

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 AEUAEL LHGAEL GAHEGLAEJ KRGNAERBI AE
AEGHAELGI HAEGOUI AENGAEB AERI OTYUGAEGHI LAEHRGAELIRGYU
AEHAELAEI 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

(Illumina 1.8+ = 41)

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

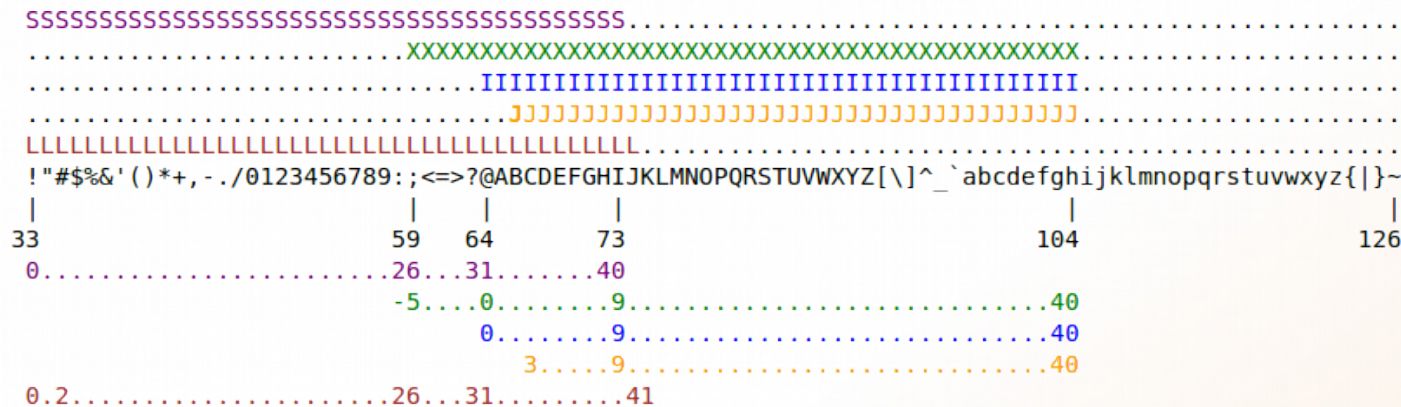
Dec	Hex	Char	Dec	Hex	Char	Dec	Hex	Char	Dec	Hex	Char
0	00	Null	32	20	Space	64	40	@	96	60	`
1	01	Start of heading	33	21	!	65	41	A	97	61	a
2	02	Start of text	34	22	"	66	42	B	98	62	b
3	03	End of text	35	23	#	67	43	C	99	63	c
4	04	End of transmit	36	24	\$	68	44	D	100	64	d
5	05	Enquiry	37	25	%	69	45	E	101	65	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)

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


- 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
```

Start position
bp chr

Sequence

Quality

Read name

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

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- Random order
- Have to sort before indexing



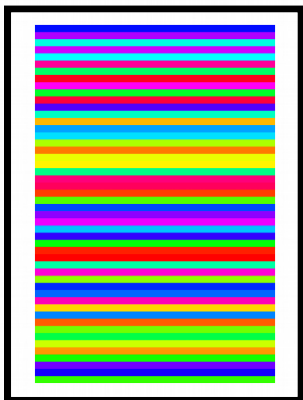
- Random order
- Have to sort before indexing



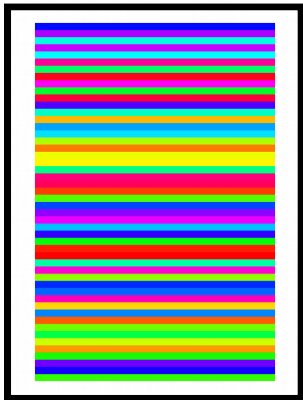
- Random order
- Have to sort before indexing



Unsorted BAM



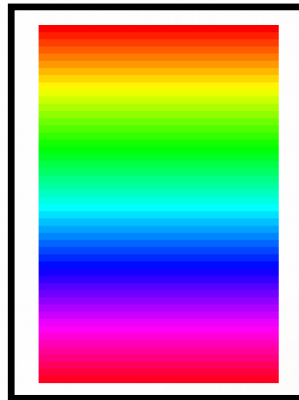
Unsorted BAM



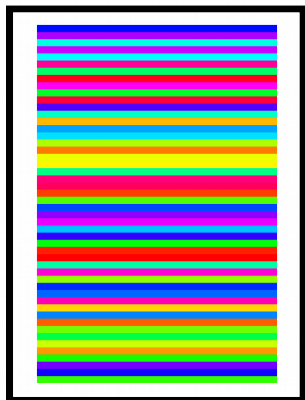
samtools sort



Sorted BAM



Unsorted BAM



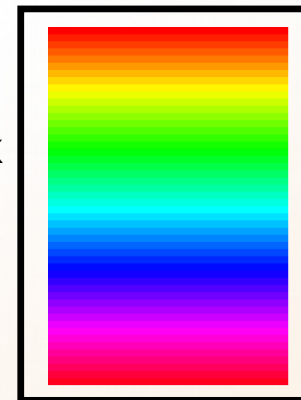
samtools sort

Sorted BAM



samtools index

Sorted BAM



BAM index

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

- Very complex format
- Used together with a reference genome

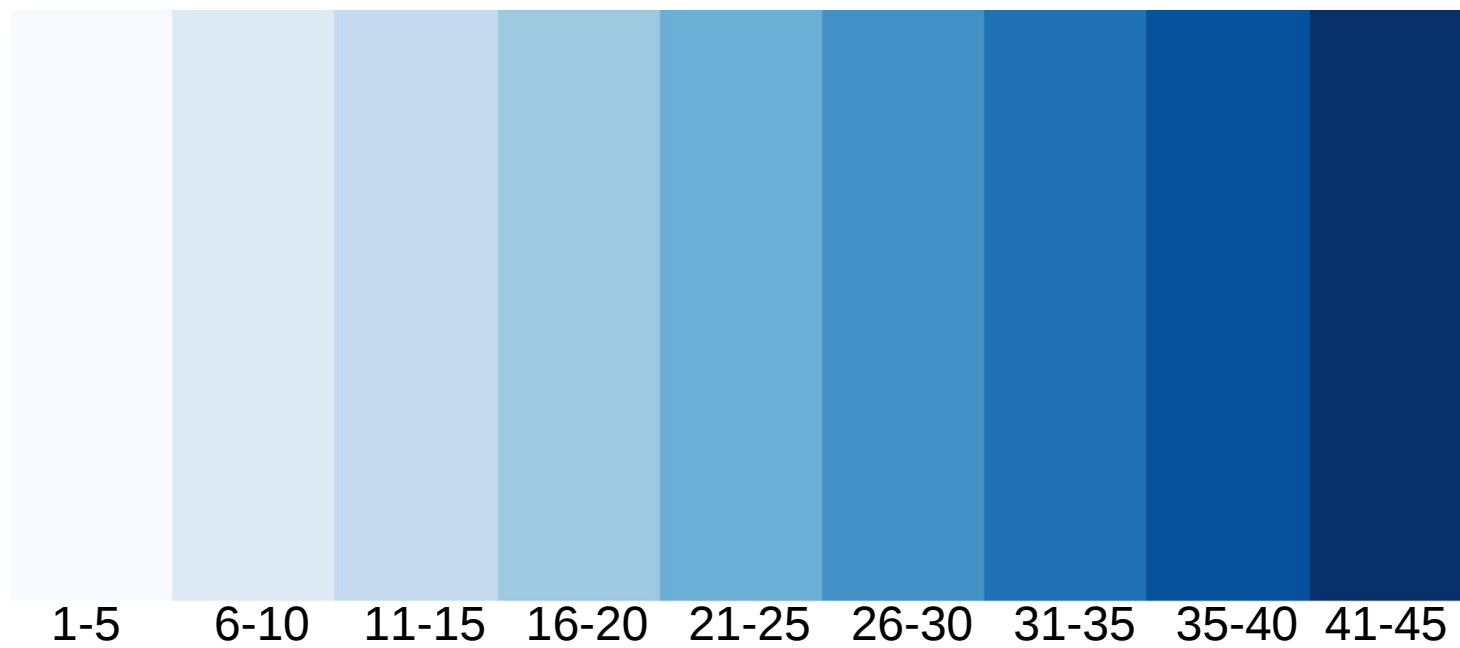
AGGCTGAGTCACGACGTGTTGAGA

TAGATCGAGGCTGAGTCACGACG

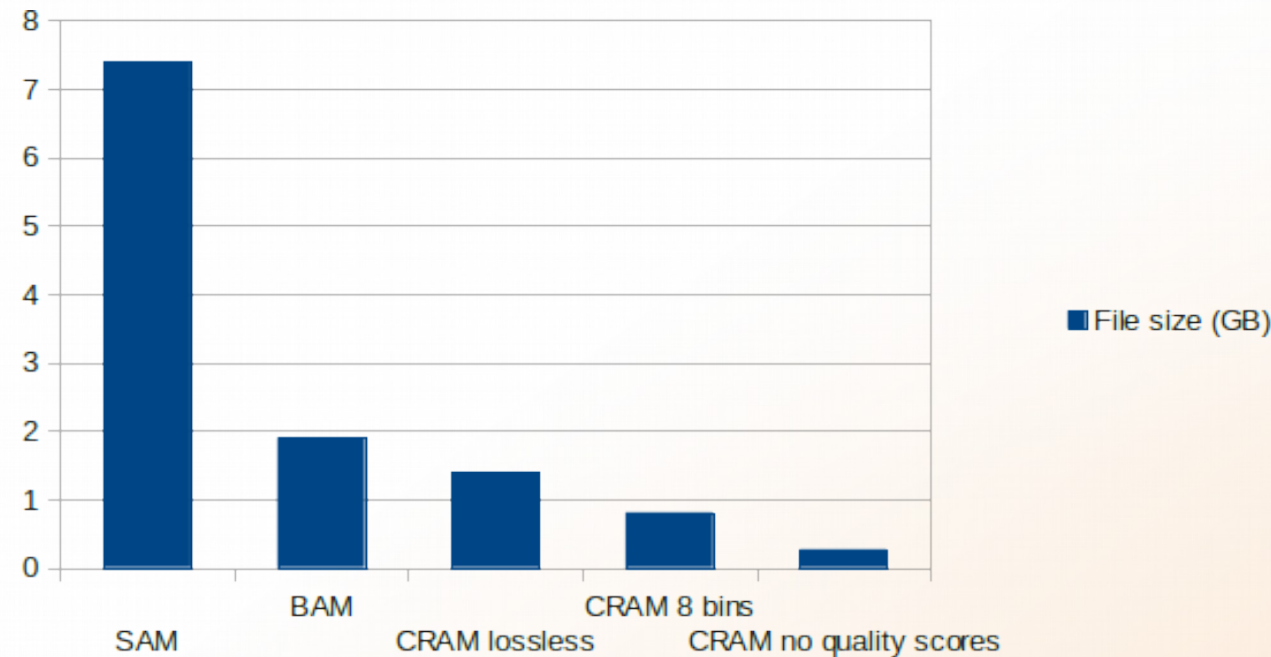
ATTCGGACGTAGATCGA**G**GCTGAG ACGTGTTG**A**GAGAGCCGTA

Ref: ATTCGGACGTAGATCGACGCTGAGTCACGACGTGTTGTGAGAGCCGTAGAC

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



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



- Not widespread, yet

- Used for: annotations
- Simple structure

- Usually:

chr start stop extra info

- Used for: annotations
- Simple structure

- Usually:

chr start stop extra info

- BED

```
chr22 1000 5000 cloneA 960 + 1000 5000 0 2 567,488, 0,3512  
chr22 2000 6000 cloneB 900 - 2000 6000 0 2 433,399, 0,3601
```

- Used for: annotations
- Simple structure

- Usually:

chr start stop extra info

- GFF

```
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)