

基生研ゲノムインフォマティックス・トレーニングコース 2020  
RNA-seq入門 – NGSの基礎からde novo解析まで–  
実践編：RNA-seq解析パイプライン  
2020.06.04–2020.06.05

# NGS基本ツールIGV

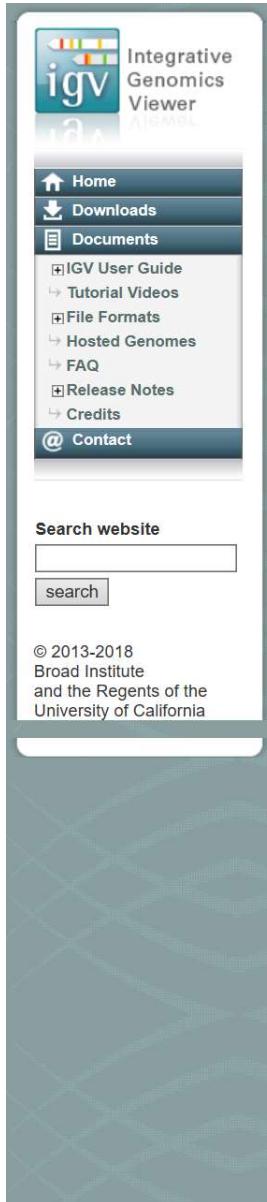
基礎生物学研究所  
生物機能解析センター  
山口勝司

## 宿題

IGVの実習は自身で用意しているパソコンを使って行います。

- ・用意したパソコンにIGVをインストールし、起動を確認せよ。
- ・以下のサイトに今回の実習に用いるdata setが用意されています。  
用意したパソコンにダウンロードせよ。

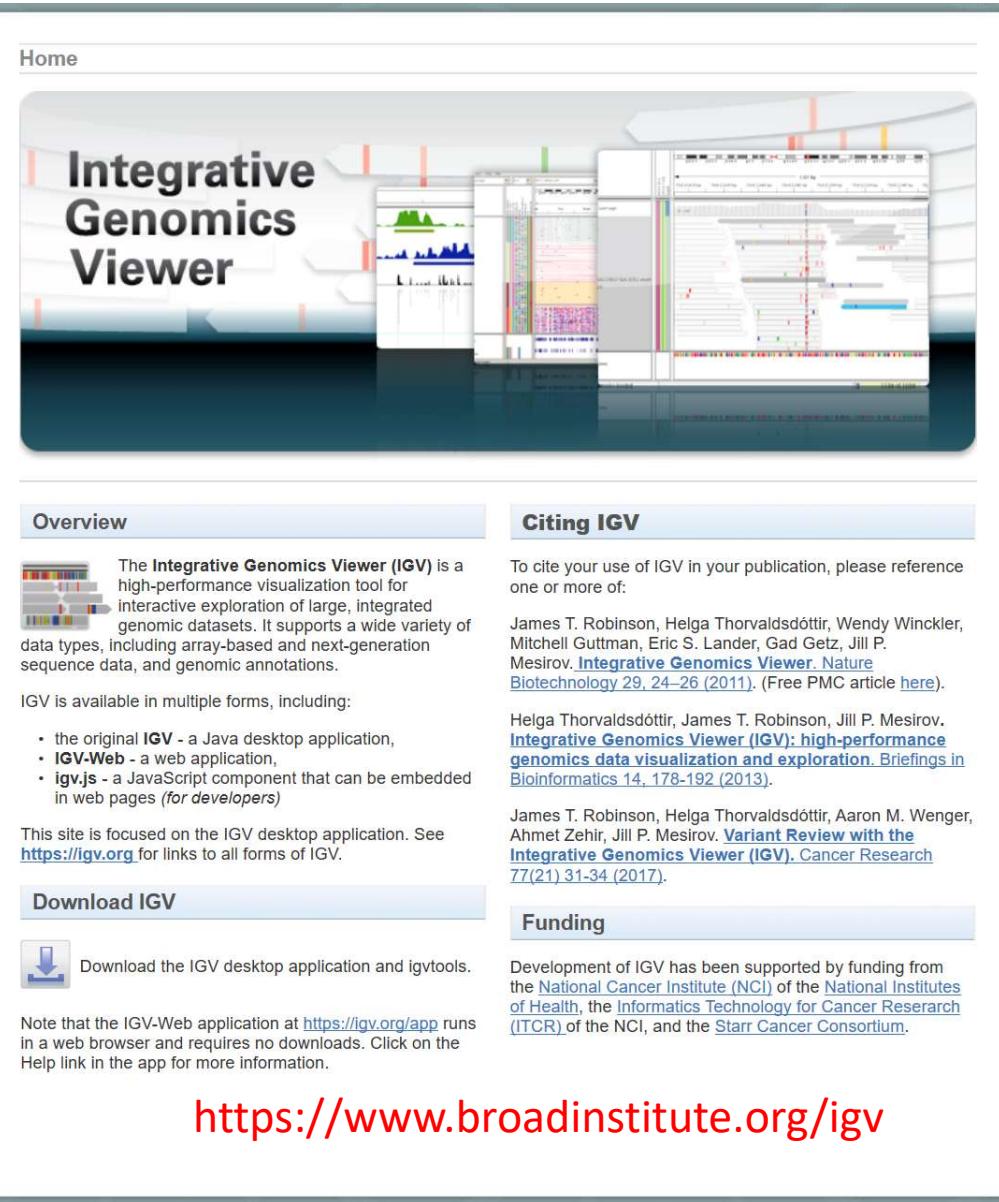
# データ可視化ツール・IGVの紹介・実習



The screenshot shows the IGV website's desktop application interface. It features a sidebar with links for Home, Downloads, Documents (including the User Guide, Tutorial Videos, File Formats, Hosted Genomes, FAQ, Release Notes, Credits, and Contact), and a search bar. The main area displays a complex genomic visualization with tracks for chromosomes, gene models, and other biological data.

## Home

# Integrative Genomics Viewer



The screenshot shows the IGV website's web application interface. It includes a large banner image of the software's interface, followed by sections for Overview, Citing IGV, Download IGV, and Funding. The Overview section provides a brief description of the tool, mentioning its availability in multiple forms (original Java application, web application, and JavaScript component). The Citing IGV section lists publications for citation. The Download IGV section provides a download link for the desktop application. The Funding section notes the support from the National Cancer Institute (NCI) and the Starr Cancer Consortium.

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

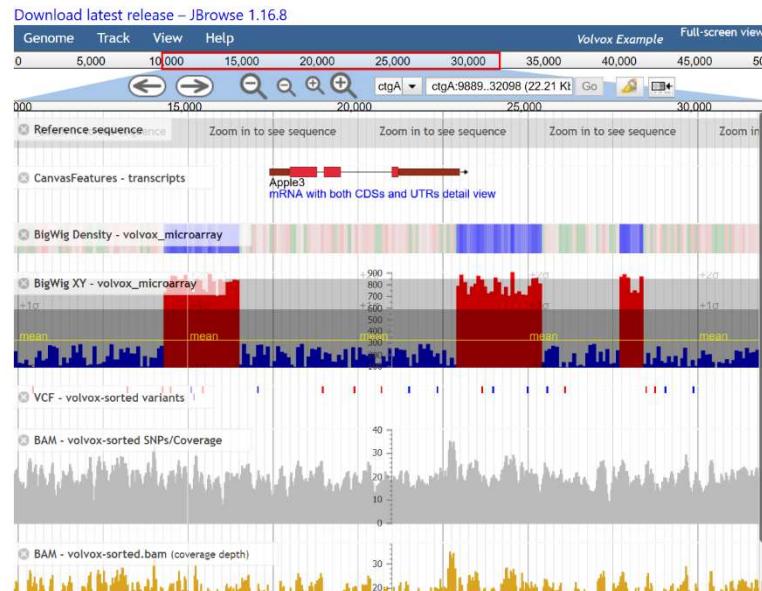
# なぜIGVを取り上げるか

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

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
- ・SNPの位置情報・頻度情報
- ・様々なデータの精度情報
- ・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. アクセスのしやすさ・使いやすさ
  - 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、サンプルデータのある
- ・WEBサーバーではなく、PCレベルでできる
- ・データ閲覧環境の共有が可能

誰もが簡便に使えるものが良い。



Integrative  
Genomics  
Viewer

ALGAR

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## Home

# Integrative Genomics Viewer



## Overview



The **Integrative Genomics Viewer (IGV)** is a high-performance visualization tool for interactive exploration of large, integrated genomic datasets. It supports a wide variety of data types, including array-based and next-generation sequence data, and genomic annotations.

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).

## 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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## 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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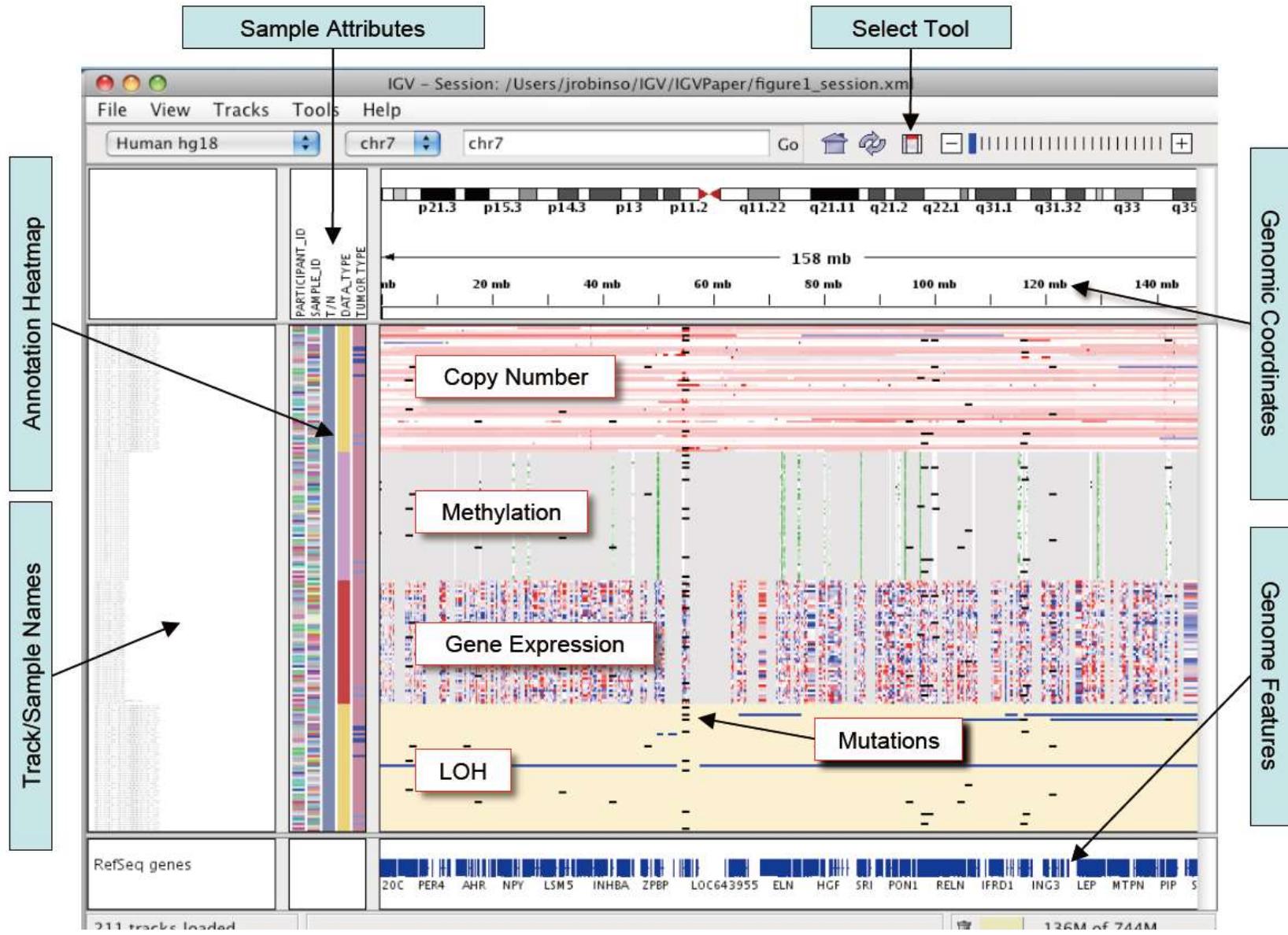
Science jobs from **naturejobs**

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



Integrative Genomics Viewer

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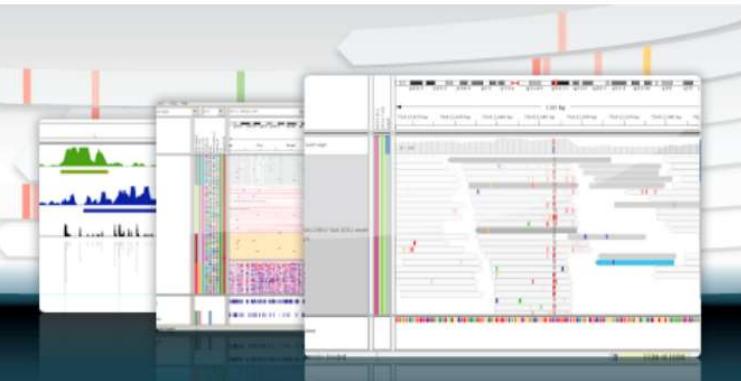
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## IGV User Guide

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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IGV Home Page

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ココをクリック

ココか

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)

## Downloads

### A message from the IGV Team.

Help our proposal for renewed funding by telling us how IGV has benefited your work and why the IGV resource should be sustained. Email us at [igv-los@broadinstitute.org](mailto:igv-los@broadinstitute.org) by May 18, 2020.

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 2.8.2

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



#### IGV Mac App

Download and unzip the Mac App Archive, then double-click the IGV application to run it. You can move the app to the *Applications* folder, or anywhere else.

**MacOS Catalina users:** We sign our Mac App as a trusted Apple developer, but it is not yet notarized by Apple (a new requirement in Catalina). To run it, right-click on the downloaded IGV app; select "Open" from the menu; and click the "Open" button in the window that pops up. After that, double-clicking on the app will also work.



#### IGV for Windows

Download and run the installer. An IGV shortcut will be created on the Desktop; double-click it to run the application.



#### IGV for Linux

Download and unzip the Archive. See the downloaded *readme.txt* for further instructions.



#### IGV and igvtools to run on the command line (all platforms)

Download and unzip the Archive. **Requires Java 11.** See the downloaded *readme.txt* and *igvtools\_readme.txt* for further instructions.

### 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

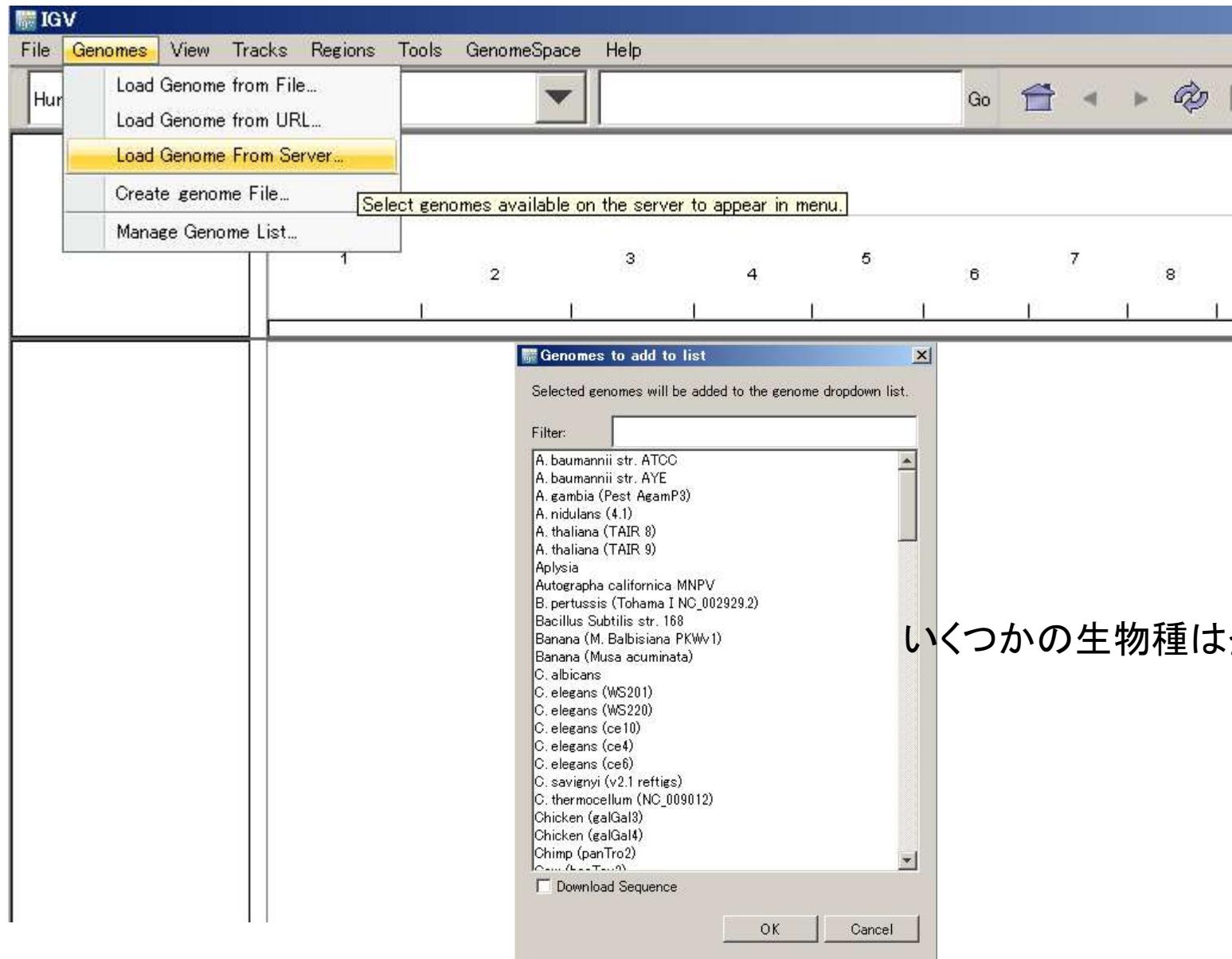
IGV is completely open for anyone to use under an [MIT open-source license](#).

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.

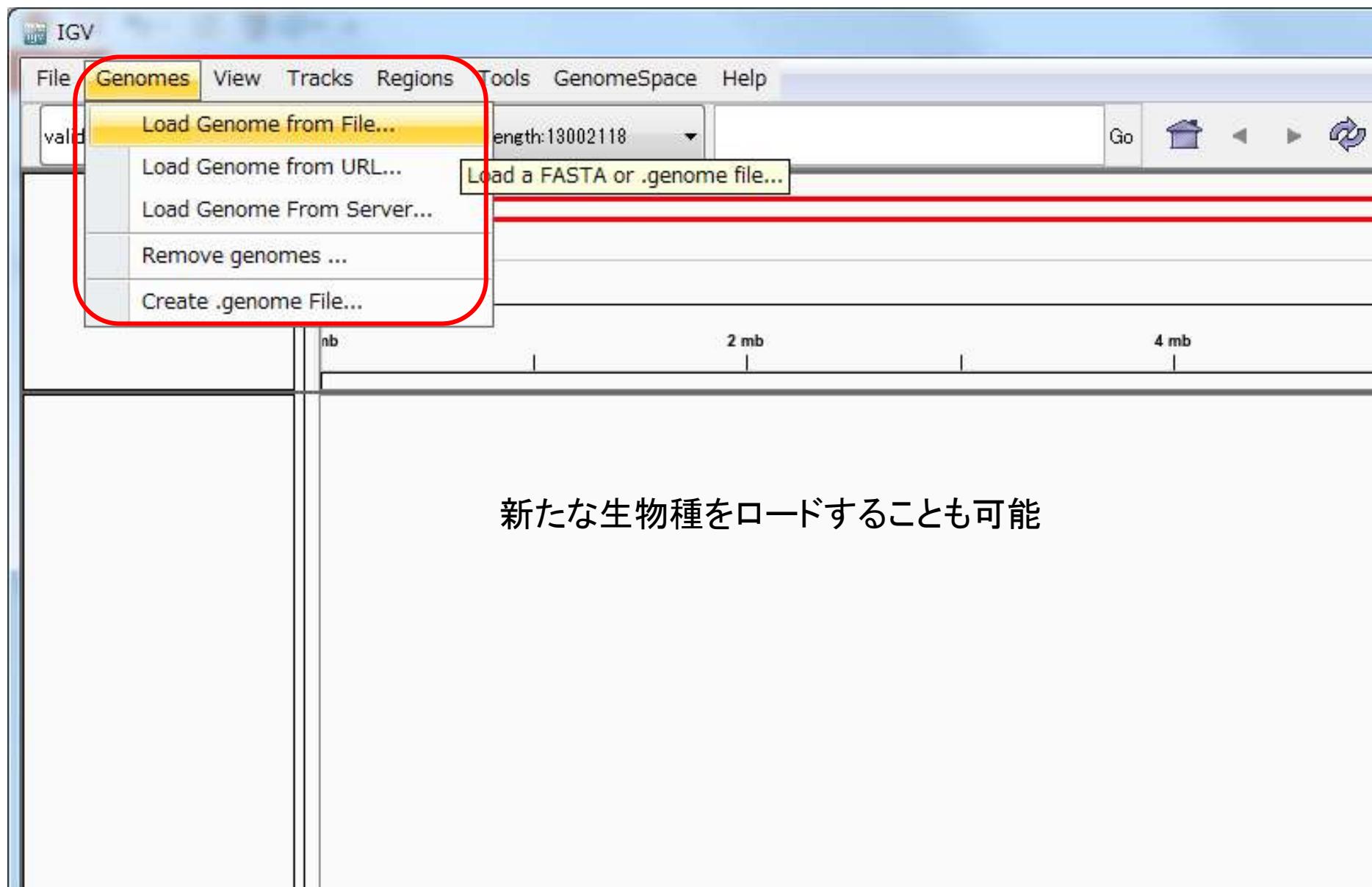
### Source Code

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

利用するパソコンの  
プラットフォームに応じて選択



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。

#### File Formats

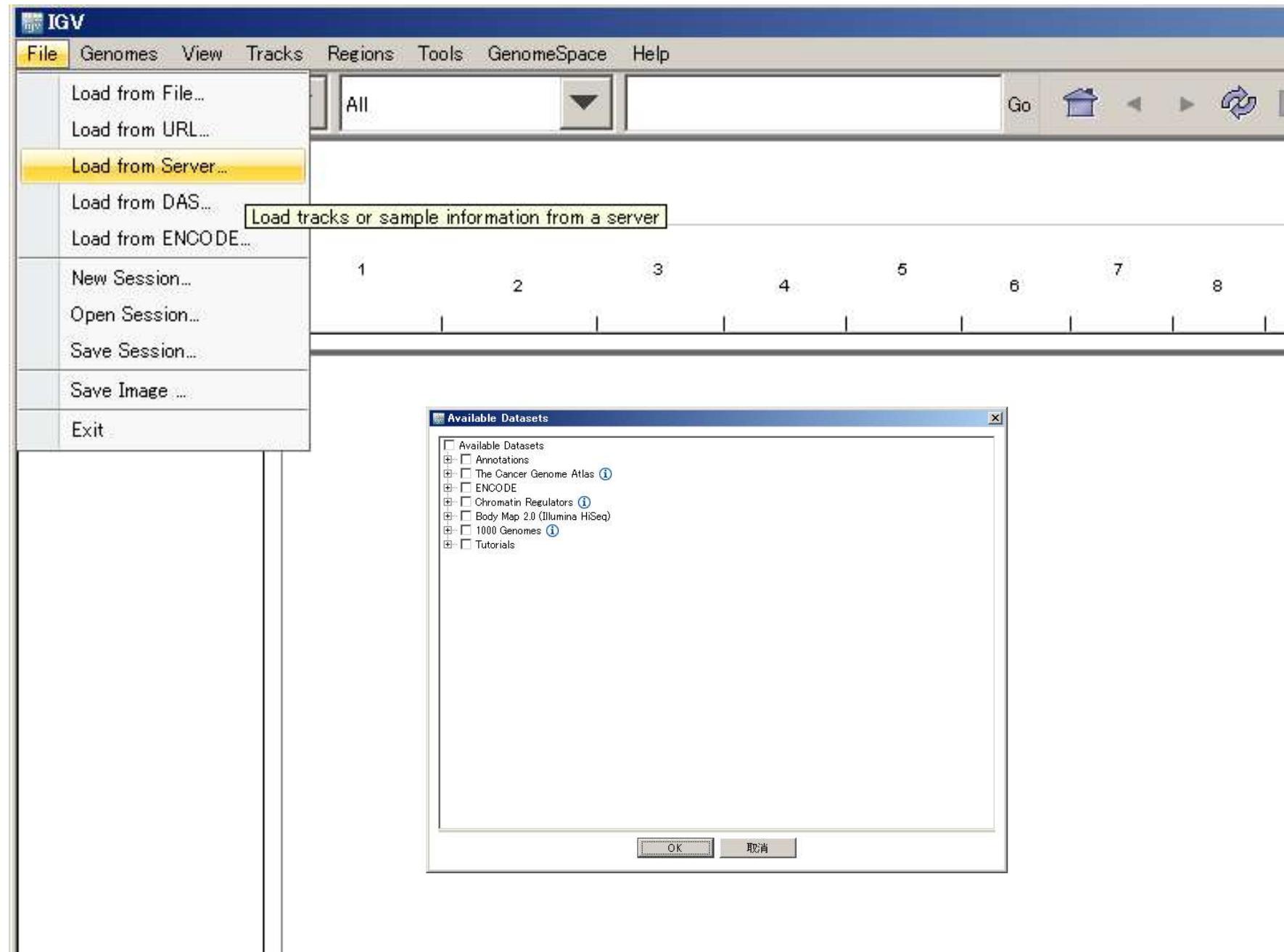
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- [Goby](#)
- [GWAS](#)
- [IGV](#)
- [LOH](#)
- [MAF \(Multiple Alignment Format\)](#)
- [MAF \(Mutation Annotation Format\)](#)
- [Merged BAM File](#)
- [MUT](#)
- [narrowPeak](#)
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- [SNP](#)
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#### 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。

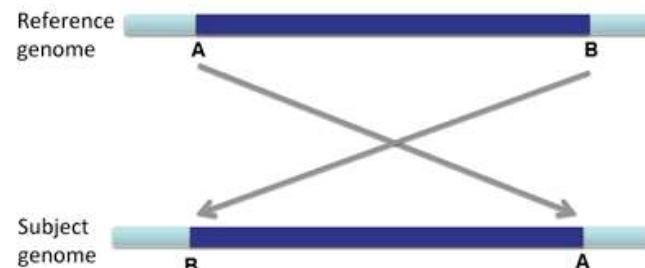
### バッチ処理

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。

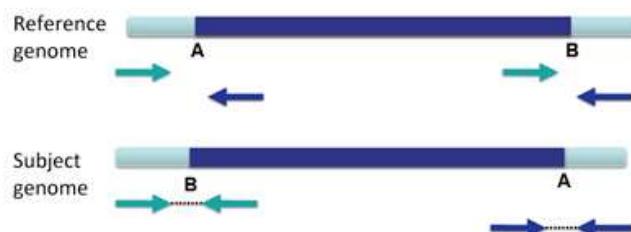
```
new
load myfile.bam
snapshotDirectory mySnapshotDirectory
genome hg18
goto chr1:65,289,335-65,309,335
sort position
collapse
snapshot
goto chr1:113,144,120-113,164,120
sort base
collapse
snapshot
```

## Inversions

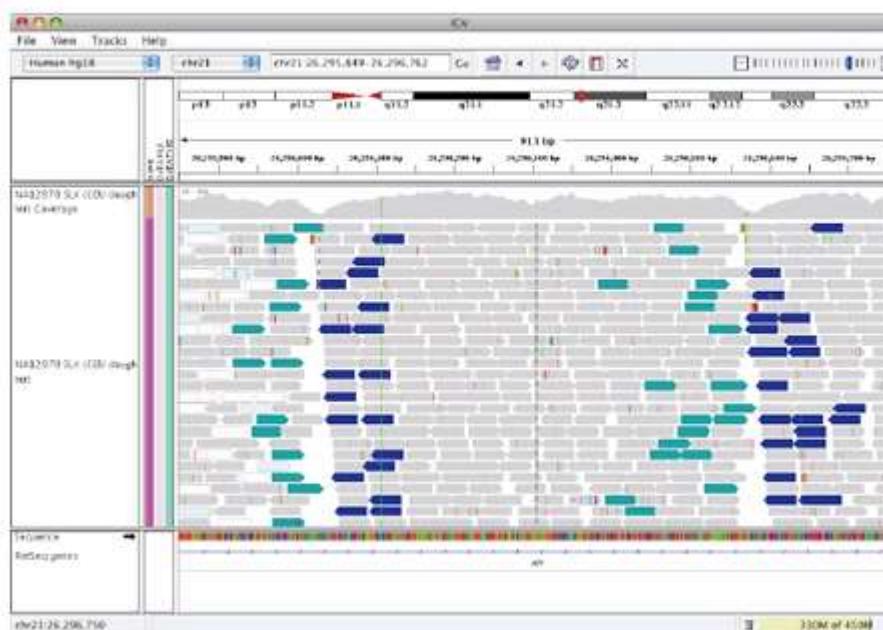
An inversion is a large section of DNA that is reversed in the subject genome compared to the reference genome.



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:

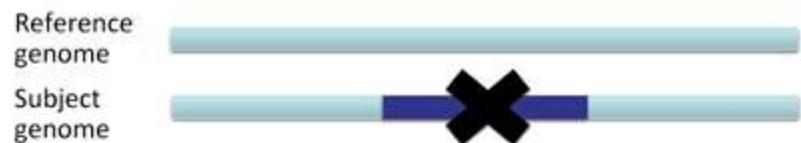
- deletions
- insertions
- inter-chromosomal rearrangements

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:

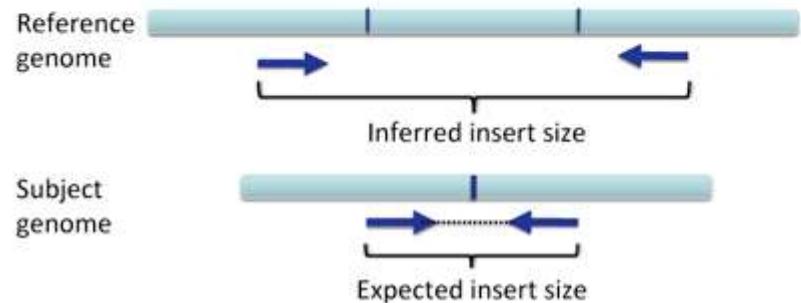
- for an insert that is larger than expected
- for an insert that is smaller than expected
- for paired end reads that are coded by the chromosome on which their mates can be found

### Deletions

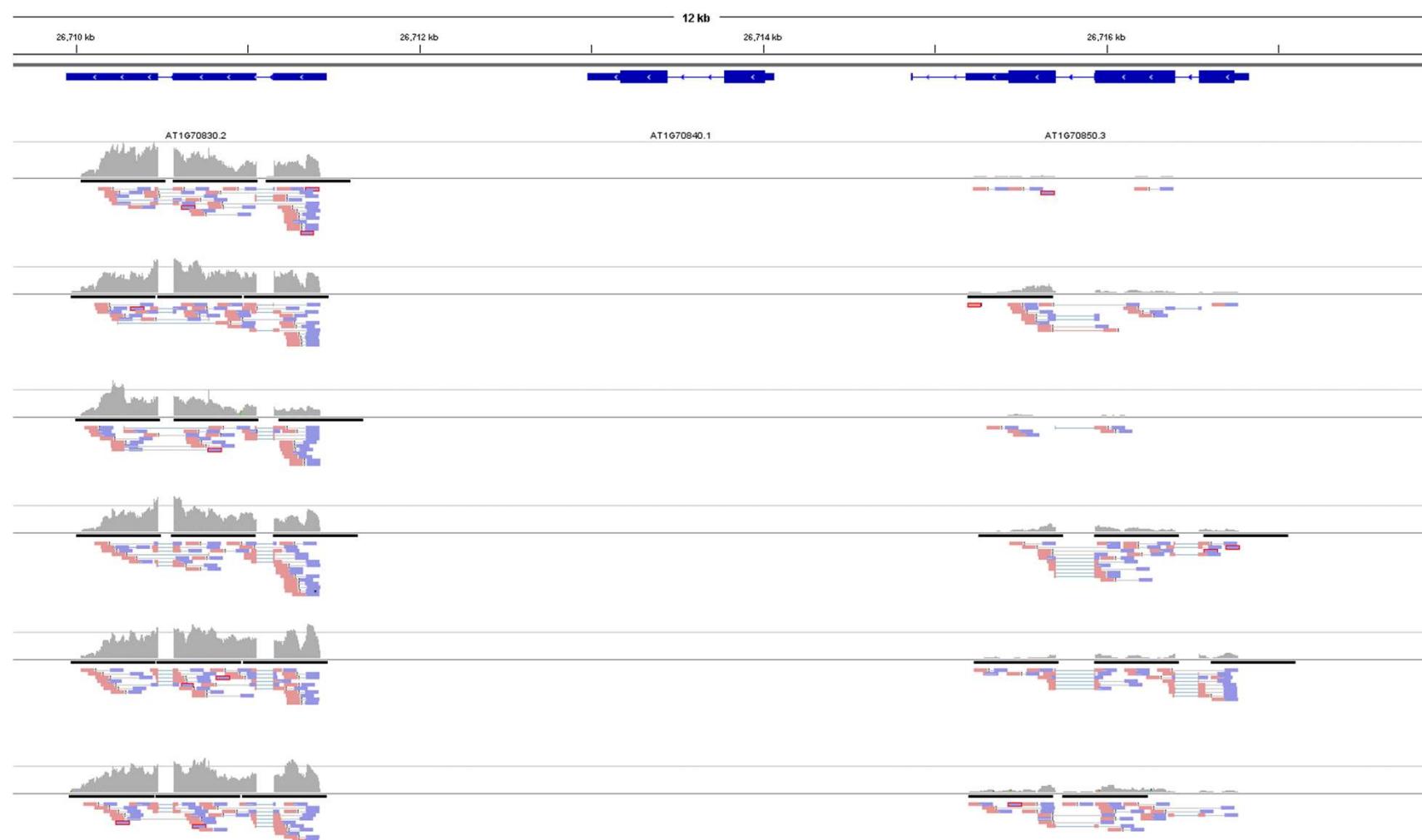
A deletion is a large section of DNA that is absent in the subject genome compared to the reference genome.



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.

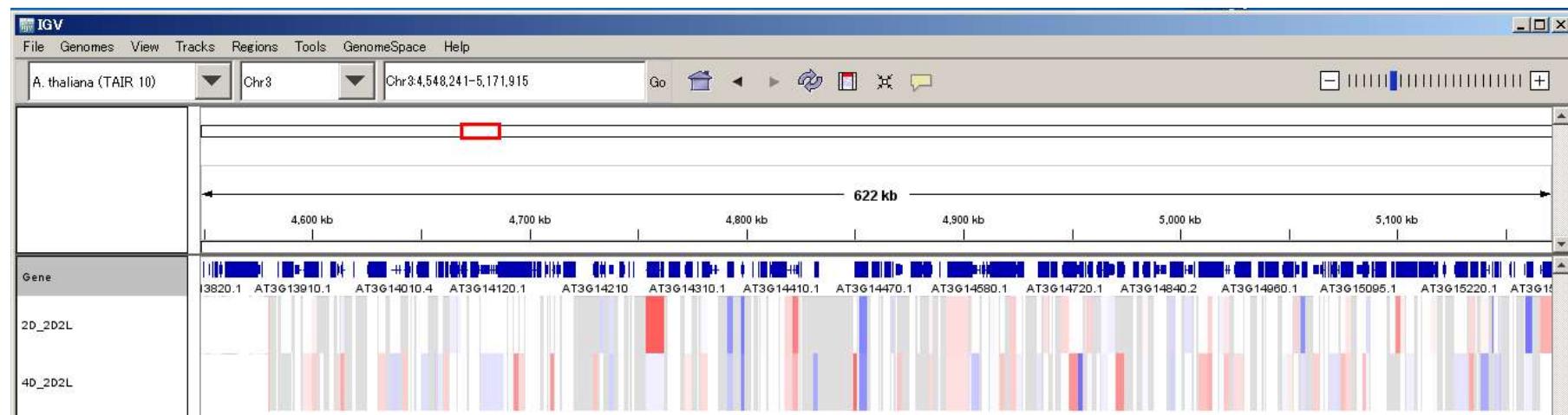


# RNA-Seqのデータ表示させる



# 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

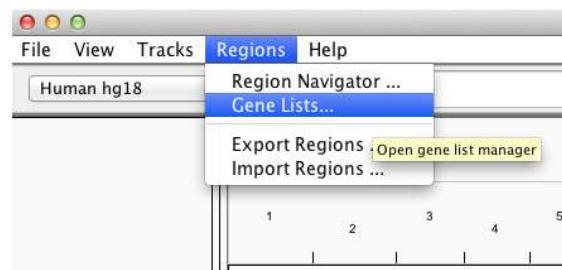
Home > IGV User Guide > Gene List View

### Gene List View

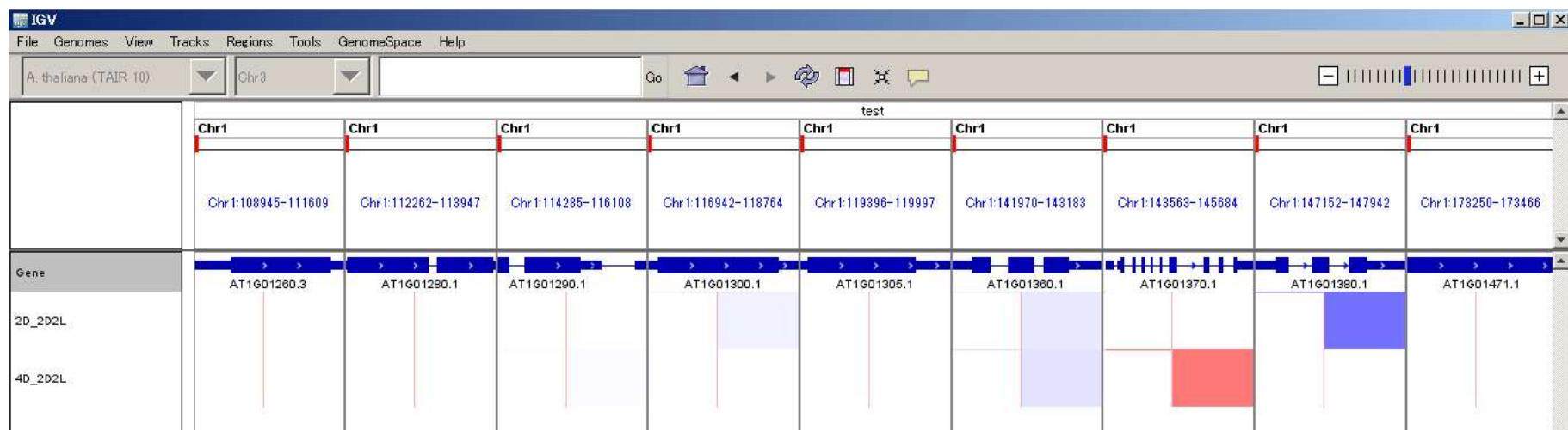
The Gene Lists functionalities in IGV allow you to view lists of genes or loci side-by-side irrespective of their genomic location.

#### 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

## Install IGV 2.8.2

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



### IGV Mac App

Download and unzip the Mac App Archive, then double-click the IGV application to run it. You can move the app to the *Applications* folder, or anywhere else.

**MacOS Catalina users:** We sign our Mac App as a trusted Apple developer, but it is not yet notarized by Apple (a new requirement in Catalina). To run it, right-click on the downloaded IGV app; select "Open" from the menu; and click the "Open" button in the window that pops up. After that, double-clicking on the app will also work.



### IGV for Windows

Download and run the installer. An IGV shortcut will be created on the Desktop; double-click it to run the application.



### IGV for Linux

Download and unzip the Archive. See the downloaded *readme.txt* for further instructions.



### IGV and igvtools to run on the command line (all platforms)

Download and unzip the Archive. **Requires Java 11.** See the downloaded *readme.txt* and *igvtools\_readme.txt* for further instructions.

## 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/>

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

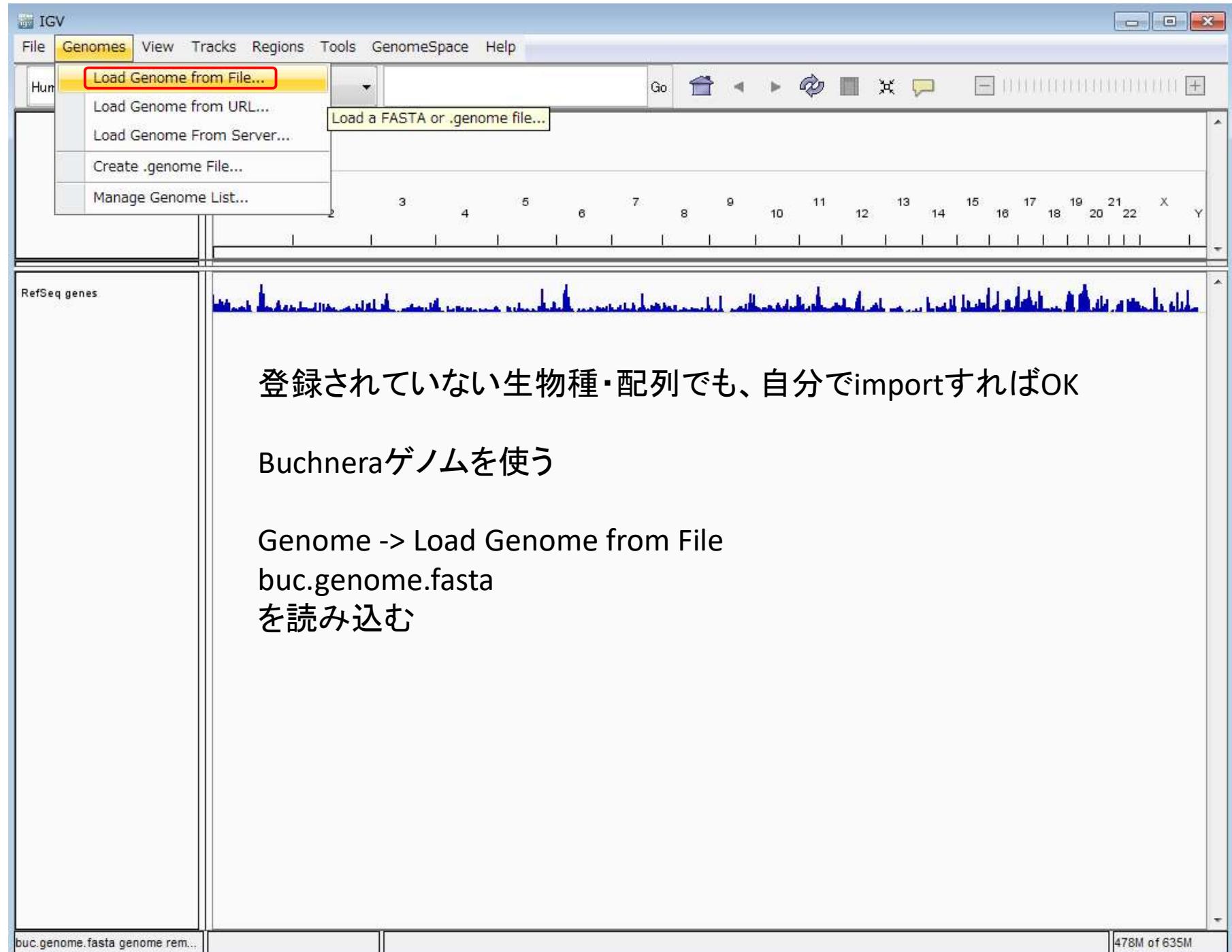
buc\_cg.wig

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 GenomeSpace Help

Load from File... (highlighted)

Load from URL... Load tracks or sample information

Load from Server...

Load from DAS...

New Session...

Open Session...

Save Session...

Save Image ...

Exit

All

gn:buc

gn:buc.p

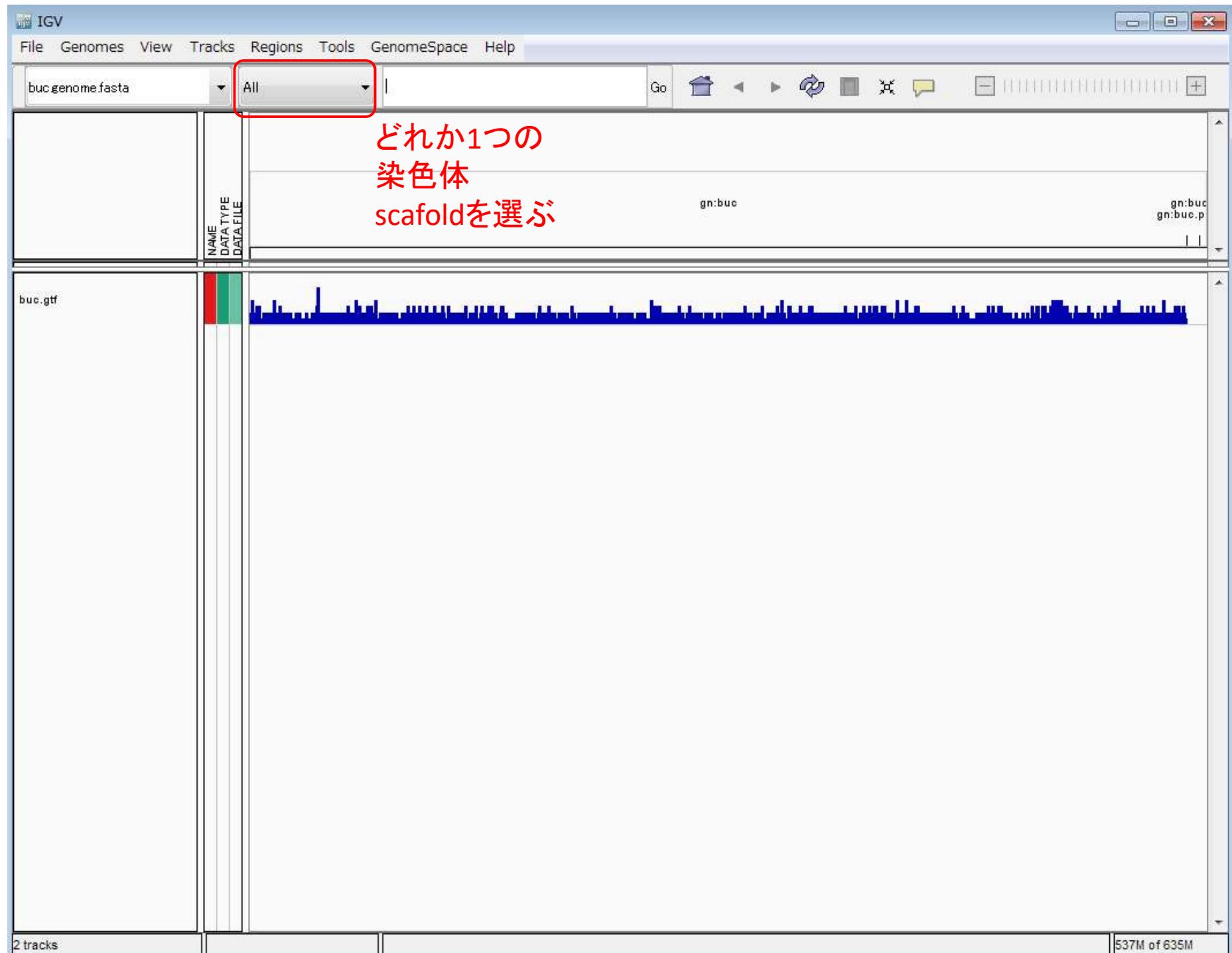
ゲノム構造を記述したgtfファイルを読み込む

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

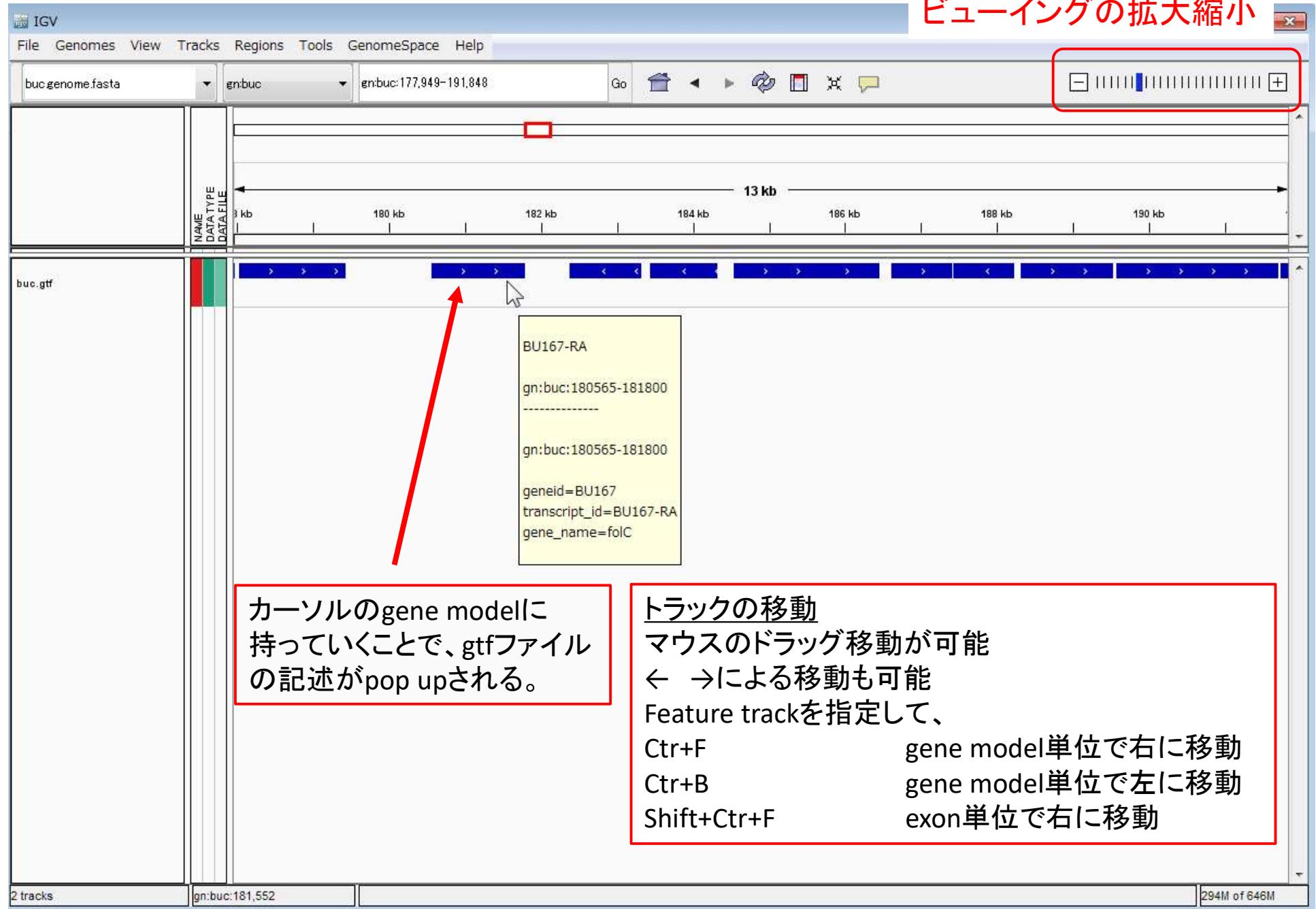
buc.gtf

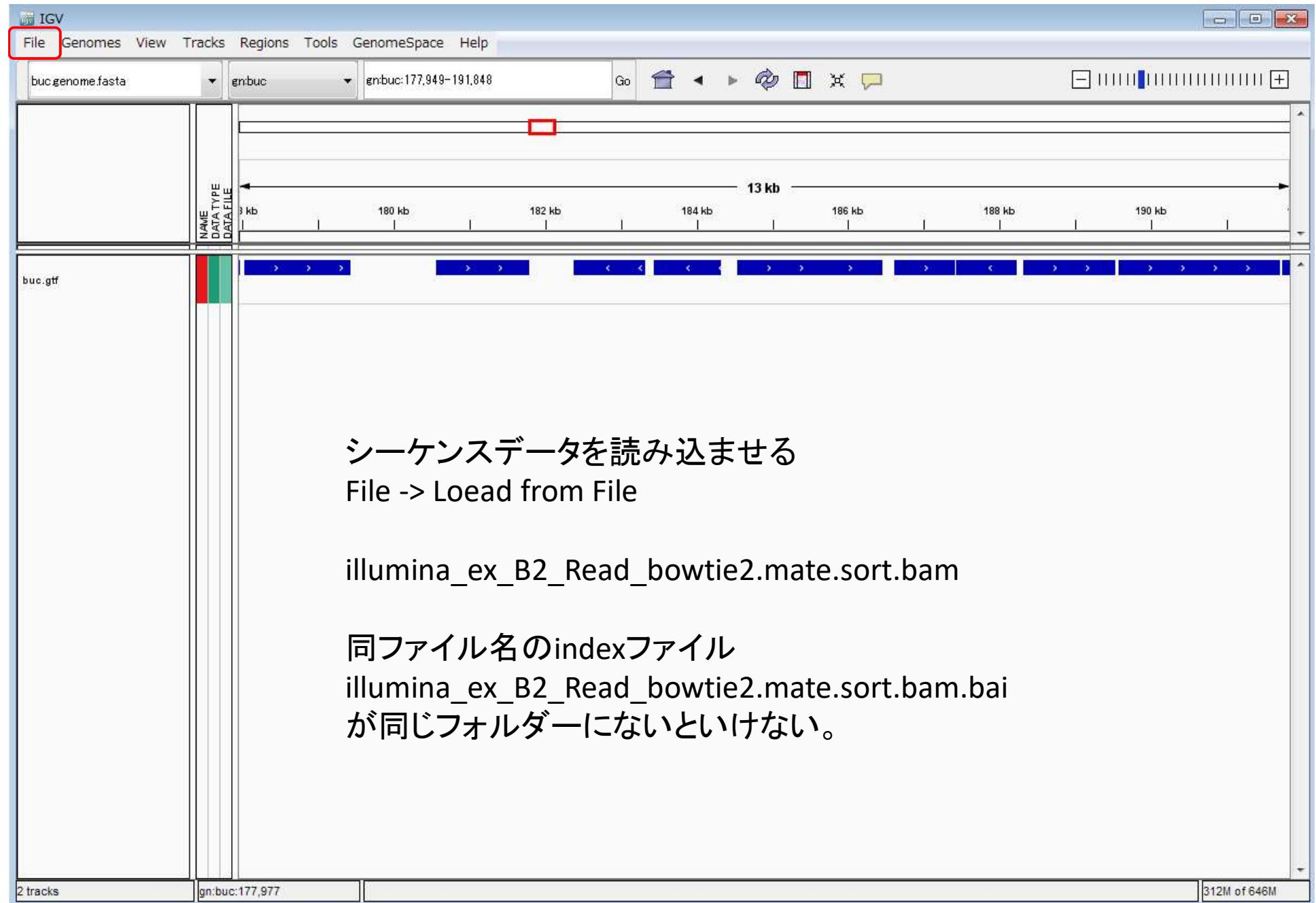
gn:buc	KEGG:GENES	CDS	197	2083	.	geneid "BU001"; transcript_id "BU001-RA"; gene_name "gidA";
gn:buc	KEGG:GENES	CDS	2278	3102	.	geneid "BU002"; transcript_id "BU002-RA"; gene_name "atpB";
gn:buc	KEGG:GENES	CDS	3139	3378	.	geneid "BU003"; transcript_id "BU003-RA"; gene_name "atpE";
gn:buc	KEGG:GENES	CDS	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 "atpG";
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 "dnA";
gn:buc	KEGG:GENES	CDS	14369	14512	.	geneid "BU013"; transcript_id "BU013-RA"; gene_name "rpmH";
gn:buc	KEGG:GENES	CDS	14525	14872	.	geneid "BU014"; transcript_id "BU014-RA"; gene_name "rnpA";
gn:buc	KEGG:GENES	CDS	15011	16609	.	geneid "BU015"; transcript_id "BU015-RA"; gene_name "yidC";

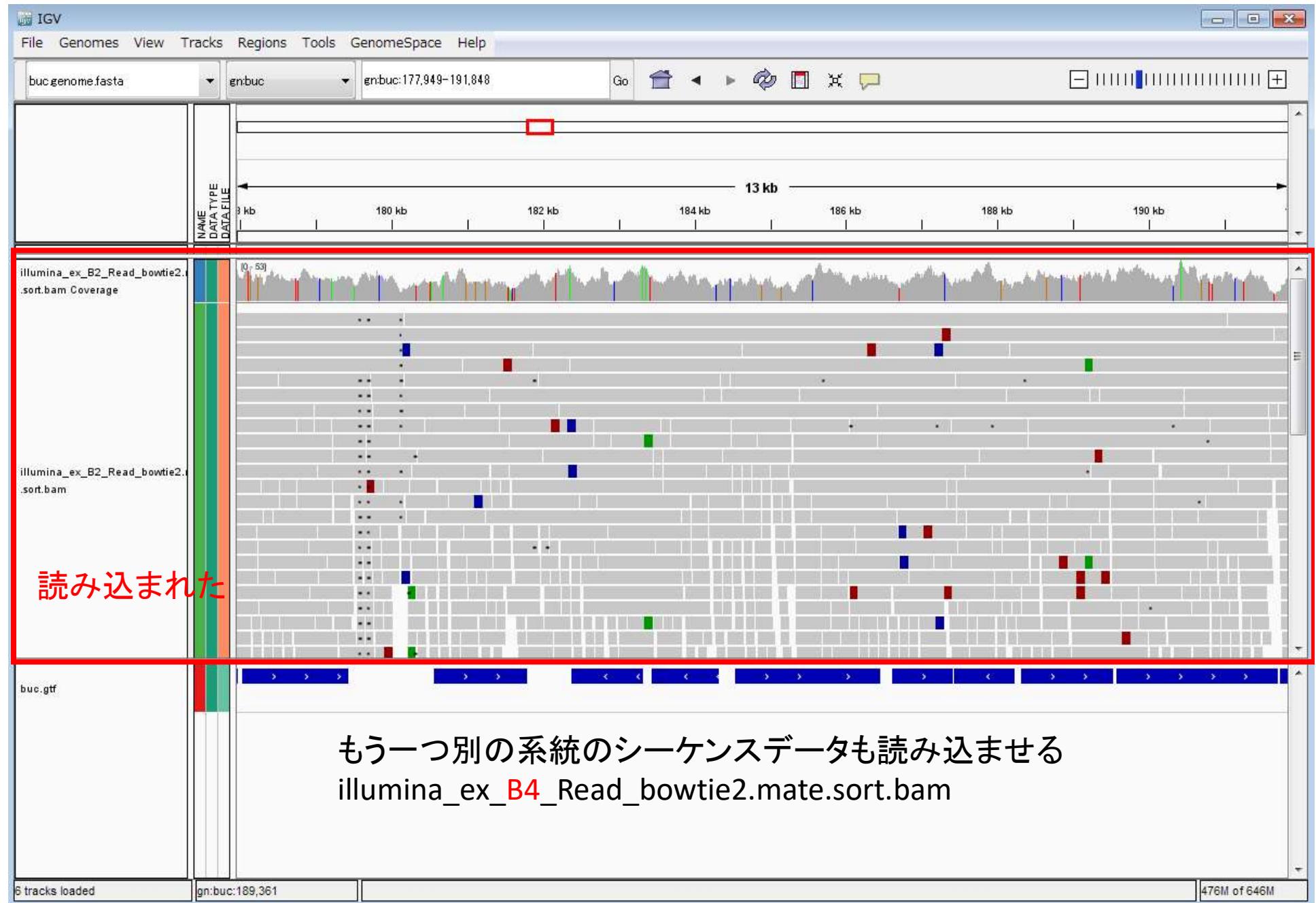
buc.genome.fasta genome rem... 511M of 635M

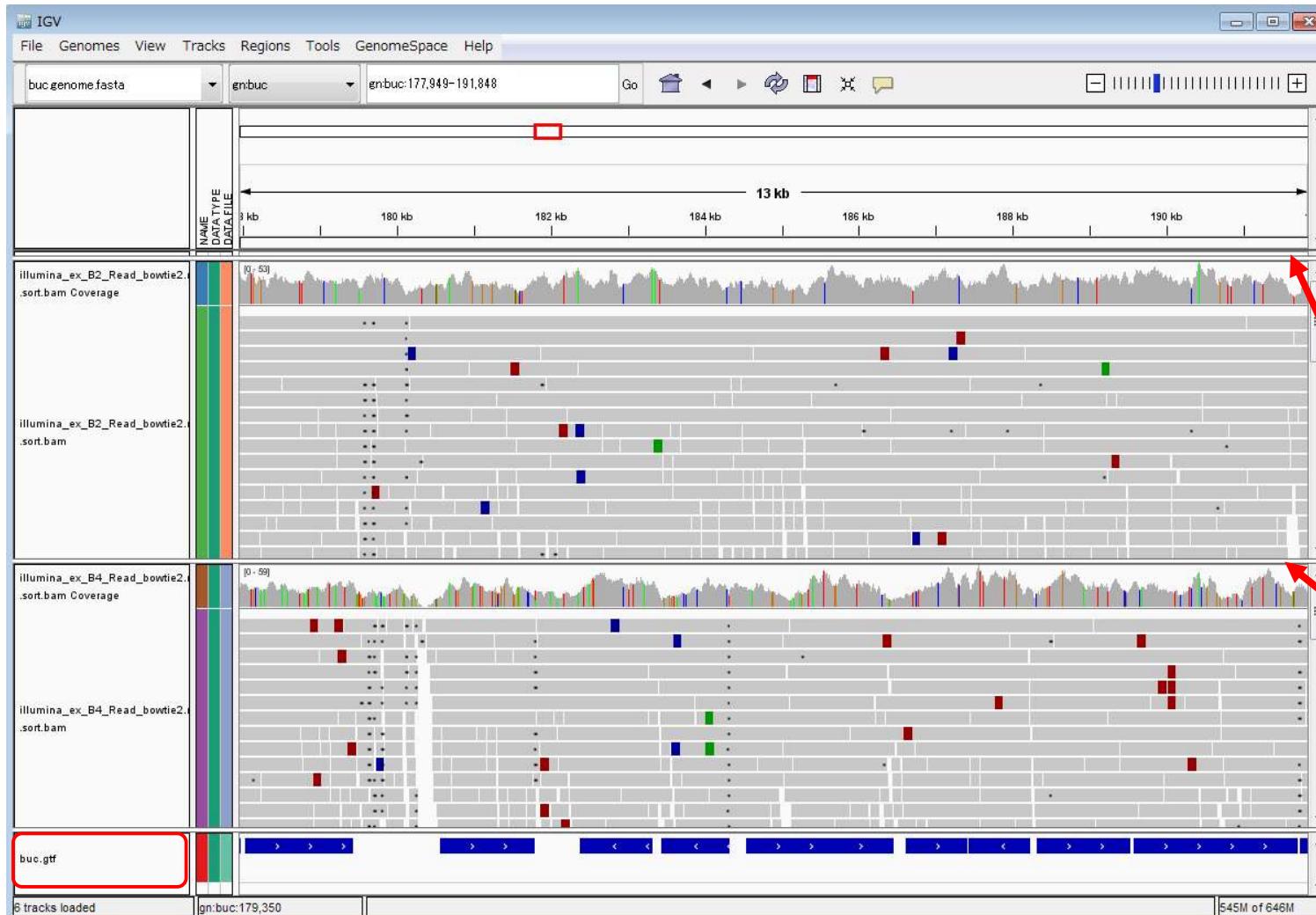


## ビューアの拡大縮小



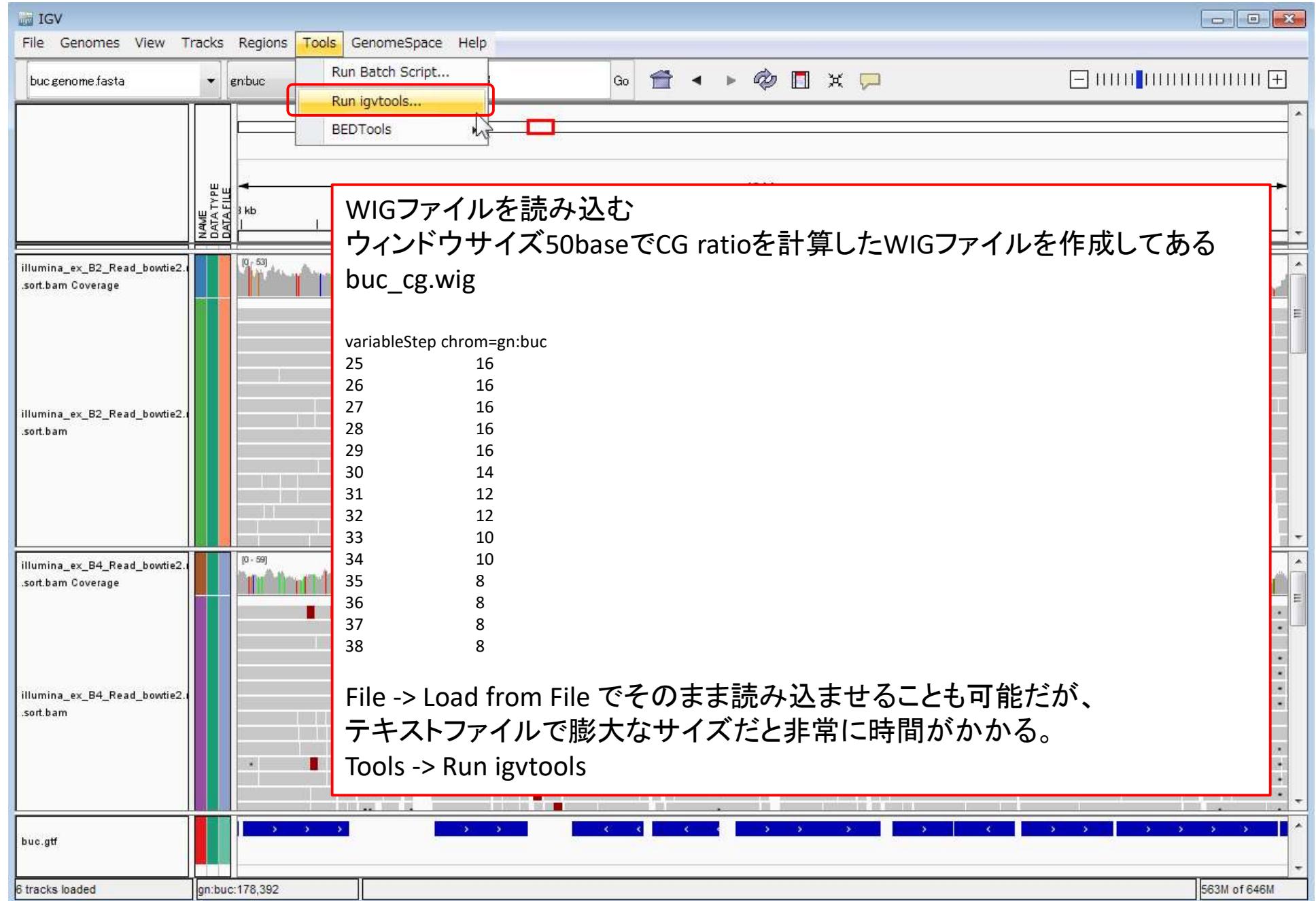


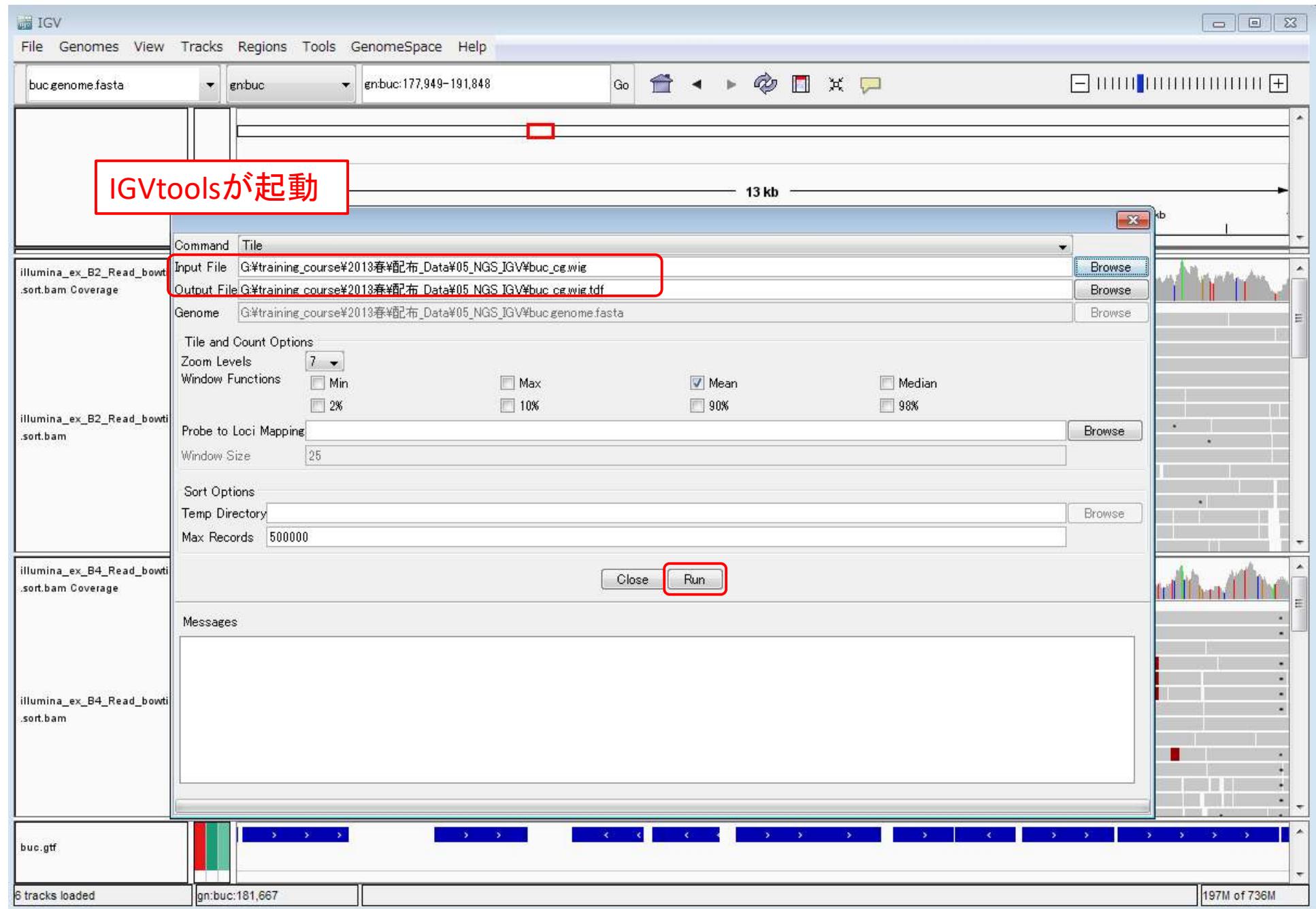


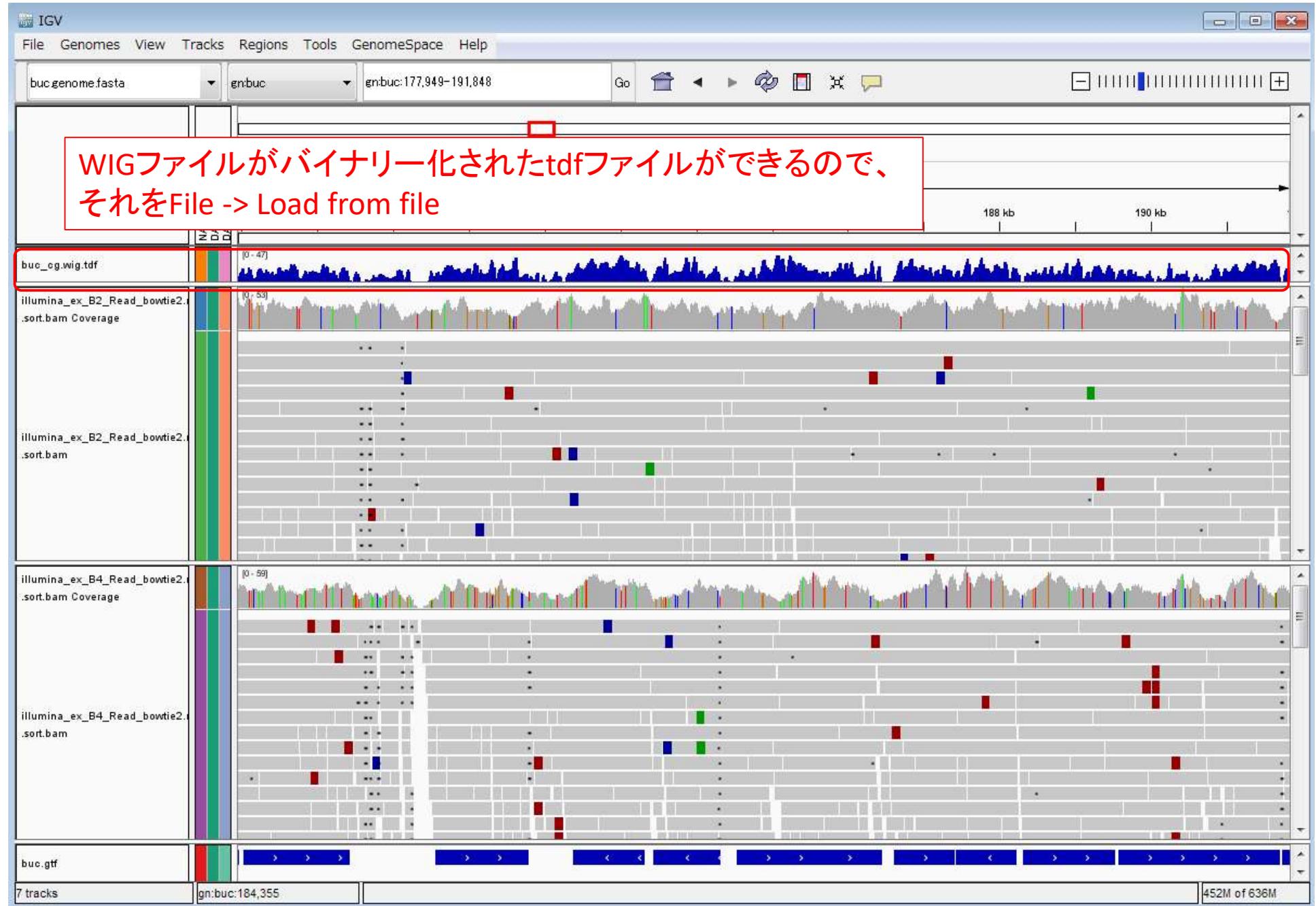


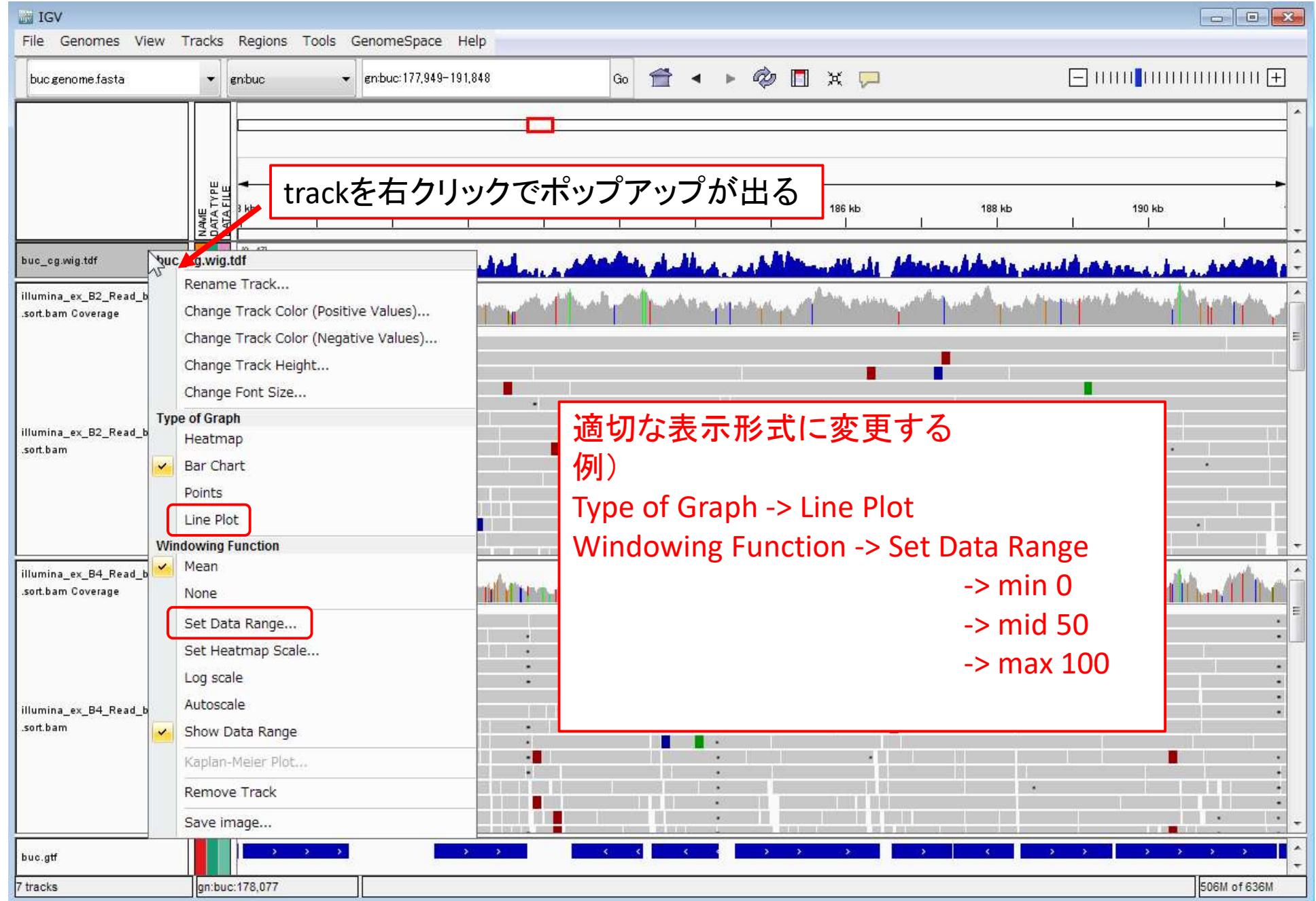
各Trackはドラッグアンドドロップで移動できる

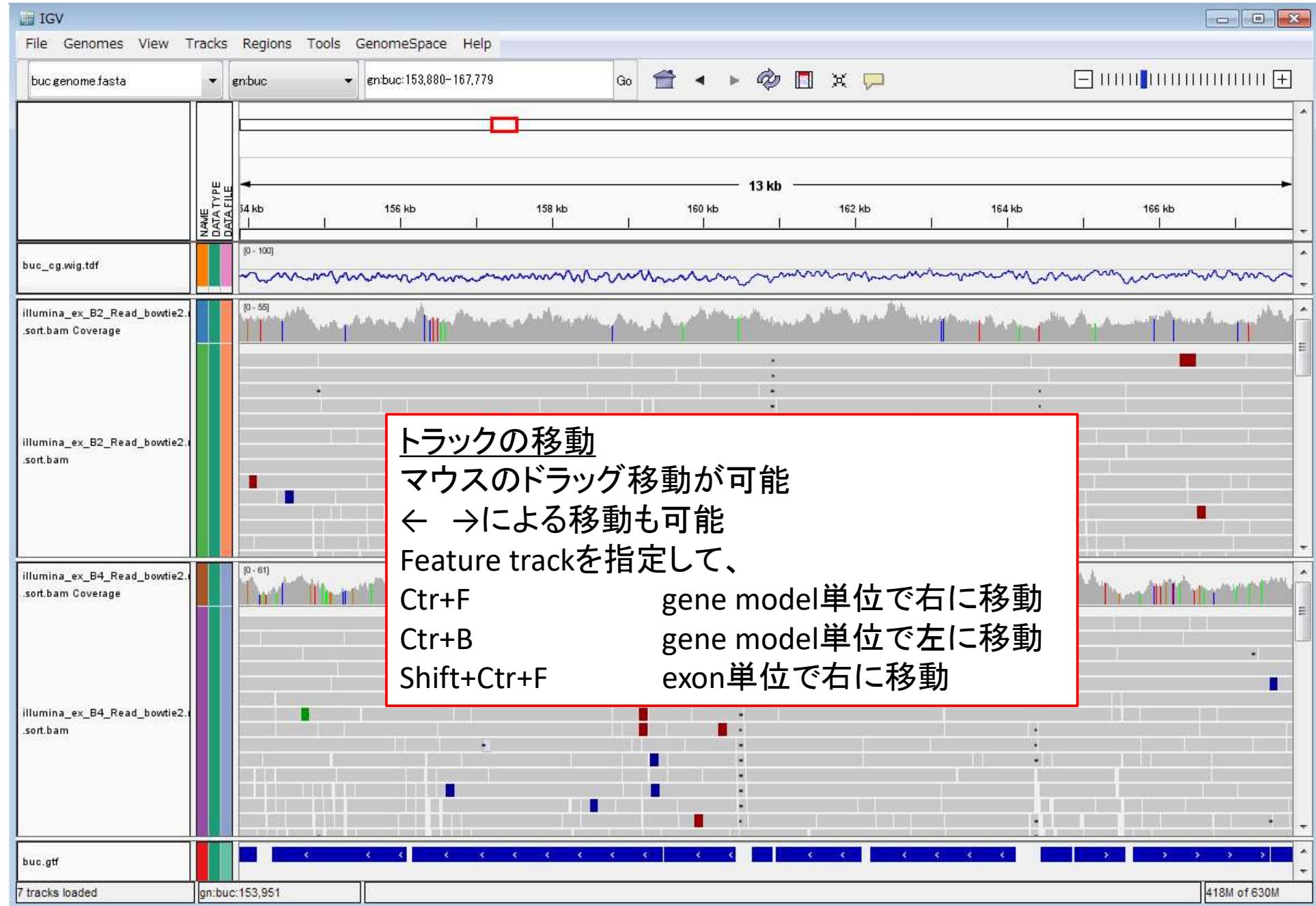
こういう所を  
クリックしたまま  
移動させることで  
幅を変更

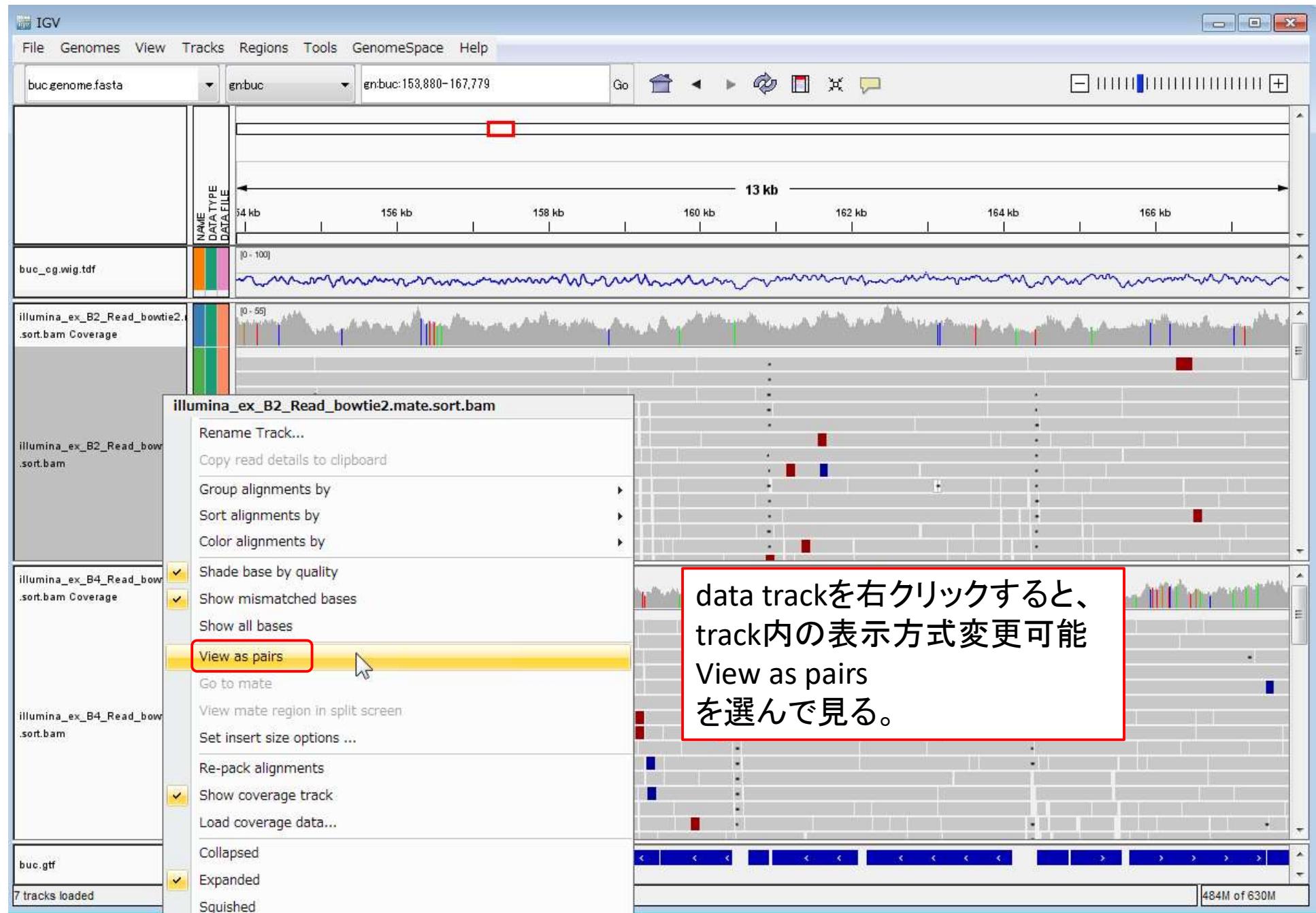


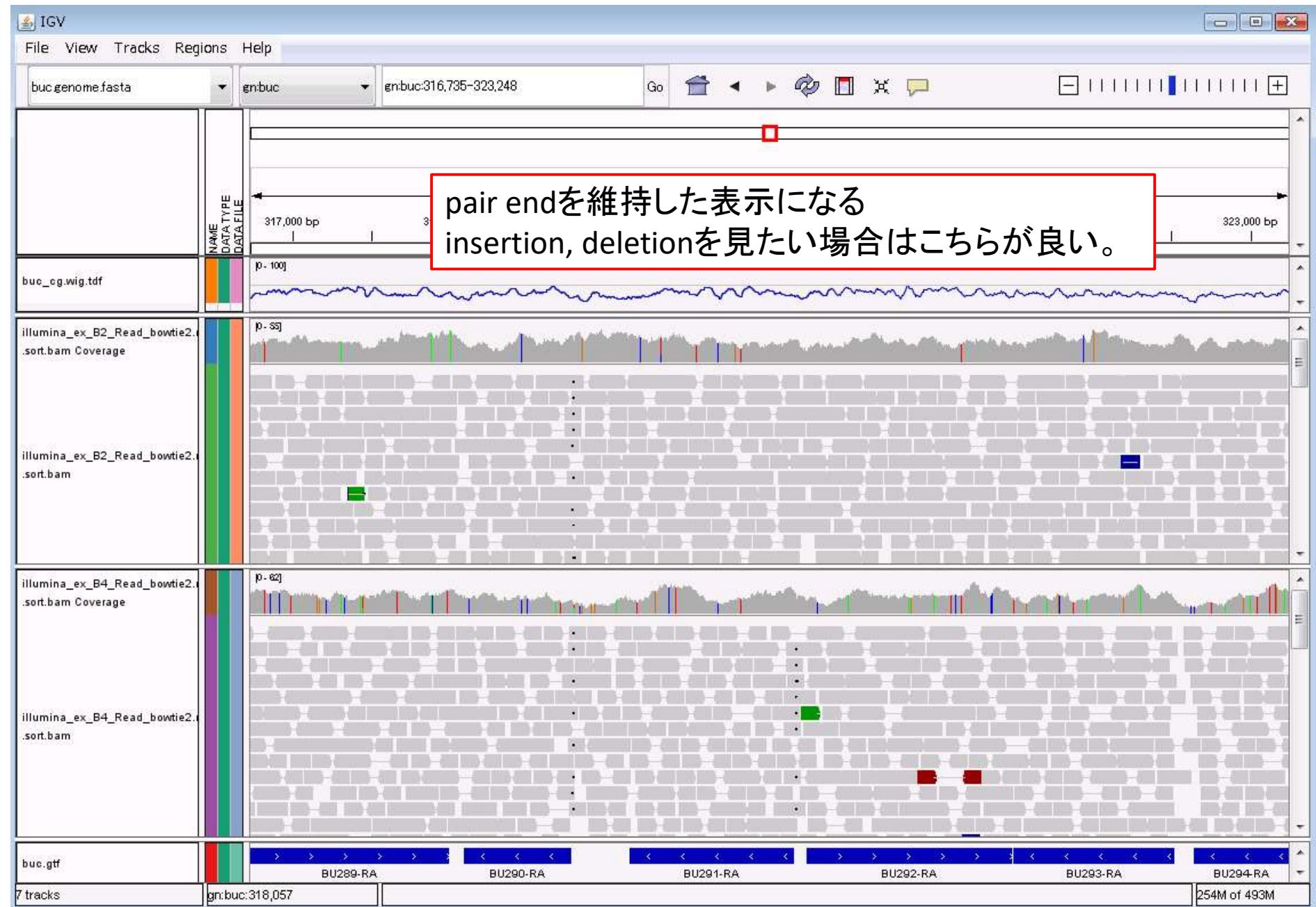


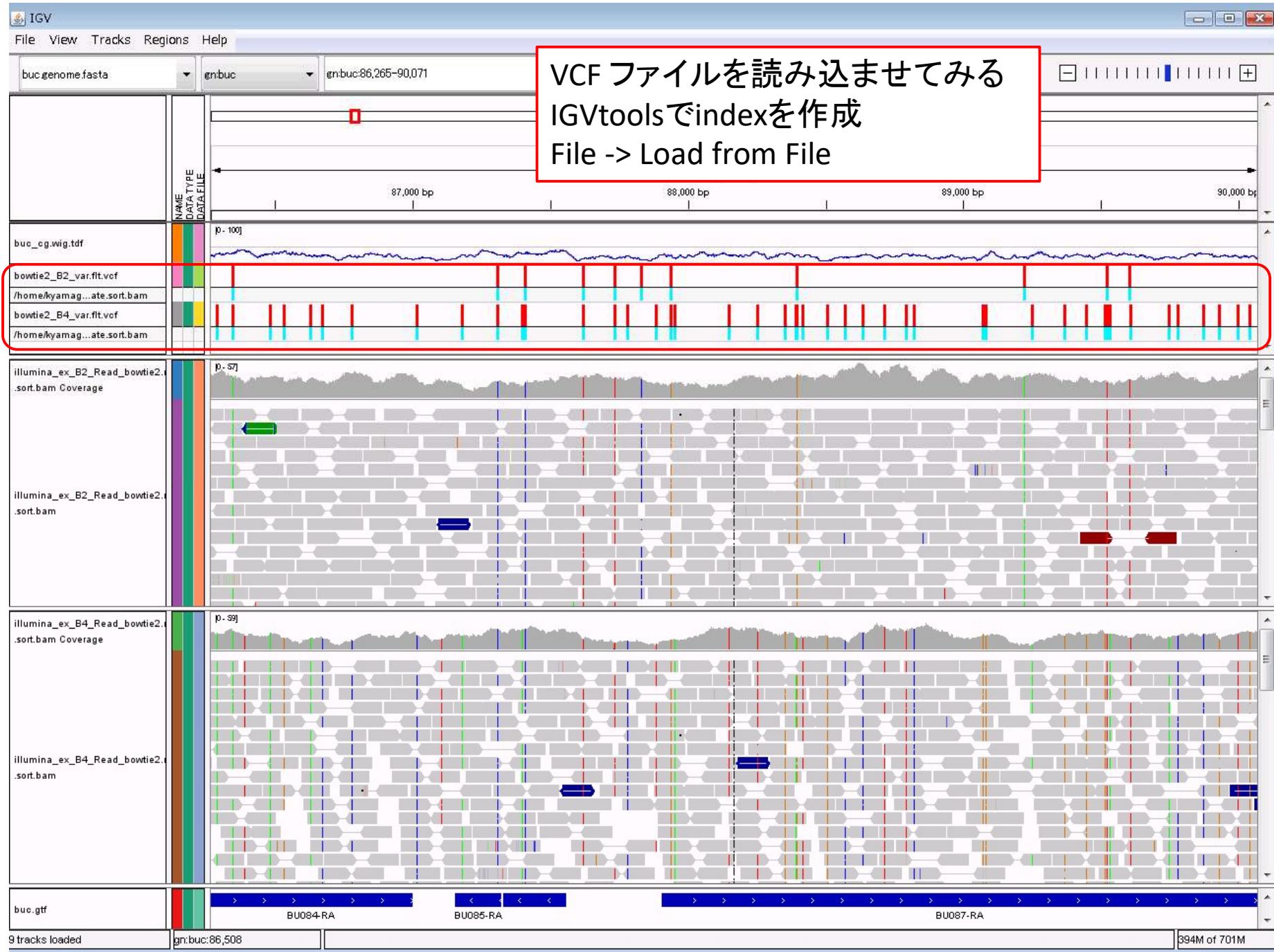


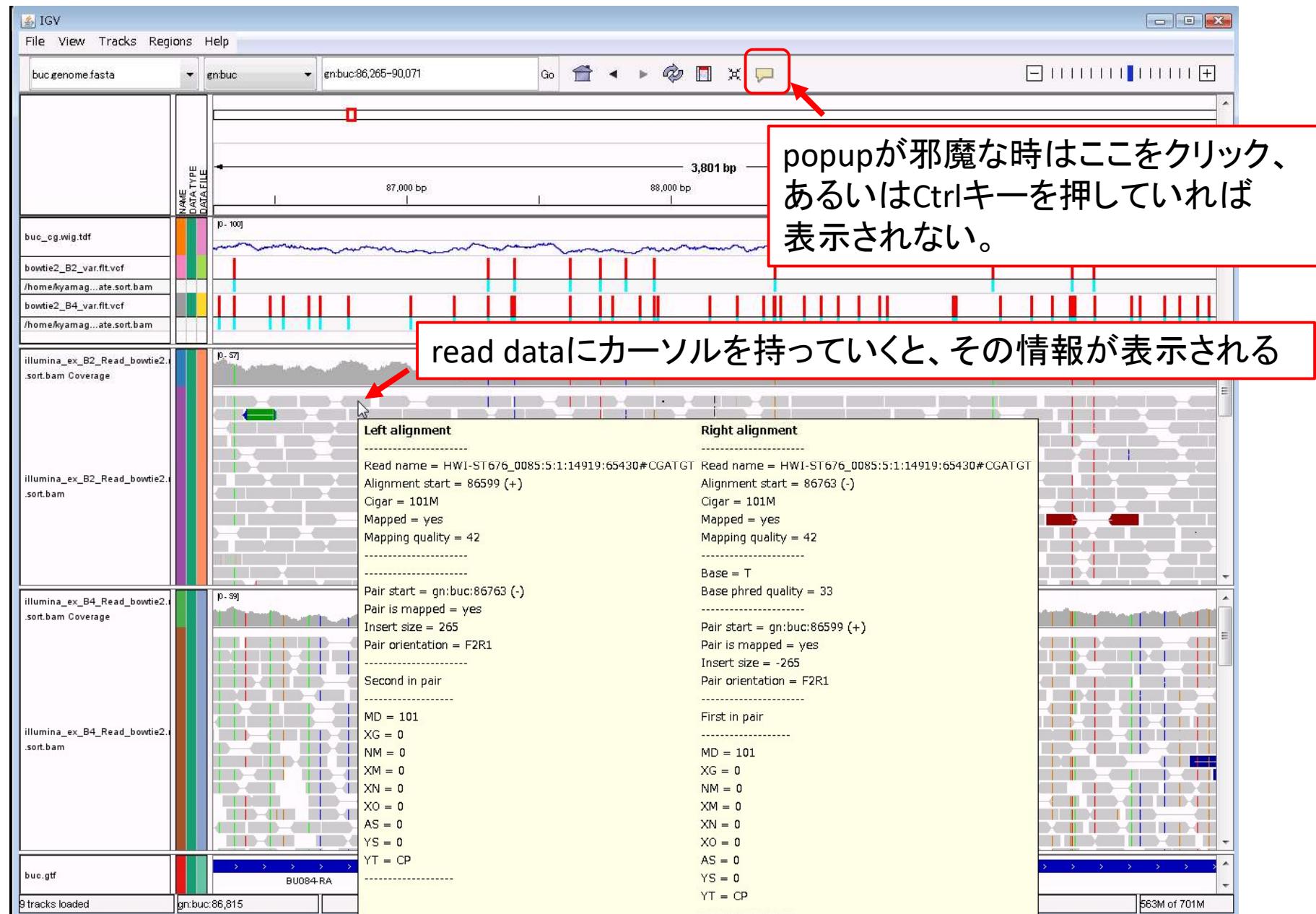












# IGV紹介のまとめ

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。