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# **pysam documentation**

***Release 0.21.0***

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Pysam is a python module for reading, manipulating and writing genomic data sets.

Pysam is a wrapper of the [htslib](#) C-API and provides facilities to read and write SAM/BAM/VCF/BCF/BED/GFF/GTF/FASTA/FASTQ files as well as access to the command line functionality of the [samtools](#) and [bcftools](#) packages. The module supports compression and random access through indexing.

This module provides a low-level wrapper around the [htslib](#) C-API as using cython and a high-level, pythonic API for convenient access to the data within genomic file formats.

The current version wraps *htslib-1.17*, *samtools-1.17*, and *bcftools-1.17*.

To install the latest release, type:

```
pip install pysam
```

See the [Installation notes](#) for details.



## 1.1 Introduction

Pysam is a python module that makes it easy to read and manipulate mapped short read sequence data stored in SAM/BAM files. It is a lightweight wrapper of the [htslib](#) C-API.

This page provides a quick introduction in using pysam followed by the API. See [Working with BAM/CRAM/SAM-formatted files](#) for more detailed usage instructions.

To use the module to read a file in BAM format, create a *AlignmentFile* object:

```
import pysam
samfile = pysam.AlignmentFile("ex1.bam", "rb")
```

Once a file is opened you can iterate over all of the read mapping to a specified region using *fetch()*. Each iteration returns a *AlignedSegment* object which represents a single read along with its fields and optional tags:

```
for read in samfile.fetch('chr1', 100, 120):
    print(read)

samfile.close()
```

To give:

```
EAS56_57:6:190:289:82      0      99      <<<7<<<;<<<<<<<8;;<7;4<;<;;;;;94<;
→69      CTCAAGGTTGTTGCAAGGGGGTCTATGTGAACAAA      0      192      1
EAS56_57:6:190:289:82      0      99      <<<<<<;<<<<<<<<<;<<;<<<<;8<6;9;;2;
→137     AGGGGTGCAGAGCCGAGTCACGGGGTTGCCAGCAC      73      64      1
EAS51_64:3:190:727:308      0      102     <<<<<<<<<<<<<<<<<<<<<<<<<::<<<844
→99      GGTGCAGAGCCGAGTCACGGGGTTGCCAGCACAGG      99      18      1
...
```

You can also write to a *AlignmentFile*:

```
import pysam
samfile = pysam.AlignmentFile("ex1.bam", "rb")
pairedreads = pysam.AlignmentFile("allpaired.bam", "wb", template=samfile)
for read in samfile.fetch():
    if read.is_paired:
        pairedreads.write(read)

pairedreads.close()
samfile.close()
```

An alternative way of accessing the data in a SAM file is by iterating over each base of a specified region using the `pileup()` method. Each iteration returns a `PileupColumn` which represents all the reads in the SAM file that map to a single base in the reference sequence. The list of reads are represented as `PileupRead` objects in the `PileupColumn.pileups` property:

```
import pysam
samfile = pysam.AlignmentFile("ex1.bam", "rb" )
for pileupcolumn in samfile.pileup("chr1", 100, 120):
    print("\ncoverage at base %s = %s" % (pileupcolumn.pos, pileupcolumn.n))
    for pileupread in pileupcolumn.pileups:
        if not pileupread.is_del and not pileupread.is_refskip:
            # query position is None if is_del or is_refskip is set.
            print('\tbase in read %s = %s' %
                  (pileupread.alignment.query_name,
                   pileupread.alignment.query_sequence[pileupread.query_position]))

samfile.close()
```

The above code outputs:

```
coverage at base 99 = 1
    base in read EAS56_57:6:190:289:82 = A

coverage at base 100 = 1
    base in read EAS56_57:6:190:289:82 = G

coverage at base 101 = 1
    base in read EAS56_57:6:190:289:82 = G

coverage at base 102 = 2
    base in read EAS56_57:6:190:289:82 = G
    base in read EAS51_64:3:190:727:308 = G
...
```

Commands available in `samtools` are available as simple function calls. For example:

```
pysam.sort("-o", "output.bam", "ex1.bam")
```

corresponds to the command line:

```
samtools sort -o output.bam ex1.bam
```

Analogous to `AlignmentFile`, a `TabixFile` allows fast random access to compressed and tabix indexed tab-separated file formats with genomic data:

```
import pysam
tabixfile = pysam.TabixFile("example.gtf.gz")
```

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```
for gtf in tabixfile.fetch("chr1", 1000, 2000):
    print(gtf.contig, gtf.start, gtf.end, gtf.gene_id)
```

*TabixFile* implements lazy parsing in order to iterate over large tables efficiently.

More detailed usage instructions is at *Working with BAM/CRAM/SAM-formatted files*.

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**Note:** Coordinates in pysam are always 0-based (following the python convention). SAM text files use 1-based coordinates.

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**Note:**

The above examples can be run in the **tests** directory of the installation directory. Type ‘make’ before running them.

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**See also:**

<https://github.com/pysam-developers/pysam>

The pysam code repository, containing source code and download instructions

<http://pysam.readthedocs.org/en/latest/>

The pysam website containing documentation

## 1.2 API

### 1.2.1 SAM/BAM/CRAM files

Objects of type *AlignmentFile* allow working with BAM/SAM formatted files.

**class** pysam.**AlignmentFile**

AlignmentFile(filepath\_or\_object, mode=None, template=None, reference\_names=None, reference\_lengths=None, text=NULL, header=None, add\_sq\_text=False, check\_header=True, check\_sq=True, reference\_filename=None, filename=None, index\_filename=None, filepath\_index=None, require\_index=False, duplicate\_filehandle=True, ignore\_truncation=False, threads=1)

A *SAM/BAM/CRAM* formatted file.

If *filepath\_or\_object* is a string, the file is automatically opened. If *filepath\_or\_object* is a python File object, the already opened file will be used.

If the file is opened for reading and an index exists (if file is BAM, a .bai file or if CRAM a .crai file), it will be opened automatically. *index\_filename* may be specified explicitly. If the index is not named in the standard manner, not located in the same directory as the BAM/CRAM file, or is remote. Without an index, random access via *fetch()* and *pileup()* is disabled.

For writing, the header of a *SAM* file/*BAM* file can be constituted from several sources (see also the samtools format specification):

1. If *template* is given, the header is copied from another *AlignmentFile* (*template* must be a *AlignmentFile*).
2. If *header* is given, the header is built from a multi-level dictionary.

3. If *text* is given, new header text is copied from raw text.
4. The names (*reference\_names*) and lengths (*reference\_lengths*) are supplied directly as lists.

When reading or writing a CRAM file, the filename of a FASTA-formatted reference can be specified with *reference\_filename*.

By default, if a file is opened in mode 'r', it is checked for a valid header (*check\_header* = True) and a definition of chromosome names (*check\_sq* = True).

#### Parameters

- **mode** (*string*) – *mode* should be *r* for reading or *w* for writing. The default is text mode (*SAM*). For binary (*BAM*) I/O you should append *b* for compressed or *u* for uncompressed *BAM* output. Use *h* to output header information in text (*TAM*) mode. Use *c* for *CRAM* formatted files.

If *b* is present, it must immediately follow *r* or *w*. Valid modes are *r*, *w*, *wh*, *rb*, *wb*, *wbu*, *wb0*, *rc* and *wc*. For instance, to open a *BAM* formatted file for reading, type:

```
f = pysam.AlignmentFile('ex1.bam', 'rb')
```

If *mode* is not specified, the method will try to auto-detect in the order 'rb', 'r', thus both the following should work:

```
f1 = pysam.AlignmentFile('ex1.bam')
f2 = pysam.AlignmentFile('ex1.sam')
```

- **template** (*AlignmentFile*) – when writing, copy header from file *template*.
- **header** (*dict* or *AlignmentHeader*) – when writing, build header from a multi-level dictionary. The first level are the four types ('HD', 'SQ', ...). The second level are a list of lines, with each line being a list of tag-value pairs. The header is constructed first from all the defined fields, followed by user tags in alphabetical order. Alternatively, an *AlignmentHeader* object can be passed directly.
- **text** (*string*) – when writing, use the string provided as the header
- **reference\_names** (*list*) – see *reference\_lengths*
- **reference\_lengths** (*list*) – when writing or opening a SAM file without header build header from list of chromosome names and lengths. By default, 'SQ' and 'LN' tags will be added to the header text. This option can be changed by unsetting the flag *add\_sq\_text*.
- **add\_sq\_text** (*bool*) – do not add 'SQ' and 'LN' tags to header. This option permits construction *SAM* formatted files without a header.
- **add\_sam\_header** (*bool*) – when outputting SAM the default is to output a header. This is equivalent to opening the file in 'wh' mode. If this option is set to False, no header will be output. To read such a file, set *check\_header=False*.
- **check\_header** (*bool*) – obsolete: when reading a SAM file, check if header is present (default=True)
- **check\_sq** (*bool*) – when reading, check if SQ entries are present in header (default=True)
- **reference\_filename** (*string*) – Path to a FASTA-formatted reference file. Valid only for CRAM files. When reading a CRAM file, this overrides both \$REF\_PATH and the URL specified in the header (UR tag), which are normally used to find the reference.

- **index\_filename** (*string*) – Explicit path to the index file. Only needed if the index is not named in the standard manner, not located in the same directory as the BAM/CRAM file, or is remote. An `IOError` is raised if the index cannot be found or is invalid.
- **filepath\_index** (*string*) – Alias for *index\_filename*.
- **require\_index** (*bool*) – When reading, require that an index file is present and is valid or raise an `IOError`. (default=False)
- **filename** (*string*) – Alternative to *filepath\_or\_object*. Filename of the file to be opened.
- **duplicate\_filehandle** (*bool*) – By default, file handles passed either directly or through File-like objects will be duplicated before passing them to `htslib`. The duplication prevents issues where the same stream will be closed by `htslib` and through destruction of the high-level python object. Set to `False` to turn off duplication.
- **ignore\_truncation** (*bool*) – Issue a warning, instead of raising an error if the current file appears to be truncated due to a missing EOF marker. Only applies to bgzipped formats. (Default=False)
- **format\_options** (*list*) – A list of key=value strings, as accepted by `-input-fmt-option` and `-output-fmt-option` in `samtools`.
- **threads** (*integer*) – Number of threads to use for compressing/decompressing BAM/CRAM files. Setting threads to `> 1` cannot be combined with *ignore\_truncation*. (Default=1)

**check\_index** (*self*)  
return True if index is present.

#### Raises

- `AttributeError` – if `htsfile` is *SAM* formatted and thus has no index.
- `ValueError` – if `htsfile` is closed or index could not be opened.

**close** (*self*)  
closes the *pysam.AlignmentFile*.

**count** (*self*, *contig=None*, *start=None*, *stop=None*, *region=None*, *until\_eof=False*, *read\_callback=u'nofilter'*, *reference=None*, *end=None*)  
count the number of reads in *region*

The region is specified by *contig*, *start* and *stop*. *reference* and *end* are also accepted for backward compatibility as synonyms for *contig* and *stop*, respectively. Alternatively, a `samtools region` string can be supplied.

A *SAM* file does not allow random access and if *region* or *contig* are given, an exception is raised.

#### Parameters

- **contig** (*string*) – reference\_name of the genomic region (chromosome)
- **start** (*int*) – start of the genomic region (0-based inclusive)
- **stop** (*int*) – end of the genomic region (0-based exclusive)
- **region** (*string*) – a region string in `samtools` format.
- **until\_eof** (*bool*) – count until the end of the file, possibly including unmapped reads as well.
- **read\_callback** (*string or function*) – select a call-back to ignore reads when counting. It can be either a string with the following values:

**all** skip reads in which any of the following flags are set: BAM\_FUNMAP, BAM\_FSECONDARY, BAM\_FQCFAIL, BAM\_FDUP

**nofilter** uses every single read

Alternatively, *read\_callback* can be a function `check_read(read)` that should return True only for those reads that shall be included in the counting.

- **reference** (*string*) – backward compatible synonym for *contig*
- **end** (*int*) – backward compatible synonym for *stop*

**Raises** `ValueError` – if the genomic coordinates are out of range or invalid.

**count\_coverage** (*self*, *contig*, *start=None*, *stop=None*, *region=None*, *quality\_threshold=15*, *read\_callback=u'all'*, *reference=None*, *end=None*)  
count the coverage of genomic positions by reads in *region*.

The region is specified by *contig*, *start* and *stop*. *reference* and *end* are also accepted for backward compatibility as synonyms for *contig* and *stop*, respectively. Alternatively, a *samtools region* string can be supplied. The coverage is computed per-base [ACGT].

#### Parameters

- **contig** (*string*) – reference\_name of the genomic region (chromosome)
- **start** (*int*) – start of the genomic region (0-based inclusive). If not given, count from the start of the chromosome.
- **stop** (*int*) – end of the genomic region (0-based exclusive). If not given, count to the end of the chromosome.
- **region** (*string*) – a region string.
- **quality\_threshold** (*int*) – quality\_threshold is the minimum quality score (in phred) a base has to reach to be counted.
- **read\_callback** (*string or function*) – select a call-back to ignore reads when counting. It can be either a string with the following values:

**all** skip reads in which any of the following flags are set: BAM\_FUNMAP, BAM\_FSECONDARY, BAM\_FQCFAIL, BAM\_FDUP

**nofilter** uses every single read

Alternatively, *read\_callback* can be a function `check_read(read)` that should return True only for those reads that shall be included in the counting.

- **reference** (*string*) – backward compatible synonym for *contig*
- **end** (*int*) – backward compatible synonym for *stop*

**Raises** `ValueError` – if the genomic coordinates are out of range or invalid.

**Returns** four array.arrays of the same length in order A C G T

**Return type** `tuple`

**fetch** (*self*, *contig=None*, *start=None*, *stop=None*, *region=None*, *tid=None*, *until\_eof=False*, *multiple\_iterators=False*, *reference=None*, *end=None*)  
fetch reads aligned in a *region*.

See `parse_region()` for more information on how genomic regions can be specified. *reference* and *end* are also accepted for backward compatibility as synonyms for *contig* and *stop*, respectively.

Without a *contig* or *region* all mapped reads in the file will be fetched. The reads will be returned ordered by reference sequence, which will not necessarily be the order within the file. This mode of iteration still requires an index. If there is no index, use `until_eof=True`.

If only *contig* is set, all reads aligned to *contig* will be fetched.

A *SAM* file does not allow random access. If *region* or *contig* are given, an exception is raised.

#### Parameters

- **`until_eof`** (*bool*) – If *until\_eof* is True, all reads from the current file position will be returned in order as they are within the file. Using this option will also fetch unmapped reads.
- **`multiple_iterators`** (*bool*) – If *multiple\_iterators* is True, multiple iterators on the same file can be used at the same time. The iterator returned will receive its own copy of a filehandle to the file effectively re-opening the file. Re-opening a file creates some overhead, so beware.

**Returns** An iterator over a collection of reads.

**Return type** `IteratorRow`

**Raises** `ValueError` – if the genomic coordinates are out of range or invalid or the file does not permit random access to genomic coordinates.

**`find_introns`** (*self*, *read\_iterator*)

Return a dictionary {(start, stop): count} Listing the intronic sites in the reads (identified by ‘N’ in the cigar strings), and their support (= number of reads).

*read\_iterator* can be the result of a `.fetch(...)` call. Or it can be a generator filtering such reads. Example `samfile.find_introns((read for read in samfile.fetch(... ) if read.is_reverse)`

**`find_introns_slow`** (*self*, *read\_iterator*)

Return a dictionary {(start, stop): count} Listing the intronic sites in the reads (identified by ‘N’ in the cigar strings), and their support (= number of reads).

*read\_iterator* can be the result of a `.fetch(...)` call. Or it can be a generator filtering such reads. Example `samfile.find_introns((read for read in samfile.fetch(... ) if read.is_reverse)`

**`get_index_statistics`** (*self*)

return statistics about mapped/unmapped reads per chromosome as they are stored in the index, similarly to the statistics printed by the `samtools idxstats` command.

CRAI indexes do not record these statistics, so for a CRAM file with a `.crai` index the returned statistics will all be 0.

**Returns** a list of records for each chromosome. Each record has the attributes ‘contig’, ‘mapped’, ‘unmapped’ and ‘total’.

**Return type** `list`

**`get_reference_length`** (*self*, *reference*)

return *reference* length corresponding to numerical *tid*

**`get_reference_name`** (*self*, *tid*)

return *reference* name corresponding to numerical *tid*

**`get_tid`** (*self*, *reference*)

return the numerical *tid* corresponding to *reference*

returns -1 if reference is not known.

**getrname** (*self*, *tid*)  
deprecated, use `get_reference_name()` instead

**gettid** (*self*, *reference*)  
deprecated, use `get_tid()` instead

**has\_index** (*self*)  
return true if htsfile has an existing (and opened) index.

**head** (*self*, *n*, *multiple\_iterators=True*)  
return an iterator over the first *n* alignments.

This iterator is useful for inspecting the bam-file.

**Parameters** **multiple\_iterators** (*bool*) – is set to True by default in order to avoid changing the current file position.

**Returns** an iterator over a collection of reads

**Return type** IteratorRowHead

**is\_valid\_tid** (*self*, *int tid*)  
return True if the numerical *tid* is valid; False otherwise.

Note that the unmapped tid code (-1) counts as an invalid.

**lengths**  
tuple of the lengths of the *reference* sequences. This is a read-only attribute. The lengths are in the same order as `pysam.AlignmentFile.references`

**mapped**  
int with total number of mapped alignments according to the statistics recorded in the index. This is a read-only attribute. (This will be 0 for a CRAM file indexed by a .crai index, as that index format does not record these statistics.)

**mate** (*self*, *AlignedSegment read*)  
return the mate of `pysam.AlignedSegment read`.

---

**Note:** Calling this method will change the file position. This might interfere with any iterators that have not re-opened the file.

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**Note:** This method is too slow for high-throughput processing. If a read needs to be processed with its mate, work from a read name sorted file or, better, cache reads.

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**Returns** the mate

**Return type** *AlignedSegment*

**Raises** `ValueError` – if the read is unpaired or the mate is unmapped

**nocoordinate**  
int with total number of reads without coordinates according to the statistics recorded in the index, i.e., the statistic printed for “\*” by the `samtools idxstats` command. This is a read-only attribute. (This will be 0 for a CRAM file indexed by a .crai index, as that index format does not record these statistics.)

**references**  
int with the number of *reference* sequences in the file. This is a read-only attribute.

**pileup** (*self*, *contig=None*, *start=None*, *stop=None*, *region=None*, *reference=None*, *end=None*, *\*\*kwargs*)

perform a *pileup* within a *region*. The region is specified by *contig*, *start* and *stop* (using 0-based indexing). *reference* and *end* are also accepted for backward compatibility as synonyms for *contig* and *stop*, respectively. Alternatively, a samtools 'region' string can be supplied.

Without 'contig' or 'region' all reads will be used for the pileup. The reads will be returned ordered by *contig* sequence, which will not necessarily be the order within the file.

Note that *SAM* formatted files do not allow random access. In these files, if a 'region' or 'contig' are given an exception is raised.

---

**Note:** 'all' reads which overlap the region are returned. The first base returned will be the first base of the first read 'not' necessarily the first base of the region used in the query.

---

### Parameters

- **truncate** (*bool*) – By default, the samtools pileup engine outputs all reads overlapping a region. If truncate is True and a region is given, only columns in the exact region specified are returned.
- **max\_depth** (*int*) – Maximum read depth permitted. The default limit is '8000'.
- **stepper** (*string*) – The stepper controls how the iterator advances. Possible options for the stepper are
  - all** skip reads in which any of the following flags are set: BAM\_FUNMAP, BAM\_FSECONDARY, BAM\_FQCFAIL, BAM\_FDUP
  - nofilter** uses every single read turning off any filtering.
  - samtools** same filter and read processing as in samtools pileup. For full compatibility, this requires a 'fastafile' to be given. The following options all pertain to filtering of the samtools stepper.
- **fastafile** (*FastaFile* object.) – This is required for some of the steppers.
- **ignore\_overlaps** (*bool*) – If set to True, detect if read pairs overlap and only take the higher quality base. This is the default.
- **flag\_filter** (*int*) – ignore reads where any of the bits in the flag are set. The default is BAM\_FUNMAP | BAM\_FSECONDARY | BAM\_FQCFAIL | BAM\_FDUP.
- **flag\_require** (*int*) – only use reads where certain flags are set. The default is 0.
- **ignore\_orphans** (*bool*) – ignore orphans (paired reads that are not in a proper pair). The default is to ignore orphans.
- **min\_base\_quality** (*int*) – Minimum base quality. Bases below the minimum quality will not be output. The default is 13.
- **adjust\_capq\_threshold** (*int*) – adjust mapping quality. The default is 0 for no adjustment. The recommended value for adjustment is 50.
- **min\_mapping\_quality** (*int*) – only use reads above a minimum mapping quality. The default is 0.
- **compute\_baq** (*bool*) – re-alignment computing per-Base Alignment Qualities (BAQ). The default is to do re-alignment. Realignment requires a reference sequence. If none is present, no realignment will be performed.

- **redo\_baq** (*bool*) – recompute per-Base Alignment Quality on the fly ignoring existing base qualities. The default is False (use existing base qualities).

**Returns** an iterator over genomic positions.

**Return type** `IteratorColumn`

**references**

tuple with the names of *reference* sequences. This is a read-only attribute

**text**

deprecated, use *references* and *lengths* instead

**unmapped**

int with total number of unmapped reads according to the statistics recorded in the index. This number of reads includes the number of reads without coordinates. This is a read-only attribute. (This will be 0 for a CRAM file indexed by a .crai index, as that index format does not record these statistics.)

**write** (*self*, *AlignedSegment read*) → int

write a single *pysam.AlignedSegment* to disk.

**Raises** `ValueError` – if the writing failed

**Returns** the number of bytes written. If the file is closed, this will be 0.

**Return type** `int`

**class** `pysam.AlignmentHeader`

header information for a *AlignmentFile* object

**Parameters**

- **header\_dict** (*dict*) – build header from a multi-level dictionary. The first level are the four types ('HD', 'SQ', ...). The second level are a list of lines, with each line being a list of tag-value pairs. The header is constructed first from all the defined fields, followed by user tags in alphabetical order. Alternatively, an *AlignmentHeader* object can be passed directly.
- **text** (*string*) – use the string provided as the header
- **reference\_names** (*list*) – see *reference\_lengths*
- **reference\_lengths** (*list*) – build header from list of chromosome names and lengths. By default, 'SQ' and 'LN' tags will be added to the header text. This option can be changed by unsetting the flag *add\_sq\_text*.
- **add\_sq\_text** (*bool*) – do not add 'SQ' and 'LN' tags to header. This option permits construction *SAM* formatted files without a header.

**as\_dict** (*self*)

deprecated, use *to\_dict()* instead

**copy** (*self*)

**from\_dict** (*type cls*, *header\_dict*)

**from\_references** (*type cls*, *reference\_names*, *reference\_lengths*, *text=None*, *add\_sq\_text=True*)

**from\_text** (*type cls*, *text*)

**get** (*self*, \**args*)

**get\_reference\_length** (*self*, *reference*)

**get\_reference\_name** (*self*, *tid*)



**get\_tid** (*self*, *reference*)

return the numerical *tid* corresponding to *reference*

returns -1 if reference is not known.

**is\_valid\_tid** (*self*, *int tid*)

return True if the numerical *tid* is valid; False otherwise.

Note that the unmapped tid code (-1) counts as an invalid.

**items** (*self*)

**iteritems** (*self*)

**keys** (*self*)

**lengths**

tuple of the lengths of the *reference* sequences. This is a read-only attribute. The lengths are in the same order as *pysam.AlignmentFile.references*

**nreferences**

int with the number of *reference* sequences in the file.

This is a read-only attribute.

**references**

tuple with the names of *reference* sequences. This is a read-only attribute

**to\_dict** (*self*)

return two-level dictionary with header information from the file.

The first level contains the record (HD, SQ, etc) and the second level contains the fields (VN, LN, etc).

The parser is validating and will raise an `AssertionError` if it encounters any record or field tags that are not part of the SAM specification. Use the *pysam.AlignmentFile.text* attribute to get the unparsed header.

The parsing follows the SAM format specification with the exception of the CL field. This option will consume the rest of a header line irrespective of any additional fields. This behaviour has been added to accommodate command line options that contain characters that are not valid field separators.

If no @SQ entries are within the text section of the header, this will be automatically added from the reference names and lengths stored in the binary part of the header.

**values** (*self*)

An *AlignedSegment* represents an aligned segment within a SAM/BAM file.

**class** *pysam.AlignedSegment* (*AlignmentHeader header=None*)

Class representing an aligned segment.

This class stores a handle to the samtools C-structure representing an aligned read. Member read access is forwarded to the C-structure and converted into python objects. This implementation should be fast, as only the data needed is converted.

For write access, the C-structure is updated in-place. This is not the most efficient way to build BAM entries, as the variable length data is concatenated and thus needs to be resized if a field is updated. Furthermore, the BAM entry might be in an inconsistent state.

One issue to look out for is that the sequence should always be set *before* the quality scores. Setting the sequence will also erase any quality scores that were set previously.

**Parameters** **header** – *AlignmentHeader* object to map numerical identifiers to chromosome names. If not given, an empty header is created.

**aend**

deprecated, use `reference_end` instead.

**alen**

deprecated, use `reference_length` instead.

**aligned\_pairs**

deprecated, use `get_aligned_pairs()` instead.

**bin**

properties bin

**blocks**

deprecated, use `get_blocks()` instead.

**cigar**

deprecated, use `cigarstring` or `cigartuples` instead.

**cigarstring**

the `cigar` alignment as a string.

The cigar string is a string of alternating integers and characters denoting the length and the type of an operation.

---

**Note:** The order length,operation is specified in the SAM format. It is different from the order of the `cigar` property.

---

Returns None if not present.

To unset the cigarstring, assign None or the empty string.

**cigartuples**

the `cigar` alignment. The alignment is returned as a list of tuples of (operation, length).

If the alignment is not present, None is returned.

The operations are:

M	BAM_CMATCH	0
I	BAM_CINS	1
D	BAM_CDEL	2
N	BAM_CREF_SKIP	3
S	BAM_CSOFT_CLIP	4
H	BAM_CHARD_CLIP	5
P	BAM_CPAD	6
=	BAM_CEQUAL	7
X	BAM_CDIFF	8
B	BAM_CBACK	9

---

**Note:** The output is a list of (operation, length) tuples, such as `[(0, 30)]`. This is different from the SAM specification and the `cigarstring` property, which uses a (length, operation) order, for example: `30M`.

---

To unset the cigar property, assign an empty list or None.

**compare** (*self*, *AlignedSegment other*)

return -1,0,1, if contents in this are binary <,<=,> to *other*

**flag**  
properties flag

**from\_dict** (*type cls, sam\_dict, AlignmentHeader header*)  
parses a dictionary representation of the aligned segment.

**Parameters** **sam\_dict** – dictionary of alignment values, keys corresponding to output from `to_dict()`.

**fromstring** (*type cls, sam, AlignmentHeader header*)  
parses a string representation of the aligned segment.

The input format should be valid SAM format.

**Parameters** **sam** – [SAM](#) formatted string

**get\_aligned\_pairs** (*self, matches\_only=False, with\_seq=False*)  
a list of aligned read (query) and reference positions.

For inserts, deletions, skipping either query or reference position may be None.

For padding in the reference, the reference position will always be None.

**Parameters**

- **matches\_only** (*bool*) – If True, only matched bases are returned - no None on either side.
- **with\_seq** (*bool*) – If True, return a third element in the tuple containing the reference sequence. For CIGAR ‘P’ (padding in the reference) operations, the third tuple element will be None. Substitutions are lower-case. This option requires an MD tag to be present.

**Returns** **aligned\_pairs**

**Return type** list of tuples

**get\_blocks** (*self*)  
a list of start and end positions of aligned gapless blocks.

The start and end positions are in genomic coordinates.

Blocks are not normalized, i.e. two blocks might be directly adjacent. This happens if the two blocks are separated by an insertion in the read.

**get\_cigar\_stats** (*self*)  
summary of operations in cigar string.

The output order in the array is “MIDNSHP=X” followed by a field for the NM tag. If the NM tag is not present, this field will always be 0.

M	BAM_CMATCH	0
I	BAM_CINS	1
D	BAM_CDEL	2
N	BAM_CREF_SKIP	3
S	BAM_CSOFT_CLIP	4
H	BAM_CHARD_CLIP	5
P	BAM_CPAD	6
=	BAM_CEQUAL	7
X	BAM_CDIFF	8
B	BAM_CBACK	9
NM	NM tag	10

If no cigar string is present, empty arrays will be returned.

**Returns** two arrays. The first contains the nucleotide counts within each cigar operation, the second contains the number of blocks for each cigar operation.

**Return type** arrays

**get\_forward\_qualities** (*self*)

return the original base qualities of the read sequence, in the same format as the *query\_qualities* property.

Reads mapped to the reverse strand have their base qualities stored reversed in the BAM file. This method returns such reads' base qualities reversed back to their original orientation.

**get\_forward\_sequence** (*self*)

return the original read sequence.

Reads mapped to the reverse strand are stored reverse complemented in the BAM file. This method returns such reads reverse complemented back to their original orientation.

Returns None if the record has no query sequence.

**get\_overlap** (*self*, *uint32\_t start*, *uint32\_t end*)

return number of aligned bases of read overlapping the interval *start* and *end* on the reference sequence.

Return None if cigar alignment is not available.

**get\_reference\_positions** (*self*, *full\_length=False*)

a list of reference positions that this read aligns to.

By default, this method only returns positions in the reference that are within the alignment. If *full\_length* is set, None values will be included for any soft-clipped or unaligned positions within the read. The returned list will thus be of the same length as the read.

**get\_reference\_sequence** (*self*)

return the reference sequence in the region that is covered by the alignment of the read to the reference.

This method requires the MD tag to be set.

**get\_tag** (*self*, *tag*, *with\_value\_type=False*)

retrieves data from the optional alignment section given a two-letter *tag* denoting the field.

The returned value is cast into an appropriate python type.

This method is the fastest way to access the optional alignment section if only few tags need to be retrieved.

Possible value types are "AcCsSiIfZHB" (see BAM format specification) as well as additional value type 'd' as implemented in htslib.

#### Parameters

- **tag** – data tag.
- **with\_value\_type** – Optional[bool] if set to True, the return value is a tuple of (tag value, type code). (default False)

**Returns** A python object with the value of the *tag*. The type of the object depends on the data type in the data record.

**Raises** `KeyError` – If *tag* is not present, a `KeyError` is raised.

**get\_tags** (*self*, *with\_value\_type=False*)

the fields in the optional alignment section.

Returns a list of all fields in the optional alignment section. Values are converted to appropriate python values. For example: [ (NM, 2) , (RG, "GJP00TM04") ]

If *with\_value\_type* is set, the value type as encode in the AlignedSegment record will be returned as well:

```
[(NM, 2, "i"), (RG, "GJP00TM04", "Z")]
```

This method will convert all values in the optional alignment section. When getting only one or few tags, please see *get\_tag()* for a quicker way to achieve this.

**has\_tag** (*self*, *tag*)

returns true if the optional alignment section contains a given *tag*.

**infer\_query\_length** (*self*, *always=False*)

infer query length from CIGAR alignment.

This method deduces the query length from the CIGAR alignment but does not include hard-clipped bases.

Returns None if CIGAR alignment is not present.

If *always* is set to True, *infer\_read\_length* is used instead. This is deprecated and only present for backward compatibility.

**infer\_read\_length** (*self*)

infer read length from CIGAR alignment.

This method deduces the read length from the CIGAR alignment including hard-clipped bases.

Returns None if CIGAR alignment is not present.

**inferred\_length**

deprecated, use *infer\_query\_length()* instead.

**is\_duplicate**

true if optical or PCR duplicate

**is\_forward**

true if read is mapped to forward strand (implemented in terms of *is\_reverse*)

**is\_mapped**

true if read itself is mapped (implemented in terms of *is\_unmapped*)

**is\_paired**

true if read is paired in sequencing

**is\_proper\_pair**

true if read is mapped in a proper pair

**is\_qcfail**

true if QC failure

**is\_read1**

true if this is read1

**is\_read2**

true if this is read2

**is\_reverse**

true if read is mapped to reverse strand

**is\_secondary**

true if not primary alignment

**is\_supplementary**

true if this is a supplementary alignment

**is\_unmapped**

true if read itself is unmapped

**isize**  
deprecated, use *template\_length* instead.

**mapping\_quality**  
mapping quality

**mapq**  
deprecated, use *mapping\_quality* instead.

**mate\_is\_forward**  
true if the mate is mapped to forward strand (implemented in terms of *mate\_is\_reverse*)

**mate\_is\_mapped**  
true if the mate is mapped (implemented in terms of *mate\_is\_unmapped*)

**mate\_is\_reverse**  
true if the mate is mapped to reverse strand

**mate\_is\_unmapped**  
true if the mate is unmapped

**modified\_bases**  
Modified bases annotations from MI/Mm tags. The output is Dict[(canonical base, strand, modification)]  
-> [ (pos,qual), ... ] with qual being (256\*probability), or -1 if unknown. Strand==0 for forward and 1 for reverse strand modification

**modified\_bases\_forward**  
Modified bases annotations from MI/Mm tags. The output is Dict[(canonical base, strand, modification)]  
-> [ (pos,qual), ... ] with qual being (256\*probability), or -1 if unknown. Strand==0 for forward and 1 for reverse strand modification. The positions are with respect to the original sequence from *get\_forward\_sequence()*

**mpos**  
deprecated, use *next\_reference\_start* instead.

**mrnm**  
deprecated, use *next\_reference\_id* instead.

**next\_reference\_id**  
the *reference* id of the mate/next read.

**next\_reference\_name**  
*reference* name of the mate/next read (None if no AlignmentFile is associated)

**next\_reference\_start**  
the position of the mate/next read.

**opt** (*self*, *tag*)  
deprecated, use *get\_tag()* instead.

**overlap** (*self*)  
deprecated, use *get\_overlap()* instead.

**pnext**  
deprecated, use *next\_reference\_start* instead.

**pos**  
deprecated, use *reference\_start* instead.

**positions**  
deprecated, use *get\_reference\_positions()* instead.

**qend**

deprecated, use `query_alignment_end` instead.

**qlen**

deprecated, use `query_alignment_length` instead.

**qname**

deprecated, use `query_name` instead.

**qqual**

deprecated, use `query_alignment_qualities` instead.

**qstart**

deprecated, use `query_alignment_start` instead.

**qual**

deprecated, use `query_qualities` instead.

**query**

deprecated, use `query_alignment_sequence` instead.

**query\_alignment\_end**

end index of the aligned query portion of the sequence (0-based, exclusive)

This the index just past the last base in `query_sequence` that is not soft-clipped.

**query\_alignment\_length**

length of the aligned query sequence.

This is equal to `query_alignment_end - query_alignment_start`

**query\_alignment\_qualities**

aligned query sequence quality values (None if not present). These are the quality values that correspond to `query_alignment_sequence`, that is, they exclude qualities of *soft clipped* bases. This is equal to `query_qualities[query_alignment_start:query_alignment_end]`.

Quality scores are returned as a python array of unsigned chars. Note that this is not the ASCII-encoded value typically seen in FASTQ or SAM formatted files. Thus, no offset of 33 needs to be subtracted.

This property is read-only.

**query\_alignment\_sequence**

aligned portion of the read.

This is a substring of `query_sequence` that excludes flanking bases that were *soft clipped* (None if not present). It is equal to `query_sequence[query_alignment_start:query_alignment_end]`.

SAM/BAM files may include extra flanking bases that are not part of the alignment. These bases may be the result of the Smith-Waterman or other algorithms, which may not require alignments that begin at the first residue or end at the last. In addition, extra sequencing adapters, multiplex identifiers, and low-quality bases that were not considered for alignment may have been retained.

**query\_alignment\_start**

start index of the aligned query portion of the sequence (0-based, inclusive).

This the index of the first base in `query_sequence` that is not soft-clipped.

**query\_length**

the length of the query/read.

This value corresponds to the length of the sequence supplied in the BAM/SAM file. The length of a query is 0 if there is no sequence in the BAM/SAM file. In those cases, the read length can be inferred from the CIGAR alignment, see `pysam.AlignedSegment.infer_query_length()`.

The length includes soft-clipped bases and is equal to `len(query_sequence)`.

This property is read-only but is updated when a new query sequence is assigned to this `AlignedSegment`.

Returns 0 if not available.

**query\_name**

the query template name (None if not present)

**query\_qualities**

read sequence base qualities, including *soft clipped* bases (None if not present).

Quality scores are returned as a python array of unsigned chars. Note that this is not the ASCII-encoded value typically seen in FASTQ or SAM formatted files. Thus, no offset of 33 needs to be subtracted.

Note that to set quality scores the sequence has to be set beforehand as this will determine the expected length of the quality score array.

This method raises a `ValueError` if the length of the quality scores and the sequence are not the same.

**query\_sequence**

read sequence bases, including *soft clipped* bases (None if not present).

Assigning to this attribute will invalidate any quality scores. Thus, to in-place edit the sequence and quality scores, copies of the quality scores need to be taken. Consider trimming for example:

```
q = read.query_qualities
read.query_sequence = read.query_sequence[5:10]
read.query_qualities = q[5:10]
```

The sequence is returned as it is stored in the BAM file. (This will be the reverse complement of the original read sequence if the mapper has aligned the read to the reverse strand.)

**reference\_end**

aligned reference position of the read on the reference genome.

`reference_end` points to one past the last aligned residue. Returns None if not available (read is unmapped or no cigar alignment present).

**reference\_id**

*reference* ID

---

**Note:** This field contains the index of the reference sequence in the sequence dictionary. To obtain the name of the reference sequence, use `get_reference_name()`

---

**reference\_length**

aligned length of the read on the reference genome.

This is equal to `reference_end - reference_start`. Returns None if not available.

**reference\_name**

*reference* name

**reference\_start**

0-based leftmost coordinate

**rlen**

deprecated, use *query\_length* instead.

**rname**

deprecated, use *reference\_id* instead.



**rnext**

deprecated, use `next_reference_id` instead.

**seq**

deprecated, use `query_sequence` instead.

**setTag** (*self*, *tag*, *value*, *value\_type=None*, *replace=True*)

deprecated, use `set_tag()` instead.

**set\_tag** (*self*, *tag*, *value*, *value\_type=None*, *replace=True*)

sets a particular field *tag* to *value* in the optional alignment section.

*value\_type* describes the type of *value* that is to be entered into the alignment record. It can be set explicitly to one of the valid one-letter type codes. If unset, an appropriate type will be chosen automatically based on the python type of *value*.

An existing value of the same *tag* will be overwritten unless *replace* is set to False. This is usually not recommended as a tag may only appear once in the optional alignment section.

If *value* is *None*, the tag will be deleted.

This method accepts valid SAM specification value types, which are:

```
A: printable char
i: signed int
f: float
Z: printable string
H: Byte array in hex format
B: Integer or numeric array
```

Additionally, it will accept the integer BAM types ('cCsSI')

For htslib compatibility, 'a' is synonymous with 'A' and the method accepts a 'd' type code for a double precision float.

When deducing the type code by the python type of *value*, the following mapping is applied:

```
i: python int
f: python float
Z: python str or bytes
B: python array.array, list or tuple
```

Note that a single character string will be output as 'Z' and not 'A' as the former is the more general type.

**set\_tags** (*self*, *tags*)

sets the fields in the optional alignment section with a list of (tag, value) tuples.

The value type of the values is determined from the python type. Optionally, a type may be given explicitly as a third value in the tuple. For example:

```
x.set_tags([(NM, 2, "i"), (RG, "GJP00TM04", "Z")])
```

This method will not enforce the rule that the same tag may appear only once in the optional alignment section.

**tags**

deprecated, use `get_tags()` instead.

**template\_length**

the observed query template length

**tid**

deprecated, use `reference_id` instead.

**tlen**

deprecated, use `template_length` instead.

**to\_dict** (*self*)

returns a json representation of the aligned segment.

Field names are abbreviated versions of the class attributes.

**to\_string** (*self*)

returns a string representation of the aligned segment.

The output format is valid SAM format if a header is associated with the AlignedSegment.

**tostring** (*self*, *htsfile=None*)

deprecated, use `to_string()` instead.

**Parameters** *htsfile* – (deprecated) AlignmentFile object to map numerical identifiers to chromosome names. This parameter is present for backwards compatibility and ignored.

**class** pysam.PileupColumn

A pileup of reads at a particular reference sequence position (*column*). A pileup column contains all the reads that map to a certain target base.

This class is a proxy for results returned by the samtools pileup engine. If the underlying engine iterator advances, the results of this column will change.

**get\_mapping\_qualities** (*self*)

query mapping quality scores at pileup column position.

**Returns** a list of quality scores

**Return type** list

**get\_num\_aligned** (*self*)

return number of aligned bases at pileup column position.

This method applies a base quality filter and the number is equal to the size of `get_query_sequences()`, `get_mapping_qualities()`, etc.

**get\_query\_names** (*self*)

query/read names aligned at pileup column position.

**Returns** a list of query names at pileup column position.

**Return type** list

**get\_query\_positions** (*self*)

positions in read at pileup column position.

**Returns** a list of read positions

**Return type** list

**get\_query\_qualities** (*self*)

query base quality scores at pileup column position.

**Returns** a list of quality scores

**Return type** list

**get\_query\_sequences** (*self*, *bool mark\_matches=False*, *bool mark\_ends=False*, *bool add\_indels=False*)

query bases/sequences at pileup column position.

Optionally, the bases/sequences can be annotated according to the samtools mpileup format. This is the format description from the samtools mpileup tool:

Information on match, mismatch, indel, strand, mapping quality and start and end of a read are all encoded at the read base column. At this column, a dot stands for a match to the reference base on the forward strand, a comma for a match on the reverse strand, a '>' or '<' for a reference skip, 'ACGTN' for a mismatch on the forward strand and 'acgtn' for a mismatch on the reverse strand. A pattern '[0-9]+[ACGTNacgtn]+' indicates there is an insertion between this reference position and the next reference position. The length of the insertion is given by the integer in the pattern, followed by the inserted sequence. Similarly, a pattern '-[0-9]+[ACGTNacgtn]+' represents a deletion from the reference. The deleted bases will be presented as '\*' in the following lines. Also at the read base column, a symbol '^' marks the start of a read. The ASCII of the character following '^' minus 33 gives the mapping quality. A symbol '\$' marks the end of a read segment

To reproduce samtools mpileup format, set all of `mark_matches`, `mark_ends` and `add_indels` to `True`.

#### Parameters

- **mark\_matches** (*bool*) – If `True`, output bases matching the reference as “.” or “,” for forward and reverse strand, respectively. This mark requires the reference sequence. If no reference is present, this option is ignored.
- **mark\_ends** (*bool*) – If `True`, add markers “^” and “\$” for read start and end, respectively.
- **add\_indels** (*bool*) – If `True`, add bases for bases inserted into or skipped from the reference. The latter requires a reference sequence file to have been given, e.g. via `pileup(fastafilename = ...)`. If no reference sequence is available, skipped bases are represented as 'N's.

**Returns** a list of bases/sequences per read at pileup column position.

**Return type** `list`

**n**

deprecated, use `nsegments` instead.

**nsegments**

number of reads mapping to this column.

Note that this number ignores the base quality filter.

**pileups**

list of reads (`pysam.PileupRead`) aligned to this column

**pos**

deprecated, use `reference_pos` instead.

**reference\_id**

the reference sequence number as defined in the header

**reference\_name**

`reference` name (None if no `AlignmentFile` is associated)

**reference\_pos**

the position in the reference sequence (0-based).

**set\_min\_base\_quality** (*self*, *min\_base\_quality*)  
set the minimum base quality for this pileup column.

**tid**  
deprecated, use *reference\_id* instead.

**class** *pysam.PileupRead*

Representation of a read aligned to a particular position in the reference sequence.

**alignment**  
a *pysam.AlignedSegment* object of the aligned read

**indel**  
indel length for the position following the current pileup site.

This quantity peeks ahead to the next cigar operation in this alignment. If the next operation is an insertion, indel will be positive. If the next operation is a deletion, it will be negation. 0 if the next operation is not an indel.

**is\_del**  
1 iff the base on the padded read is a deletion

**is\_head**  
1 iff the base on the padded read is the left-most base.

**is\_refskip**  
1 iff the base on the padded read is part of CIGAR N op.

**is\_tail**  
1 iff the base on the padded read is the right-most base.

**level**  
the level of the read in the “viewer” mode. Note that this value is currently not computed.

**query\_position**  
position of the read base at the pileup site, 0-based. None if *is\_del* or *is\_refskip* is set.

**query\_position\_or\_next**  
position of the read base at the pileup site, 0-based.  
  
If the current position is a deletion, returns the next aligned base.

**class** *pysam.IndexedReads* (*AlignmentFile samfile*, *int multiple\_iterators=True*)

Index a Sam/BAM-file by query name while keeping the original sort order intact.

The index is kept in memory and can be substantial.

By default, the file is re-opened to avoid conflicts if multiple operators work on the same file. Set *multiple\_iterators = False* to not re-open *samfile*.

#### Parameters

- **samfile** (*AlignmentFile*) – File to be indexed.
- **multiple\_iterators** (*bool*) – Flag indicating whether the file should be reopened. Reopening prevents existing iterators being affected by the indexing.

**build** (*self*)  
build the index.

**find** (*self*, *query\_name*)  
find *query\_name* in index.

**Returns** Returns an iterator over all reads with *query\_name*.

**Return type** IteratorRowSelection

**Raises** `KeyError` – if the *query\_name* is not in the index.

## 1.2.2 Tabix files

*TabixFile* opens tabular files that have been indexed with *tabix*.

**class** `pysam.TabixFile`

Random access to bgzf formatted files that have been indexed by *tabix*.

The file is automatically opened. The index file of file <filename> is expected to be called <filename>.  
tbi by default (see parameter *index*).

### Parameters

- **filename** (*string*) – Filename of bgzf file to be opened.
- **index** (*string*) – The filename of the index. If not set, the default is to assume that the index is called `filename.tbi`
- **mode** (*char*) – The file opening mode. Currently, only `r` is permitted.
- **parser** (`pysam.Parser`) – sets the default parser for this tabix file. If *parser* is `None`, the results are returned as an unparsed string. Otherwise, *parser* is assumed to be a functor that will return parsed data (see for example *asTuple* and *asGTF*).
- **encoding** (*string*) – The encoding passed to the parser
- **threads** (*integer*) – Number of threads to use for decompressing Tabix files. (Default=1)

### Raises

- `ValueError` – if index file is missing.
- `IOError` – if file could not be opened

**close** (*self*)

closes the *pysam.TabixFile*.

**contigs**

list of chromosome names

**fetch** (*self*, *reference=None*, *start=None*, *end=None*, *region=None*, *parser=None*, *multiple\_iterators=False*)

fetch one or more rows in a *region* using 0-based indexing. The region is specified by *reference*, *start* and *end*. Alternatively, a samtools *region* string can be supplied.

Without *reference* or *region* all entries will be fetched.

If only *reference* is set, all reads matching on *reference* will be fetched.

If *parser* is `None`, the default parser will be used for parsing.

Set *multiple\_iterators* to true if you will be using multiple iterators on the same file at the same time. The iterator returned will receive its own copy of a filehandle to the file effectively re-opening the file. Re-opening a file creates some overhead, so beware.

**header**

the file header.

The file header consists of the lines at the beginning of a file that are prefixed by the comment character `#`.

---

**Note:** The header is returned as an iterator presenting lines without the newline character.

---

To iterate over tabix files, use `tabix_iterator()`:

`pysam.tabix_iterator(infile, parser)`  
return an iterator over all entries in a file.

Results are returned parsed as specified by the *parser*. If *parser* is `None`, the results are returned as an unparsed string. Otherwise, *parser* is assumed to be a functor that will return parsed data (see for example `asTuple` and `asGTF`).

`pysam.tabix_compress(filename_in, filename_out, force=False)`  
compress *filename\_in* writing the output to *filename\_out*.

Raise an `IOError` if *filename\_out* already exists, unless *force* is set.

`pysam.tabix_index(filename, force=False, seq_col=None, start_col=None, end_col=None, preset=None, meta_char=u'#', int_line_skip=0, zerobased=False, int_min_shift=-1, index=None, keep_original=False, csi=False)`  
index tab-separated *filename* using tabix.

An existing index will not be overwritten unless *force* is set.

The index will be built from coordinates in columns *seq\_col*, *start\_col* and *end\_col*.

The contents of *filename* have to be sorted by contig and position - the method does not check if the file is sorted.

Column indices are 0-based. Note that this is different from the tabix command line utility where column indices start at 1.

Coordinates in the file are assumed to be 1-based unless *zerobased* is set.

If *preset* is provided, the column coordinates are taken from a preset. Valid values for preset are “gff”, “bed”, “sam”, “vcf”, “psltbl”, “pileup”.

Lines beginning with *meta\_char* and the first *line\_skip* lines will be skipped.

If *filename* is not detected as a gzip file it will be automatically compressed. The original file will be removed and only the compressed file will be retained.

By default or when *min\_shift* is 0, creates a TBI index. If *min\_shift* is greater than zero and/or *csi* is `True`, creates a CSI index with a minimal interval size of  $1 < min\_shift$  ( $1 < 14$  if only *csi* is set).

*index* controls the filename which should be used for creating the index. If not set, the default is to append `.tbi` to *filename*.

When automatically compressing files, if *keep\_original* is set the uncompressed file will not be deleted.

returns the filename of the compressed data

**class** `pysam.asTuple`  
converts a *tabix row* into a python tuple.

A field in a row is accessed by numeric index.

**class** `pysam.asVCF`  
converts a *tabix row* into a VCF record with the following fields:

Column	Field	Contents
1	contig	chromosome
2	pos	chromosomal position, zero-based
3	id	id
4	ref	reference allele
5	alt	alternate alleles
6	qual	quality
7	filter	filter
8	info	info
9	format	format specifier.

Access to genotypes is via index:

```
contig = vcf.contig
first_sample_genotype = vcf[0]
second_sample_genotype = vcf[1]
```

### **class** pysam.asBed

converts a *tabix row* into a bed record with the following fields:

Column	Field	Contents
1	contig	contig
2	start	genomic start coordinate (zero-based)
3	end	genomic end coordinate plus one (zero-based)
4	name	name of feature.
5	score	score of feature
6	strand	strand of feature
7	thickStart	thickStart
8	thickEnd	thickEnd
9	itemRGB	itemRGB
10	blockCount	number of blocks
11	blockSizes	‘,’ separated string of block sizes
12	blockStarts	‘,’ separated string of block genomic start positions

Only the first three fields are required. Additional fields are optional, but if one is defined, all the preceding need to be defined as well.

### **class** pysam.asGTF

converts a *tabix row* into a GTF record with the following fields:

Column	Name	Content
1	contig	the chromosome name
2	feature	The feature type
3	source	The feature source
4	start	genomic start coordinate (0-based)
5	end	genomic end coordinate (0-based)
6	score	feature score
7	strand	strand
8	frame	frame
9	attributes	the attribute field

GTF formatted entries also define the following fields that are derived from the attributes field:

<i>Name</i>	<i>Content</i>
gene_id	the gene identifier
transcript_id	the transcript identifier

### 1.2.3 FASTA files

**class** pysam.FastaFile

Random access to fasta formatted files that have been indexed by *faidx*.

The file is automatically opened. The index file of file <filename> is expected to be called <filename>.fai.

#### Parameters

- **filename** (*string*) – Filename of fasta file to be opened.
- **filepath\_index** (*string*) – Optional, filename of the index. By default this is the filename + “.fai”.
- **filepath\_index\_compressed** (*string*) – Optional, filename of the index if fasta file is. By default this is the filename + “.gzi”.

#### Raises

- `ValueError` – if index file is missing
- `IOError` – if file could not be opened

**close** (*self*)  
close the file.

**closed**  
bool indicating the current state of the file object. This is a read-only attribute; the close() method changes the value.

**fetch** (*self*, *reference=None*, *start=None*, *end=None*, *region=None*)  
fetch sequences in a *region*.

A region can either be specified by *reference*, *start* and *end*. *start* and *end* denote 0-based, half-open intervals.

Alternatively, a samtools *region* string can be supplied.

If any of the coordinates are missing they will be replaced by the minimum (*start*) or maximum (*end*) coordinate.

Note that region strings are 1-based, while *start* and *end* denote an interval in python coordinates. The region is specified by *reference*, *start* and *end*.

#### Returns string

**Return type** a string with the sequence specified by the region.

#### Raises

- `IndexError` – if the coordinates are out of range
- `ValueError` – if the region is invalid

**filename**  
filename associated with this object. This is a read-only attribute.



**get\_reference\_length** (*self*, *reference*)

return the length of reference.

**is\_open** (*self*)

return true if samfile has been opened.

**lengths**

tuple with the lengths of *reference* sequences.

**nreferences**

int with the number of *reference* sequences in the file. This is a read-only attribute.

**references**

tuple with the names of *reference* sequences.

## 1.2.4 FASTQ files

**class** pysam.**FastxFile**

Stream access to *fasta* or *fastq* formatted files.

The file is automatically opened.

Entries in the file can be both fastq or fasta formatted or even a mixture of the two.

This file object permits iterating over all entries in the file. Random access is not implemented. The iteration returns objects of type *FastqProxy*

### Parameters

- **filename** (*string*) – Filename of fasta/fastq file to be opened.
- **persist** (*bool*) – If True (default) make a copy of the entry in the file during iteration. If set to False, no copy will be made. This will permit much faster iteration, but an entry will not persist when the iteration continues and an entry is read-only.

### Notes

Prior to version 0.8.2, this class was called FastqFile.

**Raises** `IOError` – if file could not be opened

### Examples

```
>>> with pysam.FastxFile(filename) as fh:
...     for entry in fh:
...         print(entry.name)
...         print(entry.sequence)
...         print(entry.comment)
...         print(entry.quality)
>>> with pysam.FastxFile(filename) as fin, open(out_filename, mode='w') as fout:
...     for entry in fin:
...         fout.write(str(entry) + '\n')
```

**close** (*self*)

close the file.

**closed**

bool indicating the current state of the file object. This is a read-only attribute; the `close()` method changes the value.

**filename**

string with the filename associated with this object.

**is\_open** (*self*)

return true if samfile has been opened.

**class** pysam.FastqProxy

A single entry in a fastq file.

**get\_quality\_array** (*self*, *int offset=33*) → array

return quality values as integer array after subtracting offset.

**name**

The name of each entry in the fastq file.

**quality**

The quality score of each entry in the fastq file, represented as a string.

**sequence**

The sequence of each entry in the fastq file.

## 1.2.5 VCF/BCF files

**class** pysam.VariantFile (\*args, \*\*kwargs)

(*filename*, *mode=None*, *index\_filename=None*, *header=None*, *drop\_samples=False*, *duplicate\_filehandle=True*, *ignore\_truncation=False*, *threads=1*)

A *VCF/BCF* formatted file. The file is automatically opened.

If an index for a variant file exists (*.csi* or *.tbi*), it will be opened automatically. Without an index random access to records via *fetch()* is disabled.

For writing, a *VariantHeader* object must be provided, typically obtained from another *VCF* file/*BCF* file.

**Parameters**

- **mode** (*string*) – *mode* should be *r* for reading or *w* for writing. The default is text mode (*VCF*). For binary (*BCF*) I/O you should append *b* for compressed or *u* for uncompressed *BCF* output.

If *b* is present, it must immediately follow *r* or *w*. Valid modes are *r*, *w*, *wh*, *rb*, *wb*, *wbu* and *wb0*. For instance, to open a *BCF* formatted file for reading, type:

```
f = pysam.VariantFile('ex1.bcf', 'r')
```

If mode is not specified, we will try to auto-detect the file type. All of the following should work:

```
f1 = pysam.VariantFile('ex1.bcf')
f2 = pysam.VariantFile('ex1.vcf')
f3 = pysam.VariantFile('ex1.vcf.gz')
```

- **index\_filename** (*string*) – Explicit path to an index file.
- **header** (*VariantHeader*) – *VariantHeader* object required for writing.
- **drop\_samples** (*bool*) – Ignore sample information when reading.

- **duplicate\_filehandle** (*bool*) – By default, file handles passed either directly or through File-like objects will be duplicated before passing them to htslib. The duplication prevents issues where the same stream will be closed by htslib and through destruction of the high-level python object. Set to False to turn off duplication.
- **ignore\_truncation** (*bool*) – Issue a warning, instead of raising an error if the current file appears to be truncated due to a missing EOF marker. Only applies to bgzipped formats. (Default=False)
- **threads** (*integer*) – Number of threads to use for compressing/decompressing VCF/BCF files. Setting threads to > 1 cannot be combined with *ignore\_truncation*. (Default=1)

**close** (*self*)  
closes the *pysam.VariantFile*.

**copy** (*self*)

**fetch** (*self*, *contig=None*, *start=None*, *stop=None*, *region=None*, *reopen=False*, *end=None*, *reference=None*)  
fetch records in a *region*, specified either by *contig*, *start*, and *end* (which are 0-based, half-open); or alternatively by a samtools *region* string (which is 1-based inclusive).

Without *contig* or *region* all mapped records will be fetched. The records will be returned ordered by *contig*, which will not necessarily be the order within the file.

Set *reopen* to true if you will be using multiple iterators on the same file at the same time. The iterator returned will receive its own copy of a filehandle to the file effectively re-opening the file. Re-opening a file incurs some overhead, so use with care.

If only *contig* is set, all records on *contig* will be fetched. If both *region* and *contig* are given, an exception is raised.

Note that a bgzipped *VCF.gz* file without a tabix/CSI index (*.tbi/.csi*) or a *BCF* file without a CSI index can only be read sequentially.

**get\_reference\_name** (*self*, *tid*)  
return *reference* name corresponding to numerical *tid*

**get\_tid** (*self*, *reference*)  
return the numerical *tid* corresponding to *reference*  
returns -1 if reference is not known.

**is\_valid\_tid** (*self*, *tid*)  
return True if the numerical *tid* is valid; False otherwise.  
returns -1 if reference is not known.

**new\_record** (*self*, *\*args*, *\*\*kwargs*)  
Create a new empty *VariantRecord*.  
See *VariantHeader.new\_record()*

**open** (*self*, *filename*, *mode=u'r'*, *index\_filename=None*, *VariantHeader header=None*, *drop\_samples=False*, *duplicate\_filehandle=True*, *ignore\_truncation=False*, *threads=1*)  
open a vcf/bcf file.

If open is called on an existing *VariantFile*, the current file will be closed and a new file will be opened.

**reset** (*self*)  
reset file position to beginning of file just after the header.

**subset\_samples** (*self*, *include\_samples*)

Read only a subset of samples to reduce processing time and memory. Must be called prior to retrieving records.

**write** (*self*, *VariantRecord* *record*) → int

write a single *pysam.VariantRecord* to disk.

returns the number of bytes written.

**class** *pysam.VariantHeader*

header information for a *VariantFile* object

**add\_line** (*self*, *line*)

Add a metadata line to this header

**add\_meta** (*self*, *key*, *value=None*, *items=None*)

Add metadata to this header

**add\_record** (*self*, *VariantHeaderRecord* *record*)

Add an existing *VariantHeaderRecord* to this header

**add\_sample** (*self*, *name*)

Add a new sample to this header

**add\_samples** (*self*, *\*args*)

Add several new samples to this header. This function takes multiple arguments, each of which may be either a sample name or an iterable returning sample names (e.g., a list of sample names).

**alts**

alt metadata (*dict* ID->record).

The data returned just a snapshot of alt records, is created every time the property is requested, and modifications will not be reflected in the header metadata and vice versa.

i.e. it is just a dict that reflects the state of alt records at the time it is created.

**contigs**

contig information (*VariantHeaderContigs*)

**copy** (*self*)

**filters**

filter metadata (*VariantHeaderMetadata*)

**formats**

format metadata (*VariantHeaderMetadata*)

**info**

info metadata (*VariantHeaderMetadata*)

**merge** (*self*, *VariantHeader* *header*)

**new\_record** (*self*, *contig=None*, *start=0*, *stop=0*, *alleles=None*, *id=None*, *qual=None*, *filter=None*, *info=None*, *samples=None*, *\*\*kwargs*)

Create a new empty *VariantRecord*.

Arguments are currently experimental. Use with caution and expect changes in upcoming releases.

**records**

header records (*VariantHeaderRecords*)

**samples**

**version**

VCF version

```

class pysam.VariantRecord(*args, **kwargs)
    Variant record

    alleles
        tuple of reference allele followed by alt alleles

    alts
        tuple of alt alleles

    chrom
        chromosome/contig name

    contig
        chromosome/contig name

    copy(self)
        return a copy of this VariantRecord object

    filter
        filter information (see VariantRecordFilter)

    format
        sample format metadata (see VariantRecordFormat)

    id
        record identifier or None if not available

    info
        info data (see VariantRecordInfo)

    pos
        record start position on chrom/contig (1-based inclusive)

    qual
        phred scaled quality score or None if not available

    ref
        reference allele

    rid
        internal reference id number

    rlen
        record length on chrom/contig (aka rec.stop - rec.start)

    samples
        sample data (see VariantRecordSamples)

    start
        record start position on chrom/contig (0-based inclusive)

    stop
        record stop position on chrom/contig (0-based exclusive)

    translate(self, VariantHeader dst_header)

class pysam.VariantHeaderRecord(*args, **kwargs)
    header record from a VariantHeader object

    attrs
        sequence of additional header attributes

    get(self, key, default=None)
        D.get(k[,d]) -> D[k] if k in D, else d. d defaults to None.

```

**items** (*self*)  
D.items() -> list of D's (key, value) pairs, as 2-tuples

**iteritems** (*self*)  
D.iteritems() -> an iterator over the (key, value) items of D

**iterkeys** (*self*)  
D.iterkeys() -> an iterator over the keys of D

**itervalues** (*self*)  
D.itervalues() -> an iterator over the values of D

**key**  
header key (the part before '=', in FILTER/INFO/FORMAT/contig/fileformat etc.)

**keys** (*self*)  
D.keys() -> list of D's keys

**pop** (*self*, *key*, *default=\_nothing*)

**remove** (*self*)

**type**  
FILTER, INFO, FORMAT, CONTIG, STRUCTURED, or GENERIC  
**Type** header type

**update** (*self*, *items=None*, *\*\*kwargs*)  
D.update([E, ]\*\*F) -> None.  
Update D from dict/iterable E and F.

**value**  
header value. Set only for generic lines, None for FILTER/INFO, etc.

**values** (*self*)  
D.values() -> list of D's values

## 1.2.6 HTSFile

HTSFile is the base class for `pysam.AlignmentFile` and `pysam.VariantFile`.

**class** `pysam.HTSFile`  
Base class for HTS file types

**add\_hts\_options** (*self*, *format\_options=None*)  
Given a list of key=value format option strings, add them to an open htsFile

**category**  
General file format category. One of UNKNOWN, ALIGNMENTS, VARIANTS, INDEX, REGIONS

**check\_truncation** (*self*, *ignore\_truncation=False*)  
Check if file is truncated.

**close** (*self*)

**closed**  
return True if HTSFile is closed.

**compression**  
File compression.  
One of NONE, GZIP, BGZF, CUSTOM.

**description**

Vaguely human readable description of the file format

**format**

File format.

One of UNKNOWN, BINARY\_FORMAT, TEXT\_FORMAT, SAM, BAM, BAI, CRAM, CRAI, VCF, BCF, CSI, GZI, TBI, BED.

**get\_reference\_name** (*self*, *tid*)

return *contig* name corresponding to numerical *tid*

**get\_tid** (*self*, *contig*)

return the numerical *tid* corresponding to *contig*

returns -1 if *contig* is not known.

**is\_bam**

return True if HTSFile is reading or writing a BAM alignment file

**is\_bcf**

return True if HTSFile is reading or writing a BCF variant file

**is\_closed**

return True if HTSFile is closed.

**is\_cram**

return True if HTSFile is reading or writing a BAM alignment file

**is\_open**

return True if HTSFile is open and in a valid state.

**is\_read**

return True if HTSFile is open for reading

**is\_sam**

return True if HTSFile is reading or writing a SAM alignment file

**is\_valid\_reference\_name** (*self*, *contig*)

return True if the *contig* name *contig* is valid; False otherwise.

**is\_valid\_tid** (*self*, *tid*)

return True if the numerical *tid* is valid; False otherwise.

returns -1 if *contig* is not known.

**is\_vcf**

return True if HTSFile is reading or writing a VCF variant file

**is\_write**

return True if HTSFile is open for writing

**parse\_region** (*self*, *contig*=None, *start*=None, *stop*=None, *region*=None, *tid*=None, *reference*=None, *end*=None)

parse alternative ways to specify a genomic region. A region can either be specified by *contig*, *start* and *stop*. *start* and *stop* denote 0-based, half-open intervals. *reference* and *end* are also accepted for backward compatibility as synonyms for *contig* and *stop*, respectively.

Alternatively, a samtools *region* string can be supplied.

If any of the coordinates are missing they will be replaced by the minimum (*start*) or maximum (*stop*) coordinate.

Note that region strings are 1-based inclusive, while *start* and *stop* denote an interval in 0-based, half-open coordinates (like BED files and Python slices).

If *contig* or *region* or are \*, unmapped reads at the end of a BAM file will be returned. Setting either to . will iterate from the beginning of the file.

**Returns** a tuple of *flag*, *tid*, *start* and *stop*. The flag indicates whether no coordinates were supplied and the genomic region is the complete genomic space.

**Return type** tuple

**Raises** `ValueError` – for invalid or out of bounds regions.

**reset** (*self*)

reset file position to beginning of file just after the header.

**Returns** The file position after moving the file pointer.

**Return type** pointer

**seek** (*self*, *uint64\_t* *offset*)

move file pointer to position *offset*, see `pysam.HTSFile.tell()`.

**tell** (*self*)

return current file position, see `pysam.HTSFile.seek()`.

**version**

Tuple of file format version numbers (major, minor)

## 1.3 Working with BAM/CRAM/SAM-formatted files

### 1.3.1 Opening a file

To begin with, import the pysam module and open a `pysam.AlignmentFile`:

```
import pysam
samfile = pysam.AlignmentFile("ex1.bam", "rb")
```

The above command opens the file `ex1.bam` for reading. The `b` qualifier indicates that this is a *BAM* file. To open a *SAM* file, type:

```
import pysam
samfile = pysam.AlignmentFile("ex1.sam", "r")
```

*CRAM* files are identified by a `c` qualifier:

```
import pysam
samfile = pysam.AlignmentFile("ex1.cram", "rc")
```

### 1.3.2 Fetching reads mapped to a region

Reads are obtained through a call to the `pysam.AlignmentFile.fetch()` method which returns an iterator. Each call to the iterator will returns a `pysam.AlignedSegment` object:

```
iter = samfile.fetch("seq1", 10, 20)
for x in iter:
    print(str(x))
```





```
infile = pysam.AlignmentFile("-", "r")
outfile = pysam.AlignmentFile("-", "w", template=infile)
for s in infile:
    outfile.write(s)
```

It will also work with *BAM* files. The following script converts a *BAM* formatted file on stdin to a *SAM* formatted file on stdout:

```
infile = pysam.AlignmentFile("-", "rb")
outfile = pysam.AlignmentFile("-", "w", template=infile)
for s in infile:
    outfile.write(s)
```

Note that the file open mode needs to be changed from `r` to `rb`.

## 1.4 Using samtools commands within python

Commands available in *samtools* are available as simple function calls. Command line options are provided as arguments. For example:

```
pysam.sort("-", "output.bam", "ex1.bam")
```

corresponds to the command line:

```
samtools sort -o output.bam ex1.bam
```

Or for example:

```
pysam.sort("-m", "1000000", "-o", "output.bam", "ex1.bam")
```

In order to get usage information, try:

```
print(pysam.sort.usage())
```

Argument errors raise a `pysam.SamtoolsError`:

```
pysam.sort()

Traceback (most recent call last):
File "x.py", line 12, in <module>
    pysam.sort()
File "/build/lib.linux-x86_64-2.6/pysam/__init__.py", line 37, in __call__
    if retval: raise SamtoolsError( "\n".join( stderr ) )
pysam.SamtoolsError: 'Usage: samtools sort [-n] [-m <maxMem>] <in.bam> <out.prefix>\n'
```

Messages from *samtools* on stderr are captured and are available using the `getMessages()` method:

```
pysam.sort.getMessage()
```

Note that only the output from the last invocation of a command is stored.

In order for *pysam* to make the output of *samtools* commands accessible the stdout stream needs to be redirected. This is the default behaviour, but can cause problems in environments such as the *ipython* notebook. A solution is to pass the `catch_stdout` keyword argument:

```
pysam.sort(catch_stdout=False)
```

Note that this means that output from commands which produce output on stdout will not be available. The only solution is to run samtools commands through subprocess.

## 1.5 Working with tabix-indexed files

To open a tabular file that has been indexed with `tabix`, use `TabixFile`:

```
import pysam
tbx = pysam.TabixFile("example.bed.gz")
```

Similar to `fetch`, intervals within a region can be retrieved by calling `fetch()`:

```
for row in tbx.fetch("chr1", 1000, 2000):
    print(str(row))
```

This will return a tuple-like data structure in which columns can be retrieved by numeric index:

```
for row in tbx.fetch("chr1", 1000, 2000):
    print("chromosome is", row[0])
```

By providing a parser to `fetch` or `TabixFile`, the data will be presented in parsed form:

```
for row in tbx.fetch("chr1", 1000, 2000, parser=pysam.asTuple()):
    print("chromosome is", row.contig)
    print("first field (chrom)=", row[0])
```

Pre-built parsers are available for `bed` (`asBed`) formatted files and `gtf` (`asGTF`) formatted files. Thus, additional fields become available through named access, for example:

```
for row in tbx.fetch("chr1", 1000, 2000, parser=pysam.asBed()):
    print("name is", row.name)
```

## 1.6 Working with VCF/BCF formatted files

To iterate through a VCF/BCF formatted file use `VariantFile`:

```
from pysam import VariantFile

bcf_in = VariantFile("test.bcf") # auto-detect input format
bcf_out = VariantFile('-', 'w', header=bcf_in.header)

for rec in bcf_in.fetch('chr1', 100000, 200000):
    bcf_out.write(rec)
```

`_pysam.VariantFile.fetch()` iterates over `VariantRecord` objects which provides access to simple variant attributes such as `contig`, `pos`, `ref`:

```
for rec in bcf_in.fetch():
    print(rec.pos)
```

but also to complex attributes such as the contents to the *info*, *format* and *genotype* columns. These complex attributes are views on the underlying htslib data structures and provide dictionary-like access to the data:

```
for rec in bcf_in.fetch():
    print(rec.info)
    print(rec.info.keys())
    print(rec.info["DP"])
```

The header attribute (*VariantHeader*) provides access information stored in the *vcf* header. The complete header can be printed:

```
>>> print(bcf_in.header)
##fileformat=VCFv4.2
##FILTER=<ID=PASS,Description="All filters passed">
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=1000GenomesPilot-NCBI36
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples
With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build
129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
##contig=<ID=M>
##contig=<ID=17>
##contig=<ID=20>
##bcftools_viewVersion=1.3+htslib-1.3
##bcftools_viewCommand=view -O b -o example_vcf42.bcf
example_vcf42.vcf.gz
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001_
↪NA00002 NA0000
```

Individual contents such as contigs, info fields, samples, formats can be retrieved as attributes from header:

```
>>> print(bcf_in.header.contigs)
<pysam.cbcf.VariantHeaderContigs object at 0xf250f8>
```

To convert these views to native python types, iterate through the views:

```
>>> print(list((bcf_in.header.contigs)))
['M', '17', '20']
>>> print(list((bcf_in.header.filters)))
['PASS', 'q10', 's50']
>>> print(list((bcf_in.header.info)))
['NS', 'DP', 'AF', 'AA', 'DB', 'H2']
>>> print(list((bcf_in.header.samples)))
['NA00001', 'NA00002', 'NA00003']
```

Alternatively, it is possible to iterate through all records in the header returning objects of type *VariantHeaderRecord*:

```

>>> for x in bcf_in.header.records:
>>>     print(x)
>>>     print(x.type, x.key)
GENERIC fileformat
FILTER FILTER
GENERIC fileDate
GENERIC source
GENERIC reference
GENERIC phasing
INFO INFO
INFO INFO
INFO INFO
INFO INFO
INFO INFO
INFO INFO
FILTER FILTER
FILTER FILTER
FORMAT FORMAT
FORMAT FORMAT
FORMAT FORMAT
FORMAT FORMAT
CONTIG contig
CONTIG contig
CONTIG contig
GENERIC bcftools_viewVersion
GENERIC bcftools_viewCommand

```

## 1.7 Extending pysam

Using `pyximport`, it is (relatively) straight-forward to access pysam internals and the underlying `samtools` library. An example is provided in the `tests` directory. The example emulates the `samtools flagstat` command and consists of three files:

1. The main script `pysam_flagstat.py`. The important lines in this script are:

```

import pyximport
pyximport.install()
import _pysam_flagstat

...

flag_counts = _pysam_flagstat.count(pysam_in)

```

The first part imports, sets up `pyximport` and imports the cython module `_pysam_flagstat`. The second part calls the `count` method in `_pysam_flagstat`.

2. The cython implementation `_pysam_flagstat.pyx`. This script imports the pysam API via:

```

from pysam.libcalignmentfile cimport AlignmentFile, AlignedSegment

```

This statement imports, amongst others, `AlignedSegment` into the namespace. Speed can be gained from declaring variables. For example, to efficiently iterate over a file, an `AlignedSegment` object is declared:

```

# loop over samfile
cdef AlignedSegment read

```

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```
for read in samfile:
    ...
```

3. A `pyxbld` providing `pyximport` with build information. Required are the locations of the samtools and pysam header libraries of a source installation of pysam plus the `csamtools.so` shared library. For example:

```
def make_ext(modname, pyxfilename):
    from distutils.extension import Extension
    import pysam
    return Extension(name=modname,
                     sources=[pyxfilename],
                     extra_link_args=pysam.get_libraries(),
                     include_dirs=pysam.get_include(),
                     define_macros=pysam.get_defines())
```

If the script `pysam_flagstat.py` is called the first time, `pyximport` will compile the `cython` extension `_pysam_flagstat.pyx` and make it available to the script. Compilation requires a working compiler and `cython` installation. Each time `_pysam_flagstat.pyx` is modified, a new compilation will take place.

`pyximport` comes with `cython`.

## 1.8 Installing pysam

Pysam can be installed through `conda`, `pypi` and from the repository. The recommended way to install pysam is through `conda/bioconda`.

### 1.8.1 Conda installation

To install pysam in your current `conda` environment, type:

```
conda config --add channels r
conda config --add channels bioconda
conda install pysam
```

This will install pysam from the `bioconda` channel and automatically makes sure that dependencies are installed. Also, compilation flags will be set automatically, which will potentially save a lot of trouble on OS X.

### 1.8.2 Pypi installation

Pysam provides a python interface to the functionality contained within the `htslib` C library. There are two ways that these two can be combined, `builtin` and `external`.

#### Builtin

The typical installation will be through `pypi`:

```
pip install pysam
```

This will compile the `builtin` `htslib` source code within pysam.

`htslib` can be configured at compilation to turn on additional features such support using encrypted configurations, enable plugins, and more. See the `htslib` project for more information on these.

Pysam will attempt to configure [htslib](#) to turn on some advanced features. If these fail, for example due to missing library dependencies (*libcurl*, *libcrypto*), it will fall back to conservative defaults.

Options can be passed to the configure script explicitly by setting the environment variable `HTSLIB_CONFIGURE_OPTIONS`. For example:

```
export HTSLIB_CONFIGURE_OPTIONS=--enable-plugins
pip install pysam
```

## External

pysam can be combined with an externally installed [htslib](#) library. This is a good way to avoid duplication of libraries. To link against an externally installed library, set the environment variables `HTSLIB_LIBRARY_DIR` and `HTSLIB_INCLUDE_DIR` before installing:

```
export HTSLIB_LIBRARY_DIR=/usr/local/lib
export HTSLIB_INCLUDE_DIR=/usr/local/include
pip install pysam
```

Note that the location of the file `libhts.so` needs to be known to the linker once you run pysam, for example by setting the environment-variable `LD_LIBRARY_PATH`.

Note that generally the pysam and htslib version need to be compatible. See the release notes for more information.

### 1.8.3 Installation from repository

pysam depends on [cython](#) to provide the connectivity to the [htslib](#) C library. The installation of the source tarball (`.tar.gz`) contains pre-built C-files and cython needs not be present during installation. However, when installing from the repository, cython needs to be installed beforehand.

To install from repository, type:

```
python setup.py install
```

For compilation options, see the section on Pypi installation above.

### 1.8.4 Requirements

Depending on the installation method, requirements for building pysam differ.

When installing through [conda](#), dependencies will be resolved by the package manager. The [pip](#) installation and installation from source require a C compiler and its standard libraries as well as all requirements for building htslib. Htslib requirements are listed in the `htslib/INSTALL` file.

Installing from the repository will require [cython](#) to be installed.

## 1.9 FAQ

### 1.9.1 How should I cite pysam

Pysam has not been published in print. When referring to pysam, please use the github URL: <https://github.com/pysam-developers/pysam>. As pysam is a wrapper around htslib and the samtools package, I suggest citing [Li.2009], [Bonfield.2021], and/or [Danecek.2021], as appropriate.

### 1.9.2 Is pysam thread-safe?

Pysam is a mix of python and C code. Instructions within python are generally made thread-safe through python's [global interpreter lock \(GIL\)](#). This ensures that python data structures will always be in a consistent state.

If an external function outside python is called, the programmer has a choice to keep the GIL in place or to release it. Keeping the GIL in place will make sure that all python threads wait until the external function has completed. This is a safe option and ensures thread-safety.

Alternatively, the GIL can be released while the external function is called. This will allow other threads to run concurrently. This can be beneficial if the external function is expected to halt, for example when waiting for data to read or write. However, to achieve thread-safety, the external function needs to be implemented with thread-safety in mind. This means that there can be no shared state between threads, or if there is shared, it needs to be controlled to prevent any access conflicts.

Pysam generally uses the latter option and aims to release the GIL for I/O intensive tasks. This is generally fine, but thread-safety of all parts have not been fully tested.

A related issue is when different threads read from the same file object - or the same thread uses two iterators over a file. There is only a single file-position for each opened file. To prevent this from happening, use the option `multiple_iterators=True` when calling a `fetch()` method. This will return an iterator on a newly opened file.

### 1.9.3 pysam coordinates are wrong

pysam uses 0-based coordinates and the half-open notation for ranges as does python. Coordinates and intervals reported from pysam always follow that convention.

Confusion might arise as different file formats might have different conventions. For example, the SAM format is 1-based while the BAM format is 0-based. It is important to remember that pysam will always conform to the python convention and translate to/from the file format automatically.

The only exception is the [region](#) string in the `fetch()` and `pileup()` methods. This string follows the convention of the samtools command line utilities. The same is true for any coordinates passed to the samtools command utilities directly, such as `pysam.mpileup()`.

### 1.9.4 Calling pysam.fetch() confuses existing iterators

The following code will cause unexpected behaviour:

```
samfile = pysam.AlignmentFile("pysam_ex1.bam", "rb")

iter1 = samfile.fetch("chr1")
print(next(iter1).reference_id)
iter2 = samfile.fetch("chr2")
print(next(iter2).reference_id)
print(next(iter1).reference_id)
```

This will give the following output:

```
0
1
Traceback (most recent call last):
  File "xx.py", line 8, in <module>
    print(next(iter1).reference_id)
  File "libcalignmentfile.pyx", line 2103,
```

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```

    in pysam.libcalignmentfile.IteratorRowRegion.__next__
StopIteration

```

Note how the second iterator stops as the file pointer has moved to chr2. The correct way to work with multiple iterators is:

```

samfile = pysam.AlignmentFile("pysam_ex1.bam", "rb")

iter1 = samfile.fetch("chr1", multiple_iterators=True)
print(next(iter1).reference_id)
iter2 = samfile.fetch("chr2")
print(next(iter2).reference_id)
print(next(iter1).reference_id)

```

Here, the output is:

```

0
1
0

```

The reason for this behaviour is that every iterator needs to keep track of its current position in the file. Within pysam, each opened file can only keep track of one file position and hence there can only be one iterator per file. Using the option `multiple_iterators=True` will return an iterator within a newly opened file. This iterator will not interfere with existing iterators as it has its own file handle associated with it.

Note that re-opening files incurs a performance penalty which can become severe when calling `fetch()` often. Thus, `multiple_iterators` is set to `False` by default.

### 1.9.5 AlignmentFile.fetch does not show unmapped reads

`fetch()` will only iterate over alignments in the SAM/BAM file. The following thus always works:

```

bf = pysam.AlignmentFile(fname, "rb")
for r in bf.fetch():
    assert not r.is_unmapped

```

If the SAM/BAM file contains unaligned reads, they can be included in the iteration by adding the `until_eof=True` flag:

```

bf = pysam.AlignmentFile(fname, "rb")
for r in bf.fetch(until_eof=True):
    if r.is_unmapped:
        print("read is unmapped")

```

### 1.9.6 I can't call AlignmentFile.fetch on a file without an index

`fetch()` requires an index when iterating over a SAM/BAM file. To iterate over a file without an index, use `until_eof=True`:

```

bf = pysam.AlignmentFile(fname, "rb")
for r in bf.fetch(until_eof=True):
    print(r)

```

### 1.9.7 BAM files with a large number of reference sequences are slow

If you have many reference sequences in a BAM file, the following might be slow:

```
track = pysam.AlignmentFile(fname, "rb")
for aln in track.fetch():
    pass
```

The reason is that `track.fetch()` will iterate through the BAM file for each reference sequence in the order as it is defined in the header. This might require a lot of jumping around in the file. To avoid this, use:

```
track = pysam.AlignmentFile(fname, "rb")
for aln in track.fetch(until_eof=True):
    pass
```

This will iterate through reads as they appear in the file.

### 1.9.8 Weirdness with spliced reads in `samfile.pileup(chr,start,end)` given spliced alignments from an RNA-seq bam file

Spliced reads are reported within `samfile.pileup`. To ignore these in your analysis, test the flags `is_del == True` and `indel == 0` in the `PileupRead` object.

### 1.9.9 I can't edit quality scores in place

Editing reads in-place generally works, though there is one quirk to be aware of. Assigning to `AlignedSegment.query_sequence` will invalidate any quality scores in `AlignedSegment.query_qualities`. The reason is that samtools manages the memory of the sequence and quality scores together and thus requires them to always be of the same length or 0.

Thus, to in-place edit the sequence and quality scores, copies of the quality scores need to be taken. Consider trimming for example:

```
quals = read.query_qualities
read.query_sequence = read.query_sequence[5:10]
read.query_qualities = quals[5:10]
```

### 1.9.10 Why is there no `SNPCaller` class anymore?

SNP calling is highly complex and heavily parameterized. There was a danger that the pysam implementations might show different behaviour from the samtools implementation, which would have caused a lot of confusion.

The best way to use samtools SNP calling from python is to use the `pysam.mpileup()` command and parse the output directly.

### 1.9.11 I get an error 'PileupProxy accessed after iterator finished'

Pysam works by providing proxy objects to objects defined within the C-samtools package. Thus, some attention must be paid to the lifetime of objects. The following code snippets will cause an error:

```
s = AlignmentFile('ex1.bam')
for p in s.pileup('chr1', 1000, 1010):
    pass

for pp in p.pileups:
    print(pp)
```

The iteration has finished, thus the contents of `p` are invalid. Another variation of this:

```
p = next(AlignmentFile('ex1.bam').pileup('chr1', 1000, 1010))
for pp in p.pileups:
    print(pp)
```

Again, the iteration finishes as the temporary iterator created by `pileup` goes out of scope. The solution is to keep a handle to the iterator that remains alive:

```
i = AlignmentFile('ex1.bam').pileup('chr1', 1000, 1010)
p = next(i)
for pp in p.pileups:
    print(pp)
```

### 1.9.12 Pysam won't compile

Compiling pysam can be tricky as there are numerous variables that differ between build environments such as OS, version, python version, and compiler. It is difficult to build software that builds cleanly on all systems and the process might fail. Please see the [pysam user group](#) for common issues.

If there is a build issue, read the generated output carefully - generally the cause of the problem is among the first errors to be reported. For example, you will need to have the development version of python installed that includes the header files such as `Python.h`. If that file is missing, the compiler will report this at the very top of its error messages but will follow it with any unknown function or variable definition it encounters later on.

General advice is to always use the latest version on [python](#) and [cython](#) when building pysam. There are some known incompatibilities:

- Python 3.4 requires cython 0.20.2 or later (see [here](#))

### 1.9.13 ImportError: cannot import name csamtools

In version 0.10.0 and onwards, all pysam extension modules contain a `lib`-prefix. This facilitates linking against pysam extension modules with compilers that require to start with `lib`. As a consequence, all code using pysam extension modules directly will need to be adapted. For example,:

```
cimport pysam.csamtools
```

will become:

```
cimport pysam.libcsamtools
```

## 1.10 Developer's guide

### 1.10.1 Code organization

The top level directory is organized in the following directories:

**pysam** Code specific to pysam.

**doc** The documentation. To build the latest documentation, first install [Sphinx](#) and then type:

```
make -C doc html
```

**tests** Code and data for testing and benchmarking.

**htslib** Source code from [htslib](#) shipped with pysam. See `import.py` about importing.

**samtools** Source code from [samtools](#) shipped with pysam. See `import.py` about importing.

**bcftools** Source code from [bcftools](#) shipped with pysam. See `import.py` about importing.

### 1.10.2 Importing new versions of htslib and samtools

See instructions in `import.py` to import the latest versions of [htslib](#), [samtools](#) and [bcftools](#).

### 1.10.3 Unit testing

Unit tests are in the `tests` directory. To run all unit tests, run:

```
pytest tests
```

Most tests use test data from the `tests/*_data` directories. Some of these test data files are generated from other files in these directories, which is done by running `make` in each directory:

```
make -C tests/pysam_data  
# etc
```

Alternatively if any `tests/*_data/all.stamp` file is not already present, running the unit tests should generate that directory's data files automatically.

### 1.10.4 Benchmarking

To run the benchmarking suite, make sure that [pytest-benchmark](#) is installed. To run all benchmarks, type:

```
pytest tests/*_bench.py
```

See [Benchmarking](#) for more on this topic.

### 1.10.5 Contributors

Please see Github for a list of all contributors:

<https://github.com/pysam-developers/pysam/graphs/contributors>

Many thanks to all contributors for helping in making pysam useful.

## 1.11 Release notes

### 1.11.1 Release 0.19.1

This release wraps htlib/samtools/bcftools version 1.15.1.

- [#1104] add an `add_samples()` method to quickly add multiple samples to VCF.

### 1.11.2 Release 0.19.0

This release wraps htlib/samtools/bcftools version 1.15.

- [#1085] Improve `getopt()/getopt_long()` resetting when running samtools/bcftools commands
- [#1078] Support BAM\_CPAD in `get_aligned_pairs`
- [#1063] Run flake8 and fix some linting issues
- [#1088] Add `AlignedSegment` `is_mapped/mate_is_mapped/is_forward/mate_is_forward` properties
- Write an absent `AlignedSegment.qual` as all-bytes-0xff
- Fix `BGZFFile.read()` behaviour near or at EOF
- First API for the htlib modified bases interface

### 1.11.3 Release 0.18.0

This release wraps htlib/samtools/bcftools version 1.14.

- [#1048] and [#1060], clarify documentation of index statistics with CRAM files
- Prevent “retval may be used uninitialised” warning.
- Add new “samples” subcommand to `pysam/samtools.py`
- Introduce `TupleProxyIterator` iterator object class

### 1.11.4 Release 0.17.0

This release wraps htlib/samtools/bcftools version 1.13. Corresponding to new samtools commands, *pysam.samtools* now has additional functions *ampliconclip*, *ampliconstats*, *fqimport*, and *version*.

Bugs fixed:

- [#447] The maximum QNAME length is fully restored to 254
- [#506, #958, #1000] Don’t crash the Python interpreter on `pysam.bcftools.*()` errors
- [#603] `count_coverage`: ignore reads that have no SEQ field
- [#928] Fix `pysam.bcftools.mpileup()` segmentation fault
- [#983] Add `win32/*.ch` to MANIFEST.in
- [#994] Raise exception in `get_tid()` if header could not be parsed
- [#995] Choose TBI/CSI in `tabix_index()` via both `min_shift` and `csi`
- [#996] `AlignmentFile.fetch()` now works with large chromosomes longer than  $2^{29}$  bases

- [#1019] Fix Sphinx documentation generation by avoiding Python 2 `ur'string'` syntax
- [#1035] Improved handling of file iteration errors
- [#1038] `tabix_index()` no longer leaks file descriptors
- [#1040] `print(aligned_segment)` now prints the correct TLEN value (it also now prints RNAME/RNEXT more clearly and prints POS/PNEXT 1-based)
- `setup.py` longer uses `setup(use_2to3)` for compatibility with `setuptools >= v58.0.0`

New facilities:

- [PR #963] Additional VCF classes are exposed to pysam programmers
- [#998, PR #1001] Add `get/set_encoding_error_handler()` to control UTF-8 conversion
- [PR #1012] Running `python setup.py sdist` now automatically runs cythonize
- Running tests with `pytest` now automatically runs `make` to generate test data

Documentation improvements:

- [#726] Clarify `get_forward_sequence/get_forward_qualities` documentation
- [#865] Improved example
- [#968] `get_index_statistics` parameters
- [#986] Clarify `VariantFile.fetch` start/stop region parameters are 0-based and half-open.
- [#990] Corrected `PileupColumn.get_query_sequences` documentation
- [#999] Fix documentation for `AlignmentFile.get_reference_length()`
- [#1002] Document the default `min_base_quality` for `pileup()`

### 1.11.5 Release 0.16.0

This release wraps `htslib/bcftools` version 1.10.2 and `samtools` version 1.10. The following bugs reported against `pysam` are fixed due to this:

- [#447] Writing out QNAME longer than 251 characters corrupts BAM
- [#640, #734, #843] Setting `VariantRecord` pos or stop raises error
- [#738, #919] `FastxFile` truncates concatenated plain gzip compressed files

Additional bugfixes:

- [#840] `Pileup` doesn't work on python3 when `index_filename` is used
- [#886] `FastqProxy` raises `ValueError` when instantiated from python
- [#904] `VariantFile.fetch()` throws `ValueError` on files with no records
- [#909] Fix incorrect quoting in `VariantFile` contig records
- [#915, #916] Implement `pileup()` for unindexed files and/or SAM files

Backwards incompatible changes:

- The `samtools import` command was removed in `samtools 1.10`, so `pysam` no longer exports a `samimport` function. Use `pysam.view()` instead.

### 1.11.6 Release 0.15.4

Bugfix release. Principal reason for release is to update cython version in order to fix pip install pysam with python 3.8.

- [#879] Fix add\_meta function in libcbcf.pyx, so meta-information lines in header added with this function have double-quoting rules in accordance to rules specified in VCF4.2 and VCF4.3 specifications
- [#863] Force arg to bytes to support non-ASCII encoding
- [#875] Bump minimum Cython version
- [#868] Prevent segfault on Python 2.7 AlignedSegment.compare(other=None)
- [#867] Fix wheel building on TravisCI
- [#863] Force arg to bytes to support non-ASCII encoding
- [#799] disambiguate interpretation of bcf\_read return code
- [#841] Fix silent truncation of FASTQ with bad q strings
- [#846] Prevent segmentation fault on ID, when handling malformed records
- [#829] Run configure with the correct CC/CFLAGS/LDFLAGS env vars

### 1.11.7 Release 0.15.3

Bugfix release.

- [#824] allow reading of UTF-8 encoded text in VCF/BCF files.
- [#780] close all filehandles before opening new ones in pysam\_dispatch
- [#773] do not cache VariantRecord.id to avoid memory leak
- [#781] default of multiple\_iterators=True is changed to False for CRAM files.
- [#825] fix collections.abc import
- [#825] use bcf\_hdr\_format instead of bcf\_hdr\_fmt\_text, fix memcpy bug when setting FORMAT fields.
- [#804] Use HTSLib's kstring\_t, which reallocates and enlarges its memory as needed, rather than a fixed-size char buffer.
- [#814] Build wheels and upload them to PyPI
- [#755] Allow passing flags and arguments to index methods
- [#763] Strip 0 in header check
- [#761] Test Tabix index contents, not the compression

### 1.11.8 Release 0.15.2

Bugfix release.

- [#746] catch pileup iterator out-of-scope segfaults
- [#747] fix faidx fetch with region
- [#748] increase max\_pos to (1<<31)-1
- [#645] Add missing macOS stub files in *MANIFEST.in*, @SoapZA

- [#737] Fix bug in `get_aligned_pairs`, @bkohrn

### 1.11.9 Release 0.15.1

Bugfix release.

- [#716] raise `ValueError` if `tid` is out of range when writing
- [#697] release version using cython 0.28.5 for python 3.7 compatibility

### 1.11.10 Release 0.15.0

This release wraps `htslib/samtools/bcftools` version 1.9.0.

- [#673] permit dash in chromosome name of region string
- [#656] Support *text* when opening a SAM file for writing
- [#658] return `None` in `get_forward_sequence` if sequence not in record
- [#683] allow lower case bases in MD tags
- Ensure that `=` and `X` CIGAR ops are treated the same as `M`

### 1.11.11 Release 0.14.1

This is mostly a bugfix release, though `bcftools` has now also been upgraded to 1.7.0.

- [#621] Add a warning to `count_coverage` when an alignment has an empty `QUAL` field
- [#635] Speed-up of `AlignedSegment.find_intro()`
- treat border case of all bases in pileup column below quality score
- [#634] Fix access to `pileup.reference_sequence`

### 1.11.12 Release 0.14.0

This release wraps `htslib/samtools` versions 1.7.0.

- SAM/BAM/CRAM headers are now managed by a separate `AlignmentHeader` class.
- `AlignmentFile.header.as_dict()` returns an ordered dictionary.
- Use “stop” instead of “end” to ensure consistency to `VariantFile`. The end designations have been kept for backwards compatibility.
- [#611] and [#293] CRAM repeated fetch now works, each iterator reloads index if `multiple_iterators=True`
- [#608] pysam now wraps `htslib` 1.7 and `samtools` 1.7.
- [#580] `reference_name` and `next_reference_name` can now be set to “\*” (will be converted to `None` to indicate an unmapped location)
- [#302] providing no coordinate to `count_coverage` will not count from start/end of contig.
- [#325] @SQ records will be automatically added to header if they are absent from text section of header.
- [#529] add `get_forward_sequence()` and `get_forward_qualities()` methods



- [#577] add `from_string()` and `to_dict()/from_dict()` methods to `AlignedSegment`. Rename `tostring()` to `to_string()` throughout for consistency
- [#589] return `None` from `build_alignment_sequence` if no MD tag is set
- [#528] add `PileupColumn.__len__` method

Backwards incompatible changes:

- `AlignmentFile.header` now returns an `AlignmentHeader` object. Use `AlignmentFile.header.to_dict()` to get the dictionary as previously. Most dictionary accessor methods (`keys()`, `values()`, `__getitem__`, ...) have been implemented to ensure some level of backwards compatibility when only reading.

The rationale for this change is to have consistency between `AlignmentFile` and `VariantFile`.

- `AlignmentFile` and `FastaFile` now raise `IOError` instead of `OSError`

Medium term we plan to have a 1.0 release. The pysam interface has grown over the years and the API is cluttered with deprecated names (`Samfile`, `getname()`, `gettid()`, ...). To work towards this, the next release (0.15.0) will yield `DeprecationWarnings` for any parts of the API that are considered obsolete and will not be in 1.0. Once 1.0 has been reached, we will use semantic versioning.

### 1.11.13 Release 0.13.0

This release wraps `htslib/samtools/bcftools` versions 1.6.0 and contains a series of bugfixes.

- [#544] reading header from remote `TabixFiles` now works.
- [#531] add missing tag types H and A. A python float will now be added as 'f' type instead of 'd' type.
- [#543] use `FastaFile` instead of `Fastafile` in `pileup`.
- [#546] set `is_modified` flag in `setAttribute` so updated attributes are output.
- [#537] allow `tabix` index files to be created in a custom location.
- [#530] add `get_index_statistics()` method

### 1.11.14 Release 0.12.0.1

Bugfix release to solve compilation issue due to missing `bcftools/config.h` file.

### 1.11.15 Release 0.12.0

This release wraps `htslib/samtools/bcftools` versions 1.5.0 and contains a series of bugfixes.

- [#473] A new `FastxRecord` class that can be instantiated from class and modified in-place. Replaces `PersistentFastqProxy`.
- [#521] In `AlignmentFile`, Simplify file detection logic and allow remote index files
  - Removed attempts to guess data and index file names; this is magic left to `htslib`.
  - Removed file existence check prior to opening files with `htslib`
  - Better error checking after opening files that raise the appropriate error (`IOError` for when `errno` is set, `ValueError` otherwise for backward compatibility).
  - Report IO errors when loading an index by name.
  - Allow remote indices (tested using S3 signed URLs).

- Document `filepath_index` and make it an alias for `index_filename`.
- Added a `require_index` parameter to `AlignmentFile`
- [#526] handle unset ref when creating new records
- [#513] fix `bcf_translate` to skip deleted `FORMAT` fields to avoid segfaults
- [#516] expose IO errors via `IOError` exceptions
- [#487] add `tabix line_skip`, remove ‘pileup’ preset
- add `FastxRecord`, replaces `PersistentFastqProxy` (still present for backwards compatibility)
- [#496] upgrade to `htslib/samtools/bcftools` versions 1.5
- add `start/stop` to `AlignmentFile.fetch()` to be consistent with `VariantFile.fetch()`. “end” is kept for backwards compatibility.
- [#512] add `get_index_statistics()` method to `AlignmentFile`.

Upcoming changes:

In the next release we are planning to separate the header information from `AlignmentFile` into a separate class `AlignmentHeader`. This layout is similar to `VariantFile/VariantHeader`. With this change we will ensure that an `AlignedSegment` record will be linked to a header so that chromosome names can be automatically translated from the numeric representation. As a consequence, the way new `AlignedSegment` records are created will need to change as the constructor requires a header:

```
header = pysam.AlignmentHeader(  
    reference_names=["chr1", "chr2"],  
    reference_lengths=[1000, 1000])  
  
read = pysam.AlignedSegment(header)
```

This will affect all code that instantiates `AlignedSegment` objects directly. We have not yet merged to allow users to provide feed-back. The pull-request is here: <https://github.com/pysam-developers/pysam/pull/518> Please comment on github.

### 1.11.16 Release 0.11.2.2

Bugfix release to address two issues:

- Changes in 0.11.2.1 broke the GTF/GFF3 parser. Corrected and more tests have been added.
- [#479] Correct `VariantRecord` edge cases described in issue

### 1.11.17 Release 0.11.2.1

Release to fix release tar-ball containing 0.11.1 pre-compiled C-files.

### 1.11.18 Release 0.11.2

This release wraps `htslib/samtools/bcftools` versions 1.4.1 in response to a security fix in these libraries. Additionally the following issues have been fixed:

- [#452] add GFF3 support for `tabix` parsers
- [#461] Multiple fixes related to `VariantRecordInfo` and handling of `INFO/END`

- [#447] limit query name to 251 characters (only partially addresses issue)

VariantFile and related object fixes

- Restore VariantFile.\_\_dealloc\_\_
- Correct handling of bcf\_str\_missing in bcf\_array\_to\_object and bcf\_object\_to\_array
- Added update() and pop() methods to some dict-like proxy objects
- scalar INFO entries could not be set again after being deleted
- VariantRecordInfo.\_\_delitem\_\_ now allows unset flags to be deleted without raising a KeyError
- Multiple other fixes for VariantRecordInfo methods
- INFO/END is now accessible only via VariantRecord.stop and VariantRecord.rlen. Even if present behind the scenes, it is no longer accessible via VariantRecordInfo.
- Add argument to issue a warning instead of an exception if input appears to be truncated

Other features and fixes:

- Make AlignmentFile \_\_dealloc\_\_ and close more stringent
- Add argument AlignmentFile to issue a warning instead of an exception if input appears to be truncated

### 1.11.19 Release 0.11.1

Bugfix release

- [#440] add deprecated 'always' option to infer\_query\_length for backwards compatibility.

### 1.11.20 Release 0.11.0

This release wraps the latest versions of htslib/samtools/bcftools and implements a few bugfixes.

- [#413] Wrap HTSlib/Samtools/BCFtools 1.4
- [#422] Fix missing pysam.sort.usage() message
- [#411] Fix BGZfile initialization bug
- [#412] Add seek support for BGZFile
- [#395] Make BGZfile iterable
- [#433] Correct getQueryEnd
- [#419] Export SAM enums such as pysam.CMATCH
- [#415] Fix access by tid in AlignmentFile.fetch()
- [#405] Writing SAM now outputs a header by default.
- [#332] split infer\_query\_length(always) into infer\_query\_length and infer\_read\_length

### 1.11.21 Release 0.10.0

This release implements further functionality in the VariantFile API and includes several bugfixes:

- treat special case -c option in samtools view outputs to stdout even if -o given, fixes #315
- permit reading BAM files with CSI index, closes #370

- raise Error if query name exceeds maximum length, fixes #373
- new method to compute hash value for AlignedSegment
- AlignmentFile, VariantFile and TabixFile all inherit from HTSFile
- Avoid segfault by detecting out of range reference\_id and next\_reference in AlignedSegment.tostring
- Issue #355: Implement streams using file descriptors for VariantFile
- upgrade to htplib 1.3.2
- fix compilation with musl libc
- Issue #316, #360: Rename all Cython modules to have lib as a prefix
- Issue #332, hardclipped bases in cigar included by pysam.AlignedSegment.infer\_query\_length()
- Added support for Python 3.6 filename encoding protocol
- Issue #371, fix incorrect parsing of scalar INFO and FORMAT fields in VariantRecord
- Issue #331, fix failure in VariantFile.reset() method
- Issue #314, add VariantHeader.new\_record(), VariantFile.new\_record() and VariantRecord.copy() methods to create new VariantRecord objects
- Added VariantRecordFilter.add() method to allow setting new VariantRecord filters
- Preliminary (potentially unsafe) support for removing and altering header metadata
- Many minor fixes and improvements to VariantFile and related objects

Please note that all internal cython extensions now have a lib prefix to facilitate linking against pysam extension modules. Any user cython extensions using cimport to import pysam definitions will need changes, for example:

```
cimport pysam.csamtools
```

will become:

```
cimport pysam.libcsamtools
```

### 1.11.22 Release 0.9.1

This is a bugfix release addressing some installation problems in pysam 0.9.0, in particular:

- patch included htplib to work with older libcurl versions, fixes #262.
- do not require cython for python 3 install, fixes #260
- FastqFile does not accept filepath\_index any more, see #270
- add AlignedSegment.get\_cigar\_stats method.
- py3 bugfix in VariantFile.subset\_samples, fixes #272
- add missing sysconfig import, fixes #278
- do not redirect stdout, but instead write to a separately created file. This should resolve issues when pysam is used in notebooks or other environments that redirect stdout.
- wrap htplib-1.3.1, samtools-1.3.1 and bcftools-1.3.1
- use bgzf throughout instead of gzip
- allow specifying a fasta reference for CRAM file when opening for both read and write, fixes #280

### 1.11.23 Release 0.9.0

#### Overview

The 0.9.0 release upgrades htlib to htlib 1.3 and numerous other enhancements and bugfixes. See below for a detailed list.

**Htlib 1.3** comes with additional capabilities for remote file access which depend on the presence of optional system libraries. As a consequence, the installation script `setup.py` has become more complex. For an overview, see [Installing pysam](#). We have tested installation on linux and OS X, but could not capture all variations. It is possible that a 0.9.1 release might follow soon addressing installation issues.

The `VariantFile` class provides access to `vcf` and `bcf` formatted files. The class is certainly usable and interface is reaching completion, but the API and the functionality is subject to change.

#### Detailed release notes

- upgrade to htlib 1.3
- python 3 compatibility tested throughout.
- added a first set of bcftools commands in the `pysam.bcftools` submodule.
- samtools commands are now in the `pysam.samtools` module. For backwards compatibility they are still imported into the `pysam` namespace.
- samtools/bcftools return stdout as a single (byte) string. As output can be binary (VCF.gz, BAM) this is necessary to ensure py2/py3 compatibility. To replicate the previous behaviour in py2.7, use:

```
pysam.samtools.view(self,filename).splitlines(True)
```

- `get_tags()` returns the tag type as a character, not an integer (#214)
- `TabixFile` now raises `ValueError` on indices created by tabix <1.0 (#206)
- improve OSX installation and develop mode
- `FastxIterator` now handles empty sequences (#204)
- `TabixFile.isremote` is not `TabixFile.is_remote` in line with `AlignmentFile`
- `AlignmentFile.count()` has extra optional argument `read_callback`
- **setup.py has been changed to:**
  - install a single builtin htlib library. Previously, each pysam module contained its own version. This reduces compilation time and code bloat.
  - run `configure` for the builtin htlib library in order to detect optional libraries such as `libcurl`. `Configure` behaviour can be controlled by setting the environment variable `HTSLIB_CONFIGURE_OPTIONS`.
- `get_reference_sequence()` now returns the reference sequence and not something looking like it. This bug had effects on `get_aligned_pairs(with_seq=True)`, see #225. If you have relied on `get_aligned_pairs(with_seq=True)` in `pysam-0.8.4`, please check your results.
- improved autodetection of file formats in `AlignmentFile` and `VariantFile`.

### 1.11.24 Release 0.8.4

This release contains numerous bugfixes and a first implementation of a pythonic interface to VCF/BCF files. Note that this code is still incomplete and preliminary, but does offer a nearly complete immutable Pythonic interface to VCF/BCF metadata and data with reading and writing capability.

Potential issues when upgrading from v0.8.3:

- binary tags are now returned as python arrays
- renamed several methods for pep8 compatibility, old names still retained for backwards compatibility, but should be considered deprecated.
  - `gettid()` is now `get_tid()`
  - `gettrname()` is now `get_reference_name()`
  - `parseRegion()` is now `parse_region()`
- some methods have changed for pep8 compatibility without the old names being present:
  - `fromQualityString()` is now `qualitystring_to_array()`
  - `toQualityString()` is now `qualities_to_qualitystring()`
- `faidx` now returns strings and not binary strings in py3.
- The cython components have been broken up into smaller files with more specific content. This will affect users using the cython interfaces.

Edited list of commit log changes:

- fixes `AlignmentFile.check_index` to return `True`
- add RG/PM header tag - closes #179
- add `with_seq` option to `get_aligned_pairs`
- use `char *` inside `reconstituteReferenceSequence`
- add soft clipping for `get_reference_sequence`
- add `get_reference_sequence`
- `queryEnd` now computes length from cigar string if no sequence present, closes #176
- tolerate missing space at end of gtf files, closes #162
- do not raise `Error` when receiving output on `stderr`
- add docu about fetching without index, closes #170
- `FastaFile` and `FastxFile` now return strings in python3, closes #173
- py3 compat: relative -> absolute imports.
- add `reference_name` and `next_reference_name` attributes to `AlignedSegment`
- add function signatures to `cvcf` cython. Added note about other VCF code.
- add context manager functions to `FastaFile`
- add `reference_name` and `next_reference_name` attributes to `AlignedSegment`
- `PileupColumn` also gets a `reference_name` attribute.
- add context manager functions to `FastaFile`
- `TabixFile.header` for remote files raises `AttributeError`, fixes #157

- add context manager interface to TabixFile, closes #165
- change ctypesdef enum to typedef enum for cython 0.23
- add function signatures to cvcf cython, also added note about other VCF code
- remove exception for custom upper-case header record tags.
- rename VALID\_HEADER\_FIELDS to KNOWN\_HEADER\_FIELDS
- fix header record tag parsing for custom tags.
- use cython.str in count\_coverage, fixes #141
- avoid maketrans (issues with python3)
- refactoring: AlignedSegment now in separate module
- do not execute remote tests if URL not available
- fix the unmapped count, incl reads with no SQ group
- add raw output to tags
- added write access for binary tags
- bugfix in call to resize
- implemented writing of binary tags from arrays
- implemented convert\_binary\_tag to use arrays
- add special cases for reads that are unmapped or whose mates are unmapped.
- rename TabProxies to ctabixproxies
- remove underscores from utility functions
- move utility methods into cutils
- remove callback argument to fetch - closes #128
- avoid calling close in dealloc
- add unit tests for File object opening
- change AlignmentFile.open to filepath\_or\_object
- implement copy.copy, close #65
- add chaching of array attributes in AlignedSegment, closes #121
- add export of Fastafile
- remove superfluous pysam\_dispatch
- use persist option in FastqFile
- get\_tag: expose tag type if requested with *with\_value\_type*
- fix to allow reading vcf record info via tabix-based vcf reader
- add pFastqProxy and pFastqFile objects to make it possible to work with multiple fastq records per file handle, unlike FastqProxy/FastqFile.
- release GIL around htlib IO operations
- More work on read/write support, API improvements
- add *phased* property on *VariantRecordSample*

- add mutable properties to VariantRecord
- BCF fixes and start of read/write support
- VariantHeaderRecord objects now act like mappings for attributes.
- add VariantHeader.alts dict from alt ID->Record.
- Bug fix to strong representation of structured header records.
- VariantHeader is now mutable

### 1.11.25 Release 0.8.3

- samtools command now accept the “catch\_stdout” option.
- get\_aligned\_pairs now works for soft-clipped reads.
- query\_position is now None when a PileupRead is not aligned to a particular position.
- AlignedSegments are now comparable and hashable.

### 1.11.26 Release 0.8.2.1

- Installation bugfix release.

### 1.11.27 Release 0.8.2

- Pysam now wraps htslib 1.2.1 and samtools version 1.2.
- Added CRAM file support to pysam.
- **New alignment info interface.**
  - opt() and setTag are deprecated, use get\_tag() and set\_tag() instead.
  - added has\_tag()
  - tags is deprecated, use get\_tags() and set\_tags() instead.
- FastqFile is now FastxFile to reflect that the latter permits iteration over both fastq- and fasta-formatted files.
- A Cython wrapper for htslib VCF/BCF reader/writer. The wrapper provides a nearly complete Pythonic interface to VCF/BCF metadata with reading and writing capability. However, the interface is still incomplete and preliminary and lacks capability to mutate the resulting data.

### 1.11.28 Release 0.8.1

- Pysam now wraps htslib and samtools versions 1.1.
- Bugfixes, most notable: \* issue #43: uncompressed BAM output \* issue #42: skip tests requiring network if none available \* issue #19: multiple iterators can now be made to work on the same tabix file \* issue #24: All strings returned from/passed to the pysam API are now unicode in python 3 \* issue #5: type guessing for lists of integers fixed
- API changes for consistency. The old API is still present, but deprecated. In particular:
  - Tabixfile -> TabixFile
  - Fastafile -> FastaFile



- Fastqfile -> FastqFile
- Samfile -> AlignmentFile
- **AlignedRead -> AlignedSegment**
  - \* qname -> query\_name
  - \* tid -> reference\_id
  - \* pos -> reference\_start
  - \* mapq -> mapping\_quality
  - \* rnext -> next\_reference\_id
  - \* pnext -> next\_reference\_start
  - \* cigar -> cigartuples
  - \* cigarstring -> cigarstring
  - \* tlen -> template\_length
  - \* seq -> query\_sequence
  - \* qual -> query\_qualities, now returns array
  - \* qqqual -> query\_alignment\_qualities, now returns array
  - \* tags -> tags
  - \* alen -> reference\_length, reference is always “alignment”, so removed
  - \* aend -> reference\_end
  - \* rlen -> query\_length
  - \* query -> query\_alignment\_sequence
  - \* qstart -> query\_alignment\_start
  - \* qend -> query\_alignment\_end
  - \* qlen -> query\_alignment\_length
  - \* mrnm -> next\_reference\_id
  - \* mpos -> next\_reference\_start
  - \* rname -> reference\_id
  - \* isize -> template\_length
  - \* blocks -> get\_blocks()
  - \* aligned\_pairs -> get\_aligned\_pairs()
  - \* inferred\_length -> infer\_query\_length()
  - \* positions -> get\_reference\_positions()
  - \* overlap() -> get\_overlap()
- All strings are now passed to or received from the pysam API as strings, no more bytes.

**Other changes:**

- AlignmentFile.fetch(reopen) option is now multiple\_iterators. The default changed to not reopen a file unless requested by the user.

- `FastaFile.getReferenceLength` is now `FastaFile.get_reference_length`

Backwards incompatible changes

- Empty cigarstring now returns `None` (instead of `''`)
- Empty cigar now returns `None` (instead of `[]`)
- When using the extension classes in cython modules, `AlignedRead` needs to be substituted with `AlignedSegment`.
- `fancy_str()` has been removed
- `qual`, `qqual` now return arrays

### 1.11.29 Release 0.8.0

- **Disabled features**
  - `IteratorColumn.setMask()` disabled as `htslib` does not implement this functionality?
- **Not implemented yet:**
  - reading SAM files without header

Tabix files between version 0.7.8 and 0.8.0 are not compatible and need to be re-indexed.

While version 0.7.8 and 0.8.0 should be mostly compatible, there are some notable exceptions:

- tabix iterators will fail if there are comments in the middle or the end of a file.
- tabix raises always `ValueError` for invalid intervals. Previously, different types of errors were raised (`KeyError`, `IndexError`, `ValueError`) depending on the type of invalid intervals (missing chromosome, out-of-range, malformed interval).

### 1.11.30 Release 0.7.8

- added `AlignedRead.setTag` method
- added `AlignedRead.blocks`
- unsetting CIGAR strings is now possible
- empty CIGAR string returns empty list
- added reopen flag to `Samfile.fetch()`
- various bugfixes

### 1.11.31 Release 0.7.7

- added `Fastafire.references`, `.nreferences` and `.lengths`
- `tabix_iterator` now uses `kseq.h` for python 2.7

### 1.11.32 Release 0.7.6

- added inferred\_length property
- issue 122: MACOSX getline missing, now it works?
- seq and qual can be set None
- added Fastqfile

### 1.11.33 Release 0.7.5

- switch to samtools 0.1.19
- issue 122: MACOSX getline missing
- issue 130: clean up tempfiles
- various other bugfixes

### 1.11.34 Release 0.7.4

- further bugfixes to setup.py and package layout

### 1.11.35 Release 0.7.3

- further bugfixes to setup.py
- upgraded distribute\_setup.py to 0.6.34

### 1.11.36 Release 0.7.2

- bugfix in installer - failed when cython not present
- changed installation locations of shared libraries

### 1.11.37 Release 0.7.1

- bugfix: missing PP tag PG records in header
- added pre-built .c files to distribution

### 1.11.38 Release 0.7

- switch to tabix 0.2.6
- added cigarstring field
- python3 compatibility
- added B tag handling
- added check\_sq and check\_header options to Samfile.\_\_init\_\_
- added lazy GTF parsing to tabix

- reworked support for VCF format parsing
- bugfixes

### **1.11.39 Release 0.6**

- switch to samtools 0.1.18
- various bugfixes
- removed references to deprecated ‘samtools pileup’ functionality
- AlignedRead.tags now returns an empty list if there are no tags.
- added pnext, rnext and tlen

### **1.11.40 Release 0.5**

- switch to samtools 0.1.16 and tabix 0.2.5
- improved tabix parsing, added vcf support
- re-organized code to permit linking against pysam
- various bugfixes
- added Samfile.positions and Samfile.overlap

### **1.11.41 Release 0.4**

- switch to samtools 0.1.12a and tabix 0.2.3
- added snp and indel calling.
- switch from pyrex to cython
- changed handling of samtools stderr
- various bugfixes
- added Samfile.count and Samfile.mate
- deprecated AlignedRead.rname, added AlignedRead.tid

### **1.11.42 Release 0.3**

- switch to samtools 0.1.8
- added support for tabix files
- numerous bugfixes including
- permit simultaneous iterators on the same file
- working access to remote files

## 1.12 Benchmarking

Latest benchmarking results:

----- benchmark: 57 tests -----						
-----						
Name (time in us)	Max	IQR	Mean	StdDev	Min	
Median			Outliers	OPS	Rounds	
Iterations						
-----						
test_set_binary_tag					93.8382 (1.	
→0)	199.8786 (1.0)		96.1554 (1.0)	2.6241 (1.0)		
→95.7036 (1.0)		1.2442 (1.0)	276;303	10,399.8372 (1.0)	3170	
→1						
test_fasta_iteration_long_sequences_without_persistence					145.3292 (1.	
→55)	296.4940 (1.48)		176.5367 (1.84)	30.0896 (11.47)		
→167.2544 (1.75)		25.1532 (20.22)	840;579	5,664.5438 (0.54)	4972	
→1						
test_fasta_iteration_long_sequences_as_file					149.0638 (1.	
→59)	312.6319 (1.56)		177.2990 (1.84)	30.5218 (11.63)		
→168.6383 (1.76)		27.7516 (22.30)	669;510	5,640.1883 (0.54)	4493	
→1						
test_fasta_iteration_long_sequences					150.3211 (1.	
→60)	429.9153 (2.15)		176.7384 (1.84)	30.7021 (11.70)		
→167.5002 (1.75)		21.7482 (17.48)	682;647	5,658.0803 (0.54)	4845	
→1						
test_fasta_iteration_short_sequences_as_file					190.6604 (2.	
→03)	395.8736 (1.98)		216.6984 (2.25)	32.8710 (12.53)		
→208.9385 (2.18)		16.8057 (13.51)	291;326	4,614.7086 (0.44)	3205	
→1						
test_read_python_uncompressed					214.1297 (2.	
→28)	450.2051 (2.25)		234.6956 (2.44)	33.3642 (12.71)		
→223.4913 (2.34)		15.0129 (12.07)	140;221	4,260.8375 (0.41)	2043	
→1						
test_fastq_iteration_short_sequences_as_file					217.5961 (2.	
→32)	420.1643 (2.10)		238.0264 (2.48)	30.4469 (11.60)		
→228.8874 (2.39)		15.5573 (12.50)	209;235	4,201.2146 (0.40)	2877	
→1						
test_iterate_file_uncompressed					225.5719 (2.	
→40)	481.0989 (2.41)		249.7603 (2.60)	40.7266 (15.52)		
→239.5548 (2.50)		15.9908 (12.85)	209;327	4,003.8387 (0.38)	3627	
→1						
test_iterate_file_compressed					257.5517 (2.	
→74)	576.6843 (2.89)		287.2536 (2.99)	49.8803 (19.01)		
→277.4149 (2.90)		14.3824 (11.56)	126;248	3,481.2446 (0.33)	3023	
→1						
test_iterate_generic_uncompressed					344.8855 (3.	
→68)	712.5959 (3.57)		370.9369 (3.86)	46.2477 (17.62)		
→365.0384 (3.81)		15.7915 (12.69)	54;83	2,695.8763 (0.26)	2240	
→1						
test_iterate_parsed_uncompressed					349.3819 (3.	
→72)	765.3460 (3.83)		378.1446 (3.93)	54.2199 (20.66)		
→369.5488 (3.86)		15.4320 (12.40)	48;69	2,644.4910 (0.25)	1638	
→1						

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```

test_read_python_compressed                                462.0645 (4.
→92)      836.2234 (4.18)      493.6158 (5.13)      51.2946 (19.55)      906
→485.5469 (5.07)      11.4385 (9.19)      28;46      2,025.8670 (0.19)
→
→      1
test_iterate_parsed_compressed                            586.5730 (6.
→25)      1,050.1631 (5.25)      632.5464 (6.58)      82.6438 (31.49)
→608.1080 (6.35)      20.1799 (16.22)      115;143      1,580.9117 (0.15)      1474
→
→      1
test_iterate_generic_compressed                          587.7707 (6.
→26)      1,093.4286 (5.47)      639.6830 (6.65)      85.2593 (32.49)
→612.6408 (6.40)      19.9433 (16.03)      105;144      1,563.2743 (0.15)      1260
→
→      1
test_fasta_iteration_short_sequences_without_persistence  725.2563 (7.
→73)      1,091.6069 (5.46)      774.6549 (8.06)      53.0901 (20.23)
→751.8455 (7.86)      45.0788 (36.23)      172;110      1,290.8974 (0.12)      1276
→
→      1
test_read_binary_tag                                     817.2598 (8.
→71)      1,232.0559 (6.16)      902.7002 (9.39)      87.7807 (33.45)
→871.5261 (9.11)      17.4227 (14.00)      39;55      1,107.7875 (0.11)      331
→
→      1
test_fastq_iteration_short_sequences_without_persistence  840.3640 (8.
→96)      1,201.5756 (6.01)      870.9679 (9.06)      35.8709 (13.67)
→866.1682 (9.05)      15.9768 (12.84)      53;85      1,148.1480 (0.11)      1124
→
→      1
test_count_number_lines_from_sam_with_pysam              2,755.6140
→(29.37)      9,656.9806 (48.31)      2,963.2206 (30.82)      729.0077 (277.81)
→2,814.3115 (29.41)      106.2388 (85.38)      8;14      337.4707 (0.03)      281
→
→      1
test_fasta_iteration_short_sequences                    2,905.5942
→(30.96)      3,674.1439 (18.38)      2,982.3892 (31.02)      90.9585 (34.66)
→2,941.2108 (30.73)      96.1348 (77.26)      27;7      335.3016 (0.03)      302
→
→      1
test_fastq_iteration_short_sequences                    3,601.3145
→(38.38)      4,065.5769 (20.34)      3,671.8361 (38.19)      71.5348 (27.26)
→3,635.1625 (37.98)      81.5415 (65.53)      43;10      272.3433 (0.03)      243
→
→      1
test_count_number_lines_from_bam_with_pysam              4,178.8872
→(44.53)      12,063.5536 (60.35)      4,395.9713 (45.72)      794.3991 (302.73)
→4,240.1757 (44.31)      67.0198 (53.86)      6;19      227.4810 (0.02)      205
→
→      1
test_build_depth_from_bam_with_pysam                    6,290.2980
→(67.03)      6,788.0806 (33.96)      6,420.3862 (66.77)      126.5447 (48.22)
→6,357.6270 (66.43)      94.3104 (75.80)      35;29      155.7539 (0.01)      145
→
→      1
test_build_depth_with_filter_from_bam_with_pysam         6,935.8926
→(73.91)      8,309.5767 (41.57)      7,085.6801 (73.69)      221.5069 (84.41)
→7,005.6170 (73.20)      74.4388 (59.83)      14;27      141.1297 (0.01)      139
→
→      1
test_build_query_positions_from_bam_with_pysam           8,729.2437
→(93.02)      16,836.6525 (84.23)      9,173.2902 (95.40)      1,057.4321 (402.97)
→8,885.4395 (92.84)      264.7634 (212.79)      3;9      109.0121 (0.01)      103
→
→      1
test_build_mapping_qualities_from_bam_with_pysam        8,805.0570
→(93.83)      15,829.8910 (79.20)      9,274.7475 (96.46)      964.6925 (367.63)
→9,004.7438 (94.09)      354.9103 (285.24)      4;9      107.8196 (0.01)      106
→
→      1
test_build_query_qualities_from_bam_with_pysam           8,947.6798
→(95.35)      16,756.8158 (83.83)      9,272.9117 (96.44)      1,008.7759 (401.45)
→9,017.0493 (94.22)      217.2068 (174.57)      3;5      107.8410 (0.01)      94
→
→      1

```

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```

test_build_query_bases_from_bam_with_samtoolspysam          9,149.0448_
→ (97.50)      9,776.0092 (48.91)      9,208.3405 (95.77)      86.1889 (32.85)      _
→ 9,190.2809 (96.03)      34.1963 (27.48)      5;7      108.5972 (0.01)      84_
→      1
test_count_number_lines_from_bam_with_samtoolspipe          10,431.4052_
→ (111.16)     13,431.7447 (67.20)     11,052.2360 (114.94)     445.8241 (169.90)     _
→ 11,014.8042 (115.09)     492.6044 (395.91)      17;3      90.4794 (0.01)      _
→ 90      1
test_count_number_lines_from_bam_with_samtools          12,057.8520_
→ (128.50)     13,537.5429 (67.73)     12,595.0708 (130.99)     381.1799 (145.26)     _
→ 12,499.5783 (130.61)     457.8107 (367.94)      25;1      79.3961 (0.01)      _
→ 73      1
test_count_number_lines_from_sam_with_samtoolspipe          12,434.8756_
→ (132.51)     14,234.1983 (71.21)     13,034.1032 (135.55)     371.0373 (141.40)     _
→ 13,049.9089 (136.36)     464.8147 (373.57)      21;3      76.7218 (0.01)      _
→ 72      1
test_build_query_bases_from_bam_with_pysam          12,811.6887_
→ (136.53)     19,972.7099 (99.92)      13,268.7908 (137.99)     1,103.9459 (420.70)     _
→ 13,023.1632 (136.08)     202.3596 (162.64)      4;6      75.3648 (0.01)      _
→ 76      1
test_build_pileup_from_bam_with_samtoolspipe          13,480.7490_
→ (143.66)     15,413.4836 (77.11)      13,968.3817 (145.27)     362.1282 (138.00)     _
→ 13,917.4843 (145.42)     447.9736 (360.04)      12;3      71.5903 (0.01)      _
→ 66      1
test_count_number_lines_from_sam_with_samtools          13,963.2300_
→ (148.80)     15,936.2238 (79.73)      14,442.5627 (150.20)     340.4846 (129.75)     _
→ 14,395.4996 (150.42)     339.3036 (272.70)      12;2      69.2398 (0.01)      _
→ 59      1
test_build_pileup_from_bam_with_samtoolsshell          15,140.2969_
→ (161.34)     16,469.8567 (82.40)      15,525.9034 (161.47)     256.0741 (97.59)      _
→ 15,526.7641 (162.24)     318.5044 (255.98)      13;2      64.4085 (0.01)      _
→ 51      1
test_build_depth_from_bam_with_samtoolsshell          15,291.4841_
→ (162.96)     16,387.9916 (81.99)      15,656.5365 (162.83)     217.2420 (82.79)      _
→ 15,609.9945 (163.11)     258.9412 (208.11)      16;2      63.8711 (0.01)      _
→ 57      1
test_build_query_bases_from_bam_with_samtoolspipe          15,478.0652_
→ (164.94)     17,068.8462 (85.40)      16,072.5749 (167.15)     327.6668 (124.87)     _
→ 16,040.5049 (167.61)     428.1597 (344.11)      18;2      62.2178 (0.01)      _
→ 60      1
test_build_query_bases_from_bam_with_samtoolsshell          15,765.5794_
→ (168.01)     17,102.8059 (85.57)      16,305.4955 (169.57)     367.6865 (140.12)     _
→ 16,256.2486 (169.86)     593.9230 (477.34)      20;0      61.3290 (0.01)      _
→ 57      1
test_build_depth_from_bam_with_samtoolspipe          16,024.7944_
→ (170.77)     25,804.2309 (129.10)     17,041.3497 (177.23)     1,522.0046 (580.01)     _
→ 16,726.8887 (174.78)     767.1015 (616.52)      1;1      58.6808 (0.01)      _
→ 40      1
test_build_query_qualities_from_bam_with_samtoolspipe          16,275.7467_
→ (173.44)     18,501.0433 (92.56)      17,262.6517 (179.53)     642.2656 (244.76)     _
→ 17,255.3696 (180.30)     1,060.3429 (852.20)      20;0      57.9285 (0.01)      _
→ 51      1
test_build_pileup_from_bam_with_pysam          16,632.5681_
→ (177.25)     19,306.6560 (96.59)      17,037.7162 (177.19)     531.0751 (202.38)     _
→ 16,870.8330 (176.28)     279.1677 (224.37)      3;4      58.6933 (0.01)      _
→ 32      1
test_build_mapping_qualities_from_bam_with_samtoolspipe          17,398.3518_
→ (185.41)     18,481.6569 (92.46)      17,832.1261 (185.45)     294.9103 (continues on next page)
→ 17,837.8206 (186.39)     511.8400 (411.37)      21;0      56.0786 (0.01)      _
→ 52      1

```

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```

test_build_query_names_from_bam_with_pysam          31,534.0199_
→ (336.05) 38,316.2647 (191.70) 32,677.2804 (339.84) 1,371.6101 (522.70) _
→ 32,179.0325 (336.24) 1,392.1391 (>1000.0) 3;1 30.6023 (0.00) _
→ 30 1
test_fetch_plain          43,747.1233_
→ (466.20) 48,690.7829 (243.60) 44,573.8085 (463.56) 1,170.8588 (446.20) _
→ 44,067.5411 (460.46) 1,436.5837 (>1000.0) 3;1 22.4347 (0.00) _
→ 22 1
test_build_query_positions_from_bam_with_samtoolspipe 46,316.5548_
→ (493.58) 53,700.3577 (268.66) 48,235.6640 (501.64) 1,861.0317 (709.21) _
→ 47,625.2194 (497.63) 2,603.7074 (>1000.0) 6;1 20.7315 (0.00) _
→ 21 1
test_build_query_bases_with_reference_from_bam_with_samtoolspysam 51,565.1479_
→ (549.51) 53,523.1121 (267.78) 51,778.6650 (538.49) 424.4621 (161.76) _
→ 51,656.8925 (539.76) 110.5051 (88.81) 1;4 19.3130 (0.00) _
→ 20 1
test_build_query_bases_with_reference_from_bam_with_pysam 58,850.6740_
→ (627.15) 62,164.5451 (311.01) 60,161.6779 (625.67) 1,120.6101 (427.05) _
→ 59,595.2785 (622.71) 1,995.0196 (>1000.0) 7;0 16.6219 (0.00) _
→ 16 1
test_iterate_file_large_uncompressed          59,419.9076_
→ (633.22) 69,053.0874 (345.48) 62,825.7126 (653.38) 3,805.1150 (>1000.0) _
→ 60,805.1391 (635.35) 6,407.6949 (>1000.0) 3;0 15.9170 (0.00) _
→ 14 1
test_iterate_file_large_compressed          63,986.9571_
→ (681.89) 74,156.9120 (371.01) 68,370.8835 (711.05) 3,254.6200 (>1000.0) _
→ 67,221.2075 (702.39) 4,770.3525 (>1000.0) 5;0 14.6261 (0.00) _
→ 16 1
test_read_python_large_uncompressed          67,611.8080_
→ (720.51) 89,112.1533 (445.83) 73,631.8916 (765.76) 5,978.7052 (>1000.0) _
→ 71,702.4822 (749.21) 7,856.0165 (>1000.0) 2;1 13.5811 (0.00) _
→ 13 1
test_fetch_parsed          72,237.5344_
→ (769.81) 81,976.9856 (410.13) 74,540.4099 (775.21) 2,326.3668 (886.54) _
→ 74,016.3419 (773.39) 1,579.0667 (>1000.0) 1;1 13.4155 (0.00) _
→ 14 1
test_build_query_bases_from_bam_with_pysam_pileups 75,841.3766_
→ (808.21) 87,975.1425 (440.14) 79,393.9784 (825.68) 3,516.2458 (>1000.0) _
→ 79,346.7313 (829.09) 5,217.5033 (>1000.0) 2;0 12.5954 (0.00) _
→ 13 1
test_build_query_bases_with_reference_from_bam_with_samtoolspipe 131,162.9396 (>
→ 1000.0) 132,858.8147 (664.70) 131,838.0199 (>1000.0) 595.6090 (226.98) 131,
→ 801.5633 (>1000.0) 956.9665 (769.11) 2;0 7.5851 (0.00) 8 _
→ 1
test_iterate_parsed_large_uncompressed          132,991.8914 (>
→ 1000.0) 140,153.4099 (701.19) 134,101.5892 (>1000.0) 2,452.3161 (934.54) 133,
→ 218.1590 (>1000.0) 396.3616 (318.56) 1;1 7.4570 (0.00) 8 _
→ 1
test_iterate_generic_large_uncompressed          134,743.7277 (>
→ 1000.0) 142,329.4526 (712.08) 138,167.4954 (>1000.0) 3,346.7773 (>1000.0) 136,
→ 839.1849 (>1000.0) 6,541.1879 (>1000.0) 4;0 7.2376 (0.00) 8 _
→ 1
test_read_python_large_compressed          175,127.6311 (>
→ 1000.0) 190,855.1529 (954.86) 181,702.9339 (>1000.0) 5,756.2207 (>1000.0) 181,
→ 221.0185 (>1000.0) 8,577.6616 (>1000.0) 2;0 5.5035 (0.00) 6 _
→ 1
test_iterate_parsed_large_compressed          231,405.7611 (>
→ 1000.0) 243,728.8519 (>1000.0) 239,037.8296 (>1000.0) 5,212.7778 (>1000.0) 231,
→ 544.3324 (>1000.0) 8,062.6113 (>1000.0) 1;0 4.1834 (0.00) 5 _
→ 1

```

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```

test_iterate_generic_large_compressed                235,042.3876 (>
↪1000.0)  256,518.8371 (>1000.0)  242,535.5468 (>1000.0)  8,272.5197 (>1000.0)  240,
↪163.0748 (>1000.0)  8,360.1568 (>1000.0)          1;0      4.1231 (0.00)          5  ↪
↪      1
-----
↪-----
↪-----
↪-----

```

## 1.13 Glossary

**BAM** Binary SAM format. BAM files are binary formatted, indexed and allow random access.

**BCF** Binary *VCF*.

**BED** Browser Extensible Data format. A text file format used to store genomic *regions* as coordinates and associated notations.

**bgzip** Utility in the *htslib* package to block compress genomic data files.

**cigar** Stands for Compact Idiosyncratic Gapped Alignment Report and represents a compressed (run-length encoded) pairwise alignment format. It was first defined by the Exonerate Aligner, but was later adapted and adopted as part of the *SAM* standard and many other aligners. In the Python API, the cigar alignment is presented as a list of tuples (*operation*, *length*). For example, the tuple [ (0, 3), (1, 5), (0, 2) ] refers to an alignment with 3 matches, 5 insertions and another 2 matches.

**column**

The portion of reads aligned to a single base in the *reference* sequence.

**contig** The sequence that a *tid* refers to. For example chr1, contig123.

**CRAM** CRAM is a binary format representing the same sequence alignment information as SAM and BAM, but offering significantly better lossless compression than BAM.

**faidx** Utility in the *samtools* package to index *fasta* formatted files.

**FASTA** Simple text format containing sequence data, with only the bare minimum of metadata. Typically used for reference sequence data.

**FASTQ** Simple text format containing sequence data and associated base qualities.

**fetching** Retrieving all mapped reads mapped to a *region*.

**genotype**

An individual's collection of genes. It can also refer to the two alleles inherited for a particular gene.

**GTF**

The Gene Transfer Format is a file format used to hold information about gene structure.

**hard clipping**

**hard clipped** In hard clipped reads, part of the sequence has been removed prior to alignment. That only a subsequence is aligned might be recorded in the *cigar* alignment, but the removed sequence will not be part of the alignment record, in contrast to *soft clipped* reads.

**pileup** Pileup

**reference** Synonym for contig.

**region** A genomic region, stated relative to a *reference* sequence. A region consists of reference name ('chr1'), start (15000), and end (20000). Start and end can be omitted for regions spanning a whole chromosome. If end is missing, the region will span from start to the end of the chromosome. Within pysam, coordinates are 0-based half-open intervals, i.e., the first base of the reference sequence is numbered zero; and the base at position start is part of the interval, but the base at end is not.

When a region is written as a single string using *samtools*-compatible notation, e.g., 'chr1:15001-20000', the string's coordinates instead represent a 1-based closed interval, i.e., both (1-based) positions 15,001 and 20,000 are part of the interval. (This example denotes the same 5,000-base region as the example in the previous paragraph.)

**SAM** A textual format for storing genomic alignment information.

**sam file** A file containing aligned reads. The *sam file* can either be a *BAM* file or a *TAM* file.

**soft clipping**

**soft clipped** In alignments with soft clipping part of the query sequence are not aligned. The unaligned query sequence is still part of the alignment record. This is in difference to *hard clipped* reads.

**tabix** Utility in the *htslib* package to index *bgzip* compressed files.

**tabix file** A sorted, compressed and indexed tab-separated file created by the command line tool *tabix* or the commands `tabix_compress()` and `tabix_index()`. The file is indexed by chromosomal coordinates.

**tabix row** A row in a *tabix file*. Fields within a row are tab-separated.

**TAM** Text SAM file. TAM files are human readable files of tab-separated fields. TAM files do not allow random access.

**target** The sequence that a read has been aligned to. Target sequences have bot a numerical identifier (*tid*) and an alphanumeric name (*Reference*).

**tid** The *target* id. The target id is 0 or a positive integer mapping to entries within the sequence dictionary in the header section of a *TAM* file or *BAM* file.

**VCF** Variant Call Format.

## CHAPTER 2

---

### Indices and tables

---

#### Contents:

- [genindex](#)
- [modindex](#)
- [search](#)



## CHAPTER 3

---

### References

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**See also:**

**Information about htlib** <http://www.htlib.org>

**The samtools homepage** <http://samtools.sourceforge.net>

**The cython C-extensions for python** <https://cython.org/>

**The python language** <https://www.python.org>



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## Bibliography

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- [Bonfield.2021] *HTSlib: C library for reading/writing high-throughput sequencing data*. Bonfield JK, Marshall J, Danecek P, Li H, Ohan V, Whitwham A, Keane T, Davies RM. *GigaScience* (2021) 10(2) [giab007](#). PMID: [33594436](#).
- [Danecek.2021] *Twelve years of SAMtools and BCFtools*. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H. *GigaScience* (2021) 10(2) [giab008](#). PMID: [33590861](#).





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