Module 2: Transcription Part I: From DNA sequence to transcription unit

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Objectives

- Describe how a primary transcript (pre-mRNA) can be synthesized using a DNA molecule as the template.
- Explain the importance of the 5' and 3' regions of the gene for initiation and termination of transcription by RNA polymerase II
- Identify the beginning and end of a transcript using the capabilities of the Genome Browser (RNA-Seq, Short Match)

Prerequisites

- Understanding Eukaryotic Genes Module 1

Class Instruction

- Discuss the questions: What is transcription? What cellular proteins are required for transcription? How does it work mechanistically? What is/are the products of transcription? (students discuss in pairs, then as a class)
- Work through the Genome Browser investigation, then identify where transcription starts and ends for the *tra* gene. How long is the pre-mRNA?
- Conclude by challenging students to think about these questions:
    - How important is it for RNA polymerase II to recognize the promoter sequence?
    - Do you think it is possible for a gene to have more than one transcription start site? How would RNA polymerase II know which one to choose? When would it make a difference in the protein product, and when not?

Associated Videos and Resources

- RNA-Seq and TopHat Video
- RNA-Seq Video
- Short Match Video
- Glossary for Understanding Eukaryotic Genes

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Investigation 1: Identify the Transcription Unit

Introduction

This module will introduce you to the use of the Genome Browser to illustrate the process of transcription and help you identify regulatory elements, using the *Drosophila melanogaster* transformer (*tra*) gene as an example. You will use the UCSC Genome Browser Mirror developed by the Genomics Education Partnership (GEP), which contains RNA expression data, to identify the different parts of the gene that give rise to pre-mRNA through transcription.

Finding the transcript for tra-RA using the UCSC Genome Browser Mirror

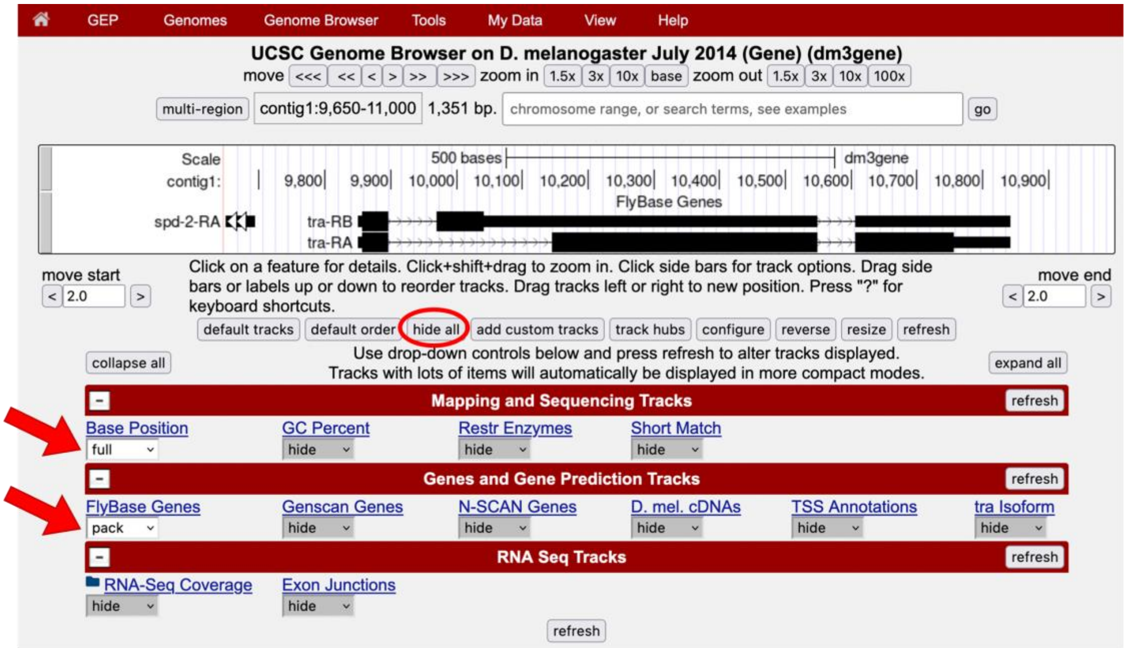
1. Open a new web browser window and go to the UCSC Genome Browser Mirror site at https://gander.wustl.edu/. Follow the instructions given in Module 1 to navigate to the contig1 project in the *D. melanogaster* "July 2014 (Gene)" assembly.
2. To navigate to the genomic region surrounding the *tra* gene, enter "**contig1:9,650-11,000**" into the "chromosome range, or search terms, see examples" field located just above the displayed tracks and then click on the "go" button. As you learned in the previous module, you can also use the buttons in the navigation controls section to zoom in, zoom out, and use the arrows to move to different parts of the contig. In addition, you can place your cursor on the "Scale" or the "Base Position" sections of the Genome Browser image and then drag your cursor from the initial position to the end position to zoom into a region of interest.
3. This region from 9,650-11,000 contains the entire *tra* (transformer) gene and the very end of the previous gene *spd-2* (spindle defective 2). As described in Module 1, the suffix (e.g., -RA)

corresponds to the name of the isoform that is associated with the gene. Hence, spd-2-RA corresponds to the A isoform of the *spd-2* gene.

4. Because the Genome Browser remembers your previous display settings, we will hide all the evidence tracks and then enable only the subset of tracks that we need: Click on the "**hide all**" button located below the Genome Browser image. Then, configure the display modes as follows:

   - Under "Mapping and Sequencing Tracks"
     - Base Position: **full**
   - Under "Gene and Gene Prediction Tracks"
     - FlyBase Genes: **pack**
   - Click on any of the "refresh" buttons to update the display (Figure 1)

Note: Depending on your screen resolution, you may need to zoom in further to see the nucleotides and amino acid translations even if you set the "Base Position" track to full.



UCSC Genome Browser on D. melanogaster July 2014 (Gene) (dm3gene)

contig1:9,650-11,000 1,351 bp.

Click on a feature for details. Click+shift+drag to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position. Press "?" for keyboard shortcuts.

Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.

Mapping and Sequencing Tracks: Base Position: full; GC Percent: hide; Restr Enzymes: hide; Short Match: hide

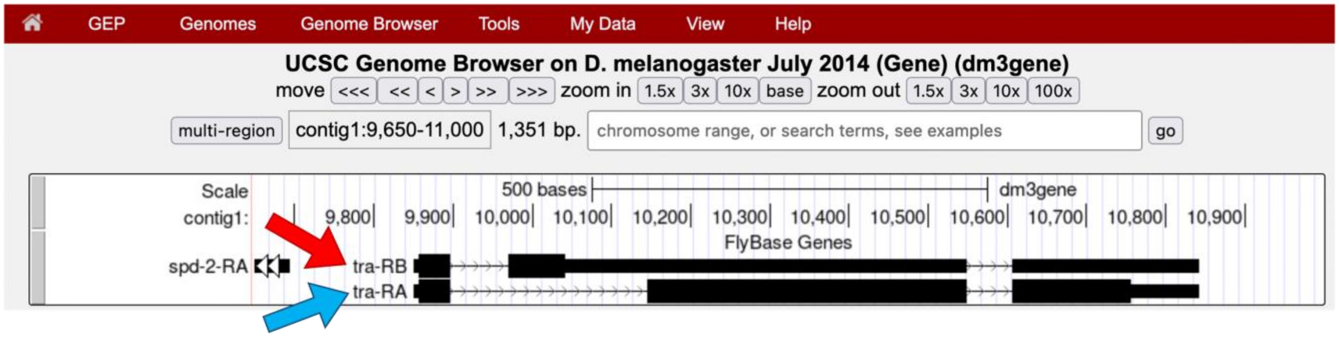
Gene and Gene Prediction Tracks: FlyBase Genes: pack; Genscan Genes: hide; N-SCAN Genes: hide; D. mel. cDNAs: hide; TSS Annotations: hide; tra Isoform: hide

RNA Seq Tracks: RNA-Seq Coverage: hide; Exon Junctions: hide

Figure 1 Configuring the display modes for the evidence tracks surrounding the *tra* gene.

Identifying the transcription unit for the *tra* gene

5. Now let's investigate how the string of As, Ts, Cs, and Gs of the DNA sequence in this genomic region give rise to the mRNAs for the *tra* gene. The "FlyBase Genes" track shows the protein-coding genes that have been annotated by FlyBase. According to this track, there are actually two different mRNAs (tra-RA and tra-RB) made from the same DNA sequence (Figure 2). These represent two alternative forms known as **isoforms** of the transformer (*tra*) gene product.



contig1:9,650-11,000 1,351 bp. chromosome range, or search terms, see examples

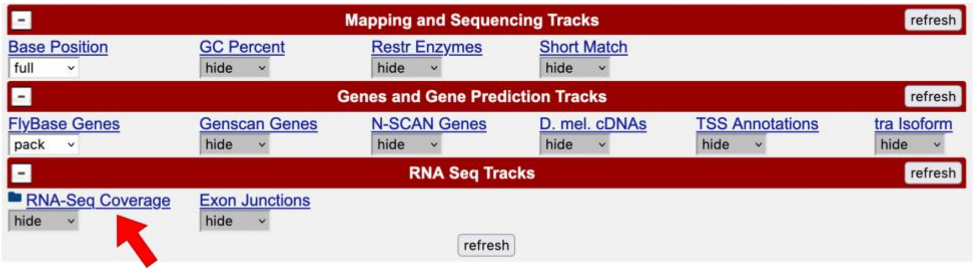
Figure 2 FlyBase annotated isoforms A (blue arrow) and B (red arrow) of *tra* in *D. melanogaster*.

6. For the moment, we will focus only on the A isoform of *tra* (tra-RA). As you learned in Module 1, the black boxes represent the exons (the part of the transcript that makes up the mRNA); the thick black boxes represent the translated regions (i.e., the parts of the exons that contain information that codes for protein) while the thinner black boxes represent untranslated regions (i.e., the part of the exons that do not contain information that codes for protein). Lines that connect multiple boxes together represent introns, the parts of the transcript that are removed in the production of a mature mRNA. Collectively, they constitute the transcription unit, the part of the gene that is read by RNA polymerase II during transcription.

We use the name "transcription unit" rather than "gene" because genes also contain regulatory sequences (promoters and both positive and negative regulatory elements) that are not transcribed. In contrast to prokaryotes, where most of the transcript codes for protein in a single open reading frame (no introns!), in eukaryotes, the transcript contains a lot of extra nucleotides that are not used to form the protein.

Q1. What is the span — the start and end base positions — of the tra-RA transcription unit?

7. The Genome Browser contains tracks that we can use to visualize the regions of the DNA that are transcribed into RNA. For example, the "RNA Seq Tracks" section contains results from sequencing (mostly mature) mRNAs and then mapping the sequences found in the RNA-Seq reads back to the genome. Hence, regions with RNA-Seq read coverage usually correspond to regions in the genome that are being transcribed. To visualize the distribution of these RNA-Seq reads, scroll down to the bottom of the page and then click on the "RNA-Seq Coverage" link under the "RNA Seq Tracks" section header (Figure 3).

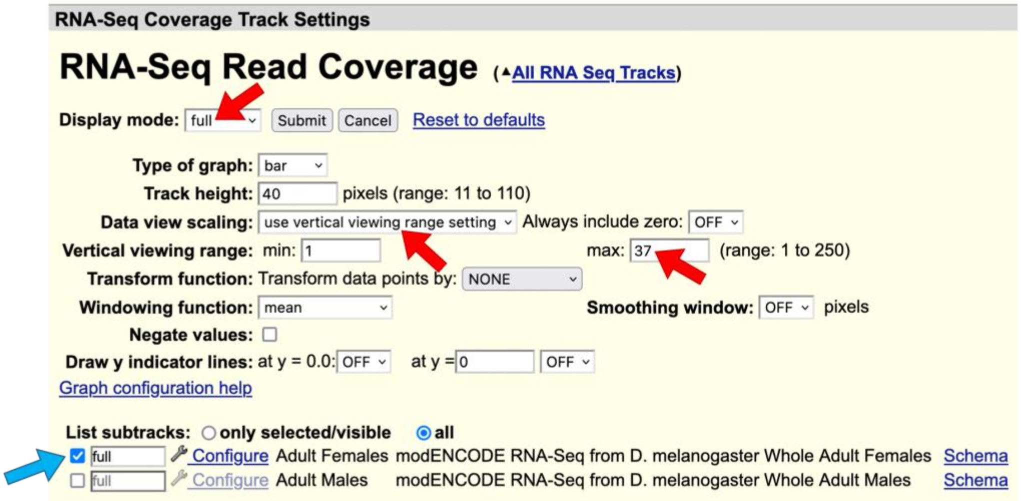

Mapping and Sequencing Tracks
refresh
Genes and Gene Prediction Tracks
refresh
RNA Seq Tracks
refresh
refresh

Base Position: full
GC Percent: hide
Restr Enzymes: hide
Short Match: hide
FlyBase Genes: pack
Genscan Genes: hide
N-SCAN Genes: hide
D. mel. cDNAs: hide
TSS Annotations: hide
tra Isoform: hide
RNA-Seq Coverage: hide
Exon Junctions: hide

Figure 3 Click on the "RNA-Seq Coverage" to configure the display settings for this evidence track.

8. Using the controls in the “RNA-Seq Read Coverage” page that comes up when you click the “RNA-Seq Coverage” link, we will modify the display settings to the following (Figure 4):
   - Change the “Display mode” field to “**full**”
   - Select the “Data view scaling” field to “**use vertical viewing range setting**”
   - Change the “max” field under “Vertical viewing range” to **37**
   - Under the “List subtracks” section, unselect the “Adult Males” track
   - Click on the “Submit” button (“Display mode” line, near the top of the page)

By default, the RNA-Seq Coverage track will auto-scale based on the read depth (that is, the number of reads) in the viewing region. The settings above override this setting and manually define the scale to be from 1 to 37. The RNA-Seq Coverage track contains data from mRNA isolated from two separate samples, adult males and adult females. Here we unselect the “Adult Males” track so that the Genome Browser will only show the RNA-Seq read coverage from adult females. We will return to the “Adult Males” track in Module 6.



RNA-Seq Coverage Track Settings

RNA-Seq Read Coverage (▲All RNA Seq Tracks)

Display mode: full Submit Cancel Reset to defaults

Type of graph: bar

Track height: 40 pixels (range: 11 to 110)

Data view scaling: use vertical viewing range setting Always include zero: OFF

Vertical viewing range: min: 1 max: 37 (range: 1 to 250)

Transform function: Transform data points by: NONE

Windowing function: mean Smoothing window: OFF pixels

Negate values: □

Draw y indicator lines: at y = 0.0: OFF at y = 0 OFF

Graph configuration help

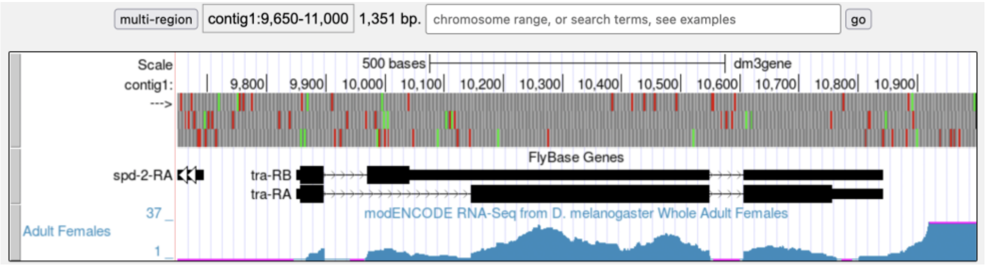
List subtracks: ○ only selected/visible ● all

☑ full Configure Adult Females modENCODE RNA-Seq from D. melanogaster Whole Adult Females Schema

□ full Configure Adult Males modENCODE RNA-Seq from D. melanogaster Whole Adult Males Schema

Figure 4 Manually define the viewing range for the RNA-Seq Read Coverage track (red arrows) and select only the subtrack of interest (i.e., Adult Females, blue arrow).

9. The Genome Browser image now includes a track in blue with peaks and valleys, labeled “**modENCODE RNA-Seq from *D. melanogaster* Whole Adult Females**” (Figure 5). The y-axis corresponds to the number of RNA-Seq reads from whole adult females that have been mapped to each genomic position of this portion of contig1.



multi-region contig1:9,650-11,000 1,351 bp. chromosome range, or search terms, see examples go

Figure 5 RNA-Seq read coverage track (blue) for the *D. melanogaster* adult female sample.

Q2. How do the peaks in the RNA-Seq Read Coverage track relate to mRNA abundance?

Q3. Most of the RNA-Seq reads come from mature (processed) RNA. Can you use this data to suggest where introns are located? Are there any regions that seem ambiguous?

In subsequent modules, we will learn more about the mRNA processing that occurs in the nucleus to remove introns prior to translation.

Investigation 2: Identify the 5' end of the transcription unit

Introduction

Previous studies have identified sequence motifs that are enriched in the region surrounding a gene's Transcription Start Site (TSS). This region is known as the core promoter. By convention, we designate the TSS as +1 and we specify the positions of the sequence motifs with respect to the TSS. For example, the initiator (**Inr**) motif is found at -2 relative to the TSS (2 bp upstream) while the **TATA box** motif is found at -31 or -30 relative to the TSS. Both of these motifs are in the same orientation as the transcript (Figure 6).

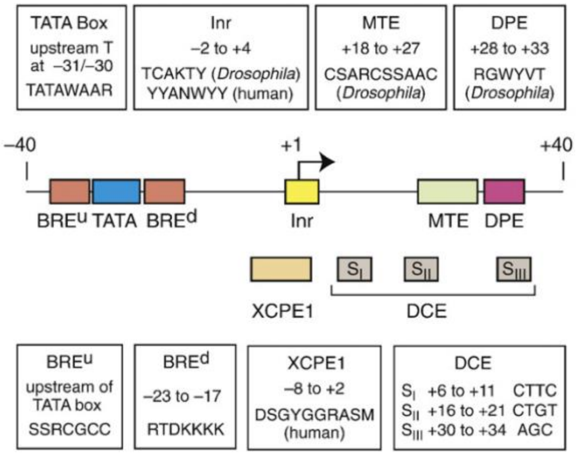


Figure 6 Motifs that are enriched near the transcription start sites of many eukaryotic genes (Juven-Gershon T and Kadonaga JT, 2010[1]). Note that the motifs are often "degenerate," N = any base, R = purine (either A or G), Y = pyrimidine (C or T), K = keto (T or G), M = amino (C or A), S = strong (G or C), W = weak (A or T), V = A/G/C (not T), D = A/G/T (not C).

In this module, we will review three lines of evidence to determine the TSS position(s) for the *tra* gene. Because RNA-Seq identifies regions of the genome that are being transcribed, we will use the RNA-Seq Coverage track to define the scope of the region to search. The start of the region with RNA-Seq read coverage is the 5' end of the transcript and corresponds to the approximate TSS site, (i.e., the

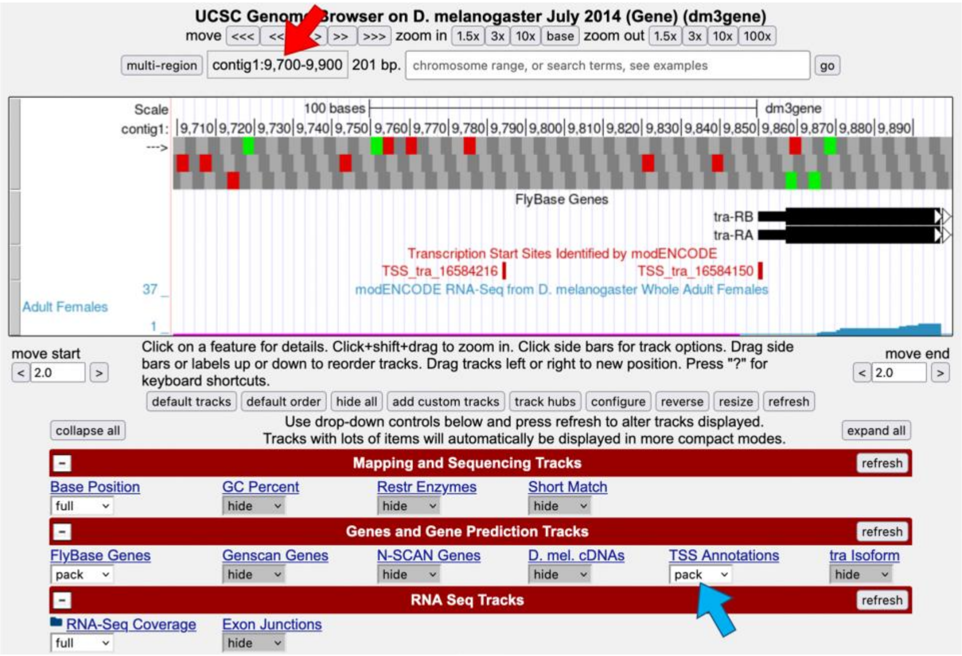
[1] Juven-Gershon T and Kadonaga JT. (2010) Regulation of gene expression via the core promoter and the basal transcriptional machinery. Developmental Biology 339:225–229

beginning of the transcription unit). RNA-Seq data hence becomes our first line of evidence to try to determine the location of the TSS. In other words, the information gathered from RNA-Seq will be used to support the choice of the TSS. To learn more about RNA-Seq, watch the RNA-Seq and TopHat video.

Q4. Examine the "RNA-Seq Coverage" and the "FlyBase Genes" tracks in the Genome Browser from left to right. At approximately which coordinate (base position) does the RNA-Seq data start for the *tra* gene? Remember that you can use the navigation controls at the top of the page to zoom in to the region of interest.

One of the first steps in mRNA processing is the addition of the 5' cap at the beginning of the transcript (we will learn more about capping in the next module). There are experimental techniques that specifically isolate the sequences that are associated with the 5' cap. These sequences or "reads" can then be mapped against the genomic assembly, and the TSSs will show higher read density than the rest of the genome. The modENCODE project summarizes these experimental data to produce a set of predicted TSSs; these predictions are shown in the "TSS Annotations" track. The TSS annotations predicted by modENCODE constitute our second line of evidence to determine the *tra* TSS location.

1. Before we turn on this track, we will zoom into the region between the end of the previous gene (*spd-2*) and the region where we see RNA-Seq data for tra-RA. Change the "chromosome range, or search terms" field to "**contig1:9,700-9,900**" and then click "go". We expect the RNA polymerase to bind and initiate transcription somewhere in this area. Scroll down to the "Gene and Gene Prediction Tracks" section and change the display mode for the "TSS Annotations" track to "pack". Click on a "refresh" button (Figure 7).



UCSC Genome Browser on D. melanogaster July 2014 (Gene) (dm3gene)

contig1:9,700-9,900 201 bp.

Click on a feature for details. Click+shift+drag to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position. Press "?" for keyboard shortcuts.

Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.

Mapping and Sequencing Tracks

- Base Position: full
- GC Percent: hide
- Restr Enzymes: hide
- Short Match: hide

Genes and Gene Prediction Tracks

- FlyBase Genes: pack
- Genscan Genes: hide
- N-SCAN Genes: hide
- D. mel. cDNAs: hide
- TSS Annotations: pack
- tra Isoform: hide

RNA Seq Tracks

- RNA-Seq Coverage: full
- Exon Junctions: hide

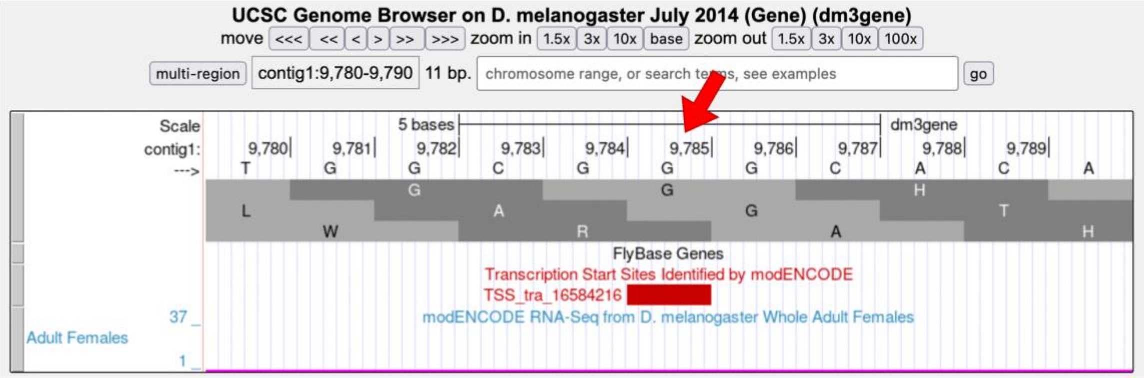
Figure 7 Annotated TSSs in the region surrounding the start of the *tra* transcripts.

Q5. How many TSS sites were identified using this technique?

Q6. Look at the labels next to each of the annotated TSSs. What are the labels for the TSS sites?

2. We will examine each of the annotated TSSs separately to determine their precise coordinates. First, let's zoom in on the feature labeled “**TSS_tra_16584216**” so you have about 11 nucleotides in view. Look at the ruler in the “Base Position” track to determine the coordinate for this TSS (Figure 8).

Q7. What is the coordinate for TSS_tra_16584216?



UCSC Genome Browser on D. melanogaster July 2014 (Gene) (dm3gene)

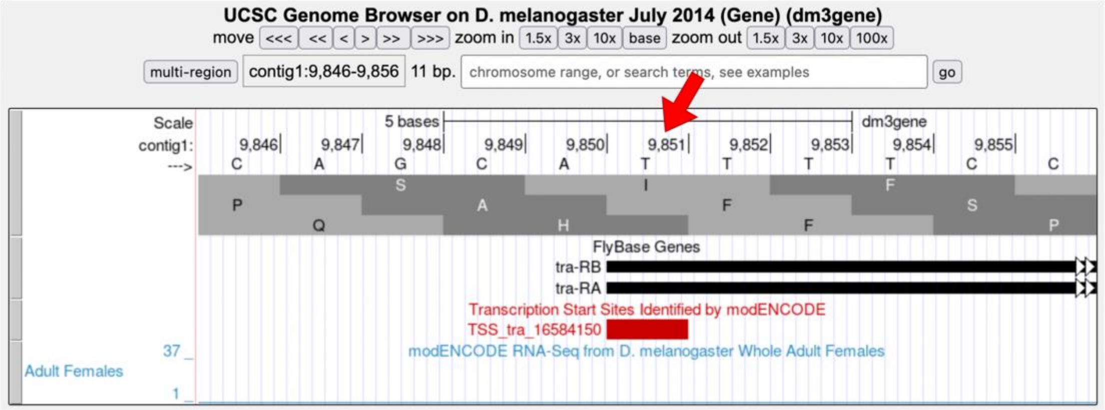
contig1:9,780-9,790 11 bp.

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
multi-region
chromosome range, or search terms, see examples
go
Scale
5 bases
dm3gene
contig1:
--->
9,780 9,781 9,782 9,783 9,784 9,785 9,786 9,787 9,788 9,789
T G G C G G G C A C A
G G H
L A G T
W R A H
FlyBase Genes
Transcription Start Sites Identified by modENCODE
TSS_tra_16584216
37
Adult Females
1
modENCODE RNA-Seq from D. melanogaster Whole Adult Females

Figure 8 Determine the position for the annotated TSS “TSS_tra_16584216”.

3. Now let’s zoom in to the second TSS site, TSS_tra_16584150 (Figure 9).

Q8. What is the coordinate for this TSS?



UCSC Genome Browser on D. melanogaster July 2014 (Gene) (dm3gene)

contig1:9,846-9,856 11 bp.

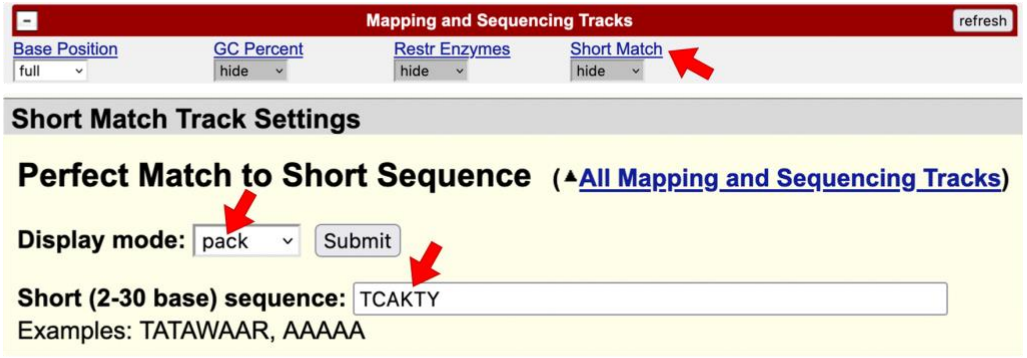
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
multi-region
chromosome range, or search terms, see examples
go
Scale
5 bases
dm3gene
contig1:
--->
9,846 9,847 9,848 9,849 9,850 9,851 9,852 9,853 9,854 9,855
C A G C A T T T T C C
S I F
P A F S
Q H F P
FlyBase Genes
tra-RB
tra-RA
Transcription Start Sites Identified by modENCODE
TSS_tra_16584150
37
Adult Females
1
modENCODE RNA-Seq from D. melanogaster Whole Adult Females

Figure 9 Determine the position for the annotated TSS “TSS_tra_16584150”.

4. We will use the Genome Browser to gather additional evidence to identify the most likely TSS. First, let's search for the Inr motif using the “**Short Match**” functionality under “Mapping and Sequencing Tracks”. Note that we expect this motif to overlap with the TSS (i.e., from -2 to +4

relative to the TSS). The presence of Inr motif in the 5' region of the gene will be our third line of evidence to support the most likely TSS location. Change the "chromosome range, or search terms" field to "**contig1:9,700-9,900**" and then click "go".

5. To learn more about the Short Match functionality, watch the Short Match video. Scroll down to the "Mapping and Sequencing Tracks" section and click on the "Short Match" link. Change the "Display mode" field to "pack" and the "Short (2-30 base) sequence field to "TCAKTY" (Figure 10). Click on the "Submit" button.
    - Note: "TCAKTY" is the consensus sequence for the Inr motif, where K (**K**eto) denotes either G or T and Y (p**Y**rimidine) corresponds to either C or T.



Mapping and Sequencing Tracks

Base Position: full; GC Percent: hide; Restr Enzymes: hide; Short Match: hide

Short Match Track Settings

Perfect Match to Short Sequence (▲All Mapping and Sequencing Tracks)

Display mode: pack

Short (2-30 base) sequence: TCAKTY

Examples: TATAWAAR, AAAAA

Figure 10 Configure the "Short Match" track to search for the Initiator (Inr) motif.

6. Each box in the "Perfect Matches to Short Sequence (TCAKTY)" track corresponds to an instance of the motif. The sign "+" or "–" next to each bar denotes the orientation of the match while the number corresponds to the first base of the motif match.

Q9. Are there any perfect matches to the Inr consensus sequence in the region between 9,700-9,900? What are the coordinates and orientation of these matches?

Q10. Which base position(s) would you assign as the TSS of the *tra* gene based on the available evidence? Describe your reasoning.

Q11. Is there any ambiguity? In other words, do the three lines of evidence (RNA-Seq tracks, TSS as predicted by the modENCODE data, and the Inr consensus sequence location) point to exactly the same position as being the TSS? If they don't, why might they differ? Could there be more than one TSS?

Let's look at a different promoter region. Navigate to the Genome Browser Gateway page by clicking on the Genomes tab at the top of the page and select the *D. melanogaster* "**Aug. 2014 (BDGP Release 6 + ISO1 MT/dm6)**" assembly. Change the "Position/Search Term" field to "**chr2R:18,867,350-18,867,430**" and then click on the "GO" button.

7. Click on "**hide all**" and then enable the following tracks under "Mapping and Sequencing Tracks":
   - Base Position: **full**
   - Short Match: **pack**

8. Search for **TCAKTY**, the Inr consensus sequence.
   - Click on the Short Match Link under "Mapping and Sequencing Tracks."
   - Type "TCAKTY" in the "Short (2-30 base) sequence" field.
   - Click on the "Submit" button.

9. Under "Genes and Gene Predictions Tracks":
   - FlyBase Genes: **pack**

10. Under "Expression and Regulation":
    - TSS (Embryonic) (R5): **pack**
    - Click on a "refresh" button. Record the position(s) and orientation(s) of any matches to the Inr motif.
    - Repeat the search for **TATAWAAR** (the TATA Box motif).

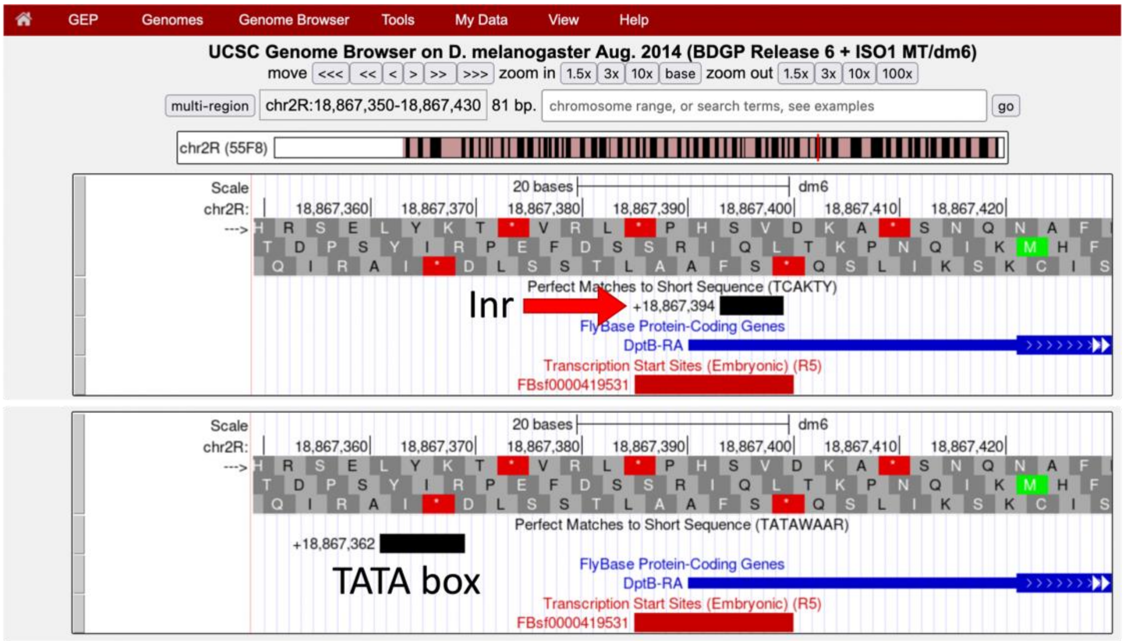

GEP
Genomes
Genome Browser
Tools
My Data
View
Help
UCSC Genome Browser on D. melanogaster Aug. 2014 (BDGP Release 6 + ISO1 MT/dm6)
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
multi-region
chr2R:18,867,350-18,867,430
81 bp.
chromosome range, or search terms, see examples
go
chr2R (55F8)
Scale
20 bases
dm6
chr2R:
18,867,360
18,867,370
18,867,380
18,867,390
18,867,400
18,867,410
18,867,420
Perfect Matches to Short Sequence (TCAKTY)
+18,867,394
Inr
FlyBase Protein-Coding Genes
DptB-RA
Transcription Start Sites (Embryonic) (R5)
FBsf0000419531
Perfect Matches to Short Sequence (TATAWAAR)
+18,867,362
TATA box

Figure 11 Use the "Short Match" track to search for the Inr and TATA box motifs.

Q12. Are there any perfect matches to the Inr consensus sequence (Figure 11)? What are the coordinates and orientation of these matches? What about the TATA Box motif? Are these signals in good agreement with the beginning of the transcription unit?

Investigation 3: Map the 3’ end of the transcription unit

Introduction

After RNA polymerase II has started transcribing a gene (**initiation**), generally with the help of various transcription factors, it will proceed (in a process called elongation) all the way to the termination signal in order to produce a molecule of pre-mRNA. Let’s review what we know about the template, and then consider termination.

RNA polymerase II will use the template DNA to synthesize a primary transcript (pre-mRNA) by pairing purine bases with pyrimidine bases. Actually, the sequence of nucleotides that you observe on the tracks in the Browser corresponds to the “coding strand” of the DNA (complementary to the template strand); the coding strand is almost identical to that pre-mRNA, except that DNA has thymine versus RNA, which has **uracil** as the pyrimidine base that pairs with A.

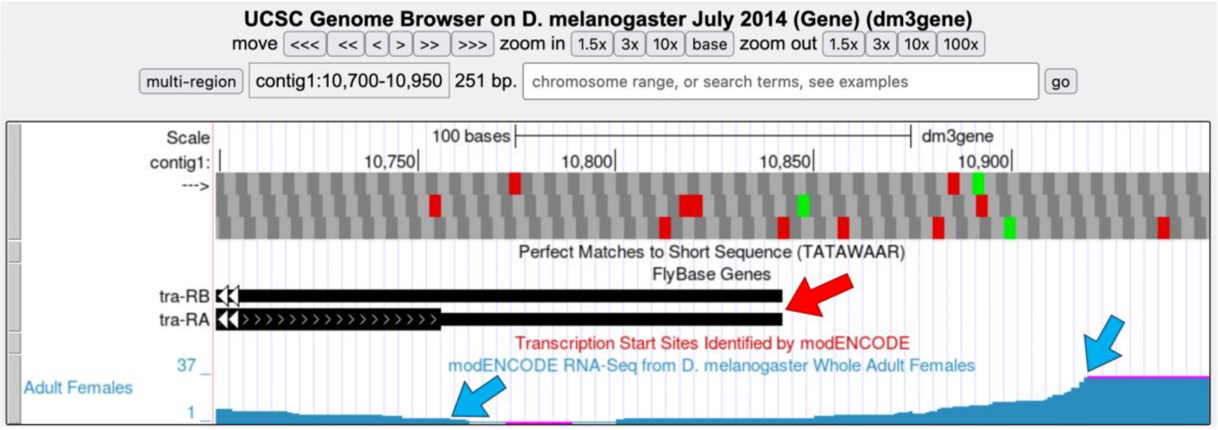
Because DNA is antiparallel, if the coding strand that you see in the browser track runs 5’ to 3’, then the template strand runs in the **3’ to 5’** direction.

RNA polymerase binds to the **promoter sequence on the template strand**, constructing the transcribed mRNA in the **5’ to 3’** direction, just like the coding DNA strand that you see on the tracks. In fact, polymerases can only add nucleotides to the 3’ end (free –OH) of the growing RNA molecule. Termination of mRNA transcription is different in eukaryotes than in prokaryotes. In eukaryotes, RNA polymerase II passes through one or more **AATAAA** sequences, which lie beyond the 3’ end of the coding region (i.e., thick black boxes in the FlyBase Genes track). The pre-mRNA molecule will thus carry the signal AAUAAA[2]. This AAUAAA signal is recognized by a special endonuclease that cuts at a site 11 to 30 nucleotides to its 3’ side. As you will learn in the mRNA processing module, a tail of polyriboadenylic acid, poly(A), is added by a special non-template-directed polymerase to the end of the transcript.

Pre-mRNA processing will be further studied in Modules 3 and 4.

1. We will try to identify the approximate end of the tra-RA transcript using the RNA-Seq data and will then search the DNA sequence for a termination signal (AATAAA). Return to the “July 2014 (Gene)” assembly, change the “chromosome range, or search terms” field to “**contig1:10,700-10,950**” and then click “go” to navigate to the 3’ end of the *tra* gene. Examine the RNA-Seq read density in the “RNA-Seq Coverage” track (Figure 12).

[2] This signal is also referred to as the poly-A signal because a poly-A tail is added to the mRNA at its 3’ end. In the next module we will refer to it as a poly-A signal.



UCSC Genome Browser on D. melanogaster July 2014 (Gene) (dm3gene)

contig1:10,700-10,950 251 bp.

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
multi-region
chromosome range, or search terms, see examples
go
Scale
100 bases
dm3gene
contig1:
10,750
10,800
10,850
10,900
--->
Perfect Matches to Short Sequence (TATAWAAR)
FlyBase Genes
tra-RB
tra-RA
Transcription Start Sites Identified by modENCODE
modENCODE RNA-Seq from D. melanogaster Whole Adult Females
37
Adult Females
1

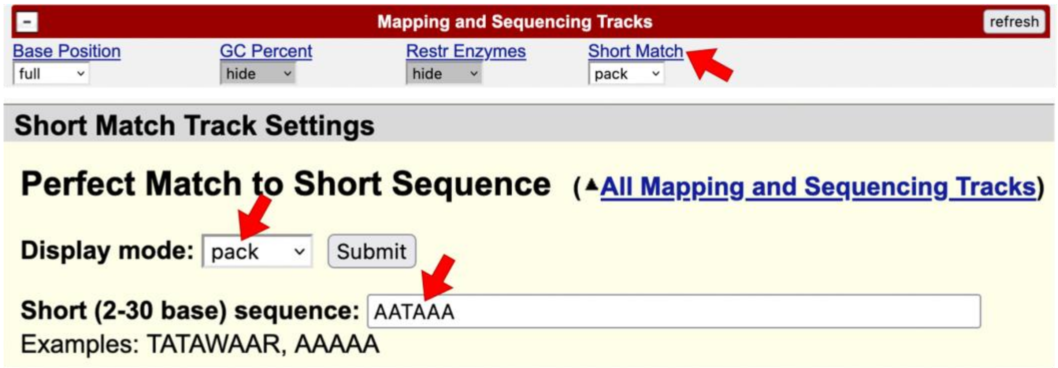
Figure 12 Comparison of the transcription end site annotated by FlyBase (red arrow) versus changes in RNA-Seq read coverage in whole adult females (blue arrows).

Q13. At which base position do you see the RNA-Seq read coverage ending in the whole adult female sample? Zoom in close to the beginning of the pink area (no RNA-Seq coverage) in the RNA-Seq track.

Q14. What is the coordinate of the 3' end of the tra-RA transcript according to the "FlyBase Genes" track? You will need to zoom in on the end of the "FlyBase Genes" track.

You may observe that after decreasing, the amount of RNA-Seq reads in this region starts to increase again, continuing at a higher level to the end of the contig. This is because there is another gene downstream (to the right) very close to *tra*. We can ignore the region (starting at around position 10,900) where the RNA-Seq reads increase.

2. We will now look for a termination signal in this 3' region of the *tra* gene. As we did when searching for the Inr consensus sequence, we can use the "Short Match" functionality to search for the AATAAA sequence.
3. Click on the "Short Match" link under the "Mapping and Sequence Tracks section". Verify that the "Display mode" is set to pack and enter the sequence "AATAAA" into the "Short (2-30 base) sequence" field (Figure 13). Click on the "Submit" button.



Mapping and Sequencing Tracks

Base Position: full

GC Percent: hide

Restr Enzymes: hide

Short Match: pack

Short Match Track Settings

Perfect Match to Short Sequence (▲All Mapping and Sequencing Tracks)

Display mode: pack

Short (2-30 base) sequence: AATAAA

Examples: TATAWAAR, AAAAA

Figure 13 Use the "Short Match" track to search for the mRNA termination signal.

Q15. How many matches are there in the search region (contig1:10,700-10,950)?

Q16. How many of these matches are on the positive (+) strand of the DNA? Remember these sequences, like the Inr consensus sequence we discussed before, are strand specific and your gene is on the + strand.

Q17. Is the sequence(s) you found in the question above contained within the 3’ untranslated region of the transcript? Remember from Module 1 that the thick black boxes in the “FlyBase Genes” track represent coding (translated) regions while the thin black boxes represent non-coding (untranslated) regions.

Q18. Based on your analysis above, which position is the best choice for the termination signal? Describe your reasoning.

Conclusion:

In this lesson, you have seen how the primary transcript (the mRNA molecule) is produced from the template DNA by an RNA polymerase interpreting different signals on the DNA. We saw that DNA sequences upstream of the 5’ end (promoter) and near the 3’ end (terminator) are important parts of the transcription unit. The pre-mRNA molecule from the TSS site to the termination signals will undergo several modifications (processing) in addition to capping that you will learn about in the next few modules.

As discussed above, the reads produced by an RNA-Seq experiment are derived primarily from processed mRNA (not the pre-mRNA). Hence, we can explore several additional questions using the RNA-Seq Coverage track:

Q19. Do you see any correlation between the areas with high RNA-Seq read coverage (high peaks) and the different boxes in the tra-RA isoform? Zoom out 10X to get an overview. Remember that the thick boxes correspond to the coding regions, the thin boxes are the untranslated regions, and the lines with arrows are introns.

Q20. Where do you see regions in the RNA-Seq coverage data with no coverage at all?

Q21. If these regions with no RNA-Seq coverage occur within an initial transcript, what could have happened to these RNA sequences?