



# 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 LHGAEI GAHEGLAEJKRGNAERBI AE  
AEGHAELGIHAEGOUIAENGAEBARI OTYUGAEGHILAEHRGAEIRGYU  
AEHAEHAEI OGAEGAERTBETHUETHIRTHJNRFS
```

- 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)

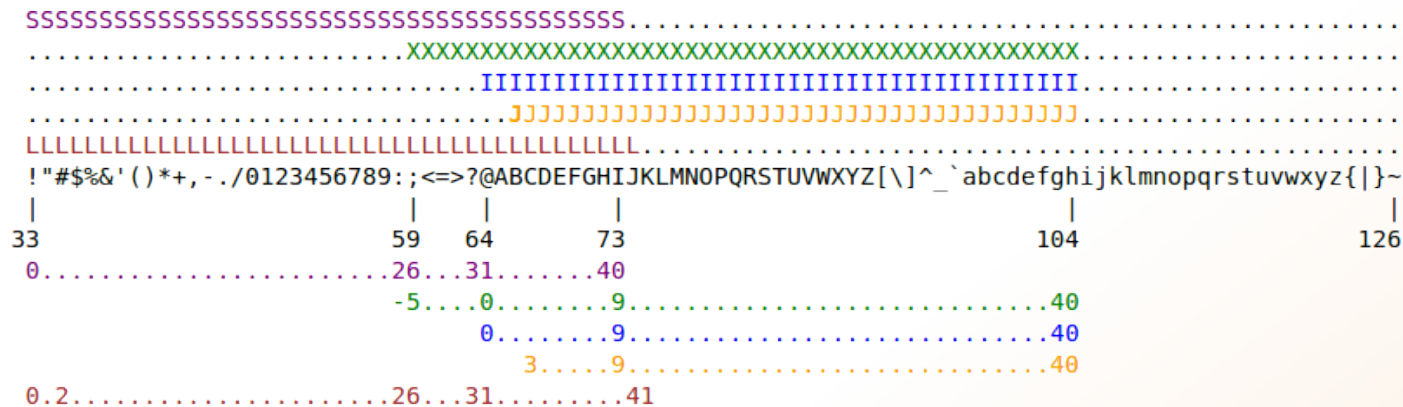
```
@SEQ_001
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%%++) (%%%! ''*(((((**%) .1***-.+*' ')) **55CC! ''*(D
@SEQ_002
GATTTGGGGTTCAAAGCAGTATTTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
!''*(((((***+))%>>CCCC%++ ((( (**).1***-.+*' ')) **55CCF>>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTTGGGGTTCAAAGCAGTAT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

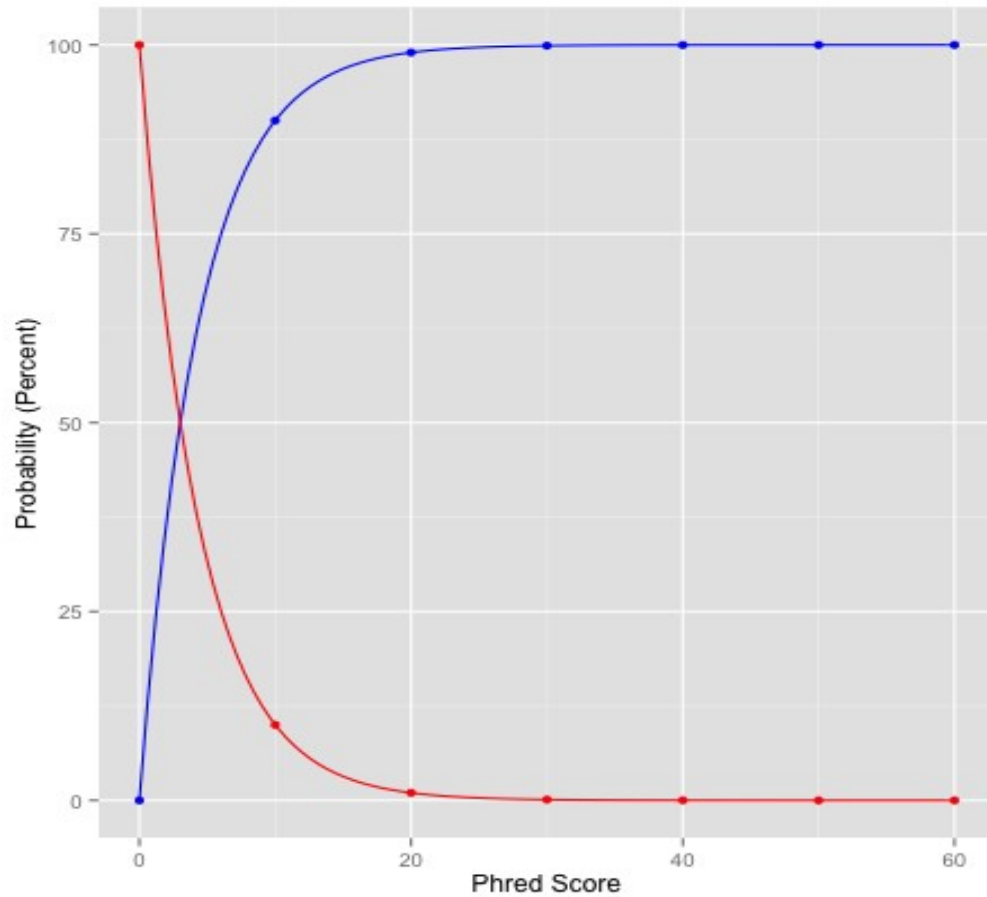
- Quality 0-40 (Illumina 1.8+ = 41)
  - 40 = best
- ASCII encoded



- 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 (Illumina 1.8+ = 41)
  - 40 = best
- ASCII encoded

```
@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 <sup>2</sup>
FLAG	[ 0-9 ]+	[0,2 <sup>16</sup> -1]	bitwise FLAG (Section 2.2.2)
RNAME	[ ^ \t\n\r@= ]+		Reference sequence NAME <sup>3</sup>
POS	[ 0-9 ]+	[0,2 <sup>29</sup> -1]	1-based leftmost POSition/coordinate of the clipped sequence
MAPQ	[ 0-9 ]+	[0,2 <sup>8</sup> -1]	MAPping Quality (phred-scaled posterior probability that the mapping position of this read is incorrect) <sup>4</sup>
CIGAR	( [ 0-9 ]+[ MIDNSHP ] )+   \*		extended CIGAR string
MRNM	[ ^ \t\n\r@= ]+		Mate Reference sequence NaMe; “=” if the same as <RNAME> <sup>3</sup>
MPOS	[ 0-9 ]+	[0,2 <sup>29</sup> -1]	1-based leftmost Mate POSition of the clipped sequence
ISIZE	-?[ 0-9 ]+	[-2 <sup>29</sup> ,2 <sup>29</sup> ]	inferred Insert SIZE <sup>5</sup>
SEQ	[ acgtnACGTN.= ]+   \*		query SEQUENCE; “=” for a match to the reference; n/N/. for ambiguity; cases are not maintained <sup>6,7</sup>
QUAL	[ !-~ ]+   \*	[0,93]	query QUALity; ASCII-33 gives the Phred base quality <sup>6,7</sup>
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  X0: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  X0: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  X0: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  X0: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      TAGCAGAGCTATTTTCAT    (((**!'!*((+))%%>AD    XT:A:U  NM:i:0  X0: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



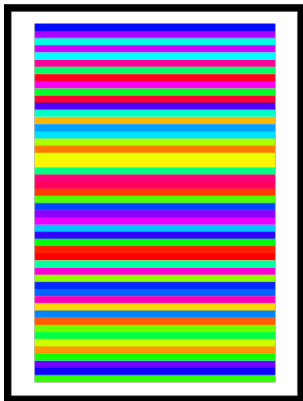
- Random order
- Have to sort before indexing



Unsorted BAM



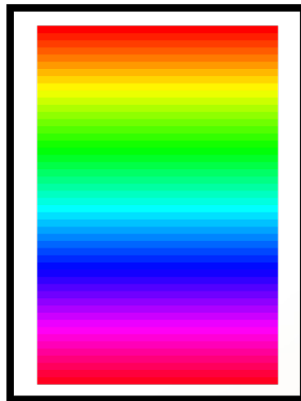
Unsorted BAM



samtools sort



Sorted BAM



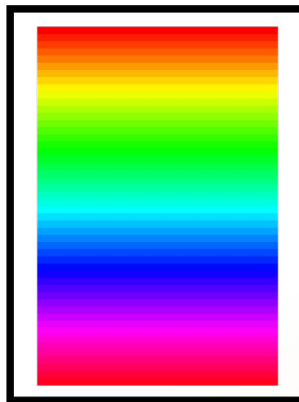
Unsorted BAM



samtools sort



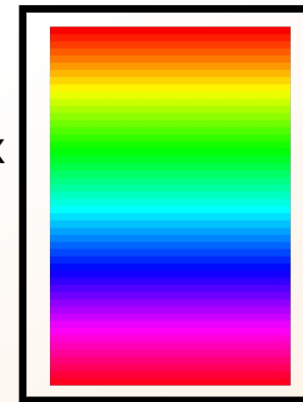
Sorted BAM



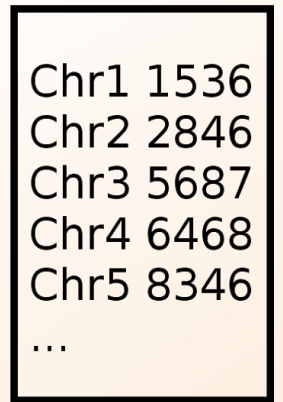
samtools index



Sorted BAM



BAM index





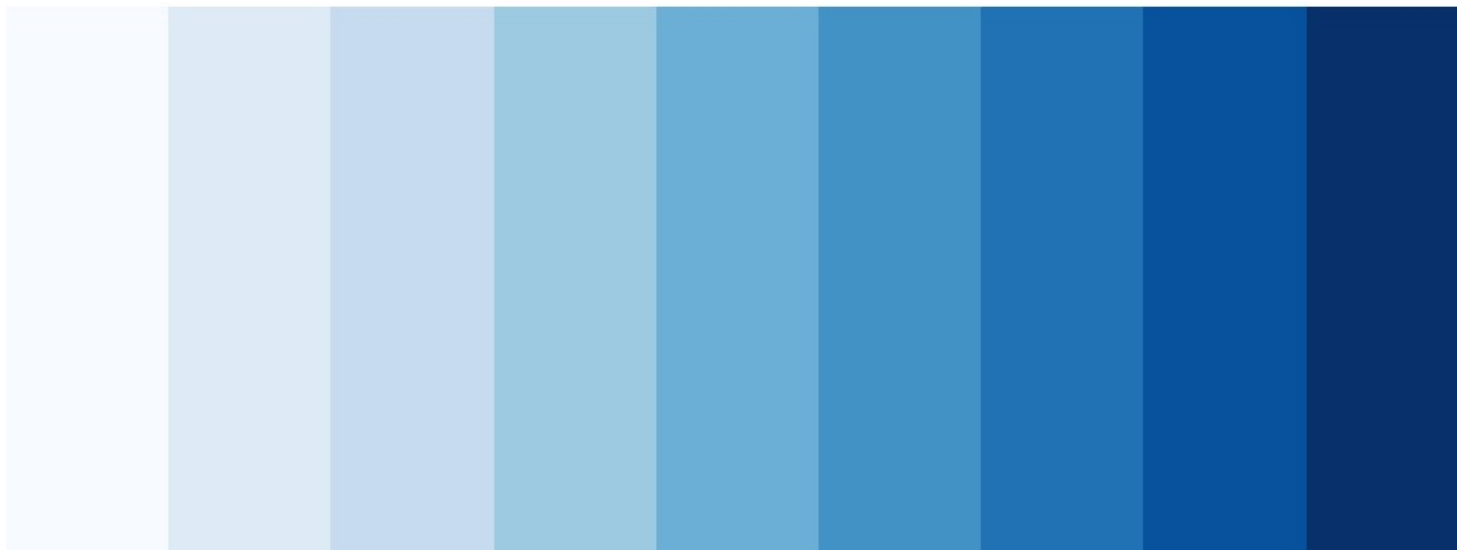
- Quality scores?
- 3 modes:
  - Lossless
  - Binned
  - No quality





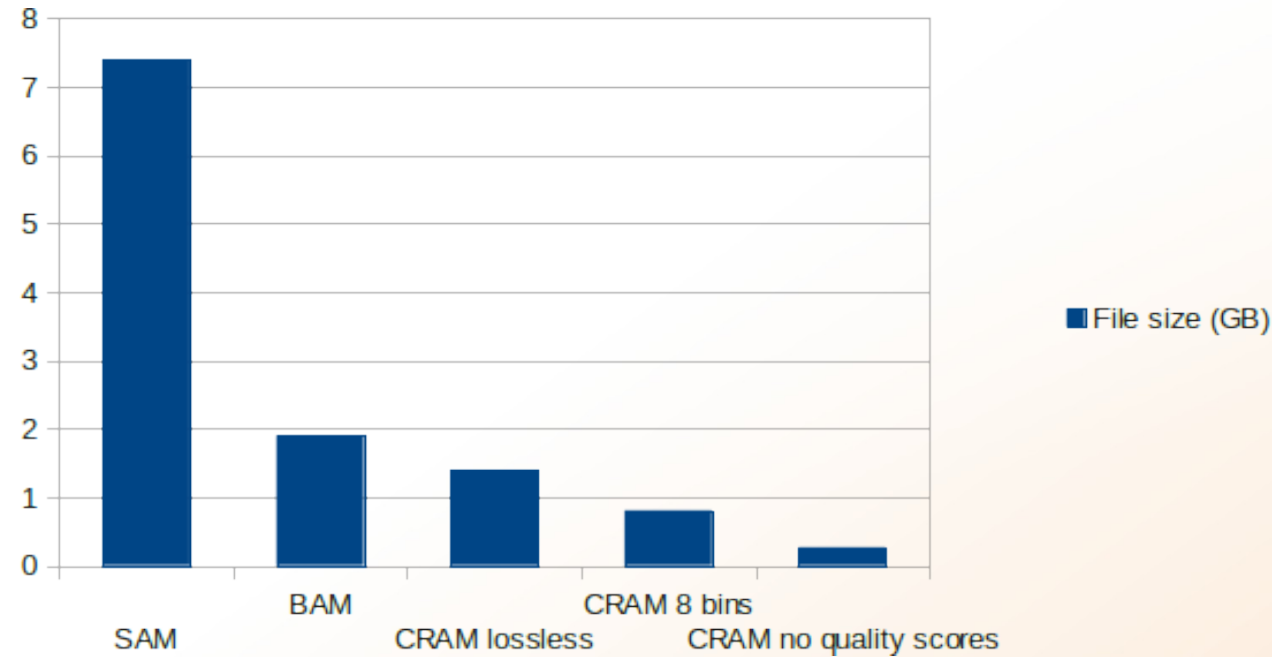
1 2 3 4 5 6 7 8 9 10 11 12 13 14 ... 32 33 34 35 36 37 38 39 40 41

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:

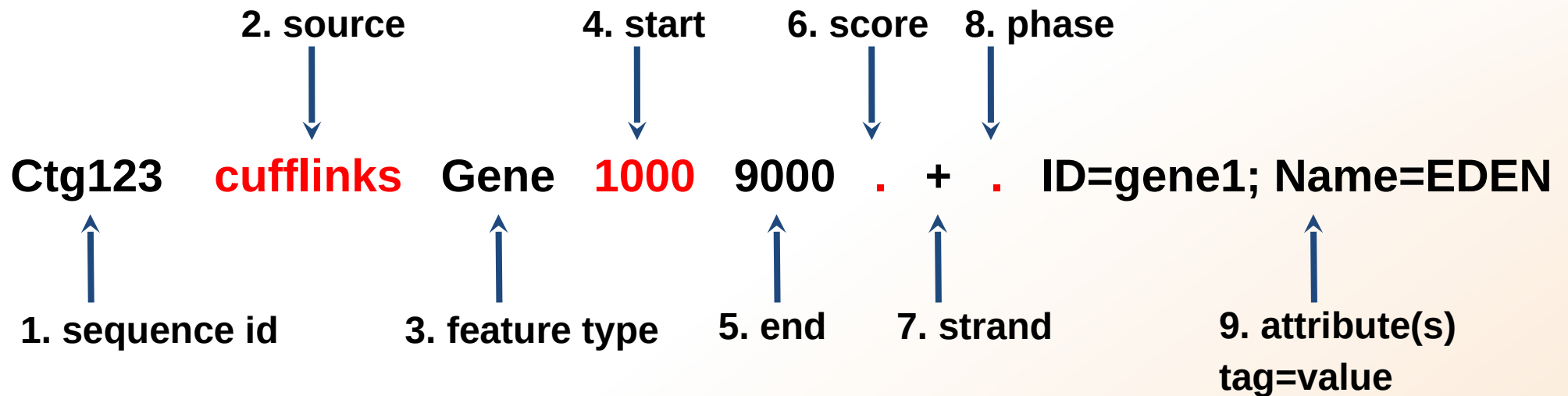
- optional fields

- 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
```

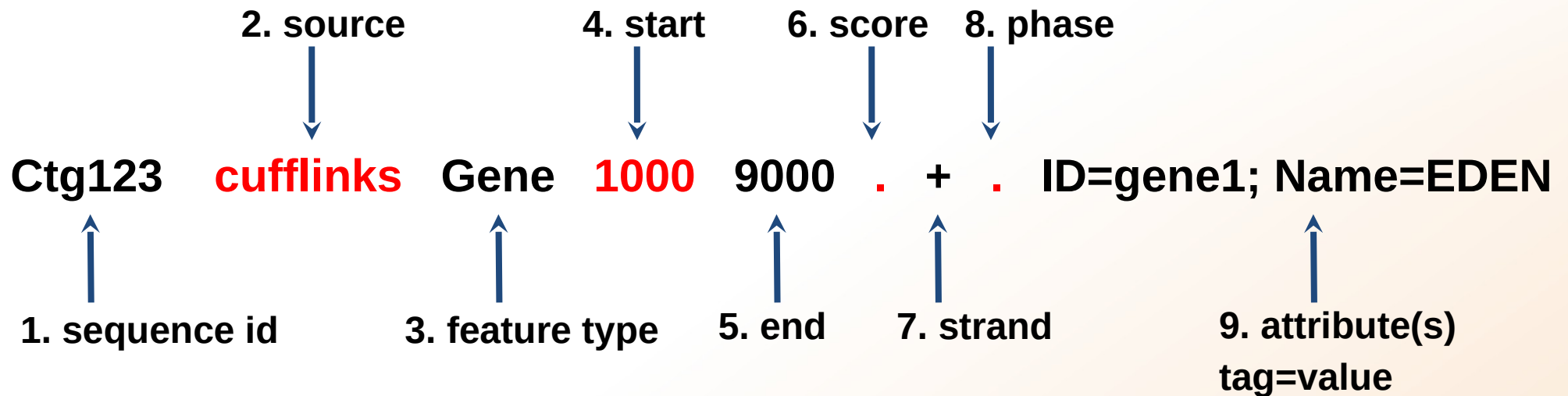
## GFF/GTF format:

- 9 columns



## 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
```

Laboratory time! (yet again)

[https://nbisweden.github.io/workshop-ngsintro/2105/lab\\_linux\\_filetypes.html](https://nbisweden.github.io/workshop-ngsintro/2105/lab_linux_filetypes.html)