BIOL199 BDB

Fall 2022

Lab 6: Understanding Eukaryotic Genomes using the Genome Browser D-E

Adapted by Melinda A. Yang, from Modules 5 and 6 of the Understanding Eukaryotic Genomes curriculum from the Genome Education Partnership (original authors: Joyce Stamm, Meg Laakso, Carina Endres Howell, and Leocadia Paliulis).

Table of Contents

D. Understanding Translation **1**

E. Understanding Isoforms **4**

Worksheet link (you also have a hard copy)

Appendix Link (http://tiny.cc/ueg_appendix, not directly needed for this section, but may be of use)

(Learning) Objectives are included with each section

Pre-lab Assignments:

1. Read the protocol and review UEG Parts A-C lab.
2. Complete the pre-lab quiz before lab at http://tiny.cc/UEG2_prelabquiz

D. Understanding Translation

(Learning) Objectives:

- Determine codons for specific amino acids and identify reading frames by looking at the Base Position track in the Genome Browser
- Assemble exons to maintain the open reading frame (ORF) for a given gene
- Define the phases of the splice donor and acceptor sites and describe how they impact the maintenance of the ORF
- Identify start and stop codons of an assembled ORF

In this exploration, we will continue to focus on the *transformer* gene (referred to as tra-RA or just *tra*), and will learn about how the *tra* mRNA is translated into a string of amino acids. Remember that there are six possible reading frames. The direction of transcription eliminates three possible reading frames. How do we determine the reading frames across the *tra* gene? Let's investigate!

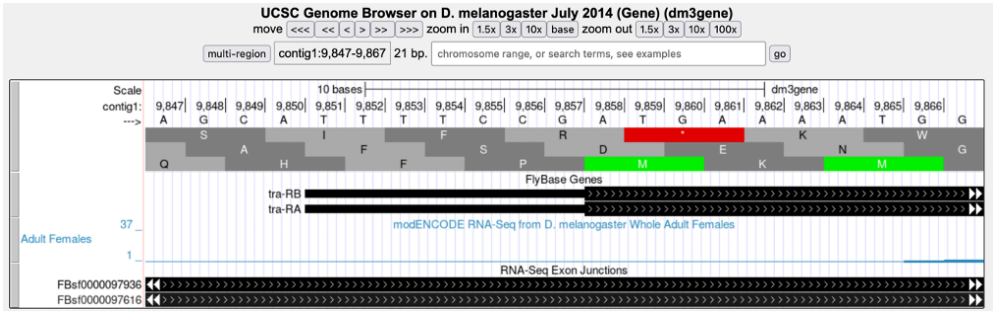
Instructions	**Comments**
1. Type "**contig1:9,840-9,900**" into the "enter position or search terms" text box and then click on the "go" button.	

2. Using the same Genome Browser page, reset the Browser by clicking on "**hide all**." Open the following tracks:

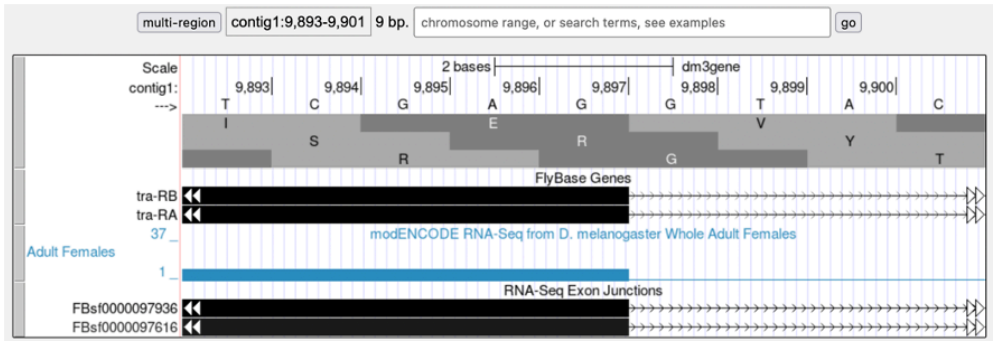
- Change base position to "**full**"
- Change FlyBase Genes to **"pack"**
- Change RNA-Seq coverage to "**full**"
- Change Exon Junctions to "**full**"
- Click "**refresh**"

3. Click on the "RNA-Seq Coverage" link under the RNA-Seq Tracks green bar. Make sure the "Data view scaling" field is set to "**use vertical viewing range setting**" and the "max" field under "Vertical Viewing range" is set to **37**. Under the "List sub-tracks" section, unselect the "**Adult Males**" track. Then, click submit.

4. Let's find the start codon for tra-RA. Zoom in on where the FlyBase Genes track shows that the translation starts (where the tracked black box gets thicker for the tra-RA isoform).



5. Now zoom in and find the last base of the first exon for tra-RA using your RNA-Seq data and Exon Junctions data.



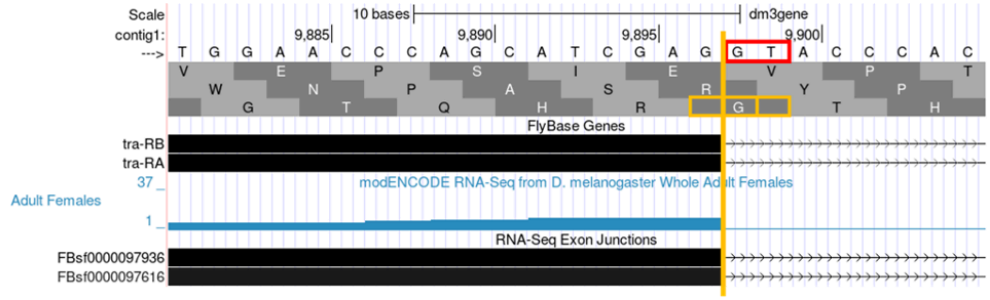
6. Look closely at reading frame 3, just before the splice site. Note that the splice site (red box) cuts off the last codon of the first CDS (orange box) after just one base. Therefore, we would say this CDS has a "**phase 1" end because there is a partial codon at the end of the CDS that is 1 base long.** (If there were a fully completed codon before the splice site, it would be in

Answer questions D1-D3

Your positions should be consistent with the values you determined in question C3a-b

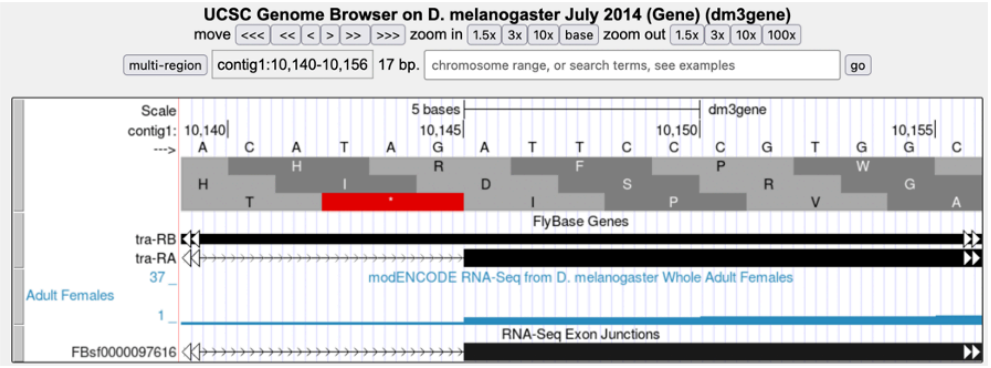
The reading frame in the next CDS may not be the same as for the previous one because of splicing. To figure out which reading frame is being translated at

phase 0, and if there were two bases before the splice site, it would be in **phase 2**.)



CDS 2, we need to check the end of the first CDS to see how many bases of the last codon are present before the 5' splice site consensus sequence.

7. Navigate to the 3' splice site of intron 1 (i.e., the location where the first intron ends and the second exon begins).



For CDS 1 with a phase 1 end, we will need two more bases from CDS 2 to complete the codon.

Your positions should be consistent with the values you determined in question C3c-d.

DISCUSS & CHECK-IN: Knowing that CDS-1 ends with a partial codon of 1 base (i.e. phase 1), what phase is the 5' end of CDS-2 at, and in what reading frame is CDS-2?

Phase at 5' end of CDS-2/exon 2: _____

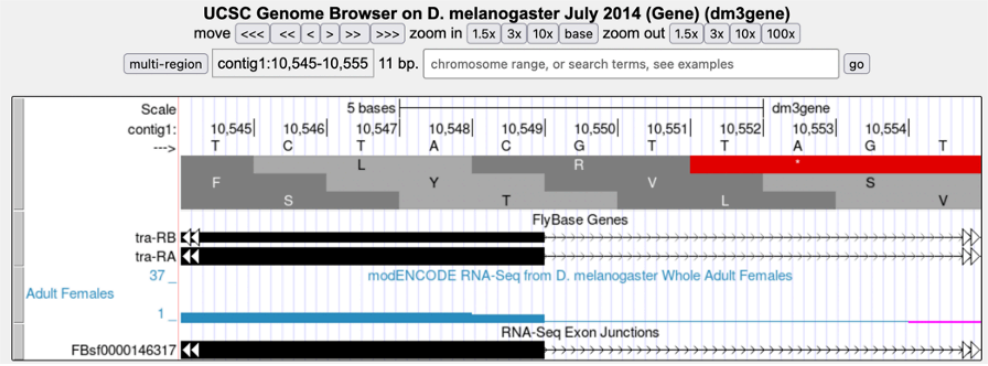
Reading frame of CDS-2: _____

8. Next, zoom out and look at the correct reading frame for all of CDS-2 (exon 2) of tra-RA. You can see that there are no stop codons in this reading frame, which lends support to our conclusion that this is the proper reading frame.

9. Now, let's do the same for the 5' splice site of intron 2 for tra-RA. Zoom in on that splice site.

Your positions should be consistent with the values you determined in question C3g.

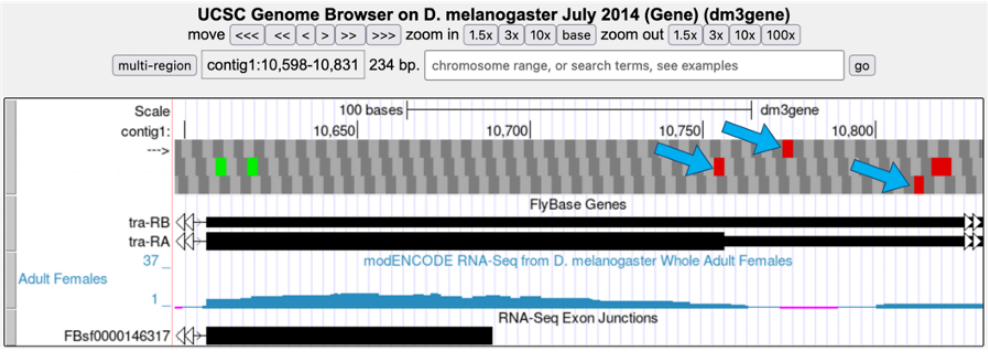
Answer question D4



10. Navigate to the start of the final exon which contains CDS-3.

Your positions should be consistent with the values you determined in question C3h-i.

11. Now locate the first stop codon in the translated reading frame. Stop codons are shown as red boxes with asterisks (red arrows).



Note that the first stop codon is not translated to an amino acid. Therefore, its positions do not correspond to any part of the amino acid sequence leading to the protein.

Answer questions D6-D7

E. Understanding Isoforms (Examining tra-RB)

(Learning) Objectives:

- Demonstrate how alternative splicing of a gene can lead to different mRNAs
- Show how alternative splicing can lead to the production of different polypeptides and result in drastic changes in phenotype

In this investigation, **we will switch from tra-RA to tra-RB**, the second isoform of the *tra* gene, and we will explore how multiple different mRNAs and polypeptides can be encoded by the same gene.

Instructions

Comments

1. Type "**contig1:9,840-9,900**" into the "enter position or search terms" text box and then click on the "go" button.

2. Using the same Genome Browser page, reset the Browser by clicking on "**hide all**." Open the following tracks:

- Change base position to "**full**"
- Change FlyBase Genes to **"pack"**
- Change RNA-Seq coverage to "**full**"
- Change Exon Junctions to "**full**"
- Click "**refresh**"

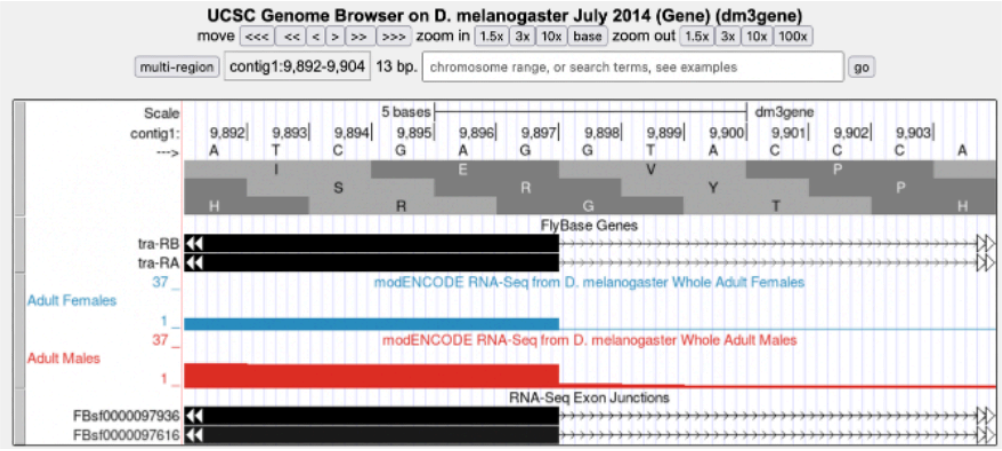
3. Click on the "RNA-Seq Coverage" link under the RNA-Seq Tracks green bar. Make sure the "Data view scaling" field is set to "**use vertical viewing range setting**" and the "max" field under "Vertical Viewing range" is set to **37**. Under the "List sub-tracks" section, make sure both "**Adult Females**" and "**Adult Males**" tracks are selected. Then, click submit.

4. Notice that tra-RB shares the same start of translation as tra-RA. Also notice that the diagrams for the first and second RNA-Seq Exon Junctions tracks have the same 5' splice site but different 3' splice sites.

DISCUSS & CHECK-IN: Discuss as a group what the RNA-Seq histograms and exon junctions show. Check in with your TA or instructor to make sure your understanding is correct.

Answer questions E1 and E2a

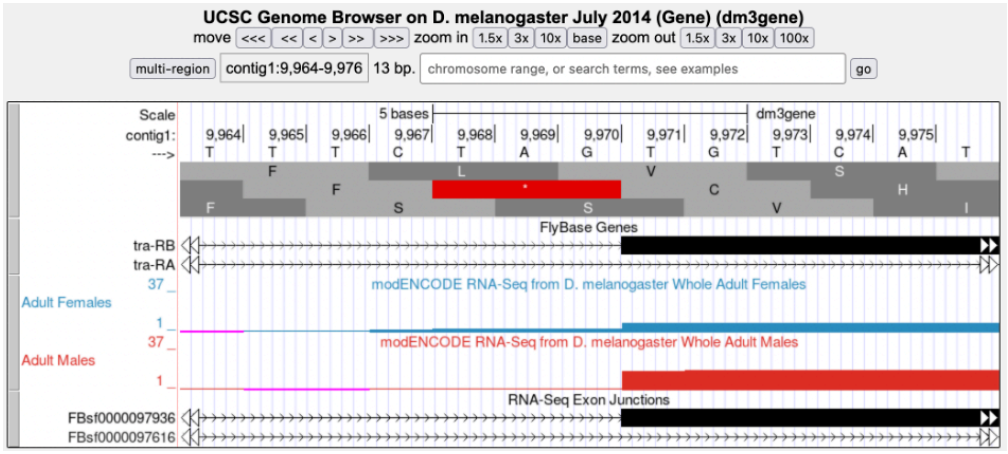
5. Now zoom in on the location of the 5' splice site at the end of the first CDS/exon in both tra-RA and tra-RB.



Answer questions E2b-d

6. Now zoom out and zoom in on the start of the second CDS/exon in tra-RB, just after the 3' splice site. We can identify exon 2 by the RNA-Seq data, in particular using the RNA-Seq Exon Junctions data. Finish out the rest of your annotation of this isoform.

Answer questions E2e-i



DISCUSS: Check your numbers with each other for worksheet question E2, and make sure you're getting the same numbers. If there's conflict in numbers, discuss and resolve where there are misconceptions amongst your group.

7. Compare your findings to what you found previously for tra-RA.
Here the splicing decision is made in a sex-specific manner; male fruit flies have targeted the spliceosome to use the first 3' acceptor site identified by the RNA-Seq Exon Junction data, while female fruit flies have targeted the spliceosome to use the second 3' acceptor site identified. This change in splicing has profound effects – in fact, it drives the programming of male and female characteristics in the developing fly.

The 3' acceptor site for the second intron in tra-RA is found inside the second exon of tra-RB. This intron is *alternatively spliced*.

8. Complete a gene model for tra-RB like you did for tra-RA.

Answer question E3

9. Answer the remaining worksheet questions.

Answer questions E4-E5