

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:

Request to Local BLAST Database

General options | Advanced options | Extension options

Select search: ☐ Search for short, nearly exact matches

Expectation value ☐ Megablast

Best hits limit: ☒ Both strands ☐ Direct ☐ Complement

Database path

Base name for BLAST DB files

▼ Save annotation(s) to

☒ Existing document

☐ New document

▼ Annotation parameters

Group name

Description

Number of CPUs being used

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.

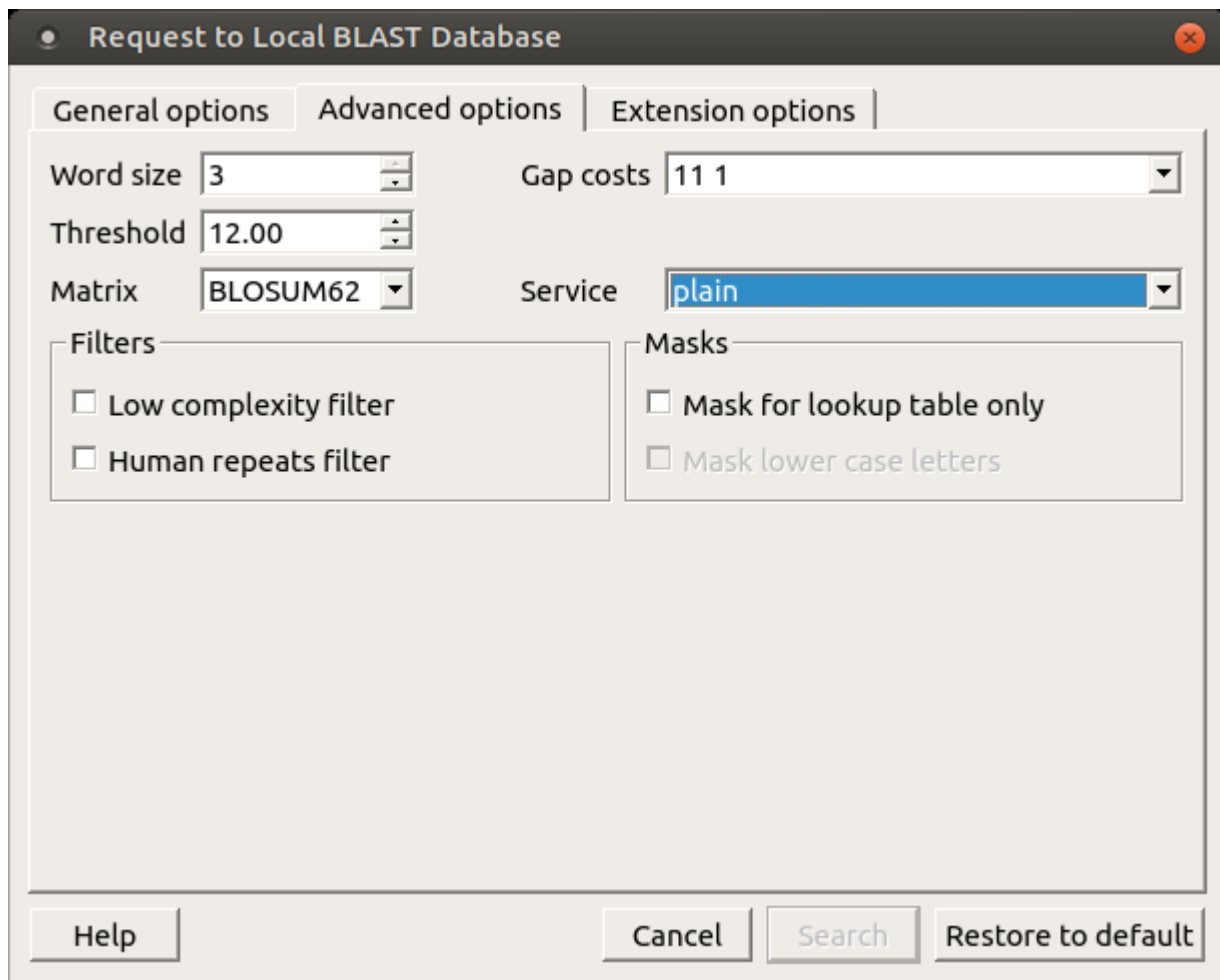
Match scores - reward and penalty for matching and mismatching bases.

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:



The image shows a software dialog box titled "Request to Local BLAST Database". It has three tabs: "General options", "Advanced options", and "Extension options". The "General options" tab is active. It contains several settings: "Word size" is set to 3, "Threshold" is 12.00, "Matrix" is BLOSUM62, "Gap costs" are 11 1, and "Service" is plain. There are two sections, "Filters" and "Masks", each with two checkboxes. In the "Filters" section, "Low complexity filter" and "Human repeats filter" are both unchecked. In the "Masks" section, "Mask for lookup table only" and "Mask lower case letters" are both unchecked. At the bottom of the dialog are four buttons: "Help", "Cancel", "Search", and "Restore to default".

Request to Local BLAST Database

General options | Advanced options | Extension options

Word size 3 Gap costs 11 1

Threshold 12.00

Matrix BLOSUM62 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.