BLAST Command Line Applications User Manual

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Introduction

Sequence similarity searching is one of the more important bioinformatics activities and often provides the first evidence for the function of a newly sequenced gene or piece of sequence. Basic Local Alignment Search Tool (BLAST) is probably the most popular similarity search tool. The National Center For Biotechnology Information (NCBI) first introduced BLAST in 1989. The NCBI has continued to maintain and update BLAST since the first version. In 2009, the NCBI introduced a new version of the stand-alone BLAST applications (BLAST+). The BLAST+ applications have a number of improvements that allow faster searches as well as more flexibility in output formats and in the search input. These improvements include: splitting of longer queries so as to reduce the memory usage and to take advantage of modern CPU architectures; use of a database index to dramatically speed up the search; the ability to save a “search strategy” that can be used later to start a new search; and greater flexibility in the formatting of tabular results.

The functionality of the BLAST+ applications is organized by search type. As an example, there is a “blastp” application that compares proteins queries to protein databases. The “blastx” application translates a nucleotide query in six frames and searches it against a protein database. This organization is different from that of the applications first released in 1997 (e.g., blastall) that supported all types of searches with one application, but it resembles that of the NCBI BLAST web site. An advantage of this design is that each application has only the options relevant to the searches it performs. Additionally, each application can compare a query to a set of FASTA sequences in a file, bypassing the need to create a BLAST databases for small and infrequently searched sets. Finally, a “remote” option permits each application to send off a search to the NCBI servers.

This manual has several sections. It provides brief installation instructions, a QuickStart, a section describing BLAST+ features in more depth, a “Cook Book” section on how to perform a number of tasks, as well as three appendices. The first appendix discusses tools to help with the transition from the older applications (e.g., blastall) to the BLAST+ applications. The second appendix documents exit codes from the BLAST+ applications. The third appendix is a table of BLAST options, the type of input required, and the default values for each application. The fourth appendix lists the scoring parameters that the blastn application supports.

An introduction to BLAST is outside the scope of this manual, more information on this subject can be found on http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastDocs.

Please feel free to contact us with any questions, feedback, or bug reports at blast-help@ncbi.nlm.nih.gov.

Installation

Entries in the BLAST Help manual provide installation instructions for Windows and LINUX/UNIX. This section provides instructions for a few cases not covered by those entries.

The BLAST+ applications are distributed both as an executable and as source code. For the executable formats we provide installers as well as tarballs; the source code is only provided as a tarball. These are freely available at ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/. Please be sure to use the most recent available version; this will be indicated in the file name (for instance, in the sections below, version 2.2.18 is listed, but this should be replaced accordingly).

Windows

Please see http://www.ncbi.nlm.nih.gov/books/NBK52637/

MacOSX

For users without administrator privileges: follow the procedure described in http://www.ncbi.nlm.nih.gov/books/NBK52640/

For users with administrator privileges and machines MacOSX version 10.5 or higher: Download the ncbi-blast-2.2.18+.dmg installer and double click on it. Double click the newly mounted ncbi-blast-2.2.18+ volume, double click on ncbi-blast-2.2.18+.pkg and follow the instructions in the installer. By default the BLAST+ applications are installed in /usr/local/ncbi/blast, overwriting its previous contents (an uninstaller is provided and it is recommended when upgrading a BLAST+ installation).

RedHat Linux

Download the appropriate *.rpm file for your platform and either install or upgrade the ncbi-blast+ package as appropriate using the commands:

```
Install:
rpm -ivh ncbi-blast-2.2.18-1.x86_64.rpm
Upgrade:
rpm -Uvh ncbi-blast-2.2.18-1.x86_64.rpm
```

Note: one must have root privileges to run these commands. If you do not have root privileges, please use the procedure described in http://www.ncbi.nlm.nih.gov/books/NBK52640/

Other Unix platforms

Please see http://www.ncbi.nlm.nih.gov/books/NBK52640/

Source tarball

Use this approach to build the BLAST+ applications yourself. Download the tarball, expand it, cd to the newly created directory, and type the following commands:

```
cd c++
./configure --without-debug --with-strip --with-mt --with-build-
root=ReleaseMT
cd ReleaseMT/build
make all_r
```

The compiled executables will be found in c++/ReleaseMT/bin.

In Windows, extract the tarball and open the appropriate MSVC solution or project file (e.g.: c++\compilers\msvc800_prj\static\build), build the -CONFIGURE- project, click on “Reload” when prompted by the development environment, and then build the -BUILD-ALL- project. The compiled executables will be found in the directory corresponding to the build configuration selected (e.g.: c++\compilers\msvc800_prj\static\bin\debugdll).

Information on using and compiling the NCBI C++ toolkit is available at http://www.ncbi.nlm.nih.gov/books/NBK7160/. Please send questions about compiling the NCBI C ++ toolkit to toolbox@ncbi.nlm.nih.gov

Quick start

A BLAST search against a database requires at least a –query and –db option. The command:

```
blastn -db nt -query nt.fsa -out results.out
```

will run a search of nt.fsa (a nucleotide sequence in FASTA format) against the nt database, printing results to the file results.out. If “-out results.out” had been left off, the results would have been printed to stdout (i.e., the screen). The blastn application searches a nucleotide query against a nucleotide database.

The BLAST+ applications print documentation when invoked with the –h or –help option. The –h option provides abbreviated help, and the –help flag provides more extensive documentation.

The BLAST databases are required to run BLAST locally and to support automatic resolution of sequence identifiers. Documentation about these identifiers can be found at http://www.ncbi.nlm.nih.gov/books/NBK7183/table/ch_demo.T5/. The databases may be retrieved automatically with the update_blastdb.pl PERL script, which is included as part of this distribution. This script will download multiple tar files for each BLAST database volume if necessary, without having to designate each volume. For example:

```
./update_blastdb.pl htgs
```

will download all the relevant HTGs tar files (htgs.00.tar.gz, …, htgs.N.tar.gz)

The script can also compare your local copy of the database tar file(s) and only download tar files if the date stamp has changed reflecting a newer version of the database. This will allow the script run on a schedule and only download tar files when needed. Documentation for the update_blastdb.pl script can be obtained by running the script without any arguments (perl is required).

RPS-BLAST ready databases are available at ftp://ftp.ncbi.nih.gov/pub/mmdb/cdd/

The BLAST taxonomy database is required in order to print the scientific name, common name, blast name, or super kingdom as part of the BLAST report or in a report with blastdbcmd. The BLAST database contains only the taxid (an integer) for each entry, and the taxonomy database allow BLAST to retrieve the scientific name etc. from a taxid. The BLAST taxonomy database consists of a pair of files (taxdb.bti and taxdb.btd) that are available as a compressed archive from the NCBI BLAST FTP site (ftp://ftp.ncbi.nlm.nih.gov/blast/db/taxdb.tar.gz). The update_blastdb.pl script can be used to download and update this archive; it is recommended that the uncompressed contents of the archive be installed in the same directory where the BLAST databases reside. Assuming proper file permissions and that the BLASTDB environment variable contains the path to the installation directory of the BLAST databases, the following commands accomplish that:

```
 # Download the taxdb archive
perl update_blastdb.pl taxdb
# Install it in the BLASTDB directory
gunzip -cd taxdb.tar.gz | (cd $BLASTDB; tar xvf - )
```

User manual

Functionality offered by BLAST+ applications

The functionality offered by the BLAST+ applications has been organized by program type, as to more closely resemble Web BLAST.

As an example, to run a search of a nucleotide query (translated "on the fly" by BLAST) against a protein database one would use the blastx application. The blastx application will also work in "Blast2Sequences" mode (i.e.: accept FASTA sequences instead of a BLAST database as targets) and can also send BLAST searches over the network to the public NCBI server if desired.

The BLAST+ package offers three categories of applications: 1.) search tools, 2.) BLAST database tools, and 3.) sequence filtering tools. The blastn, blastp, blastx, tblastx, tblastn, psiblast, rpsblast, and rpstblastn are considered search applications, as they execute a BLAST search, whereas makeblastdb, blastdb_aliastool, makeprofiledb, and blastdbcmd are considered BLAST database applications, as they either create or examine BLAST databases.

There is also a new set of sequence filtering applications described in the section Sequence filtering applications and an application to build database indices that greatly speed up megablast in some cases (see section titled Megablast indexed searches).

BLAST+ features

Tasks

The blastn and blastp applications have a –task option. This option sets the parameters (e.g., word-size or gap values) to typical values for a specific type of search. For example, the "megablast" task is optimized for intraspecies comparison as it uses a large word-size, whereas "blastn" is better suited for interspecies comparisons with a shorter word-size. These tasks resemble the "Program Selection" section of the BLAST web pages and do not preclude the user from setting other options to override those specified by the task. See Appendix C for documentation on parameter values for different tasks. The following tasks are currently available:

Program	Task Name	Description
blastp	blastp	Traditional BLASTP to compare a protein query to a protein database
	blastp-short	BLASTP optimized for queries shorter than 30 residues
blastn	blastn	Traditional BLASTN requiring an exact match of 11
	blastn-short	BLASTN program optimized for sequences shorter than 50 bases
	megablast	Traditional megablast used to find very similar (e.g., intraspecies or closely related species) sequences
	dc-megablast	Discontiguous megablast used to find more distant (e.g., interspecies) sequences

Megablast indexed searches

Indexing provides an alternative way to search for initial matches in nucleotide-nucleotide searches (blastn and megablast) by pre-indexing the N-mer locations in a special data structure, called a database index.

Using an index can improve search times significantly under certain conditions. It is most beneficial when the queries are much shorter than the database and works best for queries under 1 Mbases long. The advantage comes from the fact that the whole database does not have to be scanned during the search.

Indices can capture masking information, thereby enabling search against databases masked for repeats, low complexity, etc.

There are, however, limitations to using indexed search in blast:

- Index files are about four times larger than the blast databases. If an index does not fit into computer operating memory, then the advantage of using it is eliminated.
- Word size must be set to 16 or more in order to use an indexed search.
- Discontiguous search is not supported.

Reference: Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schäffer AA. Database Indexing for Production MegaBLAST Searches. Bioinformatics 2008, 24(16): 1757-64. PMID:18567917

BLAST search strategies

BLAST search strategies are files that encode the inputs necessary to perform a BLAST search. The purpose of these files is to be able to seamlessly reproduce a BLAST search in various environments (Web BLAST, command line applications, etc).

Exporting search strategies on the Web BLAST

Click on "download" next to the RID/saved strategy in the "Recent Results" or "Saved Strategies" tabs.

Exporting search strategies with BLAST+ applications

Add the -export_search_strategy along with a file name to the command line options.

Importing search strategies on Web BLAST

Go to the "Saved Strategies" tab, click on "Browse" to select your search strategy file, then click on "View" to load it into the submission page.

Importing search strategies with BLAST+ applications

Add the -import_search_strategy along with a file name containing the search strategy file. Note that if provided, the –query, -db, -use_index, and –index_name command line options will override the specifications of the search strategy file provided (no other command line options will override the contents of the search strategy file).

Negative GI lists

Search applications support negative GI lists. This feature provides a means to exclude GIs from a BLAST database search. The expect values in the BLAST results are based upon the sequences actually searched and not on the underlying database. For an example, see the cookbook.

Masking in BLAST databases

It is now possible to create BLAST databases that contain filtered sequences (also known as masking information or masks). This filtering information can be used as soft masking for the subject sequences. For instructions on creating masked BLAST databases, please see the cookbook.

Custom output formats for BLAST searches

The BLAST+ search command line applications support custom output formats for the tabular and comma-separated value output formats. For more details see “outfmt” in Appendix C as well as the cookbook.

Custom output formats to extract BLAST database data

blastdbcmd supports custom output formats to extract data from BLAST databases via the -outfmt command line option. For more details see the blastdbcmd options in Appendix C as well as the cookbook.

Improved software installation packages

The BLAST+ applications are available via Windows and MacOSX installers as well as RPMs (source and binary) and unix tarballs. For more details about these, refer to the installation section.

Sequence filtering applications

The BLAST+ applications include a new set of sequence filtering applications, namely segmasker, dustmasker, and windowmasker. Segmasker is an application that identifies and masks low complexity regions of protein sequences. The dustmasker application provides a similar functionality for nucleotide sequences. Windowmasker uses a genome to identify sequences represented too often to be of interest to most users. See ftp://ftp.ncbi.nlm.nih.gov/pub/agarwala/dustmasker/README.dustmasker and ftp://ftp.ncbi.nlm.nih.gov/pub/agarwala/windowmasker/README.windowmasker for more information.

Best-Hits filtering algorithm

The Best-Hit filtering algorithm is designed for use in applications that are searching for only the best matches for each query region reporting matches. Its -best_hit_overhang parameter, H, controls when an HSP is considered short enough to be filtered due to presence of another HSP. For each HSP A that is filtered, there exists another HSP B such that the query region of HSP A extends each end of the query region of HSP B by at most H times the length of the query region for B.

Additional requirements that must also be met in order to filter A on account of B are:

i evalue(A) >= evalue(B)

ii score(A)/length(A) < (1.0 – score_edge) * score(B)/length(B)

We consider 0.1 to 0.25 to be an acceptable range for the -best_hit_overhang parameter and 0.05 to 0.25 to be an acceptable range for the -best_hit_score_edge parameter. Increasing the value of the overhang parameter eliminates a higher number of matches, but increases the running time; increasing the score_edge parameter removes smaller number of hits.

Automatic resolution of sequence identifiers

The BLAST+ search applications support automatic resolution of query and subject sequence identifiers specified as GIs or accessions (see the cookbook section for an example). This feature enables the user to specify one or more sequence identifiers (GIs and/or accessions, one per line) in a file as the input to the -query and -subject command line options.

Upon encountering this type of input, by default the BLAST+ search applications will try to resolve these sequence identifiers in locally available BLAST databases first, then in the BLAST databases at NCBI, and finally in Genbank (the latter two data sources require a properly configured internet connection). These data sources can be configured via the DATA_LOADERS configuration option and the BLAST databases to search can be configured via the BLASTDB_PROT_DATA_LOADER and BLASTDB_NUCL_DATA_LOADER configuration options (see the section on Configuring BLAST).

BLAST-WindowMasker integration in BLAST+ search applications

The BLAST+ search applications support integration with the windowmasker files via the -window_masker_taxid and the WINDOW_MASKER_PATH configuration parameter (see Configuring BLAST) or via the -window_masker_db command line option.

In the first case, the WINDOW_MASKER_PATH configuration parameter should refer to a directory which contains subdirectories named after NCBI taxonomy IDs (e.g.: 9606 for human, 10090 for mouse), where the windowmasker unit counts data files should be placed with the following naming convention: wmasker.obinary (for files generated with the obinary format) and/or wmasker.oascii (for files generated with the oascii format). For an example on how to create these files, please see the Cookbook. Once these windowmasker files and the configuration file are in place, this feature can be invoked by providing the taxonomy ID to the -window_masker_taxid command line option.

Alternatively, this feature can also be invoked by providing the path to the windowmasker unit counts data file via the -window_masker_db.

Please see the Cookbook for a usage example of this feature.

DELTA-BLAST: A tool for sensitive protein sequence search

DELTA-BLAST uses RPS-BLAST to search for conserved domains matching to a query, constructs a PSSM from the sequences associated with the matching domains, and searches a sequence database. Its sensitivity is comparable to PSI-BLAST and does not require several iterations of searches against a large sequence database. See the cookbook for more information.

Concatenation of queries

BLAST works more efficiently if it scans the database once for multiple queries. This feature is known as concatenation. It speeds up MegaBLAST searches the most as they spend little time on tasks that consume CPU and most of the time streaming through the database. BLASTN and discontiguous MegaBLAST searches also run faster with concatenation, though the effect is less pronounced. BLAST+ applies concatenation on all types of searches (e.g., also BLASTP, etc.), and it can be very beneficial if the input is a large number of queries in FASTA format. BLAST+ concatenates queries by grouping them together until a specific number of letters (or "chunk size") is reached. Unfortunately, a constant chunk size for each database scan causes certain problems. For some searches the chunk size is too large, too many letters are searched at once, and the process consumes too much memory. Tests have shown that the number of successful ungapped extensions performed in the preliminary stage is a good predictor of overall memory use during a search. The BLASTN application (starting with the 2.2.28 release) takes advantage of this insight to provide an "adaptive chunk size". The application starts with a low initial chunk size of 10,000 bases and records how many successful ungapped extensions were performed during search. It adjusts the chunk size on the next database scan with a target of performing two million extensions during the search.

Query concatenation also means that BLAST will produce no output until the first set of concatenated queries have been processed. Some users find this disconcerting, but it is not a problem.

BLAST+ remote service

The BLAST+ applications can also send a search to the servers at the NCBI. In this case, the BLAST+ application is acting as a client and there is no need to install a database or provide more than minimal computing power. The BLAST+ remote service uses the same servers used by the NCBI BLAST website. The BLAST server can return a Request ID (RID) as part of the results, and that RID can be used to reformat the results with the blast_formatter or on the NCBI website. In general, the servers keep the results for an RID for 36 hours. The BLAST+ applications will use the remote service if the –remote flag is added to the command line. The BLAST+ remote service uses a shared resource (the computers at the NCBI), so only one BLAST+ application should run remote searches at a time. An example in the cookbook section demonstrates a remote search.

Configuring BLAST

The BLAST+ search applications can be configured by means of a configuration file named .ncbirc (on Unix-like platforms) or ncbi.ini (on Windows). This is a plain text file that contains sections and key-value pairs to specify configuration parameters. Lines starting with a semi-colon are considered comments. The application will search for the file in the following order and locations:

1. Current working directory
2. User's HOME directory
3. Directory specified by the NCBI environment variable

The search for this file will stop at the first location where it is found and the configurations settings from that file will be applied. If the configuration file is not found, default values will apply. The following are the possible configuration parameters that impact the BLAST+ applications:

Configuration Parameter	**Specifies**	**Default value**
BLASTDB	Path to BLAST databases.	Current working directory

DATA_LOADERS	Data loaders to use for automatic sequence identifier resolution. This is a comma separated list of the following keywords: blastdb, genbank, and none. The none keyword disables this feature and takes precedence over any other keywords specified.	blastdb,genbank
BLASTDB_PROT_DATA_LOADER	Locally available BLAST database name to search when resolving protein sequences using BLAST databases. Ignored if DATA_LOADERS does not include the blastdb keyword.	nr
BLASTDB_NUCL_DATA_LOADER	Locally available BLAST database name to search when resolving nucleotide sequences using BLAST databases. Ignored if DATA_LOADERS does not include the blastdb keyword.	nt
GENE_INFO_PATH	Path to gene information files (NCBI only).	Current working directory
WINDOW_MASKER_PATH	Path to windowmasker directory hierarchy.	Current working directory

The following is an example with comments describing the available parameters for configuration:

```
; Start the section for BLAST configuration
[BLAST]
; Specifies the path where BLAST databases are installed
BLASTDB=/home/guest/blast/db
; Specifies the data sources to use for automatic resolution
; for sequence identifiers
DATA_LOADERS=blastdb
; Specifies the BLAST database to use resolve protein sequences
BLASTDB_PROT_DATA_LOADER=custom_protein_database
; Specifies the BLAST database to use resolve protein sequences
BLASTDB_NUCL_DATA_LOADER=/home/some_user/my_nucleotide_db


; Windowmasker settings
[WINDOW_MASKER]
WINDOW_MASKER_PATH=/home/guest/blast/db/windowmasker
; end of file
```

Controlling concatenation of queries

As described above, BLAST+ works more efficiently if it scans the database once for multiple queries. This feature is knows as concatenation. Unfortunately, for some searches the concatenation values are not optimal, too many queries are searched at once, and the process can consume too much memory. For applications besides BLASTN (which uses an adaptive approach), it is possible to control these values by setting the BATCH_SIZE environment variable. Setting the value too low will degrade performance dramatically, so this environment variable should be used with caution.

Memory usage

The BLAST search programs can exhaust all memory on a machine if the input is too large or if there are too many hits to the BLAST database. If this is the case, please see your operating system documentation to limit the memory used by a program (e.g.: ulimit on Unix-like platforms). Setting the BATCH_SIZE environment variable as described above may help.

Input formats to BLAST

Multiple sequence alignment

The -in_msa psiblast option provides a way to jump start psiblast from a master-slave multiple sequence alignment computed outside psiblast. The multiple sequence alignment must contain the query sequence as one of its sequences, but it need not be the first sequence. The multiple sequence alignment must be specified in a format that is derived from Clustal, but without some headers and trailers (see example below).

The rules are also described by the following words. Suppose the multiple sequence alignment has N sequences. It may be presented in one or more blocks, where each block presents a range of columns from the multiple sequence alignment. E.g., the first block might have columns 1-60, the second block might have columns 61-95, the third block might have columns 96-128. Each block should have N rows, one row per sequence. The sequences should be in the same order in every block. Blocks are separated by one or more black lines. Within a block there are no blank lines, and each line consists of one sequence identifier followed by some whitespace followed by characters (and gaps) for that sequence in the multiple sequence alignment. In each column, all letters must be in upper case, or all letters must be in lower case.

```
# Example multiple sequence alignment file
 align1
------
26SPS9_Hs IHAAEEKDWKTAYSYFYEAFEGYdsidspkaitslkymllckimlntpedvqalvsgkla
F57B9_Ce LHAADEKDFKTAFSYFYEAFEGYdsvdekvsaltalkymllckvmldlpdevnsllsakl
YDL097c_Sc ILHCEDKDYKTAFSYFFESFESYhnltthnsyekacqvlkymllskimlnliddvkniln
YMJ5_Ce LYSAEERDYKTSFSYFYEAFEGFasigdkinatsalkymilckimlneteqlagllaake
FUS6_ARATH KNYIRTRDYCTTTKHIIHMCMNAilvsiemgqfthvtsyvnkaeqnpetlepmvnaklrc
COS41.8_Ci SLDYKLKTYLTIARLYLEDEDPVqaemyinrasllqnetadeqlqihykvcyarvldyrr
644879 KCYSRARDYCTSAKHVINMCLNVikvsvylqnwshvlsyvskaestpeiaeqrgerdsqt
YPR108w_Sc IHCLAVRNFKEAAKLLVDSLATFtsieltsyesiatyasvtglftlertdlkskvidspe
eif-3p110_Hs SKAMKMGDWKTCHSFIINEKMNGkvw----------------------------------
T23D8.4_Ce SKAMLNGDWKKCQDYIVNDKMNQkvw----------------------------------
YD95_Sp IYLMSIRNFSGAADLLLDCMSTFsstellpyydvvryavisgaisldrvdvktkivdspe
KIAA0107_Hs LYCVAIRDFKQAAELFLDTVSTFtsyelmdyktfvtytvyvsmialerpdlrekvikgae
F49C12.8_Hs LYRMSVRDFAGAADLFLEAVPTFgsyelmtyenlilytvitttfaldrpdlrtkvircne
Int-6_Mm KFQYECGNYSGAAEYLYFFRVLVpatdrnalsslwgklaseilmqnwdaamedltrlket


26SPS9_Hs lryagrqtealkcvaqasknrsladfekaltdy---------------------------
F57B9_Ce alkyngsdldamkaiaaaaqkrslkdfqvafgsf--------------------------
YDL097c_Sc akytketyqsrgidamkavaeaynnrslldfntalkqy----------------------
YMJ5_Ce ivayqkspriiairsmadafrkrslkdfvkalaeh-------------------------
FUS6_ARATH asglahlelkkyklaarkfldvnpelgnsyneviapqdiatygglcalasfdrselkqkv
COS41.8_Ci kfleaaqrynelsyksaiheteqtkalekalncailapagqqrsrmlatlfkdercqllp
644879 qailtklkcaaglaelaarkykqaakclllasfdhcdfpellspsnvaiygglcalatfd
YPR108w_Sc llslisttaalqsissltislyasdyasyfpyllety-----------------------
eif-3p110_Hs ------------------------------------------------------------
T23D8.4_Ce ------------------------------------------------------------
YD95_Sp vlavlpqnesmssleacinslylcdysgffrtladve-----------------------
KIAA0107_Hs ilevlhslpavrqylfslyecrysvffqslavv---------------------------
F49C12.8_Hs vqeqltggglngtlipvreylesyydchydrffiqlaale--------------------
Int-6_Mm idnnsvssplqslqqrtwlihwslfvffnhpkgrdniidlflyqpqylnaiqtmcphilr
```

```
26SPS9_Hs ------------------------------------------------------------
F57B9_Ce ------------------------------------------------------------
YDL097c_Sc ------------------------------------------------------------
YMJ5_Ce ------------------------------------------------------------
FUS6_ARATH idninfrnflelvpdvrelindfyssryascleylasl----------------------
COS41.8_Ci sfgilekmfldriiksdemeefar------------------------------------
644879 rqelqrnvissssfklflelepqvrdiifkfyeskyasclkmldem--------------
YPR108w_Sc ------------------------------------------------------------
eif-3p110_Hs ------------------------------------------------------------
T23D8.4_Ce ------------------------------------------------------------
YD95_Sp ------------------------------------------------------------
KIAA0107_Hs ------------------------------------------------------------
F49C12.8_Hs ------------------------------------------------------------
Int-6_Mm ylttavitnkdvrkrrqvlkdlvkviqqesytykdpitefveclyvnfdfdgaqkklrec


26SPS9_Hs RAELRDDPIISTHLAKLYDNLLEQNLIRVIEPFSRVQIEHISSLIKLSKADVERKLSQMI
F57B9_Ce PQELQMDPVVRKHFHSLSERMLEKDLCRIIEPYSFVQIEHVAQQIGIDRSKVEKKLSQMI
YDL097c_Sc EKELMGDELTRSHFNALYDTLLESNLCKIIEPFECVEISHISKIIGLDTQQVEGKLSQMI
YMJ5_Ce KIELVEDKVVAVHSQNLERNMLEKEISRVIEPYSEIELSYIARVIGMTVPPVERAIARMI
FUS6_ARATH KSNLLLDIHLHDHVDTLYDQIRKKALIQYTLPFVSVDLSRMADAFKTSVSGLEKELEALI
COS41.8_Ci QLMPHQKAITADGSNILHRAVTEHNLLSASKLYNNIRFTELGALLEIPHQMAEKVASQMI
644879 KDNLLLDMYLAPHVRTLYTQIRNRALIQYFSPYVSADMHRMAAAFNTTVAALEDELTQLI
YPR108w_Sc ANVLIPCKYLNRHADFFVREMRRKVYAQLLESYKTLSLKSMASAFGVSVAFLDNDLGKFI
eif-3p110_Hs DLFPEADKVRTMLVRKIQEESLRTYLFTYSSVYDSISMETLSDMFELDLPTVHSIISKMI
T23D8.4_Ce NLFHNAETVKGMVVRRIQEESLRTYLLTYSTVYATVSLKKLADLFELSKKDVHSIISKMI
YD95_Sp VNHLKCDQFLVAHYRYYVREMRRRAYAQLLESYRALSIDSMAASFGVSVDYIDRDLASFI
KIAA0107_Hs EQEMKKDWLFAPHYRYYVREMRIHAYSQLLESYRSLTLGYMAEAFGVGVEFIDQELSRFI
F49C12.8_Hs SERFKFDRYLSPHFNYYSRGMRHRAYEQFLTPYKTVRIDMMAKDFGVSRAFIDRELHRLI
Int-6_Mm ESVLVNDFFLVACLEDFIENARLFIFETFCRIHQCISINMLADKLNMTPEEAERWIVNLI


26SPS9_Hs LDKKFHGILDQGEGVLIIFDEPP
F57B9_Ce LDQKLSGSLDQGEGMLIVFEIAV
YDL097c_Sc LDKIFYGVLDQGNGWLYVYETPN
YMJ5_Ce LDKKLMGSIDQHGDTVVVYPKAD
FUS6_ARATH TDNQIQARIDSHNKILYARHADQ
COS41.8_Ci CESRMKGHIDQIDGIVFFERRET
644879 LEGLISARVDSHSKILYARDVDQ
YPR108w_Sc PNKQLNCVIDRVNGIVETNRPDN
eif-3p110_Hs INEELMASLDQPTQTVVMHRTEP
T23D8.4_Ce IQEELSATLDEPTDCLIMHRVEP
YD95_Sp PDNKLNCVIDRVNGVVFTNRPDE
KIAA0107_Hs AAGRLHCKIDKVNEIVETNRPDS
F49C12.8_Hs ATGQLQCRIDAVNGVIEVNHRDS
Int-6_Mm RNARLDAKIDSKLGHVVMGNNAV
```

Cookbook

Query a BLAST database with a GI, but exclude that GI from the results

```
Extract a GI from the ecoli database:
$ blastdbcmd -entry all -db ecoli -dbtype nucl -outfmt %g | head -1 | \
 tee exclude_me
1786181
Run the restricted database search, which shows there are no self-hits:
$ blastn -db ecoli -negative_gilist exclude_me -show_gis -num_alignments 0 \
 -query exclude_me | grep `cat exclude_me`
Query= gi|1786181|gb|AE000111.1|AE000111
$
```

Create a masked BLAST database

Creating a masked BLAST database is a two step process:

- **a** Generate the masking data using a sequence filtering utility like windowmasker or dustmasker
- **b** Generate the actual BLAST database using makeblastdb

For both steps, the input file can be a text file containing sequences in FASTA format, or an existing BLAST database created using makeblastdb. We will provide examples for both scenarios.

Collect mask information files

For nucleotide sequence data in FASTA files or BLAST database format, we can generate the mask information files using windowmasker or dustmasker. Windowmasker masks the over-represented sequence data and it can also mask the low complexity sequence data using the built-in dust algorithm (through the -dust option). To mask low-complexity sequences only, we will need to use dustmasker.

For protein sequence data in FASTA files or BLAST database format, we need to use segmasker to generate the mask information file.

The following examples assume that BLAST databases, listed in "Obtaining sample data for this cookbook entry", are available in the current working directory. Note that you should use the sequence id parsing consistently. In all our examples, we enable this function by including the "-parse_seqids" in the command line arguments.

Create masking information using dustmasker

We can generate the masking information with dustmasker using a single command line:

```
$ dustmasker -in hs_chr -infmt blastdb -parse_seqids \
 -outfmt maskinfo_asn1_bin -out hs_chr_dust.asnb
```

Here we specify the input is a BLAST database named hs_chr (-in hs_chr -infmt blastdb), enable the sequence id parsing (-parse_seqids), request the mask data in binary asn.1 format (-outfmt maskinfo_asn1_bin), and name the output file as hs_chr_dust.asnb (-out hs_chr_dust.asnb).

If the input format is the original FASTA file, hs_chr.fa, we need to change input to -in and -infmt options as follows:

```
$ dustmasker -in hs_chr.fa -infmt fasta -parse_seqids \
 -outfmt maskinfo_asn1_bin -out hs_chr_dust.asnb
```

Create masking information using windowmasker

To generate the masking information using windowmasker from the BLAST database hs_chr, we first need to generate a counts file:

```
$ windowmasker -in hs_chr -infmt blastdb -mk_counts \
 -parse_seqids -out hs_chr_mask.counts
```

Here we specify the input BLAST database (-in hs_chr -infmt blastdb), request it to generate the counts (-mk_counts) with sequence id parsing (-parse_seqids), and save the output to a file named hs_chr_mask.counts (-out hs_chr_mask.counts).

To use the FASTA file hs_chr.fa to generate the counts, we need to change the input file name and format:

```
$ windowmasker -in hs_chr.fa -infmt fasta -mk_counts \
 -parse_seqids -out hs_chr_mask.counts
```

With the counts file we can then proceed to create the file containing the masking information as follows:

```
$ windowmasker -in hs_chr -infmt blastdb -ustat hs_chr_mask.count \
 -outfmt maskinfo_asn1_bin -parse_seqids -out hs_chr_mask.asnb
```

Here we need to use the same input (-in hs_chr -infmt blastdb) and the output of step 1 (-ustat hs_chr_mask.counts). We set the mask file format to binary asn.1 (-outfmt maskinfo_asn1_bin), enable the sequence ids parsing (-parse_seqids), and save the masking data to hs_chr_mask.asnb (-out hs_chr_mask.asnb).

To use the FASTA file hs_chr.fa, we change the input file name and file type:

```
$ windowmasker -in hs_chr.fa -infmt fasta -ustat hs_chr.counts \
 -outfmt maskinfo_asn1_bin -parse_seqids -out hs_chr_mask.asnb
```

Create masking information using segmasker

We can generate the masking information with segmasker using a single command line:

```
$ segmasker -in refseq_protein -infmt blastdb -parse_seqids \
 -outfmt maskinfo_asn1_bin -out refseq_seg.asnb
```

Here we specify the refseq_protein BLAST database (-in refseq_protein -infmt blastdb), enable sequence ids parsing (-parse_seqids), request the mask data in binary asn.1 format (-outfmt maskinfo_asn1_bin), and name the out file as refseq_seg.asnb (-out refseq_seg.asnb).

If the input format is the FASTA file, we need to change the command line to specify the input format:

```
$ segmasker -in refseq_protein.fa -infmt fasta -parse_seqids \
 -outfmt maskinfo_asn1_bin -out refseq_seg.asnb
```

Extract masking information from FASTA sequences with lowercase masking

We can also extract the masking information from a FASTA sequence file with lowercase masking (generated by various means) using convert2blastmask utility. An example command line follows:

```
$ convert2blastmask -in hs_chr.mfa -parse_seqids -masking_algorithm repeat \
 -masking_options "repeatmasker, default" -outfmt maskinfo_asn1_bin \
 -out hs_chr_mfa.asnb
```

Here the input is hs_chr.mfa (-in hs_chr.mfa), enable parsing of sequence ids, specify the masking algorithm name (-masking_algorithm repeat) and its parameter (-masking_options "repeatmasker, default"), and ask for asn.1 output (-outfmt maskinfo_asn1_bin) to be saved in specified file (-out hs_chr_mfa.asnb).

Create BLAST database with the masking information

Using the masking information data files generated in the previous 4 steps, we can create BLAST database with masking information incorporated.

Note: we should use "-parse_seqids" in a consistent manner – either use it in both steps or not use it at all.

Create BLAST database with masking information using an existing BLAST database or FASTA sequence file as input

For example, we can use the following command line to apply the masking information, created above, to the existing BLAST database generated in Obtaining sample data for this cookbook entry:

```
$ makeblastdb -in hs_chr -input_type blastdb -dbtype nucl -parse_seqids \
 -mask_data hs_chr_mask.asnb -out hs_chr -title \
 "Human Chromosome, Ref B37.1"
```

Here, we use the existing BLAST database as input file (-in hs_chr), specify its type (-dbtype nucl), enable parsing of sequence ids (-parse_seqids), provide the masking data (-mask_data hs_chr_mask.asnb), and name the output database with the same base name (-out hs_chr) overwriting the existing one.

To use the original FASTA sequence file (hs_chr.fa) as the input, we need to use "-in hs_chr.fa" to instruct makeblastdb to use that FASTA file instead.

We can check the "re-created" database to find out if the masking information was added properly, using blastdbcmd with the following command line:

```
$ blastdbcmd -db hs_chr -info
```

This command prints out a summary of the target database:

```
Database: human chromosomes, Ref B37.1
 24 sequences; 3,095,677,412 total bases


Date: Aug 13, 2009 3:02 PM Longest sequence: 249,250,621 bases


Available filtering algorithms applied to database sequences:


Algorithm ID Algorithm name Algorithm options
 30 windowmasker


Volumes:
 /export/home/tao/blast_test/hs_chr
```

Extra lines under the "Available filtering algorithms …" describe the masking algorithms available. The "Algorithm ID" field, 30 in our case, is what we need to use if we want to invoke database soft masking during an actual search through the "-db_soft_mask" parameter.

We can apply additional masking data to an existing BLAST database with one type of masking information already added. For example, we can apply the dust masking generated above to the database generated earlier by using this command line:

```
$ makeblastdb -in hs_chr -input_type blastdb -dbtype nucl -parse_seqids \
 -mask_data hs_chr_dust.asnb -out hs_chr -title "Human Chromosome, Ref B37.1"
```

Here, we use the existing database as input file (-in hs_chr), specify its input and molecule type (-input_type blastdb -dbtype nucl), enable parsing of sequence ids (-parse_seqids), provide the dust masking data (-mask_data hs_chr_dust.asnb), naming the database with the same based name (-out hs_chr) overwriting the existing one.

Checking the "re-generated" database with blastdbcmd:

```
$ blastdbcmd -db hs_chr -info
```

we can see that both sets of masking information are available:

```
Database: Human Chromosome, Ref B37.1
 24 sequences; 3,095,677,412 total bases


Date: Aug 25, 2009 4:43 PM Longest sequence: 249,250,621 bases


Available filtering algorithms applied to database sequences:


Algorithm ID Algorithm name Algorithm options
 11 dust window=64; level=20; linker=1
 30 windowmasker
```

```
Volumes:
 /net/gizmo4/export/home/tao/blast_test/hs_chr
```

A more straightforward approach to apply multiple sets of masking information in a single makeblastdb run by providing multiple set of masking data files in a comma delimited list:

```
$ makeblastdb -in hs_chr -input_type blastdb -dbtype nucl -parse_seqids \
 -mask_data hs_chr_dust.asnb, hs_chr_mask.asnb -out hs_chr
```

Create a protein BLAST database with masking information

We can use the masking data file generated in "Create masking information using segmasker" to create a protein BLAST database:

```
$ makeblastdb -in refseq_protein -input_type blastdb -dbtype prot -
parse_seqids \
 -mask_data refseq_seg.asnb -out refseq_protein -title \
 "RefSeq Protein Database"
```

Using blastdbcmd, we can check the database thus generated:

```
$ blastdbcmd -db refseq_protein -info
```

This produces the following summary, which includes the masking information:

```
Database: RefSeq Protein Database
 7,044,477 sequences; 2,469,203,411 total residues


Date: Sep 1, 2009 10:50 AM Longest sequence: 36,805 residues


Available filtering algorithms applied to database sequences:


Algorithm ID Algorithm name Algorithm options
 21 seg window=12; locut=2.2; hicut=2.5


Volumes:
 /export/home/tao/blast_test/refseq_protein2.00
 /export/home/tao/blast_test/refseq_protein2.01
 /export/home/tao/blast_test/refseq_protein2.02
```

Create a nucleotide BLAST database using the masking information extracted from lower case masked FASTA file

We use the following command line:

```
$ makeblastdb -in hs_chr.mfa -dbtype nucl -parse_seqids \
 -mask_data hs_chr_mfa.asnb -out hs_chr_mfa -title "Human chromosomes (mfa)"
```

Here we use the lowercase masked FASTA sequence file as input (-in hs_chr.mfa), its file type (-input_type fasta), specify the database as nucleotide (-dbtype nucl), enable parsing of sequence ids (-parse_seqids), provide the masking data (-mask_data hs_chr_mfa.asnb), and name the resulting database as hs_chr_mfa (-out hs_chr_mfa).

Checking the database thus generated using blastdbcmd, we have:

```
Database: Human chromosomes (mfa)
 24 sequences; 3,095,677,412 total bases


Date: Aug 26, 2009 11:41 AM Longest sequence: 249,250,621 bases


Available filtering algorithms applied to database sequences:


Algorithm ID Algorithm name Algorithm options
 40 repeat repeatmasker lowercase


Volumes:
 /export/home/tao/hs_chr_mfa
```

The algorithm name and algorithm options are the values we provided in "Extract masking information from FASTA sequences with lowercase masking".

Obtaining Sample data for this cookbook entry

For input nucleotide sequences, we use the BLAST database generated from a FASTA input file hs_chr.fa, containing complete human chromosomes from BUILD37.1, generated by inflating and combining the hs_ref_*.fa.gz files located at:

```
ftp.ncbi.nlm.nih.gov/genomes/H_sapiens/Assembled_chromosomes/
```

We use this command line to create the BLAST database from the input nucleotide sequences:

```
$ makeblastdb -in hs_chr.fa -dbtype nucl -parse_seqids \
 -out hs_chr -title "Human chromosomes, Ref B37.1"
```

For input nucleotide sequences with lowercase masking, we use the FASTA file hs_chr.mfa, containing the complete human chromosomes from BUILD37.1, generated by inflating and combining the hs_ref_*.mfa.gz files located in the same ftp directory.

For input protein sequences, we use the preformatted refseq_protein database from the NCBI blast/db/ ftp directory:

```
ftp.ncbi.nlm.nih.gov/blast/db/refseq_protein.00.tar.gz

ftp.ncbi.nlm.nih.gov/blast/db/refseq_protein.01.tar.gz

ftp.ncbi.nlm.nih.gov/blast/db/refseq_protein.02.tar.gz
```

Search the database with database soft masking information

To enable the database masking during a BLAST search, we need to get the Algorithm ID using the -info parameter of blastdbcmd. For the database generated with windowmasker in the previous cookbook entry, we can use the following command line to activate one of the database soft masking created by windowmasker:

```
$ blastn -query HTT_gene -task megablast -db hs_chr -db_soft_mask 30 \
 -outfmt 7 -out HTT_megablast_mask.out -num_threads 4
```

Here, we use the blastn program to search a nucleotide query HTT_gene* (-query HTT_gene) with megablast algorithm (-task megablast) against the database hs_chr (-db hs_chr). We invoke the soft database masking (-db_soft_mask 30), set the result format to tabular output (-outfmt 7), and save the result to a file named HTT_megablast_mask.tab (-out HTT_megablast_mask.tab). We also activated the multi-thread feature of blastn to speed up the search by using 4 CPUs$^{\$}$ (-num_threads 4).

*This is a genomic fragment containing the HTT gene from human, including 5 kb up- and down-stream of the transcribed region. It is represented by NG_009378.

$^{\$}$ The number to use under in your run will depend on the number of CPUs your system has.

In a test run under a 64-bits Linux machine, the above search takes 9.828 seconds real time, while the same run without database soft masking invoked takes 31 minutes 44.651 seconds.

Display BLAST search results with custom output format

The –outfmt option permits formatting arbitrary fields from the BLAST tabular format. Use the –help option on the command-line application (e.g., blastn) to see the supported fields. The max_target_seqs option should be used with any tabular output to control the number of matches reported.

Example of custom output format

The following example shows how to display the results of a BLAST search using a custom output format. The tabular output format with comments is used, but only the query accession, subject accession, evalue, query start, query stop, subject start, and subject stop are requested. For brevity, only the first 10 lines of output are shown:

```
$ echo 1786181 | ./blastn -db ecoli -outfmt "7 qacc sacc evalue
qstart qend sstart send"
# BLASTN 2.2.18+
# Query: gi|1786181|gb|AE000111.1|AE000111
# Database: ecoli
# Fields: query acc., subject acc., evalue, q. start, q. end, s.
 start, s. end
# 85 hits found
AE000111 AE000111 0.0 1 10596 1 10596
AE000111 AE000174 8e-30 5565 5671 6928 6821
AE000111 AE000394 1e-27 5587 5671 135 219
AE000111 AE000425 6e-26 5587 5671 8552 8468
AE000111 AE000171 3e-24 5587 5671 2214 2130
$
```

Trace-back operations (BTOP)

The "Blast trace-back operations" (BTOP) string describes the alignment produced by BLAST. This string is similar to the CIGAR string produced in SAM format, but there are important differences. BTOP is a more flexible format that lists not only the aligned region but also matches and mismatches. BTOP operations consist of 1.) a number with a count of matching letters, 2.) two letters showing a mismatch (e.g., "AG" means A was replaced by G), or 3.) a dash ("-") and a letter showing a gap. The box below shows a blastn run first with BTOP output and then the same run with the BLAST report showing the alignments.

```
$ blastn -query test_q.fa -subject test_s.fa -dust no -outfmt "6
qseqid sseqid btop" -parse_deflines
query1 q_multi 7AG39
query1 q_multi 7A-39
query1 q_multi 6-G-A41
$ blastn -query test_q.fa -subject test_s.fa -dust no -parse_deflines
BLASTN 2.2.24+


Query= query1
Length=47


Subject=
Length=142


 Score = 82.4 bits (44), Expect = 9e-22
 Identities = 46/47 (97%), Gaps = 0/47 (0%)
 Strand=Plus/Plus


Query 1 ACGTCCGAGACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 47
 ||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct 47 ACGTCCGGGACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 93




 Score = 80.5 bits (43), Expect = 3e-21
 Identities = 46/47 (97%), Gaps = 1/47 (2%)
 Strand=Plus/Plus


Query 1 ACGTCCGAGACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 47
 ||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct 1 ACGTCCG-GACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 46
```

```
 Score = 78.7 bits (42), Expect = 1e-20
 Identities = 47/49 (95%), Gaps = 2/49 (4%)
 Strand=Plus/Plus


Query 1 ACGTCC--GAGACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 47
 |||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct 94 ACGTCCGAGAGACGCGAGCAGCGAGCAGCAGAGCGACGAGCAGCGACGA 142
```

Use blastdb_aliastool to manage the BLAST databases

Often we need to search multiple databases together or wish to search a specific subset of sequences within an existing database. At the BLAST search level, we can provide multiple database names to the “-db” parameter, or to provide a GI file specifying the desired subset to the “-gilist” parameter. However for these types of searches, a more convenient way to conduct them is by creating virtual BLAST databases for these. Note: When combining BLAST databases, all the databases must be of the same molecule type. The following examples assume that the two databases as well as the GI file are in the current working directory.

Aggregate existing BLAST databases

To combine the two nematode nucleotide databases, named “nematode_mrna” and “nematode_genomic", we use the following command line:

```
$ blastdb_aliastool -dblist "nematode_mrna nematode_genomic" -dbtype nucl \
 -out nematode_all -title "Nematode RefSeq mRNA + Genomic"
```

Create a subset of a BLAST database

The nematode_mrna database contains RefSeq mRNAs for several species of round worms. The best subset is from C. elegance. In most cases, we want to search this subset instead of the complete collection. Since the database entries are from NCBI nucleotide databases and the database is formatted with ”-parse_seqids”, we can use the “-gilist c_elegance_mrna.gi” parameter/value pair to limit the search to the subset of interest, alternatively, we can create a subset of the nematode_mrna database as follows:

```
$ blastdb_aliastool -db nematode_mrna -gilist c_elegance_mrna.gi -dbtype \
 nucl -out c_elegance_mrna -title "C. elegans refseq mRNA entries"
```

Note: one can also specify multiple databases using the -db parameter of blastdb_aliastool.

Reformat BLAST reports with blast_formatter

It may be helpful to view the same BLAST results in different formats. A user may first parse the tabular format looking for matches meeting a certain criteria, then go back and examine the relevant alignments in the full BLAST report. He may also first look at pair-wise alignments, then decide to use a query-anchored view. Viewing a BLAST report in different formats has been possible on the NCBI BLAST web site since 2000, but has not been possible with stand-alone BLAST runs. The blast_formatter allows this, if the original search produced blast archive format using the –outfmt 11 switch. The query sequence, the BLAST options, the masking information, the name of the database, and the alignment are written out as ASN.1 (a structured format similar to XML). The –max_target_seqs option should be used to control the number of matches recorded in the alignment. The blast_formatter reads this information and formats a report. The BLAST database used for the original search must be available, or the sequences need to be fetched from the NCBI, assuming the database contains sequences in the public dataset. The box below illustrates the procedure. A blastn run first produces the BLAST archive format, and the blast_fomatter then reads the file and produces tabular output.

Blast_formatter will format stand-alone searches performed with an earlier version of a database if both the search and formatting databases are prepared so that fetching by sequence ID is possible. To enable fetching by sequence ID use the –parse_seqids flag when running makeblastdb, or (if available) download preformatted BLAST databases from ftp://ftp.ncbi.nlm.nih.gov/blast/db/ using update_blastdb.pl (provided as part of the BLAST+ package). Currently the blast archive format and blast_formatter do not work with database free searches (i.e., -subject rather than –db was used for the original search).

```
$ echo 1786181 | blastn -db ecoli -outfmt 11 -out out.1786181.asn
$ blast_formatter -archive out.1786181.asn -outfmt "7 qacc sacc evalue
qstart qend sstart send"
# BLASTN 2.2.24+
# Query: gi|1786181|gb|AE000111.1|AE000111 Escherichia coli K-12 MG1655
section 1 of 400
# Database: ecoli
# Fields: query acc., subject acc., evalue, q. start, q. end,
s. start, s. end
# 85 hits found
AE000111 AE000111 0.0 1 10596 1 10596
AE000111 AE000174 8e-30 5565 5671 6928 6821
AE000111 AE000394 1e-27 5587 5671 135 219
AE000111 AE000425 6e-26 5587 5671 8552 8468
AE000111 AE000171 3e-24 5587 5671 2214 2130
AE000111 AE000171 1e-23 5587 5670 10559 10642
AE000111 AE000376 1e-22 5587 5675 129 42
AE000111 AE000268 1e-22 5587 5671 6174 6090
AE000111 AE000112 1e-22 10539 10596 1 58
AE000111 AE000447 5e-22 5587 5670 681 598
AE000111 AE000344 6e-21 5587 5671 4112 4196
AE000111 AE000490 2e-20 5584 5671 4921 4835
AE000111 AE000280 2e-20 5587 5670 12930 12847
```

Extracting data from BLAST databases with blastdbcmd

Extract lowercase masked FASTA from a BLAST database with masking information

If a BLAST database contains masking information, this can be extracted using the blastdbcmd options –db_mask and –mask_sequence as follows:

```
$ blastdbcmd -info -db mask-data-db
Database: Mask data test
 10 sequences; 12,609 total residues



Date: Feb 17, 2009 5:10 PM Longest sequence: 1,694 residues



Available filtering algorithms applied to database sequences:
```

```
Algorithm ID Algorithm name Algorithm options
 20 seg default options used
 40 repeat -species Desmodus_rotundus


Volumes:
 mask-data-db
$ blastdbcmd -db mask-data-db -mask_sequence_with 20 -entry 71022837
>gi|71022837|ref|XP_761648.1| hypothetical protein UM05501.1 [Ustilago maydis
521]
MPPSARHSAHPSHHPHAGGRDLHHAAGGPPPQGGPGMPPGPGNGPMHHPHSSYAQSMPPPPGLPPHAMNGINGPPPS
THG
GPPPRMVMADGPGGAGGPPPPPPPHIPRSSSAQSRIMEAaggpagpppagppastspavQklslANEaawvsIGsaa
etm
EdydralsayeaalrhnpysvpalsaiagvhrtldnfekavdyfqrvlnivpengdTWGSMGHCYLMMDDLQRAYTA
YQQ
ALYHLPNPKEPKLWYGIGILYDRYGSLEHAEEAFASVVRMDPNYEKANEIYFRLGIIYKQQNKFPASLECFRYILDN
PPR
PLTEIDIWFQIGHVYEQQKEFNAAKEAYERVLAENPNHAKVLQQLGWLYHLSNAGFNNQERAIQFLTKSLESDPNDA
QSW
YLLGRAYMAGQNYNKAYEAYQQAVYRDGKNPTFWCSIGVLYYQINQYRDALDAYSRAIRLNPYISEVWFDLGSLYEA
CNN
QISDAIHAYERAADLDPDNPQIQQRLQLLRNAEAKGGELPEAPVPQDVHPTAYANNNGMAPGPPTQIGGGPGPSYPP
PLV
GPQLAGNGGGRGDLSDRDLPGPGHLGSSHSPPPFRGPPGTDDRGARGPPHGALAPMVGGPGGPEPLGRGGFSHSRGP
SPG
PPRMDPYGRRLGSPPRRSPPPPLRSDVHDGHGAPPHVHGQGHGQGHGQGHGQGHGQGHGQSHGHSHGGEFRGPPPLA
AAG
PGGPPPPLDHYGRPMGGPMSEREREMEWEREREREREREQAARGYPASGRITPKNEPGYARSQHGGSNAPSPAFGRP
PVY
GRDEGRDYYNNSHPGSGPGGPRGGYERGPGAPHAPAPGMRHDERGPPPAPFEHERGPPPPHQAGDLRYDSYSDGRDG
PFR
GPPPGLGRPTPDWERTRAGEYGPPSLHDGAEGRNAGGSASKSRRGPKAKDELEAAPAPPSPVPSSAGKKGKTTSSRA
GSP
WSAKGGVAAPGKNGKASTPFGTGVGAPVAAAGVGGGVGSKKGAAISLRPQEDQPDSRPGSPQSRRDASPASSDGSNE
PLA
ARAPSSRMVDEDYDEGAADALMGLAGAASASSASVATAAPAPVSPVATSDRASSAEKRAESSLGKRPYAEEERAVDE
PED
SYKRAKSGSAAEIEADATSGGRLNGVSVSAKPEATAAEGTEQPKETRTETPPLAVAQATSPEAINGKAESESAVQPM
DVD
GREPSKAPSESATAMKDSPSTANPVVAAKASEPSPTAAPPATSMATSEAQPAKADSCEKNNNDEDEREEEEGQIHED
PID
APAKRADEDGAK
$
```

Extract all human sequences from the nr database

Although one cannot select GIs by taxonomy from a database, a combination of unix command line tools will accomplish this:

```
$ blastdbcmd -db nr -entry all -outfmt "%g %T" | \
 awk ' { if ($2 == 9606) { print $1 } } ' | \
 blastdbcmd -db nr -entry_batch - -out human_sequences.txt
```

The first blastdbcmd invocation produces 2 entries per sequence (GI and taxonomy ID), the awk command selects from the output of that command those sequences which have a taxonomy ID of 9606 (human) and prints its GIs, and finally the second blastdbcmd invocation uses those GIs to print the sequence data for the human sequences in the nr database.

Custom data extraction and formatting from a BLAST database

The following examples show how to extract selected information from a BLAST database and how to format it:

```
Extract the accession, sequence length,
and masked locations for GI 71022837:
$ blastdbcmd -entry 71022837 -db Test/mask-data-db -outfmt "%a %l %m"
XP_761648.1 1292 119-139;140-144;147-152;154-160;161-216;
```

Extract different sequence ranges from the BLAST databases

The command below will extract two different sequences: bases 40-80 in human chromosome Y (GI 13626247) with the masked regions in lowercase characters (notice argument 30, the masking algorithm ID which is available in this BLAST database) and bases 1-10 in the minus strand of human chromosome 20 (GI 14772189).

```
$ printf "%s %s %s %s\n%s %s %s\n" 13626247 40-80 plus 30 14772189 1-10 minus
\
| blastdbcmd -db GPIPE/9606/current/all_contig -entry_batch -
>gi|13626247|ref|NT_025975.2|:40-80 Homo sapiens chromosome Y genomic contig,
GRCh37.p10 Primary Assembly
tgcattccattctattctcttctACTGCATACAatttcact
>gi|14772189|ref|NT_025215.4|:c10-1 Homo sapiens chromosome 20 genomic
contig, GRCh37.p10 Primary Assembly
GCTCTAGATC
$
```

Display the locations where BLAST will search for BLAST databases

This is accomplished by using the -show_blastdb_search_path option in blastdbcmd:

```
$ blastdbcmd -show_blastdb_search_path
:/net/nabl000/vol/blast/db/blast1:/net/nabl000/vol/blast/db/blast2:
$
```

Display the available BLAST databases at a given directory

This is accomplished by using the -list option in blastdbcmd:

```
$ blastdbcmd -list repeat -recursive
repeat/repeat_3055 Nucleotide
repeat/repeat_31032 Nucleotide
repeat/repeat_35128 Nucleotide
```

```
repeat/repeat_3702 Nucleotide
repeat/repeat_40674 Nucleotide
repeat/repeat_4530 Nucleotide
repeat/repeat_4751 Nucleotide
repeat/repeat_6238 Nucleotide
repeat/repeat_6239 Nucleotide
repeat/repeat_7165 Nucleotide
repeat/repeat_7227 Nucleotide
repeat/repeat_7719 Nucleotide
repeat/repeat_7955 Nucleotide
repeat/repeat_9606 Nucleotide
repeat/repeat_9989 Nucleotide
$
```

The first column of the default output is the file name of the BLAST database (usually provided as the –db argument to other BLAST+ applications), the second column represents the molecule type of the BLAST database. This output is configurable via the list_outfmt command line option.

Use Windowmasker to filter the query sequence(s) in a BLAST search

The blastn executable can filter a query sequence using the windowmasker data files. This option can be used to mask interspersed repeats that may lead to spurious matches. The windowmasker data files should be created as discussed in step 1 of "Create masking information using windowmasker" or downloaded from the NCBI FTP site. Follow the instructions in Configuring BLAST to make sure BLAST will be able to find the windowmasker files in the examples below.

```
1. Run BLAST search using Windowmasker for sequence filtering based upon
taxid
(9606 is the taxid for human).
$ blastn -query input -db database -window_masker_taxid 9606 -out results.txt


2. Run BLAST search using Windowmasker for sequence filtering based upon the
windowmasker file name.
$ blastn –query input –db database –window_masker_db 9606/wmasker.obinary
```

Building a BLAST database with local sequences

The makeblastdb application produces BLAST databases from FASTA files. In the simplest case the FASTA definition lines are not parsed by makeblastdb and may be completely unstructured. The text in the definition line will be stored in the BLAST database and displayed in the BLAST report, but it will not be possible to fetch individual sequences using blastdbcmd or to limit the search with the –seqidlist option. Use the –parse_seqids flag when invoking makeblastdb to enable retrieval of sequences based upon sequence identifiers. In this case, each sequence must have a unique identifier, and that identifier must have a specific format. The identifier should begin right after the ">" sign on the definition line, contain no spaces, and follow the formats described in http://www.ncbi.nlm.nih.gov/books/NBK7183/?rendertype=table&id=ch_demo.T5 User supplied sequences should make use of the local or general identifiers described in the above table. A FASTA file with general IDs would look like:

```
$ cat mydb.fsa
>gnl|MYDB|1 this is sequence 1
GAATTCCCGCTACAGGGGGGGCCTGAGGCACTGCAGAAAGTGGGCCTGAGCCTCGAGGATGACGGTGCTGCAGGAAC
CCG
TCCAGGCTGCTATATGGCAAGCACTAAACCACTATGCTTACCGAGATGCGGTTTTCCTCGCAGAACGCCTTTATGCA
GAA
GTACACTCAGAAGAAGCCTTGTTTTTACTGGCAACCTGTTATTACCGCTCAGGAAAGGCATATAAAGCATATAGACT
CTT
GAAAGGACACAGTTGTACTACACCGCAATGCAAATACCTGCTTGCAAAATGTTGTGTTGATCTCAGCAAGCTTGCAG
AAG
GGGAACAAATCTTATCTGGTGGAGTGTTTAATAAGCAGAAAAGCCATGATGATATTGTTACTGAGTTTGGTGATTCA
GCT
TGCTTTACTCTTTCATTGTTGGGACATGTATATTGCAAGACAGATCGGCTTGCCAAAGGATCAGAATGTTACCAAAA
GAG
CCTTAGTTTAAATCCTTTCCTCTGGTCTCCCTTTGAATCATTATGTGAAATAGGTGAAAAGCCAGATCCTGACCAAA
CAT
TTAAATTCACATCTTTACAGAACTTTAGCAACTGTCTGCCCAACTCTTGCACAACACAAGTACCTAATCATAGTTTA
TCT
CACAGACAGCCTGAGACAGTTCTTACGGAAACACCCCAGGACACAATTGAATTAAACAGATTGAATTTAGAATCTTC
CAA
>gnl|MYDB|2 this is sequence 2
GAATTCCCGCTACAGGGGGGGCCTGAGGCACTGCAGAAAGTGGGCCTGAGCCTCGAGGATGACGGTGCTGCAGGAAC
CCG
TCCAGGCTGCTATATGGCAAGCACTAAACCACTATGCTTACCGAGATGCGGTTTTCCTCGCAGAACGCCTTTATGCA
GAA
GTACACTCAGAAGAAGCCTTGTTTTTACTGGCAACCTGTTATTACCGCTCAGGAAAGGCATATAAAGCATATAGACT
CTT
GAAAGGACACAGTTGTACTACACCGCAATGCAAATACCTGCTTGCAAAATGTTGTGTTGATCTCAGCAAGCTTGCAG
AAG
GGGAACAAATCTTATCTGGTGGAGTGTTTAATAAGCAGAAAAGCCATGATGATATTGTTACTGAGTTTGGTGATTCA
GCT
TGCTTTACTCTTTCATTGTTGGGACATGTATATTGCAAGACAGATCGGCTTGCCAAAGGATCAGAATGTTACCAAAA
GAG
CCTTAGTTTAAATCCTTTCCTCTGGTCTCCCTTTGAATCATTATGTGAAATAGGTGAAAAGCCAGATCCTGACCAAA
CAT
TTAAATTCACATCTTTACAGAACTTTAGCAACTGTCTGCCCAACTCTTGCACAACACAAGTACCTAATCATAGTTTA
TCT
CACAGACAGCCTGAGACAGTTCTTACGGAAACACCCCAGGACACAATTGAATTAAACAGATTGAATTTAGAATCTTC
CAA
>gnl|MYDB|3 this is sequence 3
GAATTCCCGCTACAGGGGGGGCCTGAGGCACTGCAGAAAGTGGGCCTGAGCCTCGAGGATGACGGTGCTGCAGGAAC
CCG
TCCAGGCTGCTATATGGCAAGCACTAAACCACTATGCTTACCGAGATGCGGTTTTCCTCGCAGAACGCCTTTATGCA
GAA
GTACACTCAGAAGAAGCCTTGTTTTTACTGGCAACCTGTTATTACCGCTCAGGAAAGGCATATAAAGCATATAGACT
CTT
GAAAGGACACAGTTGTACTACACCGCAATGCAAATACCTGCTTGCAAAATGTTGTGTTGATCTCAGCAAGCTTGCAG
AAG
```

```
GGGAACAAATCTTATCTGGTGGAGTGTTTAATAAGCAGAAAAGCCATGATGATATTGTTACTGAGTTTGGTGATTCA
GCT
TGCTTTACTCTTTCATTGTTGGGACATGTATATTGCAAGACAGATCGGCTTGCCAAAGGATCAGAATGTTACCAAAA
GAG
CCTTAGTTTAAATCCTTTCCTCTGGTCTCCCTTTGAATCATTATGTGAAATAGGTGAAAAGCCAGATCCTGACCAAA
CAT
TTAAATTCACATCTTTACAGAACTTTAGCAACTGTCTGCCCAACTCTTGCACAACACAAGTACCTAATCATAGTTTA
TCT$
```

Makeblastdb can be invoked for this file as below.

```
$ makeblastdb -in mydb.fsa -parse_seqids -dbtype nucl


Building a new DB, current time: 01/28/2011 13:39:37
New DB name: mydb.fsa
New DB title: mydb.fsa
Sequence type: Nucleotide
Keep Linkouts: T
Keep MBits: T
Maximum file size: 1073741824B
Adding sequences from FASTA; added 3 sequences in 0.00206995 seconds.
$
```

The FASTA file has three entries. All entries are part of the "MYDB" database, with the entries numbers 1, 2, and 3. Makeblastdb will store this information properly and produce an index, so that the sequences can be retrieved by these identifiers. Makeblastdb stores the title portion of the definition line (e.g., "this is sequence 1"), but will not parse it. If the first token after the ">" does not contain a bar ("|") it will be parsed as a local ID. Use the full identifier string (e.g., "gnl|MYDB|2") to retrieve sequences with a general ID

The NCBI makes databases that are searchable on the NCBI web site (such as nr, refseq_rna, and swissprot) available on its FTP site. It is better to download the preformatted databases rather than starting with FASTA. The databases on the FTP site contain taxonomic information for each sequence, include the identifier indices for lookups, and can be up to four times smaller than the FASTA. The original FASTA can be generated from the BLAST database using blastdbcmd.

Limiting a Search with a List of Identifiers

BLAST can now limit a database search by a list of text identifiers, which should be specified one per line in a text file. These identifiers, referencing the sequences to include in BLAST search, should not contain any whitespace and must be resolvable through the BLAST database ID lookup. In some cases this means that the entire bar-delimited format (specified in http://www.ncbi.nlm.nih.gov/books/NBK7183/?rendertype=table&id=ch_demo.T5) must be used. In other cases it is enough to simply specify an accession. For the "general" example from "Building a BLAST database with local sequences" a valid ID would be "gnl|MYDB|2". On the other hand, if the identifier is "gi|15674171|ref|NP_268346.1", one of the following string is sufficient:

"gi|15674171|ref|NP_268346.1", "15674171", "ref|NP_268346", "NP_268346", "NP_268346.1", etc.

When the search is limited by a list of IDs the statistics of the BLAST database are re-calculated to reflect the actual number of sequences and residuals/base included in search.

BLAST has been able to limit a search by a list of GI's for a number of years. It is important to note that the performance of a binary list of GI's will always be superior to a list of text IDs. The binary list of GI's can be formatted to require minimal conversion at run time. If all the sequences in the database have been assigned a GI, a binary list of GI's should be used rather than a list of accessions.

Multiple databases vs. spaces in filenames and paths

BLAST has been able to search multiple databases since 1997. The databases can be listed after the "-db" argument or in an alias file (see cookbook entries on blastdb_aliastool), separated by spaces. Many operating systems now allow spaces in filenames and directory paths, so some care is required. Basically, one should always have two sets of quotes for any path containing a space. Blastdbcmd is used as an example below, but the same rules apply to makeblastdb as well as the search programs like blastn or blastp.

To access a BLAST database containing spaces under Microsoft Windows it is necessary to use two sets of double-quotes, escaping the innermost quotes with a backslash. For example, Users\joeuser\My Documents\Downloads would be accessed by:

```
blastdbcmd -db "\"Users\joeuser\My Documents\Downloads\mydb\"" -info
```

The first backslash escapes the beginning inner quote, and the backslash following "mydb" escapes the ending inner quote.

A second database can be added to this command by including it within the outer pair of quotes:

```
blastdbcmd -db "\"Users\joeuser\My Documents\Downloads\mydb\" myotherdb" -info
```

If the second database had contained a space, it would have been necessary to surround it by quotes escaped by a backslash.

Under UNIX systems (including LINUX and Mac OS X) it is preferable to use a single quote (') in place of the escaped double quote:

```
blastdbcmd -db ' "path with spaces/mydb" ' -info
```

Multiple databases can also be listed within the single quotes, similar to the procedure described for Microsoft Windows.

Specifying a sequence as the multiple sequence alignment master in psiblast

The -in_msa psiblast option, unlike blastpgp, does not support the specification of a master sequence via the -query option, so if one wants to specify a sequence (other than the first one) in the multiple sequence alignment file to be the master sequence, this has to be specified via the -msa_master_idx option. For instance, in the example below, the third sequence in the multiple sequence alignment would be used as the master sequence:

```
psiblast -in_msa align1 -db pataa -msa_master_idx 3
```

Ignoring the consensus sequence in the multiple sequence alignment in psiblast

Often a consensus sequence is added to a multiple sequence alignment to be used as the master sequence in a PSI-BLAST search. The consensus sequence provides a good option to display the query-subject alignment in the output and to define which MSA columns are to be converted to PSSM. At the same time adding the consensus sequence changes the statistical properties of the original alignment. To avoid this, the -ignore_msa_master option can be used:

```
psiblast -in_msa align1 -db pataa -ignore_msa_master
```

In this case the master sequence is displayed in the output but ignored when the PSSM scores are calculated.

Performing a DELTA-BLAST search

DELTA-BLAST searches a protein sequence database using a PSSM constructed from conserved domains matching a query. It first searches the NCBI CDD database to construct the PSSM.

Download the cdd_delta database

Obtain this database from ftp://ftp.ncbi.nlm.nih.gov/blast/db using the update_blastdb.pl tool (provided as part of the BLAST+ package). Note that the cdd_delta database must be downloaded and installed to the standard BLAST database directory (see Configuring BLAST) or in the current working directory.

Execute the deltablast search

```
$ deltablast -query query.fsa -db pataa
```

Indexed megaBLAST search

The indexed megaBLAST search requires both BLAST databases as well as an index of the words found in the database. The index of words may be produced with makembindex. The example below demonstrates how to produce the index as well as perform an indexed megaBLAST search. This example assumes that the nt.00 BLAST database has been placed in the current directory (before makembindex is run) and that QUERY is a file containing a nucleotide query. Results will appear in OUTPUT. See tables C2 and C11 for information on command-line options.

```
$ makembindex -input nt.00 -iformat blastdb -old_style_index false
$ blastn -db ./nt.00 -query QUERY -use_index true -out OUTPUT
```

The BLAST databases may contain filtering (or masking) information for the database sequences. Makembindex can access this information and exclude the masked regions of the database from the index. This is demonstrated below. The first command shows how to discover the masking “Algorithm ID” from the BLAST database using blastdbcmd. In this case, the ID is 30. The second command demonstrates how to build an index that excludes the masked regions. Once the index has been built, it can be used as shown above. In the example below, the ref_contig BLAST database had been placed in the directory before makembindex was run.

```
$ blastdbcmd -db ref_contig -info
Database: ref_contig
364 sequences; 2,938,626,560 total bases


Date: Oct 7, 2011 10:34 AMLongest sequence: 115,591,997 bases


Available filtering algorithms applied to database sequences:


Algorithm ID Algorithm name Algorithm options
 30 windowmasker default options used


$ makembindex -input ref_contig -iformat blastdb -old_style_index false -
db_mask 30
creating /export/home/madden/INDEX_TEMP/ref_contig.00.idx...done
creating /export/home/madden/INDEX_TEMP/ref_contig.01.idx...done
creating /export/home/madden/INDEX_TEMP/ref_contig.02.idx...removed (empty)
```

BLAST+ remote service

The BLAST+ applications can perform a search on the NCBI servers if invoked with the "–remote" flag. All other command-line options are the same as for a stand-alone search.

The box below shows an example BLAST+ remote search using the blastn application. First, blastn searches the query against the nt database and produces a standard BLAST report. The query file (nt.u00001) contains the sequence for accession u00001 as FASTA. Second, the UNIX grep utility is used to find the RID for the search. Note that the RID can simply be found near the top of the BLAST report. Third, the RID is then used with blast_formatter to print out the results as a tabular report. Finally, the results are formatted as XML. The RID is only printed as an example and is no longer valid.

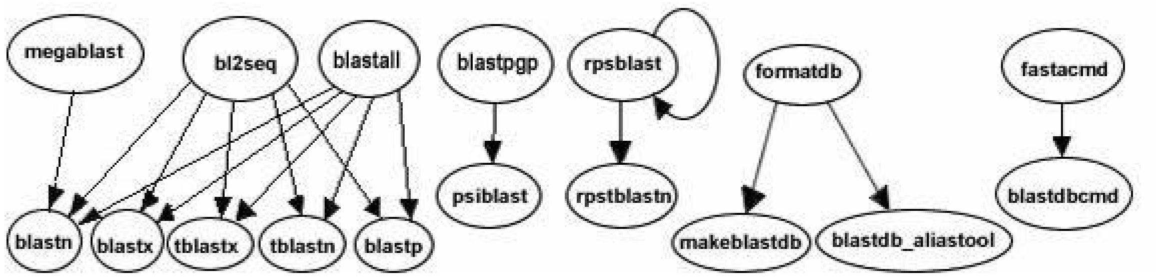
```
$ blastn –db nt –query nt.u00001 –out test.out -remote
$ grep RID test.out
RID: X3R7GAUS014


$blast_formatter –rid X3R7GAUS014 –out test.tab –outfmt 7


$blast_formatter –rid X3R7GAUS014 –out test.xml –outfmt 5
```

Appendix A. Conversion from C toolkit applications

The functionality offered by the BLAST+ applications has been organized by program type. The following graph depicts a correspondence between the NCBI C Toolkit BLAST command line applications and the BLAST+ applications:



The easiest way to get started using the BLAST+ command line applications is by means of the legacy_blast.pl PERL script which is bundled along with the BLAST+ applications. To utilize this script, simply prefix it to the invocation of the C toolkit BLAST command line application and append the --path option pointing to the installation directory of the BLAST+ applications. For example, instead of using

```
 blastall -i query -d nr -o blast.out
```

use

```
 legacy_blast.pl blastall -i query -d nr -o blast.out
--path /opt/blast/bin
```

The purpose of the legacy_blast.pl PERL script is to help users make the transition from the C Toolkit BLAST command line applications to the BLAST+ applications. This script produces its own documentation by invoking it without any arguments.

The legacy_blast.pl script supports two modes of operation, one in which the C Toolkit BLAST command line invocation is converted and executed on behalf of the user and another which solely displays the BLAST+ application equivalent to what was provided, without executing the command.

The first mode of operation is achieved by specifying the C Toolkit BLAST command line application invocation and optionally providing the --path argument after the command line to convert if the installation path for the BLAST+ applications differs from the default (available by invoking the script without arguments). See example in the first section of the Quick start.

The second mode of operation is achieved by specifying the C Toolkit BLAST command line application invocation and appending the --print_only command line option as follows:

```
$ ./legacy_blast.pl megablast -i query.fsa -d nt -o mb.out --print_only
/opt/ncbi/blast/bin/blastn -query query.fsa -db "nt" -out mb.out
$
```

Appendix B. Exit codes

All BLAST+ applications have consistent exit codes to signify the exit status of the application. The possible exit codes along with their meaning are detailed in the table below:

Exit Code	Meaning
0	Success
1	Error in query sequence(s) or BLAST options

2	Error in BLAST database
3	Error in BLAST engine
4	Out of memory
255	Unknown error

In the case of BLAST+ database applications, the possible exit codes are 0 (indicating success) and 1 (indicating failure).

Appendix C. Options for the command-line applications.

This appendix consists of several tables that list option names, types, default values, and a short description of the option. These tables were first published as an appendix to an article in BMC Bioinformatics (BLAST+: architecture and applications). They have been updated for this manual.

Table C1: Options common to all BLAST+ search applications. An option of type "flag" takes no argument, but if present is true. Some options are valid only for a local search ("remote" option not used), others are valid only for a remote search ("remote" option used).

option	**type**	**default value**	**description and notes**
db	string	none	BLAST database name.
query	string	stdin	Query file name.
query_loc	string	none	Location on the query sequence (Format: start-stop)
out	string	stdout	Output file name
evalue	real	10.0	Expect value (E) for saving hits
subject	string	none	File with subject sequence(s) to search.
subject_loc	string	none	Location on the subject sequence (Format: start-stop).
show_gis	flag	N/A	Show NCBI GIs in report.
num_descriptions	integer	500	Show one-line descriptions for this number of database sequences.
num_alignments	integer	250	Show alignments for this number of database sequences.
max_target_seqs	Integer	500	Number of aligned sequences to keep. Use with report formats that do not have separate definition line and alignment sections such as tabular (all outfmt > 4). Not compatible with num_descriptions or num_alignments.
html	flag	N/A	Produce HTML output
gilist	string	none	Restrict search of database to GI's listed in this file. Local searches only.
negative_gilist	string	none	Restrict search of database to everything except the GI's listed in this file. Local searches only.
entrez_query	string	none	Restrict search with the given Entrez query. Remote searches only.
culling_limit	integer	none	Delete a hit that is enveloped by at least this many higher-scoring hits.
best_hit_overhang	real	none	Best Hit algorithm overhang value (recommended value: 0.1)
best_hit_score_edge	real	none	Best Hit algorithm score edge value (recommended value: 0.1)
dbsize	integer	none	Effective size of the database

searchsp	integer	none	Effective length of the search space
import_search_strategy	string	none	Search strategy file to read.
export_search_strategy	string	none	Record search strategy to this file.
parse_deflines	flag	N/A	Parse query and subject bar delimited sequence identifiers (e.g., gi|129295).
num_threads	integer	1	Number of threads (CPUs) to use in blast search.
remote	flag	N/A	Execute search on NCBI servers?

outfmt	string	0	alignment view options: 0 = pairwise, 1 = query-anchored showing identities, 2 = query-anchored no identities, 3 = flat query-anchored, show identities, 4 = flat query-anchored, no identities, 5 = XML Blast output, 6 = tabular, 7 = tabular with comment lines, 8 = Text ASN.1, 9 = Binary ASN.1 10 = Comma-separated values 11 = BLAST archive format (ASN.1) Options 6, 7, and 10 can be additionally configured to produce a custom format specified by space delimited format specifiers. The supported format specifiers are: qseqid means Query Seq-id qgi means Query GI qacc means Query accesion sseqid means Subject Seq-id sallseqid means All subject Seq-id(s), separated by a ';' sgi means Subject GI sallgi means All subject GIs sacc means Subject accession sallacc means All subject accessions qstart means Start of alignment in query qend means End of alignment in query sstart means Start of alignment in subject send means End of alignment in subject qseq means Aligned part of query sequence sseq means Aligned part of subject sequence evalue means Expect value bitscore means Bit score score means Raw score length means Alignment length pident means Percentage of identical matches nident means Number of identical matches mismatch means Number of mismatches positive means Number of positive-scoring matches gapopen means Number of gap openings gaps means Total number of gap ppos means Percentage of positive-scoring matches frames means Query and subject frames separated by a '/' qframe means Query frame sframe means Subject frame btop means Blast traceback operations (BTOP) staxids means unique Subject Taxonomy ID(s), separated by a ';'(in numerical order) sscinames means unique Subject Scientific Name(s), separated by a ';' scomnames means unique Subject Common Name(s), separated by a ';' sblastnames means unique Subject Blast Name(s), separated by a ';' (in alphabetical order) sskingdoms means unique Subject Super Kingdom(s), separated by a ';' (in alphabetical order) stitle means Subject Title salltitles means All Subject Title(s), separated by a '<>' sstrand means Subject Strand qcovs means Query Coverage Per Subject qcovhsp means Query Coverage Per HSP When not provided, the default value is: 'qseqid sseqid pident length mismatch gapopen qstart qend sstart send evalue bitscore', which is equivalent to the keyword 'std'

Table C2: blastn application options. The blastn application searches a nucleotide query against nucleotide subject sequences or a nucleotide database. An option of type “flag” takes no arguments, but if present the argument is true. Four different tasks are supported: 1.) “megablast”, for very similar sequences (e.g, sequencing errors), 2.) “dc-megablast”, typically used for inter-species comparisons, 3.) “blastn”, the traditional program used for inter-species comparisons, 4.) “blastn-short”, optimized for sequences less than 30 nucleotides.

option	task(s)	type	default value	description and notes
word_size	megablast	integer	28	Length of initial exact match.
word_size	dc-megablast	integer	11	Number of matching nucleotides in initial match. dc-megablast allows non-consecutive letters to match.
word_size	blastn	integer	11	Length of initial exact match.
word_size	blastn-short	integer	7	Length of initial exact match.
gapopen	megablast	integer	0	Cost to open a gap. See appendix D.
gapextend	megablast	integer	none	Cost to extend a gap. This default is a function of reward/ penalty value. See appendix D.
gapopen	blastn, blastn-short, dc-megablast	integer	5	Cost to open a gap. See appendix D.
gapextend	blastn, blastn-short, dc-megablast	integer	2	Cost to extend a gap. See appendix D.
reward	megablast	integer	1	Reward for a nucleotide match.
penalty	megablast	integer	-2	Penalty for a nucleotide mismatch.
reward	blastn, dc-megablast	integer	2	Reward for a nucleotide match.
penalty	blastn, dc-megablast	integer	-3	Penalty for a nucleotide mismatch.
reward	blastn-short	integer	1	Reward for a nucleotide match.
penalty	blastn-short	integer	-3	Penalty for a nucleotide mismatch.
strand	all	string	both	Query strand(s) to search against database/subject. Choice of both, minus, or plus.
dust	all	string	20 64 1	Filter query sequence with dust.
filtering_db	all	string	none	Mask query using the sequences in this database.
window_masker_taxid	all	integer	none	Enable WindowMasker filtering using a Taxonomic ID.
window_masker_db	all	string	none	Enable WindowMasker filtering using this file.
soft_masking	all	boolean	true	Apply filtering locations as soft masks (i.e., only for finding initial matches).
lcase_masking	all	flag	N/A	Use lower case filtering in query and subject sequence (s).
db_soft_mask	all	integer	none	Filtering algorithm ID to apply to the BLAST database as soft mask (i.e., only for finding initial matches).
db_hard_mask	all	integer	none	Filtering algorithm ID to apply to the BLAST database as hard mask (i.e., sequence is masked for all phases of search).
perc_identity	all	integer	0	Percent identity cutoff.
template_type	dc-megablast	string	coding	Discontiguous MegaBLAST template type. Allowed values are coding, optimal and coding_and_optimal.

template_length	dc-megablast	integer	18	Discontiguous MegaBLAST template length.
use_index	megablast	boolean	false	Use MegaBLAST database index. Indices may be created with the makembindex application.
index_name	megablast	string	none	MegaBLAST database index name.
xdrop_ungap	all	real	20	Heuristic value (in bits) for ungapped extensions.
xdrop_gap	all	real	30	Heuristic value (in bits) for preliminary gapped extensions.
xdrop_gap_final	all	real	100	Heuristic value (in bits) for final gapped alignment.
no_greedy	megablast	flag	N/A	Use non-greedy dynamic programming extension.
min_raw_gapped_score	all	integer	none	Minimum raw gapped score to keep an alignment in the preliminary gapped and trace-back stages. Normally set based upon expect value.
ungapped	all	flag	N/A	Perform ungapped alignment.
window_size	dc-megablast	integer	40	Multiple hits window size, use 0 to specify 1-hit algorithm

Table C3: blastp application options. The blastp application searches a protein sequence against protein subject sequences or a protein database. An option of type "flag" takes no arguments, but if present the argument is true. Two different tasks are supported: 1.) "blastp", for standard protein-protein comparisons, 2.) "blastp-short", optimized for query sequences shorter than 30 residues. This table reflects the 2.2.27 BLAST+ release.

option	**task**	**type**	**default value**	**description and notes**
word_size	blastp	integer	3	Word size of initial match.
word_size	blastp-short	integer	2	Word size of initial match.
gapopen	blastp	integer	11	Cost to open a gap.
gapextend	blastp	integer	1	Cost to extend a gap.
gapopen	blastp-short	integer	9	Cost to open a gap.
gapextend	blastp-short	integer	1	Cost to extend a gap.
matrix	blastp	string	BLOSUM62	Scoring matrix name.
matrix	blastp-short	string	PAM30	Scoring matrix name.
threshold	blastp	integer	11	Minimum score to add a word to the BLAST lookup table.
threshold	blastp-short	integer	16	Minimum score to add a word to the BLAST lookup table.
comp_based_stats	blastp	string	2	Use composition-based statistics: D or d: default (equivalent to 2) 0 or F or f: no composition-based statistics 1: Composition-based statistics as in NAR 29:2994-3005, 2001 2 or T or t : Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, conditioned on sequence properties 3: Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, unconditionally

comp_based_stats	blastp-short	string	0	Use composition-based statistics : D or d: default (equivalent to 2) 0 or F or f: no composition-based statistics 1: Composition-based statistics as in NAR 29:2994-3005, 2001 2 or T or t : Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, conditioned on sequence properties 3: Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, unconditionally
seg	all	string	no	Filter query sequence with SEG (Format: 'yes', 'window locut hicut', or 'no' to disable).
soft_masking	blastp	boolean	false	Apply filtering locations as soft masks (i.e., only for finding initial matches).
lcase_masking	all	flag	N/A	Use lower case filtering in query and subject sequence(s).
db_soft_mask	all	integer	none	Filtering algorithm ID to apply to the BLAST database as soft mask (i.e., only for finding initial matches).
db_hard_mask	all	integer	none	Filtering algorithm ID to apply to the BLAST database as hard mask (i.e., sequence is masked for all phases of search).
xdrop_gap_final	all	real	25	Heuristic value (in bits) for final gapped alignment/
window_size	blastp	integer	40	Multiple hits window size, use 0 to specify 1-hit algorithm.
window_size	blastp-short	integer	15	Multiple hits window size, use 0 to specify 1-hit algorithm.
use_sw_tback	all	flag	N/A	Compute locally optimal Smith-Waterman alignments?

Table C4: blastx application options. The blastx application translates a nucleotide query and searches it against protein subject sequences or a protein database.

option	**type**	**default value**	**description and notes**
word_size	integer	3	Word size for initial match.
gapopen	integer	11	Cost to open a gap.
gapextend	integer	1	Cost to extend a gap.
matrix	string	BLOSUM62	Scoring matrix name.
threshold	integer	12	Minimum score to add a word to the BLAST lookup table.
seg	string	12 2.2 2.5	Filter query sequence with SEG (Format: 'yes', 'window locut hicut', or 'no' to disable).
soft_masking	boolean	false	Apply filtering locations as soft masks (i.e., only for finding initial matches).
lcase_masking	flag	N/A	Use lower case filtering in query and subject sequence(s).
db_soft_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as soft mask (i.e., only for finding initial matches).
db_hard_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as hard mask (i.e., sequence is masked for all phases of search).
xdrop_gap_final	real	25	Heuristic value (in bits) for final gapped alignment.
window_size	integer	40	Multiple hits window size, use 0 to specify 1-hit algorithm.
strand	string	both	Query strand(s) to search against database/subject. Choice of both, minus, or plus.
query_genetic_code	integer	1	Genetic code to translate query, see ftp://ftp.ncbi.nih.gov/entrez/misc/data/gc.prt

max_intron_length	integer	0	Length of the largest intron allowed in a translated nucleotide sequence when linking multiple distinct alignments (a negative value disables linking).
comp_based_stats	integer	2	Use composition-based statistics for blastx: D or d: default (equivalent to 2) 0 or F or f: no composition-based statistics 1: Composition-based statistics as in NAR 29:2994-3005, 2001 2 or T or t : Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, conditioned on sequence properties 3: Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, unconditionally Default = `2'

Table C5: tblastn application options. The tblastn application searches a protein query against nucleotide subject sequences or a nucleotide database translated at search time.

option	**type**	**default value**	**description and notes**
word_size	integer	3	Word size for initial match.
gapopen	integer	11	Cost to open a gap.
gapextend	integer	1	Cost to extend a gap.
matrix	string	BLOSUM62	Scoring matrix name.
threshold	integer	13	Minimum score to add a word to the BLAST lookup table.
seg	string	12 2.2 2.5	Filter query sequence with SEG (Format: 'yes', 'window locut hicut', or 'no' to disable).
soft_masking	boolean	false	Apply filtering locations as soft masks (i.e., only for finding initial matches).
lcase_masking	flag	N/A	Use lower case filtering in query and subject sequence(s).
db_soft_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as soft mask (i.e., only for finding initial matches).
db_hard_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as hard mask (i.e., sequence is masked for all phases of search).
xdrop_gap_final	real	25	Heuristic value (in bits) for final gapped alignment.
window_size	integer	40	Multiple hits window size, use 0 to specify 1-hit algorithm.
db_gen_code	integer	1	Genetic code to translate subject sequences, see ftp://ftp.ncbi.nih.gov/entrez/misc/data/gc.prt
max_intron_length	integer	0	Length of the largest intron allowed in a translated nucleotide sequence when linking multiple distinct alignments (a negative value disables linking).
comp_based_stats	string	2	Use composition-based statistics for tblastn: D or d: default (equivalent to 2) 0 or F or f: no composition-based statistics 1: Composition-based statistics as in NAR 29:2994-3005, 2001 2 or T or t : Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, conditioned on sequence properties 3: Composition-based score adjustment as in Bioinformatics 21:902-911, 2005, unconditionally Default = `2'

Table C6: tblastx application options. The tblastx application searches a translated nucleotide query against translated nucleotide subject sequences or a translated nucleotide database. An option of type "flag" takes no arguments, but if present the argument is true. This table reflects the 2.2.27 BLAST+ release. Only ungapped searches are supported for tblastx.

option	**type**	**default value**	**description and notes**
word_size	integer	3	Word size for initial match.
matrix	string	BLOSUM62	Scoring matrix name.
threshold	integer	13	Minimum word score to add the word to the BLAST lookup table.
seg	string	12 2.2 2.5	Filter query sequence with SEG (Format: 'yes', 'window locut hicut', or 'no' to disable).
soft_masking	boolean	false	Apply filtering locations as soft masks (i.e., only for finding initial matches).
lcase_masking	flag	N/A	Use lower case filtering in query and subject sequence(s).
db_soft_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as soft mask (i.e., only for finding initial matches).
db_hard_mask	integer	none	Filtering algorithm ID to apply to the BLAST database as hard mask (i.e., sequence is masked for all phases of search).
strand	string	both	Query strand(s) to search against database subject sequences. Choice of both, minus, or plus.
query_genetic_code	integer	1	Genetic code to translate query, see ftp://ftp.ncbi.nih.gov/entrez/misc/data/gc.prt
db_gen_code	integer	1	Genetic code to translate subject sequences, see ftp://ftp.ncbi.nih.gov/entrez/misc/data/gc.prt
max_intron_length	integer	0	Length of the largest intron allowed in a translated nucleotide sequence when linking multiple distinct alignments (a negative value disables linking)

Table C7: rpsblast application options. The rpsblast application searches a protein query against the conserved domain database (CDD), which is a set of protein profiles. Many of the common options such as matrix or word threshold are set when the CDD is built and cannot be changed by the rpsblast application. A search ready CDD can be downloaded from ftp://ftp.ncbi.nih.gov/pub/mmdb/cdd/

Option	Type	Default value	Description and notes
window_size	integer	40	Multiple hits window size, use 0 to specify 1-hit algorithm.
xdrop_ungap	real	15	Heuristic value (in bits) for ungapped extensions
xdrop_gap	real	25	Heuristic value (in bits) for preliminary gapped extensions.
xdrop_gap_final	real	40	Heuristic value (in bits) for final gapped alignment.
seg	string	12 2.2 2.5	Filter query sequence with SEG (Format: 'yes', 'window locut hicut', or 'no' to disable).
soft_masking	boolean	false	Apply filtering locations as soft masks (i.e., only for finding initial matches).

Table C8: Makeblastdb application options. This application builds a BLAST database. An option of type "flag" takes no arguments, but if present the argument is true.

option	**type**	**default value**	**Description and notes**
in	string	stdin	Input file/database name

input_type	string	fasta	Input file type, it may be any of the following: fasta: for FASTA file(s) blastdb: for BLAST database(s) asn1_txt: for Seq-entries in text ASN.1 format asn1_bin: for Seq-entries in binary ASN.1 format
dbtype	string	prot	Molecule type of input, values can be nucl or prot.
title	string	none	Title for BLAST database. If not set, the input file name will be used.
parse_seqids	flag	N/A	Parse bar delimited sequence identifiers (e.g., gi|129295) in FASTA input.
hash_index	flag	N/A	Create index of sequence hash values.
mask_data	string	none	Comma-separated list of input files containing masking data as produced by NCBI masking applications (e.g. dustmasker, segmasker, windowmasker).
out	string	input file name	Name of BLAST database to be created. Input file name is used if none provided. This field is required if input consists of multiple files.
max_file_size	string	1GB	Maximum file size to use for BLAST database.
taxid	integer	none	Taxonomy ID to assign to all sequences.
taxid_map	string	none	File mapping sequence IDs to taxonomy IDs.
logfile	string	none	Program log file (default is stderr).

Table C9: Makeprofiledb application options. This application builds an RPS-BLAST database. An option of type "flag" takes no arguments, but if present the argument is true. COBALT (a multiple sequence alignment program) and DELTA-BLAST both use RPS-BLAST searches as part of their processing, but use specialized versions of the database. This application can build databases for COBALT, DELTA-BLAST, and a standard RPS-BLAST search. The "dbtype" option (see entry in table) determines which flavor of the database is built.

option	**type**	**default value**	**Description and notes**
in	string	stdin	Input file that contains a list of scoremat files (delimited by space, tab, or newline)
binary	flag	N/A	The scoremat files are binary ASN.1
title	string	none	Title for RPS-BLAST database. If not set, the input file name will be used.
threshold	real	9.82	Threshold for RPSBLAST lookup table.
out	string	input file name	Name of BLAST database to be created. Input file name is used if none provided.
max_file_size	string	1GB	Maximum file size to use for BLAST database.
dbtype	string	rps	Specifies use for RPSBLAST db. One of rps, cobalt, or delta.
index	flag	N/A	Creates index files.
gapopen	integer	none	Cost to open a gap. Used only if scoremat files do not contain PSSM scores, otherwise ignored.
gapextend	integer	none	Cost to extend a gap by one residue. Used only if scoremat files do not contain PSSM scores, otherwise ignored.
scale	real	100	PSSM scale factor.
matrix	string	BLOSUM62	Matrix to use in constructing PSSM. One of BLOSUM45, BLOSUM50, BLOSUM62, BLOSUM80, BLOSUM90, PAM250, PAM30 or PAM70. Used only if scoremat files do not contain PSSM scores, otherwise ignored.
obsr_threshold	real	6	Exclude domains with maximum number of independent observations below this value (for use in DELTA-BLAST searches).

exclude_invalid	real	true	Exclude domains that do not pass validation test (for use in DELTA-BLAST searches).
logfile	string	none	Program log file (default is stderr).

Table C10: Blastdbcmd application options. This application reads a BLAST database and produces reports.

option	type	default value	description and notes
db	string	nr	BLAST database name.
dbtype	string	guess	Molecule type stored in BLAST database, one of nucl, prot, or guess.
entry	string	none	Comma-delimited search string(s) of sequence identifiers: e.g.: 555, AC147927, 'gnl|dbname|tag', or 'all' to select all sequences in the database
entry_batch	string	none	Input file for batch processing. The format requires one entry per line; each line should begin with the sequence ID followed by any of the following optional specifiers (in any order): range (format: 'from-to', inclusive in 1-offsets), strand ('plus' or 'minus'), or masking algorithm ID (integer value representing the available masking algorithm). Omitting the ending range (e.g.: '10-') is supported, but there should not be any spaces around the '-'.
pig	integer	none	PIG (protein identity group) to retrieve.
info	flag	N/A	Print BLAST database information.
range	string	none	Range of sequence to extract (Format: start-stop).
strand	string	plus	Strand of nucleotide sequence to extract. Choice of plus or minus.
mask_sequence_with	string	none	Produce lower-case masked FASTA using the algorithm IDs specified.
out	string	stdout	Output file name.
outfmt	string	%f	Output format, where the available format specifiers are: %f means sequence in FASTA format %s means sequence data (without defline) %a means accession %g means gi %o means ordinal id (OID) %t means sequence title %l means sequence length %T means taxid %L means common taxonomic name %S means scientific name %P means PIG %mX means sequence masking data, where X is an optional comma-separated list of integers to specify the algorithm ID(s) to display (or all masks if absent or invalid specification). Masking data will be displayed as a series of 'N-M' values separated by ';' or the word 'none' if none are available. For every format except '%f', each line of output will correspond to a sequence.
target_only	flag	N/A	Definition line should contain target GI only.
get_dups	flag	N/A	Retrieve duplicate accessions.
line_length	integer	80	Line length for output.
ctrl_a	flag	N/A	Use Ctrl-A as the non-redundant definition line separator.

Table C11: Makembindex application options. The indexed databases created by makembindex are used by production MegaBLAST software and by a new srsearch utility designed to quickly search for nearly exact matches (up to one mismatch) of short queries against a genomic database. When a FASTA formatted file is used as the input, then masking by lower case letters is incorporated in the index. Makembindex can currently build two types of indices, called "old style" and "new style" indexing. The NCBI offers full support for the new style and has deprecated the old style. A MegaBLAST search with a new style index requires that both the index and the corresponding BLAST database be present. The index structure is described in PMID:18567917. Please cite this paper in any publication that uses makembindex.

option	**type**	**default value**	**Description and notes**
input	string	stdin	Input file name or BLAST database name, depending on the value of the iformat parameter. For FASTA formatted input, this parameter is optional and defaults to the program's standard input stream.
output	string	none	The resulting index name. The index itself can consist of multiple files, called volumes, called <index_name>.00.idx, <index_name>.01.idx,... This option should not be used with new style indices.
iformat	string	fasta	The input format selector. Possible values are 'fasta' and 'blastdb'.
old_style_index	boolean	true	If set to 'false' the new style index is created. New style indices require a BLAST database as input (use -iformat blastdb), which can be downloaded from the NCBI FTP site or created with makeblastdb. The option -output is ignored for a new style index. New style indices are always created at the same location as the corresponding BLAST database.
db_mask	integer	None	Exclude masked regions of BLAST db from the index. Use makeblastdb to discover the algorithm ID to be used as input for this argument.
legacy	boolean	true	This is a compatibility feature to support current production MegaBLAST. If true, then -stride, -nmer, and -ws_hint are ignored. The legacy format must be used for BLAST.
nmer	integer	12	N-mer size to use. Ignored if –legacy is specified
ws_hint	integer	28	This is an optimization hint for makembindex that indicates an expected minimum match size in searches that use the index. If n is the value of -nmer parameter and s is the value of –stride parameter, then the value of -ws_hint must be at least n + s - 1.
stride	integer	5	makembindex will index every stride-th N-mer of the database.
volsize	integer	1536	Target index volume size in megabytes.

Appendix D. BLASTN reward/penalty values.

BLASTN uses a simple approach to score alignments, with identically matching bases assigned a reward and mismatching bases assigned a penalty. It is important to choose reward/penalty values appropriate to the sequences being aligned with the (absolute) reward/penalty ratio increasing for more divergent sequences. A ratio of 0.33 (1/-3) is appropriate for sequences that are about 99% conserved; a ratio of 0.5 (1/-2) is best for sequences that are 95% conserved; a ratio of about one (1/-1) is best for sequences that are 75% conserved [1].

For each reward/penalty pair, a number of different gap costs are supported. A gap cost includes a value to open the gap and a value to extend the gap by a base. Following the convention of the command-line applications, these costs are listed as positive numbers here. MegaBLAST uses a specialized algorithm to calculate the default gap costs for a reward/penalty pair that is described in PMID:10890397. Briefly, the default megaBLAST cost to open a gap is zero and the cost to extend a gap two letters is given by the absolute value of two mismatches minus one match. For example, given a reward of 1 and penalty of -5, the cost to extend a gap by one letter is 5.5. The default gap costs for other tasks supported by the blastn application is 5 to open a gap and 2 to extend one base.

Table D1 presents the supported reward/penalty values and gap costs.

[1] States DJ, Gish W, and Altschul SF (1991) METHODS: A companion to Methods in Enzymology 3:66-70.

Table D1: Supported reward/penalty values and gap costs for the blastn application. The left-most column presents the supported reward/penalty values. The middle column presents pairs of numbers for the cost to open and extend a gap for each reward/penalty value. Blastn also supports gap costs more stringent than those listed (e.g., for reward/penalty of 1/-3 gap costs of 5/2 or 500/2 are supported). The reward/penalty values are ordered from most to least stringent, with the more stringent values better suited for alignments with high sequence identity. The default megaBLAST gap costs are shown in the right-most column. Accurate statistics for these default megaBLAST gap costs can only be calculated for the most stringent reward/penalty values, but the values listed in the middle column can always be used.

reward/penalty	gap costs (open/extend)	default MegaBLAST gap costs (open/extend)
1/-5	3/3	0/5.5
1/-4	1/2, 0/2, 2/1, 1/1	0/4.5
2/-7	2/4, 0/4, 4/2, 2/2	0/8
1/-3	2/2, 1/2, 0/2, 2/1, 1/1	0/3.5
2/-5	2/4, 0/4, 4/2, 2/2	0/6
1/-2	2/2, 1/2, 0/2, 3/1, 2/1, 1/1	0/2.5
2/-3	4/4, 2/4, 0/4, 3/3, 6/2, 5/2, 4/2, 2/2	0/4
3/-4	6/3, 5/3, 4/3, 6/2, 5/2, 4/2	N/A
4/-5	6/5, 5/5, 4/5, 3/5	N/A
1/-1	3/2, 2/2, 1/2, 0/2, 4/1, 3/1, 2/1	N/A
3/-2	5/5	N/A
5/-4	10/6, 8/6	N/A