (gene), Name (HG970332), Symbol, Aliases, Description, Location (2473278 - 2474253 strand(+)), and Ref Sequence (HG970332). To the right of these fields are buttons for Sync name with transcript, Openlate, Annotations, and Delete. A dropdown menu is open over the Status field, which currently shows 'No status selected'. The menu contains five options: 'Finished' (with a checkmark), 'Requires Cura', 'Rejected', and 'Not Finished'. A red arrow points to the 'Finished' option.

Done! Additional information, i.e. tutorials can be found on the following site:
https://veupathdb.org/veupathdb/app/static-content/apollo_help.html