SciLifeLab
UPPSALA

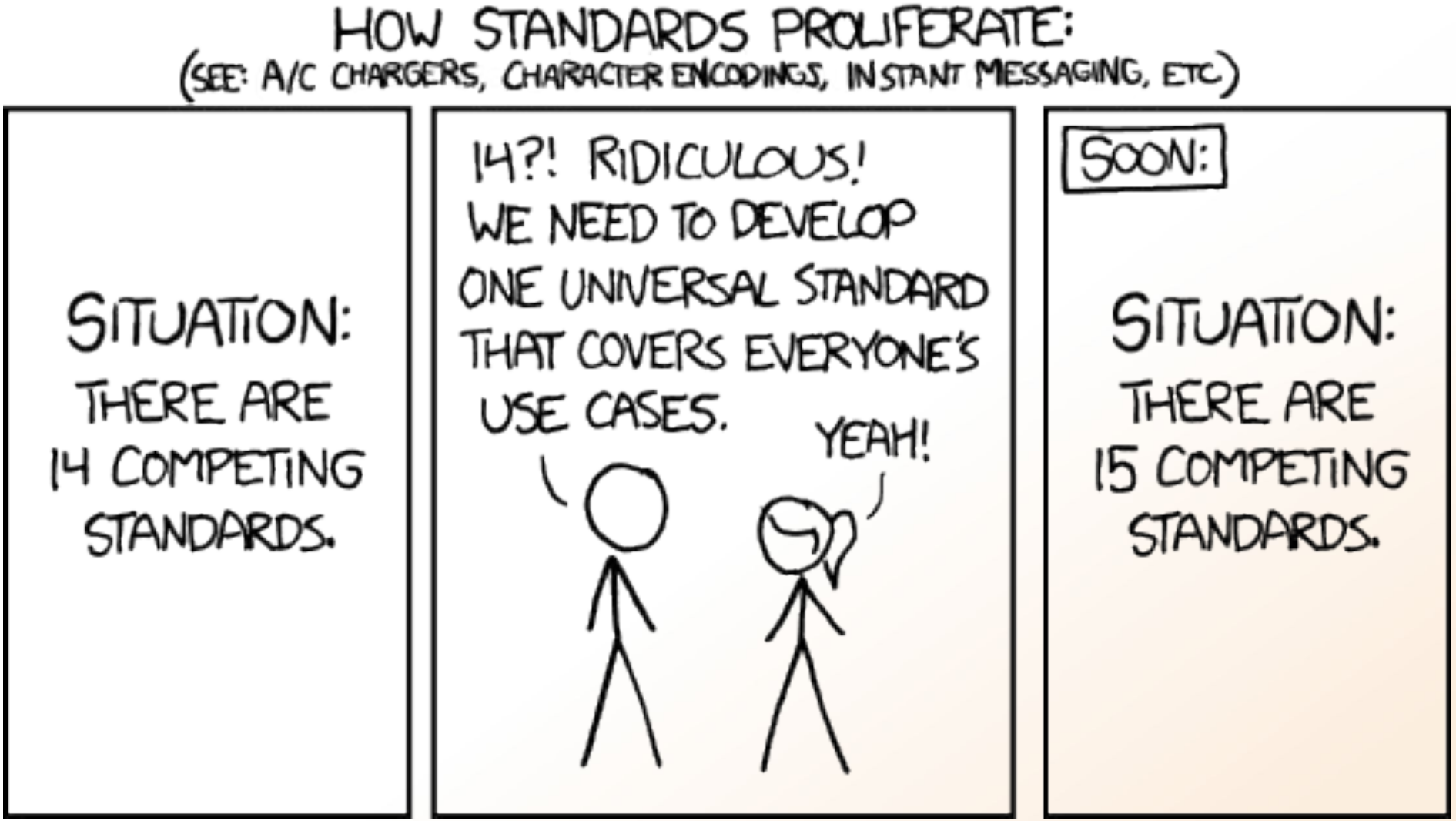
File Types in Bioinformatics

2019-09-10

Anders Sjölander
anders.sjolander@uppmax.uu.se

Enabler for Life Sciences

SciLifeLab
UPPSALA



- http://xkcd.com

SciLifeLab
UPPSALA

- 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

SciLifeLab UPPSALA

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
```

SciLifeLab UPPSALA

FASTQ

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

@ header
sequence
+
quality

SciLifeLab UPPSALA

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
```

SciLifeLab
UPPSALA

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	□

SciLifeLab UPPSALA

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)
```

SciLifeLab UPPSALA

FASTQ

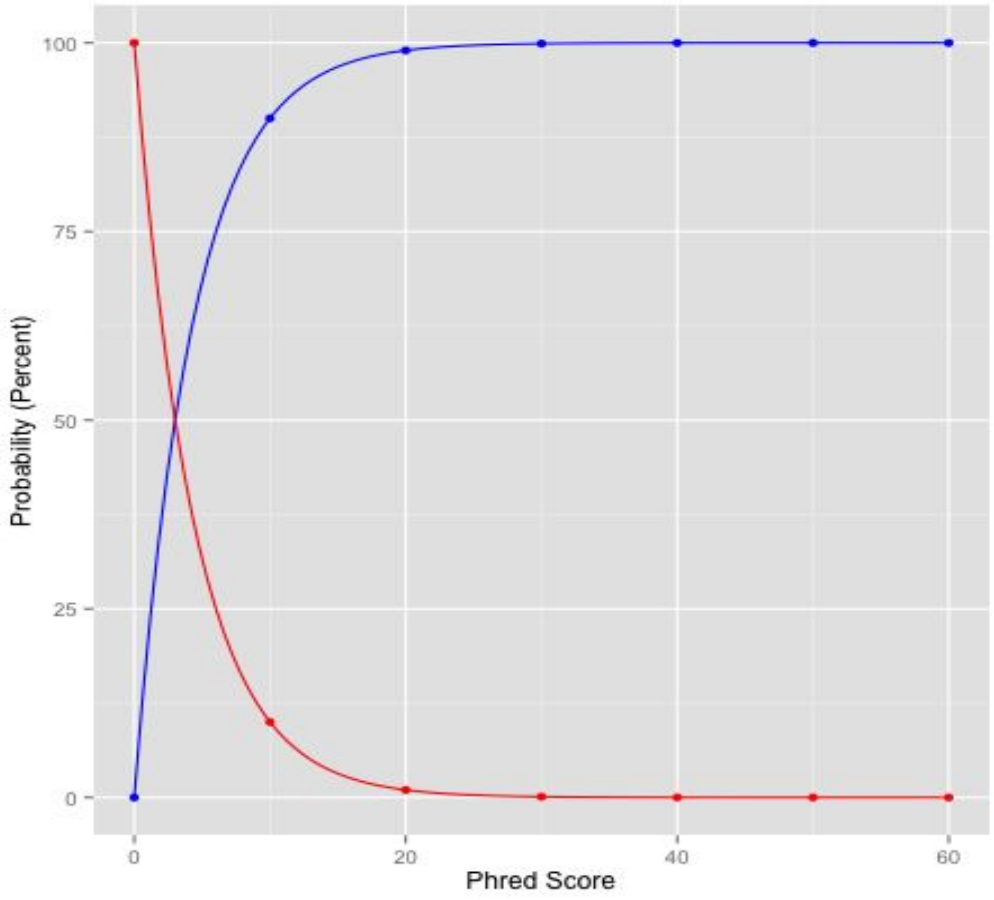
- Quality 0-40
  - 40 = best
- ASCII encoded

(Illumina 1.8+ = 41)

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

SciLifeLab UPPSALA

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%

SciLifeLab
UPPSALA

SAM

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

SciLifeLab UPPSALA

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

SciLifeLab UPPSALA

BAM

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

Contents

SciLifeLab
UPPSALA

BAM

- Random order
- Have to sort before indexing

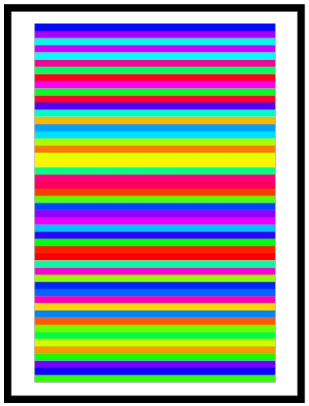
SciLifeLab UPPSALA

BAM

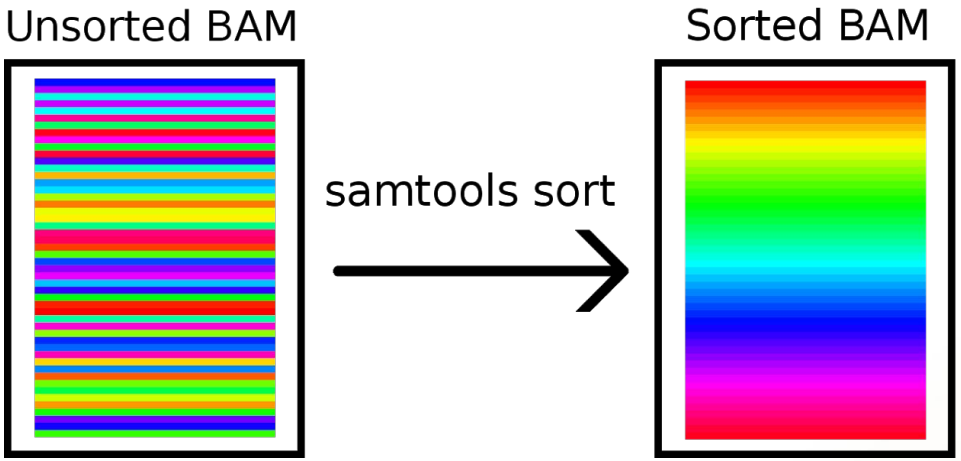
- Random order
- Have to sort before indexing

BAM

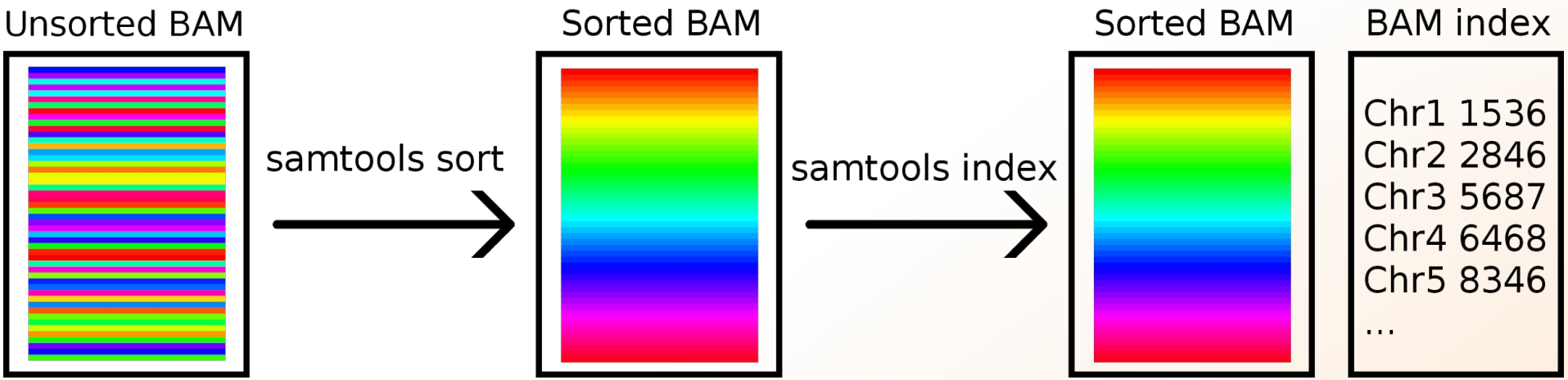
Unsorted BAM



BAM



BAM


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

SciLifeLab UPPSALA

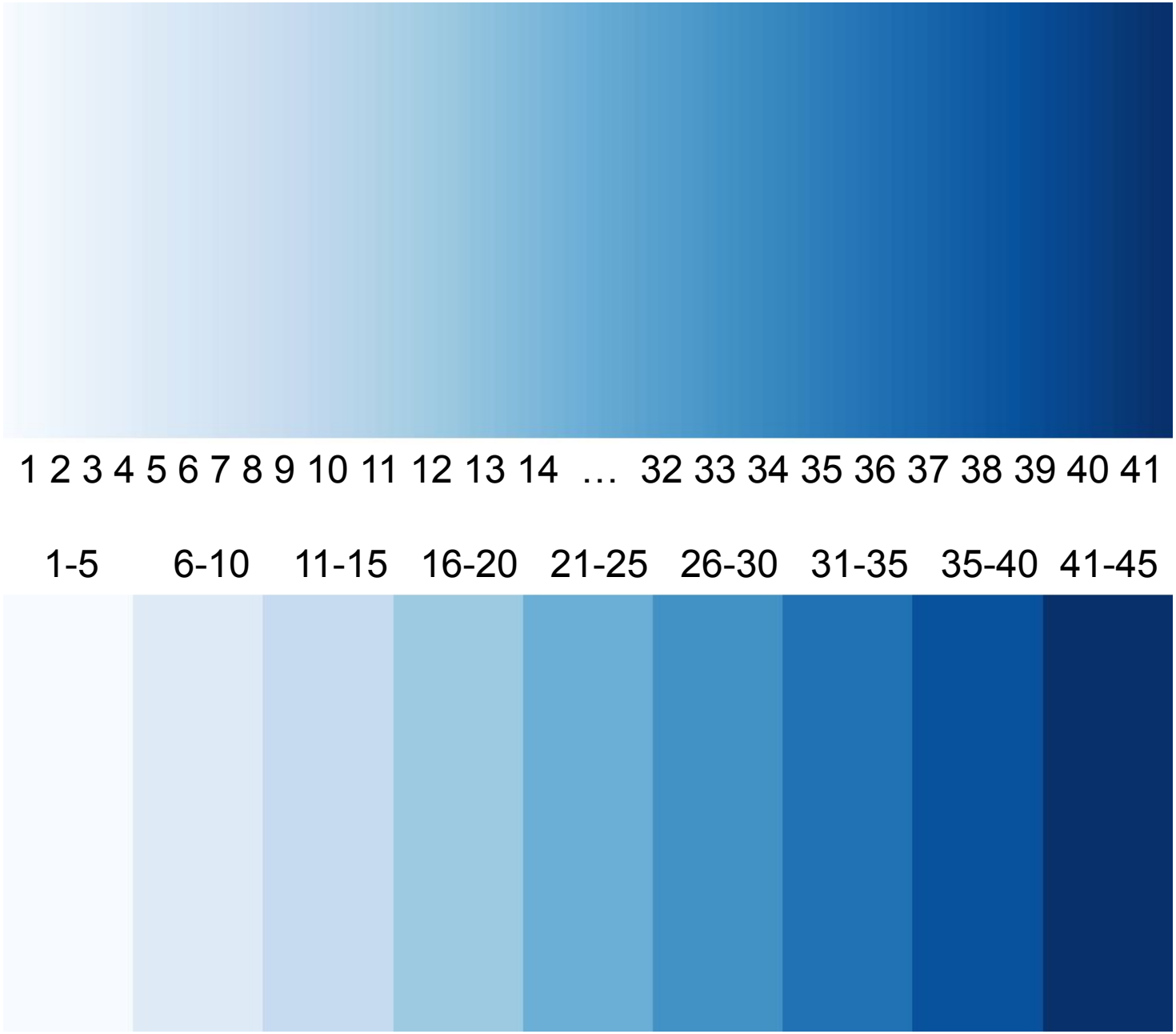
CRAM

- Very complex format
- Used together with a reference genome

SciLifeLab UPPSALA

CRAM

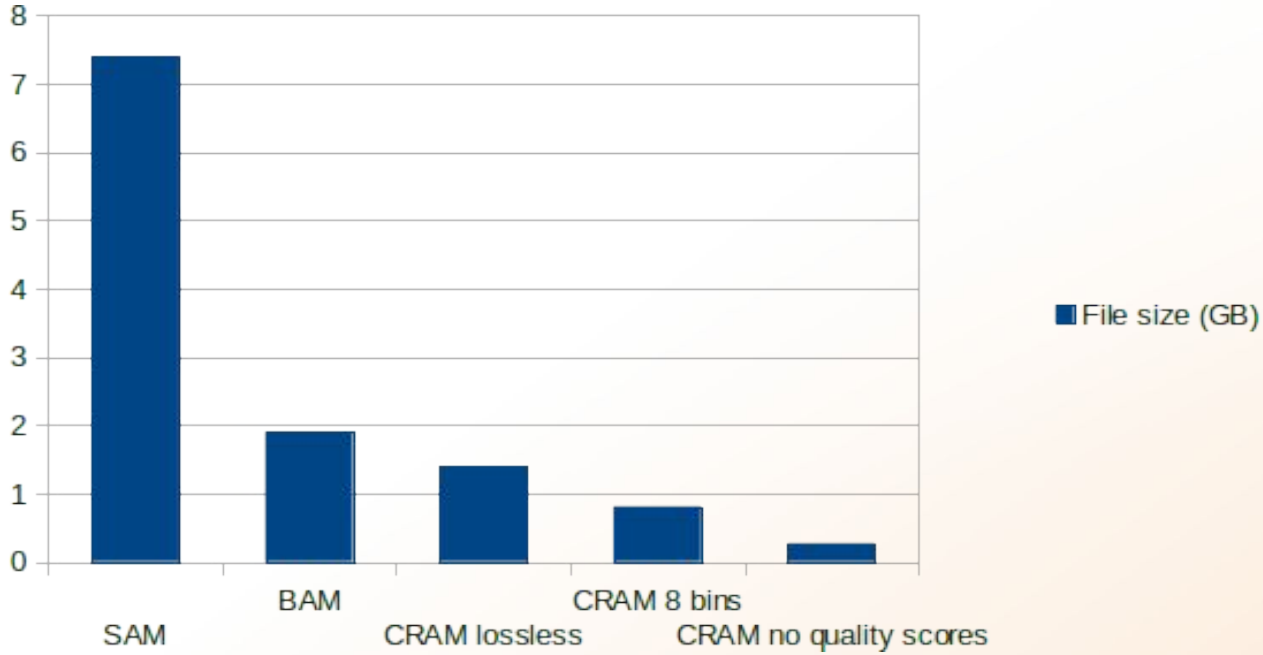
- Quality scores?
- 3 modes:
  - Lossless
  - Binned
  - No quality



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

CRAM

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



- Not widespread, yet

SciLifeLab UPPSALA

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	

SciLifeLab UPPSALA

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

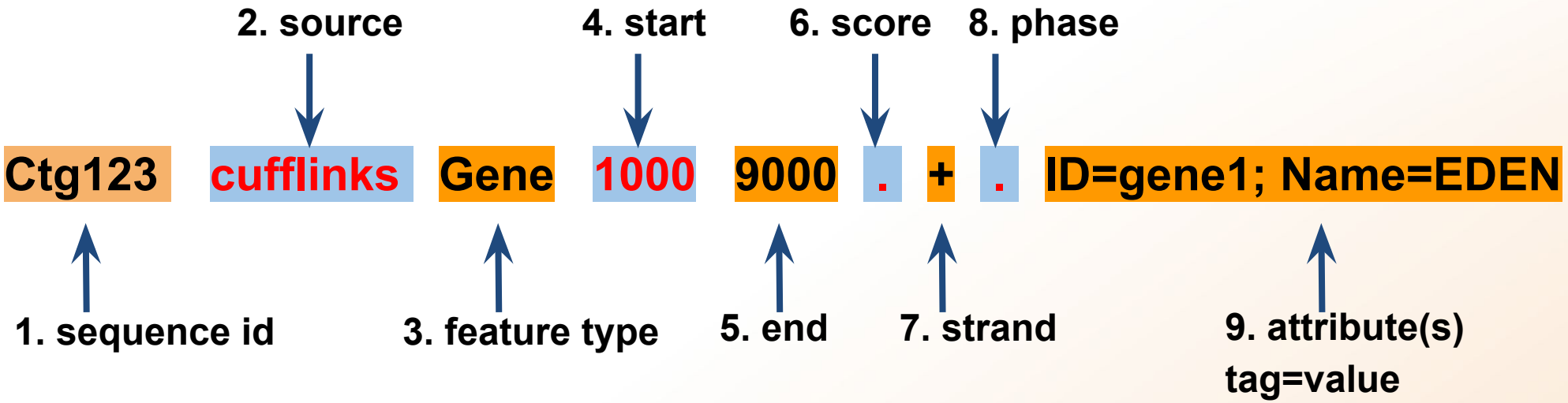
12. blockStarts - the start coordinate of each sub-element

```
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:

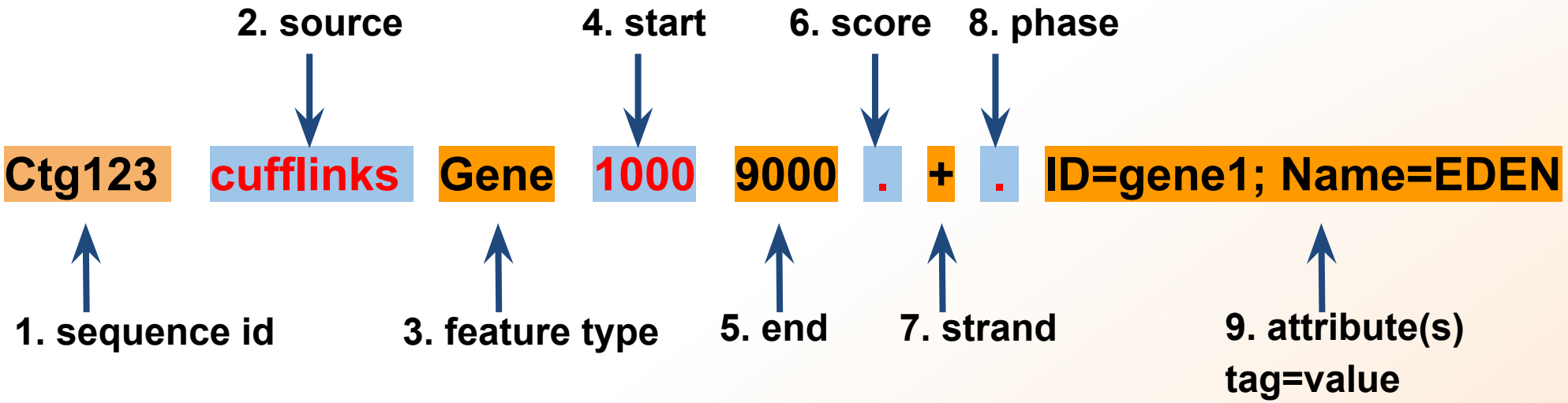
- 9 columns



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
```

SciLifeLab
UPPSALA

- Laboratory time! (yet again)