Supplementary Information
Integrative Genomics Viewer

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Comparison with other representative desktop tools

	Artemis[1]	EagleView[2]	MapView[3]	Apollo[4]	Tablet[5]	Savant[6]	IGB[7]	IGV	JBrowse[8]
Alignment viewing	X	X	X	X	X	X	X	X	X
BAM format support	X				X	X	X	X	see notes below
Array data support (expression, copy number, LOH, etc.)							X	X	
Integration of sample metadata								X	
Multi-resolution model for real-time pan and zoom of continuous data over all genomic scales						X		X	see notes below
Sharing data over the Web							QuickLoad, DAS, DAS2	HTTP, DAS	X
Hosted public datasets							X	X	

Table 1. Comparison of representative desktop tools.

Notes on table: While not a desktop application, we include JBrowse in this comparison as it can be installed locally and used to browse local data. JBrowse supports real-time pan and zoom by pre-computing images at various scales. It supports BAM files with the aid of a utility program to convert them to JSON (JavaScript Object Notation) format.

Multi-Resolution Data Model

Support of real-time pan and zoom over all genomic scales is challenging due to the size of data files produced by high-resolution array and sequencing platforms. Even when the data can be loaded, the resulting memory and processing demands can quickly bog down a visualization application. Indexed file formats, such as BAM[9] can be used to partially address this problem by enabling retrieval of data over limited genomic intervals. However, indexing alone is not sufficient to support interactive browsing over larger genomic intervals. As one zooms out, an increasingly larger portion of the underlying data must be loaded to render the view. As the density of the data exceeds the number of pixels available for display, this data must be aggregated before it can be viewed. For many datasets, the memory required to load and then aggregate this data can quickly exceed that available.

IGV addresses the “zooming-out” problem by pre-computing aggregated datasets using summary statistics such as mean, median, and percentiles for a series of fixed resolution scales. For each resolution scale (“zoom level”), the aggregated data is divided into chunks or tiles that correspond to a region viewable on a typical user display. Each tile is subdivided into bins, with the width of a bin chosen to correspond to the width represented by a pixel at that resolution scale. During the pre-computation step, data in each bin is aggregated into one or more summary statistics as specified by the user. The corresponding data tiles for each zoom level are stored in the binary Tiled Data Format, or TDF, which has been optimized for fast tile retrieval. By organizing data in this way, tile sizes for each zoom level are constant and small, containing only the data needed to render the view at the resolution supported by the screen display. Hence a single tile at the lowest resolution, which

spans the entire genome, has the same memory footprint as a tile at the very high zoom levels, which might span only a few kilobases. As the user moves across the genome and through zoom levels, only the tiles required to support the current view are accessed (Figures 1 & 2). Tiles no longer in view are discarded as needed to free memory.

Using this method, large datasets such as the Broad's Histone ENCODE dataset, (http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeBroadChipSeq/) approximately 400 GB in uncompressed WIG files, can be browsed at all resolution scales with minimal memory footprint (see section *Performance* and Figure 3 below). It is important to note that conversion to TDF is not required. Smaller data files are typically directly loaded into IGV in their original format without pre-processing (see Table 4 below).

We note that while the TDF format was developed to support the multi-resolution model, other formats could be used. The recently released bigWIG[10] format developed at UCSC is also a natural fit as it includes structures for storing pre-computed summary data by zoom level. We plan to support bigWIG as another option in addition to TDF in a future release (development work has already begun). A current limitation of bigWIG is its restriction to WIG files, precluding its use for array (multi-sample) file formats.

Savant also provides pan-and-zoom of large continuous-valued datasets over all resolution scales, following an approach similar to IGV. Data from WIG files can be pre-computed and stored at various zoom levels in binary ".savant" files. This step is mandatory as, unlike IGV, Savant does not support direct loading of WIG files. Similarly, JBrowse has an approach to support real-time pan and zoom of large quantitative data tracks using pre-computed images rather than data summarization. These images are generated from WIG files with a utility program in advance. We considered this approach early in IGV development, but we chose pre-computing the *data* rather than *images* as it allows the flexibility to dynamically specify at run time rendering parameters, such as graph type, scales, and colors. Furthermore, by utilizing pre-computed data rather than images, intermediate zoom levels can be computed on the fly, thus enabling the support of a continuous range of zooms rather than a fixed number of predetermined levels.

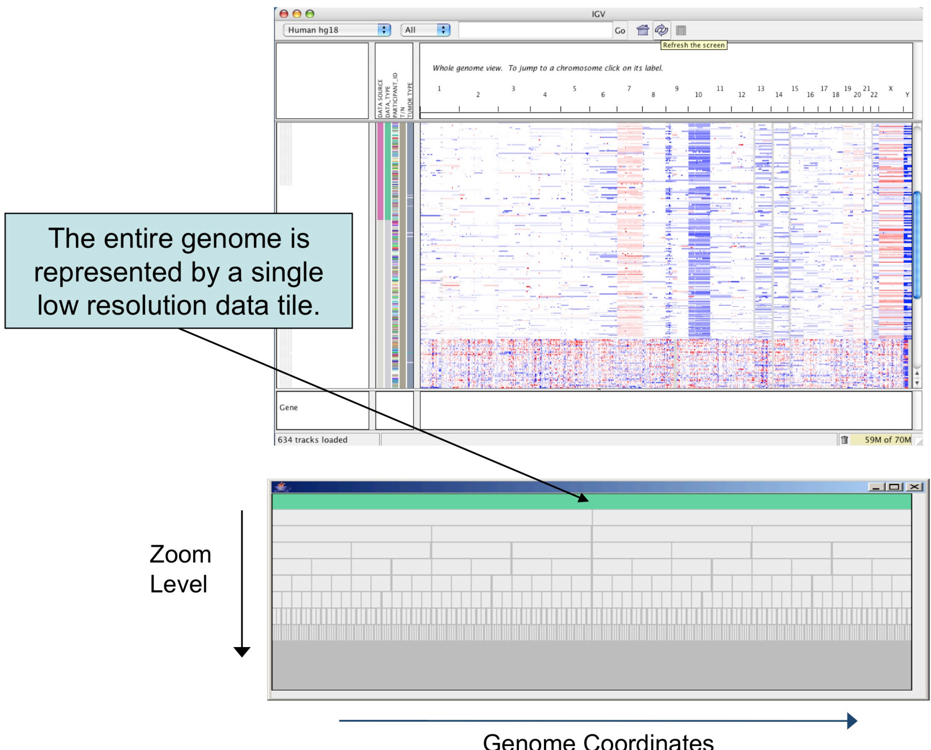

Figure 1. Multi-resolution data model: Whole-genome view. The bottom grid is a representation of the TDF file showing the tiles currently loaded in green. The entire genome is represented by a single low-resolution data tile in the TDF file.

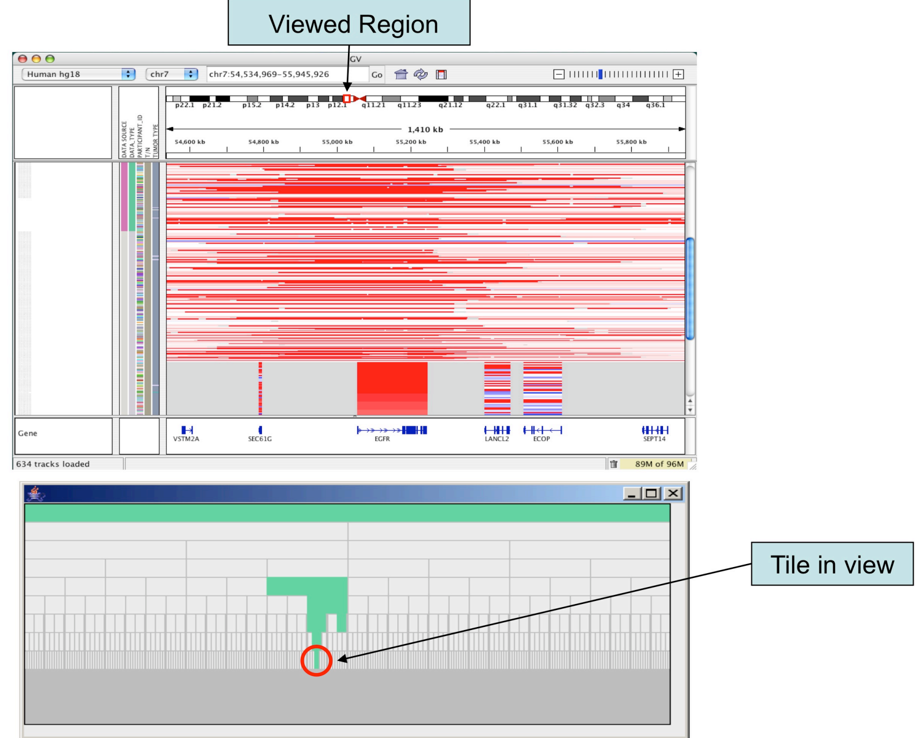

Figure 2. Multi-resolution data model: Zoomed-in view. The same data as Figure 1 is zoomed into a 1.5Mb locus around EGFR. The green blocks illustrate loaded data tiles from a series of pan and zooms. A jump was made from zoom level 0 to 4, then further panned and zoomed to arrive at the current view. The red circle highlights the tile for the current view.

Performance

Loading Numeric Data

To illustrate the advantage of pre-computing aggregate datasets we tested loading portions of a GC content (GC%) wiggle (WIG) file downloaded from UCSC in both IGV and IGB, and compared that to loading the same data converted to a TDF file in IGV. The tests were performed for 1 million, 10 million, and 50 million data points representing data windows of 5 Mbases, 50 Mbases, and 250 Mbases, respectively. Results are summarized in Table 2. As expected, load time and memory consumed increase with window size for the WIG files in both applications. The 5 Mbase window was easily handled by both applications, loading in less than three seconds and consuming minimal memory. For the 50 Mbase window approximately 200 MB of memory is required in both applications, with noticeable load times. The 250 Mbase window is pushing the usability limit for both applications. Extrapolating this result we estimate it would take approximately 6 GB of memory and at least 10 minutes to construct a whole-genome view of this track. The TDF files, by contrast, are loaded into IGV in less than 1 second in all cases and consume negligible memory. This makes interactive visual exploration possible for very large datasets, such as the Broad's ENCODE Histone dataset (http://hgdownload.cse.ucsc.edu/goldenPath/hg18/encodeDCC/wgEncodeBroadChipSeq/) consisting of 93 whole-genome ChIP-Seq tracks (Figure 3). These tracks are available as one of the hosted datasets under the "Load from server…" menu item. They load in a few seconds and take minimal memory. Zooming and panning to anywhere on the genome is near instantaneous.

Genomic Interval	WIG File		TDF File
	IGB version 6.3	IGV version 1.5.18	IGV version 1.5.18
5 Mbases (1 million data points)	3 seconds, 43 MB	2 seconds, 40 MB	< 1 sec, < 1MB
50 Mbases (10 million data points)	36 seconds, 170 MB	8 seconds, 158 MB	< 1 sec, < 1MB
250 Mbases (50 million data points)	197 seconds, 654 MB	67 seconds, 623 MB	< 1 sec, < 1MB

Table 2. Load time and memory requirements for WIG and TDF files in IGV and IGB.

Notes on table: Memory determined from memory display in lower right corner of both applications. Baseline startup memory of 34 MB for IGB and 76 MB for IGV has been subtracted from the total displayed to estimate memory required by data. The WIG files used are available for download from ftp://ftp.broadinstitute.org/pub/igv/NBT/.

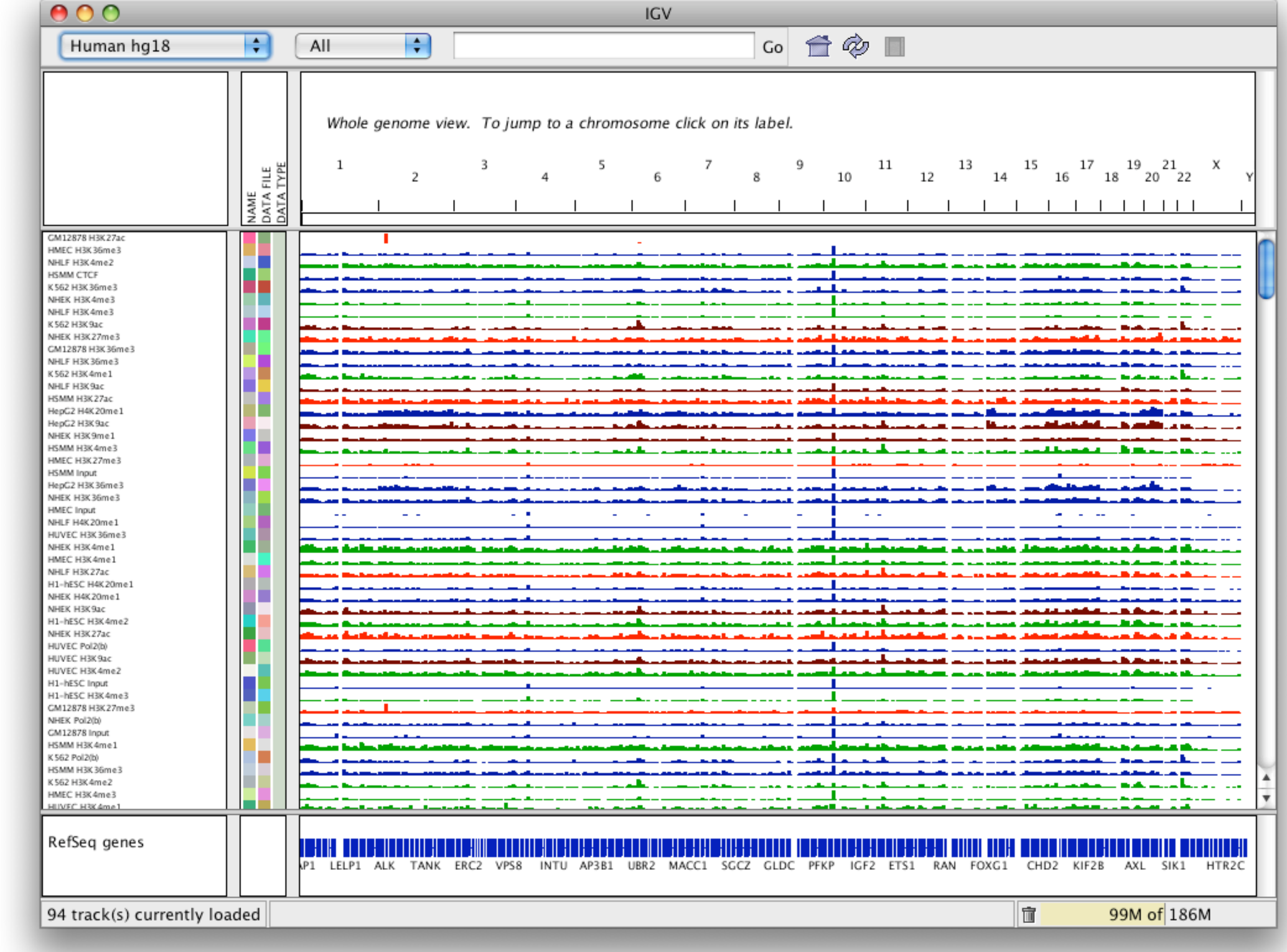

Figure 3. ENCODE Histone Data. High-density ChIP-Seq data from the ENCODE project. This data is available for loading over web to all IGV users from the "Load from server…" menu.

Loading Alignments and Genome Annotations

Zoomed out views of alignments and genome annotation features are represented by coverage or density graphs, which can be pre-computed and stored as TDF files for large tracks. Coverage graphs are used when the user zooms out past a "visibility threshold", which can vary depending on the type and density of the track. Below this threshold features are loaded on demand and drawn as individual units. Large feature tracks can be indexed, which keeps overall memory demands low.

BAM alignment files, in particular, are information rich, containing not only the alignment interval but the read bases and quality scores, indel information in the form of a "cigar" string, mate information, and other optional fields. These are memory intensive features, so the visibility threshold setting is important for good performance. The default value is 30 Kbases, which is a reasonable value for datasets in the 30X -100X coverage range. For very deep coverage datasets this window should be reduced accordingly. The memory required to load a given window size can be estimated from the formula:

$$memory = 100 + (depth * window / 30),$$

where

memory = memory required in MB
depth = average coverage depth
window = window size in Kbases.

Table 3 summarizes memory requirements in MB for various depth / window size combinations.

Coverage Depth	Window Size			
	1 Kbase	10 Kbases	30 Kbases	100 Kbases
10 X	100	103	110	133
50 X	102	117	150	267
100 X	103	133	200	433
1,000 X	133	433	1,100	3,767

Table 3. BAM file memory requirements in MB.

File Formats

IGV supports a number of different file formats for experimental data and genome annotations. For a complete list of supported formats see http://www.broadinstitute.org/igv/FileFormats. The following table shows the recommended file formats for a number of common data types.

Source Data	Recommended File Formats
ChIP-Seq, RNA-Seq	WIG, TDF
Copy number	CN, SNP, TDF, canary_calls (Birdsuite)
Gene expression data	GCT, RES, TDF
Genome annotations	GFF, BED, GTF, PSL, UCSC table format
GISTIC data	GISTIC
LOH data	LOH, TDF
Mutation data	MUT, MAF
Variant calls	VCF
RNAi data	GCT
Segmented data	SEG, CBS
Sequence alignment data	BAM, SAM, PSL
Any numeric data	IGV, WIG, TDF
Sample metatadata	Tab-delimited sample info file

Table 4. IGV file formats.

Sample Information File

A particular strength of IGV is the ability to flexibly and dynamically integrate diverse data tracks. Important to this functionality is the ability to associate arbitrary metadata to each track. These associations are defined in a tab-delimited “sample information file”. The first column of a sample information file is treated as a key identifying a track. This is often an experiment or chip identifier. Subsequent columns may contain arbitrary attributes, for example patient identifier, gender, tumor/normal, etc.

The sample information file plays an important role in integrating diverse data tracks from the same sample or patient. For example, tracks can be grouped based on the value of an attribute from the sample info file, such as a patient identifier. Similarly, sample attributes are used to overlay mutation tracks on other related tracks. Other uses of track attributes include sorting and filtering. The following table shows the contents of an example sample information file.

TRACK_ID	Data_Type	PARTICIPANT_ID	SAMPLE_ID	GENDER	T/N	Tumor_type	Treated	Primary / Secondary	Hypermutated
EX-01-001	Expression	P-01-P001	P-01-S001	M	Tumor	GBM	Y	Primary	Y
CN-01-002	CopyNumber	P-01-P001	P-01-S001	M	Tumor	GBM	Y	Primary	Y
MU-01-003	Mutation	P-01-P001	P-01-S002	M	Tumor	GBM	Y	Primary	Y
EX-01-004	Expression	P-01-P002	P-01-S003	M	Normal	GBM	Y	Secondary	Y
CN-01-005	CopyNumber	P-01-P002	P-01-S004	M	Tumor	GBM	Y	Secondary	N
EX-01-006	Expression	P-01-P002	P-01-S004	M	Tumor	GBM	Y	Secondary	N
ME-01-007	Methylation	P-01-P002	P-01-S004	M	Tumor	GBM	Y	Secondary	N
EX-01-008	Expression	P-01-P003	P-01-S006	F	Tumor	GBM	N	Primary	Y
EX-01-009	Expression	P-01-P004	P-01-S009	F	Tumor	GBM	N	Primary	Y
EX-01-0010	Expression	P-01-P005	P-01-S0011	M	Control				

Table 5. Sample information file

IGV Data Server

In addition to support for loading data via files from the local file system or URL, IGV includes an option to load data from public or private Web servers. The Broad maintains one such server, which provides access to data sets and genome annotation files from a number of genome characterization projects and public databases. These include:

- Gene expression, chromosomal copy number, loss of heterozygosity, methylation analysis, and mutation data for glioblastoma samples from The Cancer Genome Atlas project (TCGA). Note that only open-access TCGA data is made available. For more information see http://www.broadinstitute.org/igv/resources/tcga.html.
- Gene expression and chromosomal copy number data from the Multiple Myeloma Research Consortium. See http://www.broadinstitute.org/mmgp/home.
- Copy number alterations across human cancers from Beroukhim et al, *The landscape of somatic copy-number alteration across human cancers*, Nature **463**, 899-905 (18 February 2010).
- ChIP-Seq Histone data from the ENCODE project. See http://genome.ucsc.edu/ENCODE/.
- Next-generation sequencing data from the 1000 Genomes Project. See http://www.1000genomes.org/.

- ChIP-Seq Histone data from Aiden et al., *Wilms Tumor Chromatin Profiles Highlight Stem Cell Properties and a Renal Developmental Network*, Cell Stem Cell, Volume 6, Issue 6, 591-602, 4 June 2010.
- ChIP-Seq data from Ku et al., *Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains*, PLoS Genet. 2008 Oct;4(10).
- RNA-Seq data from Illumina Hi-Seq sequencing of 15 human tissues (BodyMap 2.0).
- Genome annotation data from public databases, including RefSeq, UniGene, Ensembl, UCSC Genome Bioinformatics, OMIM, COSMIC, and others.

Researchers can easily set up their own IGV servers by placing data in an HTTP accessible directory and describing that data with a simple XML file (see http://www.broadinstitute.org/igv/DataServer). This data can be optionally password protected. Standard HTTP was chosen in favor of specialized server protocols, such as DAS or QuickLoad, to minimize informatics support requirements for end users and organizations wishing to host a data server. Most organizations have a Web server, and many users have access to Web accessible directories. It is in general much easier to use this existing infrastructure than to install and maintain a specialized server. To enable Web hosting of random access formats such as BAM, TDF, and our feature index files we developed streaming classes to do binary random access over the Web using the range-byte feature of the HTTP protocol. We contributed these Java classes to the Picard project (http://picard.sourceforge.net/) for BAM files, and incorporate them internally in IGV for access to other file types.

References

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2. Huang, W. & Marth, G. *Genome Res* **18**, 1538-1543 (2008).
3. Bao, H. et al. *Bioinformatics* **25**, 1554-1555 (2009).
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10. Kent, W.J., Zweig, A.S., Barber, G., Hinrichs, A.S. & Karolchik, D. *Bioinformatics* **26**, 2204-2207 (2010).

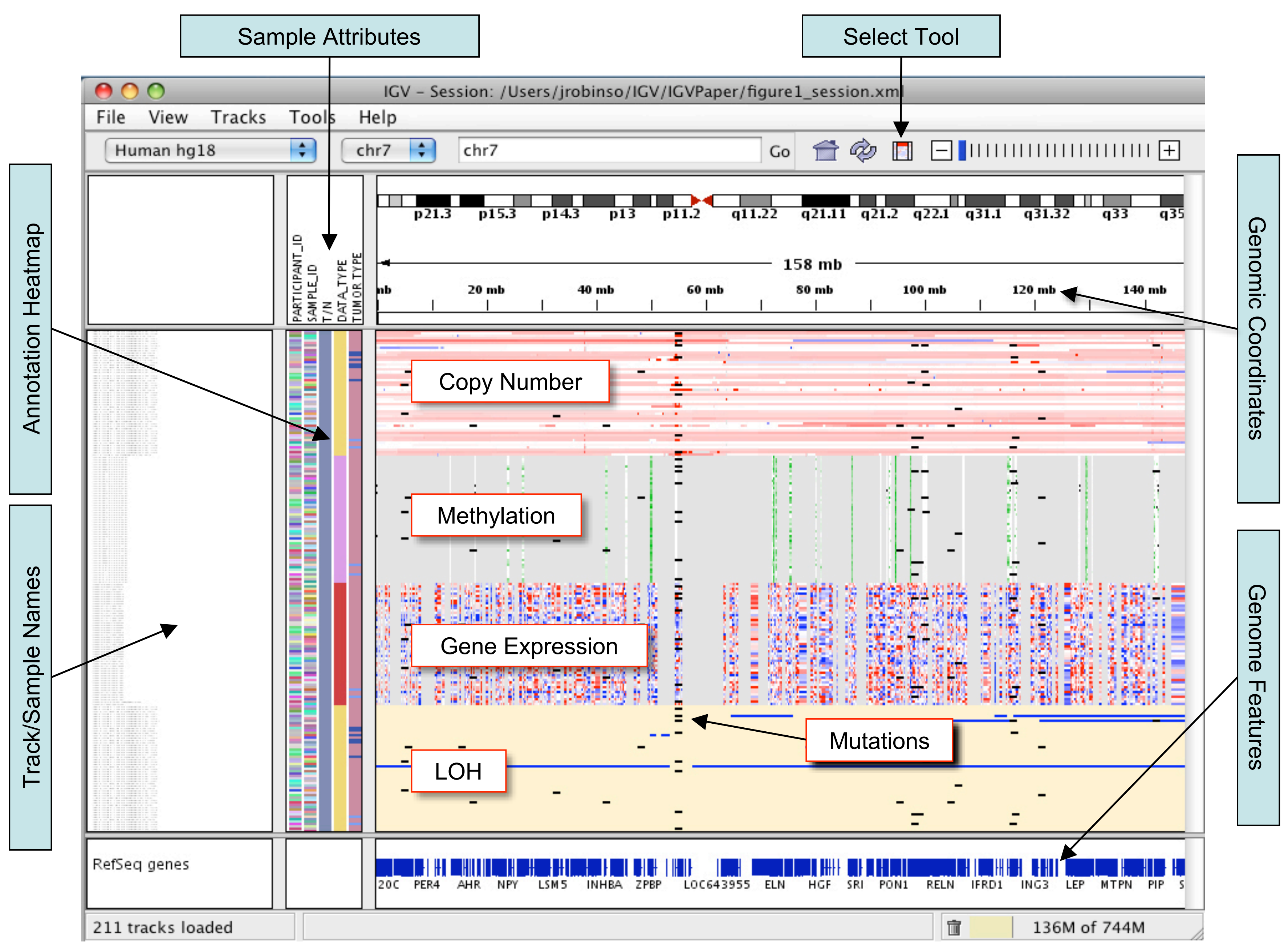


Figure S1: IGV user interface. This figure displays a screen shot of the IGV interface showing five data types (from top to bottom: Affymetrix SNP 6.0 copy number, Illumina methylation, Affymetrix gene expression, and SNP 6.0 loss of heterozygosity; mutations are overlaid with black boxes) from approximately 80 glioblastoma multiforme samples.

Figure S2: Copy number and mutation data visualized at different scales. The figure illustrates a series of views of Affymetrix SNP 6.0 copy number and sequence mutations (overlaid) for 206 glioblastoma tumor samples from the TCGA project at increasing levels of resolution. Red shading indicates the degree of copy gain; blue shading indicates copy loss. Small black squares indicate the position of point missense mutations.

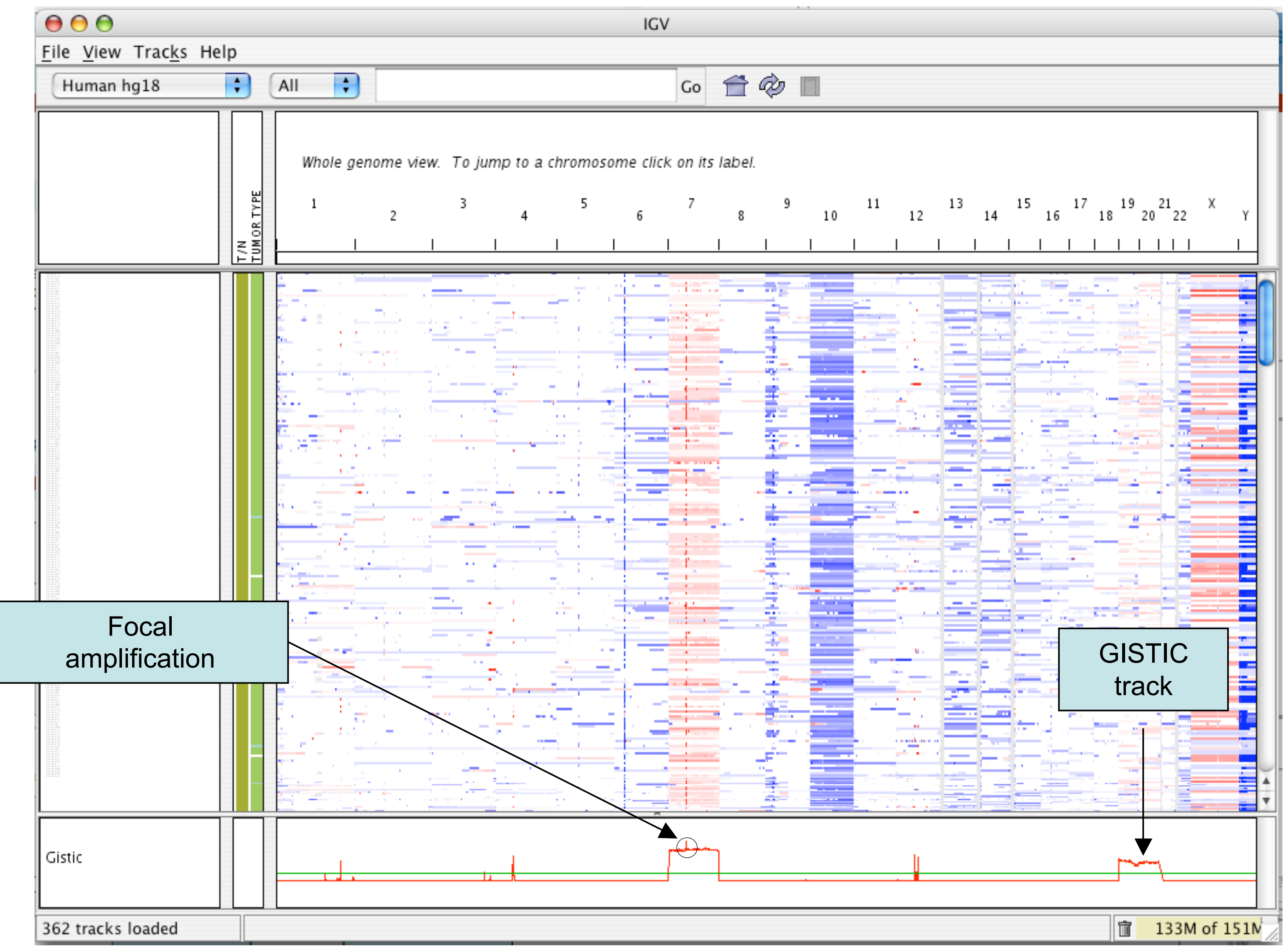


Figure S2a: Genome-wide view. Genome-wide view allowing visual assessment of general data quality as well as an overview of common alterations in this disease. From this vantage, users can identify frequent whole chromosome or chromosome arm alterations, such as gain of chromosome 7 or loss of 9p or 10.

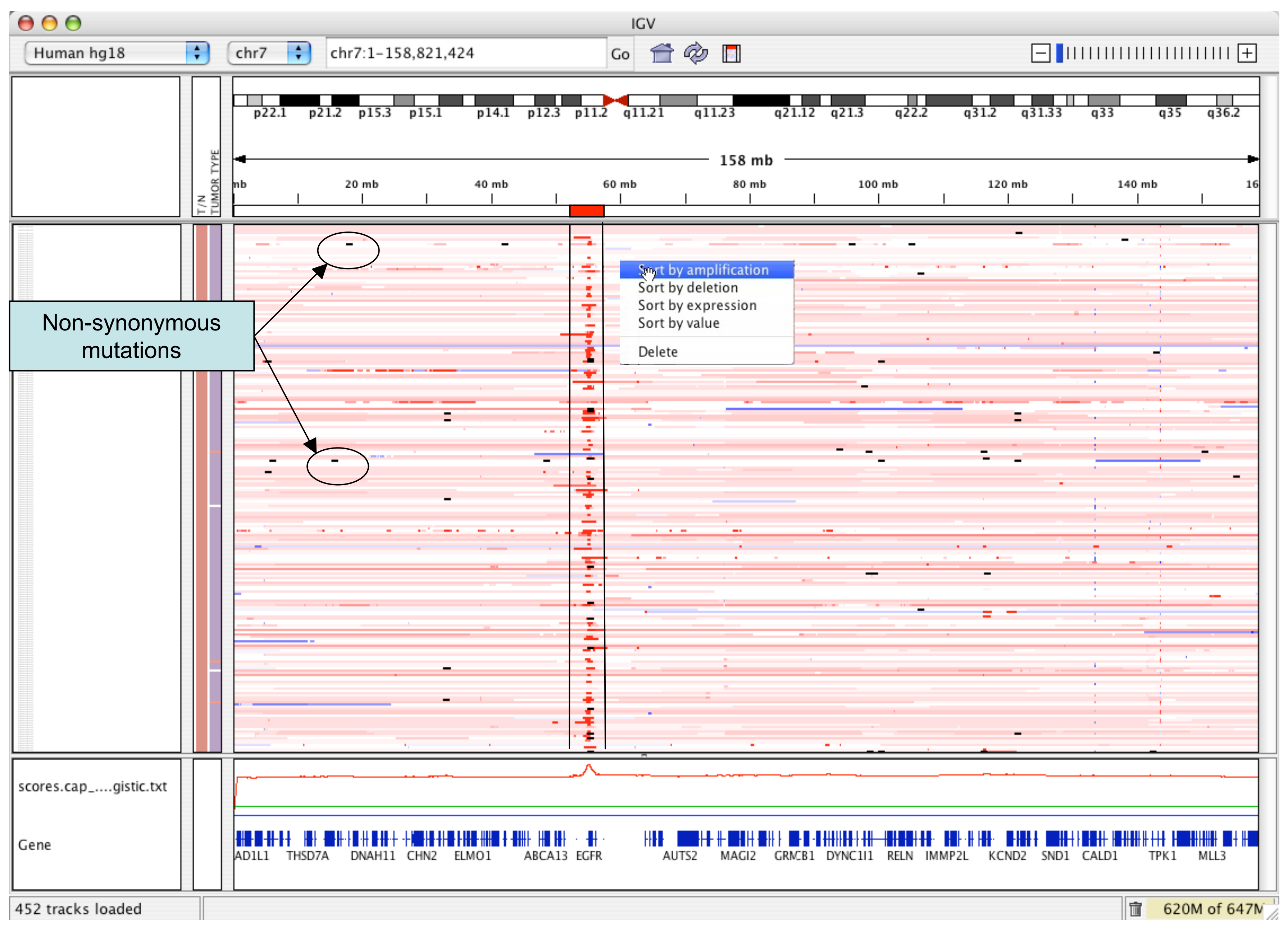


Figure S2b: Chromosome centric view of chromosome 7. Data has been overlaid with non-synonymous mutations and the user has selected a region (vertical bars) of high-level focal amplification at 7p11.

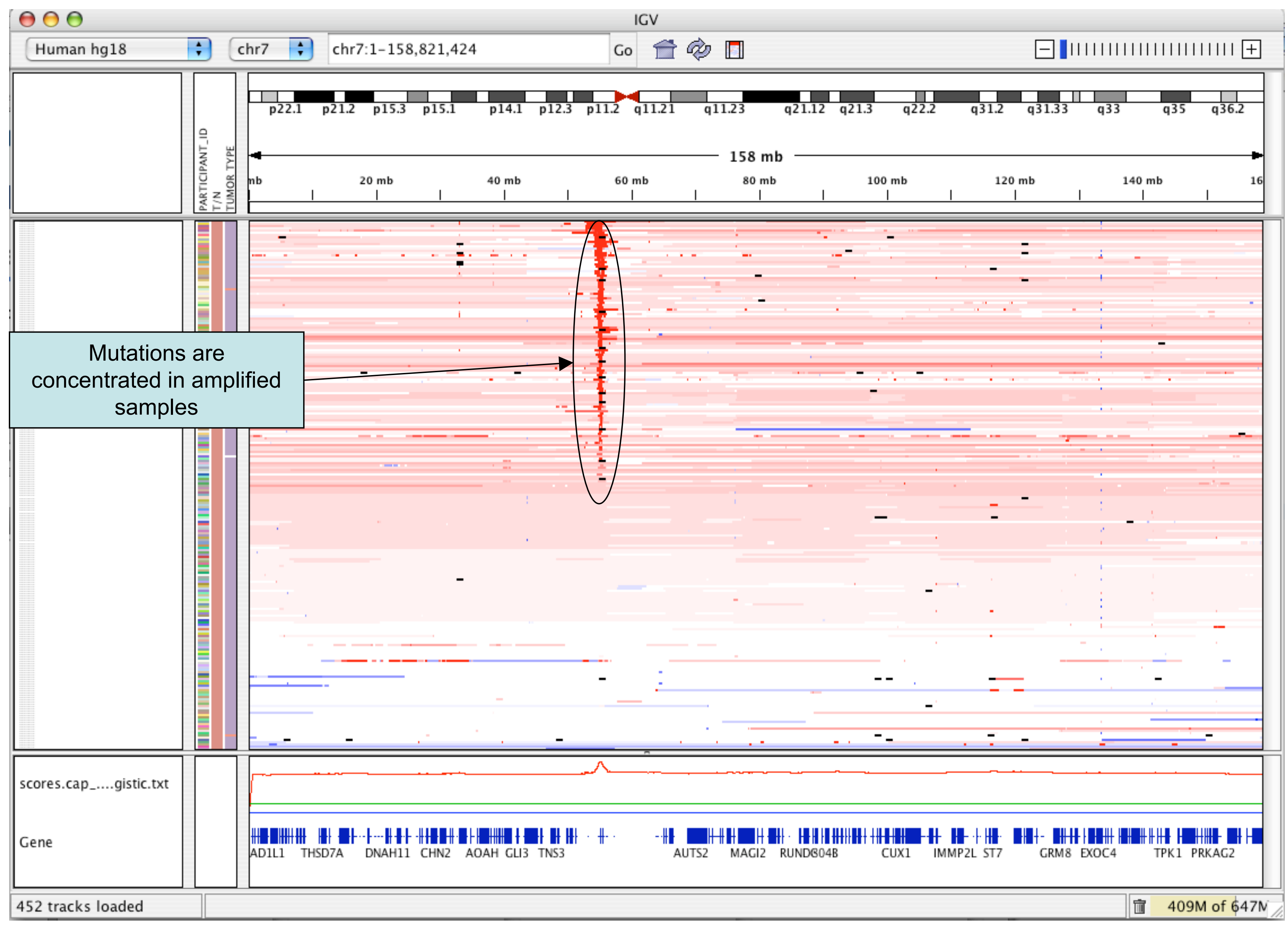


Figure S2c: Sorted by amplification in EGFR region. Samples have been sorted by amplification over the region selected in (b).

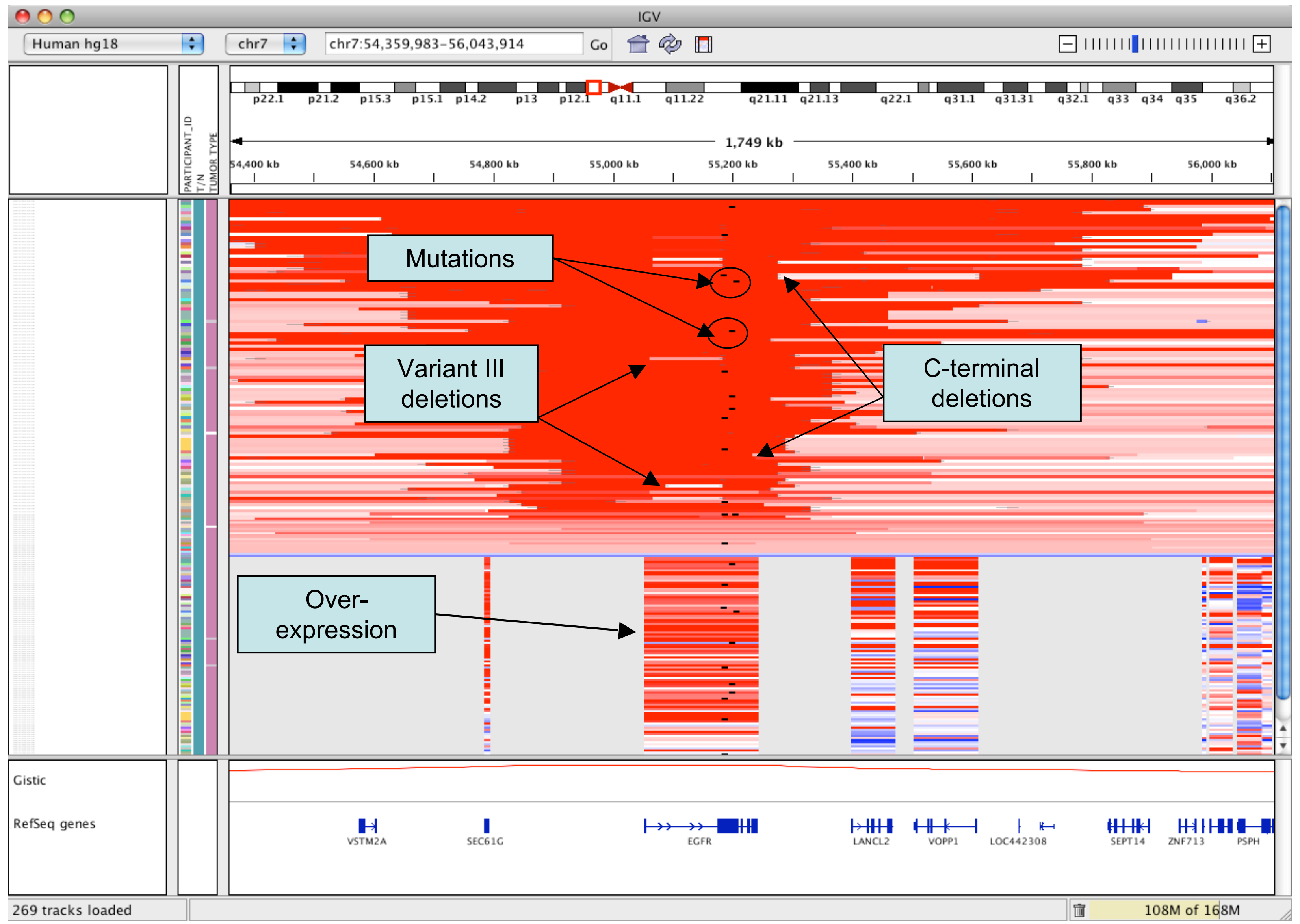


Figure S2d: Gene-centric view. Deeper exploration of the focal amplification on 7p is achieved by quickly zooming to the EGFR locus. Expression data has been added and linked to copy number tracks by means of a shared sample attribute. Here, as in the view in (b), the user has dynamically sorted samples in the region of interest by their median copy number. Expression data is automatically sorted so that sample order in the expression and copy number data is consistent. In addition to copy number and expression gains, samples with variant III deletions of exons 2-7, indicated by neutral copy number (white) in that span, are clearly visible as are two samples with c-terminal deletions.

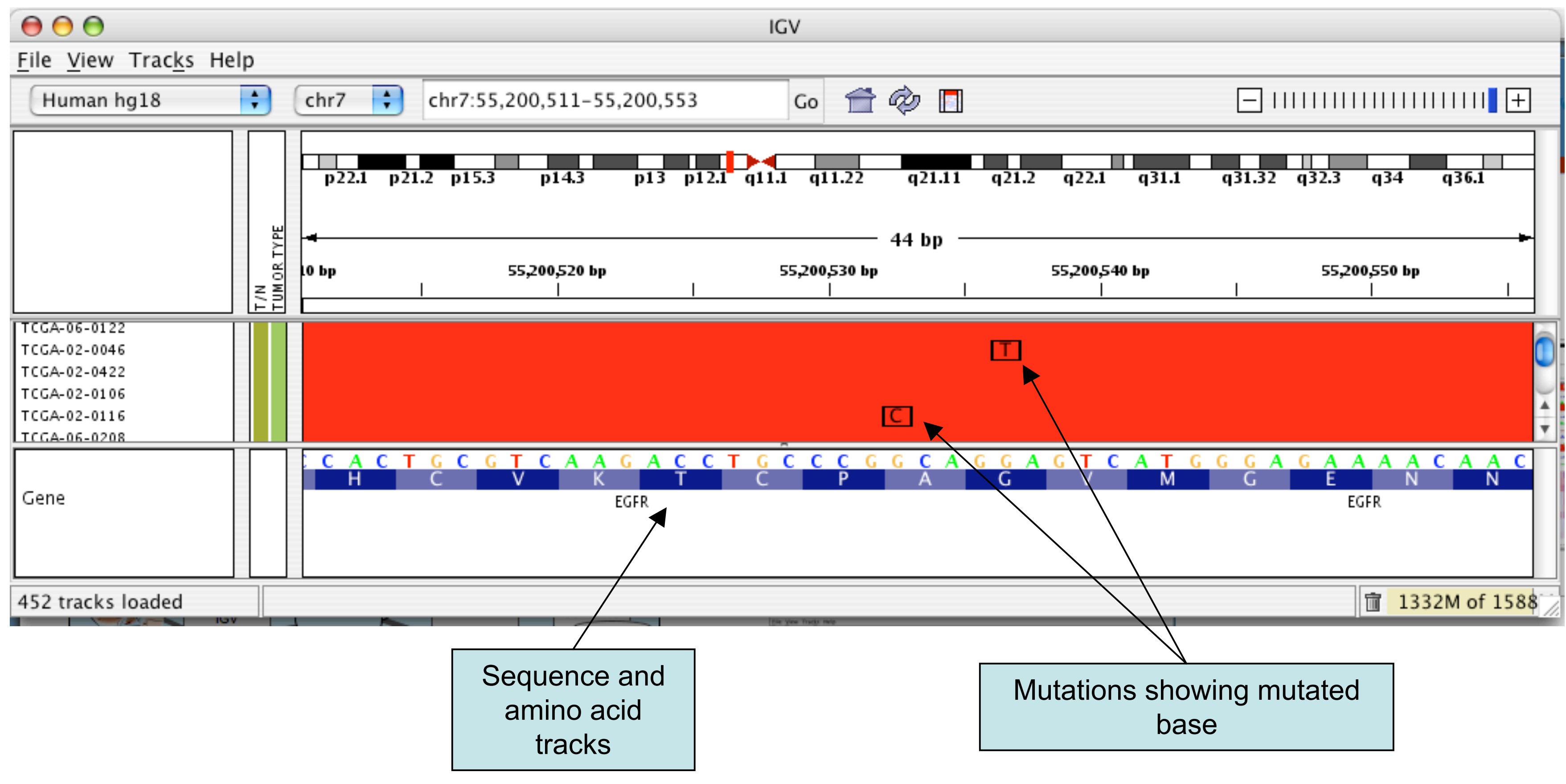


Figure S2e: Individual base pair resolution view. Zooming to single base resolution provides the reference genomic sequence and amino acids affected by these mutational events. Here we find two adjacent EGFR extracellular domain missense mutations.

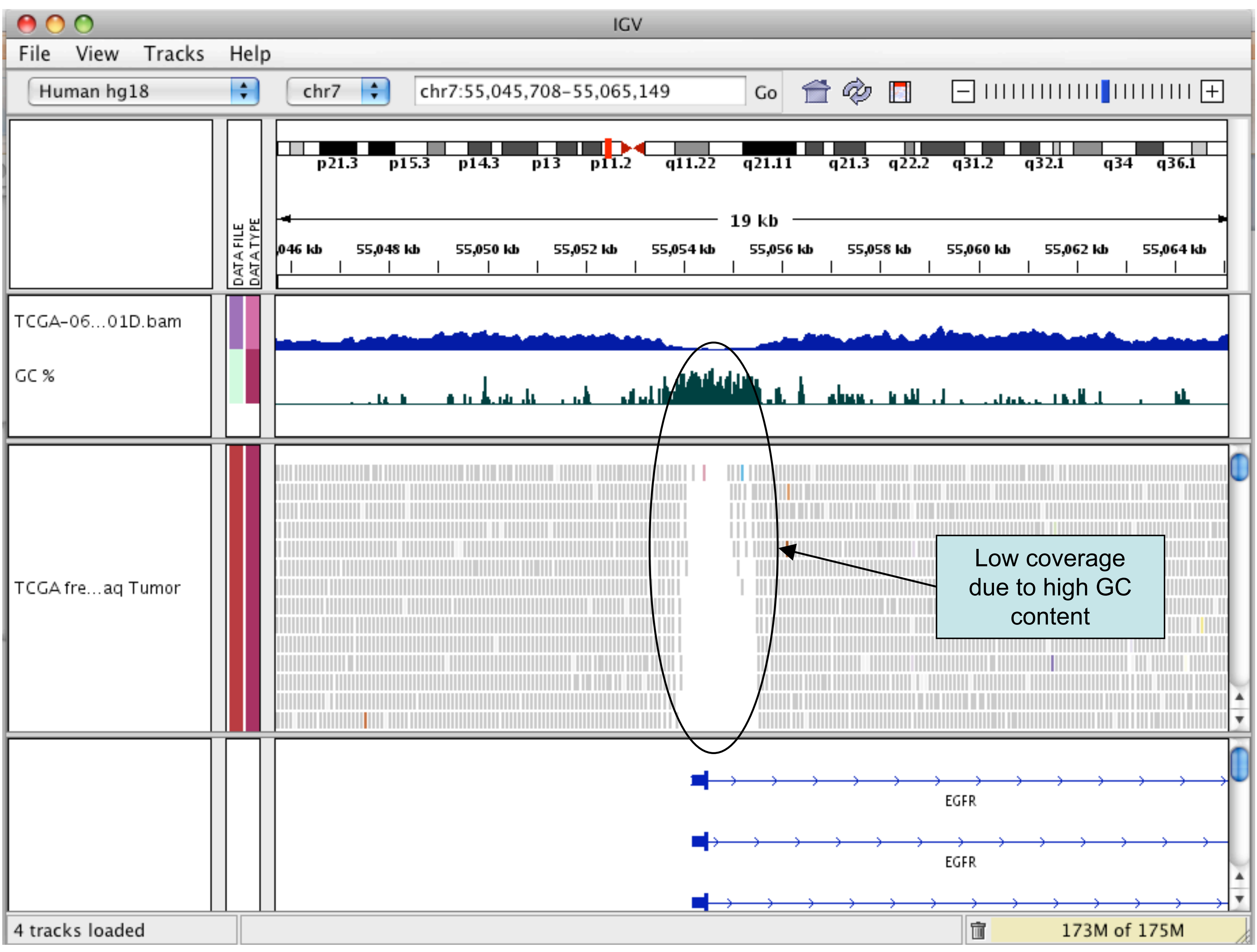


Figure S3: **Diagnosing technical issues in sequencing runs.** Using aligned reads to assess coverage in the context of GC content, it is apparent that low sequence coverage in the first exon of many genes (such as EGFR shown here) is correlated with high GC%.

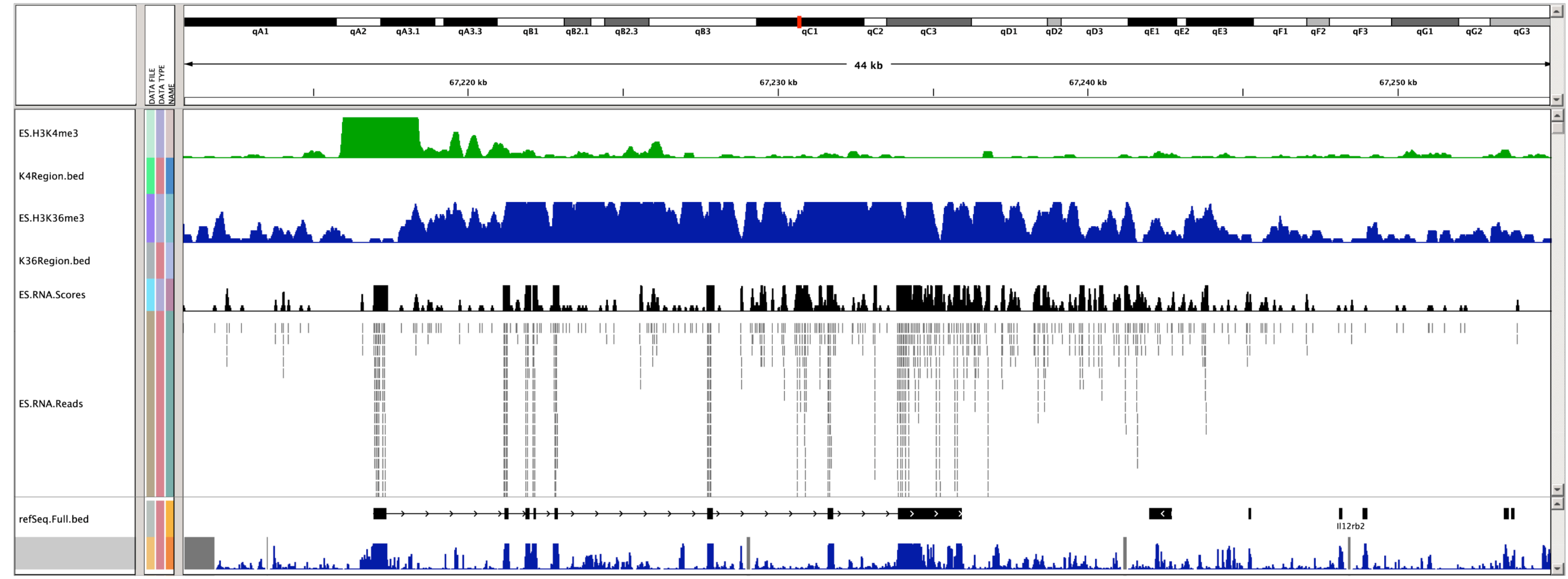
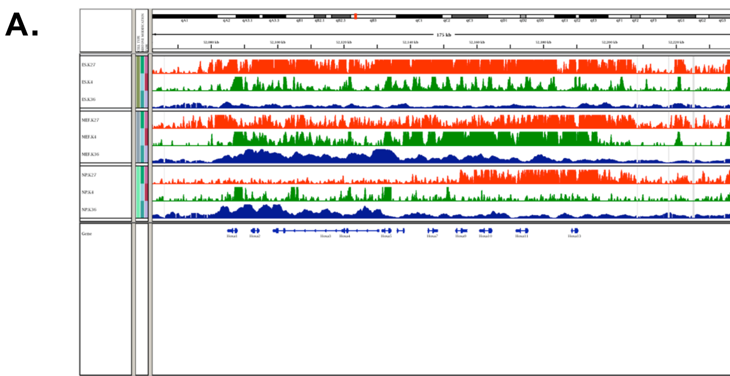
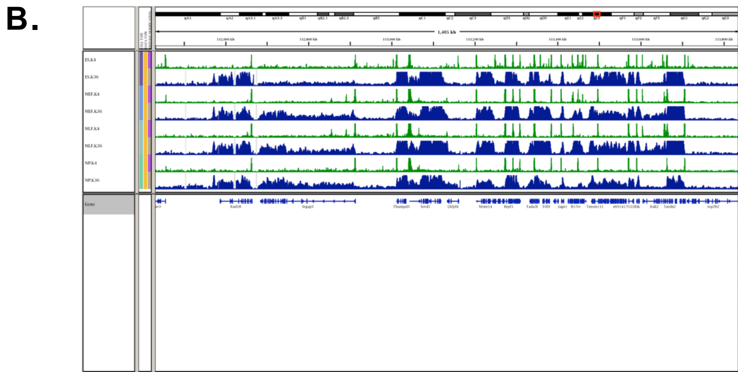


Figure S4: **Visualization of ChIP-Seq, RNA-Seq, and Conservation.** Histone modification patterns, RNA levels, and conservation levels are shown for a region. In green, H3K4me3 and in blue H3K36me3 are plotted as the number of DNA fragments obtained by ChIP-Seq at each position across the genome. H3K4me3 marks the 5' end of active genes while H3K36me3 marks the entire elongated transcript. The H3K4me3 peak is shown by the green box and the H3K36me3 region is shown with the blue box. This region corresponds to a previously annotated protein coding gene which is shown at the bottom of the panel. RNA-Seq tracks (black) are displayed from the same cell types below the histone modifications. In addition to the processed reads, the locations of the raw genome alignments are displayed in gray. The locations of these mapped reads coincide well with the previously determined exonic structure of this gene. Finally, the phastCons evolutionary conservation score is plotted for each position in the genome is shown in blue.

A.



B.



C.

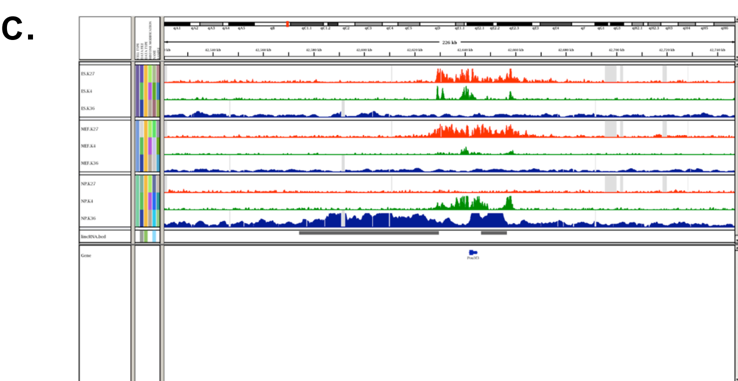


Figure S5: Dynamic exploration of chromatin data identifies known regulation and novel functional elements in the mammalian genome. Histone modifications (H3K4me3 in green, H3K36me3 in blue, and H3K27me3 in red) are plotted as the number of DNA fragments obtained by ChIP-Seq at each position across the genome. Known protein-coding genes are plotted at the bottom of the panel. The line marks the entire gene locus, boxes represent exons, and the arrow indicates the orientation of transcription (5'-3'). **(A)** The developmentally important mouse HoxA cluster of genes is shown. In mouse embryonic stem cells (mESC) these genes are "bivalent" marked with H3K4me3 (green) and H3K27me3 (red) modifications. These genes are repressed in mESC and lack H3K36me3 (blue). These genes turn on upon differentiation into mouse embryonic fibroblasts (MEF) and gain H3K36me3 enrichment over the whole cluster. In neural precursor cells (NPC) half of the cluster is expressed and marked with K4-K36 while the other half is repressed and marked with H3K27me3. (**B**) A gene dense region of the genome is shown. K4me3 (green) is shown to localize at the promoters of known protein coding genes. K36me3 (blue) is shown to mark the entire length of actively transcribed genes. **(C)** A neural specific transcription factor gene (pou3f3) locus is shown. This gene is "bivalent" in mESC and repressed in MEFs but turns on in NPC consistent with its known role in neural development. Two novel genes (gray boxes) are marked on both ends of this transcription factor. These genes are marked with K4me3 at their promoter regions and K36me3 over their length in NPC and are bivalent in mESC and repressed in MEFs.

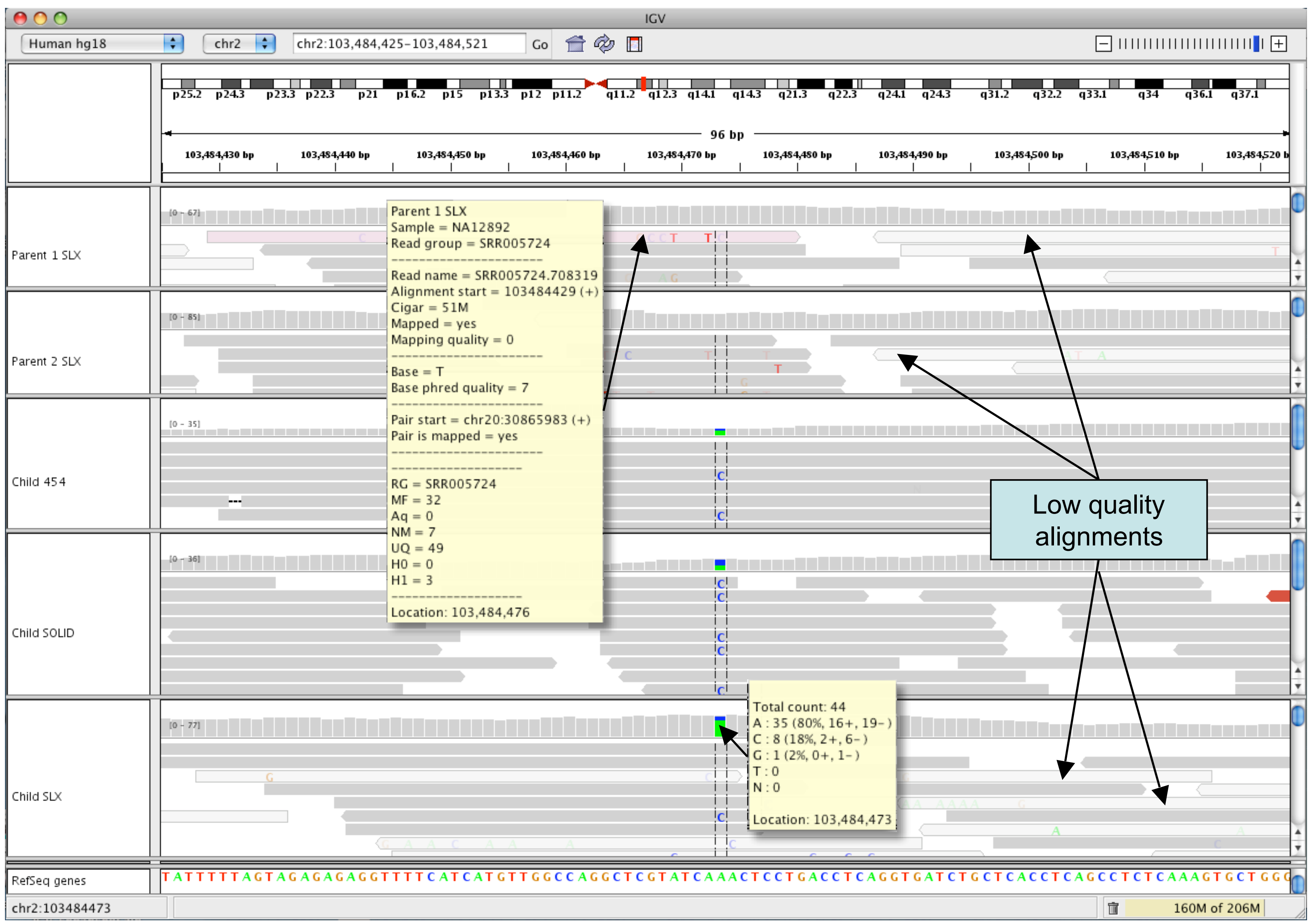


Figure S6: **Suspicious SNP call.** This figure shows aligned reads from a region on Chromosome 2 derived from whole genome sequencing of a CEU (Western European ancestry) trio. Coverage depths are approximately 30x. Parents were sequenced on the Illumina platform. Three technologies were used for the child, Illumina (SLX), 454 and SOLID. A SNP in the CEU daughter was called by an analysis tool, however visual inspection raises several flags, (1) the SNP is not present in either parent, (2) there are many nearby reads with mapping quality of zero, indicated by light grey shading with a darker outline, and (3) an unexpected (~5:1) allele imbalance in the SLX (Illumina) reads. Mousing over a feature or track reveals popup text – in this example supplying additional information for a read and the coverage plot. Note that one read is colored red, indicating that its mate is mapped to another chromosome (chr20). This is confirmed in the popup text.

Figure S7: A de novo mutation in the CEU daughter. Another region, this one on Chromosome 1, from the same trio dataset described in Figure S6. A heterozygous SNP is detected with ~50/50 allele balance in all three sequencing platforms for the CEU daughter. Both read and alignment quality scores are high as indicated by the bright base letters and solid alignment features. The SNP is not present in either parent, indicating it is de novo.

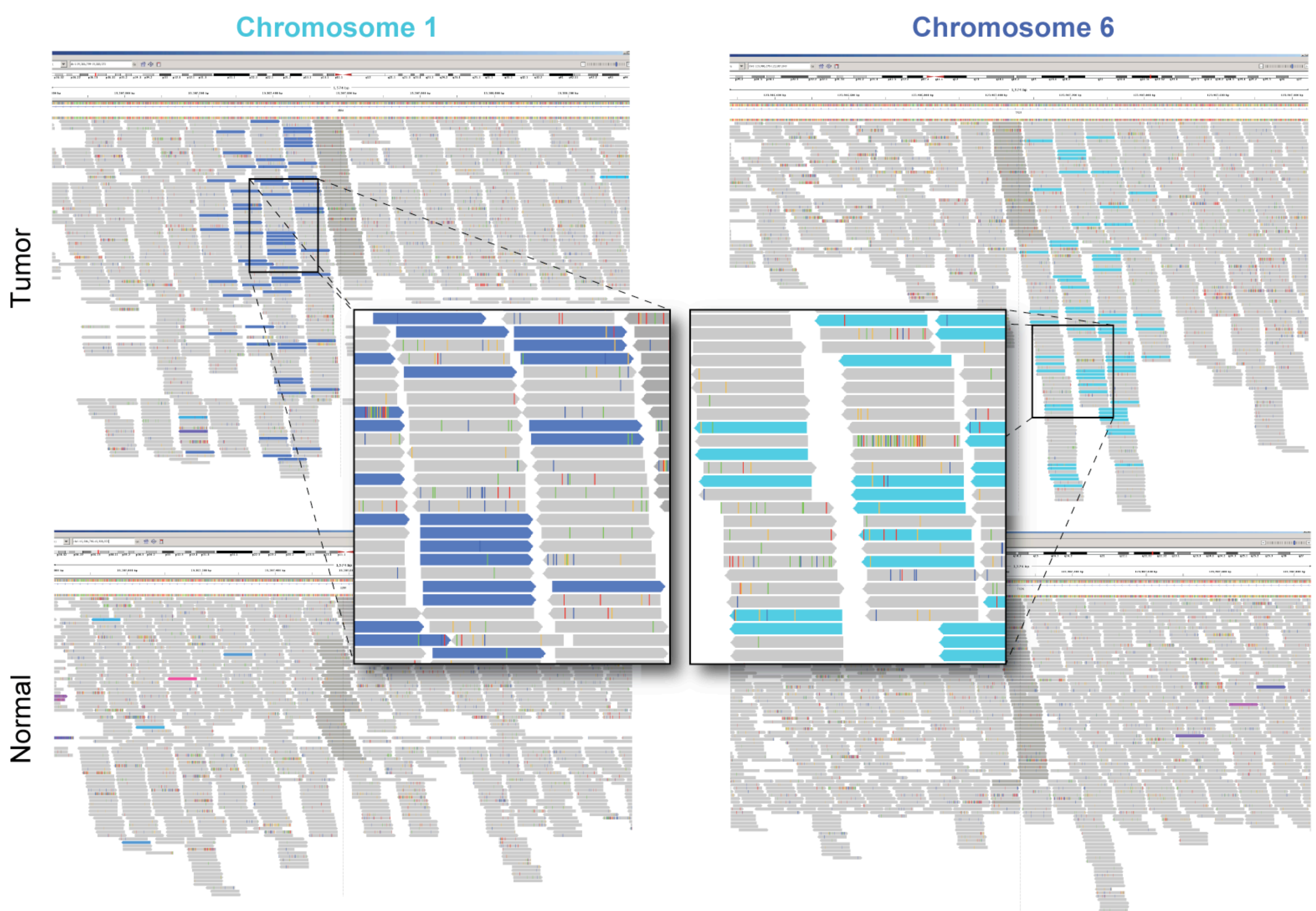


Figure S8: Paired-end sequencing data indicates a somatic intra-chromosomal rearrangement. Reads whose paired end maps to another chromosome are colored according to the chromosome of the mate. In this example read alignments on chromosome 1 are paired with mates aligned to chromosome 6, and vice versa. The large number of reads consistent in location and strand are evidence of a true rearrangement.

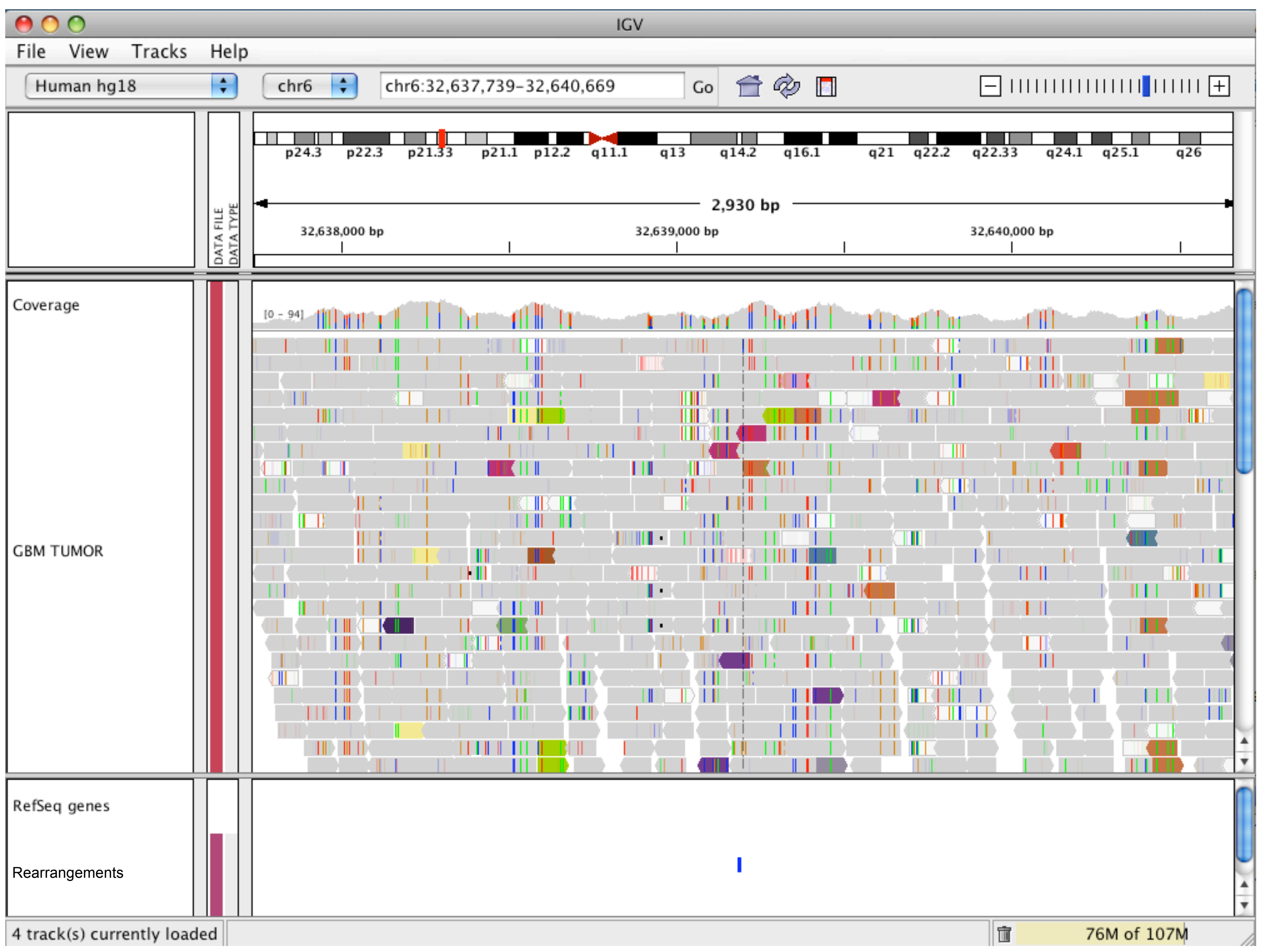


Figure S9: Putative rearrangement flagged by analysis but rejected by visual inspection. This figure shows aligned reads from a whole genome scan of a glioblastoma multifome tumor sample. Although there are a few mate-pairs that are consistent in orientation, there are many more randomly scattered around the region. Also, there are a very high number of base mismatches for a 3 kb window, as indicated by the many colored bars in the coverage graph. Taken together, the evidence points to misalignments in this region rather than a true genomic event.

IGV User Guide

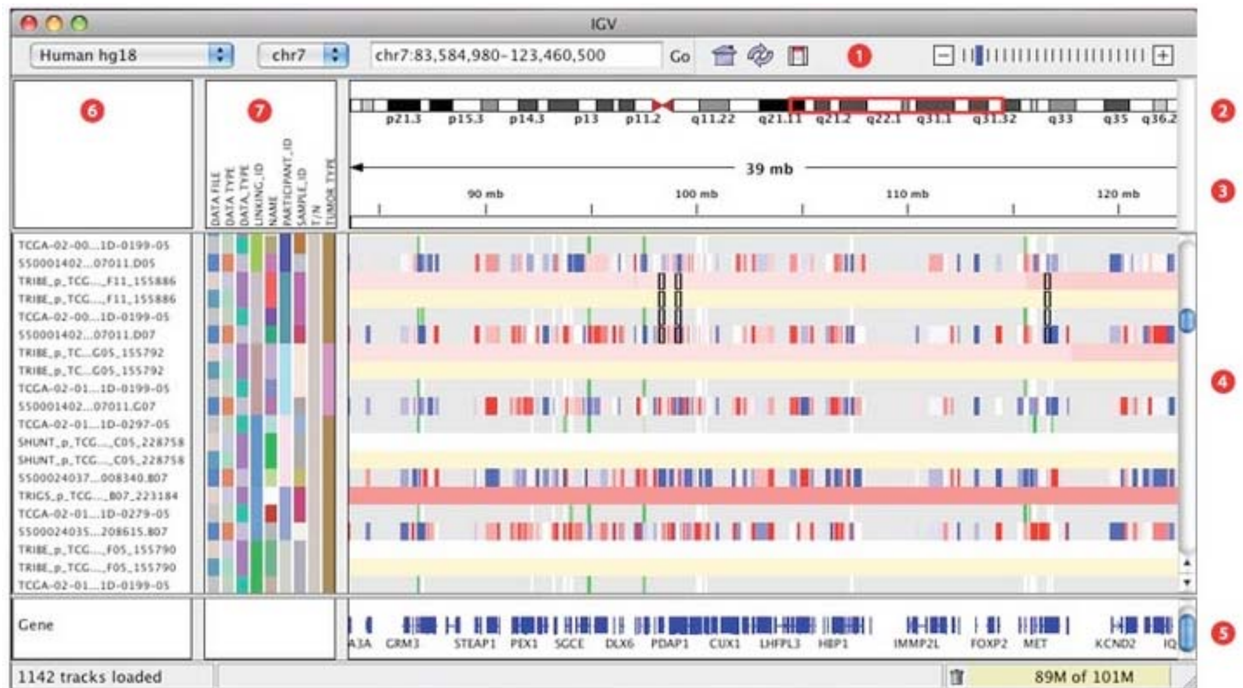
This guide fully describes the Integrative Genomics Viewer (IGV).

- To start IGV, go to the IGV downloads page: http://www.broadinstitute.org/igv/download.
- For a 10-minute hands-on introduction, see the Quick Start.

User Interface

Main Window

The following figure shows data from The Cancer Genome Atlas sorted by PARTICIPANT_ID:



1 The [tool bar](#) provides access to commonly used functions. The [menu bar](#) and [pop-up menus](#) (not shown) provide access to all other functions.

2 The red box on the chromosome ideogram indicates which portion of the chromosome is displayed. When zoomed out to display the full chromosome, the red box disappears from the ideogram.

3 The ruler reflects the visible portion of the chromosome. The tick marks indicate chromosome locations. The span lists the number of bases currently displayed.

IGV displays data in horizontal rows called *tracks*. Typically, each track represents one sample or

4. experiment. This example shows methylation, gene expression, copy number, LOH and mutation data.

5. IGV also displays features, such as genes, in tracks. By default, IGV displays data in one panel and features in another, as shown here. Drag-and-drop a track name to move a track from one panel to another. Combine data and feature panels by selecting that option on the General tab of the Preferences window.

6. Track names are listed in the far left panel. Legibility of the names depends on the height of the tracks; i.e. the smaller the track the less legible the name.

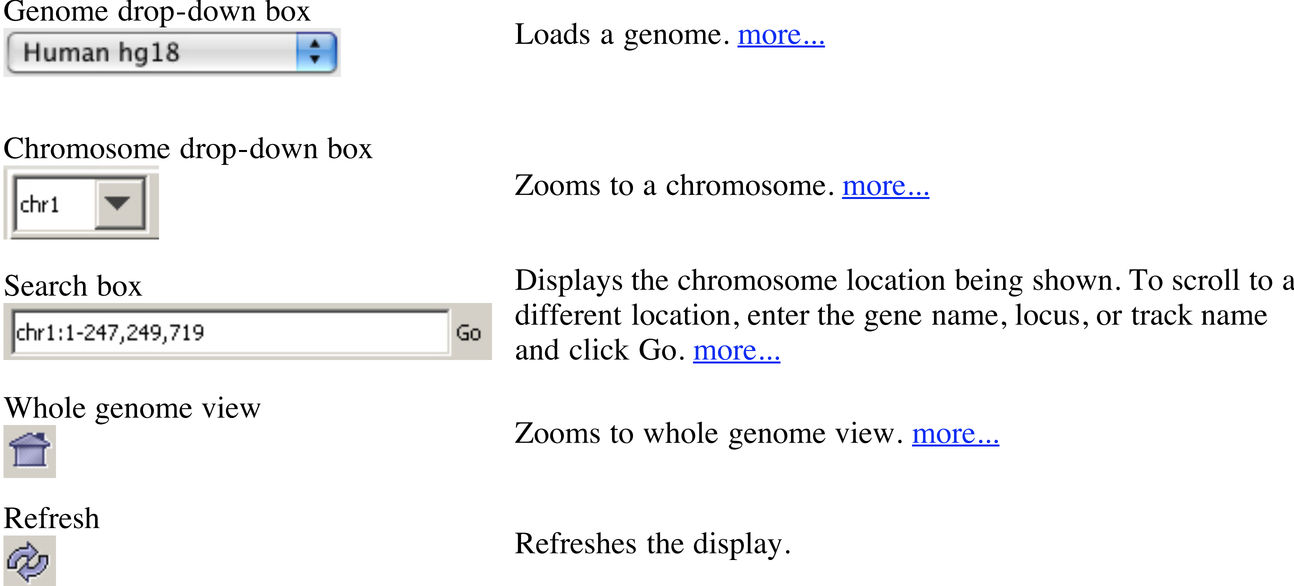
7. Attribute names are listed at the top of the attribute panel. Colored blocks represent attribute values, where each unique value is assigned a unique color. Hover over a colored block to see the attribute value. Click an attribute name to sort tracks based on that attribute value.

Menu Bar

Menu	Command	Description
File	Load from File	Displays genomic data from one or more files. more...
	Load from URL	Displays genomic data from a file identified by URL. more...
	Load from Server	Displays genomic data from the IGV data server. more...
	New Session	Unloads all currently loaded data, as if you exited and restarted IGV. more...
	Open Session	Opens a previously saved session file. more...
	Save Session	Saves your current settings to a named session file. more...
	Import Genome	Imports a genome into IGV. more...
	Remove Imported Genomes	Removes an imported genome from the Genomes drop-down list in the tool bar. more...
	Save Image	Saves a snapshot of the IGV window to a graphics file, omitting the menu bar and tool bar.
	Export Regions	Saves currently defined regions of interest to a BED file. If no regions of interest are defined, no BED file is created. more...
	Import Regions	Imports regions of interest from a BED file. more...
	Clear Regions	Removes all currently defined regions of interest. more...

	Exit	Closes IGV.
View	Preferences	Opens a tabbed menu of data display preferences. more...
	Color Legends	Displays color legends for track data, which may be modified. more...
	Show Attribute Display	Shows/hides attributes and attribute values. more...
	Select Attributes to Show	Shows/hides selected attributes and attribute values. more..
	Refresh	Refreshes the display.
Tracks	Sort Tracks	Sorts track data. more...
	Group Tracks	Groups track data. more...
	Filter Tracks	Filters track data. more...
	Fit Data to Window	Sets the track height to display all of the data, or as much data as possible. more...
	Set Track Height	Sets the track height to a specified value. more...
Help	Help	Displays the IGV User Guide.
	Tutorial	Displays the IGV Quick Start.
	About IGV	Displays IGV version and build number.

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...
Refresh	Refreshes the display.

Define a region	Defines a region of interest on the chromosome. more...
Zoom slider	Zooms in and out on a chromosome. more...

Pop-up Menus

To select tracks and display the pop-up menu, do one of the following:

- Right-click a track to select it and display the pop-up menu.
- Right-click an attribute value to select all tracks with that attribute value and display the pop-up menu. **Tip**: Keep in mind that right-clicking an attribute may select tracks that are not visible in the data panel. Scroll down the data panel to view all the selected tracks.
- Control-click track names (Mac: Command-click) to select the tracks, then right-click one of the selections to display the pop-up menu.

Commands in the track pop-up menu change the display options for the selected tracks. Most changes made via the pop-up menu are lost when you exit IGV unless you save the session. In a few cases, changing the pop-up menu also changes an option in the Preferences window; these changes are persistent.

The type of data displayed in the selected tracks determines which commands appear in the pop-up menu. This page lists commands by track type: data track, feature track, and alignment track. Use your browser's search function to find a particular command.

Data Track

Data tracks display numeric values. For an example, click File > Load from Server and select The Cancer Genome Atlas.GBM.Expression.GBM Batch 1-8 Centered and Normalized (hg18). The following commands appear in the pop-up menu for data tracks:

Command	Description
Type of Graph Heatmap Bar Chart Scatterplot Line Plot	Changes the way IGV displays track data. more...
Windowing Function 10th Percentile Median Mean 90th Percentile Maximum	Changes the value represented by each pixel of track data. At all but the lowest zoom levels, each pixel represents a significant amount of data. IGV divides the data to be displayed into "windows" of equal length each corresponding to a single pixel, summarizes the values across each window, and then displays the summarized values in the track. Select the function IGV will use to summarize the values.

Rename Track	Renames a track. more...
Set Data Range	Changes the minimum, baseline, and maximum values of the graph used to display track data. more...
Set Heatmap Scale	Changes the data range and color of the heatmaps used to display track data. more...
Change Track Height	Changes the track height for selected tracks. more...
Change Track Color (Positive/Negative Values)	Changes the track color for selected tracks. more...
Remove Tracks	Removes selected tracks from the display. more...

Feature Track

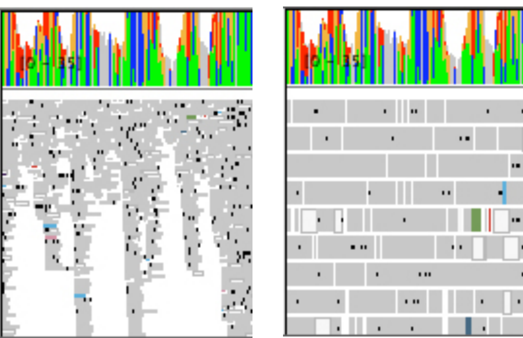
Feature tracks identify genomic features. For an example, see the Gene track, which IGV loads when you select a genome. The following commands appear in the pop-up menu for feature tracks:

Command	Description
Rename Track	Renames a track. more...
Expand Track/ Collapse Track	Displays overlapping features, such as different transcripts of a gene, on one line or multiple lines: Collapsed state (default): Expanded state:
Change Track Height	Changes the track height for selected tracks. more...
Change Track Color	Changes the track color for selected tracks. more...
Remove Tracks	Removes selected tracks from the display. more...

Alignment Track

Alignment tracks display alignments (more...). For an example, select the Human b36 genome, click File > Load from Server and select an alignment from the 1000 Genomes project. **Tip:** Zoom in to view alignments and the alignment track pop-up menu.

Command	Description
Sort alignments	Sorts alignments by start location, strand, nucleotide, mapping quality, sample, or read group (as defined in the BAM file).
Re-pack alignments	Sorts alignments to minimize gaps at the top of the track.
Shade base by quality	Uses the color intensity of a mismatched base to indicate its quality score: the darker the color the higher the score. Changing this option also changes the option on the Alignments tab of the Preferences window.
Shade alignments intersecting center	When selected, IGV shows a line at the center of the display and shades alignments that intersect that center line. Changing this option also changes the option on the Alignments tab of the Preferences window.
Copy read details to clipboard	When you hover over a read, the tool tip displays information about the read. This option copies that information and the read sequence to the clipboard.
Go to mate region	Jumps to the region of the paired read (if any).
Show all bases	By default, mismatched bases are displayed as colored letters on a gray bar that represents the read. Select this option to display all bases in the read.
Show coverage track	When selected, IGV displays the matching coverage track for the alignment track.
Load coverage data	Loads coverage data for an alignment track. To generate coverage data, use igvtools. more... Loading an alignment track from the IGV data server (File > Load from Server) automatically loads the matching coverage data.
Rename track	Renames a track.
	Changes the height of the reads to adjust the amount of information displayed. Collapsed state: Expanded state (default):

Expand track/ Collapse track	
Remove track	Removes selected tracks from the display. more...
Clear selections	Clears the outlines that show paired reads. • Control+click (Mac: Command+click) a read to outline the read and its paired mate in the same color. Colors are arbitrary but unique to each pair. A black outline indicates that the selected read has no mate. • To clear the outline for a paired read, Control+click (Command+click) either read. • To clear all outlines, right-click and select *Clear selections*.

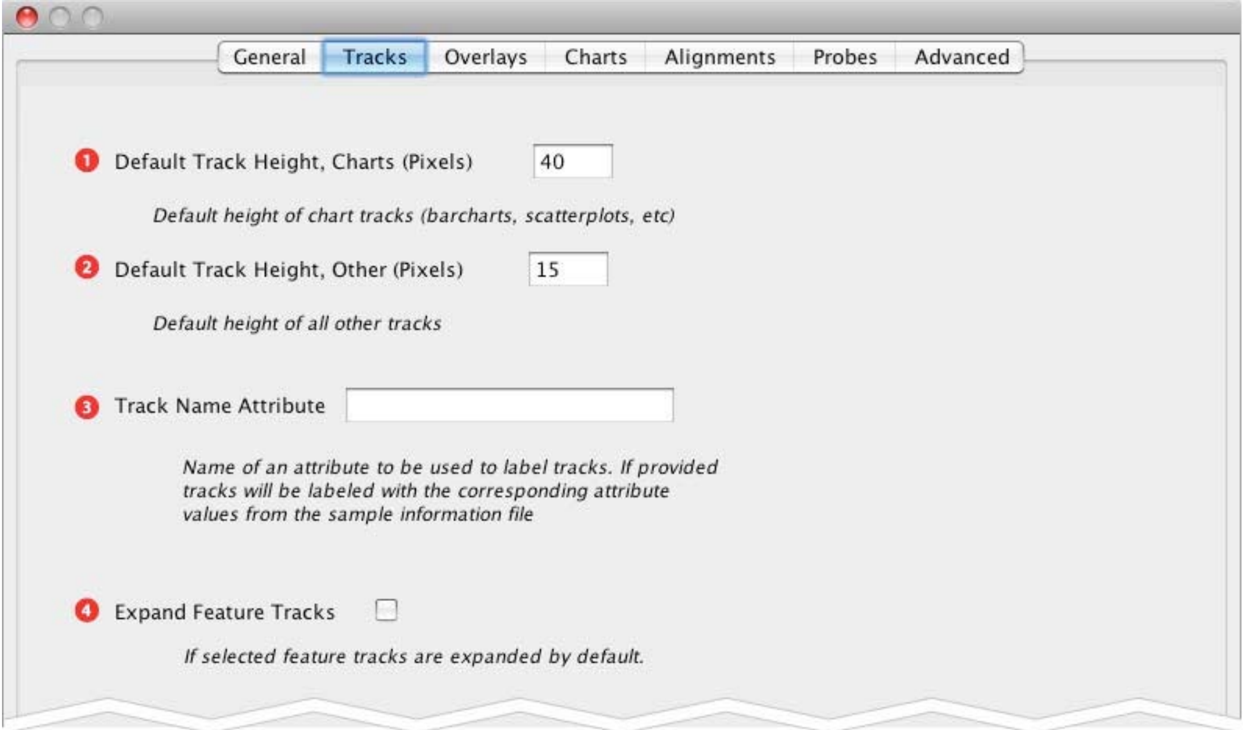
Preferences

To display the Preferences window, click View > Preferences. Preferences are preserved across sessions. To overide preferences during a session, use the track display pop-up menu. Each section on this page describes the options on a tab of the Preferences window: General, Tracks, Overlays, Charts, Alignments, Probes and Advanced.

General

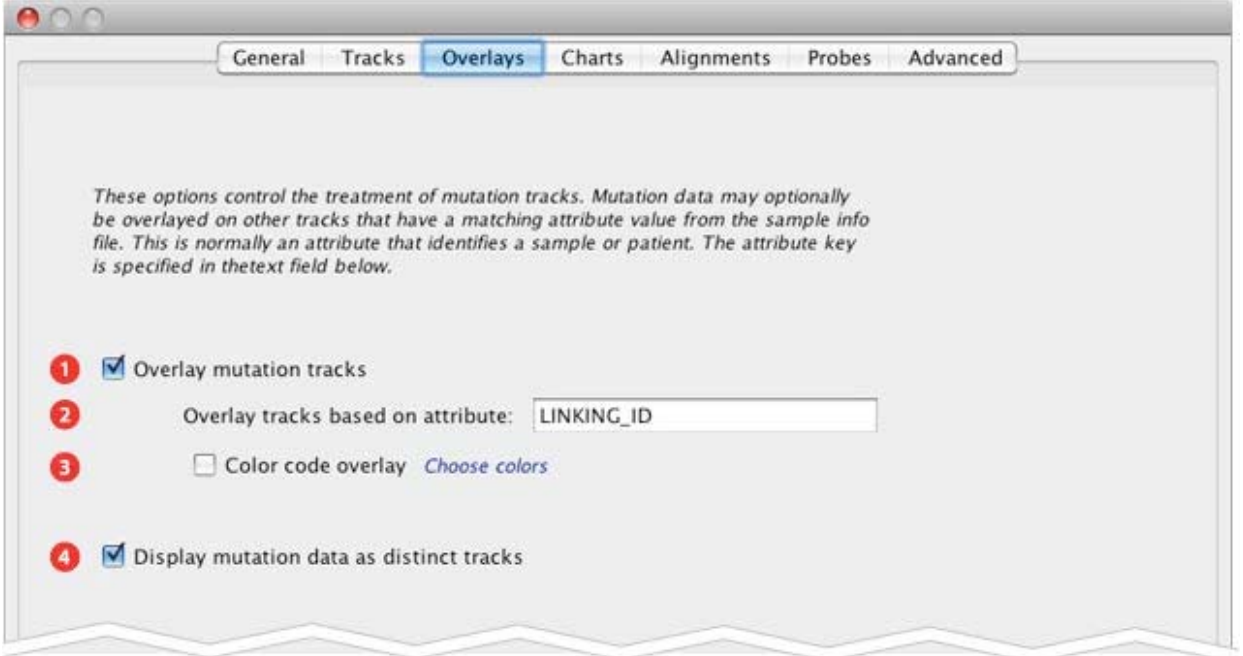
1. Select to distinguish regions with zero values (white) from regions with missing data (gray). Clear (default) to display both regions in the same way (gray). Effects only bar charts and scatter plots.
2. Select to display all tracks in a single panel. Clear (default) to display data tracks (e.g., expression data) in one panel and feature tracks (e.g., genes) in another.
3. Select (default) to fill the gaps between adjacent segments by extending the segment endpoints. Clear to leave gaps between adjacent copy number segments. Effects only tracks displaying segmented copy number.
4. Select (default) to show attributes and attribute values to the left of the data panel. Clear to hide the attributes. This option and View > Show Attribute Display have the same effect on attribute display.
5. Zoom to feature *(not shown)*. When selected the zoom level is automatically adjusted so that the target feature fills the view after a successful search. If not checked the target feature of a search is centered in the view but the zoom level is unaffected.

Tracks



1. Default track height for bar charts, scatter plots, and line plots.
2. Default track height for all other tracks.
3. Name of an attribute in the sample information file. IGV uses the corresponding attribute value as the track name.
4. Select to expand feature tracks by default.

Overlays



1. Select to overlay mutation data on other tracks. more...
2. Name of an attribute in the sample information file. IGV uses the corresponding attribute value to "link" mutation data with other track data. more...
3. Select to color-code mutation data overlaid on other tracks. Click Choose Colors (or View > Color Legends) to display the Color Legends window, which allows you to view and change mutation color codes. more...

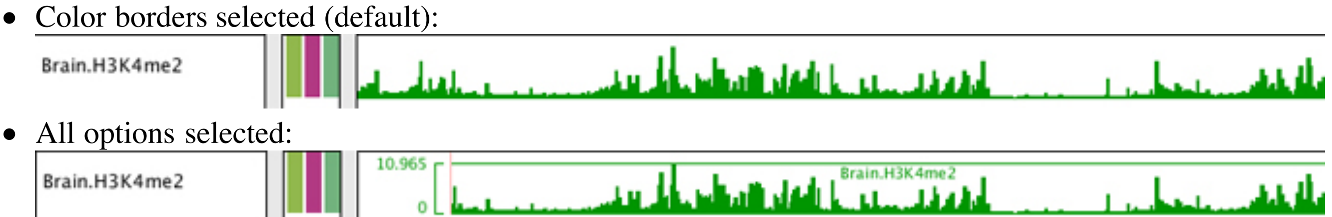
   When mutation data is displayed in a separate track, IGV color codes mutations by type (missense, silent, and so on). By default, when mutation data is overlaid on other tracks, IGV displays mutations in black for clarity.
4. Select to display mutation data in a separate track. more...

Charts

1. Adds a border at the top to the track.
2. Adds a border at the bottom of the track.
3. Colors the top and bottom borders (if any). If this option is cleared, the borders are black regardless of the track color. **Tip:** To change the track color, use the track display pop-up menu.
4. Labels the track with its name, provided the track is at least 25 pixels high.
5. Labels the y-axis with its data range.

The following figures illustrate these track display options.

- Color borders selected (default):
- All options selected:



Alignments

1. Sets the threshold at which IGV displays reads. Reads are visible only when IGV is zoomed in to display a number of bases less than or equal to this threshold.
2. Sets the maximum number of vertically stacked alignments viewable at any particular locus.
3. Sets a threshold on alignment mapping quality. Only alignments with mapping quality greater than or equal to this threshold are shown.
4. Sets a size threshold for the flagging of paired end alignments. Only paired end alignments with insert sizes greater than or equal to this threshold are flagged.
5. Select to display alignments marked as duplicate reads.
6. Select to draw a red box around any paired alignment whose mate is not mapped.
7. Select to display a coverage track for each alignment track. The coverage track is visible only when alignments are visible. It displays a gray bar chart showing the depth of the reads at each locus. If a nucleotide differs from the reference sequence in greater than 20% of quality weighted reads, IGV colors the bar in proportion to the read count of each base (A, C, G, T). Modifying this option affects the display of **subsequently** loaded alignment tracks.

Select to display the reference sequence with each alignment track. When cleared (default), IGV

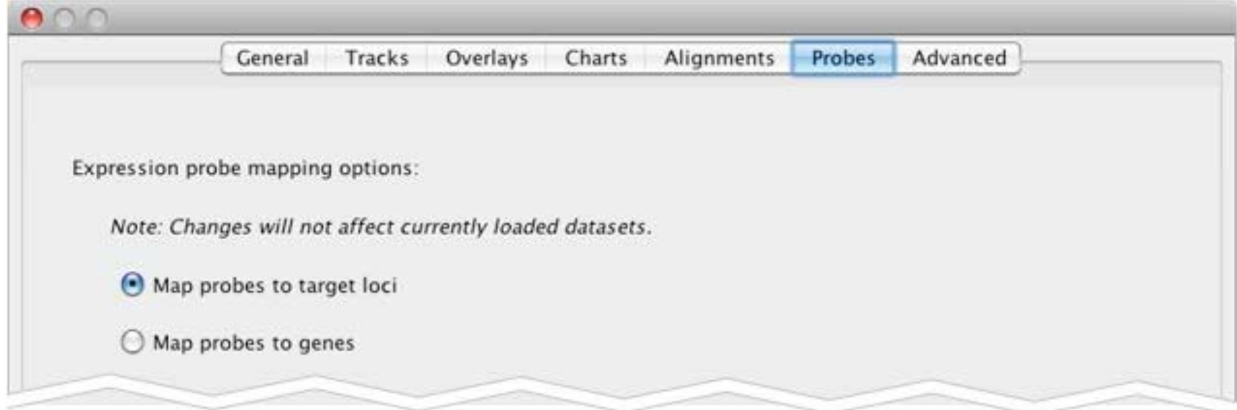
9. displays the reference sequence once. Modifying this option affects the display of **subsequently** loaded alignment tracks.

10. Select to display a line at the center of the display. When zoomed in sufficiently, IGV shades alignments that intersect the center line. At higher resolutions, the center line becomes lines that frame the aligned bases at the center of the display. Modifying this option affects the display of **subsequently** loaded alignment tracks.

11. Hide alignments that match the read groups listed in the filter file. The filter file is a text file that lists read groups one per line.

12. A colored read indicates a paired end read with a mate on another chromosome. The color of the read indicates which chromosome holds its mate.

Probes

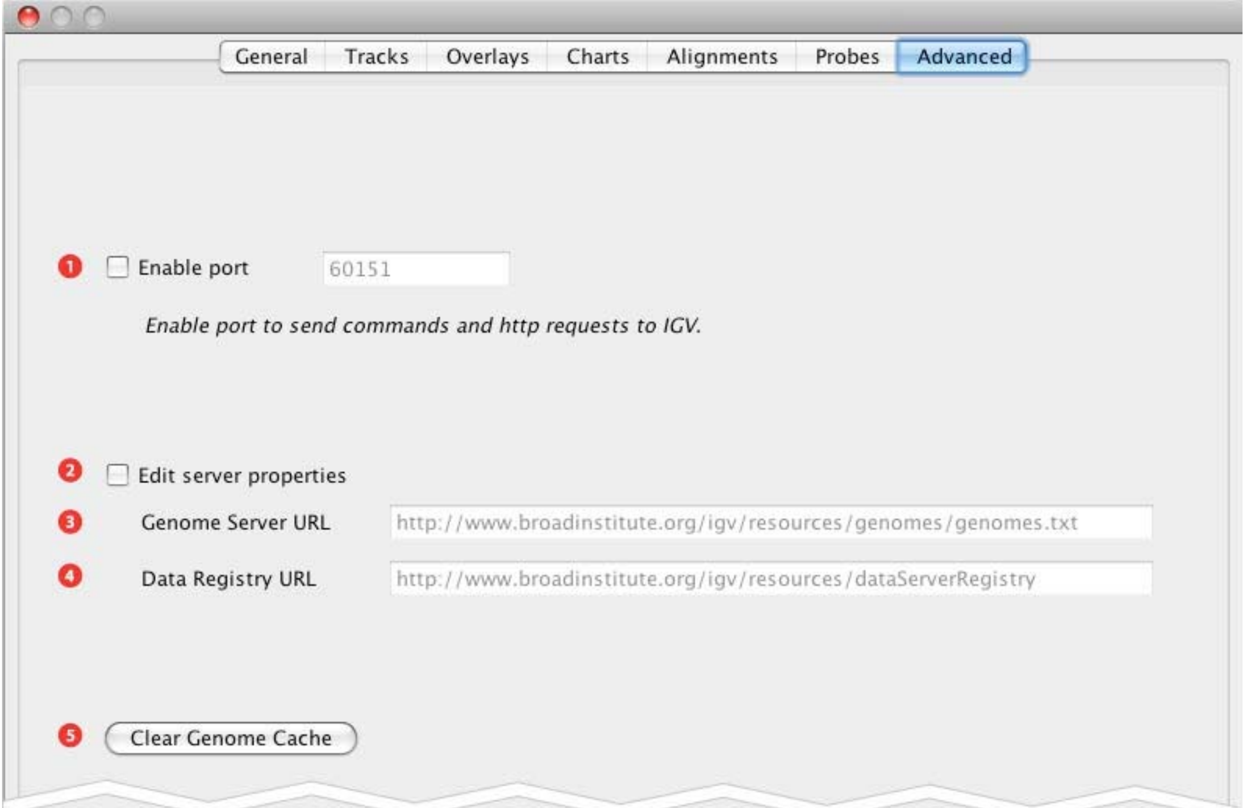


Choose an option to determine how IGV places expression data on the genome:

- Map probes to target loci: Use the probe ID to determine the probe locus and display data at that location. If that fails, map the probe ID to a gene, determine the gene locus, and display data at that location.
- Map probes to genes: Map the probe ID to a gene, determine the gene locus, and display data at that location.

Modifying this option affects the display of **subsequently** loaded alignment tracks.

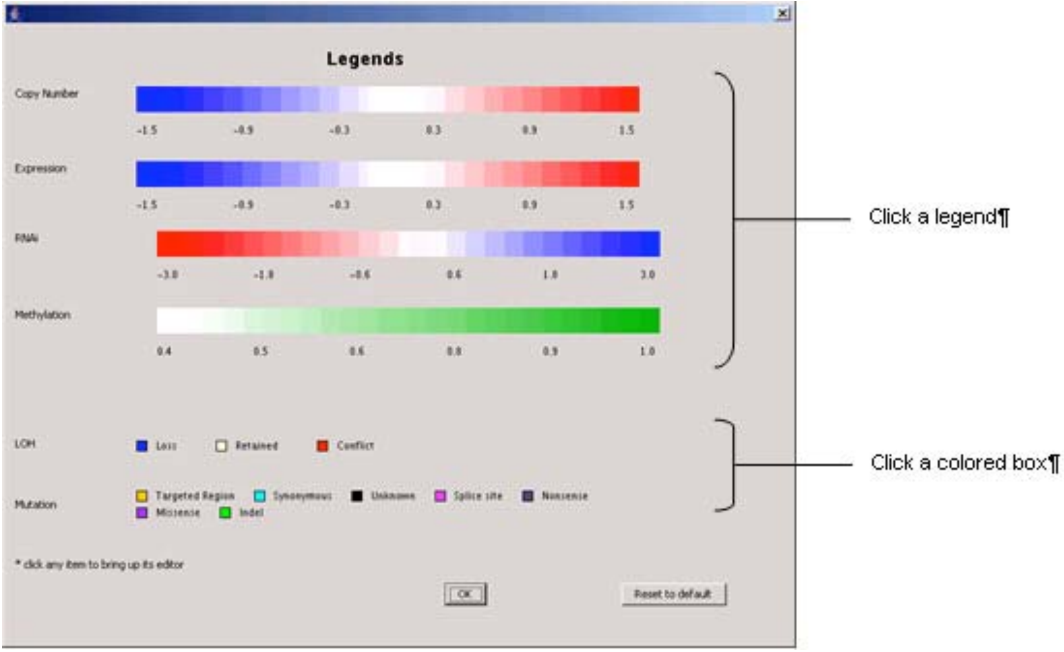
Advanced



1. Select this option to enable a port on which IGV listens for commands and http requests. Enabling the port allows control of IGV from a web browser. more...
2. Select this option to edit URLs for the IGV data and genome servers. These settings are rarely changed.
3. URL for the genome server (populates the genome drop-down list).
4. URL for the hosted datasets registry (populates File>Load from Server dialog).
5. IGV caches each genome that it loads. On rare occasions, it may be necessary to clear the cached genome file to display an updated version of the genome.

Color Legends

By default, IGV uses heatmaps to display certain types of data (see Default Display). Use the Color Legends window to change the default colors for these heatmaps.



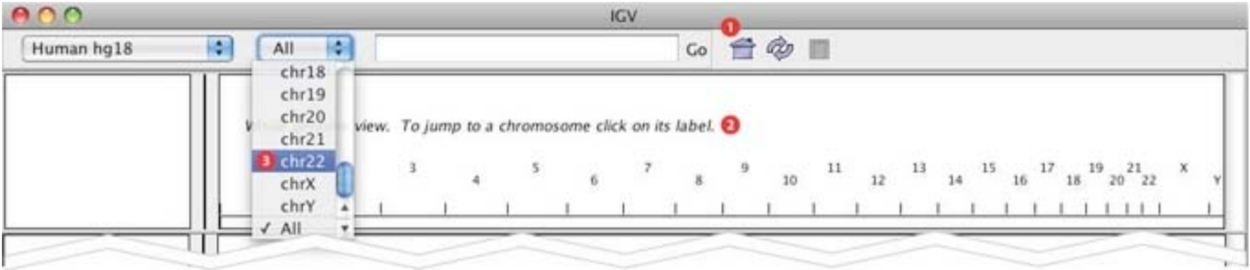
To change the default colors:

1. Click View > Color Legends to display the Color Legends window.
2. Click a heatmap legend to set its color and range.
3. For LOH and Mutation data, click a colored box to change its color.

Navigating

Zooming

Zoom out to view the whole genome, zoom in to a chromosome and continue zooming to base pair resolution. As you zoom in, the gene track shows gene names and sequence data. If the sequence data is unavailable, small blocks replace the bases. If you are using a genome stored on the IGV genome server, you must be connected to the internet to view the sequence data.



❶ Click the whole genome view icon to zoom out to the genome view.

2. From the genome view, zoom to a chromosome by clicking its label.

3. Select a chromosome from the drop-down menu to zoom to it.

To zoom in and out on a chromosome:

Zoom in	Zoom out
+	-
Double-click or shift-click the track data	Alt-click (Mac: option-click) the track data
Click a zoom level on the zoom slider	Click a zoom level on the zoom slider
Click the plus (+) icon on the zoom slider	Click the minus (-) icon on the zoom slider

Scrolling

To scroll the display:

Vertical scroll	Horizontal scroll*
Scroll bar in the IGV window	Click and drag the track data
Click and drag the track data	Click the chromosome ideogram to scroll to that location
Page Up and Page Down keys	Click the ruler to center that location
Up and down arrow keys	Left and right arrow keys
	Home and End keys (scroll by screen width)

* You cannot scroll horizontally when IGV is displaying the whole genome or a whole chromosome.

Searching

Use the search box to locate:

- A locus (for example, chr5:90,339,000-90,349,000)
- A gene symbol or other feature identifier (e.g., DPYD or NM_10000000)
- A track name (e.g., secondary_GBM_89)

IGV searches for an exact match to the name entered in the search box. For example, entering 'secondary' will not locate the 'secondary_GBM_89' track. If multiple features have the same name, IGV jumps to an arbitrary match.**Ju**

Jumping

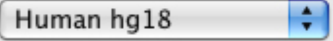
If you have a feature track loaded (e.g. Gene track, BED or GFF file), you can jump from one feature to

the next.

1. Click the track that contains the features that you want to find.
2. Jump from feature to feature:
    - Click Ctrl+F to jump forward to the next feature.
    - Click Ctrl+B to jump backward to the previous feature.

   IGV positions the start of the next (or previous) feature at the center of the display.

Loading a Genome

IGV displays annotations for one genome at a time. To load a different genome, select it from the genome drop-down list in the tool bar:


- When you switch genomes, IGV does not remap data that has already been loaded. We recommend that you remove loaded tracks (File>New Session), switch genomes, and then load the desired data files.
- The genome selected when IGV exits is automatically selected when IGV restarts.

Selecting a Hosted Genome

IGV provides several genomes, which are hosted on a server at the Broad Institute. Initially, the genome drop-down lists only these hosted genomes. If the genome you need is not available, either contact igv-help@broadinstitute.org and request that it be added or import the genome.

Importing a Genome

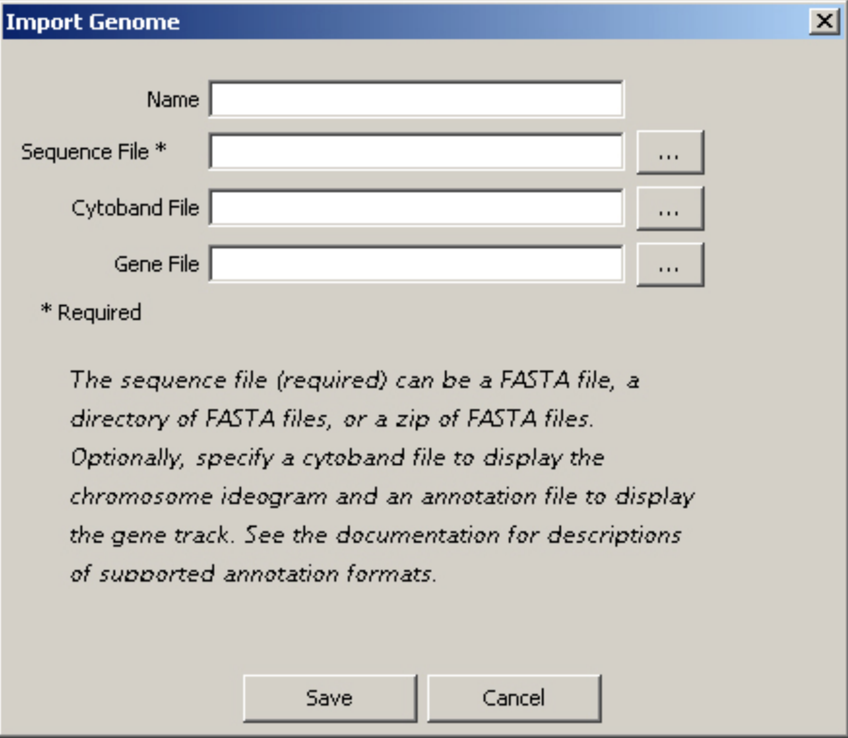
If the genome drop-down list does not include the genome that you need, you can easily import it. Imported genomes appear at the top of the genome drop-down list, above the hosted genomes.

Prerequisites:

- A FASTA file , directory of FASTA files, or zip of FASTA files that contains the sequence data for each chromosome in the genome. (Required)
- A cytoband file, which IGV uses to display the chromosome ideogram. (Optional)
- An annotation file in BED file format, the GFF file format, or any variation of the genePred table format. (Optional)

Step-by-step:

1. Click File > Import Genome. IGV displays the Import Genome window:



2. Enter a name for the genome.
3. For Sequence File, click the ellipse button and select the FASTA file (or zip of FASTA files) that contains the sequence data.
4. Optionally, specify the cytoband file and the gene track annotation (Gene File) file.
5. Click Save. IGV displays the Genome Archive window.
6. Select the directory in which to save the genome archive (*.genome) file and click Save. IGV saves the genome and loads it into IGV.

Removing an Imported Genome

To remove an imported genome:

- Click File > Remove Imported Genomes.

Loading Data and Attributes

Data and genomic annotations can be loaded from local files, http URLs, or an IGV data server.

Load from File

Load data files by browsing for files on the file system or by entering a URL. See File Formats for information about the file formats IGV accepts.

To load data from the file system:

1. Click File > Load from File. IGV displays the Select Files window.

2. Select one or more data files or sample information files, then click OK. CTRL-click (Mac: Command-click) to select multiple files.

Load from URL

To load data from an HTTP URL:

1. Click File > Load from URL.
2. Enter the HTTP URL for a data file or sample information file, then click OK.

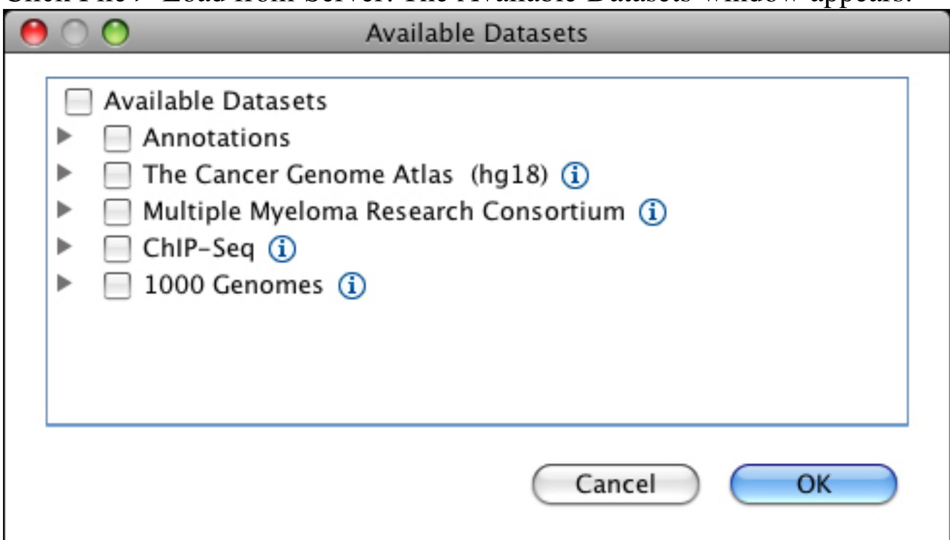
Notes:

- The following file types cannot be loaded by URL at this time: .sam, .sorted.txt, .h5 (superseded by .tdf) and .aligned (internal Broad format).
- FTP is not supported
- For .bam, .tdf, and indexed file formats the server must support byte-range requests.

Load from Server

To load data from the IGV data server:

1. Click File > Load from Server. The Available Datasets window appears:



2. Expand the tree to see the datasets.
3. Select one or more datasets. Click the check box to the left of a dataset to select it. **Warning**: Selecting a folder selects all of its subfolders and all of the datasets in those folders. This can potentially be a huge amount of data. To be sure you are loading only the data you want, open collapsed folders and select only the datasets of interest.
4. Click OK. IGV displays the genomic data.

Removing Tracks and Attributes

To remove all tracks and attributes:

- Click File > New Session. This is essentially the same as restarting IGV.

To remove specific tracks, do one of the following:

- Right-click a track name and click Remove Tracks in the pop-up menu.
- Right-click an attribute value, which selects all tracks tagged with that attribute value, and click Remove Tracks in the pop-up menu.
- Control-click track names (Mac: Command-click), then right-click one of the selections and click Remove Tracks in the pop-up menu.

Note:

- Sequence data is associated with the gene track; therefore, removing the gene track removes the sequence data.
- You cannot remove individual attributes during a session, but you can hide them.

Viewing Attributes

Attributes can be associated with tracks and used for filtering, sorting, and grouping data. By default all tracks have at least 3 attributes: Data File, Data Type, and Name. To display additional attributes, load a sample attribute file. IGV displays attribute names and values in the attributes panel.

Color-Coded Attribute Values

IGV uses color-coded blocks to represent the attribute values.

- Hover over a colored block to display the attribute value.
- Click a colored block to select all tracks with that attribute value. IGV indicates a selected track by highlighting the track name. **Tip**: Keep in mind that clicking an attribute may select tracks that are not visible in the data panel. Scroll down the data panel to view all the selected tracks.

Showing and Hiding Attributes

To show or hide selected attributes:

1. Click View > Select Attributes to Show. IGV displays a list of attributes.
2. Select (or clear) an attribute's check box to show (or hide) the attribute.
3. Click OK. IGV updates the display to show only the selected attributes.

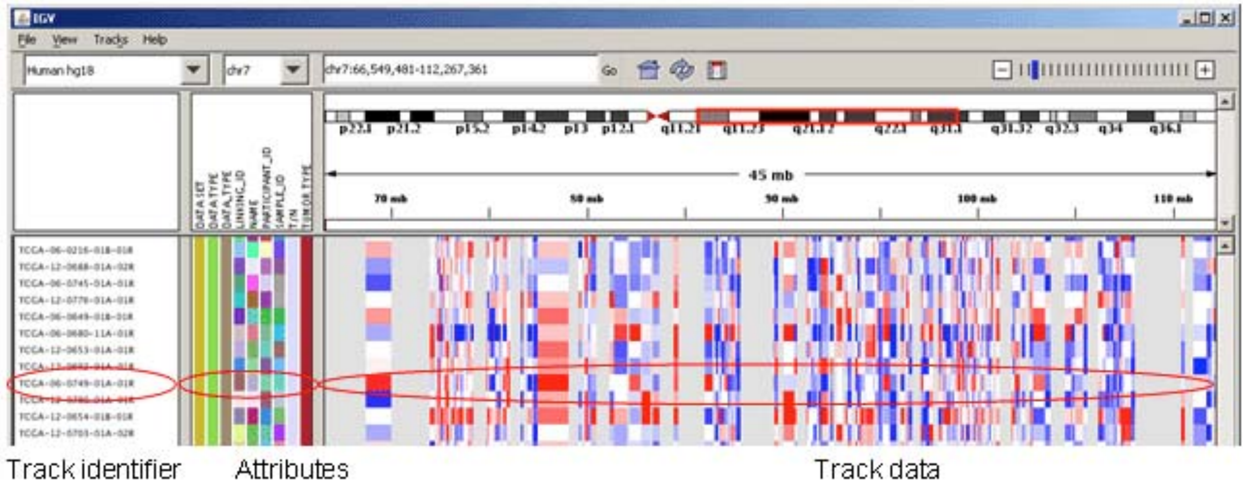
To show or hide all attributes:

- Click View > Show Attribute Display to toggle the setting. A check mark next to the menu item indicates that the attribute panel is displayed. No check mark indicates that it is hidden.
  Tip: This is a persistent setting. Toggling the menu item also toggles the corresponding setting on the General tab of the Preferences window and vice versa.

Viewing Data

Default Display

When you load genomic data, IGV displays the data in horizontal rows called tracks. Typically, each track represents one sample or experiment. For each track, IGV displays the track identifier, one or more attributes, and the data.



When loading a data file, IGV uses the file extension to determine the file format (see File Formats), the file format to determine the data type (Table 1), and the data type to determine the track default display options (Table 2).

Table 1. File Format Determines Data Type

File Format	Data Type
CBS, CN, SEG, SNP	Copy number
LOH	LOH
GCT	Gene expression or RNAi

GISTIC	GISTIC data
RES	Gene expression
SAM, BAM	Sequence alignments
BED, GFF, GFF3	Genome annotations
MUT	Mutation
IGV, WIG, HDF5 file not created with alignment processor	Other
Cytoband, FASTA	Not applicable. Cytoband and sequence files for an imported genome.

Table 2. Data Type Determines Display Options

Data Type	Default Graph Type	Default Data Range	Default Colors
Copy number	Heatmap	-1.5 to 1.5	Blue to red
Gene expression	Heatmap	-1.5 to 1.5	Blue to red
Chip	Bar chart	None, data is autoscaled	Blue
DNA methylation	Heatmap	0 to 1 (methylation score)	Green
Allele-specific copy number	Heatmap	-1.5 to 1.5	Blue to red
LOH	Heatmap	-1 to 1	Blue = LOH (1) Yellow = Retained (0) Red = Conflict (-1)
RNAi	Heatmap	-3 to 3	Red to blue
Other	Bar chart	None, data is autoscaled	Blue

Changing the Display

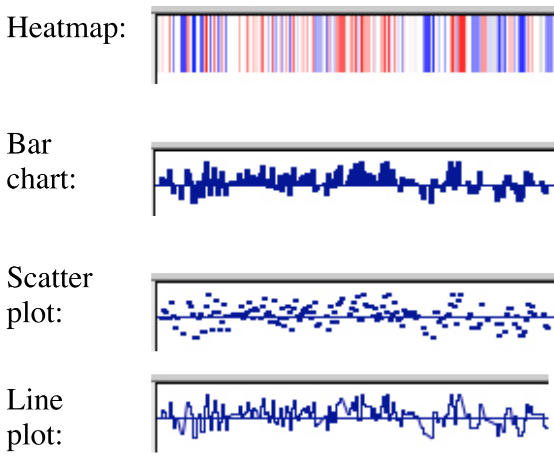
You can override IGV's default display options in several ways:

- Use the track pop-up menu to change the appearance of selected tracks.
- Use the Preferences window to set display preferences for all tracks.
- Use the Color Legends window to set the default data range and color for heatmaps, which IGV uses to display copy number, gene expression, RNAi, methylation, LOH, and mututation data (Table 2).
- For IGV and segmented (SEG, CBS) data files, add a type line to the data file to override the default data type associated with the file format and thus the default display options for the data.
- Add a track line at the top of a data file to specify the display settings for the data.
- Override the default display settings by including display attributes in the sample information file. Note that changes made with this method take precedence over the defaults prescribed by a #type line.

This section describes a few commonly used display options that apply to all (or most) tracks: graph type, data range, track color, track height, andtrack names. For information about how to load and display specific types of data, see Viewing Data. For a complete list of display options, review the options available in the pop-up menus, Preferences window, Color Legends window, and the menu bar (View and Tracks menus).

Graph Type

Most tracks are displayed using one of four graph types (the following graphs show the same data):



IGV determines the default graph type for a track as described in Default Display.

To change the graph type of selected tracks:

- Right-click a track and select a graph type from the pop-up menu.

Data Range

The *data range* for a track provides the minimum, baseline, and maximum value for the graph. IGV determines the default data range for a track as described in Default Display.

To change the data range for selected heat map tracks:

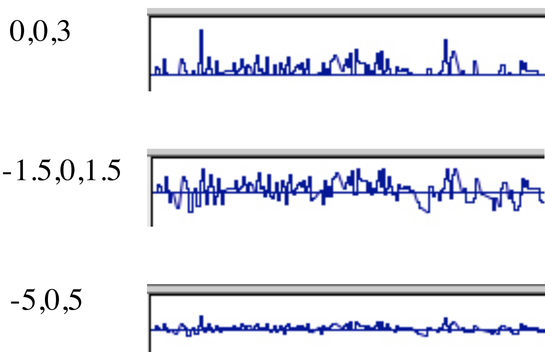
- Right-click a track and select Set Heatmap Scale from the pop-up menu.

To change the data range for other selected tracks:

- Right-click a track and select Set Data Range from the pop-up menu.

Changing the data range can significantly affect the data display:

minimum, baseline, maximum	Result



Track Color

To change the track color for selected heat map tracks:

- Right-click a track and select Set Heatmap Scale from the pop-up menu.

To change the track color for other selected tracks:

- Right-click a track and from the pop-up menu select either Change Track Color (Positive Values) or Change Track Color (Negative Values).

Track Height

To change the height of selected tracks:

- Use the track pop-up menu.

To change the height of all tracks:

- Click Tracks > Set Track Height and enter a value.

To fit the data to the window:

- Click Tracks > Fit to Window.
  IGV displays all tracks. If necessary, it sets the track height to 1 pixel and scrolls the data.

Track Names

By default, IGV displays track names to the left of the attribute panel. Legibility of the track names depends on track height; for example, track names will not be legible when track height is 1 pixel).

To select the attribute IGV uses as the track name:

- Use the Tracks tab of the Preferences window.

To display the track name as a track label:

- Use the Charts tab of the Preferences window.

To rename a track:

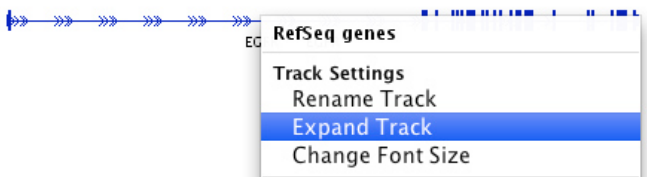
- Right-click a track or a track name, then click Rename Track in the pop-up menu.

You can only rename one track at a time. You can preserve track name changes only by saving the session.

Expanding the Track

By default all features in a track are drawn on a single line, including features that might overlap such as alternative isoforms of a transcript.

To see all overlapping features right-click on the feature track and select *Expand Track*.



This breaks expands the track to multiple rows as required so that features do not visibly overlap.

Expression Data

File Formats

For expression data, use the GCT file format. This a tab-delimited format that contains a row for each probe set ID (or gene), a column for each sample, and expression values for each feature in each sample.

Display Notes

- By default, IGV displays expression data as a blue-to-red heatmap where the data range is -1.5 to 1.5. If loaded expression data appears in tracks colored all red, check the data values and modify the data range as necessary.
- To change track display options, use the track pop-up menu. The commands that appear in the pop-up menu are those relevant to any data track.

Genomic Locations for Probes

To display expression data, IGV must first map the probe set IDs named in the expression data file to

their genomic locations. IGV displays data for all of the probes that it can map to genomic locations. If none of the probes in the file can be mapped, IGV displays an error message.

IGV determines the genomic locations for probes as follows:

1. If you use the delimiters |@ and | to specify the probe loci in the file (see the GCT file format), IGV uses the specified loci. Otherwise, it goes to the next step.
2. IGV searches all loaded annotation tracks for each probe. (This is the same as entering the ID in the first column (the Name column) of the file into the search box on the IGV tool bar and clicking Go.) If a probe is found, IGV displays the data at that location. Otherwise, it goes to the next step.
3. IGV searches its probe mapping files for each probe. If a probe is found, IGV determines the probe locus and displays the data at that location. Otherwise, it goes to the next step.
4. IGV uses its gene mapping files to map each probe ID to a gene symbol, determines the gene locus, and displays the data at that location.

Choose preferred mapping: By default, IGV uses its probe mapping files before its gene mapping files. If you prefer to map probes to genes, select the 'Map probes to genes' radio button on the Probes tab of the Preferences window.

Probe Mapping Files

Probe mapping files map probe identifiers to chromosomal locations. They are compiled from source files provided by Affymetrix, Agilent, and Illumina. The Affymetrix and Agilent mapping files are split by species due to their large size. Separate mapping files are provided for human, mouse, and other (non-mouse, non-human) species. Human probe identifiers are mapped to hg18. Depending on the vendor, mouse probe identifiers are mapped to mm9 (Affymetrix), mm5 (Agilent) or mm8 (Illumina).

Following are links to the probe mapping files:

- http://www.broadinstitute.org/igv/resources/probes/affy/affy_human_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/affy/affy_mouse_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/affy_other_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/agilent/agilent_human_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/agilent/agilent_mouse_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/agilent/agilent_other_mappings.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/illumina/illumina_allMappings.txt.gz

Gene Mapping Files

Gene mapping files map probe identifiers to gene identifiers. Following are links to the gene mapping files:

- http://www.broadinstitute.org/igv/resources/probes/affy/affy_probe_gene_mapping.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/agilent/agilent_probe_gene_mapping.txt.gz
- http://www.broadinstitute.org/igv/resources/probes/illumina/illumina_probe_gene_mapping.txt.gz

Sources for the Mapping Files

The probe and gene mapping files are compiled from source files provided by Affymetrix, Agilent, and Illumina. A list of the source files is available at http://www.broadinstitute.org/igv/resources/probes/data_sources_for_mapping.txt.

Mutation Data

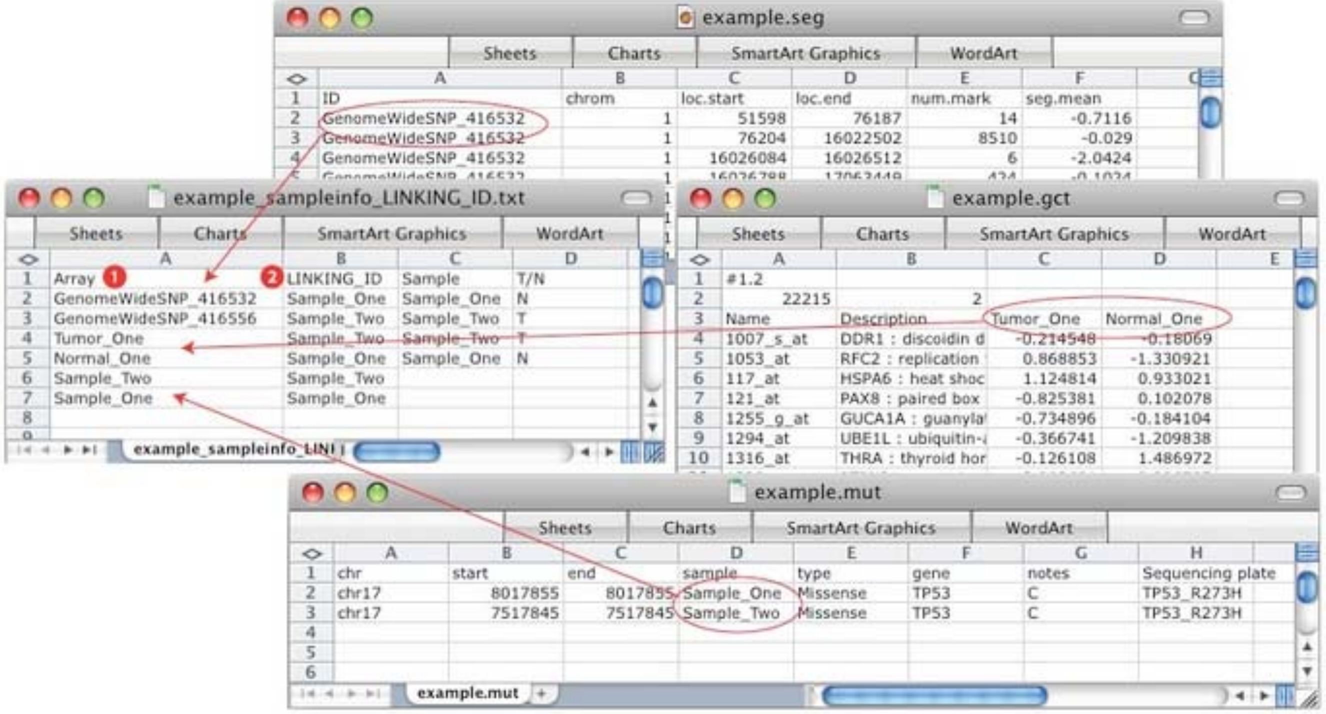
File Formats

Mutation data is loaded from a .mut file. The resulting values can be visualized as distinct tracks or overlaid on other associated tracks (e.g. expression or SNP data from the same patient). This association is specified by means of a special "linking" column in a sample information file. By default IGV looks for a column with the heading LINKING_ID for this association, but the exact heading is configurable as a user preference under the Overlays tab. Mutations are overlaid on another track when the values of this column are equal. A typical use case is to record an identifier identifying a patient, or sample, in this column.

To visualize mutation data:

1. Format mutation data using the MUT file format; e.g. example.mut.
2. Format the data from platform-specific assays using an appropriate file format; e.g. example.gct (expression data) and example.seg (segmented copy number data).
3. Define attributes and their values in a sample information file; e.g. example_sampleinfo_LINKING_ID.txt. A sample information file contains a row for each track and a column for each attribute:

1	The first column contains track identifiers. The track identifier for each mutation track and each associated data track must be included in the sample information file. The track identifiers can be found in the data files (e.g. example.mut, example.gct and example.seg).
2	Each subsequent column identifies an attribute and its value (if any) for each track. IGV uses the value of one attribute (by default, LINKING_ID) to link mutations to associated data tracks; a mutation track and data track that have the same value for this attribute are linked.


example.seg

	A	B	C	D	E	F
1	ID	chrom	loc.start	loc.end	num.mark	seg.mean
2	GenomeWideSNP_416532	1	51598	76187	14	-0.7116
3	GenomeWideSNP_416532	1	76204	16022502	8510	-0.029
4	GenomeWideSNP_416532	1	16026084	16026512	6	-2.0424

example_sampleinfo_LINKING_ID.txt

	A	B	C	D
1	Array	LINKING_ID	Sample	T/N
2	GenomeWideSNP_416532	Sample_One	Sample_One	N
3	GenomeWideSNP_416556	Sample_Two	Sample_Two	T
4	Tumor_One	Sample_Two	Sample_Two	T
5	Normal_One	Sample_One	Sample_One	N
6	Sample_Two	Sample_Two		
7	Sample_One	Sample_One		

example.gct

	A	B	C	D
1	#1.2			
2	22215	2		
3	Name	Description	Tumor_One	Normal_One
4	1007_s_at	DDR1 : discoidin d	-0.214548	-0.18069
5	1053_at	RFC2 : replication	0.868853	-1.330921
6	117_at	HSPA6 : heat shoc	1.124814	0.933021
7	121_at	PAX8 : paired box	-0.825381	0.102078
8	1255_g_at	GUCA1A : guanyla	-0.734896	-0.184104
9	1294_at	UBE1L : ubiquitin-	-0.366741	-1.209838
10	1316_at	THRA : thyroid hor	-0.126108	1.486972

example.mut

	A	B	C	D	E	F	G	H
1	chr	start	end	sample	type	gene	notes	Sequencing plate
2	chr17	8017855	8017855	Sample_One	Missense	TP53	C	TP53_R273H
3	chr17	7517845	7517845	Sample_Two	Missense	TP53	C	TP53_R273H

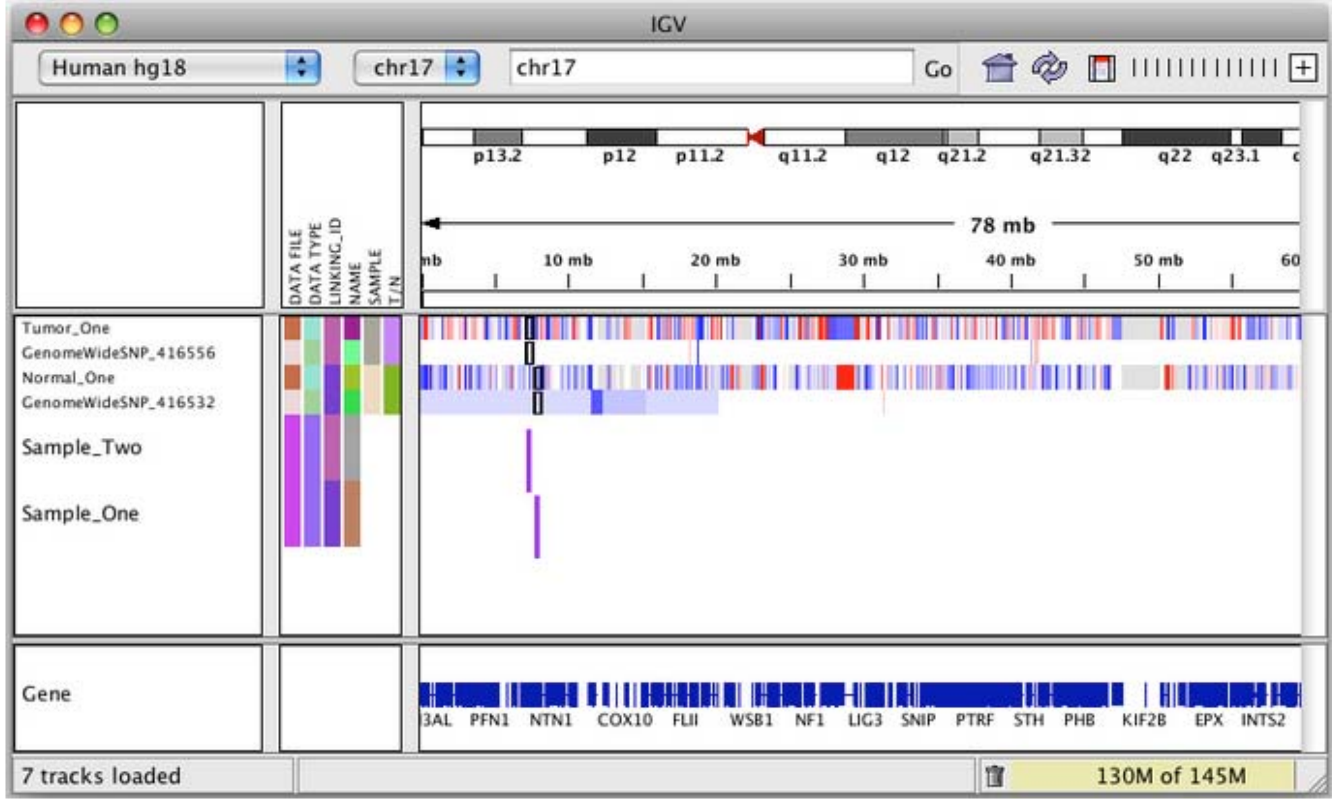
In the example sample information file, the LINKING_ID attribute (2nd column) links the mutation and data tracks. However, in practice, it might be easier to use an existing an attribute rather than adding a LINKING_ID attribute. Notice that, in this example, the LINKING_ID and Sample attributes have the same value. The LINKING_ID attribute could be removed from the sample information file and the Sample attribute used to link the mutation and data tracks. By default, IGV uses the LINKING_ID to overlay mutations on data tracks. If you use an attribute other than LINKING_ID, enter that attribute name on the Overlays tab of the Preferences window.

Display Notes

By default, IGV displays mutations (variant bases) in distinct tracks and overlaid on associated data tracks. Mutations displayed in distinct tracks are color coded by type (missense, silent, and so on). Mutations overlaid on data tracks are colored black for clarity.

Use the Color Legends window to view or change the mutation color codes.

Use the Overlays tab of the Preferences window to modify other display options for mutations.



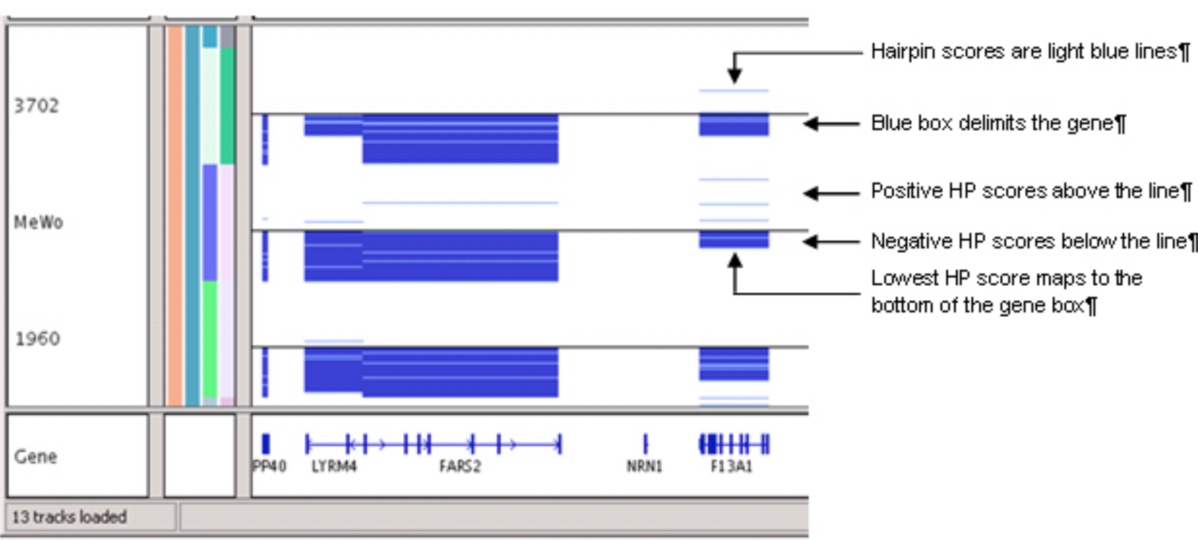
RNAi Data

IGV displays RNAi data similarly to expression data, with one exception: to facilitate analysis of hairpin scores, IGV provides a unique RNAi bar chart. To display the bar chart:

- Right-click an RNAi track and select Bar Chart from the pop-up menu.

The following figure explains how to read the bar chart.

Hover over a track to view hairpin values.



Segmented Data

File Format

For segmented data, use the SEG or CBS file format.

Display Notes

- By default, IGV displays segmented data as a blue-to-red heatmap where the data range is -1.5 to 1.5. If loaded segmented data appears in tracks colored all red, check the data values and modify the data range as necessary.
- To change track display options, use the track pop-up menu. The commands that appear in the pop-up menu are those relevant to any data track.

Gaps Between Segments

By default, IGV fills the gaps between adjacent segments by extending the segment endpoints. When zoomed in sufficiently, error bars indicate the gaps:

Alternatively, you can leave the gaps between the segments. In this case, the gaps appear gray:

To modify how gaps are displayed between adjacent segments set the "Join Adjacent CopyNumber Segments" option on the General tab of the Preferences window.

Viewing Alignments

File Formats

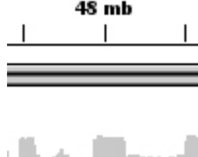
The preferred file format for viewing alignments in IGV is the BAM format, a binary form of Sequence Alignment Format (SAM). Both BAM and SAM files are described on the Samtools project page http://samtools.sourceforge.net/. IGV requires that BAM files be indexed.

For large alignment files we recommend using the BAM format. However, SAM files can be used provided the alignments are sorted by start position and indexed. The igvtools command line utilities can be used to both sort and index the files. Alternatively, if the file is already sorted an index will be automatically created upon first loading the file. IGV will attempt to store the index in the directory

where the alignment file resides with an extension of ".sai". If that fails the index is stored in the users IGV directory.

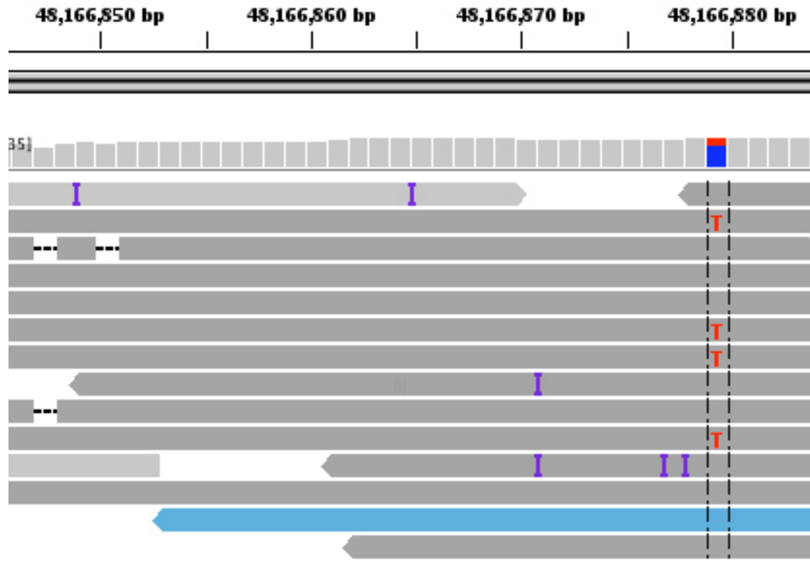
Alignments Panel

IGV displays sequence alignment data in a separate Alignments panel. The display changes as you zoom in. When zoomed out, IGV displays only coverage data, when available (more detail below).



When zoomed in to the alignment read visibility threshold (by default, 30 KB), IGV shows the reads. At this resolution the colored bars in the coverage track identify loci where more than 20% of the quality weighted reads differ from the reference. For paired end alignments, reads that have a larger than expected insert size or a mate on another chromosome are color coded to indicate the chromosome of the mate pair. (more detail below).

At higher resolutions read bases that do not match the reference are color coded, and insertions ( ), deletions relative to the reference become visible. By default, read bases that match are displayed in gray. To color code all bases, regardless of whether they are mismatched, right-click the track and select Show All Bases from the pop-up menu. In addition, mismatched bases are assigned a transparency value proportional to the read quality (phred) score. This has the effect of de-emphasizing low quality reads. Transparency shading can be turned off temporarily from the pop-up menu, or permanently from the Preferences window.

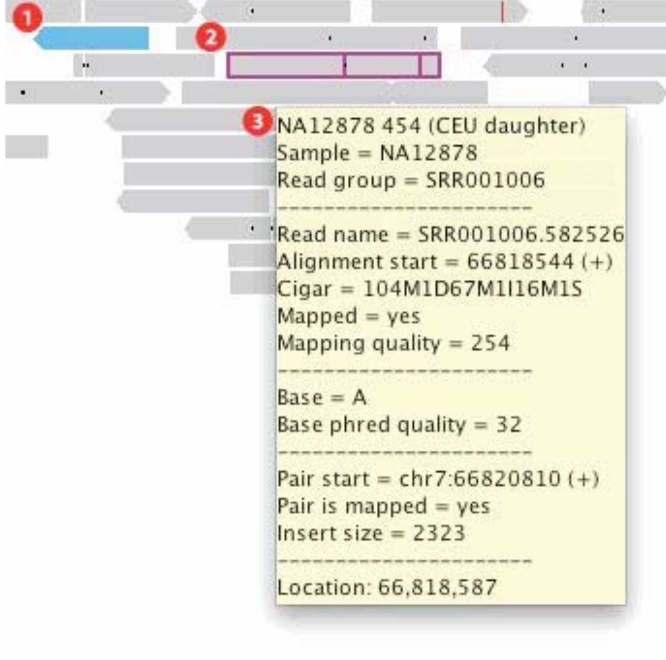


By default, when zoomed in sufficiently, IGV displays a line at the center of the display. When zoomed in further, IGV shades alignments that intersect the center line. At higher resolutions, the center line becomes two lines that frame the aligned bases at the center of the display, as shown in the figure above.

This option, along with many others, can be modified on the Alignments tab of the Preferences window.

Paired-End Alignments

IGV provides several options for working with paired-end alignments:

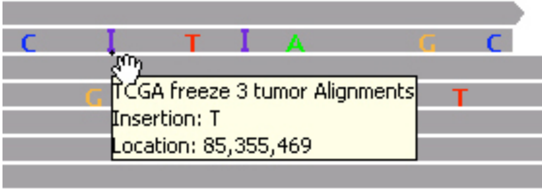


<table>
<tr><td>1</td><td>IGV colors paired-end alignments whose inferred insert size is larger than expected or whose mate read is aligned to a different chromosome. A read with a mate aligned to a different chromosome is color-coded to identify the other chromosome. A read with a large insert size is the color of its own chromosome. The chromosome color legend is on the Alignments tab of the Preferences window:

Chromosome color legend   Used to flag paired end reads with mates on other chromosomes
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y</td></tr>
<tr><td>2</td><td>Control+click (Mac: Command+click) a read to outline the read and its paired mate in the same color. Colors are arbitrary but unique to each pair. A black outline indicates that the selected read has no mate.
Control+click (Command+click) either read to clear the outline.
Right-click and select Go to Mate Region to jump to the paired mate. Note: If the paired reads have a large insert size, the paired mate will not be highlighted. This is a known issue that will be addressed in a future release.
Right-click and select Clear Selections to clear all outlines.</td></tr>
<tr><td>3</td><td>Hover over a read to view information about the read, including the location of its paired mate.</td></tr>
</table>

Insertions

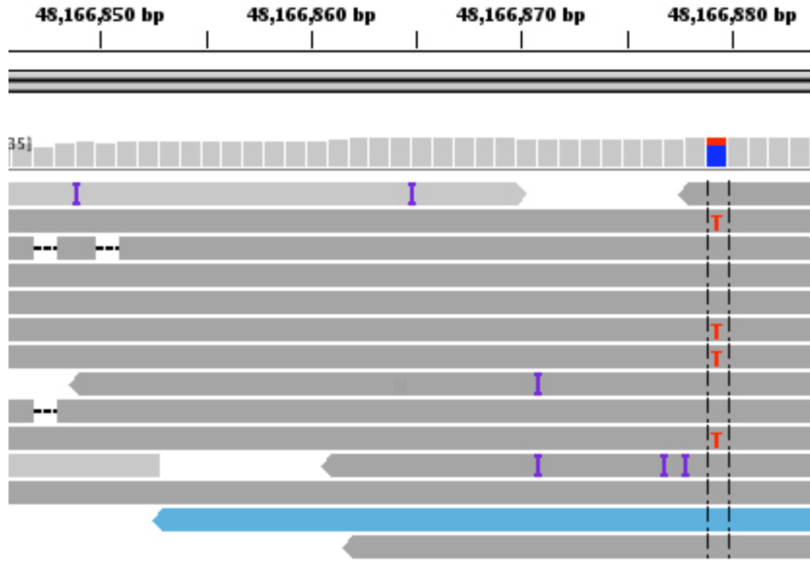
When viewing a gapped alignment, IGV indicates insertions with respect to the reference with a purple bar (I). Hover over the insertion symbol to view the inserted bases.



Read Coverage

Default Coverage Data

IGV supplements each alignment track with a coverage track. When IGV is zoomed in sufficiently to display alignments, the coverage track displays the depth of the reads displayed at each locus as a gray bar chart. If a nucleotide differs from the reference sequence in greater than 20% of quality weighted reads, IGV colors the bar in proportion to the read count of each base (A, C, G, T).



To hide the coverage track, clear the Show Coverage Track option on the Alignments tab of the Preferences window.

Extended Coverage Data

To display coverage data at the whole genome or chromosome level, use the igvtools package (count command) to generate coverage data for the alignments file. The resulting file can be associated with the alignment track by file naming convention, or loaded independently as a separate track.

To associate a coverage track using filename the track must be named as follows, and placed in the same directory as the alignment track:

<alignment file name>.tdf

For example, the coverage track for test.bam would be named test.bam.tdf.

To dynamically associate coverage data with a bam track choose the "Load Coverage Data" from either the alignment or coverage track menu. When the alignment data is loaded with its matching coverage data, the coverage track displays data at all zoom levels.

Sorting Alignments

Alignments can be sorted by start location, strand, nucleotide, mapping quality, sample, or read group.

To sort alignments:

1. Right-click a track to display the pop-up menu.
2. Choose a Sort option from the menu. IGV sorts the alignments that intersect the center line of the display.

Sorting rearranges rows so that alignements that intersect the center appear in the order specified. This can cause the alignment layout away from the center line to appear sparse. To restore the layout to an optimally packed configuration choose "Re-pack alignments" from the popup-menu.

Illumina Sequencing Support

IGV includes limited support for viewing alignments in the "sorted.txt" format from the Illumina Pipeline version 1.3, with the following restrictions.

- The contig fields (columns 12 and 19) are not supported
- The *Match chromosome* field (column 11) must either be the name of a chromosome in an IGV genome, or an entry in the seqname to chromosome mapping file defined below.

Mapping File: To view alignments from a sorted.txt file in which the chromosome names are not chromosome names in IGV a mapping file must be provided. This is a 2 column tab delimited file with chromosome names from the sorted.txt file in column 1, and corresponding IGV chromosome names in column 2. The file must be named **"sequence.map"** and placed in a specific directory, which is platform dependent:

Windows: <user home>/igv/sam
Linux: <user home>/igv/sam
Mac: <user home>/.igv/sam

On Windows computers the user home directory is normally found at

C:/Documents and Settings/<user name>

Sorting, Grouping, and Filtering

By default, IGV displays tracks in the order in which they are loaded (i.e. the order of the data in the files). Alternatively, sort the tracks by attribute, region of interest, or track list. You can also group or filter tracks.

Sorting by Attribute

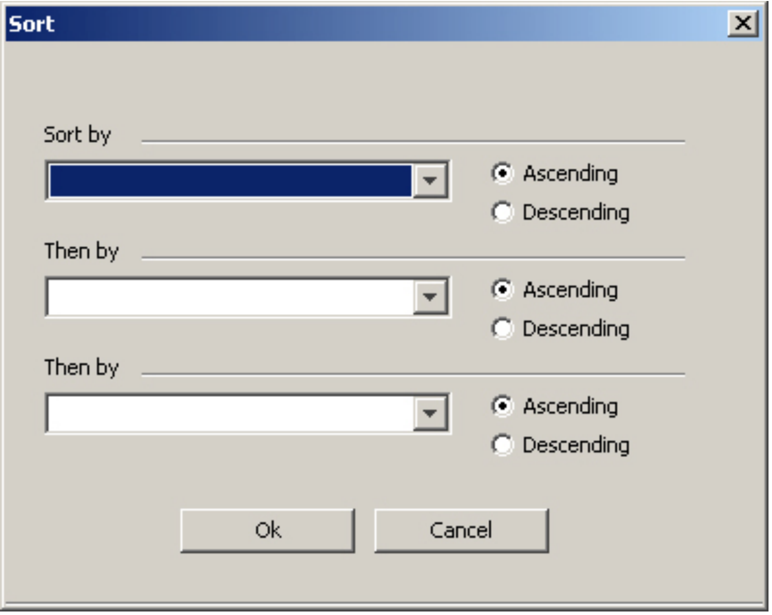
If tracks are grouped, IGV sorts the tracks in each group. To sort groups by attribute, first sort the ungrouped tracks by the desired attributes, then group the tracks.

To sort tracks based on an attribute value:

- Click the attribute name in the attributes panel. IGV sorts the tracks based on the attribute's value.

Alternatively, use the Sort Tracks command for additional options:

1. Click Tracks > Sort Tracks. IGV displays the Sort window:



2. Select the attributes to sort by and whether to sort based on ascending or descending values.

Sorting by Region of Interest

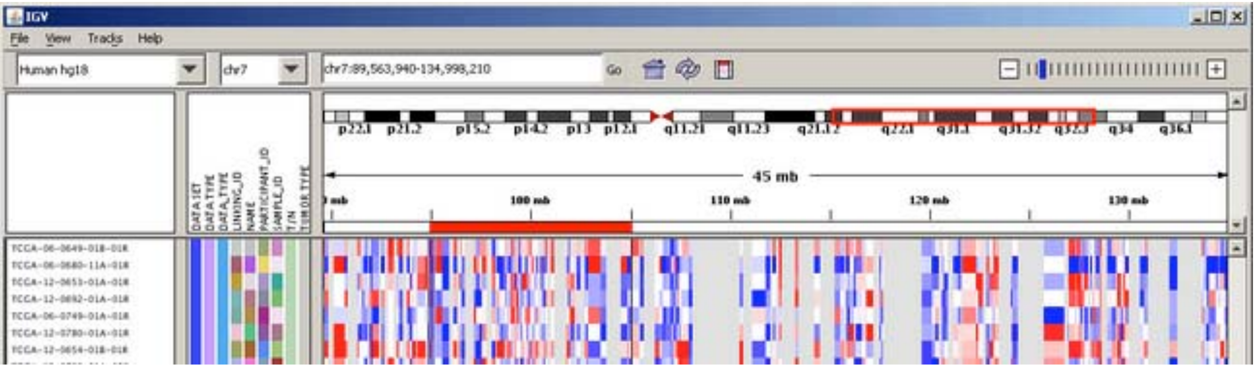
If tracks are grouped, IGV sorts the tracks in each group. It then sorts the groups using a composite score for the group, which IGV defines as the maximum score from the tracks in that group.

To sort tracks in the data panel based on a region of interest:

1. Define a region of interest on the genome, as shown below.
2. Click the red bar above the defined region and select an option from the pop-up menu:
    - Sort by amplification: Affects tracks of copy number data. Sorts tracks based on copy number values in this region, from highest to lowest.
    - Sort by deletion: Affects tracks of copy number data. Sorts tracks based on copy number values in this region, from lowest to highest.
    - Sort by expression: Affects tracks of gene expression data. Sorts tracks based on gene expression in this region, from highest to lowest.
    - Sort by value: Sorts tracks based on the values of the track data in this region, from highest to lowest.
    - Delete: Removes this region-of-interest annotation.

Defining Regions of Interest

A region of interest is a portion of the data panel that is highlighted with black lines at either side of the region and a red bar at the top:



To define a region of interest:

1. In the tool bar, click the Define a Region icon:

2. In the data panel, click the start of the region and then the end of the region. IGV draws lines delimiting the region.

To remove one region of interest from the display:

- Right-click the red bar above the region and click Delete.

To remove all regions of interest from the display:

- Click File > Clear Regions.

To export a region of interest:

- Click File > Export Regions and save the file.

To import a region of interest:

- Click File > Import Regions, navigate to the location of the exported file, and double-click it. IGV displays the lines delimiting the region.

Sorting and Filtering by Track List

To display selected tracks in a specific order:

1. Create a sample information file that contains two columns. The first column must be labeled 'Array' and lists the tracks that you want to display. The second column can be labeled 'Order' (or any other label) and lists the sort order for the tracks.
   For example, the following sample information file provides a track list for use with segmented_data_080520.seg:

Array	Order
primary_GBM_10	a
primary_GBM_20	b
primary_GBM_30	c

primary_GBM_40 d

primary_GBM_50 e

primary_GBM_60 f

primary_GBM_70 g

2. Load the data file and the sample information file that you created in step 1.
3. Apply a filter to display only those tracks that contain a value for the Order attribute. To do so, click Tracks > Filter Tracks and apply the following filter:
   Order is not equal to <leave the text field blank >
4. Sort the tracks based on the Order attribute. To do so, click Tracks > Sort Tracks and select the Order attribute.

Grouping Tracks by Attribute

To group tracks by attribute:

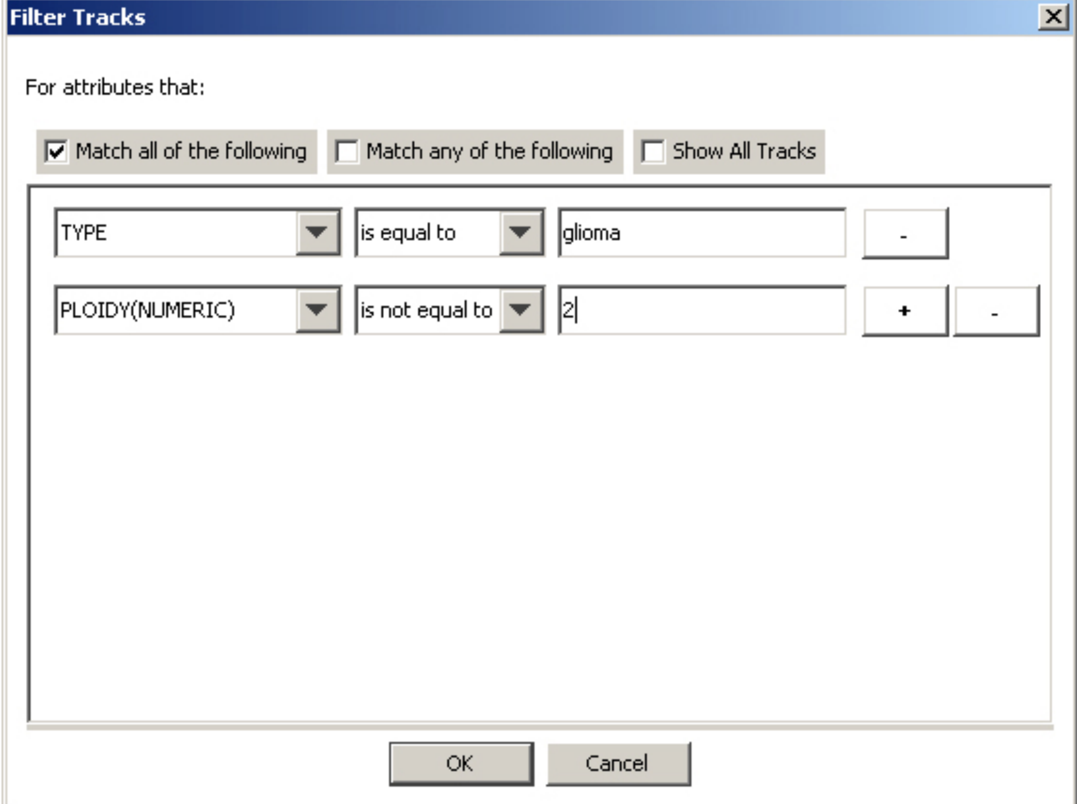
- Click Tracks > Group Tracks and select an attribute from the drop-down list. IGV organizes the tracks into groups that share the same value for that attribute.

Filtering Track Data

You can filter track data to display only tracks that meet certain criteria.

To filter tracks:

1. Click Tracks > Filter Tracks. IGV displays the Filter Tracks window:



2. Enter a filter criterion by selecting an attribute from the first drop-box and an operator from the second drop-box and entering an attribute value in the text box.
3. Optionally, add additional criteria:
   - Click the plus (+) button to enter each filter criterion.
   - Click the minus (-) button to remove a criterion.
4. Determine how the criteria should be combined by choosing one of the following, at the top of the window:
   - Match all of the following to combine the criteria using a logical AND
   - Match any of the following to combine the criteria using a logical OR
5. Click OK to apply the filter.

To clear the filter:

1. Click View > Filter Tracks.
2. At the top of the Filter Tracks window, click Show all tracks, then click OK.

Saving and Restoring Sessions

Saving and Restoring

You can save the current state of an IGV session to a named session file. You can use that file to restore the IGV session yourself or share it with colleagues, as long as they have access to the session file and any data files that were loaded when the session file was saved. For example, if the data files are loaded into IGV from a shared directory and the IGV session file is saved to that shared directory, anyone with access to the directory can restore the saved IGV session.

To save a session:

1. Click File > Save Session.
2. In the Save Session window, select a directory and session file name and click OK.

To restore a saved session:

1. Click File > Open Session.
2. In the Open Session window, select a session file and click OK. IGV ends the current session and restores the saved session.

Session File

IGV Version 1.5 and Greater

Overview

Sessions are an integral part of IGV, allowing users to share their data and views with other users simply and accurately. Session files describe the session in XML. If you wish to manually create or edit a session file, use the information below to better understand the components of each session file.

Session XML Hierarchy

- <Global>
  - <Resources>
    - <Resource>
  - <Panel>
    - <Track>
      - <DataRange>

Description of Session Components

Required - These elements are required in a session file. All session files must follow XML standards.

- <Global>: Contains information about the general state of IGV when the session was saved
  - genome= The genome id
  - locus= The genomic range selected when the session was saved
  - version= The session version (this must equal '3')
- <Resources>: An enclosing element for all Resource elements
- <Resource>: Contains the location and other important information for your data files; for instance, a Resource could be a DAS server, BED file, or sequence alignment
  - name= The name of the track for single track files
  - path= The path IGV uses to access the resource
  - url= The URL path to the resource / UCSC Track Line Url

Optional - These elements are optional in a session file and are added by IGV to help determine the placement of the data and visual style choices.

- <Panel>: Contains information about the placement of Tracks in the visual panels
  - name= The display name for the Panel
  - height= The default height for the Panel
  - width= The default width for the Panel
- <Track>: Details information about every track in a session
  - color= The default color for the data in the track
  - expand= Whether the track is expanded or not
  - height= The default height of the track
  - id= The id assigned by IGV to this track
  - name= The display name for the track
  - renderer= The renderer to be used with this Track (non-default)
  - visible= Whether the track is visible or loaded in the background
  - windowFunction= The function to be used when displaying data
- <DataRange>: A set of attributes used to determine the look of the Panel
  - baseline=
  - drawbaseline=
  - flipAxis=
  - maximum=
  - minimum=
  - type=

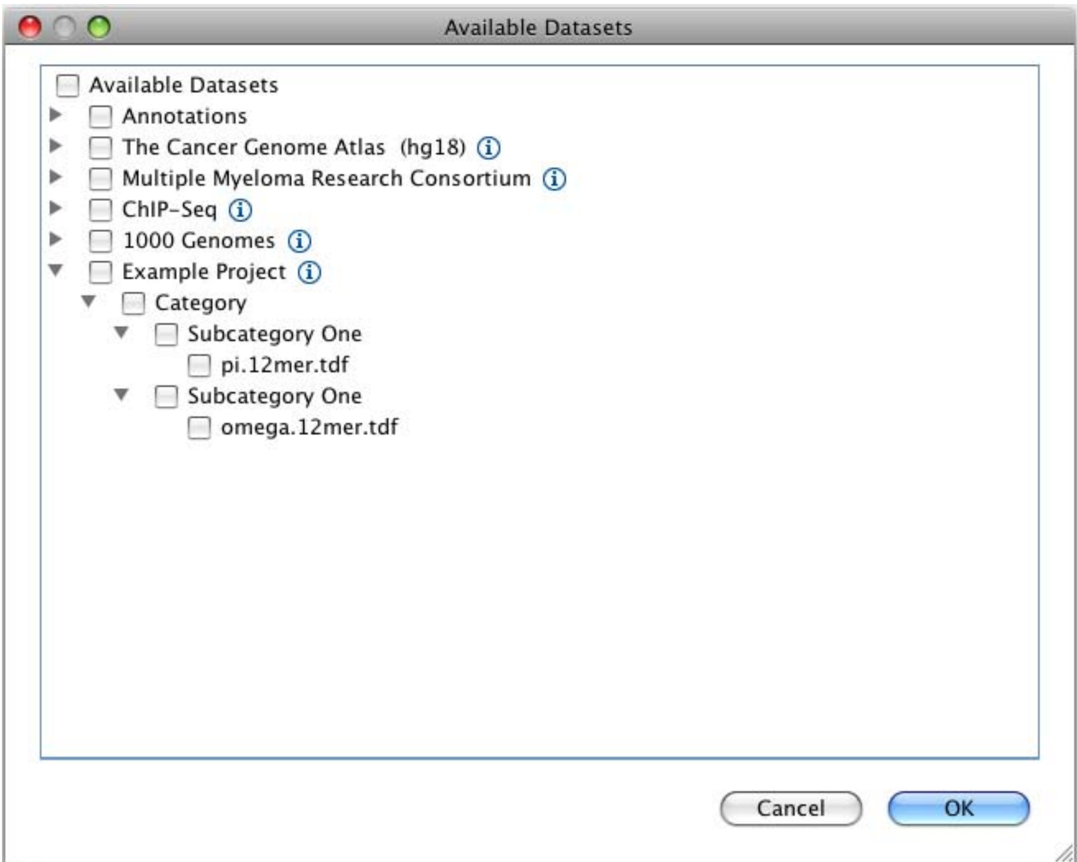
Session Example

The XML below is an example of a simple Session created by IGV

```
<?xml version="1.0" encoding="UTF-8"?>

<Global genome="hg18" locus="All" version="3">

<Resources>

<Resource hyperlink="http://genome.cse.ucsc.edu/cgi-bin/hgTrackUi?g=rnaGene" label="RNA Genes"
name="RNA Genes" path="http://www.broadinstitute.org/igvdata/annotations/hg18/rna_genes.bed"/>

<Resource hyperlink="http://genome.cse.ucsc.edu/cgi-bin/hgTrackUi?g=wgRna" label="sno/miRNA"
name="sno/miRNA" path="http://www.broadinstitute.org/igvdata/annotations/hg18/sno_mirna.bed"/>

</Resources>

<Panel height="445" name="DataPanel" width="1000">

<Track color="0,0,178" colorScale="ContinuousColorScale;0.0;20.0;255,255,255;0,0,178"
displayName="Non coding RNA" expand="false" height="45"
id="http://www.broadinstitute.org/igvdata/annotations/hg18/rna_genes.bed" name="RNA Genes"
renderer="BASIC_FEATURE" visible="true" windowFunction="count">

<DataRange baseline="0.0" drawBaseline="true" flipAxis="false" maximum="20.0" minimum="0.0"
type="LINEAR"/>

</Track>

<Track color="0,0,178" colorScale="ContinuousColorScale;0.0;20.0;255,255,255;0,0,178"
displayName="sno miRNA" expand="false" height="45"
id="http://www.broadinstitute.org/igvdata/annotations/hg18/sno_mirna.bed" name="sno/miRNA"
renderer="BASIC_FEATURE" visible="true" windowFunction="count">

<DataRange baseline="0.0" drawBaseline="true" flipAxis="false" maximum="20.0" minimum="0.0"
type="LINEAR"/>

</Track>

</Panel>

<Panel height="65" name="FeaturePanel" width="1000">

<Track color="0,0,178" colorScale="ContinuousColorScale;0.0;20.0;255,255,255;0,0,178"
displayName="RefSeq genes" expand="false" height="30" id="Genes" name="Genes"
renderer="BASIC_FEATURE" visible="true" windowFunction="count">

<DataRange baseline="0.0" drawBaseline="true" flipAxis="false" maximum="20.0" minimum="0.0"
type="LINEAR"/>

</Track>

</Panel>

</Global>
```

Configuring a Data Server

By default, the File > Load from Server option in IGV provides access to public datasets stored on the IGV data server. You can host your own web accessible datasets by creating server registry and configuration files.



To create a custom load from server menu

1. For each top level node in the hierarchy, create an XML file that describes the datafiles accessible under that node. Each datafile is specified by a Resource element which has 2 attributes, a name to be displayed to the user and a url to the file. The resources can be organized into categories, which in turn can be nested to form a tree structure. An example follows.

```
<?xml version="1.0" encoding="UTF-8"?>
<Global name="Example Project"  infolink="http://www.broadinstitute.org/igv/" version="1">
   <Category name="Category">
      <Category name="Subcategory One">
      <Resource name="pi.12mer.tdf"
            path="http://www.broadinstitute.org/igvdata/pi.12mer.tdf" />
      </Category>
      <Category name="Subcategory One">
      <Resource name="omega.12mer.tdf"
            path="http://www.broadinstitute.org/igvdata/omega.12mer.tdf" />
```

```
    </Category>
  </Category>
</Global>
```

The ***infolink*** attribute, which displays an information link for the resource, may be included in any <Global> or <Category> element.

2. Create a registry (text) file that lists the XML files. For example:

   http://www.broadinstitute.org/igvdata/annotations/hg18/hg18_annotations.xml
   http://www.broadinstitute.org/igvdata/tcga_external.xml
   http://www.broadinstitute.org/igvdata/mmgp.xml
   http://www.broadinstitute.org/igvdata/epigenetics_public.xml
   http://www.broadinstitute.org/igvdata/1KG/1KG.xml
   http://www.mycompany.org/igvdata/example_project.xml

3. In IGV, on the Advanced tab of the Preferences window, update the Data Registry URL to point to your registry file.

IGV points to exactly one data registry file. If you'd like your data server to provide access to the public datasets on the IGV data server, include them in your registry file, as shown above.

Configuring a Genome Server

To upload a new genome to your own server so you can share it with others:

1. Use the steps here ("Importing a Genome") to create an initial .genome file and sequence directory. The product of this step will be a ".genome" file and a sequence directory. The seqeuence directory contains a file for each chromosome/contig sequence in the genome.
   NOTE: The sequence directory must be placed in a web-accessible directory.
2. Copy the .genome file (which is a zip archive) to a temporary directory and unzip it.
3. Open the property.txt file in a text editor. The following properties will likely need to be edited:

   id= Unique identifier for the genome (for example, "hg18"). This property is input to some commands in igvtools.
   name= This is the name that appears in the users' pull-down genome list in IGV.
   sequenceLocation= http:// <path to sequence directory> (this is the location of the sequence directory discussed in step #1)

4. Re-zip the files from the .genome archive and name the resulting archive *<id=>.genome*. **This naming convention is mandatory for igvtools.**
5. Copy the <id=>.genome file to the folder that contains your IGV server's genome files.
6. If you haven't already done so, copy the contents of the sequence directory to http:// <path to sequence directory>.
7. To make your genome appear in the users' pull-down list, you will need to create a genomes list file. The IGV default genomes list file can be used as a starting point. Each line in the genomes list is formatted as follows:

   <name> <tab> *<URL of the .genome file>* <tab> *<id=>*

for example

Human hg18 http://iwww.broadinstitute.org/igvedata/genomes/hg18.genome hg18

8. To test in IGV, change the genome URL in the "Advanced" preferences section to: http://<path to your genomes.txt file>. You must quit IGV and restart for this preference to take effect. The genome should appear in the drop-down list.

Creating HTML Links to IGV

This section describes two forms of html links for interacting with IGV from a web page. The first can be used to launch IGV on the client machine at a specific locus with a supplied session file. The second can be used to load data and session files into IGV (after it has been launched).

Launch IGV with a Session File

The first type of html link makes use of a service running at the Broad institute to launch IGV on a specified session file. It takes the following form:

http://www.broadinstitute.org/igv/dynsession/igv.jnlp?sessionURL=***URL***&*user*=***name***&*locus*=***locus***

The base url, http://www.broadinstitute.org/igv/dynsession/igv.jnlp, is a reference to our dynamic session service. This service is neccessary to construct a custom launch file that references your session. Parameters to this service are listed below.

Parameter		Description
sessionURL	Required	URL to a session file (further described below), or a comma delimited list of data files.
locus	Optional	Locus to display. Use any syntax that is valid in the IGV search box.
user	Optional	A short name identifying your website or organization.

Example:

http://www.broadinstitute.org/igv/dynsession/igv.jnlp?sessionURL=http://www.broadinstitute.org/tumorscape/textReader/IGV/all_tumors_session.xml&locus=chr7:55054218-55206232&user=igvuser

Creating a Session File

You can create the session file from IGV, but it may be easier to create it manually. Only three elements are required, as illustrated in the example below.

```
<?xml version="1.0" encoding="UTF-8"?>
<Global genome="hg18" version="2">
```

```
  <Files>
    <DataFile name="http://www.broadinstitute.org/igvdata/pi.12mer.tdf"/>
    <DataFile name="http://www.broadinstitute.org/igvdata/omega.12mer.tdf"/>
  </Files>
</Global>
```

As illustrated in the example above data files should be accessible by http and identified by URL. This is neccessary as the session file is loaded on the client machine running IGV.

URL Commands

As of version 1.4.2, IGV can optionally listen for http requests. This option is turned off by default but can be enabled from the Advanced tab of the Preferences window. Once enabled, IGV accepts the following commands:

http://*machine*:*port*/load?file=*URL*&locus=*locus*&genome=*genome*&merge=true

http://*machine*:*port*/goto?locus=*locus*

The file parameter value can be a url or list of urls to most IGV supported data file types (exceptions listed below), or a session file. The merge parameter controls whether or not the loaded data is merged with the existing IGV session, or a loaded into a new session. If false, any data currently loaded will be unloaded after clicking this link. The default value is false.

File types not supported over http: SAM, sorted.txt, .h5.

Examples:

http://localhost:60151/load?file=http://www.broadinstitute.org/igvdata/annotations/hg18/conservation/pi.12mer.wig.tdf&locus=egfr&genome=hg18

http://localhost:60151/load?file=http://www.broadinstitute.org/tumorscape/textReader/IGV/all_tumors_session.xml&merge=true

http://localhost:60151/goto?locus=egfr

Controlling IGV through a Port

As of version 1.4, IGV can optionally listen for http requests over a port. This option is turned off by default but can be enabled from the Advanced tab of the Preferences window.

Note: IGV will write a response back to the port socket upon completion of each command. It is good practice to read this response before sending the next command. Failure to do so can overflow the socket buffer and cause IGV to freeze. See the example below for the reccommended pattern.

Commands

Command	Description
new	Create a new session. Unloads all tracks except the default genome ann

load *file*	Loads data or session files. Specify a comma delimited list of full paths or URLs.
genome *genomeId*	Selects a genome.
goto *locus*	Scrolls to a locus. Use any syntax that is valid in the IGV search box.
snapshotDirectory *path*	Sets the directory in which to write images.
snapshot *filename*	Saves a snapshot of the IGV window to an image file. If *filename* is omitted, writes a .png file with a filename generated based on the locus. If *filename* is specified, the *filename* extension determines the image file format, which must be .png or .svg.
sort *option*	Sorts an alignment track by the specified option. Recognized values for the option parameter are: base, position, strand, quality, sample, and readGroup.

Example

Example java code:

```
Socket socket = new Socket("127.0.0.1", 60151);
PrintWriter out = new PrintWriter(socket.getOutputStream(), true);
BufferedReader in = new BufferedReader(new InputStreamReader(socket.getInputStream()));


out.println("load na12788.bam,n12788.tdf");
String response = in.readLine();
System.out.println(response);

out.println("genome hg18");
response = in.readLine();
System.out.println(response);

out.println("goto chr1:65,827,301");
//out.println("goto chr1:65,839,697");
response = in.readLine();
System.out.println(response);

out.println("snapshotDirectory /screenshots");
response = in.readLine();
System.out.println(response);

out.println("snapshot");
response = in.readLine();
System.out.println(response);
```

Running IGV with a batch file

As of version 1.5, a user may load a text file from the "File" menu to execute a series of sequential tasks. The user will load a txt file that contains a list of commands, one per line, that will be run by IGV. Arguments are delimited by spaces (*note: not tabs*). Lines beginning with # or // are are skipped. See Controlling IGV through a Port for accepted commands.

Example

new
load *myfile.bam*
snapshotDirectory *mySnapshotDirectory*
genome hg18
goto chr1:65,289,335-67,427,933
sort position
collapse
snapshot
goto chr1:113,144,120-115,282,718
sort base
collapse
snapshot
goto chr4:68,457,006-69,284,218
sort strand
collapse
snapshot

The example script does the following:

1. Loads a file
2. Sets genome and snapshot directory
3. Jumps to a specified loci
4. Sorts, Collapses, and then takes a snapshot of the screen
5. Repeats these steps for other loci

Password Protected Directories

Overview

Users can access password-protected web addresses or URLs. This option only applies to those who are not using **proxy settings**.

When a user enters a password-protected URL, IGV prompts for a user name and password. If the username/password combination is incorrect, IGV will continue to ask the user to authenticate until the combination is entered correctly or the user clicks *Cancel*.

Verification and Examples

There is an example site so that users can test the password protection feature. Click http://www.broadinstitute.org/igvdata/private/. IGV should prompt for a username and password. Enter:

username: guest
password: password

After verifying that server connection and authentication, user can try the following example files in IGV by clicking *File>Load from URL*:

- http://www.broadinstitute.org/igvdata/private/SignalK562H3k36me3.tdf
- http://www.broadinstitute.org/igvdata/private/cpgIslands.hg18.bed
- http://www.broadinstitute.org/igvdata/private/snp130.bedz
- http://www.broadinstitute.org/igvdata/private/snp130.bedz.sai

If you are unable to load these files, verify that your Internet connection is working properly and contact your IT staff for help.

Setting Up a Password-protected Site

There are many ways to set up a password-protected site. The following describes one method of handling this on an Apache server.

Apache

The Apache HTTP Server is a commonly used web server; it is, for example, in use at the Broad Institute. Setting up a password requires:

- an Access File
- a Password File

The Access File (.htaccess) is located in the restricted directory. It should contain the following information:

AuthUserFile /home/[path]/.htpasswd
AuthName "Private IGV Folder"
AuthType Basic
Require valid-user

The first line should contain the path to the Password File.

The Password File (.htpasswd) should be placed in a directory that is accessible *internally*, not through the web. This is can be the home directory, but it must be a location that is not externally visible. An example password file might look like this:

user1:kJs1GPxWtLet2

The file contains the usernames and passwords for all authenticated users, with one user per line. In the example line, the username is "user1" and the password is "kJx1GPxWtLet2," which is an encrypted password representing the human-readable word, "password."

To make the authentication lines, users can contact IT staff or use one of several websites that help generate them. The one used for this line was http://www.kxs.net/support/htaccess_pw.html. This website provides a string that can be used in the .htpasswd file.

Contact your IT staff for assistance if you have any trouble. Test that a password is working by going to the password-protected directory URL. If you are not prompted for a username/password, or receive a 403 error, then the directory is not yet protected.

igvtools

Download

The igvtools utility is available on the Downloads page. This utility includes the following commands:

- **tile** converts a sorted data input file to a binary tiled data (.tdf) file.
  Supported input file formats: .wig, .cn, .snp, .igv, .gct
- **count** computes average alignment or feature density for over a specified window size across the genome.
  Supported input file formats: .sam, .bam, .aligned, .sorted.txt, .bed
- **sort** sorts the input file by start position.
  Supported input file formats: .cn, .igv, .sam, .aligned, and .bed
- **index** creates an index file for an input ascii alignment or feature file.
  Supported input file formats: .sam, .aligned, .sorted.txt, and .bed

File formats: IGVTools provides utilities for working with ascii file formats, including certain ascii alignment formats such as SAM. These utilities to not work with BAM files, for processing bam files use samtools.

Command line: All igvtools commands are run from the command line. On Windows, enter the commands at an MS-DOS prompt (click Start>Run and type: command). On Mac, enter the commands in a terminal window (click Applications>Utilities>Terminal.app).

Running igvtools

Starting with shell scripts

The igvtools utilities can invoked from one of the following scripts:

igvtools (command line version for linux and Mac OS 10.x)
igvtools_gui (gui version for linux and Mac OS 10.x)

igvtools.bat (cmmand line version for windows)
igvtools_gui.bat (gui version for windows)

The general form of the command-line version is:

igvtools [command] [options][arguments]

or

```
igvtools.bat [command] [options][arguments]
```

Recognized commands, options,arguments, and file types are described below.

Starting with java

Igvtools can also be started directly using java as shown below. This option allows more control over java parameters, such as the maximum memory to allocate. In the example below igvtools is started with 1500 MB of memory allocated

```
java -Xmx1500m  -jar igvtools.jar [command] [options][arguments]
```

To start with a gui the command is

```
java -Xmx1500m  -jar igvtools.jar -g
```

Memory settings

The scripts above allocate a fixed amount of memory. If this amount is not available on your platform you will get an obscure error along the lines of "Could not start the Virtual Machine". If this happens you will need to edit the scripts to reduce the amount of memory requested, or use the java startup option. The memory is set via a "-Xmx" parameter. For example -Xmx1500m requests 1500 MB, -Xmx1g requests 1 gigabyte.

Genome

The genome argument in the **tile** and **count** command can be either an id, or a full path to an IGV .genome file. The id for IGV supplied genomes are listed below. Genome definitions corresponding to these files are in the "genomes" subdirectory of the igvtools install. The id is derived by removing the .extension from the filename.

Current genome list: hg18, 1kg_ref, hg19, hg17, hg16, mm9, mm8, mm7, mm6, mm5, canFam2, btaurus_3.0, galGal3, cavPor3, Plasmodium_3D7_v2.1, Plasmodium_3D7_v5.5, Plasmodium_6.1, sacCer1, spombe_709, spombe_1.55, zebrafish, ce6, ce4, dm3, dm2, dmel_5.9, Aplysia, tcas_2.0, tcas_3.0, ncrassa_v3, Glamblia_2.0, me49, tair8, tair9 O_Sativa_r6, ppatens_1.2.

Note: Other genomes might be availalbe, check the "genomes" directory in the IGVTools installation folder. The id of the genome can be inferred by removing ".genome" from the name.

Commands

Tile

The "tile" command converts a sorted data input file to a binary tiled data (.tdf) file. Input file formats supported are .wig, .cn, .igv, and .gct.

Usage:

igvtools tile [options] [inputFile] [outputFile] [genome]

Required arguments:

inputFile The input file (see supported formats below).

outputFile Binary output file. Must end in ".tdf".

genome A genome id or filename. See details below. Default is hg18.

Options:

-z num Specifies the maximum zoom level to precompute. The default value is 7 and is sufficient for most files. To reduce file size at the expense of IGV performance this value can be reduced.

-f list A comma delimited list specifying window functions to use when reducing the data to precomputed tiles. Possible values are min, max, and mean. By default only the mean is calculated.

-p file Specifies a "bed" file to be used to map probe identifiers to locations. This option is useful when preprocessing gct files. The bed file should contain 4 columns:

chr start end name

where name is the probe name in the gct file.

Example:

igvtoolsh tile -z 5 copyNumberFile.cn copyNumberFile.tdf hg18

Notes:

Data file formats, with the exception of .gct files, must be sorted by start position. If neccessary files can be sorted with the "sort" command described below. Attempting to preprocess an unsorted file will result in an error.

Count

The "count" command computes average feature density over a specified

window size across the genome. Common usages include computing coverage for alignment files and counting hits in Chip-seq experiments. Supported file formats are .sam, .bam, .aligned, .sorted.txt, and .bed.

NOTE: *Bed files must include the stand column (column 6 in the bed format). This constraint will be removed in the next release.*

Usage:

igvtools count [options] [inputFile] [outputFile] [genome]

Required arguments:

inputFile The input file (see supported formats above).

outputFile Binary output file. Must end in ".tdf".

genome A genome id or filename. See details below. Default is hg18.

Options:

-z num Specifies the maximum zoom level to precompute. The default value is 7 and is sufficient for most files. To reduce file size at the expense of IGV performance this value can be reduced.

-w num The window size over which coverage is averaged. Defaults to 25 bp.

-e num The read or feature is extended by the specified distance in bp prior to counting. This option is useful for chip-seq and rna-seq applications. The value is generally set to the average fragment length of the library.

-f list A comma delimited list specifying window functions to use when reducing the data to precomputed tiles. Possible values are min, max, and mean. By default only the mean is calculated.

Notes:

The input file must be sorted by start position. The samtools package can be used to sort .bam files. Other files types can be sorted with the "sort" command (see below).

Example:

igvtools count -z 5 -w 25 -e 250 alignments.bam alignments.cov.tdf hg18

Sort

Sorts the input file by start position. This command supports the following

file formats: .cn, .igv, .sam, .aligned, and .bed.

NOTE: This command does not work with BAM files. The samtools package can be used to sort .bam files.

Usage:

igvtools sort [options] [inputFile] [outputFile]

Required arguments:

inputFile

outputFile

Options:

-t tmpdir Specify a temporary working directory. For large input files this directory will be used to store intermediate results of the sort. The default is the users temp directory.

-m maxRecords The maximum number of records to keep in memory during the sort. The default value is 500000. Increase this number if you receive "too many open files" errors. Decrease it if you experience "out of memory" errors.

Index

Creates an index for an alignment or the bed feature file formats. Indexes required for loading alignment files into IGV, and can significantly improve performance for large feature files. The input file must be sorted by start position. This command does not take an output file argument, rather the filename is generated by appending ".sai" (for alignments) or ".idx" (for features) to the input filename. IGV relies on this naming convention to find the index.

Supported file formats are .sam, .aligned, .sorted.txt, and .bed.

NOTE: This command will not index a binary (BAM) file. Use samtools to sort and index BAM files.

Usage:

igvtools index [inputFile]

Version

Prints the version to the console.

Hosted Genomes

The IGV genome server hosts several genomes. To load a hosted genome into IGV, launch IGV and select the genome from the drop-down list in the tool bar. Our thanks to the contributors.

Human

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assemblies:
  - UCSC hg19 (GCA_000001405.1), February 2009
  - UCSC hg18 (NCBI build 36.1), March 2006
  - UCSC hg17 (NCBI build 35), May 2004
  - UCSC hg16 (NCBI build 34), July 2003

Human: 1000 Genomes

- Source: 1000 Genomes, http://www.1000genomes.org/
- Assembly: b36 (1kg ref), December 2008

Mouse (*Mus musculus*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assemblies:
  - UCSC mm9 (NCBI build 37), July 2007
  - UCSC mm8 (NCBI build 36), Febuary 2006
  - UCSC mm7 (NCBI build 35), August 2005
  - UCSC mm6 (NCBI build 34), March 2005
  - UCSC mm5 (NCBI build 33), May 2004

Aplysia californica (Sea hare)

- Source: Broad Institute, http://www.broadinstitute.org/science/projects/mammals-models/vertebrates-invertebrates/aplysia/aplysia-genome-sequencing-project
- Assembly: Aplcal2.0

Arabidopsis thaliana

- Source: The Arabidopsis Information Resource, http://www.arabidopsis.org/
- Assemblies:
  - TAIR 8, November 2009

- TAIR 9, March 2010

C. elegans

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assemblies:
  - UCSC ce6, May 2008
  - UCSC ce4, January 2007

Chicken (*Gallus gallus*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assembly: UCSC galGal3 (WUSTL v 2.1), May 2006

Cow (*Bos taurus*)

- Source: University of Maryland, http://www.cbcb.umd.edu/research/bos_taurus_assembly.shtml
- Assembly: UMD 3.0

D. discoideum (Slime mold)

- Source: dictyBase, http://dictybase.org/
- Assembly: 05-13-2010

Dog (*Canis familiaris*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assembly: canFam2
- Lindblad-Toh K, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005 Dec 8;438:803-19

Drosophila melanogaster (Fruit fly)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
  - dm3, April 2006
  - dm2, April 2004
- Souce: FlyBase, http://flybase.org/
  - 5.9, June 2008
  - 5.22, October 2009

Frog (*Xenopus tropicalis*)

- Sources: Dept of Energy Joint Genome Institute, http://genome.jgi-psf.org/Xentr4/Xentr4.home.html,
- and USCS, http://hgdownload.cse.ucsc.edu/downloads.html#xenTro
- Assembly: v 4.1 (also known as xenTro2) August 2005

Giardia lamblia

- Source: GiardiaDB, http://giardiadb.org/ (GiardiaDB and TrichDB: integrated genomic resources for the eukaryotic protist pathogens Giardia lamblia and Trichomonas vaginalis.)
- Assembly: Release 2.0, November 2009

Guinea Pig (*Cavia porcellus*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu
- Assembly: CavPor3, Feb. 2008 , Broad Institute

Neurospora crassa

- Source: Broad Institute, http://www.broadinstitute.org/annotation/genome/neurospora/Home.html
- Assembly: v3

Opossum (*Monodelphis domestica*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assembly: monDom5, October 2006

Oryza sativa (Rice)

- Source: Rice Genome Annotation Project, http://rice.plantbiology.msu.edu/
- Assembly:
  - release 6, January 2009
  - release 6.1, June 2009

Physcomitrella patens

- Source: Physcome Project, http://www.cosmoss.org
- Assembly: Version 1.2, October 2009
- Rensing SA, Lang D, Zimmer AD, et al, (70 co-authors). The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. Science (2008) 319:64–69

Pig (*Sus scrofa*)

- Source: Ensembl, http://www.ensembl.org/Sus_scrofa/Info/Index
- Assembly: 9.56, September 2009

Plasmodium falciparum

- Source: Wellcome Sanger Trust, http://www.sanger.ac.uk/Projects/P_falciparum/
  - 3D7 v2.1, February 2006
  - 3D7 v5.5
- Source: PlasmoDB, http://plasmodb.org/plasmo/
  - Version 6.1, September 2009

Rat (*Rattus norvegicus)*

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/, based on version 3.4 produced by the Atlas group at Baylor Human Genome Sequencing Center (HGSC) as part of the Rat Genome Sequencing Consortium
  - UCSC Baylor 3.4/rn4 (Nov 2004)

Sea urchin (*S. purpuratus*)

- Source (assembly): Human Genome Sequencing Center, Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu/project-species-o-Strongylocentrotus purpuratus.hgsc?pageLocation=Strongylocentrotus purpuratus).
  - Spur_2.1 (Sep 2006)
  - Spur_2.5 (Feb 2010)
- Source (annotation): SpBase (http://www.spbase.org).
  - Release 4 (Oct 2009)
  - Release 5 (Jun 2010)

Stickleback (*Gasterosteus aculeatus*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/, from the Broad sequence
- Assembly: Broad Institute v1.0, Feb 2006

Toxoplasma gondii

- Source: http://toxodb.org/toxo/
  B. Gajria, A. Bahl, J. Brestelli, J. Dommer, S. Fischer, X. Gao, M. Heiges, J. Iodice, J. C. Kissinger, A. J. Mackey, et al. ToxoDB: an integrated Toxoplasma gondii database resource. *Nucleic Acids Res*., November 14, 2007; (2007).
  (http://nar.oxfordjournals.org/cgi/content/full/gkm981v1)
- Assembly: ME49

Tribolium castaneum (Red flour beetle)

- Source: Human Sequencing Genome Center at Baylor University, http://www.hgsc.bcm.tmc.edu/projects/tribolium/
- Assemblies:
  - TCas V2
  - TCas V3

Yeast (*Candida albicans*)

- Source: UCSC Genome Bioninformatics, http://www.candidagenome.org/
- Assembly: 21 (strain SC5314), April 2007

Yeast (*Saccharomyces cerevisiae*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assemblies:
  - sacCer1, October 2003
  - sacCer2, June 2008
- Source: Wellcome Trust Sanger Institute, http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html
- Assembly: sk1, February 2008

Yeast (*Schizosaccharomyces pombe*)

- Source: Wellcome Trust Sanger Institute, http://www.sanger.ac.uk/Projects/S_pombe/
- Assemblies:
  - July 2009
  - EF 1.55

Zea mays ssp. *mays* (Maize, Corn)

- Source: Maize Genome Sequencing Project, http://www.maizesequence.org/index.html
- Assembly: B73 (4a.53), November 2009

Zebrafish (*Danio rerio*)

- Source: UCSC Genome Bioninformatics, http://genome.ucsc.edu/
- Assembly:
  - ZV7/danRer5
  - ZV8/danRer6