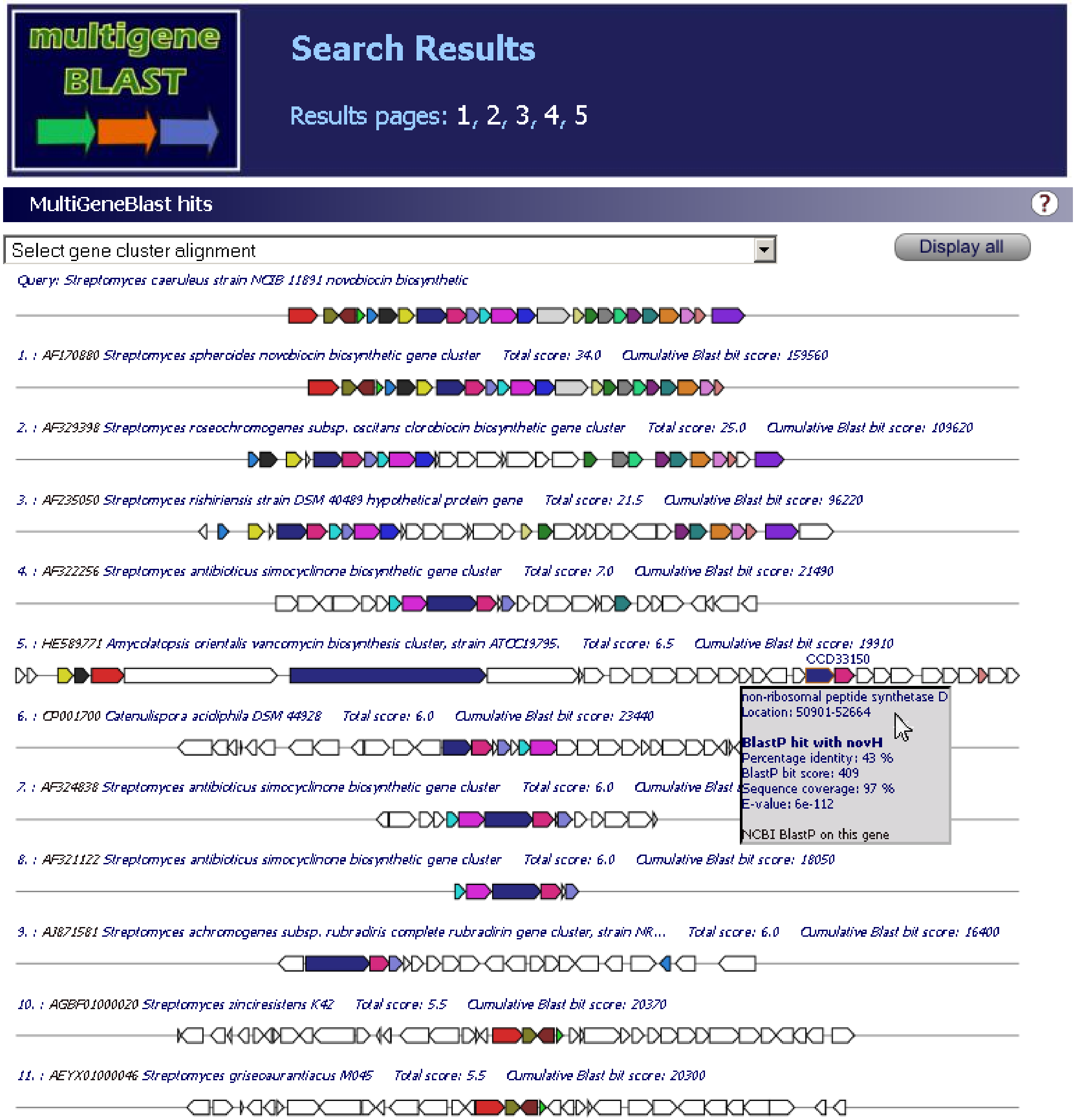
MultiGeneBlast: a Tutorial



Search Results

Results pages: 1, 2, 3, 4, 5

MultiGeneBlast hits

Query: Streptomyces caeruleus strain NCIB 11891 novobiocin biosynthetic

1. : AF170880 Streptomyces spheroides novobiocin biosynthetic gene cluster   Total score: 34.0   Cumulative Blast bit score: 159560

2. : AF329398 Streptomyces roseochromogenes subsp. oscitans clorobiocin biosynthetic gene cluster   Total score: 25.0   Cumulative Blast bit score: 109620

3. : AF235050 Streptomyces rishiriensis strain DSM 40489 hypothetical protein gene   Total score: 21.5   Cumulative Blast bit score: 96220

4. : AF322256 Streptomyces antibioticus simocyclinone biosynthetic gene cluster   Total score: 7.0   Cumulative Blast bit score: 21490

5. : HE589771 Amycolatopsis orientalis vancomycin biosynthesis cluster, strain ATCC19795.   Total score: 6.5   Cumulative Blast bit score: 19910

non-ribosomal peptide synthetase D
Location: 50901-52664

BlastP hit with novH
Percentage identity: 43 %
BlastP bit score: 409
Sequence coverage: 97 %
E-value: 6e-112

NCBI BlastP on this gene

6. : CP001700 Catenulispora acidiphila DSM 44928   Total score: 6.0   Cumulative Blast bit score: 23440

7. : AF324838 Streptomyces antibioticus simocyclinone biosynthetic gene cluster   Total score: 6.0   Cumulative Blast

8. : AF321122 Streptomyces antibioticus simocyclinone biosynthetic gene cluster   Total score: 6.0   Cumulative Blast bit score: 18050

9. : AJ871581 Streptomyces achromogenes subsp. rubradiris complete rubradirin gene cluster, strain NR...   Total score: 6.0   Cumulative Blast bit score: 16400

10. : AGBF01000020 Streptomyces zinciresistens K42   Total score: 5.5   Cumulative Blast bit score: 20370

11. : AEYX01000046 Streptomyces griseoaurantiacus M045   Total score: 5.5   Cumulative Blast bit score: 20300

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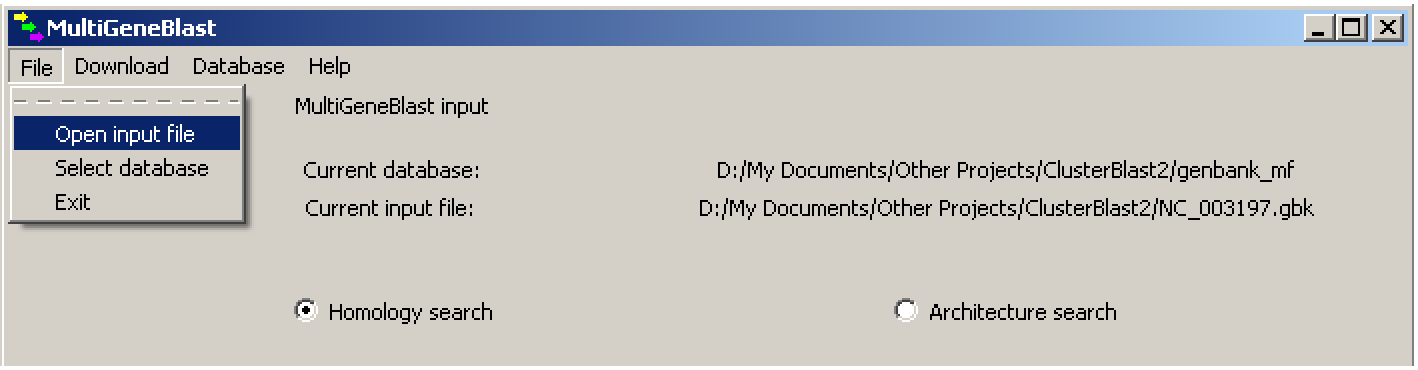
Introduction

Many biochemical pathways and systems in nature, especially in prokaryotes and fungi, are encoded by genes that are located physically close to each other on the chromosome, in operons or gene clusters. MultiGeneBlast combines Basic Local Alignment Search Tool (BLAST) searches of multiple predicted proteins and maps their hits onto their parent nucleotide scaffolds. This can either be used to find operons or gene clusters homologous to a known operon or gene cluster (with the homology search mode), or to find novel genomic loci which contain a certain user-specified combination of genes (with the architecture search mode).

MultiGeneBlast can either be run using a graphical user interface or from the command-line. The first sections of this tutorial will explain how to use the tool using the graphical user interface. A final section will explain how to use it from the command-line.

Overview: input, databases and output



MultiGeneBlast input

Current database: D:/My Documents/Other Projects/ClusterBlast2/genbank_mf

Current input file: D:/My Documents/Other Projects/ClusterBlast2/NC_003197.gbk

● Homology search ○ Architecture search

The input of a homology search consists of canonical GenBank or EMBL nucleotide entry files along with either start and end coordinates of the query region or a list of genes (locus tags or accession numbers) from the entry that will make up the search query. For an architecture search, the input consists of a FASTA file containing multiple amino acid sequence entries. When setting up a search, an input file can be loaded by selecting ‘Open input file’ in the ‘File’ menu.

The database which is searched by MultiGeneBlast is a special version of a normal NCBI Blast+ protein database, also containing files (*.cords.tar, *.pinfo.tar, *_all_descrs.txt) that contain information on the parent nucleotide entry of each protein and the location of its gene on this nucleotide entry. A database can be loaded by clicking ‘Select database’ in the ‘File’ menu.

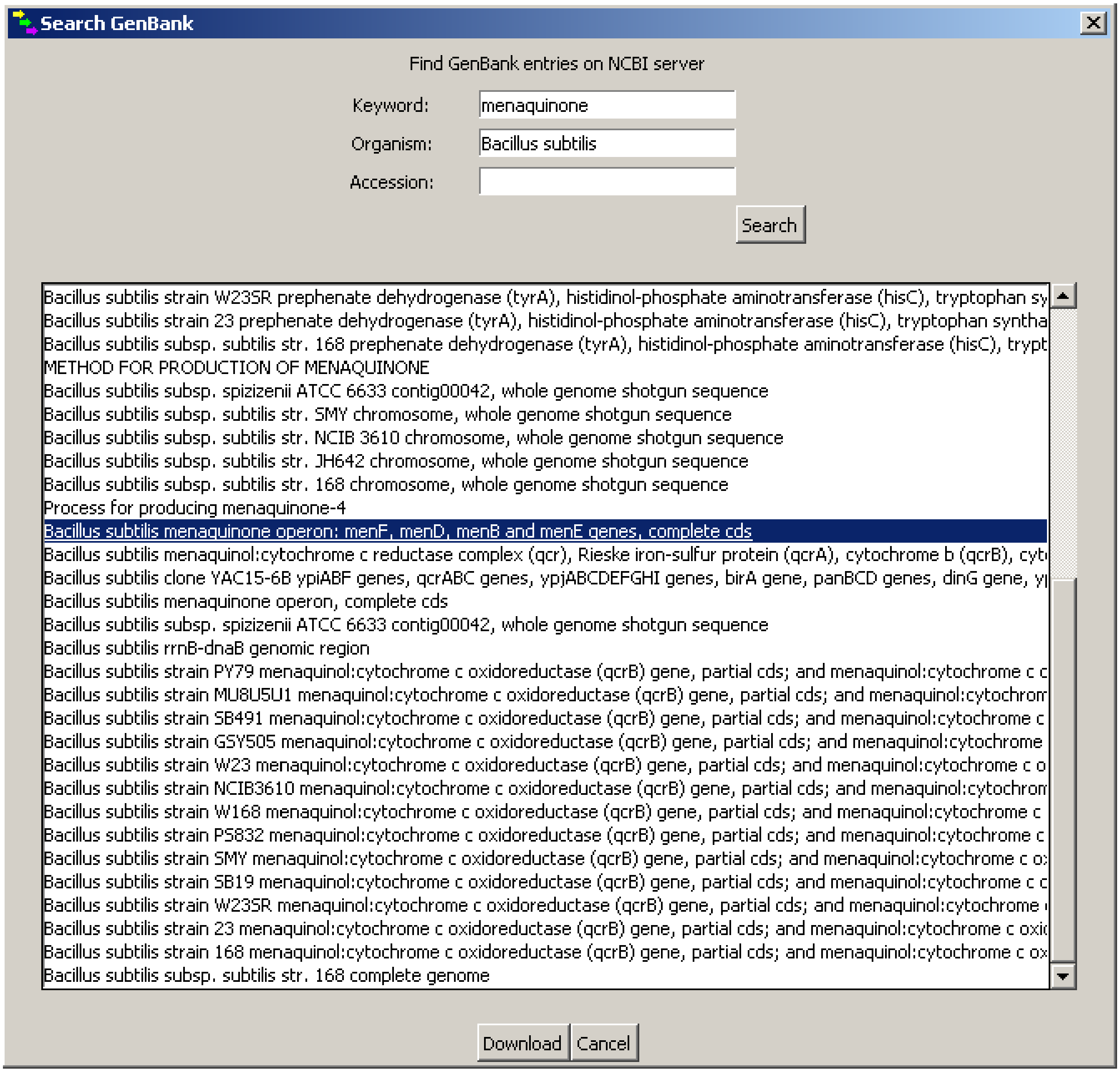
The next paragraphs will explain how to obtain input files for your search, as well as how to obtain a suitable search database.

Preparing your input file

Using an existing EMBL or GenBank file on your system

If you want to perform a homology search with a genomic region that is contained by a nucleotide EMBL or GenBank file on your system, no preparation is needed, as long as this file contains both the DNA sequence of the region and the annotations of CDS features (coding regions). If either the DNA sequence or CDS annotations are missing from the file, you can use one of the alternative modes to acquire a suitable version of your file, as stated below.

Downloading a GenBank entry of your choice



Find GenBank entries on NCBI server

Keyword: menaquinone

Organism: Bacillus subtilis

Accession:

Bacillus subtilis strain W23SR prephenate dehydrogenase (tyrA), histidinol-phosphate aminotransferase (hisC), tryptophan sy
Bacillus subtilis strain 23 prephenate dehydrogenase (tyrA), histidinol-phosphate aminotransferase (hisC), tryptophan syntha
Bacillus subtilis subsp. subtilis str. 168 prephenate dehydrogenase (tyrA), histidinol-phosphate aminotransferase (hisC), trypt
METHOD FOR PRODUCTION OF MENAQUINONE
Bacillus subtilis subsp. spizizenii ATCC 6633 contig00042, whole genome shotgun sequence
Bacillus subtilis subsp. subtilis str. SMY chromosome, whole genome shotgun sequence
Bacillus subtilis subsp. subtilis str. NCIB 3610 chromosome, whole genome shotgun sequence
Bacillus subtilis subsp. subtilis str. JH642 chromosome, whole genome shotgun sequence
Bacillus subtilis subsp. subtilis str. 168 chromosome, whole genome shotgun sequence
Process for producing menaquinone-4
Bacillus subtilis menaquinone operon: menF, menD, menB and menE genes, complete cds
Bacillus subtilis menaquinol:cytochrome c reductase complex (qcr), Rieske iron-sulfur protein (qcrA), cytochrome b (qcrB), cyt
Bacillus subtilis clone YAC15-6B ypiABF genes, qcrABC genes, ypjABCDEFGHI genes, birA gene, panBCD genes, dinG gene, y
Bacillus subtilis menaquinone operon, complete cds
Bacillus subtilis subsp. spizizenii ATCC 6633 contig00042, whole genome shotgun sequence
Bacillus subtilis rrnB-dnaB genomic region
Bacillus subtilis strain PY79 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c c
Bacillus subtilis strain MU8U5U1 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrom
Bacillus subtilis strain SB491 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c
Bacillus subtilis strain GSY505 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome
Bacillus subtilis strain W23 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c o
Bacillus subtilis strain NCIB3610 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochron
Bacillus subtilis strain W168 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c
Bacillus subtilis strain PS832 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c
Bacillus subtilis strain SMY menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c o
Bacillus subtilis strain SB19 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c c
Bacillus subtilis strain W23SR menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome
Bacillus subtilis strain 23 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c oxi
Bacillus subtilis strain 168 menaquinol:cytochrome c oxidoreductase (qcrB) gene, partial cds; and menaquinol:cytochrome c ox
Bacillus subtilis subsp. subtilis str. 168 complete genome

If the genomic region of your choice is located in a nucleotide entry in the GenBank database (as, for example, is the case for most if not all published genome sequences), you can search for the entry by clicking 'Download GenBank entry' from the 'Download' menu. If then, for example, you want to search for homologues of the menaquinone operon from *Bacillus subtilis*, which has been cloned and sequenced, you can enter 'menaquinone' as a keyword and 'Bacillus subtilis' as the organism of origin, and a number of matches will appear in the search window. After selecting the entry of your choice (which, in this case, may equally well be the complete *B. subtilis* genome sequence), you can download it by clicking the 'Download' button.

Generating an EMBL file from unannotated sequence data and an annotation table (Expert Users)

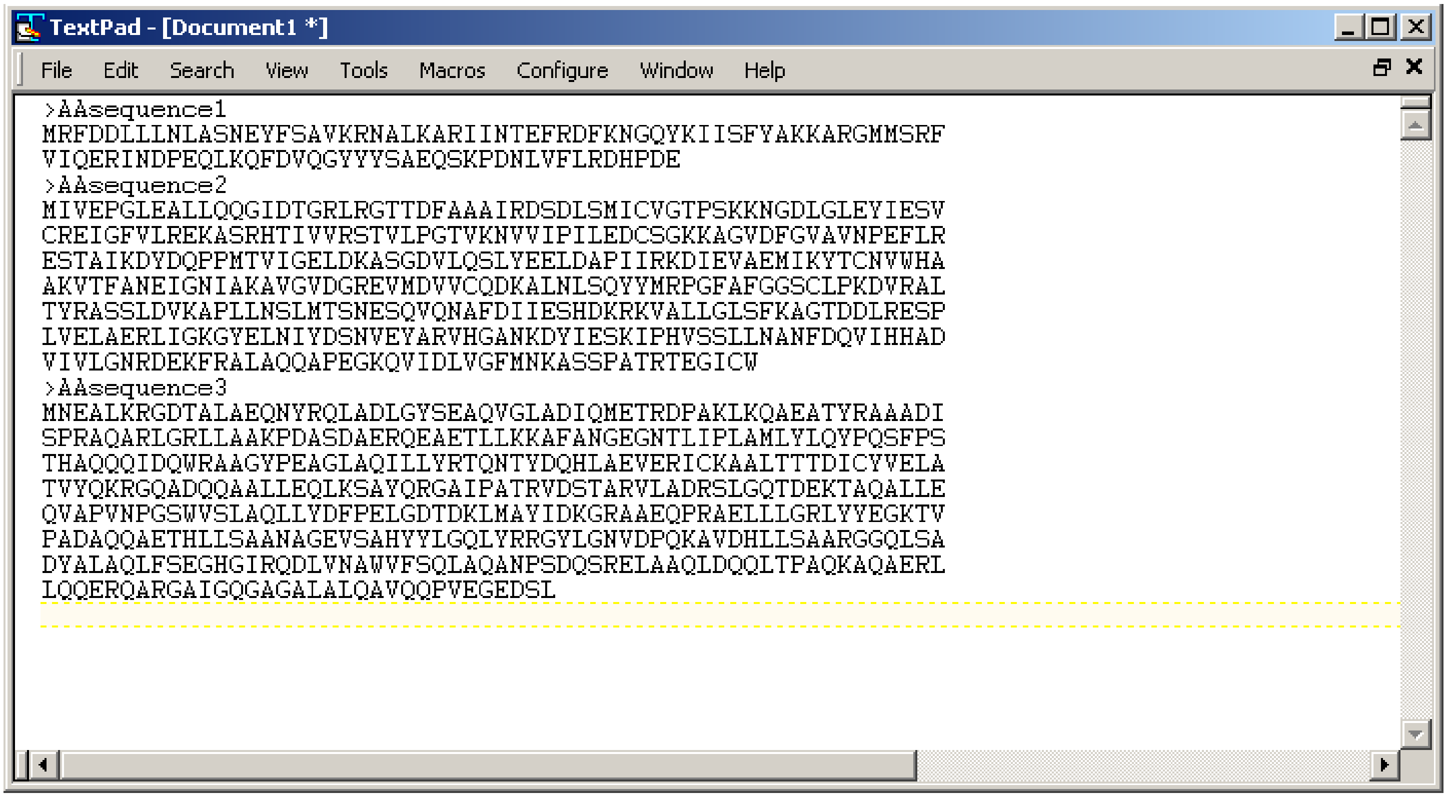
If you have your own sequence data and gene annotations, which have not been annotated in GenBank or EMBL format yet, a Python script (format_embl.py) has been provided to generate an EMBL file from a nucleotide FASTA file containing your genome sequence or contig and one TXT file containing the positions and annotations of its genes. The script is located in the 'format_embl' folder.

The script can be used as follows:

- Prepare a tab-delimited TXT file 'annotationtable.txt' containing a table with the information on each exon / gene structured in the following columns: 1) name of contig FASTA file or genome sequence, 2) gene locus tag (must be unique), 3) 5' exon or gene start, 4) 3' exon or gene end, 5) gene annotation.
- Copy 'annotationtable.txt' and the FASTA files of the contig or genome sequence into 'format_embl' folder.
- In the command line, type 'python format_embl.py'. For this command to work, you will have to have Python installed on your computer. If you use a Windows system, you will also need to add the Python installation directory to your PATH environment variable.
- If the script finishes successfully, input an EMBL file for your genome sequence or contig will have been created, which you can use as input for MultiGeneBlast.

Examples for the TXT and FASTA files are provided with the MultiGeneBlast download and are located in the 'format_embl' directory.

Preparing a multiple-entry FASTA file for an architecture search



```
>AAsequence1
MRFDDLLLNLASNEYFSAVKRNALKARIINTEFRDFKNGQYKIISFYAKKARGMMSRF
VIQERINDPEQLKQFDVQGYYYSAEQSKPDNLVFLRDHPDE
>AAsequence2
MIVEPGLEALLQQGIDTGRLRGTTDFAAAIRDSDLSMICVGTPSKKNGDLGLEYIESV
CREIGFVLREKASRHTIVVRSTVLPGTVKNVVIPILEDCSGKKAGVDFGVAVNPEFLR
ESTAIKDYDQPPMTVIGELDKASGDVLQSLYEELDAPIIRKDIEVAEMIKYTCNVWHA
AKVTFANEIGNIAKAVGVDGREVMDVVCQDKALNLSQYYMRPGFAFGGSCLPKDVRAL
TYRASSLDVKAPLLNSLMTSNESQVQNAFDIIESHDKRKVALLGLSFKAGTDDLRESP
LVELAERLIGKGYELNIYDSNVEYARVHGANKDYIESKIPHVSSLLNANFDQVIHHAD
VIVLGNRDEKFRALAQQAPEGKQVIDLVGFMNKASSPATRTEGICW
>AAsequence3
MNEALKRGDTALAEQNYRQLADLGYSEAQVGLADIQMETRDPAKLKQAEATYRAAADI
SPRAQARLGRLLAAKPDASDAERQEAETLLKKAFANGEGNTLIPLAMLYLQYPQSFPS
THAQQQIDQWRAAGYPEAGLAQILLYRTQNTYDQHLAEVERICKAALTTTDICYVELA
TVYQKRGQADQQAALLEQLKSAYQRGAIPATRVDSTARVLADRSLGQTDEKTAQALLE
QVAPVNPGSWVSLAQLLYDFPELGDTDKLMAYIDKGRAAEQPRAELLLGRLYYEGKTV
PADAQQAETHLLSAANAGEVSAHYYLGQLYRRGYLGNVDPQKAVDHLLSAARGGQLSA
DYALAQLFSEGHGIRQDLVNAWVFSQLAQANPSDQSRELAAQLDQQLTPAQKAQAERL
LQQERQARGAIGQGAGALALQAVQQPVEGEDSL
```

A multiple-entry FASTA file to be used as input for an architecture search can easily be made in any text editor, such as

Notepad (Windows) TextPad (Windows), Notepad++ (Windows), TextEdit (Mac OS X), Aquamacs (Mac OS X), Kate (Linux) or gedit (Linux). Every FASTA entry should start with a line containing the ">" sign, followed by the description. On the line or lines below that, you can then paste the amino acid sequence of your gene of interest. Protein sequences can easily be found by searching them on the NCBI Protein portal and then clicking the 'FASTA' link below the entry description. When you finished creating your FASTA input file, you save it as file type "All Files (*.*)" with the extension '.fasta'. Hence, your file name should be called something like 'yourfastafile.fasta'.

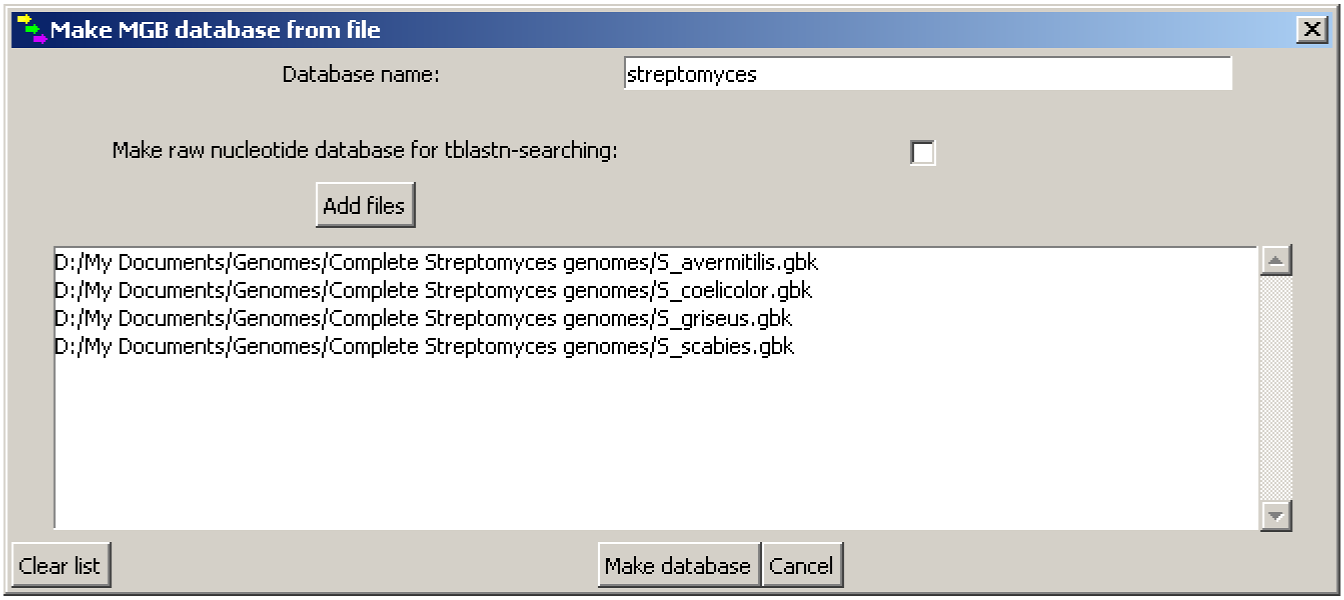
Preparing your search databases

Several options are available for downloading or creating MultiGeneBlast databases. First of all, a MultiGeneBlast database with all entries from GenBank is available for download using the gui or online. Secondly, you can create a MultiGeneBlast database from local files on your computer. Thirdly, you can design a database from multiple GenBank entries by searching for them on the NCBI server, after which MultiGeneBlast downloads them and creates a database. Finally, a database can be created by specifying a subset of GenBank divisions, after which MultiGeneBlast connects to the NCBI FTP server, downloads these divisions and constructs a MultiGeneBlast database out of them.

Downloading the general MultiGeneBlast GenBank database

For convenience, a MultiGeneBlast database containing all amino acid sequences from GenBank is available for download. This can be downloaded simply by clicking 'Download MGB Genbank database' in the 'Download' menu. Naturally, using this database has the advantage of enabling an exhaustive search of the entire available sequence space, yet it has the disadvantage that running it takes a long time and needs quite a lot of system memory.

Creating a search database from local EMBL / GenBank files



Make MGB database from file

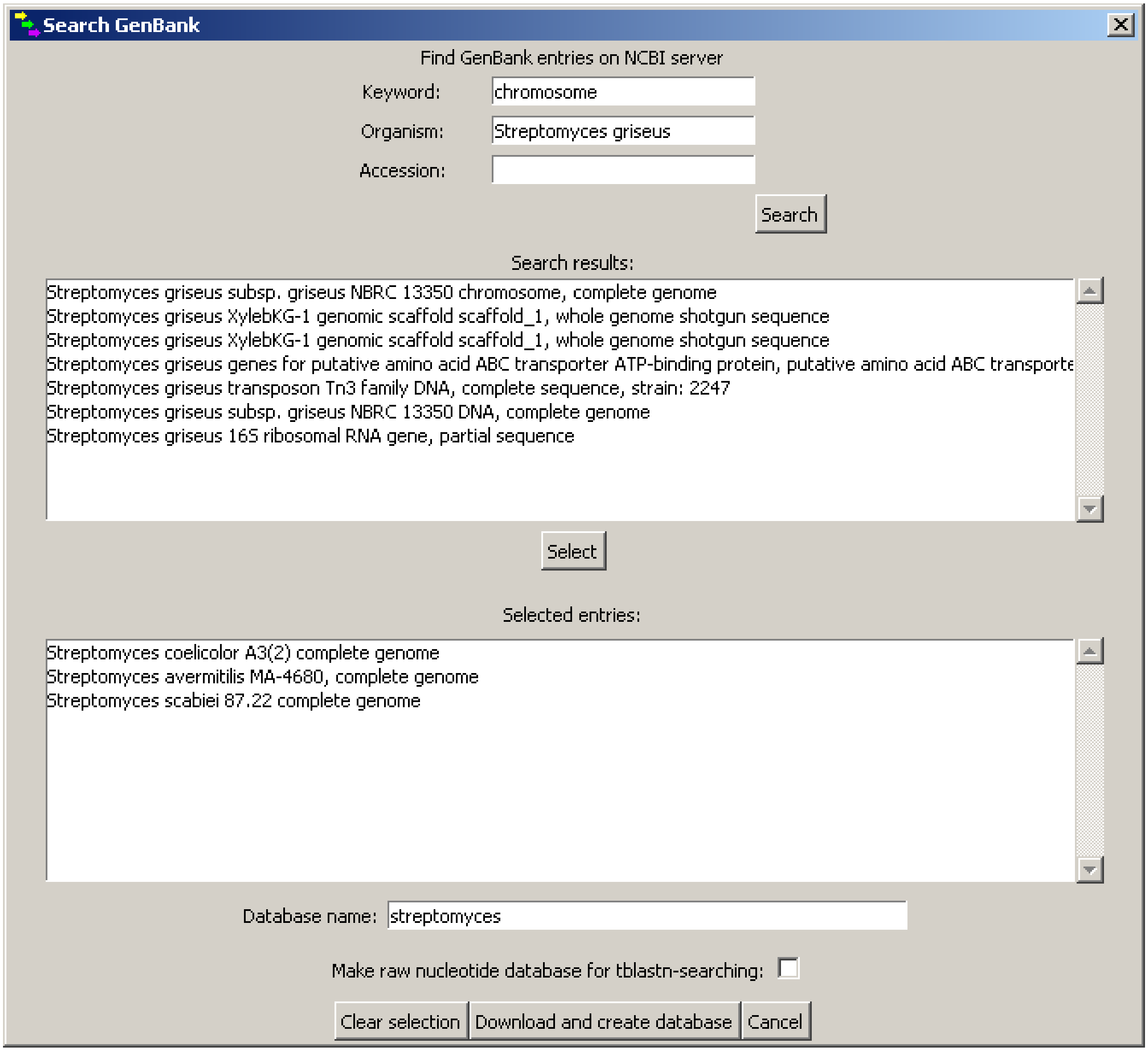
Database name: streptomyces

Make raw nucleotide database for tblastn-searching: □

D:/My Documents/Genomes/Complete Streptomyces genomes/S_avermitilis.gbk
D:/My Documents/Genomes/Complete Streptomyces genomes/S_coelicolor.gbk
D:/My Documents/Genomes/Complete Streptomyces genomes/S_griseus.gbk
D:/My Documents/Genomes/Complete Streptomyces genomes/S_scabies.gbk

If you have your own sequence data in which you want to search for certain operons or gene clusters, you can easily make a database out of your own sequence files. To do so, click 'Create database from files' in the 'Database' menu. In the window that then appears, you can click the ‘Add files’ button to select files to add to your selection. Once you are satisfied with your selection, click the ‘Make database’ button to create your database. If you want to use the tblastn algorithm directly on raw nucleotide sequences in your data instead of using the blastp algorithm to search for homology in annotated genes, you can tick the tick box at the bottom: ‘Make raw nucleotide database for tblastn-searching’. This will also allow you to add raw nucleotide FASTA sequences. The same option is also available in the other database construction windows.

Creating a search database from GenBank entries on the NCBI server



Find GenBank entries on NCBI server

Keyword: chromosome

Organism: Streptomyces griseus

Accession:

Search results:

Streptomyces griseus subsp. griseus NBRC 13350 chromosome, complete genome
Streptomyces griseus XylebKG-1 genomic scaffold scaffold_1, whole genome shotgun sequence
Streptomyces griseus XylebKG-1 genomic scaffold scaffold_1, whole genome shotgun sequence
Streptomyces griseus genes for putative amino acid ABC transporter ATP-binding protein, putative amino acid ABC transporte
Streptomyces griseus transposon Tn3 family DNA, complete sequence, strain: 2247
Streptomyces griseus subsp. griseus NBRC 13350 DNA, complete genome
Streptomyces griseus 16S ribosomal RNA gene, partial sequence

Selected entries:

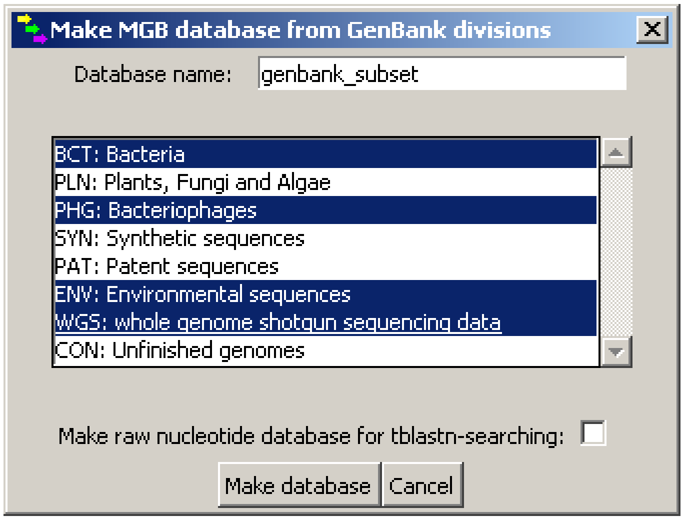
Streptomyces coelicolor A3(2) complete genome
Streptomyces avermitilis MA-4680, complete genome
Streptomyces scabiei 87.22 complete genome

Database name: streptomyces

Make raw nucleotide database for tblastn-searching: □

Often, the GenBank entries from which you would like to design your database will not be present locally on your computer. When clicking ‘Create database from online GenBank entries’ in the ‘Database’ menu, a window will appear in which you can search GenBank entries and move the ones you are interested in to a selection that is displayed at the bottom of the window. You can keep on searching and adding entries until you are satisfied with your selection, after which you can click the ‘Download and create database’ button to make MultiGeneBlast connect to the NCBI server, download the entries and compile them into a database.

Creating a search database from a subset of GenBank divisions



Database name: genbank_subset

- BCT: Bacteria
- PLN: Plants, Fungi and Algae
- PHG: Bacteriophages
- SYN: Synthetic sequences
- PAT: Patent sequences
- ENV: Environmental sequences
- WGS: whole genome shotgun sequencing data
- CON: Unfinished genomes

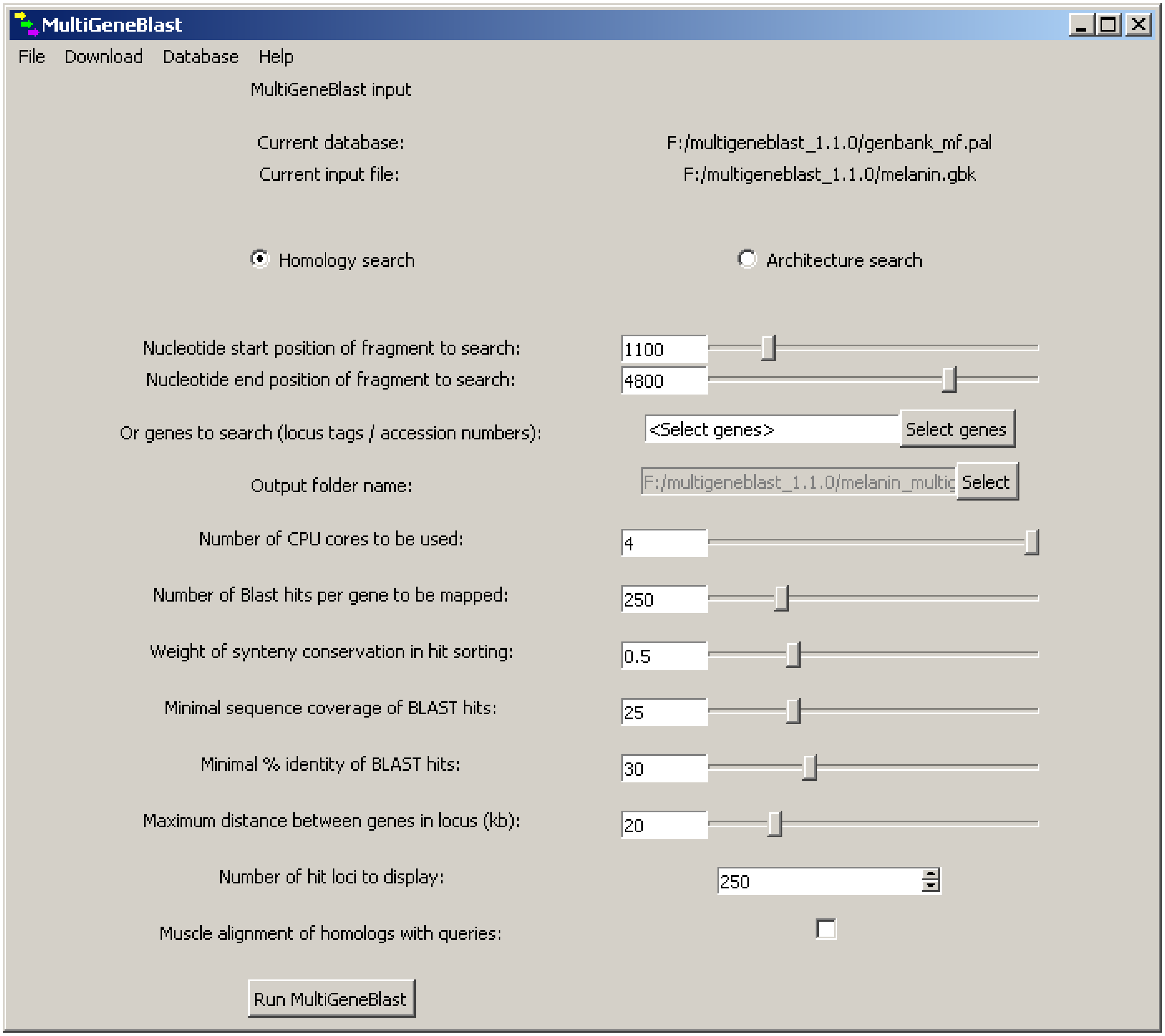
Make raw nucleotide database for tblastn-searching: ☐

In some cases, the pre-constructed GenBank MultiGeneBlast database may not entirely fit your needs, while you do want to search within a relatively global search space. For example, if you work on bacteria, you may have little interest in finding distant homologies with plant genomes. Hence, MultiGeneBlast allows you to create databases from specific subsets of GenBank divisions. When clicking ‘Create database from GenBank subdivisions’, a selection window will appear in which you can select those divisions you are interested in. After clicking ‘Make database’, MultiGeneBlast will connect to the GenBank directory on the NCBI FTP server, download the corresponding files and create a database.

Setting up your MultiGeneBlast run

When you have created or downloaded your database and have prepared your input file, you are ready to start a search. As a start, you have to select what search type you want to perform by clicking the corresponding radio button. Then, you can load the input file and database by using the corresponding options in the ‘File’ menu.

If you are setting up a homology search, this is the time when you select which region from your input file will constitute your search query. You can select the region of choice either using the scale bars for the nucleotide start position and nucleotide end position, or using the gene selection widget below that. When using genomic positions to specify your query region, you can look these up by inspecting the GenBank entry of your input online or by opening the input file in a text editor. Take care to precisely pinpoint the start and end positions, so that your search will be specific and will not consume unnecessary computational resources. When you know the gene names or locus tags covering your intended query region, it is often easier to select your query region by clicking the ‘Select genes’ button. This will open up a window in which you can select the genes of your choice. Holding the shift key while selecting allows you to select a whole array of genes at once, and holding the control key allows you to combine multiple selections. Using the gene selection to specify your search query has the advantage that you can 'skip' genes that are located in the middle of an operon or gene cluster but that are of less interest.



MultiGeneBlast input

Current database: F:/multigeneblast_1.1.0/genbank_mf.pal

Current input file: F:/multigeneblast_1.1.0/melanin.gbk

● Homology search ○ Architecture search

Nucleotide start position of fragment to search: 1100

Nucleotide end position of fragment to search: 4800

Or genes to search (locus tags / accession numbers): <Select genes>

Output folder name: F:/multigeneblast_1.1.0/melanin_multic

Number of CPU cores to be used: 4

Number of Blast hits per gene to be mapped: 250

Weight of synteny conservation in hit sorting: 0.5

Minimal sequence coverage of BLAST hits: 25

Minimal % identity of BLAST hits: 30

Maximum distance between genes in locus (kb): 20

Number of hit loci to display: 250

Muscle alignment of homologs with queries: □

Finally, after entering a name for the output directory in which the results will be stored (within the MultiGeneBlast installation folder), you can change the default of a number of search parameters:

- Number of CPU cores to be used: you can change the number of processor cores of your system that you want to devote to the MultiGeneBlast job. If you still want to use the computer for other things during the run, it is recommended to leave at least one processor free.

- Number of Blast hits per gene to be mapped: you can specify the maximum number of Blast hits per gene that will be mapped to its parent genomic regions. In most cases, 250 is enough. However, if you want to be absolutely certain that your search is exhaustive, you can select 500 or 1000. On the other hand, if you are only interested in the closest homologues, 50 may suffice, reducing the computation time of your run in the process.

- Weight of synteny conservation in hit sorting: factor that determines the weight that synteny conservation has in the sorting of hit gene clusters. A score of 0.5 gives 0.5 points to each pair of adjacent genes with conserved synteny, while the presence of homologues of the genes themselves leads to 1 point per gene.

- Minimal sequence coverage of BLAST hits: with this option, you can specify a threshold for the minimum percentage of the query gene that is involved in a Blast hit for a Blast hit to be taken into account in the analysis.

- Minimal % identity of BLAST hits: with this option, you can specify a threshold for the minimum percentage identity of a BLAST hit for it to be taken into account in the analysis.

- Maximum distance between genes in locus: this allows you to specify how far apart genes with a Blast hit are allowed to be to be counted as a single genomic locus in the results. Set this to a low value if you are looking for tightly transcriptionally coupled operons or set it to a high value if you are looking for general genomic regions.

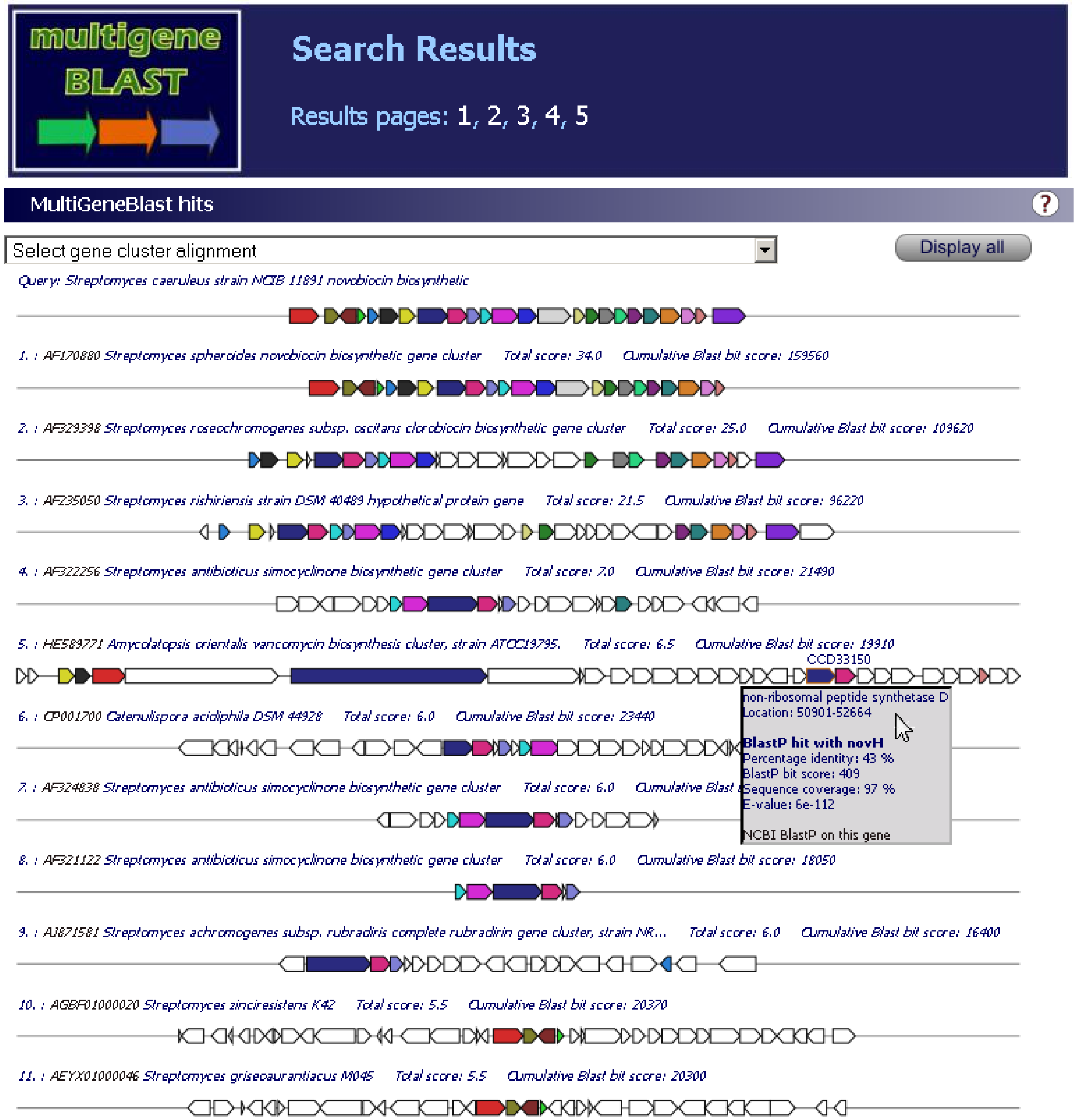
- Number of hit loci to display: this determines the maximum number of output pages (50 hits per page).

- Muscle alignment of homologs with queries: when this box is ticked, Muscle alignments of each query gene with all detected homologs will be generated. This can be a good reference to see the similarity between genes at the sequence level, and can easily be loaded into a tool such as MEGA to generate a phylogenetic tree.

When you are done setting up your run, click ‘Run MultiGeneBlast’ to start the search. A window will appear that will regularly update you on the current status of the search process.

Reading and interpreting the output

When the search has finished, a browser window will open with a visualization of the search results, with a sorted overview of the genomic regions that match best to your query. If no browser window appears, you can also manually open the file ‘displaypage1.xhtml’ in the results folder (within the MultiGeneBlast directory) with your browser.
On the following page, a screenshot of such an XHTML output is shown.



Search Results

Results pages: 1, 2, 3, 4, 5

MultiGeneBlast hits

Query: Streptomyces caeruleus strain NCIB 11891 novobiocin biosynthetic

1. : AF170880 Streptomyces spheroides novobiocin biosynthetic gene cluster Total score: 34.0 Cumulative Blast bit score: 159560

2. : AF329398 Streptomyces roseochromogenes subsp. oscitans clorobiocin biosynthetic gene cluster Total score: 25.0 Cumulative Blast bit score: 109620

3. : AF235050 Streptomyces rishiriensis strain DSM 40489 hypothetical protein gene Total score: 21.5 Cumulative Blast bit score: 96220

4. : AF322256 Streptomyces antibioticus simocyclinone biosynthetic gene cluster Total score: 7.0 Cumulative Blast bit score: 21490

5. : HE589771 Amycolatopsis orientalis vancomycin biosynthesis cluster, strain ATCC19795. Total score: 6.5 Cumulative Blast bit score: 19910

non-ribosomal peptide synthetase D
Location: 50901-52664

BlastP hit with novH
Percentage identity: 43 %
BlastP bit score: 409
Sequence coverage: 97 %
E-value: 6e-112

NCBI BlastP on this gene

6. : CP001700 Catenulispora acidiphila DSM 44928 Total score: 6.0 Cumulative Blast bit score: 23440

7. : AF324838 Streptomyces antibioticus simocyclinone biosynthetic gene cluster Total score: 6.0 Cumulative Blast

8. : AF321122 Streptomyces antibioticus simocyclinone biosynthetic gene cluster Total score: 6.0 Cumulative Blast bit score: 18050

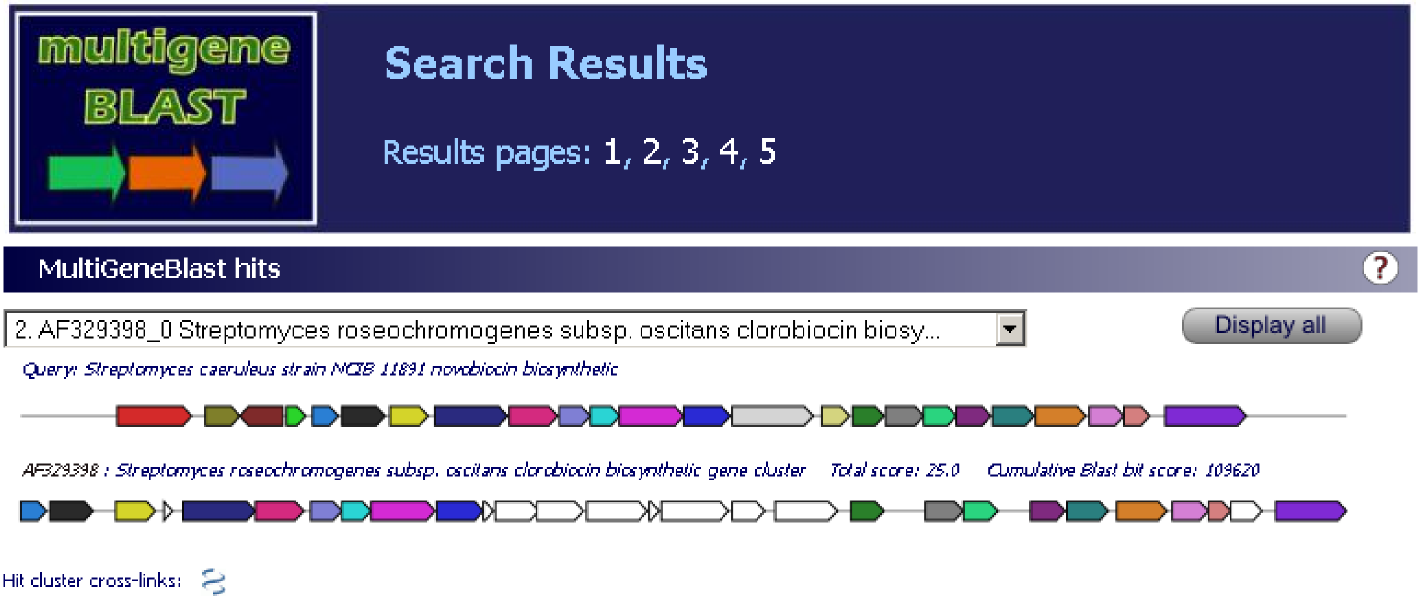
9. : AJ871581 Streptomyces achromogenes subsp. rubradiris complete rubradirin gene cluster, strain NR... Total score: 6.0 Cumulative Blast bit score: 16400

10. : AGBF01000020 Streptomyces zincirestistens K42 Total score: 5.5 Cumulative Blast bit score: 20370

11. : AEYX01000046 Streptomyces griseoaurantiacus M045 Total score: 5.5 Cumulative Blast bit score: 20300

For every hit, the output will display the total MultiGeneBlast score (composed of the number of BLAST hits matching the tresholds set and the number of contiguous gene pairs with conserved synteny) as well as the cumulative BLAST bit score. The colors of the gene arrows represent Blast homology. If multiple query genes are homologous to each other, they are grouped together with their hit genes into a single homology group with the same color. The white gene arrows represent (surrounding) genes without Blast hits to the query.

When clicking a gene, a panel will pop up that gives the annotation of the gene, its location and the details of any Blast hits on it by the query genes. Also, a link is given that opens a new browser tab to input this gene as a query in the search form for a single gene BlastP search from the NCBI server.



Search Results

Results pages: 1, 2, 3, 4, 5

MultiGeneBlast hits

2. AF329398_0 Streptomyces roseochromogenes subsp. oscitans clorobiocin biosy...

Query: Streptomyces caeruleus strain NCIB 11891 novobiocin biosynthetic

AF329398 : Streptomyces roseochromogenes subsp. oscitans clorobiocin biosynthetic gene cluster Total score: 25.0 Cumulative Blast bit score: 109620

Hit cluster cross-links:

To have a closer look at the similarities between the query gene cluster and a hit gene cluster, the selection box at the top can be used. When a gene cluster is selected, a zoomed in SVG of the query gene cluster together with this gene cluster is displayed in the browser window. To go back to the overall search results, click the ‘Display all’ button on the right. To navigate between results pages with more results, the links at the top of the output window can be used.

Using MultiGeneBlast from the command line (Expert users)

Using MultiGeneBlast from the command line follows all the same principles that were outlined above. The MultiGeneBlast executable (multigeneblast.exe on Windows or multigeneblast.py on Linux and Mac OS X) within the MultiGeneBlast directory is used to perform the searches. The executable must always be executed from the MultiGeneBlast directory itself. A list of the search parameters (as explained above) and an explanatio of how to use them can easily be obtained by typing ‘multigeneblast -h’ (Windows) or ‘./multigeneblast.py -h’ (Linux / Mac OS X) in the command prompt or terminal from within the MultiGeneBlast directory. Databases can be created from local files using ‘makedb.exe’ or ‘makedb.py’ in the ‘makedb’ subdirectory, and from GenBank divisions using ‘makegbdb.exe’ or ‘makegbdb.py’ within this same directory. For those who wish to use MultiGeneBlast as a step within a larger pipeline, the TXT output ‘clusterblast_output.txt’ in the results directory can be used for easy parsing.