NGS Data Formats

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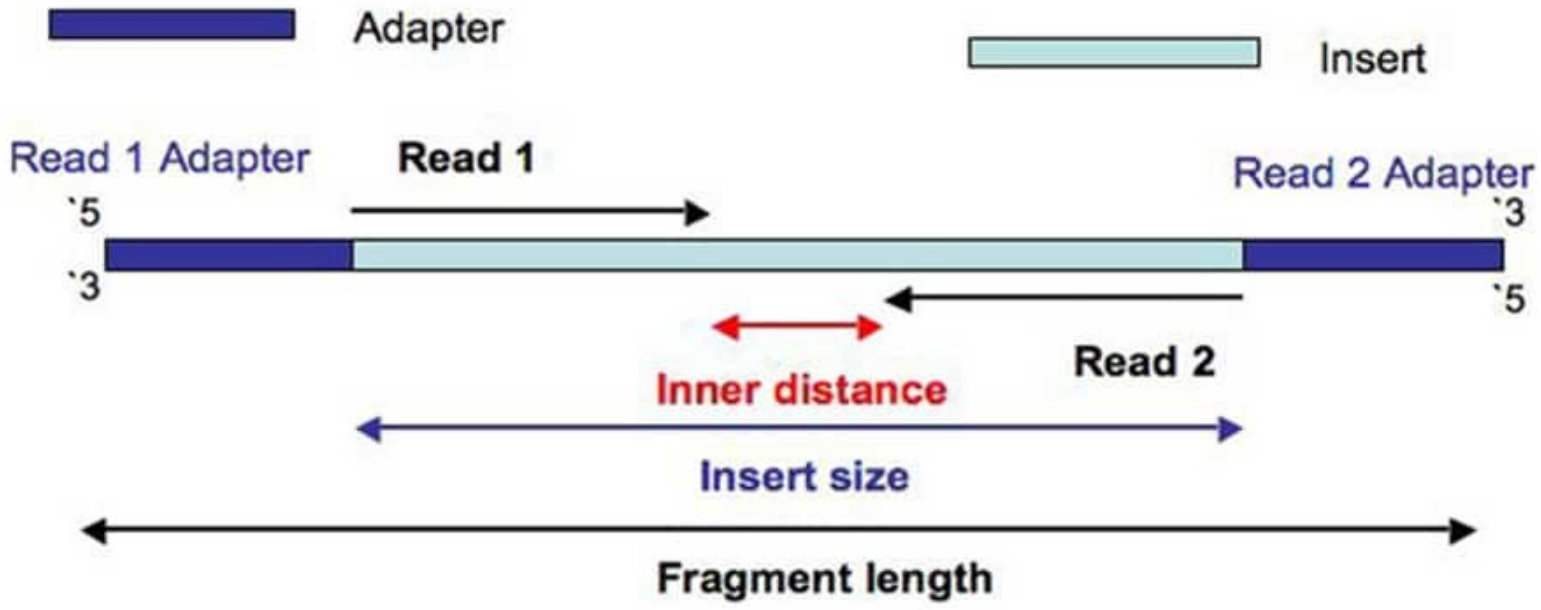
Data Formats

Illumina Sequencing Recap



Data Formats Summary

FASTQ
- Unaligned read sequences with base qualities

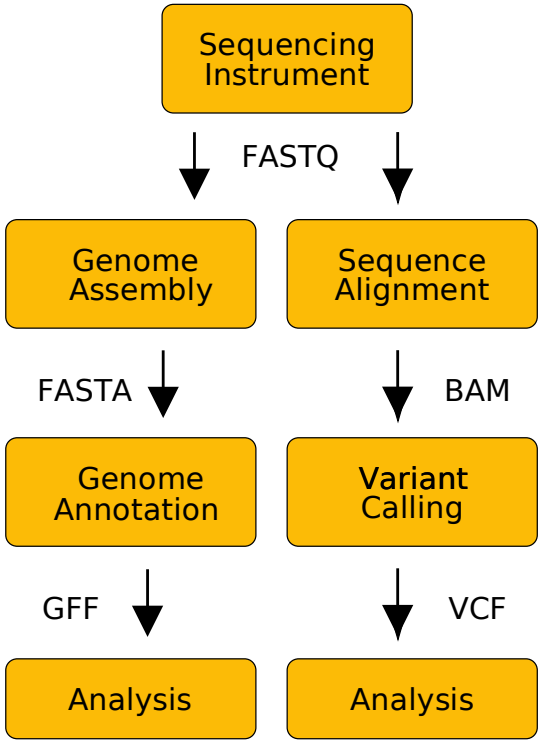
SAM/BAM
- Unaligned or aligned reads
- Text and binary formats

CRAM
- Better compression than BAM

VCF/BCF
- Flexible variant call format
- Arbitrary types of sequence variation
- SNPs, indels, structuralvariations

FASTA
- Nucleotide sequence data
- Reference genome, gene sequence

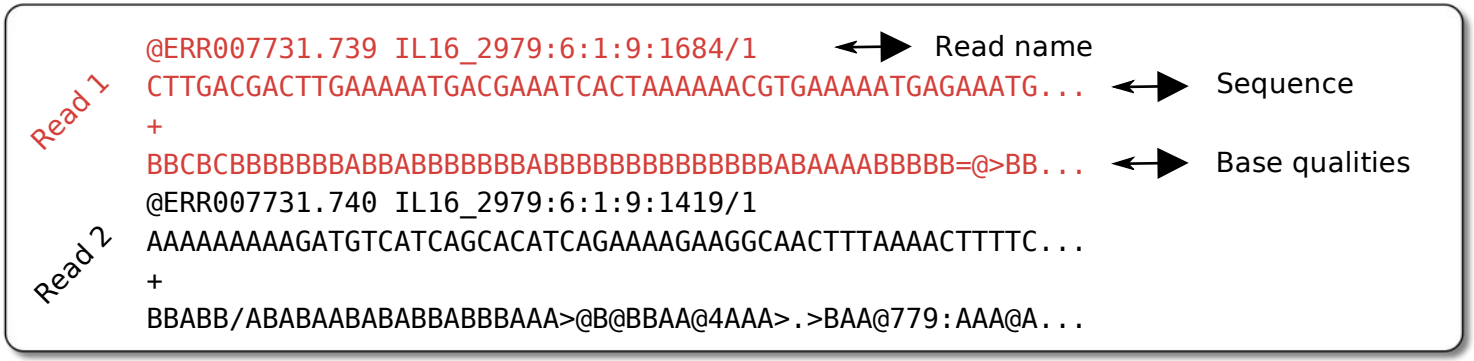
GFF
- Genes and other features of sequences
- CDS, tRNA, rRNA



FASTA - reference genome

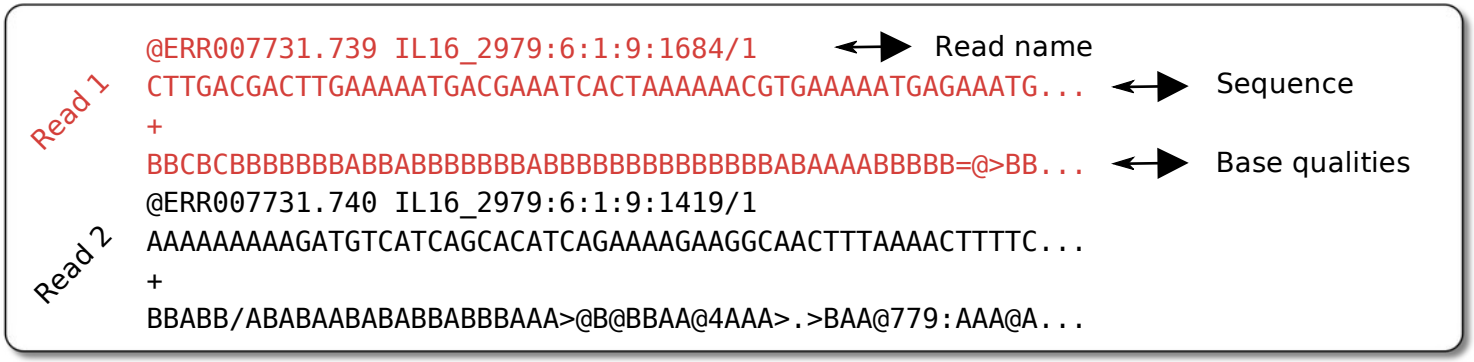
```
>1 dna:chromosome chromosome:GRCh37:1:1:249250621:1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
TATTCAAAAAATTGAGAATTTCTGACCACTTAACAAACCCACAGAAAATCCACCCGAGTG
CACTGAGCACGCCAGAAATCAGGTGGCCTCAAAGAGCTGCTCCCACCTGAAGGAGACGCG
CTGCTGCTGCTGTCGTCCTGCCTGGCGCCTTGGCCTACAGGGGCCGCGGTTGAGGGTGGG
AGTGGGGGTGCACTGGCCAGCACCTCAGGAGCTGGGGGTGGTGGTGGGGGCGGTGGGGGT
GGTGTTAGTACCCCATCTTGTAGGTCTGAAACACAAAGTGTGGGGTGTCTAGGGAAGAAG
>2
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
AAAAGCATTTATGCTACAAATTACTATGGTAATTATGCTACAAATTTATGGTACCATAAA
TTACCATAGTAATTTGTAGCATAAATTTGTACTATGGTACAAATTACATGGGAGAGTGAA
GGTGGGTTAAAACATTCATATTAAAGAACTTCCACTCAGATTGCAAGAAAAGAGAGAGGA
ATGGAGATGGTAGCACAAGTCCCTACAATAAAAGTAGATGTTTTGAGATCAGTTCTATTT
```

FASTQ



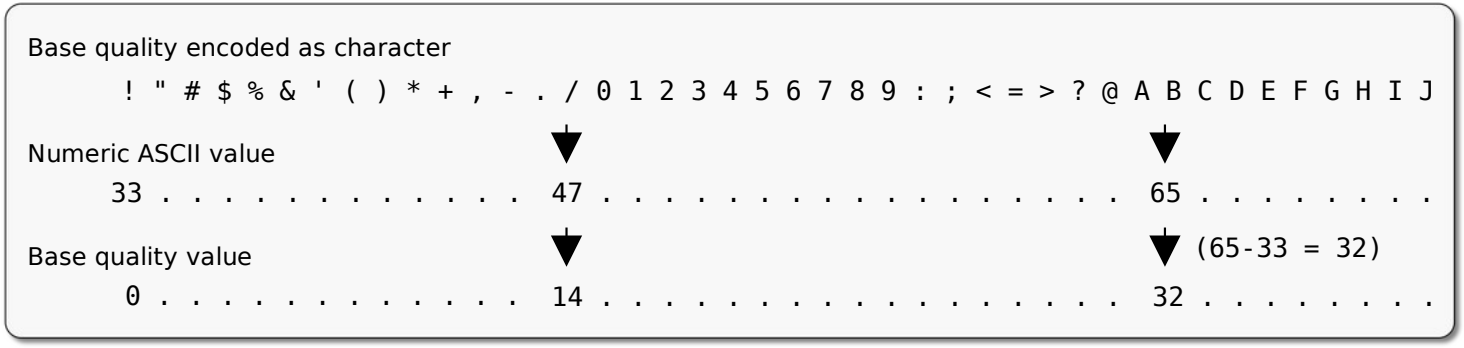
- Simple format for raw unaligned sequencing reads
- Paired-end sequencing: two FASTQ files or one interleaved file

FASTQ



- Simple format for raw unaligned sequencing reads
- Paired-end sequencing: two FASTQ files or one interleaved file

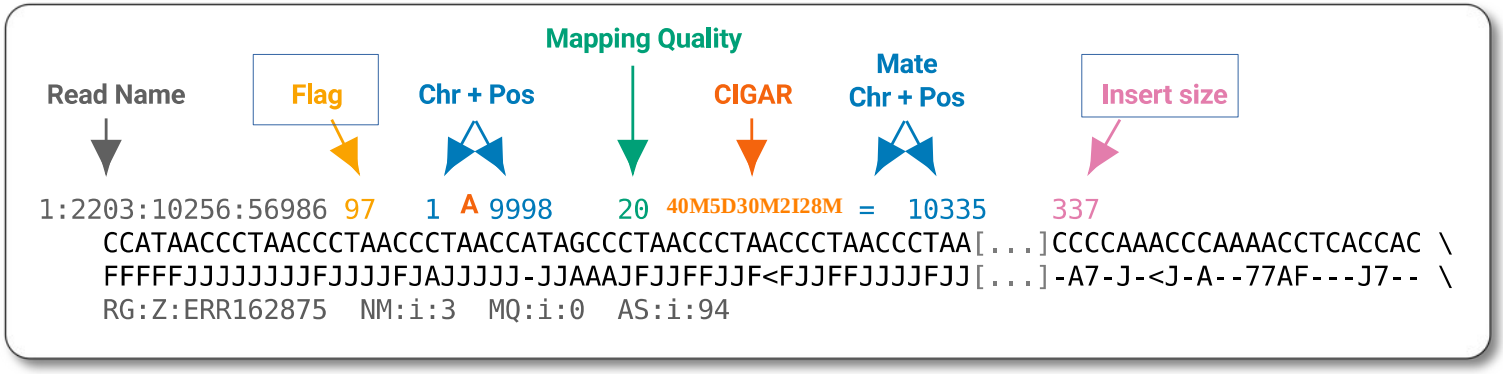
- Quality encoded in ASCII characters with decimal codes 33-126
  - ASCII code of “A” is 65, the corresponding quality is Q = 65–33 = 32

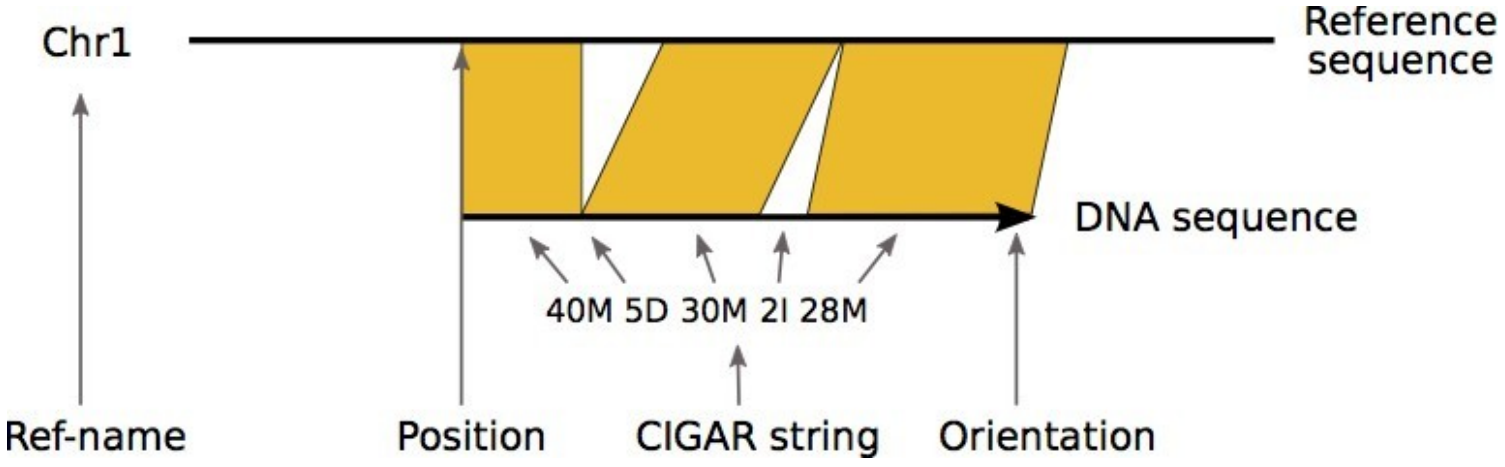


Quality = Phred-scaled probability of an error

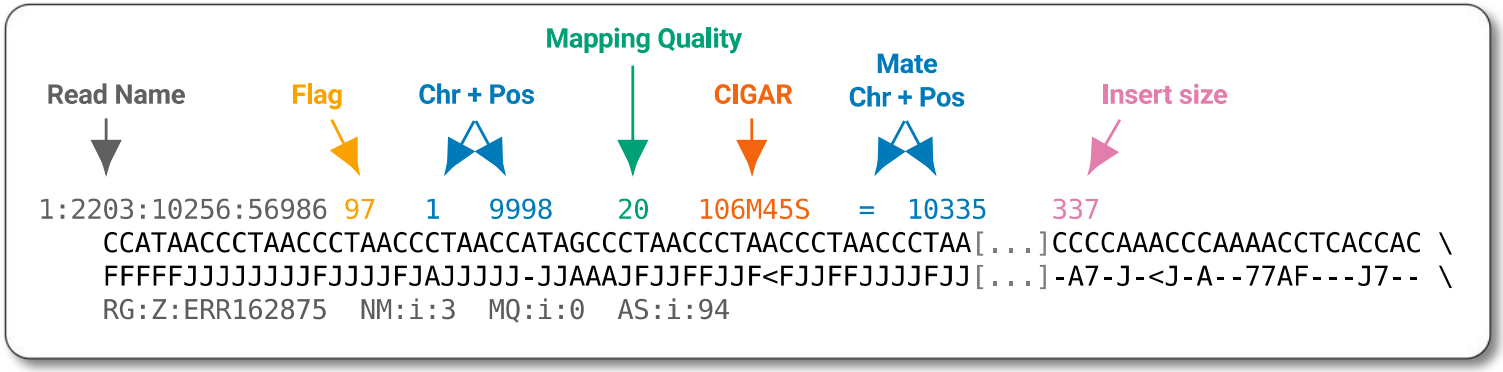
Quality	Probability of error	Accuracy
10 (Q10)	1 in 10	90%
20 (Q20)	1 in 100	99%
30 (Q30)	1 in 1000	99.9%
40 (Q40)	1 in 10000	99.99%

SAM / BAM: Sequence Alignment/Map format





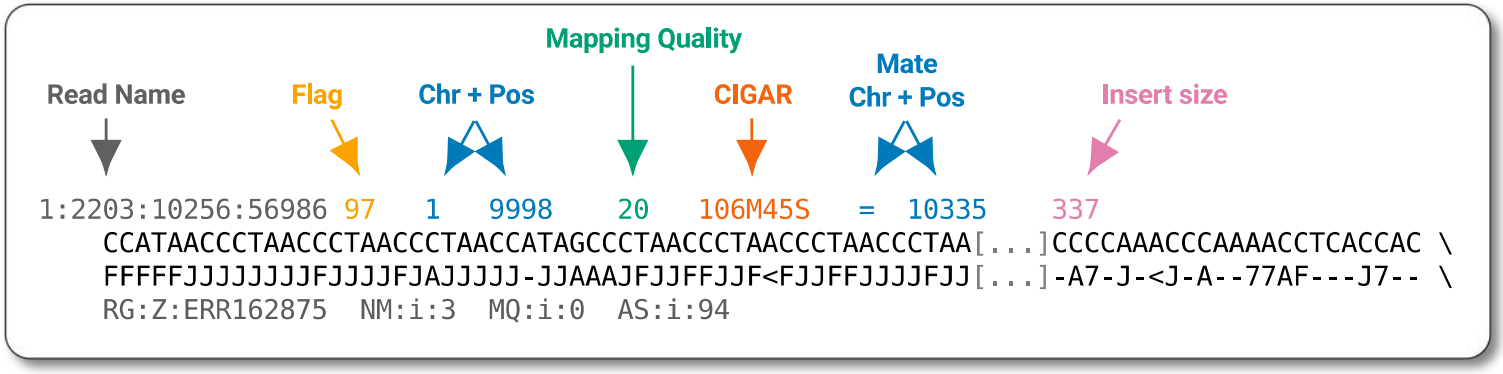
SAM / BAM: Sequence Alignment/Map format



Flag

Hex	Dec	Flag	Description
0x1	1	PAIRED	paired-end (or multiple-segment) sequencing technology
0x2	2	PROPER_PAIR	each segment properly aligned according to the aligner
0x4	4	UNMAP	segment unmapped
0x8	8	MUNMAP	next segment in the template unmapped
0x10	16	REVERSE	SEQ is reverse complemented
0x20	32	MREVERSE	SEQ of the next segment in the template is reversed
0x40	64	READ1	the first segment in the template
0x80	128	READ2	the last segment in the template
0x100	256	SECONDARY	secondary alignment
0x200	512	QCFAIL	not passing quality controls
0x400	1024	DUP	PCR or opticalduplicate
0x800	2048	SUPPLEMENTARY	supplementary alignment

SAM / BAM: Sequence Alignment/Map format



Insert size

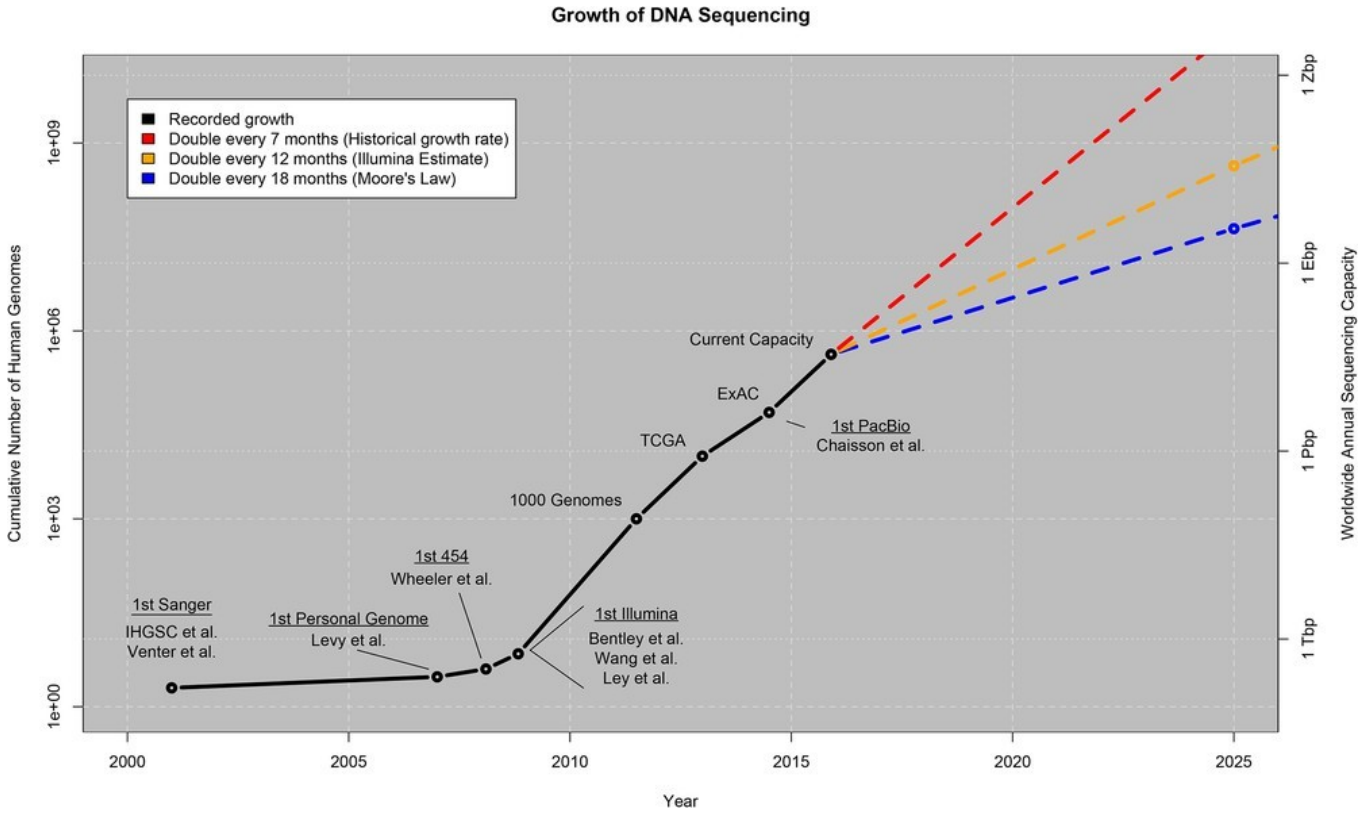
length of the DNA fragment sequenced from both ends by paired-end sequencing:

CRAM: Reference based Compression

BAM files are too large

- ~1.5-2 bytes per base pair

Increases in disk capacity are being far outstripped by sequencing technologies



Zachary D. Stephens, et al, Big Data: Astronomical or Genomical? DOI: 10.1371/journal.pbio.1002195

CRAM: Reference based Compression

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Increases in disk capacity are being far outstripped by sequencing technologies

BAM stores allof the data

- Every read base
- Every base quality
- Using a single conventional compression technique for all types of data

```
Reference sequence: ACGTACGTACGTACGTACGTACGTACGTAC
read 1:             ACGTACGTACGTACGTACGTGC
read 2:                TACGTACGCACGTACGTGCGTA
read 3:                  CGTACGCACGTACGTACGTACG
read 4:                    TACGTACGTACGTGCGTACGTA
read 5:                      CGCACGTACGTACGTACGTACG
read 6:                            TACGTGCGTACGTACGTAC
```

CRAM: Reference based Compression

BAM files are too large

- ~1.5-2 bytes per base pair

Increases in disk capacity are being far outstripped by sequencing technologies

BAM stores allof the data

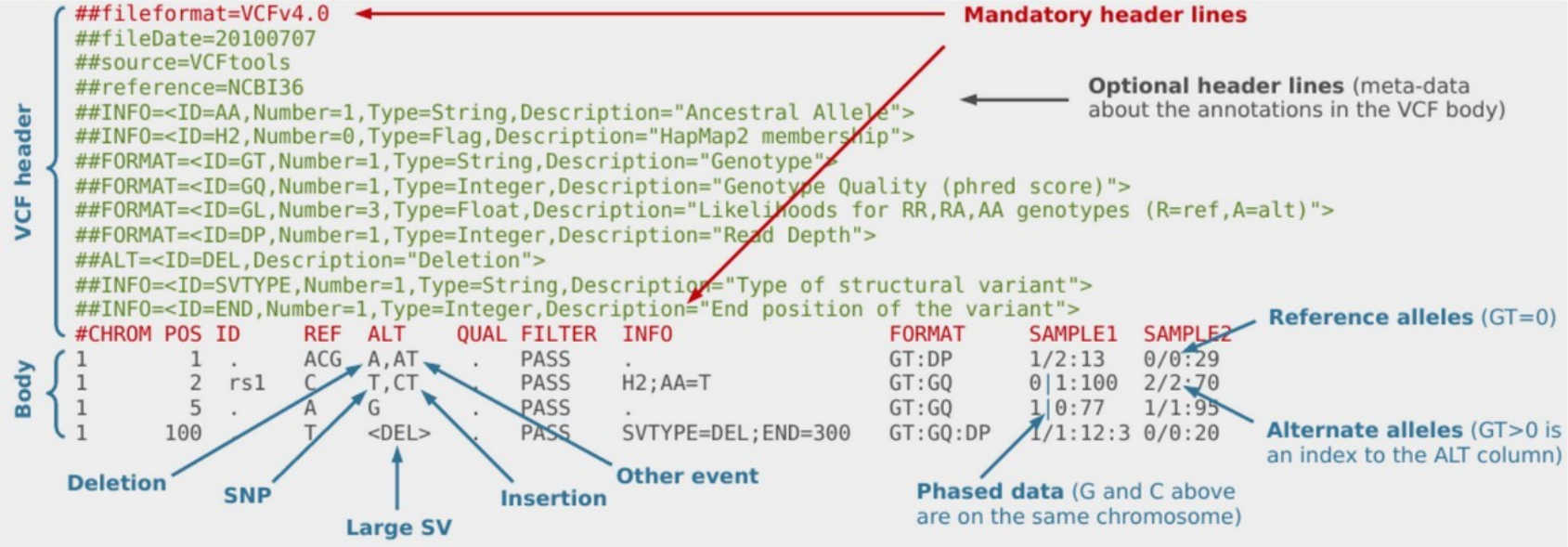
- Every read base
- Every base quality
- Using a single conventional compression technique for all types of data

```
Reference sequence: ACGTACGTACGTACGTACGTACGTACGTAC
read 1:                    ....................G.
read 2:                       ........C.............
read 3:                         ......C..............
read 4:                           .............G........
read 5:                             ..C....................
read 6:                                   .....G.............
```

CRAM: in lossless mode 60% of BAM size

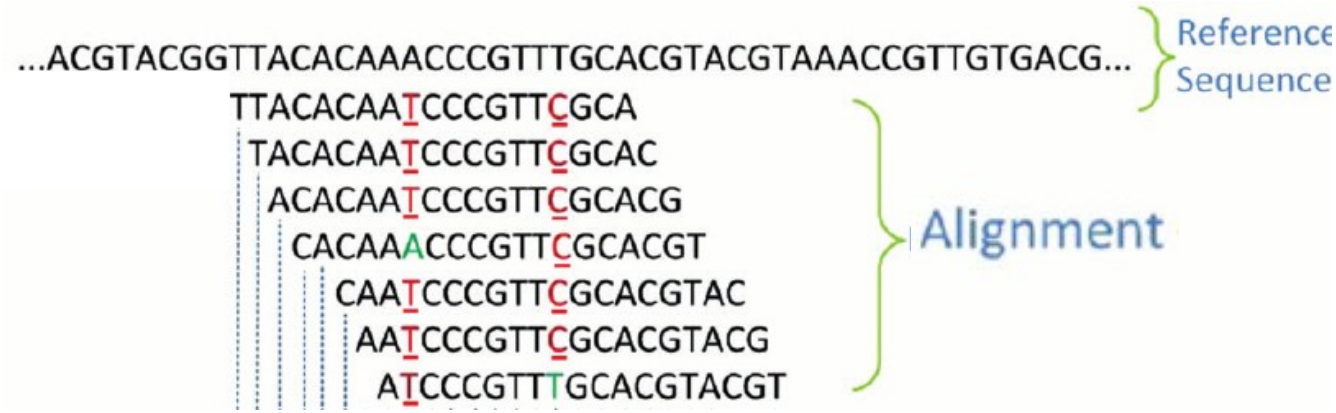
- Reference based compression
- Controlled loss of quality information
- Different compression methods for different type of data

VCF: Variant CallFormat



File format for storing variation data

- tab-delimited text, parsable by standard UNIX commands
- flexible and user-extensible
- composed of a header section and a body section



VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS  ID  REF ALT  QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11   24535  .   G   A    243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Row-oriented, tab-delimited file with eight mandatory columns (CHROM-INFO)

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">

#CHROM POS  ID  REF ALT  QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11    24535  .   G   A    243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Genomic coordinates

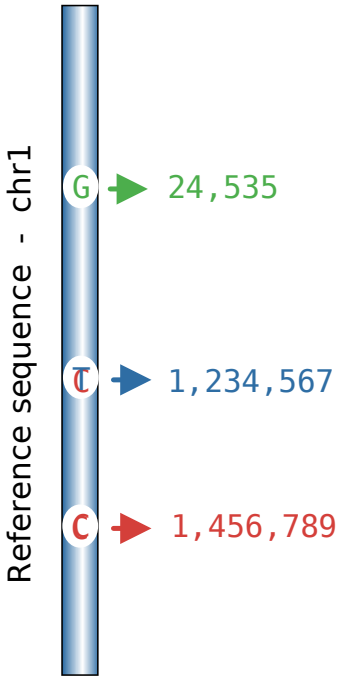
VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT   QUAL FILTER  INFO           FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11    24535  .   G   A     243   PASS    DP=221;AF=0.5  GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Arbitrary string, typically a dbSNP RefSNP id. Dot for missing value.

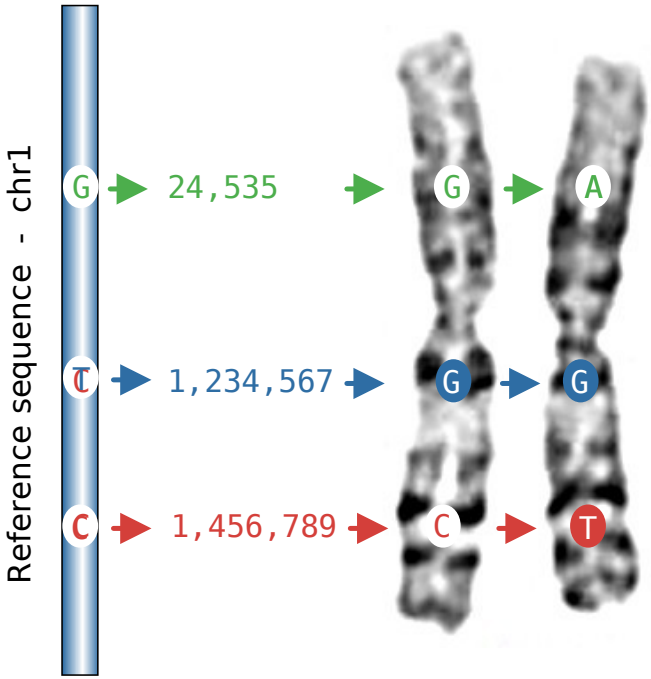
VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT  QUAL FILTER  INFO            FORMAT  SAMPLE1    SAMPLE2    SAMPLE3
11    24535  .   G   A    243  PASS    DP=221;AF=0.5   GT:AD   0/1:73,15  0/0:48,0   0/1:71,14
```



VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT  QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2    SAMPLE3
11    24535  .   G   A    243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0   0/1:71,14
```



VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT  QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2    SAMPLE3
11    24535  .   G   A    243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0   0/1:71,14
```

Although in theory phred-scaled probability, don't expect truly probabilistic interpretation in practice.

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT   QUAL FILTER INFO           FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11    24535  .   G   A     243  PASS   DP=221;AF=0.5  GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Soft-filter variants with e.g. low quality, low depth, etc.

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT   QUAL FILTER INFO          FORMAT  SAMPLE1    SAMPLE2    SAMPLE3
11    24535  .   G   A     243  PASS   DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0   0/1:71,14
```

Per-site annotations across all samples
Here **DP** is the cumulative read depth across all samples

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT   QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11    24535  .   G   A     243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Per-sample annotations. Here **GT** (genotype) and **AD** (allelic depth) will be present for each sample.

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS  ID  REF ALT  QUAL FILTER  INFO          FORMAT  SAMPLE1    SAMPLE2   SAMPLE3
11   24535  .   G   A    243  PASS    DP=221;AF=0.5 GT:AD   0/1:73,15  0/0:48,0  0/1:71,14
```

Per-sample values listed in the same order as specified in the FORMAT column, separated by a colon.

VCF anatomy

```
...
##INFO=<ID=DP,Number=1,Type=Integer,Description="Raw read depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency in population">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths (ref,alt,..)">
...
#CHROM POS   ID  REF ALT   QUAL FILTER  INFO            FORMAT  SAMPLE1    SAMPLE2    SAMPLE3
11    24535  .   G   A     243  PASS    DP=221;AF=0.5   GT:AD   0/1:73,15  0/0:48,0   0/1:71,14
12   153927  .   C   CA,T  15   LowQ    AF=0,0.1        GT      2/2        1/2        0/1
```

Multiple alternate alleles can be present in one row.

VCF vs BCF

VCFs can be very big

- compressed VCF with 3781 samples, human data:
  - 54 GB for chromosome 1
  - 680 GB whole genome

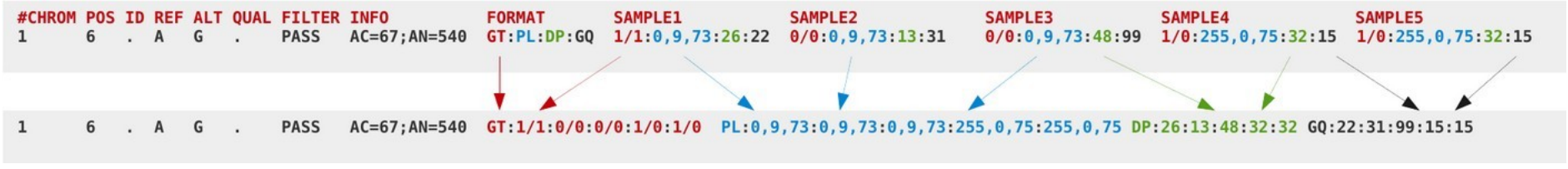
VCFs can be slow to parse

- text conversion is slow
- main bottleneck: FORMAT fields

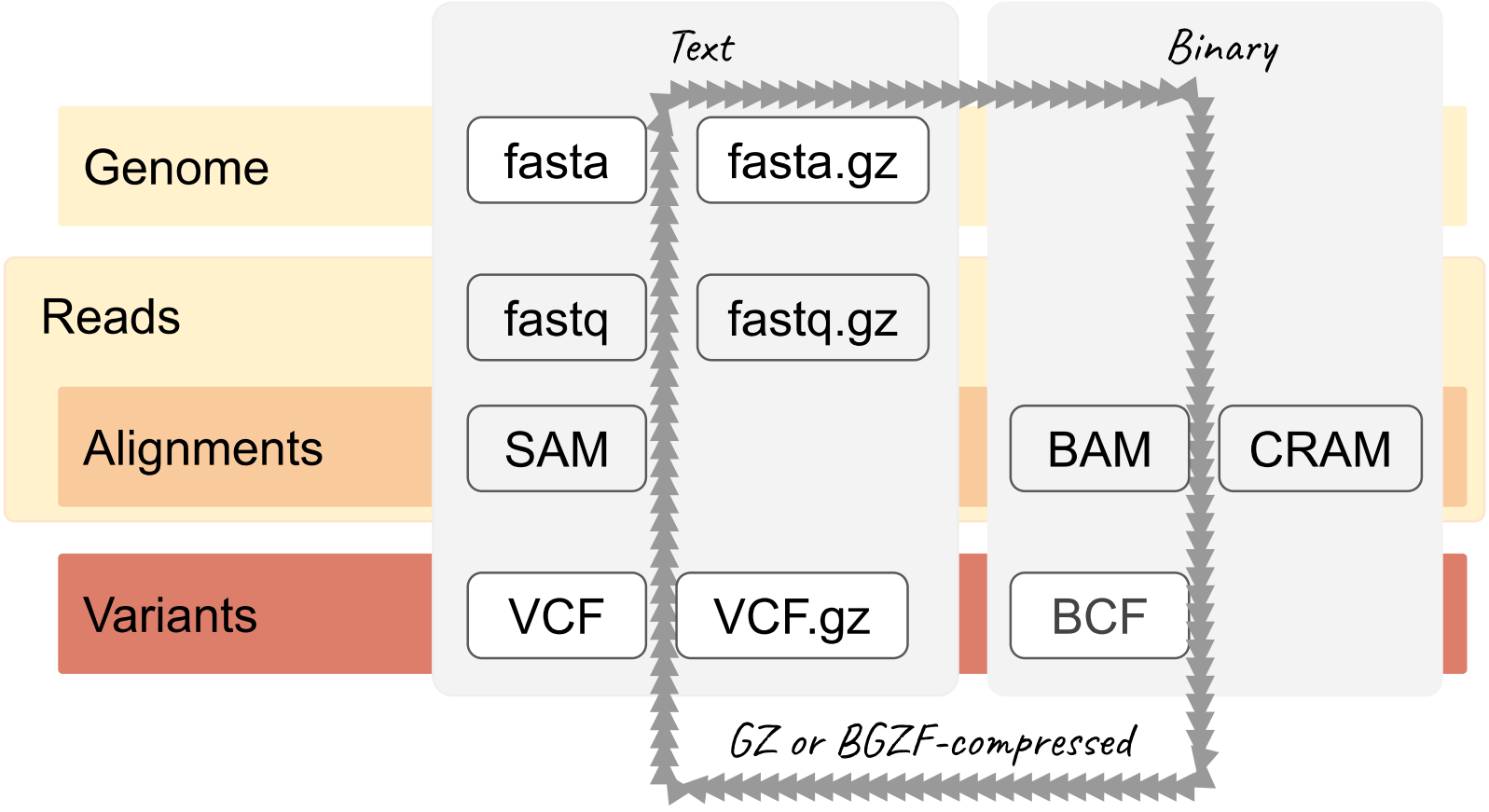
```
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT SAMPLE1 SAMPLE2
1  3  .  A  G  .  PASS  AC=67;AN=5400;DP=2809  GT:PL:DP:GQ  1/1:0,9,73:26:22    0/0:0,9,73:13:31    0/0:0,9,73:48:99  1/0:255,0,75:32:15  1/0:255,0,75:32:15
1  4  .  A  T  .  PASS  AC=15;AN=6800;DP=6056  GT:PL:DP:GQ  0/0:0,9,73:13:31    1/0:255,0,75:32:15  0/0:0,2,80:14:90  1/1:0,9,73:26:22    0/0:0,9,73:13:31
1  5  .  C  T  .  PASS  AC=20;AN=6701;DP=5234  GT:PL:DP:GQ  1/0:255,0,75:32:15  0/0:0,2,170:14:90   1/1:0,9,73:13:31  0/0:0,6,50:13:80    0/0:0,2,80:14:90
1  6  .  A  G  .  PASS  AC=67;AN=5400;DP=2809  GT:PL:DP:GQ  1/1:0,9,73:26:22    0/0:0,9,73:13:31    0/0:0,9,73:48:99  1/0:255,0,75:32:15  1/0:255,0,75:32:15
1  7  .  A  T  .  PASS  AC=15;AN=6800;DP=6056  GT:PL:DP:GQ  0/0:0,9,73:13:31    1/0:255,0,75:32:15  0/0:0,2,80:14:90  1/1:0,9,73:26:22    0/0:0,9,73:13:31
```

BCF

- binary representation of VCF
- fields rearranged for fast access



Data Formats Summary



Data Formats Summary