pós-graduação em ciências
da saúde do iamspe

Genômica Computacional

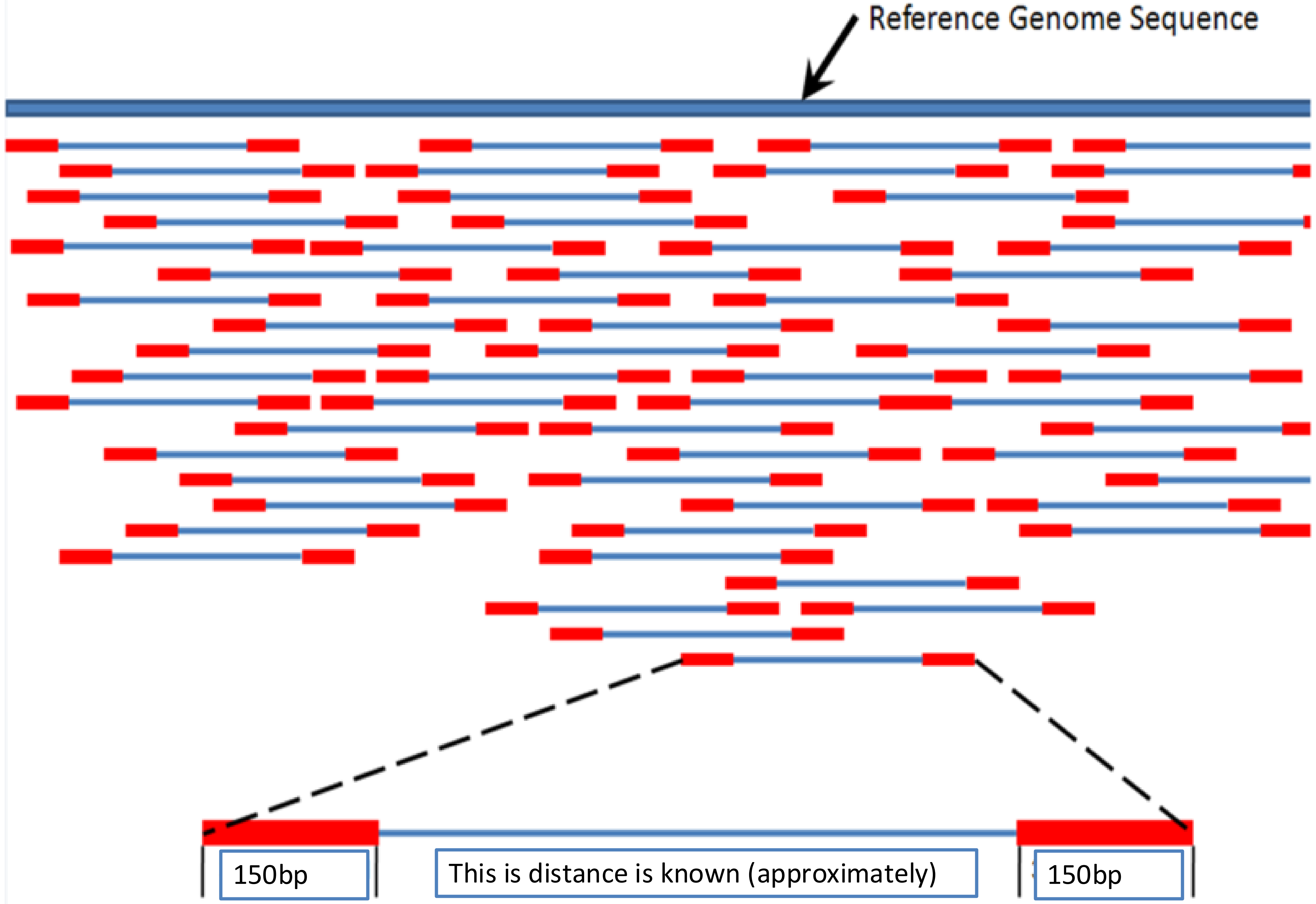
Analise de variantes

Professor: Ricardo A. Vialle

CS31 - Genômica Computacional

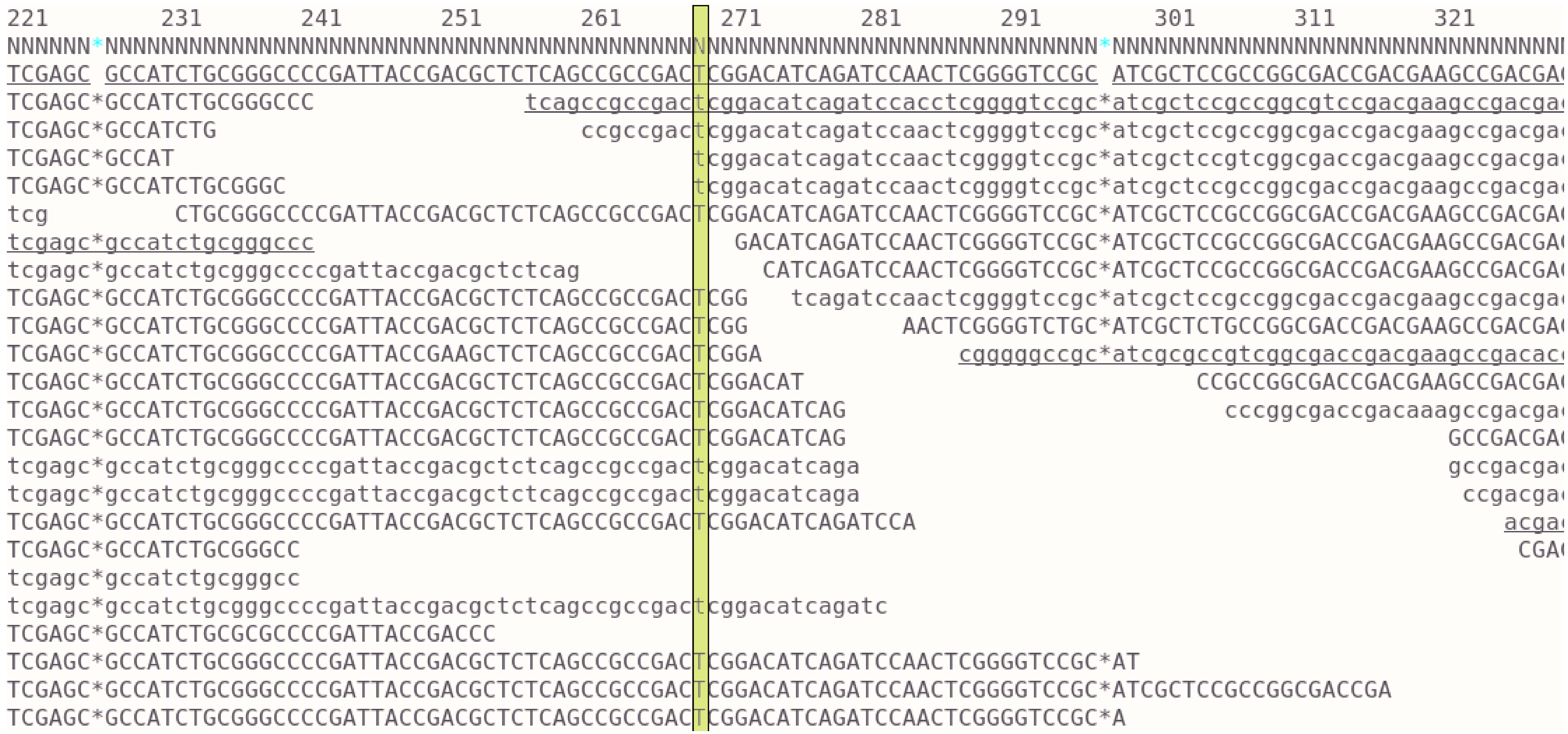
17 de Julho de 2025

Read mapping


Reference Genome Sequence
150bp
This is distance is known (approximately)
150bp

https://biocorecrg.github.io/RNAseq_course_2019/alignment.html

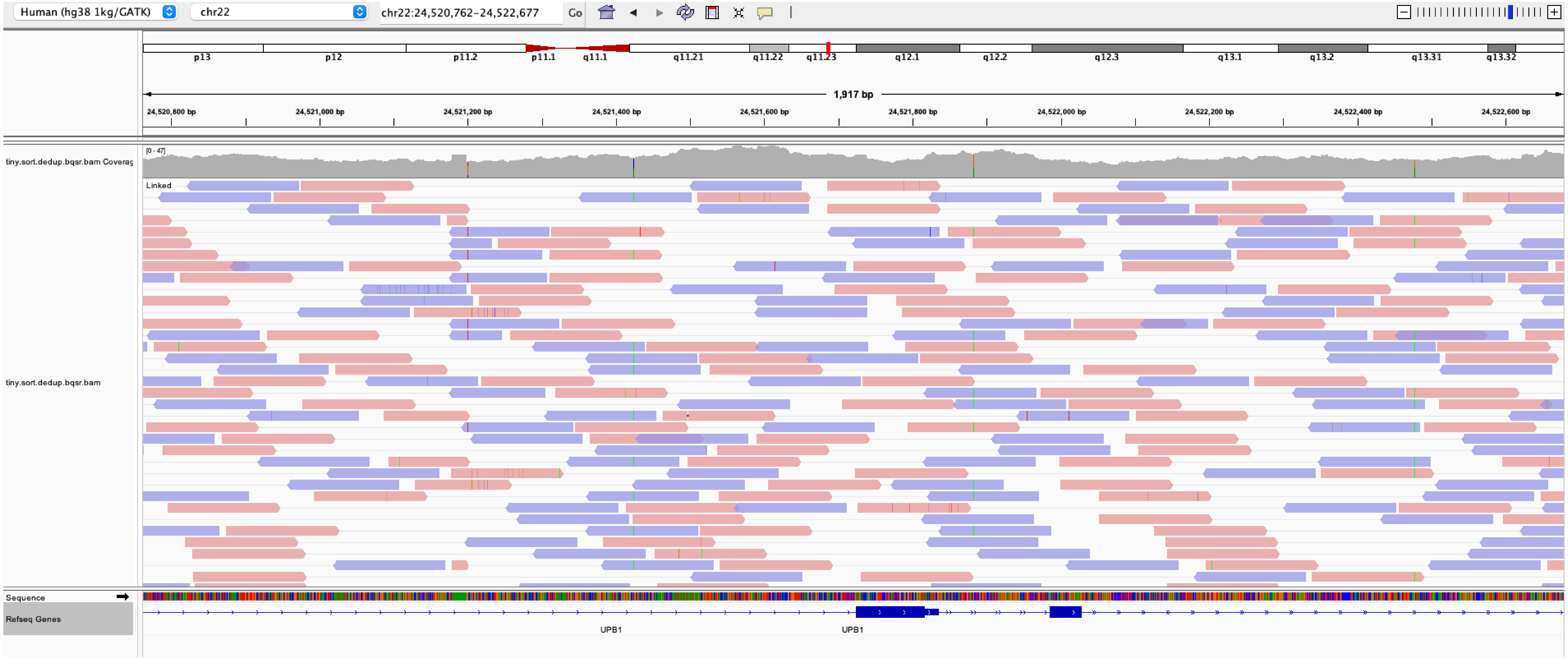
Read mapping



```
221        231       241       251       261       271       281       291        301       311       321
NNNNNN*NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN*NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
TCGAGC GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCAACTCGGGGTCCGC ATCGCTCCGCCGGCGACCGACGAAGCCGACGA
TCGAGC*GCCATCTGCGGGCCC               tcagccgccgactcggacatcagatccacctcggggtccgc*atcgctccgccggcgtccgacgaagccgacga
TCGAGC*GCCATCTG                          ccgccgactcggacatcagatccaactcggggtccgc*atcgctccgccggcgaccgacgaagccgacga
TCGAGC*GCCAT                                     tcggacatcagatccaactcggggtccgc*atcgctccgtcggcgaccgacgaagccgacga
TCGAGC*GCCATCTGCGGGC                             tcggacatcagatccaactcggggtccgc*atcgctccgccggcgaccgacgaagccgacga
tcg         CTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCAACTCGGGGTCCGC*ATCGCTCCGCCGGCGACCGACGAAGCCGACGA
tcgagc*gccatctgcgggccc                              GACATCAGATCCAACTCGGGGTCCGC*ATCGCTCCGCCGGCGACCGACGAAGCCGACGA
tcgagc*gccatctgcgggccccgattaccgacgctctcag             CATCAGATCCAACTCGGGGTCCGC*ATCGCTCCGCCGGCGACCGACGAAGCCGACGA
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGG   tcagatccaactcggggtccgc*atcgctccgccggcgaccgacgaagccgacga
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGG           AACTCGGGGTCTGC*ATCGCTCTGCCGGCGACCGACGAAGCCGACGA
TCGAGC*GCCATCTGCGGGCCCCGATTACCGAAGCTCTCAGCCGCCGACTCGGA              cgggggccgc*atcgcgccgtcggcgaccgacgaagccgacac
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACAT                            CCGCCGGCGACCGACGAAGCCGACGA
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAG                           cccggcgaccgacaaagccgacga
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAG                                           GCCGACGA
tcgagc*gccatctgcgggccccgattaccgacgctctcagccgccgactcggacatcaga                                          gccgacga
tcgagc*gccatctgcgggccccgattaccgacgctctcagccgccgactcggacatcaga                                           ccgacga
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCA                                          acga
TCGAGC*GCCATCTGCGGGCC                                                                                       CGA
tcgagc*gccatctgcgggcc
tcgagc*gccatctgcgggccccgattaccgacgctctcagccgccgactcggacatcagatc
TCGAGC*GCCATCTGCGCGCCCCGATTACCGACCC
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCAACTCGGGGTCCGC*AT
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCAACTCGGGGTCCGC*ATCGCTCCGCCGGCGACCGA
TCGAGC*GCCATCTGCGGGCCCCGATTACCGACGCTCTCAGCCGCCGACTCGGACATCAGATCCAACTCGGGGTCCGC*A
```

https://dmnfarrell.github.io/img/bam-pileup.png

IGV browser



Human (hg38 1kg/GATK)

chr22

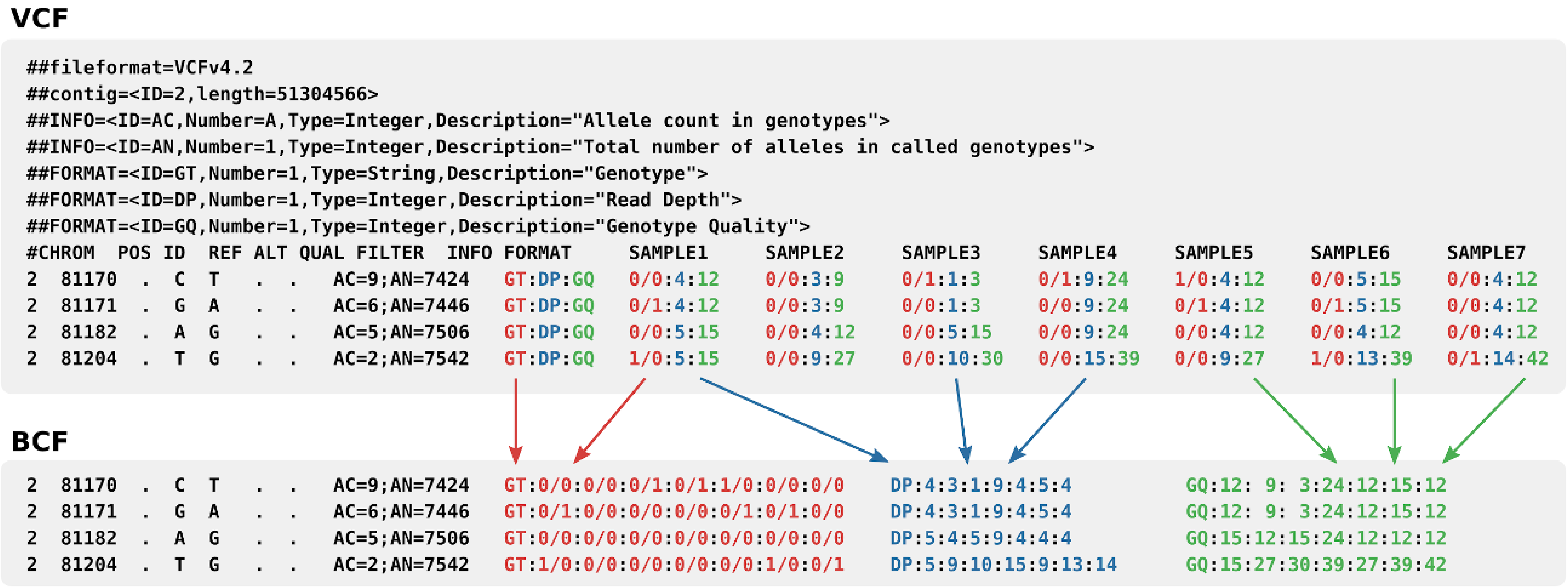
chr22:24,520,762-24,522,677

Types of genetic variation

- **99% of DNA is shared** between two individuals
- Variation in the remainder explains all our **predisposition** differences
- **Remaining** phenotypic variation: environmental/stochastic differences

Name	**Example**	**Frequency in one genome**
Single nucleotide polymorphisms (**SNPs**)	GAGGAGAACG[C/G]AACTCCGCCG	1 per 1,000 bp
Insertions/deletions (**indels**)	CACTATTC[C/CTATGG]TGTCTAA	1 per 10,000 bp
Short tandem repeats (**STRs**)	ACGGCAGTCGTCGTCGTCACCGTAT	1 per 10,000 bp
Structural variants (**SVs**) / Copy Number Variants (**CNVs**)	Large (median 5,000 bp) deletions, duplications, inversions	1 per 1,000,000 bp

VCF and BCF file format



VCF

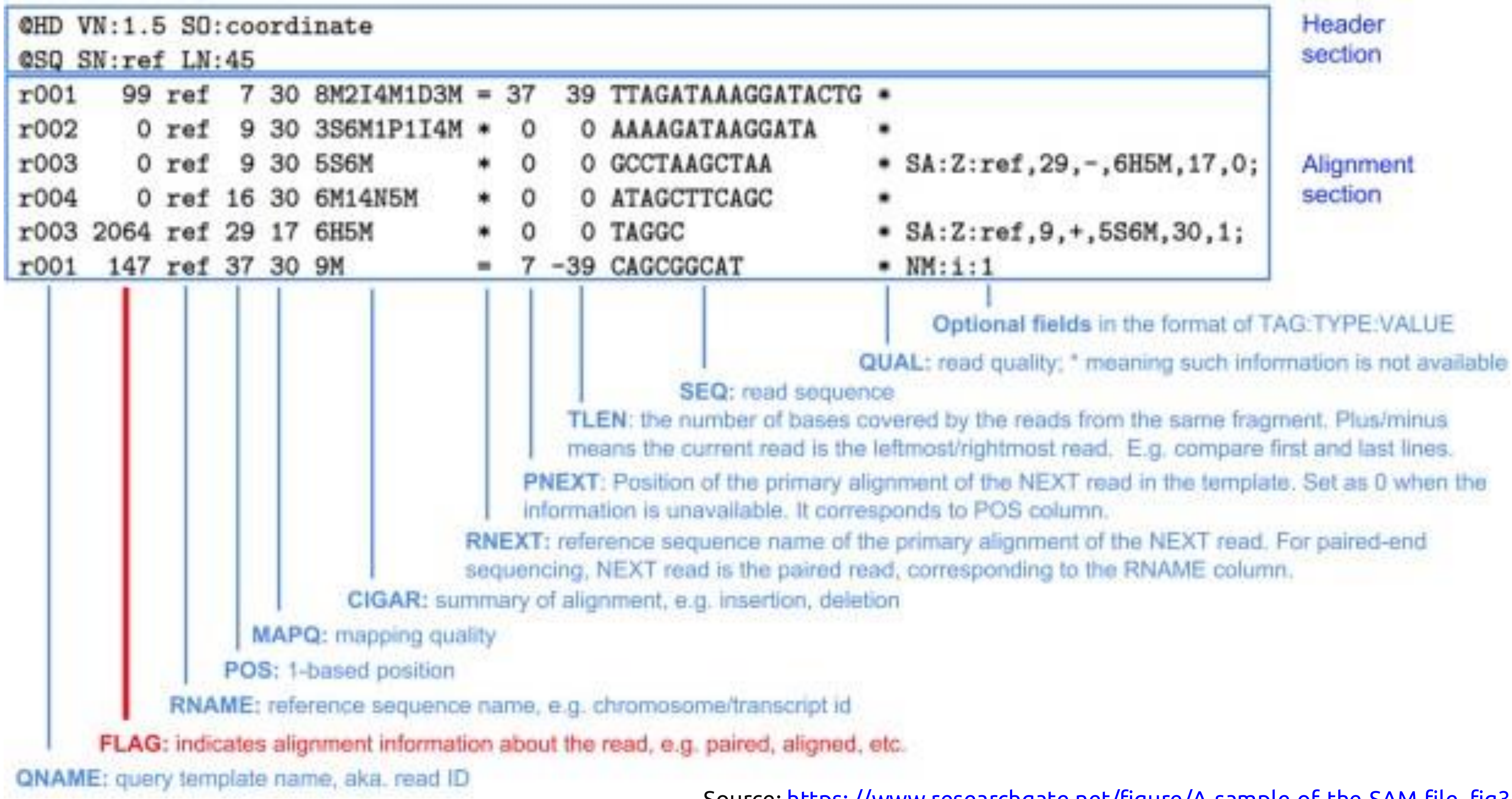
```
##fileformat=VCFv4.2
##contig=<ID=2,length=51304566>
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
#CHROM  POS ID  REF ALT QUAL FILTER  INFO FORMAT     SAMPLE1     SAMPLE2     SAMPLE3     SAMPLE4     SAMPLE5     SAMPLE6     SAMPLE7
2  81170  .  C  T   .  .   AC=9;AN=7424  GT:DP:GQ  0/0:4:12    0/0:3:9     0/1:1:3     0/1:9:24    1/0:4:12    0/0:5:15    0/0:4:12
2  81171  .  G  A   .  .   AC=6;AN=7446  GT:DP:GQ  0/1:4:12    0/0:3:9     0/0:1:3     0/0:9:24    0/1:4:12    0/1:5:15    0/0:4:12
2  81182  .  A  G   .  .   AC=5;AN=7506  GT:DP:GQ  0/0:5:15    0/0:4:12    0/0:5:15    0/0:9:24    0/0:4:12    0/0:4:12    0/0:4:12
2  81204  .  T  G   .  .   AC=2;AN=7542  GT:DP:GQ  1/0:5:15    0/0:9:27    0/0:10:30   0/0:15:39   0/0:9:27    1/0:13:39   0/1:14:42
```

BCF

```
2  81170  .  C  T   .  .   AC=9;AN=7424  GT:0/0:0/0:0/1:0/1:1/0:0/0:0/0    DP:4:3:1:9:4:5:4        GQ:12: 9: 3:24:12:15:12
2  81171  .  G  A   .  .   AC=6;AN=7446  GT:0/1:0/0:0/0:0/0:0/1:0/1:0/0    DP:4:3:1:9:4:5:4        GQ:12: 9: 3:24:12:15:12
2  81182  .  A  G   .  .   AC=5;AN=7506  GT:0/0:0/0:0/0:0/0:0/0:0/0:0/0    DP:5:4:5:9:4:4:4        GQ:15:12:15:24:12:12:12
2  81204  .  T  G   .  .   AC=2;AN=7542  GT:1/0:0/0:0/0:0/0:0/0:1/0:0/1    DP:5:9:10:15:9:13:14    GQ:15:27:30:39:27:39:42
```

https://en.wikipedia.org/wiki/Variant_Call_Format#/media/File:Binary_BCF_versus_VCF_format.png

SAM and BAM file format



```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001    99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002     0 ref  9 30 3S6M1P1I4M * 0    0 AAAAGATAAGGATA    *
r003     0 ref  9 30 5S6M       * 0    0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004     0 ref 16 30 6M14N5M    * 0    0 ATAGCTTCAGC       *
r003  2064 ref 29 17 6H5M       * 0    0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001   147 ref 37 30 9M         = 7  -39 CAGCGGCAT         * NM:i:1
```

Optional fields in the format of TAG:TYPE:VALUE

QUAL: read quality; * meaning such information is not available

SEQ: read sequence

TLEN: the number of bases covered by the reads from the same fragment. Plus/minus means the current read is the leftmost/rightmost read. E.g. compare first and last lines.

PNEXT: Position of the primary alignment of the NEXT read in the template. Set as 0 when the information is unavailable. It corresponds to POS column.

RNEXT: reference sequence name of the primary alignment of the NEXT read. For paired-end sequencing, NEXT read is the paired read, corresponding to the RNAME column.

CIGAR: summary of alignment, e.g. insertion, deletion

MAPQ: mapping quality

POS: 1-based position

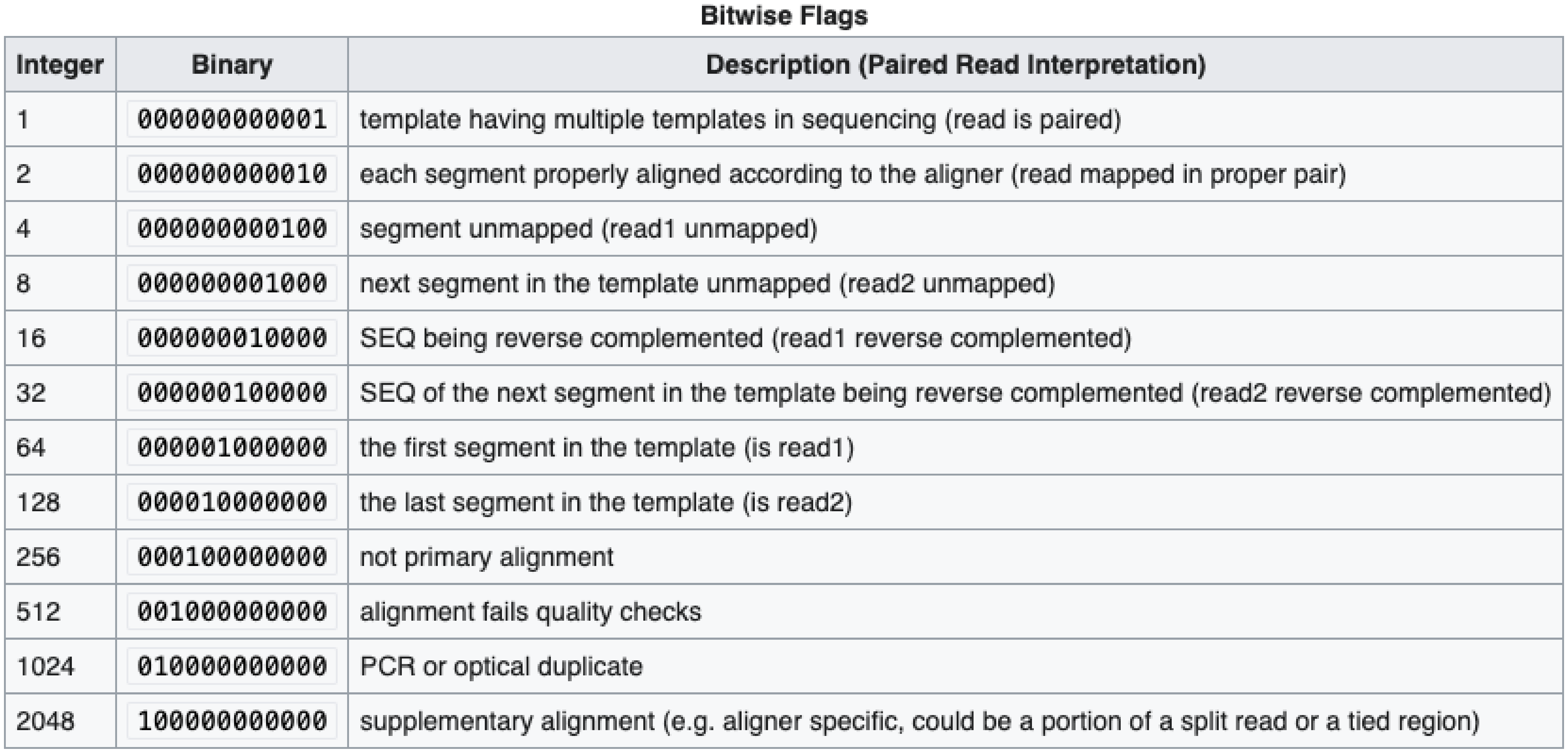
RNAME: reference sequence name, e.g. chromosome/transcript id

FLAG: indicates alignment information about the read, e.g. paired, aligned, etc.

QNAME: query template name, aka. read ID

Source: https: //www.researchgate.net/figure/A-sample-of-the-SAM-file_fig3_309134977

SAM and BAM file format (FLAG)



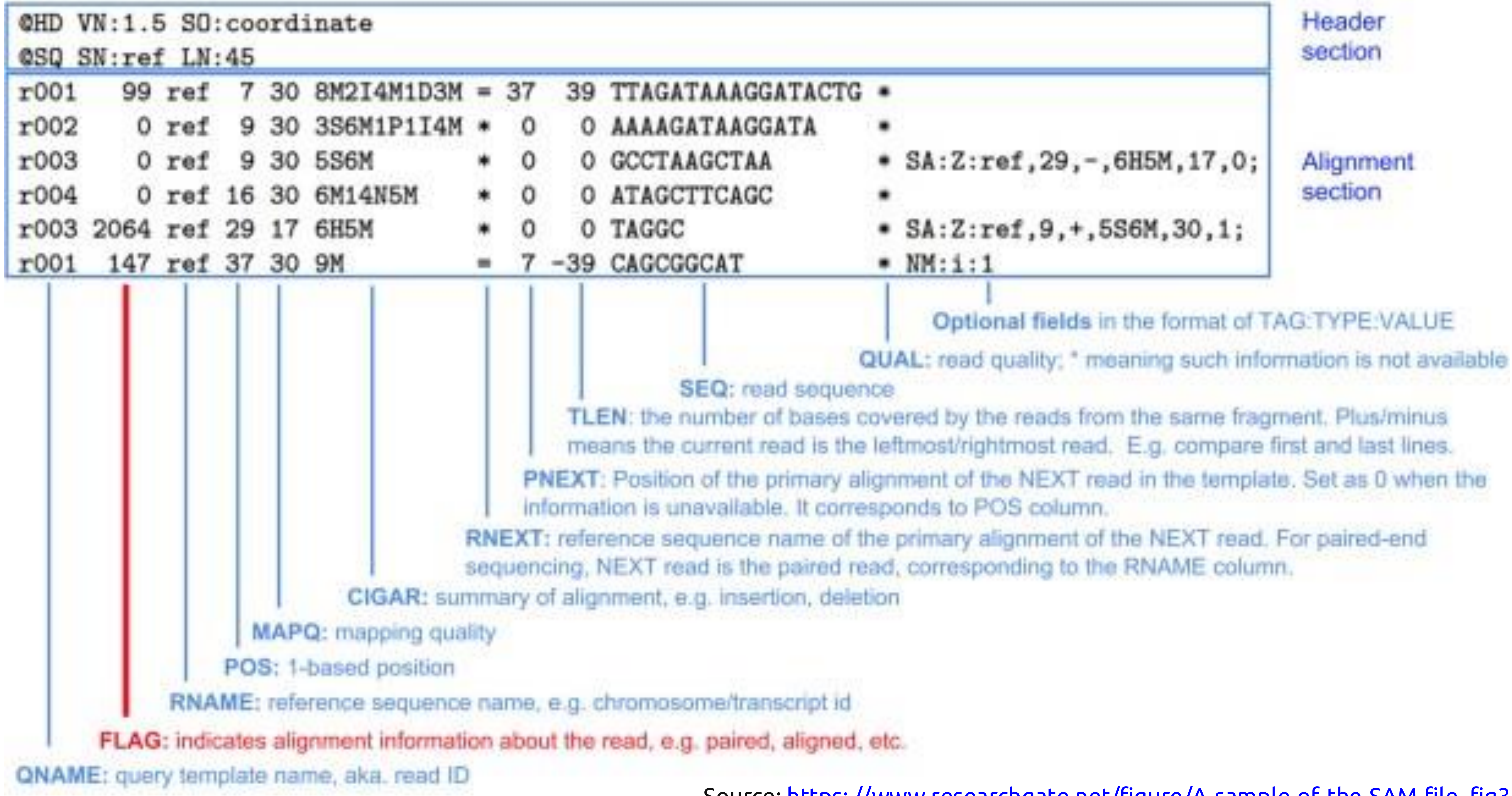
Bitwise Flags

Integer	Binary	Description (Paired Read Interpretation)
1	`000000000001`	template having multiple templates in sequencing (read is paired)
2	`000000000010`	each segment properly aligned according to the aligner (read mapped in proper pair)
4	`000000000100`	segment unmapped (read1 unmapped)
8	`000000001000`	next segment in the template unmapped (read2 unmapped)
16	`000000010000`	SEQ being reverse complemented (read1 reverse complemented)
32	`000000100000`	SEQ of the next segment in the template being reverse complemented (read2 reverse complemented)
64	`000001000000`	the first segment in the template (is read1)
128	`000010000000`	the last segment in the template (is read2)
256	`000100000000`	not primary alignment
512	`001000000000`	alignment fails quality checks
1024	`010000000000`	PCR or optical duplicate
2048	`100000000000`	supplementary alignment (e.g. aligner specific, could be a portion of a split read or a tied region)

https://en.wikipedia.org/wiki/SAM_(file_format)

Use ***samtools flagstat*** to summarize these metrics

SAM and BAM file format



```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

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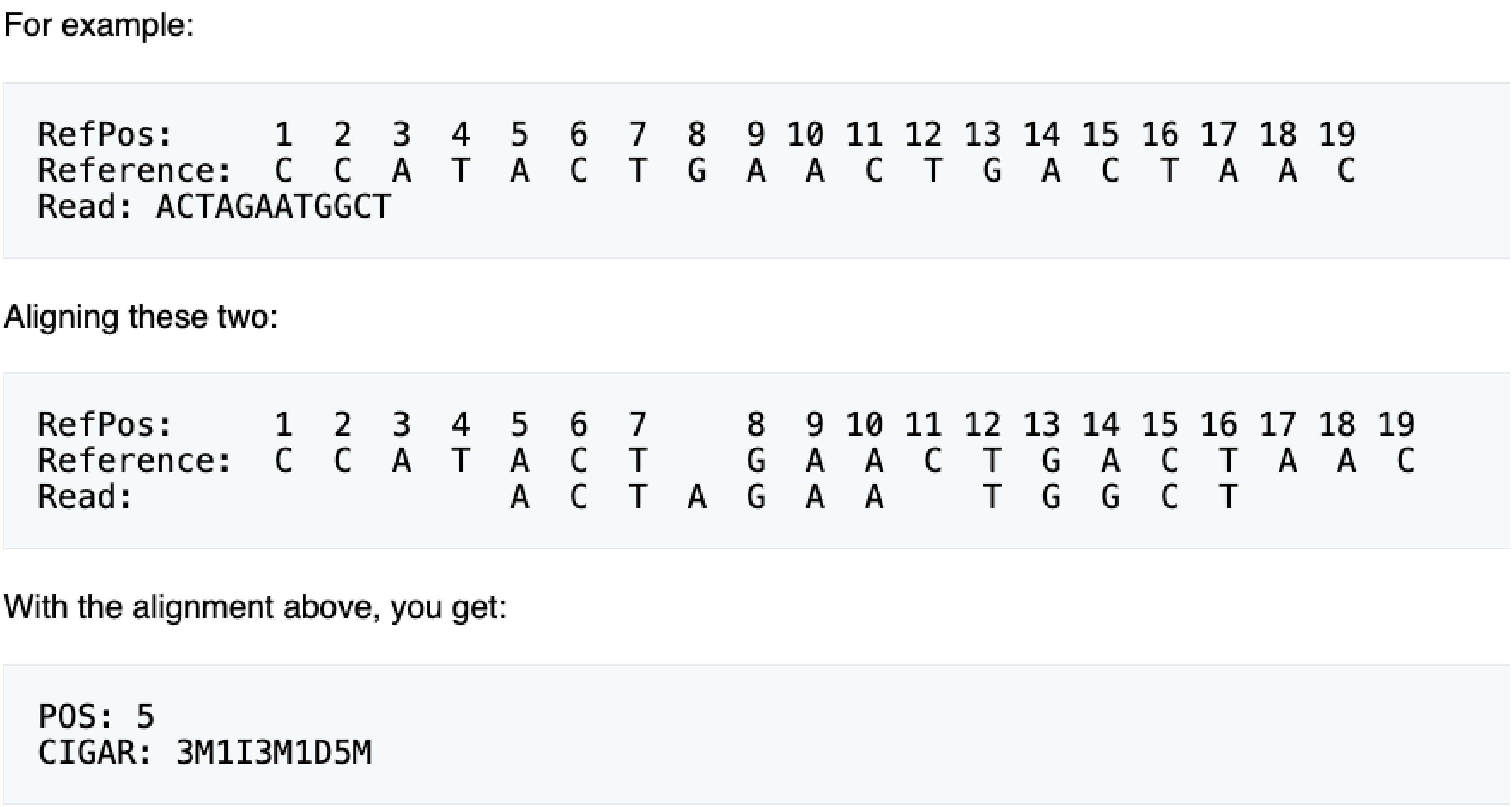
Source: https: //www.researchgate.net/figure/A-sample-of-the-SAM-file_fig3_309134977

CIGAR



For example:

```
RefPos:     1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19
Reference:  C  C  A  T  A  C  T  G  A  A  C  T  G  A  C  T  A  A  C
Read: ACTAGAATGGCT
```

Aligning these two:

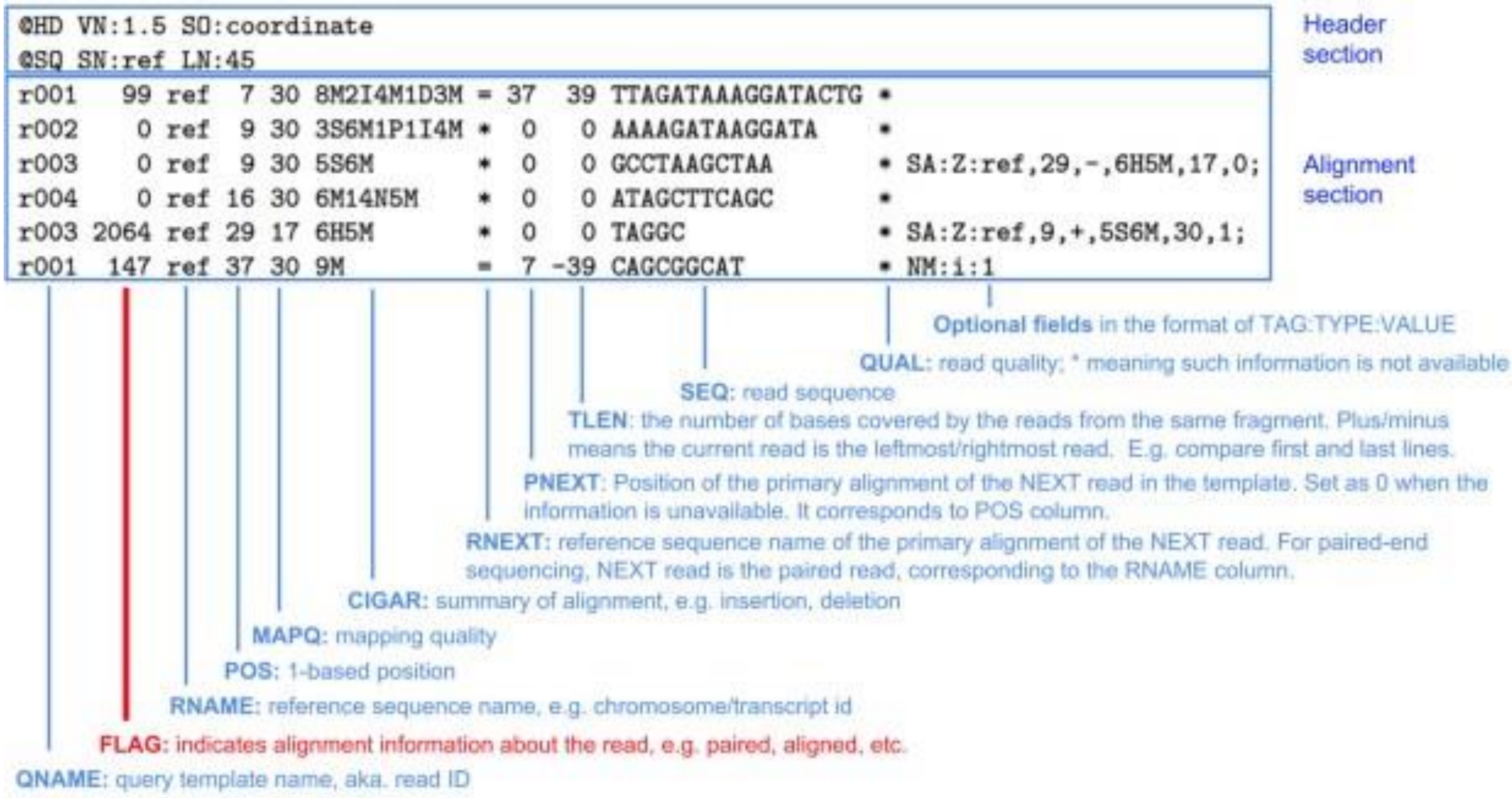
```
RefPos:     1  2  3  4  5  6  7     8  9 10 11 12 13 14 15 16 17 18 19
Reference:  C  C  A  T  A  C  T     G  A  A  C  T  G  A  C  T  A  A  C
Read:                   A  C  T  A  G  A  A     T  G  G  C  T
```

With the alignment above, you get:

```
POS: 5
CIGAR: 3M1I3M1D5M
```

https://genome.sph.umich.edu/wiki/SAM

SAM and BAM file format



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@HD VN:1.5 SO:coordinate
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r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
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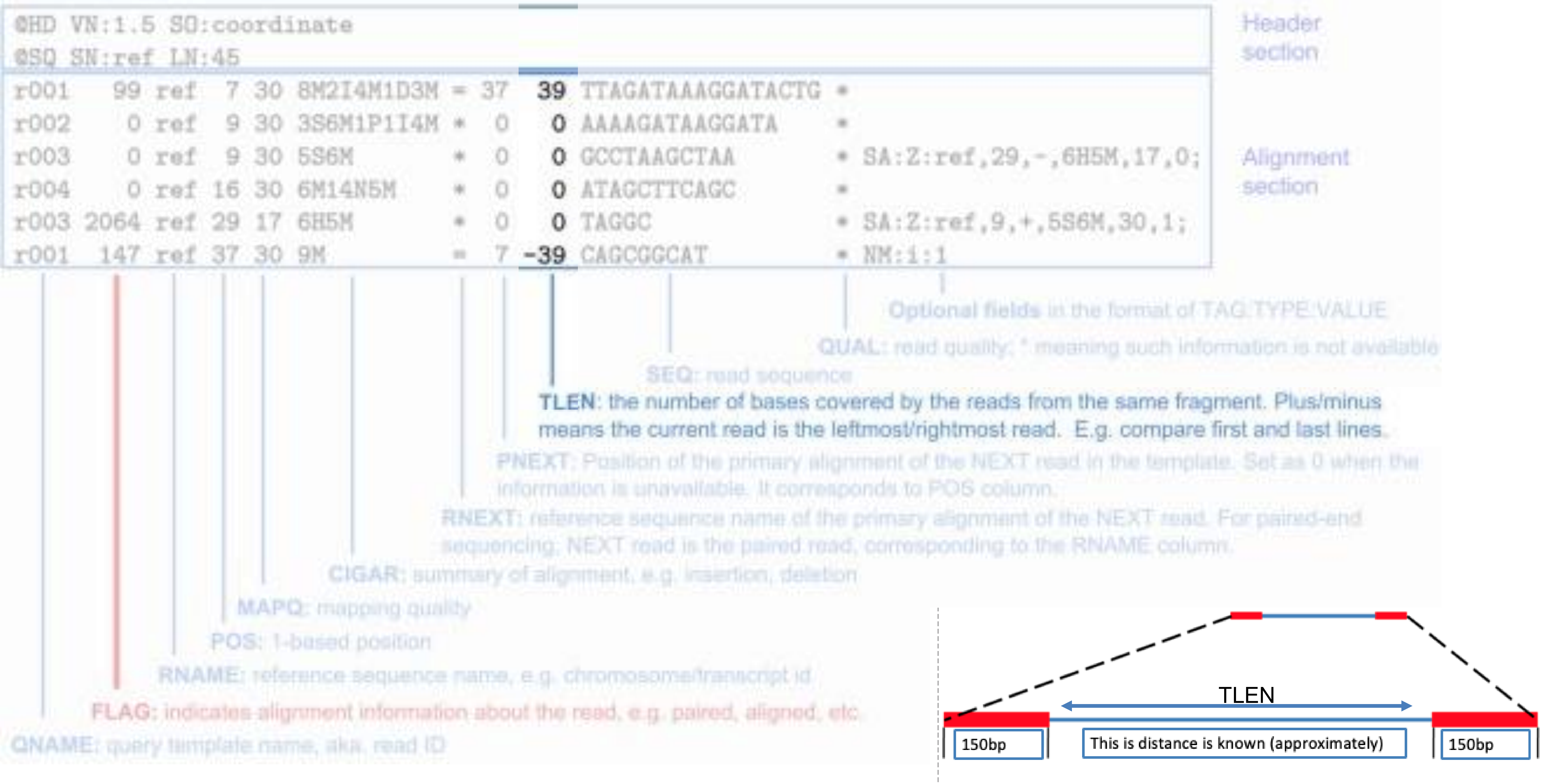

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@SQ SN:ref LN:45
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r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

Header section

Alignment section

Optional fields in the format of TAG:TYPE:VALUE

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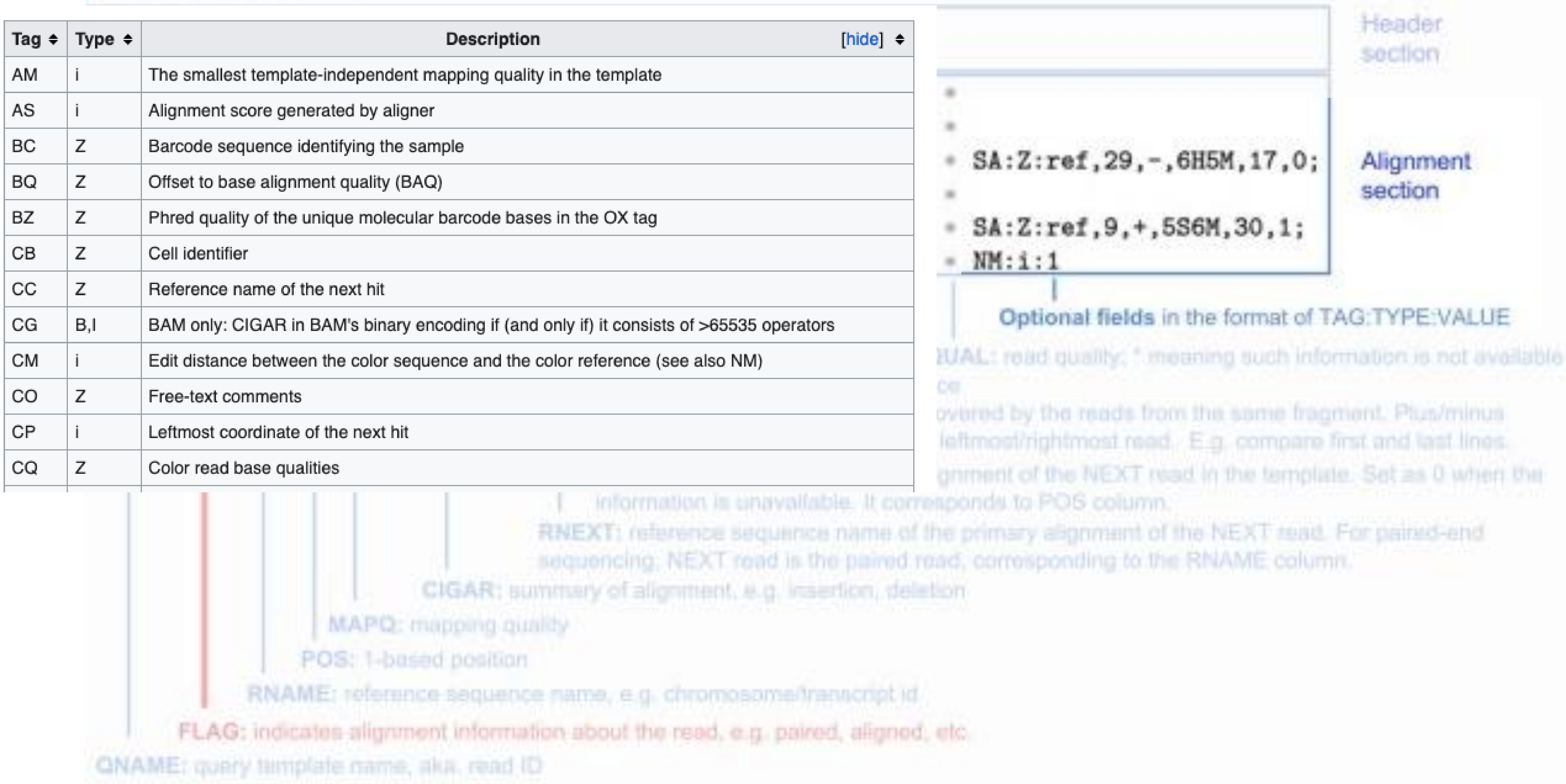

Tag	Type	Description
AM	i	The smallest template-independent mapping quality in the template
AS	i	Alignment score generated by aligner
BC	Z	Barcode sequence identifying the sample
BQ	Z	Offset to base alignment quality (BAQ)
BZ	Z	Phred quality of the unique molecular barcode bases in the OX tag
CB	Z	Cell identifier
CC	Z	Reference name of the next hit
CG	B,I	BAM only: CIGAR in BAM's binary encoding if (and only if) it consists of >65535 operators
CM	i	Edit distance between the color sequence and the color reference (see also NM)
CO	Z	Free-text comments
CP	i	Leftmost coordinate of the next hit
CQ	Z	Color read base qualities

[hide]
Header section
SA:Z:ref,29,-,6H5M,17,0;
SA:Z:ref,9,+,5S6M,30,1;
NM:i:1
Alignment section
Optional fields in the format of TAG:TYPE:VALUE
UAL: read quality; * meaning such information is not available
ce
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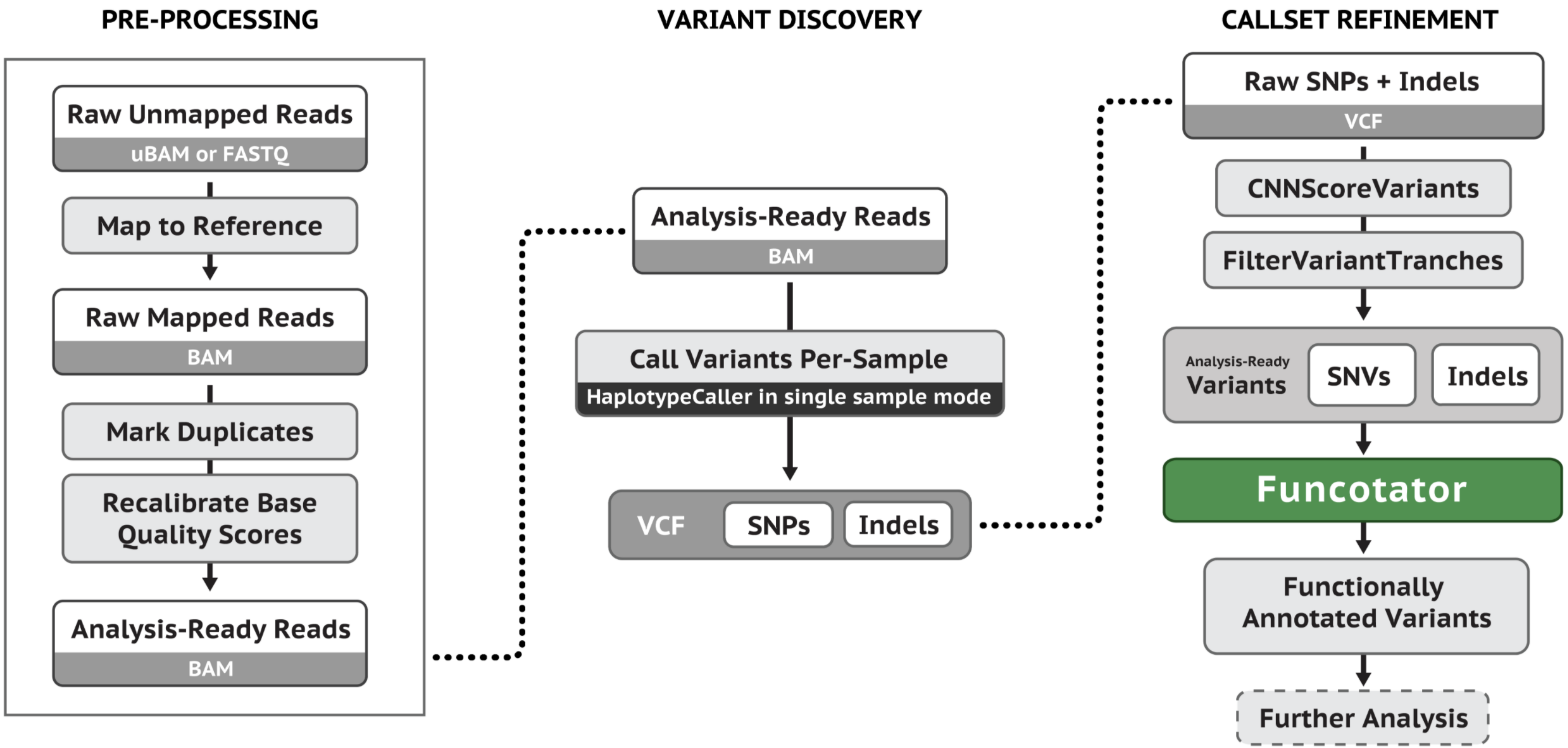
gatk



PRE-PROCESSING

Raw Unmapped Reads
uBAM or FASTQ

Map to Reference

Raw Mapped Reads
BAM

Mark Duplicates

Recalibrate Base Quality Scores

Analysis-Ready Reads
BAM

VARIANT DISCOVERY

Analysis-Ready Reads
BAM

Call Variants Per-Sample
HaplotypeCaller in single sample mode

VCF SNPs Indels

CALLSET REFINEMENT

Raw SNPs + Indels
VCF

CNNScoreVariants

FilterVariantTranches

Analysis-Ready Variants SNVs Indels

Funcotator

Functionally Annotated Variants

Further Analysis