# Sequence Alignment/Map Optional Fields Specification

## The SAM/BAM Format Specification Working Group

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The master version of this document can be found at https://github.com/samtools/hts-specs. This printing is version 080649f from that repository, last modified on the date shown above.

This document is a companion to the Sequence Alignment/Map Format Specification that defines the SAM and BAM formats, and to the CRAM Format Specification that defines the CRAM format.<sup>1</sup> Alignment records in each of these formats may contain a number of optional fields, each labelled with a tag identifying that field's data. This document describes each of the predefined standard tags, and discusses conventions around creating new tags.

# 1 Standard tags

Predefined standard tags are listed in the following table and described in greater detail in later subsections. Optional fields are usually displayed as TAG:TYPE:VALUE; the *type* may be one of A (character), B (general array), f (real number), H (hexadecimal array), i (integer), or Z (string).

Tag	Type	Description				
AM	i	The smallest template-independent mapping quality in the template				
AS	i	Alignment score generated by aligner				
BC	${f Z}$	Barcode sequence identifying the sample				
BQ	$\mathbf{Z}$	Offset to base alignment quality (BAQ)				
BZ	${f Z}$	Phred quality of the unique molecular barcode bases in the OX tag				
CB	${f Z}$	Cell identifier				
CC	${f Z}$	Reference name of the next hit				
CG	$_{\mathrm{B,I}}$	BAM only: CIGAR in BAM's binary encoding if (and only if) it consists of >65535				
		operators				
CM	i	Edit distance between the color sequence and the color reference (see also NM)				
CO	$\mathbf{Z}$	Free-text comments				
CP	i	Leftmost coordinate of the next hit				
CQ	$\mathbf{Z}$	Color read base qualities				
CR	$\mathbf{Z}$	Cellular barcode sequence bases (uncorrected)				
CS	$\mathbf{Z}$	Color read sequence				
CT	$\mathbf{Z}$	Complete read annotation tag, used for consensus annotation dummy features				
CY	$\mathbf{Z}$	Phred quality of the cellular barcode sequence in the CR tag				
DI	$\mathbf{Z}$	Duplicate Identity, for identifying the queryname that this read is a duplicate of				
DS	i	Duplicate-set Size containing the size of the duplicate set				
DT	$\mathbf{Z}$	Duplicate type, used to identifying duplicate reads as coming from the library-				
		construction (LB) or sequencing (SQ)				
E2	$\mathbf{Z}$	The 2nd most likely base calls				
FI	i	The index of segment in the template				
FS	$\mathbf{Z}$	Segment suffix				
FZ	$_{\mathrm{B,S}}$	Flow signal intensities				

<sup>&</sup>lt;sup>1</sup>See SAMv1.pdf and CRAMv3.pdf at https://github.com/samtools/hts-specs.

Tag	Type	Description
GC	?	Reserved for backwards compatibility reasons
GQ	?	Reserved for backwards compatibility reasons
GS	?	Reserved for backwards compatibility reasons
НО	i	Number of perfect hits
H1	i	Number of 1-difference hits (see also NM)
H2	i	Number of 2-difference hits
ΗI	i	Query hit index
ΙH	i	Query hit total count
LB	$\mathbf{Z}$	Library
MC	$\mathbf{Z}$	CIGAR string for mate/next segment
MD	$\mathbf{Z}$	String encoding mismatched and deleted reference bases
MF	?	Reserved for backwards compatibility reasons
MI	$\mathbf{Z}$	Molecular identifier; a string that uniquely identifies the molecule from which the record was derived
ML	$_{\mathrm{B,C}}$	Base modification probabilities
MM	$\dot{\mathbf{Z}}$	Base modifications / methylation
MN	i	Length of sequence at the time MM and ML were produced
MQ	i	Mapping quality of the mate/next segment
NH	i	Number of reported alignments that contain the query in the current record
NM	i	Edit distance to the reference
OA	$\mathbf{Z}$	Original alignment
OC	$\mathbf{Z}$	Original CIGAR (deprecated; use OA instead)
0P	i	Original mapping position (deprecated; use OA instead)
OQ	$\mathbf{Z}$	Original base quality
OX	$\mathbf{Z}$	Original unique molecular barcode bases
PG	$\mathbf{Z}$	Program
PQ	i	Phred likelihood of the template
PT	$\mathbf{Z}$	Read annotations for parts of the padded read sequence
PU	$\mathbf{Z}$	Platform unit
Q2	$\mathbf{Z}$	Phred quality of the mate/next segment sequence in the R2 tag
QT	$\mathbf{Z}$	Phred quality of the sample barcode sequence in the BC tag
QX	$\mathbf{Z}$	Quality score of the unique molecular identifier in the RX tag
R2	$\mathbf{Z}$	Sequence of the mate/next segment in the template
RG	$\mathbf{Z}$	Read group
RT	?	Reserved for backwards compatibility reasons
RX	$\mathbf{Z}$	Sequence bases of the (possibly corrected) unique molecular identifier
S2	?	Reserved for backwards compatibility reasons
SA	$\mathbf{Z}$	Other canonical alignments in a chimeric alignment
$\mathtt{SM}$	i	Template-independent mapping quality
SQ	?	Reserved for backwards compatibility reasons
TC	i	The number of segments in the template
TS	A	Transcript strand
U2	$\mathbf{Z}$	Phred probability of the 2nd call being wrong conditional on the best being wrong
υQ	i	Phred likelihood of the segment, conditional on the mapping being correct
X?	?	Reserved for end users
Υ?	?	Reserved for end users
Z?	?	Reserved for end users

# 1.1 Additional Template and Mapping data

AM:i:score The smallest template-independent mapping quality of any segment in the same template as this read. (See also SM.)

AS:i:score Alignment score generated by aligner.

**BQ:Z:qualities** Offset to base alignment quality (BAQ), of the same length as the read sequence. At the *i*-th read base,  $BAQ_i = Q_i - (BQ_i - 64)$  where  $Q_i$  is the *i*-th base quality.

CC:Z:rname Reference name of the next hit; '=' for the same chromosome.

CG:B:I,encodedCigar Real CIGAR in its binary form if (and only if) it contains >65535 operations. This is a BAM file only tag as a workaround of BAM's incapability to store long CIGARs in the standard way. SAM and CRAM files created with updated tools aware of the workaround are not expected to contain this tag. See also the footnote in Section 4.2 of the SAM spec for details.

CP:i:pos Leftmost coordinate of the next hit.

**DI:Z:rname** (For duplicate templates) The queryname of the template that is not marked as duplicate and that this template is a duplicate of.

**DS:i:count** Size of the duplicate set that the template is part of.

**DT:Z:str** (For duplicate templates) Either LB or SQ indicating the source of the duplication. Use LB if the duplication occurred during library-construction (e.g., via PCR). Use SQ if the duplication occurred during sequencing (e.g., due to imaging error, aka "optical duplicates", or due to over-aggressive bridge-amp).

#### GL:f:score

E2:Z:bases The 2nd most likely base calls. Same encoding and same length as SEQ. See also U2 for associated quality values.

FI:i:int The index of segment in the template.

FS:Z:str Segment suffix.

**H0:i:count** Number of perfect hits.

H1:i:count Number of 1-difference hits (see also NM).

**H2:i:count** Number of 2-difference hits.

**HI:**:: i Query hit index, indicating the alignment record is the i-th one stored in SAM.

IH:i:count Number of alignments stored in the file that contain the query in the current record.

MC:Z:cigar CIGAR string for mate/next segment.

## $MD:Z:[0-9]+(([A-Z]|\^[A-Z]+)[0-9]+)*$

String encoding mismatched and deleted reference bases, used in conjunction with the CIGAR and SEQ fields to reconstruct the bases of the reference sequence interval to which the alignment has been mapped. This can enable variant calling without requiring access to the entire original reference.

The  $\mathtt{MD}$  string consists of the following items, concatenated without additional delimiter characters:

- [0-9]+, indicating a run of reference bases that are identical to the corresponding SEQ bases;
- [A-Z], identifying a single reference base that differs from the SEQ base aligned at that position;
- \^[A-Z]+, identifying a run of reference bases that have been deleted in the alignment.

As shown in the complete regular expression above, numbers alternate with the other items. Thus if two mismatches or deletions are adjacent without a run of identical bases between them, a '0' (indicating a 0-length run) must be used to separate them in the MD string.

Clipping, padding, reference skips, and insertions ('H', 'S', 'P', 'N', and 'I' CIGAR operations) are not represented in the MD string. When reconstructing the reference sequence, inserted and soft-clipped SEQ bases are omitted as determined by tracking 'I' and 'S' operations in the CIGAR string. (If the

CIGAR string contains 'N' operations, then the corresponding skipped parts of the reference sequence cannot be reconstructed.)

For example, a string '10A5^AC6' means from the leftmost reference base in the alignment, there are 10 matches followed by an A on the reference which is different from the aligned read base; the next 5 reference bases are matches followed by a 2bp deletion from the reference; the deleted sequence is AC; the last 6 bases are matches.

MQ:i:score Mapping quality of the mate/next segment.

NH:i:count Number of reported alignments that contain the query in the current record.

NM:i:count Number of differences (mismatches plus inserted and deleted bases) between the sequence and reference, counting only (case-insensitive) A, C, G and T bases in sequence and reference as potential matches, with everything else being a mismatch. Note this means that ambiguity codes in both sequence and reference that match each other, such as 'N' in both, or compatible codes such as 'A' and 'R', are still counted as mismatches. The special sequence base '=' will always be considered to be a match, even if the reference is ambiguous at that point. Alignment reference skips, padding, soft and hard clipping ('N', 'P', 'S' and 'H' CIGAR operations) do not count as mismatches, but insertions and deletions count as one mismatch per base.

Note that historically this has been ill-defined and both data and tools exist that disagree with this definition.

**PQ:i:score** Phred likelihood of the template, conditional on the mapping locations of both/all segments being correct.

Q2:Z:qualities Phred quality of the mate/next segment sequence in the R2 tag. Same encoding as QUAL.

R2:Z:bases Sequence of the mate/next segment in the template. See also Q2 for any associated quality values.

SA:Z:(rname, pos, strand, CIGAR, mapQ, NM;)+ Other canonical alignments in a chimeric alignment, formatted as a semicolon-delimited list. Each element in the list represents a part of the chimeric alignment. Conventionally, at a supplementary line, the first element points to the primary line. Strand is either '+' or '-', indicating forward/reverse strand, corresponding to FLAG bit 0x10. Pos is a 1-based coordinate.

**SM:i:score** Template-independent mapping quality, i.e., the mapping quality if the read were mapped as a single read rather than as part of a read pair or template.

**TC:i:** The number of segments in the template.

TS:A:strand Strand ('+' or '-') of the transcript to which the read has been mapped.

**U2:Z:** Phred probability of the 2nd call being wrong conditional on the best being wrong. The same encoding and length as QUAL. See also E2 for associated base calls.

**UQ:**: Phred likelihood of the segment, conditional on the mapping being correct.

## 1.2 Metadata

RG:Z:readgroup The read group to which the read belongs. If @RG headers are present, then readgroup must match the RG-ID field of one of the headers.

LB:Z:library The library from which the read has been sequenced. If @RG headers are present, then *library* must match the RG-LB field of one of the headers.

PG:Z:program\_id Program. Value matches the header PG-ID tag if @PG is present.

PU:Z:platformunit The platform unit in which the read was sequenced. If QRG headers are present, then platformunit must match the RG-PU field of one of the headers.

CO:Z:text Free-text comments.

## 1.3 Barcodes

DNA barcodes can be used to identify the provenance of the underlying reads. There are currently three varieties of barcodes that may co-exist: Sample Barcode, Cell Barcode, and Unique Molecular Identifier (UMI).

- Despite its name, the Sample Barcode identifies the Library and allows multiple libraries to be combined and sequenced together. After sequencing, the reads can be separated according to this barcode and placed in different "read groups" each corresponding to a library. Since the library was generated from a sample, knowing the library should inform of the sample. The barcode itself can be included in the PU field in the RG header line. Since the PU field should be globally unique, it is advisable to include specific information such as flowcell barcode and lane. It is not recommended to use the barcode as the ID field of the RG header line, as some tools modify this field (e.g., when merging files).
- The Cell Barcode is similar to the sample barcode but there is (normally) no control over the assignment of cells to barcodes (whose sequence could be random or predetermined). The Cell Barcode can help identify when reads come from different cells in a "single-cell" sequencing experiment.
- The *UMI* is intended to identify the (single- or double-stranded) molecule at the time that the barcode was introduced. This can be used to inform duplicate marking and make consensus calling in ultradeep sequencing. Additionally, the UMI can be used to (informatically) link reads that were generated from the same long molecule, enabling long-range phasing and better informed mapping. In some experimental setups opposite strands of the same double-stranded DNA molecule get related barcodes. These templates can also be considered duplicates even though technically they may have different UMIs. Multiple UMIs can be added by a protocol, possibly at different time-points, which means that specific knowledge of the protocol may be needed in order to analyze the resulting data correctly.
- BC:Z:sequence Barcode sequence (Identifying the sample/library), with any quality scores (optionally) stored in the QT tag. The BC tag should match the QT tag in length. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the barcodes and places a hyphen ('-') between the barcodes from the same template.
- QT:Z:qualities Phred quality of the sample barcode sequence in the BC tag. Same encoding as QUAL, i.e., Phred score + 33. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the quality strings with spaces ('\(\(\(\d\_{\sigma}'\)\)) between the different strings from the same template.
- CB:Z:str Cell identifier, consisting of the optionally-corrected cellular barcode sequence and an optional suffix. The sequence part is similar to the CR tag, but may have had sequencing errors etc corrected. This may be followed by a suffix consisting of a hyphen ('-') and one or more alphanumeric characters to form an identifier. In the case of the cellular barcode (CR) being based on multiple barcode sequences the recommended implementation concatenates all the (corrected or uncorrected) barcodes with a hyphen ('-') between the different barcodes. Sequencing errors etc aside, all reads from a single cell are expected to have the same CB tag.
- CR:Z:sequence+ Cellular barcode. The uncorrected sequence bases of the cellular barcode as reported by the sequencing machine, with the corresponding base quality scores (optionally) stored in CY. Sequencing errors etc aside, all reads with the same CR tag likely derive from the same cell. In the case of the cellular barcode being based on multiple barcode sequences the recommended implementation concatenates all the barcodes with a hyphen ('-') between the different barcodes.
- CY:Z:qualities+ Phred quality of the cellular barcode sequence in the CR tag. Same encoding as QUAL, i.e., Phred score + 33. The lengths of the CY and CR tags must match. In the case of the cellular barcode being based on multiple barcode sequences the recommended implementation concatenates all the quality strings with with spaces ('\(\_{\'}\)') between the different strings.
- MI:Z:str Molecular Identifier. A unique ID within the SAM file for the source molecule from which this read is derived. All reads with the same MI tag represent the group of reads derived from the same source molecule.

- OX:Z:sequence+ Raw (uncorrected) unique molecular identifier bases, with any quality scores (optionally) stored in the BZ tag. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the barcodes with a hyphen ('-') between the different barcodes.
- BZ:Z:qualities+ Phred quality of the (uncorrected) unique molecular identifier sequence in the OX tag. Same encoding as QUAL, i.e., Phred score + 33. The OX tags should match the BZ tag in length. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the quality strings with a space ('\(\_\'\_\'\)) between the different strings.
- RX:Z:sequence+ Sequence bases from the unique molecular identifier. These could be either corrected or uncorrected. Unlike MI, the value may be non-unique in the file. Should be comprised of a sequence of bases. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the barcodes with a hyphen ('-') between the different barcodes.
  - If the bases represent corrected bases, the original sequence can be stored in OX (similar to OQ storing the original qualities of bases.)
- QX:Z:qualities+ Phred quality of the unique molecular identifier sequence in the RX tag. Same encoding as QUAL, i.e., Phred score + 33. The qualities here may have been corrected (Raw bases and qualities can be stored in OX and BZ respectively.) The lengths of the QX and the RX tags must match. In the case of multiple unique molecular identifiers (e.g., one on each end of the template) the recommended implementation concatenates all the quality strings with a space ('\_') between the different strings.

## 1.4 Original data

OA:Z:(RNAME, POS, strand, CIGAR, MAPQ, NM;)+ The original alignment information of the record prior to realignment or unalignment by a subsequent tool. Each original alignment entry contains the following six field values from the original record, generally in their textual SAM representations, separated by commas (',') and terminated by a semicolon (';'): RNAME, which must be explicit (unlike RNEXT, '=' may not be used here); 1-based POS; '+' or '-', indicating forward/reverse strand respectively (as per bit 0x10 of FLAG); CIGAR; MAPQ; NM tag value, which may be omitted (though the preceding comma must be retained).

In the presence of an existing OA tag, a subsequent tool may append another original alignment entry after the semicolon, adding to—rather than replacing—the existing OA information.

The OA field is designed to provide record-level information that can be useful for understanding the provenance of the information in a record. It is not designed to provide a complete history of the template alignment information. In particular, realignments resulting in the the removal of Secondary or Supplementary records will cause the loss of all tags associated with those records, and may also leave the SA tag in an invalid state.

OC:Z:cigar Original CIGAR, usually before realignment. Deprecated in favour of the more general OA.

OP:i:pos Original 1-based POS, usually before realignment. Deprecated in favour of the more general OA.

OQ:Z:qualities Original base quality, usually before recalibration. Same encoding as QUAL.

## 1.5 Annotation and Padding

The SAM format can be used to represent *de novo* assemblies, generally by using padded reference sequences and the annotation tags described here. See the *Guide for Describing Assembly Sequences* in the *SAM Format Specification* for full details of this representation.

**CT:Z:***strand*; *type*(; *key*(=*value*)?)\*

Complete read annotation tag, used for consensus annotation dummy features.

The CT tag is intended primarily for annotation dummy reads, and consists of a *strand*, *type* and zero or more *key=value* pairs, each separated with semicolons. The *strand* field has four values as in GFF3,<sup>2</sup> and supplements FLAG bit 0x10 to allow unstranded ('.'), and stranded but unknown strand ('?') annotation. For these and annotation on the forward strand (*strand* set to '+'), do not set FLAG bit 0x10. For annotation on the reverse strand, set the *strand* to '-' and set FLAG bit 0x10.

The *type* and any *keys* and their optional *values* are all percent encoded according to RFC3986 to escape meta-characters '=', '%', ';', '|' or non-printable characters not matched by the isprint() macro (with the C locale). For example a percent sign becomes '%25'.

PT:Z: annotag(\| annotag)\* where each annotag matches start; end; strand; type(; key(=value)?)\* Read annotations for parts of the padded read sequence.

The PT tag value has the format of a series of annotation tags separated by 'l', each annotating a sub-region of the read. Each tag consists of *start*, *end*, *strand*, *type* and zero or more *key=value* pairs, each separated with semicolons. *Start* and *end* are 1-based positions between one and the sum of the M/I/D/P/S/=/X CIGAR operators, i.e., SEQ length plus any pads. Note any editing of the CIGAR string may require updating the PT tag coordinates, or even invalidate them. As in GFF3, *strand* is one of '+' for forward strand tags, '-' for reverse strand, '.' for unstranded or '?' for stranded but unknown strand.

The type and any keys and their optional values are all percent encoded as in the CT tag.

## 1.6 Technology-specific data

FZ:B:S,intensities Flow signal intensities on the original strand of the read, stored as (uint16\_t) round(value \* 100.0).

#### 1.6.1 Color space

CM:i:distance Edit distance between the color sequence and the color reference (see also NM).

CS:Z:sequence Color read sequence on the original strand of the read. The primer base must be included.

CQ:Z:qualities Color read quality on the original strand of the read. Same encoding as QUAL; same length as CS.

## 1.7 Base modifications

Base modifications, including base methylation, are represented as a series of edits from the primary unmodified sequence as originally reported by the sequencing instrument. This potentially differs to the sequence stored in the main SAM SEQ field if the latter has been reverse complemented, in which case SAM FLAG 0x10 must be set. This means modification positions are also recorded against the original orientation (i.e. starting at the 5' end), and count the original base types.

Each modified base prediction listed also has a quality value associated with it. Given the unmodified base already has a phred likelihood, this base modification quality should be interpreted as the likelihood of this modification being correct given an assumption the original call is correct.

## MM:Z:([ACGTUN][-+]([a-z]+|[0-9]+)[.?]?(,[0-9]+)\*;)\*

The first character is the unmodified "fundamental" base as reported by the sequencing instrument for the top strand. It must be one of 'A', 'C', 'G', 'T', 'U' (if RNA) or 'N' for anything else, including any IUPAC ambiguity codes in the reported SEQ field. Note 'N' may be used to match any base rather than specifically an 'N' call by the sequencing instrument. This may be used in situations where the base modification is not a derivation of a standard base type. This is followed by either plus or minus indicating the strand the modification was observed on (relative to the original sequenced strand of SEQ with plus meaning same orientation),<sup>3</sup> and one or more base modification codes.

<sup>&</sup>lt;sup>2</sup>The Generic Feature Format version 3 (GFF3) specification can be found at http://sequenceontology.org.

<sup>&</sup>lt;sup>3</sup>Hence a tool that may reverse complement sequences does not need to understand how to manipulate the MM and ML tags.

Following the base modification codes is a recommended but optional '.' or '?' describing how skipped seq bases of the stated base type should be interpreted by downstream tools. When this flag is '?' there is no information about the modification status of the skipped bases provided. When this flag is not present, or it is '.', these bases should be assumed to have low probability of modification.<sup>4</sup>

This is then followed by a comma separated list of how many seq bases of the stated base type to skip, stored as a delta to the last and starting with 0 as the first (or next) base, starting from the uncomplemented 5' end of the SEQ field. This number series is comparable to the numbers in an MD tag, albeit counting specific base types only and potentially reverse-complemented.

For example 'C+m,5,12,0;' tells us there are three potential 5-Methylcytosine bases on the top strand of SEQ. The first 5 'C' bases are unmodified and the 6th, 19th and 20th have modification status indicated by the corresponding probabilities in the ML tag. The 12 cytosines between the 6th and 19th cytosine are unmodified. Modification probabilities for the 17 skipped cytosines are not provided.

When the '?' flag is present the tag 'C+m?,5,12,0;' tells us the modification status of the first five cytosine bases is unknown, the sixth cytosine is called (as either modified or unmodified), followed by 12 more unknown cytosines, and the 19th and 20th are called.

Similarly 'G-m,14;' indicates the 15th 'G' there might be a 5-Methylcytosine on the opposite strand (still counting using the top strand base calls from the 5' end). When the alignment record is reverse complemented (SAM flag 0x10) these two examples do not change since the tag always refers to the as-sequenced orientation. See the test/SAMtags/MM-orient.sam file for examples.

This permits modifications to be listed on either strand with the rare potential for both strands to have a modification at the same site. If SAM FLAG 0x10 is set, indicating that SEQ has been reverse complemented from the sequence observed by the sequencing machine, note that these base modification field values will be in the opposite orientation to SEQ and other derived SAM fields.

Note it is permitted for the coordinate list to be empty (for example 'MM:Z:C+m;'), which may be used as an explicit indicator that this base modification is not present. It is not permitted for coordinates to be beyond the length of the sequence.

When multiple modifications are listed, for example 'C+mh,5,12,0;', it indicates the modification may be any of the stated bases. The associated confidence values in the ML tag may be used to determine the relative likelihoods between the options. The example above is equivalent to 'C+m,5,12,0;C+h,5,12,0;', although this will have a different ordering of confidence values in ML. Note ChEBI codes cannot be used in the multi-modification form (such as the 'C+mh' example above).

If the modification is not one of the standard common types (listed below) it can be specified as a numeric ChEBI code. For example 'C+76792,57;' is the same as 'C+h,57;'.

An unmodified base of 'N' means count any base in SEQ, not only those of 'N'. Thus 'N+n,100;' means the 101st base is Xanthosine (n), irrespective of the sequence composition.

The standard code types and their associated ChEBI values are listed below, taken from Viner *et al.*<sup>5</sup> Additionally ambiguity codes 'A', 'C', 'G', 'T' and 'U' exist to represent unspecified modifications bases of their respective canonical base types, plus code 'N' to represent an unspecified modification of any base type.

<sup>&</sup>lt;sup>4</sup>The decision whether a base is assumed to be unmodified or has a probability explicitly provided is up to the modification calling program. Some programs will elide calls with modification probabilities below a threshold to provide a more compact modification tag.

<sup>&</sup>lt;sup>5</sup>Coby Viner et al., Modeling methyl-sensitive transcription factor motifs with an expanded epigenetic alphabet, https://www.biorxiv.org/content/10.1101/043794v1.

Unmodified base	$\mathbf{Code}$	Abbreviation	Name	ChEBI
С	m	5mC	5-Methylcytosine	27551
С	h	$5 \mathrm{hmC}$	5-Hydroxymethylcytosine	76792
С	f	5fC	5-Formylcytosine	76794
С	$\mathbf{c}$	5ca $C$	5-Carboxylcytosine	76793
С	$\mathbf{C}$		Ambiguity code; any C mod	
T	g	5hmU	5-Hydroxymethyluracil	16964
T	e	$5 \mathrm{fU}$	5-Formyluracil	80961
${ m T}$	b	5 caU	5-Carboxyluracil	17477
${ m T}$	${ m T}$		Ambiguity code; any T mod	
U	U		Ambiguity code; any U mod	
A	a	$6 \mathrm{mA}$	6-Methyladenine	28871
A	A		Ambiguity code; any A mod	
G	О	8oxoG	8-Oxoguanine	44605
G	G		Ambiguity code; any G mod	
N	n	Xao	Xanthosine	18107
N	N		Ambiguity code; any mod	

## ML:B:C,scaled-probabilities

The optional ML tag lists the probability of each modification listed in the MM tag being correct, in the order that they occur. The continuous probability range 0.0 to 1.0 is remapped in equal sized portions to the discrete integers 0 to 255 inclusively. Thus the probability range corresponding to integer value N is N/256 to (N+1)/256.

The SAM encoding therefore uses a byte array of type 'C' with the number of elements matching the summation of the number of modifications listed as being present in the MM tag accounting for multi-modifications each having their own probability.

For example 'MM:Z:C+m,5,12;C+h,5,12;' may have an associated tag of 'ML:B:C,204,89,26,130'.

If the above is rewritten in the multiple-modification form, the probabilities are interleaved in the order presented, giving 'MM:Z:C+mh,5,12; ML:B:C,204,26,89,130'. Note where several possible modifications are presented at the same site, the ML values represent the absolute probabilities of the modification call being correct and not the relative likelihood between the alternatives. These probabilities should not sum to above 1.0 ( $\approx$  256 in integer encoding, allowing for some minor rounding errors), but may sum to a lower total with the remainder representing the probability that none of the listed modification types are present. In the example used above, the 6th C has 80% chance of being 5mC, 10% chance of being 5hmC and 10% chance of being an unmodified C.

ML values for ambiguity codes give the probability that the modification is one of the possible codes compatible with that ambiguity code. For example MM:Z:C+C,10; ML:B:C,229 indicates a C call with a probability of 90% of having some form of unspecified modification.

## MN:i:length

The length of the SEQ field at the time the MM value was last written.

Some processing of aligned data, such as the use of hard-clipping tools, may alter SEQ sequence data. If the sequence is shortened in this manner then the base offsets in MM and ML become invalid unless they are also updated accordingly.

Some hard-clipping tools will update MM/ML but others do not, so the MN tag offers a simple sanity check. Software that wishes to validate MM should compare the length of the SEQ field with the contents of the MN tag—if they differ, the MM and ML values should be considered out-of-date. The tag is optional, but recommended, and if it is absent then there is an implicit assumption that the MM data is valid unless evidence implies otherwise (e.g., by having coordinates beyond the end of the sequence).

# 2 Draft tags

These are tags which have been proposed and are broadly accepted to become standard tags, but a review or probationary period has been deemed useful. They use the locally-defined tag namespace and processing software should consider that the tags may have local usage for other purposes.

There are currently no tags with draft status.

# 3 Locally-defined tags

You can freely add new tags. Note that tags starting with 'X', 'Y', or 'Z' and tags containing lowercase letters in either position are reserved for local use and will not be formally defined in any future version of this specification.

If a new tag may be of general interest, it may be useful to have it added to this specification. Additions can be proposed by opening a new issue at https://github.com/samtools/hts-specs/issues and/or by sending email to samtools-devel@lists.sourceforge.net.

# Appendix A Tag History

This appendix lists when standard tags were initially defined or significantly changed, and other historical events that affect how tags are interpreted or what files they may appear in.

#### September 2024

Added the MN tag for validating base modification tag consistency.

#### February 2022

Base modification tags changed to use the predefined standard names MM and ML, as their review period has finished. Programs outputting the draft Mm and Ml tags should be changed to use MM and ML instead.

#### December 2021

Amended draft Mm tag to provide hints about the modification status of skipped sequence bases.

## July 2021

Added the Mm and Ml draft tags describing base modifications.

#### March 2020

Transcript strand tag TS added, equivalent to the locally-defined XS tag produced by several RNA aligners.

## January 2019

Added the OA tag for recording original/previous alignment information. Deprecated the OC and OP tags.

### July 2018

Clarified the calculation of NM score.

## May 2018

Cellular barcode tags CB, CR, and CY added.

Removed the RT:Z tag, which was a long-deprecated synonym for BC.

#### November 2017

SAM version number VN:1.6 introduced, indicating the addition of the CG tag representation of very long CIGAR strings. Files that contain records with more than 65,535 CIGAR operators should not declare a version number lower than 1.6 in their @HD headers.

#### August 2017

Unique molecular identifier tags BZ, MI, OX, QX, and RX added. Usage of sample barcode tag BC clarified.

#### June 2017

Corrected the description of the E2 (second-most-likely bases) tag, which was previously unclear as to whether it contains bases or base qualities.

## September 2016

Predefined tags, previously listed as a brief table within the main SAM specification, have been split out into this new document. There is now space for clearer and more complete tag descriptions.

## February 2014

MC tag added.

## May 2013

SAM version number VN:1.5 introduced, with limited impact for tags other than indicating that the CT/PT annotation tag definitions are considered finalised.

SA tag added.

#### March 2012

Descriptions of CT and PT annotation tags significantly clarified.

#### October 2011

Sample barcode tags QT and RT added, with RT being identified as a deprecated alternative to BC. Read annotation tags CT and PT added.

## September 2011

FZ tag's type changed from H to B,S-array. BC and CO tags added.

## April 2011

SAM version number VN:1.4 introduced, indicating the addition of the B-array tag type. Files that contain records with B-array fields should not declare a version number lower than 1.4 in their @HD headers.

FZ tag added, with type H.

MD tag description changed to allow IUPAC ambiguity codes in addition to ACGTN.

#### March 2011

CC and CP tags reinstated with their original meanings.

## November 2010

BQ tag added.

## July 2010

The specification was rewritten as a LATEX document specifying SAM version number VN:1.3.

Tags FI, FS, OC, OP, OQ, and TC added.

Tags GC:Z, GQ:Z, and GS:Z, briefly proposed for representing repeatedly-sequenced reads, noted as reserved for backwards compatibility. Existing tags MF:i (MAQ pair flag), SQ:H (suboptimal bases), and S2:H (mate's suboptimal bases) removed and noted as reserved for backwards compatibility. CC and CP tags temporarily removed.

## July 2009

The original SAM "0.1.2-draft" specification specified version number VN:1.0 and defined a total of thirty standard tags (though SQ and S2 were already deprecated in favour of E2 and U2):

AM	CM	CS	H1	IH	MF	NM	PU	RG	$_{ m SQ}$
AS	CP	E2	H2	LB	MQ	PG	Q2	S2	U2
CC	CO	H0	HI	MD	NH	PΩ	R2	$_{\mathrm{SM}}$	UQ