

File Types in Bioinformatics

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Enabler for Life Sciences

HOW STANDARDS PROLIFERATE: (SEE: A/C CHARGERS, CHARACTER ENCODINGS, INSTANT MESSAGING, ETC.)



- Overwhelming at first
- Overview
 - FASTA – reference sequences
 - FASTQ – reads in raw form
 - SAM – aligned reads
 - BAM – compressed SAM file
 - CRAM – even more compressed SAM file
 - GTF/GFF/BED – annotations

- Used for: nucleotide or peptide sequences
- Simple structure

> header
sequence

- Used for: nucleotide or peptide sequences
- Simple structure

```
> H.Sapiens chr17:135135135-1313566
ACTCAGATCGGAATAGCATACGCATACTCAGATCGGAATAGCATACGCAT
GGATAGCTCACGACACATGACACTACAGCCAGACTACACGACTACACGAT
AAGGATATAGGACTACGACTAGCATCGACTAACTAGCTACATACG
```

```
>that random protein sequence i saw yesterday
ARGAEBAEUIRGHAERGI AEUAEL LHGAEL GAHEGLAEJKRGNAERBI AE
AEGHAELGIHAEGOUIAENGAEBARI OTYUGAEGHILAEHRGAELRGYU
AEHAELAEIOGAEGAERTBETHUETHIRTHJNRFS
```

- Just like FASTA, but with quality values
- Used for: raw data from sequencing (unaligned reads)

@ header
sequence
+
quality

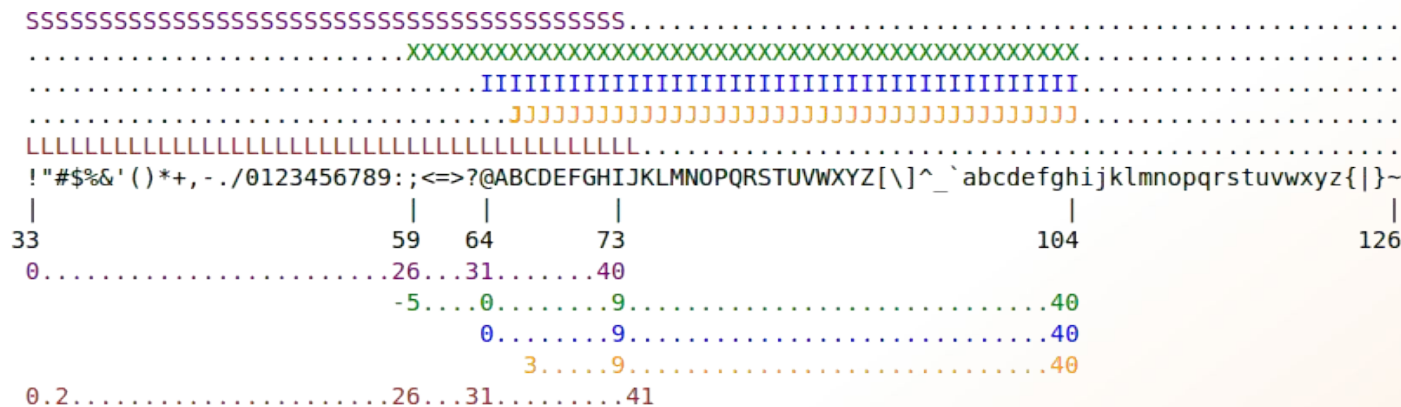
- Just like FASTA, but with quality values
- Used for: raw data from sequencing (unaligned reads)

```
@SEQ_001
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%%++)(%%%!' '*(((**%).1***-+*'')))**55CC!' '* (D
@SEQ_002
GATTTGGGGTTCAAAGCAGTATTTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
!''*(((((***+))%%>>CCCC%++(((**).1***-+*'')))**55CCF>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTTGGGGTTCAAAGCAGTAT AAGCAGTATCGATAAATCCATTTGTT
+
!''*(((((!*!''*(((((**)(%%%) .1***-+*'')))**55CCF>>>>>%%%) .1B5
```


- Quality 0-40
 - 40 = best
- ASCII encoded

| Dec | Hex | Char | Dec | Hex | Char | Dec | Hex | Char | Dec | Hex | Char |
|-----|-----|------------------|-----|-----|-------|-----|-----|------|-----|-----|------|
| 0 | 00 | Null | 32 | 20 | Space | 64 | 40 | @ | 96 | 60 | ` |
| 1 | 01 | Start of heading | 33 | 21 | ! | 65 | 41 | A | 97 | 61 | a |
| 2 | 02 | Start of text | 34 | 22 | " | 66 | 42 | B | 98 | 62 | b |
| 3 | 03 | End of text | 35 | 23 | # | 67 | 43 | C | 99 | 63 | c |
| 4 | 04 | End of transmit | 36 | 24 | \$ | 68 | 44 | D | 100 | 64 | d |
| 5 | 05 | Enquiry | 37 | 25 | % | 69 | 45 | E | 101 | 65 | e |
| 6 | 06 | Acknowledge | 38 | 26 | & | 70 | 46 | F | 102 | 66 | f |
| 7 | 07 | Audible bell | 39 | 27 | ' | 71 | 47 | G | 103 | 67 | g |
| 8 | 08 | Backspace | 40 | 28 | (| 72 | 48 | H | 104 | 68 | h |
| 9 | 09 | Horizontal tab | 41 | 29 |) | 73 | 49 | I | 105 | 69 | i |
| 10 | 0A | Line feed | 42 | 2A | * | 74 | 4A | J | 106 | 6A | j |
| 11 | 0B | Vertical tab | 43 | 2B | + | 75 | 4B | K | 107 | 6B | k |
| 12 | 0C | Form feed | 44 | 2C | , | 76 | 4C | L | 108 | 6C | l |
| 13 | 0D | Carriage return | 45 | 2D | - | 77 | 4D | M | 109 | 6D | m |
| 14 | 0E | Shift out | 46 | 2E | . | 78 | 4E | N | 110 | 6E | n |
| 15 | 0F | Shift in | 47 | 2F | / | 79 | 4F | O | 111 | 6F | o |
| 16 | 10 | Data link escape | 48 | 30 | 0 | 80 | 50 | P | 112 | 70 | p |
| 17 | 11 | Device control 1 | 49 | 31 | 1 | 81 | 51 | Q | 113 | 71 | q |
| 18 | 12 | Device control 2 | 50 | 32 | 2 | 82 | 52 | R | 114 | 72 | r |
| 19 | 13 | Device control 3 | 51 | 33 | 3 | 83 | 53 | S | 115 | 73 | s |
| 20 | 14 | Device control 4 | 52 | 34 | 4 | 84 | 54 | T | 116 | 74 | t |
| 21 | 15 | Neg. acknowledge | 53 | 35 | 5 | 85 | 55 | U | 117 | 75 | u |
| 22 | 16 | Synchronous idle | 54 | 36 | 6 | 86 | 56 | V | 118 | 76 | v |
| 23 | 17 | End trans. block | 55 | 37 | 7 | 87 | 57 | W | 119 | 77 | w |
| 24 | 18 | Cancel | 56 | 38 | 8 | 88 | 58 | X | 120 | 78 | x |
| 25 | 19 | End of medium | 57 | 39 | 9 | 89 | 59 | Y | 121 | 79 | y |
| 26 | 1A | Substitution | 58 | 3A | : | 90 | 5A | Z | 122 | 7A | z |
| 27 | 1B | Escape | 59 | 3B | ; | 91 | 5B | [| 123 | 7B | { |
| 28 | 1C | File separator | 60 | 3C | < | 92 | 5C | \ | 124 | 7C | |
| 29 | 1D | Group separator | 61 | 3D | = | 93 | 5D |] | 125 | 7D | } |
| 30 | 1E | Record separator | 62 | 3E | > | 94 | 5E | ^ | 126 | 7E | ~ |
| 31 | 1F | Unit separator | 63 | 3F | ? | 95 | 5F | _ | 127 | 7F | □ |

- Quality 0-40
 - 40 = best
- ASCII encoded

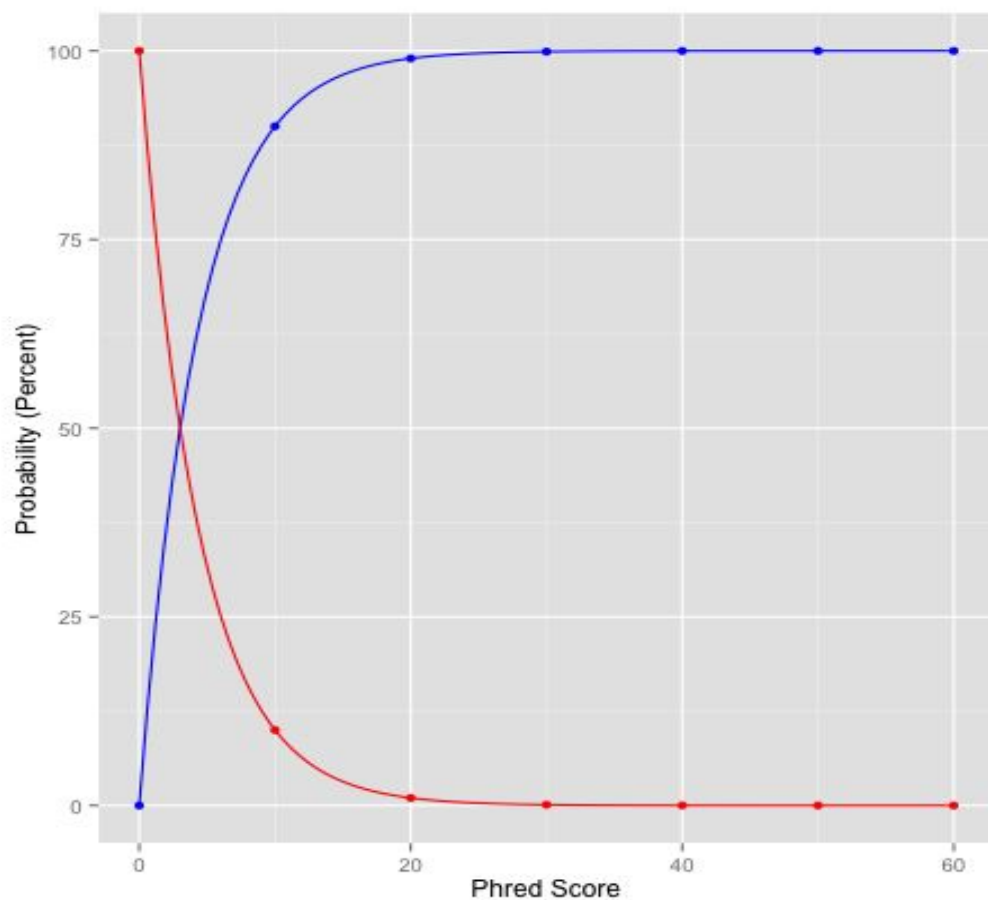


S - Sanger Phred+33, raw reads typically (0, 40)
 X - Solexa Solexa+64, raw reads typically (-5, 40)
 I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
 J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
 with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
 (Note: See discussion above).
 L - Illumina 1.8+ Phred+33, raw reads typically (0, 41)

- Quality 0-40
 - 40 = best
- ASCII encoded

(Illumina 1.8+ = 41)

```
@SEQ_001
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%>>+)(%%>>!'')*(((((**%).1***-+*')))**55CC!'')*(D
@SEQ_002
GATTTGGGGTTCAAAGCAGTATTTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
!''*(((((***+))%%>>CCCC%+((((**).1***-+*')))**55CCF>>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTTGGGGTTCAAAGCAGTAT AAGCAGTATCGATAAATCCATTTGTT
+
!''*(((((!*!''*(((((**)(%%>>).1***-+*')))**55CCF>>>>>>%%>>).1B5
```



Functions

- Accuracy
- Error

| Phred Quality Score | Error | Accuracy |
|---------------------|------------------------|----------|
| 10 | $1/10 = 10\%$ | 90% |
| 20 | $1/100 = 1\%$ | 99% |
| 30 | $1/1000 = 0.1\%$ | 99.9% |
| 40 | $1/10000 = 0.01\%$ | 99.99% |
| 50 | $1/100000 = 0.001\%$ | 99.999% |
| 60 | $1/1000000 = 0.0001\%$ | 99.9999% |

- Used for: aligned reads
- Lots of columns..

sequence string.sam

<QNAME> <FLAG> <RNAME> <POS> <MAPQ> <CIGAR> <MRNM> <MPOS> <ISIZE> <SEQ> <QUAL> [<TAG>:<VTYPE>:<VALUE> [...]]

| Field | Regular expression | Range | Description |
|-------|-------------------------------|--------------------------------------|---|
| QNAME | [^ \t\n\r]+ | | Query pair NAME if paired; or Query NAME if unpaired ² |
| FLAG | [0-9]+ | [0,2 ¹⁶ -1] | bitwise FLAG (Section 2.2.2) |
| RNAME | [^ \t\n\r@=]+ | | Reference sequence NAME ³ |
| POS | [0-9]+ | [0,2 ²⁹ -1] | 1-based leftmost POSition/coordinate of the clipped sequence |
| MAPQ | [0-9]+ | [0,2 ⁸ -1] | MAPping Quality (phred-scaled posterior probability that the mapping position of this read is incorrect) ⁴ |
| CIGAR | ([0-9]+[MIDNSHP])+ * | | extended CIGAR string |
| MRNM | [^ \t\n\r@=]+ | | Mate Reference sequence NaMe; “=” if the same as <RNAME> ³ |
| MPOS | [0-9]+ | [0,2 ²⁹ -1] | 1-based leftmost Mate POSition of the clipped sequence |
| ISIZE | -? [0-9]+ | [-2 ²⁹ ,2 ²⁹] | inferred Insert SIZE ⁵ |
| SEQ | [acgtnACGTN.=]+ * | | query SEQUENCE; “=” for a match to the reference; n/N/. for ambiguity; cases are not maintained ^{6,7} |
| QUAL | [!-~]+ * | [0,93] | query QUALity; ASCII-33 gives the Phred base quality ^{6,7} |
| TAG | [A-Z] [A-Z 0-9] | | TAG |
| VTYPE | [AifZH] | | Value TYPE |
| VALUE | [^ \t\n\r]+ | | match <VTYPE> (space allowed) |

- Used for: aligned reads
- Lots of columns..

```
@SQ      SN:31      LN:39895921
@PG      ID:bwa     PN:bwa     VN:0.7.8-r455   CL:bwa samse -f 02_sample.fq.sam /sw/data/uppnex/reference/Canis_familiaris/CanFam3/program_files/bwa/chr.31.fa 01_sample.fq.sai sample.fq
read_001 0      chr31    26546617    37    150M    *      0      0      AAAGGCTATTTCCACCT    )%%>(((**+))%%>%%>    XT:A:U NM:i:0 XO:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:150
read_002 0      chr31    26546617    37    150M    *      0      0      AGGAGAAAGGCAGATCG    '*((!''*(((((**+))%%    XT:A:U NM:i:0 XO:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:150
read_003 0      chr31    26546617    37    150M    *      0      0      AAAGGAGGCTAACGTTT    )%%>!''*((**+))%%(((*    XT:A:U NM:i:0 XO:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:150
read_004 0      chr31    26546617    37    150M    *      0      0      AGGCCATGACATCATCT    *(((((**+))%%>%%>%%    XT:A:U NM:i:0 XO:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:150
read_005 0      chr31    26546617    37    150M    *      0      0      TAGCAGAGCTATTT CAT    ((**!''*((**+))%%>AD    XT:A:U NM:i:0 XO:i:1 X1:i:0 XM:i:0 XO:i:0 XG:i:0 MD:Z:150
```

Read name

Start position
bp chr

Sequence

Quality

- Binary SAM (compressed)
- 25% of the size
- SAMtools to convert
- .bai = BAM index

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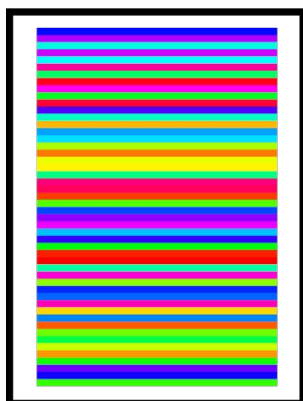
- Random order
- Have to sort before indexing



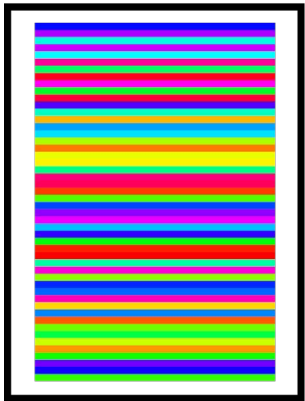
- Random order
- Have to sort before indexing



Unsorted BAM



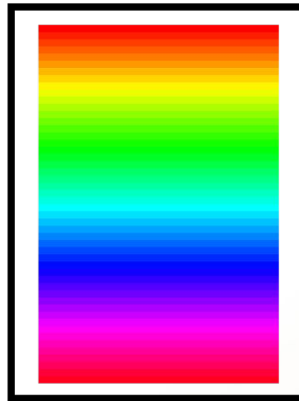
Unsorted BAM



samtools sort



Sorted BAM

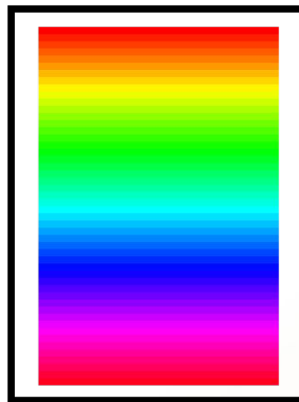


Unsorted BAM



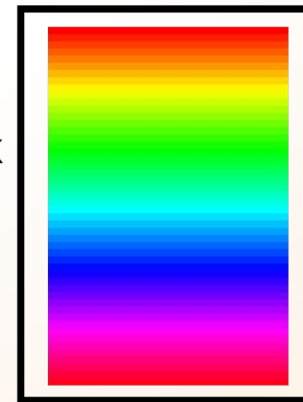
samtools sort

Sorted BAM



samtools index

Sorted BAM



BAM index

Chr1 1536
Chr2 2846
Chr3 5687
Chr4 6468
Chr5 8346
...

- # Reads

TAGATCGAGGCTGAGTCACGACG

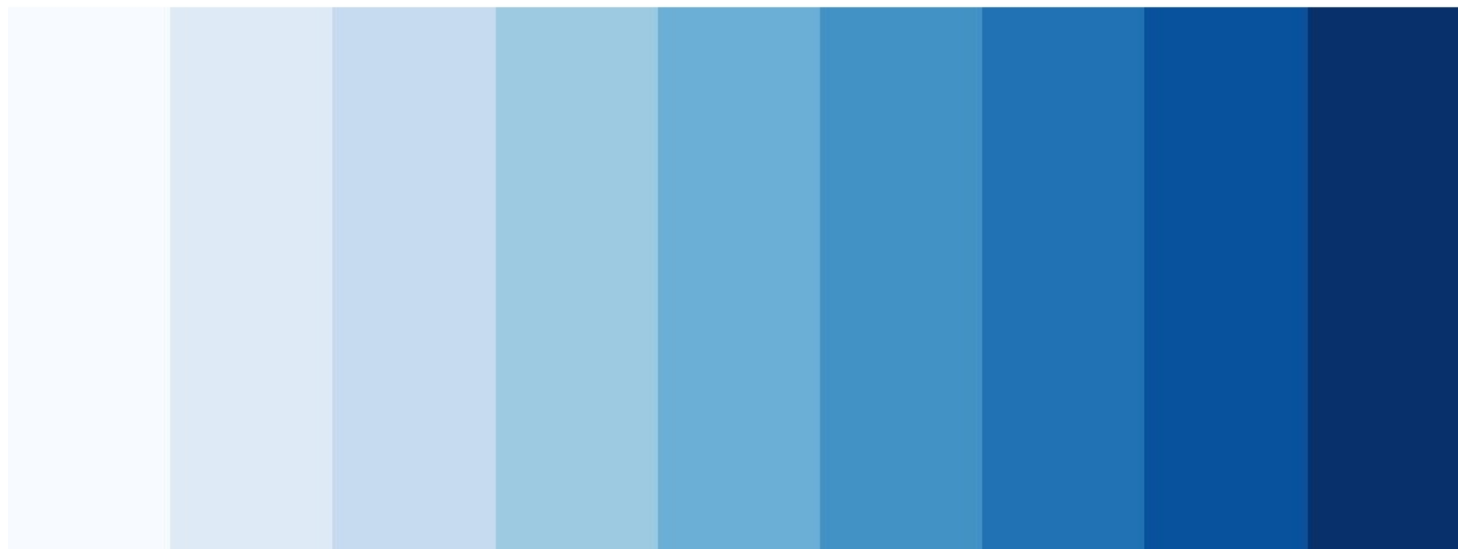
Ref: ATTCGGACGTAGATCGACGCTGAGTCACGACGTGTTGTGAGAGCCGTAGAC

- Quality scores?
- 3 modes:
 - Lossless
 - Binned
 - No quality



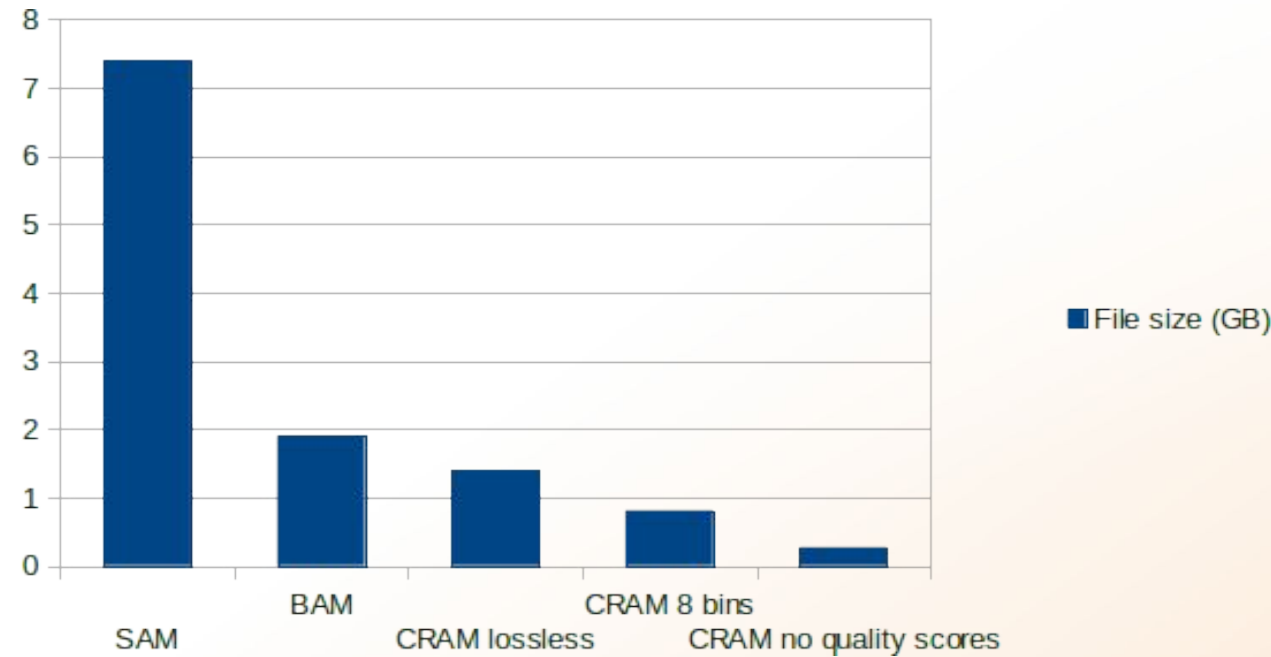
1 2 3 4 5 6 7 8 9 10 11 12 13 14 ... 32 33 34 35 36 37 38 39 40 41

1-5 6-10 11-15 16-20 21-25 26-30 31-35 35-40 41-45



=> Reducing the number of quality values increases shared blocks and improves compression.

- Quality scores?
- 3 modes:
 - Lossless
 - Binned
 - No quality



- Not widespread, yet

- Used for: annotations
- Column structure
- one line = one feature (match, exon, etc)

BED format:

- 3-12 columns
3 mandatory fields

+ 9 optional fields

| chr | start | stop | extra info |
|------------|--------------|-------------|-------------------|
| chr1 | 213941196 | 213942363 | |
| chr1 | 213942363 | 213943530 | |

BED format:

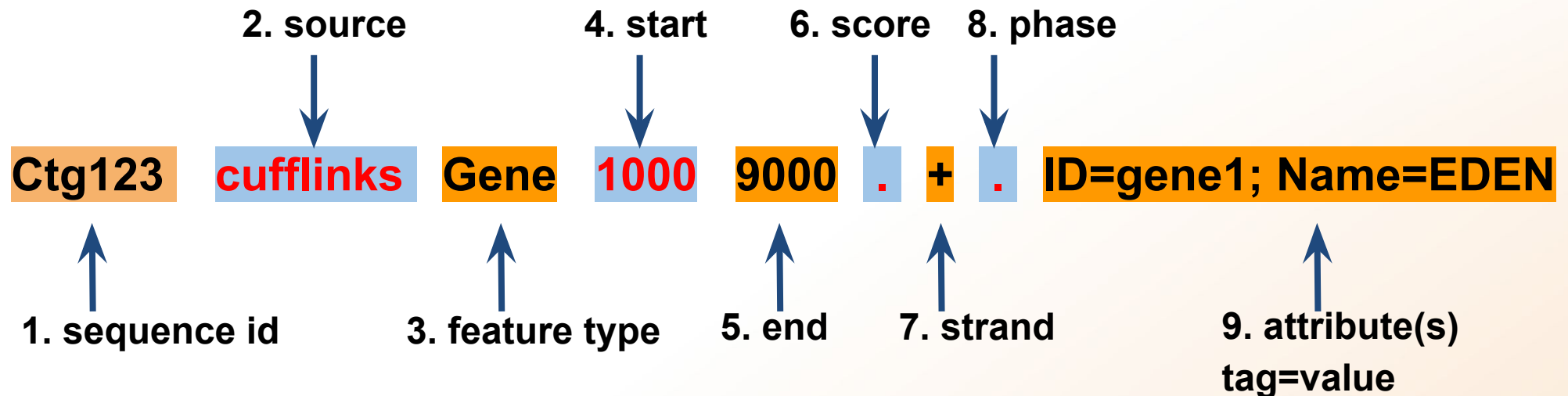
● optional fields

4. **name** - Label to be displayed under the feature, if turned on in "Configure this page".
5. **score** - A score between 0 and 1000.
6. **strand** - defined as + (forward) or - (reverse).
7. **thickStart** - coordinate at which to start drawing the feature as a solid rectangle
8. **thickEnd** - coordinate at which to stop drawing the feature as a solid rectangle
9. **itemRgb** - an RGB colour value (e.g. 0,0,255). Only used if there is a track line with the value of itemRgb set to "on" (case-insensitive).
10. **blockCount** - the number of sub-elements (e.g. exons) within the feature
11. **blockSizes** - the size of these sub-elements
12. **blockStarts** - the start coordinate of each sub-element

```
chr7    127471196    127472363    Pos1    0    +    127471196    127472363    255,0,0
chr7    127472363    127473530    Pos2    0    +    127472363    127473530    255,0,0
```

GFF/GTF format:

- 9 columns



```
chr22  TeleGene  enhancer  100000000  10001000  500  +  .  touch1
chr22  TeleGene  promoter  10010000  10010100  900  +  .  touch1
chr22  TeleGene  promoter  10020000  10025000  800  -  .  touch2
```

Laboratory time! (yet again)

https://nbisweden.github.io/workshop-ngsintro/2603/topics/linux/lab_linux_filetypes.html