

Using Apollo at the i5k Workspace@NAL

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Agenda

- Manual annotation general overview
- 15k Workspace tools for manual annotation
 - BLAST, Clustal, HMMER
 - Apollo2
- Manual annotation example: preparation
- Manual annotation live example

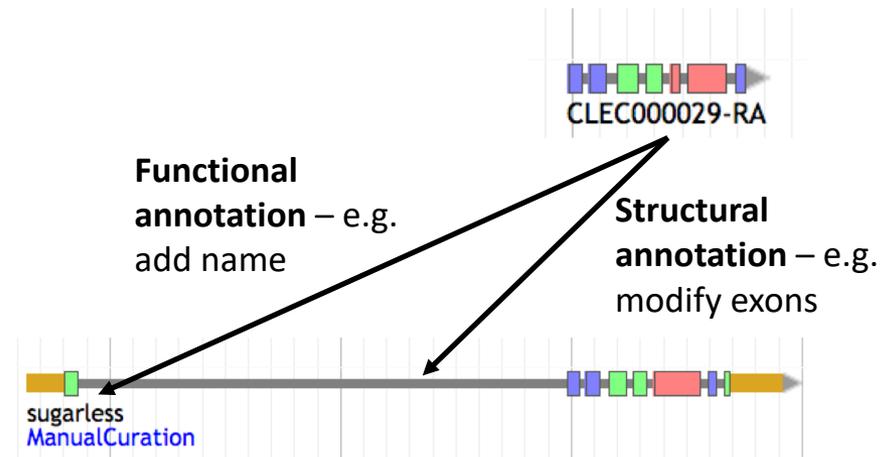
Other resources

- Monica Munoz-Torres from the Apollo group has a number of comprehensive tutorials:
 - <https://www.slideshare.net/MonicaMunozTorres/presentations>
 - I recommend these slides if you need more background:
 - <https://www.slideshare.net/MonicaMunozTorres/apollo-workshop-at-ksu-2015>
 - If you are new to Apollo, or need a refresher, I **highly recommend** that you review one of her presentations
- The official Apollo annotation guide:
 - <http://genomearchitect.org/users-guide/>
- I5k Workspace manual annotation landing page:
<https://i5k.nal.usda.gov/manual-annotation-and-apollo>
- Other manual curation tutorials:
<http://genomecuration.github.io/genometrain/d-feature-curation-crossing/>
- VEuPathDB Apollo training webinar:
<https://eupathdb.org/eupathdb/webinars.jsp#apollo>

MANUAL ANNOTATION GENERAL OVERVIEW

What is manual annotation?

- Manual review and improvement of an existing gene prediction
- Draw on external evidence (e.g. RNA-Seq, cDNA, genes from other species) to improve a computationally predicted gene model



Why manually annotate?

- Automated gene predictions are not always correct
- “Incorrect annotations poison every experiment that makes use of them ... Worse still, the poison spreads because incorrect annotations from one organism are often unknowingly used by other projects to help annotate their own genomes.”
 - Yandell and Ence 2012, doi:10.1038/nrg3174
- Link gene models to existing literature and ontologies, providing richer data

General process of manual annotation

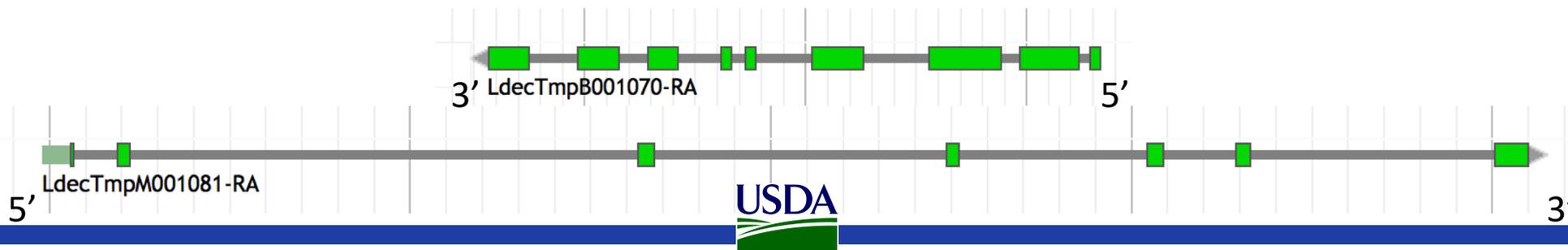
1. Select a chromosomal region of interest (e.g. scaffold)
 1. E.g. find sequence of interest from one or several other species, and align against proteins or genome sequence from your species
2. Select appropriate evidence (tracks in Apollo, or your own files)
3. Determine whether a feature in your evidence provides a reasonable starting gene model
 1. If yes: select and drag the feature to the 'user-created annotations' area, creating an initial gene model. If necessary use editing functions to adjust the model.
 2. If not – get in touch with us!
4. Edit model if necessary
5. Check your edited gene model for integrity and accuracy by comparing it with available homologs
 1. Verify that the gene model is the best representation of the underlying biology
6. Repeat steps 1 through 5 as needed to refine model
7. Add annotation details in the “Information Editor”
 1. Name, symbol, other comments

Adapted from <https://www.slideshare.net/MonicaMunozTorres/apollo-workshop-at-ksu-2015>

MANUAL ANNOTATION: 15K WORKSPACE TOOLS

First, some conventions

- HSP – High scoring pair in BLAST/BLAT alignments
 - The ‘Hits’ in an alignment result set
 - A subsection of a pair of sequences with sufficient score
 - HSPs can change based on the alignment parameters
- Five prime end and three prime end
 - Based on direction of transcription
 - Initiation site is at the five prime end
 - Stop codon is at the three prime end
- In the genome browser, arrowheads indicate direction



JBrowse and Apollo2

File: Add your own files

View: Change coloring scheme

Tools: Search using BLAT

Locate where you are on the scaffold

Search for a gene or location

Apollo2 Track selector

Revert to 'old' track selector

Zoom in/out

User-created annotations track

Find information about tracks

Log out

JBrowse is a web-based genome browser

- Visualize features that are mapped to a genome
- These features are displayed as tracks
- Many different types of data may be displayed

Apollo adds editing functions to JBrowse

- Manual gene curation
- Changes automatically saved back to server
- Edits are visible to other annotators in real-time
- Editing history is tracked

Apollo2 – Annotations Panel



Annotations panel

Annotations

Annotation Name:

Reference Sequence:

All Types:

All Users:

Go to Annotation

Name	Seq	Type	Length	Updated
OtauTmpA001262-RA	Scaffold1	gene	11,946	May 28, 2018
test gene	Scaffold5	gene	28,376	Jun 04, 2018
test mRNA		mRNA	28,376	Jun 04, 2018

Details Coding

Name: test mRNA

Description: This is a test.

Location: 2806565 - 2834941 strand(+)

Ref Sequence: Scaffold5

Owner: demo@demo.org

Annotations panel

Filter annotations

View annotation overview

Click on arrow to jump to annotation

View functional annotation details

Details Coding

Type	Start	Length
exon	2,817,179	677
exon	2,806,566	226
exon	2,832,890	2,052
CDS	2,806,608	27,094
exon	2,824,806	152
exon	2,832,682	150
exon	2,830,616	128

5' End 281717!

3' End 281785!

Strand - +

Can modify individual features via 'Coding' Panel

Apollo2 – Ref Sequence Panel

Reference sequence panel

Filter sequences

Export sequences/annotation gff3

View reference sequence list

Name	Length	Annotations
Scaffold5	4,952,630	1
Scaffold54	1,151,439	0
Scaffold527	1,104,991	0
Scaffold540	986,169	0
Scaffold58	897,936	0
Scaffold584	779,470	0
Scaffold500	685,874	0
Scaffold56	465,482	0
Scaffold52	406,915	0

Export 1 sequence(s) from Onthophagus taurus as GFF3

GFF3 GFF3 with FASTA

Export Cancel

i5k Workspace BLAST: one way to access Apollo

The screenshot shows the BLAST interface with the following elements and annotations:

- BLAST Databases:**
 - Organisms:** A list of organisms with *Eurytemora affinis* selected. Annotation: "Select organism" with an arrow pointing to the list.
 - Nucleotide:** "Genome Assembly - Eaff_11172013.genome_new_ids.fa" is selected. Annotation: "Select organism-specific database" with an arrow pointing to this option.
 - Peptide:** "Protein - EAFF_new_ids.faa" is unselected.
- Query Sequence:** A text box contains a peptide sequence: `>FBpp0070332
MDNCDQDASFRLSHIKEEVKPDISQLNDSNN
SSFSPKAESPVPFMQAMSMVHVLPGSNSASS
NNSAGDAQMAQAPNSAG
GSAAAQVQYPPNHPLSGSKHLCSICGDRA
SGKHYGVYCEGCKGFFKRTVRKDLTYACRE`. Annotation: "Paste or upload query sequence(s)" with an arrow pointing to the text box.
- Program:** "tblastn" is selected. Annotation: "Program is automatically selected" with an arrow pointing to the selected radio button.

URL: <https://i5k.nal.usda.gov/webapp/blast/>

i5k Workspace BLAST: one way to access Apollo

blastdb	qseqid	sseqid	pident	length	mismatch	gapopen	qstart
euraff	FBpp0070332	Scaffold427	36.36	77	49	0	419
euraff	FBpp0070332	Scaffold427	26.67	165	83	4	262
euraff	Eaff_11172013.genome_new_ids.fa		59.21	76	31	0	103
euraff	FBpp0070332	Scaffoldd229	56.52	92	37	1	98
euraff	FBpp0070332	Scaffoldd200	57.14	91	36	1	99
euraff	FBpp0070332	Scaffoldd12	50.57	87	39	2	104
euraff	FBpp0070332	Scaffoldd12	50.57	87	39	2	104
euraff	FBpp0070332	Scaffoldd3	85.71	35	5	0	91
euraff	FBpp0070332	Scaffoldd200	50.62	81	38	1	101

BLAST result page with 4 panels

Click on blue blastdb icon next to your favorite HSP

Annotations

0. Reference Assembly 1/3

- BLAST Results
- Gaps in assembly
- GC Content

BCM_v0.5.3/1. Gene Sets/Primary Gene Sets: Protein Coding 1/1

- EAFF_v0.5.3-Models

Blast results are displayed in Apollo

HMMER and Clustal

- Use HMMER to detect remote protein homologs
- <https://i5k.nal.usda.gov/webapp/hmmer/>
- Use Clustal to perform multiple sequence alignments
- <https://i5k.nal.usda.gov/webapp/clustal/>

Tips and Tricks

- The i5k Workspace BLAST results persist for one week
 - You can bookmark and share searches
 - BLAST HSPs are ‘draggable’ and can be used in annotations
- Jbrowse/Apollo URLs can be shared
 - Allow you to share the exact view (including active tracks) with others
- In Apollo, you can pin tracks to the top
- If you know the name or ID of the gene that you’d like to annotate, you can paste it into the search box in Apollo to navigate to it

O. brunneus and *R. dominica* information

	<i>Odontomachus brunneus</i>	<i>Rhyzopertha dominica</i>	General
Apollo registration	https://i5k.nal.usda.gov/web-apollo-registration	Email monica.poelchau@usda.gov	https://i5k.nal.usda.gov/web-apollo-registration
Apollo URL	https://apollo.nal.usda.gov/apollo/Odontomachus%20brunneus/jbrowse/	https://apollo.nal.usda.gov/apollo/Rhyzopertha%20dominica/jbrowse/	https://i5k.nal.usda.gov/available-genome-browsers
Organism page	https://i5k.nal.usda.gov/odontomachus-brunneus	NA	https://i5k.nal.usda.gov/species
Gene pages	https://i5k.nal.usda.gov/search/site/Odontomachus%2520brunneus%2520AND%2520gene	NA	NA
RNA-Seq (NAL)	Yes	Yes	No
Functional annotation (NAL)	Not yet	Yes	No
BLAST URL	https://i5k.nal.usda.gov/blast		

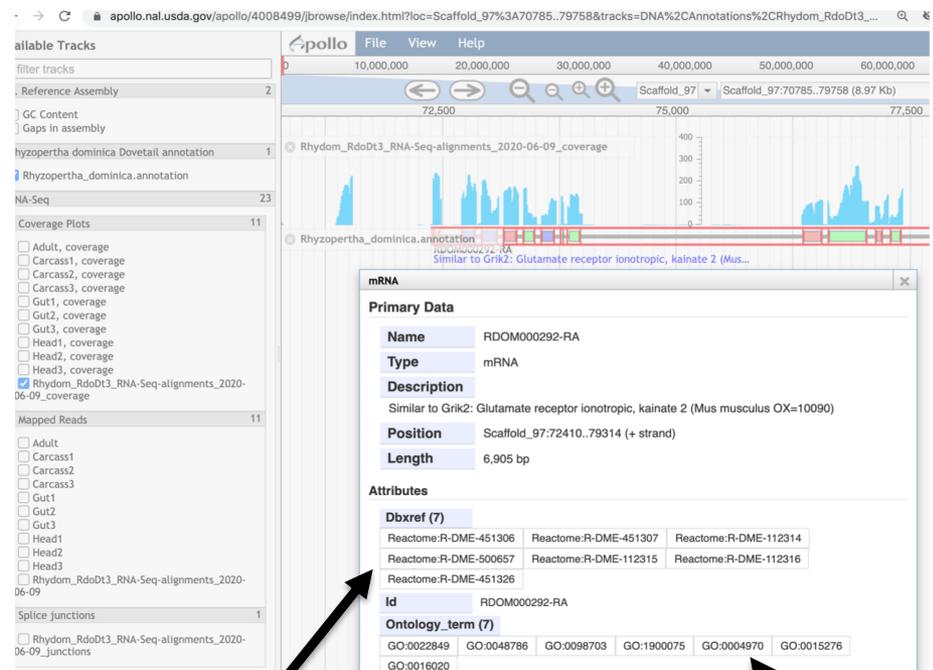
O. brunneus and *R. dominica* information – RNA-Seq

- From NAL:
Odobru_Obru_v1_RNA-Seq-alignments_2020-06-02;
Rhydom_RdoDt3_RNA-Seq-alignments_2020-06-09
- From data providers: RNA-Seq by tissue or developmental stage
- Includes coverage plots, splice junctions, and read mappings
- https://github.com/NAL-i5K/NAL_RNA_seq_annotation_pipeline/



O. brunneus and *R. dominica* information – functional annotation

- Provided in collaboration with AgBase
- GO, Kegg, Reactome
- Tab-delimited files available soon!
- <https://agbase-docs.readthedocs.io/en/latest/agbase/workflow.html>
- We would love any kind of feedback on these new resources!



Reactome pathway information

GO information (IPRscan)

MANUAL ANNOTATION EXAMPLE: PREPARATION

Annotation Example

- Glycerate kinase-like in the trap-jaw ant *Odontomachus brunneus*
- More information about the trap-jaw ant genome assembly: <https://i5k.nal.usda.gov/odontomachus-brunneus>
- *Odontomachus brunneus* Apollo URL: <https://apollo.nal.usda.gov/apollo/4006447/jbrowse/index.html?>
 - For an Apollo login, please register here: <https://i5k.nal.usda.gov/web-apollo-registration>

Choosing reference proteins: *D. melanogaster* glycerate kinase in

UniProt

Annotation score is a heuristic for annotation quality

Flybase is another great resource

The screenshot shows the UniProt entry for Q9VQC4 (GLCTK_DROME). The top navigation bar includes links for BLAST, Align, Retrieve/ID mapping, Peptide search, and SPA/QL. The entry title is "UniProtKB - Q9VQC4 (GLCTK_DROME)". Below the title, there are tabs for Display, BLAST, Align, Format, Add to basket, and History. The main content area is divided into sections: Entry (Protein: Glycerate kinase, Gene: Glyctk, Organism: Drosophila melanogaster (Fruit fly), Status: Reviewed - Annotation score: ●●○○○), Function (Catalytic activity: (R)-glycerate + ATP = (2R)-3-phosphoglycerate + ADP + H⁺, EC:2.7.1.31, Source: Rhea), and a sidebar with filters for Function, Names & Taxonomy, Subcellular location, Pathology & Biotech, and PTM / Processing.

Organism-specific databases

FlyBaseⁱ [FBgn0031428](#) CG9886

Subcellular locationⁱ

Retrieve FASTA from 'sequence' tab

Sequence (1+)ⁱ

Sequence statusⁱ: Complete.

This entry has 1 described isoform and 1 potential isoform that is computational

Q9VQC4-1 [UniParc] [FASTA](#) [Add to basket](#)

<< Hide

```
      10      20      30      40      50
MAKRQTWEQM RQIFVQAVNA VHPEKVFADF QKFDLRPQIG ENATDISIKL
      60      70      80      90     100
NGERQDISGK TCHIVGFGKA VLG MANKVQQ DLGATSAGGV LSVPVNTLKG
     110     120     130     140     150
```

Source: <https://www.uniprot.org/uniprot/Q9VQC4>

Choosing reference proteins: *Apis mellifera* glycerate kinase

The screenshot shows the HymenopteraMine v1.4 interface. The browser address bar displays the URL: 128.206.116.3:8080/hymenopteramine/report.do?id=80532493. The page title is "Gene : 411541". The gene details table is as follows:

Symbol	LOC411541	Source	RefSeq
Organism . Short Name	A. mellifera	Biotype	Protein Coding
Description	glycerate kinase		

Below the gene details is a "Quick Links" bar with tabs for Summary, Alias and DBxref, Transcript, Proteins, Function, Homology, Publications, and Other. The "Transcript" tab is selected. Under "Genome feature", the region is "gene" with a length of 2328, and the location is "LG7:12513499-12515826".

The "Transcript" section shows "All Transcripts for Gene - LOC411541 411541". It lists 1 transcript, 2 exons, and 1 CDS. The transcript table is as follows:

Transcript	Exons	CDSs
LOC411541 XM_003251169.3 2328 [FASTA...]	exon230448 exon230449	115 [FASTA...] 2163 [FASTA...]

Lots of additional information on function

FASTA available under 'Transcript' tab

Source: <http://128.206.116.3:8080/hymenopteramine/report.do?id=80532493>

Resources for learning about insect gene/protein structure and function

- UniProt: <https://www.uniprot.org/>
- OrthoDB: <https://www.orthodb.org/>
- FlyBase: <http://flybase.org/>
- VectorBase: <https://www.vectorbase.org/>
- Hymenoptera Genome Database: <http://hymenopteragenome.org/>
- AphidBase/BIPAA: <https://bipaa.genouest.org/is/>

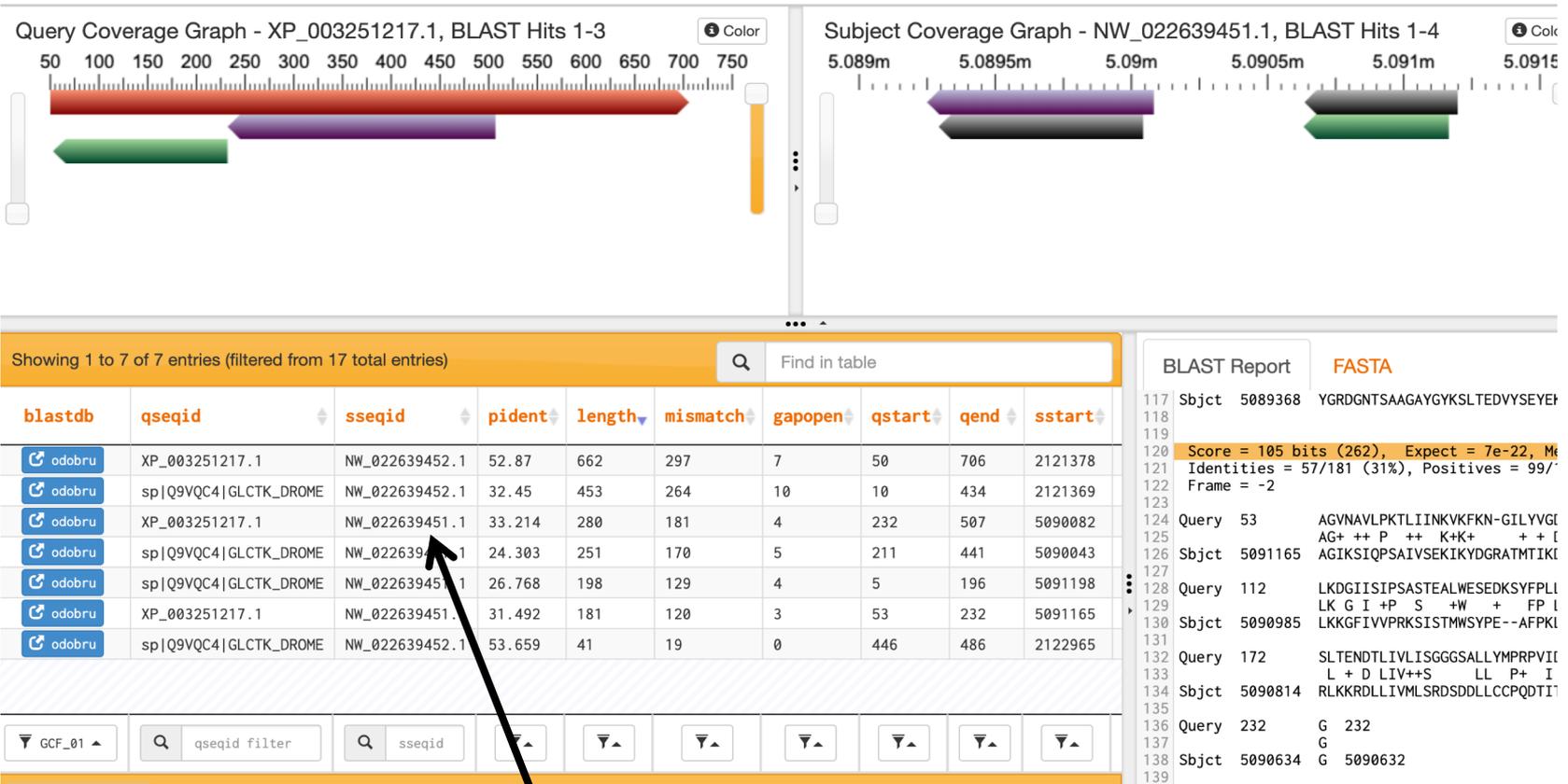
MANUAL ANNOTATION LIVE EXAMPLE

BLAST dmel, amel proteins against *O. brunneus* genome

<https://i5k.nal.usda.gov/blast/>

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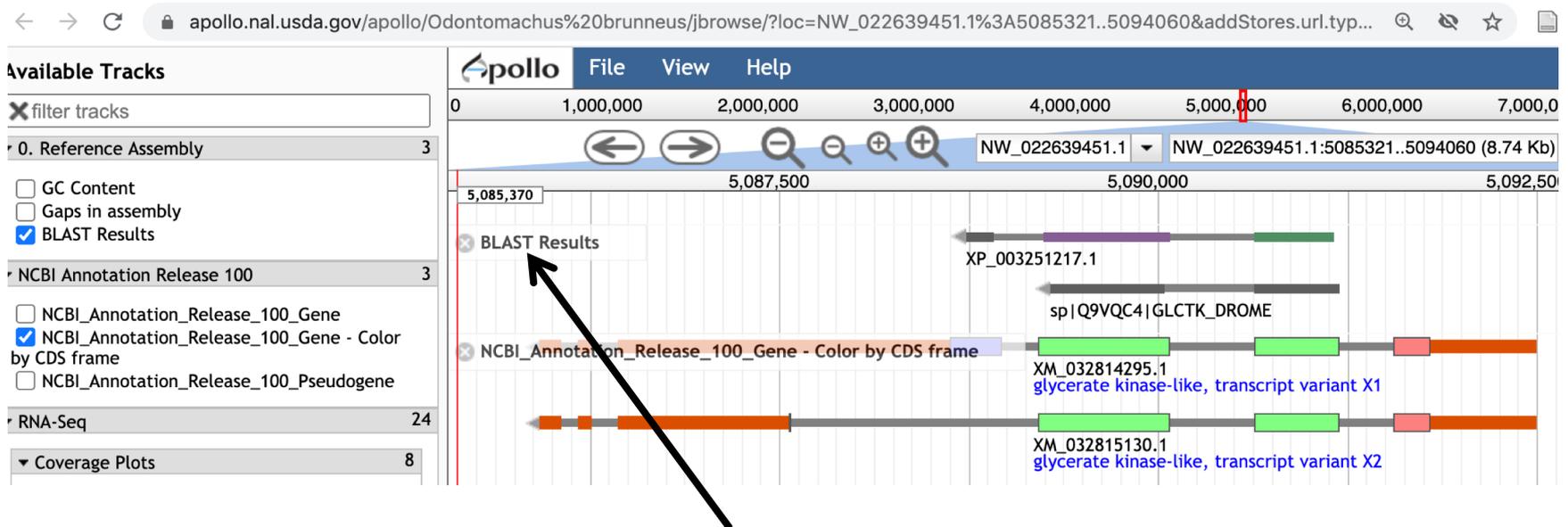


Results are filtered by e-value; we're going to focus on the second-best match here



BLAST dmel, amel proteins against *O. brunneus* genome

<https://i5k.nal.usda.gov/blast/>

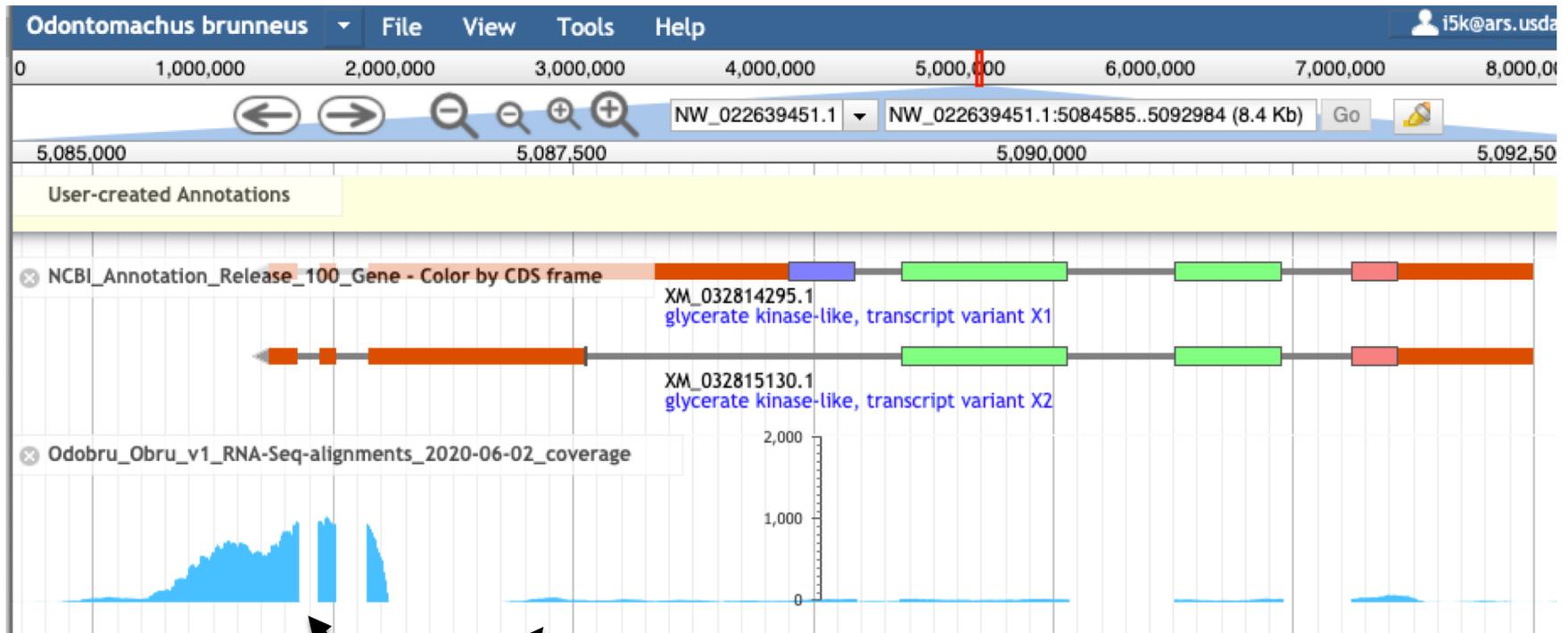


BLAST results are displayed as glyphs in browser;
can be used as annotation starting points if the
alignment is high quality

Modify *O. brunneus* model sequence in Apollo

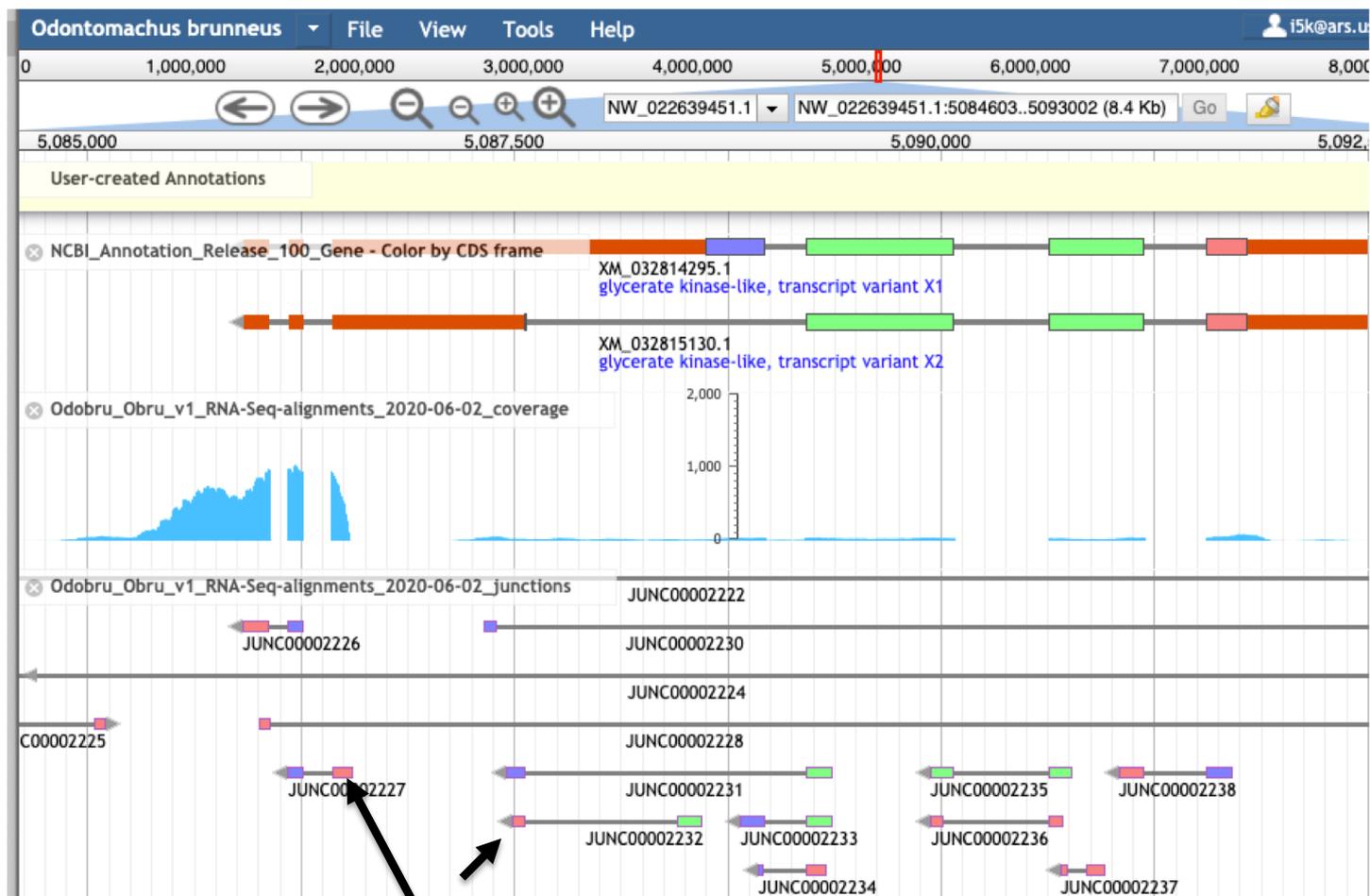
- Questions:
 - What evidence do you choose to check the integrity of the model?
 - Do you need additional evidence?
 - How do you evaluate whether the protein sequence is as complete as it can be?
 - Should you add/modify UTRs?

View available evidence



Very different coverage
between UTR and CDS

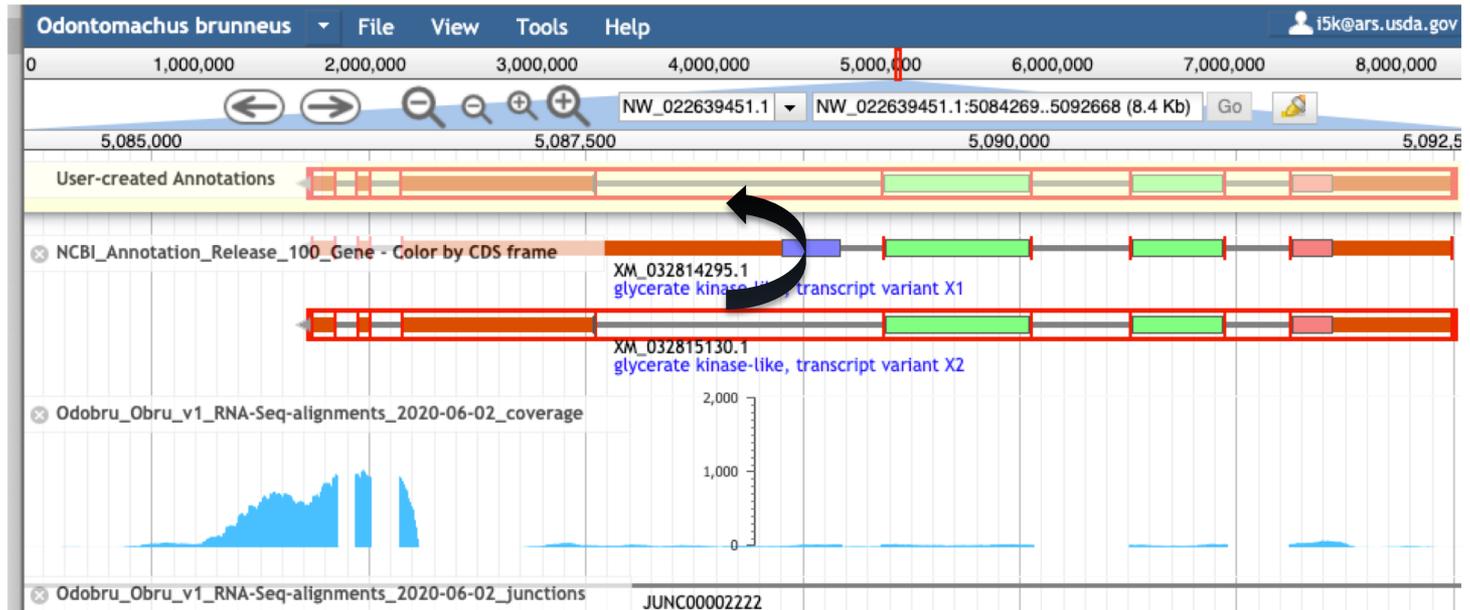
View available evidence



Junction reads don't support connection between the two expressed regions

Create new model in user-created annotations track

Drag evidence to UcA track (or right-click and select “create annotation”)



Split model

Select exons on which to split the model using the 'shift' key

Right-click on the model while continuing to hold shift to get the drop-down menu

Odontomachus brunneus File View Tools Help i5k@ars.us

1,000,000 2,000,000 3,000,000 4,000,000 5,000,000 6,000,000 7,000,000 8,000,000

5,085,000 5,085,500 5,090,000 5,092,500

User-created Annotations XM_032815130.1-00001

NCBI_Annotation_Release_100_Gene - Color by CDS frame XM_032814295.1 glycerate kinase-like, transcript variant X1 XM_032815130.1 glycerate kinase-like, transcript variant X2

Odobru_Obru_v1_RNA-Seq-alignments_2020-06-02_coverage

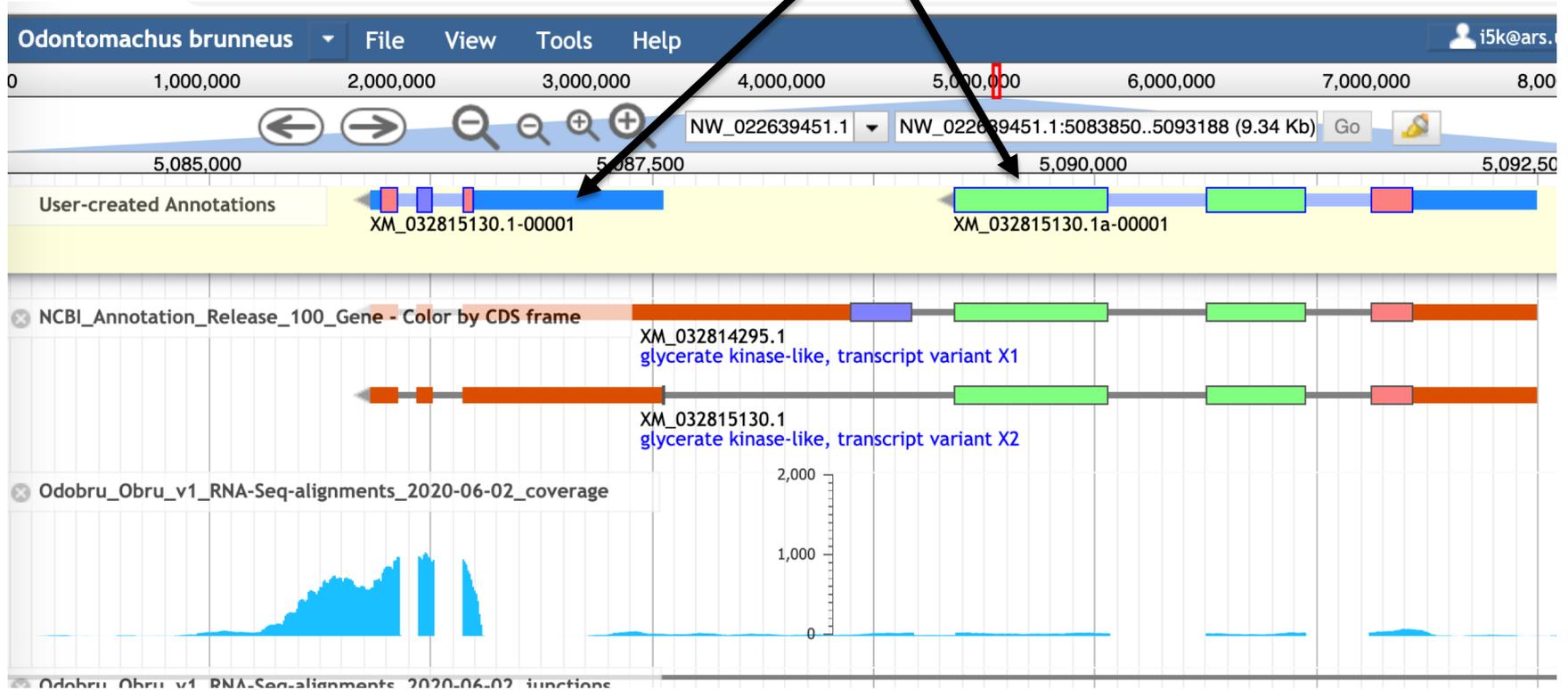
Odobru_Obru_v1_RNA-Seq-alignments_2020-06-02 junctions

- Get Sequence
- Get GFF3
- Zoom to Base Level
- View in Annotator Panel
- Edit Information (alt-click)
- Change annotation type
- Associate Transcript to Gene
- Dissociate Transcript from Gene
- Delete
- Merge
- Split**
- Duplicate
- Make Intron
- Move to Opposite Strand
- Set Translation Start
- Set Translation End

Select 'split'

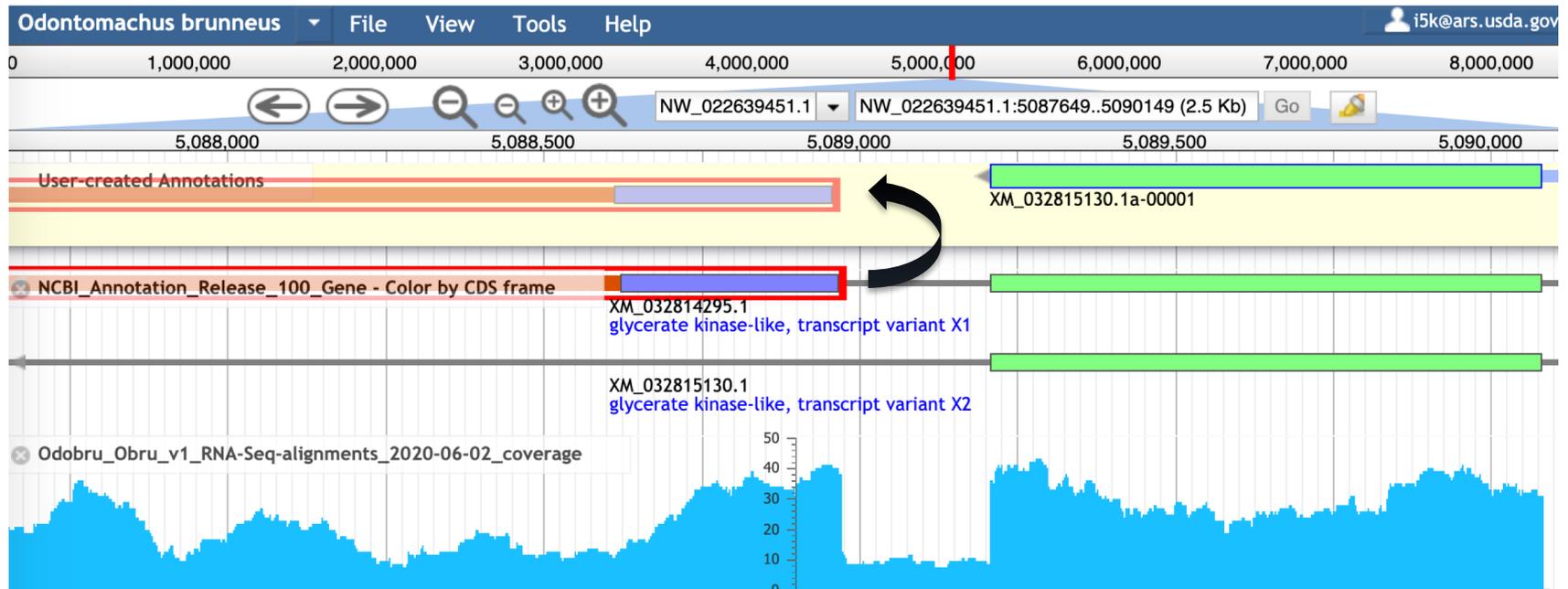
Split model

You now have 2 models! Let's start fixing the model on the right – it needs a 3' exon.



Add an exon

Zoom in, select the missing exon,
drag up to Uca track



Merge exons

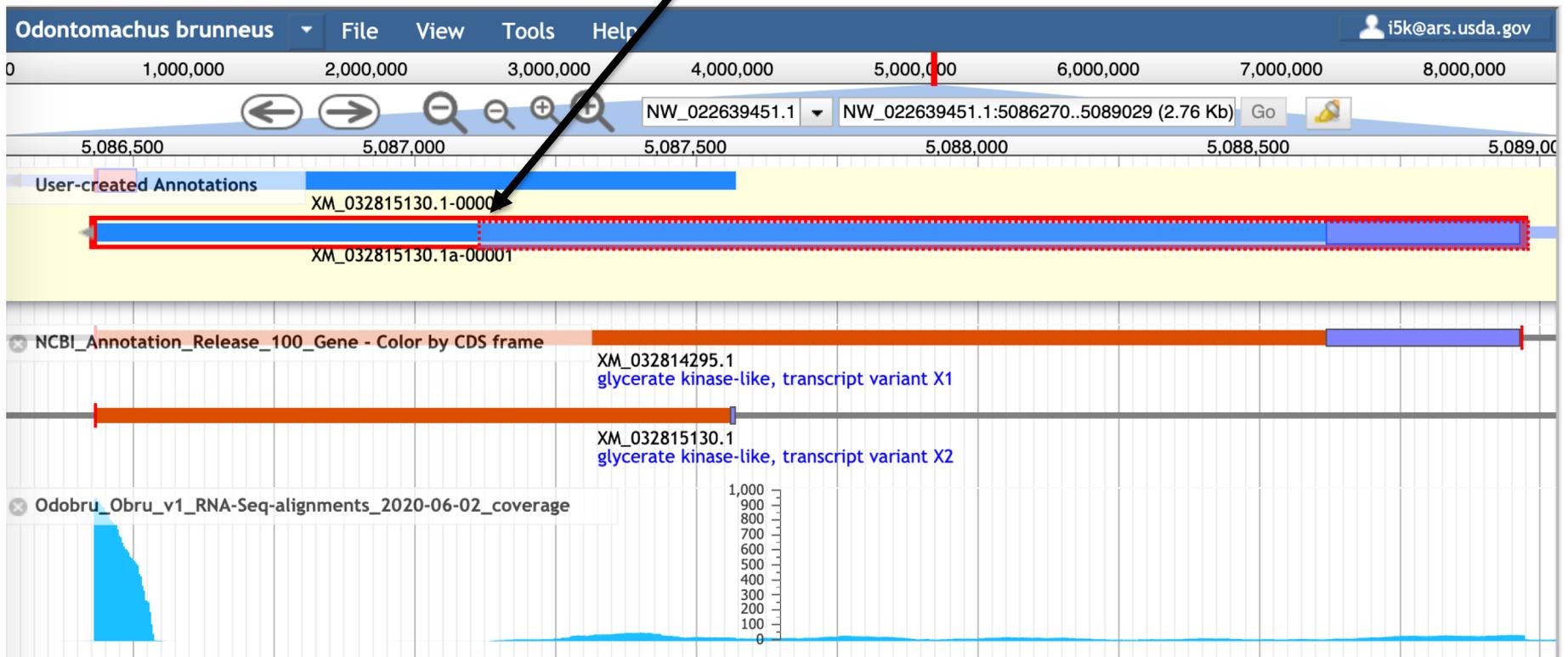
Shift-select both exons, shift-right click, then select 'merge' from the dropdown menu

The screenshot shows the GenBank/NCBI genome browser interface for *Odontomachus brunneus*. The top navigation bar includes 'File', 'View', 'Tools', and 'Help'. The main display area shows genomic coordinates from 1,000,000 to 8,000,000. A specific region is zoomed in from 5,088,000 to 5,090,000. The 'User-created Annotations' track shows two exons, XM_032814295.1-00001 and XM_032815130.1, highlighted with red boxes. The 'NCBI_Annotation_Release_100_Gene - Color by CDS frame' track shows the gene structure with exons in blue and introns in orange. The 'Odobru_Obru_v1_RNA-Seq-alignments_2020-06-02_coverage' track shows a blue histogram of RNA-seq coverage. A context menu is open over the second exon, with 'Merge' selected.

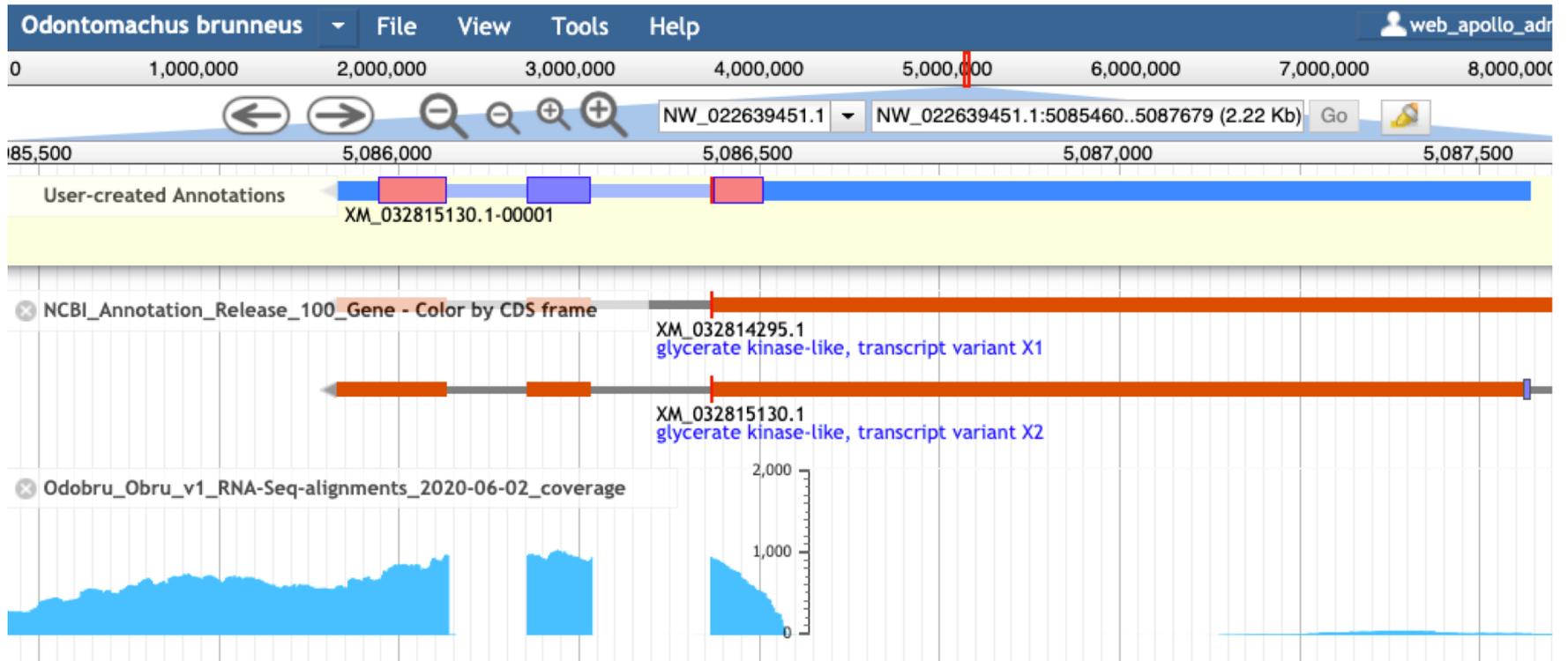
- Get Sequence
- Get GFF3
- Zoom to Base Level
- View in Annotator Panel
- Edit Information (alt-click)
- Change annotation type
- Associate Transcript to Gene
- Dissociate Transcript from Gene
- Delete
- Merge**
- Split
- Duplicate
- Make Intron
- Move to Opposite Strand
- Set Translation Start
- Set Translation End

Adjust gene boundaries

Adjust the 3' UTR to match the RNA-Seq evidence

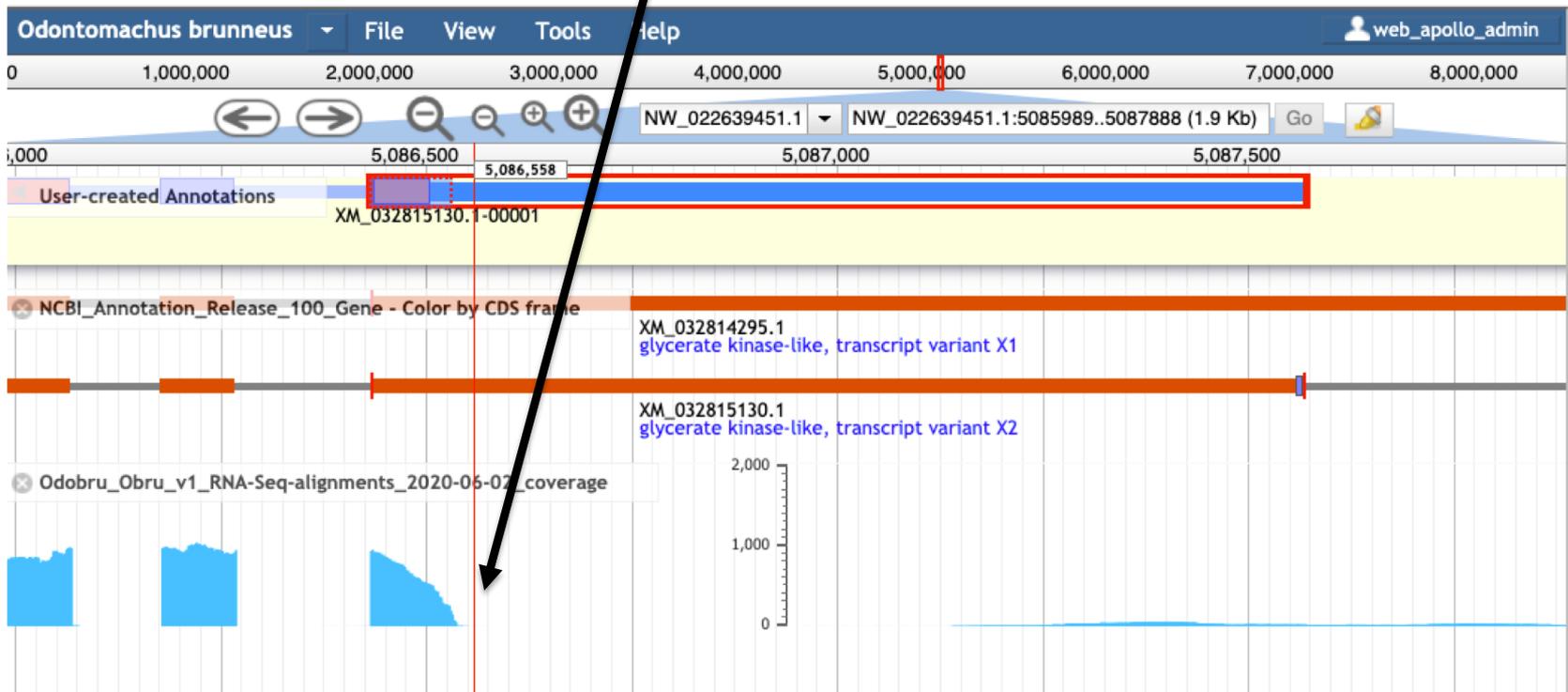


Next, the 'left' model



Adjust 5' UTR

Adjust the 5' UTR to match the RNA-Seq evidence



Set translation start

Set translation start at Methionine

The screenshot displays a genome browser interface for *Odontomachus brunneus*. The top navigation bar includes 'File', 'View', 'Tools', and 'Help'. The main view shows a genomic region from 1,000,000 to 7,000,000. A specific region is zoomed in, showing coordinates from 5,086,450 to 5,086,525. The reference sequence is displayed with amino acid translations above it. A context menu is open over the 'User-created Annotations' track, listing various actions. The 'Set Translation Start' option is highlighted in blue. A black arrow points from the text 'Set translation start at Methionine' to the 'M' in the reference sequence 'S M R K M Q N N A R Y F I R C M L L H G R D S T N Y L R'.

Reference sequence
H L H R I L H E Y E E D A E I N C E R I L H G E M Y V A A R G E R F N E L L E
L A S N I A R V G R C R I M R D F S G G A G A T G A T G T T G C T G C A C G G A G A G A T T C A A C G A A C T A C T T G A
C A C T T G C A T C G A A T A T T G C A C G A G T A T G A G G A A G A T G C A G A A T A A T G C G A G A T A C T T C A A G T G A G A T G A T G T T G C T G C A C G G A G A G A T T C A A C G A A C T A C T T G A
G T G A A C G T A G C T T A T A A C G T G C T C A T A C T C C T T C T A C G T C T T A T T A C G C T C T A T G A A G T A A C A C T C T A C A T A C A A C G A C G T G C C C T C T A A G T T G C T T G A A C T
V Q M S Y Q V L I L F I C F L A L Y K M T L H I N S C P L S E V F K
C K C R I N C S Y S S S S A S Y H S I S M Q S I Y T A A R S L N L S S S S
A S A D F I A R T H P L H L I I R S V E N H S T H Q Q V P S I R V V Q

User-created Annotations
XM_032815130.1-00001
AT A C T C C T T C T A C G T C T T A T T A C G C T C T A T G A A G T

NCBI_Annotation_Release_100_Gene - Color by CDS frame
XM_032814295.1
glycerate kinase-li

Odobru_Obru_v1_RNA-Seq-alignments_2020-06-02_coverage
900
800
700
600
500
400
300
200
100
0

Male, coverage
20,000
10,000
0

Context menu options:
Get Sequence
Get GFF3
Zoom to Base Level
View in Annotator Panel
Edit Information (alt-click)
Change annotation type
Associate Transcript to Gene
Dissociate Transcript from Gene
Delete
Merge
Split
Duplicate
Make Intron
Move to Opposite Strand
Set Translation Start
Set Translation End
Set Longest ORF
Set Readthrough Stop Codon
Set as 5' end
Set as 3' End
Set both Ends

Evaluate new protein sequence

- Blast modified sequence to NCBI's nr database
 - Make sure it doesn't match a potential contaminant
 - Get an idea whether you have the right sequence
 - Blastp home:
 - https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome
- Once contamination is ruled out, it's better to align your sequence against a smaller set of high-quality proteins
- If you notice that parts of the protein are missing, check the 'Gaps in assembly' track in the browser

Using the Information Editor

Information Editor

Select mRNA: glycerate kinase-like

gene		mRNA	
Name		Name	glycerate kinase-like
Symbol		Symbol	
Description		Description	
Created	2020-06-12	Created	2020-06-12
Last modified	2020-06-12	Last modified	2020-06-12
Status		Status	
<input type="radio"/> Approved <input type="radio"/> Delete		<input checked="" type="radio"/> Approved <input type="radio"/> Delete	

Use the mRNA/transcript side of the IE

Review our naming guidelines before naming:

<https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines>

Using the Information Editor

- Select the model in Apollo, then right-click, and select 'Edit Information' from the drop-down menu
 - Use the 'mRNA' section
 - Name: Use the i5k Workspace naming guidelines.
<https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines>
 - If a naming convention exists, use it (e.g. for gene families)
 - Name should be unique and attributed to all orthologs (as far as possible)
 - Use name from an orthologous protein if you are sure that your gene model is an ortholog.
 - Document your justification for the name in the Comments field (e.g. "88% sequence similarity via blastp to D. melanogaster pepck P20007")
 - Comments – Document what changes you performed, and your justification for the name. These notes will be visible in the OGS, so make sure that others understand them

Checklist for accuracy and integrity

- Check start, stop and exon boundaries (splice sites)
 - Try to fix non-canonical splice sites if possible
- Check if you can annotate UTRs (e.g. using RNA-Seq data)
- Check for gaps in the genome
- If you change the genome sequence, add a justification comment to the corresponding gene model
- Use BLAST or a multiple sequence aligner
 - To look at completeness of model
 - To verify the appropriateness of the gene name
- In the Information editor ***mRNA*** field
 - Update the Name if appropriate
 - Add comments that describe
 - your evidence for the annotation
 - Modifications that you made to the gene model

cf. <https://www.slideshare.net/MonicaMunozTorres/editing-functionality-apollo-workshop>

What happens to my annotation when I'm done?

- This depends on the genome project that you're working on.
 - For *O. brunneus* and *R. dominica* – we will assist with generating an OGS and submitting to GenBank
- If the genome coordinator has asked us to generate an OGS (Official Gene Set), we will do so
 - Includes submission to GenBank, where they will be archived/accessioned
 - This takes some time, but we are working on expediting it
- Otherwise, don't assume that your annotation will be archived.
 - If you need it to be, get in touch with us and we'll figure out what to do.
- Get in touch with us and the genome project coordinator if you're not sure about the status of a genome project.
- <https://i5k.nal.usda.gov/data-management-policy>

I5k Workspace ‘Etiquette’

1. Use Apollo to improve a gene model in an i5k Workspace assembly.
 1. If you just want to practice – use one of our training instances.
 1. <https://i5k.nal.usda.gov/jbrowseapollo-training>
 2. If you just want to view the data – you probably can get what you want without using Apollo. All of the data that we host is public.
2. Your annotation work is a community effort.
 1. If you notice that someone else is working on your model of choice, get in touch with them (or us) and collaborate – don’t make a 2nd model or delete the other model.
 2. Keep in mind that your work may be used by the scientific community once you’re done.
3. If you publish any of your work generated in the i5k workspace:
 1. Get in touch with the genome contact first (you can find the contact info on the organism page; <https://i5k.nal.usda.gov/species>);
 2. Please cite the i5k Workspace paper! This helps us continue to exist.
 1. <https://doi.org/10.1093/nar/gku983>

Thank you!

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- Mei-Ju Chen
- Chao-I Tuan

- i5k Coordinating Committee
- i5k Pilot Project
- Apollo & JBrowse Development Teams
- GMOD/Tripal community
- All of our users and contributors!

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