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DATA FORMATS IN NGS
INTRODUCTION TO GALAXY

Bioinformàtica per a la Recerca Biomèdica

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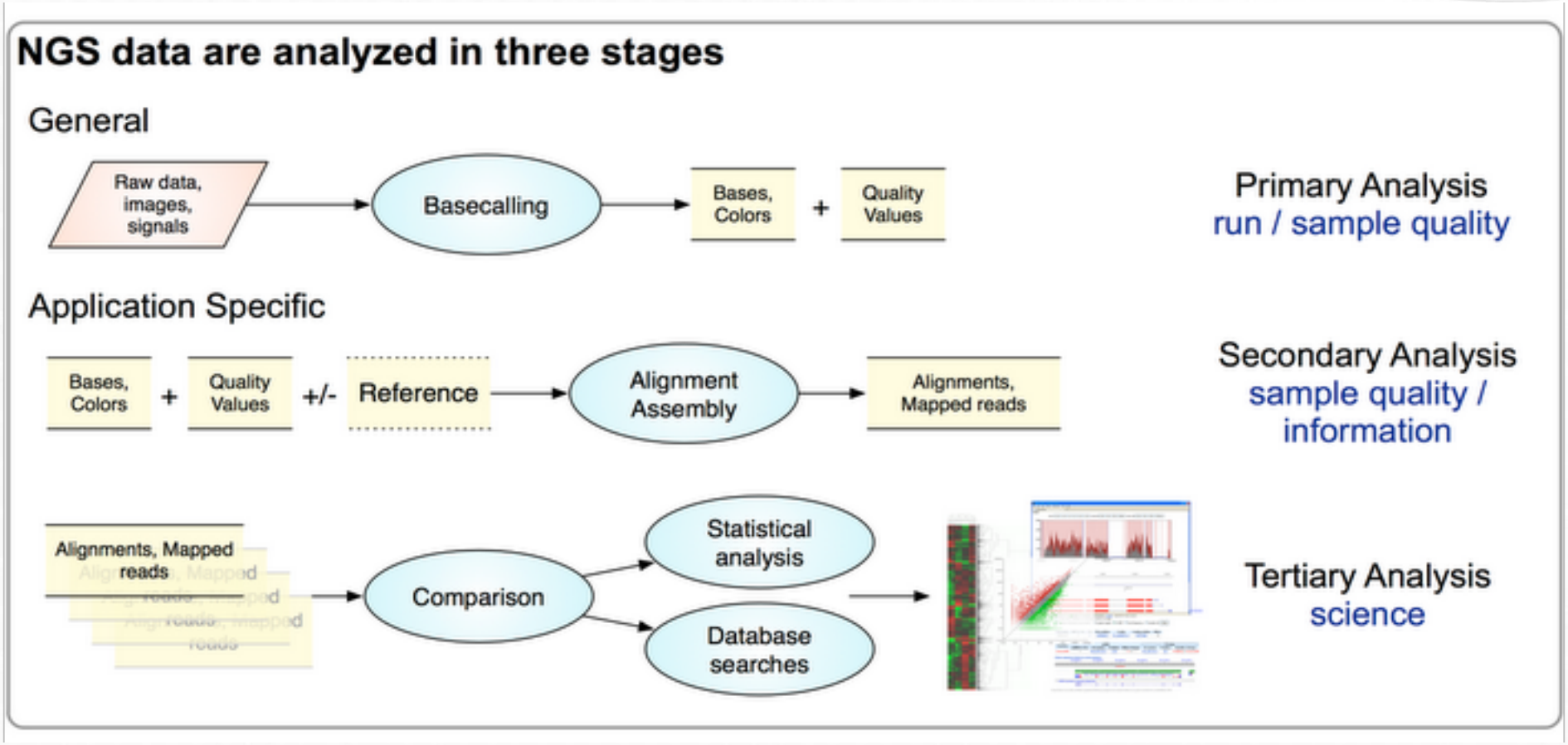
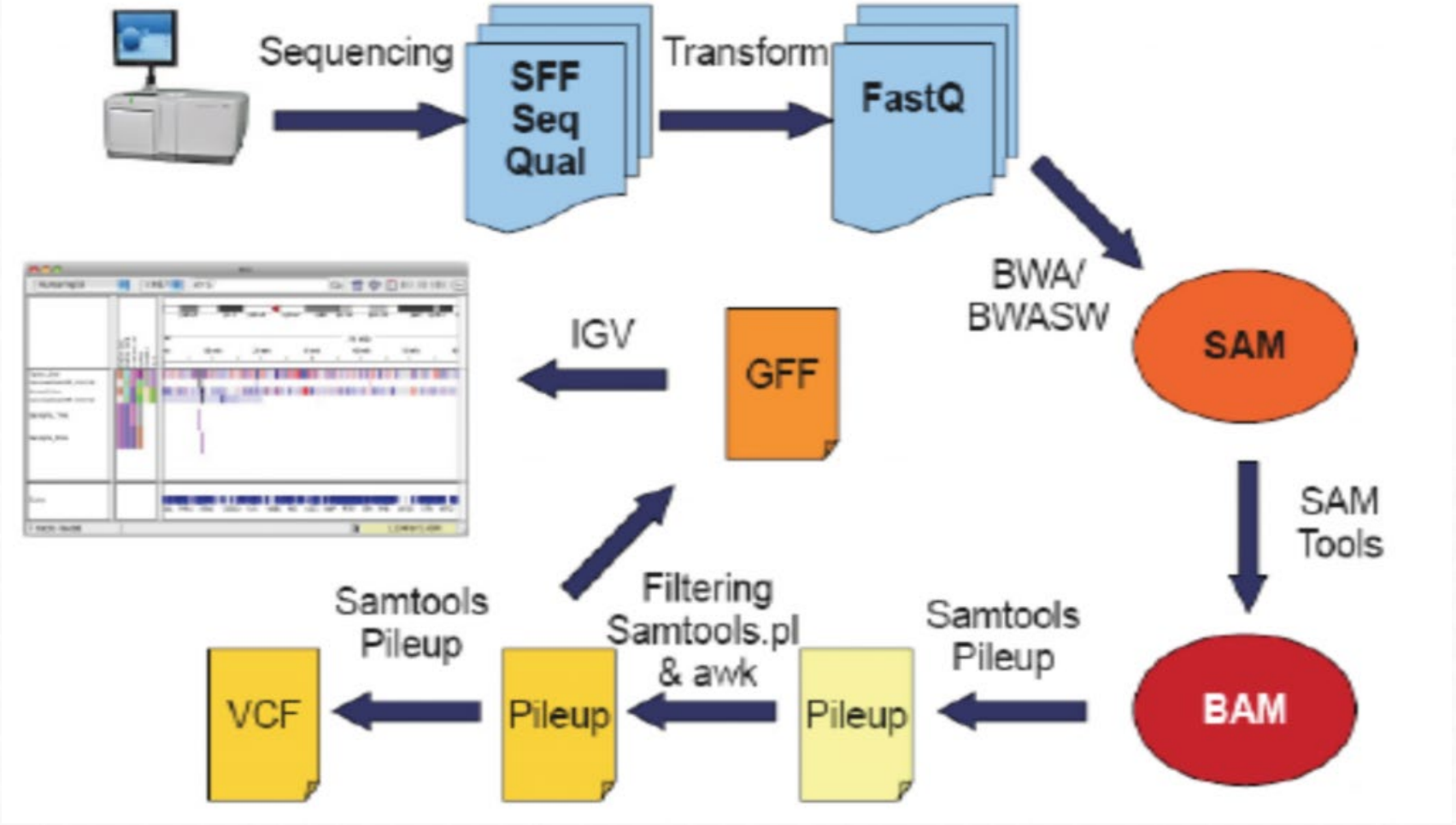
The stages of Data Analysis



Depending on the analysis step data may be stored in different formats

Data Analysis stage and file format



Data formats used in NGS

- Formats are designed to hold sequence data and other information about sequence
- There are many different types of file formats depending on:
  - Type of information they contain
    - Raw Sequence ,Co-ordinate, Parameter, Annotation, Metadata
  - Sequencing platform
  - Analysis stage
  - Data source

The FASTA format

FASTA format

- FASTA format is a text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes
- Header line starts with “>” followed by a sequence ID, and followed by lines of sequence data

```
>NG_016798.2 Homo sapiens DNA polymerase alpha 1, catalytic subunit (POLA1), RefSeqGene on
chromosome X
CCCTCAGTTGGTGCCAGTAACTGTTGTTCCCTTCTTTGTGTCCATTTGTACTCAATGTTTACCTCCCACT
TATAAGTGAGAACATGTGGTATTTGGTTTTCTGTTCCTATGTTAGTTCGCTTAGGATAATGGCTTCCAAC
TCCATCCATGTTGCTGCAGACGTGATCTCATTCTTTTTTTTTTTTTTTTTTTGAGACAGAGTCGTGCTC
TGTCGCCCAGGCTGGAGTGCAGTGGTGCGATCTCGGCTCACTGCAACCTCTGCCTCCTGGGTTGAAGTGA
CTCTCCTGGTTCAGCCTCCTGAGTAGCTAGGATTATAGGTGCCCGCCACCATGCCTGGCTAATTTTTGTA
TTTTTAGTAGAGATGGGGTTTTGCCATGTTGGCCAGGCTGATCTCGAACTCCTGACCTCAGGTGATCTGC
CCACCCAGAGTGGCCTCCCAAAGTGTTTGGAATACAGGCGTGAGCCACTGCACCTGGTTTCTTTTTTATG
GCTGTAAATTAGTTCCACCATTGTGGAAGACAGTGTGGTGATTCACATAAAAGTAGAAGTCTAAGAATCA
AACCCTAAGTCTGACTCTACCTGAGTCTTTAATCCTTCCAATATAATATTAAAGAGGACAAATTATAAAC
AAAAAGAGTCTATAATTCTATCATCCTGGCAAAATATACTCCATTTTGCATATTGCTTTCAGGTAAATAA
```

```
>NP_001365232.1 DNA polymerase alpha catalytic subunit isoform 3 [Homo sapiens]
MAPVHGDDCEIGASALSDSGSFVSSRARREKKSKKGRQEALERLKKAKAGEKYKYEVEDFTGVYEEVDEE
QYSKLVQARQDDDWIVDDDGIGYVEDGREIFDDDLEDDALDADEKGKDGKARNKDKRNVKKLAVTKPNNI
KSMFIACAGKKTADKAVDLSKDGLLGDILQDLNTETPQITPPPVMILKKKRSIGASPNPFSVHTATAVPS
GKIASPVSRKEPPLTPVPLKRAEFAGDDVQVESTEEEQESGAMEFEDGDFDEPMEVEEVDLEPMAAKAWD
KESEPAEEVKQEADSGKGTVSYLGSFLPDVSCWDIDQEGDSSFSVQEVQVDSSHLPLVKGADEEQVFHFY
WLDAYEDQYNQPGVVFLFGKVWIESAETHVSCCVMVKNIERTLYFLPREMKIDLNTGKETGTPISMKDVY
EEFDEKIATKYKIMKFKSKAEMPQLPQDLKGETFSHVFGTNTSSLELFLMNRKIKGPCWLEVKSPQLLNQ
PVSWCKVEAMALKPDLVNVIKDVSPPPLVVMAFSMKTMQNAKNHQNEIIAMAALVHHSFALDKAAPKPPF
QSHFCVVSKPKDCIFPYAFKEVIEKKNVKVEVAATERTLLGFFLAKVHKIDPDIIVGHNIYGFELEVLLQ
RINVCKAPHWSKIGRLKRSNMPKLGGRSGFGERNATCGRMICDVEISAKELIRCKSYHLSELVQQILKTE
```

The FASTQ format

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- Output of most actual sequencing platforms for raw data
- A text-based format for storing both a **nucleotide sequence** and its corresponding **quality scores**
- Standard file extension for a FASTQ file are .fq and .fastq
- FASTQ files are uncompressed and quite large because they contain the following information for every single sequencing read.
- Compressed files are also possible: fastq.gz

The FASTQ format

- File structure. 4 lines:
  - @ followed by the read ID and possibly information about the sequencing run
  - sequenced bases
  - + (perhaps followed by the read ID again, or some other description)
  - quality scores for each base of the sequence (ASCII-translated Phred scores)

```
@Seq description
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

Incise: The ASCII code

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Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char
0	0	[NULL]	32	20	[SPACE]	64	40	@	96	60	`
1	1	[START OF HEADING]	33	21	!	65	41	A	97	61	a
2	2	[START OF TEXT]	34	22	"	66	42	B	98	62	b
3	3	[END OF TEXT]	35	23	#	67	43	C	99	63	c
4	4	[END OF TRANSMISSION]	36	24	$	68	44	D	100	64	d
5	5	[ENQUIRY]	37	25	%	69	45	E	101	65	e
6	6	[ACKNOWLEDGE]	38	26	&	70	46	F	102	66	f
7	7	[BELL]	39	27	'	71	47	G	103	67	g
8	8	[BACKSPACE]	40	28	(	72	48	H	104	68	h
9	9	[HORIZONTAL TAB]	41	29	)	73	49	I	105	69	i
10	A	[LINE FEED]	42	2A	*	74	4A	J	106	6A	j
11	B	[VERTICAL TAB]	43	2B	+	75	4B	K	107	6B	k
12	C	[FORM FEED]	44	2C	,	76	4C	L	108	6C	l
13	D	[CARRIAGE RETURN]	45	2D	-	77	4D	M	109	6D	m
14	E	[SHIFT OUT]	46	2E	.	78	4E	N	110	6E	n
15	F	[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	[NEGATIVE 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 OF 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	[SUBSTITUTE]	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	[DEL]

- The ASCII code provides a simple way to express two or three digit values using a single carácter:
- For example,
  - instead of ‘110’ a lowercase n, ‘n’ can be used, or
  - Instead of a 92 a “\” can be used

What are PHRED scores

- Sequencing systems assign quality scores to each peak, that represents the error probability that an individual base call is incorrect.
- Phred scores provide log(10)-transformed error probability values:

  If p is probability that the base call is wrong the Phred score is

PHRED Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90 %
20	1 in 100	99 %
30	1 in 1000	99.9 %
40	1 in 10000	99.99 %
50	1 in 100000	99.999 %

$$Q = .10 \cdot log_{10} p$$

Base calling

- The base calling (A, T, G or C) is performed based on Phred scores.
- Ambiguous positions with Phred scores <= 20 are labeled with N.
- To assign each base a unique score identifier (instead of numbers of varying character length), Phred scores are typically represented as ASCII characters.
- Different platforms may use different ASCII ranges for Phred encoding

Description	ASCII characters		Quality score	
	Range	Offset	Type	Range
Solexa/early Illumina (1.0)	59 to 126 (; to ~)	64	Solexa	-5 to 62
Illumina 1.3+	64 to 126 (@ to ~)	64	Phred	0 to 62
Sanger standard/Illumina 1.8+	33 to 126 (! to ~)	33	Phred	0 to 93

Base call quality scores are represented with the Phred range. Different Illumina (formerly Solexa) versions used different scores and ASCII offsets. Starting with Illumina format 1.8, the score now represents the standard Sanger/Phred format that is also used by other sequencing platforms and the sequencing archives.

SAM / BAM format

- The **Sequence Alignment/Map (SAM)** format is a generic nucleotide alignment format that describes the alignment of sequencing reads to a reference.
- SAM files typically contain:
  - a short header section with information about the genomic loci of each read
  - a very long alignment section where each row represents a single read alignment.
  - Each alignment line has 11 mandatory fields for essential alignment information such as mapping position, and variable number of optional fields for flexible or aligner specific information

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

SAM / BAM : mandatory information

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Mandatory Alignment Section Fields

Position	Field	Description
1	QNAME	Query template (or read) name
2	FLAG	Information about read mapping (see next section)
3	RNAME	Reference sequence name. This should match a @SQ line in the header.
4	POS	1-based leftmost mapping position of the first matching base. Set as 0 for an unmapped read without coordinate.
5	MAPQ	Mapping quality of the alignment. Based on base qualities of the mapped read.
6	CIGAR	Detailed information about the alignment (see relevant section).
7	RNEXT	Used for paired end reads. Reference sequence name of the next read. Set to "=" if the next segment has the same name.
8	PNEXT	Used for paired end reads. Position of the next read.
9	TLEN	Observed template length. Used for paired end reads and is defined by the length of the reference aligned to.
10	SEQ	The sequence of the aligned read.
11	QUAL	ASCII of base quality plus 33 (same as the quality string in the Sanger FASTQ format).
12	OPT	Optional fields (see relevant section).

From the alignment to SAM format

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Suppose we have the following alignment with bases in lowercase clipped from the alignment. Read `r001/1` and `r001/2` constitute a read pair; `r003` is a chimeric read; `r004` represents a split alignment.

```
Coor     12345678901234  5678901234567890123456789012345
ref      AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT

+r001/1        TTAGATAAAGGATA*CTG
+r002         aaaAGATAA*GGATA
+r003       gcctaAGCTAA
+r004                      ATAGCT.............TCAGC
-r003                            ttagctTAGGC
-r001/2                                       CAGCGGCAT
```

The corresponding SAM format is:[2]

```
@HD VN:1.6 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

BAM format, a binary version of SAM

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- A **BAM file** is a binary version of a SAM file.
- Both contain identical information about reads and their mapping.
- A BAM file requires a header but a SAM file may not have one.
- Many operations (such as sorting and indexing) work only on BAM files.
- For almost any application that requires SAM input, this can be created on the fly from a BAM,
- BAM files take up much less space than SAM files.
- For archiving purposes, keep only the BAM file. The SAM file can easily be regenerated (if ever needed).

Formats for genome annotations (1) BED

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- Formats for genome annotations
- One line per genomic feature
- The **BED format** is the simplest way to store annotation tracks. It has three required fields (chromosome, start, end) and up to 9 optional fields (name, score, strand, thickStart, thickEnd, itemRgb, blockCount, blockSizes, blockStarts).

```
# 6-column BED file defining transcript loci
chr1   66999824   67210768   NM_032291 0 +
chr1   33546713   33586132   NM_052998 0 +
chr1   25071759   25170815   NM_013943 0 +
chr1   48998526   50489626   NM_032785 0 -
```

Formats for genome annotations (2) GFF/GTF

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- The **General Feature Format (GFF) and General Transfer Format (GTF)** has nine required fields; the first three fields form the basic name, start, end tuple that allows for the identification of the location in respect to the reference genome.

Sample GTF output from Ensembl data dump:

```
1 transcribed_unprocessed_pseudogene  gene        11869 14409 . + . gene_id "ENSG00000223972"; gene_name "DDX11L1"; gene_source "havana"; gene_biotype "transcribed_unprocessed_pseudogene";
1 processed_transcript                transcript  11869 14409 . + . gene_id "ENSG00000223972"; transcript_id "ENST00000456328"; gene_name "DDX11L1"; gene_sourc e "havana"; gene_biotype "transcrib
```

Sample GFF output from Ensembl export:

```
X       Ensembl Repeat  2419108 2419128 42      .       .       hid=trf; hstart=1; hend=21
X       Ensembl Repeat  2419108 2419410 2502    -       .       hid=AluSx; hstart=1; hend=303
X       Ensembl Repeat  2419108 2419128 0       .       .       hid=dust; hstart=2419108; hend=2419128
X       Ensembl Pred.trans.     2416676 2418760 450.19  -       2       genscan=GENSCAN00000019335
X       Ensembl Variation       2413425 2413425 .       +       .
X       Ensembl Variation       2413805 2413805 .       +       .
```

http://m.ensembl.org/info/website/upload/gff.html

Information fields in GFF/GTF

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1. **reference sequence**: coordinate system of the annotation (e.g., "Chr1")
2. **source**: describes how the annotation was derived (e.g., the name of the annotation software)
3. **method**: annotation type (e.g., gene)
4. **start position**: 1-based integer, always less than or equal to the stop position
5. **stop position**: for zero-length features, such as insertion sites, start equals end and the implied site is to the right of the indicated base
6. **score**: e.g., sequence identity
7. **strand**: "+" for the forward strand, "-" for the reverse strand, or "." for annotations that are not stranded
8. **phase**: codon phase for annotations linked to proteins; 0, 1, or 2, indicating the frame, or the number of bases that should be removed from the beginning of this feature to reach the first base of the next codon
9. **group**: contains the class and ID of an annotation which is the logical parent of the current one ("feature is composed of")

Variant Call Format (VCF)

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- Variant Call Format (VCF) is a text file format.
- It contains meta-information lines, a header line, and then data lines each containing information about a position in the genome.
- Also has the ability to contain genotype information on samples for each position.
- The header line names the 8 fixed, mandatory columns.

1. #CHROM
2. POS
3. ID
4. REF
5. ALT
6. QUAL
7. FILTER
8. INFO

VCF example

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```
##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
```

https://samtools.github.io/hts-specs/VCFv4.2.pdf

Summary: Main data formats used in NGS

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- **Raw data**: .fastq (.fastq.gz)
- **Aligned data**: .sam / .bam
- **Annotation data**: .gtf / .gff / .bed
- **Results data**: .vcf