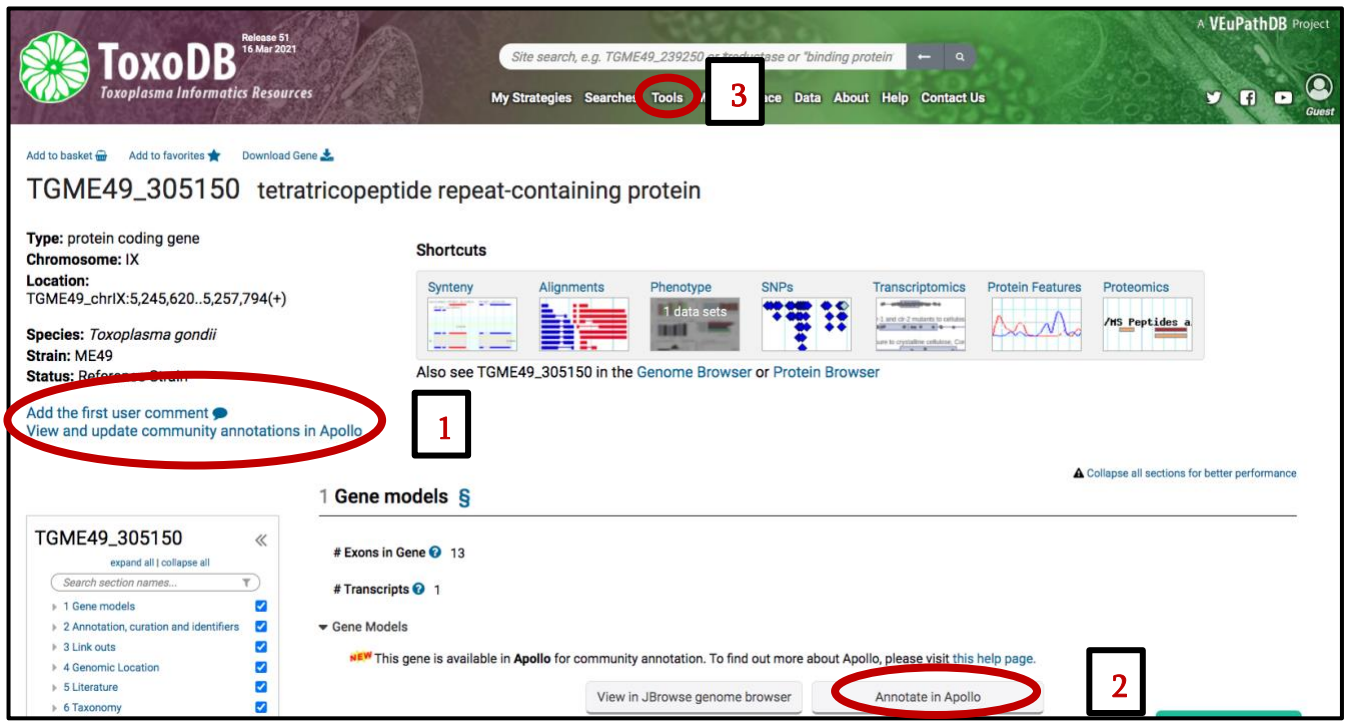
Structural annotation in Apollo
Modifying a gene model

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

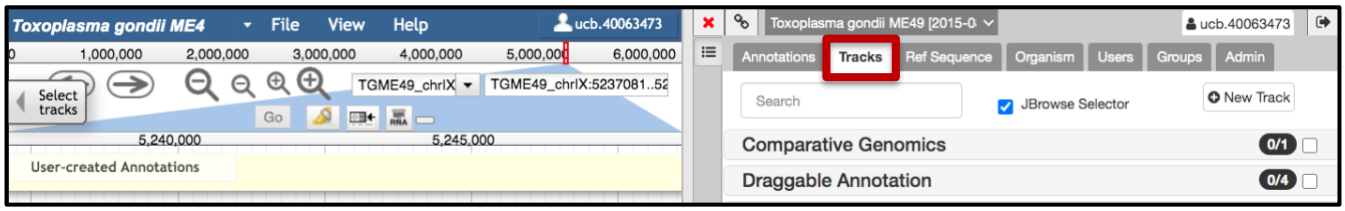
To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo** (**1**). You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo** (**2**). Alternatively, go to the **Tools**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



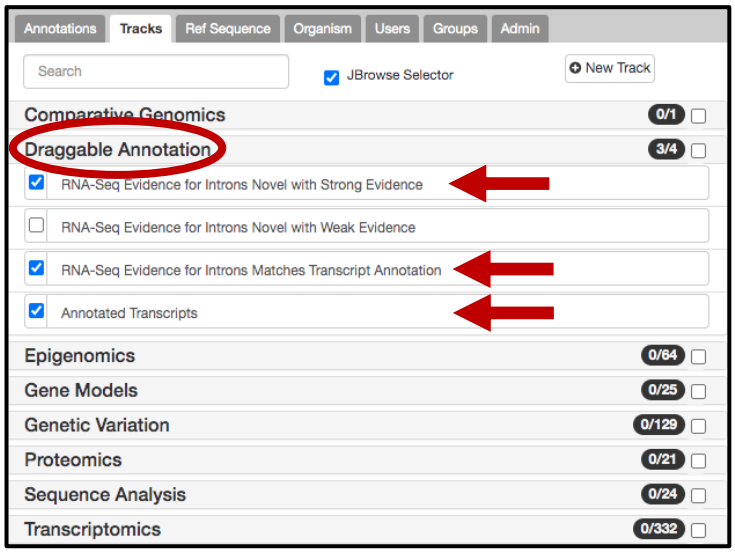
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

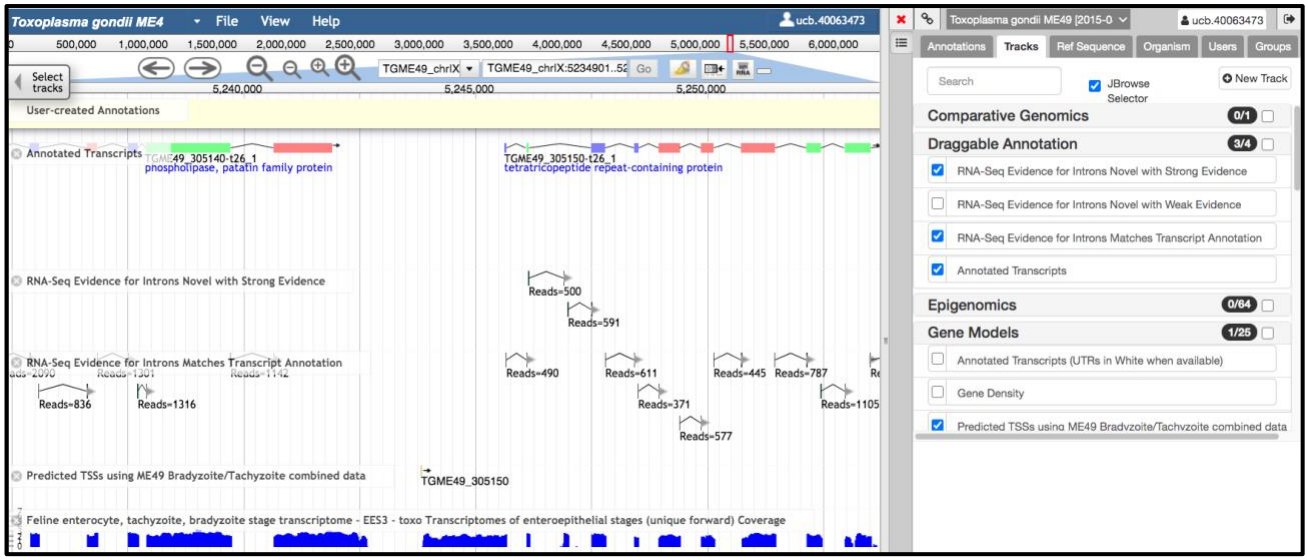
Select on the right-hand side the tab **Tracks.**



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**.

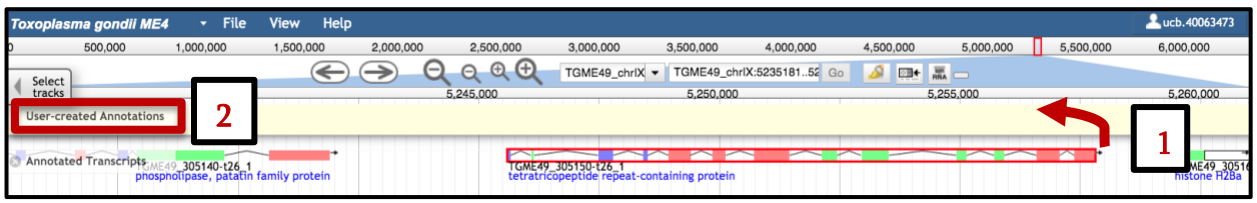


Select additional evidence, i.e. RNAseq plots or predicted TSS (transcription start sites).

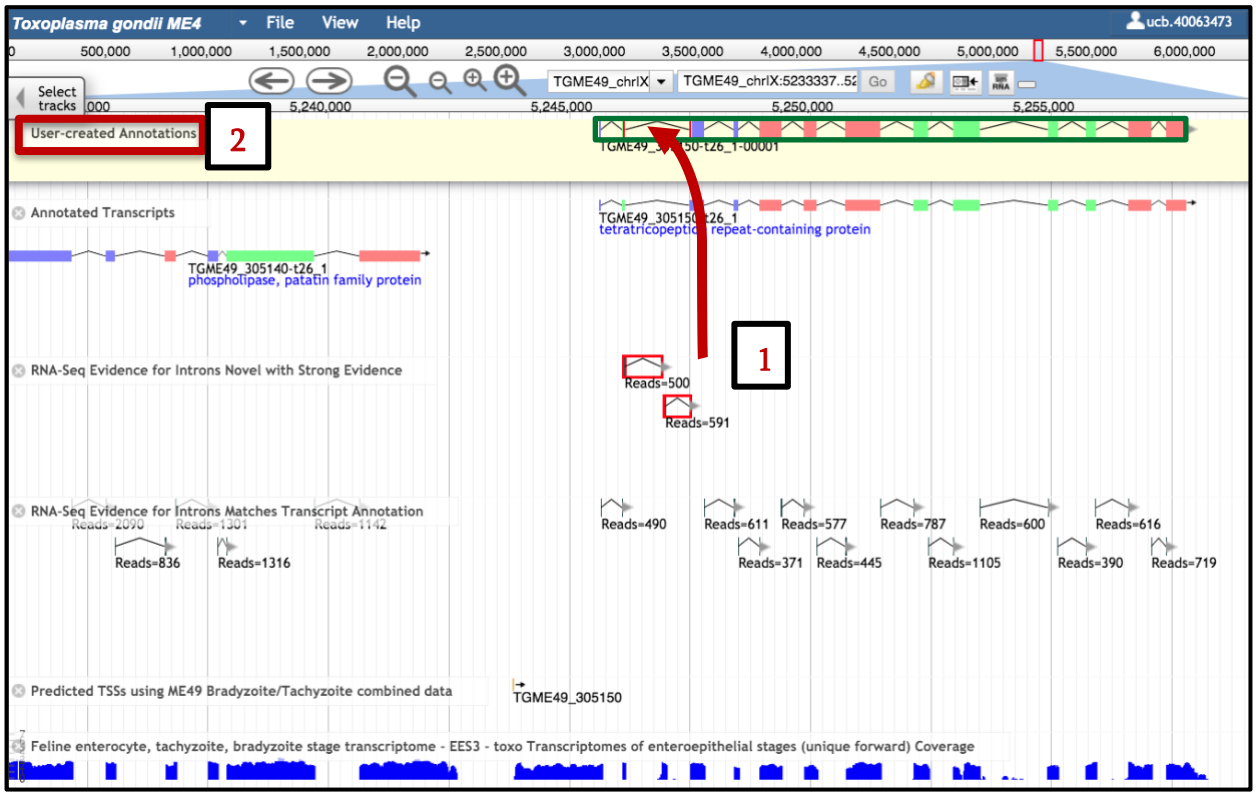


3) Modifying the gene

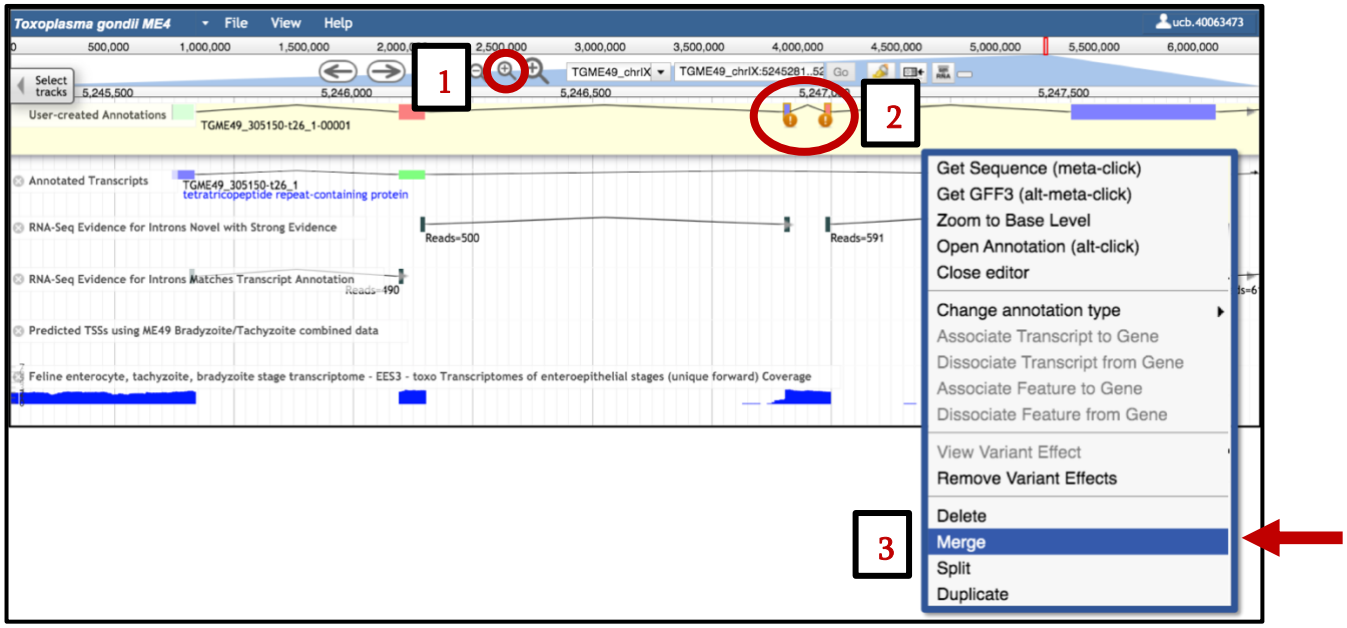
Select the gene model by clicking on one of the introns or by double clicking on the gene model (**1**). The gene model will show up with red boundaries. Drag and drop the gene into the User-created Annotations track (**2**). **Please note**: To add one-exon genes into the User-created Annotations area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



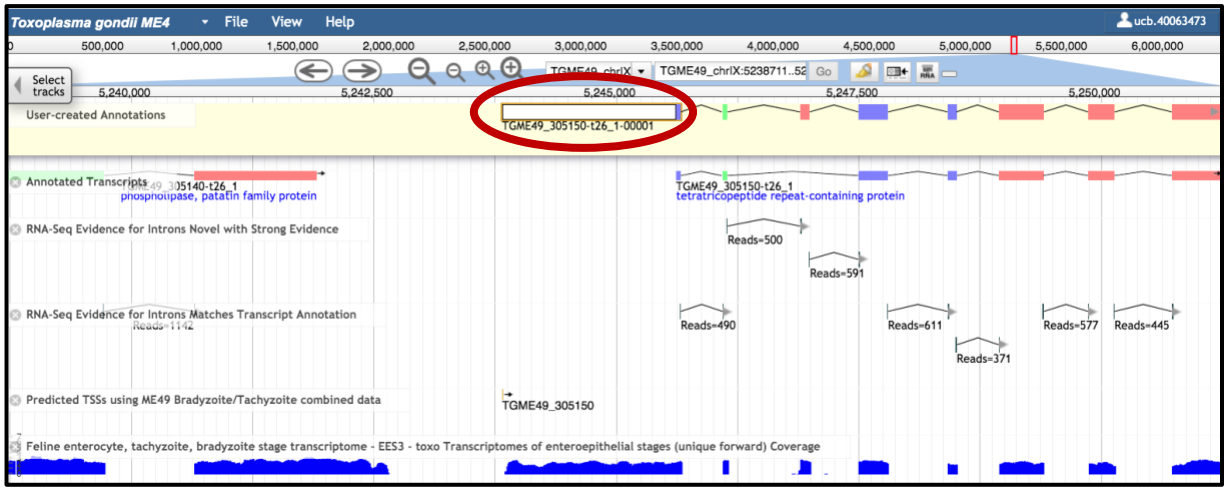
You can either select the intron junctions individually, or hold down the shift key and select both intron junctions with strong evidence (**1**), drag and drop them into the gene model (**2**). The gene will get a green box when dragging and dropping the intron evidence.



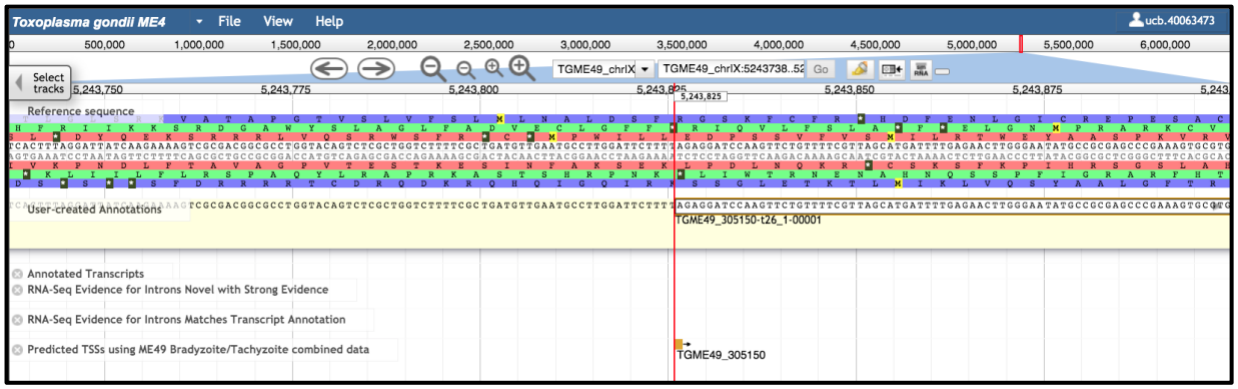
Zoom in by clicking on the **+** sign on the top (**1**). Press the Shift key and select the two small exons in the middle (**2**). With a right-click open the drop-down menu and choose **Merge** (**3**). Alternatively, select one of the exons you would like to merge, go to the edge of the feature until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



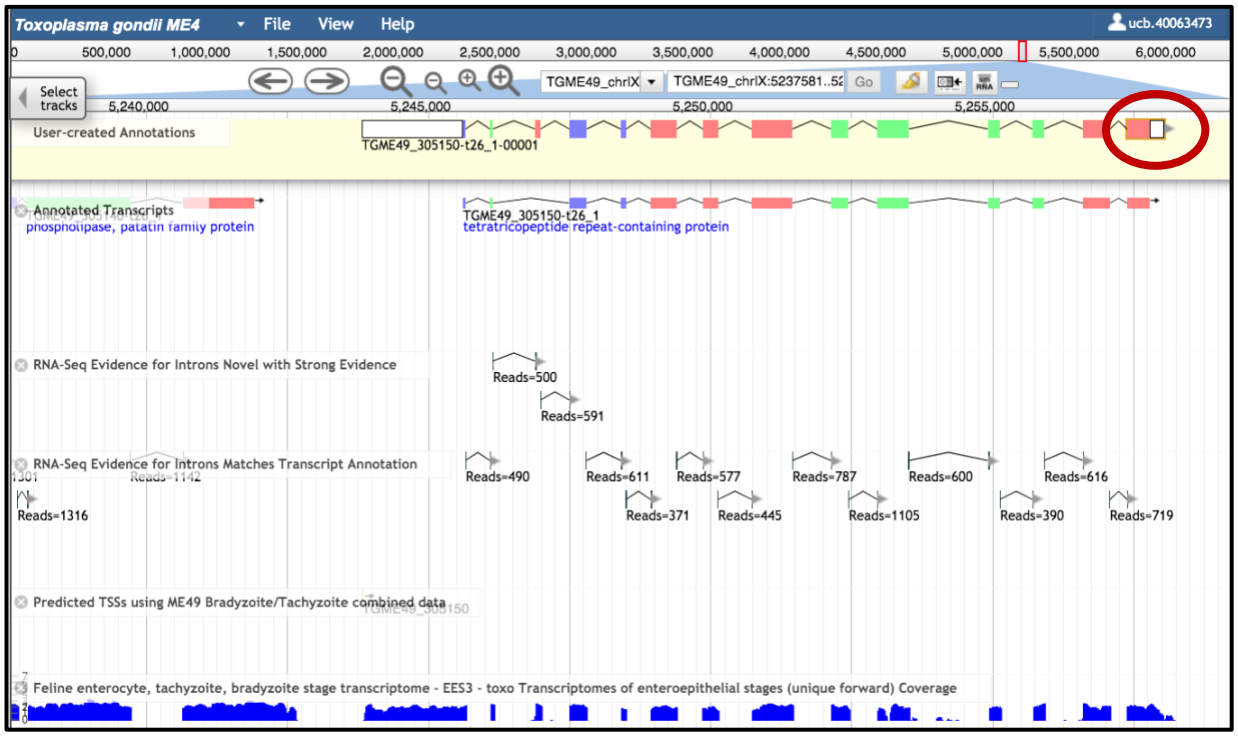
Select the first exon, point your mouse at the edge of the feature, a little arrow will appear, then extend the exon to the transcription start. Apollo will automatically create the 5'UTR!



You can zoom in to recheck the transcription start site.

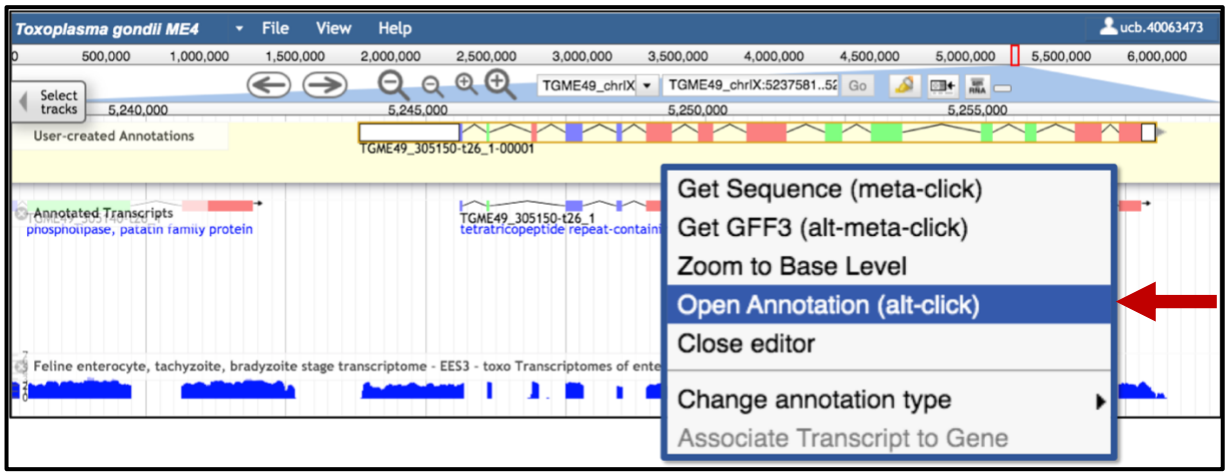


Select the last exon, point your mouse at the edge and extend the exon. Apollo will create the 3'UTR automatically!

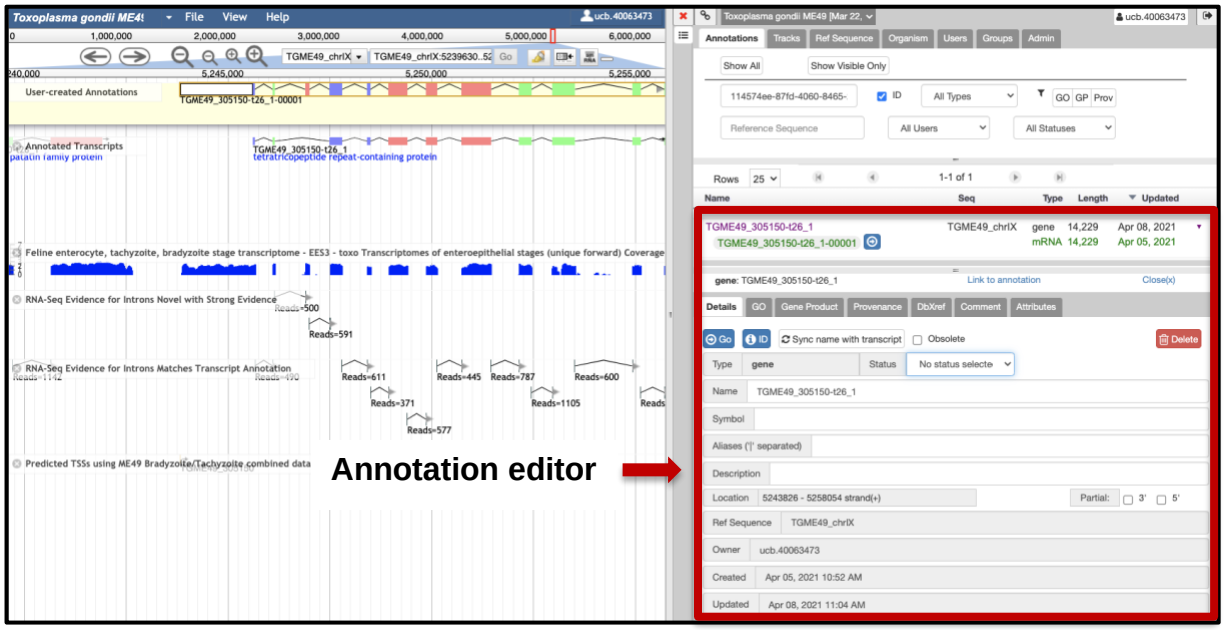


4) Opening of the Annotation editor window

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**.

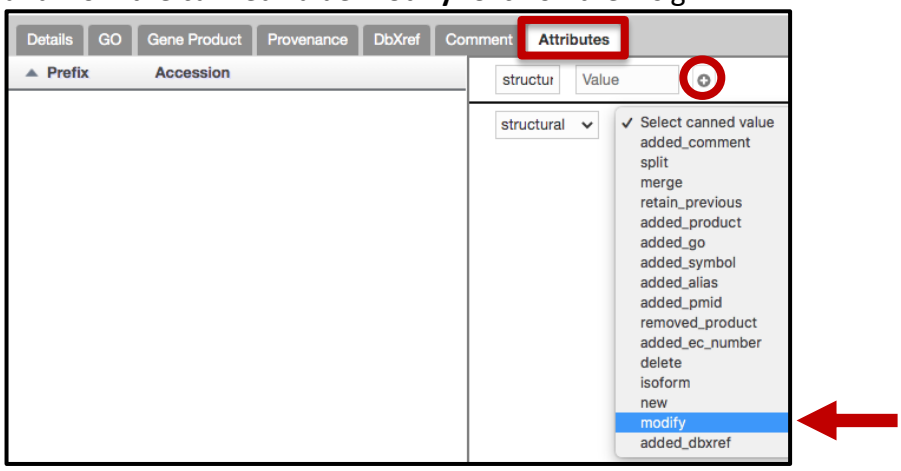


The annotation editor window is now shown on the right-hand side.

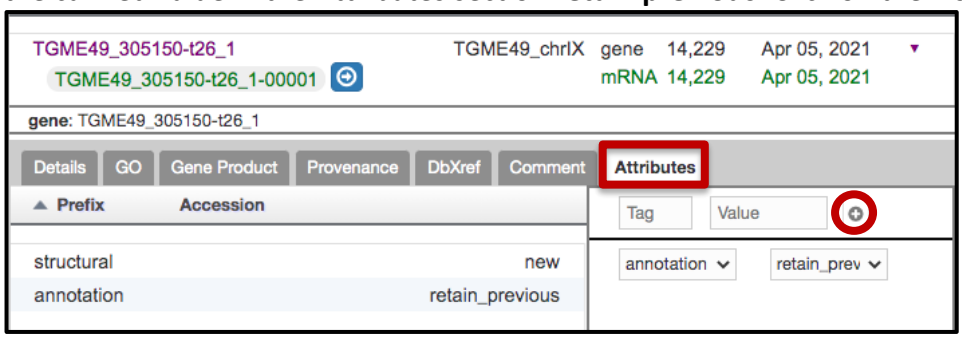


5) Finalizing the structural annotation

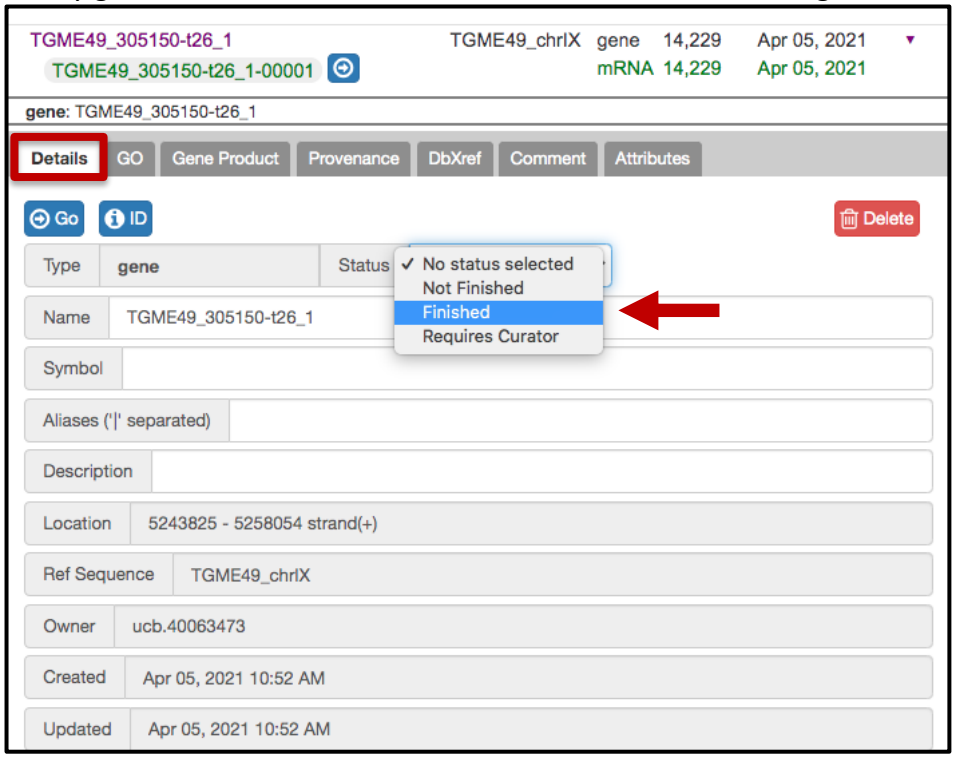
Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **modify**. Click on the **+** sign.



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.

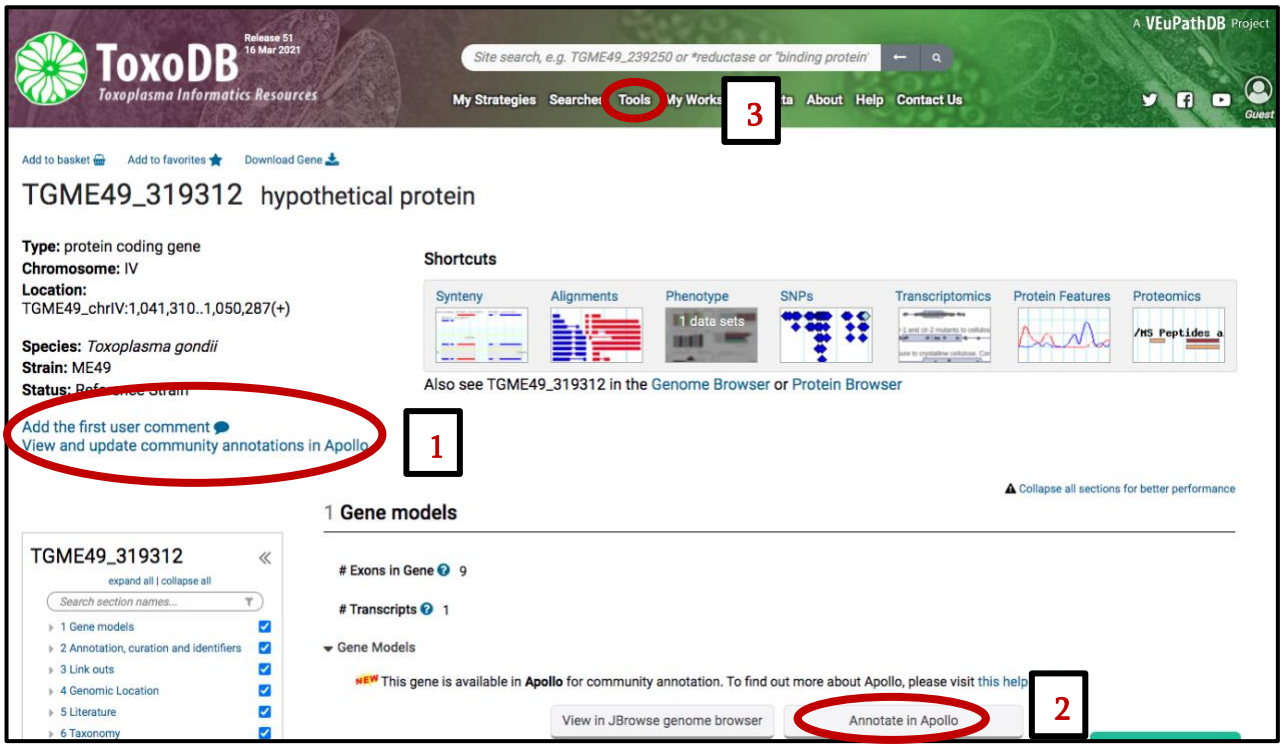
Done! For additional questions, please get in touch with the VEuPathDB help desk.

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

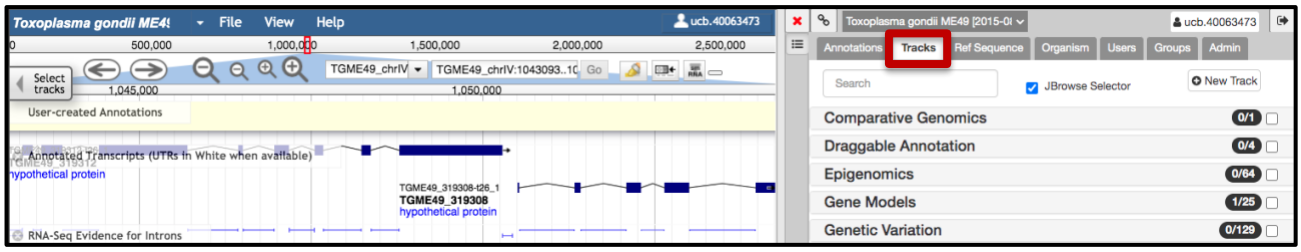
To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo** (**1**). You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo** (**2**). Alternatively, go to the **Tools**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



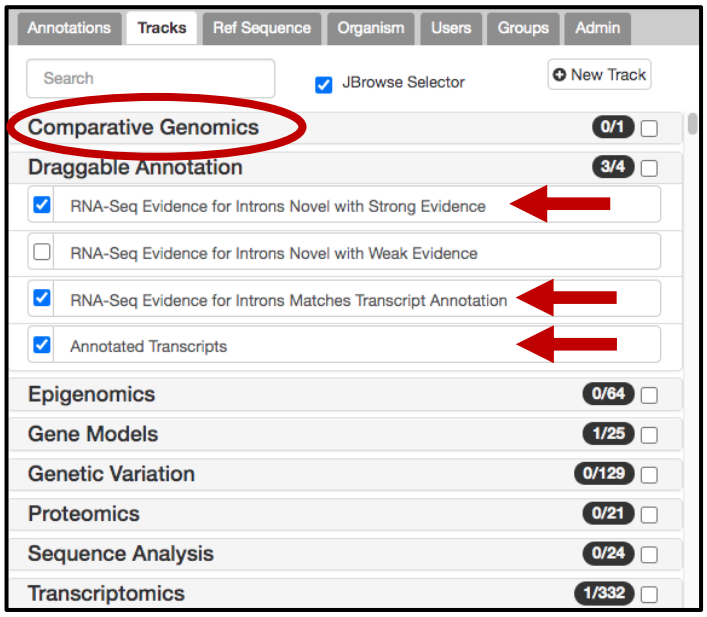
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

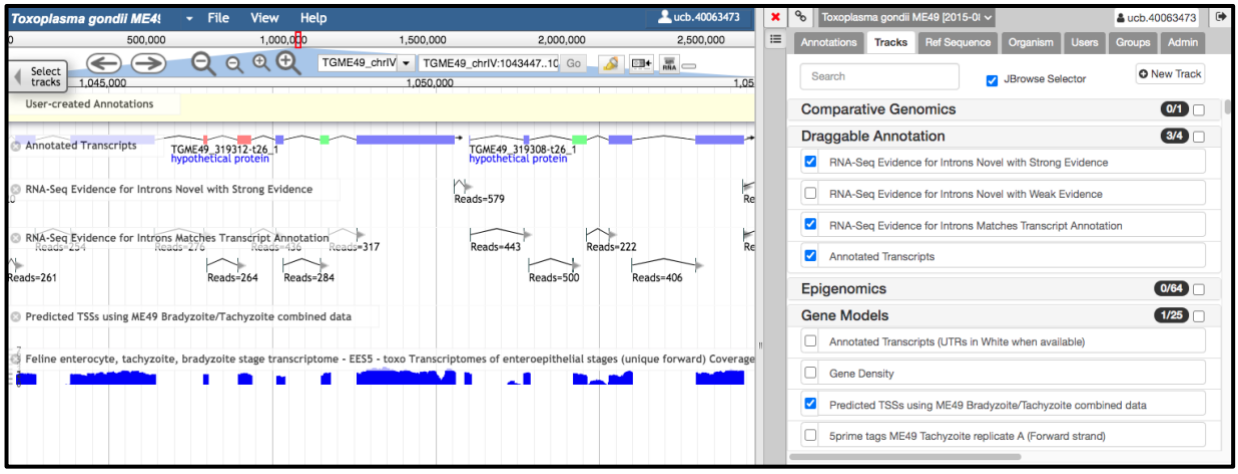
Select on the right-hand side the tab **Tracks.**



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**.

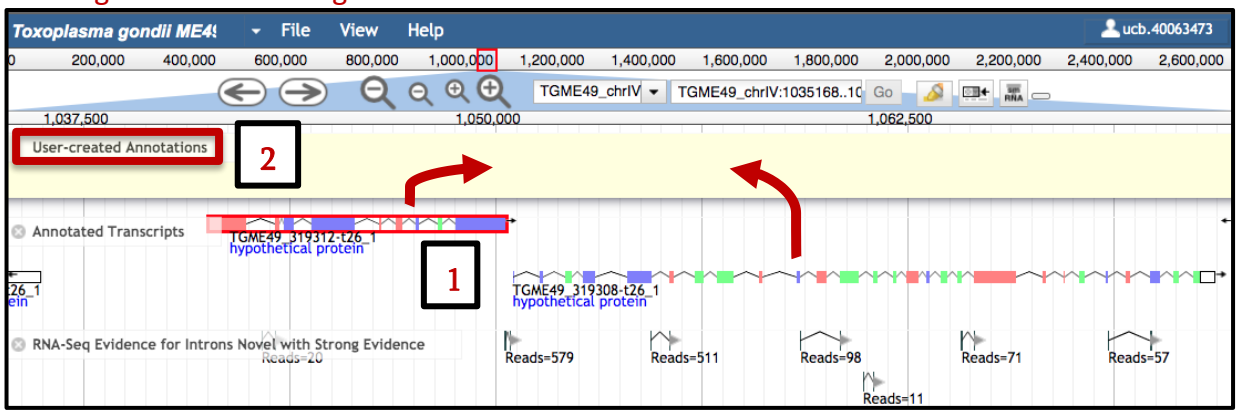


Select additional evidence, i.e. RNAseq plots and predicted TSS (transcription start sites).

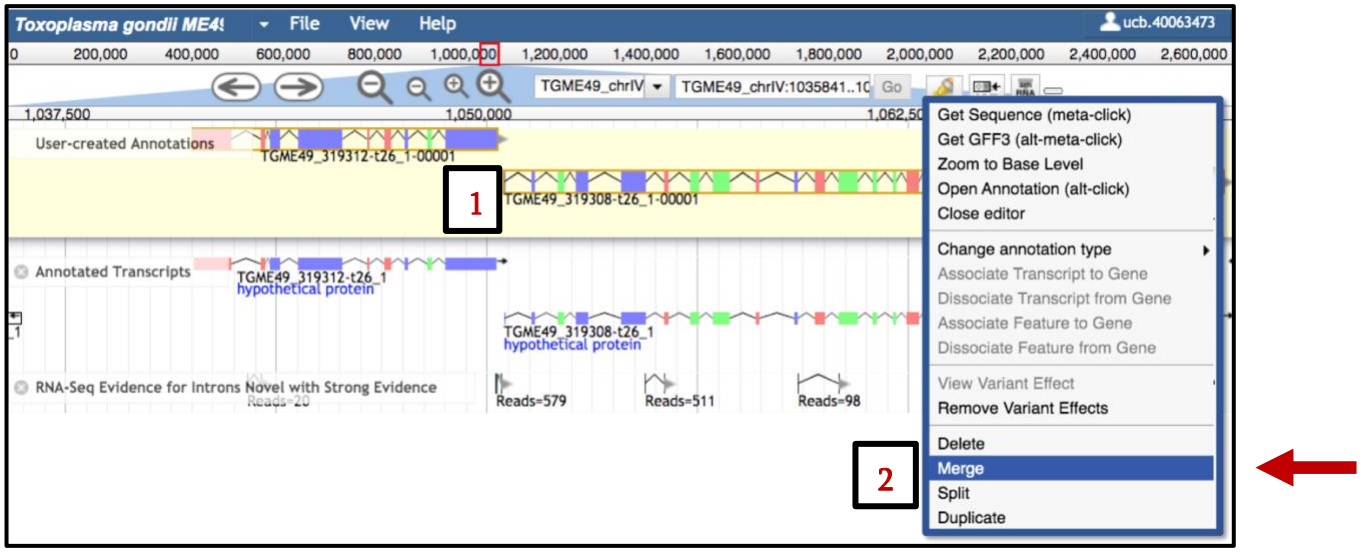


3) Merging genes

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.

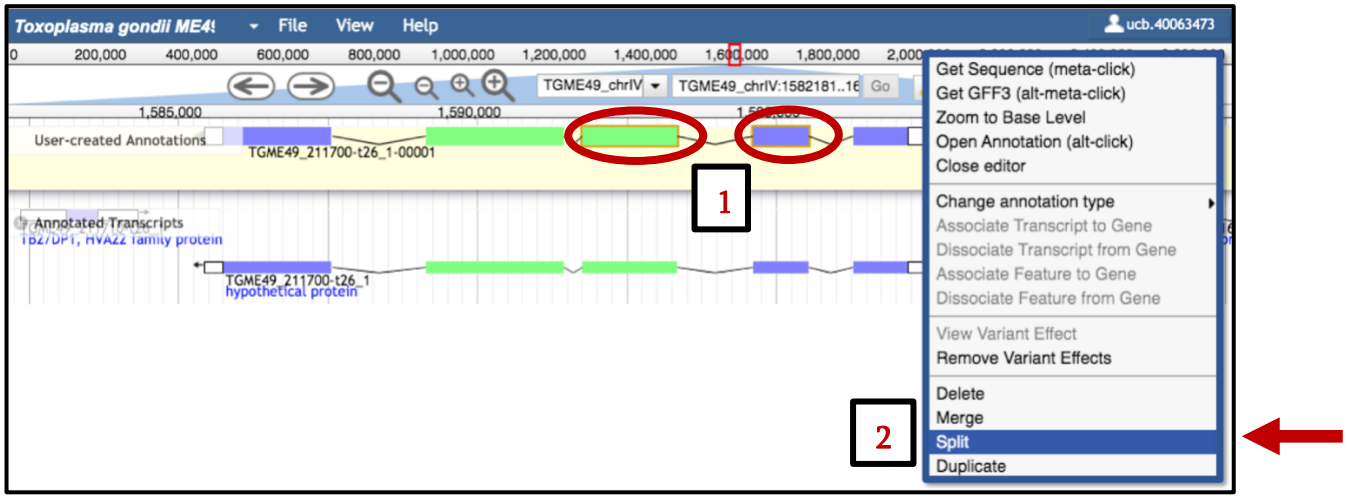


Hold down the shift key and select both gene models in the User-created Annotations track (**1**). With a right-click open the drop-down menu and choose **Merge** (**2**).



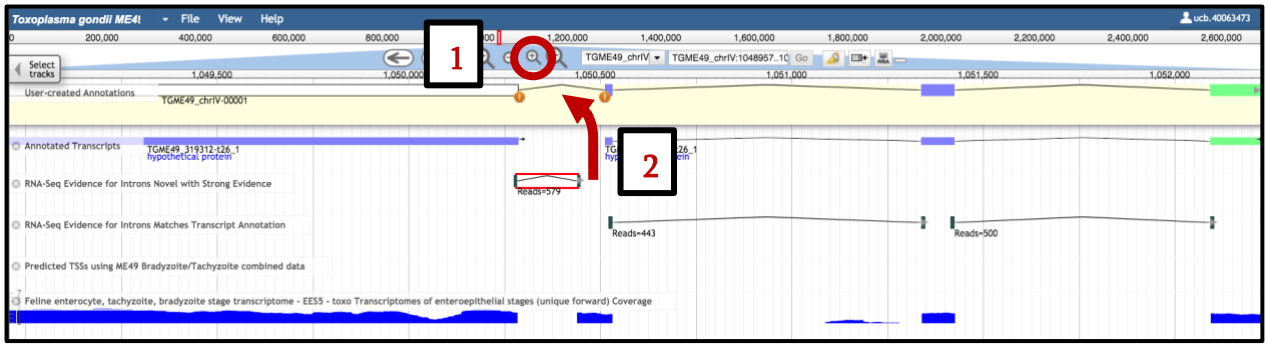
3.1) Splitting gene models

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.

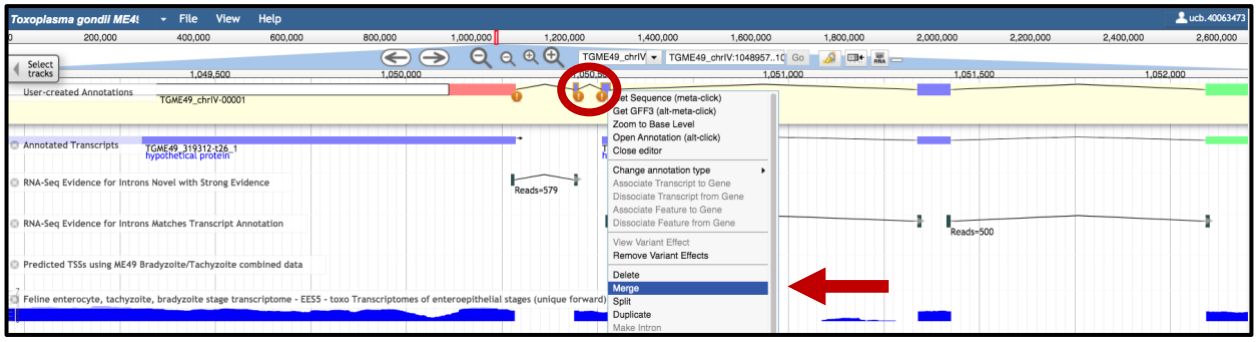


4) Correcting intron-exon boundaries

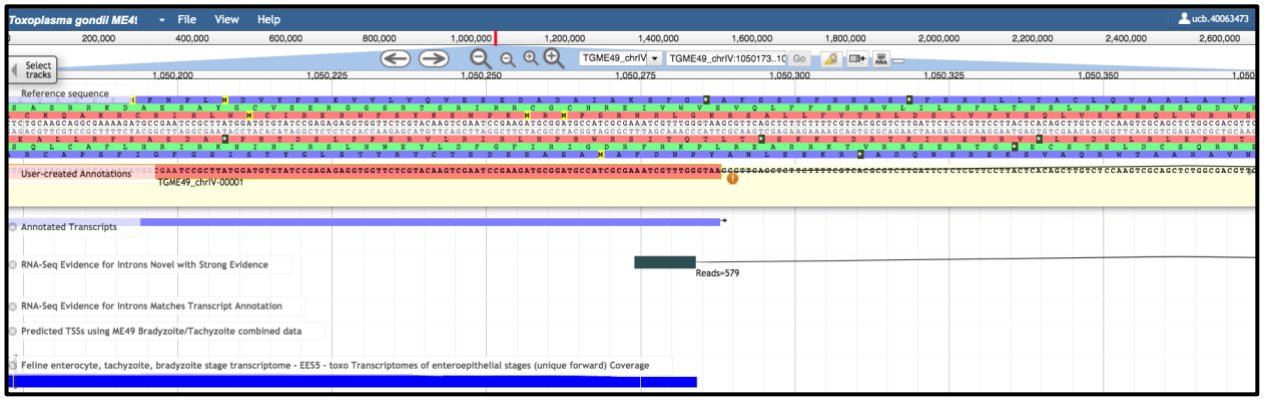
Once you've merged the gene zoom in by clicking on the **+** sign on the top (**1**). Select the new splice junction and drag it into the gene model (**2**).



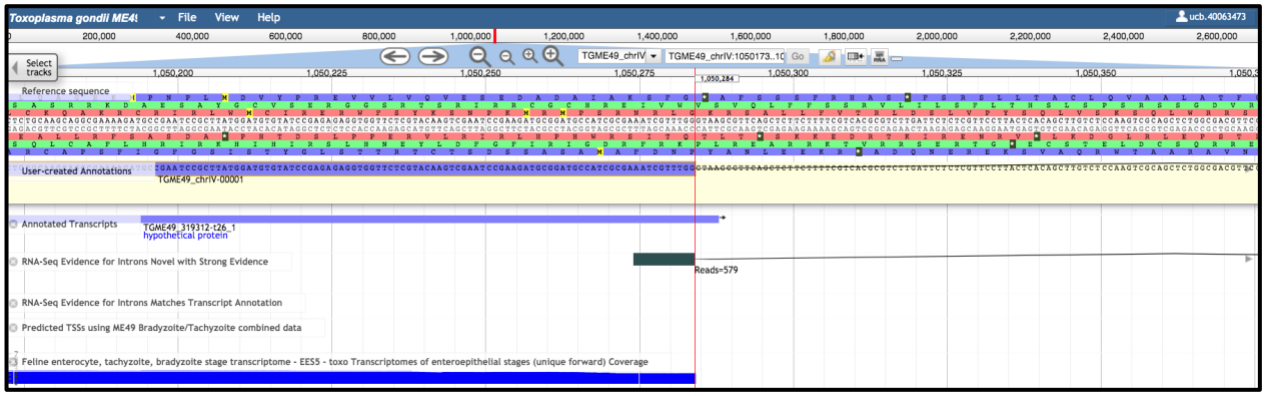
Hold down the shift key and select the two small exons. With a right-click open the drop-down menu and select merge. Hint: The exclamation mark tells you that there are non-canonical splice sites.



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

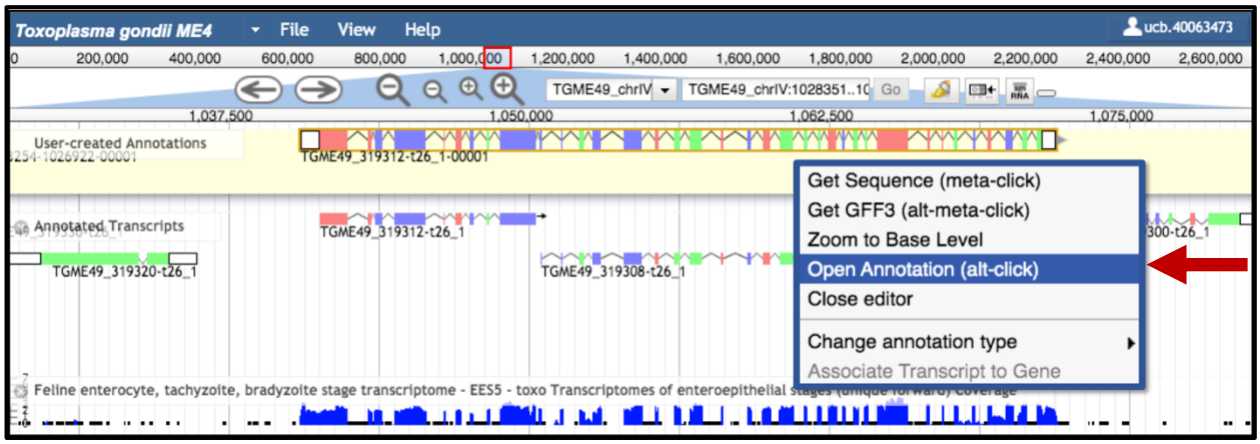


Shorten the exon to the correct splice site.

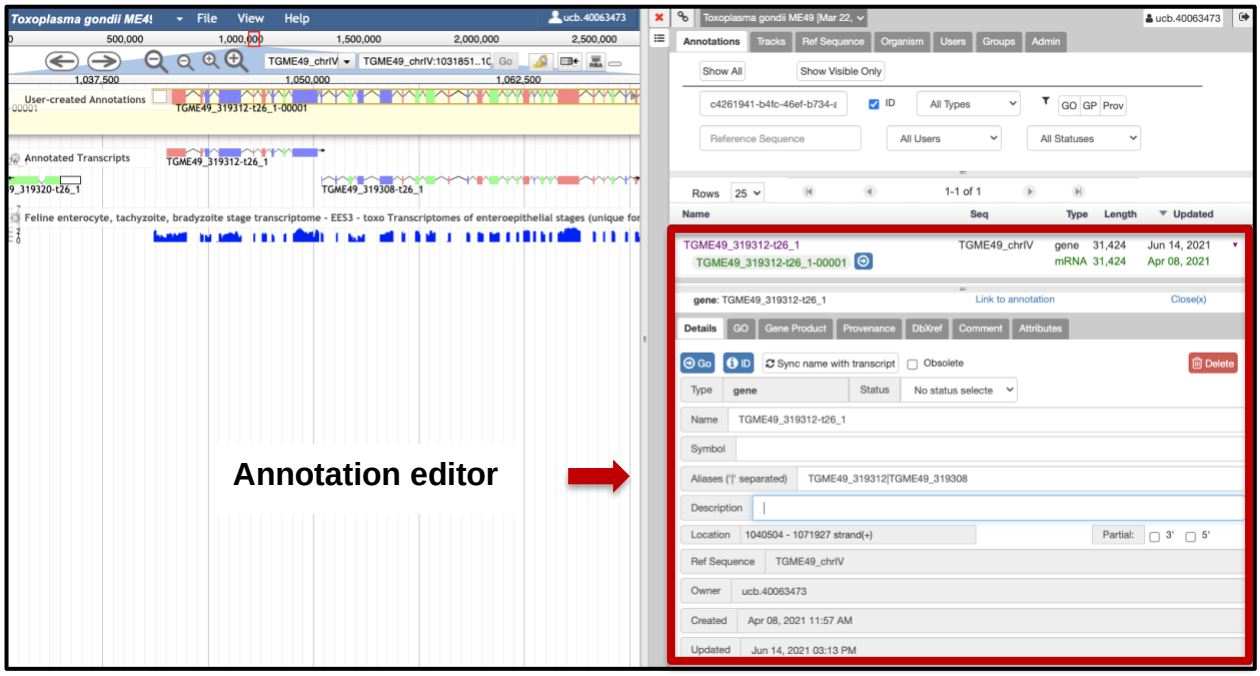


5) Opening of the Annotation editor window

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**.

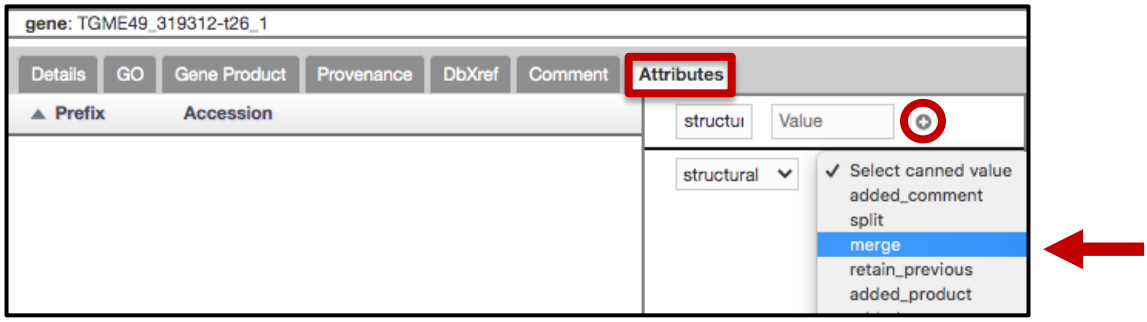


The annotation editor window is now shown on the right-hand side.

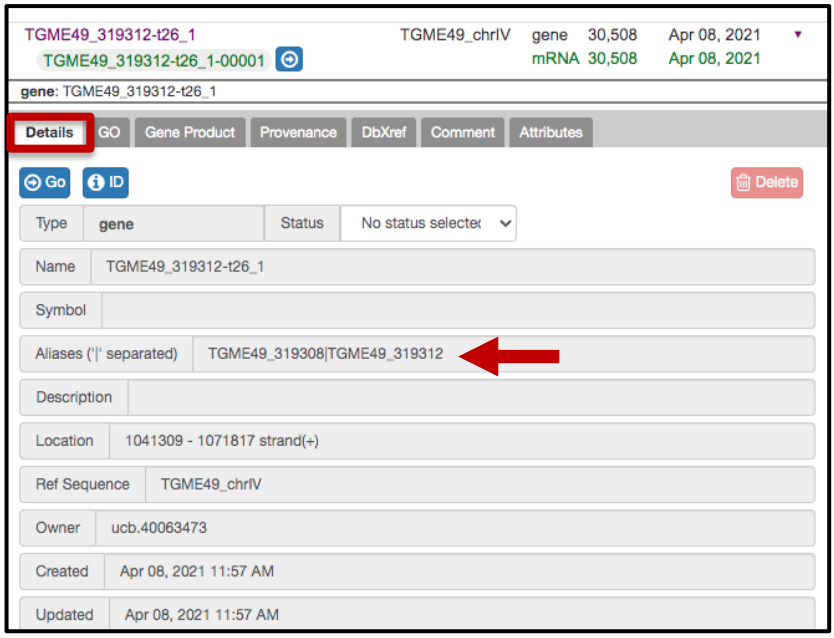


6) Finalizing the structural annotation

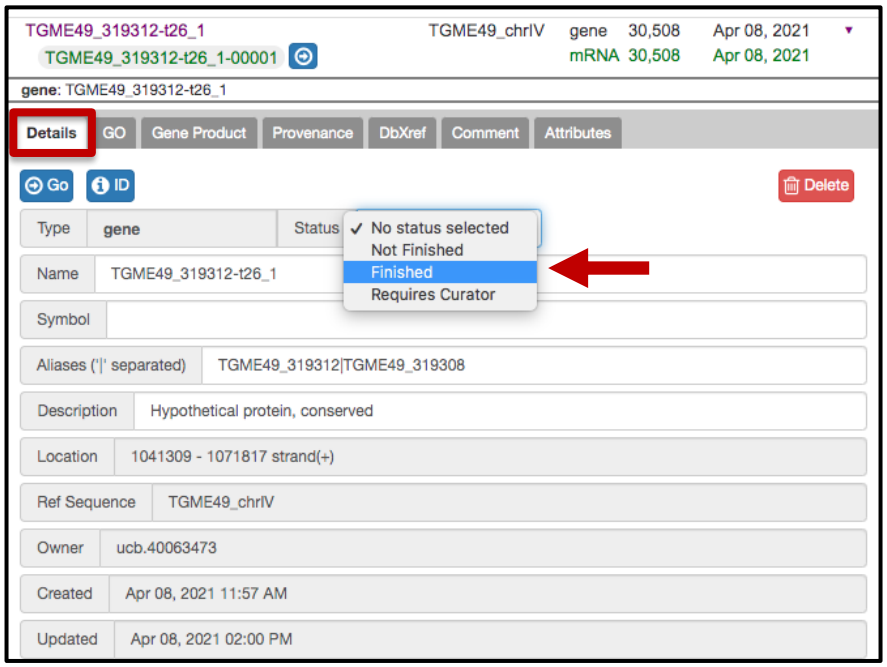
Once the annotations panel is open click on the Attributes tab, select from the canned tag **structural** and from the canned value **merge**. Click on the **+** sign.



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



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.



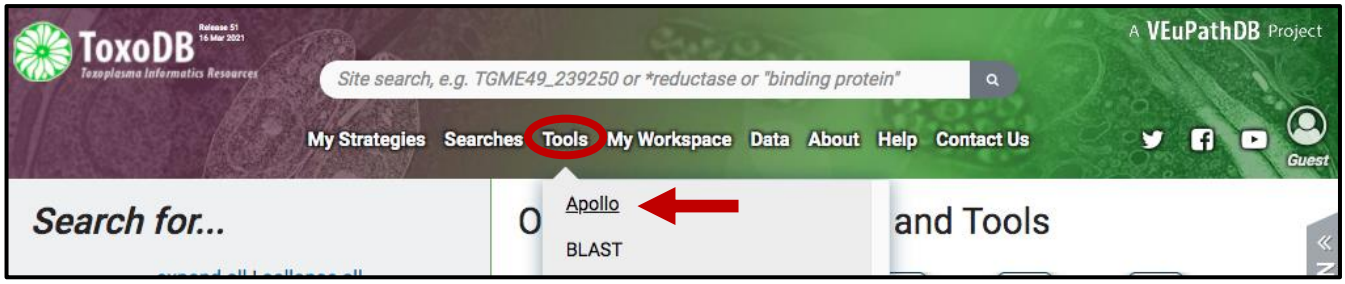
Done! For additional questions, please get in touch with the VEuPathDB help desk.

Structural annotation in Apollo
Adding a new gene

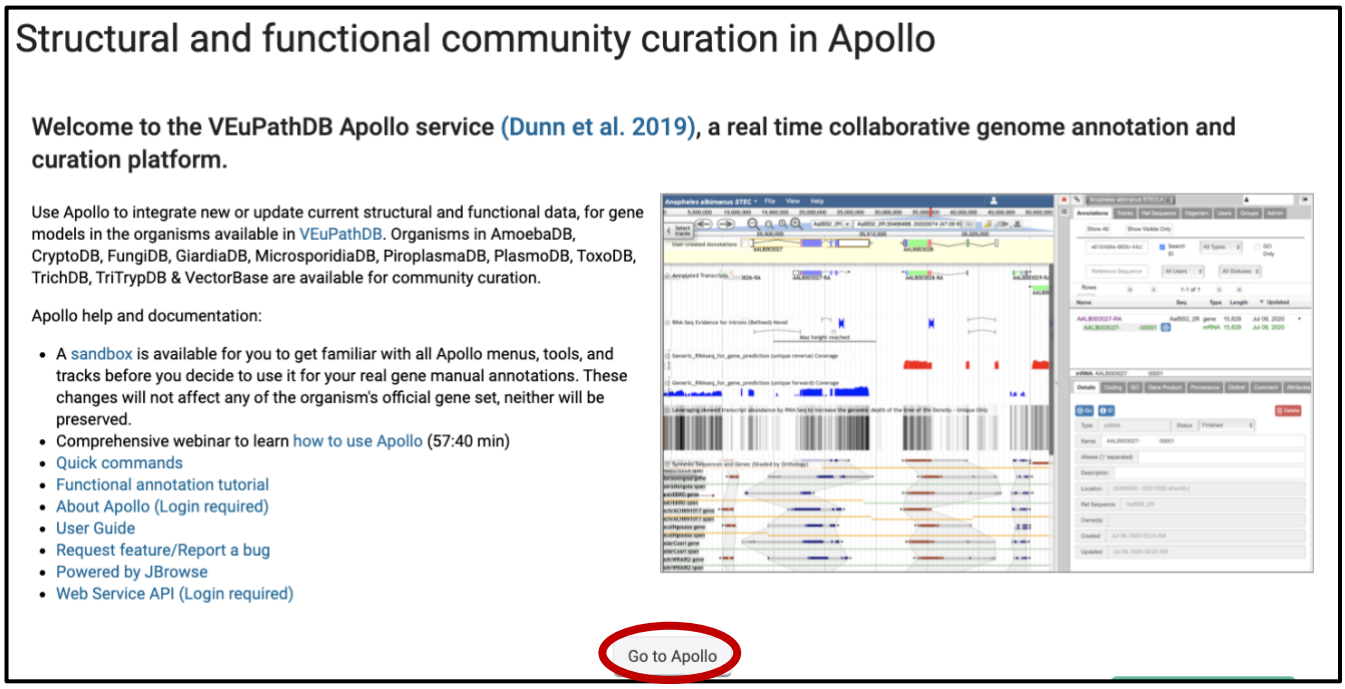
In this short tutorial we are showing you step-by-step how to add a new gene in Apollo.

1) Accessing Apollo

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



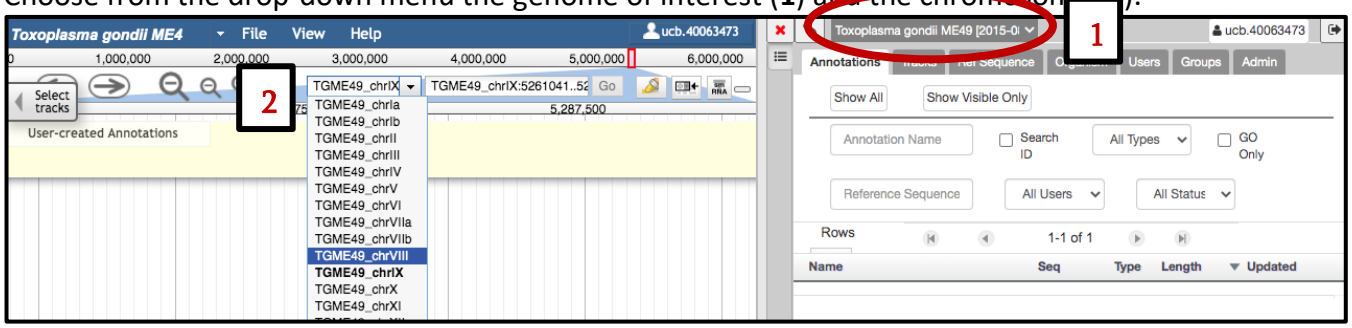
Click on the button **Go to Apollo**.



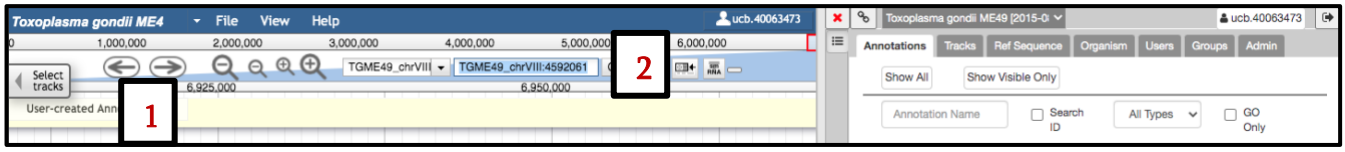
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Navigate to the genome and chromosome coordinates

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

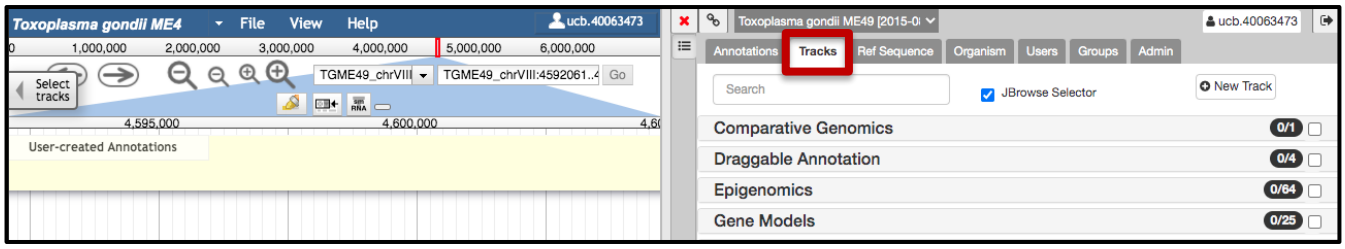


Go to the coordinates with the missing gene. Use the arrows to navigate to the coordinates (**1**), or type the coordinates in the search box (**2**).

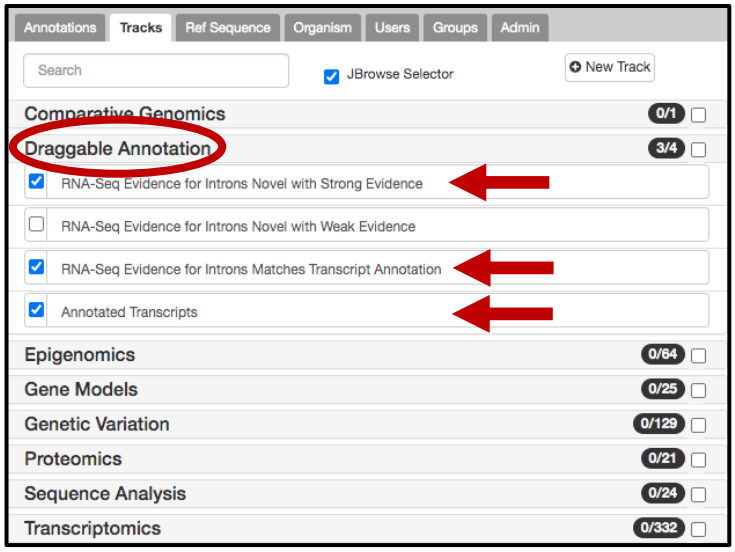


3) Adding draggable annotation and supporting evidence

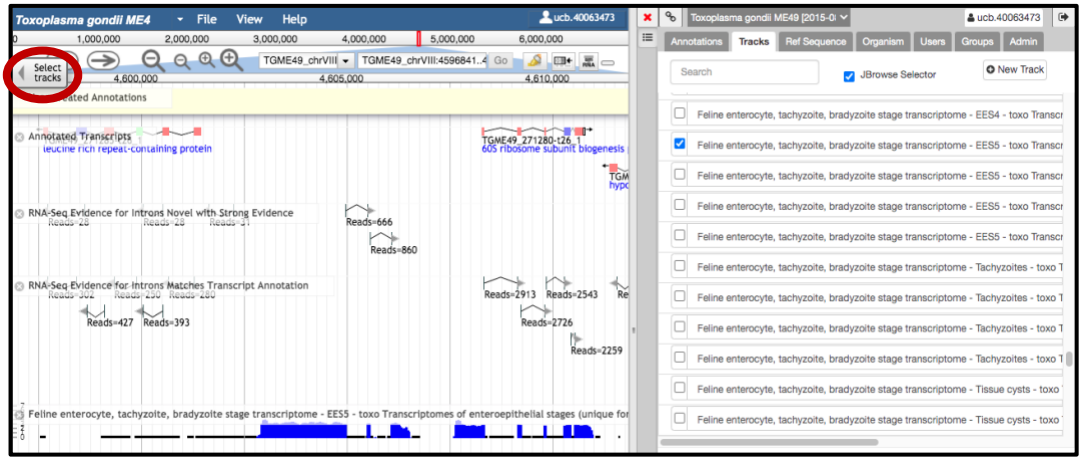
Select on the right-hand side the tab **Tracks.**



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**.

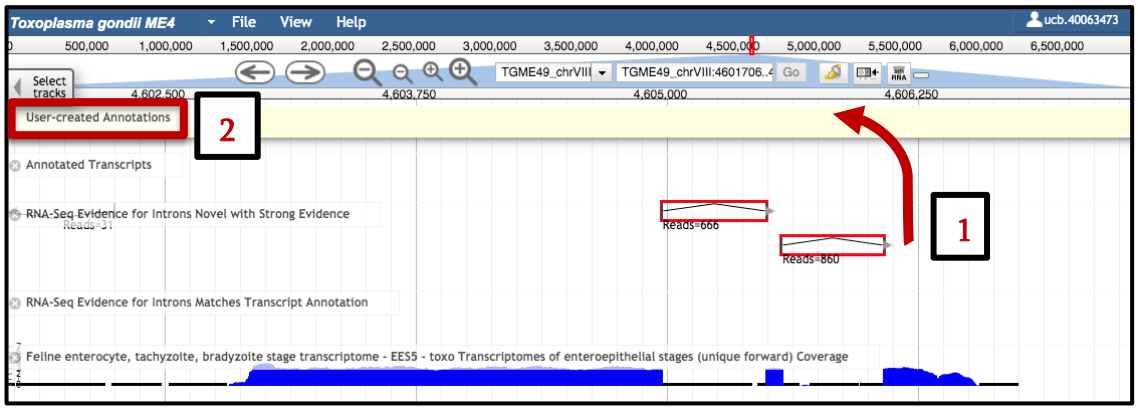


Select additional evidence, i.e. RNAseq plots from the Transcriptomics section. Alternatively, you can select evidence from the JBrowse menu by clicking on **Select tracks**.

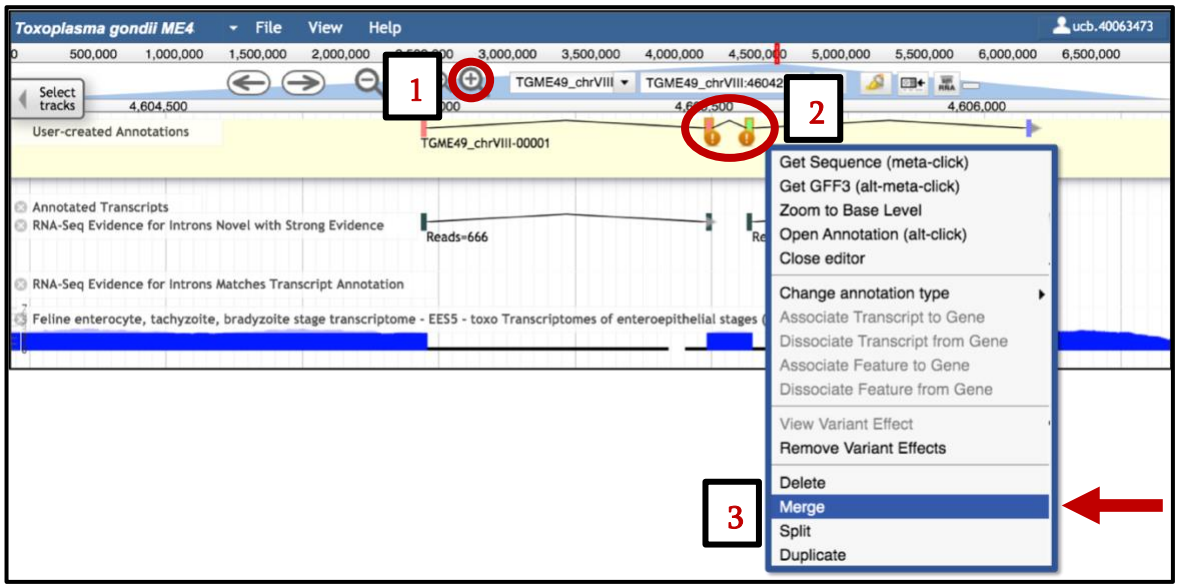


4) Building the new gene

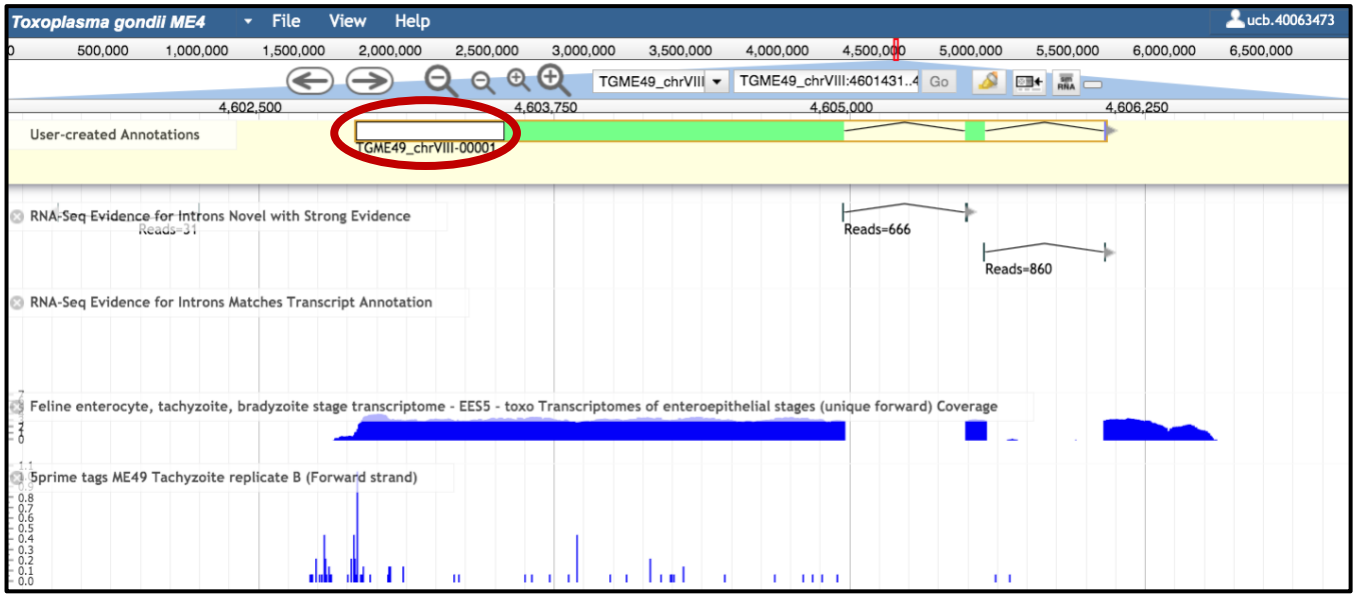
Hold down the shift key and select the introns with strong evidence (**1**), drag and drop them into the User-created Annotations track (**2**).



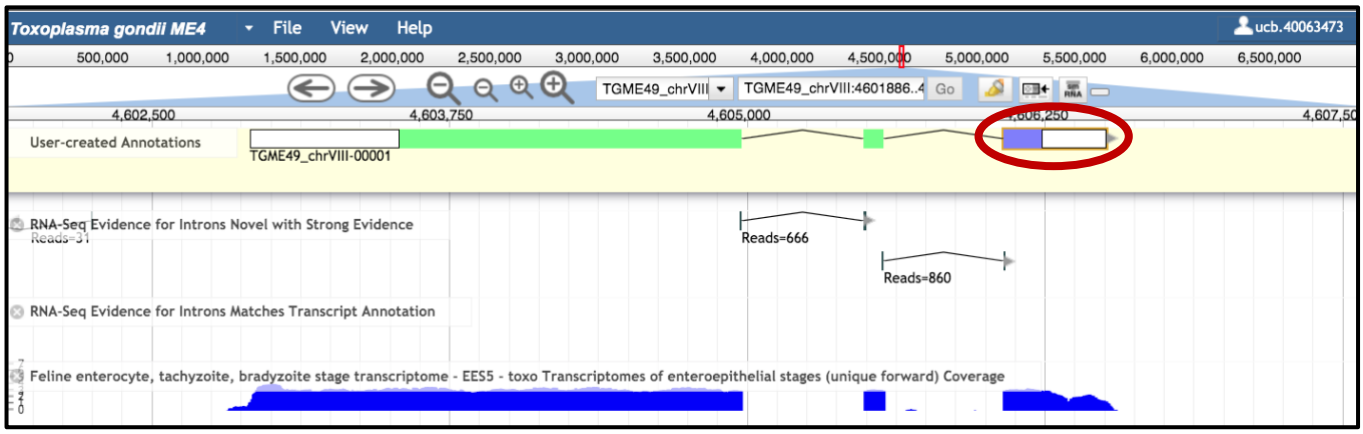
Zoom in by clicking on the **+** sign on the top (**1**). Press the Shift key and select the two small exons in the middle (**2**). With a right-click open the drop-down menu and choose **Merge** (**3**). Alternatively, select one of the exons you would like to merge, go to the edge of the feature until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



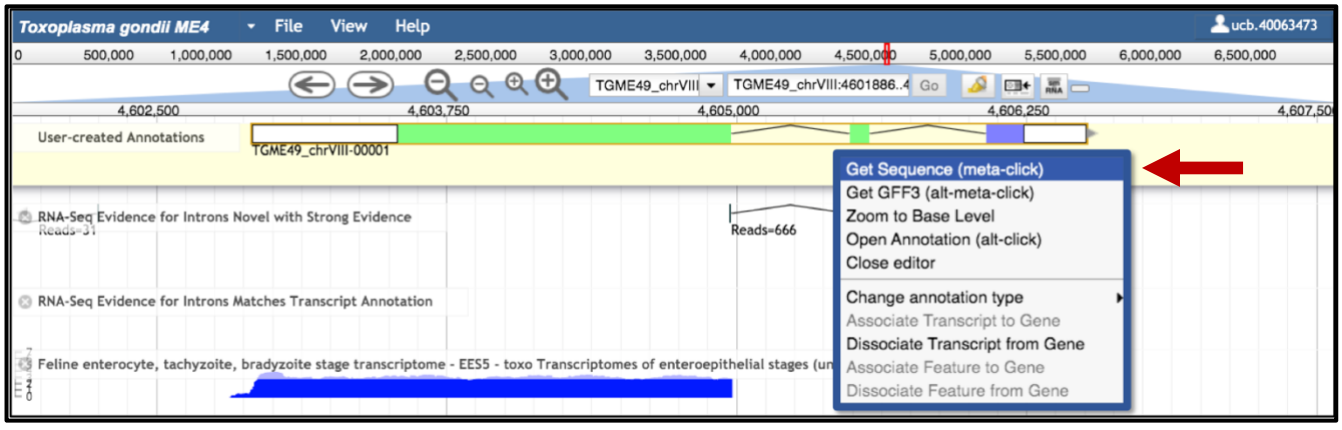
Select the first exon, point your mouse at the edge of the feature until a little arrow appears, then extend the exon to the transcription start. Apollo will automatically create the 5'UTR!



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!

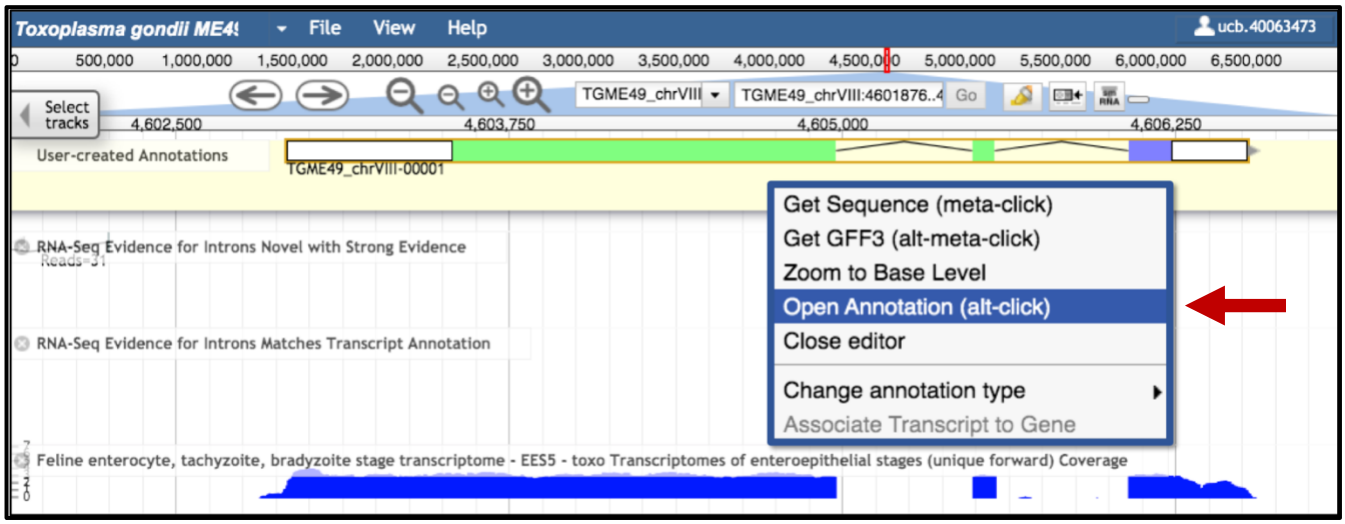


Select the new gene, with a right-click open the annotation drop-down men and choose **Get Sequence**. Copy the sequence and run blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and InterPro (https://www.ebi.ac.uk/interpro) to get additional information about the new gene.

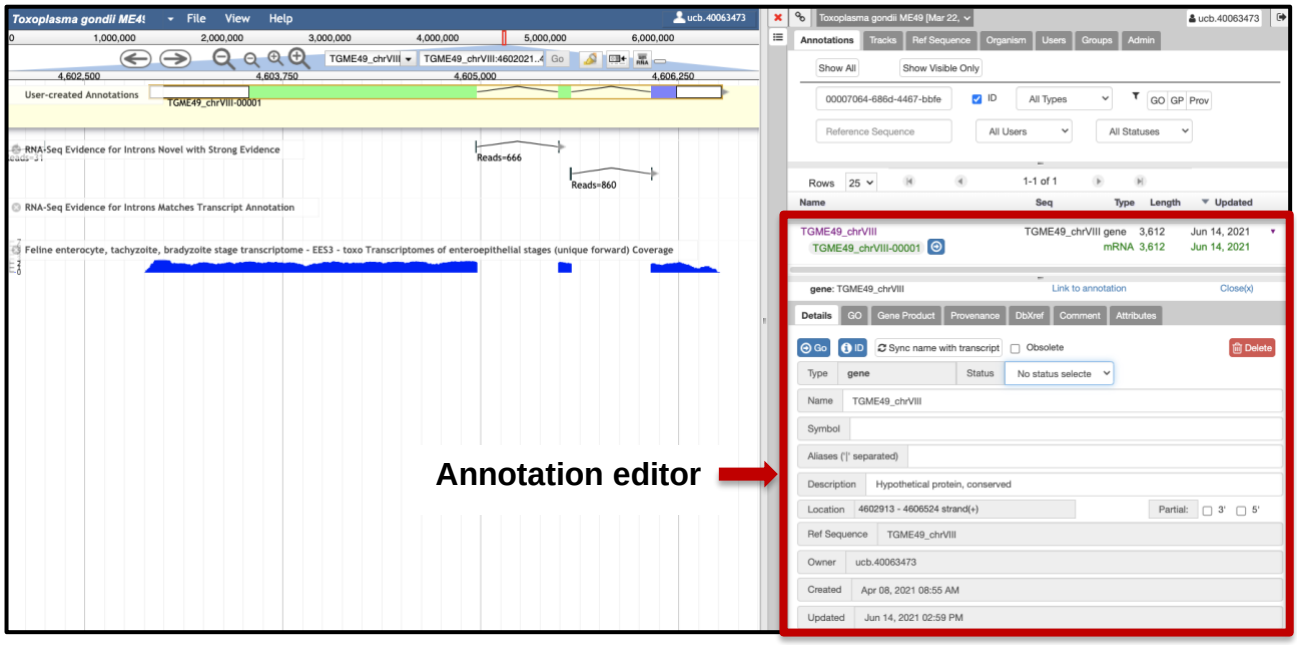


5) Opening of the Annotation editor window

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**.

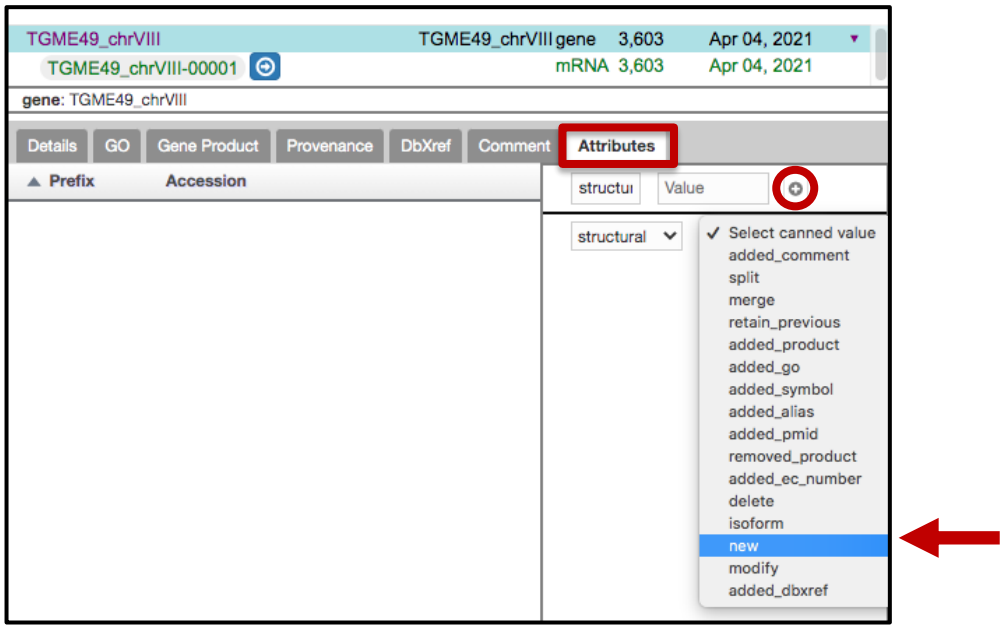


The annotation editor window is now shown on the right-hand side.

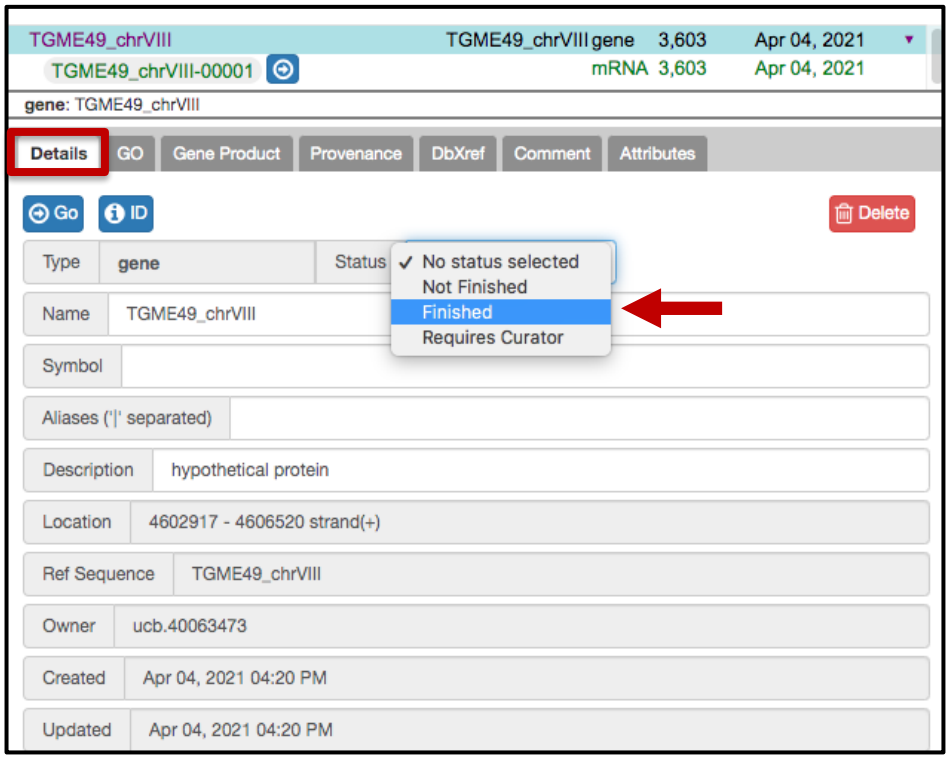


6) Finalizing the structural annotation

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.



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.



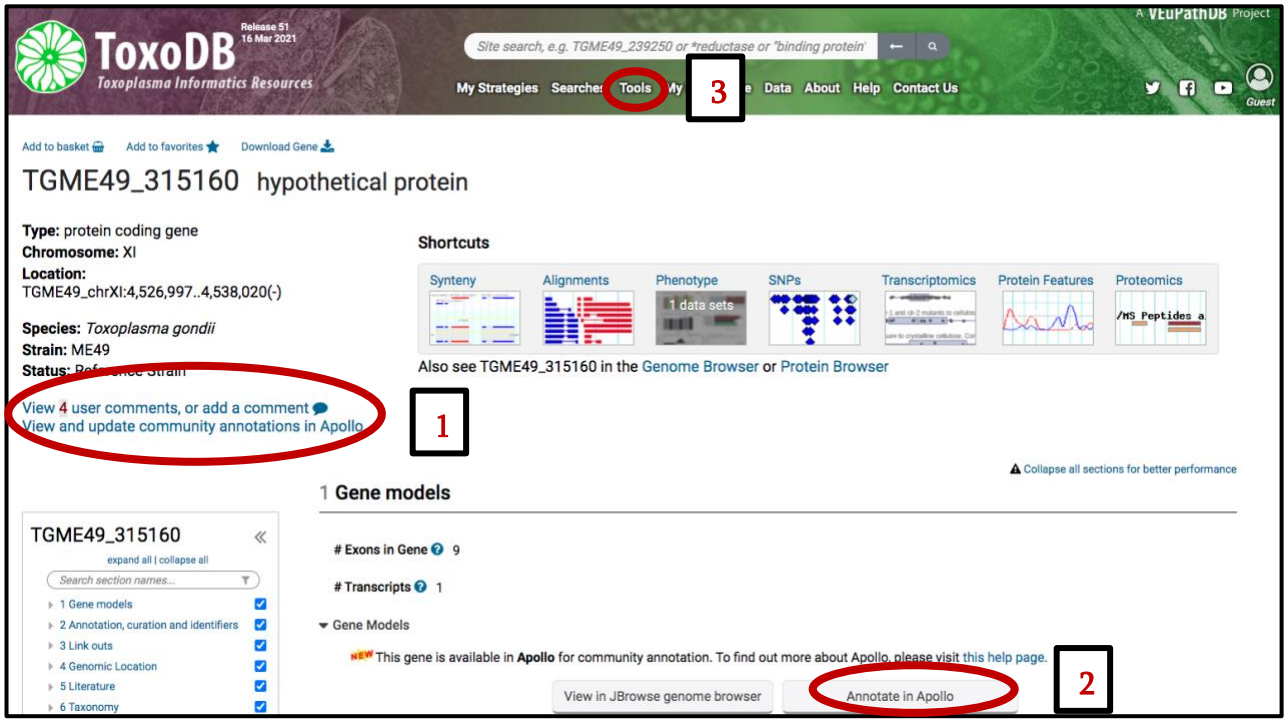
Done! For additional questions, please get in touch with the VEuPathDB help desk.

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

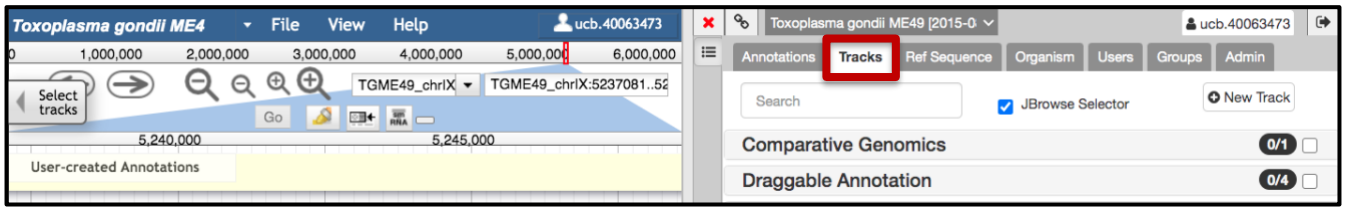
To access Apollo go to the gene record page of your gene of interest and click on the link **View and update community annotations in Apollo** (**1**). You can also access Apollo from the gene models section by clicking on the button **Annotate in Apollo** (**2**). Alternatively, go to the **Tools**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



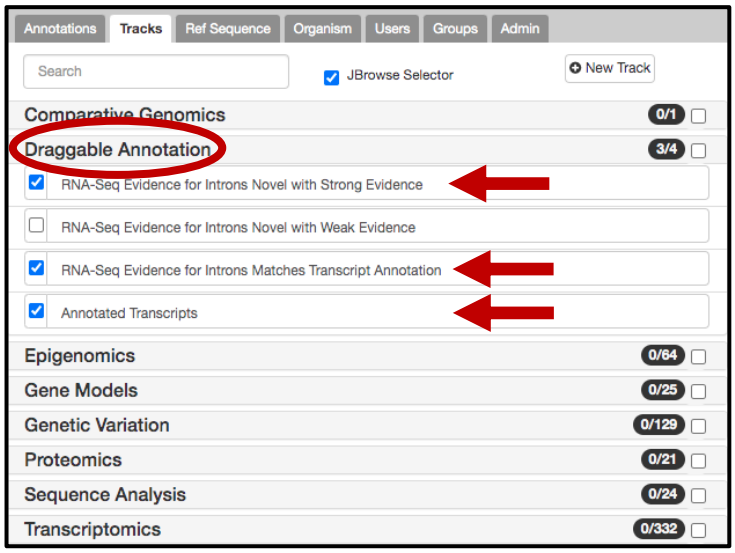
To use Apollo you need to be logged into VEuPathDB. If you have not done so yet log now into Apollo with your VEuPathDB user ID and password.

2) Adding draggable annotation and supporting evidence

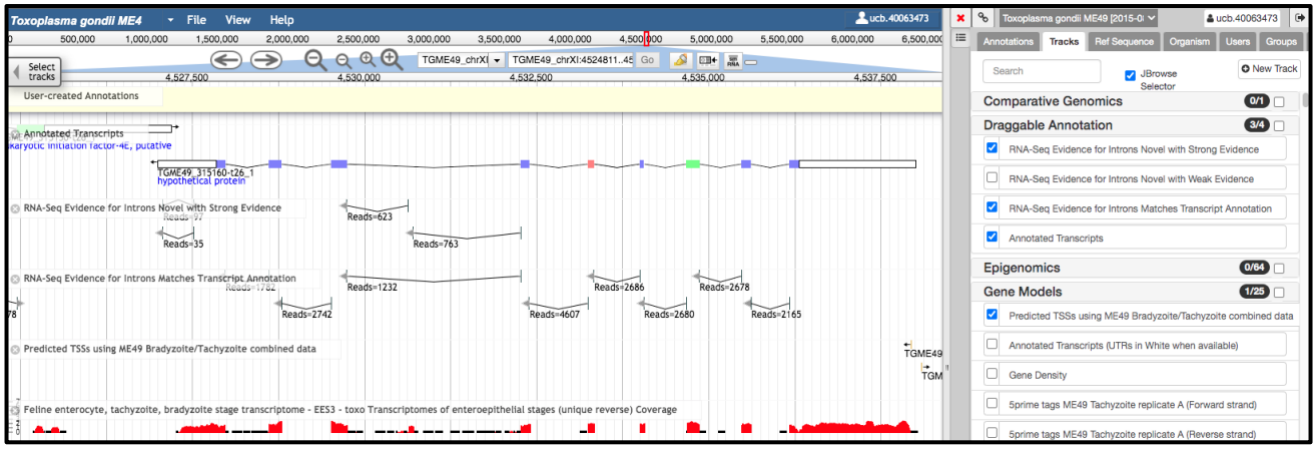
Select on the right-hand side the tab **Tracks.**



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**.

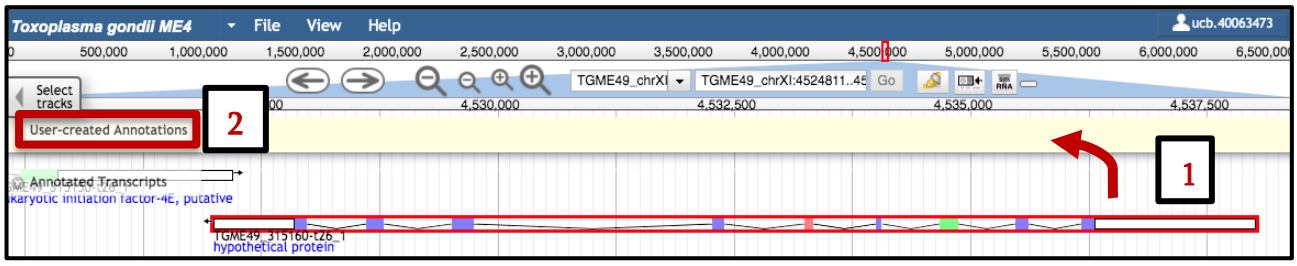


Select additional evidence, i.e. RNAseq plots and predicted TSS (transcription start sites).

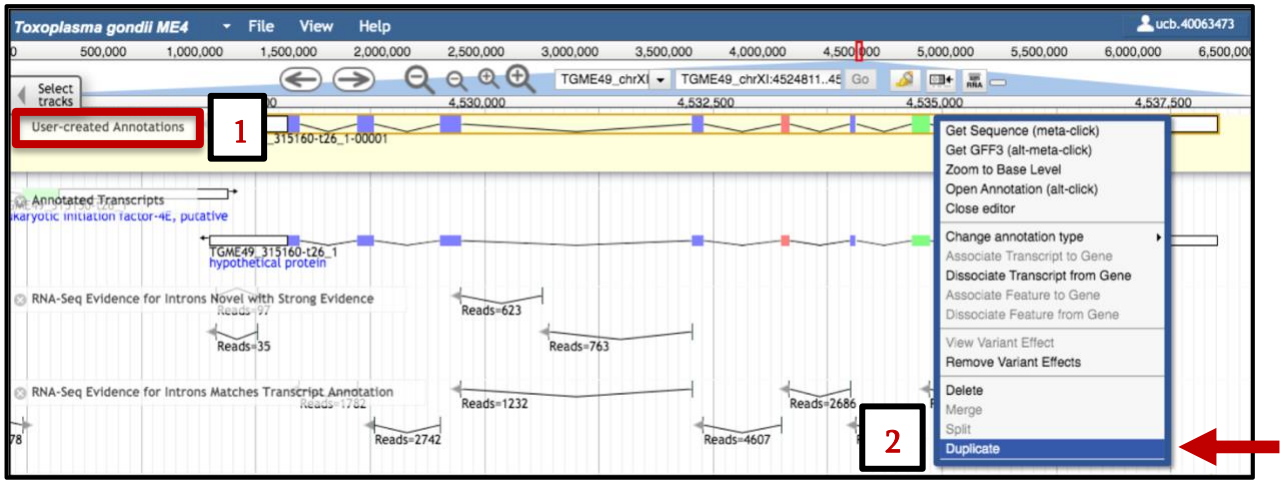


3) Adding alternative transcripts

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.

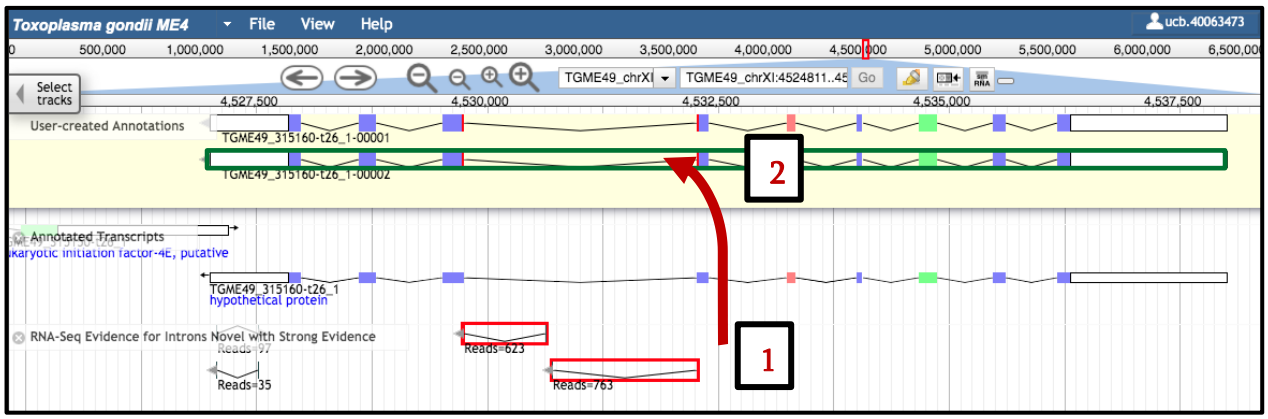


Select the gene in the User-created Annotations area (**1**). With a right-click open the annotation drop-down menu and choose duplicate (**2**).

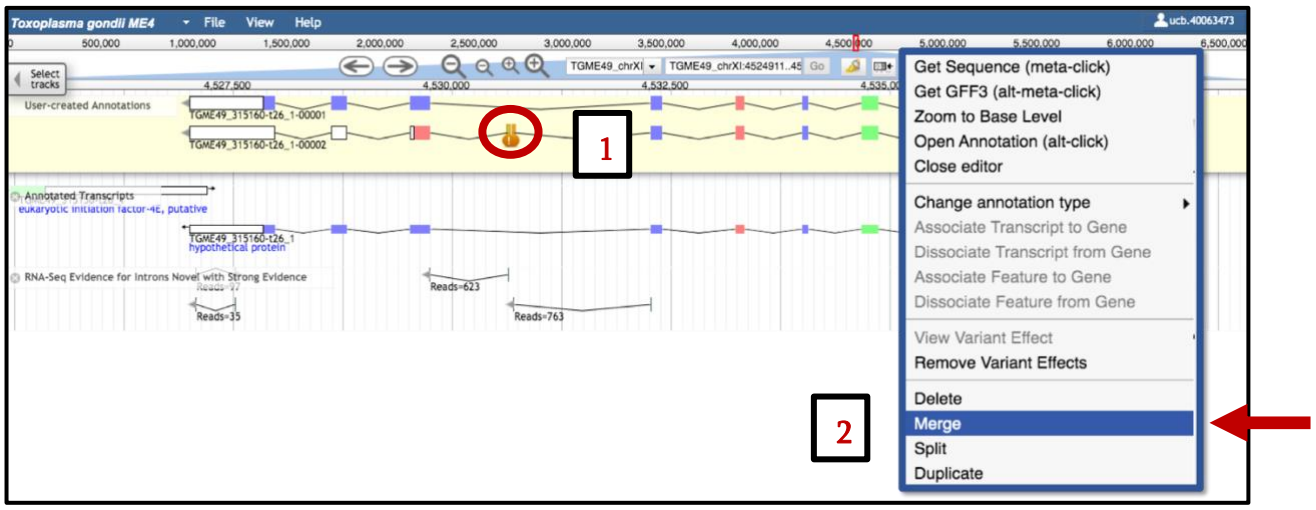


4) Modifying the alternative transcript

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.

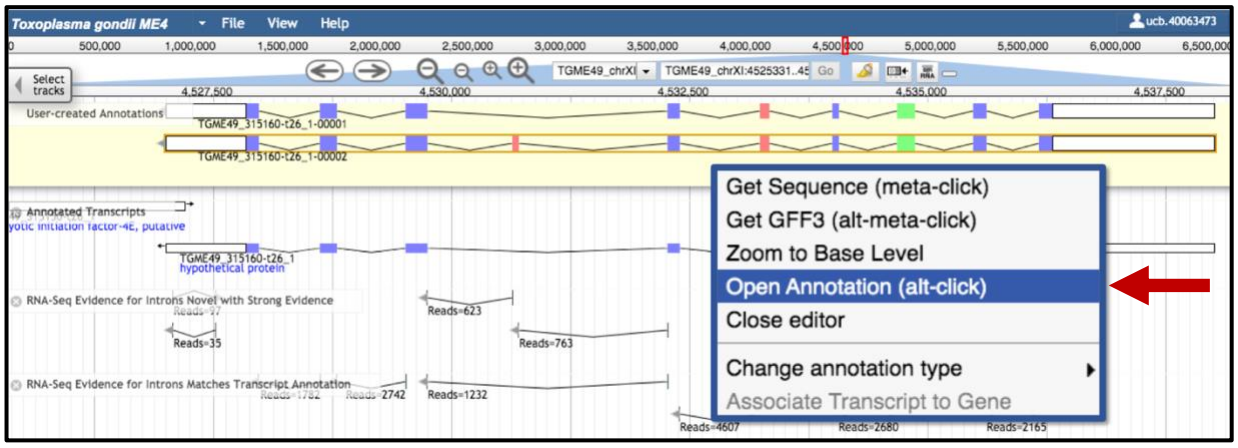


Hold down the shift key and select the two small exons. With a right-click open the drop-down menu and select merge.

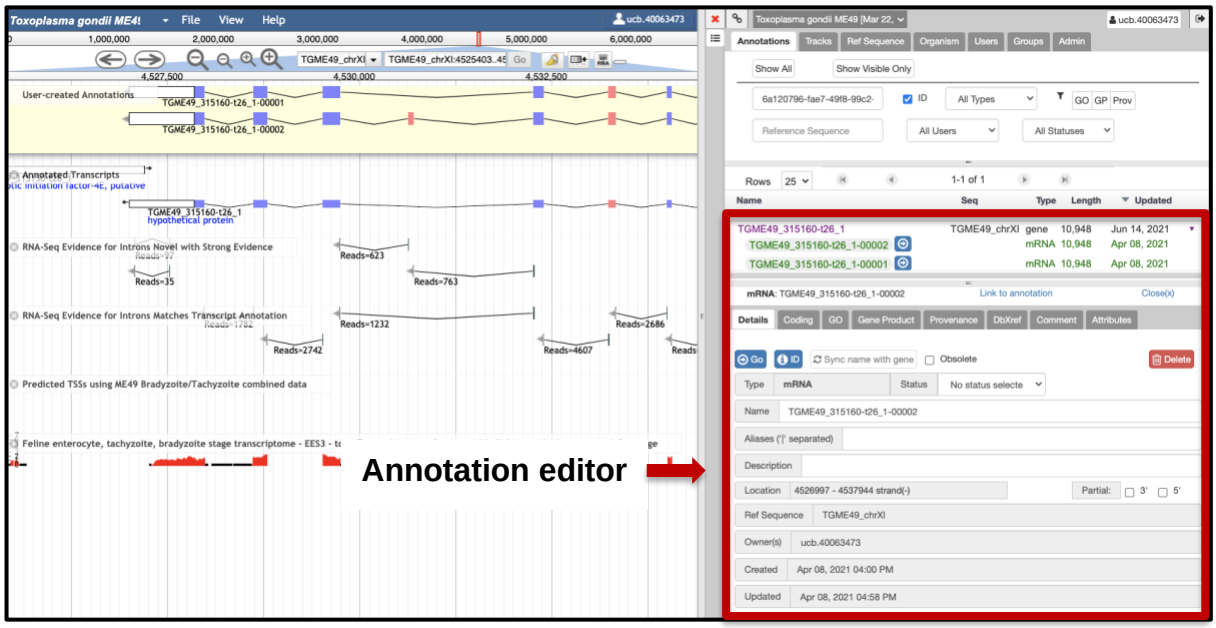


5) Opening of the Annotation editor window

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**.

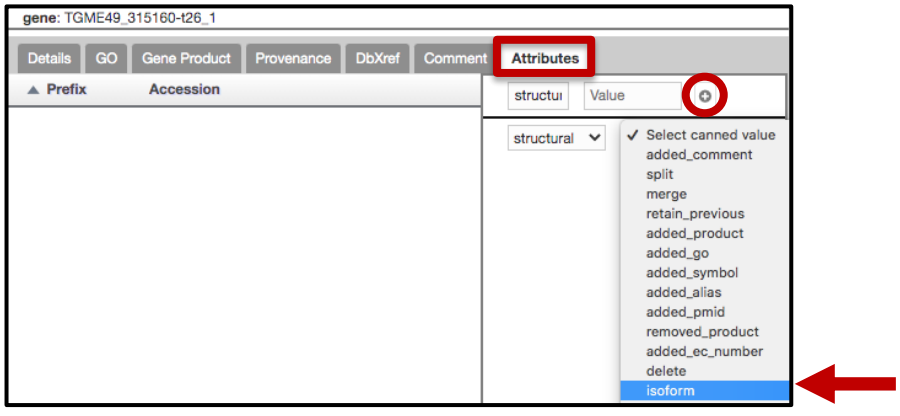


The annotation editor window is now shown on the right-hand side.

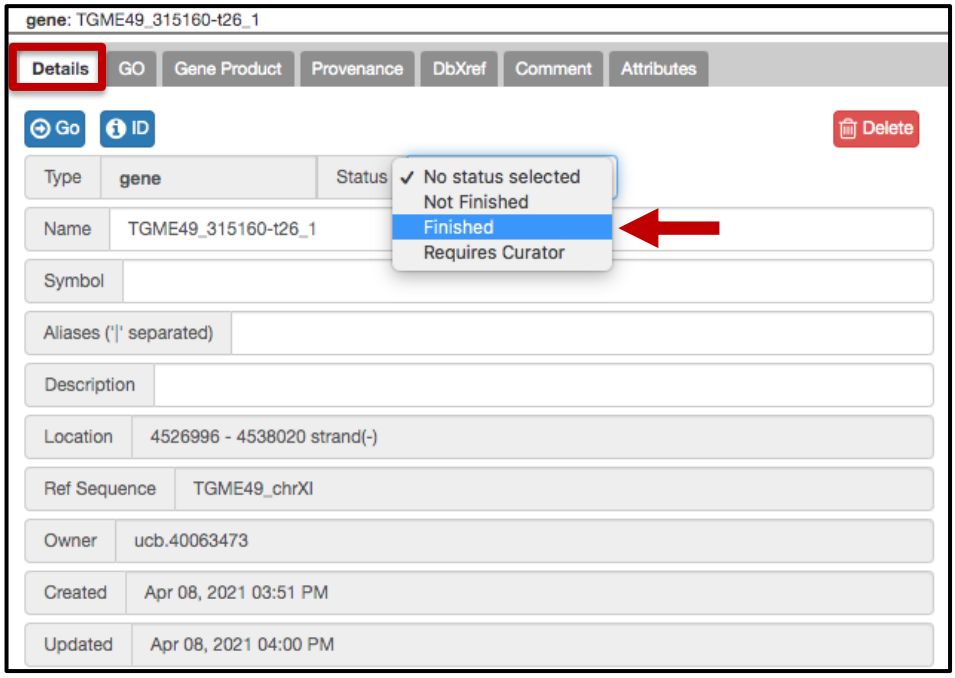


6) Finalizing the structural annotation

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.



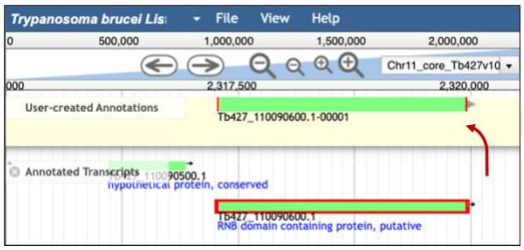
Done! For additional questions, please get in touch with the VEuPathDB help desk.

Possible problems

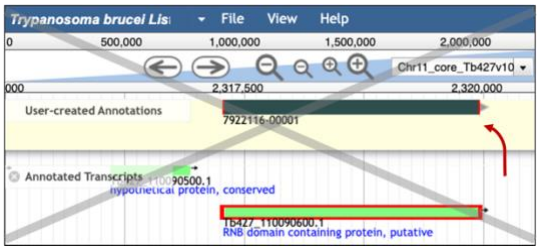
1) Problem with single exon genes

If your gene is a single exon gene, 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. Pseudogenes are shown with the colour blue, ncRNAs with the colour green. The gene in the User-created Annotation track should not be shown as a grey box.

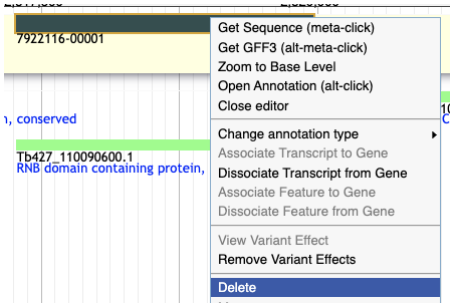
Correct



Incorrect

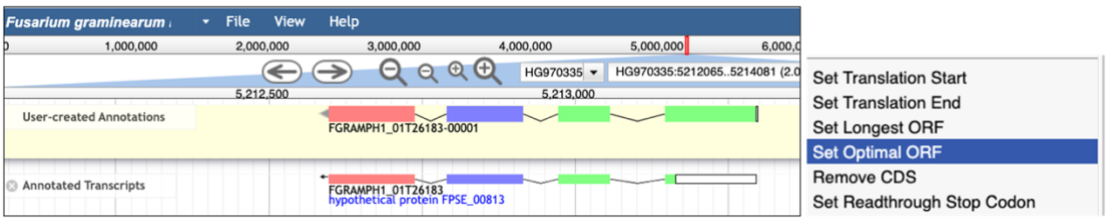


If you see a grey box when you are trying to drag a single exon gene, you need to delete it and try again. Select the grey box. With a right-click open the menu and choose Delete.

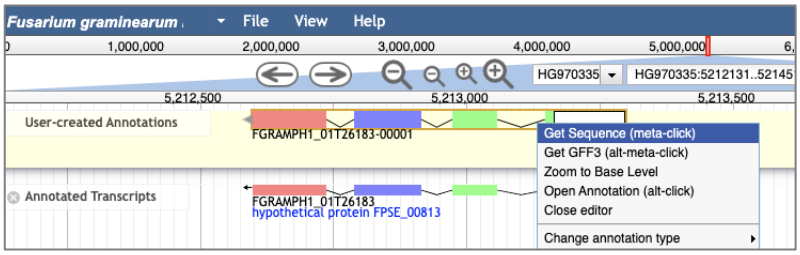


2) Missing start codon

When adding the gene model into the user-created annotations area, Apollo sometimes modifies the gene so that the start codon is lost. To correct this, use the option **Set Optimal ORF** from the right-click menu. This option will automatically create the longest ORF.



Open the menu with a right-click and select the option **Get Sequence** to recheck if your gene starts with the correct start codon.



A new window will open with the sequence. You can copy the sequence to the clipboard for further analysis.