

File Types in Bioinformatics

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Enabler for Life Sciences

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

SITUATION:
THERE ARE
14 COMPETING
STANDARDS.

14?! RIDICULOUS!
WE NEED TO DEVELOP
ONE UNIVERSAL STANDARD
THAT COVERS EVERYONE'S
USE CASES.



SOON:

SITUATION:
THERE ARE
15 COMPETING
STANDARDS.

- 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 AEUAEILHGAEI GAHEGLAEJKRGNAERBI AE
AEGHAELGI HAEGOUI AENGAEBAERI OTYUGAEGHI LAEHRGAEIRGYU
AEHAEHAEIOGAEGAERTBETHUETHIRTHJNRFS
```

- 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
GATTGGGGTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*( (( (**+) )%%%++ ) %%! ''*( (( **% ).1***-+*'' ) )**55CC! ''*( D
@SEQ_002
GATTGGGGTCAAAGCAGTATTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
!''*( (( (**+) )%%>>CCCCC%++ (( (**).1***-+*'' ) )**55CCF>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTGGGGTCAAAGCAGTAAAGCAGTATCGATAAATCCATTTGTT
+
!''*( (( *! ''*( (( **) %%% ).1***-+*'' ) )**55CCF>>>>>%%%%.1BS
```

- 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	Ø	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

```

SXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
IXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
JXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
LXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
!#$%&'()*+, -./0123456789:;<>?@ABCDEFGHIJKLMNPQRSTUVWXYZ[\]^`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

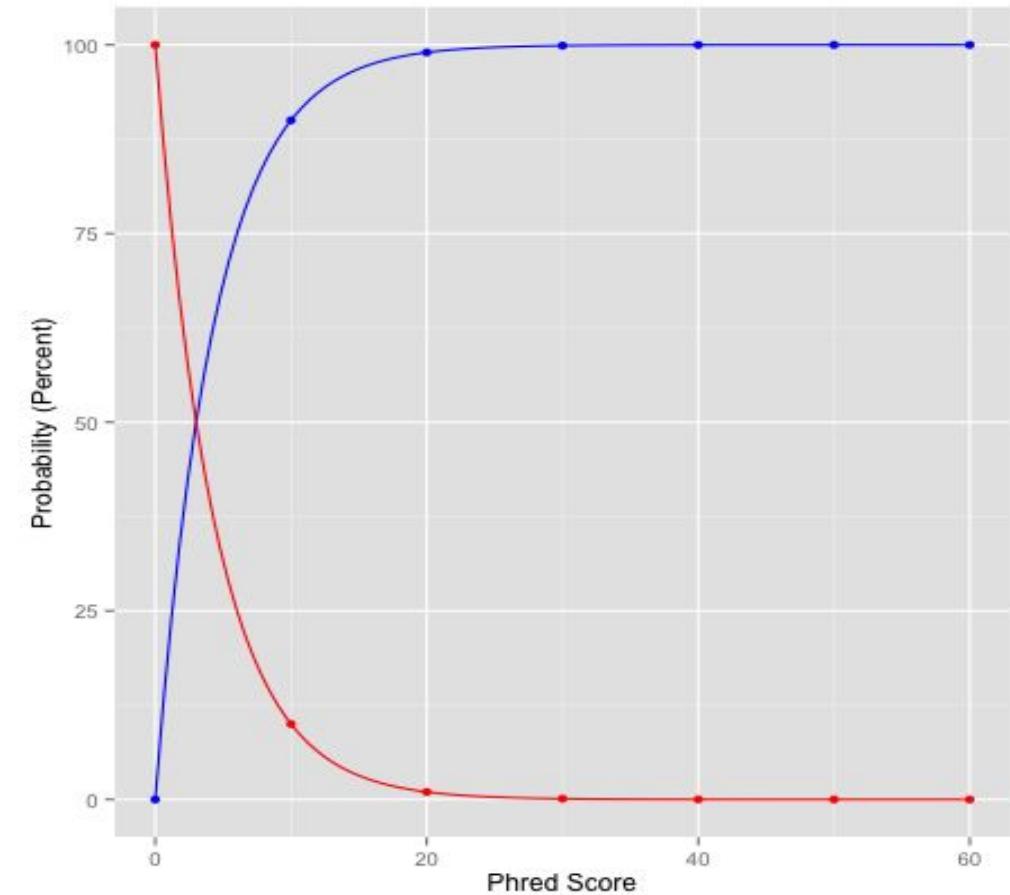
S - Sanger      Phred+33, raw reads typically (0, 40)
X - Solexa       Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
L - Illumina 1.8+ Phred+33, raw reads typically (0, 41)

  with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
  (Note: See discussion above).

```

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

```
@SEQ_001
GATTGGGGTCAAAGCAGTATCGATCAAATAGTAAATCCATTGTTCAACTCACAGTTT
+
! ' * ( ( ( ***+ ) %%%++ ) %%% ! ' * ( ( ( **% ) . 1***-+*'' ) ) **55CC! ' * ( D
@SEQ_002
GATTGGGGTCAAAGCAGTATTTGGGGTTCATTGGGGTTCATTGTTCAACTCACAGTTT
+
! ' * ( ( ( ***+ ) %%>>CCCCC%++ ( ( ( ** ) . 1***-+*'' ) ) **55CCF>>>>>C5
@SEQ_003
AAGCAGTATCGAGATTGGGGTCAAAGCAGTATAAGCAGTATCGATAAATCCATTGTT
+
! ' * ( ( ( *! ' * ( ( ( ** ) %%%% ) . 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-Z0-9]		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 AAAGGCTATTCACCT )%>((***+))%>%> XT:A:U NM:i:0 X0:i:1 X1:i:0 XM:i:0 X0: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 X0: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 X0: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 X0:i:0 XG:i:0 MD:Z:150
read_005 0 chr31 26546617 37 150M * 0 0 TAGCAGAGCTATTCAT ((**! ''*(*+))%>AD XT:A:U NM:i:0 X0:i:1 X1:i:0 XM:i:0 X0: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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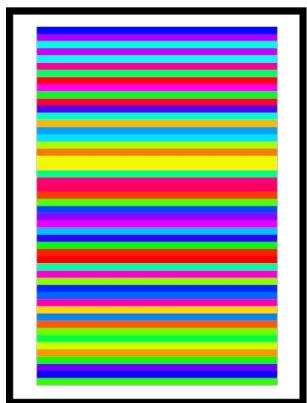
- Random order
- Have to sort before indexing



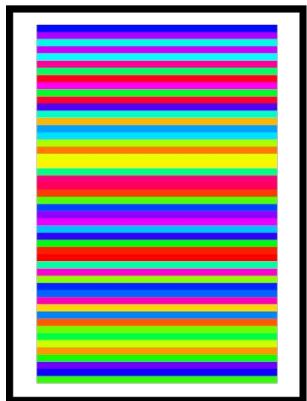
- Random order
- Have to sort before indexing



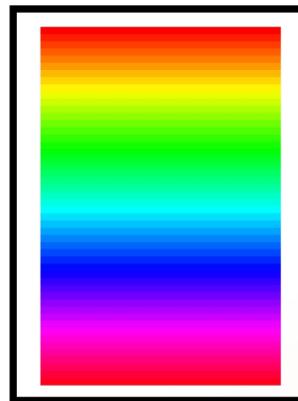
Unsorted BAM



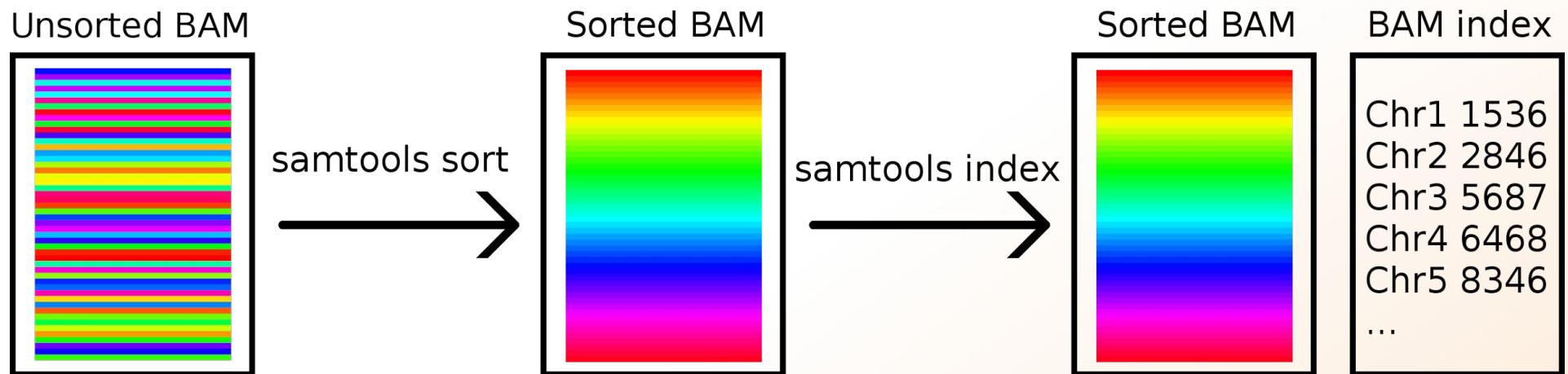
Unsorted BAM



Sorted BAM



→
samtools sort



- Very complex format
 - Used together with a reference genome

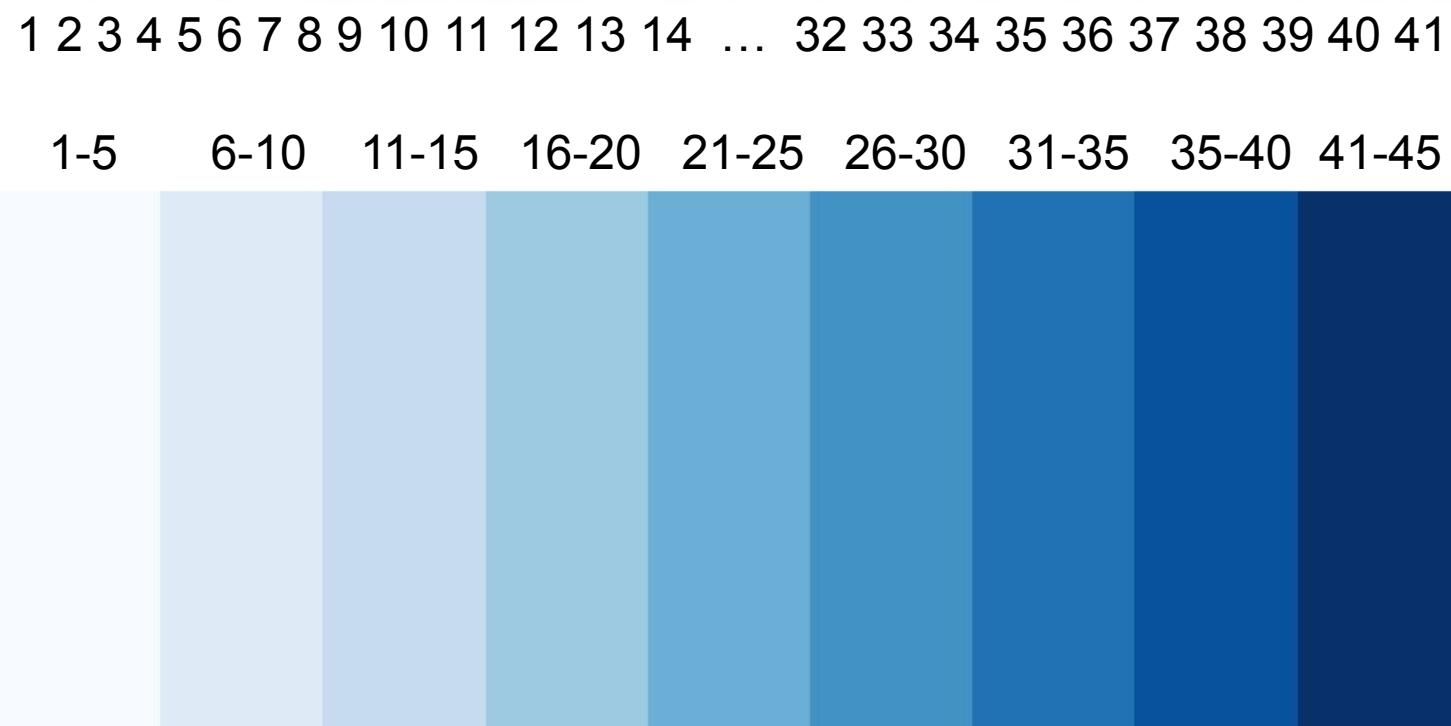
AGGCTGAGTCACGACGTGTTGAGA

TAGATCGAGG**GCTGAGTCACGACG**

ATTCGGACGTAGATCGA**GGCTGAG** **ACGTGTTGAGAGAGGCCGA**

Ref: ATTCGGACGTAGATCGACGCTGAGTCACGACGTGTTGTGAGAGGCCGTAGAC

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

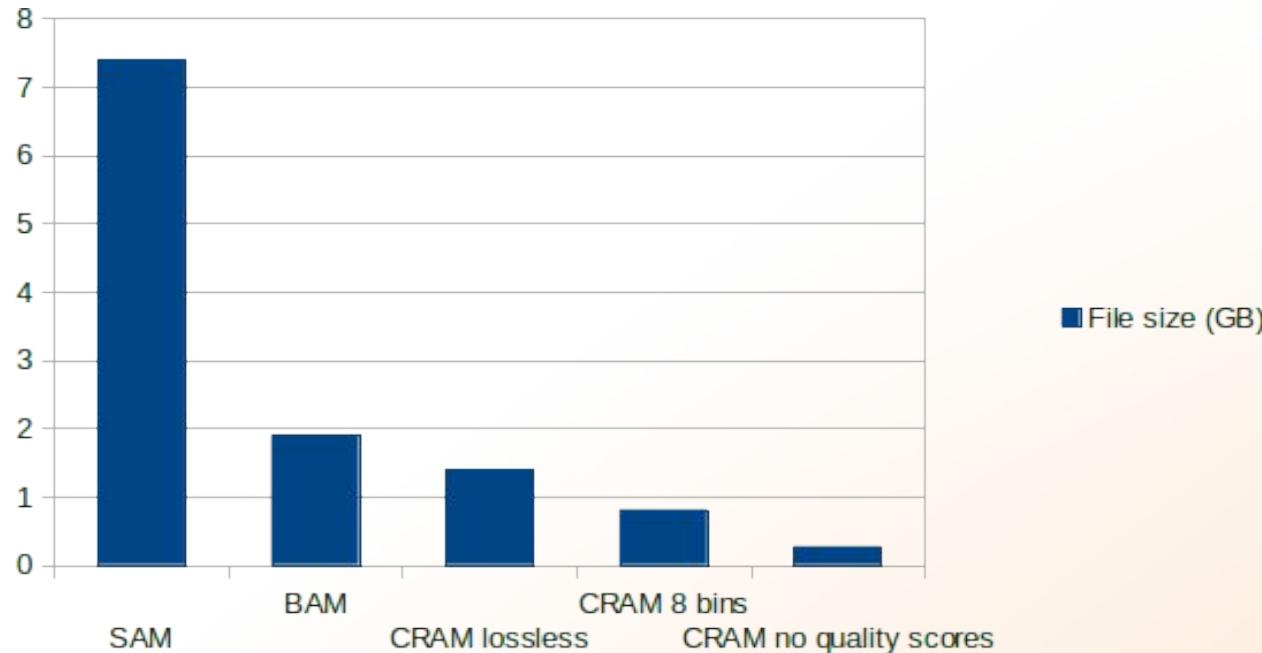


1 2 3 4 5 6 7 8 9 10 11 12 13 14 ... 32 33 34 35 36 37 38 39 40 41

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

GTF/GFF/BED

- 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:

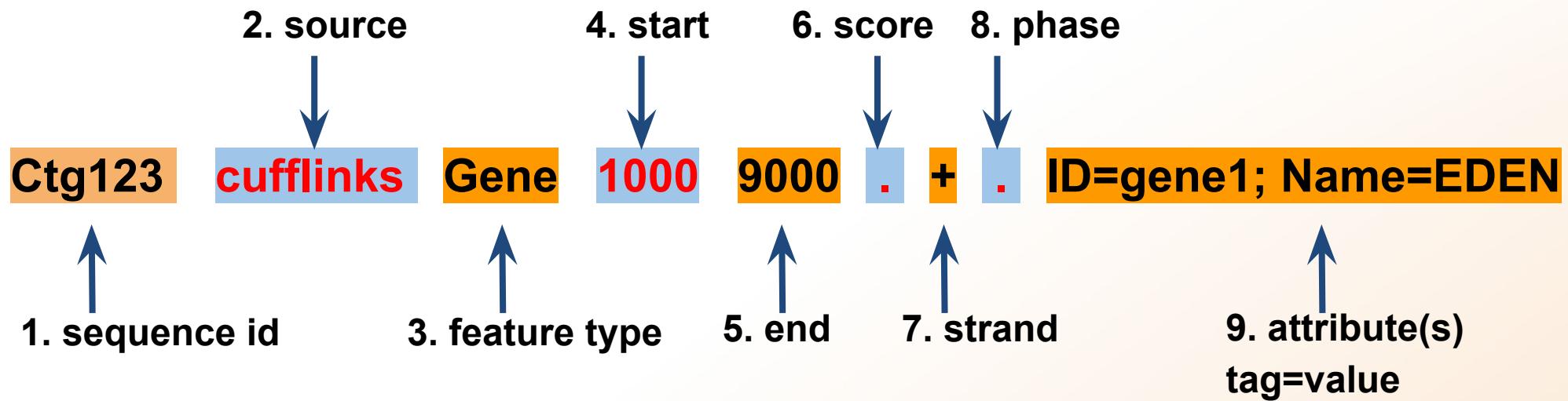
- optional fields

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	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/2603/topics/linux/lab_linux_filetypes.html