Using Apollo at the i5k Workspace@NAL

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USDA

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
- Sequence alterations and stop-codon readthroughs
- Annotating Non-coding features

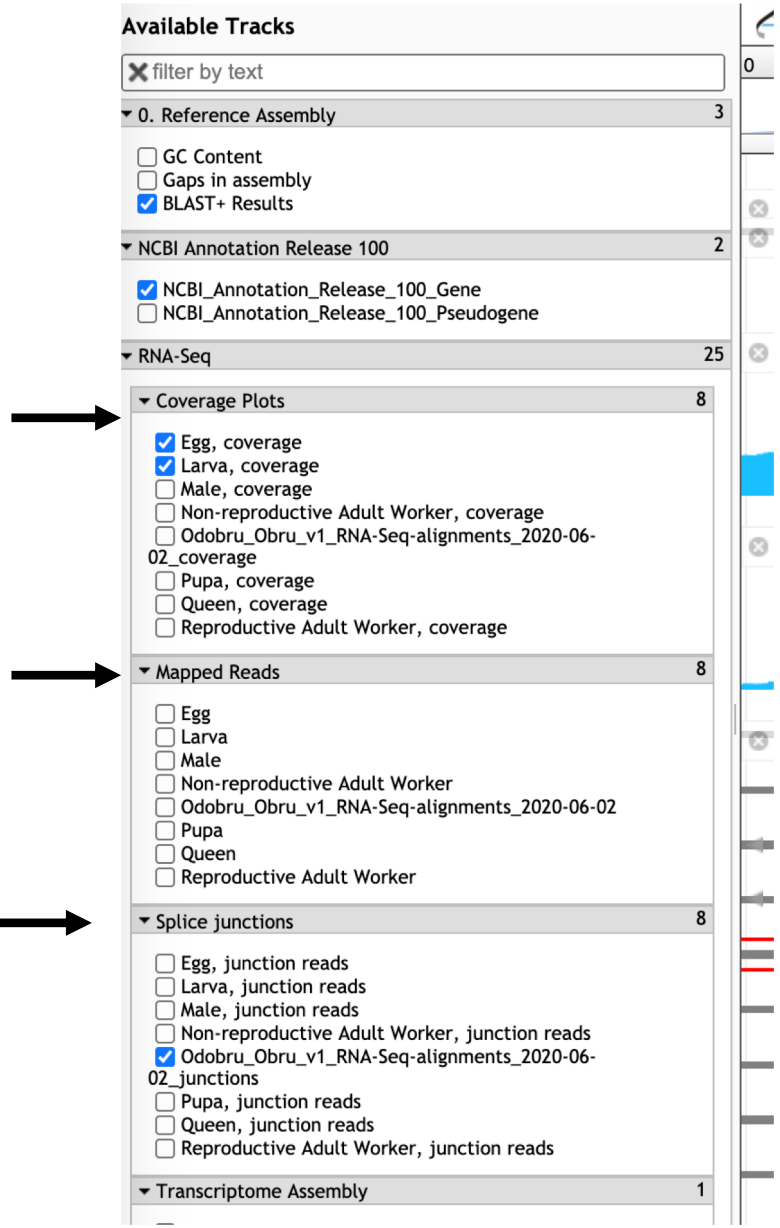
Other resources

- An additional Apollo webinar with more background: https://www.youtube.com/watch?v=dol99KExLgY&feature=youtu.be
- 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

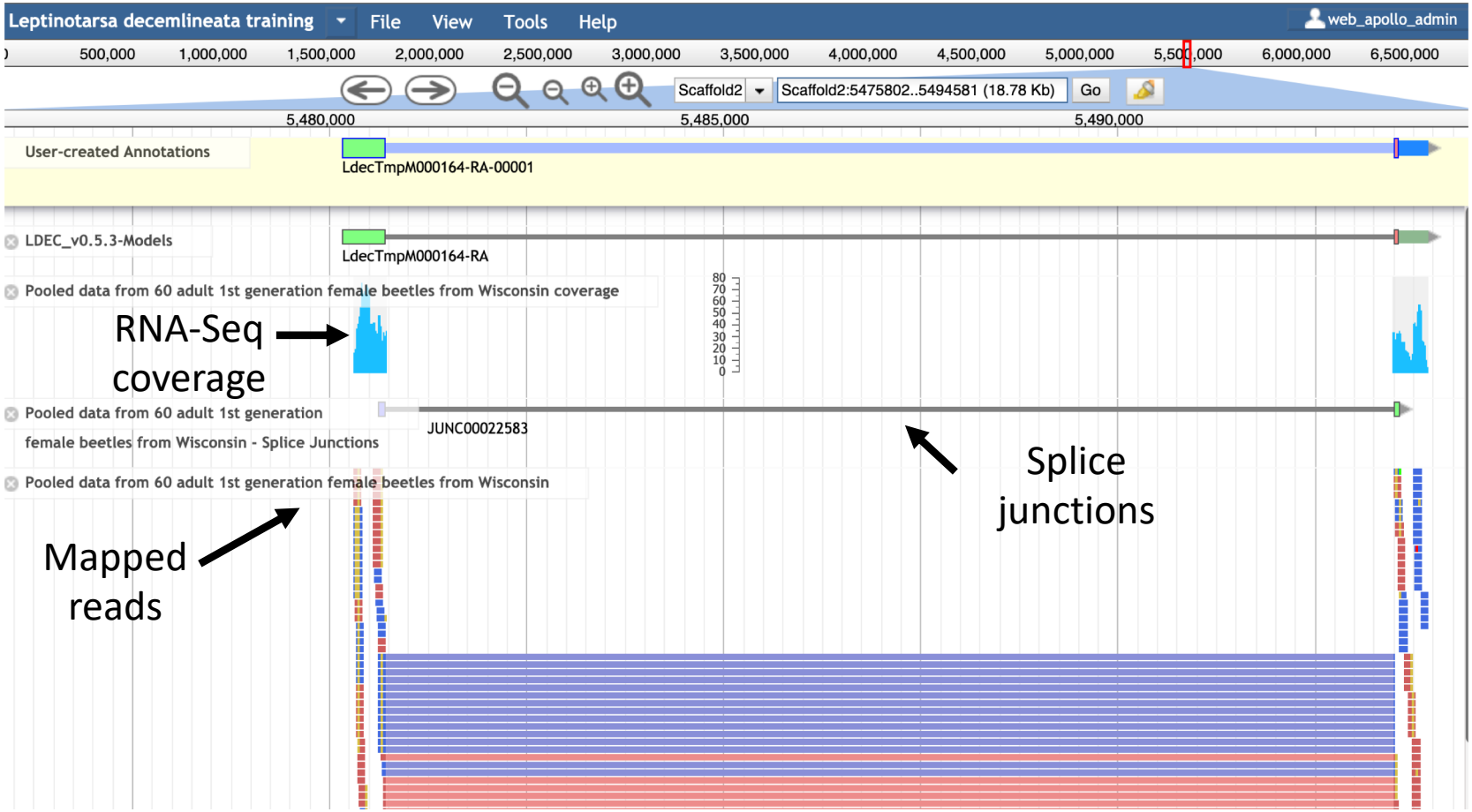
Basic RNA-Seq evaluation

RNA-Seq tracks

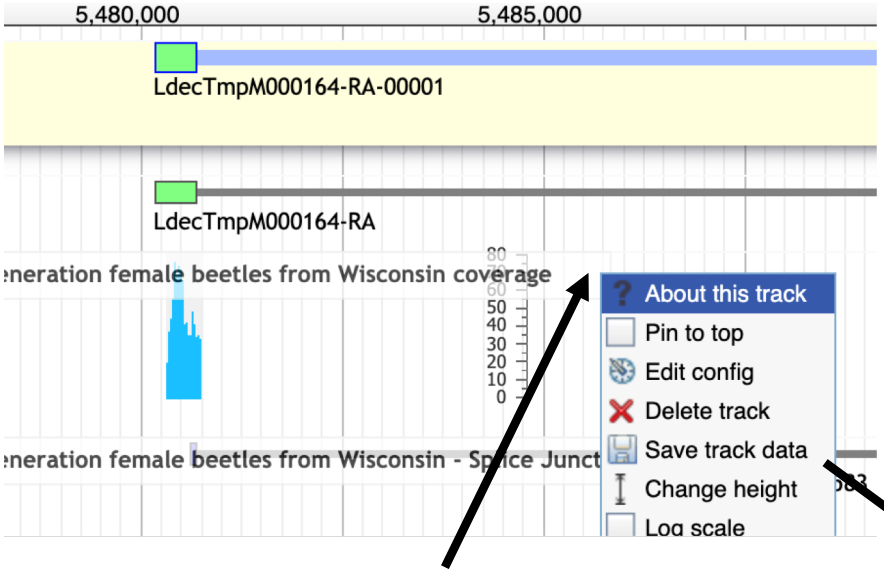
- **Coverage plots:** Histogram of the number of mappings at each nucleotide; hover over the blue area to see the value
- **Mapped reads:** Individual glyphs of each mapped read. Show mapped and spliced areas, and SNPs/indels. Informative, but hard to work with when zoomed out.
- **Junction reads:** Useful combined with coverage plots; show where mapped reads are spliced. Control-click on read and look under 'score' to see how many mapped reads support the splice junction.



A simple case

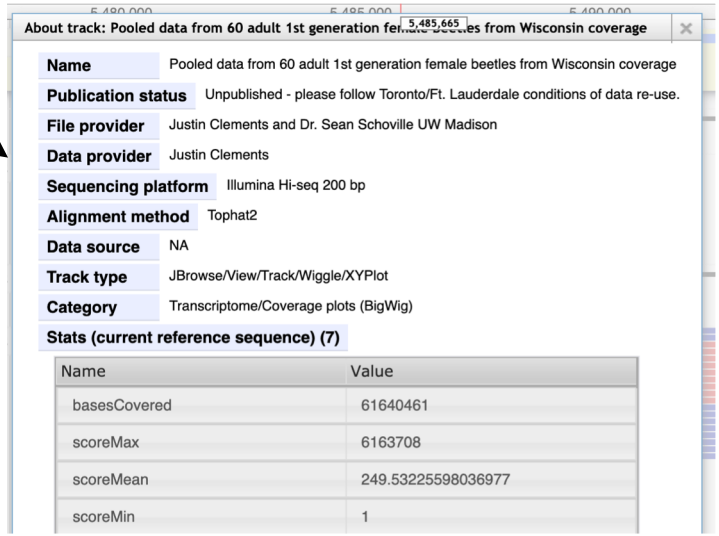


A simple case

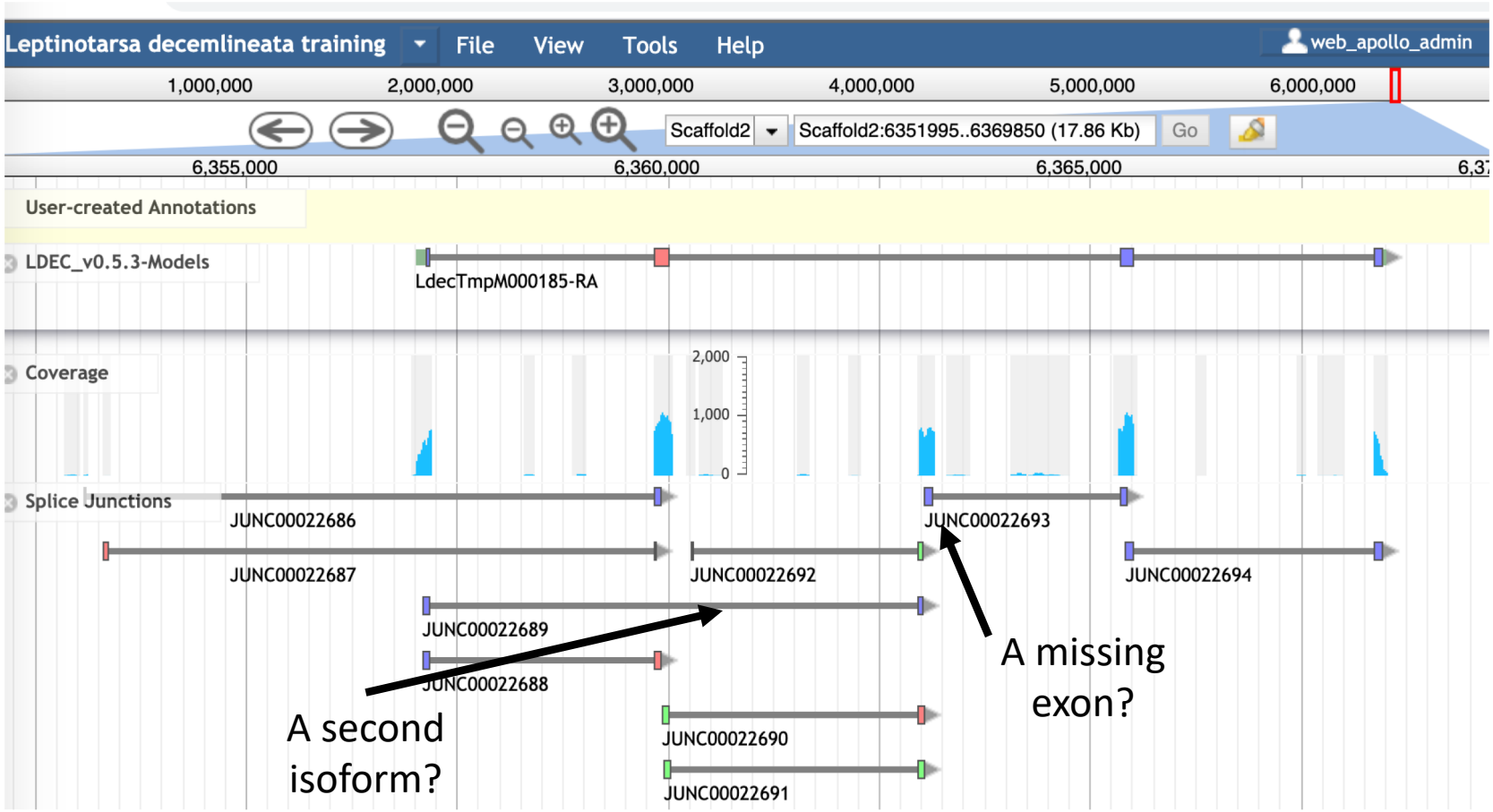


Select ‘About this track’ from drop-down menu

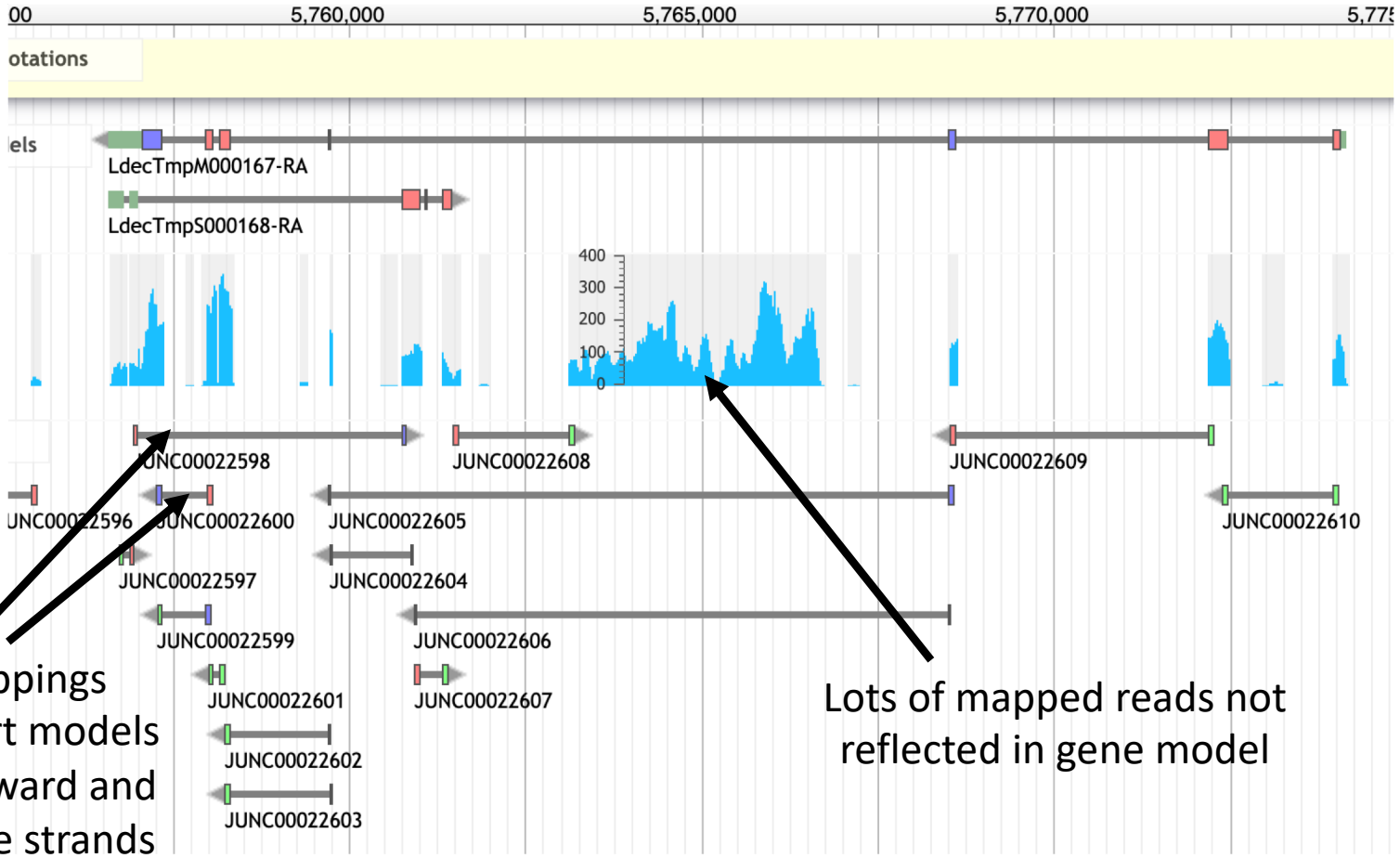
Information about methods



A more complex case



A really messy case



Mappings support models on forward and reverse strands

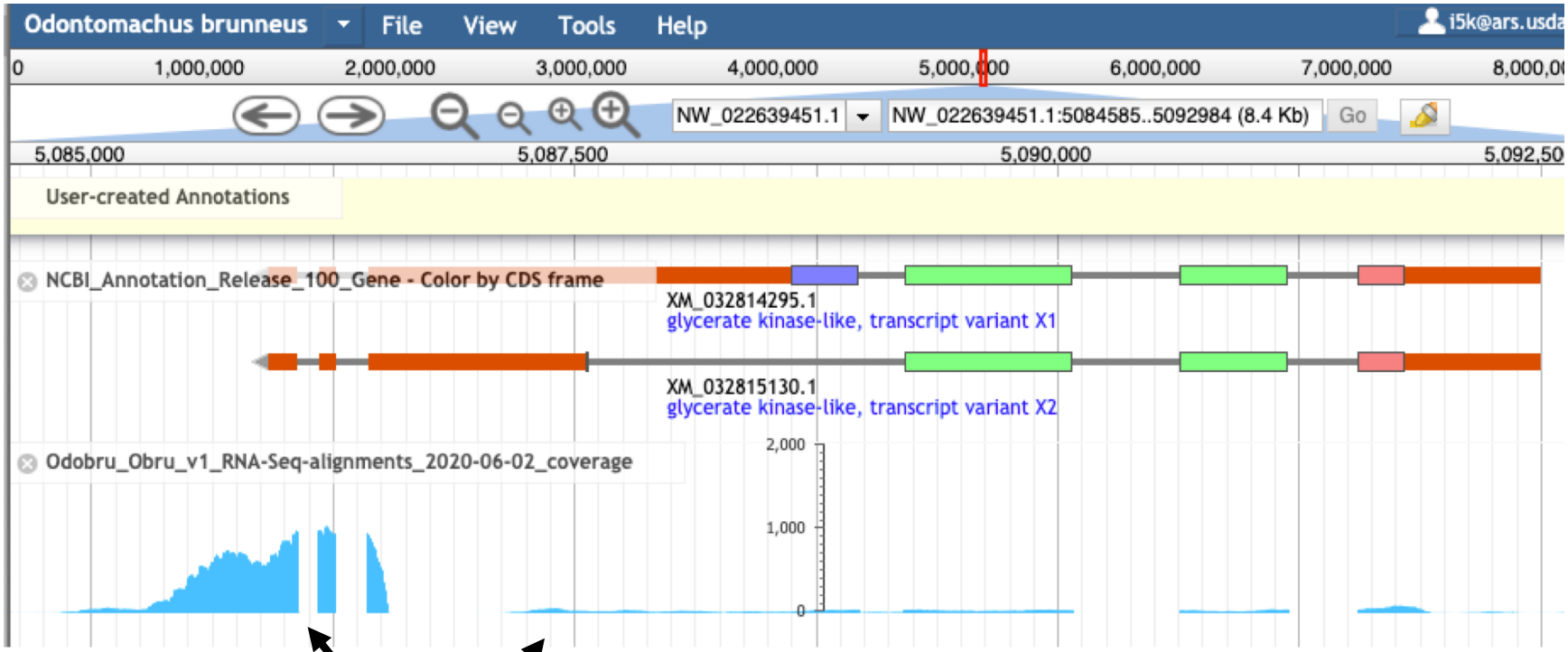
Lots of mapped reads not reflected in gene model

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

Annotation Example

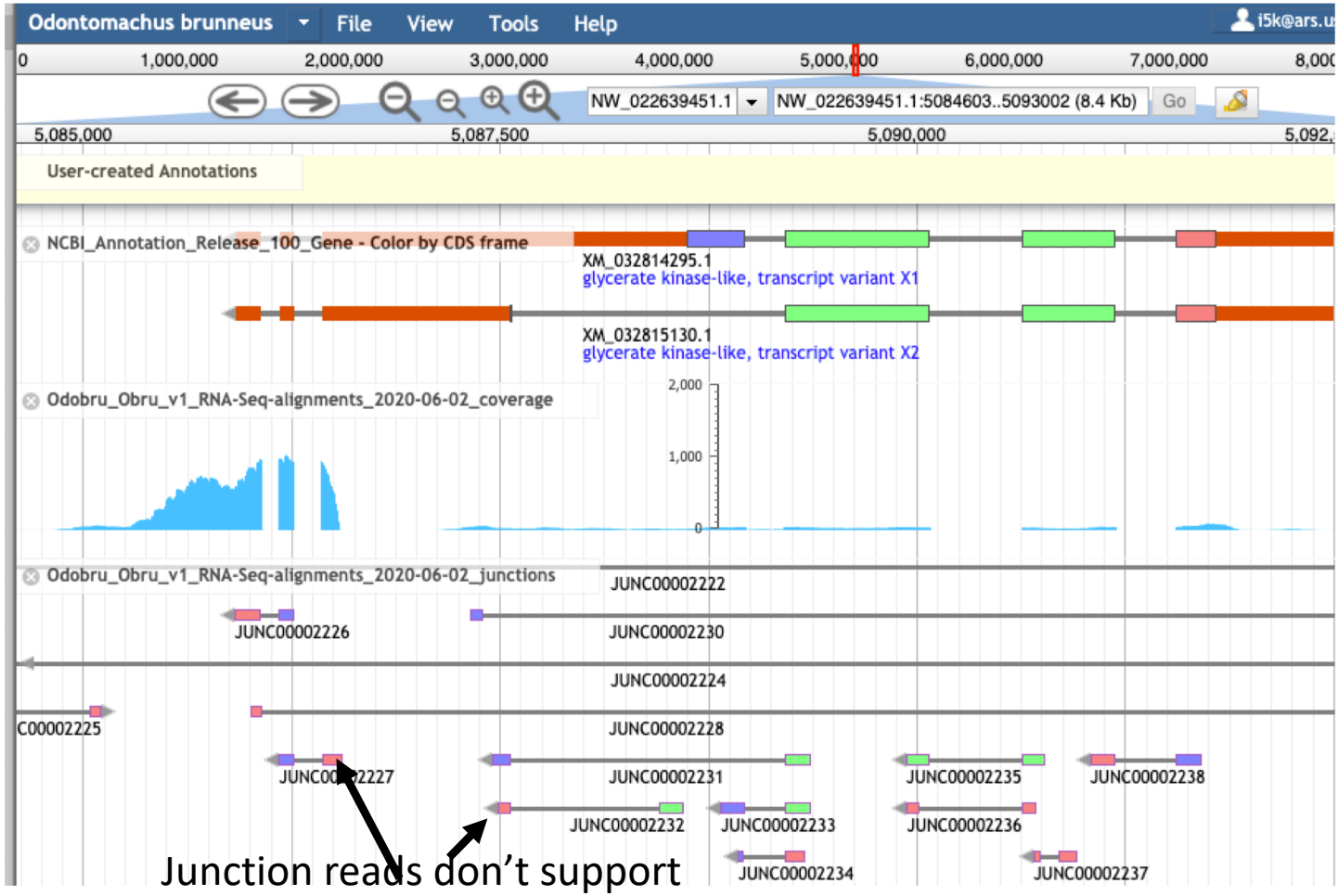
- Glycerate kinase-like in the trap-jaw ant *Odontomachus brunneus*
- More information about the trap-jaw ant genome assembly: https://i5k.nal.usda.gov/odontomachus-brunneus
- *Odontomachus brunneus* Apollo URL: https://apollo.nal.usda.gov/apollo/4006447/jbrowse/index.html?loc=NW_022639451.1%3A5084490..5093717&tracks=DNA%2CAnnotations%2CNCBI_Annotation_Release_100_Gene-CBT&highlight=

RNA-Seq evaluation



Very different coverage between UTR and CDS

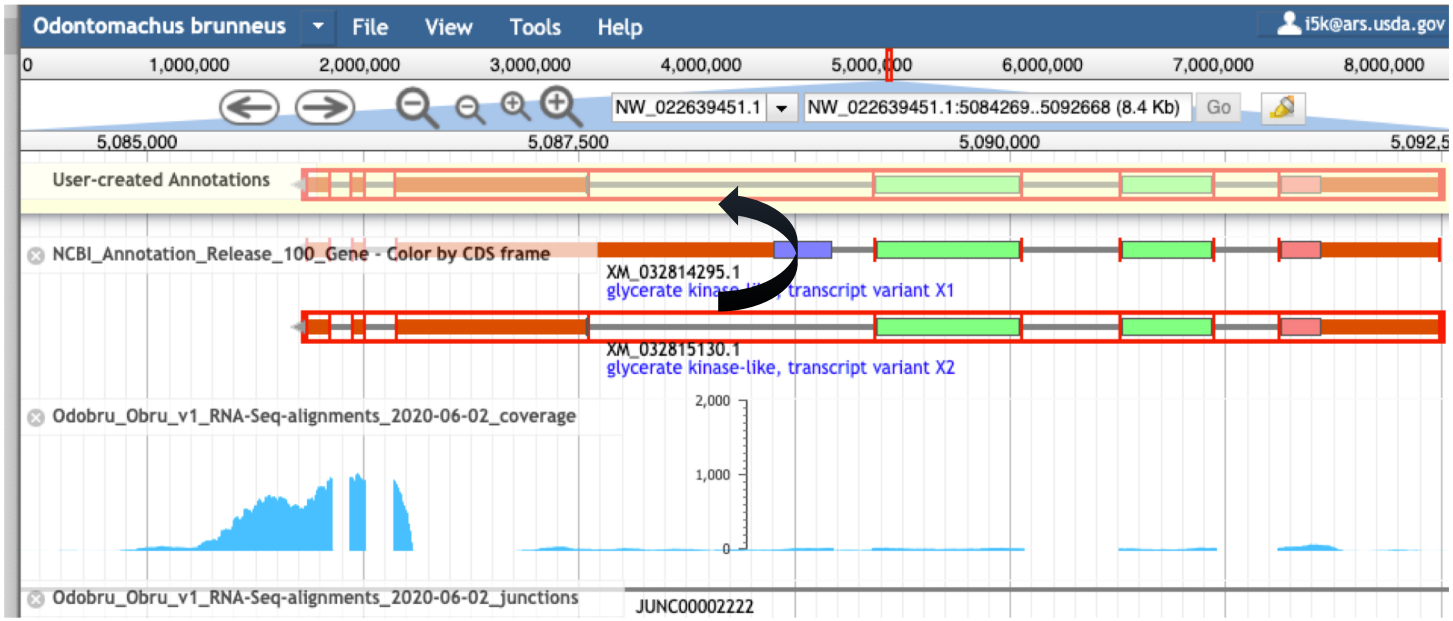
RNA-Seq evaluation



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

Create new model in user-created annotations track

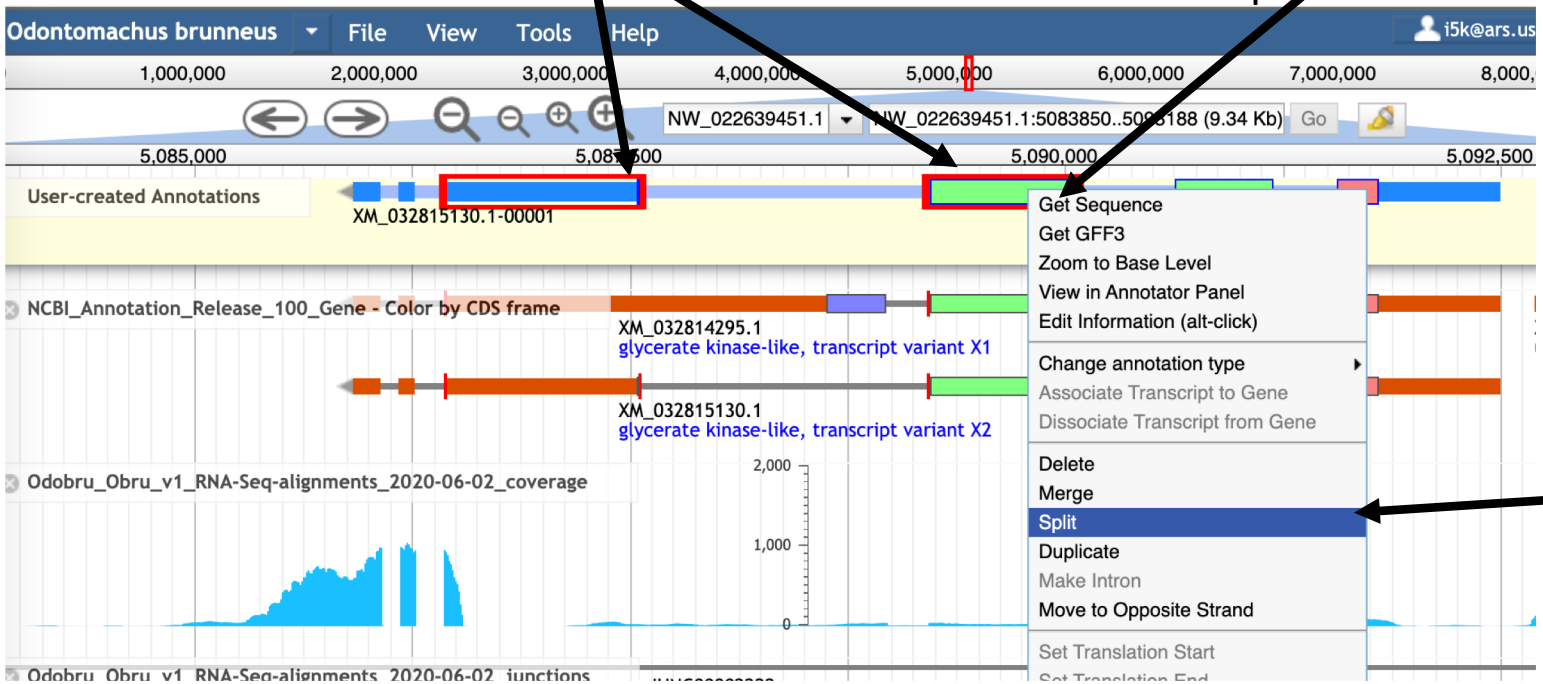
Drag evidence to UcA track (or right-click and select “create annotation”)



Split model

Select exons on which to split the model using the ‘shift’ key

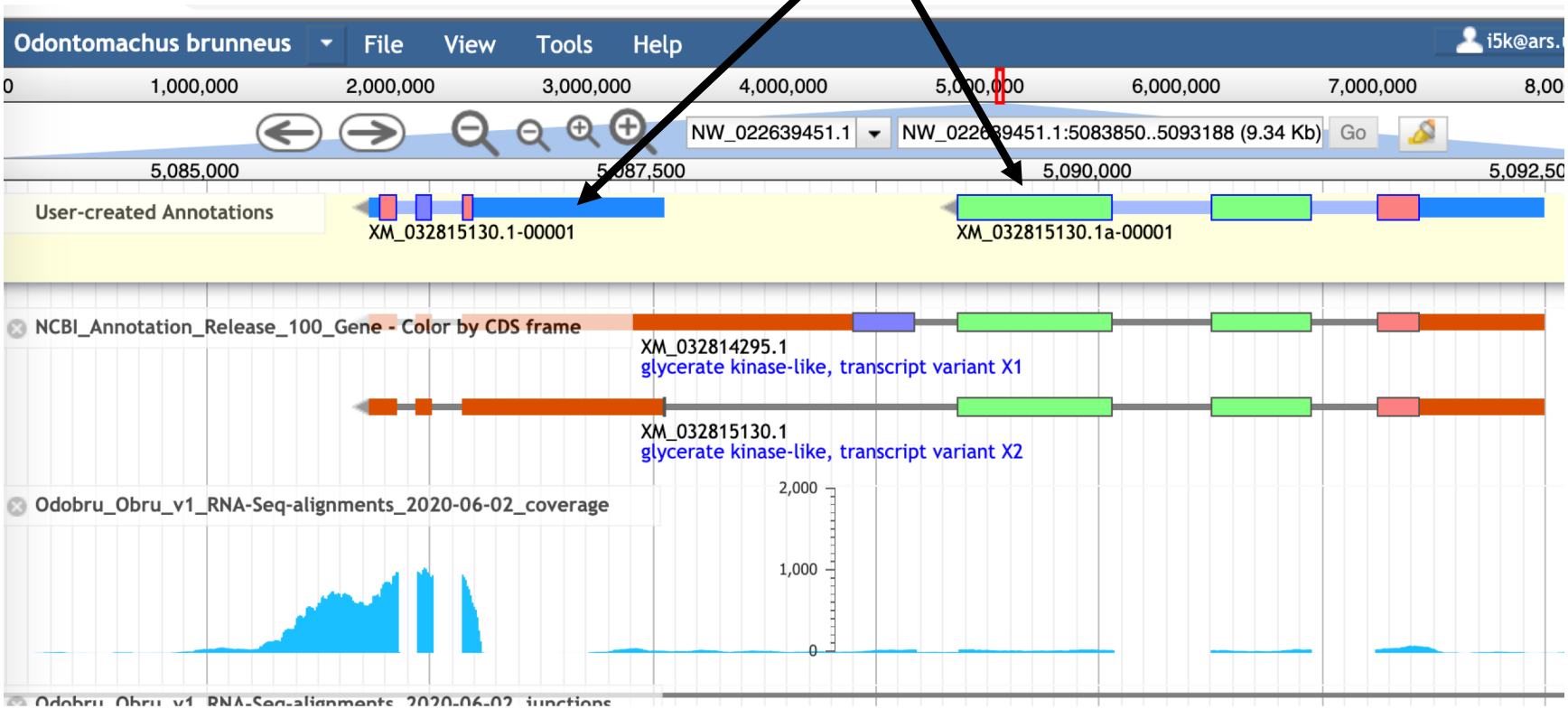
Right-click on the model while continuing to hold shift to get the drop-down menu



Select ‘split’

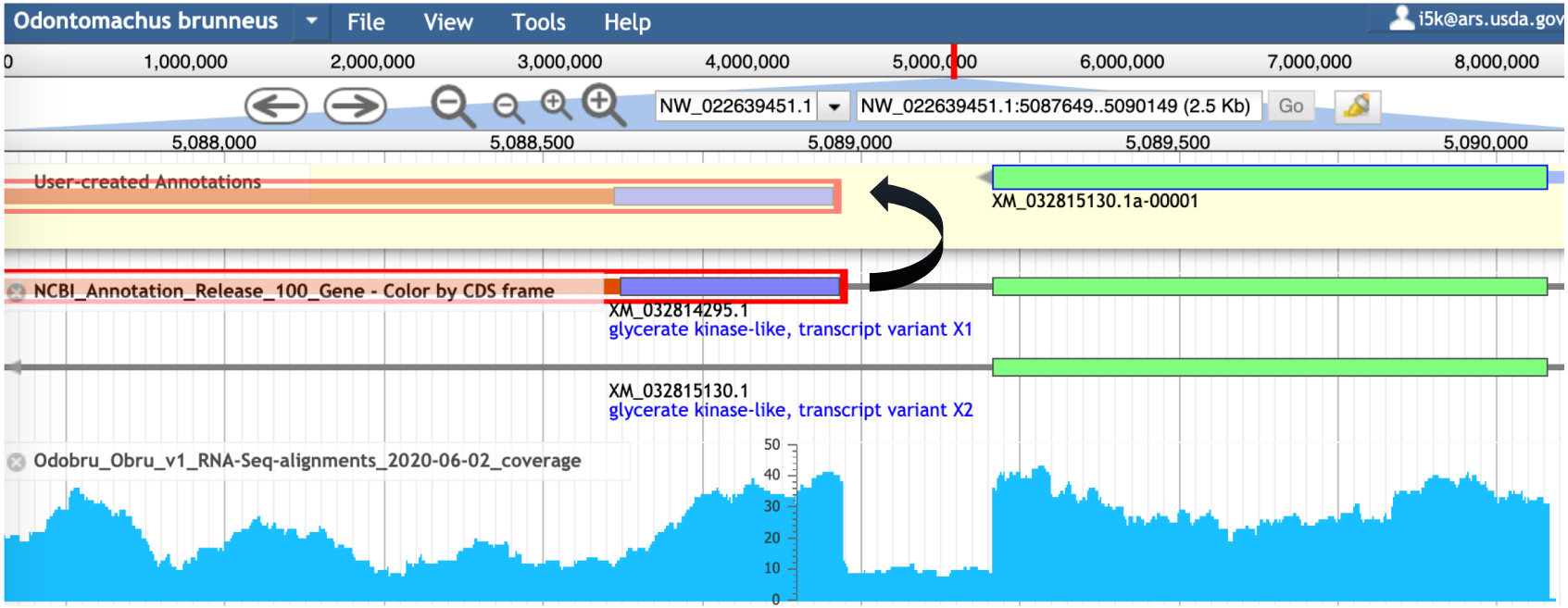
Split model

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



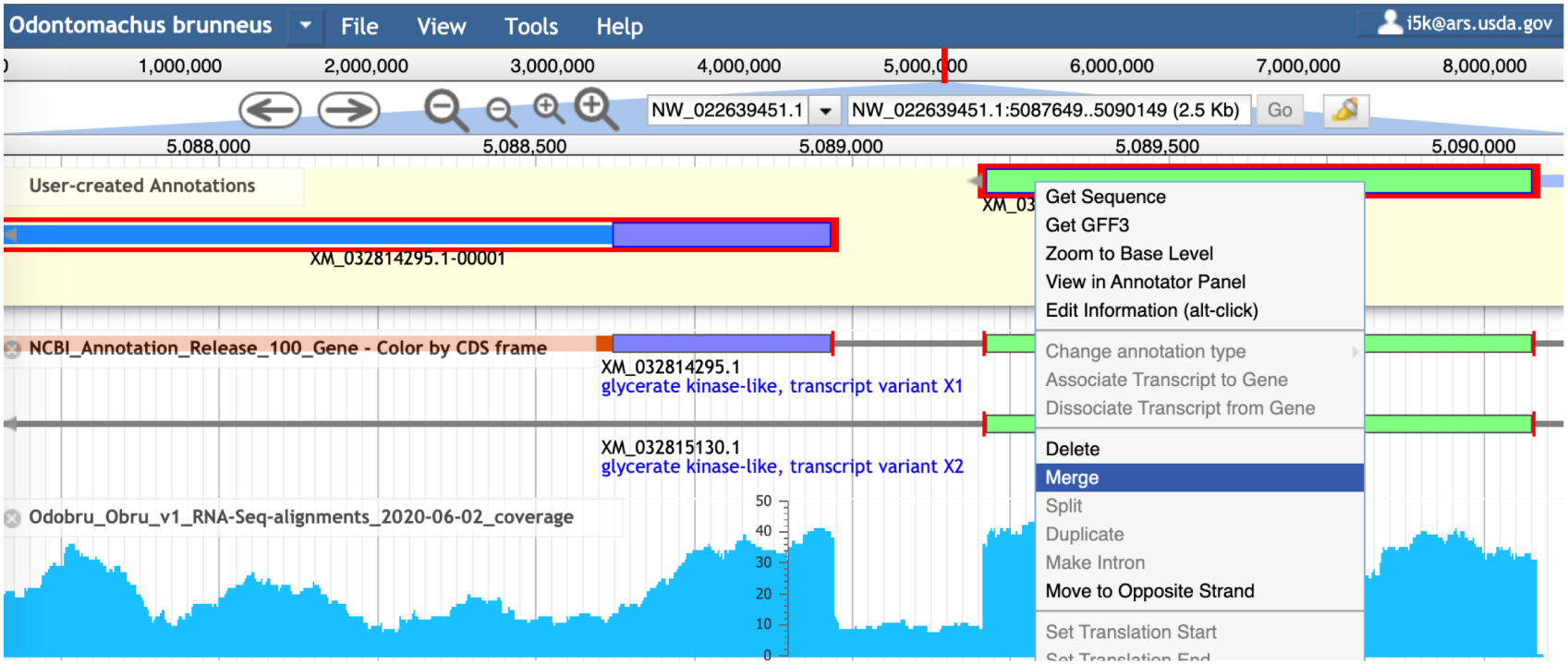
Add an exon

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



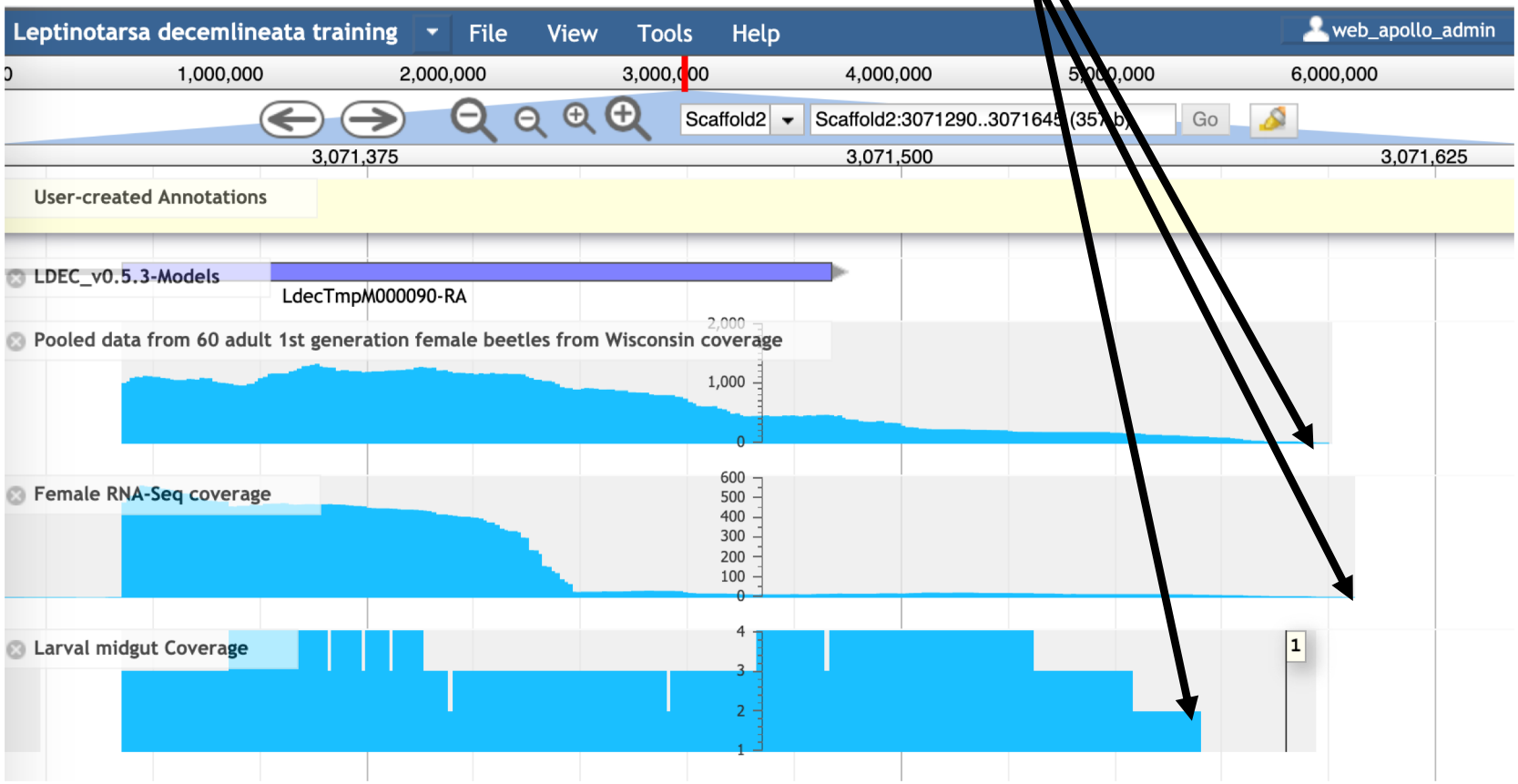
UTRs – how and when to add or adjust

Adding or adjusting UTR boundaries

- When should you add or change UTRs?
  - Only if you have RNA-Seq evidence with sufficient coverage (e.g. > 50 reads)
  - 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

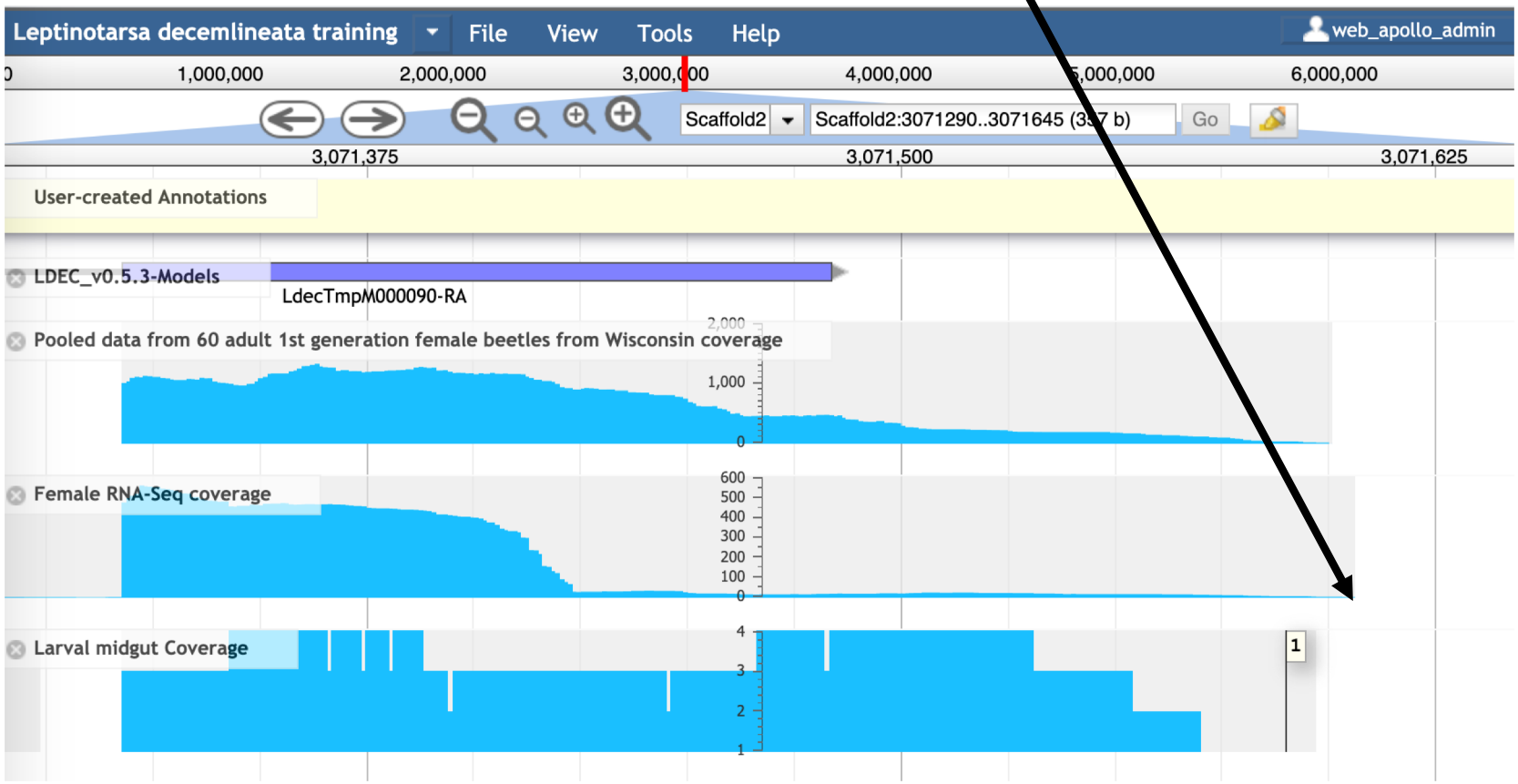
Adjusting gene boundaries

RNA-Seq evidence ends in different places for each track – how do you decide?

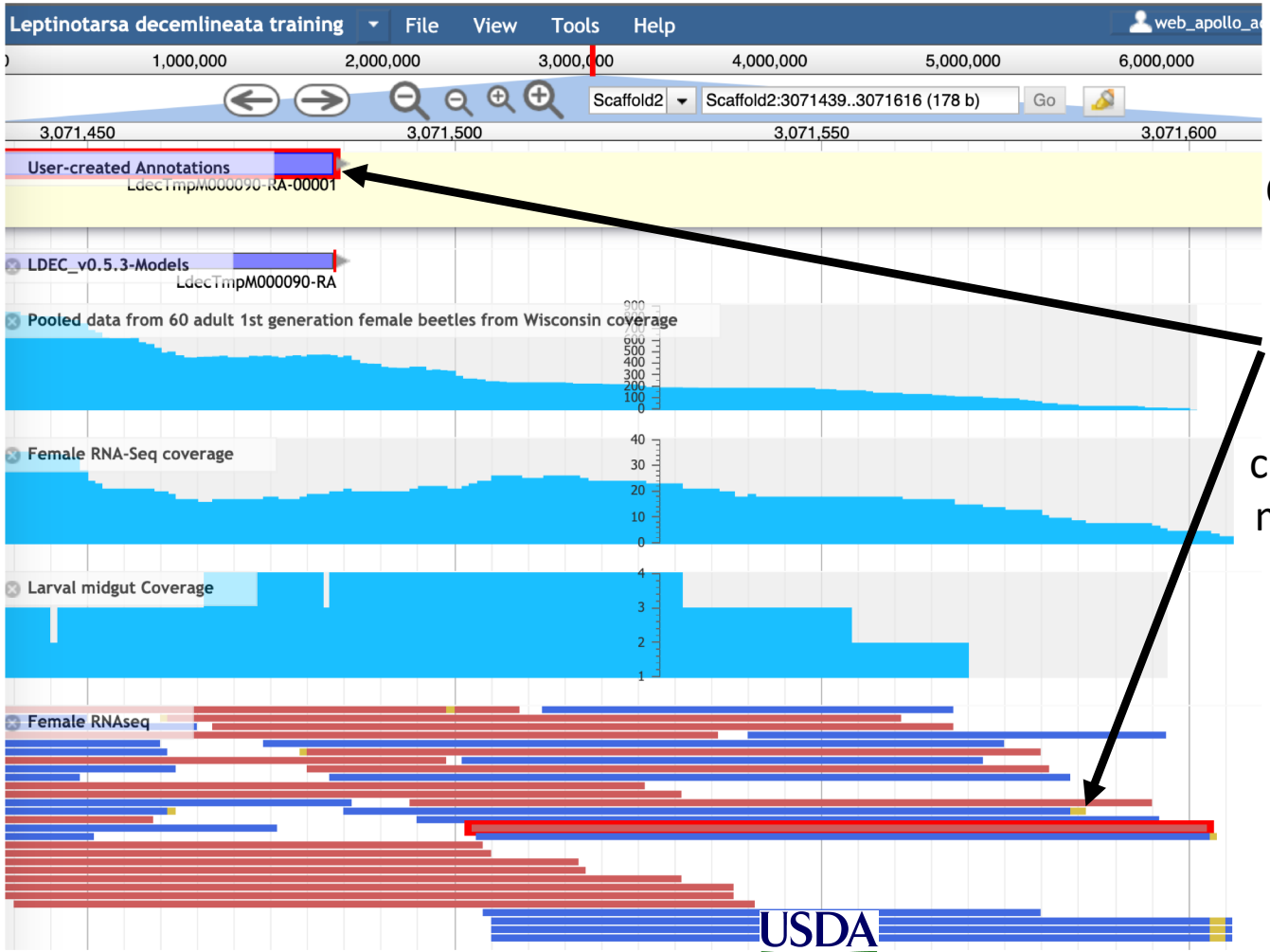


Adjusting gene boundaries

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

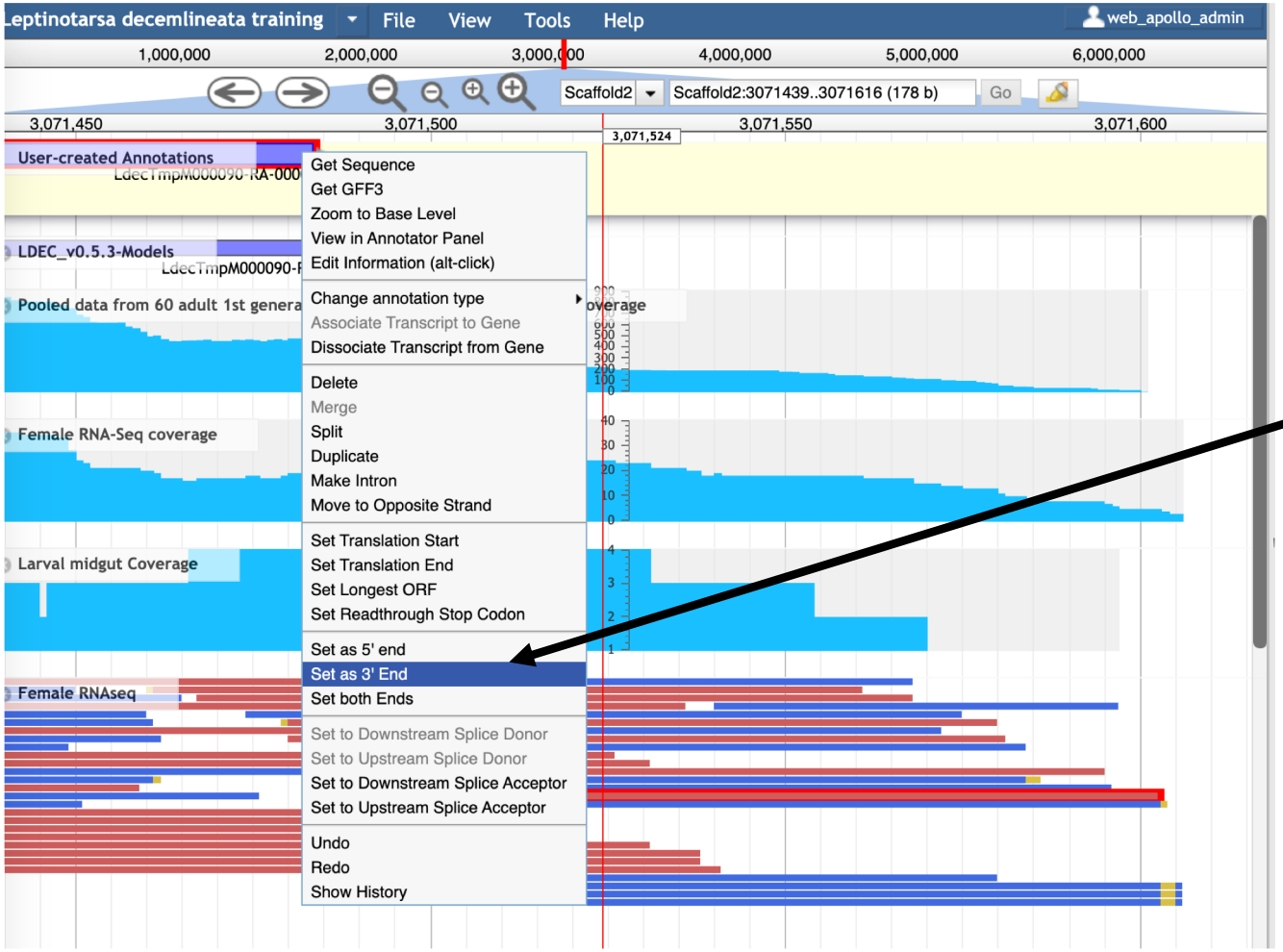


Adjusting gene boundaries



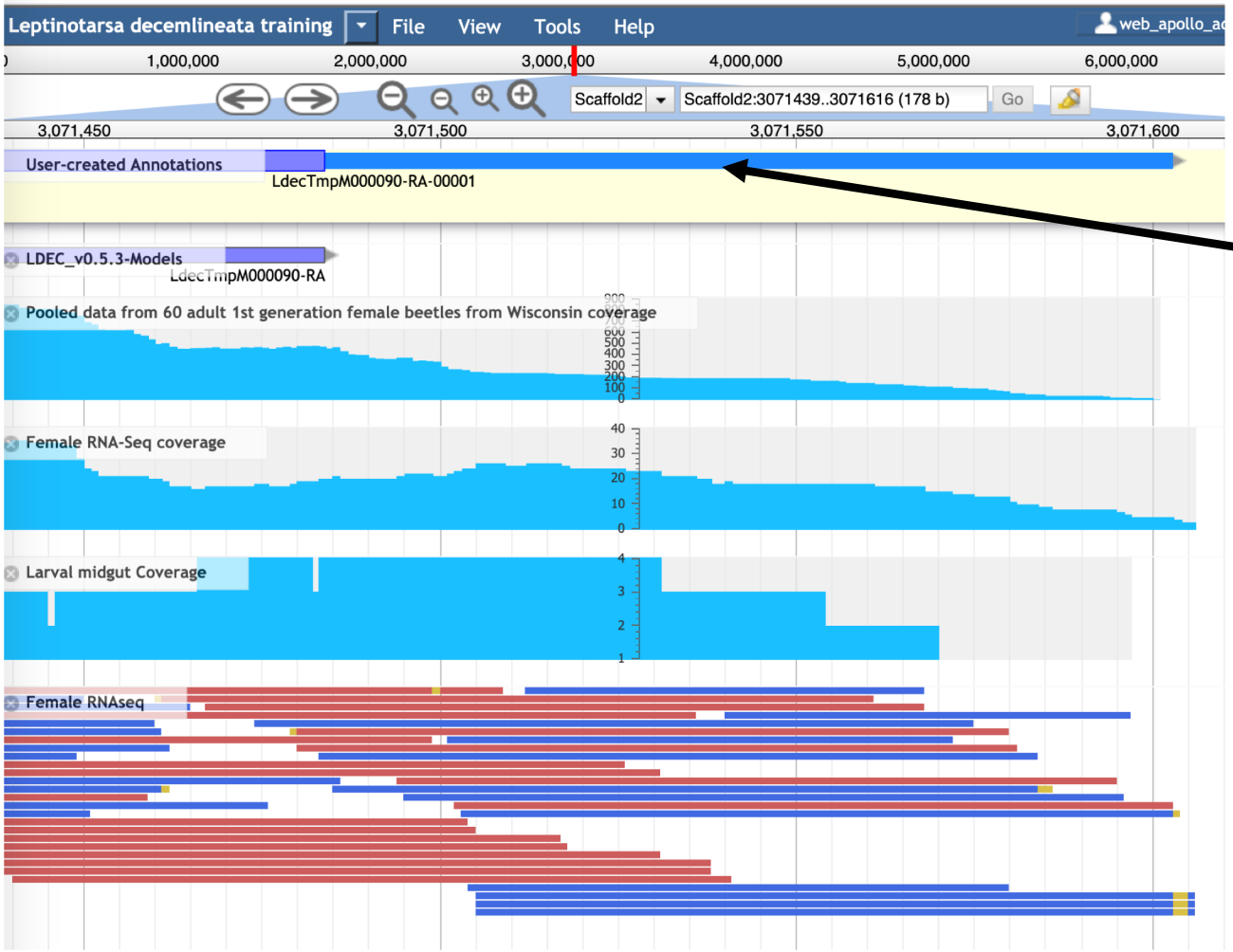
One way to change the boundary: find a mapped read on the same strand as the model; hold shift and click on the read and the model to highlight them both

Adjusting gene boundaries



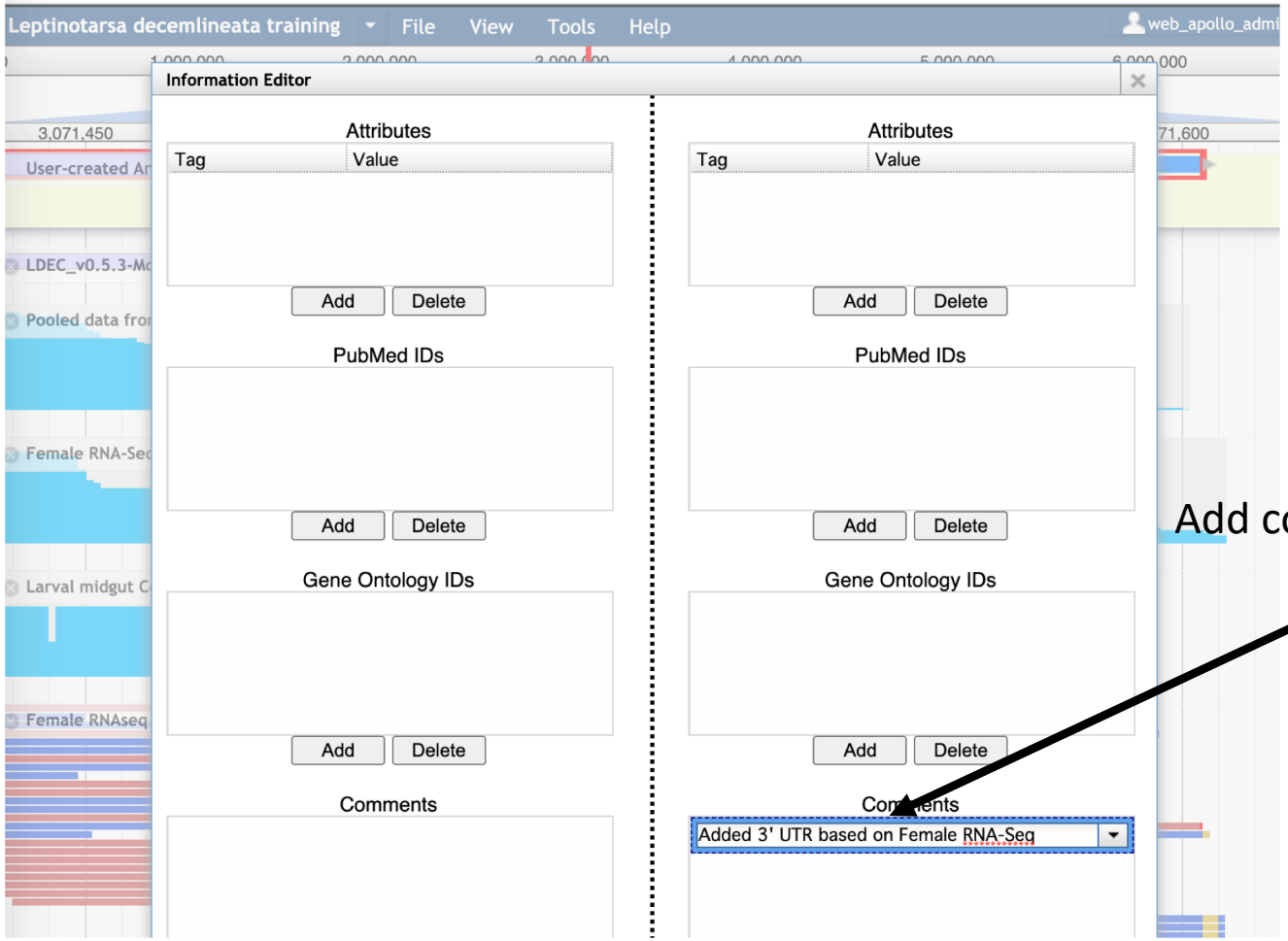
Right-click on model in user-created annotations track, and select ‘Set as 3’ end’ from the drop-down menu

Adjusting gene boundaries



New UTR is there!

Adjusting gene boundaries

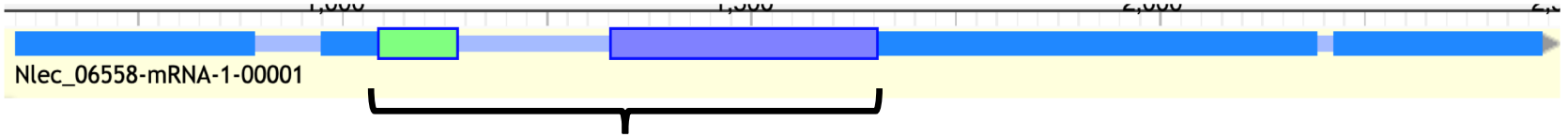


Starts, stops, open reading frames

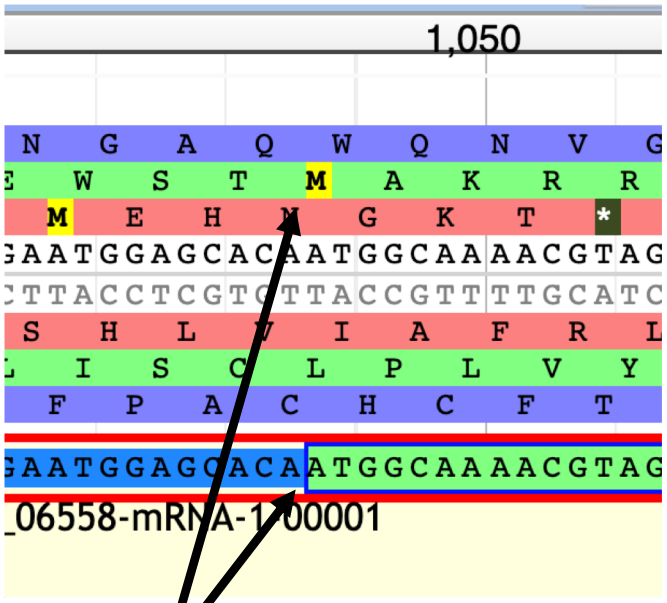
Setting the sequence start, stop, and open reading frame (ORF)

- Apollo will automatically calculate the longest possible ORF that includes canonical ‘Start’ and ‘Stop’ signals (https://genomearchitect.readthedocs.io/en/latest/UsersGuide.html)
- However, in some fringe cases, you will need to double-check
- You can change this if needed

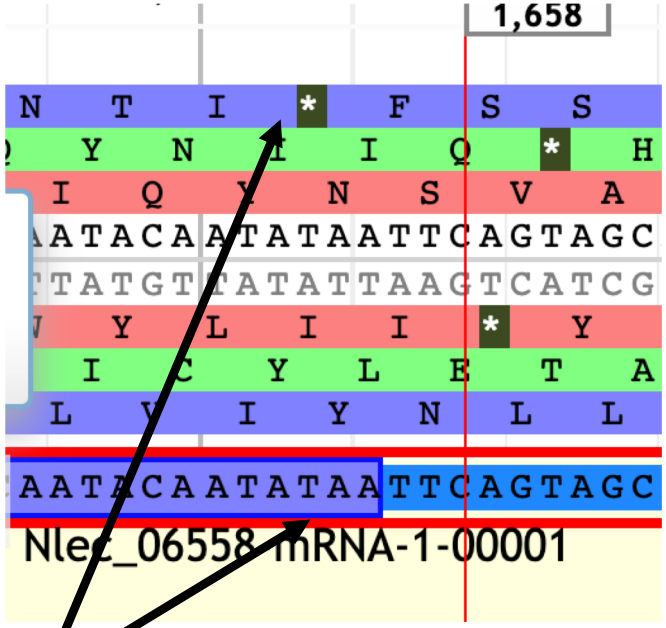
Starts, stops, ORFs



Open reading frame (ORF): translated region

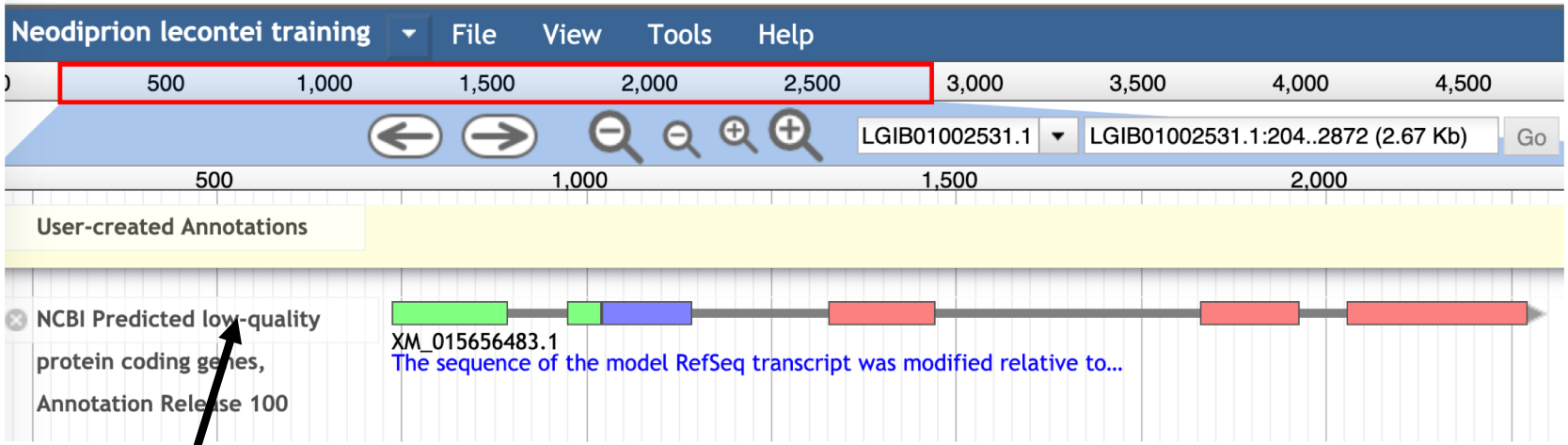


Translation start at Methionine



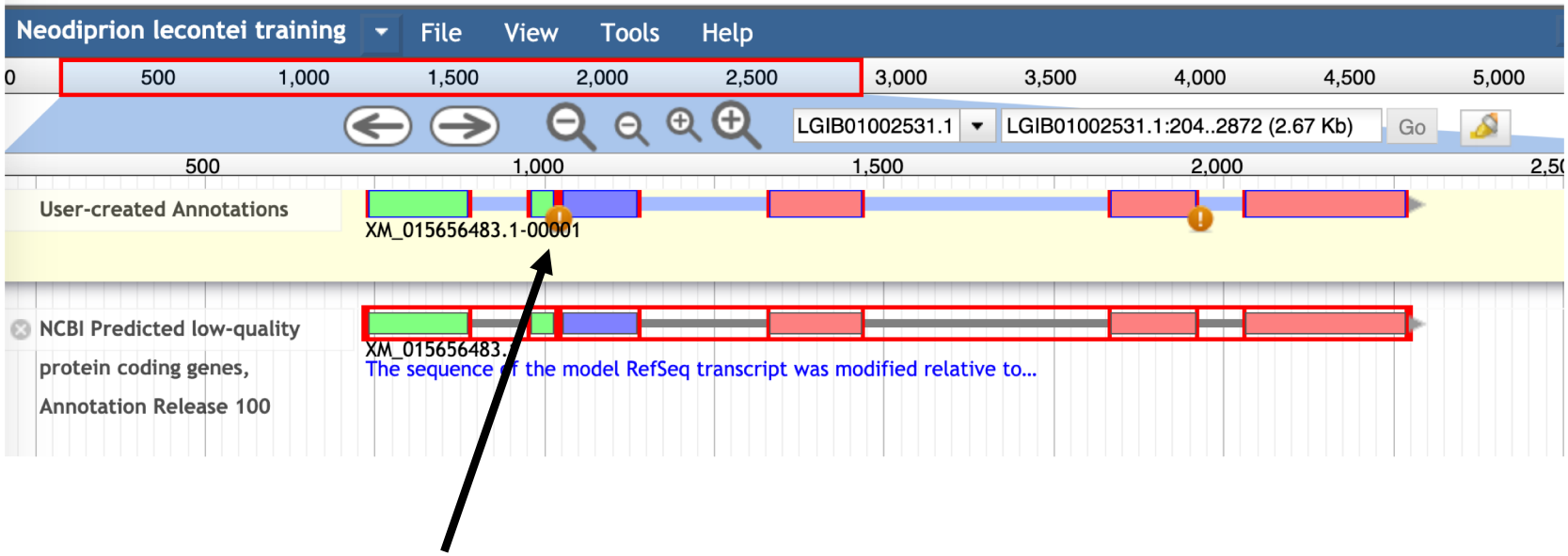
Translation stop at stop codon

Starts, stops, ORFs



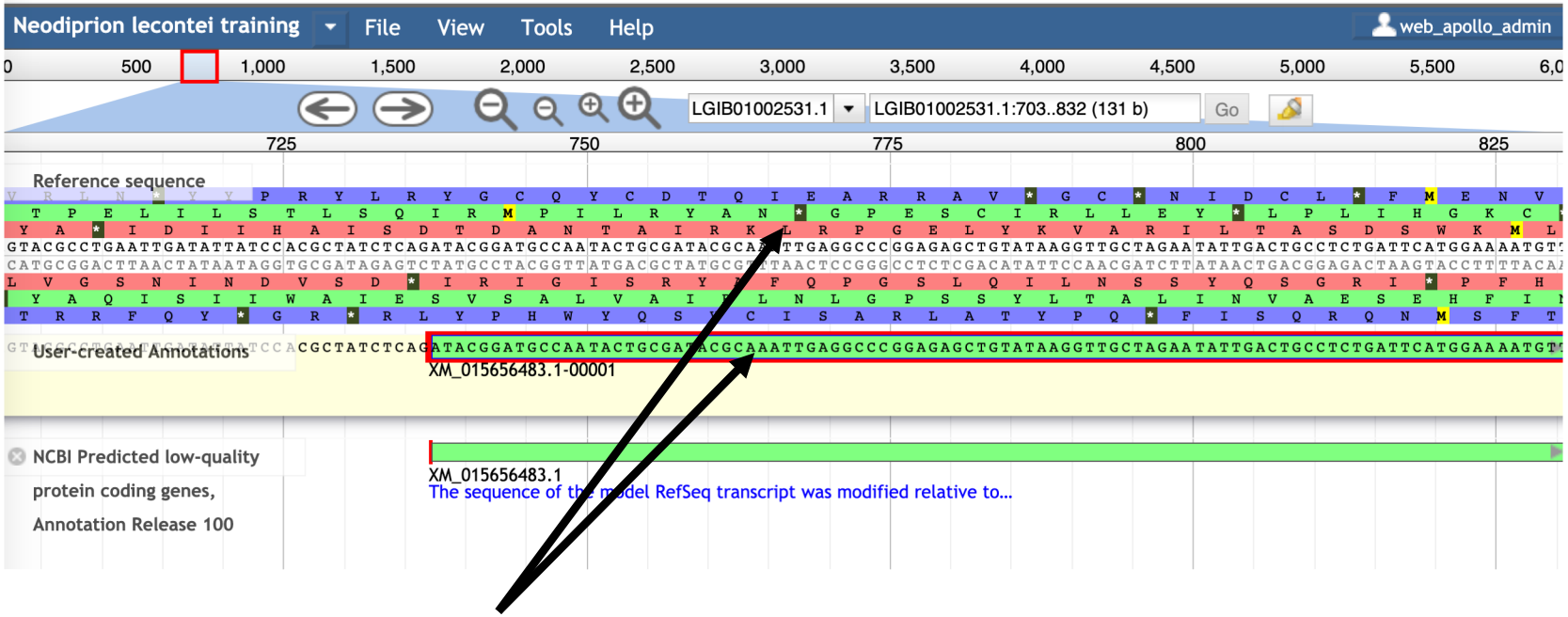
This is a ‘low quality’ protein coding gene from NCBI – it will likely show some problems in Apollo

Starts, stops, ORFs



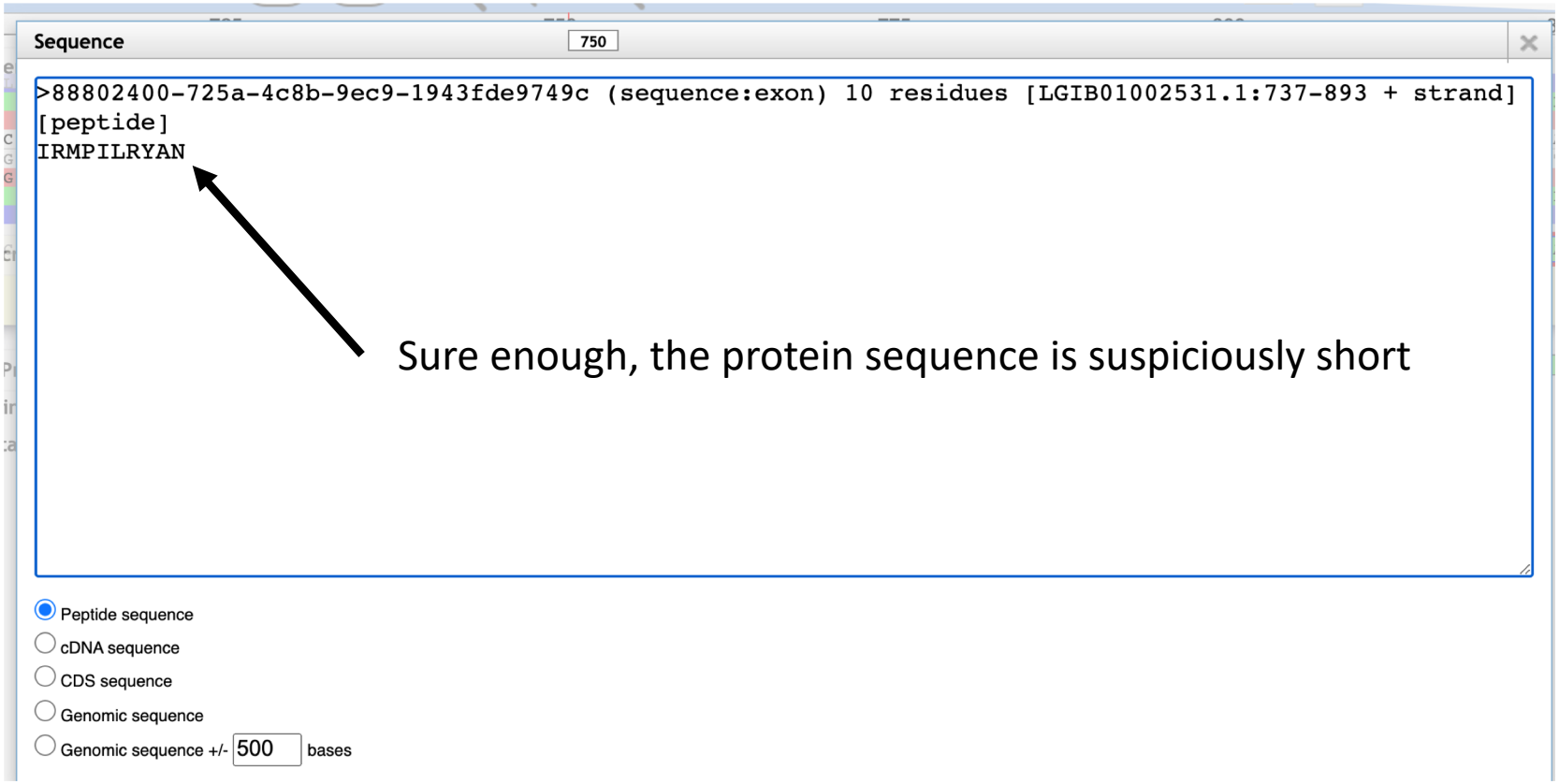
We can see a non-canonical splice site in the UcA (more on that later). Let's zoom to the start of the model.

Starts, stops, ORFs

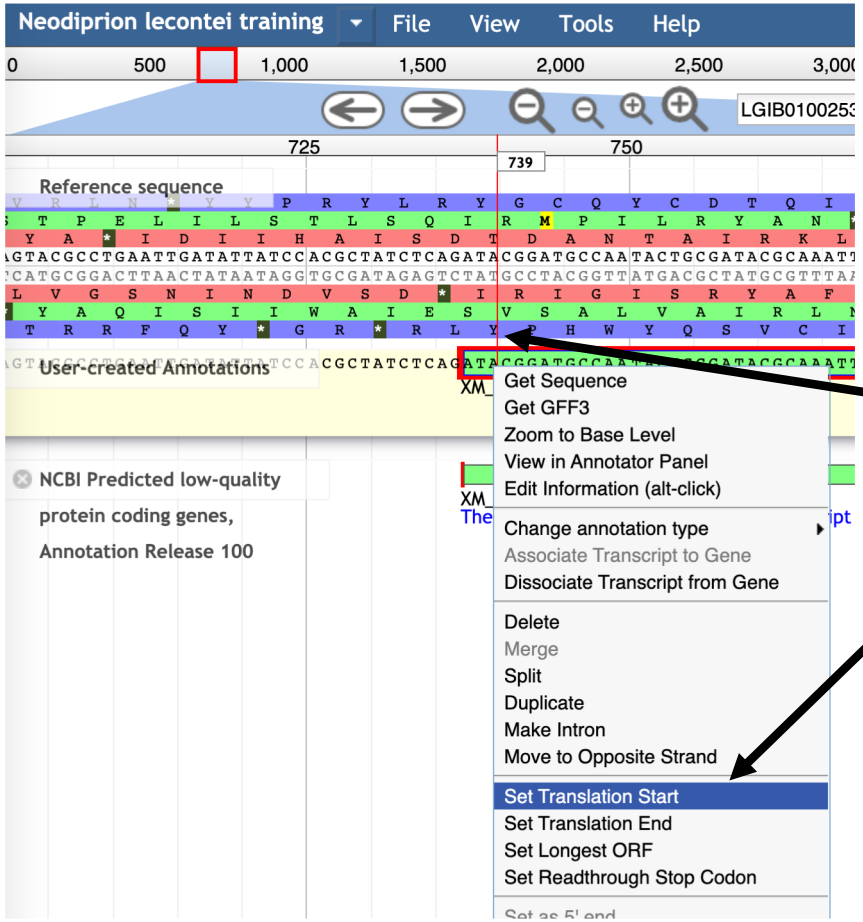


Apollo shows this model in the green reading frame – however, we can see a stop pretty early on in the genome sequence - but that's not reflected in the Apollo model! It looks like the pink reading frame doesn't have stops.

Starts, stops, ORFs

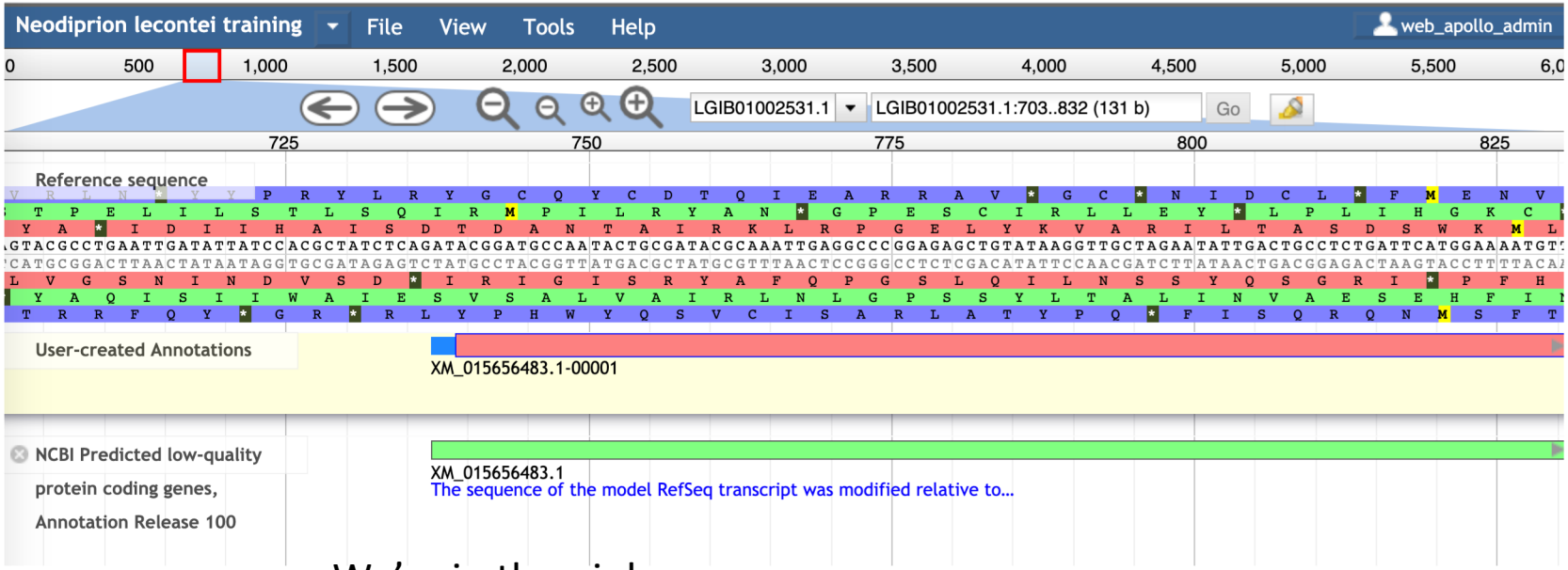


Starts, stops, ORFs



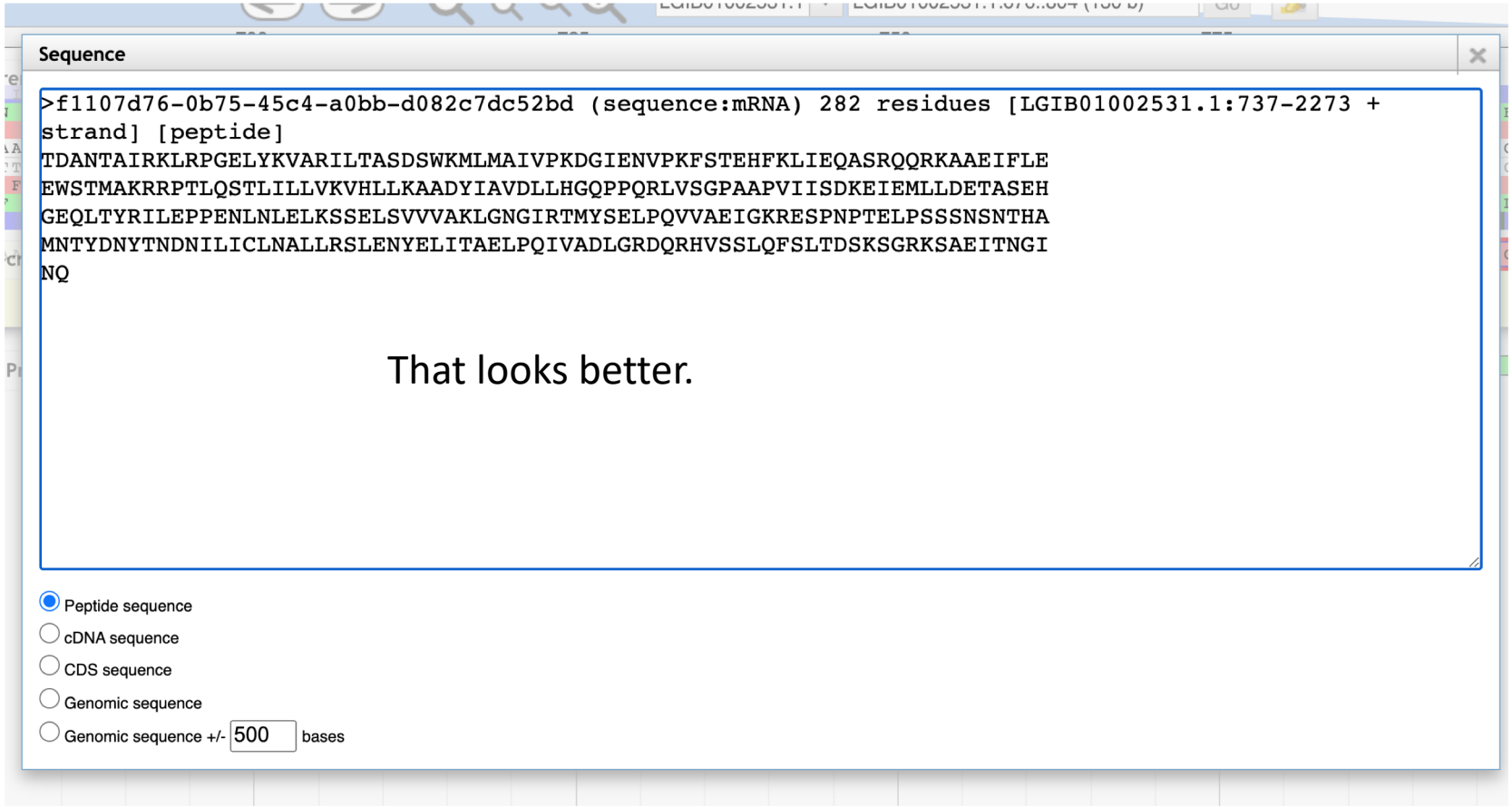
Let's set the translation start in the pink reading frame – click on the 3rd nucleotide in the UcA, right-click, and select 'Set Translation Start'

Starts, stops, ORFs

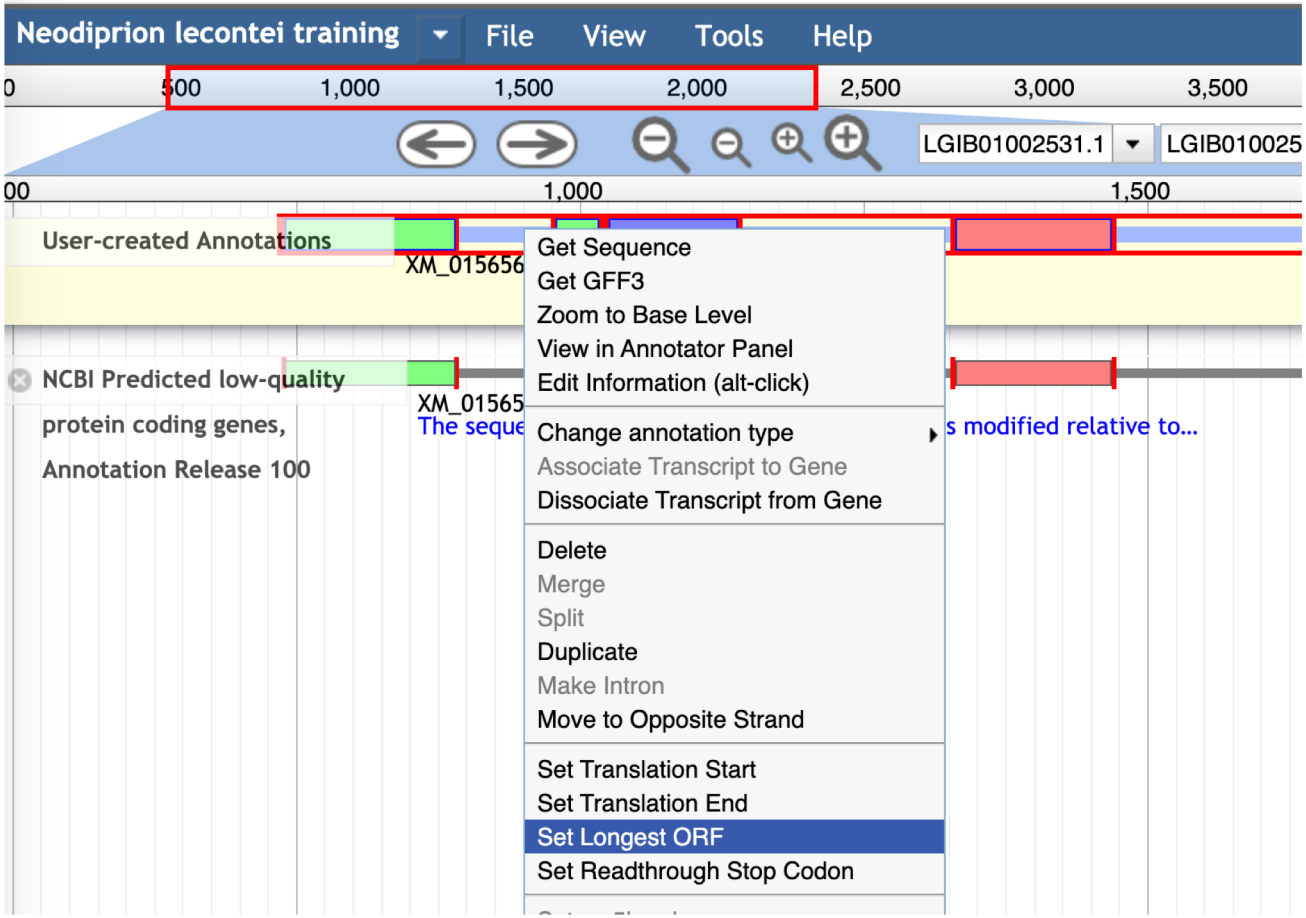


We're in the pink reading frame now – let's check the protein sequence

Starts, stops, ORFs



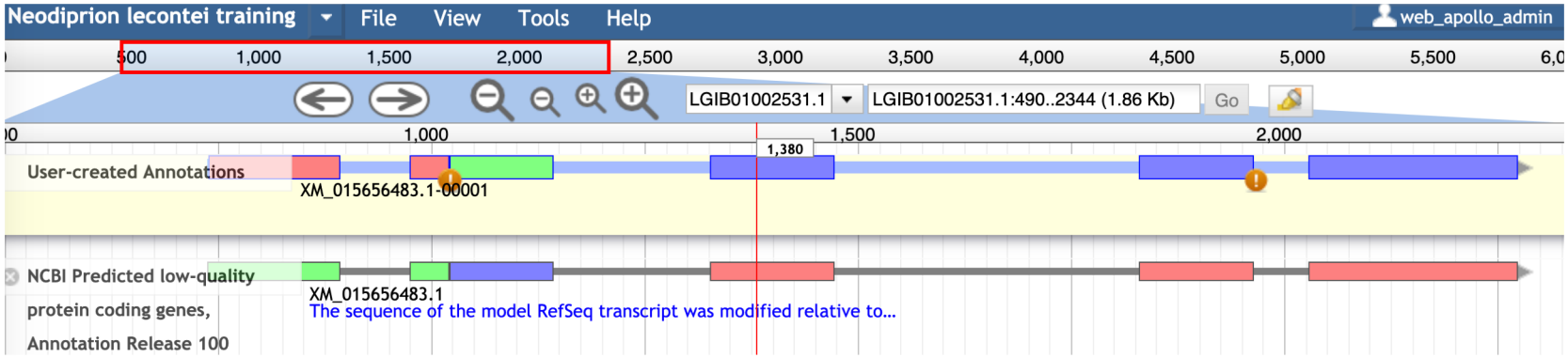
Starts, stops, ORFs

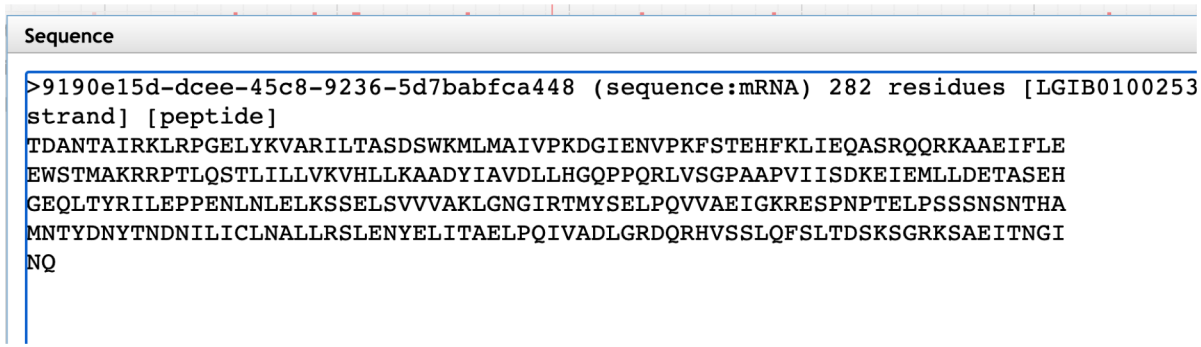


Sometimes it can be hard to tell what the protein sequence should be – in that case you can right-click and select 'Set Longest ORF'

USDA

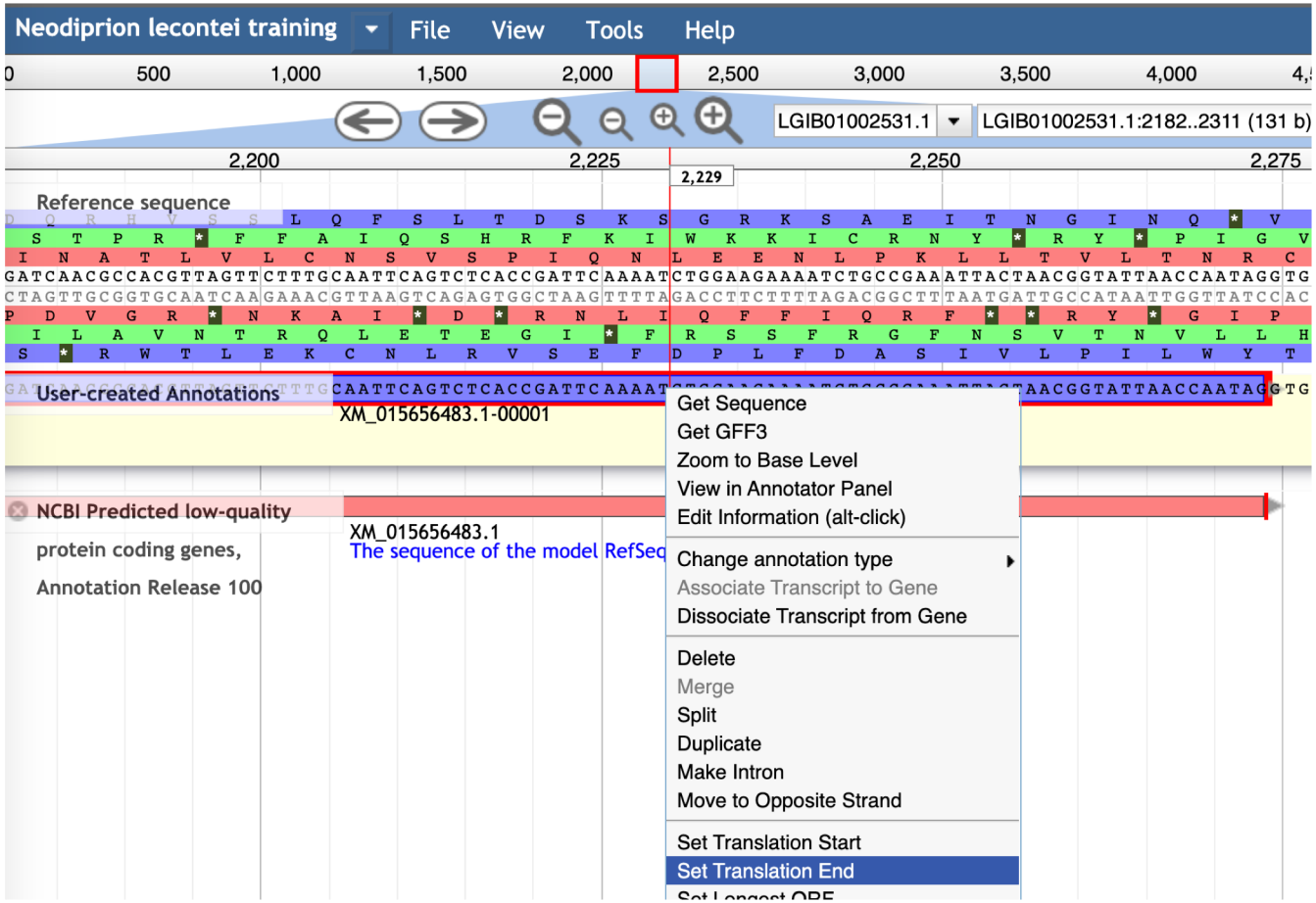
Starts, stops, ORFs





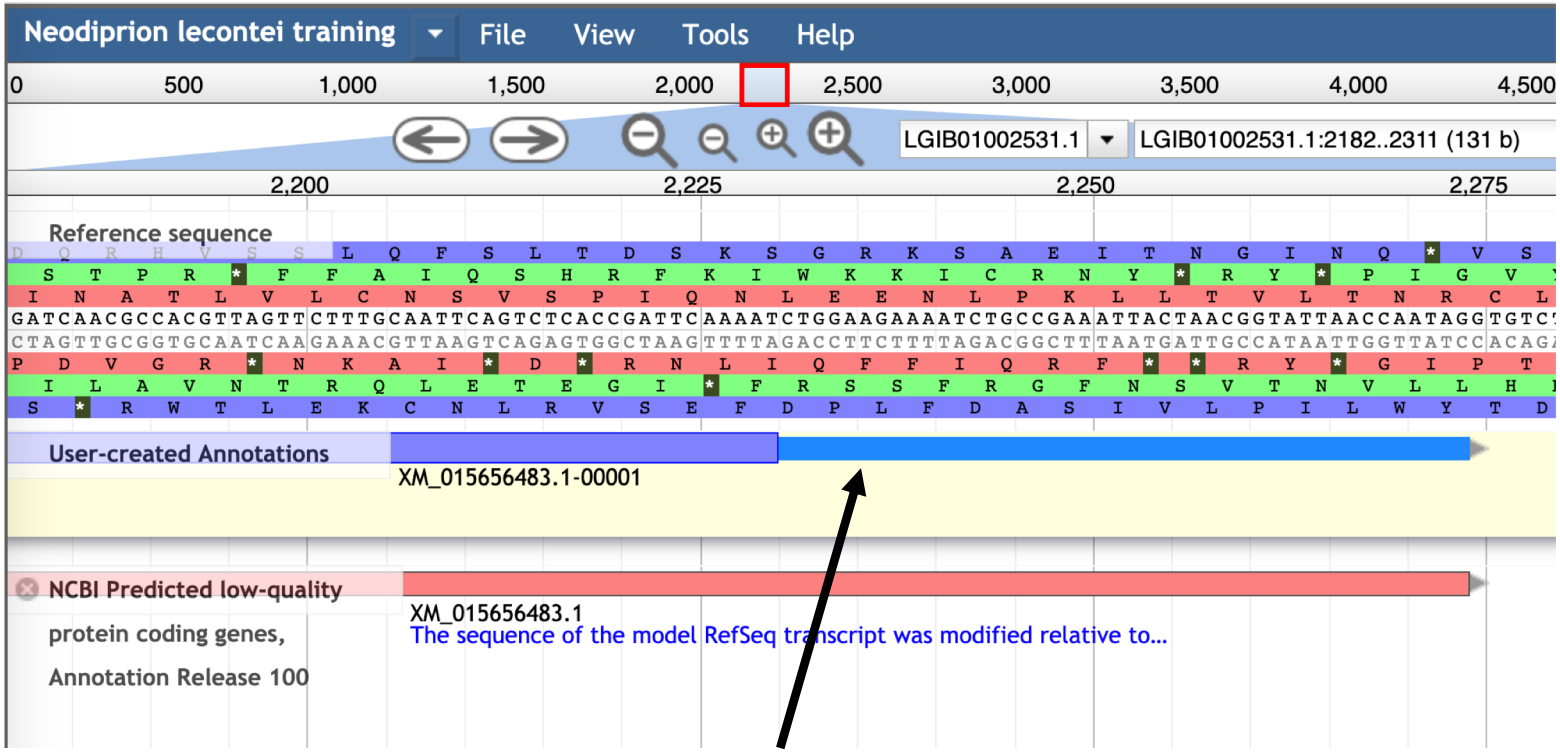
This also fixed the reading frame.

Starts, stops, ORFs



Similarly, if you have evidence to change the translation end, you can click on the corresponding nucleotide, right-click, and select ‘Set Translation End’

Starts, stops, ORFs



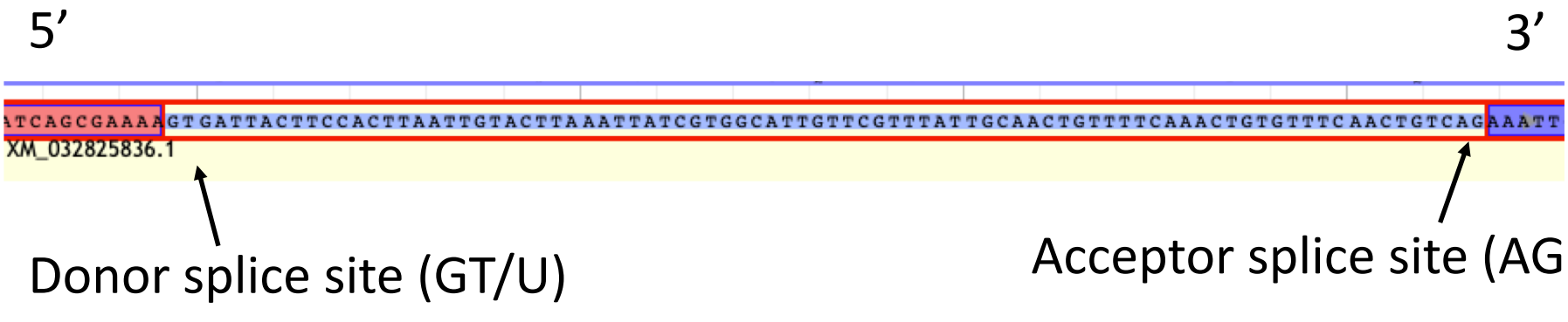
Now the sequence after the translation end is 3’ UTR.

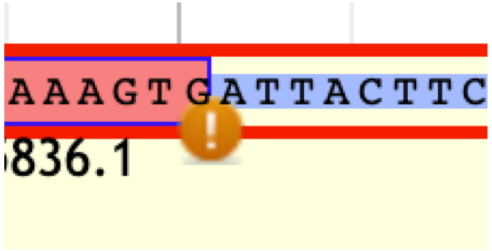
Non-canonical splice sites

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.

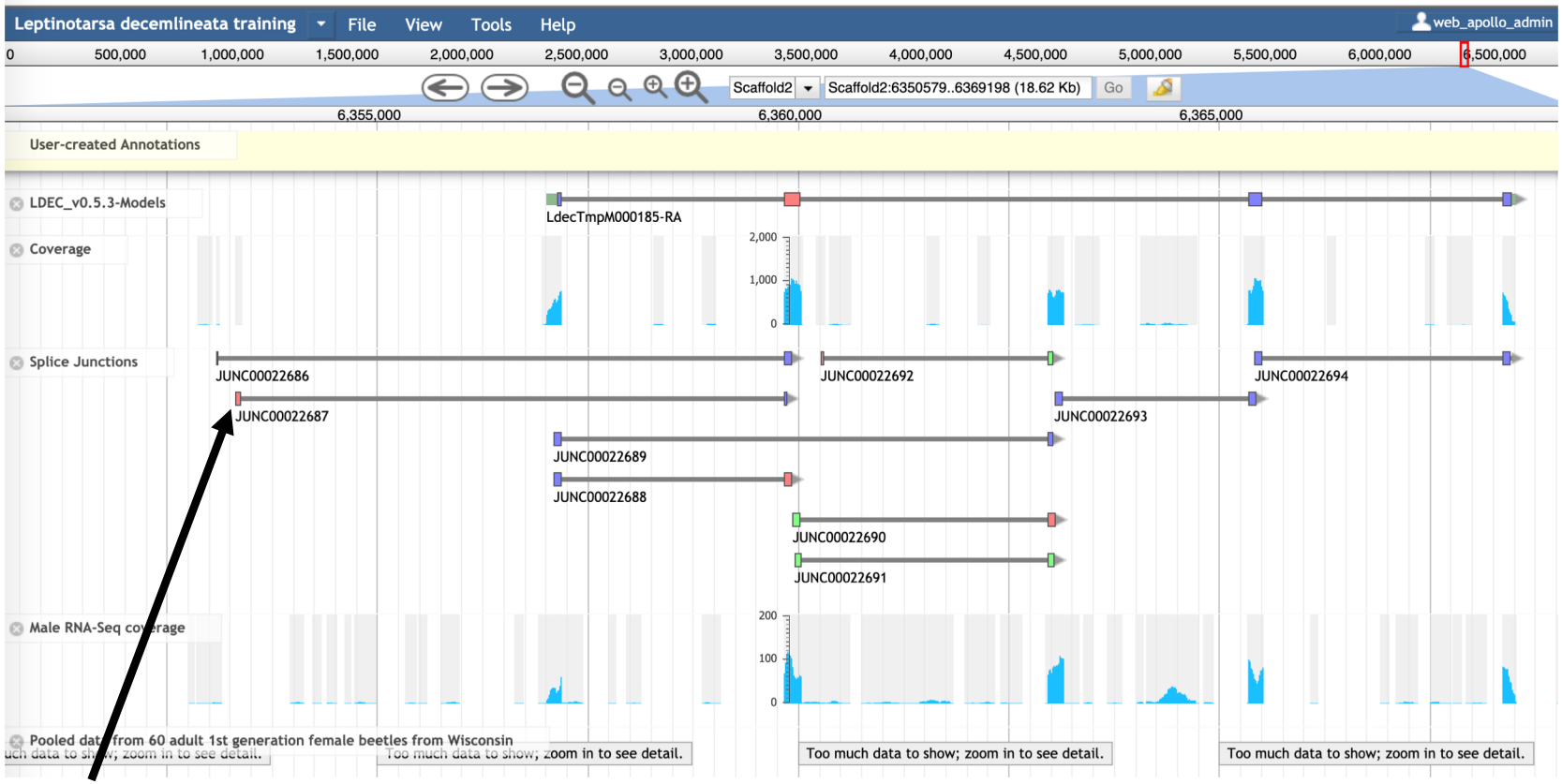
(https://www.nature.com/scitable/topicpage/rna-splicing-introns-exons-and-spliceosome-12375/)





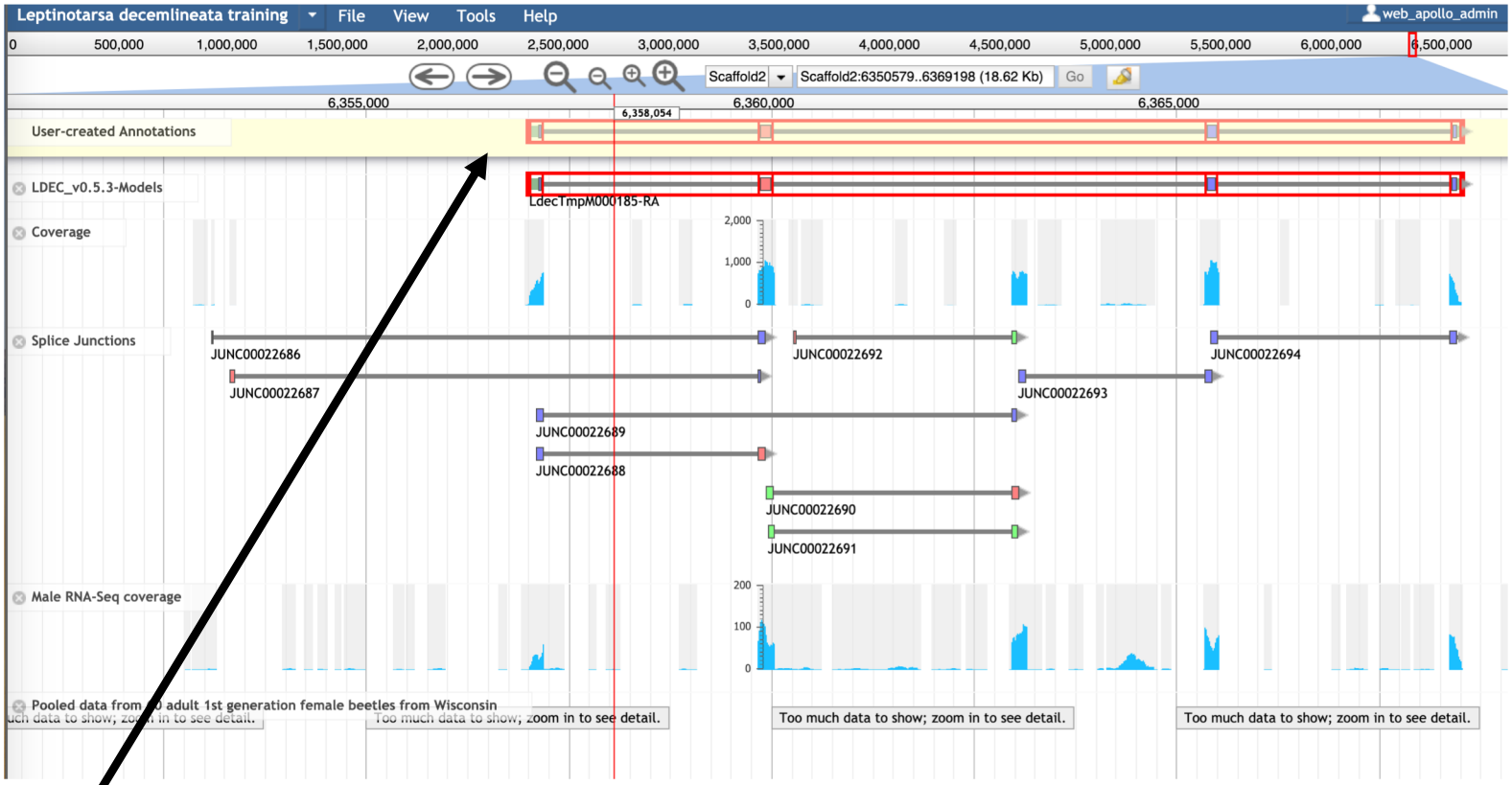
’Non-canonical’ splice sites – non-conserved, and possibly erroneous sites – are marked by an exclamation point in Apollo.

Fixing non-canonical splice sites



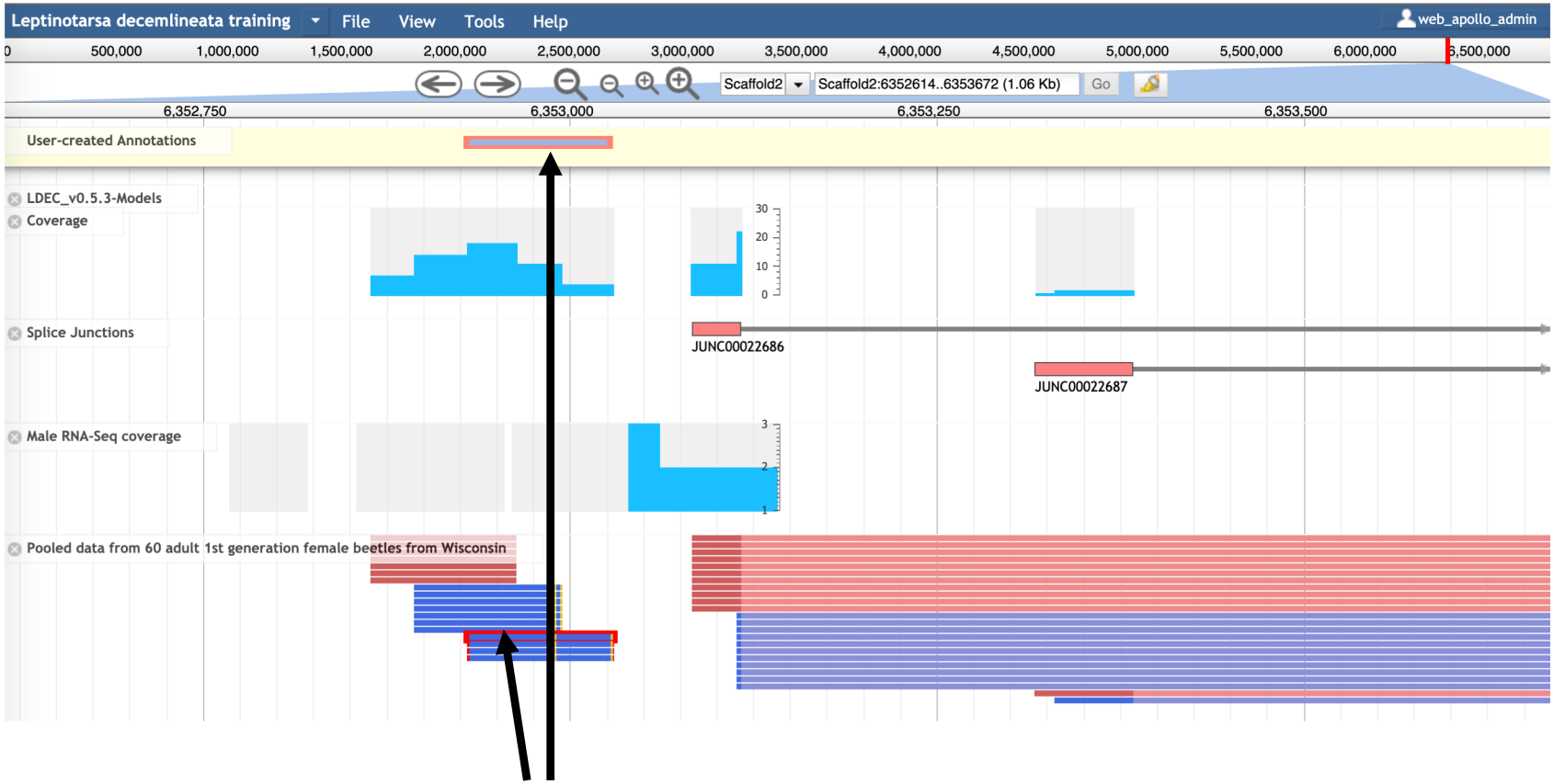
Looks like there's another isoform here – let's add an exon

Fixing non-canonical splice sites



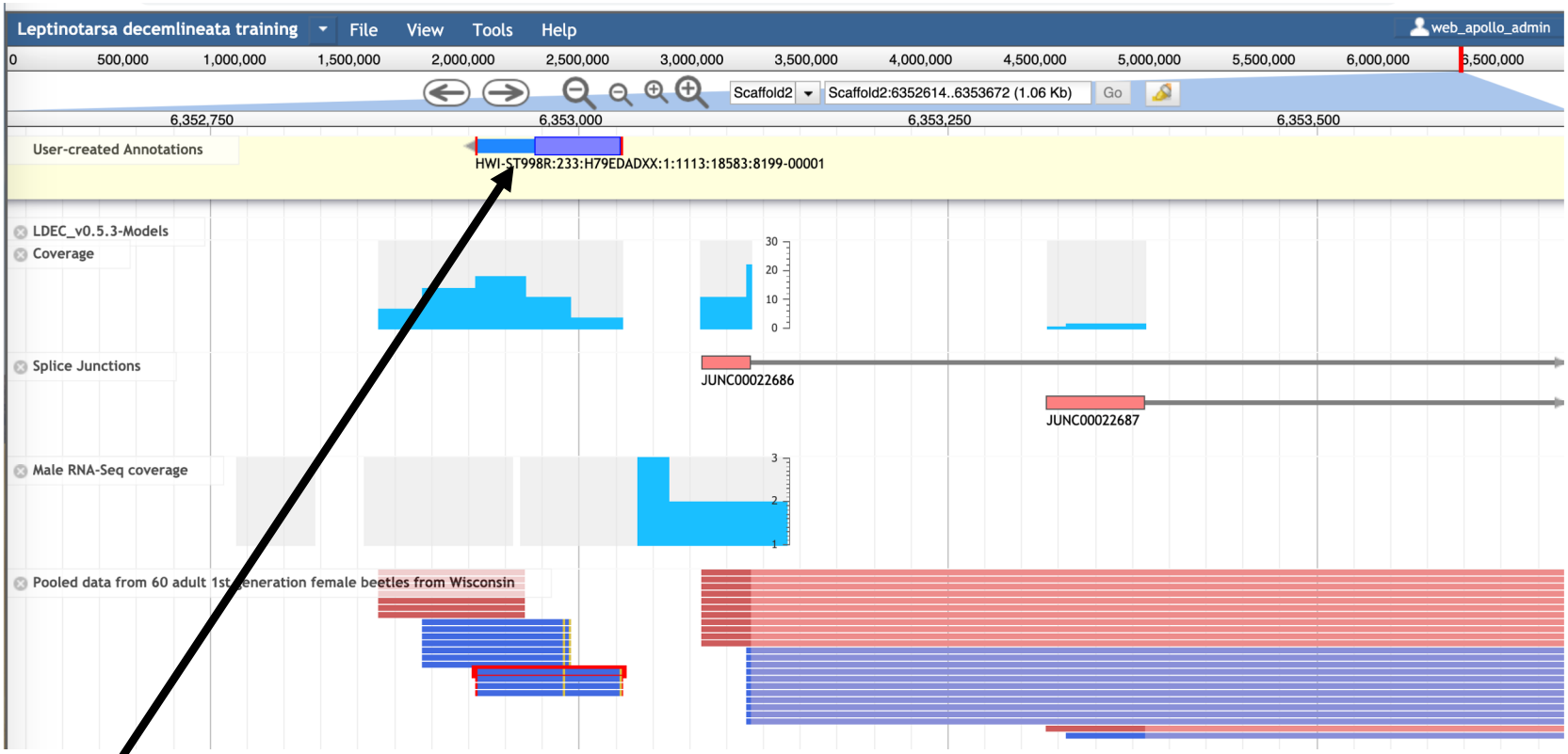
Drag model to UcA track

Fixing non-canonical splice sites



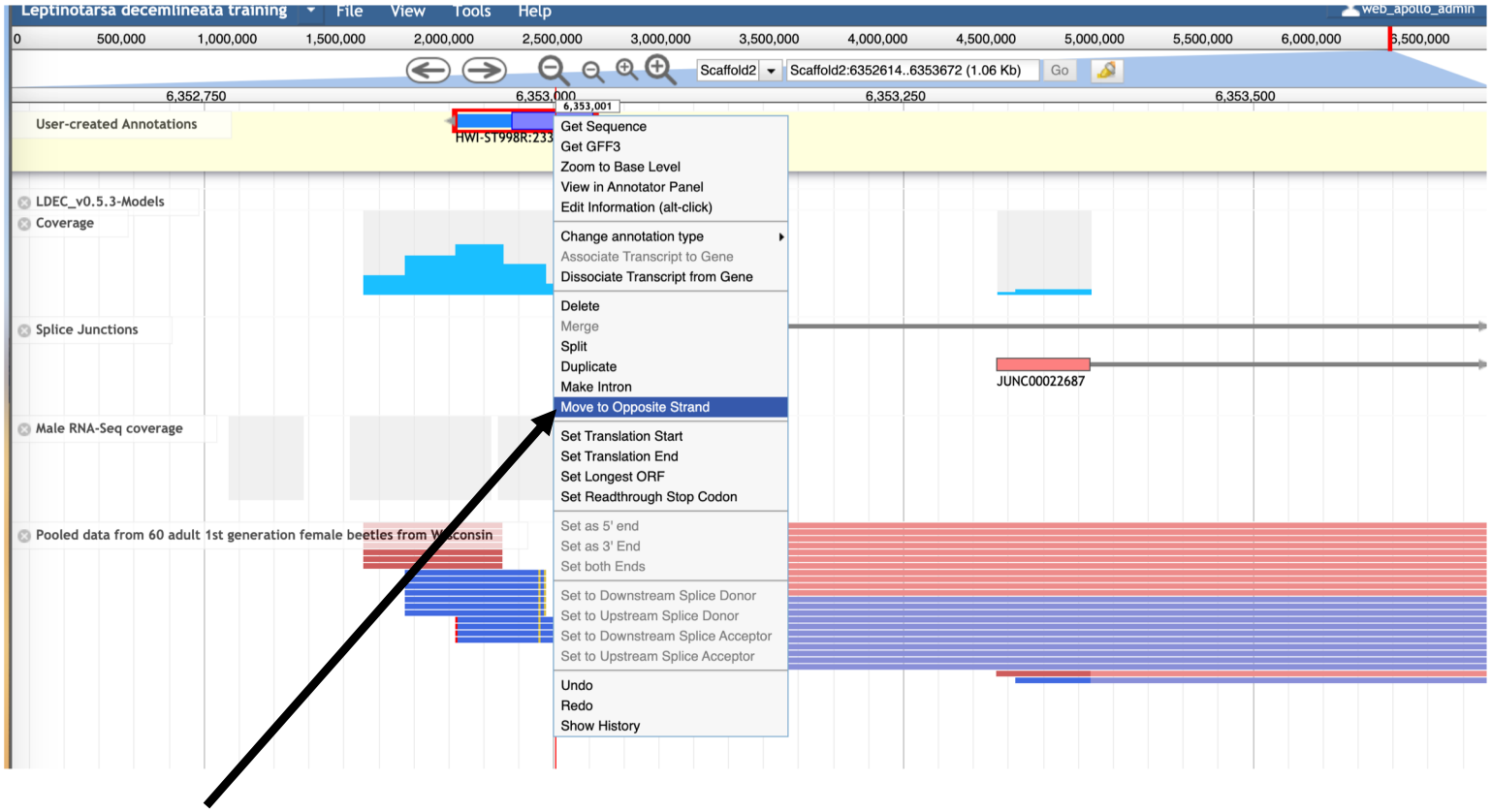
Drag evidence to UcA track to add 5’ exon

Fixing non-canonical splice sites



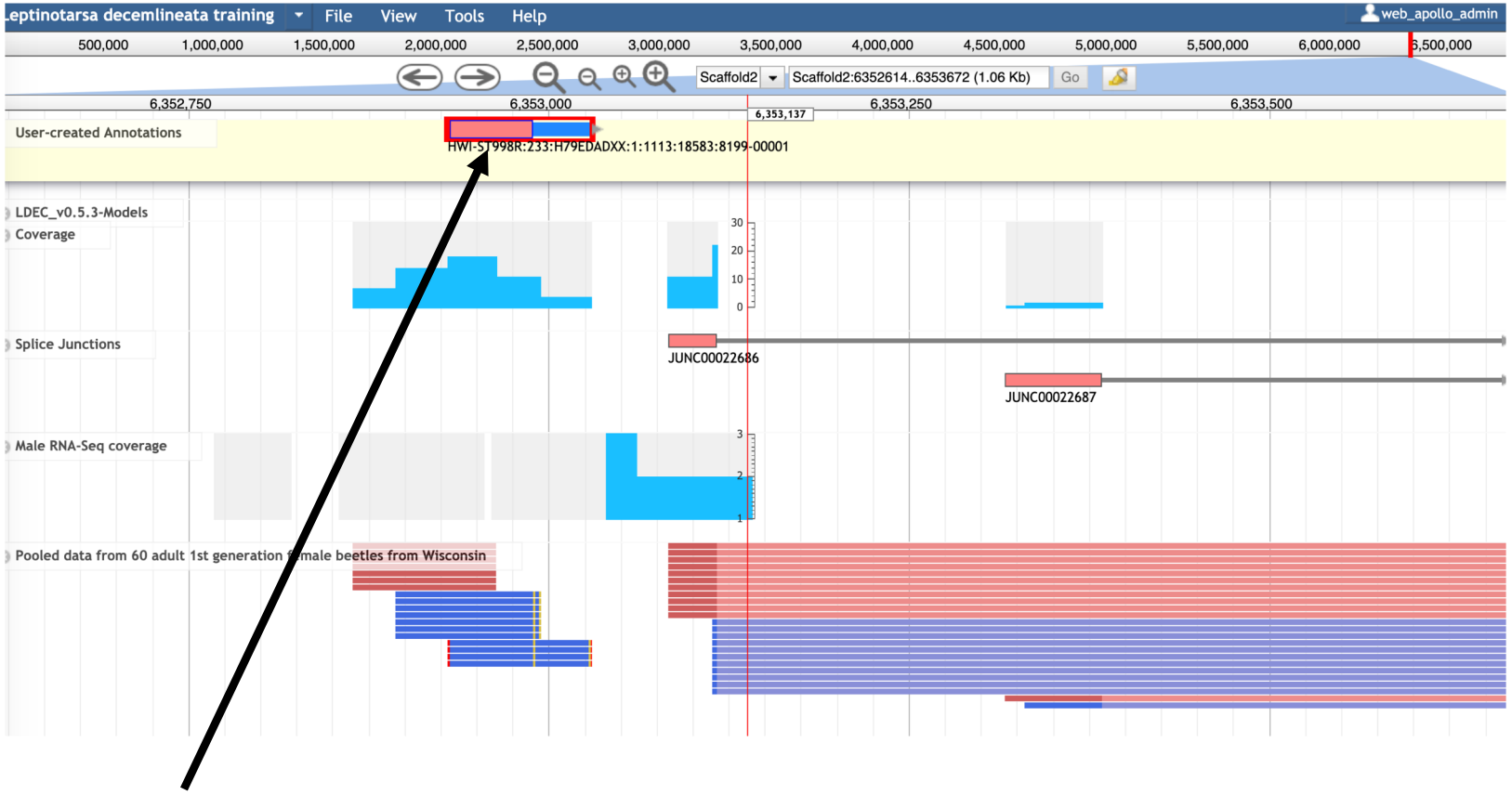
Oops, wrong strand – our model is on the forward strand

Fixing non-canonical splice sites



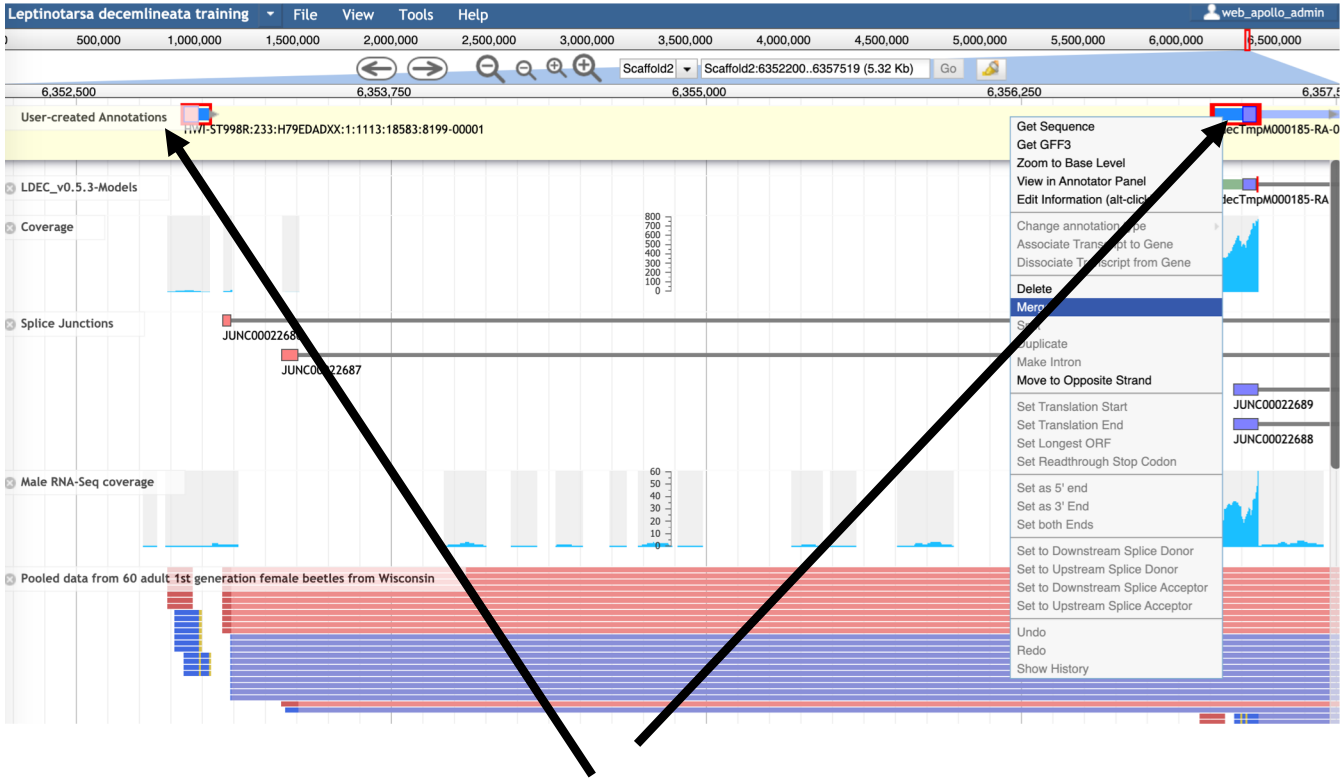
Let's flip it to the opposite strand

Fixing non-canonical splice sites



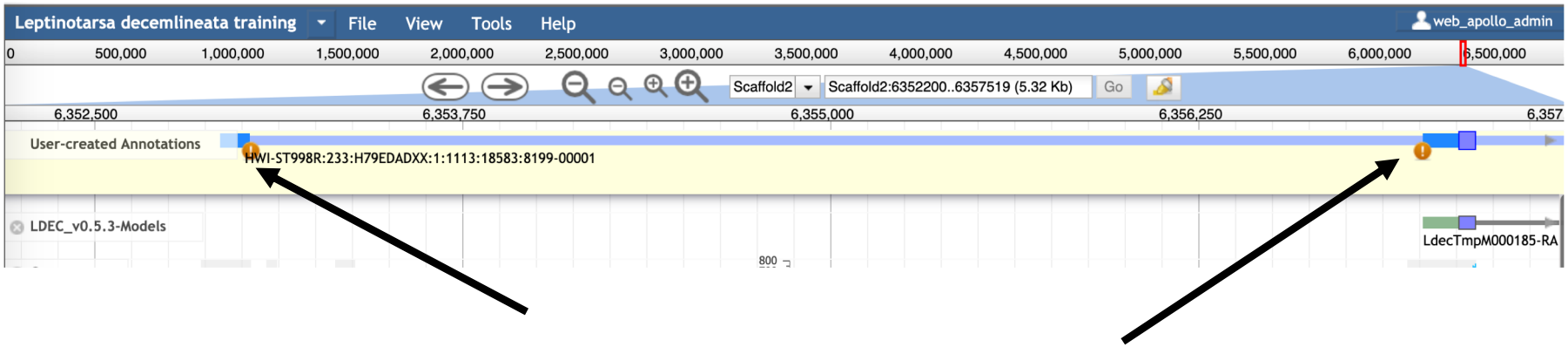
That's better.

Fixing non-canonical splice sites



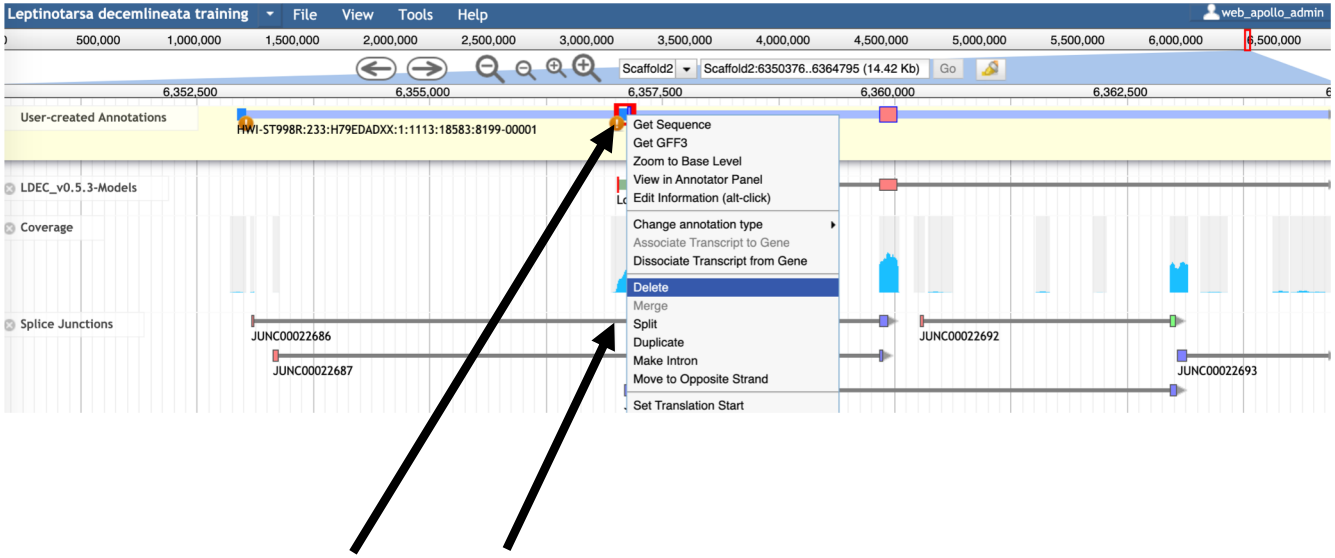
Merge the new exon to the gene model

Fixing non-canonical splice sites



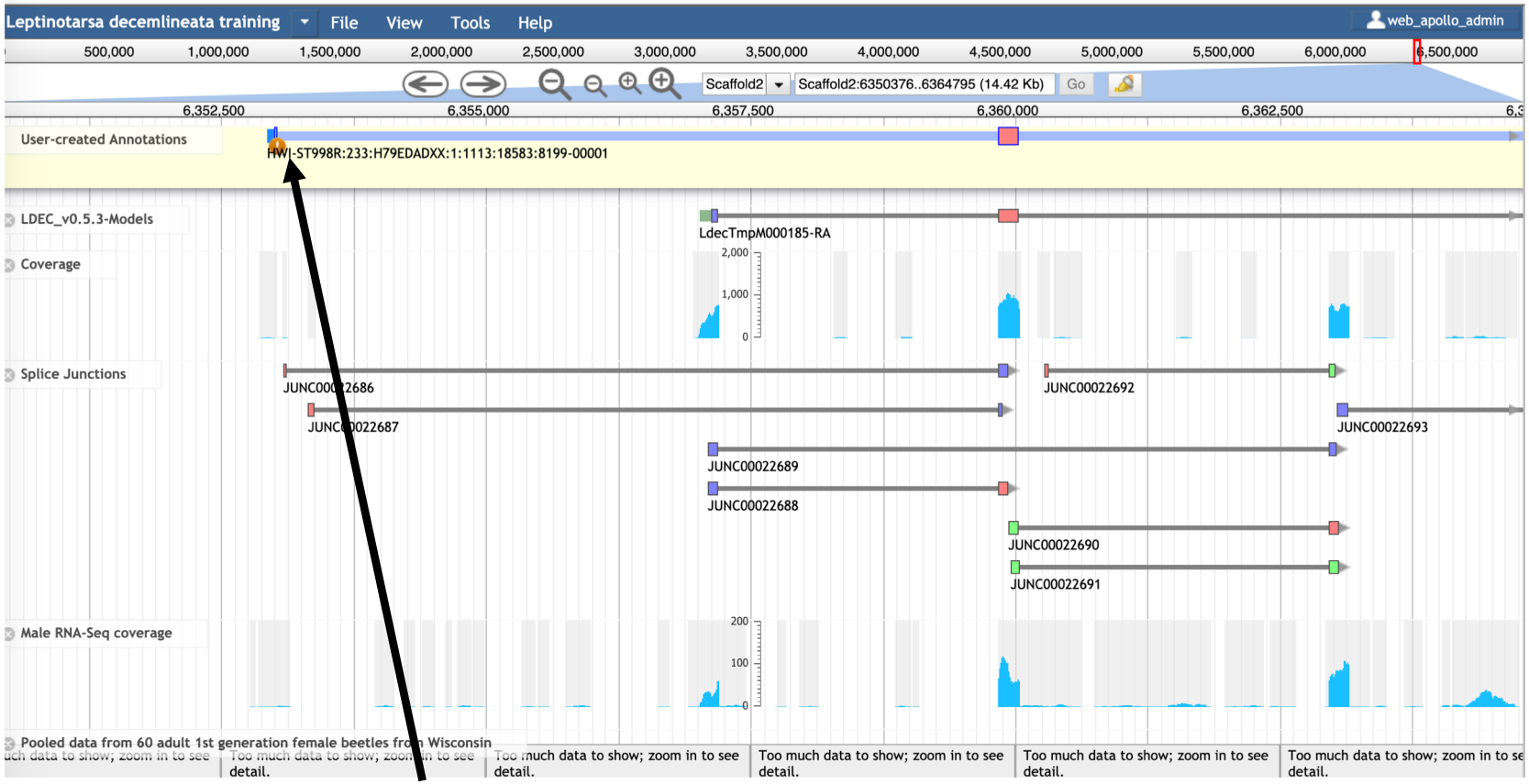
Non-canonical splice sites in merged model

Fixing non-canonical splice sites



The splice junction reads don't support the 2nd exon with the new 5' exon, so let's remove it

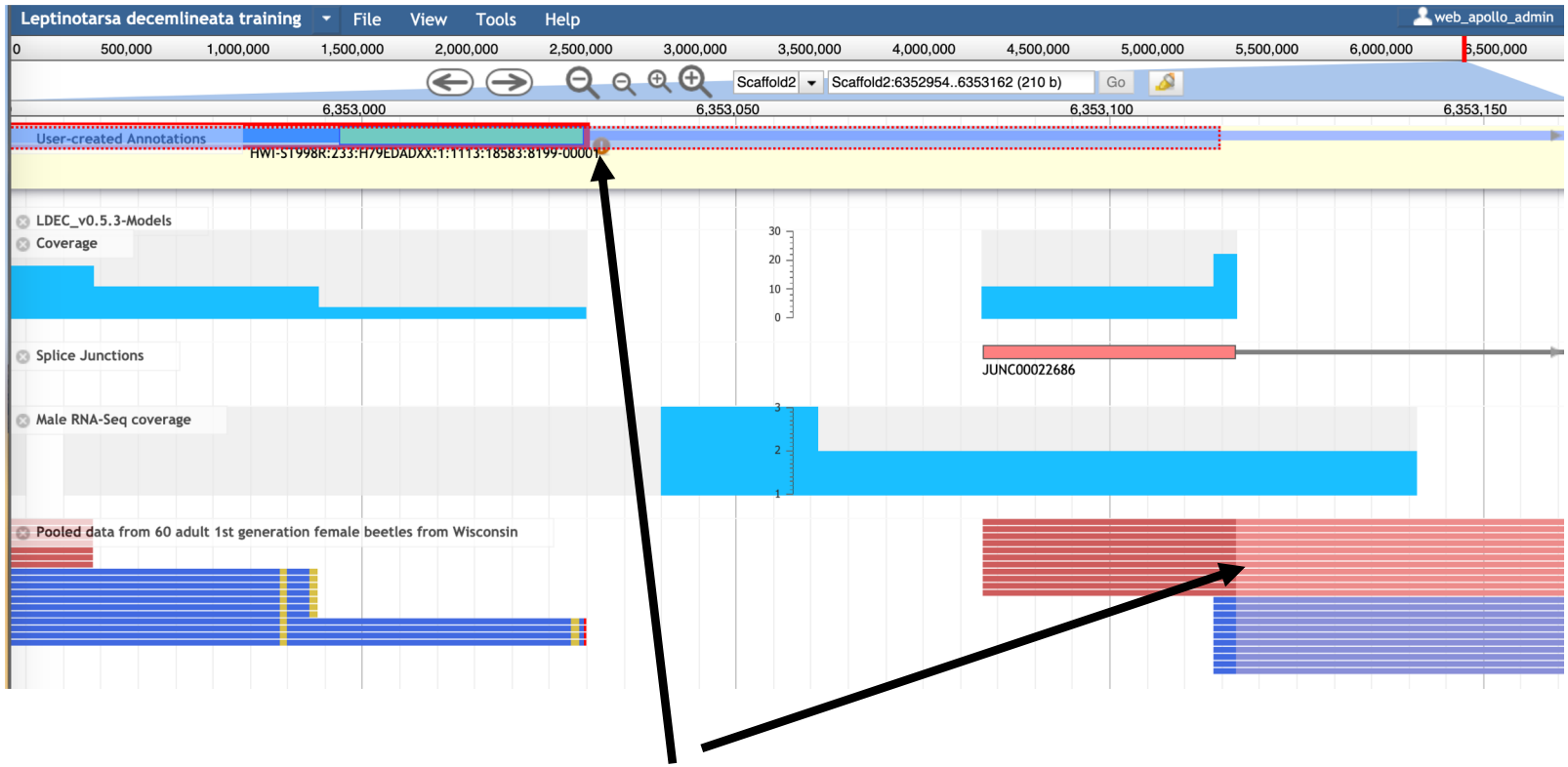
Fixing non-canonical splice sites



Only 1 non-canonical splice site left to fix

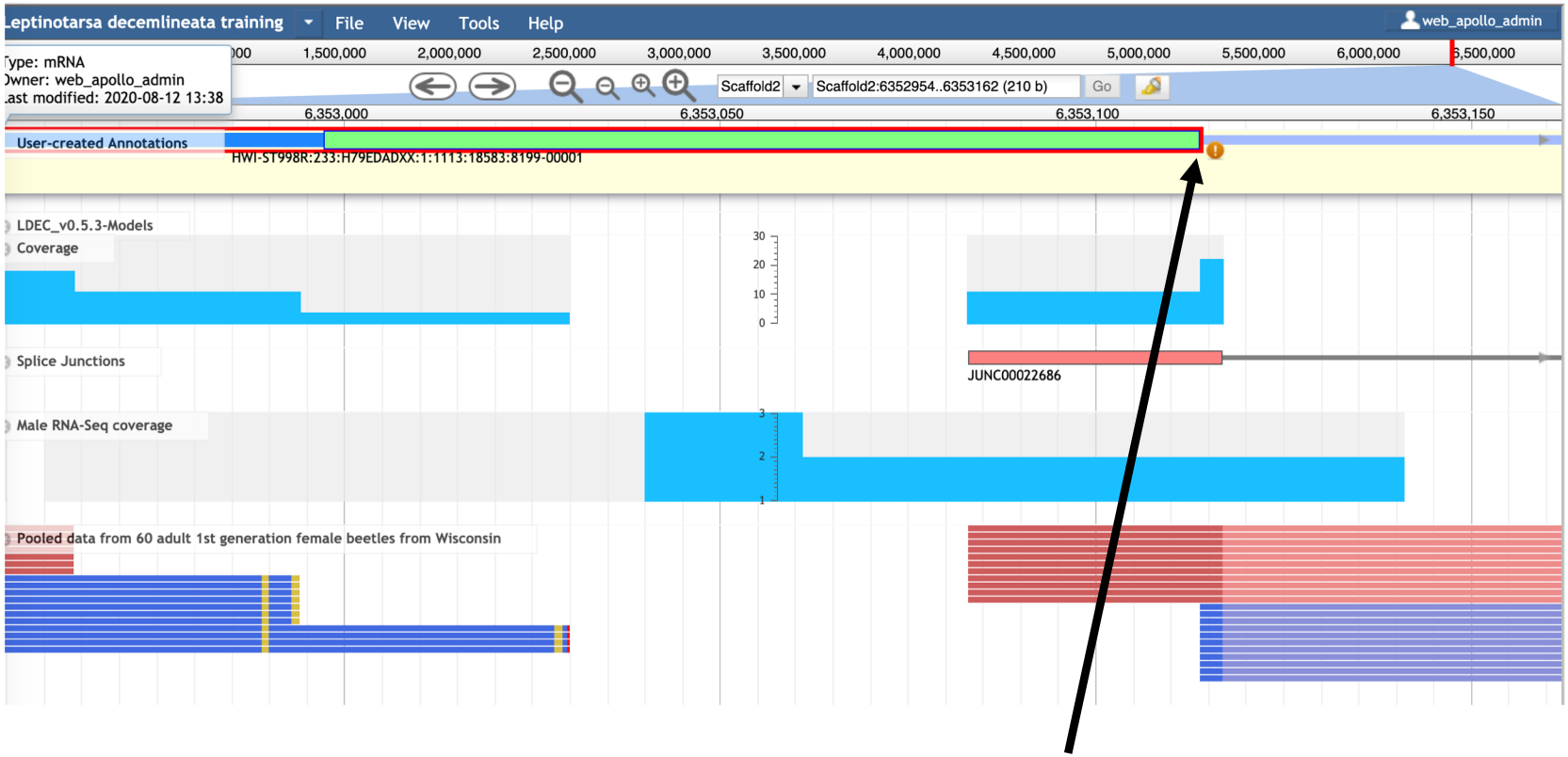
USDA

Fixing non-canonical splice sites



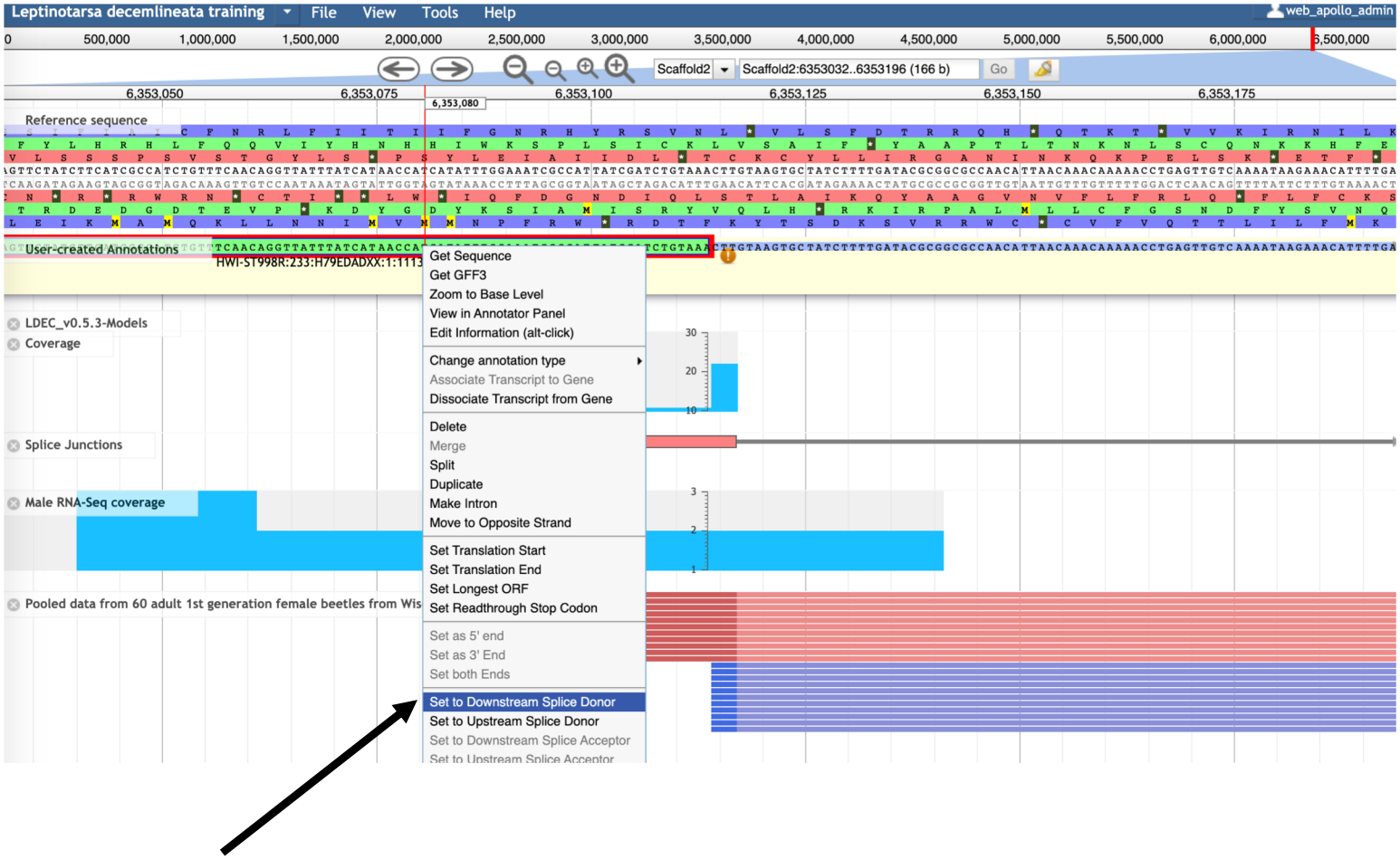
Extend exon to RNA-Seq boundary

Fixing non-canonical splice sites



Still not quite right

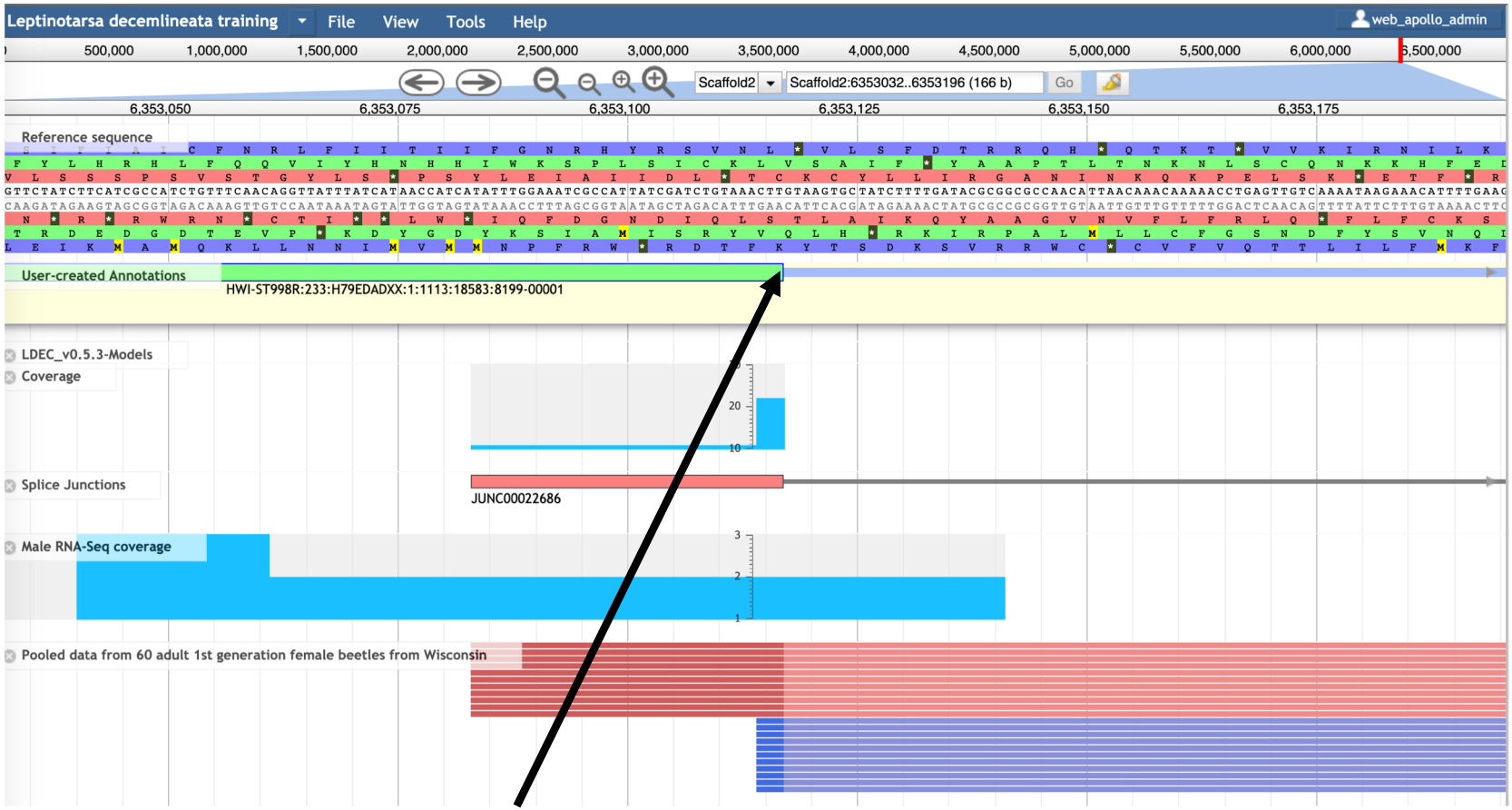
Fixing non-canonical splice sites



Let’s have Apollo find the downstream splice site donor for us

USDA

Fixing non-canonical splice sites



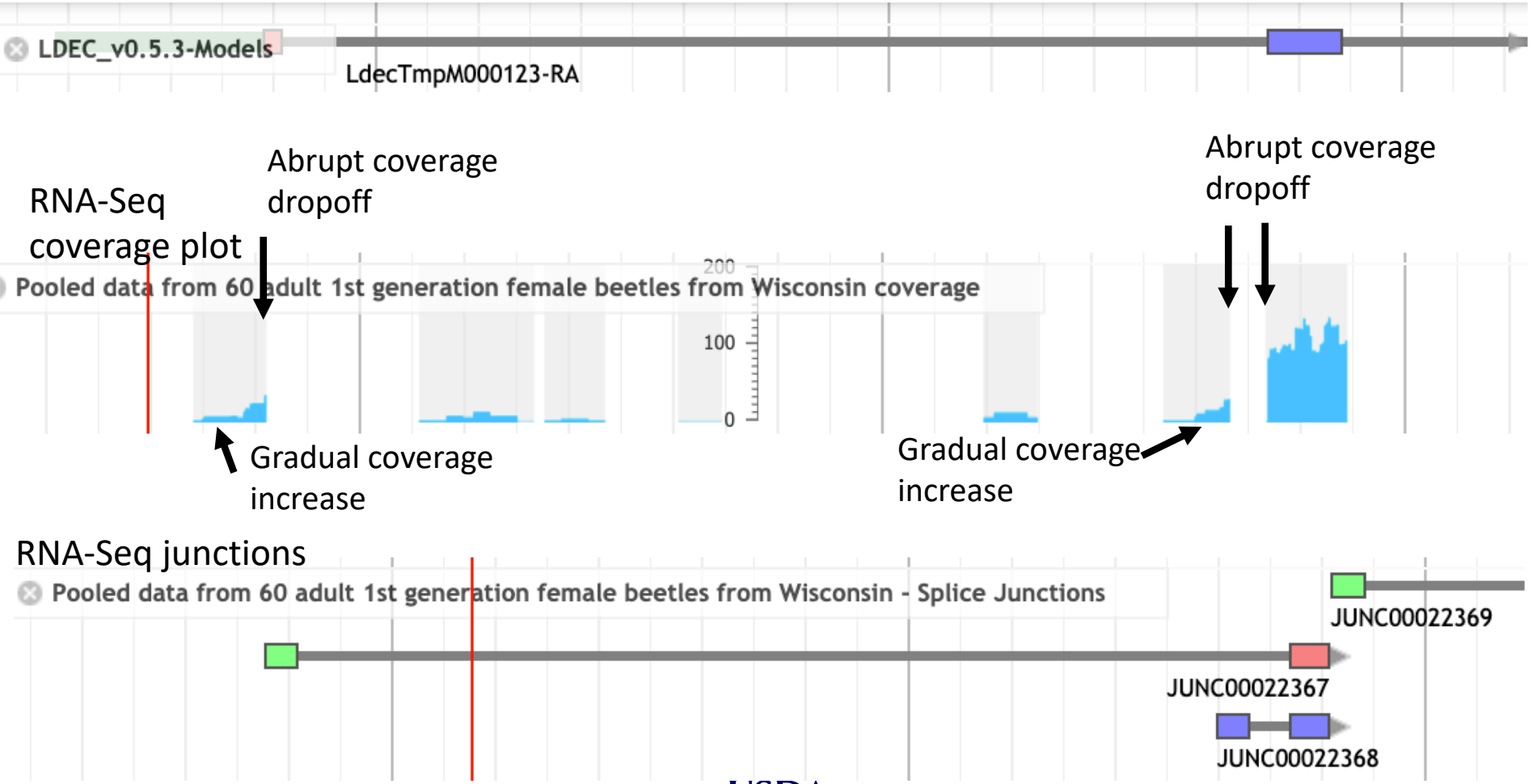
Annotating isoforms

Isoform annotation example

- 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

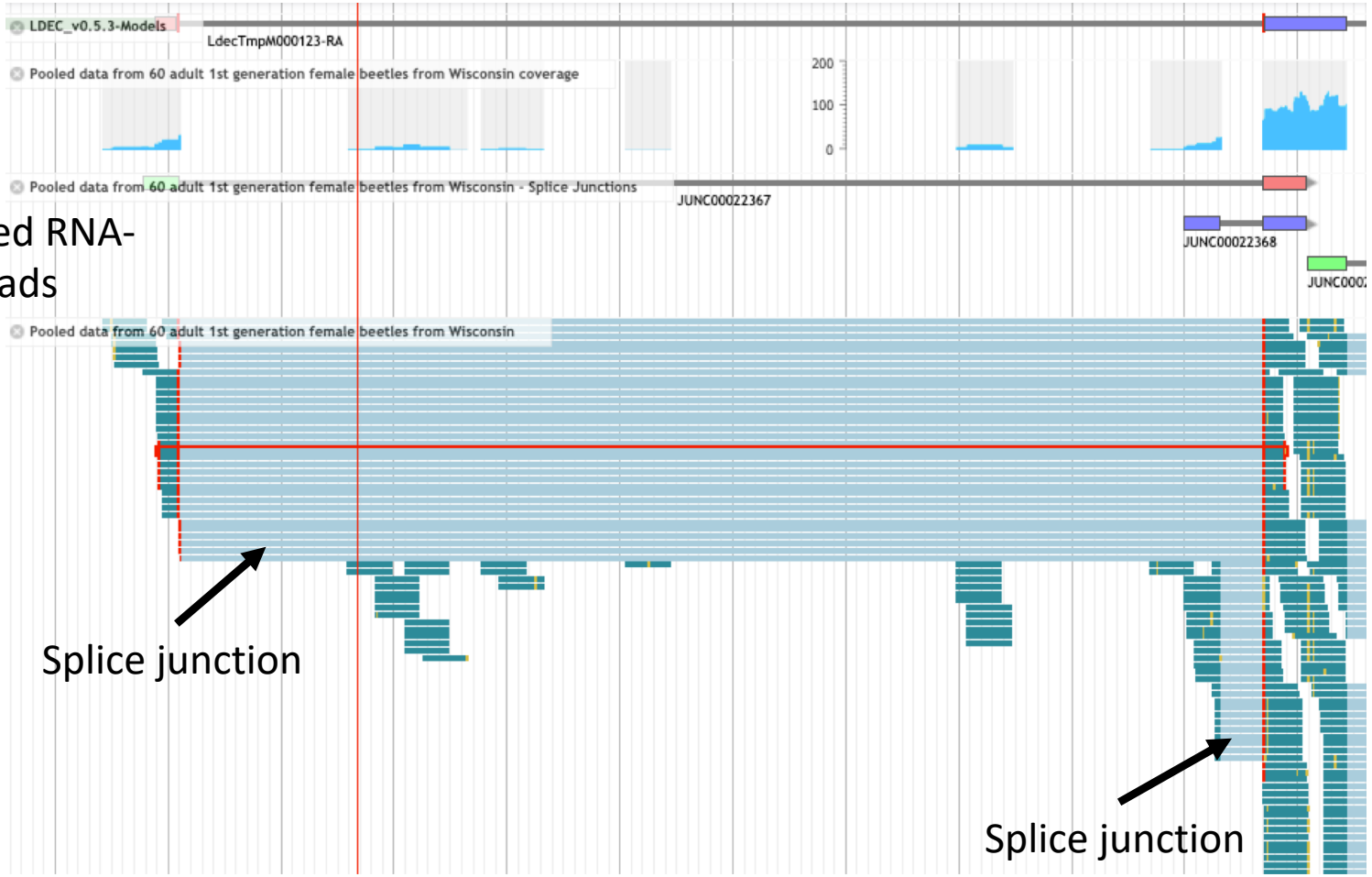
Isoform annotation example

5’ end of MAKER tyrosine protein kinase gene prediction



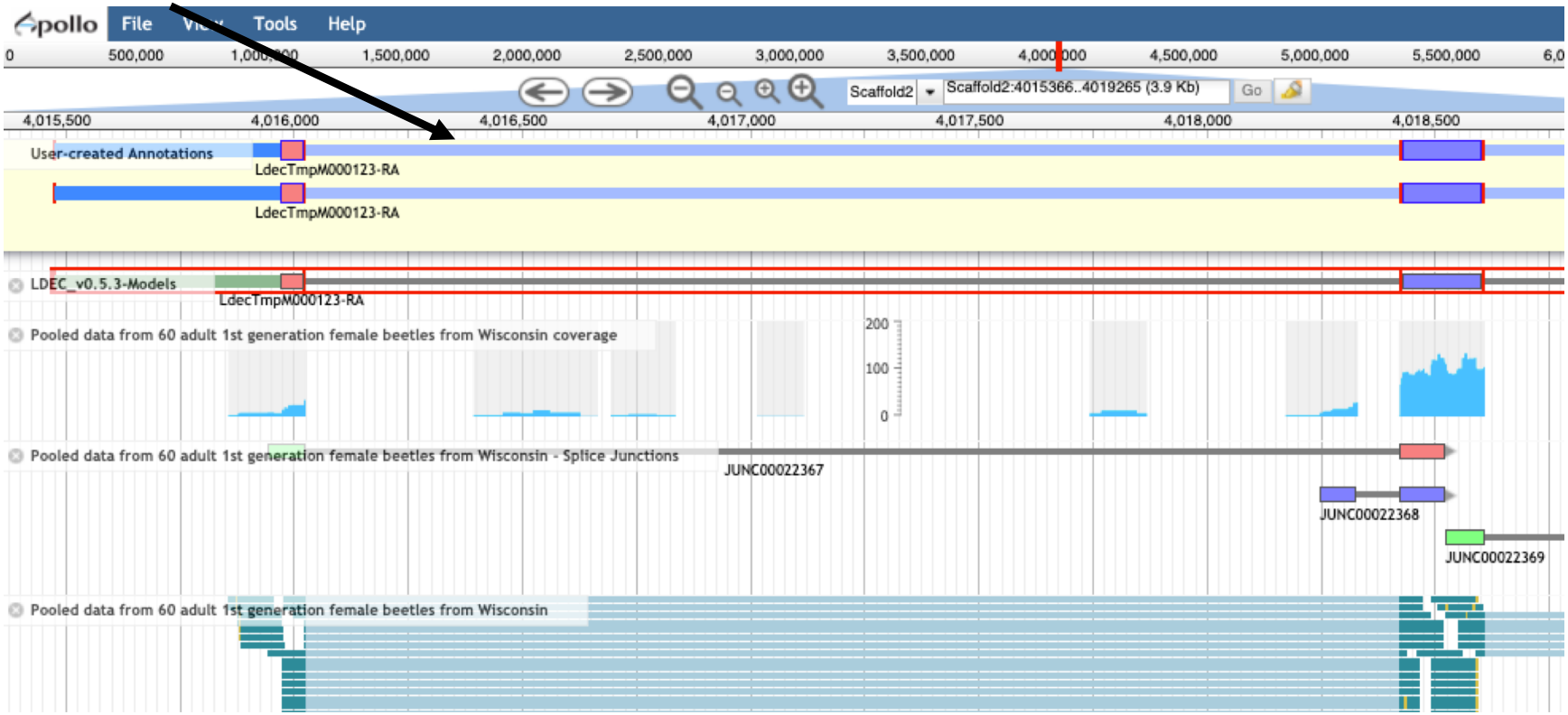
Isoform annotation example

Mapped RNA-Seq reads



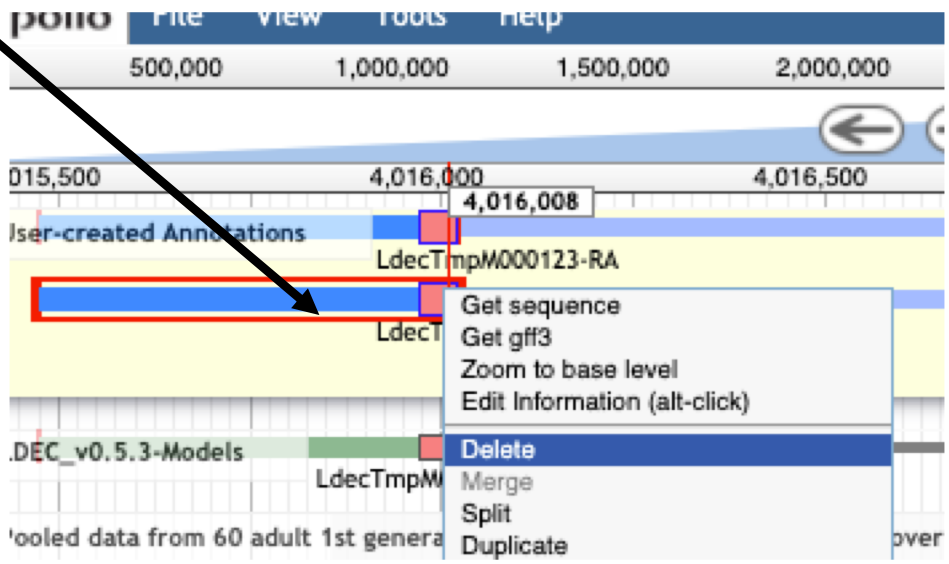
Isoform annotation example

Create 2 isoforms
from Maker model



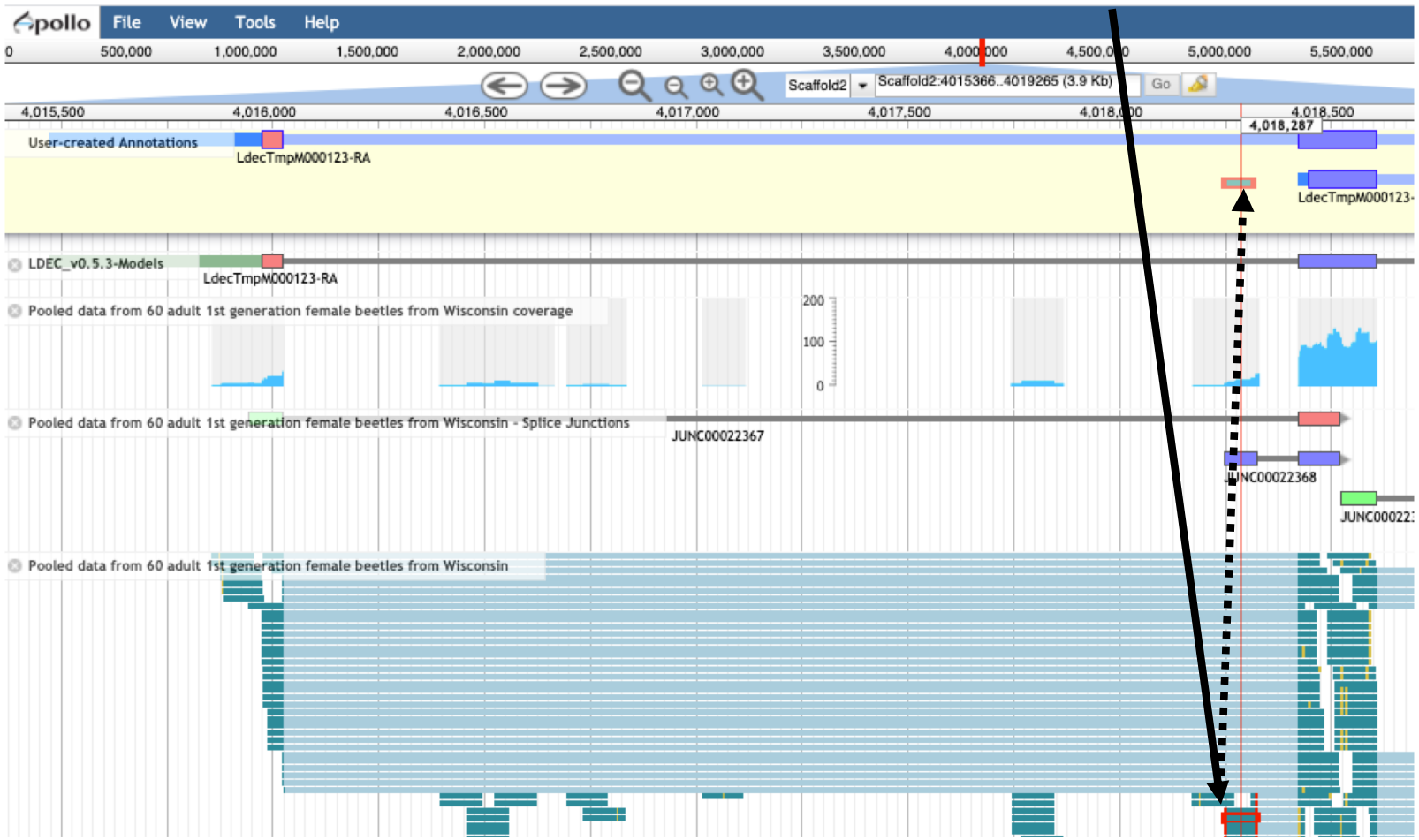
Isoform annotation example

Select and delete 5’ exon from one of the isoforms



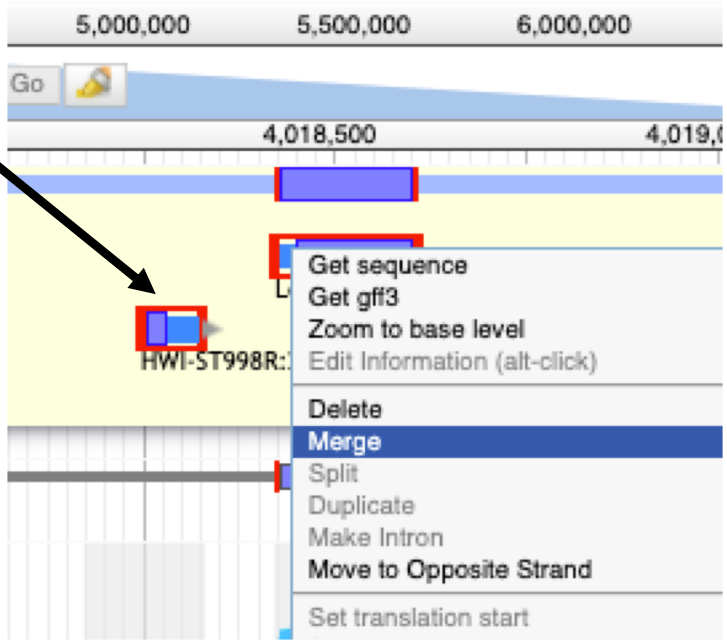
Isoform annotation example

Add a new 5' exon from mapped RNA-Seq evidence



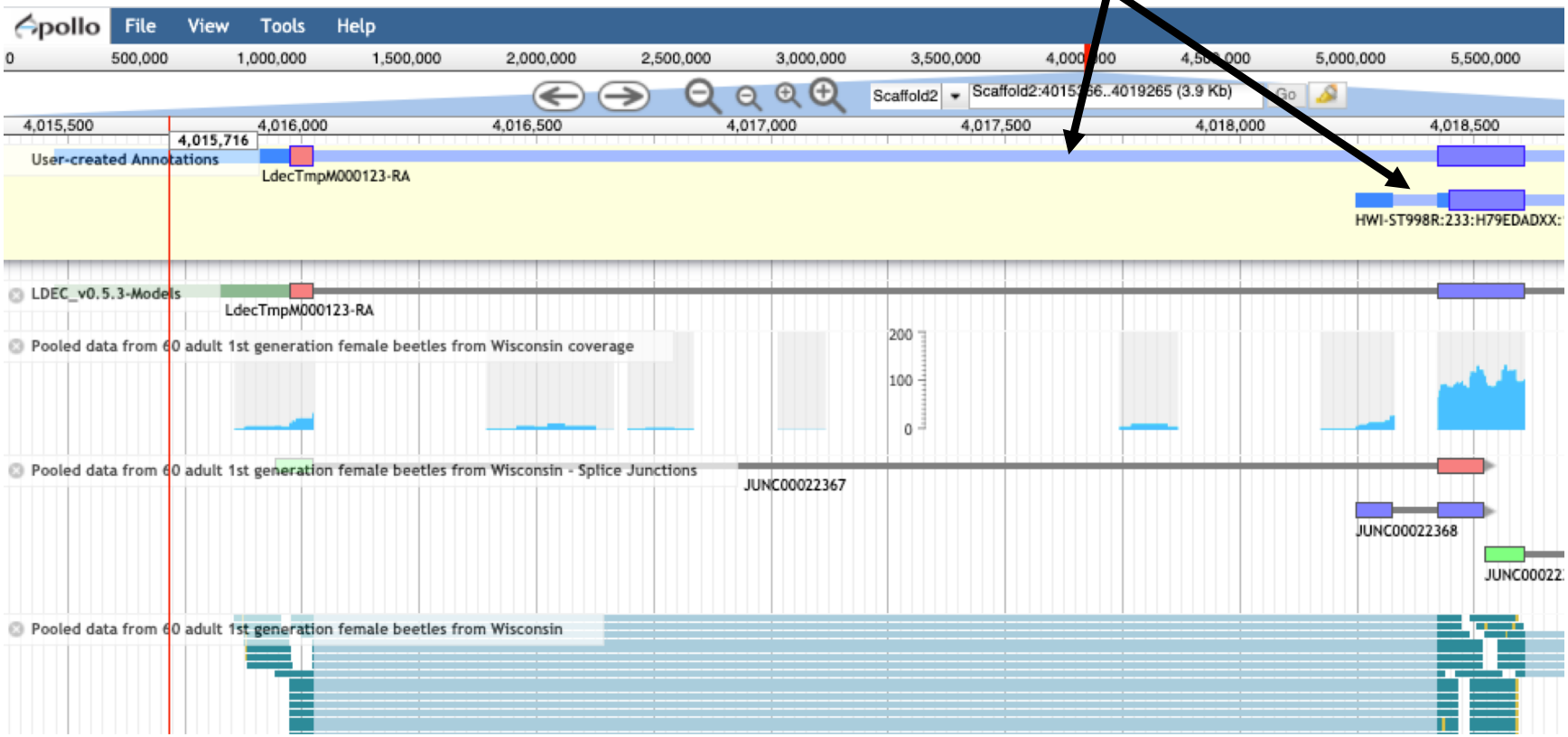
Isoform annotation example

Merge the new 5’ exon with the rest of the model



Isoform annotation example

2 isoforms supported by RNA-Seq evidence



Sequence alterations and stop-codon readthroughs

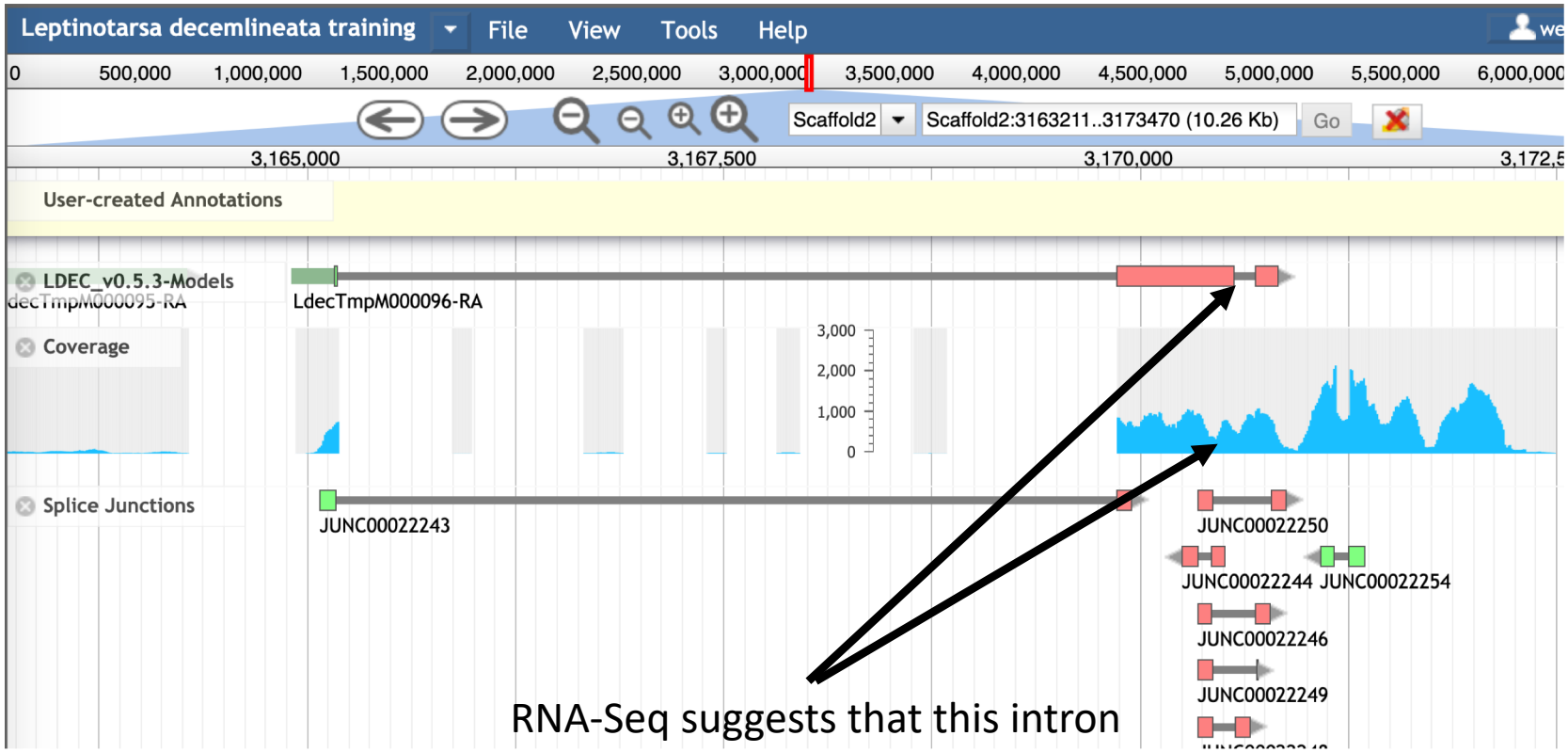
Sequence alterations

- Apollo supports annotating the genome sequence with insertions, deletions, and substitutions
- Note that this will not change the genome fasta in the sequence export – but it will allow Apollo to re-calculate a gene model's sequence
- Only add a sequence alteration in Apollo if there is evidence for it – e.g. SNPs in mapped RNA-Seq

Stop-codon readthroughs

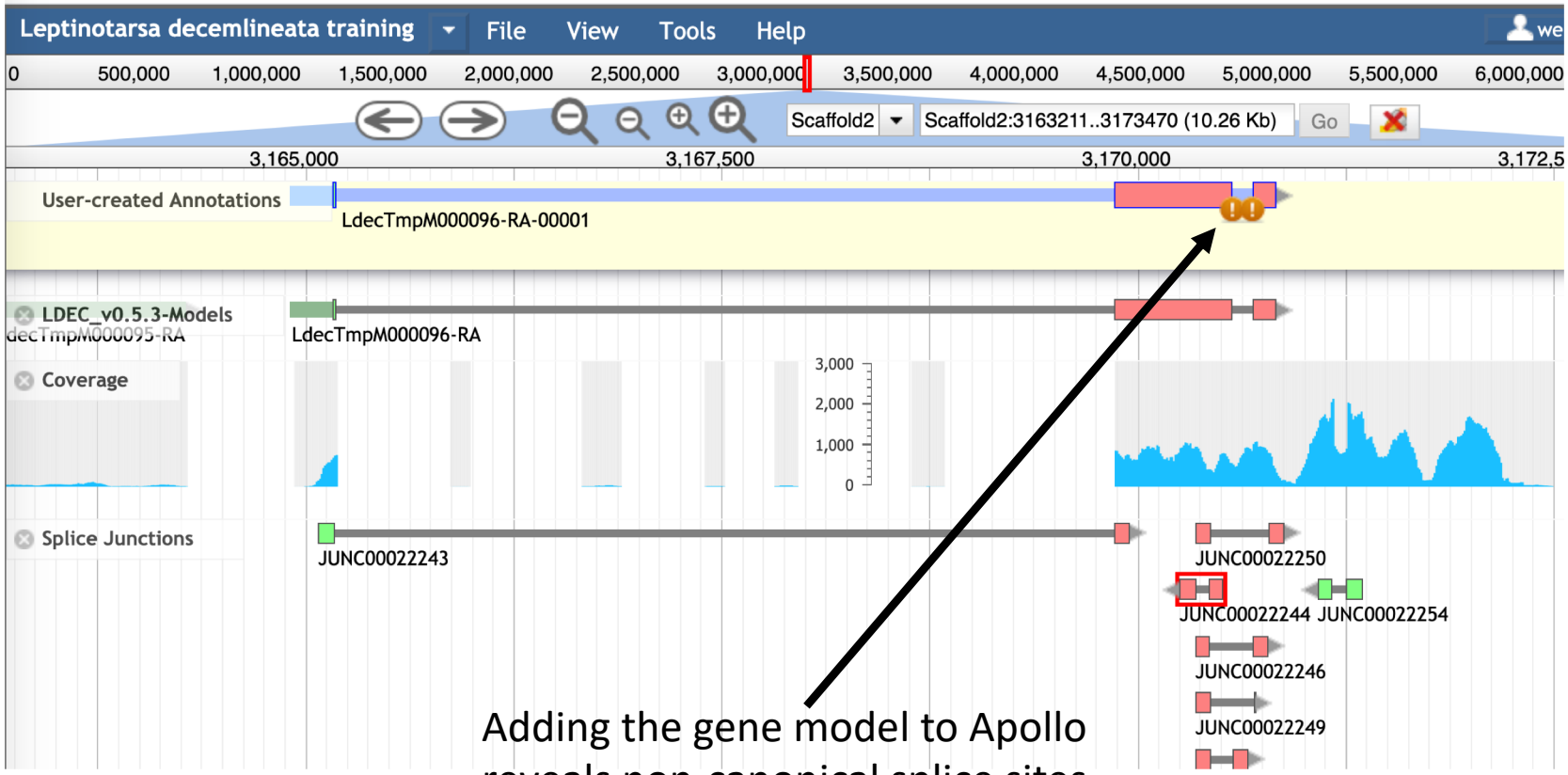
- Apollo allows you to annotate stop-codon readthrough features on the coding sequence of a gene model
- This is a special case for selenocysteine-containing proteins.
- This feature can be used in other cases – e.g. if you have evidence of errors in the genome assembly - but we don’t recommend it

Sequence alterations



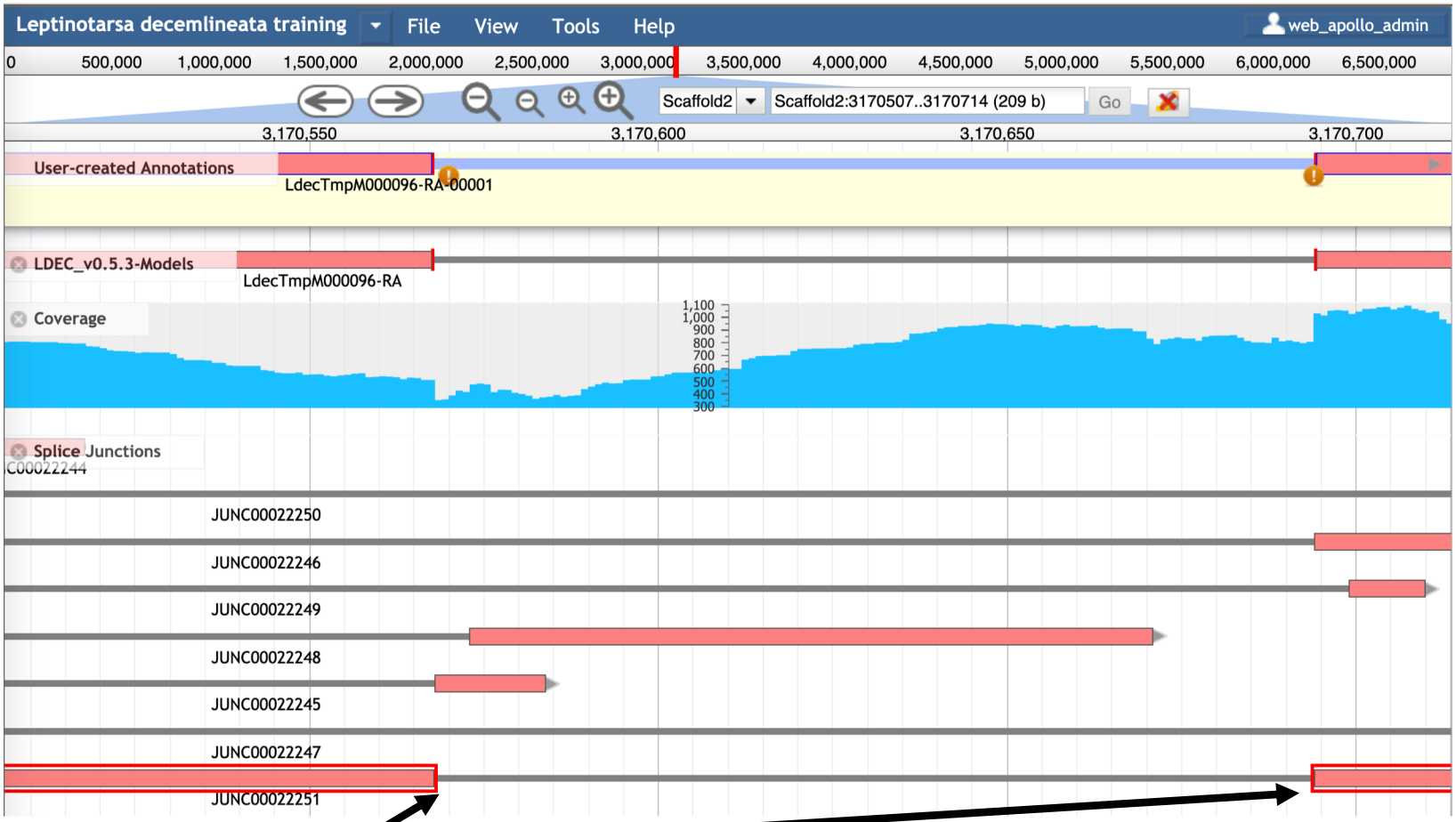
RNA-Seq suggests that this intron may not exist

Sequence alterations



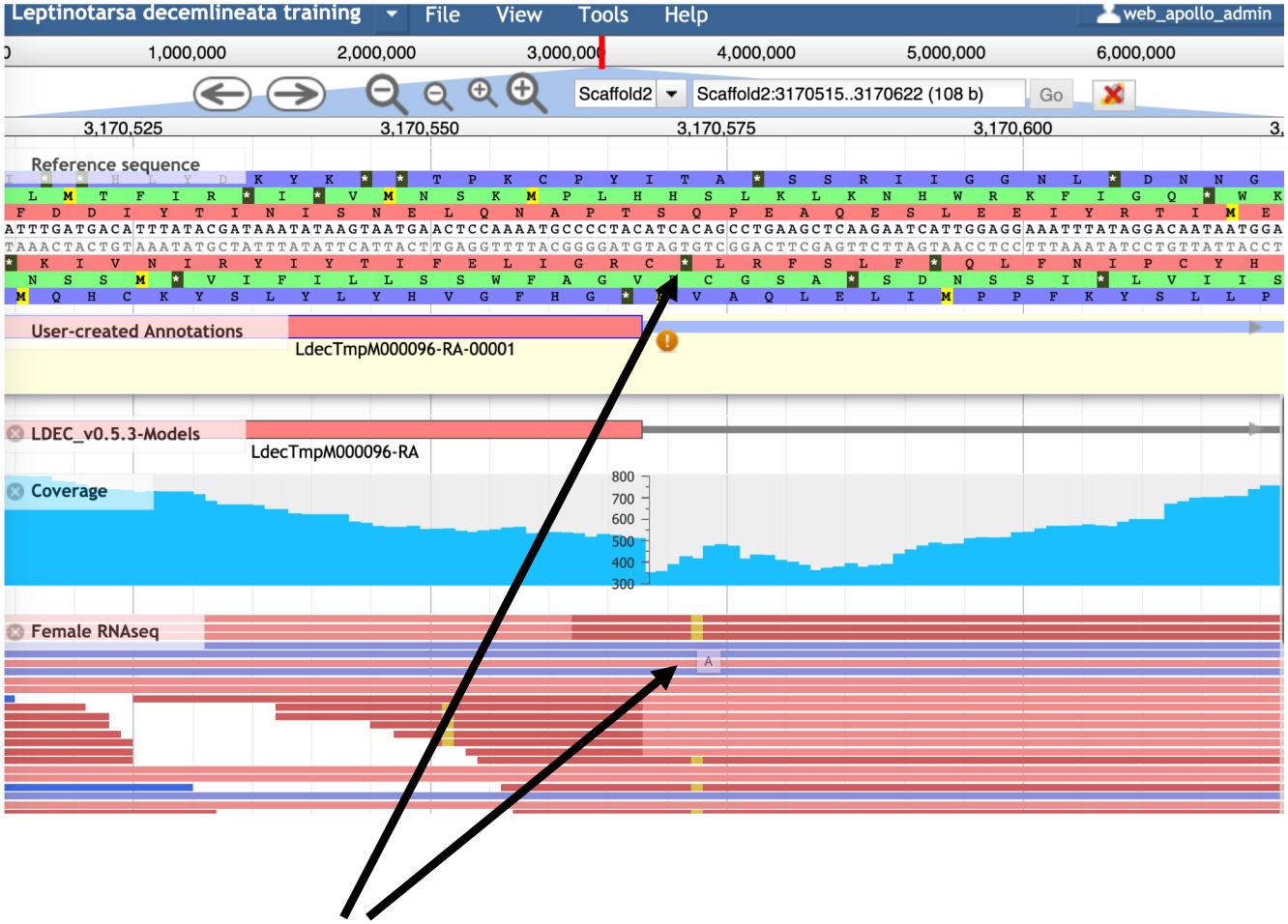
Adding the gene model to Apollo reveals non-canonical splice sites

Sequence alterations



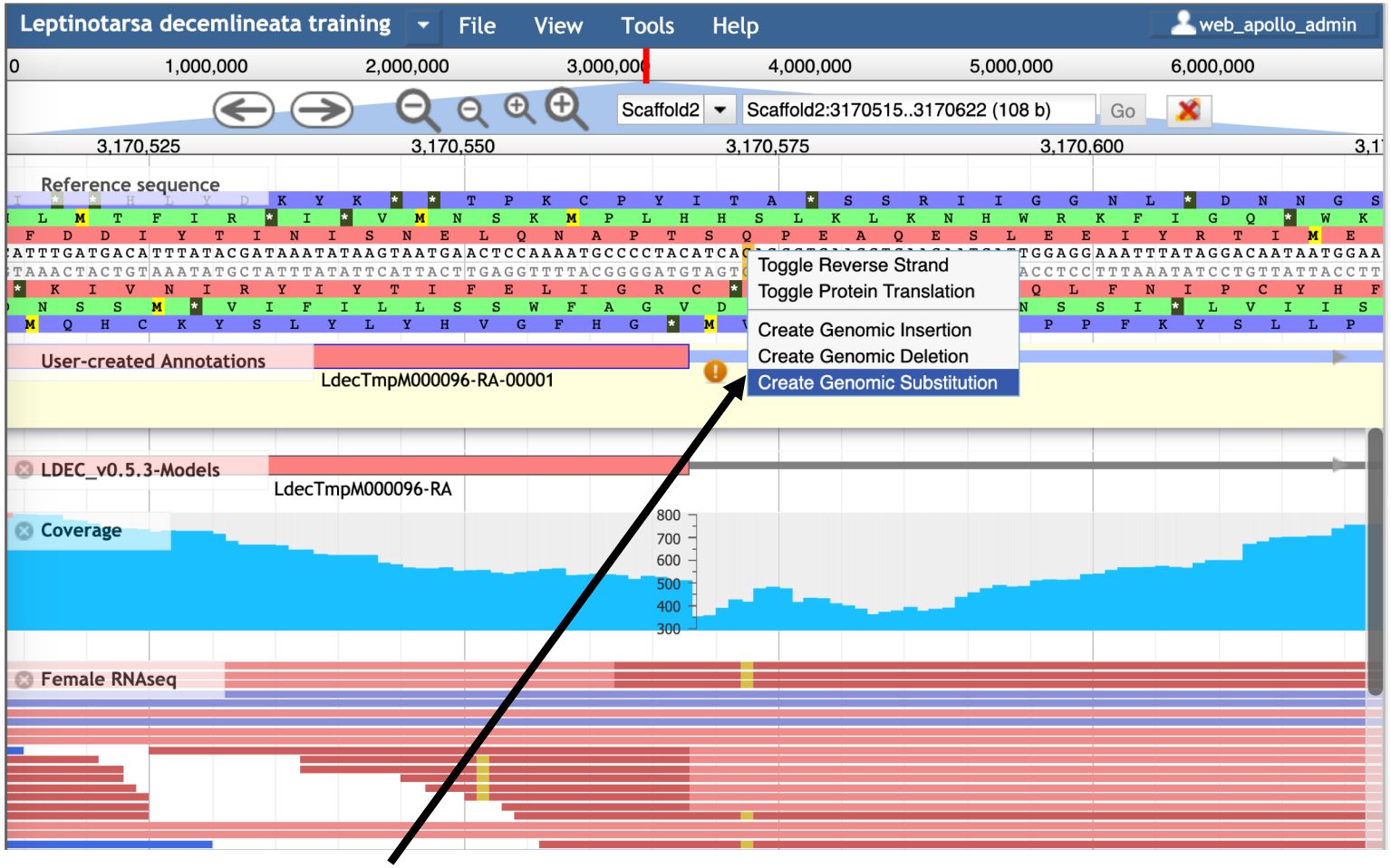
There is some support for a spliced isoform, but the RNA-Seq also suggests contiguous coding sequence

Sequence alterations



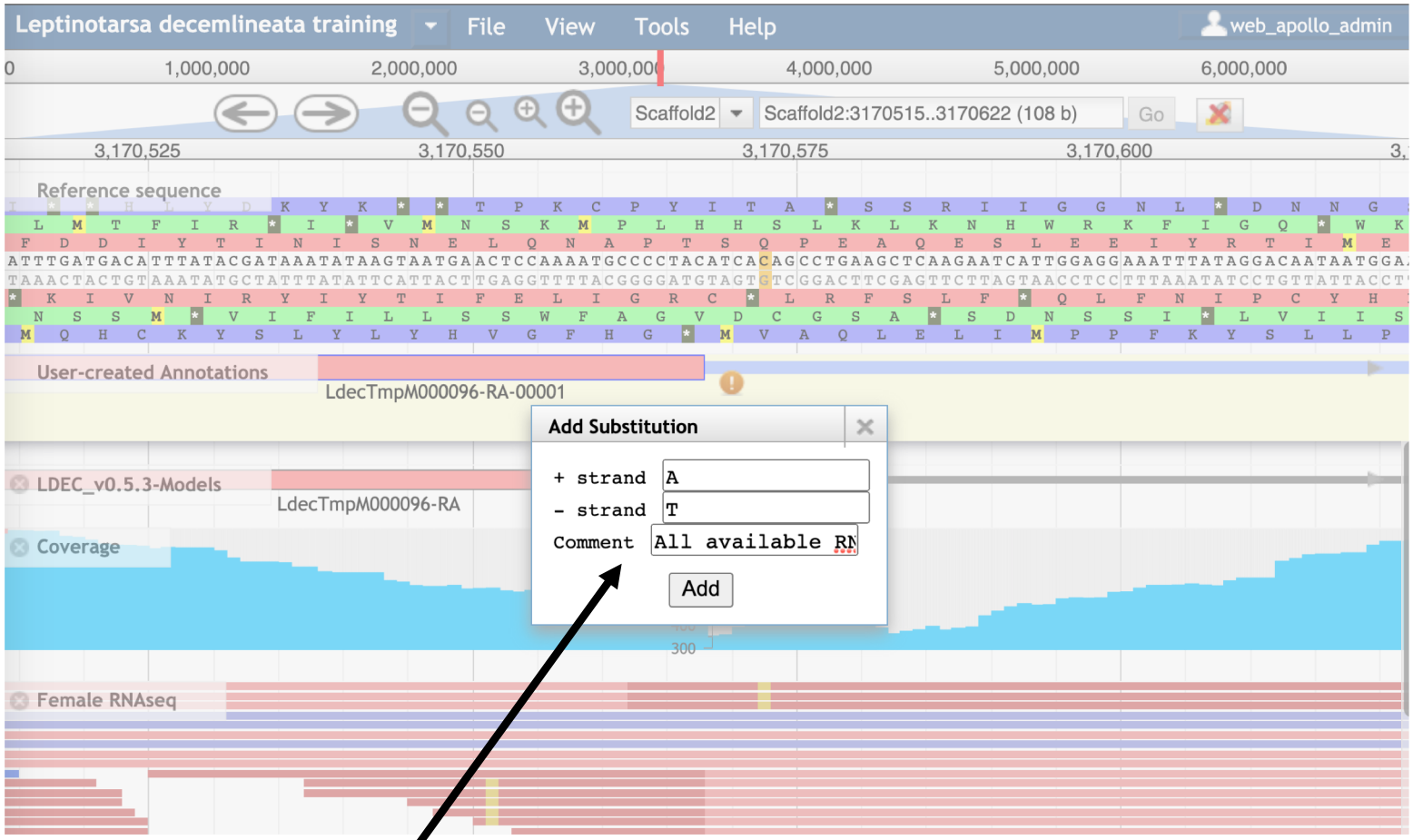
Zooming in, we see a stop codon in the ‘pink’ frame on the reverse strand, but SNPs in all the RNA-Seq reads

Sequence alterations



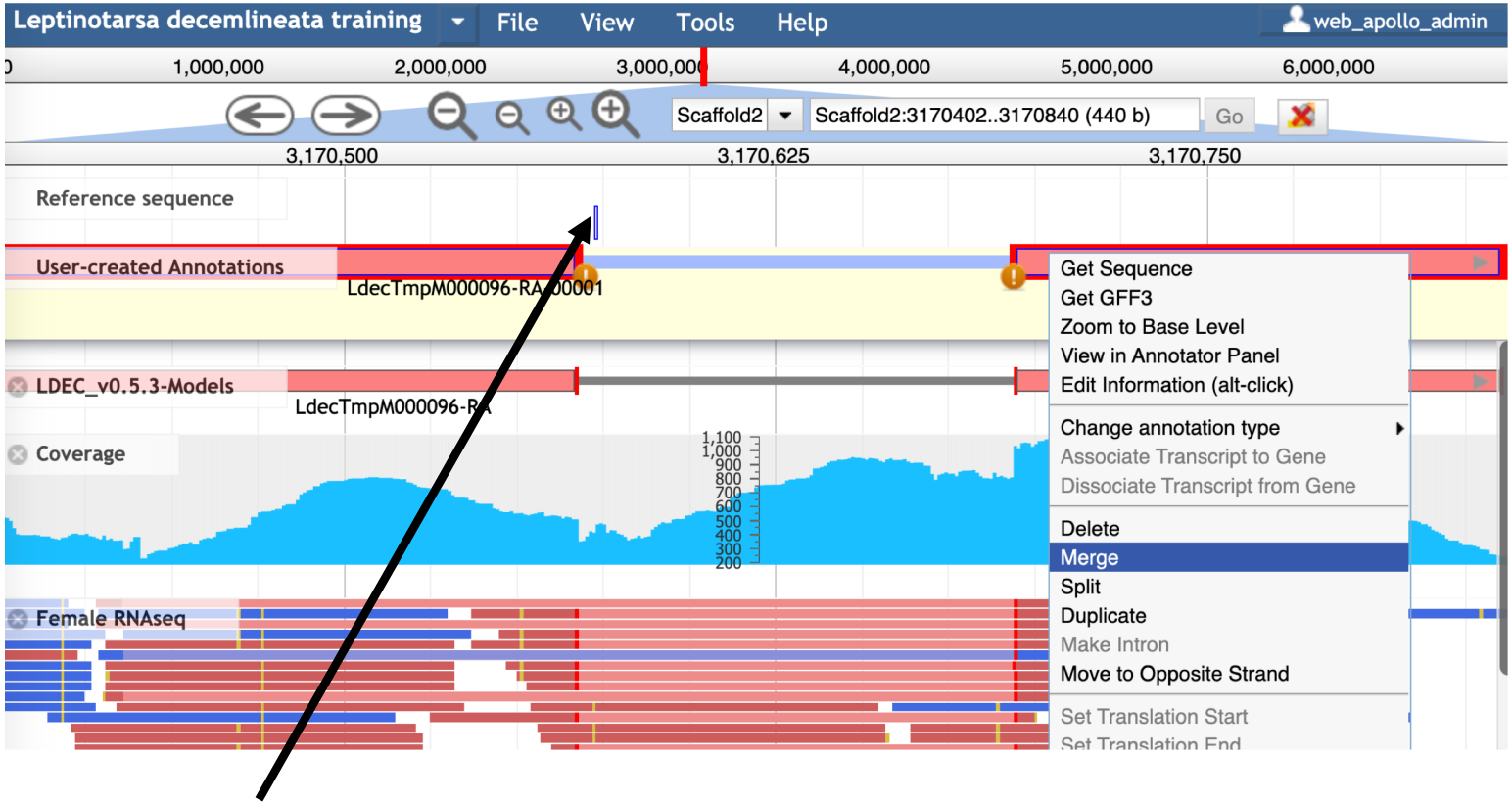
Right-click on the corresponding nucleotide in the *genome assembly* and select 'Create Genomic Substitution'

Sequence alterations



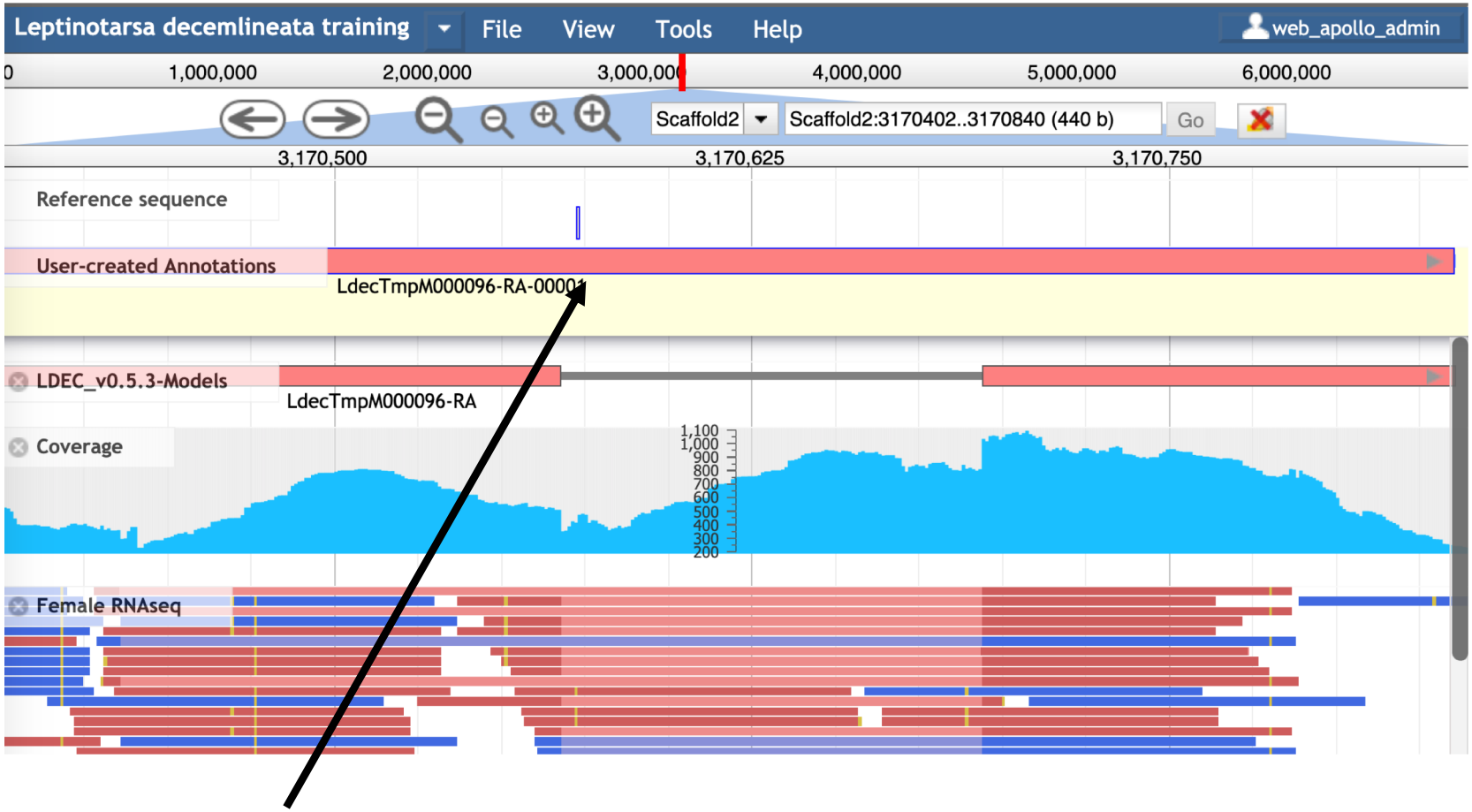
Add the substitution, and a (required) justification

Sequence alterations



Apollo added the substitution! Now, let's merge the CDS regions

Sequence alterations



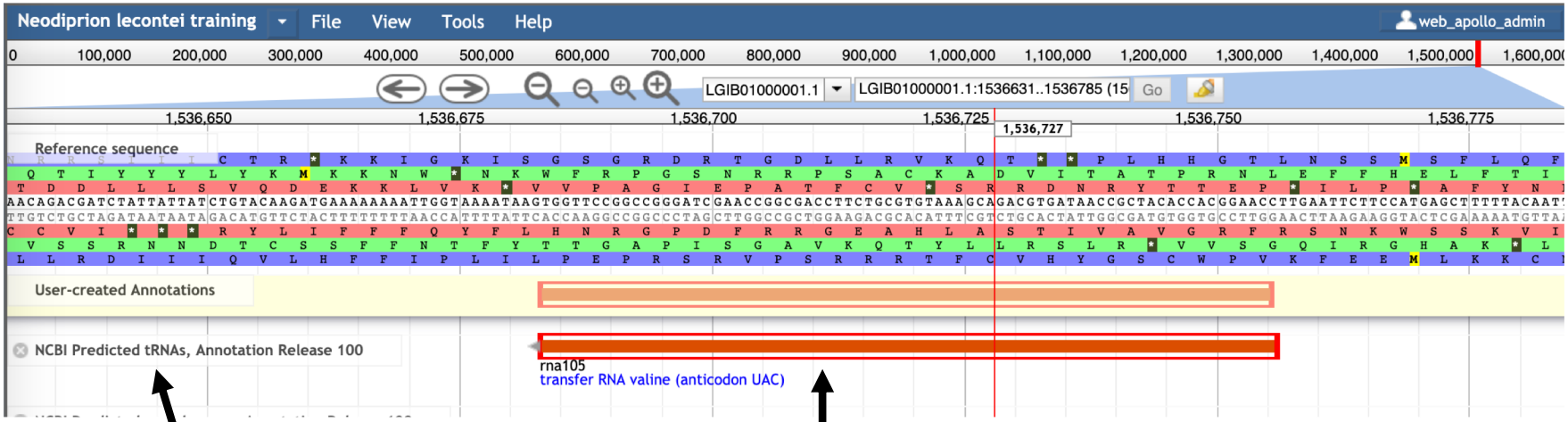
The sequence merged!

Non-coding features

Non-coding features

- Apollo supports protein-coding and non-coding features
- By default, Apollo will create protein-coding features
- For non-coding features, you can set the feature type before or after setting up the model

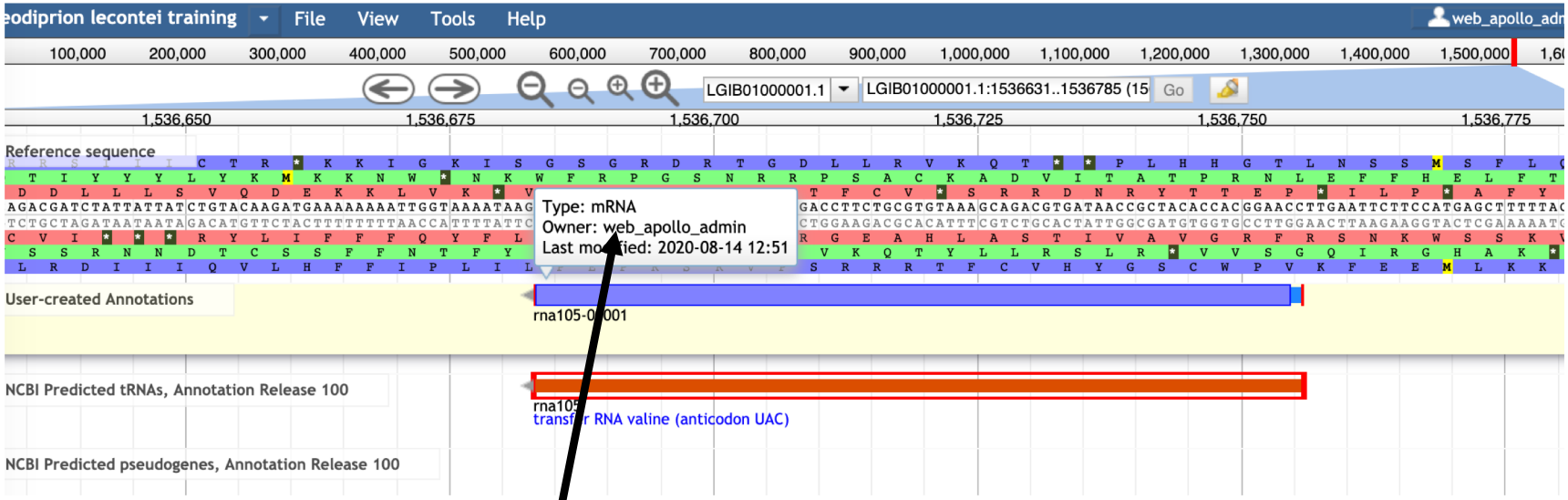
Non-coding features



This feature is a tRNA

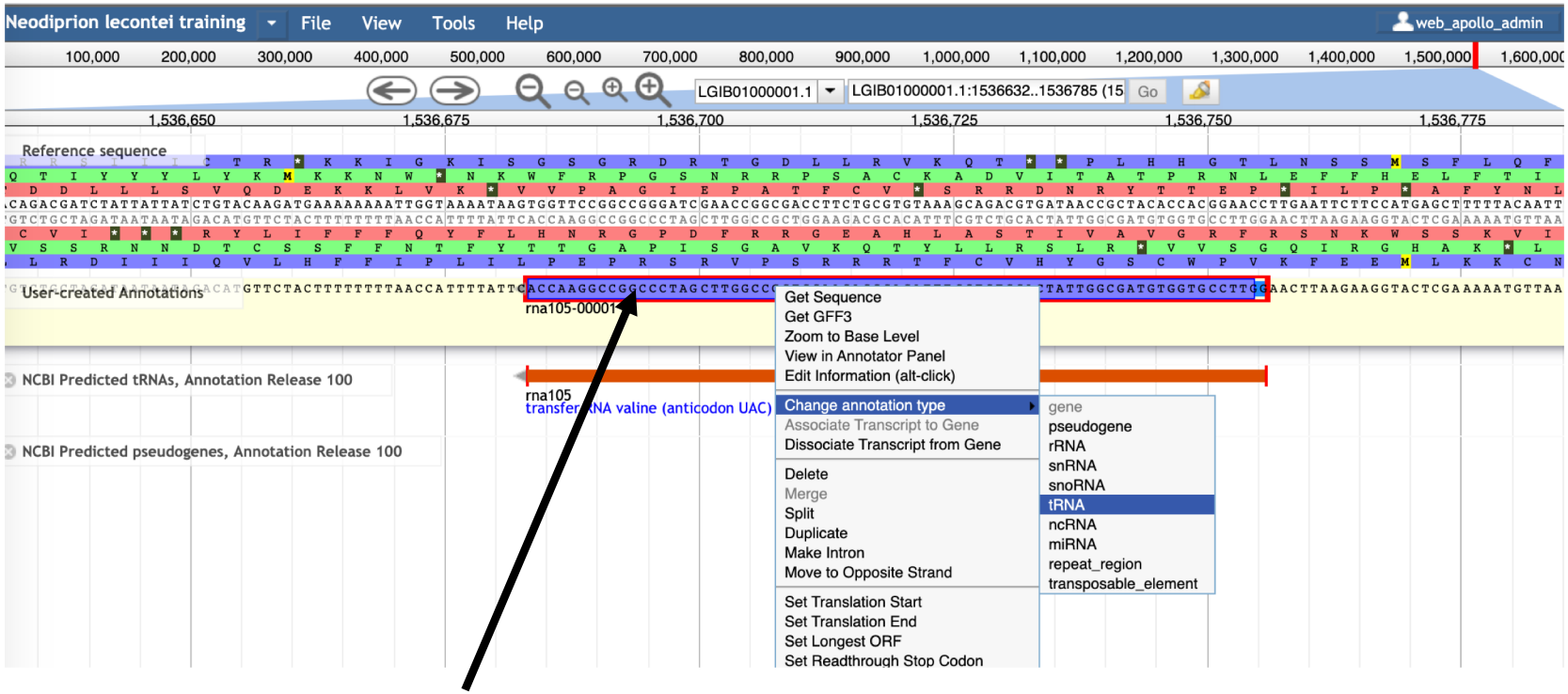
Let's drag it to the UcA track to modify it

Non-coding features



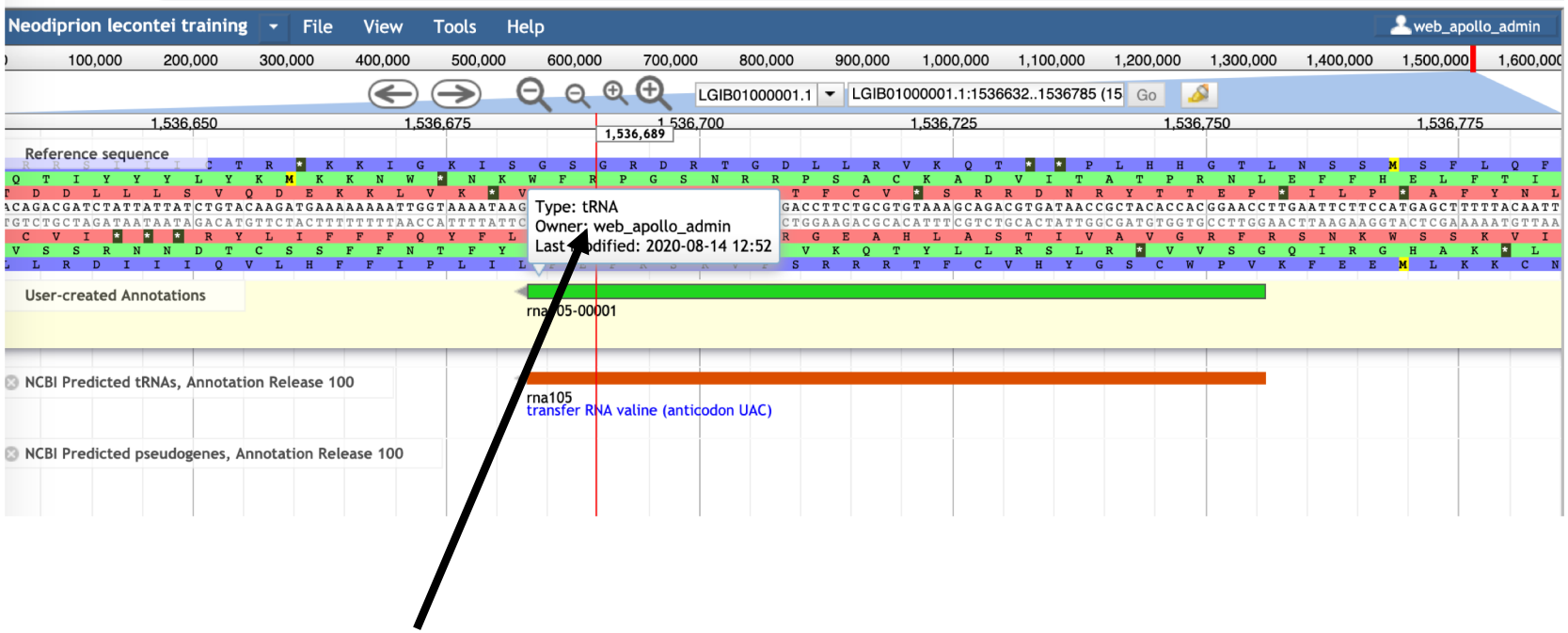
Apollo turned it into an mRNA – let's fix that

Non-coding features



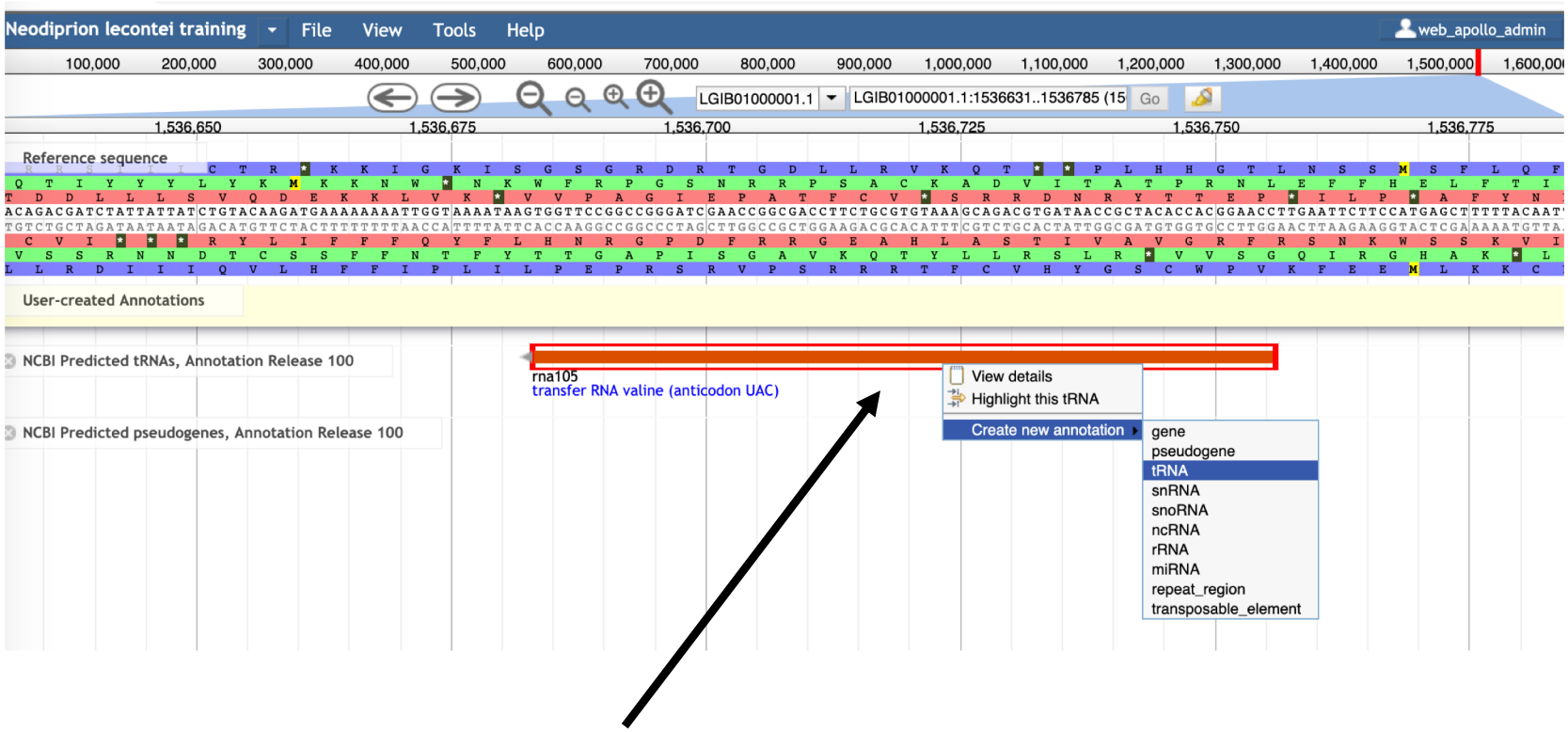
Right-click on feature, select ‘Change annotation type’, then ‘tRNA’

Non-coding features



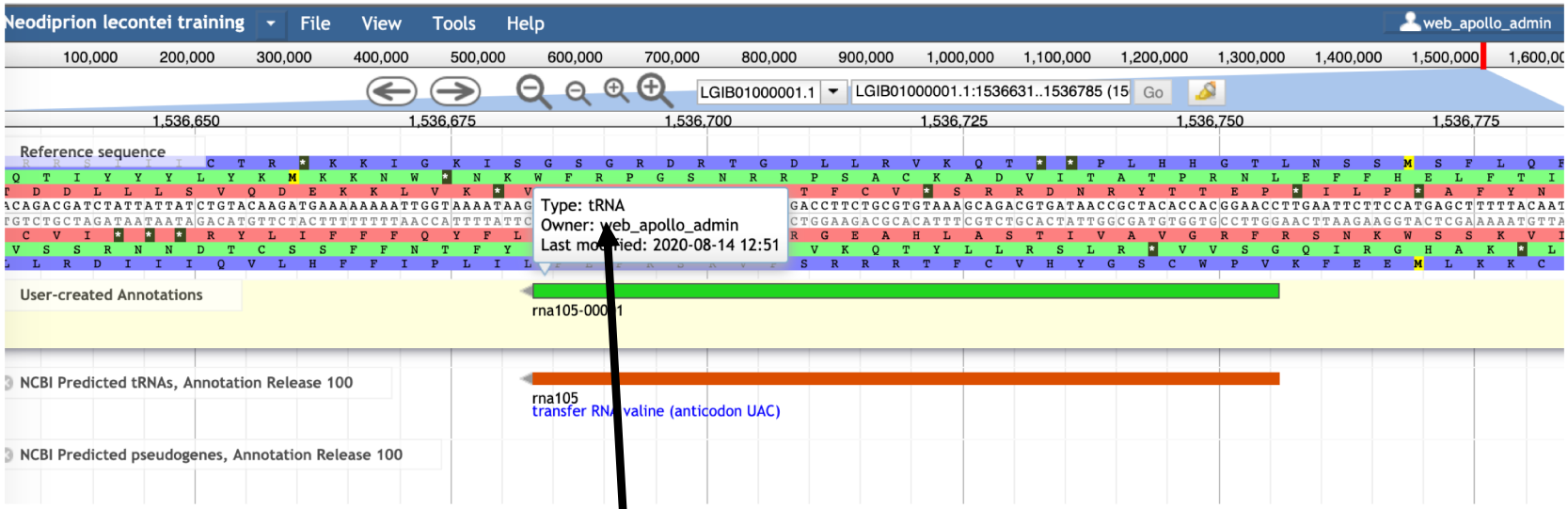
Now we have the correct feature type

Non-coding features



Another way – right-click on model *before* adding it to the UcA track, select 'Create new annotation', then select 'tRNA'

Non-coding features



Now it's a tRNA!

Thank you!

The NAL Team

- Chris Childers
- Vern Chapman
- Ming Chen
- Susan McCarthy
- Shang-Yu Chang
- Hsiu-Kang Huang

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

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