Using Apollo at the i5k Workspace@NAL

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Agenda

- Basic RNA-Seq evaluation
- Basic structural changes- splitting and merging a model, adding and removing exons
- UTRs –when and how to add and adjust
- Changing translation start and stop sites, and open reading frames
- Non-canonical splice sites
- Annotating isoforms



Other resources

- I5k Workspace manual annotation landing page: <u>https://i5k.nal.usda.gov/manual-annotation-and-apollo</u>
- An additional Apollo webinar with a worked example: <u>https://www.youtube.com/watch?v=dol99KExLgY&feature=youtu.be</u>
- Monica Munoz-Torres from the Apollo group has a number of comprehensive tutorials:
 - <u>https://www.slideshare.net/MonicaMunozTorres/presentations</u>
 - 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:
 - <u>https://genomearchitect.readthedocs.io/en/latest/UsersGuide.html</u>
- Other manual curation tutorials: <u>http://genomecuration.github.io/genometrain/d-feature-curation-crossing/</u>
- VEuPathDB Apollo training webinar: <u>https://veupathdb.org/veupathdb/app/static-content/webinars.html#apollo</u>



Basic RNA-Seq evaluation

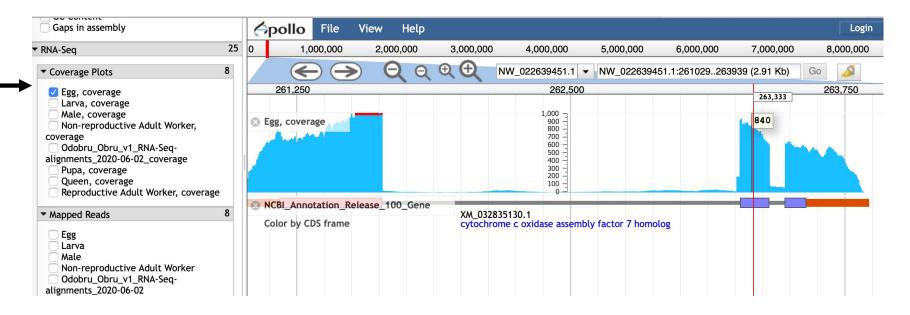


- Coverage plots: Histogram of the number of mappings at each nucleotide
- Mapped reads: Individual glyphs of each mapped read.
- Junction reads: Show where mapped reads are spliced.

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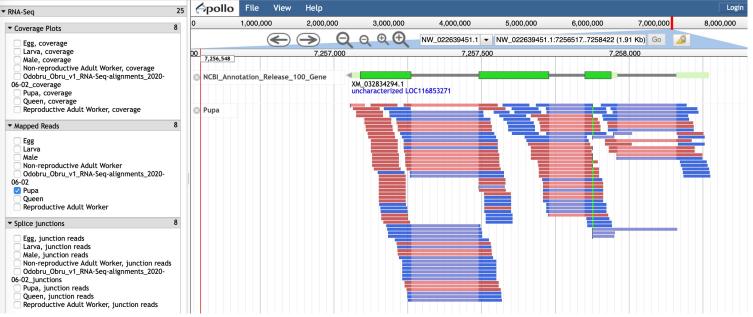


 Coverage plots: Histogram of the number of mappings at each nucleotide; hover over the blue area to see the value





 Mapped reads: Individual glyphs of each mapped read. Show mapped and spliced areas, and SNPs/indels. Informative, but hard to work with when zoomed out.





 Junction reads: Useful combined with coverage plots; show where mapped reads are spliced. The blue numbers show the 'score' – the number of mappings that support the splice junction.

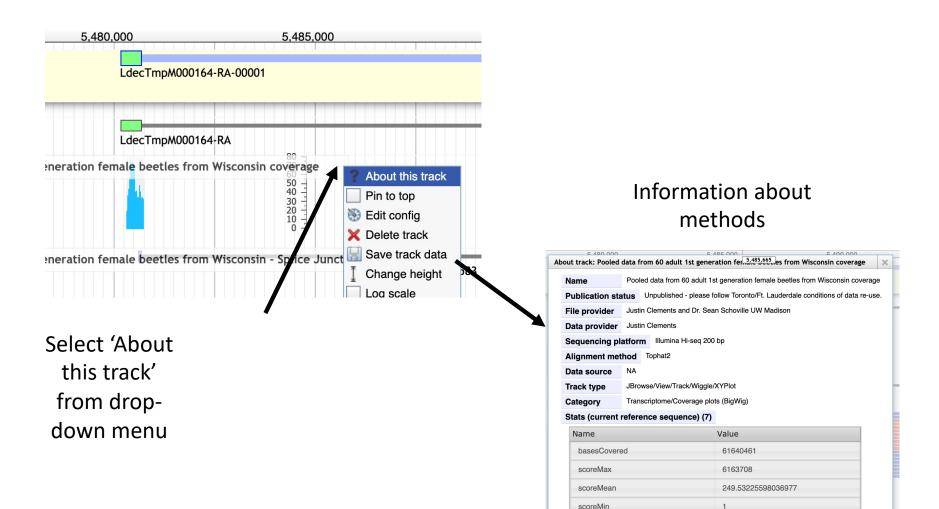
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A simple case



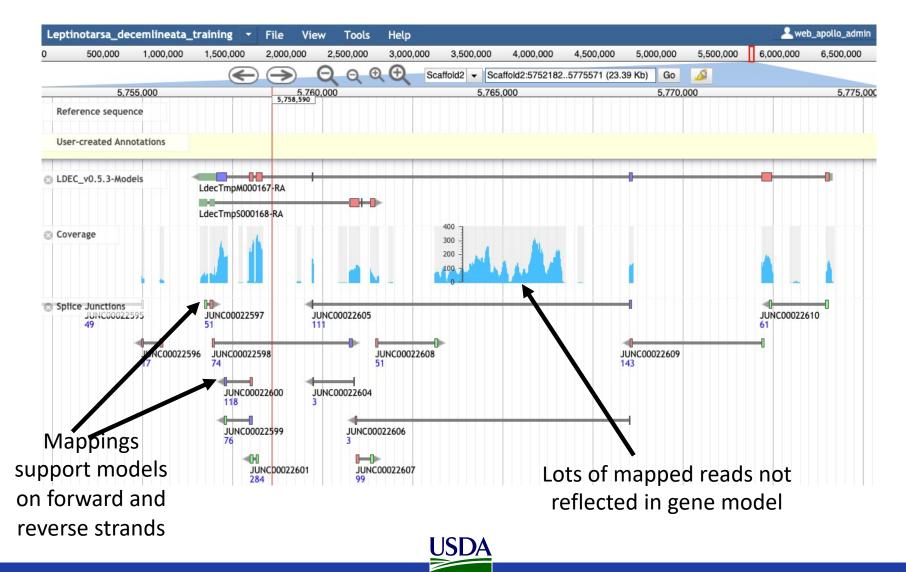


A more complex case

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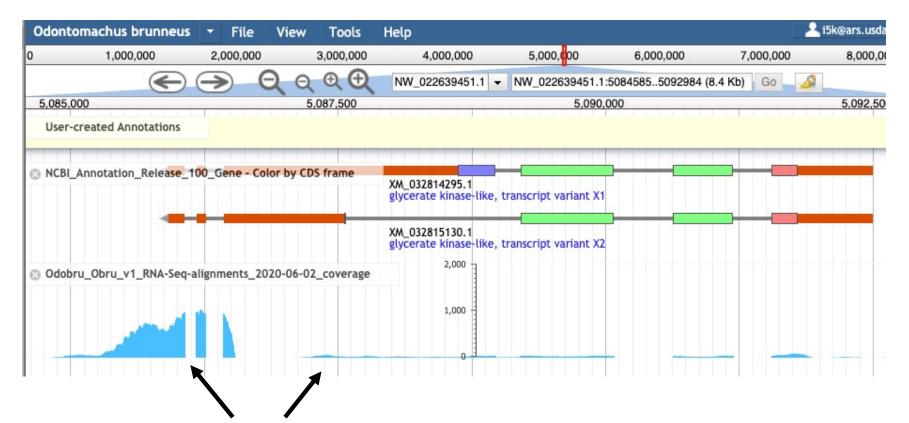
A really messy case



Basic structural changes – splitting and merging a model, adding and removing exons



RNA-Seq evaluation

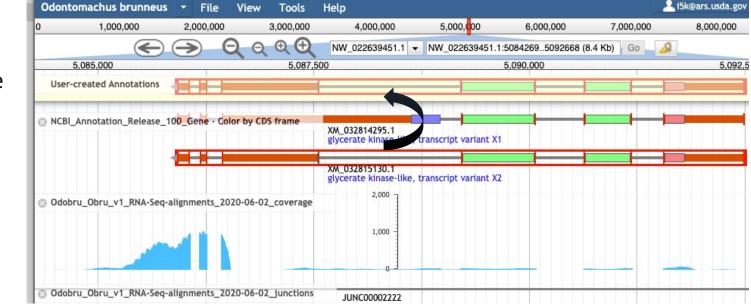


- Very different coverage between UTR and CDS
- No RNA-Seq coverage between high and low expression areas
 - 2 separate models?

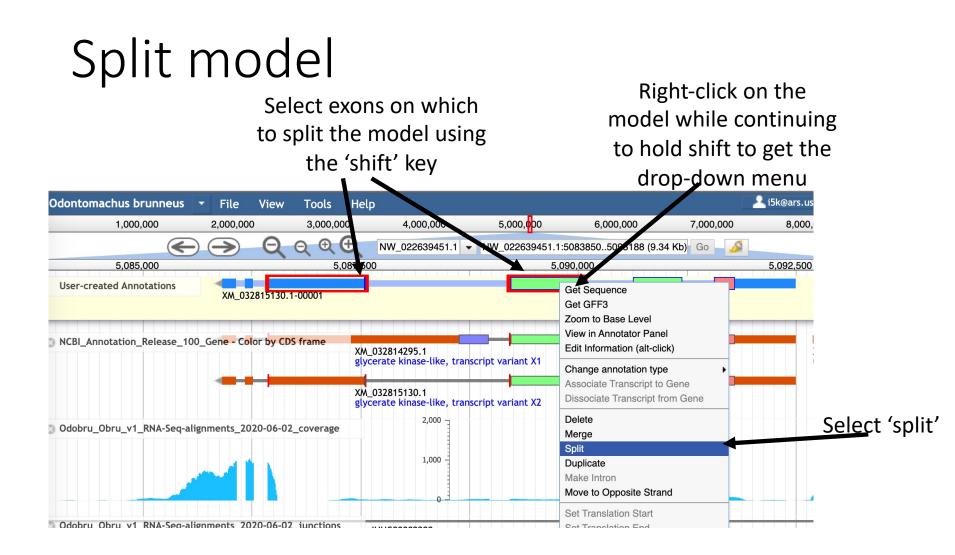


Create new model in user-created annotations track

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



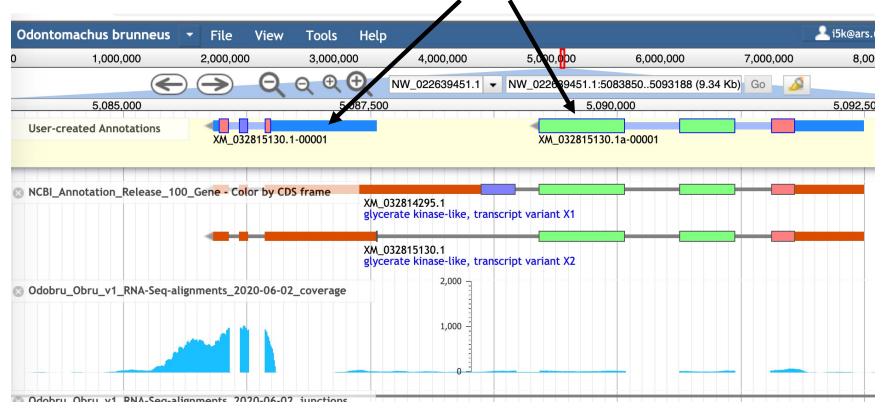






Split model

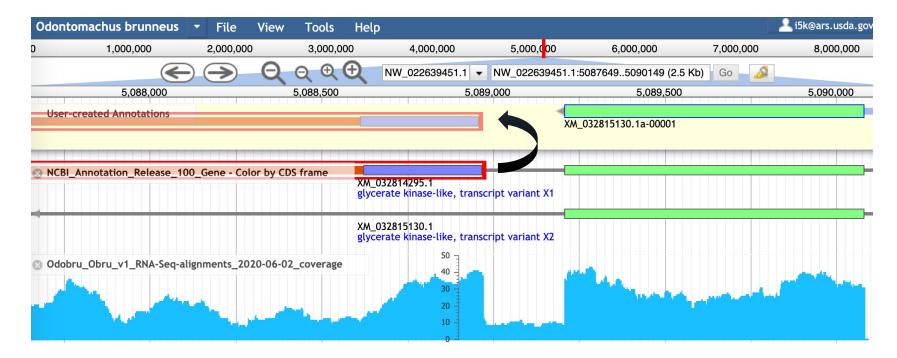
You now have 2 models! Let's start fixing the model on the right – it needs a 3' exon.





Add an exon

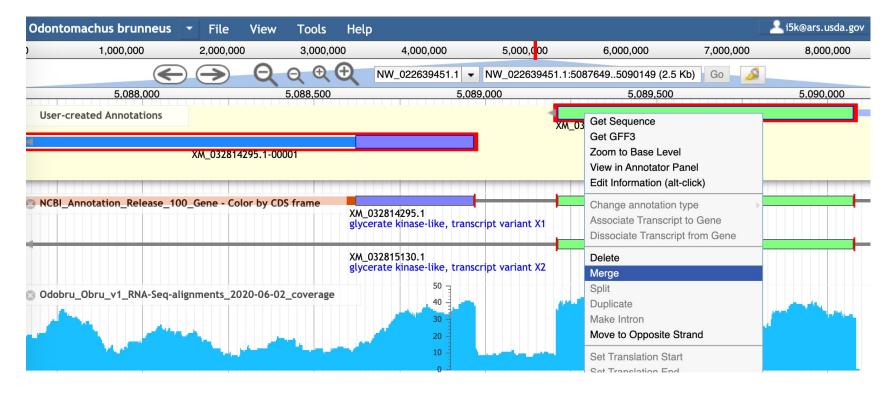
Zoom in, select the missing exon, drag up to UcA track





Merge exons

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





UTRs – how and when to add or adjust



Adding or adjusting UTR boundaries

- When should you add or change UTRs?
 - Only if you have RNA-Seq evidence with sufficient coverage relative to the rest of the model
 - Adding or changing UTRs is helpful, but not necessary if you're only interested in the protein sequence
 - Deciding where the UTR ends is usually a judgement call
- Apollo tools for gene boundary changes:
 - Manual edge-matching to available evidence
 - Automated edge-matching to available evidence



RNA-Seq evidence ends in different places for

each track – how do you decide?

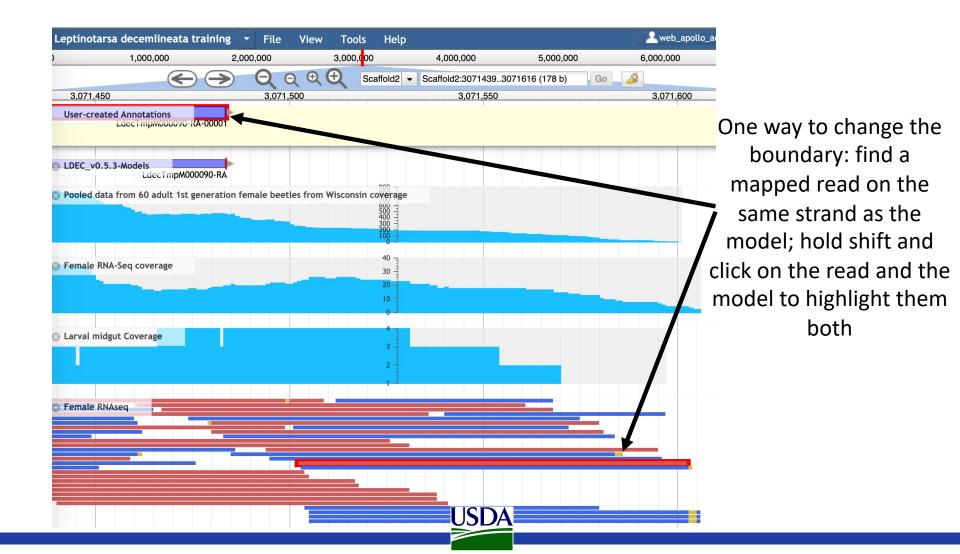
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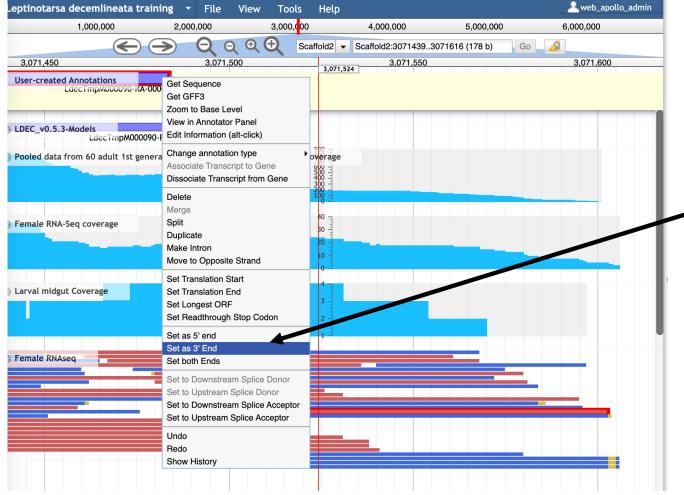


Pick the longest boundary available, and note which track you used in the 'Comments' section

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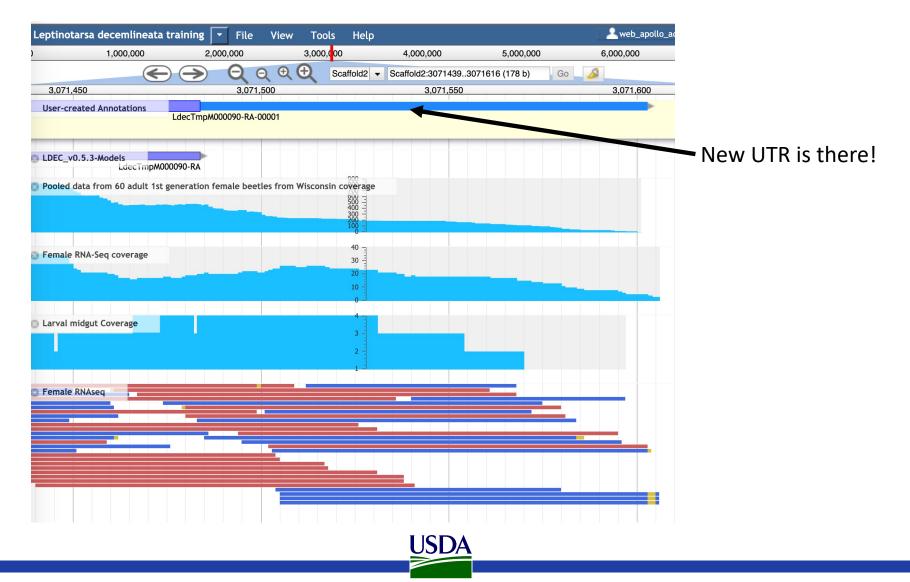


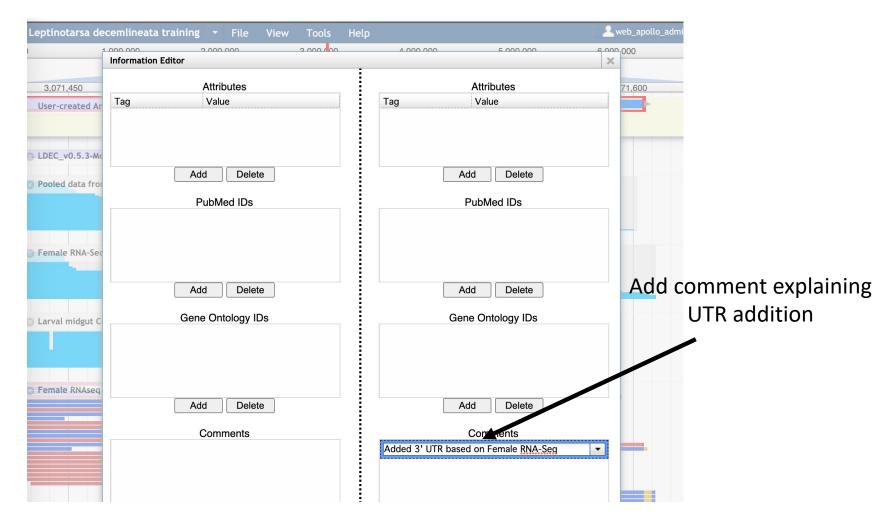




Right-click on model in user-created annotations track, and select 'Set as 3' end' from the drop-down menu









Starts, stops, open reading frames



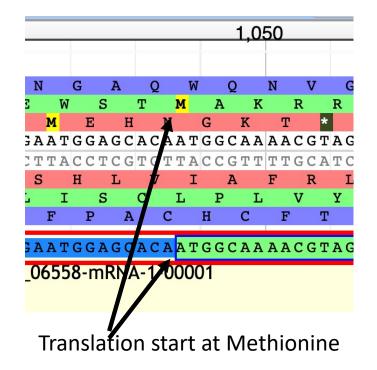
Setting the sequence start, stop, and open reading frame (ORF)

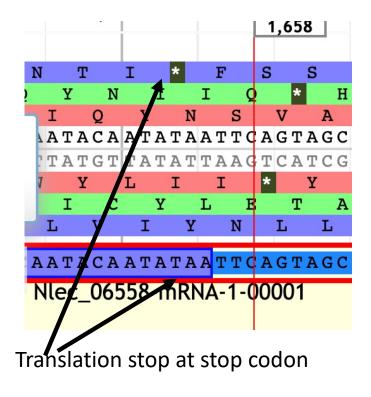
- Apollo will automatically calculate the longest possible ORF that includes canonical 'Start' and 'Stop' signals (<u>https://genomearchitect.readthedocs.io/en/latest</u> /UsersGuide.html#start-and-stop-sites)
- However, in some fringe cases, you will need to double-check
- You can change a model's start and stop sites if needed



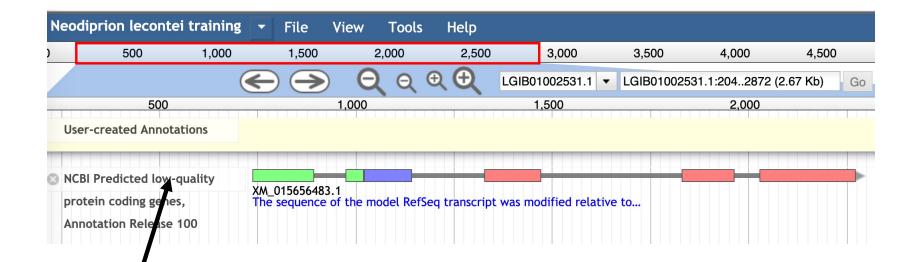


Open reading frame (ORF): translated region



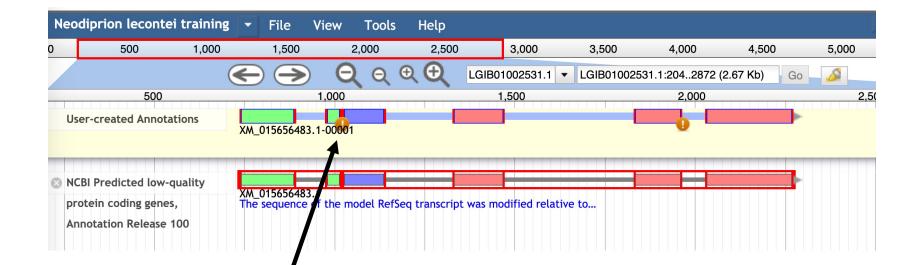






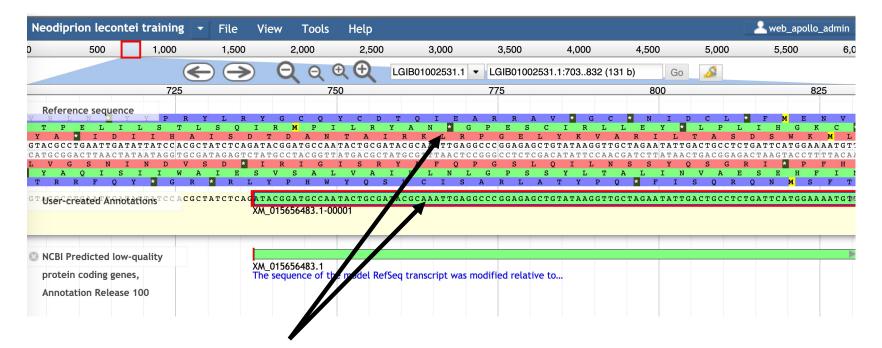
This is a 'low quality' protein coding gene from NCBI – it will likely show some problems in Apollo





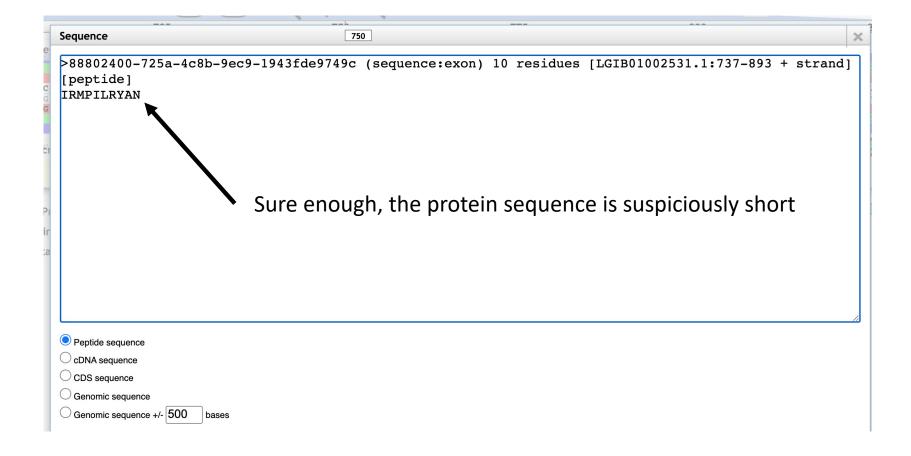
We can see a non-canonical splice site in the UcA (more on that later). Let's zoom to the start of the model.



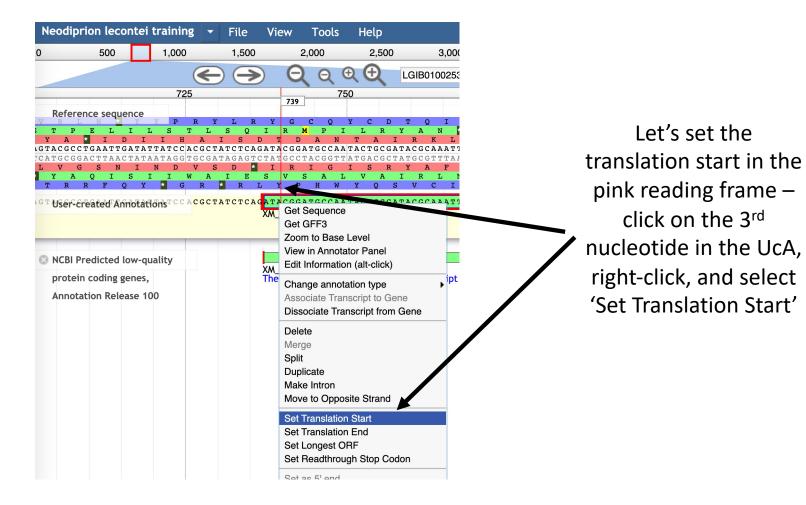


Apollo shows this model in the green reading frame – however, we can see a stop pretty early on in the genome sequence - but that's not reflected in the Apollo model! It looks like the pink reading frame doesn't have stops.





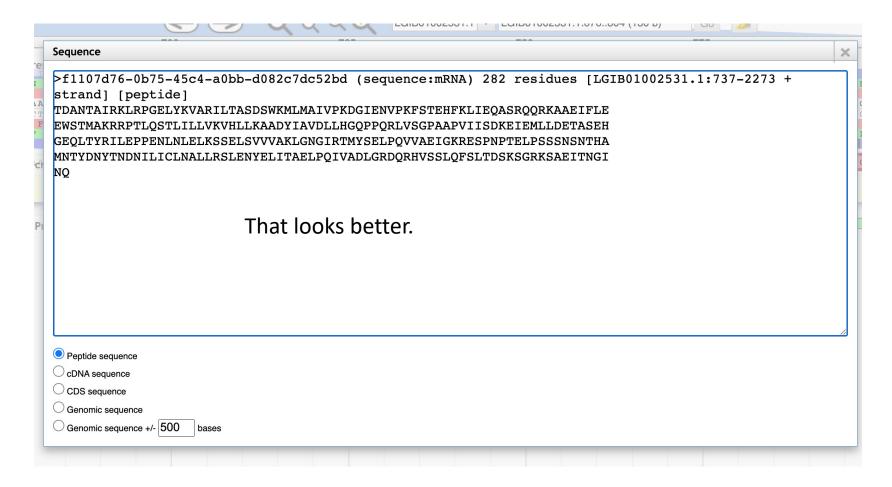




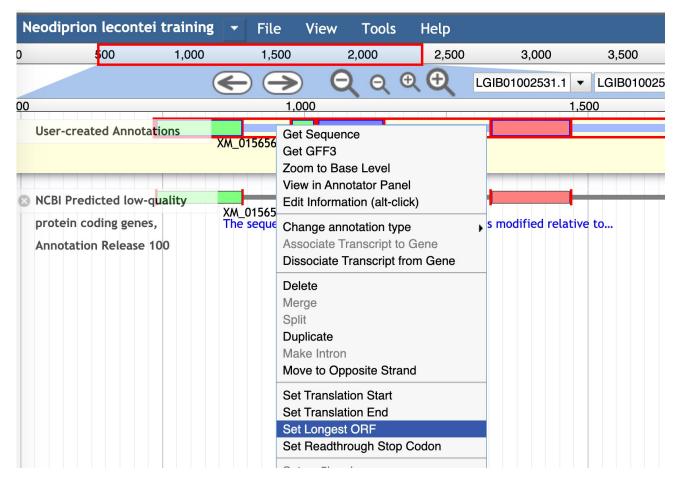


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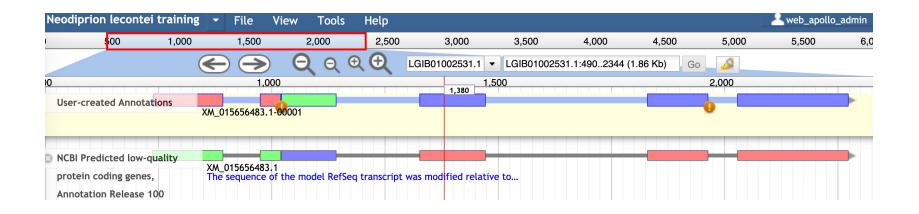






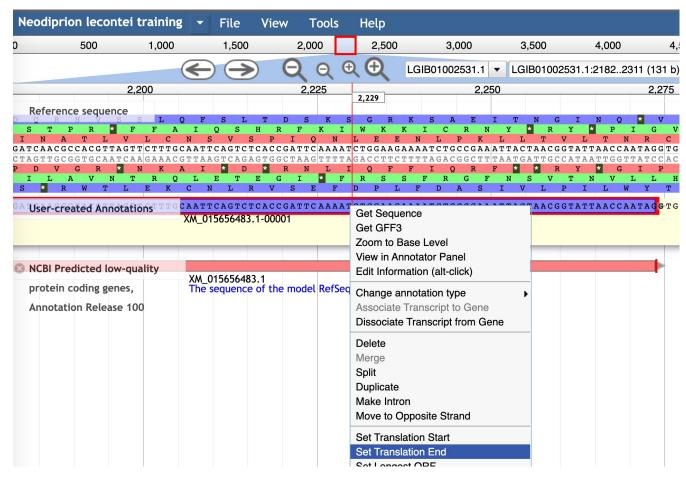
Sometimes it can be hard to tell what the protein sequence should be – in that case you can rightclick and select 'Set Longest ORF'





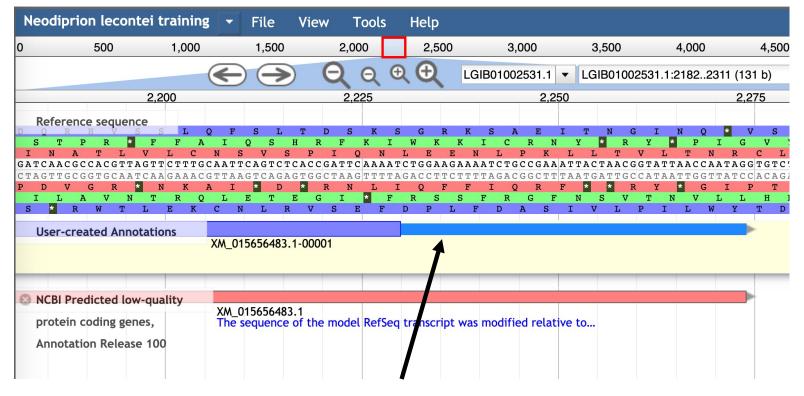
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Similarly, if you have evidence to change the translation end, you can click on the corresponding nucleotide, rightclick, and select 'Set Translation End'





Now the sequence after the translation end is 3' UTR.



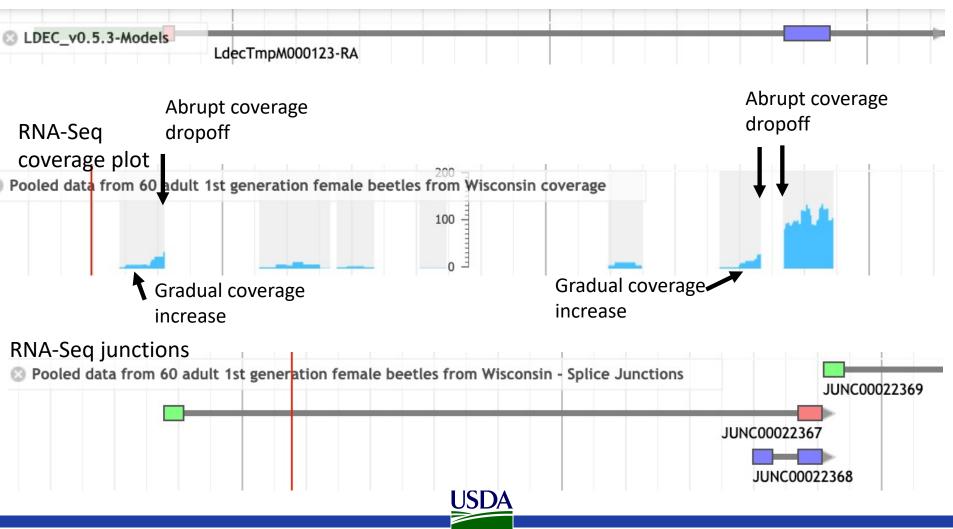
Annotating isoforms

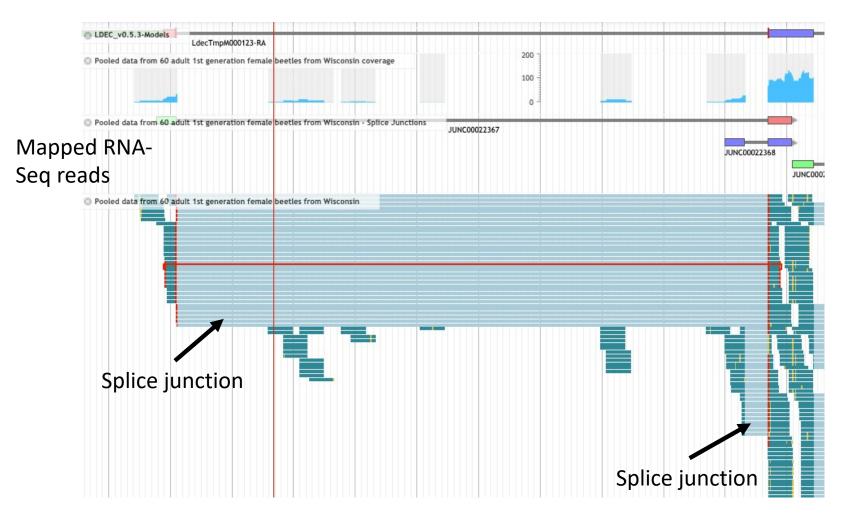


- In our experience, lots of mapped RNA-Seq reads are critical for good manual isoform annotation
- Before evaluating RNA-Seq for isoforms, it helps to understand how to interpret gradual and abrupt drops in coverage
 - Gradual usually means 5' start or 3' end of expression
 - Abrupt usually means splice junction
- Checking junction reads (if available) is incredibly useful



5' end of MAKER tyrosine protein kinase gene prediction





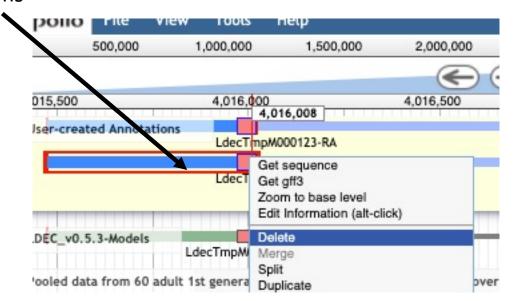


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Select and delete 5' exon from one of the isoforms



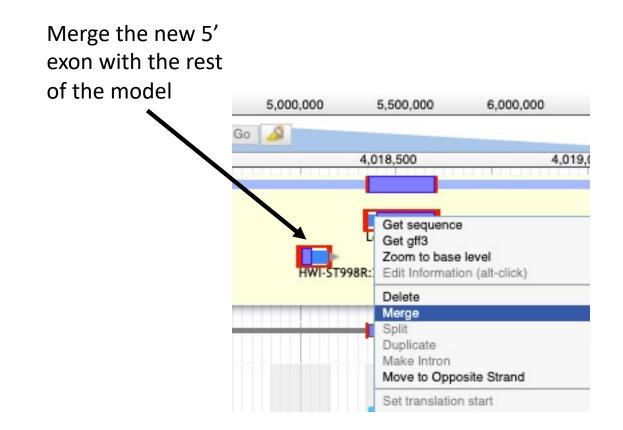


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2 isoforms supported by RNA-

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Non-canonical splice sites

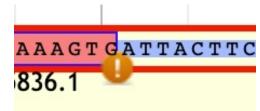


Splice sites

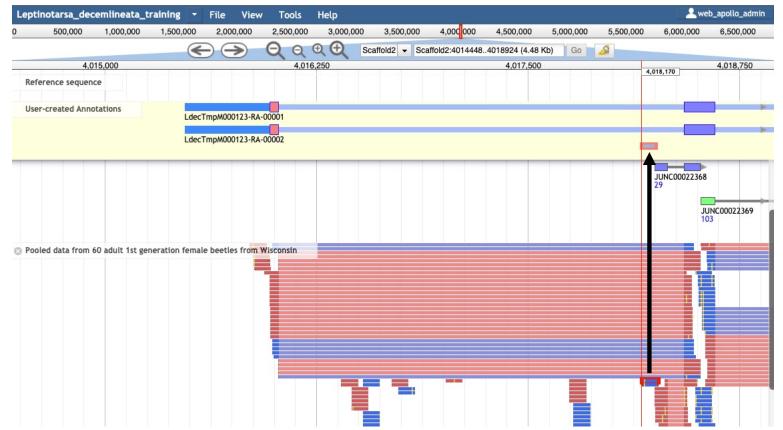
Introns are removed from primary transcripts by cleavage at conserved sequences called **splice sites**. These sites are found at the 5' and 3' ends of introns.

(https://www.nature.com/scitable/topicpage/rna-splicing-introns-exons-and-spliceosome-12375/)



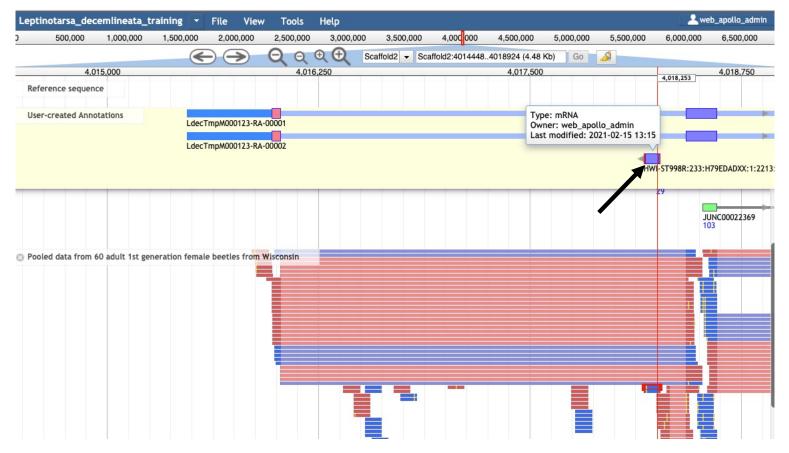


'Non-canonical' splice sites – non-conserved, and possibly erroneous sites – are marked by an exclamation point in Apollo.



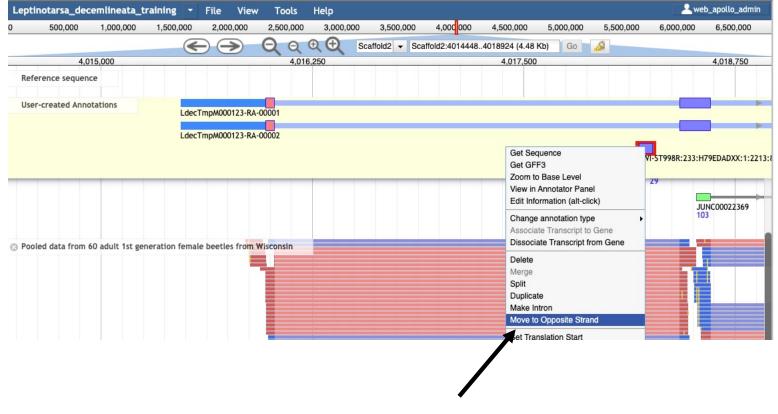
Let's revisit the previous 'Isoform annotation' example. Let's add the new exon using different starting evidence.





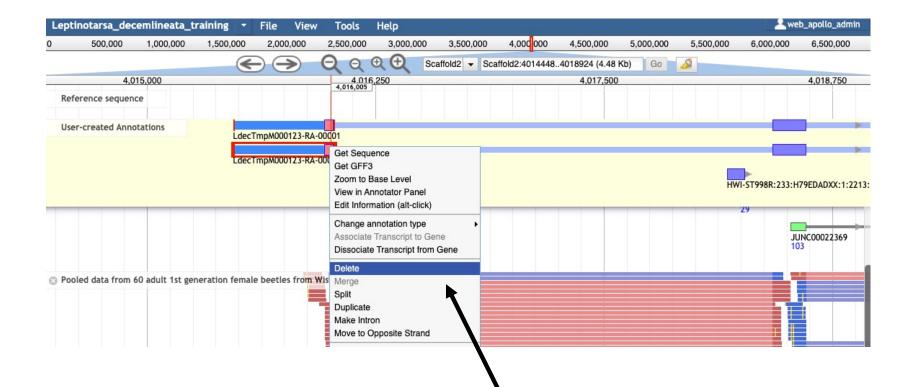
That starting evidence was mapped to the reverse strand – let's flip it to the forward strand





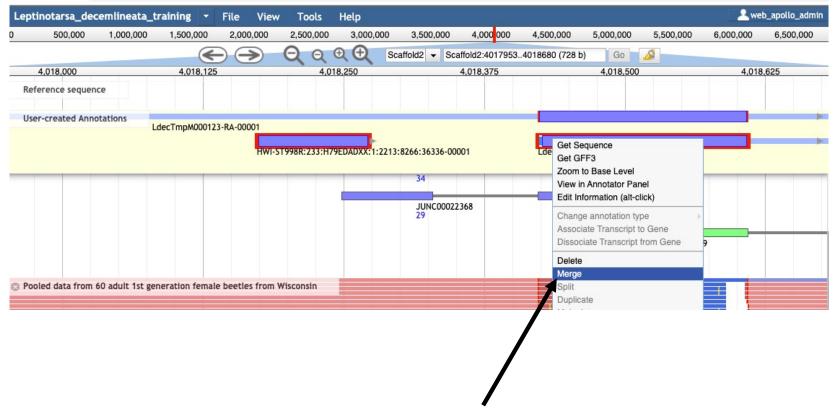
Right-click on the evidence and select 'Move to Opposite Strand'





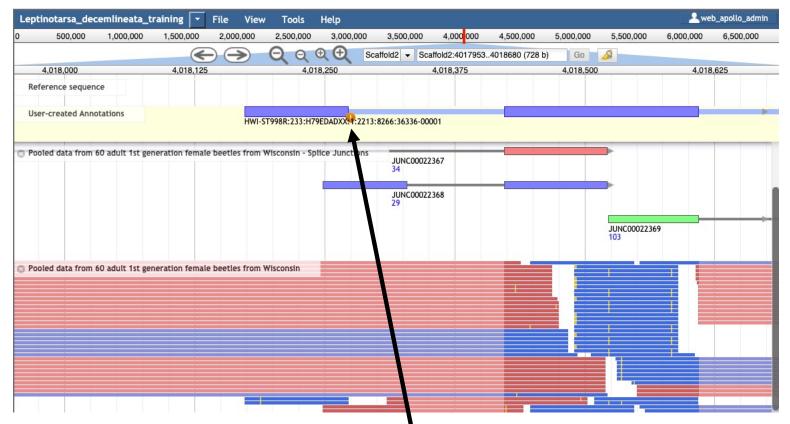
Delete the 5' exon as previously (no support for it for this isoform)





Merge the new 5' exon with the model





Now that the models are merged, Apollo shows us a non-canonical splice site



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- Canonical donor splice sites should be G(T/U) this is GC
- The coverage track shows us that the exon needs to extend further to the right

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O Poo	led data from 60	adult 1st gen	eration fema	le beetles from	Wisconsin								
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Let's use another RNA-Seq read to extend the exon to the actual splice site



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- Shift-click on the model's 5' exon and the RNA-Seq read
 - right-click to open menu
 - Select 'Set as 3' end'

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Thank you!

The NAL Team

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- Sean Buehler
- Amanda Cooksey

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

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