An introduction to the gene annotation process, from beginning to end, using a simple example from *Drosophila erecta*

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Overview

The Genomics Education Partnership (GEP) is a national, collaborative, scientific investigation of a problem in genomics, involving wet-lab generation of a large data set (e.g., sequence improvement of genomic DNA) and computer analyses of the data (including **annotation** of genes, assessment of repeats, exploration of evolutionary questions, etc.). Overall, the goal of annotation is to develop gene models for all the genes in a genome. The specific goal of the GEP is to annotate the genomes of several *Drosophila* species, using the genome of *D. melanogaster* as a reference genome. In particular, the GEP is focused on genomic regions in other species that correspond to chromosome four of *D. melanogaster*. Chromosome four is also referred to as the dot chromosome (Figure 1). Thus, the current research problem entails generating finished sequence from the fourth (dot) chromosome of various species of *Drosophila*, annotating these sequences, and making comparisons among species to discern patterns of genome organization related to the control of gene expression. Here we will concern ourselves only with the process of genome annotation.

A brief description of the scientific background of the current research problem

The scientific interest is based on observations that the dot chromosome shows a mixture of heterochromatic and euchromatic properties. The dot chromosome is like **heterochromatin** in that it stains intensely with fluorescent DNA binding dyes, has a high density of repetitious sequences, is late replicating, and exhibits very low meiotic recombination. At the same time, the distal region (i.e the region not near the centromere) of the dot chromosome is amplified in **polytene chromosomes**, which is a property of **euchromatin**, and codes for ~80 genes, a gene density similar to that found in the euchromatic arms. In most genomes, actively-transcribed regions of DNA are typically associated with euchromatic domains, while transcriptionally-silent regions of DNA are generally associated with heterochromatic domains. The control of gene expression is important to many areas of cellular and molecular biology, including the understanding of many human diseases. An understanding of chromosome organization and chromatin effects on dot chromosome gene expression in *Drosophila* will thus shed light on the mechanisms of gene regulation in general. An understanding of these chromatin effects requires careful analysis, not just of the genes present but ultimately of the type and distribution of repetitive elements and non-protein coding regions of the genome. However, we will concern ourselves here primarily with the coding regions.

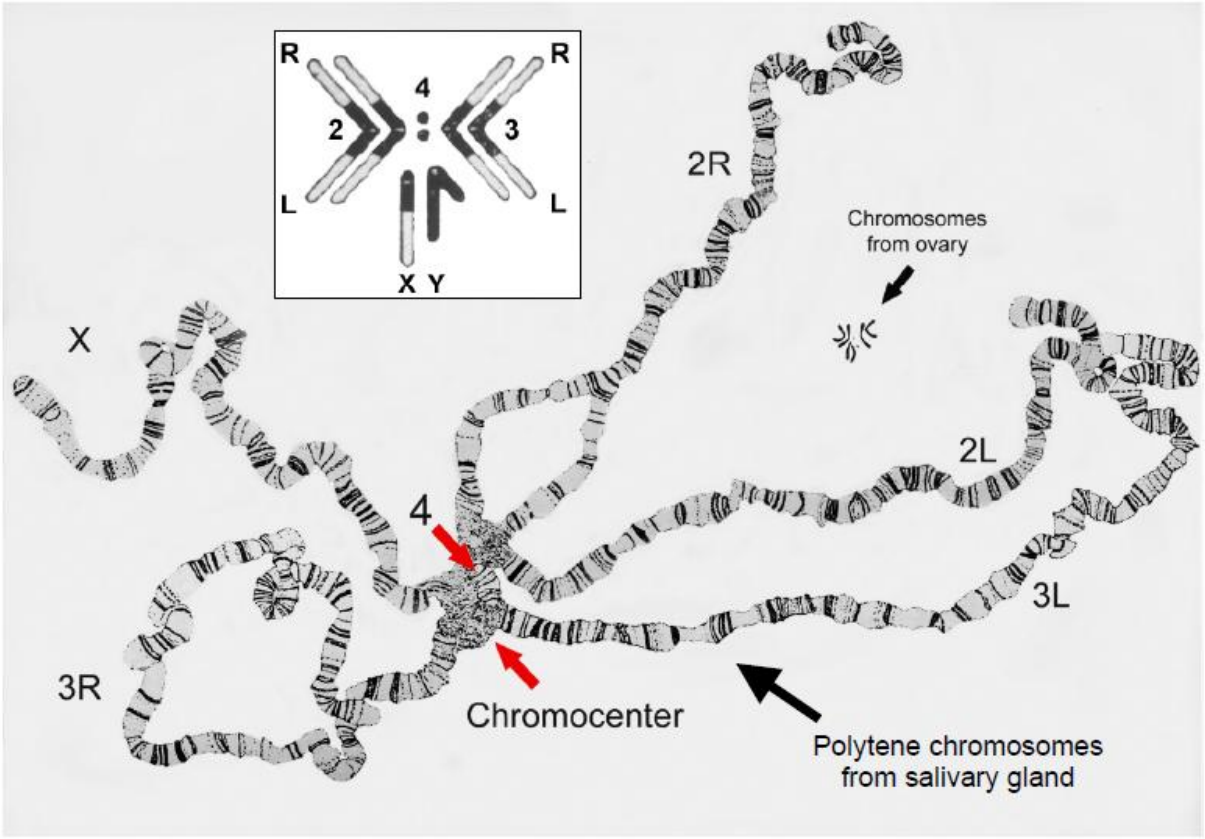


Figure 1. *Drosophila melanogaster* chromosomes (from Painter, 1934; used with permission from Oxford University Press)

What is Gene Annotation?

Gene **annotation** refers to the process of ascribing functions to various parts of a newly sequenced genome. While continually-updated and highly automated technologies are rapidly increasing the number of sequenced genomes, the process of gene annotation is nowhere near as automated and requires careful analysis by trained human annotaters (soon to be you!). In the process of annotation, decisions are made as to which particular DNA sequences within a genome contain biological information. This involves identifying **features** in the DNA sequences and, in many cases, determining which of these features are likely to be **genes.** As you know**,** the expression of protein-coding genes in cells employs mRNA intermediates and the coding regions of most protein-coding genes are interrupted, consisting of coding **exons** and non-coding **introns**. In fact, introns are often much longer than the exons of many genes, and they must be spliced out of the initial RNA transcript. Thus, gene annotation includes careful mapping of all the exon-intron boundaries, to create a gene model that results in the translation of a full-length polypeptide chain. Gene annotation is facilitated by computer programs, which scan the newly-sequenced DNA for **intron splice sites** and **open reading frames**; the programs may also search for **consensus sequences** of known gene regulatory regions (such as **promoters** and **enhancers**), which dictate when and where a gene is transcribed into RNA (Figure 2). Human annotators combine these (often contradictory) computational predictions

with sequence alignment and gene expression data (e.g. RNA-seq data) to create the best-supported gene model.

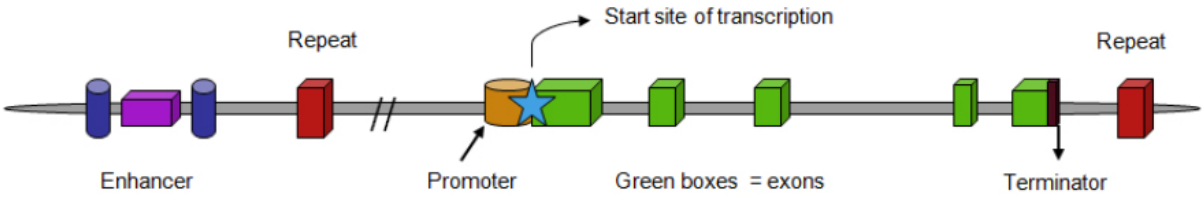


Figure 2. Diagram of a single, hypothetical eukaryotic gene. Some of the rectangles code for amino acid sequences of a protein (the exons), others contain regulatory information (promoters and enhancers), and some areas are transcribed but not translated (introns between the exons).

GEP members use a comparative genomics approach to gene annotation, comparing new genomic DNA sequences (and mRNA sequences, if available) from various *Drosophila* species to known reference DNA and mRNA sequences from *Drosophila melanogaster*. A discussion of the various rules and criteria that are used to generate gene models can be found in the **Annotation instruction sheet** on the GEP website. A copy of this document will be provided to you as a reference for future annotation projects. The following glossary of terms is also provided for your reference

Glossary of terms commonly used in the process of gene annotation

***ab initio* gene finding:** process whereby genomic DNA sequence alone is systematically searched for certain tell-tale signs of protein-coding genes (as opposed to experimental evidence in the form of an identified mRNA or protein molecule encoded by that DNA sequence

Chromatin: the DNA-protein complex found in eukaryotic chromosomes

***Euchromatin*:** chromatin that is diffuse and non-staining during interphase; may be transcribed.

***Heterochromatin*:** chromatin that retains its tight packaging during interphase; often not transcribed

Coding DNA Sequences (CDS): the subset of exon sequences that are translated into protein
cDNA (complementary DNA): a DNA copy of an mRNA molecule, manufactured using the enzyme **reverse transcriptase**

Consensus sequence: the most common nucleotide (or amino acid) at a particular position after multiple, related sequences are aligned and similar functional sequence motifs are found.

Contig: A region of **contig**uous sequence containing genes to be annotated.

Exons: gene sequences that are transcribed into RNA and are present in the mature (spliced) mRNA
molecule

Expressed Sequence Tag (EST): partial, single (e.g., one shot) sequence read of a cDNA molecule. The sequence is of relatively-low quality, usually 500 to 800 nucleotides in length.
Feature: any region of defined structure/sequence in a genomic fragment of DNA. Features would include genes, pseudogenes and repetitive elements. Most people are interested in identifying the protein-encoding genes.

Fosmid: a cloning vector, which has been manufactured to accept DNA inserts of ~40,000 base pairs (bp) [normal plasmids are able to carry only 1-20 kb]; usually propagated in *E. coli*, which can each only contain one fosmid

Homologous genes: genes that have similar sequences because they are evolutionarily related; there are two different types of homology

Orthologs: related genes in different species, which are derived from the same gene in a common ancestral species

Paralogs: related genes within a species, which have arisen by a duplication event

Introns: gene sequences that are transcribed into RNA but are removed during splicing

Isoform: any of several different forms of the same protein. Isoforms may be produced from different alleles of the same gene, from the same gene by alternative splicing, or may come from closely- related genes.

Open Reading Frame (ORF): a segment of the genome that potentially codes for a polypeptide chain (or part of a polypeptide chain). Also the part of an mRNA molecule that potentially codes for a polypeptide. ORFs are located between the start codon (ATG; AUG in the mRNA) and a stop codon (TAA, TAG, TGA; UAA, UAG, UGA in the mRNA) for translation. There are six possible reading frames for any potentially protein-encoding genomic DNA fragment: three on one strand of and three on the other strand of DNA heading in the opposite direction (Figure 3).

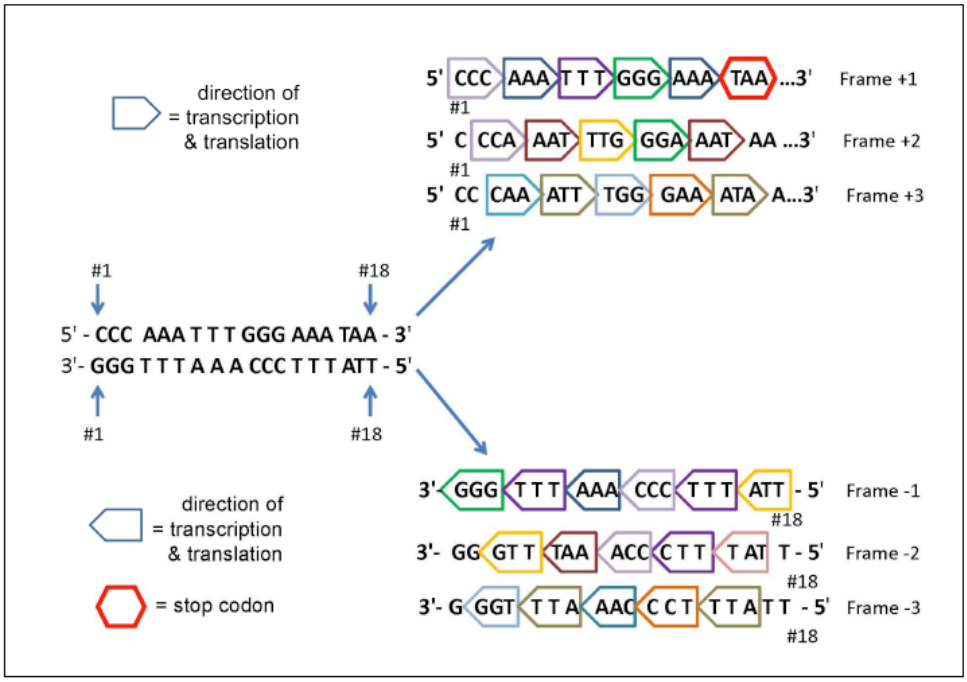


Figure 3. The six possible reading frames for any region of DNA.
Polytene chromosomes: giant chromosomes that form when multiple rounds of DNA replication occur and the sister chromatids remain attached to and aligned with each other

Splice donor: The sequence at the beginning of an intron, also called the 5' splice site. This typically has the sequence GT in DNA and GU in RNA.

Splice acceptor: The sequence at the end of an intron, also called the 3' splice site. This typically has the sequence AG in DNA and in RNA.

5' Untranslated Region (5' UTR): mRNA sequence between the 5' cap and the start codon for translation

3' Untranslated Region (3' UTR): mRNA sequence between the stop codon for translation and the poly A tail

A sample annotation project of a simple gene for *D. erecta*

The gene selected for annotation is from the *Drosophila erecta* genome, and, therefore, is relatively easy to annotate. That is, the sequence will be quite similar to that of *D. melanogaster*, so the features of the gene, such as the start codon, the intron/exon boundaries and the stop codon, should be easily recognizable. Also, this gene is well characterized in *D. melanogaster* and thus has an easily identifiable name and function (as opposed to a 'CG' gene that has been identified based simply by computer-based predictions). Keep in mind that other genes from *D. erecta* and especially genes from more distantly related species will present additional problems for annotation. Guidance for dealing with these more advanced problems can be found in other materials available on the GEP website. However, the objective here is to familiarize the student with the various tools (listed above) and basic strategies involved in the overall process of determining a gene model by annotation, checking the gene model, and submitting the result to GEP. This process can be summarized as follows, and this is the basic sequence of events covered in this document.

1. Identify and get an overview of the region to be identified in the UCSC browser mirror. The overall region is typically referred to as a fosmid or a contig, depending on the exact region. The region will consist of approximately 40,000-50,000 base pairs of DNA.
2. Download the complete DNA sequence of the fosmid or contig. This sequence will be saved as a text file and used for various BLAST searches as the region is annotated.
3. Identify the specific *D. erecta* gene to be analyzed. Ultimately, every gene from the region will be annotated, but we need to start with one.
4. Identify the ortholog *in D. melanogaster*.
5. Find the gene record of the ortholog from *D. melanogaster* using Gene Record Finder. In most cases, several versions, or is forms, will be found for the gene. We will need to annotate all is forms for each gene.
6. Compare the exon sequences from Gene Record Finder to the downloaded fosmid (or contig) DNA sequence. This is the primary method of mapping specific features of the gene (start codon, intron/exon boundaries and stop codon). This process results in a gene model consisting of a series of coordinates that correspond to the various features.
7. Use the coordinates determined in step 6 to verify the gene model using the program called Gene Model Checker.
8. Once the gene model is accepted by gene model checker, various additional checks need to be made. These include:
    1. Comparing the peptide sequence predicted by your gene model to the *D. melanogaster* peptide sequence using BLAST.
    2. Generating a 'dot plot' of the above comparison. This is a graphical representation of the alignment and is useful for identifying trouble spots.
    3. Generating files needed for the final GEP submission. These files include GFF file, a transcript file and a peptide file. These files are generated by the Gene Model Checker and need to be downloaded and submitted to GEP.
9. Complete the GEP report. This document needs to be filled out for each isoform annotated and includes all of the information described above.
10. Submission of the report using the GEP project management system.

These steps are summarized graphically in the following Figure.

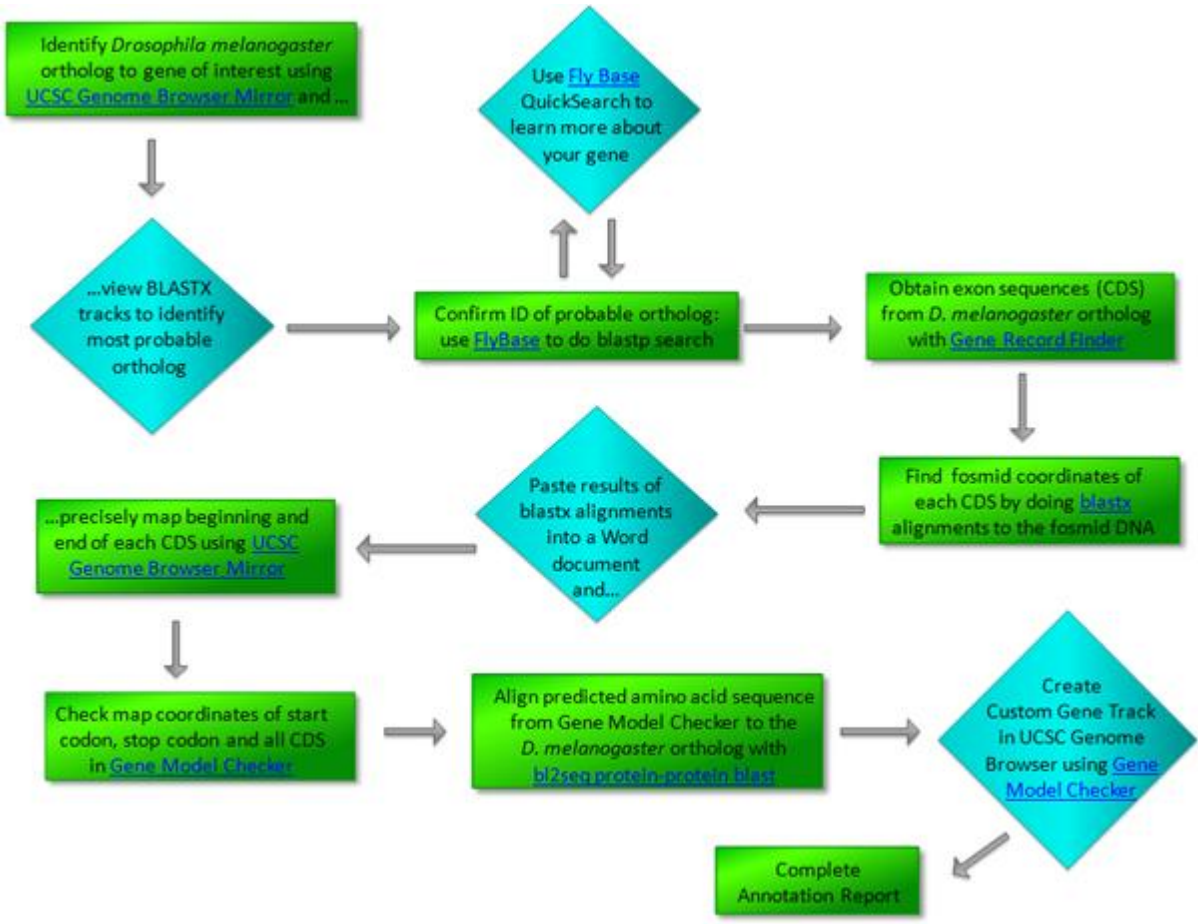


Figure 4.. Summary of steps involved in gene annotation using GEP annotation tools Reproduced from Emerson et al. 2012.

Web based annotation tools

The four major web based tools we will use are listed below. You should open each site in a separate tab in your web browser and try to keep them open as you complete this tutorial.

1. The GEP version of the UCSC genome browser: http://gander.wustl.edu/

2. The NCBI web site for using BLAST: www.ncbi.nlm.nih.gov/blast.

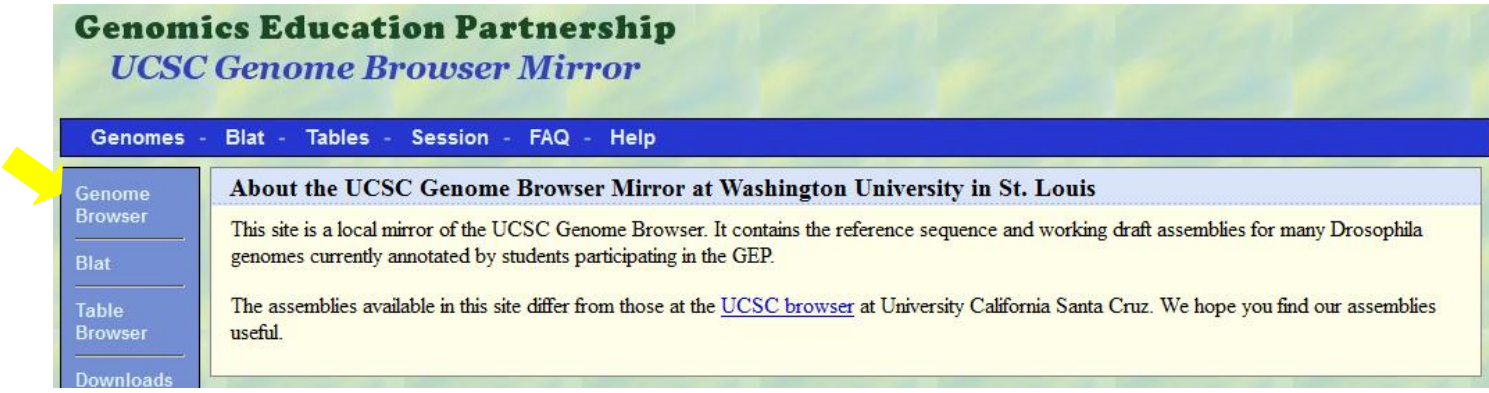
3. The Gene Record Finder tool: http://gander.wustl.edu/~wilson/dmelgenerecord/index.html

4. The Gene Model Checker tool: http://gander.wustl.edu/~wilson/genechecker/index.html

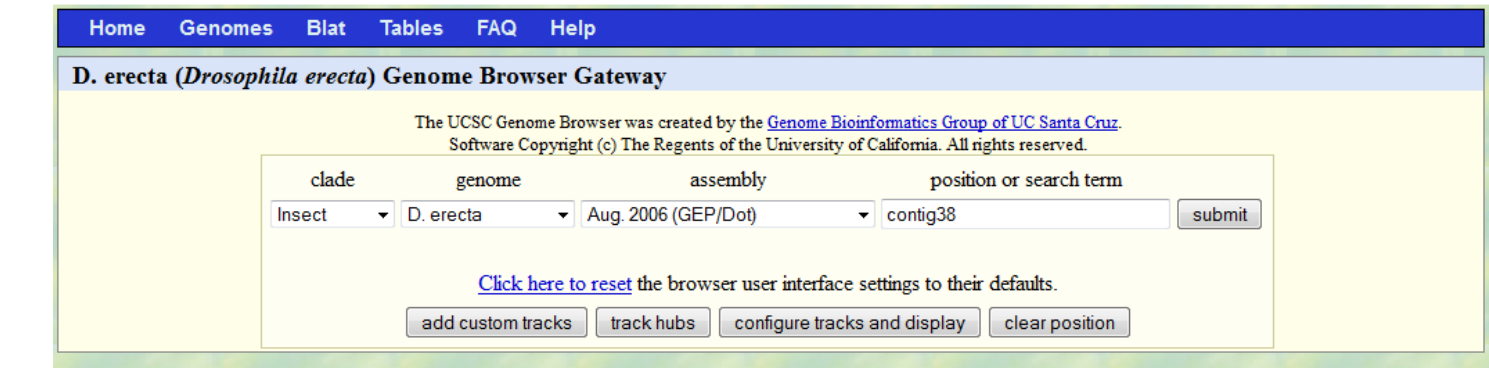
Becoming familiar with the UCSC genome browser

The UCSC (University of California-Santa Cruz) browser is a centralized source for analyzing sequences from a variety of genomes, including the human genome. We are using the GEP version of the UCSC browser, which organizes the specific *Drosophila* genome sequences we need for easier access. The GEP version can be accessed directly at gander.wustl.edu, but we will access it through the GEP website.

We will first explore the various features of the genome browser, then focus on one gene and complete the annotation and submission process for that gene. The gene we will use as an example is contained in contig38 of the *Drosophila erecta* genome sequence. This can be found through the GEP website by navigating to the following page: From the GEP site (www.gep.wustl.edu), click on **projects**, then **annotation resources**, then **GEP UCSC genome browser mirror**. This should bring you to the following screen. From here, click on **Genome Browser** (in the left hand column).



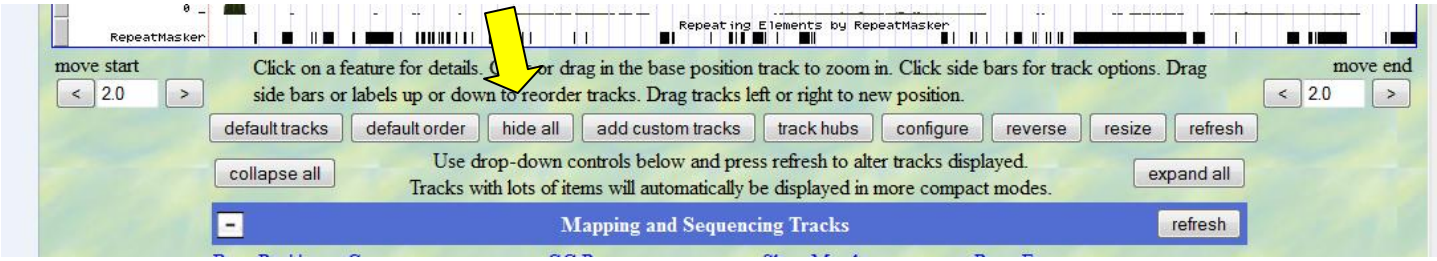
Once at the genome browser, navigate to contig38 of the *D.erecta* genome by setting the drop down windows as follows.



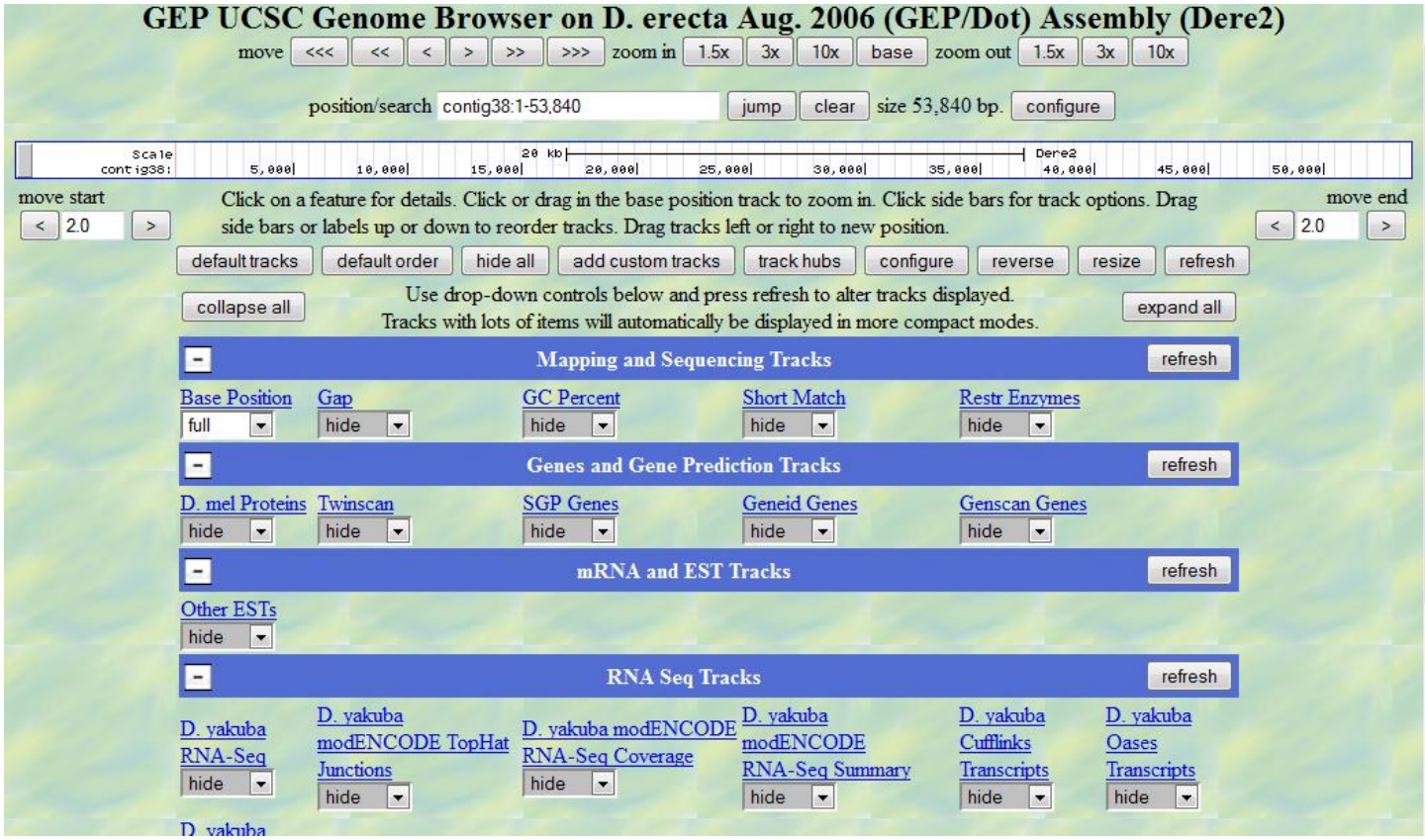
Click **submit.**

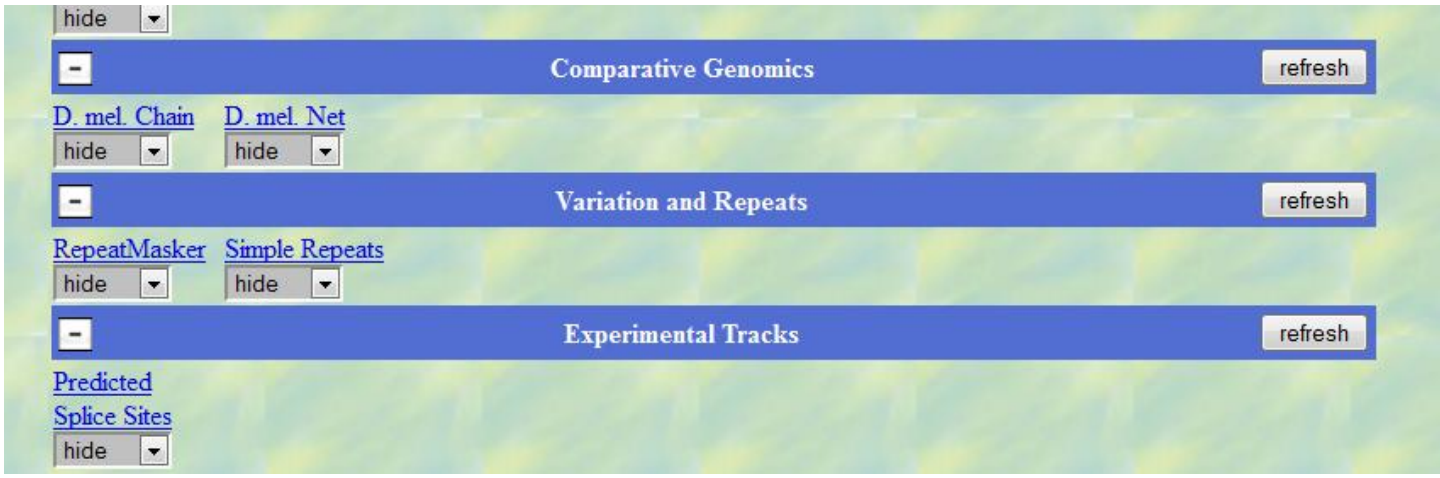
This will bring you to a screen that represents contig38. The exact appearance of this screen may vary depending on what features are selected. An example of this screen is shown below. First

notice the 'position/search' box. It should read 'contig38: 1-53,840. This tells us that the complete sequence for this region is 53,840 base pairs. To make sure we're all starting at the same place, scroll down a bit and click on 'hide all'.



Once you select 'hide all', the screen should look like that shown below. You'll probably need to scroll up and down to see everything. A description of this page is given below the figure.





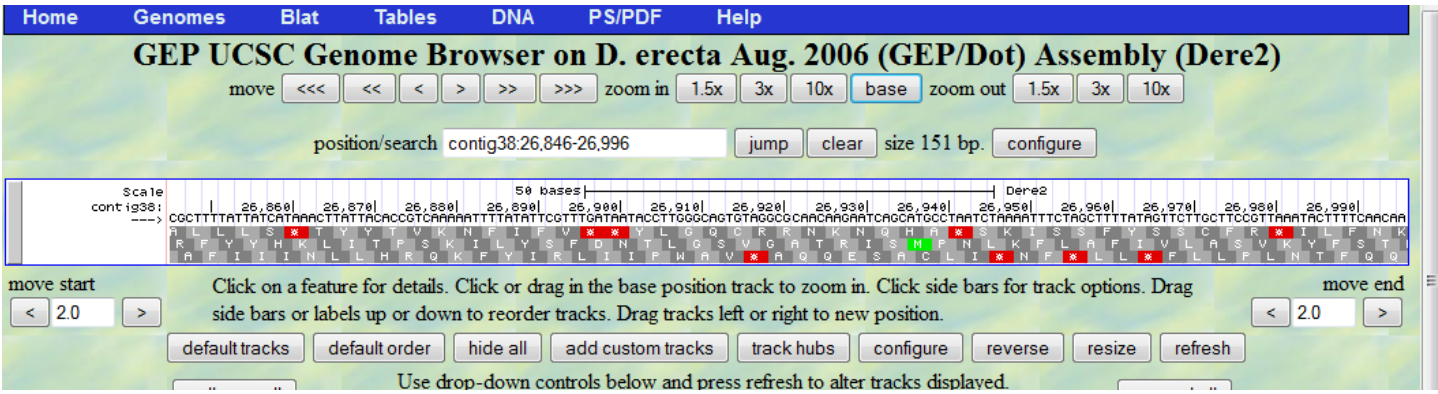
This UCSC genome browser page shows several categories of information pertaining to this genomic region. Within each category are several 'tracks' of information. We can select which tracks we want to display using the various dropdown menus. The display options for each track are: hide, dense, squish, pack, and full. These terms represent various amounts of information to display from the least (hide) to the most (full). You will need to play around with these to get an idea of what is displayed in each case. As we continue through this walkthrough we will tell you which tracks are most important for the task at hand. *Note - each time you change the settings of the drop down menus you need to click 'refresh' in order for those changes to be displayed.*

Here we give a general description of the various tracks. **You can also click on any of the hyperlinks in the browser itself to read a more thorough description of each track. Try this now. Click on the hyperlink (the blue underlined text) for 'base position' and read the description of the information contained in this track.**

The various information tracks are:

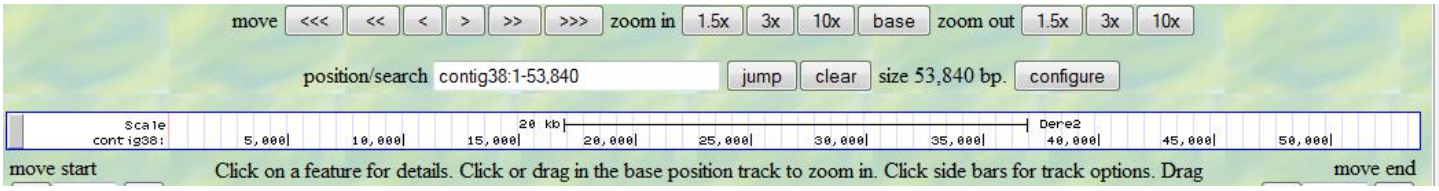
Mapping and sequencing tracks-

For this one we will mainly use the 'base position' menu. We will usually keep this set to full. You can also zoom to the base level to see the actual DNA sequence and the various translated reading frames. To do this, scroll to the top of the page, find where it says 'zoom 1.5X, 3X, 10X, and base'. Click on 'base'. The display screen should now look like this:

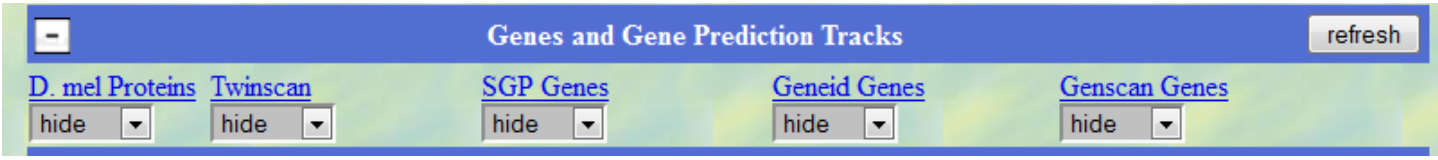


Now you can see the DNA sequence (starting with CGCTTTTA) and three possible reading frames translated from this sequence. The green M's represent start codons and the red asterisks represent stop codons. We'll be discussing reading frames more as we go.

Also notice that by zooming to the base level we have changed the amount of the sequence represented. Notice that the position/search window now reads contig38:26,846-26,996. We need to reset this to the full contig before we proceed. To do this, either click zoom out 10X several times until it reads 1-53,840, or simply type in contig38 (don't type the colon) and hit jump. That should bring you back to this screen:



Genes and gene prediction tracks:



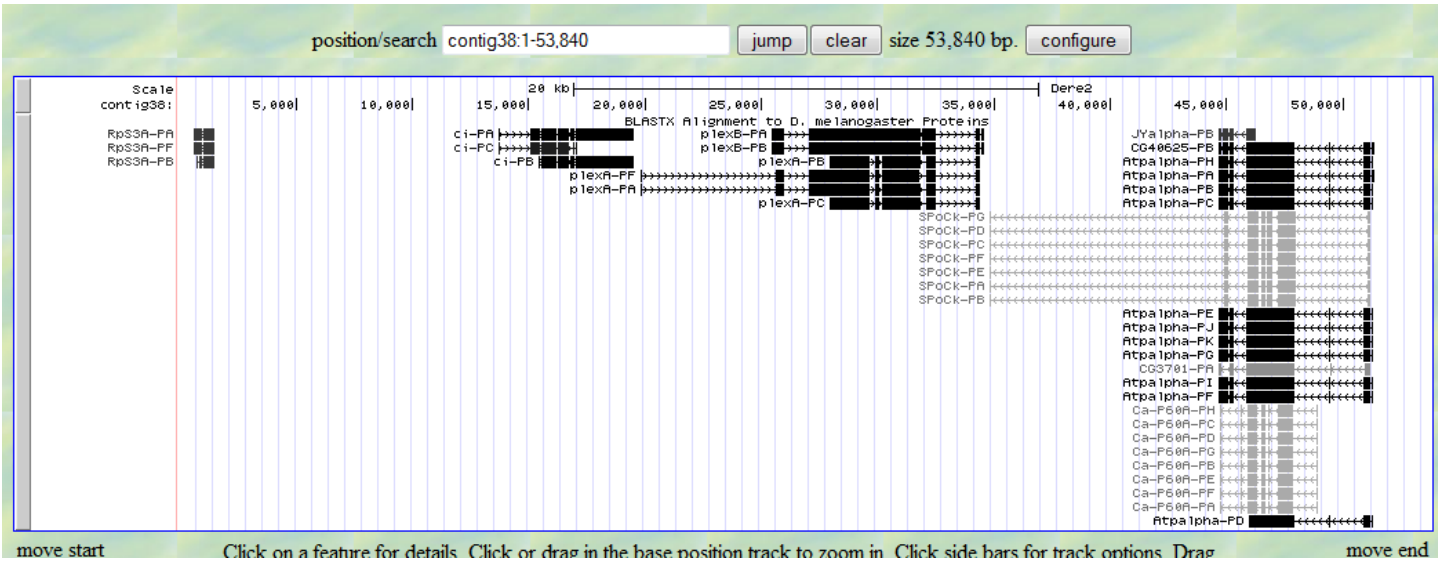
This category contains several tracks, each of which represents a different method for predicting genes in the region.

These tracks are:

D. mel proteins - This track shows potential genes based on a BLASTX comparison of the *D. erecta* contig38 to known *D. melanogaster* proteins. Recall that BLASTX translates a given DNA sequence, in this case contig38, into six possible reading frames and then compares the predicted proteins to known proteins contained in a database (in this case the *D. mel* protein database.) Only three possible reading frames are shown. These are based on the DNA sequence being transcribed and translated from left to right and are referred to as the +1, +2, and +3 reading frames. It's possible that the DNA sequence is actually transcribed and translated

from right to left, these possible reading frames are called -1, -2, and -3. If we need to we can reverse the DNA sequence by clicking the reverse arrow, but we'll deal with that when the time comes.

Change the D.mel protein menu to the various levels (hide, dense, squish, etc) to see the various amounts of information that can be displayed. Each time you change the menu, click refresh to see the change. Now, set the menu to 'pack' and click refresh. Before moving on, make sure your screen looks like this:



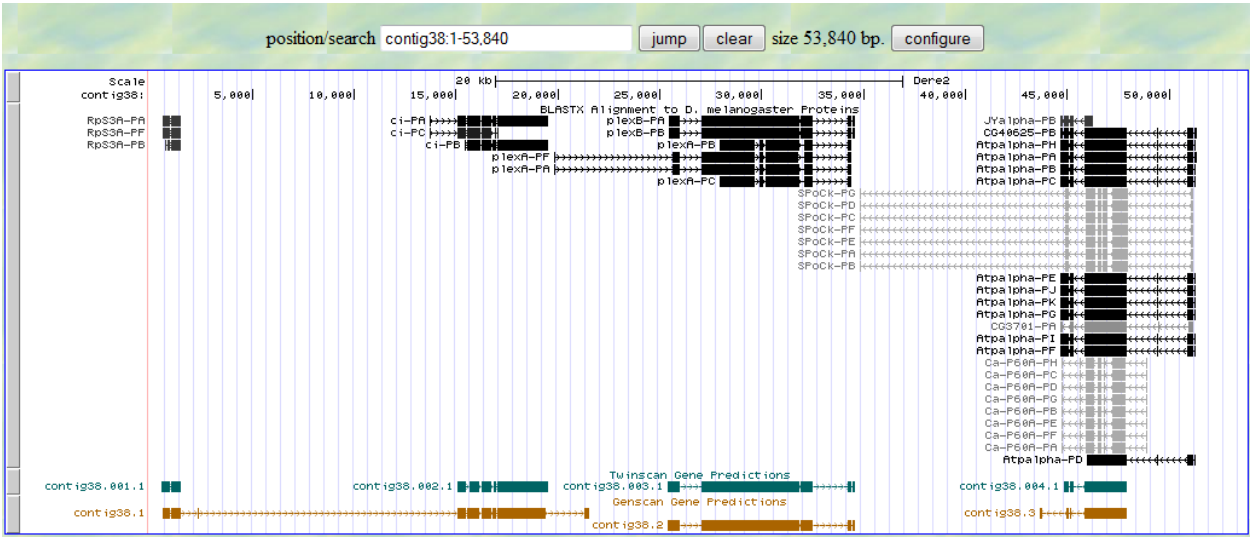
Ok, now we're getting some information. Each set of black or gray bars represents a BLASTX 'hit'. In other words each set of bars represents a gene from *D. melanogaster* that shares sequence similarity with a predicted protein from contig38 as determined using the BLASTX program. This information is very useful since the *D. melanogaster* genome is very well characterized and annotated in detail, therefore we can identify important regions (e.g. genes) in other drosophila genomes by comparing their genomic sequences to the *D. melanogaster* genome. The black bars represent highly similar BLASTX hits, and the gray bars represent genes of lower similarity.

The names of the *D. mel* genes are shown to the left of the BLASTX hits. For example the hits to the far left are labeled RpS3A-PA, RpS3A-PF, RpS3A-PB. The abbreviation RpS3A is the abbreviation for the gene. The PA, PF, and PB suffixes designate different isoforms of the gene. Recall that isoforms are different products of the same gene. For example two mRNAs that are generated by alternative splicing would be two isoforms of that gene.

Notice that the sequence to the right matches several different genes (JYalpha-PB, CG40625-PB, etc). When actually annotating a gene there are a few methods we can use to identify the best candidate for the real ortholog of the gene, but we will focus on a simple case here.

SO ... Based on what you see so far, how many genes, and how many isoforms of each, are contained within this sequence? What are the names of these genes and isoforms?

Twinscan, SGP genes, Geneid genes, and Genscan genes: These tracks all represent different computer-based gene predictions. These types of programs are sometimes referred to as *ab initio* (*ab initio* means 'from the beginning') gene predictors, and essentially predict gene features by searching the DNA sequence for recognizable gene elements (start codons, potential splice sites, etc.). The exact methods of prediction are beyond the scope of this tutorial, but you should consider these tracks as potential evidence to support your final gene model. We will focus on the Genscan gene predictor for this example, and mostly use Genscan for our annotation purposes. It's also important that these predictions are completely independent from the BASTX hits. As we accumulate evidence for a gene model, having evidence from different sources strengthens our case. To see a couple of gene predictor results set the Genscan and Twinscan tracks to 'pack', to get this screen:



The two types of gene predictions appear at the bottom of the screen. The thick bars represent exons and the thinner lines represent introns. Note that there is general agreement between the exons predicted by the two methods. However, the Genscan program predicts three genes called contig38.1, contig 38.2, and contig 38.3 and Twinscan predicts four genes. Also notice that the Genscan program has combined two genes identified by BLASTX (RpS3A and ci) into one gene called contig38.1. Although we don't yet know which of these scenarios is correct, gene predictors often make these kinds of mistakes. Part of your job as an annotator is to resolve these types of issues and provide evidence to support a final gene model.

mRNA and EST tracks: This track displays alignments of expressed sequence tags (ESTs) from species other than *D. erecta* (the reason other species are used is because the data isn't available for *D. erecta*). ESTs are single-read sequences from cDNA copies of mRNAs, and therefore usually represent fragments of transcribed genes. So ESTs are evidence that a particular region of the genome is transcribed into RNA, which is evidence of a gene in that

region. Gene expression data such as these are useful for resolving conflicting evidence from other sources; however, we will reserve further discussion of this for more complex cases.

RNA seq tracks: This track was created by mapping *D. yakuba* mRNA-Seq reads (generated by the modENCODE project) against the *D. erecta* contig sequences. RNA-seq refers to large scale next generation sequencing (NGS) methods to sequence all expressed RNAs. This is another set of data reflecting gene expression and so can be useful when trying to identify specific genes and specific exons of genes. Again, we will reserve further discussion of this for more complex cases.

Comparative genomics: *D. mel* net: This track shows the best *D. melanogaster*/*D. erecta* comparison for every part of the *D. erecta* genome. It is useful for finding orthologous regions and for studying genome rearrangement. While we won't be using these directly in our annotation process, we will include this track in our final screenshot for our final report for further analysis by the GEP.

A discussion of **"Variation and repeats"** tracks is beyond the scope of this tutorial.
Experimental tracks: The 'predicted splice site' track displays splice site predictions generated by the program GeneSplicer. It is often useful in deciding between potential splice sites when generating a gene model. We will use this later in this tutorial as we annotate one of the genes contained in this contig.

The process of gene annotation

For the purposes of this tutorial we will describe the annotation of a gene from *Drosophila erecta.* Again, it is important to realize that we have chosen a simple example in order to familiarize the student with the steps involved from the beginning to the end of the annotation for one gene. Actual annotation projects, particularly of genes from more distantly related species will present more complicated problems that will require additional approaches to resolve. There is a wealth of information available on the GEP website to help address some of these advanced problems. The goal of this tutorial is for students to go through the entire annotation process using a relatively simple example in order to get an overview of the tools and procedures involved.

Although there is no one way to annotate a gene, we will start with one particular approach and modify it as necessary when particular issues arise. In brief, this approach, as applied to *D. erecta* genes is to

1. Identify and get an overview of the region to be identified in the UCSC browser mirror.
2. Download the complete DNA sequence of the fosmid or contig.
3. Identify the specific *D. erecta* gene to be analyzed. Ultimately, every gene from the region will be annotated, but we need to start with one.
4. Identify the 'ortholog' *in D. melanogaster*.
5. Find the 'Gene Record' of the ortholog from *D. melanogaster* using 'Gene Record Finder'. In most cases, several versions, or isoforms, will be found for the gene. We will need to annotate all isoforms for each gene.

6. Compare the exon sequences from Gene Record Finder to the downloaded fosmid (or contig) DNA sequence.
7. Use the coordinates determined in step 6 to verify the gene model using the program called Gene Model Checker.
8. Once the gene model is accepted by gene model checker, various additional checks need to be made. These include:
    1. Comparing the peptide sequence predicted by your gene model to the *D. melanogaster* peptide sequence using BLAST.
    2. Generating a 'dot plot' of the above comparison. This is a graphical representation of the alignment and is useful for identifying trouble spots.
    3. Generating files needed for the final GEP submission. These files include GFF file, a transcript file and a peptide file. These files are generated by the Gene Model Checker and need to be downloaded and submitted to GEP.
9. Complete the GEP report-ugh!
10. Submit the report using the GEP project management system - yay!

Sample Annotation

Open each of the following web pages in a different tab in your browser

1. The GEP version of the UCSC genome browser: http://gander.wustl.edu/

2. The NCBI web site for using BLAST: www.ncbi.nlm.nih.gov/blast

3. The Gene Record Finder tool : http://gander.wustl.edu/~wilson/dmelgenerecord/index.html

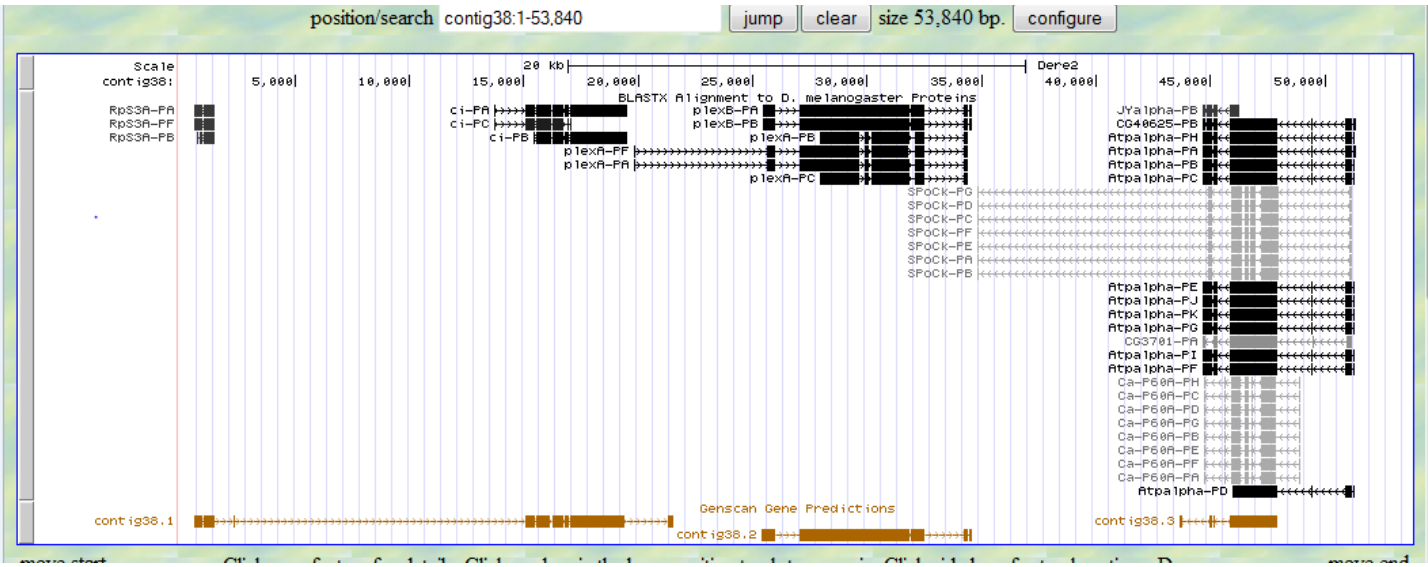
4. The Gene Model Checker tool: http://gander.wustl.edu/~wilson/genechecker/index.html

1. Inspect the various evidence tracks of the UCSC browser to identify potential genes in a particular contig of the D. ercta *genome.*

We will annotate one of the genes contained in contig38. So navigate to the appropriate region of the UCSC browser. Forgot already? Check your notes. You should be able to get to the following screen.

Click **submit**.

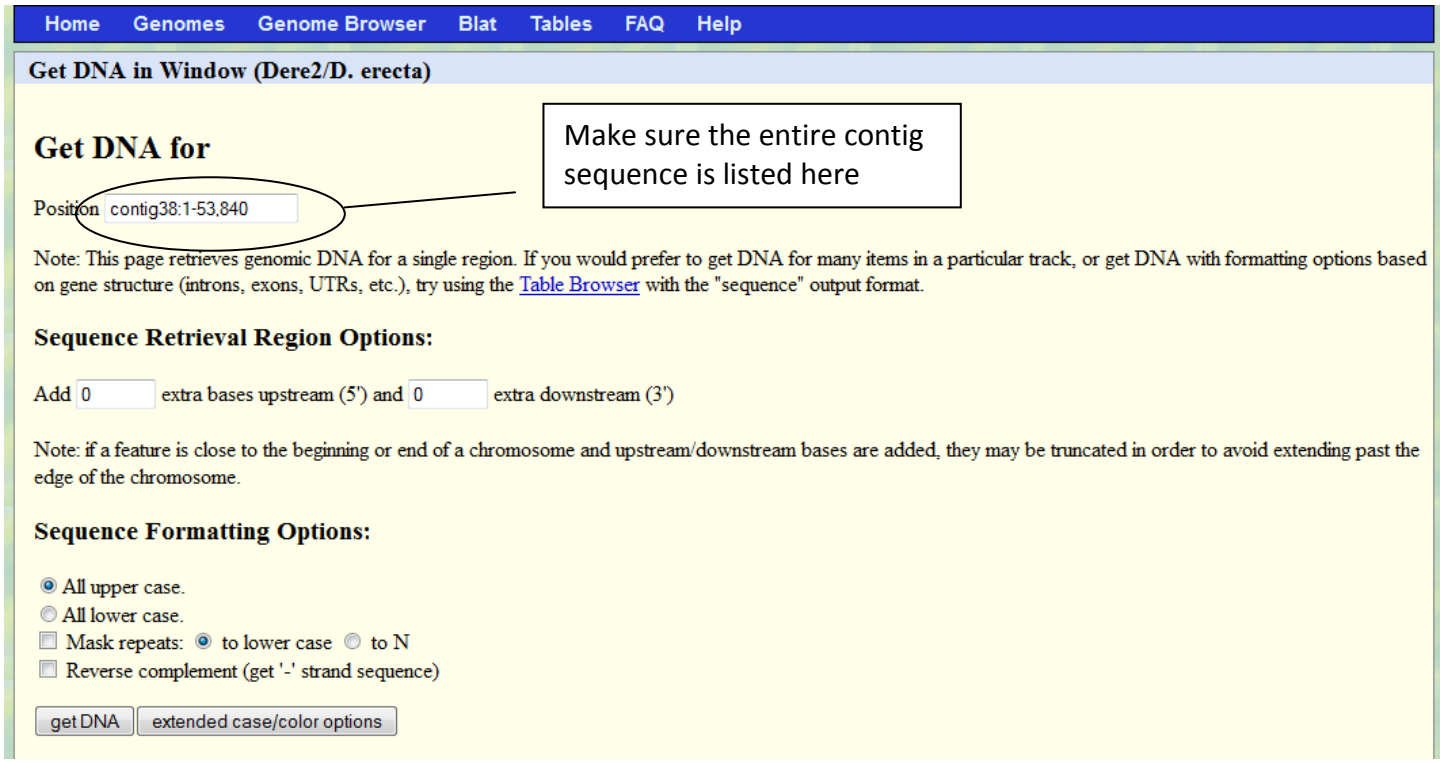
Adjust the various evidence tracks so that the screen appears as below



We will annotate the gene identified in the BLASTX search as ci. Because only one gene has been identified in the automated BLASTX search we will assume this is the ortholog. In many cases, additional steps will be required to convince yourself of the appropriate ortholog. For example, there are several hits to the rightmost gene in this fosmid and some investigation would be required to identify ortholog. From initial inspection of the BLASTX hits for ci, it appears that there are three isoforms of this gene. We will need to annotate each isoform in detail.

2. Download the complete DNA sequence of the fosmid or contig. We will download the entire contig 38 sequence from the UCSC browser page shown above. **IMPORTANT**: Make sure that the position/search window includes the entire DNA sequence (in this case bases 1-53,840).

Click on 'DNA' at the top of the page to get the following screen.

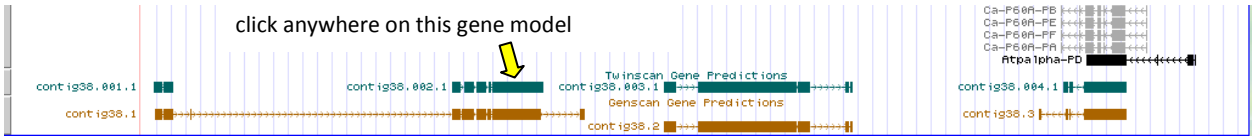


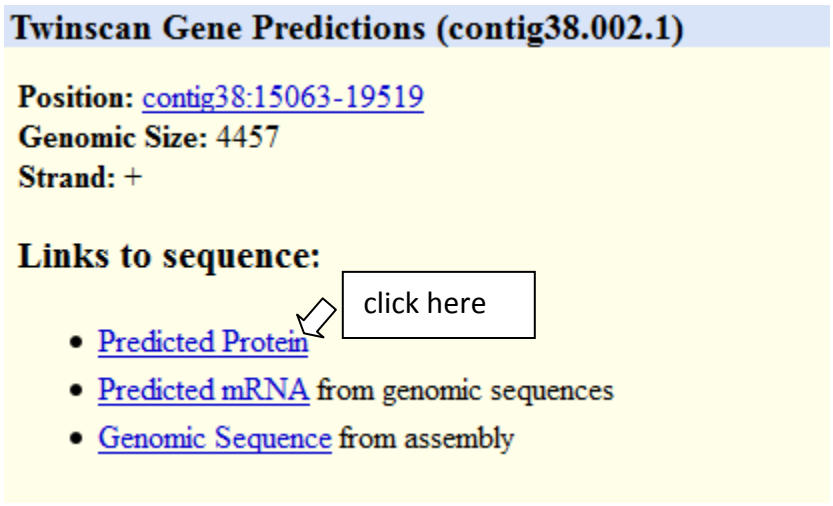
Click on 'get DNA'. This will lead to a screen with the entire 53,840 DNA sequence. Click inside the text box and hit 'ctrl A' on your keyboard to select the entire sequence. Hit 'ctrl C' to copy the entire sequence, including the first line that reads >Dere2_dna range=contig38:1-53840 5'pad=0 3'pad=0 strand=+ repeatMasking=none.

Open a notepad (or other plain text application) document (find notepad using the windows menu). Paste the entire DNA sequence into the notepad document and save it as contig38sequence.txt.

3. Identify the specific D. erecta gene to be analyzed. Ultimately, every gene from the region will be annotated, but we need to start with one. We will start with the blastX hit to the ci gene. It is relatively small and simple, but also has a few isoforms. It also has an interesting name and a well characterized function.

4. Identify the ortholog in D. melanogaster. The blastX hit shown in the browser is probably 'correct' in identifying the D. mel ortholog. However, we will independently confirm this by investigating one of the gene predictions. In this case, we will use the Twinscan prediction because it doesn't have the complication of being fused with another gene. The basic strategy here is to get the protein sequence predicted by Twinscan and use it in a blastp search of D. melanogaster proteins. To get the protein sequence click on the Twinscan prediction itself:

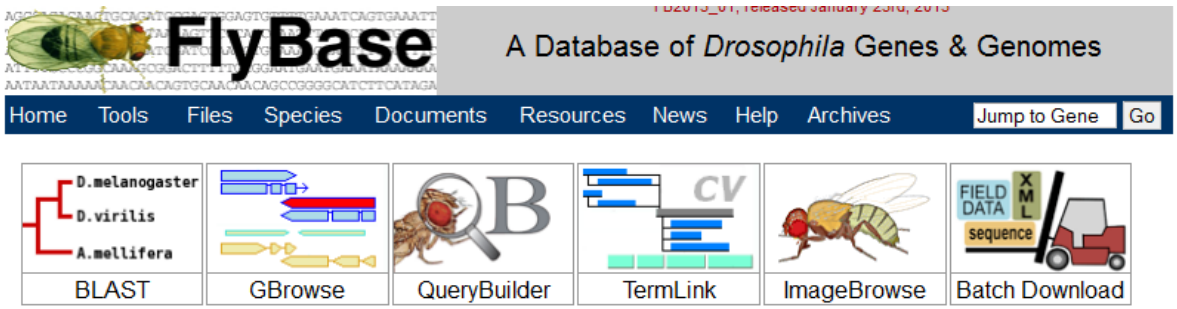




This brings up the following sequence, which is the protein sequence of the predicted Twinscan gene. This is in FASTA format. The > symbol designates this first line of the file as the name of the sequence. Select and copy this entire sequence, including the first line.

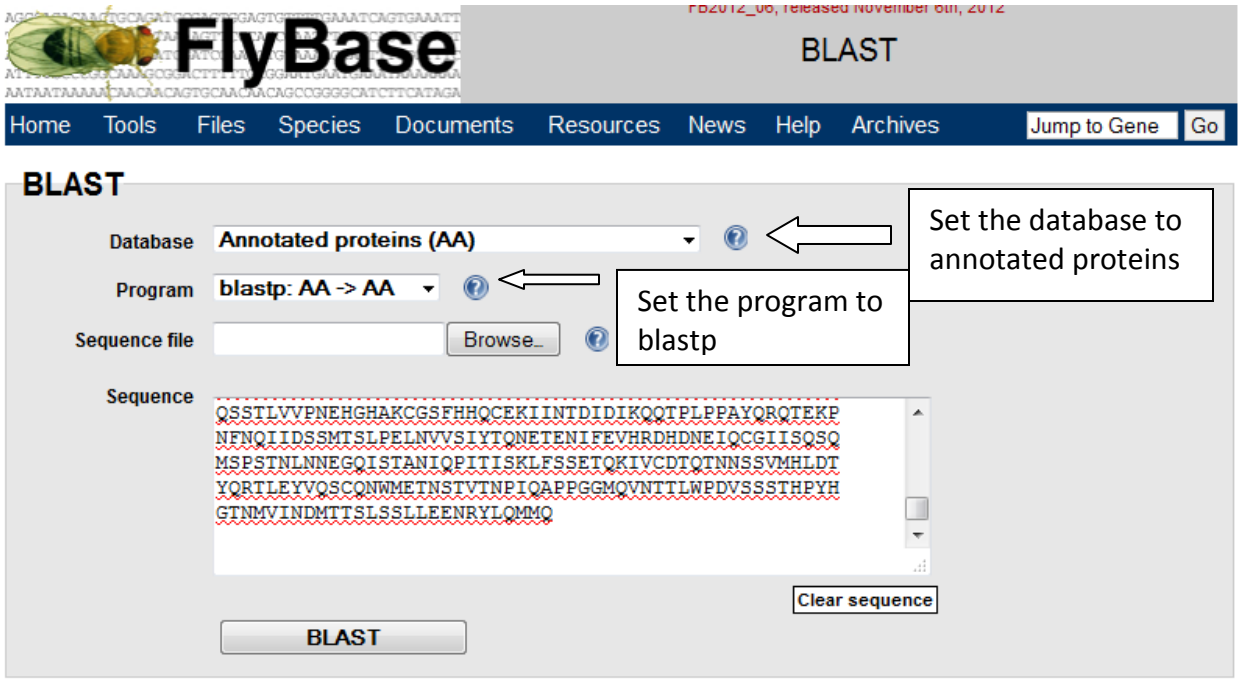
```
>contig38.002.1 length=1327
MYLFFYRYSELQFLASRRAAAVAAAATVLPGSPCINQLHPTDVSSSVTVP
SIIQTVGPSDSIKTSIQPPICNENTLANATGHQHNHQPQHVHNLNVTGQP
HVHDFHPAYRIPGYMEQLYSLQRSNSTSSFHDFHFSVDGNRPRPPGGSIR
ASISRKRALSSSPYSDSFDINSMIRFSPNSLATIMNGSRGSSTASGSYGH
ISANALNPMSHVHSTRLQQIQAHLLRASAGLLNPMTSQQVAASGFSIGHM
SASACLRVNDVHPNLSDSPSHITTSSTLKNLDDGKGKKGHFKDVVTEQPS
STSGAVAQVEADSASSHLSDRCYNNVVNNIKSIPGDIKVSTRLDEYINCG
TASTPSNEYDCANADTTDIKDEPGDFIETNCHWRSCCIEFITQDELVKHI
NNDHIQTNKKAFVCRWEDCTRGEKPFKAQYMLVVHMRRHTGEKPHKCTAY
SRLENLKTHLRSHTGEKPYTCEYPGCSKAFSNASDRAKHQNRTHSNEKPY
ICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKGLPLDDVNSRLQRD
NSHSRHNLQEHNIDSSPCSEDSHMGKILGTSSPSIKSESDISSSNHQLVN
GVRASDSLLTYSPDDVAENLNLDDGWNCDDDVDVADLPIVLRAMVNIGSG
HASASTIGGAVLARQRFRSRLQTKGINSSTIMLCNIPESNHTIGISELNQ
RITELKMEPGTAGEIKIPMPTNTAIGGFPEELLQNQGTSRNTVLNKQGIS
TASGSVQGQFRRDSQNSTASTYYGSMQSRRSSQSSQVSSIPTMRPSPTCT
TTTASFYDPISPGCSRRSSQMSNSANCYAFSSTSGLPIINKDSNNSTNAF
INKPNLGVNSVGIDNSSLPPPPSSHLIATNLKRLQGKDSENCYHNFTSGR
FCIPSCMHSLHMKNSNPVGQNEFDKVIANNTLRRQTEPVPNLNLDSLTNI
PRLSTTPNSFDITVGKTNNIASSINKDSLRKELCTVPIKADMAMTSDQHP
NERINLDEVEELILPDEMLQYLSLVKEDTNHTEKEHQTEAMGSSVYETLT
SNHYREQSNIYYSNKQILAPPSNVDIQPNTTNTIQDKFPMTAIGGSFSQR
QSSTLVVPNEHGHAKCGSFHHQCEKIINTDIDIKQQTPLPPAYQRQTEKP
NFNQIIDSSMTSLPELNVVSIYTQNETENIFEVHRDHDNEIQCGIISQSQ
MSPSTNLNNEGQISTANIQPITISKLFSSETQKIVCDTQTNNSSVMHLDT
YQRTLEYVQSCQNWMETNSTVTNPIQAPPGGMQVNTTLWPDVSSSTHPYH
GTNMVINDMTTSLSSLLEENRYLQMMQ
```

Navigate to Flybase at flybase.org

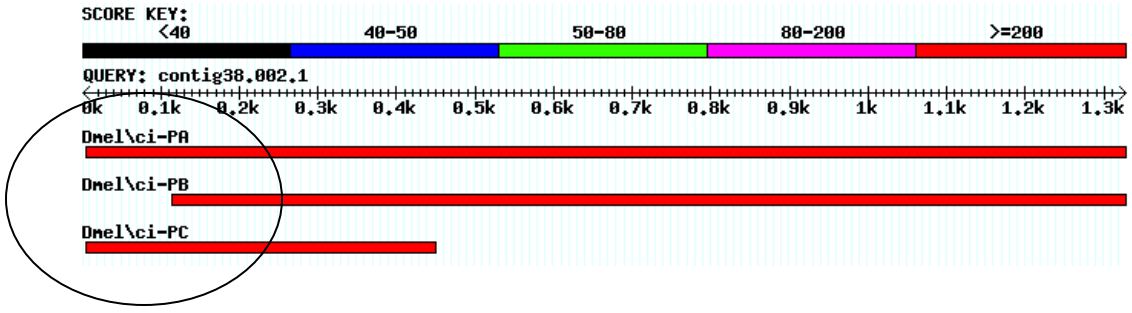


Click on BLAST

Use the protein sequence predicted by Twinscan to search the *D. melanogaster* protein database using BLASTP.



This should result in the following hits (just the top 3 are shown). Look familiar?

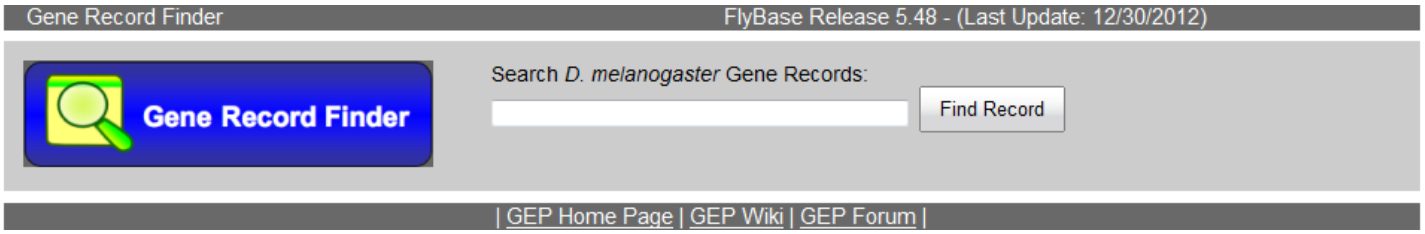


Scroll down to see the scores and E values for these alignments. Note that the E values are 0, which strongly suggests that this gene (ci) is the *D. melanogaster* ortholog of the *D. erecta* gene we are trying to annotate.

BLAST Hit Summary

Description	Species	Score	E value
ci-PA	Dmel	2182.91	0
ci-PB	Dmel	2018.82	0
ci-PC	Dmel	743.421	0

We'll return to FlyBase later. But now, to get more information about the *D. melanogaster* ci gene we will use the Gene Record Finder available at the GEP web site. To find this resource navigate to **the GEP site → projects → annotation resources →Gene Record Finder.** It should look like this:



Type ci into the search box. Note - this search is case sensitive. Remember this when you search additional genes. The Gene Record for the ci gene is shown below.

FlyBase Release 5.48 - (Last Update: 12/30/2012)

Gene Details

FlyBase ID	FlyBase Name	Chr	5' Start	3' End	Strand	Graphical Viewer
FBgn0004859	ci	4	77,667	68,336	-	View in GBrowse

mRNA Details

Select a row to display the corresponding transcript and peptide details:

FlyBase ID	FlyBase Name	Chr	5' Start	3' End	Strand	Protein ID	Graphical Viewer
FBtr0306168	ci-RB	4	77,667	68,336	-	FBpp0297298	View in GBrowse
FBtr0089178	ci-RA	4	76,957	68,336	-	FBpp0088245	View in GBrowse
FBtr0308074	ci-RC	4	76,957	68,336	-	FBpp0300417	View in GBrowse

Transcript Details | **Polypeptide Details**

Options: Export All Unique CDS's to FASTA | Export All CDS's for Selected Isoform to FASTA | Download CDS Workbook

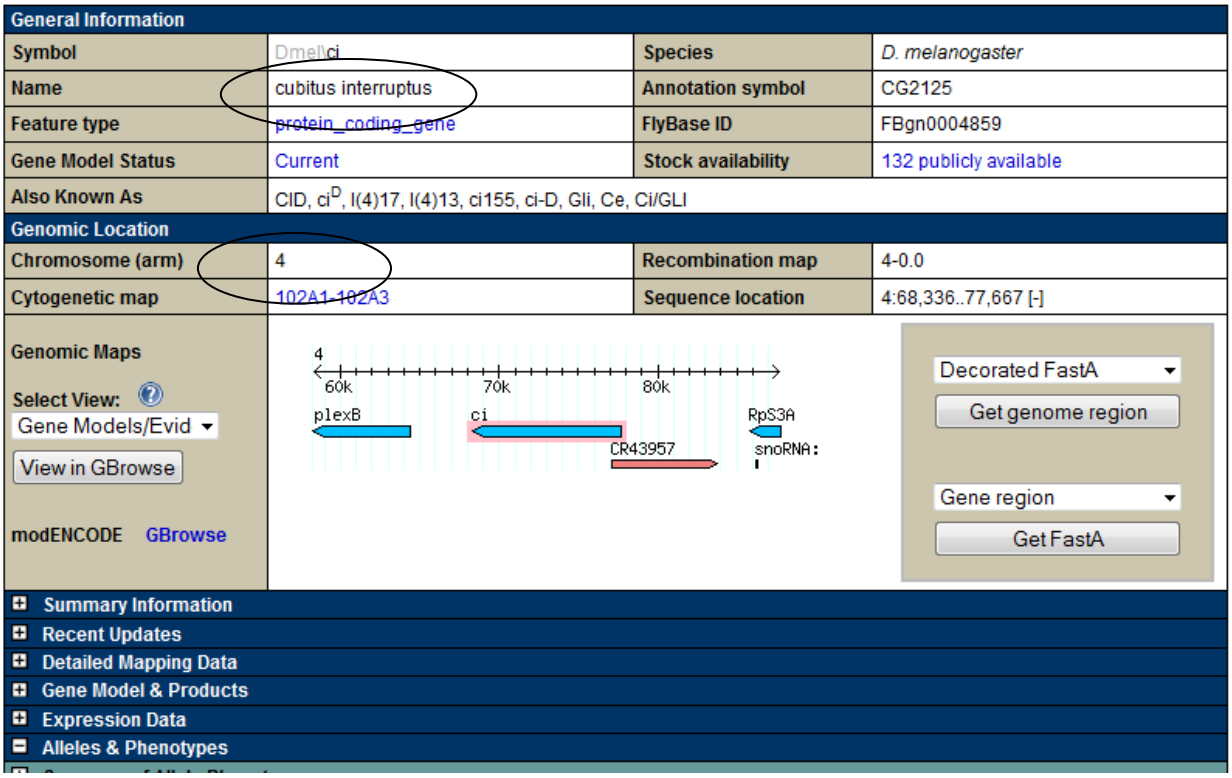
CDS usage map:

Isoform	7_1634_0	6_1634_1	6_1629_0	5_1629_2	4_1629_0	3_1629_0	2_1634_0	1_1636_1	1_1629_0
ci-RB			Y	Y	Y	Y			Y
ci-RA	Y	Y		Y	Y	Y			Y
ci-RC	Y	Y		Y	Y		Y	Y	

Select a row to display the corresponding CDS sequence:

FlyBase ID	5' Start	3' End	Strand	Phase	Length

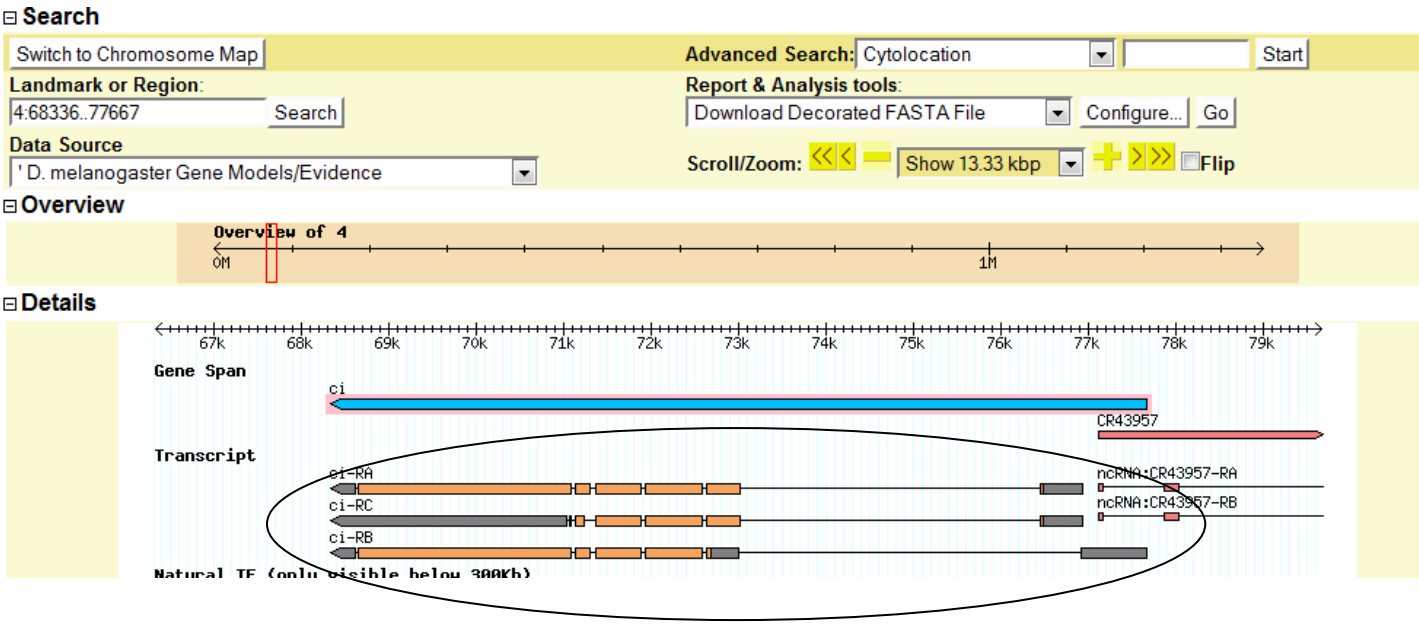
To learn more about this gene click on the link below FlyBase ID. Exploring FlyBase you should be able to easily determine the name of the gene, and can learn much more about the gene by exploring the site.



From FlyBase, amongst other things, we see the gene name is cubitus interruptus and that it is located on chromosome 4. We can also see that RpS3 and plexB are in the same region, which is consistent with the genomic region of *D. erecta* we saw in the UCSC browser. Also note that CR43957 is present in melanogaster, but not erecta. This may require further investigation, but we'll ignore it for now.

Click on summary information. Briefly, what is the function of the protein encoded by this gene?

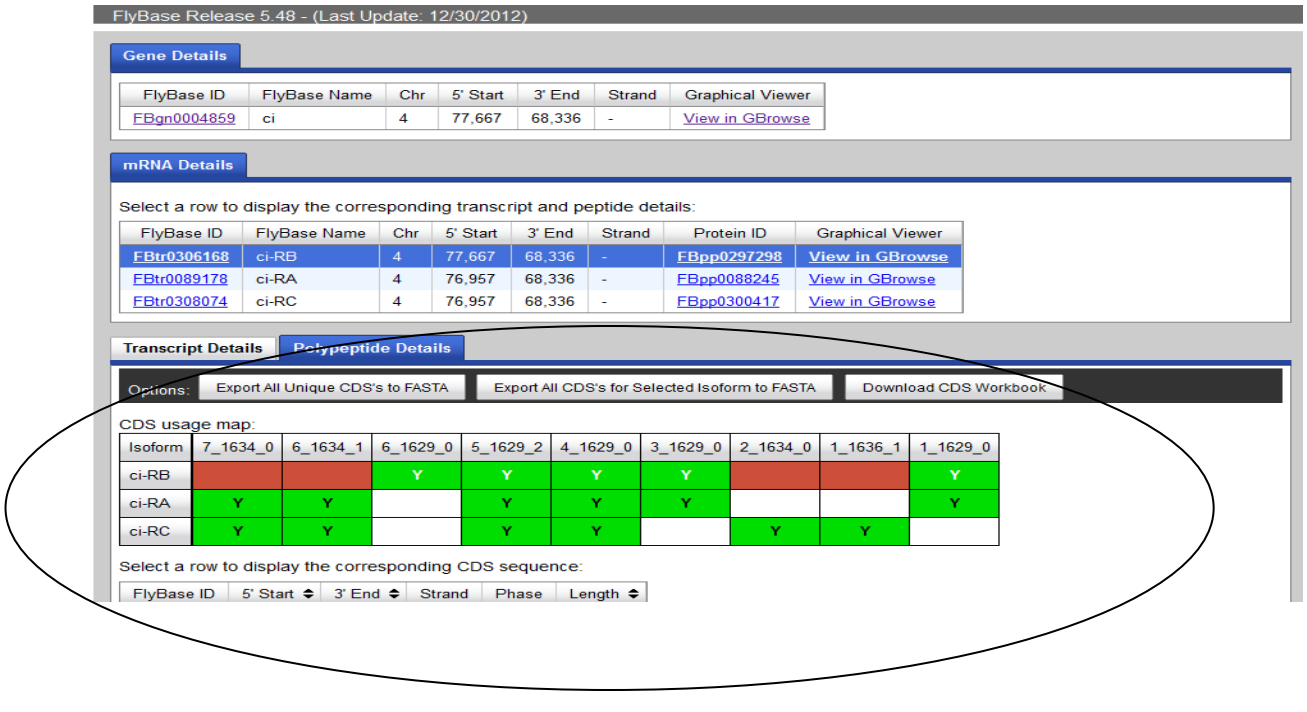
We can also learn something about the structure of the gene by clicking on GBrowse, either from the FlyBase page, or from Gene Record Finder, which yields this screen.



The GBrowse results show us the organization of translated and untranslated regions of the mRNA, which may come in handy if we need to find a small exon later. For now we will return to the Gene Record Finder page to start annotating each exon.

Mapping gene details by comparing each *D. melanogaster* ci-RB exon from Gene Record Finder to the contig38 DNA sequence.

Reopen the tab for the Gene Record Finder. Type ci into the search window to get the following:

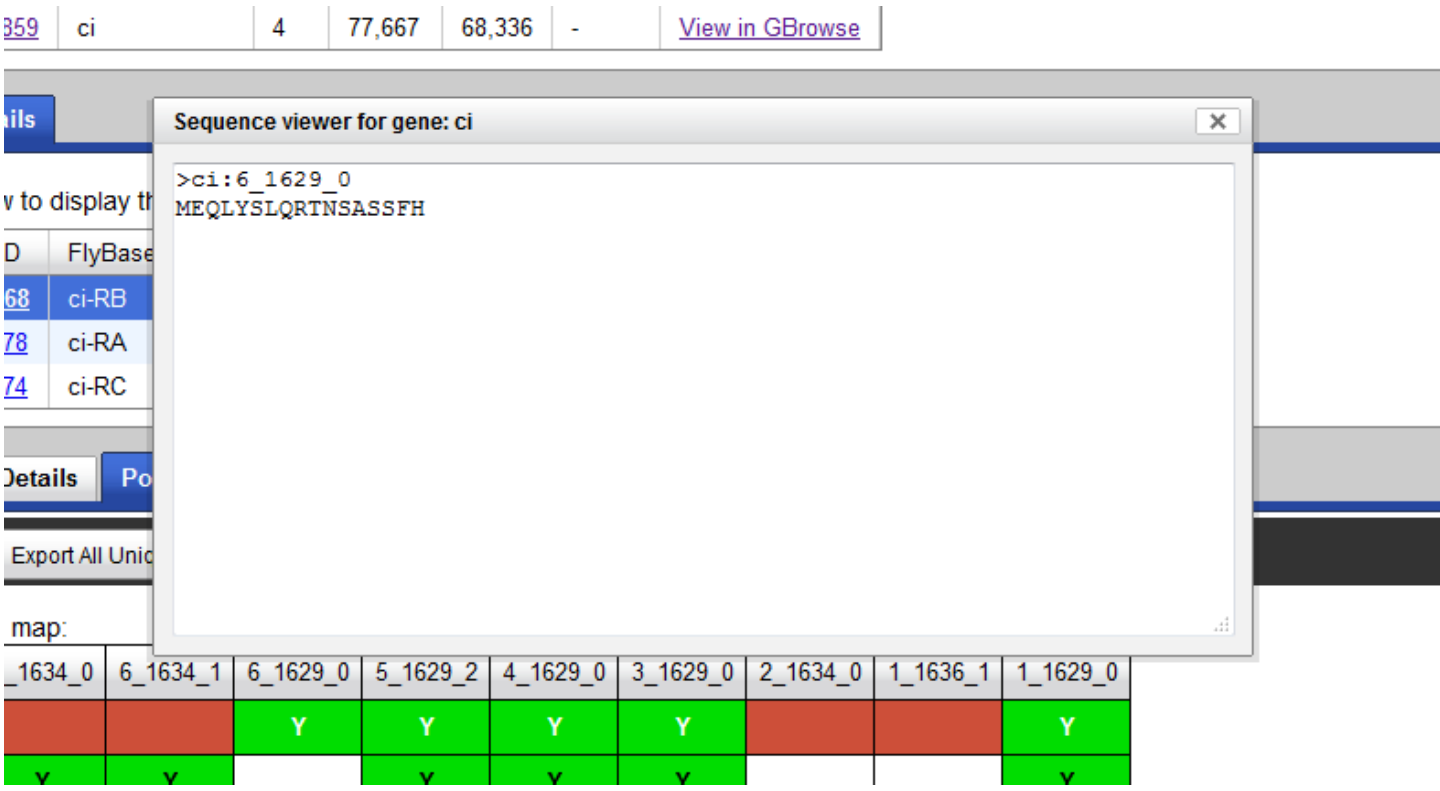


The critical information from Gene Record Finder is the organization of the exons in the D. mel gene (circled). In this view, the 'polypeptide details' tab is selected. Note there are three isoforms ci-RA, ci-RB and ci-RC. Because each of these is different in the polypeptide view, each isoform codes for a different protein isoform (technically these protein isoforms should be labeled ci-PA etc, but for some reason they're not in this case). We will need to annotate each of these. You should click on the transcript details to see the isoforms at the RNA level. In some cases there will be more isoforms in the transcript details. These differ at the RNA level, but don't affect the protein sequence. For example, two RNA isoforms might differ in the 5'-UTR, but be the same for the coding exons. We will need to report these isoforms, but they don't require any further analysis.

We will begin by annotating isoform ci-RB, which has 5 exons. Some of these exons are shared with other isoforms and will need to be annotated only once, but incorporated into the model for each isoform. Exons that are unique to the other isoforms will be annotated independently.

Each exon is designated with a number, listed in the top row of the table (7_1634_0; 6_1634_1, etc). While these numbers are rather awkward to work with, it is best to keep track of them as we proceed.

Let's start with the first exon of isoform Ci-RB. The number for this exon is 6_1629_0. Select that number in the top row. A window displaying the amino acid sequence of that exon will pop-up, as shown below.



Our goal is to locate the corresponding sequence in the *D. erecta* contig38 sequence. To do this we will perform a BLASTX comparison of the exon sequence shown above and the complete DNA sequence of contig38. Recall that the definition of a BLASTX search is to "Search a **protein** database using a **translated nucleotide** query". In this case the exon sequence will serve as the 'protein database' and the DNA sequence from the contig38 will be used to generate the 'translated nucleotide query'. This DNA sequence will be provided with the annotation package, but it can also be found using the UCSC browser. You should already have a file that contains the DNA sequence for contig38. If not, get it from the UCSC browser as described earlier in this document.

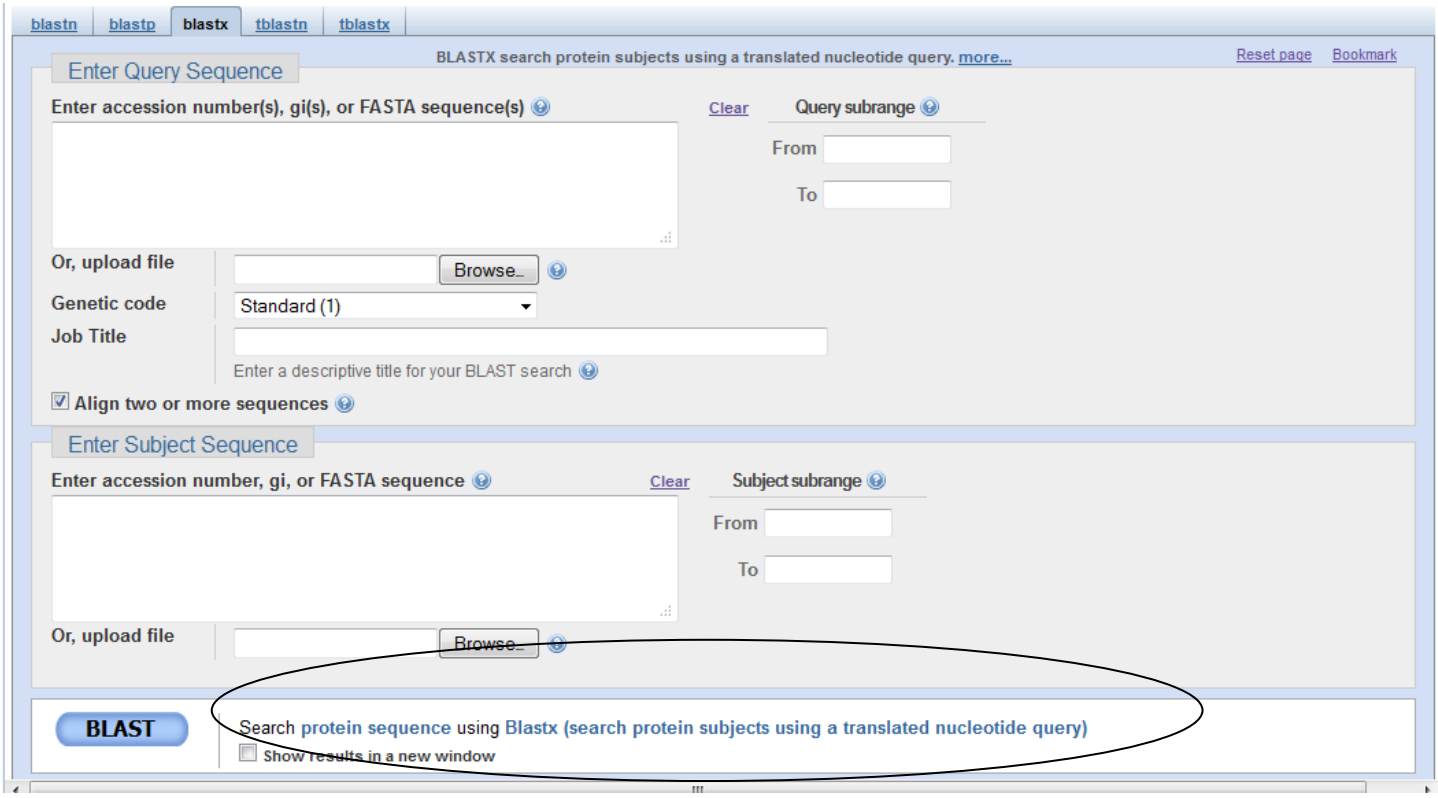
We are now ready to begin mapping exons from Gene Record Finder to the contig38 sequence.

Open a new tab. Navigate to the Blast page at http://www.ncbi.nlm.nih.gov/

Open a blastX search

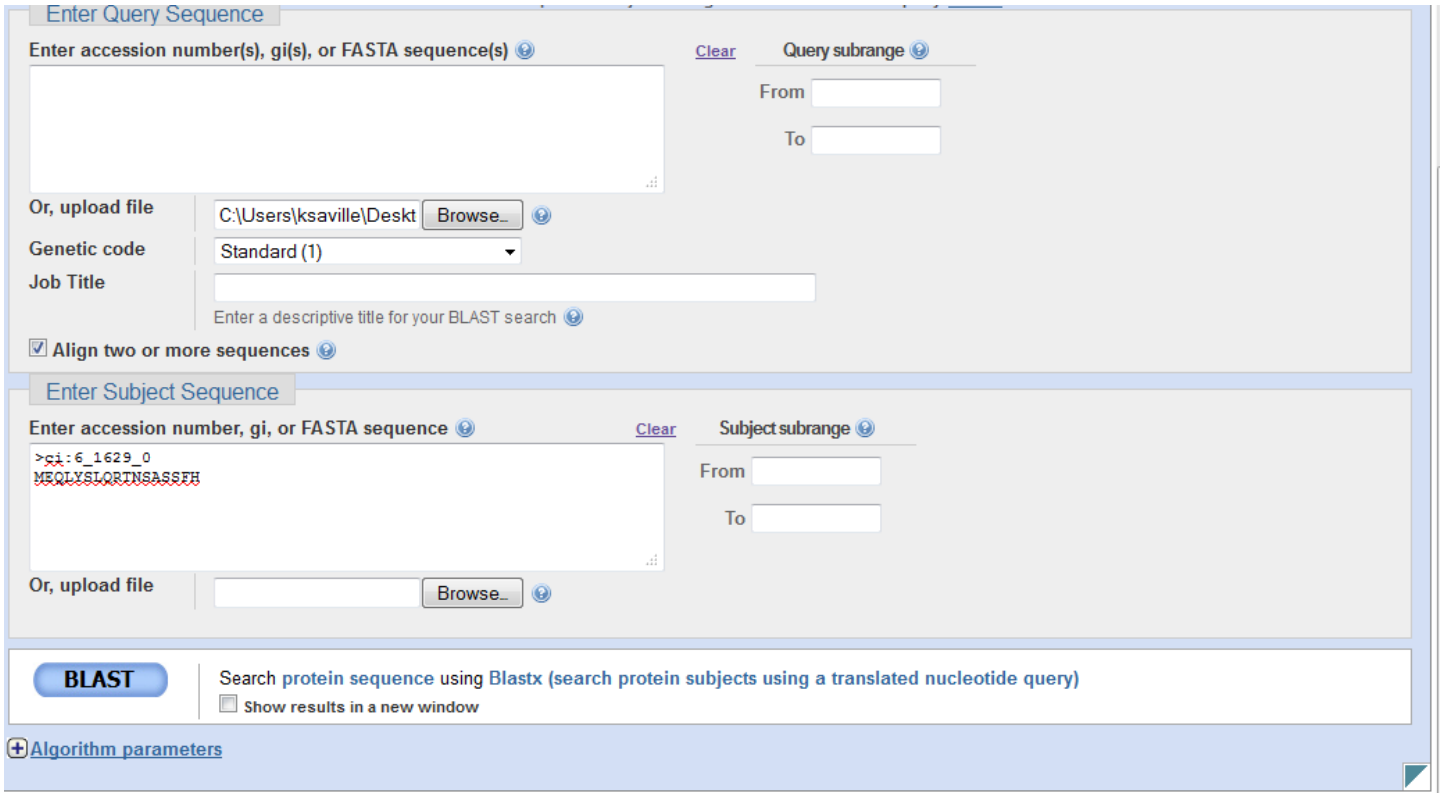
Click on 'align two or more sequences'

This should lead to this screen.



Note: You are reminded of the search strategy of blastx here. So the query (top box) needs to be DNA. Use the browse button to select your contig38sequence.txt file. For the subject, return to the Gene record Finder tab (or reopen the page if you closed it), copy the first exon from Gene

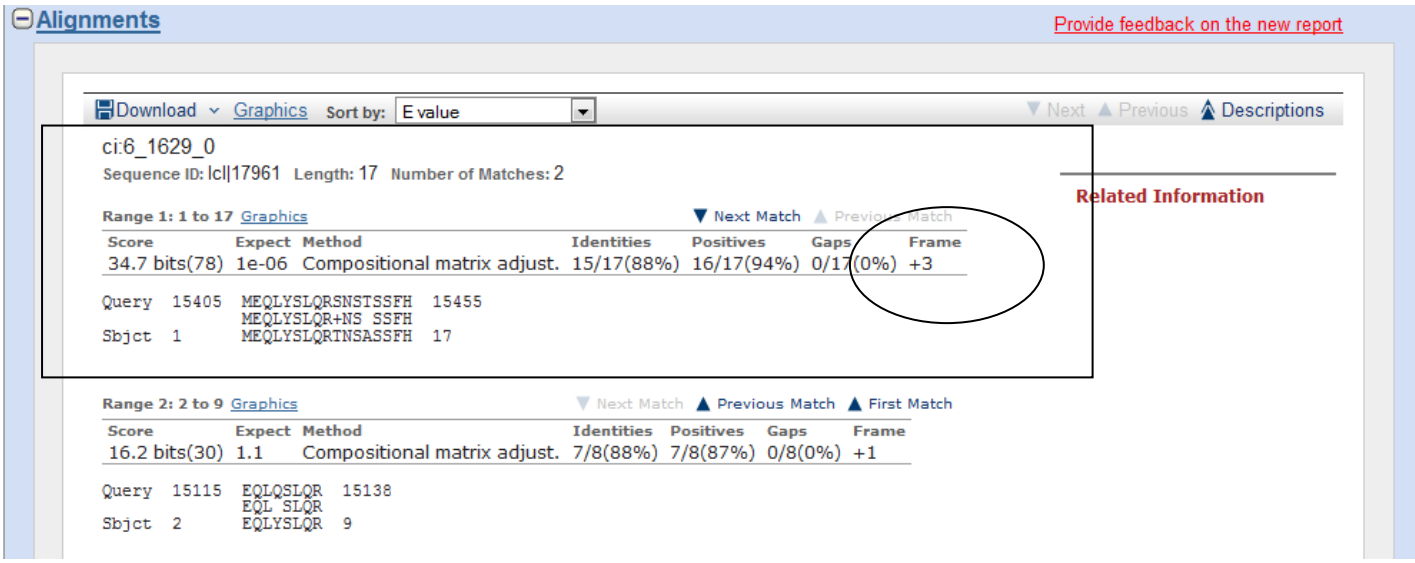
Record Finder, and paste the amino acid sequence into the subject box. The search should look like this:



Before you click BLAST, click on Algorithm parameters, find the 'low complexity filter' check box and deselect it (this just helps with the search and is covered in more detail in other Blast tutorials).

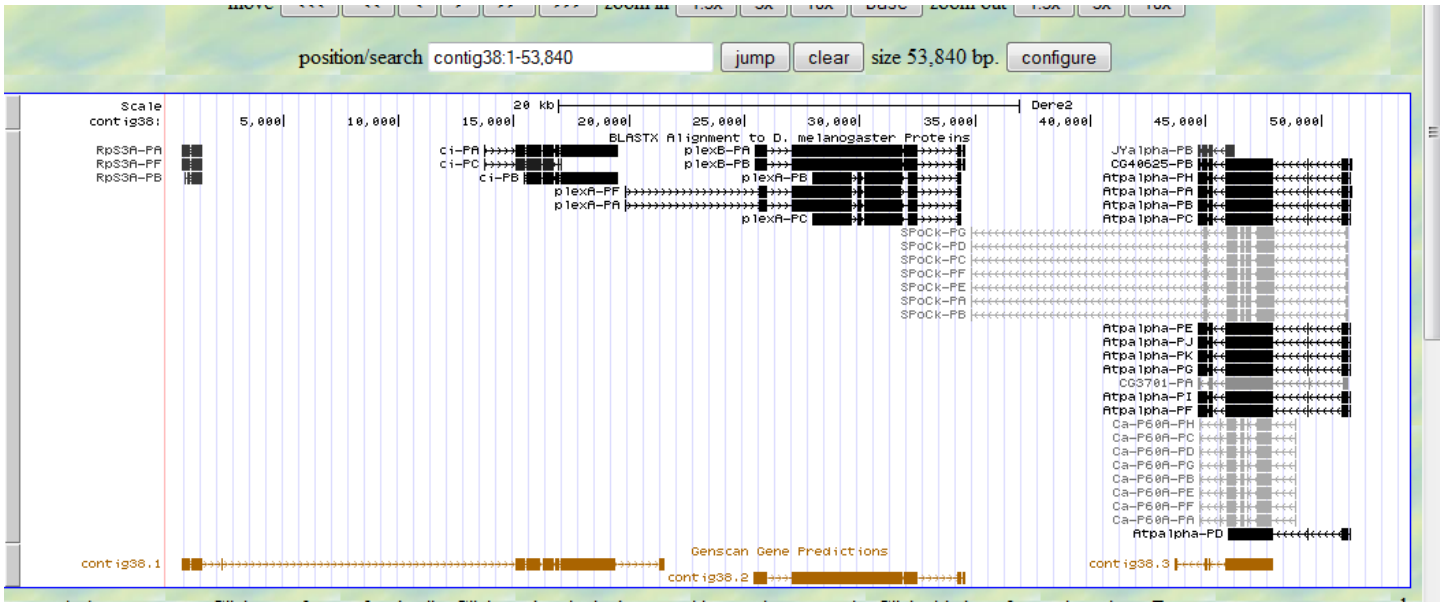
Now click BLAST

When your BLAST results are returned, scroll down a bit to find the following alignment



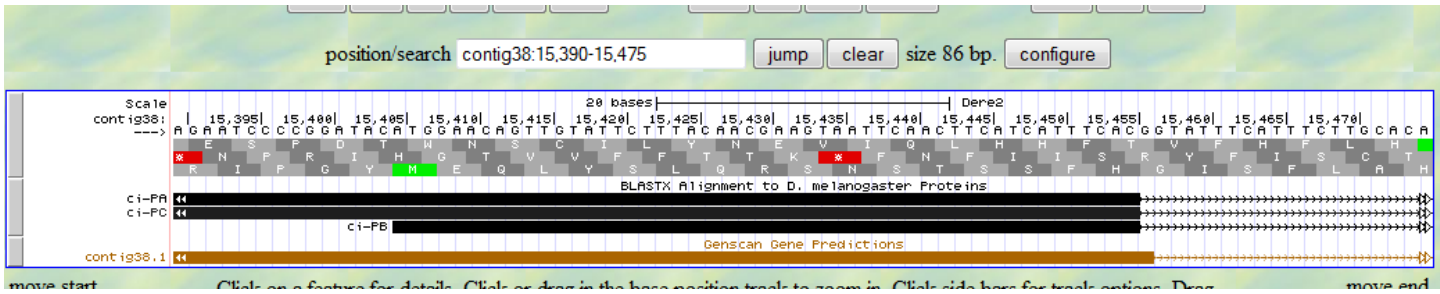
The top alignment shows where the exon sequence (subject) aligns with the contig38 sequence (query). Recall that the query sequence was the entire 53,840 base pair DNA sequence. However, the alignment is to an amino acid sequence. This is because blastx translated the DNA into all possible protein sequences, and then compared it to your exon sequence. However, the numbers (15115 and 15138) refer to the DNA sequence in contig38. We will use these numbers to locate the exon sequence in the contig. Also notice the E value of 1e-06 and, importantly, the frame of +3. The frame will be important later. Select and copy the relevant information (boxed), and paste it into a word document. This document will serve as your notebook as you annotate this gene. Notice that the 'relevant information' includes the name of the exon ci: 6_1629_0, the frame, and the alignment. In some cases the alignment will span several lines and all of the lines will need to be copied.

We will now return to the genome browser to map the specific nucleotides that correspond to this exon.



This should look familiar by now.

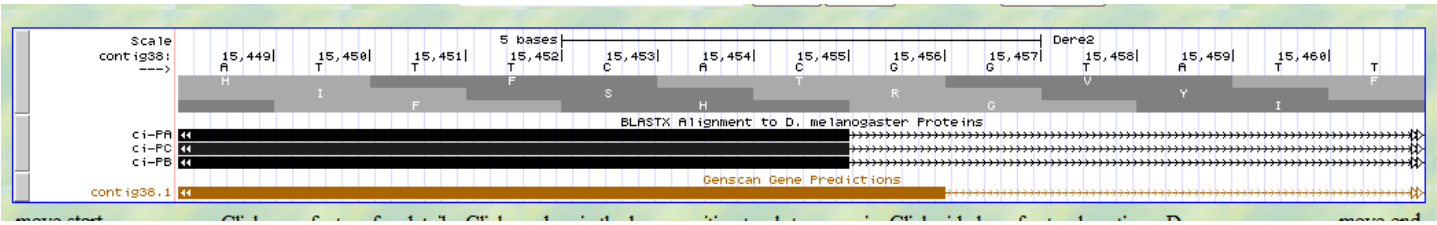
We will want to zoom in to the relevant region. Recall that the alignment was from 15405-15455, so zoom in to this general region, but include a little room on each side. One way to do this is to type the desired coordinates into the search box. Let's try 15390-15475. Click on jump, which should lead to this view:



Remember that the black bar represents an automated blast alignment and the brown bar is the Genscan predicted gene. Notice that the alignment correlates with isoform B and that isoforms A and C begin further upstream. The Genscan program did not predict the beginning of the B isoform. Also, notice the green M in frame +3 (the frames are numbered +1, +2, and +3 from top to bottom). This corresponds to nucleotide 15405 (notice the ATG beginning at that spot in the DNA sequence).

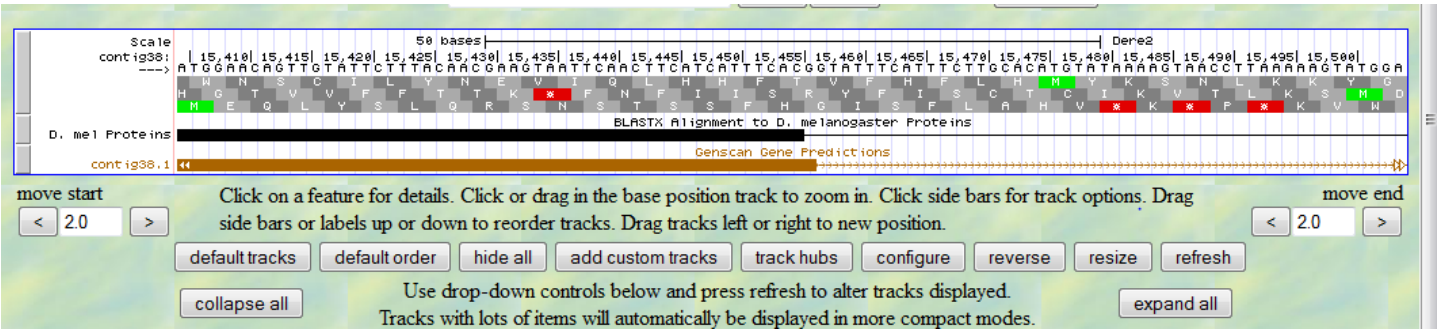
As we build our gene model, we will need to record the specific nucleotide numbers that correspond to the relevant gene features. You should record the coordinates in your Word file or create an excel file, so they can easily be cut and pasted. In this case the relevant gene feature is the start codon, and the nucleotide coordinate is **15405**, which corresponds to the A of the ATG. The end of the blastx alignment was at nucleotide 15455. If that's the end of the first exon, what DNA sequence should be present at the beginning of the intron? Is that sequence present?

OK, so the sequence at the beginning of an intron is a GU in the RNA, which corresponds to a GT in the DNA. There is a GT near the end of our alignment. We can zoom in on that area to get a better look at exactly which nucleotide corresponds to the end of the exon. We can type in coordinates that surround that area, or, you can click on the ruler bar at the top of the sequence and select the region of interest. This might take some trial and error, but once you get the hang of it, it will make navigation of the sequence much easier. Zooming to the area surrounding our proposed exon/intron junction gives this screen.



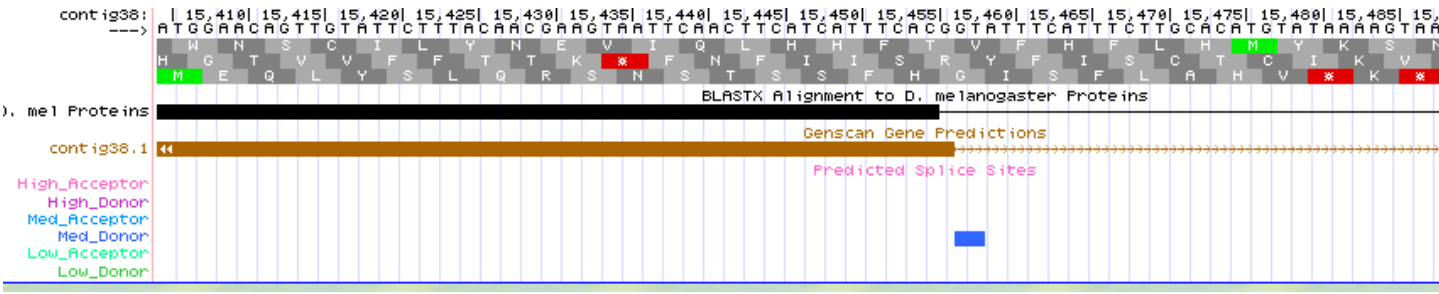
The nearest GT is at position 15457-15458. This is also the site selected by the Genscan model. Notice that the Genscan bar stops immediately before the GT. If we decide that this is indeed the proper splice site to use (and because our alignment strongly suggests it at this point, we can be reasonably confident), we would select the last nucleotide of the exon as the next coordinate for the gene model. This coordinate would be **15456**, which is the last nucleotide before the G of the GT splice site. Notice that if we had just used the blastx alignment we would have been tempted to use 15455 as the coordinate. This may seem like a trivial difference, but as you probably know, one nucleotide difference can mean a huge difference in gene function. As we annotate various genes, it is important to select the precise coordinates (if not we will find the error when using Gene Model Checker, described below, and can correct it).

Splice site predictor. Although we probably don't need additional evidence to support this choice for a splice donor site, this is a good point to introduce the splice site predictor track. Sometimes, two potential splice sites might make sense and we will need to distinguish between them. One source of additional evidence for a potential splice site is the splice site predictor track. To try this out, navigate back to the UCSC genome browser and zoom into the general region surrounding the above splice site.



Scroll down to the last track called Experimental Tracks, and set the predicted splice sites to dense. Hit refresh.

Scroll back to the top of the page, and notice the appearance of a blue box. Over to the left, a key tells us that this is a 'medium donor'. This means that the splice site predictor has identified this as a donor splice site, with a medium level of confidence. So this supports our selection as a splice donor.



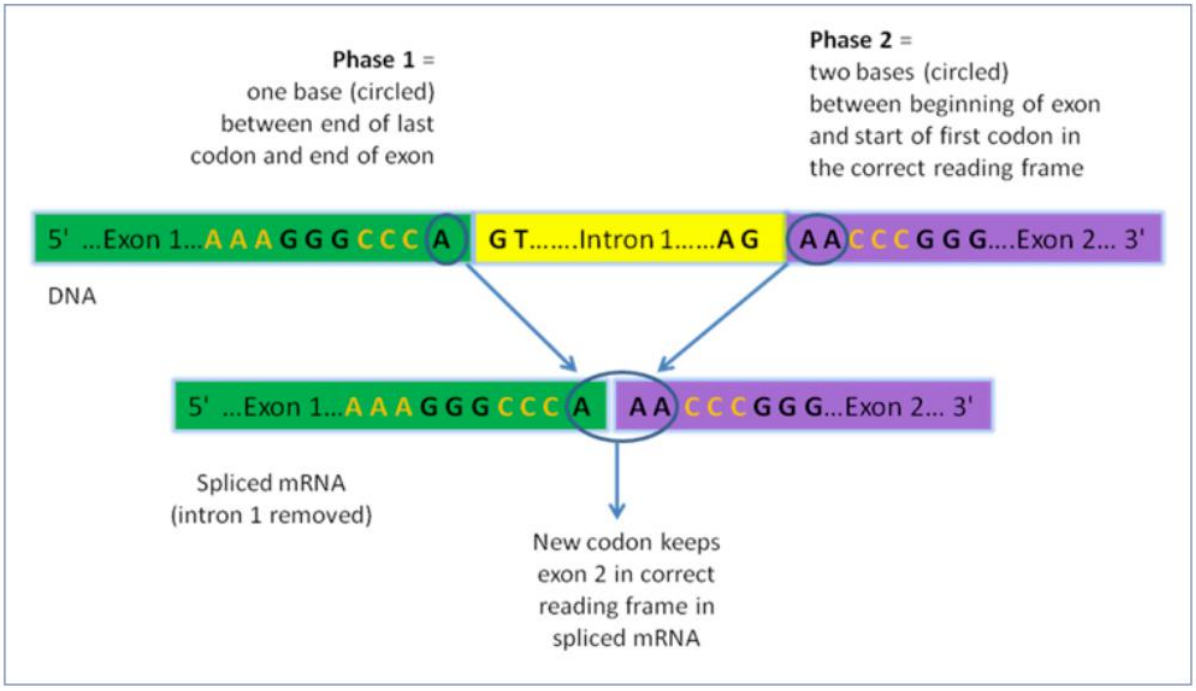
A bit of a digression to explore Reading Frames and Splicing Phases:

At this point it's worth taking a slight detour to discuss the concepts of reading frame and splicing phase. A further discussion of this can be found at the GEP web site in the document "Primer on Reading Frames and Phase." For this exon our alignment identified reading frame +3. This is the bottom of the three frames shown above. The letters in the frame refer to the single letter amino acid code and read F - H - G. Notice that the nucleotides corresponding to the last amino acid (G, which stands for the amino acid glycine) before the splice site are GGT (this would be GGU in the RNA, but we'll keep it GGT here). However, splicing would cut the RNA between the two G's as follows G|GT, removing the GT and the remaining G would then be joined with the acceptor site of the next exon. Therefore the first two nucleotides of the next exon would be used to complete the codon and this would then 'set the reading frame' for the rest of the exon'. Because one nucleotide is 'left dangling' at the end of the first exon it is said to be in phase 1. It will need to be matched with a phase 2 accepting exon that completes the codon in such a way as to maintain the proper frame.

At donor splice sites, the phase can be defined as the number of nucleotides between the end of the last codon and the GT. If there's 1, then it's phase 1, if 2 it's phase 2, if 0, phase 0. For the acceptor site, the phase is the number of nucleotides between the AG of the intron and the first full codon. If 1, then phase 1, if 2, phase 2, if 0, phase 0.

This is a bit confusing (a bit?), so let's take a look at a simplified example.

In the figure below, the splice site occurs after the first A of a codon. This means that splicing will leave an incomplete codon in the first exon. When splicing to the next exon, the first two nucleotides will need to 'complete' this codon (you complete me?). In the figure below, using the A from the first exon and the first two A's from the next codon completes the codon.



In the above example a phase 1 exon is matched with a phase 2 exon. The phases of the donor and acceptor must always match up as follows.

If the donor phase is	then the acceptor phase must be
0	0
1	2
2	1

Once these are matched up, the next codon must be in the proper frame. In the above example CCC is the next codon. Only one reading frame is shown for simplicity. In your annotation this might switch the reading frame to one that is different than the previous exon. For example, Then this sets the frame for the next codon. This might switch the reading frame from +1 to +2 or something like that, but that's OK. What's important is that the new frame must be the same as the one that identified in the BlastX search. OK - still confusing, but with some practice you'll get it.

So, where were we? Oh yeah, annotating exon 2 of the ci-RB isoform.

Performing a blastx comparison of the second exon of the ci gene and the full DNA sequence of contig38 (that is, doing the same thing for exon 2 as we did for the first exon) leads to the following alignment.

Download Graphics Sort by: E value

ci:5_1629_2

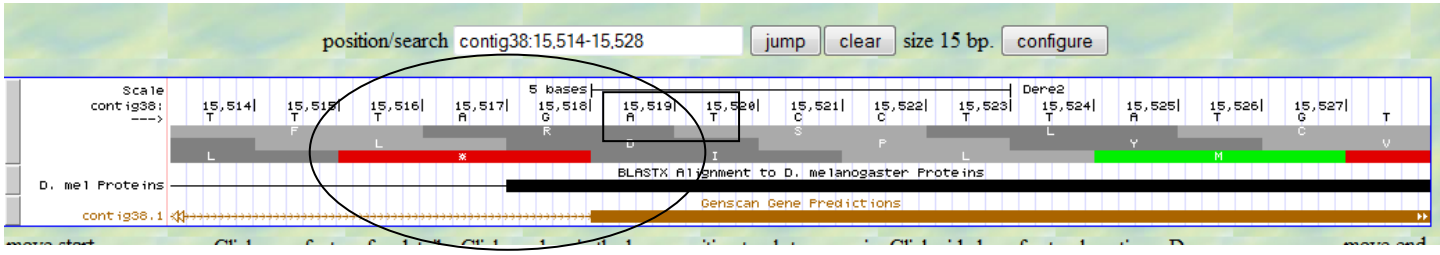
Sequence ID: lcl|51707 Length: 213 Number of Matches: 3

Range 1: 1 to 213 Graphics Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
321 bits(823)	2e-102	Compositional matrix adjust.	188/216(87%)	193/216(89%)	7/216(3%)	+2

```
Query  15521  PYVNCASAFHLAGLGLGSGDFLGSRGMSSLGELHHAAVAAAAASSLASTDFHFSVDGNR-  15697
              PYVNCASAFHLAGLGLGS DFLGSRG+SSLGELH+AAVAAAAA SLASTDFHFSVDGNR
Sbjct  1      PYVNCASAFHLAGLGLGSADFLGSRGLSSLGELHNAAVAAAAAGSLASTDFHFSVDGNRR  60

Query  15698  ---PRPPGGSIRASISRKRALSSSPYSDSFDINSMIRFSPNSLATIMNGSRGSSTASGSY  15868
                 PRPPGGSIRASISRKRALSSSPYSDSFDINSMIRFSPNSLATIMNGSRGSS ASGSY
Sbjct  61     LGSPRPPGGSIRASISRKRALSSSPYSDSFDINSMIRFSPNSLATIMNGSRGSSAASGSY  120

Query  15869  GHISANALNPMSHVHSTRLQQIQAHLLRASAGLLNPMTSQQVAASGFSIGHMSASACLRV  16048
              GHISA ALNPMSHVHSTRLQQIQAHLLRASAGLLNPMT QQVAASGFSIGHM  SA LRV
Sbjct  121    GHISATALNPMSHVHSTRLQQIQAHLLRASAGLLNPMTPQQVAASGFSIGHMPTSASLRV  180

Query  16049  NDVHPNLSDSPSHITTSSTVRLVNEDPNQIAAAALS  16156
              NDVHPNLSDS   ITTS T   V +D +Q+ AAA S
Sbjct  181    NDVHPNLSDSHIQITTSPT---VTKDVSQVPAAAFS  213
```

From this alignment it looks like the exon begins near nucleotide 15521. Notice also that the reading frame for this exon is in frame +2. Even though this is different than the +3 frame from the first exon, it's OK. We will need to choose the appropriate splice site so that the reading frame switches from +3 to +2. Zooming to the region surrounding 15521 gives us the following view. The nearest potential splice acceptor (AG) is circled.



If we were to use this AG, the first nucleotide of the next exon would be the A at position15519. Remember, that we need to use the first two nucleotides to complete the codon using the G remaining on the first exon. That is, because the previous exon ended in phase 1, we need to match it with a phase 2 acceptor site. If we do this using the previous G, then AT (boxed) to make the GAT codon, then the next codon would be CCT. Notice that this CCT corresponds to the P (for proline) in reading frame +2. This is good, because the alignment of the second exon is in the +2 frame. Also notice that the alignment begins PYV …, which is what we see at the beginning of the second reading frame. So it makes sense to use this splice site, which means the first nucleotide coordinate of this exon is 15519. Record this in the file you are using to keep track of the developing gene model.

So, so far we have three coordinates: the start codon at **15405**, the end of the first exon at **15456**, and the beginning of the second exon at **15519**. We can use these coordinates to begin generating a gene model in the following format: 15405-15456, 15519- …… We then need to continue this process to identify the coordinates for the remaining splice donor and acceptor sites and, finally, the stop codon.

You should now repeat the blastx comparisons for each remaining exon to identify the coordinates for all relevant splice sites and the stop codon.

Final Jeopardy music playing while we wait for you to complete this task ……..

OK

Use your blast results to convince yourself that these are indeed the correct coordinates. Note, when doing blast of the various exons, remember to turn off the 'low complexity filter'. This can be found in the 'algorithm parameters' section at the bottom of the blast page. The following numbers are the correct coordinates for this gene.

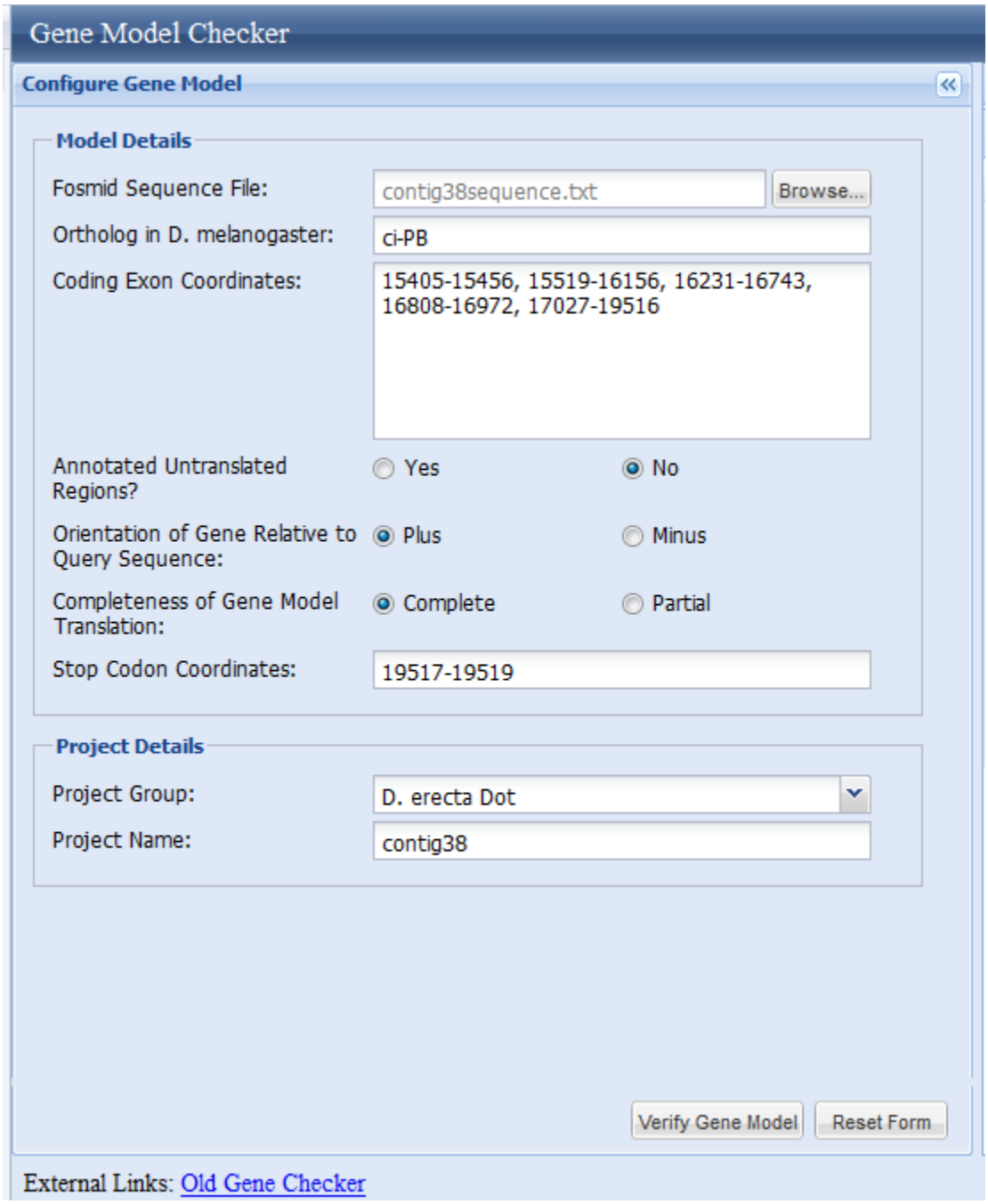
15405-15456, 15519-16156, 16231-16743, 16808-16972, 17027-19516 (19517-19519)

Next, we need to input these coordinates into the gene model checker, to see if the model is 'correct'.

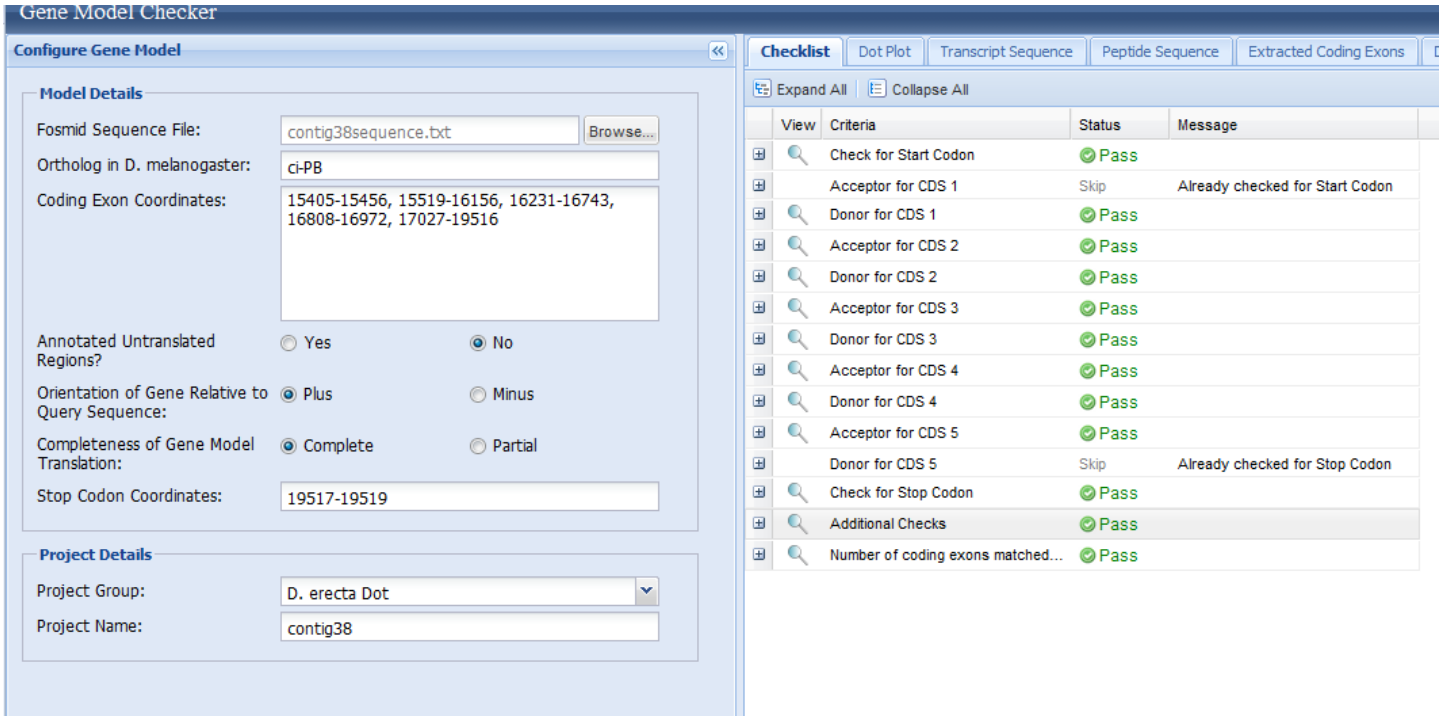
Verifying the Gene Model with Gene Model Checker

To confirm the accuracy of a gene model using the Gene Model Checker, proceed as follows:
1. Select 'Gene Model Checker' from the Projects -> Annotation Resources drop-down menu at http://gep.wustl.edu/.
2. Upload the text file of your contig sequence file into the first box on the left of the Gene Model Checker window.
3. Type in the name of the *D. melanogaster* ortholog (ci-RB) in the second box– watch for this gene to appear in the drop-down menu box as you type.
4. Enter the base number of the beginning and end of each coding sequence, in the order they appear in the in the table above, in the following format: # - #, # - #, # - #, etc.
5. *Do not include the stop codon in the last CDS since the stop codon does not code for an amino acid*. Instead, enter the stop codon coordinates in # - # format in the box lower down in the Gene Model Checker window.
6. If the information for your gene were on the (-) strand, you would select 'Minus' following 'Orientation of Gene Relative to Query Sequence.'
7. We think all the gene's coding sequences are accounted for, so select 'Complete' in the next row.
8. Use the drop-down menu to select the Project Group (*D. erecta* Dot) and type in the Project Name (contig38).
9. *Red boxes will appear around any entries that the Gene Model Checker deems incorrectly entered. Fix these before going on.*

10. Click on the 'Verify Gene Model' box at the bottom of the window. A check list will be generated to the right.



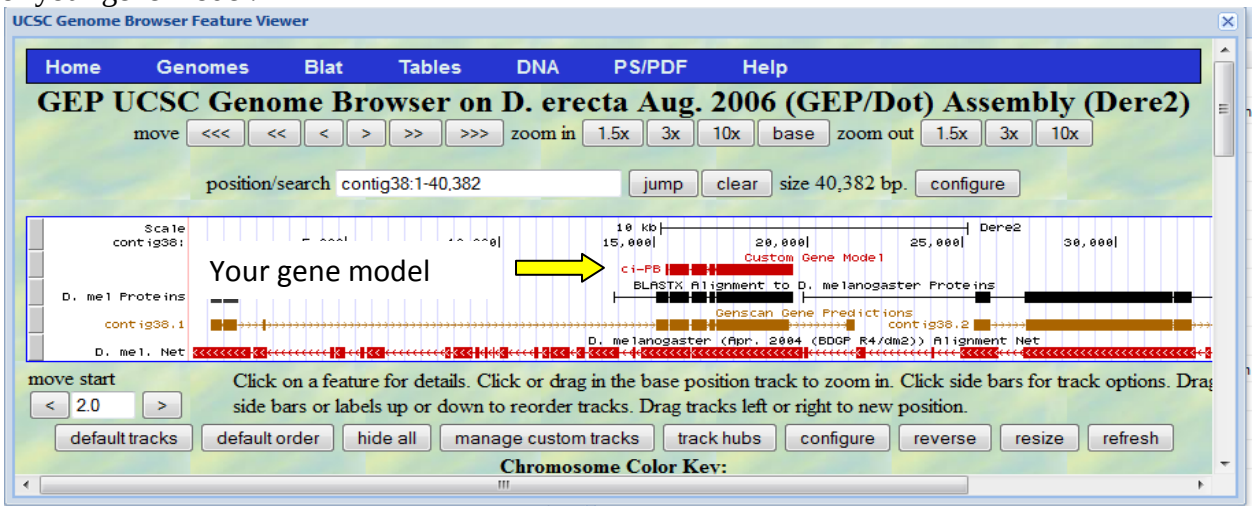
11. If there are no issues with your gene model, all lines will be green and read 'pass' (see Figure below). This just means that the coordinates you input are consistent with known splice site sequences, and there are no stop codons in the middle of your gene model. It doesn't necessarily mean that the gene model is completely correct (but it's a good sign ☺).



12. *If you passed the Gene Model Checker on the first try, intentionally change some of the coordinates to see what a failure would look like!*

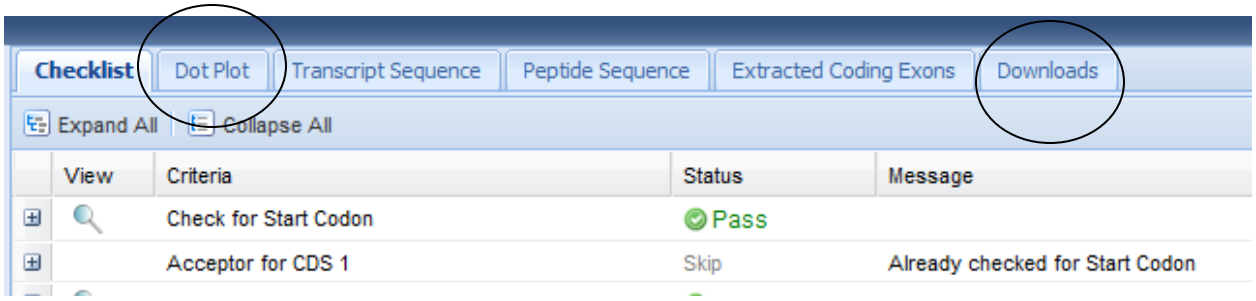
13. If there are failed parts of the Gene Model Checker, click on the small + box to the left of the failed part to get more information on the problematic sequence. A simple misreading or mistyping of intron-exon boundary coordinates is responsible for many model failures. Identify and fix these.

14. We now want to see a graphical representation of the model you created. To do this, click on a small magnifying glass to the left of any of the items in the check list. Your gene model will appear as a red bar in the UCSC browser. Zoom out and center the image to get a good view of your gene model.



Final checks and preparing information for the report.

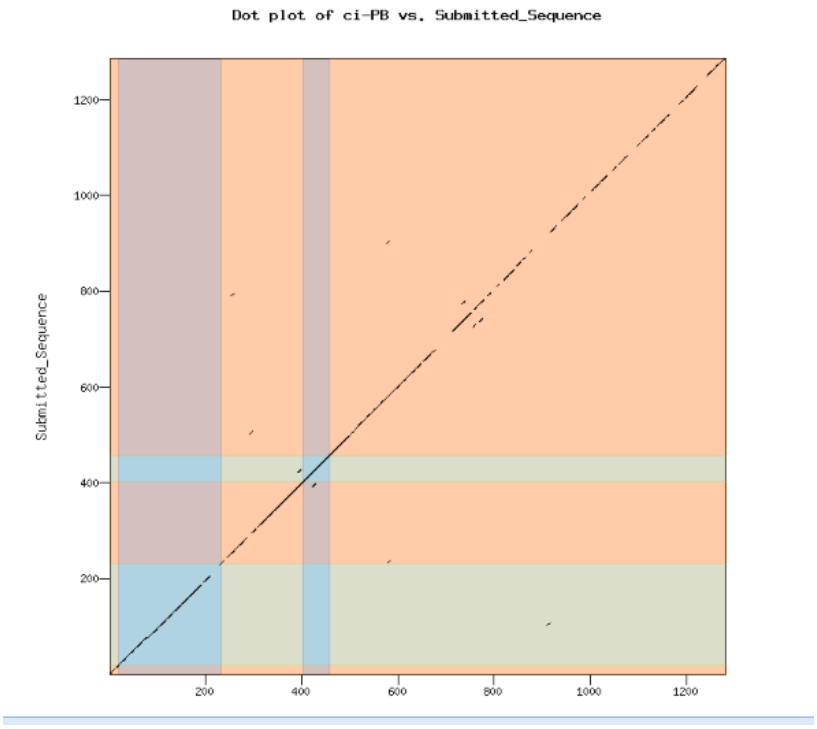
A few additional checks need to be completed and a few files need to be downloaded prior to submitting your report to the GEP Project Management System. These checks and files are generated by clicking on the various tabs in using the Gene Model Checker (circled in the figure below).



For the final report we need the information found in the Dot Plot tab and the Downloads tab.

1. Click on the Dot Plot tab to generate the following figure, then copy and paste the figure into your notebook, and ultimately into your final report. The Dot Plot is a graphical representation of the peptide sequence predicted by your gene model (submitted_sequence on the X-axis) and the D. melanogaster ci-PB protein sequence (X-axis). The diagonal line shows the positions that match between the two sequences. If there are major gaps or missing regions, these would need to be re-evaluated.

View protein alignment



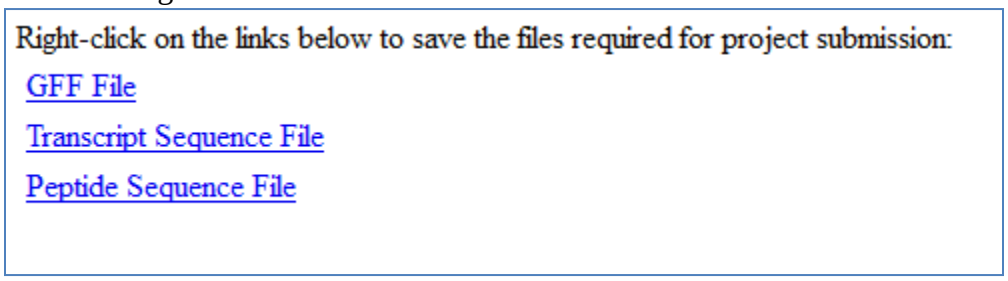
2. Generate a protein alignment by clicking the hyperlink labeled 'protein alignment' at the top of the Dot Plot page, then copy and paste this alignment into your notebook and ultimately into the final report. Note you can also click on the plain text version. You may need to play around with this a bit (adjust margins, font, etc.) to get it to look presentable in your final report. For example to get the following alignment to actually line up, the entire alignment was selected and changed to the font 'courier new' font size 10. In courier fonts, all characters are the same size, making alignments much easier. Also, while the alignment was selected, the right indent (in the ruler bar at the top of the Microsoft Word document) was adjusted slightly to the right.

```
########################################
# Program: stretcher
# Rundate: Tue  5 Feb 2013 20:08:07
# Commandline: stretcher
#    -asequence /home/wilson/public_html/genechecker/trash/130205195230.q.fasta
#    -bsequence /home/wilson/public_html/genechecker/trash/130205195230.s.fasta
#    -aformat pair
#    -outfile
/home/wilson/public_html/genechecker/trash/align_130205195230.html.txt
# Align_format: pair
# Report_file:
/home/wilson/public_html/genechecker/trash/align_130205195230.html.txt
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: ci-PB
# 2: Submitted_Sequence
# Matrix: EBLOSUM62
# Gap_penalty: 12
# Extend_penalty: 2
#
# Length: 1291
# Identity:    1107/1291 (85.7%)
# Similarity:  1174/1291 (90.9%)
# Gaps:          17/1291 ( 1.3%)
# Score: 5701
#
#
#=======================================

ci-PB              1 MEQLYSLQRTNSASSFHDPYVNCASAFHLAGLGLGSADFLGSRGLSSLGE     50
                     |||||||||:||.|||||||||||||||||||||.|||||||:|||||
Submitted_Seq      1 MEQLYSLQRSNSTSSFHDPYVNCASAFHLAGLGLGSGDFLGSRGMSSLGE     50

ci-PB             51 LHNAAVAAAAAGSLASTDFHFSVDGNRRLGSPRPPGGSIRASISRKRALS    100
                     ||:||||||||.|||||||||||||    ||||||||||||||||||||
Submitted_Seq     51 LHHAAVAAAAASSLASTDFHFSVDGNR----PRPPGGSIRASISRKRALS     96

ci-PB            101 SSPYSDSFDINSMIRFSPNSLATIMNGSRGSSAASGSYGHISATALNPMS    150
                     |||||||||||||||||||||||||||||||||.||||||||||.||||||
Submitted_Seq     97 SSPYSDSFDINSMIRFSPNSLATIMNGSRGSSTASGSYGHISANALNPMS    146

ci-PB            151 HVHSTRLQQIQAHLLRASAGLLNPMTPQQVAASGFSIGHMPTSASLRVND    200
                     |||||||||||||||||||||||||.||||||||||||..||.||||||
```

```
Submitted_Seq    147 HVHSTRLQQIQAHLLRASAGLLNPMTSQQVAASGFSIGHMSASACLRVND    196

ci-PB            201 VHPNLSDSHIQITTSPTV---TKDVSQVPAAAFSLKNLDDAREKKGPFKD    247
                     ||||||||...||||.||   .:|.:|:.|||.|||||||.:.|||.|||
Submitted_Seq    197 VHPNLSDSPSHITTSSTVRLVNEDPNQIAAAALSLKNLDDGKGKKGHFKD    246

ci-PB            248 VVPEQPSSTSGGVAQVEADSASSQLSDRCYNNVVNNITGIPGDVKVNSRL    297
                     ||.||||||||.||||||||||.||||||||||||..||||:||::||
Submitted_Seq    247 VVTEQPSSTSGAVAQVEADSASSHLSDRCYNNVVNNIKSIPGDIKVSTRL    296

ci-PB            298 DEYINCGSISIPSNEYDCANADTTDIKDEPGDFIETNCHWRSCRIEFITQ    347
                     |||||||:.|.|||||||||||||||||||||||||||||.||||||
Submitted_Seq    297 DEYINCGTASTPSNEYDCANADTTDIKDEPGDFIETNCHWRSCCIEFITQ    346

ci-PB            348 DELVKHINNDHIQTNKKAFVCRWEDCTRGEKPFKAQYMLVVHMRRHTGEK    397
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq    347 DELVKHINNDHIQTNKKAFVCRWEDCTRGEKPFKAQYMLVVHMRRHTGEK    396

ci-PB            398 PHKCTFEGCFKAYSRLENLKTHLRSHTGEKPYTCEYPGCSKAFSNASDRA    447
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq    397 PHKCTFEGCFKAYSRLENLKTHLRSHTGEKPYTCEYPGCSKAFSNASDRA    446

ci-PB            448 KHQNRTHSNEKPYICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKG    497
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq    447 KHQNRTHSNEKPYICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKG    496

ci-PB            498 LPLNDANSRLQQNNS--RHNLQEHNIDSSPCSEDSHLGKMLGTSSPSIKS    545
                     |||:|.|||||::||  ||||||||||||||||:||:||||||||||
Submitted_Seq    497 LPLDDVNSRLQRDNSHSRHNLQEHNIDSSPCSEDSHMGKILGTSSPSIKS    546

ci-PB            546 ESDISSSNHHLVNGVRASDSLLTYSPDDLAENLNLDDGWNCDDDVDVADL    595
                     |||||||||.||||||||||||||||:||||||||||||||||||||||
Submitted_Seq    547 ESDISSSNHQLVNGVRASDSLLTYSPDDVAENLNLDDGWNCDDDVDVADL    596

ci-PB            596 PIVLRAMVNIGNGNASASTIGGSVLARQRFRGRLQTKGINSSTIMLCNIP    645
                     ||||||||||:|:||||||||:||||||||.||||||||||||||||||
Submitted_Seq    597 PIVLRAMVNIGSGHASASTIGGAVLARQRFRSRLQTKGINSSTIMLCNIP    646

ci-PB            646 ESNRTFGISELNQRITELKMEPGTDAEIKIPKLPNTTIGGYTEDPLQNQT    695
                     |||.|.|||||||||||||||..|||||...||.|||:.|:.||||.
Submitted_Seq    647 ESNHTIGISELNQRITELKMEPGTAGEIKIPMPTNTAIGGFPEELLQNQG    696

ci-PB            696 SFRNTVSNKQG--TVSGSIQGQFRRDSQNSTASTYYGSMQSRRSSQSSQV    743
                     :.||||.||||  |.|||:||||||||||||||||||||||||||||||
Submitted_Seq    697 TSRNTVLNKQGISTASGSVQGQFRRDSQNSTASTYYGSMQSRRSSQSSQV    746

ci-PB            744 SSIPTMRPNPSCNST-ASFYDPISPGCSRRSSQMSNGANCNSFTSTSGLP    792
                     ||||||||:|:|.:| ||||||||||||||||||.|||.:|:||||||
Submitted_Seq    747 SSIPTMRPSPTCTTTTASFYDPISPGCSRRSSQMSNSANCYAFSSTSGLP    796

ci-PB            793 VLNKESNKSLNACINKPNIGVQGVGIYNSSLPPPPSSHLIATNLKRLQRK    842
                     ::||:||.|.||.|||||:||..|||.|||||||||||||||||||.|
Submitted_Seq    797 IINKDSNNSTNAFINKPNLGVNSVGIDNSSLPPPPSSHLIATNLKRLQGK    846

ci-PB            843 DSE--YHNFTSGRFSVPSYMHSLHIKNNKPVGENEFDKAIASNA-RRQTD    889
                     |||  |||||||||.:||.|||||:||:.|||:|||||.||:|. ||||:
Submitted_Seq    847 DSENCYHNFTSGRFCIPSCMHSLHMKNSNPVGQNEFDKVIANNTLRRQTE    896
```

```
ci-PB            890 PVPNINLDPLTNISRFSTTPHSFDINVGKTNNIASSINKDNLRKDLFTVS    939
                     ||||:|||.||||.|.||||:||||.|||||||||||||:|||:|.||.
Submitted_Seq    897 PVPNLNLDSLTNIPRLSTTPNSFDITVGKTNNIASSINKDSLRKELCTVP    946

ci-PB            940 IKADMAMTSDQHPNERINLDEVEELILPDEMLQYLNLVKDDTNHLEKEHQ    989
                     ||||||||||||||||||||||||||||||||||:|||:||||.|||||
Submitted_Seq    947 IKADMAMTSDQHPNERINLDEVEELILPDEMLQYLSLVKEDTNHTEKEHQ    996

ci-PB            990 AVPVGSNVSETIASNHYREQSNIYYTNKQILTPPSNVDIQPNTTKFTVQD   1039
                     ...:||:|.||:.|||||||||||:|||||.||||||||||||. |:||
Submitted_Seq    997 TEAMGSSVYETLTSNHYREQSNIYYSNKQILAPPSNVDIQPNTTN-TIQD   1045

ci-PB           1040 KFAMTAVGGSFSQRELSTLAVPNEHGHAKCESFHHQSQKYMNTDIGSKQQ   1089
                     ||.|||:|||||||:.|||.||||||||||.|||||.:|.:||||..|||
Submitted_Seq   1046 KFPMTAIGGSFSQRQSSTLVVPNEHGHAKCGSFHHQCEKIINTDIDIKQQ   1095

ci-PB           1090 SALPSAHQRQTEKSNYNQIIDSSMTSLPELNVDSIYPRNETENIFKVHGD   1139
                     :.||.|:|||||||.|:|||||||||||||||.|||.:|||||||:||.|
Submitted_Seq   1096 TPLPPAYQRQTEKPNFNQIIDSSMTSLPELNVVSIYTQNETENIFEVHRD   1145

ci-PB           1140 HDNEIQCGIISQSQMSPSTNLNNDGQFSTVNMQPITTSKLFPPEPQKIVC   1189
                     |||||||||||||||||||||:||.||.|:||||.||||..|.|||||
Submitted_Seq   1146 HDNEIQCGIISQSQMSPSTNLNNEGQISTANIQPITISKLFSSETQKIVC   1195

ci-PB           1190 DTQASNTSVMHLDTYQRTLEYVQSCQNWMETNNTSTNQIQSLPG-MPVNN   1238
                     |||.:|:|||||||||||||||||||||:|.||.||:.|| |.||.
Submitted_Seq   1196 DTQTNNSSVMHLDTYQRTLEYVQSCQNWMETNSTVTNPIQAPPGGMQVNT   1245

ci-PB           1239 TLFPDVSSSTHPYHGTNMVINDMTTSLTSLLEENRYLQMMQ   1279
                     ||:|||||||||||||||||||:||||||||||||||
Submitted_Seq   1246 TLWPDVSSSTHPYHGTNMVINDMTTSLSSLLEENRYLQMMQ   1286
```

3. To access files that need to be downloaded for the report, click on the Downloads tab to get the following screen.



Right click on each file and save to a folder on the desktop of your computer, or other convenient location (such as a jump drive).

The filenames will appear as follows when saved . 130128204226.fasta; 130128204226.gff; 130128204226.pep. The numbers incorporate the date the files were saved into a file name. The extensions refer the file type: .fasta is the transcript file, .pep is the peptide file, and .gff contains the coordinates of your gene model from Gene Model Checker. Sometimes the fasta extension doesn't show up.

Project Submission

Although we won't be submitting the practice annotation of the ci-RA gene, for your final project, once the gene model is complete, the information must be submitted to the GEP using the Project Management System. These steps are as follows.

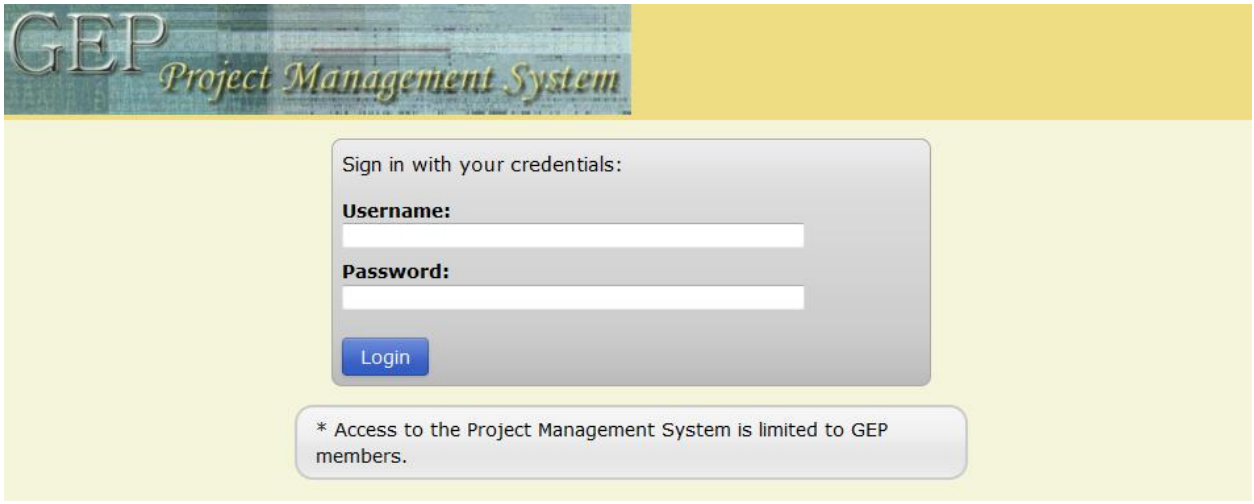
1. Navigate to the annotation submission report form via the GEP web site by following these links: **GEP →curriculum →course materials -→ Washington University → GEP specific issues →GEP annotation report.** Download the MS Word version of the report form.

2. Fill out this form. A sample report filled out for the ci-RB isoform annotated in this tutorial is included below.

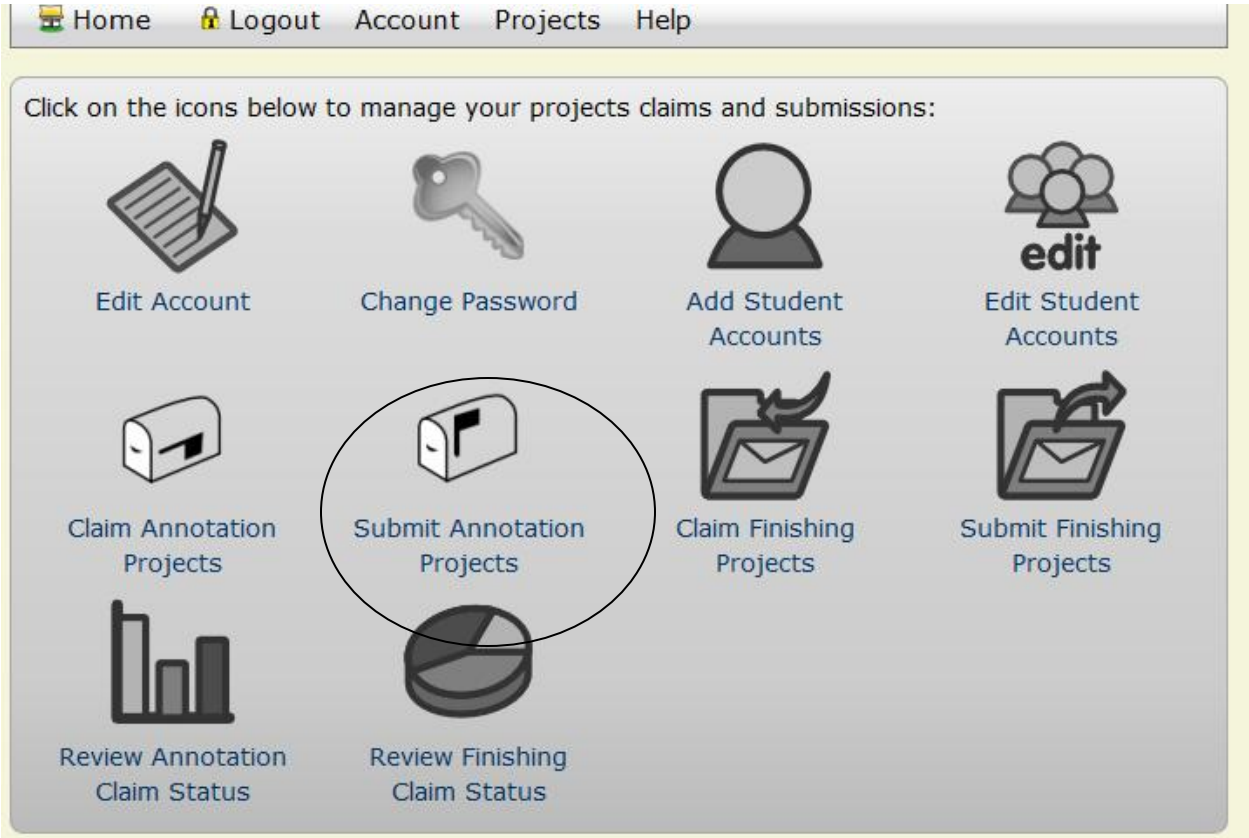
3. Download and merge all GFF, transcript and peptide files using the Annotation File Merger. **(GEP →Projects → Annotation Resources → Annotation Files Merger)**

4. Once the form is filled out, and all GFF, transcript, and peptide files have been merged navigate to the Annotation Submission form in the Project management system.

GEP →Projects → Project Management system to this screen



The GEP faculty will need to log in to this site, leading to the screen on the following page



Click on 'Summit Annotation Projects'. You will need to have claimed a project to be able to submit the results. Click on your project and follow instructions for submission and upload all files as instructed.

And you're done, now that wasn't so hard was it? ☺

ASSIGNMENT:

Repeat the annotation process for the ci-RA or ci-RC isoform.

Turn in to your professor, by email, a completed report with all necessary figures pasted in as described above. Also download the .gff, .fasta, amd .pep files and attach these along with your submission

A completed report for the ci-RB isoform is shown starting on the next page

GEP Annotation Report

Note: For each gene described in this annotation report, you should also prepare the corresponding GFF, transcript and peptide sequence files as part of your submission.

Student name: _____
Student email: _____
Faculty Advisor: _____
College/University: _____

Project details

Project name: __Derecta Dot Contig38_____
Project species: ___D. erecta_____
Date of submission: __2/5/2013_____
Size of project in base pairs: ___53,840_____
Number of genes in project: _____

Does this report cover all genes and all isoforms or is it a partial report? _____
If this is a partial report because different students are working on different regions of this sequence, please report the region of the project covered by this report:
from base ___15405 _____ to base 19519_____

Instructions for project with no genes

If you believe that the project does not contain any genes, please provide the following evidence to support your conclusions:

1. Perform a BLASTX search of the entire contig sequence against the non-redundant (*nr*) protein database. Provide an explanation for any significant (E-value < 1e-5) hits to known genes in the nr database as to why they do not correspond to real genes in the project.

2. For each Genscan prediction, perform a BLASTP search using the predicted amino acid sequence against the protein database (*nr*) using the strategy described above.

3. Examine the gene expression tracks (e.g. cDNA/EST/RNA-Seq) for evidence of transcribed regions that do not correspond to alignments to known *D. melanogaster* proteins. Perform a BLASTX search against the *nr* database using these genomic regions to determine if the region is similar to any known or predicted proteins in the *nr* database.

Complete the following Gene Report Form for each gene in your project. Copy and paste the sections below to create as many copies as needed. Be sure to create enough Isoform Report Forms within your Gene Report Form for all isoforms.

Gene report form

Gene name (i.e. *D. mojavensis eyeless*): ___*D. erecta cubitus interruptus*_____
Gene symbol (i.e. dmoj_ey): ____dere_ci_____
Approximate location in project (from 5' end to 3' end): ___15405-19519_____
Number of isoforms in *D. melanogaster:* _____3_____
Number of isoforms in this project: _____1_____

Complete the following table for all the isoforms in this project:
If you are annotating untranslated regions then all isoforms are unique (by definition)

Name of unique isoform based on coding sequence	**List of isoforms with identical coding sequences**
ci-RB	ci-RB *in some cases isoforms will differ at the RNA level, but have the same coding sequence. List all of these here. Also upload the GFF, transcript, and pep files for these isoforms*
Other isoforms will differ at the protein level, e.g. ci-RA and ci-RC in our example. List those in separate boxes here.	*If there are multiple isoforms coding for the same proteins for these, list them here. Also upload the GFF, transcript, and pep files for these isoforms*

Note: For isoforms with identical coding sequence, you only need to complete the Isoform Report Form for one of these isoforms (i.e. using the name of the isoform listed in the left column of the table above). However, you should generate GFF, transcript, and peptide sequence files for <u>ALL</u> isoforms, irrespective of whether they have identical coding sequences as

Isoform report form

Complete this report form for each unique isoform listed in the table above (copy and paste to create as many copies of this Isoform Report Form as needed):

Gene-isoform name (i.e. dmoj_ey-PA): __dere_ci-RB_____
Names of the isoforms with identical coding sequences as this isoform
_____N?A_____
_____

Is the 5’ end of this isoform missing from the end of project: ____no_____If so, how many exons are missing from the 5’ end: _____
Is the 3’ end of this isoform missing from the end of the project: _____no_____If so, how many exons are missing from the 3’ end: _____

1. Gene Model Checker Checklist

Enter the coordinates of your final gene model for this isoform into the Gene Model Checker and **paste a screenshot of the checklist results below:**

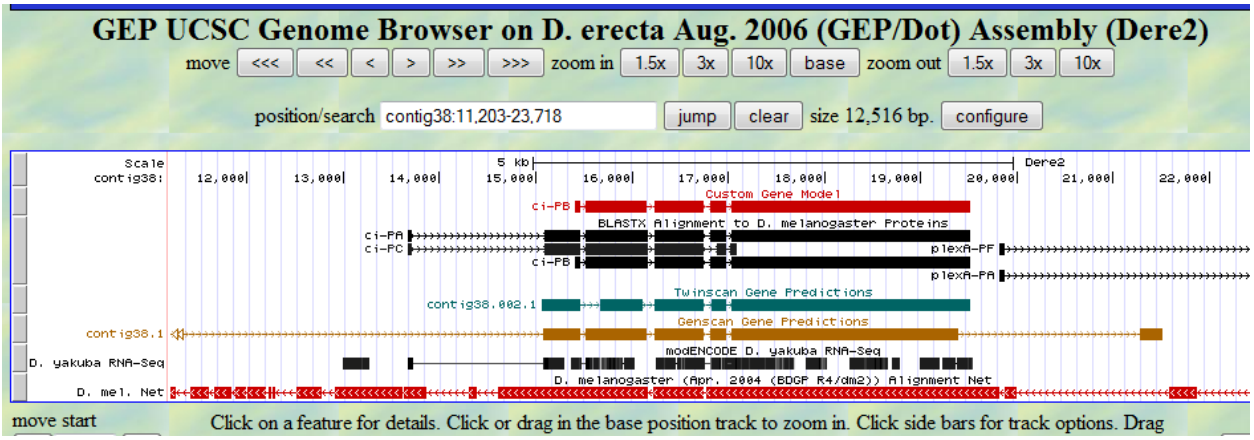
View	Criteria	Status	Message
🔍	Check for Start Codon	Pass	
	Acceptor for CDS 1	Skip	Already checked for Start Codon
🔍	Donor for CDS 1	Pass	
🔍	Acceptor for CDS 2	Pass	
🔍	Donor for CDS 2	Pass	
🔍	Acceptor for CDS 3	Pass	
🔍	Donor for CDS 3	Pass	
🔍	Acceptor for CDS 4	Pass	
🔍	Donor for CDS 4	Pass	
🔍	Acceptor for CDS 5	Pass	
	Donor for CDS 5	Skip	Already checked for Stop Codon
🔍	Check for Stop Codon	Pass	
🔍	Additional Checks	Pass	
🔍	Number of coding exons matched D. melanogaster ...	Pass	

2. View the gene model on the Genome Browser

Using the custom track feature from the Gene Model Checker (see page 10 of the Gene Model Checker user guide on how to do this; you can find the guide under “Help” -> “Documentations” -> “Web Framework” on the GEP website at http://gep.wustl.edu). Capture a screenshot of your gene model shown on the Genome Browser for your project; zoom in so that only this isoform is in the screenshot. Include the following evidence tracks in the screenshot if they are available.

1. A sequence alignment track (D. mel Protein or Other RefSeq)
2. At least one gene prediction track (e.g. Genscan)
3. At least one RNA-Seq track (e.g. RNA-Seq Alignment Summary)
4. A comparative genomics track
   (e.g. Conservation, D. mel. Net Alignment, 3-way, 5-way or 7-way multiz)

Paste the screenshot of your gene model as shown on the Genome Browser below:



3. Alignment between the submitted model and the *D. melanogaster ortholog*

Show an alignment between the protein sequence for your gene model and the protein sequence from the putative *D. melanogaster* ortholog. You can use the protein alignment generated by the Gene Model Checker or you can generate a new alignment using BLAST 2 Sequences (*bl2seq*). **Copy and paste the alignment below:**

```
ci-PB              1 MEQLYSLQRTNSASSFHDPYVNCASAFHLAGLGLGSADFLGSRGLSSLGE     50
                     |||||||||:||.|||||||||||||||||||||.|||||||:|||||
Submitted_Seq      1 MEQLYSLQRSNSTSSFHDPYVNCASAFHLAGLGLGSGDFLGSRGMSSLGE     50

ci-PB             51 LHNAAVAAAAAGSLASTDFHFSVDGNRRLGSPRPPGGSIRASISRKRALS    100
                     ||:||||||||.|||||||||||||||    ||||||||||||||||||
Submitted_Seq     51 LHHAAVAAAAASSLASTDFHFSVDGNR----PRPPGGSIRASISRKRALS     96

ci-PB            101 SSPYSDSFDINSMIRFSPNSLATIMNGSRGSSAASGSYGHISATALNPMS    150
                     ||||||||||||||||||||||||||||||||||.||||||||||.||||||
Submitted_Seq     97 SSPYSDSFDINSMIRFSPNSLATIMNGSRGSSTASGSYGHISANALNPMS    146

ci-PB            151 HVHSTRLQQIQAHLLRASAGLLNPMTPQQVAASGFSIGHMPTSASLRVND    200
                     |||||||||||||||||||||||||.|||||||||||||..||.||||||
Submitted_Seq    147 HVHSTRLQQIQAHLLRASAGLLNPMTSQQVAASGFSIGHMSASACLRVND    196

ci-PB            201 VHPNLSDSHIQITTSPTV---TKDVSQVPAAAFSLKNLDDAREKKGPFKD    247
                     ||||||||...||||.||    .:|.:|:.|||.|||||||.:.|||.|||
Submitted_Seq    197 VHPNLSDSPSHITTSSTVRLVNEDPNQIAAAALSLKNLDDGKGKKGHFKD    246

ci-PB            248 VVPEQPSSTSGGVAQVEADSASSQLSDRCYNNVVNNITGIPGDVKVNSRL    297
                     ||.||||||||.||||||||||.||||||||||||||..||||:||::||
Submitted_Seq    247 VVTEQPSSTSGAVAQVEADSASSHLSDRCYNNVVNNIKSIPGDIKVSTRL    296

ci-PB            298 DEYINCGSISIPSNEYDCANADTTDIKDEPGDFIETNCHWRSCRIEFITQ    347
                     |||||||:.|.||||||||||||||||||||||||||||||||.||||||
Submitted_Seq    297 DEYINCGTASTPSNEYDCANADTTDIKDEPGDFIETNCHWRSCCIEFITQ    346

ci-PB            348 DELVKHINNDHIQTNKKAFVCRWEDCTRGEKPFKAQYMLVVHMRRHTGEK    397
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq    347 DELVKHINNDHIQTNKKAFVCRWEDCTRGEKPFKAQYMLVVHMRRHTGEK    396
```

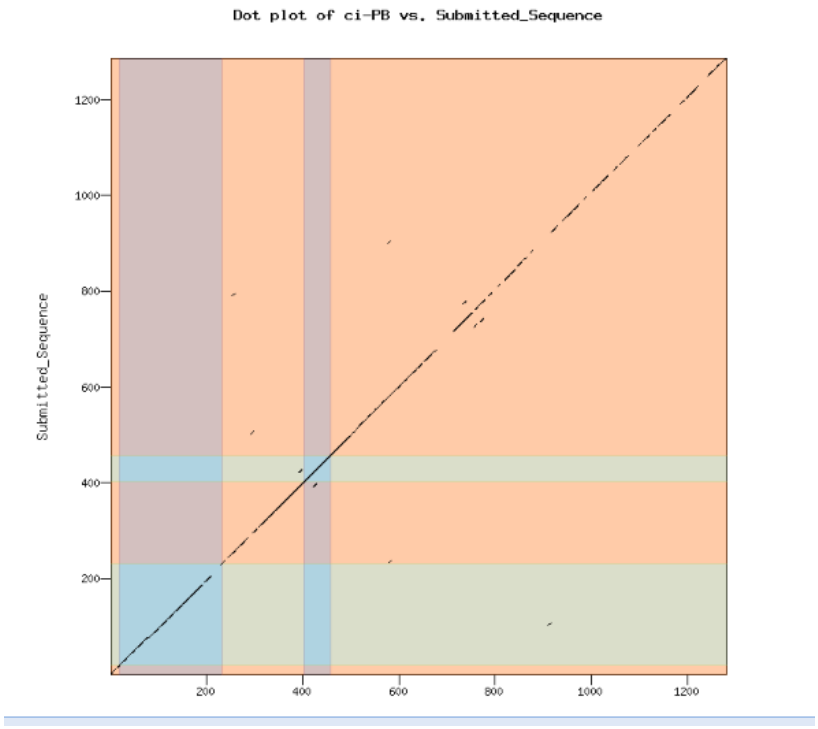
```
ci-PB           398 PHKCTFEGCFKAYSRLENLKTHLRSHTGEKPYTCEYPGCSKAFSNASDRA    447
                    ||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq   397 PHKCTFEGCFKAYSRLENLKTHLRSHTGEKPYTCEYPGCSKAFSNASDRA    446

ci-PB           448 KHQNRTHSNEKPYICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKG    497
                    ||||||||||||||||||||||||||||||||||||||||||||||||||
Submitted_Seq   447 KHQNRTHSNEKPYICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKG    496

ci-PB           498 LPLNDANSRLQQNNS--RHNLQEHNIDSSPCSEDSHLGKMLGTSSPSIKS    545
                    |||:|.||||||::||  ||||||||||||||||||:||:||||||||||
Submitted_Seq   497 LPLDDVNSRLQRDNSHSRHNLQEHNIDSSPCSEDSHMGKILGTSSPSIKS    546

ci-PB           546 ESDISSSNHHLVNGVRASDSLLTYSPDDLAENLNLDDGWNCDDDVDVADL    595
                    |||||||||.||||||||||||||||:||||||||||||||||||||||||
Submitted_Seq   547 ESDISSSNHQLVNGVRASDSLLTYSPDDVAENLNLDDGWNCDDDVDVADL    596

ci-PB           596 PIVLRAMVNIGNGNASASTIGGSVLARQRFRGRLQTKGINSSTIMLCNIP    645
                    ||||||||||:|:||||||||:||||||||.||||||||||||||||||||
Submitted_Seq   597 PIVLRAMVNIGSGHASASTIGGAVLARQRFRSRLQTKGINSSTIMLCNIP    646

ci-PB           646 ESNRTFGISELNQRITELKMEPGTDAEIKIPKLPNTTIGGYTEDPLQNQT    695
                    |||.|.|||||||||||||||..|||||...||.|||:.|:.||||.
Submitted_Seq   647 ESNHTIGISELNQRITELKMEPGTAGEIKIPMPTNTAIGGFPEELLQNQG    696

ci-PB           696 SFRNTVSNKQG--TVSGSIQGQFRRDSQNSTASTYYGSMQSRRSSQSSQV    743
                    :.||||.||||  |.|||:||||||||||||||||||||||||||||||||
Submitted_Seq   697 TSRNTVLNKQGISTASGSVQGQFRRDSQNSTASTYYGSMQSRRSSQSSQV    746

ci-PB           744 SSIPTMRPNPSCNST-ASFYDPISPGCSRRSSQMSNGANCNSFTSTSGLP    792
                    ||||||||:|:|.:| ||||||||||||||||||.|||.:|:||||||
Submitted_Seq   747 SSIPTMRPSPTCTTTTASFYDPISPGCSRRSSQMSNSANCYAFSSTSGLP    796

ci-PB           793 VLNKESNKSLNACINKPNIGVQGVGIYNSSLPPPPSSHLIATNLKRLQRK    842
                    ::||:||.|.||.|||||:||..|||.|||||||||||||||||||.|
Submitted_Seq   797 IINKDSNNSTNAFINKPNLGVNSVGIDNSSLPPPPSSHLIATNLKRLQGK    846

ci-PB           843 DSE--YHNFTSGRFSVPSYMHSLHIKNNKPVGENEFDKAIASNA-RRQTD    889
                    |||  |||||||||.:||.|||||:||:.|||:|||||.||:|. ||||:
Submitted_Seq   847 DSENCYHNFTSGRFCIPSCMHSLHMKNSNPVGQNEFDKVIANNTLRRQTE    896

ci-PB           890 PVPNINLDPLTNISRFSTTPHSFDINVGKTNNIASSINKDNLRKDLFTVS    939
                    ||||:|||.||||.|.||||:||||.|||||||||||||:|||:|.||.
Submitted_Seq   897 PVPNLNLDSLTNIPRLSTTPNSFDITVGKTNNIASSINKDSLRKELCTVP    946

ci-PB           940 IKADMAMTSDQHPNERINLDEVEELILPDEMLQYLNLVKDDTNHLEKEHQ    989
                    ||||||||||||||||||||||||||||||||||:|||:||||.|||||
Submitted_Seq   947 IKADMAMTSDQHPNERINLDEVEELILPDEMLQYLSLVKEDTNHTEKEHQ    996

ci-PB           990 AVPVGSNVSETIASNHYREQSNIYYTNKQILTPPSNVDIQPNTTKFTVQD   1039
                    ...:||:|.||:.|||||||||||:|||||.||||||||||||. |:||
Submitted_Seq   997 TEAMGSSVYETLTSNHYREQSNIYYSNKQILAPPSNVDIQPNTTN-TIQD   1045

ci-PB          1040 KFAMTAVGGSFSQRELSTLAVPNEHGHAKCESFHHQSQKYMNTDIGSKQQ   1089
                    ||.|||:|||||||:.|||.||||||||||.|||||.:|.:||||..|||
Submitted_Seq  1046 KFPMTAIGGSFSQRQSSTLVVPNEHGHAKCGSFHHQCEKIINTDIDIKQQ   1095
```

```
ci-PB           1090 SALPSAHQRQTEKSNYNQIIDSSMTSLPELNVDSIYPRNETENIFKVHGD   1139
                     :.||.|:|||||||.|:|||||||||||||||.|||.:||||||||:||.|
Submitted_Seq   1096 TPLPPAYQRQTEKPNFNQIIDSSMTSLPELNVVSIYTQNETENIFEVHRD   1145

ci-PB           1140 HDNEIQCGIISQSQMSPSTNLNNDGQFSTVNMQPITTSKLFPPEPQKIVC   1189
                     |||||||||||||||||||||||:||.||.|:||||.||||..|.|||||
Submitted_Seq   1146 HDNEIQCGIISQSQMSPSTNLNNEGQISTANIQPITISKLFSSETQKIVC   1195

ci-PB           1190 DTQASNTSVMHLDTYQRTLEYVQSCQNWMETNNTSTNQIQSLPG-MPVNN   1238
                     |||.:|:|||||||||||||||||||||||:|.||.||:.|| |.||.
Submitted_Seq   1196 DTQTNNSSVMHLDTYQRTLEYVQSCQNWMETNSTVTNPIQAPPGGMQVNT   1245

ci-PB           1239 TLFPDVSSSTHPYHGTNMVINDMTTSLTSLLEENRYLQMMQ   1279
                     ||:|||||||||||||||||||||:||||||||||||||
Submitted_Seq   1246 TLWPDVSSSTHPYHGTNMVINDMTTSLSSLLEENRYLQMMQ   1286
```

4. Dot plot between the submitted model and the *D. melanogaster ortholog*

Paste a copy of the dot plot of your submitted model against the putative *D. melanogaster* ortholog (generated by the Gene Model Checker). **Provide an explanation for any anomalies** on the dot plot (e.g. large gaps, regions with no sequence similarity).

View protein alignment



Note: Large <u>vertical and horizontal gap</u> near exon boundaries in the dot plot often indicates that an incorrect splice site might have been picked. Please re-examine these regions and provide a detail justification as to why you have selected this particular set of donor and acceptor sites.

Preparing the project for submission

For each project, you should prepare the project GFF, transcripts and peptide sequence files (for **ALL** isoforms) along with this report. You can combine the individual files generated by the Gene Model Checker into a single file using the Annotation Files Merger.

The Annotation Files Merger also allows you to view all the gene models in the combined GFF file within the Genome Browser. Please refer to the Annotation Files Merger User Guide for detail instructions on how to view the combined GFF file on the Genome Browser (you can find the user guide under "Help" -> "Documentations" -> "Web Framework" on the GEP website at http://gep.wustl.edu).

Paste a screenshot (generated by the Annotation Files Merger) with all the gene models you have annotated in this project.

For the practice annotation you are only annotating one isoforms so you don't need to merge these files. For your final project you may need to merge the files for several isoforms of a gene.

The Annotation Files Merger is found on the GEP website at

GEP →Projects → Annotation Resources → Annotation Files Merger

Have you annotated all the genes?

For each region of the project with gene predictions that do not overlap with putative orthologs identified in the BLASTX track, perform a BLASTP search using the predicted amino acid sequence against the non-redundant protein database (*nr*). **Provide a screenshot of the search results.** Provide an explanation for any significant (E-value < 1e-5) hits to known genes in the *nr* database and why you believe these hits do not correspond to real genes in your project.

*For example, if there's a genscan prediction, but no corresponding blastx hit, click on the genscan gene model, get the predicted protein sequence and use this in a blastp search of the D. melanogaster nr protein database (the **nr** data base, not the refseq database)*

References:

The GEP website: www.gep.wustl.edu

Emerson, Julia A., C. Silver Key, C. J. Alvarez, S. Mel, G. McNeil, K. J. Saville, W. Leung, C. D. Shaffer and Sarah C. R. Elgin. Introduction to the Genomics Education Partnership and Collaborative Genomics Research in Drosophila. ABLE workshop 2012