

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 AEUAIEILHGAEI GAHEGLAEJKRGNAERBIAE
AEGHAELGIHAEGOUI AENGAEBARI OTYUGAEGHILAEHRGAEIRGYU
AEHAIEHAEIOGAEGAERTBETHUETHIRTHJNRFS
```

- 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)

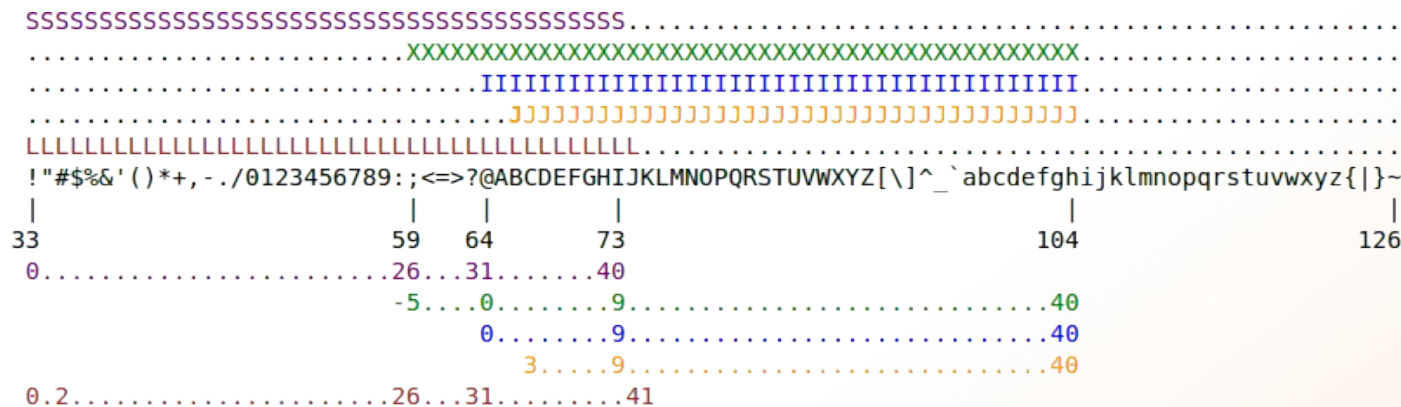
```
@SEQ_001
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%%++)(%%%!''*(((((**%).1***-+*')))**55CC!''*(D
@SEQ_002
GATTTGGGGTTCAAAGCAGTATTTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
!''*(((((***+))%%>>CCCC%++((( (**).1***-+*')))**55CCF>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTTGGGGTTCAAAGCAGTAT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

(Illumina 1.8+ = 41)

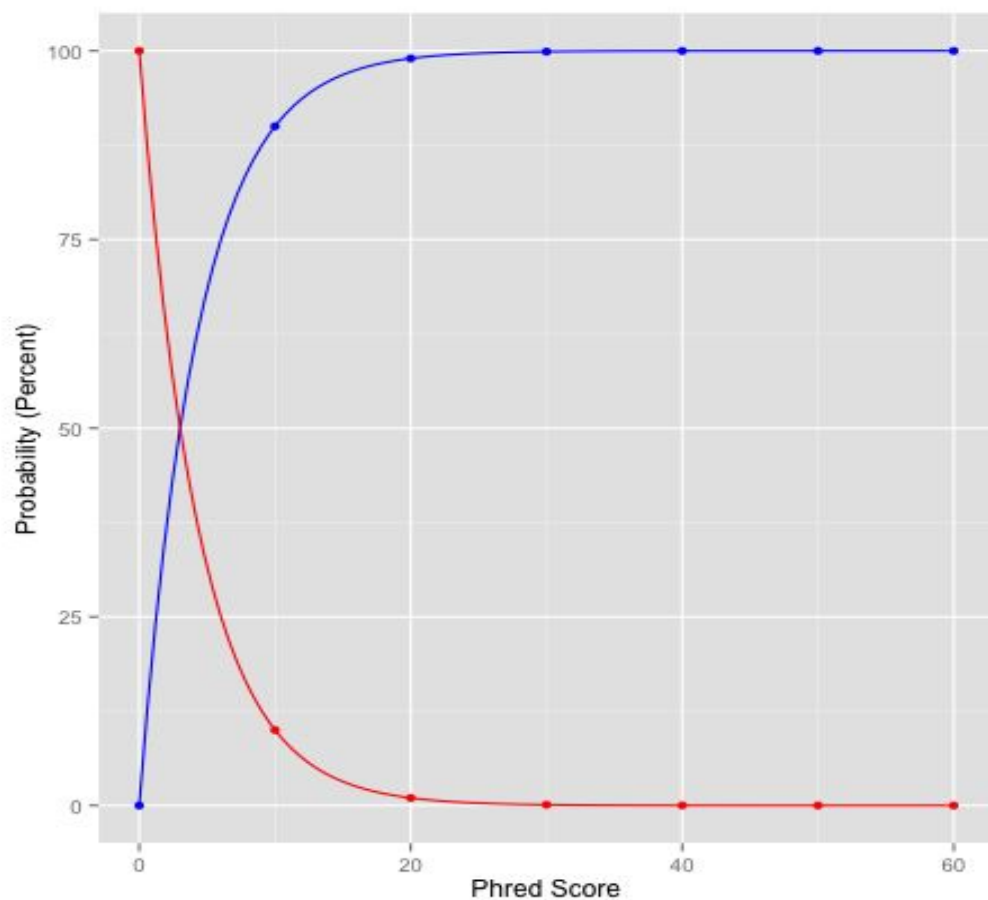


- S - Sanger Phred+33, raw reads typically (0, 40)
- X - Solexa Solexa+64, raw reads typically (-5, 40)
- I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
- J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
(Note: See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

- 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      TAGCAGAGCTATTTTCAT    ((**!' '*(((''*)%>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

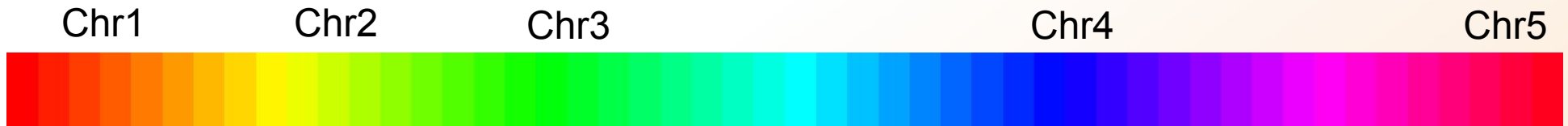
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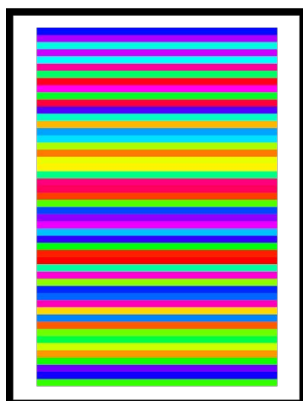
- Random order
- Have to sort before indexing



- Random order
- Have to sort before indexing



Unsorted BAM



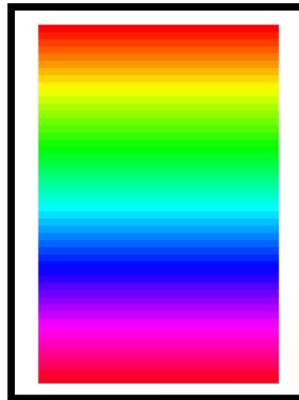
Unsorted BAM



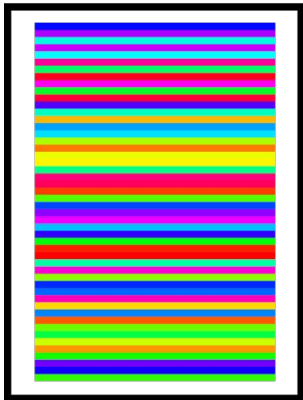
samtools sort



Sorted BAM



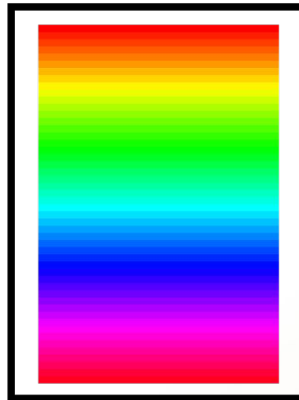
Unsorted BAM



samtools sort



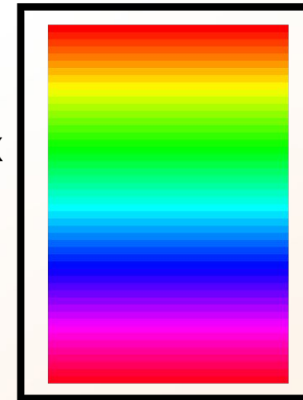
Sorted BAM



samtools index



Sorted BAM



BAM index

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

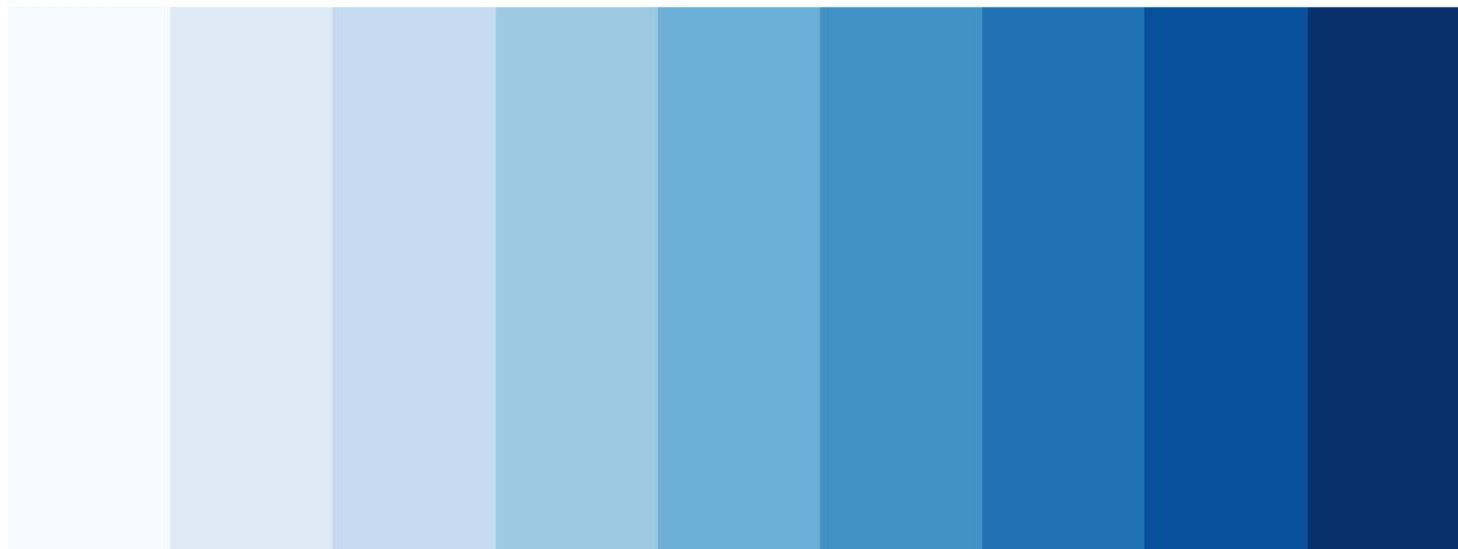
- Reads TAGATCGAGGCTGAGTCACGACG
ATTCGGACGTAGATCGAGGCTGAG ACGTGTTGAGAGAGCCGTA
- Ref: ATTCGGACGTAGATCGACGCTGAGTCACGACGTGTTGTGAGAGCCGTAGAC

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



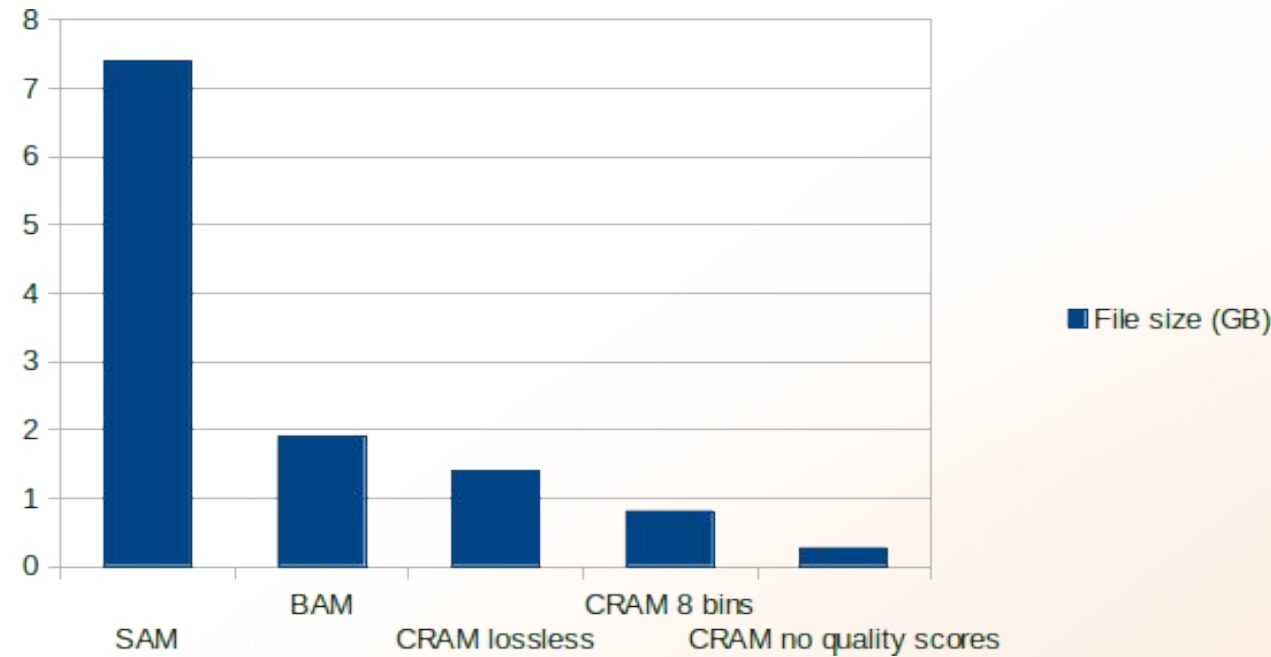
1 2 3 4 5 6 7 8 9 10 11 12 13 14 ... 32 33 34 35 36 37 38 39 40 41

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

- + optional track definition lines

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
```

BED format:

- optional track definition lines

The track line consists of the word 'track' followed by space-separated **key=value** pairs

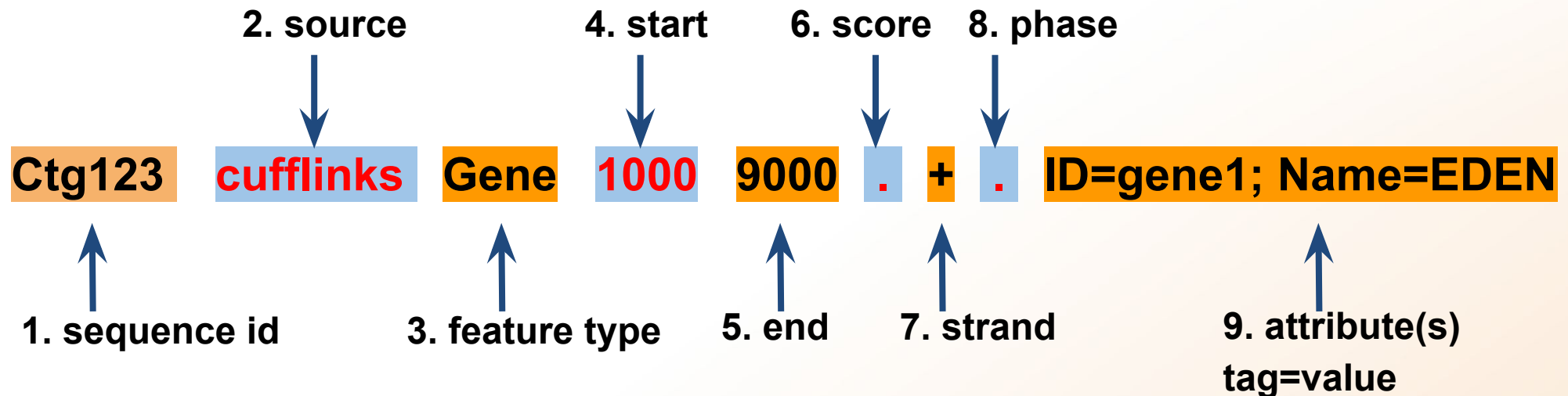
Parameters differ from databases.

Ensembl example:

```
track name="ItemRGBDemo" description="Item RGB demonstration" itemRgb="On"
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



/!\ different version 1, 2, 2.5, 3

GTF = GFF version 2

GFF3:

- Headers

```
##gff-version 3
```

```
##sequence-region ctg123 1 1497228
```

- Features

Ctg123 **cufflinks** **Gene** **1000** **9000** **.** **+** **.** **ID=gene1; Name=EDEN**

- Sequences (optional)

```
##FASTA
```

```
>ctg123
```

```
cttctgggcgtacccgattctcggagaacttgccgcaccattccgccttg
```

```
tgttcattgctgcctgcatgttcattgtctacctcggctacgtgtggcta
```

```
tctttcctcggcgcctcgtgcacggagtcgagaaaccaagaacaaaaa
```

```
aagaaattaaaatatattttgctgtggtttttgatgtgtgttttttat
```



```
##gff-version 3.2.1
```

```
##sequence-region ctg123 1 1497228
```

```
ctg123 . Gene 1000 9000 . + . ID=gene1;Name=EDEN
```

```
ctg123 . mRNA 1050 9000 . + . ID=mRNA1;Parent=gene1
```

```
ctg123 . exon 1050 1500 . + . ID=exon1;Parent=mRNA1
```

```
ctg123 . exon 7000 9000 . + . ID=exon2;Parent=mRNA1
```

```
ctg123 . CDS 1201 1500 . + 0 ID=cds1;Parent=mRNA1;Name=edenprotein.1
```

```
ctg123 . CDS 7000 7600 . + 0 ID=cds1;Parent=mRNA1;Name=edenprotein.1
```

- **Laboratory time!** (yet again)