

## NAME

vsearch — dereplicate, filter, sort, search, compare and clusterize amplicons from metagenomic projects

## SYNOPSIS

Searching:

```
vsearch [options] --vsearch_global fastafile --db fastafile (--alnout|--blast6out|--userout|--uc) outfile --id real
```

Clustering:

(in progress)

Dereplication:

```
vsearch [options] --derep_fulllength fastafile (--output|--uc) outfile
```

## DESCRIPTION

Environmental or clinical molecular studies generate large volumes of amplicons (e.g. SSU-rRNA sequences) that need to be dereplicated, filtered, sorted, searched, clusterized or compared to reference sequences. The aim of **vsearch** is to offer a all-in-one open source tool to perform these tasks, using optimized algorithm implementations and harvesting the full potential of modern computers, thus guaranteeing a fast and accurate data processing.

Nucleotidic sequence comparisons is at the core of **vsearch**. To speed up comparisons, **vsearch** implements an efficient *k*-mer filtering, and an extremely fast implementation of the Needleman-Wunsch algorithm, making use of the Streaming SIMD Extensions (SSE2) of modern x86-64 CPUs. If SSE2 instructions are not available, **vsearch** exits with an error message.

### Input

**vsearch** input is a fasta file containing one or several nucleotidic sequences. For each sequence, the sequence identifier is defined as the string comprised between the ">" symbol and the first space, or the end of the line, whichever comes first. Additionally, if the line ends with the pattern ";size=*integer*", **vsearch** will interpret *integer* as the number of occurrences (or abundance) of the sequence in the study (see the Dereplication section).

The nucleotidic sequence is defined as a string of IUPAC symbols (ACGTURYSWKMDBHVN), starting after the end of the identifier line and ending before the next identifier line or the file end. **vsearch** silently ignores ascii characters 9 to 13, and exits with an error message if ascii characters 0 to 8, 14 to 31, "." and "-" are present. All other characters are stripped and complained about in a non-blocking warning message.

**vsearch** operations are case insensitive, except when soft masking is activated. For --vsearch\_global (searching), --cluster\_fast and --cluster\_smallmem (clustering), and --maskfasta (masking) commands, the case is important if soft masking is used. Soft masking is specified with the options "--dbmask soft" (for searching) or "--qmask soft" (for searching, clustering and masking). When using soft masking, lower case letters indicate masked symbols, while upper case letters indicate regular symbols. Masked symbols are never included in the unique *k*-mers used in searching. When soft masking is not activated, all letters are converted to upper case internally and used in result files.

When aligning sequences during searching and clustering, T and U are considered identical, regardless of their case. If two symbols are non-identical, their alignment will result in the negative mismatch score (default -4), except if one or both of the symbols are ambiguous (RYSWKMDBHVN) in which case the score is zero. Alignment of two identical ambiguous symbols (e.g. R vs R) also receives a score of zero.

Optionally, **vsearch** can be compiled to accepted compressed fasta files as input (gz and bzip2 formats).

### Options

**vsearch** recognizes a large number of command-line options. For an easier navigation, options are grouped by theme (dereplication, filtering, sorting, searching, comparison, clustering). We start with general options that apply to all or most themes.

General options:

- help** display a short help and exit.
- version** output version information and exit.
- fasta\_width** *positive integer*  
fasta files produced by **vsearch** are wrapped (sequences are written on lines of *integer* nucleotides, 80 by default). Set that value to 0 to eliminate the wrapping.
- maxseqlength** *positive integer*  
all **vsearch** operations will discard sequences of length equal or greater than *integer* (50,000 nucleotides by default).
- minseqlength** *positive integer*  
all **vsearch** operations will discard sequences of length smaller than *integer* (1 nucleotide by default for sorting or shuffling, 32 nucleotides for dereplication, clustering or searching).
- notrunclabels**  
do not truncate sequence labels at first space, use the full header.
- strand** *plus/both*  
when searching, clustering or dereplicating, check the *plus* strand only (default) or check *both* strands.
- threads** *positive integer*  
number of computation threads to use (1 to 256). The number of threads should be lesser or equal to the number of available CPU cores. The default is to launch one thread per available logical core.
- uc** *filename*  
when searching, clustering or dereplicating, output results in *filename* using a uclust-like format. See <<http://www.drive5.com/usearch/manual/ucout.html>> for a description of the format.
- uc\_allhits**  
when searching, clustering or dereplicating, and when using the **--uc** option, show all hits, not just the top hit for each query.

#### Clustering options:

- centroids** *filename*  
output cluster centroid sequences to *filename* file.
- cluster\_fast** *filename*  
use the fast clustering algorithm and write the results to *filename*.
- cluster\_smallmem** *filename*  
use a slower clustering algorithm (consumes less memory) and write the results to *filename*.
- clusters** *string*  
output each cluster to a separate fasta file using the prefix *string* and a ticker (0, 1, 2, etc.) to construct the filenames.
- id** *real*  
do not add the target to the cluster if the pairwise identity with the centroid is lower than *real* (value ranging from 0.0 to 1.0 included). The pairwise identity is defined as the number of (matching columns) / (alignment length - terminal gaps). That definition can be modified by **--iddef**.
- iddef** *0/1/2/3/4*  
change the pairwise identity definition used in **--id**. Values accepted are:
  - 0. CD-HIT definition using shortest sequence as numerator.

1. edit distance.
2. edit distance excluding terminal gaps (default value).
3. Marine Biological Lab definition counting each extended gap as a single difference.
4. BLAST, same as 2 for global pairwise alignments.

**--qmask** *none/dust/soft*

mask simple repeats and low-complexity regions in sequences using the *dust* or the *soft* algorithms, or do not mask (*none*). Warning, when using *soft* masking, clustering becomes case sensitive. The default is to mask using *dust*.

**--usersort**

when using `--cluster_smallmem`, conserve the initial input order of sequences, do not sort sequences by decreasing length before clustering.

## Dereplication options:

**--derep\_fulllength** *filename*

merge strictly identical sequences contained in *filename*. Redundant sequences receive the header of the first sequence of their group. If `--sizeout` is used, the number of occurrences (i.e. abundance) is indicated at the end of the fasta header using the pattern `";size=integer;"`.

**--minuniquesize** *positive integer*

discard sequences with an abundance value smaller than *integer*.

**--output** *filename*

write the dereplicated sequences to *filename*, in fasta format.

**--sizein** take into account the abundance annotations present in the input fasta file (search for the pattern `";size=integer;"`).

**--sizeout** add abundance annotations to the output fasta file (using the pattern `";size=integer;"`).

**--topn** *positive integer*

output only the top *integer* sequences, sorted by decreasing abundance.

## Masking, shuffling and sorting options:

**--maskfasta** *filename*

mask sequences contained in *filename*.

**--maxsize** *positive integer*

when using `--sortbysize`, discard sequences with an abundance value equal to or greater than *integer*.

**--minsize** *positive integer*

when using `--sortbysize`, discard sequences with an abundance value smaller than *integer*.

**--minuniquesize** *positive integer*

when dereplicating, discard sequences with an abundance value smaller than *integer*.

**--output** *filename*

when dereplicating, sorting or shuffling, write the results to *filename*.

**--relabel** *string*

when sorting, relabel sequence headers using *string* as suffix.

**--seed** *positive integer*

when shuffling, use *integer* as seed. Set to 0 to use a pseudo-random seed.

- sizein** read abundance annotation from input
- sizeout** add abundance annotation to output
- shuffle** *filename*  
pseudo-randomly shuffle the order of sequences contained in *filename*.
- sortbylength** *filename*  
sort by decreasing length the sequences contained in *filename*.
- sortbysize** *filename*  
sort by decreasing abundance the sequences contained in *filename*.
- topn** **positive integer**  
when dereplicating, sorting or shuffling, output just the top *integer* sequences.

Searching options:

- alnout** *filename*  
write pairwise global alignments to *filename* using a human-readable format.
- blast6out** *filename*  
write search results to *filename* using a blast-like tab-separated format of twelve fields (listed below), with one line per query-target matching (or lack of matching if `--output_no_hits` is used). A similar output can be obtain with `--userout filename` and `--userfields query+target+id+alnlen+mism+opens+qlo+qhi+tlo+thi+evalue+bits`. A complete list and description is available in the section "Fields" of this manual.
  1. *query*: query label.
  2. *target*: target (database sequence or cluster centroid) label. The field is set to "\*" if there is no alignment.
  3. *id*: percentage of identity (real value ranging from 0.0 to 100.0). The percentage identity is defined as  $100 * (\text{matching columns}) / (\text{alignment length} - \text{terminal gaps})$ . See fields *id0* to *id4* for other definitions.
  4. *alnlen*: length of the query-target alignment (number of columns). The field is set to 0 if there is no alignment.
  5. *mism*: number of mismatches in the alignment (zero or positive integer value).
  6. *opens*: number of columns containing a gap opening (zero or positive integer value).
  7. *qlo*: first nucleotide of the query aligned with the target. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
  8. *qhi*: last nucleotide of the query aligned with the target. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
  9. *tlo*: first nucleotide of the target aligned with the query. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
  10. *thi*: last nucleotide of the target aligned with the query. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
  11. *evalue*: expectancy-value (not computed for nucleotidic alignments). Always set to -1.
  12. *bits*: bit score (not computed for nucleotidic alignments). Always set to 0.
- db** *filename*  
compare query sequences to the fasta-formatted target sequences contained in *filename*, using global pairwise alignment.

- dbmask** *none/dust/soft*  
mask simple repeats and low-complexity regions in target database sequences using the *dust* or the *soft* algorithms, or do not mask (*none*). Warning, when using *soft* masking search commands become case sensitive. The default is to mask using *dust*.
- dbmatched** *filename*  
write database target sequences matching at least one query sequence to *filename*, in fasta format.
- dbnotmatched** *filename*  
write database target sequences not matching query sequences to *filename*, in fasta format.
- fastapairs** *filename*  
write pairwise alignments of query and target sequences to *filename*, in fasta format.
- fulldp** dummy option. To maximize search sensitivity, **vsearch** uses a 8-way SIMD vectorized full dynamic programming algorithm (Needleman-Wunsch), whether or not **--fulldp** is specified.
- gapext** *string*  
penalties for gap extension (2I/1E)
- gapopen** *string*  
penalties for gap opening (20I/2E)
- hardmask**  
mask low-complexity regions by replacing them with Ns instead of setting them to lower case.
- id** *real* reject the sequence match if the pairwise identity is lower than *real* (value ranging from 0.0 to 1.0 included). The search process sorts target sequences by decreasing number of *k*-mers they have in common with the query sequence, using that information as a proxy for sequence similarity. That efficient pre-filtering will also prevent pairwise alignments with weakly matching targets, as there needs to be at least 6 shared *k*-mers to start the pairwise alignment, and at least one out of every 16 *k*-mers from the query needs to match the target. Consequently, using values lower than **--id** 0.5 is not likely to capture more weakly matching targets. The pairwise identity is defined as the number of (matching columns) / (alignment length - terminal gaps). That definition can be modified by **--iddef**.
- iddef** *0/1/2/3/4*  
change the pairwise identity definition used in **--id**. Values accepted are:
0. CD-HIT definition using shortest sequence as numerator.
  1. edit distance.
  2. edit distance excluding terminal gaps (default value).
  3. Marine Biological Lab definition counting each extended gap as a single difference.
  4. BLAST, same as 2 for global pairwise alignments.
- The option **--userfields** accepts the fields **id0** to **id4**, in addition to the field **id**, to produce the pairwise identity values corresponding to the different definitions.
- idprefix** *positive integer*  
reject the target sequence if the first *integer* nucleotides do not match the query sequence.
- idsuffix** *positive integer*  
reject the target sequence if the last *integer* nucleotides do not match the query sequence.

- leftjust** reject the target sequence if the alignment begins with gaps.
- match** *integer*  
score assigned to a match (i.e. identical nucleotides) in the pairwise alignment. The default value is 2.
- matched** *filename*  
write query sequences matching database target sequences to *filename*, in fasta format.
- maxaccepts** *positive integer*  
maximum number of hits to accept before stopping the search. The default value is 1. That option works in pair with maxrejects. The search process sorts target sequences by decreasing number of *k*-mers they have in common with the query sequence, using that information as a proxy for sequence similarity. If the first target sequence passes the acceptance criteria, it is accepted as best hit and the search process stops for that query. If maxaccepts is set to a higher value, more hits are accepted. If maxaccepts and maxrejects are both set to 0, the complete database is searched.
- maxdiffs** *positive integer*  
reject the target sequence if the alignment contains at least *integer* substitutions, insertions or deletions.
- maxgaps** *positive integer*  
reject the target sequence if the alignment contains at least *integer* insertions or deletions.
- maxhits** *positive integer*  
maximum number of hits to show once the search is terminated (hits are sorted by decreasing identity). The default value is 1. Set to 0 to ignore the option.
- maxid** *real*  
reject the target sequence if its percentage of identity with the query is equal or greater than *real*.
- maxqsize** *positive integer*  
reject query sequences with an abundance equal or greater than *integer*.
- maxqt** *real*  
reject if the query/target length ratio is equal or greater than *real*.
- maxrejects** *positive integer*  
maximum number of non-matching target sequences to consider before stopping the search. The default value is 32. That option works in pair with maxaccepts. The search process sorts target sequences by decreasing number of *k*-mers they have in common with the query sequence, using that information as a proxy for sequence similarity. If none of the first 32 target sequences pass the acceptance criteria, the search process stops for that query (no hit). If maxrejects is set to a higher value, more target sequences are considered. If maxaccepts and maxrejects are both set to 0, the complete database is searched.
- maxsizeratio** *real*  
reject if the query/target abundance ratio is equal or greater than *real*.
- maxsl** *real*  
reject if the shorter/longer length ratio is equal or greater than *real*.
- maxsubs** *positive integer*  
reject the target sequence if the alignment contains at least *integer* substitutions.
- mid** *real*  
reject the target sequence if its percentage of identity with the query is lower than *real* (ignoring gaps).

- mincols** *positive integer*  
reject the target sequence if the alignment length is shorter than *integer*.
- minq** *real*  
reject if the query/target length ratio is lower than *real*.
- minsize** *real*  
reject if the query/target abundance ratio is lower than *real*.
- minsl** *real*  
reject if the shorter/longer length ratio is lower than *real*.
- mintsize** *positive integer*  
reject target sequences with an abundance lower than *integer*.
- mismatch** *integer*  
score assigned to a mismatch (i.e. different nucleotides) in the pairwise alignment. The default value is -4.
- notmatched** *filename*  
write query sequences not matching database target sequences to *filename*, in fasta format.
- output\_no\_hits**  
write both matching and non-matching queries to output files (--alnout, --blast6out, and --userout; output files --uc and --uc\_allhits always feature non-matching queries). Non-matching queries are labelled "No hits" in --alnout files.
- qmask** *none/dust/soft*  
mask simple repeats and low-complexity regions in query sequences using the *dust* or the *soft* algorithms, or do not mask (*none*). Warning, when using *soft* masking search commands become case sensitive. The default is to mask using *dust*.
- query\_cov** *real*  
reject if the fraction of the query aligned to the target sequence is lower than *real*. The query coverage is computed as such:  $100.0 * (\text{matches} + \text{mismatches}) / \text{query sequence length}$ .
- rightjust**  
reject the target sequence if the alignment ends with gaps.
- rowlen** *positive integer*  
width of alignment lines in alnout output. The default value is 64. Set that value to 0 to eliminate the wrapping.
- self**  
reject the alignment if the query and target labels are identical.
- selfid**  
reject the alignment if the query and target sequences are identical.
- target\_cov** *real*  
reject if the fraction of the target sequence aligned to the query sequence is lower than *real*. The target coverage is computed as such:  $100.0 * (\text{matches} + \text{mismatches}) / \text{target sequence length}$ .
- top\_hits\_only**  
output only the hits with the highest percentage of identity with the query.
- userfields** *string*  
when using --userout, select and order the fields written to the output file. See the next section for a complete list of fields.
- userout** *filename*  
write user-defined tab-separated output to *filename*. See --userfields.

**--vsearch\_global** *filename*

*filename* of queries for global alignment search.

**--weak\_id** *real*

show hits with percentage of identity of at least *real*, without terminating the search. A normal search stops as soon as enough hits are found (as defined by --maxaccepts, --maxrejects, and --id). As --weak\_id reports weak hits that are not deduced from --maxaccepts, high --id values can be used, hence preserving both speed and sensitivity. Logically, *real* must be smaller than the value indicated by --id.

**--wordlength** *positive integer*

length of words (i.e. *k*-mers) for database indexing. The default value is 8.

## Fields:

<b>aln</b>	Print a string of M (match), D (delete, i.e. a gap in the query) and I (insert, i.e. a gap in the target) representing the pairwise alignment. Empty field if there is no alignment.
<b>alnlen</b>	Print the length of the query-target alignment (number of columns). The field is set to 0 if there is no alignment.
<b>bits</b>	Bit score (not computed for nucleotidic alignments). Always set to 0.
<b>caln</b>	Compact representation of the pairwise alignment using the CIGAR format (Compact Idiosyncratic Gapped Alignment Report): M (match), D (deletion) and I (insertion). Empty field if there is no alignment.
<b>evaluate</b>	E-value (not computed for nucleotidic alignments). Always set to -1.
<b>exts</b>	Number of columns containing a gap extension (zero or positive integer value).
<b>gaps</b>	Number of columns containing a gap (zero or positive integer value).
<b>id</b>	Percentage of identity (real value ranging from 0.0 to 100.0). The percentage identity is defined as $100 * (\text{matching columns}) / (\text{alignment length} - \text{terminal gaps})$ .
<b>id0</b>	CD-HIT definition of the percentage of identity, using the shortest sequence in the pairwise alignment as numerator (real value ranging from 0.0 to 100.0).
<b>id1</b>	The percentage of identity (real value ranging from 0.0 to 100.0) is defined as the edit distance: $100 * (\text{matching columns}) / (\text{alignment length})$ .
<b>id2</b>	The percentage of identity (real value ranging from 0.0 to 100.0) is defined as the edit distance, excluding terminal gaps. The field id2 is an alias for the field id.
<b>id3</b>	Marine Biological Lab definition of the percentage of identity (real value ranging from 0.0 to 100.0), counting each extended gap as a single difference.
<b>id4</b>	BLAST definition of the percentage of identity (real value ranging from 0.0 to 100.0), same as the field id2 for global pairwise alignments.
<b>ids</b>	Number of matches in the alignment (zero or positive integer value).
<b>mism</b>	Number of mismatches in the alignment (zero or positive integer value).
<b>opens</b>	Number of columns containing a gap opening (zero or positive integer value).
<b>pairs</b>	Number of columns containing only nucleotides. That value corresponds to the length of the alignment minus the gap-containing columns (zero or positive integer value).
<b>pctgaps</b>	Number of columns containing gaps expressed as a percentage of the alignment length (real value ranging from 0.0 to 100.0).
<b>pctpv</b>	Percentage of positive columns. When working with nucleotidic sequences, this is equivalent to the percentage of matches (real value ranging from 0.0 to 100.0).
<b>pv</b>	Number of positive columns. When working with nucleotidic sequences, this is equivalent to the number of matches (zero or positive integer value).



<b>qcov</b>	Fraction of the query sequence that is aligned with the target sequence (real value ranging from 0.0 to 100.0). The query coverage is computed as such: $100.0 * (\text{matches} + \text{mismatches}) / \text{query sequence length}$ . The field is set to 0.0 if there is no alignment.
<b>qframe</b>	Query frame (-3 to +3). That field only concerns coding sequences and is not computed by <b>vsearch</b> . Always set to +0.
<b>qhi</b>	Last nucleotide of the query aligned with the target. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
<b>ql</b>	Query sequence length (positive integer value). The field is set to 0 if there is no alignment.
<b>qlo</b>	First nucleotide of the query aligned with the target. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
<b>qrow</b>	Print the sequence of the query segment as seen in the pairwise alignment (i.e. with gap insertions if need be). Empty field if there is no alignment.
<b>qs</b>	Query segment length. Always equal to query sequence length.
<b>qstrand</b>	Query strand orientation (+ or - for nucleotidic sequences). Empty field if there is no alignment.
<b>query</b>	Query label.
<b>raw</b>	Raw alignment score (negative, null or positive integer value). The score is the sum of match rewards minus mismatch penalties, gap openings and gap extensions. The field is set to 0 if there is no alignment.
<b>target</b>	Target label. The field is set to "" if there is no alignment.
<b>tcov</b>	Fraction of the target sequence that is aligned with the query sequence (real value ranging from 0.0 to 100.0). The target coverage is computed as such: $100.0 * (\text{matches} + \text{mismatches}) / \text{target sequence length}$ . The field is set to 0.0 if there is no alignment.
<b>tframe</b>	Target frame (-3 to +3). That field only concerns coding sequences and is not computed by <b>vsearch</b> . Always set to +0.
<b>thi</b>	Last nucleotide of the target aligned with the query. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
<b>tl</b>	Target sequence length (positive integer value). The field is set to 0 if there is no alignment.
<b>tlo</b>	First nucleotide of the target aligned with the query. Nucleotide numbering starts from 1. The field is set to 0 if there is no alignment.
<b>trow</b>	Print the sequence of the target segment as seen in the pairwise alignment (i.e. with gap insertions if need be). Empty field if there is no alignment.
<b>ts</b>	Target segment length. Always equal to target sequence length. The field is set to 0 if there is no alignment.
<b>tstrand</b>	Target strand orientation (+ or - for nucleotidic sequences). Always set to "+", so reverse strand matches have tstrand "+" and qstrand "-". Empty field if there is no alignment.

## DELIBERATE CHANGES

If you are a usearch user, our objective is to make you feel at home. That's why **vsearch** was designed to behave like usearch, to some extent. Like any complex software, usearch is not free from quirks and inconsistencies. We decided not to reproduce some of them, and for complete transparency, to document here the deliberate changes we made.

During a search with usearch, when using the options `--blast6out` and `--output_no_hits`, for queries with no match the number of fields reported is 13, where it should be 12. This is corrected in **vsearch**.

The fields `qlo`, `qhi`, `tlo`, `thi` and `raw` of the `--userfields` option are not informative in usearch. This is corrected in **vsearch**.

In usearch, when using the option `--output_no_hits`, queries that receive no match are reported in `blast6out` file, but not in the alignment output file. This is corrected in **vsearch**.

**vsearch** reintroduces with `--iddef` alternative pairwise identity definitions that were removed from usearch.

## NOVELTIES

**vsearch** introduces new options not present in usearch. They are described in the "Options" section of this manual. Here is a short list:

- `shuffle`
- `fasta_width`
- `iddef`

## EXAMPLES

(in progress)

Search queries in a reference database, with a 80%-similarity threshold, take terminal gaps into account when calculating pairwise similarities:

```
vsearch --vsearch_global queries.fas --db references.fas --alnout results.aln --id 0.8 --iddef 1
```

Search a sequence dataset against itself (ignore self hits), get all matches with at least 60% identity, and collect results in a blast-like tab-separated format:

```
vsearch --vsearch_global queries.fas --db queries.fas --id 0.6 --alnout results.aln --self --blast6out results.blast6 --maxaccepts 0 --maxrejects 0
```

Clusterize with a 97% similarity threshold, collect cluster centroids, and write cluster descriptions using a uclust-like format:

```
vsearch --cluster_fast queries.fas --id 0.97 --centroids centroids.fas --uc clusters.uc
```

## LIMITATIONS

**vsearch** does not yet perform chimera detection.

## AUTHORS

Implementation by Torbjørn Rognes and Tomas Flouri, documentation by Frédéric Mahé.

## REPORTING BUGS

Submit suggestions and bug-reports at <<https://github.com/torognes/vsearch/issues>>, send a pull request on <<https://github.com/torognes/vsearch>>, or compose a friendly or curmudgeont e-mail to Torbjørn Rognes <[torognes@ifi.uio.no](mailto:torognes@ifi.uio.no)>.

## AVAILABILITY

Source code and binaries are available at <<https://github.com/torognes/vsearch>>.

## COPYRIGHT

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## SEE ALSO

**swipe**, an extremely fast Smith-Waterman database search tool by Torbjørn Rognes and available at <<https://github.com/torognes/swipe>>.

## VERSION HISTORY

New features and important modifications of **vsearch** (short lived or minor bug releases are not mentioned):

**v1.0** released November 15, 2014

First public release