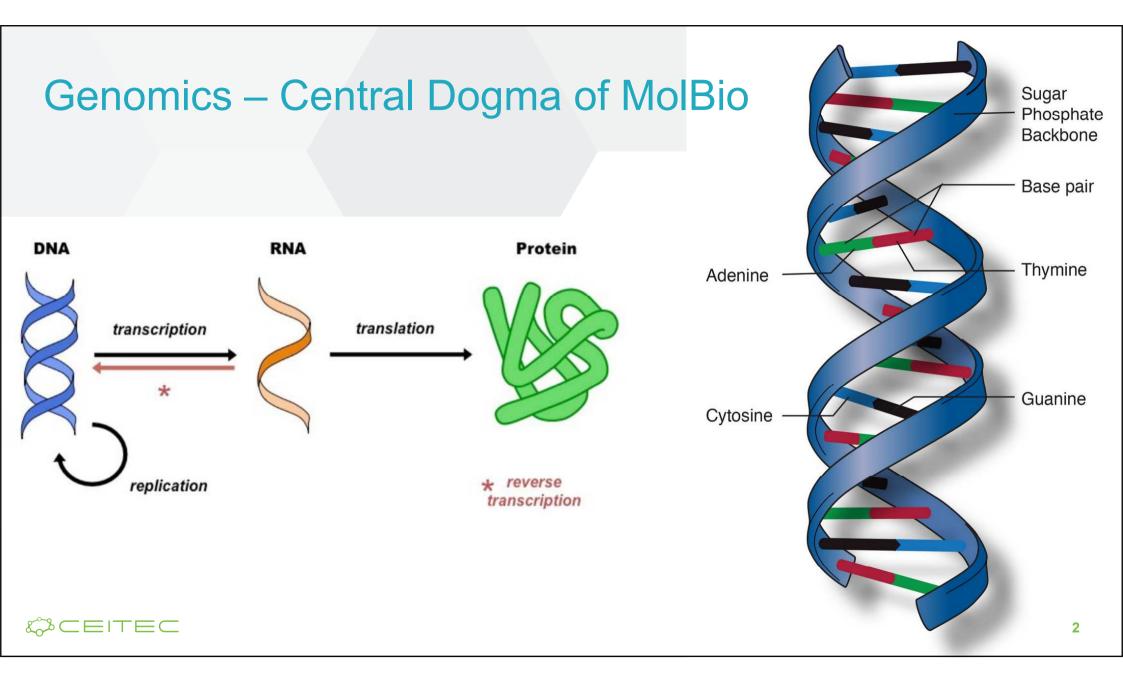
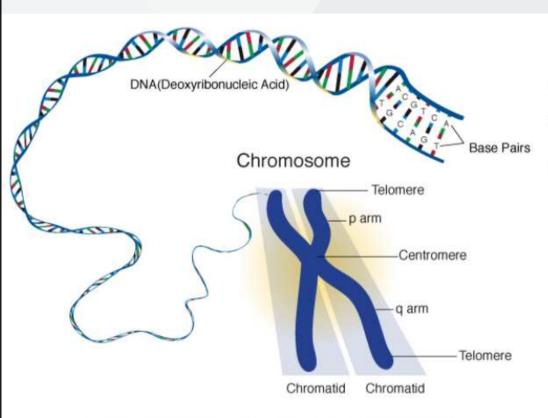
Central European Institute of Technology BRNO | CZECH REPUBLIC

Introduction to Bioinformatics (LF:DSIB01)

Week 2 : Sequence analysis



Chromosomes & Genomic Loci



Source: National Human Genome Research Institute

upstream downstream ... CCTTACTTATAATGCTCATGCTA

.. GGAATGAATATTACGAGTACGAT ..

downstream

upstream

A genomic locus is a POSITION on a chromosome or other genomic reference.

We often denote a locus by genome assembly, chr, start position, end position and strand.

A locus IS NOT A SEQUENCE even though a sequence might be associated with a locus.

\$CEITEC

Genes

- What is a gene?
 - Classical Period
 - Mendel 1866 : Zellelemente (cell elements) : some factors that determine heredity
 - Johannsen 1909: Coins the word Gene : some kind of calculating element
 - 1920s: Genes linked to chromosomes and grouped by heredity
 - Muller 1926: Gene is the basis of evolution
 - NeoClassical Period
 - 1940s: Genes have internal structure, can be dissected by recombination (1 dimension)
 - 1950s: Structure of DNA
 - 1960s: A gene is a discrete sequence that encodes a polypeptide (through RNA step)
 - 1960s-80s: cistrons; One cistron = one polypeptide
 - Modern Period
 - 1986 : Alternative Splicing
 - 1987: Multiple transcription initiation sites (One gene = many transcripts)
 - 1990s: Gene Editing (the RNA can be changed)
 - 2000s: Gene Sharing (one genomic locus can produce several widely different products)

Genes

- 1. In eukaryotic organisms, there are few if any absolute boundaries to transcription, making it impossible to establish simple general relationships between primary transcripts and the ultimate products of those transcripts.
- 2. Exons of different genes can be members of more than one transcript.
- 3. Comparably, in the organelles of microbial eukaryotes, many examples of "encrypted" genes are known: genes are often in pieces that can be found as separate segments around the genome.
- 4. In contradiction to the neoclassical definition of a gene, which posits that the hereditary information resides solely in DNA sequences, there is increasing evidence that the functional status of some genes can be inherited from one generation of individuals to the next, a phenomenon known as transgenerational epigenetic inheritance (Holliday 1987; Gerhart and Kirschner 2007; Jablonka and Raz 2009).
- "Genetic restoration" a mechanism of non-Mendelian inheritance of extragenomic information, first found in *Arabidopsis thaliana*, may also take place (Lolle *et al.* 2005).
- 6. Finally, in addition to protein coding genes, there are many RNA-encoding genes that produce diverse RNA molecules that are not translated to proteins.

The evolving definition of the term Gene (Portin and Wilkins 2017)

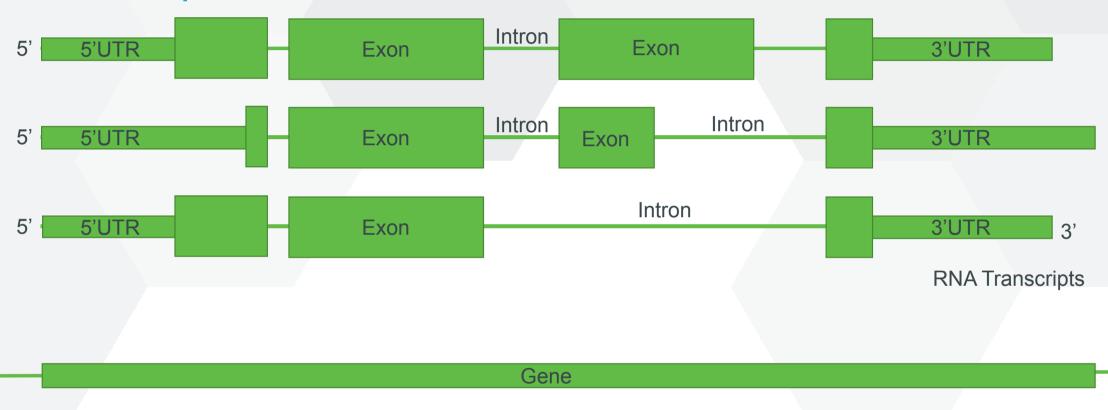
Genes

- The "Gene" cannot be simply defined
- There is no clear cut hereditary unit that acts autonomously
- A Gene is a DNA sequence (not necessarily contiguous) that specifies one or more sequence-related RNAs/proteins that are involved with some Gene Regulatory Network. (this definition pushes the 'hard' question to Network)
- A Gene is a genomic locus that produces RNAs that have been annotated as connected to each other by function or heredity (this definition might fail to include genes split across loci and is based on external annotation thus subjective)
- As a working definition of a Gene:
 A Genomic Locus that produces related* Transcripts

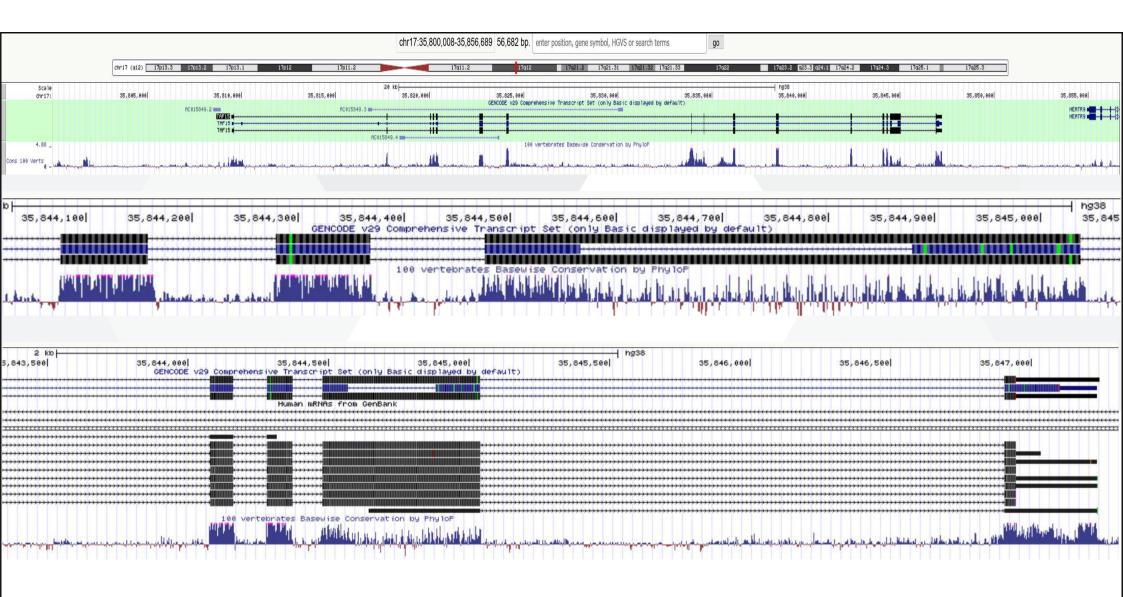
*Related implies an Annotation

\$CEITEC

Transcripts -> Genes



Gene is a locus that produces (related) Transcripts < Incomplete working definition



Genomics and Transcriptomics

- Genomics is the scientific discipline that studies heredity, genes, and genomes
- Transcriptomics is the scientific discipline that studies RNA

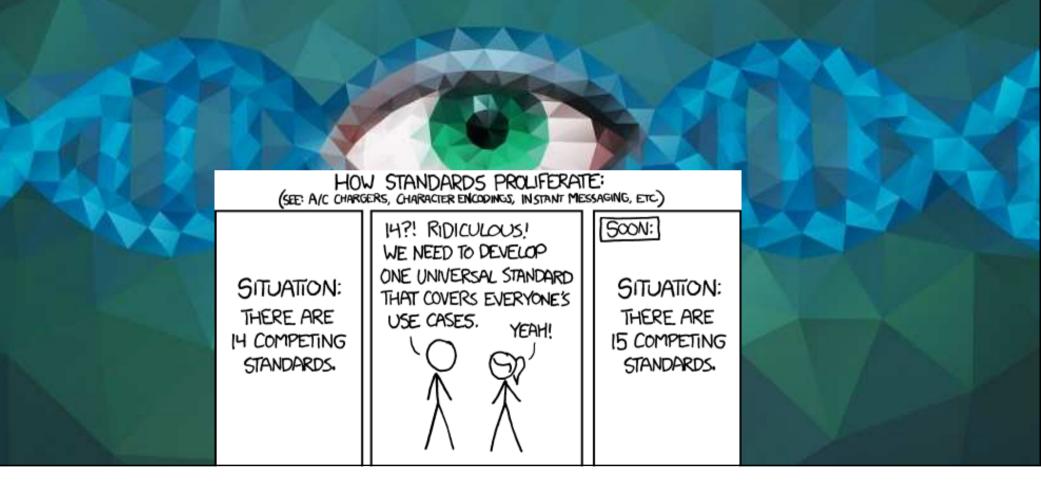
Both disciplines share techniques, analyses, and practical uses.

Genomic terms will be often used as a shortcut for talking about transcripts.



Encoding Genomic Information for Bioinformatics Use

- Location Based Formats (.bed)
- Count/Coverage Based Formats (.bedgraph .wig)
- Feature Based Formats (.gtf)
- Sequence Based Formats (.fasta .fastq)
- Multiple Alignment Files
- Alignment Based Formats (.sam)



BED (Browser Extensible Data) file format

- Tab Delimited Text File
- Number of columns consistent per line
- No Empty fields (some can have "." as N/A)

BED 3 columns : chrom, chromStart, chromEnd
BED 6 columns : BED3 + name, score, strand
BED 12 columns : BED6 + thickStart, thickEnd, itemRGB, blockCount, blockSizes, blockStarts

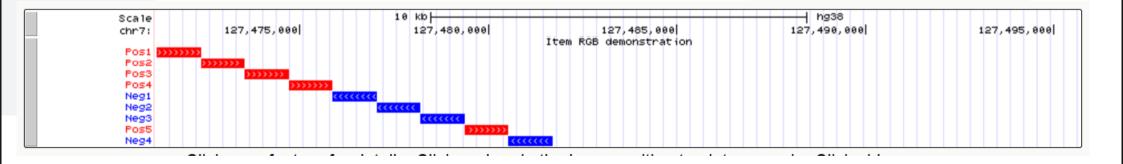
chr22 1000 5000 cloneA 960 + 1000 5000 0 2 567,488, 0,3512 chr22 2000 6000 cloneB 900 - 2000 6000 0 2 433,399, 0,3601

https://genome.ucsc.edu/FAQ/FAQformat.html#format1



chr7	127471196	127472363	Pos1	0	+	127471196	127472363	255,0,0
chr7	127472363	127473530	Pos2	0	+	127472363	127473530	255,0,0
chr7	127473530	127474697	Pos3	0	+	127473530	127474697	255,0,0
chr7	127474697	127475864	Pos4	0	+	127474697	127475864	255,0,0
chr7	127475864	127477031	Negl	0	-	127475864	127477031	0,0,255
chr7	127477031	127478198	Neg2	0	-	127477031	127478198	0,0,255
chr7	127478198	127479365	Neg3	0	-	127478198	127479365	0,0,255
chr7	127479365	127480532	Pos5	0	+	127479365	127480532	255,0,0
chr7	127480532	127481699	Neg4	0	-	127480532	127481699	0,0,255

BED 3 columns : chrom, chromStart, chromEnd BED 6 columns : BED3 + name, score, strand BED 12 columns : BED6 + thickStart, thickEnd, itemRGB, blockCount, blockSizes, blockStarts



BED pros and cons

- Generic
- Human Readable
- Useful for simple genomic loci
- Awkward handling of splice events
- Not useful for variable scores
- Must repeat BED3 info every line



bedGraph

- Used to display continuous data
- Header "browser" + Header "track" + Sorted BED lines (chr, start, stop, score)

browser position chr19:49302001-49304701 browser hide all browser pack refGene encodeRegions browser full altGraph 300 base wide bar graph, autoScale is on by default == graphing limits will dynamically change to always show full range of data in viewing window, priority = 20 positions this as the second graph Note, zero-relative, half-open coordinate system in use for bedGraph format track type=bedGraph name="BedGraph Format" description="BedGraph format" visibility=full color=200,100,0 altColor=0,100,200 priority=20 chr19 49302000 49302300 -1.0 chr19 49302300 49302600 -0.75 chr19 49302600 49302900 -0.50 chr19 49302900 49303200 -0.25 chr19 49303200 49303500 0.0 chr19 49303500 49303800 0.25 chr19 49303800 49304100 0.50 chr19 49304100 49304400 0.75

chr19 49304400 49304700 1.00

wiggle file (.wig)

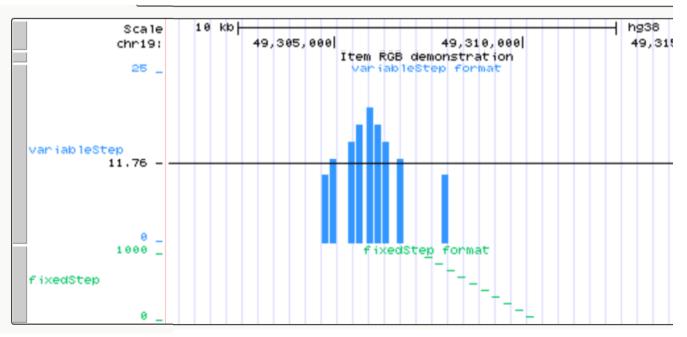
• Comes in two flavors: variablestep vs fixed step

<pre>variableStep chrom=chrN [span=windowSize] chromStartA dataValueA chromStartB dataValueB etc etc</pre>	<pre>fixedStep chrom=chrN start=position step=stepInterval [span=windowSize] dataValue1 dataValue2 etc</pre>
<pre>variableStep chrom=chr2 300701 12.5 300702 12.5 300703 12.5 300704 12.5 300705 12.5</pre>	fixedStep chrom=chr3 start=400601 step=100 11 22 33
variableStep chrom=chr2 span=5 300701 12.5	fixedStep chrom=chr3 start=400601 step=100 span=5 11 22 33
	16

browser position chr19:49304200-49310700 browser hide all 150 base wide bar graph at arbitrarily spaced positions, # threshold line drawn at y=11.76 # autoScale off viewing range set to [0:25] # priority = 10 positions this as the first graph # Note, one-relative coordinate system in use for this format # track type=wiggle 0 name="variableStep" description="variableStep format" visibility=full autoScale=off viewLimits=0.0:25.0 color=50,150,255 yLineMark=11.76 yLineOnOff=on priority=10 variableStep chrom=chr19 span=150 49304701 10.0 49304901 12.5 49305401 15.0 49305601 17.5 49305901 20.0 49306081 17.5 49306301 15.0 49306691 12.5 49307871 10.0 200 base wide points graph at every 300 bases, 50 pixel high graph # autoScale off and viewing range set to [0:1000] # priority = 20 positions this as the second graph # Note, one-relative coordinate system in use for this format # track type=wiggle 0 name="fixedStep" description="fixedStep format" visibility=full autoScale=off viewLimits=0:1000 color=0,200,100 maxHeightPixels=100:50:20 graphType=points priority=20 fixedStep chrom=chr19 start=49307401 step=300 span=200 1000 900

800

- 700
- 600
- 500
- 400
- 300 200
- 100



Wig pros and cons

- Compact
- Wide variety of values
- Difficult for human readability
- Difficult to add/subtract lines



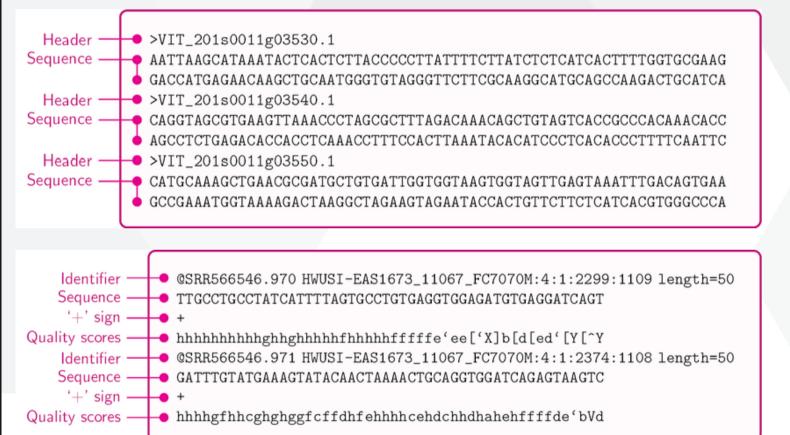
General transfer format (.gtf)

- Also commonly known as .gff (general feature format)
- 1. seqname (chr)
- 2. source (program generating seq)
- 3. feature (what is it? "CDS", "exon", "enhancer" etc)
- 4. start (position)
- 5. end (inclusive)
- 6. score (can be any float. Ideal: between 1-1000 for UCSC browser)
- 7. strand ("+", "-", ".")
- 8. frame (for exons, frame is between 0-2 representing open reading frame, else ".")
- 9. group; list of attributes (gene_id, transcript_id etc)

chr1	hg38_wgEncodeGencodeBasicV26	start_codor	111895	580 11189	<i>€</i> 582 0.	000000	+	. gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";
chr1	hg38_wgEncodeGencodeBasicV26	CDS 111	89580	11189955	0.00000	+	0	<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	exon 111	89341	11189955	0.00000	+		gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";
chr1	hg38_wgEncodeGencodeBasicV26	CDS 111	92270	11192370	0.00000	+	2	<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	exon 111	92270	11192370	0.00000	+		<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	CDS 111	93580	11193774	0.00000	+	0	gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";
chr1	hg38_wgEncodeGencodeBasicV26	exon 111	93580	11193774	0.000000	+		<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	CDS 111	94461	11194659	0.00000	+	0	<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	exon 111	94461	11194659	0.00000	+		<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	CDS 111	94854	11195020	0.00000	+	2	<pre>gene id "ENST00000376819.3"; transcript id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	stop_codon	111950	021 11195	023 0.	000000	+	<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>
chr1	hg38_wgEncodeGencodeBasicV26	exon 111	94854	11195981	0.00000	+		<pre>gene_id "ENST00000376819.3"; transcript_id "ENST00000376819.3";</pre>

11,189,	500 11,190,000 11,190,500	11,191,000	2 kb	000 11,192,500 User Supp 1	11,193,000 ied Track	11,193,500	ng38 11,194,000 11,194,500	11,195,000	,195,500 11,196,000
chr1 chr1 chr1 chr1 chr1 chr1 chr1 chr1	hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26 hg38_wgEncodeGencodeBasicV26	exon 1116 CDS 1119 exon 1119 CDS 1119 exon 1119 CDS 1119 CDS 1119 CDS 1119 Stop_codon 1119	39580 11189955 39341 11189955 32270 11192370 32270 11192370 32580 11193774 33580 11193774 34461 11194659 34461 11194659 34461 11194059 34454 11195020	.1189582 0.000 0.000000 0.000000 0.000000 0.000000	+ 0 + 2 + 0 + 0 + 0 + 0 + 0 + 2	gene_id gene_id gene_id gene_id gene_id gene_id gene_id gene_id gene_id gene_id	gene_id "ENST00000376819 "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra "ENST00000376819.3"; tra gene_id "ENST00000376819 "ENST00000376819.3"; tra	nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 nscript_id "ENST000 .3"; transcript_id	000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; 000376819.3"; ENST00000376819.3";

Fasta & Fastq



	ASCII_BASE=3					and S	anger				
	Q P_error	ASCII	0.000	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
	0 1.00000	33 !		0.07943	44 ,		0.00631	55 7	33	0.00050	66 B
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	8 0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
	9 0.12589	42 *		0.01000	53 5		0.00079	64 0	42	0.00006	75 K
	10 0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			
	ASCII_BASE=6 Q Perror		Lumina Q	P error	ASCII	Q	P error	ASCII	Q	P error	ASCII
	0 1.00000	64 @	11	0.07943	75 K	22	0.00631	86 V	33	0.00050	97 a
	1 0.79433	65 A	12	0.06310	76 L	23	0.00501	87 W	34	0.00040	98 b
	2 0.63096	66 B 67 C	13	0.05012	77 M	24	0.00398	88 X	35	0.00032	99 c
	3 0.50119 4 0.39811	67 C	14 15	0.03981 0.03162	78 N 79 O		0.00316	89 Y 90 Z	36	0.00025	100 d 101 e
	5 0.31623	69 E		0.02512	80 P	27	0.00200	91 [38	0.00016	102 f
	6 0.25119	70 F	17	0.01995	81 Q		0.00158	92 \	39	0.00013	103 g
	7 0.19953 8 0.15849	71 G 72 H	18 19	0.01585	82 R 83 S		0.00126	93] 94 ^	40 41	0.00010	104 h 105 i
	9 0.12589	73 I	20	0.01000	84 T		0.00079	95			106 j
	10 0.10000	74 J	21	0.00794	85 U	32	0.00063	96			
Identifier @SRR566546.970 HWUSI-EAS1673_11067_FC	7070M:4:1:2	299:11	09 le	ngth=5	0						
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· · · · · · · · · · · · · · · · · · ·											
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Identifier @SRR566546.971 HWUSI-EAS1673_11067_FC	7070M:4:1:2	374:11	08 le	ngth=5	0						
Sequence — GATTTGTATGAAAGTATACAACTAAAACTGCAGGTGG	ATCAGAGTAA	TC									
'+' sign → +											
Quality scores — hhhhgfhhcghghggfcffdhfehhhhcehdchhdha	hohfffdo	Nd									
	mentitude i	Jvu									

Multiple Alignment File

CLUSTAL.	W(1 83)	multiple sequence alignment
CHODIMH	w(1.05)	Marcipie bequence arigiment
IXI_234		TSPASIRPPAGPSSRPAMVSSRRTRPSPPGPRRPTGRPCCSAAPRRPQAT
IXI_235		TSPASIRPPAGPSSRRPSPPGPRRPTGRPCCSAAPRRPQAT
IXI 236		TSPASIRPPAGPSSRPAMVSSRRPSPPPPRRPPGRPCCSAAPPRPQAT
IXI 237		TSPASLRPPAGPSSRPAMVSSRR-RPSPPGPRRPTCSAAPRRPQAT
_		~
TVT 224		
IXI_234		GGWKTCSGTCTTSTSTRHRGRSGWSARTTTAACLRASRKSMRAACSRSAG
IXI_235		GGWKTCSGTCTTSTSTRHRGRSGWRASRKSMRAACSRSAG
IXI_236		GGWKTCSGTCTTSTSTRHRGRSGWSARTTTAACLRASRKSMRAACSRG
IXI_237		GGYKTCSGTCTTSTSTRHRGRSGYSARTTTAACLRASRKSMRAACSRG
IXI 234		SRPNRFAPTLMSSCITSTTGPPAWAGDRSHE
IXI 235		SRPNRFAPTLMSSCITSTTGPPAWAGDRSHE
IXI 236		SRPPRFAPPLMSSCITSTTGPPPPAGDRSHE
_		
IXI_237		SRPNRFAPTLMSSCLTSTTGPPAYAGDRSHE

Sequence Alignment Map (.sam)

<u>https://www.samformat.info/</u>

SAM stands for Sequence Alignment/Map format. It is a TAB-delimited text format consisting of a header section, which is optional, and an alignment section. If present, the header must be prior to the alignments. Header lines start with '@', while alignment lines do not. 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.

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							

Field **Brief description** Type Col 1 QNAME String Query template NAME 2 FLAG Int bitwise FLAG 3 RNAME String References sequence NAME POS 4 Int 1- based leftmost mapping POSition 5 MAPQ **MAPping Quality** Int 6 CIGAR String CIGAR String 7 RNEXT Ref. name of the mate/next read String 8 PNEXT Int Position of the mate/next read TLEN observed Template LENgth 9 Int SEQ segment SEQuence 10 String String ASCII of Phred-scaled base QUALity+33 11 QUAL

Sequence Alignment Map (.sam)

	VN:1. SN:re			ord	inate								Header section
r001		ref		30	8M2I4M1D3M	_	37	39	TTAGATAAAGGATACT	C *	1		
r002		ref			3S6M1P1I4M		0	222222	AAAAGATAAGGATA	*			
r003	0 /0.80/	ालकः उन्हारस्य र			5S6M	*	0		GCCTAAGCTAA	*	SA:Z:ref,29,-,6H5M,17	7 0.	Alignment
r004					6M14N5M	*		- 10	ATAGCTTCAGC	*		,,,,	section
1243.5.5	2064					*	0		TAGGC	*	SA:Z:ref,9,+,5S6M,30,	.1:	
r001		ref				=	7		CAGCGGCAT		NM:i:1	, = ,	
							1				 Optional fields in the formation of th	at of TA	AG:TYPE:VALUE
										QU	AL: read quality; * meaning suc	ch infor	mation is not available
									SEQ: read seque				
											rered by the reads from the sam ftmost/rightmost read. E.g. con	0	
							D				nment of the NEXT read in the to		
									tion is unavailable. It cor			empian	e. Set as 0 when the
						RN					primary alignment of the NEXT	read. I	For paired-end
						seq	uen	cing,	NEXT read is the paired	read	I, corresponding to the RNAME	columr	ı.
					CIGAR: sun	nma	ary (of alig	nment, e.g. insertion, de	letio	n		
			N	IAP	Q: mapping qua	lity							
			POS	S: 1-	based position								
		RNA	ME:	refe	rence sequence	na	ime,	e.g. o	chromosome/transcript ic	t	Γ	han	n format
	FLAG	: indi	cates	alig	nment informati	on	abo	ut the	read, e.g. paired, aligned	d, et	с.		
QNAM				120-11-122	ne, aka. read ID							(Bin	ary SAM)
	Franks af Provide		A STREET ST								L		

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