NAME

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

SYNOPSIS

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Searching:
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vsearch [options] --vsearch_global fastafile --db fastafile (--alnout|--blast6out|--userout|--uc) outputfile --id real

Clustering:

(in progress)

Dereplication:

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

Masking:

vsearch [options] --maskfasta fastafile --output outputfile

Shuffling:

vsearch [options] --shuffle fastafile --output outputfile

Sorting:

vsearch [options] (--sortbysize|--sortbylength) fastafile --output outputfile

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 *file-name*.

-- 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).
- 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 and sorted by decreasing abundance.

- **--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.

Masking options:

--hardmask

mask low-complexity regions by replacing them with Ns instead of setting them to lower case.

--maskfasta filename

mask sequences contained in filename.

--output filename

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

Shuffling options:

--output filename

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

--seed positive integer

when shuffling sequence order, use *integer* as seed. Set to 0 to use a pseudo-random seed (default behavior).

--shuffle filename

pseudo-randomly shuffle the order of sequences contained in *filename*.

--topn positive integer

output only the top integer sequences.

Sorting options:

--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*.

--output filename

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

--relabel string

relabel sequence using the prefix *string* and a ticker (1, 2, 3, etc.) to construct the new headers. Use --sizeout to conserve the abundance annotations.

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

--sortbylength filename

sort by decreasing length the sequences contained in *filename*.

--sortbysize filename

sort by decreasing abundance the sequences contained in *filename* (the pattern ";size=*integer*;" has to be present).

--topn positive integer

output only 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 --out-put_no_hits is used). A similar output can be obtain with --userout *filename* and --user-fields 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 * (matching columns) / (alignment length 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 (--vsearch_global) 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 report 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 acceptation 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 to or greater than *real*.

--maxqsize positive integer

reject query sequences with an abundance equal to or greater than integer.

--maxqt real

reject if the query/target sequence length ratio is equal to 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 acceptation 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 to or greater than real.

--maxsl real

reject if the shorter/longer sequence length ratio is equal to 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.

--minqt real

reject if the query/target sequence length ratio is lower than real.

--minsizeratio real

reject if the query/target abundance ratio is lower than real.

--minsl real

reject if the shorter/longer sequence 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 --alnout, --blast6out, and --userout output files (--uc and --uc_allhits output files 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 * (matches + mismatches) / 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 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 * (matches + mismatches) / 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. Fields are separated by "+" (e.g. query+target+id). See the next section for a complete list of fields.

--userout filename

write user-defined tab-separated output to *filename*. Select the fields with the option --userfields. If --userfields is empty or not present, *filename* is empty.

--vsearch_global filename

compare target sequences (--db) to the fasta-formatted query sequences contained in *filename*, using global pairwise alignment.

--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 accepted by the --userfields option:

aln 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.

alnlen Print the length of the query-target alignment (number of columns). The field is set to 0 if there is no alignment.

bits Bit score (not computed for nucleotidic alignments). Always set to 0.

caln 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.

evalue E-value (not computed for nucleotidic alignments). Always set to -1.

exts Number of columns containing a gap extension (zero or positive integer value).

gaps Number of columns containing a gap (zero or positive integer value).

id Percentage of identity (real value ranging from 0.0 to 100.0). The percentage identity is defined as 100 * (matching columns) / (alignment length - terminal gaps).

id0 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).

id1 The percentage of identity (real value ranging from 0.0 to 100.0) is defined as the edit distance: 100 * (matching columns) / (alignment length).

id2 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.

id3 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.

id4 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.

ids Number of matches in the alignment (zero or positive integer value).

mism Number of mismatches in the alignment (zero or positive integer value).

opens Number of columns containing a gap opening (zero or positive integer value).

pairs 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).

pctgaps Number of columns containing gaps expressed as a percentage of the alignment length (real value ranging from 0.0 to 100.0).

pctpv 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).

pv Number of positive columns. When working with nucleotidic sequences, this is equivalent to the number of matches (zero or positive integer value).

qcov 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 * (matches + mismatches) / query sequence length. The field is set to 0.0 if there is no alignment.

qframe Query frame (-3 to +3). That field only concerns coding sequences and is not computed by **vsearch**. Always set to +0.

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.

ql Query sequence length (positive integer value). The field is set to 0 if there is no alignment.

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.

qrow 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.

qs Query segment length. Always equal to query sequence length.

qstrand Query strand orientation (+ or - for nucleotidic sequences). Empty field if there is no alignment.

query Query label.

raw 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.

target Target label. The field is set to "*" if there is no alignment.

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 * (matches + mismatches) / target sequence length. The field is set to 0.0 if there is no alignment.

tframe	Target frame $(-3 \text{ to } +3)$. That field only concerns coding sequences and is not computed by vsearch . Always set to $+0$.
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.
tl	Target sequence length (positive integer value). The field is set to 0 if there is no alignment.
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.
trow	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.
ts	Target segment length. Always equal to target sequence length. The field is set to 0 if there is no alignment.
tstrand	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 extend. 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 --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>.

AVAILABILITY

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

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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