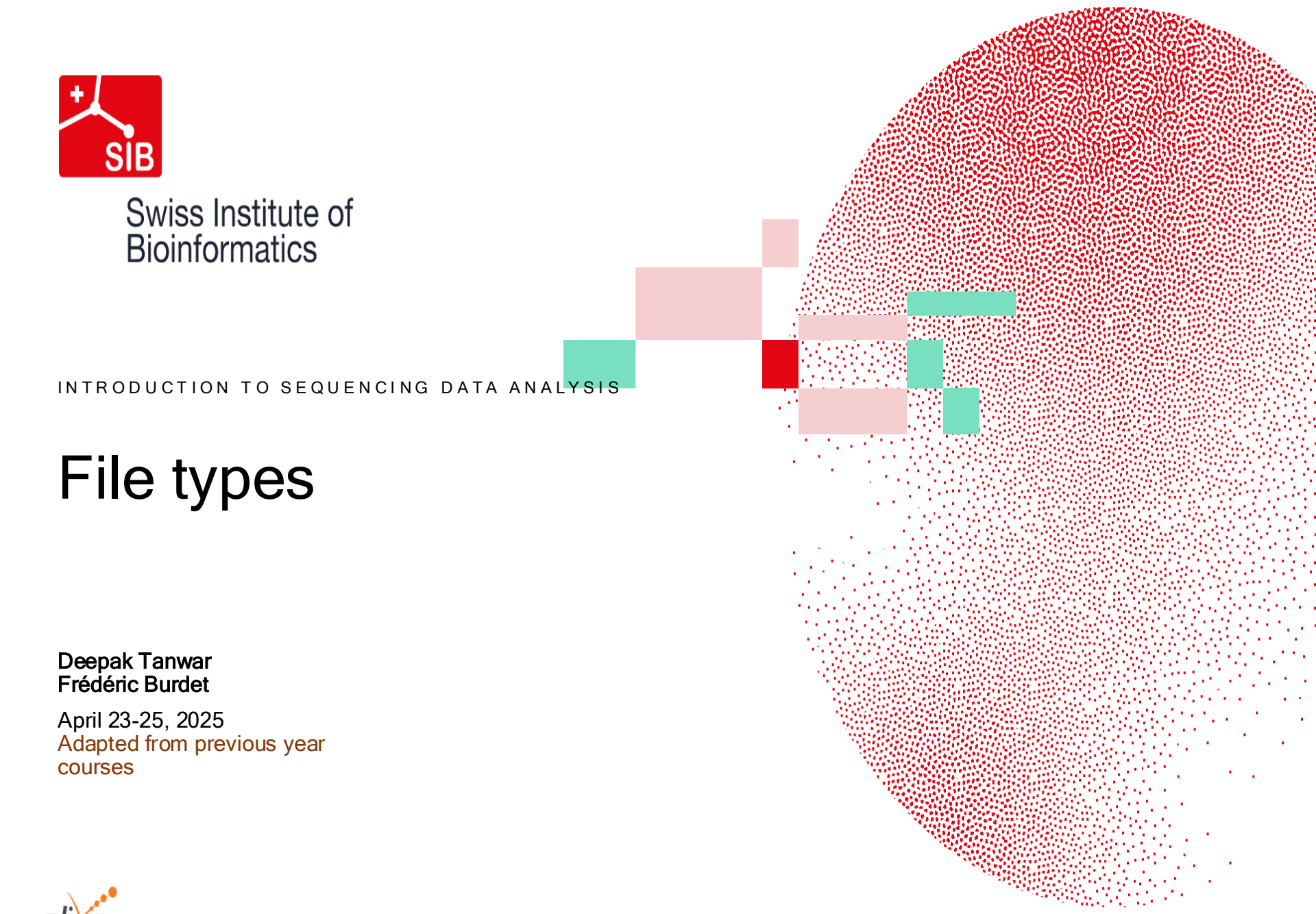

Swiss Institute of Bioinformatics

INTRODUCTION TO SEQUENCING DATA ANALYSIS

File types

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Adapted from previous year courses

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Frequently used file types

fasta	sequences
fastq	reads
sam/bam	alignments
bed	regions
gff	annotations
vcf	variants

Broad overview of file types:
https://genome-euro.ucsc.edu/FAQ/FAQformat.html

fasta

- Plain sequence: *.fasta or *.fa
- Nucleotides or amino acids (proteins)
- Useful command:

```
grep -c "^>" sequence.fasta
```

sequence.fasta

```
>sequence title1
ATCGTATCTATCGTATCT
GGTTTATCGTATCT
>sequence title2
ATGATGACGT
```

fastq files

reads.fastq

```
@D00283R:66:CC611ANXX:4:2311:2596:2330 1:N:0:TCCGGAG
ACTCTACGCTCAATAAAGATTTCTGATACGGCTCCTGAAATGCAGAATGAGT
+
B/<<<B<FFFFFFFFFBBFFFBFFFFBFFFF/FFFFFFFF/BFFFFFBFFF
```

title, starts with @

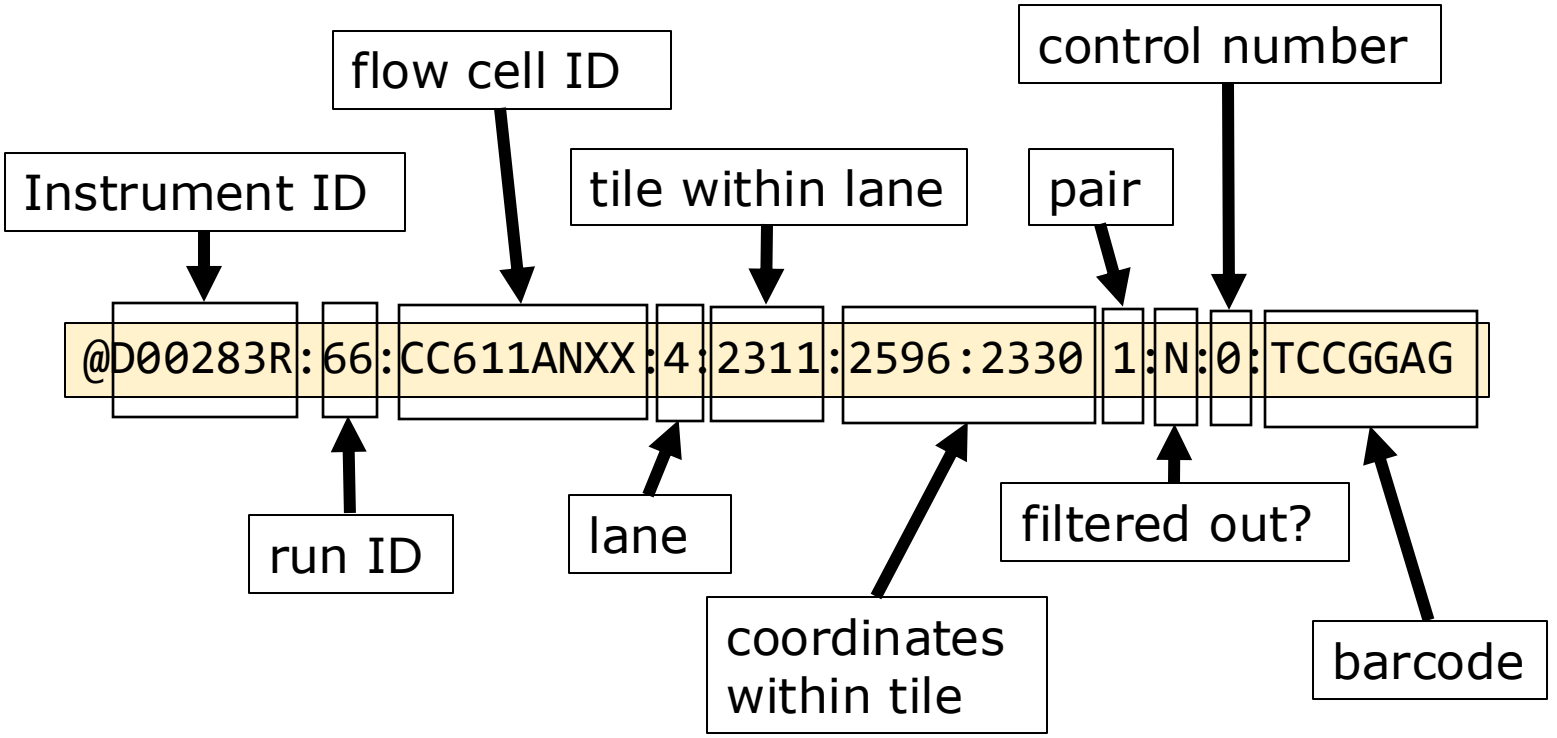
nucleotide sequence

optional description

base quality

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHI
|                              |    |        |
0.2.....................26...31........41
```

fastq header



Quiz Question 9

sam

sequence alignment format

Aim: alignments

sam header

```
@HD     VN:1.5  SO:coordinate
@SQ     SN:U00096.3     LN:4641652
@PG     ID:bowtie2      PN:bowtie2      VN:2.4.1
CL: "/opt/miniconda3/envs/ngs/bin/bowtie2-align-s \
--wrapper basic-0 \
-x /home/ubuntu/ecoli/ref_genome//ecoli-strK12-MG1655.fasta \
-1 /home/ubuntu/ecoli/trimmed_data/paired_trimmed_SRR519926_1.fastq \
-2 / home/ubuntu/ecoli/trimmed_data/paired_trimmed_SRR519926_2.fastq"
```

@SQ fields human data

```
@SQ     SN:chr1 LN:248956422
@SQ     SN:chr2 LN:242193529
@SQ     SN:chr3 LN:198295559
@SQ     SN:chr4 LN:190214555
@SQ     SN:chr5 LN:181538259
@SQ     SN:chr6 LN:170805979
@SQ     SN:chr7 LN:159345973
@SQ     SN:chr8 LN:145138636
@SQ     SN:chr9 LN:138394717
@SQ     SN:chr10        LN:133797422
@SQ     SN:chr11        LN:135086622
@SQ     SN:chr12        LN:133275309
@SQ     SN:chr13        LN:114364328
@SQ     SN:chr14        LN:107043718
@SQ     SN:chr15        LN:101991189
@SQ     SN:chr16        LN:90338345
@SQ     SN:chr17        LN:83257441
@SQ     SN:chr18        LN:80373285
@SQ     SN:chr19        LN:58617616
@SQ     SN:chr20        LN:64444167
@SQ     SN:chr21        LN:46709983
@SQ     SN:chr22        LN:50818468
@SQ     SN:chrX LN:156040895
@SQ     SN:chrY LN:57227415
@SQ     SN:chrM LN:16569
```

One aligned read from the E.coli dataset

@D00283R:66:CC611ANXX… 81 U00096.3 107 24 6M2I2M2I239M = 116 261
GCTCTTCCGATCTTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTCCA
CAACATCCATGAAACGCATTAGCACCACCATTACCCCCACCATCACCATTACCACAGGTAACGGTGCGGGCTGCCGC
GTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACA
ACCATGCGAGTGTTGAAGTT
###@<0>?4(A>:(4:>>:@>CB@>:>4@@>>8+@CCC:@4(>:3<0.7DCC>C>>::>>@8DCACC@::CCA::((+(A@4::
4C>:>><<2@AACCA@><9?:+AC@>)&50&?:3CC>:>4C@@::9?@:@CDBBB?BDDDDDDDDDBDDDDCCCC
DDDDCACCDDDDDDDDDDDDDCC>DDDDDDDDDDDDDEDDFHJJJJJIJJIJJJJJJJJJJJIJIJJJJIHFJJJJJJHHHHHFFFFF
CCC
AS:i:-44 XN:i:0 XM:i:7 XO:i:2 XG:i:4 NM:i:11 MD:Z:0T0T1A4C63A37A37A98 YS:i:-57 YT:Z:DP

SAM column	example
read name	@D00283R:66:CC61…
flag	81
reference	U00096.3
start position	107
mapping quality	24
CIGAR string	6M2I2M2I239M
reference name mate is mapped	=
start position mate	116
fragment length	261
sequence	GCTCTTCCGA
base quality	###@<0>?4(
optional	AS:i:-44
optional	XN:i:0

Tags explanation

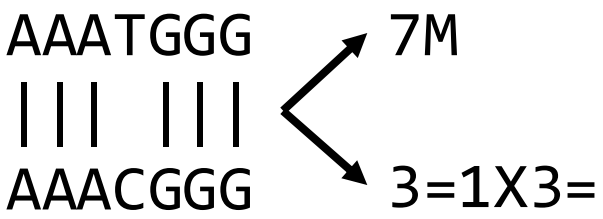
Tag	Meaning	Value	Notes
AS	Alignment score	-44	Lower = worse
XN	Ambiguous bases in ref (e.g. Ns)	0	None
XM	Mismatches	7	Align-specific
XO	Gap opens	2	2 insertions
XG	Gap bases total	4	2 insertions of 2
NM	Edit distance	11	7 mismatches + 4 indel bases
MD	Reference mismatches	0T0T1A4...	Ref-level
YS	Mate's alignment score	-57	Worse than this read
YT	Alignment type	DP	Duplicate Pair

CIGAR strings

Concise Idiosyncratic Gapped Alignment Report

Op	BAM	Description
M	0	alignment match (can be a sequence match or mismatch)
I	1	insertion to the reference
D	2	deletion from the reference
N	3	skipped region from the reference
S	4	soft clipping (clipped sequences present in SEQ)
H	5	hard clipping (clipped sequences NOT present in SEQ)
P	6	padding (silent deletion from padded reference)
=	7	sequence match
X	8	sequence mismatch

Almost never used



https://www.biostars.org/p/17043/
https://www.biostars.org/p/354942/
https://samtools.github.io/hts-specs/SAMv1.pdf

Quiz Question 10

sam flags

Bit		Description
1	0x1	template having multiple segments in sequencing
2	0x2	each segment properly aligned according to the aligner
4	0x4	segment unmapped
8	0x8	next segment in the template unmapped
16	0x10	SEQ being reverse complemented
32	0x20	SEQ of the next segment in the template being reverse complemented
64	0x40	the first segment in the template
128	0x80	the last segment in the template
256	0x100	secondary alignment
512	0x200	not passing filters, such as platform/vendor quality controls
1024	0x400	PCR or optical duplicate
2048	0x800	supplementary alignment

https://samtools.github.io/hts-specs/SAMv1.pdf

	read paired?	properly aligned?	unmapped?	mate unmapped?	flag
read1	1	1	0	0	3
read2	1	0	0	1	9
read3	1	0	1	1	13

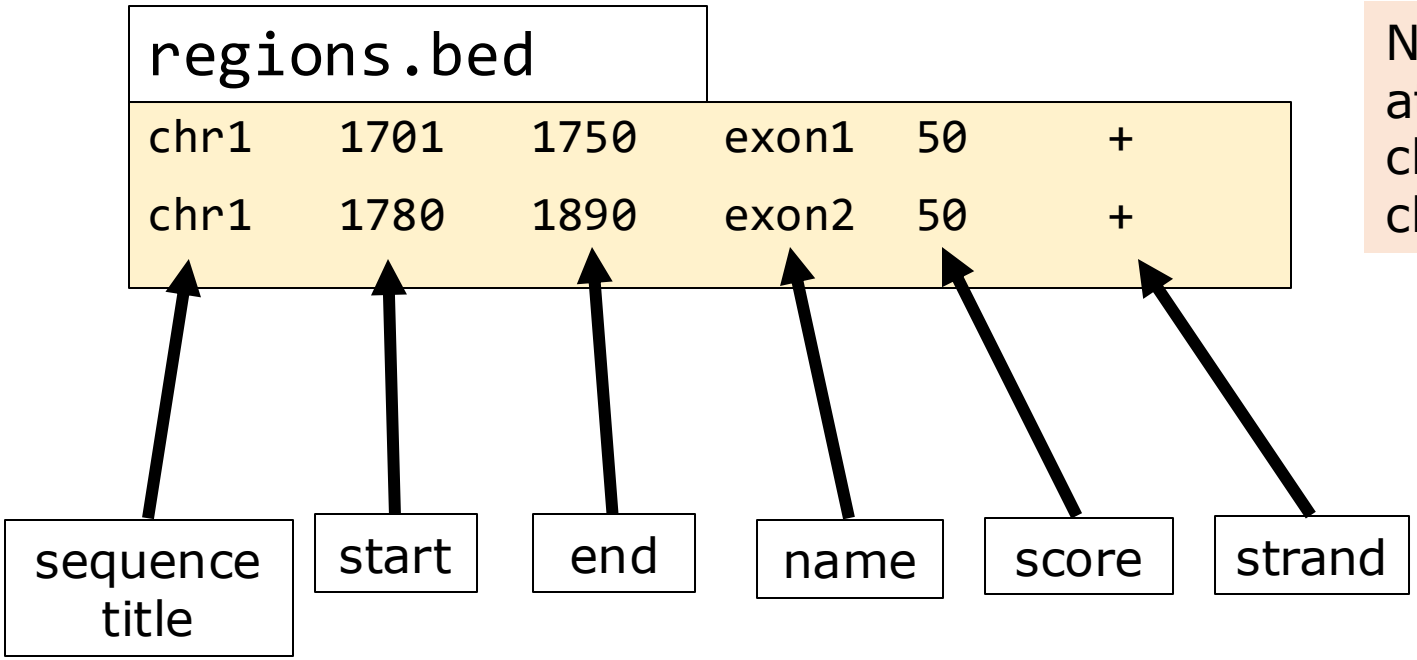
1	2	4	8

Quiz Question 11

bed

Browser Extensible Data

Aim: specify regions



Numbering starts at 0!
chr1:1702-1750
chr1:1781-1890

Simple DNA Sequence Example

- Sequence: ACTGAT

0-based Position:	0	1	2	3	4	5
1-based Position:	1	2	3	4	5	6
Base:	A	**C**	**T**	**G**	A	T

The 0-based System

- Used by: BED, BigBed, Python, C, etc.

- The first nucleotide (A) is at position 0
- Intervals are defined as [start, end) → start included, **end excluded**

- Example:
- A BED interval with start = 1, end = 4 → positions

  1, 2, 3 → C, T, G

The 1-based System

- Used by: GTF/GFF, VCF, SAM/BAM

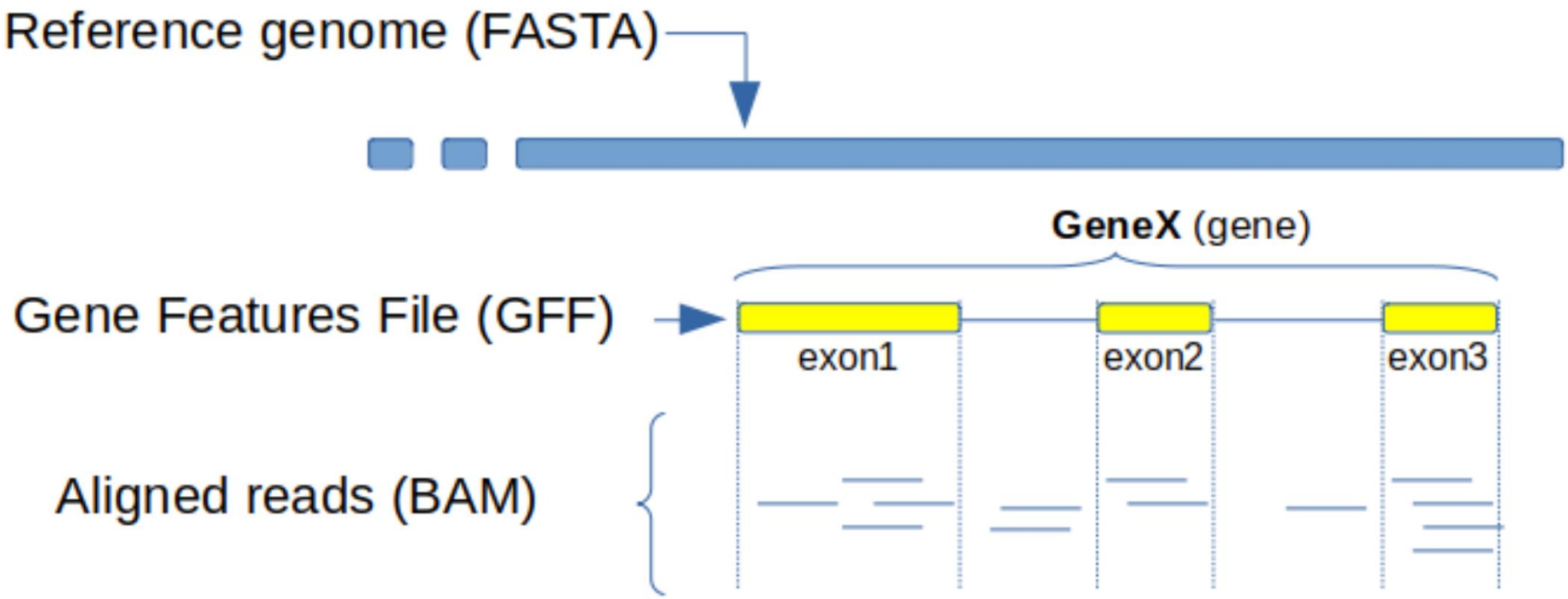
- The first nucleotide (A) is at position 1
- Intervals are defined as [start, end] → start and **end included**

- Example:
- A GTF interval with start = 2, end = 4 → positions

  2, 3, 4 → C, T, G

Question

gff



From https://bioinfogp.cnb.csic.es/tools/seqnjoy/help/

gff

General Feature Format

Aim: annotation

seq name	source	feature	start	end	score	strand	frame	attributes
1	ensembl	mRNA	339070	346959	.	-	.	ID=…;
1	ensembl	exon	339070	339312	.	-	.	Parent=; …
1	ensembl	CDS	339070	339312	.	-	0	ID=;…

```
##gff-version 3
#description: evidence-based annotation of the human genome (GRCh38), version 45 (Ensembl 111)
#provider: GENCODE
#contact: gencode-help@ebi.ac.uk
#format: gff3
#date: 2023-09-19
##sequence-region chr1 1 248956422
chr1 HAVANA gene 11869 14409 . + . ID=ENSG00000290825.1;gene_id=ENSG00000290825.1;gene_type=lncRNA
;gene_name=DDX11L2;level=2;tag=overlaps_pseudogene
chr1 HAVANA transcript 11869 14409 . + . ID=ENST00000456328.2;Parent=ENSG00000290825.1;gene_id=ENSG
00000290825.1;transcript_id=ENST00000456328.2;gene_type=lncRNA;gene_name=DDX11L2;transcript_type=lncRNA;transcript_
name=DDX11L2-
202;level=2;transcript_support_level=1;tag=basic,Ensembl_canonical;havana_transcript=OTTHUMT00000362751.1
chr1 HAVANA exon 11869 12227 . + . ID=exon:ENST00000456328.2:1;Parent=ENST00000456328.2;gene_id=ENS
G00000290825.1;transcript_id=ENST00000456328.2;gene_type=lncRNA;gene_name=DDX11L2;transcript_type=lncRNA;transcrip
t_name=DDX11L2-
202;exon_number=1;exon_id=ENSE00002234944.1;level=2;transcript_support_level=1;tag=basic,Ensembl_canonical;havana_tra
nscript=OTTHUMT00000362751.1
chr1 HAVANA exon 12613 12721 . + . ID=exon:ENST00000456328.2:2;Parent=ENST00000456328.2;gene_id=ENS
G00000290825.1;transcript_id=ENST00000456328.2;gene_type=lncRNA;gene_name=DDX11L2;transcript_type=lncRNA;transcrip
t_name=DDX11L2-
202;exon_number=2;exon_id=ENSE00003582793.1;level=2;transcript_support_level=1;tag=basic,Ensembl_canonical;havana_tra
nscript=OTTHUMT00000362751.1
chr1 HAVANA exon 13221 14409 . + . ID=exon:ENST00000456328.2:3;Parent=ENST00000456328.2;gene_id=ENS
G00000290825.1;transcript_id=ENST00000456328.2;gene_type=lncRNA;gene_name=DDX11L2;transcript_type=lncRNA;transcrip
t_name=DDX11L2-
202;exon_number=3;exon_id=ENSE00002312635.1;level=2;transcript_support_level=1;tag=basic,Ensembl_canonical;havana_tra
nscript=OTTHUMT00000362751.1
```

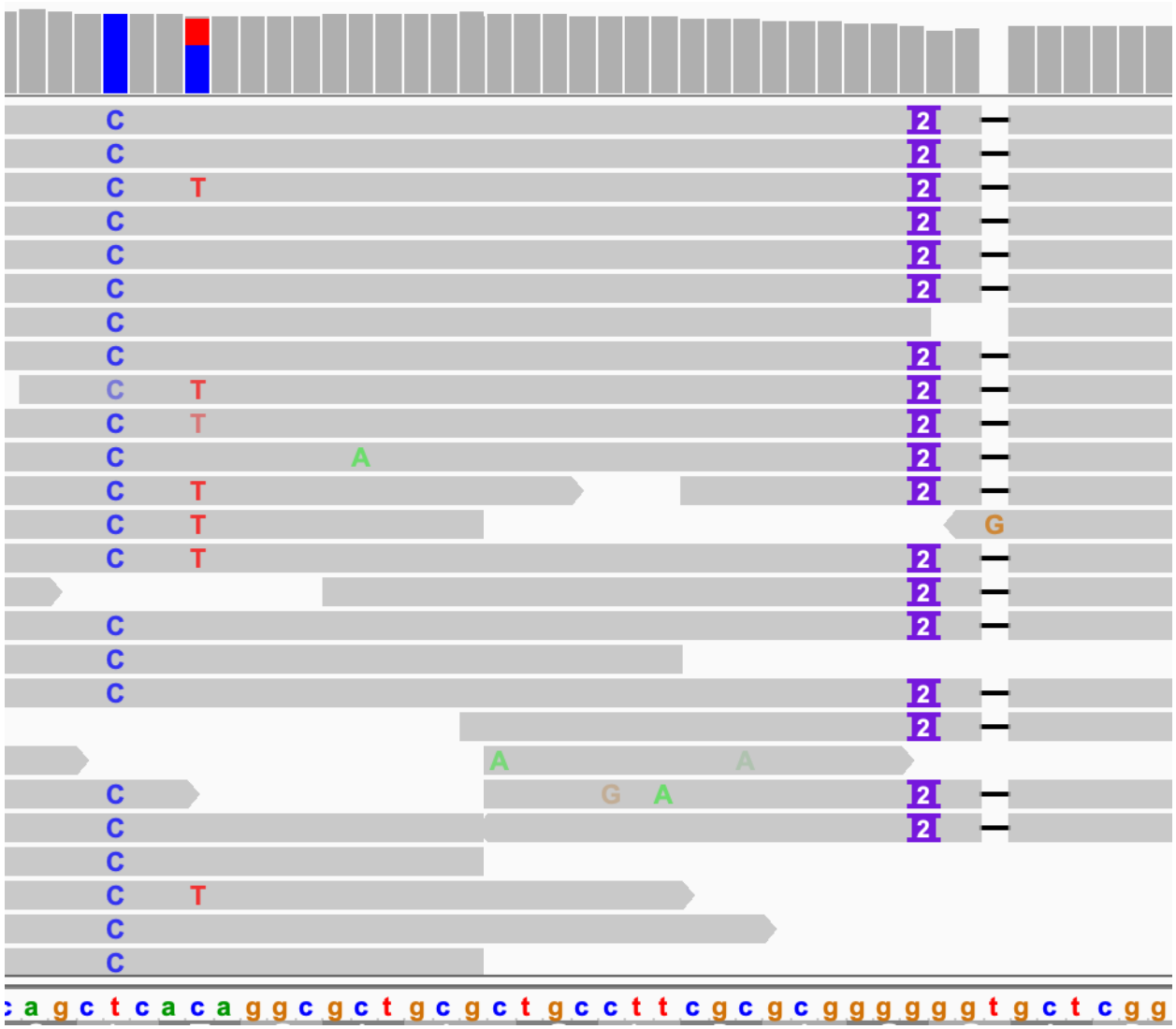
vcf

Variant Call Format

Aim: variants

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF    ALT     QUAL FILTER INFO                              FORMAT      NA00001        NA00002        NA00003
20     14370   rs6054257 G      A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:.,.
20     17330   .         T      A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3   0/0:41:3
20     1110696 rs6040355 A      G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2   2/2:35:4
20     1230237 .         T      .       47   PASS   NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20     1234567 microsat1 GTC    G,GTCT  50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2       1/1:40:3
```

SIB



vcf

```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS      ID         REF  ALT    QUAL FILTER  INFO                              FORMAT      NA00001         NA00002
20     14370    rs6054257  G    A      29   PASS    NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51  1|0:48:8:51,51
20     17330    .          T    A      3    q10     NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50  0|1:3:5:65,3
20     1110696  rs6040355  A    G,T    67   PASS    NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27  2|1:2:0:18,2
20     1230237  .          T    .      47   PASS    NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60  0|0:48:4:51,51
20     1234567  microsat1  GTC  G,GTCT 50   PASS    NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4        0/2:17:2
```

samples