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

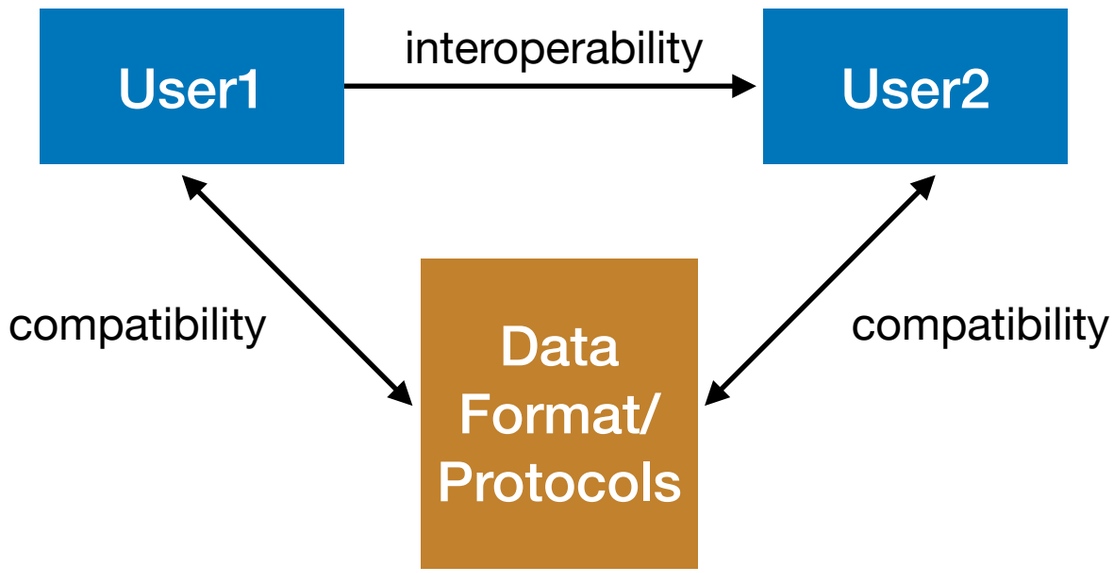
Why are (standardized) file formats important?

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]

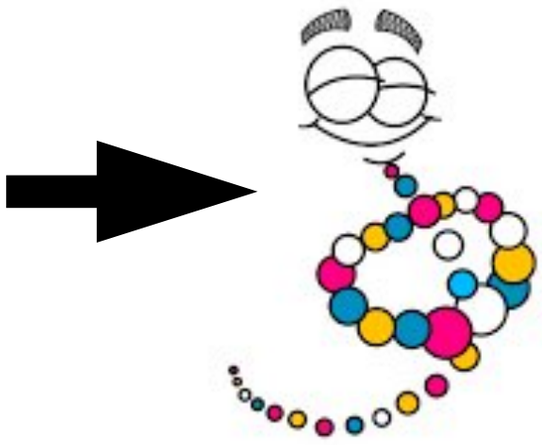


"the capability of different programs to exchange data via a common set of exchange formats, to read and write the same file formats, and to use the same protocols.... the lack of interoperability can be a consequence of a lack of attention to standardization during the design of a program"[2]

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...

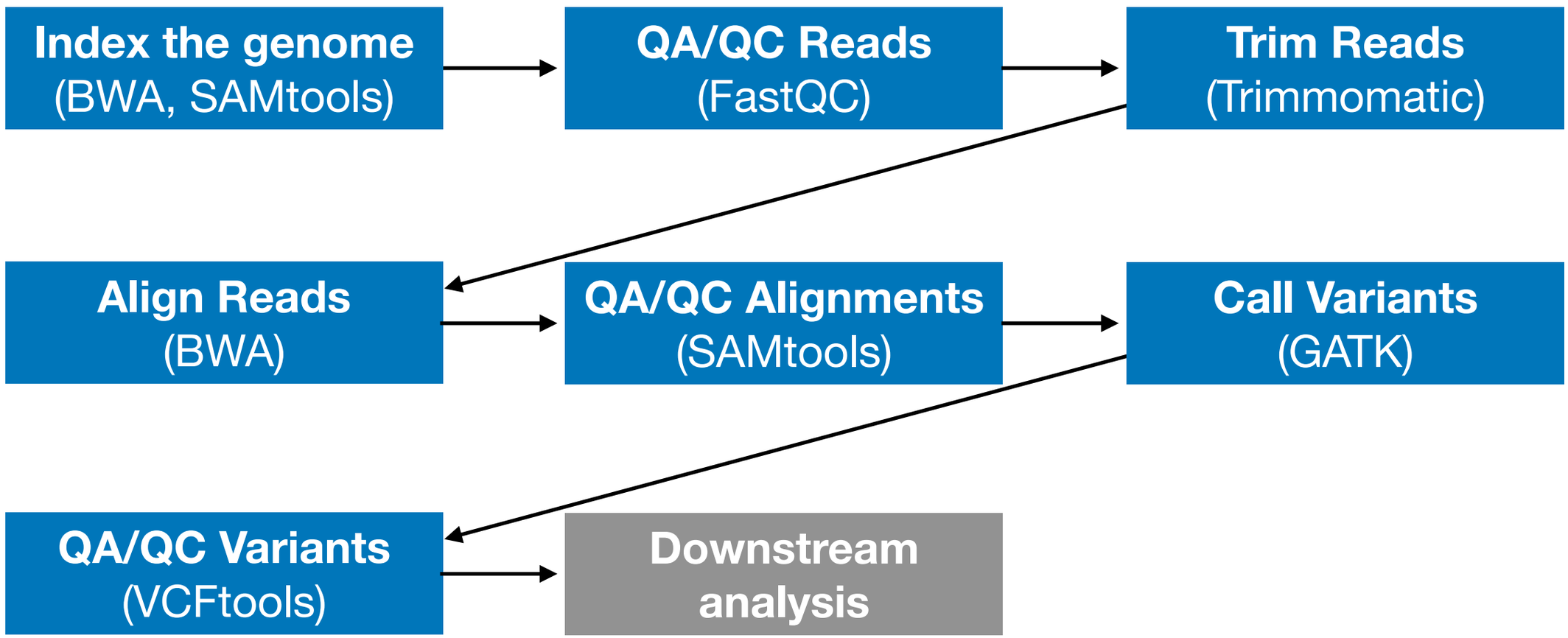
We extract DNA...



Variants

- SNPs: Single-Nucleotide Polymorphisms
- Indels: Small (≤ 50 bp) insertions-deletions
- Structural variants: mid-to-large (> 50 bp) 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
>lcl|BC064885.2_cds_AAH64885.1_1 [gene=mtpn] [protein=myotrophin] [protein_id=AAH64885.1]
ATGGGTGACAAGGAGTTCGTGTGGGCCATCAAGAACGGAGACCTGGATGCAGTGAAAGAATTCGTACTTG
GGGGCGAGGATGTGAACCGGACGCTGGATGGTGGAAGGAAACCTATGCACTACGCTGCCGACTGCGGGCA
GGATGAGGTCCTGGAGTTTCTTCTCTCGAAAGGAGCCAACATCAATGCTGCGGATAAACATGGCATCACC
CCCCTACTATCTGCCTGCTACGAGGGCCATCGCAAATGTGTCGAGTTGCTTTTATCTAAGGGAGCCGACA
CCATTAA
```

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

Should be wrapped flush, but sometimes is not

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

"At" symbol
Start of sequence portion of record

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

Whitespace only required if description present

Description/Comment optional

```
@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

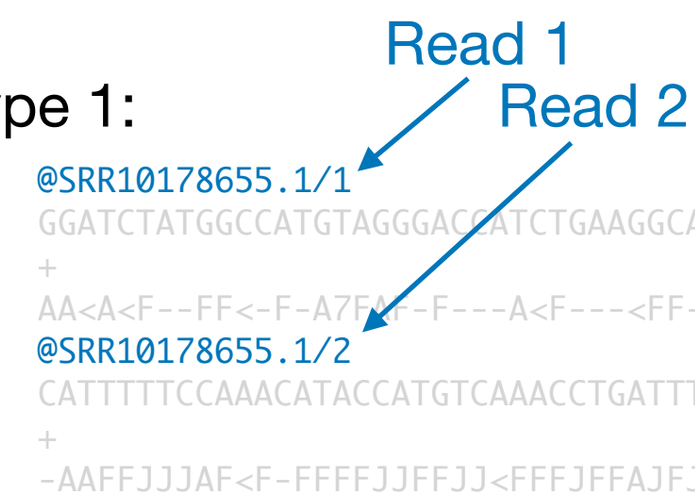
Should *not* be wrapped flush

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

FASTQ

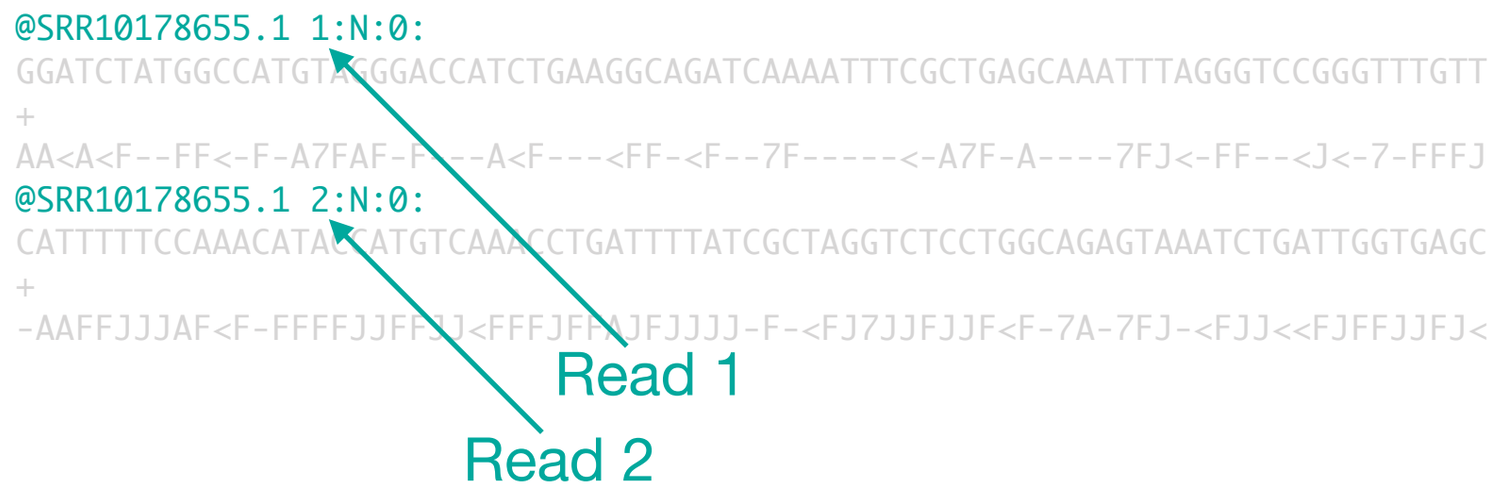
FASTQ Sequence Header: Paired-end or mate-pair reads

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
0	0	000	NUL (null)
1	1	001	SOH (start of heading)
2	2	002	STX (start of text)
3	3	003	ETX (end of text)
4	4	004	EOT (end of transmission)
5	5	005	ENQ (enquiry)
6	6	006	ACK (acknowledge)
7	7	007	BEL (bell)
8	8	010	BS (backspace)
9	9	011	TAB (horizontal tab)
10	A	012	LF (NL line feed, new line)
11	B	013	VT (vertical tab)
12	C	014	FF (NP form feed, new page)
13	D	015	CR (carriage return)
14	E	016	SO (shift out)
15	F	017	SI (shift in)
16	10	020	DLE (data link escape)
17	11	021	DC1 (device control 1)
18	12	022	DC2 (device control 2)
19	13	023	DC3 (device control 3)
20	14	024	DC4 (device control 4)
21	15	025	NAK (negative acknowledge)
22	16	026	SYN (synchronous idle)
23	17	027	ETB (end of trans. block)
24	18	030	CAN (cancel)
25	19	031	EM (end of medium)
26	1A	032	SUB (substitute)
27	1B	033	ESC (escape)
28	1C	034	FS (file separator)
29	1D	035	GS (group separator)
30	1E	036	RS (record separator)
31	1F	037	US (unit separator)

Dec	Hx	Oct	Html	Chr
32	20	040		Space
33	21	041	!	!
34	22	042	"	"
35	23	043	#	#
36	24	044	$	$
37	25	045	%	%
38	26	046	&	&
39	27	047	'	'
40	28	050	(	(
41	29	051	)	)
42	2A	052	*	*
43	2B	053	+	+
44	2C	054	,	,
45	2D	055	-	-
46	2E	056	.	.
47	2F	057	/	/
48	30	060	0	0
49	31	061	1	1
50	32	062	2	2
51	33	063	3	3
52	34	064	4	4
53	35	065	5	5
54	36	066	6	6
55	37	067	7	7
56	38	070	8	8
57	39	071	9	9
58	3A	072	:	:
59	3B	073	;	;
60	3C	074	<	<
61	3D	075	=	=
62	3E	076	>	>
63	3F	077	?	?

Dec	Hx	Oct	Html	Chr
64	40	100	@	@
65	41	101	A	A
66	42	102	B	B
67	43	103	C	C
68	44	104	D	D
69	45	105	E	E
70	46	106	F	F
71	47	107	G	G
72	48	110	H	H
73	49	111	I	I
74	4A	112	J	J
75	4B	113	K	K
76	4C	114	L	L
77	4D	115	M	M
78	4E	116	N	N
79	4F	117	O	O
80	50	120	P	P
81	51	121	Q	Q
82	52	122	R	R
83	53	123	S	S
84	54	124	T	T
85	55	125	U	U
86	56	126	V	V
87	57	127	W	W
88	58	130	X	X
89	59	131	Y	Y
90	5A	132	Z	Z
91	5B	133	[	[
92	5C	134	\	\
93	5D	135	]	]
94	5E	136	^	^
95	5F	137	_	_

Dec	Hx	Oct	Html	Chr
96	60	140	`	`
97	61	141	a	a
98	62	142	b	b
99	63	143	c	c
100	64	144	d	d
101	65	145	e	e
102	66	146	f	f
103	67	147	g	g
104	68	150	h	h
105	69	151	i	i
106	6A	152	j	j
107	6B	153	k	k
108	6C	154	l	l
109	6D	155	m	m
110	6E	156	n	n
111	6F	157	o	o
112	70	160	p	p
113	71	161	q	q
114	72	162	r	r
115	73	163	s	s
116	74	164	t	t
117	75	165	u	u
118	76	166	v	v
119	77	167	w	w
120	78	170	x	x
121	79	171	y	y
122	7A	172	z	z
123	7B	173	{	{
124	7C	174			|
125	7D	175	}	}
126	7E	176	~	~
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
```

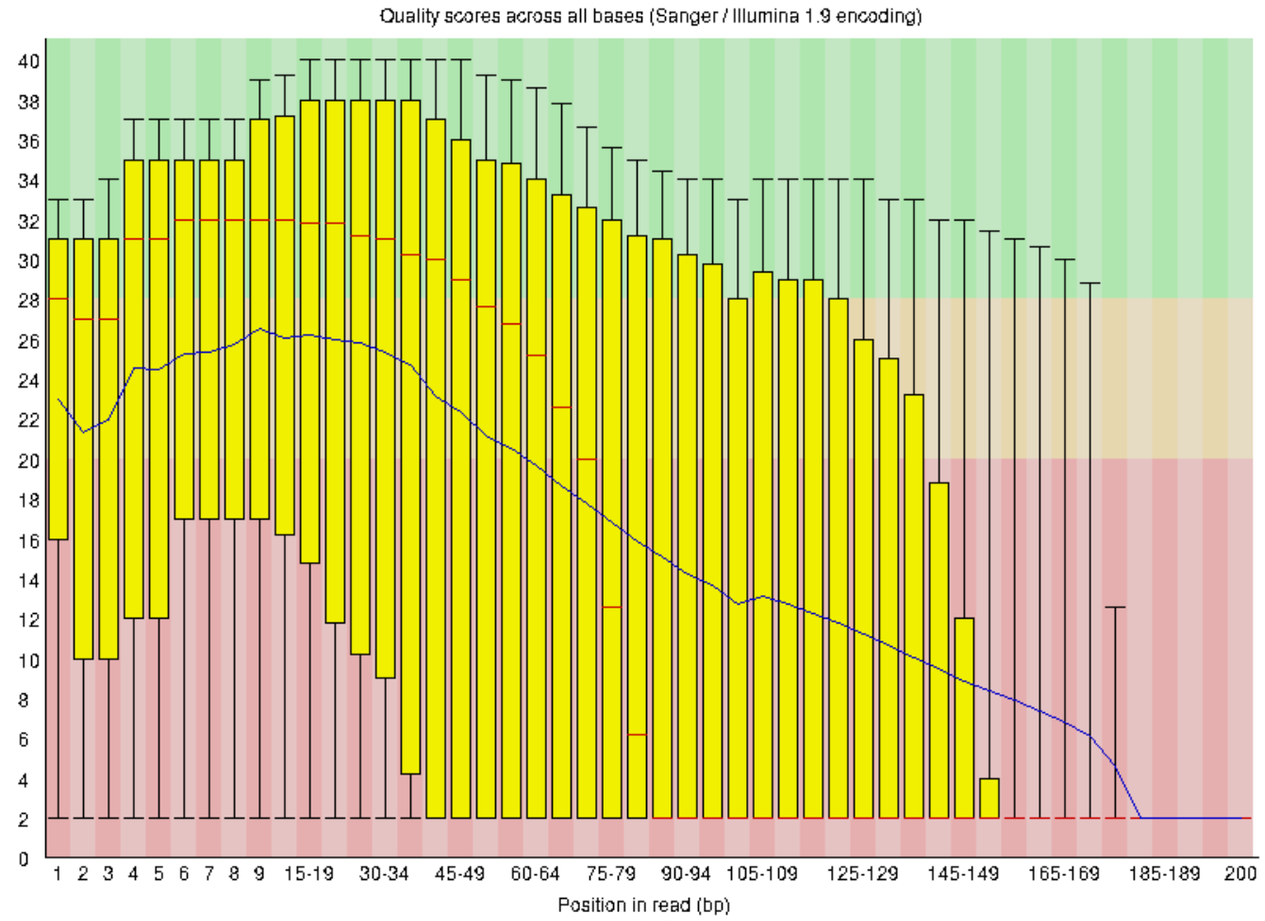
```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
 ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
 ..............................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
  !"#$%&'()*+,-./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)
```

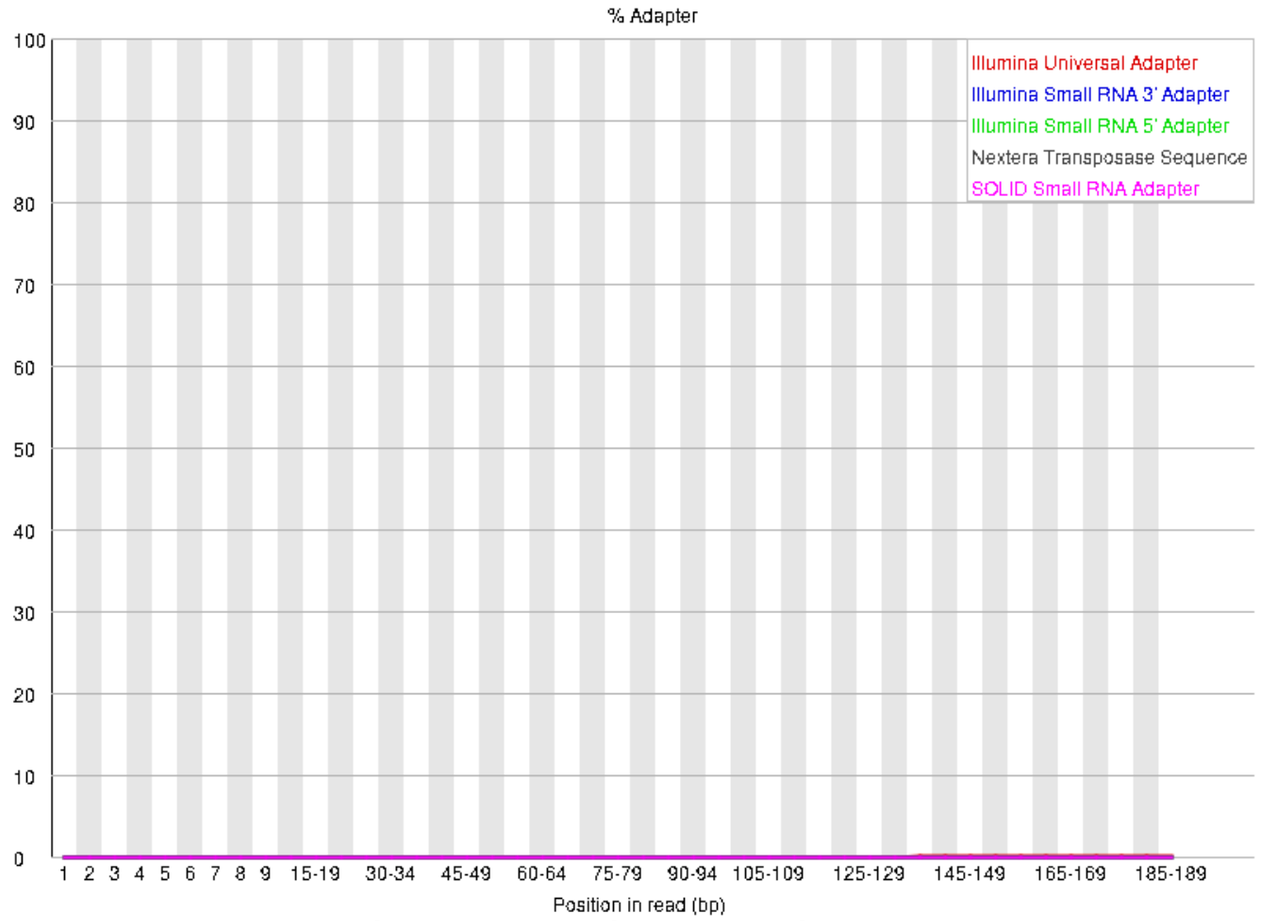
https://en.wikipedia.org/wiki/FASTQ_format

QA/QC'ing Illumina Reads

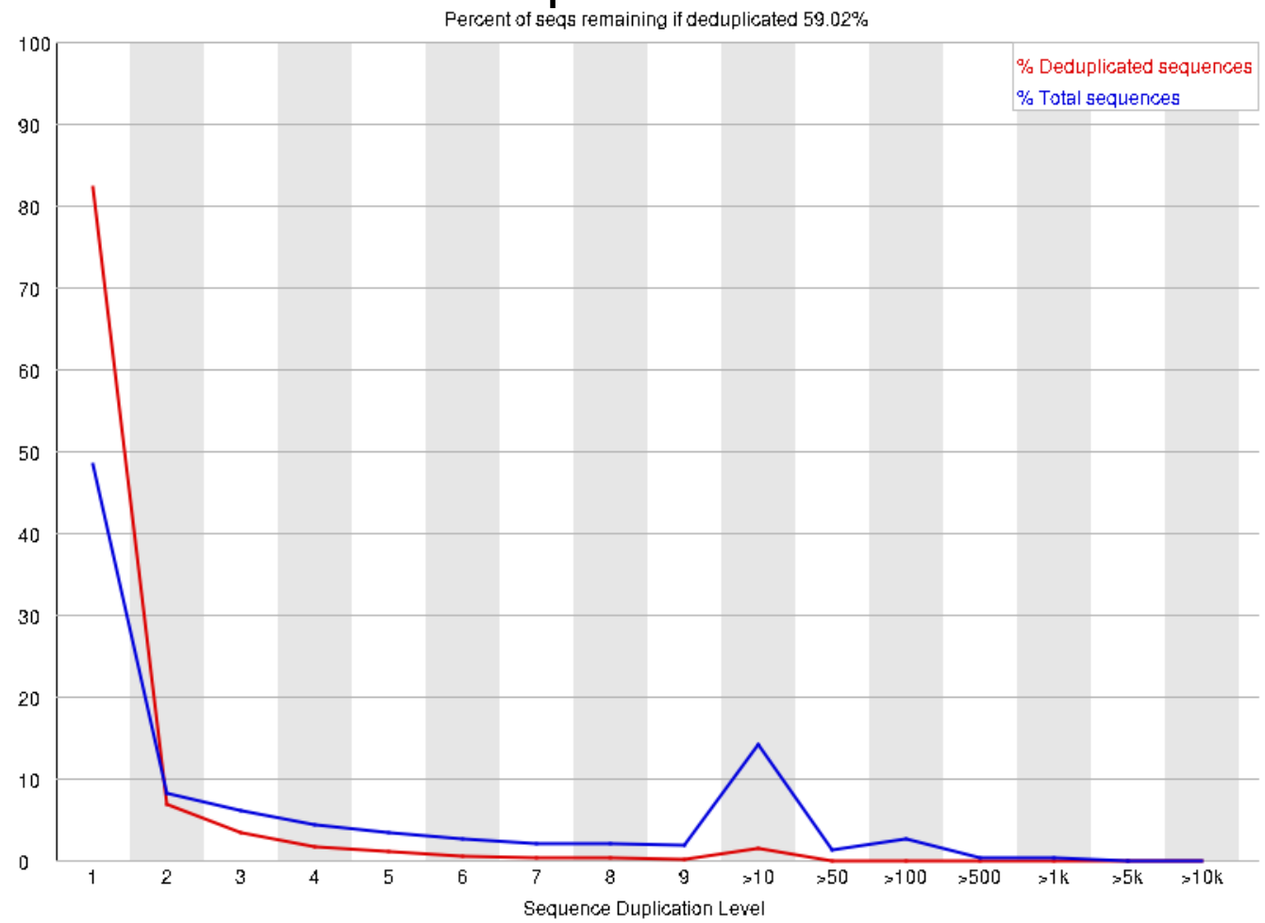
```
# Run FASTQ quality assessment tool and generate plots
$ fastqc --threads 2 --extract SRR10178655_1.fastq.gz SRR10178655_2.fastq.gz
```

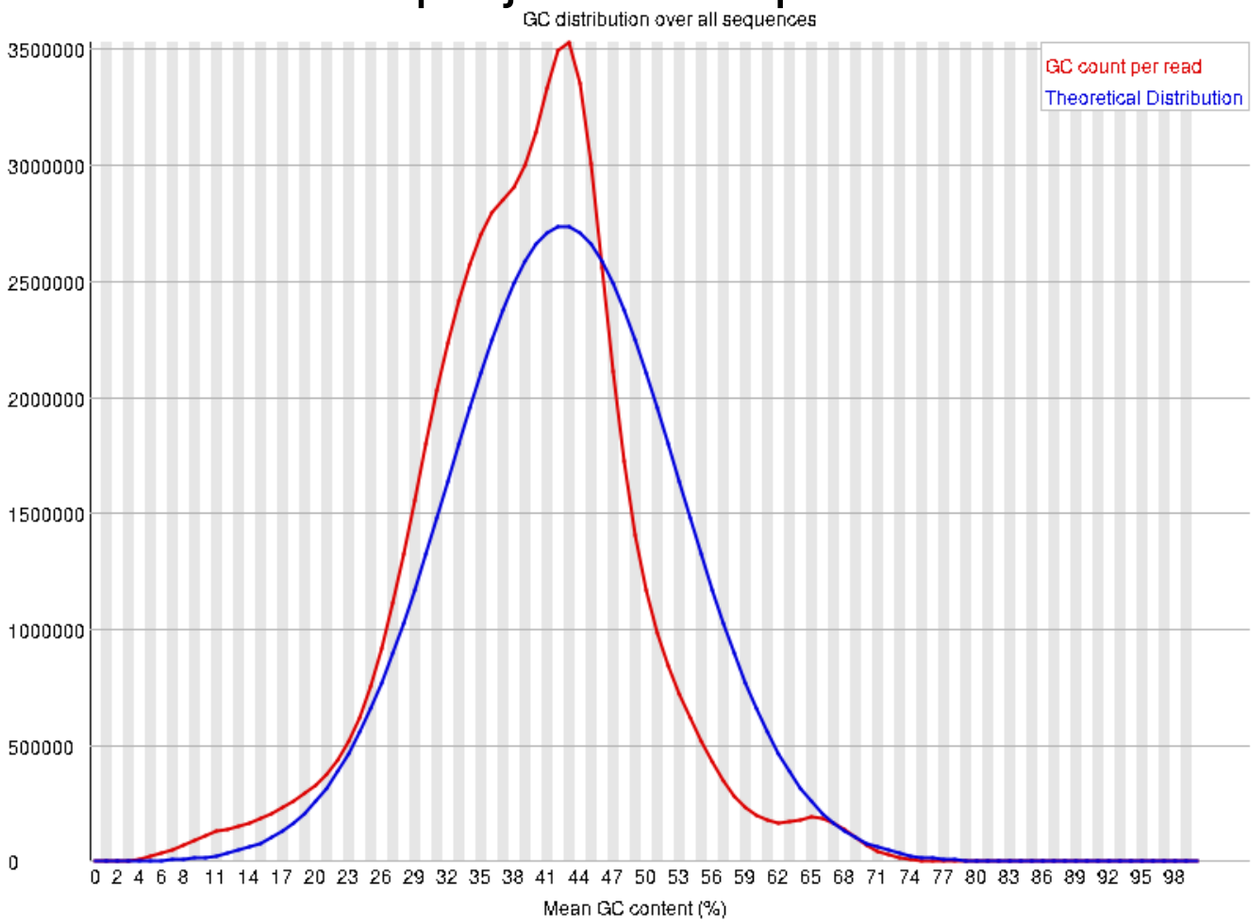
```
# View the FastQC results in Safari (Mac only):
$ open -a Safari.app SRR10178655_1_fastqc/fastqc_report.html
```





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
```

Read Group tag read group ID SaMple name LiBrary name PLatform

```
# 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
```

Read group tag: '@RG' always
Read group ID: Must be unique
Sample name: Name of sample/individual/accession
Library name: Sequencing library name
Platform: Sequencing technology

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.

Bit Flags
binary array of boolean values

Reference Sequence ID and Position
1-based alignment start coordinate of this read

Mapping Quality
Phred-scaled prob. mapping is wrong

CIGAR
describes alignment

Sequence/Read ID
same rules as in FASTQ

```
@HD  VN:1.5  SO:coordinate
@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
```

Insert Distance
Negative values mean other read in pair is upstream; positive is downstream

Read Sequence and Qualities
same rules as in FASTQ
offset = 33 *required*

Auxiliary Info Tags
Optional;
RGs almost required

Reference Sequence ID and Position
for other read in pair, if applicable

SAM/BAM/CRAM

CIGAR AND Bitwise flag field details

Useful with `samtools flags` and `samtools view -f -F`

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
but also reports mismatches

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)

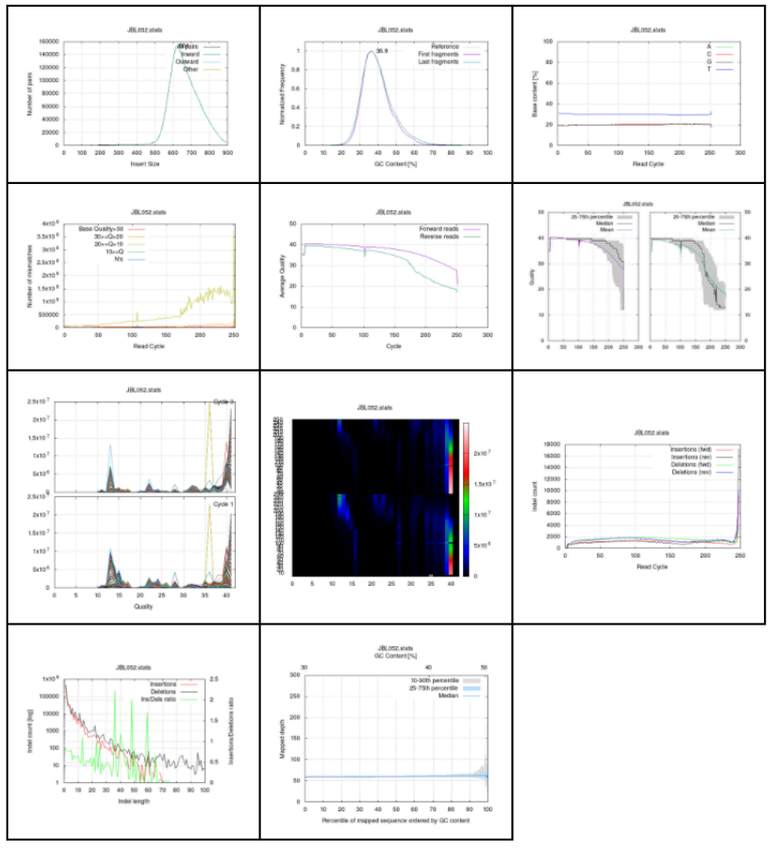
```
flags & 1024  # correct
flags > 1024  # incorrect!!
```

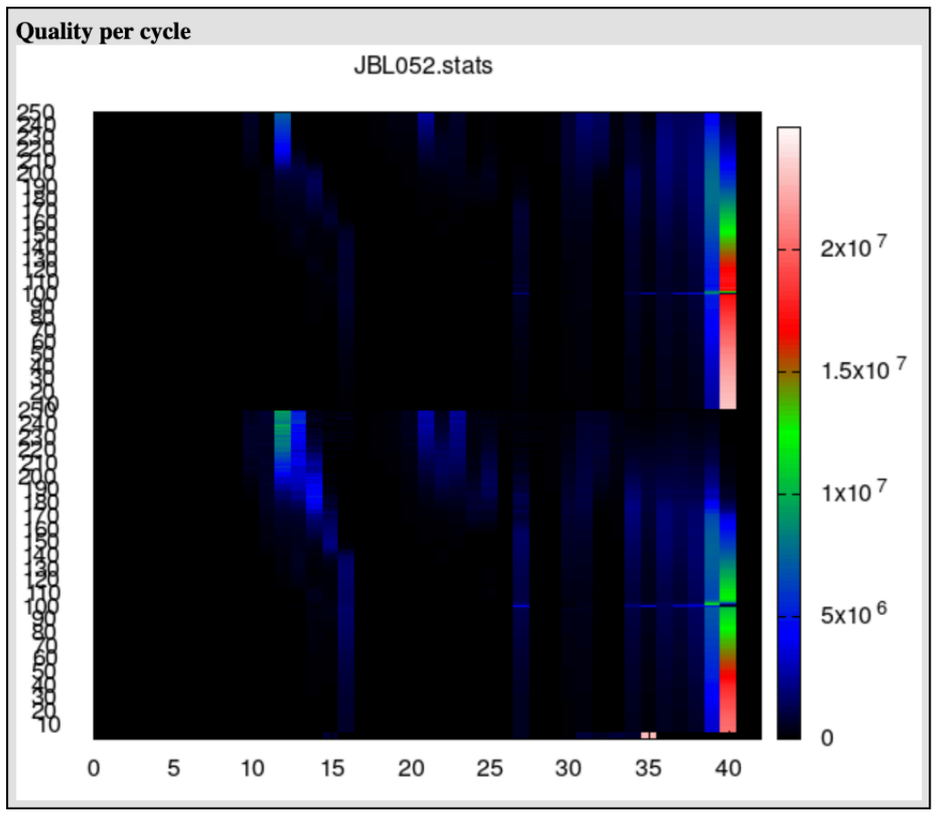
QA/QC'ing Alignments

```
# 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 --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





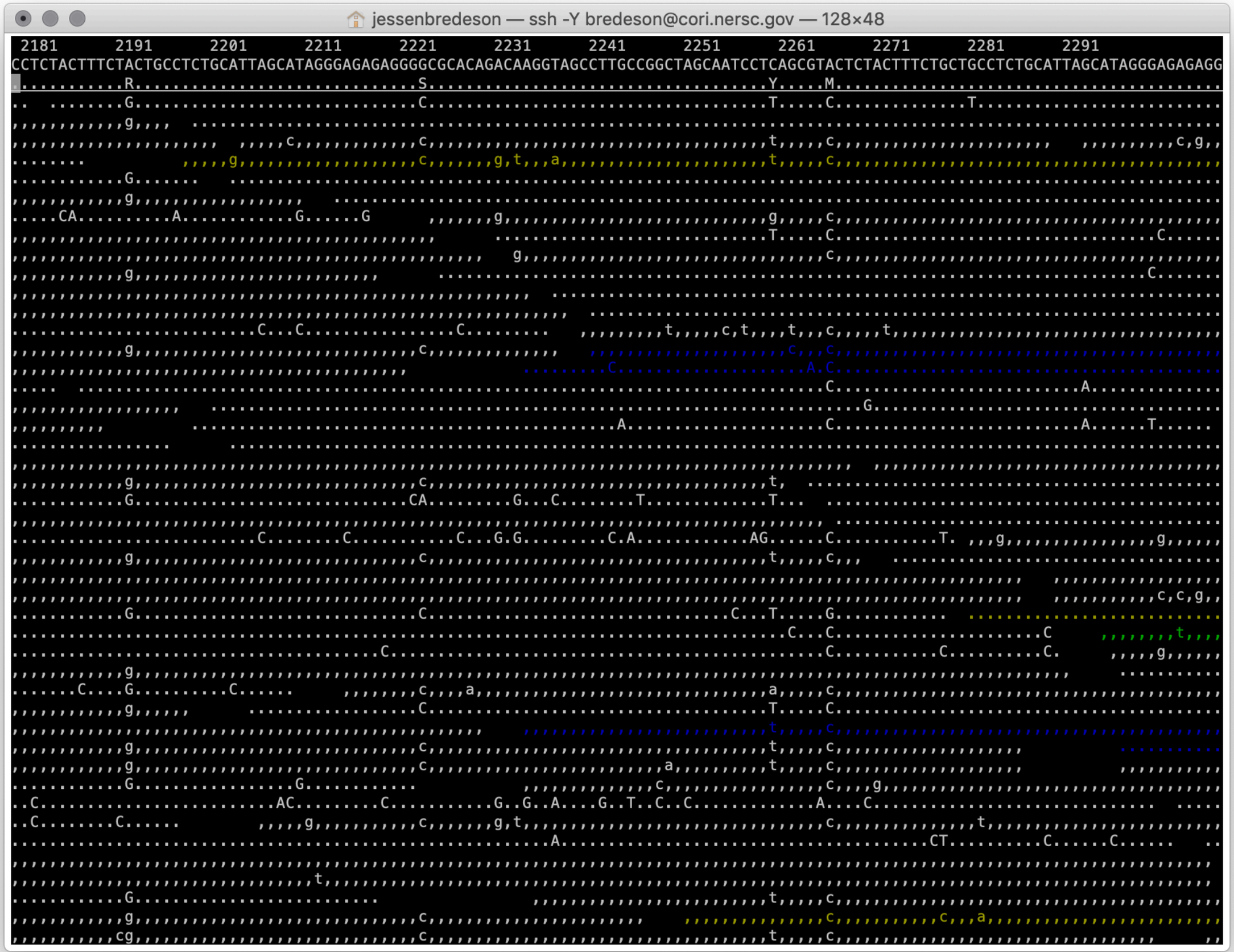
Reads

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%	

```
$ 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
```



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 30 \
    --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  0/1:99:25,25
Chr1    369655  .   CTC  CC           209.[illegible]  DP=31  GT:GQ:AD  0/1:99:10,13
Chr1    672396  .   GTT  GT,GGT       [illegible]      DP=36  GT:GQ:AD  2|1:43:0,28,8
Chr1    [illegible]  .   CG   GGTATTTTAG  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 "**.**"

Chromosome name and Position
Sequence IDs should be in contig Meta; Positions 1-based

Locus ID
if applicable
e.g., DBsnp ID, *etc.*

Locus-level Quality Score
Phred-scaled prob. that locus is not really variant

Locus-Level Meta Information
Key=Value pair info about the locus (and all samples at the locus)

```
##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 quality 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    [illegible]  .   GTT  GT,GGT   [illegible]  .        [illegible]  GT:GQ:AD   2|1:[illegible]
Chr1    [illegible]  .   GG   GGTATTT,[illegible]  .        DP=64   GT:GQ:AD   0/1:99:46,18
```

Reference and Alternate Alleles
Alleles observed in reference sequence and samples at the locus

Locus-level Soft Filter(s)
"PASS" = passes filters
"." = no filters applied
anything else = failure

Sample-Level Field Formatting
Ordered list of fields present in samples

Sample Field
Contains sample genotype and associated info at the locus

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   GGTATTTTTAG 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).

Track configuration header
Optional;
key=value pairs for configuring display preferences in a genome browser

Feature Name
May contain [!-~] characters and spaces

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

```
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  601500  BREAK5  0  +  599500  601500  213,221,213
```

Chromosome ID
Same rules apply as FASTA

Feature Start and End
0-based (just like Python lists!)
Always w.r.t. positive strand

Score
floating point value

Thick Start and End
0-based (just like Python lists!)
Always w.r.t. positive strand

RGB Feature Color
comma-separated; 0-255

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   55172   .   +   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
BioPython	Many	https://biopython.org
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	B/VCF	https://samtools.github.io/bcftools
genometools	FASTA/Q, GFF, GTF	http://genometools.org
gffread & gffcompare	GFF, GTF	https://github.com/gpertea/gffread https://github.com/gpertea/gffcompare
samtools	FASTA/Q, B/SAM	https://github.com/samtools/samtools
bamtools	B/SAM	https://github.com/pezmaster31/bamtools
vcftools	B/VCF	https://vcftools.github.io/man_latest.html
Picard	FASTA/Q, BED, B/CR/SAM, B/VCF	https://broadinstitute.github.io/picard/

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
miniprot	FASTA	https://github.com/lh3/miniprot
BWA	FASTA/Q	https://github.com/lh3/bwa
hisat2	FASTA/Q	https://daehwankimlab.github.io/hisat2/
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