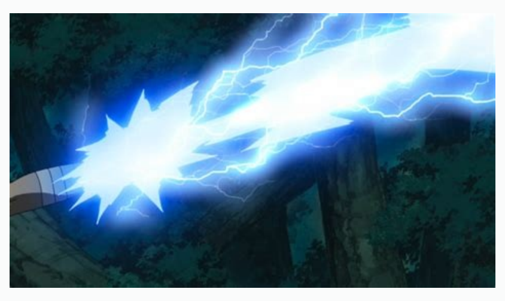
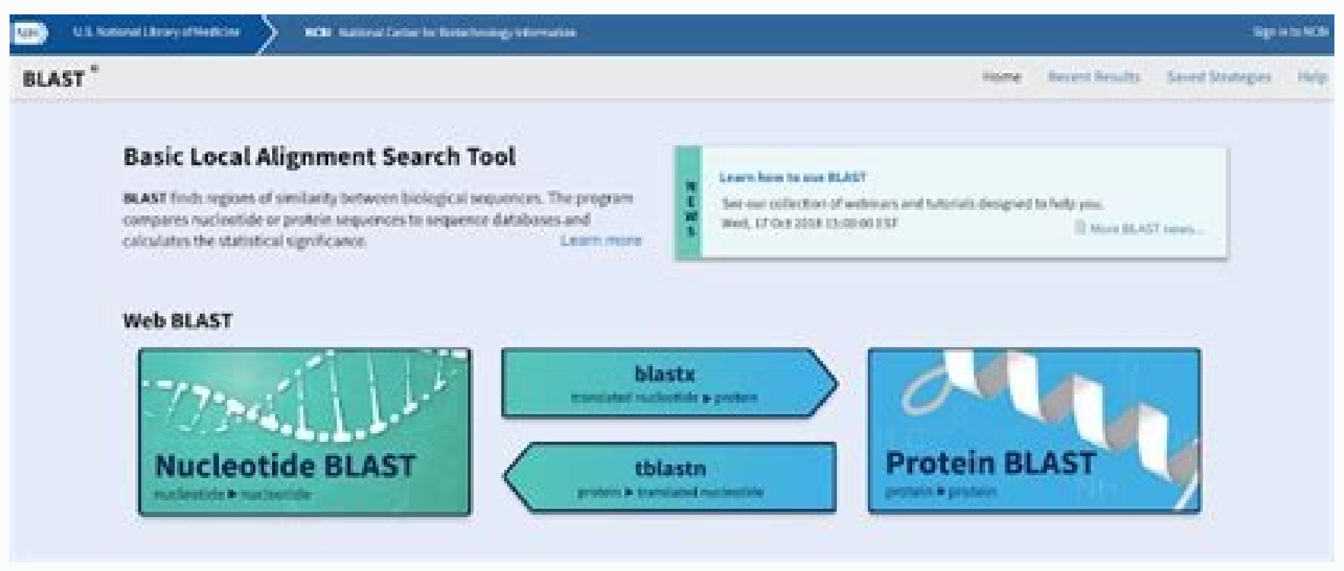
Ncbi blast user manual

I'm not robot!





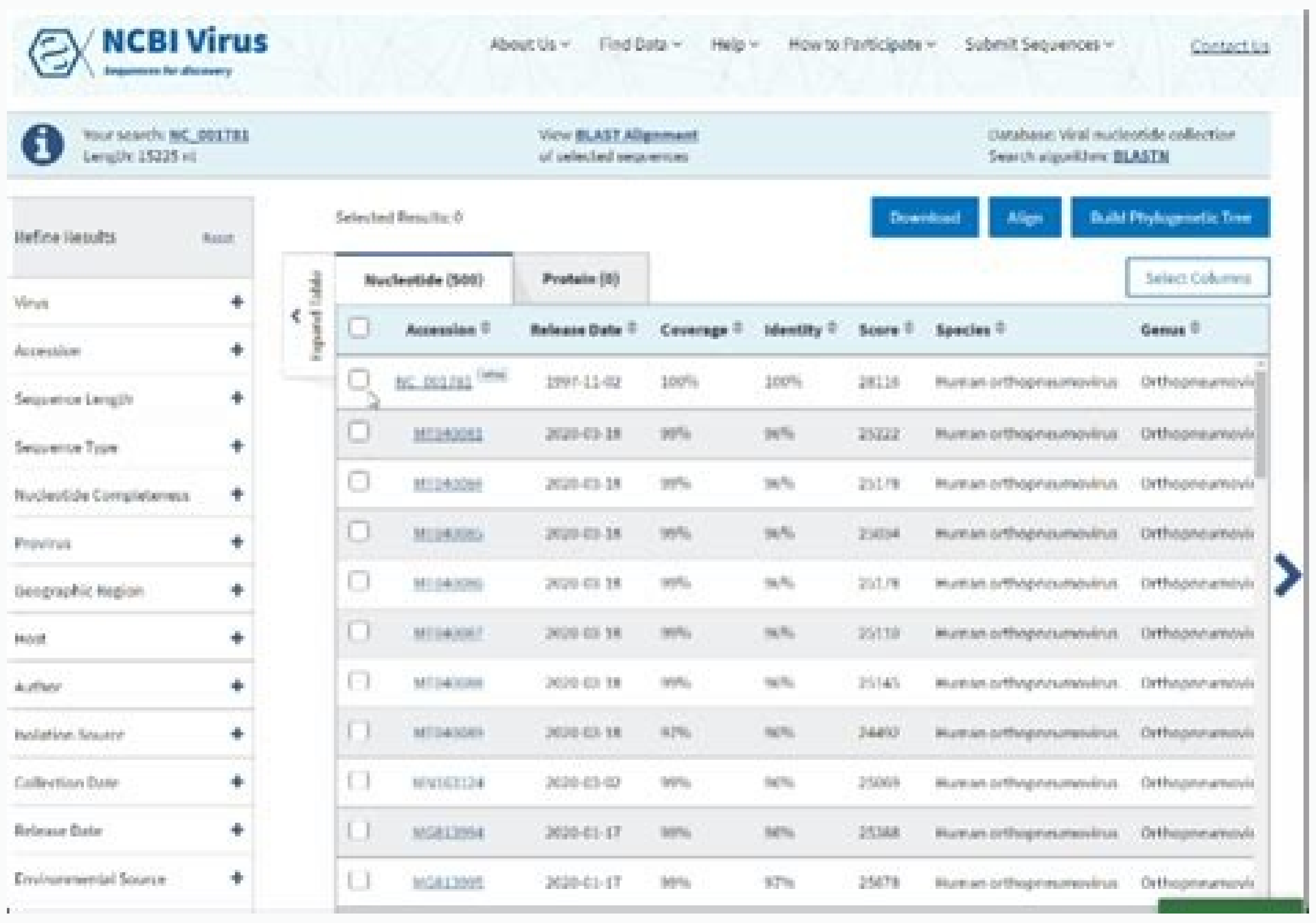
BLAST®

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. Learn more

Learn how to use BLAST

Web BLAST



NCBI Virus

Sequences for discovery

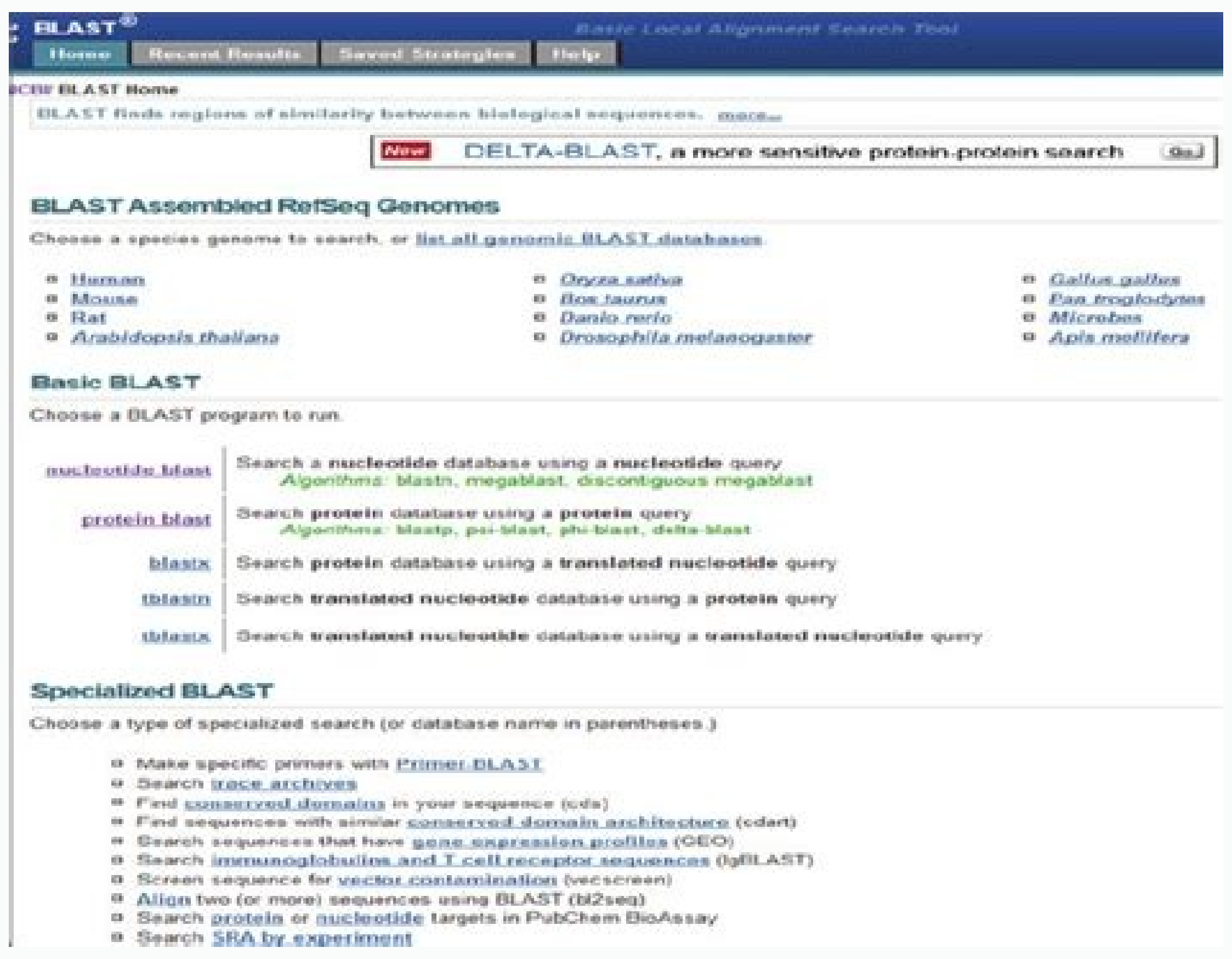
Your search: NC_001781
Length: 15225 nt

View BLAST Alignment of selected sequences

Database: Viral nucleotide collection
Search algorithm: BLASTN

Selected Results: 0

	Accession	Release Date	Coverage	Identity	Score	Species	Genus
	NC_001781	1997-11-02	100%	100%	28116	Human orthopneumovirus	Orthopneumovir
	MT040081	2020-03-18	99%	96%	25222	Human orthopneumovirus	Orthopneumovir
	MT040084	2020-03-18	99%	96%	25178	Human orthopneumovirus	Orthopneumovir
	MT040085	2020-03-18	99%	96%	25034	Human orthopneumovirus	Orthopneumovir
	MT040086	2020-03-18	99%	96%	25178	Human orthopneumovirus	Orthopneumovir
	MT040087	2020-03-18	99%	96%	25110	Human orthopneumovirus	Orthopneumovir
	MT040088	2020-03-18	99%	96%	25145	Human orthopneumovirus	Orthopneumovir
	MT040089	2020-03-18	97%	96%	24493	Human orthopneumovirus	Orthopneumovir
	MN163124	2020-03-02	99%	96%	25069	Human orthopneumovirus	Orthopneumovir
	MG813994	2020-01-17	98%	96%	25368	Human orthopneumovirus	Orthopneumovir
	MG813995	2020-01-17	99%	97%	25678	Human orthopneumovirus	Orthopneumovir



BLAST®

Basic Local Alignment Search Tool

NCBI/ BLAST Home

BLAST finds regions of similarity between biological sequences. more...

New DELTA-BLAST, a more sensitive protein-protein search Go

BLAST Assembled RefSeq Genomes

Choose a species genome to search, or list all genomic BLAST databases

- Human
- Mouse
- Rat
- *Arabidopsis thaliana*
- *Oryza sativa*
- *Bos taurus*
- *Danio rerio*
- *Drosophila melanogaster*
- *Gallus gallus*
- *Pan troglodytes*
- *Microbes*
- *Apis mellifera*

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query Algorithms: blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query Algorithms: blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

Specialized BLAST

Choose a type of specialized search (or database name in parentheses.)

- Make specific primers with Primer-BLAST
- Search trace archives
- Find conserved domains in your sequence (cds)
- Find sequences with similar conserved domain architecture (cdart)
- Search sequences that have gene expression profiles (GEO)
- Search immunoglobulins and T cell receptor sequences (IgBLAST)
- Screen sequence for vector contamination (vecscreen)
- Align two (or more) sequences using BLAST (bl2seq)
- Search protein or nucleotide targets in PubChem BioAssay
- Search SRA by experiment

Created: June 23, 2008; Last Update: March 24, 2022.Estimated reading time: 2 minutesAs part of our effort to improve BLAST+, we have implemented usage reporting to collect limited data. This information shows us whether BLAST+ is being used by the community, and therefore is worth being maintained and developed by NCBI. It also allows us to focus our development efforts on the most used aspects of BLAST+. If possible, please report your usage, so we can continue to support and develop BLAST+ to best suit your needs.You can easily opt-out of sending information about your searches if you wish by following the instructions below. This offers you greater privacy control than sending searches over the internet using web tools or the -remote option on a BLAST+ executable, which also accesses the web service.Information sent back to NCBI is limited to the name of the BLAST program, database metadata, a few BLAST parameters, as well the number and total size of your queries without any data association that might reveal the context of your research. No sequences from your queries or database are sent to the NCBI. An example set of data from a BLAST search is provided below. More information about each item is shown in the table following the list.IP=27.18.28.18comp_based_stats=2db_date=Aug 26, 2020 3:12 AMdb_length=179658219db_name=swissprotdb_num_seqs=474714evalue_threshold=10.000000exit_status=0hitlist_size=500ncbi_app=standalone-blastncbi_location=be-mdncbi_role=productionnum_queries=1num_threads=2os=UNIXoutput_fmt=11program=blastpqueries_length=656run_time=3.076507task=blastpversion=2.11.0View in own window Reported parameter Description IP The apparent IP address of the machine running BLAST comp_based_stats Composition based statistics setting See Command-line options in the manual. db_date Creation date of the BLAST database db_length Length (size) of the database in letters (bases or amino acid characters) db_name BLAST database name db_num_seqs Number of sequences in the BLAST database. evalue_threshold Expect value limit. See Command-line options exit_status BLAST program exit status. The value '0' indicates success. See the Exit codes in the manual for more information. hitlist_size Number of matches to return. This is the same value as the max_target_seqs option. See Command-line options in the manual. ncbi_app Parameter used by NCBI application logging. All BLAST programs return 'standalone-blast' ncbi_location Default parameter for BLAST. Value always 'be-me for (Bethesda, Maryland) ncbi_role Default parameter. Value always production. num_queries Number of query sequences in the BLAST search. You can opt-out of the usage reporting by adding a .ncbirc (UNIX like) or ncbi.ini (Windows) configuration file. In the configuration file you should add a line under the BLAST section to set BLAST_USAGE_REPORT to false. See here for details on setting up a configuration file.You may also opt-out of the usage reporting by setting the environment variable BLAST_USAGE_REPORT to false. In bash (under LINUX) this command would be:export BLAST_USAGE_REPORT=falseNote that this environment variable is only set in the shell (i.e., window) you are currently using and will not be set the next time you login. To permanently opt-out, this variable should be set every time a new shell is opened or with a configuration file, as described above.You can also set this environment variable, turning off usage reporting, when using BLAST+ docker by adding the -e option to your docker invocation:-e BLAST_USAGE_REPORT=falseThe NLM privacy policy is available here. 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)12 = Seqalign (JSON),13 = Multiple-file BLAST JSON,14 = Multiple-file BLAST XML2,15 = Single-file BLAST JSON,16 = Single-file BLAST XML2,17 = Sequence Alignment/Map (SAM),18 = Organism ReportOptions 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 (for all HSPs) qcovhsp means Query Coverage Per HSP qcovus is a measure of Query Coverage that counts a position in a subject sequence for this measure only once. The second time the position is aligned to the query is not counted towards this measure. 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' Created: June 23, 2008; Last Update: January 7, 2021.Estimated reading time: 2 minutesA 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.To send the search to our servers and databases, add the –remote option:blastn –db nt –query nt.fsa –out results.out -remote See more about this option in the section below, BLAST+ remote service.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. For example, use –help to get a list of output options for the –outfmt option.Create a custom database from a multi-FASTA file of sequences with this minimal command:makeblastdb –in mydb.fsa –dbtype nucl –parse_seqids See the section below, Building a BLAST database with local sequences, for more details.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 . 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 --decompress swissprot will download all the relevant swissprot tar files.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 archiveperl update_blastdb.pl taxdb# Install it in the BLASTDB directorygunzip -cd taxdb.tar.gz | (cd $BLASTDB; tar xvf -) While the previous chapters covered installing and using a few bioinformatics tools as examples of the process, there is one nearly ubiquitous tool: BLAST, or Basic Local Alignment Search Tool. Given one or more query sequences (usually in FASTA format), BLAST looks for matching sequence regions between them and a subject set. A sufficiently close match between subsequences (denoted by arrows in the figure above, though matches are usually longer than illustrated here) is called a high-scoring pair (HSP), while a query sequence is said to hit a target sequence if they share one or more HSPs. Sometimes, however, the term "hit" is used loosely, without differentiating between the two. Each HSP is associated with a "bitscore" that is based on the similarity of the subsequences as determined by a particular set of rules. Because in larger subject sets some good matches are likely to be found by chance, each HSP is also associated with an "E value," representing the expected number of matches one might find by chance in a subject set of that size with that score or better. For example, an E value of 0.05 means that we can expect a match by chance in 1 in 20 similar searches, whereas an E value of 2.0 means we can expect 2 matches by chance for each similar search. BLAST is not a single tool, but rather a suite of tools (and the suite grows over the years as more features and related tools are added). The most modern version of the software, called BLAST+, is maintained by the National Center for Biotechnology Information (NCBI) and may be downloaded in binary and source forms at ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/. This chapter only briefly covers running BLAST on the command line in simple ways. Reading the help information (e.g., with blastn --help) and the NCBI BLAST Command Line Applications User Manual at is highly recommended. The NCBI manual covers quite a few powerful and handy features of BLAST on the command line that this book does not. BLAST Types The programs in the BLAST+ suite can search for and against sequences in protein format (as we did for the HMMER example) and in nucleotide format (A's, C's, T's, and G's). Depending on what type the query and subject sets are, different BLAST programs are used. While two nucleotide sequences (N comparisons in the figure above) may be compared directly (as may two protein sequences, represented by P), when we wish to compare a nucleotide sequence to a protein sequence, we need to consider which reading frame of the nucleotide sequence corresponds to a protein. The blastx and tblastn programs do this by converting nucleotide sequences into protein sequences in all six reading frames (three on the forward DNA strand and three on the reverse) and comparing against all of them. Generally such programs result in six times as much work to be done. The tblastx program compares nucleotide queries against nucleotide subjects, but it does so in protein space with all six conversions compared to all six on both sides. Other more exotic BLAST tools include psiblast, which produces an initial search and tweaks the scoring rules on the basis of the results; these tweaked scoring rules are used in a second search, generally finding even more matches. This process is repeated as many times as the user wishes, with more dissimilar matches being revealed in later iterations. The deltablast program considers a precomputed database of scoring rules for different types of commonly found (conserved) sequences. Finally, rpsblast searches for sequence matches against sets of profiles, each representing a collection of sequences (as in HMMER, though not based on hidden Markov models). All this talk of scoring rules indicates that the specific scoring rules are important, especially when comparing two protein sequences. When comparing protein sequences from two similar species, for example, we might wish to give a poor score to the relatively unlikely match of a nonpolar valine (V) to a polar tyrosine (Y). But for dissimilar species separated by vast evolutionary time, such a mismatch might not be as bad relative to other possibilities. Scoring matrices representing these rule sets with names like BLOSUM and PAM have been developed using a variety of methods to capture these considerations. A discussion of these details can be found in other publications. Each of the various programs in the BLAST suite accepts a large number of options; try running blastn -help to see them for the blastn program. Here is a summary of a few parameters that are most commonly used for blastn et al.: -query The name (or path) of the FASTA-formatted file to search for as query sequences. -subject The name (or path) of the FASTA-formatted file to search in as subject sequences. -evalue Only HSPs with E values smaller than this should be reported. For example: -evalue 0.001 or -evalue 1e-6. -outfmt How to format the output. The default, 0, provides a human-readable (but not programmatically parseable) text file. The values 6 and 7 produce tab-separated rows and columns in a text file, with 7 providing explanatory comment lines. Similarly, a value of 10 produces comma-separated output; 11 produces a format that can later be quickly turned into any other with another program called blast_formatter. Options 6, 7, and 10 can be highly configured in terms of what columns are shown. -max_target_seqs When the output format is 6, 7, or 10 for each query sequence, only report HSPs for the first different subject sequences. -max_hsps For each query/target pair, only report the best HSPs. -out Write the output to as opposed to the default of standard output. BLAST Databases No doubt readers familiar with BLAST have been curious: aren't there databases of some kind involved in BLAST searches? Not necessarily. As we've seen, simple FASTA files will suffice for both the query and subject set. It turns out, however, that from a computational perspective, simple FASTA files are not easily searched. Thus BLAST+ provides a tool called makeblastdb that converts a subject FASTA file into an indexed and quickly searchable (but not human-readable) version of the same information, stored in a set of similarly named files (often at least three ending in .pin, .psq, and .phr for protein sequences, and .nin, .nsq, and .nhr for nucleotide sequences). This set of files represents the "database," and the database name is the shared file name prefix of these files. Running makeblastdb on a FASTA file is fairly simple: makeblastdb -in -out -dbtype -title -parse_seqids, where is one of prot or nucl, and is a human-readable title (enclosed in quotes if necessary). The -parse_seqids flag indicates that the sequence IDs from the FASTA file should be included in the database so that they can be used in outputs as well as by other tools like blastdbcmd (discussed below). Once a BLAST database has been created, other options can be used with blastn et al.: -db The name of the database to search against (as opposed to using -subject). -num_threads Use CPU cores on a multicore system, if they are available. When using the -db option, the BLAST tools will search for the database files in three locations: (1) the present working directory, (2) your home directory, and (3) the paths specified in the $BLASTDB environment variable. The tool blastdbcmd can be used to get information about BLAST databases—for example, with blastdbcmd -db -info—and can show the databases in a given path with blastdbcmd -list (so, blastdbcmd -list $BLASTDB will show the databases found in the default search paths). This tool can also be used to extract sequences or information about them from databases based on information like the IDs reported in output files. As always, reading the help and documentation for software like BLAST is highly recommended. Running a Self-BLAST To put these various tools and options to use, let's consider using blastp to look for proteins that are similar in sequence to other proteins in the yeast exome. First, we'll need to use wget to download the protein data set (after locating it at), and then gzip -d to decompress it, calling it orf_trans.fasta. In order to find sequences that are similar to others, we're going to want to blastp this file against itself. So, we'll start by creating a database of these sequences. Now we need to determine what options we will use for the blastp. In particular, do we want to limit the number of HSPs and target sequences reported for each query? Because we're mostly interested in determining which proteins match others, we probably only need to keep one hit. But each protein's best hit will likely be to itself! So we'd better keep the top two with -max_target_seqs 2 and only the best HSP per hit with -max_hsps 1. We'll also use an -evalue 1e-6, a commonly used cutoff. For the output, we'll create a tab-separated output with comment lines (-outfmt 7), creating columns for the query sequence ID, subject sequence ID, HSP alignment length, percentage identity of the alignment, subject sequence length, query sequence length, start and end positions in the query and subject, and the E value. (The coded names—qseqid, sseqid, length, etc.—can be found by running blastp -help.) Finally, we'll call the output file yeast_blastp_yeast_top2.txt and use four processors to speed the computation (which will only really help if the machine we are logged in to has at least that many). It's a long command, to be sure! This operation takes several minutes to finish, even with -num_threads 4 specified. When it does finish, we can see with less that the output file contains the columns we specified interspersed with the comment lines provided by -outfmt 7. In the output snippet above, YAL0005C has an HSP with itself (naturally), but also one with YLL024C. We'll consider basic analyses containing this sort of data—rows and columns stored in text files, interspersed with extraneous lines—in later chapters. If you don't already have the NCBI Blast+ tools installed, install them. Once you do, check the contents of the $BLASTDB environment variable. If it's not empty, use blastdbcmd to determine whether you have the "nr" database available, and any information you can determine about it (when it was downloaded, how many sequences it has, etc.) Create a new folder in your projects folder called blast. In this directory, download the p450s.fasta file and the yeast exome orf_trans.fasta from the book website. Create a database called orf_trans using makeblastdb, and use blastp to search the p450s.fasta file against it. When doing the search, use an E-value cutoff of 1e-6, keep the top one target sequences, and produce an output file called p450s_blastp_yeast_top1.blast in output format 11. Use the blast_formatter tool to convert the output format 11 file above into an output format 6 called p450s_blastp_yeast_top1.txt, with columns for: (1) Query Seq-id, (2) Subject Seq-id, (3) Subject Sequence Length, (4) Percentage of Identical Matches, (5) E Value, (6) Query Coverage per Subject, and (7) Subject title. (You may find browsing the NCBI BLAST+ manual and the output of blast_formatter -help to be informative.) The output, when viewed with less -S, should look something like this:What do these various output columns represent? The file yeast_selected_ids.txt contains a column of 25 IDs identified as interesting in some way. Use blastdbcmd to extract just those sequence records from the orf_trans database as a FASTA file named yeast_selected_ids.fasta. (Again, browsing the BLAST+ manual and the output of blastdbcmd -helpwill be useful.)

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