

Making Request to Database

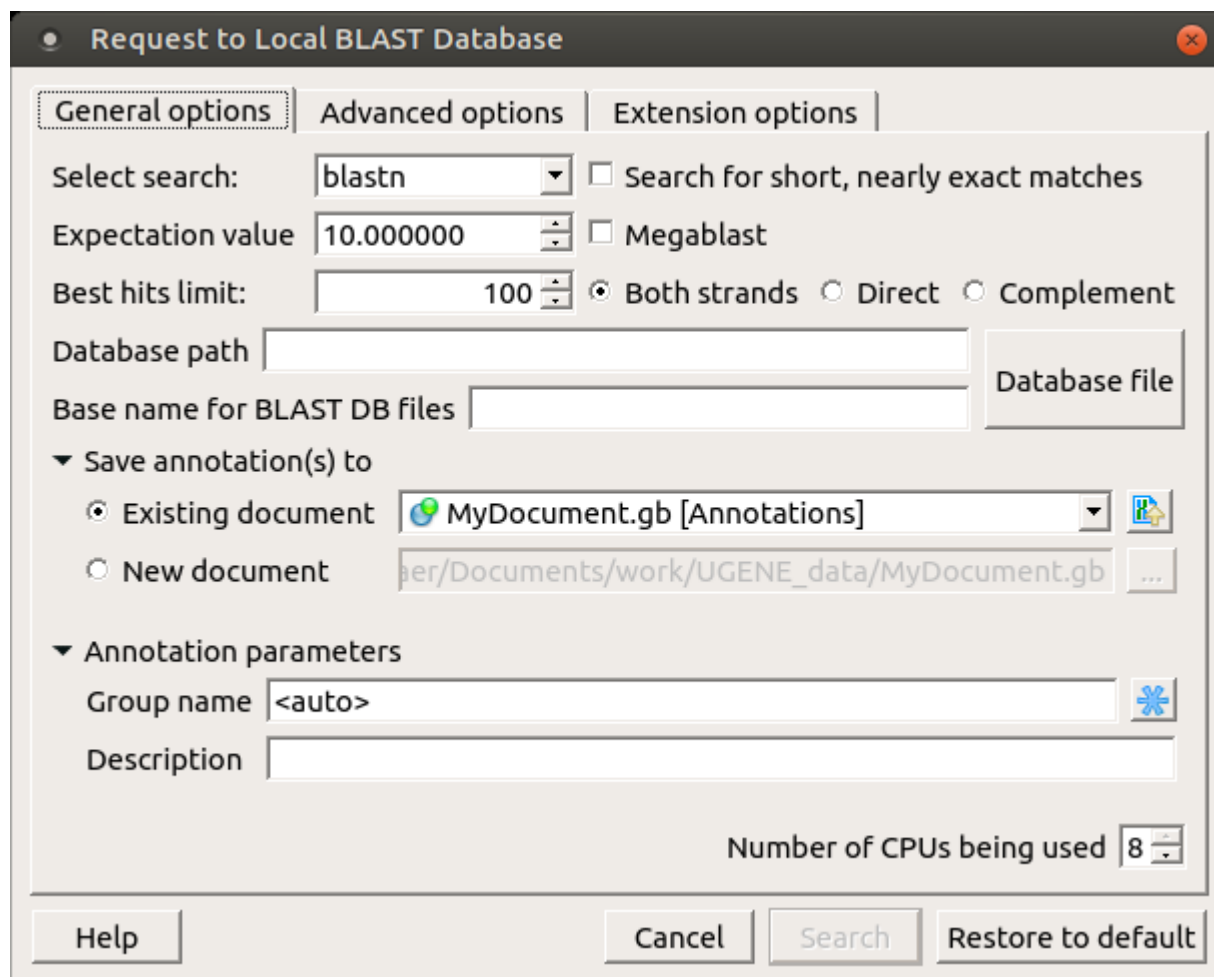
To make a request to a local BLAST database do the following:

- If you're using *BLAST* open *Tools BLAST BLAST Search*.
- If you're using *BLAST+* open *Open Tools BLAST BLAST+ Search*.

If there is a sequence opened you can also initiate the request to a local BLAST database from the *Sequence View*:

- If you're using *BLAST* select the *Analyze Query with BLAST* item in the context menu or in the *Actions* main menu.
- If you're using *BLAST+* select the *Analyze Query with BLAST+* item in the context menu or in the *Actions* main menu.

The *Request to local BLAST database* dialog will appear:



The dialog box titled "Request to Local BLAST Database" features three tabs: "General options", "Advanced options", and "Extension options". The "General options" tab is active, showing fields for "Select search:" (a dropdown menu with "blastn" selected), "Expectation value" (a text box with "10.000000"), and "Best hits limit:" (a text box with "100"). To the right of these fields are checkboxes for "Search for short, nearly exact matches" and "Megablast", and radio buttons for "Both strands" (selected), "Direct", and "Complement". Below these are text boxes for "Database path" and "Base name for BLAST DB files", followed by a "Database file" button. A section titled "Save annotation(s) to" contains radio buttons for "Existing document" (selected) and "New document". The "Existing document" option is linked to a dropdown menu showing "MyDocument.gb [Annotations]" and a file icon button. The "New document" option is linked to a text box showing a file path and a file icon button. Below this is a section titled "Annotation parameters" with a "Group name" text box containing "<auto>" and a "Description" text box. At the bottom right, there is a "Number of CPUs being used" text box with the value "8". At the bottom of the dialog are four buttons: "Help", "Cancel", "Search", and "Restore to default".

The following general options are available:

Select search - here you should select the tool you would like to use. If the query sequence is a nucleotide sequence then *blastn*, *blastx* and *tblastx* items are available. For a protein sequence the items are *blastp* and *tblastn*.

Expectation value - this option specifies the statistical significance threshold for reporting matches against database sequences. Lower expectation thresholds are more stringent, leading to fewer chance matches being reported.

Culling limit - the maximum number of hits that will be shown (not equal to number of annotations). The maximum available number is 5000.

Search for short, nearly exact matches - automatically adjusts the word size and other parameters to improve results for short queries.

Megablast - select this option to compare query with closely related sequences. It works best if the target percent identity is 95% or more, but it is very fast.

Database path - path to the database files.

Base name for BLAST DB files - base name for the BLAST database files.

You can see the description of the annotation saving parameters [here](#).

The following advanced parameters are available:

Request to Local BLAST Database

General options | **Advanced options** | Extension options

Word size: 11

Gap costs: 2 2

Match scores: 1 -3

Filters

- ☐ Low complexity filter
- ☐ Human repeats filter

Masks

- ☐ Mask for lookup table only
- ☐ Mask lower case letters

Help Cancel Search Restore to default

Word size - the size of the subsequence parameter for the initiated search.

Gap costs - costs to create and extend a gap in an alignment. Increasing the Gap costs will result in alignments which decrease the number of Gaps introduced.

Match scores - reward and penalty for matching and mismatching bases.

Filters - filters for regions of low compositional complexity and repeat elements of the human's genome.

Masks for lookup table only — this option masks only for purposes of constructing the lookup table used by BLAST so that no hits are found based upon low-complexity sequence or repeats (if repeat filter is checked).

Mask lower case letters — with this option selected you can cut and paste a FASTA sequence in upper case characters and denote areas you would like filtered with lower case.

The view of the *Advanced options* tab depends on the selected search. For the *blastn* search it looks like on the picture above. When the *blastx* search is selected in the general options, the view of the *Advanced options* tab is the following:

Request to Local BLAST Database

General options | Advanced options | Extension options

Word size: 3

Threshold: 12.00

Matrix: BLOSUM62

Gap costs: 11 1

Service: plain

Filters

- ☐ Low complexity filter
- ☐ Human repeats filter

Masks

- ☐ Mask for lookup table only
- ☐ Mask lower case letters

Help Cancel Search Restore to default

As you can see there is no *Match scores* option, but there are *Threshold*, *Matrix*, *Composition-based statistics* and *Service* options.

Threshold - threshold for extending hits.

Matrix — key element in evaluating the quality of a pair-wise sequence alignment is the "substitution matrix", which assigns a score for aligning any possible pair of residues.

Service — blastp service which needs to be performed: plain, psi or phi.

Composition-based statistics - composition-based statistics.

When the *tblastx* search is selected in the general options, the view of the *Advanced options* tab is the following:

Request to Local BLAST Database

General options | Advanced options | Extension options

Word size

Threshold

Matrix Service

Filters

- ☐ Low complexity filter
- ☐ Human repeats filter

Masks

- ☐ Mask for lookup table only
- ☐ Mask lower case letters

Help Cancel Search Restore to default

The following extension options are available:

Request to Local BLAST Database

General options | Advanced options | Extension options

X dropoff value (in bits)

For gapped alignment	0
For ungapped extensions	7.00
For final gapped alignment	0

Multiple Hits Window Size

40

☒ Perform gapped alignment

Help Cancel Search Restore to default

For gapped alignment - X dropoff value (in bits) for gapped alignment.

For ungapped alignment - X dropoff value (in bits) for ungapped alignment.

For final gapped alignment - X dropoff value (in bits) for final gapped alignment.

Multiple hits window size - multiple hits window size.

Perform gapped alignment - performs gapped alignment.