Short Read Alignment
&
Variant Calling

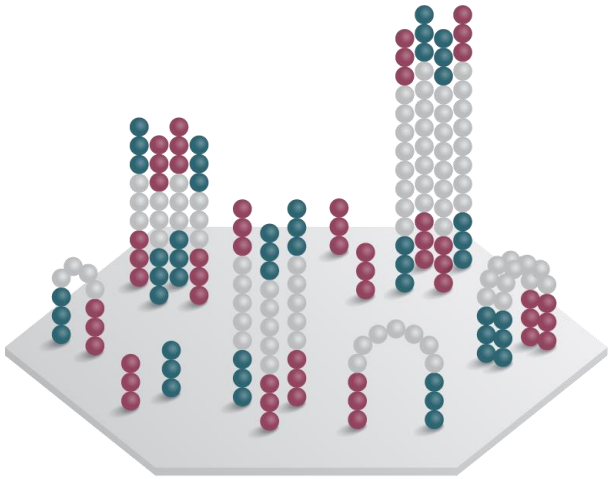
- Loo...ts of **Google/ChatGPT** search
- Proper **data** & **directory** management
- Perform **background study**
- Be **persistent**

Workflow


Short Read Alignment
Refine Alignment
Variant Calling
Variant Filtration
Annotation

Short Read Alignment

Basic Concepts and Common Terminologies



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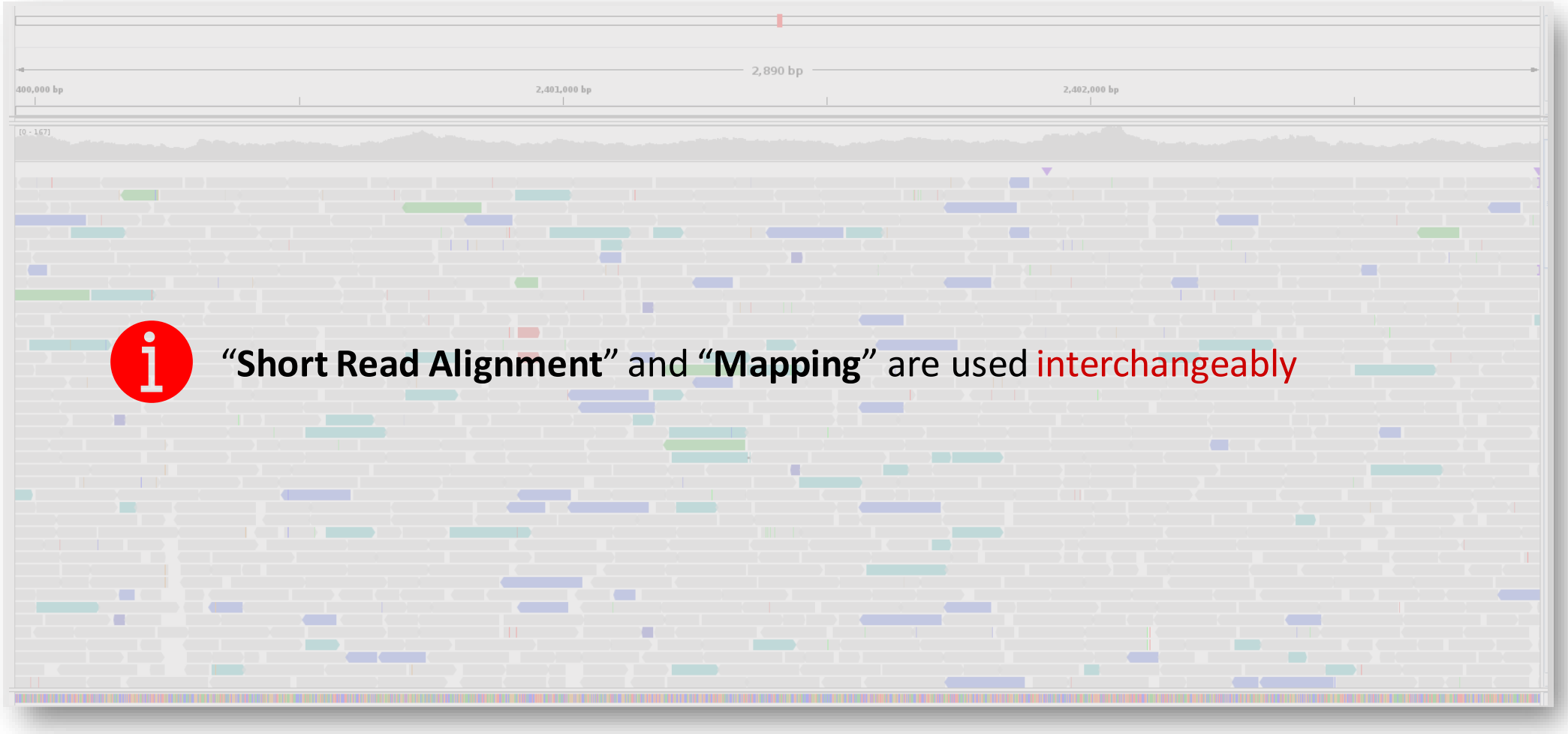
Short Read Alignment / Mapping

Determine the most likely **location** and **orientation** of each read within the reference sequence.



Commonly Used Tools...

bwa

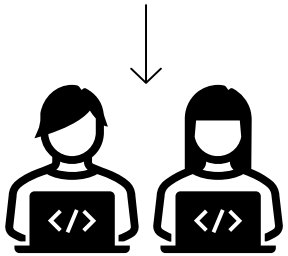
minimap2

BWA-MEM2

bowtie2

HISAT2

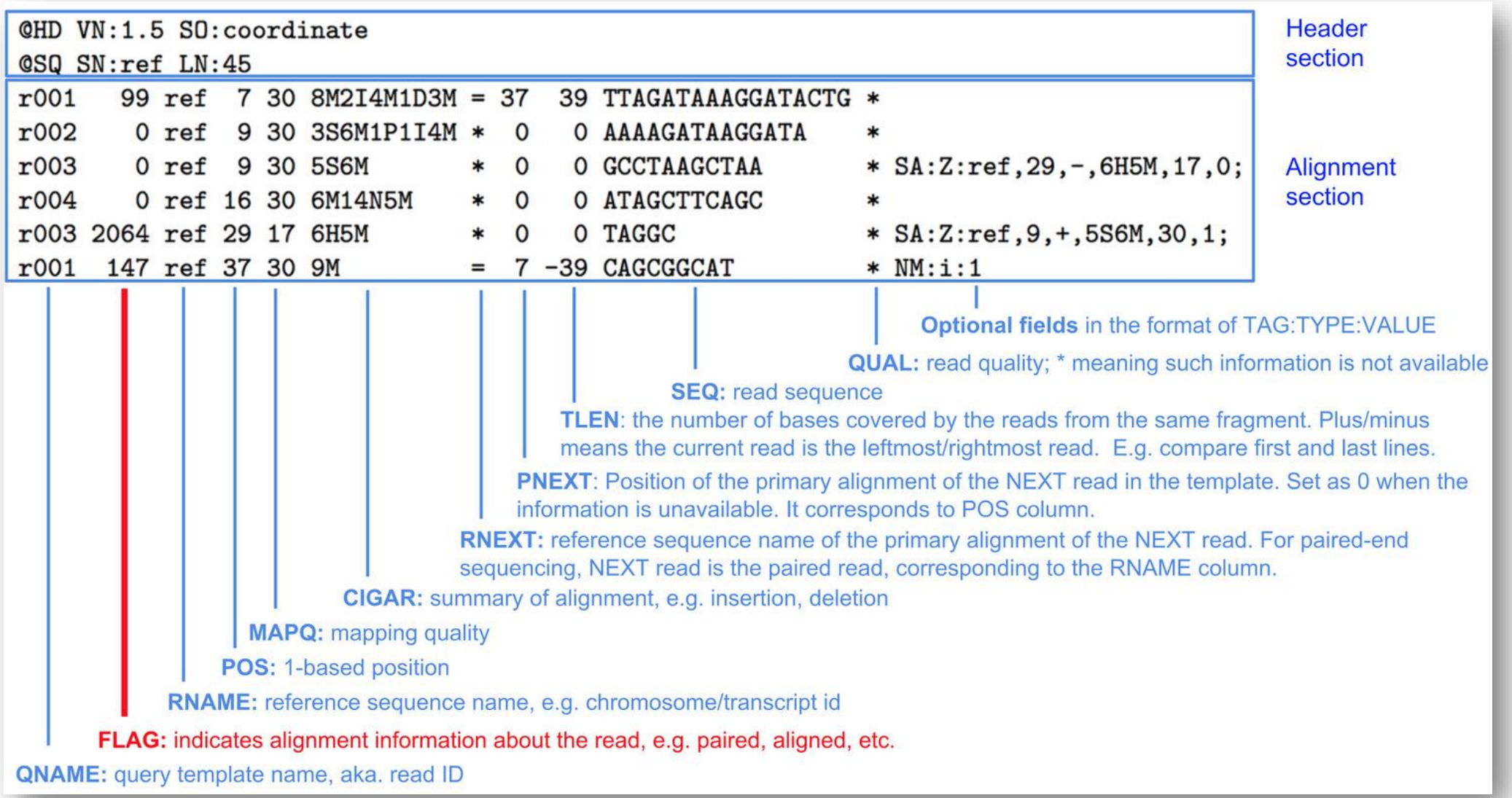
STAR



SAM File

```
@SQ     SN:NC_003198.1  LN:4809037
@PG     ID:bwa  PN:                                                    .fastq.gz data/STY_0014_R
2.fastq.gz
VH00293:1:AAAM7FVM5                                                     2329299 -168    CTGGCCGC
AAATGATTTGCGTCAGGCC                                                    GTGCGCAGAATACAGATCCCCACCG
GCGCGGAGGCAACGATTTT                                                    CCCCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCCCCCCCCC                                                      AS:i:150        XS:i:0
VH00293:1:AAAM7FVM5                                                     2329317 168     ATCAACAA
AGTTGACCTGCTGGCCGCA                                                    ATATTGACACCATCAATAGTGCGCA
GAATACAGATCCCCACCGC                                                    CCCCCCCCCCC;C-CCCCCCCCCCC
CCCCCCC;CCCCCCCC;CC                                                     AS:i:151        XS:i:0
VH00293:1:AAAM7FVM5                                                     2873318 -35     CCCCCCCC
CCCCCCCCCCACACCACCC                                                    CCAGAAATTCTCTTATTGTCAGAAT
AGTGAGCGTGCCTTACCGA                                                    CCCCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCC;CCCCCCCCCC                                                     XS:i:0
VH00293:1:AAAM7FVM5                                                     =       2873318 35      G
TTCACTTTCCAGAAATTCT                                                    CTATAGGTGTAGATCTCCGTGGTCG
CCGTTTGTGTTATAACAGC                                                    CCCCCCCCCCCCC---CC--C---C
C;C--CC-C-CC-;C;CC;                                                     AS:i:36 XS:i:0
VH00293:1:AAAM7FVM5                                                     3459458 -75     CTTTTTCA
AGCAGAAGACGGCATACGA                                                    GGTAGCATATTGTCATGCTAACGAC
GTAACCCAACATATGGATC                                                    CCCCCCCCCCC-CCCCCCCCCC-CC
CCCCCCCCCCCCCC;CCCC                                                     XS:i:0
VH00293:1:AAAM7FVM5                                                     3459458 75      AAAAAATA
TGCCTCCCCTTGTAAGGGC                                                    TTTGTTAACCACTATCGGTGTAGAT
CTCGGTGGGGGCCGTATCATTAA CCCCCCCCCCCCCCCCCCCC;-CCCCCCC;CCCCCCCCCCCCCCCCCCCCCCCCCCCC;CCCCC-CCCCCCCCCCCC-CCCCCCCCCCCCCCCC-;
```

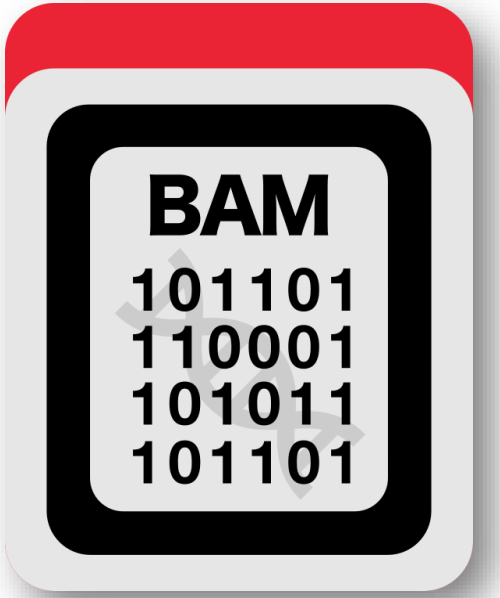
SAM (Sequence Alignment/Map) File



Source: https://www.samformat.info/sam-format-flag

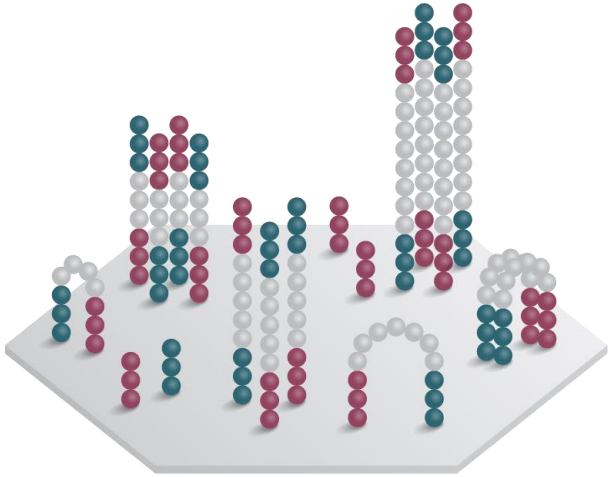
BAM (Binary Alignment/Map file) File

- **Binary, compressed (and almost always sorted) representation of the SAM information**
  - Sorting by coordinate allows fast query on the information by location.



Variant Calling

Basic Concepts and Common Terminologies



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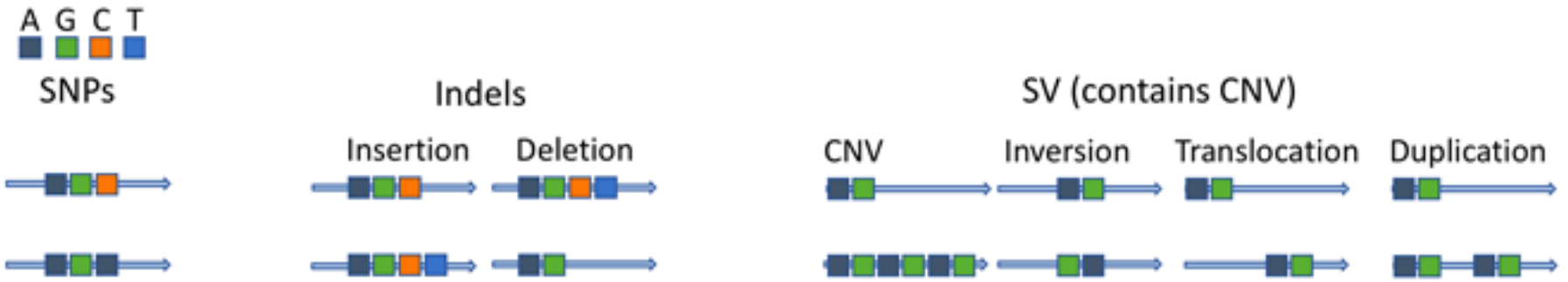
Variant Calling

Variant calling is the process of identifying genetic variations or differences, such as single nucleotide polymorphisms (**SNPs**) or insertions/deletions (**indels**), in a subject's genome compared to a reference genome.



Source: https://www.csc.fi/-/crunching-ngs-data-on-pouta-cloud

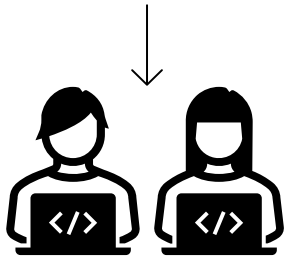
Commonly Used Variant Callers

bcftools

FreeBayes

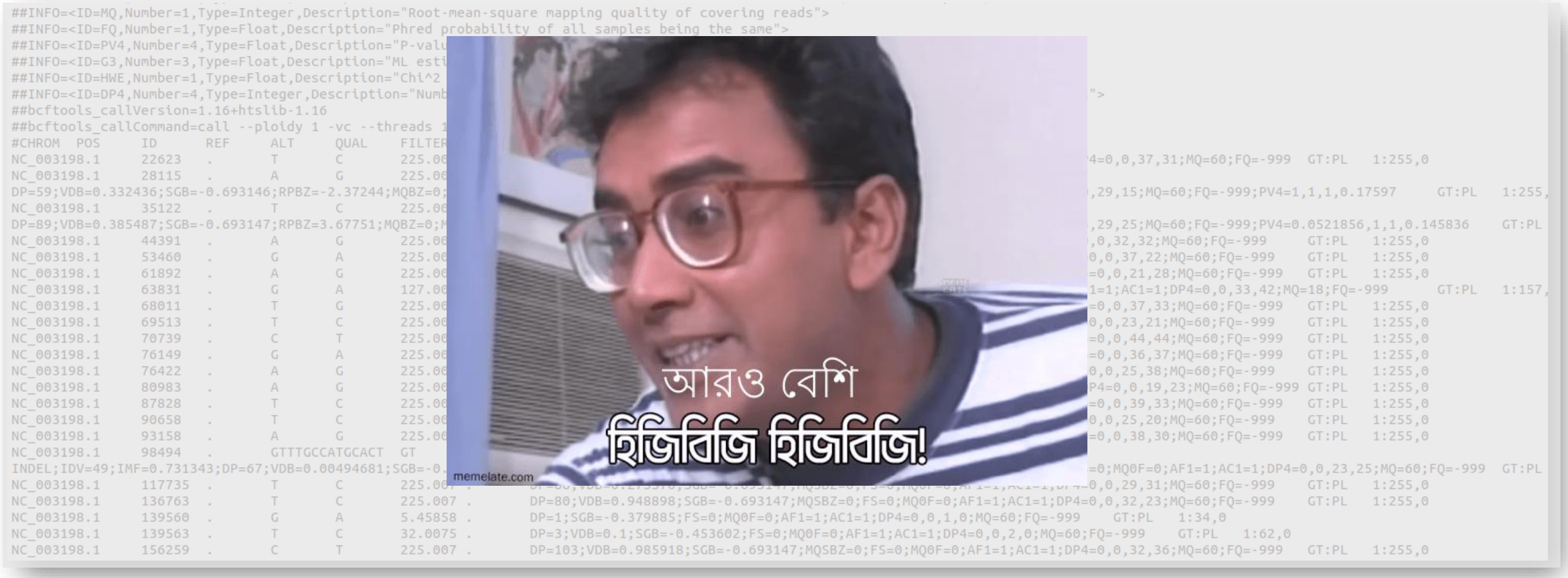
VarScan2

GATK



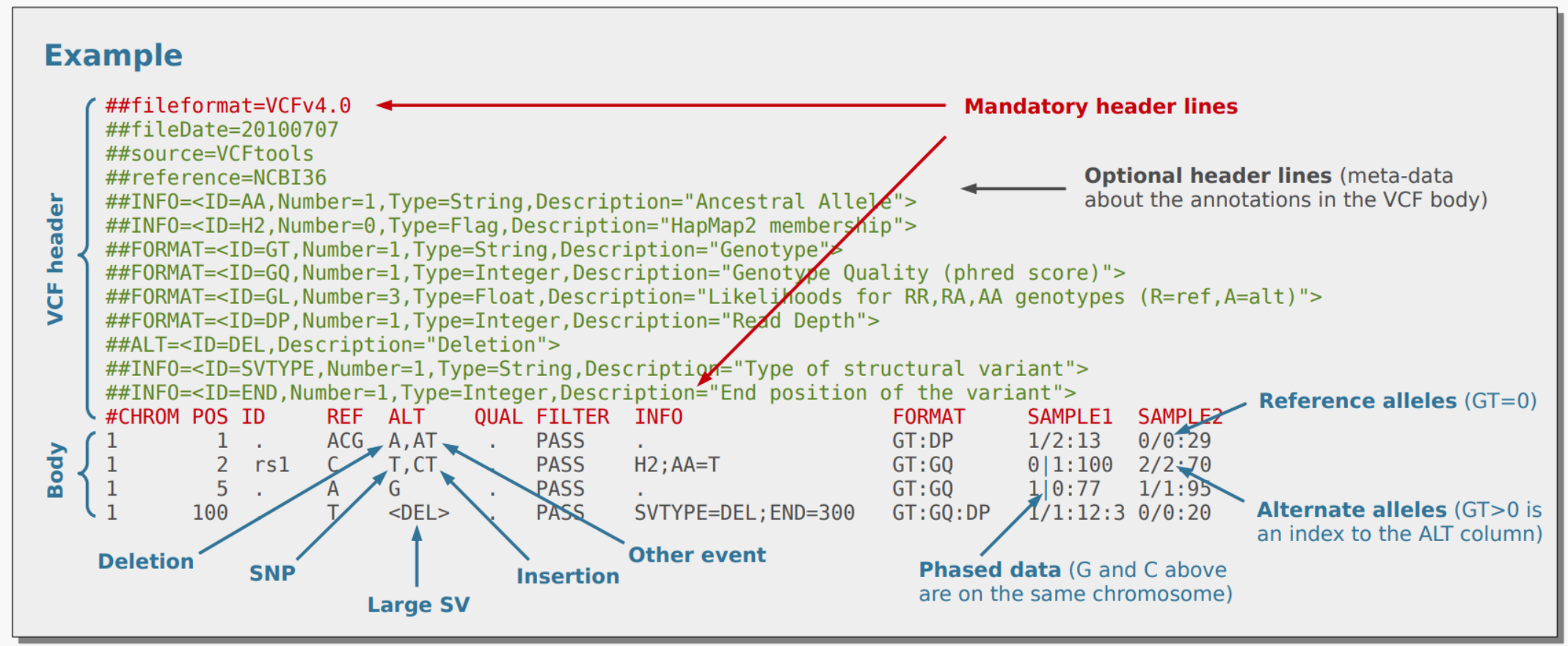
VCF file format

Data representation format used to describe **variations** in the genome



VCF file format

Data representation format used to describe **variations** in the genome



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Source: https://vcftools.sourceforge.net/VCF-poster.pdf

Happy Learning