Making Request to Database

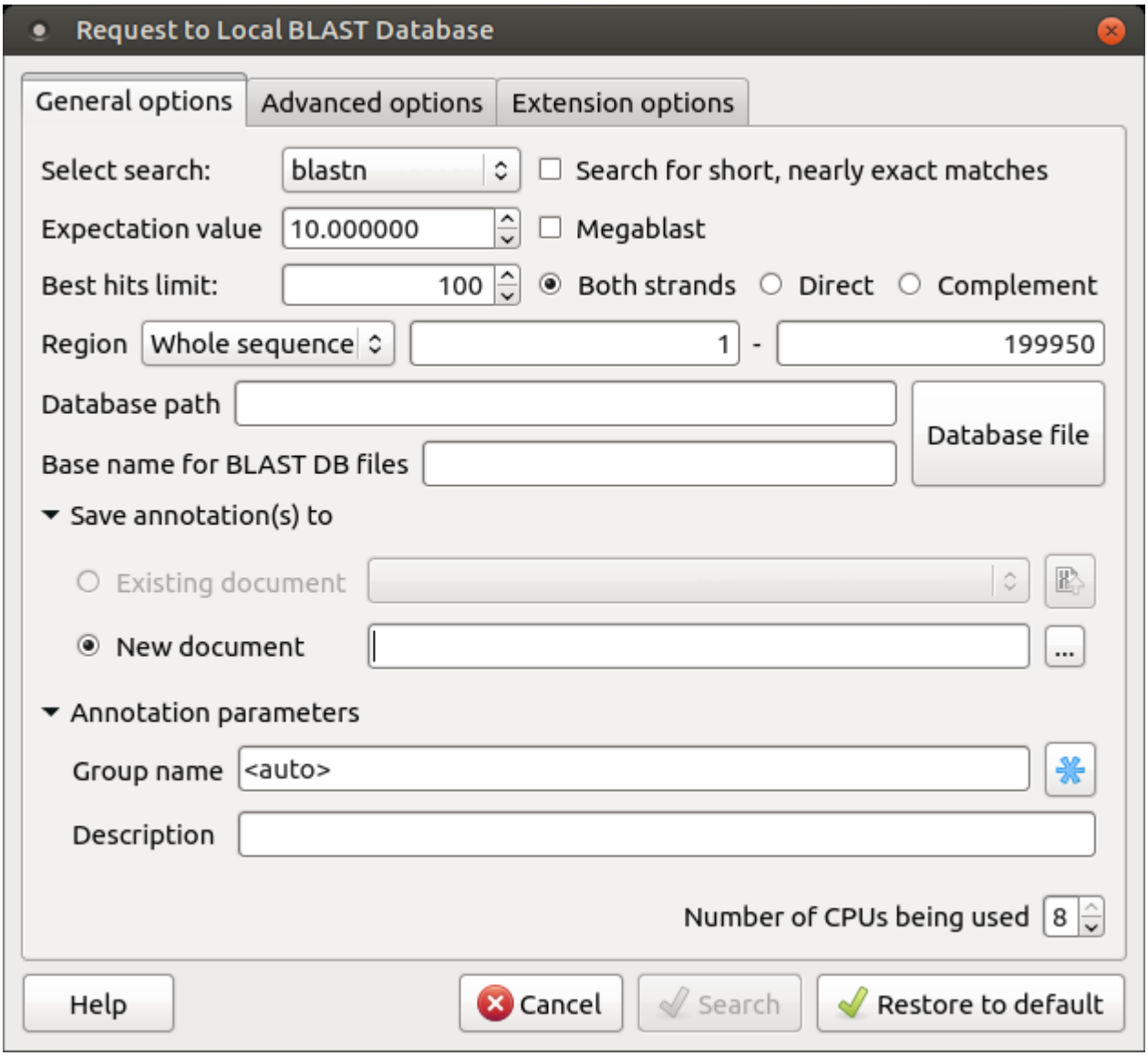
To make a request to a local BLAST database do the following:

- 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*:

- Select the *Analyze Query with BLAST+* item in the context menu or in the *Actions* the main menu.

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



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* it ems 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 expect 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 a 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.

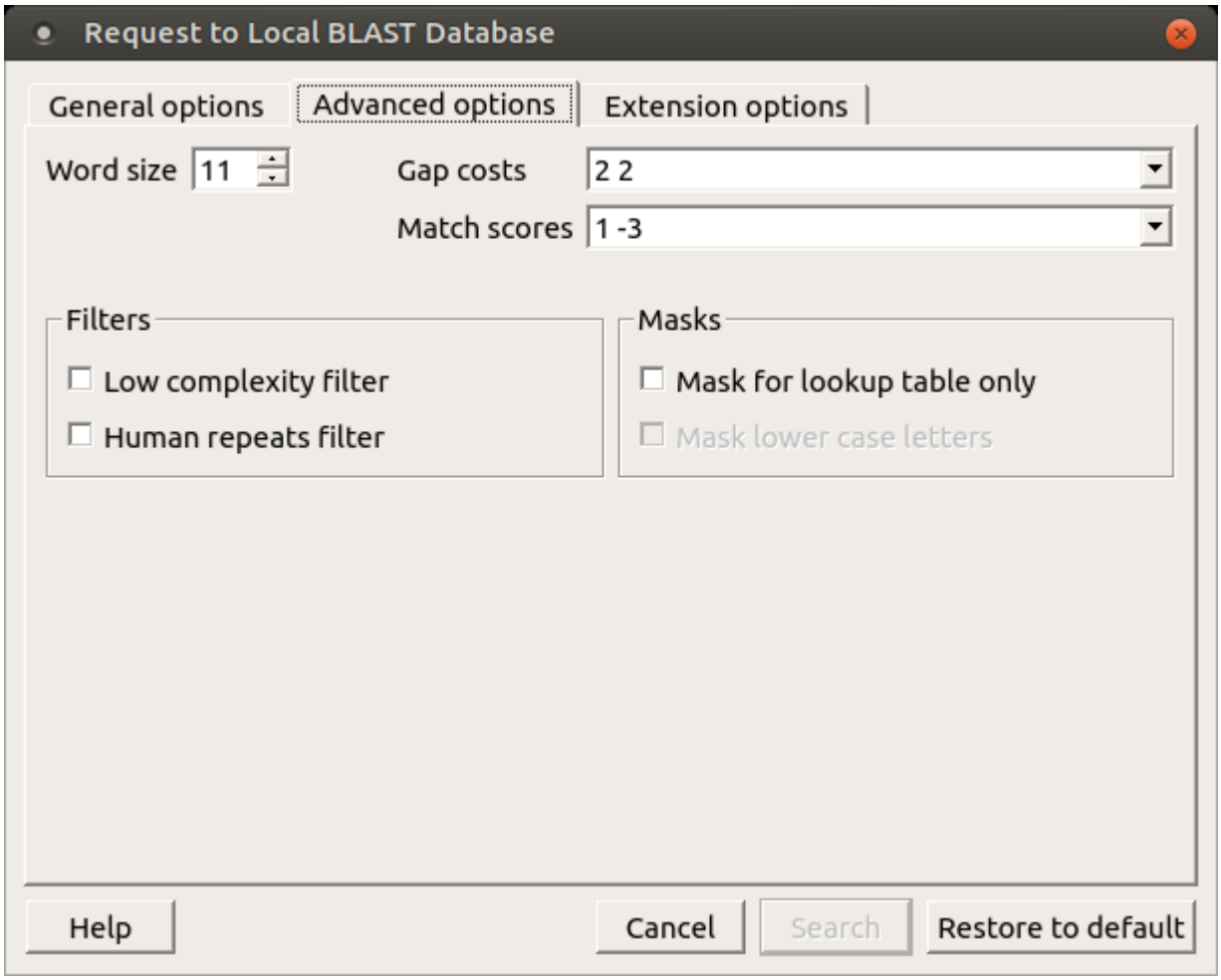
Megablast - select this option to compare queries with closely related sequences. It works best if the target percent identity is 95% or more, but it is very fast.

Database path - path to the database files.

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

You can see the description of the annotation saving parameters *here*.

The following advanced parameters are available:



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.

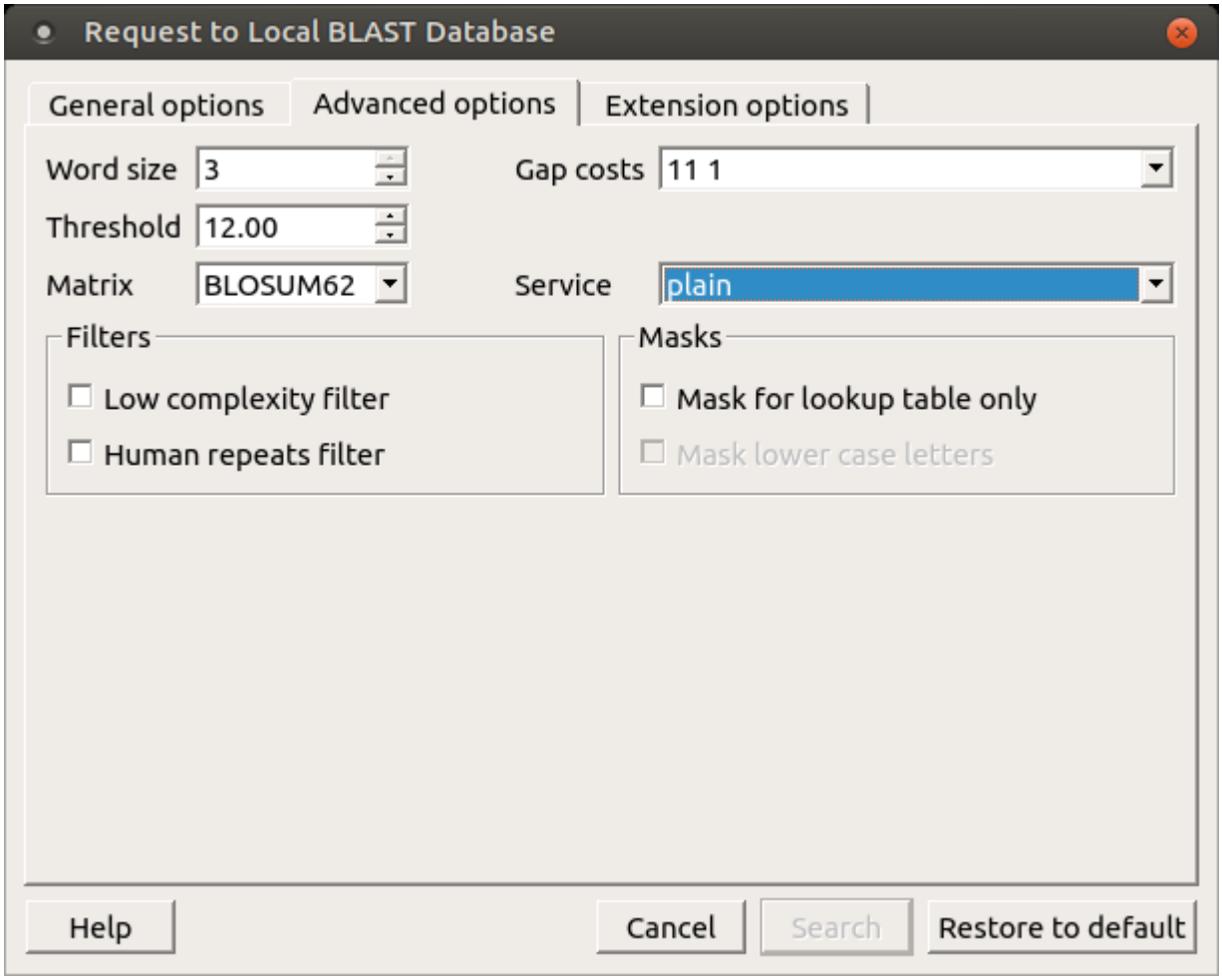
Match scores - reward and penalty for matching and mismatching bases.

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 in the picture above. When the *blastx* search is selected in the general options, the view of the *Advanced options* tab is the following:



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

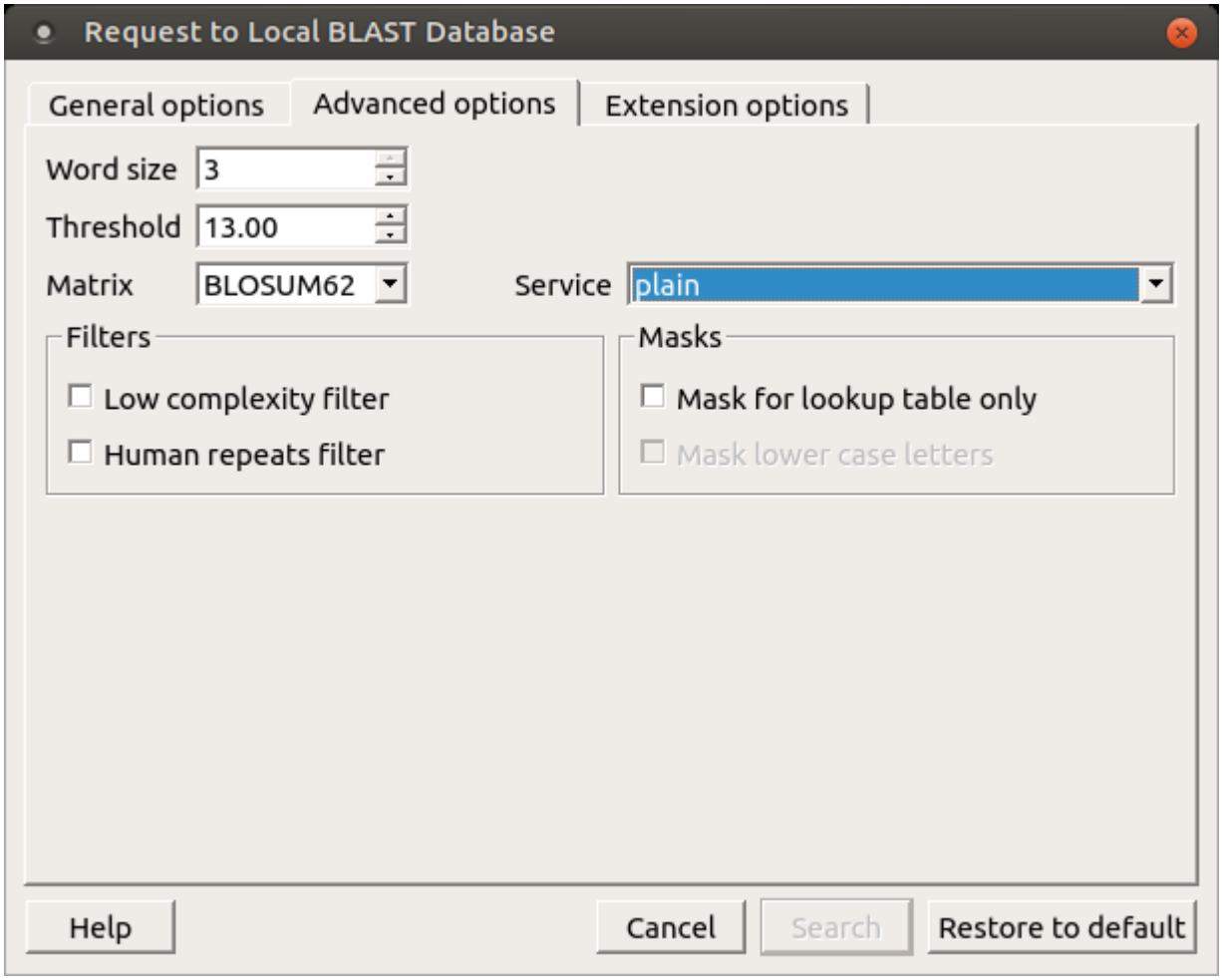
Threshold - threshold for extending hits.

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

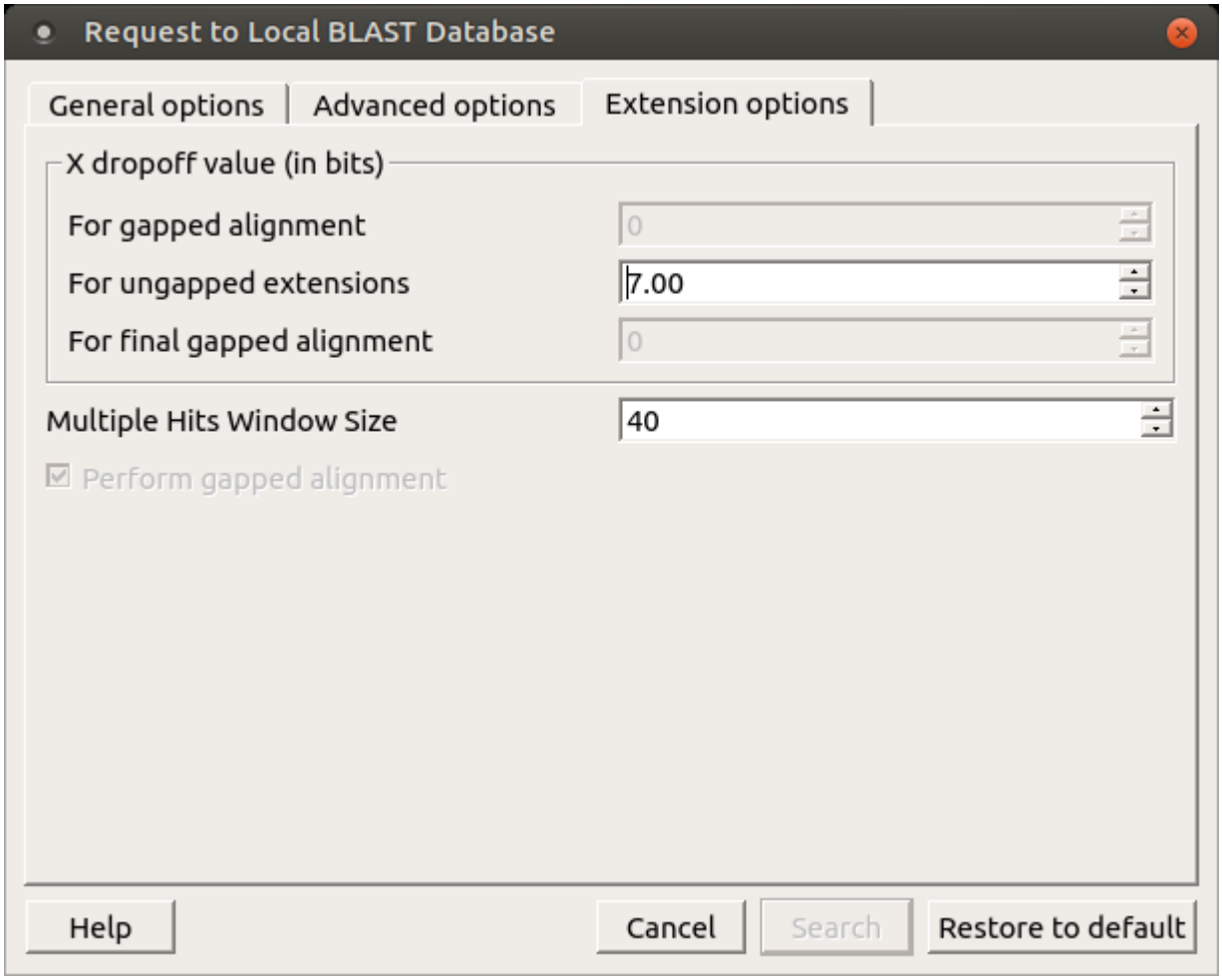
Service — blastp service which needs to be performed: plain, psi or phi.

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:



The following extension options are available:



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.