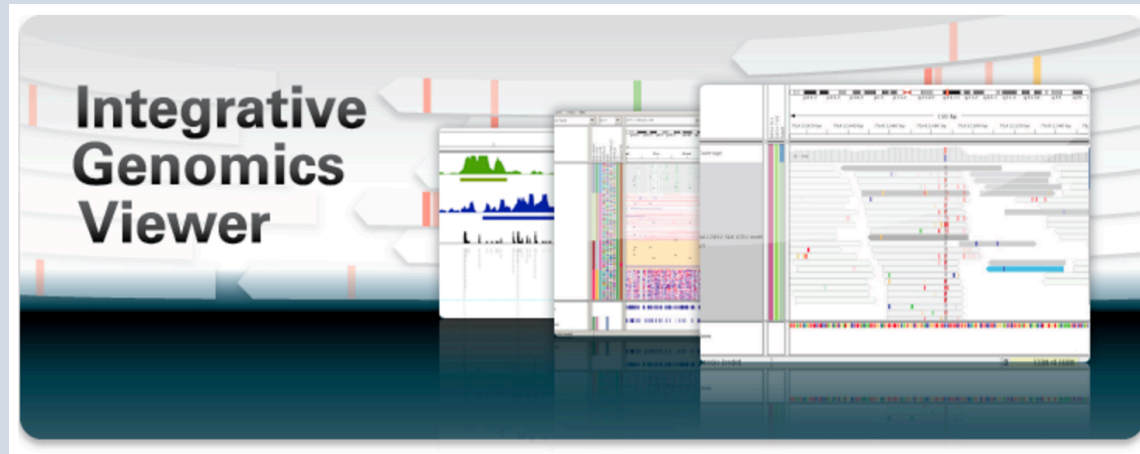




Cold
Spring
Harbor
Laboratory

Introduction to IGV The Integrative Genomics Viewer

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Advanced Sequencing Technologies & Applications
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Visualization Tools in Genomics

- there are **over 40 different genome browsers**, which to use?
- depends on
 - task at hand
 - kind and size of data
 - data privacy

HT-seq Genome Browsers



Integrative
Genome
Viewer



UCSC
Genome Browser
Cancer Genome Browser



Trackster
(part of Galaxy)

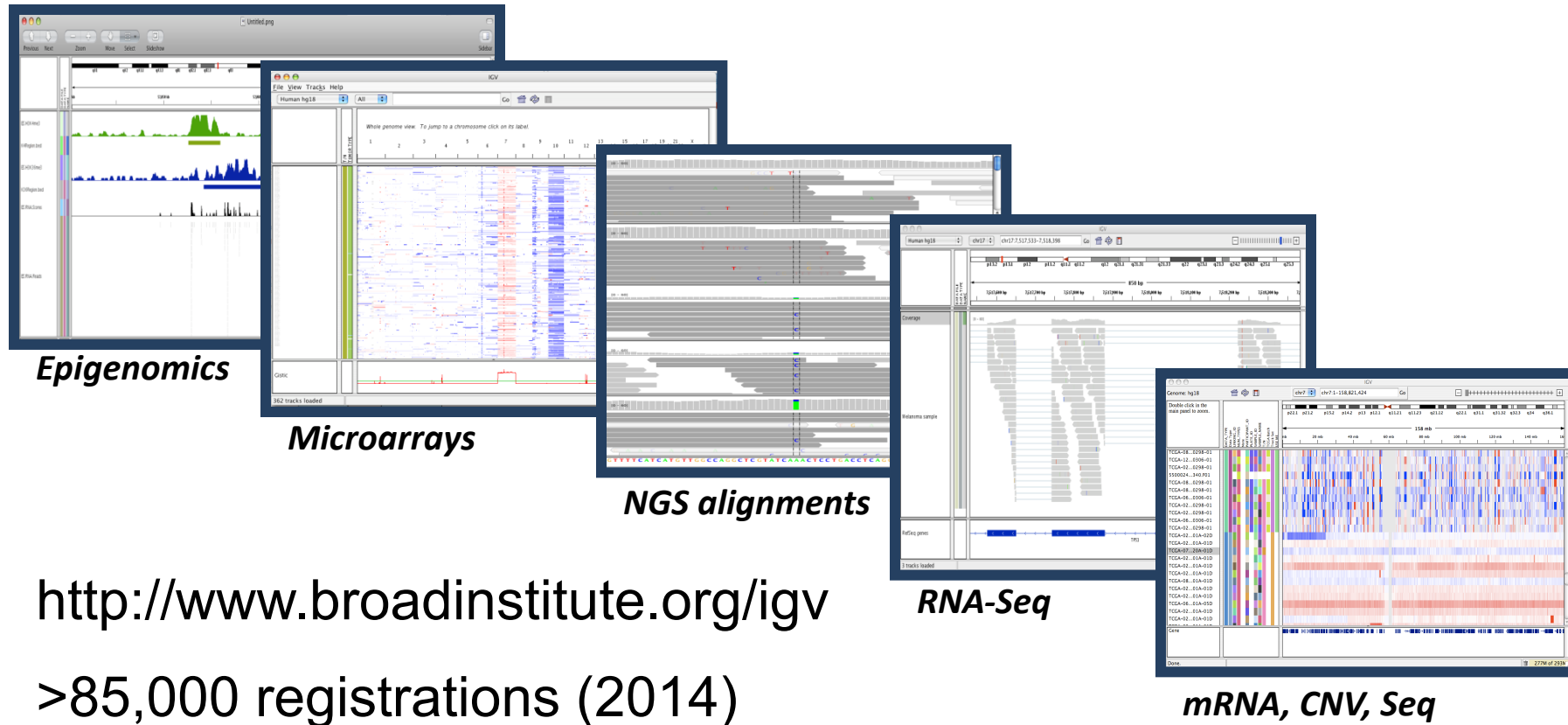


Savant
Genome
Browser

- task at hand : visualizing HT-seq reads, especially good for inspecting variants
- kind and size of data : large BAM files, stored locally or remotely
- data privacy : run on the desktop, can keep all data private
- UCSC Genome Browser has been retro-fitted to display BAM files
- Trackster is a genome browser that can perform visual analytics on small windows of the genome, deploy full analysis with Galaxy

Integrative Genomics Viewer (IGV)

Desktop application for the interactive visual exploration of integrated genomic datasets

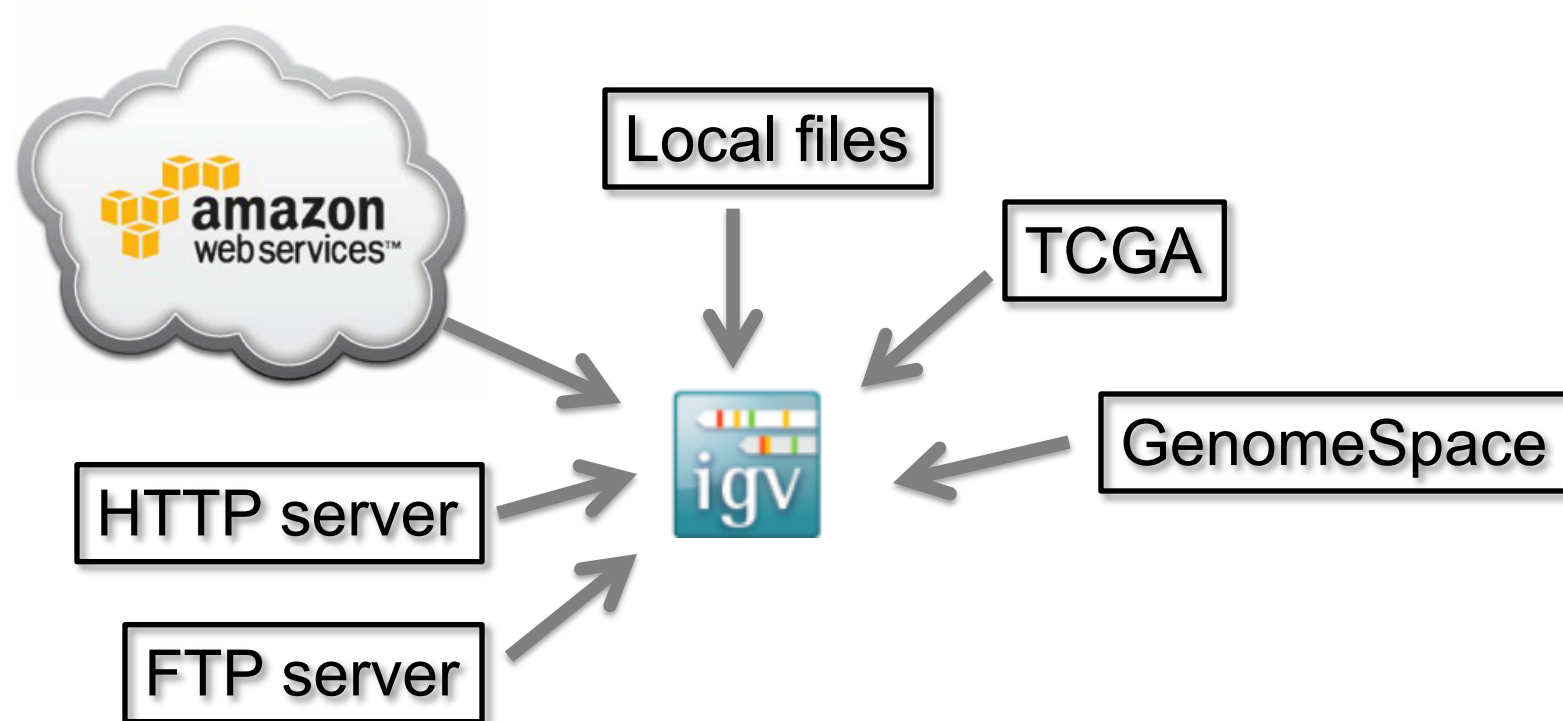


Features

With IGV you can...

- Explore large genomic datasets with an intuitive, easy-to-use interface.
- Integrate multiple data types with clinical and other sample information.
- View data from multiple sources:
 - local, remote, and “cloud-based”.
- Automation of specific tasks using command-line interface

IGV data sources



- View **local** files without uploading.
- View **remote** files without downloading the whole dataset.

Using IGV: the basics

- Launch IGV
- Select a reference genome
- Load data
- Navigate through the data
 - WGS data
 - SNVs
 - structural variations

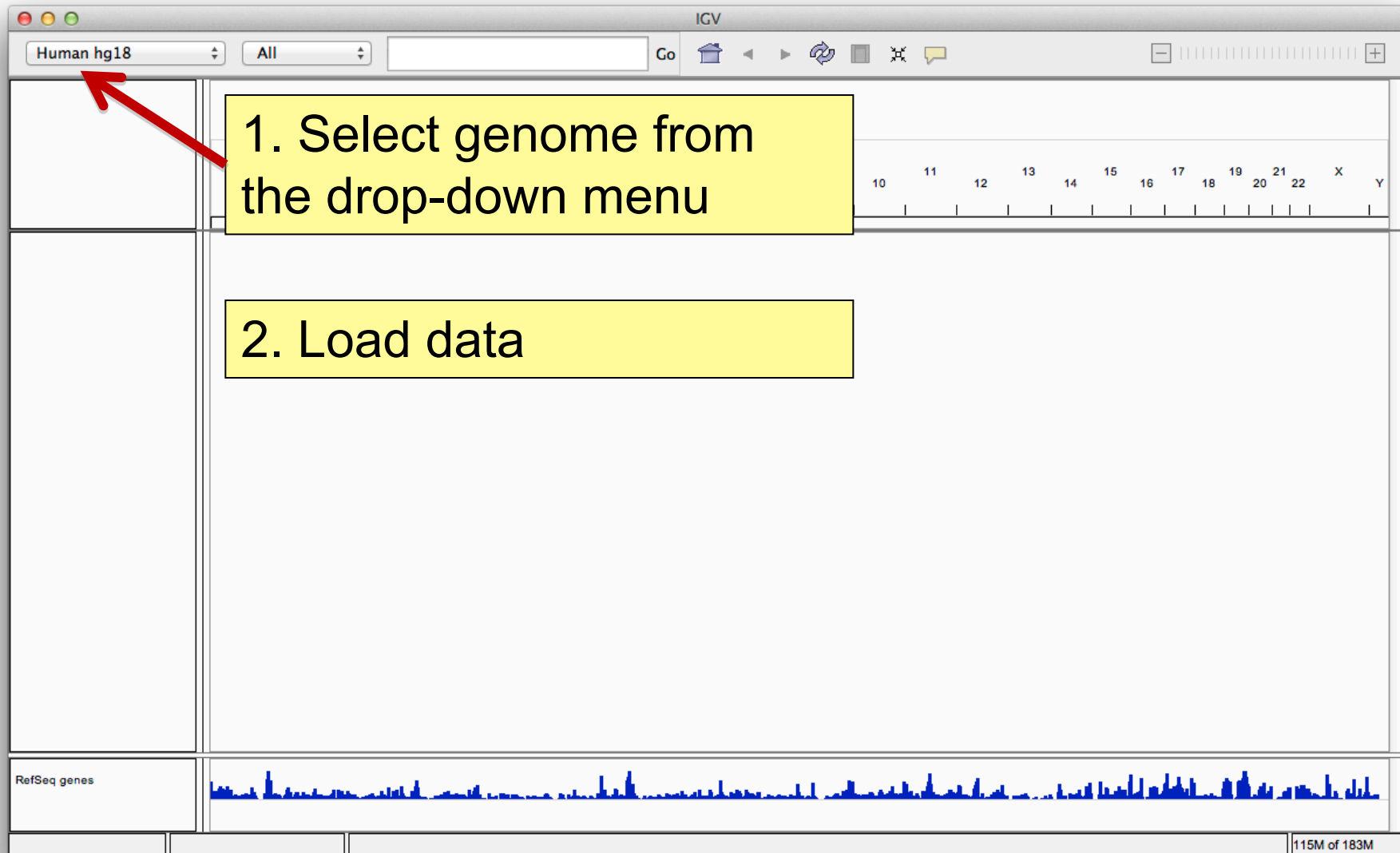
Launch IGV

The image shows a sequence of steps to launch IGV:

- Home Page:** The 'Downloads' link in the top navigation bar is highlighted with a red circle.
- Registration Page:** The 'Registration' page is shown, where users must agree to the GNU Lesser General Public License (LGPL).
- Downloads Page:** The 'Downloads' page provides instructions for different operating systems. The 'Download Mac App' button is highlighted with a red circle.
- Launch Options:** A table of launch options is shown, with the 'Launch' buttons for various memory configurations highlighted with a red circle.

Launch	Launch	Launch	Launch
Launch with 700 MB	Launch with 1.2 GB	Launch with 2 GB	Launch with 4 GB
Maximum usable memory for Windows OS with 32-bit Java.	Maximum usable memory for 32-bit MacOS.	For large memory datasets with 64-bit Java.	

Launch IGV



Human hg18 All Go

1. Select genome from the drop-down menu

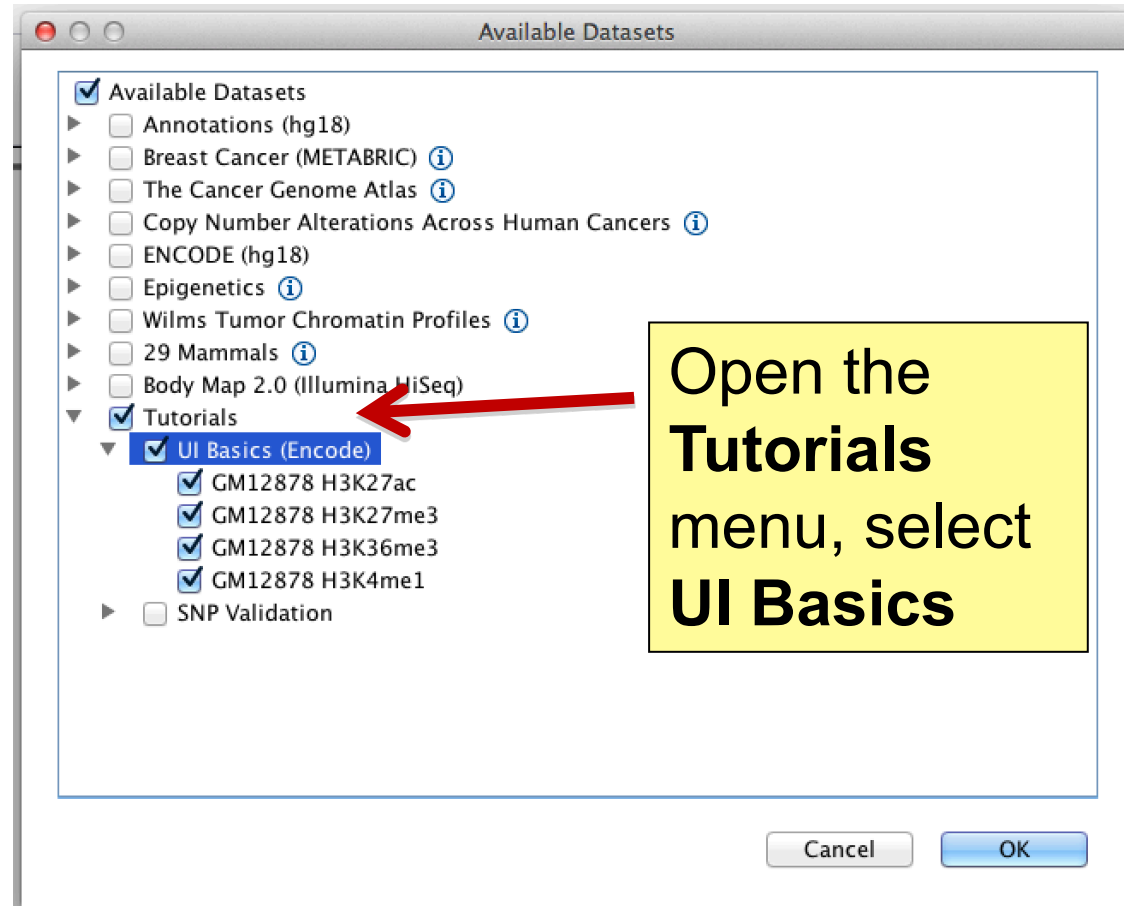
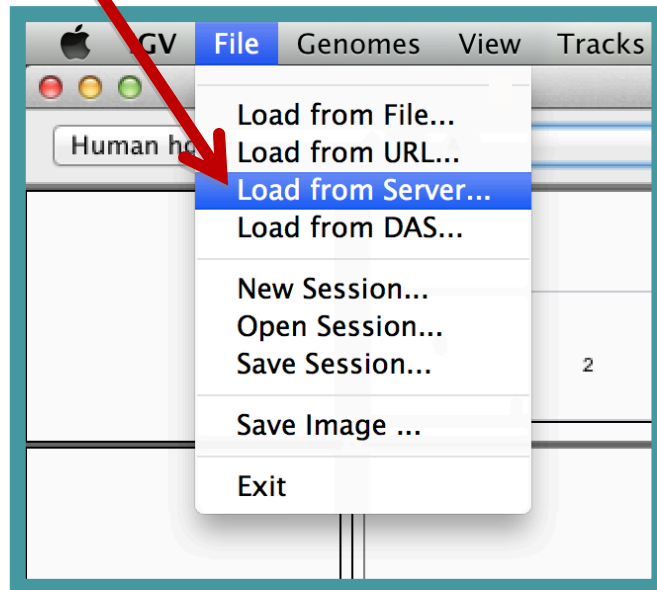
2. Load data

RefSeq genes

115M of 183M

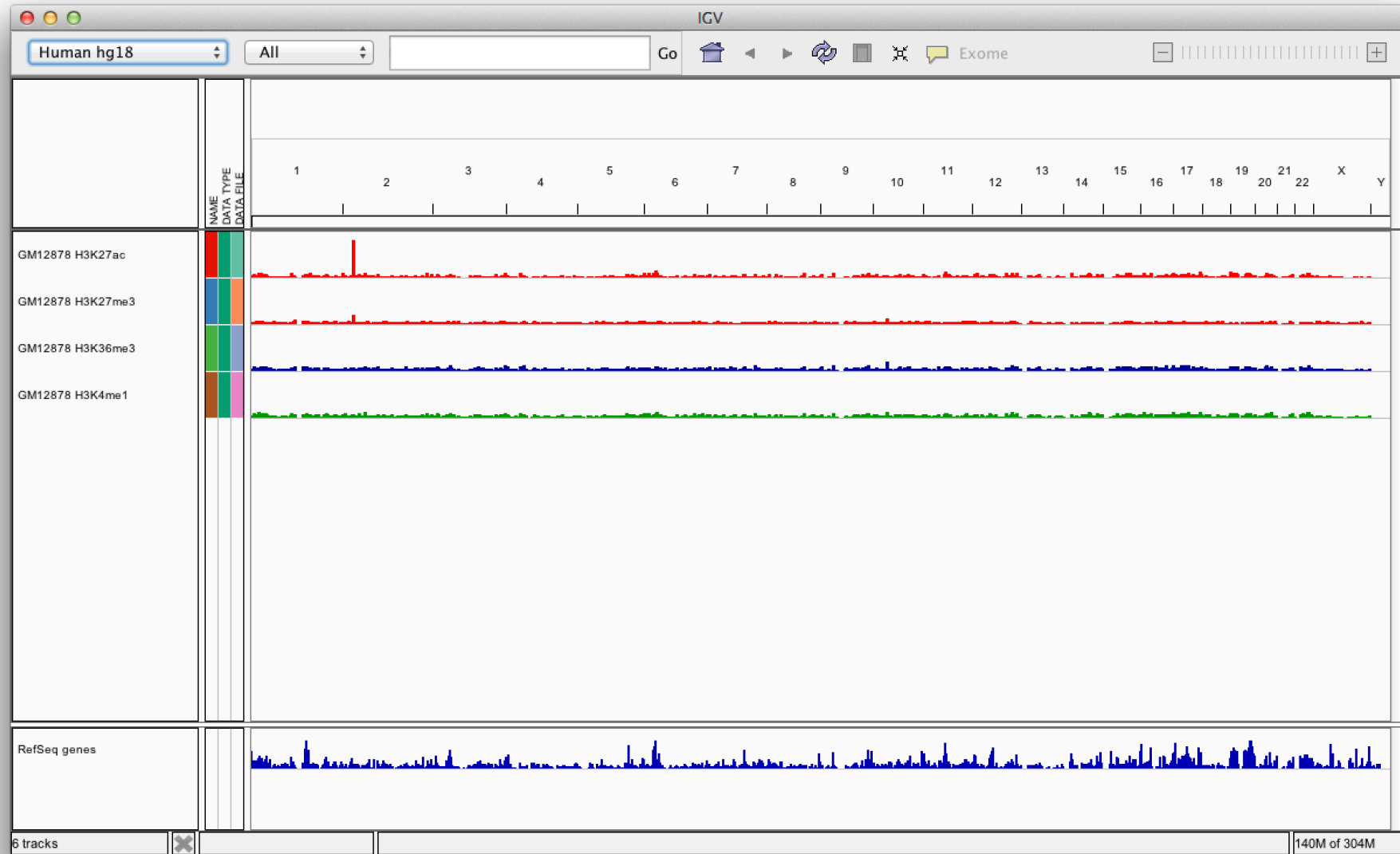
Load data

Select **File > Load from Server...**

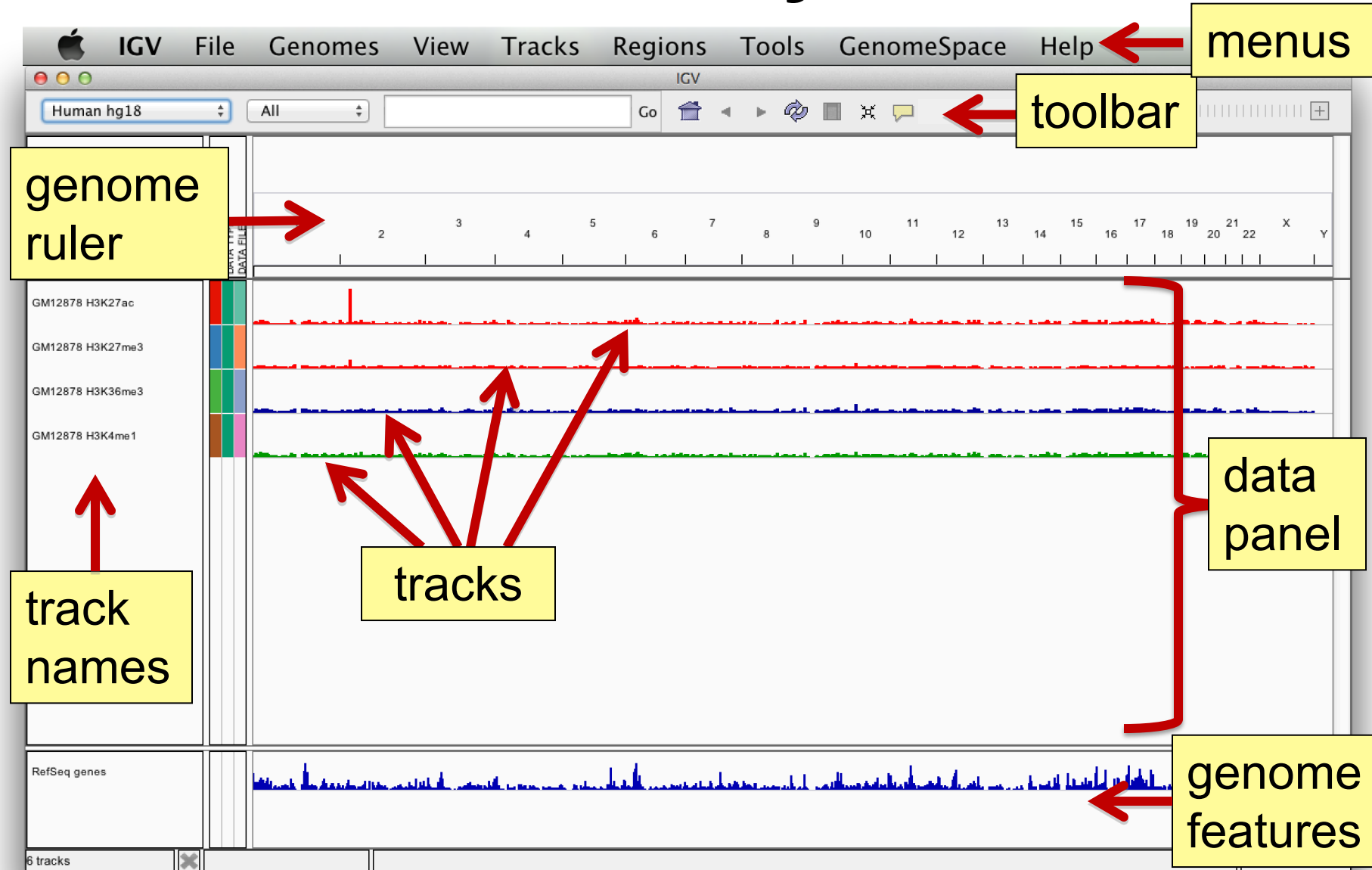


Open the **Tutorials** menu, select **UI Basics**

Screen layout



Screen layout



File formats and track types

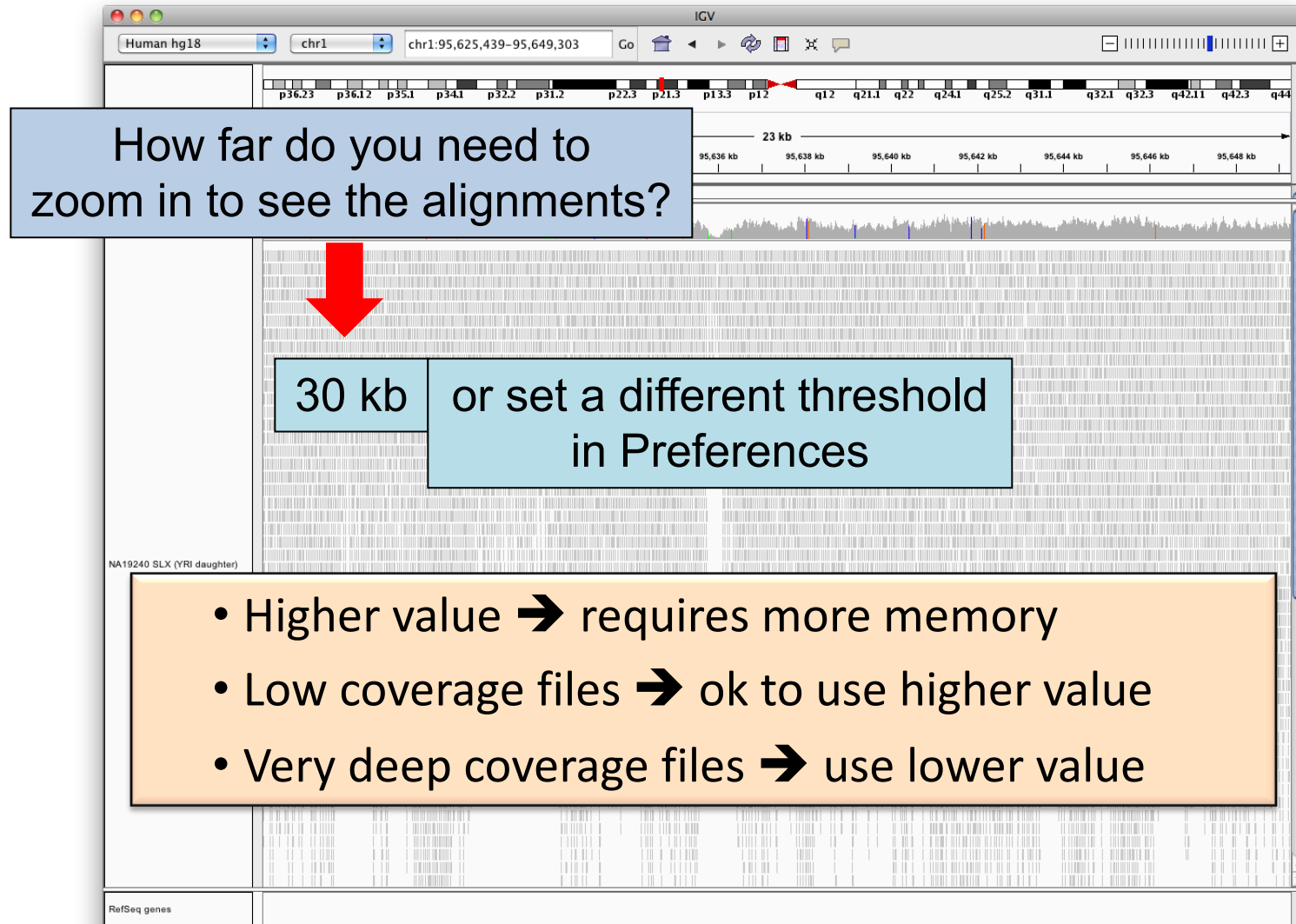
- The **file format** defines the track type.
- The **track type** determines the display options
 - [BAM](#)
 - [BED](#)
 - [BedGraph](#)
 - [bigBed](#)
 - [bigWig](#)
 - [Birdsuite Files](#)
 - [CBS](#)
 - [CN](#)
 - [Cufflinks Files](#)
 - [Custom File Formats](#)
 - [Cytoband](#)
 - [FASTA](#)
 - [GCT](#)
 - [genePred](#)
 - [GFF](#)
 - [GISTIC](#)
 - [Goby](#)
 - [GWAS](#)
 - [IGV](#)
 - [LOH](#)
 - [MAF](#)
 - [Merged BAM File \(.bam.list\)](#)
 - [MUT](#)
 - [PSL](#)
 - [RES](#)
 - [SAM](#)
 - [Sample Information](#)
 - [SEG](#)
 - [SNP](#)
 - [TAB](#)
 - [TDF](#)
 - [Track Line](#)
 - [Type Line](#)
 - [VCF](#)
 - [WIG](#)
- For current list see: www.broadinstitute.org/igv/FileFormats

Viewing alignments

Whole chromosome view



Viewing alignments – Zoom in



Human hg18 chr1 chr1:95,625,439-95,649,303

How far do you need to zoom in to see the alignments?

23 kb

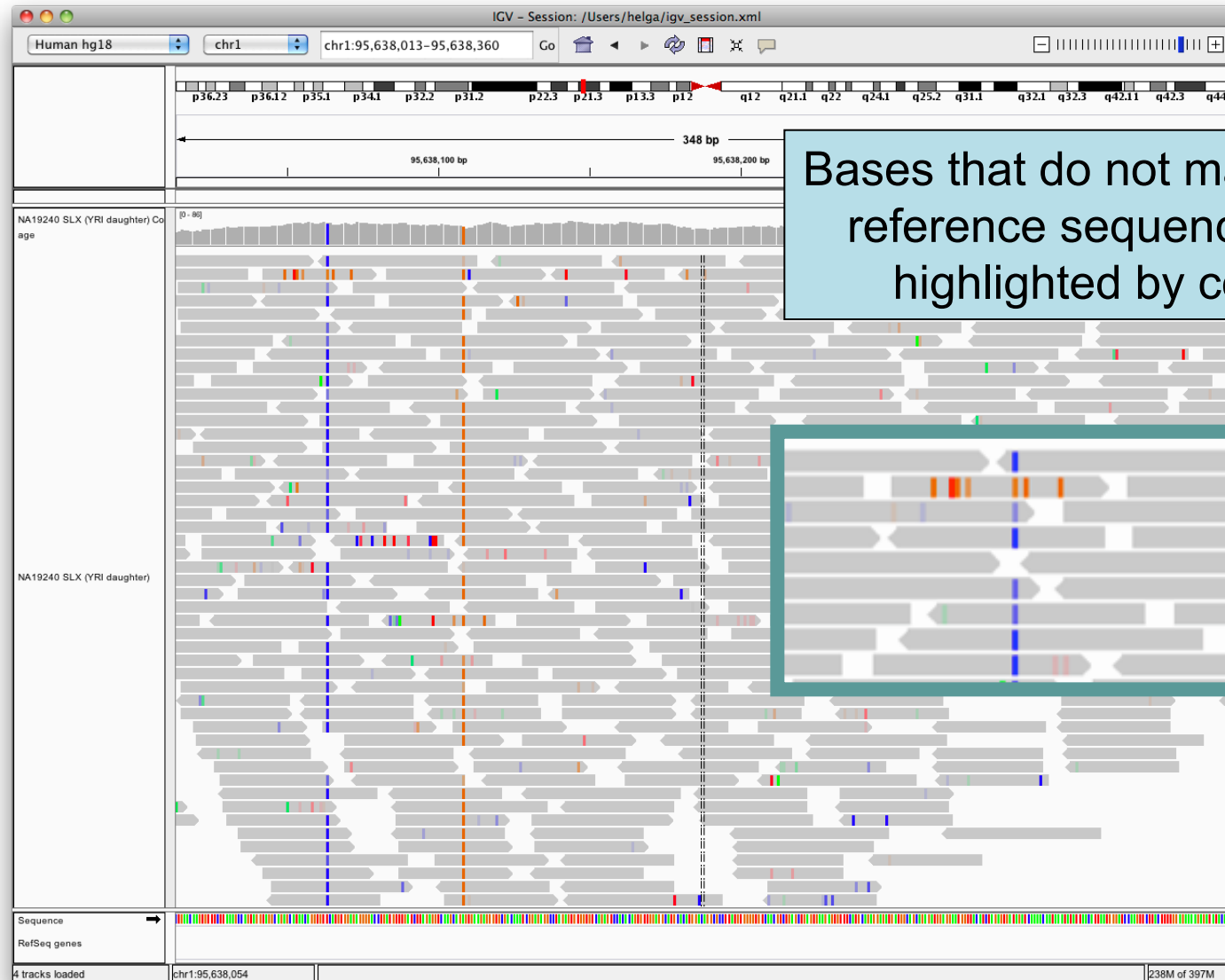
30 kb or set a different threshold in Preferences

- Higher value → requires more memory
- Low coverage files → ok to use higher value
- Very deep coverage files → use lower value

NA19240 SLX (YRI daughter)

RefSeq genes

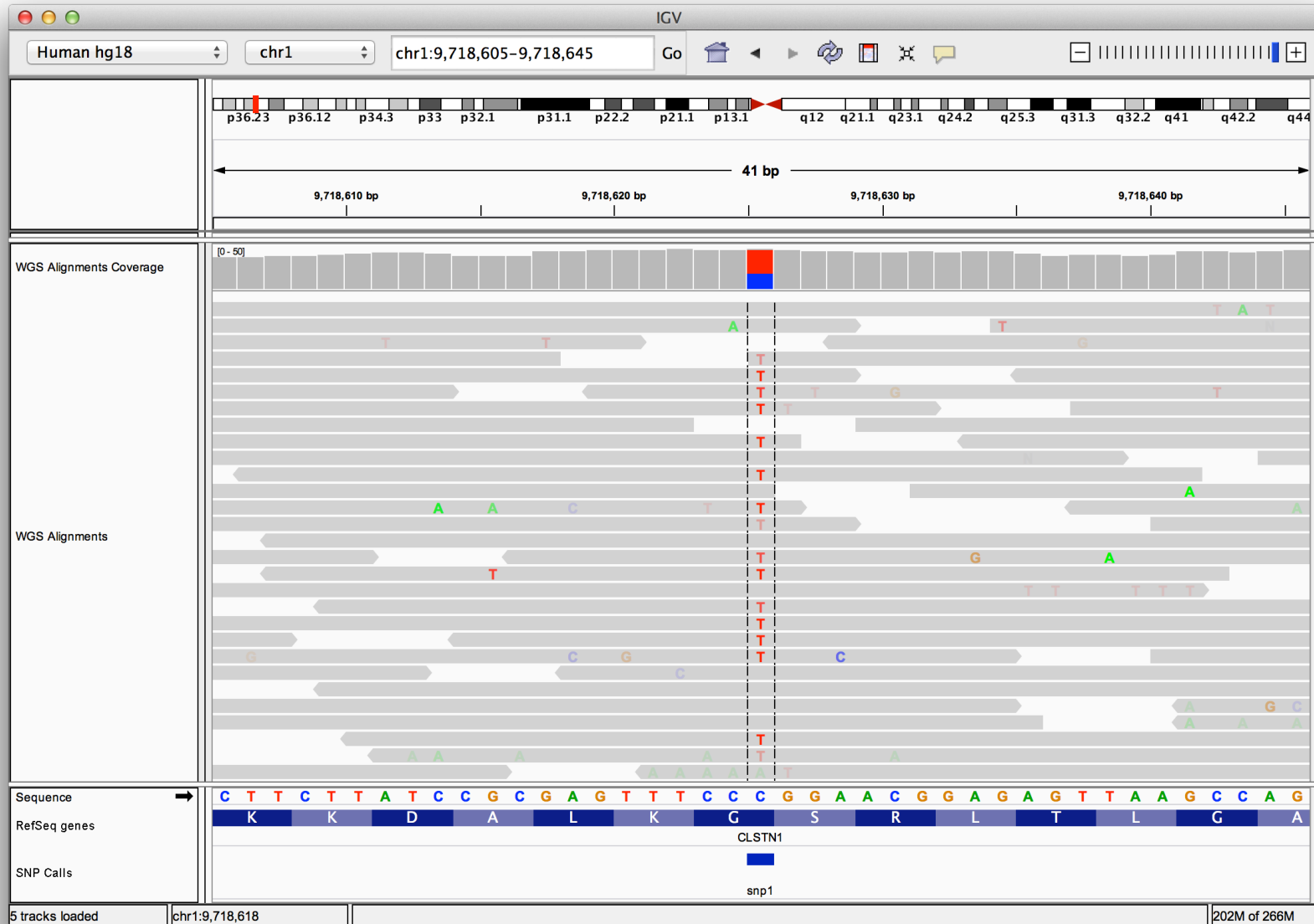
Viewing alignments – Zoom in



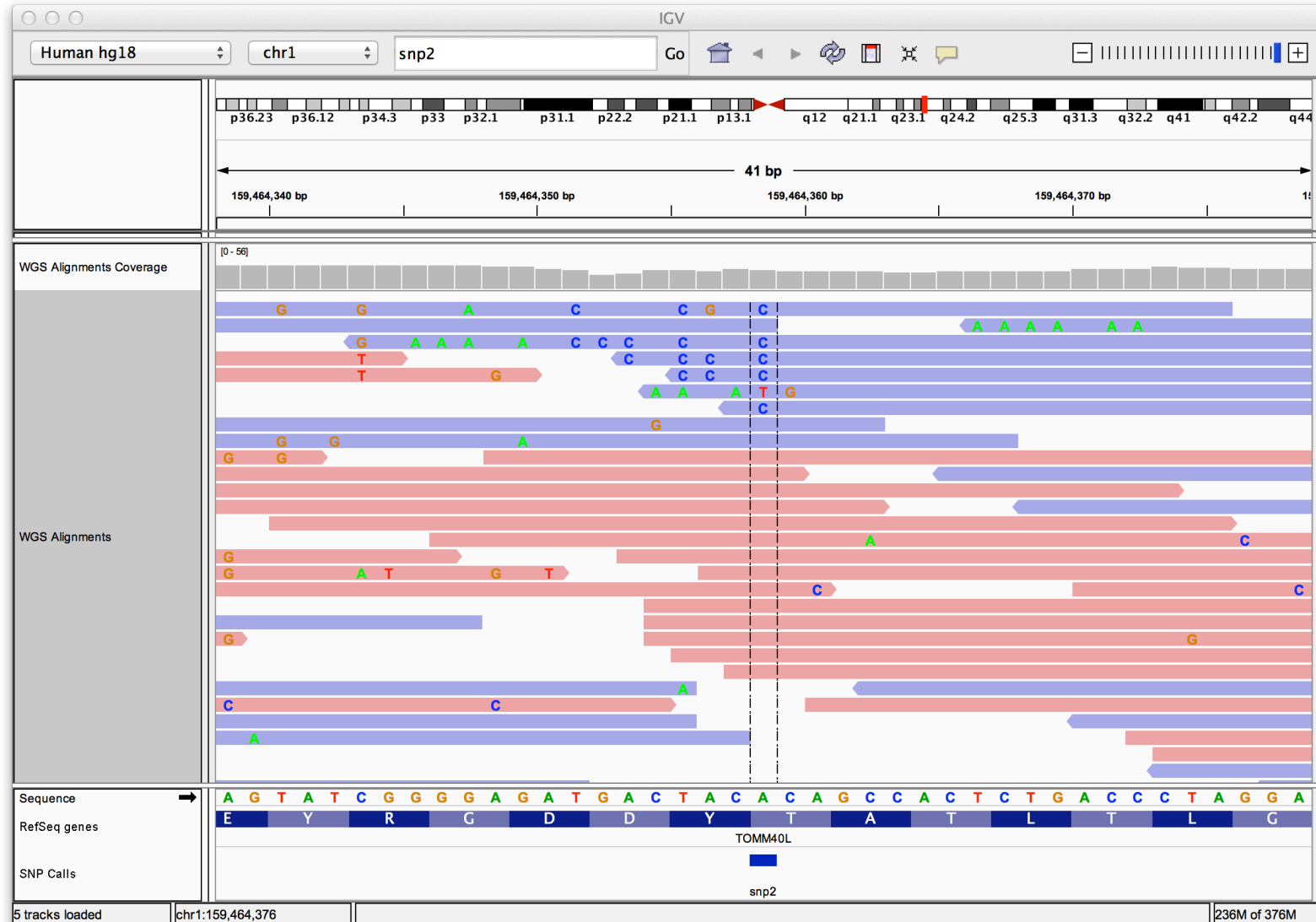
SNVs and Structural variations

- Important metrics for evaluating the validity of SNVs:
 - Coverage
 - Amount of support
 - Strand bias / PCR artifacts
 - Mapping qualities
 - Base qualities
- Important metrics for evaluating SVs:
 - Coverage
 - Insert size
 - Read pair orientation

Viewing SNPs and SNVs



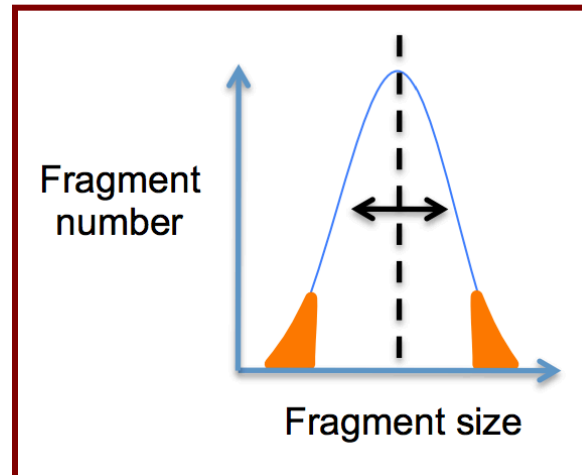
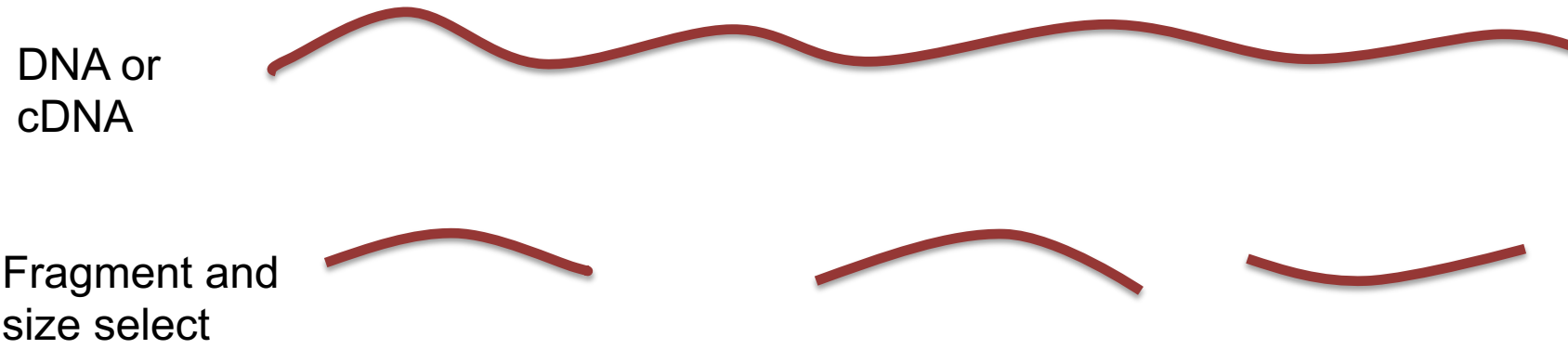
Viewing SNPs and SNVs



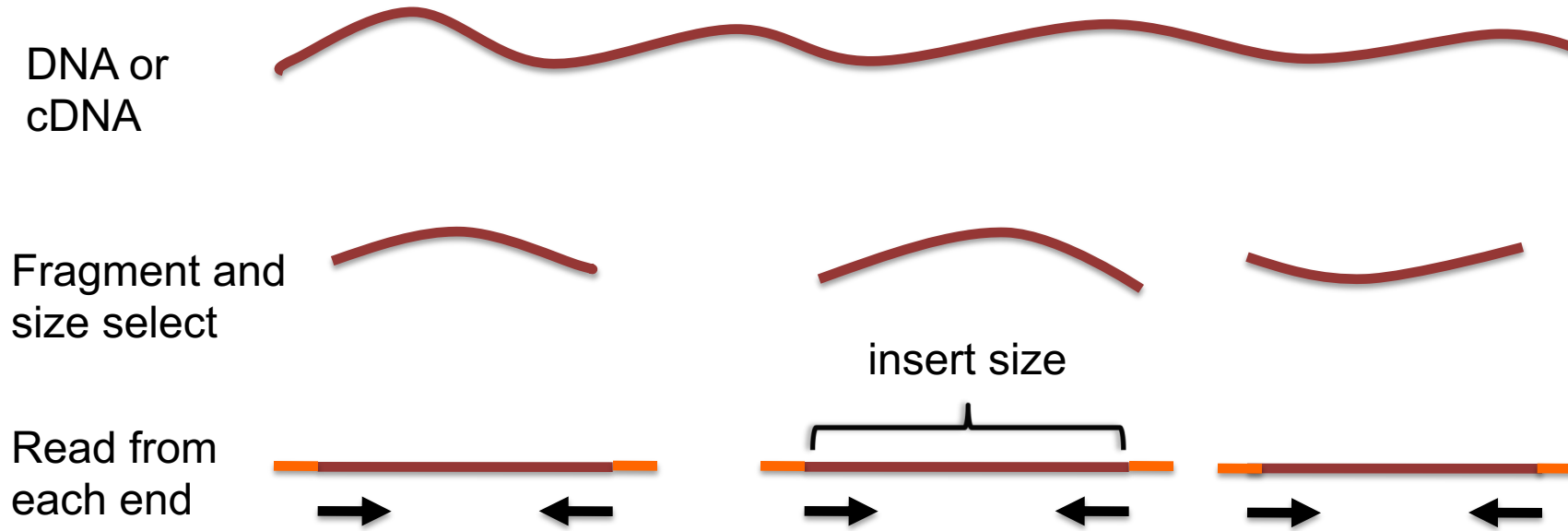
Viewing Structural Events

- Paired reads can yield evidence for genomic “structural events”, such as deletions, translocations, and inversions.
- Alignment coloring options help highlight these events based on:
 - Inferred insert size (template length)
 - Pair orientation (relative strand of pair)

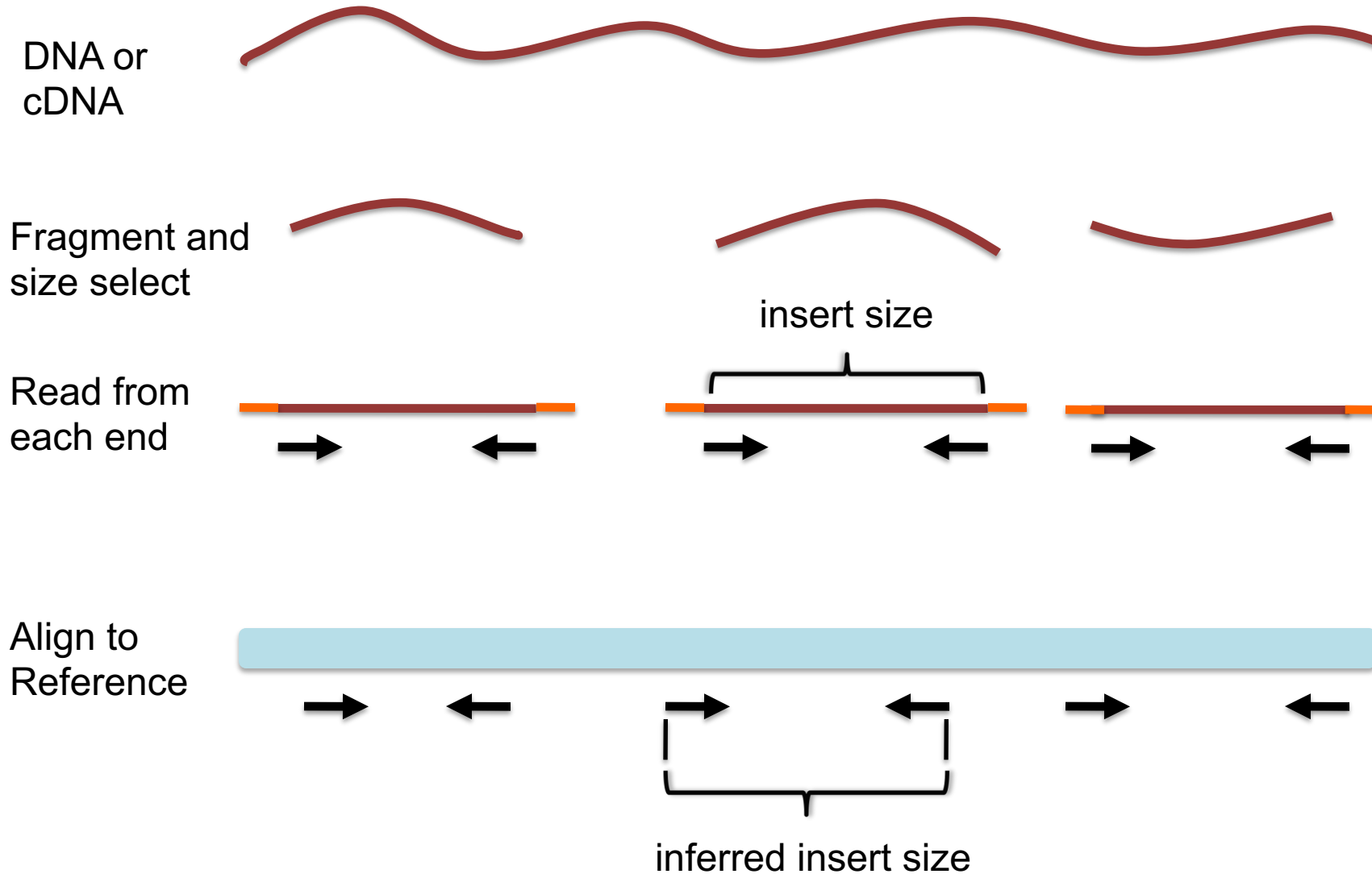
Paired-end sequencing



Paired-end sequencing



Paired-end sequencing



Interpreting inferred insert size

The “inferred insert size” can be used to detect structural variants including

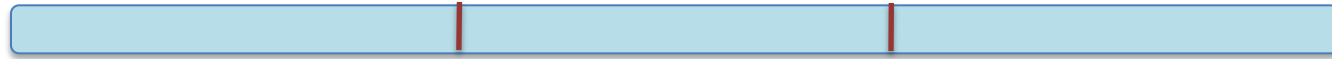
- Deletions
- Insertions
- Inter-chromosomal rearrangements: (Undefined insert size)

Deletion

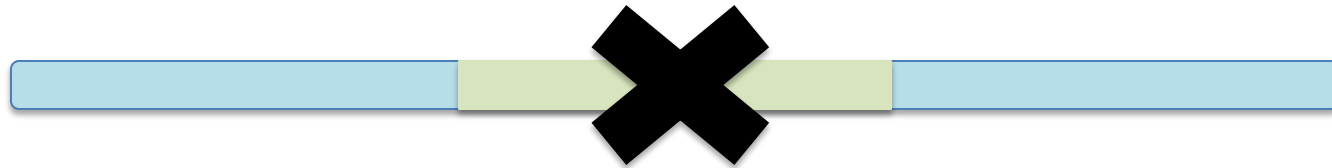
What is the effect of a deletion on inferred insert size?

Deletion

Reference
Genome

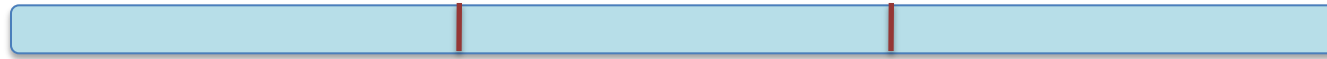


Subject



Deletion

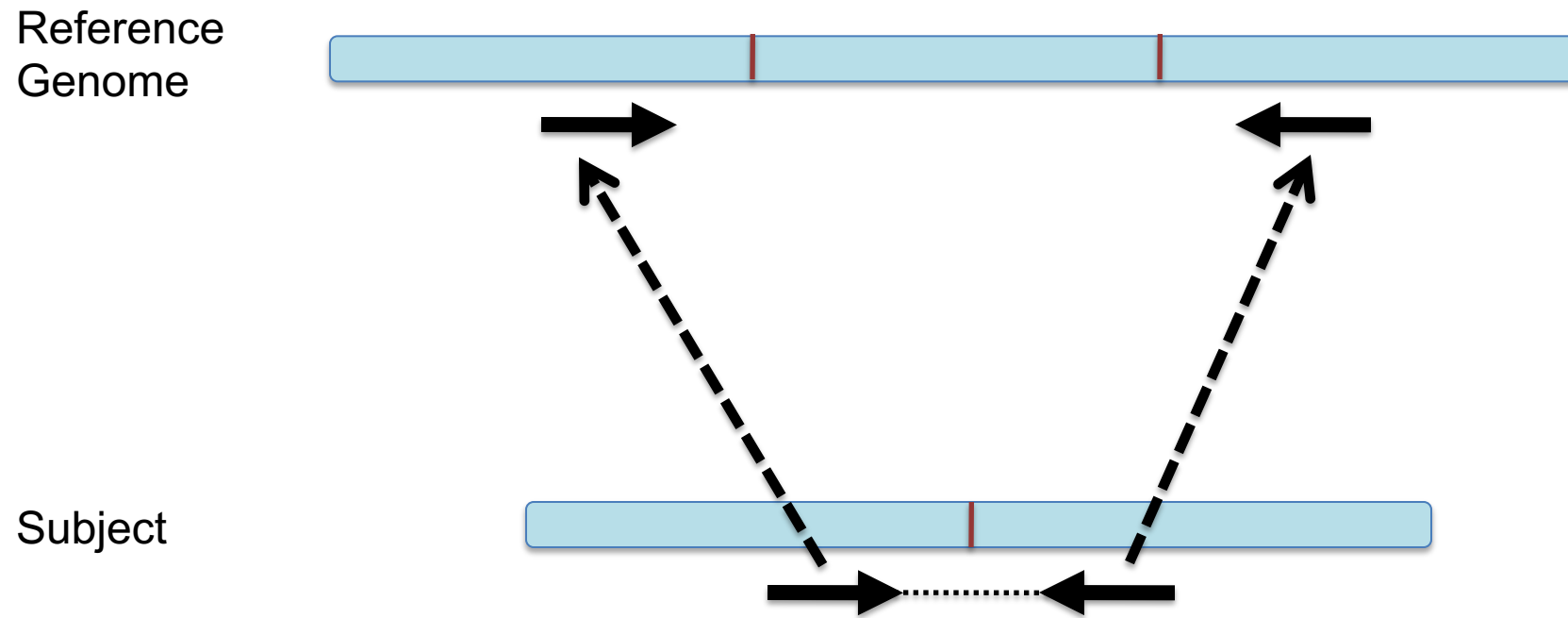
Reference
Genome



Subject



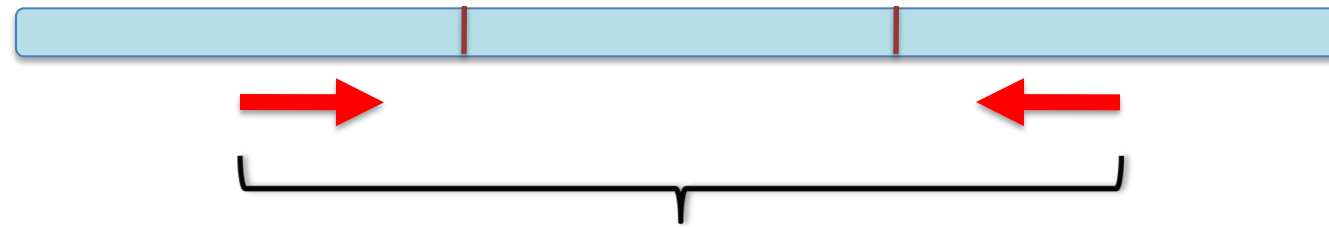
Deletion



Deletion

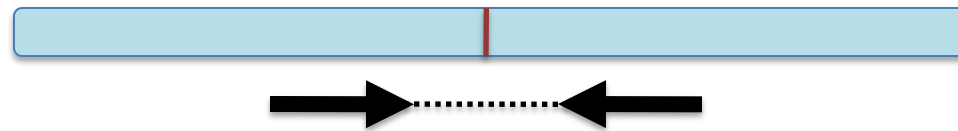
Inferred insert size is $>$ expected value

Reference
Genome



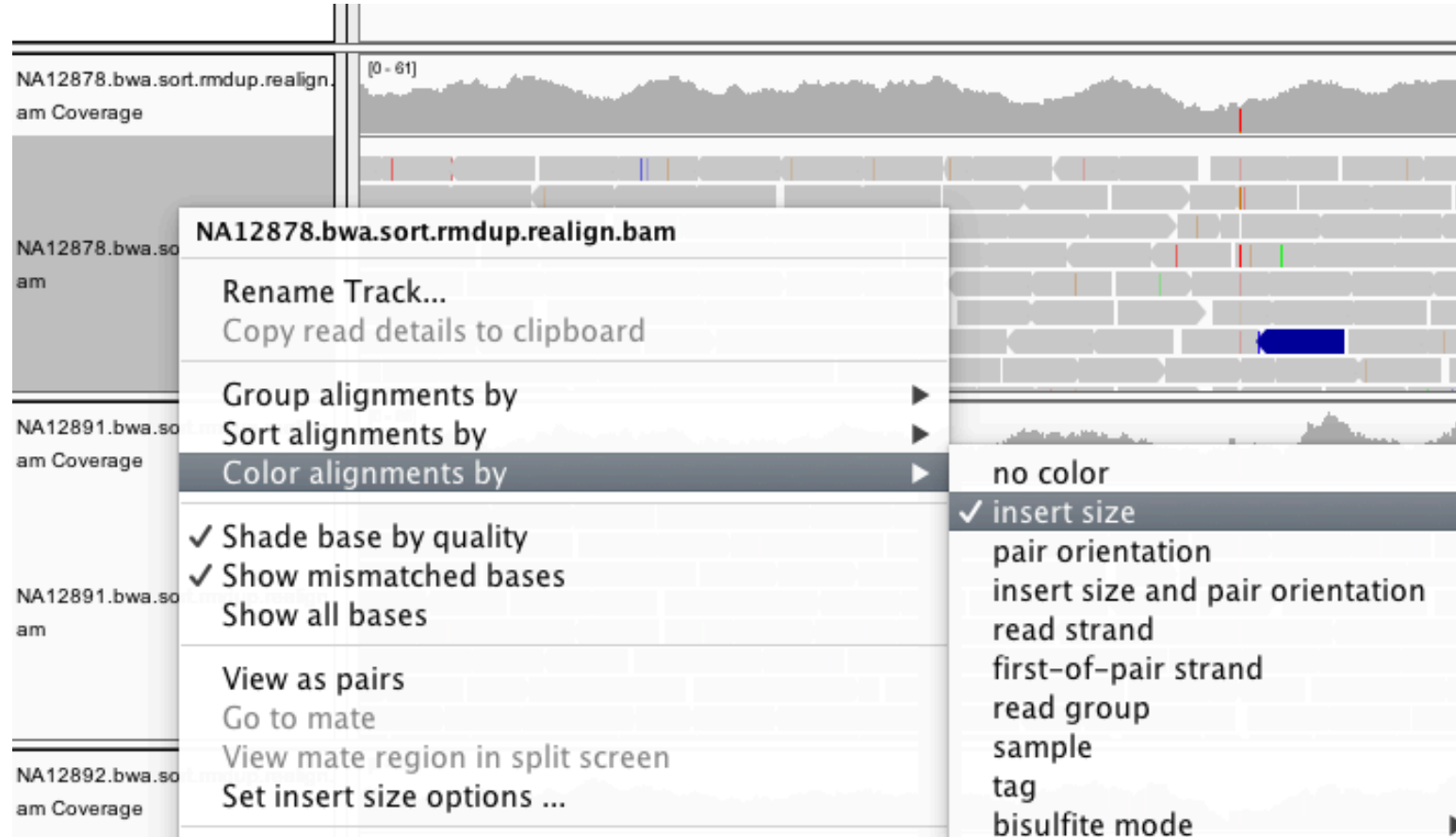
inferred insert size

Subject



expected insert size

Color by insert size

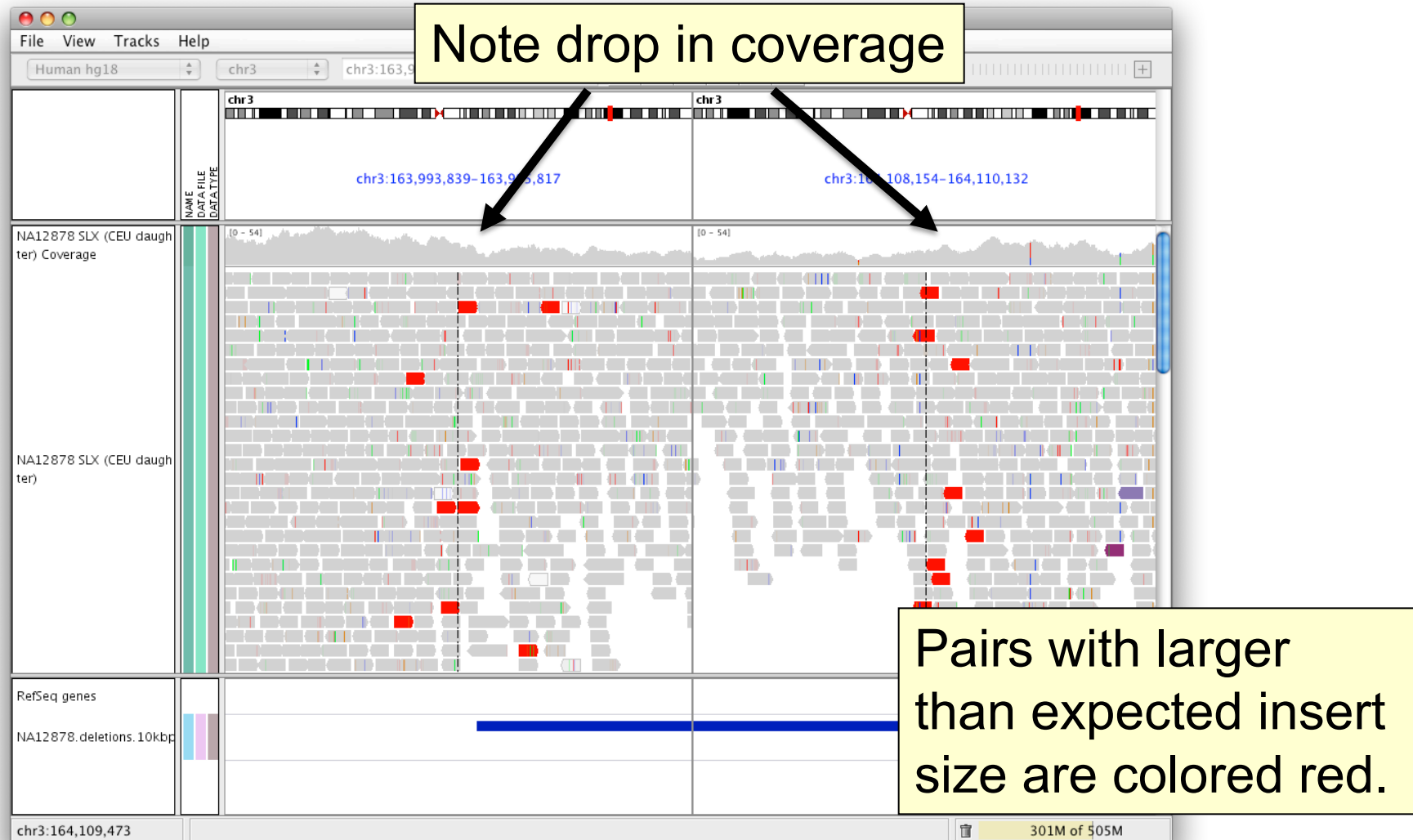


The screenshot shows a genome browser interface with several tracks. The top track is labeled 'NA12878.bwa.sort.rmdup.realign.am Coverage' and shows a coverage plot. Below it are alignment tracks for 'NA12878.bwa.sort.rmdup.realign.am', 'NA12891.bwa.sort.rmdup.realign.am Coverage', 'NA12891.bwa.sort.rmdup.realign.am', and 'NA12892.bwa.sort.rmdup.realign.am Coverage'. A context menu is open over the 'NA12878.bwa.sort.rmdup.realign.am' track, showing options for 'Color alignments by'. The 'insert size' option is selected and highlighted in blue.



Context menu options for 'Color alignments by':

- no color
- insert size
- pair orientation
- insert size and pair orientation
- read strand
- first-of-pair strand
- read group
- sample
- tag
- bisulfite mode

Deletion



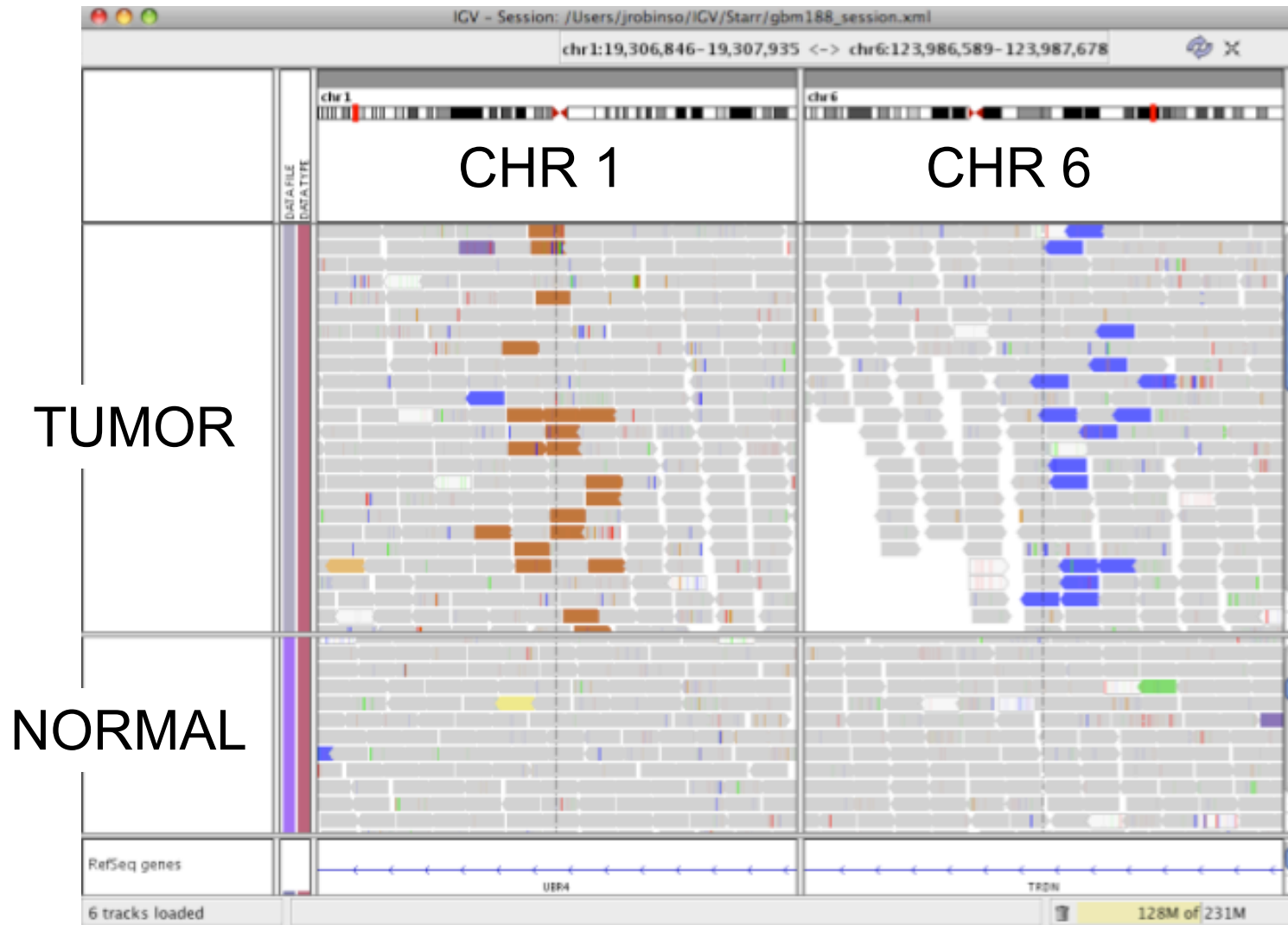
Insert size color scheme

- Smaller than expected insert size: 
- Larger than expected insert size: 
- Pairs on different chromosomes

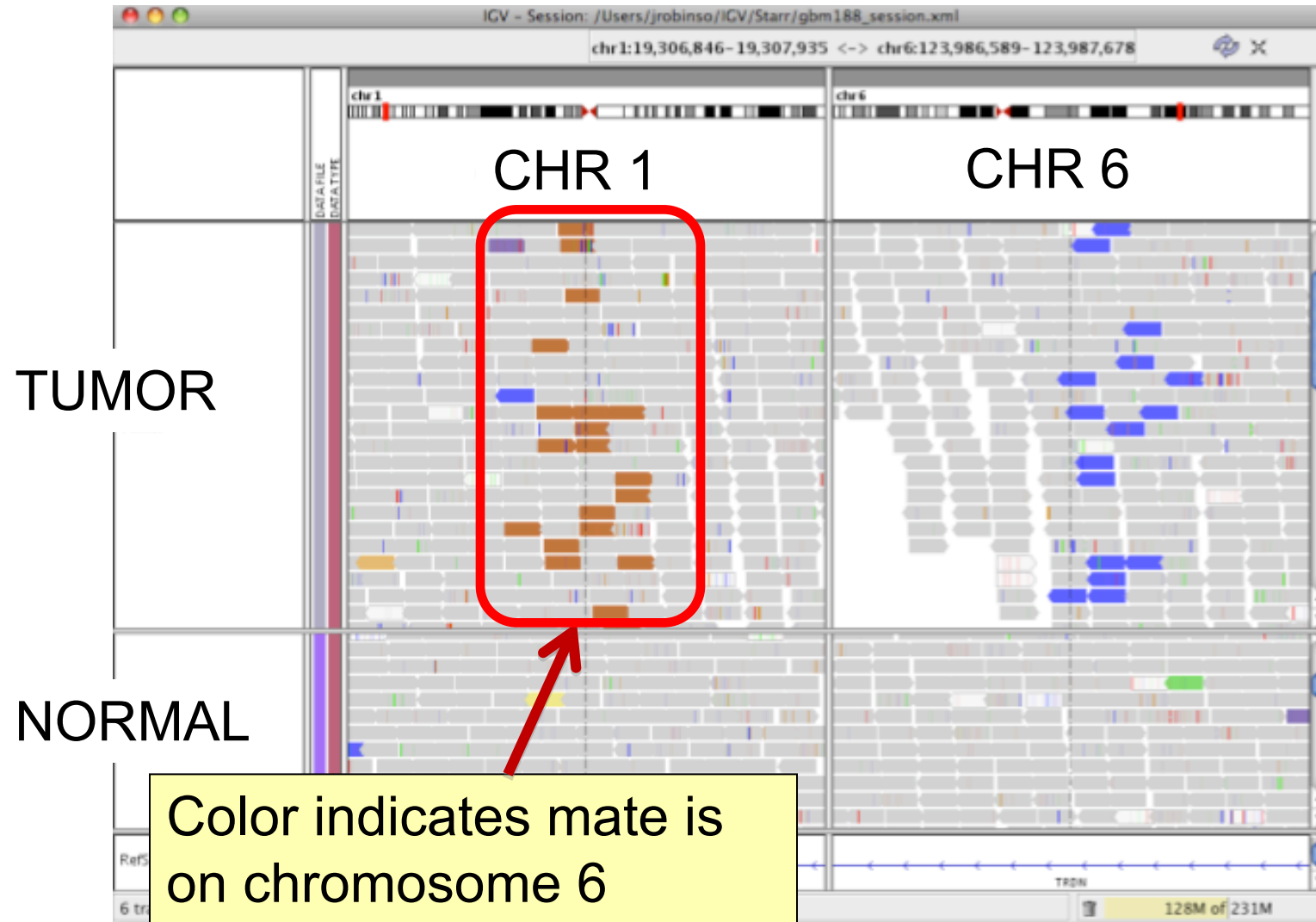
Each end colored by chromosome of its mate



Rearrangement



Rearrangement



Interpreting Read-Pair Orientations

Orientation of paired reads can reveal structural events:

- Inversions
- Duplications
- Translocations
- Complex rearrangements

Orientation is defined in terms of

- read strand, left *vs* right, *and*
- read order, first *vs* second

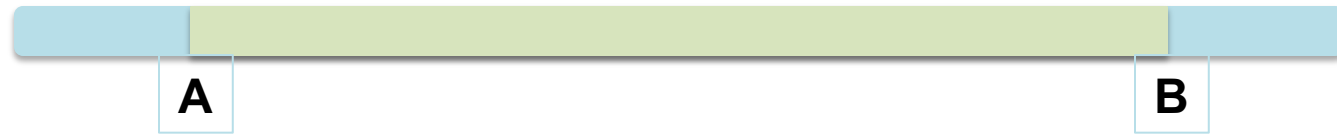
Inversion

Reference
genome

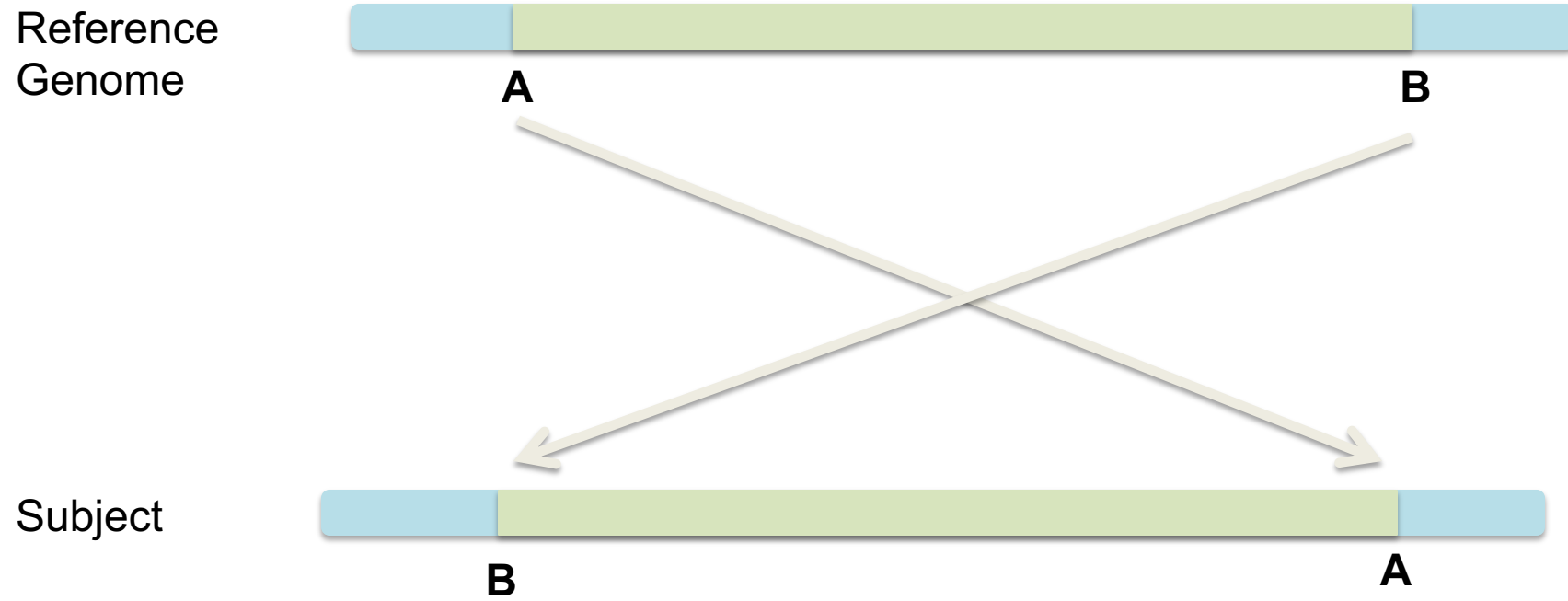


Inversion

Reference
genome

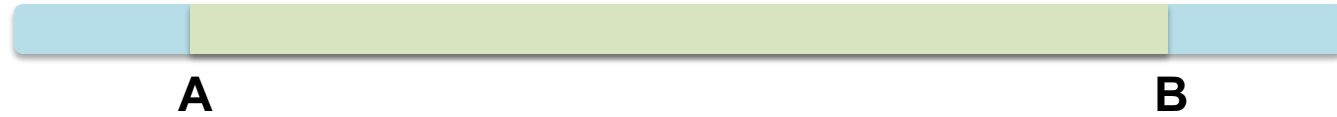


Inversion

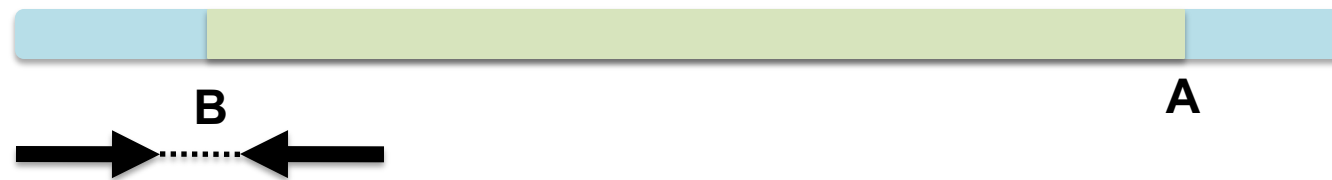


Inversion

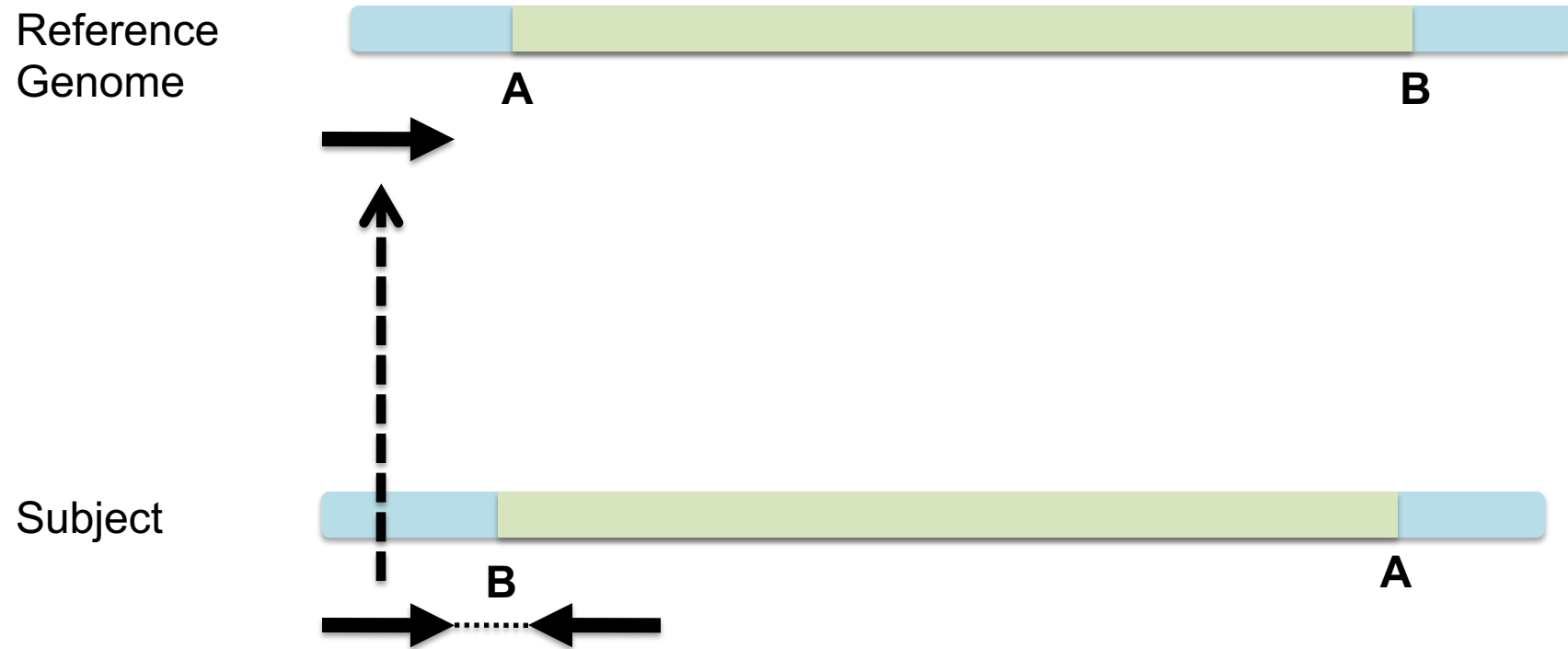
Reference
Genome



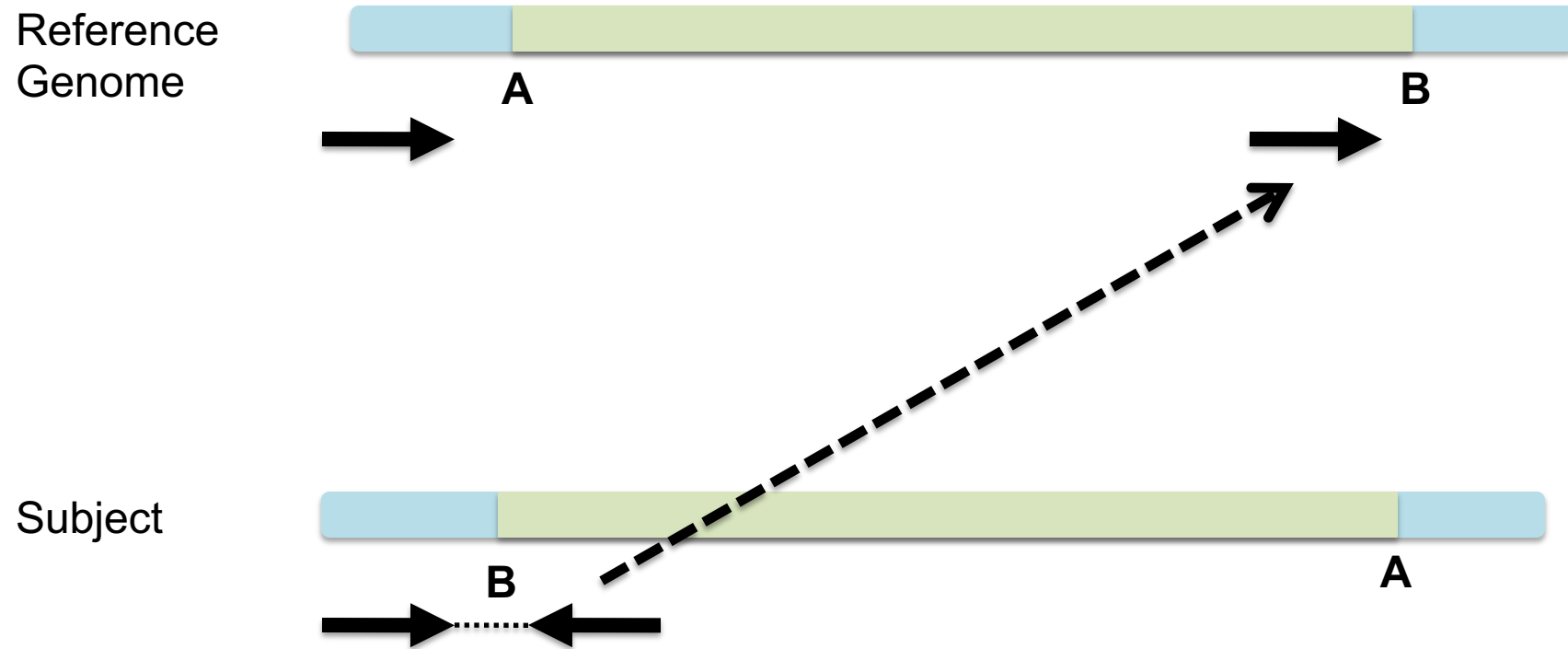
Subject



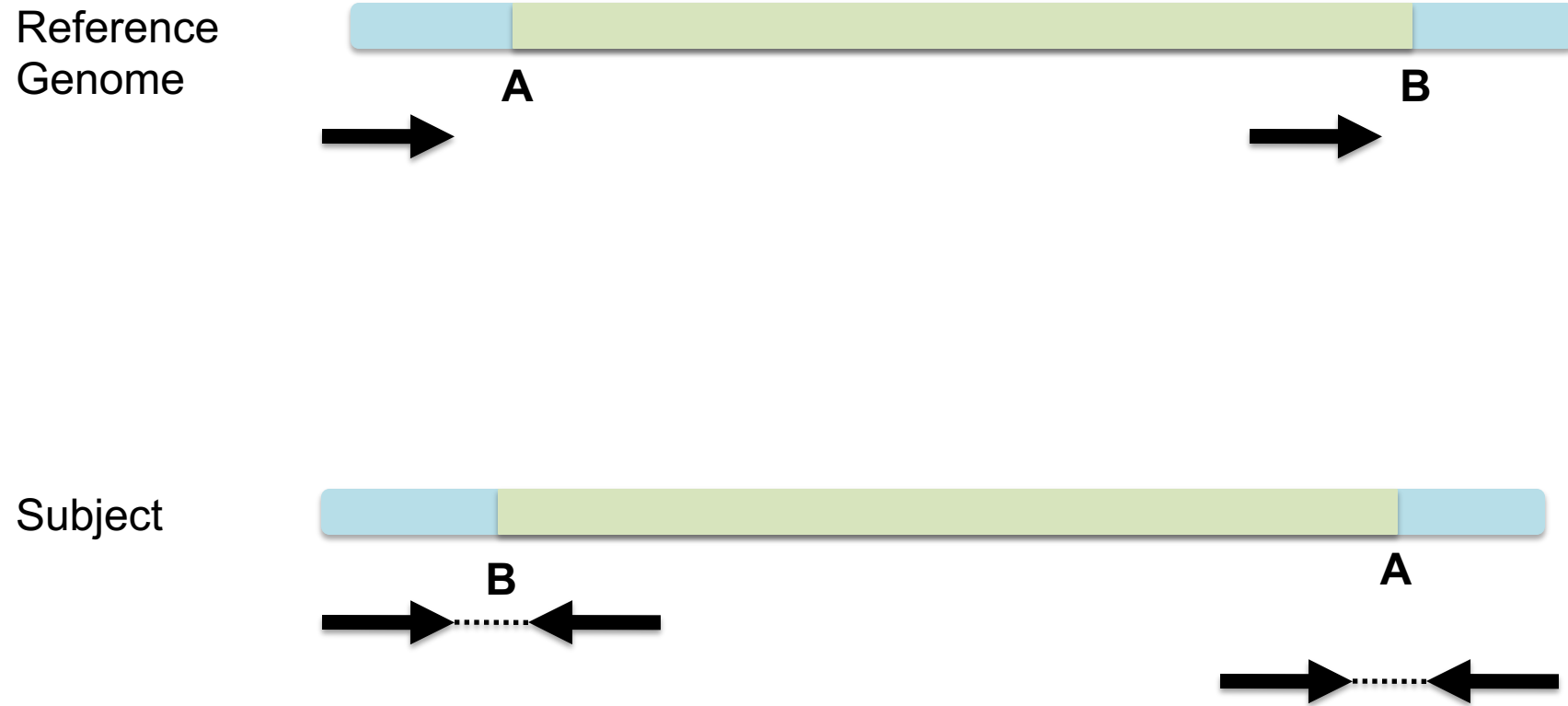
Inversion



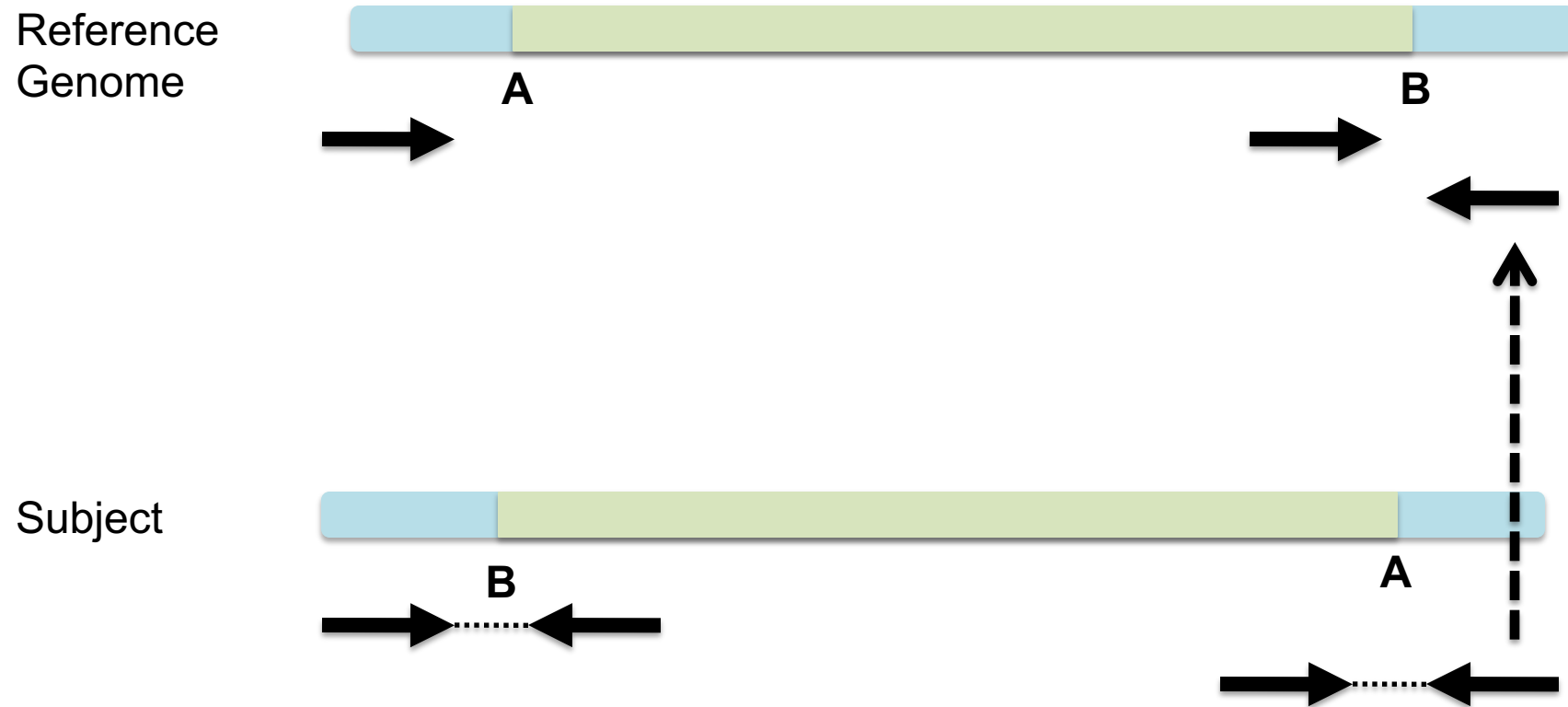
Inversion



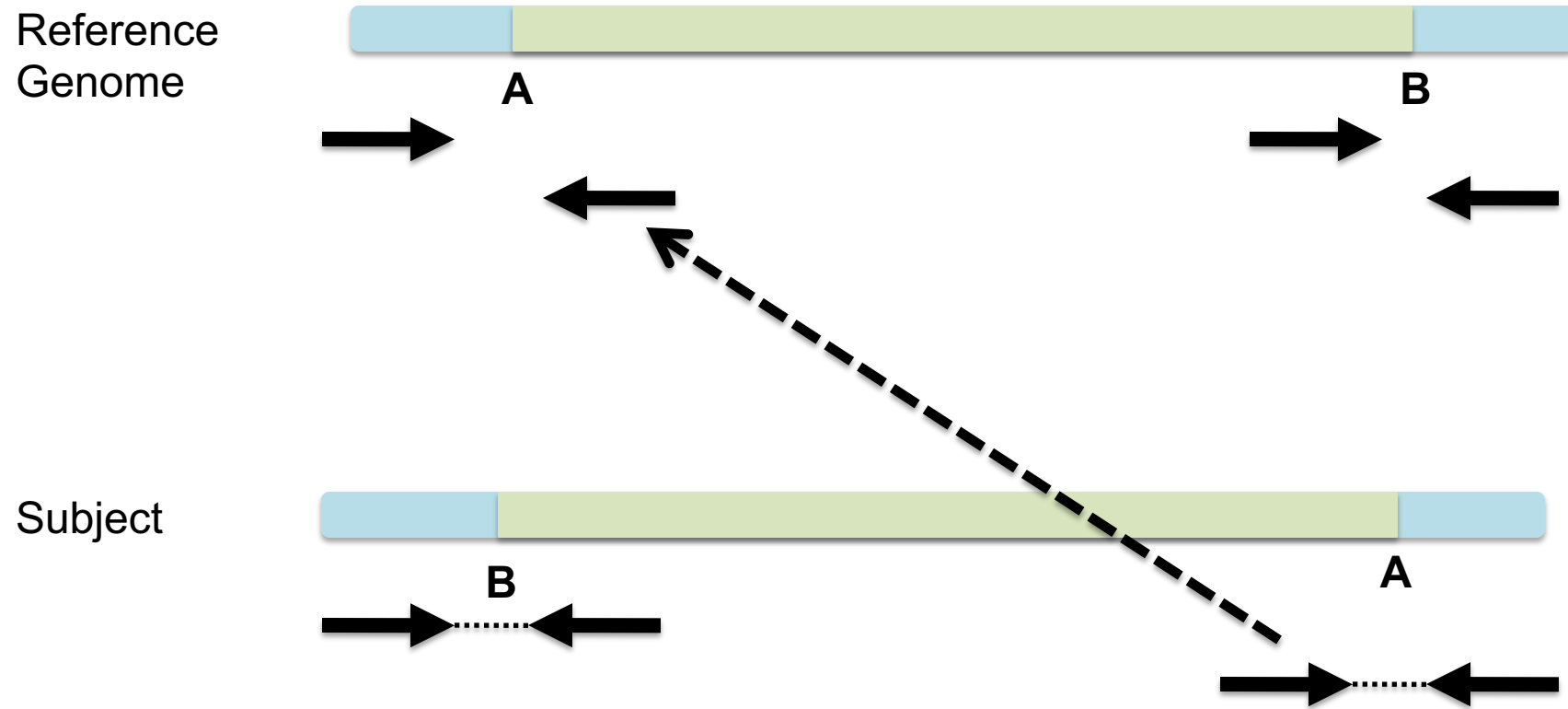
Inversion



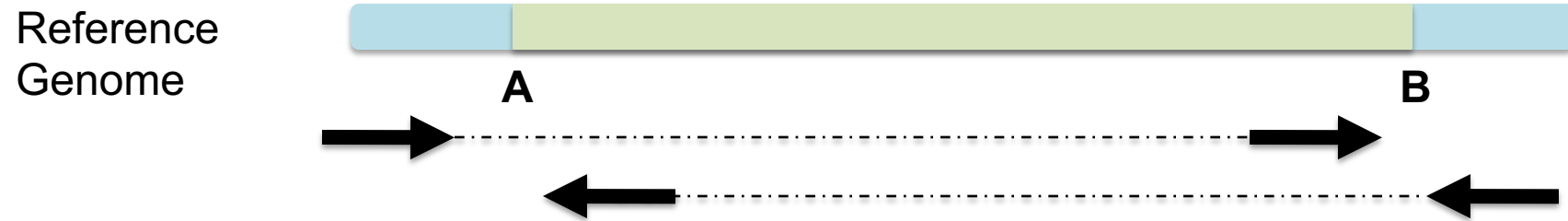
Inversion



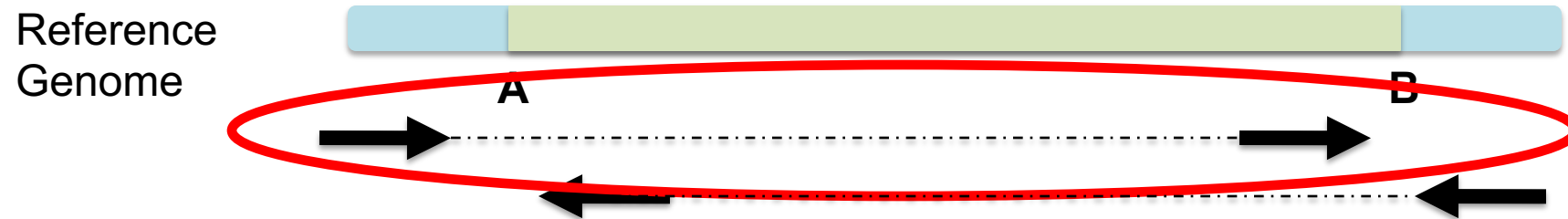
Inversion



Inversion



Inversion



Anomaly: expected orientation of pair is inward facing ($\longrightarrow \longleftarrow$)

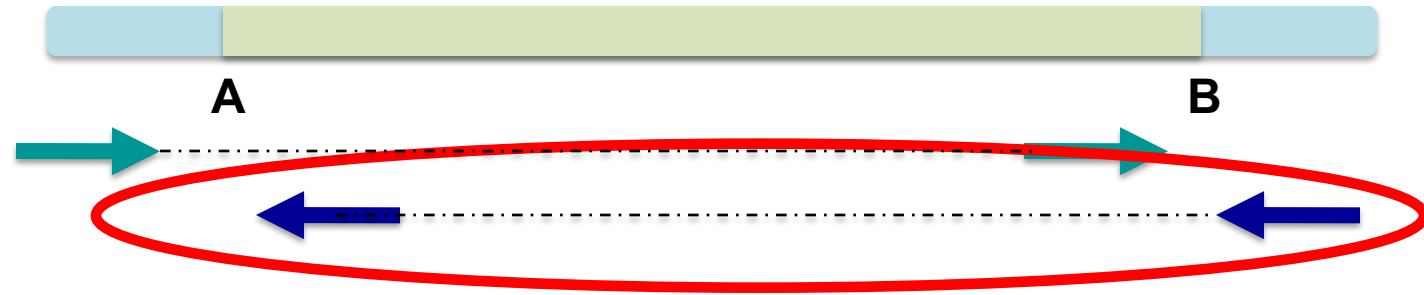
Inversion



“Left” side pair

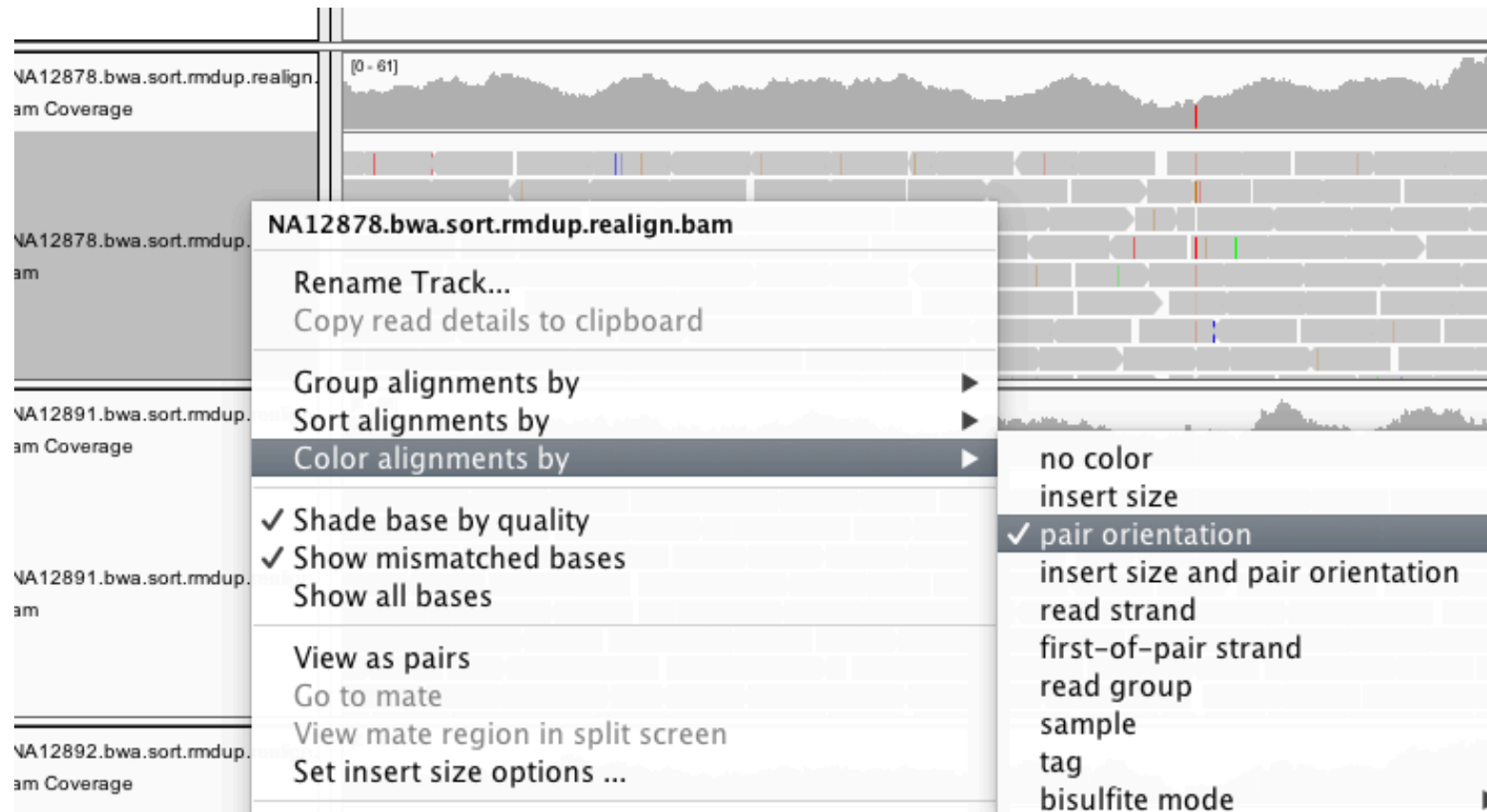
Inversion

Reference
Genome

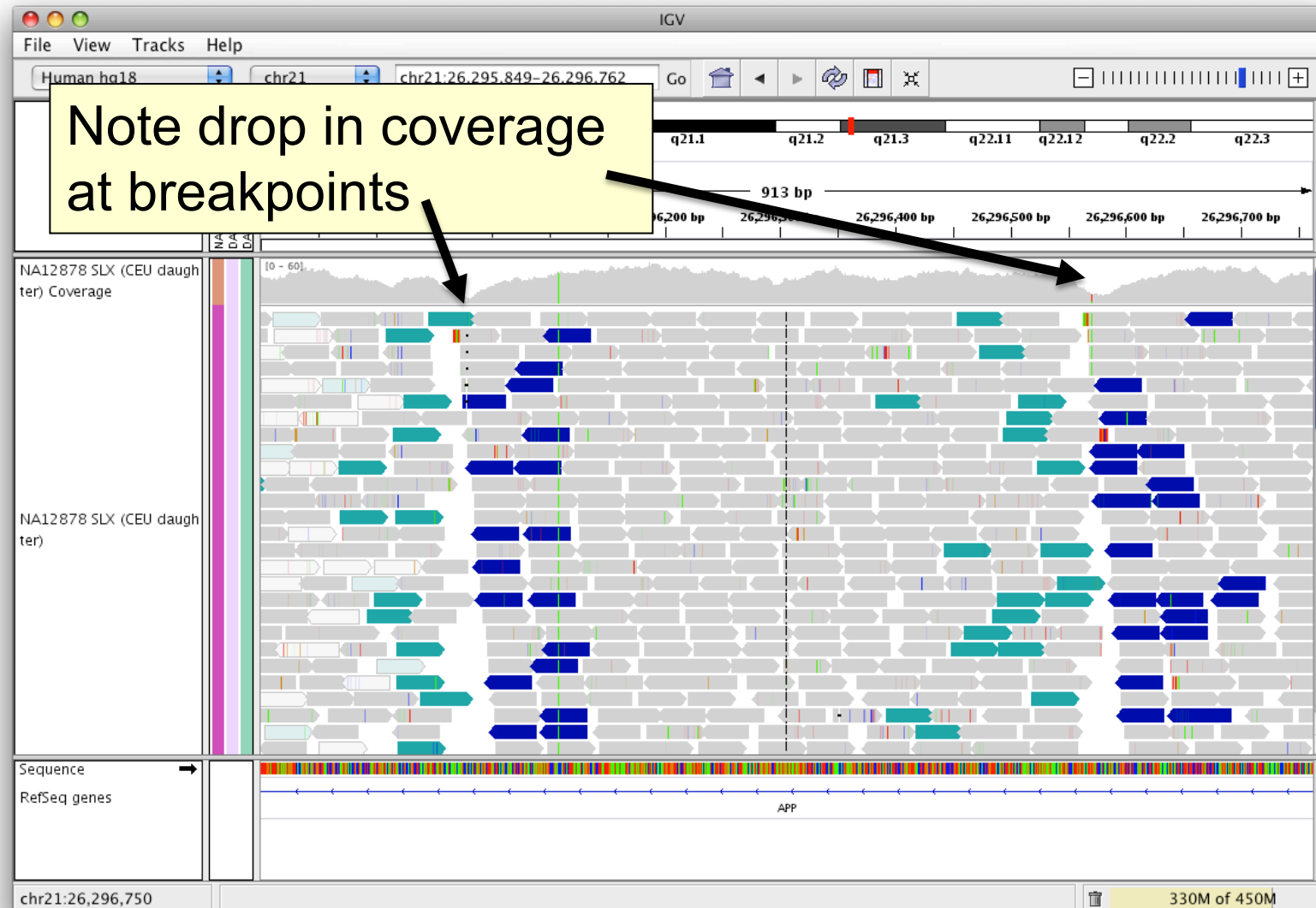


“Right” side pair

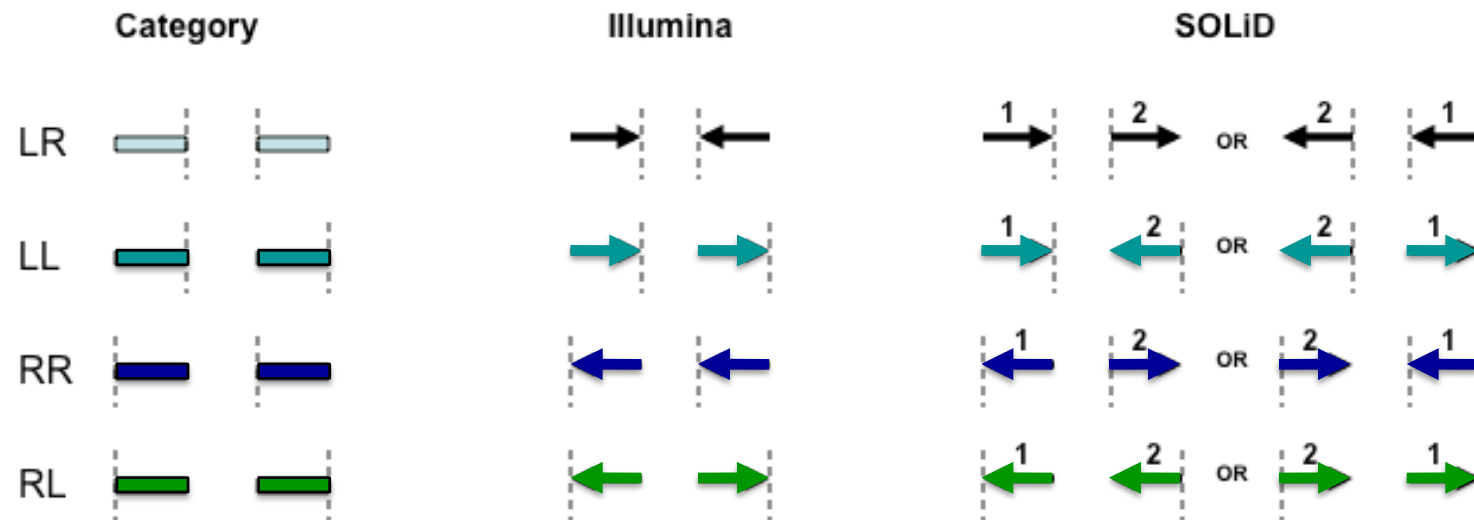
Color by pair orientation



Inversion



Interpretation of read pair orientations

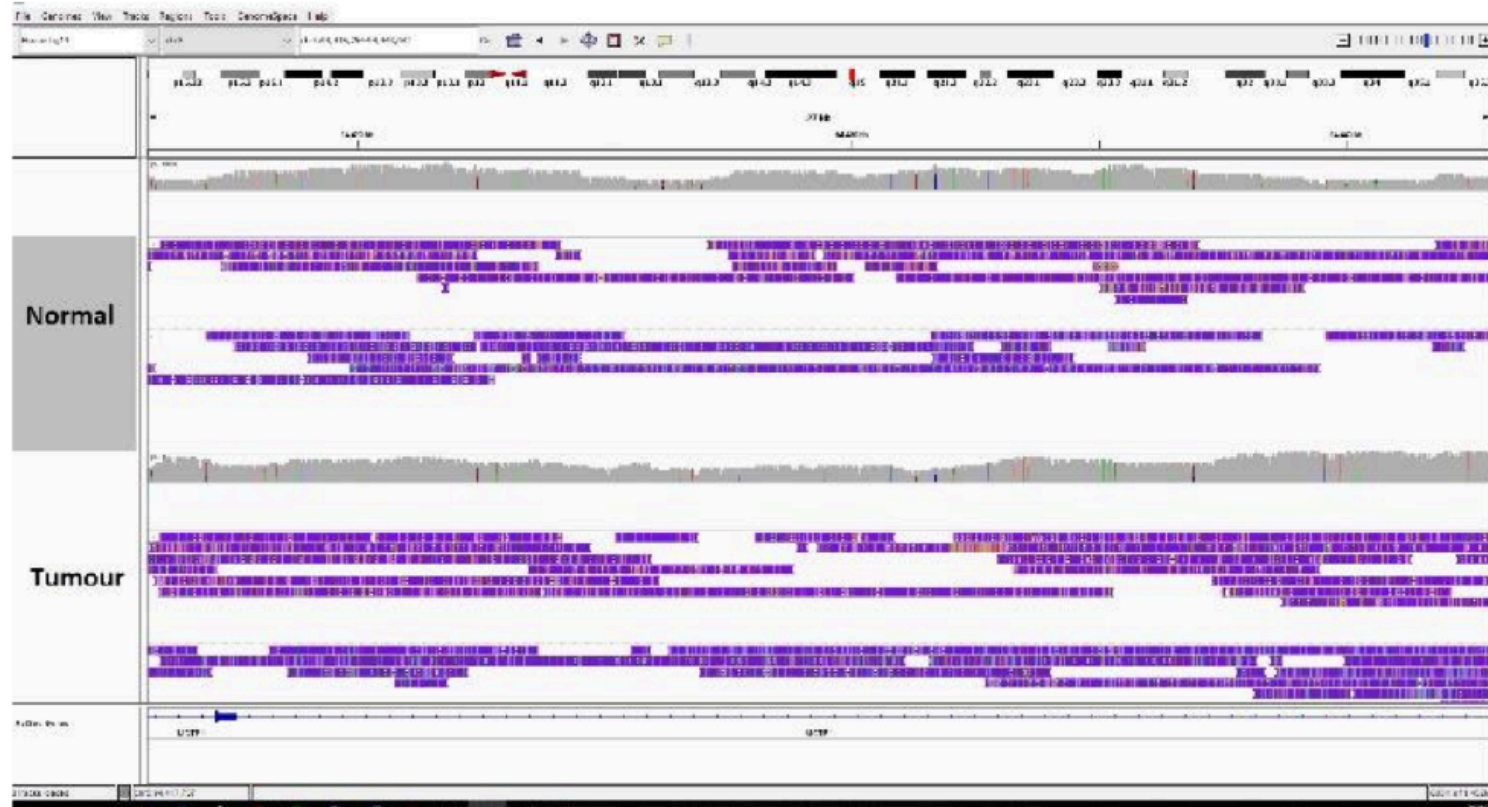


- LR Normal reads.
The reads are left and right (respectively) of the unsequenced part of the sequenced DNA fragment when aligned back to the reference genome.
- LL,RR Implies inversion in sequenced DNA with respect to reference.
- RL Implies duplication or translocation with respect to reference.

These categories only apply to reads where both mates map to the same chromosome.

Figure courtesy of Bob Handsaker

Long read considerations



- Commonly see lots of small indels and single base errors that are simply noise
- Can be removed to be able to view the data more cleanly

Long read considerations

The image shows a screenshot of a genome browser interface with the 'Alignments' track options dialog open. The 'View' menu is open, showing 'Preferences...' and 'Color Legends ...'. The 'Alignments' tab is selected in the dialog, showing various options for track display and alignment filtering. A yellow box on the left contains the text 'Setting an indel threshold hides noise from small indels' with an arrow pointing to the 'Hide indels < 20 bases' checkbox in the 'Alignment Track Options' section.

Setting an indel threshold hides noise from small indels

Alignments Track Options

- On initial load show: Alignment Track Coverage Track Splice Junction Track
- Visibility range threshold (kb): 1000 *Range at which alignments become visible*
- Downsample reads Max read count: 100 per window size (bases): 50
- Shade mismatched bases by quality: 5 to 20
- Mapping quality threshold: 0
- Label indels > 1 bases
- Flag clipping > 0 bases
- Hide indels < 20 bases
- Filter duplicate reads
- Filter vendor failed reads
- Filter secondary alignments
- Show center line
- Flag unmapped pairs
- Show soft-clipped bases
- Quick consensus mode
- Filter supplementary alignments
- Hidden SAM ta... SA,MD,XA,RG

Coverage Track Options

- Coverage allele-fraction threshold: 0.2 Quality weight allele fraction

Splice Junction Track Options

- Show flanking regions Min flanking width: 0 Min junction coverage: 1

Insert Size Options

- Defaults Minimum (bp): 50 Maximum (bp): 1000
- Compute Minimum (percentile): 0.5 Maximum (percentile): 99.5

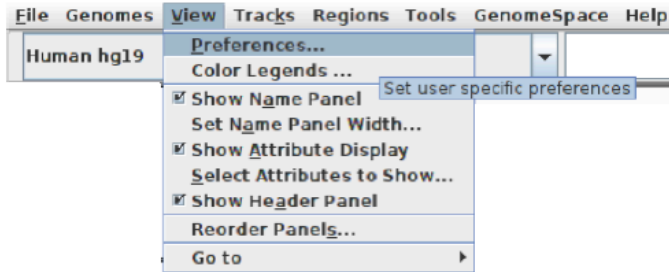
OK Cancel

Long read considerations

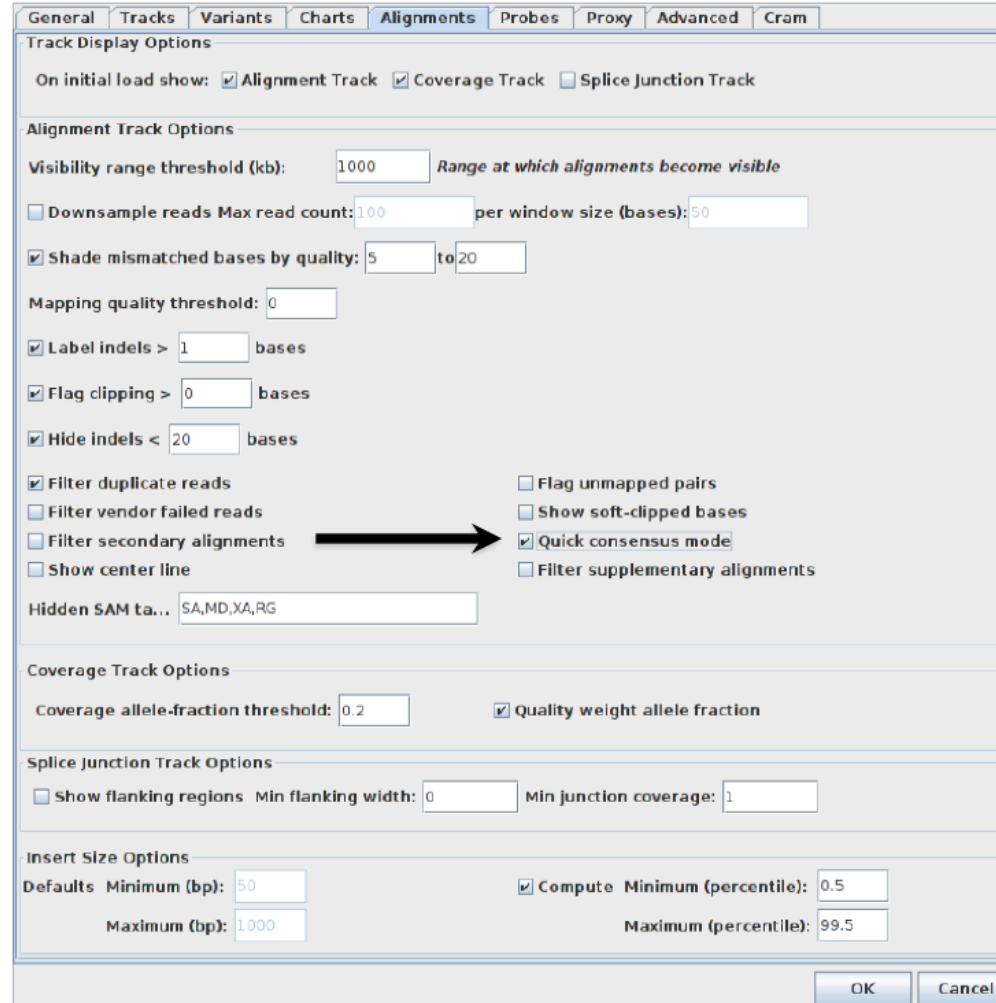


- Reads are not all purple dashes
- Next step would be to call a consensus at each position

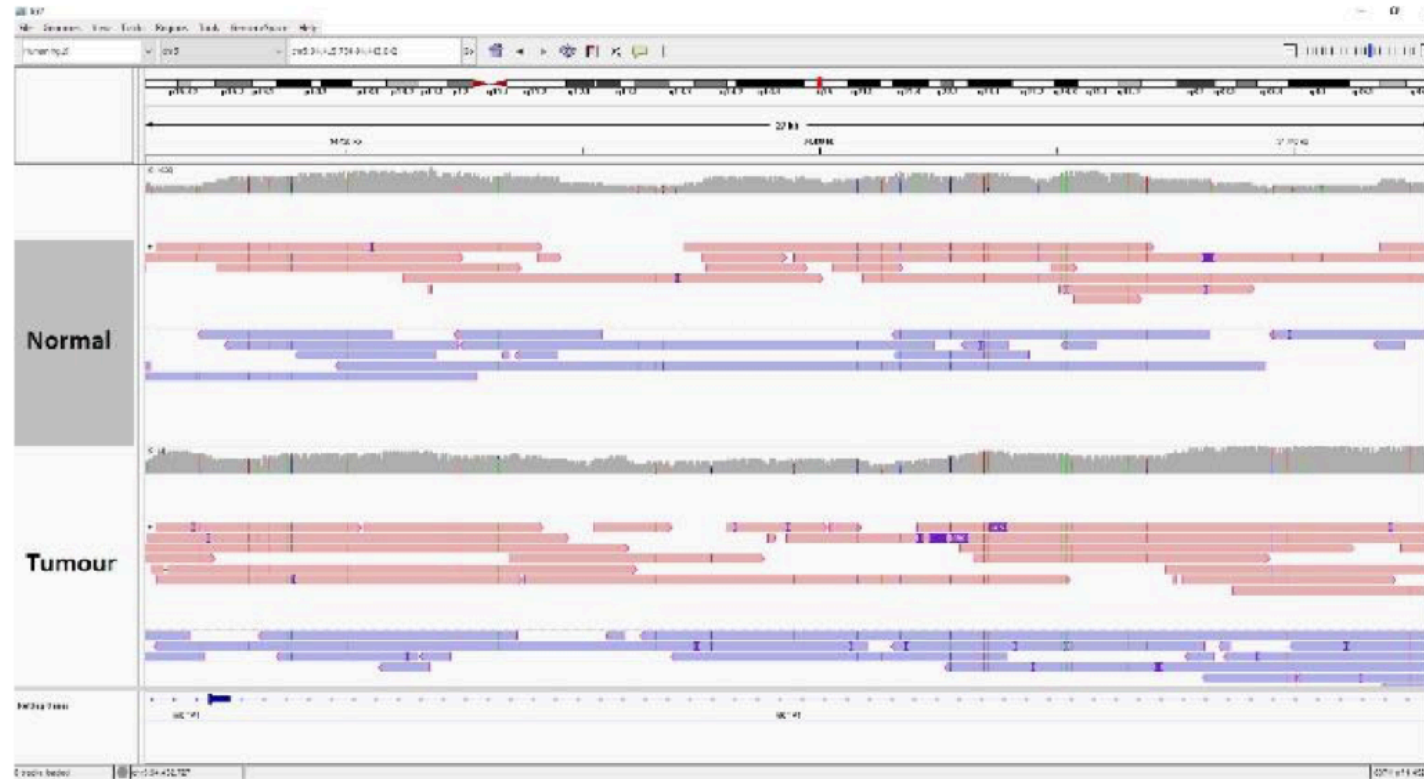
Long read considerations



Option for
generating
consensus
sequences



Long read considerations



- Much easier to parse through the genomic data
- Large insertions and deletions are also labelled now

Manual Review Standard Operating Procedure (SOP) paper

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ARTICLE | Genetics
in Medicine

Open

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples

Erica K. Barnell, BS¹, Peter Ronning, BS¹, Katie M. Campbell, BS¹, Kilannin Krysiak, PhD^{1,2}, Benjamin J. Ainscough, PhD^{1,3}, Lana M. Sheta¹, Shahil P. Pema¹, Alina D. Schmidt, BS¹, Megan Richters, BS¹, Kelsy C. Cotto, BS¹, Arpad M. Danos, PhD¹, Cody Ramirez, BS¹, Zachary L. Skidmore, MEng¹, Nicholas C. Spies, BS¹, Jasreet Hundal, MS¹, Malik S. Sediqzad¹, Jason Kunisaki, BS¹, Felicia Gomez, PhD¹, Lee Trani, BS¹, Matthew Matlock, BS¹, Alex H. Wagner, PhD¹, S. Joshua Swamidass, MD/PhD^{4,5}, Malachi Griffith, PhD^{1,2,3,6} and Obi L. Griffith, PhD^{1,2,3,6}

Purpose: Following automated variant calling, manual review of aligned read sequences is required to identify a high-quality list of somatic variants. Despite widespread use in analyzing sequence data, methods to standardize manual review have not been described, resulting in high inter- and intralab variability.

Methods: This manual review standard operating procedure (SOP) consists of methods to annotate variants with four different calls and 19 tags. The calls indicate a reviewer's confidence in each variant and the tags indicate commonly observed sequencing patterns and artifacts that inform the manual review call. Four individuals were asked to classify variants prior to, and after, reading the SOP and accuracy was assessed by comparing reviewer calls with orthogonal validation sequencing.

Results: After reading the SOP, average accuracy in somatic variant identification increased by 16.7% (p value = 0.0298) and average interreviewer agreement increased by 12.7% (p value < 0.001). Manual review conducted after reading the SOP did not significantly increase reviewer time.

Conclusion: This SOP supports and enhances manual somatic variant detection by improving reviewer accuracy while reducing the interreviewer variability for variant calling and annotation.

Genetics in Medicine (2018) <https://doi.org/10.1038/s41436-018-0278-z>

Keywords: somatic variant refinement; manual review