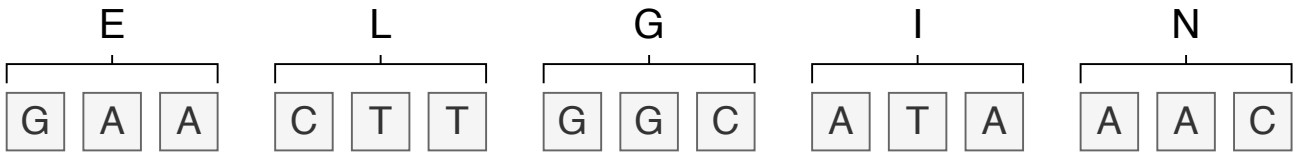
Determining CDS Coordinates



tblastn

query - CDS for *D. melanogaster* exon
subject - nucleotide (nt) sequence of interest (turn off low complexity filter and use no compositional adjustment)

For **highly conserved alignments**: look for splice sites nearest the exon boundaries
For **less conserved alignments**: attempt to extend the exon boundaries to include additional sequence from the Open Reading Frame (ORF), while still maintaining correct splice phases; conserve exon size

Exon

5' end

Is the splice acceptor site AG?

YES

Check phase compatibility with previous splice donor site

Check for predicted acceptor splice site markers in the GEP UCSC Genome Browser

Acceptor site must be AG; look for another possibility

NO

3' end

What is the splice donor site?

GT

Note the phase and make sure it aligns with the next acceptor site

Check for predicted donor splice site markers in the GEP UCSC Genome Browser

Check for nearest GT that will fit the donor phase

If no GT possible because it violates minimum intron size rule (~40 nt) when looking downstream or leads to loss of conserved sequence when looking upstream

GC

Look for more evidence to support this non-canonical, alternative splice site

Go to flybase.org to check the intron sequence of interest:
Is the non-canonical splice site present for this gene in *D. melanogaster*?

NO

YES

Continue looking for other evidence to justify GC

Other lines of evidence possible to use to verify one GT is better than another

1. If available, check RNA-Seq evidence to prove canonical or alternative splice site exists (e.g., TopHat or regtools junctions)

2. Use the gene prediction tracks in the GEP UCSC Genome Browser to support alignment to selected site or demonstrate alternative solution

3. Use the *blastx* track to verify level and length of conservation

4. Check closely-related species for choice of splice site (BLAT analysis): sequencing error?

5. Perform Clustal Omega searches to test level of conservation between species

Adapted from Jeannette Wong 08/05/2010
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