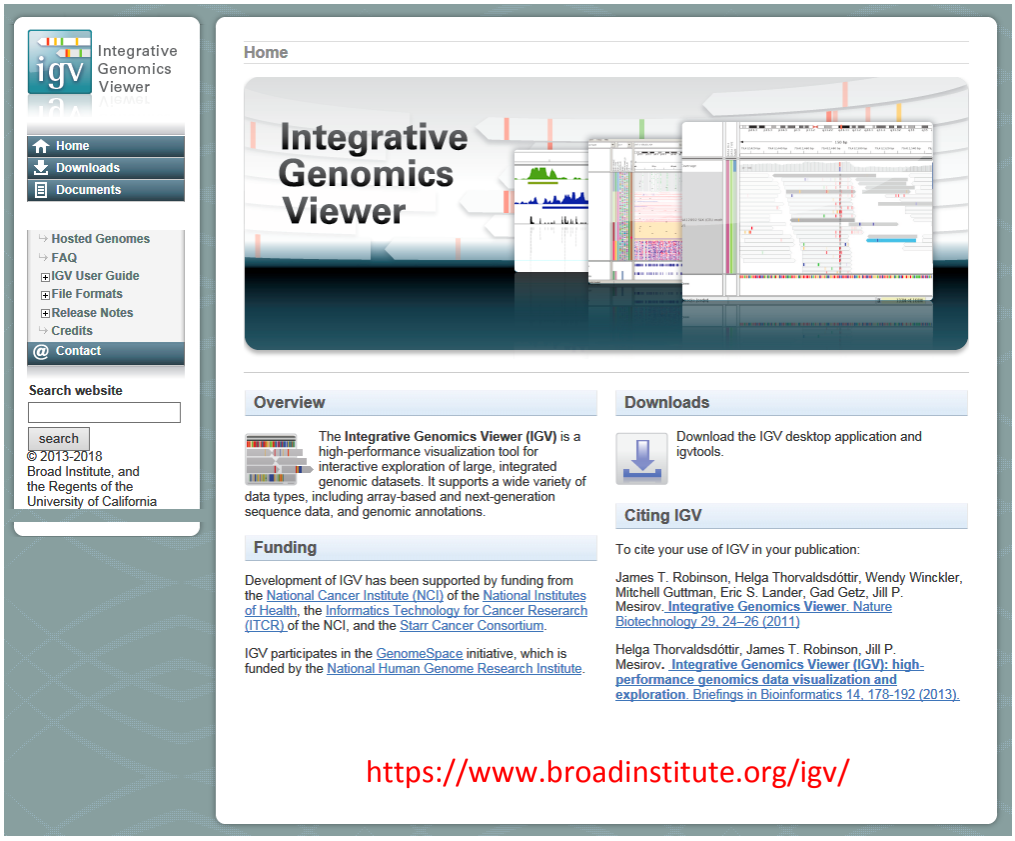
基生研ゲノムインフォマティックス・トレーニングコース　2019春
RNA-seq入門 – NGSの基礎からde novo解析まで–
実践編 ： RNA-seq解析パイプライン
2018.03.14-2018.03.15

NGS基本ツールIGV

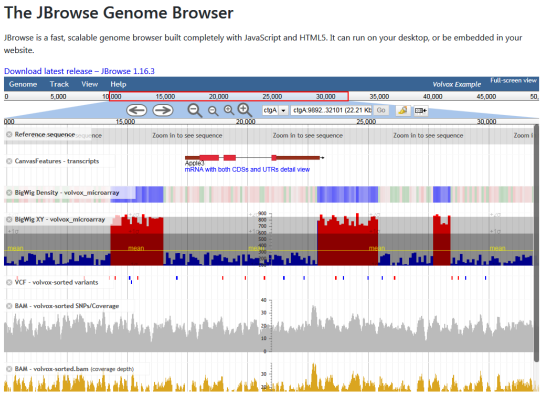
基礎生物学研究所
生物機能解析センター
山口勝司

データ可視化ツール・IGVの紹介・実習



なぜIGVを取り上げるか

データ可視化ツール
・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



後者はコミュニティーで広く利用、
あるいはウェブ公開を目的とするには
良いが、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。

より大容量なデータに対応できる。

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ようにするか
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

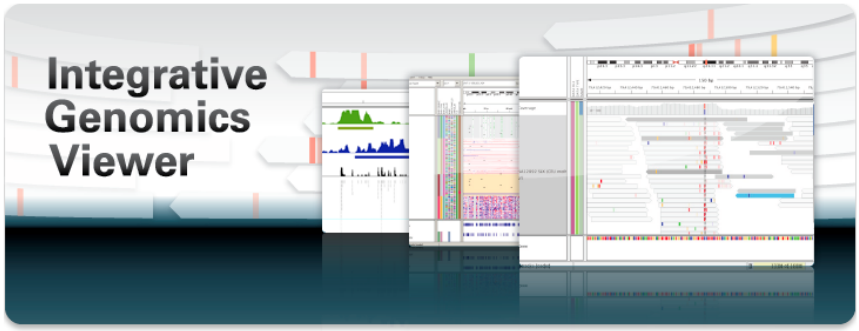
Home
Downloads
Documents

Hosted Genomes
FAQ
IGV User Guide
File Formats
Release Notes
Credits
Contact

Search website

search

Home



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.

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 Reserarch (ITCR) of the NCI, and the Starr Cancer Consortium.

IGV participates in the GenomeSpace initiative, which is funded by the National Human Genome Research Institute.

Downloads

Download the IGV desktop application and igvtools.

Citing IGV

To cite your use of IGV in your publication:

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)

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).

Integrative genomics viewer

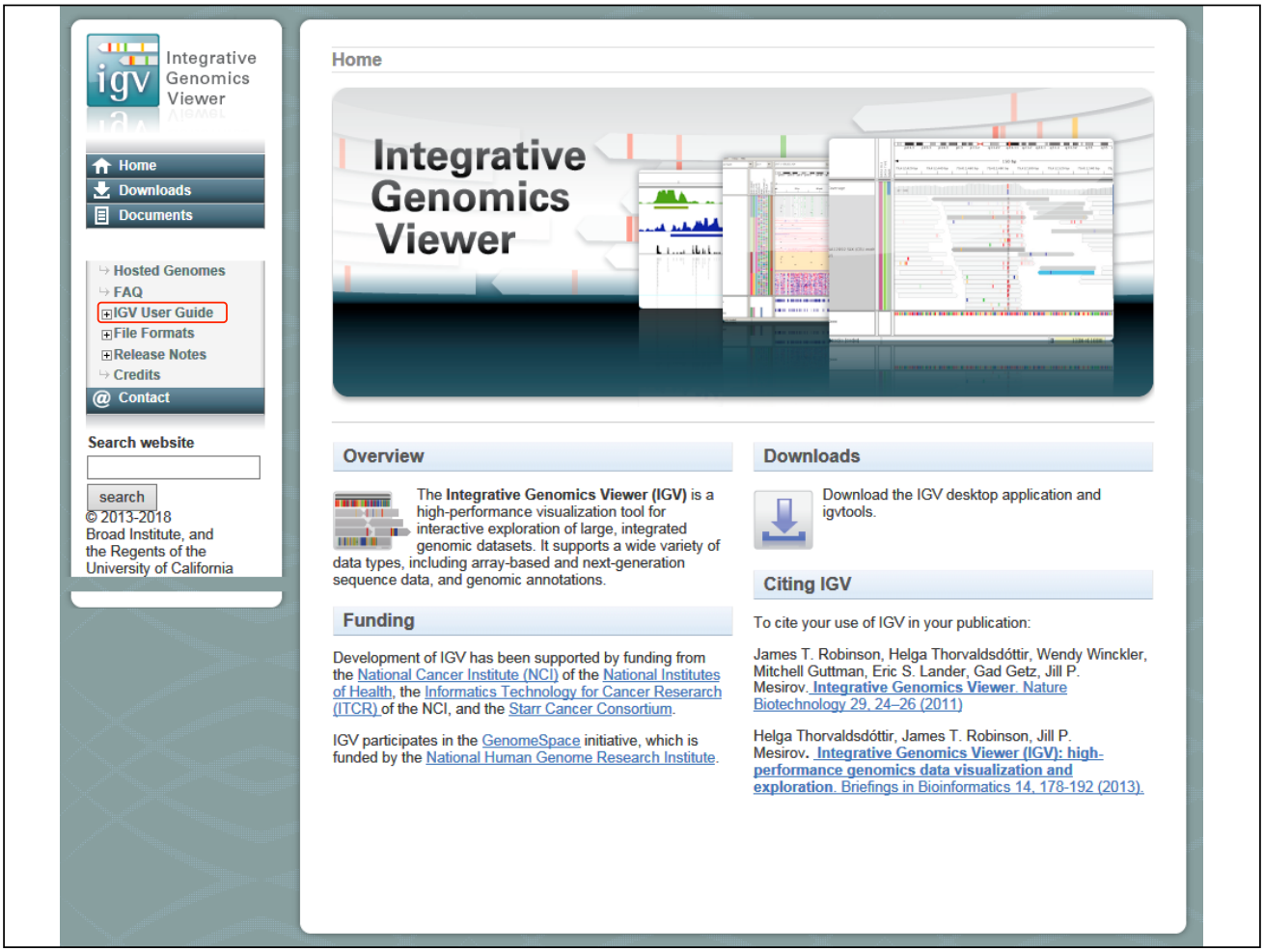
James T Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S Lander, Gad Getz & Jill P Mesirov

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.


Integrative Genomics Viewer
Home
Downloads
Documents
Hosted Genomes
FAQ
IGV User Guide
File Formats
Release Notes
Credits
Contact
Search website
search
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Home
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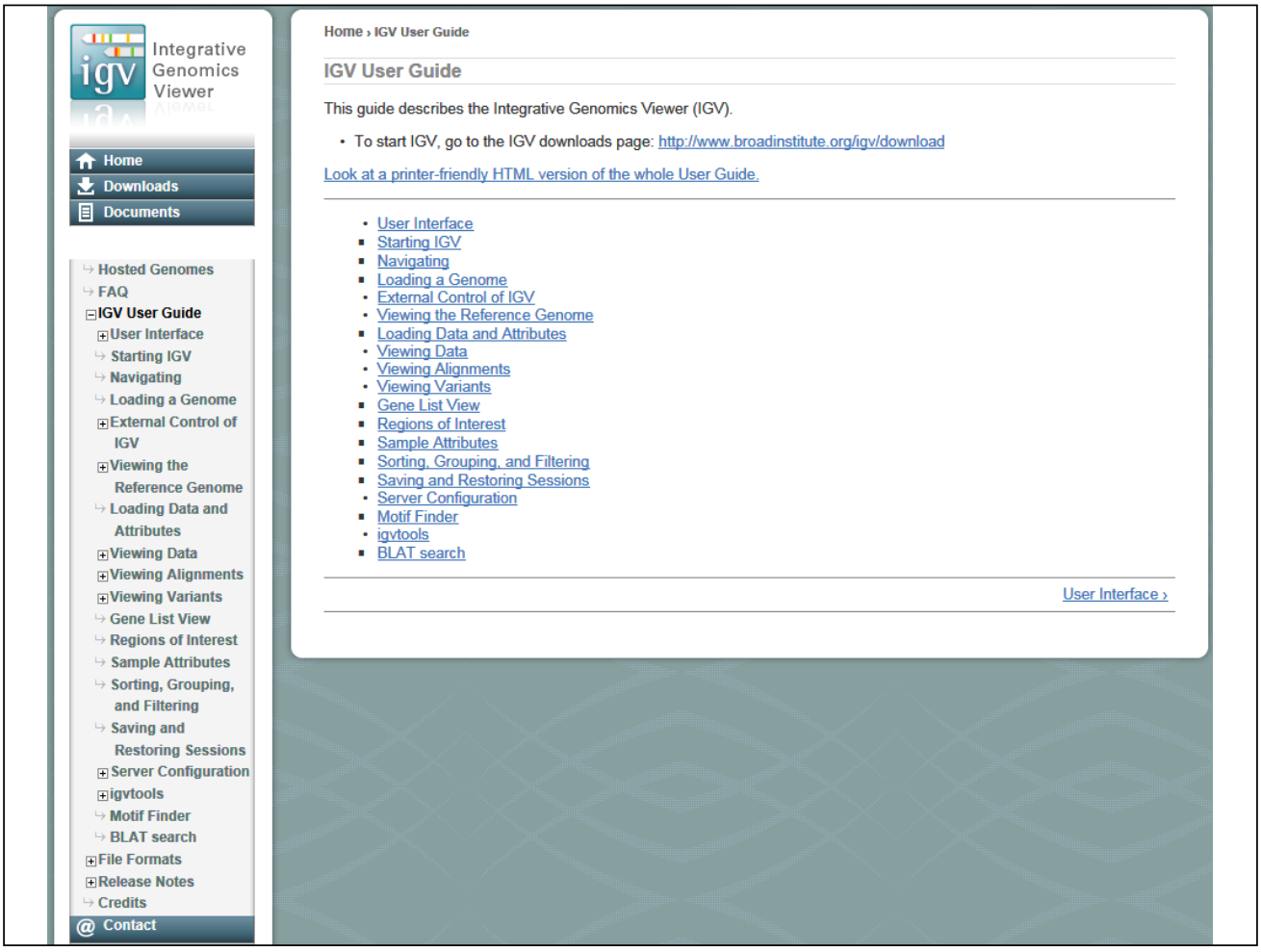
Integrative Genomics Viewer
Home
Downloads
Documents
Hosted Genomes
FAQ
IGV User Guide
User Interface
Starting IGV
Navigating
Loading a Genome
External Control of IGV
Viewing the Reference Genome
Loading Data and Attributes
Viewing Data
Viewing Alignments
Viewing Variants
Gene List View
Regions of Interest
Sample Attributes
Sorting, Grouping, and Filtering
Saving and Restoring Sessions
Server Configuration
igvtools
Motif Finder
BLAT search
File Formats
Release Notes
Credits
Contact
Home › IGV User Guide
IGV User Guide
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Look at a printer-friendly HTML version of the whole User Guide.
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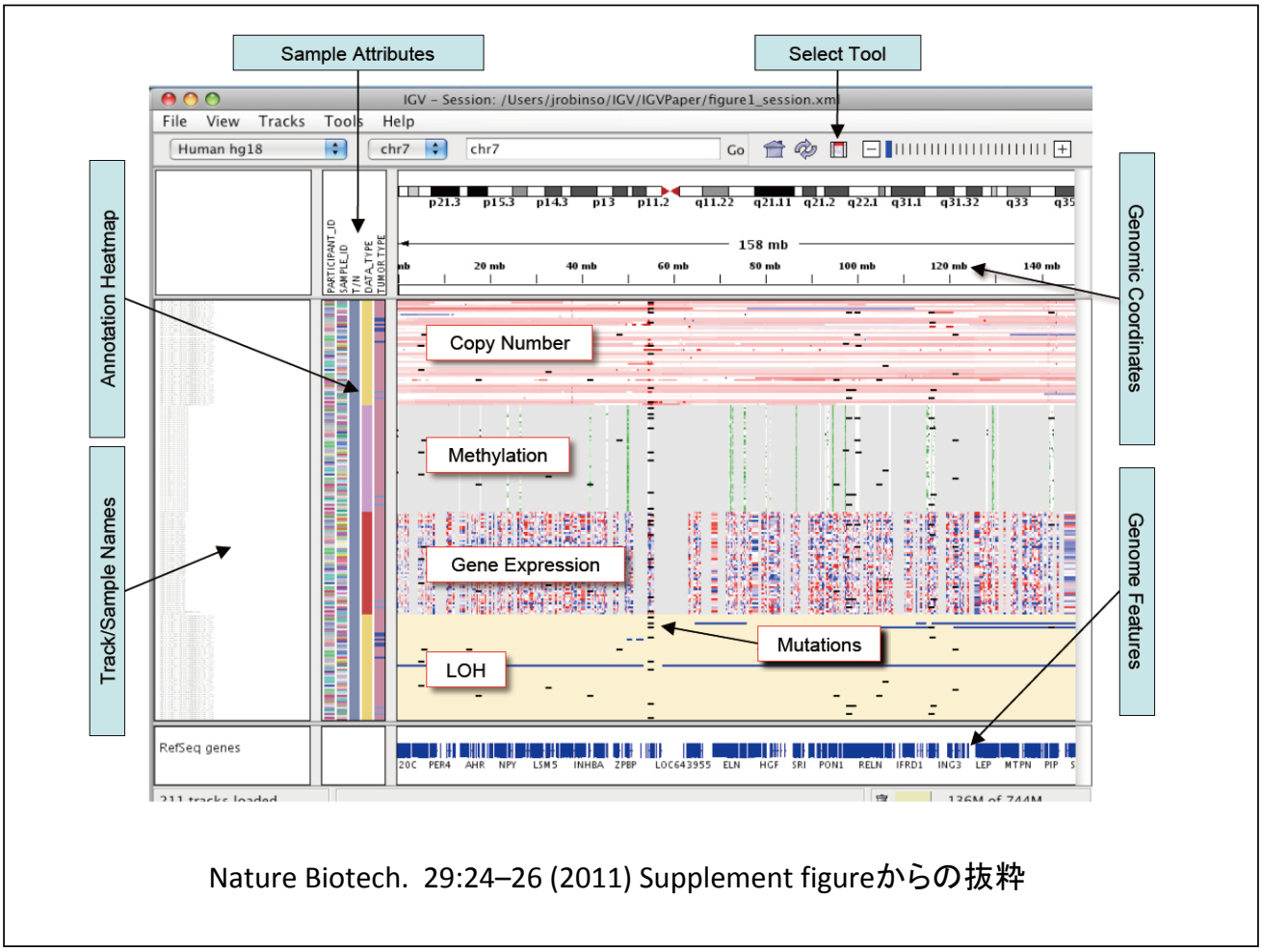
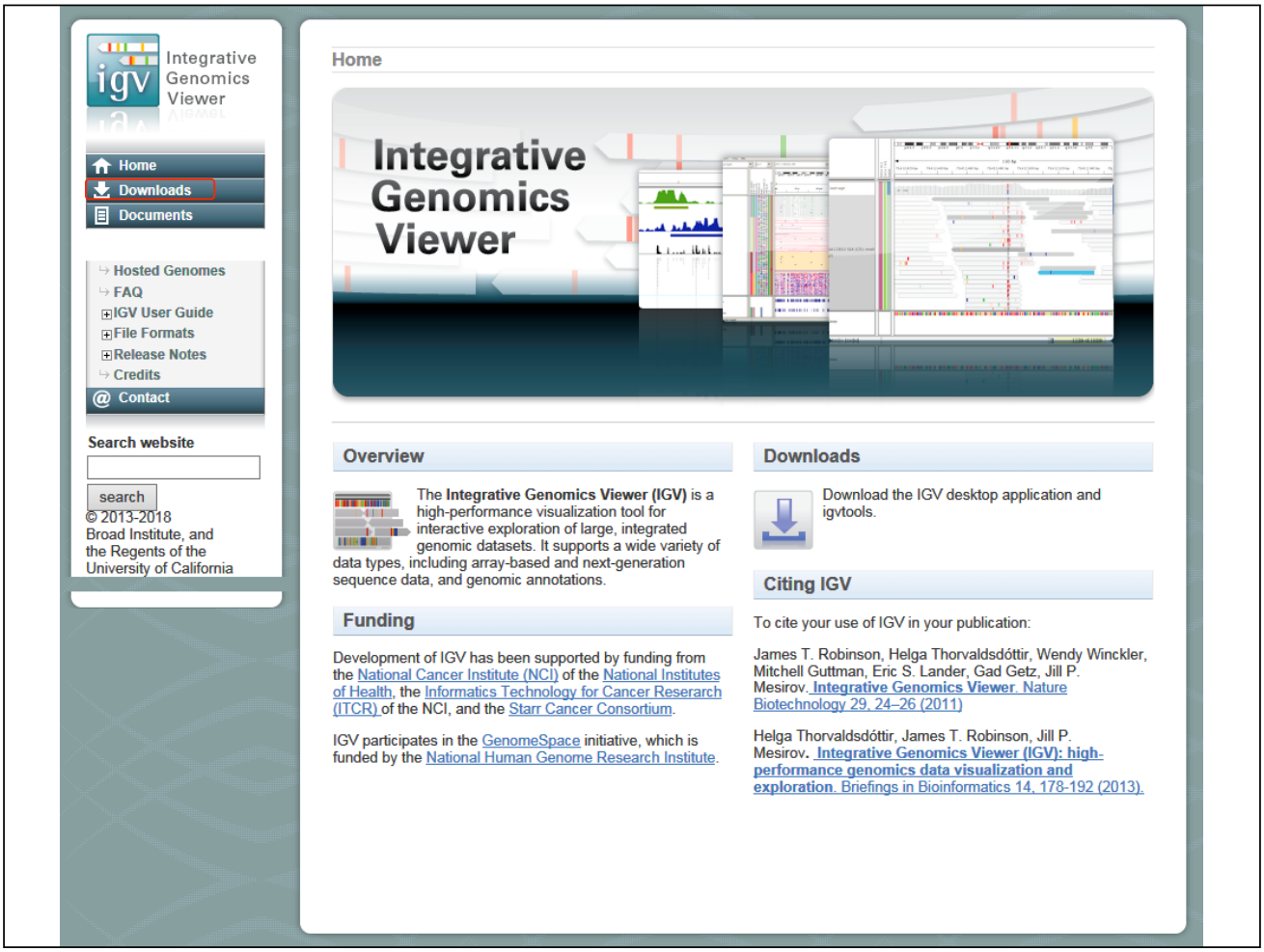


Nature Biotech. 29:24–26 (2011) Supplement figureからの抜粋



Downloads

Integrative Genomics Viewer - IGV 2.4

NOTE: IGV 2.4.x releases require Java 8. For Java 11 see the development snapshot build.

Install IGV

Download IGV Mac App

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

Download IGV on Windows

Download and unzip the Archive, then double-click the *igv.bat* file to run IGV. See *readme.txt* to run IGV from the command line

For high DPI screens: Use the development snapshot build of IGV.

Download IGV to run on Linux / MacOS command line

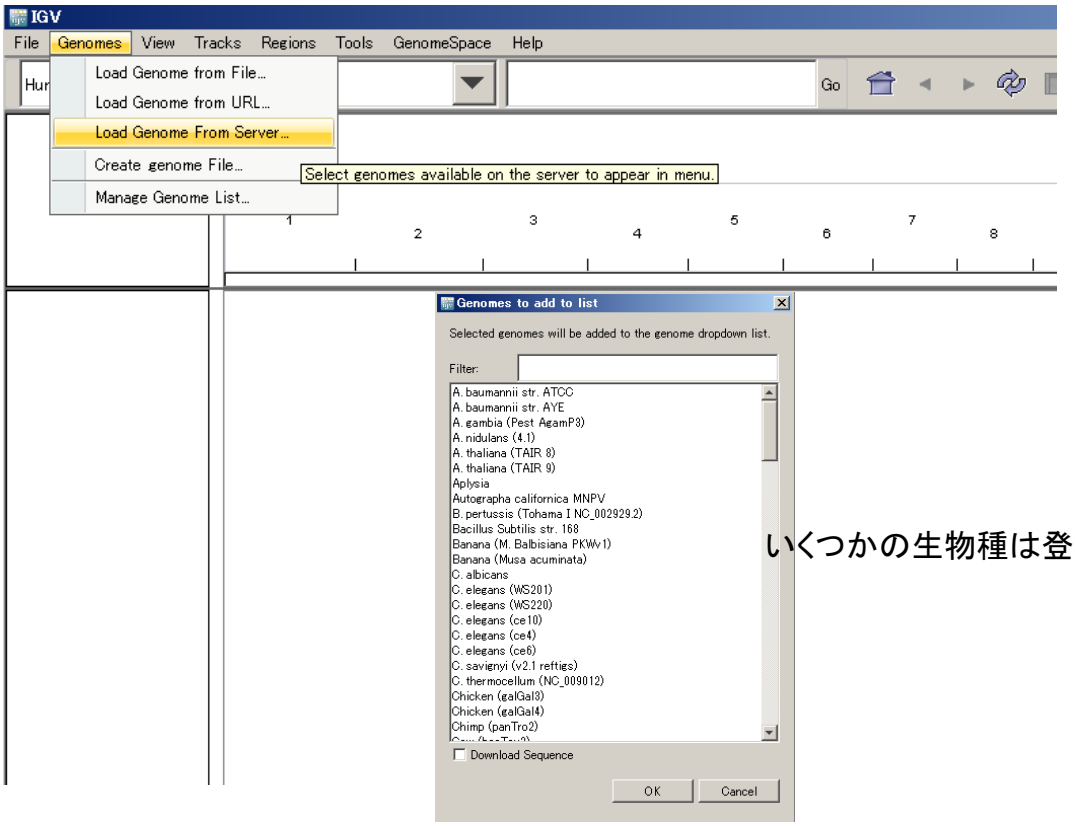
Download and unzip the Archive. See the downloaded *readme.txt* for further instructions.

Launch IGV using Java Web Start

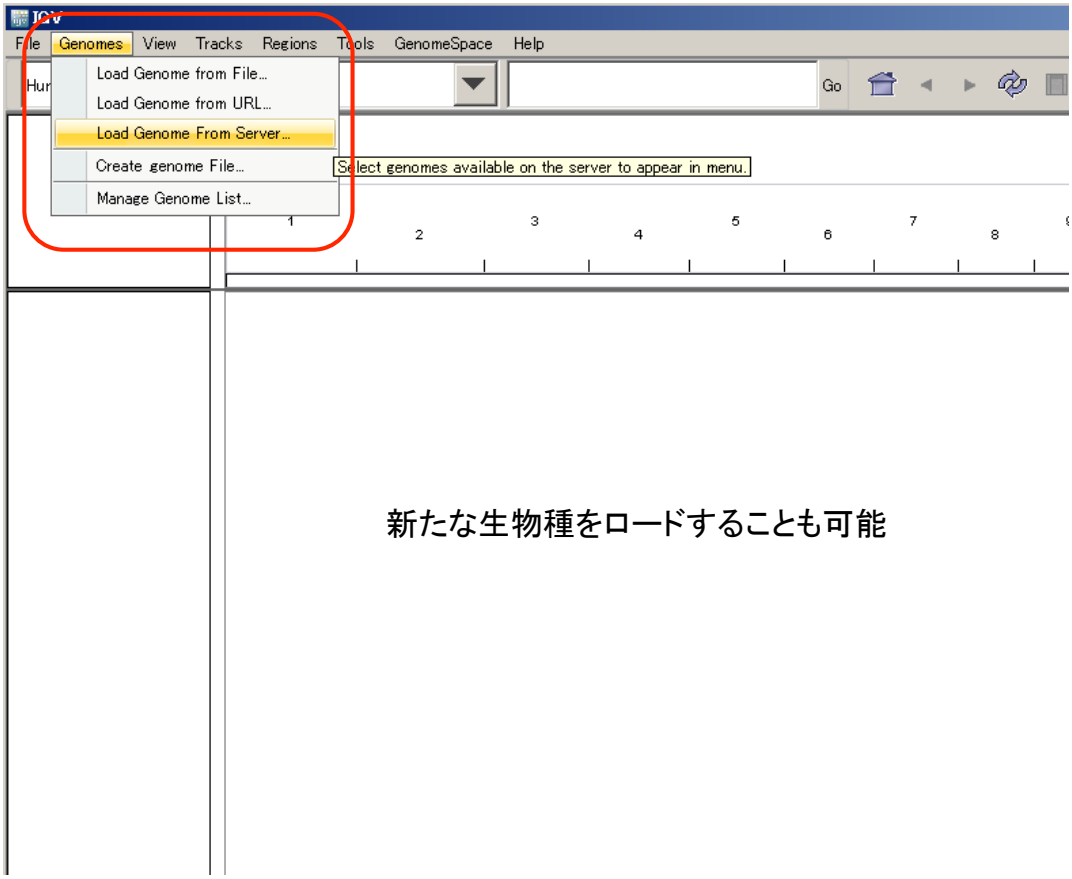
Launch	Launch	Launch
1.2 GB	2 GB	10 GB
Max memory for Windows with 32-bit Java		Only for large memory machines with 64-bit Java

Mac users note: If you are notified of security errors that prevent launching IGV, try right-clicking on the downloaded .jnlp file and select *Open With > Java Web Start*.

Windows users note: Most Windows installs do not include 64-bit Java by default, even if the operating system is 64-bit. Attempting to use the 2GB or greater launch options with 32-bit Java will result in the error *"could not create virtual machine"*.



いくつかの生物種は登録されている



新たな生物種をロードすることも可能

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

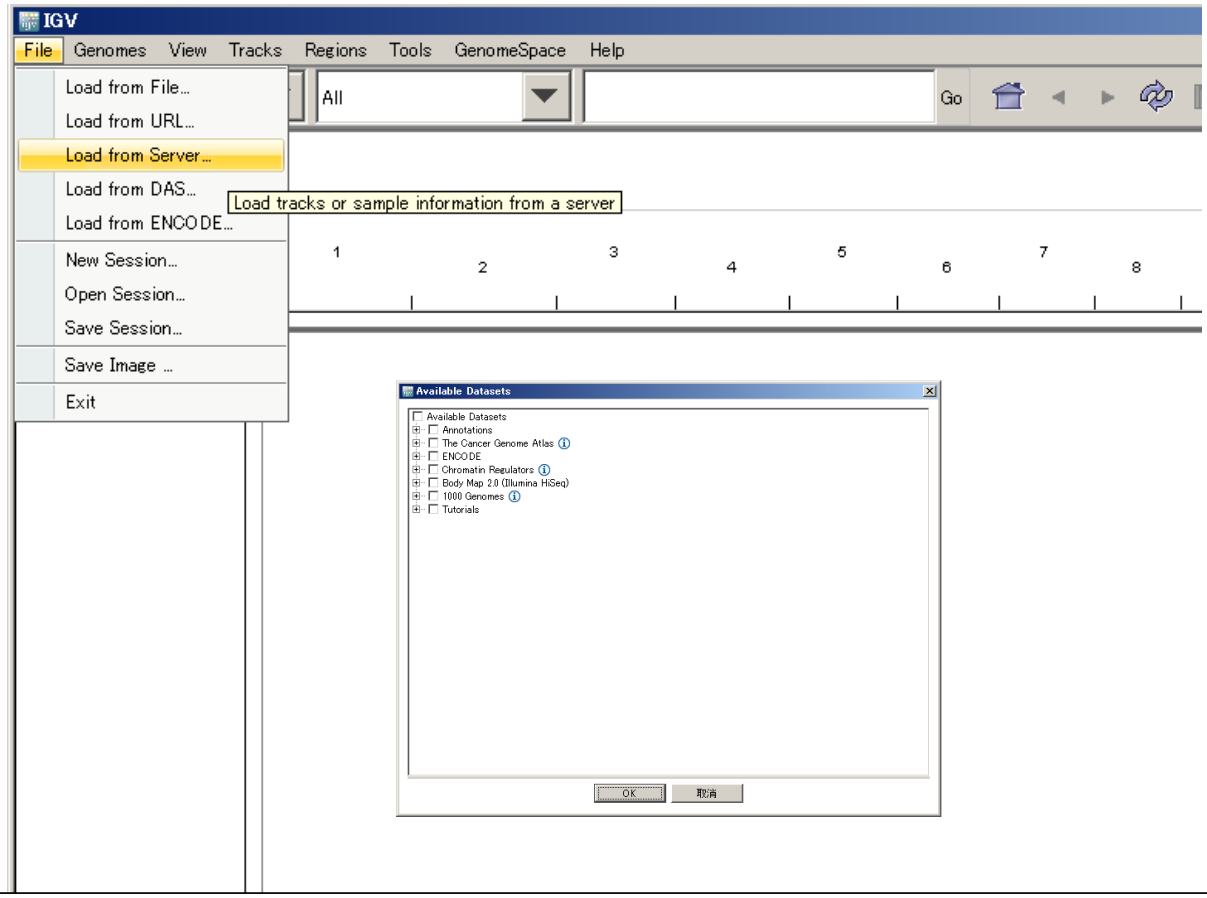
- File Extension Identifies Format
- Recommended File Formats
- BAM
- BED
- BedGraph
- bigBed
- bigWig
- Birdsuite Files
- broadPeak
- CBS
- Chemical Reactivity Probing Profiles
- chrom.sizes
- CN
- Custom File Formats
- Cytoband
- FASTA
- GCT
- CRAM
- genePred
- GFF/GTF
- GISTIC
- Goby
- GWAS
- IGV
- LOH
- MAF (Multiple Alignment Format)
- MAF (Mutation Annotation Format)
- Merged BAM File
- MUT
- narrowPeak
- PSL
- RES
- RNA Secondary Structure Formats
- SAM
- Sample Info (Attributes) file
- SEG
- SNP
- TAB
- TDF
- Track Line
- 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。

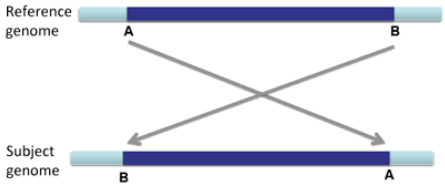
バッチ処理

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。

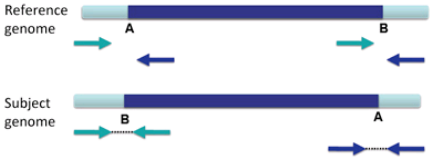
```
new
load myfile.bam
snapshotDirectory mySnapshotDirectory
genome hg18
goto chr1:65,289,335-65,309,335
sort position
collapse
snapshot
goto chr1:113,144,120-113,164,120
sort base
collapse
snapshot
```

Inversions

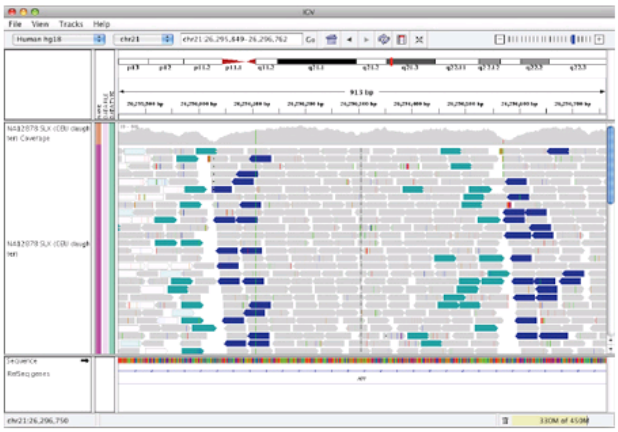
An inversion is a large section of DNA that is reversed in the subject genome compared to the reference genome.



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:

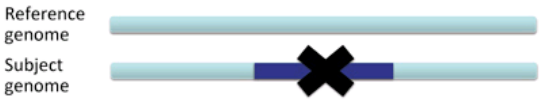
- deletions
- insertions
- inter-chromosomal rearrangements

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:

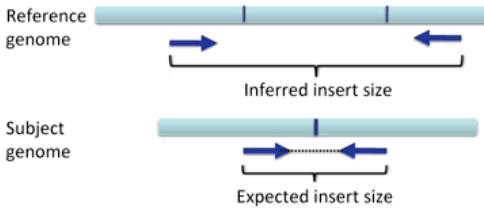
- for an insert that is larger than expected
- for an insert that is smaller than expected
- 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y for paired end reads that are coded by the chromosome on which their mates can be found

Deletions

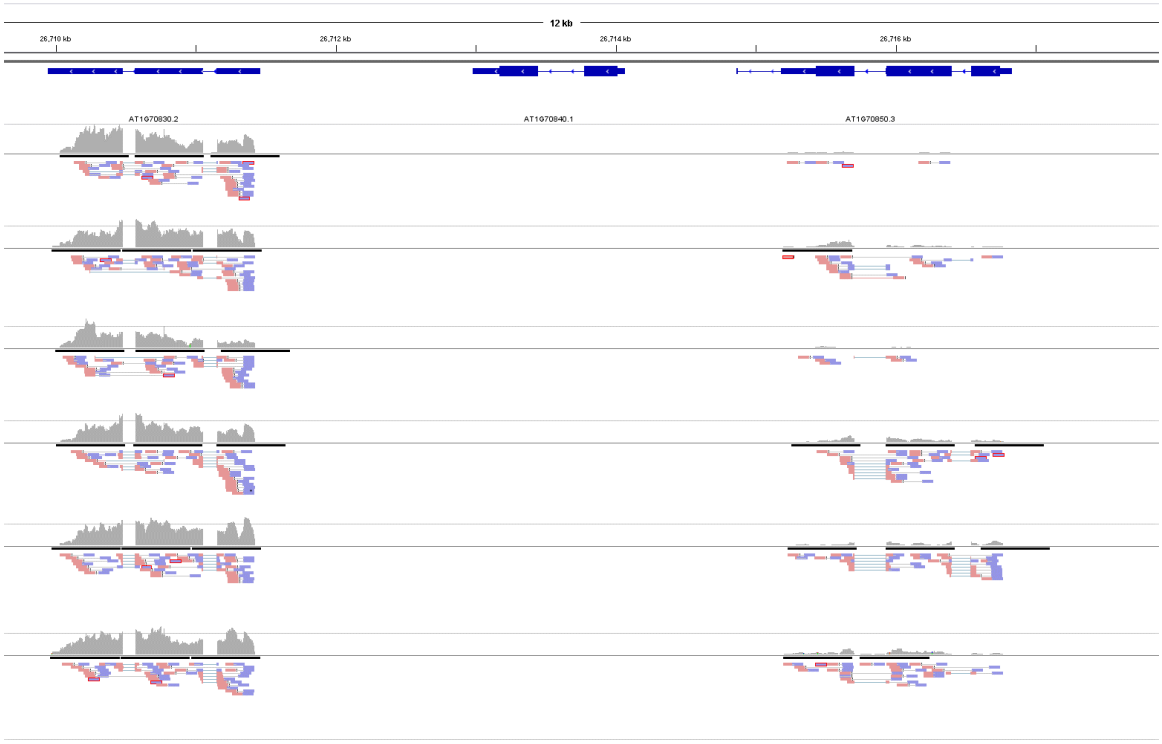
A deletion is a large section of DNA that is absent in the subject genome compared to the reference genome.



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.

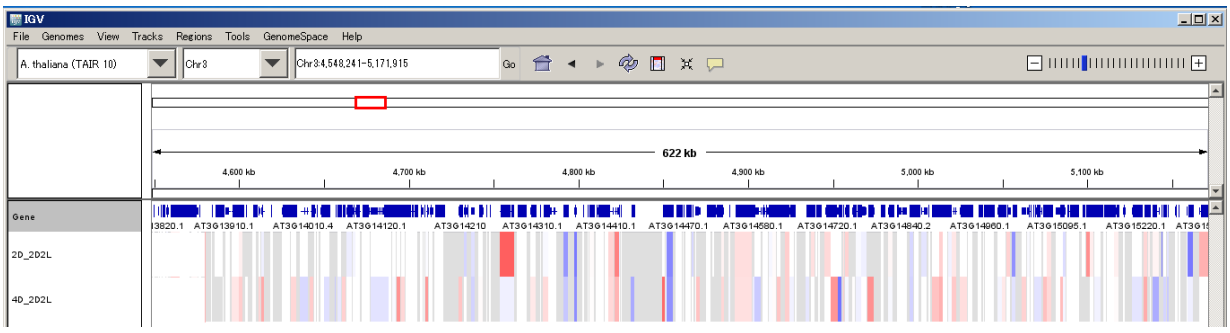


RNA-Seqのデータ表示させる

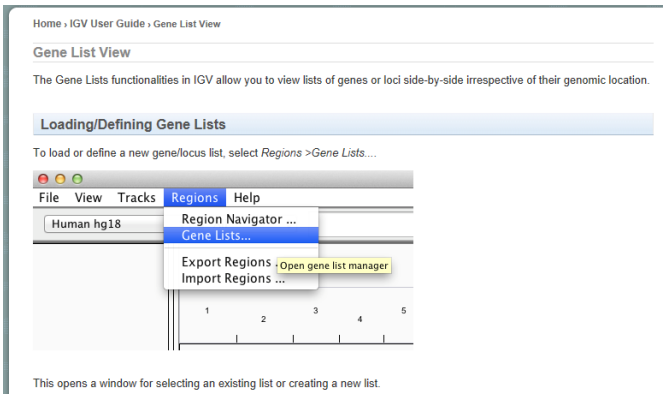


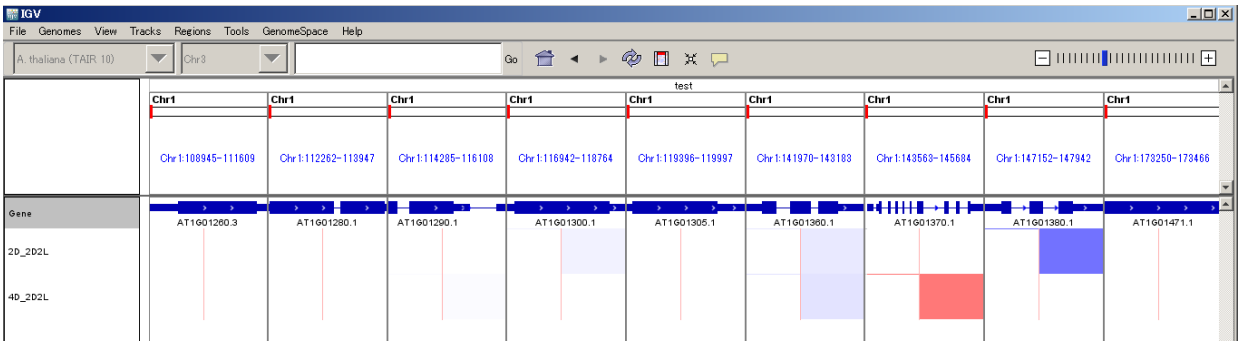
GCTファイルでgene ローカスの発現情報を図示

```
#
#
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



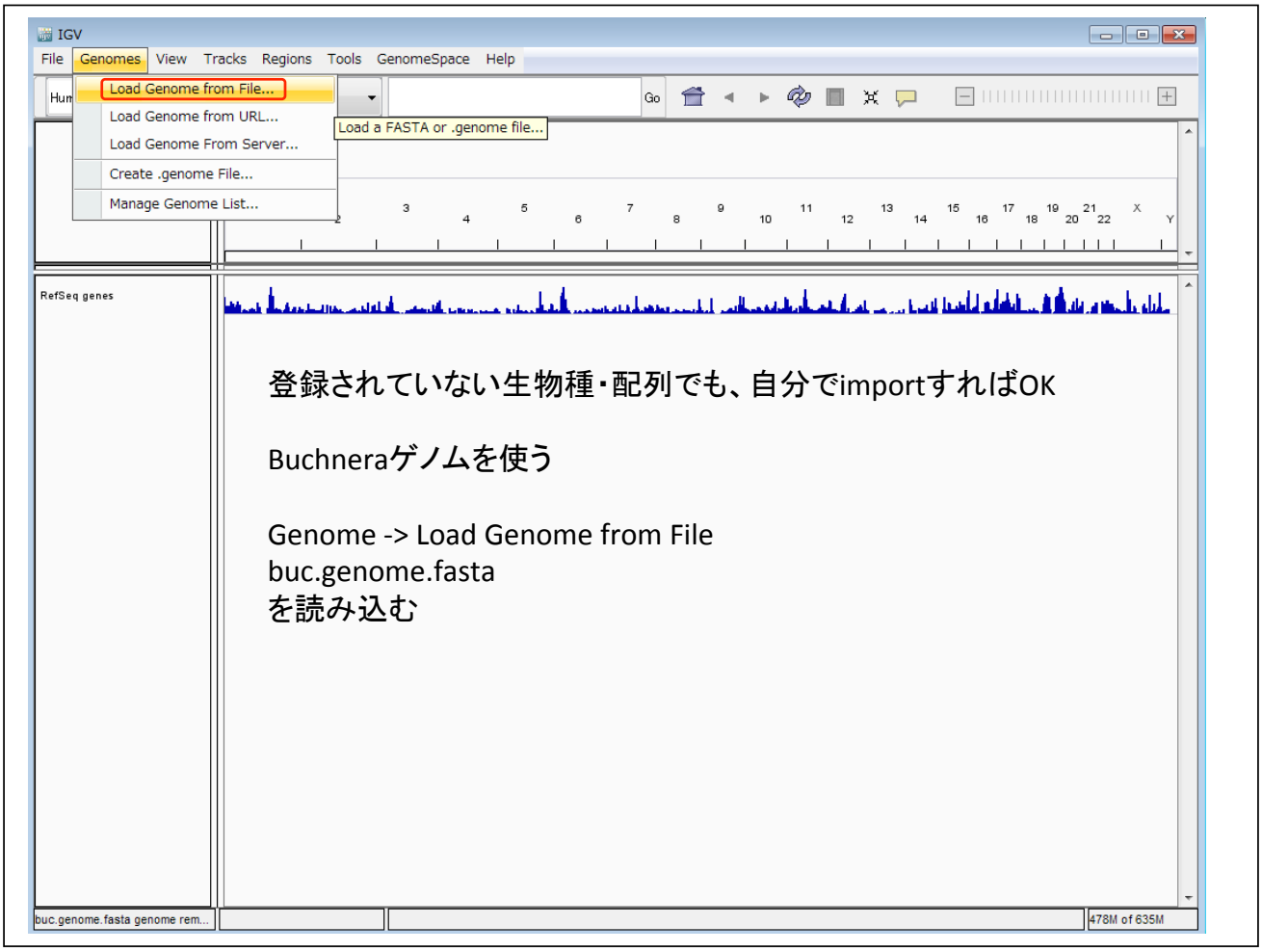


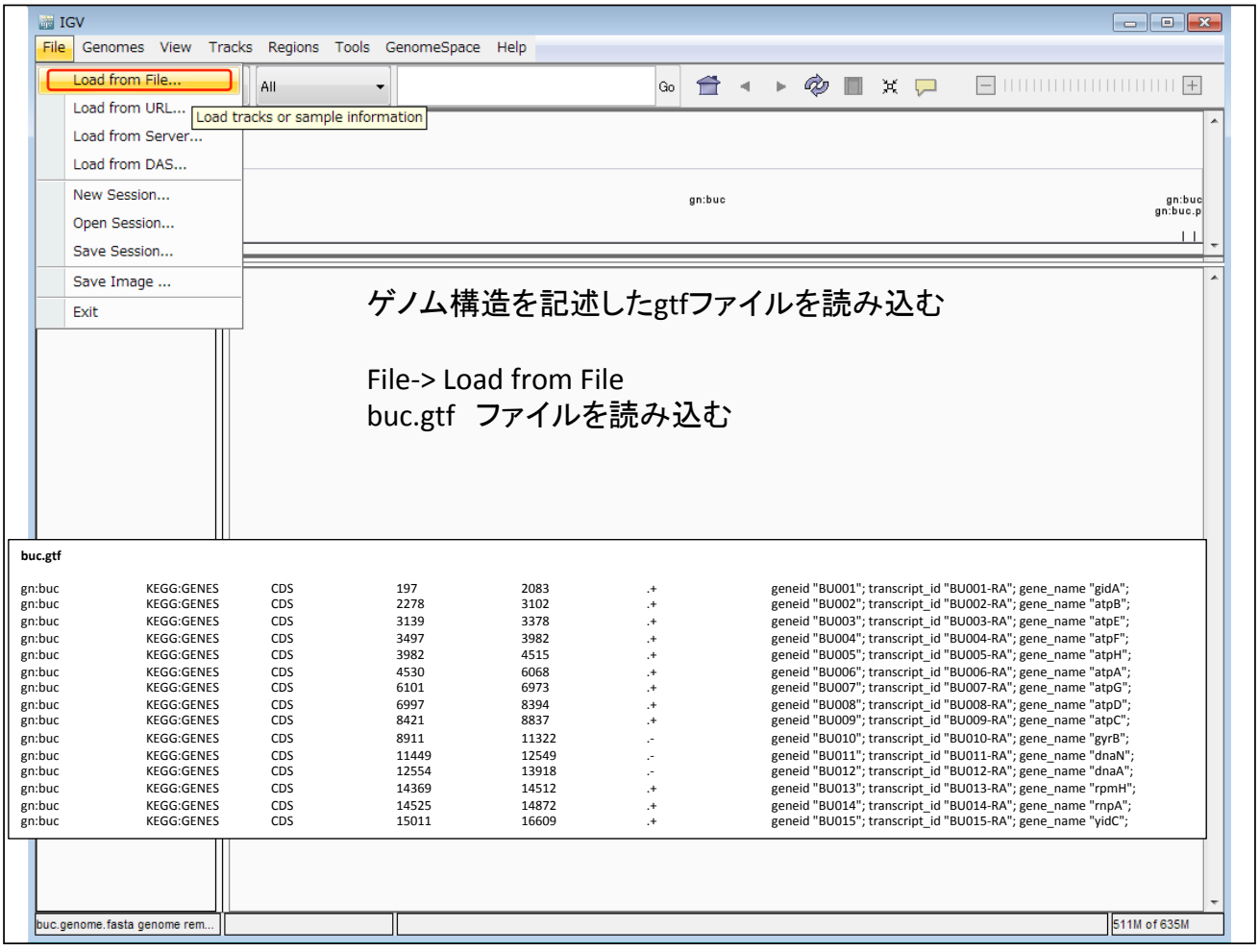
IGV実習

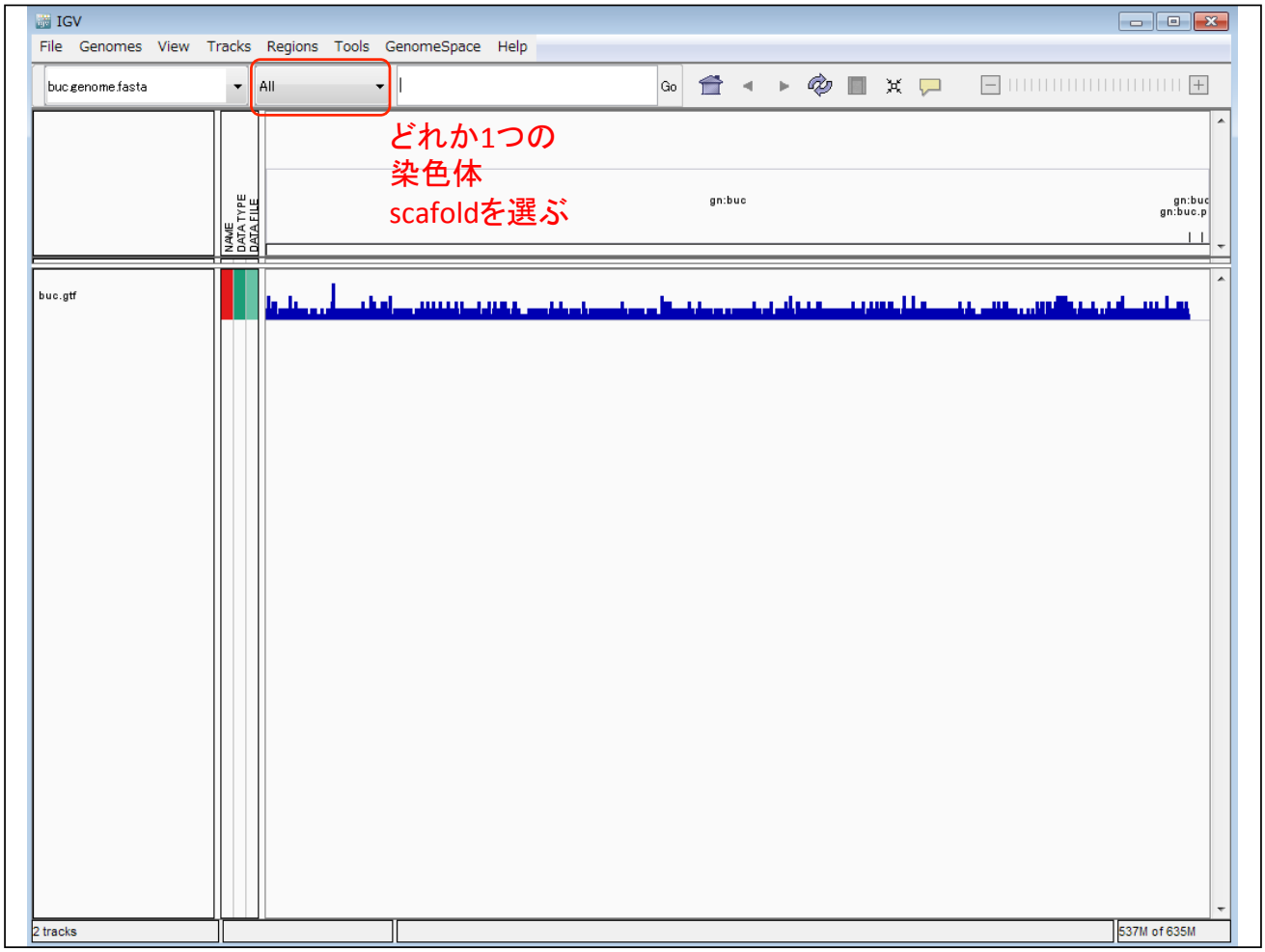
IGVの使用法を学ぶと共に
先のファイルフォーマットも
確認しよう

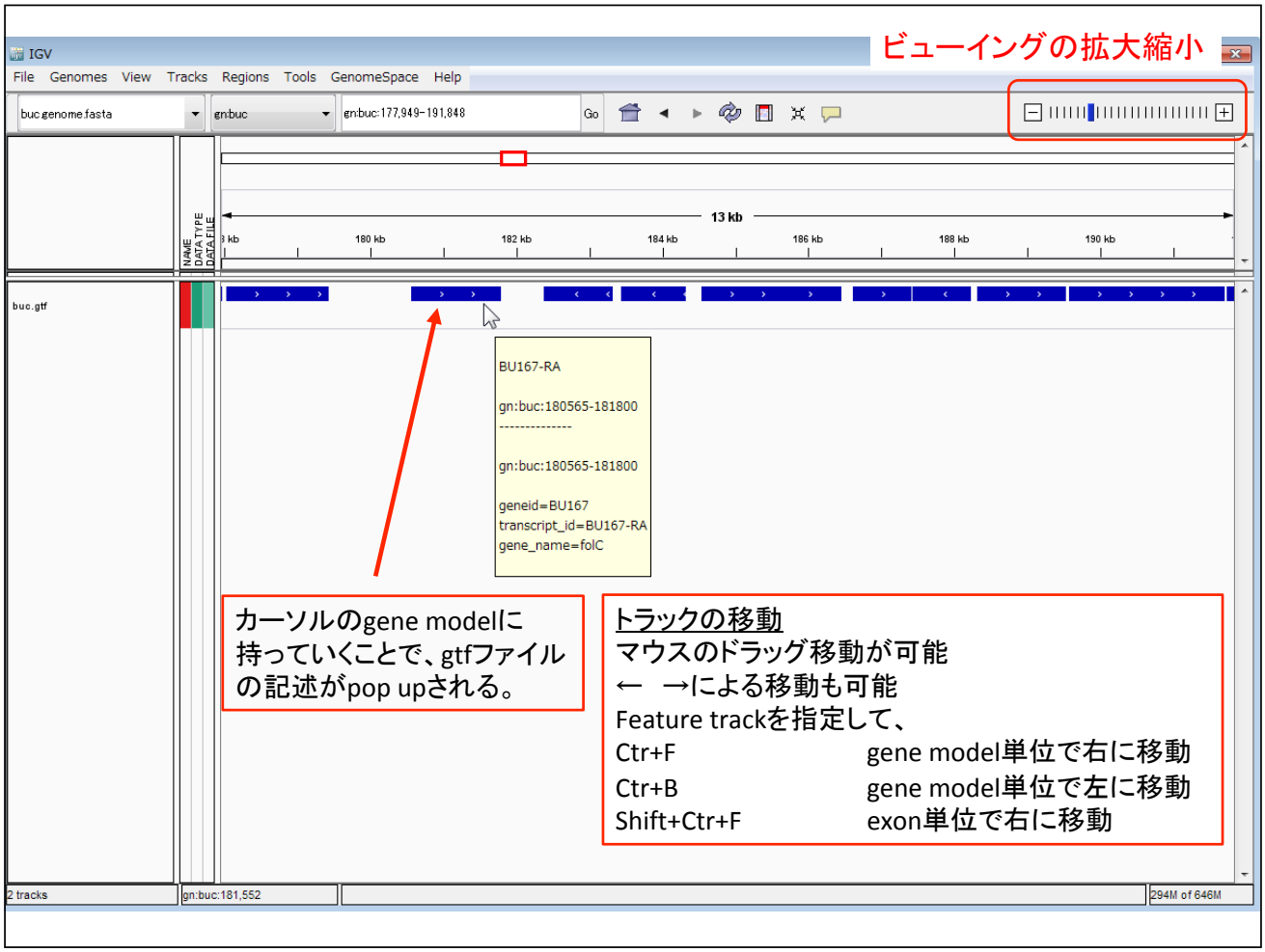
以下のファイルを確認

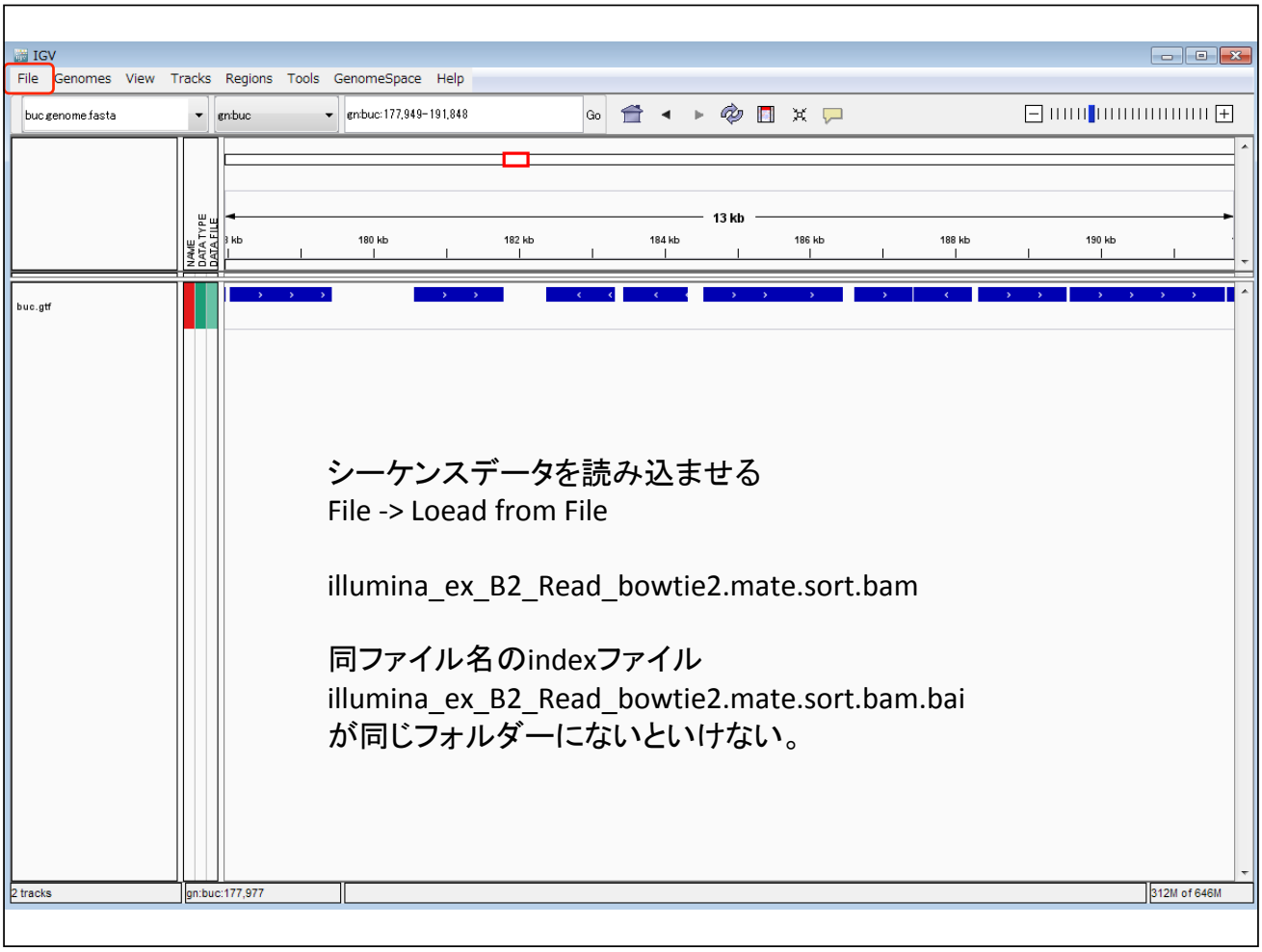
buc.genome.fasta
buc.gtf
buc_cg.wig
illumina_ex_B2_Read_bowtie2.mate.sort.bam
illumina_ex_B2_Read_bowtie2.mate.sort.bam.bai
illumina_ex_B4_Read_bowtie2.mate.sort.bam
illumina_ex_B4_Read_bowtie2.mate.sort.bam.bai


IGV
File
Genomes
View
Tracks
Regions
Tools
GenomeSpace
Help
Load Genome from File...
Load Genome from URL...
Load Genome From Server...
Create .genome File...
Manage Genome List...
Load a FASTA or .genome file...
Go
RefSeq genes
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
buc.genome.fasta genome rem...
478M of 635M


IGV
File
Genomes
View
Tracks
Regions
Tools
GenomeSpace
Help
Load from File...
Load from URL...
Load from Server...
Load from DAS...
New Session...
Open Session...
Save Session...
Save Image ...
Exit
Load tracks or sample information
All
Go
gn:buc
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 "dnaA";
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


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
All
Go
どれか1つの
染色体
scafoldを選ぶ
gn:buc
buc.gtf
2 tracks
537M of 635M

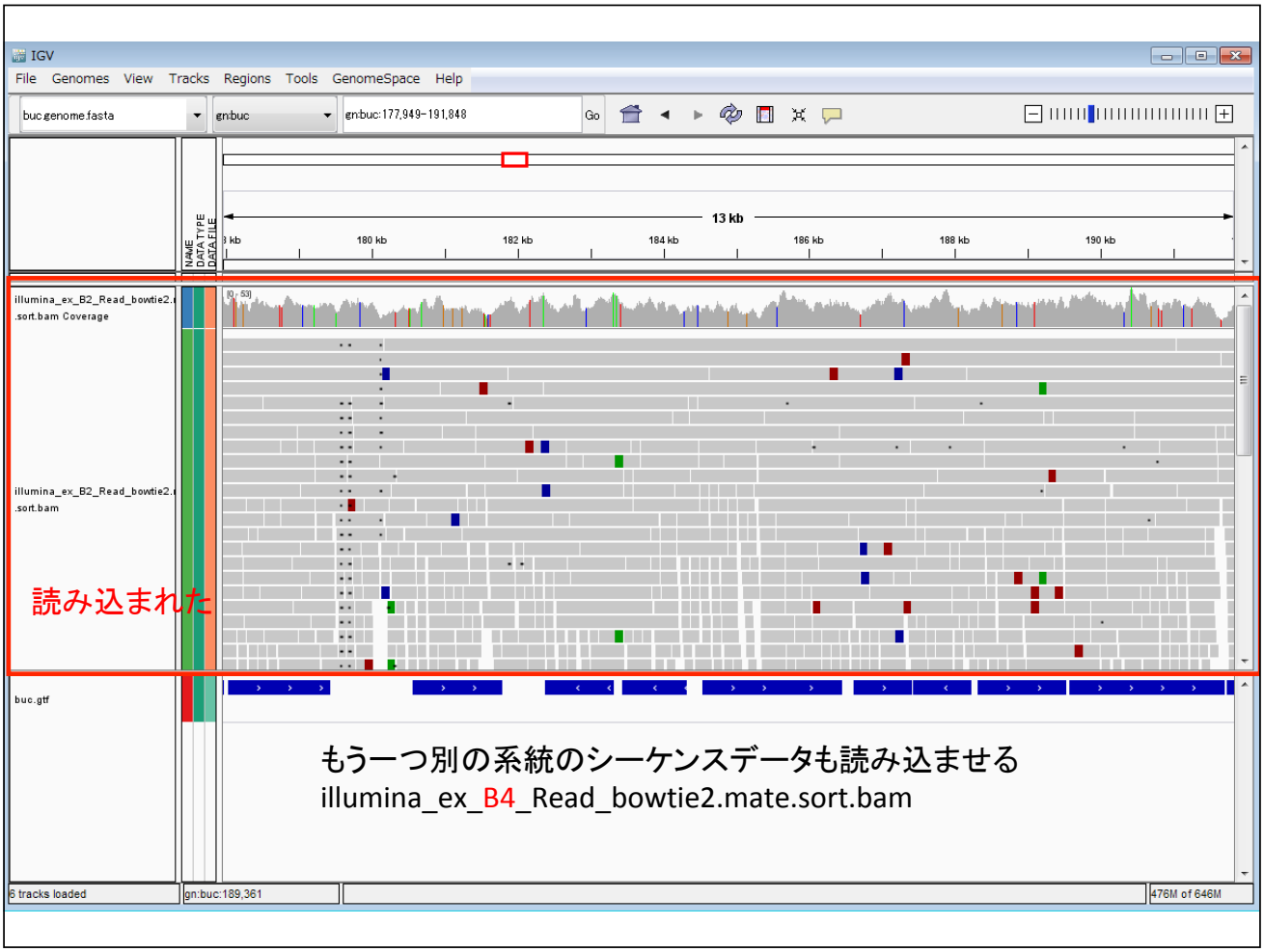

ビューイングの拡大縮小
IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:177,949-191,848
Go
13 kb
180 kb
182 kb
184 kb
186 kb
188 kb
190 kb
buc.gtf
BU167-RA
gn:buc:180565-181800
gn:buc:180565-181800
geneid=BU167
transcript_id=BU167-RA
gene_name=folC
カーソルのgene modelに
持っていくことで、gtfファイル
の記述がpop upされる。
トラックの移動
マウスのドラッグ移動が可能
← →による移動も可能
Feature trackを指定して、
Ctr+F gene model単位で右に移動
Ctr+B gene model単位で左に移動
Shift+Ctr+F exon単位で右に移動
2 tracks
gn:buc:181,552
294M of 646M



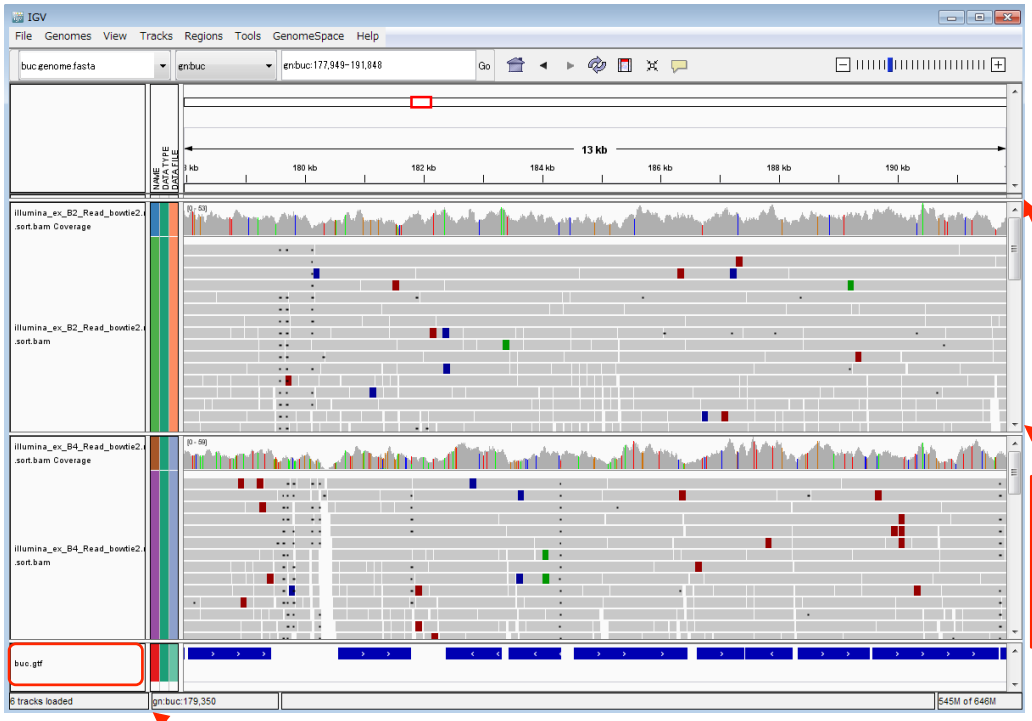
シーケンスデータを読み込ませる
File -> Loead from File

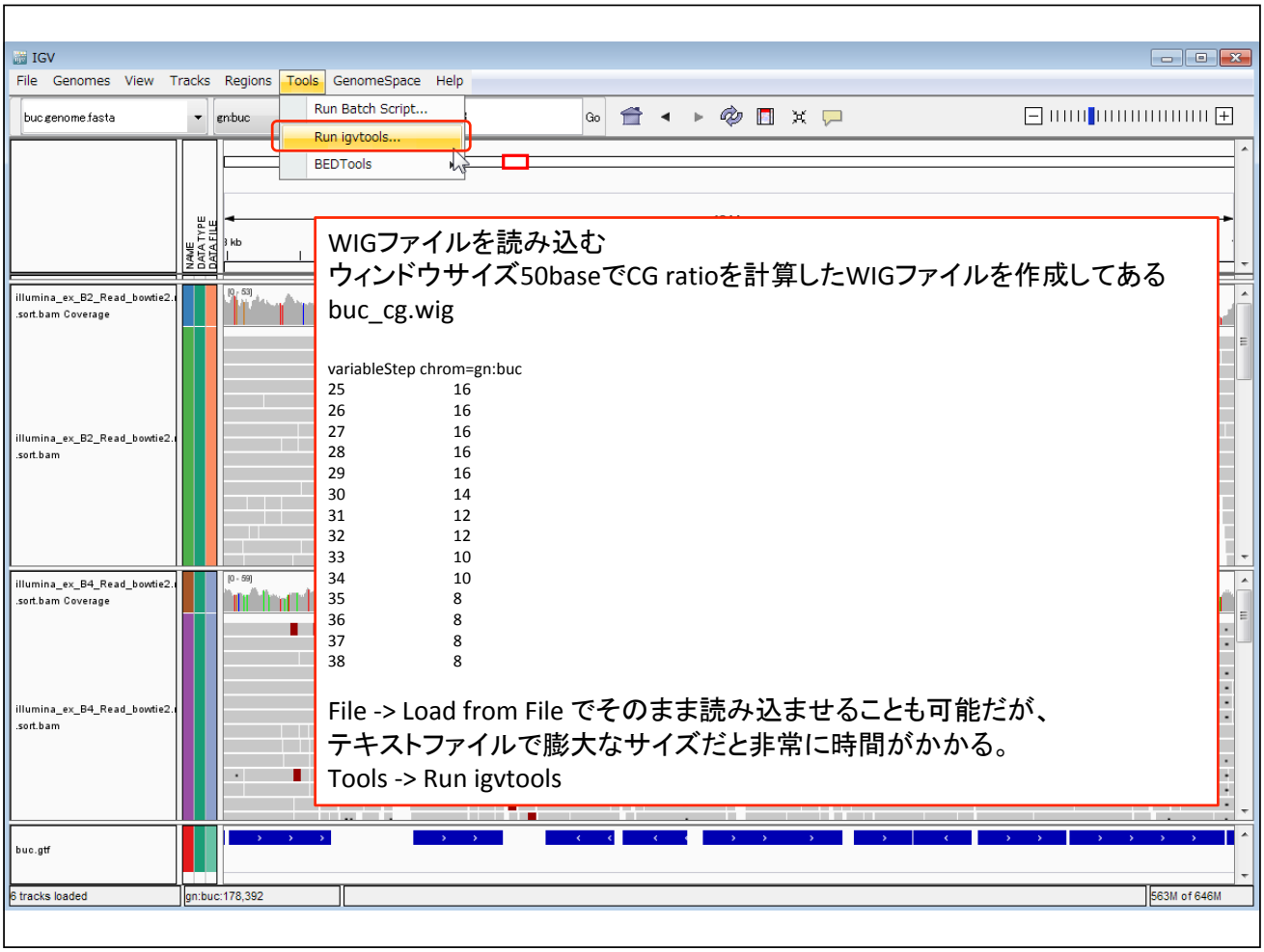
illumina_ex_B2_Read_bowtie2.mate.sort.bam

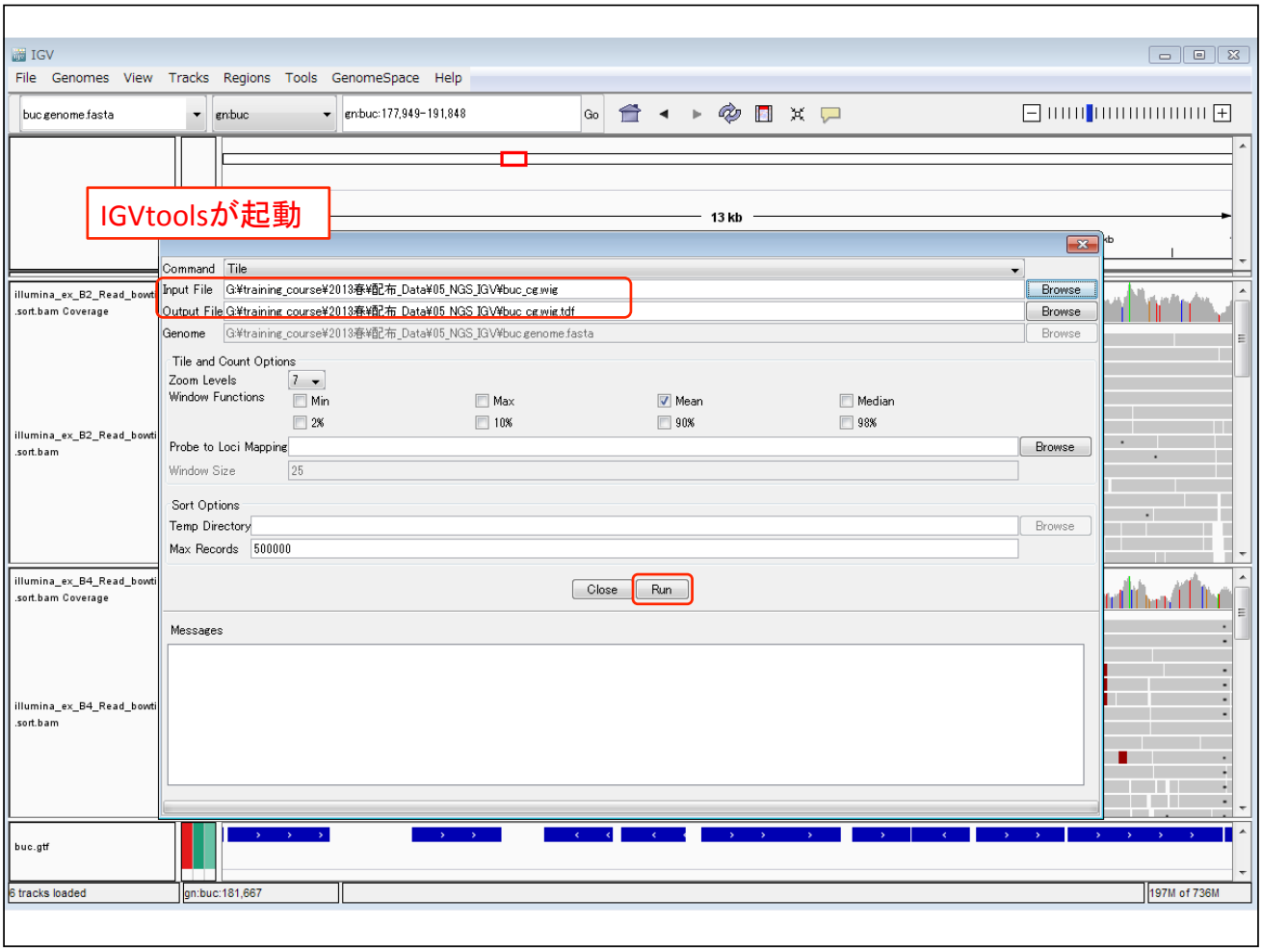
同ファイル名のindexファイル
illumina_ex_B2_Read_bowtie2.mate.sort.bam.bai
が同じフォルダーにないといけない。

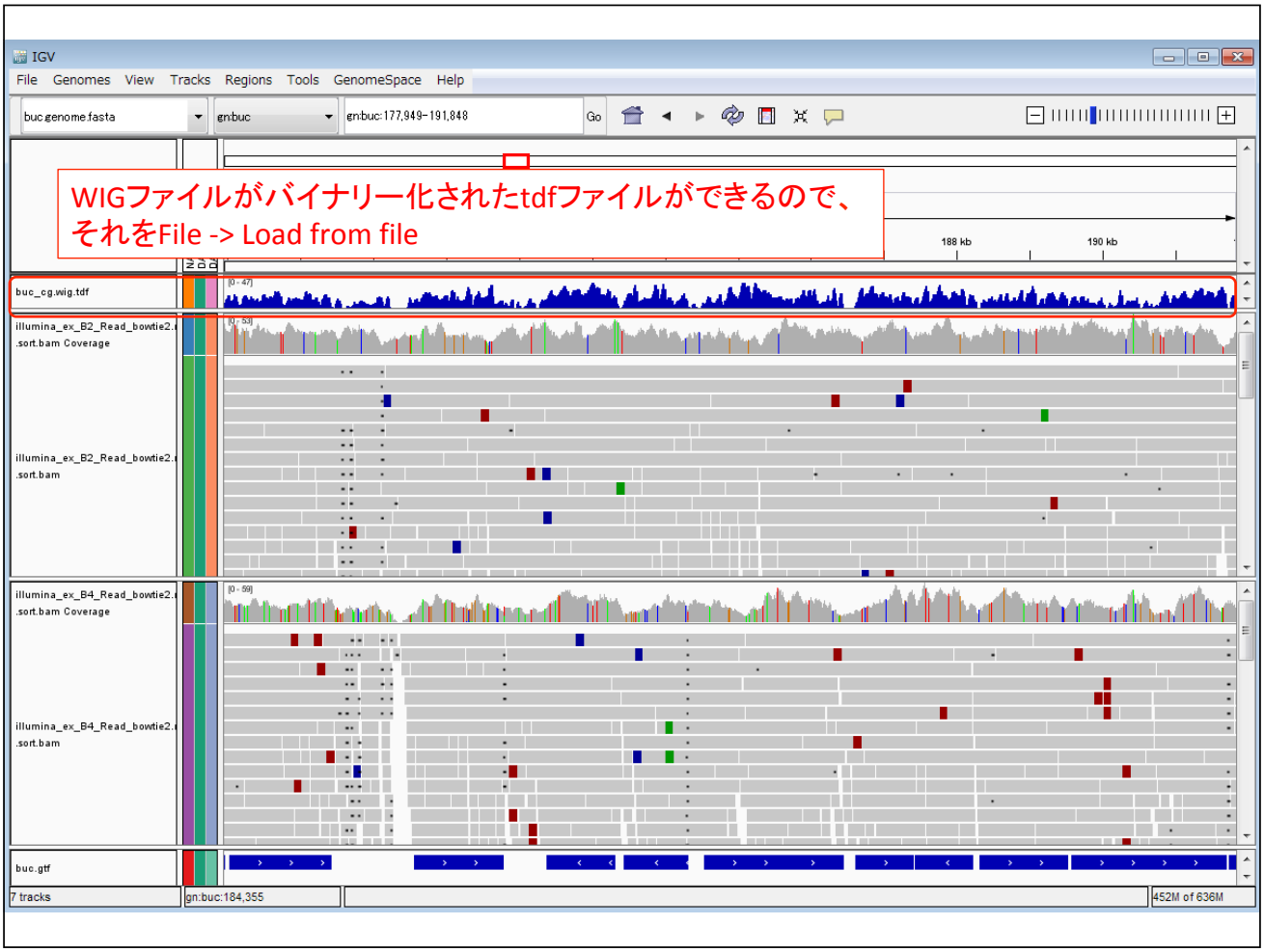


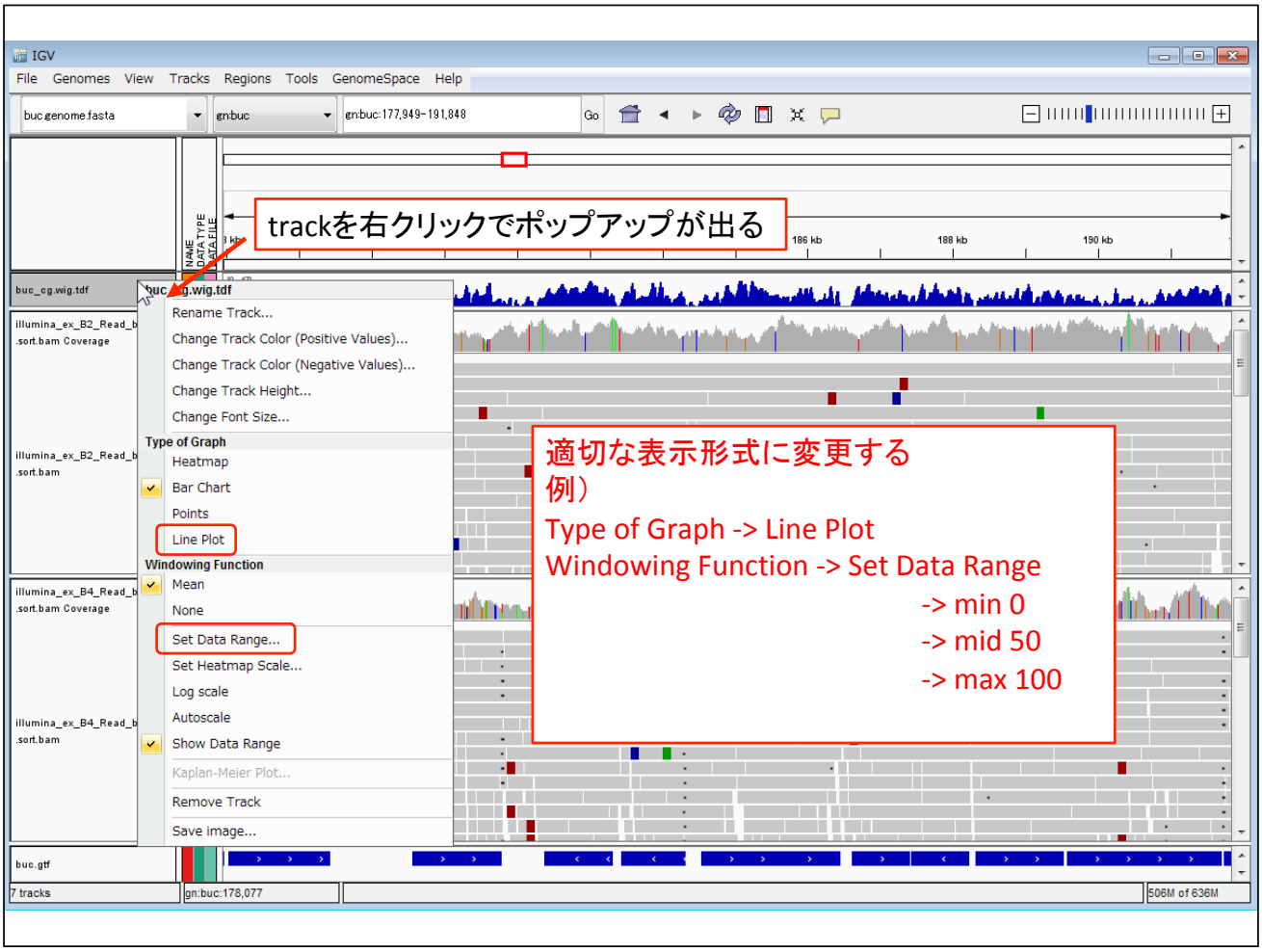
もう一つ別の系統のシーケンスデータも読み込ませる
illumina_ex_B4_Read_bowtie2.mate.sort.bam

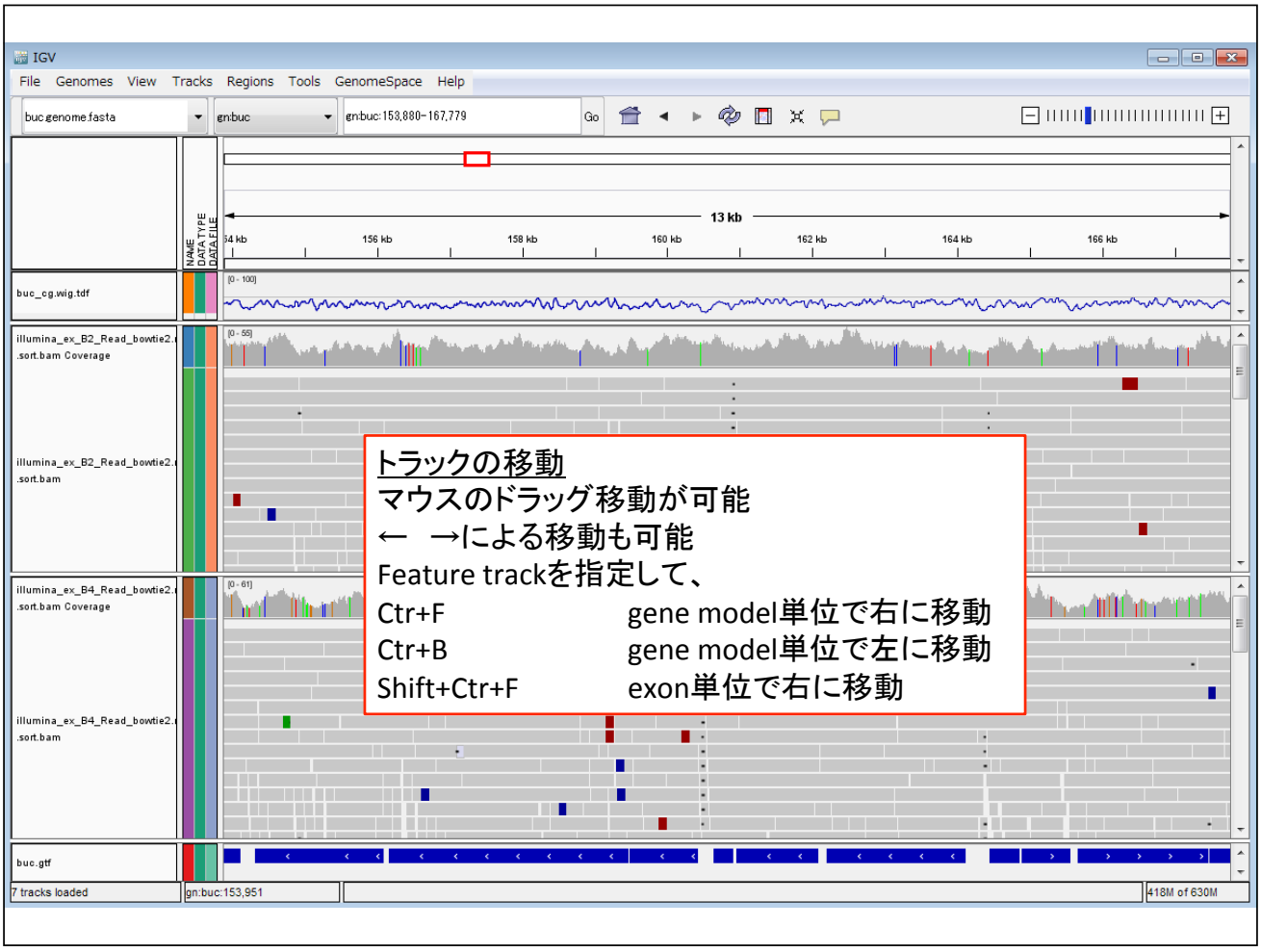

こういう所を
クリックしたまま
移動させることで
幅を変更
各Trackはドラッグアンドドロップで移動できる

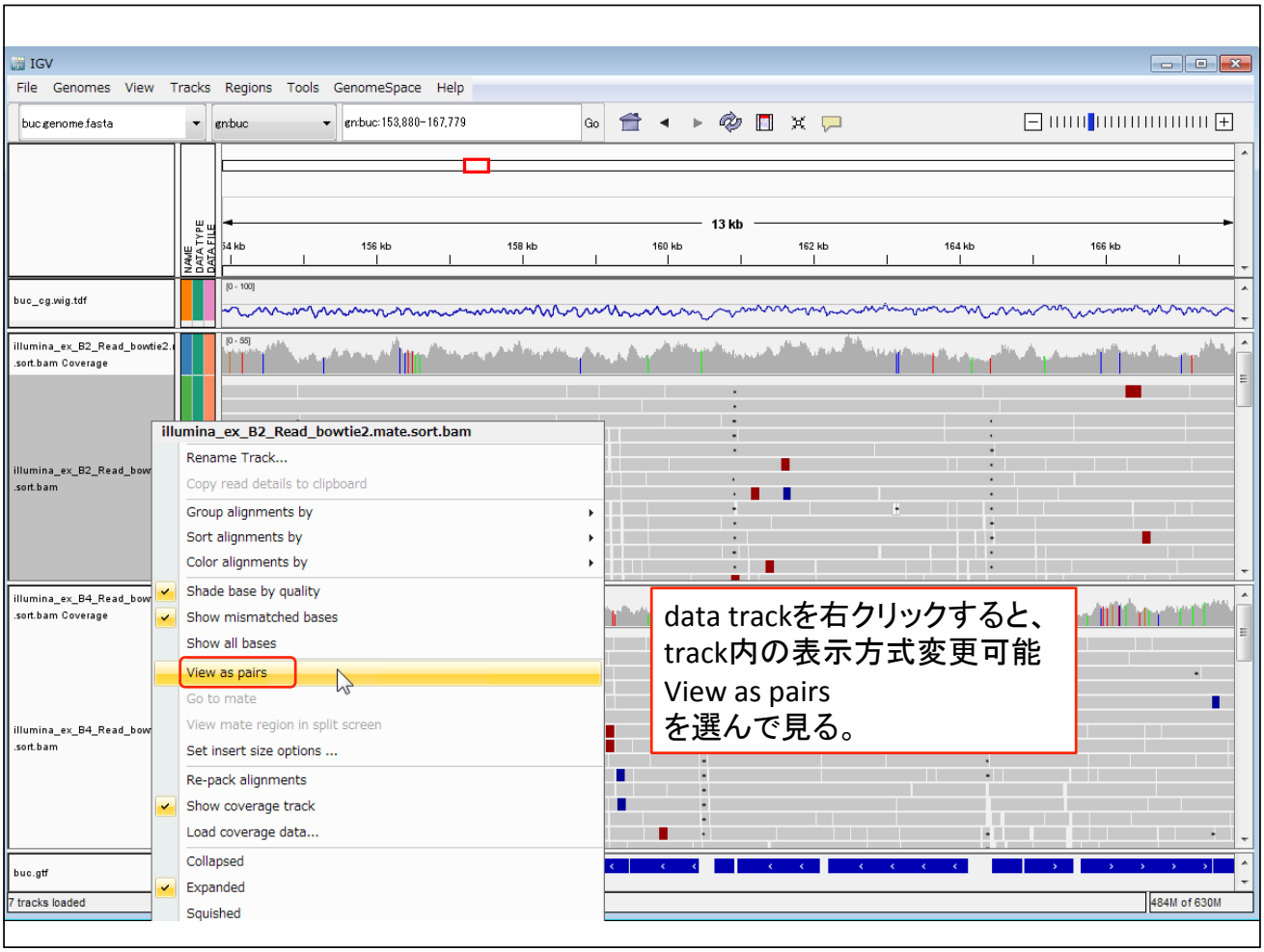

Run Batch Script...
Run igvtools...
BEDTools
WIGファイルを読み込む
ウィンドウサイズ50baseでCG ratioを計算したWIGファイルを作成してある
buc_cg.wig
variableStep chrom=gn:buc
25 16
26 16
27 16
28 16
29 16
30 14
31 12
32 12
33 10
34 10
35 8
36 8
37 8
38 8
File -> Load from File でそのまま読み込ませることも可能だが、
テキストファイルで膨大なサイズだと非常に時間がかかる。
Tools -> Run igvtools

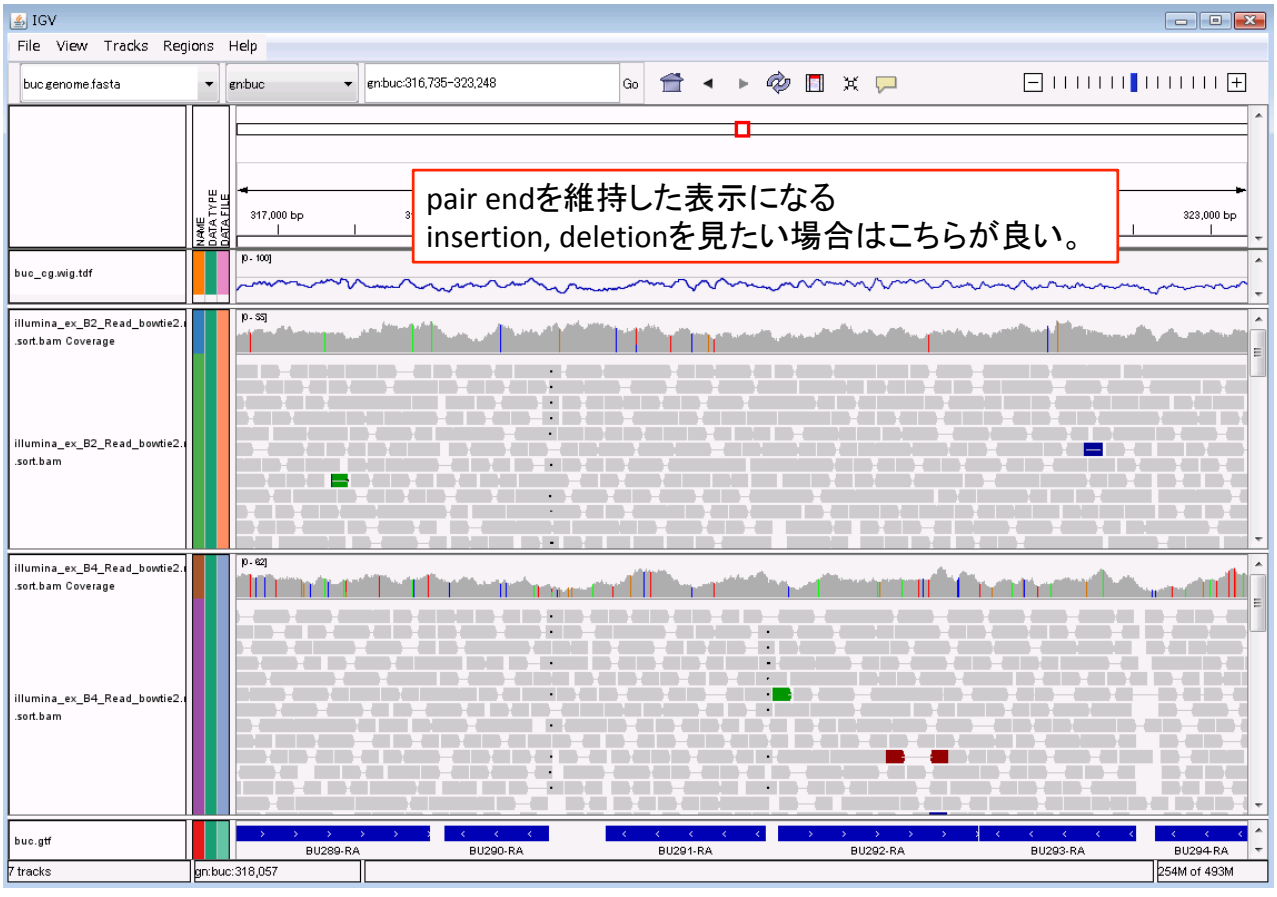

IGVtoolsが起動
IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:177,949-191,848
Go
13 kb
Command Tile
Input File G:¥training_course¥2013春¥配布_Data¥05_NGS_IGV¥buc_cg.wig
Output File G:¥training_course¥2013春¥配布_Data¥05_NGS_IGV¥buc_cg.wig.tdf
Genome G:¥training_course¥2013春¥配布_Data¥05_NGS_IGV¥buc.genome.fasta
Browse
Tile and Count Options
Zoom Levels 7
Window Functions
Min
Max
Mean
Median
2%
10%
90%
98%
Probe to Loci Mapping
Window Size 25
Sort Options
Temp Directory
Max Records 500000
Close
Run
Messages
illumina_ex_B2_Read_bowti
.sort.bam Coverage
illumina_ex_B2_Read_bowti
.sort.bam
illumina_ex_B4_Read_bowti
.sort.bam Coverage
illumina_ex_B4_Read_bowti
.sort.bam
buc.gtf
6 tracks loaded
gn:buc:181,667
197M of 736M

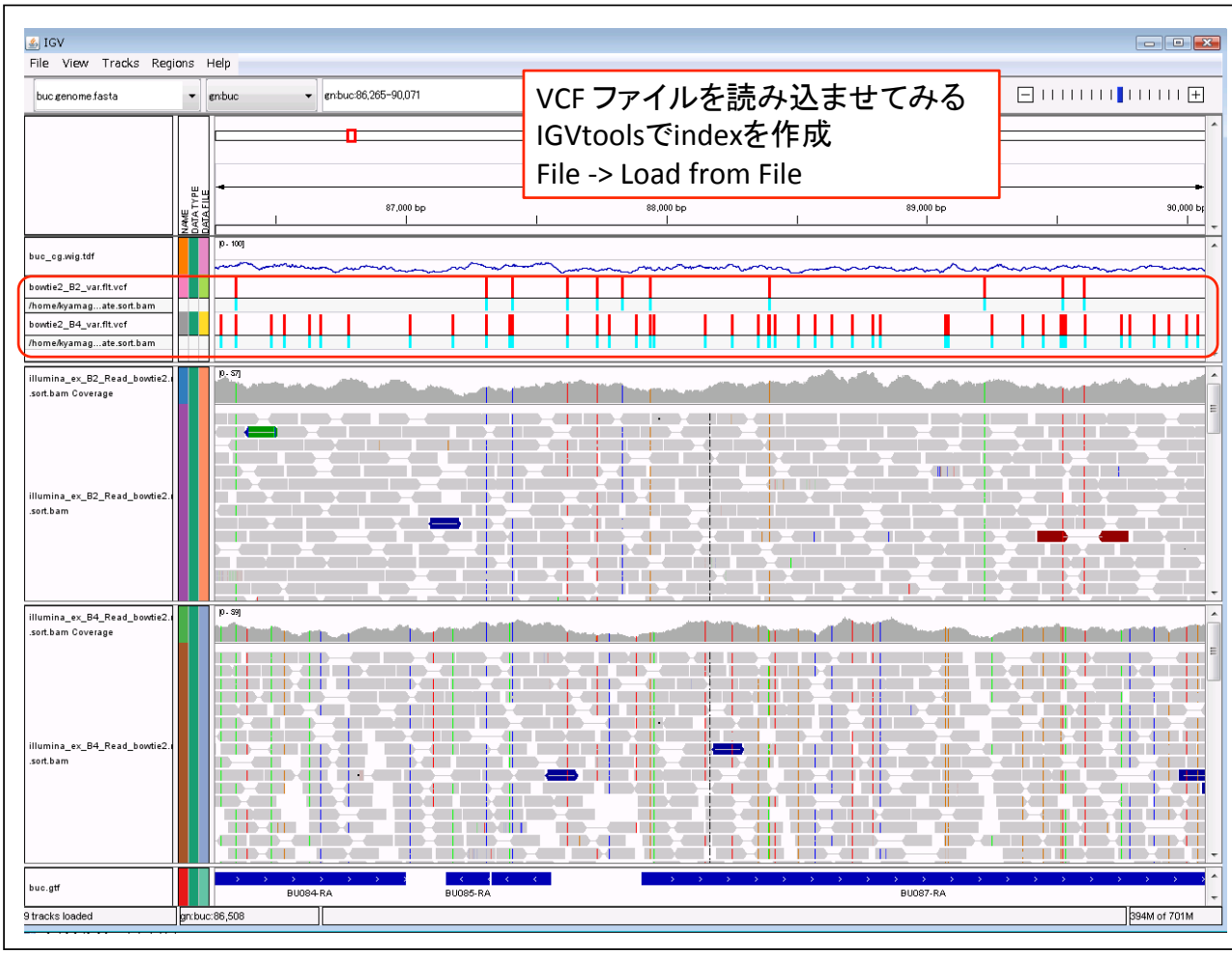

WIGファイルがバイナリー化されたtdfファイルができるので、
それをFile -> Load from file
IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:177,949-191,848
Go
188 kb
190 kb
buc_cg.wig.tdf
illumina_ex_B2_Read_bowtie2.
.sort.bam Coverage
illumina_ex_B2_Read_bowtie2.
.sort.bam
illumina_ex_B4_Read_bowtie2.
.sort.bam Coverage
illumina_ex_B4_Read_bowtie2.
.sort.bam
buc.gtf
7 tracks
gn:buc:184,355
452M of 636M

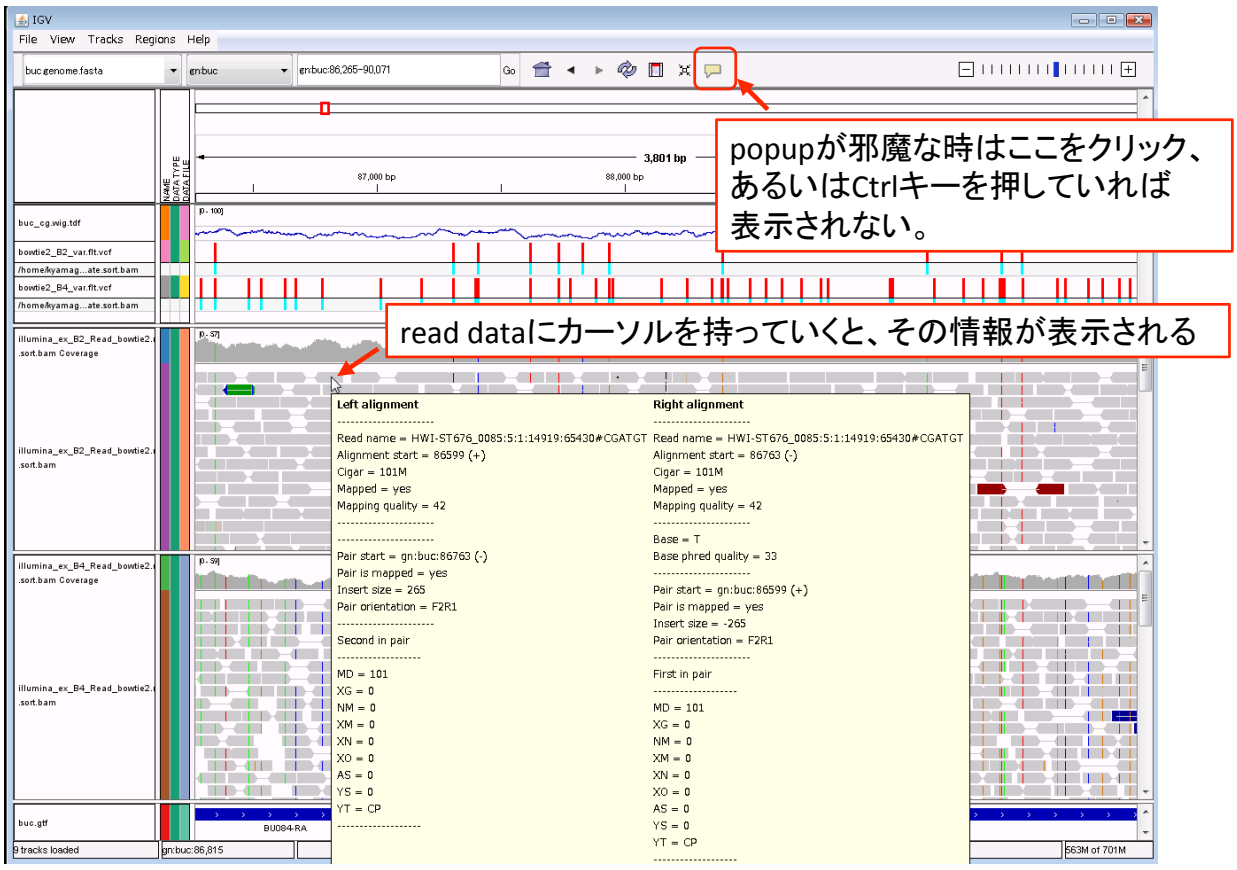

IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:177,949-191,848
Go
trackを右クリックでポップアップが出る
buc_cg.wig.tdf
Rename Track...
Change Track Color (Positive Values)...
Change Track Color (Negative Values)...
Change Track Height...
Change Font Size...
Type of Graph
Heatmap
Bar Chart
Points
Line Plot
Windowing Function
Mean
None
Set Data Range...
Set Heatmap Scale...
Log scale
Autoscale
Show Data Range
Kaplan-Meier Plot...
Remove Track
Save image...
適切な表示形式に変更する
例）
Type of Graph -> Line Plot
Windowing Function -> Set Data Range
-> min 0
-> mid 50
-> max 100
buc.gtf
7 tracks
gn:buc:178,077
506M of 636M


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:153,880-167,779
Go
13 kb
buc_cg.wig.tdf
トラックの移動
マウスのドラッグ移動が可能
← →による移動も可能
Feature trackを指定して、
Ctr+F gene model単位で右に移動
Ctr+B gene model単位で左に移動
Shift+Ctr+F exon単位で右に移動
buc.gtf
7 tracks loaded
gn:buc:153,951
418M of 630M


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
buc.genome.fasta
gn:buc
gn:buc:153,880-167,779
Go
13 kb
154 kb
156 kb
158 kb
160 kb
162 kb
164 kb
166 kb
NAME
DATA TYPE
DATA FILE
buc_cg.wig.tdf
illumina_ex_B2_Read_bowtie2.
.sort.bam Coverage
illumina_ex_B2_Read_bow
.sort.bam
illumina_ex_B4_Read_bow
.sort.bam Coverage
illumina_ex_B4_Read_bow
.sort.bam
buc.gtf
7 tracks loaded
484M of 630M
illumina_ex_B2_Read_bowtie2.mate.sort.bam
Rename Track...
Copy read details to clipboard
Group alignments by
Sort alignments by
Color alignments by
Shade base by quality
Show mismatched bases
Show all bases
View as pairs
Go to mate
View mate region in split screen
Set insert size options ...
Re-pack alignments
Show coverage track
Load coverage data...
Collapsed
Expanded
Squished
data trackを右クリックすると、
track内の表示方式変更可能
View as pairs
を選んで見る。


IGV
File View Tracks Regions Help
buc.genome.fasta
gn:buc
gn:buc:316,735-323,248
Go
317,000 bp
323,000 bp
NAME
DATA TYPE
DATA FILE
buc_cg.wig.tdf
illumina_ex_B2_Read_bowtie2.
.sort.bam Coverage
illumina_ex_B2_Read_bowtie2.
.sort.bam
illumina_ex_B4_Read_bowtie2.
.sort.bam Coverage
illumina_ex_B4_Read_bowtie2.
.sort.bam
buc.gtf
BU289-RA
BU290-RA
BU291-RA
BU292-RA
BU293-RA
BU294-RA
7 tracks
gn:buc:318,057
254M of 493M
pair endを維持した表示になる
insertion, deletionを見たい場合はこちらが良い。


VCF ファイルを読み込ませてみる
IGVtoolsでindexを作成
File -> Load from File


popupが邪魔な時はここをクリック、
あるいはCtrlキーを押していれば
表示されない。
read datalにカーソルを持っていくと、その情報が表示される

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。