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Why did (or didn't) UCLUST assign sequence Q to target S?

1. Check UCLUST's idea of the identity of Q and S.
2. The alignment looks bad.
3. The identity is above the threshold, but Q wasn't assigned to S.
4. Q was assigned to T which has lower identity than S.
5. Quickly reproducing the state when Q was processed.

Memory requirements

Reduce redundancy

Trim sequence labels

Split the database

Two-level search

Command-line options

Input options

Output options
Introduction
UBLAST and USEARCH are database search algorithms that are hundreds of times faster than BLAST in some applications.

UCLUST is a clustering algorithm based on USEARCH that is significantly better than CD-HIT: it is faster, uses less memory, is more sensitive, allows clustering at lower identities, can cluster much larger datasets and produces higher-quality clusters (higher average identity between member sequence and the 'seed' sequence that defines the cluster).

UBLAST, USEARCH, UCLUST and some other algorithms are implemented in a program called uclust. It combines functionality roughly equivalent to BLASTN, BLASTP, MEGABLAST, BLASTCLUST, CD-HIT, CD-HIT-EST, CD-HIT-2D and CD-HIT-EST-2D in a single program. The clustering method was implemented first, which is why the program name and manual tends to emphasize UCLUST rather than USEARCH. But the search algorithm is more fundamental and I believe it will probably turn out to be more widely used in practice, so I'm "re-positioning" UCLUST as USEARCH.

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Installation
The uclust program is distributed as a stand-alone binary (executable file). The binary is self-contained: it does not require configuration files, environment variables, third-party libraries or other external dependencies. There is no setup script or installer because they're not needed. All you need to do is download or copy the binary file to a directory that is accessible from the computer where you want to run the code. For more information, please see http://drive5.com/cmdline.html.
**UCLUST overview**

UCLUST is a flexible program that can be used in many different ways, which can be confusing to new users. Here is an overview of how UCLUST works to help you get a picture of what's going on.

**Searching**

The core step is a search algorithm that searches a database stored in memory.

A query sequence matches a database sequence if the similarity is high enough. Similarity is calculated in one of two ways:

1. Identity computed from a global alignment, i.e. an alignment that includes all letters from both sequences.

2. An expectation value (E-value) computed from a local alignment.

A database sequence is sometimes called a target or a seed, because a cluster of similar sequences can be grown from it.

The minimum identity is set by the --id option, e.g. --id 0.97 means that the global alignment must have at least 97% identity. By default, identity is computed as the number of matching (identical) letters divided by the length of the shorter sequence, as shown below.

Other definitions of identity are also supported; see the --iddef option.
Local alignments are gapless high-scoring segment pairs (HSPs). Similarity for HSPs is defined by an E-value computed using Karlin-Altschul statistics.

UBLAST seeks local alignments of the query that have low enough E-values. By default, UBLAST stops searching when it finds a match; generally you will want to modify this behavior by setting command-line options.

USEARCH seeks a global alignment of the query to a target that exceeds the given identity threshold and, optionally, also has a low enough E-value.

By default, UBLAST and USEARCH stop searching when a match is found. Usually, UBLAST and USEARCH find the best match first, or something close, but this is not guaranteed, especially for when similarities are low.

If it is important to find the best possible match, then you can increase the --maxaccepts option, which defaults to 1. If you want all matches to be reported rather than the best match, then you can use the --allhits option, which will have no effect unless you also set maxaccepts > 1.

UBLAST and USEARCH may also stop searching if they fail to find a match. By default, they quit after eight failed attempts. Database sequences are tested in an order of decreasing number of words in common with the query. The number of common words correlates well with identity. This means that the first hit found is likely to be good, and conversely that the more sequences are tested without finding a match, the less likely it is that a match will be found later. So, giving up early doesn't miss a potential hit very often, though this becomes increasingly likely at low identities. You can set the maximum number to try using the --maxrejects option. With very high and very low identity thresholds, increasing maxrejects can significantly improve sensitivity. Here, a rule of thumb is that low identity is below 60% for amino acid sequences or 80% for nucleotides, high identity is 98% or more.

**Clustering**

The UCLUST algorithm uses USEARCH internally in order to assign sequences to clusters. Each cluster is defined by a representative sequence called a seed. Each sequence in a cluster matches the seed according to the identity threshold, e.g. 97%.

![Seed and clusters](image)

UCLUST performs de novo clustering by starting with an empty database in memory. Query sequences are processed in input order. If a match is found to a database sequence, then the query is assigned to that cluster (first figure below), otherwise the query becomes the seed of a new cluster (second figure below).
Only the seeds need to be stored in memory because other cluster members are irrelevant for processing new query sequences. This is an advantage for large datasets because the amount of memory needed and the number of sequences to search are reduced. However, this design may not be ideal in some scenarios because it allows parts of non-seed sequences in the same cluster to fall below the identity threshold. I plan to add other clustering methods to future versions of UCLUST: these will probably be slower for large datasets, but may be useful in some applications. Please let me know if there are new features you would like to have.
Read initial database from library (optional)

```
--lib library.fasta
```

Get next query sequence \( Q \) from input file

```
--input seqs.fasta
```

Match found?

Search only? \(--libonly\)

Add \( Q \) to database

Write 'S' (new seed) record

Write 'N' (no match) record

Write 'H' (hit) record

```
UCLUST flowchart
```
Basic usage
In the following, UCLUST refers to the program in general, and uclust stands for the binary file name. When typing a command, you should replace uclust with the binary file name on your system, e.g. uclust2.1.598_linuxi86_64.

UBLAST: local database search
UBLAST requires two input files: the database (specified by the --db option) and the query set (specified by the --ublast option). Each sequence in the query set is compared with the database, which is searched for a match exceeding a specified E-value (default 10.0). For example,

```
  uclust --ublast query.fasta --db database.fasta --blast6out results.b6 --evalue 0.1 --maxtargets 100 --maxaccepts 0 --maxrejects 0
```

The output file format is based on the -outfmt 6 option to NCBI BLAST. All HSPs found that exceed the E-value threshold are written to the output file. In most cases, you will want to set the --maxtargets option to a large number, say 100 or 1000 and the E-value relatively high, say to 0.1. A high E-value is needed to find distant matches, but you would usually prefer to keep going and look for a better match if there is one, which is accomplished by setting --maxtargets.

There is no need to pre-format or index the database, so there is no command equivalent to formatdb or makeblastdb.

PLEASE NOTE
The implementation of UBLAST in v3.0 is an experimental prototype. This algorithm is under rapid development, and its design and behavior is likely to change in the near future. So please don't assume forward-compatible behavior or command lines in your pipelines or scripts.

USEARCH: global database search
USEARCH requires two input files: the database and the query set. Each sequence in the query set is compared with the database, which is searched for a match exceeding a specified identity threshold. For example,

```
  uclust --query query.fasta --db db.fasta --uc results.uc --id 0.90
```

Results are written to a UCLUST (.uc) format file. The format of this file is explained later.

You can optionally specify an E-value:

```
  uclust --query query.fasta --db db.fasta --uc results.uc --id 0.90 --evalue 1.0
```

Giving an E-value causes matches with E-values above this threshold to be rejected. The E-value is currently computed by extracting the optimal gapless HSP from the global alignment. I believe the E-value is over-estimated in this version, so a high value (say, 1.0) may be preferred.

PLEASE NOTE
The method for computing E-values is subject to change, and it is likely that you will need to adjust your thresholds for future versions of USEARCH.
**UCLUST: De novo clustering**

UCLUST generates clusters containing similar sequences. A similarity threshold is specified, say --id 0.9 which means 90% identity. Each cluster has a representative sequence (its seed); all sequences in a cluster are required to have at least the given identity with the seed. Typical usage is:

```
uclust --sort seqs.fasta --output seqs_sorted.fasta
uclust --input seqs_sorted.fasta --uc results.uc --id 0.90
```

The first step is to sort the sequences in a way that is appropriate for your data. Decreasing length is appropriate when both fragments and full-length sequences are present in your data. Sorting by decreasing length can be done using the --sort option command, as shown above. Very large datasets can be sorted using --mergesort if --sort runs out of memory.

If you sort by some other criteria, then you must specify the --usersort option to the clustering step.

**UCLUST+USEARCH: Simultaneous search and clustering**

In UCLUST+USEARCH mode, sequences that do not match the database (here called a library, and specified by the --lib option) become seeds for new clusters. Later sequences in the input file may be matched to these new seeds. Input sequences should be sorted appropriately as for de novo clustering. For example,

```
uclust --input seqs_sorted.fasta --lib database.fasta --uc results.uc --id 0.90
```

The FASTA file for the database is not updated if new seeds are added. New seeds are indicated by 'S' records in the .uc file. See the discussion of the --uc2fasta option for how to create an updated database file.

**USTAR: Fast, approximate multiple alignment of clusters**

UCLUST can create a multiple alignment of each cluster. This requires three steps: 1. clustering, 2. conversion to FASTA (--uc2fasta), 3. inserting additional gaps (--staralign).

```
uclust --input seqs_sorted.fasta --uc results.uc --id 0.90
uclust --uc2fasta results.uc --input seqs_sorted.fasta --output results.fasta
uclust --staralign results.fasta --output aligned.fasta
```

This method emphasizes speed over alignment quality. It is not intended to replace slower but more accurate methods like MUSCLE. When sequence identity is reasonably high, the alignment will be good enough to be informative. Note that in addition to creating a multiple alignment, a consensus sequence is generated for each cluster. This can be useful for high-throughput evaluation of cluster quality. See below ("Clumping") for a method that can create high-quality alignments of very large sets.

**UHIRE: Hierarchical clustering**

Hierarchical clustering can be performed by repeatedly re-clustering at decreasing identities. Each time, the input sequences are the seeds found in the previous iteration. For example, the following commands cluster at 99, 97 and 90% identity.

```
uclust --input reads.sorted.fasta --id 0.99 --uc 99.uc
uclust --uc2fasta 99.uc --types S --input reads.sorted.fasta --output seeds99.fasta
uclust --input seeds99.fasta --id 0.97 --uc 97.uc
uclust --uc2fasta 97.uc --types S --input seeds99.fasta --output seeds97.fasta
uclust --input seeds97.fasta --id 0.90 --uc 90.uc
uclust --uc2fasta 90.uc --types S --input seeds97.fasta --output seeds90.fasta
```
Equivalent results can be obtained in a single step using the --uhire command, as follows.

```
uclust --uhire reads.sorted.fasta --hireout results.hire --ids 99,97,90
```

This command is explained in more detail in a later section. The --ids option specifies a series of percent identity thresholds to use. Since commas are usually significant to command shells, the --ids option should usually be quoted, e.g.:

```
uclust --uhire reads.sorted.fasta --hireout results.hire --ids "99,97,90"
```

Note that clusters at levels below the first may be more diverse than you expect. Please read the warning in later section that describes UHIRE in more detail.

**Clumping**

UCLUST supports a type of clustering I call *clumping*, which to the best of my knowledge has not previously been described, though the idea is simple and is probably not new. The goal is to create clusters ("clumps") of a given size. Members of a given clump should be more similar to each other than to members of other clumps. The motivation is to divide the input into subsets that are tractable for more expensive methods, say those requiring all-vs.-all comparisons. The subsets should be as large as possible to leverage the accuracy of the expensive method. One application of clumping is the creation of very large multiple alignments. More details are given in later sections.

**UCHIME: Chimera detection**

UCLUST implements algorithms for detecting chimeric sequences. This can be done de novo, or by using a reference database that is assumed to be free of chimeras. More details are given in a later section.

**Input data**

Input to UCLUST is generally in the form of FASTA files containing nucleotide or amino acid sequences. The database (sometimes called the library) is stored in memory. Query sequences are processed in the order they appear, allowing files of arbitrary size to be read sequentially with minimal use of memory.

**Sorting input sets for clustering**

Query sequences for de novo clustering should be ordered so that the most appropriate seed sequence for a cluster is likely to be found before other members. For example, ordering by decreasing length is desirable when both complete and fragmented sequences are present, in which case full-length sequences are generally preferred as seeds since a fragment may attract longer sequences that are dissimilar in terminal regions which do not align to the seed, as in the following example.

```
Seed: THESEED
First hit: THESEED INSERTED
Second hit: THESEED TERMINAL
```

The two hits are both 100% identical to the seed in a pair-wise alignment (see later sections for a more detailed discussion of identity). However, the hits are extended with different terminal regions (red) and therefore have only about 50% identity to each other.

In other cases, long sequences may make poor seeds. For example, with some high-throughput sequencing technologies longer reads tend to have higher error rates, and in such cases sorting by decreasing read quality score may give better results. For 16S or 18S sequences, sorting by decreasing abundance may give significantly better results (see below for more on this topic).
If new seeds may be identified (de novo clustering with or without database matching), then UCLUST checks that input sequences are sorted by decreasing length. This check can be disabled by specifying the --usersort option, which specifies that input sequences have been pre-sorted in a way that might not be decreasing length. This check is a defense against users who expect UCLUST to sort automatically because CD-HIT does. UCLUST does not sort automatically for several reasons, most important is that a length sort is not optimal for some types of data.

**Sorting input by length**

If required, sorting by decreasing length is done in a separate step as follows:

```
  uclust --sort input.fasta --output input_sorted.fasta
```

The current implementation of --sort loads all sequences into memory for faster speed, so requires that the available memory be at least as big as the input file. Larger sets can be sorted using a merge sort:

```
  uclust --mergesort input.fasta --output input_sorted.fasta --split 500.0
```

The --split option (default 1000.0) specifies the number of megabytes to use for each partition of the input file. Typically, the maximum RAM needed for the sort will be a bit more than this, but in a worst-case scenario can be closer to 2x the --split value, so a conservative choice is to use about half the physically available memory. Smaller values tend to give slower speeds.

There is no need to sort query sequences for UBLAST or USEARCH, because no new seeds are created.

If you want to sort by some other criteria, then you will need to write your own program or script to do this.

**OTU identification and sorting input by abundance**

The advantage of sorting by decreasing length is that this tends to prevent fragments from becoming seeds. However, in some applications this is not optimal because the longest sequences may have anomalous insertions, for example due to sequencing errors or a diverged gene that is not typical of its family. If the goal of clustering is to identify taxonomic units such as species ("OTUs") in a high-throughput sequencing experiment based on a single gene (e.g. 16S), then a better solution may be to sort by decreasing abundance. The most abundant sequence is likely to be a true biological sequence, while less common sequences may be artifacts due to sequencing error or PCR artifacts such as chimeras, as illustrated in the following figure. This shows the cluster for a single species; the red dot represents reads of the true sequence of the species. A dot indicates a unique sequence, the size of the dot indicates its abundance, i.e. the number of identical (or very similar) reads having that sequence. The longest sequence in the figure is likely to be one of the outliers, and will give a less accurate OTU—imagine drawing a circle of radius of size 97% around one of the outlying dots and you will see that some reads that belong to the species will be incorrectly excluded.
Sorting by abundance can be accomplished as follows. First cluster at a high identity, say \(--id 1.0 \) or 0.99. The size of the clusters can then be obtained from the C records in the .uc file (see below for more information on the .uc file format). This can be done using a series of commands like the following.

```
uclust --sort reads.fasta --output reads.sorted.fasta
uclust --input reads.sorted.fasta --id 0.99 --uc 99.uc
grep "^C" 99.uc | sort -nrk3 > c.uc # sort by decreasing cluster size
uclust --uc2fasta c.uc --types C --output c.fasta
```

Grepping on the first letter (grep "^C" above) selects a single type of record from the .uc file. For details of the .uc file format, see below. Finally, OTUs can be identified by clustering, e.g. for species 97\% is typically used:

```
uclust --input c.fasta --id 0.97 --uc 97.uc --usersort
```

Note the \(--usersort\) option, which is required for de novo clustering when input sequences are not sorted by decreasing length.

**UCLUST file format**

The native UCLUST format (.uc) is a tab-separated text file. Each line is either a comment (starts with \#) or a record. Each query sequence generates at least one record; additional record types give information about clusters. The cluster number appears in every record. If an input sequence matched a target sequence, then the alignment and the identity computed from that alignment are also provided. A compressed representation of the alignment is used to save disk space. Records are appended to the output file as they are generated in order to minimize memory use, and sequences therefore appear in the same order as the input file.

Some example records:

<table>
<thead>
<tr>
<th>Type</th>
<th>Cluster</th>
<th>Size</th>
<th>%Id</th>
<th>Strand</th>
<th>Qlo</th>
<th>Tlo</th>
<th>Alignment</th>
<th>Query</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>0</td>
<td>292</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>AH70_12410</td>
<td>*</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>292</td>
<td>99.7</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>292M</td>
<td>EN70_12566</td>
<td>AH70_12410</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>292</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>EX70_12567</td>
<td>*</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>292</td>
<td>98.2</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>292M</td>
<td>AH70_12410</td>
<td>EX70_12567</td>
</tr>
</tbody>
</table>
Each record has ten fields, separated by tabs.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cluster</th>
<th>Size</th>
<th>%Id</th>
<th>Strand</th>
<th>Qlo</th>
<th>Tlo</th>
<th>Alignment</th>
<th>Query</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>Library</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>New seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Hit, also known as an accept; i.e. a successful match.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Library cluster.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>New cluster.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Not matched (a sequence that didn't match library with --libonly specified).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Reject (generated only if --output_rejects is specified).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Record types are:

- **L**: Library seed (generated only if a match is found to this seed).
- **S**: New seed.
- **H**: Hit, also known as an accept; i.e. a successful match.
- **D**: Library cluster.
- **C**: New cluster.
- **N**: Not matched (a sequence that didn't match library with --libonly specified).
- **R**: Reject (generated only if --output_rejects is specified).

Records of type C and D are used when clustering. The Size field contains the cluster size, i.e. the number of sequences in the cluster including the seed, and %Id is the average identity of non-seed sequences to the seed. Otherwise, Size is the sequence length and %Id is the identity of the pair-wise alignment of this sequence to the seed. For Library clusters (D), records are only output if Size > 1, i.e. library sequences with no matches are not output. A library seed records (L) are output only if a hit is found to this seed. This saves writing a large number of records for library sequences that are not matched, but means that cluster numbers in the .uc file may not be consecutive (because UCLUST internally assigns a cluster number to every library seed, whether or not it is matched).

Rejections (R) are sequences that were aligned to a seed but found to have an identity below the threshold. Rejections are not output unless --output_rejects is specified. Using --output_rejects may increase the size of the .uc file significantly. Rejection records are mainly useful when trouble-shooting unexpected results.

The alignment is compressed using run-length encoding, as follows. Each column in the alignment is classified as M, D or I:

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Query sequence</th>
<th>Seed sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Match</td>
<td>Letter</td>
<td>Letter</td>
</tr>
<tr>
<td>D</td>
<td>Delete</td>
<td>Gap</td>
<td>Letter</td>
</tr>
<tr>
<td>I</td>
<td>Insert</td>
<td>Letter</td>
<td>Gap</td>
</tr>
</tbody>
</table>

Here, "match" simply means a letter-letter column; the letters may or may not be identical. If there are \( n \) consecutive columns of type C, this is represented as \( nC \). For example, 123M is 123 consecutive
matches. As a special case, if $n=1$ then $n$ is omitted. So for example, D5M2I3M represents an alignment of this form:

<table>
<thead>
<tr>
<th>Query sequence</th>
<th>-XXXXXXXXXX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed sequence</td>
<td>XXXXXXX--XXX</td>
</tr>
<tr>
<td>Column type</td>
<td>DMMMMMIIIMMM</td>
</tr>
</tbody>
</table>

If a line in the output file starts with #, it is a comment and parser scripts should ignore it.

Records in the .uc file appear in the same order as the input sequences. You can sort the file using the standard Linux sort command, as follows:

```
sort -nk2 results.uc > results_sorted.uc
```

You can sort first by cluster number then by identity using:

```
sort -n -k2 -k4 results.uc > clusters.sorted.uc
```

UCLUST can also do the sort:

```
uclust --sortuc results.uc --output results_sorted.uc
```

However, the current implementation reads the entire file into memory, so may fail for very large sequence sets.

**E-values in the .uc file**

If the --uc_evalue option is specified, then a bit score and E-value is written to .uc records. The %id field is used, and is formatted as follows: 55.1/144.8/1.2e-6. Here 55.1 is the identity, 144.8 is the bit score and 1.2e-6 is the E-value. The bit score and E-value is computed from the optimal gapless HSP in the global alignment. This method appears to over-estimated E-values, and is subject to change in future versions.

**FASTA format**

UCLUST re-formats both labels and sequences when generating FASTA format output.

Labels look like this:

```
>43|99.7%|AH70_12410
```

Here, 43 is the cluster number and 99.7% is the identity to the seed. The identity will be shown as * for the seed:

```
>43|*|AH70_12200
```

If a .uc record has an alignment, then the query sequence is re-formatted to indicate its pair-wise alignment to the seed. Gaps indicate deletions relative to the seed, lower-case indicates insertions relative to the seed. Here is an example:
This represents the following pair-wise alignment:

<table>
<thead>
<tr>
<th>TheSeed</th>
<th>SEQVEN-CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NonSeed</td>
<td>S-QLENnCE</td>
</tr>
</tbody>
</table>

You can convert UCLUST to FASTA format as follows:

```
uclust --uc2fasta results.uc --input seqs.fasta --output results.fasta [--types XYZ...]
```

Here, seqs.fasta must be the same input file used when generating results.uc. The --types option specifies which record types to convert, default is SH (seeds and hits). It is not valid to use L or D in --types because these refer to library sequences and the current implementation extracts sequences from the input set only. Using --types enables some convenient idioms, as in the following examples.

**Create non-redundant database**

```
uclust --input seqs.fasta --uc results.uc --id 0.90
uclust --uc2fasta results.uc --types S --output nr.fasta
```

**Assign to non-redundant database, cluster unmatched sequences and create new library**

```
uclust --input seqs.fasta --lib nr.fasta --uc results.uc --id 0.90
uclust --uc2fasta results.uc --types S --output newlib.fasta
```

**Multiple alignment FASTA format**

(See Clumping for information on creating high-quality alignments). Alignments generated by --uc2fasta are saved in a specialized FASTA format. You can convert to a more conventional multiple alignment format by using --staralign:

```
uclust --staralign results.fasta --output star.fasta
```

The results.fasta file must be sorted by cluster number.

Gaps are added so that each sequence in a given cluster has the same length. Letters that are aligned to the same position in the seed appear in the same column and are in upper case. Deletions relative to the seed are indicated by dashes. Insertions relative to the seed are indicated in lower-case, and should not be considered aligned to each other. The seed sequence is the last sequence is a special case that represents a consensus sequence. If the position is 100% conserved, i.e. if all letters in that column are identical, then an upper case letter is used. Otherwise, seed letters are lower-case.
**BLAST6 format**

Local alignments (HSPs) are reported if the --blast6out filename option is given. The format follows the NCBI BLAST -outfmt 6 option. It is a tab-separated text file with one line per HSPs. By convention I use the .b6 extension for files in this format. There are twelve fields, as shown in the following table.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Query label</td>
</tr>
<tr>
<td>2</td>
<td>Target label</td>
</tr>
<tr>
<td>3</td>
<td>Percent identity</td>
</tr>
<tr>
<td>4</td>
<td>Alignment length</td>
</tr>
<tr>
<td>5</td>
<td>Number of mismatches</td>
</tr>
<tr>
<td>6</td>
<td>Number of gap-opens</td>
</tr>
<tr>
<td>7</td>
<td>1-based position of start in query</td>
</tr>
<tr>
<td>8</td>
<td>1-based position of end in query</td>
</tr>
<tr>
<td>9</td>
<td>1-based position of start in target</td>
</tr>
<tr>
<td>10</td>
<td>1-based position of end in target</td>
</tr>
<tr>
<td>11</td>
<td>E-value</td>
</tr>
<tr>
<td>12</td>
<td>Bit score</td>
</tr>
</tbody>
</table>

**BLAST-like alignment format**

Alignments generated during clustering or database search can be saved in a human-readable BLAST-like format by using the --blastout option, e.g.:

```
  uclust --query seqs.fasta --db greengenes.fasta --blastout hits.blast
```

Since this format is rather verbose, the file size will be much larger than the corresponding .b6 or .uc file. This format may be changed in future versions, so it is recommended that parsers use the FASTA output generated by --fastapairs instead (see next).

**FASTA alignment format**

Alignments generated by UCLUST and USEARCH can be saved in FASTA format by using the --blastout option, e.g.:

```
  uclust --query seqs.fasta --db greengenes.fasta --fastapairs hits.fasta
```

This format is probably the most convenient for parsers that need to derive information from explicit alignments. Pairs are separated by blank lines, to make the file easier to inspect visually. The query sequence is first, the target (seed, database) sequence is second. If the input sequences are nucleotides, then a + or - is appended to the label of the target sequence to indicate the strand. If the strand is - (reverse strand match), then the target sequence is reverse-complemented.
**CD-HIT format**
The CD-HIT .clstr format is supported for the benefit of code already written for that format. You can convert UCLUST format to and from .clstr as follows:

```
  uclust --uc2clstr results.uc --output results.clstr [--amino]
  uclust --clstr2uc results.clstr --output results.uc
```

The --amino option to --uc2clstr specifies that sequence lengths in the .clstr file should be written with the 'aa' suffix, e.g.:

```
>Cluster 83
0  297aa, >DTS1061058149802r_891_1_1... *
```

By default, the 'nt' suffix is used, so this would appear as:

```
>Cluster 83
0  297nt, >DTS1061058149802r_891_1_1... *
```

**“Exact” and “optimal” clustering**
An "optimal" variant of the algorithm is specified by --optimal, which is equivalent to these options:

```
  --maxaccepts 0 --maxrejects 0 --nowordcountreject
```

This guarantees that every seed will be aligned to the query, and that every sequence will therefore be assigned to the highest-identity seed that passes the identity threshold ($t$). All pairs of seeds are guaranteed to have identity < $t$. The number of seeds is guaranteed to be the minimum that can be discovered by greedy list removal, though it is possible that the number of clusters could be reduced by using a different set of seeds.

An "exact" variant of the algorithm is selected by --exact, which is equivalent to:

```
  --maxaccepts 1 --maxrejects 0 --nowordcountreject
```

This guarantees that a match will be found if one exists, but not that the best match will be found.

The exact and optimal variants are guaranteed to find the minimum possible of clusters and both guarantee that all pairs of seeds have identities < $t$. Exact clustering will be faster, but may have lower average identity of non-seeds to seeds. "Optimal" and "exact" are misleading names that ideally should be changed. They are retained for backwards compatibility.

**Searching with reverse-complement (minus) strand**
By default, nucleotide database searches are made in the same orientation (i.e., plus strand only). You can enable both plus and minus strand matching by using --rev. In the current implementation, using --rev approximately doubles memory use but results in only small increases in execution time. Optimizations are possible that would avoid most of the increase in memory, but would be a fair amount of work to implement and so far do not appear to be worth the effort.
The UIRE algorithm for hierarchical clustering

The --uhire command performs hierarchical clustering, as follows.

```
uc lust --uhire reads.sorted.fasta --hireout results.hire --clumpout results.clump --clumpfasta filenameprefix --maxclump 1000 --ids id1,id2...,idN
```

At least one output option must be given, i.e. at least one of --hireout, --clumpout or --clumpfasta. The --maxclump option gives the maximum number of sequences in a clump (default 1000). The --ids option gives the percent identities of each level in the hierarchy. The default is 99,98,95,90,85,80,70,50,35. Note that --ids uses percentages (0 to 100), compared to --id which uses fractional identities (0.0 to 1.0). Values are separated by commas. Since commas are significant to most command shells, the value of the --ids argument should usually be quoted. If the --clumpfasta option is given, each clump is written to a file named clump.0, clump.1, clump.2 etc., prefixed by the --filenameprefix option. This will typically be a directory name. E.g., you might do this:

```
uc lust --uhire reads.sorted.fasta --clumpfasta myclumpdir/ --maxclump 256
```

Note the '/' at the end of the prefix. This is not required, but if present specifies that clump files are to be stored in the given directory, which must exist. Sequences for each clump will be stored in these files:

```
myclumpdir/clump.0
myclumpdir/clump.1
..etc..
```

In addition to the clumps, a file named ‘master’ will also be written. This contains the longest sequence in each clump. It can be used for creating large multiple alignments, as explained shortly below.

**WARNING**

This method was primarily designed to support clumping (see below). Note that clusters at levels below the first (highest identity) level will tend to be more diverse than clusters obtained in a single step. Say the first two levels are 99% and 98%. The 98% step uses seeds from the 99% step as input. Suppose a cluster at 99% includes two sequences S and A where S is the seed and A is another sequence such that \( \text{pctid}(A,S) \geq 99\% \). A is discarded when the 98% clustering is done. Now suppose T is the seed at 98%, so \( \text{pctid}(S,T) \geq 98\% \). There is no guarantee that A has \( \geq 98\% \) id with T, it may be less, and in fact we should expect such cases because A can be ‘further’ from T than S is. So clustering all sequences including A at 98% will tend to give different numbers of clusters than the hierarchical method.

The .hire file format

The .hire format is designed to be easily parsed by a scripting language and to avoid very long lines as found in mothur files. If you would like a script to convert .hire to mothur format, please let me know.

A .hire file is a text file.

The first line is the number of levels (K), i.e. the number of ids specified in the --ids option.

The second line is the number of sequences (N).

The following N lines specify sequences. Each line contains three tab-separated fields, for example:
The first field is the sequence ID, an integer 0, 1 ... (N-1). This is redundant, but should be used by parsers to check that they are in synch with the file.

The second field is the sequence length in letters.

The third field is the sequence label from the FASTA file.

Following the last sequence (ID=N-1) will be K levels. Each level is specified as follows.

The first line in a LEVEL is a record with four fields, for example:

```
LEVEL 6 9 70.0
```

The first field is always the text "LEVEL". The remaining fields are:

- Level number, a zero-based level number 0, 1 ... K-1.
- Number of input sequences at this level.
- Percent identity for this level.

This is followed by one line per sequence. Each line has three fields. Here is a complete example of a level.

```
LEVEL 6 9 70.0
6 0 *
6 61 *
6 565 0
6 726 *
6 1542 *
6 4408 61
6 4858 0
6 4879 *
6 9366 *
```

In the lines following the LEVEL record, the first field is the level number. As for sequence records, this field is redundant but should be used by parsers to verify consistency with the file. The second field is the sequence ID, referring back to the sequence records at the beginning of the file. Sequence IDs are the same for all levels. A given level will have only the subset of IDs that correspond to seeds discovered in the previous level. The third field is either a second sequence ID, indicating a match, or an asterisk '*', indicating no match. A match means that the sequence was assigned to a cluster, an asterisk means that this sequence becomes a new seed at this level. So the above example has six seeds that would be passed down to the next level and three matches, two to seed ID=0 and one to seed ID=61. If there is a 7th level, it will have six input sequences which are the seeds identified at level 6.

**Creating large, high-quality multiple alignments**

**MUSCLE** can create alignments of up to perhaps 10,000 to 20,000 sequences. Larger sets can be aligned using a divide and conquer strategy based on clumping. This may be advantageous even in cases where MUSCLE can align the complete set as the resulting alignments tend to be more compact, having fewer columns and thus fewer gaps, which may be preferred for some types of analysis.
In outline, the strategy is as follows.

1. Create clumps, i.e. clusters that are small enough for MUSCLE to align.
2. Create a 'master' set containing the longest sequence from each clump.
3. Align each clump.
4. Align the master set.
5. Merge the clumps into a final alignment, using the master alignment as a guide.

The first step is to create clumps. Anecdotally, I have found that a clump size of around 5000 gives good results, but this may vary depending on your data. I recommend experimenting with different clump sizes and examining the results. Typical commands would be:

```bash
mkdir myclumps
uclust --uhire seqs.sorted.fasta --clumpfasta myclumpdir/ --maxclump 5000
```

The clumps and the master set are then aligned using MUSCLE. For example (bash syntax):

```bash
mkdir clumpalns
cd myclumpdir
for filename in clump.* master
do
    muscle -in $filename -out ../clumpalns/$filename -maxiters 2
done
cd..
```

I recommend the -maxiters 2 option to MUSCLE as a good compromise between speed and accuracy for larger sets. Any multiple alignment method can be used in place of MUSCLE if desired.

The alignments are combined using the --mergeclumps command, as follows.

```bash
uclust --mergeclumps clumpalns/ --output aligned.fasta
```

Sequences in the master file are required to have their labels formatted to indicate the clump number. This is done automatically if the --clumpfasta option is used; if you use some other method to select the master set then you must take care to follow the label formatting convention. The clump ID (0, 1... N-1) is indicated by a prefix like >M123 | where 123 is the clump ID. For example, this is a valid FASTA label for the master sequence of clump 28:

```fasta
>M28|GF2FOAC01AU7TA
```

Clump 8 must contain an identical sequence with label >GF2FOAC01AU7TA, this correspondence is used to merge the alignments of each clump into a single multiple alignment.

**UCHIME: Chimeric sequence detection**

UCHIME searches for chimeric sequences. Like UCLUST, UCHIME leverages the high speed of the USEARCH algorithm, which is used as a subroutine. The basic command-line is:

```bash
uclust --uchime myseqs.fasta --report myseqs.rep --reportx myseqs.repx
[--lib ref.fasta] [--minh 0.6]
```
The --lib option specifies a trusted reference database. If not specified, de novo detection is performed. In de novo mode, chimeric triples are reported and additional evidence is required to determine which of the three is the true chimera. A good heuristic is to designate the least abundant sequence as the chimera. Input and reference sequences should be in unaligned FASTA format (no gaps). The --minh option specifies the minimum score to be considered a hit. Default is --minh 0.6, which gives an error rate < 1% on full-length simulated sequences.

**SNPs and voting**

UCHIME constructs a three-way alignment of two putative step-parents (A and B) to the query sequence (Q). A is always the first step-parent that models the left side of the query, B is the second (right side). UCHIME counts columns in the three-way alignment containing differences (SNPs). SNPs in which two sequences agree and the third differs either support or contradict the model. SNPs in which all three sequences differ do not provide direct evidence but could reduce confidence by indicating noise (sequence errors, inaccurate alignment or divergence between the true parent and the step-parent).

**Summary report format (--report option)**

In the summary report produced by the --report <filename> option, the top hit (T) is also reported. The top hit is the most similar sequence to the query, and acts as a control: the closer the top hit is to the model, the lower the confidence in the prediction. Often, but not always, the top hit is one of the step-parents in the model. In the Labels column, there are either three or four labels: Q(A,B) or Q(A,B,T). The top hit is indicated by a + prefix on the label. Here is some sample output.

<table>
<thead>
<tr>
<th>H</th>
<th>Div</th>
<th>L.SNPs y/n/?</th>
<th>R.SNPs y/n/?</th>
<th>IdP</th>
<th>IdT</th>
<th>IdAB</th>
<th>IdM</th>
<th>Labels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>1.712</td>
<td>1.010</td>
<td>A14/B0/N0/70</td>
<td>B17/A0/N0/7</td>
<td>99.0%</td>
<td>99.2%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>1.100</td>
<td>1.023</td>
<td>A55/B0/N0/70</td>
<td>B22/A0/N0/70</td>
<td>97.6%</td>
<td>99.5%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>1.058</td>
<td>1.009</td>
<td>A17/B0/N0/70</td>
<td>B12/A0/N0/70</td>
<td>99.2%</td>
<td>99.2%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.866</td>
<td>1.008</td>
<td>A136/B0/N0/70</td>
<td>B12/A0/N0/70</td>
<td>99.2%</td>
<td>99.2%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.855</td>
<td>1.008</td>
<td>A55/B0/N0/70</td>
<td>B11/A0/N0/70</td>
<td>99.2%</td>
<td>99.2%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.791</td>
<td>1.007</td>
<td>A75/B0/N0/70</td>
<td>B10/A0/N0/70</td>
<td>99.3%</td>
<td>99.3%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.790</td>
<td>1.007</td>
<td>A55/B0/N0/70</td>
<td>B10/A0/N0/70</td>
<td>99.3%</td>
<td>99.3%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.748</td>
<td>1.007</td>
<td>A10/B0/N0/70</td>
<td>B19/A2/N0/70</td>
<td>99.0%</td>
<td>99.0%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.736</td>
<td>1.006</td>
<td>A55/B0/N0/70</td>
<td>B9/A0/N0/70</td>
<td>99.6%</td>
<td>99.6%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.651</td>
<td>1.006</td>
<td>A21/B0/N0/70</td>
<td>B6/A0/N0/70</td>
<td>99.5%</td>
<td>99.5%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.641</td>
<td>1.005</td>
<td>A3/B0/N0/70</td>
<td>B2/A0/N0/70</td>
<td>99.5%</td>
<td>99.5%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.628</td>
<td>1.005</td>
<td>A7/B0/N0/70</td>
<td>B5/A2/N0/70</td>
<td>99.4%</td>
<td>99.4%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Y</td>
<td>0.544</td>
<td>1.005</td>
<td>A8/B0/N0/70</td>
<td>B10/A0/N0/70</td>
<td>99.2%</td>
<td>99.2%</td>
<td>96.2%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Detailed report format (--reportx option)
The detailed report shows three-way alignments and summary statistics for each model. Here is an example alignment.

Query ( 209 nt) FW1022_2nd_NO3Lac_F3SGFHA01AFKGD
ParentA ( 207 nt) FW1023_2nd_NO3Lac_F3SGFHA01DNNB3
ParentB ( 208 nt) FW1022_2nd_NO3Lac_F3SGFHA01CZS9S

A     1 tgcgcaggcggttatataagacagatgtgaaatccccgggctcaaccttggaactgcattagtgactgtatagctagag 79
Q     1 tgcgcaggcggttatataagacagatgtgaaatcccccgggctcaacctgggaactgcattagtgactgtatagctagag 80
B     1 tgcgcaggcggttatataagacagatgtgaaatccccgggctcaaccttggaactgcattagtgactgtatagctagag 79

SNPs                 A A        AA                                AA A       A A
Model   .............AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

A    80 tgcggcaaggggtgagaatttccaacggtgtagcagtgaaatgcgtGgaTatgCggaggaacaccgatggcgaaggca 158
Q    81 tgcggcaaggggtgagaatttccaacggtgtagcagtgaaatgcgtagagatgtggaggaacaccgatggcgaaggca 160
B    80 tAcggcagagggggtgagaatttccaacggtgtagcagtgaaatgcgtagagatgtggaggaacaccgatggcgaaggca 159

SNPs     A            A         B                    B  B   B                         BB
Model   AAAAAAAAAAAAAAAxxxxxxxxxBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB

A   159 cccctgggccTGCactgacgctcatgcacgaaagcgtgggGagcaaaca 207
Q   161 cccctgggccgatactgacgctcatgcacgaaagcgtgggtagcaaaca 209
B   160 cccctgggccgatactgacgctcatgcacgaaagcgtgggtagcaaaca 208

SNPs              BBB                           B
Model   BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB........

Score 2.838
Ids.  QA 95.2%, QB 94.7%, AB 89.9%, QModel 100.0%, Div. +5.1%
SNPs    Left A=11(100%)/b=0/N=0/?=0, Right B=10(100%)/a=0/N=0/?=0

SNP classification
Letters that agree are shown in lower-case, disagreements are shown in upper-case (in order to stand out -- I think upper case stands out better against lower-case than vice versa). SNPs are classified as follows. Here, a column is written as AQB, and upper case indicates disagreement. These are the possible types of column.

<table>
<thead>
<tr>
<th>aqb</th>
<th>Q=A=B</th>
<th>Not a SNP.</th>
</tr>
</thead>
<tbody>
<tr>
<td>aQB</td>
<td>Q=A, Q!=B</td>
<td>Votes for model if on the left (symbol 'A') or against model if on right (symbol 'a').</td>
</tr>
<tr>
<td>Aqb</td>
<td>Q=B, Q!=A</td>
<td>Votes for model if on the right (symbol 'B') or against model if on left (symbol 'b').</td>
</tr>
<tr>
<td>aQb</td>
<td>A=B, Q!=A, Q!=B</td>
<td>Indicates SNP in query vs. (step-)parents. Could be a read error, or a change in one of the true parents vs. the closest step-parent. Symbol 'N'.</td>
</tr>
<tr>
<td>AQB</td>
<td>All different.</td>
<td>Not directly informative, indicates noise. Symbol '?'.</td>
</tr>
</tbody>
</table>

Pros and cons of UCHIME
Please note that UCHIME is a work in progress. I am hoping to improve the algorithm in the near future. This is a brief discussion of the current version.

Advantages of UCHIME
Compared with previous methods, UCHIME is very fast: it can process hundreds or thousands of sequences per minute, depending on length. Also, unlike some other methods it does not require a trusted (i.e., chimera-free) reference database or a pre-built multiple alignment of trusted sequences. Preliminary tests suggest that on full-length 16S rRNA sequences, UCHIME has better sensitivity and
lower error rates than any other method. UCHIME provides a score for each predicted chimera which allows the user to set a score threshold. Adjusting the threshold increases sensitivity or reduces the false-positive rate, similar to a BLAST e-value. At reasonable thresholds, the false-positive rate is consistently low.

**Disadvantages of UCHIME**

Like most other methods, UCHIME currently only searches for "bimeras" (two parent sequences), but "multimeras" can also be important in practice [doi 10.2144/000113219]. Preliminary tests suggest that Chimera Slayer has significantly better sensitivity than UCHIME in two scenarios: (i) with short reads, and (ii) when parent sequences are not available in the reference database so that related sequences ("step-parents") are needed to construct a model of the query sequence. Here, "short" means less than about 500 nucleotides.

While several avenues for improving detection accuracy remain to be explored, I believe UCHIME can, and therefore should, at least match the performance of Chimera Slayer on bimeras before moving on. This is because UCHIME currently uses a similar strategy to Chimera Slayer that constructs a three-way alignment of putative step-parents to a query sequence. If UCHIME is less sensitive than Chimera Slayer, then one or more phases of UCHIME must be sub-optimal (search for candidate step-parents, alignment construction or model scoring). Optimizing the "three-way / bimera" algorithm will inform attempts to develop a better "multi-way / multimera" algorithm, which is my long-term goal.

If you are working with short reads, then a practical compromise could be to use UCHIME to find candidate chimeras using a threshold designed to maximize sensitivity at the expense of a higher error rate. The candidates could then be passed to the much slower Chimera Slayer for final discrimination. (Note that the complete CMCS pipeline should be used, not the Chimera Slayer perl script alone).

**Search parameter tuning**

UCLUST offers a number of parameters for adjusting speed and sensitivity. The characteristics of datasets found in practice are highly variable, and it is therefore challenging to set universally appropriate defaults or to develop simple guidelines to assist users in setting the best parameter values for particular applications. With these considerations in mind, the following procedure is suggested for tuning parameters. (See also the section below Evaluating fast alignment performance).

A dataset is first clustered at low identity (say, 50% for proteins or 80% for nucleotides) using default parameters, giving an initial set $S$ of clusters. A subset $F$ is then extracted from $S$ for performance tuning analysis. The redundancy of $F$ at higher identities, e.g. 90%, will typically be similar to $S$, meaning that the average cluster size will be similar. If $F$ is small enough, the exact or optimal variants can be used as a reference against which faster but potentially less sensitive parameters can be compared. Redundancy is the most important factor in determining elapsed time (which scales roughly linearly in the number of sequences $N$ and the number of clusters $M$) and memory use (which scales roughly linearly in $M$), unless $M$ becomes very large. Time or memory use on $S$ with a given set of parameters can therefore be estimated as $|S|/|F| \times$ (time or memory on $F$). If the exact variant is prohibitively expensive on $F$, an alternative is to use parameters designed for high sensitivity while retaining some speed heuristics, e.g.

```
--maxaccepts 0 --maxrejects 0 --maxtargets 128 --step 0 --bump 0.
```

See also the --check_fast option.
**Gap penalties**

UCLUST supports a rich set of gap penalty options. Up to 12 separate penalties can be specified: all combinations of query / target, left / interior / end, and open / extend.

The following table gives the penalties that UCLUST uses by default.

<table>
<thead>
<tr>
<th>Penalty</th>
<th>Default</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior gap open</td>
<td>10.0 (nucleotide)</td>
</tr>
<tr>
<td></td>
<td>17.0 (amino acid)</td>
</tr>
<tr>
<td>Terminal gap open</td>
<td>1.0</td>
</tr>
<tr>
<td>Interior gap extend</td>
<td>1</td>
</tr>
<tr>
<td>Terminal gap extend</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Terminal gaps are penalized much less than interior gaps, which is typically appropriate when fragments are aligned to full-length sequences. These defaults can be changed using the --gapopen and --gapext options. The nucleotide defaults would be set using these options:

```
--gapopen 10.0I/1.0E --gapext 0.5
```

A numerical value for a penalty is optionally followed by one or more letters that specify particular types of gap. Here, "10.0I" means "Interior gap=10.0", and "1.0E" means "End gap=1.0". If no letters are given after the numerical value, then the penalty applies to all gaps. More than one letter can be specified, so for example "0.5IE" means "Interior and End gaps=0.5", which is the same as all gaps. Following are valid letters: I=Interior, E=End, L=Left, R=Right, Q=Query and T=Target. If more than one numerical value is specified, then they must be separated by a slash character '/'. White space is not allowed. If a star ('*') is used as the numerical value, then the gap is forbidden. Using * in an open penalty means that the gap will never be allowed, using * in an extension penalty means that gaps longer than one will be forbidden. So, for example, *LQ in --gapopen means "left end-gaps in the query are not allowed". A sign (plus or minus) is not allowed in the numerical value, which can be integer or floating-point (in which case a period '.' must be used for the decimal point). The --gapopen and --gapext options are interpreted first by setting the defaults, then by scanning the string left-to-right. Later values override previous values.

The final settings are written to the --log file, and I strongly recommend that you use this information to check that your options are correctly formatted. Here is another set of example options.

```
--gapopen 10.0QL/*QL/2.0TE/1.0QR --gapext 0.5I/0.1E
```
The resulting penalties appear as follows in the log file.

<table>
<thead>
<tr>
<th>Penalty</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00</td>
<td>Open penalty (query, internal)</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>Open penalty (query, left end)</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>Open penalty (query, right end)</td>
<td></td>
</tr>
<tr>
<td>10.00</td>
<td>Open penalty (target, internal)</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>Open penalty (target, left end)</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>Open penalty (target, right end)</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>Ext. penalty (query, internal)</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>Ext. penalty (query, left end)</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>Ext. penalty (query, right end)</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>Ext. penalty (target, internal)</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>Ext. penalty (target, left end)</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>Ext. penalty (target, right end)</td>
<td></td>
</tr>
</tbody>
</table>

**Considerations when using non-standard gap penalties**

The --gapopen and --gapext options do not always work well with the fast alignment heuristics that are enabled by default. In some cases, especially if some gap types are forbidden, then this can cause UCLUST to crash.

If possible, the best thing to do is to disable the heuristics by using --nofastalign. Then the gap penalties should work well. If you have very large datasets and heuristics are needed, then I recommend testing on small datasets and reviewing the --blastout file to make sure that the alignments look reasonable for your application.

A compromise that often works well is to disable HSPs by using --hsp 0. In typical applications, banding will still give improvements in speed without significantly degrading alignment accuracy or estimates of identity.

**Evaluating fast alignment performance**

The --check_fast option performs an automated analysis of the --fastalign heuristics. With --check_fast, whenever a pair-wise alignment is constructed, UCLUST compares the alignment with and without fast heuristics (HSPs and banding). Results are written to the --log file. Here is an example.

Pair-wise alignment statistics

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alignments</td>
<td>1183407</td>
</tr>
<tr>
<td>Hits</td>
<td>1113873 (94.1%)</td>
</tr>
<tr>
<td>Fast alignments same as slow (hits)</td>
<td>718023 (64.5%)</td>
</tr>
<tr>
<td>Fast alignments same as slow (all)</td>
<td>774833 (65.5%)</td>
</tr>
<tr>
<td>No HSPs found</td>
<td>4302 (0.4%)</td>
</tr>
<tr>
<td>Alignments with HSPs not in slow</td>
<td>188 (0.0%)</td>
</tr>
<tr>
<td>Rejected by low HSP id</td>
<td>3131 (0.3%), 0 are FPs (0.0%), 1 are FNs (0.0%)</td>
</tr>
<tr>
<td>Heuristic %id &gt; 1% error vs. slow</td>
<td>6319 (0.5%)</td>
</tr>
<tr>
<td>Heuristic false-positive hits</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Heuristic false-negative hits</td>
<td>4163 (0.4%)</td>
</tr>
<tr>
<td>CPU time for slow alignments</td>
<td>2.65e+012</td>
</tr>
<tr>
<td>CPU time for fast alignments</td>
<td>1.45e+011</td>
</tr>
<tr>
<td>Alignment time speedup by using heuristics</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Here, 'slow' means without fast heuristics, i.e. using full dynamic programming as with --nofastalign. 1.1M alignments were made, and 94% of these were hits. This shows the effectiveness of the UCLUST index and filtering algorithm: almost all alignments confirmed putative hits. About 2/3 of alignments
were the same with and without the --fastalign heuristics: 64.5% of alignments for hits, and 65.5% overall. So about 1/3 of alignments were sub-optimal in terms of maximizing the objective score. However, the statistics show that these sub-optimal alignments make little difference in estimating the query-target identity. There was just one false negative (FN) due to rejection of a target sequence based on the low identity of its HSP(s), and no false positives (FPs). In only 6.3k/1.1M cases (0.5%) was the identity estimated using a fast heuristic alignment more than 1% different from the identity computed from the full dynamic programming alignment. There were no false positive hits and only 4.2k/1.1M false negative hits, i.e. 0.4%. In most cases, this low "error" rate is well worth the 18x speed improvement achieved by using --fastalign. And in general, it is not certain that the full dynamic programming alignments are really better than the heuristic alignments, so it is not clear whether these are true biological "errors".

**Sequence identity: the good, the bad and the ugly**

UCLUST was originally designed as a clustering tool for reducing redundancy in large sets of sequences. In such applications, sequence identity, defined as a percentage of identical letters, is commonly used as a measure of sequence similarity. UCLUST and USEARCH have been rapidly adopted in a wide range of applications, and this has exposed a number of issues related to the use of identity as a measure of similarity.

*High identity for taxonomy ("OTU-picking")*

UCLUST is commonly used to cluster reads of single genes, e.g. bacterial 16S rRNA. Here, clusters are interpreted as operational taxonomic units (OTUs), most often as species. The rule of thumb that sequences with ≥ 97% identity belong to the same bacterial species has been widely adopted. This rule was developed prior to the development of next-generation short-read sequencing technologies, which are often used to sequence a partial gene rather than the full-length gene. The sequenced region is typically more variable than the average over the full-length gene, and this means that divergence of more than 97% might occur within strains of a single species. Conversely, though less likely, different species might also have ≥ 97% by chance. Further, clustering based on a 97% identity threshold with a representative sequence (seed) means that cluster members may be diverged by 6% or more, raising the question of which clustering criteria are optimal for OTU identification -- a topic that is debated. So far, I'm not convinced that other methods work better than UCLUST, but I'm open to discussion and would be happy to add new features to UCLUST if it makes sense. In the case of the seed-based clustering method that is currently the only option in UCLUST, sorting by abundance probably gives better OTUs and is strongly recommended. Finally, it should be noted that there will be bad sequences due to sequencing errors and chimeras produced during amplification. Smaller clusters will therefore tend to be either "rare biosphere" species or bad reads, with an increasing number of bad reads towards the tail of the cluster size distribution.

*Low identity for homology recognition (database search)*

USEARCH is sometimes used to search for homologs in protein databases, e.g. to assign function to predicted ORFs in metagenomic shotgun reads. This presents a different set of challenges. The sensitivity of USEARCH declines with low identity. Also, local alignment may have advantages over global alignment for detecting distant homologs, though there are arguments both ways (e.g., local alignment may detect isolated domains in common in proteins that have different domain organizations and hence quite different functions). Below 30-40%, identity is an increasingly bad predictor of homology and function (see D. Lee et al. *Nat. Rev. Mol. Cell Biol.* 8, 995-1005). Better is to use an expectation value derived from a local alignment score and the effective database size (S.E. Brenner et al. *PNAS* 95, 6073-78).
Identity as a measure of evolutionary distance
Estimating evolutionary distance is a complex topic. Identity can be used to define a distance \( (d) \), e.g. \( d = 1 - f \), where \( f \) is fractional identity (e.g. \( f=0.97 \) for 97%). For high identities, i.e. \( f \) close to 1, this is roughly a parsimony distance and, for example, should be approximately additive. However, identity-based distance measures do not consider the possibility of multiple substitutions occurring at a single site, or differences in rates for different types of substitutions (e.g., transitions vs. transversions for nucleotides. Depending on the precise definition of identity, a distance measure may ignore insertions and deletions, or may treat them as equivalent to substitutions, which is not biologically realistic.

Defining sequence identity
Sequence identity can be defined in many different ways; see for example this web page and its literature references: [http://openwetware.org/wiki/Wikiomics:Percentage_identity](http://openwetware.org/wiki/Wikiomics:Percentage_identity). Identity is usually defined to be a ratio where the numerator is the number of identical letters in an alignment. Many choices are possible for the denominator, each of which has pros and cons in different applications. Common choices include:

- The number of columns in the alignment (terminal gaps may be included or excluded).
- The length of the shorter sequence.
- The length of the longer sequence.
- The average sequence length.
- The number of columns containing letter pairs (i.e., gaps are ignored).

Terminal gaps
Some definitions of identity treat terminal gaps as special cases. This is important, e.g. if fragments are being aligned to full-length sequences, in which case terminal gaps are experimental artifacts rather than evidence of insertions or deletions. It should be noted that definitions of identity that count terminal gaps differently from internal gaps are more sensitive to details of the algorithm used to generate the alignment, and in particular to gap penalties. Problems may be caused if a short motif is mis-aligned close to a terminal, like this.

Query:   -XX--------XXXXXXXXXXXXXX------
Target:  XXXXXXXXXXXXXXXXXXXXXXXXXXXX

Presumably, the correct alignment would be more like this:

Query:   ------ XXXXXXXXXXXXXXXX------
Target:  XXXXXXXXXXXXXXXXXXXXXXXXXXXX

If gapped columns count as differences and terminal gaps are discarded, then the first alignment may have much lower identity.

The --iddef option
UCLUST supports several definitions of identity, which may be chosen via the --iddef \( n \) option, where \( n \) is 0, 1 ... etc. The default is --iddef 0.
**Default definition of identity**
The default definition, --iddef 0, uses the length of the shorter sequence as the numerator. Since all gap columns are discarded, this can report 100% identity despite gaps in the shorter sequence. Consider the following example.

```
Query: SEQ-ENCE
Target: SEQVENCE
```

Here, there are 7 identities and the length of the shorter sequence is also 7, giving $Id = \frac{7}{7} = 100\%$.

**All-diffs definition**
The all-diffs definition (--iddef 1) considers every gap column and every mismatch to be a difference, which is achieved by using the number of columns in the global alignment as the numerator. This is essentially equivalent to the definition of edit distance. In the above example, there are 8 columns in the alignment, so $Id = \frac{7}{8} = 87.5\%$.

**Internal diffs definition**
The internal diffs definition (--iddef 2) is similar to all-diffs, except that terminal gaps are not included in the alignment length. See above *(Terminal gaps)* for a discussion of a potential problem with this definition. This may be more appropriate if fragment sequences (e.g., partial 16S genes from a short-read sequencing experiment) are aligned to full-length sequences (complete genes in a reference database). Consider this example.

```
Query: ---V-NC-
Target: SEQVENCE
```

Here, there are 4 columns after terminal gaps are discarded, so the internal diffs $Id = \frac{3}{4} = 75\%$, while the default $Id = \frac{3}{3} = 100\%$ and the all-diffs $Id = \frac{3}{8} = 37.5\%$.

**Marine Biology Lab definition**
The MBL definition (--iddef 3) is similar to all-diffs, except that a gap of any length (i.e., consecutive series of gap columns) counts as a single difference. Both internal and terminal gaps are counted.

Identity is defined as:

$$1.0 - \frac{\text{mismatches} + \text{gaps}}{\text{longer_sequence_length}}$$

Notice that unlike other definitions, this does not use the number of matches as the numerator. Consider the following example.

```
Query: --QVDNC-
Target: SEQVENCE
```

Here, mismatches = 1 and gaps = 2 so $Id = 1 - \frac{1 + 2}{8} = 72.5\%$. In theory, this expression can be negative, in which case it is set to zero by UCLUST.

**Computing all-vs-all pair-wise identities**
You can compute all pair-wise identities for a set of input sequences as follows:

```
 uclust --query segs.fasta --db segs.fasta --usersort --allhits
       --maxaccepts 0 --maxrejects 0 --id 0 --uc allpairs.uc
```
Why did (or didn’t) UCLUST assign sequence Q to target S?
This is the most common question I get about UCLUST results. The following notes walk you through the process of figuring out why UCLUST didn't do what you expected.

Check UCLUST's idea of the identity of Q and S
The first thing to check is the identity of Q and S according to the UCLUST alignment. If this is above (below) the threshold, this would explain why it was (was not) assigned to the target. First make a file qs.fa containing just Q and S, then:

```bash
uclust --input qs.fa --usersort --id 0.0 --blastout qs.blast --uc qs.uc
```
You can check the identity in the .uc file, and see the alignment and identity calculation in the .blast file.

The alignment looks bad
If the .blast alignment looks bad, this may be caused by the heuristics (HSPs and banding) used by UCLUST to make fast alignments. You can check this manually by repeating with --nofastalign:

```bash
uclust --input qs.fa --usersort --id 0.0 --blastout qs.blast --uc qs.uc --nofastalign
```
UCLUST can do this automatically by using --check_fast, in which case you should use the same --id that you used for clustering, say:

```bash
uclust --input qs.fa --usersort --id 0.90 --check_fast --log qs.log
```
A report will be written to the log file. See the section "Evaluating fast alignment heuristics" for more information.

You can reduce the number of bad alignments by using --nofastalign, which does full global dynamic programming alignments with no heuristics. This will be slower, but will produce alignments of comparable quality to programs like MUSCLE and CLUSTALW.

If --nofastalign is unacceptably slow, you can compromise by tweaking the heuristics. E.g., you can increase the band radius by using the --band option.

The identity is above the threshold, but Q wasn’t assigned to S
There are two cases: Q was assigned to a different target (next section below), or no match was found.

If no match to S was found, there are three possible explanations:

1. S was rejected because the word count was too low.
2. S was rejected because the HSP identity was too low.
3. S was not tested because the search was abandoned before reaching S.

Cases 1 and 2 can be checked by clustering a file containing just S and Q. Make sure S comes first so that it becomes the seed. Then:

```bash
uclust --input qs.fa --usersort --id ...
```
Use the same --id and other search options that you used in your original run. If Q is assigned to S, this means that in your original run, the search must have been abandoned before reaching S. If Q is not
assigned to S, but has a high enough identity in your first test, then S must have been rejected because
the word count or HSP identity was too low. You can check whether it was the word count by setting the
--nowordcountreject option. If S was rejected because of a low word count, now Q should be assigned
to S.

You can check rejections by repeating your original run with --output_rejects. If repeating your original
run is time-consuming, a later section below explains how to reproduce what happened to Q more
quickly. With output_rejects, Reject records (R in column 1) are written to the .uc file. If there is a reject
record for S, this is due to case 1 or 2 above, or because the identity calculated from an alignment is too
low (as discussed earlier). If there is no reject record for S, then S was never tested and the search was
abandoned too early. This is expected to happen in rare cases because target sequences are tested in an
order that correlates approximately with identity, but not exactly. It can therefore happen that UCLUST
gives up the search when in fact there is match lower in the list. The frequency of this type of false
negative can be reduced by increasing the --maxrejects option.

Q was assigned to T which has lower identity than S
This also happens occasionally because of the order in which UCLUST checks target sequences. Since the
order does not exactly correlate with identity, there may be a better match lower in the list, but is not
found because the first match found is accepted by default. The frequency of these sub-optimal hits can
be reduced by increasing --maxaccepts, which defaults to 1. Generally you should increase --maxrejects
also, otherwise UCLUST will give up after only 8 rejections, which limits the total number of sequences
tested and prevents UCLUST from getting deep into the list of potential hits.

Quickly reproducing the state when Q was processed
If you are using --libonly, you can make an input file containing just Q. The database in memory doesn't
change, so Q should be processed in exactly the same way as in your full run. It should then be very fast
to try the following techniques with the entire database instead of just one or two targets.

Use --blastout to review the alignment and identity calculation.

Use --nofastalign or --check_fast to compare alignments with and without heuristics.

Use --output_rejects to review rejections.

If you are not using --libonly, then the database grows over time and you need to know exactly which
sequences were in the database when Q was processed. The can be determined from the .uc file. Each
new seed is indicated by an S record. So extract all S records in the .uc file that appear before Q, and call
this file seeds.uc. Convert seeds.uc to a FASTA library:

    uclust --uc2fasta seeds.uc --input seqs.fa --output seeds.fa --types S

If you used --lib, add that in too:

    cat lib.fa >> seeds.fa

Now you can process Q in exactly the same way as your original run without waiting for other sequences
to be processed first. Put Q into a file q.fa, then:

    uclust --input q.fa --lib seeds.fa ...other options as in original run...
Memory requirements
The amount of memory needed is approximately 10x the size of a FASTA file containing the database (seeds). A better estimate is

(9x the number of letters in all sequences) + (1x the number of letters in all labels)

The amount of memory required can be reduced in a number of ways, as follows.

Reduce redundancy
If you have very similar sequences in your database, say at least 98% identical, then it could pay to reduce redundancy by clustering at a high identity, say 98% or 99%. This, of course, can be done using uclust itself to pre-process your database.

Trim sequence labels
Sequence labels, i.e. the characters following '>' in a FASTA file, are stored as-is in memory. If your labels are long and your sequences are short, then the amount of memory required for labels may be a significant fraction of the total memory requirement. If so, it pays to reduce the label size. For example, you could label your sequences with an integer or some other short string that can be used as a key for retrieving longer annotations in a post-processing step.

Split the database
You can split the database into smaller pieces. This allows you to parallelize a search (e.g. by running the query against N pieces in parallel on N machine in a cluster, or to serialize (by running one piece after the other on a single machine). Splitting the database may also have the advantageous side-effect of improving sensitivity. The very high speed of the USEARCH algorithm is achieved by limiting explicit sequence comparisons to a small subset of the database having the most unique words in common with the query sequence. As the database size grows, more spurious sequences will tend to appear in this subset and sensitivity may be reduced as a result.

Two-level search
If finding the closest possible match to a very large database is important in your application, then you can combine the "reduce redundancy" and "split" strategies to achieve improved speed, reduced memory use and (usually, but not always) higher sensitivity. The idea is to search first in a low-redundancy database (LRD). Sequences in the LRD are annotated with the name of a second-level database (SLD) which has more closely-related sequences. There are several SLDs that, when combined, contain the full set of sequences. In the second pass, the query is searched against the SLD identified in the first search.
This picture is over-simplified: we don't want a separate SLD for every sequence in the low-redundancy DB. There are two reasons for this: if the SLD is too small, we lose the advantage of the high search speed of USEARCH because there will be too much overhead setting up each query. Also, we want to group related families into a single SLD because otherwise the hit to the LRD may not correctly identify the SLD with the closest possible match.

To create the databases, I suggest the following approach.

1. Cluster at a fairly low identity; say 50% for proteins or 80% for nucleotides.

2. Pick a desired size for an SLD, say 1/N of the full database. If a cluster from step 1 is larger than this, you can split it by clustering at a higher identity, or go back and re-cluster the entire database at a higher identity.

3. Merge clusters from step 1 to create the SLDs (SLD1, SLD2 ... SLDN). This can be done by a simple greedy algorithm which can be implemented in a script, let me know if you'd like help with this. Label each sequence with the name of its SLD (this is so that the SLD name is available in step 5 below where the LRD is created).

4. Cluster each SLD at a high identity; say 98% for nucleotides or 90% for proteins.

5. Combine all the seeds from step 4 above, this produces the LRD.

To run a two-pass query, first search the query sequences against the LRD. Then divide them according to the SLD identified by the LRD hit and run each subset against its SLD; this of course can be done serially or in parallel.
### Command-line options

#### Input options

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>--input filename</td>
<td>Input file containing query sequences. FASTA format. By default, must be sorted by decreasing sequence length (see --usersort).</td>
</tr>
<tr>
<td>--query filename</td>
<td>Same as --input, except implies --libonly.</td>
</tr>
<tr>
<td>--db filename</td>
<td>Same as --lib if used with --query.</td>
</tr>
<tr>
<td>--lib filename</td>
<td>Library file. FASTA format. This is used to initialize the search database in memory. By default, the initial database is empty.</td>
</tr>
<tr>
<td>--usersort</td>
<td>By default, if new seeds may be created, then UCLUST requires that the input file is sorted by decreasing sequence length, and will fail if it is not. Specify --usersort to indicate that file is sorted by some other criteria (or is not sorted at all but you think this is OK). For de novo clustering, input should be sorted so that a suitable seed sequence will appear before other members of the cluster. If the input includes both full-length sequences and fragments, then sorting by decreasing length is usually the best approach.</td>
</tr>
<tr>
<td>--maxlen L</td>
<td>Ignore query sequences that are longer than L. Default 10000. UCLUST is currently not designed to handle very long sequences. If you increase this value significantly, then UCLUST may fail due to lack of memory or for other reasons. Let me know if you have applications that need longer sequence lengths.</td>
</tr>
<tr>
<td>--minlen L</td>
<td>Ignore query sequences that are shorter than L. Default 16.</td>
</tr>
<tr>
<td>--amino</td>
<td>Specifies that input sequences use the 20-letter amino acid alphabet. By default, UCLUST 'guesses' this from the frequencies of AGCTU in the first few sequences.</td>
</tr>
<tr>
<td>--nucleo</td>
<td>Specifies that the input sequence use a nucleotide alphabet. By default, UCLUST 'guesses' this from the frequencies of AGCTU in the first few sequences.</td>
</tr>
</tbody>
</table>

#### Output options

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>--trunclabels</td>
<td>Truncate FASTA labels at the first whitespace character.</td>
</tr>
<tr>
<td>--allhits</td>
<td>Write all hits to the .uc file. By default, only the best hit found is written. To get more than one hit, you must specify --allhits and set --maxaccepts</td>
</tr>
<tr>
<td>Option</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>--[no]output_rejects</td>
<td>Write reject records to the .uc file. By default, rejects are not written (--nooutputrejects). This is mainly useful for trouble-shooting unexpected results.</td>
</tr>
<tr>
<td>--log filename</td>
<td>Write a log file with miscellaneous information. I recommend that you try this and take a look at the output, you might find some of it helpful.</td>
</tr>
<tr>
<td>--blastout filename</td>
<td>Output file for human-readable alignments in a BLAST-like format. This format may be changed in future versions, so it is recommended that parsers use the FASTA output generated by --fastapairs instead.</td>
</tr>
<tr>
<td>--fastapairs filename</td>
<td>Output file for pair-wise alignments in FASTA format. Pairs are separated by blank lines, to make the file easier to inspect visually. The query sequence is first, the target (seed, database) sequence is second. If the input sequences are nucleotides, then a + or - is appended to the label of the target sequence to indicate the strand. If the strand is - (reverse strand match), then the target sequence is reverse-complemented.</td>
</tr>
<tr>
<td>--rowlen n</td>
<td>Row length for --blastout file. Default 64.</td>
</tr>
<tr>
<td>--idchar c</td>
<td>Character annotating identities in --blastout file. Default '</td>
</tr>
<tr>
<td>--diffchar c</td>
<td>Character annotating differences in --blastout file. Default blank . Nucleotide alignments only.</td>
</tr>
<tr>
<td>--uc_evalue</td>
<td>Write E-value to .uc file. The percent-id field is then formatted as follows: 55.1/144.8/1.2e-6, where 55.1 is the identity, 144.8 is the bit score and 1.2e-6 is the E-value. The bit score and E-value is computed from the optimal gapless HSP in the global alignment.</td>
</tr>
</tbody>
</table>

**Search options**

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>--id f</td>
<td>Minimum identity to accept a hit. Floating point number in range 0.0 to 1.0. Default 0.9.</td>
</tr>
<tr>
<td>--maxaccepts n</td>
<td>Stop searching if n hits have been found, then report the best. Default 1. Zero means infinity, i.e. don't stop however many matches have been found (but will still stop if the maximum number of rejects has occurred). Use --maxaccepts 0 --maxrejects 0 to force a search of the entire database with every query, this guarantees that the best hit will be found, if one is found.</td>
</tr>
<tr>
<td>Option</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>--maxrejects n</td>
<td>Stop searching if n rejects have occurred, then report a failure to find a hit. Default 8. Zero means infinity, i.e. keep searching until all a hit is found or database sequences have been tested.</td>
</tr>
<tr>
<td>--maxtargets t</td>
<td>Keep searching until t targets have been examined, then report the best hit found or a failure to find a hit. If you set this option, then you should probably also set --maxaccepts 0 and --maxrejects 0 as the first terminating condition encountered wins.</td>
</tr>
<tr>
<td>--w n</td>
<td>Word length for unique word index. Default is 8 for nucleotides, 5 for amino acids. It is not clear whether these defaults are good in all applications; further research is needed to understand this better.</td>
</tr>
<tr>
<td>--k n</td>
<td>Word length for HSP finding. Default is 5 for nucleotides, 3 for amino acids. It is not clear whether these defaults are good in all applications; further research is needed to understand this better.</td>
</tr>
<tr>
<td>--wordcountreject</td>
<td>By default, --wordcountreject is enabled so that target sequences are rejected if they have too few unique words in common with the query sequence. The threshold is estimated using heuristics. This improves speed, but may also reduce sensitivity. Using --nowordcountretract disables word count rejection.</td>
</tr>
<tr>
<td>--bump n</td>
<td>By default, an optimization called &quot;threshold bumping&quot; is used to reduce the search space when many target sequences are found to pass the word count threshold. This may reduce sensitivity slightly, and may increase the probability that the top hit is not found, but often improves speed significantly when the database is large. Default is --bump 50. Use --bump 0 to disable bumping.</td>
</tr>
<tr>
<td>--stepwords n</td>
<td>By default, an optimization called &quot;stepping&quot; is used to speed up database searching. This is effective when the number of words in common between the query and target is expected to be large. Then it is expensive to check all words, and stepping selects a subset of words in the query. By default, --stepwords is 8. This means that the number of query words is chosen so that approximately 8 words are expected to be found in the target sequence. Use --stepwords 0 to disable stepping. As with bumping, stepping may reduce sensitivity and may reduce the probability that the best hit is found first.</td>
</tr>
<tr>
<td>--rev</td>
<td>By default, UCLUST searches only the plus strand for nucleotide sequences. If --rev is specified, then UCLUST will also search the reverse-complemented sequence.</td>
</tr>
<tr>
<td>Option</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>--libonly</td>
<td>By default, if no hit is found, UCLUST will add the query sequence as a new seed. If --libonly is specified, this does not happen. Using --lib and --libonly is appropriate for database search applications.</td>
</tr>
<tr>
<td>--self</td>
<td>Used for searching a database against itself, e.g. to reduce redundancy. If the target and query labels are identical, the target is ignored. Typical use is uclust --input lib.fa --lib lib.fa --libonly --self --uc results.uc.</td>
</tr>
<tr>
<td>--idprefix n</td>
<td>Require that the first n letters of query and target are identical. Default zero.</td>
</tr>
<tr>
<td>--exact</td>
<td>Same as --maxrejects 0 --nowordfilter. Guarantees that a hit will be found if one exists.</td>
</tr>
<tr>
<td>--optimal</td>
<td>Same as --maxrejects 0 --maxaccepts 0 --nowordfilter. Similar to --exact but also guarantees that the best hit will be found.</td>
</tr>
</tbody>
</table>

**Alignment options**

<table>
<thead>
<tr>
<th>Option</th>
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</tr>
</thead>
<tbody>
<tr>
<td>--match s</td>
<td>Match score for nucleotides. Default 2.0.</td>
</tr>
<tr>
<td>--mismatch s</td>
<td>Mismatch score for nucleotides. Default -1.0.</td>
</tr>
<tr>
<td>--gapopen s</td>
<td>Gap open penalty specification. Format is described elsewhere in this manual.</td>
</tr>
<tr>
<td>--gapext s</td>
<td>Gap extension penalty specification. Format is described elsewhere in this manual.</td>
</tr>
<tr>
<td>--[no]fastalign</td>
<td>Default is --fastalign. Specify --nofastalign to disable fast alignment heuristics (HSPs and banding).</td>
</tr>
<tr>
<td>--hsp</td>
<td>Minimum length for an HSP. Default 32. Specify zero to disable HSPs.</td>
</tr>
<tr>
<td>--hspscore s</td>
<td>Minimum score/column for an HSP. Default 1.0.</td>
</tr>
<tr>
<td>--band n</td>
<td>Radius of band for banded dynamic programming between HSPs. Default 16. Specify zero to disable banding.</td>
</tr>
<tr>
<td>--check_fast</td>
<td>Compare results using alignments with and without fast heuristics and generate a report in the --log file.</td>
</tr>
</tbody>
</table>

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## Miscellaneous options

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>--quiet</td>
<td>Don't write progress messages to standard error.</td>
</tr>
<tr>
<td>--version</td>
<td>Write version to standard output and exit.</td>
</tr>
<tr>
<td>--help</td>
<td>Write command-line help to standard output and exit.</td>
</tr>
</tbody>
</table>