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Home

About IGB

New sequencing technologies are making it much easier for labs to produce huge volumes of short- and long-read sequencing data. At the same time, more labs are using DNA microarrays to study expression patterns, identify sites bound by transcriptional factors, and explore the epigenome. To understand these new data sets, researchers need to view their data alongside other known features of the genomic landscape.

The Integrated Genome Browser (IGB, pronounced Ig-Bee) aims to meet this need. First developed at Affymetrix in 2003 for their tiling array products, IGB provides an advanced, highly customizable environment for exploring and analyzing large-scale genomic data sets.

Using IGB, you can:

- View your RNA-Seq, ChIP-chip or ChIP-seq data alongside genome annotations and sequence.
- Investigate alternative splicing, regulation of gene expression, epigenetic modifications of DNA, and other genome-scale questions.
- · View results from aligning short-read sequences onto a target genome, identify SNPs, and check alignment quality.
- Copy and paste genomic sequences for further analysis into other tools, such as primer design and promoter analysis tools.
- Create high-quality images for publication in a variety of formats.

If you use IGB in your research, please cite:

Nicol JW, Helt GA, Blanchard SG Jr, Raja A, Loraine AE.

The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets. Bioinformatics. 2009 Oct 15;25(20):2730-1. Epub 2009 Aug 4. PubMed PMID: 19654113; PubMed Central PMCID: PMC2759552. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2759552

IGB features

IGB lets you view results from your own experiments or computational analyses alongside public domain gene annotations, sequences, and genomic data sets, thus making it easier for you to determine how your experiments agree or disagree with current thinking and models of genomic structure.

Some of the features IGB offers include:

- Animated zooming. Most genome browsers implement "jump zooming," in which you click a zoom button (or other type of control) and then wait for the display to re-draw. In IGB, zooming is animated, allowing you to easily and quickly adjust the zoom level as needed.
- Simple Data Sharing System QuickLoad. IGB implements a very simple, easy-to-use system for sharing data called QuickLoad. You can use the QuickLoad system to set up a Web site you can use to share your data with colleagues, reviewers, and the public.
- Draggable graphs. You can display genome graphs data (e.g., "bar" and "wiggle" files) alongside and even on top of reference genome annotations, thus making it easier to see how your experimental results match up to the published reference genome annotations. You can reset your graphs to "floating" and click-drag them over annotations to compare your results with annotations and others' experiments.
- Edge-matching across tracks. When you click an item in the display, the edges of other items in the same or different tracks with identical boundaries light up, highlighting interesting similarities or differences across gene models, sequence reads, or other features.
- Integration with local and remote external data sources. IGB can load data from a variety of sources, including Distributed Annotation Servers, QuickLoad servers, ordinary Web sites, and local files.
- Intron-trimming sliced view. In many species, introns are huge when compared to the exonic (coding) regions of genes. IGB provides a Sliced View tab that trims uninformative regions from introns.
- Web-controls. IGB can be controlled from a web browser or any other program capable of sending HTTP requests. Via IGB links, you can create Web pages that direct IGB to scroll to a specific region and load data sets from local files or servers.
- Scripting. IGB understands a simple command language that allows users to write simple scripts directing IGB to show a genome, zoom and scroll to specific regions, and other functions.
- Open source. All development on IGB proceeds via a 100% open source model. The license allows developers to incorporate IGB (and its components) into new applications.

About the User's Guide

- Introduction
- Credits
- Document Conventions

Introduction

The first IGB User's Guide appeared in 2003 when Affymetrix released IGB for the first time. For many years, the User's Guide was published in PDF format. In 2010, we converted the User's Guide to wiki format to allow more people to contribute to the Guide and update it as the IGB application changes and grows.

Credits

The wiki software we are using to publish the current IGB User's Guide is from Atlassian Software, which gave us a license to use their software.

Many people have edited and added content to the User's Guide, including Ann Loraine, John Nicol, Archana Raja, Hiral Vora, Shira Stav, Vikram Bishnoi, Alyssa Gulledge, Richard Linchangco and many others.

Thank you to Atlassian and to IGB User's Guide authors for helping scientists and educators learn to use IGB in for research and teaching.

Document Conventions

On-screen text (i.e., menus, button labels, folder names, field values, etc.) is indicated by **bold** font.

An item followed by a "greater than" symbol followed by another item means "click the first item; the second item will become visible; then click the second item."

Examples:

- File > Open File... means "click on the File menu and choose Open File..."
- View > Set AutoLoad Threshold to Current View means "click on the View menu and then click the Set AutoLoad Threshold to Current View button"

Note that some images are from earlier versions of IGB and may differ slightly from the latest, released version.

Unless otherwise noted, the manual assumes you are using a two button mouse (right and left buttons). Operations in this document that require right-clicking can be performed on single-button mice (e.g., Apple) by holding down the control key while clicking the mouse button.

Quick start

- Introduction
- Step 1: Get and start IGB
- Step 2: Choose species and genome version
- Step 3. Open data sets
- Step 4: Zoom in
 - Other ways to zoom
- Step 5: Load data
- Step 6: Configure tracks

Introduction

As you learn to use IGB, you'll find it offers many features that make it one of the best tools available for visualization and exploration of genomic data sets.

If you are new to IGB, the following Quick Start Guide will help you get started.

Step 1: Get and start IGB

- Go to bioviz.org/igb/download.html IGB Download Page
- Download and launch IGB

If you have trouble starting IGB, see Troubleshooting or ask for help on BioStars. (Please include the IGB tag to ensure we'll see your question.)

Step 2: Choose species and genome version

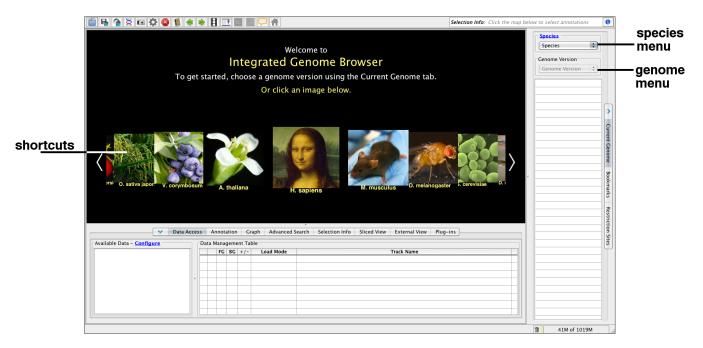
To choose a species and genome version

· Click a shortcut image (loads most recent genome)

or

• Choose Species and Genome Version using the Current Genome tab.

IGB start screen



If your species or genome version is not listed, you can enter it as a custom species and genome when you open a data file. For more information, see Custom Genomes

Step 3. Open data sets

Open data sets from remote data sources (Data Access tab) or by opening local files.

To open a data set from a remote data source

- 1. Select Data Access
- 2. Select data sets in the Available Data sub-panel

To open local files on your computer

- 1. Select File > Open File... or File > Open URL...
- 2. Enter file name or URL

When you select a data sets or a file, IGB will add a new empty track to the main view and list it in the **Data Management** table. Regions that do not have data loaded have a darker background color.

IGB window after opening a data set

		Chromosome 1 (Arabidopsis thaliana TA	IR9) - Integrated Genome Browser 7.0.0	
new	🖹 🖶 🎓 🗷 🛠 🔕 🐔 🗏 🤅	♦ ● 🗄 🛄 📰 📰	Selection Info: Click the map below	w to select annotations
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			1	Arabidopsis th
	TAIR10 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			(8) Seque Length chr1 30,427,671 chr2 19,698,289 chr3 23,459,850
	Coordinates	10,000,000	20,000,000 34	chr3 23,459,656 chr5 26,975,502 chrC 154,478
	TAIR10 mRNA (-)	8,881,820		chrM 366.924 genome Pertection of
	Outa Access Selection Info Available Data - Configure	Advanced Search Sliced View Anno Data Management Table	tation Graph External View Plug-ins	new
data		FG BG +/- Load Mode	Track Name	track
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sets		Cenome Cenome	TAIR10 mRNA X	
			1	179M of 4039M

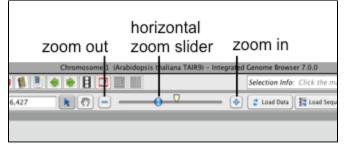
Step 4: Zoom in

Because many data files contain too much data to view all at once, IGB does not load data into the viewer until you click the Load Data button.

Before loading data, zoom in to a region.

- 1. Click a location in the main view
- 2. Drag the horizontal zoom slider or use plus and minus buttons

IGB horizontal zooming controls



Other ways to zoom

Other ways to zoom include

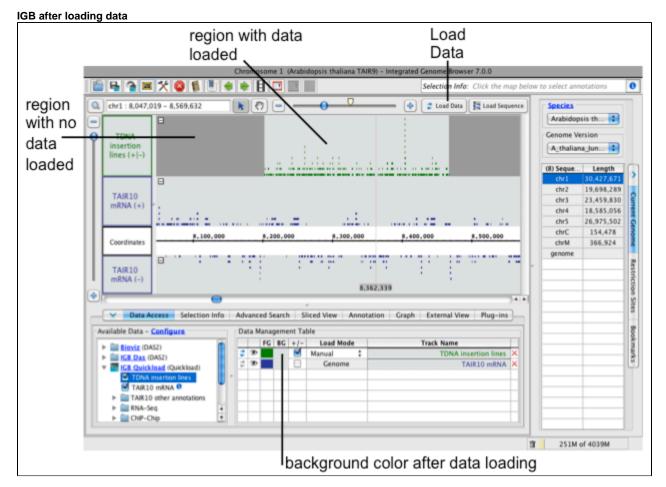
- Search for a gene by name
- Double-click a feature
- Click-drag within the coordinate axis

See also:

- Zooming
- Panning the display

Step 5: Load data

To load data, click Load Data button. Regions with loaded data show the selected background color; areas without loaded data appear darker.



See also:

- Main View
- Loading Data

Step 6: Configure tracks

You can reorder the tracks by dragging the Track Label (the Data Management Table reflect changes).

To change style elements of a track, click the track label and use the **Annotation** or **Graph** tab to change to change color, track height, annotation label, amount of data shown (stack height), and other options.

See also:

- Customizing tracks
- Preferences
- Annotation tab
- Graph Tab

Get IGB

- Introduction
- IGB System Requirements
- Get IGB from bioviz.org Java Web Start
- Get IGB from SourceForge.net
 - Get igb.zip
 - Open the igb folder
- Compile and run IGB from the source code

Introduction

You can get IGB in two ways:

- Java Web start from bioviz.org
- Download igb.zip from sourceforge

IGB System Requirements

IGB can run on any computer that has the Java Runtime Environment (JRE) version 1.6 or higher. (This is also called Java 6.) IGB is developed and tested mostly on Macintosh and Windows, but should work on any computer with Java enabled.

The amount of RAM memory and disk space required depends on the type of data you intend to view. We recommend running IGB with at least 2 Gb RAM.

Get IGB from bioviz.org Java Web Start

To get the latest version of IGB from bioviz.org, visit http://bioviz.org and follow the links to the IGB download page. If your computer has Java Web Start installed, then you should be able to run IGB by clicking one of the download links.

Get IGB from SourceForge.net

Get igb.zip

The IGB distribution available from SourceForge (igb.zip) includes a compiled "jar" (java archive) file called **igb_exe.jar**, which you can use to run IGB on almost any system.

• Download igb.zip file and unpack it.

Open the igb folder

Inside this folder you will find three files: run_igb.command (for Mac), run_igb.bat (for Windows) and run_igb.sh (for other UNIX systems).

On Windows or Mac systems, you can double-click the file to launch IGB.

You can also run IGB from the command line by typing

```
$ run_igb.command
```

Compile and run IGB from the source code

This is a good option if you want to use the most up-to-date version of IGB.

See the IGB Developer's Guide for how to obtain and compile the source code.

IGB terminology

- Genomes and Chromosomes
- Tracks
 - Annotations
 - Graphs
 - Sequences
 - Alignments
 - Probe set alignments

Genomes and Chromosomes

chromosome: In IGB, chromosome refers to any single sequence. Typically this corresponds to the sequence of a physical chromosome. Alternatively, it may represent an assembled contig corresponding to part of a physical chromosome. All chromosomes in IGB are assumed to be DNA, rather than RNA sequences. No two chromosomes within the same genome version can have the same name.

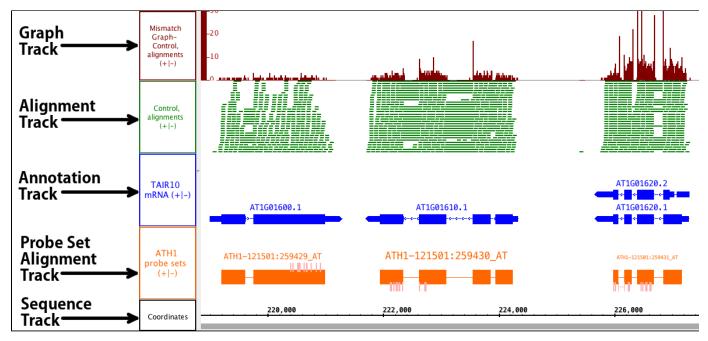
genome, genome version, assembly: These terms refer to a group of chromosome sequences that you or another group assembled and made available. IGB designates these using the month and year they were published or made publicly available. For example, NCBI versions 35 and 36

of the human genome are considered to be two separate genome assemblies. Each one contains multiple chromosome sequences, including the expected chromosomes 1 to 22, X and Y. Other sequences, such as "chr22_random" are also considered distinct chromosomes for the purposes of display in IGB.

Every sequence in IGB is identified by its genome version and chromosome names, which must therefore be distinct.

Tracks

Tracks are rows of data that appear in the IGB interface. When you open a file, the data within the file will appear in one or more tracks. Older versions of IGB refered to tracks as "tiers" and so you may see this term used elsewhere in the User's guide.

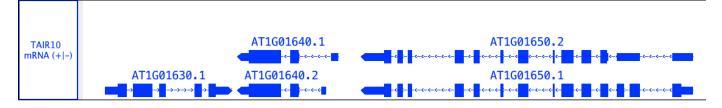


Annotations

Annotations indicate the known or suspected locations of genomic features such as genes, exons, promoter regions, pseudogenes, and so forth. Annotations may consist of a single coordinate, a single span with a start and end positions, or a collection of spans. Most annotations reside on either the plus or minus strand of a chromosome, but some do not.

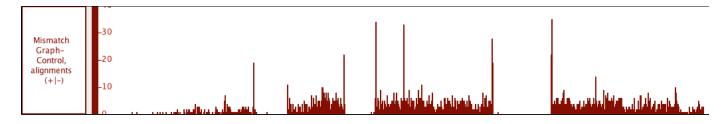
Examples of annotations include:

- single-coordinate feature: splice sites
- single-span feature, with strand: an exon
- single-span feature, no strand: a restriction site
- multi-span feature, with strand: a gene model



Graphs

Graphs consists of numeric data associated with regions or single-base positions in a chromosome.



Sequences

Sequences are sets of DNA residues comprising a chromosome. Sequences can be fully or partially loaded from local files, QuickLoad sites, or DAS servers. IGB allows you to examine sequence data in a separate window called the Sequence Viewer.

	219,000	219,020	219,040	219,060	219,080
Coordinates	CCGATAACAACGGAGGTTCA	T T A A T G A G G G T T T G A T T A T G	A T A C T T A C G A G T G A C A G A T T	GT GA A T A T C A A A G A G A A G C T	TCTTCCTCCCT

Alignments

Alignments represent how sequences obtained from an experiment (such as reads from an RNA-Seq experiment) align onto the reference genomic sequence. At low zoom they look like annotations, but with marks representing mismatches between the read and the reference genome whenever these data are available. At higher zoom, the aligned sequence bases become visible. File formats for alignments include BAM and PSLX.



Probe set alignments

Probe set alignments consists of a target sequence aligned onto a chromosome and the location of probes relative to the aligned target sequence. This data type is Affymetrix-specific.



IGB main window

- Introduction
- IGB start screen
- IGB window
- Navigation tools
- Data display area
 - Annotation tracks
 - Zoom stripe

Introduction

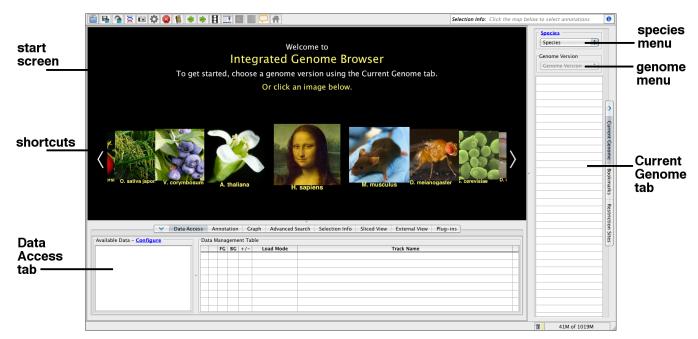
The IGB main window contains tabbed panels, the main data display, toolbar, and navigation tools.

IGB start screen

When IGB first launches, the IGB window contains

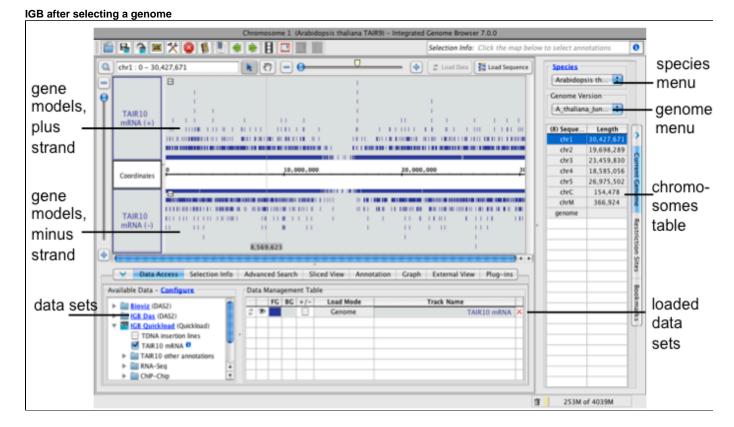
- Start screen with shortcuts to the latest genome versions for popular species
- Current Genome tab on the right
- Data Access tab on the lower part of the IGB window.

IGB start screen



When you choose a genome, a view of the first chromosome listed in the Current Genome tab replaces the IGB start screen.

If available, reference gene models annotations for the selected genome will load from a remote IGB data source.

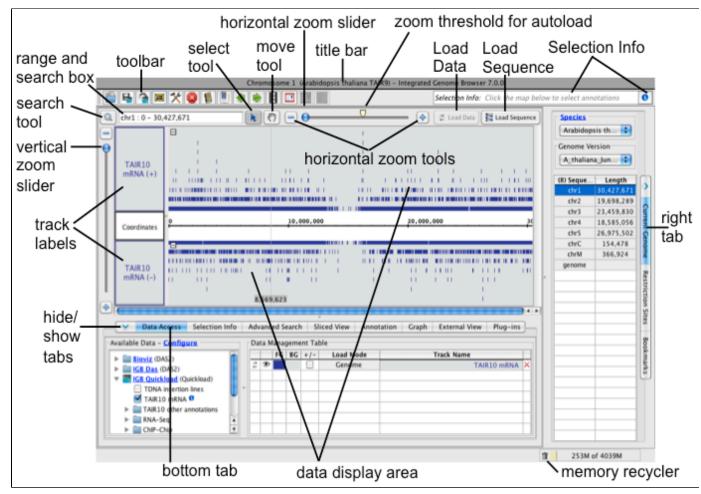


IGB window

The IGB window contains

- · Data display area with central coordinates axis and tracks
- Title bar indicating the chromosome and genome version on display

- Toolbar (new in IGB 7.0) with shortcuts offering one-click access to commonly used functions
- Selection Info box which shows information about selected annotations or data sets
- Load Data button which triggers loading of data into the viewer. See Loading data for details
- Load Sequence button which loads genomic sequence bases for the region in view
- Navigation tools including zoom sliders, scrollbars



Navigation tools

The **range box and search box** shows the chromosome and genomic coordinate range currently in view. Use the **range and search box** to search loaded annotations by name or keyword.

Horizontal and vertical zoom sliders control the zoom level. Use the horizontal and vertical zoom sliders to control zoom level. Zooming in shows less data in greater detail and zooming out shows more data in less detail. Use the + and - buttons to zoom incrementally.

The horizontal zoomer also has an AutoLoad Threshold mark showing the zoom level at which IGB will automatically load data from data sets whose Load Mode setting is to Auto. (Details are in Loading data)

Use the move tool (hand icon) move the data display field in any direction.

Use the **select tool** select tiems in the data display field. Click or click-drag over items in the display to select them or or click-drag selection tool over the coordinate axis to zoom in. See Selecting Items.

Data display area

The data display area tracks and the coordinate axis. It is split vertically, with the track labels on the left and data on the right.

Track labels show track names. Right-clicking a track label opens a menu with options to change track appearance, save tracks, and other functions. You can also click and drag track labels to change the vertical ordering of tracks.

The coordinates track displays genomic coordinates and separates minus and plus strand tracks. The coordinates track contains

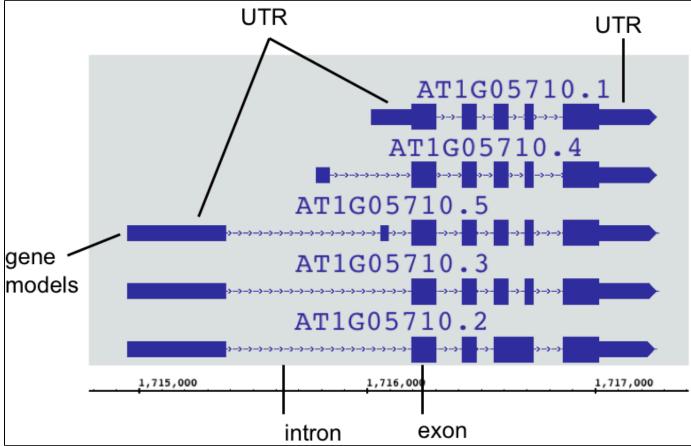
• A cytobands track with chromosome ideogram, where available

- A number line representing the genomic sequence axis
- The reference genomic sequence, which when loaded appears below below the number line.

Annotation tracks

IGB depicts gene annotations as blocks representing exons linked by lines representing introns. Translated regions (CDSs) appear as taller rectangles, and untranslated regions (UTRs) are indicated by thinner exons, as in the following example.

Example gene model



When space permits, IGB displays labels next to annotations, depending on settings for the track. To see labels, uses the vertical or horizontal zoom sliders to zoom in. Once annotations are large enough, labels appear.

To change the appearance of gene models in a track

- 1. Select the track by clicking its track label
- 2. Select the Annotation tab
- 3. Select options under Style and Labels and Strand

Not every gene model has annotated UTRs. When UTRs are not annotated, all exons are the same height.

Zoom stripe

The zoom stripe indicates the location of the most recent click. It indicates the focus for zooming, the position in the display that remains stationary when you zoom horizontally.

The zoom strip label indicates the location of the zoom stripe.

zoom stripe				-				
	١,	70	7,6	20				<u> </u>
	:	С	A	G	G	G	С	Т
zoom stripe								
label —	-	1	1,7	07	,62	3		

Loading data

- Introduction
 - Choose Species and Genome Version
- Loading data sets
- Data Management Table
- Load data to visualize

Introduction

IGB aims to be an integrated genome browser, meaning it can display data from a variety of diverse data sources, all merged into the same view. This includes data sets loaded from your computer, from URL sites, or from various public (and private) DAS, DAS2 and Quickload servers. IGB can also display data from many file types, including:

- Short read alignments data, aka "next generation sequence data" (BAM format)
- Standard annotations such as RefSeq annotations, provided by public repositories
- Alignments of Affymetrix probe sets to the genome, provided by the NetAffx group at Affymetrix (link.psl format).
- Alignments of ESTs or mRNAs produced by blat (psl formats)
- Tilling array graphs, from the TAS program from Affymetrix
- Copy number graphs from the CNAT program Affymetrix
- Data generated from other Affymetrix software tools, such as GCOS, Expression Console and ExACT.
- Annotation and graph files prepared by any method in any of the supported formats.

A list of supported file formats is here.

Choose Species and Genome Version

The first step to loading data is to choose **Species** and **Genome Version**. IGB uses this information to offer data sources with relevant data sets. To set the species and version, select them in the **Current Genome** panel.

Alternatively, if you chose to open a file from File > Open File... or Open URL... the file selection widow will permit you to specify species and genome using the drop down menus at the bottom of the window.

00	Open
Cin chp	
Name	Date Modified
ArabidopsisATH1-121502.CHP.	.gz Thursday, October 21, 2010 1:42 PM
TisMap_Brain_01_v1_WTGene1.	rma-ge Thursday, October 21, 2010 1:42 PM
File Format:	Known Types
	Cancel Open
Choose: Arabidopsis thaliana	A_thaliana_Jun_2009

While some file types contain information specifying their species and genome version, most do not. However, if you load a file that has the species and genome information, IGB will open the proper species and genome, even if it is not the one you have chosen.

Loading data sets

IGB does NOT immediately display loaded files. Many of today's next-gen sequencing files are too big to display all at once. IGB handles this issue by waiting to visualize data sets until you ask it to load data.

While you can immediately refresh to visualize most files, many larger file types, such as BAM and WIG should first have a defined, smaller region selected prior to refreshing the image.

There are several ways to get data sets into IGB, from servers/sources, from URLs and from the local computer. To load data from a server, locate the data set in the folders of the **Data Sources** panel. Put a check in the box next to the data you are interested in. This file will be entered into the **Data Management Table**.

For files loaded from URL or from the local computer, just drag and drop into the IGB interface; the file will immediately appear in the **Data Management Table**. Alternatively, use the **File > Open File..** or **Open URL...** to find and load the file(s) you want. Be sure to set the species and genome at the bottom of the file selection window.

Data Management Table

IGB is capable of loading and displaying whole genomes, whole chromosomes or just portions of a data file, depending on the file type. Therefore, although there are many load options, not every file type can be loaded the same way. There is always the option of **Don't Load**, which will simply not load the individual file but will maintain it in the list.

- Don't Load is the setting for not loading any data, but remaining in the Data Management Table
- Genome is primarily used with sequence files; this is the default load that IGB uses for the reference sequences for most model organisms (e.g. TAIR10 mRNA)
- Manual is the default Load Mode for IGB. This setting tells IGB to load only the region in view (e.g. CoolI1T1.sm.wig). For large files, such as .bam, .wig or other short read associated files, we strongly recommend that you zoom into a small region of interest, usually about <100Kb depending on your read density, and then Load Data to see this region. This is the default setting for most files.
- Auto is an option for BAM and SAM files (e.g. Cooll1T1.sm.bam), which will allow IGB to automatically load the data in view, if the view is zoomed below the Auto threshold, marked with a yellow arrow head (red arrow). The Auto zoom level can be adjusted to your needs. First, set the main view to the zoom level you want. Then, View > Set AutoLoad Threshold to Current View. This will change the needed zoom level AND will move the yellow arrow to indicate the new threshold setting.

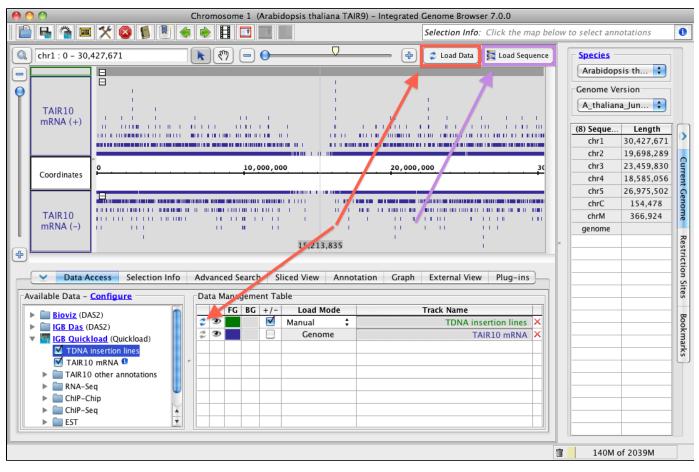
If you set the Autoload threshold too high, IGB will try to load all of the indicated data, which could exceed the memory limit.



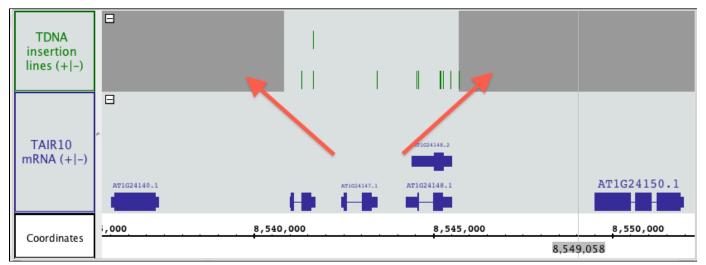
Load data to visualize

(II)

If the data is set to Load Mode > Manual, simply click Load Data. If you want all the tracks to load data at once, use the Load Data button (red box, arrow); to load a single track use the Load Data icon in the Data Management Table (red arrow). Sequence can be loaded from the Load Sequence button (purple box, arrow).

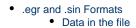


You might load a track and then zoom out to show a greater region of the data sets. IGB will show gray where the track is not loaded yet (red arrows).



File Formats

- Introduction
- Table of supported file formats
- Files Types Supported Through Plugins
- About GFF and its variants
- About the .wig format (also called bedGraph)
- Partial data loading using tabix indexed files
- Sequence File Formats



Introduction

IGB supports multiple file formats in both compressed and uncompressed formats. See the table below for details and links (when available) to resources describing each format.

IGB also supports some file formats using third-party plug-ins.

See below for details.

⚠

For IGB to recognize and open a file, the file name must end with one of the supported file format extensions, listed below.

Table of supported file formats

Туре	Extension	Description			
Affymetrix XML	.axml	A mostly-obsolete XML format used internally at Affymetrix.			
BAM	.bam	A binary indexed version of the SAM format used for displaying alignment data. See SAMtools for more details. Note: Be sure you've indexed your BAM file (you should have a .bai file as well). The index file must reside in the same folder as the BAM File. Supported in IGB 6.3 and higher.			
SAM	.sam	Plain text version of BAM format. Supported in IGB 6.6 and higher. We recommend using this only for smaller files.			
BAR	.bar	Binary graph format developed by Affymetrix. Generated from tiling arrays by TAS (Tiling Analysis Software) from Affymerix, cisGenome from the Hongkai Ji Research group, and others.			
BED	.bed	A tabular format developed for use with the UCSC genome browser. IGB supports four, twelve, and fourteen column BED format. In IGB, the thirteenth and fourteenth columns of fourteen-column BED format (also called BED detail format) are interpreted as gene name and description, respectively.			
BEDGRAPH	.bedgraph	Same as the wiggle format. See below for details.			
BigBED	.bigbed	bigBed format stores annotation items that can either be simple, or a linked collection of exons, much as bed files do. Bed files are created initially from bed type files, using the program bedToBigBed. The resulting bigBed files are in an exed binary format. The main advantage of the bigBed files is that only the portions of the files needed to display a licular region are loaded into IGB, so for large data sets bigBed is considerably faster than regular bed files. See http://g me.ucsc.edu/goldenPath/help/bigBed.html.			
BigWIG	.bigwig	Like the bigBED format, this is an indexed form of a WIG file leading to the ability to partially load data, and much faster load times. See http://genome.ucsc.edu/goldenPath/help/bigWig.html.			
BGR	.bgr	nary graph format developed by Affymetrix.			
Binary Files	.bps, .bgn, .brs, .bsnp, .brpt, .bp1	inary annotation formats developed specifically for IGB by Affymetrix. These are generally not documented anywhere re being retired. If you are using any of these formats, we recommend using tabix-indexed, bgzip-compressed BED de fourteen column BED) files instead.			
BNIB	.bnib	binary format for sequence data originally developed for IGB by Affymetrix to speed up loading sequence data over the twork. This format is no longer used. Starting with IGB 7.0, we will no longer support this file format. We recommend sing 2bit instead of BNIB for sequence files.			
СНР	.chp	Binary files generated by Affymetrix software. Signal values and presence calls for probesets on Affymetrix microarrays. There are multiple sub-formats, identifiable from the file contents. IGB can read most, but not all of these formats			
CN_SEGMENTS	.cn_segments	Output from Affymetrix Genotyping Console representing a summary of all chromosomal region exhibiting copy number change.			
Copy Number Analysis	.cnt	The Affymetrix CNT file format is a tabular format representing output from the Affymetrix CNAT program.			
Cytoband	.cyt	A text format for representing chromosome band (ideogram) data. Examples are available from the IGBQuickLoad.org site under human genome directories.			
DAS XML files	.das, .dasxml, .das2xml	XML formats returned from DAS servers. See http://www.biodas.org. See DAS/1 specification and DAS/2 specification			
Expression Graphs	.egr, .egr.txt, .sin	EGR is a tabular format for associating arbitrary numbers of scores with scored genomic intervals. The extension ".egr" is preferred. The other extensions are kept for backwards compatibility. Files generated from Affymetrix GeneChip Operating Software (GCOS) or ExACT (Exon Array Computational Tool) software.			
FASTA	.fa, .fasta	Sequence data in a simple ASCII format. See here . Recommended only for short sequences. Otherwise, use 2Bit (see below) or follow these instructions to convert to the ".bnib" format. Note : IGB does not support the use of the Control-A character in the header lines.			

GenBank	.gb, .gen	NCBI's file format. Experimentally supported in IGB 6.4.			
GFF (General Feature Format)	.gff, gtf, .gff3	General Feature Format. There are several types of GFF file that use incompatible syntax. The original GFF format is GFF1. A variant called GTF is also used. GFF3 has been proposed to extend on GFF and to constrain the specification more tightly to avoid mutually-incompatible versions of GFF. If IGB has difficulty reading your GFF file, make sure the header includes the GFF version, as indicated in the GFF specification documents.			
GR	.gr	Tab-delimited graph format. A simple text format containing two columns of numbers separated by a single space or tab. The first number is the base position; the second number is the score. Because this format does not include chromosome names, we recommend you use .sgr or .wig formats instead.			
PSL	.psl, .psl3, .link.psl	PSL is a tabular format used for representing alignments in UCSC's BLAT tool.			
PSLX	.pslx	PSLX is an extension to the PSL format that shows sequence data in each alignment. Supported in IGB 6.4.			
Scored Intervals	.sin, .egr, .egr.txt	See EGR.			
Scored Map	.map	An outdated format, replaced now by EGR files.			
SGR	.sgr	Tab-delimited graph format. Sequence graph files that show base coordinate scores. These files are generated by CNAT the Affymetrix Chromosome Copy Number Analysis Tool software). The format of .sgr text files is: chromosome dentification, then two columns of numbers separated by a single space or a tab. The first number is the base position; the second number is the score			
SIN	.sin	See Expression Graphs format.			
TALLY	.tally	Tally files are created by the bam_tally program (using options -P -B 0), and contain mismatch pileup information. The display is identical to the MisMatch Pileup view mode. The Tally files contain the sequence reference. The plugin will use a tabix index if available.			
USeq	.useq	USeq is a binary indexed format used to display graph and annotation data. Supported in IGB 6.2. For more information about it, see: http://useq.sourceforge.net.			
Wiggle	.wig	This is a text format for graphical data designed for the UCSC genome browser. IGB supports all 3 subtypes: BED, variableStep, fixedStep. For more information, see the UCSC Web page describing the format: http://genome.ucsc.edu/gold enPath/help/wiggle.html. Files in wiggle format can use UCSC track lines to specify colors and other properties.			
2Bit	.2bit	2Bit is a compact format for DNA sequences developed by UCSC. Supported in IGB 6.3. See http://genome.ucsc.edu/FAQ/ FAQformat.html#format7 for more information about it.			

Files Types Supported Through Plugins

Some file formats can be read after installing the appropriate plug-in. See Plug-ins.

About GFF and its variants

GFF stands for 'general feature format' or 'gene finding format'; it is a tab-delimited file with 9 columns. There are several types of GFF files that use incompatible syntax. The original GFF format is GFF1. A variant called GTF is also used. GFF3 has been proposed to extend on GFF and to constrain the specification more tightly to avoid mutually-incompatible versions of GFF. Some GFF files created by Affymetrix make use of extensions to GFF that are specific to IGB. These are indicated in the file headers by lines beginning with "##IGB-".

IGB can handle most versions of GFF/GTF, but may have difficulty with some rarely-used advanced features. IGB does not read any FASTA data that is included in some GFF3 files. If IGB has difficulty reading your GFF file, make sure that there is a line in the header similar to ##gff-vers ion 2 that identifies the correct format number 1, 2 or 3.

The GFF format is described at http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

The GTF format is described here http://genes.cs.wustl.edu/GTF2.html

The GFF3 format is described here http://song.sourceforge.net/gff3-jan04.shtml

About the .wig format (also called bedGraph)

A "wig" file is a data file that associates numerical values (e.g., read coverage) with a region of the genome. For example, here is an excerpt:

```
track type=bedGraph name="Pollen RNA-Seq coverage"
chr1 0 3722 0
chr1 3722 3797 1
chr1 3797 5890 0
chr1 5890 5893 4
chr1 5893 5897 5
chr1 5897 5902 6
chr1 5902 5910 7
chr1 5910 5939 8
chr1 5939 5944 9
chr1 5944 5957 10
```

Note that the top line of the file contains information (meta-data) about the data set, including its name. When you open the file in IGB, you'll see this "name" attribute again in the track label.

Partial data loading using tabix indexed files

IGB supports partial data loading of several file types using tabix indexing. Supported file types include SAM, BED, BEDGRAPH, PSL, and PSLX.

Indexed files allow for faster searching and loading. The indexed file and its index (.tbi file) must reside in the same folder either on your local computer or on a server. More about tabix can be found here.

Sequence File Formats

IGB supports fasta and 2bit formats. Older versions of IGB also support an IGB-specific format called bnib. Newer versions of IGB will probably still open bnib files, but as of IGB 7.0, we are no longer including the bnib format in our testing process.

FASTA files contain sequence data in a simple ASCII format. For details, Google search fasta.

We recommend using FASTA for short sequences only. For loading data into IGB and setting up a QuickLoad site, we use the 2bit sequence format.

BNIB is an older format developed for IGB that makes it possible to represent sequence data in a very compact format. 2bit, developed at UCSC is also a compact, binary format for representing sequence data, but a number of open source tools are available for working with this format and so for this reason, IGB now uses 2bit instead of bnib.

.egr and .sin Formats

EGR (also known as Scored Interval, .sin, format.files) are TAB-delimited files with a header. They can contain one or more scores associated with named annotations or with named or unnamed genomic regions. They have an optional header section which is a list of tag-value pairs, one per line, in the form: **# tag = value** Currently the only tags used by the parser are of the form **score\$i** (score name tags are optional). If score name tags are present, then score number **\$i** will be named according to the value of the **score\$i** tag. If any score name tags are missing, default names will be created.

It is recommended that a tag value pair with the genome version, such as **#genome_version = H_sapiens_May_2004**, be included to indicate which genome assembly the sequence coordinates are based on. This will ensure that the file is being compared to other annotations from the same assembly.

Data in the file

There are three versions of this format. They can all be described this way, where the parentheses indicate optional elements:(annot_id) ((seqid) min_coord max_coord strand) [score]*

- 1. seqid is word string [a-zA-Z_0-9]+
- 2. min_coord is int
- 3. max_coord is int
- 4. strand can be '+', '-', or '.' for "unknown"
- 5. score is float
- 6. annot_id is word string [a-zA-Z_0-9]+

All lines must have same number of columns. Format 1 has tab-delimited lines with 4 required columns, any additional columns are scores:seqid min_coord max_coord strand [score]* Format 2 has tab-delimited lines with 5 required columns, any additional columns are scores:annot_id seqid min_coord max_coord strand [score]* Format 3 has tab-delimited lines with 1 required column, any additional columns are scores:annot_id [score]* The IGB parser should be able to distinguish between these, based on combination of number of fields, and presence and position of the strand field. For use in IGB, EGR version 3 is dependent on prior loading of annotations with matching ids.

Examples

- Format 1:# genome_version = H_sapiens_Apr_2003
- 1. score0 = A375
- 2. score1 = FHS gene1 chr22 14433291 14433388 + 140.642 175.816 gene2 chr22 14433586 14433682 + 52.3838 58.1253 gene3 chr22 14434054 14434140 + 36.2883 40.7145
- Format 2:# genome_version = H_sapiens_Apr_2003

```
    score0 = A375
    score1 = FHS
chr22 14433291 14433388 + 140.642 175.816
chr22 14433586 14433682 + 52.3838 58.1253
chr22 14434054 14434140 + 36.2883 40.7145
```

- Format 3:(assumes annotations with the names gene1, gene2, and gene3 are already loaded.)# genome_version = H_sapiens_Apr_2003
- 1. score0 = A375 2. score1 = FHS gene1 140.642 175.816 gene2 52.3838 58.1253 gene3 36.2883 40.7145

Zooming and panning the display

Introduction

To make it easy for you to move through a genome, IGB implements a visualization technique called animated one-dimensional semantic zooming.

This means we provide separate controls for vertical and horizontal zooming - the "one-dimensional" part. "Semantic zooming" means that when you zoom in or out, objects on display change their appearance depending on the zoom level. Also, when you zoom in or out, the position of your last click (indicated by the zoom stripe graphic) stays put while the display stretches or contracts around it.

As a result, zooming in IGB evokes the feeling of effortless flight through a genome; and just as importantly, you can easily adjust the zoom level to match the genes, sequence reads, or other items you're investigating.

One you've set the zoom level, you can use IGB horizontal and vertical scrollbars to move the display right and left or up and down. IGB also features a move tool that lets you move in any direction.

Panning the Display

- Introduction
- Grab tool
- Scrollbars
- Click-drag left or right
- Arrow toolbar shortcuts
- Auto Scroll

Introduction

Moving the IGB display without changing zoom level is called "panning," a term borrowed from film. Panning is also called "scrolling."

When you are zoomed in, only part of your data are visible and much of it is off-screen. The data display area is like the viewfinder of a camera that only shows you part of the surrounding scene. Use IGB's panning controls to move the data display area and view a different part of the genomic data scene.

Because moving through the data scene is an key aspect of data exploration and analysis, IGB offers many ways to pan the display.

To pan the display in IGB, you can

- Click-drag using the Move tool
- Use the horizontal or vertical scrollbars

- Click-drag the selection tool right or left
- Click the toolbar panning shortcuts
- Turn on Auto scroll

Grab tool

The **Move** tool allows you to move in any direction by click-dragging the display.

To use the Move tool



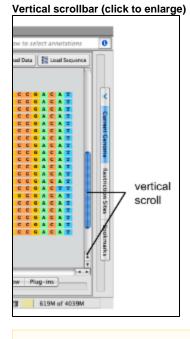
Select the Move tool
 Click and drag the display to move in any direction

Scrollbars

The horizontal and vertical scrollbars move the display up and down, similar to how scrollbars function in other applications.

To pan the display using the scrollbars, click the gutter or click-drag the thumb from left to right. You can also click the ends of the scrollbar.

Horizontal scrollbar (click to enlarge)				
	AT4G19110.1 AT4G19110.2 AT4G19112.1 BIBBBBBBBC 4 C	AT4G19120.1	AT4619130.1	
10,450,000		10,460,000	10,4	
Sliced View	Craph	External View	Plug-ins	
			576M of 4039M	
	horiz	ontal scro	oll	



Horizontal scroll works best when you're zoomed out.

Click-drag left or right

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Click-drag the Selection tool into the left or right side of the data display area to move left or right.

To move left or right using the Selection tool



- 2. Click and hold the mouse in an empty area of the data display area
- 3. Drag the mouse into the left or right edge of the display
- 4. Release the mouse to stop scrolling

Arrow toolbar shortcuts

You can use the right and left arrow shortcuts to pan left or right.



Auto Scroll

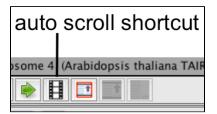
Use Auto Scroll to scan through large regions at medium zoom levels. IGB will move through the image, from left to right, at a speed you define.

To activate autoscroll

• Click the Autoscroll icon in the toolbar

or

• Select Tools > Start Auto Scroll.



To set auto scroll speed

- 1. Select Tools > Configure Auto Scroll...
- 2. Enter values
- 3. Click Apply to apply the new speed settings
- 4. Click Start to start auto scroll

Auto Scroll configuration window

OOO Au	toScroll F	Parameters	
Scroll increment (p	ixels)	4	
Time interval (milli	seconds)	20	
Megabases per min	ute:	0.528	
Total minutes for s	eq:	35.19897	
Can	cel (Apply	Start

Zooming

Introduction

- Zoom Stripe
- Horizontal zooming
- Vertical zooming
- Vertical zooming examples
- Jump zooming

Introduction

Zooming means: changing the scale of the display. It is essential to be able to change scale quickly when viewing and analyzing genomic data because the scale of what you need to view can vary by many orders of magnitude. You will find that as you work with large genomic data sets, you often need to "zoom" from chromosomes to exons and back again.

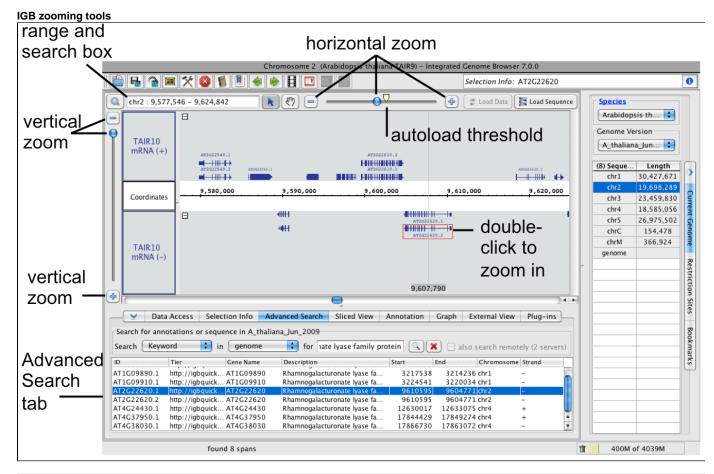
To make exploring data easier, IGB implements a visualization technique called animated, one-dimensional semantic zooming. Animated zooming means that during zooming, the scale of the display changes in real time as you operate IGB's vertical and horizontal zoom sliders. Semantic zooming means that the appearance of data objects changes depending on how much area they occupy and the scale of the display. One-dimensional zooming means that you can change horizontal and vertical scaling independently from each other.

When you use the zooming sliders in IGB, the display appears to stretch or contract in real time about a fixed location, called the "zoom focus," which you set by clicking the display. This helps you keep track of your location during zooming. The zoom stripe, which is unique to IGB, marks the horizontal position of the zoom focus and helps you keep track of your location during zooming. It also serves as a pointer at very high zoom levels, making it easy to point out individual bases or compare edges of overlapping objects.

Because changing scale is such a critical element of exploring a genomic data set, IGB provides many tools for zooming, including:

These include:

- Zoom sliders and buttons
- Keyboard and mouse wheel shortcuts
- · Double-clicking to zoom in on a feature
- Click and drag the coordinate axis to zoom in on feature
- Searching to zoom in on a gene



The autoload threshold icon shows the zoom level at which data sets with Auto load mode automatically load into IGB.

(i)

Zoom Stripe

The **Zoom Stripe**, a semi-transparent vertical bar in the image, marks the current focus of zooming, typically the last place you clicked in the display. The zoom stripe label at the bottom of the display indicates its genomic location.

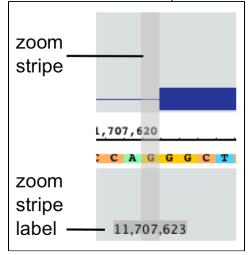
When you zoom horizontally, the zoom stripe remains in place as the display stretches or contracts around it. To set the zoom stripe position

• Click a location in the IGB data display area

To hide or show the zoom stripe or the zoom stripe label

• Select View > Show Zoom Stripe or View > Show Zoom Stripe Label

Zoomed-in view of the zoom stripe



Horizontal zooming

Use the horizontal zoom slider to change the scale of the display in real time, or click the "+" and "-" buttons to the right and left of the zoom slider to zoom in small increments.

You can also use keyboard shortcuts for horizontal zooming. Default keyboard shortcuts for zooming include:

- META SHIFT RIGHT zoom in horizontally
- META SHIFT LEFT zoom out horizontally

Vertical zooming

Use the vertical zoom slider to stretch or compress the display in the vertical dimension. Zooming vertically will expand or compress the vertical dimension of the entire display, with one exception: If you have locked the size of a track, its height will remain the same.

Default keyboard shortcuts for vertical zooming include:

- META SHIFT DOWN zoom in vertically
- META SHIFT RIGHT zoom out vertically

To change keyboard shortcuts, select File > Preferences > Toolbar.

You can also click the buttons flanking the vertical zoom slider to zoom in or out.

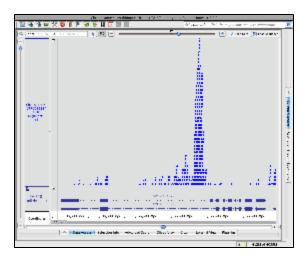
Vertical zooming examples

The following examples demonstrate how you can use vertical zooming to stretch or compress a track.

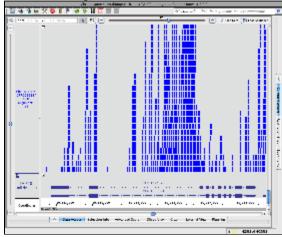
These images show the AG locus of Arabidopsis thaliana and a track containing read alignments from a ChIP-Seq experiment investigating binding sites for LFY, a transcription factor that regulates this locus.

In each example, the height of the track containing reference gene models (TAIR10 mRNA) is locked and the vertical zoom settings are different. Note how in the second example, the vertical zoom stretches the LFY IP track but does not affect the locked gene models track. (To hide or show the lock indicator, clear **View > Show Locked Track Icon**.)

No vertical zoom



Vertical zooming



Annotation tab settings for gene models track

(Select All) (Clear Track) (Save Track) (Delete Track) (Restore Default) (Other Options.,)
Style Foroground Frack Label Track Label Fone: 14 0	Stack Height 2 (ADDIV) (Optimize) -Track Height U Lock Track Height (Pools)	Labels and Strand Label Teld: 10 2 Strand: Strand: Calor by Strand: Calor by Strand:	Operation Single-Track: Copy # (Apply) Multi-Track 1 (Apply)

Jump zooming

Often, you'll want to jump to a region or feature.

To jump-zoom to a feature:

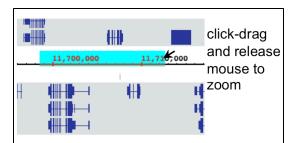
• Double click on it

To jump-zoom to a region:

- Select the selection tool
 Click-drag the select tool along the coordinates axis; you should see a blue box indicating the selected region
- 3. Release the mouse button

The display will "jump" to the region you selected.

Jump zooming



Selecting Items

- Introduction
- How to select an item (or items) in IGB
- Selecting items activates edge matching

Introduction

Most objects in the IGB display can be selected by clicking on them. These include annotations, graphs, results from search sequence, and entire tracks.

When an item is selected, IGB draws a red box around it.

IGB also supports selecting and deselecting more than one item at once.

How to select an item (or items) in IGB

To select items, click the pointer icon to activate the selection tool:

The following are ways to select items in the Main View window using the selection tool; selections are outlined in red.

To do this:	Do this:			
Select a single annotation or feature.	Click it.			
Select a multi-exon gene model	Click a line connecting exons or on the label (if active).			
Select a single-exon gene model	Click an exon, right-click and choose Select parent.			
Add to the current selection	SHIFT-click to add individual items or shift and drag to add multiple items.			
Select multiple items in the display	Click-drag the mouse over all items you wish to select. Start the drag in an empty area. SHIFT-click or SHIFT-drag to add to current selection.			
	O Click-dragging selects entire annotations rather than individual parts.			
	IGB reports the number of selected items in the Selection Info box. Use this to count aligned sequences in an alignments track.			
Deselect 1 item from a group selection.	Type SHIFT+CNTRL and click the item you want to de-select			
Select a sequence region.	Click-drag over the sequence bases you want to select. To select residues, begin dragging in the axis tier.			
Deselect everything.	Click an empty region anywhere in the main view.			
Select a floating graph.	Click its handle - the rectangle at the left inside the data display area			
Select an entire annotation track.	Click its track label in the left-hand panel.			

Selecting items activates edge matching

When you select items in the display, the edges of the selected item and all other items with identical edges are highlighted.

This feature helps in comparing splice variation, exon length, read location, etc.

You can change the edge match color or turn it off. See Other Options.

Using the mouse to select or move

Selection and Move modes

You can use the mouse to select items or move the display, depending on which mouse mode you've selected. In **Move** mode, click-dragging on the main data display moves it from side to side, or up and down. In **Selection** mode, clicking or click-dragging on the display selects items, allowing you to find out more information about them, run Google searches, and more.

To set the mouse mode, click the Selection Tool or the Move tool buttons:



To change to **Selection** mode, click the **Selection** tool. You can now select items in IGB by clicking or click-dragging. You can also jump-zoom to a region by click-dragging a region of the sequence axis.

To change to Move mode, click the Move tool.

Shortcut

To temporarily switch between mouse modes, click and hold the **ALT** button. When you release the mouse button, you'll return to the currently selected mouse mode.

Right-click menus

- Introduction
- Right-clicking annotations
- Right-clicking alignments
- Track labels
 - Annotation track right-click menu
 - Short read (alignment) track right-click menu
 - Graph track right-click menu

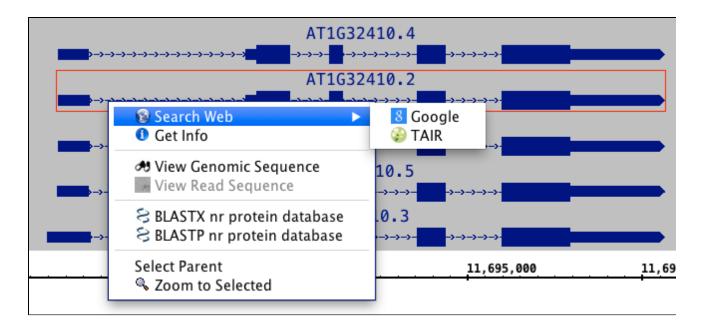
Introduction

IGB displays context menus when you right-click track labels or items in the data display area. These menus are called "context menus" because the menu options you'll see depend on what you clicked.

Right-clicking annotations

When you right-click an annotation, you'll see a context menu with the following options:

- Select Parent selects the feature of which the right-clicked item is a part. For example, if you right-click an exon and choose Select Parent, the entire gene model is selected.
- Zoom to Selected zooms to the right-clicked item. Choosing this option is the same as double-clicking.
- View Genomic Sequence loads genomic sequence (if not already loaded) and displays it in a new Sequence Viewer window.
- Search Web opens your web browser to Web pages that have more information about the selected model.
- Get Info displays the Selection Info panel containing information relevant to your selection.
- BLASTX nr protein database searches a non-redundant protein database using a translated nucleotide query.
- BLASTP nr protein database searches a non-redundant protein database using a protein query.



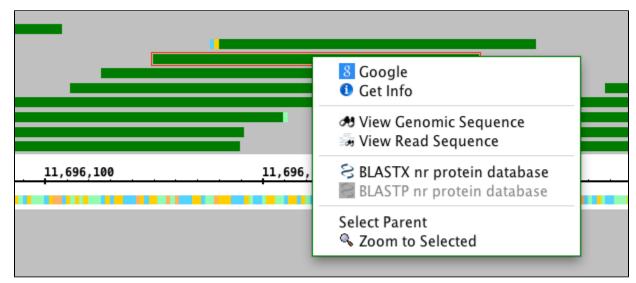
Right-clicking alignments

When you right-click an alignment, you'll see the same options as in the preceding section plus new options specific to alignments. Context menu items for alignments include:

View Read Sequence opens the aligned sequence in the Sequence Viewer.

Restore Read sequence superimposes the read sequence on the alignment blocks. Ordinarily, if the genomic sequence has been loaded, only mismatched bases are drawn.

For more information about alignments, see Visualizing read alignments.



Track labels

Right click a track label to view options and operations.

The menu you see will vary depending on the track type.

Annotation track right-click menu

TAIR10 mRNA (+ -)	TAIR10 mRNA Optimize Stack Height Set Stack Height Hide Show Collapse Customize Show mismatched bases Shade base by quality Track Operations Color by Filt	AT1G18730.4 AT1G18730.3 AT1G18730.2 AT1G18730.2 Copy Depth Graph (All) Depth Graph (Start)
Coordinates	Filter Save Selected Annotations as File Save Track As #S	Not Summary
	Clear Data Delete Track	

• Optimize Stack Height Set stack height so that all available annotations in the currently visible region are shown.

- Set Stack Height Open a window that allows you to set the stack height.
- Hide Hide the track.
- **Show** Show hidden tracks.
- **Collapse** Compress the track so that all annotations occupy a single row.
- Customize Open the Preferences > Tracks tab window.
- Track Operations Perform operations on a track. See Annotation track operations
- Color by Score Change annotation color depending on score
- Set Range of Color by Score Configure color options.
- Save Selected Annotations as File... Saves selected annotations to a file
- Save Track As... Save all the data in the track as a file.

Short read (alignment) track right-click menu

RNA–Seq (+ –)	RNA-Seq Optimize Stack Height Set Stack Height Hide Show Collapse Customize ✓ Show mismatched bases Shade base by quality	
	Track Operations	Сору
	Color by Filter	Depth Graph (All) Depth Graph (Start) Find Junctions
	Save Selected Annotations as File Save Track As #S Show as Paired	Find Junctions (TopHat) ► Mismatch Graph Mismatch Pileun Graph
Coordinates	Clear Data Delete Track	Not Summary

Graph track right-click menu

_	-5,000	
Cold	Cold Optimize Stack Height Set Stack Height Hide Show Collapse Customize	
Contro	Track Operations Color by Score Set Range of Color by Score Save Selected Annotations as File Save Track As	Copy Inverse Inverse Log 10 Inverse Log 2 Inverse Natural Log Log 10
		Log 2 Natural Log →→→ Power0 Sqrt →→→
		AT1G43170.4

Tabbed panels

- Introduction
- Hide or show tabs
- Changing tabbed panel locations

Introduction

IGB tabbed panels provide an easy-to-use way to access multiple functions. Each tab contains information and/or functions involving loading data, exploring data, working with graphs, etc.

By default, the tabbed panels are part of the IGB main window, but you can also display them in separate windows. You can also change their orientation or location within the main IGB display.

Hide or show tabs

To hide or show tabbed panels

- Click arrow at left end of bottom tabbed panels
- Click arrow at top end of side tabbed panels

To show a tabbed panel

· Click its tab

When you first launch IGB, the Data Access and Current Genome tabbed panels are open by default.

Changing tabbed panel locations

To open all or some tabbed panels in a new window or change their location, select the Tabs menu.

Tabs menu (click to enlarge)

Tabs	Bookmarks Help		
Curi	rent Genome	•	
Exte	rnal View	►	
Data	a Access	•	✓ Bottom
Slice	ed View	►	Window
Ann	otation	►	Hide
Adv	anced Search	►	
Gra	oh	►	
Boo	kmarks	•	
Sele	ction Info	•	
Plug	–ins	•	
Rest	riction Sites	•	
	n Current Left Tab in New Window		
-	n Current Right Tab in New Window		
Ope	n Current Bottom Tab in New Window		
Ope	n Left Tabbed Panes in New Window		
	n Right Tabbed Panes in New Window		
Ope	n Bottom Tabbed Panes in New Window	N	
	•		

Data Access

- Introduction
- Data Access sub-panels
 - Available Data
 - Data Management table

Introduction

This tab provides an interface for loading and configuring data sets from local files or from data source listed in the Available Data sub-panel.

Use the Data Access tabbed panel to

- open or close data sets listed in the Available Data sub-panel
- get more information about available data sets or data source
- manage track appearance and loading behavior

Data Access	panel ((shown	in its	own	window)
Dulu A00000	punci	(5110 1111		~	minaon,

O O O Data Access									
Available Data - Configure		Da	ta M	lanag	jeme	nt Tal	ole		
▶ ■ IGB Das (DAS2)				FG	BG	+/-	Load Mode	Track Name	
V G IGB Quickload (Quickload)		2	۹				Genome	RefGene	×
Cytobands		4	۲				Genome	Cytobands	×
RefGene 1									
mRNA 🔨	^								
EST 🟮									
UCSC (DAS)									

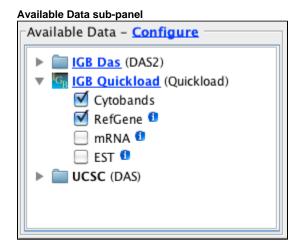
Data Access sub-panels

Available Data

The Available Data sub-panel provides access to data sets hosted on IGB data source sites, including IGBQuickLoad sites, DAS1 sites, and other compatible data servers.

- · Click the checkboxes to load or unload data sets hosted on IGB data sources
- Click the "i" icons to get more information about a data set

- Click Data Source links (e.g., IGB QuickLoad) to visit the data source in your Web browser
- · Click Configure to add or remove data servers (opens the Data Sources tab in the Preferences window)



Data Management table

Because many data sets are very large, IGB allows you to control how and when data from a file or data set loads. The Data Management table allows you to set the IGB Load Mode, hide or show tracks, delete tracks, and configure track annotation and background colors, and combined or separate plus and minus tracks. Click in the column labeled **Track Name** to edit a track name.

Possible load modes include:

- Manual Data sets load into the viewer only when you click the Load Data button
- Genome Data sets load all at once
- Auto Data sets load only when the zoom level exceeds the threshold indicated by the autoload threshold marker on the horizontal zoomer

Γ	Data Management Table											
			FG	BG	+/-	Load Mode	Track Name					
	47	۲				Genome	RefGene	×				
	47	۲				Genome	Cytobands	×				
2												
L												

(i) You can halt data loading by clicking the stop loading icon on the bottom left of the IGB window

Selection Info

- Introduction
- · View information about selected items
- View or copy table contents

Introduction

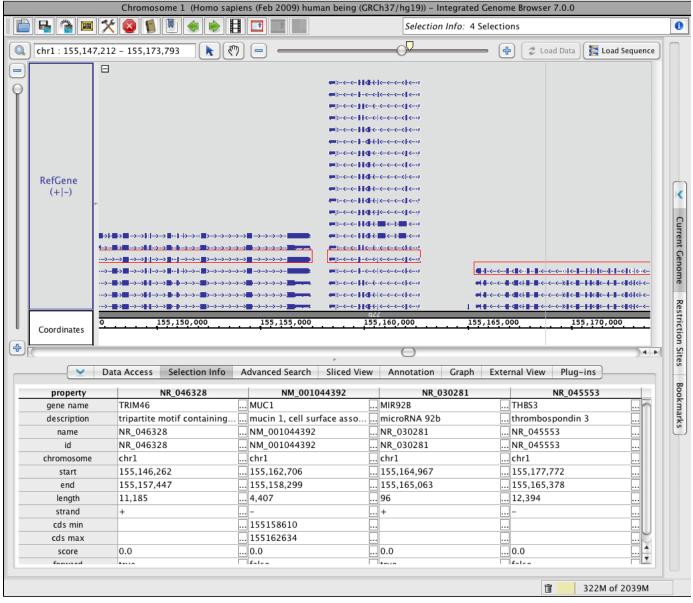
The **Selection Info** tab shows information about selected items in the display, including tracks and features within tracks. Information is shown in a table where each column in the table represents a selected item.

View information about selected items

To use the tab

- Select one or more items in the main window.
- Click the Selection Info tab

Selection Info tab with four items selected



View or copy table contents

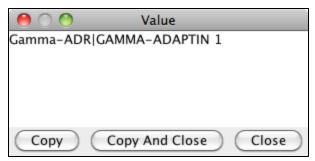
To view and copy the contents of individual cells

Click the '...' icon

This will open a new window showing the cell contents.

To copy data from the cell

Click button labeled Copy or Copy and Close



Advanced Search

- Introduction
- Search types
 - Search by ID or Name or Keyword
 - Searching sequence
 - Searching for multiple residues simultaneously
- Regular expression and wild cards

Introduction

Use the Advanced Search tab to search sequence residues or annotations. Both search types support regular expressions and wild card characters.

Using advanced search you can

- · look up genes or other annotations by keyword, name, or id
- find for instances of a transcription factor binding sites
- display locations of PCR primers

Search results will appear in the Advanced Search results table. Double-click a row in the table to view the result in the main IGB window.

If you search for sequence, IGB will also display color-coded bars in the coordinates track indicating the matched sequence.

Search types

The Advanced Search supports:

- **ID or name** find features by name
- Keyword find features by keywrod
- Residues find patterns in genomic sequence

Search by ID or Name or Keyword

To find a feature or annotation by ID or name

- 1. Select IDs or names from the Search menu.
- 2. Choose "genome" or a chromosome to search from the in menu.
- 3. Enter ID or gene name you want to find (for textbox).
- 4. Press <Enter> key or click **Search** button

Only data already loaded into the IGB viewer will be searched unless also search remotely is checked.

IDs or Names search will search IDs and names of annotations.

Keyword search, similar to ID or name search, will search annotation names, but it will also search other information associated with annotations, such as descriptions or other attributes. Choose Keyword to search descriptions, names, ids, and other attributes.

Results will appear in the Search Results table. Double-click a row in the table to zoom to that feature.

Advanced Search tabbed panel after searching the human genome

	Data Acces	ss Selection Info	Advanced Search Sliced View Annota	tion Graph	External View Plug-ins	
Search for annota	ations or sequence in I	H_sapiens_Feb_20	09			
Search IDs or r	names 🛟 in geno	ome 🛟 for	r ada		🔍 🗶 🗹 also search	remotely (1 server)
ID	Tier	Gene Name	Description	Start	End Chromosome	Strand
NR_033387	http://igbquickload.		ADARB2 antisense RNA 1	1568824	1599179 chr10	+
NR_036460	http://igbquickload.	ADAT1	adenosine deaminase, tRNA-specific 1	75657221	75632246 chr16	
NR_036636	http://igbquickload.	ADAM1A	ADAM metallopeptidase domain 1A, pseud	112336866	112339706 chr12	+
NR_037933	http://igbquickload.	ADAM20P1	ADAM metallopeptidase domain 20 pseud	70950473	70935595 chr14	
NR_038264	http://igbquickload.	ADAMTS9-AS2	ADAMTS9 antisense RNA 2	64670545	64997143 chr3	+
NR_048577	http://igbquickload.	ADAM15	ADAM metallopeptidase domain 15	155023747	155035252 chr1	+
NR_048578	http://igbquickload.	ADAM15	ADAM metallopeptidase domain 15	155023747	155035252 chr1	+
NR_048579	http://igbquickload.	ADAM15	ADAM metallopeptidase domain 15	155023747	155035252 chr1	+
Search for *ada	* on genome: 141 loca	al matches, 1936 r	emote matches			

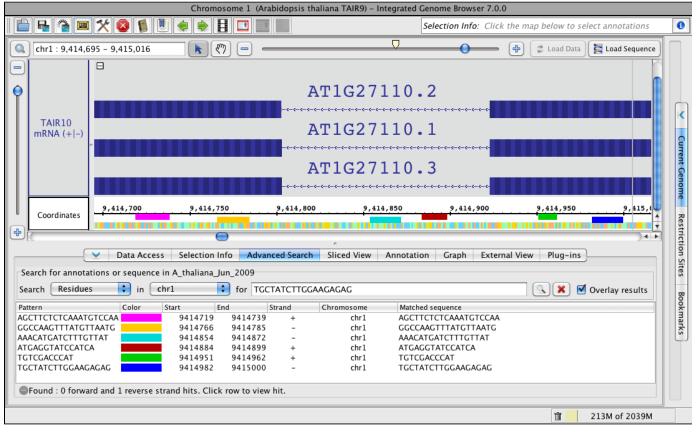
Searching sequence

To find all instances of a sequence or regular expression

- 1. Select Residues from the Search menu
- 2. Choose chromosome to search from in menu.
- 3. Enter sequence you want to find (for textbox).
- 4. Check overlay results to preserve results from previous searches
- 5. Press <Enter> key or click Search button

IGB displays matches in the results table and as colored bars underneath the coordinates axis. Matches on the minus strand appear in a slightly lower position.

Residue search results.



Searching for multiple residues simultaneously

To search for multiple residues simultaneously enter residues in advanced search box separated with the pipe (|) symbol.

Example: atgttc|atggc

This will return a search for atgttc and atggc separately.

Regular expression and wild cards

IGB searching supports regular expressions and wild cards. This is especially useful when searching for sequence motifs, such as transcription

factor binding sites.

Example queries:

Pattern	Represents	Example	Finds
	any single nucleotide	ACCT.T	ACCTTT, ACCTAT, ACCTGT, and ACCTCT (4 possibilities)
	any two nucleotides	ACCTT	ACCTAAT, ACCTATT, ACTAGT, Etc. (4 x 4 possibilities)
[CG]	a C or a G	ACCT[CG]TC	ACCTCTC and ACCTGTC
T{1,n}	1 to n T's	ACGGT{1,3}C	ACGGTC, ACGGTTC, ACGGTTTC
T*	Zero or more T's	ACGGT*C	ACGGC, ACGGTTTTTC, Etc.
.*?	a string of any length containing any nucleotides	TCGGGGTTAA.*?CTGGACTC	Many possibilities.
.*	the longest possible string of any length containing any nucleotides	TCGGGGTTAA.*CTGGACTC	Differs from the search above in that the longest possible result(s) will be found
(?i)	use case-insensitive matching	(?i)A[CG]T	ACT, agt, acT, AGt, etc.

More information about regular expressions is available from http://docs.oracle.com/javase/7/docs/index.html.

Sliced View

- Introduction
- Display sliced view of a gene
- Sliced View options
 - Slice by Selection
 - Slice Buffer
 - Analyze ORFs
 - Min ORF Length
- Viewing deletions and insertions
 - Insertion example
 - Deletion example
- Showing ORFs and stop codons

Introduction

The **Sliced View** tab displays an alternative view of items from in the Main View. In the **Sliced View**, introns are shown as having the same size, set in the **Slice Buffer** box). The **Sliced View** also can show open reading frames (ORFs).

Sliced view example (click to enlarge)

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Display sliced view of a gene

To view a sliced view of a gene

- · Click the Slicked View tab to make it the active bottom tab
- Open the Sliced View tab in a separate window (this is optional) Tabs > Open Current Bottom Tab in New Window
- Click a gene model in the Main View
- Observe that the same gene model appears in the Sliced View but intronic regions are removed

Sliced View options

The bottom row of the Sliced View panel shows options for controlling Sliced View appearance.

Sliced View options (click to enlarge)

Slice By Selection	Slice Buffer:	100	Analyze ORFs	Min ORF Length: 10	0

Slice by Selection

This option controls when the **Sliced View** updates. Check this option to ensure that whatever is selected in the Main View is shown in the **Sliced View** display.

Slice Buffer

The **Slice Buffer** setting controls the size of introns drawn in the **Sliced View** display. The "buffer" consists of intronic sequence on the five and three prime ends of exons.

Analyze ORFs

Check this option to activate display of ORFs (open reading frames) underneath the Sliced View coordinate axis track.

Min ORF Length

This option controls the size of ORFs shown when Analyze ORFs is checked.

Viewing deletions and insertions

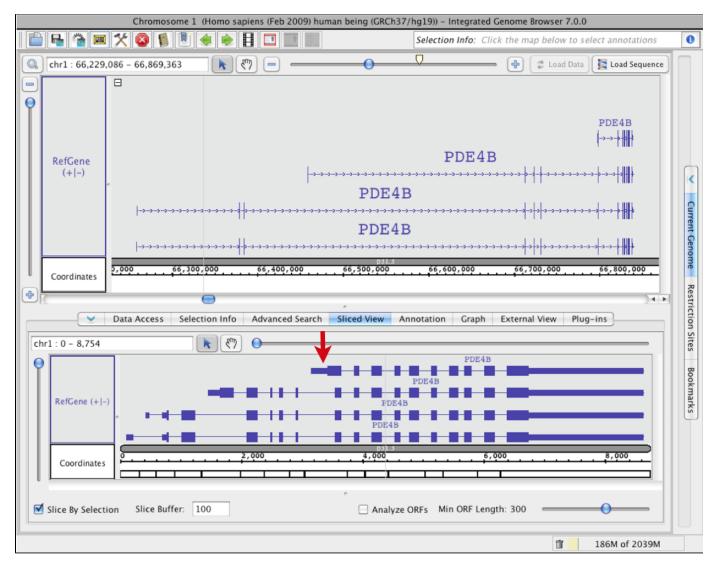
The Sliced View can help you see where sequences present in one transcript are absent from the others due to alternative splicing.

To examine alternate splicing using the Sliced View

- · select one or more transcripts in the Main View
- deletion icons (X characters) IGB draws on the other transcripts
- insertions are shown as larger introns

Insertion example

In this example, all four gene models were selected in the main view when the sliced view was created. In the sliced view, an arrow points to an exon that is present in all four models but is larger in the first one. Introns overlapping this exon in the other three models are larger.



Deletion example

In this example, the bottom gene was selected in the main view when the sliced view was created. An arrow points to an exon in the main view that is present in the middle gene model but absent in the other two. Since the third model which doesn't include this exon was used to create the sliced view, the skipped exon is indicated using an X character in the **Sliced View** tab.



Showing ORFs and stop codons

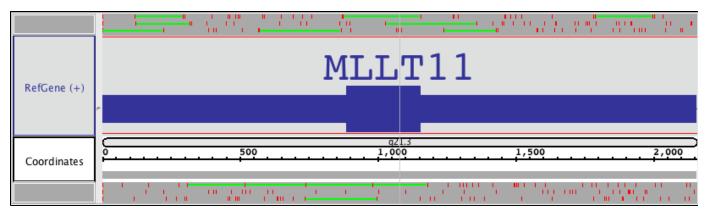
The Sliced View panel can show open reading frames and stop codons.

To visualize open reading frames and stop codons

- Select an annotation in the Main View
- Click the Sliced View tab
- Check the Analyze ORFs checkbox
- Use the Min ORF slider to adjust the mininum length of ORFs to show (in base pairs)

ORFs will appear for all six translation frames. Stop codons are shown as red marks. ORFs that are as long as or longer than the minimum ORF setting will be shown as green lines. Stop codon and ORF colors can be set using **Preferences > Other Options**.

Sliced View ORF display.



Annotation

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- Introduction
 - Changing track appearance
 - Style sub-panel
 - Stack Height sub-panel
 - Track Height sub-panel
 - Labels and Strand
- Operations

Introduction

The Annotation tab provides convenient access to annotation track configuration options and operations. To use Annotation tab,

- 1. Select one or more tracks by clicking track labels
- 2. Select Annotation tab
- 3. Change settings in Annotation tab sub-panels

Annotation tab

000	Bo	ttom Tabbed Panes			
Data Access Selection Info Advanced Search Sliced View Annotation Graph External View Plug-ins					
Select All	Select All Clear Track Save Track Delete Track Restore Default Other Options				
Style	Stack Height	Labels and Strand	Operations		
Foreground	3 (Apply) (Optimize)	Label Field: id 🛟	Single-Track:		
Background	Track Height	Change I.	Copy 🗘 (Apply)		
Track Label	Lock Track Height (Pixels)	Strand: ☑ +/- ☑ Arrow	Multi-Track:		
Track Label Font:	Set Height (Pixels):	Color by Strand:	(Apply)		
18 🛟	Apply	⊠ + ⊠ -			

Changing track appearance

Annotation tab sub-panels on the left of the Annotation tab offer options for changing track appearance. The following sections describe track appearance configuration options and how to set them.

Click Other Options... as a shortcut to opening the Tracks tab in the Preferences window. Clicking Other Options is the same thing as selecting File > Preferences > Tracks.

Clicking Restore Defaults restores how a track appeared when first loaded into IGB.

Also see Tracks

Style sub-panel

Use the **Style** sub-panel settings to change track colors and track label font. Changing the background color changes background color for both track and track label. Changing the foreground color changes the color of annotations within a track and the font color of the track label. Changing the track label color changes the color of the track label only.

To change color and font settings

- 1. Select one or more tracks
- 2. Select options in the **Style** sub-panel

Stack Height sub-panel

Stack height is the number of annotations that can appear stacked in individual rows within a track. If a location in the genome contains more annotations than can be stacked in their own rows, IGB places them in a summary row. Annotations in the summary row are darker than annotations stacked in individual rows.

Change the stack height setting to change the number of annotations that can be stacked into individual rows.

To change stack height so that every annotation in the current view appears in its own row and no annotations are shown in the summary row

- 1. Select one or more tracks
- 2. Click Optimize

To change stack Height to a specific value

- 1. Select one or more tracks
- 2. Enter a new value in the Stack Height box
- 3. Click Apply or type ENTER

Track Height sub-panel

Beginning in IBG 7.0, you can specify the absolute height (in pixels) for one or more tracks. To specify the height of a track

- 1. Select one or more tracks
- 2. Select Lock Track Height
- 3. Enter a value in the Set Height box
- 4. Click **Apply** or type ENTER

To change the track height, Lock Track Height option must be selected.

Labels and Strand

Use the Label Field menu to specify which annotation property IGB display next to each annotation in a track.

To find out what properties are available to use as annotation labels

- 1. Select an annotation
- 2. Click the Selection Info button in the top right of the IGB window

A window will open listing annotation properties in the left column and their values in the right column.

To use a property's value as the annotation label in an annotation track

- 1. Select a track by clicking the track label
- 2. Select Annotation tab
- 3. Select the property from the Label Field menu

Use the Strand checkboxes to determine how strand will be indicated in a track. You can choose any combination of options in the Strand sub-pa nel.

Available options for indicating strand include the following:

- +/- Selecting this option combines plus and minus strand annotations into the same tier. De-selecting this option separates plus and minus strand annotations into separate tiers.
- Arrow Selecting this option causes annotations to be drawn with arrows pointing in the direction of transcription.
- Color by Strand Selecting this option causes annotations to be colored according to their strand. This option over-rides the foreground color set in the Style sub-panel.

Operations

You can perform operations on tracks by

- Click operations buttons in the top row of the Annotation tab
- Using the **Operations** sub-panel

The **Operations** sub-panel contains operations you can perform on one or more annotations tracks.

For details, see Annotation track operations.

The operations buttons in the top row of the Annotation tab include

- Save track contents to a file
 - Delete a track, which removes the track from the IGB data display area
- Clear a track, which removes data from a track without deleting the track from the IGB data display area

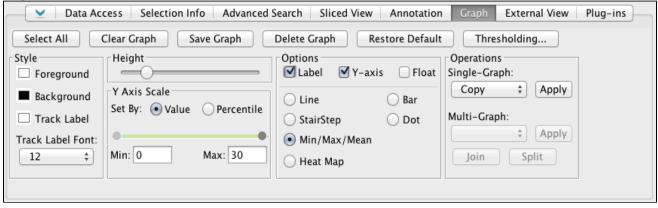
Graph

- Introduction
 - Changing the appearance of graphs
 - Graph Labels
 - Y Axis and Scaling
 - Floating Graphs
 - Change the height of a graph
 - Change graph styles
 - Change graph color

Introduction

Use the Graph tab to change graph appearance and perform operations on graphs.

Graph tab in IGB 7



Changing the appearance of graphs

Most graphs are similar to the annotation tracks; you can modify many properties of graph tracks in the same way you modify properties of annotation tracks.

To change Right-click on the track handle and choose from the menu. See Customize track appearance. Floating graphs do not appear in such a track. Settings and adjustments that are specific for graphs can be made using the **Graph** tab; to enable most of these options, a graph track must first be selected.

To change the settings for one or more graphs that are displayed in IGB:

- 1. Click the Graph Adjuster tab.
- 2. Select the graph(s) to change by doing one of the following:
 - a. Click the track label to select a graph.
 - b. Shift-click to select additional graphs.
 - c. To change the settings for all graphs that are currently displayed, click the Select All button in the Graph Adjuster panel.
- 3. Make changes to the graph settings by typing in new values or by operating sliders in the Graph Adjuster panel.

Any changes you make to the values in the Graph Adjuster panel will apply to all currently selected graphs.

Graph Labels

You can show the track name, and y-axis min, max on graphs by checking the Label option in the Manipulations panel.

Y Axis and Scaling

You can set the Y axis to be displayed by checking the Y Axis box. The axis markings will appear at the left side of the track.

The **Y-axis scale** can be altered using the **Y-Axis Scale** sliders. Scaling is an important issue to consider when viewing expression values because the distribution of these values across the range defined by their minimum and maximum may be highly asymmetrical. For example, the majority of values could range between 100 and 1,000, with just one or two percent exceeding 100,000. These extremely high values (relative to the rest) would tend to dwarf the others if displayed to scale because the vertical axis would be forced to show a range of 1 to 100,000. Although

technically accurate, such a graph would not be very informative because most of the information – the relative differences in value between different base pair positions – would be impossible to discern visually.

Setting minimum and maximum bounds **By Value** allow the user to set the cut-off values based on the actual **Y-axis** number. This does not take into consideration where most of the actual data lies. **By Percentile** offers thresholds defined by the actual percentage of data. For instance, using our theoretical 1 to 100,00, if you set **By Percentile** with **Max:** 90, and 90% of your data is below 1000, the **Y-axis** range would run from 0 to 1000. Any extreme values that are above or below the chosen thresholds are truncated from the display, and the values in between are displayed across a range that allows their differences to be noted.

To set these visible bounds, use the Y-axis scale section of the Graph tab:

✓ Data Access Selection Info Advanced Search Sliced View Annotation Graph External View Plug-ins					
Select All	Select All Clear Graph Save Graph Delete Graph Restore Default Thresholding				
Style Foreground	Height	Options Cabel Y-axis Float	Operations Single-Graph:		
Background	Y Axis Scale	⊖ Line ⊖ Bar	Copy ‡ Apply		
Track Label	Set By: • Value OPercentile	🔿 StairStep 🔹 🔿 Dot	Multi-Graph:		
Track Label Font:	Min: 0 Max: 30	• Min/Max/Mean	Apply Join Split		
12 +		🔾 Heat Map	Join Spire		

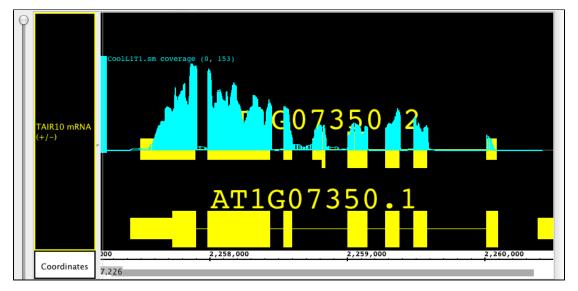
- To set specific values, select **By Value**. The chosen minimum and maximum value will be applied to each selected graph. You are free to set maximum and minimum values that cover a range smaller or larger than the actual range of your data. Either enter the number in the entry box or use the sliders.
- To set values by percentile, select **By Percentile**. Percentile ranges will be computed separately for each graph, which means that the absolute minimum and maximum range values for each selected graph may be different. Either enter the number in the entry box or use the sliders.

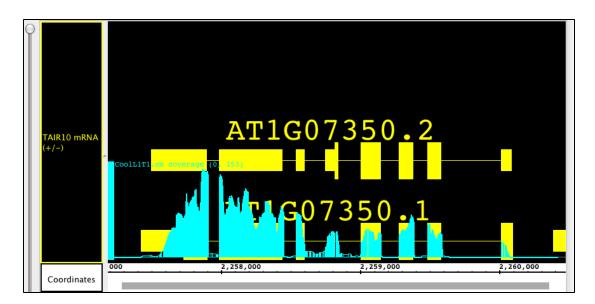
To display the full range of the data, choose By Percentile and set the Min to 0 and Max to 100.

Floating Graphs

In the **Options** panel, check the option **Floating**. By selecting this option, the graph track becomes 'disengaged' from the track framework. This allows a graph track to be moved any where (vertically) within the main image, AND overlay whatever it is placed over. By placing this quantitative track (graph) over or nearer to annotation tracks, you might find it easier to understand and analyze the data. If the graph is floating, you can change it's height by holding down the shift key and dragging your mouse up or down on the colored bar at the left end of the graph (this block is the 'graph handle').

To move or select a floating graph, click the graph-handle. In the pictures below you can see how the floating graph can be moved to overlay two different gene models, showing the alignment of exons and introns with the depth graph (.wig) from a next-generation sequencing experiment.





Change the height of a graph

There are several ways to change the vertical height of a graph. Select the graphs you want to change by clicking on their track label, then drag the **Height** slide bar in the **Graph** tab. Alternatively, move your cursor to the boundary between track labels, and shift the edge of the track label up or down.

Change graph styles

Graphs can be shown in various representational styles; the type of graph that is most appropriate depends on the type of question being asked about the data. For example, when comparing trends and patterns, it is very useful to use the line graph display method. The number of expression intervals being shown also can affect the graph display choice. The user is encouraged to experiment with the different display types to find out which method works best for specific purposes.

To change the graph style, select the graph track, then select the type of graph in the Style panel.

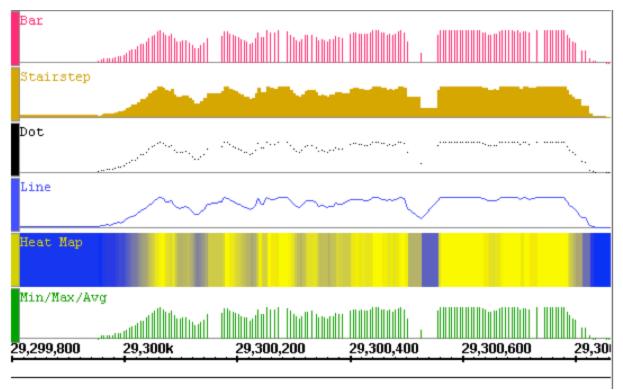
	Data Access Se	lection Info Search Sliced View Graph Adjuster	External View Plug-ins
Select All Save Selected Delete Selected Thresholding Style Foreground: Background: Bar Line Obt Min/Max/Avg Heat Map StairStep Black/White Image: StairStep Max: 14 Maipulations Manipulations Manipulations Image: Copy Max: M	Style Foreground: Background: Foreground: Stire Bar Line Dot Min/Max/Avg Heat Map StairStep	Height Y-Axis Scale By Value By Percentile Min: 0	Label Y Axis Floating Transform Copy Co Base: Operation Diff Co Go

The following graph styles are available in IGB:

- Bar -- Individual values are shown as vertical bars that are one base wide for position graphs and of variable width for interval graphs.
- Line -- Subsequent values are linked with a line. Even if the input file was not sorted, the values will be connected in order along the genomic coordinate axis.
- Dot -- Shows a single dot for each data value. For interval graphs, horizontal lines will be connect the start and end points.
- Min/Max/Avg -- This is the default style for most graph data types and is usually fine for most purposes. It is especially useful for showing very densely populated graphs with data points for large numbers of positions. When IGB is zoomed all the way in, the display is equivalent to the Bar style. When zooming out, IGB starts to summarize values. When the scale of the display reaches the point where individual x-values are associated with multiple score values, IGB picks the maximum and minimum values and draws a vertical bar between them. In addition, IBG draws lines through the average of all the data points represented at each x value.
- Stairstep -- Similar to the bar graph style, except that bar widths along the horizontal axis are stair-stepped. For example, if position 100 has a value of 50 and position 200 has a value of 75 and there are no values in between, then IGB will draw a bar of height 50 that starts at position 100 and stops at position 200. Then, at position 200, IGB will draw a new bar of height 75 that terminates at the next location with a value. This style is particularly useful for viewing .egr and .sgr files, ESTs, or other high-density data.
- Heat map -- Instead of showing relative intensity via the height of the line at each pixel or coordinate as in most other graph styles, a heat map shows expression levels via color or brightness of the line at each pixel or coordinate. This graph style is useful if you want

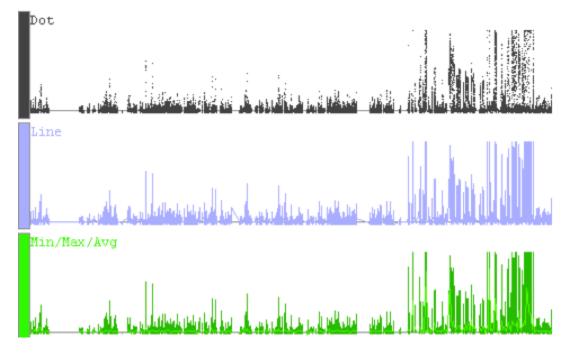
areas of greater expression to jump out at you. If a graph does not render or is hard to see, adjust the visible bounds of the graph until features are readily visible. Several heat map color maps are available to choose from. Transparent color maps are available and may be useful for floating graphs.

The following image illustrates how a single data set would look in each of the graph styles. The graph used for this image is a simple position graph. An example of an interval graph was given in the introduction to this chapter.



Note that with the heat map style for position graphs, the background (here in white) does not show through in regions where there are no data points. This is because the heat map first applies the stair-step function on the data before choosing colors to display. Compare the widths of the color bands in the heat map graph to those in the stair-step graph. For interval graphs displayed in the heat map style, the background will show through in regions with no data.

Due to the high zoom level in the above image, the Min/Max/Avg graph style is displayed exactly the same as the Bar style. When zoomed out, it reverts to showing the minimum, maximum and average values of all the data in the whole range of coordinates under each horizontal pixel width.



Change graph color

To change graph color, like changing graph style, select graph, then use the color boxes in the Style panel.

For graphs in the heat map style, the chosen color will only be used on graph labels and handles. The heatmap colors come from the chosen heat map.

External View

- Introduction
- · View a region in an external genome browser
- Import UCSC genoe browser settings
- Advice on region sizes

Introduction

Use this tab to view the currently visible region in the Ensembl or UCSC Genome Browsers.

Use this tab to

- See an alternative view on the same data
- Find tracks to download and import into the IGB environment

Both Ensembl and UCSC Genome Bioinformatics sites are accessible via DAS1 data sources in IGB. And the UCSC Genome site has a Table Browser that allows you to download Genome Browser data tracks in formats IGB supports.

So if you see something worth further investigation, you can import the data directly into IGB.

View a region in an external genome browser

Depending on the genome, you can use External View tab to view the currently visible region in the UCSC or Ensembl genome browsers.

To view a region

- Click External View tab to open it
- Choose external browser from the menu
- Click Update

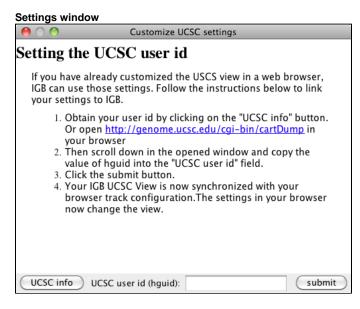
If you navigate to a new region, click the Update button.

External View showing TP53 in IGB and the Ensembl Genome Browser

		Chromo	osome 17 (Homo sapie	ns (Feb 2009) human bein	g (GRCh37/hg19	9)) – Integrated Genom	ne Browser 7.0.0	
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Import UCSC genoe browser settings

If you have used a web browser to access the UCSC server and have changed the UCSC view through the browser, you can import those settings into IGB, permitting IGB to display the view in the same way as the web browser. Click the **settings** button (blue box in previous image) and follow the directions in the window.



Advice on region sizes

While you can access information on very large regions (spanning 10-15 genes), a smaller region or specific gene may return more data due to more details being available. To use ENSEMBL you must have a fairly zoomed in picture (<100,000 bp) and it must be a species that ENSEMBL

curates. The same is true for UCSC, although it can handle slightly larger regions of the chromosome. A smaller region or specific gene may return more data due to more details being available.

Larger regions take longer to load.

Plug-ins

- Introduction
- How to install or un-install a plug-in
 - Adding new plug-in repositories
- Updating plug-ins

Introduction

Plug-ins are small programs you can install while IGB is running to add new functionality. Use the Plug-ins tab to find out what plug-ins are available, install or un-install them, update installed plug-ins, and add new plug-in servers.

How to install or un-install a plug-in

- Click the Plug-ins tab
- Click checkbox in Installed column

Plug-ins tab

istalled	Name	Description	Version	Repository
	TallyHandler 🚺	plugin to allow the user to load tally files	1.0.0	Bioviz
	TranscriptIsoform 🚺	BETA release of transcript isoform evidence visualization	0.0.9	
	TutorialHelper 🕕	plugin to allow the user to see widget ids to help develop tutorials	0.0.9	Bioviz
	SampleSelection 🕕	tab to allow the user to select sample data	1.0.0	Bioviz

Adding new plug-in repositories

To add a new plug-in repository

- Click Repositories
- Enter URL of the repository

Updating plug-ins

IGB shows you the version number of a plug-in in the Version column. The Update All button will become available (no longer be grayed out) if newer versions of a currently installed plug-in are available.

Click Update Selected or Update All to update selected or all plug-ins.

Geometric Mean

Primer Design

Tutorial Helper

Current Genome

- Introduction
- Species link
- Choosing species and genome version
- Choose a sequence to view
- View the entire genome

Introduction

Use the Current Genome tab to select the species, genome version, and chromosome to view in IGB.

Species link

Clicking the Species link (blue arrow) returns you to the IGB start screen.

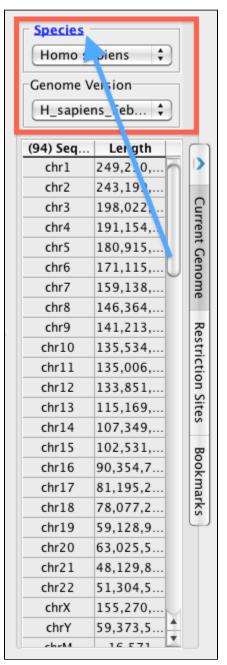
Choosing species and genome version

All species and genome versions supported by IGB data sources appear in the Species and Genome Version menus.

If you have added your own custom genome, it will also be listed here.

To view a new genome

- Select a species from the Species menu (red box)
- · Select a genome version from the Genome Version menu



Choose a sequence to view

The Current Genome tab contains a table listing chromosomes and chromosome sizes for the current genome version.

Use this table to sort chromosomes by name or size or to choose a new chromosome to view.

To change to a new sequence

• Click its row in the table

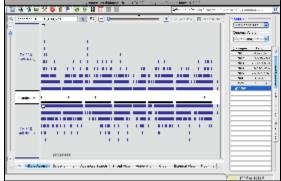
To sort by size or name

• Click the column title

View the entire genome

The chromosome table contains a row named **genome.** Clicking this row activates a whole-genome view in which all chromosomes are shown in IGB.

Whole genome view (click to enlarge)



Restriction Sites

- Introduction
- How to map sites
- Viewing results

Introduction

The Restriction Sites tab maps restriction enzyme sites within the reference genomic sequence.

Restriction Sites tabbed panel

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Avrll		
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Bbyl	T	Ŧ
	Map Sites	
\square	Clear	\supset

How to map sites

To display restriction sites for an enzyme (or group of enzymes)

- Click the enzyme name
- Click Map Sites

Multiple selections are possible by SHIFT- or CONTROL-clicking (platform dependent)

Viewing results

Sites are shown underneath the coordinate axis in the main view. To zoom in on a site, double-click it.

Zoomed-out view of sites recognized by Not1 (a rare cutter) mapped onto Arabidopsis chr1 (Click to enlarge)



Zoomed-in view of a single Not1 site (click to enlarge)

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Click the Clear button to remove sites.

Bookmarks

• Introduction

- · Bookmarks tab overview
- Returning to a bookmarked region
- Creating new bookmarks
 - Bookmark types
- Managing bookmarks
- Exporting and Importing bookmarks
 Viewing bookmark properties (for advanced users)
- IGB bookmarks file

Introduction

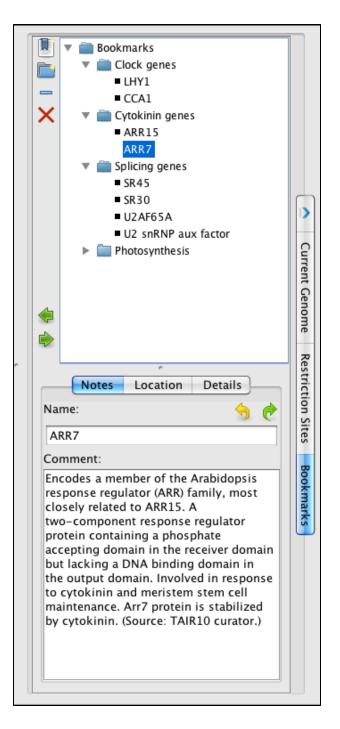
Use IGB bookmarks to:

- Jump to a bookmarked location and re-load data.
- Make notes on a genomic scene.
- Share analysis results with other users.

Bookmarks tab overview

The Bookmark tab allows you easy access to the bookmark list and provides tools for managing and sharing bookmarks.

IGB bookmarks tab with example bookmarks



Returning to a bookmarked region

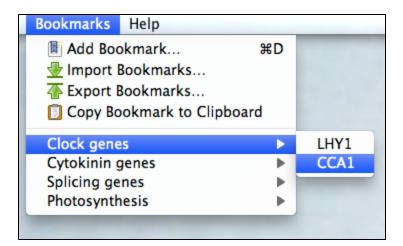
To return to a bookmarked region,

Double-click it

or

• Choose the bookmark from the Bookmarks menu

Bookmarks menu



Creating new bookmarks

To bookmark a location and data scene

- Choose Bookmarks > Add Bookmark
- or
- Click Bookmarks tab
- Click add the New Bookmark icon

A window will appear with places to enter a name, notes, and bookmark type.

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🗌 Use Defau	lt Name		
Name:			
ARR15			
Comment:			
Type–A response regulator; negative regulator of cytokinin signaling. RNA–Seq suggests it is up–regulated by cold.			
Position Only			
Position and Data			
	Cancel OK		

Bookmark types

Position Only bookmarks record genome, current position and zoom level.

Position and Data bookmarks record genome, current position, zoom level, and data sets.

Managing bookmarks

You can sort your bookmarks into folders and associated comments with comments on folders.

To edit the name or comments associated with a bookmark or folder

- Click Bookmarks tab.
- Click to select a bookmark or folder.
- Edit name or comments in the Notes sub-tab.

To delete a bookmark or folder

- · Click to select it
- Click the delete bookmark icon (red X) or press Delete

Exporting and Importing bookmarks

To save a copy of the bookmarks to a file

• Choose Bookmarks > Export Bookmarks...

To import another user's bookmarks

• Choose Bookmarks > Import Bookmarks...

Bookmarks are saved as a single file in an HTML-based format. Use caution if you choose to edit the file.

You can import multiple sets of bookmarks during the same IGB session. Imported bookmarks will be added to the end of the list of existing bookmarks.

Viewing bookmark properties (for advanced users)

To see when a bookmark was created and the region it refers to

- Click Bookmarks tab
- Click the bookmark to select it
- Click Location sub-tab

To see other properties of a bookmark

- Click Bookmarks tab
- Click the bookmark to select it
- Click **Details** sub-tab

or

- Click **Bookmarks** tab
- Right-click a bookmark
- Select Properties..

A new window will open that reports information about the bookmark and how IGB represents it internally.

To see how IGB combines these properties into an IGB bookmark, export your bookmarks and open the bookmarks file in a text editor to inspect bookmark syntax.

IGB bookmarks file

IGB saves your bookmarks at the end of each session in a file named bookmarks.html in a folder named .igb in your home folder.

Visualizing read alignments

- Introduction
- Open short-read alignment (BAM or SAM) file
 - Set the load mode (optional)
 - Manual (the default)
 - Auto
- Zoom in on a region and load data
- Load sequence data to view mismatches
- Adjust stack height

Introduction

Starting with IGB version 6.3, you can use IGB to visualize sequence alignment files alongside annotations and genome sequence.

IGB supports visualization of alignment files in

- BAM format (binary alignment format)
- SAM format (plain text version of BAM)
- PSLX format (output from the blat alignment tool)
- VCF (with the appropropriate plug-in)

As with other file formats, IGB can open files from your local hard drive, from a Web site, or from a QuickLoad data source.

If you are loading alignments from a Web site, IGB will first download your index (BAI) file and then use it to determine which part of the much larger BAM file to download whenever you click the **Load Data** button.

A Each BAM file requires an index file (BAI) located in the same location (same folder, same website, etc.) as the BAM file. SAM files do not require indexes.

Open short-read alignment (BAM or SAM) file

If your species and genome version of interest are available, first select it using menus in the Current Genome tab or click a species shortcut image on the IGB start screen.

Next, open the file. You can open a file in IGB by

- using File > Open File menu (allows you to enter a custom species and genome)
- using File > Open URL.. menu
- · click-dragging from your desktop into the IGB window
- click-dragging a URL from a Web page
- or loading from a QuickLoad server

When you first open a BAM file, a new empty track will appear in the main IGB window, labeled with the name of the file or the name of the data set if loaded from an IGBQuickLoad site.

A new row representing the newly opened file or data set will also appear in the Data Management Table in the Data Access tab.

If your genome of interest is not available, you can enter it in the file chooser window.

Set the load mode (optional)

Manual (the default)

When you first open a BAM file, its **Load Mode** will first be set to **Manual**, meaning that to load the data into IGB, you click the **Load Data** button. Also, only data for the currently shown region will load into IGB.

Before clicking the Load Data button, first zoom in to a region or search for a gene of interest.

Attempts to load data for large regions will prompt a warning from IGB.

Since alignment data sets are typically very large, it is a good idea to only load a few genes' worth of data, depending on the file and the experiment.

Auto

The Auto load mode setting triggers automatic loading of data when the display is zoomed in past the **Autoload** threshold, indicated by the yellow marker on the horizontal zoom control.

Drag the horizontal zoom control to the right of the Autoload marker to activate automatic loading of data sets.

To change the Autoload threshold to the current zoom level, choose View > Set Autoload Threshold to Current View

Zoom in on a region and load data

- Use the slider, double-click a gene model, run a search, or click-drag the sequence axis to zoom in.
- Click the Load Data button to load data into the display.

IGB will then load data for the currently visible region.

If genomic sequence data have not already been loaded for the current region, IGB will show the sequence bases of reads when zoomed in, as in the image below.

Read alignments (click to enlarge)

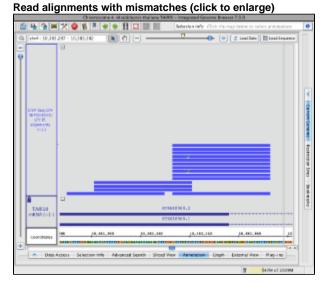
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Load sequence data to view mismatches

To view just mismatches between reads and the reference genome

• Click Load Sequence in View in the upper right corner of the IGB display

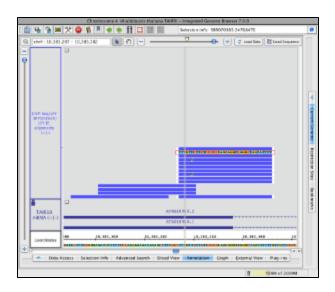
This will trigger loading of genomic sequence and allow IGB to show just the mismatches rather than the full sequence of each aligned read.



To restore read sequence

• Right click a read and select Restore Read Sequence.

Read alignment with read sequence (click to enlarge)



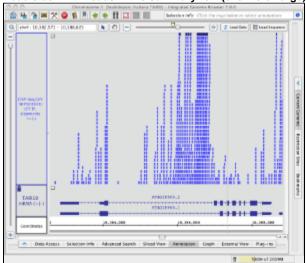
Adjust stack height

Note that in the image below showing read alignments from a ChIP-Seq experiment, there is a row of reads at the top of the track which appear darker than the rest. The darker row indicates there is some number of reads that are not being shown because they exceed the current stack height setting for the track.

The darker row of reads in the top row of the track are called the "summary row." To count them, click-drag the arrow cursor over the summary row. In this case there are more than 1,000 reads in the summary row.

The number of rows shown below the summary row is determined by the stack height setting. To change the stack height setting

· Right-click a track label and choose Set Stack Height or Optimize Stack Height



Read stack with reads in the summary row (click to enlarge)

The image below shows the same scene as in the image above after using the **Optimize Stack Height** feature. Note that the height of the annotation track (TAIR10 mRNA) is locked to ensure that increasing the read track's stack height would not affect the annotation track's height, which is set to 100 pixels.

Read stack with all reads showing (click to enlarge)

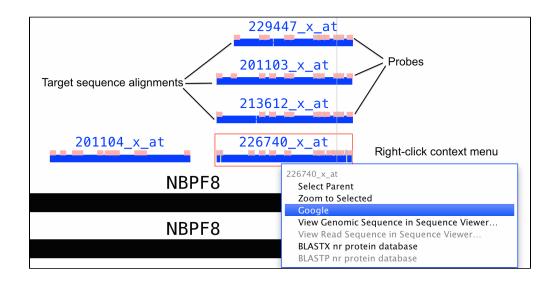
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Visualizing probe sets

- Probe set alignments link.psl files
- About probe set alignment visualizations
- Why this is useful

Probe set alignments - link.psl files

IGB can display Affymetrix probe sets aligned onto a reference genome - it can show probe set design sequences aligned onto a genome with the locations of the probes indicated as blocks.



When IGB was first developed at Affymetrix, the company distributed probe set alignment files for its catalog 3' IVT arrays. In recent years, however, they've stopped updating these files. So for some genomes, the alignment files you can find on the Affymetrix Web site reference obsolete reference genomes. If you need to work with more up-to-date genomes, we recommend you create your own alignment files or request them from the IGB team. For some genomes, we've added probe set alignments to the main IGBQuickLoad.org site. Mainly we've done this at the request of individual researchers, and so if you would like to request an array, let us know. If probe set alignments are available from our site, you'll typically find them in a folder named **Affymetrix** under the **Data Sources** section of the **Data Access** panel.

You can also make your own probe set alignment files using blat, tabix, and a python script we wrote. For more information, see this Bitbucket repository.

About probe set alignment visualizations

Depending on when the arrays were designed, Affymetrix typically used expressed sequences from GenBank to select probes for probe sets these expressed sequences were sometimes called "exemplar" or "consensus" sequences. They then selected individual probes from regions near the 3' end of the expressed sequence. Affymetrix (as of 2014) distributes probe and target sequences on their Web site, where "target sequences" contain the 3' end regions from which the probes were selected.

Probe set visualizations in IGB show the alignments of target, exemplar, or consensus sequences onto the genome. They also show the locations of probes that were selected from the design sequence. See the preceding figure for an example.

Because probes were selected from the expressed sequences, sometimes a probe will be shown as split across an intron. Also, sometimes probes overlap. And sometimes probes may be missing. If the target sequence contains a region that can't align onto the reference, and if this unaligned sequence contains a probe, then that probe will not be shown.

If you have questions about what you see in a probe set alignment, let us know.

Why this is useful

Often multiple, seemingly redundant probe sets interrogate one gene. This situation mainly arises when a gene has multiple, alternative three-prime ends due to alternative splicing or alternative termination sites. If an experiment identifies genes where redundant probe sets are differentially expressed with different fold-changes or in opposite directions, this can indicate that the treatment affects splicing as well as the overall abundance of RNAs arising from the gene.

Thus if you observe redundant probe sets that give different or contradictory results, it's a good idea to view them in IGB and compare their alignment to annotated genes and transcripts.

Sequence Viewer

Introduction

The Sequence Viewer gives users the option to view a region of sequence in a separate window with many options. The viewer will display the nucleotide sequence of selected sequence, exons, gene models, cytobands, or short reads. When you right-click a selected item in the display, such as a gene model, you'll see a menu offering **View Genomic Sequence**. Alternatively, you can access the same command from the **View** me nu.

The viewer also has a variety of ways to display protein translations, and the ability to copy/export nucleotide sequence, annotated nucleotide sequence, protein sequence, etc.

View Sequence

Œ

By default, the viewer depicts bases in yellow on a black background. Use the **Colors** menu to change the color scheme to **Blue on white** (exons in blue and introns in black)

Selected Sequence or Cytobands

You can look at the selected sequence highlighted in the refseq, or (if present) the sequence of DNA contained in a cytoband. While this method loses the advantage of annotations/ exon designations, it does allow you to select DNA from introns, upstream and downstream regions, or any other area which may contain sequence of interest (i.e. promoters, enhancer binding elements, etc.). Select the sequence you wish to view by click-dragging the cursor over the sequence bar at any zoom level. A red outline will appear around the selected sequence.

This method of genomic DNA selection also allows a direct copy to the clipboard, if you wish to skip the Sequence viewer. Simply right click within the selected sequence, and chose **Copy**.

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										• • •
Coordinates	16,228,980	16,229,000	16,229,020	16,229,040	16,229,060	16,229,080	16,229,100	16,229,120	16,229,140	16,229,16
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	~			Copy Selected	Sequence to Cli nic Sequence	pboard %C				

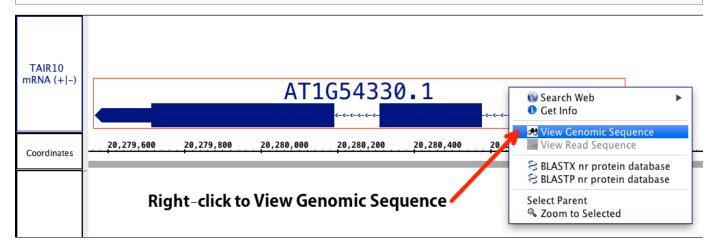
Right click within the selected area or open the **View** menu and chose **View Genomic Sequence**. The sequence viewer will open, and the title will show the genomic range of the sequence, the genome version, and the chromosome number. The **Show cDNA** option will be disabled, but all other options will remain. The DNA sequence will always be presented in the + (plus) direction. However, if your sequence of interest is the other strand, **Show > Complement** will provide the complement of the sequence (this is usable with all sequences).

The sequence viewer also allows you to save the reverse complement of the sequence you are viewing if you wish. Go to File > Save As FASTA (Reverse Complement).

Gene Models or Other Annotation

For gene models/ annotations, right click on the annotation or open View menu and choose option View Genomic Sequence.

To choose a single exon (part of) a gene model, right-click the exon. To choose the entire gene model, right-click any of the introns or the label, if available.



Choosing **View Genomic Sequence** opens the Sequence Viewer as shown below. By default, the viewer depicts exonic bases in yellow and intronic bases in white against a black background. The start codon (ATG) is shown with a green box around it, and the stop codon has a red box around it.



The title bar shows the name of the selected item. For instance, the image below shows genomic sequence for gene model AT1G54330.1, from the A_thaliana_Jun_2009 genome version, on chromosome 1.

0) 💮 👘			AT1G54330.1 : A_thaliana_Jun_2009 : chr1	
File	Edit	Show	Colo	lors Show cDNA	
	0 C1	TAATC	АСТТ	TTAATTAATCTCTCTCATCTCTTTCTCTCTCGTTTTCTAAC	ſ
	50 <mark>G</mark> (CATACA	астт	TT GAATGGCGCCCAT GAGTTT GCCTCCAGGATTCAGGTTTC	

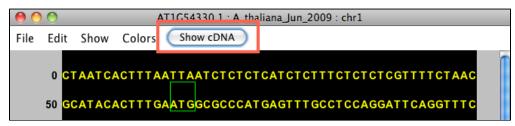
Short Reads/ Alignments

The Sequence Viewer has a special option for short reads. If you select a short read and select **View Genomic Sequence** you will see the genomic sequence that the read is aligning to. The option **View Read Sequence** will show the sequence of the read itself, including any inserted sequence. If a deletion is present, the deleted nucleotide(s) will appear as a dash (-).

Additional Features for Annotated Gene Models

Show cDNA

After selecting a gene model, the Sequence Viewer allows you to select **Show cDNA**, which will eliminate introns from the displayed sequence. We recommend selecting this option before using the translation feature. If an individual exon is selected, this button will be available, but will not change the sequence.



After selecting this feature, you'll see just the exonic (block) sequence, and the button will read Show Genomic.



Show translation

Translation options are accessed from the **Show** menu. **Reverse Complement** provides the reverse complement in place (changes what is displayed). The **Complement** provides the complement under the main sequence. The + (forward) translations appears over the sequence; the - (reverse) translations below. To copy these translations, see **Copy** below

Reverse Complement Complement
+1 Translation +2 Translation +3 Translation -1 Translation -2 Translation -3 Translation
All + Translations All – Translations

Copying and Saving Sequence

As mentioned, you can copy selected sequence to the clipboard directly from the main window of IGB. Additionally, you can copy a selection or all of your sequence from the sequence viewer. Highlight the sequence of interest (as shown below) and use **Copy > Copy Selected DNA Sequence**. Then open a text editor of choice and paste the sequence.

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	0	СТА	АТ	CA	стт	TA	ΔΤΤ		гст	ст	стс	ATC	тст	ттс	тст	стс	3 T T 1	гтс	ΤΔΔ	с	ſ
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You can also **Copy DNA with Lowercase Introns and Start/Stop Codons** (NEW!) which copies the DNA sequence with the annotations. When you paste this sequence, you will be able to determine intron and exon boundaries, and find the annotated start and stop codons within the nucleotide sequence. This can be very helpful for PCR primer design or alternative splicing studies.

Copy Annotated Translation (NEW!) will copy the annotated translation for the selected gene model - no highlighting needed.

Copy Selected Translation(s) (NEW!) requires that you **Show** 1-6 translations. The user then highlights the region of interest (possibly the whole sequence). This function then copies the translation(s). When pasted each translation is concatenated so that all of +1 shows together, then all of +2, then all of +3, etc. with a label indicating which translation is which.

You also have the option to save the sequence directly from the viewer into a fasta file. Fasta files can be shared and used for bioinformatics in this file format, but fasta files can also be opened and viewed from any text editor to access the sequence directly. The file will contain the sequence as well as a line with all of the relevant information from the title of the sequence viewer window. We allow you to save the sequence directly or in the reverse complement, as you need. Open File > Save As Fasta or Save As Fasta (reverse complement).

Finally, you can export an image of the viewer directly using the Save Image function.

Genome graph tracks

Introduction

Introduction

Genome graphs consist numerical data associated with base pair positions. You can open and view genome graph data files in IGB and manipulate them using functions in the **Graph** tab.

Using the **Graph** tab settings, you can display graphs as line graphs or heat graphs, change their scale, convert them to floating (draggable) graphs, perform different types of transformations, and even compute averages or sums of multiple graphs.

You can also create graphs from annotation tracks, such as an RNA-Seq coverage graph showing the number of read alignments starting at each position.

Examples of genome graphs include:

- · Probe intensity values from genome tiling arrays.
- · Percentage methylation across chromosomes
- GC content along chromosomes
- · Measures of conservation between two genomes

There are two basic types of genomes graphs:

- Position graphs associate scores with single genomic positions.
- · Interval graphs associate scores with ranges of genomic positions

Advanced Graph Manipulation

- Introduction
- Duplicating graphs
- Transforming
- Joining and Splitting graphs
- Combining Graphs Arithmetically

Introduction

IGB allows you to manipulate graph tracks in multiple ways, both by transforming an individual track or by combining two graph tracks in a mathematical way. Controls for transforming and combining graphs can be found in the **Graph** tab. Any track that has been transformed or combined can be saved as **WIG**, **BEDGRAPH**, or **EGR** (see File Formats for more details).

Data Access Selection Info Advanced Search Sliced View Annotation Graph External View Plug-ins								
Select All Clear Graph Save Graph	Delete Graph Restore Default	Thresholding						
Style Height Foreground Y Axis Scale Track Label Set By: • Value Percentile Track Label Font: Min: 0	Options Label Y-axis Float Line Bar StairStep Dot Min/Max/Mean Heat Map	Operations Single-Graph: Copy ‡ Apply Multi-Graph: \$ Apply Join Split						

Duplicating graphs

You may want to duplicate a graph to experiment with different graph adjustments without losing adjustments that you have already made. The first choice in the **Track Operations** drop down menu is **Copy** (see image of **Graph** tab), which makes a copy of the graph without any transformation. This can be useful if you want to explore the effects of showing one graph in two graph styles at the same time.

Select the graph, set Track Operations *to *Copy.

Transforming

By default, all graphs display in linear scale, but IGB allows you the option to transform a graph into other scales. Select the graph(s) and open the **Graph** tab. Select the type of transformation you would like to perform from the pull-down menu labeled **Single-Graph** in the **Operations** pan el.

The picture below includes all of the options available in IGB.

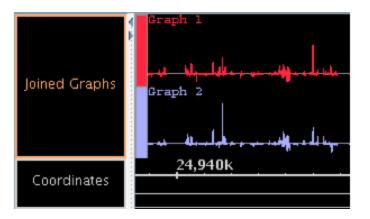
Inverse Ln	2
Inverse Log	\square
Inverse Log10	
Inverse Log2	
Log	
Log10	
Log2	
Natural Log	•

IGB duplicates your graph in a new track and applies the selected scale to the new graph; your original graph is not deleted.

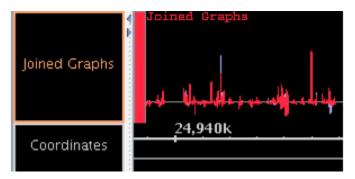
Joining and Splitting graphs

It is possible to join two or more graphs into a single track. The two (or more) graphs will then have a single track tier label and can be moved and adjusted as a group. To join multiple graphs, select them, then press the **Join** button in the **Operations** panel of the **Graph** tab. To separate these graphs, select the joined track and press the **Split** button. To switch between the expanded and collapsed state, right-click in the joined track label, and choose between **Expand** and **Collapse**.

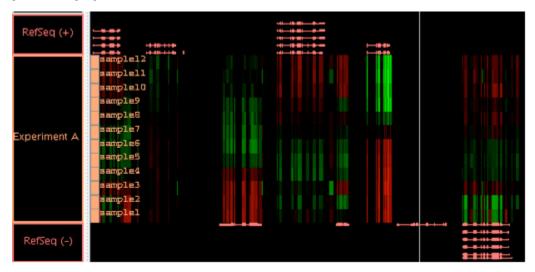
When the track is in the expanded state, the multiple graphs are shown in separate non-overlapping bands.



If the tier is collapsed, all graphs are shown in the same, overlapping band. When joined graphs are collapsed, any opaque regions of the top graph will hide corresponding features of the lower graph or graphs.



Joining graphs into a single annotation track can be very useful for expression data from multiple experiments. Here, data from several experiments at different times during the development of an organism are shown together in a single track, and it is easy to see that expression of genes is being regulated over time.



By selecting the single tier label, here labeled "Experiment A", you can easily set the graph style and data range for all these graphs to be identical.

Combining Graphs Arithmetically

IGB allows you to work with numerical/graph data, allowing you to do quantitative comparisons between data sets. This is especially useful for comparing experimental results between a control and treatment group, for instance. You may need to compare the relative differences in expression levels, or you may need to generate an average. For example, a graph of cancerous cells will show what's turned on or off when compared to a graph of normal cells. Results that show significantly more or less activity than a control graph for the same region may indicate an area of interest. While you can use the Combine Annotations functions to qualitatively locate regions of interest, combining graphs will give you quantitative answers.

IGB allows you to work with 2 graph tracks at a time. The first track selected will be given a temporary label of 'A'. Shift click selection of the second track will give it the temporary label 'B'. After the two tracks have been selected, you choose the mathematical function from the **Multi-Gra**

ph option of the Operations panel.

\checkmark	Diff
	Max
	Mean
	Median
	Min
	Product
	Ratio
	Sum

IGB will open an new track for the newly created graph. It will be titled as the name of the function performed, and then each of the original tracks in parenthesis. The new track can be adjusted for style and display just as any other graph track.

Applying thresholds to graphs

- Introduction
- The Thresholding Window
 - Direction
 - Changing the threshold
 - Specify offsets
 - Control gaps
- Make an Annotation Track of the Threshold Results

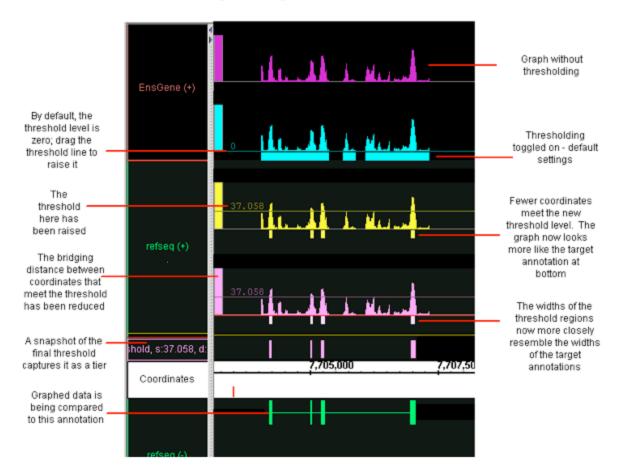
Introduction

A common analysis step is to determine meaningful regions on a sequence based on graph values being above or below a certain threshold. To screen out non-meaningful values and to view meaningful features of a graph as annotations, use the thresholding feature in IGB. Thresholding displays graph features as annotation-like bars in locations where the graph value at the coordinate meets your defined threshold.

You can convert this data into a standard annotation track in order to analyze it using all of the features in IGB for working with and comparing annotations.

In some cases, you may want to adjust your graph thresholds so the bars correlate to existing annotations; any threshold bars visible at that point that don't have known annotation counterparts (or vice-versa) may indicate areas for further investigation.

Use thresholding to compare graphed data with known annotations



The Thresholding Window

To use thresholding, select one (or more graphs). Click the **Thresholding** button in the **Graph** tab panel. This will open the **Graph Thresholds** window.

00	Graph Thresholds
	Visibility On 🛟 Make Track
	Direction: \bigcirc > thresh \bigcirc <= thresh
By Value:	572.5
By Percentile:	74.6%
	Offsets for Thresholded Regions
	Start 12 End 13
Max Gap <= 1	00
Min Run > 30	

The first thing you will need to do is set the visibility to 'On'. By default, the threshold is set midway between the graph minimum and maximum values. To obtain more meaningful thresholds, adjust the threshold settings using the methods described in the following sections.

Direction

You can tell IGB to show you either the data above the threshold (> thresh) or below the threshold (<= thresh). For example, for expression results graph score represents the level of hybridization activity. For a hybridization activity level to be considered significant, the score must be

above a certain threshold. So you would choose the **> thresh** option and set your desired threshold value. If you need help to pick a threshold level, toggle the Y-axis on to see the graph's scale on the Y-axis. See Graph.

Changing the threshold

To change the thresholds of selected graphs so that more or fewer points meet the threshold limit, modify these settings in the Graph Thresholds window:

- By Value specifies the exact threshold value.
- By Percentile sets the threshold based on the percentage of graph values greater or less than your threshold.

Specify offsets

The **Offsets for Thresholded Regions** feature is designed specifically for working with tiling array data. Captured data from tiling array probes should usually be shifted for display. Since the probes are 25 base pairs long, but the x-coordinates given represent the starting coordinate, you should shift the threshold data so that it starts at 12 base pairs past the given beginning, and ends at 13 base pairs past the beginning. This is the default placement of all graph threshold bars; if you are viewing typical tiling array data, IGB default is set to the correct parameters. If these offsets are not correct for the data you are analyzing, you should change them.

When you view non-tiling array data in IGB, you should adjust the offset to 0 (set Start to 0 and End to 1) to correctly align the threshold bars with their actual coordinates.

Control gaps

Experimental data can be noisy. If you are looking for general trends and want to ignore small local variations, you can use the Max Gap and Min run settings. Slide the sliders to adjust, or enter values into the boxes for each parameter:

Max Gap: Groups of coordinates that meet the threshold may be separated by gaps where the threshold is not met. By default, thresholding bridges such gaps if they are less than 100 bps long. For example, if there are 300 bps that meet the threshold, then 99 bps that don't, followed by 100 that do, the threshold bar would bridge the 99 bps to produce a single threshold annotation 499 bps long. To change the length of this bridge, enter a different value into the Max Gap text-entry field. Often times, if you have short introns in your species/ gene models, you might need to adjust this lower to have potential introns drawn correctly.

Min run: minimum number of bases in a row that must meet the threshold before an annotation bar will appear. For example, by default the Min run is 30 base pairs, so a sequence of 29 base pairs that meet the threshold will not be marked by an annotation bar. Again if you species/ gene models typically have very short exons, you may need to reduce this number. On the other hand, if your species has a minimum size for exon length to be meaningful, you can adjust this higher.

Make an Annotation Track of the Threshold Results

As shown above, while you are working with threshold values, the 'bars' appear in the same track as the graph itself. However, you may wish to record the threshold results before changing values again; you may wish to work with the thresholding results as a separate track; you may with to save the results to share with others. For all of these reasons, you can create an annotation (NOT a graph) track of the thresholding results. After you have captured threshold "annotations" from a graph or other set of data, you can examine these "annotations" using all of the tools in IGB for working with and comparing annotations.

After you have set the threshold levels to yield results, you click the **Make Track** button. After an annotation track has been created with **Make Track**, it is frozen and is no longer linked to changes in the **Graph Thresholds** window. However, you can continue to modify the criteria in the **G raph Thresholds** window, altering the threshold results for the same graph track and then create another annotation track.

This track will be given a default label containing all of the relevant parameters, which you can later change if desired. An example name is

"threshold, [60 to infinity] offsets: (+12, +13), max_gap=20, min_run=30, graph: depth: Hotl2T1"

The name of the new track will always start with 'threshold' and end with the name of the graph track that was used (in this case, the graph was a depth graph made from a .bam track called Hotl2T1). The value '60 to infinity' shows that the threshold level was set to 60 and looked at > thresh . The offsets were NOT corrected as they should have been, and are set to the tiling array values. Finally, the max gap and min run values are recorded.

Creating Graph Tracks from Annotation Tracks

- Creating Graphs
- Depth Graph
- Mismatch Graph
- Mismatch Pileup Graph

Creating Graphs

IGB can create several useful graphs from annotation tracks. Although the **Depth** graph can be utilized for all annotations, the usefulness shines, in combination with the **Mismatch** graph, with .bam and other short read alignment files. Here, you can see how many reads are present at each nucleotide position (**Depth**) and where mismatches occur as compared to the genomic sequence file.

In IGB 7.0 we had added the benefit that after these graphs are created, they will dynamically update if you load more data into the parent tracks. Just move around and hit **Load Data**; the tracks will update and so will the IGB track.

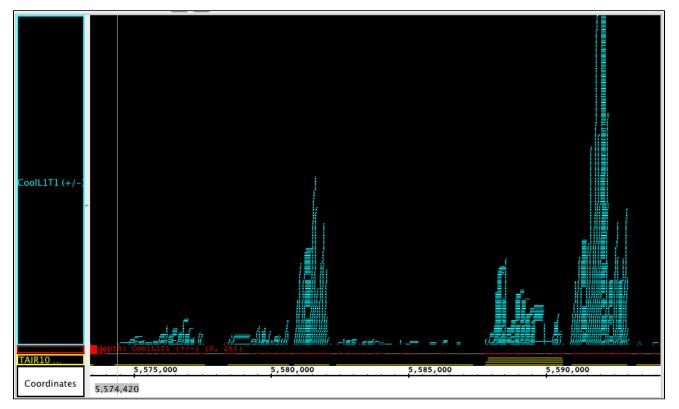
Graph tracks, such as these, can be saved as .wig formatted files.

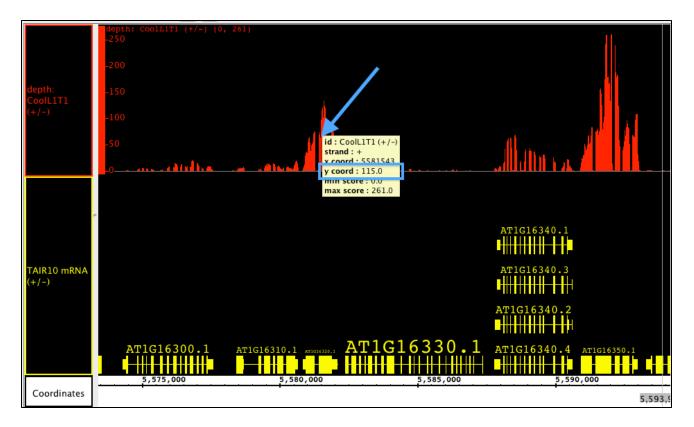
Depth Graph

You can generate an overview of the relative density of annotation coverage across an entire chromosome. This feature dynamically graphs the number of annotations in a track that are present at each nucleotide across the genomic sequence. When you are completely zoomed in, and each pixel represents a single nucleotide base, the graph shows actual values for the number of reads aligned at that genomic location. Using the **Select** tool to hover over a particular point in the graph will open a tooltip with the actual number of reads at that point.

Right-click on the annotation in the label panel and choose Track Operations > Depth.

The picture below shows a fully expanded **BAM** file, showing the difficulty of seeing any details. The second picture shows just the depth graph (*BAM *track is 'hidden') with the tool tip showing the number of reads aligned onto that specific genomic location.





Mismatch Graph

The **Mismatch** graph is specific to short read alignment files, such as BAM. The graph shows only the number of mismatched nucleotides across all reads at a specific genomic location, which can be very helpful in the detection of allelic variation, SNP identification and also error checking. Right click in the label to access the menu; choose **Track Operations > Mismatch Graph**. This function requires that the genomic sequence be loaded; if it is not loaded, IGB will load the sequence for you.

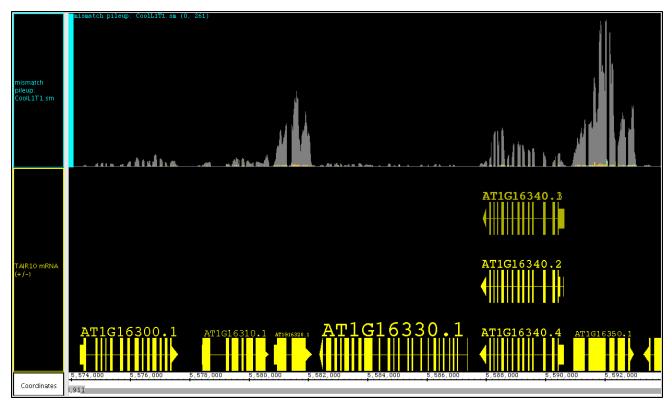
If you hover the **Select** tool (arrow cursor) over an individual bar of the graph, you will get a tool tip showing the total number of reads, and then the number of reads broken down by nucleotide. In the picture below, you can see that the total number of reads at the specified position is 260 (b lue arrow). Of those reads, 257 at at 'T', which you can see is the matching nucleotide (purple arrows). 3 of those reads contain a 'G' instead; since G is a mismatch, the graph shows a bar with height of 3 (red arrows).



Mismatch Pileup Graph

In the **Mismatch Pileup**, you have the **Depth** portion represented as a dark gray graph, with the **Mismatch** overlayed in the matching nucleotide colors. The colors show which and how many of each nucleotide(s) are present at each mismatch position. The **Mismatch Pileup** graph, like the **Mismatch** graph, is also specific to short read alignment files, such as BAM. Right click in the label to access the menu; choose **Track Operations > Mismatch Pileup Graph**. This function requires that the genomic sequence be loaded; if it is not loaded, IGB will load the sequence for you.

In the image below, you can see the gray depth portion matches the **Depth** graph from above, but you can also see the mismatched nucleotides (very small numbers in this section), which are color coded by G, A, T and C.



Annotation tracks

Annotation tracks contain gene models, short read alignments, EST alignments, or other genomic features. IGB allows you to change how annotations appear - you can change colors, change the Track Label names, and so on.

IGB also allows you to create new tracks by combining existing tracks. For example, you can create a new track that represents the intersection of two existing tracks. This is useful when looking for new novel genes or splicing variants, for example.

Customize track appearance

- Introduction
- Track Colors
- Track label text
- Track label width
- Edge Match color
- Annotation labels
- Strand controls
 - Combine or separate plus and minus strand annotations
 - Use Arrows to indicate strand
 - Use color to indicate strand
- Collapsing and expanding tracks
- Stack height
- Using color to represent scores

Introduction

IGB offers many options to customize the appearance of annotation tracks.

IGB remembers your customizations from session to session.

Some of the available customizations include:

- Annotation color
- Track background color
- Track label text, color
- Track height (pixels)
- Annotation stack height
- Annotations labels (e.g., using score or gene name)
- Changing track height and position in the display
- Changing strandedness of the tracks
- Changing the amount of data shown within the track (Stack Height)

You can change appearance of annotation tracks

- By clicking Annotation tab
- By right-clicking a track label
- By opening File > Preferences > Tracks

You can also set the default values for newly opened tracks based on file type using the Track Defaults tab in the Preferences window.

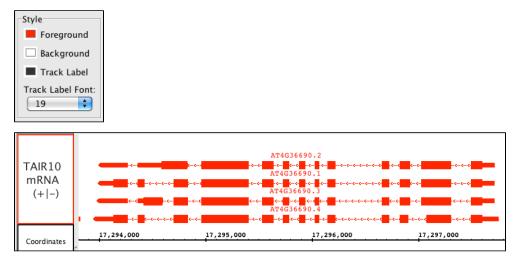
Track Colors

In IGB you can modify the color schemes for annotation and graph tracks. Using controls in the Annotation and Track Preferences tabs, you can

- change annotation and graph colors (foreground, FG)
- change background color (BG)
- change the color of the track labels

Unless you change it, the track label font color always matches the foreground color.

The following images shows the **Style** settings from the **Annotation** tab for a selected track. Note that the track label outline is red, indicated the track is selected.



Track label text

All annotation tracks have a track label in the panel on the left side of the display. You can change several aspects of how the label appears. You can

- change the text (called the "display name") displayed on the label
- change the track label text font size and color

To change the text in a track label, select the track by clicking its track label and

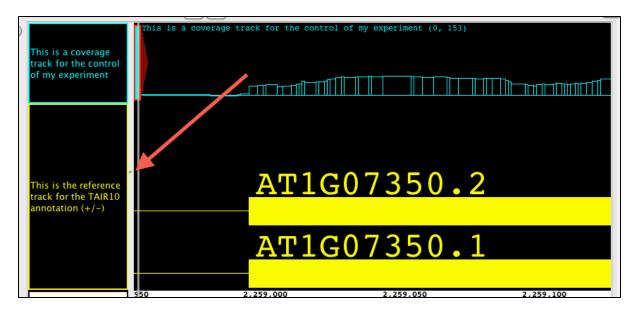
- choose Preference > Tracks tab and enter new text under the Track Name column
- or choose the Data Access panel and enter new text under the Track Name column in the Data Management Table

To change the track label font size, select the track by clicking its track label and

- choose Preferences > Tracks and enter a new value using the Track Label Font menu
- or choose the Annotation tab and enter a new value in the Style box

Track label width

You can also change the width of a track label. To increase or decrease track label width, drag the vertical bar that separates the labels from the data. The red arrow in the image below shows the 'grab point' which can be used to change the label column width.



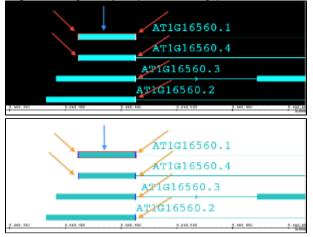
Edge Match color

If you use white or light gray shades for backgrounds, you may want to change the color of the Edge Match graphics so that it is easier to see.

To change the edge match color

- Open File > Preferences > Other Options
- Click on the swatch labeled Edge Match Color

Edge matching examples (click to enlarge)



Annotation labels

Every annotation has a set of associated attributes, such as its ID, score, name, gene name, description, start and end positions, and so on.

You can configure IGB to display any one of these attributes next to an annotation.

To change what values are shown as the annotation label

- Select the track by clicking its track label
- Click Annotation tab
- Choose an option in the Label Field menu



Strand controls

Combine or separate plus and minus strand annotations

IGB can show plus and minus strand features in separate tracks or combined into one track. To combine plus and minus strand annotation into a single track

- Select the track by clicking its track label
- Click Annotation tab
- Click +/- checkbox in the Labels and Strand sub-panel

Use Arrows to indicate strand

IGB can draw arrows within introns that indicate strand. To use arrows to indicate strand

- Select the track by clicking its track label
- Click Annotation tab
- Click Arrow checkbox in the Labels and Strand sub-panel

Use color to indicate strand

IGB can indicate annotation strand using color. To turn this option on or off or choose colors to use for minus and plus strand feature

- · Select the track by clicking its track label
- Click Annotation tab
- · Click Color by Strand checkbox in the Labels and Strand sub-panel
- Select colors by clicking the Color by Strand swatches

Collapsing and expanding tracks

Collapsing a track merges all annotations in the track into a single row.

To collapse a track

- right-click the track label and choose Collapse
- click the collapse track icon in the top left corner of the track

Stack height

The stack height setting controls the number of annotation rows that appear within a track. If there are more annotations than can be shown, IGB draws these in a single summary row in the top or bottom row of the track, depending on whether plus or minus strand features are being shown.

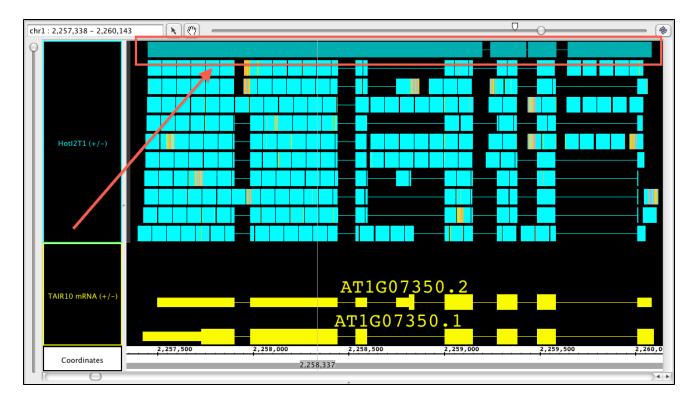
To change stack height, right-click the track label and

- choose Set Stack Height
- choose **Optimize Stack Height** to set a value that is optimal for the current view, e.g., ensure that all annotations will be drawn individually as part of a stack

or

• click the track label (to select the track) and click the corresponding stack height icons in the toolbar

In the image below, the Stack Height is set to 10. The red arrow points to the summary row.



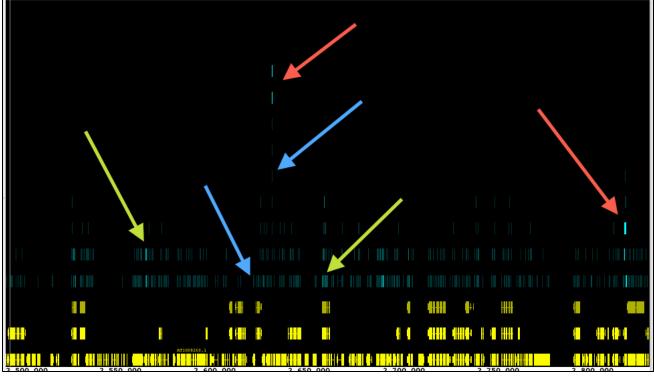
Using color to represent scores

For annotation data that contains scores, IGB can display the data with color intensity reflective of the scores. The scores must have been included in the data when it was loaded. Files in GFF and BED format allow the specification of scores. To turn color-by-score on or off, right-click the track name and choose **Color by Score**.

The default range that IGB uses is 0 to 1000+. New for IGB 7.0, we have added the ability to Set Range of Color by Score. This allows the user to set both the top, as well as bottom scores to best display their data. You can set the lower value so that any data with a score below your threshold will be barely visible; you can set the upper score to the highest score in your data, or any other value. After setting the lower and upper values, IGB will scale the color intensity across the new range so that the highest values are most intense, and the lowest values are the least intense (see image below).

This function has no effect on data that doesn't contain scores.

In this image, exons are the scored value. Exons with the highest scores will display in the chosen foreground color (red arrows). Exons with the lowest scores will display in a color intensity that blends with the background color (blue arrows). All other scores will be displayed at color intensities associated with their numerical score, where mid range numbers will show with mid range color intensity (green arrows).



Annotation track operations

- Introduction
- Single track functions
 - Not
 - Copy
- Multi-track functions
 - Intersect
 - Union
 - A not B, B not A
 - XOR

Introduction

IGB contains several functions that allow you to create all new annotation and graph tracks from existing tracks.

These functions can help identify new genes, new splicing variants, and help expose biologically meaningful aspects of your data. You can save the new tracks as BED format files, which you can re-open later in IGB and share with others.

There are two types of track functions

- Single-track functions that apply to one track only
- Multi-track functions that apply to two or more tracks

Single track functions

To access single-track functions

- Select a track
- Click Annotations sub-panel
- Choose a function from the **Operations** sub-panel

Annotation tab

Operations Single-Track: Copy	Apply
Multi-Track:	Apply

OR

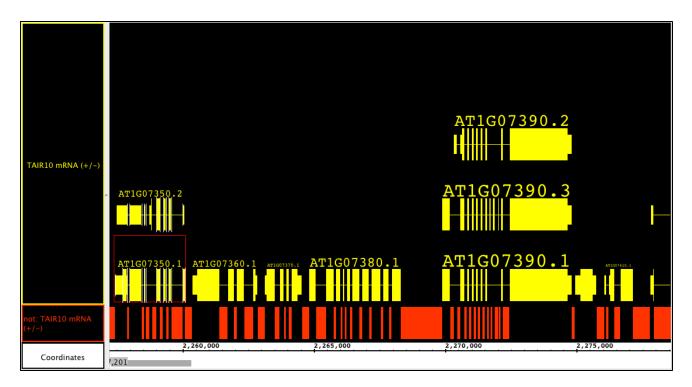
- Right-click a track labelChoose Track Operations

Right-click menu

RefGen	RefGene	
	Optimize Stack Height Set Stack Height	
	Hide Show	p31.1 71,400,000
Coordir	Collapse	
	Customize	<-<-<-<-<-<-<-<-<-<-<-<-<-<-<-<-<-<-<-
	Track Operations	Сору
RefGen	✓ Color by Score	Depth
Referen	Set Range of Color by Score	Not
	Save Selected Annotations as File	Summary
	Save Track As #S	
	\bigcirc	

Not

Not creates a new track with annotations representing empty regions from the selected track.



Сору

This function simply produces a copy of the original, with no changes to the annotations.

Multi-track functions

To access multi-track functions

By performing the functions below, IGB can provide new annotation tracks showing a uniquely specified/analyzed data set. This can be merged annotations, or annotations of areas where only one or the other track have data This powerful set of functions can help discover new features of all types within the data. For instance, selecting a RNA-seq experimental track followed by a reference sequence track and choosing **A not B** will show you all of the places where there are RNA reads that do not align to the annotated genome; all of the annotations in the newly created track could represent retained introns, new exons, new exon-intron boundaries, and even whole new genes.

These actions are specific to the tracks and strands that you selected, i.e. you can select just the + strand or just the - strand of a data set. This can be used to you advantage; for instance, if you want to know where on the chromosome there are annotations that overlap on the forward and reverse strands, then choose the + strand and the - strand and use the **Intersect** operation.

Another place where performing track comparisons is useful is when you are comparing treatments of samples versus controls. Depending on the function chosen, the new tracks will show differences or similarities, or even regions left completely unaffected. This can even be used with thresholding tracks created from graphs. By comparing 2 different tracks with different threshold settings, these operations can show you what is different between the two cases. To capture the graph thresholds in a track, see Applying thresholds to graphs.

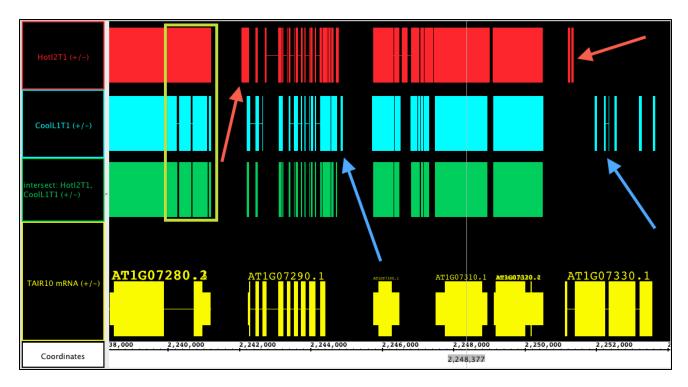
To combine tracks:

- Select two or more tracks by clicking and SHIFT-clicking the track labels. The first track selected is designated 'A' and the second track is 'B'.
- Right-click a selected track label and choose Track Operations.

Choose one of the options listed. The effects of each option is described below.

Intersect

Yields only the overlapping portions of A and B. This is good for looking at similarities between tracks. Although not quantitative, this is a good way to find out what similar elements are present or not. The picture below shows the intersection track, in green, of two .bam files (shown collapsed) and their reference sequence (also collapsed). Here you can see that they generally have the same genes expressed, even though they may be expressed at different levels. The red arrows are pointing out where ONLY the treatment track has reads; blue arrows show only control reads. The green box highlights the fact that if either track has gaps (such as introns) they will show in the intersection track.



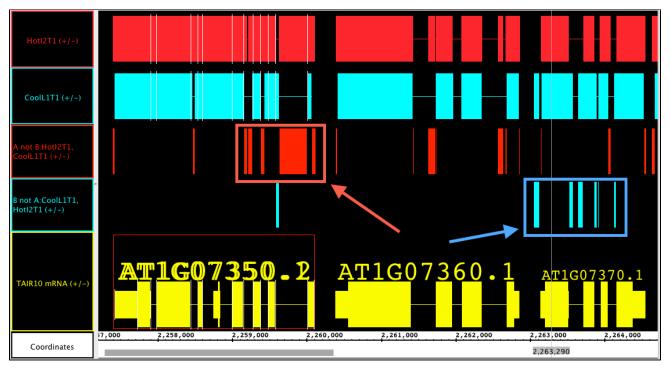
Union

Yields all bases that are in either A OR B

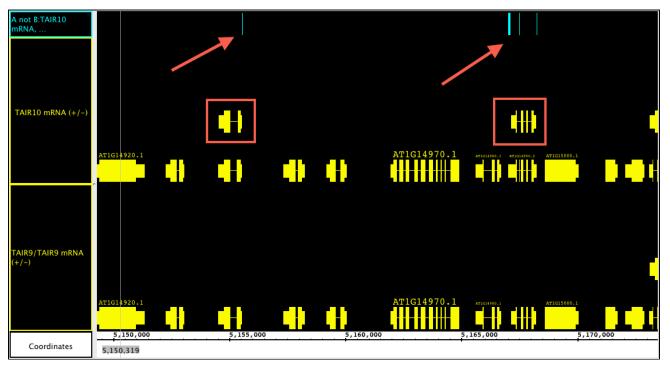
A not B, B not A

A not B and B not A tracks show the part of track 'A' that does not overlap with track 'B' and vice versa. These tools are incredibly powerful for finding differences between two tracks. You can find differences due to treatments, between expressed sequences (RNA-seq) and annotated tracks (reference sequences) or between different versions of data sets. There are many other applications as well.

To use these functions in IGB, select track 'A' first, and then track 'B'. The new track label indicates the function and the order of the track selection, i.e. in the image below the track label "A not B:Hotl2T1, Cool L1T1(+/-)" indicates **A not B** was used to generate the track and that Hotl2 is 'A' and CoolL1 is 'B'. In this image, the **A not B** track shows all of the instances where there is data present in Hotl2 that are not matched in CoolL1 (red box, red arrow). Meanwhile, the **B not A** track shows CoolL1 data that is not present in Hotl2 blue box, blue arrow).

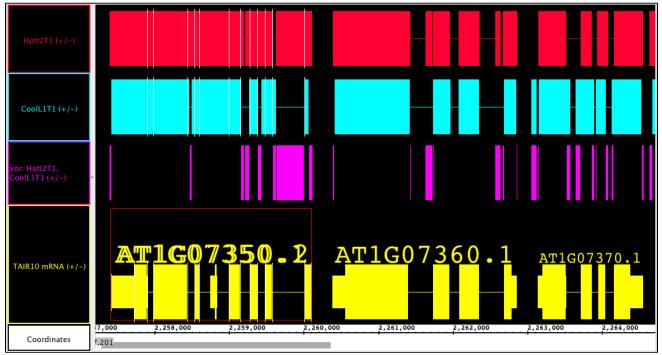


In this track, we compare TAIR10 annotations to older TAIR9 annotations to find differences. You can see that there are 2 new splice variants included in this region, but that they do not look very different from the original by a quick glance. the **A not B** track identifies exactly where they are different.



XOR

XOR tracks shows the union of the material that is in A or B but not both; this is like a summary track of both **A not B** and **B not A**. Order of track selection is irrelevant here. While this track summarizes the differences into one track, you cannot determine which of the original tracks contains the difference, but it makes and excellent way to skim across data looking for general regions of difference.



Customize annotation colors using Color By

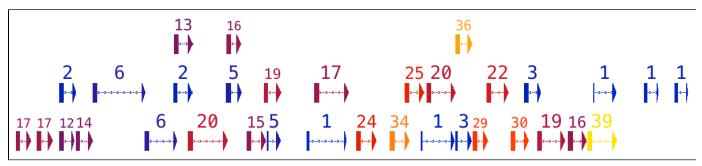
- Introduction
- · How to customize annotation colors using Color By
- Attributes and Options for setting annotation color

- Color by Property
- · Coloring by numeric values: Using the heatmap editor
 - Heatmap Handle Settings panel

Introduction

The Color By feature in IGB lets you customize annotation color based on annotation attributes like length, property, RGB, and score.

For example, here is an image from IGB showing splice junction features colored by their score. Redder features have higher scores and blue features have smaller scores.

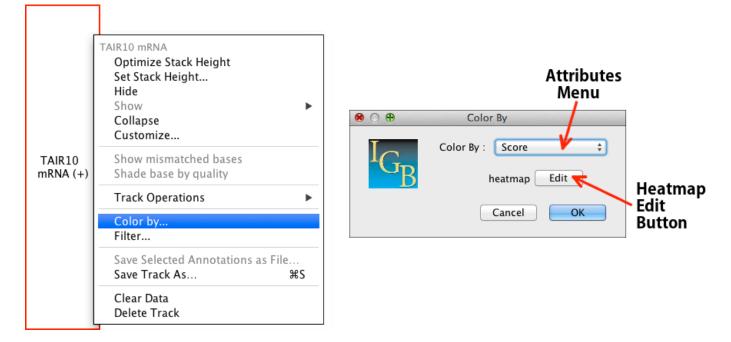


How to customize annotation colors using Color By

To customize annotation colors in a track

- Right-click the track label
- Select Color by to open the **Color by** dialog
- Choose an attribute from the menu
- Select options and colors. Note that available options depend on attribute type.

Figure 1. Right-click annotation track label and select Color by... to open Color by dialog



Attributes and Options for setting annotation color

The menu in the **Color By** dialog shows annotation **attributes** you can use to configure annotation colors. The attribute names you see will depend on the type of data set (or file) you loaded into the track. When you select an attribute, the **Color by** dialog will then show you options for choosing colors. The options you see after selecting an attribute will depend on the **attribute** type - whether it's a number (like score) or a

category (like name).

Color by Property

Coloring by **Property** is unique in that it lets you select a property from a drop down menu, enter a value for the property, and then select a color that indicates a match or a mismatch.

To select a color, click a color swatch and then choose a color from the color palette that appears. Click the OK button to confirm your selection.

Color By Color By: Property property gene name value EMB76 match Cancel OK	TAIR10 mRNA (+ -)	AT1G01040.2 AT1G01060.2 AT303200.1 AT1G01060.2 AT303200.1 AT1G01060.3 AT303200.2
		AT1G01040.1 AT1G01060.3 AT10000.2

Coloring by numeric values: Using the heatmap editor

You select a numeric attribute (like score) to color by, then the **Color by** dialog will show a heatmap **Edit** button that lets you color annotations using customizable heatmap.

To use the the Heat Map Editor:

- Click Edit in the Color by dialog to open the Heatmap Editor
- Use the color handles (triangles above the heatmap) to configure the heat map.
- To change a color range, click-drag a color handle triangle to a new location or edit its settings in the Handle Settings panel.
- Add new handles by clicking the Add button.
- To remove a handle, select the handle and click the **Delete** button.
- When the editor has at least two handles a gradient appears as in the example below.

00	Configure Heatmap		
◀ ▼	∇	▼	\bigtriangledown
112.5550	512.1550	766.9000968.3	650 /
Min=1.0	Range	Max=1	1000.6
Handle Settings			
Attribute Value 968.365 🗘	Set Range	Add [Delete
Color			
		Cancel	ОК



Heatmap Handle Settings panel

Handle Settings			
Attribute Value 0 1 Color 5	Set Range	Add 3	Delete 4

- 1. Attribute Value displays the value for the selected handle. Enter a new value to change the setting.
- 2. Set Range sets the Min and Max values of the heatmap.
- 3. Add Handle adds a new handle to the editor.
- 4. **Delete Handle** deletes the selected handle.
- 5. Color swatch lets you choose a new color for the selected handle.

*Note: Population of the Attributes Menu is currently *file format* driven rather than *data* driven. Therefore, if the attribute is not present in your file, the Color By feature will not actually be applied when the OK button is clicked. Make sure the attribute you are trying to color by exists for your file!

Deleting tracks

There are three ways to remove (delete) a track.

To delete either a graph or annotation track:

- Right-click the track label and select Delete track
- Select the Data Access tab and click the red "x" next to the track name in the Data Management Table
- Select the track, select Annotation or Graph tab, and click the Delete button

Reference sequence

- Introduction
- Loading reference genomic sequence data
- What to do if your genome is not supported
- Loading sequence into a new track
 - Viewing the reference sequence
 - Examples
 - Copy and paste sequence bases in IGB

Introduction

IGB display reference genomic sequence in the Coordinates track. IGB supports many interactions and views on genomic sequence, including copying sequence, viewing sequence in the **Sequence Viewer**, and running BLAST searches. Other features may be available via IGB plug-ins.

Loading reference genomic sequence data

IGB does not load genomic sequence until you request it, giving you total control over how much data is loaded and when.

To load genomic sequence

• Click Load Sequence button

Sequence data will then load from a remote data source or local file, depending on whether you're viewing a genome version supported by an external data source or if you're viewing a genome you loaded using the Load Custom Genome option.

If you are working with a genome that is not supported by the IGBQuickLoad data source, let us know. It is easy to add new genome versions to IGBQuickLoad.org.

Some external sequence data servers will reject requests for residues from a region that is too large. Others will return the data, but only after a very long wait. If you have problems, let us know.

What to do if your genome is not supported

If you have a sequence file in one of the formats that IGB can read (File Formats), you can use that sequence as a reference sequence.

To use your own file as the reference genome sequence

- Choose File > Open Custom Genome
- · Enter a species name into the Species text box
- · Enter a genome version name into the Genome Version text box
- Use the "..." button to browse and open your reference sequence file
- Click OK

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IGB will then open the file and it will appear in the Data Management table.

To load data, click Load Sequence button (NOT Load Data) to view the sequence in the coordinate axis.

If the chromosome names do not follow standard synonym conventions you may need to create a personal synonyms file. See: Person al Synonyms.

If the sequence length contradicts your annotation files, IGB will not be able to display them together. Please contact us if you need help.

Loading sequence into a new track

If you want to load an additional sequence as a track rather than as a reference sequence (perhaps a substrain or an individual), open it as if it were an ordinary file.

• You can drag and drop the file into IGB from either a desktop folder or from a URL.

or

- You can also choose File > Open File... or Open URL....
- Click Load Data to load the sequence into the track

As with any other track, a new empty track will appear labeled with the name of the file you opened. The file name will appear in the **Data Management** table in the **Data Access** tab.

Viewing the reference sequence

At low zoom (many bases are shown) loaded sequence is shown as a gray bar beneath the coordinates axis.

When you are zoomed in, the sequence bases become visible. If sequence has not yet been loaded, '-' (hyphen) characters are shown. If the sequence is loaded, the nucleotide base letters are shown. By default, IGB uses cool colors to represent A and T bases and warmer colors to represent G and C bases.

Change base colors in the Preferences > Other Options tab.

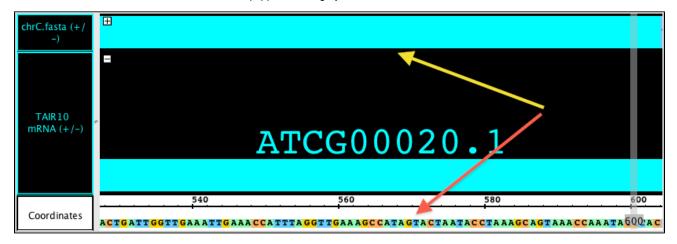
Note that depending on the sequence data server, file, or URL that supplied the sequence data, bases may appear in upper or lower case.

Examples

In the first image, you can see a sequence loaded as a track (red arrow). Notice that the refseq has not yet been loaded (blue arrow) so it appears gray with '-' marks; when you are zoomed out, the sequence track shows a gray bar (default color), and the coordinate axis is empty.

chrC.fasta (+/ -)	⊞ ACTGATT GGTT GAAATTG AAA CCATTTA GGTTGAAAGC CATA GTA CTAATAC CTAAAGC AGTAAA CCAAATA (CCTA C
TAIR10		
mRNA (+/-)	ATCG00020.1	
Coordinates	540 560 580	600
coordinates		500

After loading the refseq (red arrow), the sequence in the track (yellow arrow) changes to the FG color for that track (cyan in this case) where ever it matches the refseq. In this state, it will only highlight differences/mismatches with a letter and a color change. Zoomed out, the track appears as a solid bar in the FG color for the track. The refseq appears as a gray bar in the coordinate axis.



Copy and paste sequence bases in IGB

You can copy a sequence of bases into another application, such as Notepad.

To capture a sequence of bases:

- Make sure that the sequence residues are already loaded.
- Either select a single annotation or click and drag in the Coordinates track to select the desired coordinate range.
- Choose Edit menu > Copy Selected Residues to Clipboard.
- Paste the text string into the target application or location.

If you have selected an annotation that has introns, the selected residues will only include the exon regions.

If you have selected a negative-strand annotation, the selected residues will be reverse-complemented.

If you want to capture all the residues including the intron regions, select the region by dragging in the Coordinates track

Custom Genomes (Genomes not in IGB)

Introduction

 \oslash

- How to show a new genome in IGB
- Viewing reference gene model annotations
- · For advanced users Naming genomes and genome versions
 - Naming a Genome
 - Adding a Common Name for a Species

Introduction

IGB already supports many species and genome versions, not just the species shown on the start screen. To check whether a genome is

available in IGB, click the Current Sequence tab and use the Species and Genome Version menus to look for your genome of interest.

However, if your genome is not available, you can still use IGB. Here's how:

How to show a new genome in IGB

- 1. Return to the IGB Start Screen. To return to the start screen, click the **Home** icon in the **Toolbar**. You can also select "Species" in the **Sp** ecies menu in the **Current Genome** tab.
- 2. Select File > Custom Genome to open the Custom Genome window
- 3. Enter a species name and genome version name.
- 4. Select a reference sequence file.
- 5. Click OK.

000	Open	Custom Genome
I_{C}	Species	Equus unicornis
B	Genome Version	My first genome
	Reference Sequence	mysequence.fa
		Cancel OK

Viewing reference gene model annotations

If you have annotations for your genome, use File > Open File... to open and view them in IGB.

Note: The sequence names in your gene models file and reference sequence file need to match. If they don't, then you can create a chromosom e synonyms file that IGB will use to match names.

For advanced users - Naming genomes and genome versions

Naming a Genome

If you are building a genome for display in IGB, we recommend that you give it an IGB-friendly name, consisting of the month and year of release combined with genus and species, following the pattern: G_species_mon_yyyy, where G is the first letter of the genus, mon is the three-letter English abbreviation for the month the genome was released, and year is the year of the release.

For example, A_thaliana_Jun_2009 is the *Arabidopsis thaliana* genome assembly and annotation published in June of 2009; or H_sapiens_Feb_2009 which is the *Homo sapiens* genome assembly published February of 2009. Using this scheme will ensure that IGB displays the latest genome first in the genome version menu under the **Current Genome** panel.

If you are working with a subspecies or strain, you can also include the subspecies name.

For example, if you are working with Helicobacter pylori 26695 (released July 2012) then enter species Helicobacter pylori 26695 and genome version H_pylori_26695_Jul_2012.

Adding a Common Name for a Species

When users operate the **Species** menu, a tooltip appears that lists the species common name. This information comes from a file named species.txt that is packaged with IGB application.

If you are adding a new species, contact the IGB developers and ask to have your common name added to the species.txt file.

If you are distributing your own data sets via a **QuickLoad** site, you can also place your own custom species.txt file in the root directory of the **Qui ckLoad** site.

For more information about the file and its format, see: How IGB recognizes species and genome versions (species.txt)

Saving images and printing

- Saving images
- Image export options
- Printing

Saving images

To save an image, select File > Save Image.... A window similar to the image below will open.

/Users/auser/exp	ort nng			Browse
/ Users/ auser/ exp	orc.prig			browse
Portable Network	Graphics	(*.png)	‡ Ca	ncel OK
Image Size				
	1,217	Height:	874 🛊	pixels 🕴
		Resolution	: 300 ‡	Refresh
Preview				
Whole Fram	e			
O Main View				÷ .
0			<u>e 8 </u>	
🔵 Main View (with Labels	5)		
Sliced View	(with Labe	(s)		

Image export options

- Image format IGB supports PNG, JPEG, and SVG.
- Image size Select image size. Default values are the current dimensions of the IGB window. Aspect ratio is locked. To change the aspect ratio, resize the IGB window and click the **Refresh** button.
- Export options (Preview)
 - Whole Frame includes the entire IGB window
 - Main View (with Labels) includes tracks and track labels only.
 - Sliced View (with Labels) is available only when the Sliced View tab is open. It will export the image from the Sliced View tab.

Use the View menu to show or hide parts track decorations and other visual tools, such as the zoom stripe, edge matching marks, or the zoom stripe label.

View menu options (click to enlarge)

- ♦ Clear Visual Tools
 ♦ Show All Visual Tools
 ✓ I Show Zoom Stripe
 ✓ I Show Zoom Stripe Label
 ✓ I Show Properties in tool tip
- V N? Show Properties in tool t
- Show Collapse Option
- ✓ Show IGB Track Mark
- ✓ Show Edge Matching
- Show Locked Track Icon

Printing

• File > Print Main View with Labels... prints tracks and track labels only

• File > Print Whole Frame... prints the entire IGB window.

Editing IGB-generated SVG files

- Introduction
- How to crop in Adobe Illustrator.
- How to crop in Inkscape (free)

Introduction

SVG files generated by IGb sometimes include vector graphics that extend outside image area. This is due to problems with the SVG export library IGB uses, and we aim to fix this in future versions of IGB.

Until then, you'll need to use a vector graphics editor to remove the overhanging data (see below) if necessary.

How to crop in Adobe Illustrator.

- 1. Open the SVG file.
- 2. Adobe may display the message: "Clipping will be lost on roundtrip to Tiny." If yes, click OK.
- 3. Select the Rectangle Tool.
- 4. Draw a rectangle over the part of the image you wish to use.
- 5. Change the Opacity to 0%.
- 6. Select the Selection Tool.
- 7. Highlight both the rectangle and the image.
- 8. Select Object > Clipping Mask > Make.

How to crop in Inkscape (free)

- 1. Open the SVG file.
- 2. Select the Create rectangles and squares tool.
- 3. Place a rectangle over the part of the image you wish to use.
- 4. Under the Fill and Stroke tab, change Opacity to 0%.
- 5. Select the Select and transform objects tool.
- 6. Highlight both the rectangle and the image.
- 7. Select Object > Clip > Set

IGB Gallery

Introduction

See links below for images from the IGB software showing a variety of data types and data sets.

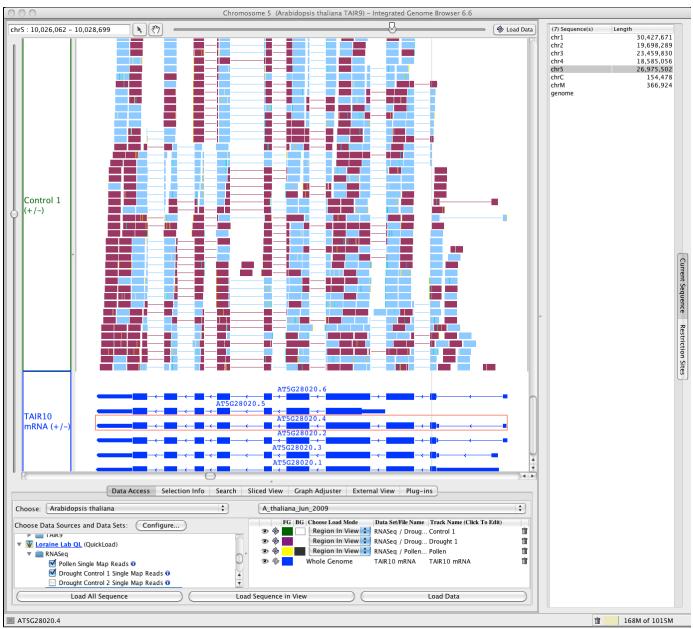
Most of these images are from data sets created in the Loraine Lab.

Some examples (e.g., the tiling array examples) come from data harvested from the Gene Expression Omnibus.

Color-coding read alignments by strand

IGB Preferences allows configuring display of read alignments.

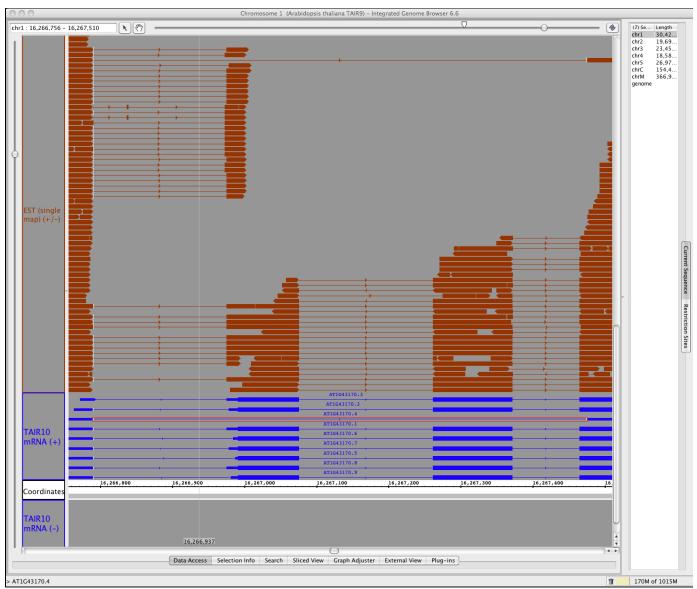
Sometimes it is useful to use color to indicate strand in RNA-Seq reads. The image below shows IGB configured to display plus and minus strand reads using different colors. Users can choose the colors on a track by track basis. These data were loaded from a custom QuickLoad site.



Edge matching ESTs to assess alternative splicing

Edge matching ESTs to assess alternative splicing

Edge matching is a specialized visualization technique for genomics data that makes it easy to compare boundaries. Edge matching is especially useful for genes that undergo alternatives splicing. We can assess the degree to which individual splicing choices are made by looking at overlapping data from EST alignments or RNA-Seq data. The example below shows a visualization in IBG 6.6, which introduces a new iconography for gene models that uses arrows to indicate strand. Arrows pointing right mean the annotation is on the plus (forward) strand and arrows pointing left indicate minus strand annotations. Note how in this case that one of the gene models suggests an exon skipping event but only one overlapping EST supports it.

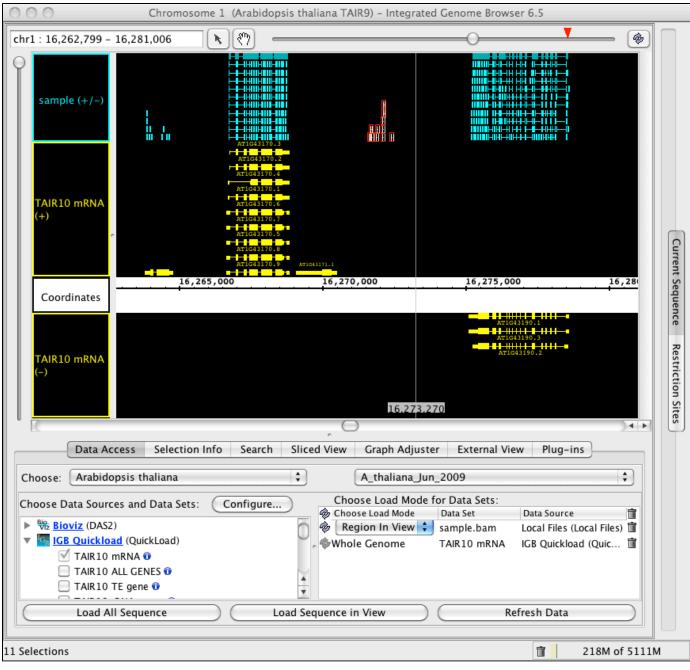


Counting reads with selection tool

Counting reads in a region with no annotated protein-coding gene

This image illustrates using the selection tool (the arrow icon) to select a set of reads over a region of the *Arabidopsis* genome with no annotated protein-coding gene. This region might contain a gene of another type, e.g., an "other RNA" gene. To check this, load the TAIR10 ALL data set.

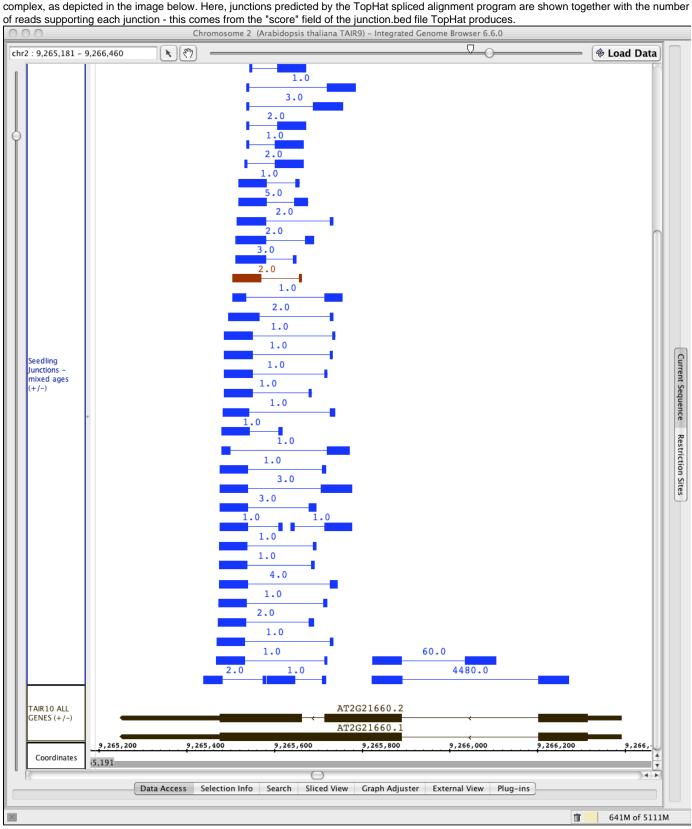
Note that the number of selected items appears in the lower left corner of the IGB window. The version of IBG is 6.5., released in April, 2011.



Alternative splicing in GRP7 (AT2G21660)

TopHat analysis of RNA-Seq data reveals alternative splicing in Arabidopsis thaliana GRP7 (AT2G21660)

This image from IGB 6.6 shows how IGB can capture a rich data scene and convey details about alternative splicing. The image depicts Arabidopsis thaliana GRP7 (AT2G21660) encoding "a small glycine-rich RNA binding protein" that "regulates..circadian oscillations of its own transcript." (Functional annotation comes from The Arabidopsis Information Resource locus page for AT2G21660.) Splicing pattern of GRP7 is



Using IGB to view effects of kmer on assembly quality

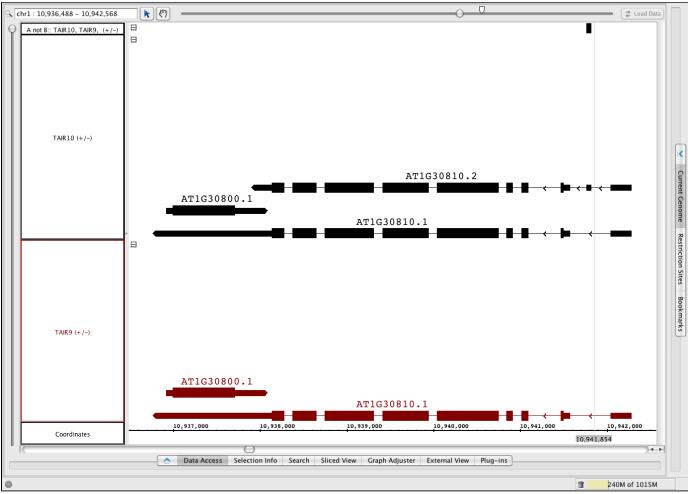
This image from IGB 6.6 shows a close-up view of two nearby A thaliana Jun 2009 genes on opposite strands. Tracks shown in yellow display alignments of transcriptome assemblies of RNA-Seq data from Arabidopsis seedlings undergoing a severe drought stress and unstressed controls. Transcriptome assemblies were performed using Oases run with the same parameters and data, but with different kmer values. Note how different kmer values yield very different results. Note also how the assembly performed using kmer 21 includes a transcript model that appears to merge adjacent genes from opposite strands.



Comparing new and old annotation releases

This image from IGB 6.7 demonstrates track operation "A not B" that creates a new track containing annotated regions present in A but absent from B. An "A not B" track can highlight differences between runs of the same analysis software using different parameters, new additions to an annotated gene set, and even differential expression across RNA-Seq data sets.

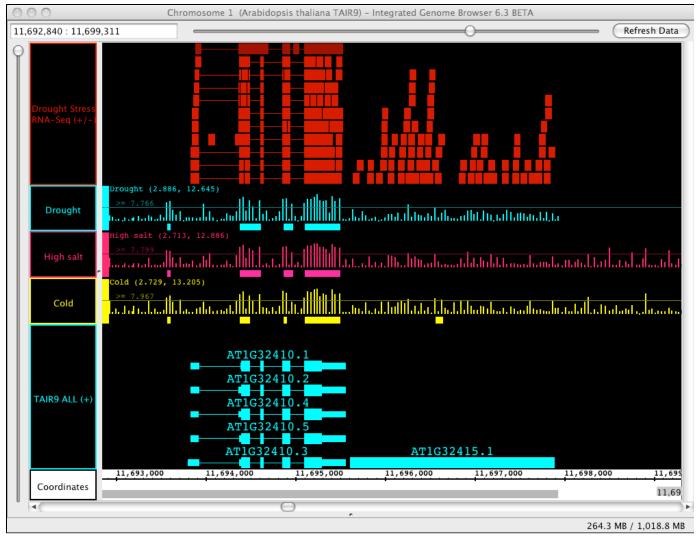
Here, the "A not B" track occupies the top row of the IGB display.



Viewing RNA-Seq and expression tiling array data in the same view

Viewing RNA-Seq and expression tiling array data in the same view in IGB 6.3

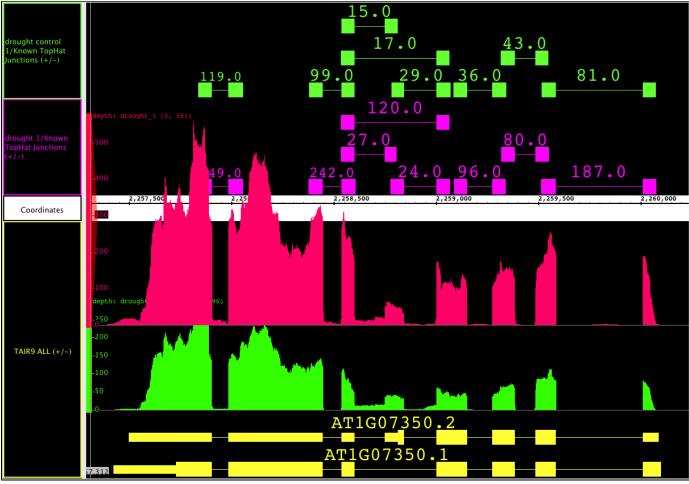
In this image, you see three different *Arabidopsis* expression tiling array data sets corresponding to cold (GSM243694), high salt (GSM243703), and drought (GSM243707) treatments assayed using the Affymetrix AtTile1R tiling array platform. The data were loaded in simple graph format, where probe intensities are shown as vertical bars. The graphs were then configured via the Graph Adjuster tab's Graph Thresholding option to display a bar underneath groups of consecutive probes with intensity values above a certain threshold. Note how the bars seem to correspond to know exons in the gene models displayed in the TAIR9 track. At the top of the display are short read Illumina RNA-Seq data (75 bases per read) from plants undergoing severe drought stress. Note that graph thresholding seems to suggest that this gene contains a previously undiscovered five prime exon. However, the RNA-Seq data contain no reads that support this idea. This example illustrates some of the ambiguities that can arise from using data from high-throughput methods - like tiling arrays and Illumina sequencing. Consider that the data sets are enormous and noisy! Thus, purely through chance we will observe at least some genes adjacent to what tiling arrays seem to suggest are unannotated exons. If the RNA-Seq data supported the idea that this region of high probe intensity is indeed an exon, then we would be much more likely to believe the conclusion, because the odds of two entirely different expression measurement technologies giving the same spurious result are very small.



Visualizing output from TopHat and BowTie

Visualizing output from TopHat and BowTie

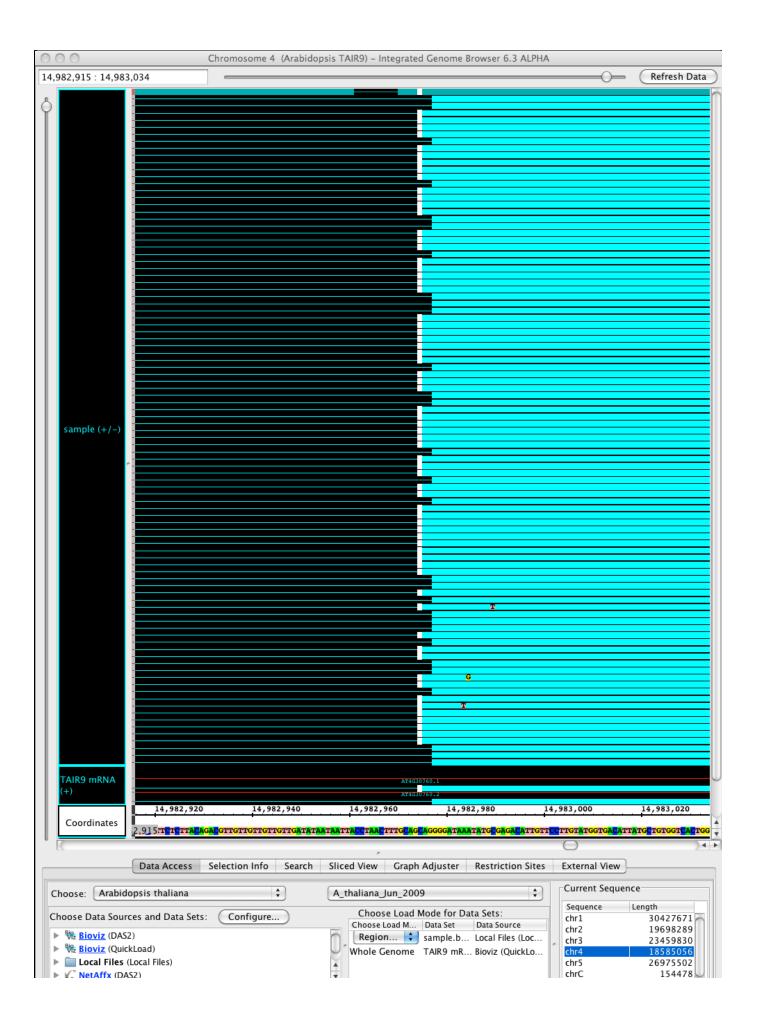
The image below shows output from TopHat and BowTie, programs that align short reads from Illumina sequencing experiments onto a genome. The top two tracks are from a BED format file that TopHat creates in which each line of the BED file represents a splicing choice and the score field indicates the number of reads that supported that choice. The two floating graphs (red and green) are from WIG files the programs produce that summarize the number of reads covering individual regions. In this particular experiment, the control sample (green) produced many more reads than the treatment sample (red), and so the fact that there are more reads overall in the treatment (red) sample tells us this gene is up-regulated under the treatment.



Visualizing RNA-Seq reads aligned onto a genome

Visualizing RNA-Seq reads aligned onto a genome

This image from IGB shows short read sequences aligned onto the Arabidopsis A_thaliana_Jun_2009 (TAIR9) genome. The track above the Coordinates track presents part of two Arabidopsis gene models, with sequence data loaded. Note how the reads seem to support two alternative splicing variations in the overlapping gene. Also note the nucleotide differences between the aligned reads (upper track) and the reference sequence.

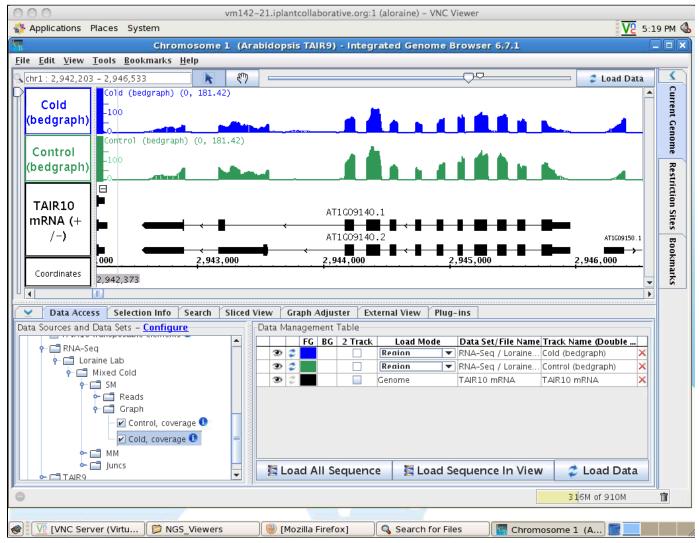


Load All Sequence	Load Sequence In View	Refresh Data ge	M 366924 A

96.2 MB / 2,042.8 MB

IGB 6.7.1 running in iPlant Atmosphere

iPlant Atmosphere offers a nifty way you can run IGB on an iPlant hosted virtual machine. To make this image, I logged into Atmosphere, requested a new virtual machine, and then used a free software tool called VNC Viewer to log in. From there, I downloaded and launched IGB from http://bioviz.org/igb just as I would do on my local desktop or laptop computer. What's great about this is that now I can use IGB to access files I've made in the iPlant Discovery Environment or files I've made by running software running on the VM itself. I can also load data from the IGBQuickLoad site in the usual way, letting me compare my results to publicly available data sets, like the one shown below. The data shown here come from an RNASeq experiment from the Loraine lab that we deployed the the publicly-accessible IGBQuickLoad site at UNC Charlotte.



Advanced Features

Scripting and the IGB command language

- Introduction
- Syntax
 - Commands
 - Specify a genome version
 - Go to a region
 - Add a new data set to IGB's list of active data sets
 - Change the load mode for an active (opened) data set
 - Load data from currently active data sets
 - Make images
 - Select genes
 - Wait (sleep) for commands to finish



Introduction

Starting with IGB 6.3, you can control IGB from scripts, text files containing simple commands for navigation, loading data, and making snapshots.

You can run an IGB script:

- By opening a script file using File > Open. Script files should end with .igb or .js for javascript.
- From IGB links. See: Controlling IGB using IGB Links
- By drag-and-drop, dragging the file into an open IGB window

Note: A protoype plug-in enabling python scripts (.py extension) was developed but is not yet released. If you would like to know more, please get in touch.

Syntax

The script file has a simple, space-delimited syntax. Each command should occupy a separate line.

Commands

Specify a genome version

genome <version>

Specify a genome version (e.g., H_sapiens_Mar_2006). Any genome version or synonym that's accepted by IGB is accepted here.

Go to a region

goto <region>

Go to a region of a chromosome (e.g., "chr1:40000-60000"). Any format that's accepted by IGB is accepted here.

Add a new data set to IGB's list of active data sets

load <file or URL>

Load the specified file (identified by full path) or URL into IGB. The data set will appear in the user's Data Management table upon completion.

Change the load mode for an active (opened) data set

loadmode <NO_LOAD | REGION_IN_VIEW | CHROMOSOME | GENOME> <file or URL>

Change the load mode for the specified data set or file, identified by full path.

Load data from currently active data sets

refresh

This is equivalent to clicking the Load Data button in IGB. This triggers loading of data from the active data sets available in IGB, as specified in the load command.

Make images

snapshot <filename>

snapshotwholeFrame <filename>

snapshotmainView <filename>

snapshotmainViewWithLabels <filename>

snapshotslicedViewWithLabels <filename>

Export the entire frame, main view, main view with labels, or sliced view, respectively, to a an image file.

The filename extension determines the format.

Supported formats are those supported by IGB Export function. The filename defaults to "<genome_version><timestamp>.png" if not specified.

Select genes

select <geneids>

Selects the given gene IDs, which are passed in as a comma-separated list.

Wait (sleep) for commands to finish

sleep <milliseconds>

Sleep the application for x milliseconds.

IGB is multi-threaded, which means that the user may be able to operate IGB while data are being loaded. If your IGB script includes loading data from remote sites (e.g, RNASeq data from the IGBQuickLoad.org site) then you should include a sleep command to give IGB time to load the data.

Comments

Lines starting with the IGB script comment character '#' are ignored.

Example script

```
# select genome
genome A_thaliana_Jun_2009
# go to a region in chr4
# commas are optional
goto chr4:8,860,484-8,872,096
# load a file
load
http://igbquickload.org/quickload/A_thaliana_Jun_2009/cold_stress/cold_control.sm.bam
# load the data
refresh
```

Controlling IGB using IGB Links

- Introduction
- IGB links syntax
 - Show a region
- Other options
 - Scriptfile
 - Scriptfile examples
 - Load a species and navigate to a region
 - Open a data set

Introduction

You can control IGB by embedding IGB links in Web pages. This is useful when you need to manually inspect a large number of regions. By clicking links in an IGB links page, you can quickly move to a new region.

IGB links syntax

(i)

When IGB launches, it starts a "bookmark server" at localhost, port 7085.

If you launch a second instance of IGB while one is already running, the new instance will listen to a different port. For this reason, bookmark links will only work for the first IGB instance only.

IGB uses this internal server to handle HTTP requests.

An IGB link base URL is structured as

http://localhost:7085/UnibrowControl?[parameter]

Show a region

IGB link syntax to show a region is:

```
http://localhost:7085/UnibrowControl?version=[version]&seqid=[seqid]
    start=[start]&end=[end]
```

Parameters include:

- version genome version
- · start start position of the region to show
- end end position of the region to show

seqid - chromosome sequence

Example:

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=A_thaliana_Jun_2009

A Other parameters are also available. If you're developing IGB links, it helps to open up an exported IGB bookmarks file in a text editor and take a look at how the links are structured.

Other options

Scriptfile

As of IGB 6.3, you can use the scriptfile parameter to send commands to IGB, where scriptfile designates a local file or URL

http://localhost:7085/UnibrowControl?scriptfile=[file or URL]

• For IGB command line syntax, see Scripting and the IGB command language.

Scriptfile examples

Load a species and navigate to a region

http://127.0.0.1:7085/UnibrowControl?scriptfile=http://wiki.transvar.org/confluence/download/attachments/17269487/load_and_navigate.igb

Open a data set

http://127.0.0.1:7085/UnibrowControl?scriptfile=http://wiki.transvar.org/confluence/download/attachments/17269487/add_new_data.igb

Command Line Arguments

- Run IGB from the command line
- IGB command line options and arguments

Run IGB from the command line

To run IGB at the command line, download the latest release (igb.zip) from http://sourceforge.net/projects/genoviz/.

Uncompress igb.zip to make a new directory igb which contains the igb_exe.jar, which you can run at the command line.

The following command runs IGB with 1 Gb of RAM.

\$ java -Xmx1g -jar igb_exe.jar [IGB options]

IGB command line options and arguments

-prefs [file|URL]

• Load the preferences file referenced by file or URL.

-offline

· Do not attempt to contact remote servers for data, only use cached data.

-href [URL]

• Open a IGB control bookmark.

-tile_width [pixels]

Horizontal resolution for IGB windows when using a multi-monitor instance of IGB. Default is 1024 pixels wide.

-tile_height [pixels]

· Vertical resolution for IGB windows when using a multi-monitor instance of IGB. Default is 768 pixels high.

-tile_columns [num]

• Number of columns of IGB windows to open when using a multi-monitor instance of IGB. Default is 4.

-tile_rows [num]

• Number of rows of IGB windows to open when using a multi-monitor instance of IGB. Default is 2.

-port [port_number]

• Port number to listen.

Track lines for configuring track appearance

- Introduction
- Track line syntax
- Supported track line properties

Introduction

For many text-based file formats, it is possible to configure the track name, color, and display properties through the use of track lines in the file header or comments.

Track lines were invented for use in the UCSC genome browser, and the full format is described on the UCSC Genome Bioinformatics web site.

IGB understands a subset of the full format, and will ignore property settings that it doesn't use.

Track line syntax

In ".wig", ".bed", ".psl", or "gff" format (version 1 or 2), the track line begins with "track".

In ".gff" format version 3, the track line must begin with "##track" to indicate it as a meta-directive, since track lines would otherwise disobey the GFF3 specification.

GFF3 has its own rich methods of specifying annotation styles. A future version of IGB may make use of this.

Here is a sample track line that can be understood by IGB:

track name="Example" description="Example Description" visibility=full color=200,100,0 useScore=1 url="http://sample.com/search?id=\$\$"

Supported track line properties

IGB understands these track line properties:

name	Short track name.
color	Track color, in the format r,g,b; where r, g, and b are numbers from 0 to 255.
description	Human-readable short description of the track.
visibility	In IGB, visibility=1 and visibility=dense will cause the track to be displayed in "collapsed" mode. Otherwise, the track displays in "expanded" mode.
url	A URL for linking to an outside website. Any instances of \$\$ in the URL will be replaced with the item id. See Configuring Web links (LinkOuts) to external sites.
useScore	Whether to convert score data to color values. Valid values are useScore=0 and useScore=1 Files in ".bed" and ".gff" format can contain scores.
itemRgb	If itemRgb=On, use the data in column 9 of ".bed" files to determine the colors of individual annotations. Using this function requires additional memory.
graphType	Sets the graph type for graph files in the "wiggle" format. Can be graphType=bar or graphType=points.

How IGB recognizes species and genome versions (species.txt)

- How IGB recognizes species and genome versions
- Species.txt

How IGB recognizes species and genome versions

IGB is distributed with a tab-delimited file called "species.txt" that lists binomial names for species with sequence genomes along with their common names.

IGB uses these data to populate the Species menu in the Current Genome tab.

When IGB accesses a QuickLoad site, it will also attempt to retrieve a species.txt file from the QuickLoad root directory. If available, it will use those data to add new species to the Species menu.

If you are building a QuickLoad site for a newly sequenced genome, create a species.txt file containing your newly added species to ensure that the IGB Species menu will display the genus, species, and common names properly.

Species.txt

The species.txt file that is distributed with IGB is version-controlled and resides in the igb/resource directory in the project repository.

Here is a version that was current as of December, 2014.

Interactive tutorials

IGB now includes a Getting Started tutorial can run from the IGB Help Menu.

To see available tutorials

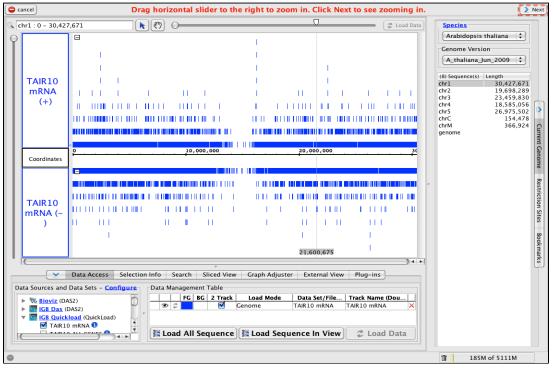
• Click Help > Tutorials

Tutorials offer a simple way to get acquainted with basic IGB functions, such as zooming, searching for features, loading sequence, and so on.

When you start a tutorial, IGB will display instructions on on what to do next.

Here you see an image from the tutorial, already under way.

Instructions appear in the top of the main window (in red) and hints on what to do next are outlined in dashed red lines.



Configuring Web links (LinkOuts) to external sites

- Introduction
- LinkOut contents
- How to create a new LinkOut
- Links to documentation on Java regular expressions

Introduction

When users right-click a gene model or other item in the display, IGB displays a menu with links to external Web sites. These links to external Web sites are called "LinkOuts."

You can create new LinkOuts using the Web links tool.

LinkOut contents

A LinkOut consists of

- URL the Web page IGB will open when users right-click an item
- Name the LinkOut name displayed in the right-click menu
- Pattern the pattern IGB matches against track id or item id to create the LinkOut

Every item in the IGB display has an id or identifier.

IGB uses this id to create the URL.

How to create a new LinkOut

Click Tools > Web Links to open the Web Links tool.

Use the Web Links tool to create new LinkOuts. Use the existing LinkOuts as a guide to making new ones.

To find out an item's id

- Click the item to selected it
- Click the Selection Info tab or Info button in the Selection Info box (top right)

Links to documentation on Java regular expressions

Java Regular expression: http://docs.oracle.com/javase/1.4.2/docs/api/java/util/regex/Pattern.html Regular Expression Online Generator: http://txt2re.com/

Regular Expression Online tester: http://www.regexplanet.com/advanced/java/index.html

Controlling IGB using IGB Links with Genome Synonyms

IGB recognizes commonly-used synonyms for a variety of genomes.

If you are creating IGB bookmarks, you can use these synonyms to refer to genome versions.

Examples:

Example 1. To load Arabidopsis thaliana TAIR9 for chr1: 20,633,626 - 20,636,669

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=A_thaliana_Jun_2009

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=TAIR9

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=Tair9

Example 2. To load Homo sapiens Feb 2009 for chr1 : 52,065,515 - 52,267,144

http://localhost:7085/UnibrowControl?seqid=chr1&end=52267144&start=52065515&version=H_sapiens_Feb_2009

http://localhost:7085/UnibrowControl?seqid=chr1&end=52267144&start=52065515&version=hg19

http://localhost:7085/UnibrowControl?seqid=chr1&end=52267144&start=52065515&version=human_ncbi37

Example 3. To load Mus musculus July 2007 for chr1 : 120,520,690 - 120,583,464

 $http://localhost: 7085/UnibrowControl?seqid=chr1&end=120583464&start=120520690&version=M_musculus_Jul_20078464&start=120520690&version=M_musculus_Jul_2007864&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=120520690&version=M_musculus_Jul_200786&start=1205206&start=1205206&start=1205206&start=1205206&start=1205206&start=1205206&start=120520&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=12052&start=1205&star$

http://localhost:7085/UnibrowControl?seqid=chr1&end=120583464&start=120520690&version=mm9

http://localhost: 7085/UnibrowControl?seqid=chr1&end=120583464&start=120520690&version=Mm:NCBI37044&start=120520690&version=Mm:NCBI3704&start=120520&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=12050&start=1

Example 4. To load Caenorhabditis elegans May 2008 for chrll : 4,082,693 - 4,089,972

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=C_elegans_May_2008

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=ce6

http://localhost:7085/UnibrowControl?seqid=chr1&end=20636664&start=20633631&version=WS190

Example 5. To load Bos taurus Oct 2007 for chr1 : 50,368,385 - 50,708,462

http://localhost:7085/UnibrowControl?seqid=chr1&end=18659361&start=18080205&version=B_taurus_Oct_2007

http://localhost:7085/UnibrowControl?seqid=chr1&end=18659361&start=18080205&version=bosTau4

http://localhost:7085/UnibrowControl?seqid=chr1&end=18659361&start=18080205&version=Cow_Oct_2007

Personal Synonyms

- Introduction
- Synonym file format
- Creating personal synonym files
- Loading synonym files

Introduction

Different data sources often refer to the same genome version or chromosome by different names. For instance, the 2004 assembly of the human genome has several names: NCBI uses "human genome build 35", UCSC uses "hg17", ENSEMBL uses "ensembl1834", and IGB uses "H_sapiens_May_2004". Also, chromosomes can have multiple names, such as "chromosome 2", "chr2", "chrom 2" or "2".

IGB integrates this various data by using synonym files, which recognize common names for a single genome version or single chromosome. The synonym file for genome versions allows data with different version names to be displayed together. The synonym file for chromosomes allows data with different chromosome names to be displayed together. However, if a data set has an unrecognized or mismatched name, IGB cannot display it with other data files. This is a problem that can sometimes occur with data sets from species-specific databases or with private data.

For example, let's consider two files for E. coli K-12 subtr. MG1655, downloaded from GenBank; the sequence file (.fa) refers to the chromosome sequence as "gi]49175990|ref|NC_000913.2|", but the gene model annotation file (.gb) refers to the chromosome sequence as "NC_000913". Since these are 2 different names for the same chromosome sequence, IGB will not be able to show the data from these files in the same view. A synonym file would make "gi]49175990|ref|NC_000913.2|" equivalent to "NC_000913", permitting both to be shown in the same view.

Synonym file format

Both the genome version and chromosome synonym files are tab-delimited text files. Each row contains a display name in the first position and one or more synonyms, separated into columns by tabs.

The first listed synonym is used as a display name in the **Current Genome** tab, either in the **Genome Version** list or as the name of a chromosome under **Sequence(s)**, unless there is already an IGB display name, which has priority*. **Using the previous example, if** "NC_000913" was typed the first column, and "gi|49175990|ref|NC_000913.2|" was typed in the second column, the name shown in *Current Sequences would be "NC_000913". If a more user-friendly name was entered in the first column (e.g. "chr1"), and the other names were entered in the next 2 columns, Current Sequences would show "chr1" as the name of the sequence.

Synonyms for genome versions could include short identifiers, such as "hg19", or "pantro3". If you are creating a synonym file for a species not currently listed, we encourage you to use the IGB format for the first synonym; first letter of the genus, the full species name, the three letter designation for month and then the year the genome was released, e.g. G_species_Mon_20XX. Synonyms for chromosomes could include "chr 1" (with a space), "chrl" (Roman numeral 1), or a reference name such as "gi|33338594".

Creating personal synonym files

You can create a personal synonym file for genome versions or for chromosomes when different names prevent 'matching' data sets from being displayed together.

To create a synonym file:

- 1. Open a new file in a plain text editor (such as Notepad).
- 2. Enter the display name (column 1).
- 3. Press TAB.
- 4. Enter the next synonym.
- 5. Repeat step 3 and 4 until all synonyms are entered.
- 6. (Optional) Repeat procedure on a new line for another genome version or chromosome number as appropriate.
- 7. Save file as chromosome.txt for chromosome synonym file. Save as synonym.txt for genome version synonym file.

We strongly encourage you to proofread your files; if your synonym file contradicts itself or IGB's built-in synonyms, unpredictable behavior may result, e.g. if you name "chr 2" as a display name and then mistakenly add "chr 1" as a synonym, this will disrupt internal synonyms. The IGB internal synonym files for genome versions and chromosomes are available for reference.

Loading synonym files

Synonym files are loaded from the **Data Sources** page in **Preferences**. When a new file is loaded, or changes are made to a loaded file while IGB is already opened, IGB will require a restart to recognize the new synonyms.

To load a synonym file:

- 1. Open File, choose Preferences, click Data Sources page.
- 2. Under Personal Synonyms, click . . . (browse) next to Version Synonyms File to add the genome version file or next to Chromosome Synonyms File to add the chromosome file.
- 3. Choose and open file.
- 4. Restart IGB.

If you clear your preferences, you will need to reload your synonym files.

Personal Synonyms	
Version Synonyms File	
Chromosome Synonyms File	

FindJunctions

- Introduction
- How to get FindJunctions
- Using the jar file to run FindJunction

Introduction

The FindJunctions program is a Java program that uses spliced alignments to identify exon-exon junctions in RNA-Seq data. When given a BAM file, it produces a BED file that summarizes every spliced aligned alignment identified in the BAM file. If also given a reference genomic sequence file (in .2bit format) it attempts to identify the strand of origin for each junction by looking for canonical intron splice junction sequences.

How to get FindJunctions

To obtain a copy of FindJunction, you can either download a copy of the compiled "jar" file or get a copy of the source code and compile the program yourself.

To download a compiled copy, select **Tools > Attachments** (top right of this Web page). See the attachment notes for the subversion repository revision number of the compiled "jar" file.

To get a copy of the source code, check it out from the Genoviz repository and then use ant to compile it, as follows:

```
$ svn co http://svn.code.sf.net/p/genoviz/code/trunk/tools/FindJunctions
$ cd FindJunctions
$ ant release
```

This will create a new file named dist/FindJunction_exe.jar. Use this file to run the program.

Using the jar file to run FindJunction

To run the program from the command line, you would do something like:

```
java -Xmxlg -jar FindJunction_exe.jar -u -n 5 -b Genome.2bit -o FJ.bed
sample1.bam,sample2.bam
```

In this example, the -Xmx1g option specifies that the program can run with up to 1 Gb of computer memory (RAM) using the code in jar file (-jar) FindJunction_exe.jar. The -u option (for unique) indicates that only uniquely mapping spliced reads with NH tag equal to 1 will be used to construct junctions. The -n option is the number of bases that must map to either side of a putative intron for a spliced read to be used to create or support a junction feature. The -b option gives the full path to the .2bit genomic sequence file that will be used to identify junction strand. The -o (output) option gives the name of the junctions.bed file that will be written. The final argument is a comma-separated list of the BAM files containing spliced alignments.

The output file (FJ.bed) is BED12 format. The name field contains a name constructed from the location of the junction and the score field contains the number of spliced alignments used to create the junction feature.

Creating QuickLoad Sites

- Introduction
- How to Set Up a QuickLoad Site
 - Step One: Create a QuickLoad root directory.
 - Step Two: Create one or more genome directories.
 - Step Three: Create contents.txt file.
 - Optional Step: Create a synonyms.txt file.
 - Step Four: Create genome.txt files for each genome.
 - Optional: Create liftall.lft file.
 - Step Five: Create annots.xml files.
 - Annots.xml options specify track color, annotation style, and more
 - Step Six: Add your annotation files.
 - Step Seven: Add sequence data.
 - Step Eight: Add your new QuickLoad site to IGB.

Introduction

IGB QuickLoad (QL) is a simple file-based system for users to access annotation, alignment, or sequence data.

Any file that IGB can open (using the File menu) can be accessed from a QL site.

QuickLoad sites can reside on your computer hard drive or on a remote Web (http) or FTP site.

A QL site contains

- meta-data files describing available genome versions, annots
- data files, including sequence data, alignments, genome graphs, and annotations

You can set up a QL site on your local computer or on a web server.

To view an example, see the IGB QuickLoad site.

How to Set Up a QuickLoad Site

Step One: Create a QuickLoad root directory.

Create a folder on your local computer or on a Web server. This folder will contain your genome directories and a "meta-data" file called **contents**.**txt**.

If you are hosting a QuickLoad site using the Apache Web server, you can configure QuickLoad to demand a user name and password from anyone who accesses the data by adding an .htacess file to the top level directory. See: http://httpd.apache.org/docs/2.0/howto/auth.html.

Step Two: Create one or more genome directories.

Create genome directories for each of the genome versions you want to make available via QuickLoad.

To ensure the genome will be displayed correctly in the **Current Genome** tabbed panel menus, the names of these directories should follow the IGB format of:

• (First letter of the genus, capitalized) _ (species, all lower case) _ (3 letters indicating month of release, first letter capitalized) _ (4 digits, the year).

For example, A_thaliana_Jun_2009 is the TAIR10 Arabidopsis thaliana genome assembly that was released in June 2009.

You can also indicate subspecies or cultivated varieties by including additional suffixes.

For example, there are two *Oryza sativa* (rice) subspecies in wide cultivation: japonica and indica. The first rice sequence published was from the Nipponbare variety which is a member of the japonica subspecies. Because an indica genome sequence is now available, we need names that distinguish them. We designate assemblies of the japonica genome sequence using prefix O_sativa_japonica and the month and year of release: **O_sativa_japonica_Jun_2009**. Annotations and sequence data reside in genome version folder O_sativa_japonica_Jun_2009 on the main IGB QuickLoad site - view its contents by visiting http://igbquickload.org/quickload/O_sativa_japonica_Jun_2009

Tip: If you set up your QuickLoad site in the Web directories of an Apache server, you can modify and fine-tune how files are displayed by adding directives to the **.htaccess** file in that directory. You can configure Apache to allow users to view the contents of your QuickLoad directory and include a short description of each file or data set. Here is an example:

Options Indexes
IndexOptions FancyIndexing IgnoreCase FoldersFirst DescriptionWidth=*
AddDescription "Arabidopsis thaliana TAIR9 genome release" A_thaliana_Jun_2009
AddDescription "Arabidopsis thaliana TAIR8 genome release" A_thaliana_Apr_2008
AddDescription "Arabidopsis thaliana TIGRv5/TAIR7 genome release" A_thaliana_Jan_2004
AddDescription "TAIR9 annotation file" TAIR9_*.gz
AddDescription "Annotation file list" annots.[txt xml]
AddDescription "Chromosome lengths and assembly information" genome txt
AddDescription "Available genome assemblies" contents.txt
AddDescription "Mitochondrial Genome (Fasta format)" [Cc]hrM.fa*
AddDescription "Chloroplast Genome (Fasta format)" [Cc]hrC.fa*

Step Three: Create contents.txt file.

Create a simple, plain text file called **contents.txt** and add it the top-level directory.

This file should be plain text, tab-delimited; all columns must be separated by a tab character. If you create this file by hand, be sure to use a text editor that inserts tab characters when you type a tab.

The first column should list names of the genome directories you have created and want to share.

The second column is optional - it contains text IGB will display on the title bar when you open IGB.

View contents.txt on IGB QuickLoad: http://igbquickload.org/quickload/contents.txt

Note: You can include other directories and other files in your QL site folder; IGB will simply ignore anything that isn't listed in your contents.txt file. Also, any changes in the name of the genome directory must be updated in the contents.txt file or IGB will not recognize it.

Optional Step: Create a synonyms.txt file.

This is a list of synonyms for genomes. This list allows you to match names across different QuickLoad sites and also the Galaxy workflow site. For example, if a QuickLoad site at another location uses different names to refer to the same genome version, you can specify these in the synonyms.txt. Each line contains any number of synonyms for a genome, separated by tabs.

Here is an example: http://igbquickload.org/quickload/synonyms.txt

Step Four: Create genome.txt files for each genome.

For each genome directory, create a **genome.txt** (formerly, **mod_chromInfo.txt**) file that lists all the chromosomes in your genome, together with their sizes.

As with the **contents.txt** file, it should be tab delimited:

The first column lists the chromosome names and the second column lists their sizes. IGB uses this file to create the sequence selection table under the **Data Access Panel**.

Here is an example*: *http://igbquickload.org/quickload/A_thaliana_Jun_2009/genome.txt

Note: You can create your genome.txt file from a sequence 2bit file (see below) using twoBitInfo, available from http://hgdownload.cse.ucsc.edu/a dmin/exe/. See Step Seven: Add sequence data.

Optional: Create liftall.lft file.

This describes how contigs are assembled into chromosomes.

Each line contains: CONTIG_START tab CONTIG_NAME tab CONTIG_LENGTH tab CHROMOSOME_NAME tab CHROMOSOME_LENGTH

Note: This is a relatively old feature in IGB and we are not entirely sure it still works.

Step Five: Create annots.xml files.

Each genome version subdirectory needs an **annots.xml** file that is either empty or contains a listing of annotations you want to appear the **Avail able Data Sets** section of IGB's **Data Access** tabbed panel.

Here is a simple example:

```
<files>
<file name="TAIR10.bed.gz"
title="TAIR10 ALL"
description="All TAIR10 genome models"
label_field="id"
background="000000"
foreground="00FFFF"
max_depth="10"
name_size="12"/>
</files>
```

Note that the **name** attribute of the **file** tag indicates the physical location of the file relative to the directory where the annots.xml file resides. However, you can also use a URL to indicate the location of the file. This means that you if you want to reference other resources hosted on other machines, you can do it.

Also note that if you are hosting BAM files, the BAM file index ".bai" file needs to reside in the same directory as the BAM file. Otherwise, IGB will display an error message when users try to access the data.

When loaded into IGB, each file will create one or more tracks. (Some file types, such as BED and GFF3, can specify multiple tracks.)

Annots.xml options - specify track color, annotation style, and more

An annots.xml file contains one or more file tags, enclosed in a files element. The file tag has attributes that will dictate how the data will look when displayed in IGB. Most of these options correspond to options users configure using the Tracks tab in the IGB Preferences window. (See F ile > Preferences > Tracks.)

attribute	optional	Description
name	Required	The name of the file on your file system or a URL. The URL can point to any file, anywhere, provided it's accessible via the internet.
title	Optional	User-friendly text IGB will show in the Data Access Panel as the title of the data set. If you don't provide a title, IGB will display the name attribute instead.
description	Optional	IGB will display this text as a tooltip when users hover the mouse over the data set title in the Data Access tab.
url	Optional	Use this tag to specify the location of a Web page describing the data set. If provided, IGB will display an "info" icon next to the data set title. When users click the icon, their Web browser will open showing the contents of the URL you provide.
load_hint	Optional	If used, its value should be "Whole Sequence". Using this tag will force IGB to load the entire file when users select the genome version, which is usually appropriate only for reference gene model annotations or other equally small data sets.
label_field	Optional	Use this field to indicate the annotation property (e.g., "score" or "id") that should be used in IGB to label individual annotations. For gene models, "id" is best.
background	Optional	Use this field to define track background color (e.g. background="000000")
foreground	Optional	Use this field to define annotation or graph colors (e.g. foreground="00FFFF")
max_depth	Optional	Use this field to define the default max depth value, the number of annotations that can be shown individually in a stack (max_depth="10" or max_depth="0" for unlimited)
name_size	Optional	Use this field to define the default track label name font size (e.g. name_size="12")
connected	Optional	Use this field to define the default boolean value for the connected field (e.g. connected="true" or connected="false")
show2tracks	Optional	Use this field to define the default boolean value for the show2Tracks field (e.g. show2tracks="true" or show2tracks="false")
direction_type	Optional	Use this field to dictate whether annotations or alignments will be shown using arrows and/or color to indicate direction. (e.g. direction_type="arrow", direction_type="color", direction_type="both", direction_type="none")
positive_strand_color	Optional	Use this field to define the default positive strand color (e.g. positive_strand_color="CCFFFF")
negative_strand_color	Optional	Use this field to define the default negative strand color (e.g. negative_strand_color="33FFFF")

Step Six: Add your annotation files.

Place your annotation, BAM, or graph (bedgraph or bigwig) files into the appropriate genome sub-directories. You can you use any format IGB

supports.

Starting with IGB 6.6, IGB QuickLoad can support BED, bedgraph, and GFF files that have been sorted, compressed, and indexed using the bgzip and tabix utility. Doing this helps speed up data loading in IGB and allows loading data by region. For an example of how we used tabix to distribute coverage and junction files from an RNA-Seq data set, see Creating a new genome release for IGB QuickLoad from the IGB Developer's Guide.

Step Seven: Add sequence data.

You may not need to do this if you are working with a genome that other QuickLoad sites support. IGB may be able to retrieve sequence data for your genome from other sites if it can find another site that supports the same genome version and contains sequence data. However, if you are working with a newly sequenced genome or don't want to use other groups' servers, you can support IGB's sequence visualization functionality by setting up your own sequences to distribute.

To do this, you will need to obtain sequence files and convert them to a binary format (twobit) that allow IGB to quickly obtain all or part of the sequence data when users press the **Get Sequence** button, run a blast search, or open the **Sequence Viewer** window.

The twobit format was first developed for the blat cDNA-to-genome alignment tool. IGB 2bit uses to support efficient retrieval of sequence data. To convert a fasta format file to twobit, use faToTwoBit available from http://hgdownload.cse.ucsc.edu/admin/exe/.

To convert a fasta file to 2bit, do something like this:

\$ faToTwoBit A_thaliana_Jun_2009.fa A_thaliana_Jun_2009.2bit

Note: The 2bit sequence file should have the same name as your genome version directory and should have file extension "2bit," e.g., A_thaliana_Jun_2009.2bit.

However, before you create the sequence files, check that the names of sequences in your fasta file match the names used in your annotation files. For example, if your annotations file lists sequence name chr1, then the fasta file should contain a sequence record with fasta header ">chr1" and nothing else. If they don't match, you'll need to either edit your fasta headers or create a sequence synonyms files.

Note: You can make your genome.txt file (Step Four above) using twoBitInfo, another program available from http://hgdownload.cse.ucsc.edu/ad min/exe/. To create a genome.txt file from a 2bit sequence file, do something like this:

\$ twoBitInfo A_thaliana_Jun_2009.2bit genome.txt

Step Eight: Add your new QuickLoad site to IGB.

Tell IGB to use your new QuickLoad site.

Follow the directions in Adding and Managing Data Source Servers To add the local server, click the "..." button and select the folder which contains the **contents.txt** file, e.g. "Quickload servers".

Preferences

Preferences

IGB offers many options to customize track appearance, add new data servers, and change data loading behavior.

Most customization options are available via the **Preferences** window which contains several tabbed panels devoted to different types of preferences.

To open the Preferences window

- Choose File > Preferences or
- Click the Preferences shortcut icon in the Toolbar

The following links contain descriptions of each tab in the Preferences window.

Tracks

- Introduction
- Track List
- Properties
- Show Strand

• Restore to Default

Introduction

Use **Preferences > Tracks** to configure the appearance of loaded tracks. Changes take effect immediately if **Auto Refresh** is selected or when you click the **Refresh** button. You can also change many aspects of track appearance using the **Annotation** tab or by right-clicking a track label.

For more about changing the appearance of tracks, read Customizing track appearance

0 0	Pref	ferences					
Tracks	Track Defaults	Toolbar	Ot	her Optior	ns 🕨		
	Track Name 10 other annotations/ALL C TAIR10 mRNA Coordinates aine Lab / Mixed Cold / SM		BG	FG	Size 14 14 12 12		
	RNA-Seq / Loraine Lab / Mixed Cold / SM / Reads / Co 12 RNA-Seq / Loraine Lab / Mixed Cold / SM / Graph / Co 12						
Select All)		_	store to D			
Properties Track Name: Foreground: Anno		Appl Track Label:		how Stran Arrow +/- Color - Auto Re	efresh		

Track List

The **Tracks** tab has a list showing all loaded tracks (all tracks listed in the **Data Access > Data Management** table). You can select a track by clicking on it in this list. This table allows you to select any number of tracks, so that you can apply changes to one, all or a subset.

To select multiple tracks

• Click a track while pressing CNTRL (on Mac, press CMD)

Properties

Track Name can be changed by typing in the entry box. This can also be changed from the Data Access > Data Management Table.

To change the Foreground color (FG), the Background color (BG) or the Track Label color, the color square and choose a color. To change the

Track Label Font size, use the font size dropdown box. Color and font size can also be changed from the Annotation or Graph tabs; color can be changed from the Data Access panel.

The **Annotation Label Field** box allows you to have a specific label displayed over your annotations. The default option is gene name, however you can use any property available within the file including, name, ID, gene descriptions or score. The **Annotation Label Field** box dynamically adjusts to offer you all options for a selected track. You can turn off the labels by setting **Label Field** to *none*.

Show Strand

IGB allows you to control the strand display. Checking **Arrow** will show directional arrow heads within introns, and will put a pointed end onto the last exon of each gene model. +/- allows the user to combine both plus and minus strands into a single track, as opposed to the default which draws the + strand above the coordinate axis, and the - strand below the axis. Checking **Color** will change the color of all annotations from the **Fo** reground color to the + color or the - color. The colors can be used as default or changed to your preference by clicking on the color box(es). You can all options at the same time, and these options can be used whether or not the strands are combined.

Restore to Default

Sometimes after changing the appearance of a track in a variety of ways, colors and font sizes, etc., you want to return it to the original settings. R estore to Default will restore the track to the Preferences > Track Default settings.

If the default has been changed or updated *after* loading the selected track, using the **Restore to Default** button will change all the setting to be in line with the *current* **Track Default**.

Track Defaults

- Introduction
- Setting defaults by file type

Introduction

Use Track Defaults to set defaults for how data from different file types will appear once you open them.

000	Prei	ferences	and a second second		
Tracks	Track Defaults	Toolbar	Oth	er Optior	ns 🕨 🕨
Track Default L	ist				
	File Type		BG	FG	Size
	* default *				12
	bam				12
	bedgraph				12
Add			1		Delete
	Background: 🗌 Track Label Font: 12.0	Apply Track Label:)		
Annot	ation Label Field: * no	ne * 🛟			

Setting defaults by file type

To pre-configure how data from a file type will appear once opened and loaded into IGB

- 1. Select a file type from the table or use the **Add** button to add new file type to the table
- 2. After selecting, make the style changes such as color, label, max expand and strand options.

This will not affect any files already opened and loaded into IGB.

Also, if you change settings for a track after loading it, your settings will over-ride these defaults.

The Add button lists file formats that IGB supports.

🤭 🔿 🔿 Add Track Default	
Enter value:	
2bit 📉	
bam 🔍	
bar	
bed	
bedgraph	
bgn	
bgr	
bnib	
bp1	
bp2	
Cancel OK	
	_

Toolbar

- Introduction
- Adding Shortcuts to the Toolbar

Introduction

The IGB 7.0 introduced the **Toolbar**, which provides one-click access to most IGB functions. To see a list of IGB functions you can add to the **Toolbar**, select **File > Preferences > Toolbar**. Use the Toolbar tab in the IGB to view or change keystroke shortcuts for these and other IGB functions.

Adding Shortcuts to the Toolbar

To select a shortcut function to add to the **Toolbar**, check its box in the **Toolbar**? column. To the left you can see the icon as it will appear in the **Toolbar**, and to the right is the name of the function. If you would like to add a function to the **Toolbar** that does not have an icon, let us know! If you would like a function that does not appear in the list at all, let us know! We are always happy to help out with new features and options.

If you would prefer to set and use **Key Stroke** short cuts, click in the **Key Stroke** field for your selected shortcut, and the cursor will appear in an open text box. Type the command key(s) you want to use (CONTROL/META, ALT or SHIFT, or combinations) + a letter or number key. Type ENTER after you set the key stroke combination.

1	Toolbar	Other Options Plugin Repositories	Data Sources
	Toolbar ?	Action	Key Stroke
		About Integrated Genome Browser	
]		Add Bookmark	
	-	Anchor Floating Track	
6		Cancel Script	
1		Change BackGround Color	
A		Change Font Size	
1		Change ForeGround Color	
1		Change Height	
	✓	Change Stack Height for Selected Annotation Tracks	
(Change Track Label Color	
	-	Change Track Name	
	-	Clamp to View	
	-	Collapse	
	-	Combine Strands	
ì		Configure Auto Scroll	
9		Configure Web Links	
ŝ	✓	Configure	
[]		Copy Selected Sequence to Clipboard	META C
		Delete Selected Track	
	✓	Exit	META Q
	-	Expand	
		Export Bookmarks	
	-	Export Preferences	
	-	Float Track	
		Get help or report a problem	
	\checkmark	Go to IGB User's Guide	F1
Į		Hide Visual Tools	
۶		Hide	
ŀ		Home	META H

Key Stroke shortcuts for zooming and scrolling the view will apply both in the main view and in the sliced view. The effect can be confusing when both views are visible in the same window at the same time. Opening the **Sliced View** tab in a separate window to disconnect the two views.

Other Options

- Introduction
- Coordinates
- Residue Colors
 - ORF (Open Reading Frame) Analyzer
- Edge Match
- Codon Display
- Track Resize Behavior
- Data loading and exit behaviors
 Confirmations
- Reset all preferences to defaults

Introduction

Select File > Preferences > Other Options to configure the following:

- · Coordinates track number format
- Sequence base colors
- ORF analyzer
- Edge matching
- Gene model translations
- Track resizing
- · IGB exit and data set loading behaviors

Preferences Other options

	Pret	erences	
Tracks Tr	ack Defaults	Toolbar	Other Options
Coordinates			
Number format:	СОММА 🛟		
Residue Colors A: T:	G :	C:	Other:
ORF Analyzer (Slice	d View tab)		
Stop Codon:	Dynamic	ORF:	Background:
Edge Match			
Color:		🗹 Show E	dge Matching
Codon Display			
🔘 Hide	💽 One	Letter	○ Three Letter
Track Resize Behavi	or		
🔘 Adjust All Track	s	🖲 Ac	ljust Adjacent Tracks
🗹 Ask before exit	V	Confirm before	e loading large data set
✓ Confirm before a		Display Errors	
(Posot profe	rence to default	

Coordinates

These are options for changing number appearance. **FULL** shows the number with no thousands separators and the unit 'kb'. **COMMA** shows the numbers with commas at every 10³ (U.S. style) and with no unit designations. This is the default. **ABBREV** will show the numbers as abbreviated as is reasonable, with unit designations. For example, when zoomed out, numbers appear as 10M, 20M etc. Changes made here to **Number Format** take effect immediately.

Residue Colors

IGB displays letters representing DNA bases (residues) when zoomed-in. Residues are shown in alignment tracks, sequence tracks, and in underneath the coordinate axis.

By default, IGB uses cool colors for A and T residues and warmer colors for G and C residues. **Other** refers to residues that are either 'N', 'X' or '-' (missing) and can be set to a bright color if you are looking for deletions in short read sequences; otherwise you may wish to leave it as the neutral gray.

Click a color swatch to change base colors.

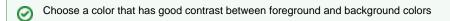
ORF (Open Reading Frame) Analyzer

These preferences affect colors used to indicate stop codons, the ORFs and the background of the ORF tracks that are shown in the Sliced View tab.

Edge Match

Edge matching highlights features that have identical boundaries. Edge matching becomes active when you click items in the display.

Click the swatch to change the edge match color. The selected color applies all annotation tracks.



Codon Display

The **Codon Display** is activated when the sequence of a gene model is loaded and the user zooms in close enough to see the sequence. The codon translation appears within the gene model. User can set the amino acids to appear in one letter format (default), three letter format or be hidden. The colors is based on the chosen foreground color for the track.



Codon display is active only for tracks loaded from 14-column BED detail files.

Track Resize Behavior

Track Resize Behavior is how IGB adjusts other tracks when a specific track height is changed. **Adjust All Tracks** means that as a single track is increased in height, all other tracks are reduced by a commensurate amount. **Adjust Adjacent Tracks** (default) means that all added or subtracted height is removed/added to the bordering track, leaving the rest of the display unaffected.

Data loading and exit behaviors

Confirmations

IGB warns you when you are about to exit the program and before you delete a file or files. Each of these warnings has the option to not be shown again. To turn off these warnings or reset your previous choice, check (or uncheck) these options.

Reset all preferences to defaults

Click Reset preferences to defaults to reset all the preferences to the IGB defaults. This is sometimes useful if you need to troubleshoot IGB.

Be careful, as this action will reset ALL preferences and will also cause IGB to forget the location you were last viewing.

Data Sources Tab

- Introduction
- Data sources
- Data sources order
- Adding new data sources
 - Enter user names and passwords
- Data loading settings
 - Personal synonyms files
 - Genome synonyms File
 - Chromosome Synonyms File
- Cache Settings and clear the cache

Introduction

Use the Data Sources Preferences tab to

- view, add, or edit data source sites from which IGB can retrieve data.
- load personal genome version and chromosome synonyms files
- change how IGB caches data from remote and local sites

When you choose a species and genome version, IGB downloads meta-data files about available genomes from each site listed the Data Sources list. If a site contains data for the currently displayed genome, then IGB lists those sites in the **Available Data Sources/Data Sets** sectio n of the **Data Access** tab. And when you load sequence data into IGB by clicking the Load Sequence button, IGB attempts to obtain the sequence data from the data sources in the same order they appear in the Data Sources list. To open the Data Sources Preferences tab

• Select Data Access > Configure

or

• Select File > Preferences > Data Sources

Data Sources

Graphs	Other Options	Plugin Repositories	Data Source
Data Sources			
Name	Type URL		En
IGB Das		ckload.org/das2/genome	¥ 1
Bioviz UofUtahBioinfo		.org/das2/genome	
NetAffx		ver.hci.utah.edu:8080/DAS2 xdas.affymetrix.com/quicklo	
IGB Quickload		ckload.org/quickload/	ad_data/ ♥ ♥ /dsn ♥
HughesLab		slab.ccbr.utoronto.ca/igb/	
UCSC		ne.cse.ucsc.edu/cgi-bin/das	/dsn 🗖
Ensembl		nsembl.org/das/dsn	
BioVizServerCa.			
Your server na	. Quick file:/Users/a	user / Declaters / IC B9/2 Otection	%20and
		user/Desktop/IGB%20testing	/02 Vanu
		user/Desktop/IGB%20testing	
	<u></u>		Auto Load Data
ſ	Add	Authentication	
(Auto Load Data
(▲ 💌 (Add		Auto Load Data
ersonal Synon	▲ 💌 (Add		Auto Load Data
,	Add		Auto Load Data
Personal Synon Version Synony	Add		Auto Load Data
Version Synony	Add yms rms File		Auto Load Data
,	Add yms rms File		Auto Load Data
Version Synony Chromosome S	Add yms rms File		Auto Load Data
Version Synony	Add yms rms File		Auto Load Data
Version Synony Chromosome S Cache Settings	Add yms yms File		Auto Load Data
Version Synony Chromosome S	Add yms yms File		Auto Load Data
Version Synony Chromosome S Cache Settings	Add yms yms File		Auto Load Data

Data sources

The **Data sources** table lists all active and inactive data sources that have been added to IGB. Active data sources have a check in the active column. When you select a genome version, IGB contacts each active server to determine if the server has any data sets for the selected genome. If a server has data sets for the selected genome, the available data sets will appear as options in the **Available Data Sources/Data Sets** section of the **Data Access** tab.

Data sources order

For some genome versions, more than one data source may provide sequence data. When you click the **Load Sequence** button (top right), IGB loads sequence directly from the first server on the list that supports the currently selected genome. Starting with IGB 6.6, you can change the order of data sources listed in the **Data Sources** preferences tab.

To change sequence order, click **Up** and **Down** buttons (red box).

Name	Type	URL	En
NetAffx	DAS2	http://netaffxdas.affymetrix.com/das2/genome	\checkmark
NetAffx	QuickL	http://netaffxdas.affymetrix.com/quickload_data/	<u></u>
Bioviz	DAS2	http://bioviz.org/das2/genome	\checkmark
IGB Quickload	QuickL	http://igbquickload.org/quickload/	\checkmark
UCSC	DAS	http://genome.cse.ucsc.edu/cgi-bin/das/dsn	
Ensembl	DAS	http://www.ensembl.org/das/dsn	
HughesLab	QuickL	http://hugheslab.ccbr.utoronto.ca/igb/	
BioVizServerCa	QuickL	http://bioviz.org/cached/	\checkmark
UofUtahBioinfo	DAS2	http://bioserver.hci.utah.edu:8080/DAS2DB/gen	
		<i>d</i>	
_		🗹 Auto Load	l Dat

Adding new data sources

Use Add.. to add a new data source. A new window will open with boxes and a menu for you to specify a new data sources.

Enter user names and passwords

Click Authentication.. to enter or change the user name and password IGB uses to access private data sources.

Most data sources don't require authentication. If you select a public (open) data source and click Authentication, IGB will allow you to enter a user name and password but won't use it when contacting the data source.

Data loading settings

Some servers are configured to automatically load certain foundation data sets, such as the RefSeq gene models, into IGB as soon as the corresponding genome is selected. To block this behavior, uncheck **Auto Load Data**.

Personal synonyms files

Different data sources often refer to the same genome version or chromosome by different names.

For example, hg17, ensembl1834 and H_sapiens_May_2004 all refer to the same genome assembly and sequence. Similarly, some data providers use **chr1** to refer to chromosome one while others may use **1** to mean the same thing.

IGB provides a system for designating synonyms for sequences and genome versions.

Genome synonyms File

A version synonyms file matches genome versions and allows you to display data from multiple sources in the same window.

Each time IGB restarts, synonyms will be loaded and merged from all the servers in use as well as from any personal synonyms file that you have specified.

A genome version synonyms file follows a simple tab-delimited format in which synonyms for the same genome appear in the same line, separated by tab characters.

Chromosome Synonyms File

A chromosome synonyms file is tab-delimited. Each line lists possible names for the same sequence.

Each time IGB starts, synonyms will be loaded and merged from all the servers in use as well as from any personal chromosome synonyms file entered here.

Personal Synonyms	
Version Synonyms File	
Chromosome Synonyms File	

Cache Settings and clear the cache

IGB caches data that you have loaded over the web in order to speed the loading process the next time you use it. Files loaded from your local file system will not be cached.

Clicking the Empty Cache button forces IGB to clear its local cache of downloaded data sets.

Tip. If you suspect that the cache files have somehow been corrupted, which may happen if a download was halted unexpectedly, unexpected behavior may result. Click **Empty Cache** if data sets you've opened from a QuickLoad site don't appear to have any data or cause IGB to freeze.

	Cache Settings	
click to empty cache	Cache Behavior Normal Empty Cache Cleared	•

Adding and Managing Data Source Servers

- Data Sources
- Server Priority
- Add...
- Edit...
- Refreshing a data source

Data Sources

- The Data Sources panel shows a list of servers including Name, Type, URL, and an Enabled checkbox. To turn a source off or on, click the Enabled checkbox. When data sources are disabled, IGB will show them as grayed out.
- The Auto Load Data feature (when checked) will allow IGB to automatically load some data sets. (Only data sets configured with annots.xml load hint Whole Genome will autoload into IGB.)

 IGB Quickload Quick http://igbquickload.org/quickload/ IGB Das DAS2 http://igbquickload.org/das2/genome Bioviz DAS2 http://bioviz.org/das2/genome UofUtahBioinf DAS2 http://bioserver.hci.utah.edu:8080/DAS2DB/g NetAffx Quick http://netaffxdas.affymetrix.com/quickload_da HughesLab Quick http://hugheslab.ccbr.utoronto.ca/igb/ 		ſ
Bioviz DAS2 http://bioviz.org/das2/genome UofUtahBioinf DAS2 http://bioserver.hci.utah.edu:8080/DAS2DB/g NetAffx Quick http://netaffxdas.affymetrix.com/quickload_da	₫	
 UofUtahBioinf DAS2 http://bioserver.hci.utah.edu:8080/DAS2DB/g NetAffx Quick http://netaffxdas.affymetrix.com/quickload_da 	-	11
NetAffx Quick http://netaffxdas.affymetrix.com/quickload_da		
	\sim	Ш
HughesLab Quick http://hugheslab.ccbr.utoronto.ca/igb/		1
		-
UCSC DAS http://genome.cse.ucsc.edu/cgi-bin/das/dsn	☑	A
Ensembl DAS http://www.ensembl.org/das/dsn		¥
Auto Load	d Da	ata

Server Priority

Server order affects the order in which IGB retrieves genomic sequence data. When you click the Load Sequence button, IGB will request sequence data from servers in the order in which they are listed. Once it finds a server that can deliver the data, it uses that server's data and ignores the rest of the data sources on the list.

Re-order the list using the up and down arrows. Select the server, and click the arrows to move it up and down in the priority list.

Add...

Use the Add button to add a new DAS, DAS/2, or QuickLoad server.

To add a data source:

• Click the Add... button to open the Add Data Source window.

$\bigcirc \bigcirc \bigcirc$	Add Data Source
Name	Your server name
Type	QuickLoad
URL	http://
	Cancel Add Server

• Choose a unique name for this new data source. This is the name that will appear in the Data Access panel.

- Choose the server type (DAS/1, DAS/2, or QuickLoad).
- The URL name should start with protocol strings "file://", "http://", or "https://".
 - If this is a local QuickLoad server, you can use the Local Directory button ('...') to select a folder on your local file system.
 - A URL for a local directory on a Windows machine might look something like this: "file:///C:/Documents and Settings/QuickLoad/"
- Click the Add Server button.
- If the server is private and requires authentication, IGB will next ask you to enter a user name and password.

Edit...

To edit the name or URL associated with a data source, select the source you want to edit and click **Edit...** A new window will open identical to the **Add...** window. Use it to enter new values. (You will not be able to edit any of the default IGB data sources.)

After completing the edit, click the load icon to the left the server name to refresh server settings. This will also force IGB to re-read the server's configuration files.

Refreshing a data source

If you are developing a new data source for IGB, you may need to force IGB to re-read its configuration files. Click the Refresh button to the left of a data source to force IGB to re-read the data source server's configuration files.

Plugin Repositories Tab

With the inclusion of Plugins in IGB, we provide an interface for including new Plugin repository sites. IGB supported repositories are shown.

00	Pr	eferences	
Graphs	Other Options	Plugin Repositories	Data Sources
lugin Repositorie	5		
Name 🔺	URL		Enab
Bioviz	http://bioviz.org	/igbserver	
Genentech	http://research-	pub.gene.com/igb_plugins	\checkmark
Genentech devel	http://research-	pub.gene.com/igb_plugins/	develo 📃
		Add	Remove

Repositories can be accessed by checking the Enabled box; they can be added by clicking the Add button, and the following window opens:

00			
Name	Your server name		
URL	http://		
		Cancel	Add Server

Provide the information, and be sure the new repository has **Enabled** checked.

Repositories can be made inaccessible by unchecking the Enabled box; they can be completely removed by selecting the repository and clicking the **Remove** button.

Sharing Preferences

Importing and Exporting Preferences

You can set each preference manually, or you can export and import preferences to an XML file. This can be done to save them or share them among a group of people who are working on similar or related projects. This is also useful if you want to move IGB from one computer to another, or if you would like to share preferences with other users.

In earlier versions of IGB, preferences were set by the user by editing a file called "igb_prefs.xml". That file is deprecated, though it is still used by some users for certain advanced functions.

Exporting Preference Settings

You can export preference settings for use in different instances of IGB or to share them with other IGB users.

- Choose File > Preferences.
- In the Preferences dialog box, choose Preferences menu > Export Preferences.
- Choose the destination and filename to which you want to save your preference settings.
- Click Save.

/!\

Importing Preference Settings

You can import preference settings that you or another IGB user has exported. You can freely exchange preferences between Windows, Linux, and Macintosh systems. Preferences in the file that you import are merged with your existing preferences.

Be sure you trust the provider of the file.

- Choose File menu > Preferences.
- In the Preferences dialog box, choose Preferences menu > Import Preferences.
- Choose the saved preferences file and click **Open**.

Troubleshooting

See the links below for common problems and their solutions.

Note: To run IGB, you will need Java installed on your computer. Most computer types already have Java, but if you need to install it yourself, visit the Java download page. To find if you already have Java, click the link labeled Do I have Java?

IGB can't be opened because it is from an unidentified developer (Mac)

If you are a Mac user, you may see the following message when you attempt to download and run IGB from the IGB download page:



The IGB download page uses Java Web Start to distribute IGB, and so you may see this message if your Mac is configured to block Java Web Start applications developed by non-Apple verified developers.

There are at least three (maybe more!) workarounds. Each one has benefits and drawbacks. If you have questions, let us know.

Workarounds

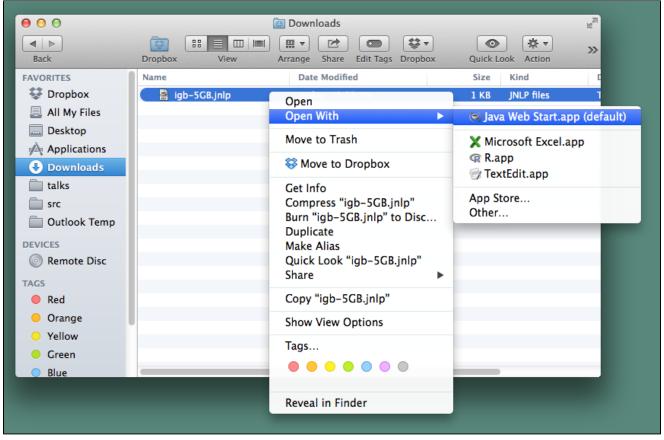
- Use Java Web Start app to open IGB JNLP file
- Download igb.zip and run IGB using an IGB script
- Modify the security settings on your Mac

Use Java Web Start app to open IGB JNLP file

When you download or launch IGB from the BioViz.org download page, your browser downloads a special file called a "JNLP" file. If you open this file using Java Web Start.app on your computer, then IGB will download and run.

- 1. Open your Downloads folder and locate the JNLP file downloaded from BioViz.
- 2. CNTRL-click (or right-click) the file.
- 3. Select Open With > Java Web Start.app

This is probably the safest option but may be less convenient than the other options listed in the following section. Also it requires that Java Web Start be installed on your system.



Download igb.zip and run IGB using an IGB script

One big disadvantage of this workaround is that you won't automatically get newer versions (including bug fix releases) when they become

available on the BioViz site. The major benefit is that it nearly always works no matter how your system is configured.

- 1. Go to the IGB downloads page and download igb.zip (See the section titled Other ways to get IGB)
- 2. Unzip igb.zip by double-clicking or using "unzip" command in Terminal. This creates a new a folder named igb.
- 3. Open the igb folder and double-click a run_igb.command script.
- 4. A new Terminal will open and IGB will launch. Don't close the Terminal window or IGB will shut down without saving any bookmarks you've made or giving the opportunity to save your session.

Modify the security settings on your Mac

This is least safe option, but the most convenient. Don't do this unless you're confident you can avoid mistakenly running programs downloaded from the Internet.

See the section titled **Disable GateKepper's Unidentified Application** in this documentation for instructions on how to modify the security settings on your Mac.

Unable to launch the application

If you typically download and run IGB using the Bioviz Java Web Start launch pages (the Download IGB page), you may be experiencing problems with old-of-date files or preference settings. Alternatively, your organization may be blocking certain types of internet-based applications (like IGB) from downloading and running on your desktop.

If you are unable to download and launch IGB from the Bioviz site, try the following:

- Verify that your you're using Java 6 or higher. IGB can run only on computers with Java 6 (or higher) installed. If you're using Java Web Start (e.g., from the IGB download page) to start IGB, also verify your Web browser is also using Java 6. See http://www.javatester.org/version.html to find out if you are running Java 1.6 or higher.
- Clear your Web browser cache and re-open your browser. Sometimes your browser will attempt to run IGB using an out-of-date, cached version, which affects various elements of IGB including memory requirements. For example, IGB uses a deployment descriptor file that can get out of date. Deleting your browser cache gets rid of out-of-date files, allowing you to get the latest versions, which then will allow you to run IGB.

For instructions see How do I clear my web browser's cache from Indiana University IT Knowledge Base.

- Clear your Java Cache. The Java cache may contain outdated or corrupted copies of the deployment descriptor or libraries. This affects application start-up and arguments passed to the application. See https://elearn.usu.edu/java/clear-java-cache.html for instructions on how to clear the Java cache on Windows or Mac OS X. For Linux, simply delete the directory ~/.java
- Delete the IGB Cache folder. This will clear all cached data, as well as remove any corrupted data that could be blocking IGB from running. These directories are hidden; you will need to enable viewing of hidden files, or delete them from the command line. Warning: D o not delete ENTIRE .igb directory because if you do, you will lose your bookmarks along with cached data. Only remove the IGB cache folder.
 - MacOSX: Delete folder UserHome/.igb/cache
 - Windows: Delete folder UserHome/Application Data/IGB/cache
 - Linux: Delete folder ~/.igb/cache
- Clear the plist (For Mac users only). IGB stores the users' Preferences information in different locations on different platforms. On Windows, Preferences are stored in the windows registry. On Linux, preferences are stored in ".xml" files. In both of these cases, we recommend against editing these files directly. In Mac OS, Preferences are stored in a file called a "plist," which you can edit using a Mac utility called the Property List Editor. To edit your plist using the Property List Editor, open a new Finder window and open Library > Preferences in your home folder. Double-click the file named com.affymetrix.igb.plist, which should launch the Property List Editor. Select the item called "/com/affymetrix/igb" and click "Delete Item." (See image below.) This will re-set all your preferences to their defaults. Wa rning: This action will also delete all data sources and passwords you've added; you will need to add them back manually once IGB is able to run.

000	📄 com.af	fymetrix.igb.plist 🛛 💬
Add Item Delete Item		
Кеу	Туре	Value
▼ Root	Dictionary	(1 item)
/com/affymetrix/igb/	Dictionary 🌲	(32 items) +

- Download IGB executable. If none of the above solutions help, it's possible that your organization is preventing Java applications (like IGB) that are downloaded from the internet via Java Web Start from running on yours and other users' computers. If this is the case, then you should download IGB directly from the Sourceforge Web site as a "zip" file, unpack it, and then use the "run_igb" scripts we've developed for users who can't run IGB from Java Web Start. To do this:
 - Visit http://sourceforge.net/projects/genoviz/files/.
 - Choose the latest release of IGB (e.g., IGB 6.4.1 as of this writing.)
 - Download the file named igb.zip
 - Once it downloads, unpack it. (On most systems, all you have to do is double-click the file, and on some systems, it will unpack automatically.)
 - · Once unpacked, you should see a folder called "igb"
 - Open the folder and double-click run_igb.sh (on Mac or Linux) or run_igb.bat (Windows.)
 - IGB should then launch and run.

Java Web Start starts, but fails to launch IGB

The IGB download page uses Java Web Start to download and launch IGB on your computer. The way this works is that your browser downloads what's called a "JNLP" (java network launch protocol) file, which is also sometimes called a "deployment descriptor." When your browser downloads the file, it then launches a second program called Java Web Start on your computer that initiates the IGB download and starts IGB for you. (It may also ask if you would like to create an IGB shortcut on your desktop.)

If Java Web start launches and then fails to start IGB, try the following steps to trouble-shoot:

• Java Web Start fails with an error like "Cannot grant permissions to unsigned jars."

Likely this is caused by a recent bug introduced in OpenJDK. Easiest workaround is to use the Sun JDK for Java Webstart. See http://sourceforge.net/tracker/?func=detail&aid=3063085&group_id=129420&atid=714744 for more details about this bug in OpenJDK.

• Under Windows, the Java Web Start version of IGB fails without presenting a window or small loading window flashes on the screen.

Reset your browser cache. For details, see: How do I clear my web browser's cache from Indiana University IT Knowledge Base. This is caused by a bug in a stale copy of the deployment descriptor. One possible causes is that the deployment descriptor requested more memory than 32-bit Windows could allocate and causes Java to crash.

- Java Web Start fails with an error like "Java virtual machine unable to start". You may have requested more memory for IGB than your machine can handle. Clear your browser cache, close and reopen your browser, and try the "small memory" version of IGB. For details on how to clear the cache, see How do I clear my web browser's cache fr om Indiana University IT Knowledge Base.
- Under Macintosh OS X with Firefox, the Java Web Start version of IGB fails. Error message says "java.lang.UnsupportedCla ssVersionError: Bad version number in .class file". This is a Firefox bug. Firefox will not correctly launch IGB once the Java Plug-In has been started. Restart Firefox and try again or use a different browser to launch IGB.

• Under Macintosh OS X 10.8 (Mountain Lion), the Java Web Start version of IGB fails. Error message says "The digital signature is not trusted. Java will not allow any access to this application." With 10.8, Apple has introduced new security feature i.e. Gatekeepter. By default Gatekeeper is enabled. This will not allow to run any apps that has not been download from Mac app store. Here's the link on how to disable it.http://answers.uchicago.edu/page.php?id=2548

IGB runs, but acts strangely.

If you are able to run IGB but are observing strange behaviors, try the following:

- Verify that your you're using Java 6 or higher. IGB can run only on computers with Java 6 (or higher) installed. If you're using Java Web Start (e.g., from the IGB download page) to start IGB, also verify your Web browser is also using Java 6. See http://www.javatester. org/version.html to find out if you are running Java 1.6.
- Try running a lower memory option of IGB. If you are running an older computer and you try to launch the higher memory options of IGB, you may see IGB try to start up and then quit, reporting an error like "Java virtual machine unable to start." If this happens, you may have requested more memory for IGB than your machine can provide. If you see this error, clear your browser cache, close and reopen your browser, and try a "small memory" version of IGB.
- Clear your browser cache and re-open your browser. Sometimes your browser will attempt to run IGB using an out-of-date, cached version, which affects various elements of IGB including memory requirements. For example, IGB uses a deployment descriptor file that can get out of date. Deleting your browser cache gets rid of out-of-date files, allowing you to get the latest versions, which then will allow you to run IGB. For instructions see How do I clear my web browser's cache from Indiana University IT Knowledge Base.
- Clear your Java Cache. The Java cache may contain outdated or corrupted copies of the deployment descriptor or libraries. This
 affects application start-up and arguments passed to the application. See https://elearn.usu.edu/java/clear-java-cache.html for
 instructions on how to clear the Java cache on Windows or Mac OS X. On Linux, simply delete the directory ~/.java
- Clear your IGB cache. This will clear any cached data, but will also remove any corrupted data that could be causing crashes or odd behavior while the application is running. Go to File > Preferences > Data Sources and click the 'Empty Cache' button at the bottom of the screen.
- Reset Preferences to default. You should use this as a last resort, because this will remove all saved preferences, including
 servers and passwords you've entered. *To do this, go to *File > Preferences > Other Options and click the 'Reset all preferences
 to defaults' button at the bottom of the screen.

I can't see my data!

If you are having trouble viewing your data, see the following:

- · Ensure that your file is in a format we support
- Click Load Data button
- .bam files need a .bai file
- I opened my sequence file (".fasta" or ".bnib" or "twobit") but all I see is a gray bar and dashes.

Ensure that your file is in a format we support

IGB can open and read nearly all of the common data and graph files used with genomics data. For more information about supported file types, please see File Formats. We also take requests - if you would like us to support a new type of file format, please let us know.

Click Load Data button

IGB allows you to open files from your desktop, local servers, URLs or other data sources.

However, IGB will not instantly start loading the data into the display.

When you open files, IGB adds them to the list of opened files in the **Data Management** table in the **Data Access** tab. Note that the Data Management table lists a Load Mode for each opened file or data set. By default, IGB adds most files and data sets using the **Region in View** loa d mode. Data sets and files with load mode **Region in View** will load into the main data display when you click the **Load Data** button. To display data from these files, zoom in to a region you want to see, and click **Load Data**. Whatever data in the file that overlaps with the current region in view will appear in the display.

Tip: If your data file is not large, change Load Mode to Genome. If you do that, IGB will then read and display all the data in the file. Examples of data sets that work best when loaded in the Genome mode include: gene models for the entire human genome or significant regions identified in a ChIP-Seq experiment.

For more about loading files, please see Loading data.

.bam files need a .bai file

IGB can display alignments data from .bam files. However, it requires a .bam index file (called a .bai) to be present in the same folder as the .bam file.

I opened my sequence file (".fasta" or ".bnib" or "twobit") but all I see is a gray bar and dashes.

To save memory, IGB doesn't load sequence data until you click the **Load Sequence** button. To view the bases, click the **Load Sequence** button and then zoom in.

Unable to detect proxy setting

If you have downloaded IGB as igb.zip

Open 'run_igb.sh' in a text editor. Look up for following lines.

```
# Use 32MiB as initial heap size
echo $VMARGS[*] | grep -q '\-Xms\|\-ms' || VMARGS[${#VMARGS[*]}]="-Xms32m"
```

Add following lines after it.

```
# Use proxy serverecho
$VMARGS[*] | grep -q '\-DproxySet' || VMARGS[${#VMARGS[*]}]="-DproxySet=true"
# Replace the IP address of the server to the name/IP of your proxy-serverecho
$VMARGS[*] | grep -q '\-Dhttp.proxyHost' ||
VMARGS[${#VMARGS[*]}]="-Dhttp.proxyHost=wwwproxyhost"
# Replace the port to the port that is used at your proxy-serverecho
$VMARGS[*] | grep -q '\-Dhttp.proxyPort' ||
VMARGS[${#VMARGS[*]}]="-Dhttp.proxyPort' ||
```

If you are running IGB using a jnlp file.

Open jnlp file in a text editor. Look up for following lines.

```
<argument>-prefs</argument>
<argument>http://www.bioviz.org/igb/releases/igb-6.4/igb_prefs.xml</argument>
```

Add following lines after it.

```
<argument>-DproxySet</argument>
<argument>true</argument>
<argument>-Dhttp.proxyHost</argument>
<argument>wwwproxyhost</argument>
<argument>-Dhttp.proxyPort</argument>
<argument>wwwproxyport</argument>
```

Replace wwwproxyhost and wwwproxyport with the IP/name of your working www proxy and proxy port respectively.

e.g wwwproxyhost can be replaced by 192.168.0.1 and wwwproxyport can be replaced by 3128

Note: This solution is not yet verified by IGB team.

My data isn't there

When you open or drag a file into IGB, the file will appear in the **Data Management Table** on the **Data Access** panel. Be sure that your file was properly opened, and that it appears in the list. IGB will also open an empty track for this file with a grayer background, waiting for you to load the data.

There can be several reasons that your data has not appeared:

• Data not yet loaded

IGB does not immediately load your data when you open a file or a URL. Most of today's data sets are to large to visualize all at once. After you zoom in to a more refined area, click **Load Data**. Your data will be loaded and appear in the **Data Display Area**.

• The file names for genome version or chromosomes do not match

Often times, different files will use different names for either the genome version (e.g. "human genome build 35" is also named "hg17", "ensembl1834", or " H_sapiens_May_2004") or chromosome name (e.g. "chromosome 1" is also named "1", "chr1", "chr 1", "chr1" or "chrom1"). If the files have mismatched names, IGB is not able to align them. This can be easily corrected the instructions on the Personal Synonyms page to adjust the names.

• You are in an area where there is no data

Sometimes, while zoomed into low data areas, it will seem like your data is not there. However, if the background color in your data track is the selected **BG** color (and not a darker/grayer color) IGB has loaded all information in the region. Zoom out or scroll to a new region and click **Load Data**.

The IGB menus have disappeared

There is a Java bug (in version 1.7, update 13) in which opening a pop-up window from within IGB causes the IGB menus to disappear. This issue has been seen with the survey, Preferences and even sequence viewer. Until the next Java update, this will likely remain an issue.

The simple fix is to minimize and reopen the IGB window. All of the menus will be restored.

Where is my IGB download? (Safari)

If you are using Safari as your web browser, you may be wondering where your IGB JNLP file is located after downloading IGB. This is because Safari downloads JNLP files into an uncommon directory (/private/var/.../T).

Ċ	Reader O		Safari
Download Now	Downloads	Clear	

Make Safari download JNLP files into the Downloads directory

- 1. Open a Safari window and select Preferences from the Safari menu located at the top of your screen.
- 2. Select the General Tab.
- 3. Uncheck the box next to Open "safe" files after downloading.
- 4. Quit and then reopen Safari.

JNLP files will now download into the Downloads folder.

Safari	File	Edit	View	History	Bookmarks	Develop	Window	Help		_			
About Safari	Safari Extensi	ons											
Prefere	ences		Ж,	←1.∶	Select	Prefere	ences						
	e Brows Safari					000			A		General	CV2	
Service	25		•		1	General Tabs	AutoFill I	Passwords	Security Pr	rivacy	Notifications Extensions Adva	inced	10000
Hide S Hide C Show /	thers		жн жн							_	Firefox (32.0.3)	•	
Quit S			жQ					Default s	earch eng	line:	Google	\$	
Quit St	ararr							Safa	ri opens w	vith:	A new window	\$	
			2	. Sele	ct the		N	ew windo	ws open w	vith:	Top Sites	\$	
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									Homepa	age: 🖡	http://www.apple.com/star	tpage/	
											Set to Current Page		
								Remove	history ite	ems:	After one year	\$	
								То	p Sites sho	ows:	12 sites	\$	
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							Remo	ve downlo	oad list ite	ems:	Manually	\$	
				3. Un	check	this bo	»х —		;		Open "safe" files after dov "Safe" files include movies, pic PDF and text documents, and	tures, sounds,	?

Installing and Updating Java

Running IGB requires Java. Your system already may have Java installed. If not, follow the link below for instructions on how to install or update Java on your computer.



Click here for instructions on installing Java (version 8) on your computer.

Tutorials

Welcome

To help new and veteran users get acquainted with IGB features, we've developed the following tutorials.

If you have ideas for improvement or new tutorials, please let us know.

Email your suggestions to:

Ann Loraine aloraine – at – uncc.edu

Thank you and best of luck in your research,

The IGB Team

Assessing splicing - Cold-induced alternative splicing of Arabidopsis SR45

• Introduction

- Setting up
 - Open read alignment files
 - Customize the tracks
- Analyze Splicing
 - · Background on SR45, a plant-specific, putative splicing regulator
 - A note on nomenclature AGI locus codes
 - SR45 mutant phenotype
 - View SR45 in IGB
 - Use the search tab to search for AT1G16610 (SR45 AGI locus code)
 - View genomic sequence and protein translations for SR45 splice variants
 - Load RNA-Seq reads from cold stress experiment
 - Assess relative abundance of SR45 splicing isoforms
- Finishing up export an image showing what you found

Introduction

The Arabidopsis and other plant genomes include many genes predicted to encode multiple splice variants.

However, it isn't always clear which of these splicing variants are the most highly expressed in specific cell types, organ structures, or conditions.

One way to assess which isoform is most abundant is to visualize aligned reads from an RNA-Seq experiment.

If the read lengths are sufficiently long (75 or more bases) and the gene of interest has sufficient coverage, you can usually tell which variant is the most abundant.

And if you have data from experiments that investigate different treatments, you can sometimes also find out if the ratio of isoforms has changed as a result of the treatment. This is called condition-dependent alternative splicing, which just means: a treatment has changed the pattern of splicing at a locus.

In this tutorial, you'll learn how to use IGB to investigate splicing using RNA-Seq data. Using data from a cold-stress experiment in Arabidopsis thaliana, you'll learn how to:

- Identify the dominant isoform for a gene
- Observe condition-dependent alternative splicing

This tutorial will feature SR45/AT1G16610 from Arabidopsis thaliana, a putative splicing regulator that is itself alternatively spliced.

Setting up

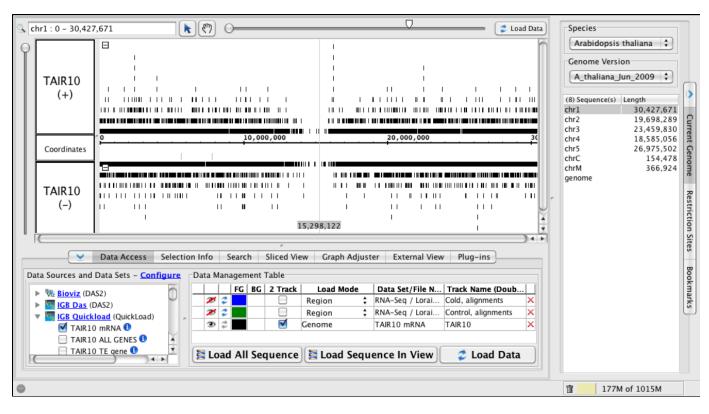
Follow these instructions to configure IGB to get started looking at a cold stress RNA-Seq data from Arabidopsis thaliana:

- Start IGB
 - Download and launch IGB using Java Web Start from http://www.bioviz.org/igb/download.shtml
 - Or download igb.zip from http://sourceforge.net/projects/genoviz/files/.
 - To launch IGB from igb.zip, double-click igb.zip to unpack it, open folder igb, and double-click run_igb.bat (Windows) or run_igb.command (Mac)
- Choose species Arabidopsis thaliana, Genome Version A_thaliana_Jun_2009

Note that the gene models should automatically load.

Tip: Color the gene track black (foreground) and white (background) to make attractive images for slides and printing. Just click the color swatches (squares) in the Data Management table to change colors.

IGB 6.7 showing protein-coding (TAIR10 mRNA) chr1 gene models, with gene models changed to black and background changed to white.



Open read alignment files

Now start opening read alignment files from chilled and unchilled samples.

- Under the Data Access tabbed panel, open
 - Data Source IGB Quickload > RNASeq > Loraine Lab > Mixed Cold > SM > Reads
- Click the checkboxes next to data sets named:
 - Control, alignments
 - Cold, alignments

Please Note: When you click the checkboxes, IGB will access the files to make sure they are readable, but it won't immediately start loading the data. The reason for this is that many genomic data sets, especially next-generation sequencing data, are very large. To save memory and time, IGB gives you a chance to zoom and pan to a region you are most interested in viewing before loading the data into the viewer.

If it was able to access the files, IGB will add the data sets to the **Data Management Table** under the **Data Access Tab** and will also create new (empty) tracks for the data sets. Once the data are loaded, it will appear in these new tracks.

Customize the tracks

If you don't like the default color scheme, take a moment to customize your track settings colors.

Foreground and background color schemes are set by the IGBQuickLoad server, but you can change them in IGB if you like. IGB will
 remember your color choices between sessions.

Click the color squares in the **Data Management Table** (FG is foreground, BG is background) or choose **File > Preferences > Tracks** to open a new Window that let's you customize different aspects of the display at several at once. Click each row in the table and give each row a white background. Choose colors for individual rows by clicking on them and then selecting options.

Tip: Customize multiple tracks at once by choosing File > Preferences > Tracks

Tip: Simplify the track name and increase track name label size to make the Track Labels easier to read.

Analyze Splicing

The remainder of the tutorial will focus on one gene - SR45, also known as AT1G16610.

Background on SR45, a plant-specific, putative splicing regulator

SR45 encodes a putative splicing regulator resembling members of the so-called SR protein family, a group of proteins that contain one or more RNA-recognition motifs (RRM) and regions enriched with serine-arginine (SR) or arginine-serine (RS) amino acid repeats.

The function of the RS/SR repeats is not well-understand, but one idea is that they help stabilize interactions between pre-mRNA transcripts and proteins during splicing or spliceosome assembly.

- Read about SR45 at the Arabidopsis Information Resource
- Read about SR45 in Entrez Gene at the National Center for Biotechnology Information (USA)

A note on nomenclature - AGI locus codes

Like most Arabidopsis genes, SR45 has a second name (AT1G16610) called an AGI locus code that was first assigned by the Arabidopsis Genome Initiative, which sequenced and annotated early versions of the Arabidopsis genome. Use the AGI code to locate the gene in Integrated Genome Browser. (More on this later.)

SR45 mutant phenotype

One mutant line (called sr45-1) carrying a disruption in the SR45 gene has been characterized. These plants exhibit multiple defects, including misshapen petals and reduced root growth. SR45a expresses at least three known splicing variants.

An article titled **Two alternatively spliced isoforms of the Arabidopsis SR45 protein have distinct roles during normal plant development** i nvestigated how Variants 1 and 2 differ with respect to SR45 function.

The authors found that transforming the sr45-1 plants with splice variant 1 corrected the petal phenotype, while transforming with variant 2 corrected the root phenotype. (Variant 3 was not tested.)

The difference between the variants was not large, but clearly the two different splice forms affect development in different ways.

Another article titled **The plant-specific SR45 protein negatively regulates glucose and ABA signaling during early seedling development in Arabidopsis** reported that SR45 plays a role in sugar sensing and ABA pathways during early seedling development. (ABA is a plant hormone that helps keep seeds from sprouting prematurely.) This article represents one of the first discoveries of a link between a splicing regulator and a hormone response pathway in plants.

- Read Two alternatively spliced isoforms of the Arabidopsis SR45 protein have distinct roles during normal plant development
- Read The plant-specific SR45 protein negatively regulates glucose and ABA signaling during early seedling development in Arabidopsis

View SR45 in IGB

Use the search tab to search for AT1G16610 (SR45 AGI locus code)

To view the SR45 gene in IGB, click the Search tab and enter the AGI locus code for SR45: AT1G16610.

Three rows of results will appear. Double-click one to navigate to the SR45 region.

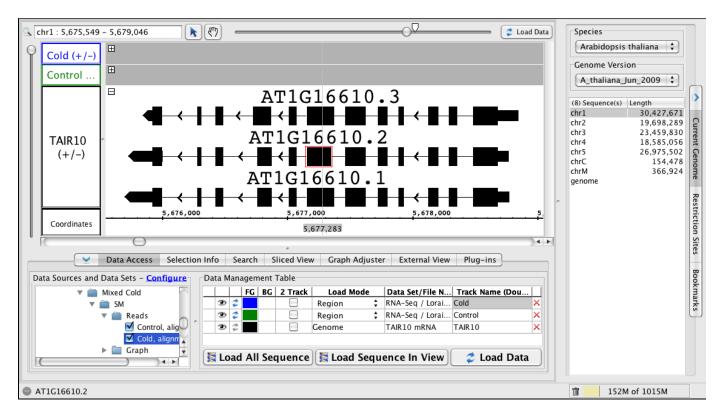
You'll see three variants. Note that transcription proceeds from right to left - the promoter is on the right in this case because the gene resides on the minus strand of the chromosome.

Let's customize the display even further - change the "max stack depth" and show all gene models in one track:

- Right-click (or control-click on Mac) the TAIR10 track label
 - Choose Change > Adjust Max Stack Depth and enter 3 to remove extra white space above the gene models. (Note that if you zoom out, gene models from genes with more than three gene models may be drawn on top each other.)
 - Choose Change > Show 1 track (+/-) to combine plus and minus strand gene models into the same track

When you're done, you'll see something like the image below.

Note that one exon is selected in variant AT1G16610.2. This is the differentially spliced exon that differs between variants 1 and 2. In variant 2, the exon has a different five prime end. It encodes a slightly shorter protein than Variant 1.



View genomic sequence and protein translations for SR45 splice variants

- Right-click (or control-click on Mac) variant 1 AT1G1660.1.
- Choose View Genomic Sequence in Sequence Viewer
 - When the viewer opens, click **Show cDNA**
 - Click Show > +2 Translation to view the amino acids encoded by variant 1.

Note how the SR45 protein contains C-terminal and N-terminal RS/SR motifs. This is unusual for an SR protein and some researchers believe that this unusual structure makes SR45 a plant-specific SR protein, and others argue that it should not properly be considered an SR protein.

 Read this review article on SR proteins: Implementing a rational and consistent nomenclature for serine/arginine-rich protein splicing factors (SR proteins) in plants.

Genomic sequence viewer with amino acid translation of splice variant AT1G16601.1:

T V C L S V S L L * S F L S A R V L K Q G F S R F S R 0 CACAGTCTGTCTGTCTGTCTCTCCTCTAATCTTTTTTTTCCGCTAGGGTTTTGAAGCAAGGTTTCTCGCGATTTTCTC
Y L S P M A K P S R G R R S P S V S G S S S R S S S 80 GATATCTCTCGCCCATGGCGAAACCAAGTCGTGGCCGTCGTTCCCCCTCCGTGTCTGGCTCGTCATCTCGTTCCAGTTCC
R S R S G S S P S R S I S R S R S R S R S L S S S S 160 AGATCTCGTTCGGGTTCGAGCCCCTCCAGGTCTATTTCCCGCTCACGCTCCCGTTCTAGATCGCTCTTCATCTTCATC
PSRSVSSGSRSPPRRGKSPAGPARRG 240 TCCTTCGCGAAGCGTCAGTTCTGGGAGTCGCAGTCCTCCACGTCGTGGAAAAAGTCCTGCTGGACCTGCTAGGCGAGGTC
S P P P P S K G A S S P S K K A V Q E S L V L H V 320 GCTCTCCTCCTCCACCATCTAAAGGAGCATCTTCACCTTCCAAGAAAGCTGTTCAAGAATCTCTTGTTCTCCATGTT
D S L S R N V N E A H L K E I F G N F G E V I H V E I 400 GATTCTCTTAGCAGGAATGTGAATGAAGCCCATCTCAAAGAAATCTTTGGCAACTTTGGTGAAGTTATACATGTCGAAAT
A M D R A V N L P R G H G Y V E F K A R A D A E K A Q 480 TGCGATGGATCGAGCTGTTAATCTTCCAAGAGGGACATGGTTATGTTGAGTTCAAGGCAAGAGCTGATGCTGAGAAAGCTC
L Y M D G A Q I D G K V V K A T F T L P P R Q K V S 560 AGCTCTACATGGATGGTGCCCAAATTGATGGAAAGGTTGTTAAAGCAACGTTCACACTACCACCTCGTCAGAAAGTATCT
S P P K P V S A A P K R D A P K S D N A A A D A E K D 640 TCACCCCTAAACCTGTCTCAGCTGCACCAAAAAGAGATGCTCCAAAATCTGATAATGCTGCTGCTGACGCTGAGAAAGA
G G P R R P R E T S P Q R K T G L S P R R R S P L P R 720 TGGTGGTCCCAGGCGCCCAAGAGAGACATCTCCTCAACGGAAAACAGGGCTTTCTCCGAGAAGGCGATCACCTCTACCTA
R G L S P R R R S P D S P H R R R P G S P I R R R G 800 GGAGAGGCTTATCACCTAGAAGGCGATCACCTGATTCTCCCCATCGCCGGAGGCCGGGCTCTCCTATCCGCCGTCGCGGT
D T P P R R R P A S P S R G R S P S S P P P R R Y R S 880 GATACACCTCCTAGACGTAGGCCAGCATCACCATCTAGAGGCCGTTCTCCATCTTCTCCCCCTCCAAGACGATATAGATC
PPRGSPRRIRGSPVRRRSPLPLRRRSP 960 TCCTCCAAGGGGGCTCCCTAGAAGAATTCGTGGCAGTCCTGTTCGAAGAAGGTCTCCTCTTCCTCTAAGACGAAGGTCTC
PPRRLRSPPRRSPIRRSSPIRRSPIRRSPIRRPG 1040 CACCTCCAAGGAGACTACGCAGTCCTCCCAGAAGATCTCCAATCCGTAGGCGTAGCCGATCCCCAATTCGTAGGCCTGGT
R S R S S S I S P R K G R G P A G R R G R S S S Y S S 1120 CGCTCTCGTTCAAGTTCCATCTCACCCCGCAAGGGACGAGGTCCAGCTGGGAGACGTGGGAGGTCATCTTCTTACTCTAG
S P S P R R I P R K I S R S R S P K R P L R G K R S S 1200 TTCACCAAGTCCCAGAAGGATTCCAGGAAGATTTCAAGGAGGCCGCAGTCCTAAGAGGGCCACTGAGAGGAGAAAAAGAAGAAGCA
S N S S S S S S P P P P P P P R K T ⁺ R ⁺ ⁺ K L K K 1280 gcagtaacagcagcagcagcagttcaccgccaccaccacctcgtaaaact <u>taa</u> aggtgataaaaattaaaaaaa

Load RNA-Seq reads from cold stress experiment

If you have already accessed the read alignment files, you should see two empty tracks in the IGB main view (as in the images above.) To load the reads mapping to the SR45 region, click the **Load Data** button.

- Click Load Data to load reads for the region
- Right-click (or control-click) the Control and Cold Treatment track labels
 - Choose Change > Adjust Max Stack Depth and enter "0" to ensure that all reads will be shown
 - Use the vertical slider to stretch the display vertically to stretch the reads and gene models

About Max Stack Depth: The Max Stack Depth setting takes some getting used to, but as you learn to use IGB, you'll find it gives you extra power to configure the IGB display and fine-tune images you create for publication, slides, and reports.

The Max Stack Depth setting allows you to control the number items (reads, gene models, or other annotations) that can be stacked on top of each other in a given track.

This is a useful setting when you're working with BAM files (read alignments) because for some genes, there will be tens, hundreds, or even thousands of aligned reads that overlap the same region of the genome.

If you set the **Max Stack Depth** to a number other than 0, then IGB will never build "stacks" of overlapping reads taller than that number. Any extra reads that would make the stack too tall (and exceed the Max Stack Depth setting) will be drawn on top of each other in a special overlap row at the very top of the track (for plus strand reads) or at the bottom of the track (for minus strand reads.)

This special "slop" row of extra reads or annotations will be drawn in a slightly darker color than the individually stacked items in the rest of the track.

You can interact with both the individually stacked and "slop" reads or annotations; you can click on them, click-drag across them to select them, and so on, which is useful when you need to count the number of reads overlapping a gene or a an intron. (More on this later.)

Assess relative abundance of SR45 splicing isoforms

The SR45 gene is annotated as producing three possible variants. Which of these is the most highly expressed in the cold treatment and control samples?

To answer this question, use the aligned reads from the two RNA-Seq samples.

Focus on the differentially spliced region:

- Double-click the alternative exon in variant 1 (sixth from the left) to zoom in on it. Note it has two possible boundaries and that the intron to its immediate right (5-prime side relative to transcription) is an alternatively spliced intron.
- Scroll up through the treatment and control read alignments. Observe how many reads support the the V1 versus the V2 version of the alternative intron. Which form is more common? Is the same form more common in the treatment or in the control sample?

To answer this question, we have to look at reads that align across the intron immediately to the right of the alternative exon.

The intron has two alternative 3-prime boundaries, also called "acceptor sites." Remember that the gene is transcribed from right to left (see arrows in IGB) because it is located on the minus strand of the chromosome.

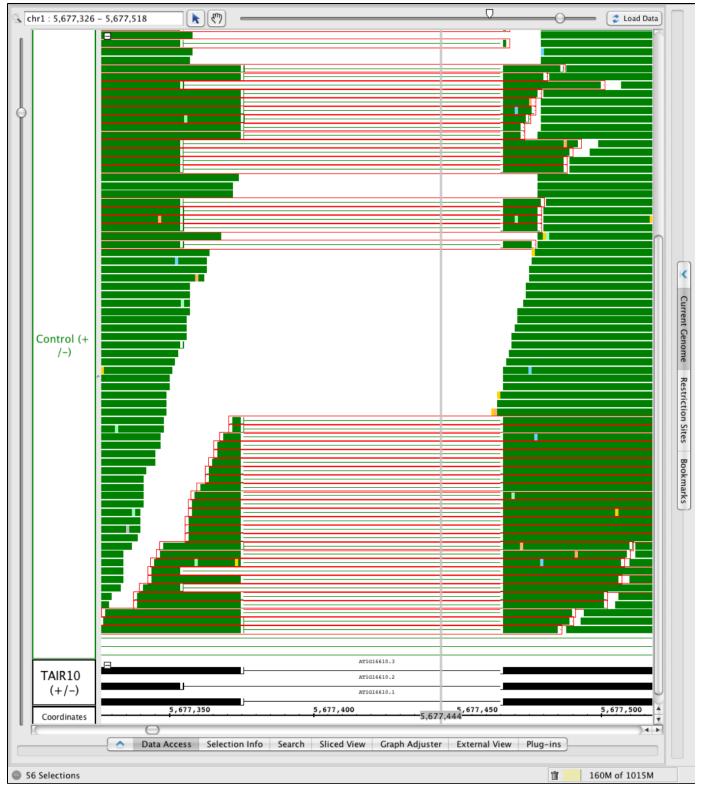
With respect to splicing, the most informative reads are spliced reads, reads that align across the alternative intron. These spliced reads can support one or the other choice, but not both.

You can count all the reads that overlap the intron simply by selecting them. When you click-drag over a region using the arrow (selection cursor), then anything you capture in the drag will be added to the list of currently selected items.

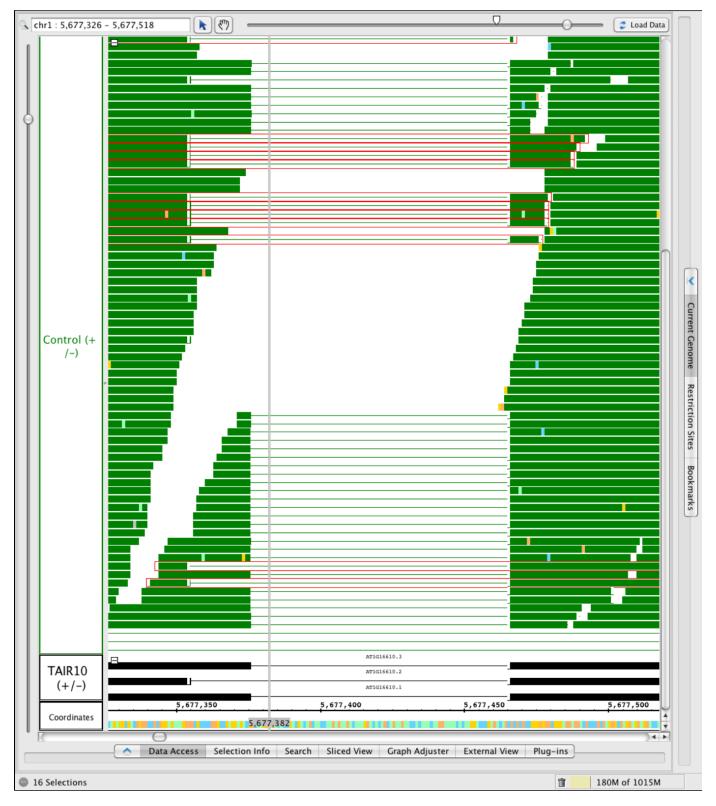
- Select reads by click-dragging over a region
- · To add an item to the selection list, SHIFT-CLICK it
- To remove an item to the selection list, CONTROL-SHIFT-CLICK it.

Use selection to count the number of reads that support V1 or V2.

IGB with 56 reads selected. Selected reads include just the reads that were spliced across the alternative intron - selections include reads supporting either V1 or V2.



IGB with just the V2 supporting reads selected:



Based on this analysis, it appears that the V1 form (AT1G16610.1) is more abundantly expressed in the control sample.

That is, out of 56 spliced reads, only 16 (around 25%) supported V2 and the rest supported V1.

- Repeat the process for the Cold-treated sample.
 - Were there fewer or more reads overlapping the alternative intron in the cold-treated sample?
 - What percentage of reads supported V2?

What preliminary conclusions can you draw about cold stress and splicing in SR45?

Finishing up - export an image showing what you found

If you would like to make images like the ones you see above, choose File > Export image. A new window will appear giving you the option to create image files in various formats, set image size and resolution, and save either the whole IGB window, the main view, or the main view with labels.

• Use File > Export Image to create a "souvenir" image from your session with IGB.

RNA-Seq Coverage graphs - Visualizing data from maize reproductive transcriptomes

- Introduction
 - About the data
 - What's a coverage graph?
- Setting up
 - Setting IGB Preferences The preferences window from IGB 6.7.0
 - Rescale the graphs, add y-axis
- View coverage graphs from four samples
 - Explore the data.
 - Use IGB's zoom controls to zoom around in the data
 - Stretch the display vertically to show more detail, including gene model labels
 - Jump-zoom to a gene model by double-clicking on it.
 - · Examples of new genes and/or differential expression across sample types
 - A multi-kilobase view of chromosome 1
 - Gene expression high in ovule and lower in leaves and embryo samples.
 - A single-exon gene expressed in pollen but not the other sample types.
 - A new gene?
- · Get more info from Phytozome, maizegenome.org, or google.
- A complex locus with multiple predicted splicing variants.
- Data loaded for all of chromosome one

Introduction

A paper published in 2011 titled **Utility of RNA Sequencing for Analysis of Maize Reproductive Transcriptomes** describes a study that used RNA-Seq to characterize genes expressed in reproductive structures from maize.

About the data

The study sequenced fourteen libraries prepared from fourteen sample types, including pollen, ovules, tassels, anthers, etc. One replicate per sample type was included.

To find out more about the study,

- Read the paper (PDF)
- · View sequencing records from the Short Read Archive

In this tutorial, you'll learn how to load these data into IGB, view genome coverage graphs for a subset of samples, and identify genes that are expressed primarily in ovules, pollen, or other sample types.

To make these data available for visualization in IGB, we downloaded the sequence data from the Short Read Archive and then aligned the sequences onto a maize reference genome assembly using the spliced alignment tool TopHat. We used a program called wiggles (from the TopHat distribute) to generate coverage graph files for each sample. We then set up the files on IGB's QuickLoad site at http://www.igbquickload. org/quickload.

For a description of how we set up the data in the IGB QuickLoad system, please see this page from the IGB Developer's Guide:

• Creating a new genome release for IGB - an example from Zea mays

What's a coverage graph?

A coverage graph in an RNA-Seq experiment is a type of genome graph that reports the number of reads overlapping regions in the genome. In a coverage graph, the x-coordinates are the genomic base pairs and the y coordinates represent the number of reads overlapping each genomic base.

In this experiment, graphs represent read counts; no normalization has been done. Thus simply comparing graph heights "by eye" can be misleading; you will need to take into account the fact that different samples produced different numbers of mapped reads. However, the number of mapped reads per sample type was similar across the different samples and so the relative heights of coverage graphs can give a reasonably good approximation of gene expression levels per sample.

Here is an example of the first few lines from a bedgraph file:

```
0
     6
        1
1
  6 9 2
1
  9 10 4
  10 35 5
1
1
  35
      41 4
```

The first column reports the name of the sequence (chromosome 1), the next two columns give the start and end coordinates of the first region, and the third number reports the number of reads that overlap that region. Note that the coordinates are in interbase.

• Information about interbase: Introduction to Chado - Interbase Coordinates.

When we processed these data to make them available via IGB QuickLoad, we also computed read alignment distributions for each library. You can view the read alignment distribution files in the same Web directories where the coverage graphs and read alignment files reside. Click the IGB Quickload Data Source to view the data repository and navigate to this folder:

http://igbquickload.org/quickload/Z_mays_B73_Mar_2010/SRP006463/

Setting up

Follow these instructions to configure IGB to get started looking at gene expression in maize reproductive tissues.

- Start IGB
 - Download and launch IGB using Java Web Start from http://www.bioviz.org/igb/download.shtml •
 - Or download igb.zip from http://sourceforge.net/projects/genoviz/files/.
 - To launch IGB from igb.zip, double-click igb.zip to unpack it, open folder igb, and double-click run_igb.bat (Windows) or run_igb.command (Mac)
- Choose species Zea mays B73, Genome Version Z_mays_B73_Mar_2010

Note that the gene models for maize should automatically load.

- · Customize gene models appearance
 - Right-click (or control-click on Mac) the Zea mays gene models track and choose
 - Choose Change > Show track (+/-) to combined plus and minus gene models into the same track
 - Note that when you zoom in far enough, you'll see arrows appear that indicate the direction of transcription
 - Change background color to white and foreground color to black to maximize readability for printouts and slides

Now start opening coverage graphs from different samples from the RNA-Seq experiment.

- Under the Data Access tabbed panel, open
- Data Source IGB Quickload > RNASeq > SRP006463 Reproductive > Single-map reads
- Click the checkboxes next to data sets or click-drag them over to the Data Management Table.
 - coverage SRR189770 Ovule
 - coverage SRR189771 Pollen
 - coverage SRR189766 Embryo
 - coverage SRR189761 Leaves

The four data sets will automatically be added to the Data Management Table. Don't worry if you accidentally add a different data set. You can remove it by unchecking the box or clicking the red x.

Take a moment to customize the colors. Click the color squares in the Data Management Table (FG is foreground, BG is background) or choose File->Preferences->Tracks to open a new Window that let's you customize different aspects of the display at several at once. Click each row in the table and give each row a white background. Choose colors for individual rows by clicking on them and then selecting options.

Tip: Simplify the track name to make the Track Labels easier to read.

Tip: Color the gene track black (foreground) and white (background) to make attractive images for slides and printing.

Setting IGB Preferences - The preferences window from IGB 6.7.0

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	nscripts 5b release				10
Coordinat	25				12
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Name S				View	Mode ‡
Max St	ack Depth: 10 L	abel Field:	A T	✓ Au	ito Refresh
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Once you've set up the tracks, zoom in on a region.

• Enter 1:51631058-61254530 into the Search/Range Box (upper left corner) and type Return.

IGB should then scroll and zoom to the region you entered. Next, click **Load Data** and and proceed from the first example below, after adjusting Y-axis scales as described in the next section.

TIP: You can also use IGB links from a Web site to navigate a genome in IGB. An IGB link looks like this: http://localhost:7085/UnibrowControl?se qid=1&end=61254530&start=51631058&version=Z_mays_B73_Mar_2010. When you click the link, IGB should scroll and zoom to the location indicated in the link, provided your computer allows access to non-standard ports. (This may not work on every computer.)

Once the data load, you'll see a view that looks a bit like the image below. To match the image below, you'll need need to rescale the graphs, add a y-axis, and so.

Note to advanced users: If you are running the high-memory version of IGB and have a fast internet connection, you can probably load all the data for chromosome 1 for all four graphs. However, if you try this and out of memory, you can always delete a graph (using the red X) and click the trashcan icon at the bottom right of IGB to force what is called "garbage collection," a programming term that means recovering memory.

Rescale the graphs, add y-axis

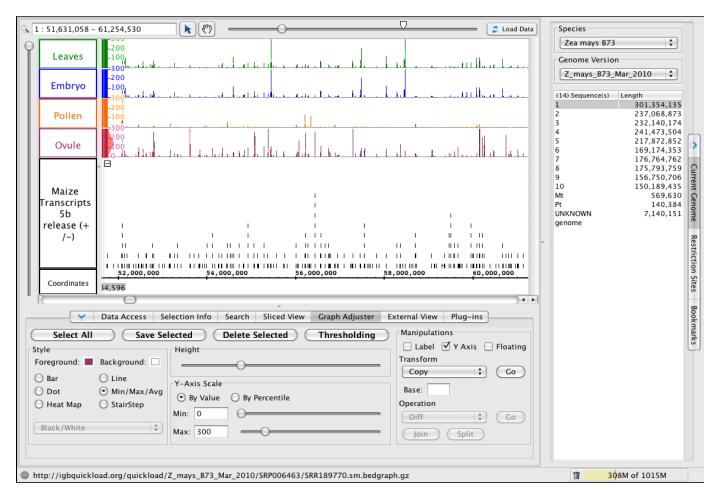
Use the **Graph Adjuster** tab to put all the graphs into the same scale, thus make it possible to compare them. Click the **Graph Adjuster** tab and then click the **Select All** button to select all the graphs. While all are selected to the following:

- Click Y Axis to add y axis scale to every graph.
- Unclick Label to hide the label for each graph.
- Under Y-Axis Scale, click By Value and then enter a new maximum. 300 is a good place to start for these data.
- Use the Height slider to increase the height for each graph.

After setting the colors and configuring the Graphs, you should see something like the following image. (The Ovule graph is selected here to show its **Graph Adjuster** settings.)

View coverage graphs from four samples

- To jump to this region, enter 1:51631058-61254530 (same as above) into the Search/Range Box (upper left corner) and type Return.
- Click Load Data button (top right) to load the data into IGB.



Explore the data.

Now that the graphs use the same scale, you can zoom and pan around the chromosome and observe how different genes are expressed in different samples.

Use IGB's zoom controls to zoom around in the data

To zoom in, click the place you want to zoom - the vertical stripe (called a zoom stripe) will hop to the location of your most recent click and also will focus zooming so that when you zoom in or out, the zoom stripe will remain stationary while the scene around it expands or contracts. To zoom in, drag the horizontal slider (top right) to the right. To zoom out, drag the horizontal slider to the left.

Stretch the display vertically to show more detail, including gene model labels

To stretch the display in the vertical dimension, drag the the vertical slider (left side of the IGB window) up. To compress the display (and make it fit inside the IGB main data display), drag the vertical slider down.

To zoom in a region, click-drag your mouse along the number line. During the drag, you'll see a blue highlight. When you release the mouse, you'll jump zoom to the region you selected.

Jump-zoom to a gene model by double-clicking on it.

To zoom in on a gene model, just double-click it. This shortcut will help you quickly inspect interesting genes and their structure.

Scroll and zoom around the chromosome, making note of places where one or more samples appear to have more reads covering a particular region and double-click on gene models to zoom in for a closer look.

Also, keep your eyes open for regions with reads but no annotated gene. When you find one, click the corresponding read alignments file for the sample whose graph contained expression data. Load the reads to get an idea of the intron/exon structure for the unannotated gene.

Visit the following regions (see below) to see examples of new genes and differential expression across sample types.

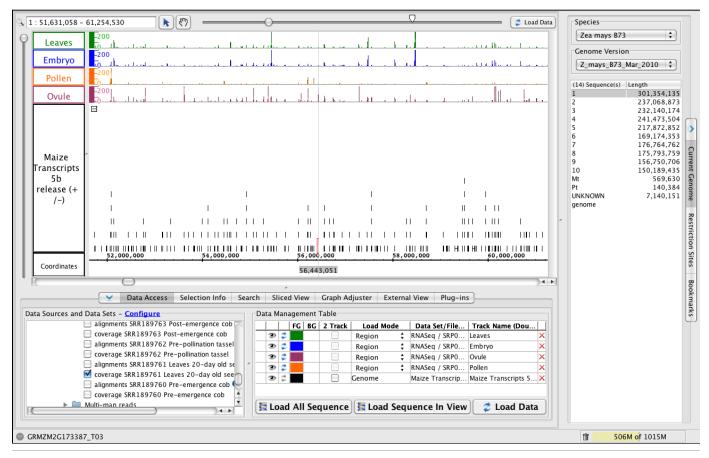
Tip: To navigate quickly to the regions shown below, enter the regions (as shown) in the upper left text box and press the Return key on your keyboard. IGB will then jump to the region you entered. You can also search for gene models by name using the **Search** tab.

Examples of new genes and/or differential expression across sample types

A multi-kilobase view of chromosome 1

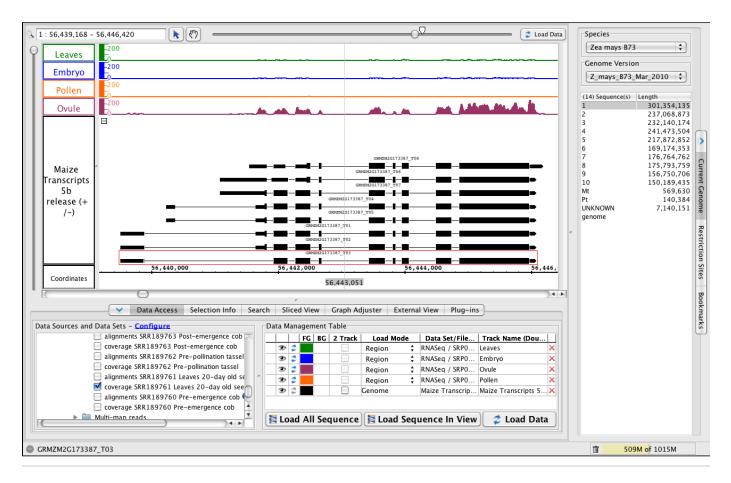
• To jump to this region, paste 1:51,631,058-61,254,530 into the Search/Range box and hit Enter.

Note how different graphs have peaks at different regions, but all graphs use the same scale.



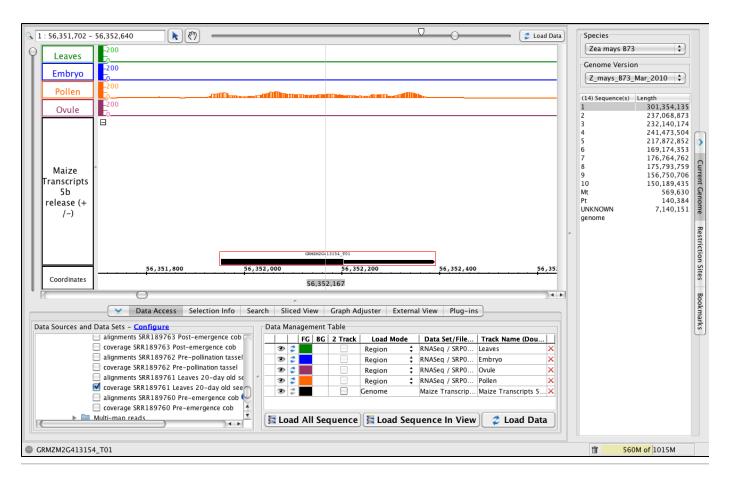
Gene expression high in ovule and lower in leaves and embryo samples.

• To jump to this region, paste 1:56,439,168-56,446,420 into the Search/Range box and hit Enter.



A single-exon gene expressed in pollen but not the other sample types.

• To jump to the region, paste 1:56,351,702-56,352,640 into the Search/Range box and hit Enter.

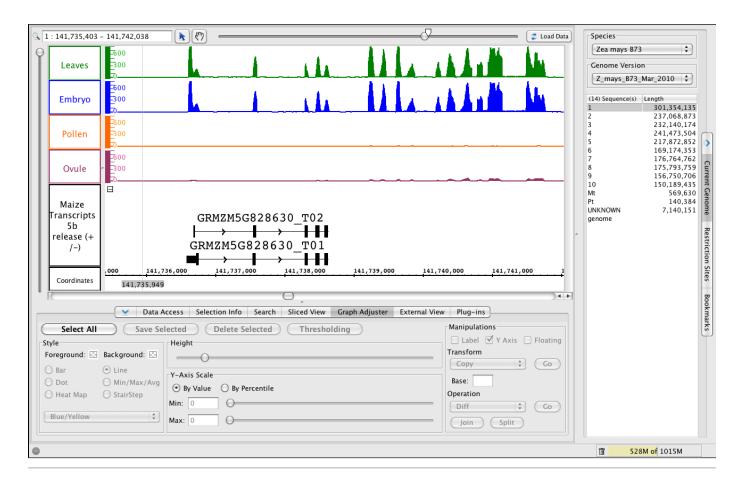


A new gene?

- To jump to the region, paste 1:141,735,403-141,742,038 into the Search/Range box and hit Enter.
- Click Load Data to load the data for this region, which is outside the range of data you loaded earlier in the tutorial.

To replicate this image, modify the Max Stack Depth setting for this track, which controls the amount of vertical space above the two gene models.

- Right-click the Maize Transcripts 5b track label and choose **Change > Adjust Max Stack Depth**. Enter 2. This will tell IGB to never make a "stack" of individually drawn gene models that is larger than 2. Any other gene models will be drawn on top of each other in the very top row (the "slop" row) at the top of the track.
- When you look at a gene with more than two gene models, use Change > Adjust Max Stack Depth to change this setting to a larger number to see all the gene models individually, or enter 0 to allow unlimited stacking.



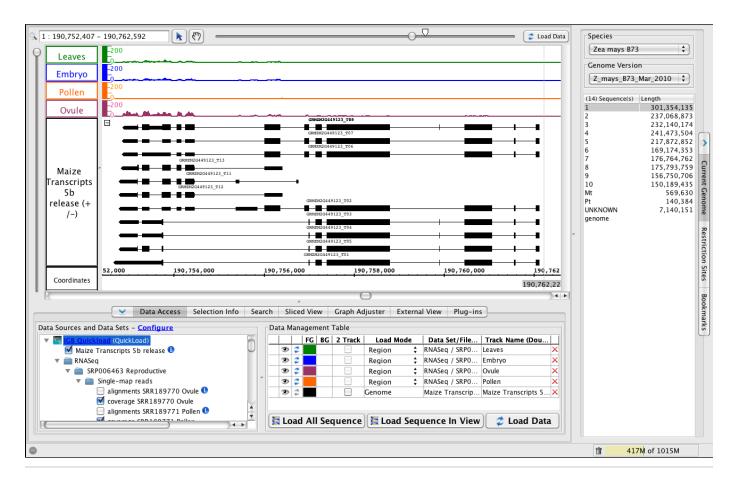
Get more info from Phytozome, maizegenome.org, or google.

- To jump to the region, paste 1:80,185,398-80,190,414 into the Search/Range box and hit Enter.
- Click Load Data to load graphs data for this region, which is outside the first region you loaded.
- Right-click the gene model (as shown below) and choose Get More Info to search Phytozome, MaizeGenome.org, or Google for information about the selected gene.

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	Evan An Sequence E Load Sequence in view 2 Load Data	
GRMZM2G131853_T01		1015M

A complex locus with multiple predicted splicing variants.

- To jump to the region, paste 1:190,752,407-190,762,592 into the Search/Range box and hit Enter.
 Click Load Data to load graphs data for the region.
- To view all the variants, change the **Max Stack Depth** for the track.
 - Right-click the Maize transcripts 5b track label and choose Change > Adjust Max Stack Depth. Enter 13 or 0 for unlimited.



Data loaded for all of chromosome one

- Warning: Loading all of the data from the remote server (igbquickload.org) may take some time! Also, your computer may not have sufficient memory to display all four graphs. But if you would like to try it and view all the data at once, use the horizontal slider (top right) to zoom all the way out. Then, click Load Data. If the loaded data take up too much memory (e.g., close to your maximum memory limit shown on the lower right corner of IGB), then you can delete a data sets by clicking the red X or unchecking it in the Data Sources and Data Sets folder tree.
- To recover memory, click the trash can icon in the bottom right of the display.
- To halt data loading, click the minus symbol that appears in the bottom left whenever data are being accessed from a server or local file.

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View a bacterial genome in IGB

This tutorial describes how to import sequence and annotations into IGB when your genome of interest is not already available from an IGB QuickLoad or Distributed Annotation site. In this tutorial, we'll demonstrate importing a bacterial genome (E. coli) using files downloaded from NCBI.

Get genome data from NCBI

To view a bacterial genome in IGB, first visit NCBI to retrieve the sequence data and annotations.

• Go to the NCBI GenBank record for E. coli K-12 subtr. MG1655: http://www.ncbi.nlm.nih.gov/nuccore/NC_000913.2

Download annotations file

Click the Send menu (on the right side of the record) and choose Complete Record, File, GenBank, and Create File.

Save the file to your computer; by default, it will be named "sequence.gb".

A If you change the name, be sure to use the ".gb" file extension so that IGB can recognize the file and the file format.

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Next, click on the FASTA link just under the name and reference of the record. This will load up the sequence for this genome. In the picture below, after FASTA has been selected, the Display Settings indicate that the record shown is the FASTA; the options below the name and the NCBI Reference Sequence number offer GenBank and Graphics.

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Again, using the Send function select Complete record, File, FASTA (default), Create file.

This will create a new file called 'sequence.fasta'. This file contains the reference sequence. Make sure that the extensions for the fasta file is either '.fasta' or '.fa'. If the extension is '.txt', you can safely change it to '.fa'.

Step 2: Synonyms

When IGB shows these files, it uses the header of each file to identify the 'chromosome' that is being displayed. Unfortunately (especially with NCBI files), the headers for each file type (FASTA sequence files, annotation files, .bam/alignment files, etc.) can ALL be different for the exact same chromosome/sequence! IGB will see different headers and treat each one as a separate chromosome; they will not be visualized together, even though they are all the same length.

To overcome this, we have the ability to enter 'synonyms'; IGB already has some internal synonyms so that "1" and "chr1" are all equivalent. However, the NCBI reference numbers are specific to each chromosome, for each organism. You will need to know the header of each of these files; if you are not sure, a quick way is to drag all of the files into IGB at the same time.

In the picture below, we have dragged (in order) the gene models (.gb) and the sequence file (.fasta). In the **Current Genome** panel we have "NC_000913" and "gi|49175990|ref|NC_000913.2|". Notice that both **Sequences** have the same **Length** in the Current Genome panel. If we select "NC_000913", and then **Load Data**, we can show the annotations but we can NOT show the sequence.

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Alternatively, if we select the 'gi' number and hit load, the sequence file loads (as a track) but the models cannot be seen.

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To fix this issue, we will create a 'personal synonym' file. This is a simple text file that is tab separated. All terms included in a single line will be 'equivalent' and IGB will know that they are to be shown together. To create and load the synonym file is very simple:

- Open a text editor; this must be simple text (.txt), and not rich text format (.rtf)
 Type in 1 and then a tab. Type in chr1 and then a tab. (1 and chr1 are standard names for the first chromosome, and many files use Type in the name of the first file (i.e. NC_000913) and then hit the TAB key.
 Type the second name (i.e. gi|49175990|ref|NC_000913.2|)

- Save this file with the name chromosome.txt

0) 🔿		chromosome.txt	
1	chr1	NC_000913	gi 49175990 ref NC_000913.2	
				11.

Just that easy! If you are making a synonym file for a multi-chromosomal organism, then make a <u>new</u> line in the file for each chromosome, and just add all of the 'names' associated with it (make sure that there is a 'tab' between each name!). If you include a file that has a new name, open the chromosome.txt, and add the name to the proper line (be sure to include the tab between the last name and the new name!).

• Now, while IGB is open, choose Configure from the Data Access panel or open File > Preferences > Data Sources. Click the '...' butto n next to Chromosome Synonyms File, select your chromosome.txt file and click open. The file path will appear in the open box, showing that it is loaded. You can close the preferences window at this point, and IGB will remember to load your chromosome.txt file for you every time it opens. This means if you make a change in your chromosome.txt while IGB is open, you will need to re-open IGB to see those changes take place.

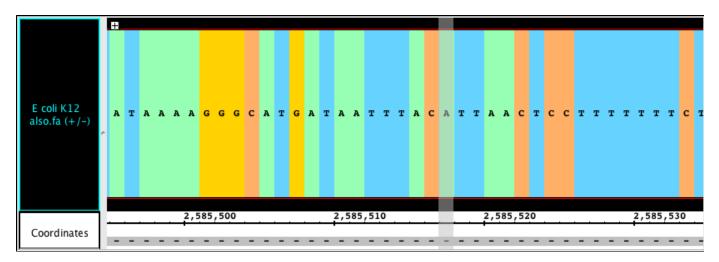
	O O Preferences				
Shortcuts	Graphs	Other Options	Plugin Repositories	Data Sources	
Data Sources					
Name	Type	URL		Enabled	
IGB Das	DAS2	http://igbquickload.o			
Bioviz	DAS2	http://bioviz.org/das			
UofUtahBioinfoCore	DAS2		utah.edu:8080/DAS2DB/gen	ome 📃	
NetAffx	QuickLoad		ymetrix.com/quickload_data	/	
IGB Quickload	QuickLoad	http://igbquickload.o			
HughesLab	QuickLoad	http://hugheslab.ccb			
UCSC Ensembl	DAS		csc.edu/cgi-bin/das/dsn	A RADA	
BioVizServerCache	DAS	http://www.ensembl			
BIOVIZSERVERCache	QuickLoad	http://bioviz.org/cac	ned/		
Pars and Supanyme		Add.			
ersonal Synonyms		Add.			
Personal Synonyms Version Synonyms I	File	Add.			
Version Synonyms I	_		Authentication	Remove	
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IGB will need to be restarted to properly load and recognize your chromosome.txt; close this instance of IGB and then reopen IGB.

Step 3: Visualizing the Reference Sequence and Models

At this point, we have the sequence file and we have the gene models. When you open the new instance of IGB, your synonyms will be loaded for you.

We will now open these two files so that you can begin analysis of your own data. The first step will be to open the sequence file as a references sequence rather than as a track. This will allow the sequence to load into the 'sequence' area of the **Coordinate Axis**, and IGB will use it for sequence comparison with your data. If a sequence file is loaded as a track (and there is NO reference sequence) it shows the actual nucleotide sequence (the reference sequence line shows dashes and it greyed out) but it cannot be used for comparison to you .bam files or other data:



If the reference sequence is loaded, however, the sequence in the track will ONLY show mismatches, but otherwise appear as a solid bar. The references sequence line in the Coordinate Axis area will now have the nucleotides show (when zoomed in).

E coli K12		
also.fa (+/-)		T
	2,585,500 2,585,510	2,585,520 2,585,530
Coordinates	A T A A A <mark>G G G C A T G</mark> A T A A T T T A C A	TTAACTCCTTTTTTTC-

To open up the sequence and annotation files:

- File > Open Reference Sequence...
- Chose the 'sequence.fasta' file.
- Drag the sequence.gb (gene models) into IGB.
- Click the Load Data button. NOTE: you need to click the Load Data button and NOT the Load Sequence button to visualize your sequence file!

Both the sequence and the models will load. You will be zoomed out, so the sequence will appear as a grey bar; as you zoom in the colors and nucleotides will become visible. At this point, you can drag in your files of interest and begin viewing them with the models and sequence. Keep in mind that if the name of the chromosome(s) is different in **your** files, you will have to add the name to the chromosome.txt and then reopen IGB so it can load in the new information.

Focus on a Feature Live On-line Tutorials

To help new and experienced IGB users get more out of using IGB to analyze data, we are hosting a series of live, on-line tutorials called "Focus on a Feature."

The next Focus on a Feature tutorial will be in February, 2015.

To view recordings from previous tutorials, view them under Video Tutorials or visit the IGB Channel at YouTube.

Video Tutorials

- IGB Quickies
- Focus on a Feature
- In-Depth Analysis

IGB Quickies

Short how-to videos demonstrating specific features within IGB.

Focus on a Feature

Previously recorded on-line tutorials demonstrating in depth use of IGB.

In-Depth Analysis

Longer videos describing more in-depth analysis of data and features in IGB.

IGB Quickies

- IGB Quickies
 - Quick Start
 - Zooming
 - Semantic Zooming
 - Panning the Display
 - Adjusting Vertical Data Display
 - Adjusting Track Height and Track Order
 - Adding a New or Custom Genome
 - Exporting Images
 - Creating Bookmarks

IGB Quickies

Quick Start

Zooming

Semantic Zooming

Panning the Display

Adjusting Vertical Data Display

Adjusting Track Height and Track Order

Adding a New or Custom Genome

Exporting Images

Creating Bookmarks

Focus on a Feature

- Focus on a Feature
 - ChIP-Seq Alignment and Visualization
 - All New Features in IGB 8.2
 - RNA-Seq Alignment and Visualization
 - Alternative Splicing and Primer Design
 - Working with Sequence Data in IGB
 - Working with Graphs in IGB

Focus on a Feature

ChIP-Seq Alignment and Visualization

All New Features in IGB 8.2

RNA-Seq Alignment and Visualization

Alternative Splicing and Primer Design

Working with Sequence Data in IGB

Working with Graphs in IGB

In-Depth Analysis

- In-Depth Analysis
 - Viewing RNA-Seq Data

In-Depth Analysis

Viewing RNA-Seq Data

Older Versions of IGB Getting Older Versions of IGB System Requirements for Older Versions Troubleshooting Older Versions

See the links below for common problems and their solutions.

Note: To run older versions of IGB, you will need Java installed on your computer. Most computer types already have Java, but if you need to install it yourself, visit the Java download page. To find if you already have Java, click the link labeled Do I have Java?

Release notes for older versions

New in IGB 6.5

Hello and welcome to IGB 6.5!

The following new features debuted in IGB 6.5, released in April 2011.

Most of these new features come from you - the IGB user and developer community.

As always, the IGB team relies on you, the user, to suggest ways we can improve the IGB software. Please keep sharing your ideas!

Sequence Viewer

For the release of IGB 6.5, we have created a **Sequence Viewer**. When you right-click an item in the main IGB data display, you'll see a new option offering to display genomic sequence. Choosing this option opens the **Sequence Viewer**, shown below.

The **Sequence Viewer** can display all six frames of translation (**Show** menu), a spliced cDNA (**Show cDNA** button), or change the color scheme. Exons and introns in gene models appear as different colors, and the viewer also marks start and stop codons, if available. You also have the option to view complementary strand sequence. You can copy and paste sequence or save it to a file using the **File** menu.

IGB 6.5 also makes it easier to work with genomic sequence bases. Just load the sequence in the usual way and then click-drag across the sequence of interest to select it. Right-click to open the **Sequence Viewer** or just press Control-C to copy.

For more information, read Genome sequence viewer.



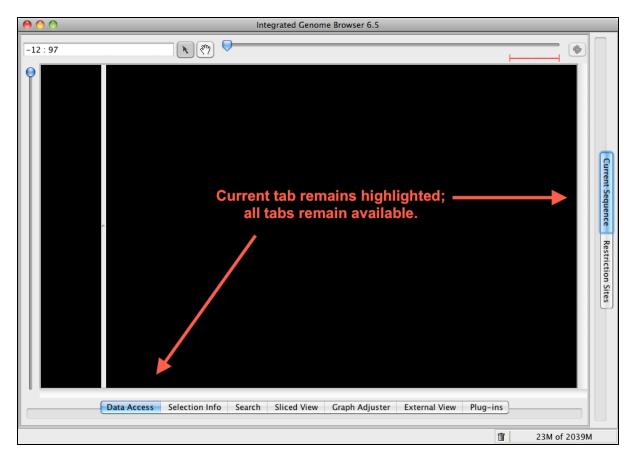
Tabbed Panels and Tray Layouts

To give you greater flexibility in setting up your personal IGB environment, we have made some changes in the main IGB window. Upon start up, you will now see panels on the right as well as the bottom of the main window. These panels (also called trays) contain all of the usual tabs. The main difference is that you can move them out of the way by clicking the active tab, freeing more space for data exploration.

As always, clicking any tab will open it in the usual way. As with previous versions, you can open tabs in a new window. Now, however, you can move them to left, right, or bottom trays, depending on the tab.

For more information, read Tabbed panels.

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Coordinates Data Access Sele	14,700,000 rction Info Search Slice	14,750,000	14,800,000 ter External View	14,850,0 Plug-ins	00			Current Sequence Restriction Sites
Choose: Arabidopsis thaliana	•	A_thaliana_Ju	n_2009		•			ites
Choose Data Sources and Data S ▶ [®] 2 Bioviz (DAS2) ▼ M GB Quickload (QuickLoad) ✓ TAIR10 mRNA [®] Load All Sequence		Choose Lo Choose Loa Choose Loa Whole Gen	ome TAIR10 mRN/	s: Data Source A IGB Quickload (Qu resh Data				
						1	109M of 2039M	_



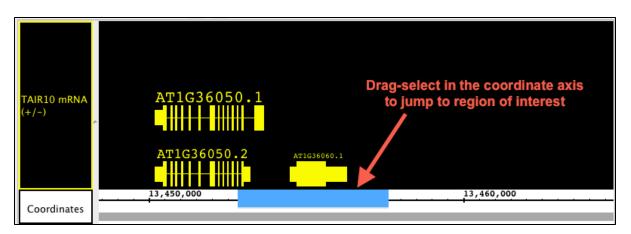
More Zooming and Panning Options

IGB 6.5 introduces several new ways to pan (scroll) and zoom.

IGB now includes two tool buttons, **Select** and **Grab**, next to the horizontal zoomer. Click the 'hand' button (outlined in red below) to choose the **G** rab tool. Then click and drag the tool on the display to move left or right, and up or down. To select items, choose the **Select** tool (arrow cursor).

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		▋╢╢┤╺┫┇╢╦═┾╺╪╌┥╺╋	 	A71G53050.1	A71G53090.2
0	19,740,000 19	,750,000 19,760	,000	19,770,000	19,780,000
Coordinates					19,78
			\bigcirc) 4 🕨

A new zoom feature allows you to "jump zoom" to a region by click-dragging (using the **Select** tool) over the central axis - the number line in the center of the display. Just click-drag over the region of interest, and release. IGB will jump and zoom to the highlighted region.



For more information, read Panning the Display or Zooming.

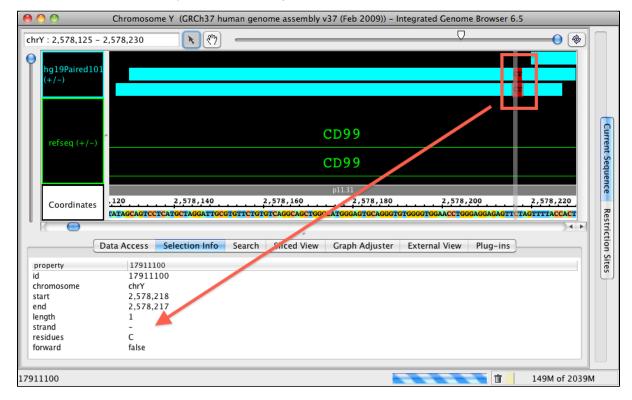
Autoload for BAM files

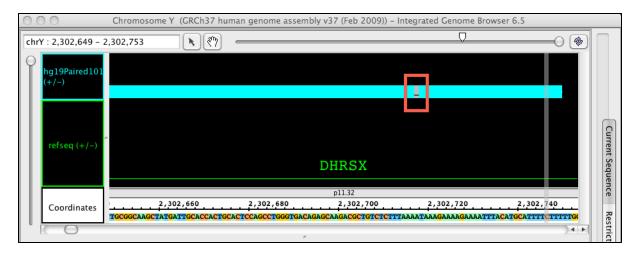
A new optional **Autoload Mode** (for BAM files) tells IGB to automatically load data from a file or URL once you've zoomed in far enough. A marker appears above the horizontal zoomer indicating the zoom level that triggers automatic loading of data.

Chromosome	1 (Arabidopsis thaliana TAIR9) - Integrated	Cono	me Browser 6.5
R 🐑 —	0	∇	(\$)

Visualize insertions and deletions

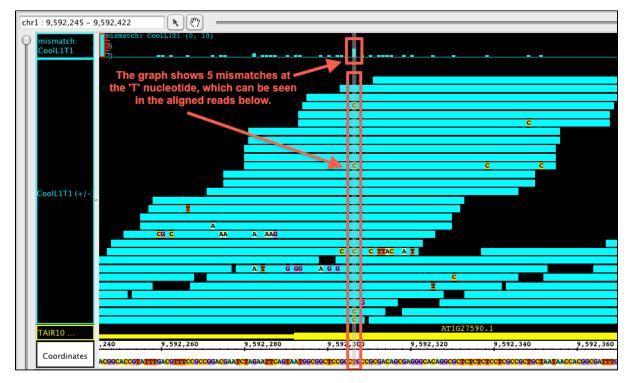
Another feature is that IGB can now show both insertions and deletions in short reads. Insertions appear as a pair of red residues, indicating the point of insertion. Details about the insertion can be gotten from the **Selection Info** panel or by hovering the mouse over the red residues to activate the tooltip; this information contains the length and sequence of the insertion. Deletions appear as a 'dash', highlighted by default as gray; by opening **File > Preferences > Other Options**, you can set the 'other nucleotides' to appear as any color you like. This will highlight the deletions, as well as unloaded sequence, or 'N' reads in your selected color.





Create a mismatch graph

IGB now provides the opportunity to create a mismatch graph from BAM files. This bar graph shows how many mismatched reads are present at each nucleotide. You simply select the the track, right click in the label and open the menu; you are now offered the option of **Make Mismatch Graph**, along with the regular option of **Make Annotation Depth Graph**. By hovering over a position in the mismatch graph, a tooltip with information about nucleotide distribution will appear. This allows a user to quickly and easily find out how many mismatched nucleotides in total, as well as how many of which type, are represented in the reads at any single genomic location. This can be very useful in SNP identification and allelic determination. For more information on making either graph type, read Creating Graph Tracks from Annotation Tracks.



Plug-Ins

We have instituted the ability for IGB to accept plug-ins, allowing developers and users to create small 'add in' programs that customize the functionality of IGB. Each plug-in can perform a function or series of functions for a specific purpose. By selecting the right plug-ins to add, the user expands the usefulness of IGB and tailors it to their needs, without cluttering IGB with unnecessary functions. Some plug-ins, such as **Invers eTransformer** and **PowerTransformer** are already available in the IGB program. These can be activated from the **Plug-ins** tab. The IGB developers will continue to author and make available some plug-ins; users can also write and use their own plug-ins, suited to their needs.

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Name InverseTransformer	Description Graph Transformer that takes the inverse of the input – 1/x	Version 1.0.0	Installed
PowerTransformer	Graph Transformer that takes the input to a given power – x^a	1.0.0	
Show Installed	Show Uninstalled Update all Update selected	Repo	sitories)

Expanded Support for Plant Genomics

IGB continues to allow access to publicly available DAS servers that support some plant genomes, but with version 6.5, the IGB team debuted a new IGB QuickLoad site with several sequenced plant or algal genomes not previously supported. We also moved the site to a faster server with improved internet connectivity, which should make your experience with IGB feel faster and more responsive.

To view the new IGB QuickLoad site, and see the full list of genomes we directly host, visit http://www.igbquickload.org/quickload.

New in IGB 6.6

New Features for 6.6

Welcome to IGB 6.6! (Did you see our new welcome screen?) We have made many changes and improvements to make IGB even easier for users. Many changes, such as the Data Panel redesign and the Preference tab Changes are immediately obvious, but there are many more new and exciting functions you should explore.

Redesign of the Data Access Panel

The **Data Access** panel has been redesigned to allow users to easily change the foreground and background colors, plus change the name of the track. The **Delete** icon (trashcan) and the **Reload** icon (arrows) are joined by the **Hide** icon (open eye); **Hide** can be used to hide tracks from view in the main window without deleting the track. A new warning icon indicates when your more recent **Load Data** request failed to add anything to the display.

More subtle is a new feature affecting the Data Sources tree; you can now remove an track in the display by clicking its checkbox in the **Data** sources and **Data Sets** panel.

if loading a file is taking too long (or it might fill the memory) a user can stop the loading process by using the X box at the bottom of the main window.

Here is a close-up of the redesigned **Data Access** panel showing the '**No Data**!' alert, the **Hide** function activated (red line through eye), the color change options, and the now-editable **Track Name** column.

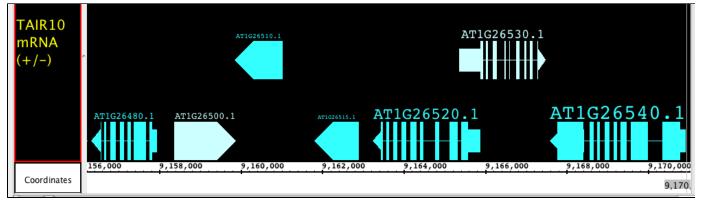
Data Access Selection Info Choose: Arabidopsis thaliana	Search	Slice			aph Adjuster Extension	ernal View Plug-i	ns	\$	
Choose Data Sources and Data Sets: Configure ► ¹⁰ / ₁₀ <u>Bioviz</u> (DAS2) ▼ IGB Quickload (QuickLoad) ■ TAIR10 mRNA ① ■ TAIR10 ALL GENES ① ■ TAIR10 TE gene ① ■ TAIR10 TRNA genes ① ■ TAIR10 rRNA genes ① ■ TAIR10 miRNA genes ①		0 0 0 0		FG BC	Choose Load Moo Region In View Region In View Region In View Region In View Region In View Whole Genome	CoolL1T1.bed CoolL1T1.sm.bam CoolL1T1.sm.wig Hotl2T1.sm.bam	CoolL1T1.sm coverag Hotl2T1.sm	>	
E Load All Sequence	🧮 Loa	d Se	quer	ce in	View	🖗 Load Data			

New Options for Changing Track Appearance

Including the redesign of the Data Access panel, we have made changing the style of a track much simpler and we have expanded the track appearance options. Track parameters can be changed through the right-click on the label menu (current method), **Data Access** panel (new) or the newly enhanced **Tracks** tab in the **Preferences** panel. The **Tracks** tab now has many great features to help you adjust the presentation of the tracks. As always, colors, name, track depth and label field can be accessed, but in addition, users can now change the font size of labels, or

highlight strand differences (see red box in the image below). This is a new set of feature which allows the user to show direction of gene models. The options include **Arrow**, which uses an arrow head to indicate direction of transcription, and **Color**, which uses 2 colors (can be changed) to indicate + or - strand. This feature is an excellent way to show strandedness when + and - strands are shown as a single track (see second image).

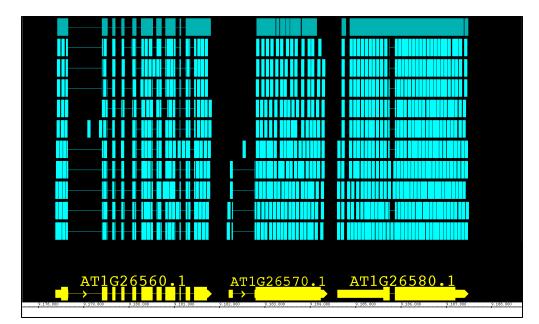
00		Preference	s		
Fracks Tra	ack Defaults	Shortcuts	Graphs	Other Options	►
Select Track- Display N. CoolL1T1.s CoolL1T j TAIR10 mR Coordinate	m unctions NA	Backgr	o Foregro	. Track Name 12 12 12 12 12 12	
Properties Track Name:	CoolL1T1.sr	n		Show Strand	
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Name Size:	12.0	Apply To All T	racks	View Mode default	
Max Depth:	10 La tracks (+/-)	bel Field: Connected	Collapsed	Auto Refresh	

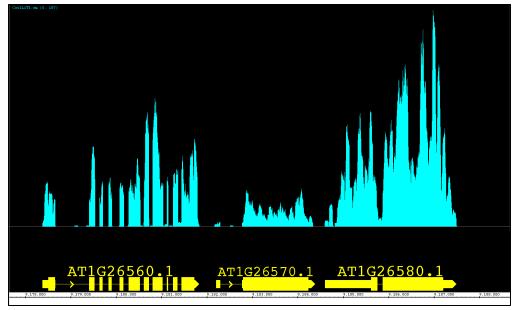


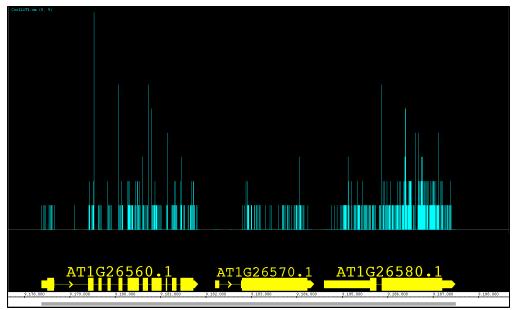
Prototype: View Mode

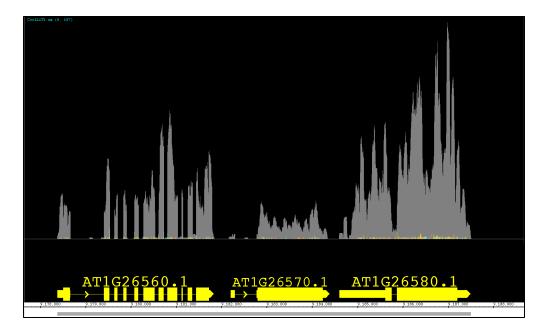
We have been developing a fantastic new feature called **View Mode**. We have been so pleased with this feature we are releasing it as a prototype with version 6.6. This feature allows you to get more information out of your annotation tracks without having to create several independent graphs! Accessible from the right-click menu or the **Preferences >Tracks** tab, you can set an annotation track to show **depth**, as if you created a depth graph. For BAM files you can change the **View Mode** to show **mismatches** as if it were a mismatch graph. We have also introduced a new view (also available as a **Create Graph** option) called **Mismatch Pileup**; this new function highlights mismatches (color coordinated to each nucleotide) overlayed on a dark gray depth graph, giving a lot of information compacted into a single track. Unlike graphs, you can readily change the view options at will, getting graphical information and returning to a default representation if you choose. As we continue to expand this feature, it will gain more options for adjusting the graphical display and possibly even additional views! Feel free to contact us with your ideas.

The pictures below show Default view, Depth view, Mismatch view and Mismatch Pileup view.









Improved Preference Tabs

Almost all of our **Preferences** tabs have been redesigned to make easier to use. This includes the incredibly expanded functions of the **Tracks** ta b (see *Track Appearance* below for details), the simplification of the **Other Options** tab, and the addition of source ordering buttons in the **Data Sources** (see *Faster Performance* below for details). We have also created the entirely new **Track Defaults** tab where you can now set the default colors, font size, label, max depth and strand determination for different annotation file formats. You can also set the colors and label size for different graph file types.

		s Sho	ortcuts	Graphs	Other Options
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New Features for the Main View

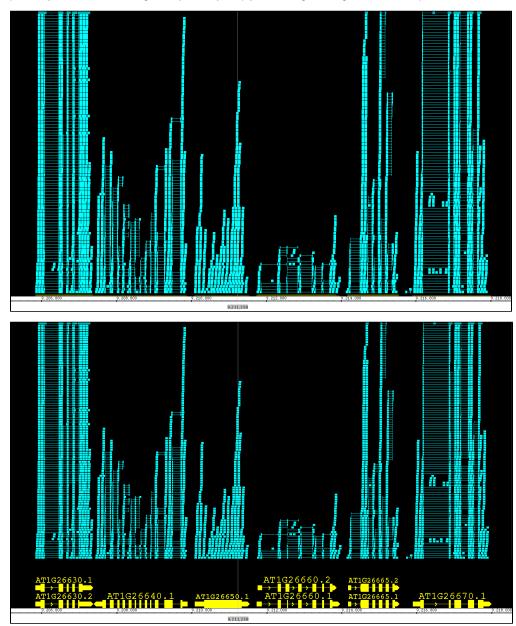
There are many new and improved features to enhance the main view in IGB. We have made the **Zoom Stripe** translucent so that you can easily see your data at the same time you can see point of focus, but both the **Zoom Stripe Label** and the **Zoom Stripe** itself can be turned off from the

View menu. Another fantastic feature accessible from the right-click menu is the Focus On Selected Track option, which will allow the user to 'zoom in' and fill the viewable space with the track of interest. We have also changed the sequence to a dynamic font which both allows you to zoom in on the track just a little more and increases visibility of each nucleotide. By installing the MergeAnnotationOperator Plugin, you can now join annotations into a single track, just as the Join function works for graphs.

Prototype: Track Height Adjustment

Here is another experimental function that was just too useful to leave out! You can now adjust the track height for annotation and graph tracks. Move the cursor over the boundary between two track labels until the height adjustment cursor icon appears. Click and drag to the new height. As this feature is still under development, it may exhibit some quirky behavior, but we found it to be so useful overall that we included it with this release. Look for this feature to be even better in future releases!

In the pictures below you can see a common situation, where a short reads file (BAM) overwhelms the reference sequence models. In the second picture, you can see the image readjusted by simply increasing the height of the ref seq track.



Improvements to Search

Now when using the **Search** tab for sequences, IGB provides a tabular output in the **Search** panel as well as more visible (green) hash marks on the sequence bar. A search that might be running too long or was incorrectly entered can be stopped using the **X** box below table.

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New Features for Graphs

Our new **Mismatch Pile-up** (described in **View Mode**) graph shows both the **Depth** AND **Mismatch** graphs as one over-layed graph, and is readily adjusted by using the **Graph Adjuster** tab. We also have redesigned the Manipulations portion of our Graph Adjuster tab to accommodate the functions provided by Plugins. Three new Plugin functions include **Geometric Mean, Power Transformation** and **Inverse Transformation**; after installing from the **Plugins** tab, these functions can be found in the **Transform** drop down box.

New File Types, Faster Performance

With more and more data being present in more and more formats, we have expanded the types of files IGB can accept; users can load SAM files (with or without matching index files), fasta files that have been indexed and traditional BED, PSL, SAM and WIG that have been indexed using tabix. After installing the appropriate **Plugins**, IGB can additionally use BigBed, BigWig and VCF formats. We have made many behind the scenes changes to speed up file handling, but we now also permit the user to prioritize the order in which IGB queries servers for data; this can allow IGB to preferentially go to faster servers first OR to get sequences from a preferred server (**Preference > Data Sources**).

Improved Bookmarks

We have added two new features to **Bookmarks**. We have activated tooltips for bookmarks, so that you can find the chromosome and nucleotide range for each bookmark without needed to open it. We have also introduced a **History** function; using the 'fast forward' and the 'rewind' buttons in the **Bookmarks** tab, you can scan back and forth through your bookmark browsing history, letting you go back for a second look, without losing your place.

New in IGB 6.7

New Features in IGB 6.7

Welcome to IGB 6.7. We have made many changes and improvements to make IGB even easier to use.

Many changes, such as moving the **Species** and **Genome Version** to **Current Sequences** tab and the redesigned **Bookmarks** tab will be immediately obvious to IGB veterans, but there are many more new and exciting functions you should explore in IGB 6.7.

Major thanks are due to IGB software developer Hiral Vora, who led the effort and kept us all on track for the current release. Thank you Hiral!

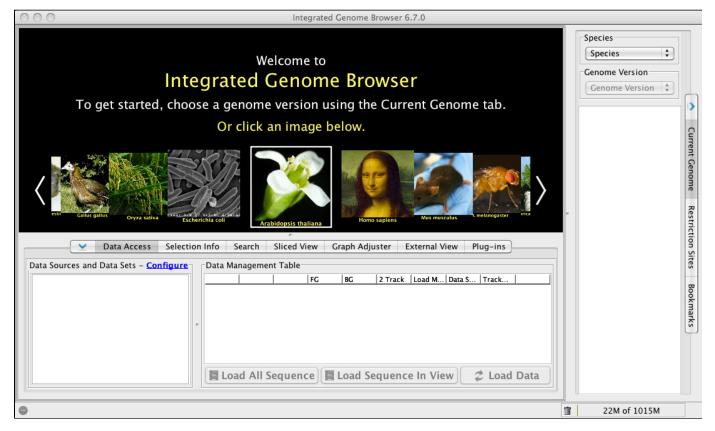
Panels and tabs redesigned

To make IGB easier to use, we re-configured the user interface for several IGB tabs and panels including

- · Bookmarks tab
- the Export File window
- the Web Links Tools interface.

We also re-designed the IGB start screen. Now, when you start IGB for the first time, you'll see a new start screen that provides quick and easy access to a variety of model organisms, helping new users jump right in.

Credit for this new interface go to Dr. Jeremy Villalobos and Graduate Research Assistant David Norris.



Better Bookmarks tab - and support for comments

The Bookmarks tab now supports adding comments to bookmarks and bookmark folders.

When you create a new bookmark, IGB will give you the option to add a free text comment. (As before, you can name bookmarks whatever you like or just use the IGB-supplied default.)

The Add Bookmark function offers the option of making one of two types of bookmarks:

- · a Position Only bookmark which records genomic location
- or a Position and Data bookmark, which also records data sets currently being displayed

Returning to a Position Only bookmark returns you to the bookmarked genomic position but won't load data.

Returning to a **Position and Data** bookmark returns you to the bookmarked genomic position and also re-loads data sets that were in the original scene (provided they are available).

Right clicking a bookmark will open a new window displaying bookmark attributes.

Thank you to Graduate Research Assistants Zhong Ren and David Norris for programming and also to Dr. Alyssa Gulledge for design and usability evaluation.

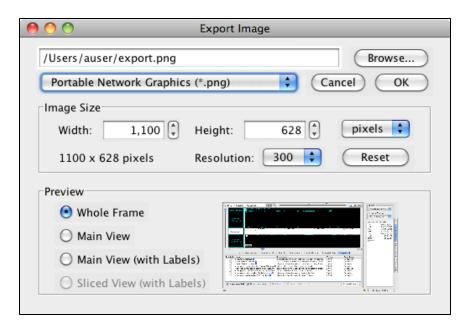
New options for exporting image files

Along with a redesign to improve ease of use, the **Export** interface also features new options not previously available. Users can now specify image size and file type for export. You can still specify which part of the IGB display you'd like to export, e.g, the main display image, the main image with the track labels, or the entire window with tabbed panels (such as **Selection Info**) showing. IGB can currently export images in PNG or JPEG formats.

We are also added a new feature that allows you to set image resolution/DPI. IGB now makes it easier to create high-quality images of 300 dpi or better (the minimum required for images destined for publication in printed journals).

Note to current users: Unfortunately, we had to remove export to PDF and other vector graphics formats. There were a number of show-stopping bugs in the external libraries we were using to support export in those formats. We are looking for new libraries that will be able to support those formats and hope to aim this functionality in the next release.

Thank you to Zhong Ren (CS Masters student) for programming the Export Image window.



Autoload for features that use sequence data

A number of IGB ffeatures that require access to sequence data, such as **Restriction Site** mapping, the **Sequence Viewer** for gene models and **Search Residues** functions. Previously, IGB would ask you before loading sequence data. Starting with IGB 6.7, IGB will automatically load sequence data from available IGB QuickLoad or DAS sites, speeding the process of retrieving information you need for analysis and visualization. However, you can still **Load Sequence in View** or **Load All Sequence** information from the **Data Access** panel.

Tip: You can control which server IGB will use for accessing sequence data by changing the ordering of **Data Sources** in the **Data Sources** Pref erences tab. Data providers differ on whether it is better to deliver masked versus unmasked genomic sequence to users, and so we decided to give users the ability to control which data source provides the reference genome sequence. Thanks are due to developer Lance Frohman (Genentech) who added this feature.

Expanded residue Search function displays multiple sequences

With IGB 6.7, we have introduced the ability to overlay multiple sequence Search results (up to 7 different searches).

The basic **Search Residue** function hasn't changed; as before, you type in the sequence you are searching for and IGB returns a list of 'hits' and adds colored bars to the sequence indicating the location of matches.

However, if you check the new **Overlay** option, subsequent residue searches will be added to the list and highlighted in new colors without removing previous search results.

Use this new feature when you need to display multiple sequence features in the same view, such as forward and reverse primers for PCR primers, transcription factor binding sites, and so on.

Thanks are due to Dr. Alyssa Gulledge for designing and testing this new feature.

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This image shows the 3 primers needed for determining splice variant choice for the AT1G07940 gene of Arabidopsis thaliana, all shown in the same view.

Expanded file types and increased save options

We are always expanding our ability to support new file types. With IGB 6.7, we can now support BedDetail files and tabix indexed GFF files. If there is a file type we don't support yet, let us know! IGB has allowed users to save annotation tracks (including tracks created through **Track Operations**) as .bed files. Now, IGB can now save graph tracks (including tracks generated with IGB tools in the **Graph Adjuster** panel!) as .bedgraph files.

NEW in IGB 7.0

- Welcome to IGB 7.0
 - New features and improvements a short list
 - IGB 7.0 highlights
 - Intron arrows in gene models
 - Protein translations for BED Detail files
 - Search tips
 - Selection Info textbox
 - Saving and loading sessions
 - Saving tracks and annotations
 - More control over Color by Score
 - · Features for creating publication-quality images
 - New and improved tabbed panels
 - Data Access panel
 - Annotation panel
 - Graph panel
 - Selection Info panel
 - New Tab Menu
 - Toolbar shortcuts
 - Locking and setting track height (in pixels)

- Stack Height shortcuts
- Auto Scroll Updates
- New genomes supported in IGBQuickLoad
- Retiring Affymetrix DAS2 data source in progress
- New features for QuickLoad sites ٠

Web Link link-out builder tool improved

Welcome to IGB 7.0

We are excited to bring you many new and improved features with the release of IGB 7.0.

As always, we strive to improve all aspects of IGB, including functionality, usability, and connectivity.

IGB 7.0 start screen (click to enlarge)



New features and improvements - a short list

IGB 7.0 has includes new features for data analysis and exploration, including:

- · Better-looking, more informative gene models
 - Protein translation display when zoomed in
 - Better indicators for strands (intron arrows)
- Easier, faster searching to help you find genes by gene name or keyword
- New Sequence Viewer features, including the ability to export conceptual translations of gene models or other features
- A new Selection Info textbox that reports the name or number of selected features
- A way to save and load IGB sessions, which helps you save your place in a long-term analysis or share your analysis with others
- Expanded Save Track features, including the ability to save selected annotations and/or tracks as BED detail file format
- Support for BED detail format (replaces Affymetrix "BRS" and "BGN" formats) • More control over the numerical range of the Color by Score feature
- · Several improvements to help you make publication quality images

We redesigned the UI to make IGB easier to use for advanced and beginning IGB users. New features include:

- A new Annotation tab for configuring and styling annotation tracks (reads, gene models, ESTs, mRNAs)
- A new Toolbar allows users to customize their IGB environment with one click access to favorite shortcut functions
- Enhanced Stack Height and Track Height options give more control over the amount and size of data to be shown
- Improved Auto Scroll feature makes scanning through large amounts of data smoother and easier

IGB was designed from the start to allow sharing and accessing data sets from diverse providers. IGB 7.0 has new features to promote and enable data integration, including:

- Many reference genomes data sets updated to Nov 2012 versions (replacing many out-of-date Affymetrix NetAffx DAS data sets) New plant species add to the IGB data source IGBQuickLoad.org
- · Continued support and improvements to QuickLoad sites
- Improved Configure Web Links tool for building customized IGB linkout patterns, useful for data providers setting up their own QuickLoad sites

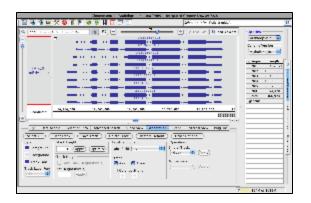
See below for highlights and images showing the new IGB 7.0 interface.

IGB 7.0 highlights

Intron arrows in gene models

To make it easier to identify strand of origin for gene models, we augmented the Arrow strand style to include arrow marks along the entire length of introns. (This change was inspired in part by UCSC Genome Browser, which uses greater-than symbols to indicate strand.)

New arrow strand style (click to enlarge)



Protein translations for BED Detail files

We have expanded the ability to include protein translation during analysis in two ways. In the main view, users see translations overlaid on gene models, where amino acids are either displayed as single letter or three letters (or hidden, if you prefer). Simply click **Load Sequence** and zoom in on the gene; the amino acid sequence will appear according to the annotated start and stop of the gene model.

Close-up on a gene model (click to enlarge)

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The Sequence Viewer features new options for copying gene model conceptual translations and intron/exon sequences.

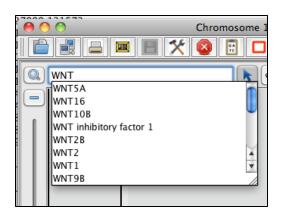
New Sequence Viewer menu items (click to enlarge)



Search tips

IGB 7.0 introduces search tips. Start typing in either the Quick Search/Coordinates display (top left) or in the Advanced Search tab and IGB will suggest search terms.

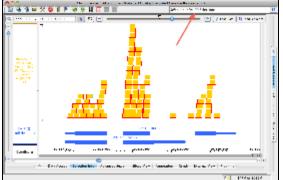
Search tips



Selection Info textbox

In addition to the added copy functions in the **Selection Info** tab, we have also added a new **Selection Info** textbox (top right), which reports the name of a currently selected item or the number of items if more than one is selected. If you have selected a region of genomic sequence, the number of selected bases pairs is shown. Clicking the "i" icon opens a new window showing information about the selected item.





Selection info window (click to enlarge)

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Saving and loading sessions

Now you can easily "save your place" in a lengthy IGB session and also share your sessions with collaborators. A new **Save Session** feature creates a simple .xml file that remembers your location, files you've opened, data you've loaded, track preferences, and more.



Saving tracks and annotations

IGB now supports saving selected annotations as well as entire tracks. IGB also supports more formats for saving annotations to local files, including BED Detail, bedgraph (wig), and more.

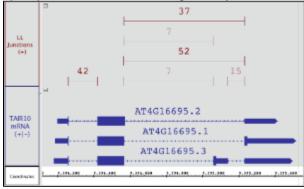
More control over Color by Score

Color by Score allows you to make annotations appear darker or lighter based on their score. This feature aids in identifying outliers and assessing the distribution of scores in a large data set. However, prior to this release, the scale of values was pre-set as 1 to 1000.

Starting with IGB 7.0, you can use the Set Range for Color by Score feature to designate your own range of values.

In the example below, higher-scoring splice junctions features with greater support (more supporting reads) appear darker than less well-supported junctions. You can also tell IGB to display the score above each feature by choosing "score" as the Label Field.

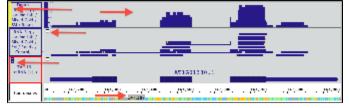
Splice junction feature shown using Color by Score



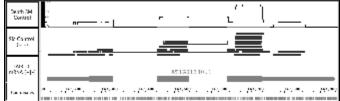
Features for creating publication-quality images

- You can now set the track label font color and font size independently from track data.
- IGB now supports larger image resolution settings. You can export an image with resolution up to 1000 DPI.
- A new feature Clear Visual Tools and Show All Visual tools (View menu) hides graphical elements such as the zoom stripe, the IGB Track Mark, Locked Track Mark, etc.

Image showing visual tools you can hide using the new Clear Visual Tools feature (click to enlarge)



Publication-quality, grayscale version of the image shown above (click to enlarge)



IGB Export Image window (click to enlarge)

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New and improved tabbed panels

Data Access panel

The order, background color, and font color of data sets in the **Data Management Table** now reflects their appearance and ordering in the IGB main window display. We right-justified the text in the **Data Management Table**, making long file paths easier to read. File information is still available via the **Selection Info** tab.

We also updated and simplified data set Load Mode settings, which now include Auto, Manual and Genome load modes.

- Manual means data only load when you request it by pressing the Load Data button
- Auto means that data load automatically whenever the zoom level passes the AutoLoad threshold.
- Set the zoom level AutoLoad threshold using the View menu
 Genome means data for the entire genome load at once

Annotation panel

The new Annotation tabbed panel provides easy access to annotation track settings and operations.

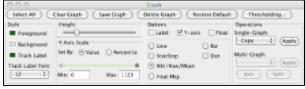
Annotation tab (click to enlarge)

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Graph panel

The Graph panel now includes a new double-headed slider for setting the lower and upper bounds on the visible range of graphs.

The new graph panel (click to enlarge)



Selection Info panel

Each cell in the **Selection Info** table now contains a button you can click to view and copy cell contents. This is useful when the cells contain a large amount of text or text you would like to copy into a Google search or other tool.

New Tab Menu

The new **Tab** menu makes it easier to access tab control features. Tabs, as always, can be shown or hidden, can be displayed as part of the IGB main window (as IGB "trays") or as separate windows.

Portrait (side) tabs can be shown in on the left or right side of the main window.

Tabs menu (click to enlarge) Tabs Bookmarks Help Open Current Left Tab in New Window Open Current Right Tab in New Window Open Current Bottom Tab in New Window Open Left Tabbed Panes in New Window Open Right Tabbed Panes in New Window Open Bottom Tabbed Panes in New Window Data Access Selection Info ► Advanced Search ь Sliced View Þ Annotation Þ Graph External View Plug-ins Current Genome ь Restriction Sites Bookmarks

Toolbar shortcuts

We added a new toolbar that allows users to access commonly-used functions with one click of a button. This is personalized by the user: open the **Preferences > Toolbar** tab, and select the functions you want in the **Toolbar**! This saves the need to continually flip between tabs for a commonly used function. This has been added in addition to **Key Stroke** shortcuts, which can still be personalized as well.

As part of this new feature, **Preferences > Toolbar** has been redesigned. Any function that can be put in the **Toolbar** has an icon and a checkbox; simply check the box on and the button will appear. After filling your **Toolbar** with functions you like, they can be rearranged into an order that suits you by simply clicking on the icon and dragging it to the new position. If you decide you do not want a function in the **Toolbar**, uncheck it from the **Preferences > Toolbar** menu. All of the functions in **Preferences > Toolbar** remain available for Key Stroke shortcuts as well.

Did we miss your favorite function? Let us know! Use Request a Feature or a Shortcut... to get to our request forum!



IGB Toolbar Preferences (click to enlarge)

C	10	Preferences		
	Tracks	Track Defaults Toolbar	Other Options	1
	Toolbar?	Action	Key Stroke	T
1		Copy Selected Sequence to Clipboard	meta C	1
×		Delete Selected Track		
8	~	Ext	meta Q	
	-	Expand		
F		Export Bookmarks		
	~	Export Image		ľ
	-	Export Preferences		1
	-	Float Track		1
?		Get Help at IG8 Help Forum		1
1	1	Go to IGB User's Guide	F1	1
ø	8	Hide		1
ð		Import Bookmarks		
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		Print Main View with Labels		

Locking and setting track height (in pixels)

Lock Track Height is a new feature that allows locking the vertical dimension (in pixels) of a track. No matter how tall the other tracks become, a locked track's height never varies. Lock Track Height and Set Height for an annotation track using the new Annotation tab.

Stack Height
4 Apply Optimize
Track Height
🗹 Lock Track Height (Pixels)
Set Height (Pixels):
150 Apply

Stack Height shortcuts

IGB allows users to set the number of overlapping annotations that appear in "stacks" along the genomic sequence axis. If the number of annotations overlapping a region exceeds the track's stack height setting, the extra annotations are draw in a single summary row at the top of the track. (IGB draws them in a darker color to signal they are part of the summary row.)

Previously, you could change the stack depth by entering new values in the Adjust Max Stack Depth window.

Starting with IGB 7.0, we added new Optimize Stack Height shortcuts to the Toolbar and Annotation tabs.

These shortcuts re-set the stack height for a track so that all annotations appear in a stack, with no "left overs" in the summary row.

Auto Scroll Updates

We simplified configuring and running the animated IGB Auto Scroll feature, which scrolls tracks scroll from left to right automatically.



New genomes supported in IGBQuickLoad

We added many new genomes and species to the IGBQuickLoad site, which is version controlled via publicly accessible subversion repository located at https://svn.transvar.org/repos/genomes/trunk/pub/quickload.

The IGBQuickLoad data repository allows us to document data sets using subversion log messages and allows everyone to retrieve older versions of data sets when needed, a key feature for ensuring reproducible research in complex data analyses.

Retiring Affymetrix DAS2 data source in progress

We are in the process of retiring the Affymetrix DAS2 data source, which the company has not updated in more than a year. For each data set available via the Affymetrix NetAffx DAS2 source, we are setting up sequence data and reference genome annotations in the IGBQuickLoad.org site. To support Affymetrix probe set alignments, we created a variant of Affymetrix' link.psl format that allows indexing by the tabix utility, which allows partial, region-based manual loading of these data possible.

New features for QuickLoad sites

Users can easily make Quickload sites to hold their data or make it easy to share their data with others. We have improved their ability to control the Quickload environment and to control how data loaded from the site appears.

File attribute in annots.xml now supports URLs

Annotation files listed in annots.xml files can now point to remote URLs instead of local files. To use this feature, replace the "name" attribute with a URL. IGB will then attempt to load the data from the remote site. This feature allows QuickLoad data providers to integrate data sets stored on diverse sites into a single Data Source under the IGB Data Access panel.

Continued support for foreground, background, and other style attributes QuickLoad data providers can continue to specify how data sets will appear once loaded into IGB using file element attributes. Once loaded, of course, users can change all of these elements to personalize their data analysis experience.

QuickLoad site-specified Web links

Data providers can now specify link-out patterns by including a file named preferences.xml at the top level of the QuickLoad root directory.

Web Link link-out builder tool improved

The **Web Links Tool** is an easy way for you to create and test new link-out patterns. Use the **Web Links Tool** to specify regular expressions for data sets or annotation ids; web links are used by IGB to access external Web sites when users choose the **Get More Info** option (right-click menu). IGB built-in links (e.g., Google searches, links to NCBI) provide examples of how to build your own link-out patterns.

		Web Links Tool	
Server Provided We	b Link		
Name	Regular Expression	URL Pattern	Matches
Dictyflase Gene	C0^DD8_C\d+5	http://dictybase.org/gene/\$\$	Annotation ID
DictyBase	(?0^008\d+\$	http://www.dictybase.org/db/ogi-bin/sea	Annotation ID
DictyBase	0%*dicty8ase:.*\$	http://dictybase.org/db/cgi-bin/search/s	Annotation ID
Entrez cross-data	(?)."NCBL"	http://www.ncbi.nlm.nih.gov/gquery?term	Annotation ID
Entrez	0%4GeneID*\$	http://www.ncbi.nlm.mh.gov/gene/55	Annotation ID
Flybase	(?0AFLYBASE:.*\$	http://flybase.org/.bin/fbidg.html?\$\$	Annotation ID
GeneDB	(?0."sanger_pombe5	http://www.genedb.org/genedb/Search?n.	Track Name
Google		http://www.google.com/search?g=\$\$	Annotation ID
Name NCRI Evample	Regular Expression	URI. Paltern herr: //achi aim aib agu/auggaga ISS	
Name NCBI Example	Regular Expression (20(EST))ImRNA)(R	UIB. Pattern http://ncbi.nim.nih.gov/nuccore/55	Matches Track Nam
			Matches Track Nam
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NCBI Example	OkestijimRNAijiR	http://incbi.nim.nih.gov/nuccore/55	Track Nam
NCBI Example Create New Web Link Builder Name: NCBI Exa	OkestijimRNAijiR	http://ncbl.nim.nih.gov/nuccore/55 (Impor	Track Nam

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