Filetypes for Annotation & Alignment

J Fass | 19 June 2018

Filetypes

- Fasta
- Fastq
- GTF / GFF
- SAM / BAM / CRAM
- BED

See *https://genome.ucsc.edu/FAQ/FAQformat.html*

Fasta

```
>sequenceName | plus other junk | few maintain a standard here
AGTGGAGCAAGCACAGAGAAGAAACTGCAGTCAGGACATAAAGTAAAGTA
ATTAATCTAAAAAATAGTCTGAGCAGTCTTCTCTGCTGAANNNNNNNNNN
NNNNNNNNNNNNNNNNNNNAATTTCCTTACTAGGAGGTCTTTAGTACAGA
TTCCTGATATGTAATTAATCACTAAATGTCTTTAATGGGATCTCTTTCTA
TTGAGATATTTGTAAACTTTCTTCATGTGATTGGTTTACAGATATTCAGG
TTTCTGCAAATGGGTGCTGTCTATATTATAGAATTTTTAGTTGAAATTTT
CAAAATACTCTTTGagtattctcttgtaattatattactttacaaggttt
gtggggcatctctttcatttgtgattacatggttgcagtattctttttgt
tcttagtcagactgtataattgtctgtgaagtccagtaaacttttgaaag
```

Assembly gap?

Soft-masked repetitive sequence? Low confidence assembly?

Fastq

```
@K00188:264:HG3WJBBXX:1:1101:6289:1595 1:N:0:TAGCTT
GGACTGCCTTTCAGCCCGTCGCAGAGGGAATGGGAGCCTCTGGAGCGGGTGCAGAGGCTCAGCAG
+
AAFFFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJAJJJJJJ

@header
<sequence>
+(sometimes header?)
<base qualities>
```

Fastq

```
@K00188:264:HG3WJBBXX:1:1101:6289:1595 1:N:0:TAGCTT
GGACTGCCTTTCAGCCCGTCGCAGAGGGAATGGGAGCCTCTGGAGCGGGTGCAGAGGCTCAGCAG
+
AAFFFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
```

Oct	Dec	Hex	Char	Oct	Dec	Hex	Char
000	0	00	NUL '\0' (null character)	100	64	40	@
001	1	01	SOH (start of heading)	101	65	41	A
002	2	02	STX (start of text)	102	66	42	B
003	3	03	ETX (end of text)	103	67	43	C
004	4	04	EOT (end of transmission)	104	68	44	D
005	5	05	ENQ (enquiry)	105	69	45	E
006	6	06	ACK (acknowledge)	106	70	46	F
007	7	07	BEL '\a' (bell)	107	71	47	G
010	8	08	BS '\b' (backspace)	110	72	48	H
011	9	09	HT '\t' (horizontal tab)	111	73	49	I
012	10	0A	LF '\n' (new line)	112	74	4A	J
013	11	0B	VT '\v' (vertical tab)	113	75	4B	K

Fastq

Oct	Dec	Hex	Char
112	74	4A	J

74 - 33 = 41

Probability of error = 10 ^ (-41 / 10) ~ 0.0001

41 is the “phred-scaled Q-value”

Standard FASTQ encodes qualities using “phred + 33” quality characters. See https://en.wikipedia.org/wiki/FASTQ_format for a good graphic about current and older encodings.

Common QC question: “how many reads have average of at least Q30?”

GTF / GFF

Gene Transfer Format / Gene Feature Format … describes gene locations within the genome. If we align reads to the genome, reads that align to gene regions must have come from mRNA from that gene, yes?

GTF / GFF

- Human/mouse: GENCODE (uses Ensembl IDs) (http://www.gencodegenes.org/), but may need some manipulation to work with certain software
- Ensembl genomes (http://ensemblgenomes.org/) and Biomart (http://www.ensembl.org/biomart/martview/)
- Illumina igenomes (http://support.illumina.com/sequencing/sequencing_software/igenome.html) provides indexes for some software, and files with extra info for Tophat/cufflinks.
- NCBI genomes (http://www.ncbi.nlm.nih.gov/genome/)
- Many specialized databases (Phytozome, Patric, VectorBase, FlyBase, WormBase)
- “Do it yourself” genome assembly and gene-finding (don’t forget functional annotation)

GTF / GFF

chr12	unknown	exon	4382902	4383401	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	CDS	4383207	4383401	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	start_codon	4383207	4383209	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	CDS	4385171	4385386	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	exon	4385171	4385386	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	CDS	4387926	4388085	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	exon	4387926	4388085	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	CDS	4398008	4398156	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	exon	4398008	4398156	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	CDS	4409026	4409172	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	exon	4409026	4414522	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";
chr12	unknown	stop_codon	4409173	4409175	.	+	.	gene_id "CCND2"; gene_name "CCND2"; p_id "P6197"; transcript_id "NM_001759"; tss_id "TSS231";

The left columns list source, feature type, and genomic coordinates

The right column includes attributes, including gene ID, etc.

```
chr12	unknown	CDS	3677872	3678014	.	+	2	gene_id "PRMT8"; gene_name "PRMT8"; p_id "P10933"; transcript_id "NM_019854"; tss_id "TSS4368";
```

GTF

Sequence Name (i.e., chromosome, scaffold, etc.)	**chr12**
Source (program that generated the gtf file or feature)	**unknown**
Feature (i.e., gene, exon, CDS, start codon, stop codon)	**CDS**
Start (starting location on sequence)	**3677872**
End (end position on sequence)	**3678014**
Score	**.**
Strand (+ or -)	**+**
Frame (0, 1, or 2: which is first base in codon, zero-based)	**2**

Attribute (";"-delimited list of tags with additional info)

gene_id "PRMT8"; gene_name "PRMT8"; p_id "P10933";
transcript_id "NM_019854"; tss_id "TSS4368";

GTF file with questionable attributes …

```
gene_id "AAEL005599";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "1 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "2 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "3 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "4 of 4";
transcript_id "AAEL005599-RA";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "1 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "2 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "3 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA"; exon_number "4 of 4";
gene_id "AAEL005599"; transcript_id "AAEL005599-RA";
gene_id "AAEL016379"; transcript_id "AAEL005599-RA"; exon_number "5 of 4";
gene_id "AAEL016380"; transcript_id "AAEL005599-RA"; exon_number "6 of 4";
```

SAM / BAM / CRAM!

http://www.htslib.org/

See also samtools man page: *http://samtools.sourceforge.net/*

SAM spec grew out of 1000 Genomes Project (see Li et al. 2009 *Bioinformatics* 25:2078)

SAM is plain text; BAM is binary, compressed version of SAM; CRAM is further compressed but not widely used / recognizable by many tools.

SAM

Header lines (start with "@")

```
[...]
@SQ     SN:ctg103993     LN:217
@SQ     SN:ctg103994     LN:222
@SQ     SN:ctg103995     LN:205
@SQ     SN:ctg103996     LN:210
@PG     ID:bwa  PN:bwa  VN:0.7.13-r1126 CL:bwa mem -t 4 -M ../../01_Reference/Transcriptome-Contigs-Build2.fna
../../02-Cleaned/3E/3E_SE.fastq
@PG     ID:bwa-7BC92A6F PN:bwa  VN:0.7.13-r1126 CL:bwa mem -t 4 -M ../../01_Reference/Transcriptome-Contigs-Build2.fna
../../02-Cleaned/3E/3E_R1.fastq ../../02-Cleaned/3E/3E_R2.fastq
K00188:264:HG3WJBBXX:1:1116:14692:35180#0      121     ctg2      128     58      101M =       128     0
AAGTCTCGACCAAGTGGTTCAGATGGTGACACAGATGTTAGCCCCATCCACCATTCAGTTGCCGTTTTGATAGCTGGAAATCCTGTAAACACAAT
GCTGAG   FJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   NM:i:10
K00188:264:HG3WJBBXX:1:1116:14692:35180#0      181     ctg2      128     0      *             =       128     0
TTTAGTTTTAATTTTTGACTTTGAATAGCGGGAGTCCAGATCGTGTGAACACAGCAGACTGAGCACTCCATTGACAGCCTTCTTCTGTACTTTAGC
TATCC   FJFJJFAAJF7F7JJJJAFFFAF<7<AFFJJJFJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJFAJJJJJJJJFFFJJJJJJJJJJFFJJJJJJJFFFAA   AS:i:0  XS:i:0
K00188:264:HG3WJBBXX:1:1202:11028:9596#0       121     ctg5      45      60      101M   =       45      0
TTCTTTTTTCTACAGTTCATTGTCTGTATAAAGTATGCATCAGGAACAATCTGACTAGGAAGGTAAATAATGTAAAACAGATGATTATTGTATGAAA
GTTG  JJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   NM:i:8
K00188:264:HG3WJBBXX:1:1202:11028:9596#0       181     ctg5      45      0      *             =       45      0
TCAGCTGTATTAGTAATTTAGTAGAAAAGGTCTTGAGAGAATTATGTTTTTTAAAAATCCACATCACTTCAAACAAAAAGCCCCATTAGAATGGAGG
GCCA   FJFJJJJJFJJJJJFFJJJJFJAJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFF-JFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   AS:i:0
[...]
```

Alignment line (one line per alignment)

SAM

```
[...]
@SQ     SN:ctg103993     LN:217
@SQ     SN:ctg103994     LN:222
@SQ     SN:ctg103995     LN:205
@SQ     SN:ctg103996     LN:210
@PG     ID:bwa  PN:bwa  VN:0.7.13-r1126 CL:bwa mem -t 4 -M ../../01_Reference/Transcriptome-Contigs-Build2.fna ../../02-Cleaned/3E/3E_SE.fastq
@PG     ID:bwa-7BC92A6F PN:bwa  VN:0.7.13-r1126 CL:bwa mem -t 4 -M ../../01_Reference/Transcriptome-Contigs-Build2.fna ../../02-Cleaned/3E/3E_R1.fastq ../../02-Cleaned/3E/3E_R2.fastq
K00188:264:HG3WJBBXX:1:1116:14692:35180#0     121     ctg2     128     58     101M =     128     0     AAGTCTCGACCAAGTGGTTCAGATGGTGACACAGATGTTAGCCCCATCCACCATTCAGTTGCCGTTTTGATAGCTGGAAATCCTGTAAACACAATGCTGAG   FJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   NM:i:10
K00188:264:HG3WJBBXX:1:1116:14692:35180#0     181     ctg2     128     0     *     =     128     0     TTTAGTTTTAATTTTTGACTTTGAATAGCGGGAGTCCAGATCGTGTGAACACAGCAGACTGAGCACTCCATTGACAGCCTTCTTCTGTACTTTAGCTATCC   FJFJJFAAJF7F7JJJAFFFAF<7<AFFJJJFJJJJJJJJJJJJJJJJJJJJFJJJJJJFAJJJJJJJFFFJJJJJJJJJJFFJJJJJJFFFAA   AS:i:0  XS:i:0
K00188:264:HG3WJBBXX:1:1202:11028:9596#0     121     ctg5     45     60     101M   =     45     0     TTCTTTTTTCTACAGTTCATTGTCTGTATAAAGTATGCATCAGGAACAATCTGACTAGGAAGGTAAATAATGTAAAACAGATGATTATTGTATGAAAGTTG  JJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   NM:i:8
K00188:264:HG3WJBBXX:1:1202:11028:9596#0     181     ctg5     45     0     *     =     45     0     TCAGCTGTATTAGTAATTTAGTAGAAAAGGTCTTGAGAGAATTATGTTTTTTAAAAATCCACATCACTTCAAACAAAAAGCCCCATTAGAATGGAGGGCCA   FJFJJJJJFJJJJJFFJJJJFAJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFF-JFJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   AS:i:0
[...]
```

Read Pair (identical headers)

SAM

```
K00188:264:HG3WJBBXX:1:1116:14692:35180#0        121      ctg2       128      58       101M       =        128      0
AAGTCTCGACCAAGTGGTTCAGATGGTGACACAGATGTTAGCCCCATCCACCATTCAGTTGCCGTTTTGATAGCTGGAAATCCTGTAAACACAATGCTGAG
FJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFFFAA   NM:i:10
```

Col	Field	Type	Regexp/Range	Brief description
1	QNAME	String	[!-?A-~]{1,254}	Query template NAME
2	FLAG	Int	[0,2^{16}-1]	bitwise FLAG
3	RNAME	String	*|[!-()+-<>-~][!-~]*	Reference sequence NAME
4	POS	Int	[0,2^{31}-1]	1-based leftmost mapping POSition
5	MAPQ	Int	[0,2^{8}-1]	MAPping Quality
6	CIGAR	String	*|([0-9]+[MIDNSHPX=])+	CIGAR string
7	RNEXT	String	*|=|[!-()+-<>-~][!-~]*	Ref. name of the mate/next read
8	PNEXT	Int	[0,2^{31}-1]	Position of the mate/next read
9	TLEN	Int	[-2^{31}+1,2^{31}-1]	observed Template LENgth
10	SEQ	String	*|[A-Za-z=.]+	segment SEQuence
11	QUAL	String	[!-~]+	ASCII of Phred-scaled base QUALity+33

SAM

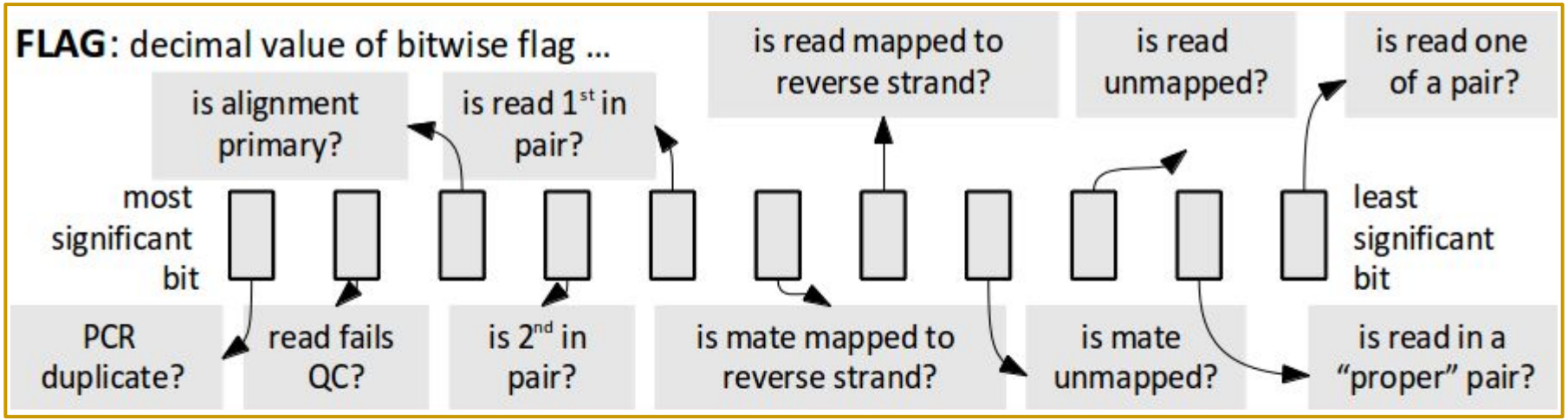
QNAME: Query name

Read IDs are truncated at first whitespace (spaces / tabs), which can make them *non-unique*. Illumina reads with older IDs have trailing “/1” and “/2” stripped (this information is recorded in the next field). Illumina reads with newer IDs have second block stripped (read number is recorded in the next field).

@FCC6889ACXX:5:1101:8446:45501#CGATGTATC/1 ⇒ @FCC6889ACXX:5:1101:8446:45501

@HISEQ:153:H8ED7ADXX:1:1101:1368:2069 1:N:0:ATCACG ⇒ @HISEQ:153:H8ED7ADXX:1:1101:1368:2069

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```




```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

99 (decimal) = 00001100011 (binary) (0 / NO .. 1 / YES)

… so, (from right to left): read is in a pair; the pair is proper; read *is* mapped (double neg); mate *is* mapped (double neg); read is mapped to forward strand (double neg); mate is mapped to reverse strand; read is 1st in pair … *remaining bits not used*

SAM

FLAG: still confused?

https://broadinstitute.github.io/picard/explain-flags.html

Common flags for SR (single reads): 0, 4, 16, sometimes 20 (hmm..)

Common flags for PE (paired ends): 99/147, 83/163, 77/141, 65/129, 81/161 ...

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

RNAME: reference sequence name

Reference sequence ID (from fasta header), *possibly truncated at first whitespace (still unique??)*

>chromosome 1
… *becomes* …
chromosome
… (!)

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

POS: 1-based *leftmost* position of (post-clipping) aligned read

```
      ... 4,773,680 4,773,690 4,773,700 4,773,710 ...
                  |         |         |         |
REF:...TACCCAATGGGGATGACATAAGGTGCCATCTGTGGGCTGGTGATTCCATAGTAGAC...
READ:                        GGTGCCATCTGTGGGCTGGTGATCCCATAGTAGAC...
```

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

POS: 1-based *leftmost* position of (post-clipping) aligned read

```
      ... 4,773,680 4,773,690 4,773,700 4,773,710 ...
                  |         |         |         |
REF:   ...CCAATGGGGATGACATAAGTGCCATCTGTGGGCTGGTGATCAGTAGAC...
READ:                        GTGCCATCTGTGGGCTGGTGATCAGTAGAC...
```

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
49H101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

POS: 1-based *leftmost* position of (post-clipping) aligned read

```
      ... 4,773,680 4,773,690 4,773,700 4,773,710 ...
                  |         |         |         |
REF:    ...CCAATGGGGATGACATAAGTGCCATCTGTGGGCTGGTGATCAGTAGAC...
READ:                       GCCGTGCCATCTGTGGGCTGGTGATCAGTAGAC...
```

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
3S101M
=
4773721
132
GCCGTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBBBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:GCC101(?)  YT:Z:UU XS:A:-  NH:i:1
```

SAM

MAPQ: mapping quality (phred scaled)

Mapping quality is used by some aligners, in different ways. It's generally a function of the edit distance (mismatches, indels), and the uniqueness of the alignment. Multiple equivalent best alignments yield a mapping quality of zero; alignments with an edit distance close to the best alignment lower the mapping quality.

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

CIGAR: extended CIGAR string (Compact Idiosyncratic Gapped Alignment Report)

Format: [0-9][MIDNSHP][0-9][MIDNSHP]...
M = match / mismatch (!), I/D = insertion / deletion, N = skipped bases on reference, S/H = soft / hard clip (hard clipped bases no longer appear in the sequence field), P = padding
… e.g. “101M” means that all bases in the read align to bases in the reference, starting with position (4,773,690), always in the order of the reference.

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

MRNM: reference sequence to which the *mate* of this read is aligned

"=" … mate is aligned to the same reference sequence is this read

"*" … this is a single read; no mate exists

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

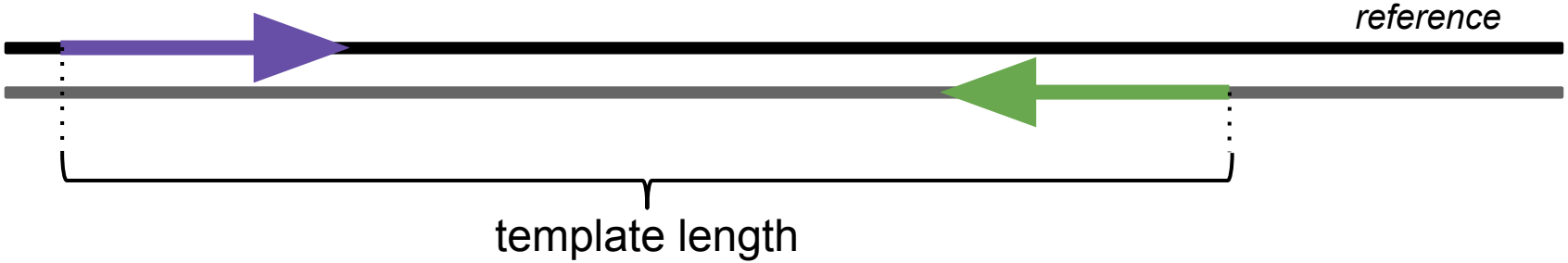
MPOS: 1-based, left-most position of 1st (post-clipping) nucleotide of mate read

"0" … no mate exists

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

TLEN: inferred insert size / template length … “0” if no mate … “-#” if second read(?)




```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM

SEQ and **QUAL:** read's nucleotides and base qualities, *always in the order of the reference (forward, top) strand!* … includes any insertions, deletions, etc. present in the read.

Reads aligned to reverse strand appear in reverse, with reversed base qualities.

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

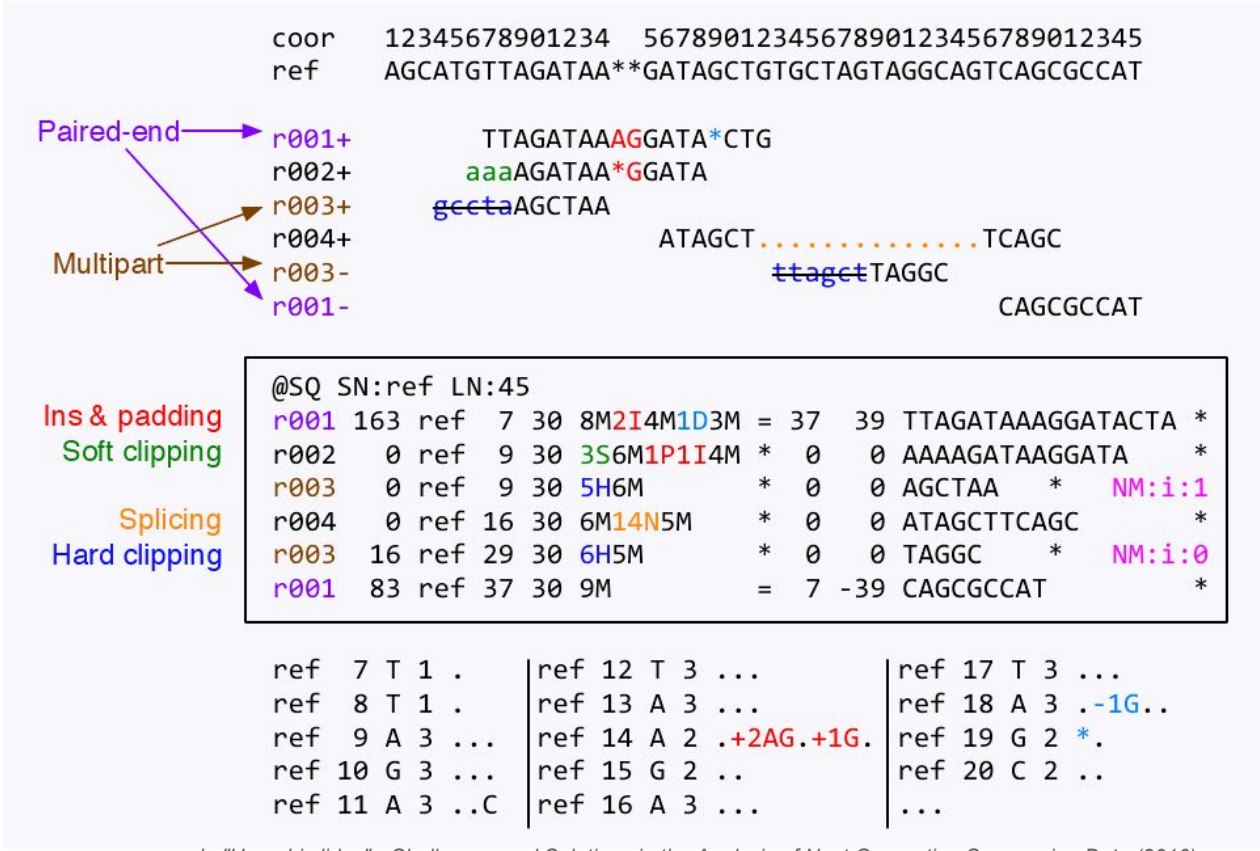
SAM

OPT: various pre-defined and user-defined tags in the format TAG:VTYPE:VALUE … VTYPE is one of [A (printable character); i (signed integer); f (floating point); z (printable string); H (hex string)].

e.g.: NM:i:0 means zero mismatches in this alignment
e.g.: XS:A:- was set by TopHat, RNA that was read was coded by the reverse strand
e.g.: NH:i:1 means that the number of hits for this read was 1 (would be more for repeat)

```
HISEQ:153:H8ED7ADXX:1:1104:8193:69947
99
chr1
4773690
50
101M
=
4773721
132
GTGCCATCTGTGGGCTGGTGATC[...]AGCAGCATGCTCCATGGTCTCTACATG
BBBFFFFFFBFFFFBFFFBFFBB[...]FFBFFBBBBBBBBBBBBBBBBBBBBB<
AS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:101  YT:Z:UU XS:A:-  NH:i:1
```

SAM - quick summary



google "Heng Li slides" - Challenges and Solutions in the Analysis of Next Generation Sequencing Data (2010)

BAM

BAMs are compressed SAMs (so, binary, not human-readable text … don't look directly at them!). They can be indexed to allow rapid extraction of information, so alignment viewers do not need to uncompress the whole BAM file in order to look at information for a particular read or coordinate range, somewhere in the file.

Indexing your BAM file, myCoolBamFile.bam, will create an index file, myCoolBamFile.bam.bai, which is needed (in addition to the BAM file) by viewers and other downstream tools. An occasional downstream tool will require an index called myCoolBamFile.bai (notice that the “.bai” replaces the “.bam”, instead of being appended after it).

CRAM

Available as of SAMtools 1.0, and is a binary format like BAM. Uses data-specific compression tools (i.e. compressing letters is different than compressing numbers), *specifically* reference-based compression (e.g. for aligned reads, only *mis-matching* bases need to be stored). Also can employ *lossy* compression of base qualities, which appears to have a negligible effect on, say, variant calling (see Illumina *white paper*).

Indexing your CRAM file, myCoolBamFile.cram, will create an index file, myCoolBamFile.cram.crai, which is needed (in addition to the CRAM file) by viewers and other downstream tools.

This is still a ***relatively recent development***, so it may be a while before many tools are CRAM-capable.

?’s …?