Bioinformatics file formats

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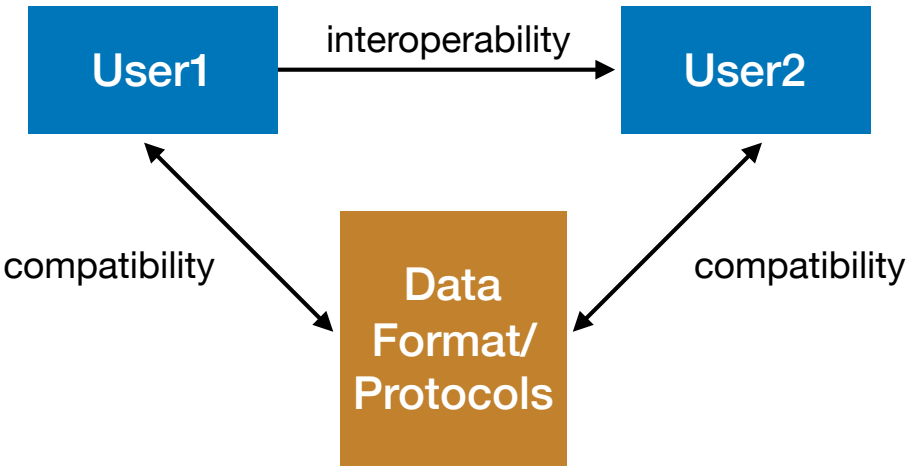
Goals and outline

- Understand importance of standardized file formats
- Introduce you to commonly-used formats in bioinformatics
- Resources for manipulating or parsing them yourself

Why are (standardized) file formats important?

Data sharing and collaboration

File standards provide:
a common language for data sharing,
promote collaboration,
ensure data reusability,
reduce user errors



Syntactic and semantic interoperability

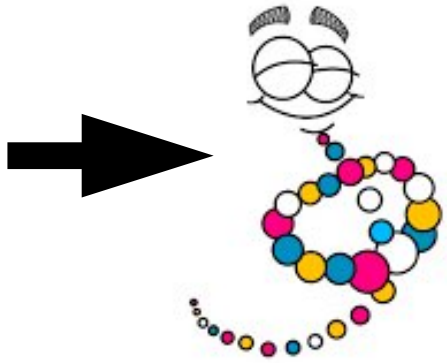
"The capability to communicate, execute programs, or transfer data among various functional units in a manner that requires the user to have little or no knowledge of the unique characteristics of those units"[1]

"the capability of different programs to exchange data via a common set of exchange formats, to read and write the same file formats, and to use the same protocols.... the lack of interoperability can be a consequence of a lack of attention to standardization during the design of a program"[2]

1. ISO/IEC 2382-01 *Information Technology Vocabulary, Fundamental Terms*
2. Gordon and Hernandez, *The Official Guide to the SSCP Book*

We have a specimen of interest...

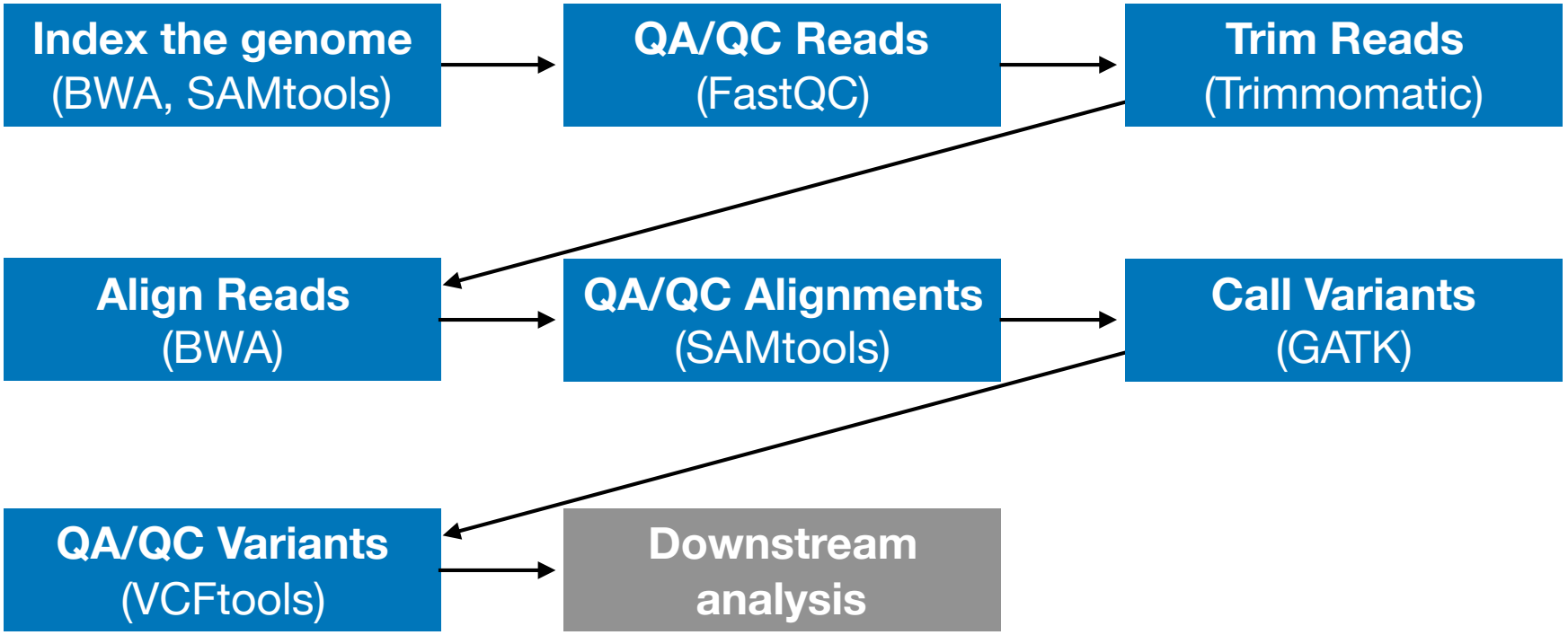
We extract DNA...



Variants

- SNPs: Single-Nucleotide Polymorphisms
- Indels: Small insertions-deletions
- Structural variants: mid-to-large sized insertions, deletions, rearrangements

Variant-calling workflow



We have data, now what?

```
$ ls
Trex_genome.fasta
Trex_genome.annotation.gff3
SAMPLE_NoIndex_R1_001.fastq.gz
SAMPLE_NoIndex_R2_001.fastq.gz

$ head -3 Trex_genome.fasta
>Chr1
ATGCGTACGTATTCTGCGCTATCGCTATCAGCTATCTAGCTACGATCGATCAGCTATCGTACGATCACT
ACTAGCTGACTGATCGATATCTCTTCGAGATTCTCTCTCTCTTTTTTTTTTCGATACGACGTACGATGCG

$ gunzip -c SAMPLE_NoIndex_R1_001.fastq.gz | head -4
@HS3:SAMPLE:1:1:1
ATCGCGAGCTTACGACTACGATCGATCGATTCGGCTACTATCGATCGTAGCTACGTAGTCTCGCGTATC
+
AAFFFJFJJFJJJJJJJJJJJJJJJAJAFFJJJJFJJAJ<<JJJJJJJJFFJJFJ700+)'&%######
```

Index the genome

```
$ bwa index Trex_genome.fasta  # creates the following files:
$ ls Trex_genome.fasta*
Trex_genome.fasta
Trex_genome.fasta.bwt
Trex_genome.fasta.pac
Trex_genome.fasta.ann
Trex_genome.fasta.amb
Trex_genome.fasta.sa

$ samtools faidx Trex_genome.fasta  # creates Trex_genome.fasta.fai
$ head -3 Trex_genome.fasta.fai
Chr1  217471166  141        100  101
Chr2  181034961  219646160  100  101
Chr3  153873357  402491612  100  101

$ samtools dict Trex_genome.fasta >Trex_genome.dict
$ head -3 Trex_genome.dict
@HD  VN:1.0   SO:unsorted
@SQ  SN:Chr1  LN:217471166  M5:56d95ce6647ea9087b857b1efa6d00dd
@SQ  SN:Chr2  LN:181034961  M5:20852c561ea38c67aa67e6d655cfebf2
```

FASTA/Pearson

https://en.wikipedia.org/wiki/FASTA_format

```
>U31202.1 Human noggin (NOGGIN) gene, complete cds
GAGCTCCGGCGGGTCAGCCGGACTGTCGGCTTCCCGGGGCATCTGGGTCCGGCGGGGCACAGCCCTGGGC
GCTGCCGAAGCCGCCGCCGCCGCCTCCGCGGCGAGTACAGGCGGCTTCCCCCGGAGCCTGTGCAGCTCCA
GCTCCTCGGGGGTGGAGAAGTGGGGGGTGGGGGTGATGTATGGGGGGAAGAAGGGGGAGGGGCCAACCCC
GAGAGAGTCAGTGGTTTCCATGGTGATGGAGCTGAAAGTGCAGGAAATTTAAAGGCTTGGACCCTGCGAG
ACAGACAAACCGGTGCCAACGTGCGCGGACGCCGCCGCCGCCGCCGCCGCTGGAGTCCGCCGGGCAGAGC
CGGCCGCGGAGCCCGGAGCAGGCGGAGGGAAGTGCCCCTAGAACCAGCTCAGCCAGCGGCGCTTGCACAG
AGCGGCCGGNCGAAGAGCAGCGAGAGGAGGAGGGGAGAGCGGCTCGTCCACGCGCCCTGCGCCGCCGCCG
GCCCGGGAAGGCAGCGAGGAGCCGGCGCCTCCCGCGCCCCGCGGTCGCCCTGGAGTAATTTCGGATGCCC
AGCCGCGGCCGCCTTCCCCAGTAGACCCGGGAGAGGAGTTGCGGCCAACTTGTGTGCCTTTCTTCCGCCC
CGGTGGGAGCCGGCGCTGCGCGAAGGGCTCTCCCGGCGGCTCATGCTGCCGGCCCTGCGCCTGCCCAGCC
TCGGGTGAGCCGCCTCCGGAGAGACGGGGGAGCGCGGCGGCGCCGCGGGCTCGGCGTGCTCTCCTCCGGG
GACGCGGGACGAAGCAGCAGCCCCGGGCGCGCGCCAGAGGCATGGAGCGCTGCCCCAGCCTAGGGGTCAC
CCTCTACGCCCTGGTGGTGGTCCTGGGGCTGCGGGCGACACCGGCCGGCGGCCAGCACTATCTCCACATC
CGCCCGGCACCCAGCGACAACCTGCCCCTGGTGGACCTCATCGAACACCCAGACCCTATCTTTGACCCCA
AGGAAAAGGATCTGAACGAGACGCTGCTGCGCTCGCTGCTCGGGGGCCACTACGACCCAGGCTTCATGGC
CACCTCGCCCCCCGAGGACCGGCCCGGCGGGGGCGGGGGTGCAGCTGGGGGCGCGGAGGACCTGGCGGAG
CTGGACCAGCTGCTGCGGCAGCGGCCGTCGGGGGCCATGCCGAGCGAGATCAAAGGGCTAGAGTTCTCCG
AGGGCTTGGCCCAGGGCAAGAAGCAGCGCCTAAGCAAGAAGCTGCGGAGGAAGTTACAGATGTGGCTGTG
GTCGCAGACATTCTGCCCCGTGCTGTACGCGTGGAACGACCTGGGCAGCCGCTTTTGGCCGCGCTACGTG
AAGGTGGGCAGCTGCTTCAGTAAGCGCTCGTGCTCCGTGCCCGAGGGCATGGTGTGCAAGCCGTCCAAGT
CCGTGCACCTCACGGTGCTGCGGTGGCGCTGTCAGCGGCGCGGGGGCCAGCGCTGCGGCTGGATTCCCAT
CCAGTACCCCATCATTTCCGAGTGCAAGTGCTCGTGCTAGAACTCGGGGGCCCCCTGCCCGCACCCGGAC
ACTTGATCCTCGAGCTC
>lcl|BC064885.2_cds_AAH64885.1_1 [gene=mtpn] [protein=myotrophin] [protein_id=AAH64885.1]
ATGGGTGACAAGGAGTTCGTGTGGGCCATCAAGAACGGAGACCTGGATGCAGTGAAAGAATTCGTACTTG
GGGGCGAGGATGTGAACCGGACGCTGGATGGTGGAAGGAAACCTATGCACTACGCTGCCGACTGCGGGCA
GGATGAGGTCCTGGAGTTTCTTCTCTCGAAAGGAGCCAACATCAATGCTGCGGATAAACATGGCATCACC
CCCCTACTATCTGCCTGCTACGAGGGCCATCGCAAATGTGTCGAGTTGCTTTTATCTAAGGGAGCCGACA
AGACGGTGAAGGGCCCAGACGGACTCAATGCTTTGGAATCTACAGACAACCAGGCTATCAAAGATTTGCT
CCATTAA
```

FASTA/Pearson

FASTA Defline: Sequence ID + Description on same line, sequence string on the next

"greater than"
Start of record

Whitespace only required if description present

Description/Comment
Optional;
Free-form text

Sequence ID
Required;
Any printable non-whitespace characters: [!-~]

```
>U31202.1 Human noggin (NOGGIN) gene, complete cds
GAGCTCCGGCGGGTCAGCCGGACTGTCGGCTTCCCGGGGCATCTGGGTCCGGCGGGGCACAGCCCTGGGC
GCTGCCGAAGCCGCCGCCGCCGCCTCCGCGGCGAGTACAGGCGGCTTCCCCCGGAGCCTGTGCAGCTCCA
GCTCCTCGGGGGTGGAGAAGTGGGGGGTGGGGGTGATGTATGGGGGGAAGAAGGGGGAGGGGCCAACCCC
GAGAGAGTCAGTGGTTTCCATGGTGATGGAGCTGAAAGTGCAGGAAATTTAAAGGCTTGGACCCTGCGAG
ACAGACAAACCGGTGCCAACGTGCGCGGACGCCGCCGCCGCCGCCGCCGCTGGAGTCCGCCGGGCAGAGC
CGGCCGCGGAGCCCGGAGCAGGCGGAGGGAAGTGCCCCTAGAACCAGCTCAGCCAGCGGCGCTTGCACAG
AGCGGCCGGNCGAAGAGCAGCGAGAGGAGGAGGGGAGAGCGGCTCGTCCACGCGCCCTGCGCCGCCGCCG
GCCCGGGAAGGCAGCGAGGAGCCGGCGCCTCCCGCGCCCCGCGGTCGCCCTGGAGTAATTTCGGATGCCC
AGCCGCGGCCGCCTTCCCCAGTAGACCCGGGAGAGGAGTTGCGGCCAACTTGTGTGCCTTTCTTCCGCCC
CGGTGGGAGCCGGCGCTGCGCGAAGGGCTCTCCCGGCGGCTCATGCTGCCGGCCCTGCGCCTGCCCAGCC
TCGGGTGAGCCGCCTCCGGAGAGACGGGGGAGCGCGGCGGCGCCGCGGGCTCGGCGTGCTCTCCTCCGGG
GACGCGGGACGAAGCAGCAGCCCCGGGCGCGCGCCAGAGGCATGGAGCGCTGCCCCAGCCTAGGGGTCAC
CCTCTACGCCCTGGTGGTGGTCCTGGGGCTGCGGGCGACACCGGCCGGCGGCCAGCACTATCTCCACATC
CGCCCGGCACCCAGCGACAACCTGCCCCTGGTGGACCTCATCGAACACCCAGACCCTATCTTTGACCCCA
AGGAAAAGGATCTGAACGAGACGCTGCTGCGCTCGCTGCTCGGGGGCCACTACGACCCAGGCTTCATGGC
CACCTCGCCCCCCGAGGACCGGCCCGGCGGGGGCGGGGGTGCAGCTGGGGGCGCGGAGGACCTGGCGGAG
CTGGACCAGCTGCTGCGGCAGCGGCCGTCGGGGGCCATGCCGAGCGAGATCAAAGGGCTAGAGTTCTCCG
AGGGCTTGGCCCAGGGCAAGAAGCAGCGCCTAAGCAAGAAGCTGCGGAGGAAGTTACAGATGTGGCTGTG
GTCGCAGACATTCTGCCCCGTGCTGTACGCGTGGAACGACCTGGGCAGCCGCTTTTGGCCGCGCTACGTG
AAGGTGGGCAGCTGCTTCAGTAAGCGCTCGTGCTCCGTGCCCGAGGGCATGGTGTGCAAGCCGTCCAAGT
CCGTGCACCTCACGGTGCTGCGGTGGCGCTGTCAGCGGCGCGGGGGCCAGCGCTGCGGCTGGATTCCCAT
CCAGTACCCCATCATTTCCGAGTGCAAGTGCTCGTGCTAGAACTCGGGGGCCCCCTGCCCGCACCCGGAC
ACTTGATCCTCGAGCTC
```

FASTA Body/ Sequence string
Nucleotide, amino acid, IUPAC codes, alignment characters [-*]

Should be wrapped flush, but sometimes is not

```
>lcl|BC064885.2_cds_AAH64885.1_1 [gene=mtpn] [protein=myotrophin] [protein_id=AAH64885.1]
ATGGGTGACAAGGAGTTCGTGTGGGCCATCAAGAACGGAGACCTGGATGCAGTGAAAGAATTCGTACTTG
GGGGCGAGGATGTGAACCGGACGCTGGATGGTGGAAGGAAACCTATGCACTACGCTGCCGACTGCGGGCA
GGATGAGGTCCTGGAGTTTCTTCTCTCGAAAGGAGCCAACATCAATGCTGCGGATAAACATGGCATCACC
CCCCTACTATCTGCCTGCTACGAGGGCCATCGCAAATGTGTCGAGTTGCTTTTATCTAAGGGAGCCGACA
CCATTAA
```

FASTA files are *best* suffixed with ".fasta" or ".fa"; some tools *require* this.

FASTQ

https://en.wikipedia.org/wiki/FASTQ_format

```
@SRR10178655.1 0:N:0:
GGATCTATGGCCATGTAGGGACCATCTGAAGGCAGATCAAAATTTCGCTGAGCAAATTTAGGGTCCGGGTTTGTT
+
AA<A<F--FF<-F-A7FAF-F---A<F---<FF-<F--7F-----<-A7F-A----7FJ<-FF--<J<-7-FFFJ
@SRR10178655.2 0:N:0:
ATAAAAAAAAATTAATAATCTATTCTTTATTTAAAACTAATTTTTAAATTAATTGGTTTTTGTGGAATGGTATTA
+
AAFFFJFJAJJJFFA<JF-7FJF<JJJJ--<<FJ-J<A7---<-FFJFJFJJAJ-F<F<<-F-7---7-<-<FFA
@SRR10178655.3 0:N:0:
CATTATATACGTCGCCACTCTTAATTTCCTTTTCCATAAGAGCGTATAATCTTGTAATACAATGTCTTCTCCAAC
+
AAAFFJJJJAFJJJJAFJF<JFAFJJJF-<FAJJFJ-F-F-<7-7-<FJJJJF<JA-FF---<-7<F-F<-7-<F
@SRR10178655.4 0:N:0:
AAGTTATTCTGCCTCTAATGCGATAACTGTAATCTTTAATTGTGTAATTTCTTTTTCACAATCTGAGCCACGCCA
+
AAAAAJF<-A<-7FJFJJJFJFJJJJJ<FJ--7<FF-7-<--7-A<7FFJAJFFJJJAJ7FF-F7FA-7<-A-7-
@SRR10178655.5 0:N:0:
ATATATTAATTAATAATTAATTTATAATAAATATATGATATTAATTAATATATATATATATAATATATTTAATAA
+
AAFFFJFJJJJJJ<FJJJAFJJ<FFAJFJ--FJJJJ-FFF-<FFJFA-FJJ-AJ-<<-FFFAFJJJJJAJ-7---
```

FASTQ

FASTQ Sequence Header: Sequence ID + Description on same line, sequence string on the next

Whitespace only required if description present

"At" symbol
Start of sequence portion of record

Description/Comment optional

Sequence ID
Required;
Any printable non-whitespace characters
[!-~]

```
@SRR10178655.1 0:N:0:
GGATCTATGGCCATGTAGGGACCATCTGAAGGCAGATCAAAATTTCGCTGAGCAAATTTAGGGTCCGGGTTTGTT
+
AA<A<F--FF<-F-A7FAF-F---A<F---<FF-<F--7F-----<-A7F-A----7FJ<-FF--<J<-7-FFFJ
@SRR10178655.2 0:N:0:
ATAAAAAAAAATTAATAATCTATTCTTTATTTAAAACTAATTTTTAAATTAATTGGTTTTTGTGGAATGGTATTA
+
AAFFFJFJAJJJFFA<JF-7FJF<JJJJ--<<FJ-J<A7---<-FFJFJFJJAJ-F<F<<-F-7---7-<-<FFA
@SRR10178655.3 0:N:0:
CATTATATACGTCGCCACTCTTAATTTCCTTTTCCATAAGAGCGTATAATCTTGTAATACAATGTCTTCTCCAAC
+
AAAFFJJJJAFJJJJAFJF<JFAFJJJF-<FAJJFJ-F-F-<7-7-<FJJJJF<JA-FF---<-7<F-F<-7-<F
@SRR10178655.4 0:N:0:
AAGTTATTCTGCCTCTAATGCGATAACTGTAATCTTTAATTGTGTAATTTCTTTTTCACAATCTGAGCCACGCCA
+
AAAAAJF<-A<-7FJFJJJFJFJJJJJ<FJ--7<FF-7-<--7-A<7FFJAJFFJJJAJ7FF-F7FA-7<-A-7-
@SRR10178655.5 0:N:0:
ATATATTAATTAATAATTAATTTATAATAAATATATGATATTAATTAATATATATATATATAATATATTTAATAA
+
AAFFFJFJJJJJJ<FJJJAFJJ<FFAJFJ--FJJJJ-FFF-<FFJFA-FJJ-AJ-<<-FFFAFJJJJJAJ-7---
```

FASTQ Sequence
Nucleotide, amino acid, IUPAC codes

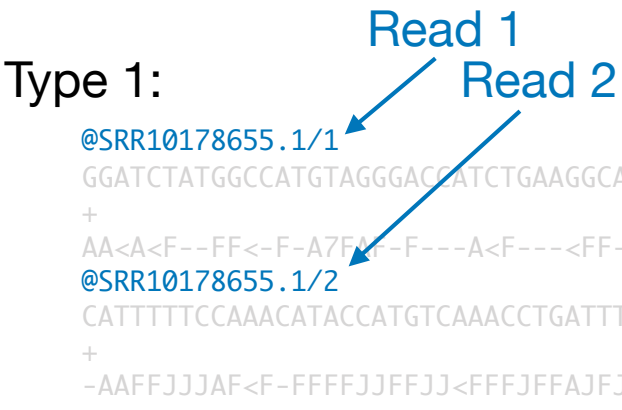
Should *not* be wrapped flush

FASTQ files are *best* suffixed with ".fastq" or ".fq", some tools *require* this.

FASTQ

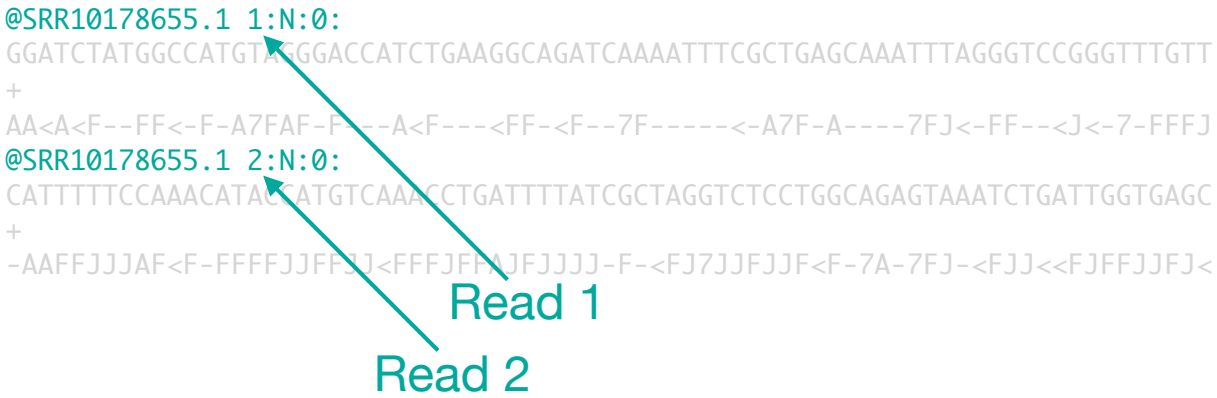
FASTQ Sequence Header: Paired-end or mate-pair reads

Type 1:



Paired (or mated) reads may be interleaved into same file or separate files. If in separate files, Read 1 and Read 2 sequences *must* be in same order.

Type 2:



FASTQ

FASTQ Qualities Header: Same as Sequence Header, or absent completely

"Plus" symbol
Start of qualities portion of record

Qualities ID
Optional;
If present, typically same as Sequence ID; Must follow same rules

```
@SRR10178655.1 0:N:0:
GGATCTATGGCCATGTAGGGACCATCTGAAGGCAGATCAAAATTTCGCTGAGCAAATTTAGGGTCCGGGTTTGTT
+
AA<A<F--FF<-F-A7FAF-F---A<F---<FF-<F--7F-----<-A7F-A----7FJ<-FF--<J<-7-FFFJ
@SRR10178655.2 0:N:0:
ATAAAAAAAAATTAATAATCTATTCTTTATTTAAAACTAATTTTTAAATTAATTGGTTTTTGTGGAATGGTATTA
+
AAFFFJFJAJJJFFA<JF-7FJF<JJJJ--<<FJ-J<A7---<-FFJFJFJJAJ-F<F<<-F-7---7-<-<FFA
@SRR10178655.3 0:N:0:
CATTATATACGTCGCCACTCTTAATTTCCTTTTCCATAAGAGCGTATAATCTTGTAATACAATGTCTTCTCCAAC
+
AAAFFJJJJAFJJJJAFJF<JFAFJJJF-<FAJJFJ-F-F-<7-7-<FJJJJF<JA-FF---<-7<F-F<-7-<F
@SRR10178655.4 0:N:0:
AAGTTATTCTGCCTCTAATGCGATAACTGTAATCTTTAATTGTGTAATTTCTTTTTCACAATCTGAGCCACGCCA
+
AAAAAJF<-A<-7FJFJJJFJFJJJJJ<FJ--7<FF-7-<--7-A<7FFJAJFFJJJAJ7FF-F7FA-7<-A-7-
@SRR10178655.5 0:N:0:
ATATATTAATTAATAATTAATTTATAATAAATATATGATATTAATTAATATATATATATATAATATATTTAATAA
+
AAFFFJFJJJJJJ<FJJJAFJJ<FFAJFJ--FJJJJ-FFF-<FFJFA-FJJ-AJ-<<-FFFAFJJJJJAJ-7---
```

FASTQ Qualities
ASCII+*offset* encoded "Phred" scores.

Must be same length as sequence.

Should *not* be wrapped flush

https://en.wikipedia.org/wiki/FASTQ_format

FASTQ

$$\text{Phred} = -10 \bullet \log_{10}(P)$$

P = fractional probability that the base call is wrong

```
ascii_char = chr(Phred + offset);     Phred = ord(ascii_char) - offset
```

Dec	Hx	Oct	Char	Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr
0	0	000	NUL (null)	32	20	040	&#32;	Space	64	40	100	&#64;	@	96	60	140	&#96;	`
1	1	001	SOH (start of heading)	33	21	041	&#33;	!	65	41	101	&#65;	A	97	61	141	&#97;	a
2	2	002	STX (start of text)	34	22	042	&#34;	"	66	42	102	&#66;	B	98	62	142	&#98;	b
3	3	003	ETX (end of text)	35	23	043	&#35;	#	67	43	103	&#67;	C	99	63	143	&#99;	c
4	4	004	EOT (end of transmission)	36	24	044	&#36;	$	68	44	104	&#68;	D	100	64	144	&#100;	d
5	5	005	ENQ (enquiry)	37	25	045	&#37;	%	69	45	105	&#69;	E	101	65	145	&#101;	e
6	6	006	ACK (acknowledge)	38	26	046	&#38;	&	70	46	106	&#70;	F	102	66	146	&#102;	f
7	7	007	BEL (bell)	39	27	047	&#39;	'	71	47	107	&#71;	G	103	67	147	&#103;	g
8	8	010	BS (backspace)	40	28	050	&#40;	(	72	48	110	&#72;	H	104	68	150	&#104;	h
9	9	011	TAB (horizontal tab)	41	29	051	&#41;	)	73	49	111	&#73;	I	105	69	151	&#105;	i
10	A	012	LF (NL line feed, new line)	42	2A	052	&#42;	*	74	4A	112	&#74;	J	106	6A	152	&#106;	j
11	B	013	VT (vertical tab)	43	2B	053	&#43;	+	75	4B	113	&#75;	K	107	6B	153	&#107;	k
12	C	014	FF (NP form feed, new page)	44	2C	054	&#44;	,	76	4C	114	&#76;	L	108	6C	154	&#108;	l
13	D	015	CR (carriage return)	45	2D	055	&#45;	-	77	4D	115	&#77;	M	109	6D	155	&#109;	m
14	E	016	SO (shift out)	46	2E	056	&#46;	.	78	4E	116	&#78;	N	110	6E	156	&#110;	n
15	F	017	SI (shift in)	47	2F	057	&#47;	/	79	4F	117	&#79;	O	111	6F	157	&#111;	o
16	10	020	DLE (data link escape)	48	30	060	&#48;	0	80	50	120	&#80;	P	112	70	160	&#112;	p
17	11	021	DC1 (device control 1)	49	31	061	&#49;	1	81	51	121	&#81;	Q	113	71	161	&#113;	q
18	12	022	DC2 (device control 2)	50	32	062	&#50;	2	82	52	122	&#82;	R	114	72	162	&#114;	r
19	13	023	DC3 (device control 3)	51	33	063	&#51;	3	83	53	123	&#83;	S	115	73	163	&#115;	s
20	14	024	DC4 (device control 4)	52	34	064	&#52;	4	84	54	124	&#84;	T	116	74	164	&#116;	t
21	15	025	NAK (negative acknowledge)	53	35	065	&#53;	5	85	55	125	&#85;	U	117	75	165	&#117;	u
22	16	026	SYN (synchronous idle)	54	36	066	&#54;	6	86	56	126	&#86;	V	118	76	166	&#118;	v
23	17	027	ETB (end of trans. block)	55	37	067	&#55;	7	87	57	127	&#87;	W	119	77	167	&#119;	w
24	18	030	CAN (cancel)	56	38	070	&#56;	8	88	58	130	&#88;	X	120	78	170	&#120;	x
25	19	031	EM (end of medium)	57	39	071	&#57;	9	89	59	131	&#89;	Y	121	79	171	&#121;	y
26	1A	032	SUB (substitute)	58	3A	072	&#58;	:	90	5A	132	&#90;	Z	122	7A	172	&#122;	z
27	1B	033	ESC (escape)	59	3B	073	&#59;	;	91	5B	133	&#91;	[	123	7B	173	&#123;	{
28	1C	034	FS (file separator)	60	3C	074	&#60;	<	92	5C	134	&#92;	\	124	7C	174	&#124;	|
29	1D	035	GS (group separator)	61	3D	075	&#61;	=	93	5D	135	&#93;	]	125	7D	175	&#125;	}
30	1E	036	RS (record separator)	62	3E	076	&#62;	>	94	5E	136	&#94;	^	126	7E	176	&#126;	~
31	1F	037	US (unit separator)	63	3F	077	&#63;	?	95	5F	137	&#95;	_	127	7F	177	&#127;	DEL

Source: www.LookupTables.com

P	Phred
1×10^{0}	0
1×10^{-1}	10
1×10^{-2}	20
1×10^{-3}	30
1×10^{-4}	40
1×10^{-5}	50
1×10^{-6}	60

FASTQ

$$\text{Phred} = -10 \bullet \log_{10}(P)$$

P = fractional probability that the base call is wrong

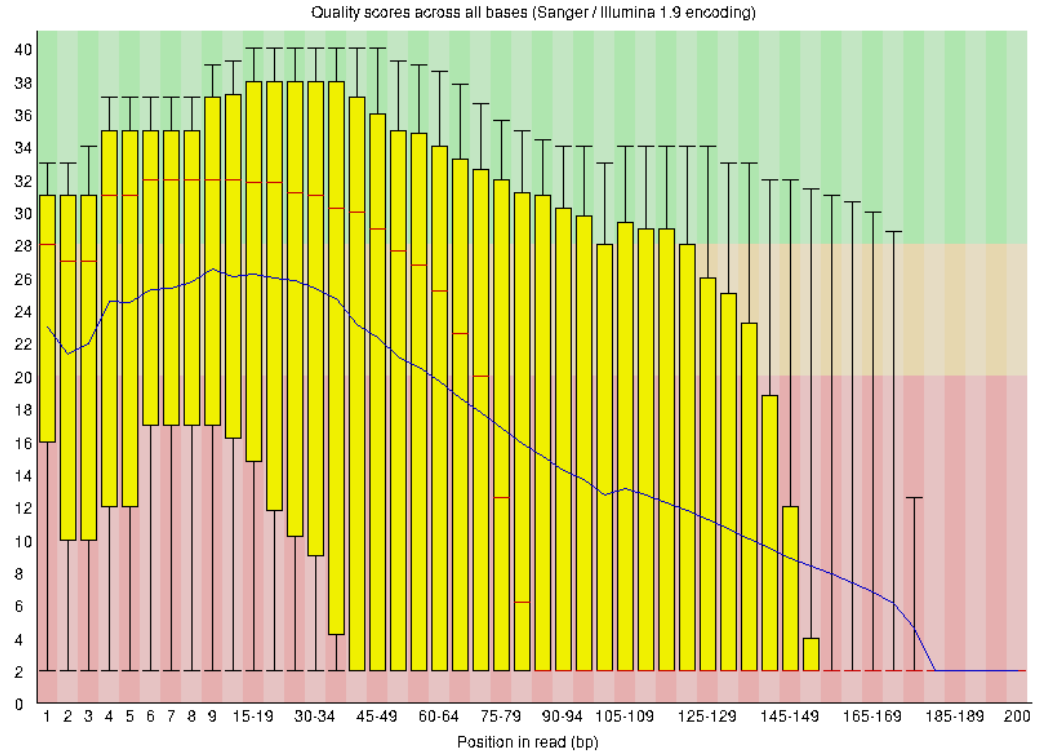
`ascii_char = chr(Phred + offset);` `Phred = ord(ascii_char) - offset`

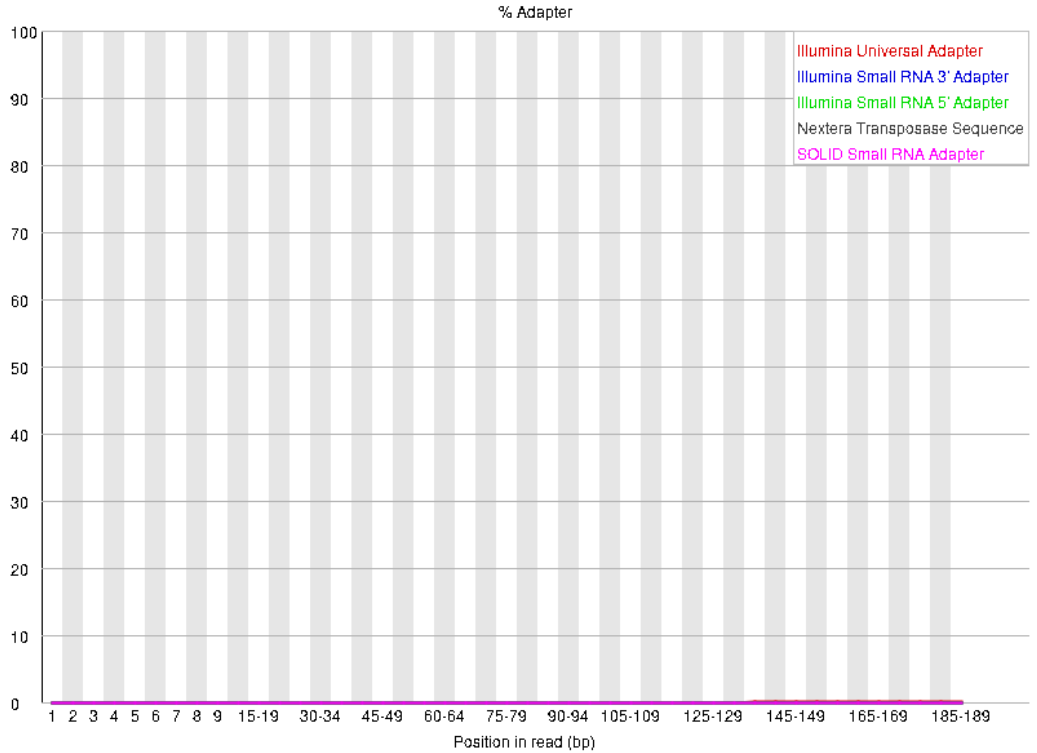
```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX......................
 ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII......................
 ................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ......................
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                          |    |        |                              |                     |
33                        59   64       73                            104                   126
 0........................26...31.......40
                           -5....0........9.............................40
                                 0........9.............................40
                                    3.....9.............................41
 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, 41)
    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)
```

https://en.wikipedia.org/wiki/FASTQ_format

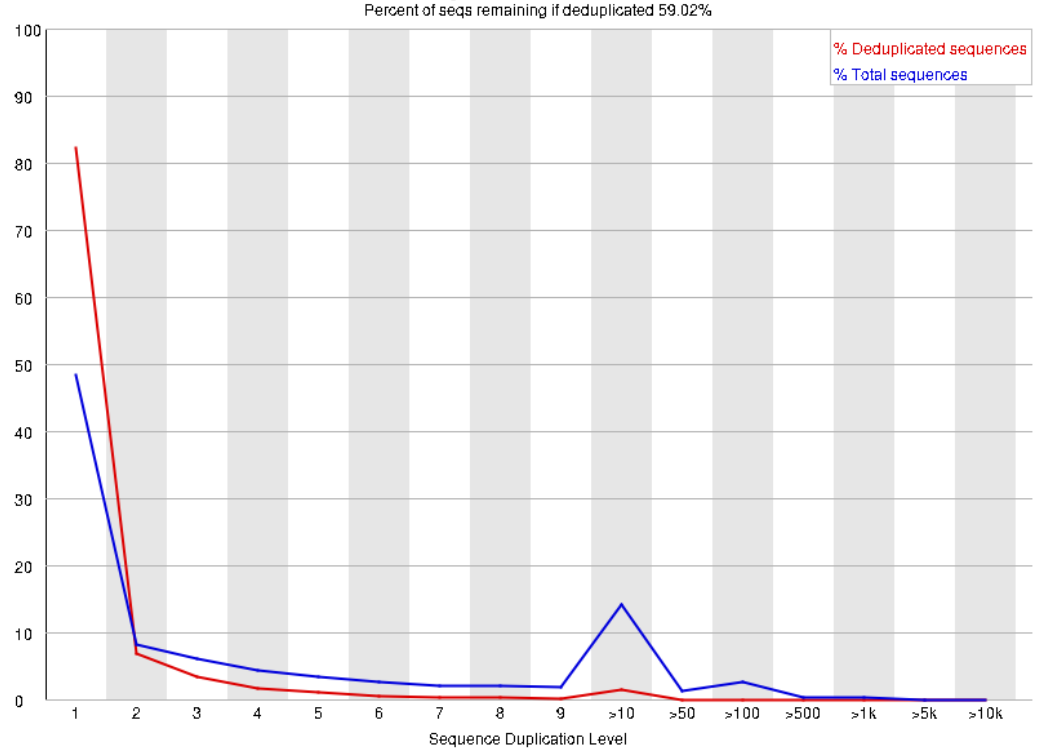
QA/QC'ing Illumina Reads

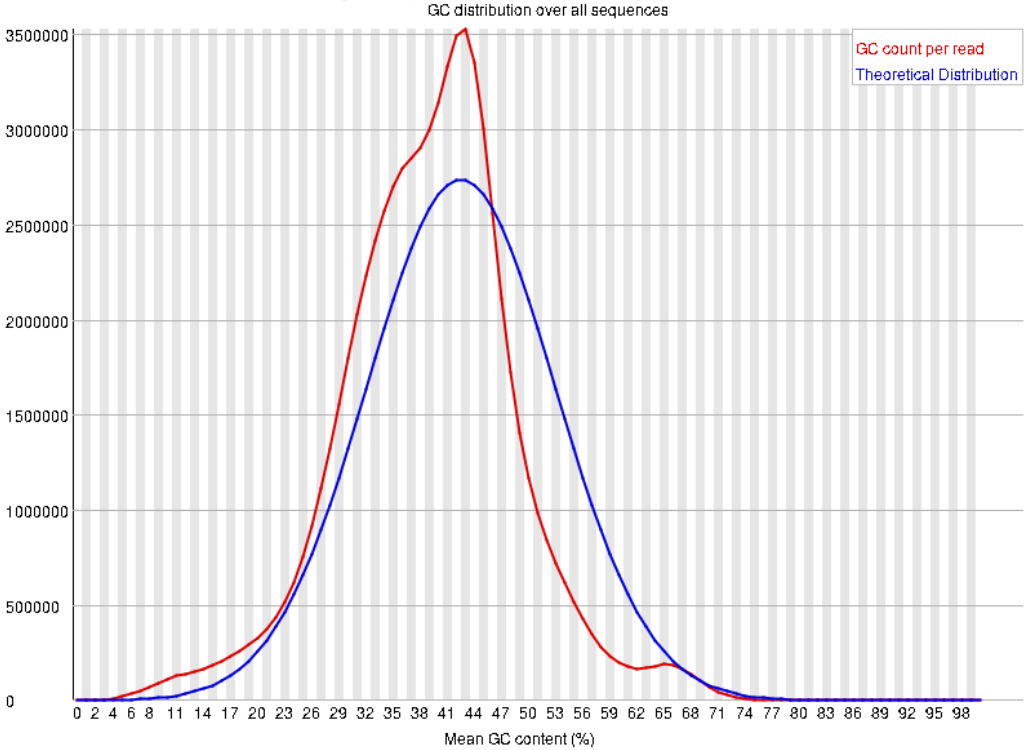
```
# Run FASTQ quality assessment tool and generate plots
$ fastqc --threads 2 --extract SRR10178655_1.fastq.gz SRR10178655_2.fastq.gz

# View the FastQC results in Safari (Mac only):
$ open -a Safari.app SRR10178655_1_fastqc/fastqc_report.html
```





https://www.bioinformatics.babraham.ac.uk/projects/fastqc/





Trim and align Reads

```
# Find adapter sequences in your reads and trim them off
$ java -Xmx500m -jar ./Trimmomatic-0.39/trimmomatic-0.39.jar PE -phred33 \
   -summary SRR10178655.summary SRR10178655_1.fastq.gz SRR178655_2.fastq.gz \
   SRR10178655_1_passed.fastq.gz SRR10178655_1_failed.fastq.gz \
   SRR10178655_1_passed.fastq.gz SRR10178655_2_failed.fastq.gz MINLEN:100 \
   ILLUMINACLIP:./Trimmomatic-0.39/adapters/NexteraPE-PE.fa:2:30:10:2:keepBothReads

# Align the reads to the genome
$ bwa mem -R '@RG\tID:SRR10178655\tSM:Trex\tLB:HAMMOND01\tPL:ILLUMINA' \
   Trex_genome.fasta SRR10178655_1_passed.fastq.gz SRR10178655_2_passed.fastq.gz | \
   samtools view -b - >SRR10178655.bam

# Sort the read alignments by genome coordinate
$ samtools sort -m 1g -o SRR10178655.srt.bam SRR10178655.bam

# Index the BAM file for fast search (creates SRR10178655.srt.bam.bai)
$ samtools index SRR10178655.srt.bam
```

SAM/BAM/CRAM

http://samtools.github.io/hts-specs/SAMv1.pdf
http://samtools.github.io/hts-specs/SAMtags.pdf

SAM: Sequence Alignment/Map format
BAM: Binary SAM
CRAM: Reference-Compressed SAM (also binary)

```
@HD  VN:1.3  SO:coordinate
@SQ  SN:ref|NC_001133| LN:230218
@SQ  SN:ref|NC_001134| LN:813184
@SQ  SN:ref|NC_001148| LN:948066
@SQ  SN:ref|NC_001224| LN:85779
@PG  ID:bwa  PN:bwa  VN:0.7.15-r1140 CL:bwa mem ...
@RG  ID:SRR10178655  SM:Trex  LB:HAMMOND01  PL:ILLUMINA
SRR10178655.85923  163  ref|NC_001133|  1    30  2S7M  =  383  392
  ACATTACTC  AAA))-*##  NM:i:0  MD:i:7  AS:i:7  RG:Z:SRR10178655
SRR10178655.85923  83   ref|NC_001133|  383  60  9M    =  1    -392
  ACCTCACAT  7JFFFFFAA  NM:i:0  MD:Z:9  AS:i:9  RG:Z:SRR10178655
```

SAM/BAM/CRAM

SAM Header: Meta information describing file format and data within. Header lines must start with "@" symbol (and read IDs must not). Tab separated. Reference IDs cannot be "*", "0", or "="; they have special meaning.

Header format version and sort order

Sequence
Reference sequence IDs and lengths; listed in same order as in FASTA

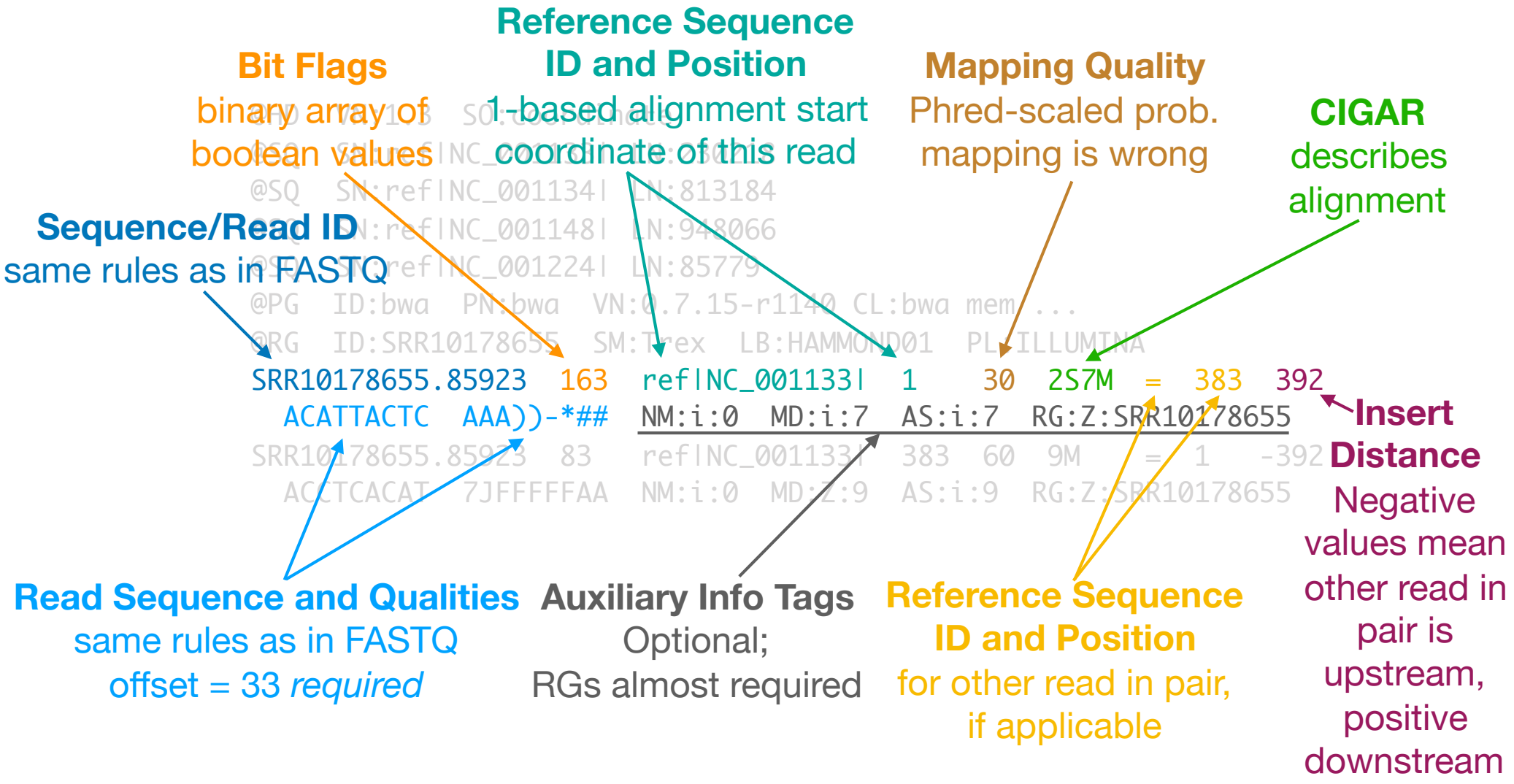
Program
processing history (with commands)

Read Group
Almost required; ID, sample name, and library names, sequencing platform

```
@HD   VN:1.3  SO:coordinate
@SQ   SN:ref|NC_001133|  LN:230218
@SQ   SN:ref|NC_001134|  LN:813184
@SQ   SN:ref|NC_001148|  LN:948066
@SQ   SN:ref|NC_001224|  LN:85779
@PG   ID:bwa  PN:bwa  VN:0.7.15-r1140 CL:bwa mem ...
@RG   ID:SRR10178655  SM:Trex  LB:HAMMOND01  PL:ILLUMINA
SRR10178655.85923  163  ref|NC_001133|  1    30  2S7M  =  383  392
  ACATTACTC  AAA))-*##  NM:i:0  MD:i:7  AS:i:7  RG:Z:SRR10178655
SRR10178655.85923  83   ref|NC_001133|  383  60  9M    =  1    -392
  ACCTCACAT  7JFFFFFAA  NM:i:0  MD:Z:9  AS:i:9  RG:Z:SRR10178655
```

SAM/BAM/CRAM

SAM Body: Describes mapping and alignment without the reference. Eleven required fields. Tab separated. Undefined values: "0" for numeric field, a "*" for non-numeric.



SAM/BAM/CRAM

CIGAR AND Bitwise flag field details

CIGAR operators

Op	Meaning
M	Match
I	Insertion
D	Deletion
=	Sequence match
X	Sequence mismatch
N	Forward-skip query on reference (intron)
H	Query hard clipping
S	Query soft clipping
P	Padded reference
B	Backward-skip query on reference

Example:

```
Q: ATGACAGGACAGAT-GAGG
   ||| |||| ||||| ||
R: ATG-CAGGCCAGATTGATA
```

3M 1I 10M 1D 2S
describes same alignment as
3= 1I 4= 1X 5= 1D 2S

Bit Flags

n	2^n	Meaning
0	1	Read is paired
1	2	Read is part of proper pair
2	4	Read is unmapped
3	8	Other read in pair is unmapped
4	16	Read is rev complemented
5	32	Other read is rev complemented
6	64	Read is R1
7	128	Read is R2
8	256	Alignment is a secondary hit
9	512	Read fails QA/QC
10	1024	Read is duplicate
11	2048	Alignment is split/supplementary

To add or test for flags, use 2^n values with bitwise operations:

Add flag(s)

```
flags |= 2**0
flags |= 2**1
flags |= 2**6
```

Test for flag(s)

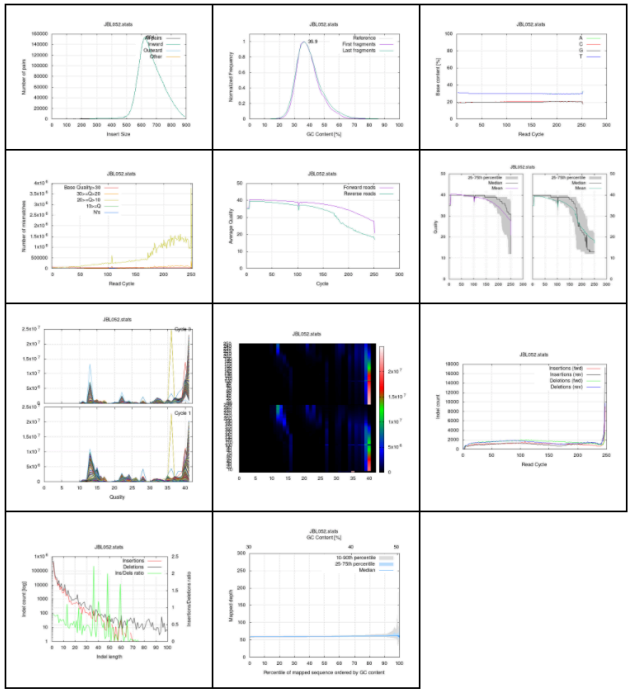
```
flags & 1024  # correct
flags > 1024  # incorrect!!
```

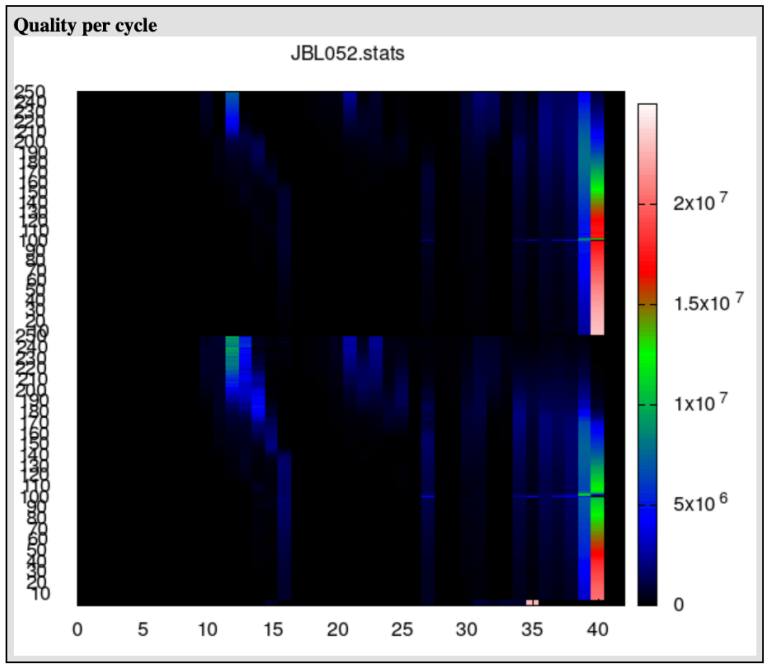
QA/QC'ing Alignments

```
# Mark optical and PCR duplicate read pairs (reduce bias)
$ gatk MarkDuplicates --java-options '-Xmx1G' \
   -MAX_FILE_HANDLES 2000 \
   -I SRR10178655.srt.bam \
   -O SRR10178655.srt.mdup.bam \
   -M SRR10178655.metrics

# Calculate QA/QC metrics for read quality etc.
$ samtools stats -@4 --ref-seq Trex_genome.fasta \
    SRR10178655.srt.mdup.bam >SRR10178655.stats

# Generate the plots
$ plot-bamstats -s Trex_genome.fasta >Trex_genome.gc

$ plot-bamstats -r Trex_genome.gc -p SRR10178655 SRR10178655.stats

# View the FastQC results in Safari (Mac only):
$ open -a Safari.app SRR10178655.html
```

QA/QC'ing Alignments

file:///Users/jessenbredeson/Downloads/JBL052.html





Reads

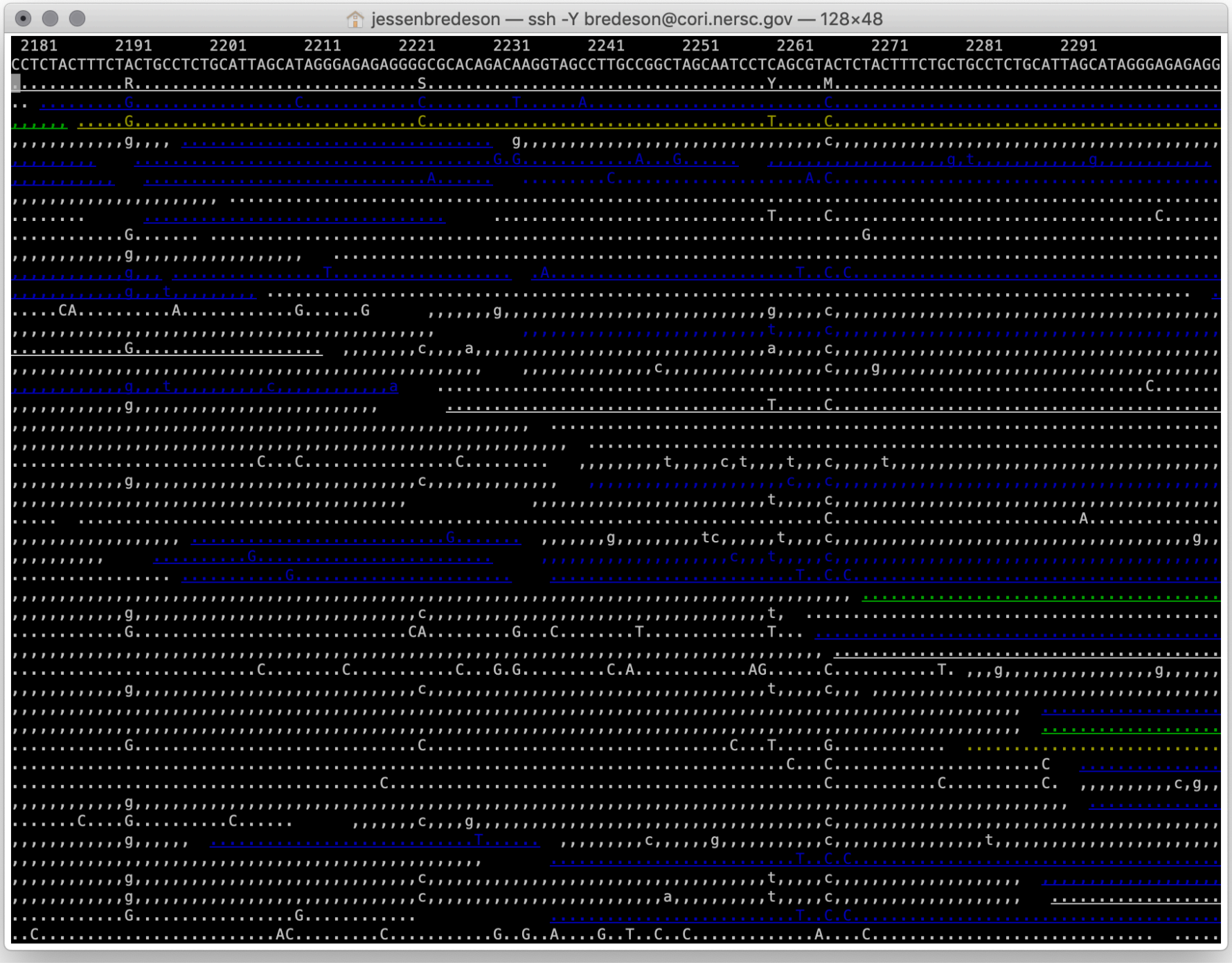
total:	54,968,582	
filtered:	0	(0.0%)
non-primary:	377,364	
duplicated:	0	(0.0%)
mapped:	54,878,503	(99.8%)
zero MQ:	3,226,009	(5.9%)
avg read length:	251	

Bases

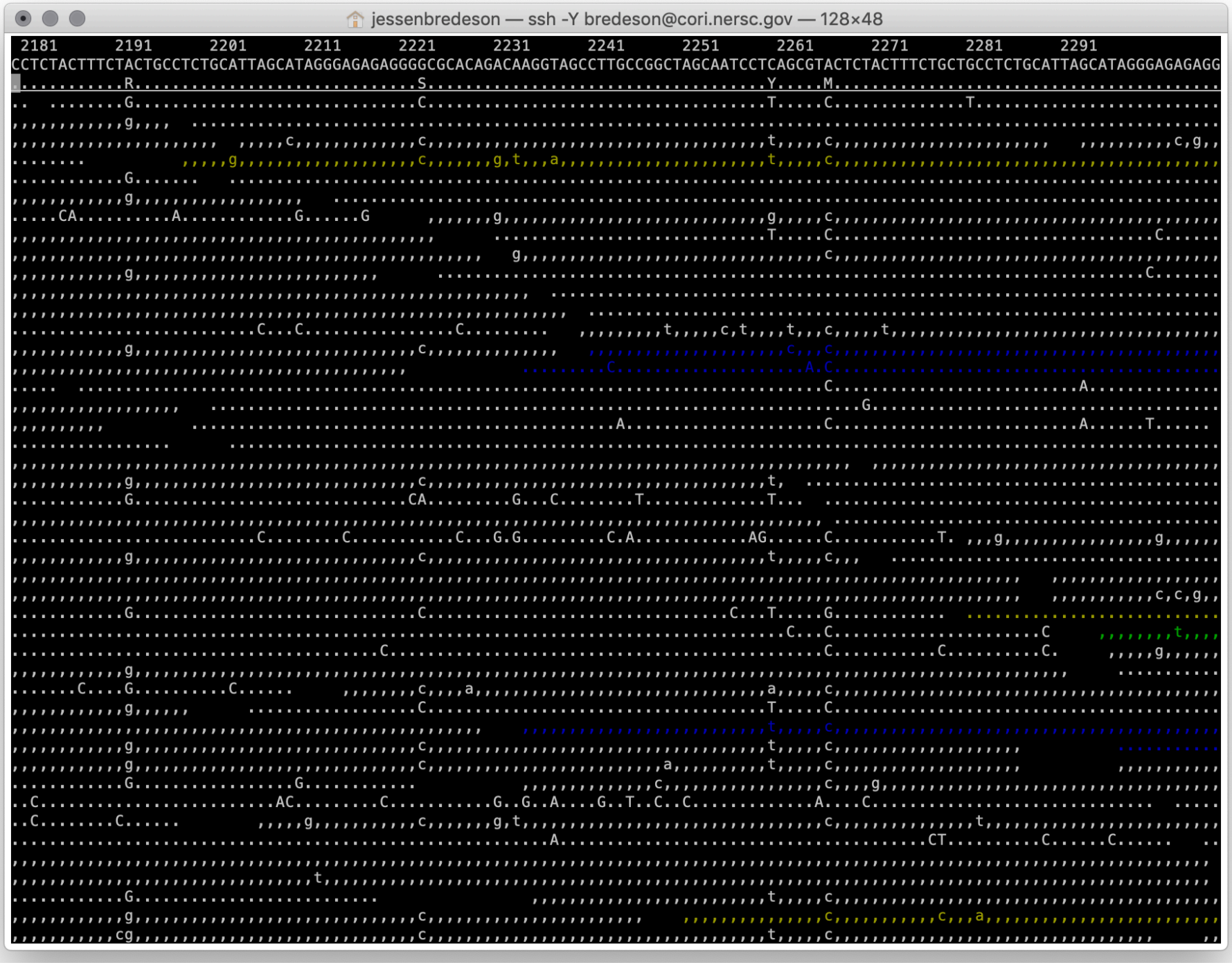
total:	13,797,114,082	
mapped:	12,862,420,009	(93.2%)
error rate:	1.21%	

Press "?" for help

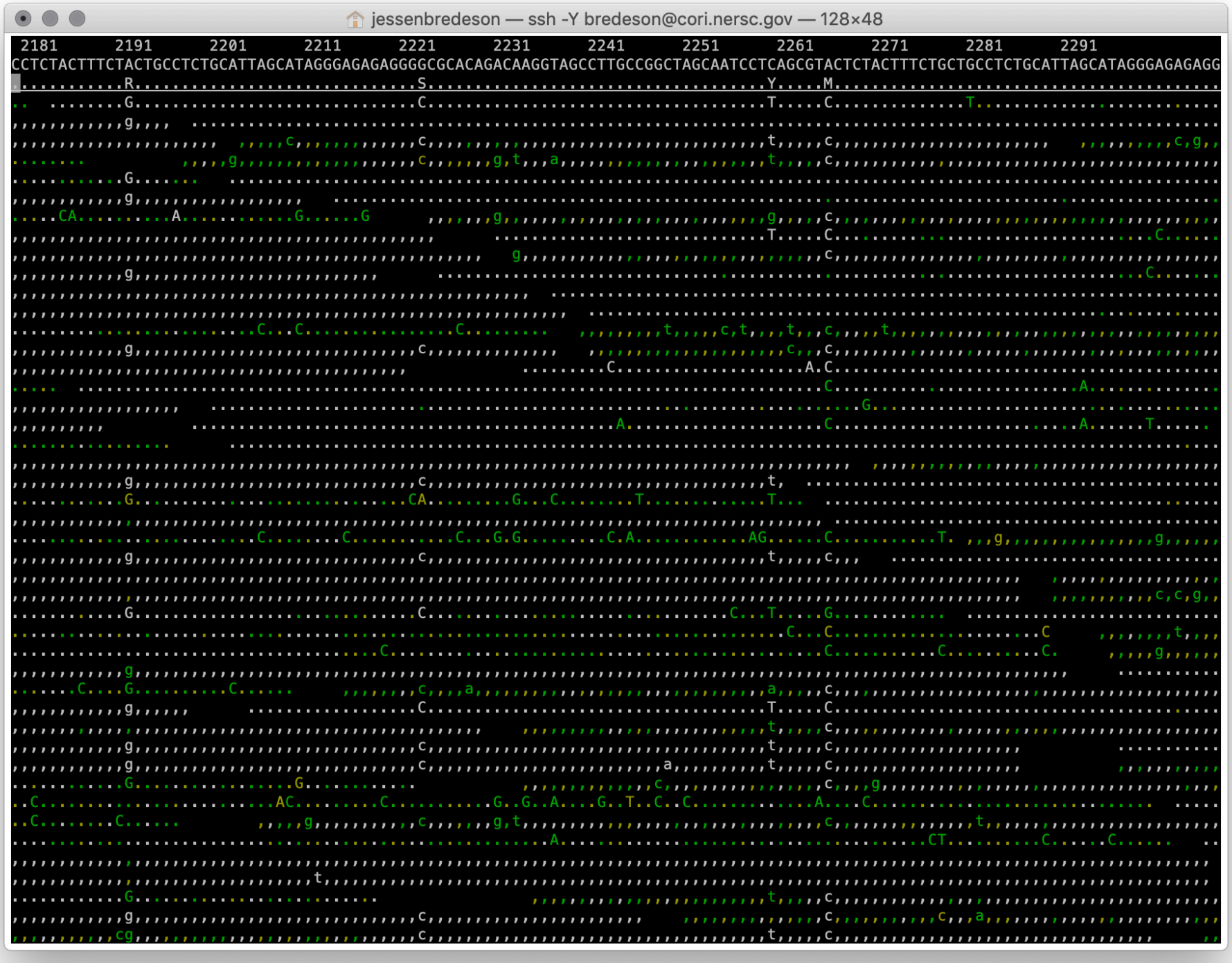
```
$ samtools tview SRR10178655.srt.mdup.bam Trex_genome.fasta
```



```
$ samtools view -b -f3 -F3852 SRR10178655.srt.mdup.bam > SRR10178655.srt.mdup.proper.bam
$ samtools index SRR10178655.srt.mdup.proper.bam
$ samtools tview SRR10178655.srt.mdup.proper.bam Trex_genome.fasta
```



```
$ samtools tview SRR10178655.srt.mdup.proper.bam Trex_genome.fasta
```



Call Variants

```
# Use local assembly of reads on the genome to calculate SNPs and Indels
gatk HaplotypeCaller \
    --minimum-mapping-quality 30 \
    --min-base-quality-score 20 \
    --read-validation-stringency SILENT \
    --reference Trex_genome.fasta \
    --input SRR10178655.srt.mdup.proper.bam \
    --output SRR10178655.vcf
```

VCF/BCF

http://samtools.github.io/hts-specs/VCFv4.3.pdf

VCF: Variant Call Format
BCF: Binary VCF

```
##fileformat=VCFv4.2
##FILTER=<ID=LowQual,Description="Locus is low quality">
##FILTER=<ID=PASS,Description="Locus passes all filters">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="The Phred-scaled prob. of the genotype">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Number of observation for each allele">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##contig=<ID=Chr1,length=217471166>
##contig=<ID=Chr2,length=181034961>
#CHROM  POS      ID  REF  ALT          QUAL     FILTER   INFO   FORMAT    Trex
Chr1    534      .   T    A            8.826    LowQual  DP=1   GT:GQ:AD  ./.:0:0,1
Chr1    1315     .   A    G            564.103  PASS     DP=51  GT:GQ:AD  1|0:99:26,25
Chr1    369655   .   CTC  CC           209.026  .        DP=31  GT:GQ:AD  0|1:99:19,12
Chr1    672396   .   GTT  GT,GGT       912.199  .        DP=36  GT:GQ:AD  2|1:43:0,28,8
Chr1    2192815  .   GG   GGTATTTTTAG  253.597  .        DP=64  GT:GQ:AD  0/1:99:46,18
```

VCF/BCF

VCF Metadata Lines: For humans and computers. Required by most tools to pre-declare how to parse file body correctly.

fileformat Meta
Required on first line;
Tells tools how to interpret rest of file

FILTER Meta
explicitly defines soft filters one expects to see in the FILTER column

```
##fileformat=VCFv4.2
##FILTER=<ID=LowQual,Description="Locus is low quality">
##FILTER=<ID=PASS,Description="Locus passes all filters">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="The Phred-scaled prob. of the genotype">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Number of observation for each allele">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##contig=<ID=Chr1,length=217471166>
##contig=<ID=Chr2,length=181034961>
#CHROM  POS     ID  REF  ALT          QUAL     FILTER   INFO   FORMAT    Trex
Chr1    534     .   T    A            8.826    LowQual  DP=1   GT:GQ:AD  ./.:0:0,1
Chr1    1315    .   A    G            564.103  PASS     DP=51  GT:GQ:AD  0/1:99:25
Chr1    369655  .   CTC  CC           209.[illegible]  DP=31  GT:GQ:AD  0/1:99:10,12
Chr1    672396  .   GTT  GT,GGT       [illegible]  DP=36  GT:GQ:AD  2|1:43:0,28,8
Chr1    210381  .   CG   GGTATTTTTAG  253.597  DP=64  GT:GQ:AD  0/1:99:46,18
```

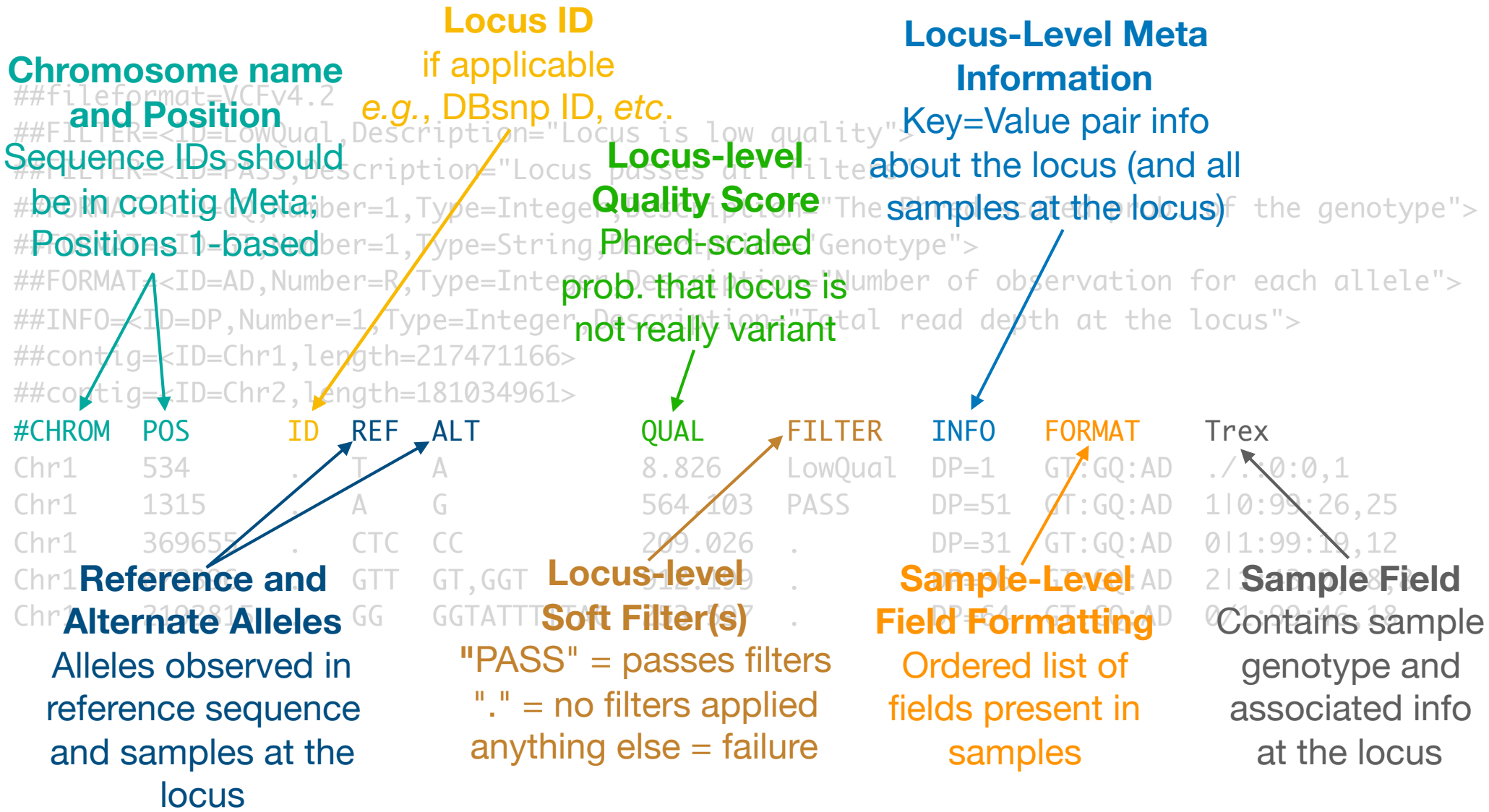
contig Meta
Optional, encouraged;
Describes reference sequences observed in CHROM column

INFO Meta
Explicitly defines the types of Key=Value data to be observed in INFO column

FORMAT Meta
Explicitly defines the types data to be observed in sample column(s)

VCF/BCF

VCF Header Line: Defines columns, including the sample names. Required by most tools to parse file correctly; undefined fields set to "."



VCF/BCF

VCF Loci: Tab-delimited columns. Alleles indexed from 0 (REF) to N (ALT) alleles. Genotypes represented with those indices

```
##fileformat=VCFv4.2
##FILTER=<ID=LowQual,Description="Locus is low quality">
##FILTER=<ID=PASS,Description="Locus passes all filters">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="The Phred-scaled prob. of the genotype">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Number of observation for each allele">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##contig=<ID=Chr1,length=217471166>
##contig=<ID=Chr2,length=181034961>
#CHROM  POS      ID  REF  ALT        QUAL     FILTER  INFO   FORMAT     Trex
Chr1    534      .   T    A          8.826    LowQual DP=1   GT:GQ:AD   ./.:0:0,1
Chr1    1315     .   A    G          564.103  PASS    DP=51  GT:GQ:AD   1|0:99:26,25
Chr1    369655   .   CTC  CC         209.026  .       DP=31  GT:GQ:AD   0|1:99:19,12
Chr1    672396   .   GTT  GT,GGT     912.199  .       DP=36  GT:GQ:AD   2|1:43:0,28,8
Chr1    2192815  .   GG   GGTATTTTAG 253.597  .       DP=64  GT:GQ:AD   0/1:99:46,18
```

Substitution locus

Complex locus
Multi-allele;
Deletion *and* substitution!

No-call or hard-filtered genotype

Deletion locus

Insertion locus

Phased genotypes

Unphased genotype

Allele Depth
Read count for each allele

Annotation files

BED

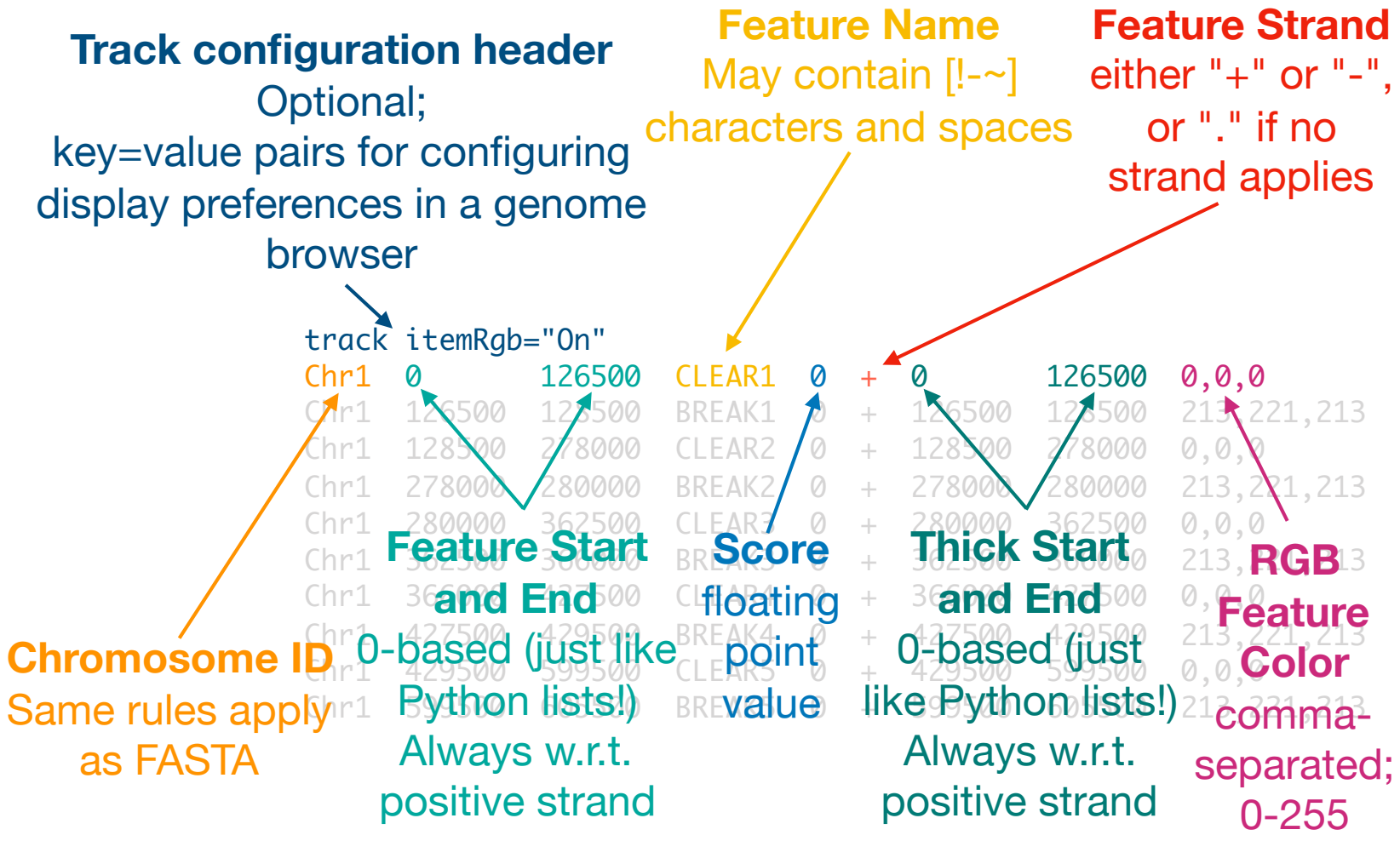
https://genome.ucsc.edu/FAQ/FAQformat.html#format1

BED: Browser Extensible Data format

```
track itemRgb="On"
Chr1  0       126500  CLEAR1  0  +  0       126500  0,0,0
Chr1  126500  128500  BREAK1  0  +  126500  128500  213,221,213
Chr1  128500  278000  CLEAR2  0  +  128500  278000  0,0,0
Chr1  278000  280000  BREAK2  0  +  278000  280000  213,221,213
Chr1  280000  362500  CLEAR3  0  +  280000  362500  0,0,0
Chr1  362500  366000  BREAK3  0  +  362500  366000  213,221,213
Chr1  366000  427500  CLEAR4  0  +  366000  427500  0,0,0
Chr1  427500  429500  BREAK4  0  +  427500  429500  213,221,213
Chr1  429500  599500  CLEAR5  0  +  429500  599500  0,0,0
Chr1  599500  605500  BREAK5  0  +  599500  605500  213,221,213
```

BED

BED: Columns tab-delimited. First three required, all others optional (first 6 typical).



GFF3

https://github.com/The-Sequence-Ontology/Specifications/blob/master/gff3.md

GFF: Generic Feature Format

```
##gff-version 3
##species http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=436495
##genome-build RexBase Trex1
##sequence-region Chr1 1 217471166
# Note Trex_genome.fasta, complete genome
Chr1  Gnomon  gene         43895  78350  .  +  .  ID=gene32251;Name=LOC101732307
Chr1  Gnomon  mRNA         43895  78350  .  +  .  ID=rna61088;Name=XM_012954515.1;Parent=gene32251
Chr1  Gnomon  CDS          43895  43947  .  +  0  ID=rna61088.1.CDS;Parent=rna61088
Chr1  Gnomon  exon         43895  43947  .  +  .  ID=rna61088.1.exon;Parent=rna61088
Chr1  Gnomon  start_codon  43895  43897  .  +  0  ID=rna61088.1.start_codon;Parent=rna61088
Chr1  Gnomon  CDS          48839  49007  .  +  1  ID=rna61088.2.CDS;Parent=rna61088
Chr1  Gnomon  exon         48839  49007  .  +  .  ID=rna61088.2.exon;Parent=rna61088
Chr1  Gnomon  CDS          53889  54000  .  +  0  ID=rna61088.3.CDS;Parent=rna61088
Chr1  Gnomon  exon         53889  54000  .  +  .  ID=rna61088.3.exon;Parent=rna61088
Chr1  Gnomon  CDS          55055  55173  .  +  2  ID=rna61088.4.CDS;Parent=rna61088
Chr1  Gnomon  exon         55055  55173  .  +  .  ID=rna61088.4.exon;Parent=rna61088
```

GFF3

GFF Header: Pragma begin with "##", comments with "#". Format pragma required for GFF3, highly-recommended for GFF2/GTF.

Pragma/Directives

Pre-declared set of pragma with specific formats/definitions. Mostly for computers/browsers.

```
##gff-version 3
##species http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=436495
##genome-build RexBase Trex1
##sequence-region Chr1 1 217471166
# Note Trex_genome.fasta, complete genome
Chr1  Gnomon  gene          43895  78350  .  +  .  ID=gene32251;Name=LOC101732307
Chr1  Gnomon  mRNA          43895  78350  .  +  .  ID=rna61088;Name=XM_012954515.1;Parent=gene32251
Chr1  Gnomon  CDS           43895  43947  .  +  0  ID=rna61088.1.CDS;Parent=rna61088
Chr1  Gnomon  exon          43895  43947  .  +  .  ID=rna61088.1.exon;Parent=rna61088
Chr1  Gnomon  start_codon   43895  43897  .  +  0  ID=rna61088.1.start_codon;Parent=rna61088
Chr1  Gnomon  CDS           48839  49007  .  +  1  ID=rna61088.2.CDS;Parent=rna61088
Chr1  Gnomon  exon          48839  49007  .  +  .  ID=rna61088.2.exon;Parent=rna61088
Chr1  Gnomon  CDS           53889  54000  .  +  0  ID=rna61088.3.CDS;Parent=rna61088
Chr1  Gnomon  exon          53889  54000  .  +  .  ID=rna61088.3.exon;Parent=rna61088
Chr1  Gnomon  CDS           55055  55173  .  +  2  ID=rna61088.4.CDS;Parent=rna61088
Chr1  Gnomon  exon          55055  55173  .  +  .  ID=rna61088.4.exon;Parent=rna61088
```

Format Version Pragma/Directive

Required for GFF3, highly-recommended for GFF2/GTF formats

Comments

Free-form text for humans, ignored by parsers.

GFF3

GFF Features: Nine tab-delimited fields required. Null values a "."

Reference ID
Chromosome/scaffold ID
May only contain characters in set:
[a-zA-Z0-9.:^*$@!+_?-|]

Feature Type
Must be SO term or accession number

Score
floating point number

Feature Strand
either "+" or "-", or "." if no strand applies

Feature Attributes
Semi-colon separated Key=Value pairs; reserved keys begin with capitals letters; "Parent" attribute defines feature hierarchy; must use URL-escaping for forbidden characters

```
##gff-version 3
##species http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=436495
##genome-build RexBase Trex1
##sequence-region Chr1 1 217471160
# Note Trex_genome.fasta, complete genome
Chr1  Gnomon  gene        43895  78350  .  +  .  ID=gene32251;Name=LOC101732307
Chr1  Gnomon  mRNA        43895  78350  .  +  .  ID=rna61088;Name=XM_012954515.1;Parent=gene32251
Chr1  Gnomon  CDS         43895  43947  .  +  0  ID=rna61088.1.CDS;Parent=rna61088
Chr1  Gnomon  exon        43895  43947  .  +  .  ID=rna61088.1.exon;Parent=rna61088
Chr1  Gnomon  start_codon 43895  43897  .  +  0  ID=rna61088.1.start_codon;Parent=rna61088
Chr1  Gnomon  CDS         48839  49007  .  +  1  ID=rna61088.2.CDS;Parent=rna61088
Chr1  Gnomon  exon        48839  49007  .  +  .  ID=rna61088.2.exon;Parent=rna61088
Chr1  Gnomon  CDS         53889  54000  .  +  0  ID=rna61088.3.CDS;Parent=rna61088
Chr1  Gnomon  exon        53889  54000  .  +  .  ID=rna61088.3.exon;Parent=rna61088
Chr1  Gnomon  CDS         55055  55173  .  +  2  ID=rna61088.4.CDS;Parent=rna61088
Chr1  Gnomon  exon        55055  55173  .  +  .  ID=rna61088.4.exon;Parent=rna61088
```

Source
Usually the program or organization that generated the annotations

Start and End Positions
1-based coordinates on "+" strand

Codon Phase
either 0, 1, or 2; Offset to next codon position

Resources

File manipulation/filtering

pysam (API)	FASTA/Q, BED, B/CR/SAM, B/VCF	https://pysam.readthedocs.io/en/latest/api.html#sam-bam-cram-files
pyFaidx (API)	FASTA	https://doi.org/10.7287/peerj.preprints.970v1
Seqtk	FASTA/Q	https://github.com/lh3/seqtk
Seqkit	FASTA/Q	https://doi.org/10.1371/journal.pone.0163962
seqmagick	Many	https://seqmagick.readthedocs.io
bedtools	BAM, BED, GFF, VCF	https://bedtools.readthedocs.io
bcftools	VCF/BCF	https://samtools.github.io/bcftools
genometools	FASTA/Q, GFF3	http://genometools.org
samtools	FASTA/Q, B/SAM	https://github.com/samtools/samtools
vcftools	VCF/BCF	https://vcftools.github.io/man_latest.html

Resources

QA/QC, Adapter and Quality trimming

trimmomatic	FASTQ	http://usadellab.org/cms/?page=trimmomatic
FastQC	FASTQ, B/SAM	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Sickle	FASTA/Q	https://github.com/ucdavis-bioinformatics/sickle
Scythe	FASTA/Q	https://github.com/ucdavis-bioinformatics/scythe
Sabre	FASTA/Q	https://github.com/najoshi/sabre
cutadapt	FASTA/Q	https://cutadapt.readthedocs.io/en/stable/

Alignment

minimap2	FASTA/Q	https://github.com/lh3/minimap2
BWA	FASTA/Q	https://github.com/lh3/bwa
bowtie2	FASTA/Q	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
STAR	FASTQ	https://github.com/alexdobin/STAR
GMAP	FASTA/Q	http://research-pub.gene.com/gmap/
exonerate	FASTA	https://www.ebi.ac.uk/about/vertebrate-genomics/software/exonerate

Resources

Variant calling

FreeBayes	BAM, VCF	https://github.com/ekg/freebayes
GATK4	FASTA/Q, B/CRAM, VCF	https://software.broadinstitute.org/gatk/documentation
DeepVariant	FASTA/Q	https://github.com/google/deepvariant
vg	FASTA/Q	https://github.com/vgteam/vg

Common file issues

- Non-printable characters
- Non-ASCII encoded characters
- Incorrect formatting (spaces instead of tabs)
- Truncated files

od -c