VARIANT CALLING: Detecting Mutations in Bacterial Genomes

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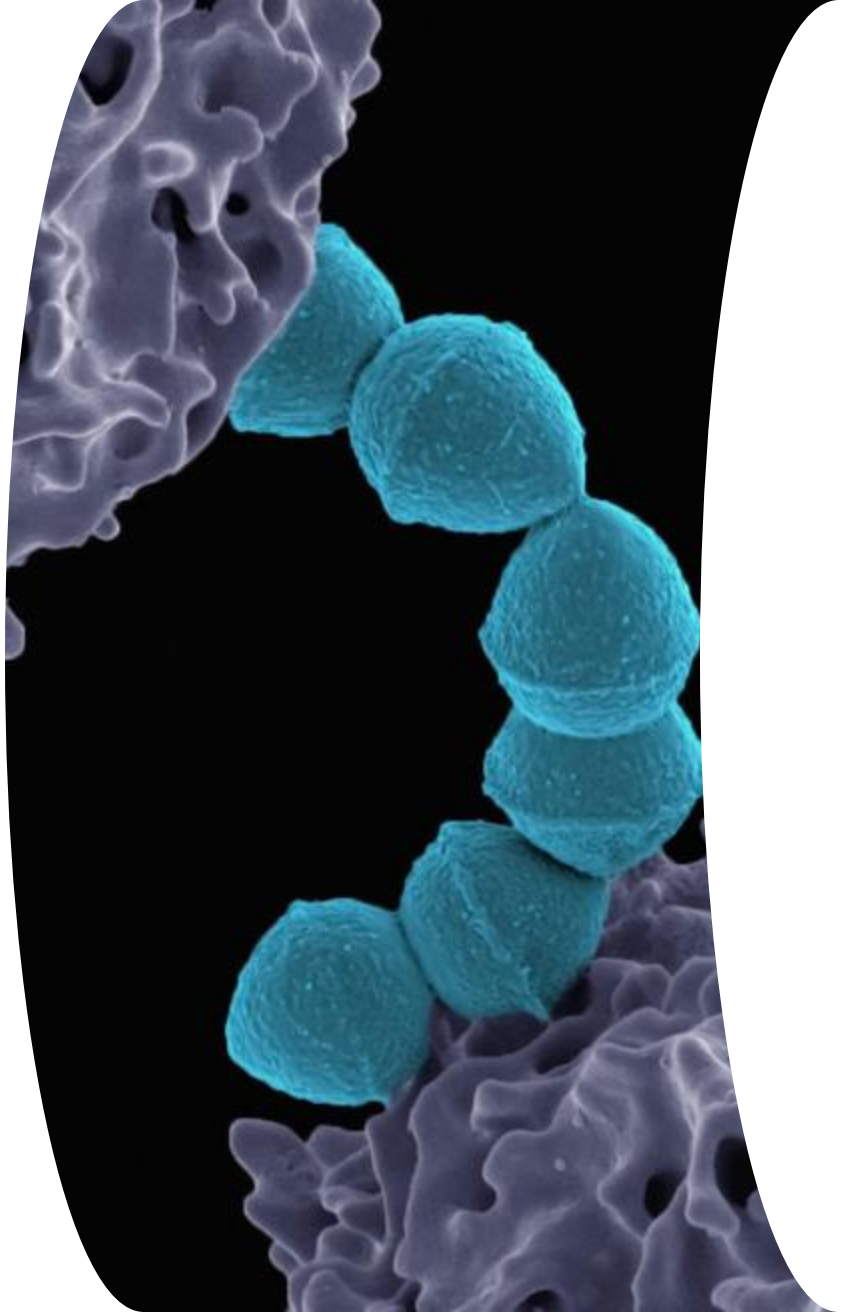


Streptococcus Pyogenes
Photo by National Institute of Allergy and Infectious Diseases on Unsplash

Background

- Next-generation sequencing (NGS) technologies have transformed genomic research by providing high-throughput sequencing capabilities with unparalleled speed and accuracy.
- The generated sequencing reads hold the key to unlocking valuable insights into genetic variations and disease mechanisms.
- The raw sequencing data from NGS platforms requires sophisticated computational analysis to extract meaningful information.

https://pharmafeatures.com/cracking-genetic-ciphers-secondary-ngs-analysis/

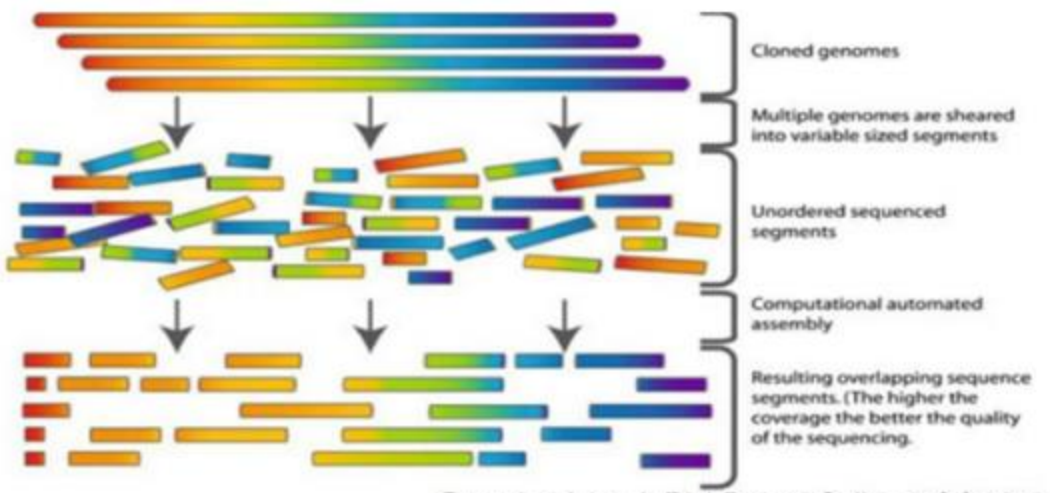
Background

- Secondary or Downstream Analysis
- Read alignment and variant calling are *critical steps that bridge the gap between raw data and actionable genetic insights*.
- By understanding the complexities and nuances of these analysis techniques, researchers can make informed decisions to harness the full potential of NGS data.

https://pharmafeatures.com/cracking-genetic-ciphers-secondary-ngs-analysis/

Learning Objectives

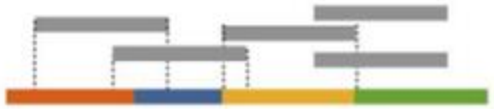
- Learn the principles and methods of detecting genetic variations in bacterial genomes.
- Explore tools and software for variant calling
- Gain knowledge of variant calling and its applications.
- Learn about challenges in variant calling.



Commins J. et al, Biol Proced Online 11(1) 2015

Mapping to reference sequence

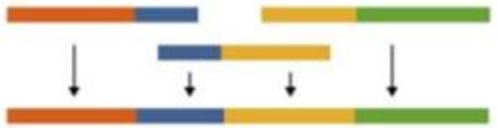
Recreate the genome with using prior knowledge as reference



Mapping is as good as reference used

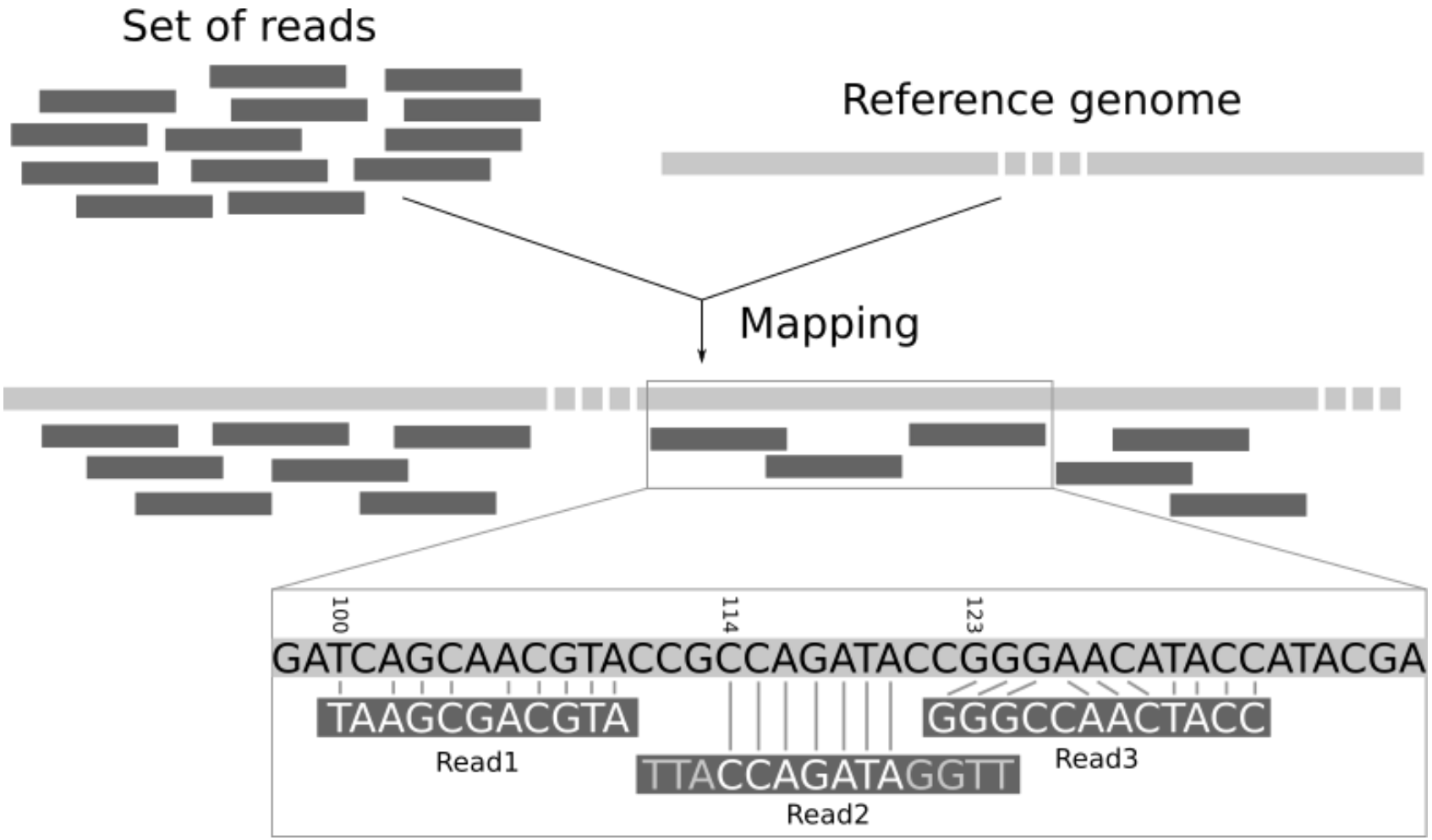
De Novo assembly

Recreate the genome with no prior knowledge



Problem with repeated regions, high coverage and long

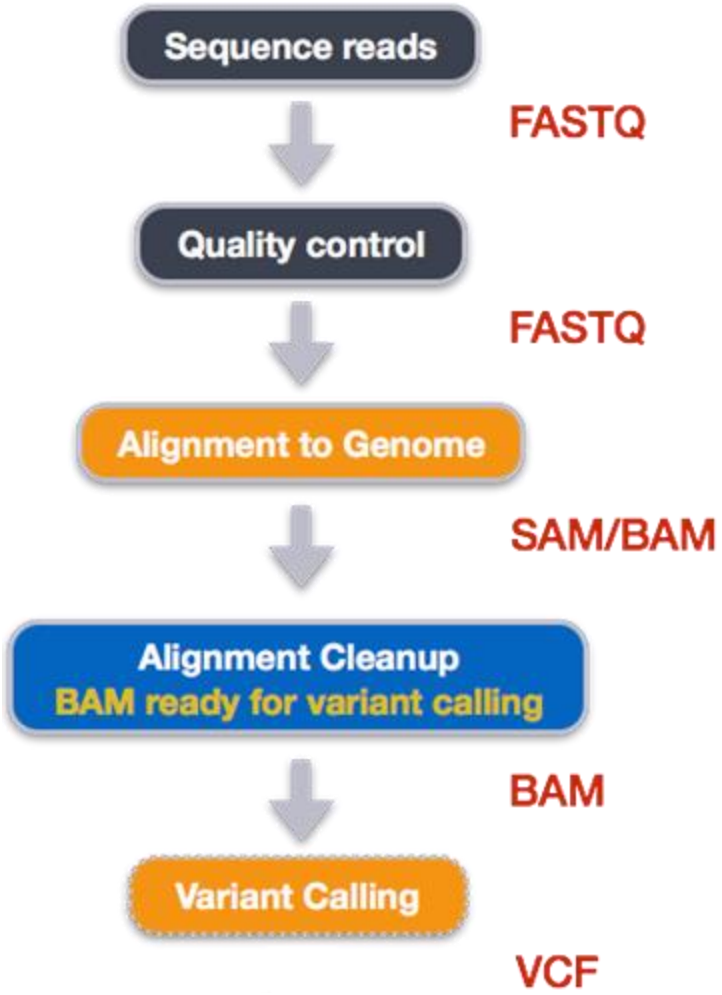
https://pharmafeatures.com/cracking-genetic-ciphers-secondary-ngs-analysis/



Review Read Mapping principles from our earlier ACORN course:
https://zenodo.org/records/12805691

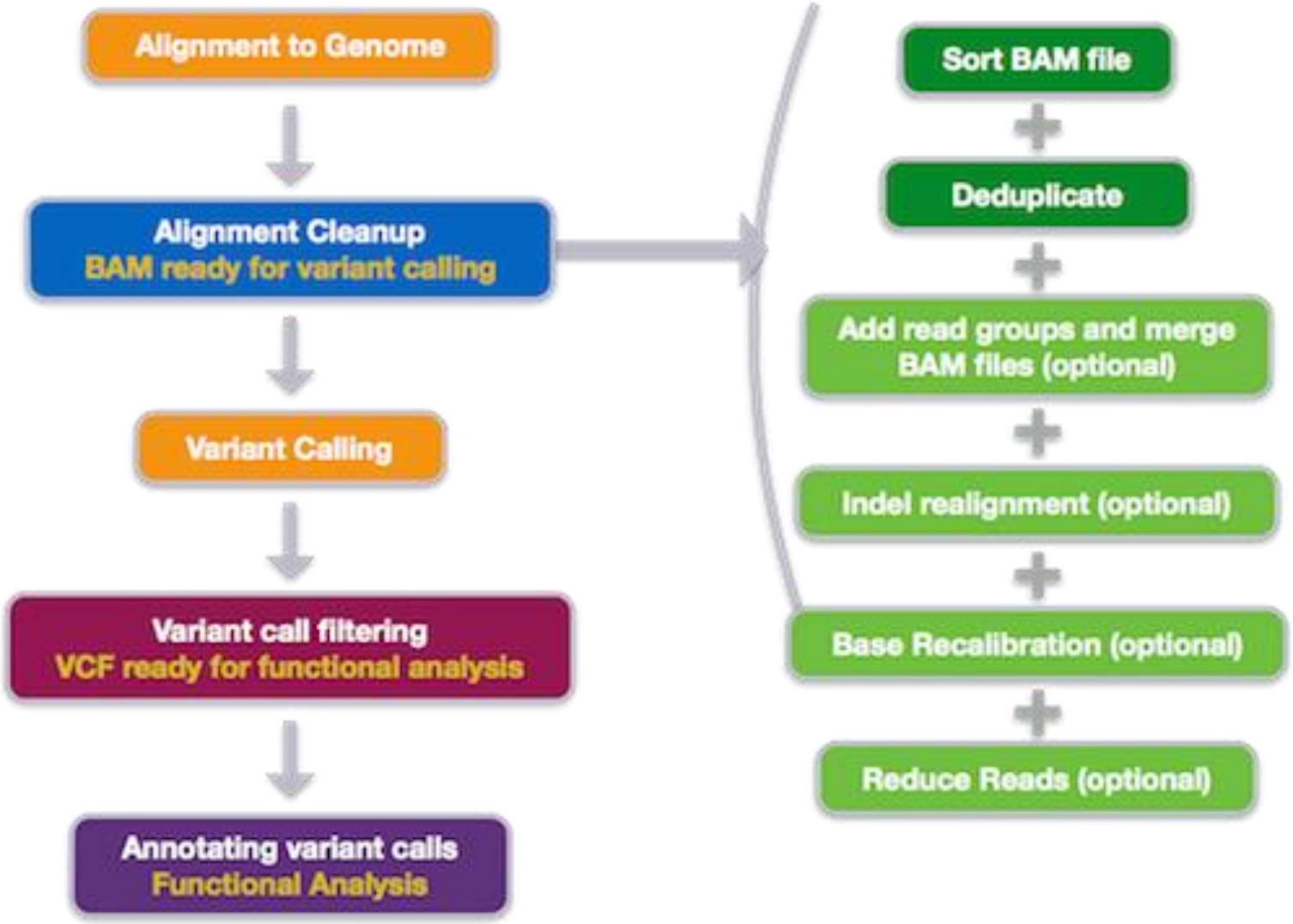
https://training.galaxyproject.org/training-material/topics/sequence-analysis/tutorials/mapping/tutorial.html

Variant Calling Workflow



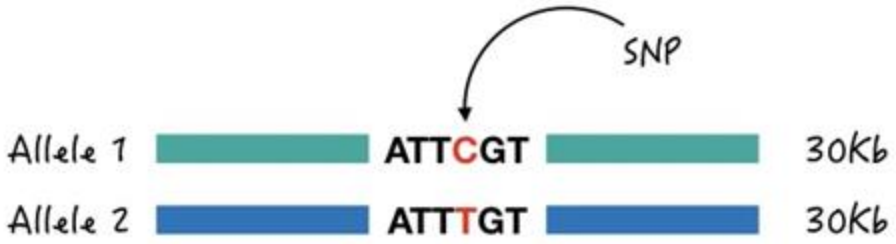
https://datacarpentry.org/wrangling-genomics/04-variant_calling.html

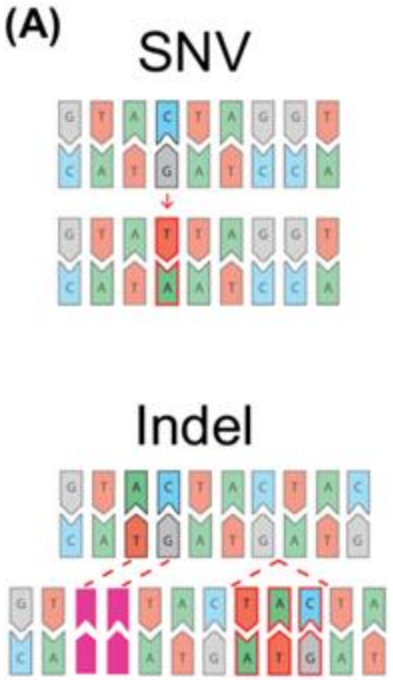
Variant Calling Workflow

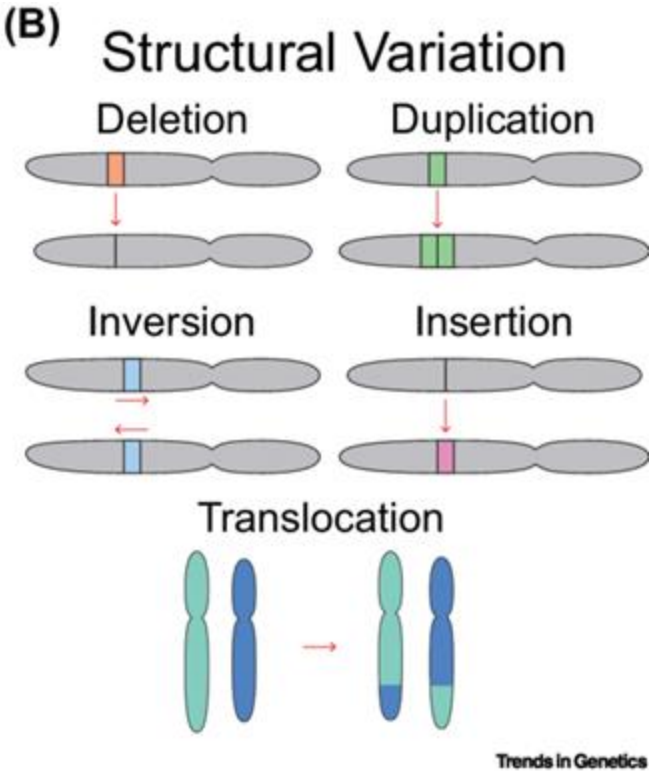


https://hbctraining.github.io/In-depth-NGS-Data-Analysis-Course/sessionVI/lessons/01_alignment.html

SNV/SNP vs SV







https://geneticeducation.co.in/snp-vs-snv/
https://www.researchgate.net/publication/346898467_Hotspots_of_Human_Mutation/figures?lo=1

SNPs

Transitions (Ti): Point mutations where a purine (adenine, A, or guanine, G) is replaced by another purine, or a pyrimidine (cytosine, C, or thymine, T) is replaced by another pyrimidine. Thus, there are two types of transitions:

- **A ↔ G** (purine to purine)
- **C ↔ T** (pyrimidine to pyrimidine)

Transversions (Tv): Point mutations where a purine is replaced by a pyrimidine or vice versa. There are four possible transversions:

- **A ↔ C**
- **A ↔ T**
- **G ↔ C**
- **G ↔ T**

$$\text{Ti/Tv ratio} = \frac{\text{Number of Transitions (Ti)}}{\text{Number of Transversions (Tv)}}$$

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.

Ti/Tv > 1

Evolutionary Implications

- **Indicator of Evolutionary Conservation:** A high Ti/Tv ratio might indicate that a sequence has undergone relatively few mutational changes over time or that there is strong selective pressure to maintain certain genetic sequences. In such cases, transitions are more likely to be retained because they often result in less functional change (e.g., synonymous substitutions or conservative amino acid changes).
- **Reflects Mutation and Repair Biases:** The ratio greater than 1 suggests that the natural mutation processes and DNA repair mechanisms in the organism tend to favor the occurrence or retention of transitions over transversions.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.

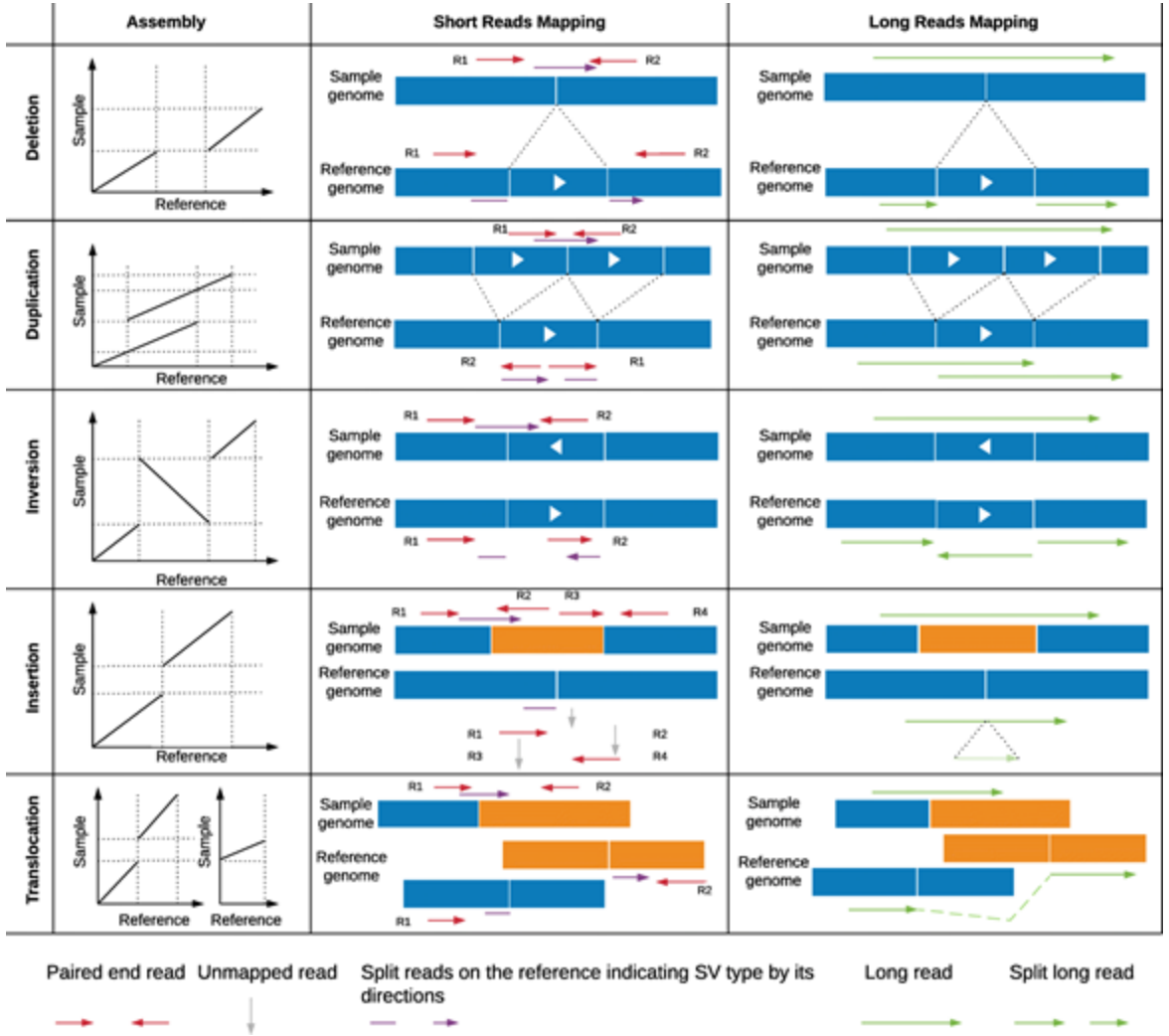
Ti/Tv < 1

Evolutionary Implications

- **Unusual Evolutionary Scenarios**: A Ti/Tv ratio less than 1 might be seen in unusual evolutionary scenarios, such as:
  - **Species or Population with Unusual Mutation Patterns**: Some organisms or specific populations may have mutational biases that lead to a higher rate of transversions. For example, some viruses or bacteria that have been exposed to certain mutagens may show such patterns.
  - **Extreme Selective Pressures**: Environments with extreme selective pressures (e.g., high levels of radiation or chemical exposure) may lead to increased transversion rates if those mutations provide some survival advantage or occur more frequently due to specific types of DNA damage.
- **Recent Rapid Evolution or Divergence**: If the sequence under study represents a lineage that has recently undergone rapid evolution or divergence, the Ti/Tv ratio might temporarily be lower than expected due to unique evolutionary pressures or recent changes in mutational processes.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.

De novo assembly, short-read and long-read mapping approaches to identify structural variants



Genome Biology **volume 20**, Article number: 246 (2019). DOI: https://doi.org/10.1186/s13059-019-1828-7.

Variant Calling Workflow: Summary

1. Get Illumina / Nanopore data – processed BAM files
2. Call SNPs & indels with **FreeBayes**
3. Call SNPs & indels with **BCFtools**
4. Screening SNPs & indels with **BCFtools** & **vcfutils.pl**
5. Visualise data with **IGV**
6. Evaluate VCF files

Metrics

Tech	Illumina				Nanopore			
Sample	ERR9975619	ERR9975619	ERR9975619	ERR9975619	ERR6319263	ERR6319263	ERR6319263	ERR6319263
Mapper	Minimap2	Minimap2	Bwa	Bwa	Minimap2	Minimap2	Bwa	Bwa
SNPcaller	BCFtools	FreeBayes	BCFtools	FreeBayes	BCFtools	FreeBayes	BCFtools	FreeBayes
Ti	41	36	43	37	24	1438	24	1469
Tv	15	18	17	18	14	104	14	100
Ti/Tv	2.7	2.0	2.5	2.1	1.7	13.8	1.7	14.7
Insertion	2	3	2	2	4	427	3	410
Deletion		8		8		1076		1053
SNP	56	56	60	57	38	1681	38	1053
Complex	NA	3	NA	3	NA	102	NA	98

Example results for 2 samples with differing mappers & SNP callers

Evaluate VCF files

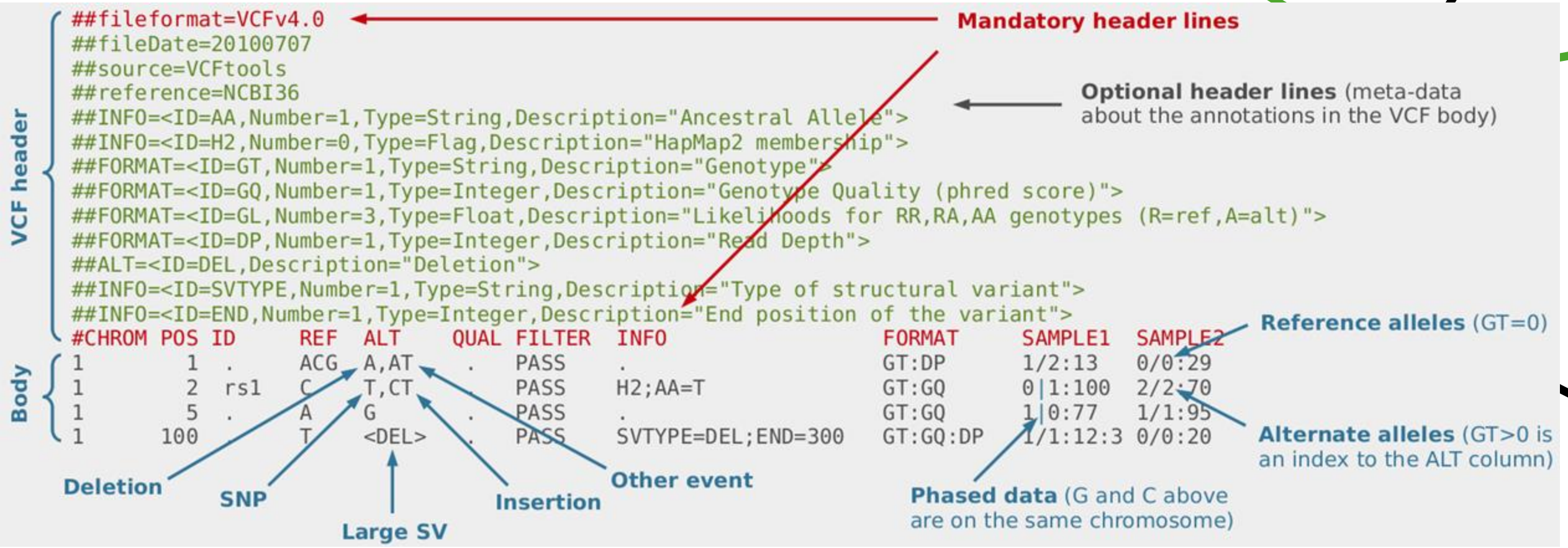
Variant Call Format (VCF) files are the standard way of representing mutation data

Developed originally in 2008-10 for 1000 Genomes Project

VCF files vary – exact info depends on SNP caller used to make them, and era (VCF file versions)

https://www.nature.com/articles/s41525-022-00295-z

Evaluate VCF files



https://www.nature.com/articles/s41525-022-00295-z

Evaluate VCF files

The header info has some valuable info, eg for depth DP:

```
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth of reads passing MAPQ filter">
```

```
##INFO=<ID=AC,Number=R,Type=Integer,Description=”Total number
  of alleles in called genotypes">
```

https://www.nature.com/articles/s41525-022-00295-z

Evaluate VCF files

First 34 lines = column info for SNPs
lines 35+ = SNPs (one per line)

"DP" at start of lines with SNPs

```
grep -c "DP" *.vcf
```

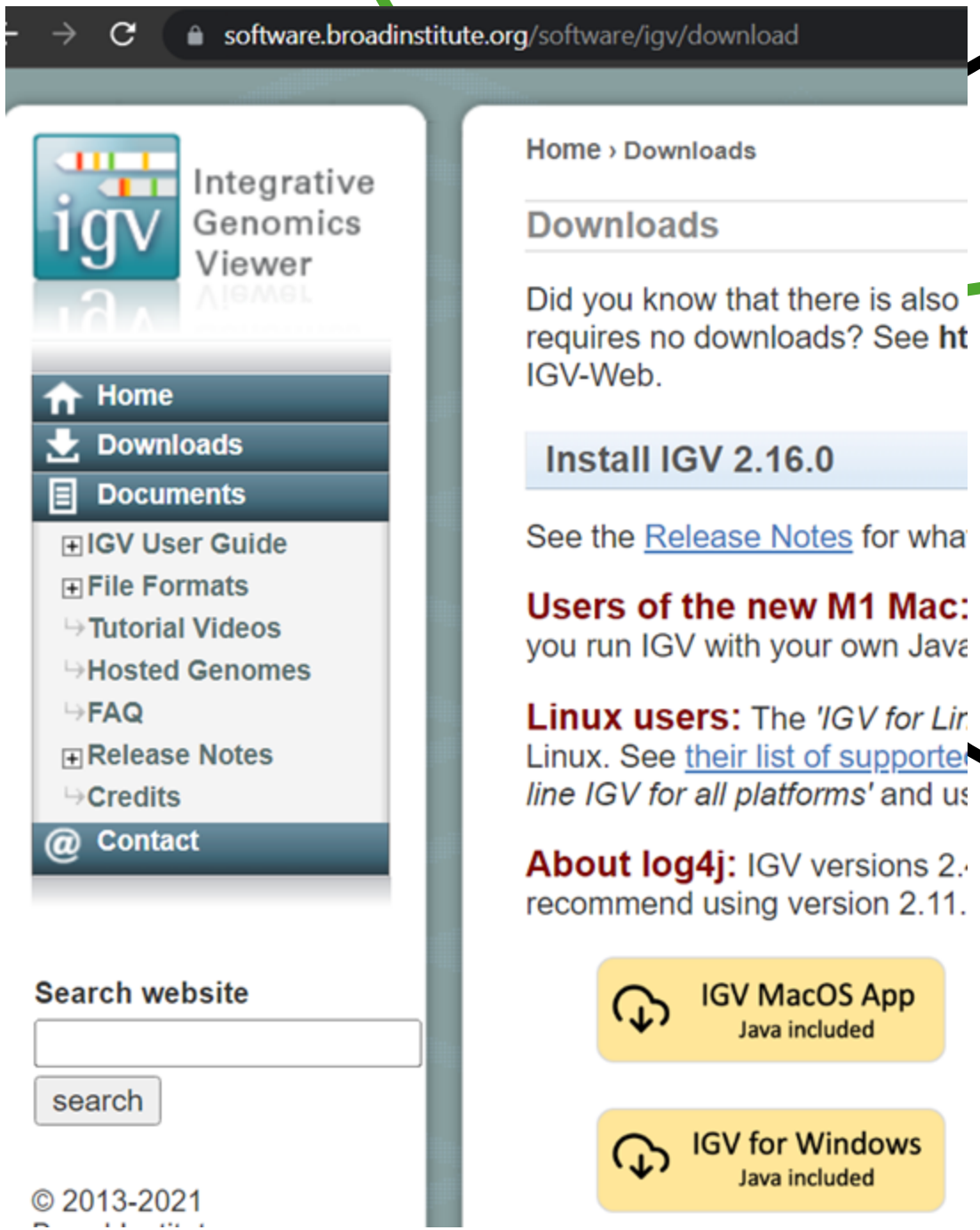
Sample	#SNPs
G3682.vcf	6
G3735.vcf	18
G3771.vcf	18
NoName.vcf	17

https://www.nature.com/articles/s41525-022-00295-z

Visualisation: IGV

Download IGV (with Java)
Install it on your own computer

See: https://software.broadinstitute.org/software/igv/download



Visualisation: IGV

Alternatively:

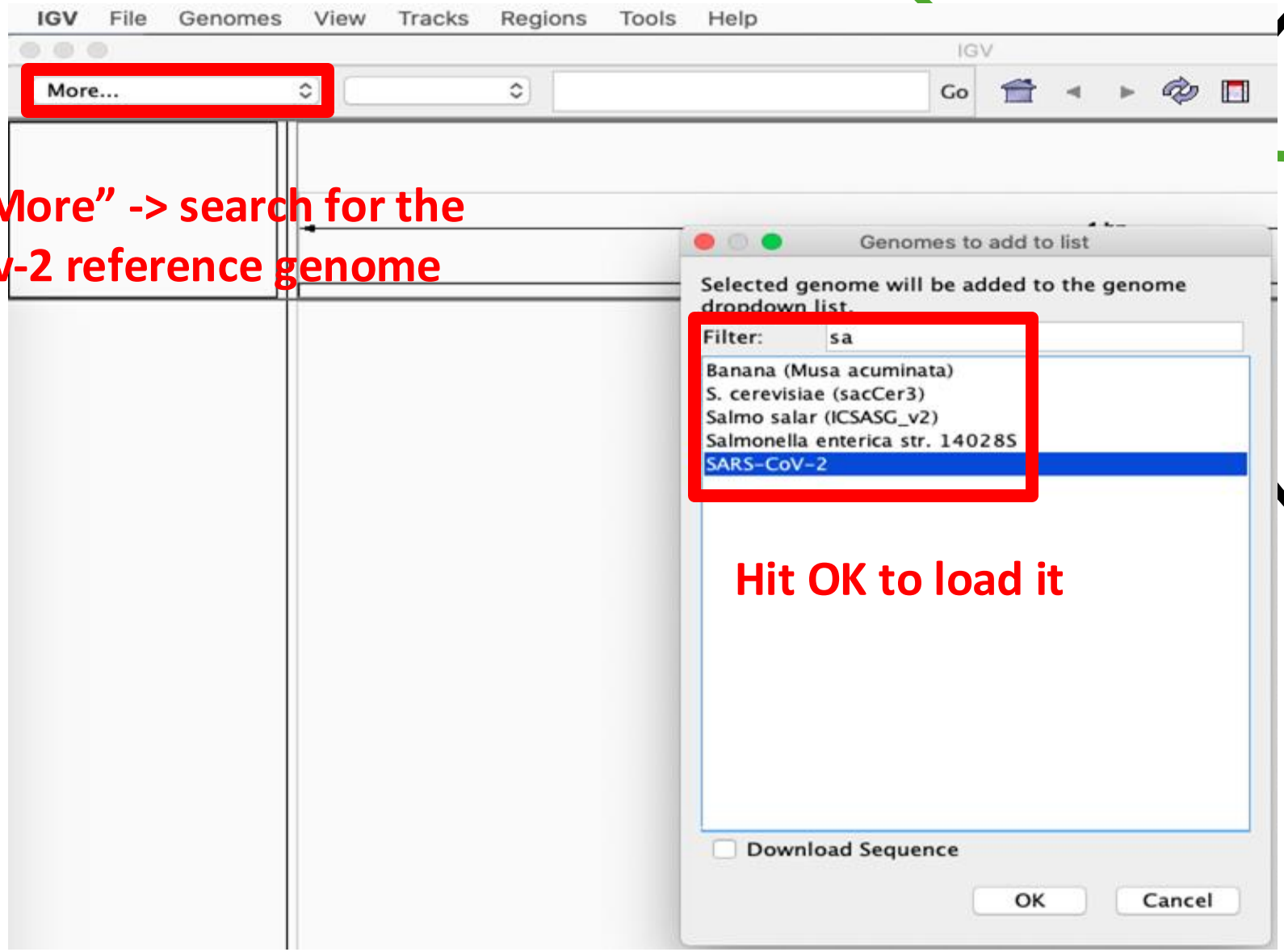
Install it locally in your server area:

```
wget https://data.broadinstitute.org/igv/projects/downloads/2.16/IGV_2.16.1.zip

unzip IGV_2.16.1.zip

cd IGV_2.16.1/

./igv.sh
```

Visualisation: IGV

Launch IGV application

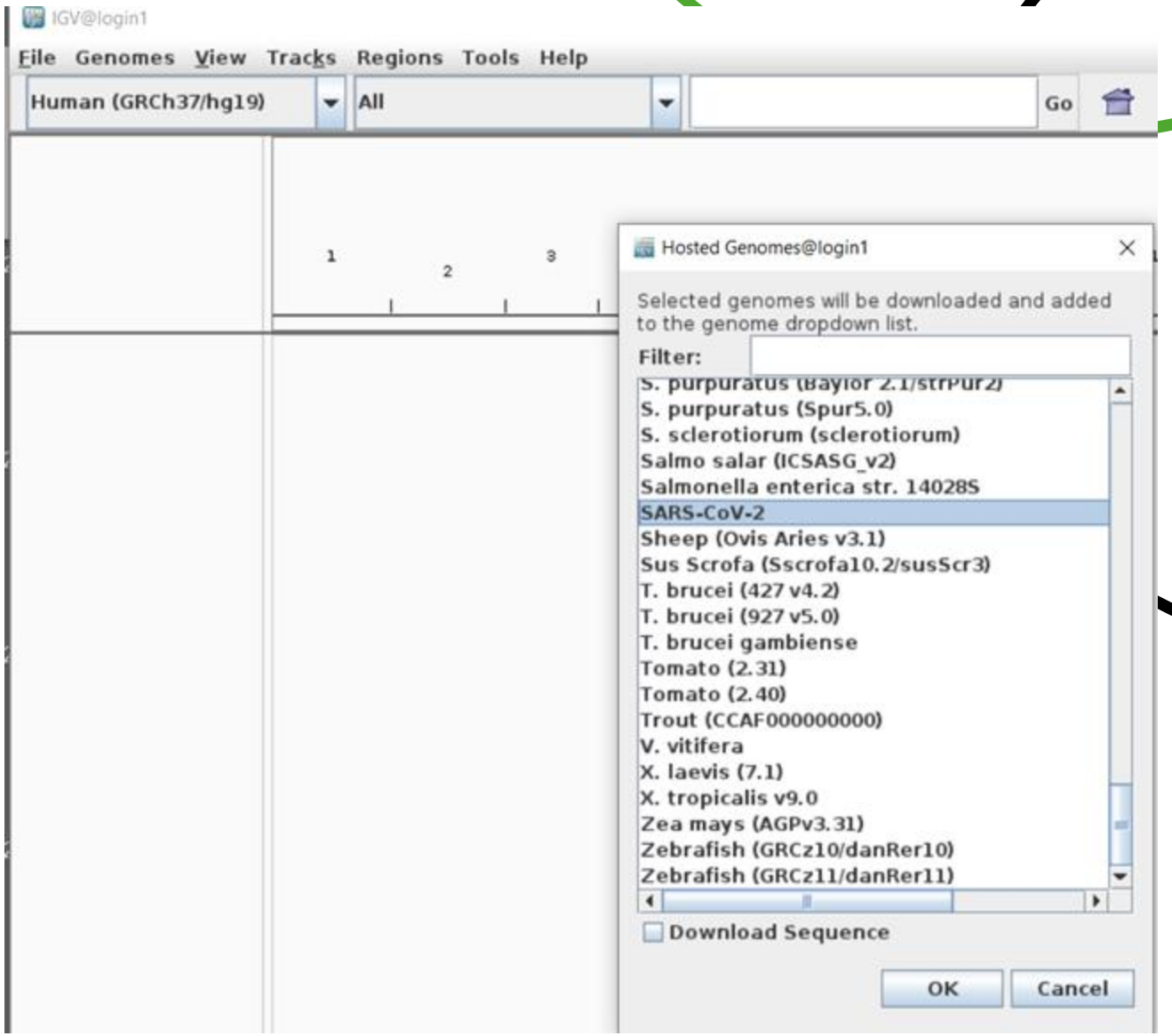
Go to “More” -> search for the Sars-Cov-2 reference genome



Visualisation: IGV

Go to “File” and select the reference genome you want

e.g. Sars-Cov-2

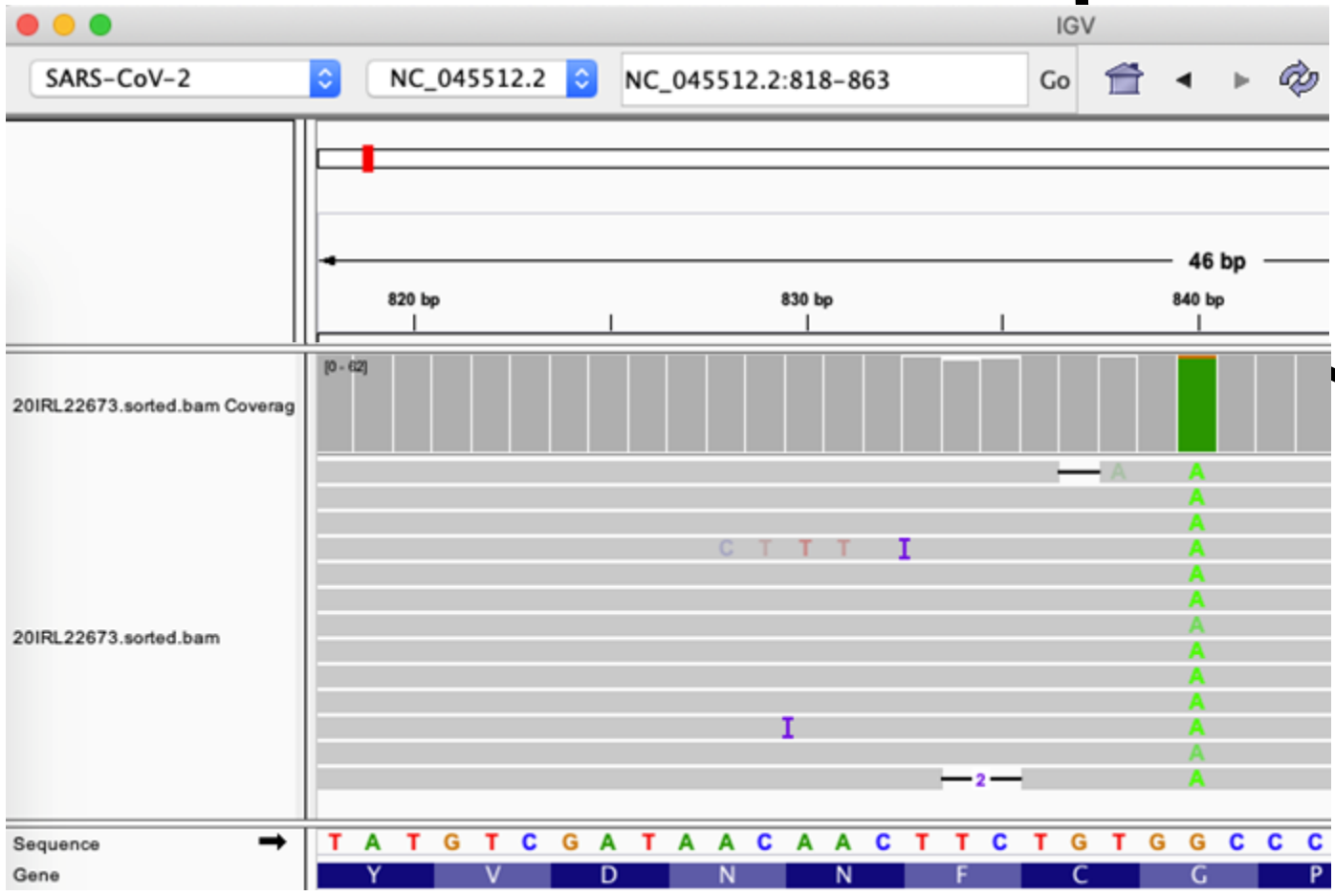


Visualisation: IGV

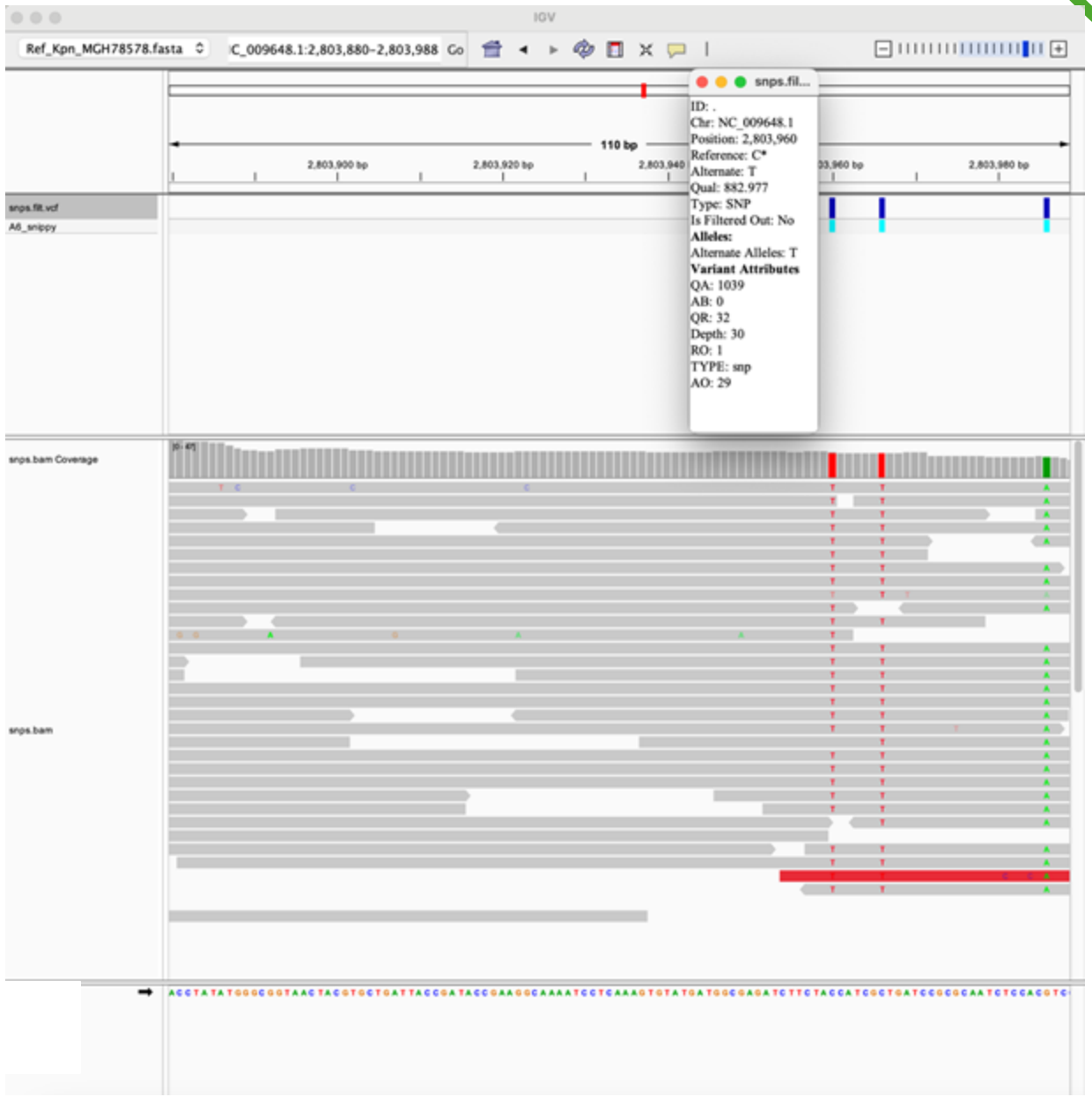
Check some mutations, e.g.:

Go to the sites
eg 840 here
GGC -> GAC
Gly -> Asp

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
MN908947.3	840	.	G	A	500	PASS	DP=62;A



Visualisation: IGV



Visualisation: IGV

More details during Tutorial Session

Tutorial (Make a copy or Download):
https://colab.research.google.com/drive/1zorsPRI2ioDcfH6QgBVDaSpM2yVtcjtk#scrollTo=593cOtwoO4RO

Quick guide:
https://github.com/aedecano/ACORN_Variant_Calling