Atelier scRNA-seq

Check your signal with a genome browser
IGV

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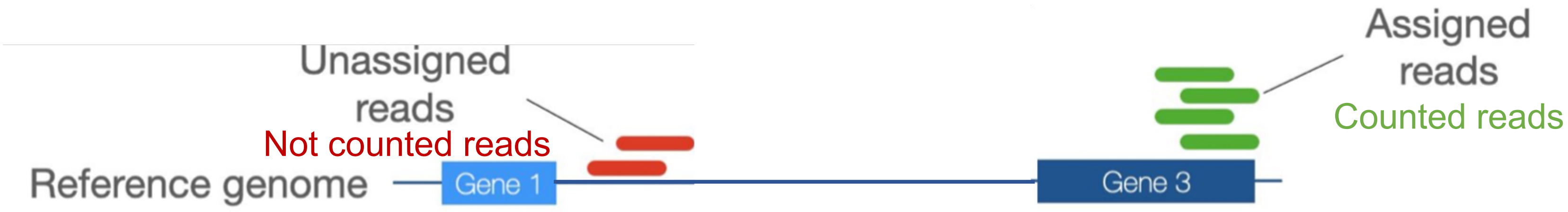
Organisation of the scRNA-seq course

- From cells to nucleotide sequences (reads)
  - focus on the 10X genomics technology
  - how are the reads organised
- Preprocessing : from reads to raw count matrix
  - quality check (FASTQC)
  - mapping (STAR)
  - how is annotation used
  - barcode and UMI treatment
  - visualizing the reads
  - constructing the count matrix
  - call cells / empty droplets filtering

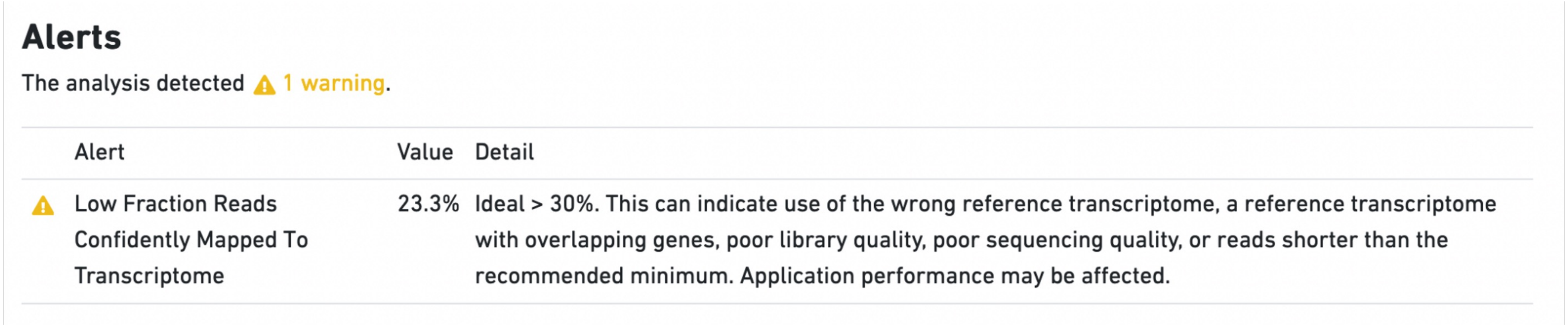
Single cell analysis is about counts
so why visualizing the reads in a genome browser ?

- You do not understand the counts on your favourite gene ?
- The global results look weird ?

→ Your reads may not overlap the gene positions…



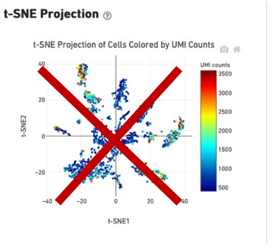
Bad counts result in poor and even fake results



Alerts

The analysis detected ⚠ 1 warning.

	Alert	Value	Detail
⚠	Low Fraction Reads Confidently Mapped To Transcriptome	23.3%	Ideal > 30%. This can indicate use of the wrong reference transcriptome, a reference transcriptome with overlapping genes, poor library quality, poor sequencing quality, or reads shorter than the recommended minimum. Application performance may be affected.

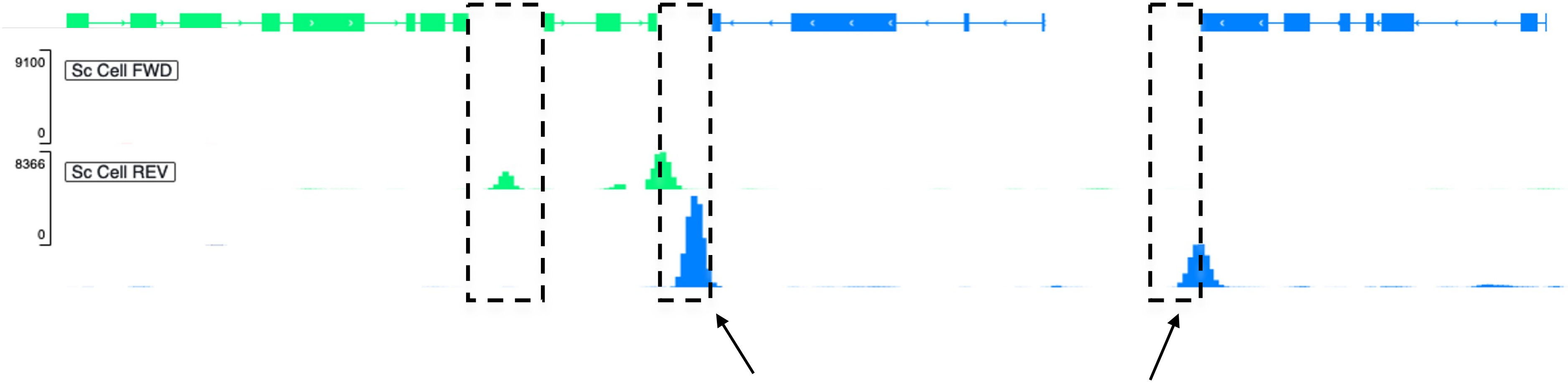


→ The clusters are then based on a very limited amount of reads and will not be reliable

→ If the counted reads are low, the estimated number of cells will be smaller (relies on a smaller amount of BC+UMIs)

What is not annotated cannot be counted

- ❖ If the **gene annotations** are **deduced from the protein annotations, the 5’ and 3’ UTRs** (10x data) are **not included**
- ❖ If you work on a **not so popular model organism** or **cancer data, annotations may not fit** your data
  - ➢ It’s clearly **an issue for single cell analyses**



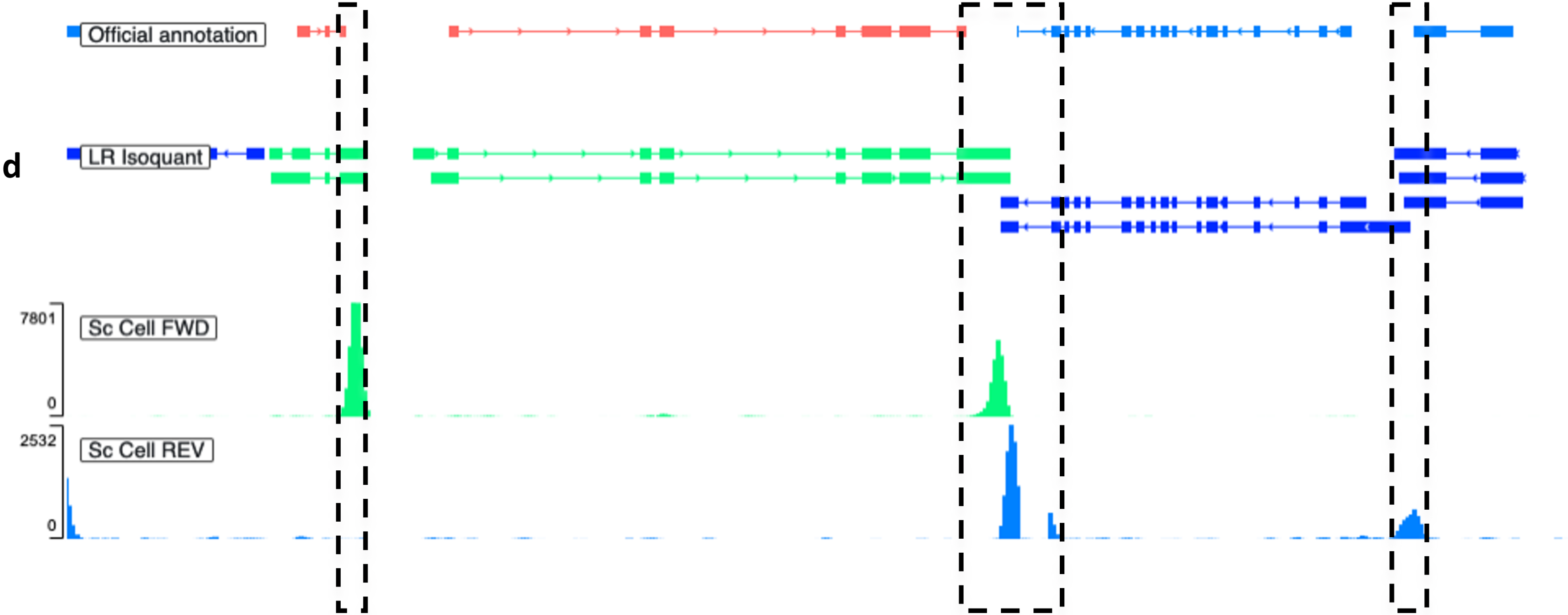
Only the reads out of the rectangles are counted
→ Most of the signal is excluded

Compute a new annotation using bulk RNASeq data (short and long reads)

You can build a **new annotation** using either **long or/and short read protocoles** (but stranded if possible) and tools such as **Isoquant**, **Stringtie2,** etc…

Computed annotations from long read RNASeq

SingleCell Signal (FWD/REV)

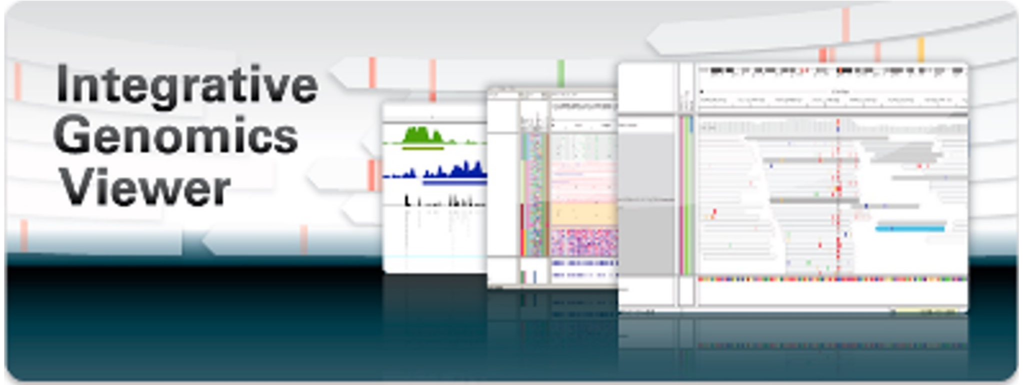


Before and after re-annotation analyses

	Official annotation	**Isoquant annotation**
Estimated Number of Cells	**2114**	**2624**
Reads Mapped to Genome	82	82
Reads Mapped Confidently to Genome	79,9	80,2
Reads Mapped Confidently to Intergenic Regions	**46,3**	**11,4**
Reads Mapped Confidently to Intronic Regions	3,6	1,4
Reads Mapped Confidently to Exonic Regions	**30**	**67,4**
Reads Mapped Confidently to Transcriptome	**23,3**	**66,1**
Reads Mapped Antisense to Gene	0,5	2

Integrative Genome viewer (IGV) is the most popular Genome Browser

- IGV is a java **multiplatform tool** : It will work under **Linux, macOSX and Windows**
- IGV is **open, free, lively** and maintained at the Broad Institute



IGV is available in multiple forms

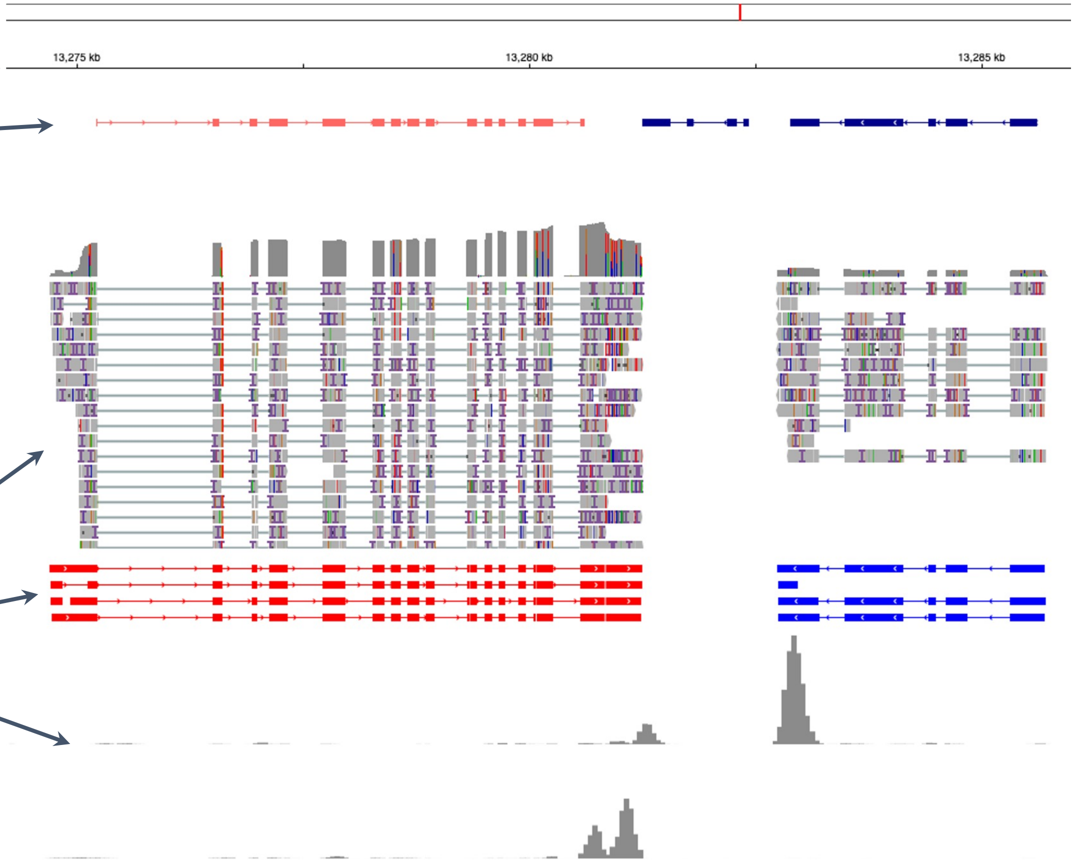
- **the original IGV** - a Java **desktop application**
- **IGV-Web** - a **web application**

https://igv.org

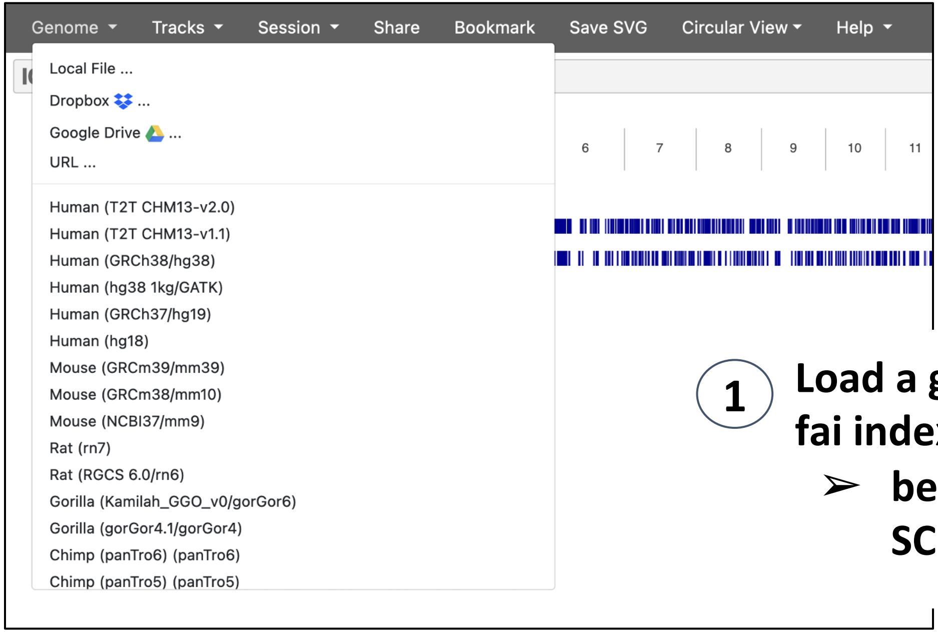
What do you need to use IGV ?

- A **reference** genome (fasta file)
- An **annotation** file (gtf or gff file)
  - Already used to perform your SC analysis

- The files resulting from **your alignements**
  - **bam** files (and bai index files)
  - **bed** files (read position files)
  - **bedgraph** files (coverage files)

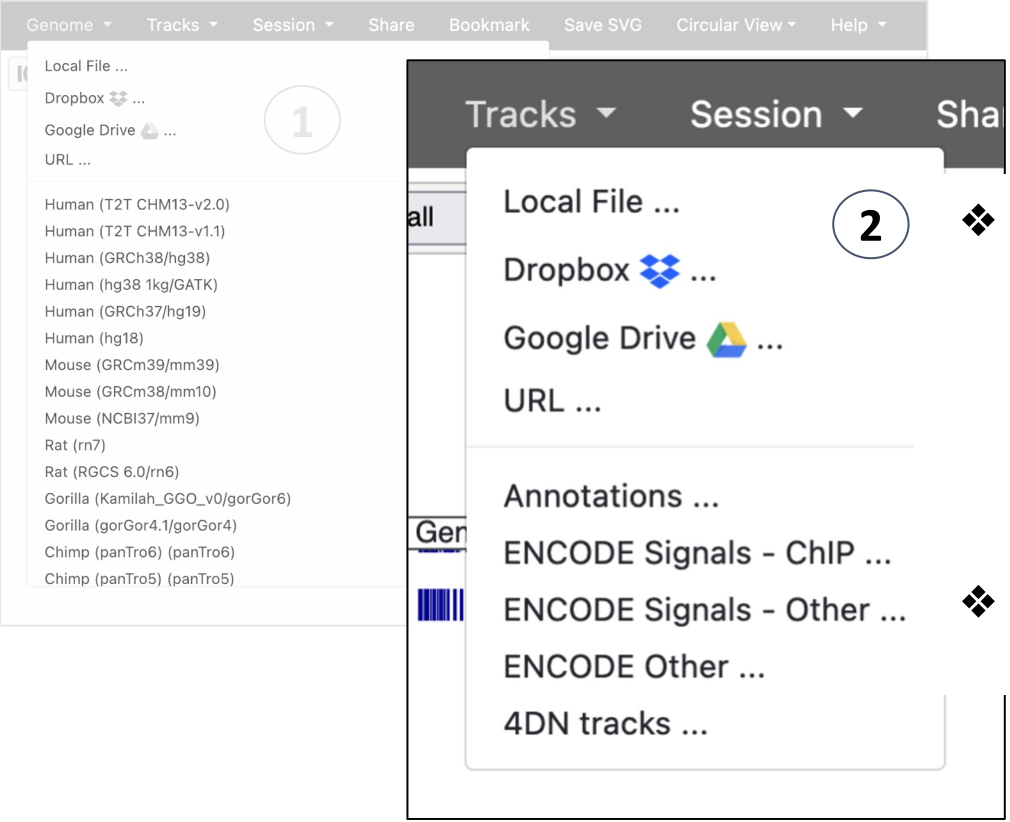


How to begin with IGV ?



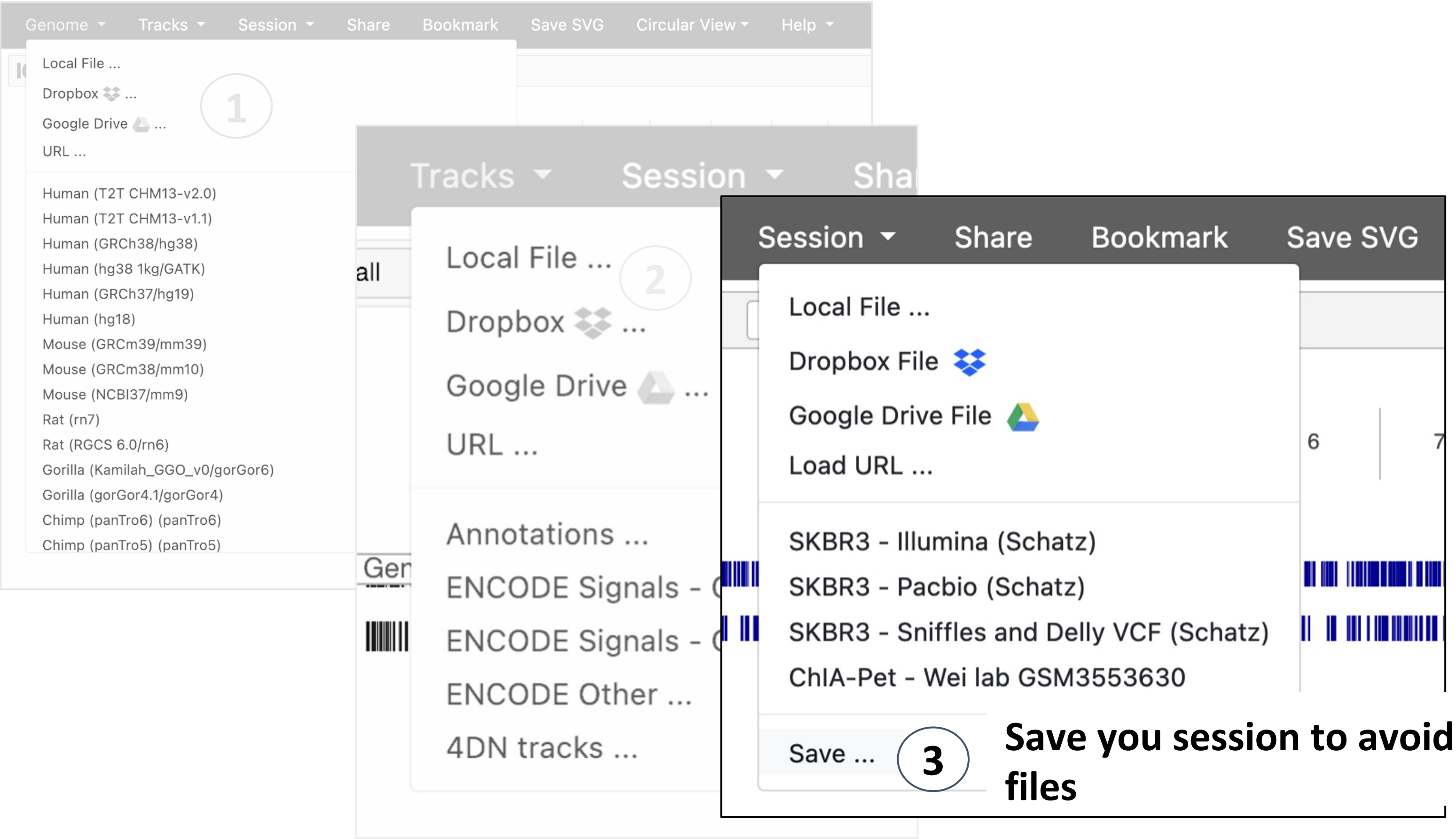
1. **Load a genome from the list or upload a fasta file (with fai index file)**
   - ➢ **be sure it’s the same as the genome used for your SC analysis**

How to begin with IGV ?



- ❖ Load an annotation from the list or upload a gtf file
  - ➢ Again be sure it’s the same as the annotation used for your SC analysis
  - ➢ Be sure it goes with your genome file (Chromosome name…)
- ❖ Load your bam, bed, bedgraph files

How to begin with IGV ?



Save you session to avoid reloading your files

References

Single cell analysis failure and gene annotation

- Pool, AH., Poldsam, H., Chen, S. *et al.* Recovery of missing single-cell RNA-sequencing data with optimized transcriptomic references. *Nat Methods* **20**, 1506–1515 (2023).

Isoquant

- Prjibelski, A.D., Mikheenko, A., Joglekar, A. *et al.* Accurate isoform discovery with IsoQuant using long reads. *Nat Biotechnol* **41**, 915–918 (2023)
- https://github.com/ablab/IsoQuant

Stringtie2

- Shumate A, Wong B, Pertea G, Pertea M Improved transcriptome assembly using a hybrid of long and short reads with StringTie, *PLOS Computational Biology* 18, 6 (2022)
- https://github.com/gpertea/stringtie

IGV

- 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)
- https://igv.org/doc/desktop/