SciLifeLab
UPPSALA

# File Types in Bioinformatics

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Enabler for Life Sciences

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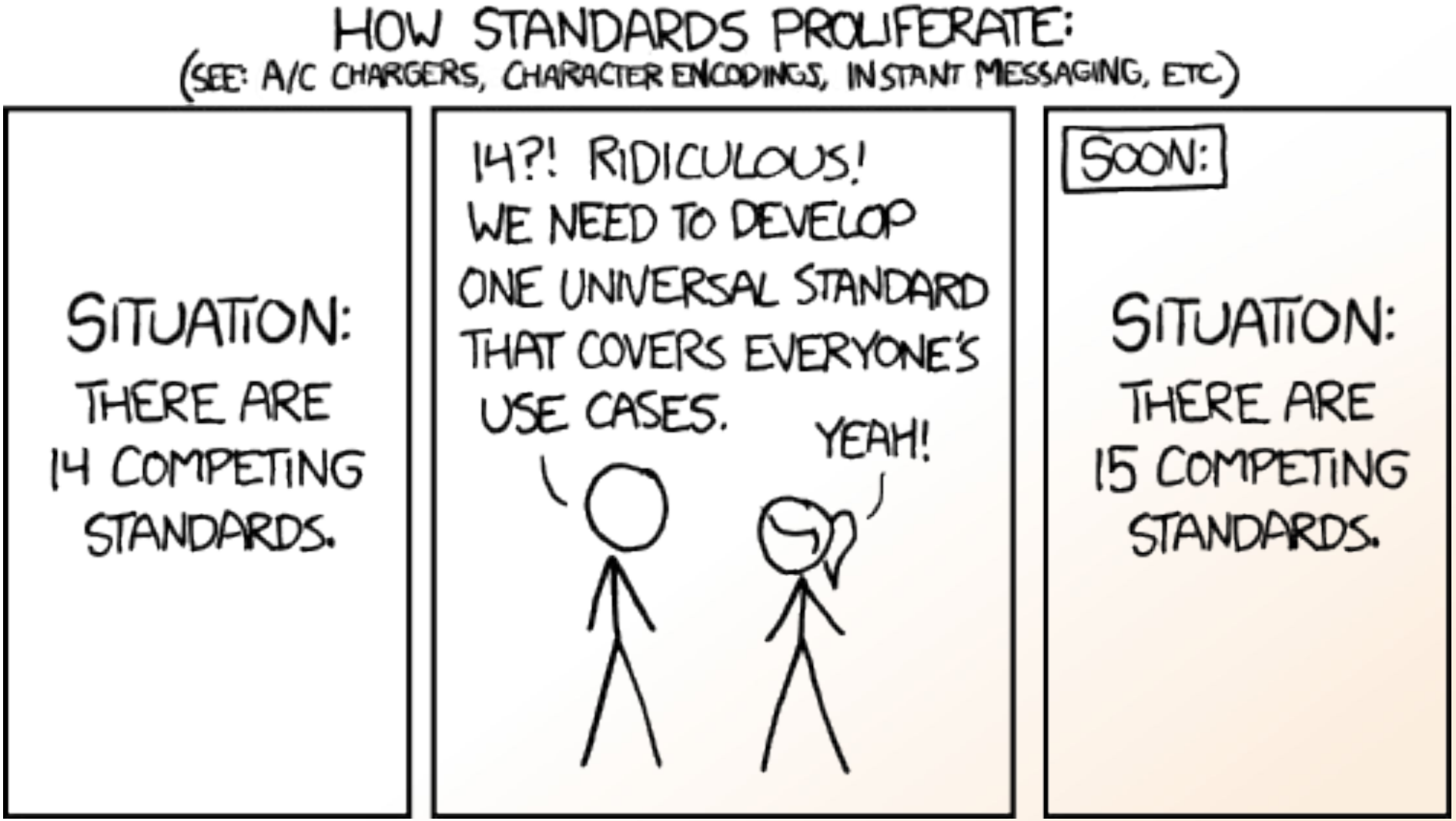


- http://xkcd.com

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- 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

# FASTA

- Used for: nucleotide or peptide sequences
- Simple structure

> header
sequence

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# FASTA

- Used for: nucleotide or peptide sequences
- Simple structure

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

>that random protein sequence i saw yesterday
ARGAEBAEUIRGHAERGIAEUAEILHGAEIGAHEGLAEJKRGNAERBIAE
AEGHAELGIHAEGOUIAENGAEBAERIOTYUGAEGHILAEHRGAEIRGYU
AEHAEHAEIOGAEGAERTBETHUETHIRTHJNRFS
```

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# FASTQ

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

@ header
sequence
+
quality

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# FASTQ

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

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

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# FASTQ

- 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 | □ |

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# FASTQ

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

(Illumina 1.8+ = 41)

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
 ...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
 ................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                         |    |        |                              |                     |
33                        59   64       73                            104                   126
 0........................26...31.......40
                          -5....0........9.............................40
                               0........9.............................40
                                  3.....9.............................40
 0.2......................26...31.........41

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)
```

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# FASTQ

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

(Illumina 1.8+ = 41)

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

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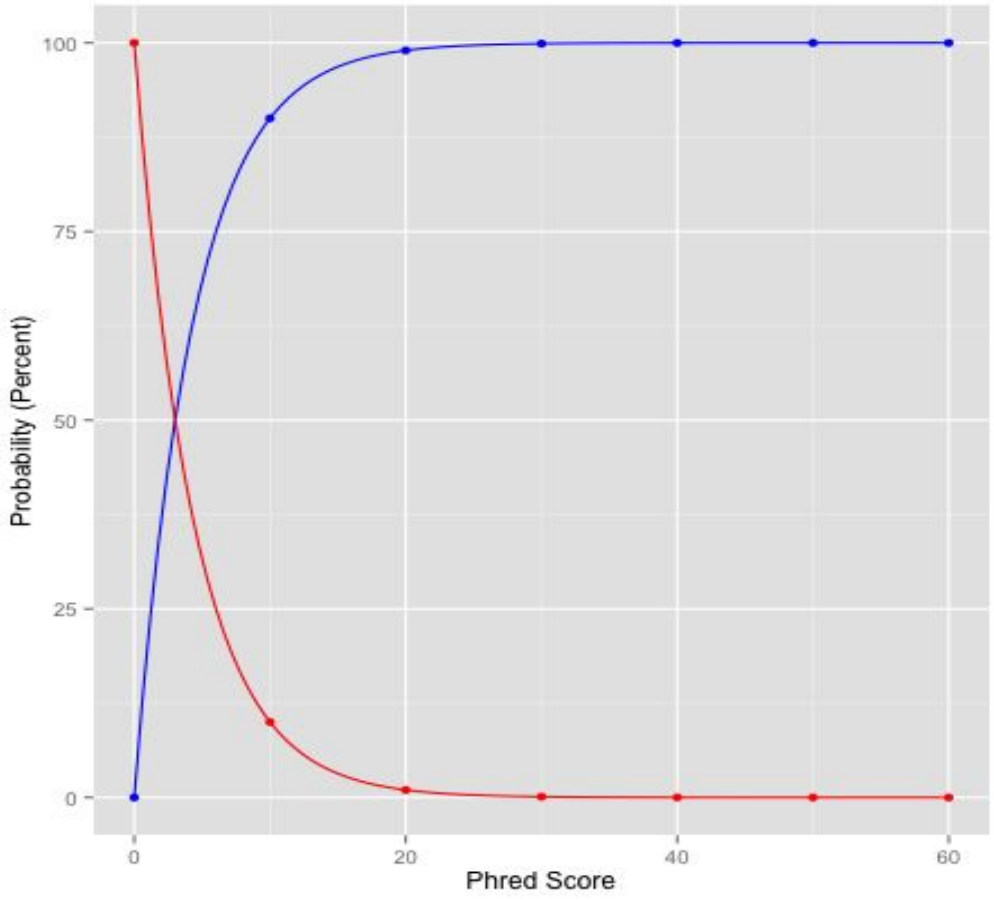
# FASTQ




| 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% |

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# SAM

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

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# SAM

seguence_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-Z0-9] | | TAG |
| VTYPE | [AifZH] | | Value TYPE |
| VALUE | [^\t\n\r]+ | | match <VTYPE> (space allowed) |

# SAM

- 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       TAGCAGAGCTATTTCAT       ((**!''*((*+))%%>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
```

Start position
bp chr

Sequence

Quality

Read name

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# BAM

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

## Contents

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# BAM

- Random order
- Have to sort before indexing

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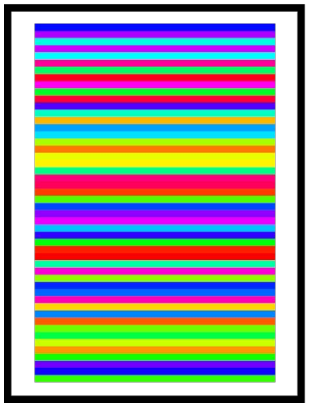
# BAM

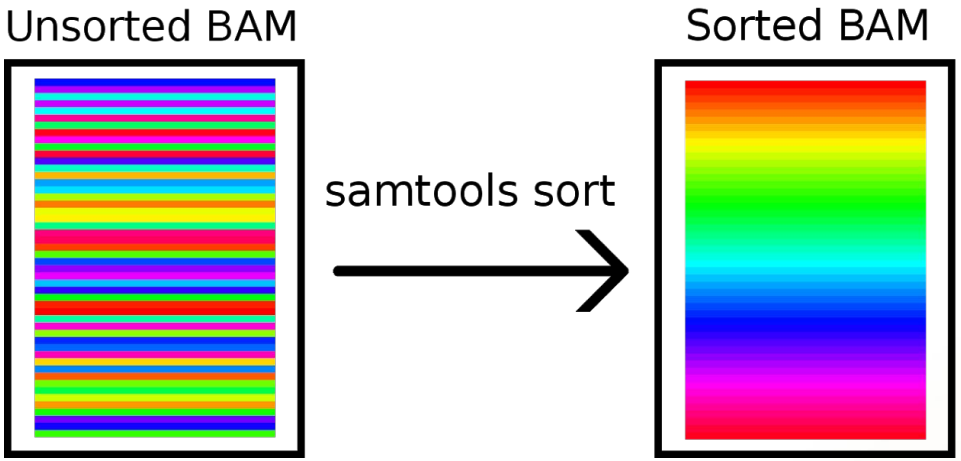
- Random order
- Have to sort before indexing

# BAM

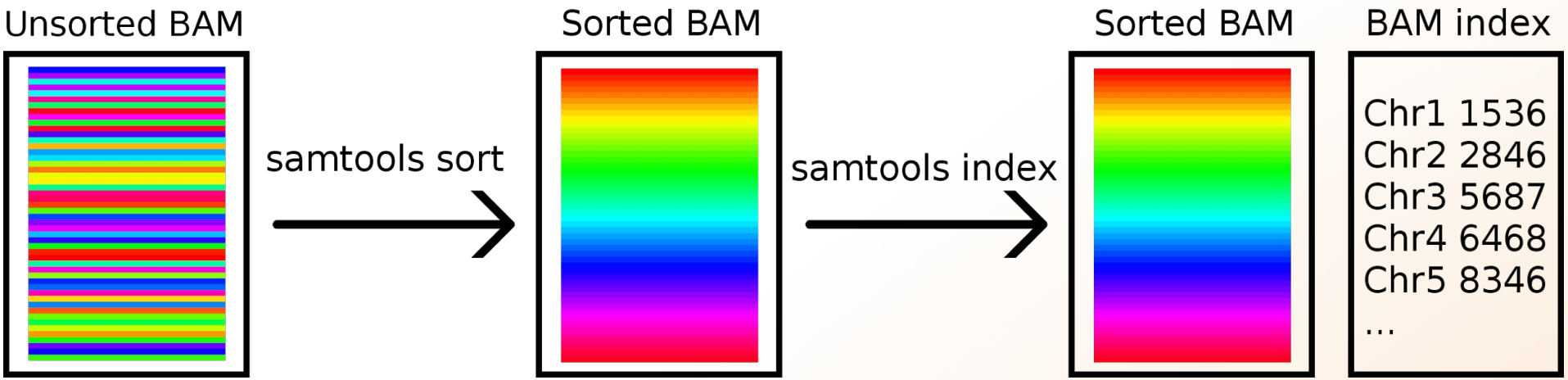
Unsorted BAM



# BAM



# BAM


Unsorted BAM
samtools sort
Sorted BAM
samtools index
Sorted BAM
BAM index
Chr1 1536
Chr2 2846
Chr3 5687
Chr4 6468
Chr5 8346
...

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# CRAM

- Very complex format
- Used together with a reference genome

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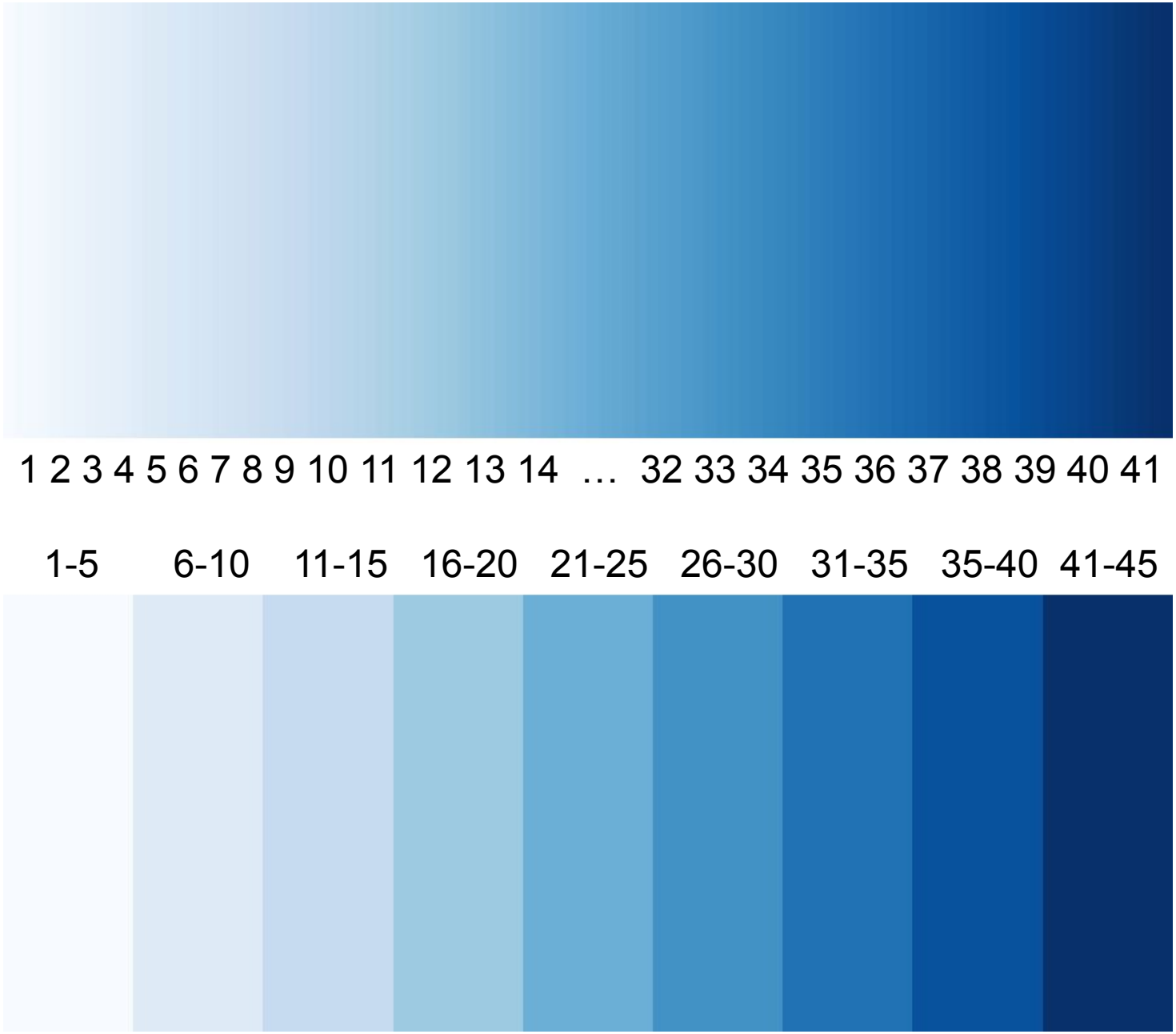
# CRAM

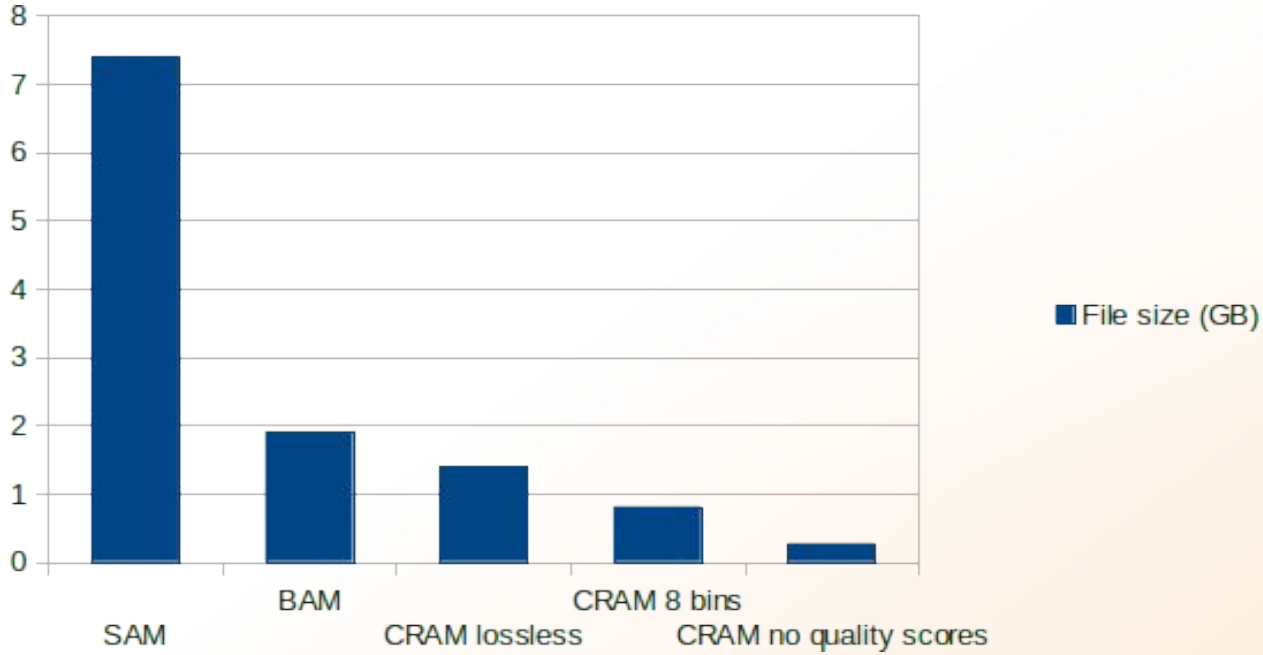
- Quality scores?
- 3 modes:
  - Lossless
  - Binned
  - No quality




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

# CRAM

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




- Not widespread, yet

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# GTF/GFF/BED

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

# GTF/GFF/BED

BED format:

- 3-12 columns

  3 mandatory fields + 9 optional fields

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

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# GTF/GFF/BED

## 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

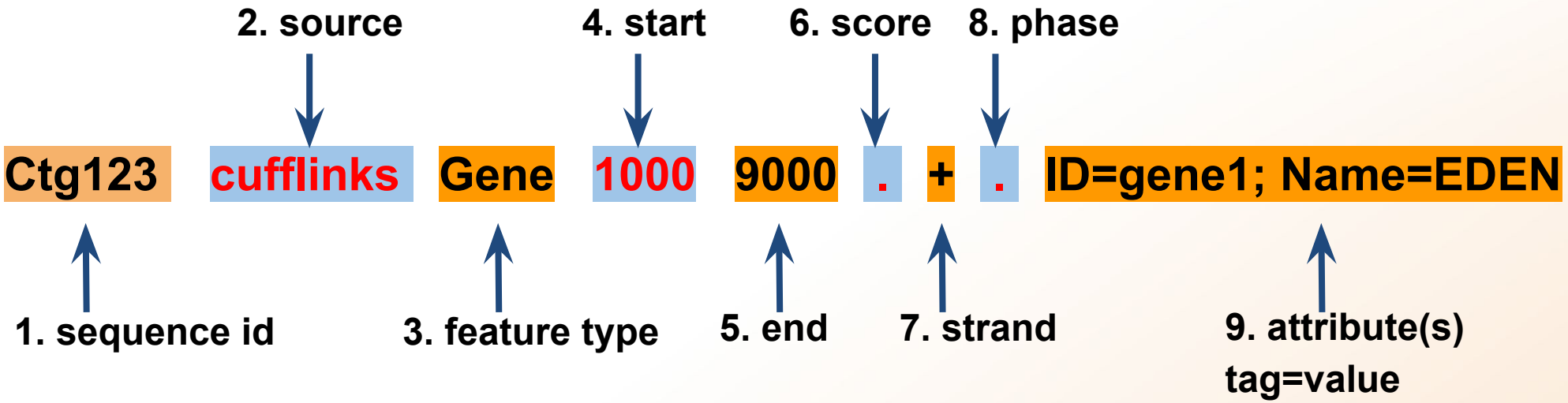
**12. blockStarts** - the start coordinate of each sub-element

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

# GTF/GFF/BED

GFF/GTF format:

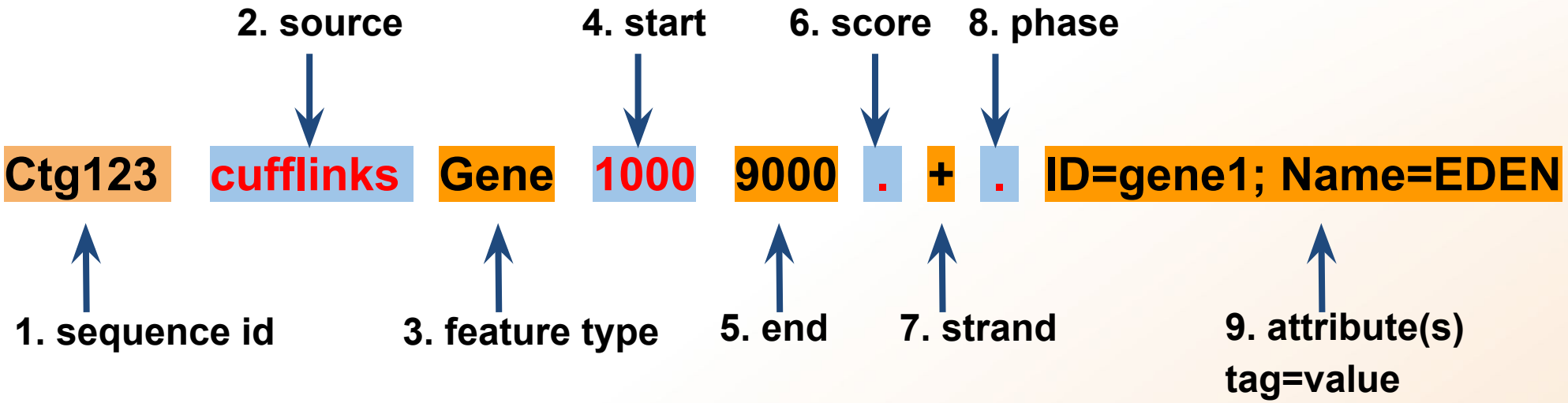
- 9 columns



# GTF/GFF/BED

## GFF/GTF format:

- 9 columns




```
chr22  TeleGene enhancer  10000000  10001000  500 +  .  touch1
chr22  TeleGene promoter  10010000  10010100  900 +  .  touch1
chr22  TeleGene promoter  10020000  10025000  800 -  .  touch2
```

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# Laboratory time! (yet again)

https://nbisweden.github.io/workshop-ngsintro/2011/lab_linux_filetypes.html