Viewing alignments with IGV

Ester Feldmesser

SAM format

HWI-ST808:87:C068VACXX:2:1101:1234:2199 16 chr5 177482768 2 100M * 0 0
TGACGGTCCATTCCCGGGCTCGATGCCGGAAAAACCCCTTGGCCCGCCGGAAGGGCAGGCACATGGGCATAGGTAAGCGGAAGGGTACAGCCAATGCACG
######@CA?5&DBB@@9BA99<7@98:(?@?5)5(@?<807DCBHFHGBIIIHHHEF@@FB?3GIIIIGGGGEIGFIIIHEFBIGFFFBHFEDDAA:=:
AS:i:-29 XS:i:-32 XN:i:0 XM:i:6 XO:i:0 XG:i:0 NM:i:6 MD:Z:35A26G3C7G3A18C2 YT:Z:UU

Alignments are reported in a compact representation: SAM format

```
0       61G9EAAXX100520:5:100:10095:16477  (read name)
1       83  (FLAGS stored as bit fields; 83 = 00001010011 )
2       chr1  (alignment target)
3       51986 (position alignment starts)
4       38
5       46M  (Compact description of the alignment in CIGAR format)
6       =
7       51789
8       -264
9       CCCAAACAAGCCGAACTAGCTGATTTGGCTCGTAAAGACCCGGAAA   (read sequence, oriented according to the forward alignment)
10      ###CB?=ADDBCBCDEEFFDEFFFDEFFGDBEFGEDGCFGFGGGGG   (base quality values)
11      MD:Z:67
12      NH:i:1
13      HI:i:1
14      NM:i:0
15      SM:i:38          (Metadata)
16      XQ:i:40
17      X2:i:0
```

SAM format specification: http://samtools.sourceforge.net/SAM1.pdf

More output formats

SAM format

```
HWI-ST808:87:C068VACXX:2:1101:1234:2199 16   chr5 177482768 2        100M  *        0     0
TGACGGTCCATTCCCGGGCTCGATGCCGGAAAAACCCCTTGGCCCGCCGGAAGGGCAGGCACATGGGCATAGGTAAGCGGAAGGGTACAGCCAATG
######@CA?5&DBB@@9BA99<7@98:(?@?5)5(@?<807DCBHFHGBIIIHHHEF@@FB?3GIIIGGGGEIGFIIIHEFBIGFFFBHFED
AS:i:-29  XS:i:-32  XN:i:0    XM:i:6    XO:i:0    XG:i:0    NM:i:6    MD:Z:35A26G3C7G3A18C2
     YT:Z:UU
```

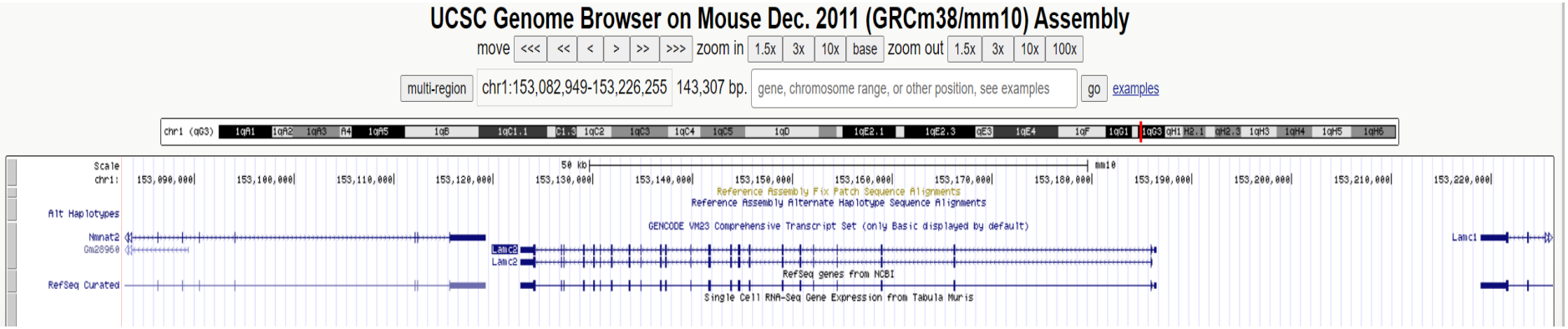
BAM format – Binary format of the SAM file, it saves disk space.

BAM.bai format – Index for the bam format

For viewing BAM files, an index file must be found in the same directory as the BAM file. The index should be named by appending "**.bai**" to the BAM file name.

Genome browser

- It is a graphical interface to display genomic data information from a biological database
- Usually the genome is represented horizontally
- Feature such as gene definitions, mRNAs, gene prediction and structure, proteins, expression, regulation, variation, comparative analysis can be displayed

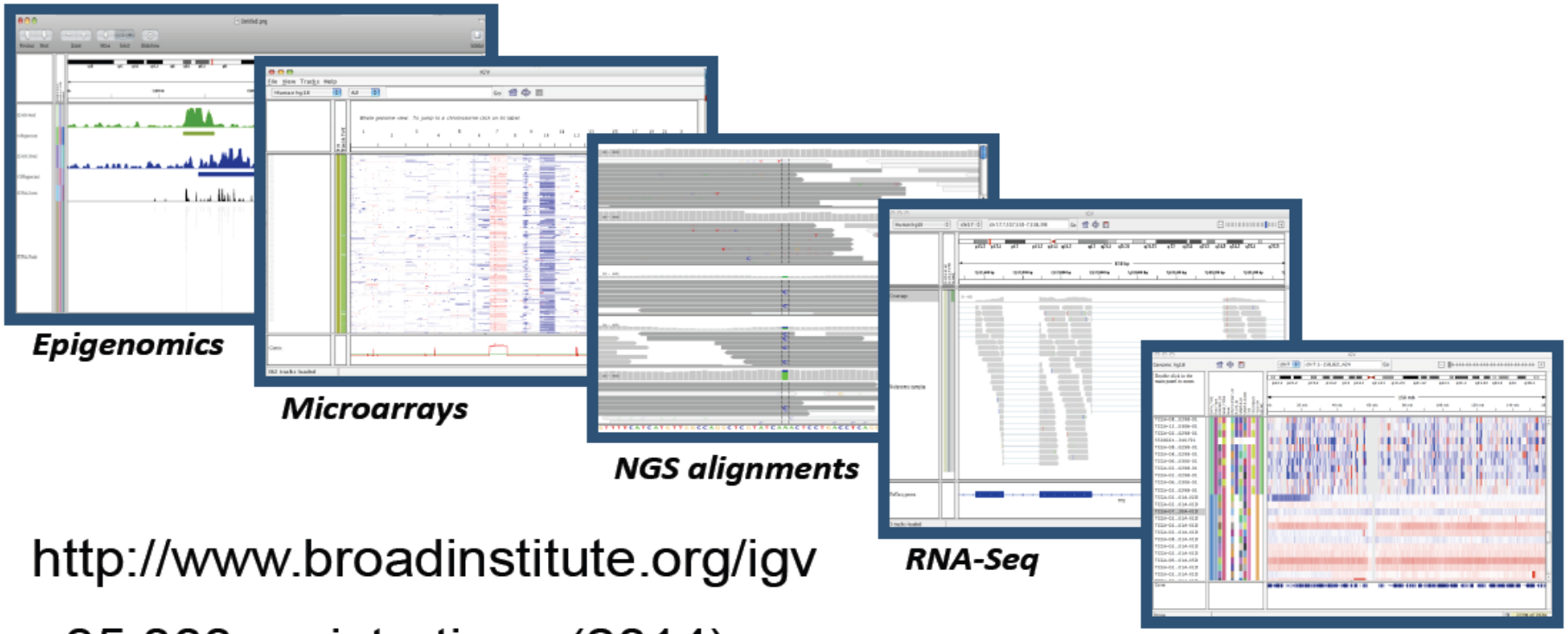


Integrated Genomics Viewer (IGV)

- The **Integrative Genomics Viewer (IGV)** is a high-performance visualization tool for interactive exploration of large, integrated genomic datasets.
- It was developed at the Broad Institute (http://software.broadinstitute.org/software/igv/home).
- It supports a wide variety of data types, including next-generation sequence data, and genomic annotations.
- It supports a wide variety of file formats to upload data (http://software.broadinstitute.org/software/igv/FileFormats).

Integrative Genomics Viewer (IGV)

Desktop application for the interactive visual exploration of integrated genomic datasets



http://www.broadinstitute.org/igv

>85,000 registrations (2014)

Opening IGV

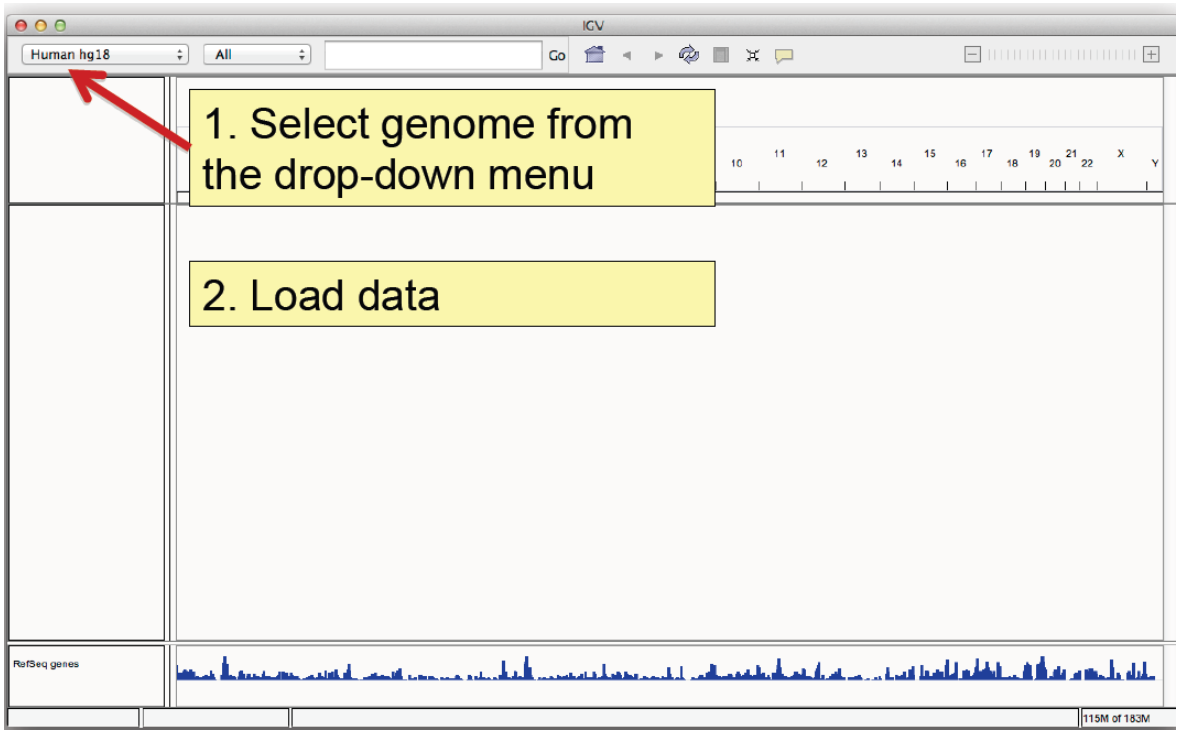
1. Go to https://software.broadinstitute.org/software/igv/download
2. You might be requested to register if you are not
3. Click on the arrow to download the IGV installer
4. Save the installer
5. Double-click the installer to run the installation
6. An IGV shortcut will be created on the Desktop
7. Double-click it to run the application.
8. Allow Java to run if requested

9. Note: Most files that you load need to be indexed

Using IGV

User guide:
https://software.broadinstitute.org/software/igv/book/export/html/6

A genome will be loaded when you open IGV. You can change the genome by clicking the drop down menu in the upper-left.

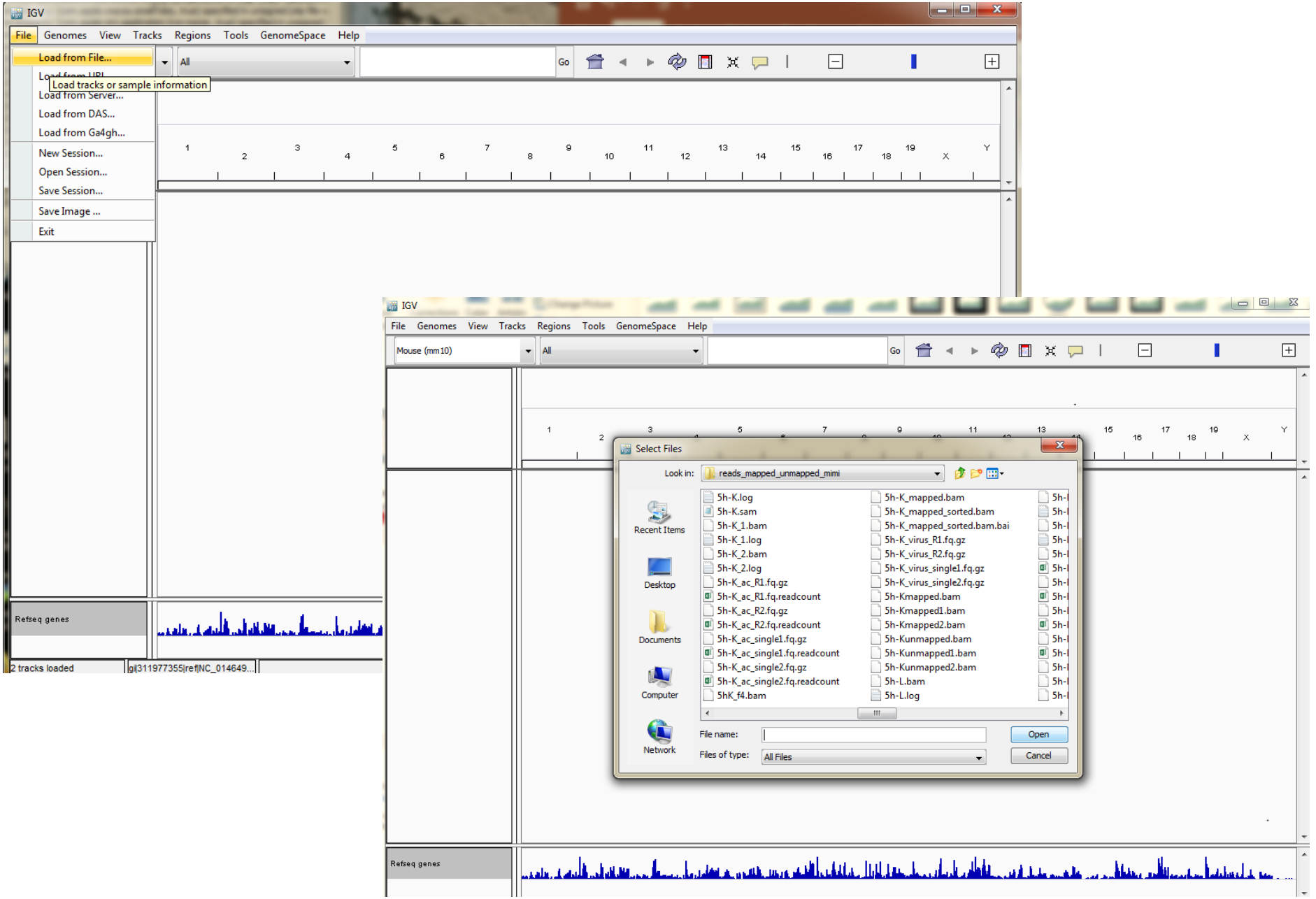


File Formats

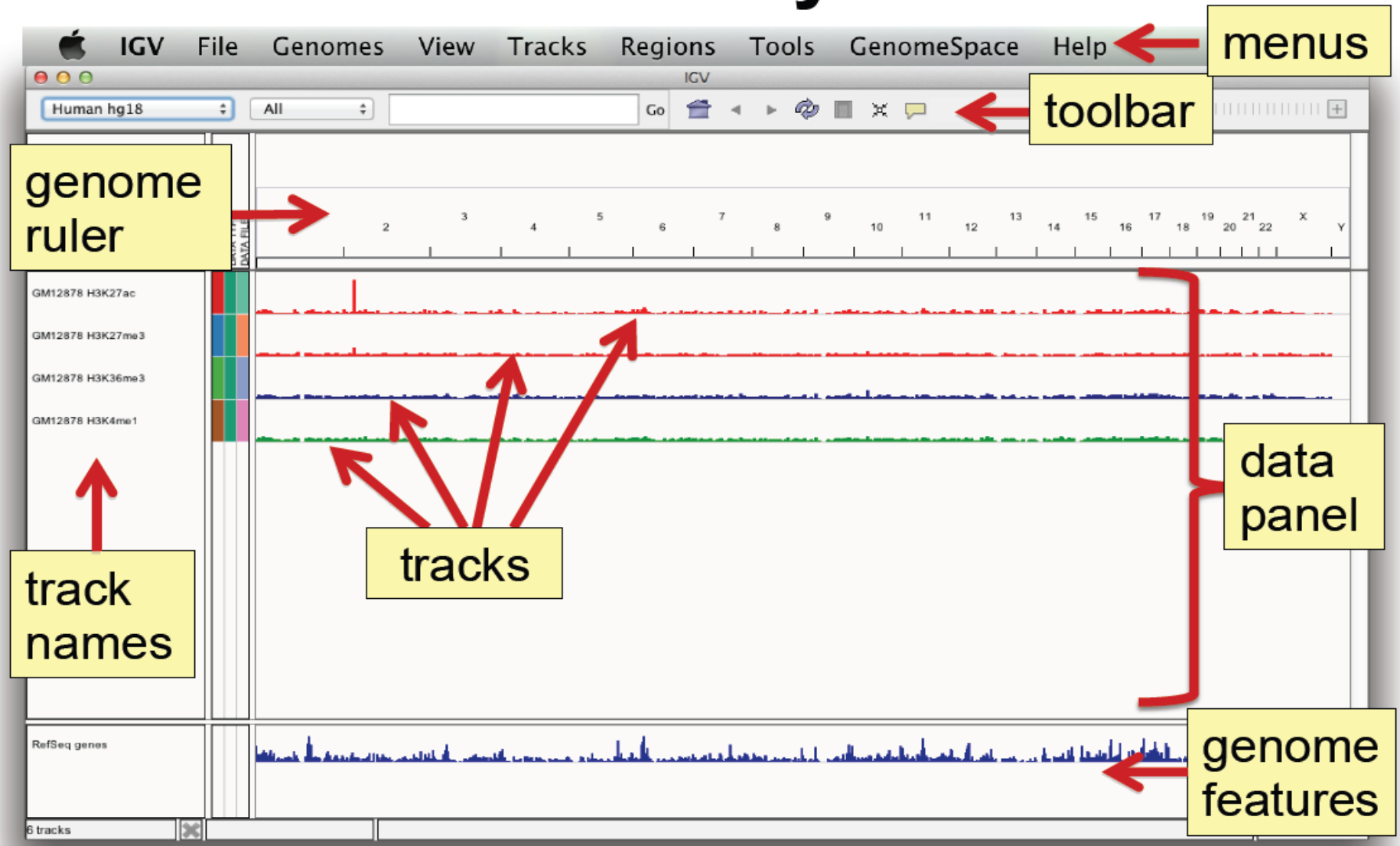
Most files formats need an index

- BAM
- BED
- broadPeak
- FASTA
- GFF/GTF
- Merged BAM File
- narrowPeak
- PSL
- SNP
- TAB
- TDF
- VCF

Load the data

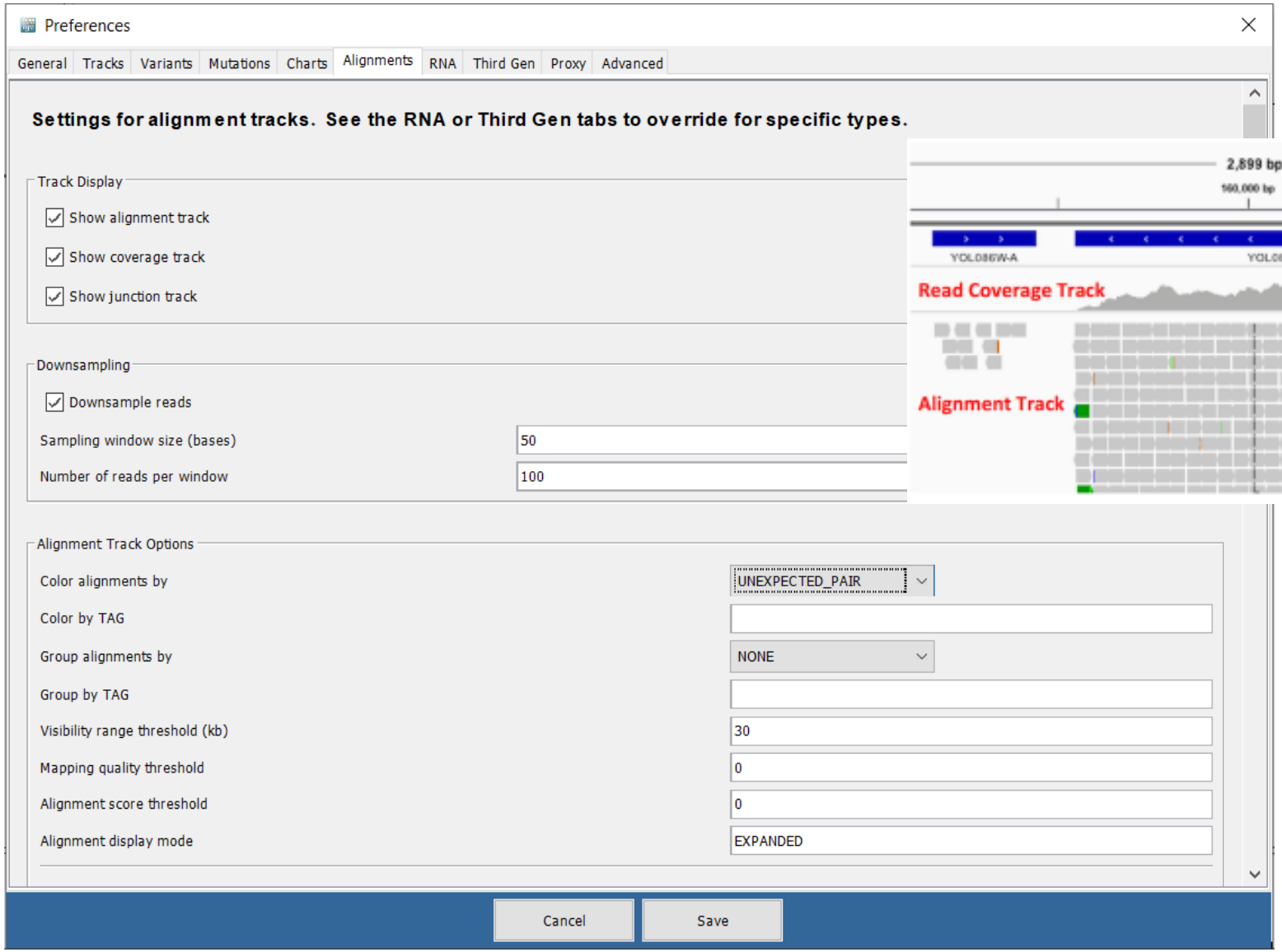


Screen layout

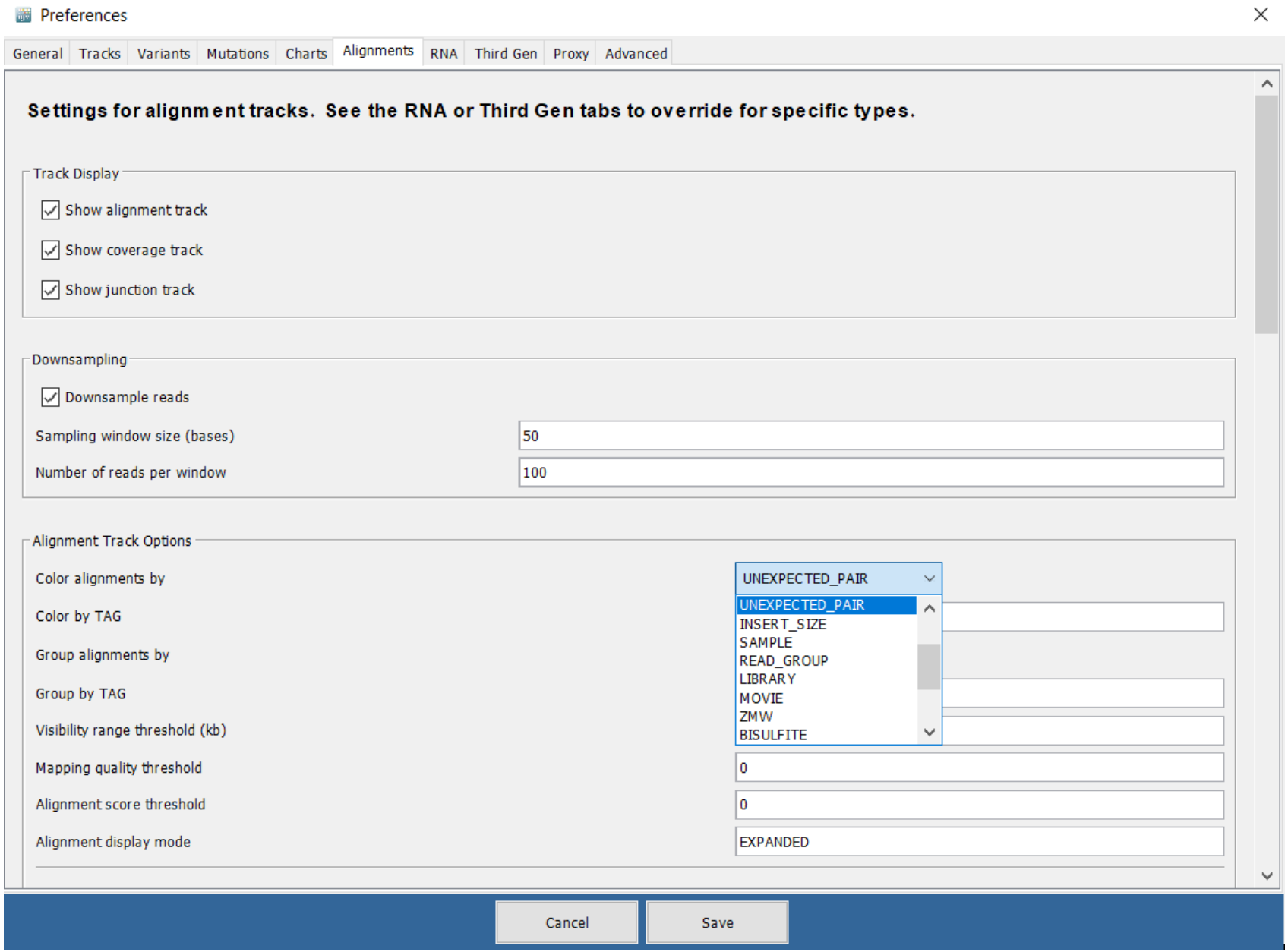


Preferences

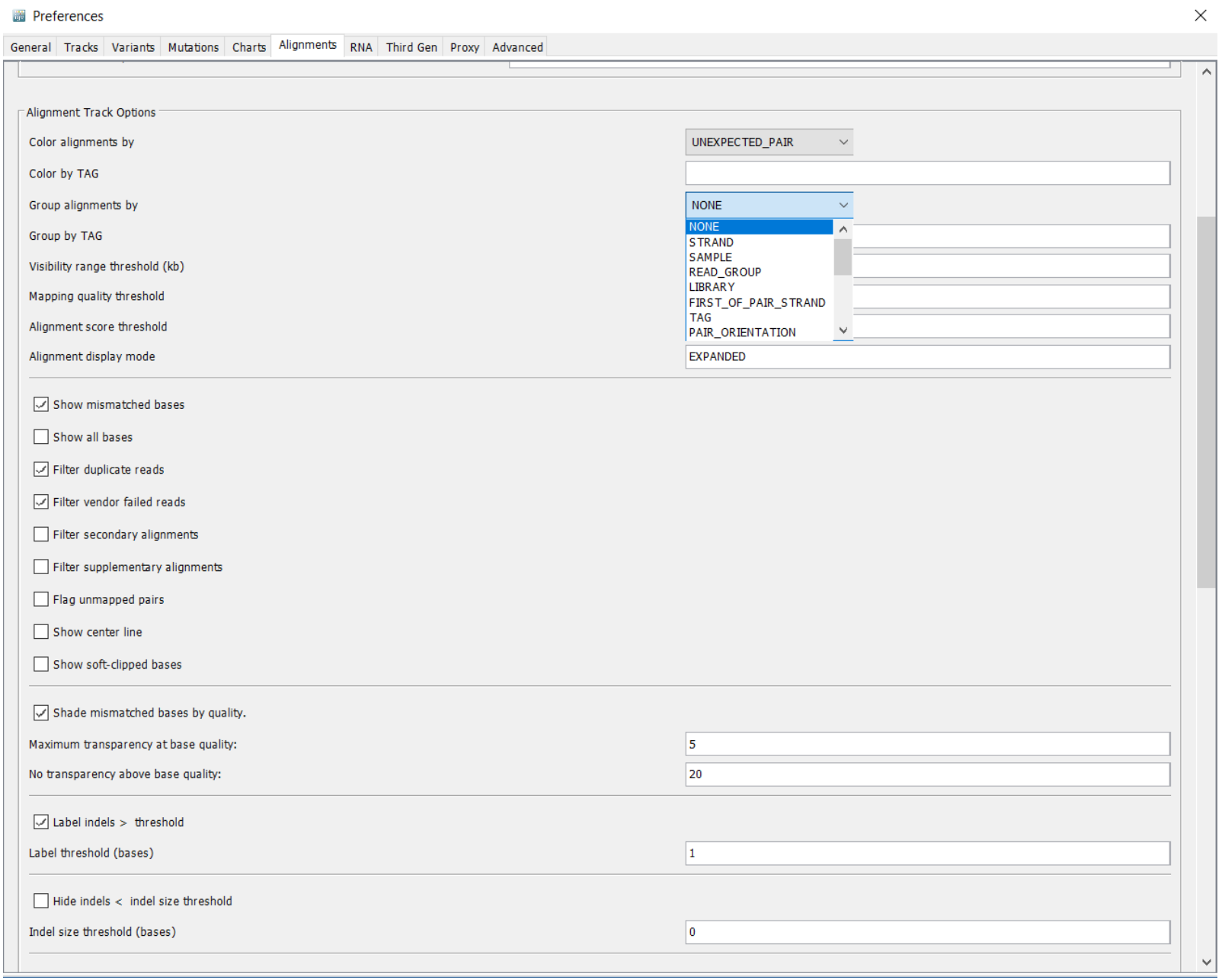
To display the Preferences window, click *View>Preferences*.



Preferences

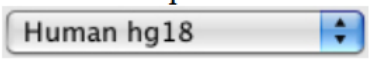


Preferences



Tool Bar

Genome drop-down box

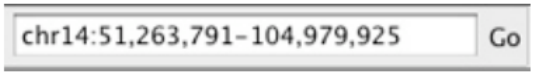


Loads a genome. more...

Chromosome drop-down box

Zooms to a chromosome. more...

Search box



Displays the chromosome location being shown. To scroll to a different location, enter the gene name, locus, or track name and click Go. more...

Whole genome view

Zooms to whole genome view. more...

Moves backward and forward through views of the genome like the back and forward buttons in a web browser.

Refresh

Refreshes the display.

Define a region

Defines a region of interest on the chromosome. more...

Reduces the row height on all tracks to fit all data for the region in view into the window; will also expand tracks (to their maximum preferred size) to fill the view, if needed.

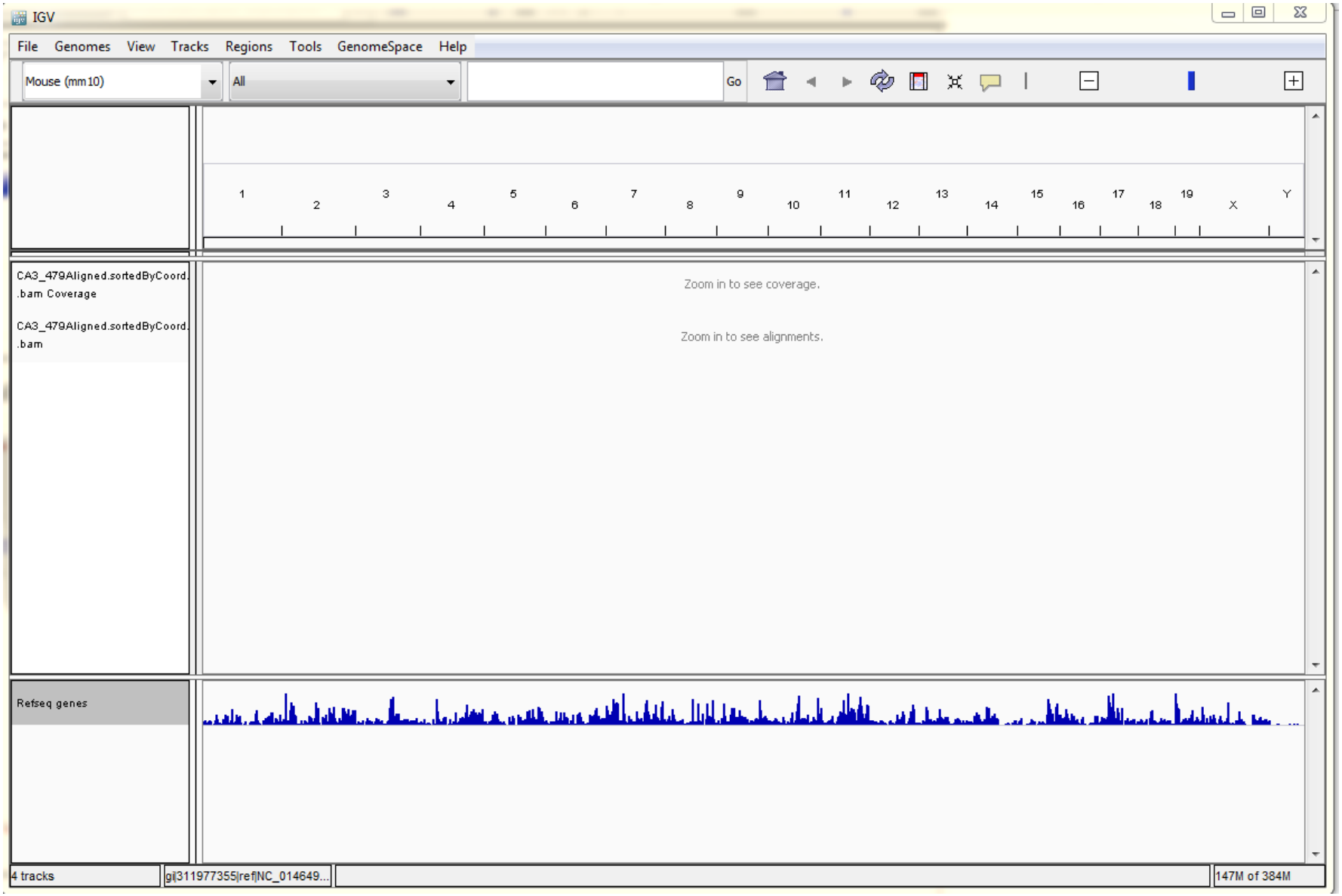
Controls the popup information behavior. Options include displaying the information as the cursor hovers over an item, or when the item is clicked. The popup can also be disabled.

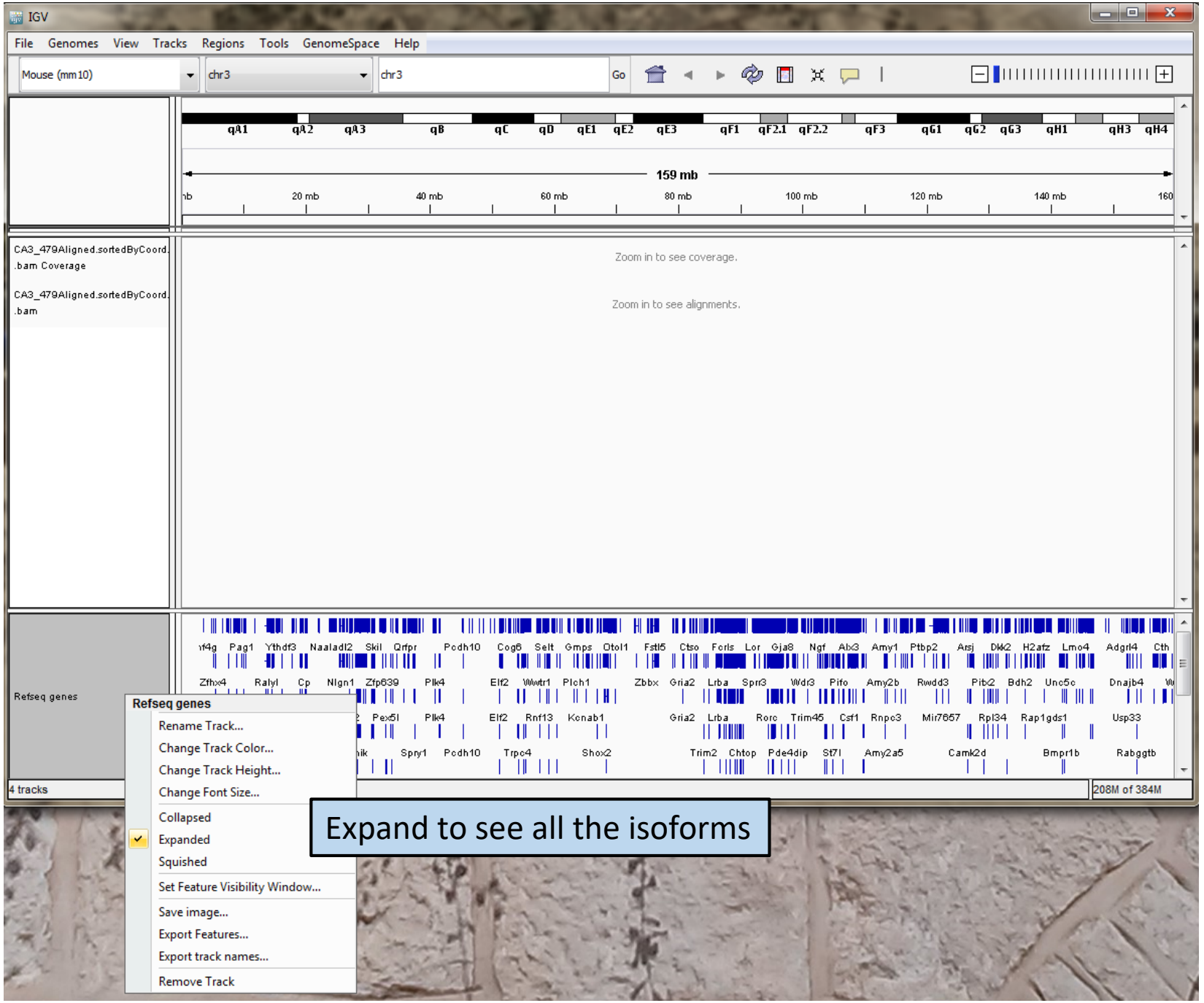
Zoom slider

Zooms in and out on a chromosome. Sometimes referred to as the "railroad track." more...

https://software.broadinstitute.org/software/igv/book/export/html/

Viewing alignments – Zoom in




IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
Mouse (mm10)
chr3
chr3
Go
159 mb
CA3_479Aligned.sortedByCoord.bam Coverage
CA3_479Aligned.sortedByCoord.bam
Zoom in to see coverage.
Zoom in to see alignments.
Refseq genes
Refseq genes
Rename Track...
Change Track Color...
Change Track Height...
Change Font Size...
Collapsed
Expanded
Squished
Set Feature Visibility Window...
Save image...
Export Features...
Export track names...
Remove Track
4 tracks
208M of 384M
Expand to see all the isoforms

Feature Track Options

Viewing Options for the Feature Track

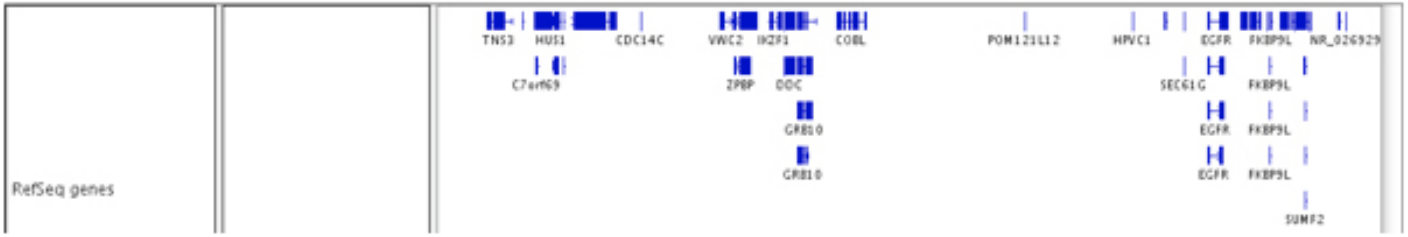
There are 3 different options for viewing the feature track. These allow you to display overlapping features, such as different transcripts of a gene, on one line or multiple lines

To change the view of the feature track, right-click on the feature track and select one of the options:

Collapsed:

Squished:

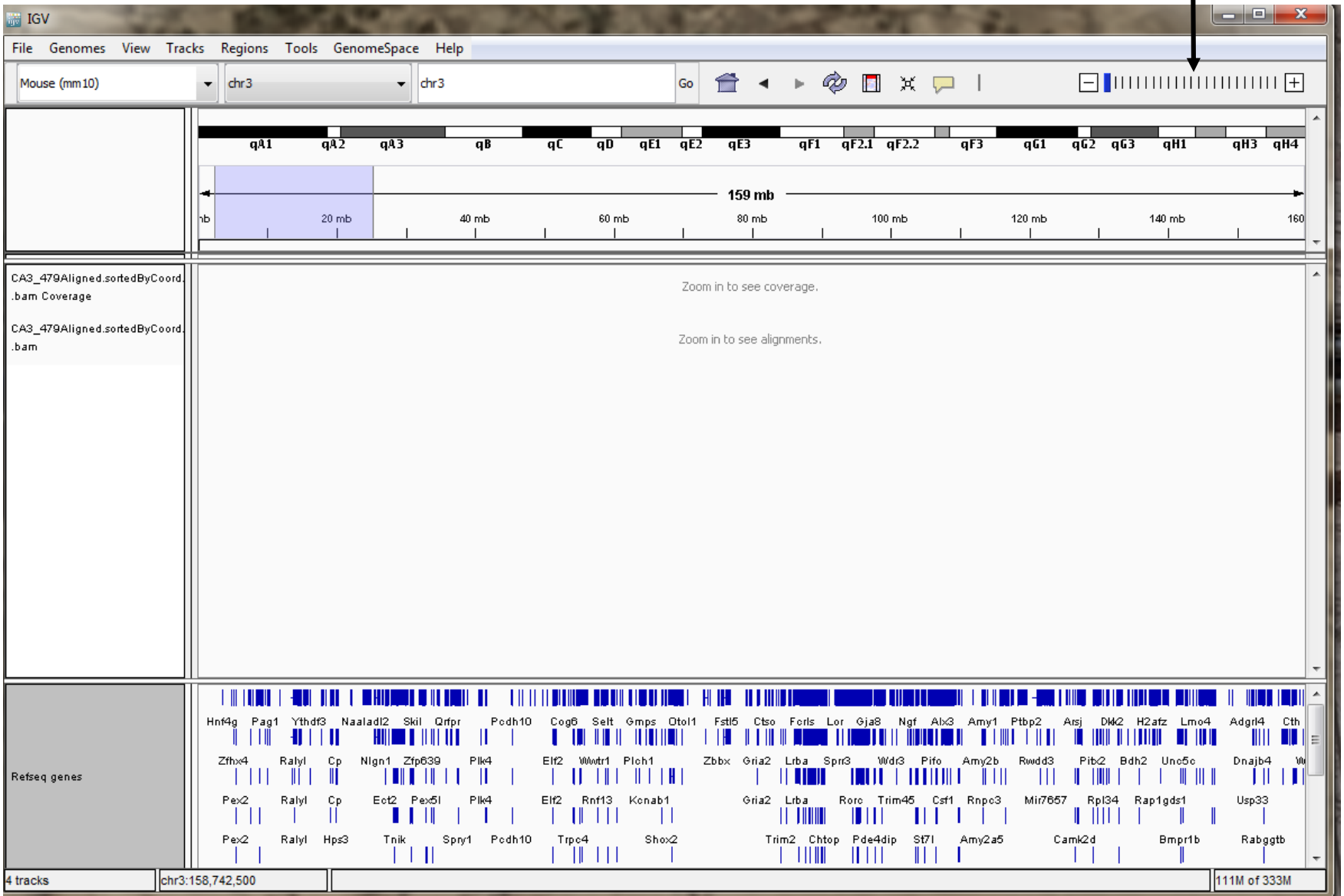
Expanded:



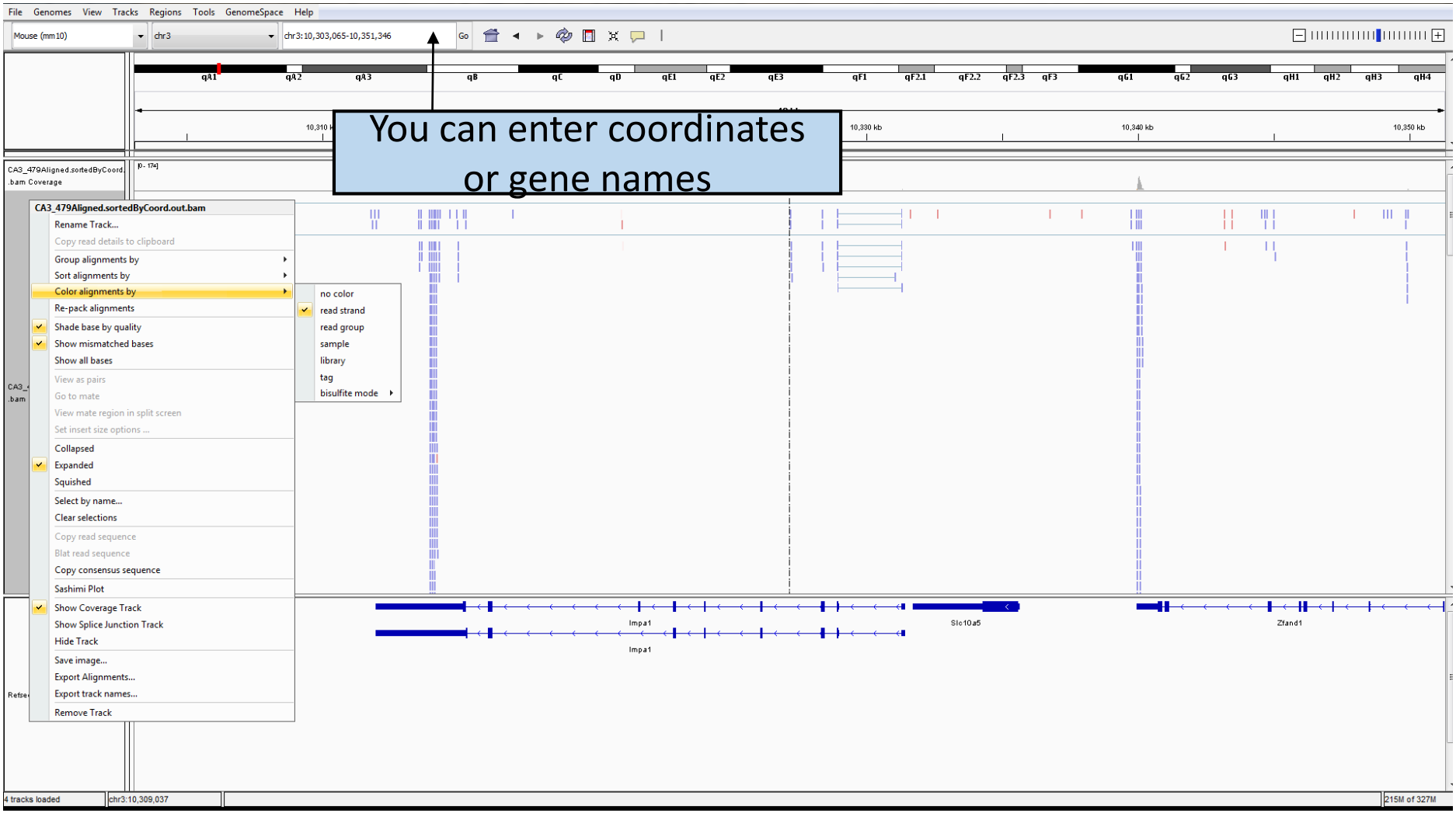
Exon Jumping

This feature is similar to feature jumping. To feature-jump, you select a feature track and press Ctrl-F for forward, Ctrl-B for back. To exon-jump, you select a feature track and press SHIFT-Ctrl-F to center the next exon in your view, SHIFT-Ctrl-B to move back one exon.

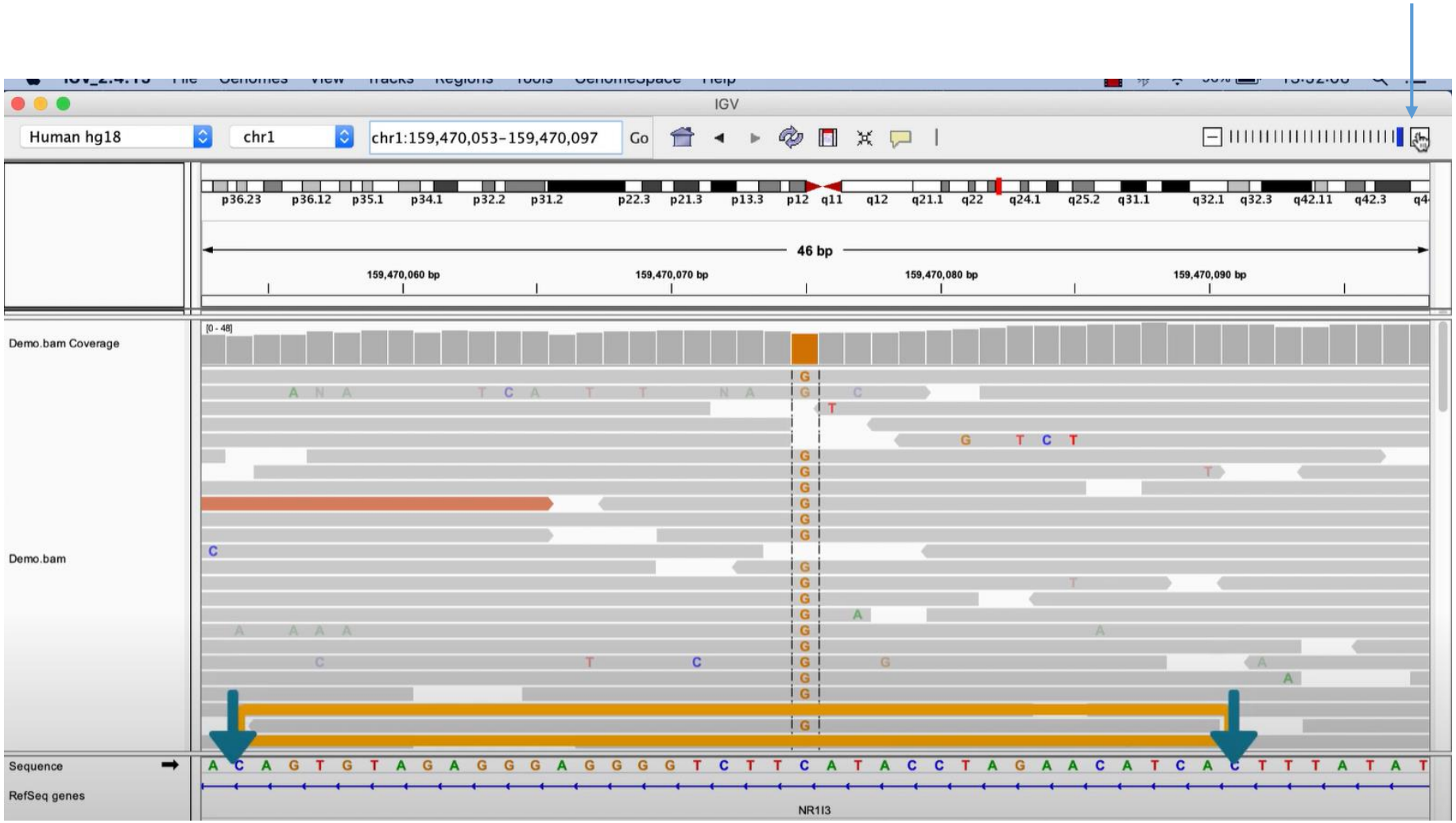
Zooming in


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
Mouse (mm10)
chr3
chr3
Go
qA1 qA2 qA3 qB qC qD qE1 qE2 qE3 qF1 qF2.1 qF2.2 qF3 qG1 qG2 qG3 qH1 qH3 qH4
159 mb
20 mb 40 mb 60 mb 80 mb 100 mb 120 mb 140 mb
CA3_479Aligned.sortedByCoord.
.bam Coverage
CA3_479Aligned.sortedByCoord.
.bam
Zoom in to see coverage.
Zoom in to see alignments.
Refseq genes
Hnf4g Pag1 Ythdf3 Naaladl2 Skil Qrfpr Pcdh10 Cog6 Selt Gmps Otol1 Fstl5 Ctso Fcrls Lor Gja8 Ngf Alx3 Amy1 Ptbp2 Arsj Dlk2 H2afz Lmo4 Adgrl4 Cth
Zfhx4 Ralyl Cp Nlgn1 Zfp639 Plk4 Elf2 Wwtr1 Plch1 Zbbx Gria2 Lrba Sprr3 Wdr3 Pifo Amy2b Rwdd3 Pibx2 Bdh2 Unc5c Dnajb4
Pex2 Ralyl Cp Ect2 Pex5l Plk4 Elf2 Rnf13 Kcnab1 Gria2 Lrba Rorc Trim45 Csf1 Rnpc3 Mir7657 Rpl34 Rap1gds1 Usp33
Pex2 Ralyl Hps3 Tnik Spry1 Pcdh10 Trpc4 Shox2 Trim2 Chtop Pde4dip St7l Amy2a5 Camk2d Bmpr1b Rabggtb
4 tracks
chr3:158,742,500
111M of 333M

Viewing reads aligned to genes



Individual reads (zoom in)



Gene model

Genes are represented as lines and boxes. Lines represent intronic regions, and boxes represent exonic regions. The arrows indicate the direction/strand of transcription for the gene. When an exon box become narrower in height, this indicates a UTR.

Find exon coordinates

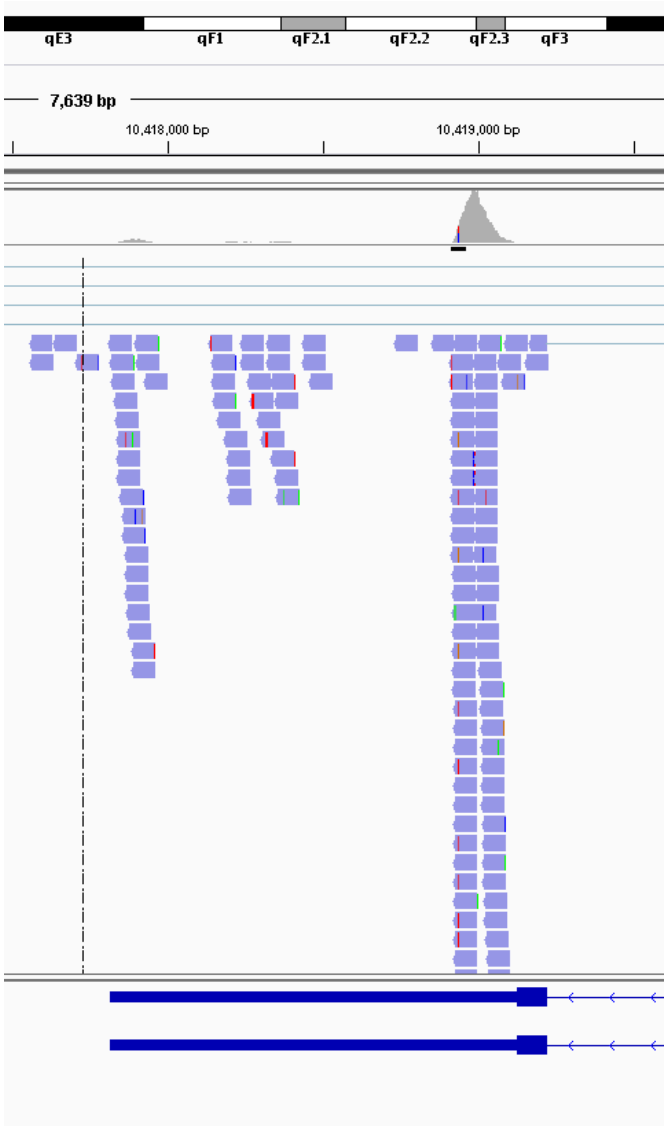
It will not work with introns, introns are not considered features in IGV.

Mismatches

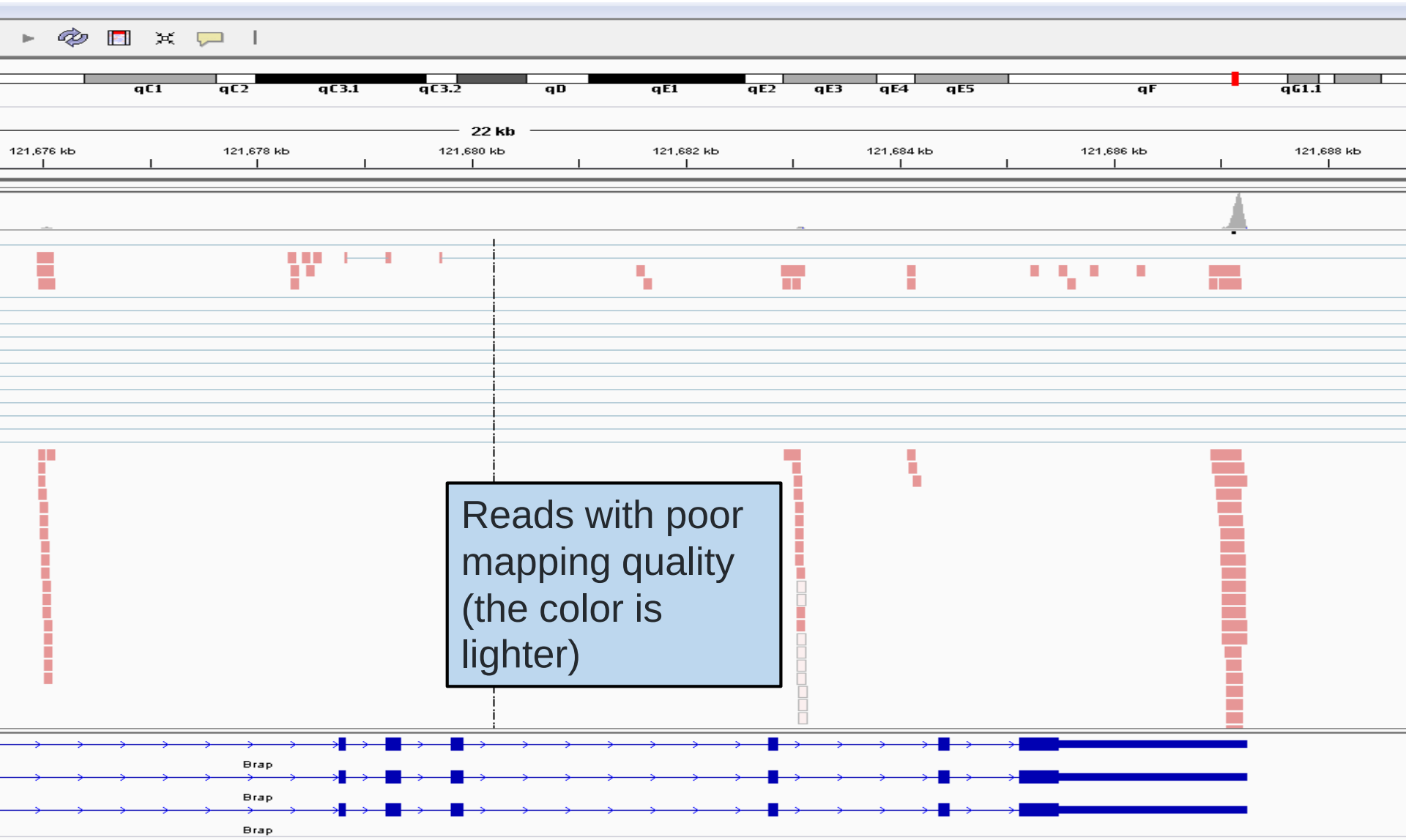
Bases that do not match the reference sequence are highlighted by color

Unless you zoom in a lot, the genome sequence is not shown

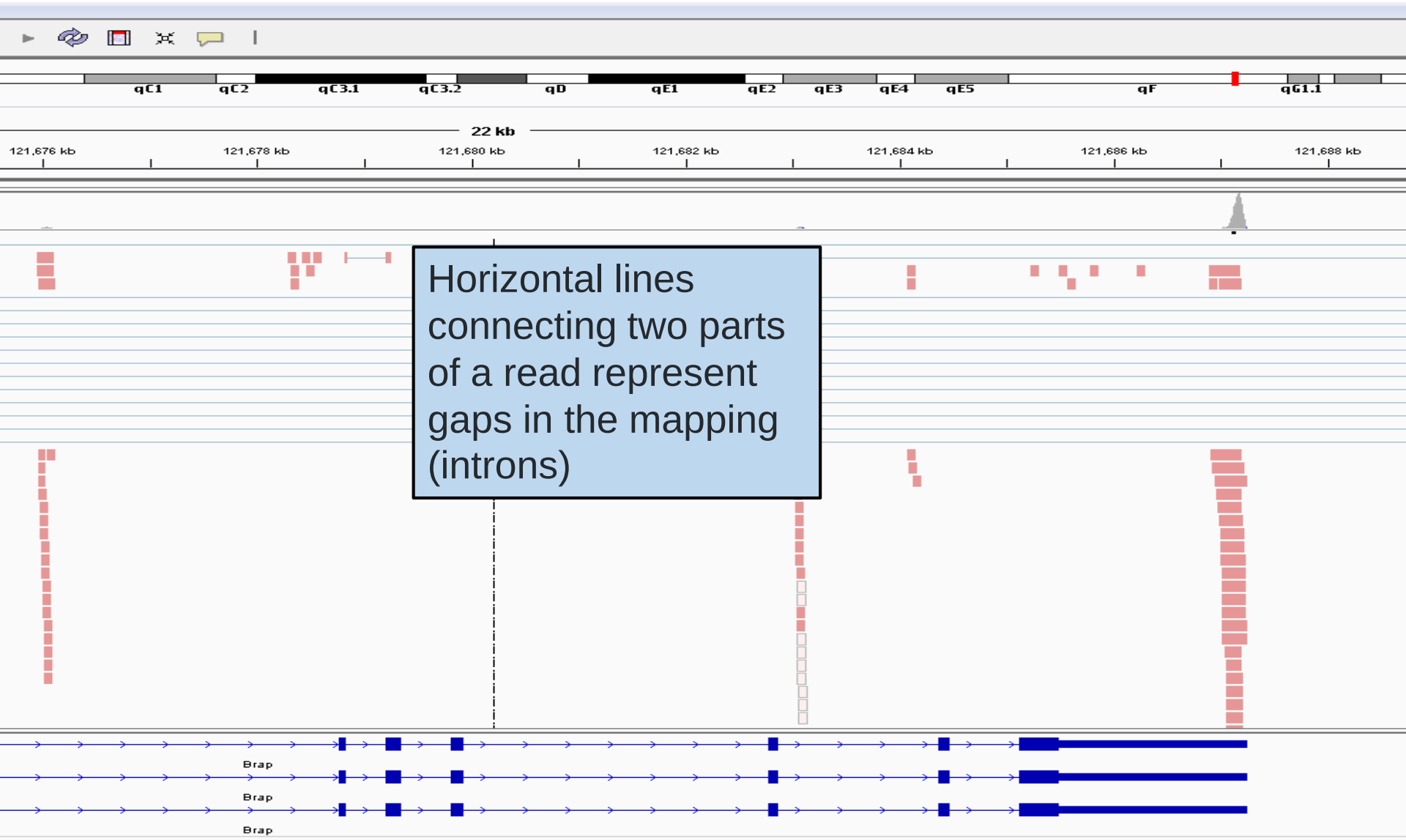
Genome feature are shown according to their coordinates



Mapping quality



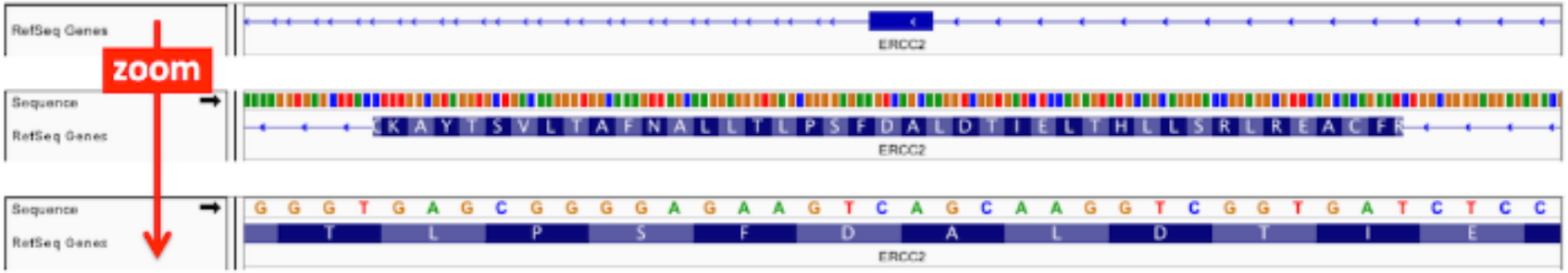
Spliced reads



Sequence Track Options

When zoomed in sufficiently, the reference genome *Sequence* track appears at the top of the lower panel above the *Genes* track, if any, in the IGV display as shown in the **Screenshot** (2015.04.01). The sequence is represented by colored bars or colored letters, depending on zoom level, with adenine in green, cytosine in blue, guanine in yellow, and thymine in red (**A**, **C**, **G**, **T**).

- To change this default nucleotide coloring scheme see the Modify the prefs.properties file page.



Flipping the Strand

You can change the strand that is displayed by clicking on the arrow in the title to the left of the track. Note that the sequence and the arrow are only displayed when zoomed in to a sufficiently small region.

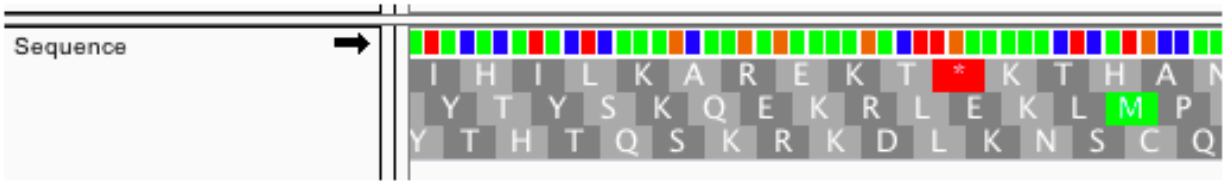
- Alternatively, right-click on *Sequence* track to select *Flip strand* from the pop-up menu.

The direction of the arrow indicates which strand is currently displayed. An arrow pointing left indicates that the negative strand is showing. This strand will show the complement nucleotides and reverse complement translations.

Sequence Translation

With the reference genome sequence track, you can optionally display a 3-band track that shows a 3-frame translation of the amino acid sequence for the corresponding nucleotide sequence. The translation is shown for the strand indicated.

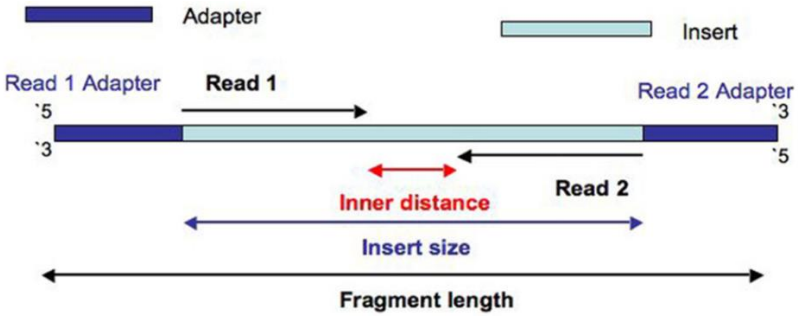
- Right-click on *Sequence* track to select *Show translation* from the pop-up menu and to select a *Translation Table*.
- Selecting *Save image* from the right-click pop-up menu save the lower display panel containing the *Sequence* track as an image.

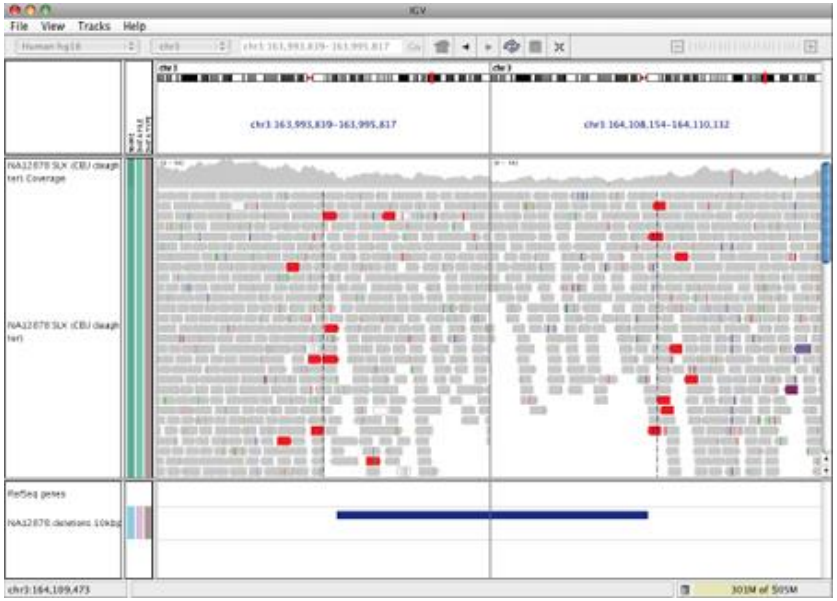


Amino acids are displayed as blocks colored in alternating shades of gray. Methionines are colored green, and all stop codons are colored red. When you zoom all the way in, the amino acid symbols will appear.

You can toggle the display of this translation track by clicking once, anywhere in the sequence or translation track, or by toggling *Show Translation* in the track popup menu.

Interpreting Color by Insert Size





Coloring by insert size is for DNA alignments and is not designed to indicate RNA-Seq paired read mate distances. It is based on set base pair values or computed from the size distribution of a library against the reference genome as defined in the Alignment Preferences Panel.

The inferred insert size can be used to detect structural variants, such as:

- deletions
- insertions
- inter-chromosomal rearrangements

IGV uses color coding to flag anomalous insert sizes. When you select *Color alignments>by insert size* in the popup menu, the default coloring scheme is:

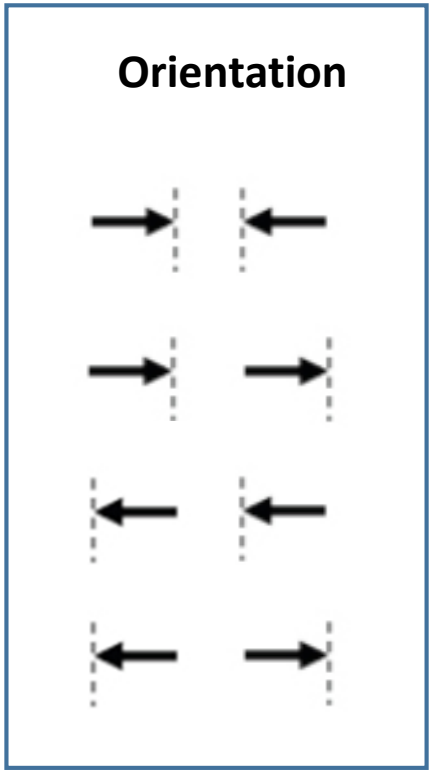
- for an inferred insert size that is larger than expected (possible evidence of a deletion)
- for an inferred insert size that is smaller than expected (possible evidence of an insertion)

Interpreting Color by Pair Orientation

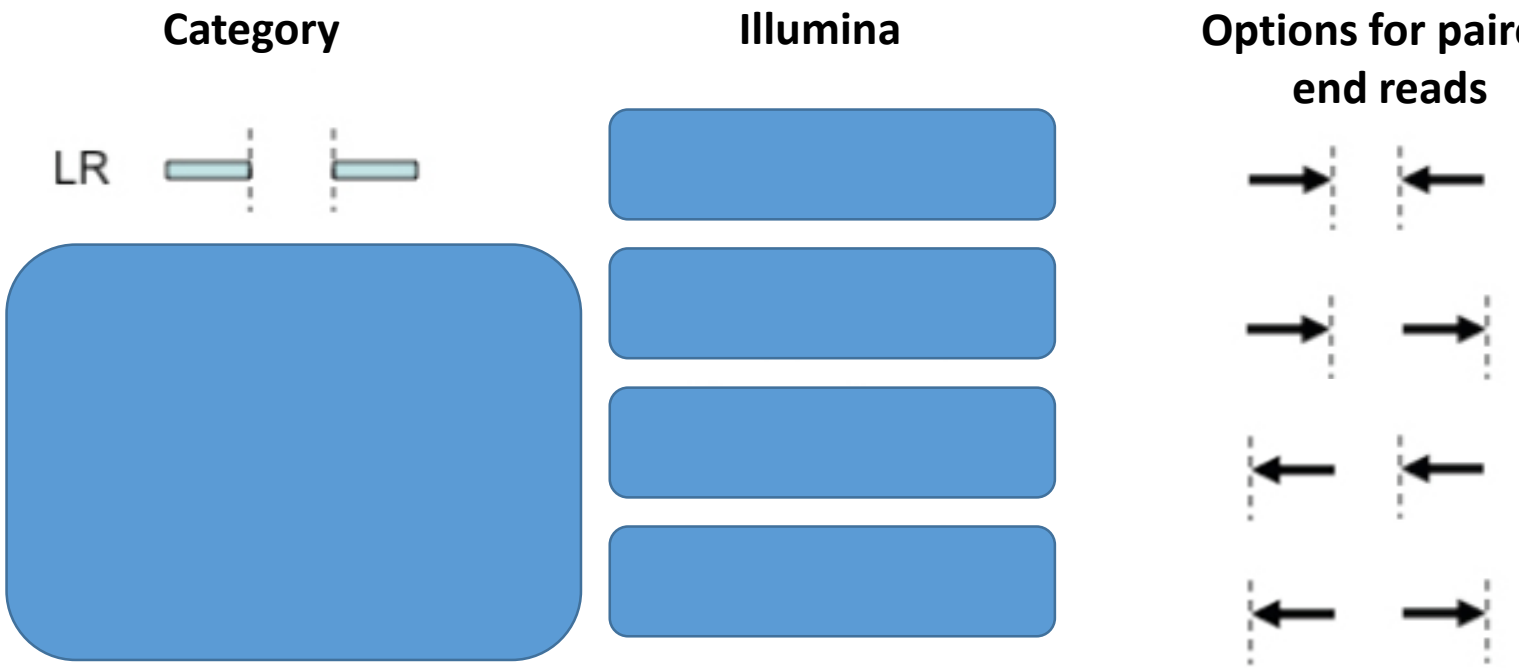
Read orientation of paired reads is defined in terms of read-strand: left versus right, and first read versus second read of a pair.

The orientation can be used to detect structural events including:

- Inversions
- Duplications
- Translocations



By selecting *Color alignments>by pair orientation*, you can flag anomalous pair orientations in IGV.



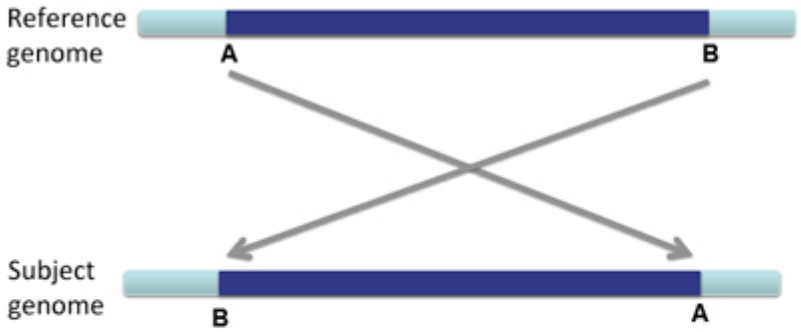
LR	Normal reads. The reads are left and right (respectively) of the unsequenced part of the sequenced DNA fragment when aligned back to the reference genome.
LL,RR	Implies inversion in sequenced DNA with respect to reference.
RL	Implies duplication or translocation with respect to reference.

(figure courtesy of Bob Handsaker)

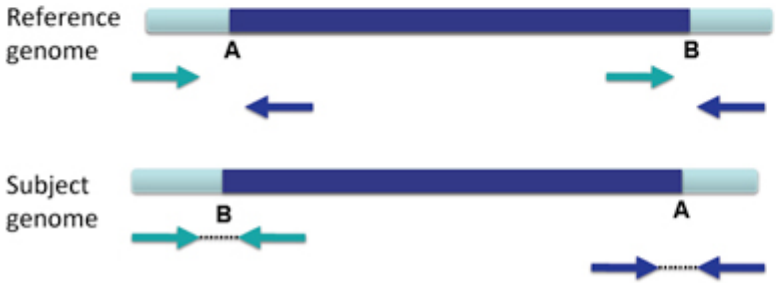
https://software.broadinstitute.org/software/igv/interpreting_pair_orientations

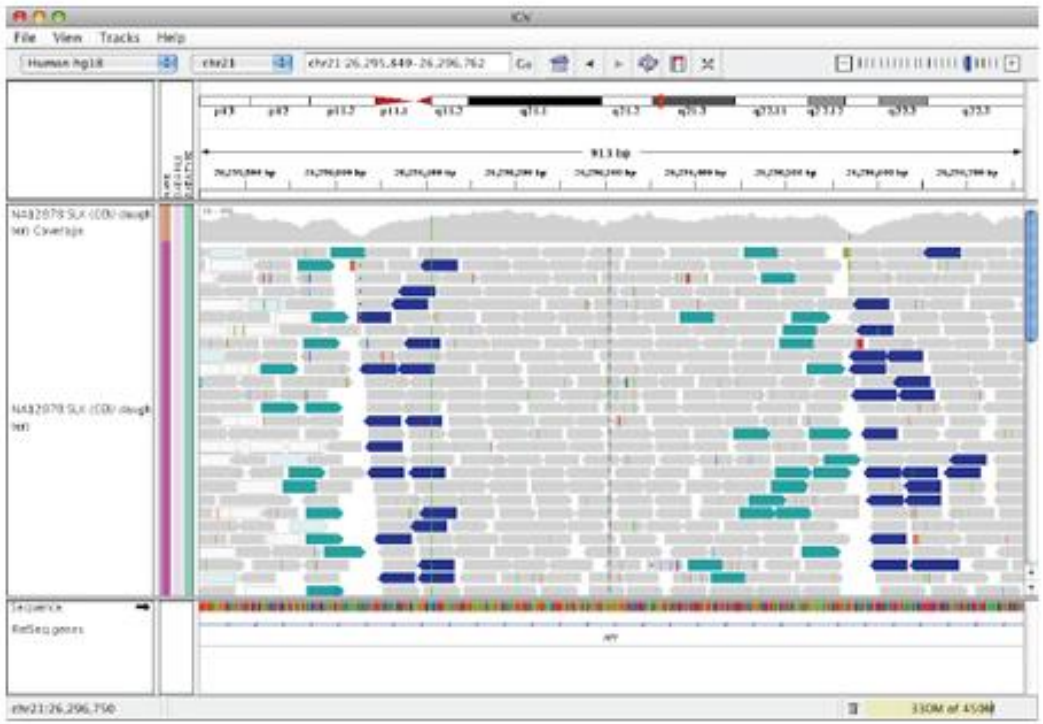
Inversions

An inversion is a large section of DNA that is reversed in the subject genome compared to the reference genome.



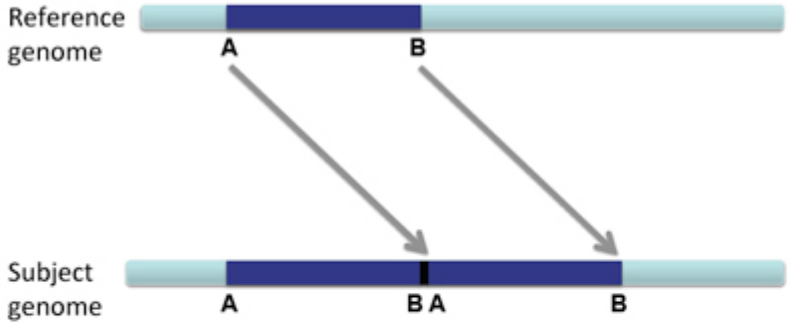
When an inversion shows up in paired-end reads, the reads are distinctively variant from the reference genome.



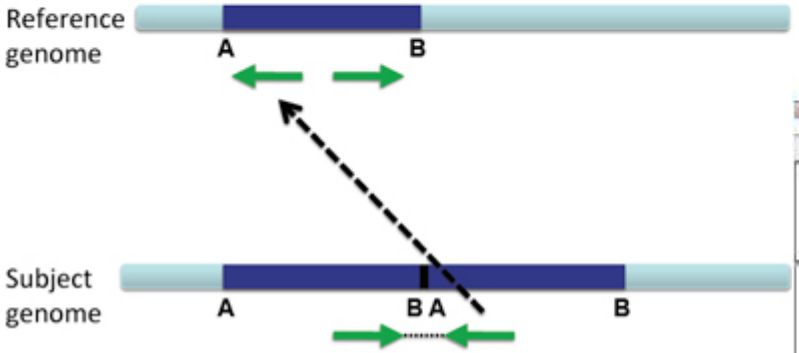


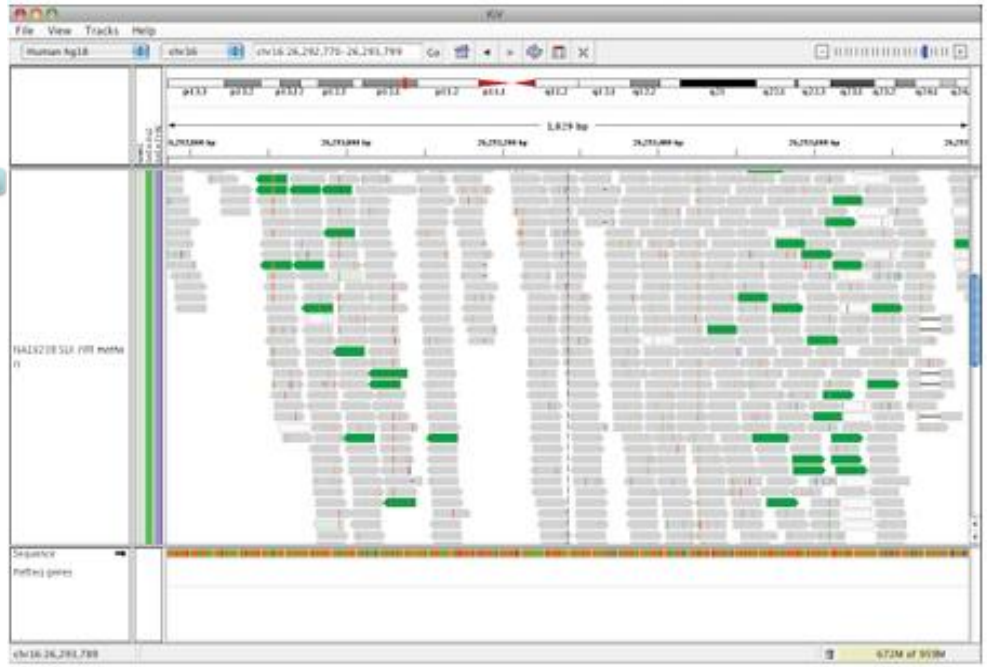
Tandem Duplication

When a large section of DNA is duplicated and inserted into the genome next to the original sequence, this is called a tandem duplication.



The reads will not only be duplicated, but also be arranged as shown below.





QUESTIONS?

Thank you