

基礎生物学研究所ゲノムインフォマティックス・トレーニングコース 2021夏
RNA-seq入門：RNA-seq解析パイプライン
2021.09.15–2021.09.16

NGS基本ツールIGV

基礎生物学研究所
生物機能解析センター
山口勝司

データ可視化ツール・IGVの紹介・実習

The screenshot shows the homepage of the Integrative Genomics Viewer (IGV) website. The header features the IGV logo and navigation links for Home, Downloads, Documents, and Contact. A search bar is available for website search. The main content area includes a large banner image of the IGV software interface displaying genomic tracks. Below the banner are sections for Overview, Citing IGV, Download IGV, and Funding. The Overview section provides a brief description of IGV as a high-performance tool for visual exploration of genomic data. The Citing IGV section lists publications and bioRxiv articles. The Download IGV section offers links for desktop application and igvtools. The Funding section acknowledges support from the National Institutes of Health.

<https://www.broadinstitute.org/igv>

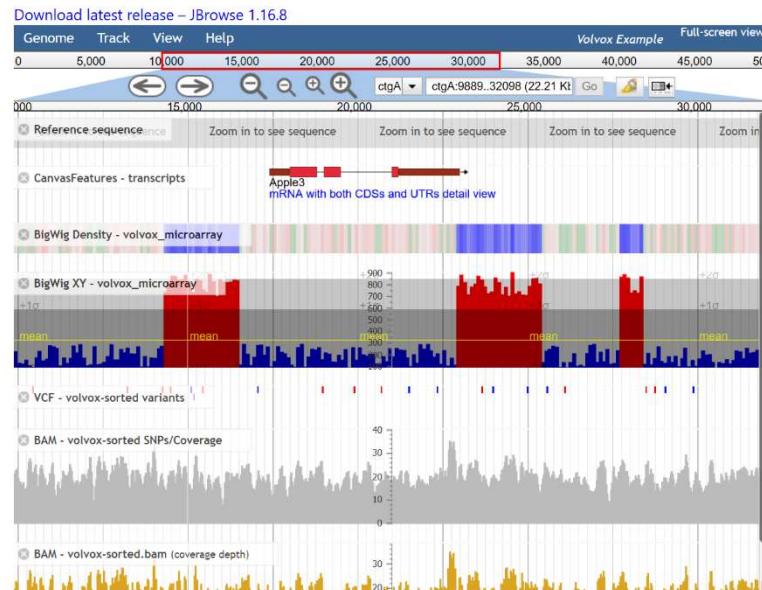
なぜIGVを取り上げるか

データ可視化ツール 2つのタイプ

- ・自分のパソコン(ローカル環境)にインストールして使うタイプ
- ・サーバーに構築して、ネットワーク上で使うタイプ

The JBrowse Genome Browser

JBrowse is a fast, scalable genome browser built completely with JavaScript and HTML5. It can run on your desktop, or be embedded in your website.



後者はコミュニティーで広く利用する目的
ウェブ公開を目的とするには良い。
より大容量なデータに対応できる。

ネットワーク・情報セキュリティの高度な
知識も要求される。

管理者的な人がいて、その人がやって
くれるなら、これも良いが…

もっとお手軽なものとしてIGVを紹介

可視化ツールに求められるものは何か

膨大なデータを、如何に直感的に理解できるか
sortや絞り込みができる表データと対比双璧になる

多様なデジタル情報

- ・配列、GC ratio、遺伝子情報
- ・遺伝子発現情報
- ・遺伝子多型の位置情報・頻度情報
- ・様々なデータの精度情報
- ・RNA-seq, ChIP-seq, RAD-seq, BS-seq…

レファレンス配列 / gene model / gene annotation と
NGSデータを並べて比較
複数のデータセットを並べて比較

様々なスケールで比較・統合的に解釈できるようにしたい

ゲノムviewerに自分のデータを乗せ、
統合的直感的に比較・解釈できること

可視化ツールをどう選ぶか

選択の基準

genome data viewing に求められるもの

取捨選択の基準

1. 無料 / 有料 / 基本無料
2. 個人的レベルの使用 / コミュニティーレベルの使用
3. 見るだけ/自分から色々工夫
4. アクセスibilitiy・ユーザビリティ
 - 導入に必要なコンピュータスペック
 - マニュアルは分かりやすいか
 - 情報の多さ
 - 利用の簡便さ
 - 使っている人が近くにいるか

Integrative Genomics Viewer(IGV)

お手軽ツール

- ・アカデミックウェアで無料
- ・コミュニティーでの利用者が多いから、情報も多い
- ・javaのプログラムなので、オールプラットフォーム対応
- ・マニュアルは親切、サンプルデータもある
- ・WEBサーバーではなく、PCレベルで利用出来る
- ・データ閲覧環境の共有も可能

誰もが簡便に使えるものが良い。

igv

Integrative Genomics Viewer

IGV

Home

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- IGV User Guide
- Tutorial Videos
- File Formats
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- Release Notes
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Search website

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University of California

Home

Integrative Genomics Viewer



Overview

The Integrative Genomics Viewer (IGV) is a high-performance, easy-to-use, interactive tool for the visual exploration of genomic data. It supports flexible integration of all the common types of genomic data and metadata, investigator-generated or publicly available, loaded from local or cloud sources.

IGV is available in multiple forms, including:

- the original IGV - a Java desktop application,
- IGV-Web - a web application,
- igv.js - a JavaScript component that can be embedded in web pages (*for developers*)

This site is focused on the IGV desktop application. See <https://igv.org> for links to all forms of IGV.

Download IGV

Download the IGV desktop application and igvtools.

Note that the IGV-Web application at <https://igv.org/app> runs in a web browser and requires no downloads. Click on the Help link in the app for more information.

Citing IGV

To cite your use of IGV in your publication, please reference one or more of:

James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. [Integrative Genomics Viewer](#). *Nature Biotechnology* 29, 24–26 (2011). (Free PMC article [here](#)).

Helga Thorvaldsdóttir, James T. Robinson, Jill P. Mesirov. [Integrative Genomics Viewer \(IGV\): high-performance genomics data visualization and exploration](#). *Briefings in Bioinformatics* 14, 178–192 (2013).

James T. Robinson, Helga Thorvaldsdóttir, Aaron M. Wenger, Ahmet Zehir, Jill P. Mesirov. [Variant Review with the Integrative Genomics Viewer \(IGV\)](#). *Cancer Research* 77(21) 31-34 (2017).

James T. Robinson, Helga Thorvaldsdóttir, Douglass Turner, Jill P. Mesirov. [igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer \(IGV\)](#). *bioRxiv* 2020.05.03075499.

Funding

Development of IGV has been supported by funding from the [National Cancer Institute \(NCI\)](#) of the [National Institutes of Health](#), the [Informatics Technology for Cancer Research \(ITCR\)](#) of the NCI, and the [Starr Cancer Consortium](#).



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◀ previous abstract next abstract ▶

NATURE BIOTECHNOLOGY | OPINION AND COMMENT | CORRESPONDENCE

Integrative genomics viewer

James T Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S Lander, Gad Getz & Jill P Mesirov

[Affiliations](#) | [Corresponding authors](#)

Nature Biotechnology 29, 24–26 (2011) | doi:10.1038/nbt.1754

Published online 10 January 2011

To the Editor:

Rapid improvements in sequencing and array-based platforms are resulting in a flood of diverse genome-wide data, including data from exome and whole-genome sequencing, epigenetic surveys, expression profiling of coding and noncoding RNAs, single nucleotide polymorphism (SNP) and copy number profiling, and functional assays. Analysis of these large, diverse data sets holds the promise of a more comprehensive understanding of the genome and its relation to human disease. Experienced and knowledgeable human review is an essential component of this process, complementing computational approaches. This calls for efficient and intuitive visualization tools able to scale to very large data sets and to flexibly integrate multiple data types, including clinical data. However, the sheer volume and scope of data pose a significant challenge to the development of such tools.

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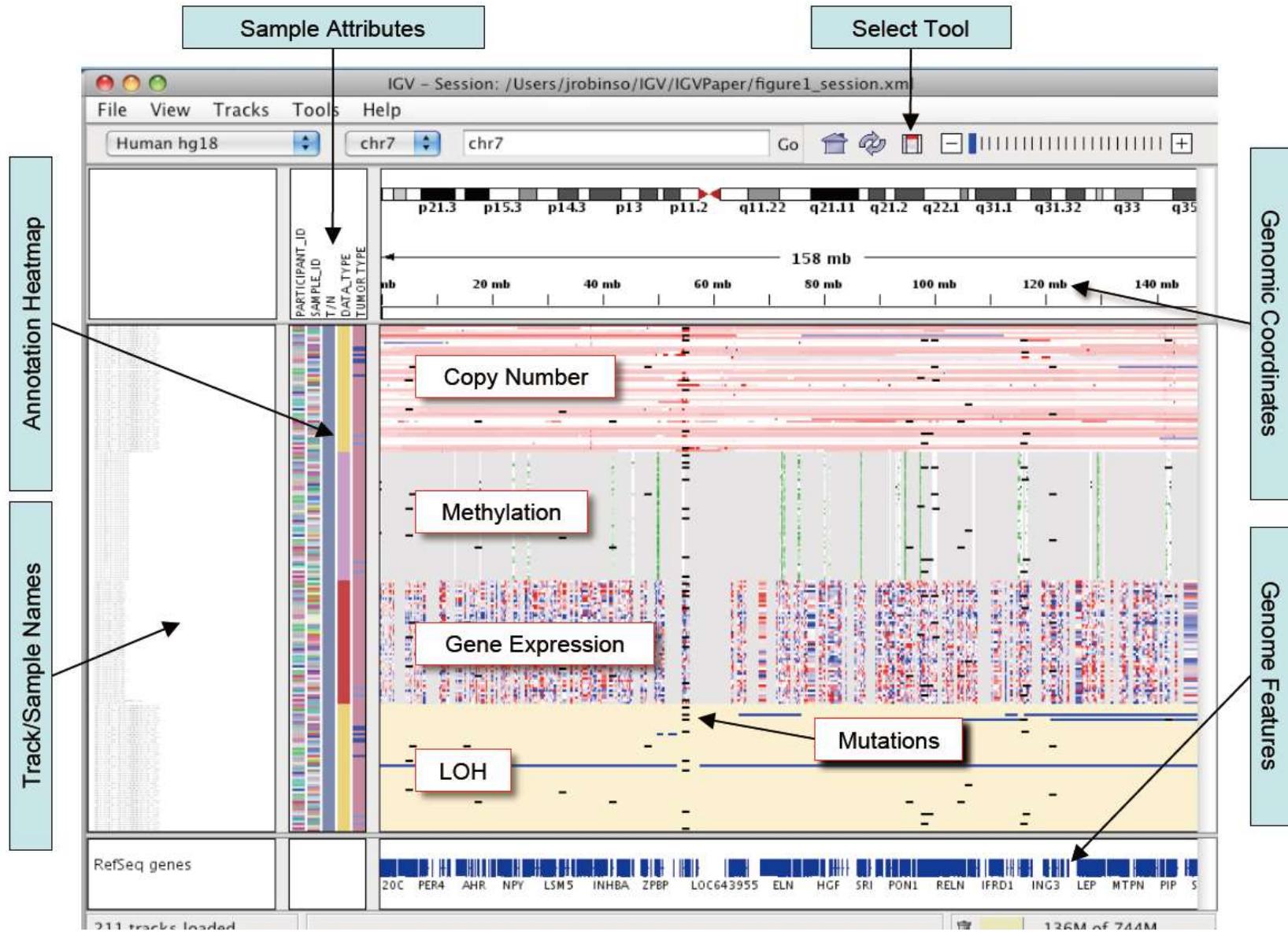
Science jobs from **naturejobs**

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Harvard Medical School

[Ramalingaswami Re-Entry Fellowship](#)

Ministry of Science & Technology, Government of India



Nature Biotech. 29:24–26 (2011) Supplement figureからの抜粋

 Integrative Genomics Viewer

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Home

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Integrative
Genomics
Viewer
ALMEL

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IGV User Guide

This guide describes the Integrative Genomics Viewer (IGV).

- To start IGV, go to the IGV downloads page: <http://www.broadinstitute.org/igv/download>

[Look at a printer-friendly HTML version of the whole User Guide.](#)

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[User Interface >](#)

ココか

The screenshot shows the IGV website's sidebar menu. A blue arrow points to the 'Downloads' option, which is highlighted with a red box. The menu also includes 'Home', 'Documents', 'IGV User Guide', 'Tutorial Videos', 'File Formats', 'Hosted Genomes', 'FAQ', 'Release Notes', 'Credits', and '@ Contact'. Below the menu is a search bar with a 'search' button and copyright information for 2013-2021, Broad Institute, and the Regents of the University of California.

Integrative Genomics Viewer

The homepage features a large image of the IGV software interface, showing multiple tracks of genomic data. Below the image are sections for 'Overview' and 'Citing IGV'. The 'Overview' section contains a brief description of the tool and its availability in different forms. The 'Citing IGV' section provides citation information for publications related to the tool.

ココをクリック

A blue arrow points to the 'Download IGV' button. Below it, a text box says 'Download the IGV desktop application and igvtools.' and features a download icon with a red box around it. A note at the bottom states that the IGV-Web application runs in a web browser and requires no downloads; a blue arrow points to the 'Help link in the app for more information.'

現在はウェブアプリ版もある
(今回の実習では使いません)

The 'Funding' section at the bottom right discusses the funding support for the development of IGV from the National Cancer Institute (NCI) and other organizations.

Downloads

Did you know that there is also an **IGV web application** that runs only in a web browser, does not use Java, and requires no downloads? See <https://igv.org/app>. Click on the [Help](#) link in the app for more information about using IGV-Web.

Install IGV 2.11.0

See the [Release Notes](#) for what's new in each IGV release.

NOTE for users of the new M1 Mac: Apple's Rosetta software is required to run the IGV MacOS App that includes Java. If you run IGV with your own Java installation, Rosetta may not be required if your version of Java runs natively on M1.

NOTE for Linux users: The 'IGV for Linux' download includes *AdoptOpenJDK 11 for x64 Linux with glibc 2.12 or higher*. If this does not work on your version of Linux, use the 'Command line IGV' download with your own Java installation.



利用するパソコンの
プラットフォームに応じて選択

Other IGV Versions

[Development Snapshot Build](#). Latest development snapshot; built at least nightly

[Archived Versions](#). Old releases going back to IGV 2.0

This Downloads page is for the IGV desktop version. There are also other versions of IGV:

- If you are looking for the **IGV-Web application**, see <https://igv.org/app>
- If you are a developer looking for information about the embeddable *igv.js component*, see <https://github.com/igvteam/igv.js>
- If you want to use IGV in your Jupyter Notebooks, see <https://github.com/igvteam/igv-jupyter>

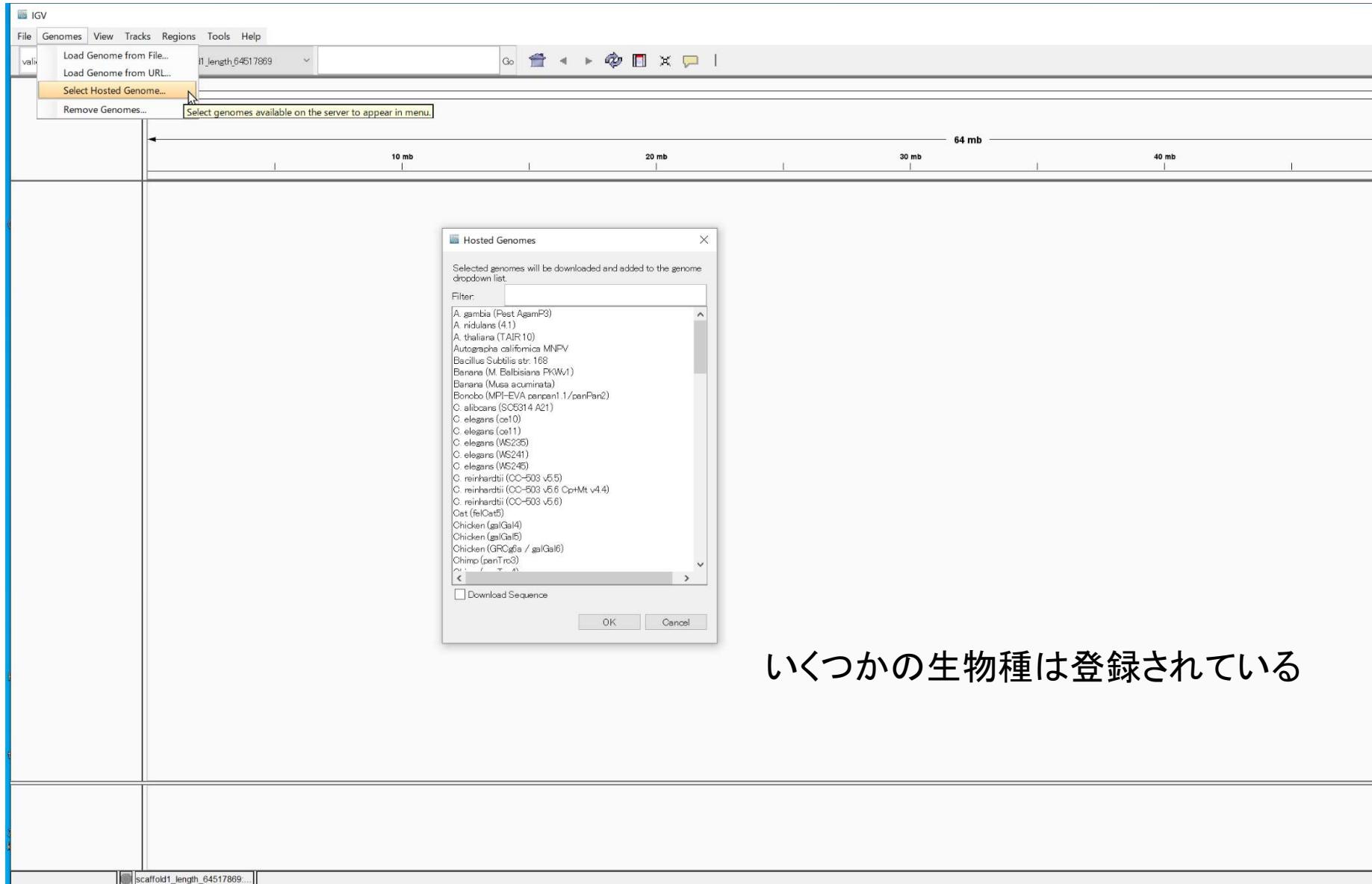
License

IGV is completely open for anyone to use under an [MIT open-source license](#).

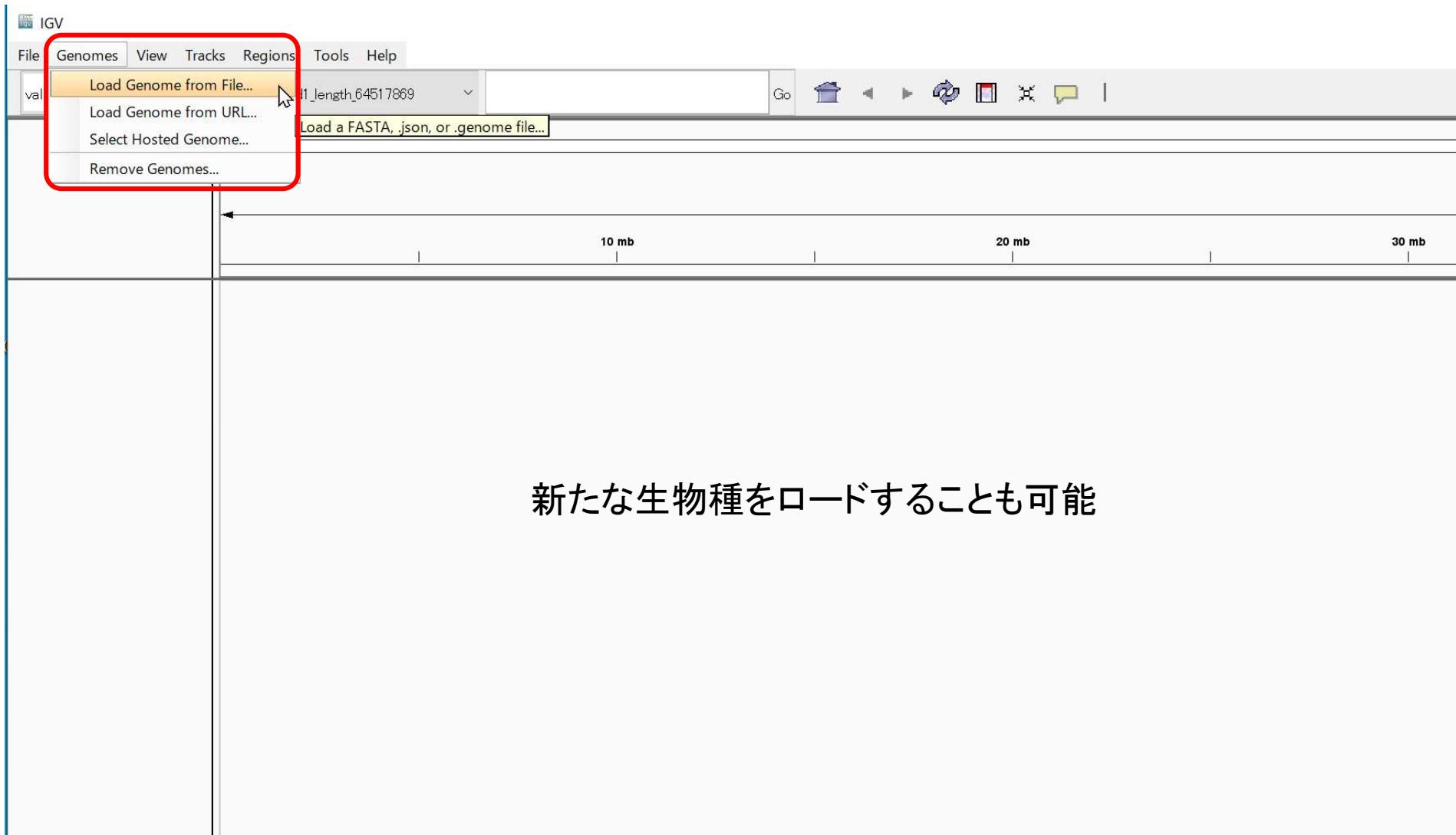
IGV development, maintenance, and support is funded by grants and it is important to be able to show that it is useful to the scientific community. Please see the [home page](#) for information on how to cite IGV.

Source Code

The source code repository for the IGV desktop application is hosted on GitHub at <https://github.com/igvteam/igv/>



いくつかの生物種は登録されている



ゲノムViewerなので次世代DNAシーケンサーのデータに限定されない。
マイクロアレイの結果や、ゲノムアノテーションの情報も随時表示できる。

対応するファイル形式に応じて、表示方法が決まる。

Home > File Formats

File Formats

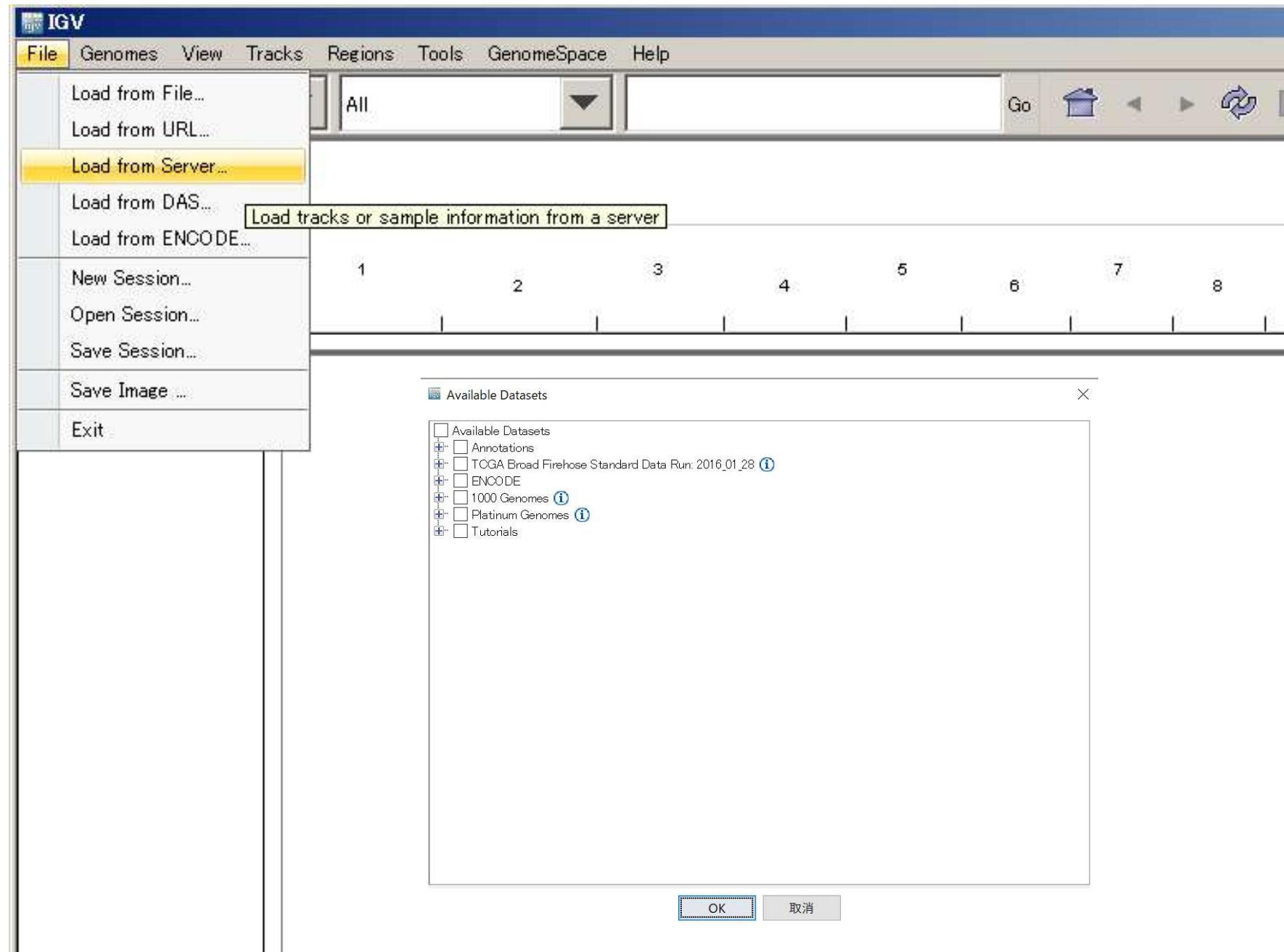
- [BAM](#)
- [BED](#)
- [BEDPE](#)
- [BedGraph](#)
- [bigBed](#)
- [bigWig](#)
- [Birdsuite Files](#)
- [broadPeak](#)
- [CBS](#)
- [Chemical Reactivity Probing Profiles](#)
- [chrom.sizes](#)
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- [Custom File Formats](#)
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- [FASTA](#)
- [GCT](#)
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- [GFF/GTF](#)
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- [MAF \(Multiple Alignment Format\)](#)
- [MAF \(Mutation Annotation Format\)](#)
- [Merged BAM File](#)
- [MUT](#)
- [narrowPeak](#)
- [PSL](#)
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- [TDF](#)
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- [Type Line](#)
- [VCF](#)
- [WIG](#)

File Formats

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

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

公開情報のviewerとして



その他の便利機能

セッションの保存

表示しているデータの読み込み状況を、それごと保存。

セッションをロードすることで、意図した画面を表示できる。

データセットが揃っていること、フォルダー構造が同一である必要がある。

Ref配列のコピー

Define a region of interestのアイコンを押す。

カーソルが十字となる。

配列の取り出したい領域の開始と終了ポジションをクリック。

指定された領域が赤色になり、右クリックしてCopy Sequence

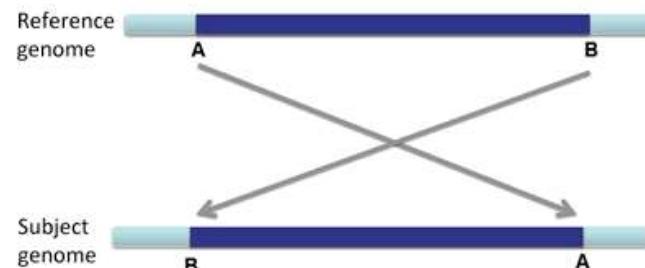
バッチ処理

重要領域の画面スナップショットを自動で取ったりできる。

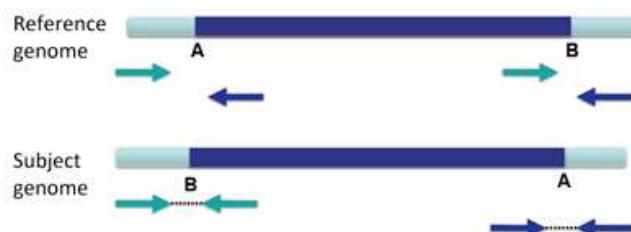
```
new
load myfile.bam
snapshotDirectory mySnapshotDirectory
genome hg18
goto chr1:65,289,335-65,309,335
sort position
collapse
snapshot
goto chr1:113,144,120-113,164,120
sort base
collapse
snapshot
```

Inversions

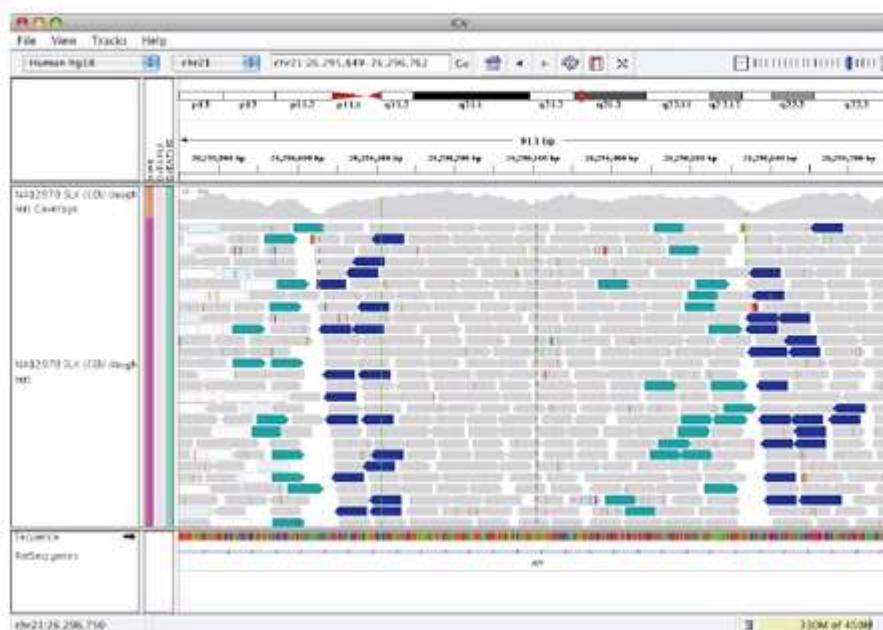
An inversion is a large section of DNA that is reversed in the subject genome compared to the reference genome.



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



This appears in IGV as shown below.



Interpreting Color by Insert Size

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

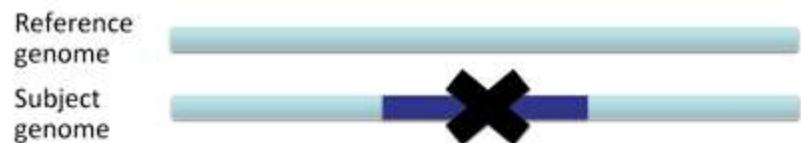
- deletions
- insertions
- inter-chromosomal rearrangements

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

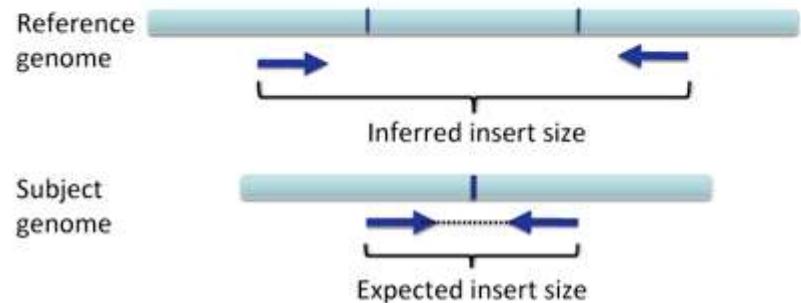
- for an insert that is larger than expected
- for an insert that is smaller than expected
- for paired end reads that are coded by the chromosome on which their mates can be found

Deletions

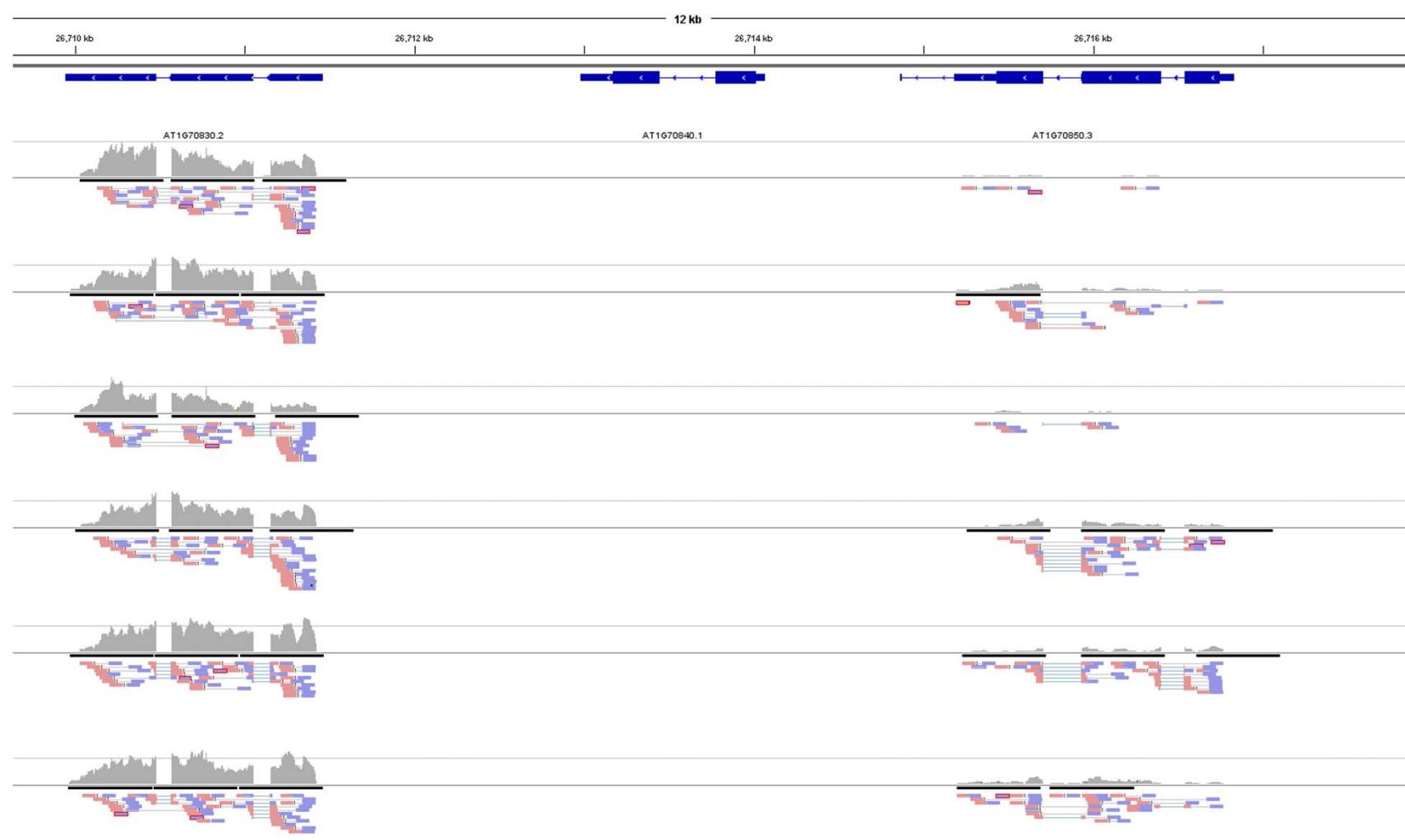
A deletion is a large section of DNA that is absent in the subject genome compared to the reference genome.



The "expected" insert size is the insert size obtained in sequencing the subject genome. The "inferred" insert size is the insert size that would result in the reference genome, assuming the same pair of reads.

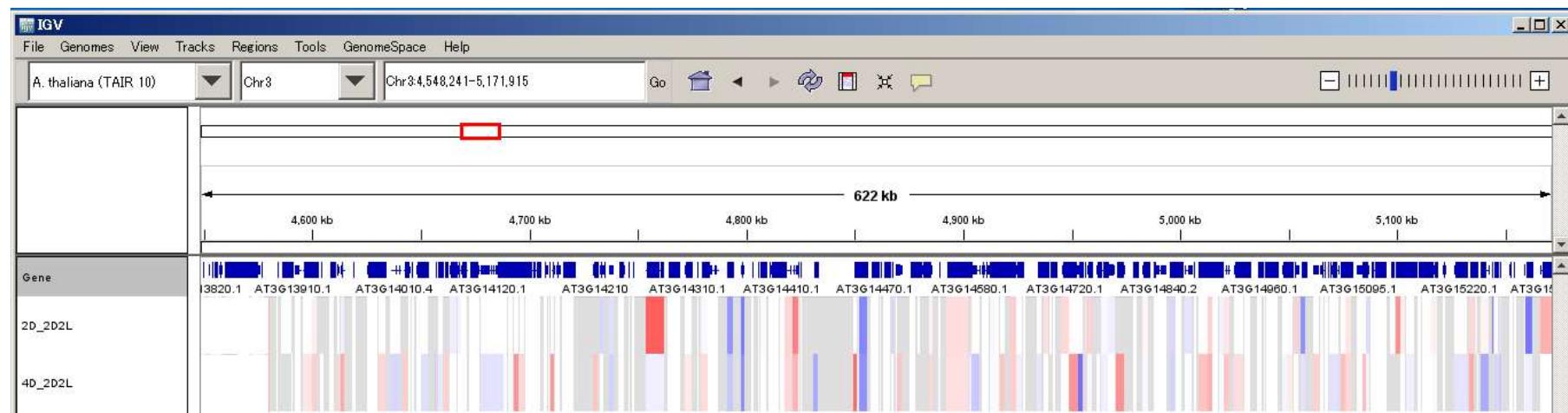


RNA-Seqのデータ表示させる



GCTファイルでgene ローカスの発現情報を図示

```
#  
#  
Name      Description          2D_2D2L    4D_2D2L  
ANAC001   |@Chr1:3630-5899     -2.60184   -2.60956  
DCL1      |@Chr1:23145-33153    -0.742675  -1.5642  
MIR838A   |@Chr1:23145-33153    0          0  
AT1G01073  |@Chr1:44676-44787    0          0  
IQD18     |@Chr1:52238-54692    -1.93871   -1.13128  
AT1G01115  |@Chr1:56623-56740    0          0  
GIF2      |@Chr1:72338-74737    -0.251287  -0.616679  
AT1G01180  |@Chr1:75582-76758    0.45929   -0.809567  
AT1G01210  |@Chr1:88897-89745    1.6964    0.857196  
FKGP      |@Chr1:91375-95651    -0.174589  0.725947  
AT1G01240  |@Chr1:99893-101834   -0.226384  -0.936641  
AT1G01260  |@Chr1:108945-111609   -0.161848  0.315699  
CYP703A2   |@Chr1:112262-113947  0          0  
CNX3      |@Chr1:114285-116108   0.111249   -0.551359  
AT1G01300  |@Chr1:116942-118764   -0.68348   0.108578
```



Gene listを定義して
サンプルごと
条件ごと
の発現・発現変動を
カラーマップできる

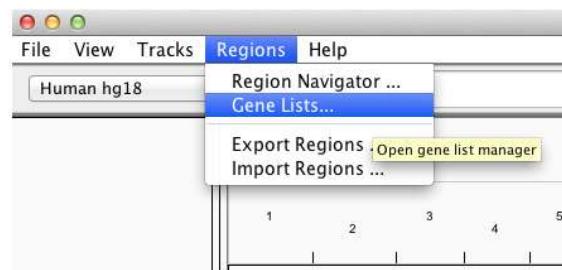
Home > IGV User Guide > Gene List View

Gene List View

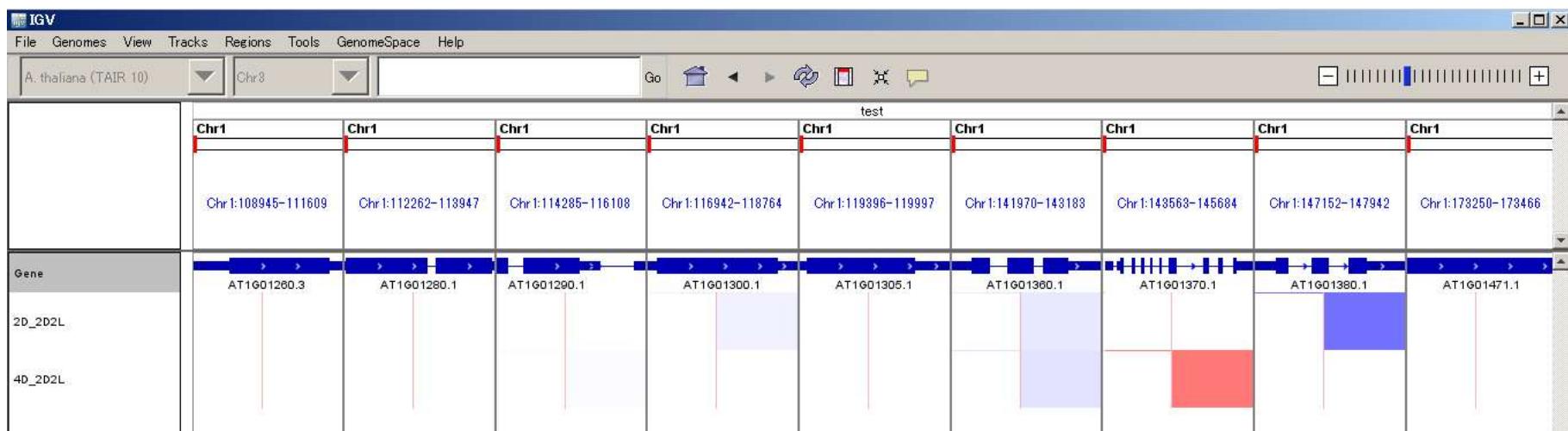
The Gene Lists functionalities in IGV allow you to view lists of genes or loci side-by-side irrespective of their genomic location.

Loading/Defining Gene Lists

To load or define a new gene/locus list, select Regions >Gene Lists....



This opens a window for selecting an existing list or creating a new list.



IGV実習

Home > Downloads

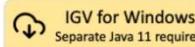
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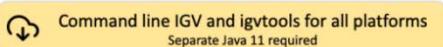
Install IGV 2.9.1

See the [Release Notes](#) for what's new in each IGV release.

 IGV MacOS App Java included
 IGV MacOS App Separate Java 11 required

 IGV for Windows Java included
 IGV for Windows Separate Java 11 required

 IGV for Linux Java included

 Command line IGV and igvtools for all platforms Separate Java 11 required

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- If you are looking for the **IGV-Web application**, see <https://igv.org/app>
- If you are a developer looking for information about the embeddable *igv.js* component, see <https://github.com/igvteam/igv.js>
- If you want to use IGV in your Jupyter Notebooks, see <https://github.com/igvteam/igv-jupyter>

License

IGV is completely open for anyone to use under an [MIT open-source license](#).

IGV development, maintenance, and support is funded by grants and it is important to be able to show that it is useful to the scientific community. Please see the [home page](#) for information on how to cite IGV.

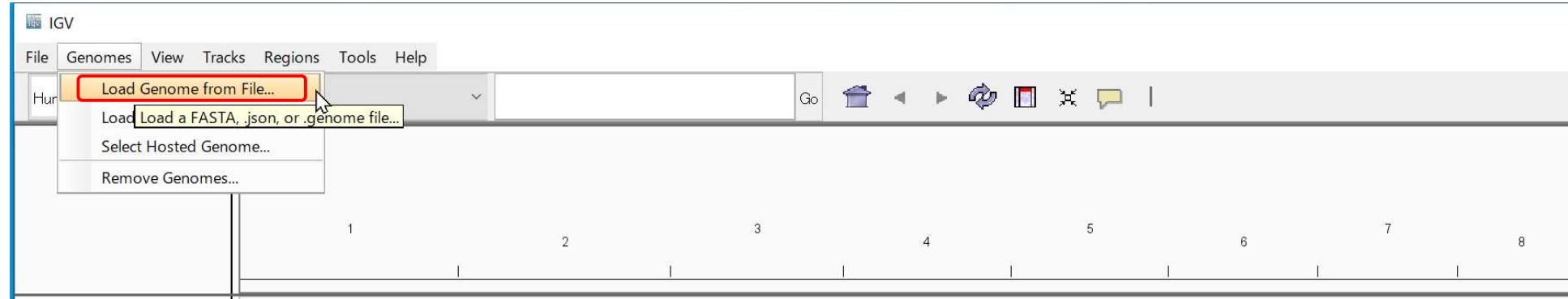
Source Code

The source code repository for the IGV desktop application is hosted on GitHub at <https://github.com/igvteam/igv/>

IGVの使用法を学ぶと共に
先のファイルフォーマットも
確認しよう

以下のファイルを確認

buc.genome.fasta
buc.gtf
buc_cg.wig
illumina_ex_B2_Read_bowtie2.mate.sort.bam
illumina_ex_B2_Read_bowtie2.mate.sort.bam.bai
illumina_ex_B4_Read_bowtie2.mate.sort.bam
illumina_ex_B4_Read_bowtie2.mate.sort.bam.bai



IGV

File Genomes View Tracks Regions Tools Help

Hu... Load Genome from File...

Load Load a FASTA, json, or .genome file...

Select Hosted Genome...

Remove Genomes...

1 2 3 4 5 6 7 8

登録されていない生物種・配列でも、自分でimportすればOK
Buchneraゲノムを使う
Genome -> Load Genome from File
buc.genome.fasta
を読み込む

IGV

File Genomes View Tracks Regions Tools Help

Load from File... Load from File...

Load from URL... Load tracks or sample information

Load from Server...

Load from ENCODE (2012)...

Reload Tracks

New Session...

Open Session...

Save Session...

Reload Session

Save PNG Image ...

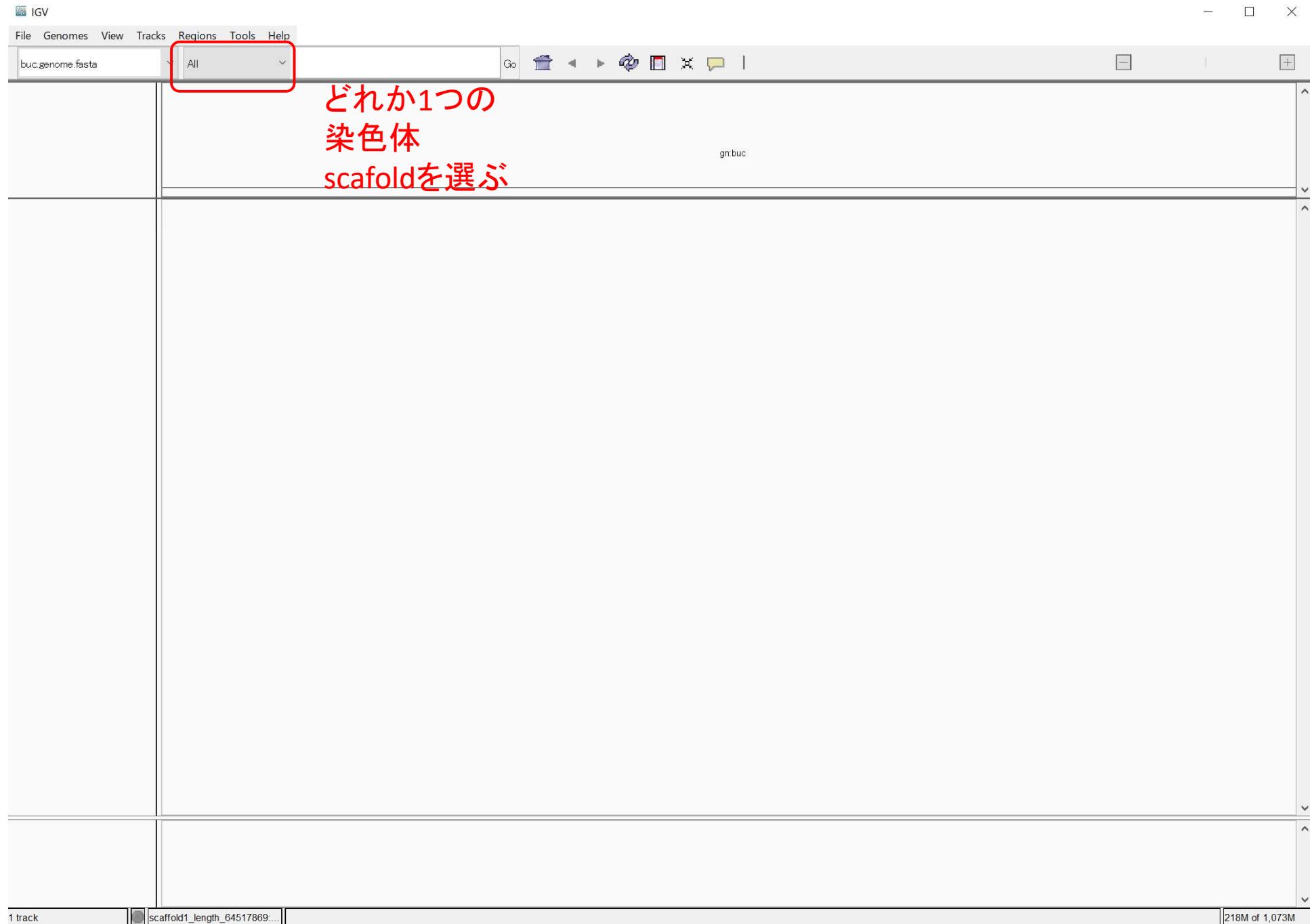
Save SVG Image ...

Exit

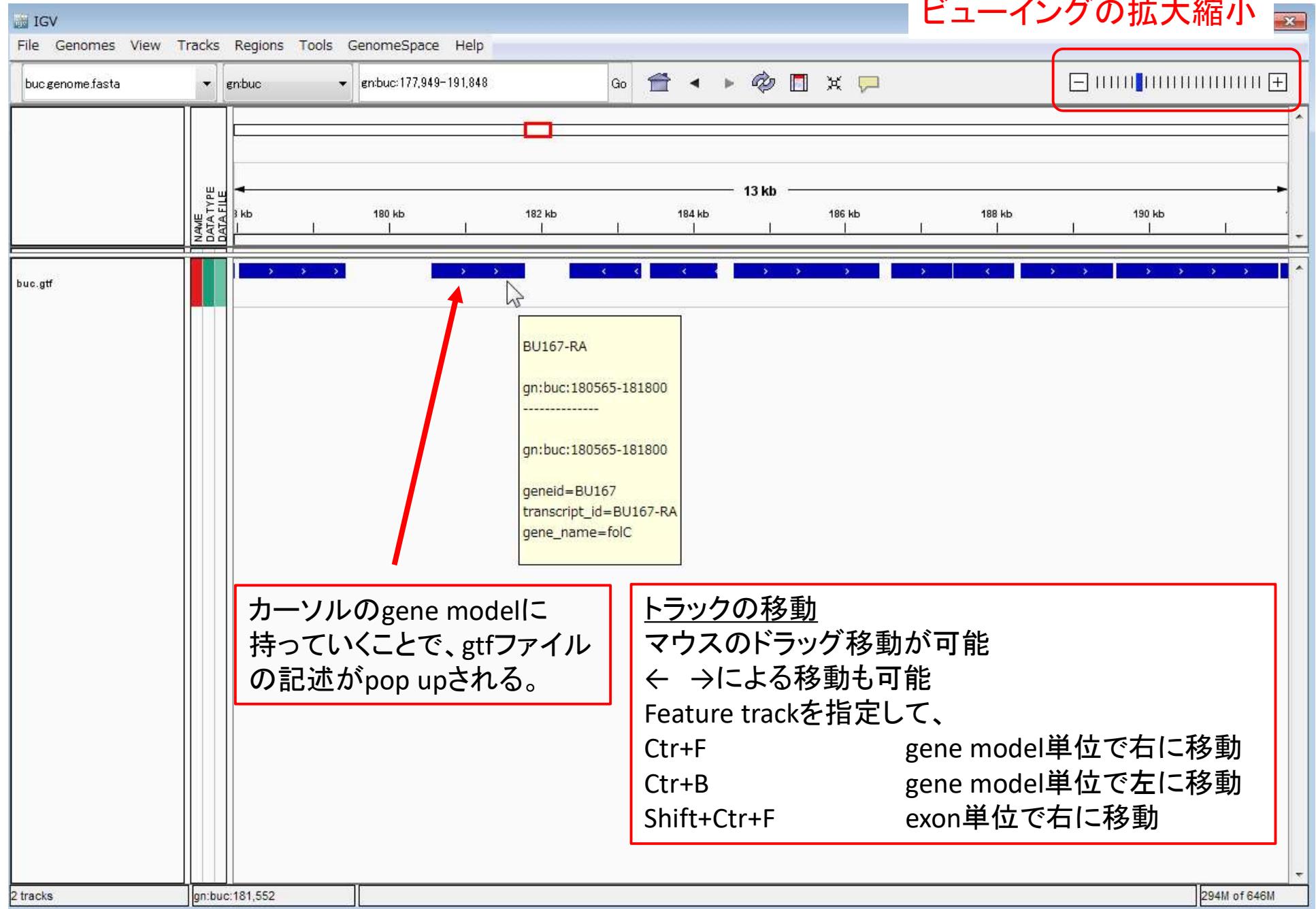
1 ゲノム構造を記述したgtfファイルを読み込む 5 7
2 6

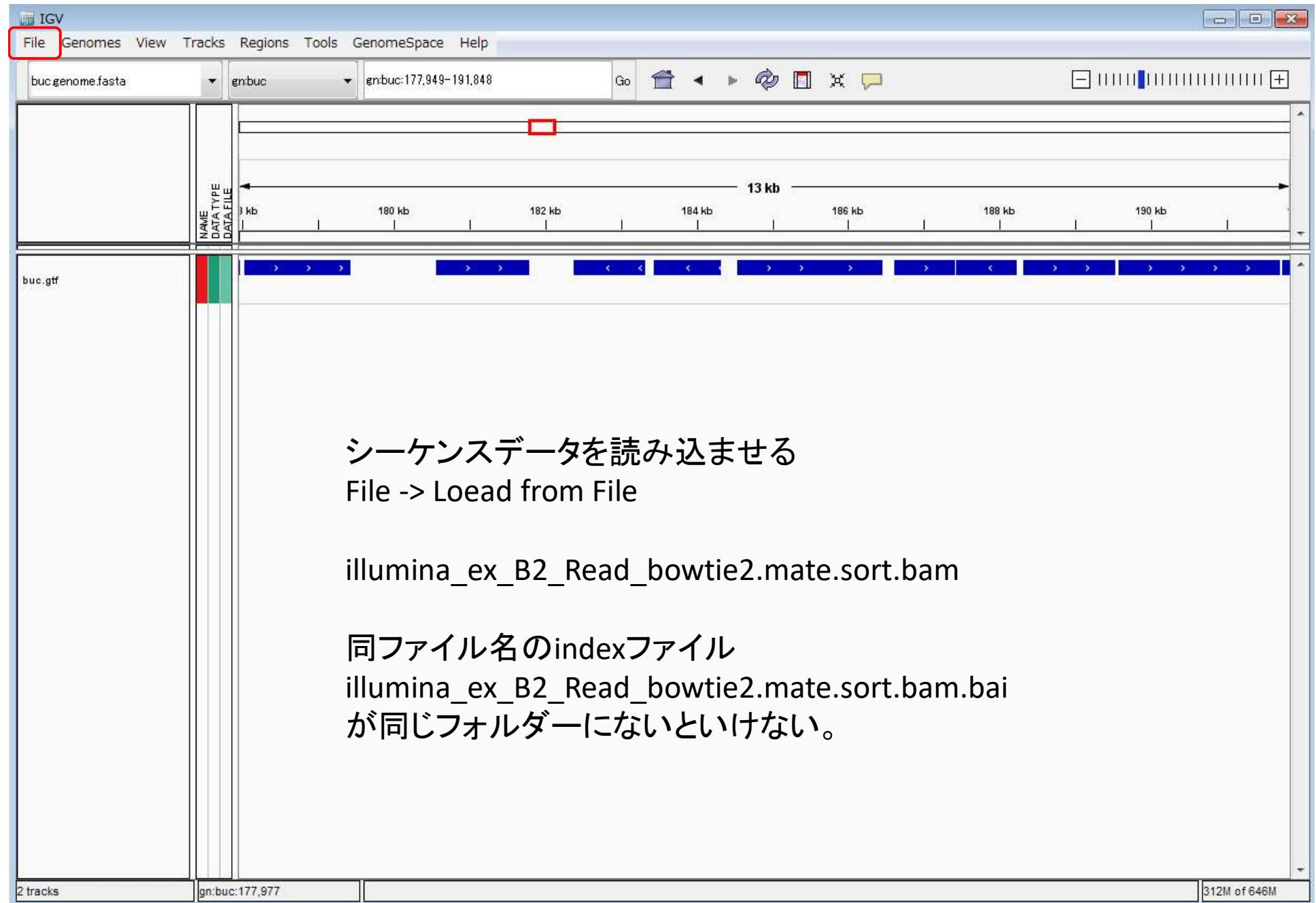
File-> Load from File
buc.gtf ファイルを読み込む

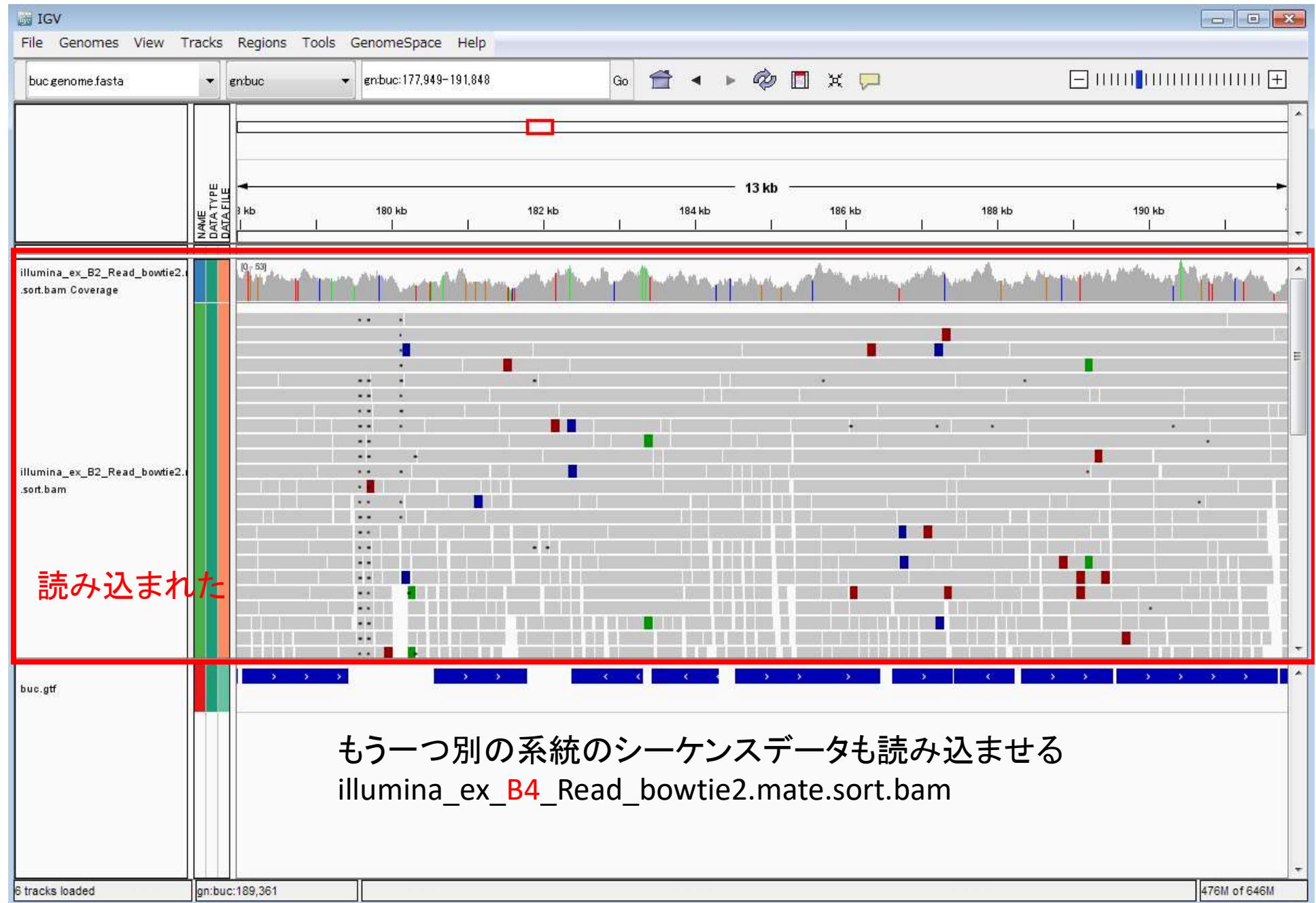
buc.gtf						
gn:buc	KEGG:GENES	CDS	197	2083	.+	geneid "BU001"; transcript_id "BU001-RA"; gene_name "gidA";
gn:buc	KEGG:GENES	CDS	2278	3102	.+	geneid "BU002"; transcript_id "BU002-RA"; gene_name "atpB";
gn:buc	KEGG:GENES	CDS	3139	3378	.+	geneid "BU003"; transcript_id "BU003-RA"; gene_name "atpE";
gn:buc	KEGG:GENES	CDS	3497	3982	.+	geneid "BU004"; transcript_id "BU004-RA"; gene_name "atpF";
gn:buc	KEGG:GENES	CDS	3982	4515	.+	geneid "BU005"; transcript_id "BU005-RA"; gene_name "atpH";
gn:buc	KEGG:GENES	CDS	4530	6068	.+	geneid "BU006"; transcript_id "BU006-RA"; gene_name "atpA";
gn:buc	KEGG:GENES	CDS	6101	6973	.+	geneid "BU007"; transcript_id "BU007-RA"; gene_name "atpC";
gn:buc	KEGG:GENES	CDS	6997	8394	.+	geneid "BU008"; transcript_id "BU008-RA"; gene_name "atpD";
gn:buc	KEGG:GENES	CDS	8421	8837	.+	geneid "BU009"; transcript_id "BU009-RA"; gene_name "atpC";
gn:buc	KEGG:GENES	CDS	8911	11322	.-	geneid "BU010"; transcript_id "BU010-RA"; gene_name "gyrB";
gn:buc	KEGG:GENES	CDS	11449	12549	.-	geneid "BU011"; transcript_id "BU011-RA"; gene_name
"dNan";						
gn:buc	KEGG:GENES	CDS	12554	13918	.-	geneid "BU012"; transcript_id "BU012-RA"; gene_name "dNanA";
gn:buc	KEGG:GENES	CDS	14369	14512	.+	geneid "BU013"; transcript_id "BU013-RA"; gene_name
"rmpH";						
gn:buc	KEGG:GENES	CDS	14525	14872	.+	geneid "BU014"; transcript_id "BU014-RA"; gene_name "rmpH";
gn:buc	KEGG:GENES	CDS	15011	16609	.+	geneid "BU015"; transcript_id "BU015-RA"; gene_name "yidC";



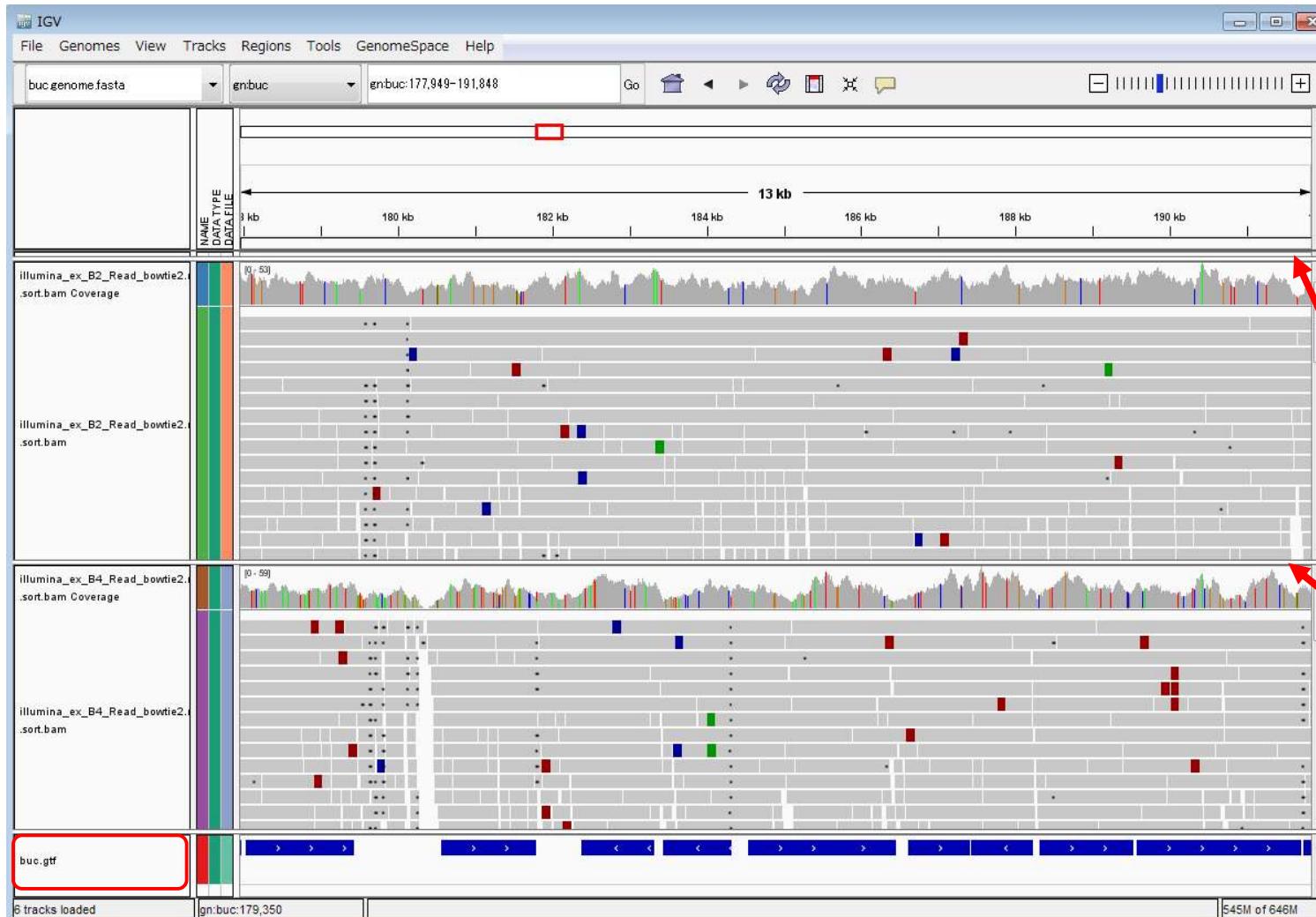
ビューアの拡大縮小





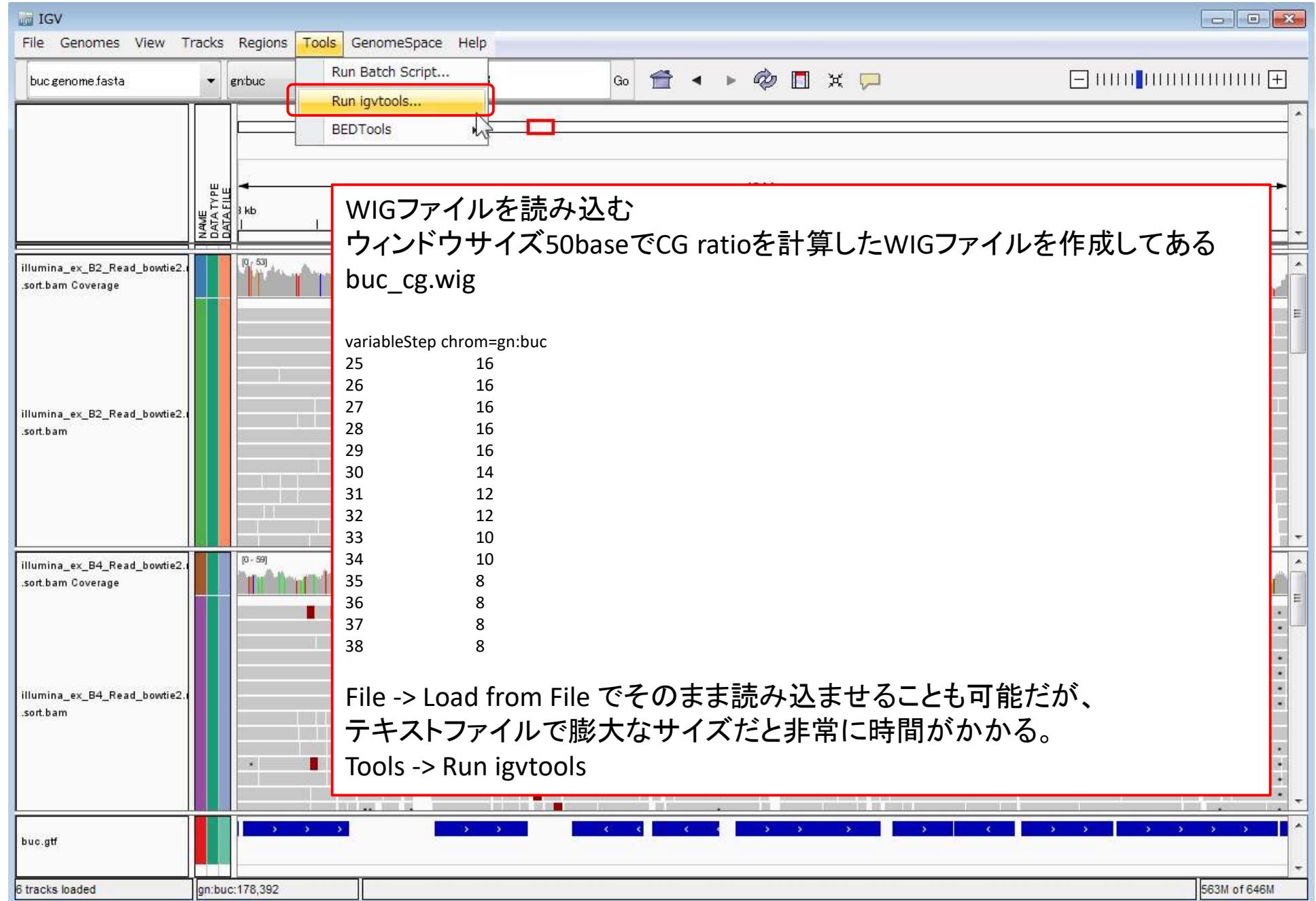


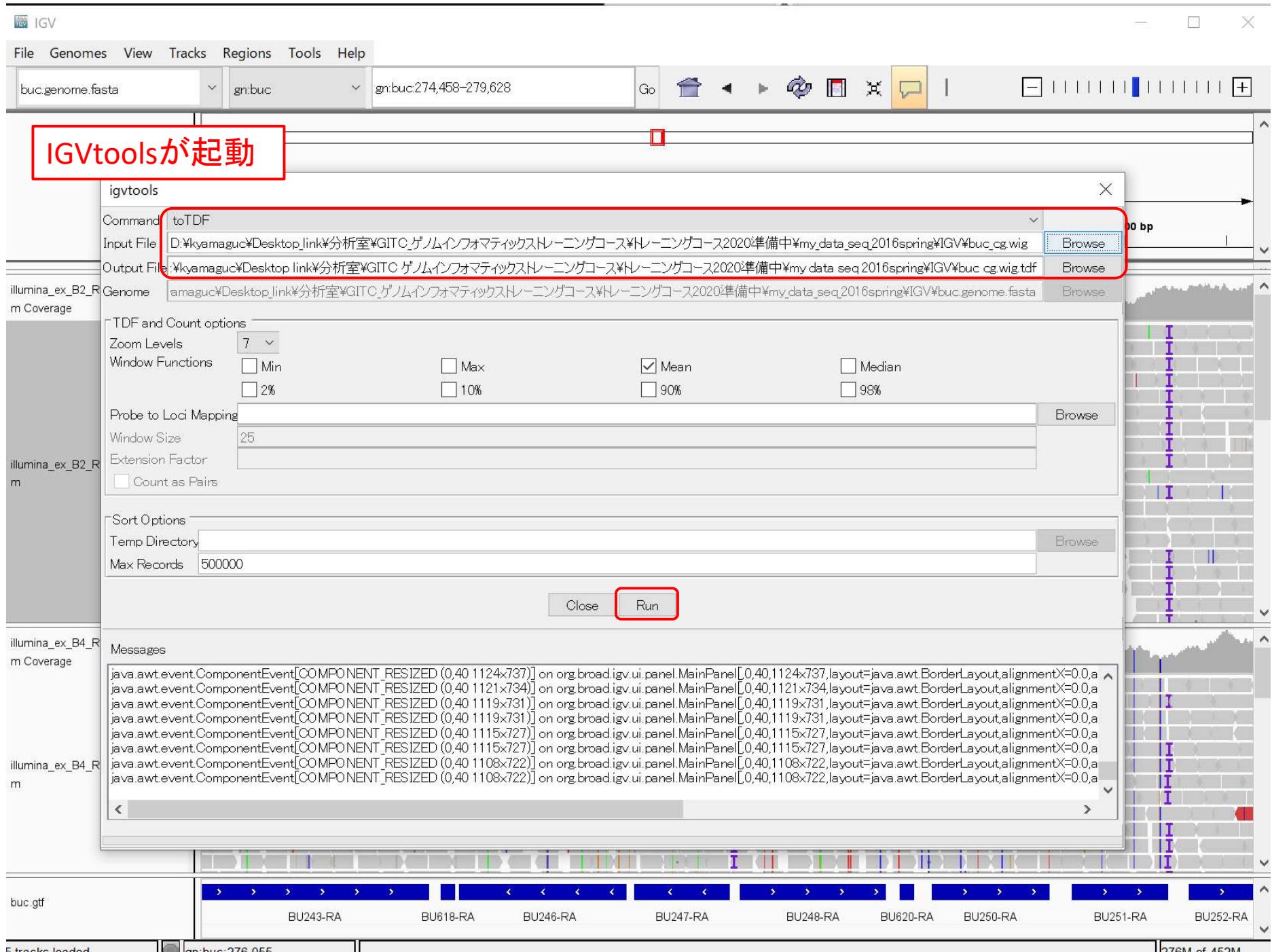
もう一つ別の系統のシーケンスデータも読みませる
illumina_ex_B4_Read_bowtie2.mate.sort.bam

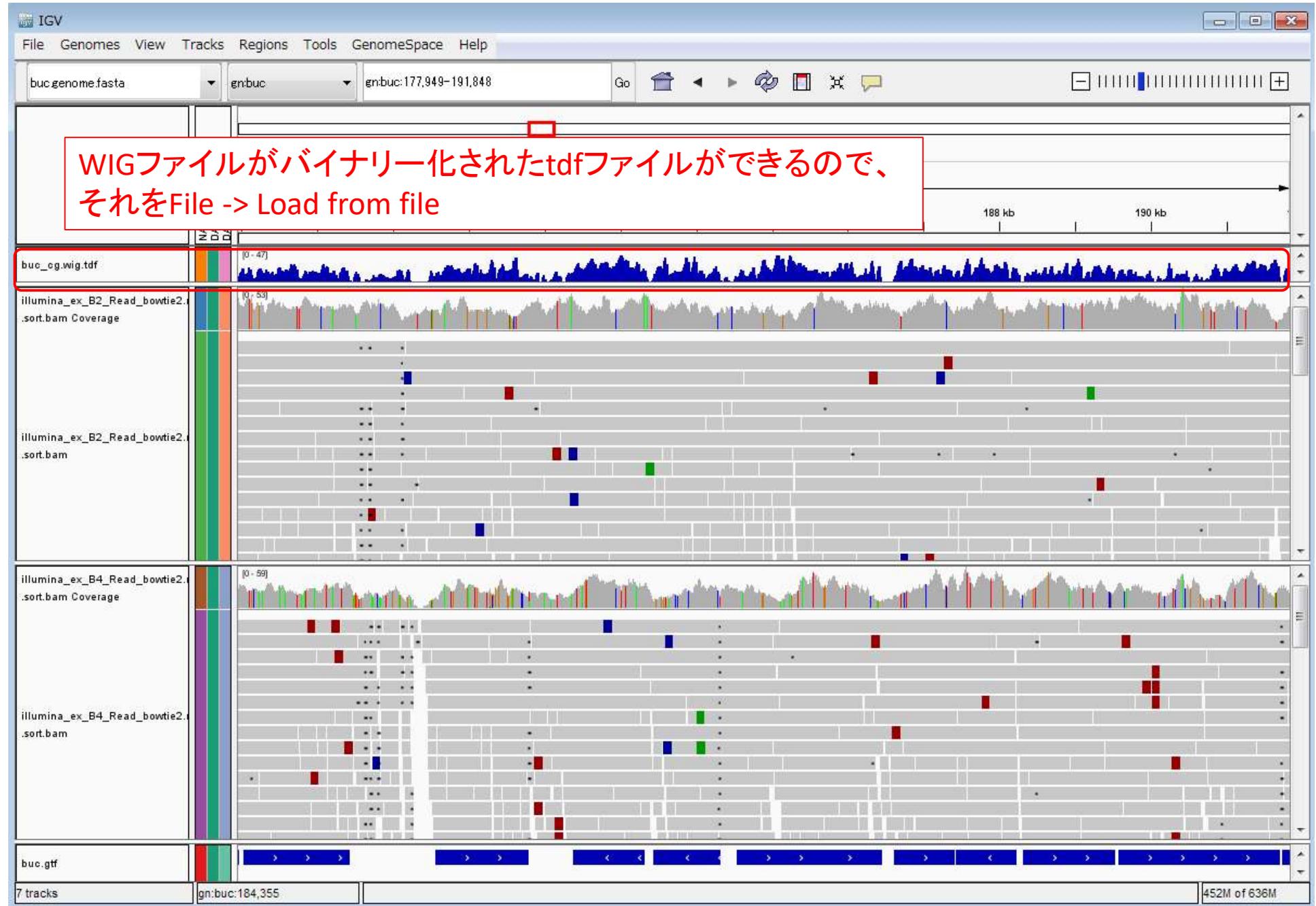


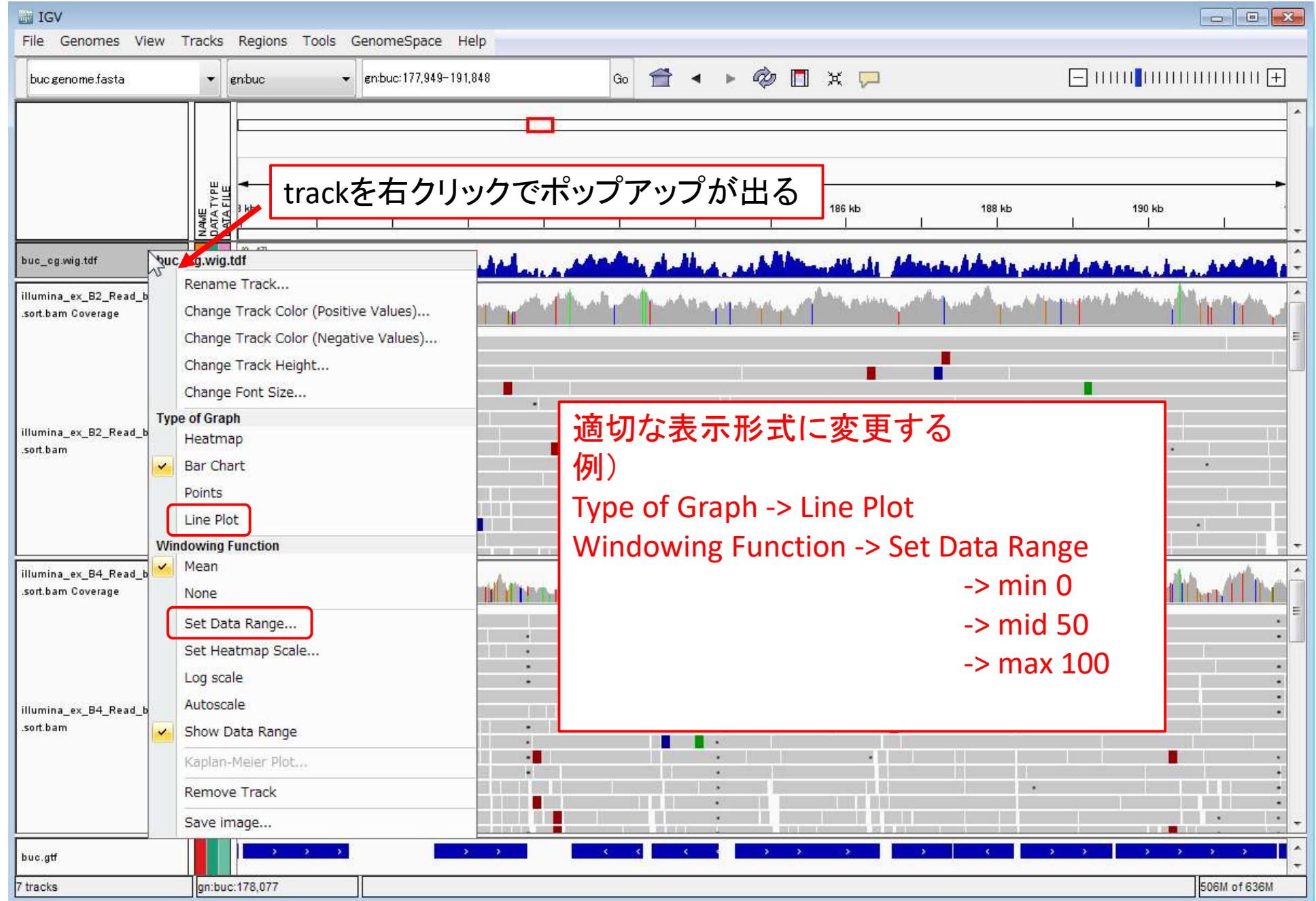
各Trackはドラッグアンドドロップで移動できる

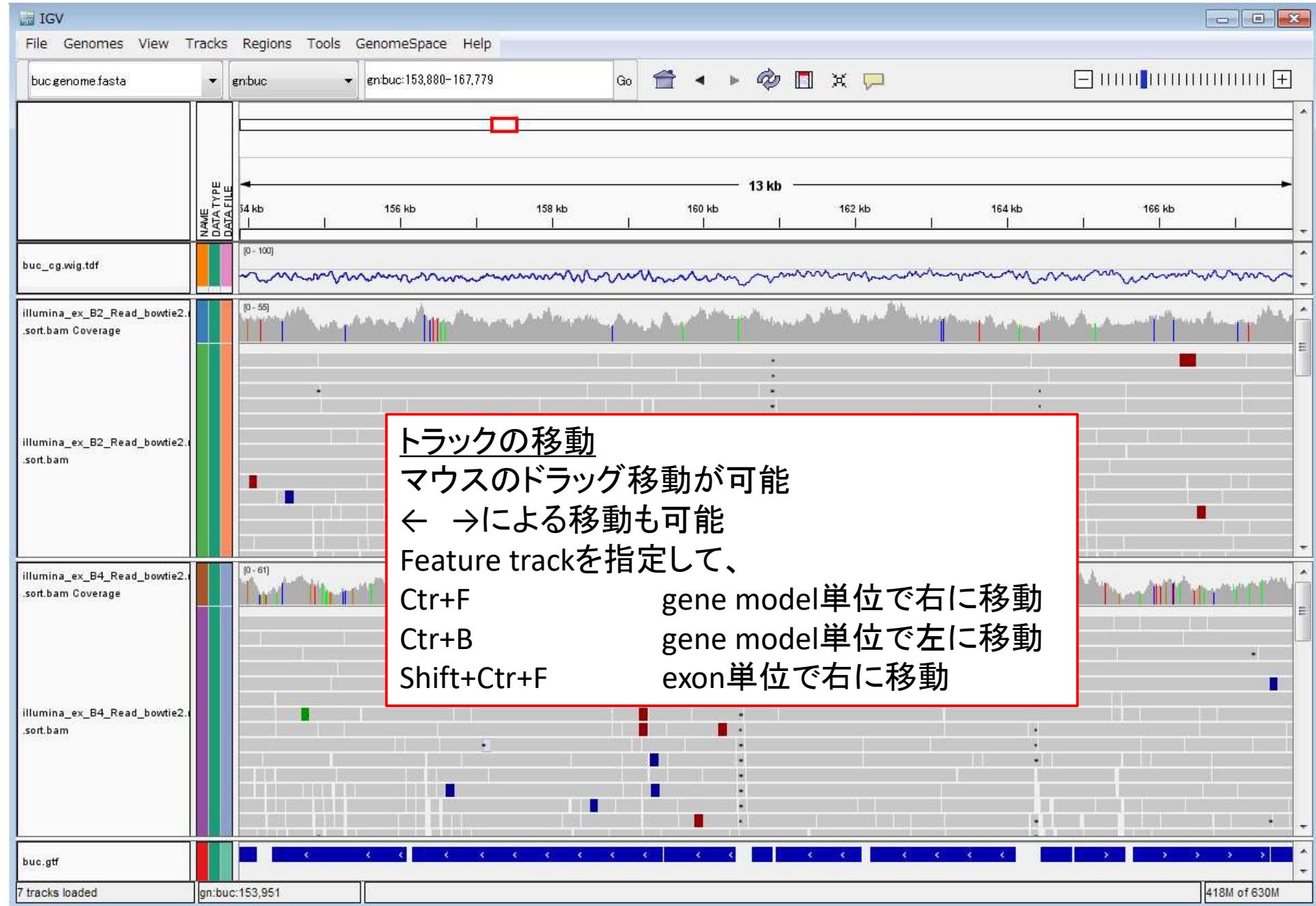
こういう所を
クリックしたまま
移動させることで
幅を変更

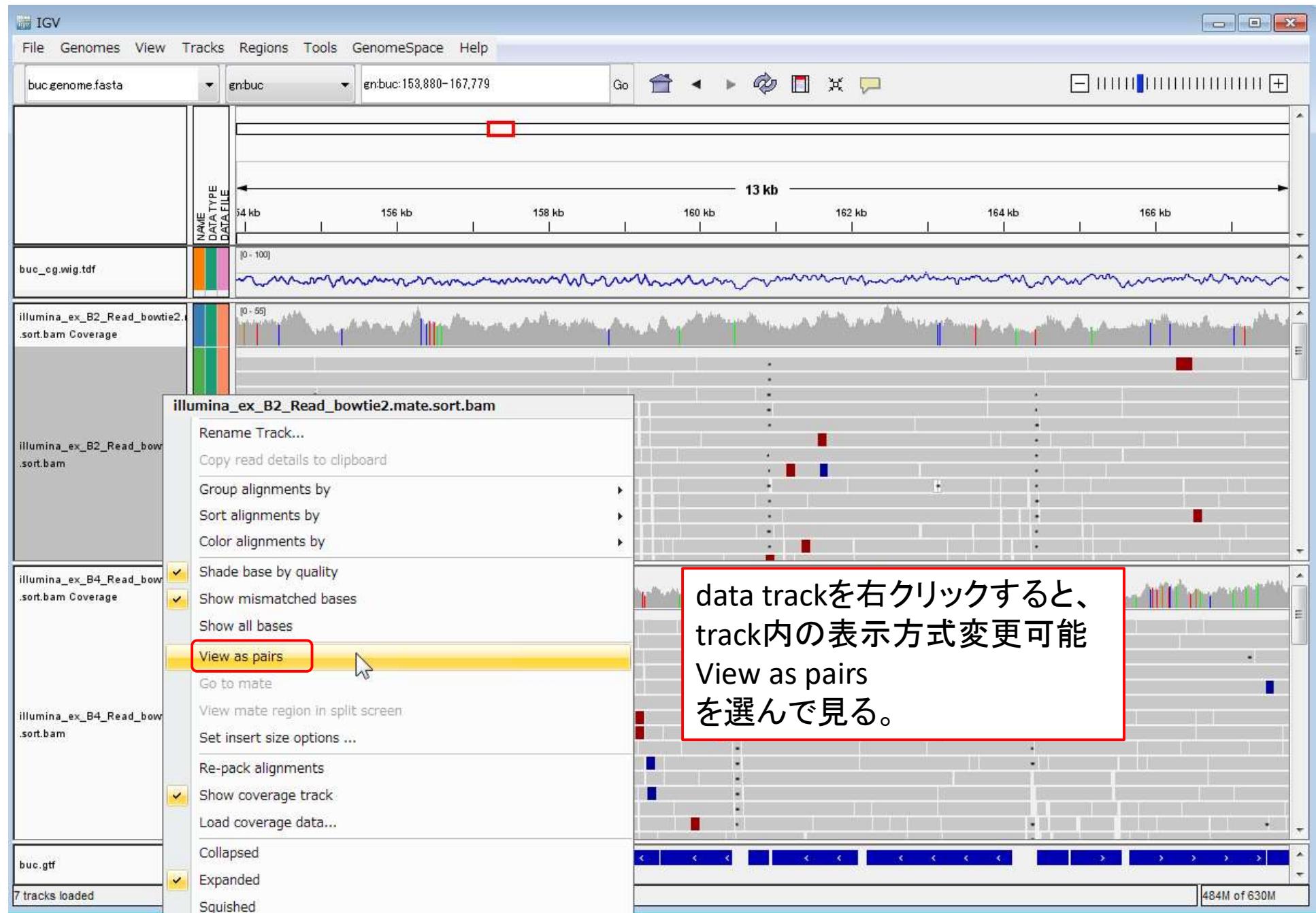




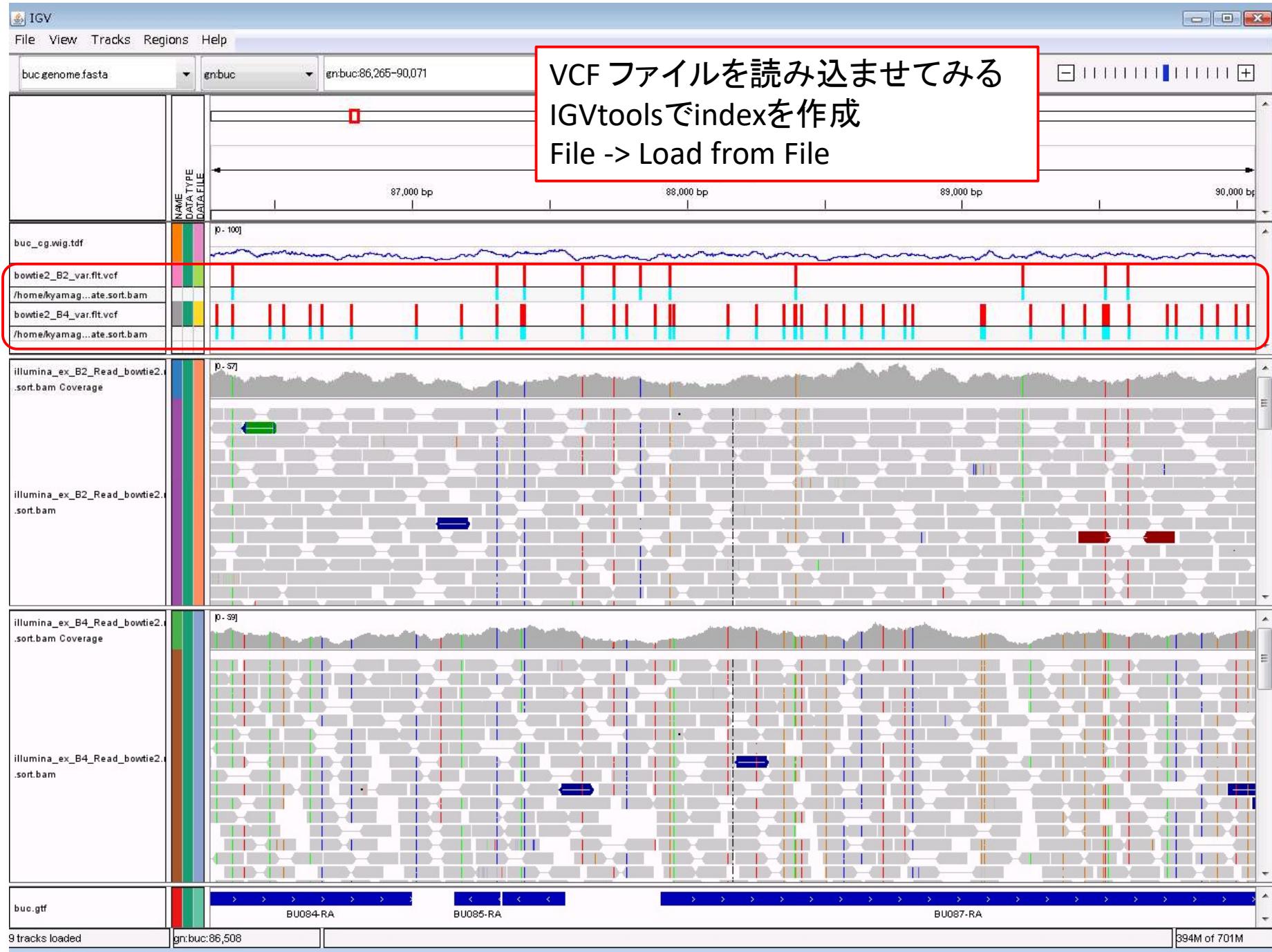


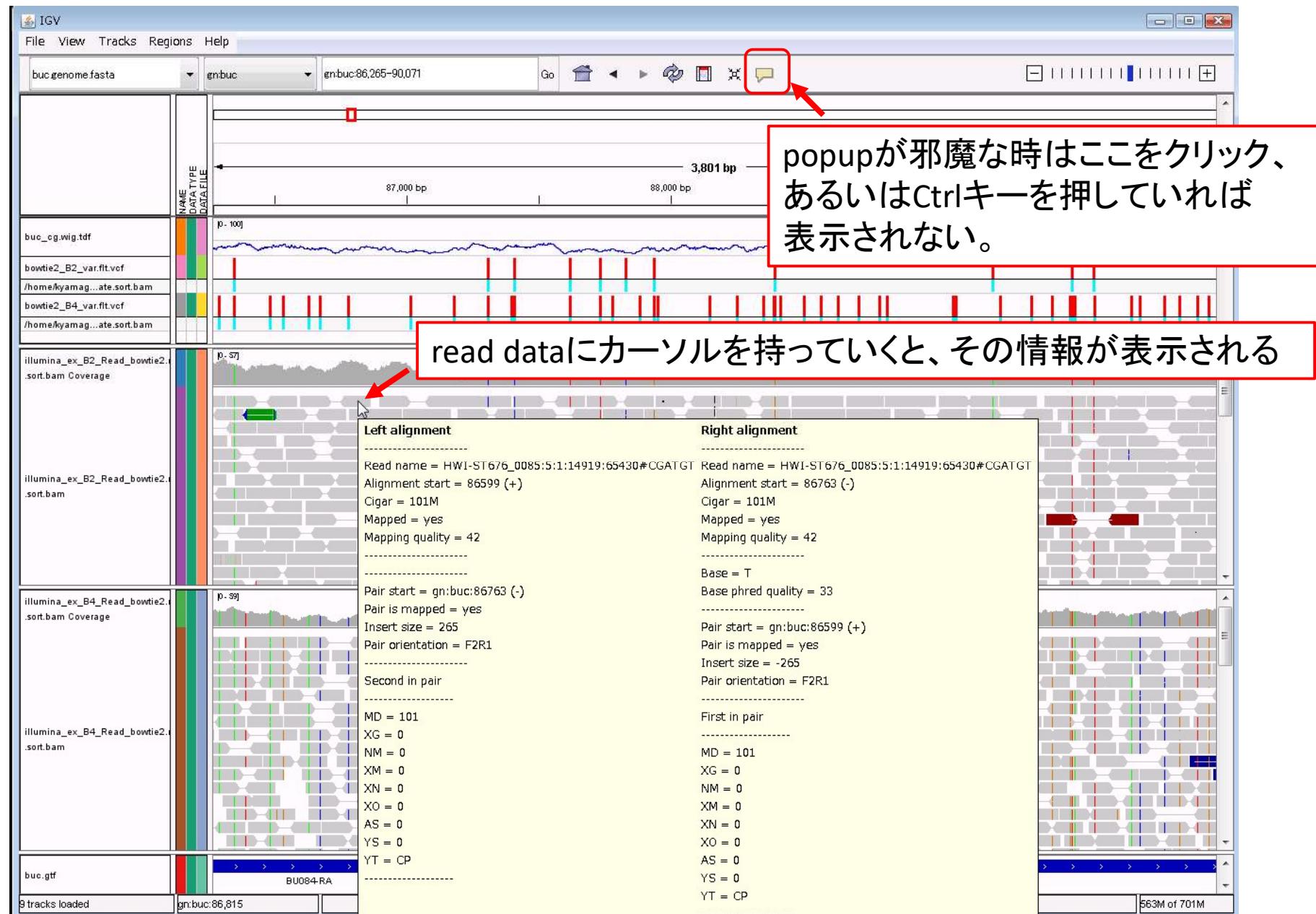












IGV紹介のまとめ

可視化ツールとして十分な機能を持つ

- ・無料
- ・比較的簡単・お手軽
- ・自分で見るためにも良し、人に見せるためにも良し
- ・利用範囲は次世代DNAシーケンサーに限定しない
広くゲノミクスの解析に有用

ごく一部のみの機能を紹介しました。
ウェブサイトを見ながら復習をお勧めします。