



Unipro UGENE Manual

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Unipro UGENE Online User Manual

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About Unipro

Established in 1992 Unipro company has its headquarters located in Novosibirsk Akademgorodok (the home of Siberian Branch of Russian Academy of Sciences). The company's primary activity is IT outsourcing solutions. To learn more about the company, please, visit the [company website](#).

About UGENE

Unipro UGENE is a free cross-platform genome analysis suite. It is distributed under the terms of the [GNU General Public License](#).

To learn more about UGENE visit [UGENE website](#).

It works on Windows, macOS or Linux and requires only a few clicks to install.

- [Key Features](#)
- [User Interface](#)
- [High Performance Computing](#)
- [Cooperation](#)

Key Features

- Creating, editing and annotating **nucleic acid** and **protein** sequences
- Search through online databases: **NCBI**, **ENSEMBL**, **PDB**, **SWISS-PROT**, **UniProtKB/Swiss-Prot**, **UniProtKB/TrEMBL**, **UniProt(DAS)**, **Ensembl Human Genes (DAS)**
- Multiple sequence alignment: **ClustalW**, **ClustalO**, **MUSCLE**, **Kalign**, **MAFFT**, **T-Coffee**
- Online and local **BLAST** and **BLAST+** search
- Restriction analysis with integrated **REBASE** restriction enzyme database
- Integrated **Primer3** package for **PCR** primers design
- Search for direct, inverted and **tandem repeats** in DNA sequences
- Constructing **dotplots** for nucleic acid sequences
- Search for transcription factor binding sites (**TFBS**) with **weight matrix** and **SITECON** algorithms
- Aligning short reads with **Bowtie**, **Bowtie 2**, **BWA**, **BWA-SW** and **UGENE Genome Aligner**
- Contig assembly with **CAP3**
- Search for **ORFs**
- **Cloning in silico**
- **3D structure viewer** for files in **PDB** and **MMDB** formats, anaglyph view support
- Protein secondary structure prediction with **GOR IV** and **PSIPRED** algorithms
- **HMMER2** and **HMMER3** packages integration
- Building (using integrated **PHYLIP** and **MrBayes** packages) and viewing **phylogenetic trees**
- Local sequence alignment with optimized **Smith-Waterman** algorithm
- Combining various algorithms into custom workflows with **UGENE Workflow Designer**
- Search for a pattern of various algorithms' results in a nucleic acid sequence with **UGENE Query Designer**
- Visualization of **next generation sequencing data (BAM files)** using **UGENE Assembly Browser**
- PCR in silico
- Spade de novo assembler

User Interface

- Visual and interactive genome browsing including circular plasmid view
- Multiple alignment editor
- Chromatograms visualization
- 3D viewer for files in PDB and MMDB formats with anaglyph stereo mode support
- Phylogenetic tree viewer
- Easy to use Workflow Designer for custom computational workflows
- Easy to use Query Designer for analyze a nucleotide sequence using different algorithms at the same time
- Assembly Browser for visualize and efficiently browsing large next generation sequence assemblies

High Performance Computing

- Complete support of modern multicore processors and SSE instructions
- Out of the box support of modern GPUs using NVIDIA CUDA and ATI Stream
- Integrated solutions for Cell Broadband Engine

Cooperation

- Can be used for education purposes in schools and universities
- Features to be included into the next release are initiated by users
- UGENE team is ready for collaboration in related projects, both free and commercial

Download and Installation

UGENE is compatible with the three most common operating systems: Windows, macOS, and Linux. It has some [minimum system requirements](#). If your system fits these requirements, you're welcome to download UGENE from <http://ugene.net/download>. The program can be used and distributed under the terms of [GPLv2](#).

Follow [these recommendation](#) to choose which UGENE package to download.

Below you can also find links to the guides on UGENE installation on different operating systems.

- [System Requirements](#)
- [UGENE Packages](#)
- [Installation on Windows](#)
- [Installation on macOS](#)
- [Installation on Linux](#)
 - [Native Installation on Ubuntu](#)
 - [Native Installation on Fedora](#)
- [Data Analysis Tools](#)
- [Reference Data for NGS](#)
 - [Configure Data for Metagenomics Classification](#)
 - [Configure ChIP-Seq Analysis Data](#)

System Requirements

- **Operating system (64-bit or 32-bit):**
 - Windows 7+
 - macOS 10.7+
 - Linux
- **RAM:**
 - 512 Mb RAM is required.
 - At least 2 Gb RAM is recommended.
- **Disk space:**

The minimum required disk space depends on the [UGENE package](#):

 - Standard package: 200-300 Mb
 - Online installer (with default settings), Full package: 500-900 Mb
 - NGS package (with ChIP-Seq data): 21-24 Gb
 - UGENE with all components installed (data for metagenomics classification and ChIP-Seq): 250Gb
- **Display:**
 - It is recommended to set the screen resolution to 1280 x 720 or greater.
- **Internet:**
 - Internet connection is required for some tasks like loading data from online databases.



UGENE takes care to use the capabilities of your system: the more RAM and cores you have, the more quickly you'll get results of your calculations.

Also, if you have an OpenCL-capable video card, you can use GPU-optimized versions of the following tools:


- [Smith-Waterman Search](#)
- [UGENE Genome Aligner](#)

UGENE Packages

The following package types are currently available:

- **Online Installer:** if the Internet connection is available on the target computer, it is recommended to use this type of package. The installation wizard allows one to add required components or update them, when a new UGENE version is released.
- **Standard Package:** the package includes UGENE main executables, [embedded tools](#), sample data, etc.
- **Full Package:** the same as in the Standard Package + [external tools](#) (including tools for NGS data analysis) – this package is recommended for offline installation.
- **NGS Package:** the same as in the Full Package + ChIP-Seq data analysis additions (RScript, reference data)

Also, different package delivery types are available depending on the operating system.

 Note that the number of package types will be decreased in the next releases.

Installation on Windows

The following UGENE package delivery types are available on Windows:

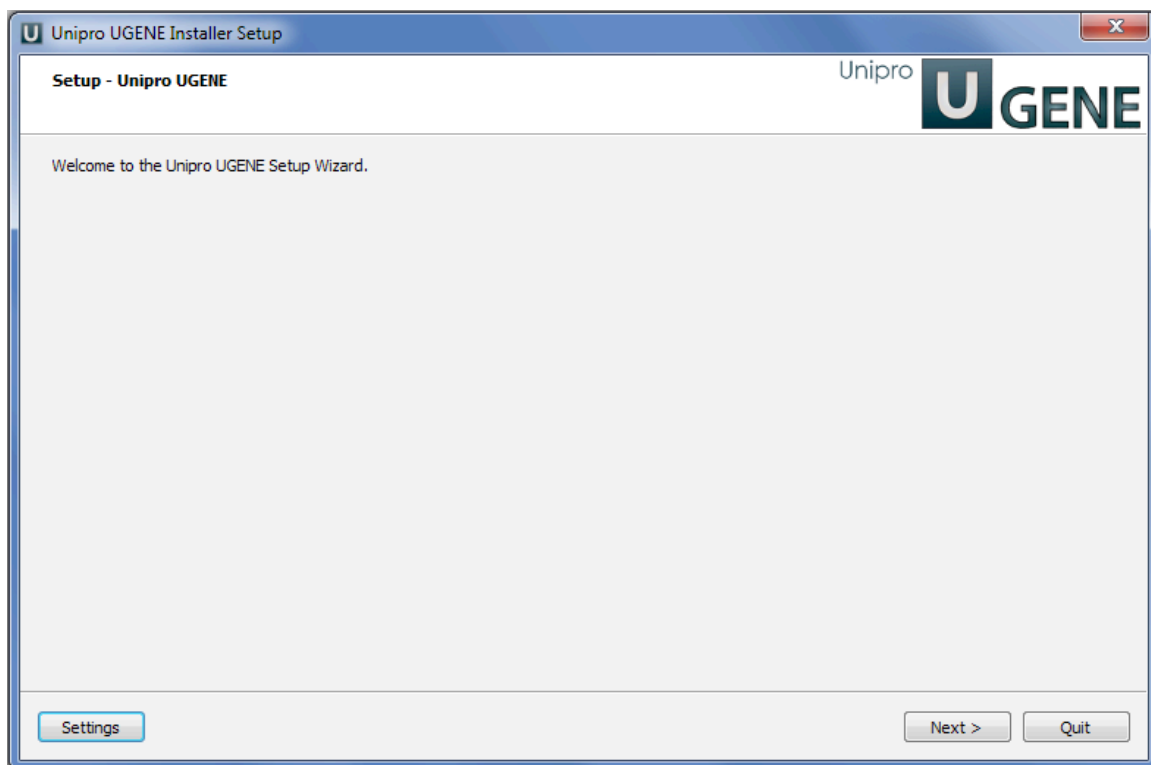
- **Online installer:** recommended, provides closer integration with the operating system, requires administrative rights, updates to new versions are supported
- **Offline installer:** provides closer integration with the operating system, administrative rights are required, does not require Internet connection to be installed
- **Zip archive:** a portable version, does not require administrative rights, do not require Internet connection to be installed

Current UGENE version is available for Windows 7 and higher. The online installer is universal for 64-bit and 32-bit platforms. For other types be careful to select an appropriate link on the "[Download UGENE and components](#)" page.

For obsolete Windows versions (XP, Vista) an old UGENE version 1.29 is only available.

Installation using online installer

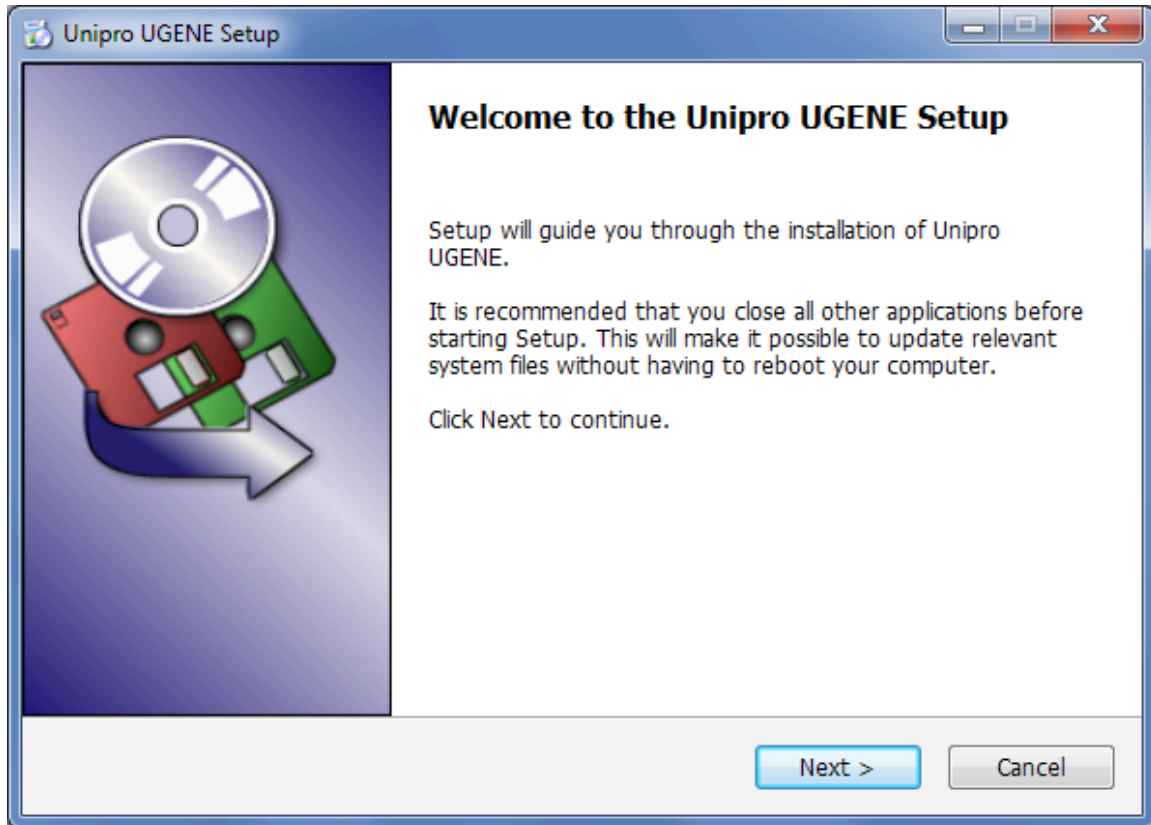
- Download the online installer executable file.
- When installing the package, make sure the Internet connection is available.
- Run the executable file with administrative rights.
- Follow the installation wizard.



On a new UGENE version release, a notification will appear with an option to update the package.

Installation using offline installer

- Download the offline installer executable file for your platform (64-bit or 32-bit). Choose **Standard** or **Full** package.
- Run the executable file with administrative rights.
- Follow the installation wizard.



On a new UGENE version release, a notification will appear. However, to install the new version, it is required to manually download the package again.

Installation using zip archive

- Download the zip archive for your platform (64-bit or 32-bit). Choose [Standard](#), [Full](#), or [NGS package](#) (the NGS package is available for 64-bit platform only).
- Unpack the archive.
- Open the unpacked folder and run "ugeneui.exe" (i.e. UGENE User Interface).

On a new UGENE version release, a notification will appear. However, to install the new version, it is required to manually download the package again.

Installation on macOS

UGENE delivery type on macOS is a DMG installer. There are two options:

- **Online installer:** recommended, updates to new versions are supported
- **Offline installer:** does not require Internet connection to be installed

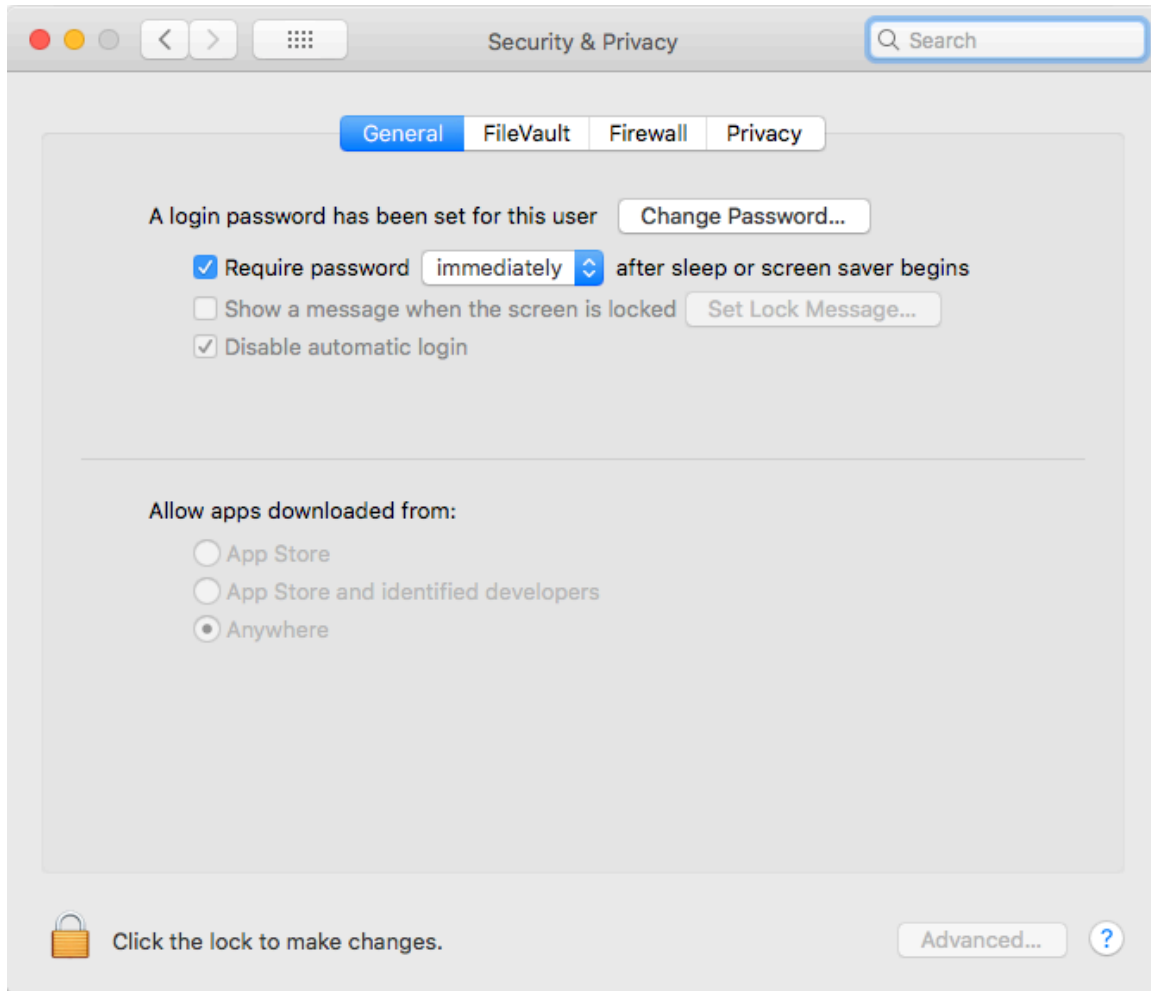
Current UGENE version is available for operating system version 10.7 or higher. Old UGENE packages are also provided for earlier OS versions.

All download links are available on the "[Download UGENE and components](#)" page.

Configure system preferences

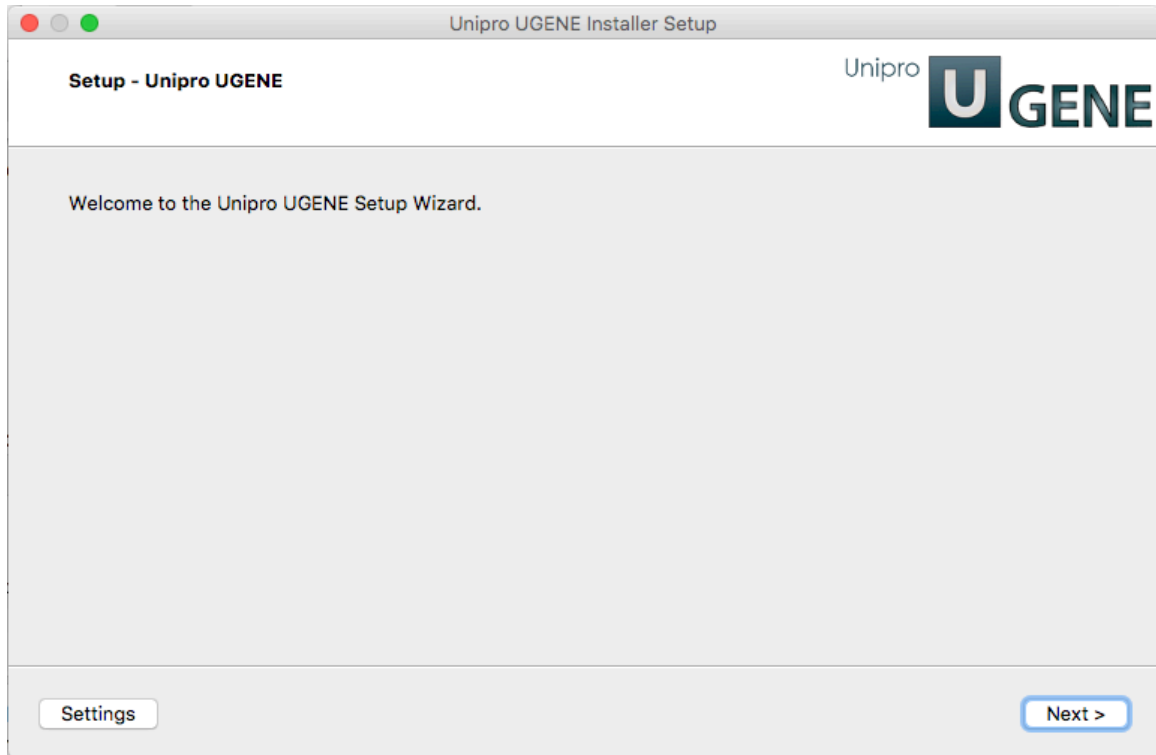
It may be required to configure macOS settings to install UGENE:

- Select "System Preferences -> Security & Privacy".
- Unlock the settings to make changes.
- Under "Allow apps downloaded from" select "Anywhere".



Installation using online installer

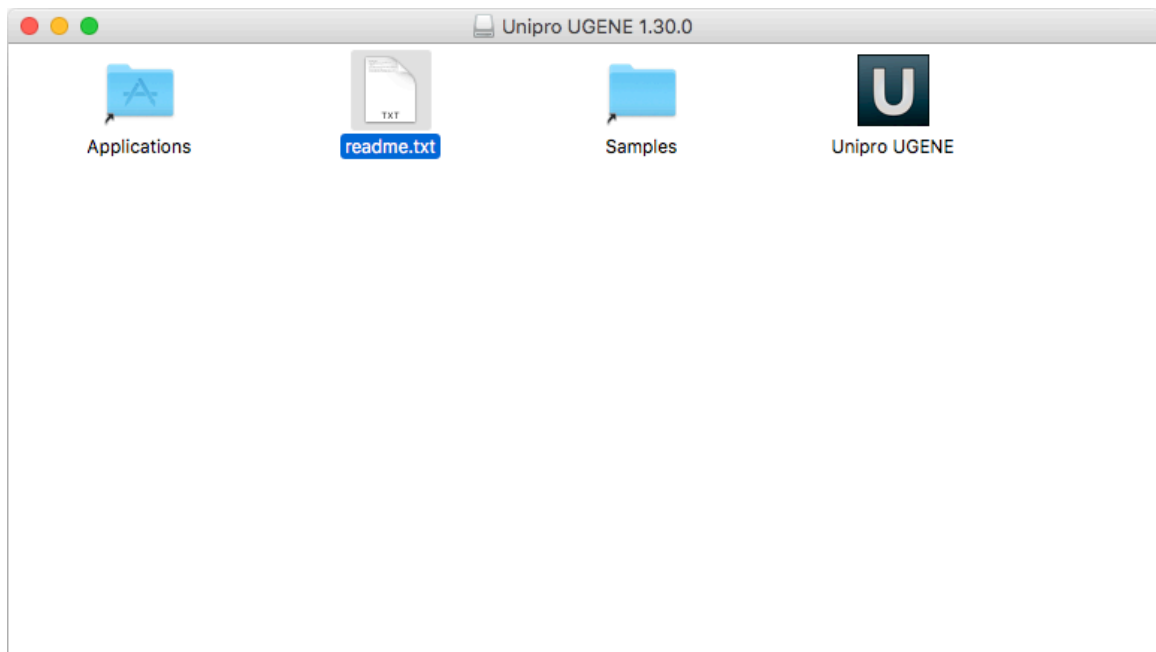
- Download the online installer DMG archive.
- When installing the package, make sure the Internet connection is available.
- Double-click on the archive to unpack it.
- Run the unpacked executable file.
- Follow the installation wizard.



On a new UGENE version release, a notification will appear with an option to update the package.

Installation using offline installer

- Download the offline installer DMG archive. Choose [Standard](#), [Full](#), or [NGS package](#).
- Double-click on the archive.
- UGENE is provided under free GNU GPL v2 license. Accept the license terms to install the package.
- Move "Unipro UGENE" application bundle to the "Applications" folder on macOS or to another appropriate location.



On a new UGENE version release, a notification will appear. However, to install the new version, it is required to manually download the package again.

Installation on Linux

The following UGENE package delivery types are available on Linux:

- **Online installer:** updates to new versions are supported

- **TAR.GZ archive:** does not require Internet connection to be installed
- **Native installation:** available for Ubuntu and Fedora only, provides closer integration with the operating systems, requires administrative rights

Download links for the online installers and TAR.GZ archives are available on the "[Download UGENE and components](#)" page. Be careful to select a link for correct platform (64-bit or 32-bit).

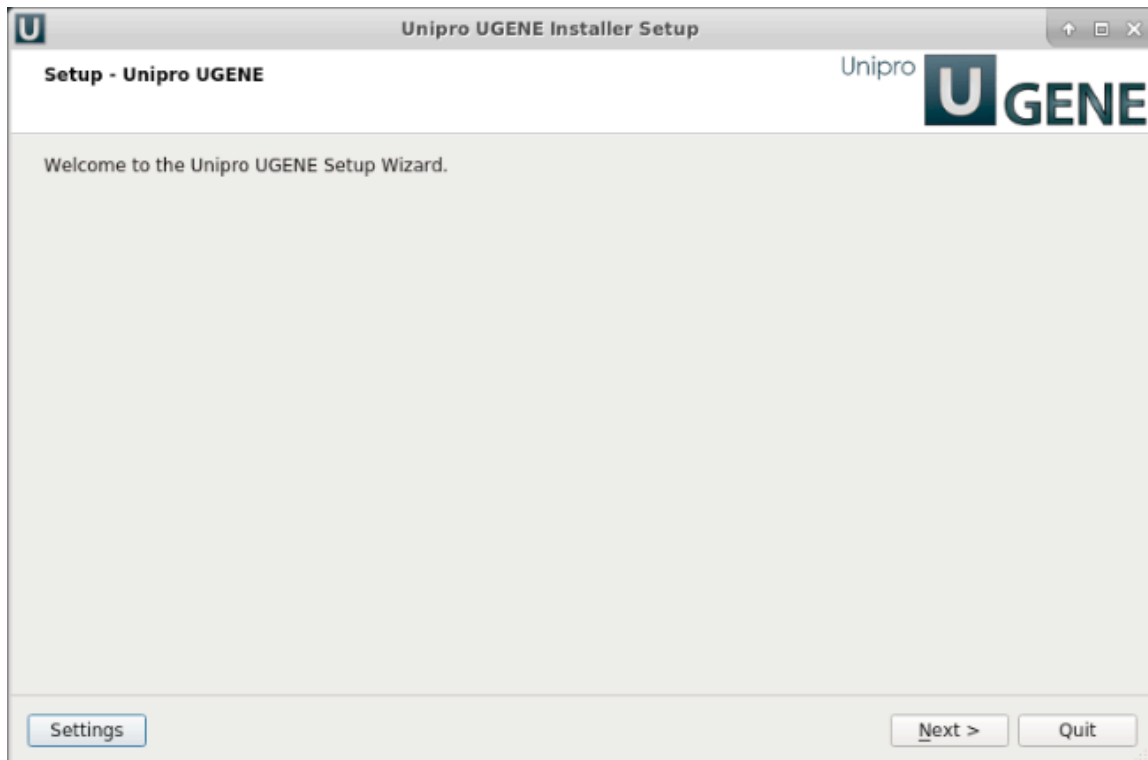
Information about native installation on Ubuntu and Fedora is provided below.

Installation using online installer

- Download the online installer file for your platform (64-bit or 32-bit).
- Make the file executable - in the Terminal run "chmod +x *installer_file_name*".
- Execute the file: "*./installer_file_name*". For example:

```
cd ~/Downloads
chmod +x ugeneInstaller_64bit
./ugeneInstaller_64bit
```

- Follow the installation wizard.



On a new UGENE version release, a notification will appear with an option to update the package.

Installation using TAR.GZ archive

- Download the required TAR.GZ archive. Choose [Standard](#), [Full](#), or [NGS package](#) for your platform (64-bit or 32-bit). Note that the NGS package is available for 64-bit platform only.
- Unpack the archive - in the Terminal run "tar -xf *archive_file*".
- From the unpacked folder run "*./ugene -ui*" (here "ui" states for "User Interface"). For example:

```
cd ~/Downloads
tar -xf ugene-current-version-x86-64.tar.gz
cd ugene-current-version
./ugene -ui
```

On a new UGENE version release, a notification will appear. However, to install the new version, it is required to manually download the package again.

Native installation

UGENE is a part of Ubuntu and Fedora distributions. Follow the instructions below to install it on one of these systems.

- [Native Installation on Ubuntu](#)
- [Native Installation on Fedora](#)

Native Installation on Ubuntu

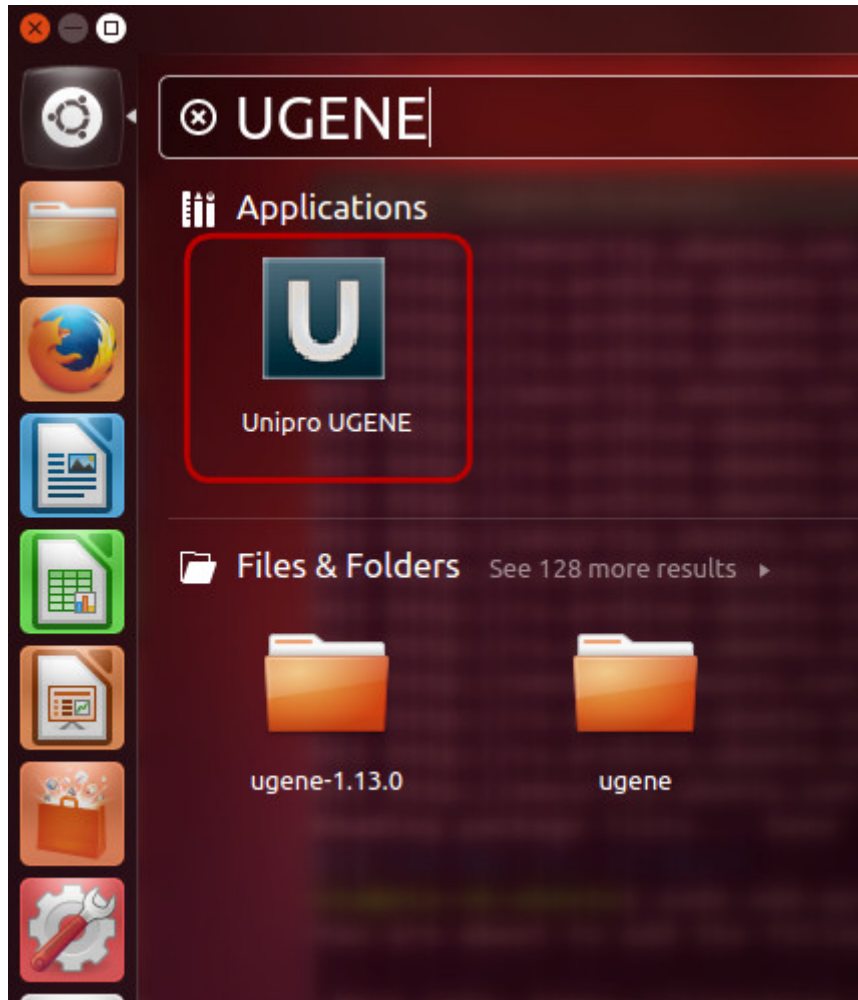
- UGENE packages for different Ubuntu versions are available on the Personal Package Archives (PPA). Add "iefremov/ppa" repository and pull down the latest list of software available. In the Terminal run:

```
sudo add-apt-repository ppa:iefremov/ppa
sudo apt-get update
```

- Install UGENE, tools with non-free licenses, and sample data:

```
sudo apt-get install ugene
sudo apt-get install ugene-non-free
sudo apt-get install ugene-data
```

UGENE will appear in the applications list.



Native Installation on Fedora

On Fedora UGENE is available through yum software package manager.

- To install UGENE in the Terminal run:

```
sudo yum install ugene
```

The latest available UGENE version will appear in the applications list.

Data Analysis Tools

UGENE provides GUI for a lot of different bioinformatics tools. A tool is supplied either as embedded or external:

- Embedded tool:** the tool is integrated into the UGENE source code. Such tools are always available in UGENE independently on the UGENE package type and operating system.
- External tool:**
 - Integrated (supported) external tool:** the tool executable file (or files) is provided with UGENE. It is launched when one uses the tool in the UGENE GUI. The result is also shown in the UGENE GUI. Note that the tool may be available on some operating systems only.
 - Custom external tool:** it is possible to [add any custom external tool](#) as a workflow element in the Workflow Designer. See "[Custom External Tools](#)" documentation chapter for details.

Specify integrated external tools in the installed package

The external tools (if available on the target operating system) are provided by default in the Online Installer, Full and NGS UGENE packages.

One can specify the tools manually:

- Download the tools executables, see links on the "[Download UGENE and components](#)" page:
 - External Tools Package:** the package contains all external tools except JRE (i.e. "java") and RScript.

- **Java Runtime Environment (JRE):** although UGENE is not a Java application, a few tools (e.g. FastQC, Trimmomatic) require JRE. Make sure **JRE 8** or higher is installed.
 - **RScript:** R is currently used in UGENE for ChIP-Seq data analysis only.
2. Configure the tools in UGENE: [open the UGENE Application Settings dialog](#), select the "External Tools" page and specify the external tools executables.

Integrated external tools


See the list of the currently integrated external tools, information about their availability on different operating systems and version included into the corresponding External Tools Package in the table below.

External tool	Windows (64-bit)	Windows (32-bit)	macOS (64-bit)	Linux (64-bit)	Linux (32-bit)
bedGraphToBigWig	4	N/A	4	4	N/A
bedtools	2.20.1	2.20.1	2.20.1	2.20.1	2.20.1
BLAST	2.2.26	2.2.26	2.2.26	2.2.26	2.2.26
BLAST+	2.2.30	2.2.30	2.2.30	2.2.30	2.2.30
Bowtie	0.12.7	0.12.7	1.0.0	1.0.0	1.0.0
Bowtie2	2.3.4.3	2.1.0	2.3.4.3	2.3.4.3	2.1.0
BWA	0.7.12	0.7.12	0.7.12	0.7.12	0.7.12
CAP3	12/21/07	12/21/07	12/21/07	12/21/07	12/21/07
CEAS	1.0.2	1.0.2	1.0.2	1.0.2	N/A
CLARK	N/A	N/A	1.2.4 (UGENE-customized)	1.2.4 (UGENE-cust omized)	N/A
ClustalO	1.2.1	1.2.1	1.2.1	1.2.1	1.2.1
ClustalW	2.1	2.1	2.1	2.1	2.1
ConductGO	Version not specified	Version not specified	Version not specified	Version not specified	N/A
conservation_plot	0.1.0	0.1.0	0.1.0	0.1.0	N/A
Cufflinks tools	N/A	N/A	2.0.2	2.0.2	N/A
Cutadapt	1.7.1	1.7.1	1.7.1	1.7.1	1.7.1
DIAMOND	N/A	N/A	0.9.22	0.9.22	N/A
FastQC	0.11.2	0.11.2	0.11.2	0.11.2	0.11.2
JRE (java)	1.8.0	1.8.0	N/A	N/A	N/A
HMMER	3.1b2	3.1b2	3.1b2	3.1b2	3.1b2
Kraken	N/A	N/A	1.0	1.0	N/A
MACS	1.4.2	1.4.2	1.4.2	1.4.2	N/A
MAFFT	7.212	7.212	7.212	7.212	7.212
MetaPhlan2	N/A	N/A	2.0.0	2.0.0	N/A
MrBayes	3.2.3	3.2.3	3.2.3	3.2.3	3.2.3
peak2gene	1.02	1.02	1.02	1.02	N/A
perl	5.24.3	5.24.0	N/A	N/A	N/A
PhyML	20120412	20120412	20120412	20120412	20120412

python	2.7.5	2.7.5	2.7.5	2.7.5	2.7.5
Rscript	R is not included into the External Tools Packages. However, it may be installed using the UGENE Online Installer package.				
samtools	0.1.19	0.1.19	0.1.19	0.1.19	0.1.19
SeqPos	1.01	1.01	1.01	1.01	N/A
SnpEff	4.3i	4.3i	4.3i	4.3i	4.3i
SPAdes	N/A	N/A	3.12.0	3.12.0	N/A
Spidey	Version not specified	Version not specified	N/A	Version not specified	Version not specified
StringTie	N/A	N/A	1.3.4d	1.3.4d	N/A
T-Coffee	8.99	8.99	11.00	11.00	11.00
TopHat	N/A	N/A	2.1.1	2.1.1	N/A
Trimmomatic	0.38	0.38	0.38	0.38	0.38
vcftools	0.1.12b	0.1.12b	0.1.12b	0.1.12b	0.1.12b
WEVOTE	N/A	N/A	Version not specified	Version not specified	N/A

Reference Data for NGS


To run some NGS data analysis (metagenomics classification or ChIP-Seq) it is required to provide reference data (e.g. RefSeq databases, NCBI taxonomy files, etc.).

 Both taxonomy classification and ChIP-Seq tools are available on macOS and Linux operating systems only. Also, make sure there is enough disk space on the target computer.

It is recommended to use the Online Installer to download these data, however, one can also download them directly (see links on the "[Download UGENE and components](#)" page). In the latter case it is required to additionally manually configure an installed UGENE package, see instructions below.

Configure Data for Metagenomics Classification

UGENE integrates a set of tools for taxonomy classification of microorganisms using whole-genome shotgun sequencing data. The tools are Kraken, CLARK, DIAMOND, etc. See, for example, "[Parallel NGS Reads Classification](#)" sample workflow that allows one to classify input FASTQ files with these tools working in parallel and then join their output with another tool WEVOTE.


 The tools are available on 64-bit macOS or Linux operating systems only. Also, as the tools are quite resource-consuming, it is recommended to have at least 16 Gb of RAM available.

To use these tools one should provide appropriate taxonomy data and reference data, specific for a tool. Some reference databases are provided for each of the tool. One can use these data or build a custom database (see, for example, workflow element "[Build Kraken Database](#)").

It is recommended to use the UGENE [Online Installer](#) package to install and automatically configure the data. However, if the Internet is not available on the target computer, or it is required to use another UGENE package for some other reason, follow the instructions below on how to download and configure the data.

Download data for metagenomics classification

Use links in the "Data for NGS metagenomics classification" section on the "[Download UGENE and components](#)" page to download the data.

 Make sure to have enough disk space on the target computer.

See the list of the available downloads in the table below.

Data	Archive size	Unpacked data size	Description	Data source
NCBI taxonomy classification	2.5 Gb	31 Gb	This includes a set of taxonomy data files from NCBI. These data should be present for any type the NGS classification analysis.	Original data were downloaded from the NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/pub/taxonomy/).
NCBI RefSeq bacterial genomes	130 Gb	132 Gb	<p>The data can be used to build a database for CLARK-I (light version of CLARK), CLARK, or Kraken.</p> <p>As UGENE integrates modified version of CLARK/CLARK-I, it is possible to provide *.gz archives as input for building the database. In particular, "CLARK-I DB: RefSeq bacterial+viral genomes" (see below) was generated using the archived data.</p> <p>Also, keep in mind that changing of some parameters of the "Classify Sequences with CLARK" element may cause re-building of the reference database. The reference data should be present in this case!</p> <p>For building a Kraken database usage of *.gz archives is not supported, it is required to unpack each *.gz file, so even more disk space will be required.</p>	Original data were downloaded from the NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/refseq/release/bacteria/bacteria*.genomic.fna.gz)
NCBI RefSeq viral genomes	77 Mb	77 Mb	<p>Similarly to "NCBI RefSeq bacterial genomes", although the size of the data is rather small.</p> <p>The reference data are included into "CLARK-I DB: RefSeq bacterial+viral genomes" and "CLARK-I DB: RefSeq viral genomes" databases.</p>	Original data were downloaded from the NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/refseq/release/viral/viral*.genomic.fna.gz).

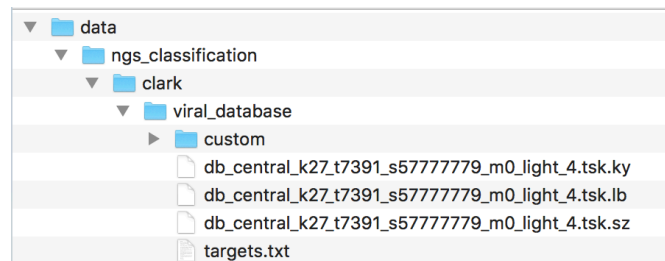
<p>NCBI RefSeq GRCh38 human genome</p>	<p>837 Mb</p>	<p>838 Mb</p>	<p>Similarly to "NCBI RefSeq bacterial genomes".</p> <p>The data are not included into any database, but provided in case one would like to use them when building a custom database.</p>	<p>Original data were downloaded from the NCBI FTP (ftp://ftp.ncbi.nih.gov/genomes/H_sapiens/CHR_*/hs_ref_GRC*chr*.fa.gz).</p>
<p>Kraken DB: MiniKraken 4Gb database</p>	<p>2.5 Gb</p>	<p>4.3 Gb</p>	<p>A sample reference database provided in UGENE for Kraken.</p> <p>It is a pre-built 4 GB database constructed from complete bacterial, archaeal, and viral genomes in RefSeq (as of Oct. 18, 2017). This can be used by users without the computational resources needed to build a Kraken database. However this contains only 2.7% of kmers from the original database.</p>	<p>Original data were downloaded using a link on the Kraken web site (https://ccb.jhu.edu/software/kraken/).</p>
<p>CLARK-I DB: RefSeq bacterial+viral genomes</p>	<p>7.4 Gb</p>	<p>11 Gb</p>	<p>One of the reference databases provided in UGENE for CLARK-I.</p> <p>The database was build using archived RefSeq bacterial and viral genomes.</p>	<p>See above.</p>
<p>CLARK-I DB: RefSeq viral genomes</p>	<p>16 Mb</p>	<p>72 Mb</p>	<p>One of the reference databases provided in UGENE for CLARK-I.</p> <p>The database was build using archived RefSeq viral genomes.</p>	<p>See above.</p>
<p>DIAMOND DB: UniRef50</p>	<p>5.2 Gb</p>	<p>13 Gb</p>	<p>One of the reference databases provided in UGENE for DIAMOND.</p> <p>Note that unlike Kraken and CLARK, DIAMOND requires protein reference sequences as input.</p>	<p>Original data were downloaded from the Uniprot FTP (ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/uniref/uniref50/uniref50.fasta.gz). Then a DIAMOND database was built.</p>
<p>DIAMOND DB: UniRef90</p>	<p>13 Gb</p>	<p>34 Gb</p>	<p>One of the reference databases provided in UGENE for DIAMOND.</p>	<p>Original data were downloaded from the Uniprot FTP (ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/uniref/uniref90/uniref90.fasta.gz). Then a DIAMOND database was built.</p>

MetaPhlan2 embedded DB: mpa_v20_m200	1 Gb	1.2 Gb	This database is provided with the MetaPhlan2 tool. It was built on ~1M unique clade-specific marker genes identified from ~17,000 reference genomes (~13,500 bacterial and archaeal, ~3,500 viral, and ~110 eukaryotic).	The database was downloaded here: https://bitbucket.org/biobakery/metaphlan2/downloads/ .
Total:	162 Gb	227 Gb		

Configure data

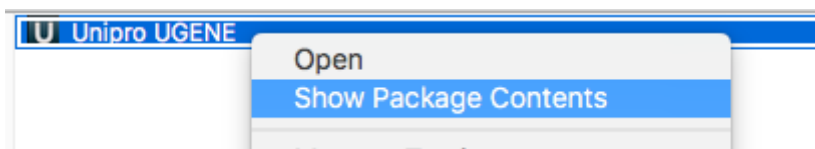
Data described above are stored as 7zip archives. After a file download, unpack it using an appropriate file archiver (for example, [Keka](#) on macOS).

The unpacked data are stored in a folders structure with the root folder called "data". For example, for "NCBI RefSeq viral genomes" the archive is called "ngs_classification.clark.viral_database.7z" and the unpacked data look as follows:



It is required to move these data to the [UGENE data folder](#), following the hierarchical data structure:

- On Linux it is "data" folder, located in the UGENE installation folder.
- On macOS the "data" folder is located inside the "Unipro UGENE.app" bundle. Right-click on the bundle, select "Show Package Contents", select "Contents -> MacOS -> data" folder.



Thus, all required data will be placed to the "ngs_classification" sub-folder of the UGENE data folder.



Kraken, CLARK, DIAMOND and WEVOTE are integrated as [external tools](#). So, also make sure the tools executables are set in the UGENE Application Settings.

Configure ChIP-Seq Analysis Data

UGENE integrates the [Cistrome workflow](#) for analysis of ChIP-Seq data. The workflow requires reference data to be present in the UGENE data folder. Reference data for human are provided in the [NGS package](#) and in the Online Installer. To configure them manually download the data from the "[Download UGENE and components](#)" page and unpack to the "cistrome" folder inside the UGENE data folder.

Note that RScript [external tool](#) should also be provided.

Basic Functions

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UGENE Terminology

Project

Storage for a set of data files and visualization options.

Document

A single file (can be stored on a local hard drive or be a remote web page). Each *document* contains a set of *objects*.

Object

A minimal and complete model of biological data. For example: a single sequence, a set of annotations, a multiple sequence alignment.

Task

A process, usually asynchronous, that works in background. For example: some computations, loading and writing files.

Plugin

A dynamically loaded module that adds new functionality to UGENE.

Object View

A graphical view for a single or a set of *objects*.

Project View

A visual component used to manage active *project*.

Task View

A visual component used to manage active *tasks*.

Log View

A visual component used to show logs.

Notifications

A visual component used to show notifications. Generally it is used to open tasks reports.

Plugin Viewer

A visual component used to manage *plugins*.

Sequence View

An *Object View* aimed to visualize DNA, RNA or protein sequences along with their properties like annotations, chromatograms, 3D models, statistical data, etc.

Annotation

Additional information about a sequence, identified by its name and the sequence region.

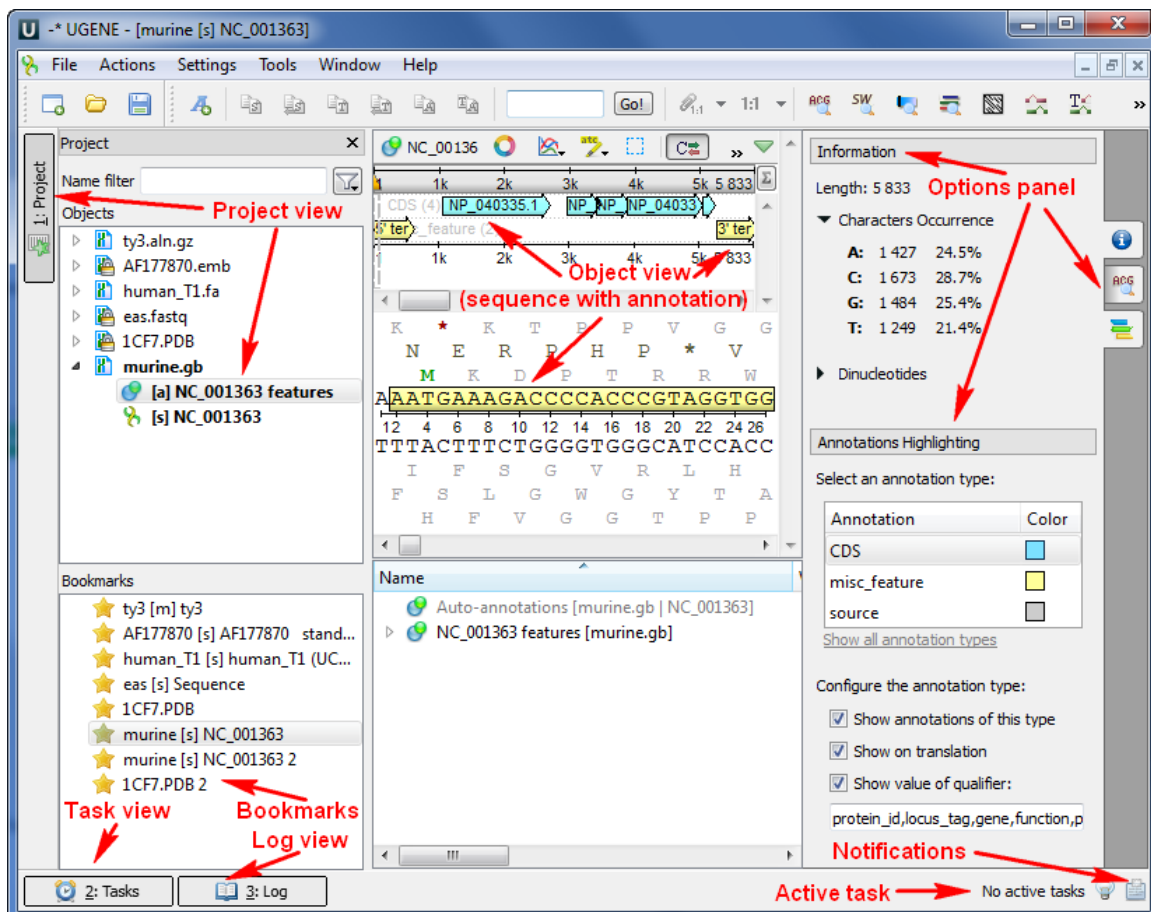
Alignment Editor

An *Object View* used to visualize and edit DNA, RNA or protein multiple sequence alignments.

Options Panel

An *Options Panel* it is the panel with different information tabs and tabs with settings for *Sequence View* and *Assembly Browser*.

In the image below you can see a typical UGENE window with a *Project View* and a single *Object View* window opened:



UGENE Window Components

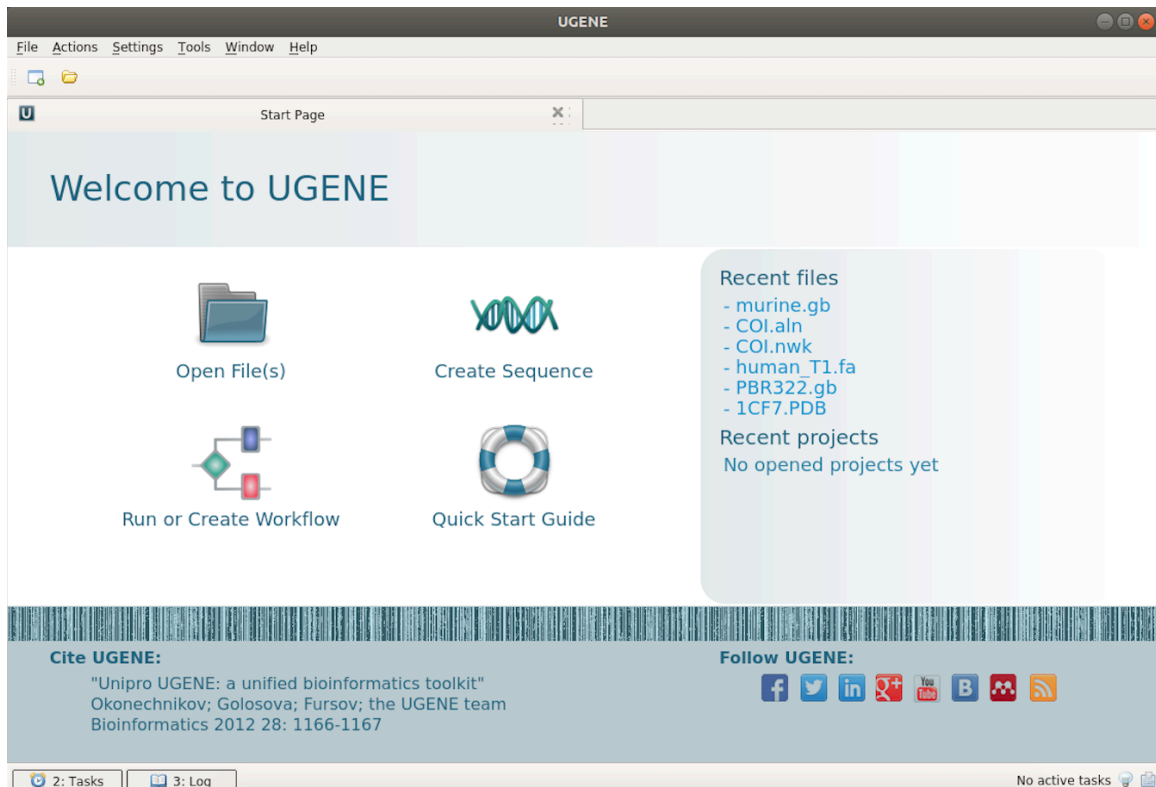
This chapter describes UGENE main window components *Project View*, *Task View*, *Log View* and the *Notifications* popup window.

- Start Page
- Project View
- Task View
- Log View
- Notifications

Start Page

The *Start Page* is the first page that appear when UGENE is launched. From this page you can open a file, create a sequence, create a workflow (use a sample workflow or create a new one), open the Quick Start Guide, and open recent files directly.

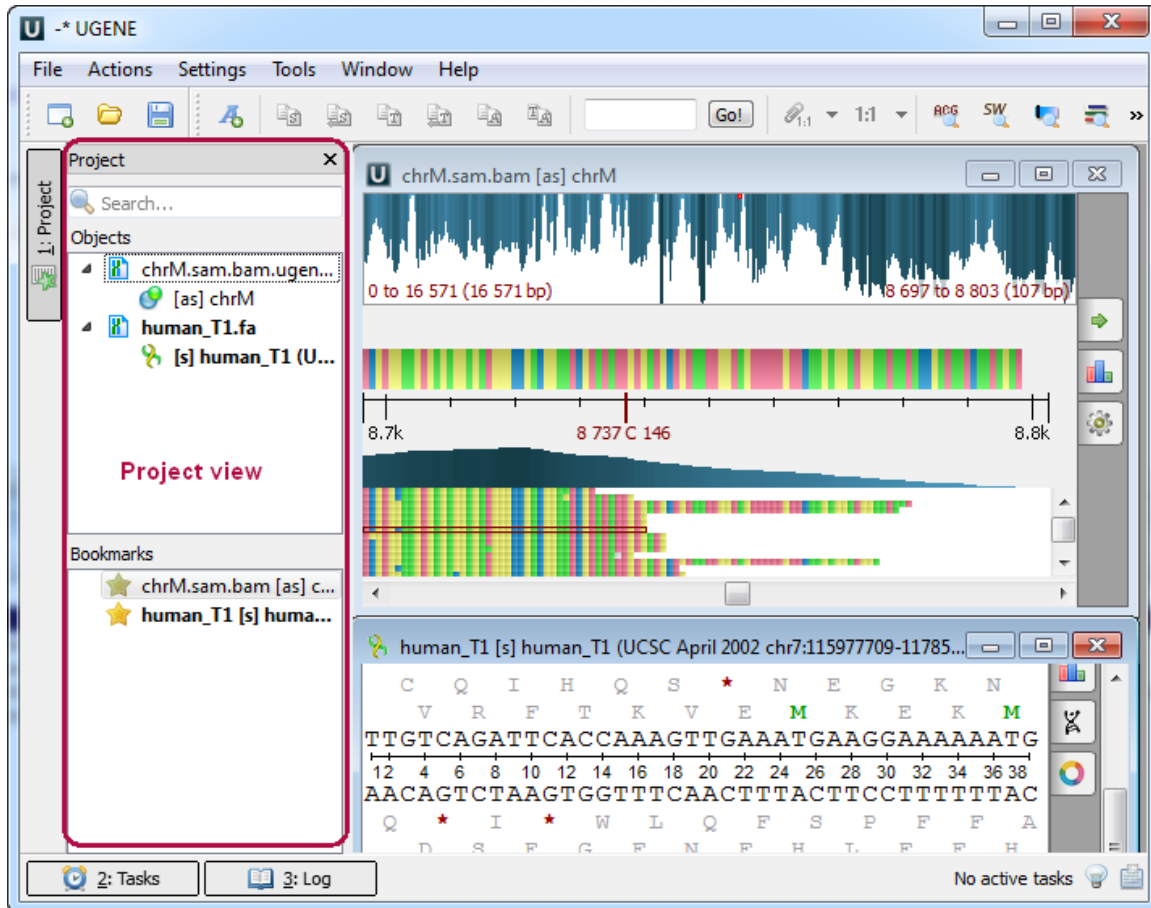
To return to the *Start Page* at any time select *Help->Open Start Page* in the main menu.



Project View

The *Project View* shows documents and bookmarks of the current *project*. The documents are files added to the project. And the bookmarks are visual view states of the documents. Read *Using Bookmarks* to learn more about bookmarks.

To show/hide the *Project View*, click the *Project* button in the main UGENE window:



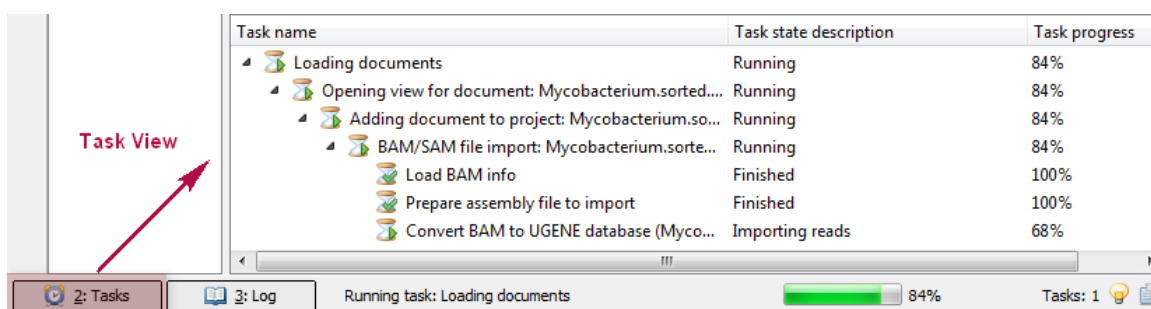
You can also use the Alt+1 hotkey to show/hide the *Project View*.

To create a new project, refer to [Creating New Project](#). Note that if you have no project created when opening file with a sequence, an alignment or any other biological data, a new anonymous project is created automatically.

Task View

The *Task View* shows active tasks, for example, algorithms computations.

To show/hide the *Task View*, click the *Tasks* button in the main UGENE window:



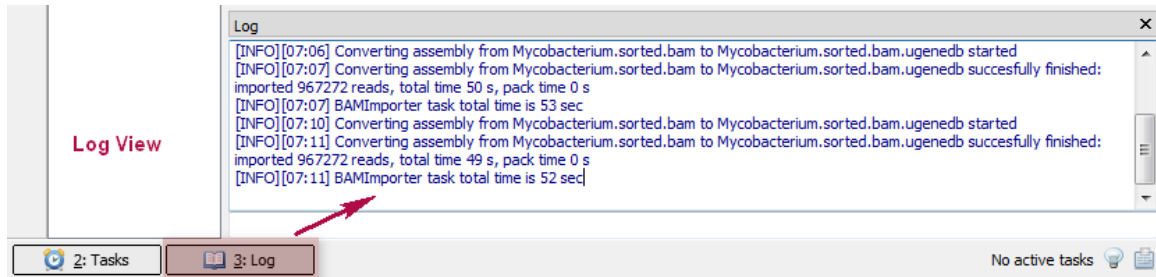
The hotkey for showing/hiding the *Task View* is Alt+2.

The *Task name* column of the *Task View* shows the tasks names. *Task state description* shows the status of the active tasks: Started, Running, Finished and so on. The *Task progress* column shows the percentage of the tasks progress. If you want to cancel a task, click the red cross button in the *Actions* column for the task.

Log View

The *Log View* shows the program log information.

To show/hide the *Log View* click the *Log* button in the main UGENE window:

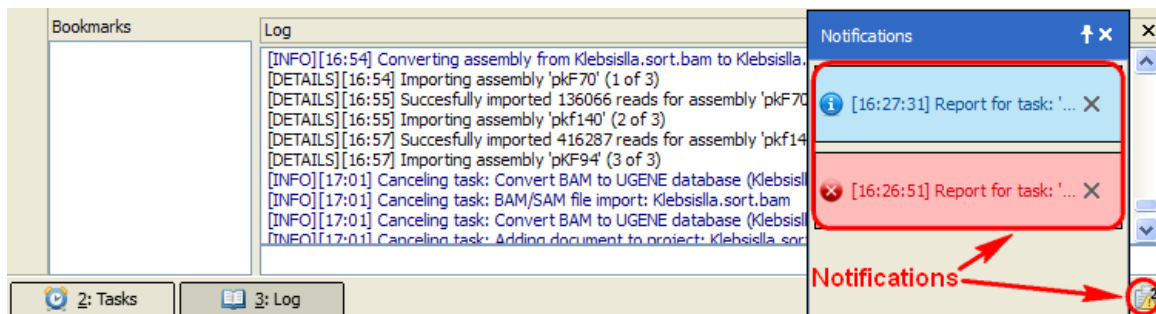


The hotkey for this action is Alt+3.

It is possible to configure the *Log View* settings: the level of the log to show (ERROR, INFO, DETAILS, TRACE), the category (Algorithms, Tasks, etc.), and the format of the log messages (format of the dates, etc.). This settings can be configured in the UGENE *Application Settings*.

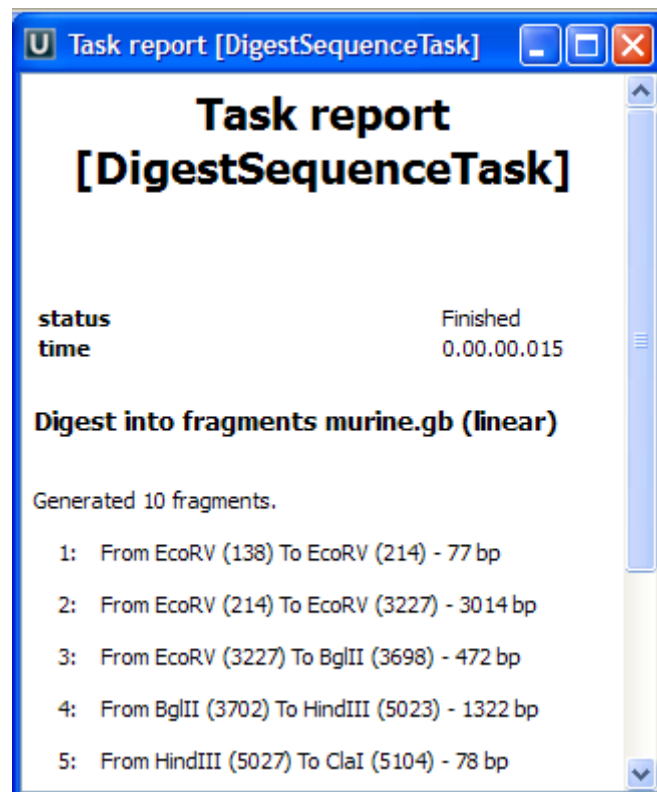
Notifications

The *Notifications* component shows notifications for tasks reports.



If a task has finished without errors, the notification is blue. If an error has occurred during the task execution, the notification is red. If a warning has occurred during the task execution, the notification is yellow.

To open a task report, click on the corresponding notification. See an example of a task report below:



To remove a notification from the *Notifications* popup window, click the notification cross button.

Note that you can click on the clip button of the *Notifications* popup window to show the window always on top.

Main Menu Overview

Menu	Description
File	A set of project level operations. List of operations: new project , new document from text , new workflow , open , open as , open from clipboard , access remote database , search NCBI genbank , connect to UGENE shared database , save all , save project as , export project , close project , recent files , recent projects , exit .
Actions	Various actions associated with the active window. List of operations: go to position , add , copy/paste , analyze , align , cloning , export , remove , edit (for the <i>Sequence View</i>); go to position , add , copy/paste , colors , highlighting , edit , align , tree , statistics , view , export , advanced , consensus mode , close active window (for the <i>Alignment Editor</i>).
Settings	Preferences and plugin settings.
Tools	Various tools. This menu is extended by different plugins. List of operations: sanger data analysis , NGS data analysis , BLAST , multiple sequence alignment , cloning , primer , search for TFBS , HMMER tools , build dotplot , random sequence generator , show counters , expert discovery , query designer , workflow designer .
Window	A list of active windows and basic manipulations with the windows. List of operations: window layout (multiple or tubbed), close active view , close all windows , tile windows , cascade windows , next window , previous window .
Help	Application help and check for updates. List of operations: open UGENE user manual , open workflow designer manual , open query designer manual , view UGENE documentation online , visit UGENE website , check for updates , open start page , about .
Unipro UGENE (macOS only)	List of operations: about Unipro UGENE , preferences , services , hide Unipro UGENE , hide others , show all , quit Unipro UGENE .

The menus can be dynamically populated with new actions added by plugins. Check the [Plugins](#) documentation to learn how each plugin affects global and context menus.

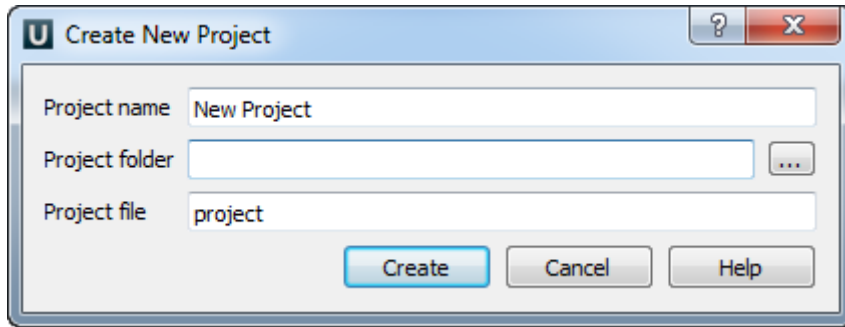
Creating New Project

A *project* store links to the data files, cross-file data associations, and visualization settings.

Below is the description of how to create a new project manually. Note that if you have no project created when opening a file with a sequence, an alignment or any other biological data, a new anonymous project is created automatically.

To create a new project select the *File New project* menu or click the *New project* button on the main toolbar.

The dialog will appear:

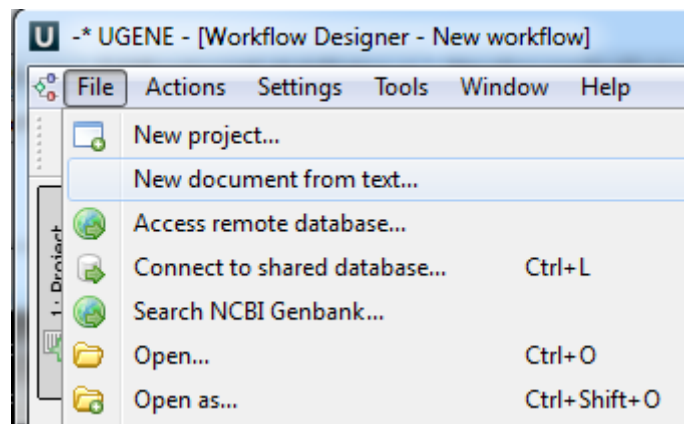


Here you need to specify the visual name for the project and the directory and file to store it.

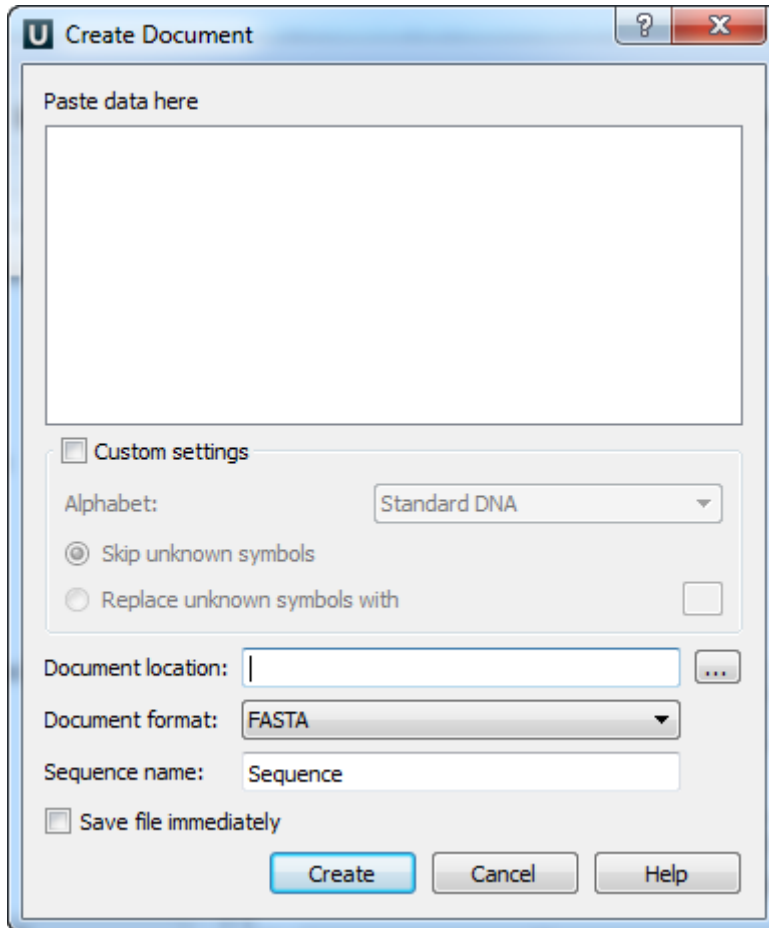
After you click the *Create* button the *Project View* window is opened.

Creating Document

To create a new sequence file from a text, select the *File New document from text* main menu item:



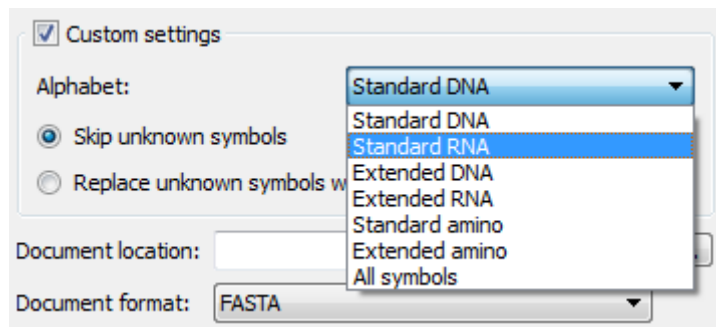
The *Create Document* dialog appears:



You can input the created sequence to the *Paste data here* field. You can type or paste sequences in FASTA or text format.

The following *Custom settings* are available:

Alphabet — here you can select the alphabet:



The following alphabets are available: Standard DNA, Standard RNA, Extended DNA, Extended RNA, Standard amino, Extended amino.

Skip unknown symbols / *Replace unknown symbols with* — you can select either to skip unknown input symbols or to replace them with the specified symbol.

Document location — a location of the created document.

Document format — a format of the created document. Currently available formats are FASTA and Genbank.

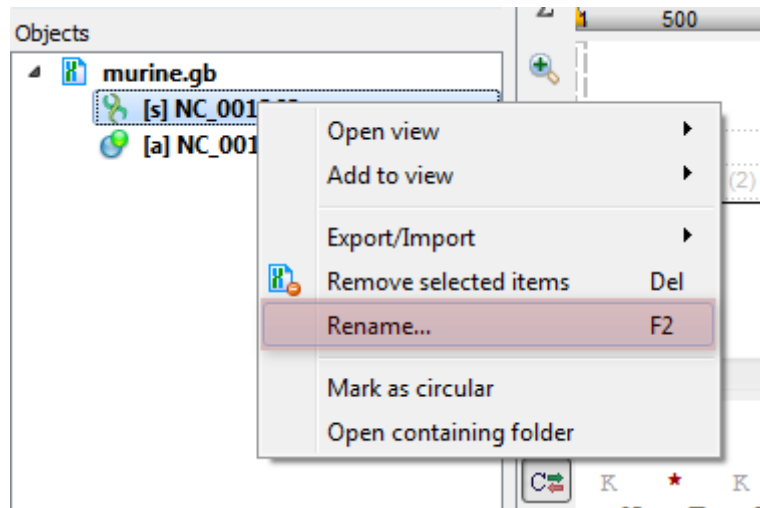
Sequence name — a name of the sequence in the created document.

Save file immediately — check this option if you want to save the document immediately after the *Create* button is pressed.

The created document will be added to the current project and opened in the *Sequence View*.

Renaming Object

To rename an object in the project tree view use the F2 shortcut or *Rename* context menu item:



Opening Document

UGENE stores information about *documents* you are working with in a *project*. Once a *document* has been opened, the information about it is saved in the current *project*.

- [Opening for the First Time](#)
 - [Advanced Dialog Options](#)
- [Opening Document Present in Project](#)
- [Opening Several Documents](#)
- [Opening Unloaded Documents](#)
- [Opening from Clipboard](#)

Opening for the First Time

To open a *document* that is not yet presented in the current *project* use either an advanced *Open* dialog, a simple open file dialog or just drag the document to the UGENE window.

UGENE automatically detects the *format* of the *document*, but if you use the advanced dialog you can choose the format manually.

To open the advanced dialog select one of the following:

- *Add Existing document* item in the *Project View* context menu
- *File Open As* item in the main menu

To simply open the document select one of the following:

- *Open* item in the main toolbar
- *File Open* item in the main menu

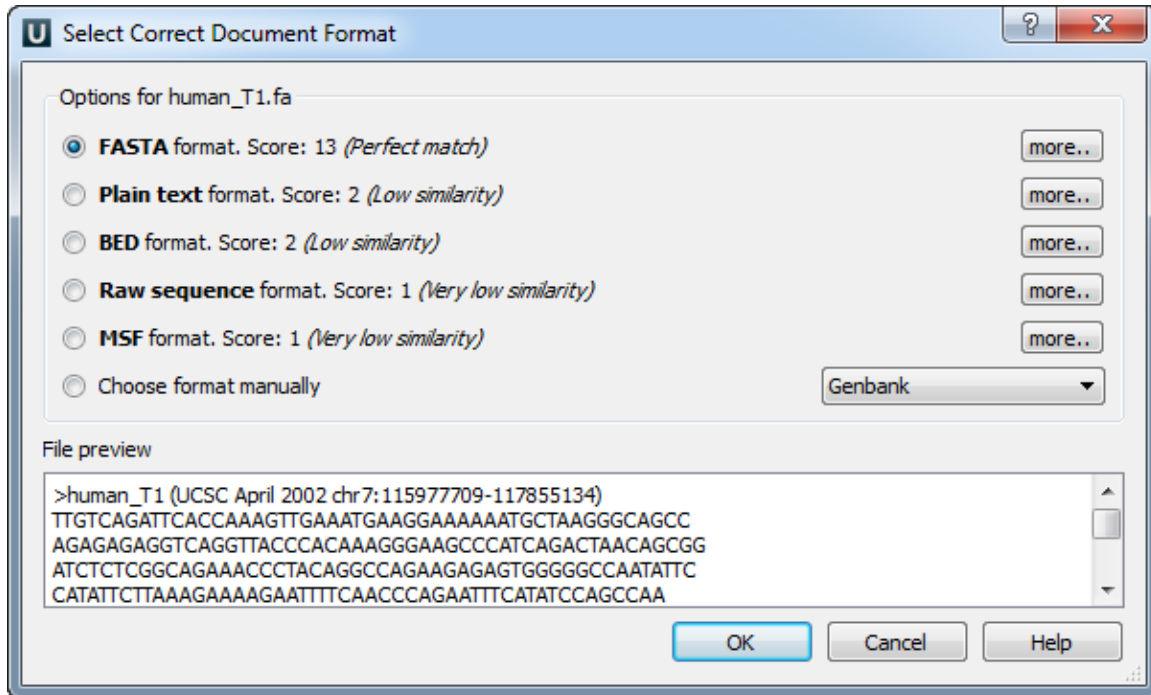
or drag the file to the UGENE window. Also it is able to drag and drop documents (not objects) between opened UGENEs.

⚠ Documents created not by UGENE are *locked*. To be able to edit the document you should save a copy of the document and continue working with the copy.

- [Advanced Dialog Options](#)

Advanced Dialog Options

Open the *Select Correct Document Format* dialog by *Add Existing document* item in the *Project View* context menu or by *File Open As* item in the main menu. The following dialog will appear:



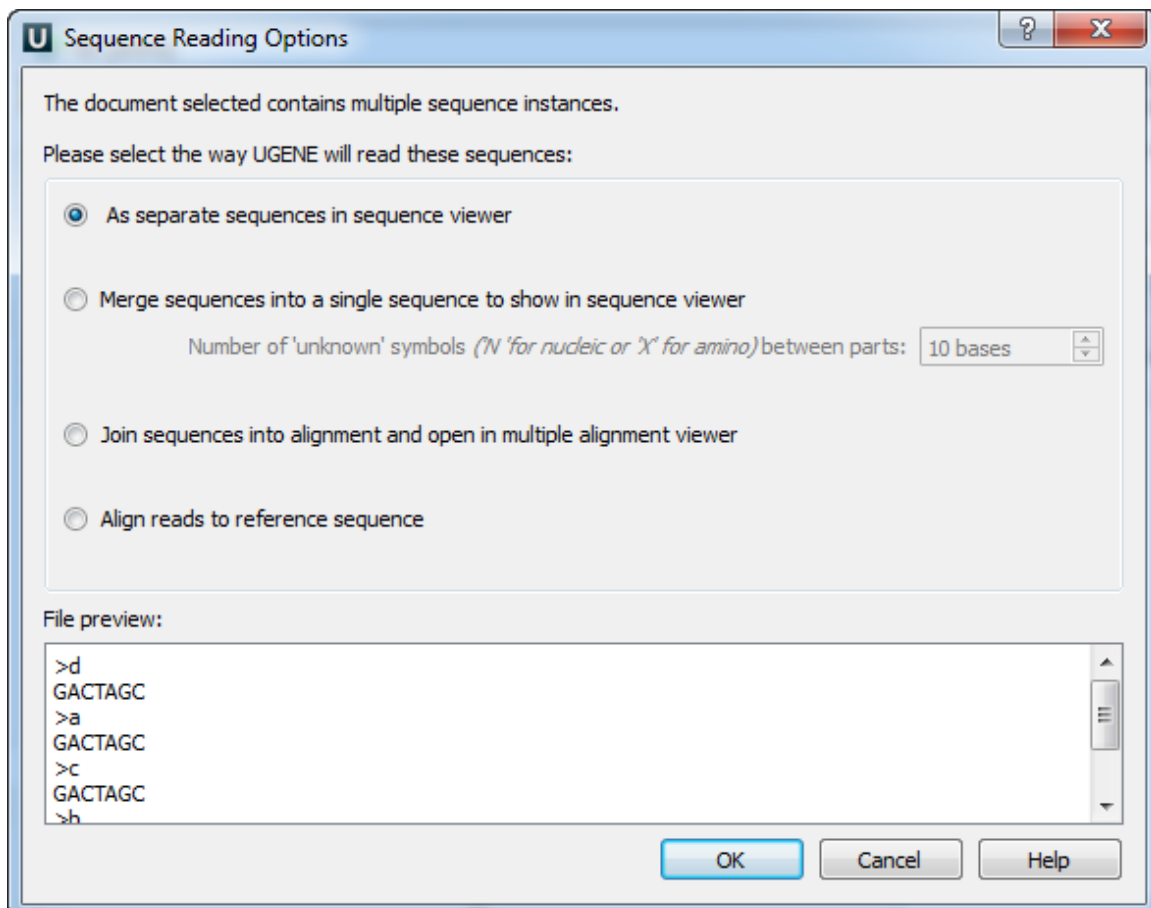
Here you can choose how to interpret the data stored in the file. The format is detected automatically, but you can select it manually.

Opening Document Present in Project

To open a *document* that is already present in the current *project* select it in the *Project View* and click Enter, double-click on it or drag it to an empty space of the UGENE window.

Opening Several Documents

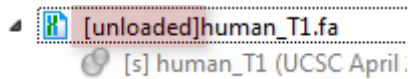
To open several documents that are not yet presented in the current *project* use the *File Open* item in the main menu. The *Select files open* dialog will appear. Select the documents with a help of the *Ctrl* button and click on the *Open* button. The following dialog will appear:



Select the reading options and click on the *OK* button.

Opening Unloaded Documents

The [unloaded] prefix indicates that document is unloaded:



To open unloaded document use the *Load selected document* context menu, *Enter* key or double click on the document. To unload the document use the corresponding context menu item.

Opening from Clipboard

It is possible in UGENE opening text data and files by pasting them into the *Project View* from the clipboard.

Select text data in a browser or in a text editor or file(s) from your system and press *Ctrl+C* (*Cmd+C* for macOS).

Switch to UGENE with an opened project, click the project area and press *Ctrl+V* (*Cmd+V*).

The copied data or file(s) will be opened in UGENE.

Another way for the file(s) is drag-drop. Using your mouse, drag and drop the files from the file system to UGENE, and they will be opened by the program.

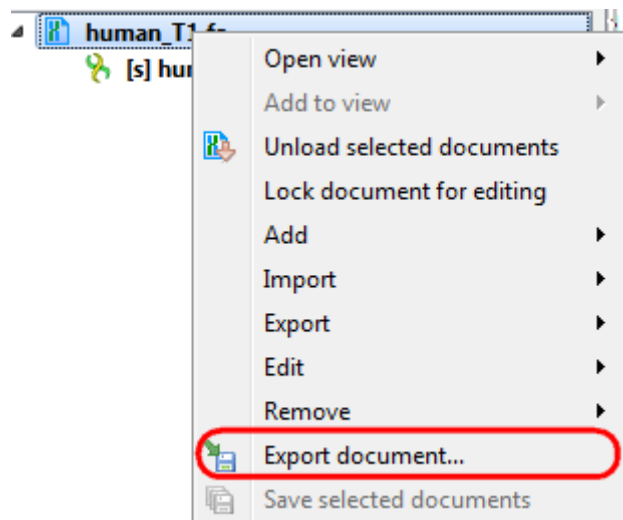
Opening Containing Folder

To open a containing folder of the *document* that is already present in the current *project* select it in the *Project View* and click on the *Open containing folder* context menu item.

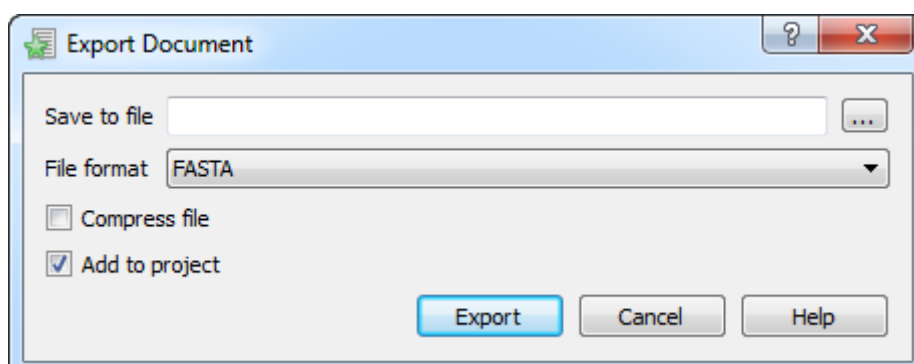
Exporting Documents

If a document has a format that supports writing in UGENE (see the *Supported File Formats* chapter), you can export the document to a new document in a required format.

To do it use the *Export document* item in the context menu:



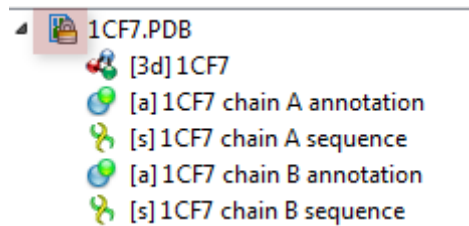
The following dialog appears:



Here you may select the name of the output file in the *Save to file* field and, optionally, choose the format of the output file in the *File format* field. Use the *Compress file* checkbox to compress the file. The *Add to project* checkbox, checked by default, adds the output file to the current project. After choosing all parameters click the *Export* button.

Locked Documents

The lock icon in the document element indicates that the document can't be modified:



UGENE does not allow modification of some formats that were created not by UGENE.

If UGENE is able only to read a document (see the [Supported File Formats](#) chapter), you can export the document objects to a file. To do it use the *built-in export utilities*.

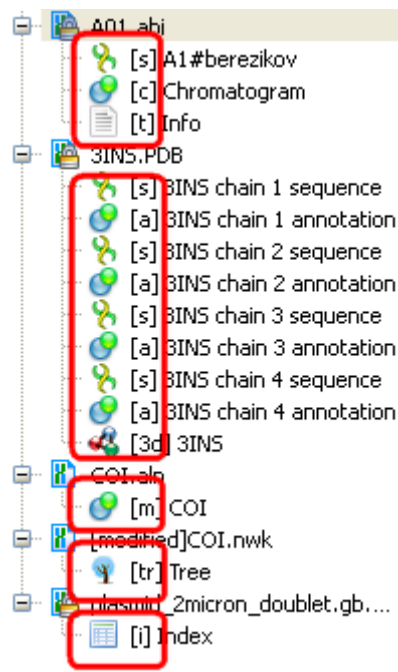
Also, you can *export* the document objects of unlocked documents.

Using Objects and Object Views

The document always contains one or more *objects*. An *object* is a structured biological data that can be visualized by different *Object Views*.

A single *Object View* can visualize one or several objects of different types. For example a single view can show a sequence, annotations for the sequence, 3D model for the part of the sequence or its chromatogram simultaneously.

The type of an object is indicated by the symbol in the square brackets and the icon near the object:



Below is the list of object types supported by the current version of UGENE.

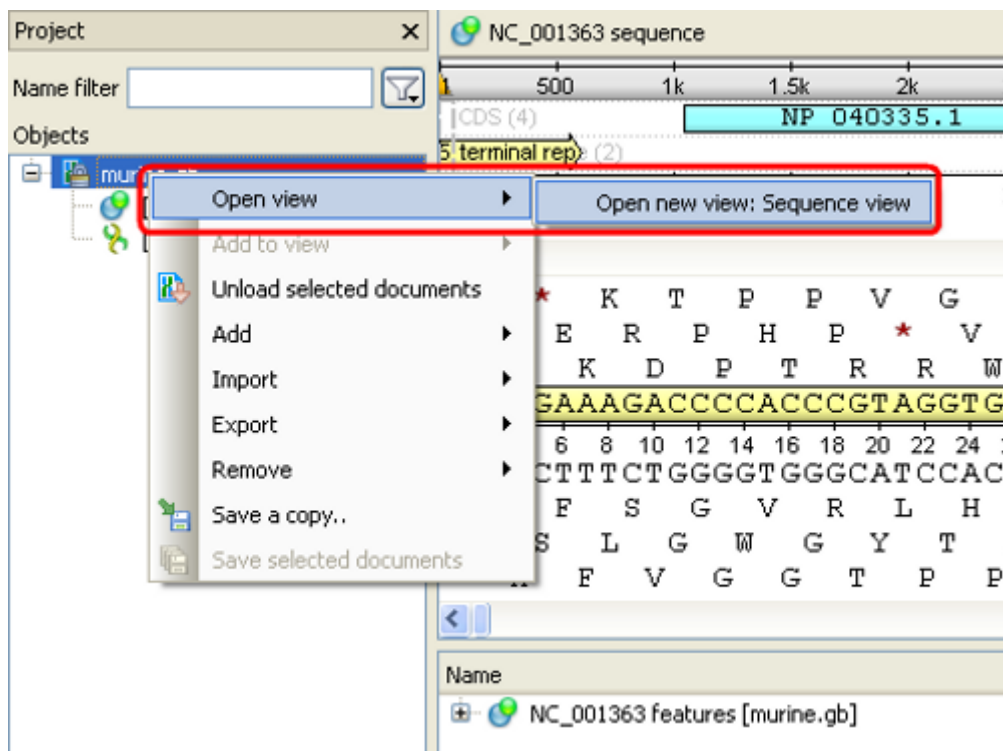
Object types:

Symbol	Icon	Description
[3d]		A 3D model.
[a]		Annotations for DNA sequence regions.
[as]		An assembly.

[c]		Chromatogram data.
[i]		A file with index information for a set of other, usually large files.
[m]		A multiple sequence alignment.
[s]		A nucleic, protein or raw sequence.
[t]		A plain text.
[tr]		A phylogenetic tree.

You can edit names of particular objects, such as sequence objects, by selecting them in the *Project View* and then pressing F2. To be able to do so, the document containing the target object must be unlocked.

To see the list of all available views for a given object select the object and activate the context menu inside the *Project View* window and select the *Open view* submenu:



The picture above illustrates an option to visualize the selected DNA sequence object using the *Sequence View* — a complex and extensible *Object View* that focuses on visualization of sequence objects in combination with different kinds of related data: sequence annotations, graphs, chromatograms, sequence analysis algorithms. Note, that the *Sequence View* is described in more details in the separate [document](#) [ation section](#).

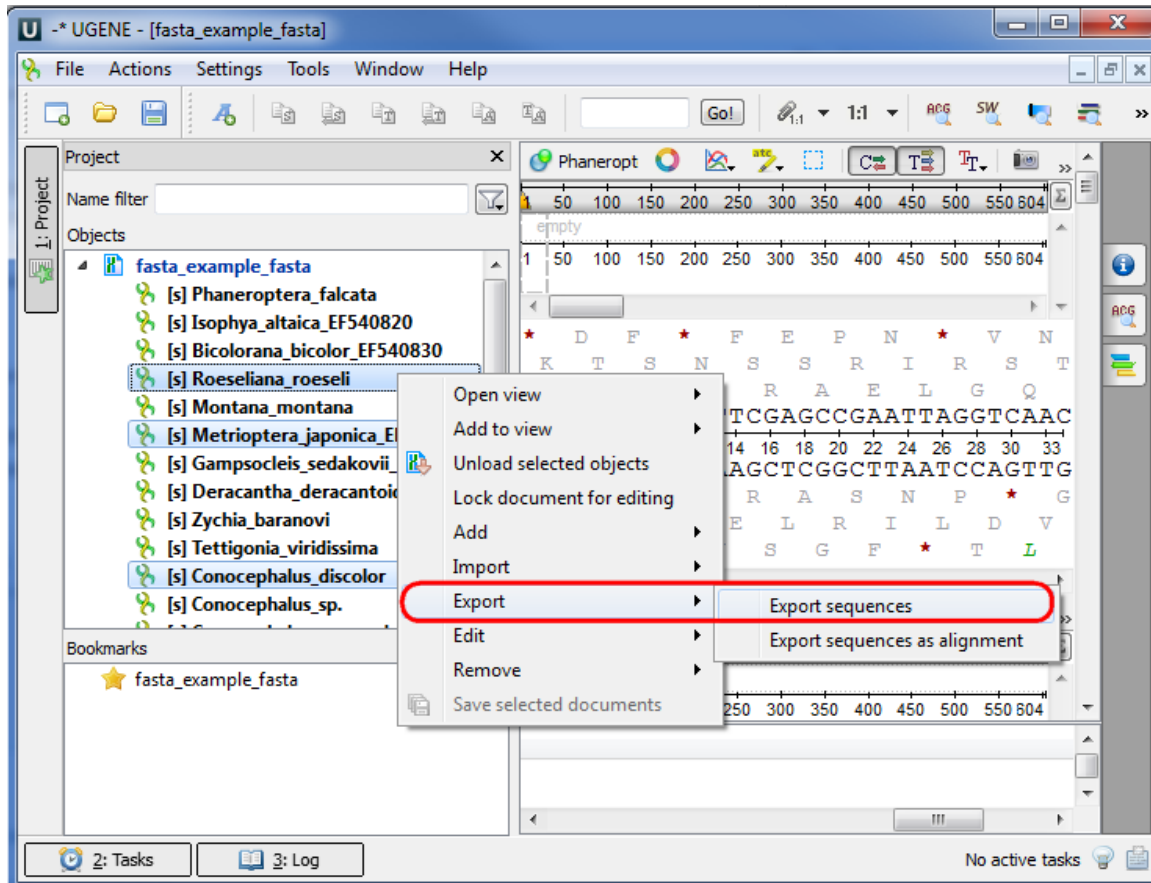
Exporting Objects

The document *objects* can be exported into a new document. For more details see the following chapters:

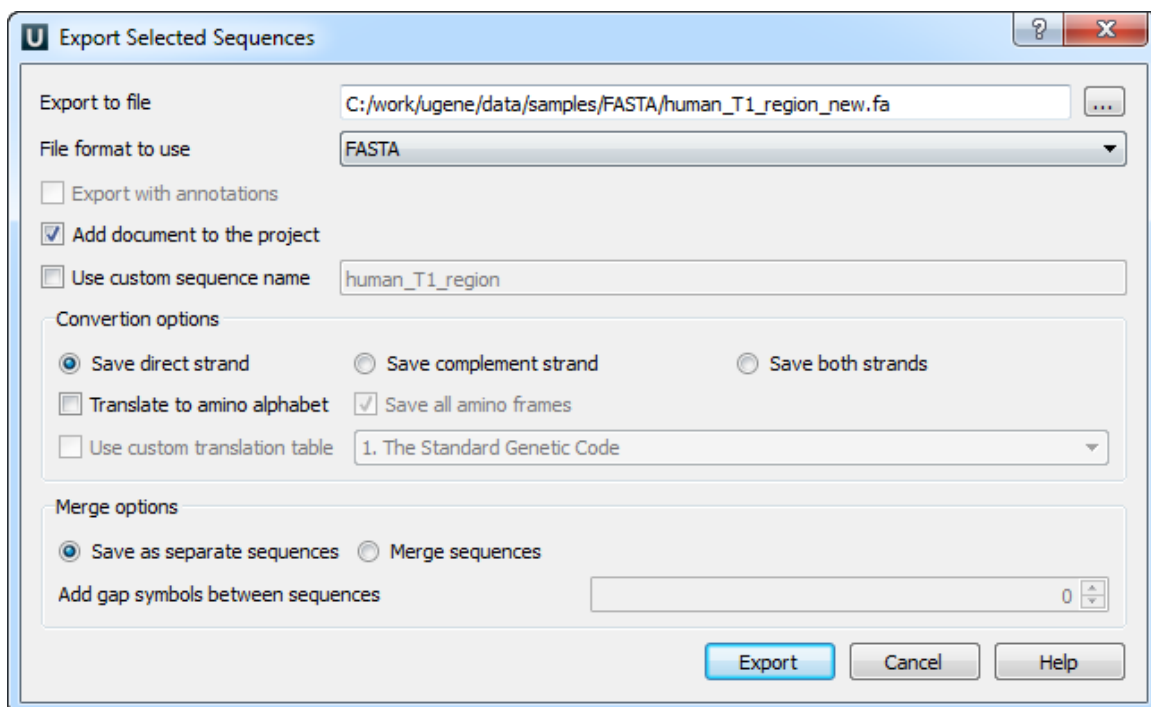
- [Exporting Sequences to Sequence Format](#)
- [Exporting Sequences as Alignment](#)
- [Exporting Alignment to Sequence Format](#)
- [Exporting Nucleic Alignment to Amino Translation](#)
- [Export Sequences Associated with Annotation](#)

Exporting Sequences to Sequence Format

Select a single or several sequence objects in the *Project View* window and click the *Export* *Export sequences* context menu item:



The *Export Selected Sequences* dialog will appear:



Here you can select the location of the result file and a sequence file format. You can choose to add newly created document to the current project and use custom sequence name. To do it check the corresponding checkboxes.

Use the *Conversion options* to choose a strand for saving sequence(s). Also you can translate sequence(s) to amino alphabet.

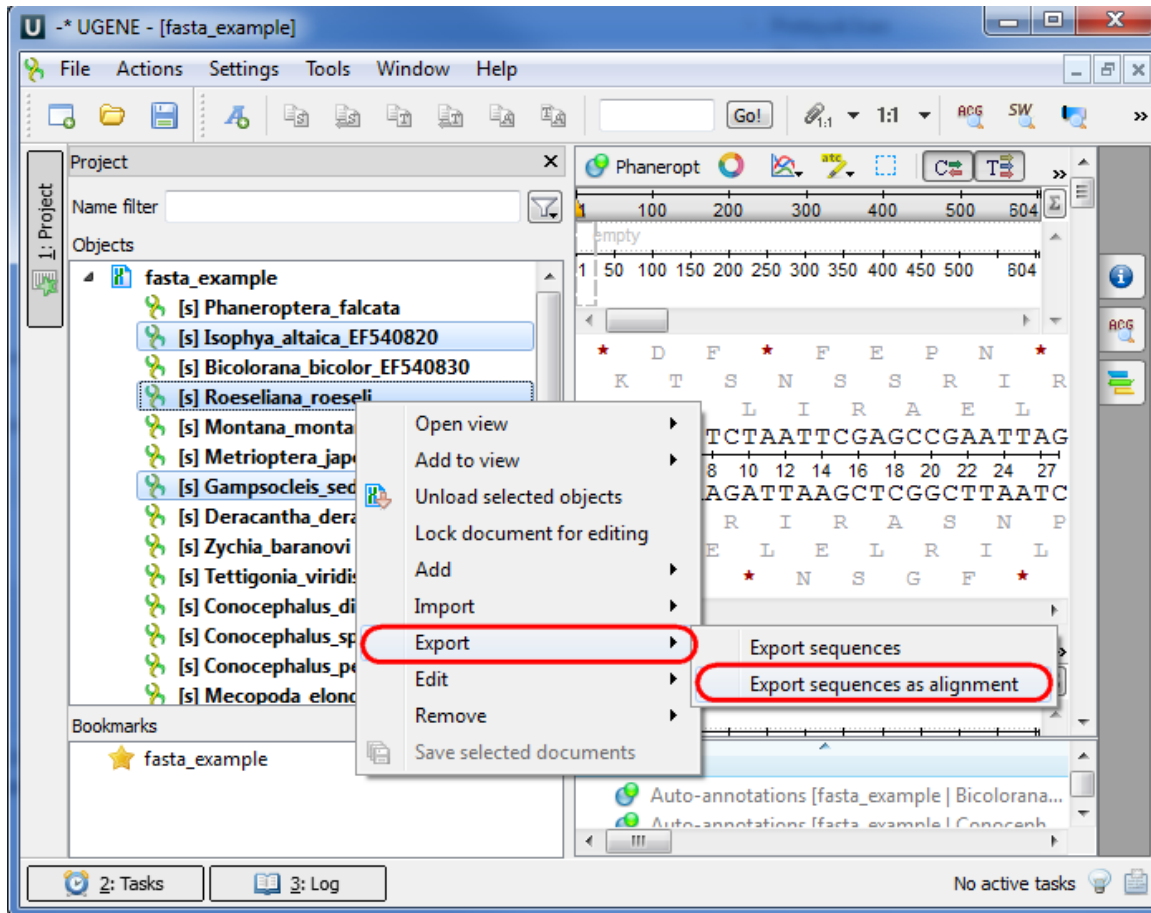
Also it is possible to specify whether to merge the exported sequences into a single sequence or store them as separate sequences. If you merge the sequences, you're allowed to select the gap symbols between sequences. This is the length of the insertion region between sequences that contain **N** symbols for nucleic or **X** for protein sequences.

Export sequence with annotations

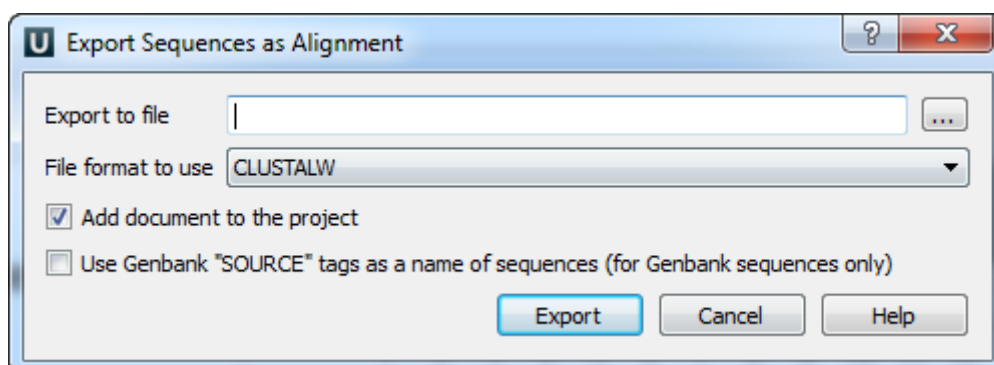
To export sequence with annotations choose Genbank or GFF format. The *Export with annotations* checkbox will be available. Check the checkbox and sequence will be exported with annotations .

Exporting Sequences as Alignment

Suppose, we want to interpret FASTA file as multiple alignment. To do this, select a single or several sequence objects in the *Project View* window, click right mouse button to open the context menu and select the *Export* *Export sequences as alignment* item:

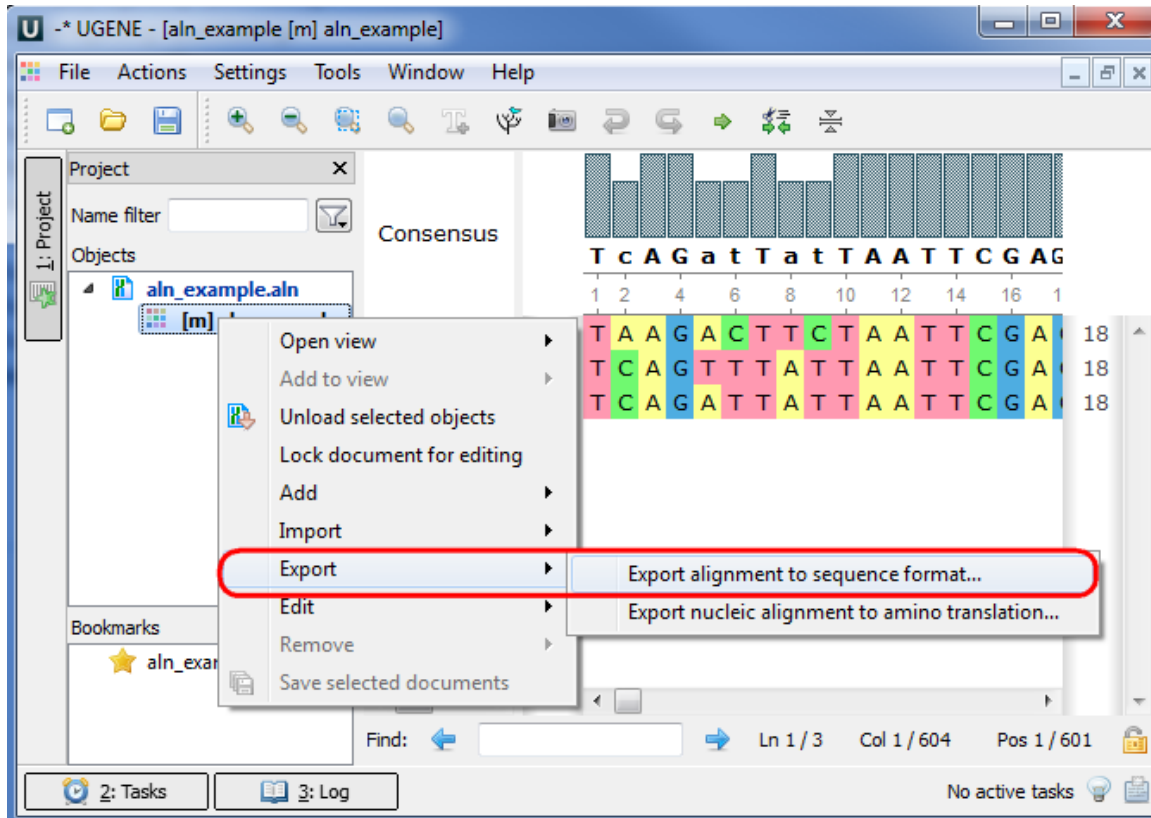


The *Export Sequences as Alignment* dialog will appear where you can point the result alignment file location, to select a multiple alignment file format, to use Genbank "SOURCE" tags as a name of sequences for Genbank sequences and optionally add the created document to the current project:

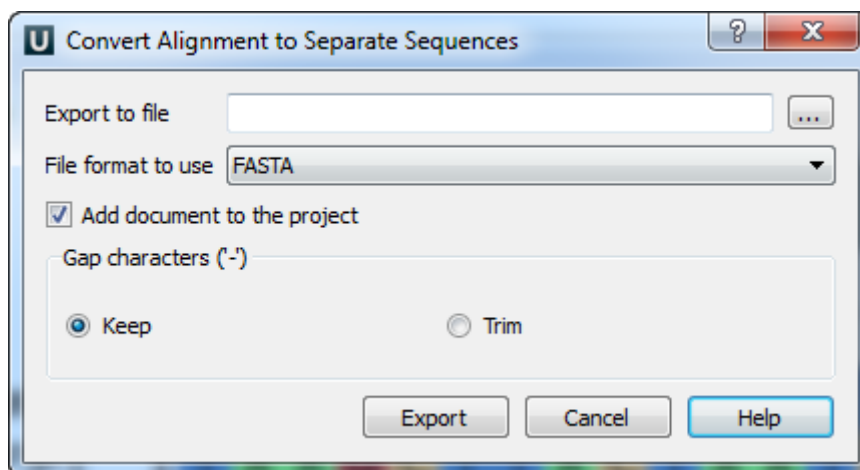


Exporting Alignment to Sequence Format

Select a single object with a sequence alignment in the *Project View* window and click the *Export* *Export alignment to sequence format* context menu item:



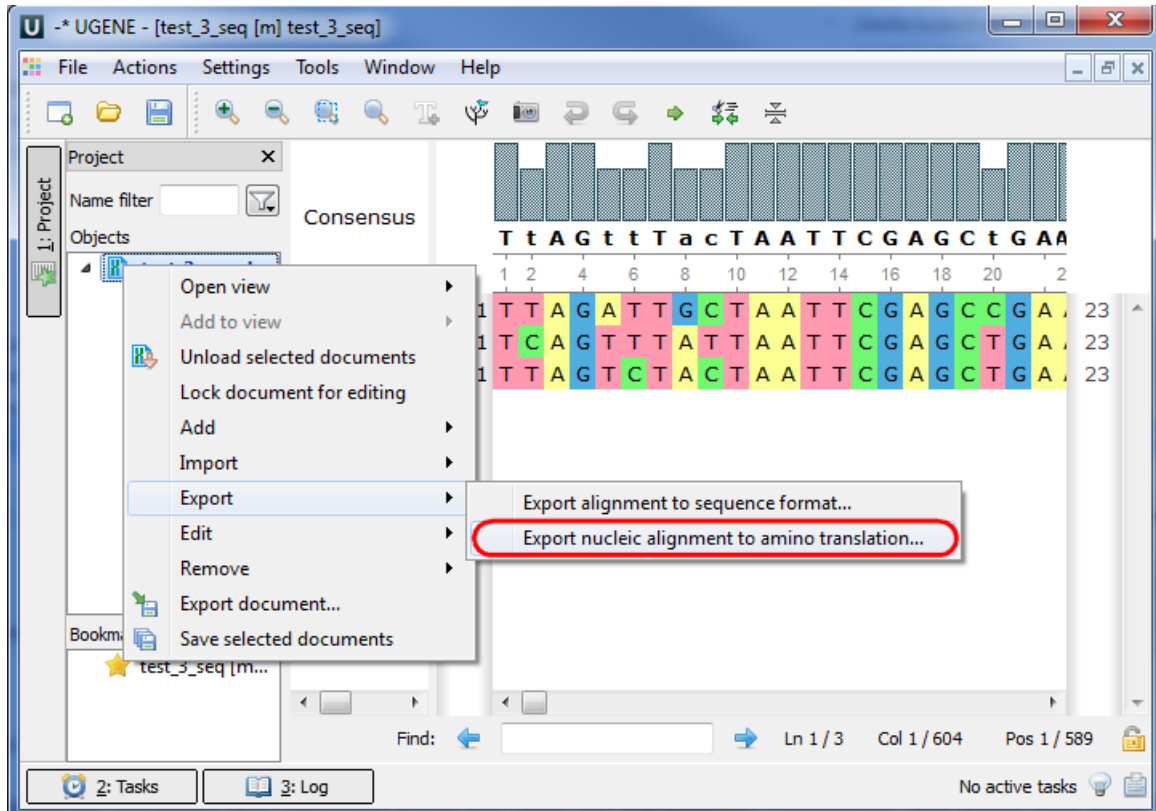
The *Convert Alignment to Separate Sequences* dialog will appear:



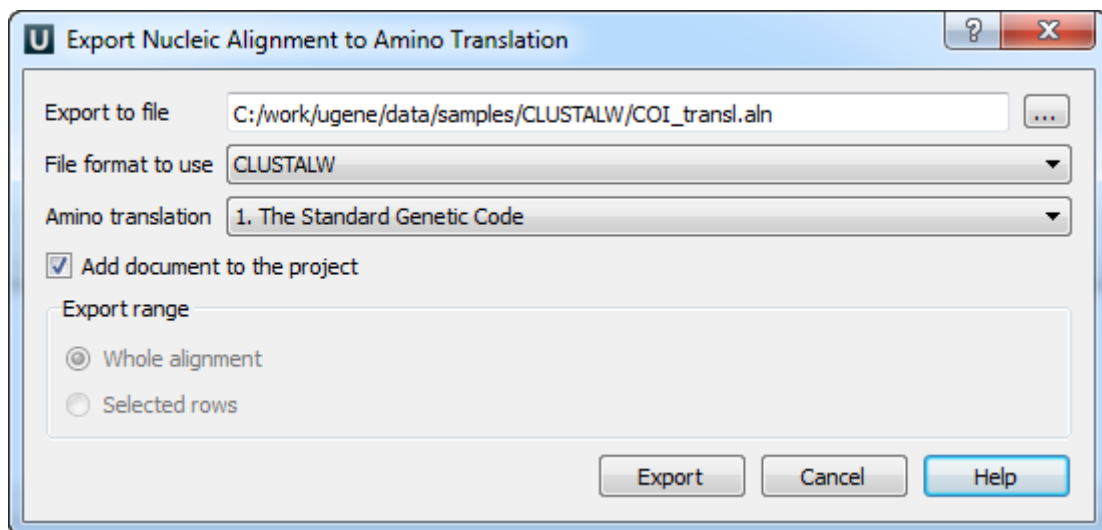
Here it is possible to specify the result file location, to select a sequence file format, to define whether to keep or remove gaps ('-' chars) in the aligned sequences and optionally add the created document to the current project.

Exporting Nucleic Alignment to Amino Translation

Select a single object with a nucleic sequence alignment in the *Project View* window and click the *Export* *Export nucleic alignment to amino translation* context menu item:



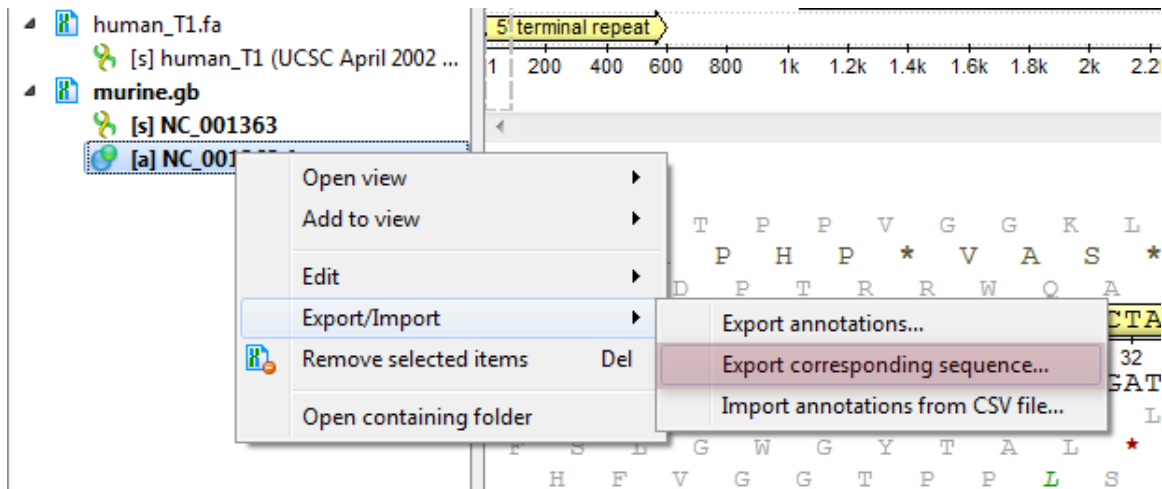
The *Export Nucleic Alignment to Amino Translation* dialog will appear:



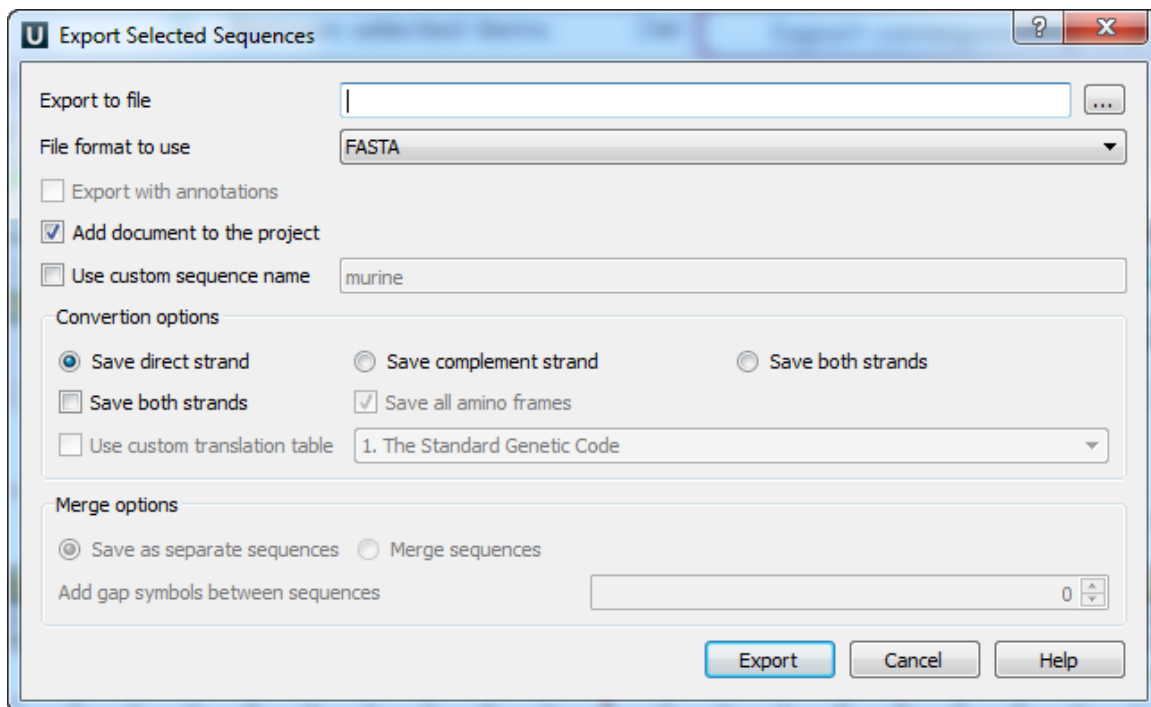
Here it is possible to specify the result file location, to select a file format and an amino translation, to export whole alignment or selected rows and optionally add the created document to the current project.

Export Sequences Associated with Annotation

In UGENE you can export a sequence associated with an annotation. To do it select the annotation in the *Project View* window and click the *Export/Import Export corresponding sequence* context menu item:



The *Export Selected Sequences* dialog will appear:



Here you can select the location of the result file and a sequence file format. You can choose to add newly created document to the current project and use custom sequence name. To do it check the corresponding checkboxes.

Use the *Conversion options* to choose a strand for saving sequence(s). Also you can translate sequence(s) to amino alphabet.

Also it is possible to specify whether to merge the exported sequences into a single sequence or store them as separate sequences. If you merge the sequences, you're allowed to select the gap symbols between sequences. This is the length of the insertion region between sequences that contain **N** symbols for nucleic or **X** for protein sequences.

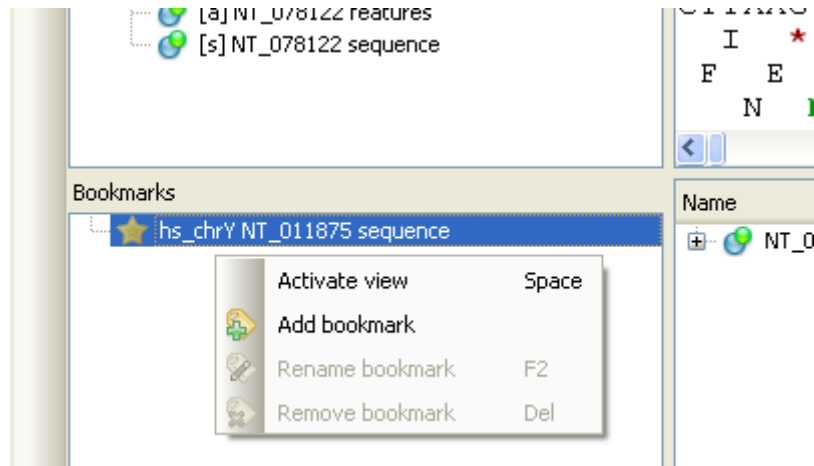
Export sequence with annotations

To export sequence with annotations choose Genbank or GFF format. The *Export with annotations* checkbox will be available. Check the checkbox and sequence will be exported with annotations.

Using Bookmarks

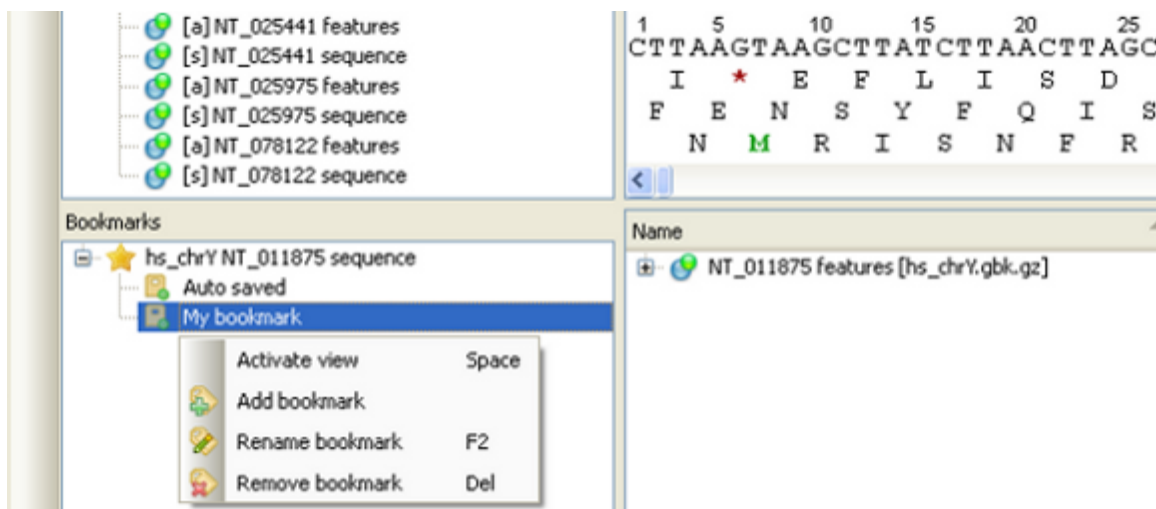
One of the most important features supported by most *Object Views* is an ability to save and restore visual view state. Saving and restoring visual state of an *Object View* enables rapid switching between different data regions and is similar to bookmarks used in Web browsers.

Initially an *Object View* is created as *transient*. It means that its state is not saved. To save current state of a view select an item with the view name in the *Bookmarks* part of the *Project View* windows and select the *Add bookmark* item in the context menu:



For every persistent view UGENE automatically saves the state of the view in the *Auto saved* bookmark when the view is closed.

Now, by activating bookmarks you can restore the original view state. For example for the *Sequence View* bookmarks you can store a visual position and zoom scale for the sequence region.

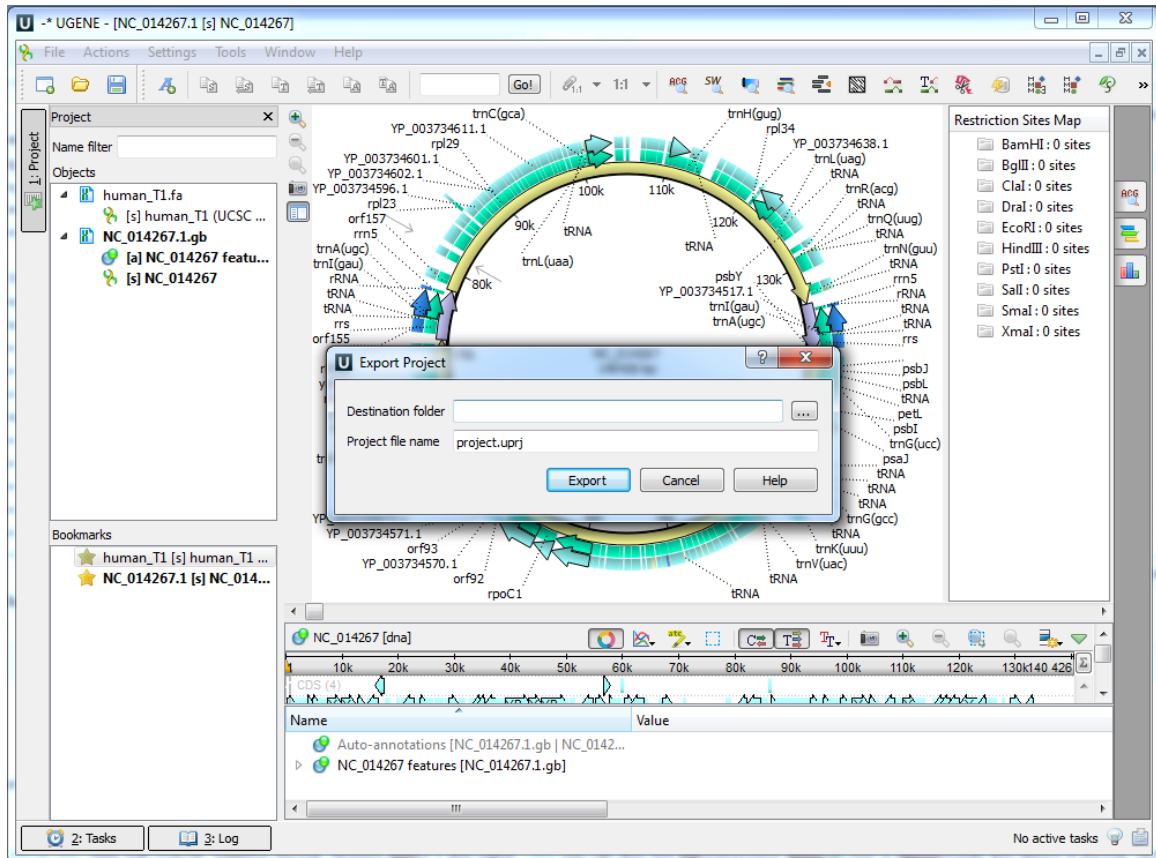


Use the F2 keyboard shortcut to rename a bookmark. To remove a bookmark press the Delete key.

UGENE has limited set of built-in *Object Views*. Extensions modules or plugins can be used to adjust the existing views or to add new views to the tool.

Exporting Project

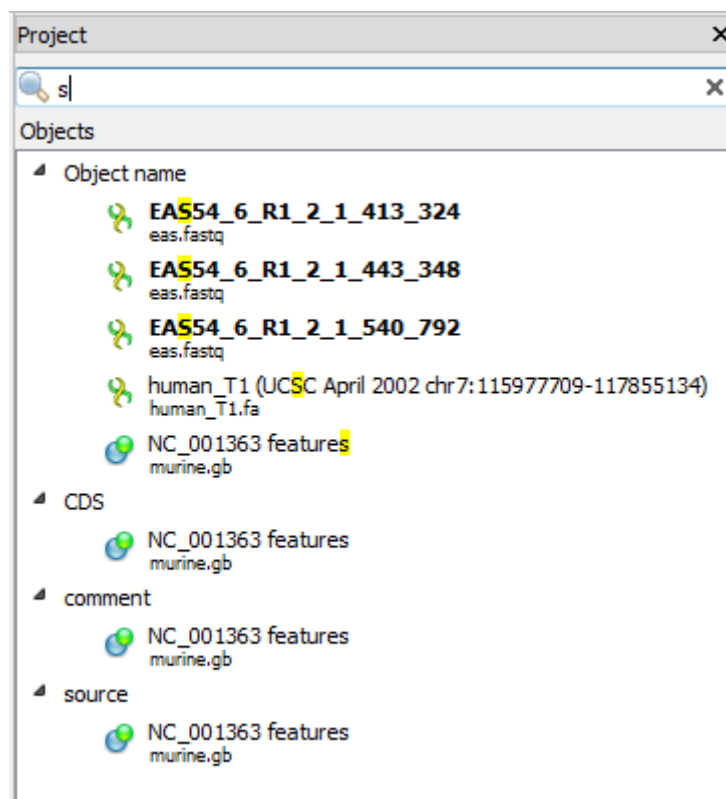
All the opened documents and bookmarks (along with the corresponding views states) can be saved within a project file. To do so, select *File Export Project*. It will invoke the *Export project* dialog, where you can select the destination folder and the project file name.



To load a saved project later, select *File Open* and specify the path to the project file.

Search in Project

Use the search field in the project view to search in the whole project:



Options Panel

The *Options Panel* is available in the *Sequence View* and in the *Assembly Browser*. By default, it is closed. To open a tab of the Options Panel click on the corresponding icon at the right side of a *Sequence View* or *Assembly Browser* window. To close the tab click again on the

tab icon.

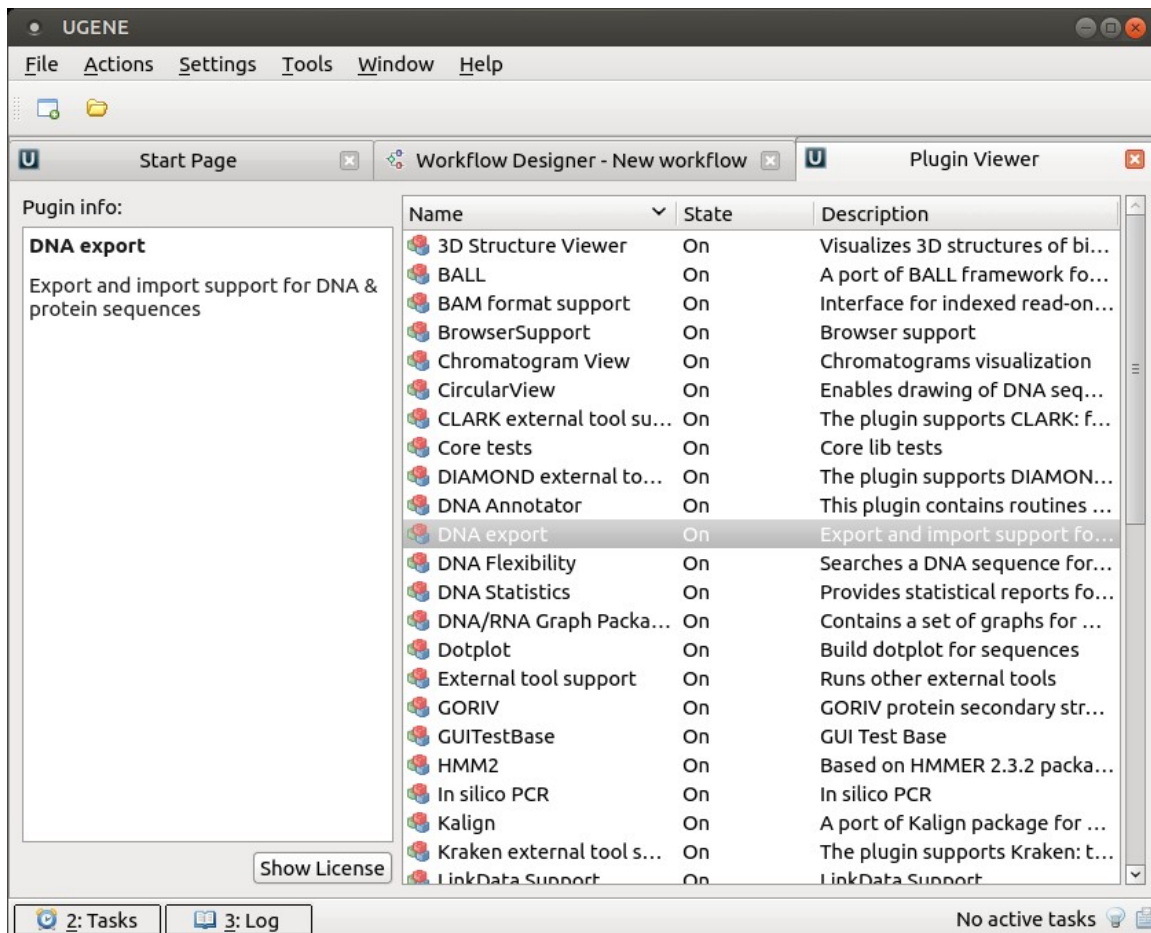
More detailed information about different *Options Panel* tabs can be found in the following chapters:

- *Options Panel in Sequence View*
 - *Information about Sequence*
 - *Search in Sequence*
 - *Highlighting Annotations*
- *Options Panel in Assembly Browser*
 - *Navigation in Assembly Browser*
 - *Assembly Browser Settings*
 - *Assembly Statistic*

Plugins Viewer

A *plugin* is a dynamically loaded module that adds new functionality to UGENE.

To manage plugins select the *Settings Plugins* main menu item. The *Plugin Viewer* window will appear:



The window shows the list of available plugins. Use the *Show License* button to show license each of them.

Searching NCBI Genbank

UGENE allows searching data in NCBI GenBank remote database. To do this open the following dialog by *File->Search NCBI Genbank* main menu:

To search data in the nucleotide or protein databases enter a general text query to the search field, select the database and click on the *Search* button. You can use a protein name, gene name, or gene symbol directly. Searching for a submitter or author name in the following format will produce the best results.

Use the boolean operator AND to find records that contain every one of your search terms, the intersection of search results.

Use the boolean operator OR to find records that include one of several search terms, the union of search results.

Use the boolean operator NOT to exclude records matching a search term.

To limit results use the *Result limit* field.

After you click the *Search* button, UGENE searches the biological objects and shows it in the *Results* field. You can download the object(s). Select one or several objects (for selecting several objects use the *Ctrl* button) and click the *Download* button. The dialog will appear:

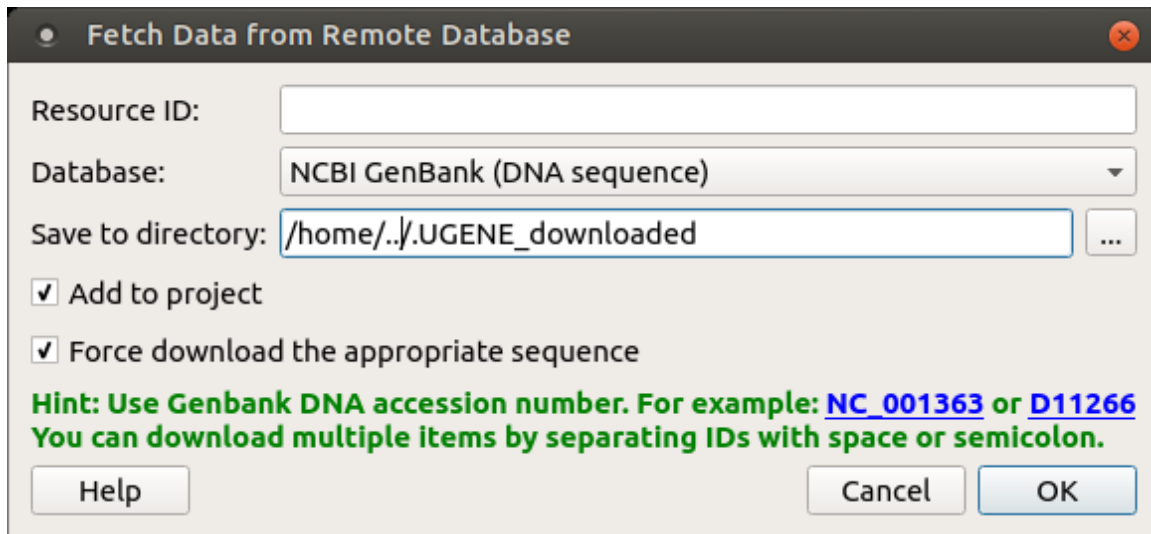
After you click the *OK* button, UGENE downloads the biological objects and adds it to the current *project*.

Fetching Data from Remote Database

UGENE allows fetching data from remote biological databases such as NCBI GenBank, NCBI protein sequence database, and some others.

To fetch data select the *File Access remote database...* item in the main menu.

The dialog will appear:



Here you need to enter the unique id of the biological object and choose a database. The following databases are available: NCBI Genbank (DNA sequence), NCBI protein sequence database, ENSEMBL, PDB, SWISS-PROT, UniProtKB/Swiss-Prot, UniProtKB/TrEMBL. Unique identifiers are different for various databases. For example, for NCBI GenBank such unique id could be [Accession Number](#) or [NCBI GI number](#). Optionally, you can add the document to a project and browse for a directory to save the fetched file to.

After you click the *OK* button, UGENE downloads the biological object (DNA sequence, protein sequence, 3d model, etc.) and adds it to the current *project*.

If something goes wrong check the [Log View](#), it will help you to diagnose the problem.

UGENE Application Settings

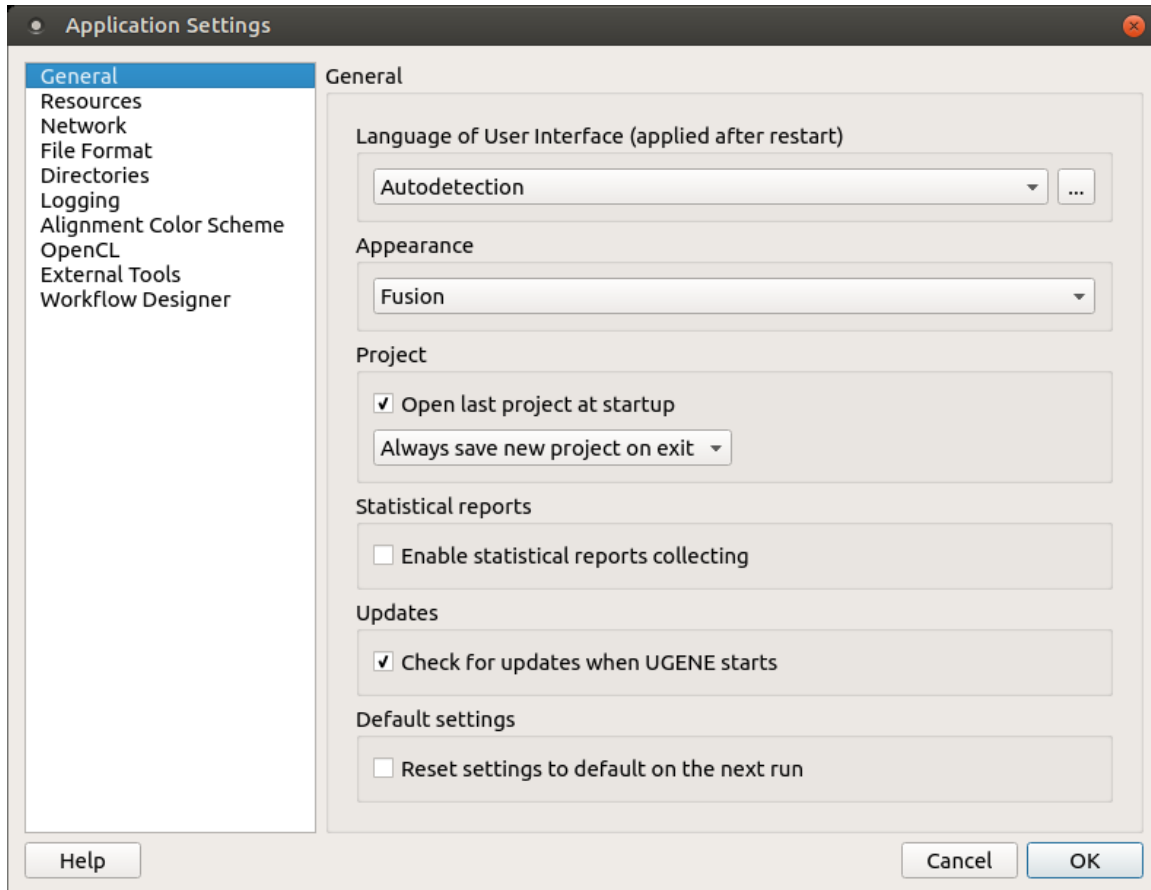
To open the UGENE *Application Settings* dialog:

- On Windows or Linux: select "*Settings Preferences*" in the main menu.
- On macOS: select "*Unipro UGENE Preferences*" in the main menu

The following settings are available:

- General
- Resources
- Network
- File Format
- Directories
- Logging
- Alignment Color Scheme
- External Tools
 - Supported External Tools
 - Custom External Tools
- Workflow Designer Settings
- OpenCL

General



The following settings are available on the tab:

Language of User Interface (applied after restart) — here you can select UGENE localization. Currently, available localizations are EN and RU. The default value (*Autodetection*) specifies that UGENE should use the operating system regional options to select the localization. This setting is applied only after UGENE is reopened.

Appearance — defines the appearance of the application.

Open last project at startup — if the option is checked, the last project is opened when UGENE is started. Also, you can choose default settings for a saving project.

Enable statistical reports collecting — collects information about UGENE usage and sends it to the UGENE team to help improve the application.



The collected information includes:

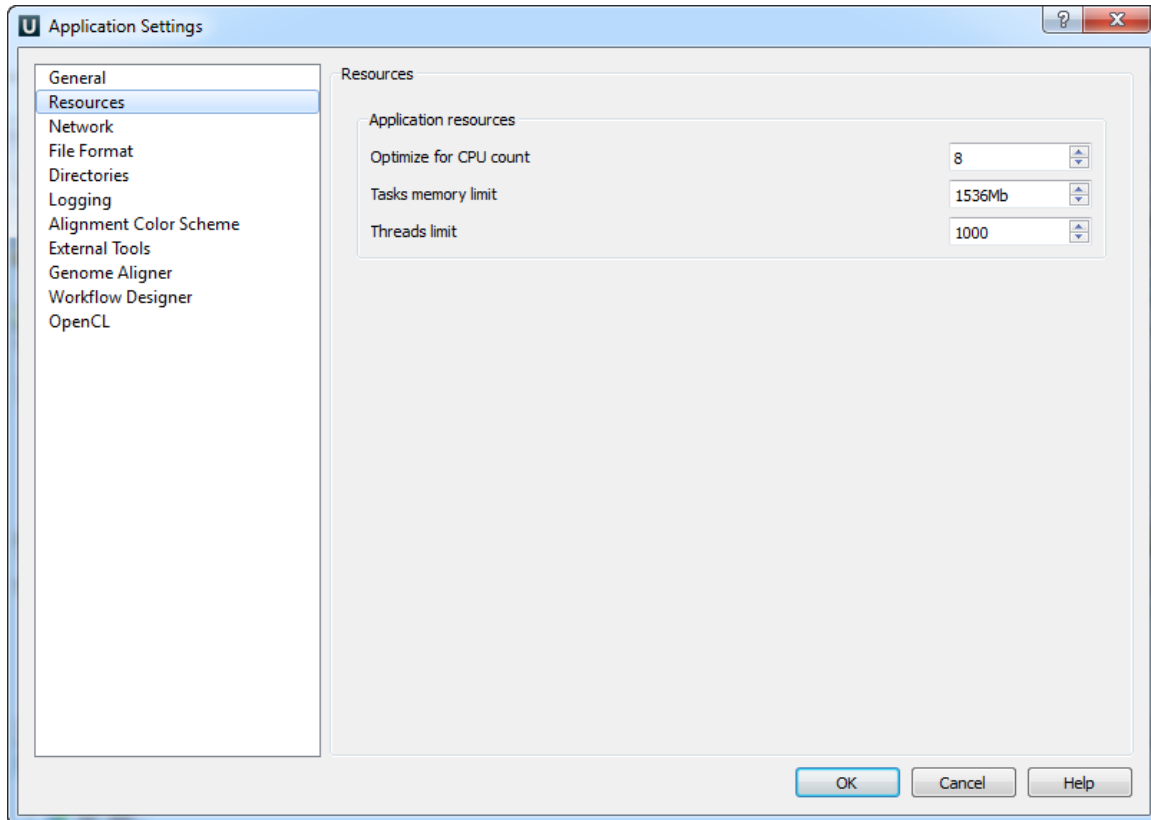
1. System info: UGENE version, OS name, Qt version, etc.
2. Counters info: number of launches of certain tasks (e.g. HMM search, MUSCLE align).

The collected information DOESN'T include any personal data.

Check for updates when UGENE starts - checks for updates when UGENE starts.

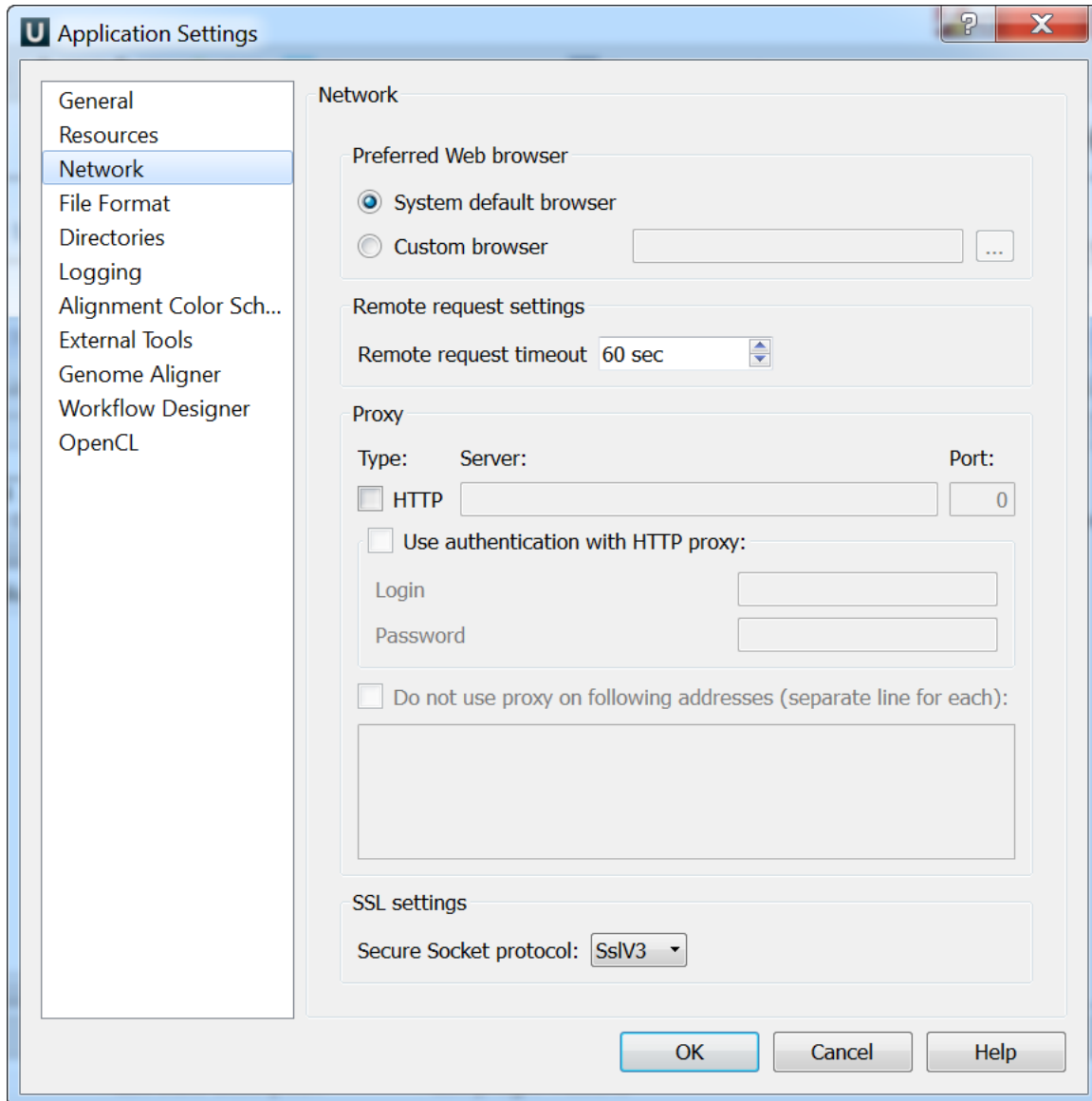
Default settings — this option resets the default settings on the next run.

Resources



On the *Resources* tab you can set resources that can be used by the application: *Optimize for CPU count*, *Tasks memory limit* and *Threads limit*.

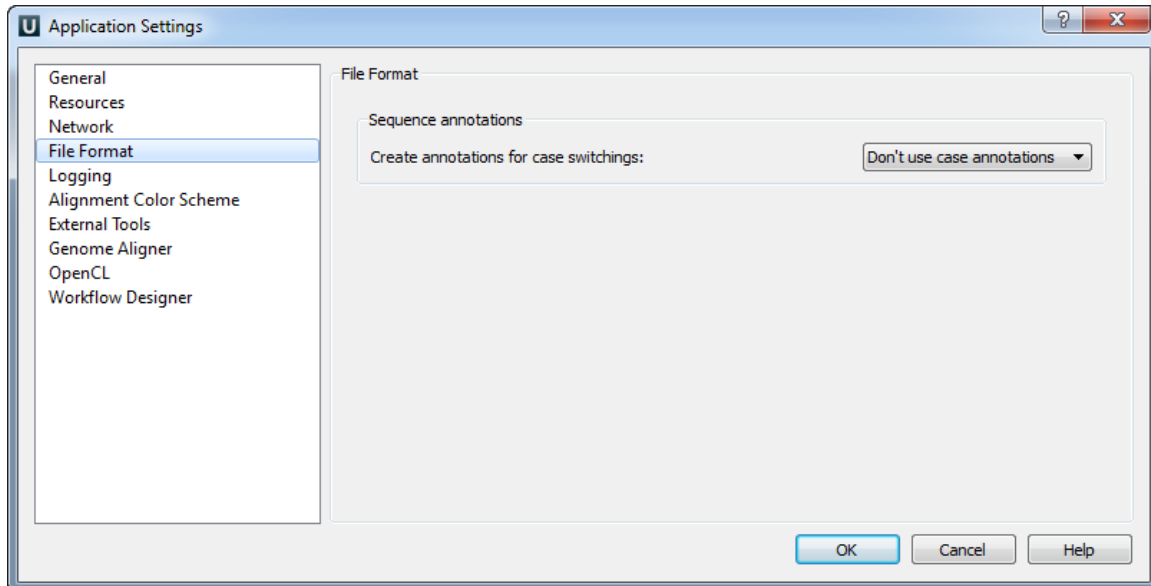
Network



On the *Network settings* tab of the dialog you can specify *Proxy* server parameters, select *SSL settings* and configure the *Remote request timeout*.

Preferred Web browser — you can use either *System default browser* or specify some other browser.

File Format

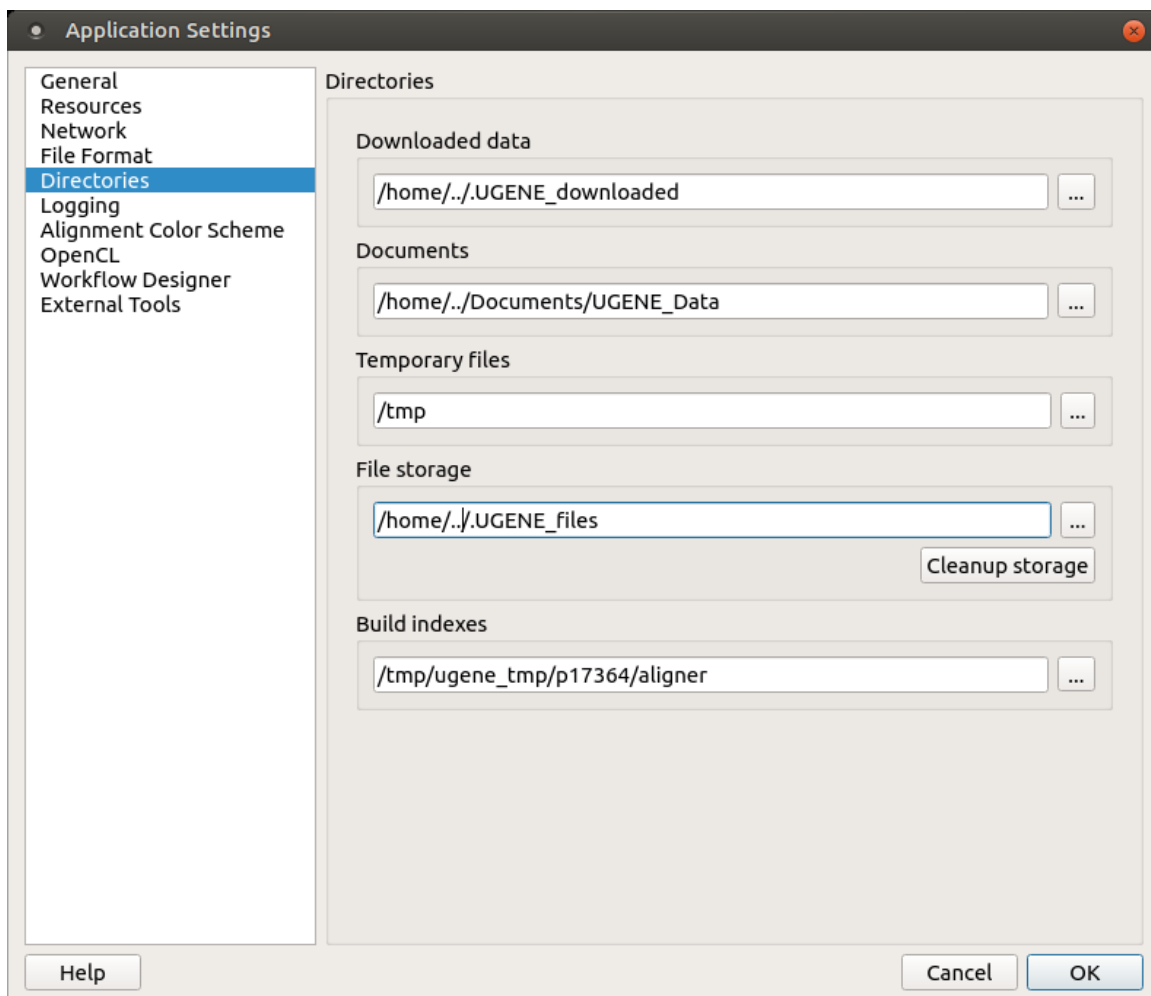


The *Sequence Annotations* settings allows to use upper/lower case annotations during the file reading process.

Format options:

1. *Don't use case annotations* (default mode) — usual sequence reading and writing.
2. *Use lower case annotation* — sequences are read and annotations with names *lower_case* are added. When these sequences are written to file then the case becomes like original the file case (the case is saved).
3. *Use upper case annotation* — there is a similar behavior but with “upper_case” annotations.

Directories



The following settings are available on the tab:

Downloaded data — specifies the path where files downloaded from the remote databases will be stored.

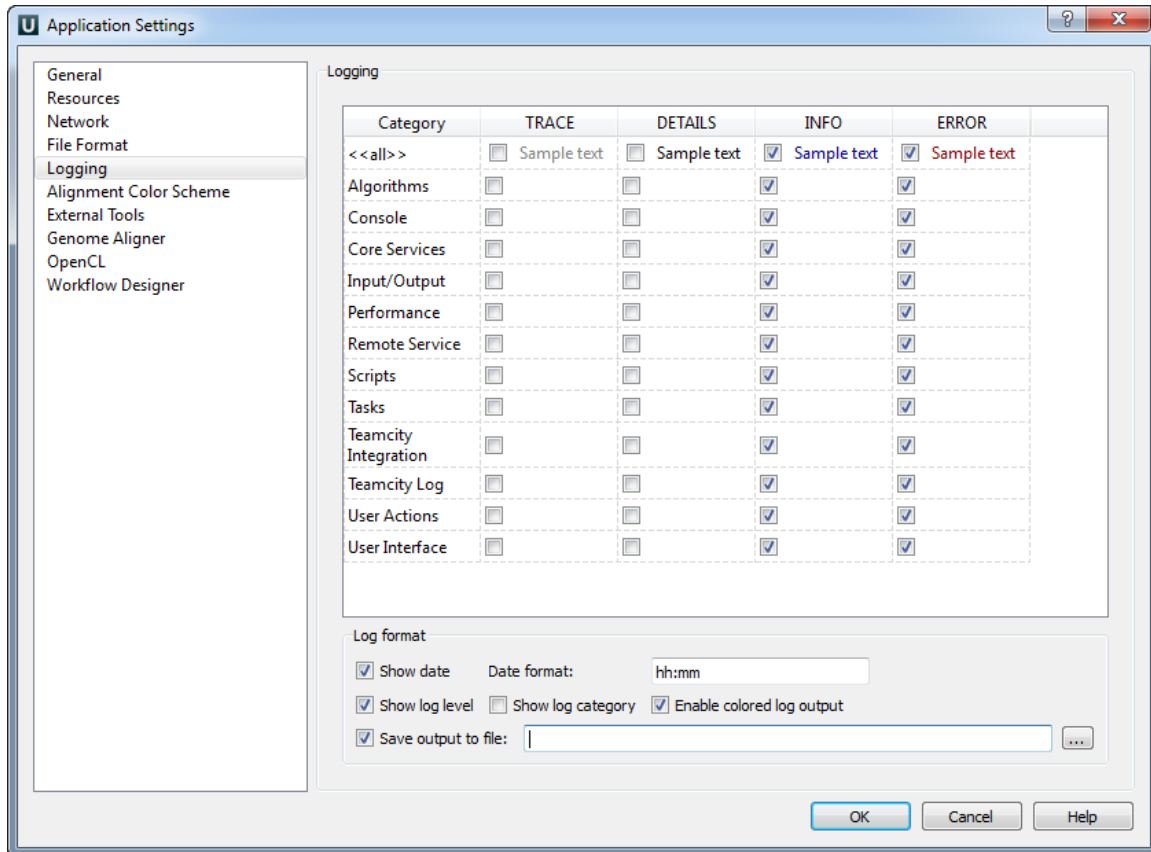
Documents - the path where will be stored UGENE data.

Temporary files — the path where will be stored temporary files.

File storage — the path where will be stored UGENE files.

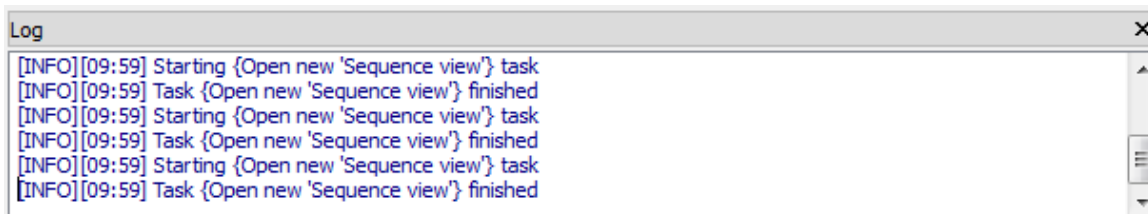
Build indexes - the path where will be stored UGENE Genome Aligner indexes.

Logging

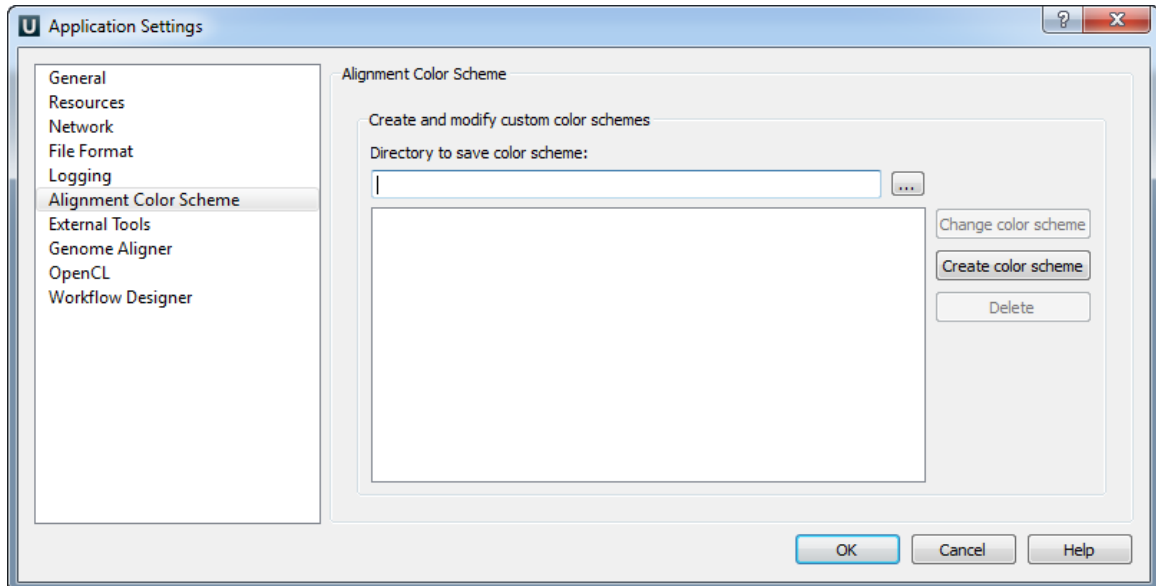


On the *Logging* tab you can select type of log information (*ERROR*, *INFO*, *DETAILS*, *TRACE*) for each *Category* that will be output to the *Log View*.

You can select format for each log message by checking the *Show date*, *Show log level* and *Show log category* options.



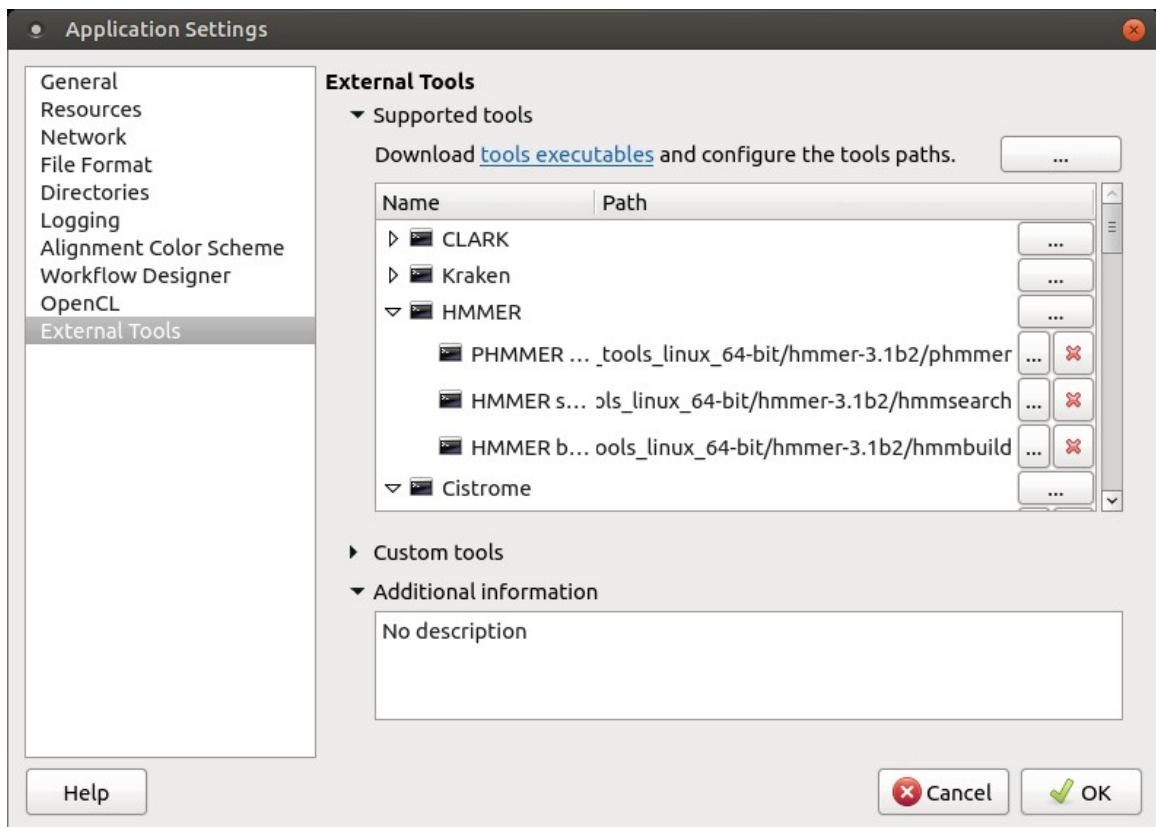
Alignment Color Scheme



On the *Alignment Color Scheme* tab you can create, change and delete custom color schemes.

External Tools

Set executable files for external tools:



Supported External Tools

Executable paths for external tools, already integrated into UGENE, can be configured in the "Supported tools" group of the "External tools" page of the "Application Settings" dialog. See the full list of the supported tools in the "[Data Analysis Tools](#)" chapter of the documentation.

Custom External Tools

A custom tool can be integrated into the UGENE GUI as a workflow element in the [Workflow Designer](#) - a UGENE component that allows joining tools into workflows.

As described in the "[Custom Elements with External Tools](#)" chapter of the Workflow Designer documentation, there are two options when one creates such workflow element:

- Set a local executable path
- Set an integrated external tool

The first option is intended for cases when a quick temporary integration of a tool into a workflow is enough.

The second option, although requires additional configuration steps, provides a more close integration with UGENE. It can be used by advanced UGENE users for providing a tool for their colleagues.

Below the preparatory configuration steps for the second option are described.

Create an XML configuration file for custom external tool

Create an XML file with the custom external tool description. The file should match the following XML schema:

```
<?xml version = "1.0" encoding = "UTF-8"?>
<xs:schema xmlns:xs = "http://www.w3.org/2001/XMLSchema">

<xs:element name = "ugeneExternalToolConfig">
  <xs:complexType>
    <xs:sequence>
      <xs:element name = "name" type = "xs:string" />
      <xs:element name = "id" type = "xs:string" />
      <xs:element name = "executableName" type = "xs:string" />
      <xs:element name = "executableFullPath" type = "xs:string" minOccurs
="0" />
      <xs:element name = "description" type = "xs:string" minOccurs = "0" />
      <xs:element name = "version" type = "xs:string" minOccurs = "0" />
      <xs:element name = "launcherId" type = "xs:string" minOccurs = "0" />
      <xs:element name = "dependencies" type = "xs:string" minOccurs = "0" />
    </xs:sequence>
    <xs:attribute name = "version" type = "xs:string" use="required" />
  </xs:complexType>
</xs:element>

</xs:schema>
```

These XML elements have the following meaning:

- **name** - the external tool name as it should be displayed in the UGENE GUI. This XML element is mandatory.
- **id** - the external tool unique identifier. The following characters are allowed: letters ('A'-'Z'), digits ('0'-'9'), '_', '-'. This XML element is mandatory.
- **executableName** - the name of the tool executable file (with the file extension, if it is present). This XML element is mandatory.
- **executableFullPath** - a full path to the external tool executable. This XML element is optional. If the "executableFullPath" value is not specified, UGENE searches for a file with the specified "executableName" name in the same folder as the XML config file and sets the tool path accordingly. One can also specify either an absolute path to a file or a path, relative to the XML config. The path is displayed near the tool in the "Application Settings" dialog. It can be also manually set up by a user.
- **description** - a detailed description of the external tool. This XML element is optional.
- **version** - a version of the tool executable. This XML element is optional.
- **launcherId** - if the tool is supposed to be run with some script interpreter or it is a java application, then this element should contain an appropriate ID. Currently, UGENE supports the following launchers IDs: **USUPP_PYTHON2**, **USUPP_PERL**, **USUPP_RSCRIPT**, **USUPP_JAVA**. If the launcher is specified, it would be added to the launch command explicitly whenever you try to launch the external tool. The path to the launcher will be taken from the "Application Settings" (see the corresponding tools in the "Supported tools" group). If the element is not specified or it is empty, no additional launcher will be added to the command that will run the tool. This XML element is optional.
- **dependencies** - a comma-separated list of other external tool IDs the new tool is dependent from. If the dependency is not satisfied, then the dependent tool is marked as "not valid" in UGENE. This XML element is optional.

See also the following examples:

- A minimalistic config:

```
<?xml version = "1.0" encoding = "UTF-8"?>
<ugeneExternalToolConfig version = "1.0">
  <name>An imported custom tool</name>
  <id>CUSTOM_TOOL</id>
  <executableName>my_tool.py</executableName>
</ugeneExternalToolConfig>
```

This config describes only the tool name, the tool ID and the tool executable file path. If the XML config is imported from the same folder as the python script, the path will be set to the python script. The tool won't have any dependencies, its version will be "unknown".

- A config without dependencies and a launcher:

```
<?xml version = "1.0" encoding = "UTF-8"?>
<ugeneExternalToolConfig version = "1.0">
  <name>RAXML</name>
  <id>RAXML</id>
  <executableName>raxmlHPC</executableName>
  <executableFullPath>./my_folder/raxmlHPC</executableFullPath>
  <description>RAXML - Randomized Axelerated Maximum
Likelihood</description>
  <version>8.2.12</version>
</ugeneExternalToolConfig>
```

This config describes an external tool with a description and a version. Path to the tool executable is relative: it is supposed that the folder structure is the following:

```
my_config.xml
my_folder/
  raxmlHPC
```

- A config with a dependency and a launcher:

```
<?xml version = "1.0" encoding = "UTF-8"?>
<ugeneExternalToolConfig version = "1.0">
  <name>HISAT2</name>
  <id>HISAT2</id>
  <executableName>hisat2</executableName>
  <executableFullPath>d:\work\hisat2.1\hisat2</executableFullPath>
  <description>HISAT2 is a fast and sensitive alignment program for
mapping next-generation sequencing reads (both DNA and RNA) to a
population of human genomes (as well as to a single reference
genome).</description>
  <toolkitName>HISAT2</toolkitName>
  <version>2.1.0</version>
  <launcherId>USUPP_PERL</launcherId>
  <dependencies>USUPP_PERL</dependencies>
</ugeneExternalToolConfig>
```

This config describes a tool that is supposed to be run by the Perl interpreter. There is no Perl by default on Windows, but the UGENE installer provides a possibility to install Perl. The tool can notify UGENE that it should be launched by Perl, so UGENE will add a path to Perl to the command that runs the tool. The XML config also specifies an absolute full path to the executable file.

Prepare a package with the XML configuration file, the tool executable and, optionally, other files

The easiest way to "pack" the custom external tool is to:

- create a new folder with all files required for running the tool,

- specify a relative path in the XML configuration file described above,
- put the XML configuration file into the same folder as the main tool executable.

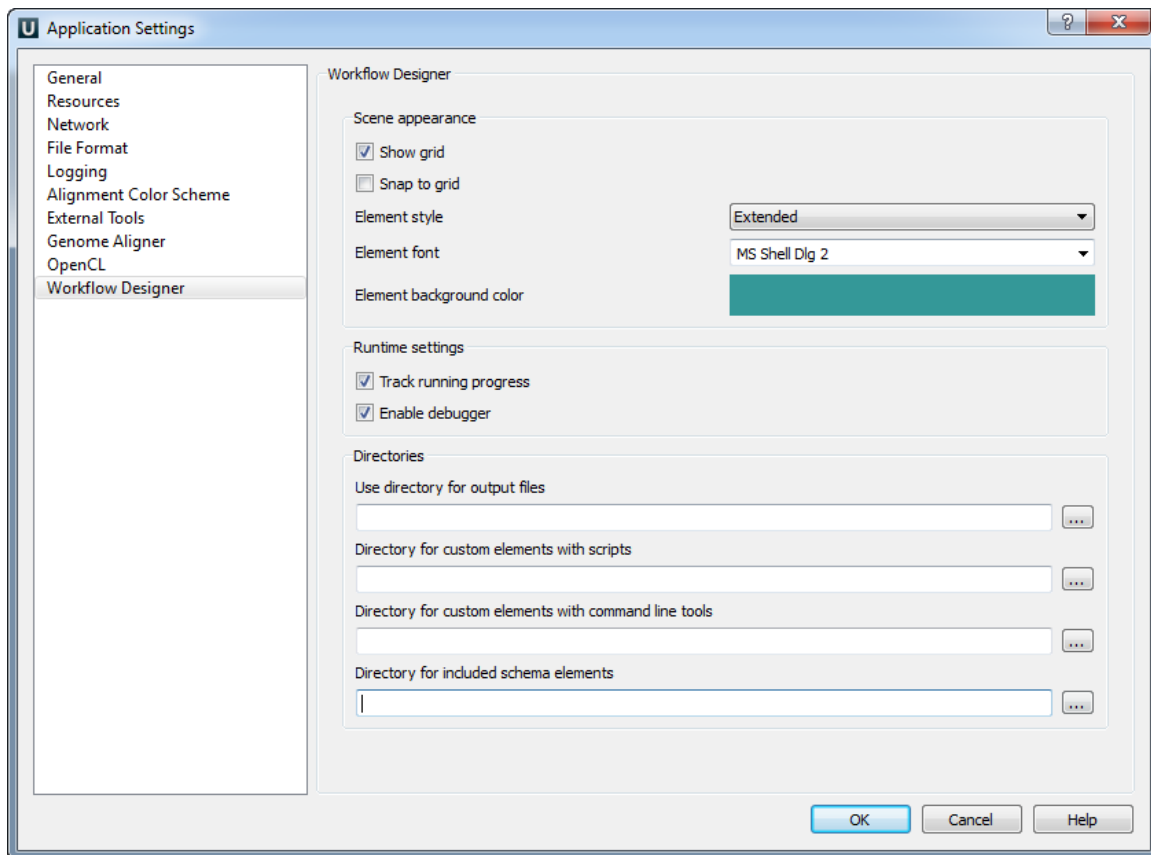
This "package" can be shared with other users, if required.

Import the tool into UGENE

To add the custom external tool one needs to import its XML configuration file in the "Custom tools" group on the "External Tools" page of the "Application Settings" dialog.

Workflow Designer Settings

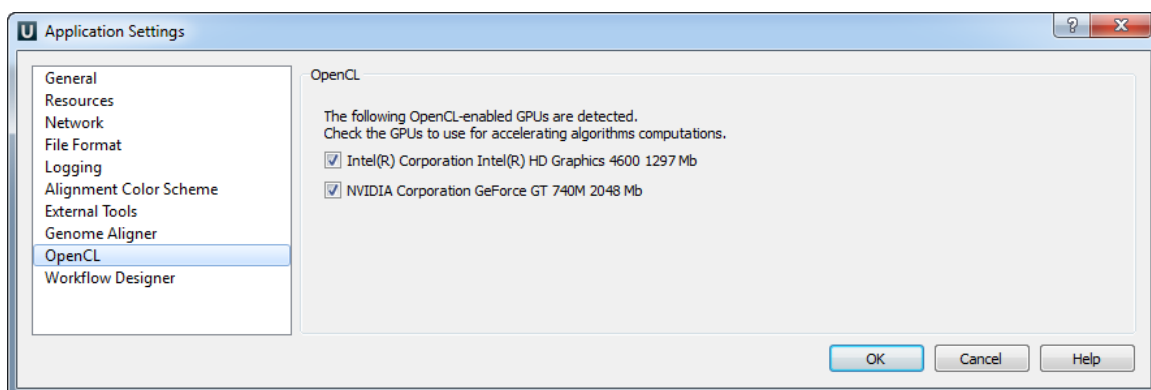
Use this tab to configure the Workflow Designer settings:



OpenCL

If you have a video card that supports OpenCL you can use it to speed up some calculations in UGENE.

To do it install the latest video card driver and check the corresponding check box:



Now you can, for example, use OpenCL optimization for the *Smith-Waterman algorithm*.

Sequence View

- Sequence View Components
- Global Actions
- Sequence Toolbars
- Sequence Overview
- Zoom View
- Details View
- Information about Sequence
- Manipulating Sequence
 - Show/Hide Sequence View Components
 - Showing Sequence in Multiple Lines
 - Translating Nucleotide Sequence
 - Zooming Sequence
 - Creating New Ruler
 - Going To Position
 - Selecting Sequence Region
 - Copying and Pasting Sequence
 - Editing Sequence
 - Searching in Sequence
 - Load Patterns from File
 - Search Algorithm
 - Search in
 - Other Settings
 - Annotations Settings
 - Exporting Selected Sequence Region
 - Exporting Sequence of Selected Annotations
 - Exporting Sequence Image
 - Locking and Synchronize Ranges of Several Sequences
 - Multiple Sequence Opening
- Annotations Editor
 - "db_xref" Qualifier
 - Automatic Annotations Highlighting
 - The "comment" Annotation
- Manipulating Annotations
 - Creating Annotation
 - Selecting Annotations
 - Editing Annotation
 - Highlighting Annotations
 - Annotations Color
 - Annotations Visibility
 - Show on Translation
 - Captions on Annotations
 - Creating and Editing Qualifier
 - Adding Column for Qualifier
 - Copying Qualifier Text
 - Finding Qualifier
 - Deleting Annotations and Qualifiers
 - Importing Annotations from CSV
 - Exporting Annotations

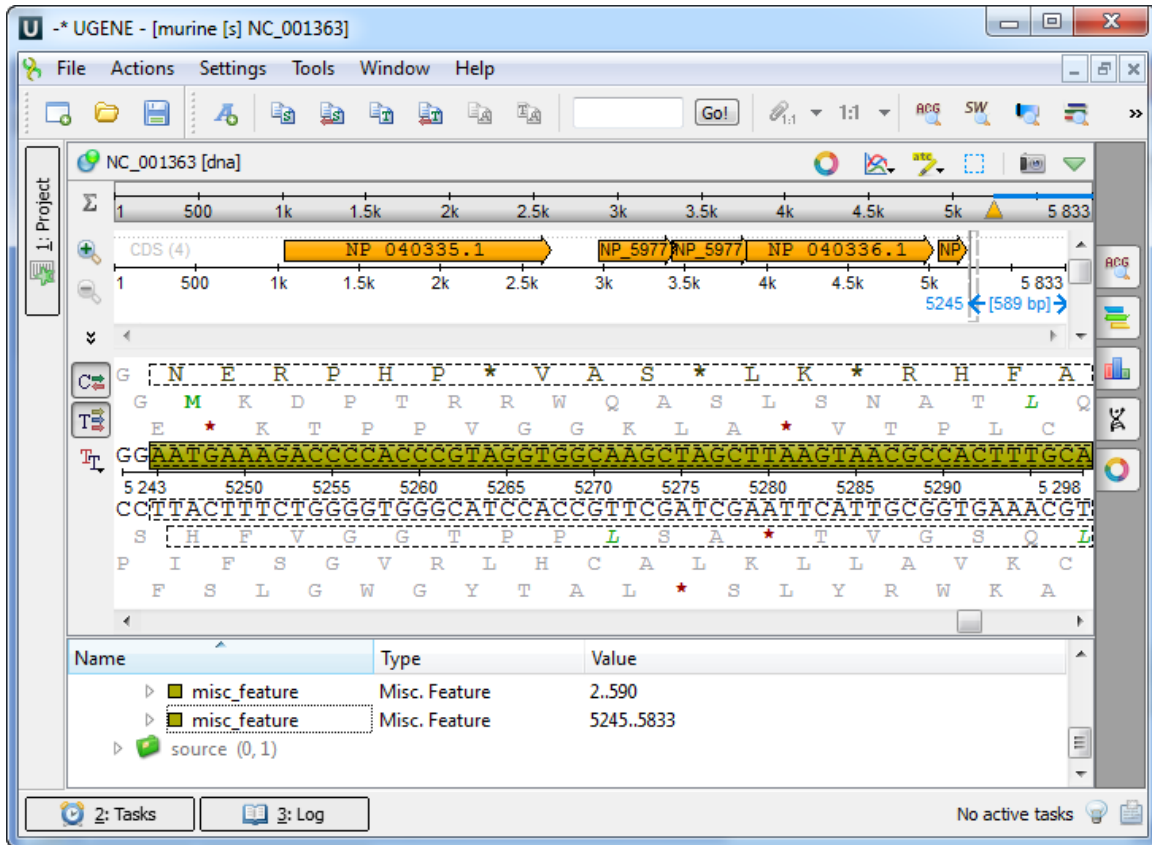
Sequence View Components

The *Sequence View* is one of the major *Object Views* in UGENE aimed to visualize and edit DNA, RNA or protein sequences along with their properties like annotations, chromatograms, 3D models, statistical data, etc.

For each file UGENE analyzes the file content and automatically opens the most appropriate view.

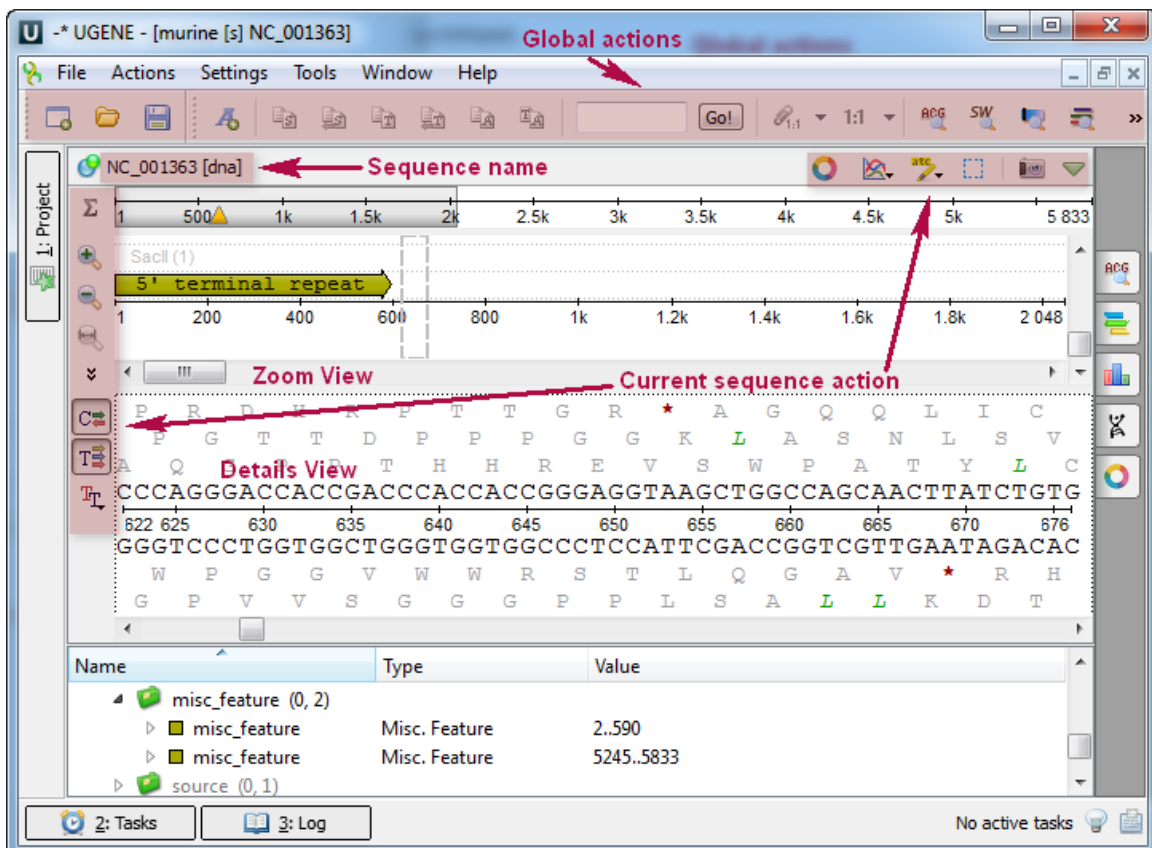
To activate the *Sequence View* open any file with at least one sequence. For example you can use the \$UGENE/data/samples/EMBL/AF177870.emb file provided with UGENE.

After opening the file in UGENE the *Sequence View* window appears:

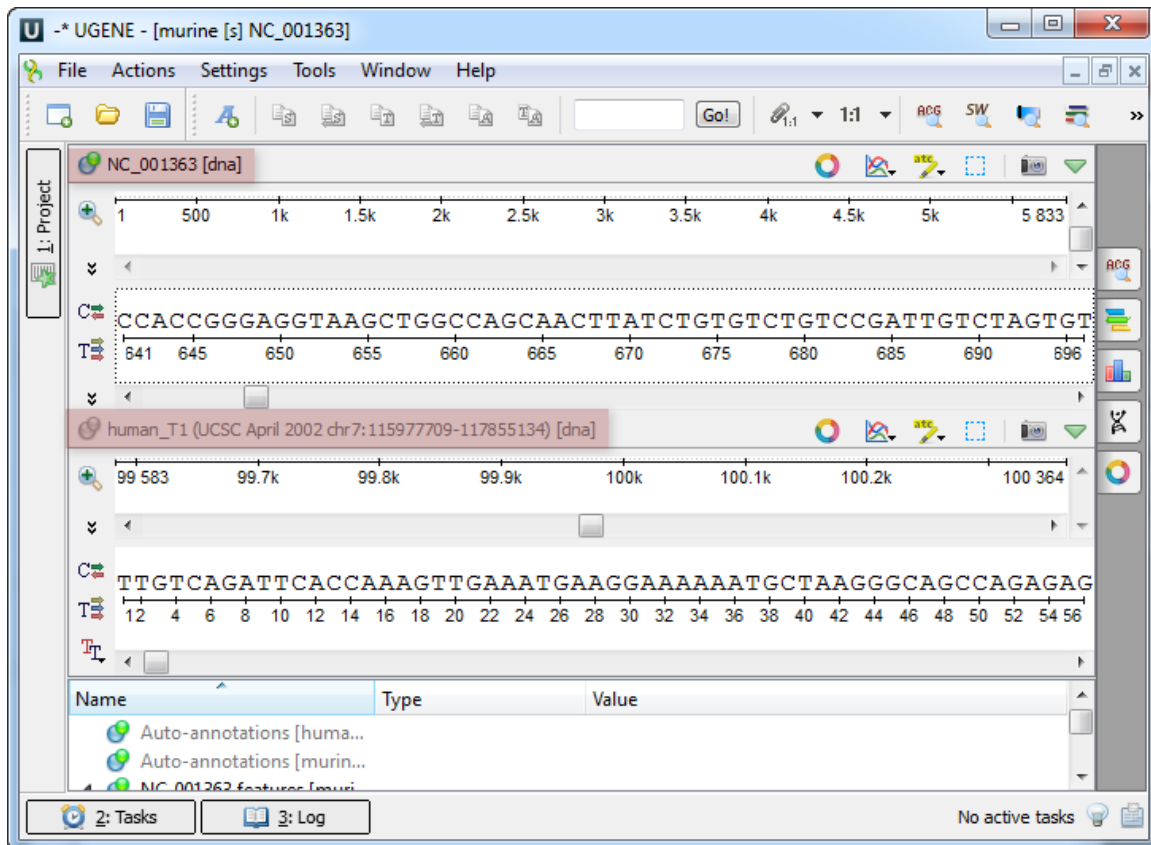


After the view is opened you can see a set of new buttons in the toolbar area. The actions provided by these buttons are available for all sequences opened in the view. In the picture below these buttons are pointed by the “Global actions” arrow.

Below the toolbar there is an area for a single or several sequences. For each sequence a smaller toolbar with actions for the sequence and the following areas are available:



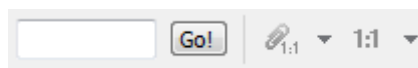
An example of the *Sequence View* with several sequences:



You can change the focus by clicking on the corresponding sequence area. All sequences that are not in focus have the sequence name and icon disabled.

The bottom area of the *Sequence View* is the *Annotations Editor*. It contains a tree-like structure of all annotations available for all sequences shown in the *Sequence View* and can be used to perform various actions on annotations: create a new annotation, modify the existing one, group, sort, etc.

Global Actions

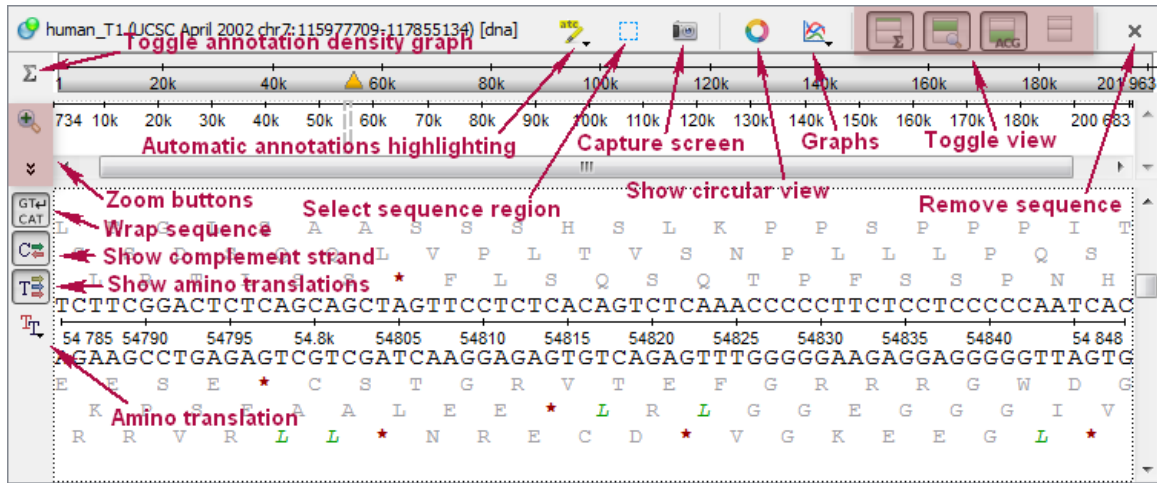


The global action toolbar provides possibility to go to the specified position (in all sequences at the same time).

Also it allows to lock or adjust ranges of sequences in the same *Sequence View*. See [this paragraph](#) for details.

Sequence Toolbars

A brief description of the sequence toolbars buttons is shown on the picture below:

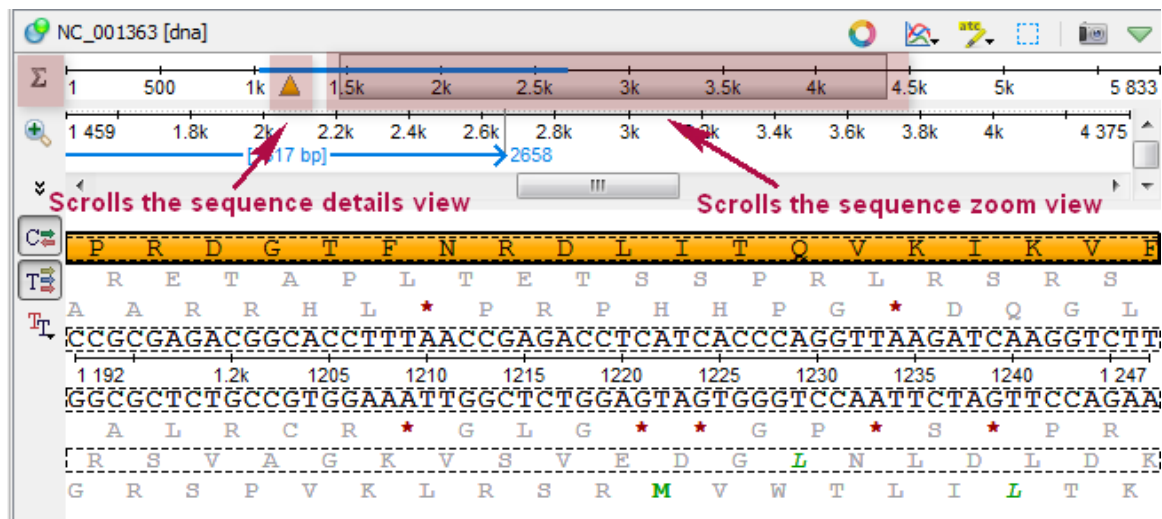


See also:

- [Toggling Views](#)
- [Capturing Screenshot](#)
- [Zooming Sequence](#)
- [Showing and Hiding Translations](#)
- [Selecting Sequence Region](#)
- [Showing Sequence in Multiple Lines](#)

Sequence Overview

The *Sequence overview* is an area of the *Sequence View* below the sequence toolbar. It shows the sequence in whole and provides handy navigation in the *Sequence zoom view* and the *Sequence details view*.



When the sigma button (in the left part of the *Sequence overview*) is pressed, density of annotations in the sequence is shown. For example in the picture below there are annotations in the parts of the sequence that are marked with dark grey color:



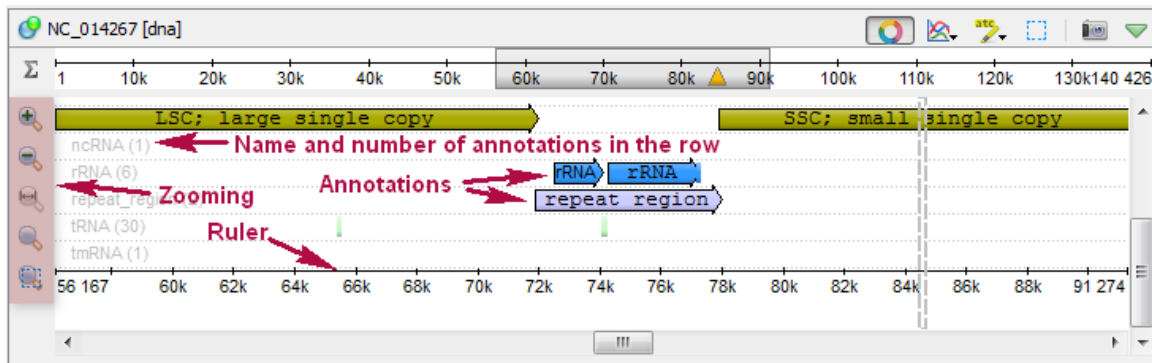
See also:

- [Sequence Zoom View](#)
- [Sequence Details View](#)

Zoom View

The *Sequence zoom view* is designed to provide flexible tools for navigation in large annotated sequence regions.

The most *Sequence zoom view* space is used to visualize annotations for the sequence. The annotations are organized in rows by their names. If two annotations with the same name overlap, an extra row is created. For every row the name and the total number of annotations in the row are shown with a light grey text at the left part of the area.

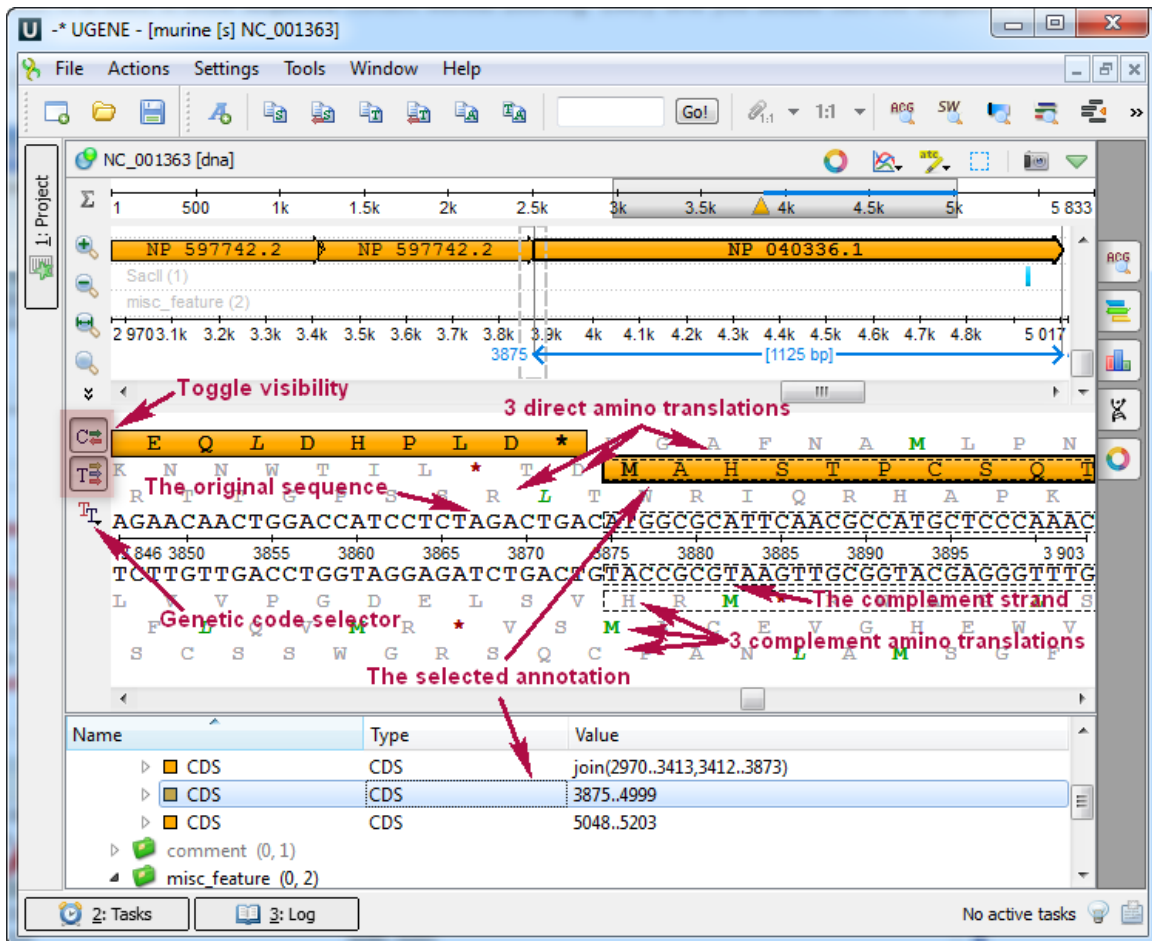


Below the annotation rows there is a ruler to show coordinates in the sequence.

Details View

The *Sequence details view* is a supplementary component of the *Sequence overview*. It is used to show sequence content without zooming. Every time you double click the sequence in the *Sequence overview* area or select an annotation, the corresponding sequence position is made visible in the *Sequence details view*.

For a DNA sequence the *Sequence details view* automatically shows complement DNA strand and 6 amino translation frames.



See also:

- [Navigating the Details View using the Sequence Overview](#)
- [Translating Nucleotide Sequence](#)

Information about Sequence

Context information about a sequence can be found on the *Statistics* tab in the *Options Panel*. All information is contextual, i.e. it shows statistics about the currently selected region (on the selected sequence). The tab includes information about:

- Common statistics
 - Length - number of bases in the analyzed sequence
 - GC content - the molar percentage of guanine and cytosine bases in an oligonucleotide sequence
 - Molar weight - is the sum of the atomic masses of the constituent atoms for 1 mole of oligonucleotide
 - Molar ext. coefficient - the molar extinction coefficient is a physical constant that is unique for each sequence and describes the amount of absorbance at 260nm (A_{260}) of 1 mole/L DNA solution measured in 1 cm path-length cuvette
 - Melting T_M - melting temperature is the temperature at which an oligonucleotide duplex is 50% in single-stranded form and 50% in double-stranded form
 - nmole/ OD_{260} - the amount of oligonucleotide in nanomoles that, when dissolved in 1 mL volume, results in 1 unit of absorbance at 260 nm with a standard 1 cm path-length cuvette
 - g/ OD_{260} - the amount of oligonucleotide in micrograms that, when dissolved in 1 mL volume, results in 1 unit of absorbance at 260 nm with a standard 1 cm path-length cuvette
- Characters occurrence
- Dinucleotides occurrence (for sequences with the standard DNA and RNA alphabets)

The screenshot displays the Unipro UGENE software interface. The main window shows a DNA sequence with a ruler at the top indicating positions from 1 to 5,833. A selected region is highlighted in green. Below the sequence, a table lists annotations:

Name	Type	Value
Auto-annotations [murine.gb NC_001363]		
NC_001363 features [murine.gb]		
CDS (0, 4)		
CDS	CDS	1042..2658
CDS	CDS	join(2970..3413,...
CDS	CDS	3875..4999
CDS	CDS	5048..5203
comment (0, 1)		
misc_feature (0, 2)		
misc_feature	Misc. Feature	2..590
misc_feature	Misc. Feature	5245..5833
source (0, 1)		

On the right side, the *Statistics* panel is visible, showing the following data:

- ▼ Common Statistics
 - Length:** 589
 - GC Content:** 52.12%
 - Molar Weight:** 181766.49 Da
 - Molar Ext. Coef:** 6352900 l/mol
 - Melting T_M :** 85.13 C
 - nmole/ OD_{260} :** 0.16
 - µg/ OD_{260} :** 28.61
- Characters Occurrence
- ▼ Dinucleotides
 - AA:** 50
 - AC:** 28
 - AG:** 44
 - AT:** 29
 - CA:** 41
 - CC:** 54
 - CG:** 27
 - CT:** 41
 - GA:** 37
 - GC:** 38
 - GG:** 37
 - GT:** 32
 - TA:** 22
 - TC:** 43
 - TG:** 36
 - TT:** 29

To copy the statistical information about a sequence select it on the *Options Panel* and choose the copy item in the context menu, or use the Ctrl+C shortcut.

Manipulating Sequence


- Show/Hide Sequence View Components
- Showing Sequence in Multiple Lines
- Translating Nucleotide Sequence
- Zooming Sequence
- Creating New Ruler
- Going To Position
- Selecting Sequence Region
- Copying and Pasting Sequence
- Editing Sequence
- Searching in Sequence
 - Load Patterns from File
 - Search Algorithm
 - Search in
 - Other Settings
 - Annotations Settings
- Exporting Selected Sequence Region
- Exporting Sequence of Selected Annotations
- Exporting Sequence Image
- Locking and Synchronize Ranges of Several Sequences

- Multiple Sequence Opening

Show/Hide Sequence View Components

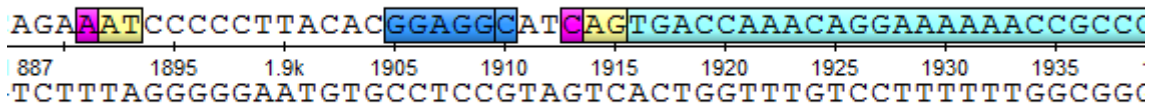
To show or hide a *Sequence View* component (*Sequence Overview*, *Zoom View*, or *Details View*) use the following buttons on a sequence toolbar. The fourth button allows one to show/hide all the components at once.



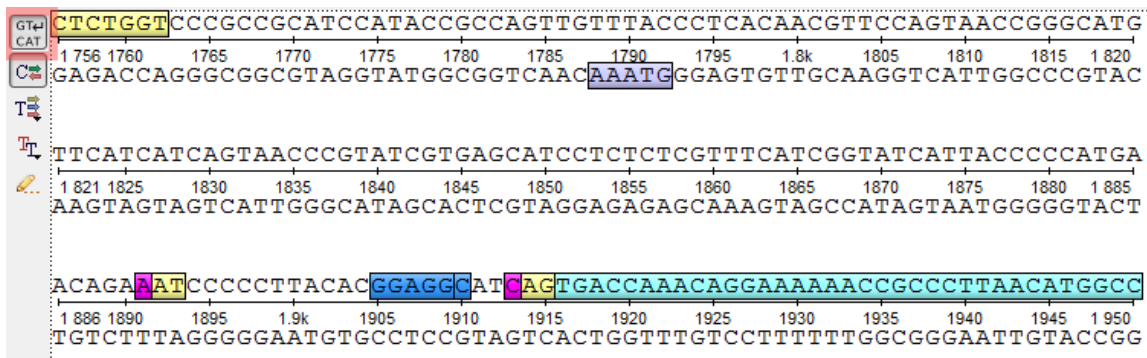
If several sequences are opened in the *Sequence View*, click the cross button  on a sequence toolbar to hide the sequence.

Showing Sequence in Multiple Lines

By default, a sequence in the *Details View* is shown in one line:



To show the sequence in multiple lines, click the *Wrap sequence* button on the left toolbar of the *Details View*.

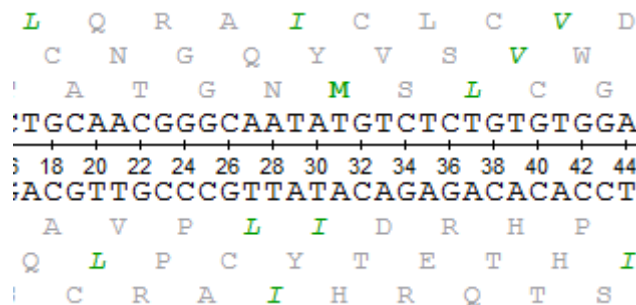


Translating Nucleotide Sequence

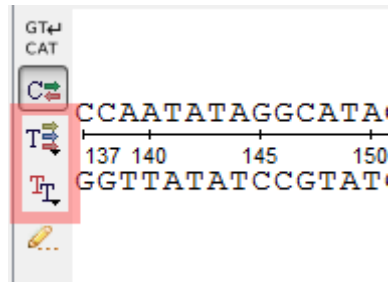
When a nucleotide sequence is opened in the *Sequence View*, the sequence and its complementary sequence are shown by default in the *Details View*.

Showing/hiding the amino acid sequences

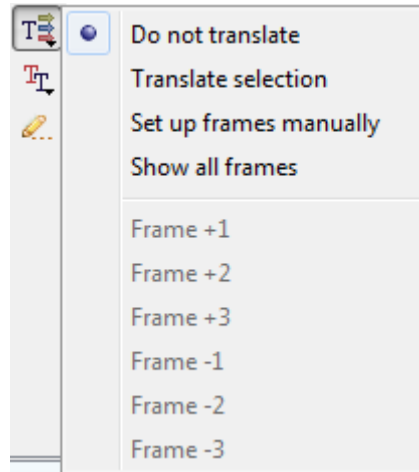
It is possible to translate them and show also the corresponding amino acid sequences:



The translation settings are available on the left toolbar of the *Details View*.

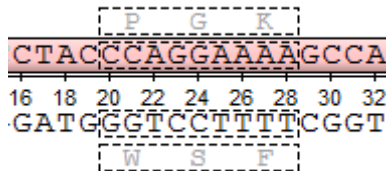


The *Show/hide amino acid translations* menu allows one to set up the mode of the amino acid sequences visualization:

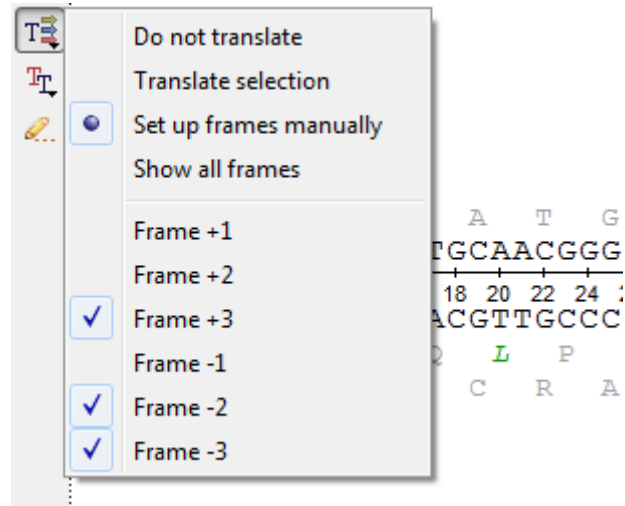


The following options are available:

- *Do not translate*— hide the amino acid sequences.
- *Translate selection* — translate only a selected region of the sequence and the complementary sequence.



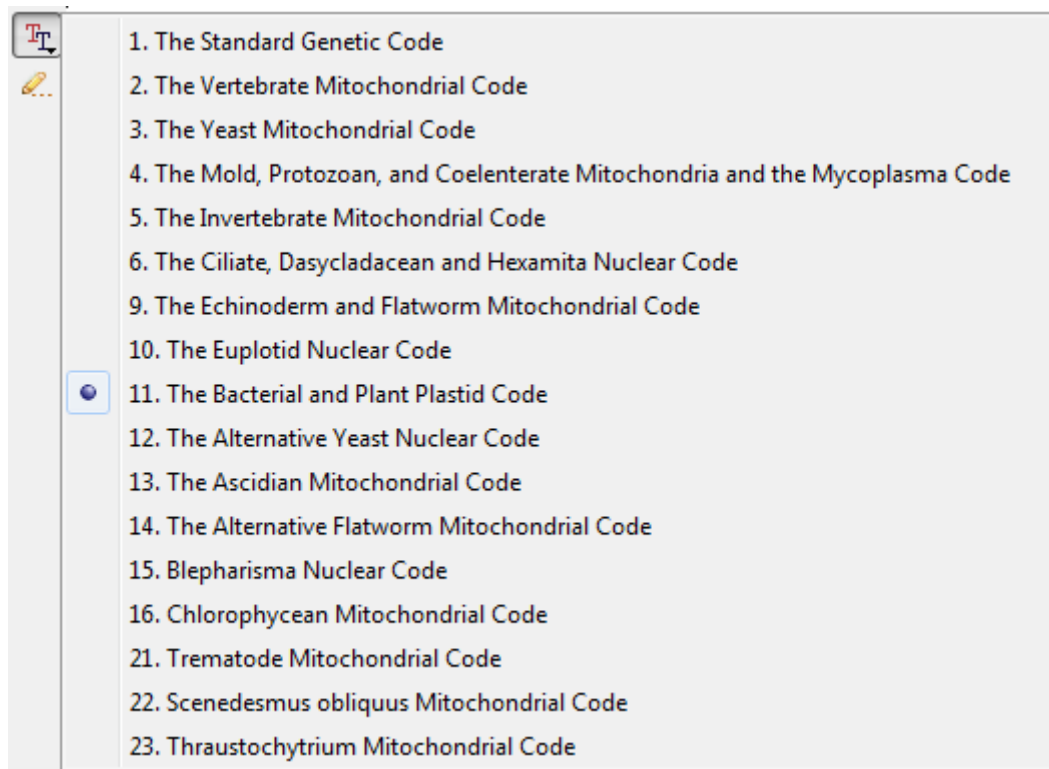
- *Set up frames manually* — select the [reading frames](#) to show. There are three frames for the sequence ("+1", "+2", "+3") and three frames for the complementary sequence ("-1", "-2", "-3"). Note that the complementary frame items are hidden in the menu, if the complementary sequence is hidden.



- *Show all frames* — show all amino acid sequences.


Setting the genetic code

The default value for the *genetic code* for a nucleotide sequence translation is read by UGENE from the sequence file when it is available. One can also set up the genetic code for the sequence using the *Select genetic code* menu:



 All analysis routines (like HMMER, ORF finding, etc.) will use this code by default.

Codon table

To refresh the knowledge about the amino acid codes, select the *Show codon table* button  on the *Sequence View* global toolbar. The codon table appears at the upper part of the window:

1st base	2nd base								3rd base		
	U		C		A		G				
U	UUU	Phenylalanine (Phe, F)	UCU	Serine (Ser, S)	UAU	Tyrosine (Tyr, Y)	UGU	Cysteine (Cys, C)	U		
	UUC		UCC			UAC		UGC	C		
	UUA		UCA			UAA	Stop codon (*)	UGA	Stop codon (*)	A	
	UUG		UCG			UAG		UGG	Tryptophan (Trp, W)	G	
C	CUU	Leucine (Leu, L)	CCU	Proline (Pro, P)	CAU	Histidine (His, H)	CGU	Arginine (Arg, R)	U		
	CUC				CCC		CAC			CGC	C
	CUA				CCA		CAA		Glutamine (Gln, Q)	CGA	A
	CUG				CCG		CAG			CGG	G
A	AUU	Isoleucine (Ile, I)	ACU	Threonine (Thr, T)	AAU	Asparagine (Asn, N)	AGU	Serine (Ser, S)	U		
	AUC				ACC		AAC		AGC	C	
	AUA				ACA		AAA	Lysine (Lys, K)	AGA	A	
	AUG		Methionine (Met, M)		ACG		AAG		AGG	R	
G	GUU	Valine (Val, V)	GCU	Alanine (Ala, A)	GAU	Aspartic acid (Asp, D)	GGU	Glycine (Gly, G)	U		
	GUC				GCC		GAC			GGC	C
	GUA				GCA		GAA		Glutamic acid (Glu, E)	GGA	A
	GUG				GCG		GAG			GGG	G

Zooming Sequence

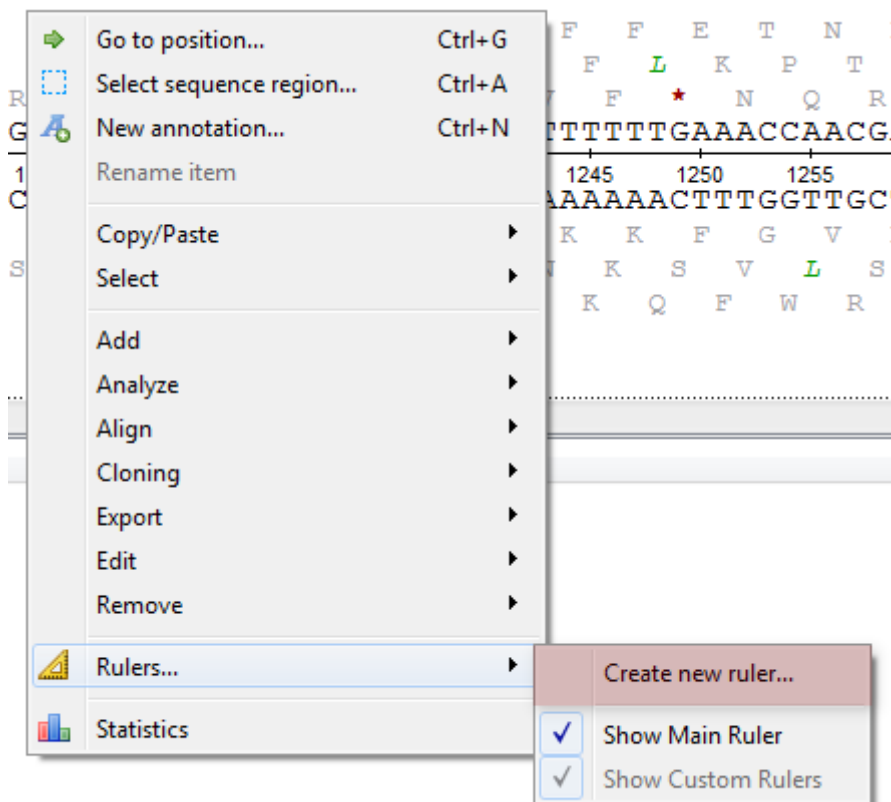
To zoom a sequence in the *Sequence zoom view* you can use one of the zoom button on the sequence toolbar:



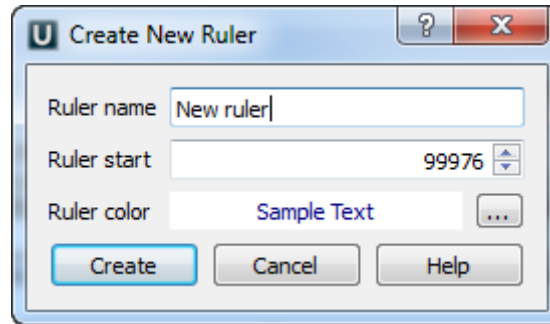
There are standard *Zoom In* and *Zoom Out* buttons. Additionally you can zoom to a selected region using the *Zoom to Selection* button. To restore the default view of the *Sequence zoom view* (when the sequence is not zoomed) use the *Zoom to Whole Sequence* button.

Creating New Ruler

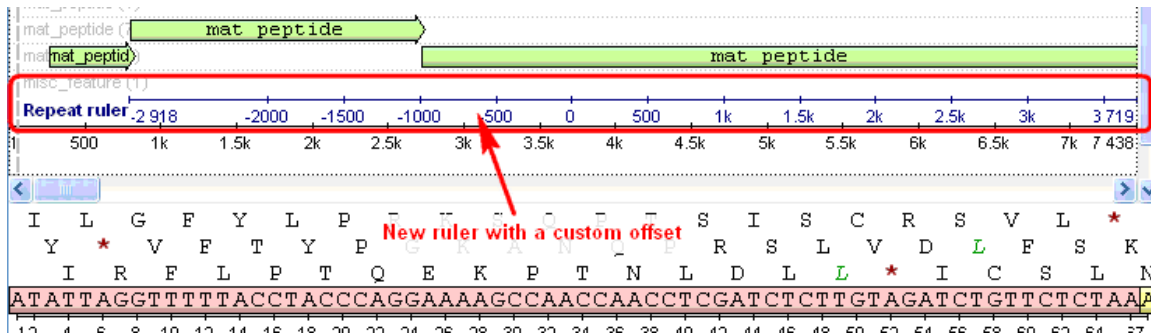
You can create any number of additional rulers by clicking the *Ruler Create new ruler* context menu item:



The following dialog will appear:

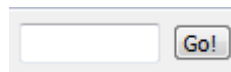


The new ruler will be shown right above the default one:

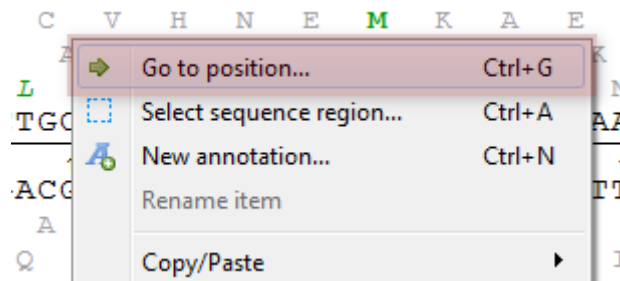


Going To Position

To go to a position, use the global actions toolbar:



Or use the *Go to position* context menu or the *Actions* main menu item.



Also you can use the shortcut Ctrl-G.

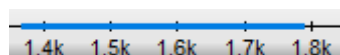
Selecting Sequence Region

Selection in the Sequence View components

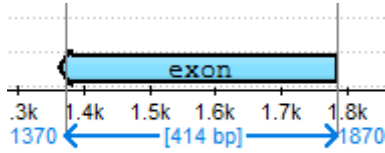
Holding the left mouse button down and moving the mouse, one can select a sequence region in the *Zoom View* or in the *Details View* components of the *Sequence View*. A sequence region that correspond to an annotation can also be selected by double-clicking on the annotation in the *Zoom View*, *Details View*, or *Annotations Editor*.

Selection of a sequence region is synchronized between different *Sequence View* components.

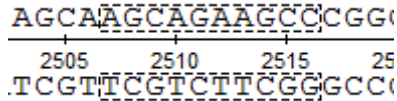
In the *Sequence Overview* the selection is displayed as a blue line:



In the *Zoom View* a blue line with coordinates and the length of the selected region is shown:



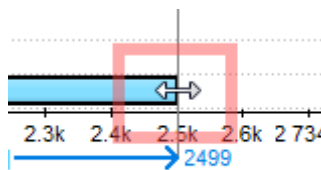
In the *Details View* a rectangle with a dashed border is shown around the selected region:




Selecting a region using the mouse

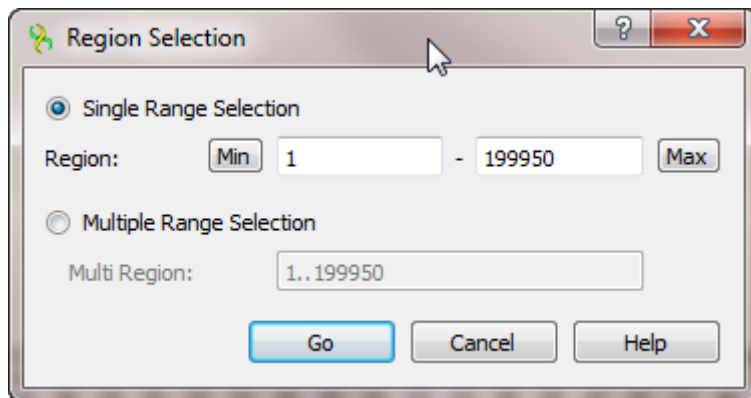
It is possible to select a region of a sequence using the mouse in each component of the *Sequence View*.

In the *Zoom View* and the *Details View* the selection can be adjusted by dragging its left or right border:



Selecting a region using the dialog

If information about exact coordinates of a region is available, click the *Select sequence region* button  on the toolbar or *Select->Sequence region* in the context menu of the *Sequence View*. Input the coordinates in the *Region Selection* dialog:



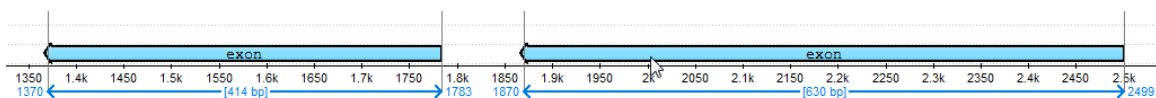
It is possible to input:

- *Single Range Selection* — a common region with a single range. Use the *Min* and *Max* buttons to automatically specify the beginning and the end of the sequence in the corresponding fields.
- *Multiple Range Selection* — a region that consists of several join elements, for example, exons of a gene. See "[The DDBJ/ENA/GenBank Feature Table Definition](#)" for details.

Selecting a region around or between annotations

To select a region between two annotations, for example, an intron region between two exons:

- Click on the annotations, holding the *Ctrl/Cmd* key:



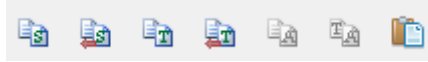
- Click **Select**→*Sequence between selected annotations* in the context menu of the *Sequence View*.

To select a region that contains the annotations in a single range, choose the *Sequence around selected annotations* item in the menu.

Copying and Pasting Sequence

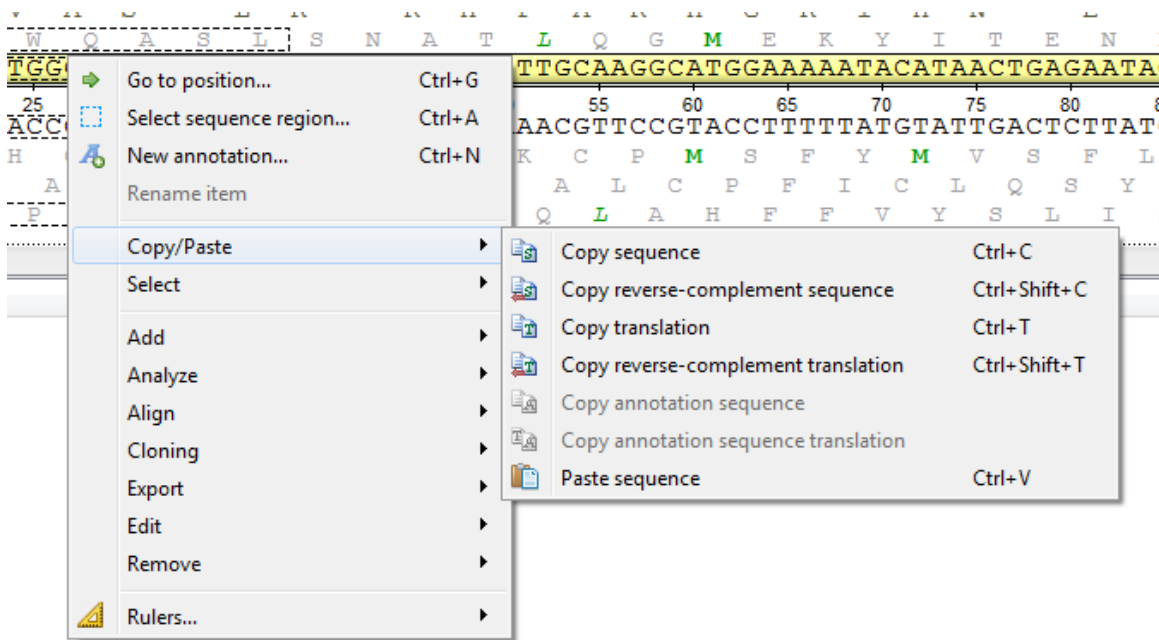
The selected sequence region, an annotation sequence or their amino translations can be copied to clipboard:

- By pressing the corresponding buttons in the global toolbar.



- Using the following shortcuts:
 - Ctrl-C — copies direct sequence strand
 - Ctrl-T — copies direct amino translation
 - Ctrl-Shift-C — copies reverse-complement sequence
 - Ctrl-Shift-T — copies reverse-complement amino translation

- Using the *Copy* submenu of the context menu:



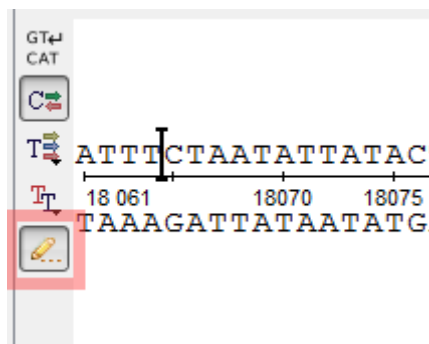
To paste sequence use the corresponding context menu item or *Ctrl+V* shortcut.

Editing Sequence

If the corresponding document is not locked, it is possible to edit a sequence.

Editing mode and annotations settings

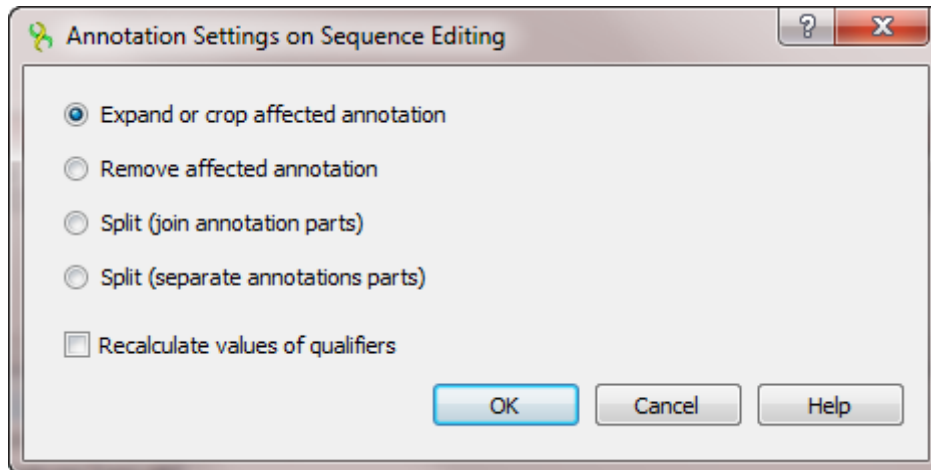
To switch on the editing mode for the sequence, select the *Edit sequence* button on the left toolbar of the *Details View*:



A special cursor appears in this case, and the sequence can be edited as in a text editor:

- To insert characters to the sequence, move the cursor to the required location and type the characters or paste them using *Ctrl + V / Cmd + V* keyboard shortcut.
- To replace a sequence region, select it and insert the new characters.
- To remove a character from the sequence, move the cursor to the required location and press *Backspace* or *Delete*. To remove a region, select it and press one of these shortcuts.

One can also configure the way how annotations located in an edited region should be modified. To open a dialog with the settings select *Edit -> Annotations settings on sequence editing* in the *Actions* main menu or in the context menu of the *Sequence View*.



Select one of the following:

- *Expand or crop affected annotation* — an annotation located in an edited region is expanded in case of characters insertion or cropped in case of characters deletion.
- *Remove affected annotation* — all annotations in an edited region are removed.
- *Split (join annotation parts)* — an annotation is split into two join elements (see "[The DDBJ/ENA/GenBank Feature Table Definition](#)" for details).
- *Split (separate annotation parts)* — an annotation is split into two annotations.

It is also possible to check the *Recalculate values of qualifiers* option in the dialog. If it is selected, qualifiers of all annotations are parsed on the sequence editing. Qualifiers values that specify coordinates (like "100..200") are re-calculated accordingly to the sequence modification. For example, the value might become "104..204", if four characters have been inserted before the corresponding annotation.

Getting reverse-complement, reverse, or complement sequence

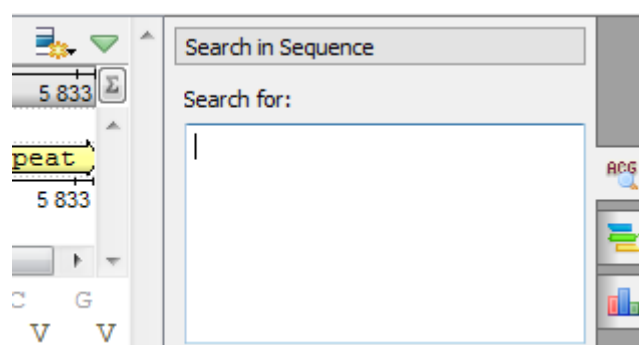
To replace a sequence with its reverse-complement sequence, select *Edit -> Reverse-complement sequence* in the *Actions* main menu or in the context menu of the *Sequence View*. The keyboard shortcut for this action is *Ctrl + Shift + R / Cmd + Shift + R*.

Use the *Reverse sequence* or *Complement sequence* items in the same menus to replace the sequence with its reverse or complement sequence.

Searching in Sequence

To search for a pattern(s) in a sequence go to the *Search in Sequence* tab of the the *Options Panel* in the *Sequence View*.

Input the value you want to search in the text field and click the *Search* button. To search multiple patterns input the patterns separated by a new line in the pattern text field. To add a new line symbol *Ctrl+Enter* may be used. You can input the value as sequence or name of the sequence in the FASTA format and sequence after that.



By default, **misc_feature** annotations are created for regions that exactly match the pattern. Find below the description of the available settings.

- Load Patterns from File
- Search Algorithm
- Search in
- Other Settings
- Annotations Settings

Load Patterns from File

A screenshot of a software interface showing a checked checkbox labeled "Load patterns from file". Below it is a text input field labeled "Path:" followed by a button with three dots (a file selection button).

Use this checkbox to load patterns from file. When this option is active the *Search for* field is disabled.

Search Algorithm

A screenshot of a software interface showing a dropdown menu titled "Search algorithm". The current selection is "Exact".

This group specifies the algorithm that should be used to search for a pattern. The algorithm can be one of the following:

- *InsDel* — there could be insertions and/or deletions, i.e. a pattern and the searched region can vary in their length. You can specify the percentage of the pattern and a searched region match in the field nearby. Note that this value also depends on the pattern length and is disabled when the pattern hasn't been specified.
- *Substitute* — a pattern may contain characters different from the characters in the searched region. When this algorithm has been selected you can also specify the match percentage and additionally it is possible to take into account ambiguous bases.
- *Regular expression* — a regular expression may be specified instead of a pattern. For example character '.' matches any character, '*' matches zero or more of any characters. There is also the *Limit result length* option that specifies the maximum length of a result.
- *Exact* - find a place where one or several patterns are found within a larger pattern.

Search in

A screenshot of a software interface showing three dropdown menus under the heading "Search in":

- "Strand" is set to "Both".
- "Search in" is set to "Sequence".
- "Region" is set to "Whole sequence".

In this group you can specify where to search for a pattern: in what region and in which strand (for nucleotide sequences). Also for nucleotide sequences it is possible to search for a pattern on the sequence translations.

Strand — for nucleotide sequences only. Specifies on which strand to search for a pattern: *Direct*, *Reverse-complementary* or *Both* strands.

Search in — for nucleotide sequences you can select the *Translation* value for this option. In this case the input pattern will be searched in the amino acid translations.

Region — specifies the sequence range where to search for a pattern. You can search in the whole sequence, specify a custom region or search in the selected region.

Other Settings

A screenshot of a software interface showing a section titled "Other settings" with two checkboxes:

- Remove overlapped results
- Limit results number to:

 Below the second checkbox is a text input field containing the number "100000" and a small spinner control.

This group contains additional common settings:

Remove overlapped results — annotates only one of the overlapped results.

Limit results number to — limits number of the searched results to the specified value.

Annotations Settings

In the *Save annotation(s) to* group you can set up a file to store annotations. It could be either an existing annotation table object or a new annotation table.

In the *Annotation parameters* group you can specify the name of the group and the name of the annotation. If the group name is set to `<auto>` UGENE will use the group name as the name for the group. You can use the `'/'` characters in this field as a group name separator to create subgroups. If the annotation name is set to *by type* UGENE will use the annotation type from the *Annotation type:* table as the name for the annotation. Also you can add a description in the corresponding text field. To use a pattern name for the annotations check the corresponding checkbox.

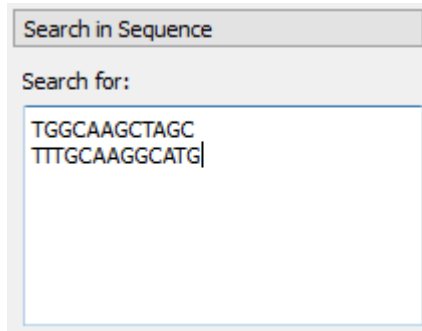
After that click the *Create annotations* button. The annotations will be created. Also you can see the result statistic and navigation under the *Search for:* field:

Searching for one or several patterns and names of the result annotations

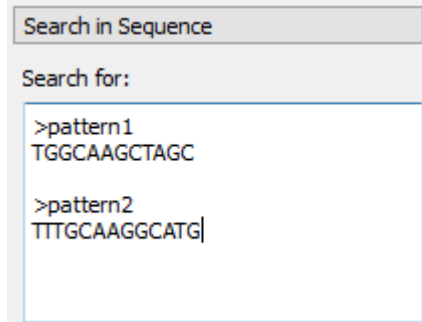
If you search for one pattern only, than input the required name into the *Annotation name* field and leave the *Use pattern name* check box unchecked.

You can also search for several patterns at a time by:

- Inputting several patterns into the search field (click `<Ctrl>` + `<Enter>` keys to insert to a new line):



- Inputting several patterns into the search field in FASTA format:



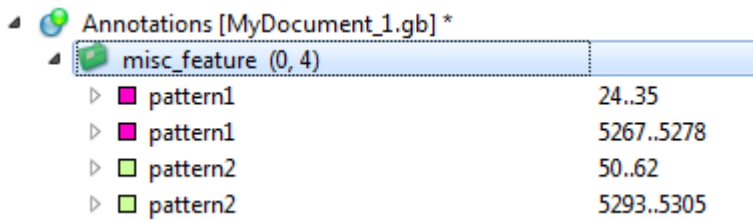
- Loading patterns from a FASTA file

Even when you search for several patterns, names of the found annotations will be identical by default (the name is specified in the *Annotation name* field).

If you want to assign different names to annotations found for different patterns, then you should:

- Input the patterns in FASTA format (the latter two cases above)
- Check the *Use pattern name* checkbox in the *Annotation parameters* group

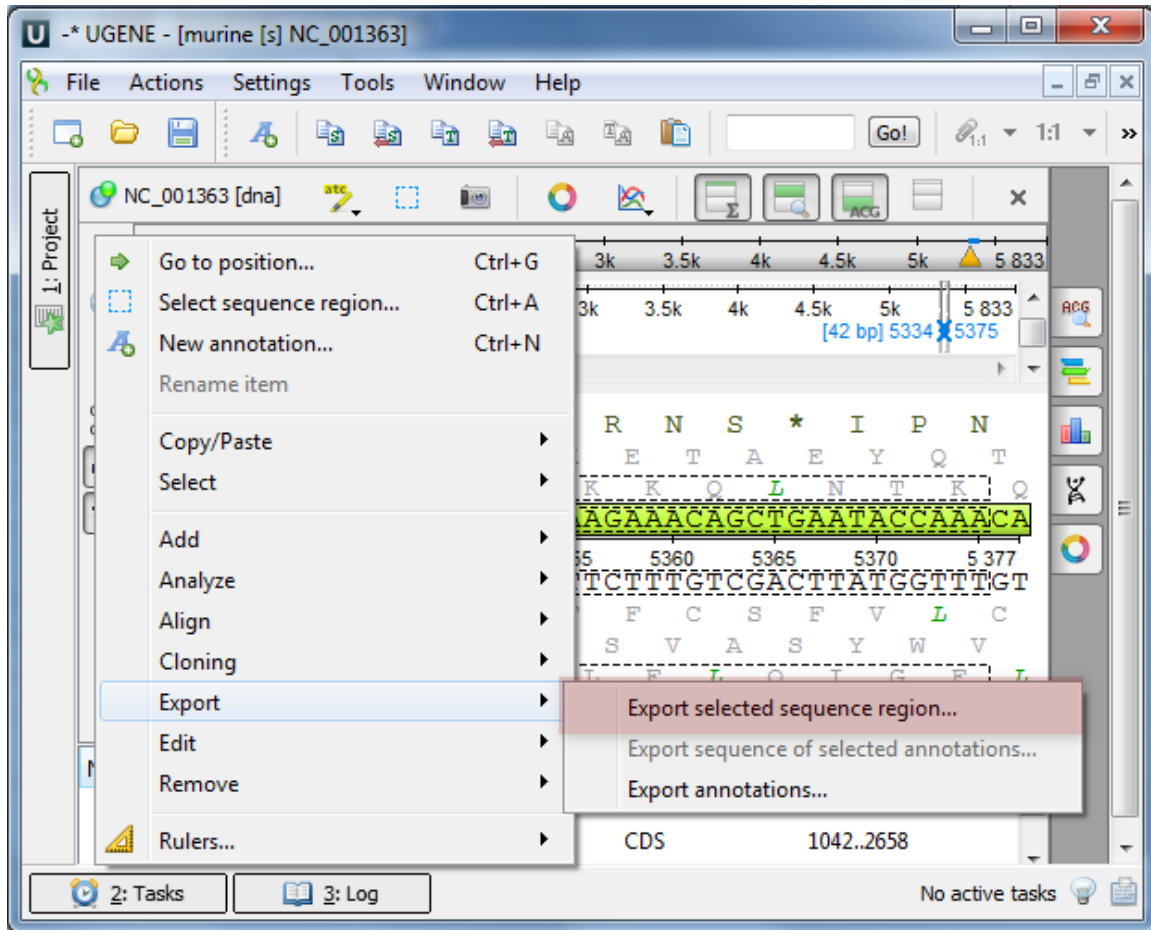
Here is an example of the found annotations in the *Annotations Editor*:



Exporting Selected Sequence Region

Open a sequence object in the *Sequence View* and select a region by pressing and moving the left mouse button over the sequence.

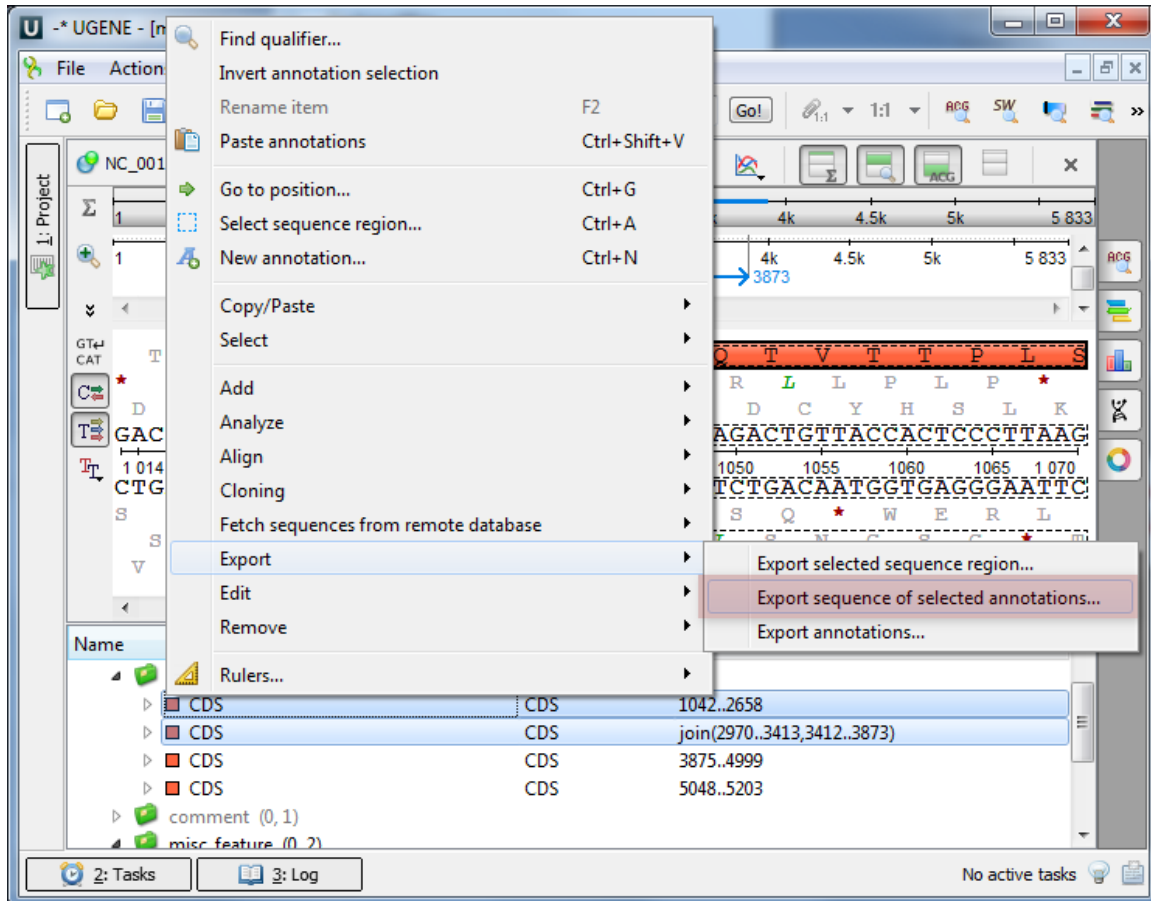
Use the *Export > Export selected sequence region* context menu item to save selection into a file of a sequence format.



The *Export Selected Sequence Region* dialog will appear which is similar to the *Export Selected Sequences* dialog described [here](#).

Exporting Sequence of Selected Annotations

Open the *Sequence View* with document that contains annotations. A good candidate here could be any file in Genbank format with both sequence and annotations. Select a single or several annotations or annotation groups in the *Annotation editor*, click the right mouse button to open the context menu and select the *Export Export sequence of selected annotations* item:



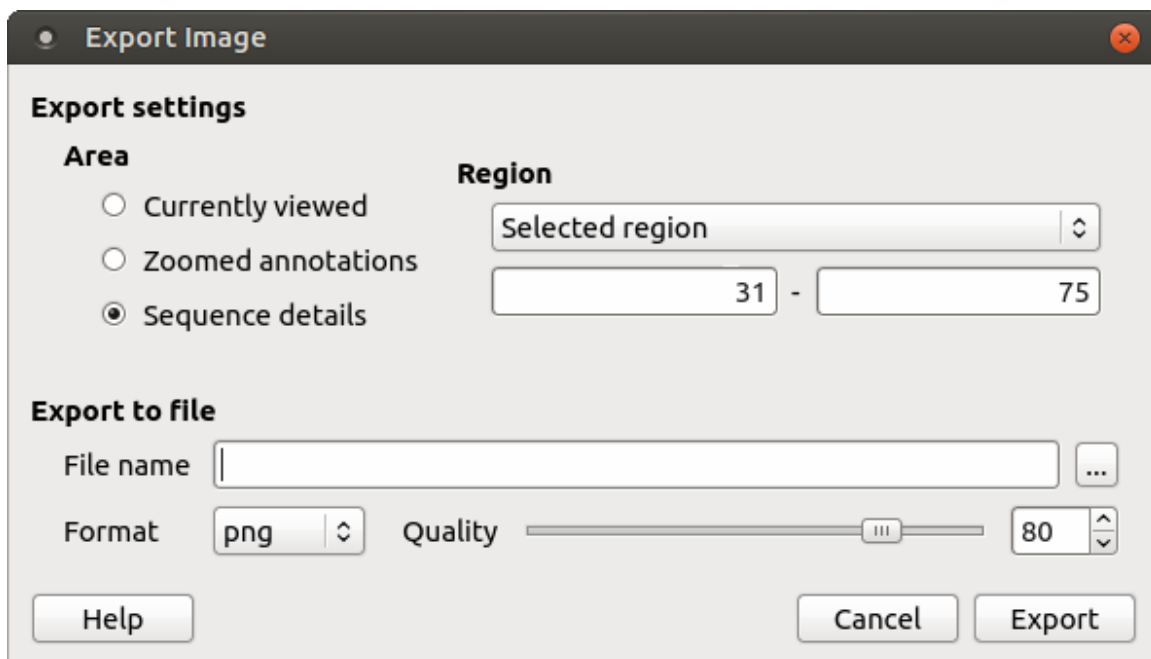
The *Export Sequence of Selected Annotations* dialog will appear which is similar to the *Export Selected Sequences* dialog described [here](#).

Exporting Sequence Image

Use a sequence toolbar *Export image* button to save a screenshot of the sequence:



The *Export Image* dialog will appear where you should set name, location, export settings and format of the picture:



UGENE supports export to the BMP, JPEG, JPG, PNG, PPM, TIF, TIFF, XBM, XPM and SVG image formats. You can export currently viewed, zoomed annotations or sequence details areas. Also you can export whole sequence or custom region. Use the *Region* settings to

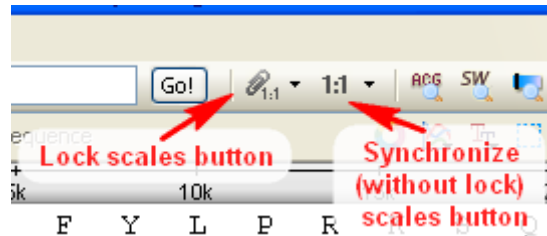
do it.

Locking and Synchronize Ranges of Several Sequences

An important feature of the *Sequence zoom view* is the ability to synchronize and lock visual ranges of different sequences shown in the *Sequence View*.

This feature is available when there are two or more sequences opened in the same *Sequence View*.

If we click the *Lock scales* button the second sequence scale will be adjusted to be the same as the focused sequence scale and is locked. Now if we move a scrollbar or use zoom buttons for any of the sequence, visual ranges for the rest sequences will also be adjusted.



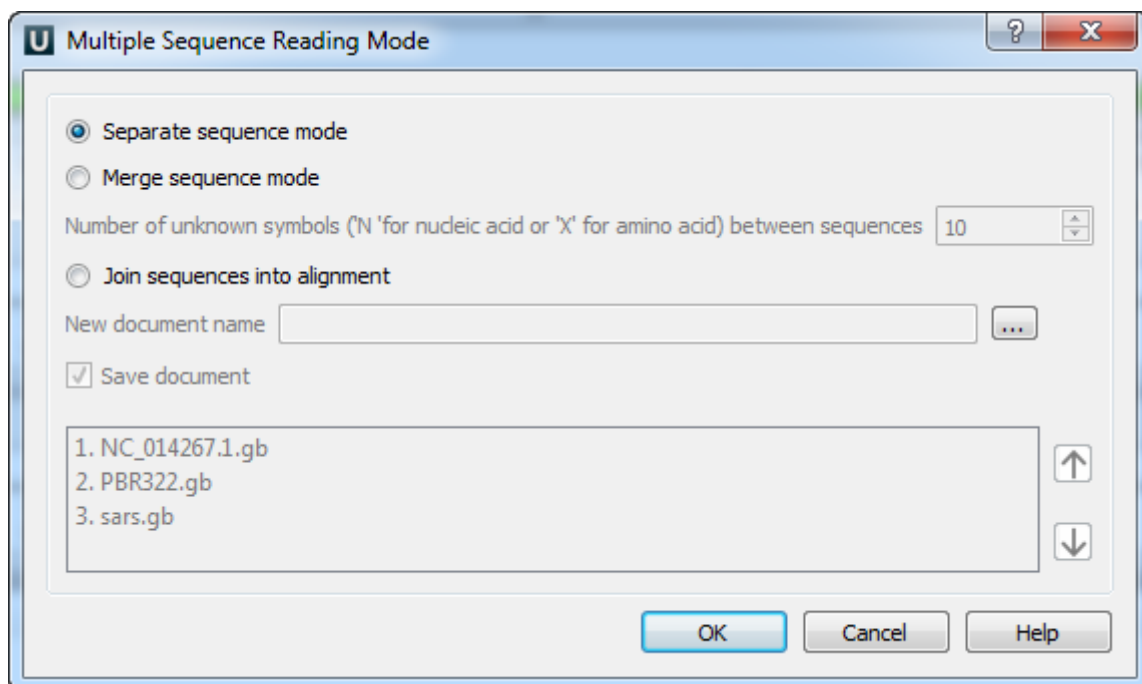
To unlock the scales click the same button again.

You may use the *Adjust scales* button to synchronize scales without locking them.

Note, that if you have a selected sequence region or a selected annotation the scales will be synchronized by the start position of the region or the annotation. If there are no active selection the regions are synchronized by the first visible sequence position on the screen.

Multiple Sequence Opening

To open several sequences use the *File->Open* menu item or *Open* toolbar button and using *Ctrl* select the several sequences and click the *Open* button. The following dialog will appear:



The following parameters are available:

Separate sequence mode - opens the sequences as separate sequences.

Merge sequence mode - merges sequences into one sequence with selected number of unknown symbols between sequences.

Join sequences into alignment - joins sequences into alignment.

Save document - save document to the selected document.

Also you can change the order of the sequences by up and down arrows.

Choose the parameters and click the *Open* button. Sequences will be opened in the selected mode.

In the *Separate sequence mode* sequences will be opened as separate sequences in selected order. You can change the sequences order

by drag and drop in the sequence view.

Annotations Editor

The *Annotations editor* contains tools to manipulate annotations for a sequence. It provides a convenient way to organize, view and modify a single annotation as well as annotation groups.

An annotation for a sequence consists of:

- *Name* (or key) — indicates the biological nature of the annotated feature.
- *Location* — coordinates in the sequence.
- *The list of qualifiers* — qualifiers are the general mechanism for supplying information about annotation. Qualifiers are stored as pairs of (name, value) strings.

Below is the default layout of the *Annotations editor* with an extra column for the “note” qualifier added:

Name	Value	note
Auto-annotations [murine.gb NC_001363]		
NC_001363 features [murine.gb]		
CDS (0, 4)	1042..2658	
CDS	join(2970..3413,3412..3873)	Predicted by GeneMark; artific
CDS	3875..4999	
CDS	5048..5203	Predicted by GeneMark
misc_feature (0, 2)		
misc_feature	2..590	5' terminal repeat
note	5' terminal repeat	
misc_feature	5245..5833	3' terminal repeat
source (0, 1)		

There are usually several objects with annotations in the *Annotations editor*. A special *Auto-annotations* object is always presented for each sequence opened. It contains annotations automatically calculated for the sequence (see *below* for details).

An object contains groups of annotations used by UGENE for logical organization of the annotations. An annotation must always belong to some group.

For documents created not by UGENE annotations are grouped by their names. For annotations created in UGENE it is possible to use arbitrary group names.

Groups can contain both annotations and other groups. The numbers in the brackets after a group name in the *Annotations editor* are the count of subgroups and annotations in the current group.

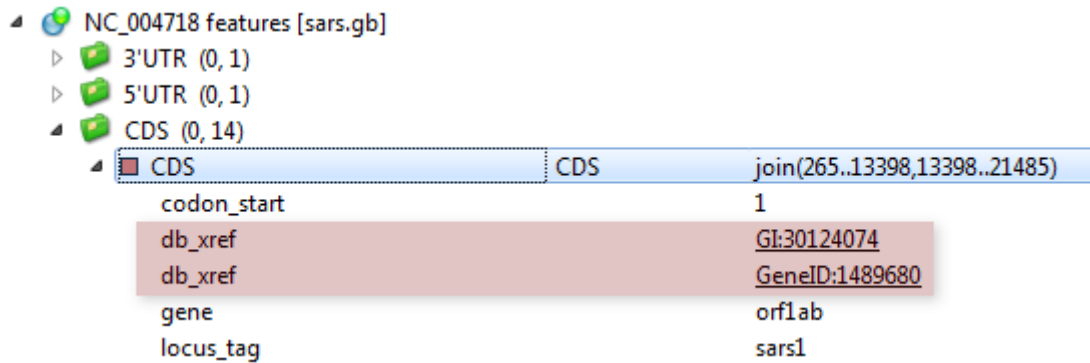
A single annotation is allowed to be presented in several groups simultaneously. An annotation is physically removed from the document when it does not belong to any group.

- "db_xref" Qualifier

- Automatic Annotations Highlighting
- The "comment" Annotation

"db_xref" Qualifier

Some files in Genbank format contain the *db_xref* qualifier. A value of this qualifier is a reference to a database.



When you click on the value a web page is opened or a file is loaded specified in the reference. The loaded file is added to the current *project*.

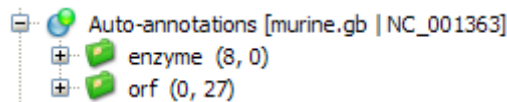
Automatic Annotations Highlighting

Enabling the automatic annotations highlighting allows you to automatically calculate and highlight annotations on each nucleotide sequence opened.

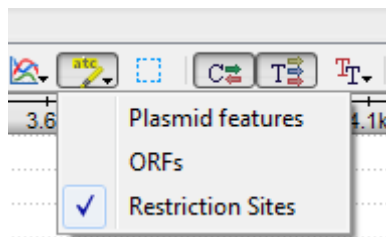
Currently, the following annotations types support the automatic highlighting:

- Open reading frames
- Restriction sites
- Plasmid features

The corresponding groups of annotations found are stored in the *Auto-annotations* object in the *Annotations editor*, for example:



To disable/enable the automatic annotations calculations use the *Automatic Annotations Highlighting* menu button on the *Sequence View* toolbar:



To create a permanent annotation click on the *Make auto-annotations persistent* context menu item and choose the annotation parameters in the *Create Permanent Annotation* dialog.

The "comment" Annotation

General information about a file in GenBank or Vector NTI Sequence format, stored in the COMMENT sections of the file, is shown in UGENE in a special *comment* annotation in the *Annotations Editor*.

The information, for example, may include the name of the file author, creation date and last modification date for the file, and so on:

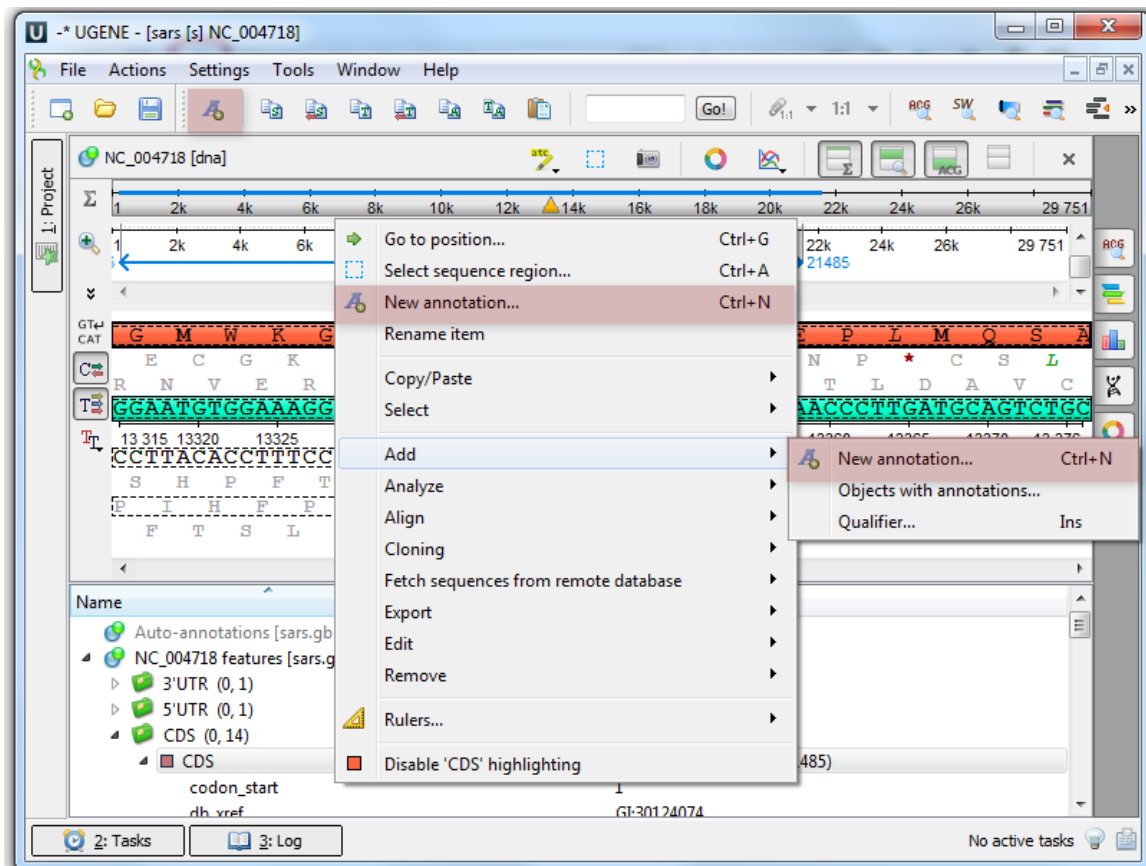
2xCyPET-3xFLAG-6 features [2xCyPet-3xFLAG-...		
CDS (0, 2)		
comment (0, 1)		
comment	Comment	1..4340
01		This file is created by Vector NTI http://www.invitrogen.com/
Author name		Demo User
Creation date		Thu Dec 23 13:29:41 2010
Last modification date		Thu Dec 23 13:29:41 2010
Object name		2xCyPET-3xFLAG-6xHIS/pDONRP2R-P3 (p3')
Owner		
misc_feature (0, 11)		

Manipulating Annotations

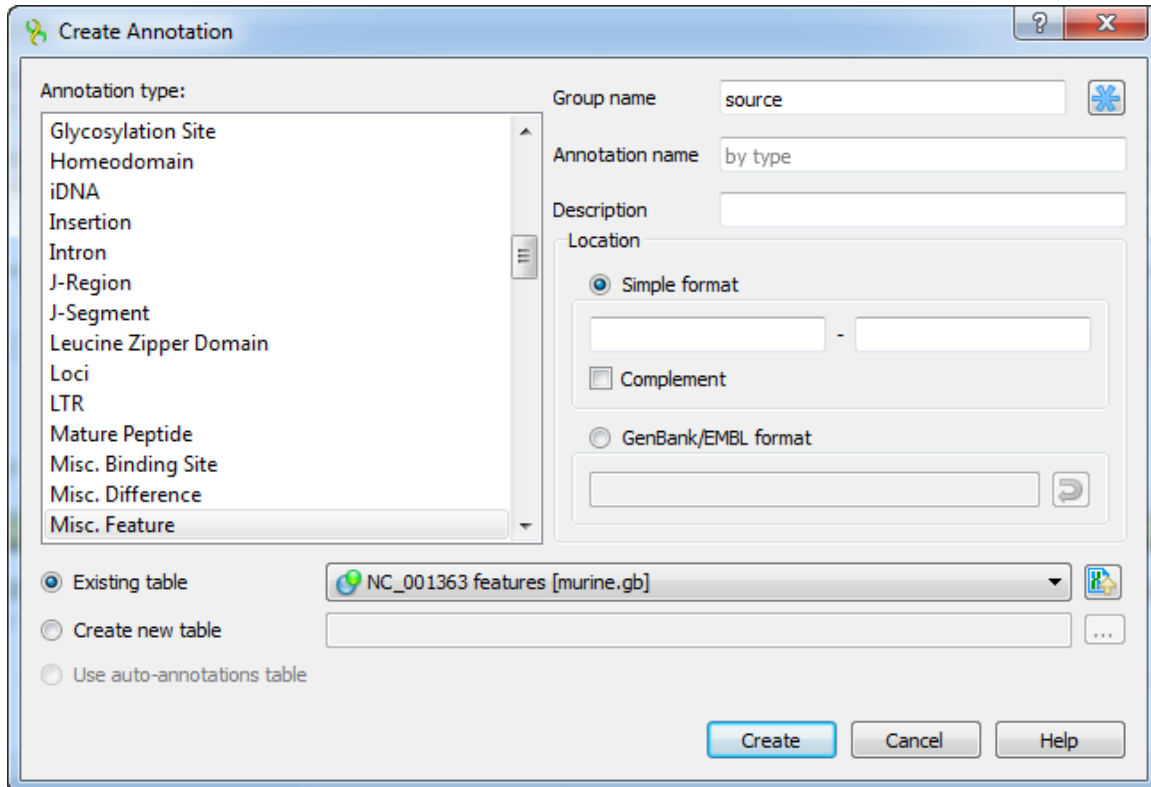
- Creating Annotation
- Selecting Annotations
- Editing Annotation
- Highlighting Annotations
 - Annotations Color
 - Annotations Visibility
 - Show on Translation
 - Captions on Annotations
- Creating and Editing Qualifier
- Adding Column for Qualifier
- Copying Qualifier Text
- Finding Qualifier
- Deleting Annotations and Qualifiers
- Importing Annotations from CSV
- Exporting Annotations

Creating Annotation

To create a new annotation for the active sequence press the Ctrl-N key sequence, select the *New annotation* toolbar button or use the *Add New annotation* or *New annotation* context menu item:



This will activate a dialog where to set up annotation parameters:



The dialog asks where to save the annotation. It could be either an existing annotation table object, a new annotation table or auto-annotations table (if it is available).

You can also specify the name of the group and the name of the annotation. If the group name is set to <auto> UGENE will use the group name as the name for the group. You can use the '/' characters in this field as a group name separator to create subgroups. If the annotation name is set to *by type* UGENE will use the annotation type from the *Annotation type:* table as the name for the annotation. Also you can add a description in the corresponding text field.

The *Location* field contains annotation coordinates. The coordinates must be provided in the Genbank or EMBL file formats. If you want to annotate complement strand sequence check the corresponding checkbox for the simple format or surround the coordinates with the "complement()" word or press the last button in the corresponding row to do it automatically.

Note, that by default the *Location* field contains the coordinates of the selected sequence region.

Once the *Create* button is pressed the annotation is created and highlighted both in the *Sequence overview* and the *Sequence details view* as reas:

Name	Value
Annotations [MyDocument.gb] *	
misc_feature (0, 1)	
misc_feature	9..39
NC_001363 features [NC_001363.gb] *	
CDS (0, 4)	

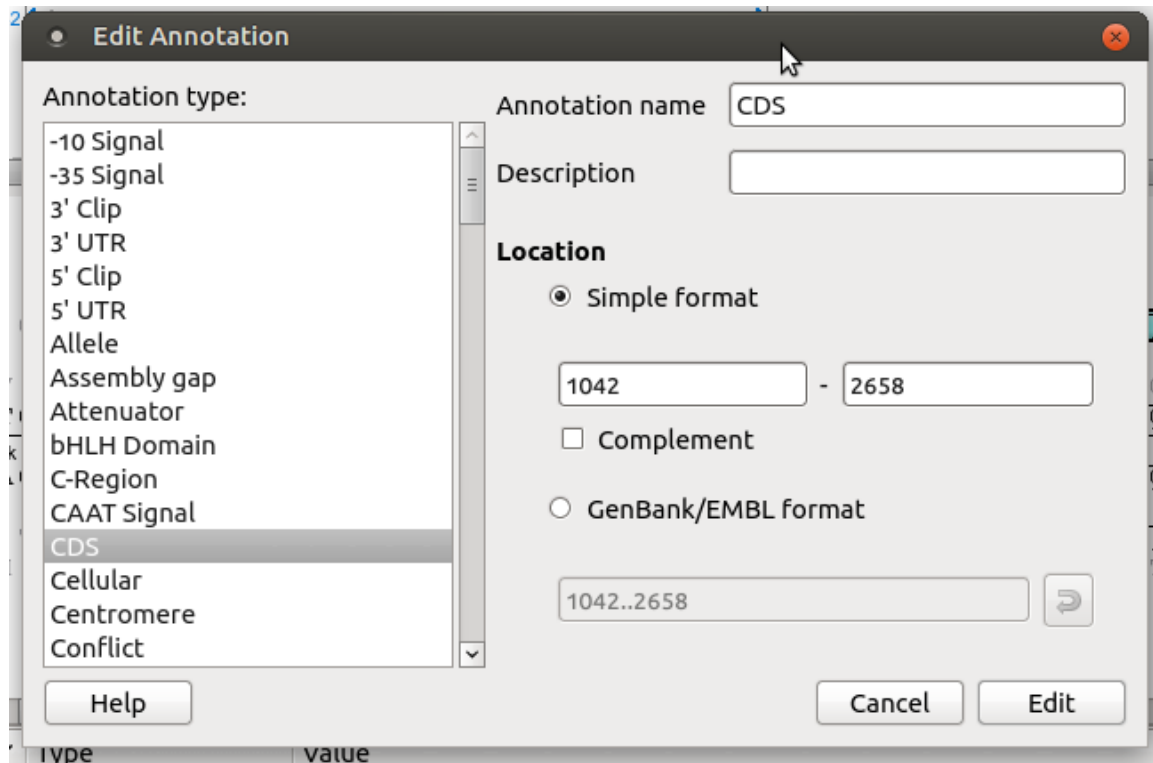
Selecting Annotations

To select one annotation click on it. To select several annotations hold *Ctrl* key while clicking on the annotations. To invert the selection use the *Invert annotation selection* item in the *Annotations editor* context menu.

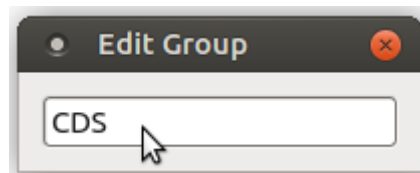
Double-clicking on an annotation also selects the corresponding sequence region.

Editing Annotation

If the document is not locked, it is possible to edit an annotation or an annotation group using the *Rename item* context menu from the *Annotation Editor* or from the *Sequence View* or with a help F2 key in the *Annotation Editor*. The result of pressing for an annotation:



The result of pressing for an annotation group:



Highlighting Annotations

To configure settings of annotation names go to the *Annotation Highlighting* tab in the *Options Panel*.

By default the tab shows annotations names of the opened *Sequence View*.

The screenshot displays the Unipro UGENE software interface. The main window shows a genomic track for NC_00136 with a scale from 1 to 5.833 kb. A specific region is highlighted, showing DNA sequence and protein translations. Annotations are visible as colored boxes above the sequence. On the right, the 'Annotations Highlighting' panel is open, showing a table of annotation types and their colors, and configuration options for displaying annotations.

Annotation	Color
BadI	Light Green
BaeGI	Orange
BaeI	Purple
CDS	Green
SacII	Cyan
comment	Pink
misc_feature	Yellow
source	Grey

Configure the annotations:

- Show annotations
- Show on translation
- Show value of qualifier: label,note

If you want to see all annotation names, click the *Show all annotation names* link. The *Previous annotation* and *Next annotation* buttons seek to the previous or to the next annotation of the view correspondingly.

Find below information about annotations names' properties that you can configure.

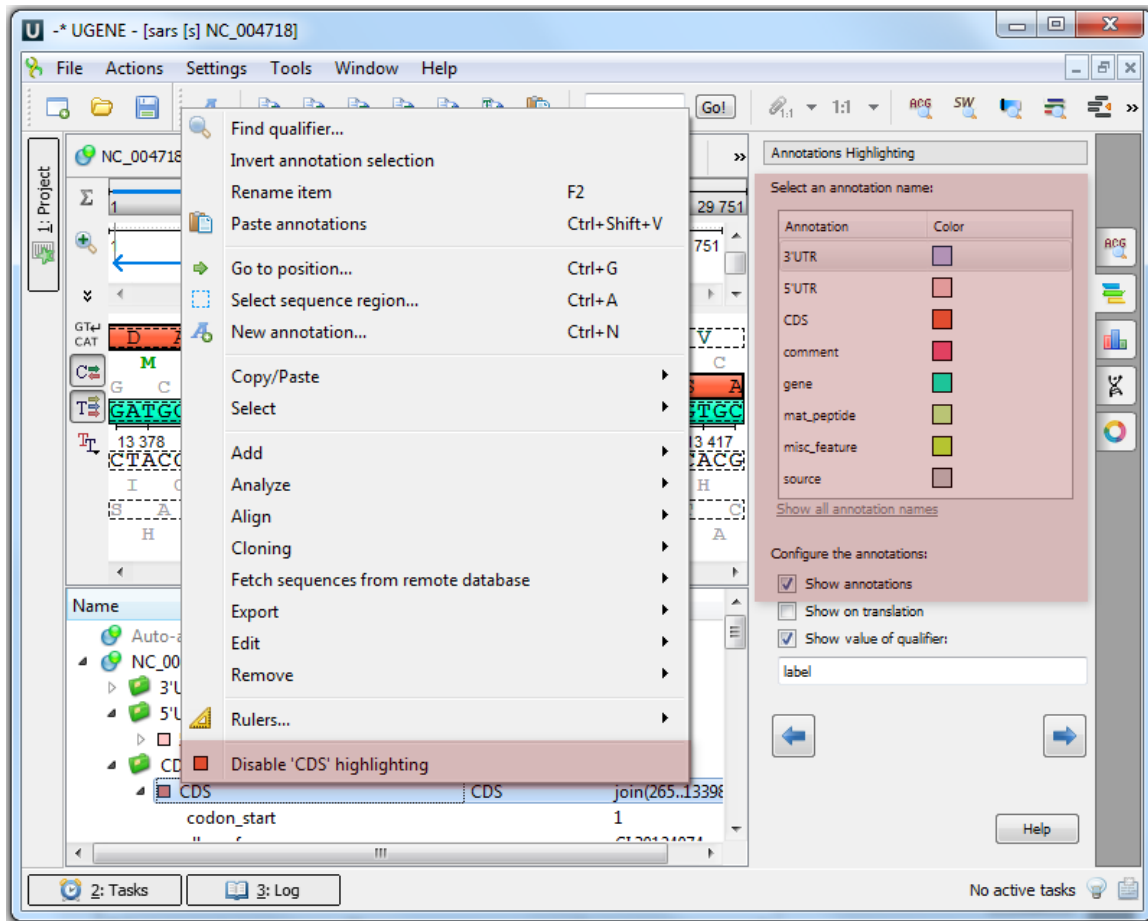
- Annotations Color
- Annotations Visibility
- Show on Translation
- Captions on Annotations

Annotations Color

To change a color of all annotations of a certain type click on the corresponding color box in the annotations types table and select the required color in the appeared *Select Color* dialog.

Annotations Visibility

To show/hide annotations with a certain name, select this name in the annotations names table and check/uncheck the *Show annotations* checkbox below. Another way to show/hide the annotations is to select the *Enable/Disable highlighting* item in the context menu of an annotation.



Show on Translation

This option is available for nucleotide sequences only. It specifies to show the annotation on the corresponding amino sequence instead of the original nucleotide sequence in the *Sequence Detailed View*, for example:

```

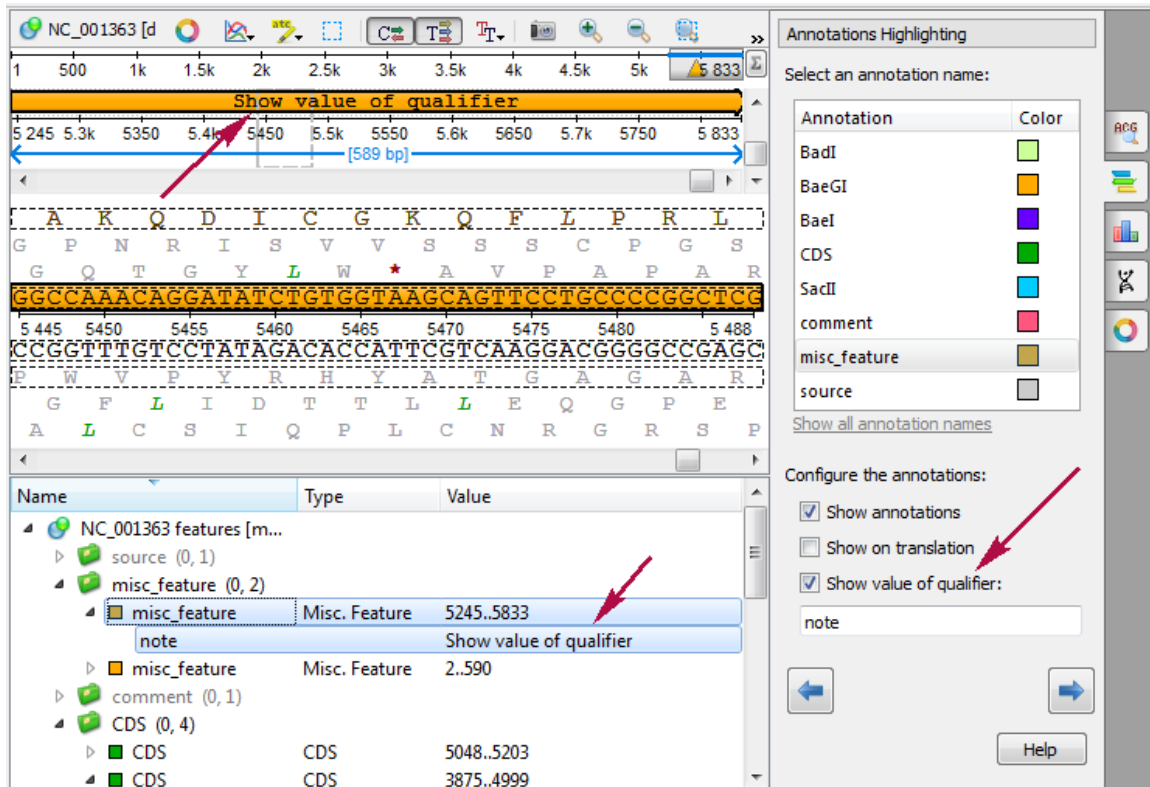
.....
  D  S  P  K  L  K
  |  |  |  |  |  |
  2  I  H  Q  S  *  N
  |  |  |  |  |  |
  R  F  T  K  V  E
  |  |  |  |  |  |
  A  G  A  T  T  C  A  C  C  A  A  G  T  T  G  A  A
  |  |  |  |  |  |  |  |  |  |  |  |  |
  3  8 10 12 14 16 18 20 22 24
  |  |  |  |  |  |  |  |  |  |
  C  T  A  A  G  T  G  G  T  T  T  C  A  A  C  T  T  T
  |  |  |  |  |  |  |  |  |  |
  I  *  W  L  Q  F
  |  |  |  |  |  |
  S  E  G  F  N  F

```

You can enable/disable this option by checking/unchecking the *Show on translation* checkbox.

Captions on Annotations

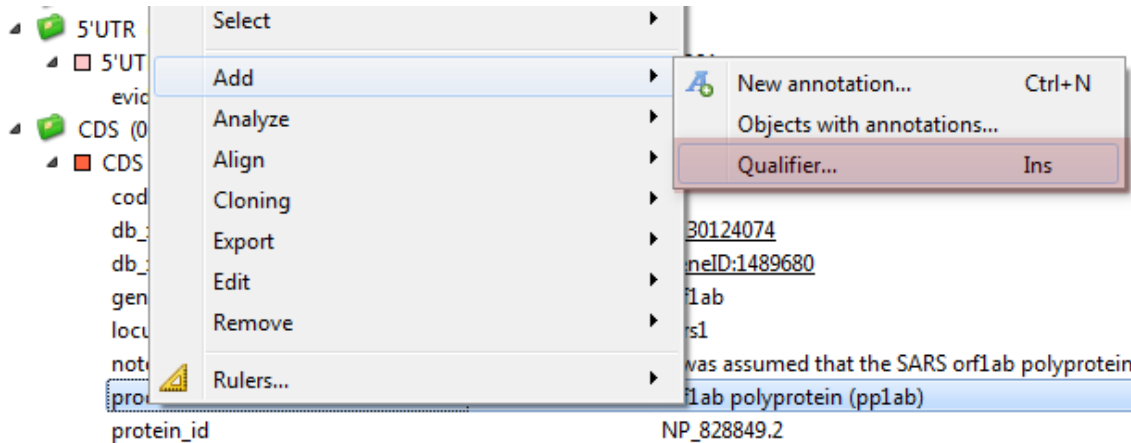
It is possible to show a value of a qualifier of an annotation instead of the annotation type name in the *Sequence Zoom View*. To enable this option for an annotation type check the *Show value of qualifier* check box and input the values of the required qualifiers in the text field nearby this check box. See the image below.



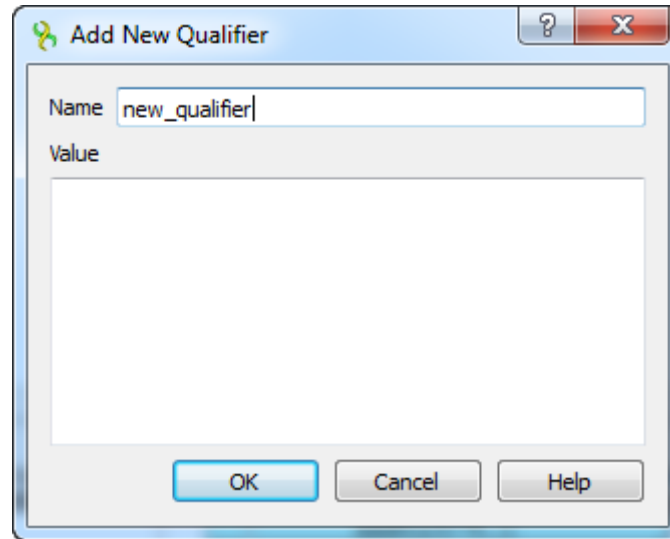
If you input several qualifiers names (separated by comma), then the first found qualifier is taken into account and shown on the annotation.

Creating and Editing Qualifier

To add a qualifier to an annotation select it in one of the *Sequence View* subviews and press the Insert key, or use the *Add Qualifier* context menu or the *Actions* main menu item.

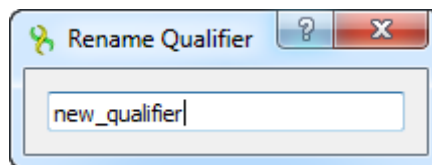


The dialog will appear:

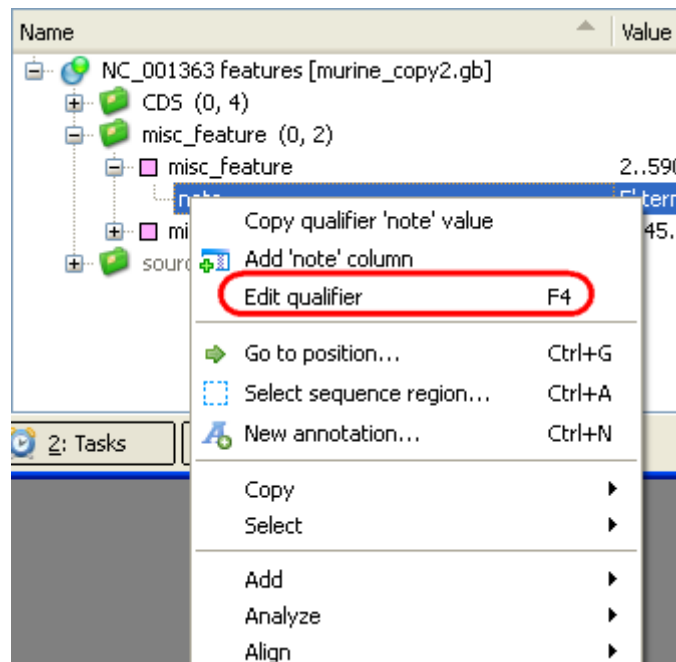


Here you can specify the name and the value of the qualifier.

You can use the F2 key to rename a qualifier:



To edit a qualifier, select the qualifier and press the F4 key or use the *Edit qualifier* context menu item:



Adding Column for Qualifier

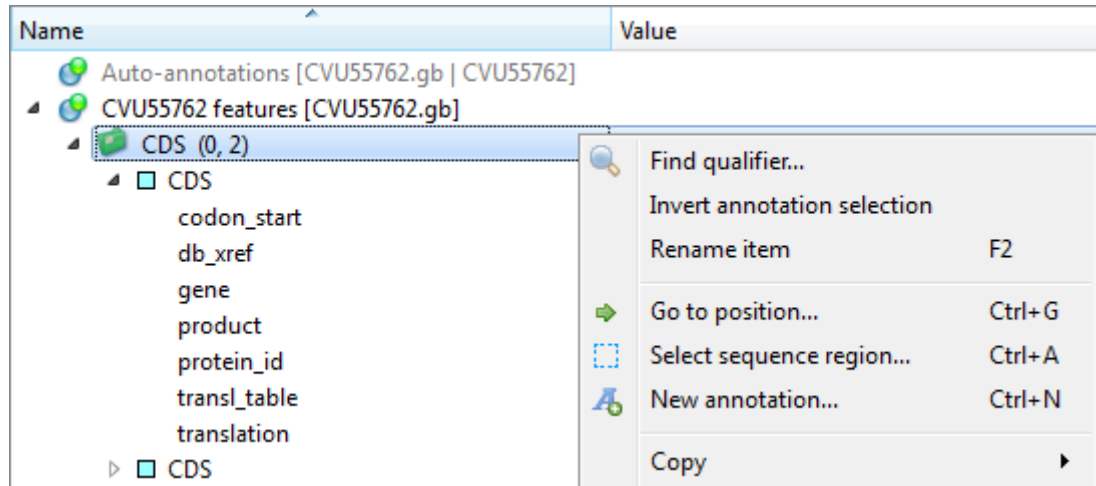
It is possible to add a column with the qualifier values to the *Annotations editor*. To add the column, select the *Add [the qualifier name] column* qualifier context menu item.

Copying Qualifier Text

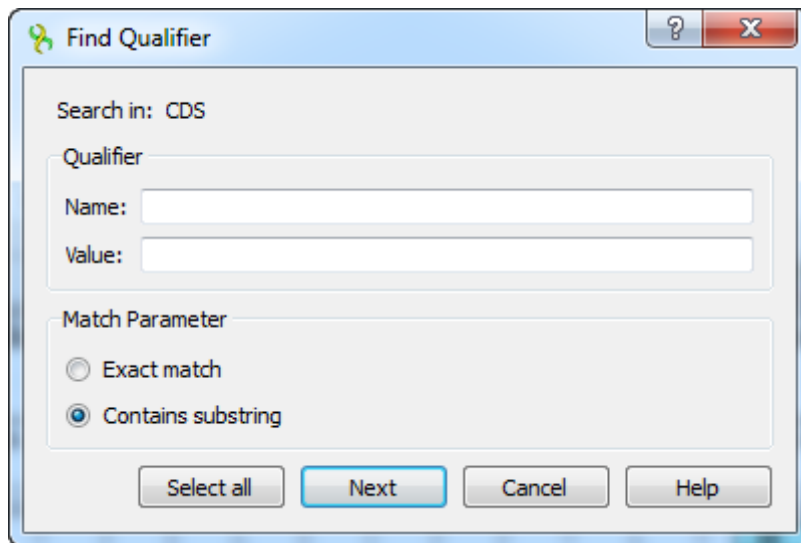
Use the *Copy qualifier [the qualifier name] text* qualifier context menu item to copy the qualifier value.

Finding Qualifier

To find a qualifier select annotation(s) or group(s) of annotations and use the *Find qualifier* context menu.



The dialog will appear:



Here you can specify the name and the value of the qualifier and select the searching parameter: *Exact match* or *Contains substring*.

Deleting Annotations and Qualifiers

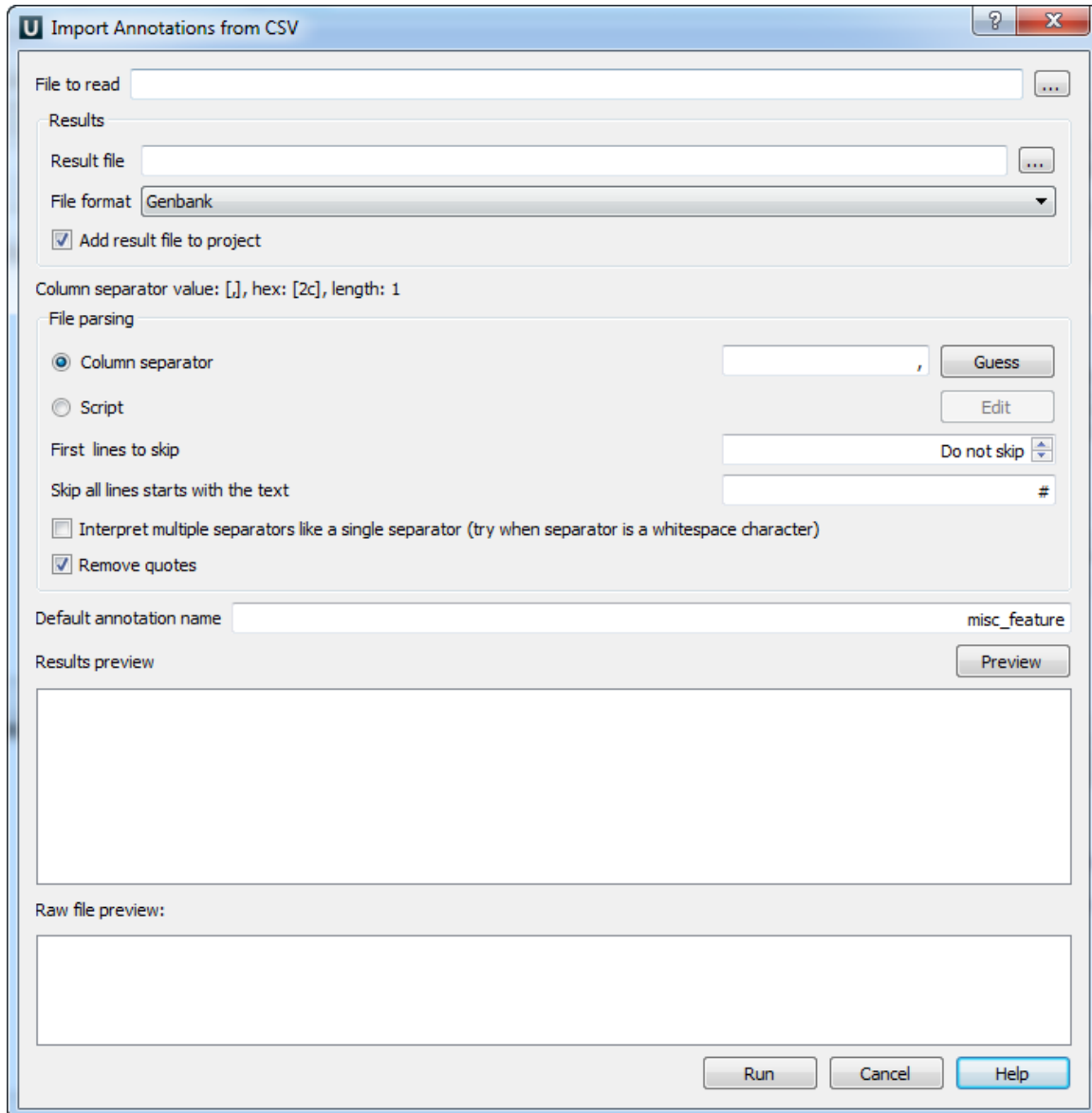
Selected annotations, groups and qualifiers can be deleted using the Delete key.

To remove an annotation object from the active view, select the object in the *Annotations editor* and press the Shift-Delete. Note that the object will not be removed from the project, but just from the active *Sequence View*. To add object again just drag and drop it to the *Sequence View*.

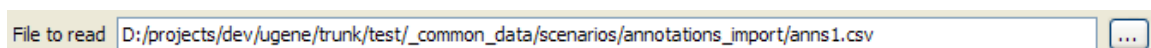
Importing Annotations from CSV

It is possible to import annotations for a sequence from an annotations table stored in the CSV format.

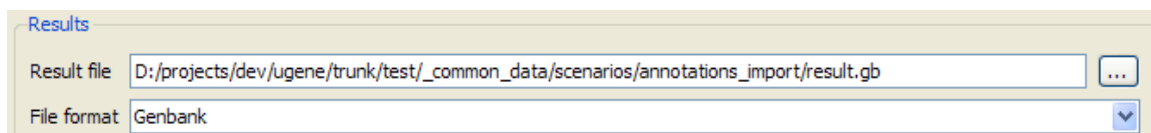
To import annotations from a CSV file, right-click on a *Project View* and select *Import Import annotations from CSV*. The following dialog box will appear:



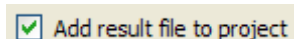
Basically you need to specify the file to read annotations table from (*required*):



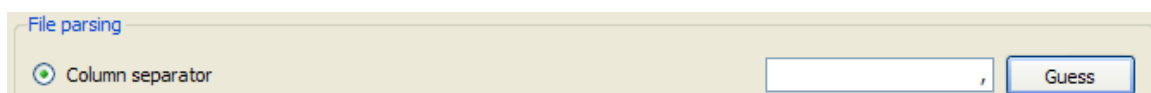
And the format of and the path to the file to write the annotations table into (*required*):



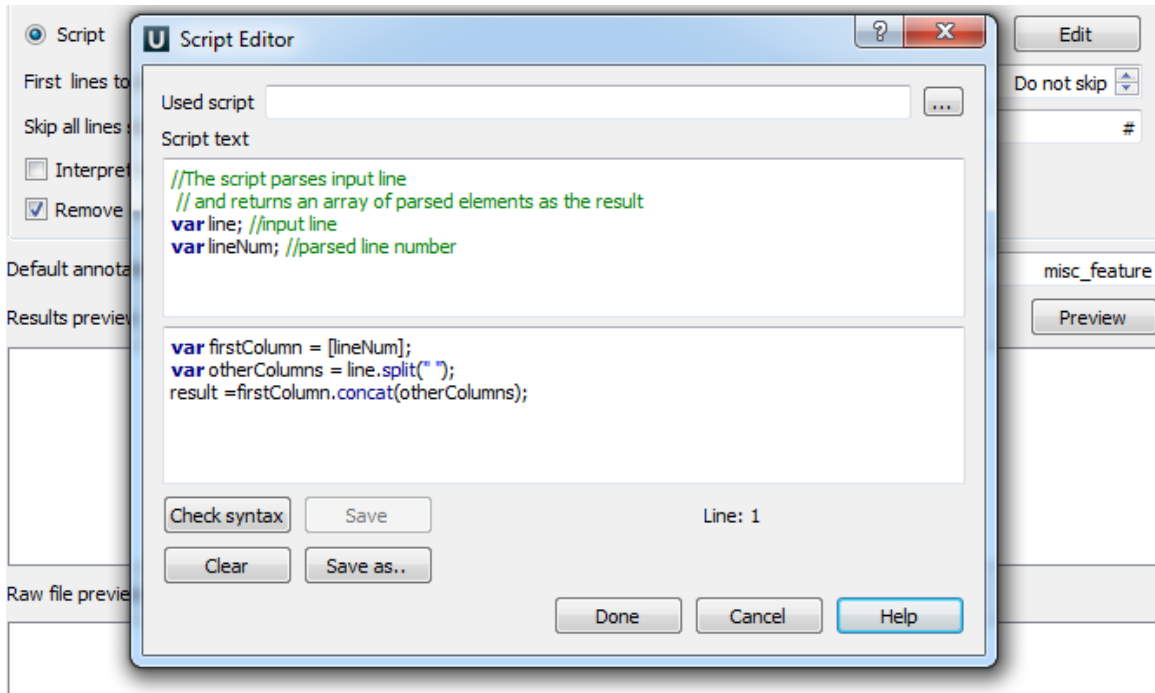
Check *Add result file to project* to link the annotations to the currently opened sequence.



To use a separator to split the table, check the *Column separator* item and specify the separator symbols. Also you can press *Guess* to try to detect the separator from the input file.



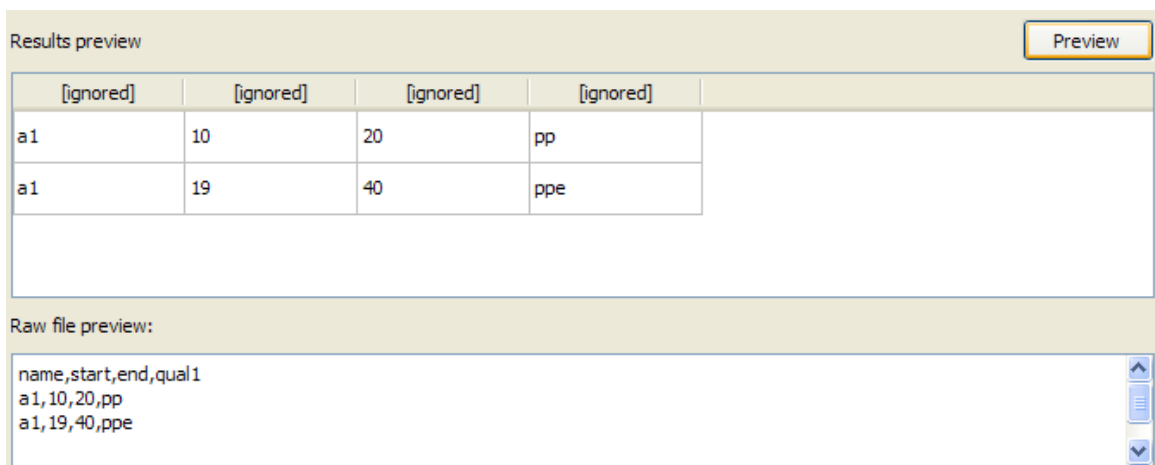
Alternatively, you can press *Edit* and edit the script which will specify the separator for each parsed line. It is possible to use line number in the script.



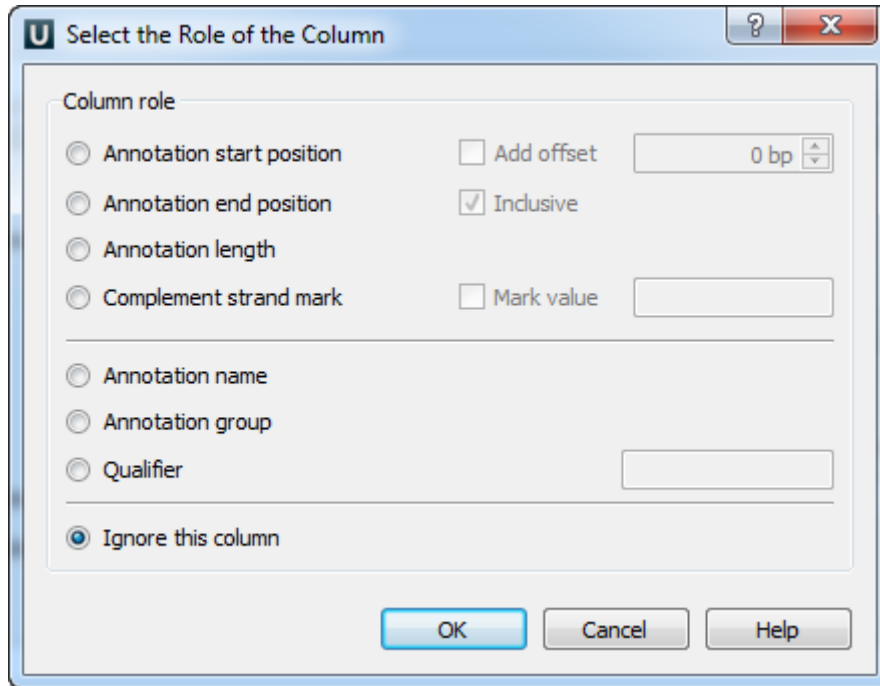
Using the arrows, you exclude the necessary number of lines at the beginning of the document from parsing. You can also skip all lines that start with the specified text.



By pressing *Preview* one can bring up the view of the current annotations table (which is produced from the input file with the specified parameters values). The input file contents will also be shown at the bottom part of the dialog.

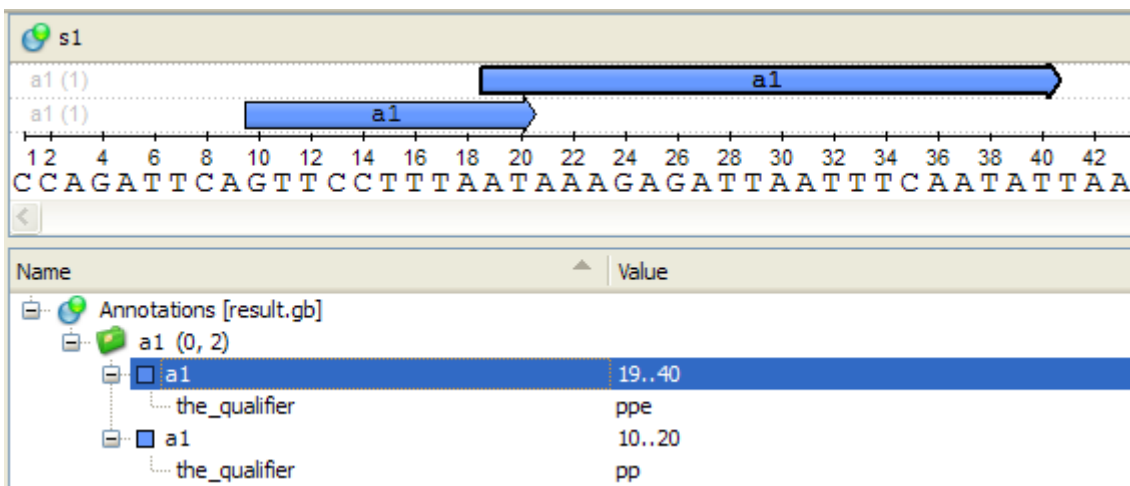


The preview table headline indicates the types of the information contained in the corresponding columns. By default the values are *[ignored]*. To specify a column role, click on the corresponding headline element:



The annotation start and end positions must be specified. It is possible to add an offset to every read start position by checking the *Add offset* checkbox, and to shorten annotations by one from the end by unchecking the *Inclusive* checkbox.

When all the roles are specified, press *Run*. With the *Add to project* checkbox specified and a *Sequence View* opened, on success you will see the *Sequence View* with annotations linked:

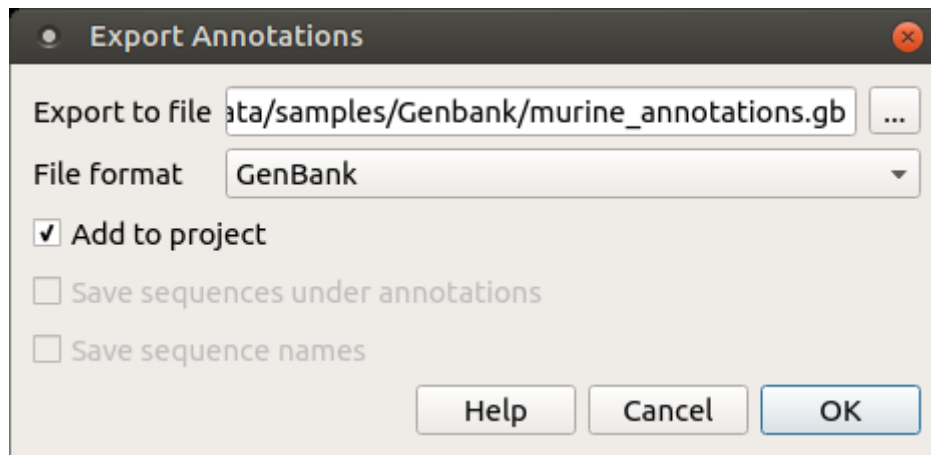


Exporting Annotations

To export annotation(s) open the *Sequence View* with a document that contains annotations. Select a single or several annotations or annotation groups in the *Annotation editor*, select the *Export > Export annotations* context menu item.

Also, you can export annotation(s) from the *Project View* by the *Export > Export annotations* context menu item.

The *Export Annotations* dialog will appear:

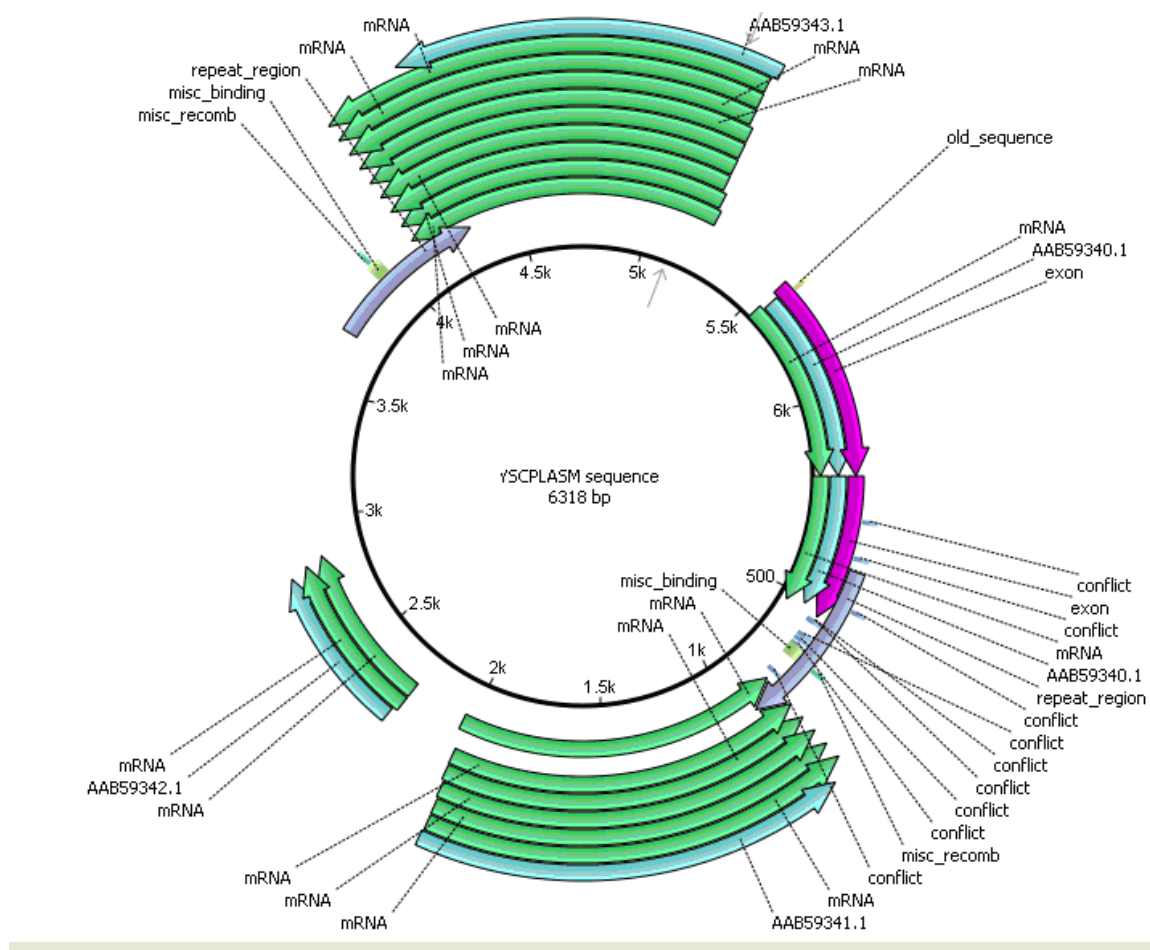


Here you can add the document to project, set the path to the file, choose the file format and optionally for CSV format you can save the sequence along with annotations and save sequence names.

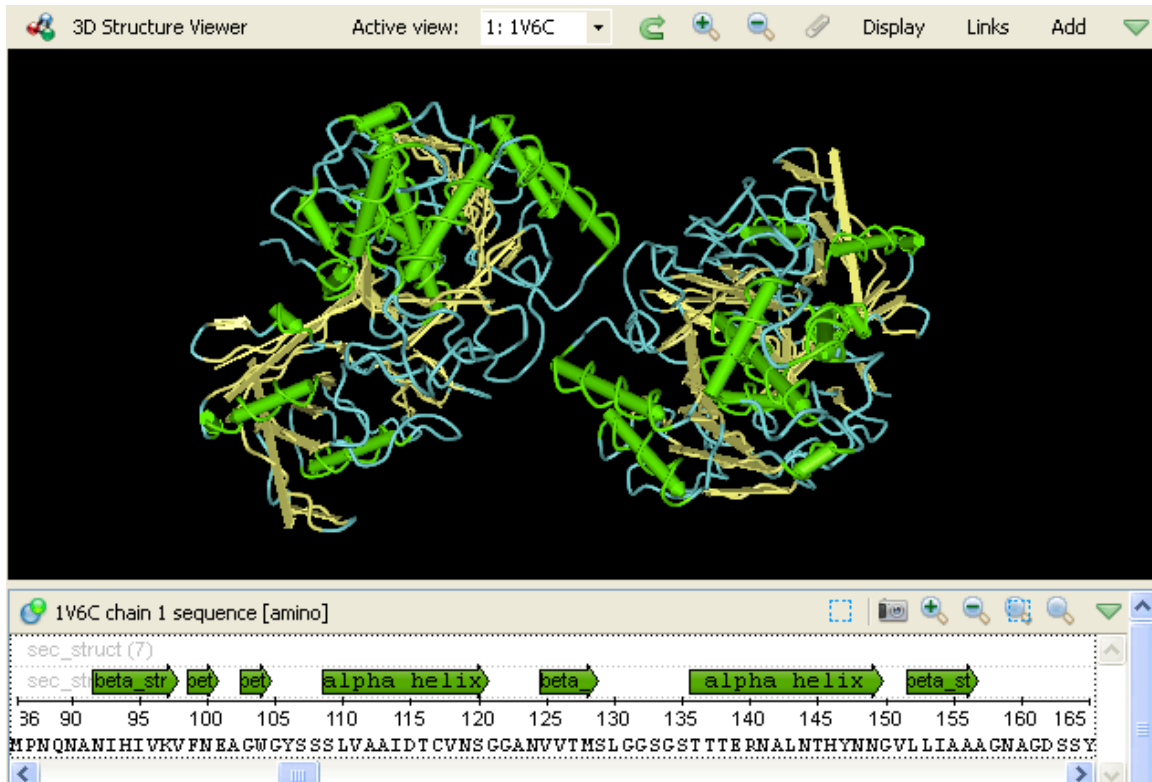
Sequence View Extensions

The functionality of the *Sequence View* can be significantly increased with *Sequence View Extensions*. Below is the demonstration its functionality.

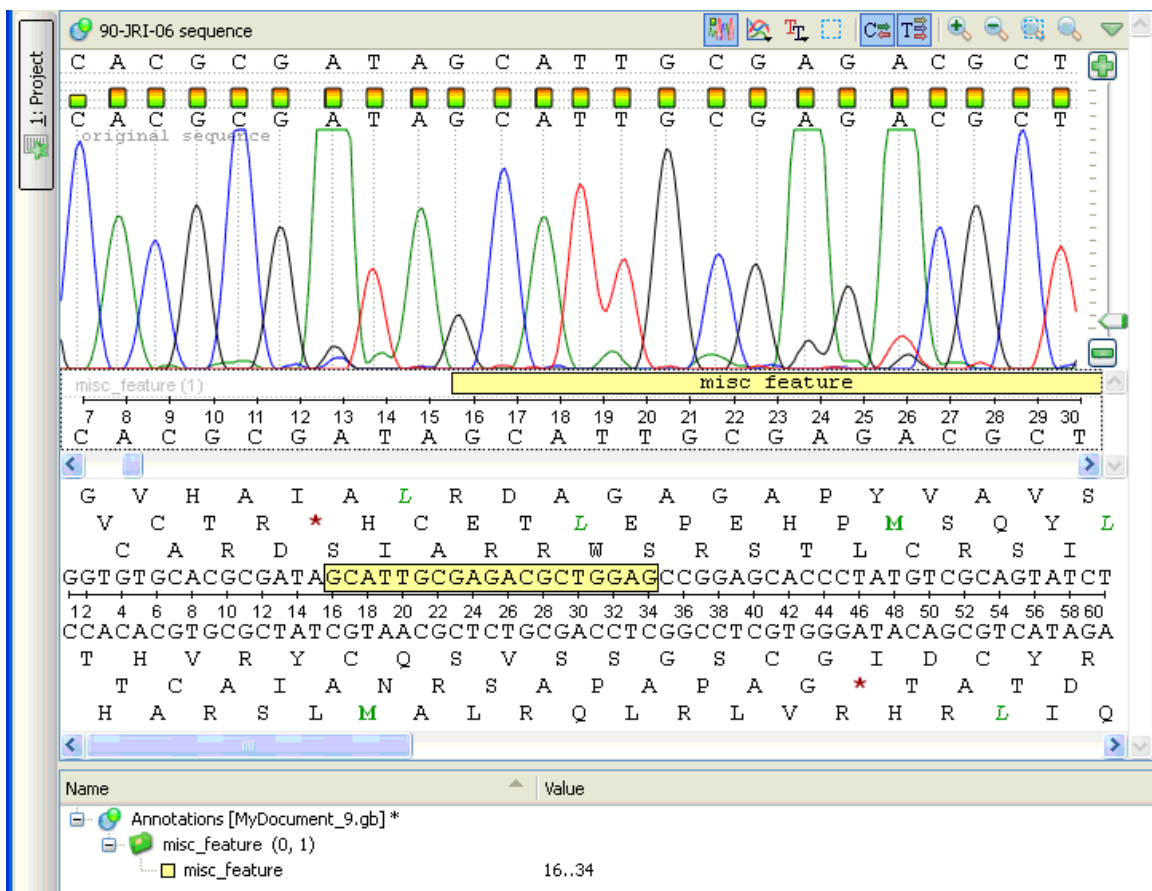
The *Circular Viewer* shows the circular view of a sequence:



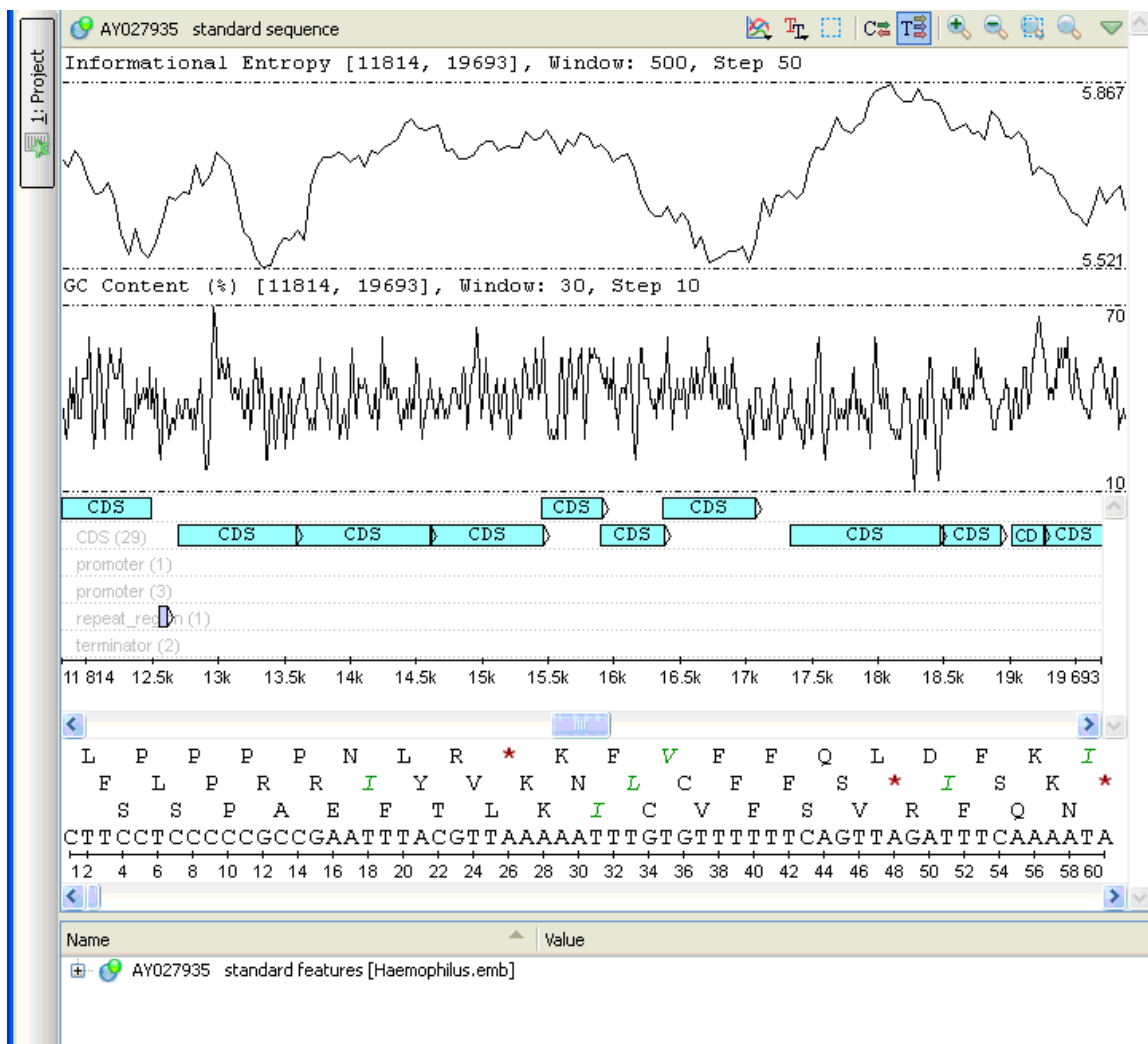
The *3D Structure Viewer* adds 3D visualization for PDB and MMDB files:



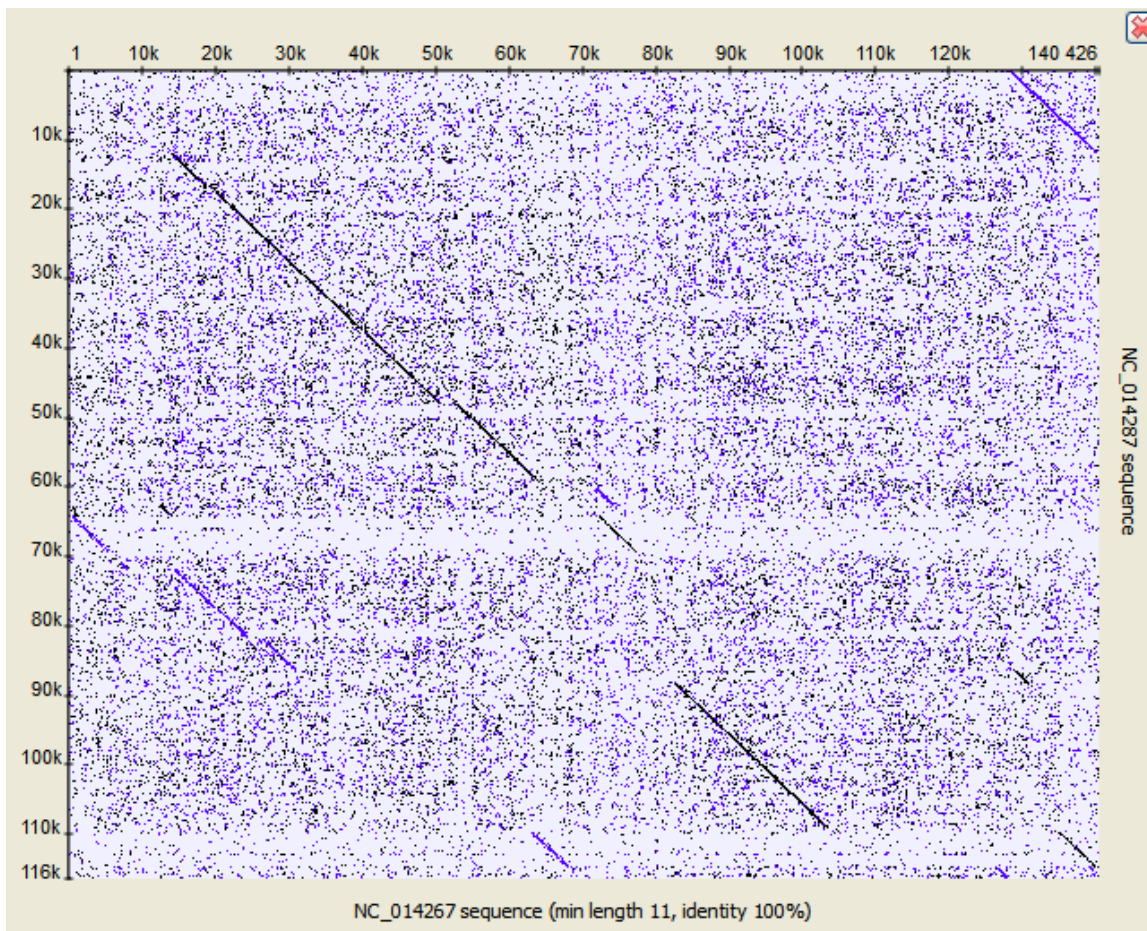
The *Chromatogram Viewer* adds support for chromatograms visualization and editing:



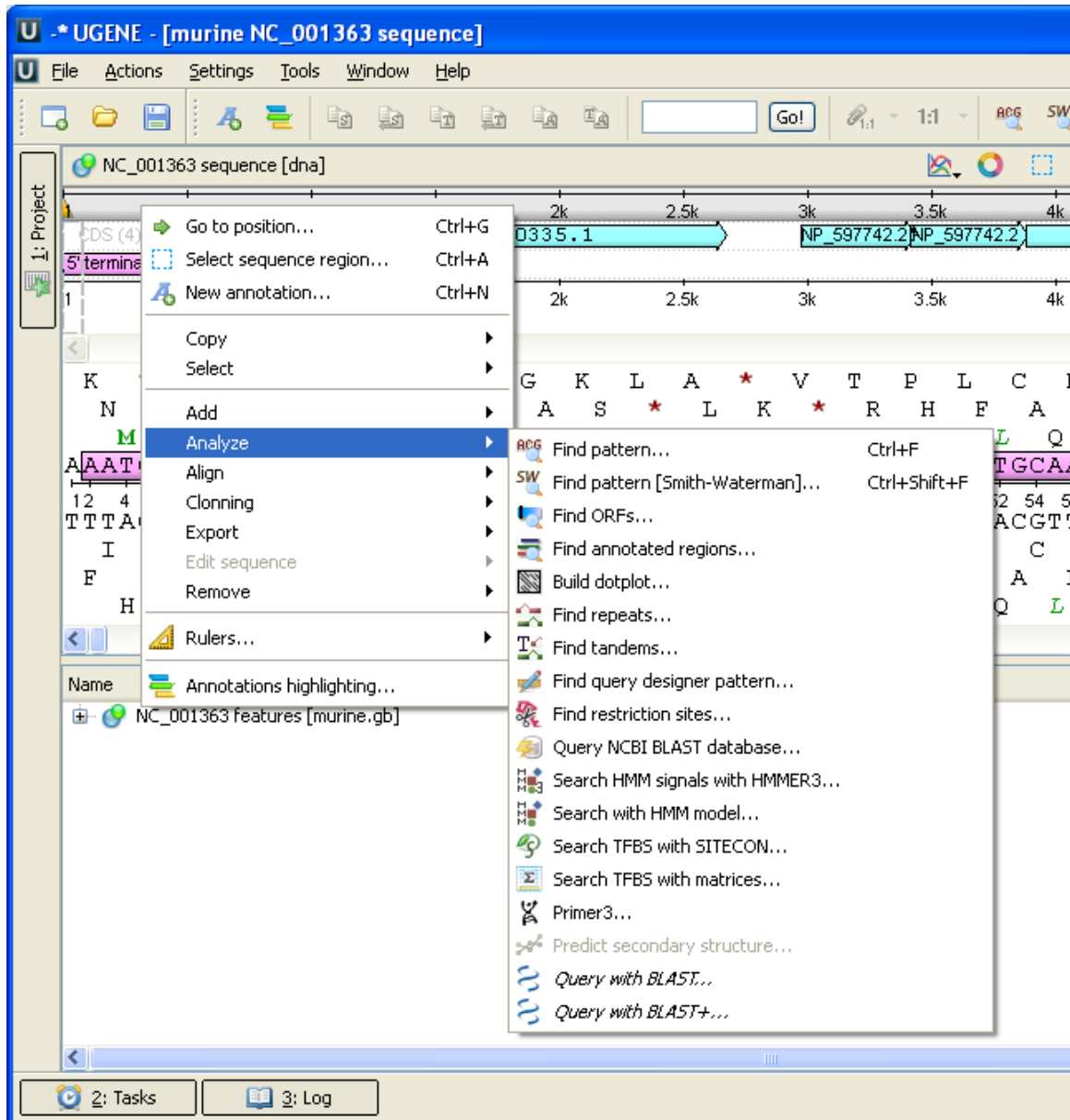
The *DNA Graphs Package* shows various graphs for sequences:



The *Dotplot* provides a tool to build dotplots for DNA or RNA sequences.



A number of other instruments add the graphical interface for popular sequence analysis methods:



For details see the next sections of the documentation:

- Circular Viewer
 - Circular View Settings
- 3D Structure Viewer
 - Opening 3D Structure Viewer
 - Changing 3D Structure Appearance
 - Selecting Render Style
 - Selecting Coloring Scheme
 - Calculating Molecular Surface
 - Selecting Background Color
 - Selecting Detail Level
 - Enabling Anaglyph View
 - Moving, Zooming and Spinning 3D Structure
 - Highlight Region on 3D Structure
 - Selecting Models to Display
 - Structural Alignment
 - Exporting 3D Structure Image
 - Working with Several 3D Structures Views
- Chromatogram Viewer
 - Exporting Chromatogram Data
 - Viewing Two Chromatograms Simultaneously
- Graphs Package
 - Description of Graphs
 - Graph Settings
 - Saving Graph Cutoffs as Annotations
- Dotplot
 - Creating Dotplot
 - Navigating in Dotplot

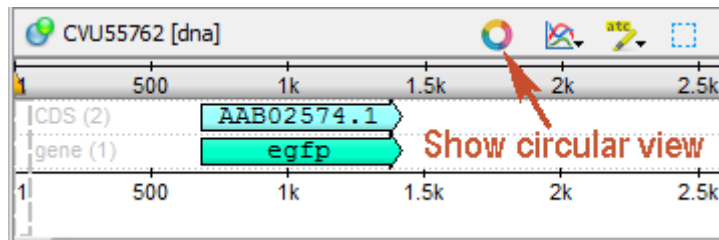
- Zooming to Selected Region
- Selecting Repeat
- Interpreting Dotplot: Identifying Matches, Mutations, Inversions, etc.
- Editing Parameters
- Filtering Results
- Saving Dotplot as Image
- Saving and Loading Dotplot
- Building Dotplot for Currently Opened Sequence
- Comparing Several Dotplots

Circular Viewer

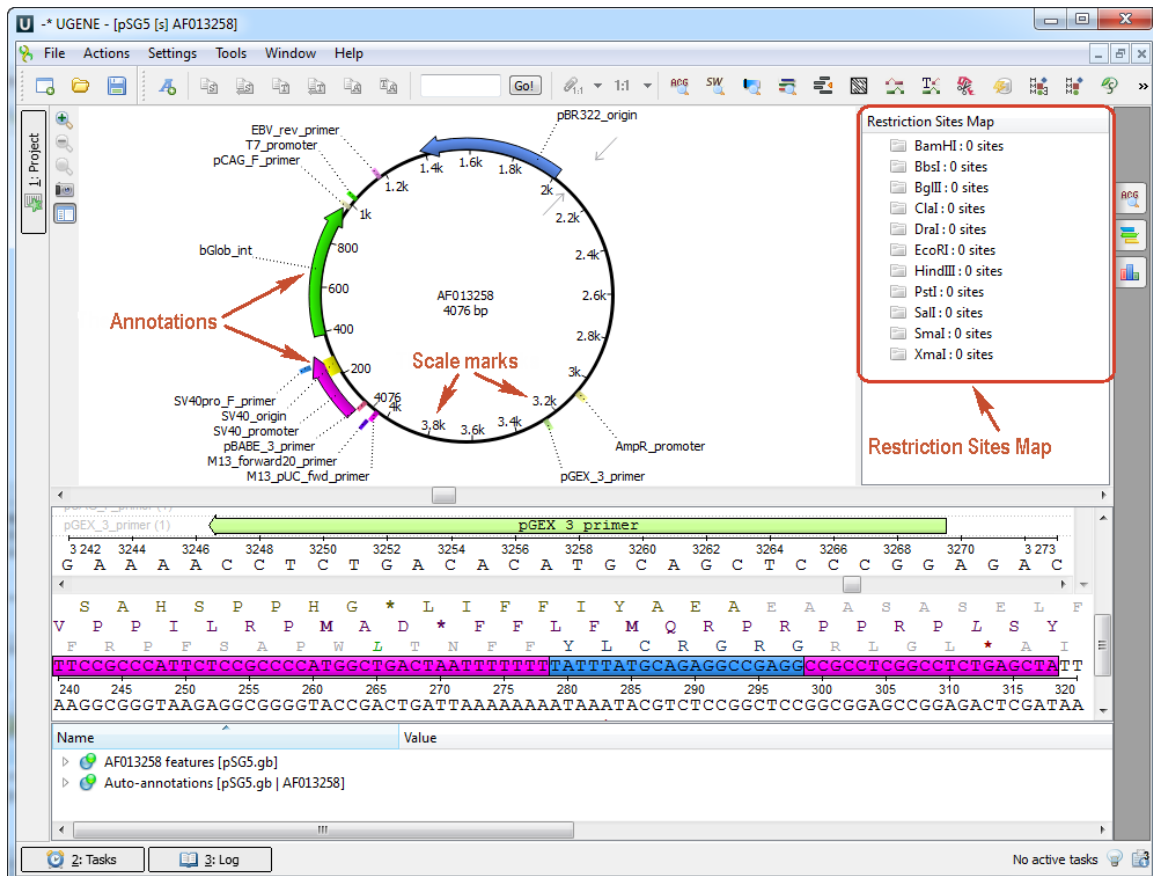
The *Circular Viewer* plugin provides capability to show the circular view of a nucleotide sequence.

Usage example:

Open a nucleotide sequence object in the *Sequence View*. The *Show circular view* button is available on the sequence toolbar:



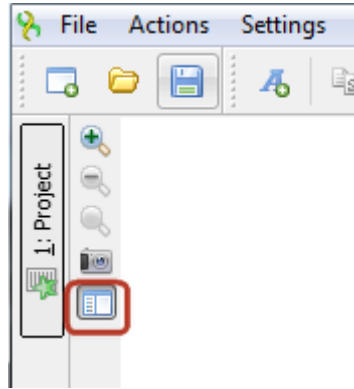
Pressing the button will show the circular view of the sequence:




If you work with file with many sequences the button closes circular views if some circular views are opened and if all circular views are closed, it opens all of them.

Also you can mark sequences as circular in UGENE by the *Mark as circular* sequence context menu item. When the sequences are marked as *Circular*, the Circular View is automatically opened for them in all opened Sequence View windows.

The *Restriction Sites Map* will appear automatically. To show restriction sites the *Show Restriction Sites* menu should be checked. To hide the map click on the following button:

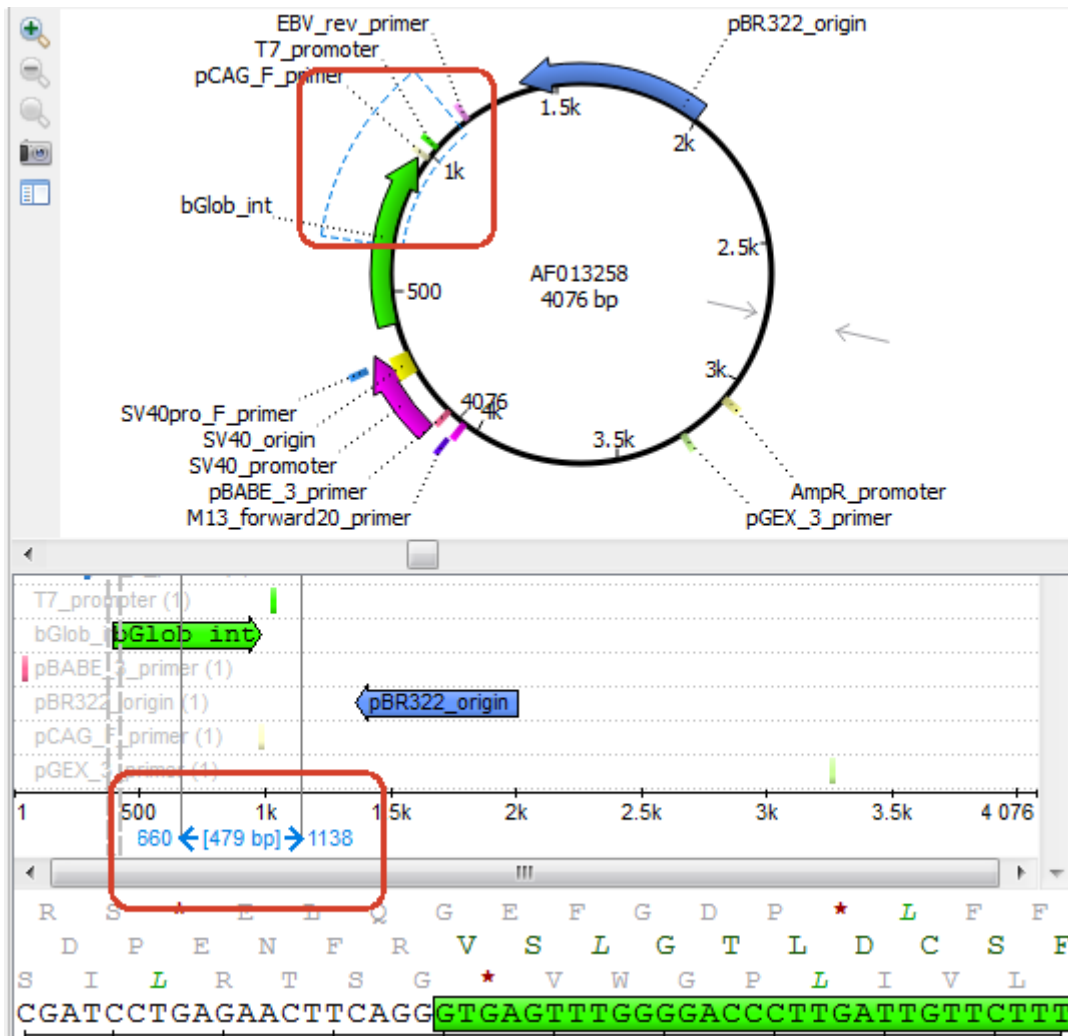


 The *Circular Viewer* is opened automatically when the *Sequence View* is opened for a plasmid.

The inner circle represents the sequence clockwise and the scale marks show the corresponding sequence positions. The sequence annotations are represented as curved colored regions at the outer side of the circle.

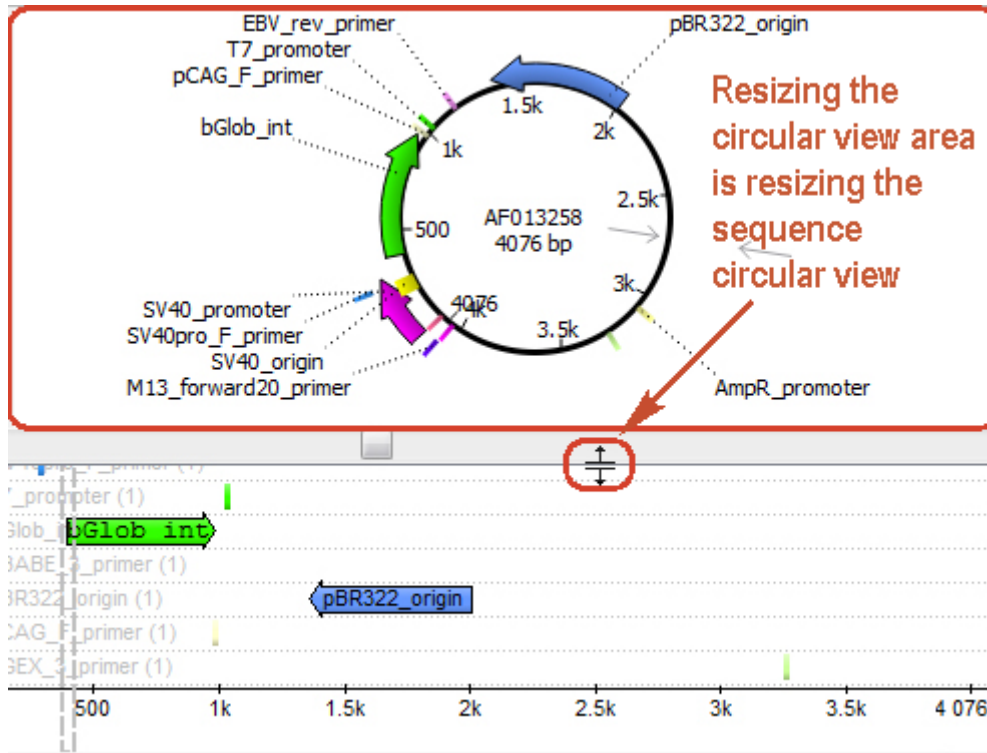
The *Circular Viewer* helps to navigate within the sequence. You can select an annotation on the circular view and the annotation will also be focused and highlighted in all *Sequence View* areas: *Sequence overview*, *Sequence zoom view*, *Sequence details view* and *Annotations editor*.

You can also select a sequence region:



This will also affect the *Sequence View*. You can select a sequence region with *Ctrl* and the selection will be inverted.

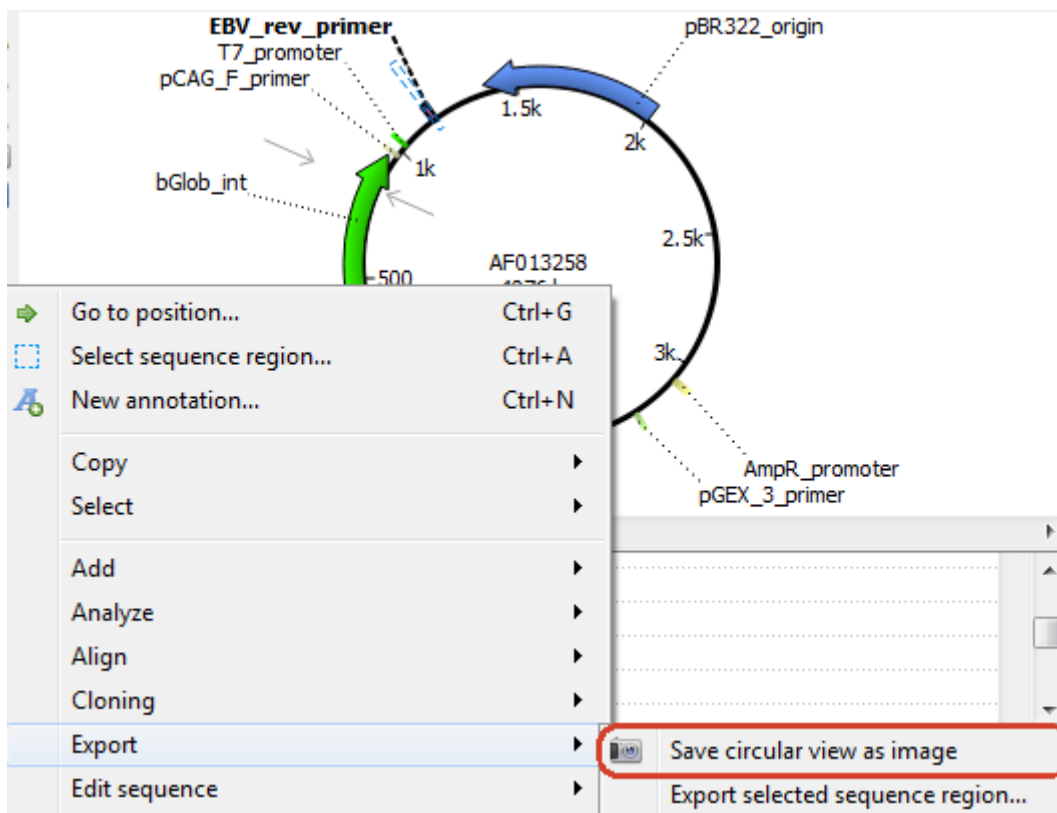
Note that the circular view is zoomed automatically when the *Circular Viewer* area is resized:



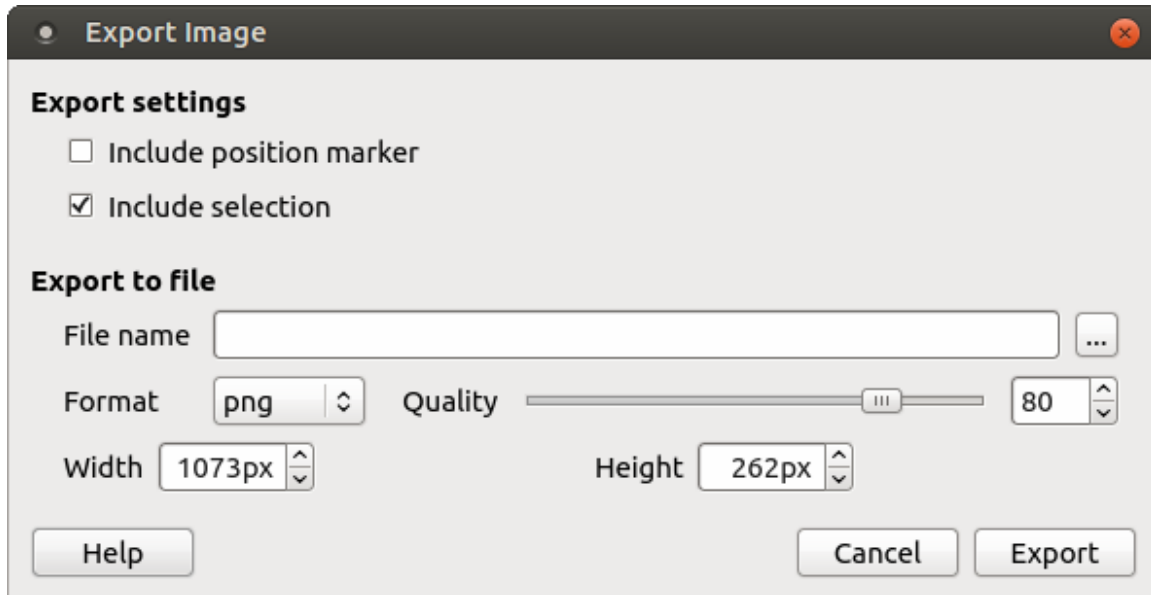
So you can adjust it to an appropriate size.

It is possible to rotate the circular view using the mouse wheel. Also it is possible to shift the start point of a circular molecule by *Edit sequence* -> *Set new sequence origin* context menu item.

Use the *Export* -> *Save circular view as image* context menu or the *Actions* main menu item to save the image of the circular view.

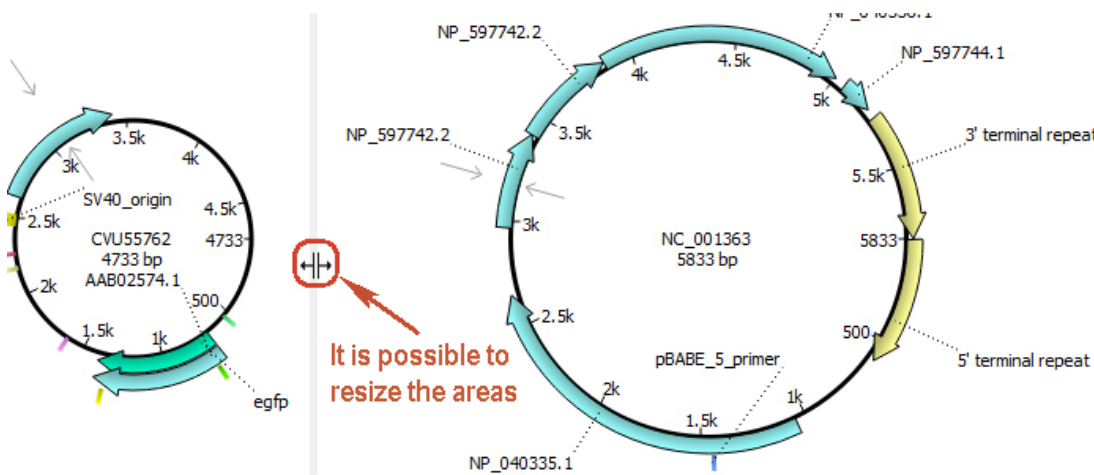


The *Export Image* dialog will appear:



Here you can browse for the file name, select the width, height and resolution of the image as well as its format: svg, ps, pdf, bmp, jpeg, jpg, png, ppm, tif or tiff. Also you can include position and selection markers to the image by the corresponding checkboxes.

Note, that if a sequence file contains several sequences it is possible to view the circular views of the sequences in the same *Circular Viewer* area.

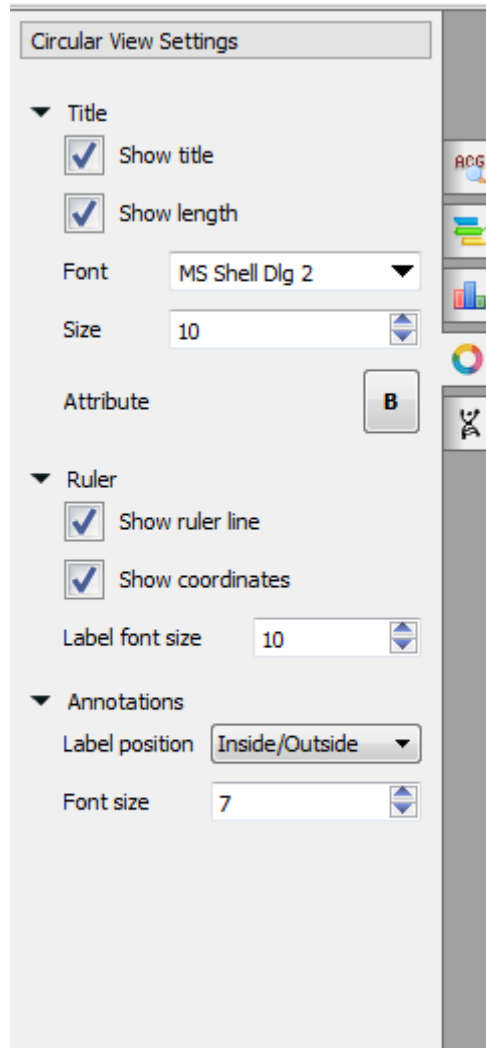


You can work with these circular views at the same time.

Circular View Settings

To configure circular view settings go to the *Circular View Settings* tab in the *Options Panel*.

Activate the circular view for a sequence and the following settings will appear:



In the title section you can show or hide title and length, change font, size and attribute.

In the ruler section you can show or hide ruler line and coordinates and change the label font size.

In the annotation section you can select the label position and change the label size.

The following label positions are available:

- inside - all labels are inside of the annotations
- outside - all labels are outside of the annotations
- inside/outside - if the label can fit the annotation and it is not auto-annotation, it's located inside. Otherwise outside.
- none - no labels at all

3D Structure Viewer

The 3D Structure Viewer is intended for visualization of 3D structures of biological molecules.

Using the 3D Structure Viewer you can work with data from the Protein Data Bank (PDB) - a repository for the 3D structural data of large biological molecules, such as proteins and nucleic acids, maintained by the [Worldwide Protein Data Bank \(wwPDB\)](#).

You can work as well with data from the NCBI [Molecular Modeling DataBase \(MMDB\)](#), also known as "Entrez Structure", a database of experimentally determined structures obtained from the [RCSB Protein Data Bank](#).

Find the description of the 3D Structure Viewer' features below.

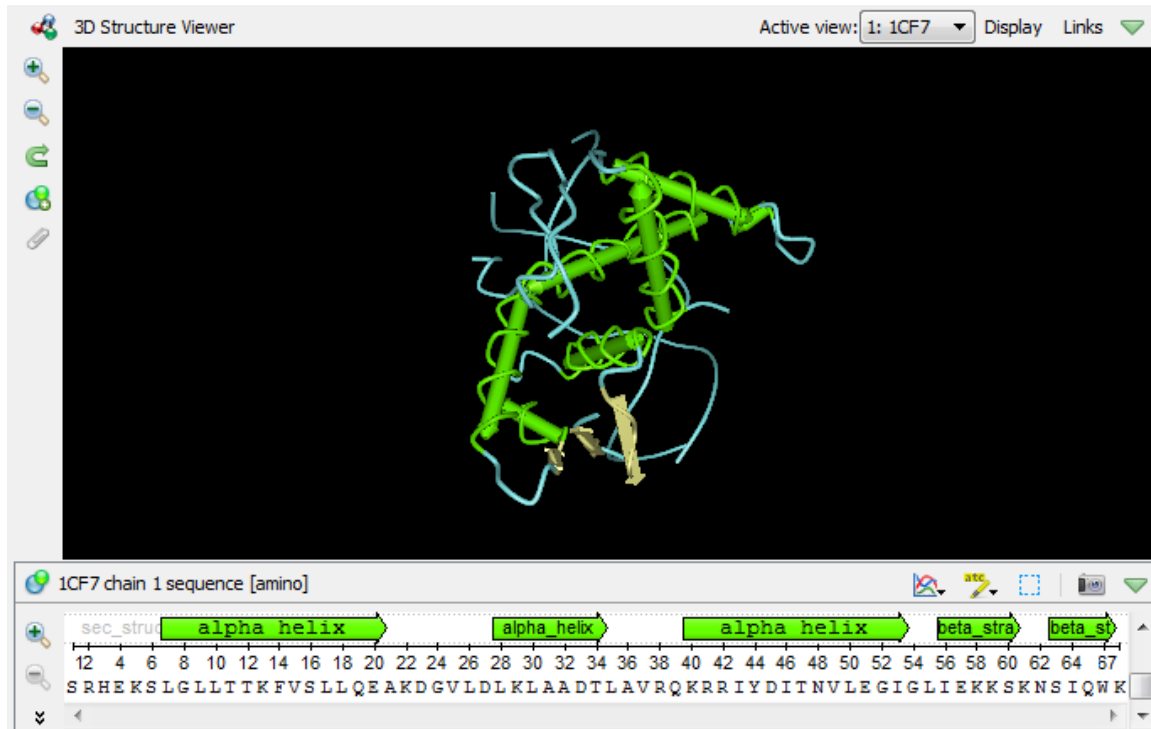
- [Opening 3D Structure Viewer](#)
- [Changing 3D Structure Appearance](#)
 - [Selecting Render Style](#)
 - [Selecting Coloring Scheme](#)
 - [Calculating Molecular Surface](#)
 - [Selecting Background Color](#)
 - [Selecting Detail Level](#)

- Enabling Anaglyph View
- Moving, Zooming and Spinning 3D Structure
- Highlight Region on 3D Structure
- Selecting Models to Display
- Structural Alignment
- Exporting 3D Structure Image
- Working with Several 3D Structures Views

Opening 3D Structure Viewer

The *3D Structure Viewer* is opened automatically when you open a PDB or MMDB file.

For example, *open* \$UGENE/data/samples/PDB/1CF7.PDB. The 3D Structure Viewer adds a view to the upper part of the *Sequence View*.



Notice the *Links* button on the toolbar. When you click the button the menu appears with quick links to online resources with detailed information about the molecule opened:

- PDB Wiki
- RSCB PDB
- PDBsum
- NCBI MMDB

Note that if you're online, you can access the Protein Data Bank directly from UGENE and load a required file by its PDB ID (see *Fetching Data from Remote Database* for details).

Hint

Don't forget to select the correct database (PDB) while fetching.

Changing 3D Structure Appearance

This chapter describes how you can change a 3D structure appearance.

- Selecting Render Style
- Selecting Coloring Scheme
- Calculating Molecular Surface
- Selecting Background Color
- Selecting Detail Level
- Enabling Anaglyph View

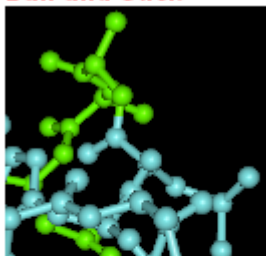
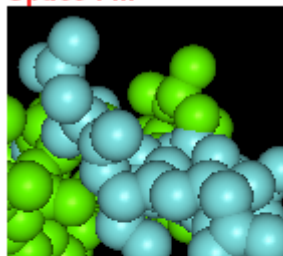
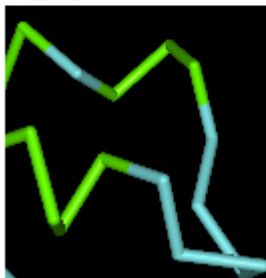
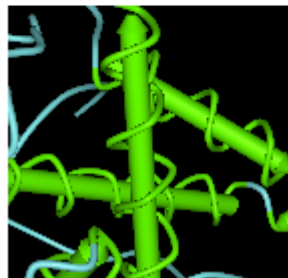
Selecting Render Style

The following render styles are available:

- *Ball-and-Stick*
- *Space Fill*

- *Tubes*
- *Worms*

To change the render style select an appropriate item in the *Render Style* menu (it can be found either in the 3D Structure Viewer context menu or in the the *Display* menu on the toolbar).

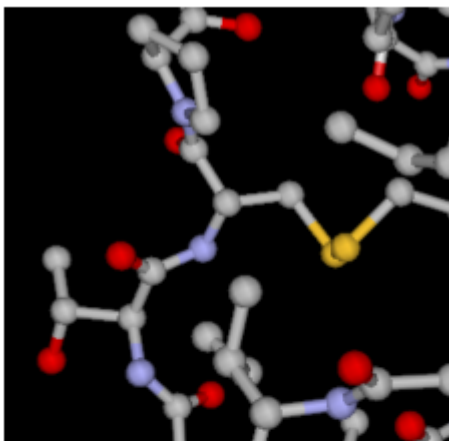
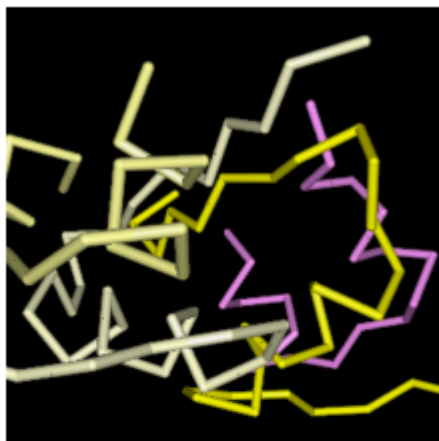
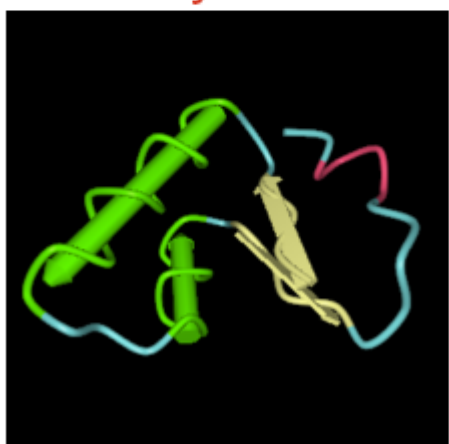
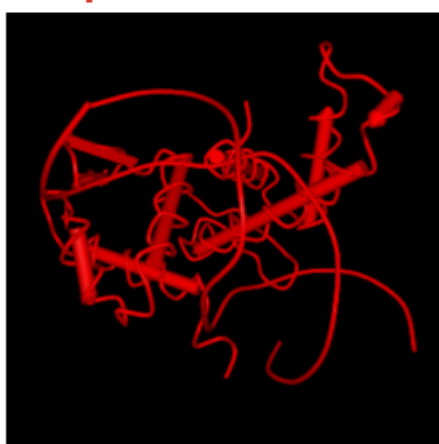
Ball-and-Stick**Space Fill****Tubes****Worms**

Selecting Coloring Scheme

You can select one of the following coloring schemes:

- *Chemical Elements*
- *Molecular Chains*
- *Secondary Structure*
- *Simple colors*

To change the coloring scheme open the *Coloring Scheme* menu (available in the context menu and in the *Display* menu on the toolbar).

Chemical elements**Molecular chains****Secondary structure****Simple colors****Calculating Molecular Surface**

To calculate the molecular surface of a molecule select the *Molecular Surface* item in the 3D Structure Viewer context menu or in the *Display* menu on the toolbar and check one of the following items:

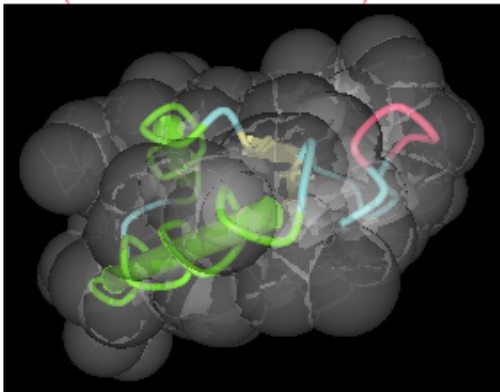
- SAS (solvent-accessible surface)
- SES (solvent-excluded surface)
- vdWS (van der Waals surface)

To remove the molecular surface that has already been calculated select the *Off* item.

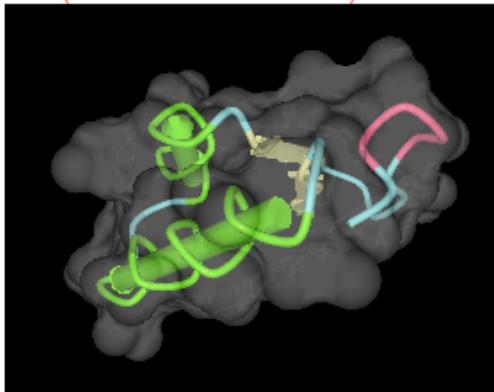
You can also select the *Molecular Surface Render Style* to modify the calculated molecular surface appearance:

- *Convex Map*
- *Dots*

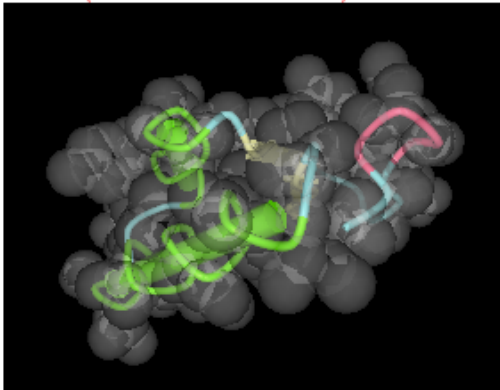
SAS (solvent-accessible surface)



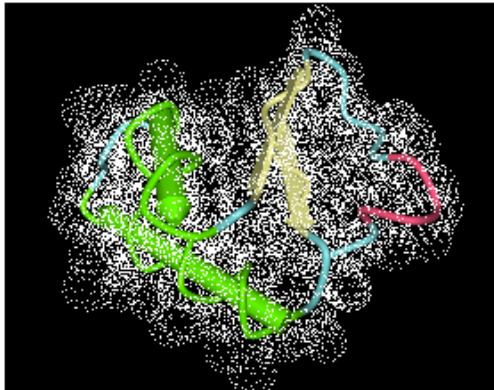
SES (solvent-excluded surface)



vdWS (van der Waals surface)



vdWS with dots



Selecting Background Color

To change the background color open the *Settings* dialog (choose the *Settings* item in the 3D Structure Viewer context menu or in the *Display* menu on the toolbar), press the *Set background color* button and select a color in the dialog appeared.

Selecting Detail Level

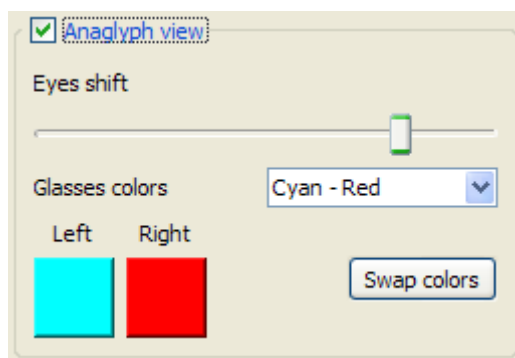
To select the detail level of a 3D Structure representation open the *Settings* dialog of the 3D Structure Viewer and drag the *Detail level* slider.

Enabling Anaglyph View

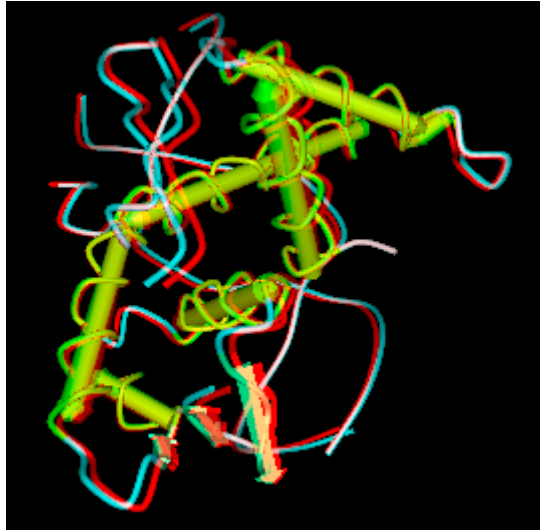
UGENE allows you to view a molecule in the anaglyph mode. To enable the anaglyph view open the *Settings* dialog of the 3D Structure Viewer and check the *Anaglyph view* check box.

You can modify the color settings: select one of the available *Glasses colors* or set custom colors, swap the colors.

The offset of the color layers can be adjusted by dragging the *Eyes shift* slider.



See the result the anaglyph view is applied to a molecule below:



Moving, Zooming and Spinning 3D Structure

A 3D structure can be easily spinned, moved and resized:

- To spin the 3D structure drag the mouse on the 3D structure while holding the left mouse button.
- To move the 3D structure hold the Ctrl keyboard button and drag the mouse with the left button pressed.
- To resize the 3D structure either use the mouse wheel or *Zoom In* and *Zoom Out* buttons on the toolbar.

At any time you can restore the default view by pressing the *Restore Default View* button on the toolbar.



You can also overview the whole structure by spinning it automatically. Select the *Spin* item either in the 3D Structure Viewer context menu or in the *Display* menu on the toolbar to do it.

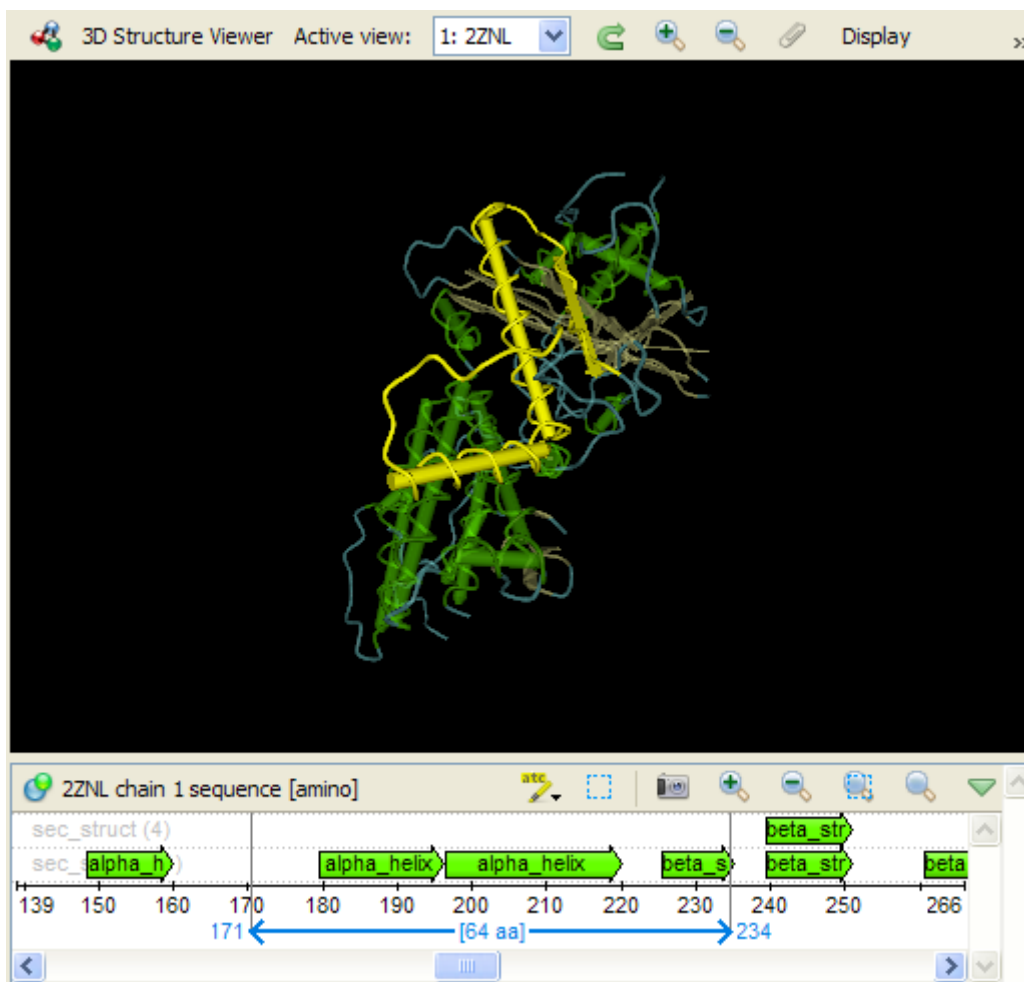
To stop the spinning uncheck the *Spin* item.

Highlight Region on 3D Structure

When you *are selecting a region of a sequence* e.g in the *Sequence zoom view* the corresponding region on the 3D structure is being highlighted while the rest regions of the 3D structure are being shaded.

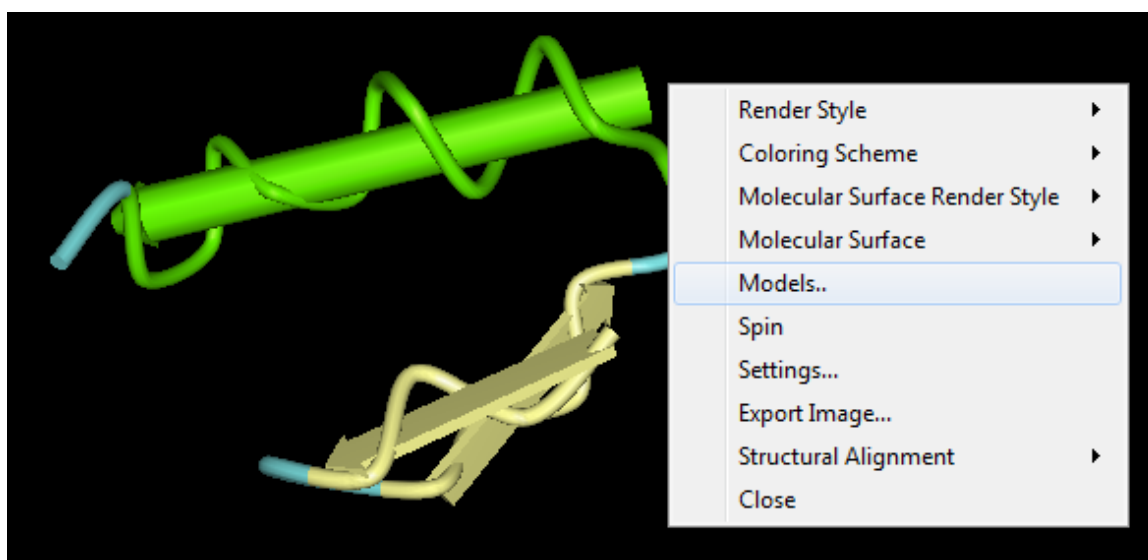
To configure the color of a region selected open the *Settings* dialog (press the *Settings* item in the 3D Structure Viewer context menu or in the *Display* menu on the toolbar to do it), press the *Set selection color* button and select a color in the dialog appeared.

To adjust the shading drag the *Unselected regions shading* slider in the *Settings* dialog.

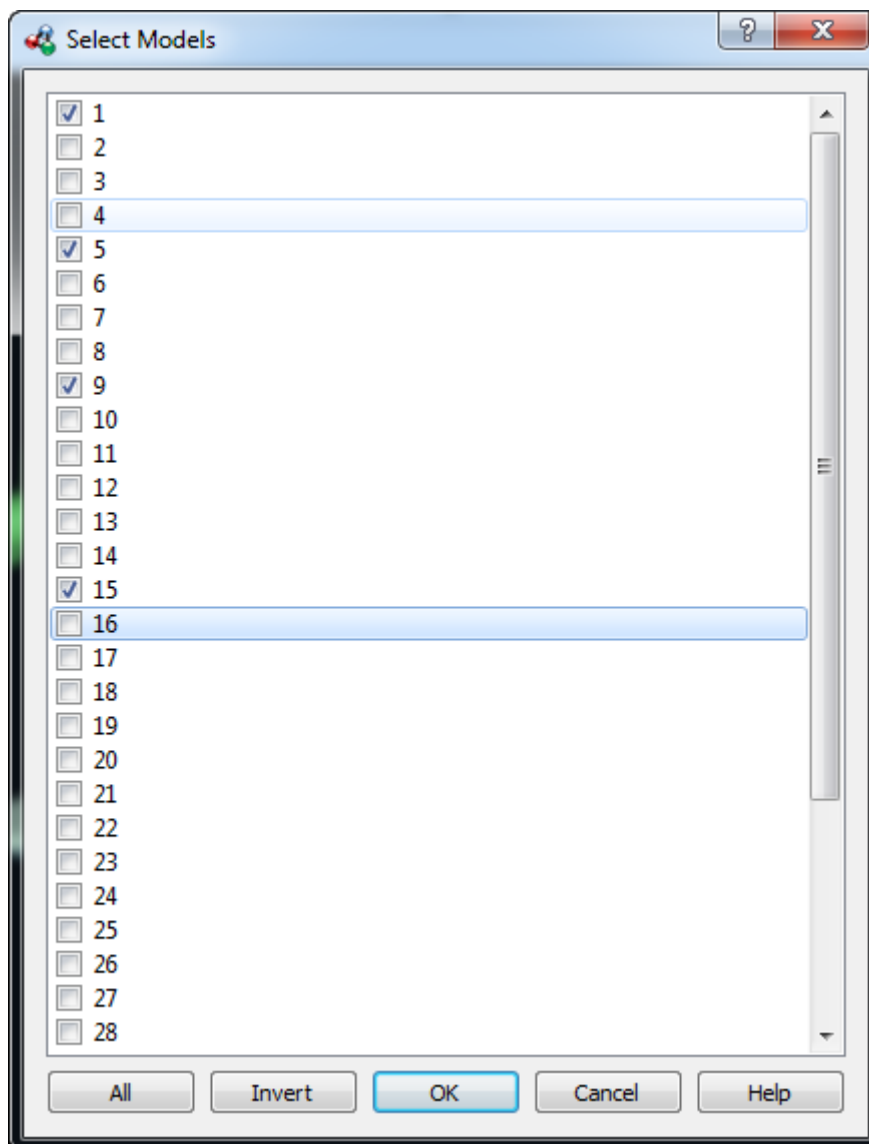


Selecting Models to Display

When a molecular structure contains multiple models (e.g. NMR ensembles of models), the *Models* item appears in the 3D Structure Viewer context menu and in the *Display* menu on the toolbar. For example, for protein with PDB ID 1ZNF the menu looks as follows:



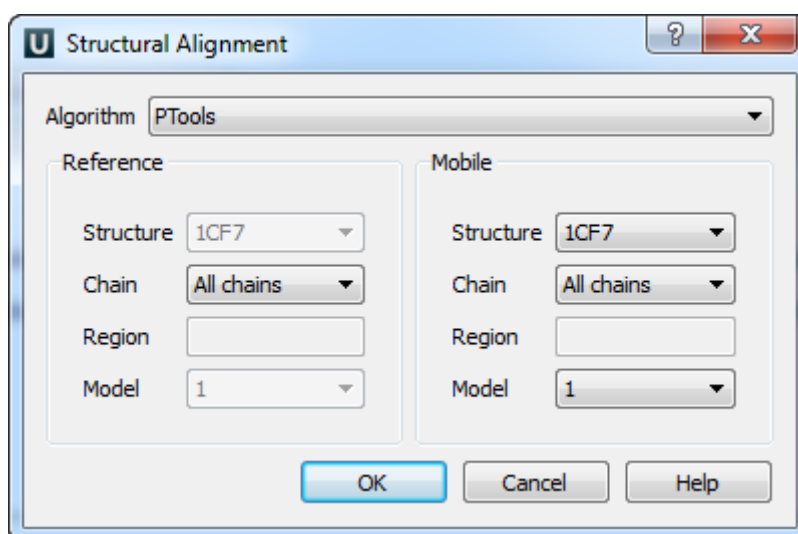
The dialog will appear:



To show all the models check the *All* item. To show only one model check the item and click the *OK* button. To show several models select it and click *OK* button. To show the inverted selection click the *Invert* button and click *OK* button.

Structural Alignment

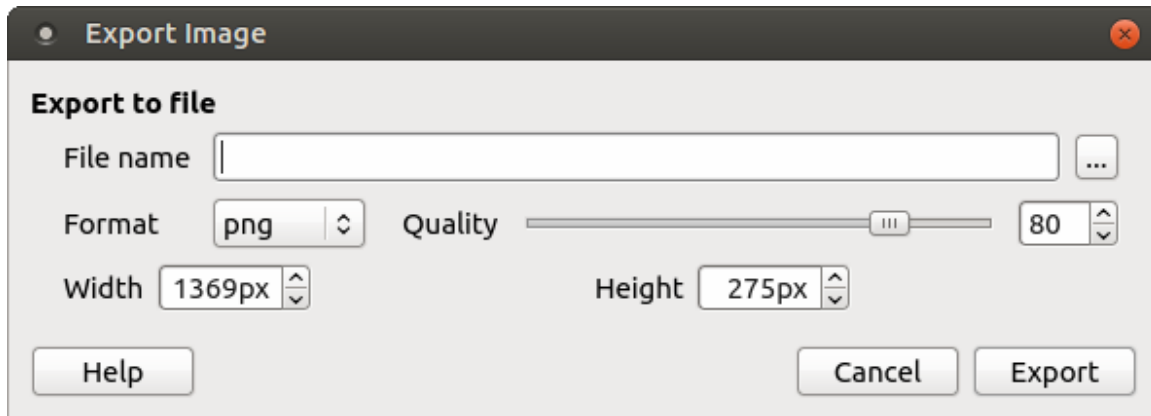
To use the structural alignment call the *Structural alignment->Align with* context menu item. The following dialog will appear:



Here you can change reference and mobile settings. After that click on the *OK* button. To reset structural alignment call the *Structural alignment->Reset* context menu item.

Exporting 3D Structure Image

To export a 3D structure image select the *Export Image* item in the 3D Structure Viewer context menu or in the *Display* menu on the toolbar. The *Export Image* dialog will appear:

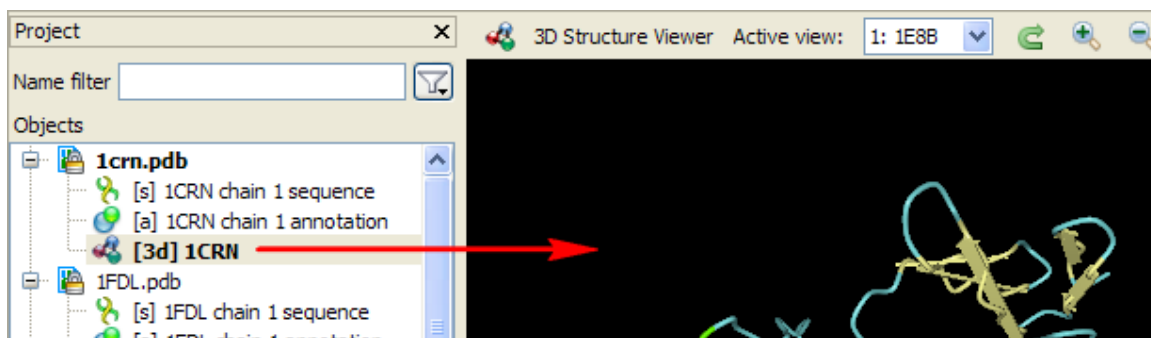


Here you can browse for the file name, select the width and height of the image as well as its format: svg, png, ps, jpg, jpeg, tiff, tif, pdf, bmp or ppm. For jpg, jpeg formats the quality score parameter is available.

Working with Several 3D Structures Views

To add another view to the 3D Structure Viewer you can:

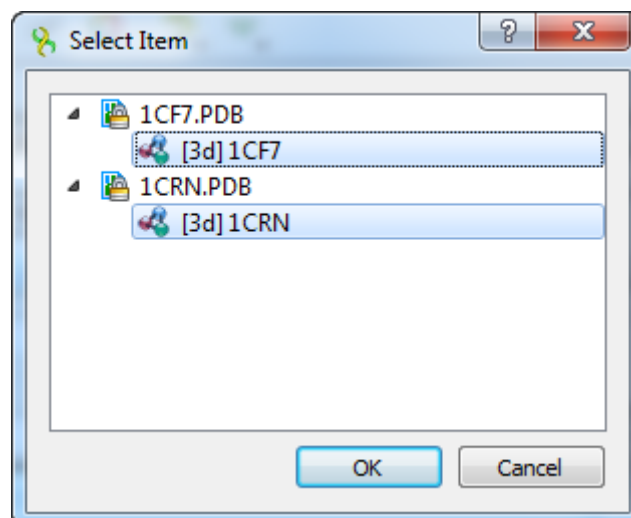
- Drag a required [3d] object from the *Project View* to the 3D Structure Viewer.



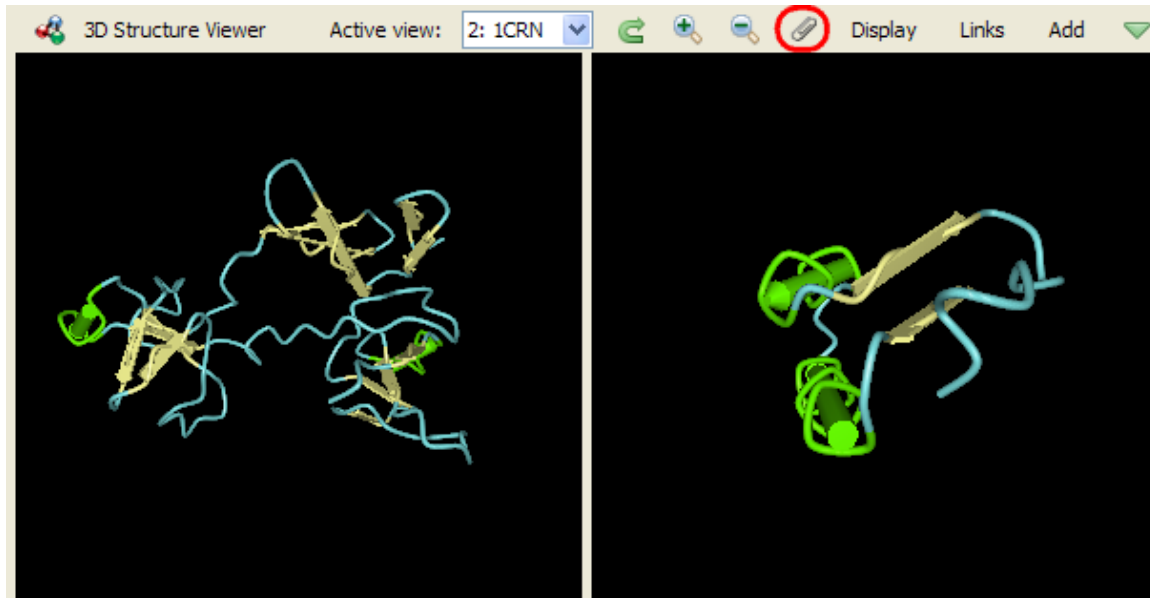
- Press the *Add* button on the toolbar. The *Select Item* dialog will appear. Select [3d] objects to add.

Hint

Use the Ctrl keyboard button to select several objects.



Below you can see the 3D Structure Viewer with two views:

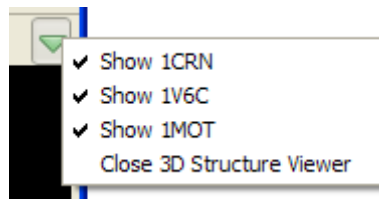


To select an active view click on the view area or select an appropriate value in the *Active view* combo box on the toolbar.

To synchronize the views press the *Synchronize 3D Structure Views* sticky button on the toolbar (see the image above). When the button has been pressed the 3D structures are *moved, zoomed and spinned* synchronously. Press the button again to stop the views synchronization.

The views that are no more required can be closed by selecting the *Close* button in the 3D Structure Viewer context menu.

Also you can hide/show views for a while. Use the menu of the green arrow button on the toolbar to do it:



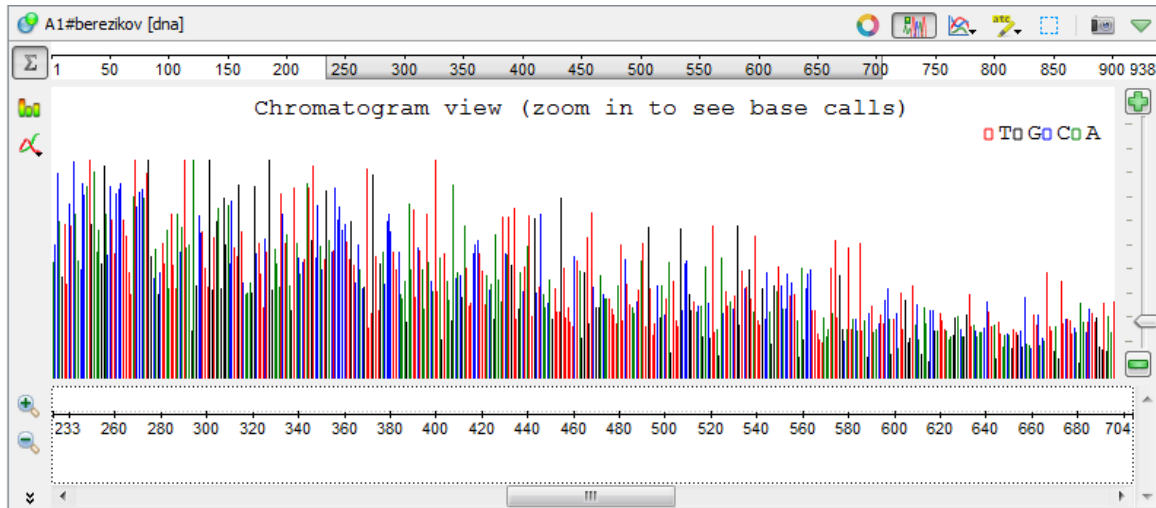
Notice that the 3D Structure Viewer can be closed from this menu.

Chromatogram Viewer

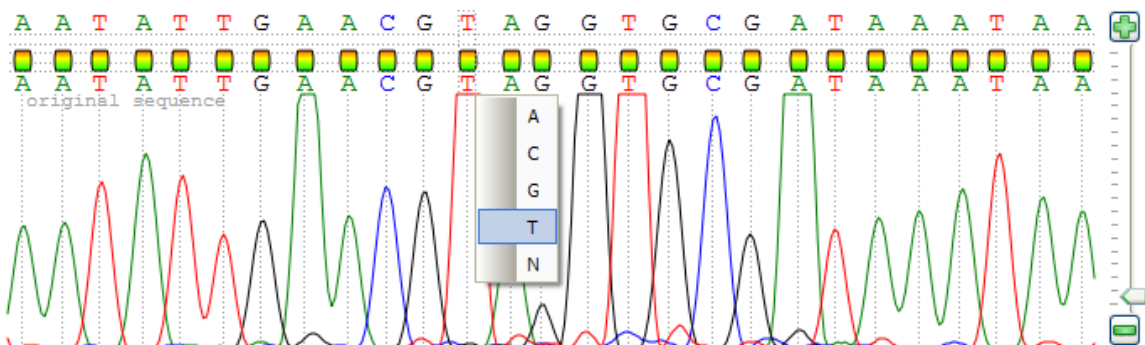
The *Chromatogram Viewer* plugin brings DNA chromatogram data viewing and editing capabilities into UGENE.

Currently supported chromatogram file formats are ABIF and SCF.

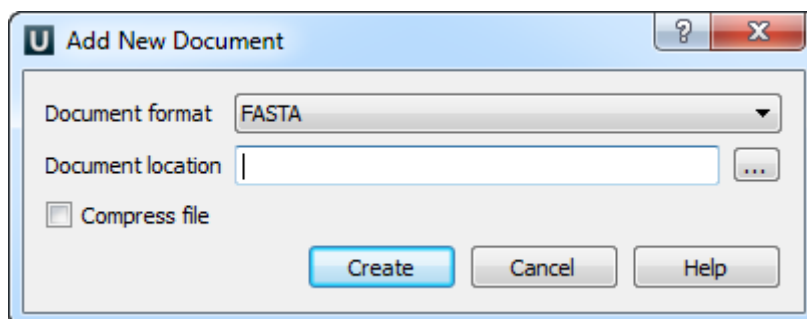
To view a chromatogram, just open an interesting file in UGENE by standard means (e.g. drag&drop the file or press the Ctrl-O shortcut). The *Chromatogram Viewer* is automatically embedded into the generic *Sequence View* if chromatogram data are found, as on the screenshot below:



After zooming in, more chromatogram details are available:



To edit a sequence data, right-click on the chromatogram view and select the *Edit new sequence* item in the appeared context menu. The following dialog will appear:

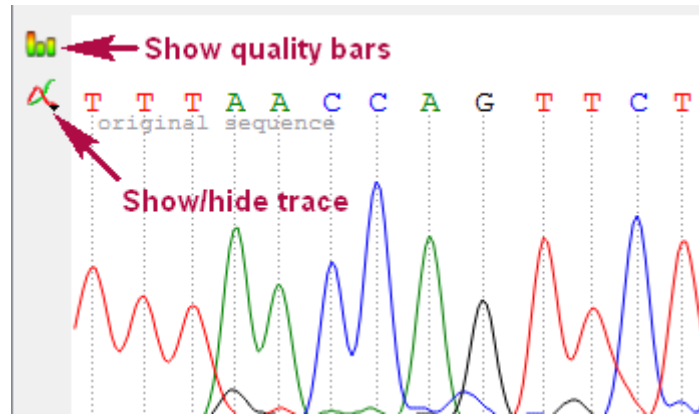


Select new document format and location and click on the *Create* button.

The original DNA sequence is not allowed to be changed; however you can add and modify a new sequence stored in a separate file.

The sequence being edited is displayed right above the original one. Symbols can be changed by clicking on interesting value, modifications are shown in bold.

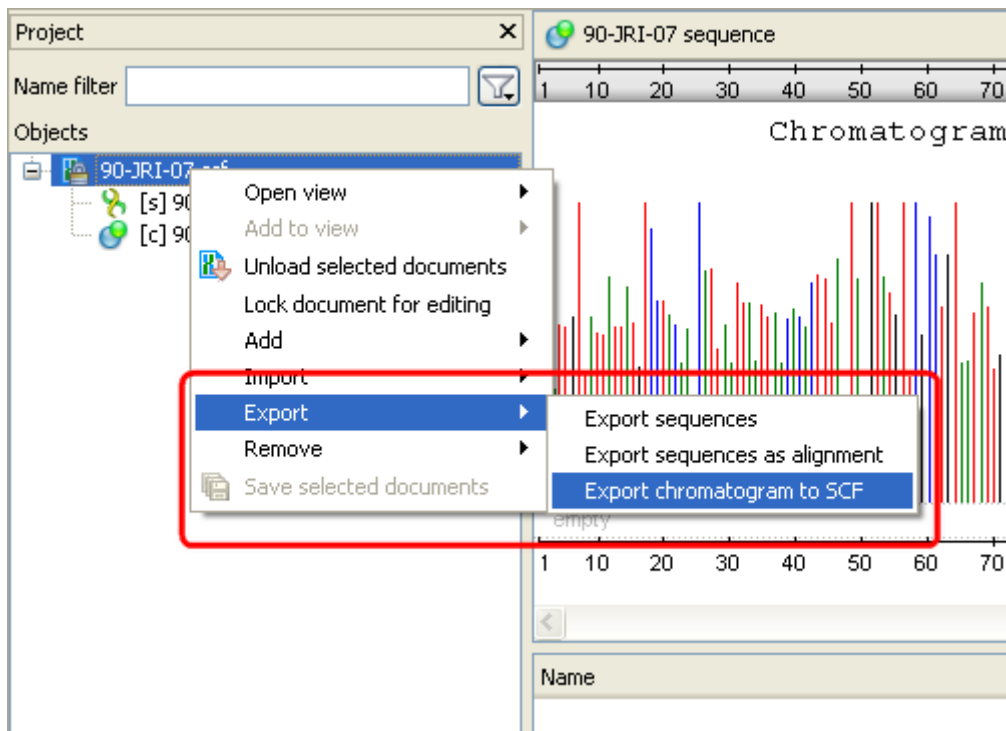
Also you can show/hide different signals of chromatogram and quality bars with a help of the *Show/hide trace* and *Show quality bars* toolbar buttons correspondingly:



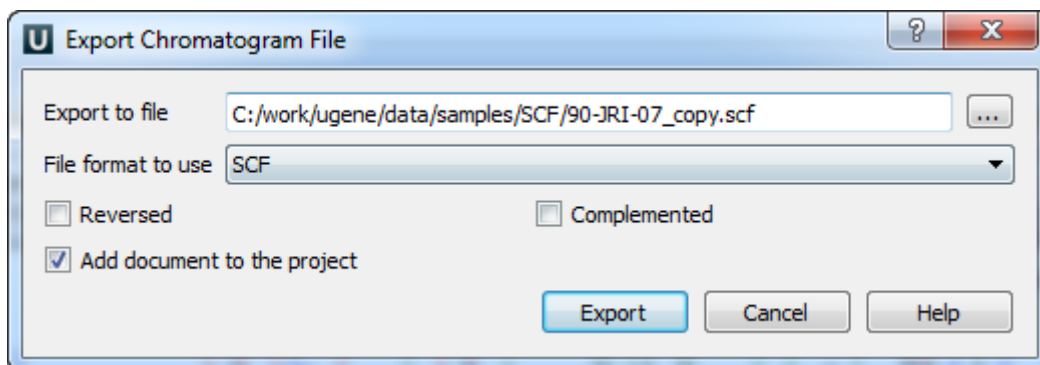
- Exporting Chromatogram Data
- Viewing Two Chromatograms Simultaneously

Exporting Chromatogram Data

Open, for example, the \$UGENE/data/samples/SCF/90-JRI-07.srf file. In the *Project View* context menu there is *Export chromatogram to SCF* item:



After clicking on the item, the *Export chromatogram file* dialog will appear:

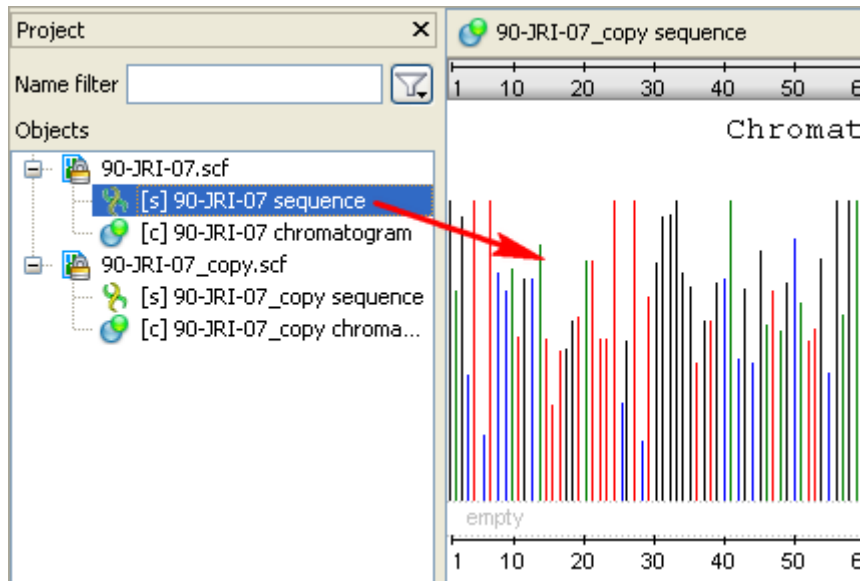


Check the *Reversed* and *Complemented* options if you want to create a reverse and complement chromatogram. Press the *Export* button.

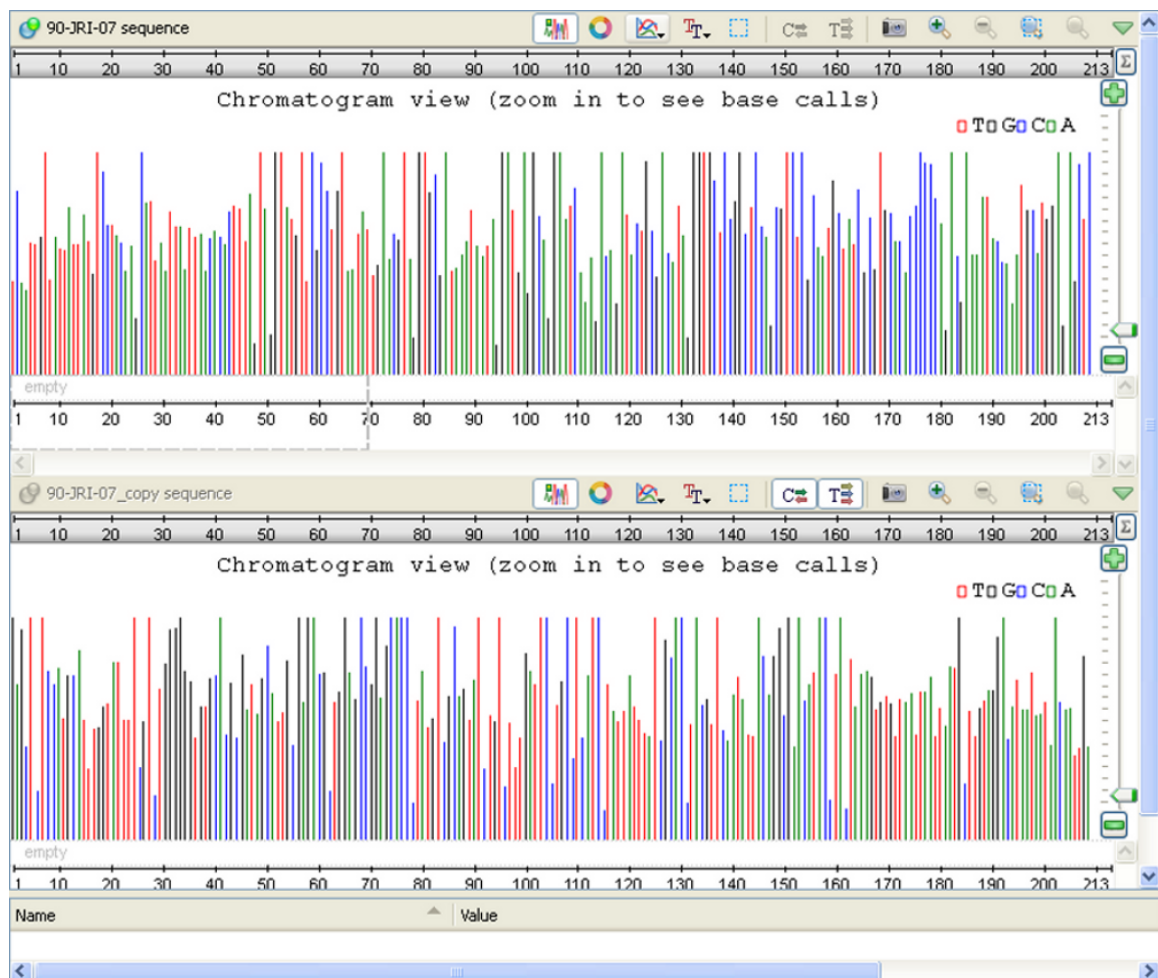
The exported file will be opened in the *Sequence View*.

Viewing Two Chromatograms Simultaneously

To add another sequence to the *Sequence View*, drag the required sequence object from the *Project View* and drop it in the *Sequence View* area. (Note that the dragged object is the sequence object, not the chromatogram object.)

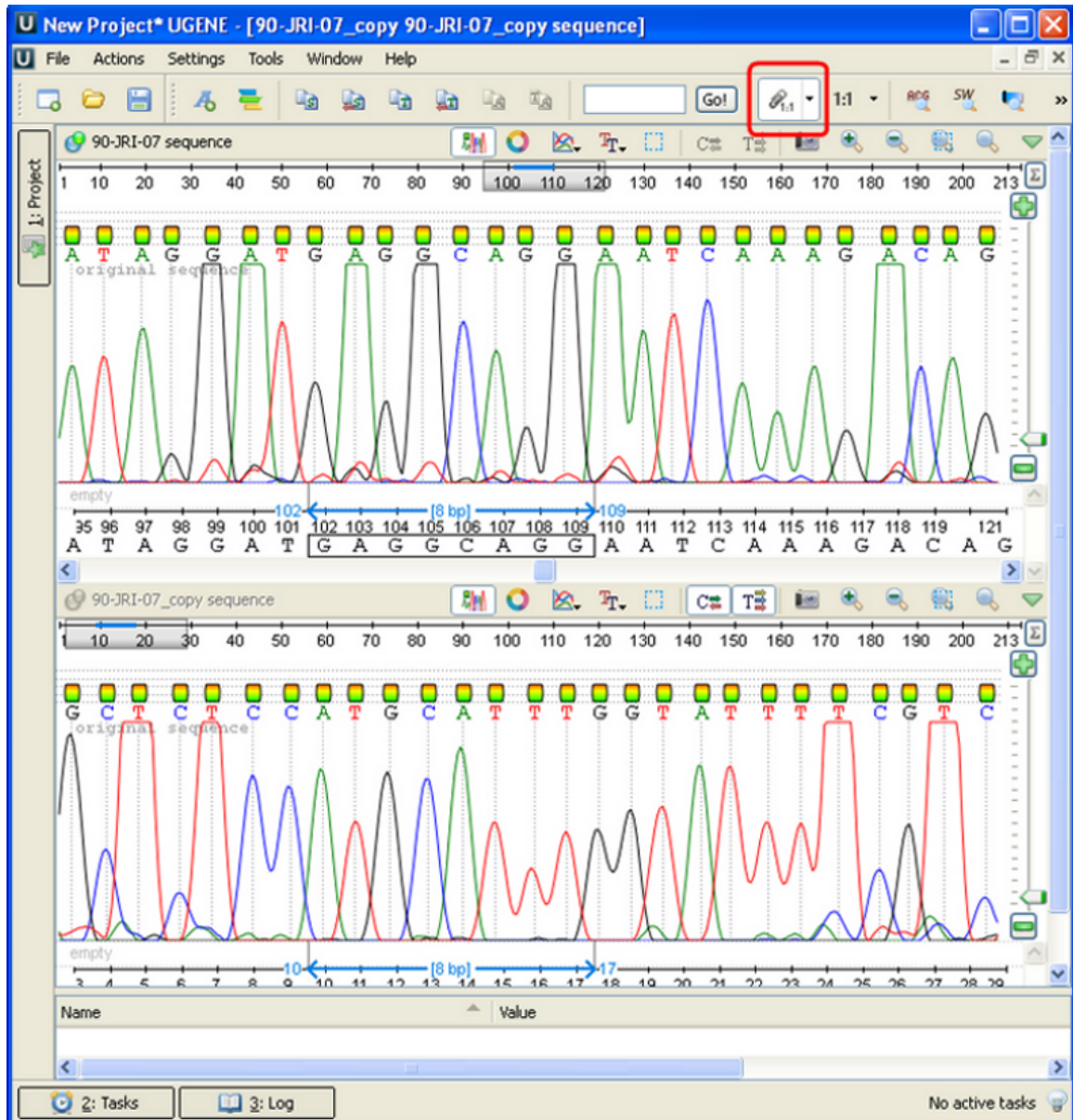


The result will look like this:



You can also use the *Lock scales* and *Adjust scales* global actions for the chromatograms.

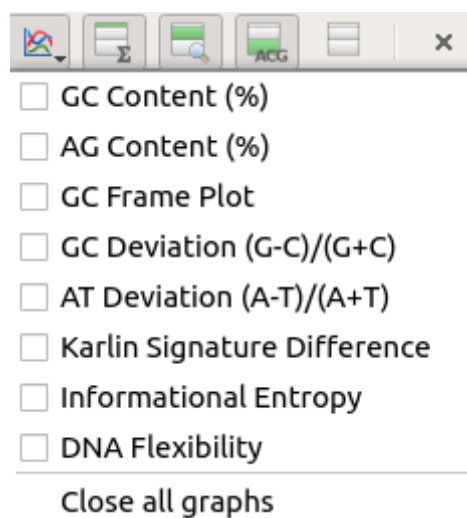
For example if you lock the scales you are able to scroll the sequences simultaneously. Also when you select a sequence region in one sequence, the same region is selected in the second sequence.



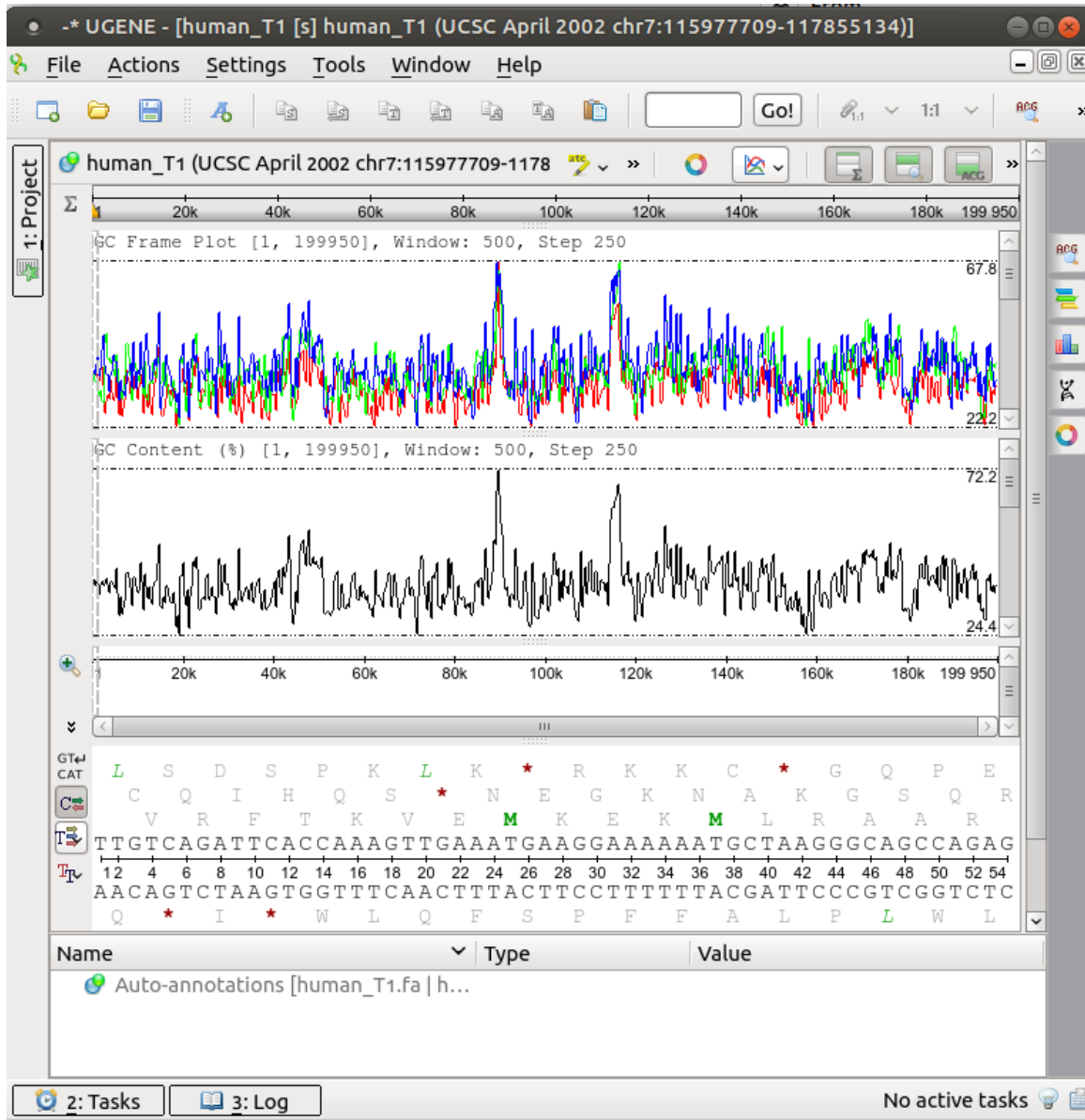
Graphs Package

The *DNA/RNA Graphs Package* draws contextual graphs for sequences. The *DNA/RNA Graphs Package* is available for the Standard DNA and Standard RNA alphabets.

Open a sequence in the *Sequence View* and click the *Graphs* icon on the toolbar. The popup menu appears:

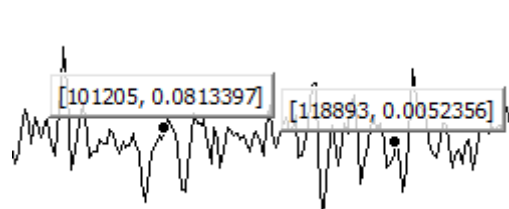


To see a graph select the corresponding graph item in the popup menu. A new area with the graph appears right above the *Sequence zoom view*.



Each point on a graph is calculated for a window of a specified size. The window is moved along the sequence by a step. See [Graph Settings](#) for instructions on how to modify these parameters.

It is possible to get information about each point of a graph. When a mouse is moved in the *Graphs* area, a small circle shows on the graph. A coordinates hint shows above it. When you hold Shift and click on a graph, the circle and the hint locks:



To remove it click on the hint. Also, you can delete all labels by *Graph->Delete all labels* context menu. To select all extremum points use the *Graph->Select all extremum points* context menu item.

All graphs are always aligned with the range shown in the *Sequence zoom view*. It means that if you change the visible range in the overview (either by zooming or scrolling) the graph will also be updated. The minimum and maximum values of the visible range are shown at the right lower and upper corners of the graph.

To close a graph, uncheck its item in the popup menu.

- [Description of Graphs](#)
- [Graph Settings](#)
- [Saving Graph Cutoffs as Annotations](#)

Description of Graphs

Find below the detailed description of each graph. Note that characters A, C, G and T in the formulas denote the number of corresponding nucleotide in a window.

- *DNA Flexibility* — searches for regions of high DNA helix flexibility in a DNA sequence. The average *Threshold* in a window is calculated by the following formula:

$$(\text{sum of flexibility angles in the window}) / (\text{the window size} - 1)$$

For more detailed information see [DNA Flexibility](#) paragraph.

- *GC Content (%)* — shows the percentage of nitrogenous bases (either guanine or cytosine) on a DNA molecule. It is calculated by the following formula:

$$(G+C) / (A+G+C+T) * 100$$

- *AG Content (%)* — shows the percentage of nitrogenous bases (either adenine or guanine) on a DNA molecule. It is calculated by the following formula:

$$(A+G) / (A+G+C+T) * 100$$

- *GC Frame Plot* — this graph is similar to the GC content graph but shows the GC content of the first, second and third position independently. It is most effective in organisms with GC rich genomic sequence but it also works on all microbial sequences.
- *GC Deviation (G-C)/(G+C)* — shows the difference between the “G” content of the forward strand and the reverse strand. *GC Deviation* is calculated by the following formula:

$$(G-C) / (G+C)$$

- *AT Deviation (A-T)/(A+T)* — shows the difference between the “A” content of the forward strand and the reverse strand. *AT Deviation* is calculated by the following formula:

$$(A-T) / (A+T)$$

- *Karlin Signature Difference* — dinucleotide absolute relative abundance difference between the whole sequence and a sliding window. Let:

$$\begin{aligned} f(XY) &= \text{frequency of the dinucleotide } XY \\ f(X) &= \text{frequency of the nucleotide } X \\ p(XY) &= f(XY) / f(X) * f(Y) \\ p_{\text{seq}}(XY) &= p(XY) \text{ for the whole sequence} \\ p_{\text{win}}(XY) &= p(XY) \text{ for a window} \end{aligned}$$

The *Karlin Signature Difference* for a window is calculated by the following formula:

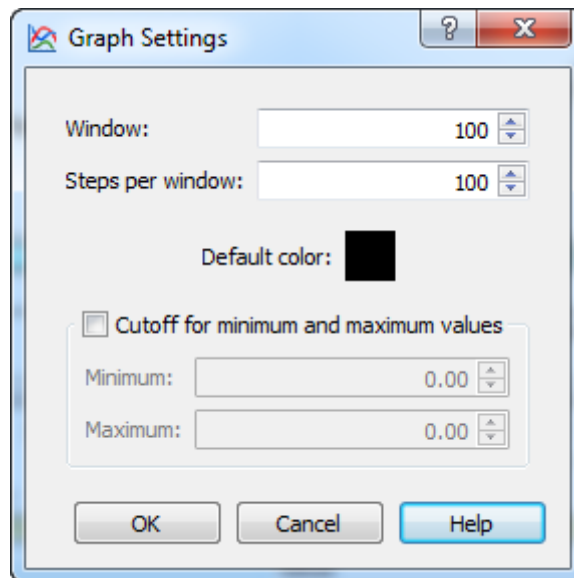
$$\text{sum}(p_{\text{seq}}(XY) - p_{\text{win}}(XY)) / 16$$

- *Informational Entropy* — is calculated from a table of overlapping DNA triplet frequencies. The use of overlapping triplets smooths the frame effect. *Informational Entropy* is calculated by the following formula:

$$-(\text{triplet frequency}) * \log_{10}(\text{triplet frequency}) / \log_{10}(2)$$

Graph Settings

To change settings of a graph, select the *Graph->Graph settings* item in the graph context menu. The *Graph Settings* dialog appears:



The following parameters are available:

Window — the number of bases in a window.

Steps per window — the number of steps in window. The *Step* is calculated as *Window / Steps per window*.

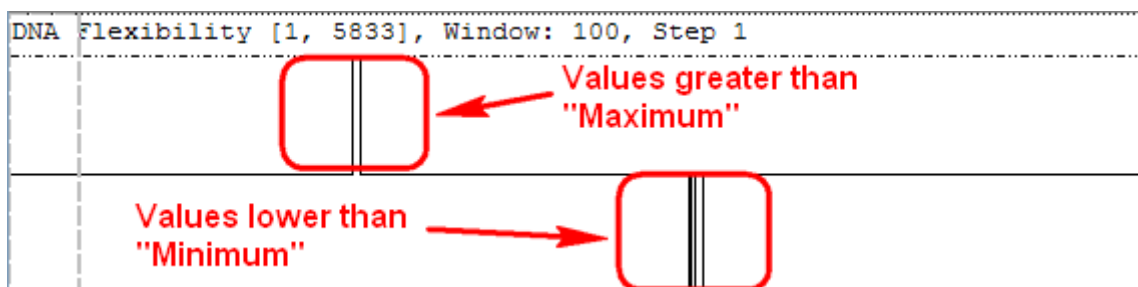
Default color — the default color of line of graph (or lines of graphs for *GC Frame Plot*).

Checking of the *Cutoff for minimum and maximum values* checkbox enables the following settings:

Minimum — the minimum value for cutoff.

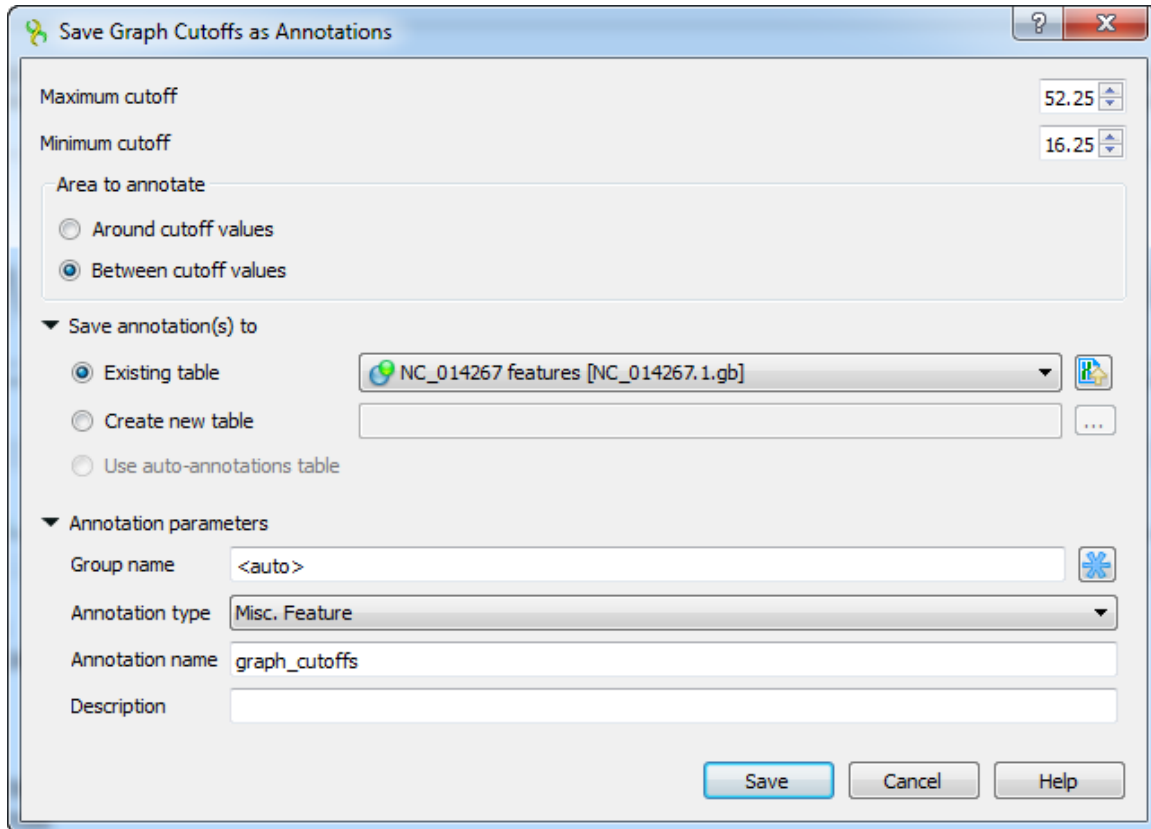
Maximum — the maximum value for cutoff.

Select an appropriate minimum and maximum value and click the *OK* button to show the graph of cutoffs. The graph is divided into 2 parts. The upper part shows values greater than the specified *Maximum* value. The lower part of the graph shows values lower than the specified *Minimum* value. For example:



Saving Graph Cutoffs as Annotations

To save graph cutoffs as annotations select the *Graph->Save cutoffs as annotations* item in the graph context menu. The following dialog will appear:



The following parameters are available:

Maximum cutoff - maximum cutoff value.

Minimum cutoff - minimum cutoff value.

Around cutoff values - saves the values around cutoffs values.

Between cutoff values - saves the values between cutoffs values.

In the *Save annotation(s) to* group you can set up a file to store annotations. It could be either an existing annotation table object, a new annotation table or auto-annotations table (if it is available).

In the *Annotation parameters* group you can specify the name of the group and the name of the annotation. If the group name is set to <auto> UGENE will use the group name as the name for the group. You can use the '/' characters in this field as a group name separator to create subgroups. If the annotation name is set to *by type* UGENE will use the annotation type from the *Annotation type*: table as the name for the annotation. Also you can add a description in the corresponding text field.

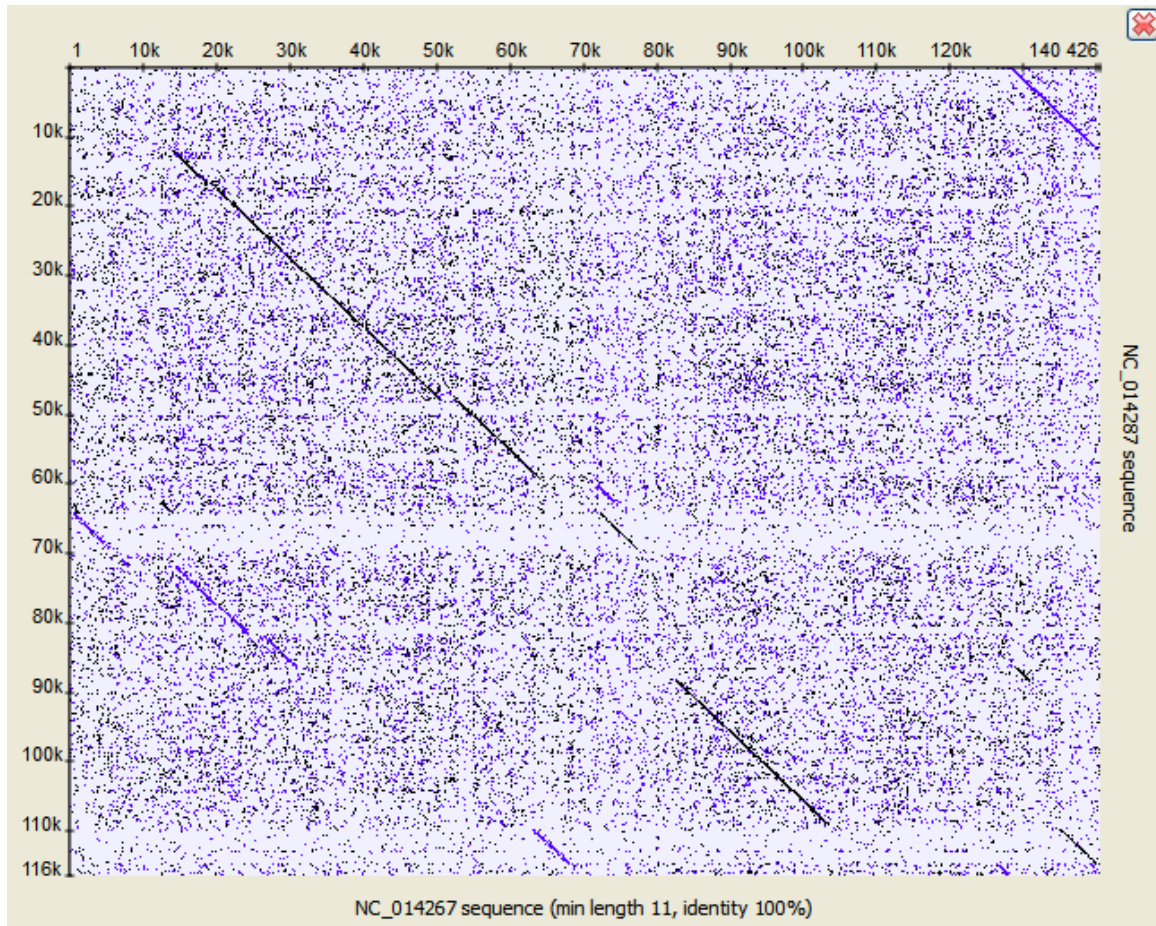
Select the parameters and click on the *Save* button. The corresponding annotations will be saved.

Dotplot

The *Dotplot* plugin provides a tool to build dotplots for DNA or RNA sequences. This allows comparing these sequences graphically. Using a dotplot, you can easily identify such differences between sequences as mutations, inversions, insertions, deletions and low-complexity regions.

Also the plugin provides advanced features: comparing multiple dotplots, navigation in a dotplot, dotplots synchronization, saving and loading a dotplot, etc.

An example of a dotplot view:



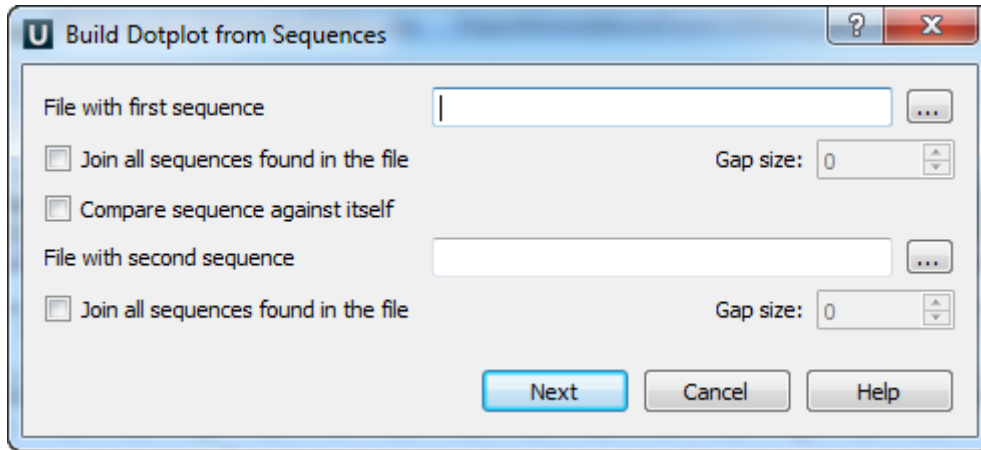
The *Dotplot* plugin uses the *Repeat Finder* plugin to build a dotplot, make sure you have the *Repeat Finder* plugin installed.

The *Dotplot* features are described in more details below.

- Creating Dotplot
- Navigating in Dotplot
- Zooming to Selected Region
- Selecting Repeat
- Interpreting Dotplot: Identifying Matches, Mutations, Inversions, etc.
- Editing Parameters
- Filtering Results
- Saving Dotplot as Image
- Saving and Loading Dotplot
- Building Dotplot for Currently Opened Sequence
- Comparing Several Dotplots

Creating Dotplot

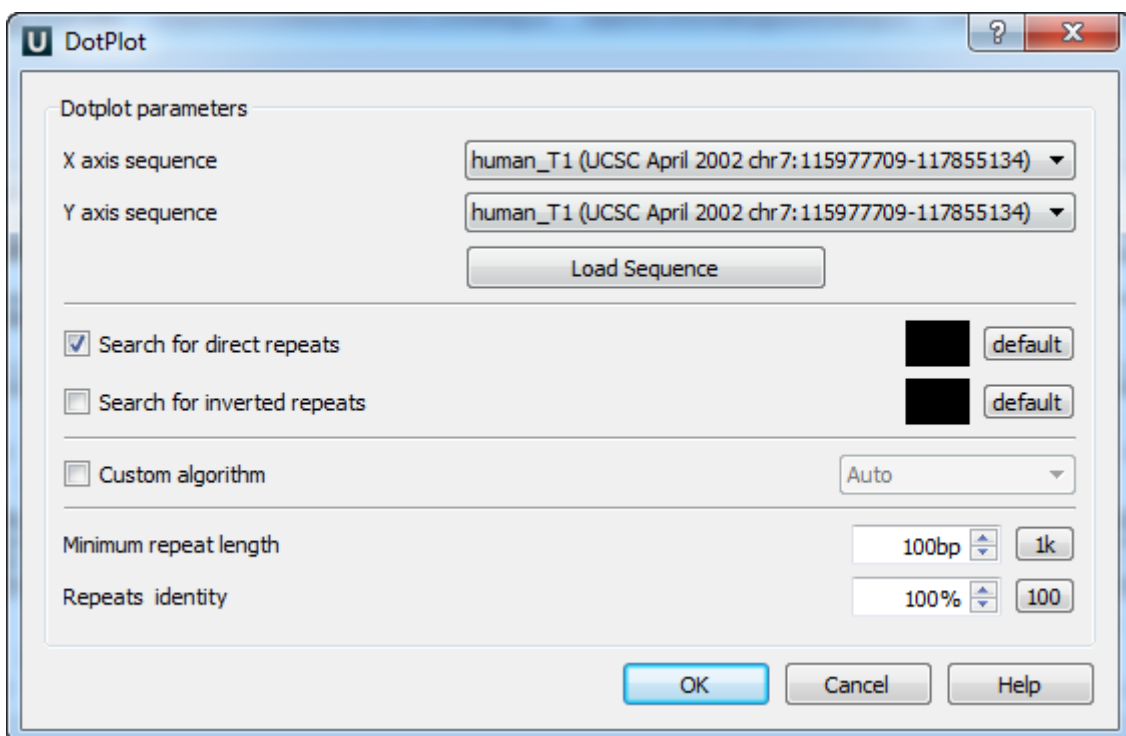
To create a dotplot select the *Tools Build dotplot* main menu item. The *Build dotplot from sequences* dialog will appear:



Here you should specify the *File with first sequence*. Also you should either check the *Compare sequence against itself* option or select the *File with second sequence*.

Optionally you can select to *Join all sequences found in the file* (for the first and/or for the second file). If you select to join the sequences you can also select the *Gap size*. The gap of the specified size will be inserted between the joined sequences.

After you press the *Next* button, the dialog to configure the dotplot parameters will appear:



The following parameters are available:

X axis sequence — the sequence for the X dotplot axis.

Y axis sequence — the sequence for the Y dotplot axis.

If there are several sequences in the specified (the first or the second) file and you haven't selected to join the sequences in the previous dialog, then you can select a sequence in these fields.


If you have selected to *Join all sequences found in the file*, then you can't select a separate sequence from the file, the joined *Sequence* can be selected instead.

Search direct repeats — check this option to search for direct repeats in the specified sequences. You can also select the color with which the repeats will be displayed in the picture. The *default* button sets the default color.

Search inverted repeats — check this option to search for inverted repeats in the specified sequences. You can also select the color with which the repeats will be displayed in the picture. The *default* button sets the default color.

Custom algorithm — optionally you can select an algorithm to calculate the repeats:

- Auto
- Suffix index
- Diagonals

 The specified algorithm is provided to the *Repeat Finder* plugin as an input parameter. In most cases the *Auto* value is appropriate.

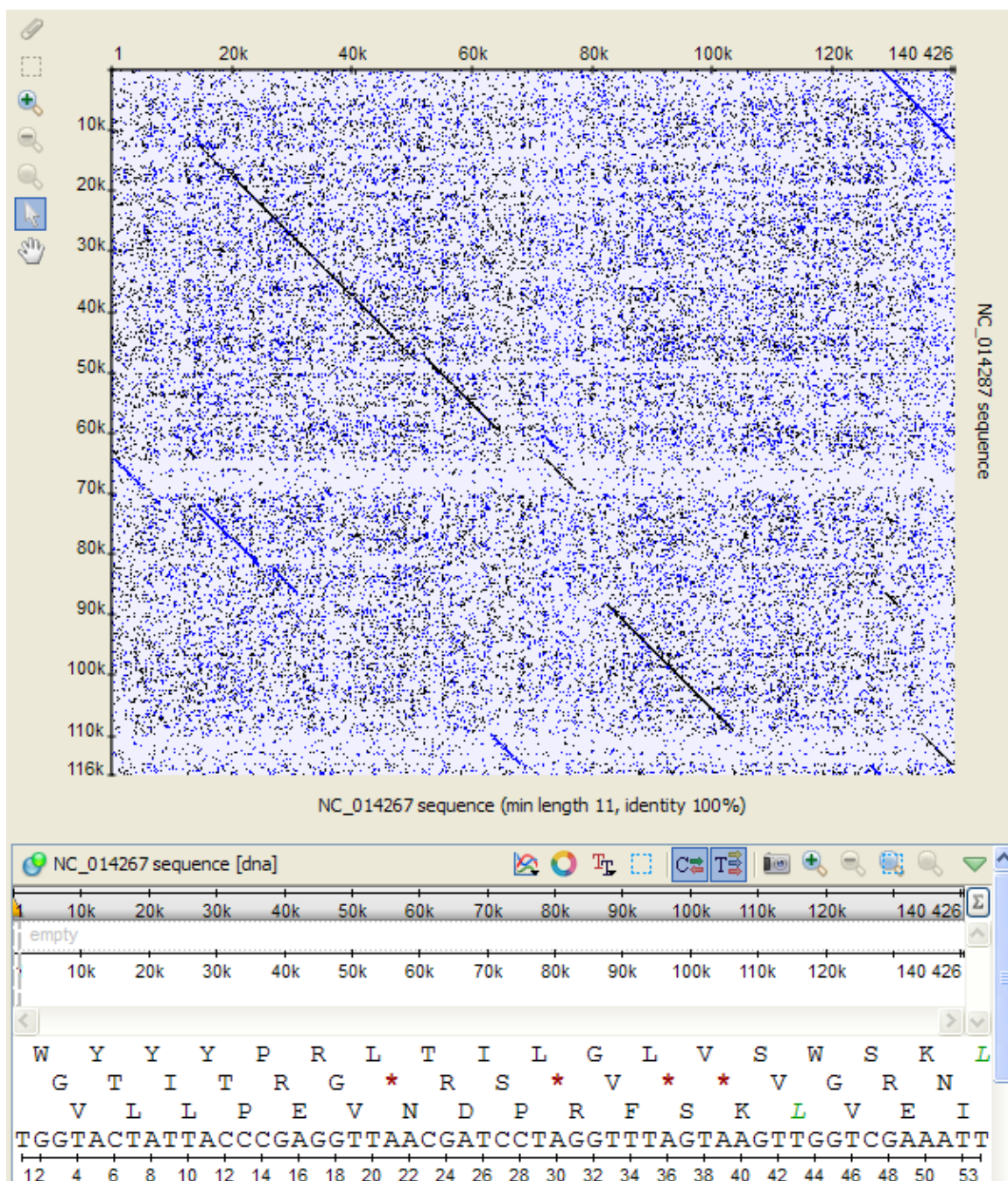
Minimum repeat length — allows to draw only such matches between the sequences that are continuous and long enough. For example if it equals to *3bp*, then only repeats will be found that contain 3 and more base symbols.

Press the *1k* button to automatically adjust the *Minimum repeat length* value. Such value will be set, that there will be about 1000 repeats found.

Repeats identity — specifies the percents of the repeats identity.

Press the *100* button to set the *100%* identity.

After the parameters are set, press the *OK* button. The dotplot will appear in the *Sequence View*.



It is a two-dimensional plot consisted of dots.

Each dot on the plot corresponds to a matched base symbol at the “x” position of the horizontal sequence and the “y” position of the vertical sequence.

Visible diagonal lines indicate matches between sequences in the given particular region.

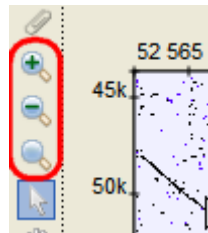
See also:

- [Interpreting Dotplot: Identifying Matches, Mutations, Inversions, etc.](#)
- [Building Dotplot for Currently Opened Sequence](#)

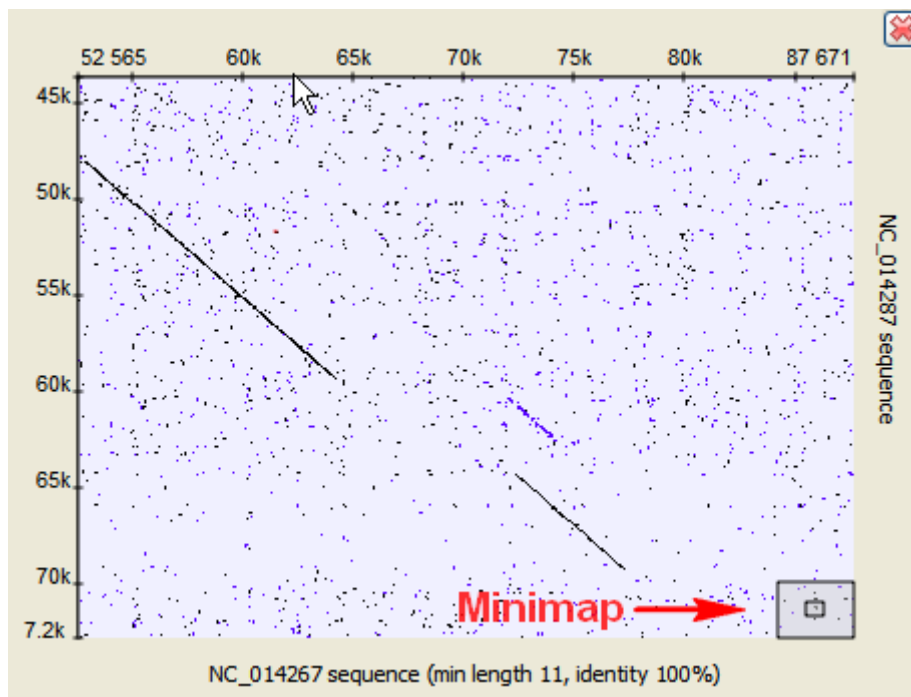
Navigating in Dotplot

To zoom in / zoom out a dotplot you can:

- Rotate the mouse wheel.
- Press corresponding zoom buttons located on the left:



To move the zoomed region you can:



- Hold the middle mouse button and move the mouse cursor over the zoomed region of the dotplot.
- Click on the desired region of the *minimap* in the right bottom corner.
- Activate the *Scroll tool*, hold the left mouse button and move the mouse cursor over the zoomed region:



Zooming to Selected Region

To select a dotplot region activate the *Select tool*:



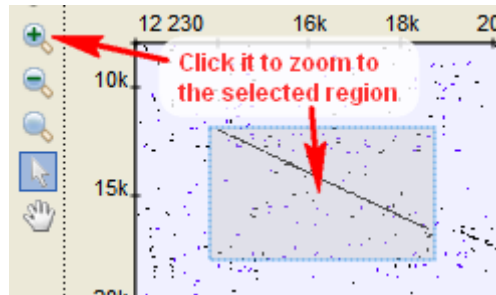
hold down the left mouse button and drag the mouse cursor over the dotplot.

When you select a region on a dotplot the corresponding region is also selected in other *Sequence View* areas (*Sequence details view*, *Sequ*

ence zoom view, etc.).

The opposite is true as well: if you select a region in a *Sequence View* area, the corresponding region is also selected in the dotplot view.

To zoom to the region selected click the *Zoom in* on the left.



Selecting Repeat

To select a repeat activate the *Select tool*:



and click on the repeat:



To deselect the repeat either click on other repeat or hold Ctrl and click somewhere on the dotplot.

Interpreting Dotplot: Identifying Matches, Mutations, Inversions, etc.

Using a dotplot graphic, you can identify such the following differences between the sequences:

1. Matches

A *match* between sequences looks like a diagonal line on the dotplot graphic, representing the continuous match (or repeat).

2. Frame shifts

a. Mutations

Mutations are distinctions between sequences. On the graphic they are represented by gaps in diagonal lines. They interrupt matches.

b. Insertions

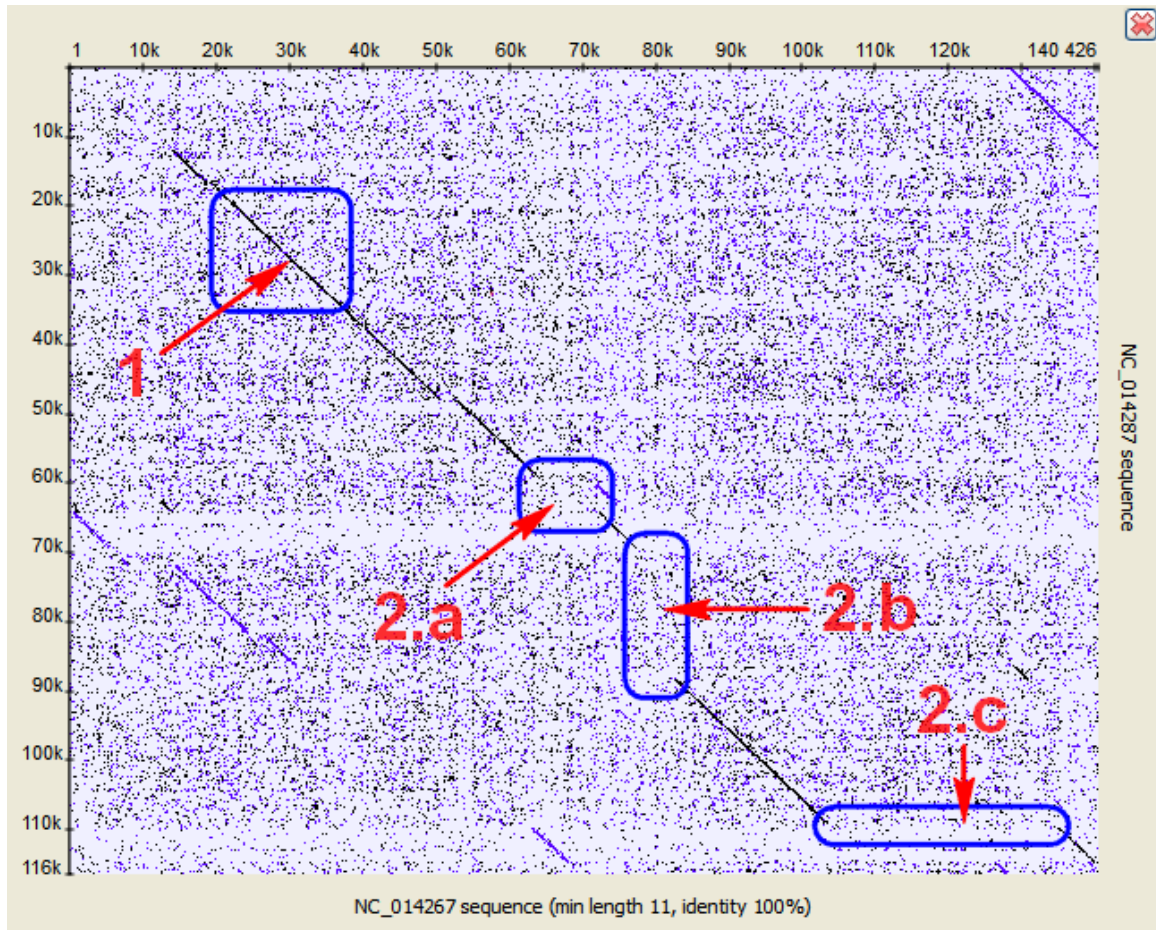
Insertions are parts of one sequence that are missed in the another, while the surrounding parts match. In other words, an insertion is a subsequence that was inserted into a sequence.

Graphically, insertions are represented by gaps which lie only on one axis. A little shift towards the other axis indicates a mutation involved.

c. Deletions

A deletion is a subsequence that was deleted from a sequence.

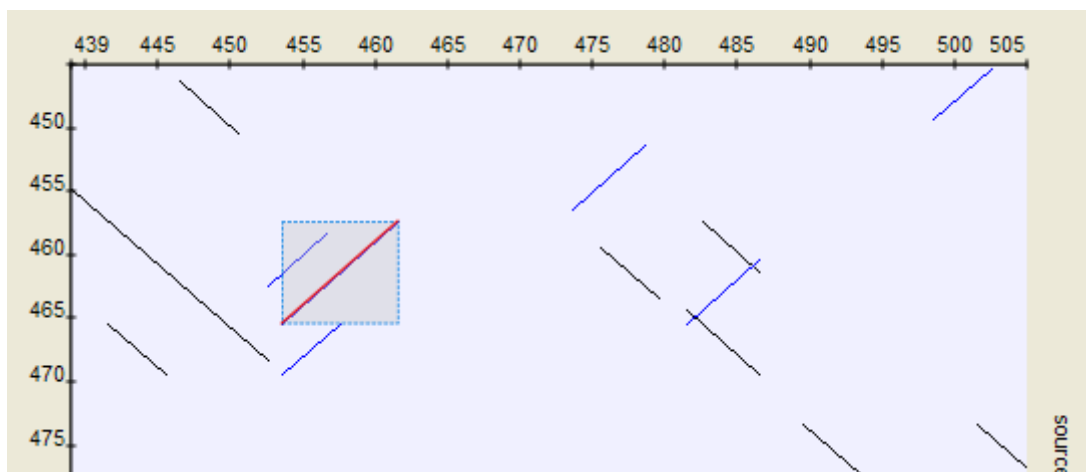
A deletion from sequence A found in sequence B can be considered as an insertion into sequence B and contained in sequence A.



3. Inverted repeats

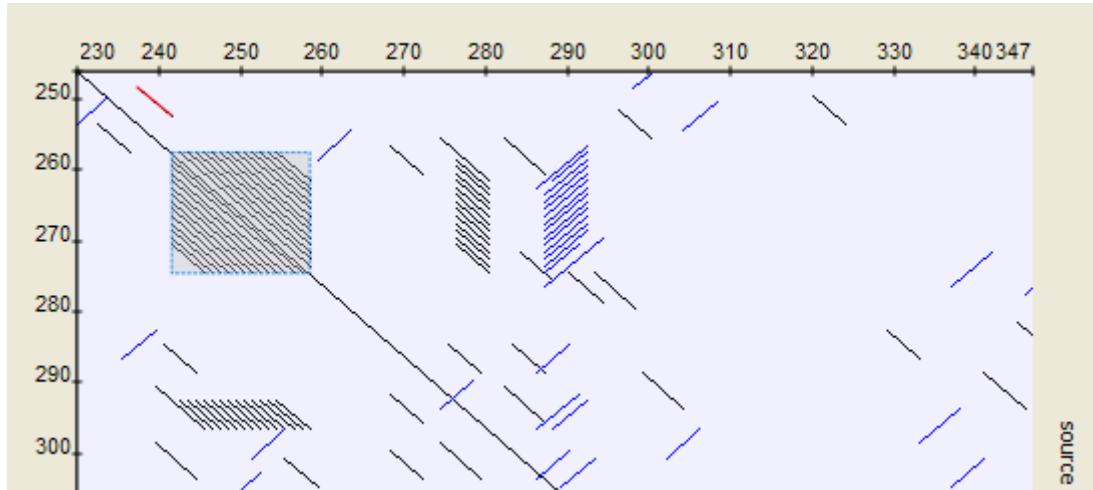
The *Dotplot* plugin allows to search for inverted repeats as well. Inverted repeats are shown contrary to the direct repeats.

Use the *Search direct repeats* and *Search inverted repeats* options of the *Dotplot* parameters dialog to select which repeats to draw (the dialog is described [here](#)).



4. Low-complexity regions

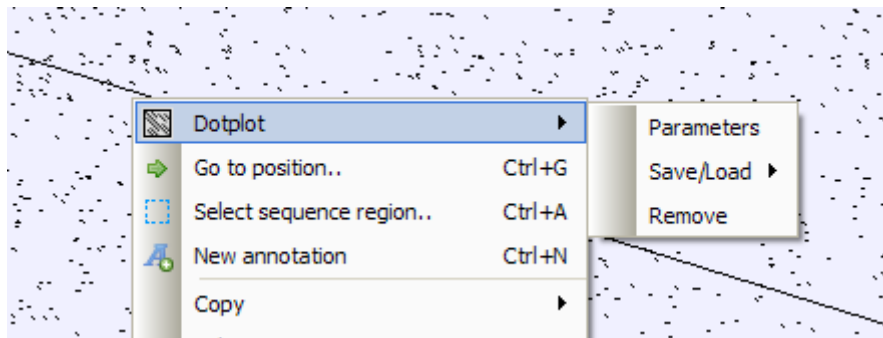
A low-complexity region is a region produced by redundancy in a particular part of the sequence. It is represented on a plot as a rectangular area filled with the matches.

**Hint**

Compare sequence with itself to easily find low-complexity regions in it.

Editing Parameters

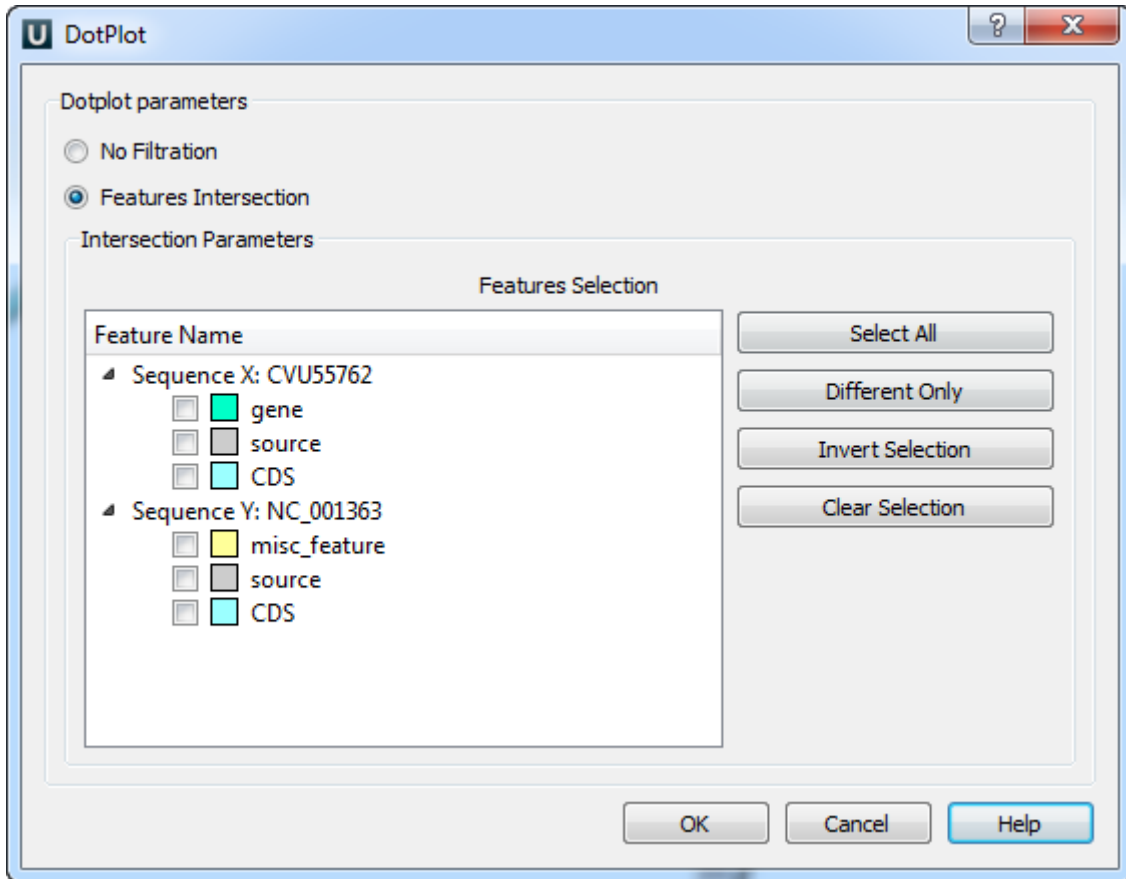
It is possible to edit parameters of a built dotplot. Right-click on the dotplot and select the *Dotplot Parameters* context menu item:



The parameters dialog will be re-opened. See description of the available parameters [here](#).

Filtering Results

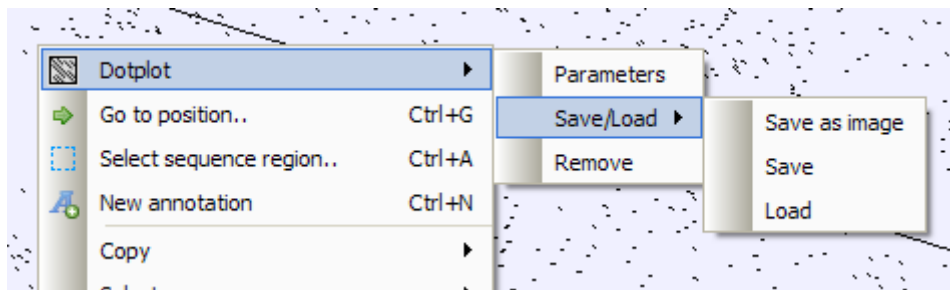
It is possible to find features intersections and filter dotplot results. Right-click on the dotplot and select the *Dotplot Filter results* context menu item. The following dialog will appear:



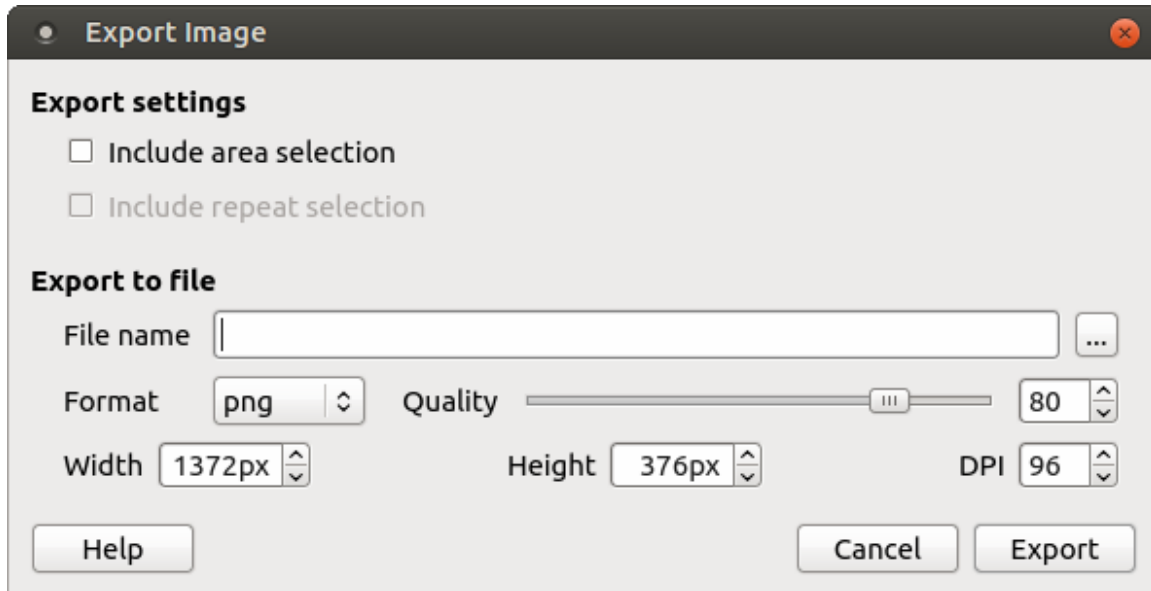
Select features and click OK button. The filtered dotplot will appear.

Saving Dotplot as Image

To save a dotplot as image right-click on the dotplot and select the *Dotplot Save/Load Save as image* context menu item:



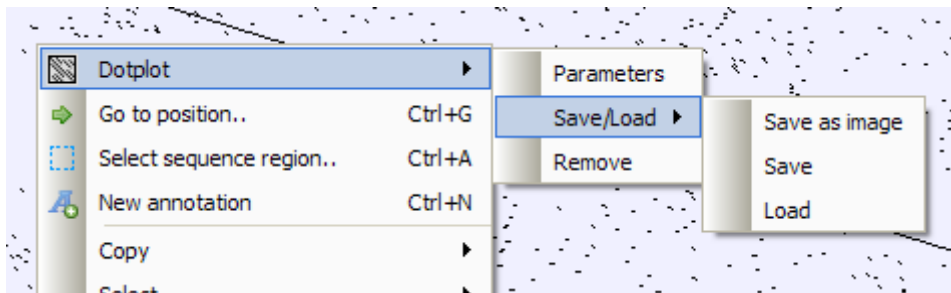
The following dialog will appear:



Available formats are *.png, *.jpg, *.bmp, *.jpeg, *.ppm, *.tif, *.tiff, *.xbm and *.xpm.

Saving and Loading Dotplot

To save a dotplot in a native format, right-click on the dotplot and select the *Dotplot Save/Load Save* context menu item:



The *Save Dotplot* dialog will appear. A dotplot is saved in a file with the *.dpt extension.

Later the dotplot can be loaded using the *Dotplot Save/Load Load* context menu item.

Building Dotplot for Currently Opened Sequence

To build a dotplot for currently opened sequences, create a multiple view containing these sequences. It can be arranged by dragging the corresponding sequence objects (the items started with the “[s]”) into the same *Sequence View*.

Then right-click on the created view and select the *Analyze Build dotplot* item in the context menu. Every sequence from the current multiple sequence view can be used to build a dotplot.

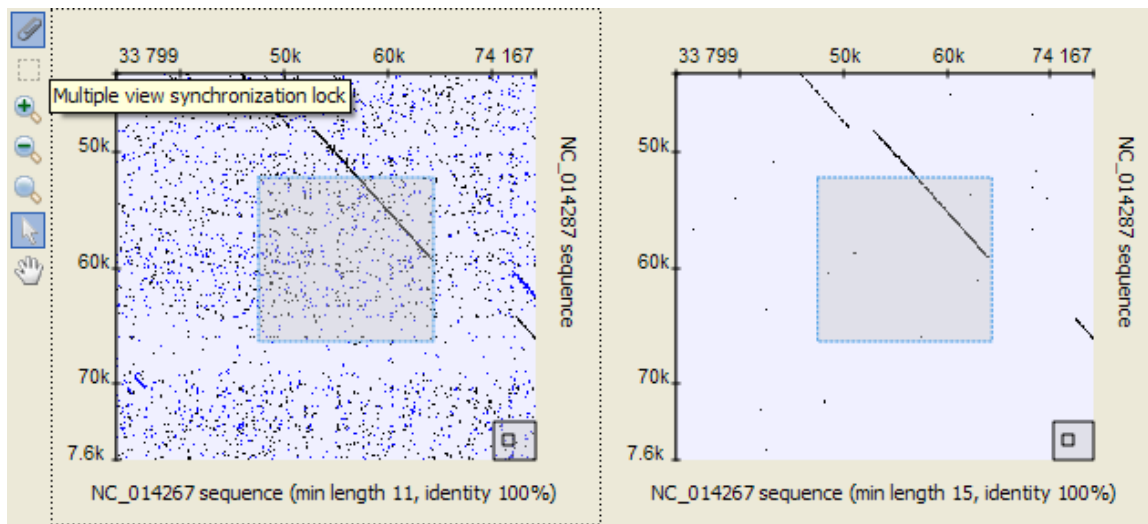


If you need to compare a sequence with itself, you can activate the menu from a single *Sequence View*.

Comparing Several Dotplots

Dotplots created for the same view are shown at the same view.

If the horizontal and vertical sequences of several dotplots are the same correspondingly, it is possible to lock all zooming and navigating operations for these dotplots. Press the *Multiple view synchronization lock* button on the left.



Alignment Editor

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 - Replacing with Reverse-Complement
 - Replacing with Reverse
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 - MrBayes
 - PhyML Maximum Likelihood

Overview

This chapter gives an overview of the *Alignment Editor* components and explains basic concepts of browsing an alignment.

- Alignment Editor Features
- Alignment Editor Components
- Navigation
- Coloring Schemes
 - Creating Custom Color Scheme
- Highlighting Alignment
- Zooming and Fonts
- Searching for Pattern
- Consensus
 - Export Consensus
- Alignment Overview

Alignment Editor Features

The *Alignment Editor* is a powerful tool for visualization and editing DNA, RNA or protein multiple sequence alignments. The editor supports different multiple sequence alignment (MSA) formats, such as ClustaW, MSF, and Stockholm. The full list of file formats supported in

UGENE is [here](#).

The editor provides the interactive visual representation which includes:

- Navigation through an alignment;
- Optional coloring schemes (for example Clustal, Jalview like, etc.);
- Flexible zooming for large alignments;
- Export publication-ready images for alignment;
- Several consensus calculation algorithms.

Using the *Alignment Editor* you can:

- Perform multiple sequence alignment using integrated MUSCLE and KAlign algorithms;
- Edit an alignment: delete/copy/paste symbols, sequences, and subalignments;
- Build phylogenetic trees;
- Generate grid profiles;
- Build Hidden Markov Model profiles to use with HMM2/HMM3 tools.

Alignment Editor Components

Here is the default layout of the editor:



The *Alignment Editor* components:

For example, let's assume that the coordinate of the first visible base of the row is N , but the row contains K gaps before the position N . The starting offset value will be $N-K$. The same rule is true for the ending offset.

You can turn off the *Sequence offsets* by unchecking the *Actions View Show offsets* main menu item or *View Show offsets* context menu item.

Navigation

The *Sequence area* provides several flexible ways to navigate through an alignment. The simplest way is to use the mouse and the scrollbars.

Alternatively, you can use arrow keys on the keyboard to navigate.

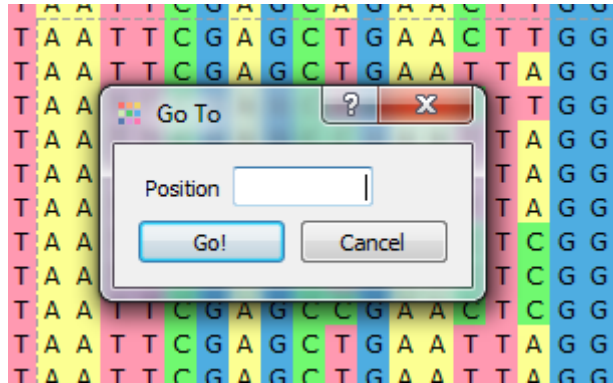
The list of hotkeys for quick navigation:

- PageUp — to move one screen left.
- PageDown — to move one screen right.
- Home — to center the starting columns of the alignment.
- End — to move to the trailing columns of the alignment

Hint

if you use Shift key with the hotkeys above you will navigate through the rows. For example, Shift-PageDown will move one screen down.

Finally, you can use the *Go to position* dialog from the *Actions* menu, the context menu or the editor toolbar.

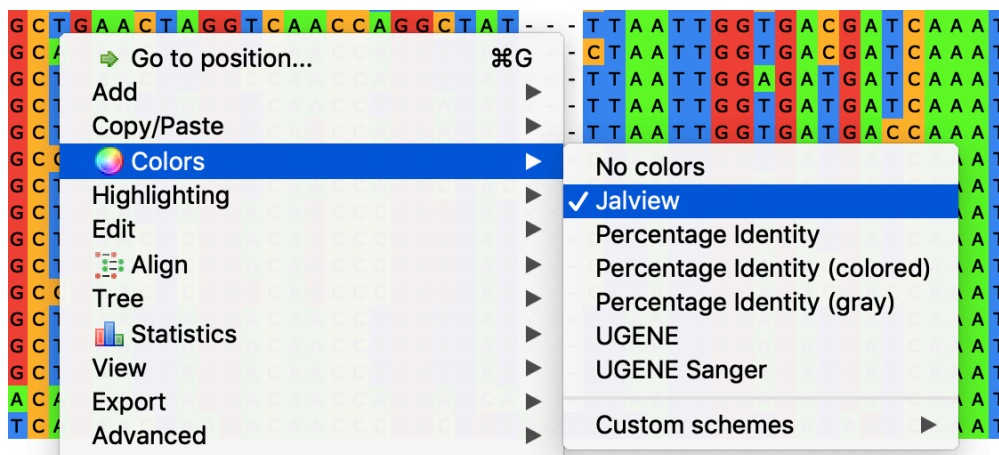


Enter the column number (base coordinate) and the view will be centered to the corresponding base.

Coloring Schemes

It is possible to apply different coloring schemes for an alignment, so that nucleotide or amino acid residues are colored depending on some rules.

To set another coloring scheme, select the required item in the main (*Actions Colors*) or context menu (*Colors*) of the *Alignment Editor*, or in the *Color* combo box on the *Highlighting* tab on the *Options Panel*.



Note that the coloring schemes setting may interfere with other alignment highlighting settings (see e.g. *Highlighting* combo box on the *Highlighting* tab of the *Options Panel*). Thus, the colors may vary in this case from the described below.

Coloring Schemes for Nucleotides

The following schemes options are available for a nucleotide alignment:

- *No colors* — do not color the residues background (i. e. all residues have white background and black font).
- *Jalview* — use the following background colors: A - green, C - orange, G - red, T/U - blue. Gaps and characters of the extended nucleotide alphabet are not colored.
- *Percentage identity* — a residue background color depends on its presence in the column of the alignment: <=40% - white, 41-60% - light slate blue, 61-80% - medium slate blue, 81-100% dark slate blue.
- *Percentage identity (colored)* — colors depend on the residues percentage presence in a column. The following rules are applied (in the order of priority):
 - If all residues (100%) are the same and there are no gaps, the residues in the column have yellow background and red font.
 - If there are 50% of one residue in the column and 50% of another (gaps are not taken into account), one half of the residues has green background and black font (the half to be colored is determined based on the following priorities:

T>U>G>C>A>B>D>H>K>M>R>S>V>W>Y>N).

- If there is one or several residues in a column with percentage number more or equal to the threshold parameter (see the *Threshold* parameter on the *Highlighting* tab of the *Options Panel*), then most frequent residues are colored as follows: light blue background color and blue font. Note that gaps are taken into account when calculating percentage number in the column in this case. Also, if required, the same residues priorities are applied as in the previous rule.
- If the rules above are not applied for a residue, then it remains uncolored, i.e. white background color and black font are used.
- *Percentage identity (gray)* — this coloring scheme is similar to *Percentage identity* scheme, but shades of gray color are used instead of the slate blue color.
- *UGENE* — this is the default coloring scheme, the following background colors are used: A - yellow, C - light green, G - light blue, T/U - light red, gap - white. Other colors may be used for characters of the extended nucleotide alphabet.
- *UGENE Sanger* — this coloring scheme is used in the *UGENE Sanger Reads Editor*, the background colors correspond to the peaks of Sanger chromatogram peaks: A - green, C - blue, G - light gray, T - red. The orange background color is used for a gap, magenta background color is used for characters of the extended nucleotide alphabet.
- A *custom scheme* can be created and used with colors that depend on a residue.

Coloring Schemes for Amino Acids

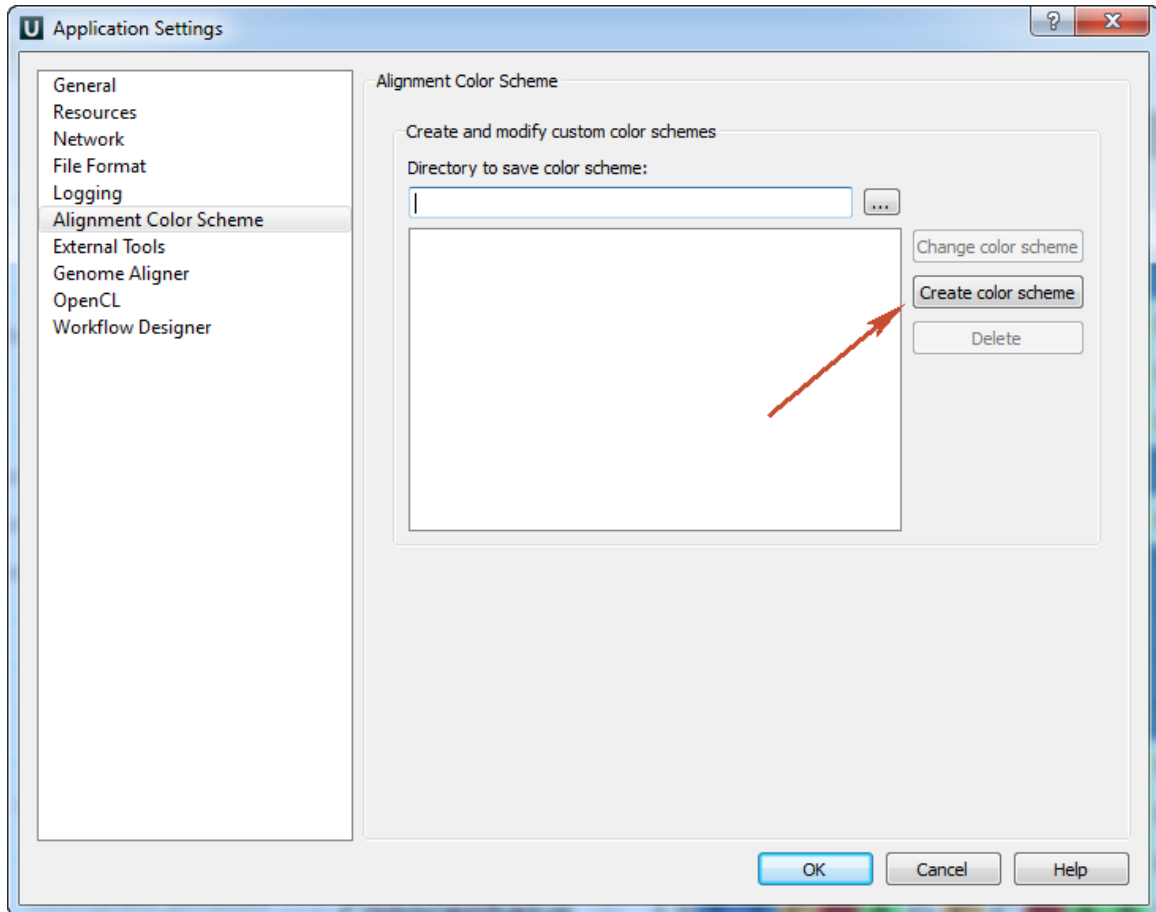
The following schemes options are available for an amino acids alignment:

- *No colors* — do not color the residues background (i. e. all residues have white background and black font).
- *Buried index* — depending on the frequency of occurrence inside a globule the amino acids residues are colored from light green (less frequent) to blue (most frequent).
- *Clustal X* — an emulation of the default color scheme in Clustal X, a graphical interface for the ClustalW multiple alignment program.
- *Helix propensity* — depending on helix propensity the residues are colored from magenta (the highest helix propensity) to green (the lowest one).
- *Hydrophobicity* — residues are colored based on their hydrophobicity. Most hydrophobic residues are red, most hydrophilic are blue.
- *Percentage identity* — a residue background color depends on its presence in the column of the alignment: <=40% - white, 41-60% - light slate blue, 61-80% - medium slate blue, 81-100% dark slate blue.
- *Percentage identity (gray)* — this coloring scheme is similar to *Percentage identity* scheme, but shades of gray color are used instead of the slate blue color.
- *Strand propensity* — residues with highest strand propensity are yellow, with lowest are blue.
- *Tailor* — see [this publication](#) by William R. Taylor.
- *Turn propensity* — residues with highest turn propensity are red, with lowest are cyan.
- *UGENE* — this is the default coloring scheme, the following background colors are used for the residues: positive (KRH) - yellow, aromatic (FWY) - green, large aliphatic hydrophobic (ILM) - blue, small hydrophobic (ST) - rose, GP - red, EX - gray.
- *Zappo* — amino acids residues are colored according to their physicochemical properties: aliphatic/hydrophobic (residues ILVAM) - rose, aromatic (FWY) - orange, positive (KRH) - dark blue, negative (DE) - red, hydrophilic (STNQ) - light green, conformationally special (PG) - magenta, cysteine (C) - yellow.
- A *custom scheme* can be created and used with colors that depend on a character.

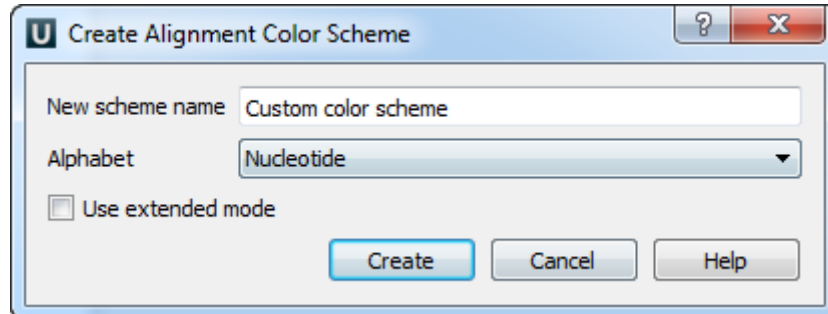
- [Creating Custom Color Scheme](#)

Creating Custom Color Scheme

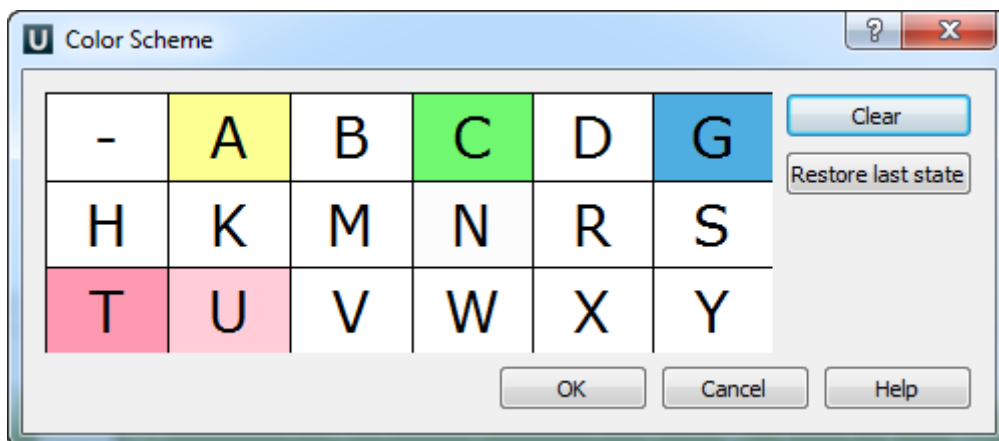
To create custom color scheme use the *Colors->Custom schemes->Create new color scheme* context menu item. The *Application Settings* dialog will appear. Click on the *Create color scheme* button:



The following dialog will appear:



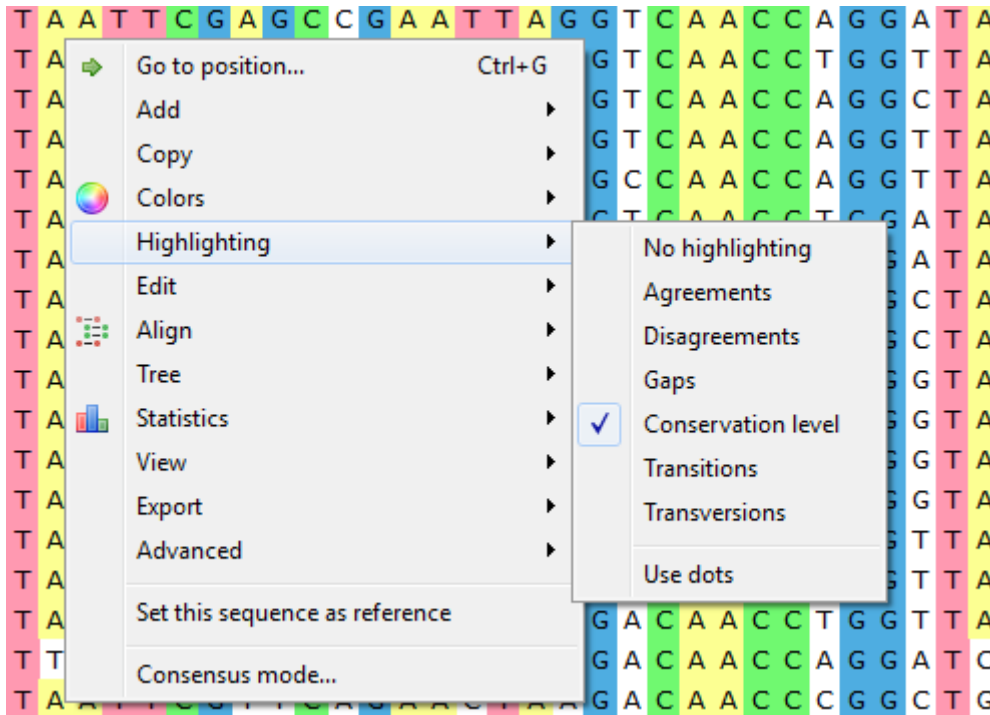
Select the new scheme name, alphabet and click on the *Create* button. The next dialog will appear for nucleotide extended mode:



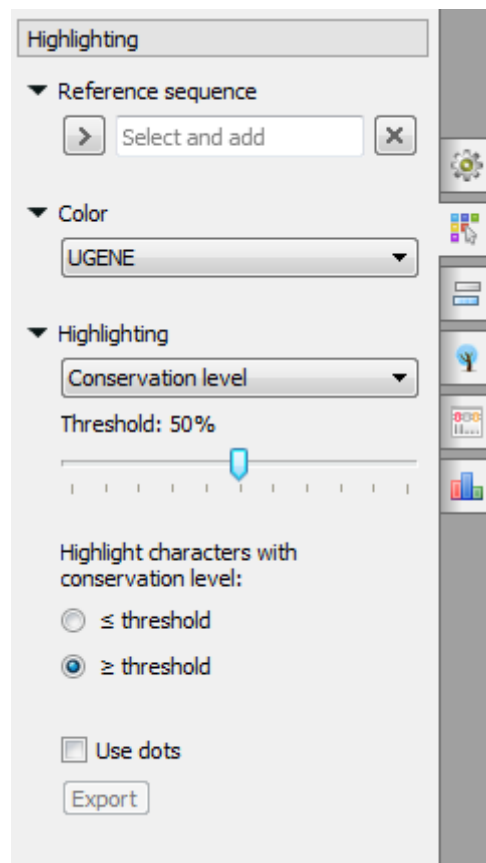
Here you can select a color for each element. Click on the element for it. The new scheme will be created after clicking the *OK* button. The new custom scheme will be available in the *Colors->Custom schemes* context menu.

Highlighting Alignment

To apply an alignment highlighting mode, select it in the *Highlighting* context menu:



or on the *Highlighting* tab of the *Options Panel*:



The following modes are available:

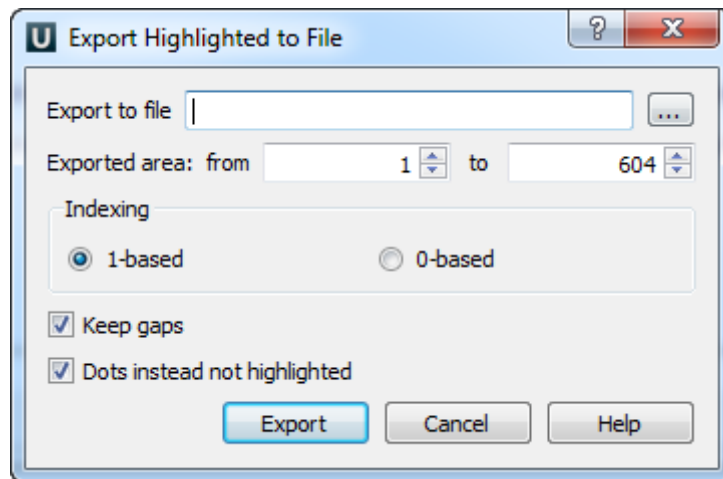
- *Agreements* — highlights symbols that coincide with the reference sequence.
- *Disagreements* — highlights nucleotides that differ from the reference sequence.
- *Gaps* - highlights gaps.
- *Conservation level* - highlights conservation level of symbols in a multiple alignment \geq or \leq threshold. To select the conservation parameters use the *Highlighting* Options Panel tab.

- *Transitions* - highlights transitions.
- *Transversions* - highlights transversions.

To use dots instead of symbols which are not highlighted check the *Use dots* checkbox in the *Options Panel* or use the *Highlighting->Use dots* context menu item.

To select a reference sequence use the *Set this sequence as reference* context menu or *Reference sequence* field in the *Highlighting* tab of the *Options Panel*.

Also you can export highlighting with a help of the *Export* button in the *Options Panel* or by the *Export->Export highlighted* context menu item. The following dialog will appear:



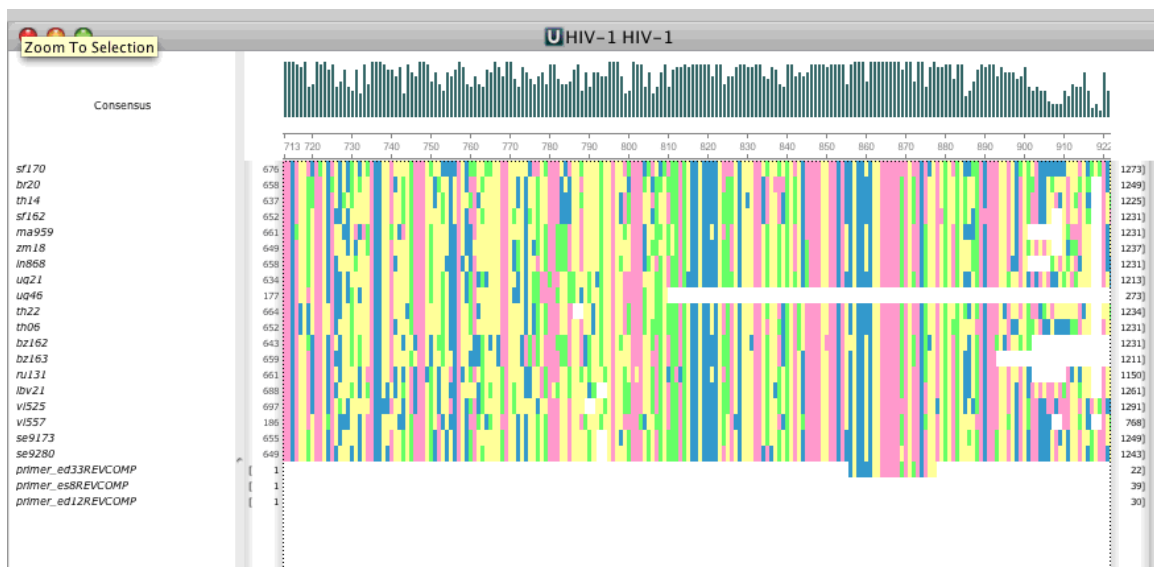
Select file to export, exported area and click on the *Export* button. The task report will appear in the *Notifications*.

Zooming and Fonts

To perform zoom operations use the corresponding buttons on the editor toolbar.



By default, the base characters are visible when zooming. But for rather long sequences there is another zoom mode available. In this mode the bases are not shown. This allows viewing very large sequence regions (up to 500 bp).



You can zoom to the selected region by clicking the *Zoom to selection* button. It is very convenient operation, when the alignment size is rather large. For example, you can zoom out to some percentage, select an interesting region and then zoom to the selection.

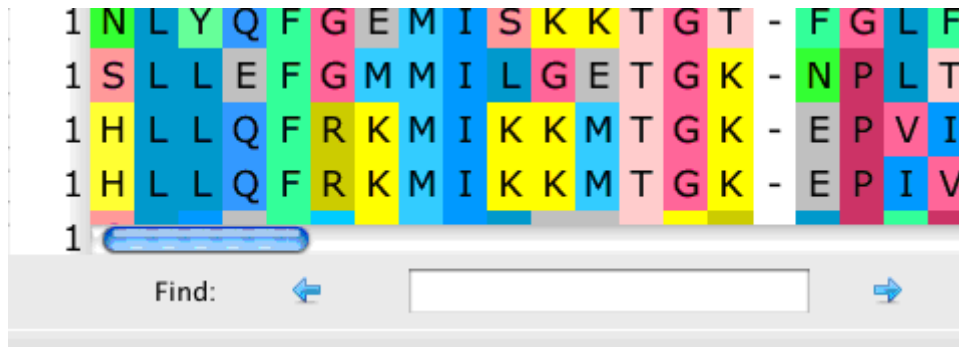
You can change font by clicking the *Change font* button.

To reset zoom and font click the *Reset zoom* button.

Searching for Pattern

You can search for a pattern inside an alignment.

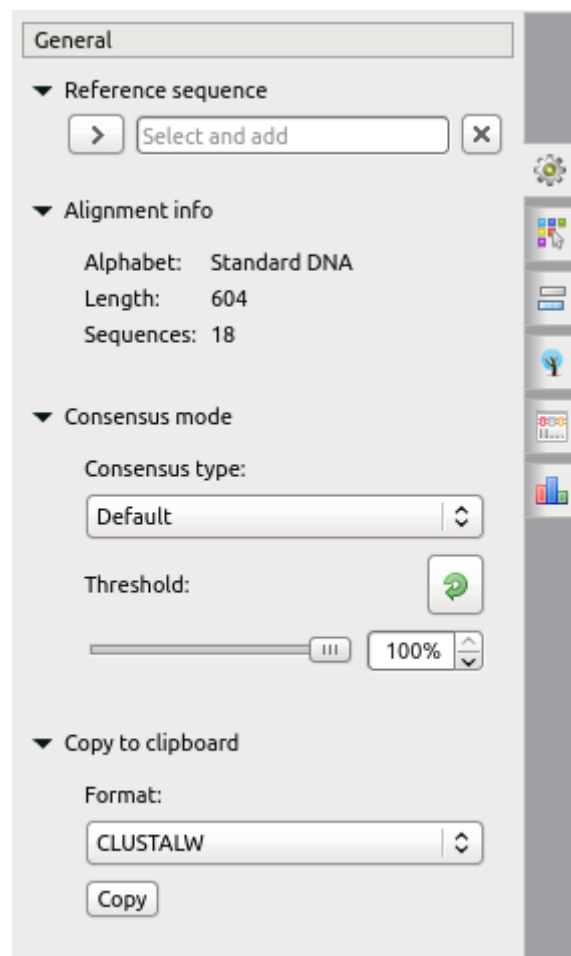
Enter a query string in the edit box under the *Sequence area*.



Press the right arrow to search in the direction "From left to right, from top to bottom". Press the left arrow to search in the direction "From right to left, from bottom to top". If the pattern is found, the result will be focused and highlighted in the *Sequence area*. You can continue the search in any direction from this position.

Consensus

Each base of a consensus sequence is calculated as a function of the corresponding column bases. There are different methods to calculate the consensus. Each method reveals unique biological properties of the aligned sequences. The *Alignment Editor* allows switching between different consensus modes. To switch the consensus mode go to the *General tab* of the *Options Panel* or activate the context menu (using the right mouse button) or the *Actions* menu and select the *Consensus mode* item and *General tab* will be opened automatically:



There are several consensus modes:

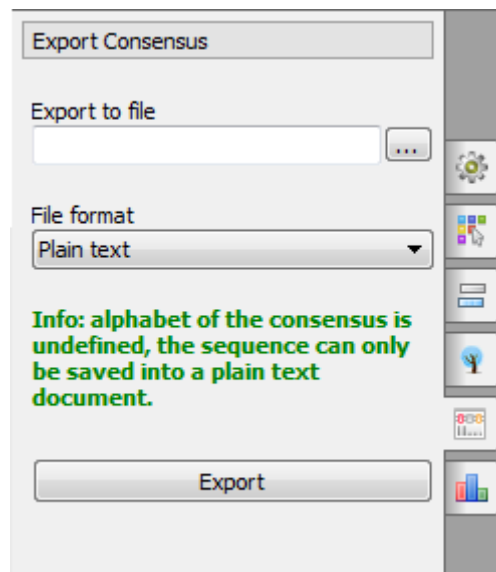
- **JalView (Default)** — the mode is based on the JalView algorithm. A character in the consensus is calculated based on the characters in the corresponding column and the value of the threshold:
 - If the percentage value of a character is greater than the threshold, the character is shown in upper case in the consensus.
 - If there are two characters with high frequency in a column, the '+' sign is shown.
 - If the percentage value of a character is high, but lower than the specified threshold, the character is shown in lower case in the consensus.
- **ClustalW** — emulates the ClustalW program behavior.
- **Levitsky** — proposed by Victor Levitsky, this mode calculates the consensus of a DNA alignment, taking into account frequencies of characters in the whole alignment, i.e. not only one column. The algorithm includes these steps:
 - Collect global alignment frequencies for every character using the [extended DNA alphabet](#).
 - For every column select the rarest character in the whole alignment with the percentage in the column greater or equal to the threshold value.
- **Simple extended** — a character of the consensus is calculated based on the characters in the corresponding column. The extended DNA alphabet is used for the result consensus, the threshold value is also taken into account.
- **Strict** — if a character percentage in a column is higher than the specified threshold, the character is shown in the consensus at this position. Otherwise, a gap character ('—') is shown.

Also the *General tab* shows the general information about an alignment and allows one to set up a reference sequence. The following chapter describes how to export a consensus sequence:

- [Export Consensus](#)

Export Consensus

To export a consensus sequence use the *Export consensus* tab of the *Options Panel*:



The following parameters are available:

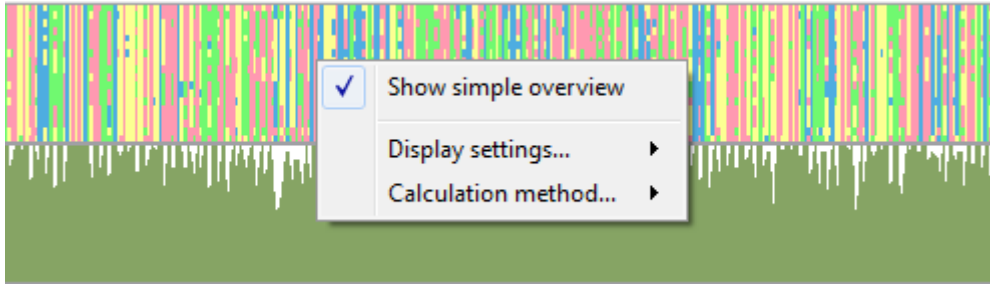
Export to file - the output file path.

File format - format of the output file. The possible values depend on the mode, selected for the consensus calculation. For *Default* and *ClustalW* modes the *Plain text* value is only available. For *Levitsky*, *Simple extended* and *Strict* consensus modes sequence formats FASTA and GenBank are also available.

Click on the *Export* button to export the consensus sequence to the specified output file.

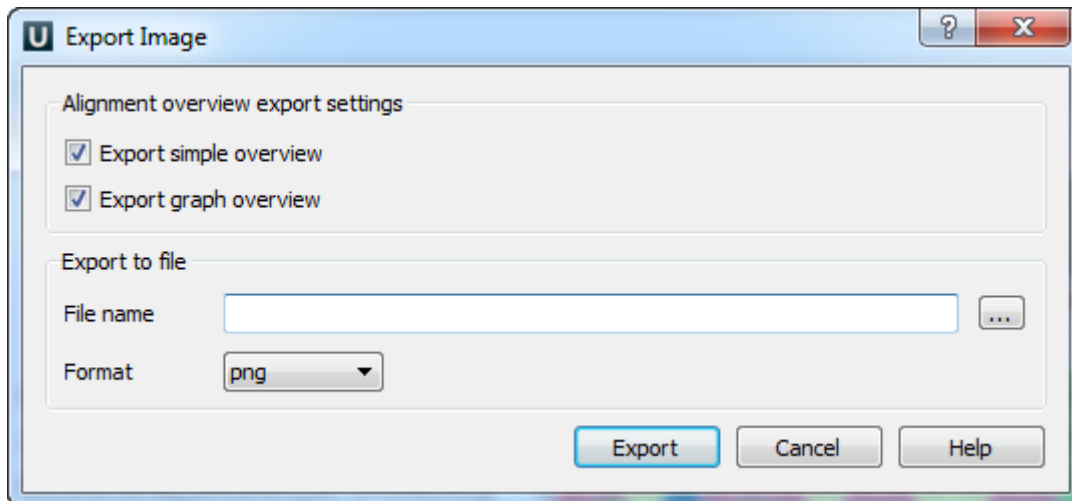
Alignment Overview

The alignment overview is shown automatically in the Alignment Editor. To close the overview click on the *Overview* toolbar button. To show the simple alignment overview use the *Show simple overview* context menu item of the overview.



The following settings of the alignment overview are available:

Export as image - you can export multiple alignment overview and simple alignment overview as image. Use this context menu item to do it. In the following dialog select the required parameters and click on the *Export* button:



Display settings:

Graph type - sets the graph type: histogram, line graph or area graph.

Orientation - sets the orientation: top to bottom or bottom to top.

Set color - sets the graph color.

Calculation method - sets the calculation method: strict, gaps, clustal or highlighting.

To use these settings go to the corresponding context menu items of the alignment overview.

Working with Alignment

This chapter explains how to work efficiently with the *Alignment Editor*. You will learn how to modify an alignment, remove gaps, align sequences, copy and paste regions, add new sequences and extract subalignments as new alignments.

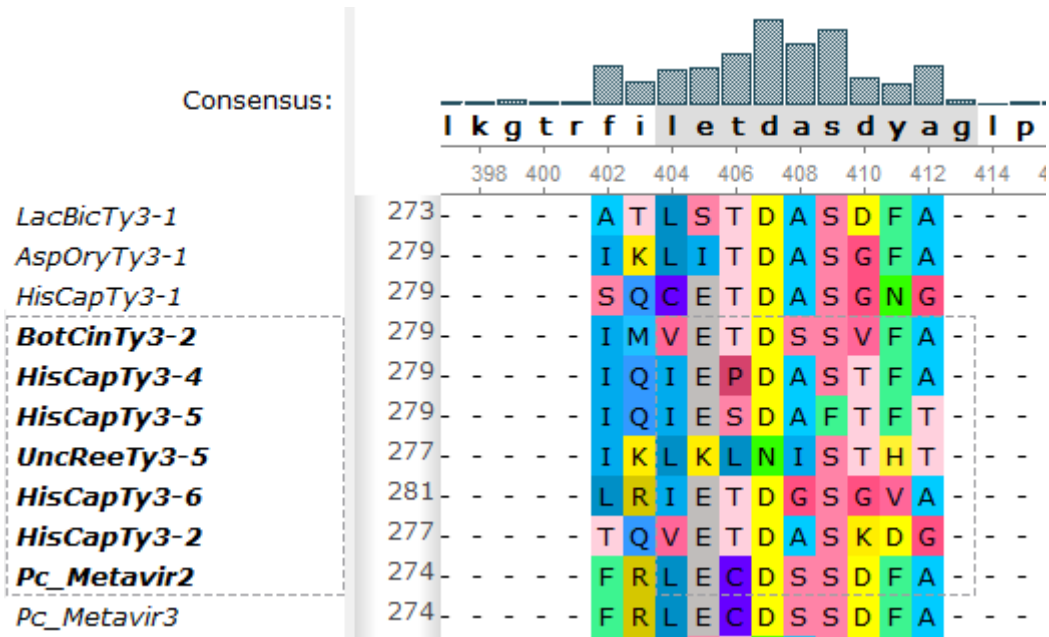
- Selecting Alignment Region
- Moving Subalignment
- Copying and Pasting Subalignment
- Editing Alignment
 - Removing Selection
 - Filling Selection with Gaps
 - Replacing Selected Character
 - Replacing with Reverse-Complement
 - Replacing with Reverse
 - Replacing with Complement
 - Removing Columns of Gaps
 - Removing Sequence
 - Removing All Gaps
 - Undo and Redo Framework
- Saving Alignment
- Aligning Sequences
- Aligning Sequence to this Alignment
- Pairwise Alignment
- Working with Sequences List
 - Adding New Sequences
 - Renaming Sequences
 - Sorting Sequences

- Shifting Sequences
- Collapsing Rows
- Copying Sequences
- Exporting in Alignment
 - Extracting Selected as MSA
 - Exporting Sequence from Alignment
 - Exporting Alignment as Image
- Importing APR and ACE Files

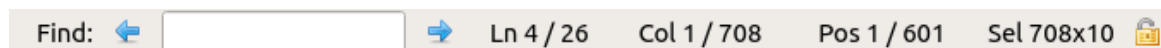
Selecting Alignment Region

Selection in the Alignment Editor components

In the *Name List* and the *Sequence Area* selection is visualized as a rectangle with a dashed border. In the *Consensus Area* the consensus sequence character of the corresponding columns are shown with light gray background:



Information about selection is shown in the statistic bar under alignment.



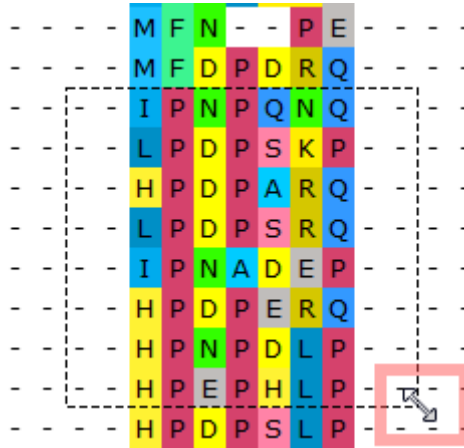
Selecting a region using the mouse and keyboard shortcuts

Using the mouse one can select:

- Several consequent sequences in the *Name List*.
- Several consequent columns in the *Consensus Area*.
- An alignment region in the *Sequence Area*.

By holding the *Shift* key it is possible to select a region between two coordinates of a mouse click. For example, if there is a big alignment, one can click on a character (the left upper coordinate of the region), press *Shift*, scroll to the required location of the alignment, and click on another character (the right bottom coordinate of the region).

It is possible to modify a selection in the *Sequence Area* by dragging the selection rectangle border:



Press the *Esc* key to clear the selection.

Moving Subalignment

To move subalignment there are different ways:

1. Select a subalignment and drag and drop it. The subalignment will be moved.
2. With a help of the *Space*, the subalignment will be moved to the right by the size of the selection. With a help of the *Backspace*, the subalignment will be returned to the first state.
3. With a help of the *Ctrl+Space*, the subalignment will be moved to the right by one column. With a help of the *Ctrl + Backspace*, the subalignment will be returned to the first state.
4. With a help of the *Ctrl*, you can join the parts of alignment. When there is a fragment of characters, surrounded by gaps in a row, select continuous block without gaps on *Ctrl* pressed and move the selection to the right/left.

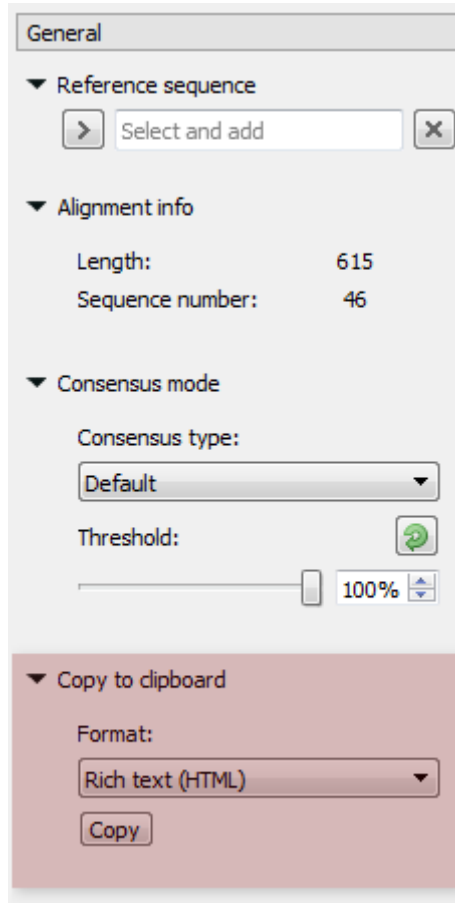
Copying and Pasting Subalignment

To copy current selection click the *Copy/Paste Copy selection* item in the *Actions* main menu or the context menu. The hotkey for this action is *Ctrl-C*.

Also you can copy selection in different formats, for example FASTA, CLUSTALW, RICH TEXT(HTML), NEXUS, MSF.

To copy subalignment in selected format do the following:

- Select the subalignment;
- Select the *Copy/Paste->Copy formatted* context menu item in the context menu or use hotkey *Ctrl+Shift+C*.
- The global Options Panel tab will open:

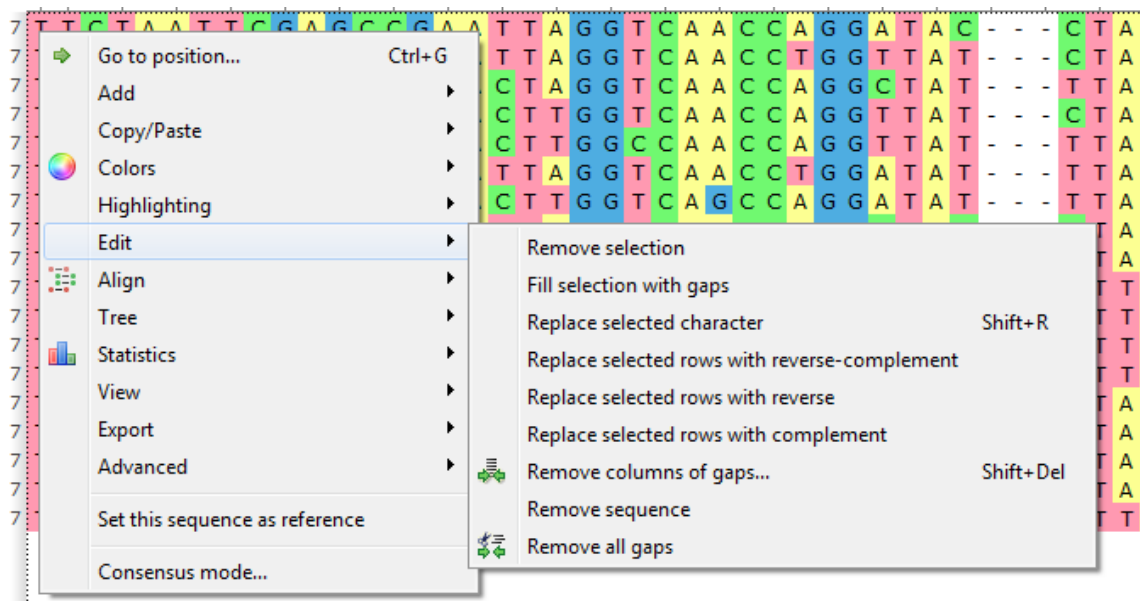


- Select format and press the *Copy* button.

To paste any of copied selection use the *Copy/Paste->Paste* context menu item or *Ctrl+V* shortcut.

Editing Alignment

Select the *Edit* submenu in the *Alignment Editor* context menu:



The actions available from this menu are described below.

- Removing Selection
- Filling Selection with Gaps
- Replacing Selected Character
- Replacing with Reverse-Complement
- Replacing with Reverse

- Replacing with Complement
- Removing Columns of Gaps
- Removing Sequence
- Removing All Gaps
- Undo and Redo Framework

Removing Selection

To remove a subalignment select it and choose the *Edit Remove selection* item in the context menu or press the Delete key. For macOS use the Fn+Delete key instead of the Delete key.

Filling Selection with Gaps

Select a region in the alignment and choose the *Edit Fill selection with gaps* item in the context menu or press the Spacebar. The region is filled with gaps shifting the subalignment from the region to the right.

Replacing Selected Character

To replace a symbol in the alignment select the nucleotide and press *Shift+R* for switching the edit mode on.

While the selection is blinking, press a keyboard character that you want to replace the nucleotide with.

After that the edit mode is switched off. For replacing another nucleotide press *Shift+R* again.

You always can cancel the changes pressing *Ctrl+Z*.

Replacing with Reverse-Complement

To replace sequence(s) in the alignment with reverse-complement select it and use the *Edit->Replace with reverse-complement* item in the context menu.

Replacing with Reverse

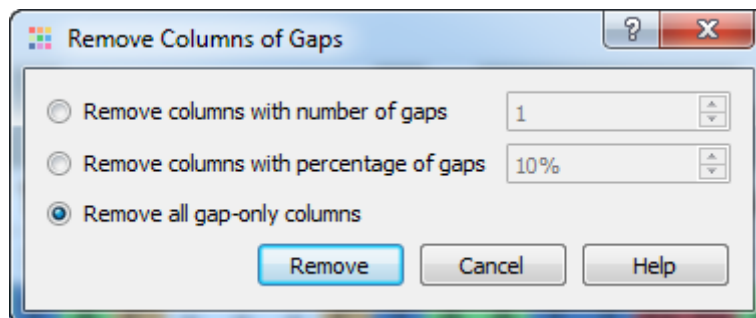
To replace sequence(s) in the alignment with reverse select it and use the *Edit->Replace with reverse* item in the context menu.

Replacing with Complement

To replace sequence(s) in the alignment with complement select it and use the *Edit->Replace with complement* item in the context menu.

Removing Columns of Gaps

To remove columns containing certain number of gaps select the *Edit Remove columns of gaps* item in the context menu. The dialog appears:



There are the following options:

Remove columns with number of gaps — removes columns with number of gaps greater than or equal to the specified value.

Remove columns with percentage of gaps — removes columns with percentage of gaps greater than or equal to the specified value.

Remove all columns of gaps — this option is selected by default. It specifies to remove columns from the alignment if they entirely consist of gaps.

Select the option required and press the *Remove* button.

Removing Sequence

Select a sequence and call the *Edit Remove sequence(s)* item in the *Actions* main menu or in the context menu to remove sequence from the alignment.

Removing All Gaps

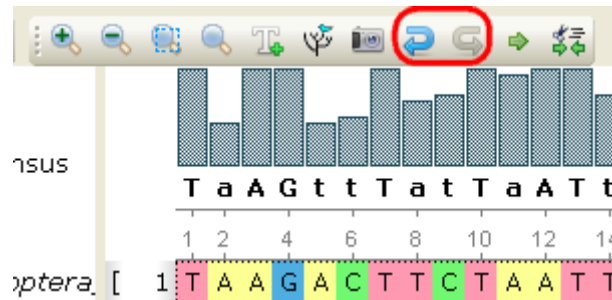
Use the *Edit Remove all gaps* item in the *Actions* main menu or in the context menu to remove all gaps from the alignment.

Undo and Redo Framework

The editor tracks all modifications of the aligned sequences.

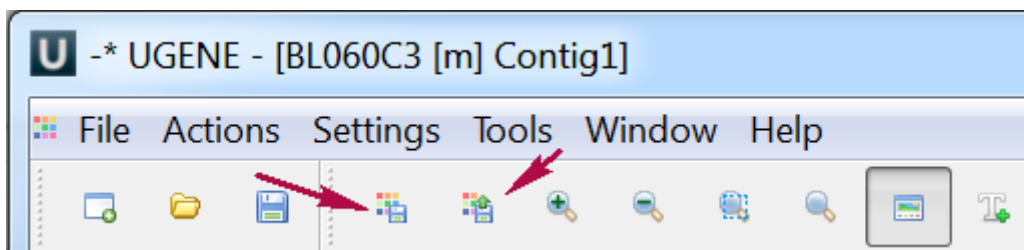
When a modification happens the current state of the multiple sequence alignments object is being recorded.

You can apply any previous state and redo the modifications using the corresponding buttons on the toolbar:



Saving Alignment

To save current alignment click the *Save alignment* button, to the the alignment into another file click the *Save alignment as* button.



Aligning Sequences

The *Alignment Editor* integrates several popular multiple sequence alignment algorithms. Below is the list of available algorithms and links to the documentation:

- Port of the popular *MUSCLE3* algorithm.
- KAlign plugin: effective work with huge alignments.
- ClustalW and MAFFT: these algorithms appeared in the version 1.7.2 of UGENE with the *External Tools* plugin.
- T-Coffee: this alignment algorithm is available since version 1.8.1 of UGENE with the *External Tools* plugin.
- ClustalO: Clustal Omega is the latest addition to the Clustal family. It offers a significant increase in scalability over previous versions, allowing hundreds of thousands of sequences to be aligned in only a few hours.

To align sequences choose a preferred alignment method in the *Actions* main menu, in the context menu or by *Align* main toolbar button .

Also you may find useful the following video tutorials devoted to the multiple sequence alignment:

- [Making a multiple sequence alignment from FASTA file](#)
- [Working with large alignments in UGENE](#)
- [Performing profile-to-profile and profile-to-sequence MUSCLE alignments](#)
- [Running remote MUSCLE task](#)

Aligning Sequence to this Alignment

To align a sequence to an opened alignment, click the *Align sequence to this alignment* toolbar button:

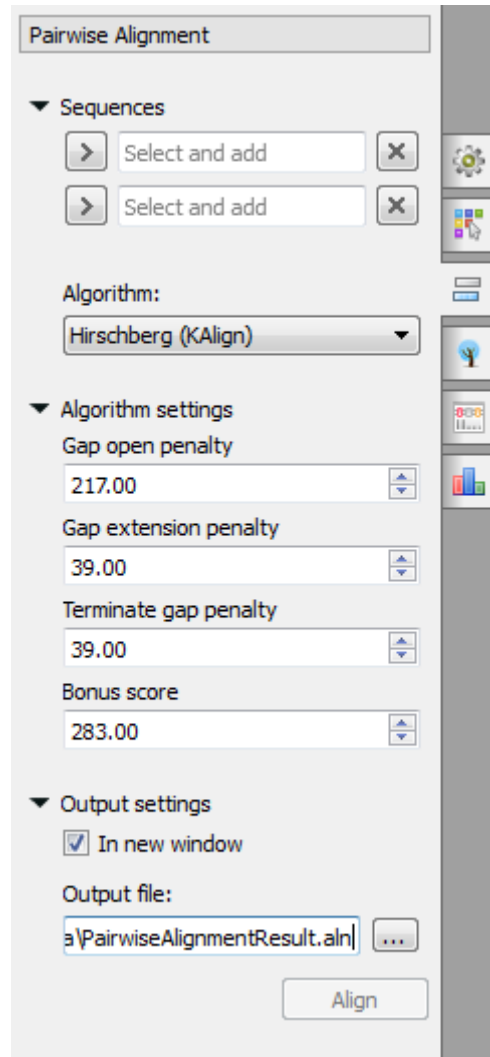


Choose a file with the sequence from the files system, and click *Open*.

Also, you can add an already opened sequence or sequences to the alignment. To do it, select the sequence object(s) in the *Project View* and click the *Align sequence to this alignment* toolbar button. The sequence(s) will be aligned to the alignment automatically.

Pairwise Alignment

To align two sequences go to the *Pairwise Alignment* tab of the *Options Panel*:



Select two sequence from the original alignment, select the parameters and click on the *Align* button. The following parameters are available:

Algorithm - algorithm of the pairwise alignment. There are two algorithms:

Hirschberg (KAlign) - algorithm has the following parameters:

Gap open penalty - indicates the penalty applied for opening a gap. The penalty must be negative.

Gap extension penalty - indicates the penalty applied for extending a gap.

Terminate gap penalty - the penalty to extend gaps from the N/C terminal of protein or 5'/3' terminal of nucleotide sequences.

Bonus score - a bonus score that is added to each pair of aligned residues.

Smith-Waterman - the following parameters are available:

Algorithm version - version of the algorithm implementation. Non-classic versions produce the same results as classic but much faster. To use these optimizations our system must support these capabilities: OPENCL, SSE2 or SW_classic.

Scoring matrix - scoring matrix.

Gap open penalty - penalty for opening a gap.

Gap extension penalty — penalty for extending a gap.

Output settings - settings of the output file.

Working with Sequences List

- Adding New Sequences
- Renaming Sequences
- Sorting Sequences

- Shifting Sequences
- Collapsing Rows
- Copying Sequences

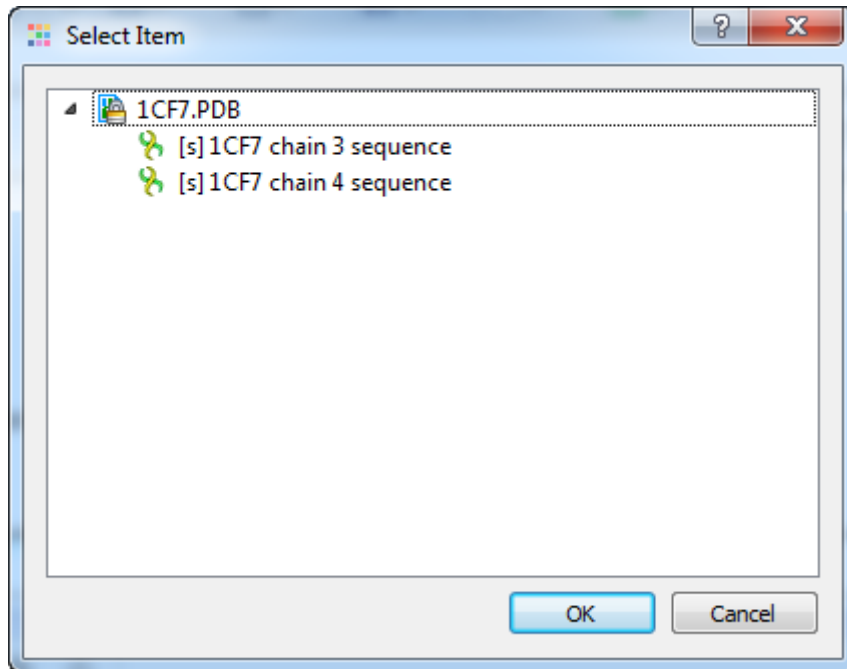
Adding New Sequences

You can add new sequences to an alignment using the *Add* submenu in the *Actions* main menu or the context menu.

There are two ways to add a new sequence to the current alignment:

- From a file in the compatible format (FASTA, GenBank etc.). The list of the supported data formats can be found [here](#).
- From the current project.

If you activate this item, the following dialog will appear:



You will see the *Project View* tree filtered to show only appropriate sequences. Select the items to add and press the *Ok* button.

Renaming Sequences

To rename a sequence double click on the name of this sequence and enter a new sequence name in the dialog.

Sorting Sequences

To sort sequences by name in the alphabetical order choose the *View Sort sequences by name* item from the *Actions* main menu or the context menu.

Shifting Sequences

To change an order of sequences in a multiple sequence alignment do the following:

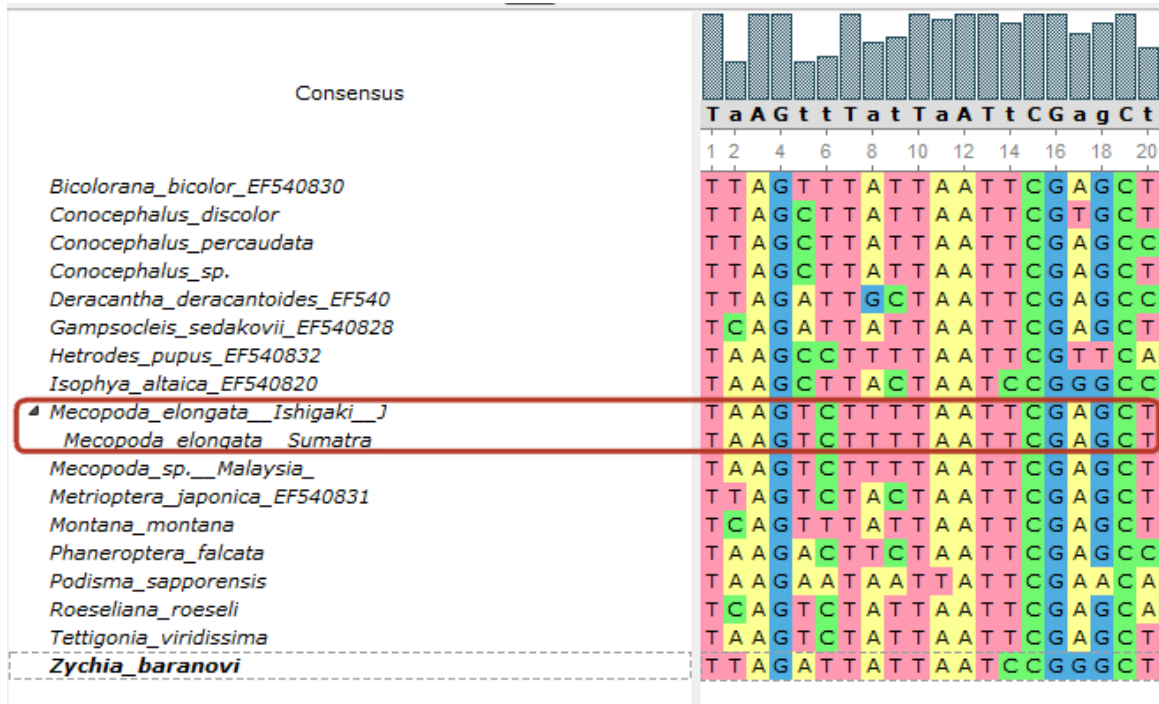
- select sequence or sequences in the sequences names list by click or by click and drag correspondingly.
- click and drag on selected region to shift it.

Collapsing Rows

It is able to collapse the sequential rows. To collapse rows click on the *Switch on/off collapsing* main toolbar button:



The triangle will appear near collapsed sequences. Click on the triangle to show the whole tree of the collapsed rows.

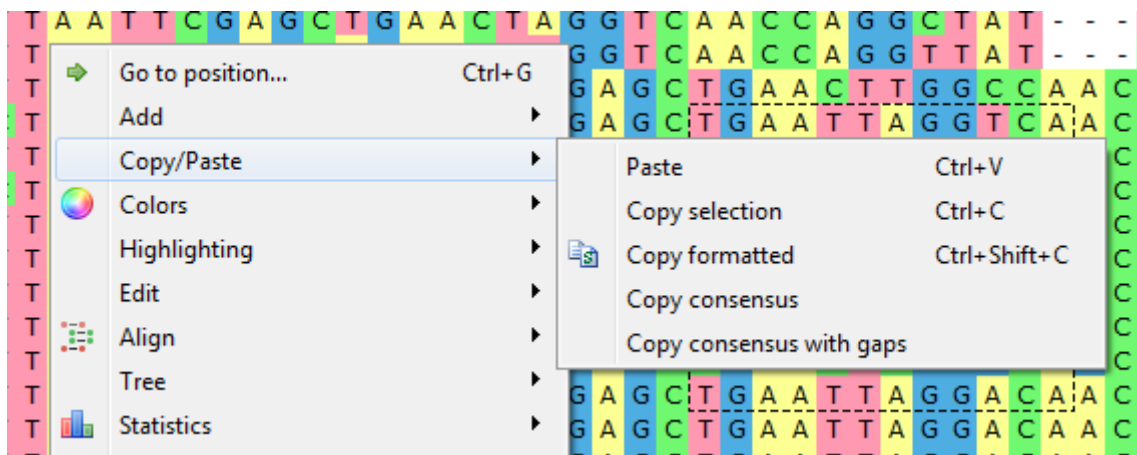


To update the collapsed groups click on the corresponding main toolbar button .

Copying Sequences

To copy one or several sequences do the following:

- Select the sequences in the *Sequence list* area;
- Select the *Copy/Paste Copy selection* context menu item in the *Sequence area* or use hot key combination. Note, that if you activate context menu in the *Sequence list* area you will lose your current selection.



To copy consensus sequence use the *Copy/Paste Copy consensus* item. To copy consensus with gaps use the corresponding menu item.

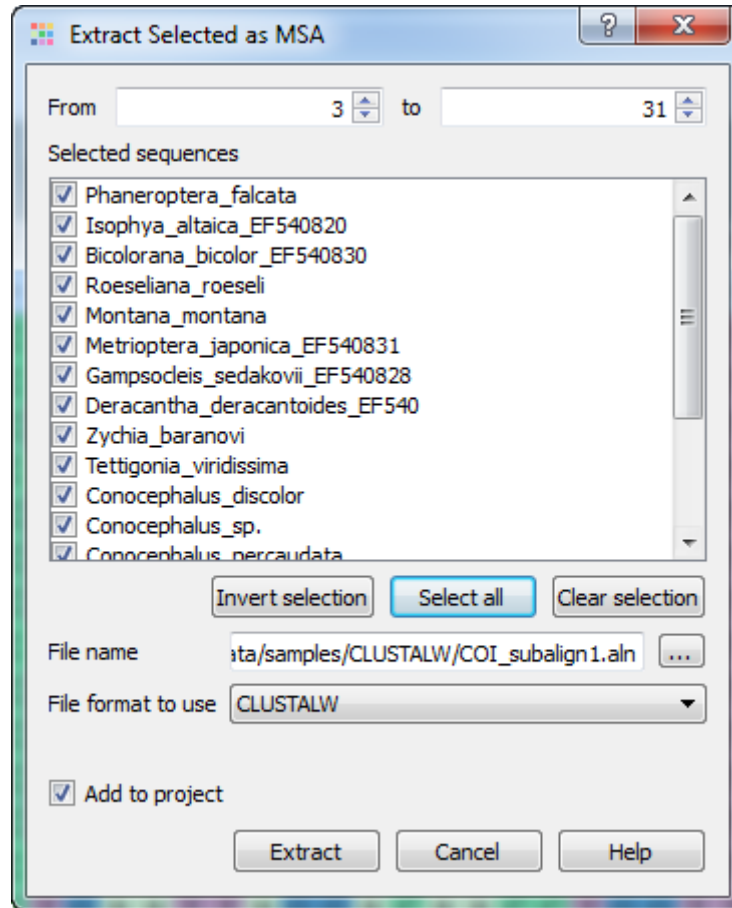
Exporting in Alignment

- Extracting Selected as MSA
- Exporting Sequence from Alignment
- Exporting Alignment as Image

Extracting Selected as MSA

It is possible to extract a subalignment and save it as new multiple sequence alignment (MSA).

Select a subalignment and choose the *Export Save subalignment* item in the *Actions* main menu or in the context menu. The following dialog appears:



Specify the name and format of the new MSA file in the *File name* and *File format to use* fields. The currently selected region is extracted by default when you press the *Extract* button.

You can change the columns to be extracted using the *From* and *to* fields. And change the rows to be extracted by checking/unchecking required sequences in the *Selected sequences* list.

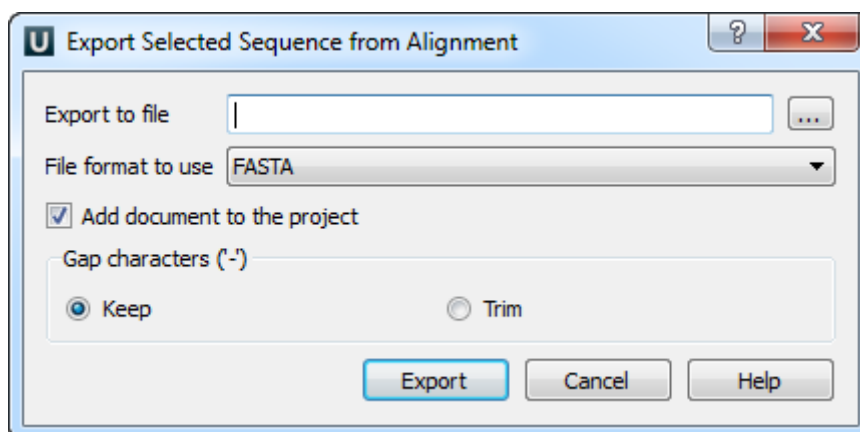
Use buttons:

- *Invert selection* — to invert the selection of the sequences.
- *Select all* — to select all sequences.
- *Clear selection* — to clear the selection of all sequences.

The *Add to project* check box specifies to add the MSA file created from the subalignment to the active project.

Exporting Sequence from Alignment

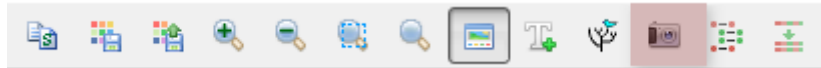
To export one sequence from an alignment select the sequence in the sequence list or in the sequence area and use the *Export->Save sequence* context menu item. The following dialog will appear:



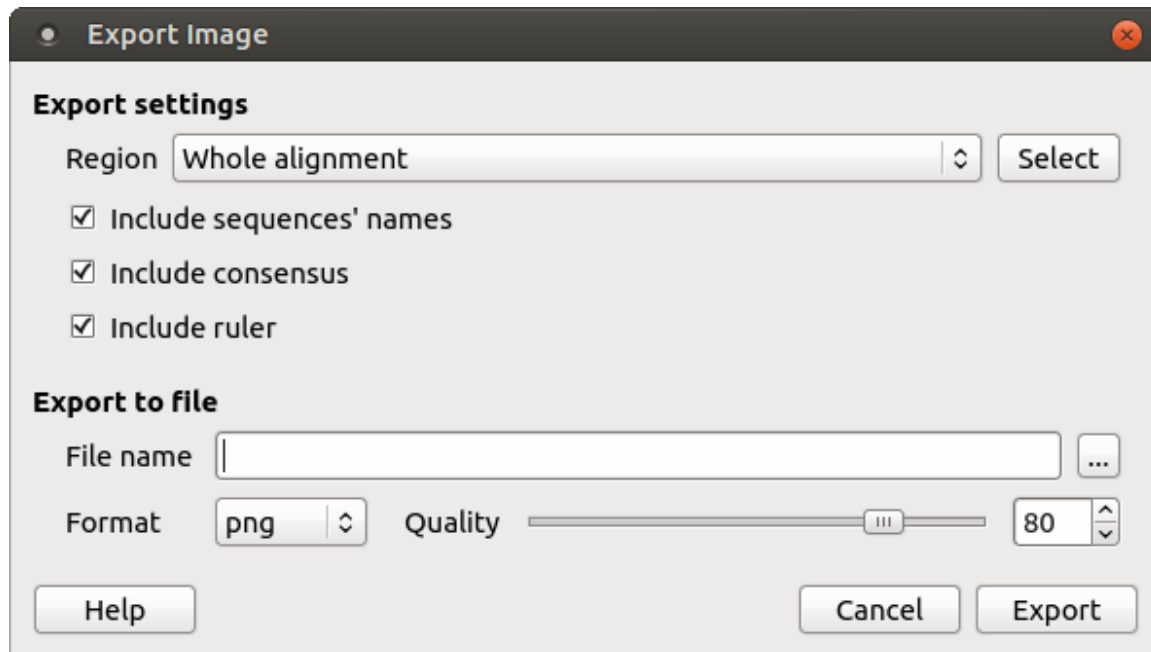
Here it is possible to specify the result file location, to select a sequence file format, to define whether to keep or remove gaps ('-' chars) in the sequence and optionally add the created document to the current project.

Exporting Alignment as Image

To export an alignment as image click the *Export as image* button on the editor toolbar or call the *Export->Export as image* context menu item.



The Export Image dialog will appear where you should set name, location, export settings and format of the picture:

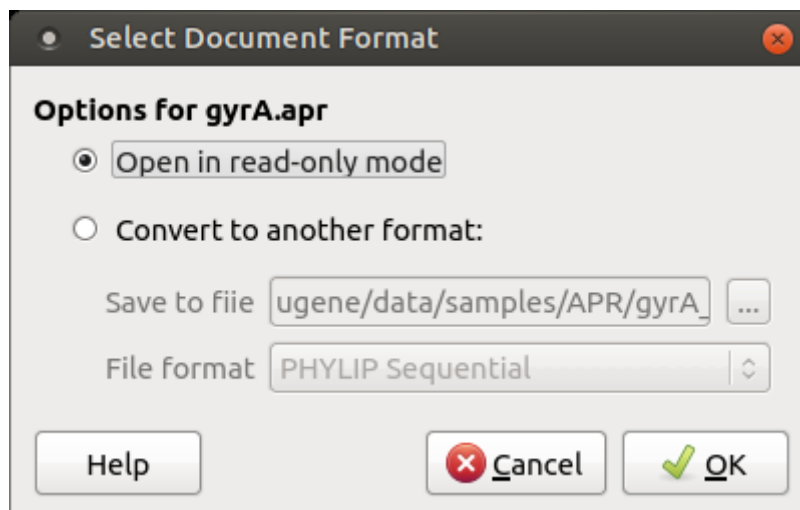


UGENE supports export to the BMP, CUR, DDS, ICNS, JP2, JPEG, JPG, PBM, PGM, PNG, PPM, TIF, TIFF, WBMP, WEBP, XBM, XPM and SVG image formats. You can export whole alignment or custom region. To select the custom region click on the *Select* button.

Importing APR and ACE Files

To start working with APR file you can open it in the Alignment Editor in read-only mode or convert it to another format.

To do this, *open* the *.apr file. The following dialog will appear:

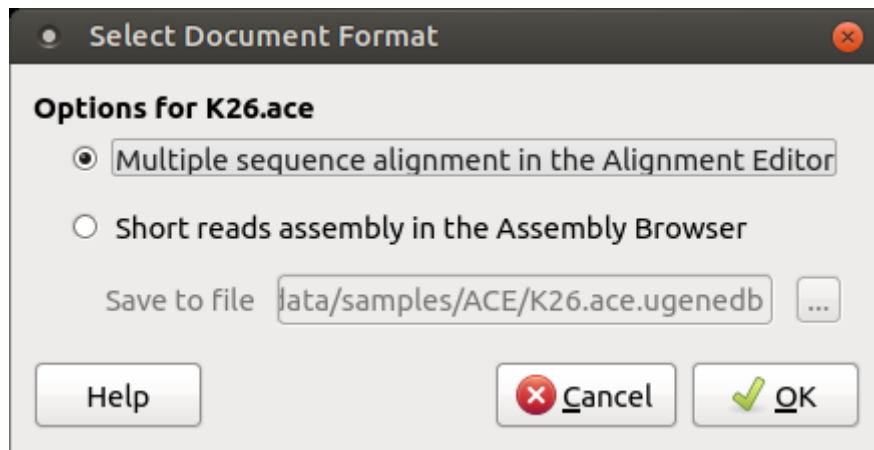


If you choose the first option the file will be opened in the Alignment Editor as multiple sequence alignment in read-only mode. If you choose the second option the file will be converted to a corresponding format and open in UGENE.

The following formats are available: CLUSTALW, FASTA, Mega, MSF, NEXUS, Phylip Interleaved, Phylip Sequential, Stockholm.

To start working with ACE file you can open it in the Alignment Editor or import it to the UGENE database file.

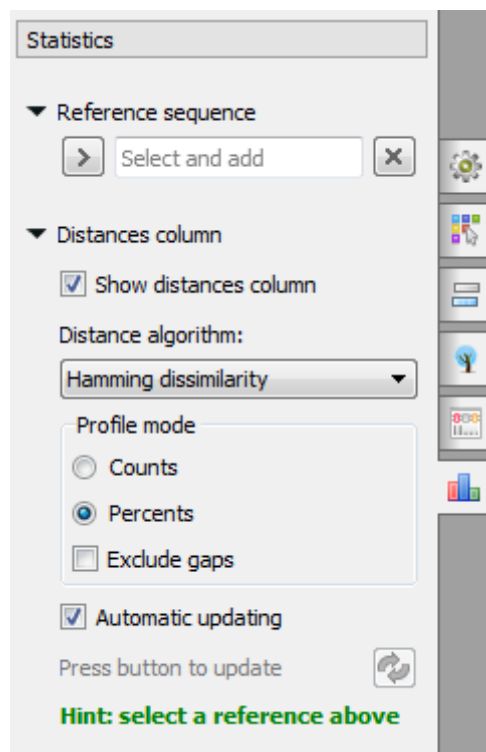
To do this, *open* the *.ace file. The following dialog will appear:



If you choose the first option the file will be opened in the Alignment Editor as multiple sequence alignment. If you choose the second option the file will be opened in Assembly Browser.

Statistics

To show statistics use the *Statistic* tab of the *Options Panel*:



Here you need to select a reference sequence.

You can show/hide distance column by *Show distances column* checkbox.

Also you can change the distance algorithm, select the profile mode and exclude gaps.

To update the column use *Automatic update* checkbox or press corresponding button to update.

To generate distance matrix and grid profile see the documentation below:

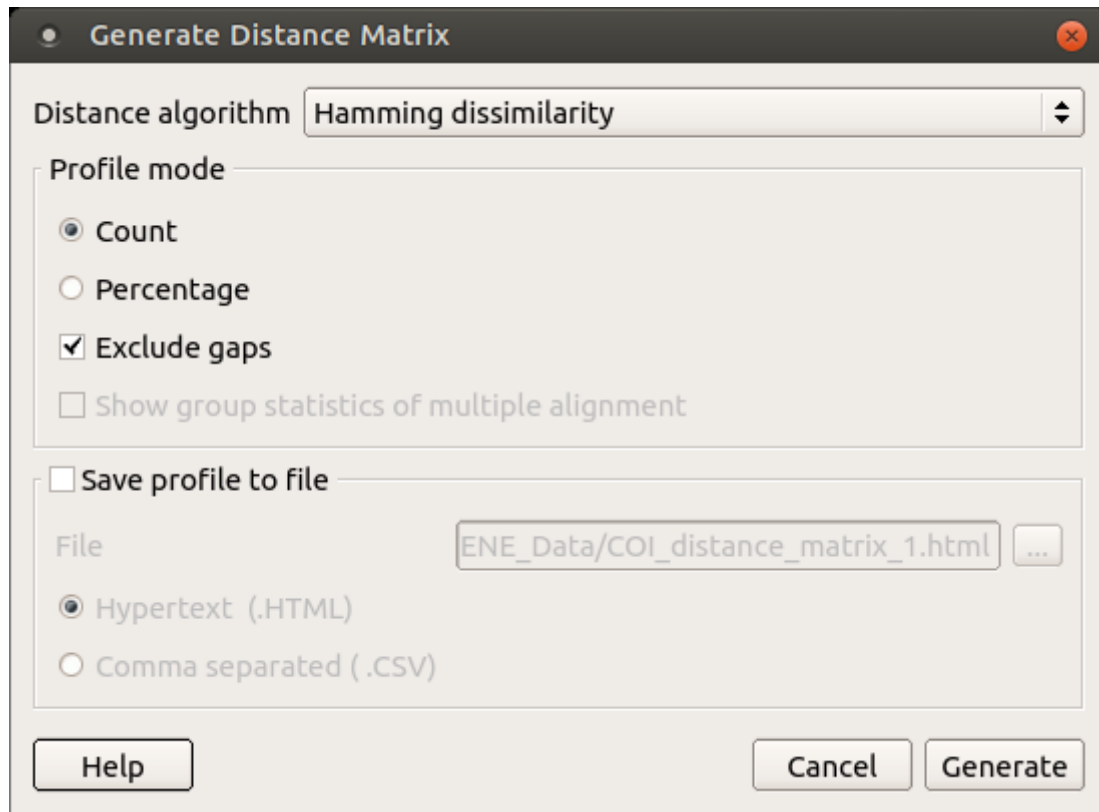
- [Distance Matrix](#)
- [Grid Profile](#)

Distance Matrix

Using the *Alignment Editor* you can also create a distance matrix of a multiple sequence alignment.

To create a distance matrix, use the *Statistics Generate distance matrix* item in the *Actions* main menu or in the context menu.

The dialog will appear:



The following parameters are available:

Distance algorithm - there are two distance algorithms: "Hamming distance" for dissimilarity and "Simple similarity" for similarity.

Profile mode: Counts/Percents — select the *Percents* to have scores shown as percents in the report. Also, you can *Exclude gaps*.

Show group statistics of multiple alignment - shows group statistics when the collapsing is switched on.

Save profile to file — allows saving the profile to a file in the HTML or CSV format. The CSV format is convenient for further processing in worksheets editors like Excel.

The resulting profile in the HTML mode:

Distance matrix for COI

Table content: Hamming dissimilarity

	Phaneroptera_falcata	Isophya_altaica_EF540820	Bicolorana_bicolor_EF540830	Roeseliana_1
Phaneroptera_falcata	0	106	118	115
Isophya_altaica_EF540820	106	0	115	119
Bicolorana_bicolor_EF540830	118	115	0	54
Roeseliana_roeseli	115	119	54	0
Montana_montana	116	118	85	75
Metrioptera_japonica_EF540831	113	115	84	72
Gampsocleis_sedakovii_EF540828	128	125	101	97
Deracantha_deracantoides_EF540	110	109	91	92
Zychia_baranovi	100	114	109	112
Tettigonia_viridissima	114	110	104	99
Conocephalus_discolor	123	115	110	116
Conocephalus_sp.	122	114	110	114
Conocephalus_percaudata	130	121	123	120
Mecopoda_elongata_Ishigaki_J	103	100	107	100
Mecopoda_elongata_Sumatra_	103	100	107	100
Mecopoda_sp._Malaysia_	102	101	102	98
Podisma_sapporensis	116	128	120	116
Hetrodes_pupus_EF540832	152	162	154	146

Legend: 10% 25% 50% 70% 90%

Grid Profile

Using the *Alignment Editor* you can create a statistic profile of a multiple sequence alignment.

The alignment grid profile shows positional amino acid or nucleotide counts highlighted according to the frequency of symbols in a row.

To create a grid profile, use the *Statistics Generate grid profile* item in the *Actions* main menu or in the context menu.

To learn more about this feature, refer to the *DNA Statistics* plugin documentation.

Advanced Functions

This chapter is devoted to the advanced functions of the *Alignment Editor*. You will learn how to build a grid profile, export a picture of an alignment and build HMM profiles.

- Building HMM Profile

Building HMM Profile

The editor has capabilities to build a Hidden Markov Model profile based on the multiple sequence alignment.

This functionality is based on the [Sean Eddy's HMMER](#) package.

To build a HMM profile select the *Advanced Build HMMER2 profile* or the *Advanced Build HMMER3 profile* item in the *Actions* main menu or in the context menu.

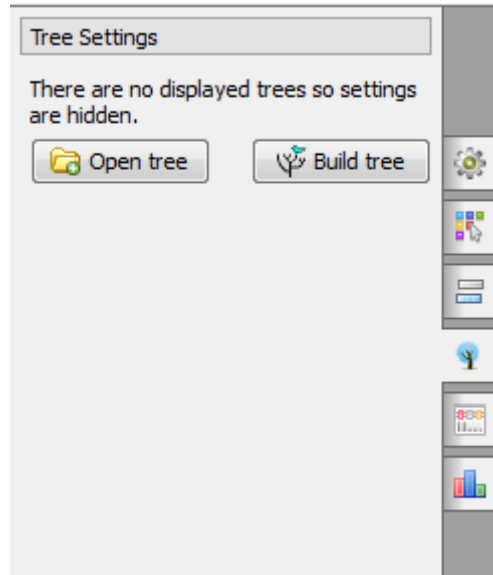
Learn more about the HMM tool in the documentation pages of the *HMM2* and the *HMM3* plugins.

Building Phylogenetic Tree

To build a tree from an alignment either press the *Build Tree* button on the toolbar, select the *Tree Build Tree* item in the alignment context menu or the *Actions Tree Build Tree* item in the main menu.



Also you can use *Tree Settings* tab of the *Options Panel*:



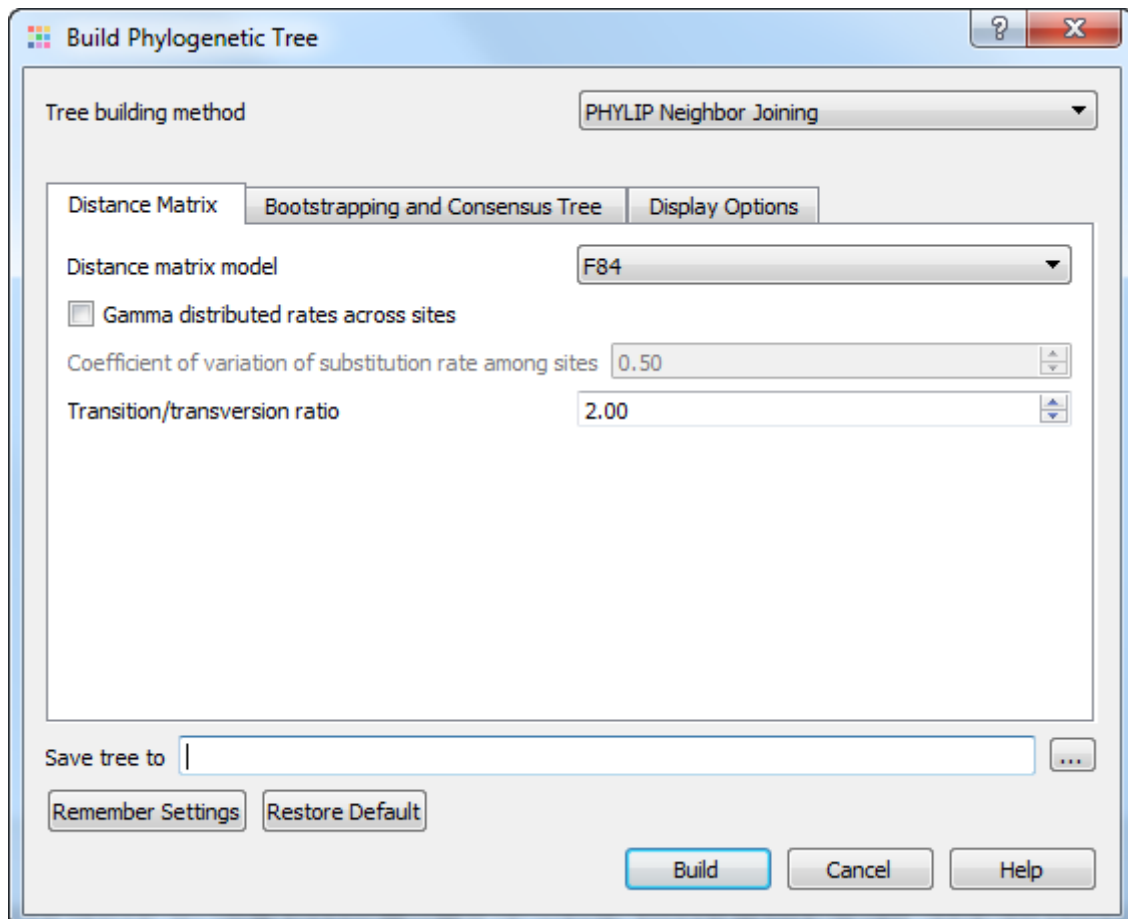
Three methods for building phylogenetic trees are supported:

1. The *PHYLIP Neighbour-Joining* method. The *PHYLIP* package implementation of the method is used under the hood.
2. The *MrBayes* external tool. Check [MrBayes Web Site](#) for more details.
3. PhyML Maximum Likelihood method. Check [PhyML Maximum Likelihood Web Site](#) for more details.

- [PHYLIP Neighbor-Joining](#)
- [MrBayes](#)
- [PhyML Maximum Likelihood](#)

PHYLIP Neighbor-Joining

The *Building Phylogenetic Tree* dialog for the *PHYLIP Neighbour-Joining* method has the following view:



The following parameters are available:

Distance matrix model — model to compute a distance matrix. The following values are available for a nucleotide multiple sequence alignment:

- F84
- Kimura
- Jukes-Cantor
- LogDet

The following models are available for a protein alignment:

- Jones-Taylor-Thornton
- Henikoff/Tillier PMB
- Dayhoff PAM
- Kimura

Gamma distributed rates across sites — specifies to take into account unequal rates of change at different sites. It is assumed that the distribution of the rates follows the Gamma distribution.

Coefficient of variation of substitution rate among sites — becomes available if the *Gamma distributed rates across sites* parameter is checked. Specifies the coefficient of the distribution of the rates.

Transition/transversion ratio — expected ratio of transitions to transversions.

To enable bootstrapping check the *Bootstrapping and Consensus Trees* group check box. The following parameters are available:

Number of replicates — number of replicate data sets.

Seed — random number seed. By default, it is generated automatically. You can manually change this value in order to make results of different runs (of a tree building) reproducible. The should must be an integer greater than zero and less than 32767 and which is of the form $4n+1$, that is, it leaves a remainder of 1 when divided by 4. Any odd number can also be used, but may result in a random number sequence that repeats itself after less than the full one billion numbers. Usually this is not a problem.

Consensus type — specifies the method to build the consensus tree. Select one of the following:

- *Strict* — specifies that a set of species must appear in all input trees to be included in the strict consensus tree.
- *Majority Rule (extended)* — specifies that any set of species that appears in more than 50% of the trees is included. The program then considers the other sets of species in order of the frequency with which they have appeared, adding to the consensus tree any which are compatible with it until the tree is fully resolved. This is the default setting.
- *M1* — includes in the consensus tree any sets of species that occur among the input trees more than a specified fraction of the time (see the *Fraction* parameter below). The *Strict* consensus and the *Majority Rule* consensus are extreme cases of the MI consensus, being for fractions of 1 and 0.5 respectively.
- *Majority Rule* — specifies that a set of species is included in the consensus tree if it is present in more than half of the input trees.

Fraction — becomes available when the *Consensus type* parameter is set to *M1*. Specifies the fraction.

Display tree in new window - displays tree in new window.

Display tree with alignment editor - displays tree with alignment editor.

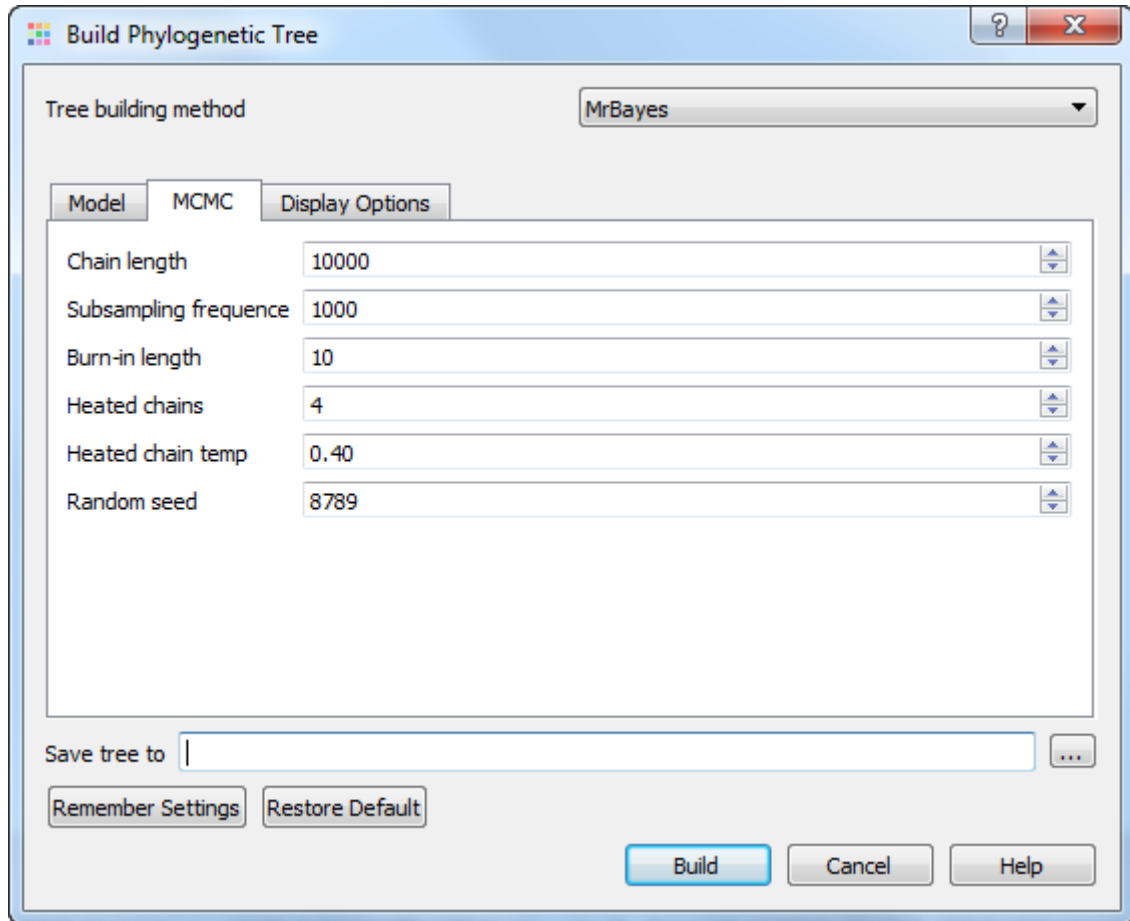
Synchronize alignment with tree - synchronize alignment and tree.

Save tree to — file to save the tree built.

Press the *Build* button to build a tree with the parameters selected.

MrBayes

The *Building Phylogenetic Tree* dialog for the *MrBayes* method has the following view:



There are two steps to a phylogenetic analysis using *MrBayes*:

1. Set the evolutionary model.
2. Run the Markov chain Monte Carlo (MCMC) analysis.

The evolutionary model is defined by the following parameters:

Substitution model — specifies the general structure of a DNA substitution model. This parameter is available for the nucleotide sequences. It corresponds to the Nst setting of MrBayes. You may select one of the following:

- JC69 (Nst=1)
- HKY85 (Nst=2)
- GTR (Nst=6)

Rate matrix (fixed) — specifies the fixed-rate amino-acid model. This parameter is available for amino-acid sequences. The following models are available:

- poisson
- jones
- dayhoff
- mtrev
- mtmam
- wag
- rtrev
- cprev
- vt
- blosum
- equaline

The following parameters are common for nucleotide and amino-acid sequences:

Rate — sets the model for among-site rate variation. Select one of the following:

- equal — no rate variation across sites.
- gamma — gamma-distributed rates across sites. The rate at a site is drawn from a gamma distribution. The gamma distribution has a single parameter that describes how much rates vary.
- propinv — a proportion of the sites are invariable.

- *invgamma* — a proportion of the sites are invariable while the rate for the remaining sites are drawn from a gamma distribution.

Gamma — sets the number of rate categories for the gamma distribution.

You can select the following parameters for the MCMC analysis:

Chain length — sets the number of cycles for the MCMC algorithm. This should be a big number as you want the chain to first reach stationarity, and then remain there for enough time to take lots of samples.

Subsampling frequency — specifies how often the Markov chain is sampled. You can sample the chain every cycle, but this results in very large output files.

Burn-in length — determines the number of samples that will be discarded when convergence diagnostics are calculated.

Heated chains — number of chains will be used in Metropolis coupling. Set 1 to use usual MCMC analysis.

Heated chain temp — the temperature parameter for heating the chains. The higher the temperature, the more likely the heated chains are to move between isolated peaks in the posterior distribution.

Random seed — a seed for the random number generator.

Display tree in new window - displays tree in new window.

Display tree with alignment editor - displays tree with alignment editor.

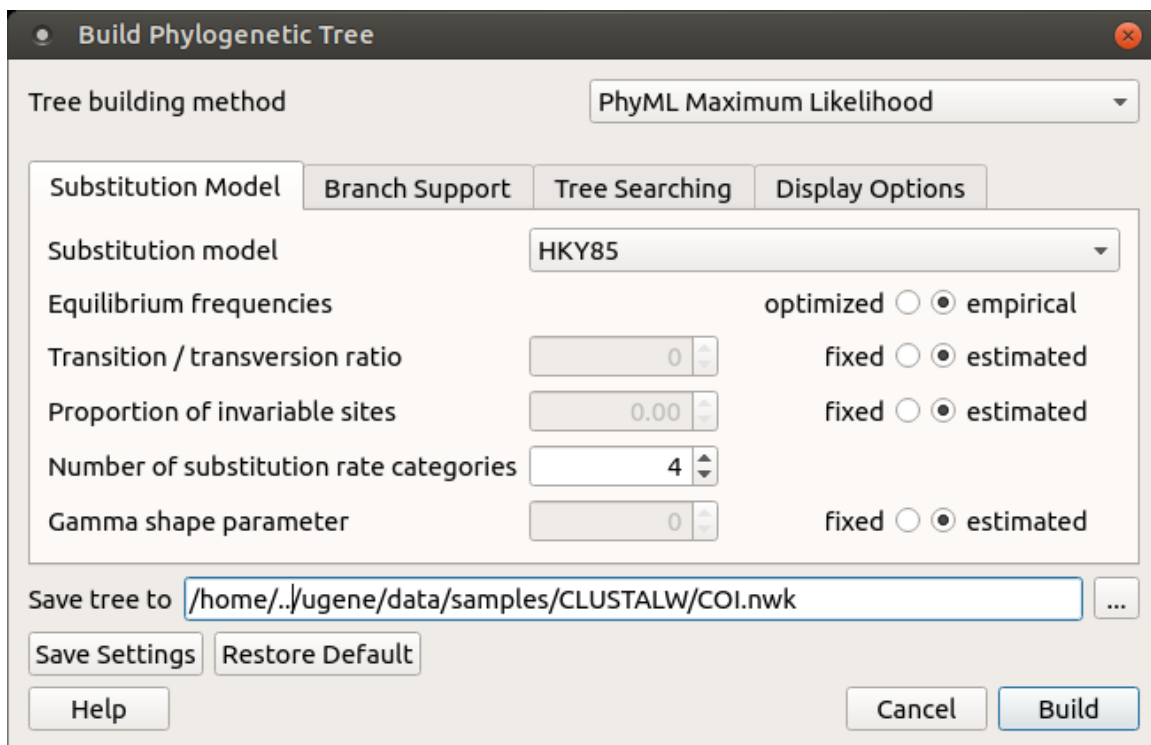
Synchronize alignment with tree - synchronize alignment and tree.

Save tree to — file to save the built tree.

Press the *Build* button to run the analysis with the parameters selected and build a consensus tree.

PhyML Maximum Likelihood

The *Building Phylogenetic Tree* dialog for the *PhyML Maximum Likelihood* method has the following view:



The following parameters are available:

Substitution model parameters - a selection of the Markov model of substitution:

Substitution model - model of substitution.

Equilibrium frequencies - equilibrium frequencies.

Transition/transversion ratio - fix or estimate the transition/transversion ratio in the maximum likelihood framework.

Proportion of invariable sites - the proportion of invariable sites, i.e., the expected frequency of sites that do not evolve, can be

fixed or estimated.

Number of substitution rate categories - the number of substitution rate categories.

Gamma shape parameter - the shape of the gamma distribution determines the range of rate variation across sites.

Branch support parameters - the selection of the method that is used to measure branch support:

Use fast likelihood method - use fast likelihood method.

Perform bootstrap - the support of the data for each internal branch of the phylogeny can be estimated using non-parametric bootstrap.

Tree searching parameters - the selection of the tree topology searching algorithm:

Make initial tree automatically - initial tree automatically.

Type of tree improvement - type of tree improvement.

Set number of random starting tree - the number of random starting tree.

Optimize tree topology - the tree topology is optimised in order to maximise the likelihood.

Optimize branch lengths - optimize branch lengths.

Optimise substitution rate - substitution rate parameters are optimized.

Display tree in new window - displays tree in the new window.

Display tree with alignment editor - displays tree with alignment editor.

Synchronize alignment with tree - synchronize alignment and tree.

Save tree to - file to save the built tree.

Press the *Build* button to run the analysis with the parameters selected and build a consensus tree.

Sanger Reads Editor

- Sanger Reads Editor Overview
 - Sanger Reads Editor Features
 - Sanger Reads Editor Components
- Working with Chromatogram
 - Mapping Reads to Reference
 - Alignment Statistics
 - Alignment Appearance
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 - Navigation in Sanger Reads Alignment
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 - Removing Gap at the Left
 - Removing All Columns of Gaps
 - Trimming Left End
 - Trimming Right End
 - Renaming Read
 - Removing Read
 - Undo and Redo
 - Exporting Alignment without Chromatograms

Sanger Reads Editor Overview

This chapter gives an overview of the *Sanger Reads Editor* components and explains basic concepts of browsing a chromatogram.

- Sanger Reads Editor Features
- Sanger Reads Editor Components

Sanger Reads Editor Features

Sanger Reads Editor is dedicated to DNA sequence analysis and manipulation. You can trim, map to reference and view Sanger sequencing trace files and create consensus sequences.

The editor provides interactive visual representation which includes:

- Navigation through an chromatogram;
- Flexible zooming;
- Several consensus calculation algorithms.

Using the *Sanger Reads Editor* you can:

- Export alignment without chromatogram;
- Edit chromatogram: remove/replace/trim.

Sanger Reads Editor Components

Here is the default layout of the editor:

The screenshot shows the UGENE software interface. The main window displays a Sanger reads alignment view. The reference sequence is KM099231: G C T T G T G C C G G C C C A T C A C T T T C A C G A G C T. The consensus sequence is G C T T G T G C C C C C A T C A C T T T C A C G A G C T. The chromatogram area shows signal peaks for each base. The overview bar at the bottom shows the alignment of reads across a genomic region. The right sidebar contains a 'Consensus' panel with options for consensus mode, type, threshold, and export settings.

Working with Chromatogram

This chapter explains how to work efficiently with the *Sanger Reads Editor*.

- Mapping Reads to Reference
- Alignment Statistics
- Alignment Appearance
- Overview and Show and Hide Chromatogram
- Sanger Reads Consensus
 - Export Chromatogram Consensus
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 - Trimming Right End
 - Renaming Read
 - Removing Read
 - Undo and Redo
- Exporting Alignment without Chromatograms

Mapping Reads to Reference

To map Sanger sequencing reads to a reference use the *Tools*→ *Sanger data analysis*→ *Map reads to reference* main menu item. The following dialog appears:

Map Sanger Reads to Reference

Input data

Reference
 ...

Reads

Settings

Trimming quality threshold ^ v

Mapping min similarity ^ v

Read name in result alignment v

Result alignment

...

Add to project

It is required to input:

Reference sequence — a file with a single DNA sequence of any supported file format (e.g. FASTA or GenBank). This parameter is mandatory.

Reads — a set of files in *.ab1 or *.scf format with the Sanger sequencing data to be mapped to the reference sequence. It is required to add at least one read. A read orientation (forward or reverse) will be automatically detected during the mapping.

During the mapping task execution, these data are processed as follows. First of all, to enhance the further mapping sensitivity, the [low-quality](#) ends of the reads are trimmed off. Then the reads are mapped using a UGENE original method that works in two steps: rough mapping of a read using BLAST+ and enhancement of the read alignment with the Smith-Waterman algorithm. After that the percentage similarity of the two sequences is calculated, that is the number of all edit operations (i.e., insertions, deletions, and substitutions) required to transform a read sequence into the corresponding region of the reference sequence. Reads that have low similarity with the reference sequence are filtered out.

One can configure the following parameters of the mapping task

Trimming quality threshold — all bases at the ends of the reads with quality lower than the specified value are trimmed. To skip the trimming, set this value to zero.

Mapping min similarity — all reads mapped to the reference with lower percentage similarity than the specified value are filtered out.

Read name in result alignment — reads in the resulting alignment can be named either by names of the sequences in the input files or by the input files names. Set this value to "File name", for example, if the sequences in the input *.ab1 files have the same name, this will help in distinguishing of the reads in the resulting alignment.

The resulting alignment is stored in a native UGENEDB format. One can set up the file location and name in the *Result alignment* field. Note that thereafter it is also possible to [export data to standard alignment formats without chromatograms](#) such as FASTA, ClustalW, etc.

To initiate the mapping task execution click the *Map* button in the dialog. Note that when the task is finished, the task statistics can be found in a report, available by clicking the corresponding [notification](#):

Task report [Map Sanger reads to reference]

Status Finished
Time 0h 00m 03.652s

Details

Reference sequence: KM099231

Mapped reads (16):

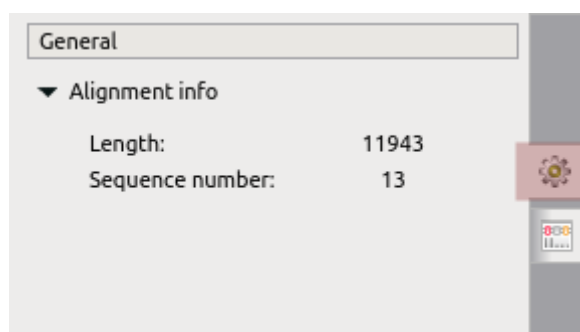
← SZYD_Cas9_5B70	similarity 99%
→ SZYD_Cas9_5B71	similarity 93%
← SZYD_Cas9_CR50	similarity 98%
← SZYD_Cas9_CR51	similarity 98%
← SZYD_Cas9_CR52	similarity 99%
← SZYD_Cas9_CR53	similarity 99%
← SZYD_Cas9_CR54	similarity 99%
← SZYD_Cas9_CR55	similarity 98%
← SZYD_Cas9_CR56	similarity 99%
→ SZYD_Cas9_CR60	similarity 99%
→ SZYD_Cas9_CR61	similarity 98%
→ SZYD_Cas9_CR62	similarity 99%
→ SZYD_Cas9_CR63	similarity 99%
→ SZYD_Cas9_CR64	similarity 99%
→ SZYD_Cas9_CR65	similarity 100%
→ SZYD_Cas9_CR66	similarity 98%

Filtered by low similarity (4):

SZYD_Cas9_CR49	similarity 0%
SZYD_Cas9_CR57	similarity 58%
SZYD_Cas9_CR58	similarity 0%
SZYD_Cas9_CR59	similarity 48%

Alignment Statistics

To show statistics use the *General* tab of the *Options Panel*:



Alignment Appearance

To perform zoom operations use the corresponding buttons on the editor toolbar.

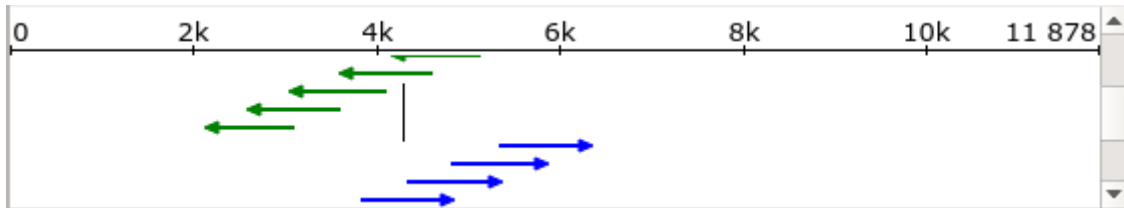


You can zoom in and zoom out by the corresponding toolbar buttons. To reset zoom use the *Reset zoom* button. To increase/decrease peaks height use the corresponding buttons.

You can change font by *Appearance*→*Change characters font* context menu item or by the main *Actions*→*Appearance* menu item.

Overview and Show and Hide Chromatogram

The chromatogram overview is shown automatically in the *Sanger Reads Editor*. To close the overview click on the *Show overview* toolbar button. Drag and drop vertical cursor to change the current position.



To show/hide chromatogram click on the corresponding toolbar button:



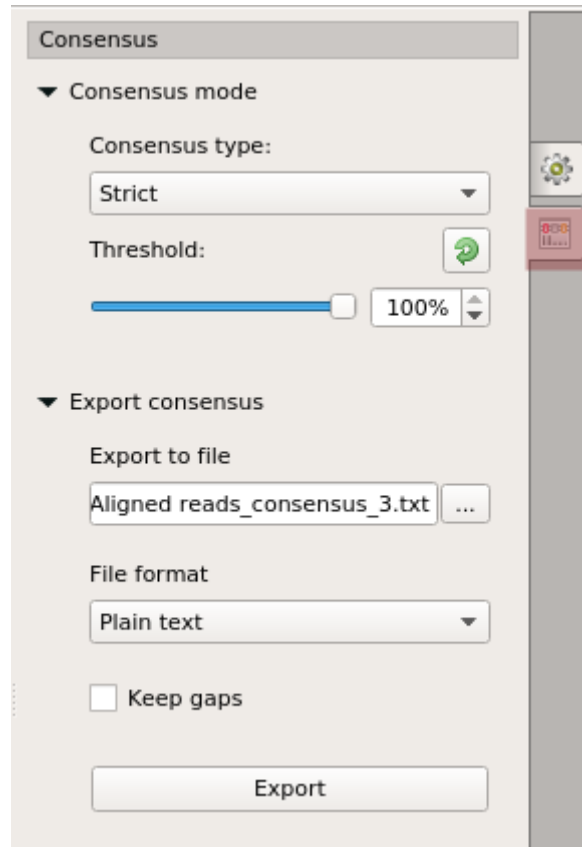
Sanger Reads Consensus

Each base of a consensus sequence is calculated as a function of the corresponding column bases. The *Sanger Reads Editor* allows switching between different consensus modes: Simple extended and Strict.

The Simple extended algorithm selects the best character from the extended DNA alphabet. Only bases with frequencies which are greater than a threshold value are taken into account.

The Strict algorithm returns gap character ('-') if symbol frequency in a column is lower than threshold specified.

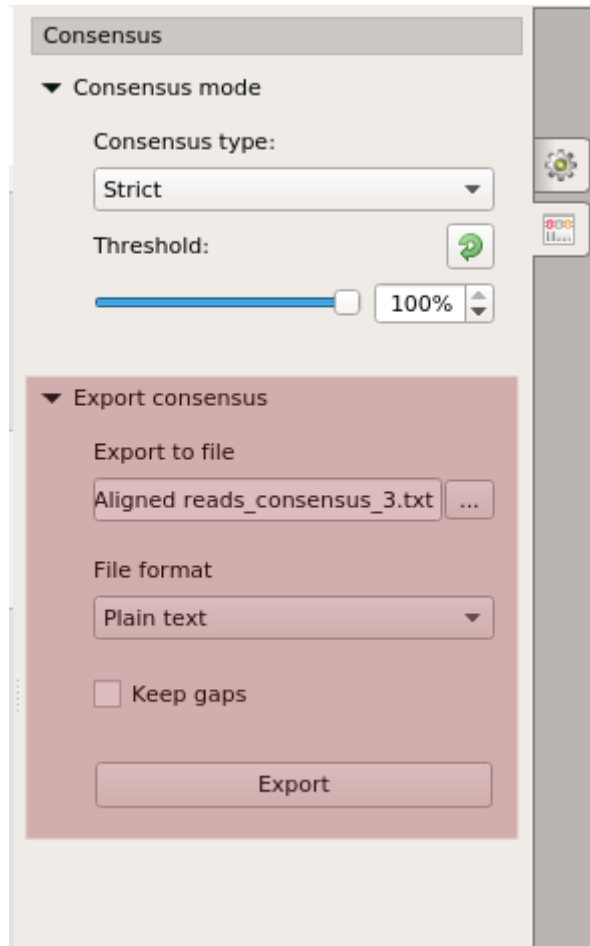
To switch the consensus mode go to the *Consensus tab* of the *Options Panel*.



- [Export Chromatogram Consensus](#)

Export Chromatogram Consensus

To export consensus sequence use the *Consensus* tab of the *Options Panel*:



The following parameters are available:

Export to file - here you need to select path for the output file.

File format - format for the output file.

Keep gaps - set this parameter if the result consensus must keep gaps.

When you click on the *Export* button the consensus sequence will be exported into selected output file.

Navigation in Sanger Reads Alignment

You may jump to previous/next ambiguous character in the reads alignment and edit the characters.

After you finish the editing, use the navigation by previous/next variations to find differences between the reference sequence and the consensus.

Use the main *Actions*→ *Navigation* menu, context menu or the following toolbar buttons:



Editing Sanger Reads

Select the *Edit* submenu in the *Sanger Reads Editor* context menu.

The actions available from this menu are described below:

- Inserting Character
- Replacing Character and Gap
- Removing Character and Gap
- Inserting Gap
- Removing Gap at the Left
- Removing All Columns of Gaps
- Trimming Left End
- Trimming Right End
- Renaming Read
- Removing Read
- Undo and Redo

Inserting Character

To insert a nucleotide into a read in the *Sanger Reads Editor*, select a nucleotide or a gap in the corresponding location in the *Chromatogram Area* and switch to the editing mode by pressing *Shift+I*, selecting *Edit->Insert character/gap* in the context menu, or selecting *Actions->Edit->Insert character/gap* in the main menu. While the cursor is blinking, press a character on the keyboard that you want to insert. After that the editing mode is switched off.

Note that if you press the '-' character on the keyboard while being in the editing mode, a gap is inserted. The other way to insert a gap is by pressing *Space*, see the [Inserting Gap](#) chapter.

You can always cancel the changes by pressing *Ctrl+Z*.

Replacing Character and Gap

To replace read symbol or gap in the *Sanger Reads Editor* select the nucleotide and press *Shift+R* or *Edit->Replace character/gap* context menu for switching the edit mode on.

While the selection is blinking, press a keyboard character that you want to replace.

After that the edit mode is switched off. For replace another symbol press *Shift+R* again.

You always can cancel the changes pressing *Ctrl+Z*.

Removing Character and Gap

To delete read symbol or gap in the *Sanger Reads Editor* select the nucleotide and press *Del* or *Edit->Remove character/gap* context menu for switching the edit mode on.

While the selection is blinking, press a delete button.

After that the edit mode is switched off. For remove another symbol press *Del* again.

You always can cancel the changes pressing *Ctrl+Z*.

Inserting Gap

To insert a gap into a read in the *Sanger Reads Editor* select a character or a gap in the corresponding location in the *Chromatogram Area* and press *Space*, select *Edit->Insert gap* in the context item, or *Actions->Edit->Insert gap* in the main menu.

Thus, by holding the *Space* key, one can shift a Sanger read to the right. To shift the read to the left hold the *Backspace* key, see the [Removing Gap at the Left](#) chapter.

You can always cancel the changes by pressing *Ctrl+Z*.

Removing Gap at the Left

To remove a gap in the *Sanger Reads Editor* select the next character or gap after the gap you want to remove and press *Backspace*. You can also select *Edit->Remove gap at the left* in the context menu or *Actions->Edit->Remove gap at the left*.

If there is a region of gaps or it is the beginning of a read, by holding the *Backspace* key, one can shift a Sanger read to the left. To shift the read to the right hold the *Space* key, see the [Inserting Gap](#) chapter.

You can always cancel the changes by pressing *Ctrl+Z*.

Removing All Columns of Gaps

Use the *Shift+Del* or *Edit Remove all columns of gaps* item in the *Actions* main menu or in the context menu to remove all columns of gaps from the alignment. This can be useful, for example, when an insertion in a read turned out not to be real, it was replaced by a gap, and a column of gaps was left in the alignment.

Trimming Left End

To trim left end in the *Sanger Reads Editor* select the next symbol after trimming part and use *Ctrl+Shift+Backspace* or *Edit->Trim left end* context menu for switching the edit mode on.

After that the edit mode is switched off.

You always can cancel the changes pressing *Ctrl+Z*.

Trimming Right End

To trim right end in the *Sanger Reads Editor* select the previous symbol before trimming part and use *Ctrl+Shift+Del* or *Edit->Trim right end* context menu for switching the edit mode on.

After that the edit mode is switched off.

You always can cancel the changes pressing *Ctrl+Z*.

Renaming Read

To rename read use *F2* or *Rename read* context menu.

Removing Read

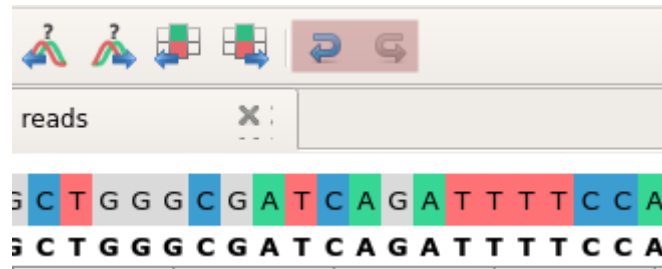
To remove read use *Delete* keyboard button or *Remove read* context menu.

Undo and Redo

The editor tracks all modifications.

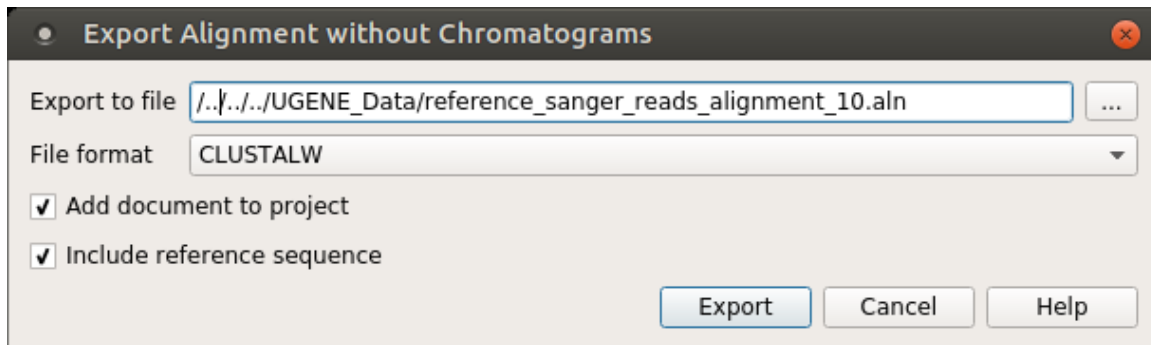
When a modification happens the current state is being recorded.

You can apply any previous state and redo the modifications using the corresponding buttons on the toolbar:



Exporting Alignment without Chromatograms

To export alignment without chromatogram use the *Actions*→*Alignment*→*Export alignment without chromatogram* main menu or the same context menu item. The following dialog will appear:



Here it is possible to specify the result file location, to select a sequence file format, optionally include consensus and add the created document to the current project.

Assembly Browser

The UGENE Assembly Browser project started in 2010 was inspired by [Illumina iDEA Challenge 2011](#) and multiple requests from UGENE users. The main goal of the Assembly Browser is to let a user visualize and efficiently browse large next generation sequence assemblies.

Currently supported formats are SAM (Sequence Alignment/Map) and BAM, which is a binary version of the SAM format. Both formats are produced by SAMtools and described in the following specification: [SAMtools](#). Support of other formats is also planned, so please send us a request if you're interested in a certain format.

To browse an assembly data in UGENE, a BAM or SAM file should be imported to a UGENE database file. After that you can convert the UGENE database file into a SAM file. The import to a UGENE database file has both advantages and disadvantages. The disadvantages are that the import may take time for a large file and there should be enough disk space to store the database file.

On the other hand, this allows one to overview the whole assembly and navigate in it rather rapidly. In addition, during the import you can select contigs to be imported from the BAM/SAM file. So, there is no need to import the whole file if you're going to work only with some contigs. Note that in the future there are plans to support the other approach as well, namely, when a BAM/SAM file is opened directly.

The Assembly Browser has been tested on different BAM/SAM files from the [1000 Genomes Project](#) and other sources.

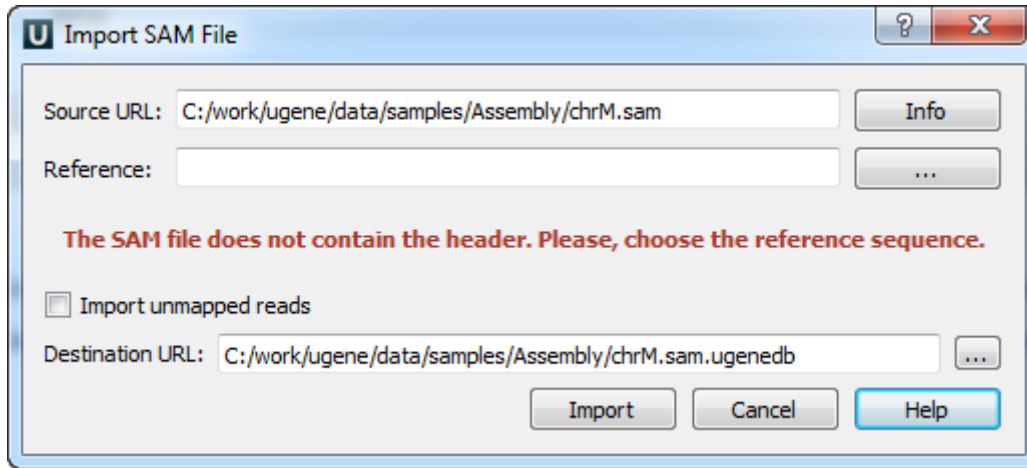
Read the documentation below to learn more about the Assembly Browser features.

- [Import BAM and SAM Files](#)
- [Browsing and Zooming Assembly](#)
 - [Opening Assembler Browser Window](#)
 - [Assembly Browser Window](#)
 - [Assembly Browser Window Components](#)
 - [Reads Area Description](#)
 - [Assembly Overview Description](#)
 - [Ruler and Coverage Graph Description](#)
 - [Go to Position in Assembly](#)
 - [Using Bookmarks for Navigation in Assembly Data](#)
- [Getting Information About Read](#)
- [Short Reads Visualization](#)
 - [Reads Highlighting](#)
 - [Reads Shadowing](#)
- [Associating Reference Sequence](#)
- [Associating Variations](#)
- [Consensus Sequence](#)
- [Exporting](#)
 - [Exporting Reads](#)
 - [Exporting Visible Reads](#)
 - [Exporting Coverage](#)
 - [Exporting Consensus](#)
 - [Exporting Consensus Variations](#)
 - [Exporting Assembly as Image](#)
 - [Exporting Assembly Region](#)
- [Options Panel in Assembly Browser](#)
 - [Navigation in Assembly Browser](#)
 - [Assembly Statistics](#)
 - [Assembly Browser Settings](#)
- [Assembly Browser Hotkeys](#)
 - [Assembly Overview Hotkeys](#)
 - [Reads Area Hotkeys](#)

Import BAM and SAM Files

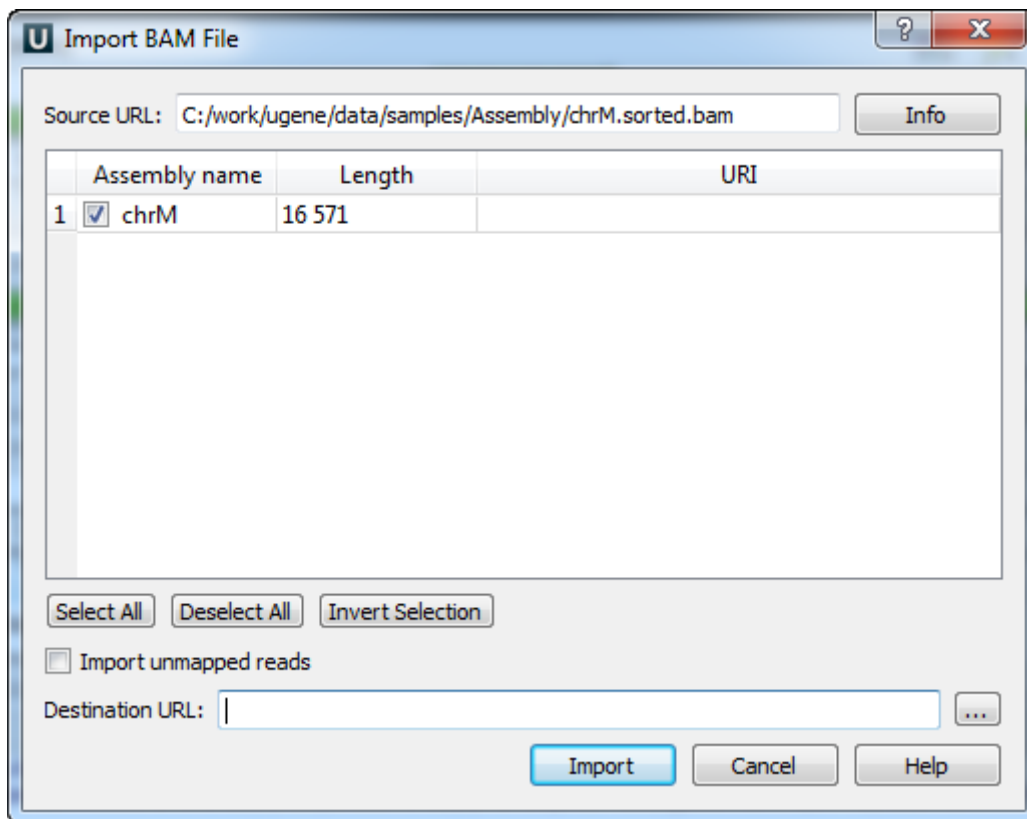
To start working with an assembly import it to the UGENE database file. To do this, *open* the assembly file.

For assembly file without header you need to choose a reference sequence:



Select the reference sequence and click *Import* button.

For other assembly files the following dialog appears:



The *Source URL* field in the dialog specifies the file to import. The *Info* button nearby can be used to obtain additional information about the file.

There is a list of contigs below the *Source URL*. Check the contigs that you want to import to the database. You can use the *Select All*, *Deselect All* and *Invert Selection* buttons to manage the selection.

The *Destination URL* field specifies the output database file.

If you check the *Import unmapped reads*, then all unmapped reads in the assembly (i.e. read with the unmapped flag or without CIGAR) are imported. Note, however, that they are not visualized in the current UGENE version.

To start the import, click the *Import* button in the dialog. You can see the progress of the import in the *Task View*. To export a UGENE database file into the SAM format, select the *Actions Export assembly to SAM format* item in the main menu.

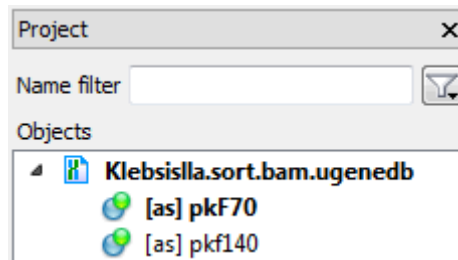
Browsing and Zooming Assembly

- Opening Assembler Browser Window
- Assembly Browser Window
- Assembly Browser Window Components
- Reads Area Description

- [Assembly Overview Description](#)
- [Ruler and Coverage Graph Description](#)
- [Go to Position in Assembly](#)
- [Using Bookmarks for Navigation in Assembly Data](#)

Opening Assembler Browser Window

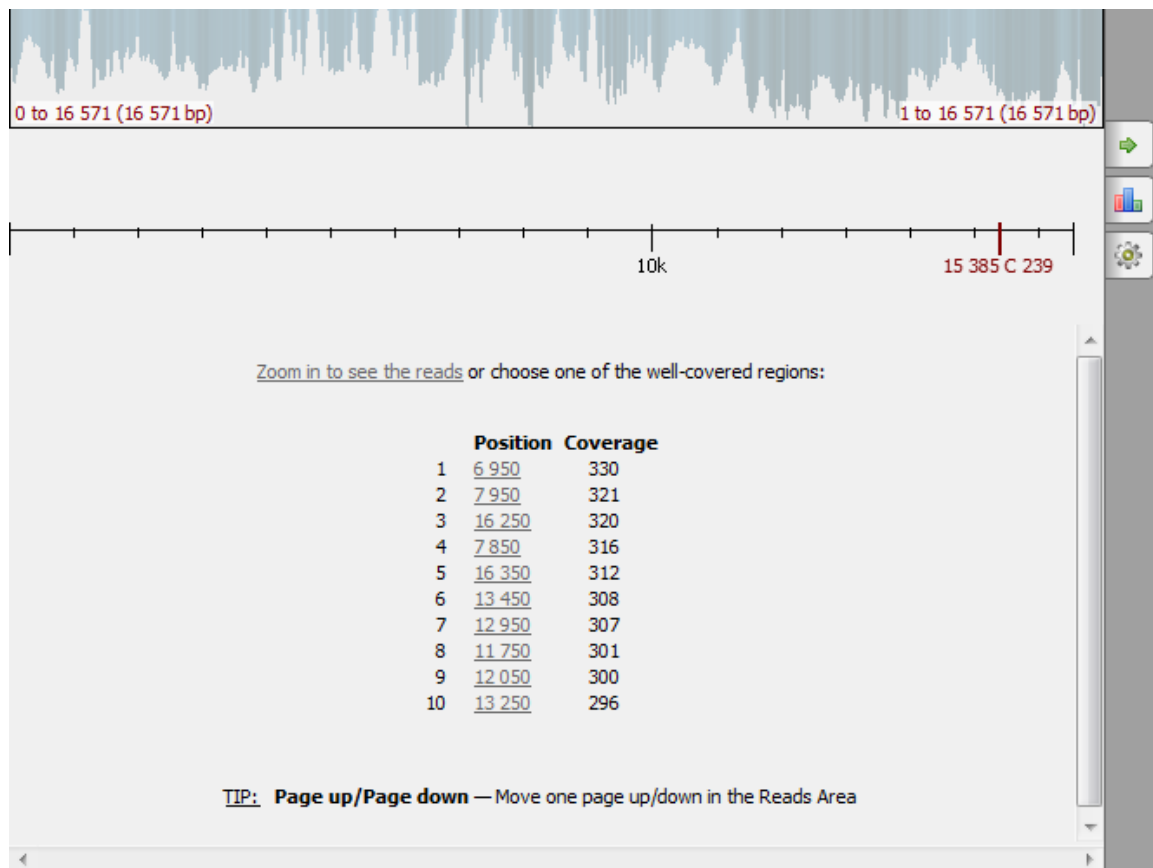
An imported assembly added to the project is shown in the *Project View* as follows:



Each [as] object corresponds to an imported contig. When you double-click on an [as] object a new Assembly Browser window with the assembly data is opened. A window for the first assembly object in the list is opened automatically after the import.

Assembly Browser Window

The opened window contains the list of well-covered regions of the assembly:



Note that for large assemblies it may take some time to calculate the overview and the well-covered regions.

To see the reads, either select a region from the list or zoom in, for example, by clicking the link above the well-covered regions or by rotating the mouse wheel.

You can also use the hotkeys. Tips about hotkeys are shown under the list of well-covered regions. To learn about available hotkeys refer to [Assembly Browser Hotkeys](#).

Assembly Browser Window Components

An Assembly Browser window consists of:

Assembly Overview

By default, shows the whole assembly overview. Can be resized to provide an overview of an assembly part.

Reference Area

Shows the reference sequence.

Consensus Area

Shows the consensus sequence.

Ruler

Shows the coordinates in the *Reads Area*.

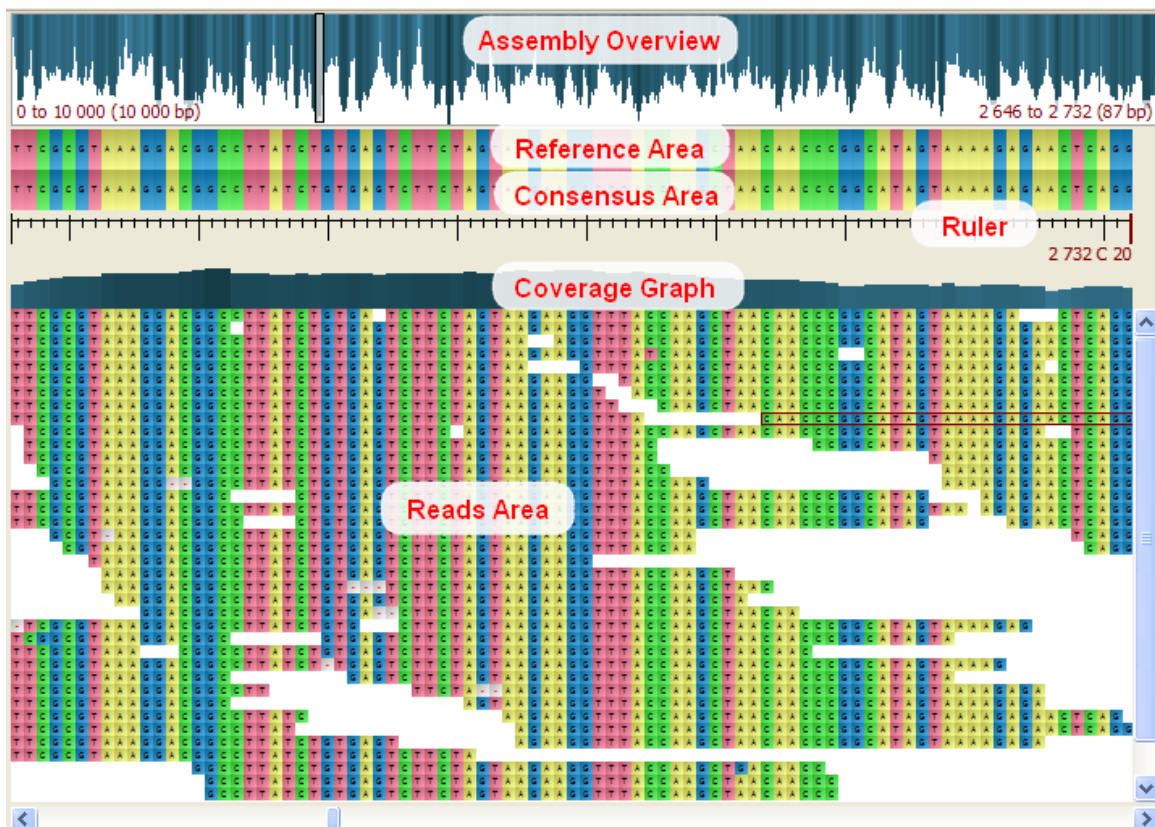
Reads Area

Displays the reads.

Coverage Graph

Shows the coverage of the Reads Area.

See the example below:



Reads Area Description

The *Reads Area* provides a visualization of reads of an assembly part. To zoom in or zoom out, rotate the mouse wheel.

To perform zooming you can also use the *Zoom In* and *Zoom Out* buttons on the toolbar or the *Actions Zoom In* and *Actions Zoom Out* items in the main menu.

Also, when you double-click on a read it is zoomed in and moved to the center of the window.

By dragging the mouse while holding the left mouse button you can navigate in the Reads Area.

To navigate long distances in the Reads Area use the *Assembly Overview* described [below](#).

Other ways to navigate in the assembly are:

- Use the horizontal and vertical scroll bars of the Reads Area
- [Go to a specified position in an assembly](#)

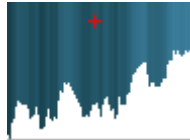
To learn about available hotkeys refer to [Assembly Browser Hotkeys](#).

By default, assembly rendering is optimized while scrolling. While you are moving across an assembly, it shows the assembly in gray color, but when you stop it shows the assembly in different colors. To disable this option uncheck the *Optimize the rendering while scrolling* item in the context menu of the *Reads Area* or *Optimize scrolling* item on the *Assembly Browser Settings* tab of the *Options Panel*.

Assembly Overview Description

The *Assembly Overview* shows a coverage overview of the assembly. The longer the depth of a line in the overview and the deeper the color, the more reads are located in this region.

To open a region of the assembly in the *Reads Area* click on it in the Assembly Overview. On the overview, the selected region is displayed either as a gray rectangle, a red cross or a red rectangle. For example:

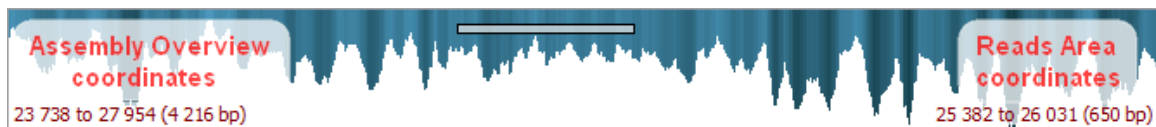


If you hold Shift and select a region on the overview, the overview is zoomed to the selection.

Note that when the Assembly Overview is in focus and you use either the zoom buttons on the toolbar, the zoom items in the *Actions* main menu, or a mouse wheel, the Reads Area is resized appropriately.

The Assembly Overview can also be resized. To zoom in the overview, select either the *Zoom in* or the *Zoom in 100x* item in the Assembly Overview context menu. You can scroll the resized overview by dragging the mouse while pressing down the mouse wheel. To zoom out the overview, select the *Zoom out* item in the context menu. The *Restore global overview* item in the context menu restores the default overview size when the whole contig overview is shown.

Notice that the Assembly Overview shows the coordinates of the assembly areas visible in the Reads Area and in the Assembly Overview:



To scroll the resized overview, drag the mouse while pressing down the mouse wheel.

To learn about available hotkeys refer to [Assembly Browser Hotkeys](#).

Ruler and Coverage Graph Description

The *Ruler* shows the coordinates in the *Reads Area*. When you move the mouse cursor in the Reads Area the coordinate of the selected location with the coverage of reads is shown on the ruler in dark red. The Coverage Graph shows the exact coverage of the sequence at each position. For example on the image below the coordinate is 9168 and the coverage of reads is 251.



To show/hide the coordinates on the ruler you can click the following button on the toolbar:



To show/hide the coverage on the ruler you can click the following button on the toolbar:



Alternatively, you can use the *Show coordinates* and *Show coverage under cursor* check boxes located on the *Assembly Browser Settings* tab of the *Options Panel*.

Go to Position in Assembly

To go to the required position in an assembly use the following field located on the Assembly Browser toolbar.



Input the location and click the *Go!* button. A similar *Go!* field is also available on the *Navigation* tab of the *Options Panel*.

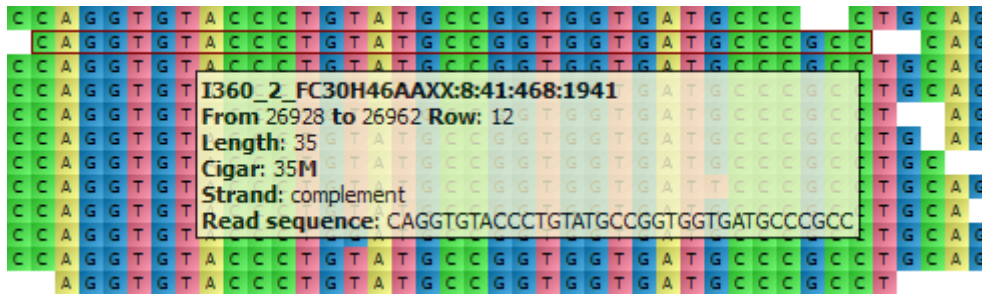
Using Bookmarks for Navigation in Assembly Data

Use *bookmarks* to save and restore visual state of an assembly, for example, position in the assembly, zoom scale, etc.

Getting Information About Read

A read displayed in the *Reads Area* consists of the bases (A, C, G, T). It may also contain the N character that stays for an ambiguous base. Depending on the value of the *Cigar* parameter, the read can be shown partially or gaps can be inserted inside the read (see below).

By default when a read is hovered over in the *Reads Area* a hint appears:



To disable this behaviour click the following button on the toolbar:



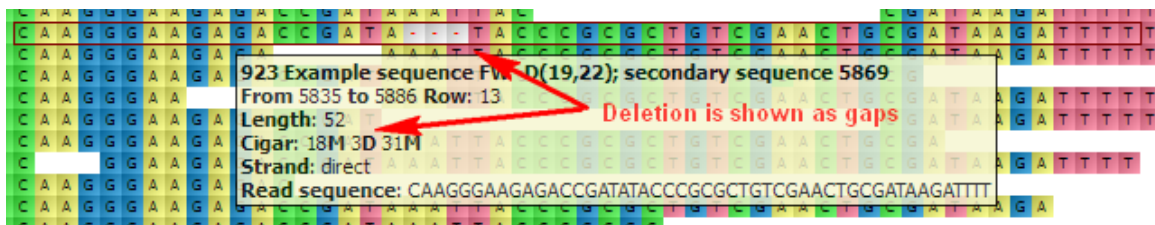
Or uncheck the *Show pop-up hint* check box on the *Assembly Browser Settings* tab of the *Options Panel*.

The hint shows the following information about the read:

- Read name
- Location
- Length
- Cigar
- Strand
- Read sequence

The operations in the *Cigar* parameter are described as follows:

- **M** — Alignment match (can be a sequence match or mismatch).
- **I** — Insertion to the reference. Skipped when the read is aligned to the reference, i.e. it is not shown in the Reads Area, but is present in the read sequence.
- **D** — Deletion from the reference. Gaps are inserted to the read when the read is aligned to the reference. For example:



- **N** — Skipped region from the reference. Behaves as **D**, but has a different biological meaning: for mRNA-to-genome alignment it represents an intron.
- **S** — Soft clipping (clipped sequences are present in the read sequence, i.e. behaves as **I**).
- **H** — Hard clipping (clipped sequences are not present in the read sequence).
- **P** — Padding (silent deletion from padded reference).
- **=** — Exact match to the reference.
- **x** — Reference sequence mismatch.

To copy the information about the read to the clipboard, select the *Copy read information to clipboard* item in the Reads Area context menu. Now you can paste it in any text editor.

To copy the current position of the read select the *Copy current position to clipboard* item in the *Reads Area* context menu.

Short Reads Vizualization

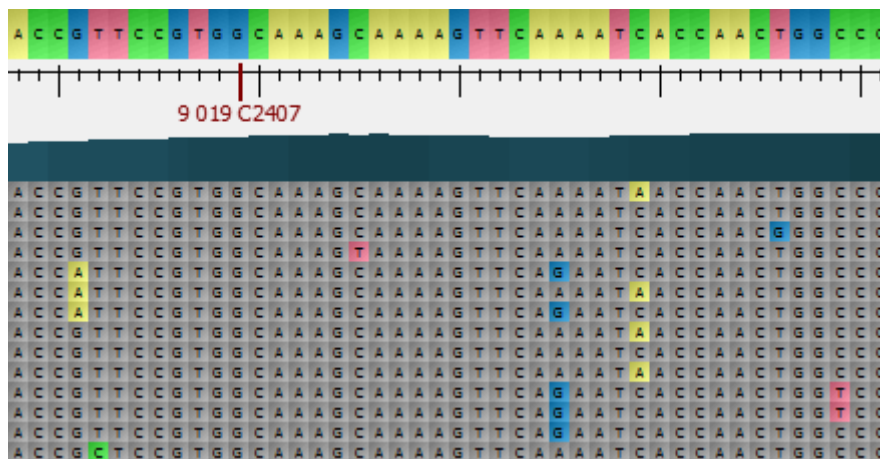
There are various modes of reads highlighting and shadowing.

- Reads Highlighting
- Reads Shadowing

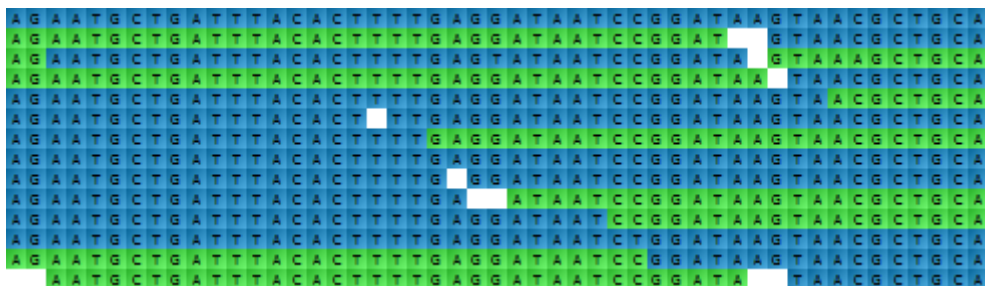
Reads Highlighting

To apply a reads highlighting mode, select it in the *Reads highlighting* menu of the *Reads Area* context menu or on the *Assembly Browser Settings* tab of the *Options Panel*. The following modes are available:

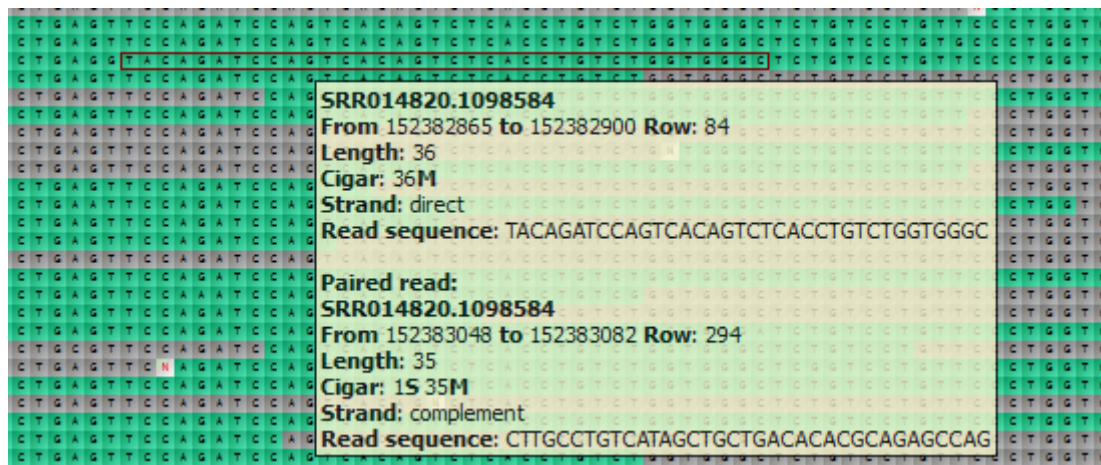
- *Nucleotide* — shows all nucleotides in different colors. It is used by default.
- *Difference* — highlights gaps and nucleotides that differ from the reference sequence. You should add a reference first for correct displaying of this highlighting.



- *Strand direction* — highlights reads located on the direct strand in blue and reads on the complement strand in green.



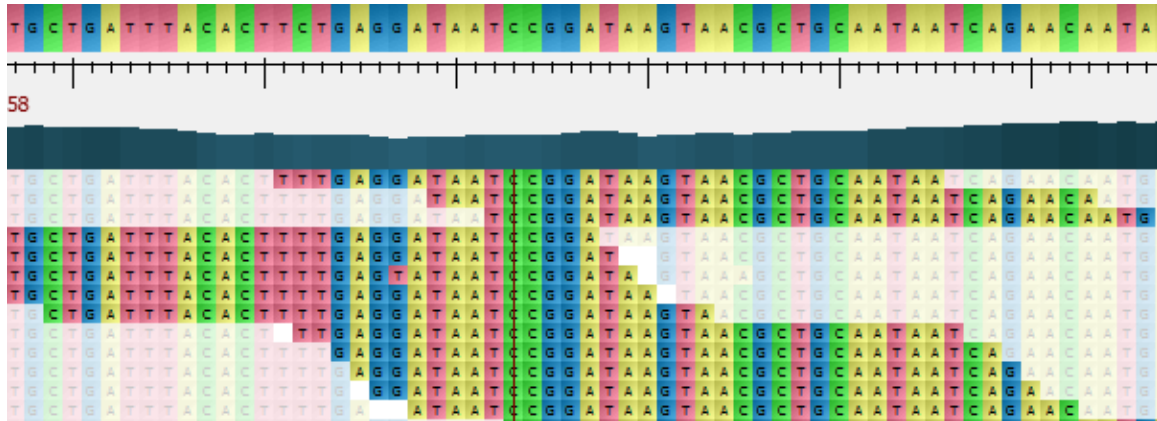
- *Paired reads* — highlights all paired reads in green. Note that the information about the pair is shown in the hint.



Reads Shadowing

Various modes of column highlighting are available from the *Reads shadowing* item in the context menu of the *Reads Area*:

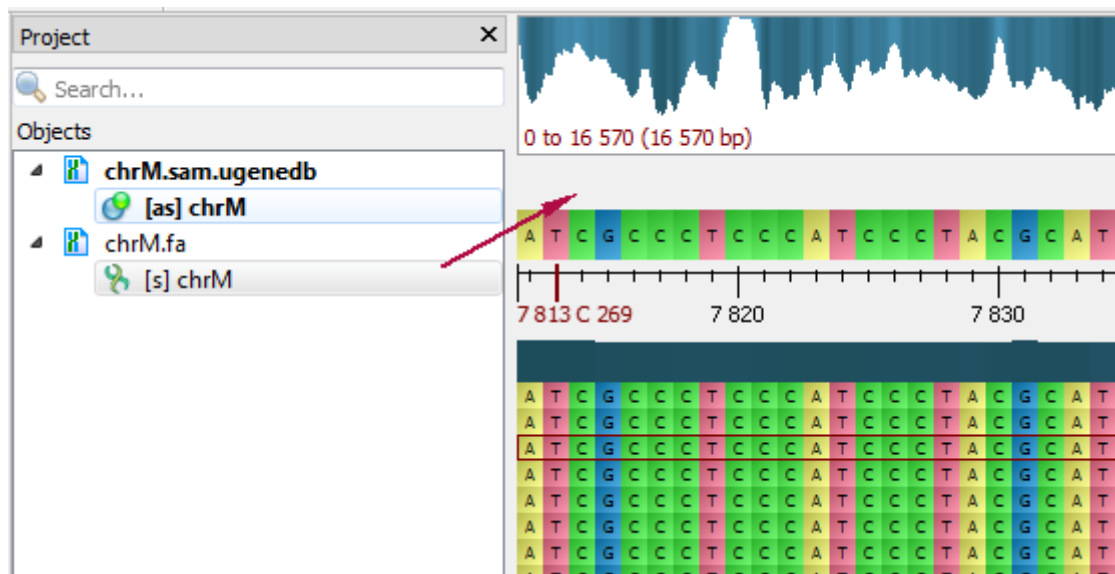
- *Disabled* — highlights all columns of nucleotides.
- *Free* — highlights all reads that intersect a given column. In this mode you can lock a position. Click the *Lock here* item in the context menu to do it. To return to a locked position, select the *Jump to locked base* item in the context menu.
- *Centered* — highlights all reads that intersect the column in the center of the screen.



Associating Reference Sequence

To associate a reference sequence with the assembly use one of the following ways:

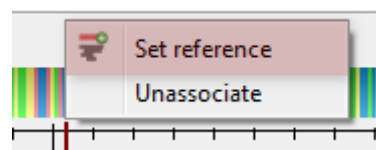
1. *Open the sequence* (the sequence must be loaded) and drag it to the *Assembly Reference Area*:



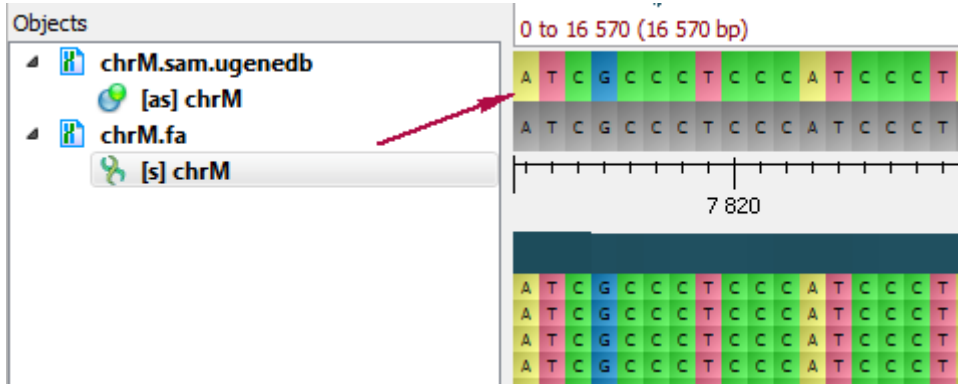
2. Use the corresponding toolbar button:



3. Use the corresponding context menu item:



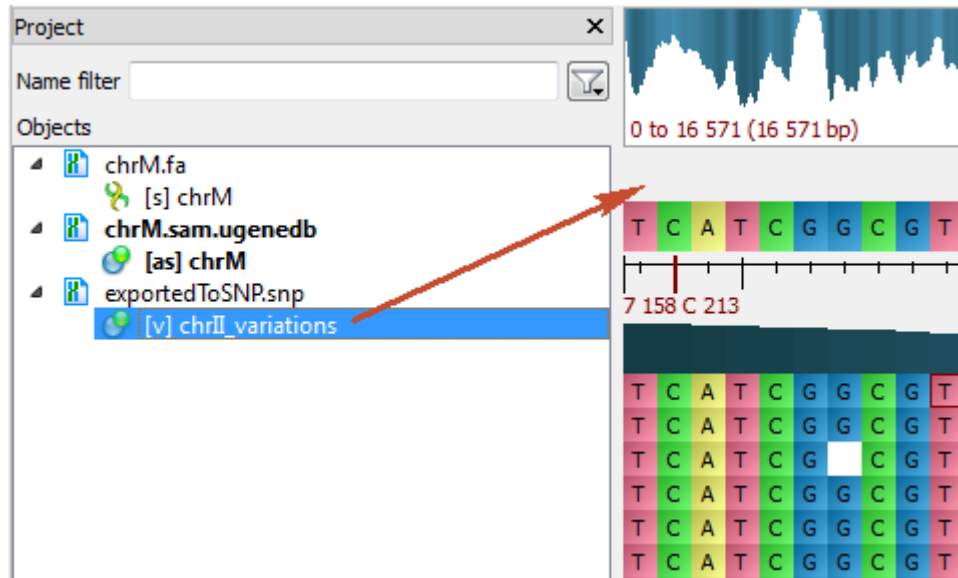
The reference sequence will appear in the *Reference Area*:



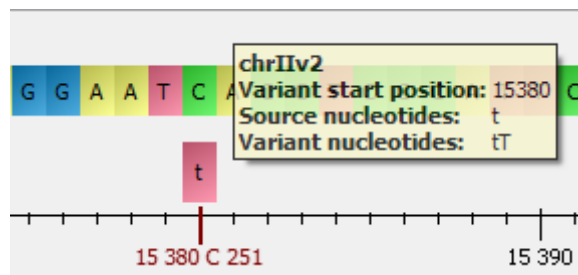
To remove the association, select the *Unassociate* item in the *Reference Area* context menu.

Associating Variations

To associate variations with the assembly, *open the sequence* (the sequence must be loaded) and drag it to the *Assembly Reference Area*:



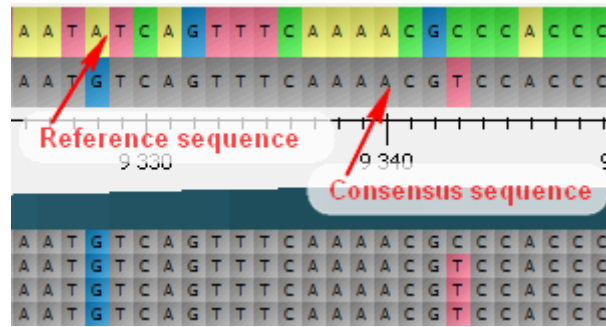
The variations will appear under the *Consensus Sequence*:



To remove the association, select the *Remove track from the view* item in the *Variations Area* context menu.

Consensus Sequence

A consensus sequence can be found in the *Consensus Area* under a reference sequence. It refers to the most common nucleotide at a particular position.

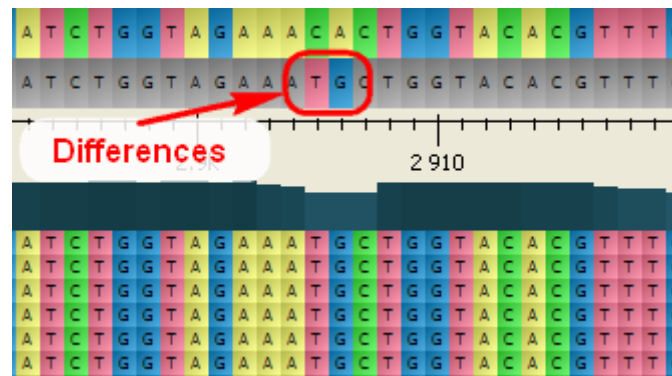


To choose a consensus algorithm select the *Consensus algorithm* item either in the context menu of the *Consensus Area*, in the context menu of the *Reads Area* or on the *Assembly Browser Settings* tab of the *Options Panel*.

The following algorithms are currently available:

- *Default* — shows the most common nucleotide at each position. When there is equal numbers of different nucleotides in a position, the consensus sequence resulting nucleotide is selected randomly from these nucleotides.
- *SAMtools* — uses an algorithm from the SAMtools Text Alignment Viewer to build the consensus sequence. The algorithm takes into account quality values of reads and nucleotides and works with the extended nucleotide alphabet.

To leave only differences between the reference and the consensus sequences highlighted on the consensus sequence, select the *Show difference from reference* item in the context menu of the *Consensus Area* or the *Difference from reference* item on the *Assembly Browser Settings* tab of the *Options Panel*.



To export a *Consensus Sequence*, right-click on it in the *Consensus Area* and select the *Export Export consensus* item in the context menu. For more information about consensus exporting see [Exporting Consensus](#).

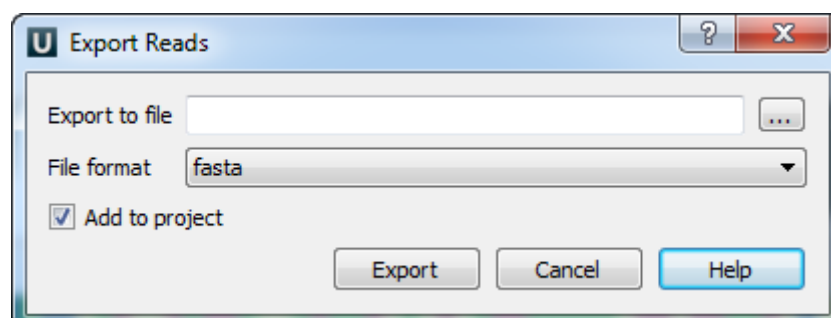
Exporting

- Exporting Reads
- Exporting Visible Reads
- Exporting Coverage
- Exporting Consensus
- Exporting Consensus Variations
- Exporting Assembly as Image
- Exporting Assembly Region

Exporting Reads

To export a read, right-click on it in the *Reads Area* and select the *Export Current read* item in the context menu.

The *Export Reads* dialog appears:



Select a file to export the read to and the file format. The read can be exported either to a FASTA or FASTQ file.

When the parameters are set click the *Export* button.

The read is exported to the file and if the *Add to project* check box has been checked it is added to the current *project* from where you can open it.

Exporting Visible Reads

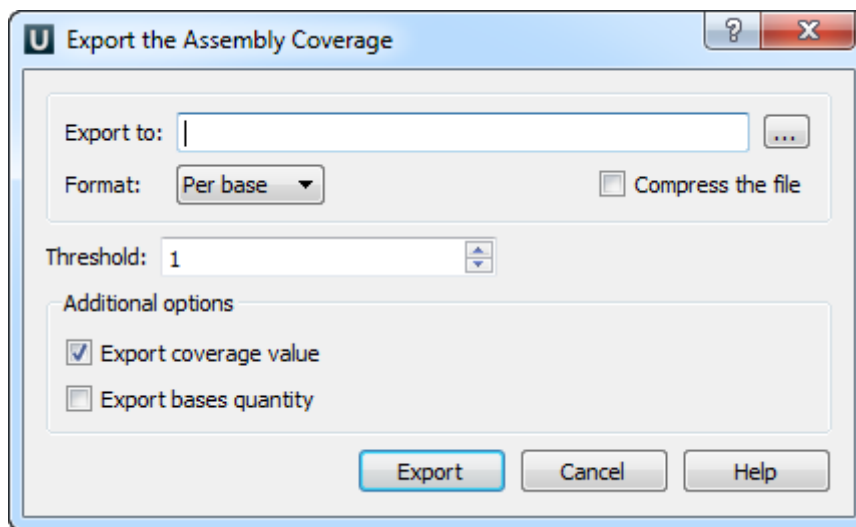
To export all reads visible in the *Reads Area* select the *Export Visible reads* item in the *Reads Area* context menu.

The *Export Reads* dialog appears. The dialog is described in the *Exporting Read* section.

Exporting Coverage

To export a coverage of the assembly, select either the *Export coverage* item in the *Consensus Area* context menu.

The *Export Coverage* dialog appears:



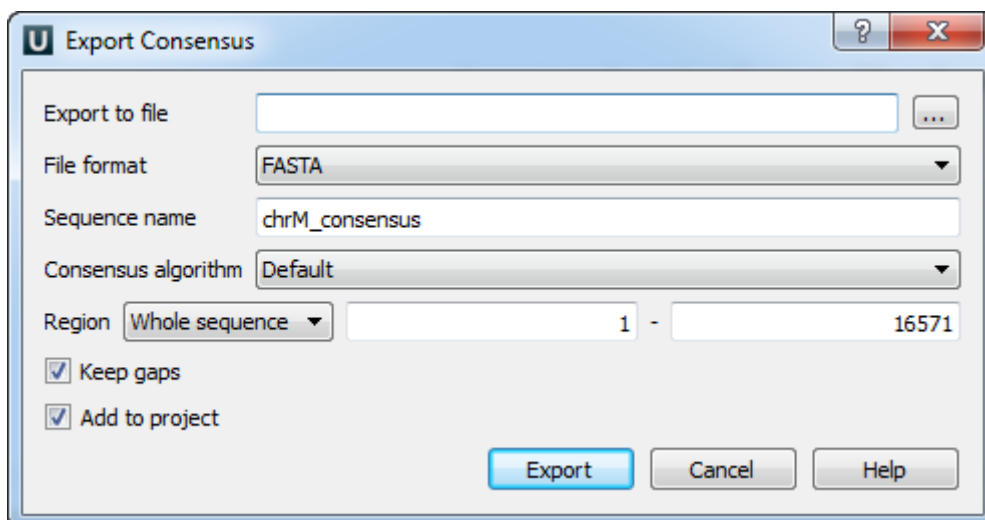
Select a file, threshold and format: *Histogram*, *Per base* or *Bedgraph*. Where *threshold* is the minimum coverage value to export. For *Per base* format the additional options are available: *Export coverage value* or *Export bases quantity* or both of them.

When all the parameters are set click the *Export* button.

Exporting Consensus

To export a consensus sequence of the assembly, select either the *Export consensus* item in the *Consensus Area* context menu or the *Export Consensus* item in the *Reads Area* context menu.

The *Export Consensus* dialog appears:



Select a file and the file format. The consensus can be exported to a FASTA, FASTQ, GFF or GenBank file.

Modify, if required, the exported sequence name and choose the *consensus algorithm*.

The consensus is exported with gaps if the *Keep gaps* check box has been checked.

Also you can select the exporting region. It can be either a *Whole sequence*, a *Visible* region, or a *Custom* region.

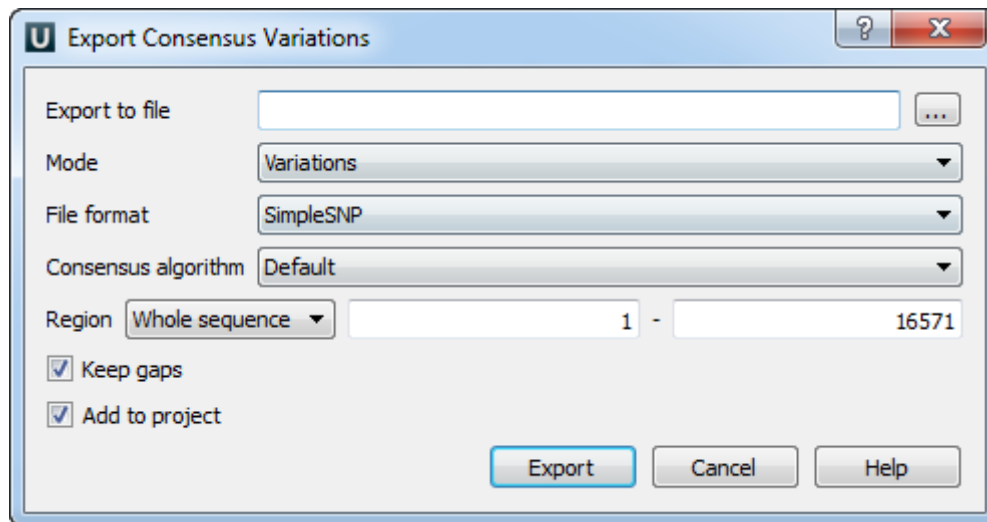
When all the parameters are set click the *Export* button.

The consensus sequence is exported to the file and if the *Add to project* check box has been checked it is added to the current *project* and opened.

Exporting Consensus Variations

To export a consensus sequence variations of the assembly, select the *Export consensus variations* item in the *Consensus Area* context menu.

The following dialog will appear:



Select a file, mode and the file format. The following modes are available: *Variations*, *Similar* and *All*. *Variations* can be exported as to a SimpleSNP or VCFv4 file.

Modify, if required, the *consensus algorithm*.

The consensus is exported with gaps if the *Keep gaps* check box has been checked.

Also you can select the exporting region. It can be either a *Whole sequence*, a *Visible* region, or a *Custom* region.

When all the parameters are set click the *Export* button.

The consensus sequence is exported to the file and if the *Add to project* check box has been checked it is added to the current *project* and opened.



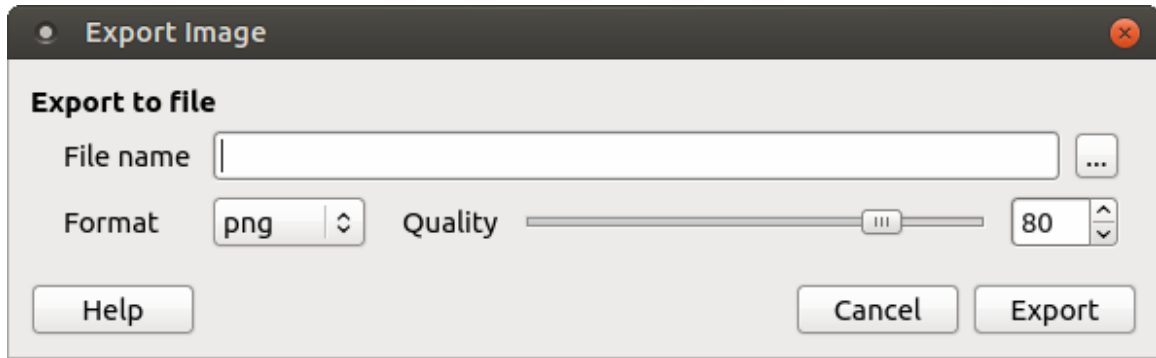
The *Export consensus variations* feature is available when the reference sequence is associated with assembly.

Exporting Assembly as Image

To export the visible part of the assembly as an image, select either the *Actions Export as image* item in the main menu or the following button on the toolbar:



The *Export Image* dialog appears:



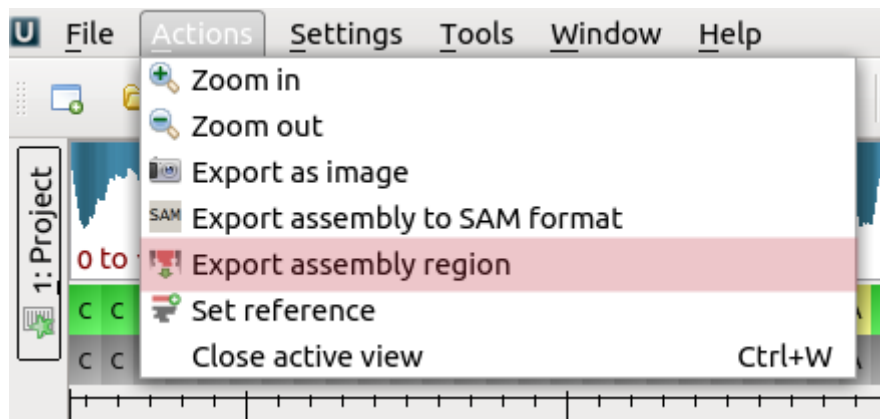
In the dialog you can select the image file name and its format (bmp, jpeg, png, etc.). For some file formats the *Quality* parameter also becomes available.

When the parameters are set click the *OK* button.

Exporting Assembly Region

To export an assembly region use one of the following ways:

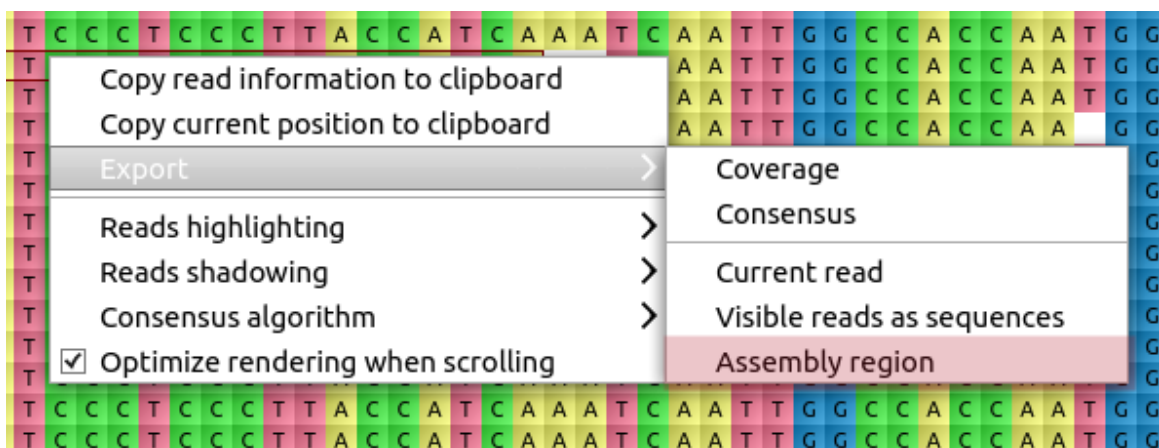
1. Use the *Actions->Export assembly region* main menu item:



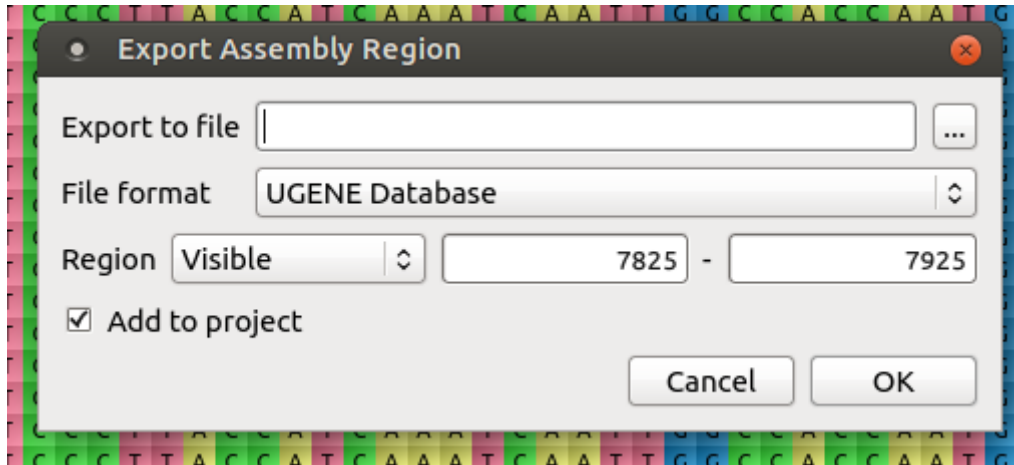
2. Use the corresponding toolbar button:



3. Use the corresponding context menu item:



The *following* dialog will appear:



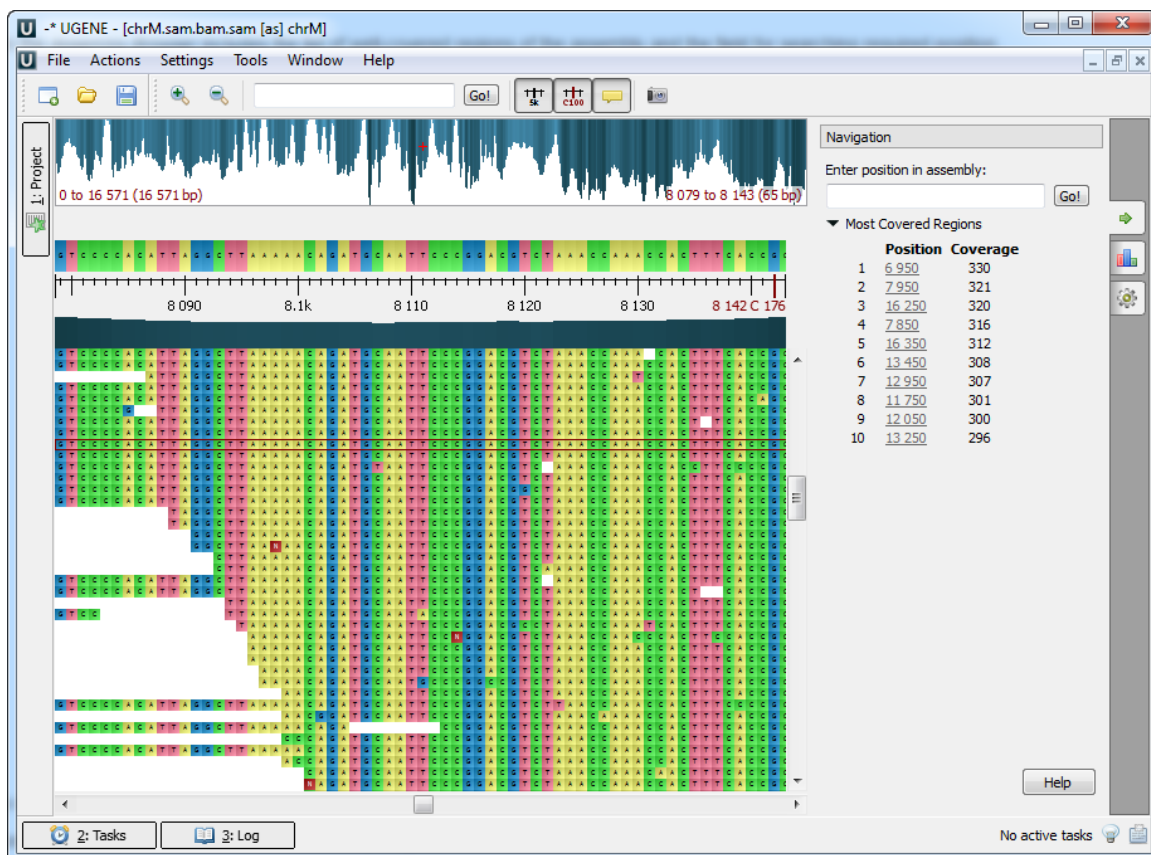
Select a file, region and the file format.

Options Panel in Assembly Browser

- Navigation in Assembly Browser
- Assembly Statistics
- Assembly Browser Settings

Navigation in Assembly Browser

The *Navigation* tab of the *Options Panel* in the *Assembly Browser* includes the list of well-covered regions of the assembly and the field for searching required position.



To learn more about well-covered regions refer to the *Assembly Browser Window* chapter.

To learn more about searching required position refer to the *Go to Position in Assembly* chapter.

Assembly Statistics

The *Assembly Statistics* tab includes the following *Assembly Information*:

- Name — the name of the opened assembly.
- Length — the length of the assembly.

- Reads — the number of reads in the assembly.

Also the tab can include the *Reference Information* if it is available in the assembly file. For example:

- MD5
- Species
- URI



Assembly Browser Settings

The *Assembly Browser Settings* tab includes *Reads Area*, *Consensus Area* and *Ruler* settings.



To learn more about *Reads Area* settings refer to the [Reads Area Settings](#) chapter.

To learn more about *Consensus* see the [Consensus Sequence](#) chapter.

To learn more about *Ruler* see the [Browsing and Zooming Assembly](#) chapter.

Assembly Browser Hotkeys

- [Assembly Overview Hotkeys](#)
- [Reads Area Hotkeys](#)

Assembly Overview Hotkeys

The following hotkeys are available for the *Assembly Overview*:

Hotkey	Action
Shift + move mouse	Zoom the Assembly Overview to selection
Ctrl + wheel	Zoom the Assembly Overview
Alt + click	Zoom the Assembly Overview in 100x
wheel + move mouse	Move the Assembly Overview

Reads Area Hotkeys

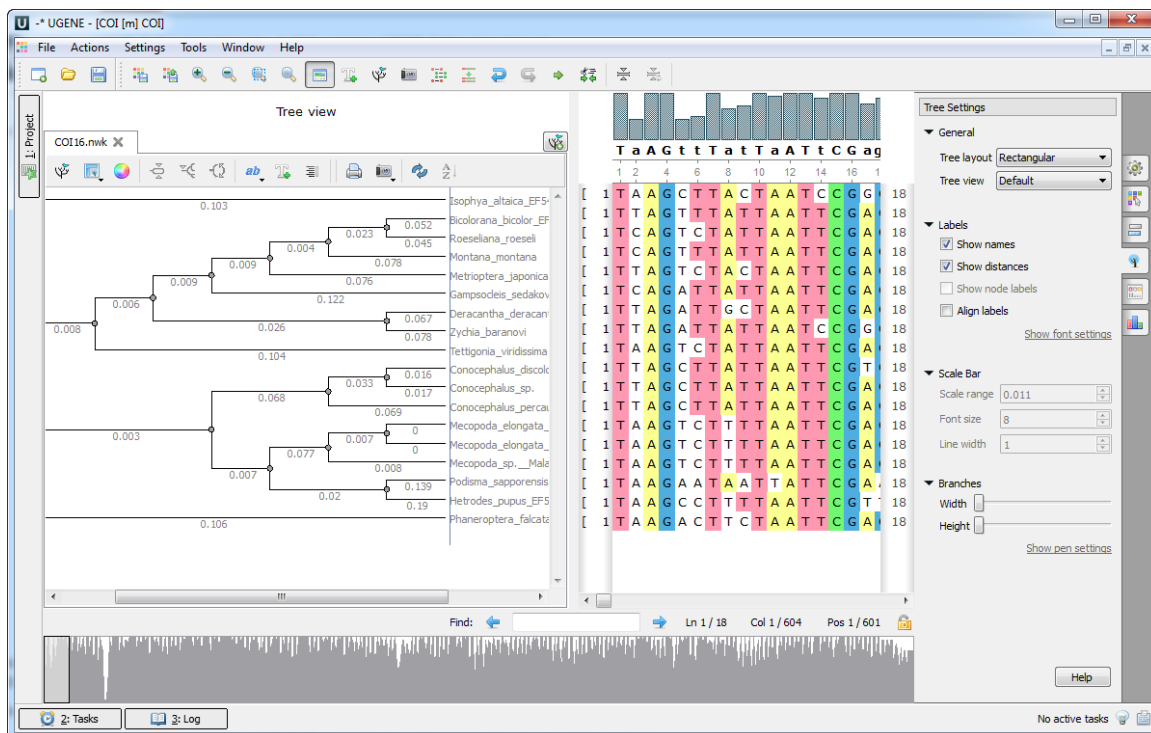
The following hotkeys are available for the *Reads Area*:

Hotkey	Action
wheel	Zoom the Reads Area
double-click	Zoom in the Reads Area
+ / -	Zoom in / zoom out the Reads Area
click + move mouse	Move the Reads Area
arrow	Move one base in the corresponding direction in the Reads Area

Ctrl + arrow	Move one page in the corresponding direction in the Reads Area
Page Up / Page Down	Move one page up / down in the Reads Area
Home / End	Move to the beginning / end of the assembly in the Reads Area
Ctrl+G	Focus to the <i>Go to position</i> field on the toolbar

Phylogenetic Tree Viewer

The *Phylogenetic Tree Viewer* is intended to display a phylogenetic tree built from an alignment or loaded from a file (e.g. a Newick file).



To load a tree from a file follow the instruction described in the [Opening Document](#) paragraph or use the *Tree settings* tab of the *Options Panel*. For example, you may open the \$UGENE\data\samples\Newick\COI.nwk sample file provided within UGENE package.

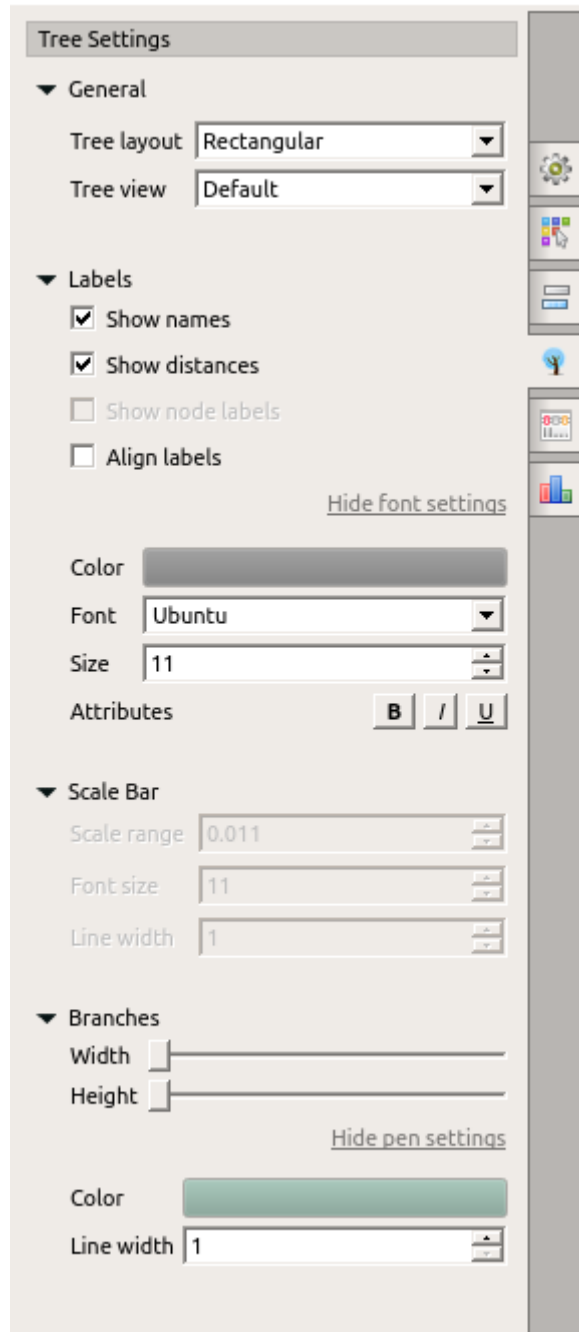
To build a tree from a multiple sequence alignment see the [Building Phylogenetic Tree](#) paragraph.

To learn what you can do with a tree using UGENE Phylogenetic Tree Viewer read the documentation below.

- **Tree Settings**
 - Selecting Tree Layout and View
 - Modifying Labels Appearance
 - Showing and Hiding Labels
 - Aligning Labels
 - Changing Labels Formatting
 - Adjusting Branch Settings
- **Zooming Tree**
- **Working with Clade**
 - Selecting Clade
 - Collapsing and Expanding Branches
 - Swapping Siblings
 - Zooming Clade
 - Adjusting Clade Settings
 - Changing Root
- **Exporting Tree Image**
- **Printing Tree**

Tree Settings

To adjust a tree settings select either the *Tree Settings* toolbar button or the *Tree settings* tab of the *Options Panel*. The *Tree settings* tab:



Detailed information about tree setting see below:

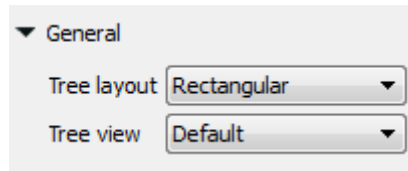
- [Selecting Tree Layout and View](#)
- [Modifying Labels Appearance](#)
 - [Showing and Hiding Labels](#)
 - [Aligning Labels](#)
 - [Changing Labels Formatting](#)
- [Adjusting Branch Settings](#)

Selecting Tree Layout and View

You can select one of the following tree layouts:

- *Rectangular*
- *Circular*
- *Unrooted*

To do it press the *Layout* toolbar button and check the required item in the appeared menu or select it in the *Tree settings Options Panel* tab:



See the example of the *Circular* layout:



Also you can select one of the following tree view:

- *Default*
- *Phylogram*
- *Cladogram*

Modifying Labels Appearance

From this paragraph you can learn how to show/hide taxon and distance labels, align them and change their formatting (font, color, etc.).

- [Showing and Hiding Labels](#)
- [Aligning Labels](#)
- [Changing Labels Formatting](#)

Showing and Hiding Labels

When you open a tree all available labels are shown by default.

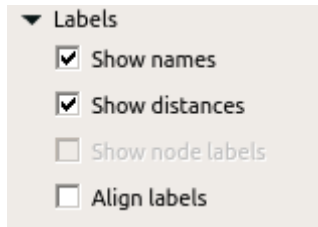
To hide the taxon (sequence name) labels select the *Show labels* toolbar button or in the *Tree settings Options Panel* tab uncheck the *Show names* item.

To hide the distance labels uncheck the *Show distances* item.

To show/hide node labels (if it is available) use the *Show node labels* item.

To show the labels again check an appropriate item.

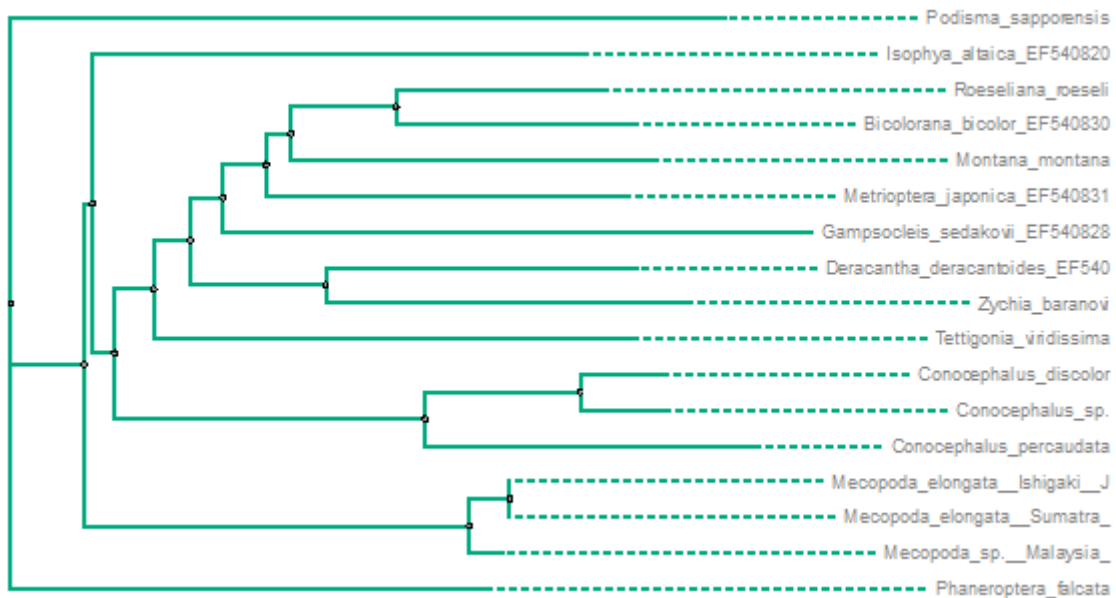
Labels settings in the *Options Panel*:



Aligning Labels

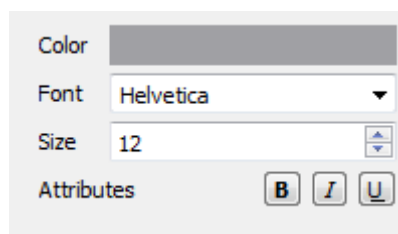
To align a tree labels press the *Align labels* toolbar button or in the *Tree settings Options Panel* tab check the *Align label* item.

See the example of aligning labels below:



Changing Labels Formatting

To change formatting of a tree labels select the *Labels Formatting* toolbar button or the *Tree settings Options Panel* tab:



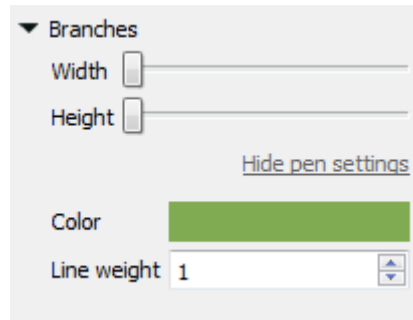
Here you can select color, font, size and attributes (bold, italic, etc.) of the labels.

Note that when *a clade has been selected* the labels formatting settings are applied to the clade only.

Adjusting Branch Settings

To adjust branch settings select the *Branch Settings* toolbar button, the *Branch Settings* context menu item or the *Tree settings Options Panel* tab.

The following settings are available:



Here you can select the color and the line width of the tree branches.

Note that when *a clade has been selected* the branch settings are applied to the clade only.

Zooming Tree

To change the size of a tree use the *Zoom In* and *Zoom Out* toolbar button. You can use the *Restore Zooming* toolbar button to set the default size.

Or use the corresponding items in the *Actions* main menu.

See also: [Zooming Clade](#).

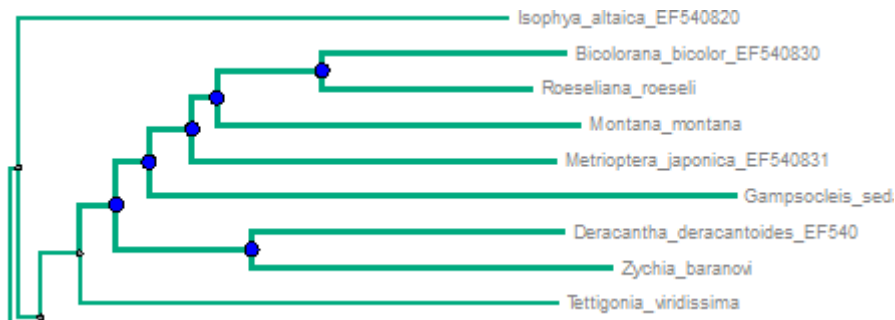
Working with Clade

This paragraph describes how to select a clade and modify it's appearance.

- [Selecting Clade](#)
- [Collapsing and Expanding Branches](#)
- [Swapping Siblings](#)
- [Zooming Clade](#)
- [Adjusting Clade Settings](#)
- [Changing Root](#)

Selecting Clade

To select a clade click on it's root node:

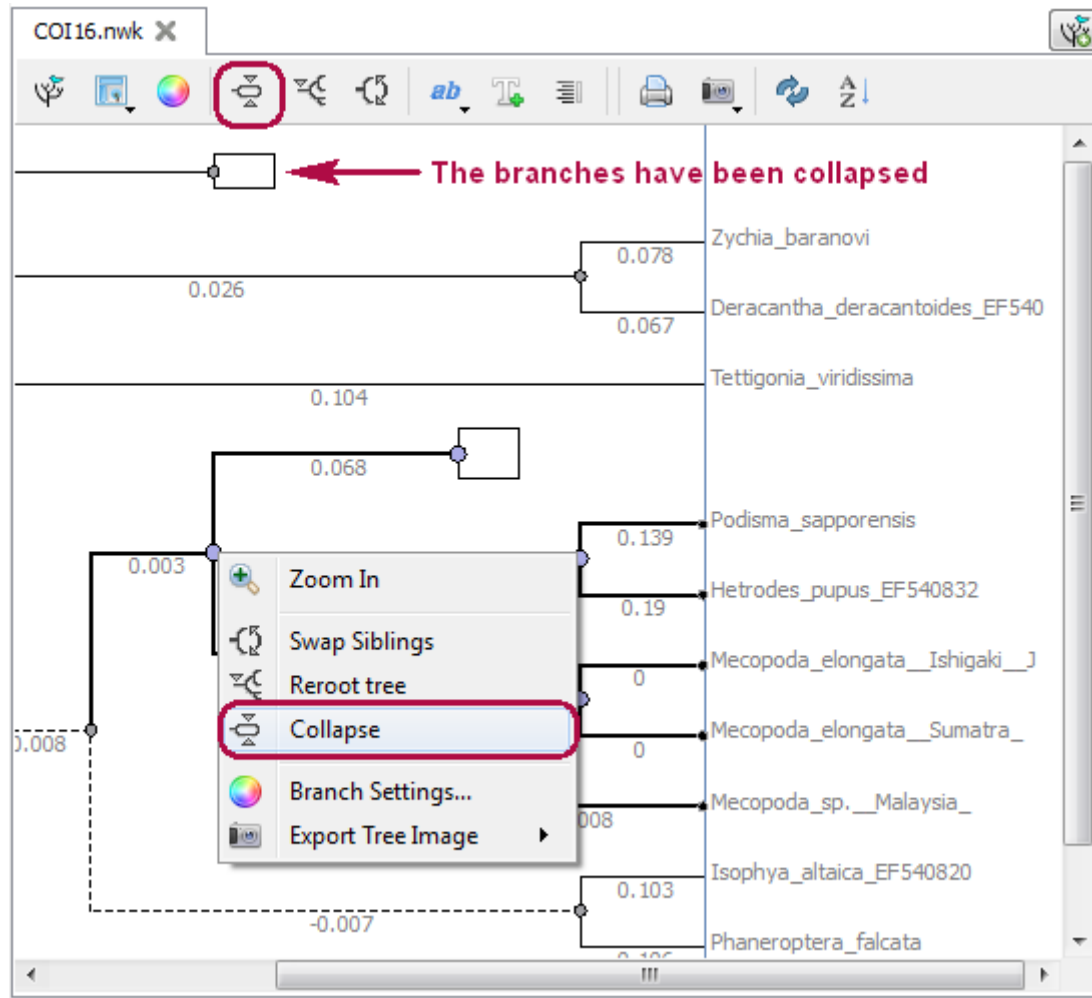


You can see that the corresponding branches are highlighted.

To select several clades at the same time hold the Shift key and click on the root nodes of the clades.

Collapsing and Expanding Branches

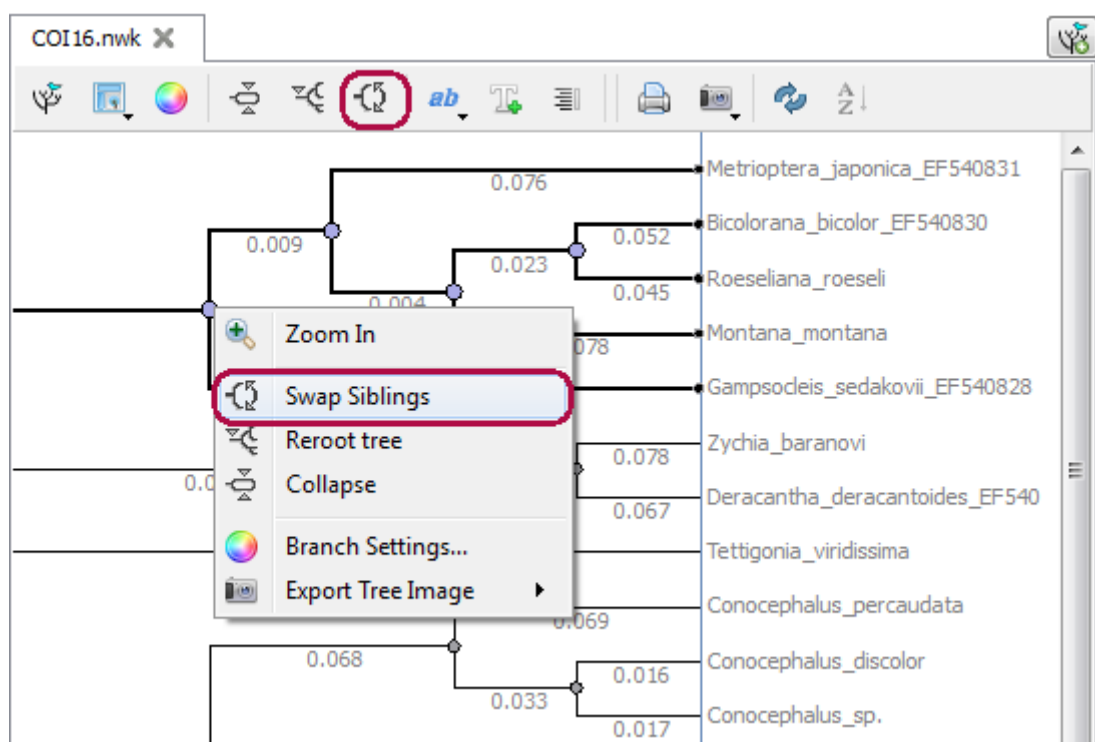
You can hide branches of a clade by selecting the *Collapse* item in the context menu of the clade's root node or use the *Collapse* button on the tree toolbar:



To show the collapsed clade select the *Expand* item in the node's context menu.

Swapping Siblings

To rearrange two branches of an internal node, select the *Swap Siblings* item in the node context menu or click the *Swap Siblings* button on the tree toolbar, while the node is selected:



Zooming Clade

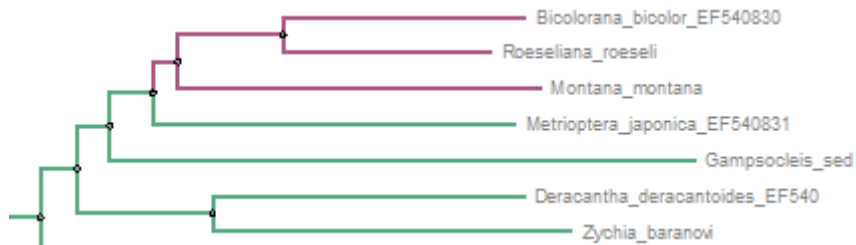
Additionally to other *zooming options* you can use the *Zoom In* item in the context menu of the root node of a clade.

Adjusting Clade Settings

When a clade is selected the *branch* and the *labels formatting* settings are applied to the clade only.

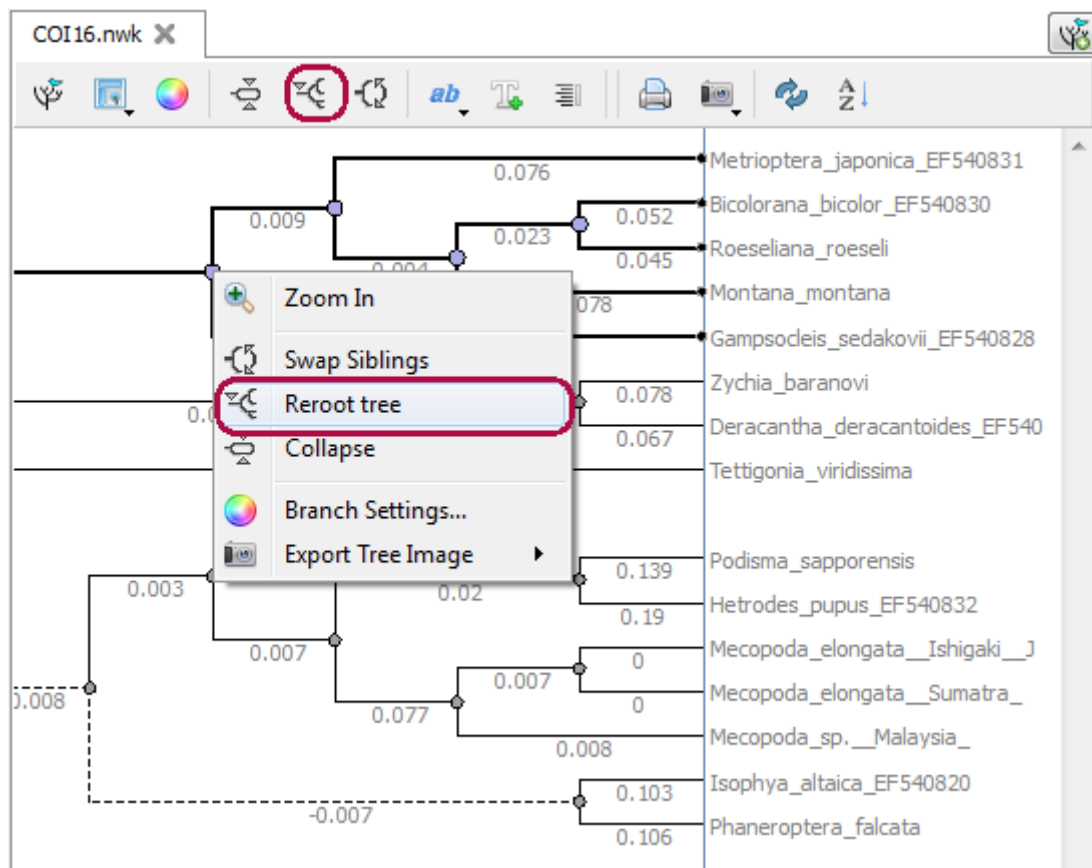
Note that the settings are not applied to the *collapsed* branches (if any).

See an example of changing branch settings for a clade:



Changing Root

To change root of a tree select the root and call the *Reroot tree* context menu item or use the *Reroot tree* button on the tree toolbar:



Exporting Tree Image

A tree image can be exported to a raster format (.png, .jpg, .bmp, etc.) or to a vector format (.svg).

Select either the *Export Tree Image* toolbar button or the *Actions Export Tree Image* item in the main menu.

In the submenu appeared select the *Screen Capture* item to save the tree image to a raster format. The standard *Save As* dialog will appear where you can select the file name and format.

To export a tree image to a vector format select the *As SVG* item in the *Export Tree Image* submenu.

Printing Tree

To print a tree select either the *Print Tree* toolbar button or the *Actions Print Tree* item in the main menu.

The standard print dialog will appear where you can select a printer to use and specify other settings.

Extensions

- Workflow Designer
- DNA Annotator
- DNA Flexibility
 - Configuring Dialog Settings
 - Result Annotations
- DNA Statistics
- DNA Generator
- ORF Marker
- Remote BLAST
 - Exporting BLAST Results to Alignment
 - Fetching Sequences from Remote Database
- BLAST and BLAST+
 - Creating Database
 - Making Request to Database
 - Fetching Sequences from Local BLAST Database
- Repeat Finder
 - Repeats Finding
 - Tandem Repeats Finding
 - Tandem Repeats Search Result
- Restriction Analysis
 - Selecting Restriction Enzymes
 - Using Custom File with Enzymes
 - Filtering by Number of Hits
 - Excluding Region
 - Circular Molecule
 - Results
- Molecular Cloning in silico
 - Digesting into Fragments
 - Creating Fragment
 - Constructing Molecule
 - Available Fragments
 - Fragments of the New Molecule
 - Changing Fragments Order in the New Molecule
 - Removing Fragment from the New Molecule
 - Editing Fragment Overhangs
 - Reverse Complement a Fragment
 - Other Constuction Options
 - Output
 - Creating PCR Product
- In Silico PCR
 - Primers Details
 - Primer Library
- Secondary Structure Prediction
- SITECON
 - SITECON Searching Transcription Factors Binding Sites
 - Types of SITECON Models
 - Eukaryotic
 - Prokaryotic
 - Building SITECON Model
- Smith-Waterman Search
- HMM2
 - Building HMM2 Model
 - Calibrating HMM2 Model
 - Searching Sequence Using HMM2 Profile
- HMM3
 - Building HMM Model
 - Searching Sequence Using HMM Profile
 - Searching Sequence Against Sequence Database
- uMUSCLE
 - MUSCLE Aligning
 - Aligning Profile to Profile with MUSCLE
 - Aligning Sequences to Profile with MUSCLE
- ClustalW
- MAFFT
- T-Coffee
- Bowtie
 - Bowtie Aligning Short Reads
 - Building Index for Bowtie
- Bowtie 2
 - Bowtie 2 Aligning Short Reads
 - Building Index for Bowtie 2
- BWA
 - Aligning Short Reads with BWA
 - Building Index for BWA
- BWA-SW
 - Aligning Short Reads with BWA-SW

- Building Index for BWA-SW
- BWA-MEM
 - Aligning Short Reads with BWA-MEM
 - Building Index for BWA-MEM
- UGENE Genome Aligner
 - Aligning Short Reads with UGENE Genome Aligner
 - Building Index for UGENE Genome Aligner
 - Converting UGENE Assembly Database to SAM Format
- CAP3
- SPAdes
- Weight Matrix
 - Searching JASPAR Database
 - Building New Matrix
- Primer3
 - RTPCR Primer Design
- Spliced Alignment mRNA and cDNA
- External Tools Plugin
 - Configuring External Tool
- Query Designer
- Plasmid Auto Annotation
- ClustalO
- Kalign Aligning

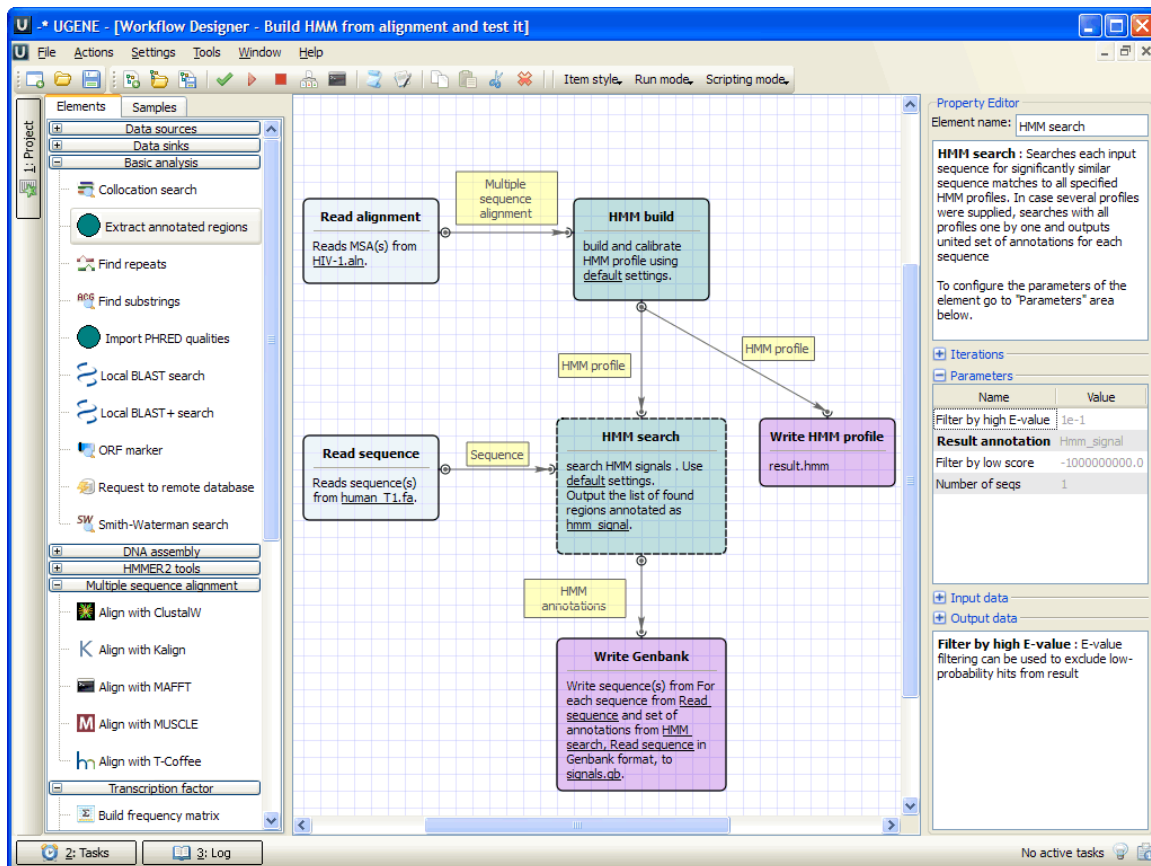
Workflow Designer

The *Workflow Designer* allows a molecular biologist to create and run complex computational workflow schemas even if he or she is not familiar with any programming language.

The workflow schemas comprise reproducible, reusable and self-documented research routines, with a simple and unambiguous visual representation suitable for publications.

The workflow schemas can be run both locally and remotely, either using graphical interface or launched from the command line.

The elements that a schema consists of corresponds to the bulk of algorithms integrated into UGENE. Additionally you can create custom workflow elements.



To learn more about the *Workflow Designer* read the Workflow Designer Manual (follow the link on the UGENE documentation page).

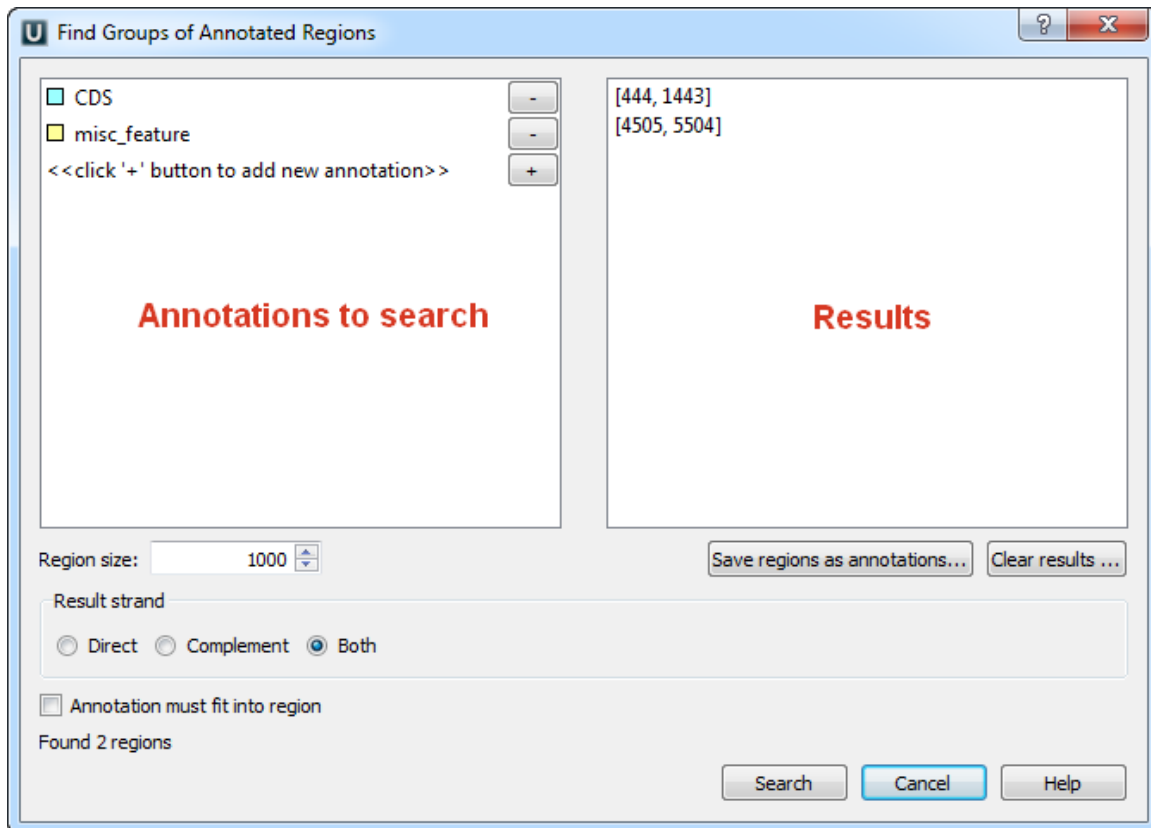
DNA Annotator

The *DNA Annotator* plugin provides an algorithm to search for sequence regions that contain a predefined set of annotations.

Usage example:

Open the *Sequence View* for a sequence that has annotations. A good candidate here could be any file in Genbank format with a rich set of annotations.

Select the *Analyze Find annotated regions* item in the context menu. The dialog will appear:



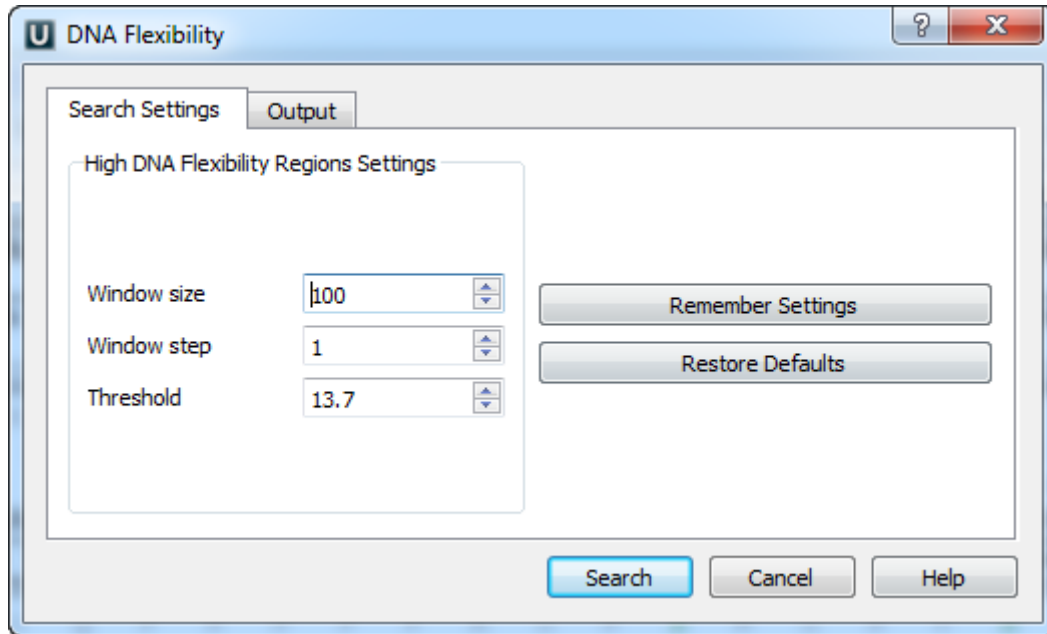
Using this dialog you can search for DNA sequence regions that contain every annotation from the list on the left side. The found regions are displayed on the right side of the dialog.

Use the *Save regions as annotations* button to store the regions as new annotations to the sequence.

DNA Flexibility

To search for regions of high DNA helix flexibility in a DNA sequence, open the sequence in the *Sequence View* and select the *Analyze Find high DNA flexibility regions* item in the context menu. Note that only standard DNA alphabet is supported, i.e. the sequence should consist of characters A, C, G, T and N.

The following dialog appears:



The calculation is made for overlapping windows along a given sequence. If there are two or more consecutive windows with an average flexibility threshold (in each window) greater than the specified *Threshold* parameter, such area is marked by an *annotation*.

The average threshold in a window is calculated by the following formula:

$$(\text{average window threshold}) = (\text{sum of flexibility angles in the window}) / (\text{the window size} - 1)$$

The following flexibility angles are used during the calculation:

Dinucleotide	Angle	Dinucleotide	Angle
AA	7.6	CA	14.6
AC	10.9	CC	7.2
AG	8.8	CG	11.1
AT	12.5	CT	8.8
GA	8.2	TA	25
GC	8.9	TC	8.2
GG	7.2	TG	14.6
GT	10.9	TT	7.6

A minimum value is used when N characters is present in a dinucleotide:

- **CN, NC, GN, NG, NN**: 7.2
- **AN, NA, TN, NT**: 7.6

- [Configuring Dialog Settings](#)
- [Result Annotations](#)

Configuring Dialog Settings

In the dialog you can setup the corresponding parameters:

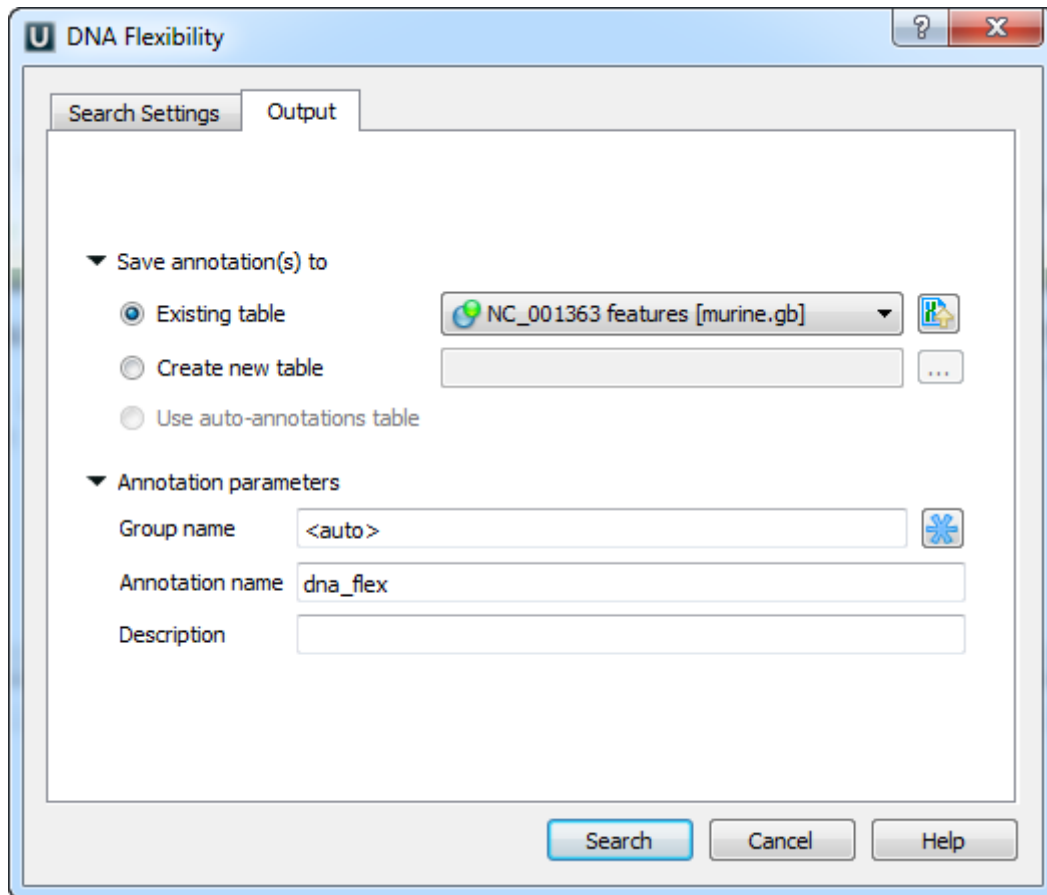
Window size — the number of bases in a window. The window size should be greater than 2. The default value is 100 bp.

Window step — the number of bases used to shift a window. The *Window step* should be a positive integer. The default value is 1 bp.

Threshold — the threshold value of the twist angle (see above). The default value is 13.7.

You can remember the input values or restore the default values using the *Remember Setting* and the *Restore Defaults* buttons.

The annotations names and other parameters can be changed on the *Output* tab of the dialog:



Once the *Search* button has been pressed, the *annotations* for the regions of the high DNA flexibility are created.

Result Annotations

Each annotation has the following qualifiers:

- *area_average_threshold* — average window threshold in the area (i.e. *total_threshold* / *windows_number*)
- *total_threshold* — sum of all window thresholds in the area
- *windows_number* — number of windows in the area

■ dna_flex	144..156
... area_average_threshold	14.672
... total_threshold	58.689
... windows_number	4



Using the *DNA Graphs Package* you can see the flexibility graph of a DNA sequence.

DNA Statistics

The *DNA Statistics* plugin provides exportable statistic reports.

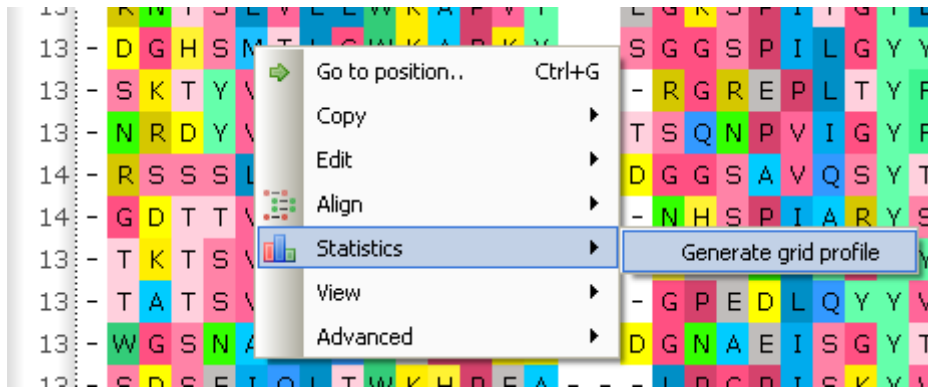
In the current UGENE version, the *DNA Statistics* plugin provides only *Alignment Grid Profile* report. The *Alignment Grid Profile* shows positional amino acid or nucleotide counts highlighted according to the frequency of symbols in a row.

The original idea of the MSA Grid Profile is described in the following paper:

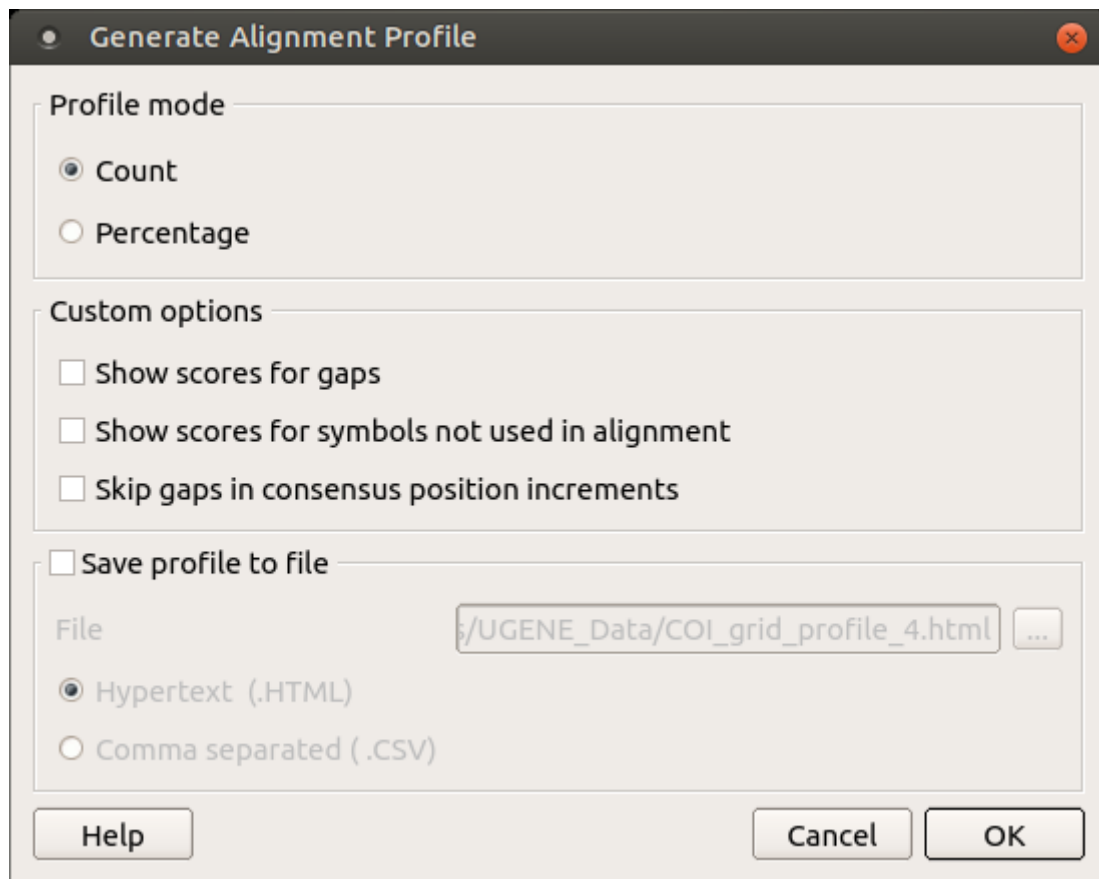
“Alberto Roca, Albert Almada and Aaron C Abajian: ProfileGrids as a new visual representation of large multiple sequence alignments: a case study of the RecA protein family, BMC Bioinformatics 2008, 9:554”

Usage example:

Open a sequence alignment in the *Alignment Editor* and use the *Statistics Generate grid profile* context menu item.



The dialog will appear:



Here is a brief description of the options that can be set in the dialog:

Profile mode: Counts/Percents — select the *Percents* to have scores shown as percents in the report.

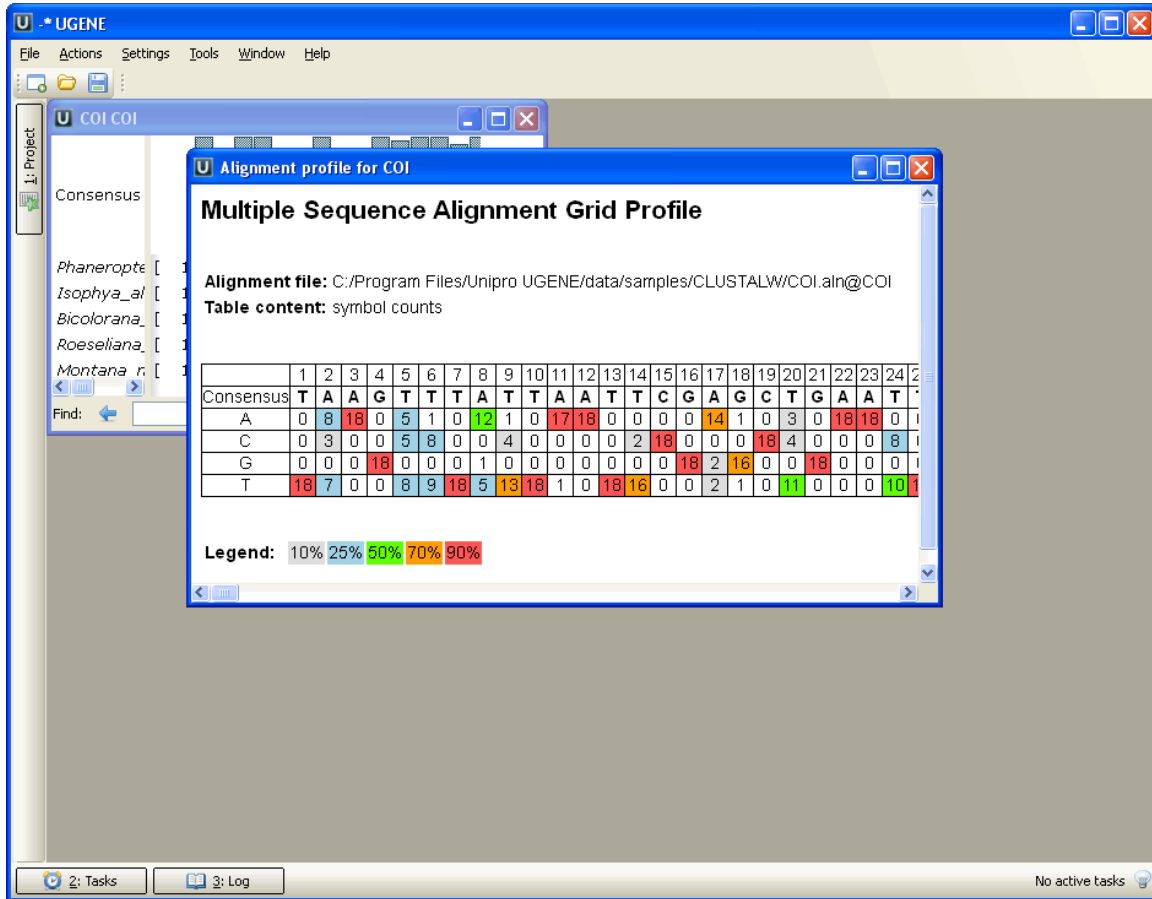
Show scores for gaps — check this item if you want gap characters ('—') statistics to be shown in the report.

Show scores for symbols not used in alignment — if a symbol is not used in the alignment at all it won't be shown in the report. Check this item to make all symbols of alignment alphabet reported.

Skip gaps in consensus position increments — consensus ruler configuration. If checked the gaps in consensus will not lead to ruler increments.

Save profile to file — allows saving profile to a file in the HTML or CSV format. The CSV format is convenient for further processing in worksheets editors like Excel.

The resulting profile in the HTML mode:



DNA Generator

DNA sequence generator is a tool that generates a random DNA sequence with specified nucleotide content. To generate a random DNA sequence select the *Tools->Generate* sequence item in the main menu. The dialog will appear:

The following parameters are available:

Length - length of the resulted sequence(s) (using '1000' bp by default).

Window size - the size of a window where set content (using '1000' by default).

Number of sequences - the number of sequences to generate (using '1' by default).

Initialize random generator manually - value to initialize the random generator.

Reference - path to the reference file (could be a sequence or an alignment).

Base content - set the base content percents manually.

GC Skew - set the GC skew of the resulted sequence.

Output file - output file.

Format - output file format (using 'fasta' by default).

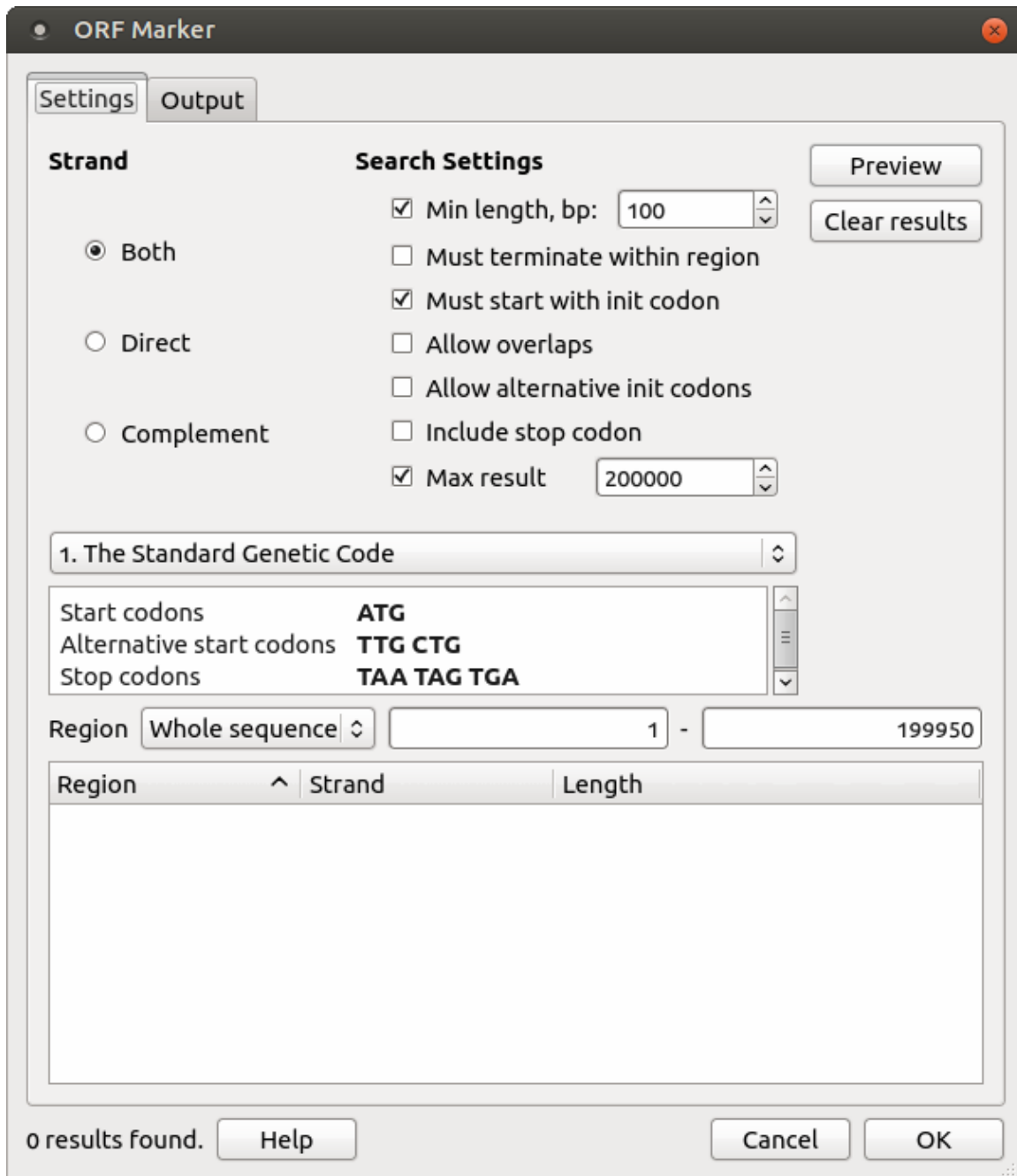
Add to project - adds the generated sequence(s) to project.

Once the *Search* button has been pressed, the sequence(s) are created.

ORF Marker

From this chapter you can learn how to search for Open Reading Frames (ORF) in a DNA sequence. The ORFs found are stored as automatic annotations. This means that if the automatic annotations highlighting has been enabled then ORFs are searched and highlighted for each sequence opened. Refer [Automatic Annotations Highlighting](#) to learn more.

To open the *ORF Marker* dialog, select the *Analyze Find ORFs* item in the context menu.



The following search settings are available:

Min length — ORFs with length lower than *Min length* value will not be found.

Must terminate within region — this option ignores boundary ORFs located beyond the search region.

Must start with init codon — item switches the ORF Marker algorithm to the mode when any non-stop amino acid code is interpreted as region start position.

Allow overlaps — alternative (downstream) initiators, when another start codon is located within a longer ORF, i.e. all possible ORFs will be found, not only the longest ones.

Allow alternative init codon — option includes ORFs starting with alternative initiation codons, accordingly to the current translation table.

Include stop codon — includes stop codons into resulting annotations.

The other available parameters are:

DNA-to-Amino translation table defines the way start, alternative start and stop codons are encoded.

Strand — where to search the ORFs: in the direct strand, in the complement strand or in both strands.

Preview — allow to preview the regions, strands and lengths of the found ORFs.

Clear results — becomes available when some results have been found, clears these results.

To set the saving parameters go to the *Output* tab of the dialog.

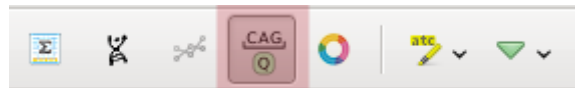
Here you can modify *the annotations saving* parameters (*Group name*, *Description* and a file to save the annotation to).

Results:

When the search parameters has been selected and the *OK* button has been pressed in the dialog, the *auto-annotating* becomes enabled. In the *Annotations editor* the ORFs annotations can be found in the *Auto-annotations/orf* group.

After the search has been finished you can browse the results, sort them by length, strand or start position and save as annotations to the original sequence in the Genbank format.

For more information about codons use the codon table. To show or hide the table use *Ctrl+T* shortcut or click the *Show codon table* toolbar button menu:



The codon table will appear:

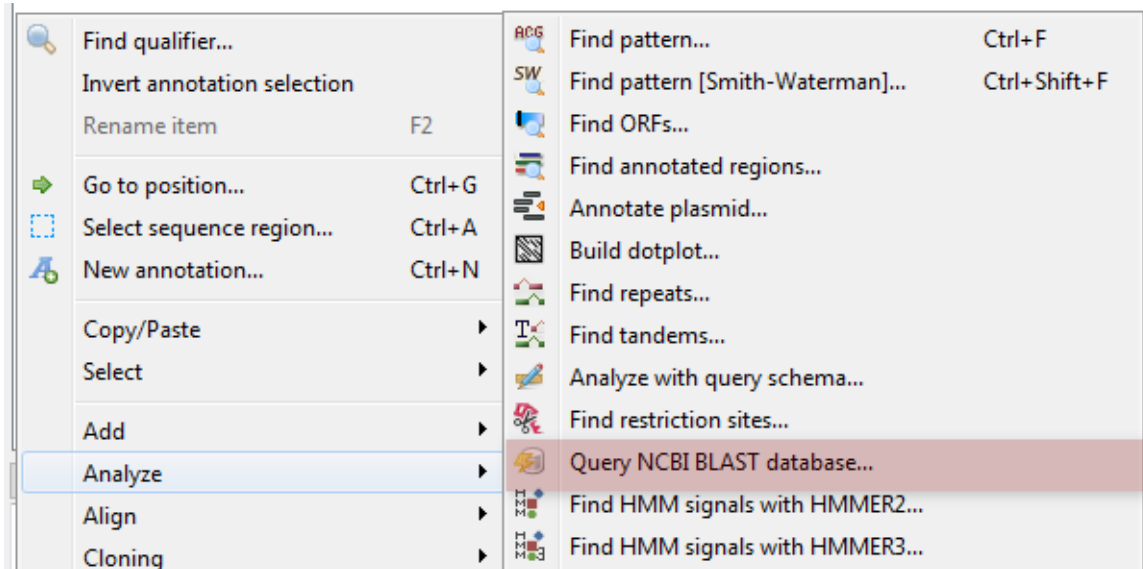
1st base		2nd base					
		U		C		A	
U	UUU	Phenylalanine (Phe, F)		UCU	Serine (Ser, S)	UAU	Tyrosine (Tyr, Y)
	UUC			UCC		UAC	
	UUA			UCA		UAA	Stop codon (*)
	UUG			UCG		UAG	
C	CUU	Leucine (Leu, L)		CCU	Proline (Pro, P)	CAU	Histidine (His, H)
	CUC			CCC		CAC	
	CUA			CCA		CAA	Glutamine (Gln, Q)
	CUG			CCG		CAG	
A	AUU	Isoleucine (Ile, I)		ACU	Threonine (Thr, T)	AAU	Asparagine (Asn, N)
	AUC			ACC		AAC	
	AUA			ACA		AAA	Lysine (Lys, K)
	AUG	Methionine (Met, M)		ACG		AAG	
G	GUU	Valine (Val, V)		GCU	Alanine (Ala, A)	GAU	Aspartic acid (Asp, D)
	GUC			GCC		GAC	
	GUA			GCA		GAA	Glutamic acid (Glu, E)
	GUG			GCG		GAG	

Clicking on a codon name redirects you to Wikipedia to give you a brief description of the corresponding amino acid. Cells of the table are colored according to classes of amino acids.

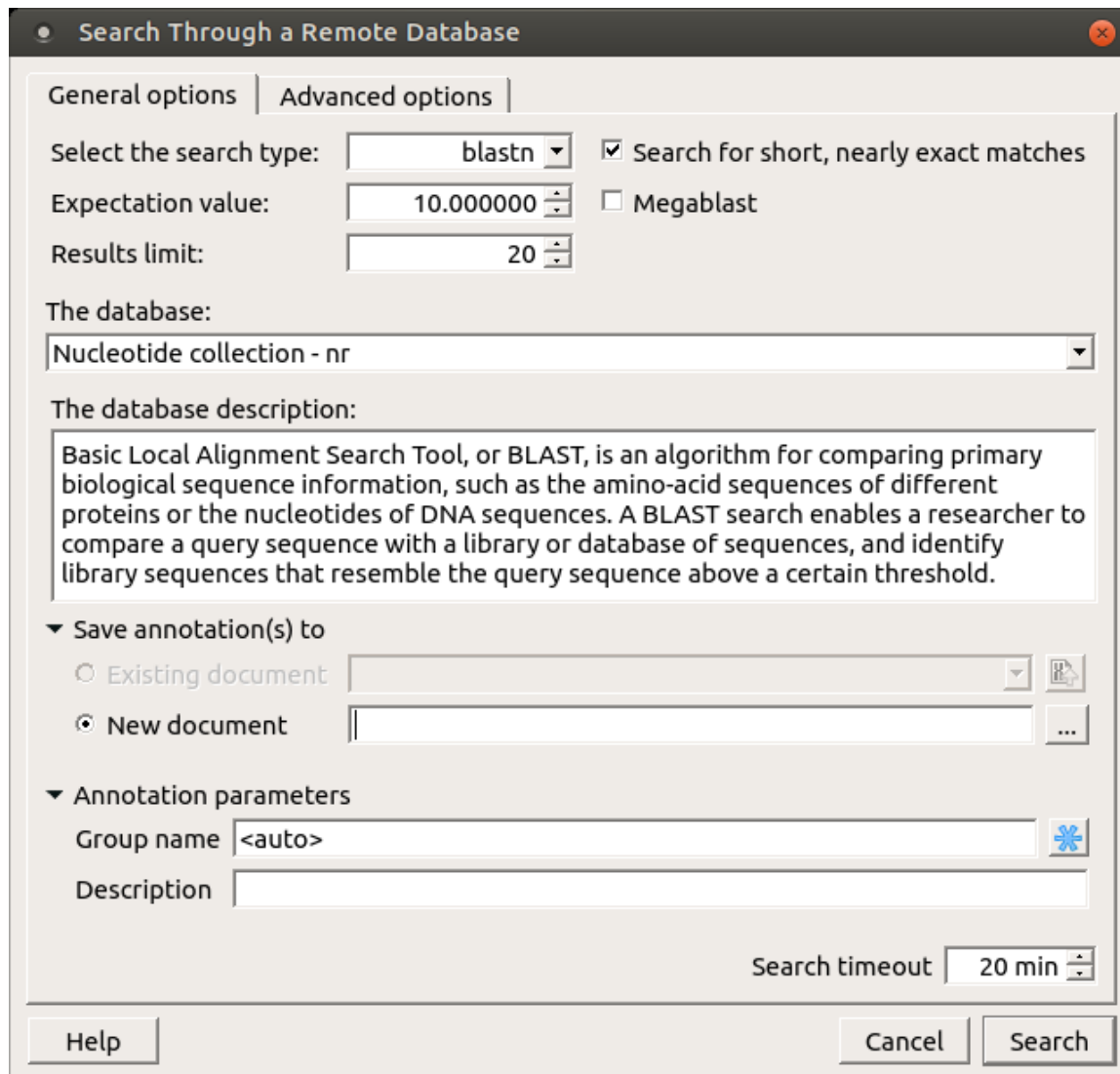
Remote BLAST

The *Remote BLAST* plugin provides a capability to annotate sequences with information stored in the *NCBI BLAST* remote database.

To perform a remote database search open a *Sequence View*, select a sequence region to analyze and click the *Analyze Query NCBI BLAST database* context menu item. If a region is not selected the whole sequence will be analyzed.



The following dialog will appear where you can choose the search options:



General options are:

Select the search type — in the remote databases the *blastn* search is used for nucleotide sequences, *blastp* and *cdd* searches are used for amino sequences.

UGENE also provides a way to use *blastp* and *cdd* searches for nucleotide sequences. This is achieved by translating the nucleotide sequence into the amino sequences.

When a sequence is translated the translation table from the active *Sequence View* is used. Finally, all 6 translations are used to query the remote database with the selected *blastp* or *cdd* search.

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.

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

Database — the target database.

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.

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

Search timeout — the remote task terminated if the timeout is reached.

✔ There is a little difference in default values of parameters between [NCBI Nucleotide BLAST](#) web interface and UGENE:

- The web interface uses the *megablast* option by default: the search is fast, but only highly similar sequences are found.
- UGENE ignores the option by default: the search may take more time, but all somewhat similar sequences are found.

Check the *Megablast* option, if you want exactly the same results to be found in UGENE as you had in the NCBI web interface.

Also there is *Advanced options* tab:

Search Through a Remote Database

General options | **Advanced options**

Word size: 11 | Gap costs: 5 2

Match scores: 2-3

Entrez query:

Filters

Low complexity filter

Human repeats filter

Masks

Mask for lookup table only

Mask lower case letters

Filter results

Filter by

accession

def (filter by definition of annotations)

id

Select result by

EValue

Score

Help | Cancel | Search

The view of the *Advanced options* tab depends on the selected search. For the *blastn* search it looks like on the picture above.

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.

Entrez query — a BLAST search can be limited to the result of an *Entrez query* against the database chosen. This restricts the search to a subset of entries from that database fitting the requirement of the *Entrez query*. Examples are given below:

protease NOT hiv1[organism] — this will limit a BLAST search to all proteases, except those in HIV 1.

1000:2000[slen] — this limits the search to entries with lengths between 1000 to 2000 bases for nucleotide entries, or 1000 to 2000 residues for protein entries.

Mus musculus[organism] AND biomol_mrna[properties] — this limits the search to mouse mRNA entries in the database. For common organisms, one can also select from the pulldown menu.

10000:100000[mlwt] — this is yet another example usage, which limits the search to protein sequences with calculated molecular weight between 10 kD to 100 kD.

src specimen voucher[properties] — this limits the search to entries that are annotated with a */specimen_voucher* qualifier on the source feature.

all[filter] NOT envirommental sample[filter] NOT metagenomes[orgn] — this excludes sequences from metagenome studies and uncultured sequences from anonymous environmental sample studies.

For help in constructing *Entrez queries* see the [Entrez Help document](#).

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

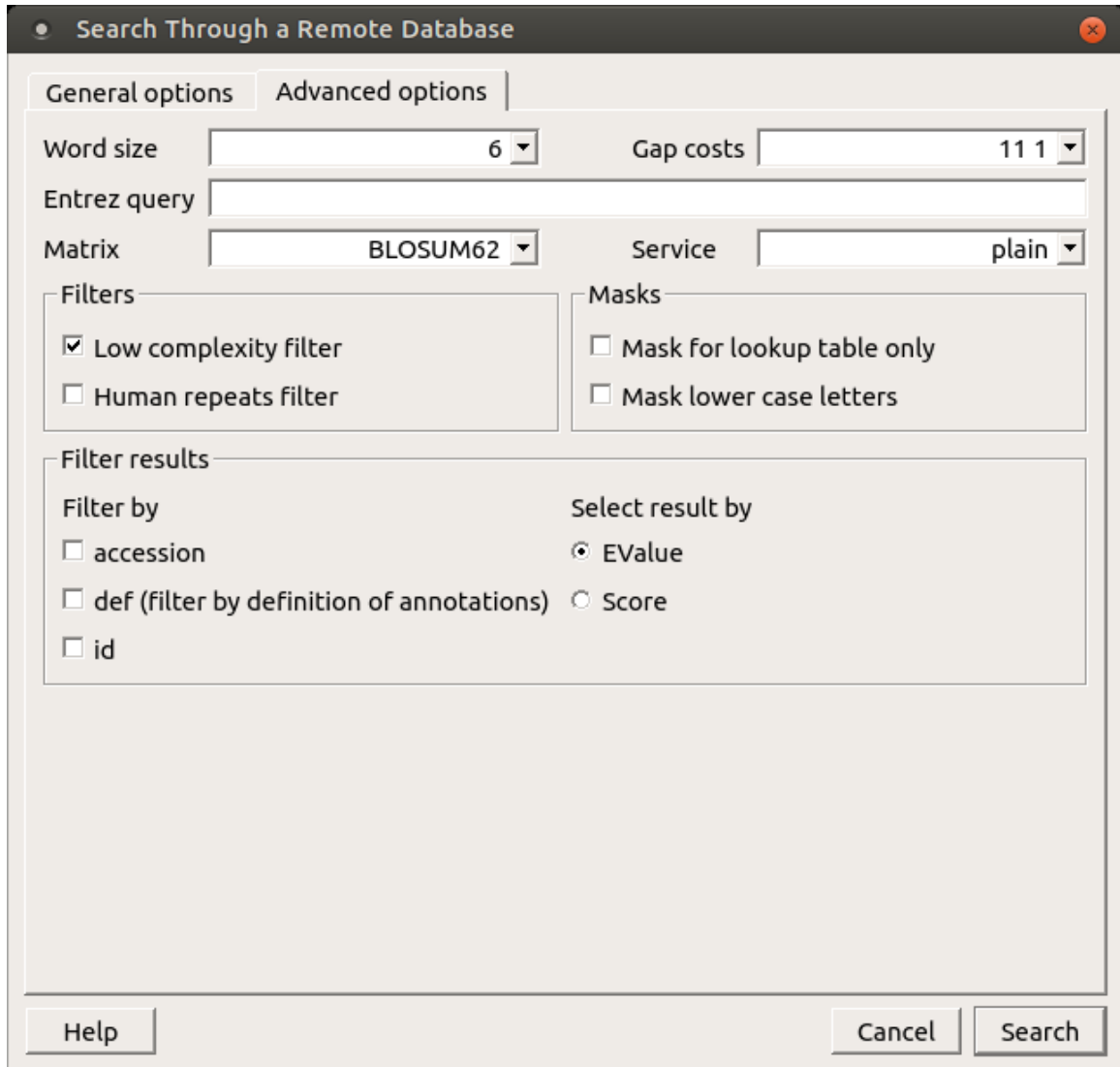
Masks for lookup table only — this option masks only for purposes of constructing the lookup table used by BLAST so that no hits are found based upon low-complexity sequence or repeats (if repeat filter is checked).

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.

Filter by — filters results by accession, by definition of annotations or by id.

Select result by — selects results by EValue or by score.

When the *blastp* 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 *Matrix* and *Service* options.

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.

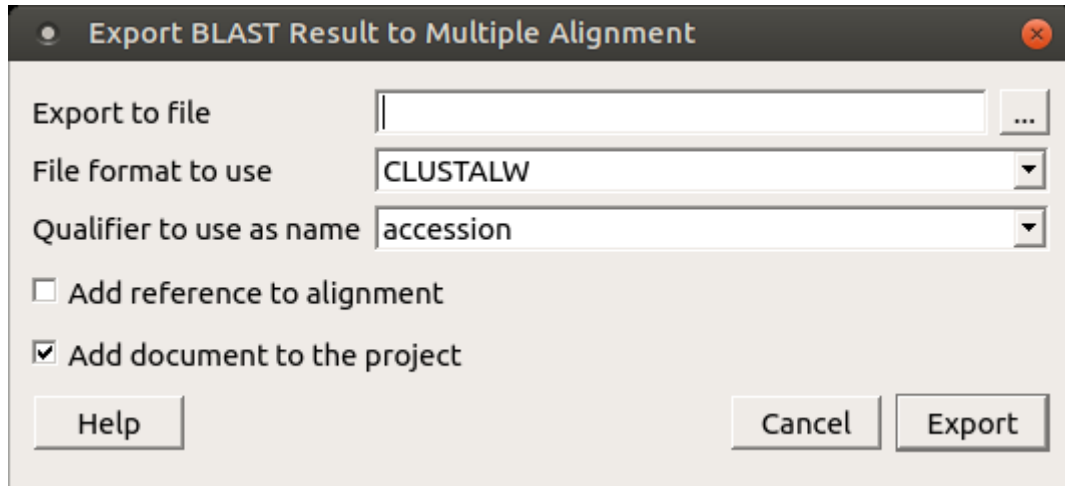
Service — blastp service which needs to be performed: plain, psi or phi.

The *Advanced options* tab is not available when the *cdd* search is selected.

- [Exporting BLAST Results to Alignment](#)
- [Fetching Sequences from Remote Database](#)

Exporting BLAST Results to Alignment

To export BLAST results as alignment select the results in the *Annotations Editor* and call the *Export->Export BLAST result to alignment* context menu item. The following dialog will appear:



The following parameters are available:

Export to file - name of the new file.

File format to use - format of the new file. The following formats are available: CLUSTALW, FASTA, MSF, MEGA, NEXUS, PHYLIP Interleaved, PHYLIP Sequential, Stockholm.

Qualifier to use as name - name of the qualifier. The following qualifiers are available: accession, def, id.

Add reference to alignment - adds a reference to alignment.

Add document to the project - adds the new document to the project.

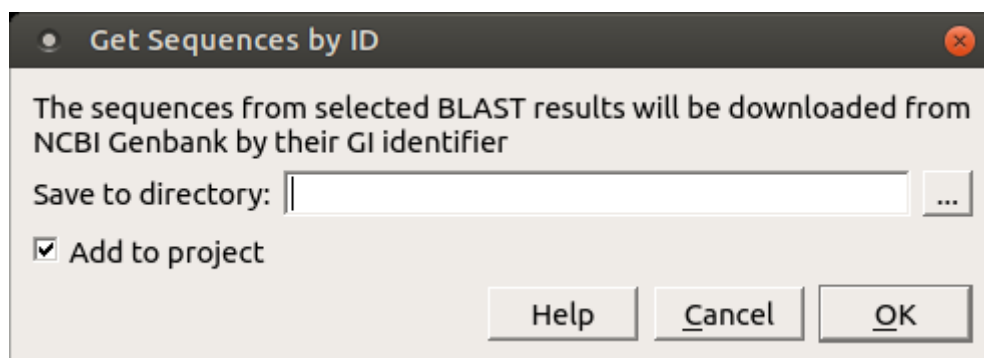
Select the options and click on the *Export* button.

Fetching Sequences from Remote Database

Each result annotation found with the *remote BLAST* in UGENE has "accession" and "id" qualifiers that can be used to fetch the corresponding sequences from the NCBI. The prompt way to fetch the sequences of several annotations is the following:

- Select the annotations in the *Annotations Editor*.
- Open the context menu.
- Choose the *Fetch sequences from remote database->Fetch sequences by 'id' from 'blast result' item* or *Fetch sequences from remote database->Fetch sequences by 'accession' from 'blast result' item*.

The following dialog will appear:



Select an output path in the dialog and click the *OK* button.

BLAST and BLAST+

The Basic Local Alignment Search Tool (*BLAST*) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. *BLAST* can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

BLAST+ is a new version of the *BLAST* package from the NCBI.

From UGENE you can use the following tools of the old *BLAST* package:

- **blastall** — the old program developed and distributed by the NCBI for running BLAST searches.
- **formatdb** — formats protein or nucleotide source databases before these databases can be searched by **blastall**.

And the following tools of the new BLAST+ package:

- **blastn** — searches a nucleotide database using a nucleotide query.
- **blastp** — searches a protein database using a protein query.
- **blastx** — searches a protein database using a translated nucleotide query.
- **tblastn** — compares a protein query against a translated nucleotide database (the all six reading frames).
- **tblastx** — translates the query nucleotide sequence in all six possible frames and compares it against the six-frame translations of a nucleotide sequence database.
- **makeblastdb** — — formats protein or nucleotide source databases before these databases can be searched by other *BLAST+* tools

BLAST home page: http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome

To make *BLAST* (or *BLAST+*) tools available from UGENE:

1. Install the required version of *BLAST* (or *BLAST+*) on your system.
2. Set the paths to the executables, you are going to use, on the *External tools* tab of UGENE *Application Settings* dialog.

After you've finished this configuration you can access the tools from the *Tools BLAST* submenu of the main menu.

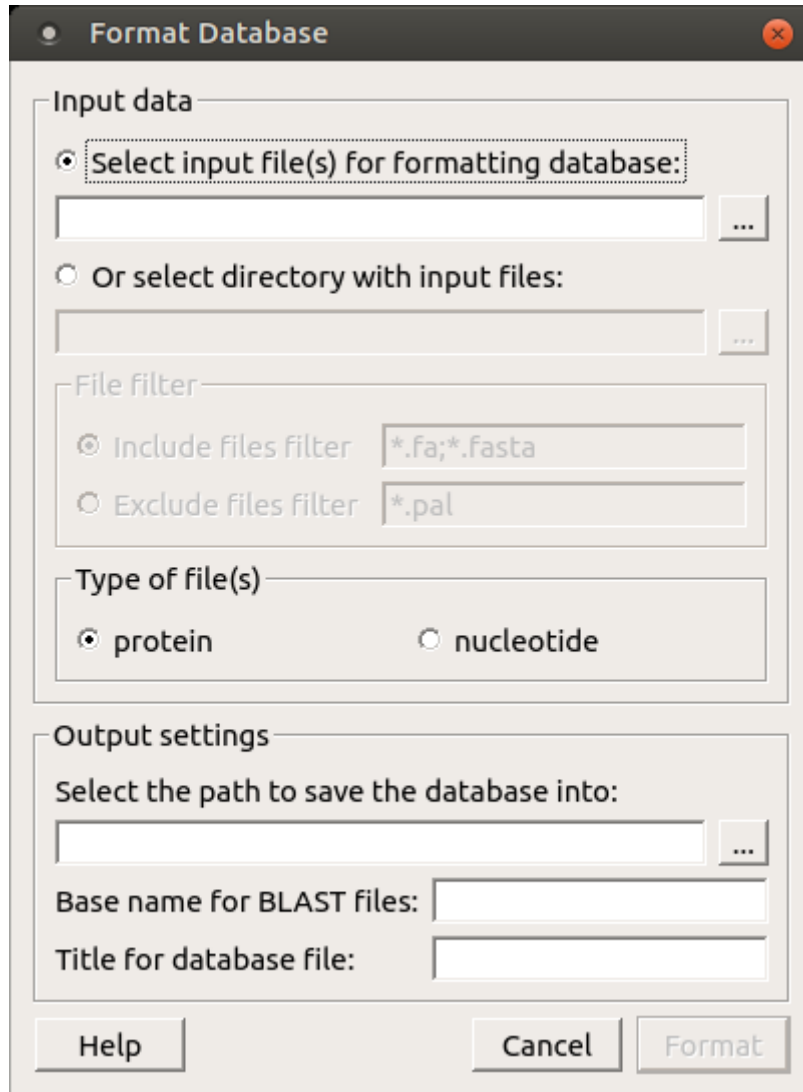
- [Creating Database](#)
- [Making Request to Database](#)
- [Fetching Sequences from Local BLAST Database](#)

Creating Database

To format a BLAST database do the following:

- If you're using *BLAST* open *Tools BLAST FormatDB*.
- If you're using *BLAST+* open *Tools BLAST BLAST+ make DB*.

The *Format database* dialog appears:



Here you must select the input files. If all the files you want to use are located in one directory, you can simply select the directory with the files. By default only the files are taken into account with *.fa and *.fasta extensions. You can change this by specifying either *Include files filter* or *Exclude files filter*.

You can choose either *protein* or *nucleotide* type of the files.

Then you must select the path to save the database file and specify a *Base name for BLAST files* and a *Title for database file*.

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* the 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

Region -

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 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.

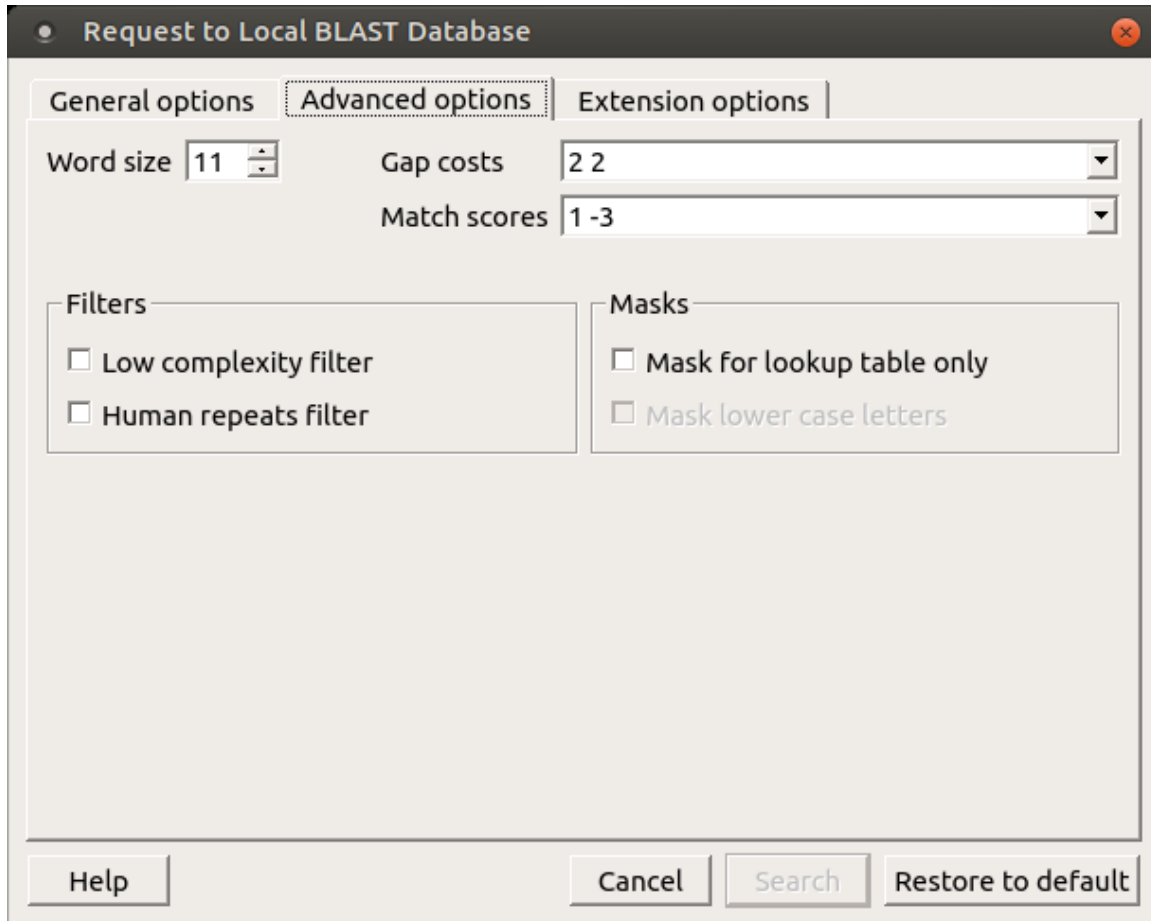
Megablast - select this option to compare query with closely related sequences. It works best if the target percent identity is 95% or more, but it is very fast.

Database path - path to the database files.

Base name for BLAST DB files - 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.

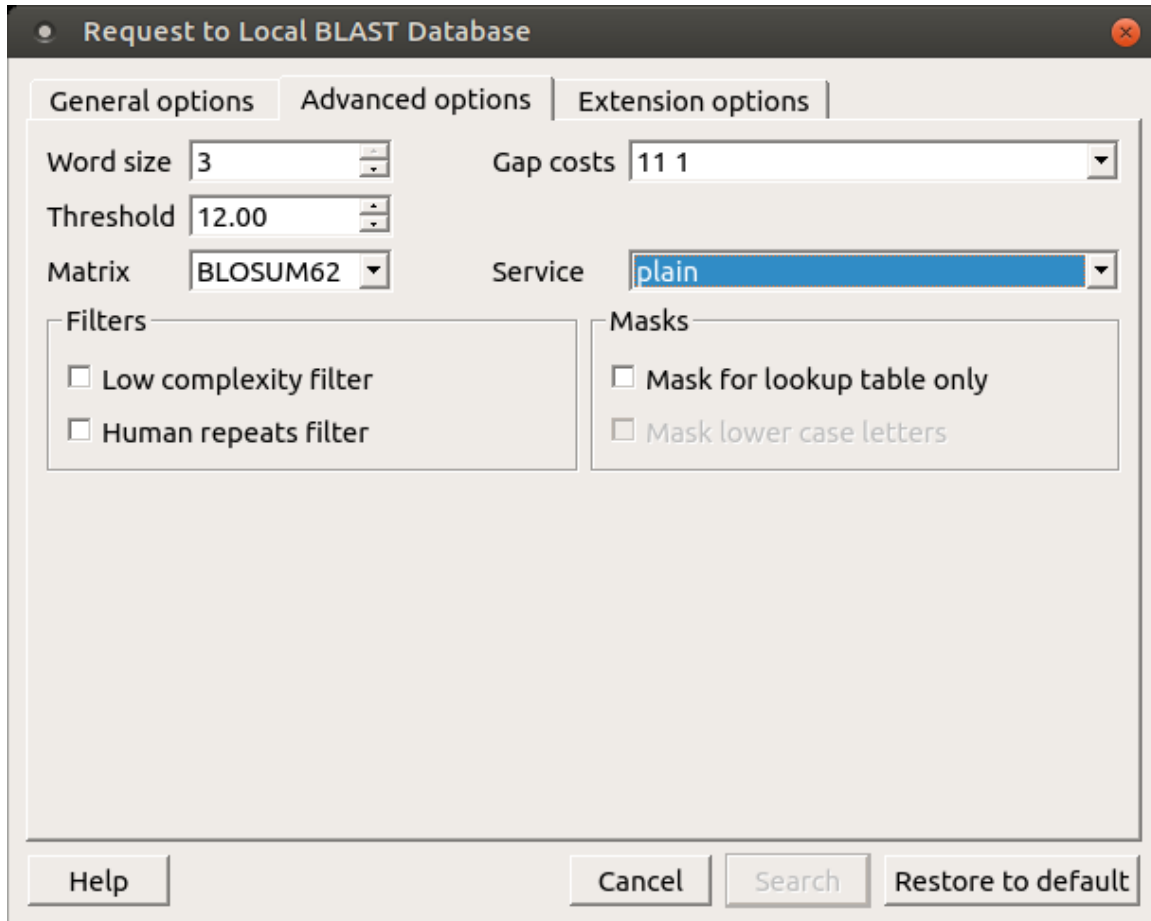
Match scores - reward and penalty for matching and mismatching bases.

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 on 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.

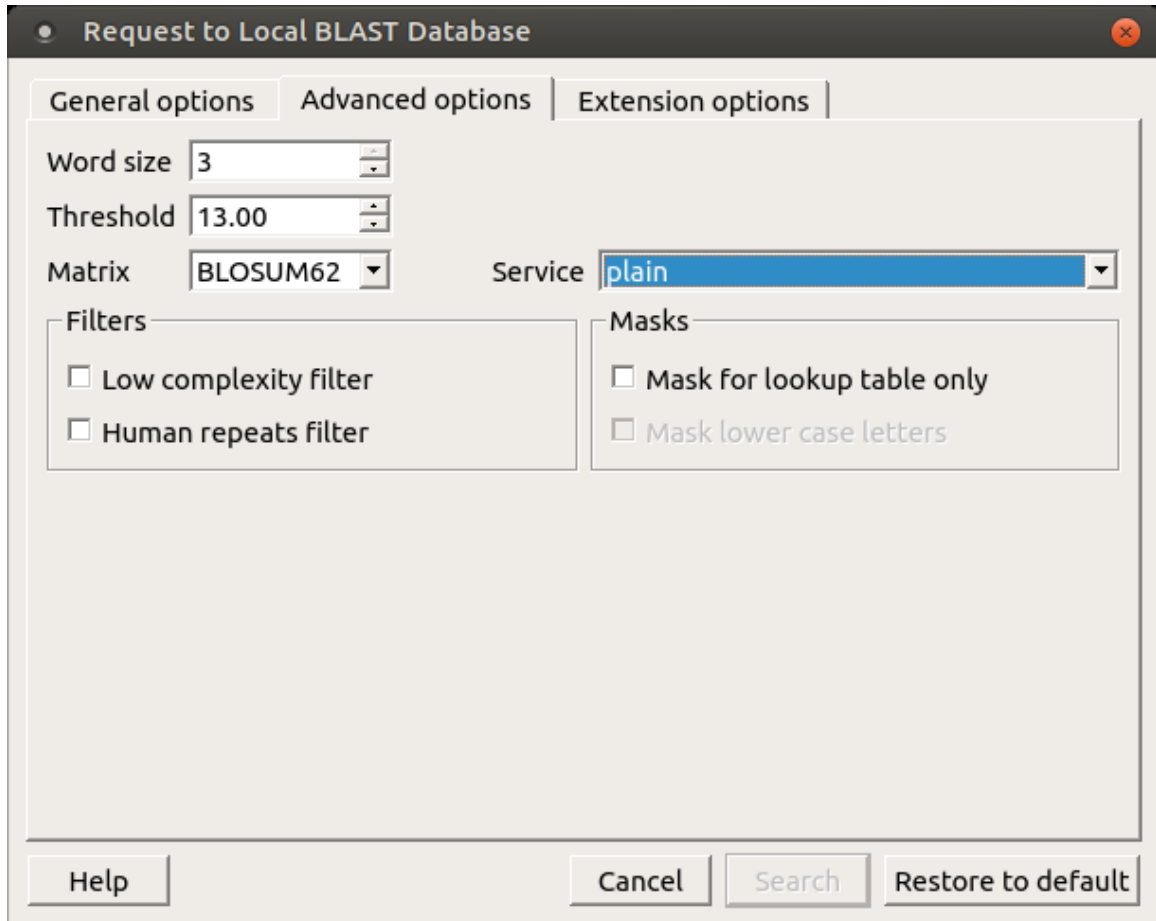
Threshold - threshold for extending hits.

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

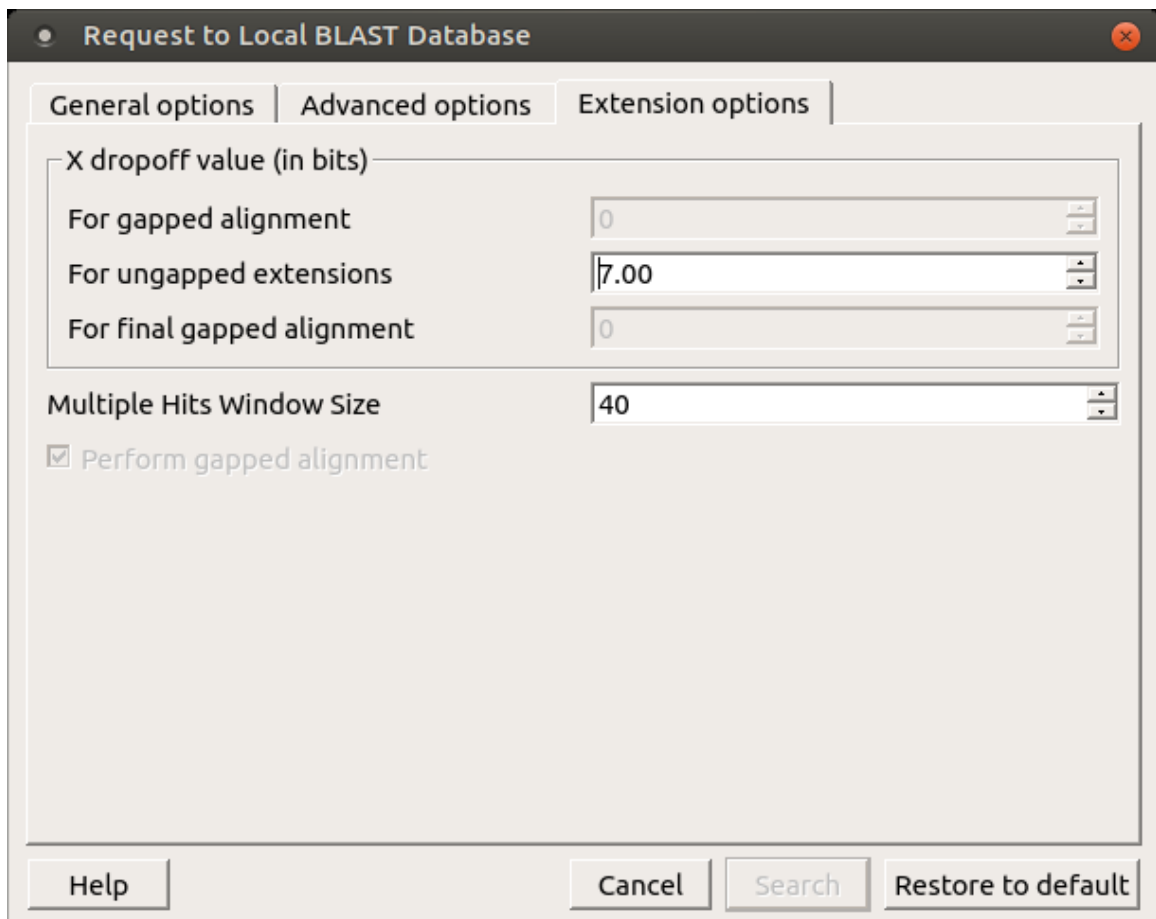
Service — blastp service which needs to be performed: plain, psi or phi.

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:



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.

Fetching Sequences from Local BLAST Database

To fetch sequences from local BLAST database use the *Fetch sequences from local BLAST database->Fetch sequences by 'id' from 'blast result'* context menu item of the blast result. The following dialog will appear:

Here you need select a query ID, database, type of file(s) and output path. After that click on the *Fetch* button. To fetch sequences for several annotations at the same time select the blast results with *Ctrl* key and call the *Fetch sequences from local BLAST database->Fetch sequences by 'id' from 'blast result'* context menu item.

Repeat Finder

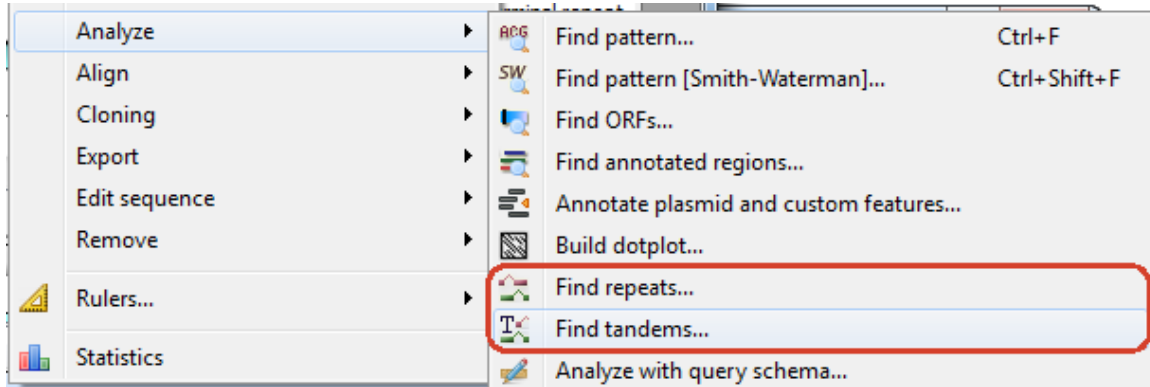
The *Repeat Finder* plugin provides a tool to search for direct and invert repeats in a DNA sequence. Also it allows to search for tandem repeats.

- Repeats Finding
- Tandem Repeats Finding
 - Tandem Repeats Search Result

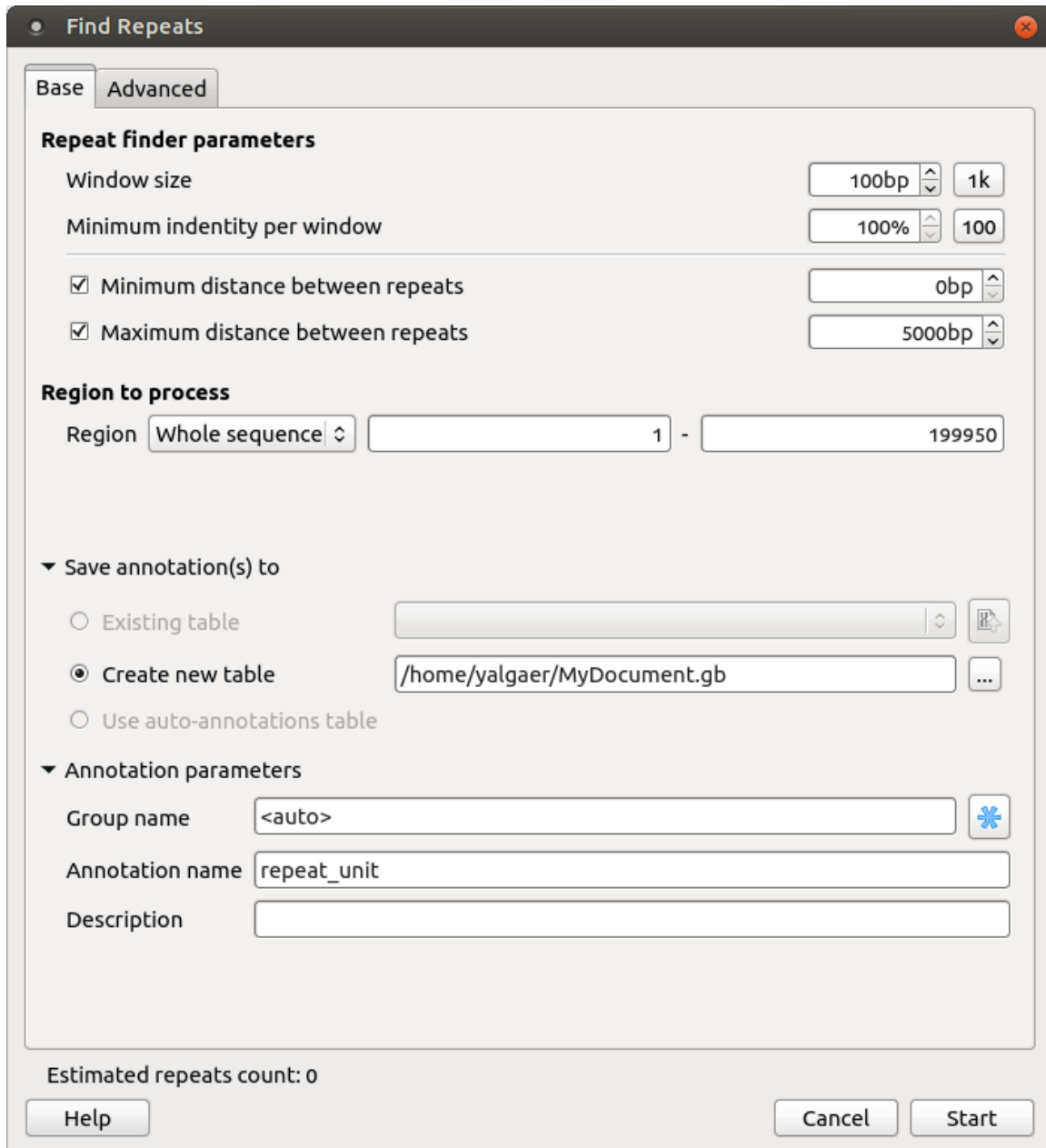
Repeats Finding

Usage example:

Open a DNA sequence in the *Sequence View* and select the *Analyze Find repeats...* context menu item:

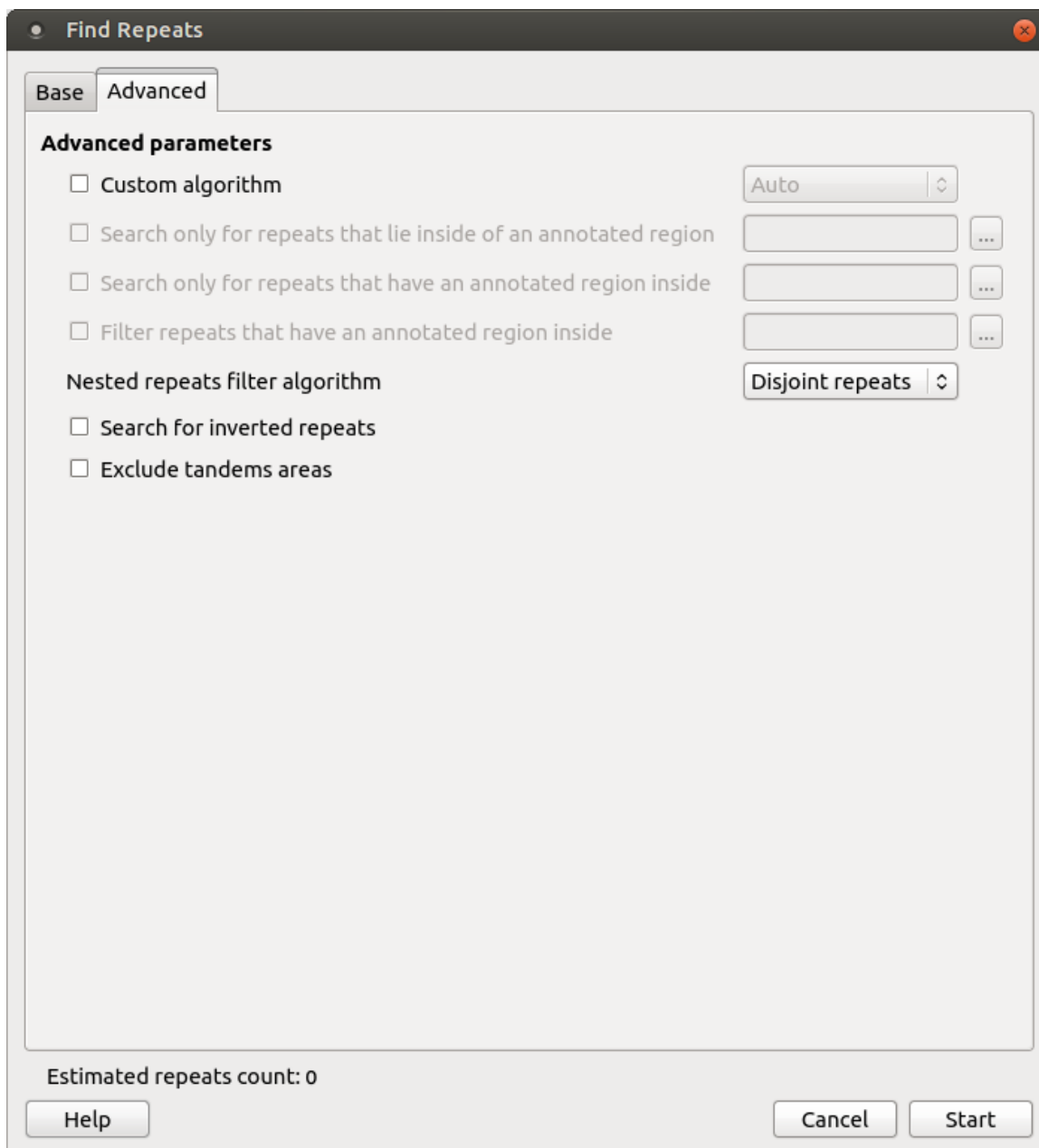


The dialog will appear that allows specifying repeat parameters and the annotations table document to save the results into:



The dialogues status line displays approximate repeats number that will be found with the current settings.

The *Advanced* tab provides additional repeats finding options:



The found repeats are saved and displayed as annotations to the DNA sequence:

Name	Value
Annotations [MyDocument_1.gb] *	
repeat_unit (0, 4)	
repeat_unit	join(19912..19956,19990..20034)
repeat_unit	join(63727..63804,65754..65831)
repeat_unit	join(185499..185538,189058..189097)
repeat_unit	join(190533..190577,190640..190684)

Tandem Repeats Finding

To find tandem repeats, select the *Analyze Find tandem repeats...* context menu item in the *Sequence View* window.

In the opened dialog you can specify the tandem search parameters, the region to search in and the result parameters:

Find Tandem Repeats

General Output

Settings

Preset settings: All

Algorithm: Suffix array (op)

Min length of repeated sequence: 1 n

Max length of repeated sequence: 1000000 n

Min length of tandem repeat: 9

Min number of repeats: x3

Show overlapped tandems

Search in

Region: Whole sequence 1 - 199950

Help Cancel Start

The dialog parameters:

- Present Settings* — specify the tandem repeats parameters with predefined values by selecting the available preset.
- Algorithm* — the algorithm parameter allows to select the search algorithm. The default and a fast one is optimized suffix array algorithm.
- Min length of repeated sequence* - the minimum acceptable repeat length measured in base symbols.
- Max length of repeated sequence* - the maximum acceptable repeat length measured in base symbols.
- Min length of tandem repeat* - the minimum tandem size sets the limit on the minimum acceptable length of the tandem, i.e. the minimum total repeats length of the searched tandem.
- Min number of repeats* - the minimum number of repeats of a searched tandem.
- Show overlapped tandems* - check if the plugin should search for the overlapped tandems, otherwise keep unchecked.
- Search in* - specify the region to search in the whole sequence, a custom region or the region of the current selection (if any).

The output settings can be found in the *Output* tab:

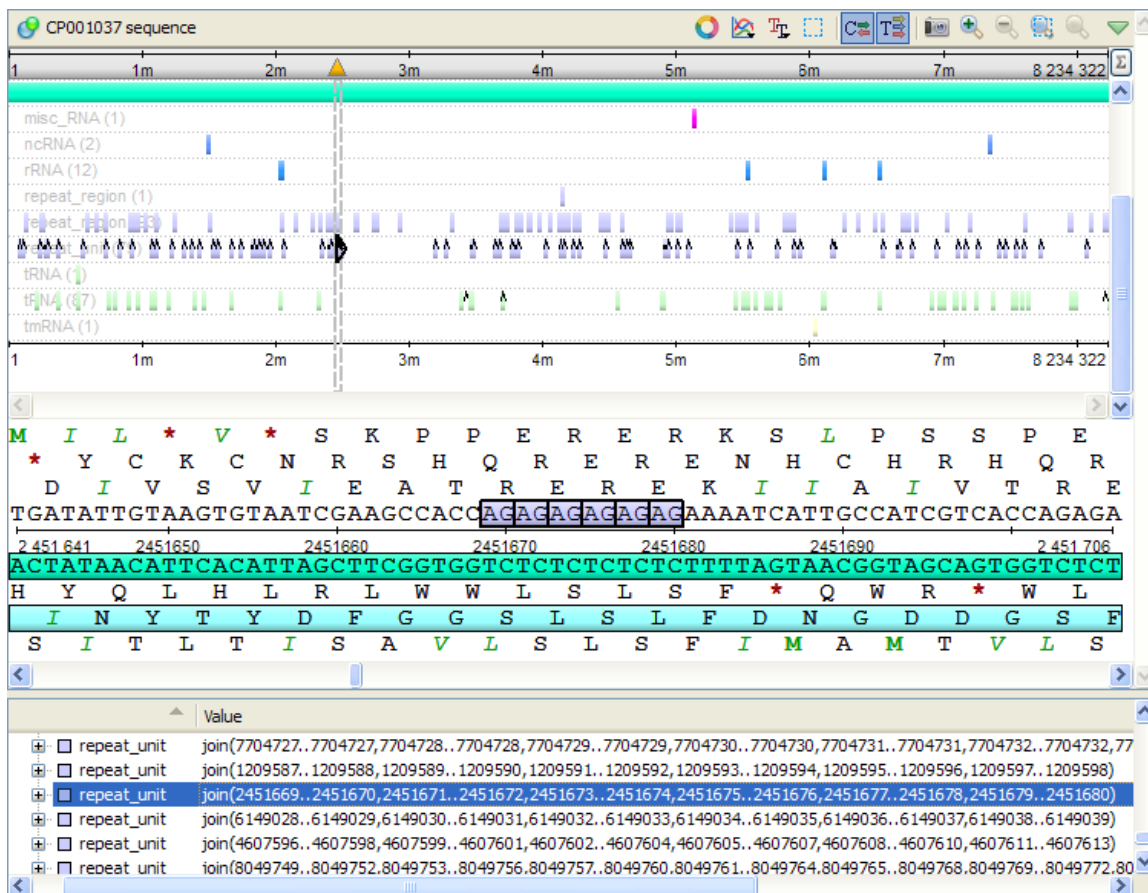
In the *Save annotation(s) to* group, you can set up a file to store annotations. It could be either an existing annotation table object, a new annotation table or auto-annotations table (if it is available).

In the *Annotation parameters* group, you can specify the name of the group and the name of the annotation. If the group name is set to <auto> UGENE will use the group name as the name for the group. You can use the ‘/’ characters in this field as a group name separator to create subgroups. If the annotation name is set to *by type* UGENE will use the annotation type from the *Annotation type:* table as the name for the annotation. Also, you can add a description in the corresponding text field.

- [Tandem Repeats Search Result](#)

Tandem Repeats Search Result

An example of the search results for the micro-satellite preset:



The tandem repeats annotations are located side by side.

Restriction Analysis

From this chapter you can learn how to search for restriction sites on a DNA sequence.

The restriction sites found are stored as automatic annotations. This means that if the automatic annotations highlighting is enabled then the restriction sites are searched and highlighted for each nucleotide sequence opened. Refer [Automatic Annotations Highlighting](#) to learn more.

Open a DNA sequence in and click the following button on the *Sequence View* toolbar:



Alternatively, select either the *Actions Analyze Find restriction sites* item in the main menu or the *Analyze Find restriction sites* item in the context menu.

The *Find restriction sites* dialog appears:

Find Restriction Sites

Filter by name:

Name	Accession	Type	Sequence	Organizm / Details
▷ A (0, 264)				Aaal .. AxyI
▷ B (2, 917)				BacI .. BvuBI
▷ C (1, 193)				Cacl .. CvnI
▷ D (1, 31)				Daql .. Dsp1I
▷ E (2, 325)				Eacl .. EspHK30I
▷ F (0, 63)				F-CphI .. F-TevIV
▷ G (0, 23)				Gall .. Gsul
▷ H (1, 312)				H-DreI .. Hsul
▷ I (0, 61)				I-AchMI .. I-Vdi141I

Selected enzymes:
BamHI,BglII,ClaI,DraI,EcoRI,EcoRV,HindIII,PstI,Sall,SmaI,XmaI

Filter by number of results:
 Minimum hits: Maximum hits:

Search in:
 Region: -
 Exclude -

Total number of enzymes: 4862, selected 11

Buttons: Help, Cancel, OK, Open enzymes, Export enzymes, Select All, Select None, Select by length, Invert selection, Load selection, Save selection, REBASE Info

You can see the list of restriction enzymes that can be used to search for restriction sites. Also you can set a region to search for. The information about enzymes was obtained from the [REBASE](#) database. For each enzyme in the list a brief description is available (the accession ID in the database, the recognition sequence, etc.). If you're online you can get more detailed information about an enzyme selected by clicking the *REBASE Info* button.

- [Selecting Restriction Enzymes](#)
- [Using Custom File with Enzymes](#)
- [Filtering by Number of Hits](#)
- [Excluding Region](#)
- [Circular Molecule](#)
- [Results](#)

Selecting Restriction Enzymes

To select an enzyme check it in the list. Notice that the enzyme appears in the *Selected enzymes* area of the dialog.

You can also use the *Select All* button to select all the enzymes available, the *Select None* button to deselect all the enzymes.

To select all enzymes with recognition sequence length shorter than the specified value click the *Select by length* button and input the minimum length in the dialog appeared.

To invert selection click the *Invert selection* button.

As soon as enzymes are selected you can click the *OK* button to search for corresponding restriction sites in the sequence.

Using Custom File with Enzymes

To load a custom file with enzymes click the *Enzymes file* button and browse for the file. The file must be of the Bairoch format.

For details about the format refer <http://rebase.neb.com/rebase/rebase.f19.html>.

To export enzymes use the *Export enzymes* button. You can also save the currently selected enzymes to a file and load saved selection. Click the *Save selection* and *Load selection* buttons correspondingly to do it.

Filtering by Number of Hits

To filter the results by the number of restriction sites found for an enzyme check the *Filter by number of results* check box and input the minimum value and the maximum value of hits.

Excluding Region

To exclude a sequence region from the search check the *Exclude region* check box and input the start and the end positions of the region. If a subsequence has been selected before opening the dialog you can click the *Selected* button to automatically fill the values with the selected subsequence's start and end positions.

Circular Molecule

To consider the sequence as circular and be able to search for restriction sites between the end and the beginning of the sequence check the *Circular molecule* option.

Example: Let's consider:

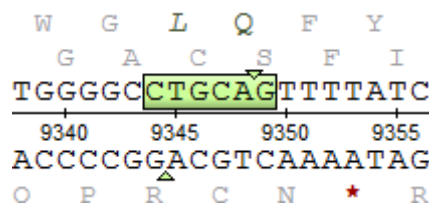
- The sequence is "CTGC ... CAC".
- *AarI* restriction enzyme (with recognition sequence "CACCTGC") has been checked.

In this case if the *Circular molecule* option has been checked, the restriction site will be found. If it hasn't been checked, the restriction site won't be found (in this position).

Results

When at least one enzyme has been selected and the *OK* button has been pressed in the dialog, the *auto-annotating* becomes enabled. In the *Annotations editor* the Restriction Sites annotations can be found in the Auto-annotations\enzyme group.

The direct and complement cut site positions are visualized as triangles on an annotation in the *Sequence details view*.



Molecular Cloning in silico

This chapter describes a set of tools in UGENE to perform molecular cloning experiments *in silico*.

This allows you to digest a molecule into fragments, create a fragment from a sequence region and ligate fragments into a new molecule.

- Digesting into Fragments
- Creating Fragment
- Constructing Molecule
 - Available Fragments
 - Fragments of the New Molecule
 - Changing Fragments Order in the New Molecule
 - Removing Fragment from the New Molecule
 - Editing Fragment Overhangs
 - Reverse Complement a Fragment
 - Other Constuction Options
 - Output
- Creating PCR Product

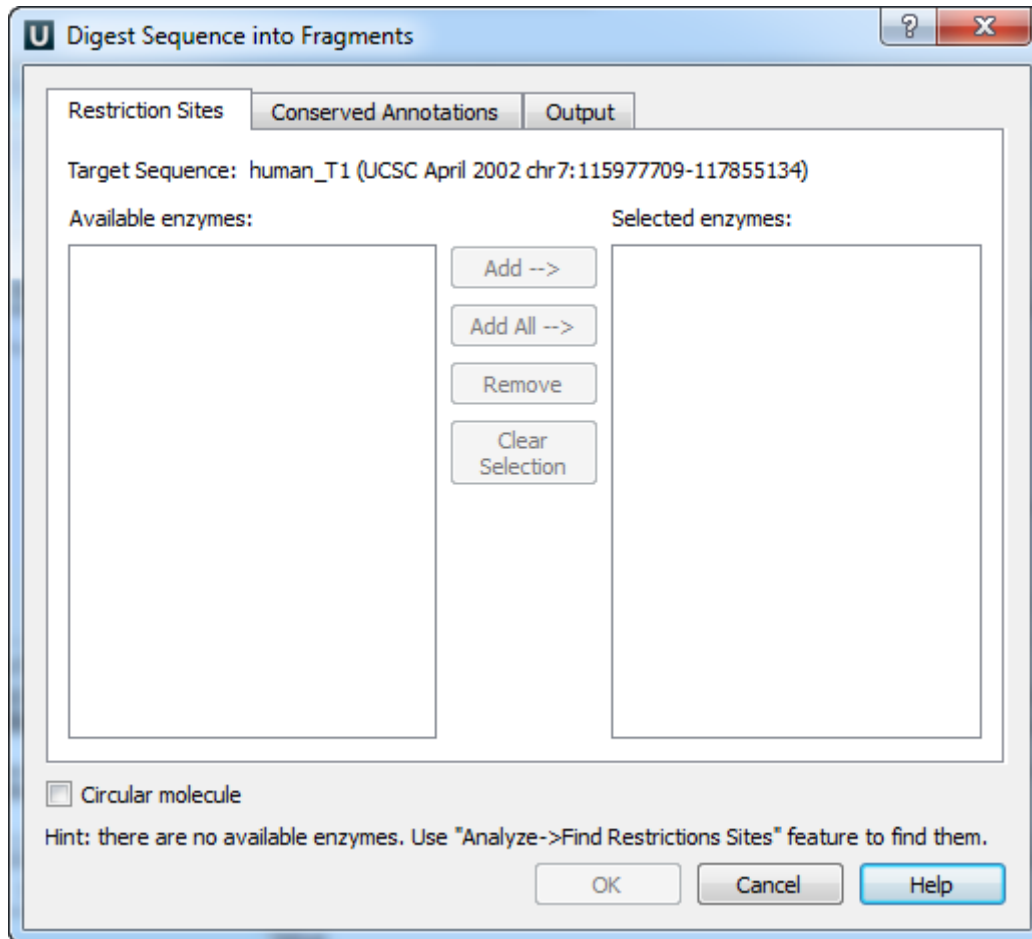
Digesting into Fragments

Open a DNA molecule you want to cut into fragments.

Digestion into fragments is performed using restriction enzymes. So before continuing make sure that the restriction analysis has been performed. Refer chapter [Restriction Analysis](#) for details.

Select either the *Tools Cloning Digest into Fragments* item or the *Actions Cloning Digest into Fragments* item in the main menu or the *Cloning Digest into Fragments* item in the context menu.

The *Digest Sequence into Fragments* dialog appears:



On the *Restriction Sites* tab of the dialog you can see the name of the molecule, the list of restriction enzymes found during the restriction analysis that can cut the molecule and the list of enzymes selected to perform the digestion.

To digest the sequence into fragments you should select at least one enzyme.

To move an enzyme to the *Selected enzymes* list click on it in the *Available enzymes* list and press the *Add* button. Note that you can select several items in a list by holding the Ctrl key while clicking on the items.

To select all available enzymes press the *Add All* button.

To remove enzymes from the *Selected enzymes* list select them in the list and press the *Remove* button.

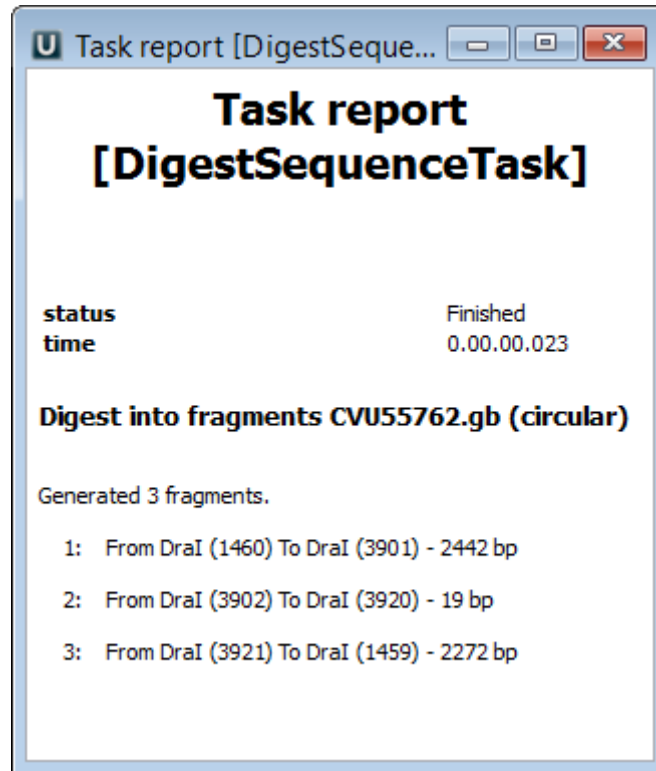
To remove all items from the *Selected enzymes* list press the *Clear Selection* button.

On the *Conserved Annotations* tab of the dialog you can select the annotations that must not be disrupted during cloning.

On the *Output* tab of the dialog you can select the file to save the new molecule to.

As soon as the required parameters are selected press the *OK* button. The fragments will be saved as annotations.

Also all the generated fragments are available in the task report:

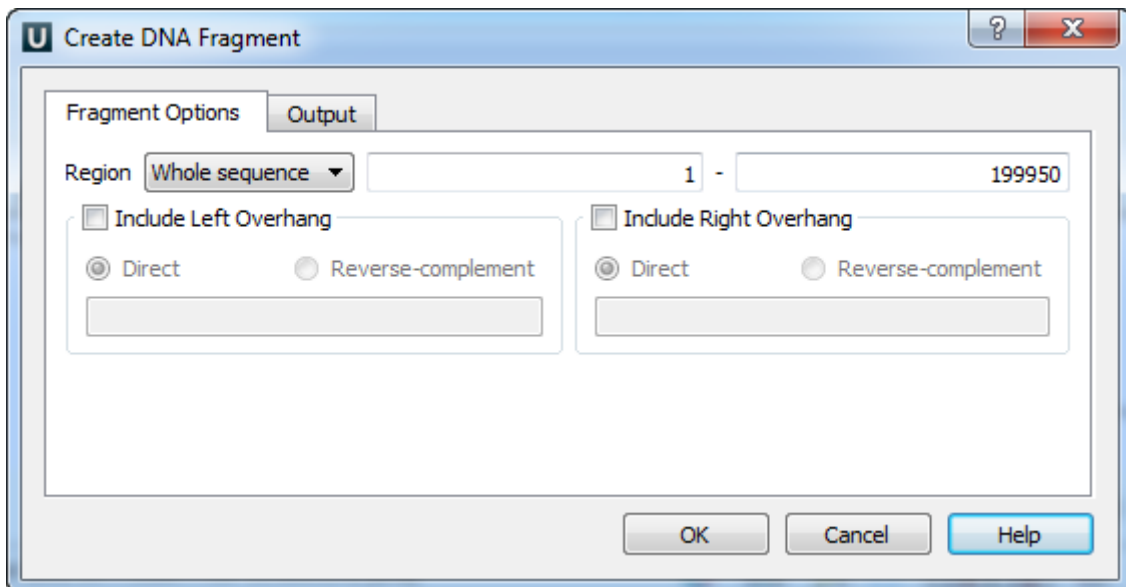


Refer to *Notifications* to learn more about task reports.

Creating Fragment

To create a DNA fragment from a sequence region activate the *Sequence View* window and select either the *Actions Cloning Create Fragment* item in the main menu or the *Cloning Create Fragment* item in the context menu.

The *Create DNA Fragment* dialog appears:



If a region has been selected you can choose to create the fragment from this region. Otherwise you can either choose to create the fragment from the whole sequence or choose the *Custom* item and input the custom region.

To add a 5' overhang to the direct strand check the *Include Left Overhang* check box and input the required nucleotides. To add a 5' overhang to the reverse strand in addition to the described steps select the *Reverse-complement* item in the same group box.

Similarly, to add a 3' overhang check the *Include Right Overhang* check box, input the required overhang and select either the direct or the reverse-complement strand.

On the *Output* tab of the dialog you can optionally modify the annotations output settings.

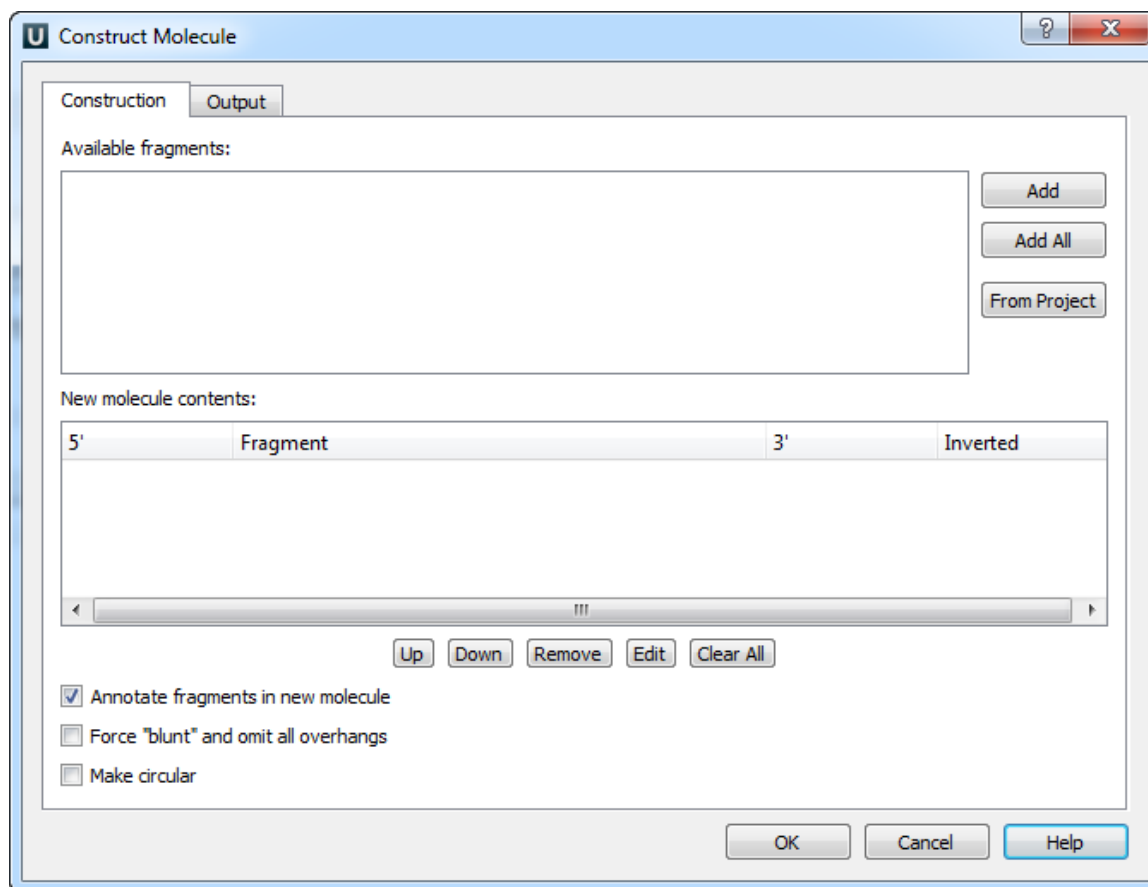
Finally, press the *OK* button to create the fragment. The fragment will be saved as an annotation.

Constructing Molecule

To construct a new molecule from fragments select the *Tools Cloning Construct Molecule* item in the main menu.

If a *Sequence View* window is active you can also select either the *Actions Cloning Construct Molecule* item in the main menu or the *Cloning Construct Molecule* item in the context menu.

The *Construct Molecule* dialog appears:



- Available Fragments
- Fragments of the New Molecule
- Changing Fragments Order in the New Molecule
- Removing Fragment from the New Molecule
- Editing Fragment Overhangs
- Reverse Complement a Fragment
- Other Construction Options
- Output

Available Fragments

All the fragments available in the current project are shown in the *Available fragments* list.

You can automatically create a fragment from a DNA molecule from the current UGENE *project*. Click the *From Project* button to do so. The *Select Item* dialog appears with the sequence objects available. Select a sequence and press the *OK* button. After that create a fragment in the appeared *Create DNA Fragment* dialog as described in the *Creating Fragment* paragraph. The fragment created from the sequence appears in the list of available fragments.

Fragments of the New Molecule

The next step is to add required fragments to the new molecule contents.

To add fragments select them in the list of available fragments and click the *Add* button or by double-click on a fragment.

To add all the fragments click the *Add All* button.

Changing Fragments Order in the New Molecule

To change the order of fragments in the new molecule select a fragment in the new molecule contents list and click either the *Up* or the *Down* button to move the fragment in the corresponding direction.

Removing Fragment from the New Molecule

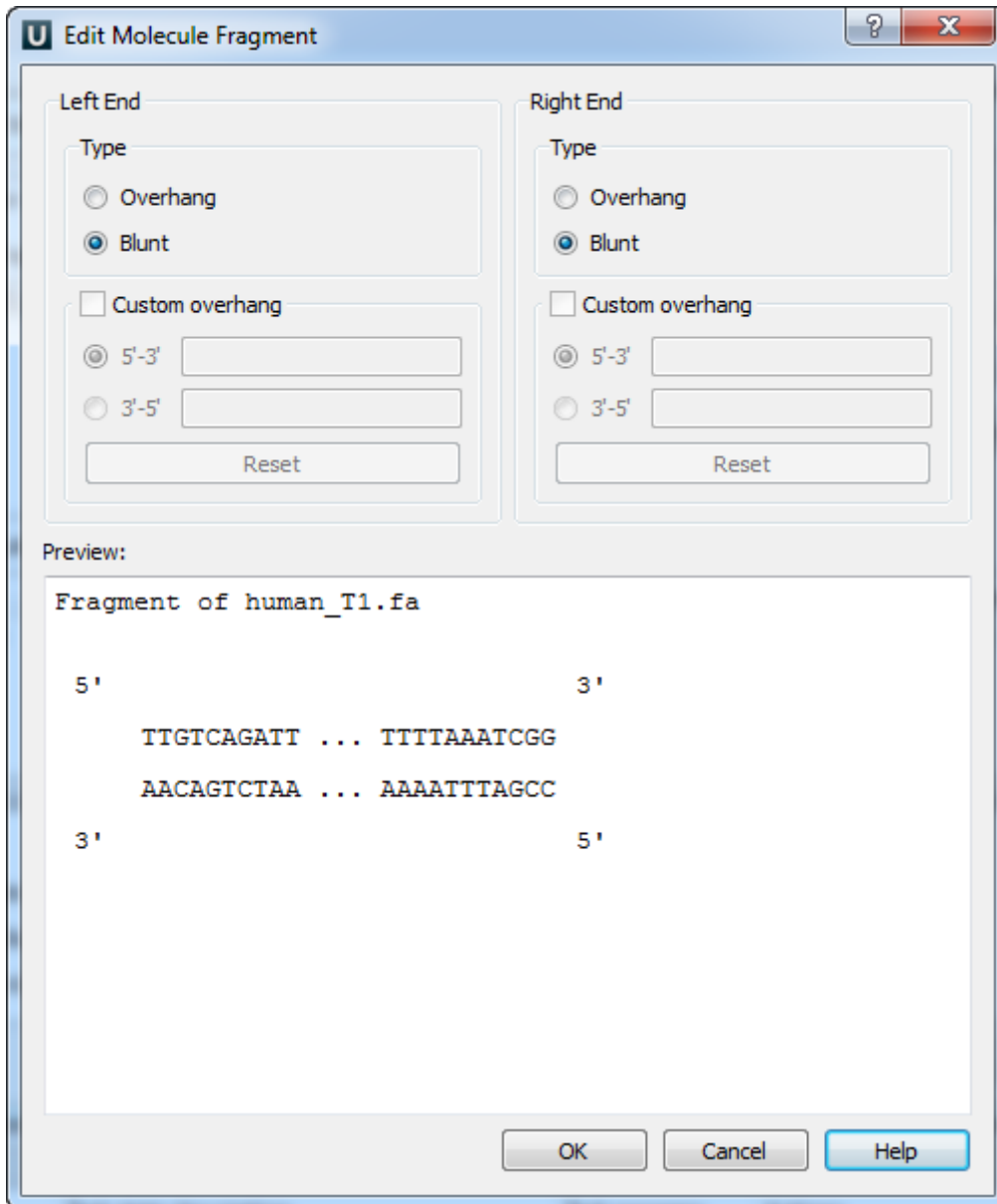
To remove a fragment from the new molecule select it in the new molecule contents list and click the *Remove* button.

To remove all the fragments click the *Clear All* button.

Editing Fragment Overhangs

To edit a fragment's overhangs select the fragment in the new molecule contents list and click the *Edit* button.

The *Edit Molecule Fragment* dialog appears:



Here you can select the type of each DNA end and even input a custom overhang.

The changes you've made are shown in the *Preview* area of the dialog.

To confirm the changes and close the dialog click the *OK* button.

Reverse Complement a Fragment

To reverse complement a fragment check the *Inverted* check box for the fragment in the new molecule contents list.

Other Constuction Options

To save the fragments of the new molecule as annotations check the *Annotate fragments in new molecule* check box.

To make all DNA ends blunt check the *Force "blunt" and omit all overhangs* check box. All overhangs would be cut in this case.

Check the *Make circular* check box to make the new molecule circular.

Output

On the *Output* tab of the dialog you can select the file to save the new molecule to.

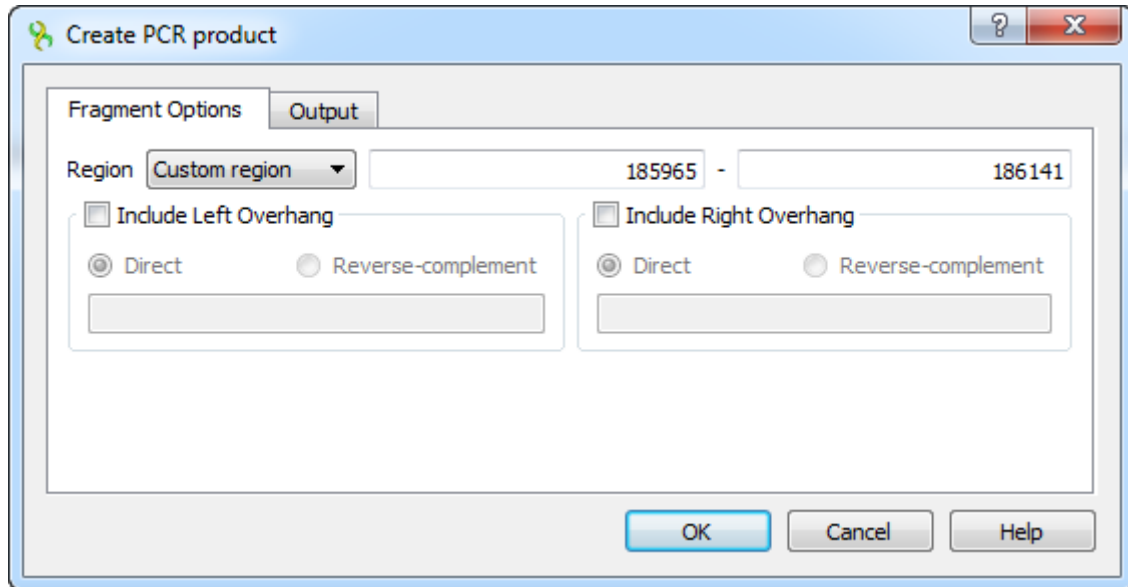
The molecule is opened by default as soon as it is created. To modify this behavior uncheck the *Open view for new molecule* check box on the same tab.

To save the molecule file to the hard disk immediately after it is created check the *Save immediately* check box. Otherwise it would be stored in memory until you save or remove it.

Creating PCR Product

To create a PCR product from a primer use the *Cloning->Create PCR product* context menu of primer annotation.

The *Create PCR Product* dialog appears:



If a primer has been selected you can choose to create the PCR product from this primer. Otherwise you can either choose to create the PCR from the whole sequence or choose the *Custom* item and input the custom region.

To add a 5' overhang to the direct strand check the *Include Left Overhang* check box and input the required nucleotides. To add a 5' overhang to the reverse strand in addition to the described steps select the *Reverse-complement* item in the same group box.

Similarly, to add a 3' overhang check the *Include Right Overhang* check box, input the required overhang and select either the direct or the reverse-complement strand.

On the *Output* tab of the dialog you can optionally modify the annotations output settings.

Finally, press the *OK* button to create the PCR product. The PCR product will be saved as an annotation.

In Silico PCR

In Silico PCR Overview

In silico PCR is used to calculate theoretical polymerase chain reaction (PCR) results using a given set of primers (probes) to amplify DNA sequences.

UGENE provides the In silico PCR feature only for nucleic sequences with Standard DNA and Extended DNA alphabets. To use it in UGENE open a DNA sequence and go to the *In silico PCR* tab of the Options Panel:

In Silico PCR

▼ Forward primer
 ACGTTACGTACGTACTACGTACGTGC
 Tm = 59.54°C, 26-mer
 Mismatches 0 bp

▼ Reverse primer
 AAAAACGTACGTCGT
 Tm = 38.25°C, 16-mer
 Mismatches 0 bp

▼ Settings
 3' perfect match 15 bp
 Maximum product 5000 bp
 Extract annotations Inner

[Show primers details](#)

Warning:
Self-dimer can be formed:
Delta G: -15.4 kcal/mole
Base Pairs: 11

Find product(s) anyway

There are the following parameters:

Forward primer - forward primer.

Reverse primer - on the opposite strand from the forward primer.

Mismatches - mismatches limit.

3' perfect match - specify the number of nucleotides at the 3' end that must not have mismatches.

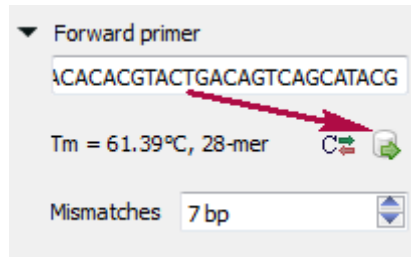
Maximum product - maximum size of the amplified sequence.

Extract annotations - specify the type of extracted annotations: *Inner*, *All intersected* or *None*.

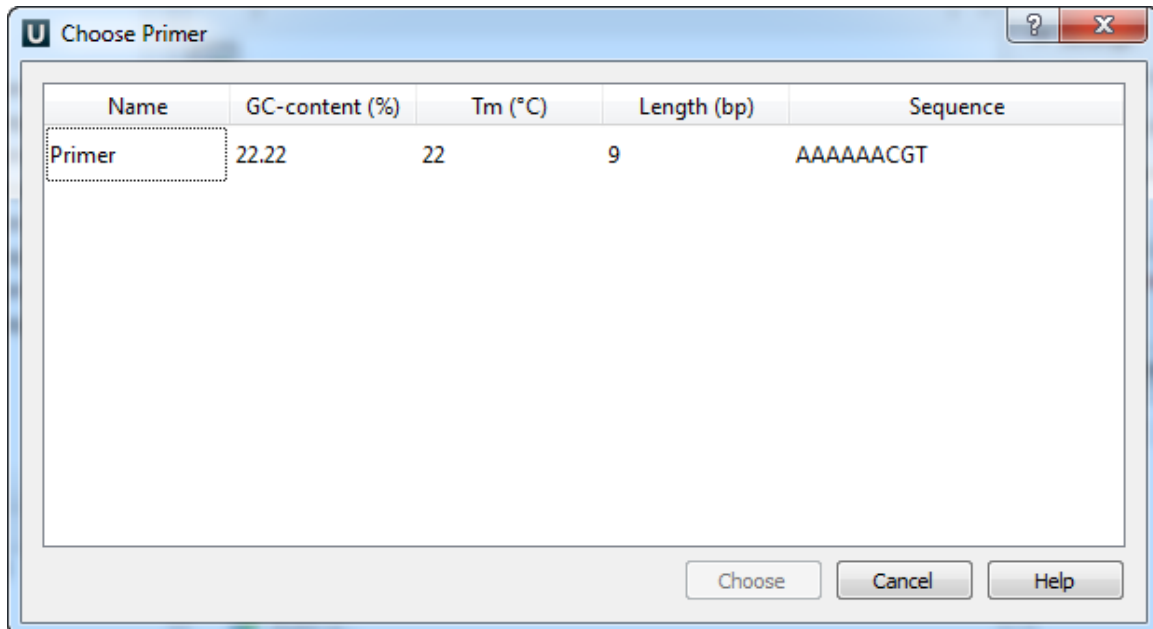
- Value *Inner* is selected by default. When this value is selected, the extracted PCR product contains annotations from the original sequence, located within the extracted region.
- Value *All intersected* specifies that all annotations of the original sequence that intersect the extracted region must be extracted as well.
- Value *None* specifies that annotations from the original sequence must not be extracted.

Choosing primers

Type two primers for running In Silico PCR. If the primers pair is invalid for running the PCR process then the warning is shown. Also, primers for the running In silico PCR can be chosen from a [primer library](#). Click the following button to choose a primer from the primers library:

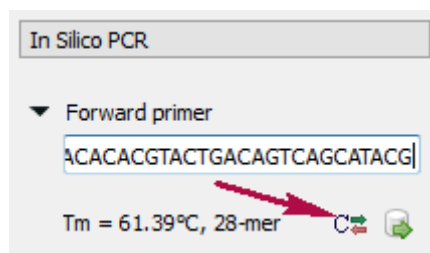


The following dialog will appear:



The table consists of the following columns: name, GC-content (%), Tm, Length (bp) and sequence. Select primer in the table and click the *Choose* button.

Click the *Reverse-complement* button for making a primer sequence reverse-complement:



Click *Show primers details* for seeing *statistic details* about primers.

When you run the process, the predicted PCR products appear in the products table.

Products table

There are three columns in the table:

- region of product in the sequence
- product length
- preferred annealing temperature

Click the product for navigating to its region in the sequence.

Click the *Extract product(s)* button for exporting a product(s) in a file or use double click for that.

Region	Length	Ta
60822 - 63999	3178	58.56

- Primers Details
- Primer Library

Primers Details

Click *Show primers details* for seeing statistic details about primers.

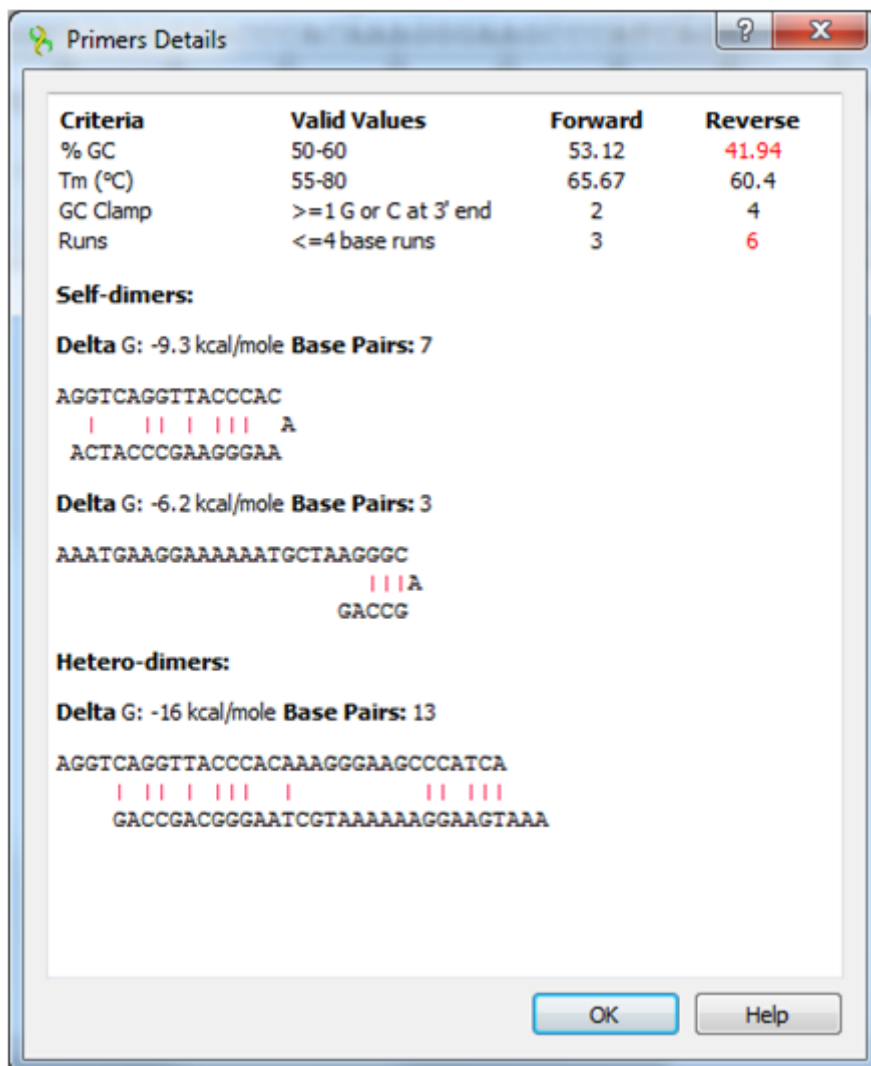
Mismatches

▼ Settings

Maximum product

[Show primers details](#)

The following dialog will appear:

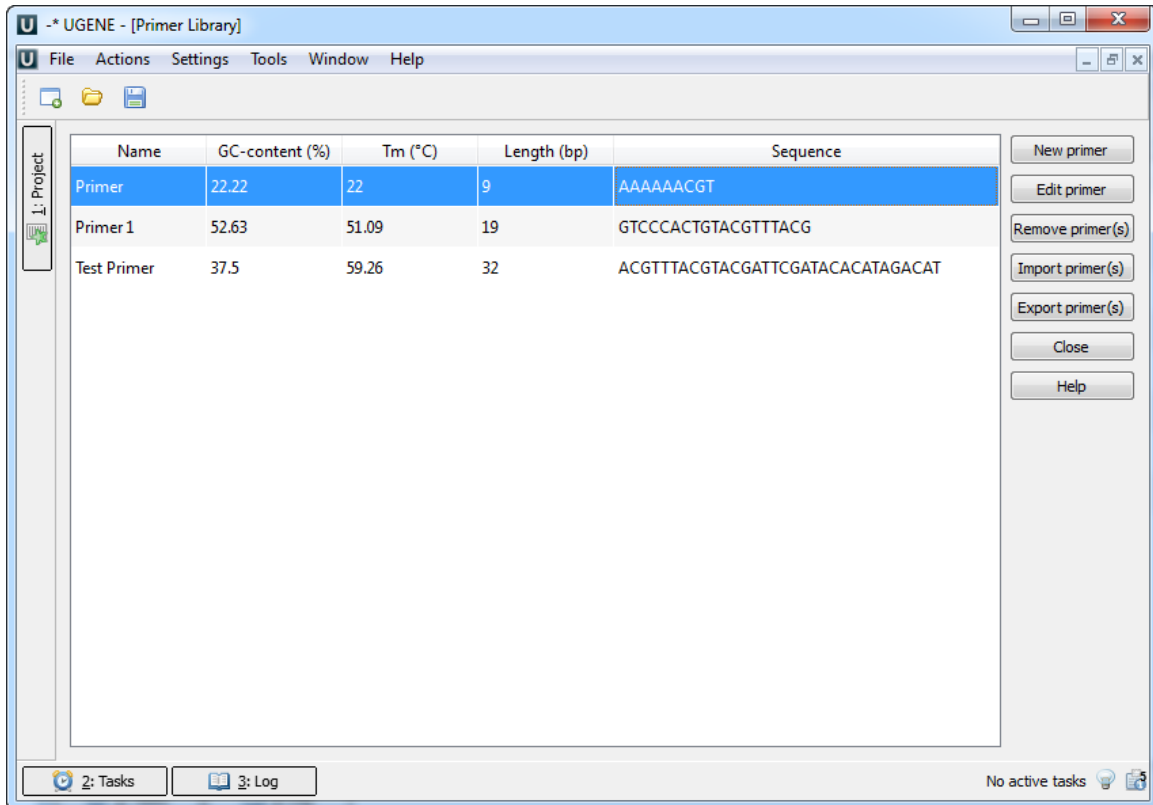


This is a dialog with statistic details about primers: melting temperature, GC content, dimers, self-dimers, etc. If a value is not correct for its criteria then it is colored in red.

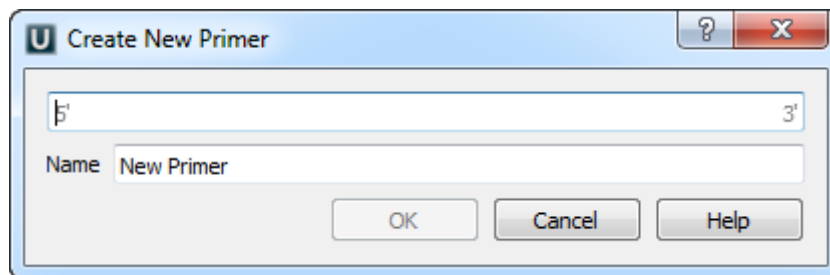
Primer Library

The primer library is a storage for keeping user primers. The added primers are stored between UGENE sessions.

Go to the *Tools->Primer->Primer library* context menu to configure the primer library. The following window will appear:



Click the *New primer* button to add a new primer. The following dialog will appear:

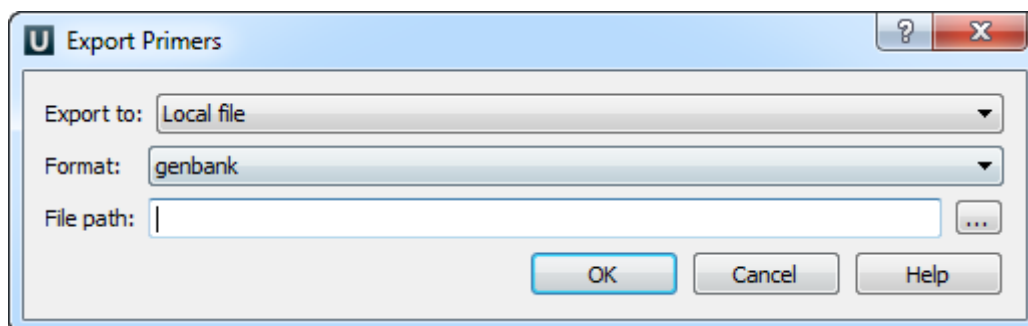


Input the primer sequence and primer name and click on the *OK* button.

Select the primer and click the *Edit primer* button to edit primer.

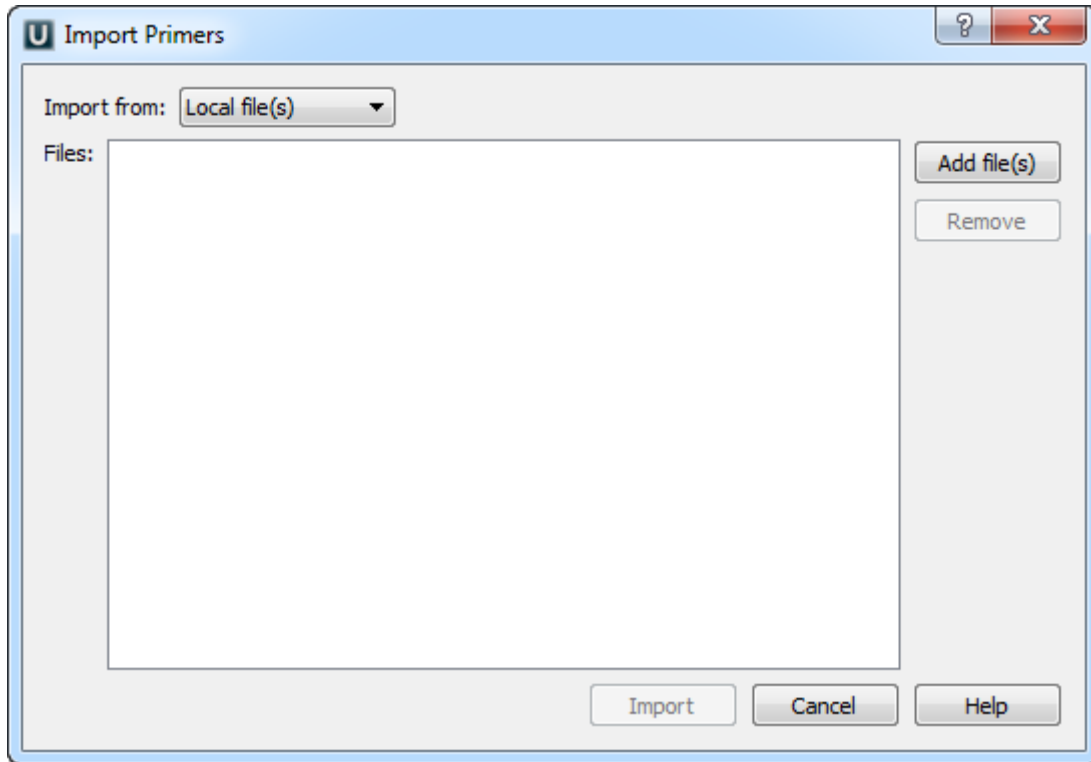
Select the primer in the table (you can use Ctrl and Shift) and click *Remove primer(s)* button to remove primer(s).

To export primer(s) select it and click the *Export primer(s)* button. The following dialog will appear:



Select file and file format and click on the *OK* button.

To import primer(s) click the *Import primer(s)* button. The following dialog will appear:



Add one or several files with primer sequences. Note that all sequence formats, supported by UGENE, can be imported, for example, FASTA, GenBank, etc. But the sequences must consist of ACGT characters only.

Click the *Import* button to import the added files into the primers library.

Secondary Structure Prediction

The *Secondary Structure Prediction* plugin provides a set of algorithms for the protein secondary structure (alpha-helix, beta-sheet) prediction from a raw sequence.

Currently, available algorithms are:

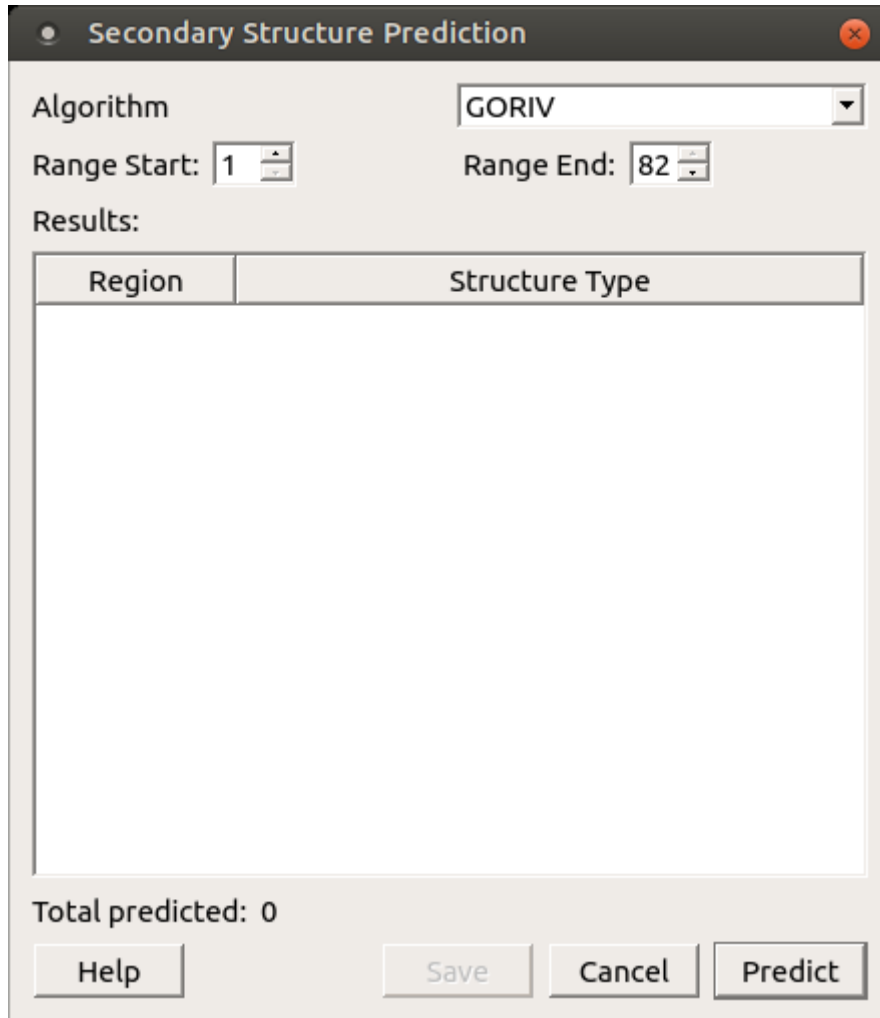
- **GORIV** Jean Garnier, Jean-Francois Gibrat, and Barry Robson, "GOR Method for Predicting Protein Secondary Structure from Amino Acid Sequence", in *Methods in Enzymology*, vol.266, pp. 540 - 553, (1996).

The improved version of the GOR method in J. Garnier, D. Osguthorpe, and B. Robson, *J. Mol. Biol.*, vol. 120, p. 97 (1978).

- **PsiPred** Bryson K, McGuffin LJ, Marsden RL, Ward JJ, Sodhi JS. & Jones DT. (2005) Protein structure prediction servers at University College London. *Nucl. Acids Res.* 33(Web Server issue): W36-38.

Jones DT. (1999) Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.* 292: 195-202.

You can access these analysis capabilities for a protein sequence using the *Analyze Predict secondary structure...* context menu item. The dialog will appear:

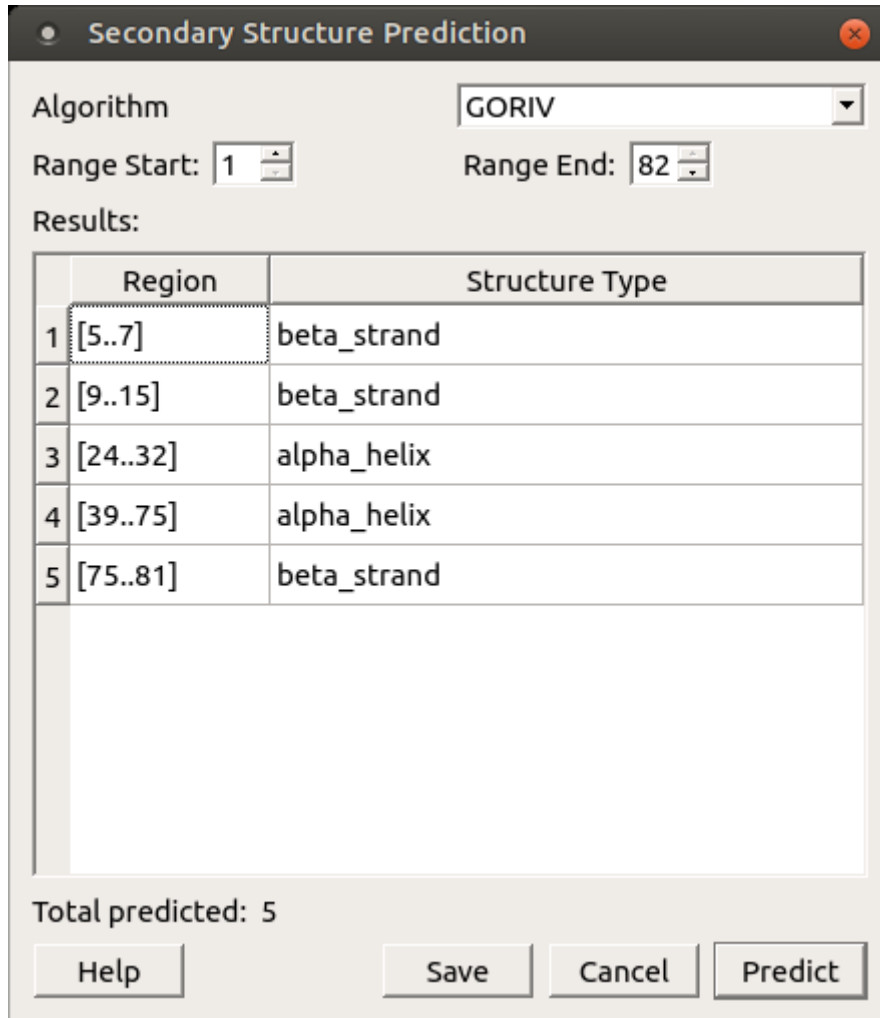


It supports the following options:

Algorithm — you can choose the preferred algorithm. Currently, “GORIV” and “PsiPred” algorithms are available.

Range start / Range end — select the sequence range for prediction.

Results — the visual representation of the prediction results, for example:



Save as annotation — select this button to save the results as annotations of the current protein sequence.

SITECON

SITECON — is a program package for recognition of potential transcription factor binding sites basing on the data about conservative conformational and physicochemical properties revealed on the basis of the binding sites sets analysis.

To cite *SITECON* use the following article:

“Oshchepkov D.Y., Vityaev E.E., Grigorovich D.A., Ignatieva E.V., Khlebodarova T.M. *SITECON*: a tool for detecting conservative conformational and physicochemical properties in transcription factor binding site alignments and for siterecognition. //Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W208-12.”

UGENE version of *SITECON* provides a tool for recognition of potential binding sites for over *90 types* of transcription factors. Also UGENE version of *SITECON* provides a tool for recognition of potential binding sites basing site alignment proposed by user. For the detailed method description see the [original SITECON site](#).

Data about used context-dependent conformational and physicochemical properties are available in the [PROPERTY Database](#).

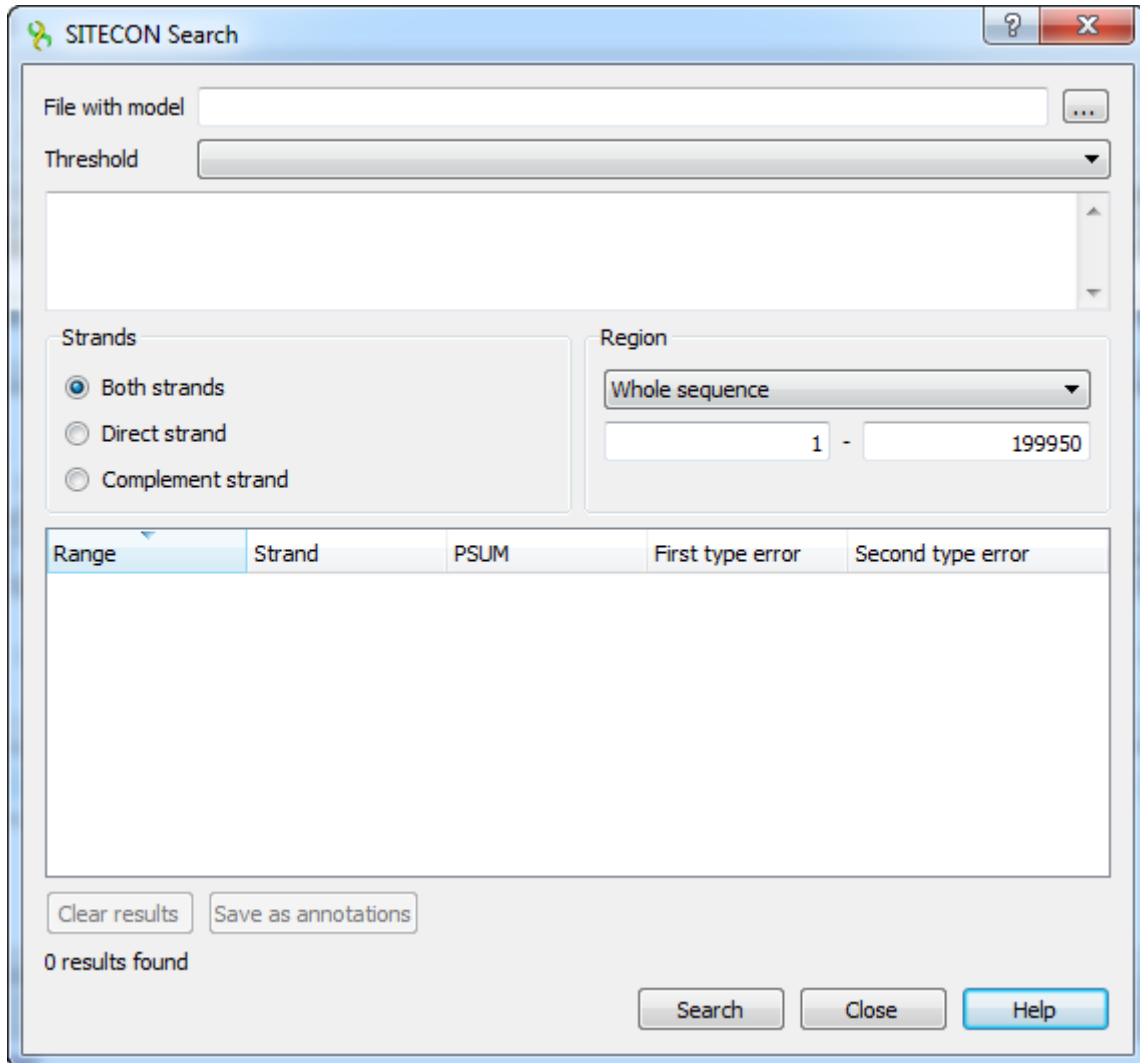
- [SITECON Searching Transcription Factors Binding Sites](#)
- [Types of SITECON Models](#)
 - Eukaryotic
 - Prokaryotic
- [Building SITECON Model](#)

SITECON Searching Transcription Factors Binding Sites

To search transcription factor binding sites in a DNA sequence select the *Analyze Search TFBS with SITECON...* context menu item.

In the appeared search dialog you must select a file with TFBS profile. The profiles supplied with UGENE are placed in the \$UGENE/data/sitecon_models folder.

After the profile is loaded the threshold-filter is populated with values read from profile. You can use the filter to remove low-scoring regions from the result.



The regions found by SITECON algorithm can be saved as annotations to the DNA sequence in the Genbank format.

Every *SITECON* profile supplied with UGENE contains complete information about calibration settings provided to UGENE team by the author of *SITECON*.

The original TFBS alignments used to calculate profiles can be requested directly from the author of *SITECON*.

Types of SITECON Models

- Eukaryotic
- Prokaryotic

Eukaryotic

Name	Description
CEBP_a	CCAAT-enhancer-binding protein_alpha
CEBP_all	CCAAT-enhancer-binding proteins
CLOCK	Circadian Locomotor Output Cycles Kaput
cMyc_can	Myc (c-Myc) is a regulator gene that codes for a transcription factor. A mutated version of Myc is found in many cancers.
CRE	Cyclic AMP response element
E2F1	Transcription factor E2F1 is a protein that in humans is encoded by the E2F1 gene.
E2F1/DP1sel1	E2F factors bind to DNA as homodimers or heterodimers in association with dimerization partner DP1.

EGR1	Early growth response protein 1
EKLF	Erythroid Kruppel-like Factor
ER2	Estrogen receptor beta
GATA_all	GATA transcription factors are a family of transcription factors characterized by their ability to bind to the DNA sequence "GATA"
GATA-1	GATA-binding factor 1
GATA-2	GATA-binding protein 2
GATA-3	Trans-acting T-cell-specific transcription factor GATA-3
HMG-1	High-mobility group protein 1
HNF-1	Hepatocyte nuclear factor 1
HNF-3	Hepatocyte nuclear factor 3
HNF-4	Hepatocyte nuclear factor 4
IRF	Interferon regulatory factors
isre	Interferon stimulation response element
MyoD	MyoD belongs to a family of proteins known as myogenic regulatory factors (MRFs)
MyOGsel3	Myogenin
NF-1	Neurofibromin 1
NF-E2	Transcription factor NF-E2 45 kDa subunit is a protein that in humans is encoded by the NFE2 gene.
NFATp	Pre-existing component of the NFAT(Nuclear factor of activated T-cells) transcription complex.
NFkB_all	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFkB_hetero	The p50 (NFkB1)/p65 (RELA) heterodimer is the most abundant form of NF-kB
NFkB_homo	The c-Rel protein is a member of the NF-kB family of transcription factors and contains a Rel homology domain
Nfy	Nuclear transcription factor Y
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
Oct-1	Octamer transcription factor 1
Oct_all	Octamer transcription factors
p53	Protein 53
PPRF	Paramedian pontine reticular formation
Pu1	Is a protein that in humans is encoded by the SPI1 gene
setCREB	cAMP response element-binding
setCREBzag	cAMP response element-binding
SRE_san	Serum response element
SRF	Serum response factor
STAT1	Signal Transducer and Activator of Transcription 1
STAT	Signal Transducer and Activator of Transcription

TTF1	Thyroid transcription factor 1
USF	Upstream stimulatory factors
yy1	Is a protein that in humans is encoded by the YY1 gene

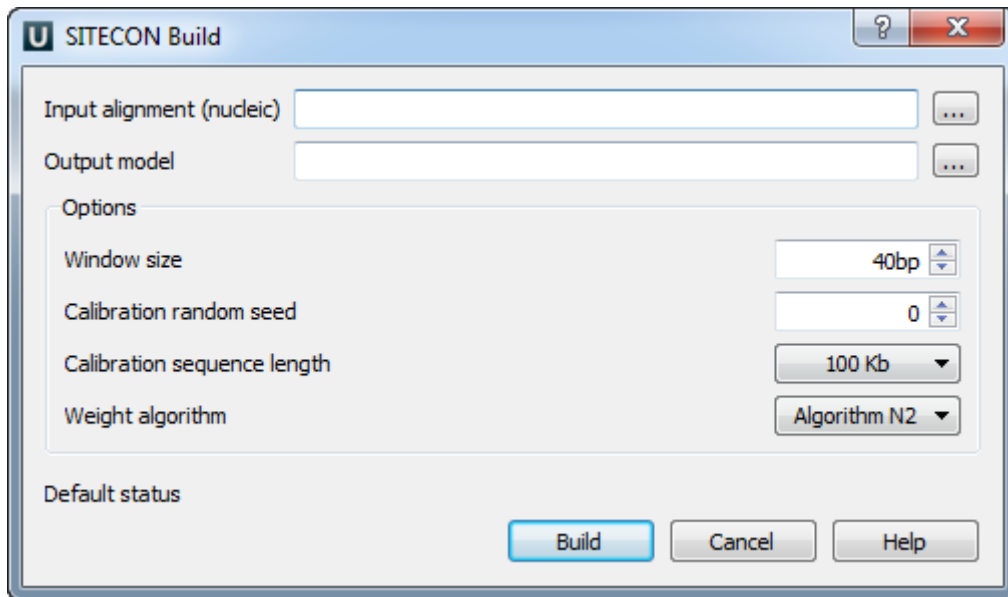
Prokaryotic

Name	Description
AgaR	N-acetylgalactosamine repressor, AgaR, negatively controls the expression of the aga gene cluster
AgaC	AgaC is the Enzyme IIC domain of a predicted N-acetylgalactosamine-transporting PEP-dependent phosphotransferase system
ArcA	ArcA transcriptional dual regulator
ArgR	ArgR complexed with L-arginine represses the transcription of several genes involved in biosynthesis and transport of arginine, transport of histidine, and its own synthesis and activates genes for arginine catabolism.
CpxR	DNA-binding response regulator in two-component regulatory system with CpxA
Crp	cAMP receptor protein
CysB	Cysteine B
CytR	Cytidine Regulator
DeoR	Deoxyribose Regulator
DnaA	DnaA is the linchpin element in the initiation of DNA replication in E. coli.
FadR	Fatty acid degradation Regulon
fis	Factor for inversion stimulation
FliHDC	Operon that encodes two transcriptional regulators
Fnr	FNR is the primary transcriptional regulator that mediates the transition from aerobic to anaerobic growth through the regulation of hundreds of genes.
FruR	Fructose repressor
FUR	Ferric Uptake Regulation
GALR	Galactose repressor
GALS	Galactose isorepressor
GLPR	sn-Glycerol-3-phosphate repressor
Gntp	Is a member of the GntP family transporters
HNS	Histone-like nucleoid structuring protein
ICLR	Isocitrate lyase Regulator
IHF	Integration host factor
ISCR1	Iron-sulfur cluster Regulator 1
ISCR3	Iron-sulfur cluster Regulator 3
LEXA	LexA represses the transcription of several genes involved in the cellular response to DNA damage or inhibition of DNA replication

Lrp	Leucine-responsive regulatory protein
MALT	Maltose regulator
MARA	Multiple antibiotic resistance
MELR	Melibiose regulator
MEtJ	MetJ represses the expression of genes involved in biosynthesis and transport of methionine
MetR1	MetR participates in controlling several genes involved in methionine biosynthesis [Weissbach91] and a gene involved in protection against nitric oxide
MLC	DgsA, better known as Mlc, "makes large colonies," is a transcriptional dual regulator that controls the expression of a number of genes encoding enzymes of the Escherichia coli phosphotransferase (PTS) and phosphoenolpyruvate (PEP) systems
MODE	Molybdate-responsive transcription factor
NAC	Nitrogen assimilation control
NAGC_new2	N-acetylglucosamine
NANR	N-acetyl-neuraminic acid regulator
NARL2	Nitrate/nitrite response regulator NarL
NARL	Nitrate/nitrite response regulator NarL
NARP	Nitrate/nitrite response regulator NarP
NIRC	NirC is a nitrite transporter which is a member of the FNT family of formate and nitrite transporters
OmpC	OmpC is a member of the GMP family
OxyR	Oxidative stress regulator
PHOB	PhoB is a dual transcription regulator that activates expression of the Pho regulon in response to environmental Pi
PHOP	Member of the two-component regulatory system phoQ/phoP involved in adaptation to low Mg ²⁺ environments and the control of acid resistance genes
PurR	PurR dimer controls several genes involved in purine nucleotide biosynthesis and its own synthesis
RcsB_1	Regulator capsule synthesis B
RcsB_2	Regulator capsule synthesis B
Rob2	Right origin-binding protein
ROB	Right origin-binding protein
soxS	SoxS is a dual transcriptional activator and participates in the removal of superoxide and nitric oxide and protection from organic solvents and antibiotics
TORR	TorR response regulator
TRPR	Tryptophan (trp) transcriptional repressor
TyrR	Tyrosine repressor

Building SITECON Model

To build a new SITECON model call the *Tools->SITECON->Build new SITECON model from alignment* main menu item. The following dialog will appear:

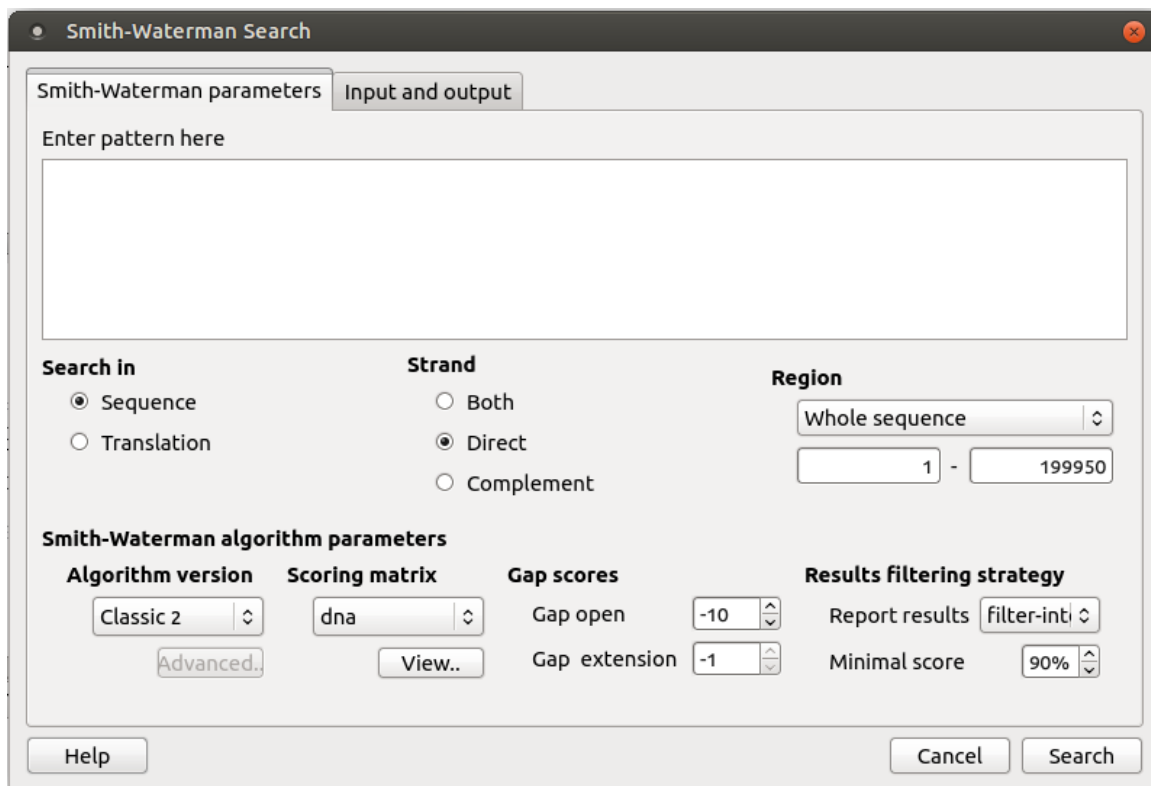


Here you need to select a nucleotide alignment and an output model. Optionally, you can change other parameters. After that click on the *Build* button.

Smith-Waterman Search

The *Smith-Waterman Search* plugin adds a complete implementation of the Smith-Waterman algorithm to UGENE.

To use the plugin open a nucleotide or protein sequence in the *Sequence View* and select the *Analyze Find pattern [Smith-Waterman]* item in the context menu. The *Smith-Waterman Search* dialog appears:



First of all you need to specify the pattern to search for. The rest parameters are optional:

Search in — select either to search in the sequence or in its translation.

Strand — select the strand to search in: direct, complementary or both strands.

Region — specifies the region of the sequence that will be used to search for the pattern. By default, if a subsequence has been

selected when the dialog has been opened, then the selected subsequence is searched for the pattern. Otherwise, the whole sequence is used. You can also input a custom range.

Algorithm version — version of the algorithm implementation. Non-classic versions produce the same results as classic but much faster. To use these optimizations our system must support these capabilities.

- Classic 2
- SSE2
- CUDA
- OPENCL

Scoring matrix — can be chosen from a bunch of matrices supplied with UGENE. To view a matrix selected click the *View* button.

Gap open — penalty for opening a gap.

Gap extension — penalty for extending a gap

Report results — simple heuristic which allows to filter intersected hits. If it is set to *none*, the algorithm may report large set of almost identical results in the same region.

Minimal score — another simple heuristic which measures sequences similarity. It is more convenient than using some abstract scores. If set to 100%, the algorithm will search for exact substring match.

The results of the search are saved as annotations or as multiple alignment. To set the saving parameters go to the *Input and output* tab of the dialog.

If you want to save the results as annotations input *the annotations saving* parameters (*Annotation name*, *Group name*, *Annotation type*, *Description* and a file to save the annotation to). Also you can add qualifier with corresponding pattern subsequences to result annotations. Check the corresponding checkbox for it.

If you want to save the results as multiple alignment select the following parameters:

The image shows a dialog box titled "Smith-Waterman Search" with two tabs: "Smith-Waterman parameters" and "Input and output". The "Input and output" tab is active. It contains the following fields and controls:

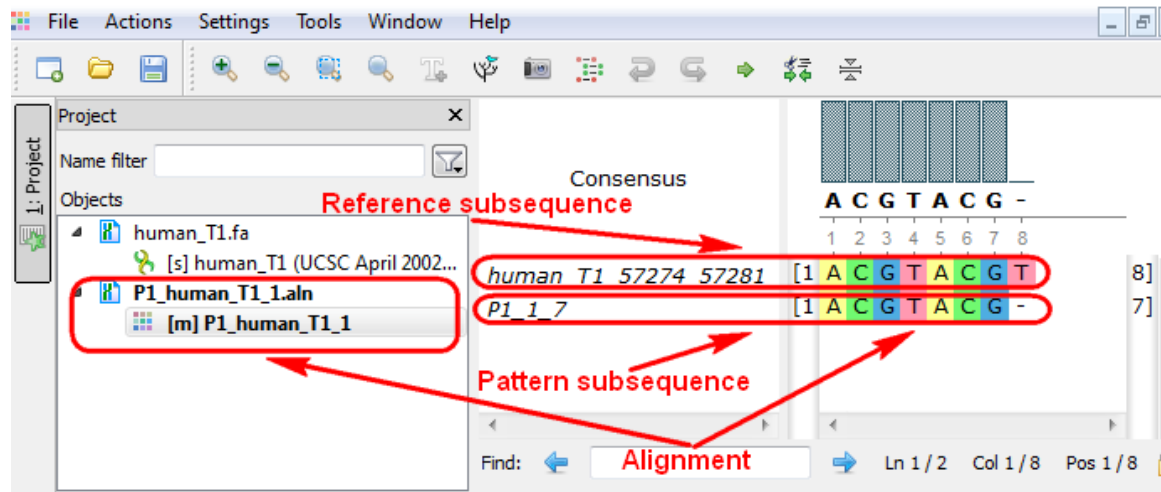
- Save results as:** A dropdown menu set to "Annotations".
- Save annotation(s) to:** A section with three radio buttons:
 - Existing table
 - Create new table (with a text field containing "/home/yalgaer/MyDocument.gb" and a file selection icon)
 - Use auto-annotations table
- Annotation parameters:** A section with four text fields:
 - Group name:** "<auto>" (with a refresh icon)
 - Annotation type:** "Misc. Feature" (with a dropdown arrow)
 - Annotation name:** "by type"
 - Description:** (empty)
- Add qualifier with corresponding pattern subsequences to result annotations
- Buttons at the bottom: "Help", "Cancel", and "Search".

Here you can select a file to save the alignment to (*Alignment files directory path* parameter).

Using the *Set advanced options* checkbox you can select the saving options.

You can set the different templates for files names: create your own or create by using the following: [E] — adds a subsequence end position, [hms] — adds a time, [MDY] — adds a date, [S] — adds a subsequence start position, [L] — adds a subsequence length, [SN] — adds a reference sequence name prefix, [PN] — adds a pattern sequence name prefix, [C] — adds a counter.

You can create templates for alignment files names, reference subsequence names, pattern subsequence names and for pattern sequence name:



HMM2

The *HMM2* plugin is a toolkit based on the Sean Eddy's HMMER2 package.

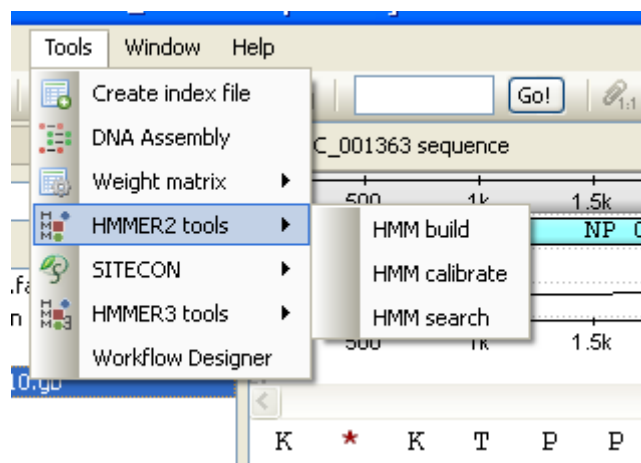
While working on this plugin we were guided by the following principles:

- Make the HMMER2 tools accessible to a wider user audience by providing graphical interface for all supported utilities for most of the platforms.
- Be compatible with the original HMMER2 package.
- Create the high-performance solution utilizing modern multi-core processors and SIMD instructions.

The current version of UGENE provides user interface for three HMM2 tools: *HMM build*, *HMM calibrate* and *HMM search*.

In the original program the corresponding commands are: "hmmbuild", "hmmcalibrate" and "hmmsearch".

To access these tools select the *Tools HMMER2 tools* submenu of the program main menu:



We highly recommend reading the [original HMMER2 documentation](#) to learn how to use utilities provided by the plugin.



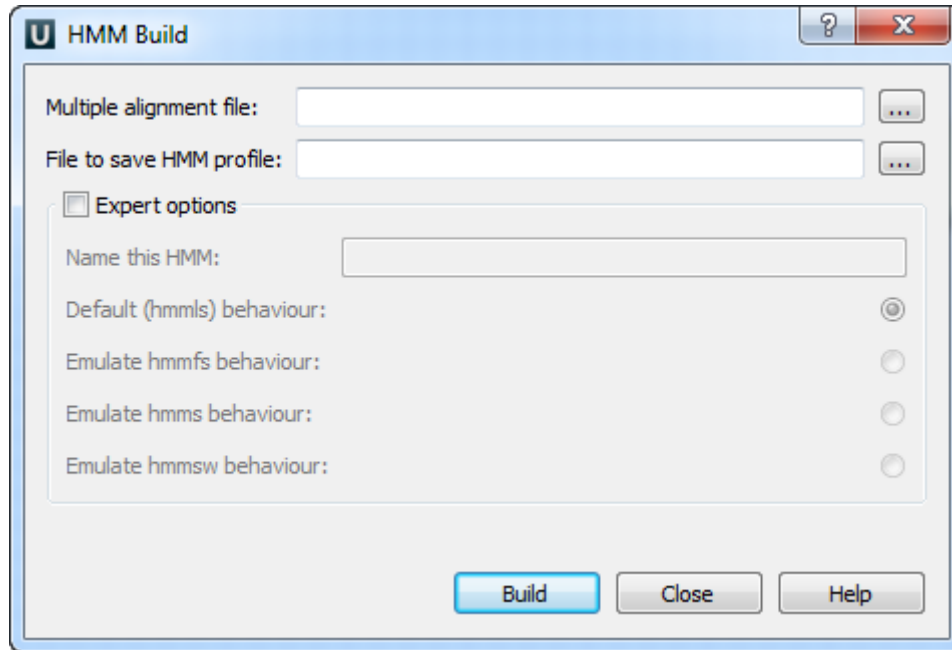
SSE2 algorithm is implemented by Leonid Konyaev, Novosibirsk State University. Use of the SSE2 optimized version of the *HMM search* algorithm with quad-core CPU gives >30x performance boost when compared with the original single-threaded algorithm (single sequence mode).

- Building HMM2 Model
- Calibrating HMM2 Model
- Searching Sequence Using HMM2 Profile

Building HMM2 Model

HMM build tool is used to build a new HMM profile from a multiple alignment.

You can use any alignment file formats supported by UGENE. The output HMM profile format is compatible with the HMMER2 package.

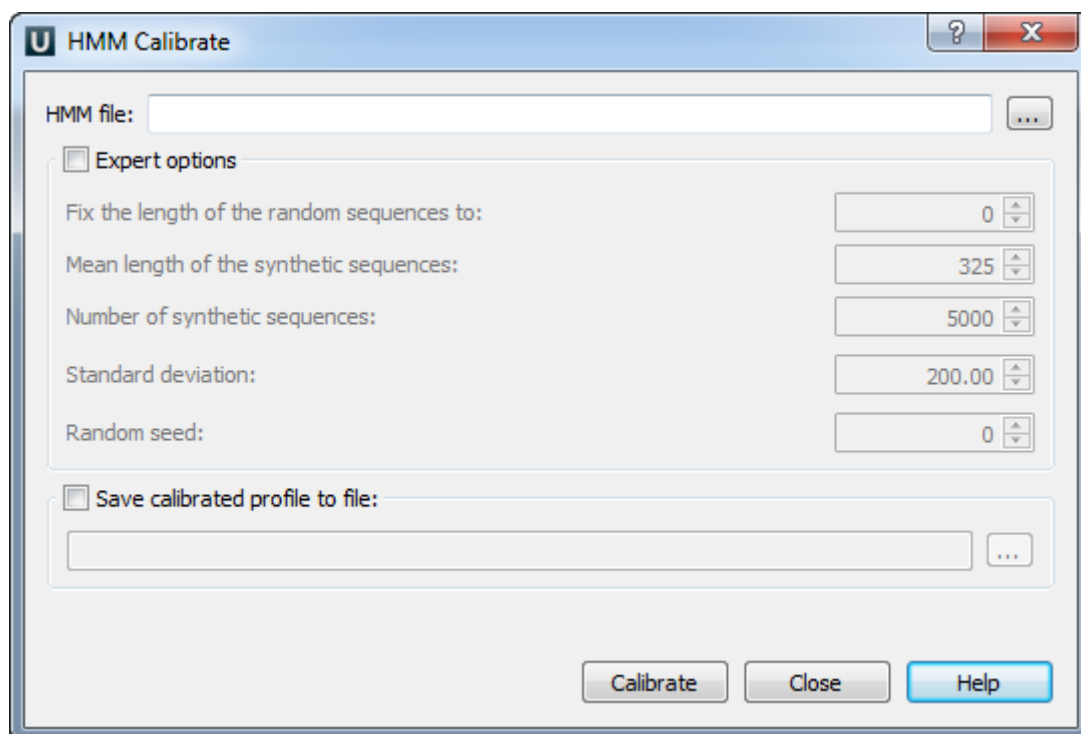


 The *HMM build* tool does not automatically calibrate a profile. Use the *HMM calibrate* tool to calibrate the profile.

Calibrating HMM2 Model

The *HMM calibrate* tool reads a HMM profile file, scores a large number of synthesized random sequences with it, fits an extreme value distribution (EVD) to the histogram of those scores, and re-saves the hmm file including the EVD parameters.

To avoid modification of the original HMM file you can select a new location for the calibrated profile.



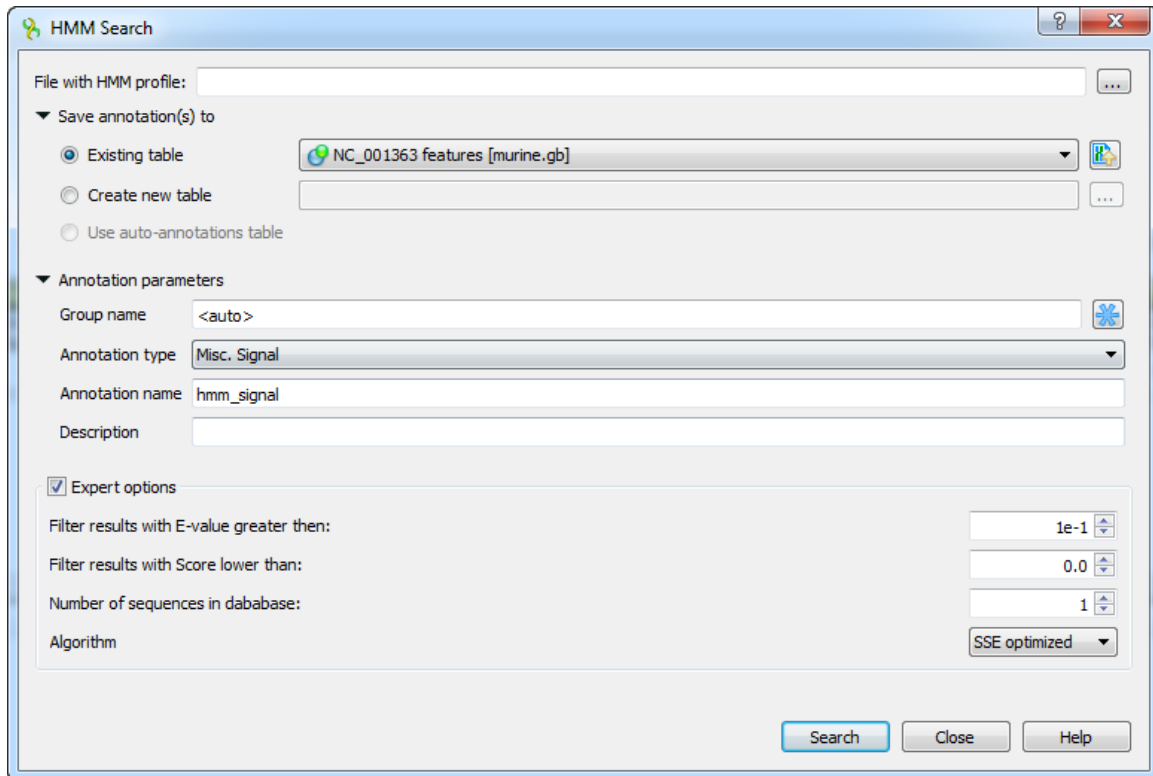
Searching Sequence Using HMM2 Profile

The *HMM search* tool reads a HMM profile from a file and searches the sequence for significantly similar sequence matches.

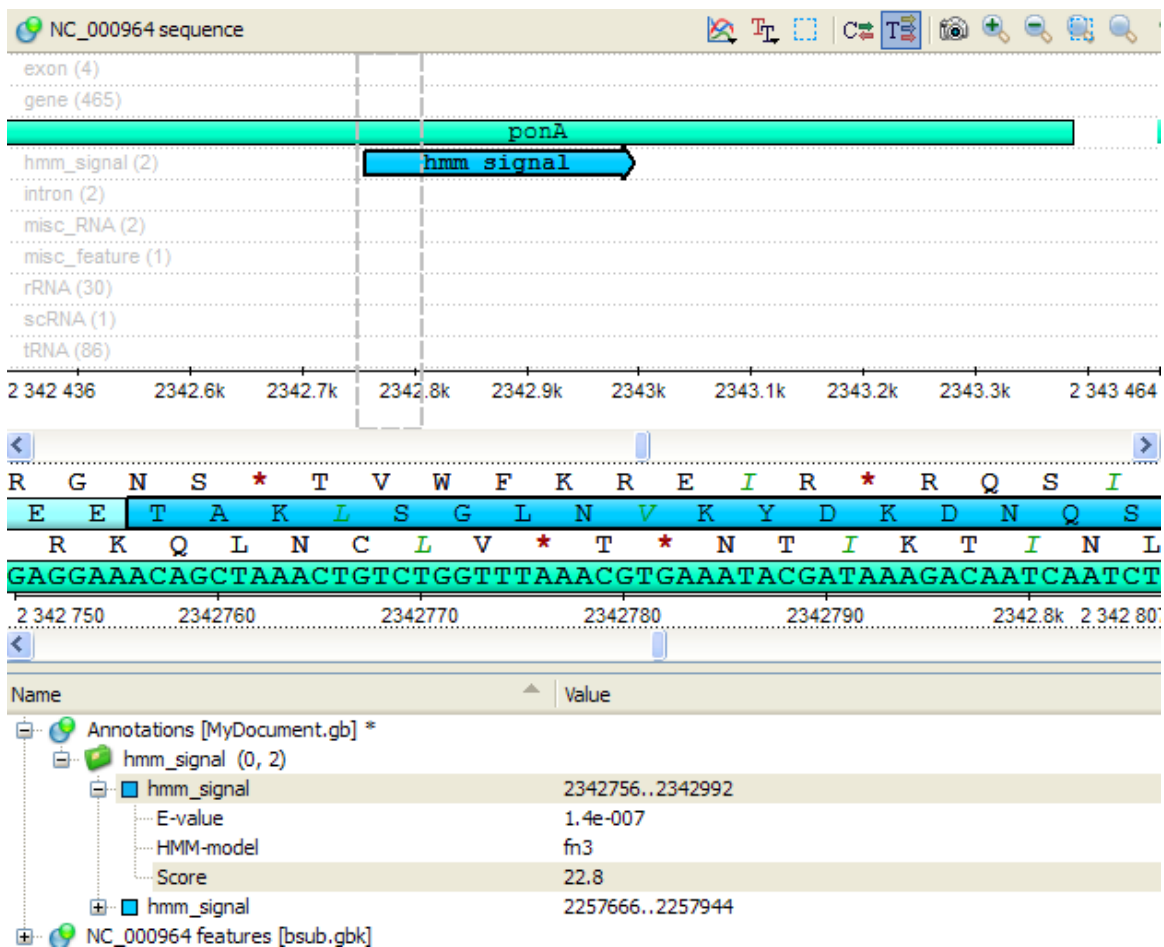
The sequence must be selected in the *Project View* or there must be an active *Sequence View* window opened.


If the selected sequence is nucleic and the HMM profile is built for amino alignment, the sequence is automatically translated and all 6 translations are used to search in.

If a HMM profile is built for nucleic alignment, the search is performed for both strands (direct and complement).



The search results are stored as sequence annotations in the Genbank file format.



 All HMM2 UGENE tools work only with files that contain a single HMM model.

HMM3

The *HMM3* plugin is a toolkit based on the Sean Eddy's [HMMER3 package](#).

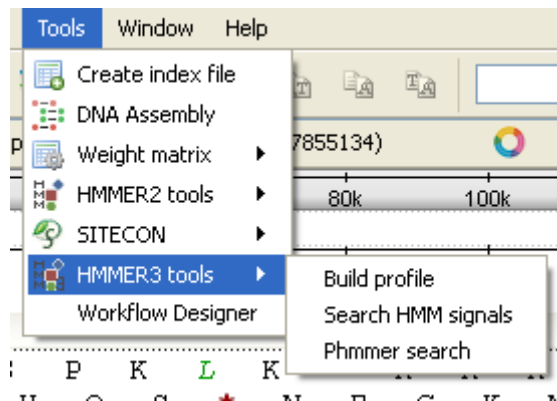
While working on this plugin we were guided by the following principles:

- Make the HMMER3 tools accessible to a wider user audience by providing graphical interface for all supported utilities for most of the platforms.
- Be compatible with the original HMMER3 package.
- Create the high-performance solution utilizing modern multi-core processors.

The current version of UGENE provides user interface for three HMM3 tools: *HMM3 build*, *HMM3 search* and *Phmmer search*.

In the original program the corresponding commands are: “hmmbuild”, “hmmsearch” and “phmmer”.

To access these tools select the *Tools HMMER3 tools* submenu of the program main menu:



We highly recommend reading the original HMMER3 documentation to learn how to use utilities provided by the plugin.

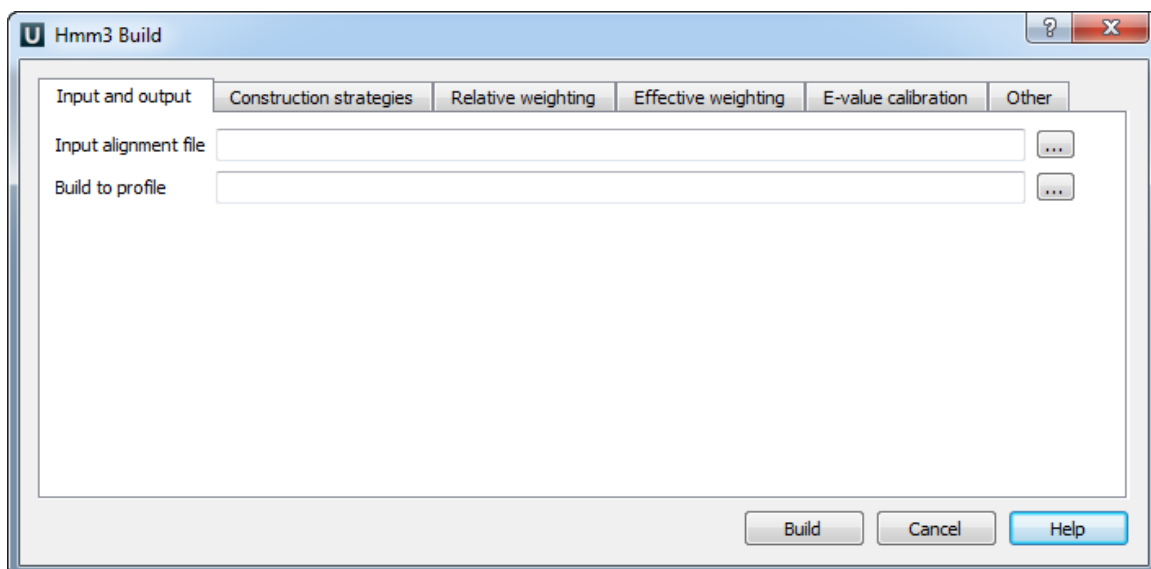
- [Building HMM Model](#)
- [Searching Sequence Using HMM Profile](#)
- [Searching Sequence Against Sequence Database](#)

Building HMM Model

The *HMM3 build* tool is used to build a new HMM profile from a multiple alignment. You can use any alignment file formats supported by UGENE.

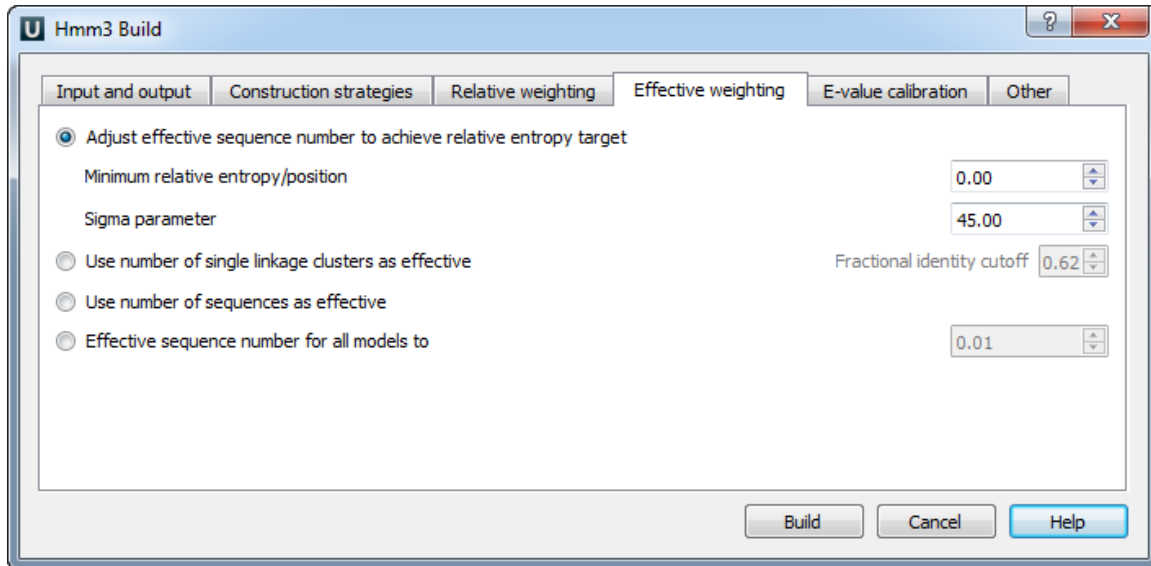
The output HMM profile format is compatible with the HMMER3 package, but it is not compatible with the HMMER2.

The *HMM3 build* automatically calibrates the target model.



The HMM3 configuration dialog provides an easy way to set appropriate search parameters.

Here you can see effective weighting strategies options:



Searching Sequence Using HMM Profile

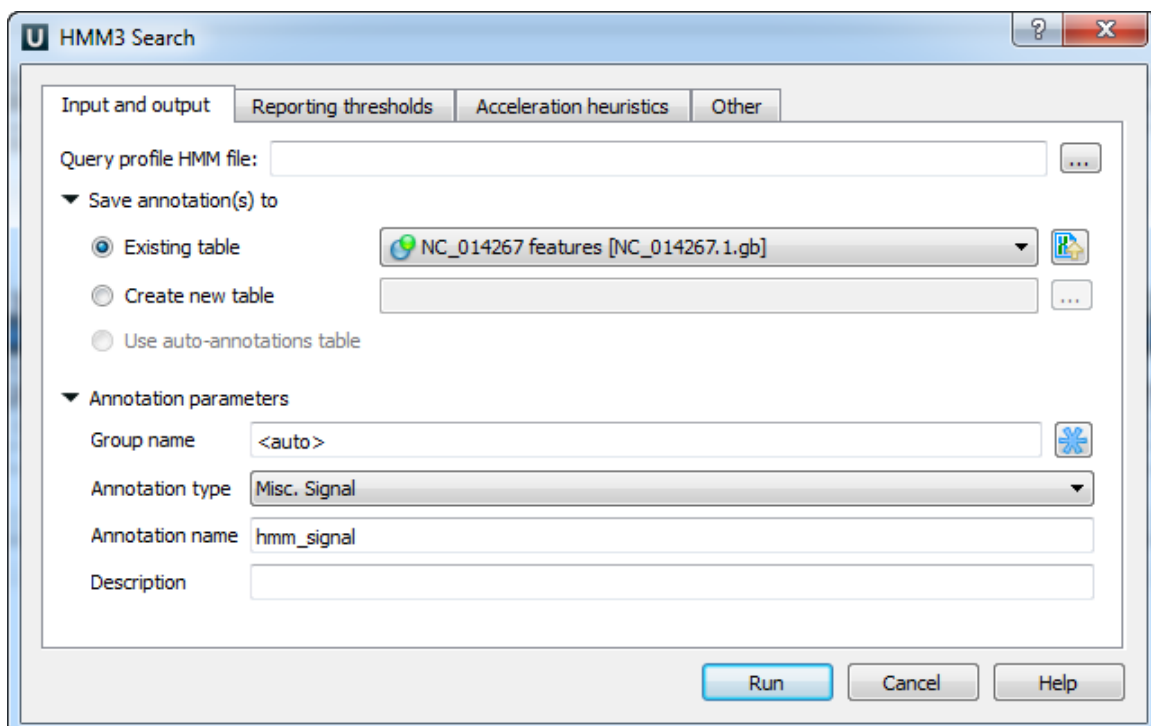
The *HMM3 search* tool reads a HMM profile from a file and searches a sequence for significantly similar sequence matches.

The sequence must be selected in the *Project View* or there must be an active *Sequence View* window opened.

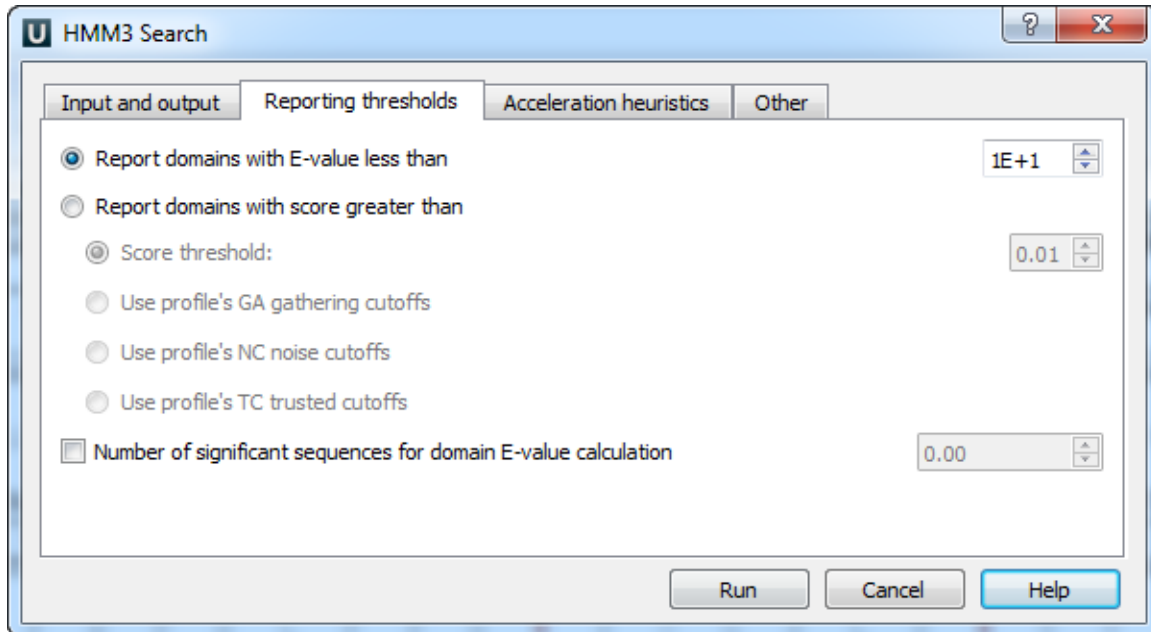
If the selected sequence is nucleic and profile HMM is built from amino alignment, the sequence will be automatically translated and searched in all possible frames (6 totally).

If a profile HMM is built for nucleic alignment, the search is performed for both strands (direct and complement).

The *HMM3 search* accepts the HMMER2 HMM profiles (amino only) as a backward compatibility feature. An interesting post about using the HMMER2 models with the HMMER3 is available on the [Sean Eddy's blog](#).




For example, reporting thresholds options can be configured using the dialog:



The search results are stored as sequence annotations in the Genbank file format.

Name	Value
Annotations [MyDocument_3.gb]*	
hmm_signal (0, 24024)	
hmm_signal	6594..6679
hmm_signal	6695..6781
hmm_signal	6796..6882
hmm_signal	6992..7076
hmm_signal	7092..7177
Accuracy per residue	9.76351e-01
Bias	3.53754e-02
Conditional e-value	5.96204e-17
Envelope of domain location	7091...7177
HMM model	fn3 Accession number in PFAM database: PF00041
HMM region	1...87
Independent e-value	1.89874e-17
Score	49.864132
hmm_signal	7288..7372
hmm_signal	7387..7473

 The HMM3 search works only with files that contain a single HMM model.

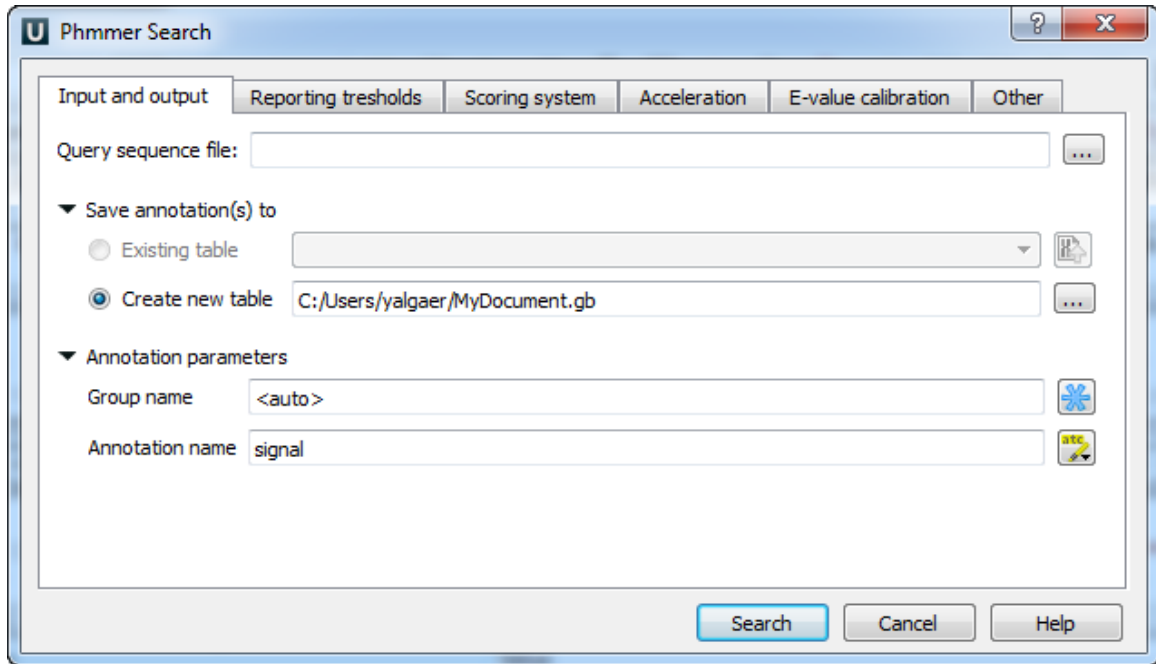
Searching Sequence Against Sequence Database

The *Phmmer* search tool searches for query sequence matches in sequence database, much as BLASTP or FASTA would do.

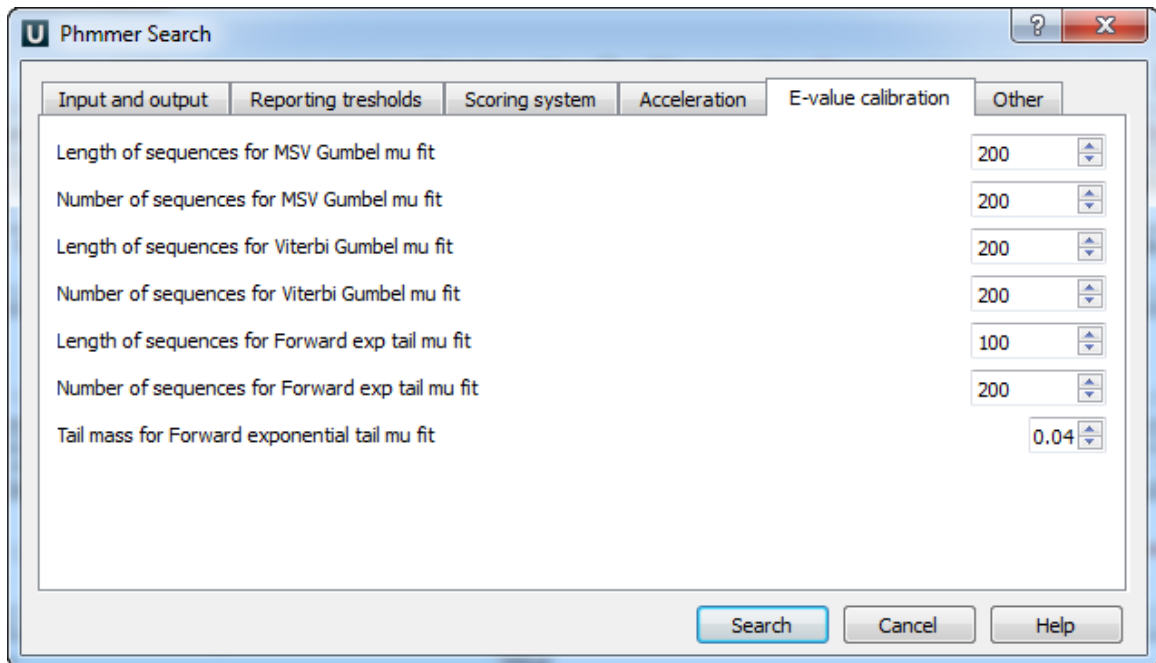
The *Phmmer* search works essentially like the *HMM3* search does, except you provide a query sequence instead of a query profile HMM.

The database sequence must be selected in the *Project View* or there must be an active *Sequence View* window opened.

Select the query sequence in the *Phmmer* search dialog:



You can set options of the *Phmmer search* by choosing the needed dialog tab. Here you can see the e-value calibration options:



The results are stored as sequence annotations in the Genbank file format.

The screenshot displays the Unipro UGENE interface. At the top, a genomic track shows a sequence of pink vertical bars representing a signal. Below the track, a sequence of amino acid residues is shown: LHEGMEYTFRVSAENKYGVGEGLEKSEPIVARHPFDVDPDAPPPPNIVDVRHDS. A pink box highlights the segment GVGEGLEKSEPIVARHPFDVDPDAPPPPNIVDVRHDS. Below the sequence, a table lists annotations for this region.

Name	Value
Annotations [MyDocument_3.gb] *	
signal (0, 546)	
signal	9781..9856
signal	10471..10549
signal	13660..13717
signal	36707..36782
signal	37397..37475
signal	40586..40643
Accuracy per residue	8.02474e-01
Bias	1.38025e-02
Conditional e-value	1.34634e-01
Envelope of domain location	40579...40645
HMM region	804...858
Independent e-value	1.34634e-01
Query sequence	fibronectin_1.2_1
Score	-3.513604
signal	63633..63708
Accuracy per residue	7.36235e-01
Bias	4.17314e-03

! The *Phmmer* search works only with single-sequence databases.

uMUSCLE

UGENE contains graphical ports of the Robert C. Edgar's MUSCLE tool for multiple alignment.

! MUSCLE4 is not supported since UGENE version 1.7.2.

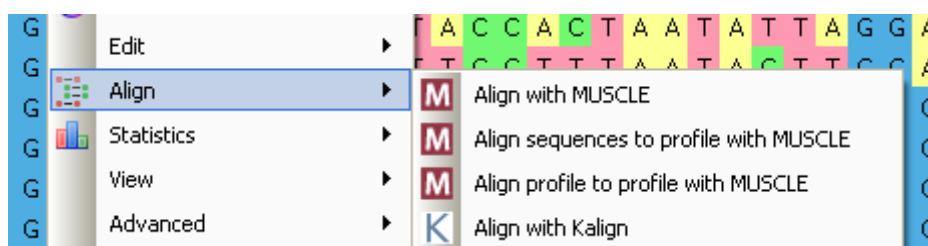
The package is integrated completely, so there is no need in extra files for using it. It is possible to run several multiple alignment tasks in parallel, check the progress and cancel the running tasks safely.

! The k-mer clustering part of the MUSCLE algorithm was optimized for multicore systems by Timur Tleukenov, Novosibirsk State Technical University.

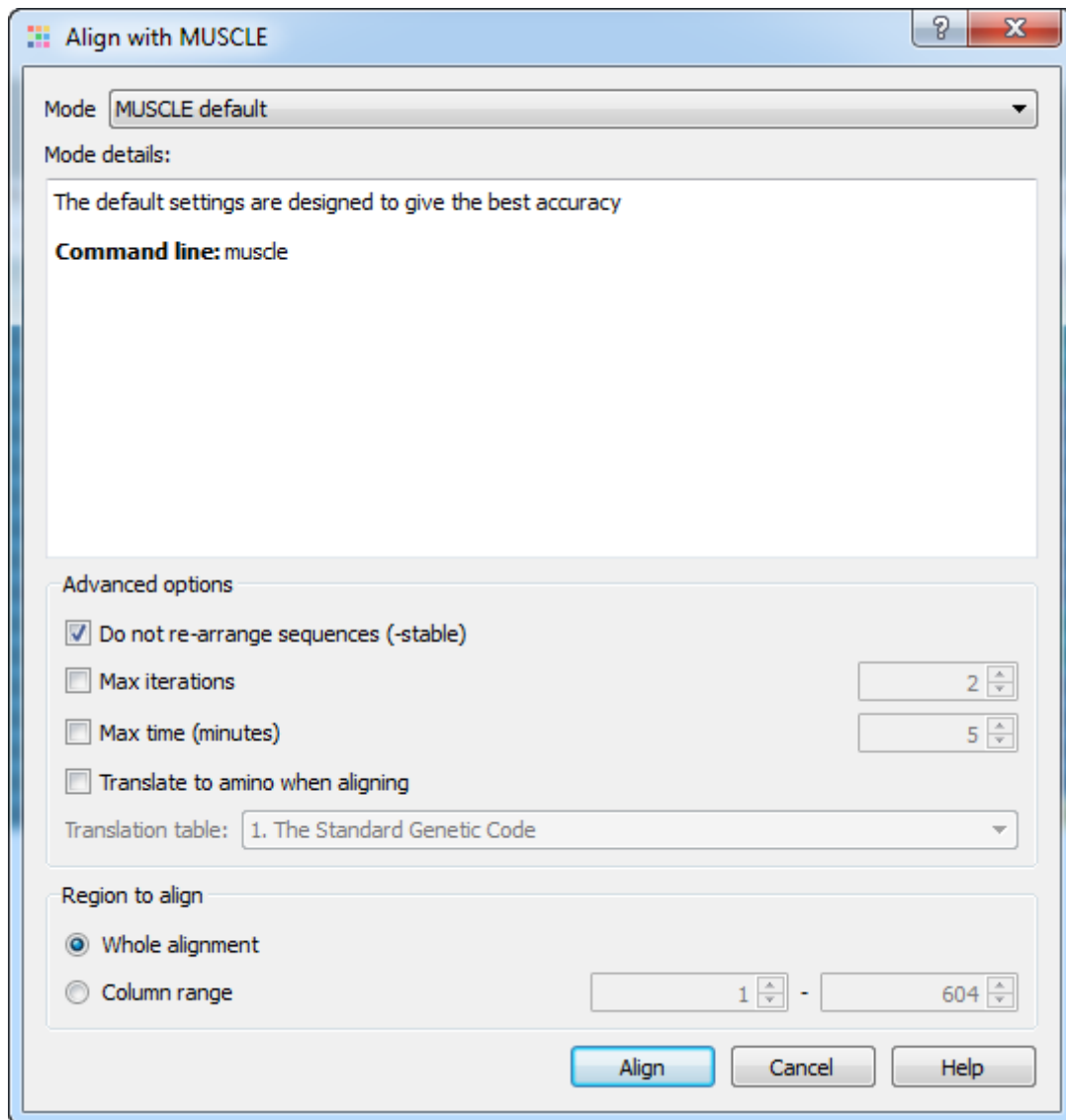
- MUSCLE Aligning
- Aligning Profile to Profile with MUSCLE
- Aligning Sequences to Profile with MUSCLE

MUSCLE Aligning

To run the classic MUSCLE use the *Align* *Align with MUSCLE* context menu item in the *Alignment Editor*.



The dialog contains the list of MUSCLE modes: *MUSCLE default*, *Large alignment*, *Refine only*.



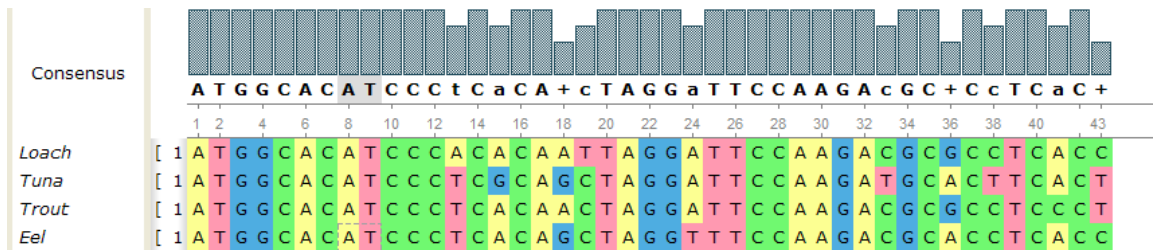
⚠ By default UGENE does not rearrange sequence order in an alignment, but the original MUSCLE package does. To enable sequence rearrangement uncheck the *Do not re-arrange sequences (-stable)* option in the dialog.

One of the improvements to the original MUSCLE package is the ability to align only a part of the model. When the *Column range* item is selected the region of the specified columns is only passed to the MUSCLE alignment engine. The resulted alignment is inserted into the original one with gaps added or removed on the region boundaries.

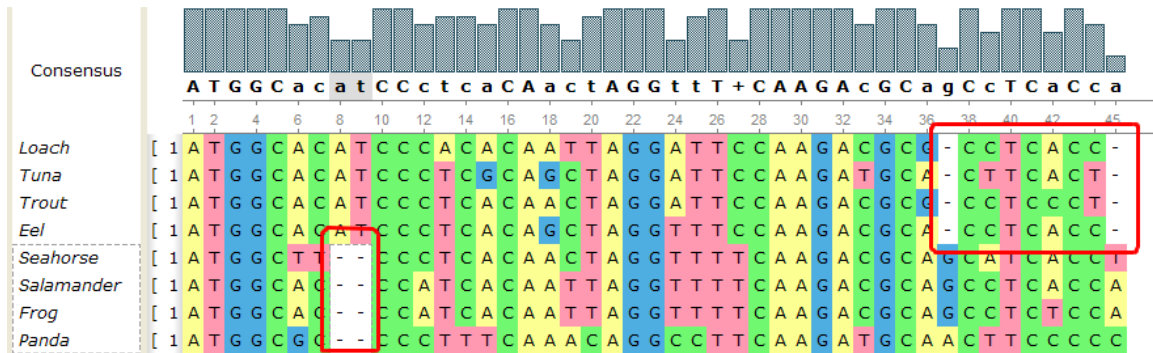
⚠ To visually select the column range to align, make a selection in the alignment editor first. Then invoke the MUSCLE plugin. Its column range boundary values will automatically match the given selection.

Aligning Profile to Profile with MUSCLE


The *Align > Align profile to profile with MUSCLE* context menu item allows to align an existing profile to an active alignment. During this process the MUSCLE does not realign the profiles, but inserts columns with gaps characters only ('—' characters). For example, the alignment in the picture below could be used as a profile:



The same profile after profile-to-profile alignment:



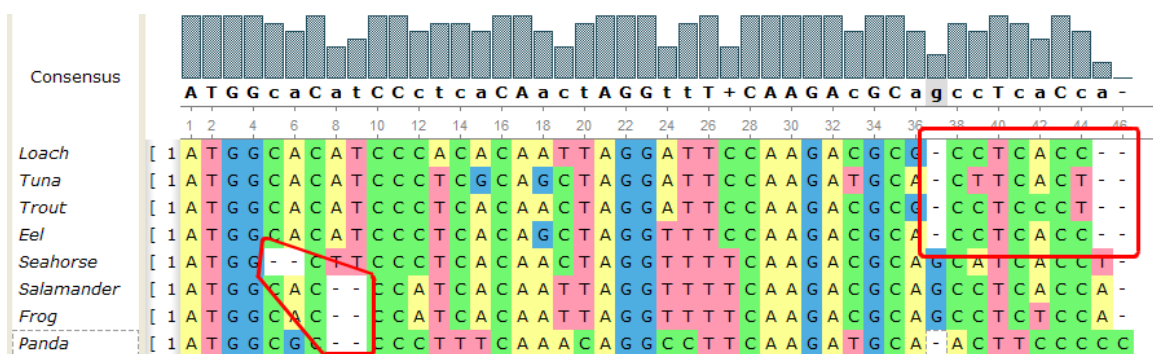
There are two gap columns inserted into the source profile, and two gap columns inserted into the added one. Therefore the profiles columns kept intact and the alignments haven't been changed.

 Aligning a profile to the active alignment you will modify the original alignment file, since it will contain 2 profiles after the operation is completed.

Aligning Sequences to Profile with MUSCLE

Another feature provided by the plugin is aligning a set of unaligned sequences to an existing profile. To use this feature select the *Align sequences to profile with MUSCLE* context menu item.

This option is not available in the original MUSCLE package (v3.7) and is a new functionality for original MUSCLE users. In this mode each sequence from the input file is aligned to the active profile separately and is merged to the result alignment only after all sequences are processed. For example, the alignment in the picture above can be used as a profile again. And the added profile can be used as a set of sequences. The result of such sequences-to-profile alignment is presented on the picture below:



The original alignment is not modified, only columns with gap ('—') character can be inserted.

The second profile was considered as a set of sequences and therefore is modified.

Note that if a file with another alignment is used as a source of unaligned sequences, the gap characters are removed and each input sequence is processed independently.

This method is quite fast, for example an alignment of 3000 sequences (1000 bases each) to the existing profile takes about 5 minutes on the usual Core2Duo computer.

ClustalW

Clustal is a widely used multiple sequence alignment program. It is used for both nucleotide and protein sequences. *ClustalW* is a command-line version of the program.

Clustal home page: <http://www.clustal.org>

If you are using Windows OS, there are no additional configuration steps required, as *ClustalW* executable file is included to the UGENE distribution package. Otherwise:

- Install the *Clustal* program on your system.
- Set the path to the *ClustalW* executable on the *External tools* tab of UGENE *Application Settings* dialog.

Now you are able to use *Clustal* from UGENE.

Open a multiple sequence alignment file and select the *Align with ClustalW* item in the context menu or in the *Actions* main menu. The *Align with ClustalW* dialog appears (see below), where you can adjust the following parameters:

Gap opening penalty — cost of opening up a new gap in the alignment. Increasing this value will make gaps less frequent.

Gap extension penalty — cost of every item in a gap. Increasing this value will make gaps shorter.

Weight matrix — specifies a single weight matrix for nucleotide sequences or series of matrices for protein sequences.

For nucleotide sequences the weight matrix selected defines the scores assigned to matches and mismatches (including IUB ambiguity codes), it can take values:

- *IUB* — default scoring matrix used by BESTFIT for the comparison of nucleic acid sequences. X's and N's are treated as matches to any IUB ambiguity symbol. All matches score 1.9; all mismatches for IUB symbols score 0.
- *CLUSTALW* — previous system used by ClustalW, in which matches score 1.0 and mismatches score 0. All matches for IUB symbols also score 0.

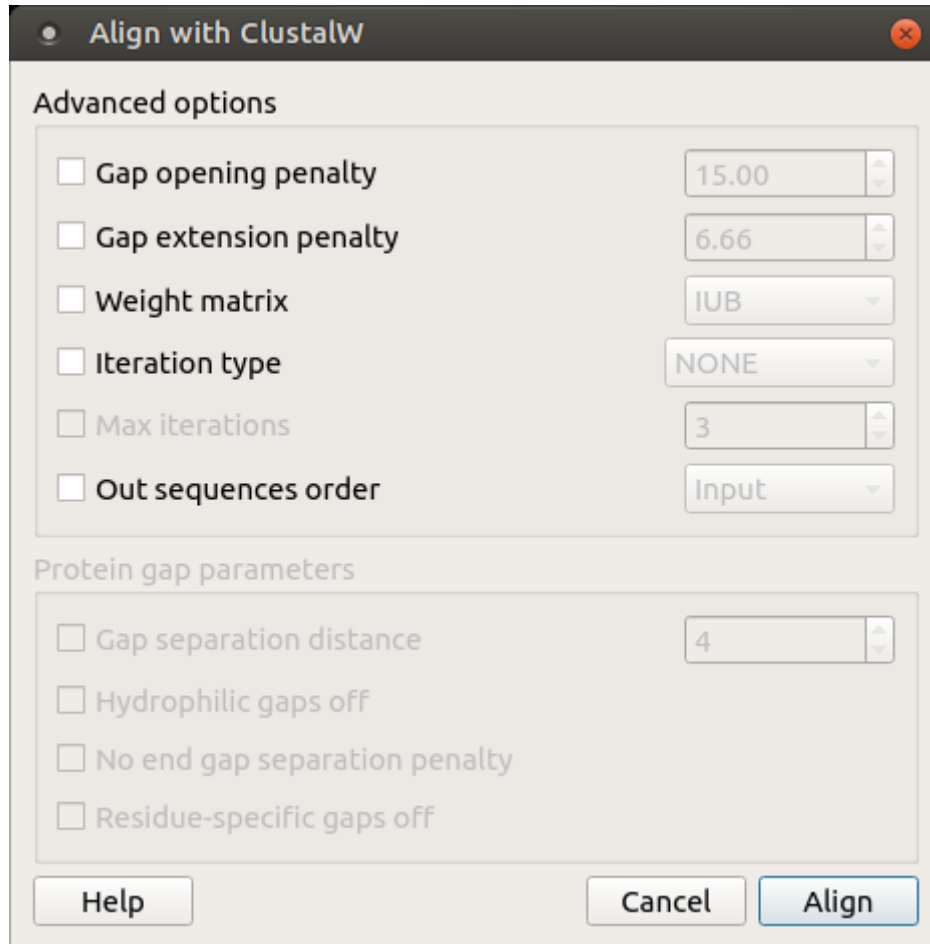
For protein sequences it describes the similarity of each amino acid to each other. The following values are available:

- *BLOSUM* — **B**LO**C**k**S** of **A**mino **A**cid **S**UBstitution **M**atrices first introduced in a paper by Henikoff and Henikoff. These matrices appear to be the best available for carrying out data base similarity (homology searches).
- *PAM* — **P**oint **A**ccepted **M**utation **M**atrices introduced by Margaret Dayhoff. These have been extremely widely used since the late '70s.
- *GONNET* — these matrices were derived using almost the same procedure as the Dayhoff one (above) but are much more up to date and are based on a far larger data set. They appear to be more sensitive than the Dayhoff series.
- *ID* — identity matrix which gives a score of 1.0 to two identical amino acids and a score of zero otherwise.

Iteration type — specifies the iteration type to use. During the iteration step each sequence is removed in turn and realigned. It is kept if the resulting alignment is better than the one has been made before. This process is repeated until the score converges or until the maximum number of iterations is reached. Available values are:

- *NONE* — specifies not to use iterations.
- *TREE* — specifies to iterate at each step of the progressive alignment.
- *ALIGNMENT* — specifies to iterate on the final alignment.

Max iterations — maximum number of iterations.



The following parameters are only available for protein sequences:

Gap separation distance — tries to decrease the chances of gaps being too close to each other. Gaps that are less than this distance apart are penalized more than other gaps. This does not prevent close gaps; it makes them less frequent, promoting a block-like appearance of the alignment.

Hydrophilic gaps off — increases the chances of a gap within a run of hydrophilic amino acids.

No end gap separation penalty — treats end gaps just like internal gaps to avoid gaps that are too close.

Residue-specific gaps off — amino acid specific gap penalties that reduce or increase the gap opening penalties at each position in the alignment or sequence. For example, positions that are rich in glycine are more likely to have an adjacent gap than positions that are rich in valine.

MAFFT

Originally, MAFFT is a multiple sequence alignment program for UNIX-like operating systems. However, currently, it is available for macOS, Linux, and Windows. It is used for both nucleotide and protein sequences.

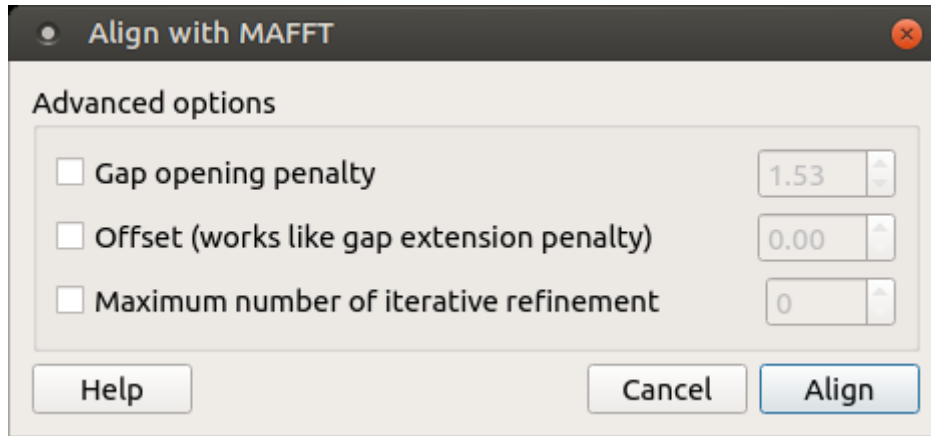
MAFFT homepage: <http://mafft.cbrc.jp/alignment/software>

To make *MAFFT* available from UGENE:

- Install the *MAFFT* program on your system.
- Set the path to the *MAFFT* executable on the *External tools* tab of UGENE *Application Settings* dialog.

For example, on Windows, you need to specify the path to the *mafft.bat* file.

To use *MAFFT* open a multiple sequence alignment file and select the *Align with MAFFT* item in the context menu or in the *Actions* main menu. The following dialog appears:



The following parameters are available:

Gap opening penalty — Gap opening penalty at group-to-group alignment.

Offset (works like gap extension penalty) — offset value, which works like the gap extension penalty, for group-to-group alignment.

Maximum number of iterative refine — specifies the number of cycles of iterative refinement to perform.

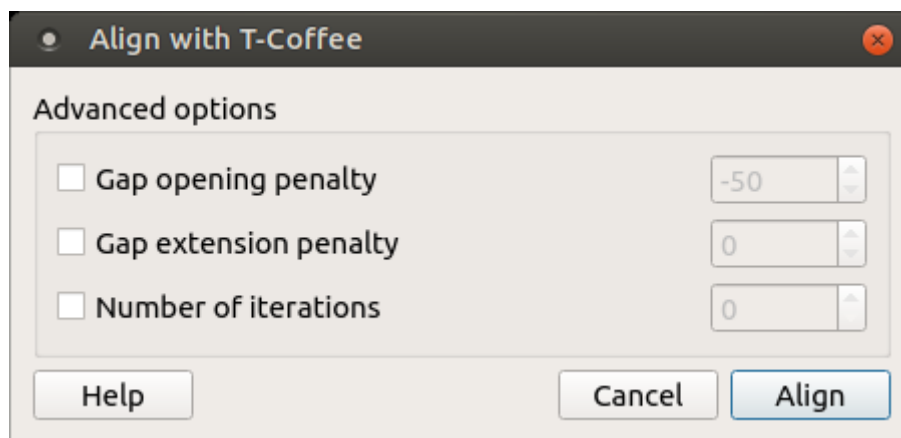
T-Coffee

T-Coffee is a multiple sequence alignment package.

T-Coffee home page: [T-Coffee](#)

To make *T-Coffee* available from UGENE see the [External Tools](#).

To use *T-Coffee* open a multiple sequence alignment file and select the *Align with T-Coffee* item in the context menu or in the *Actions* main menu. The following dialog appears:



The following parameters are available:

Gap opening penalty — indicates the penalty applied for opening a gap. The penalty must be negative.

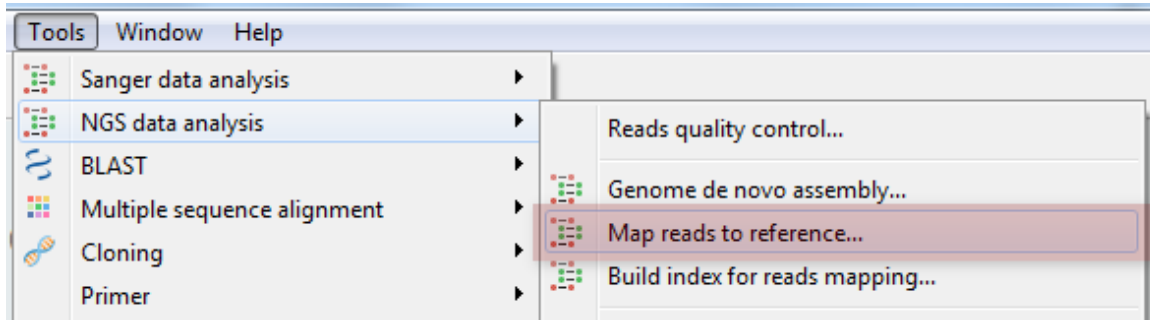
Gap extension penalty — indicates the penalty applied for extending a gap.

Number of iterations — specifies the number of iterations.

Bowtie

Bowtie is a popular short read aligner. Click [this link](#) to open *Bowtie* homepage. *Bowtie* is embedded as an *external tool* into UGENE.

Open *Tools* *DNA Assembly* submenu of the main menu.

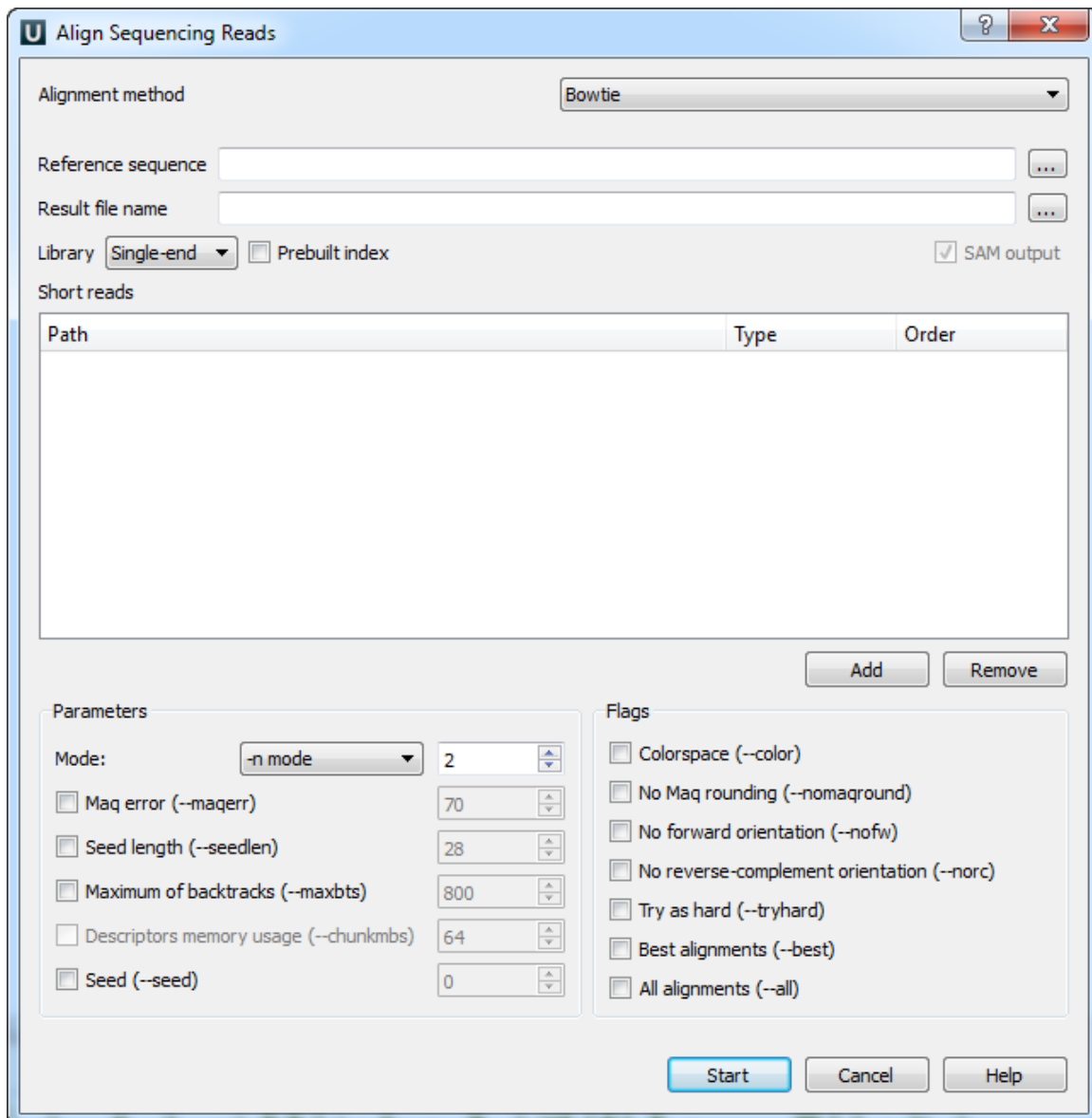


Select the *Align short reads* item to align short reads to a DNA sequence using *Bowtie*. Or select the *Build index* item to build an index for a DNA sequence which can be used to optimize aligning of the short reads to the sequence.

- [Bowtie Aligning Short Reads](#)
- [Building Index for Bowtie](#)

Bowtie Aligning Short Reads

When you select the *Tools DNA Assembly Align short reads* item in the main menu, the *Align Short Reads* dialog appears. Set value of the *Align short reads method* parameter to *Bowtie*. The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

Library - single-end or paired-end reads.

Prebuilt index — check this box to use an index file instead of a source reference sequence. The index is a set of 6 files with suffixes .1.ebwt, .2.ebwt, .3.ebwt, .4.ebwt, .rev.1.ebwt, and .rev.2.ebwt. The index is created during the alignment. Also you can *build it manually*.

SAM output — always save the output file in the SAM format (the option is disabled for *Bowtie*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.



Short reads length for *Bowtie* can't be more than 1024.

You can also configure other parameters. They are the same as in the original *Bowtie* (you can read detailed description of the parameters on the [Bowtie manual page](#)).

Select one of the following alignment modes:

The *-n* alignment mode:

When the *-n mode* is selected, *Bowtie* determines which alignments are valid according to the following policy. Alignments may have no more than N mismatches (where N is a number 0-3) in the first L bases (where L is a number 5 or greater, set with *Seed length*) on the high-quality (left) end of the read. The sum of the Phred quality values at all mismatched positions (not just in the seed) may not exceed E (set with *Maq error*). Where qualities are unavailable (e.g. if the reads are from a FASTA file), the Phred quality defaults to 40.

The *-v* alignment mode:

In *-v mode*, alignments may have no more than V mismatches, where V may be a number from 0 through 3. Quality values are ignored. The *-v mode* is mutually exclusive with the *-n mode*.

The following parameters are available:

Maq error (*-maqerr*) — maximum permitted total of quality values at all mismatched read positions throughout the entire alignment, not just in the “seed”. The default is 70. By default, *Bowtie* rounds quality values to the nearest 10 and saturates at 30. Note that the rounding can be disabled with *No Maq rounding*.

Seed Length (*-seedlen*) — the number of bases on the high-quality end of the read to which the *-n* applies. The lowest permitted setting is 5 and the default is 28.

Maximum of backtracks (*-maxbts*) — the maximum number of backtracks (default: 125 without *Best*, 800 with *Best*). A “backtrack” is the introduction of a speculative substitution into the alignment.

Descriptors memory usage (*-chunkmbs*) — the number of megabytes of memory a given thread is given to store path descriptors in the *Best* flag. Default: 64. This parameter is available if the *Best* flag is checked.

Seed (*-seed*) — pseudo-random number generator.

The following flags are available:

Colorspace (*-color*) — the input is read in colorspace, colors are encoded as characters A/C/G/T (A=blue, C=green, G=orange, T=red).

No Maq rounding (*-nomaqround*) — Maq (Mapping and Assembly with Quality) accepts quality values in the Phred quality scale, but internally rounds values to the nearest 10, with a maximum of 30. By default, *Bowtie* also rounds this way. *No Maq rounding* prevents this rounding in *Bowtie*.

No forward orientation (*-nofw*) — do not attempt to align against the forward reference strand.

No reverse-complement orientation (*-norc*) — do not attempt to align against the reverse-complement reference strand.

Try as hard (*-tryhard*) — try as hard as possible to find valid alignments when they exist, including paired-end alignments.

Best alignments (*-best*) — make *Bowtie* guarantee that reported singleton alignments are “best” in terms of stratum (i.e. number of mismatches, or mismatches in the seed for the case of *-n mode*) and in terms of the quality values at the mismatched position(s).

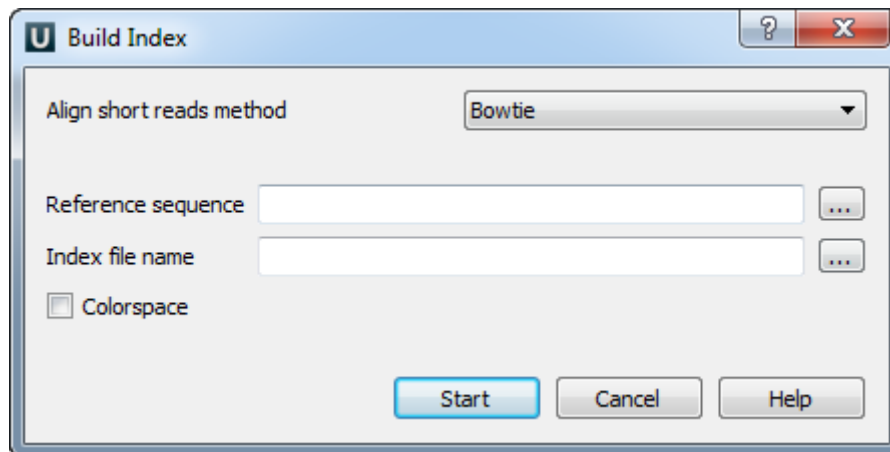
All alignments (*-all*) — report all valid alignments per read or pair. Validity of alignments is determined by the alignment policy (combined effects of *-n mode*, *-v mode*, *Seed length*, and *Maq error*).

Select the required parameters and press the *Start* button.

Building Index for Bowtie

To build *Bowtie* index select the *Tools NGS data analysis Build index for reads mapping* item in the main menu. The *Build Index* dialog appears. Set the *Align short reads method* parameter to *Bowtie*.

The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

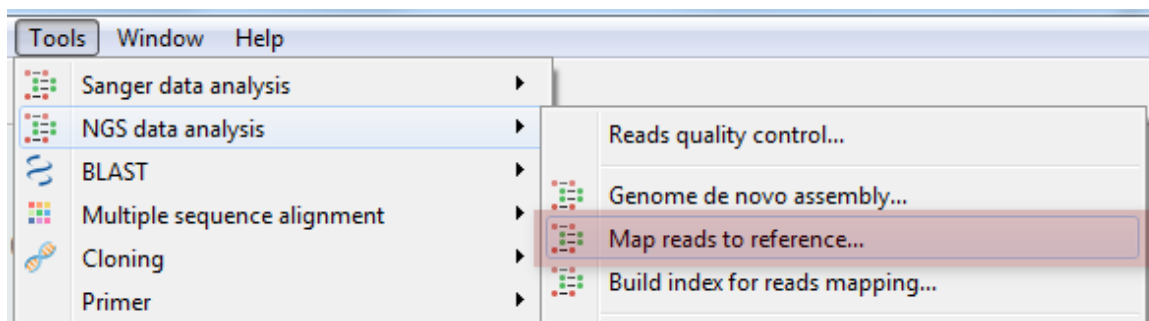
Index file name — a file to save the created index to. This parameter is required.

Colorspace (-color) — the input is read in colorspace, colors are encoded as characters A/C/G/T (A=blue, C=green, G=orange, T=red).

Bowtie 2

Bowtie 2 is a popular ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences. [Click this link](#) to open *Bowtie 2* homepage. *Bowtie 2* is embedded as an *external tool* into UGENE.

Open *Tools Align to reference* submenu of the main menu.



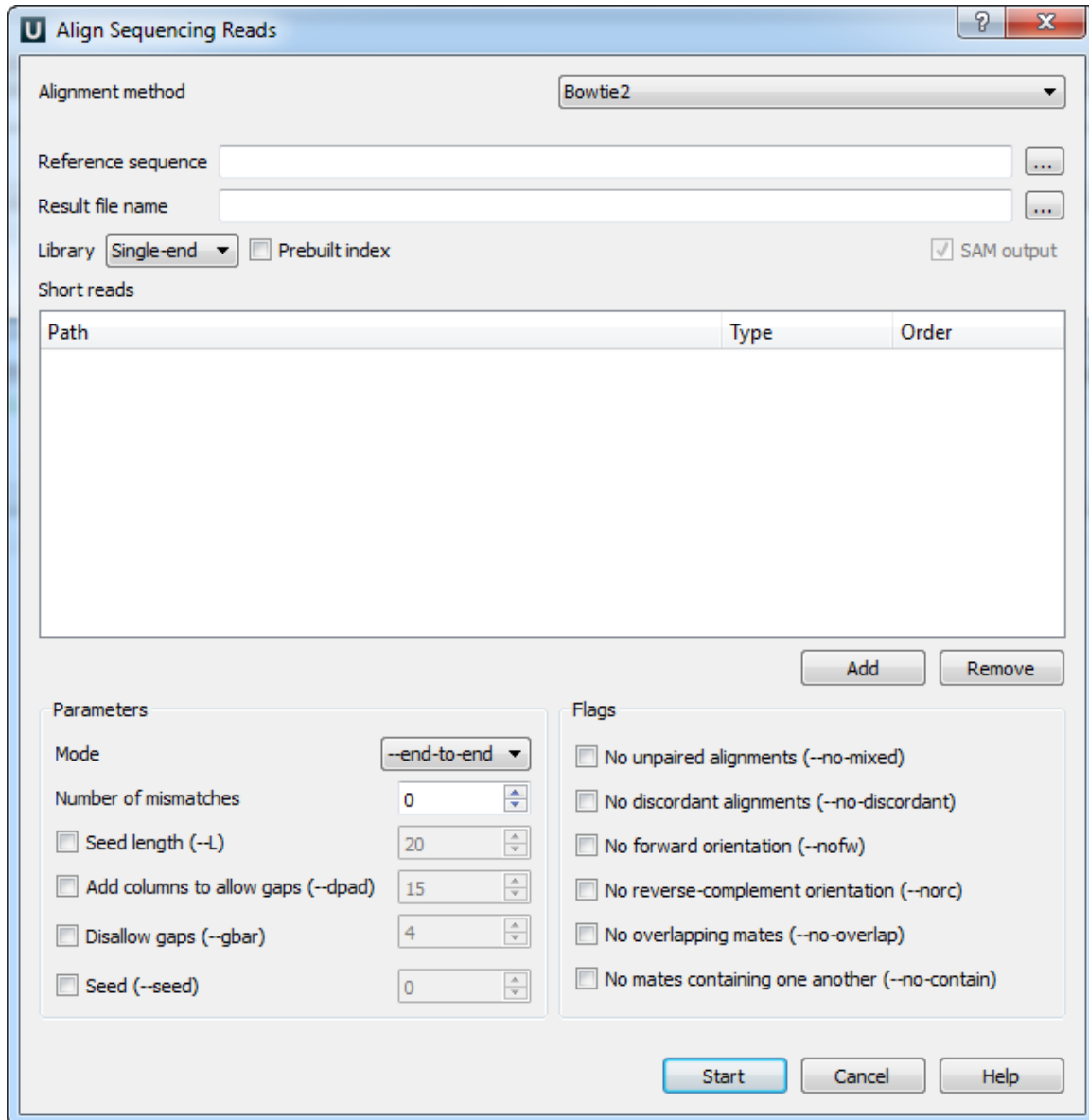
Select the *Align short reads* item to align short reads to a DNA sequence.

Or select the *Build index* item to build an index for a DNA sequence which can be used to optimize aligning of the short reads to the sequence:

- [Bowtie 2 Aligning Short Reads](#)
- [Building Index for Bowtie 2](#)

Bowtie 2 Aligning Short Reads

When you select the *Tools Align to reference Align short reads* item in the main menu, the *Align Sequencing Reads* dialog appears. Set value of the *Align short reads method* parameter to *Bowtie 2*. The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

Library - single-end or paired-end reads.

Prebuilt index — check this box to use an index file instead of a source reference sequence. The index is a set of 6 files with suffixes .1.ebwt, .2.ebwt, .3.ebwt, .4.ebwt, .rev.1.ebwt, and .rev.2.ebwt. The index is created during the alignment. Also you can *build it manually*.

SAM output — always save the output file in the SAM format (the option is disabled for *Bowtie*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.

You can also configure other parameters. They are the same as in the original *Bowtie 2* (you can read detailed description of the parameters on the *Bowtie 2 manual page*).

Select one of the following alignment modes:

The `--end-to-end` alignment mode:

By default, *Bowtie 2* performs end-to-end read alignment. That is, it searches for alignments involving all of the read characters. This is also called an "untrimmed" or "unclipped" alignment.

When the `--local` option is specified, *Bowtie 2* performs local read alignment. In this mode, *Bowtie 2*

might "trim" or "clip" some read characters from one or both ends of the alignment if doing so maximizes the alignment score.

The following parameters are available:

Number of mismatches (--N) — sets the number of mismatches to allowed in a seed alignment during **multiseed alignment**. Can be set to 0 or 1. Setting this higher makes alignment slower (often much slower) but increases sensitivity.

Seed length (--L) — Sets the length of the seed substrings to align during **multiseed alignment**. Smaller values make alignment slower but more sensitive.

Add columns to allow gaps (--dpad) — "Pads" dynamic programming problems by <int> columns on either side to allow gaps.

Disallow gaps (--gbar) — disallow gaps within <int> positions of the beginning or end of the read.

Seed (--seed) — use <int> as the seed for pseudo-random number generator.

The following flags are available:

No unpaired alignments (--no-mixed) — by default, bowtie2 cannot find a concordant or discordant alignment for a pair, it then tries to find alignments for the individual mates. This option disables that behavior.

No discordant alignments (--no-discordant) — by default, bowtie2 looks for discordant alignments if it cannot find any concordant alignments. A discordant alignment is an alignment where both mates align uniquely, but that does not satisfy the paired-end constraints. This option disables that behavior.

No forward orientation (--nofw) — if --nofw is specified, bowtie2 will not attempt to align unpaired reads to the forward (Watson) reference strand.

No reverse-complement orientation (--norc) — if --norc is specified, bowtie2 will not attempt to align unpaired reads against the reverse-complement (Crick) reference strand.

No overlapping mates (--no-overlap) — if one mate alignment overlaps the other at all, consider that to be non-concordant.

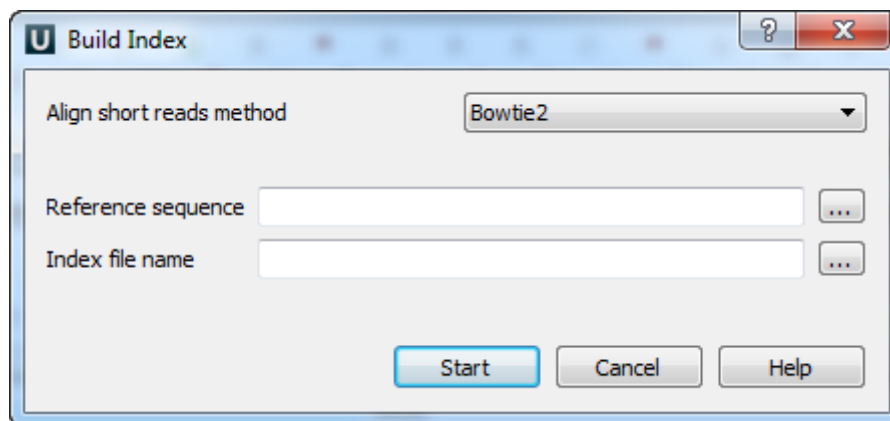
No mates containing one another (--no-contain) — if one mate alignment contains the other, consider that to be non-concordant.

Select the required parameters and press the *Start* button.

Building Index for Bowtie 2

To build *Bowtie 2* index select the *Tools NGS data analysis Build index for reads mapping* item in the main menu. The *Build Index* dialog appears. Set the *Align short reads method* parameter to *Bowtie 2*.

The dialog looks as follows:



There are the following parameters:

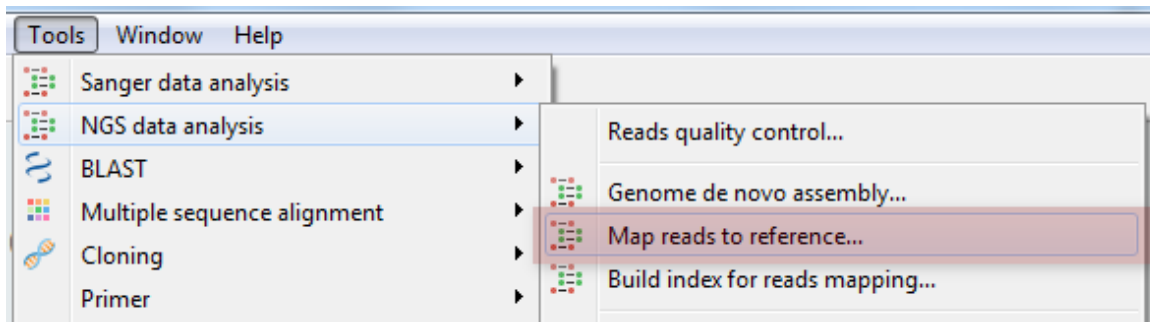
Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

Index file name — a file to save the created index to. This parameter is required.

BWA

BWA is a fast light-weighted tool that aligns relatively short reads to a reference sequence. Click [this link](#) to open *BWA* homepage. *BWA* is embedded as an *external tool* into UGENE.

Open *Tools DNA assembly* submenu of the main menu.

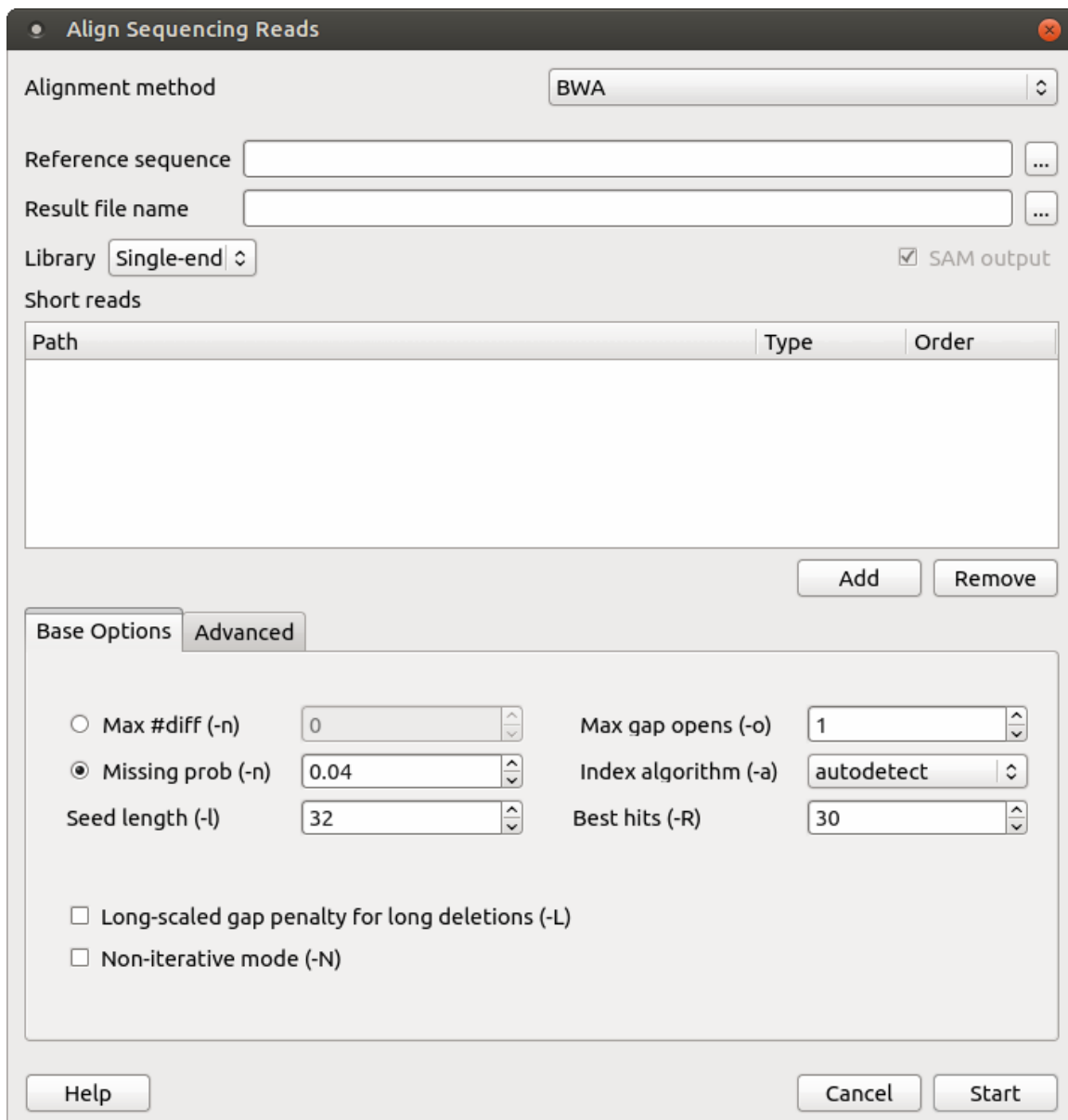


Select the *Align short reads* item to align short reads to a DNA sequence using *BWA*. Or select the *Build index* item to build an index for a DNA sequence which can be used to optimize aligning of short reads.

- [Aligning Short Reads with BWA](#)
- [Building Index for BWA](#)

Aligning Short Reads with BWA

When you select the *Tools DNA Assembly Align short reads* item in the main menu, the *Align Short Reads* dialog appears. Set value of the *Align short reads method* parameter to *BWA*. The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

Library - single-end or paired-end reads.

Prebuilt index — check this box to use an index file instead of a source reference sequence. Also you can *build it manually*.

SAM output — always save the output file in the SAM format (the option is disabled for *BWA*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.

You can also configure other parameters. They are the same as in the original *BWA* (you can read detailed description of the parameters on the [BWA manual page](#)). Select one of the following parameters, that correspond to the *-n* option in the original *BWA*.

Max #diff (-n) — maximum edit distance. An integer value should be input.

Missing prob (-n) — the fraction of missing alignments given 2% uniform base error rate. A float value is used.

Seed length (-l) — take the subsequence of the specified length as seed. If the specified length is larger than the query sequence, seeding will be disabled. For long reads, this option is typically ranged from 25 to 35.

Max gap opens (-o) — maximum number of gap opens.

Index algorithm (-a) — algorithm for constructing *BWA* index.

It implements three different algorithms:

- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwtsw* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Best hits (-R) — *proceed with suboptimal alignments if there are no more than specified number of equally best hits. This option only affects paired-end mapping. Increasing this threshold helps to improve the pairing accuracy at the cost of speed, especially for short reads (~32bp).*

Long-scaled gap penalty for long deletion (-L) — long-scaled gap penalty for long deletion.

Non-iterative mode (-N) — disable iterative search. All hits with no more than *Max #diff* differences will be found. This mode is much slower than the default.

You can also configure the following advanced parameters:

Enable long gaps — checking this box allows one to set the *Max gap extensions* parameter.

Max gap extensions (-e) — maximum number of gap extensions.

Indel offset (-i) — disallow insertions and deletions within the specified number of base pairs towards the ends.

Max long deletion extensions (-d) — disallow a long deletions within the specified number of base pairs towards the 3'-end.

Max queue entries (-m) — maximum queue entries.

Barcode length (-B) — length of barcode starting from the 5'-end. When the specified length is positive, the barcode of each read will be trimmed before mapping and will be written at the BC SAM tag. For paired-end reads, the barcode from both ends are concatenated.

Threads (-t) — number of threads.

Max seed differences (-k) — maximum edit distance in the seed.

Mismatch penalty (-M) — *BWA* will not search for suboptimal hits with a score lower than the specified value.

Gap open penalty (-O) — gap open penalty.

Gap extension penalty (-E) — gap extension penalty.

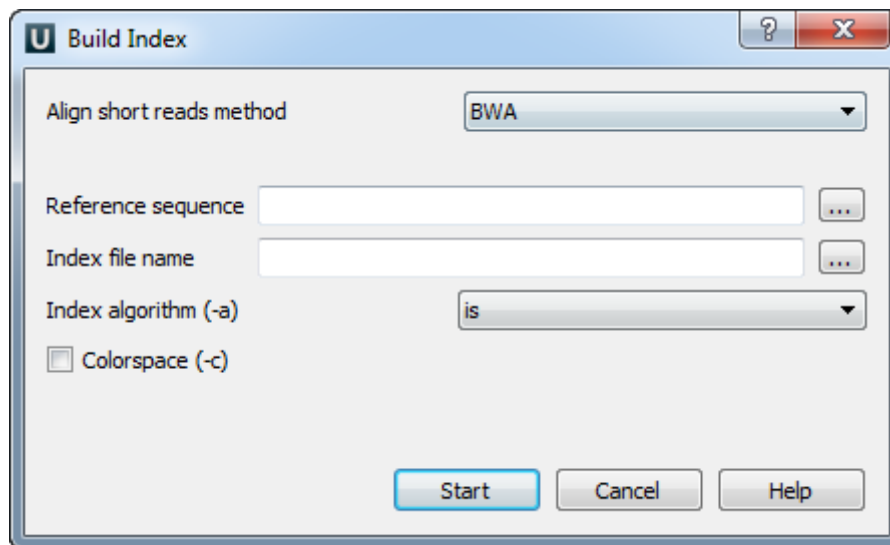
Quality threshold (-q) — parameter for read trimming.

Select the required parameters and press the *Start* button.

Building Index for BWA

To build *BWA* index select the *Tools* *NGS data analysis* *Build index for reads mapping* item in the main menu. The *Build Index* dialog appears. Set the *Align short reads method* parameter to *BWA*.

The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

Index file name — file to save index to. This parameter is required.

Index algorithm (-a) — Algorithm for constructing BWA index. Available options are:

It implements three different algorithms

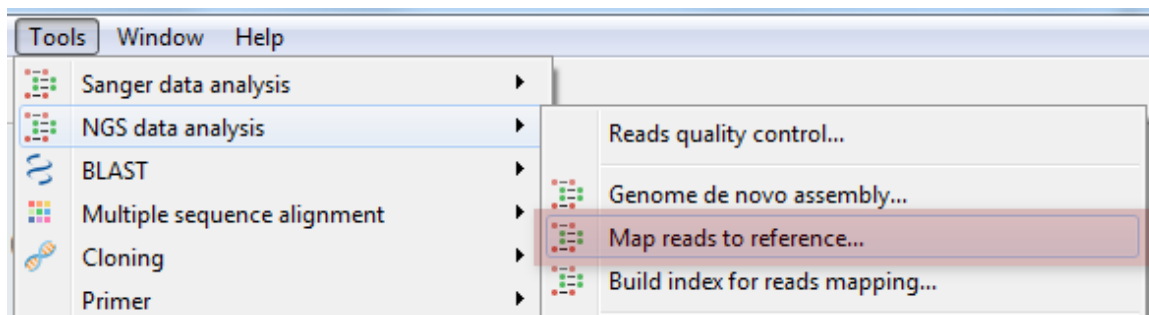
- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwtsw* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Colospace (-color) — the input is read in colospace, colors are encoded as characters A/C/G/T (A=blue, C=green, G=orange, T=red).

BWA-SW

BWA is a fast light-weighted tool that aligns relatively short reads to a reference sequence. Click [this link](#) to open *BWA* homepage. *BWA-SW* share similar features such as long-read support and split alignment. *BWA-SW* is embedded as an *external tool* into UGENE.

Open *Tools* *Align to reference* submenu of the main menu.

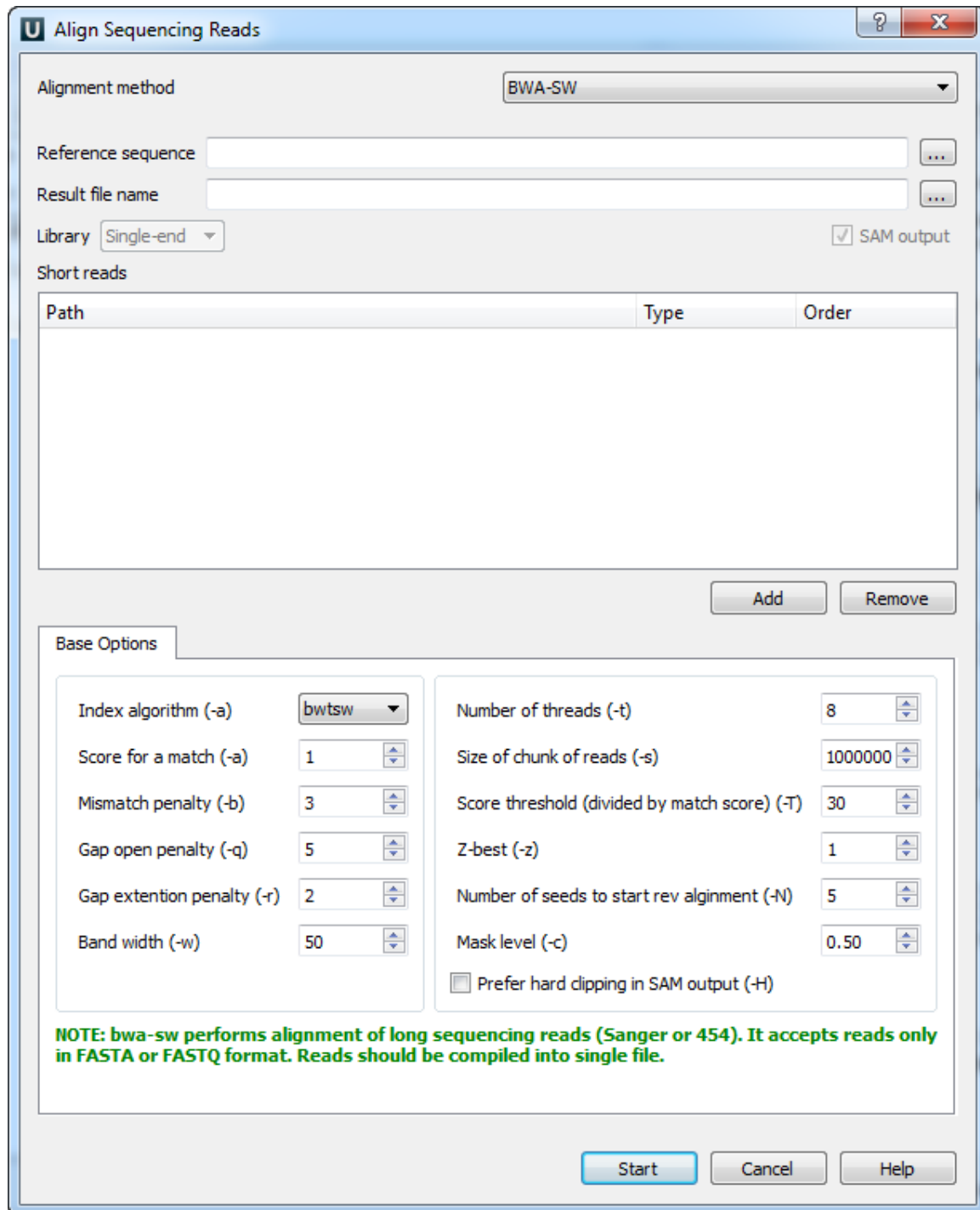


Select the *Align short reads* item to align short reads to a DNA sequence using *BWA-SW*. Or select the *Build index* item to build an index for a DNA sequence which can be used to optimize aligning of short reads.

- [Aligning Short Reads with BWA-SW](#)
- [Building Index for BWA-SW](#)

Aligning Short Reads with BWA-SW

When you select the *Tools* *Align to reference* *Align short reads* item in the main menu, the *Align Sequencing Reads* dialog appears. Set value of the *Align short reads method* parameter to *BWA-SW*. The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

SAM output — always save the output file in the SAM format (the option is disabled for *BWA*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.

You can also configure other parameters.

Index algorithm (-a) — algorithm for constructing BWA-SW index.

It implements three different algorithms:

- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwts* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Score for a match (-a) — score of a match.

Mismatch penalty (-b) — mismatch penalty.

Gap open penalty (-q) — gap open penalty.

Gap extension penalty (-r) — Gap extension penalty. The penalty for a contiguous gap of size k is $q+k*r$.

Band width (-w) - Band width in the banded alignment.

Number of threads (-t) - Number of threads in the multi-threading mode.

Size of chunk of reads (-s) - Maximum SA interval size for initiating a seed. Higher *-s* increases accuracy at the cost of speed.

Score threshold (divided by much score) (-T) - minimum score threshold.

Z-best (-z) - Z-best heuristics. Higher *-z* increases accuracy at the cost of speed.

Number of seeds to start rev alignment (-N) - Minimum number of seeds supporting the resultant alignment to skip reverse alignment.

Mask level (-c) - Coefficient for threshold adjustment according to query length. Given an l -long query, the threshold for a hit to be retained is $a*\max\{T, c*\log(l)\}$.

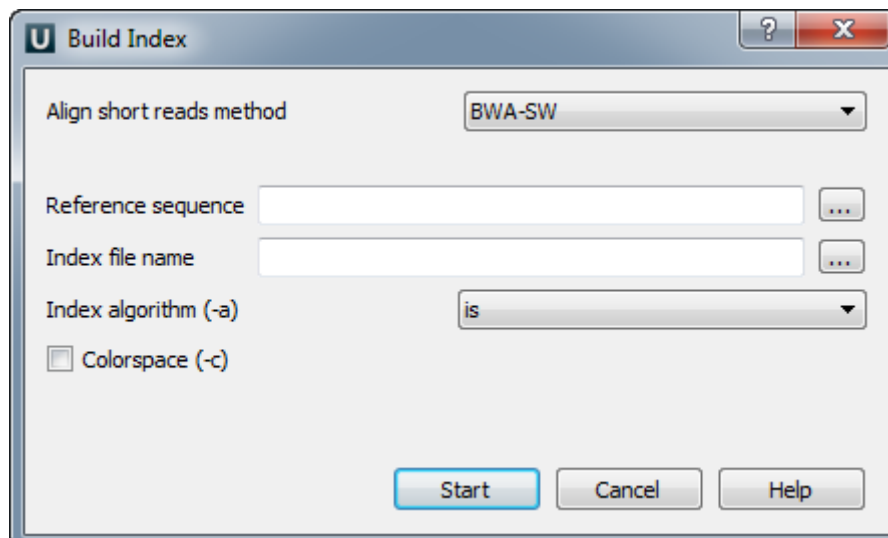
Prefer hard clipping in SAM output (-H) - use hard clipping in the SAM output. This option may dramatically reduce the redundancy of output when mapping long contig or BAC sequences.

Select the required parameters and press the *Start* button.

Building Index for BWA-SW

To build *BWA-SW* index select the *Tools NGS data analysis Build index for reads mapping* item in the main menu. The *Build Index* dialog will appear. Set the *Align short reads method* parameter to *BWA-SW*.

The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

Index file name — file to save index to. This parameter is required.

Index algorithm (-a) — Algorithm for constructing BWA index. Available options are:

It implements three different algorithms

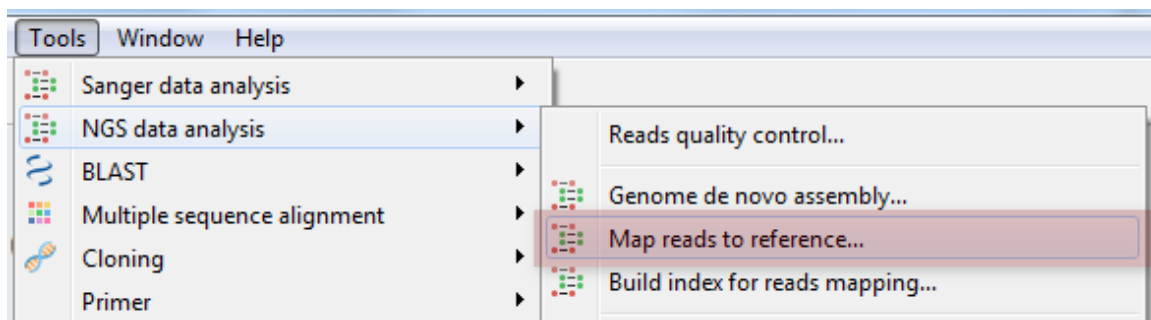
- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwtsv* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Colorspace (-color) — the input is read in colorspace, colors are encoded as characters A/C/G/T (A=blue, C=green, G=orange, T=red).

BWA-MEM

BWA is a fast light-weighted tool that aligns relatively short reads to a reference sequence. Click [this link](#) to open *BWA* homepage. *BWA-MEM* is generally recommended for high-quality queries as it is faster and more accurate. *BWA-MEM* also has better performance than *BWA-backtrack* for 70-100bp Illumina reads.

Open *Tools* *Align to reference* submenu of the main menu.



Select the *Align short reads* item to align short reads to a DNA sequence using *BWA-MEM*. Or select the *Build index* item to build an index for a DNA sequence which can be used to optimize aligning of short reads.

- [Aligning Short Reads with BWA-MEM](#)
- [Building Index for BWA-MEM](#)

Aligning Short Reads with BWA-MEM

When you select the *Tools* *Align to reference* *Align short reads* item in the main menu, the *Align Sequencing Reads* dialog appears. Set value of the *Align short reads method* parameter to *BWA-MEM*. The dialog looks as follows:

There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

Prebuilt index — check this box to use an index file instead of a source reference sequence. Also you can *build it manually*.

SAM output — always save the output file in the SAM format (the option is disabled for *BWA*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.

You can also configure other parameters.

Index algorithm (-a) — algorithm for constructing BWA index.

It implements three different algorithms:

- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwts* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Number of threads (-t) — number of threads.

Min seed length (-k) — minimum seed length. Matches shorter than *INT* will be missed. The alignment speed is usually insensitive to this value unless it significantly deviates 20.

Band width (-w) — band width. Essentially, gaps longer than *INT* will not be found. Note that the maximum gap length is also affected by the scoring matrix and the hit length, not solely determined by this option.

Dropoff (-d) — off-diagonal X-dropoff (Z-dropoff). Stop extension when the difference between the best and the current extension score is above $|i-j|*A/INT$, where *i* and *j* are the current positions of the query and reference, respectively, and *A* is the matching score. Z-dropoff is similar to BLAST's X-dropoff except that it doesn't penalize gaps in one of the sequences in the alignment. Z-dropoff not only avoids unnecessary extension, but also reduces poor alignments inside a long good alignment.

Internall seeds length (-r) - trigger re-seeding for a MEM longer than $minSeedLen*FLOAT$. This is a key heuristic parameter for tuning the performance. Larger value yields fewer seeds, which leads to faster alignment speed but lower accuracy.

Skip seeds threshold (-c) - discard a MEM if it has more than *INT* occurrence in the genome. This is an insensitive parameter.

Drop chain threshold (-D) - drop chains shorter than *FLOAT* fraction of the longest overlapping chain.

Rounds of mate rescues (-m) - perform at most *INT* rounds of mate rescues for each read.

Skip mate rescue (-S) - skip mate rescue.

Skip pairing (-P) - in the paired-end mode, perform SW to rescue missing hits only but do not try to find hits that fit a proper pair.

Score for a match (-A) - matching score.

Mismatch penalty (-B) - mismatch penalty. The sequence error rate is approximately: $\{.75 * \exp[-\log(4) * B/A]\}$.

Gap open penalty (-O) - gap open penalty.

Gap extension penalty (-E) - gap extension penalty. A gap of length *k* costs $O + k*E$ (i.e. *Gap open penalty* is for opening a zero-length gap).

Penalty for clipping (-L) - clipping penalty. When performing SW extension, BWA-MEM keeps track of the best score reaching the end of query. If this score is larger than the best SW score minus the clipping penalty, clipping will not be applied. Note that in this case, the SAM AS tag reports the best SW score; clipping penalty is not deducted.

Penalty unpaired (-U) - penalty for an unpaired read pair. BWA-MEM scores an unpaired read pair as $scoreRead1+scoreRead2-INT$ and scores a paired as $scoreRead1+scoreRead2-insertPenalty$. It compares these two scores to determine whether we should force pairing.

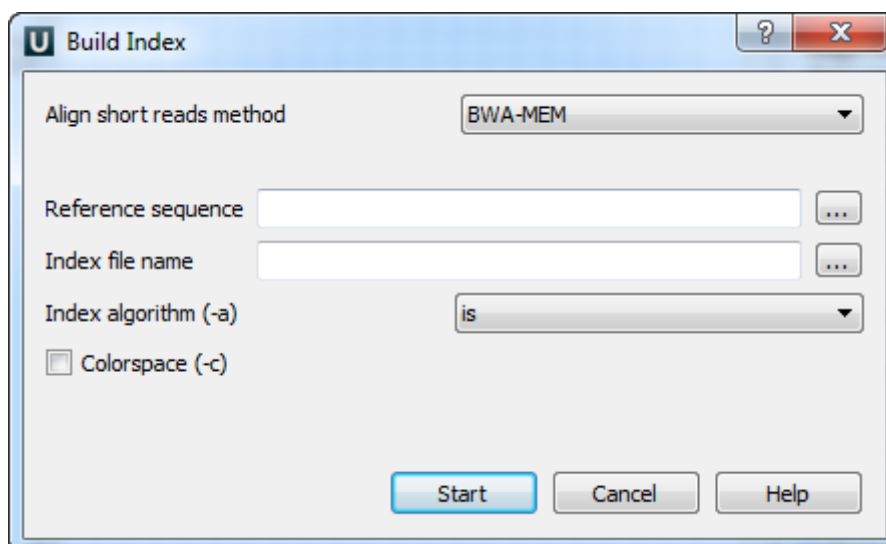
Score threshold (-T) - don't output alignment with score lower than score threshold. This option only affects output.

Select the required parameters and press the *Start* button.

Building Index for BWA-MEM

To build *BWA-SW* index select the *Tools NGS data analysis Build index for reads mapping* item in the main menu. The *Build Index* dialog will appear. Set the *Align short reads method* parameter to *BWA-MEM*.

The dialog looks as follows:



There are the following parameters:

Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

Index file name — file to save index to. This parameter is required.

Index algorithm (-a) — Algorithm for constructing BWA index. Available options are:

It implements three different algorithms

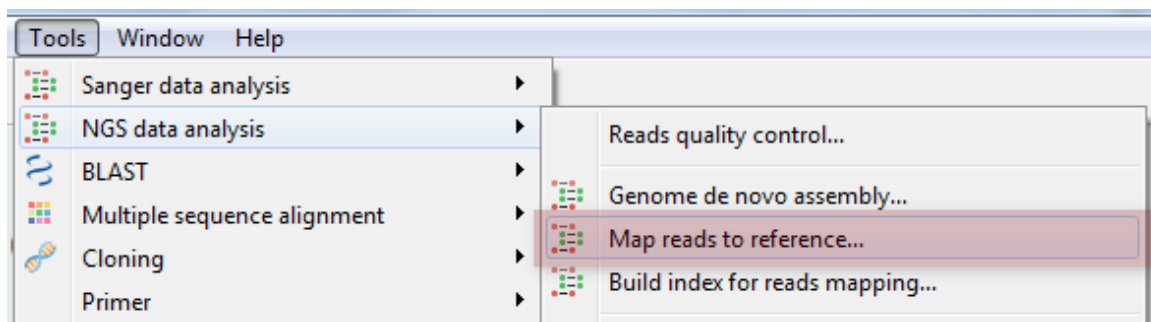
- *is* — designed for short reads up to ~200bp with low error rate (<3%). It does gapped global alignment w.r.t. reads, supports paired-end reads, and is one of the fastest short read alignment algorithms to date while also visiting suboptimal hits.
- *bwtsw* — is designed for long reads with more errors. It performs heuristic Smith-Waterman-like alignment to find high-scoring local hits. Algorithm implemented in *BWA-SW*. On low-error short queries, *BWA-SW* is slower and less accurate than the *is* algorithm, but on long reads, it is better.
- *div* — does not work for long genomes.

Colorspace (-c) — the input is read in colorspace, colors are encoded as characters A/C/G/T (A=blue, C=green, G=orange, T=red).

UGENE Genome Aligner

The [UGENE Genome Aligner](#) is a fast short read aligner. It aligns DNA sequences of various lengths to the reference genome with configurable mismatch rate.

It is available from the *Tools DNA assembly* submenu of the main menu.

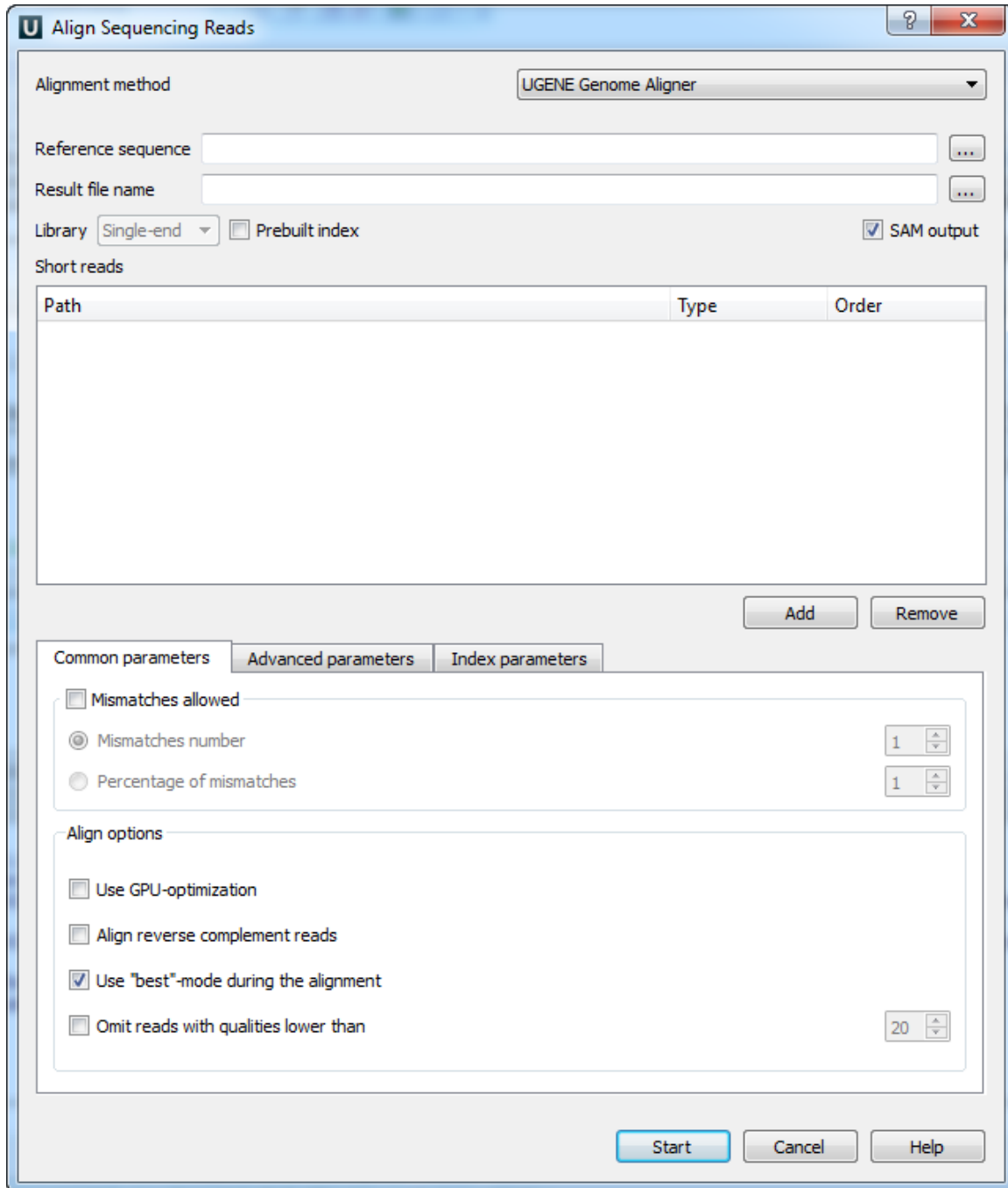


Select the *Align short reads* item to align short reads to a DNA sequence or *Build index* item to build an index for a DNA sequence which can be used to optimize aligning short reads to the sequence.

- [Aligning Short Reads with UGENE Genome Aligner](#)
- [Building Index for UGENE Genome Aligner](#)
- [Converting UGENE Assembly Database to SAM Format](#)

Aligning Short Reads with UGENE Genome Aligner

When you select the *Tools DNA Assembly Align short reads* item in the main menu, the *Align Short Reads* dialog appears. Set the *Align short reads method* parameter to *UGENE Genome Aligner*. The dialog looks as follows:



The following parameters are available:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in UGENE database format or SAM format (if the box *SAM output* check), to write the result of the alignment into. This parameter is required.

Prebuilt index — check this box to use an index file instead of a reference sequence. Also you can *build it manually*.

SAM output — checking this box allows one to save output files in the SAM format. The default format of output files is the UGENE database format (ugenedb).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.



The *Aligning Short Reads with UGENE Genome Aligner* has no limitation on short reads length.

Common parameters:

Mismatches allowed — check this box to allow mismatches between the reference sequence and a short read. Select one of the following:

- *Mismatches number* to set the number of mismatched nucleotides allowed. This parameter can take values: 1, 2 and 3.
- *Percentage of mismatches* to set the number of mismatches in percents. Note, that in this case the absolute number of mismatches can vary for different reads. This parameter can take values: 1 - 10 %.

Align options:

- *Use GPU-optimization* — use an openCL-enabled GPU during the alignment (the corresponding hardware should be available on your computer).
- *Align reverse complement reads* — use both: a read and its reverse complement during the alignment.
- *Use "best"-mode during the alignment* — report only about best alignments (in terms of mismatches).
- *Omit reads with qualities lower than* — omit all reads with qualities lower than the specified value. Reads that have no qualities are not omitted.

Advanced parameters:

Maximum memory for short reads — maximum memory usage for short reads. This parameter allows one to decrease the load on the computer on one side and to increase the computer speed of the task on the other side.

- *Total memory usage* — shows the total memory usage.
- *System memory size* — shows the total system memory size.

Index parameters:

Reference fragmentation — this parameter influences the number of parts the reference will be divided. It is better to make it bigger, but it influences the amount of memory used during the alignment.

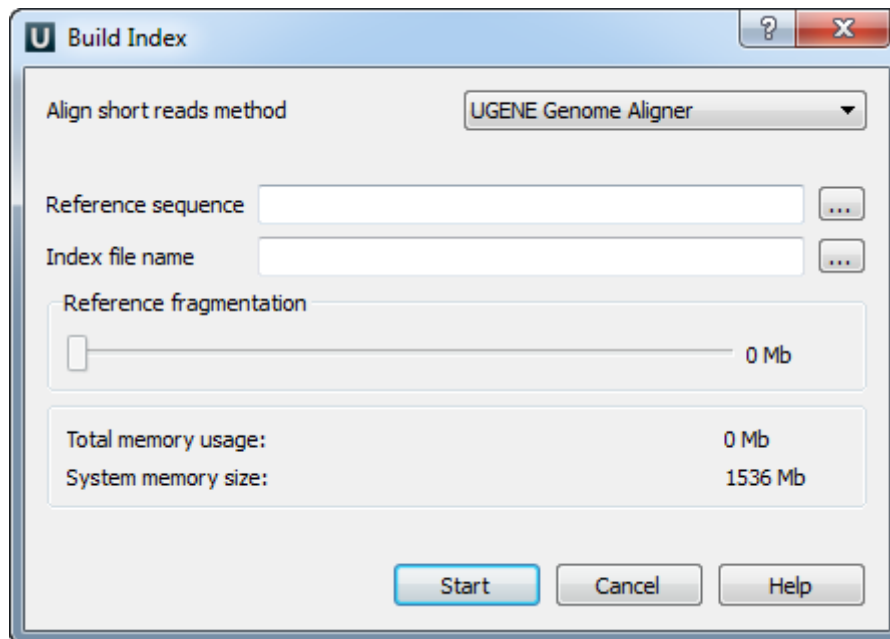
- *Index memory usage size* — shows the index memory usage.
- *Directory for index files* — temporary directory for saving index files.

You can choose a temporary directory for saving index files for the reference that will be built during the alignment. If you need to run this algorithm one more time with the same reference and with the same reference fragmentation parameter, you can use this prebuilt index that will be located in the temporary directory.

Building Index for UGENE Genome Aligner

You can build an index to optimize short reads alignment using *UGENE Genome Aligner*. To open the *Build Index* dialog, select the *Tools NGS data analysis Build index for reads mapping* item in the main menu. Set value of the *Align short reads method* parameter to *UGENE Genome Aligner*.

The dialog looks as follows:



The parameters are the following:

Reference sequence — DNA sequence to which short reads would be aligned to. This parameter is required.

Index file name — file to save index to. This parameter is required.

Reference fragmentation — this parameter influences the amount of parts the reference will be divided. It is better to make it bigger,

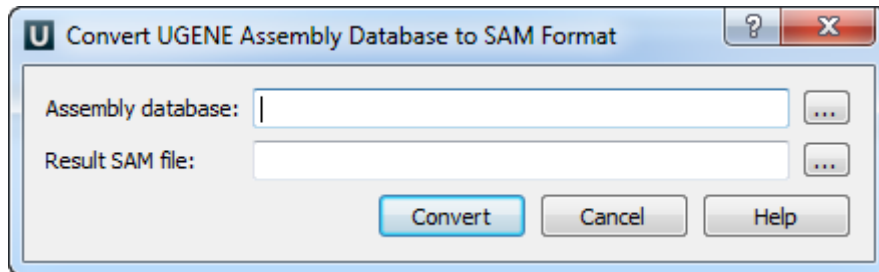
but it influences the amount of memory used during the alignment.

Total memory usage — shows the total memory usage.

System memory size — shows the total system memory size.

Converting UGENE Assembly Database to SAM Format

To convert UGENE data base to SAM format click on the *Tools->DNA Assembly->Convert UGENE assembly database to SAM format* context main menu item. The following dialog will appear:



Select assembly and result files and click on the *Convert* button.

CAP3

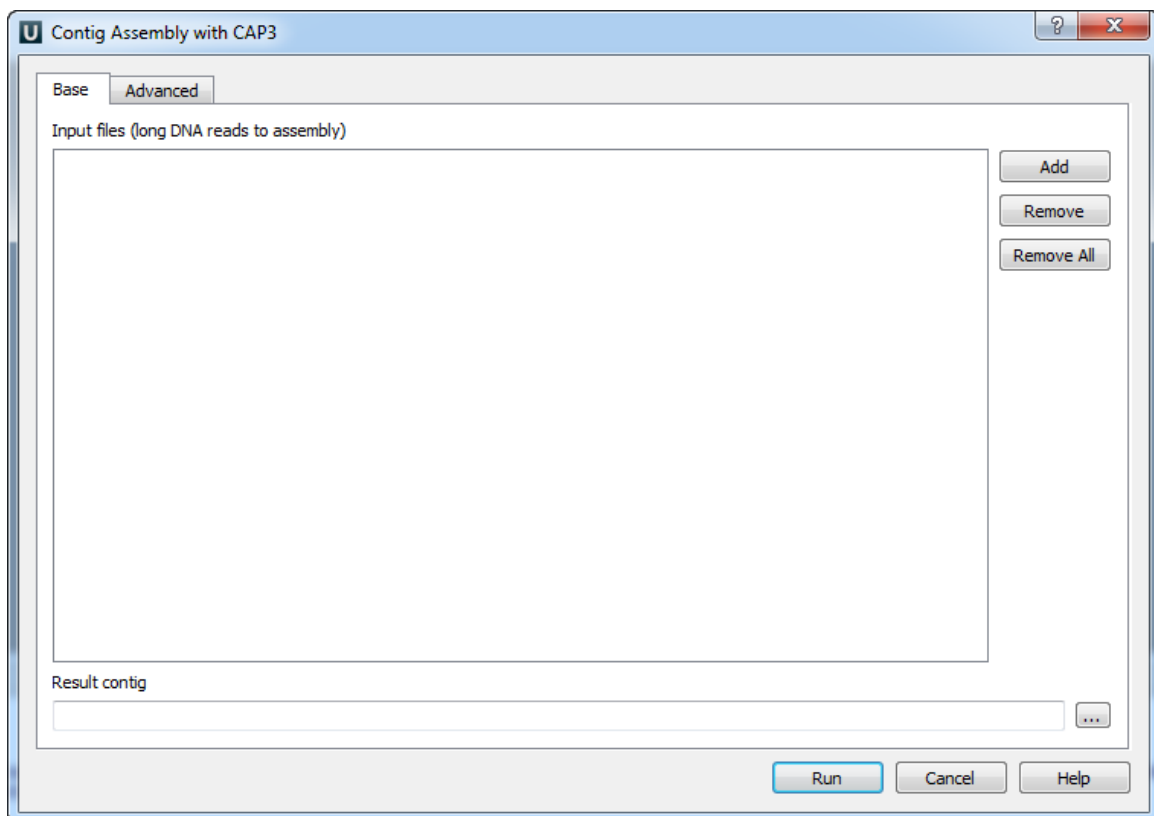
CAP3 (CONTIG ASSEMBLY PROGRAM Version 3) is a sequence assembly program for small-scale assembly with or without quality values. Click [this link](#) to open CAP3 homepage. CAP3 is embedded as an *external tool* into UGENE.

Open *Tools Sanger data analysis* submenu of the main menu.



Select the *Reads de novo assembly (with CAP3)* item to use the CAP3.

The *Contig Assembly With CAP3* dialog appears.



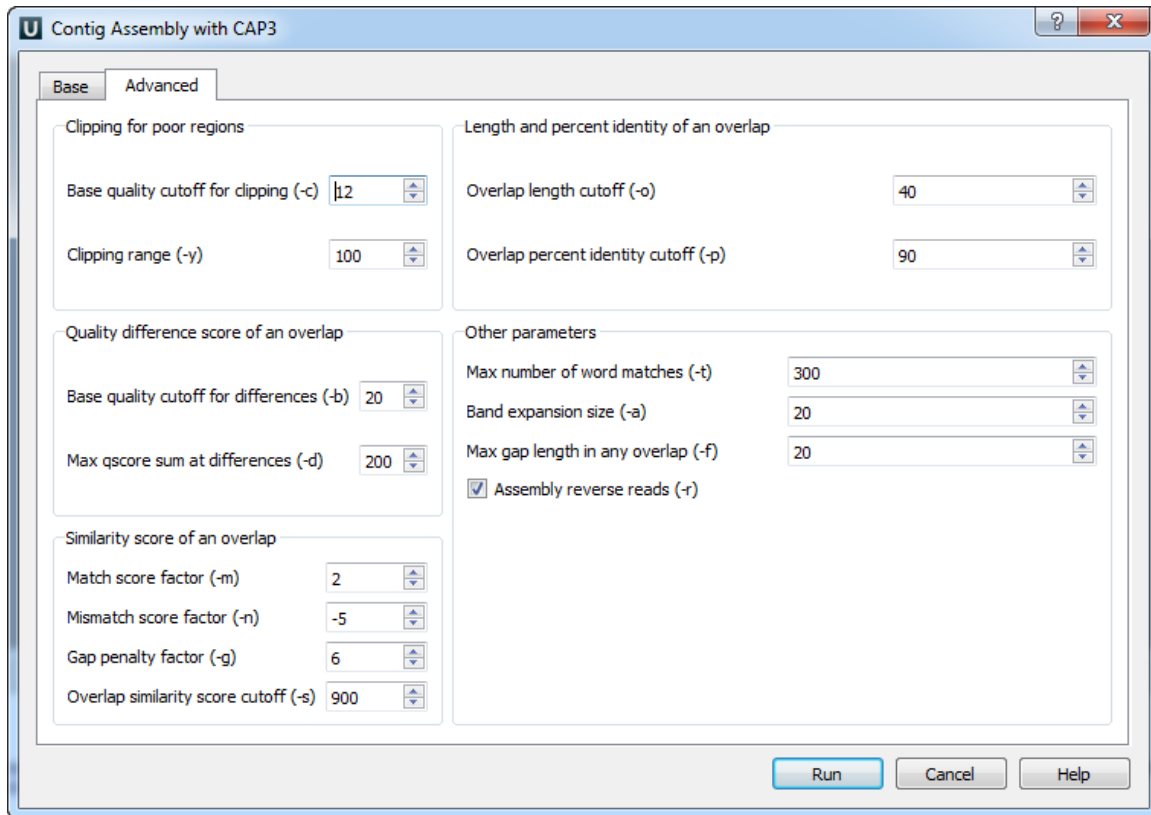
You can add or remove input files using *Add* and *Remove* buttons. To remove all files click the *Remove all* button. *Input files* are files with a long DNA reads in FASTA, FASTQ, SCF or ABI formats. At least one input file should be added. Input a *Result contig* name and press the *Run* button. CAP3 produces assembly results in the ACE file format (".ace"). The file contains one or several contigs assembled from the input

reads.



The quality scores for FASTA sequences can be provided in an additional file. The file must be located in the same folder as the original sequences and have the same name as FASTA file, but another extension: **.qual**.

Also you can change the following advanced parameters:



Clipping for poor regions parameters:

Clipping of a poor end region of a read is controlled by parameters *Base quality cutoff for clipping (-c)* (the specified value should be more than 5), and *Clipping range (-y)* (the specified value should be more than 5).

Quality difference score of an overlap parameters:

Base quality cutoff for differences (-b) — if an overlap contains a difference at bases of quality values q_1 and q_2 , then the score at the difference is $\max(0, \min(q_1, q_2) - b)$, where b is the specified value. The specified value should be more than 15. The difference score of an overlap is the sum of scores at each difference.

Max qscore sum at differences (-d) — remove an overlap if its difference score is greater than the specified value. The specified value should be more than 20.

Similarity score of an overlap parameters:

The following parameters are used to calculate the similarity score of an overlapping alignment:

Match score factor (-m) — a match at bases of quality values q_1 and q_2 is given a score of $m * \min(q_1, q_2)$, where m is the specified value. The specified value should be more than 0.

Mismatch score factor (-n) — a mismatch at bases of quality values q_1 and q_2 is given a score of $n * \min(q_1, q_2)$, where n is the specified value. The specified value should be less than 0.

Gap penalty factor (-g) — a base of quality value q_1 in a gap is given a score $-g * \min(q_1, q_2)$, where g is the specified value; q_2 is the quality value of the base in the other sequence right before the gap. The specified value should be more than 0.

The similarity score is calculated as the sum of scores of each match, each mismatch and each gap. Based on this value and the following value some overlaps are removed:

Overlap similarity score cutoff (-s) — remove overlaps with similarity scores less than the specified value. The specified value should be more than 250.

Length and percent identity of an overlap parameters:

Overlap length cutoff (-o) — minimum length of an overlap (in base pairs). The specified value should be more than 15 base pairs.

Overlap percent identity cutoff (-p) — minimum percent identity of an overlap. The specified value should be more than 65%.

Other parameters:

Maximum number of word matches (-t) — an upper limit of word matches between a read and other reads. Increasing the value would result in more accuracy, however this could slow down the program. The specified value should be more than 0.

Band expansion size (-a) — a number of bases to expand a band of diagonals for an overlapping alignment between two sequence reads. The specified value should be more than 10.

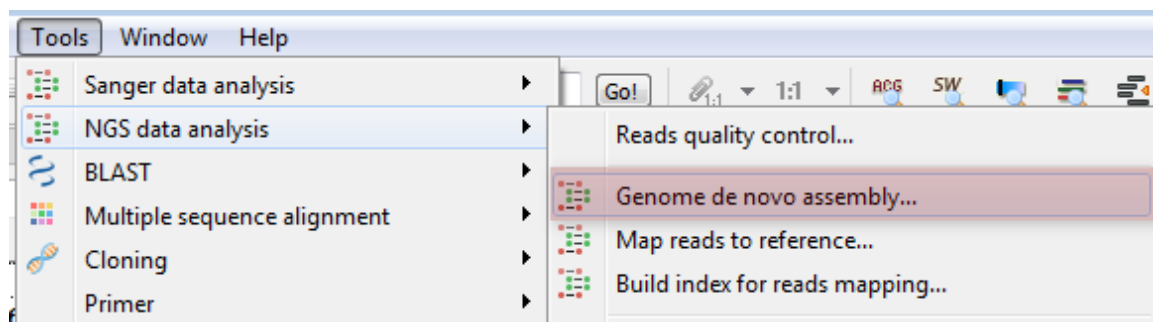
Max gap length in any overlap (-f) — reject overlaps with a gap longer than the specified value. A small value may cause the program to remove true overlaps and to produce incorrect results. This option may be used by the user to split reads from alternative splicing forms into separate contigs. The specified value should be more than 1.

Assembly reverse reads (-r) — consider reads in reverse orientation for assembly. The default value is “checked”.

SPAdes

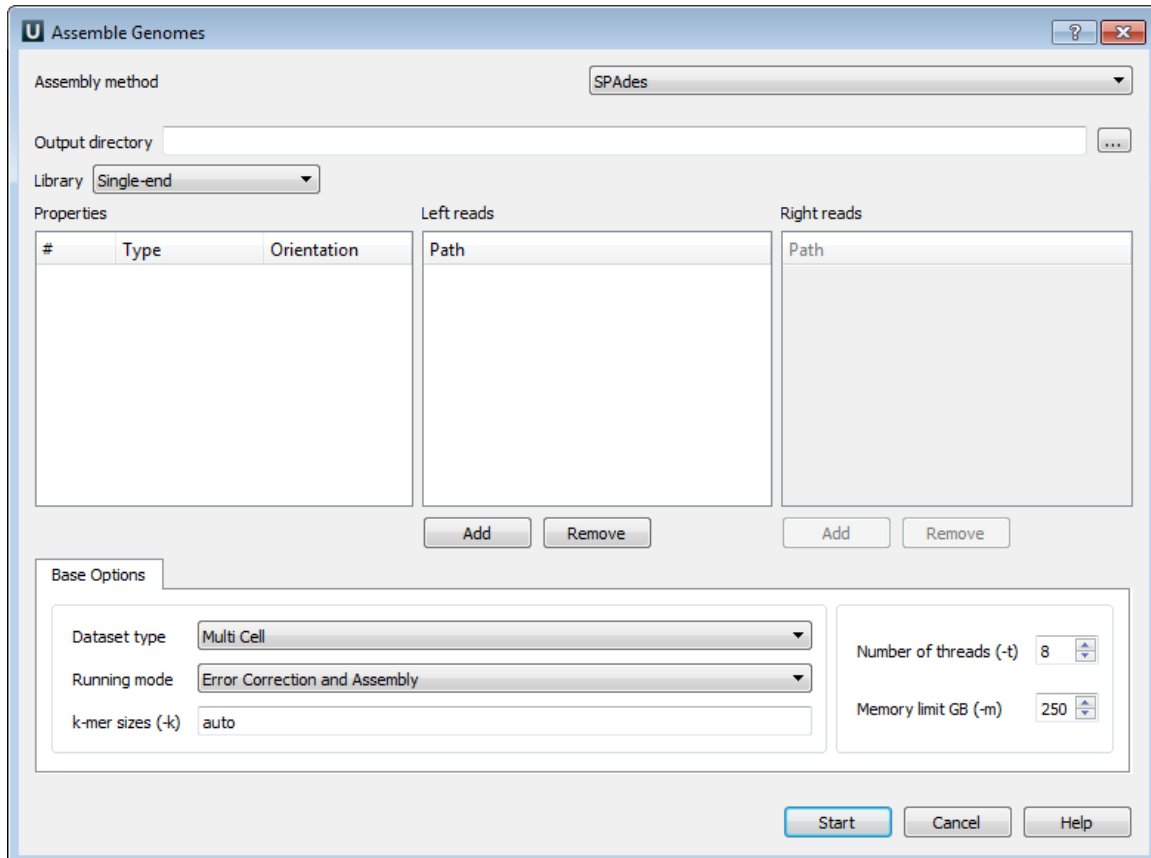
SPAdes – St. Petersburg genome assembler. Click [this link](#) to open SPAdes homepage. SPAdes is embedded as an *external tool* into UGENE.

Open *Tools* *NGS data analysis*.



Select the *Genome de novo assembly* item to use the *SPAdes*.

The *Assemble Genomes* dialog will appear.



The following parameters are available:

Output directory - SPAdes stores all output files in output directory, which is set by the user.

Library - to run SPAdes choose one of the following libraries:

- Single-end
- Paired-end
- Paired-end (Interplaced)
- Paired-end (Unpaired files)
- Sanger
- PacBio

Left reads - file(s) with left reads.

Right reads - file(s) with right reads.

For each dataset in the paired-end libraries you can change type and orientation.

Datasesst type - dataset type.

Running mode - running mode.

k-mer sizes (-k) - k-mer sizes.

Number of threads (-t) - number of threads.

Memory limit GB (-m) - memory limit.

Weight Matrix

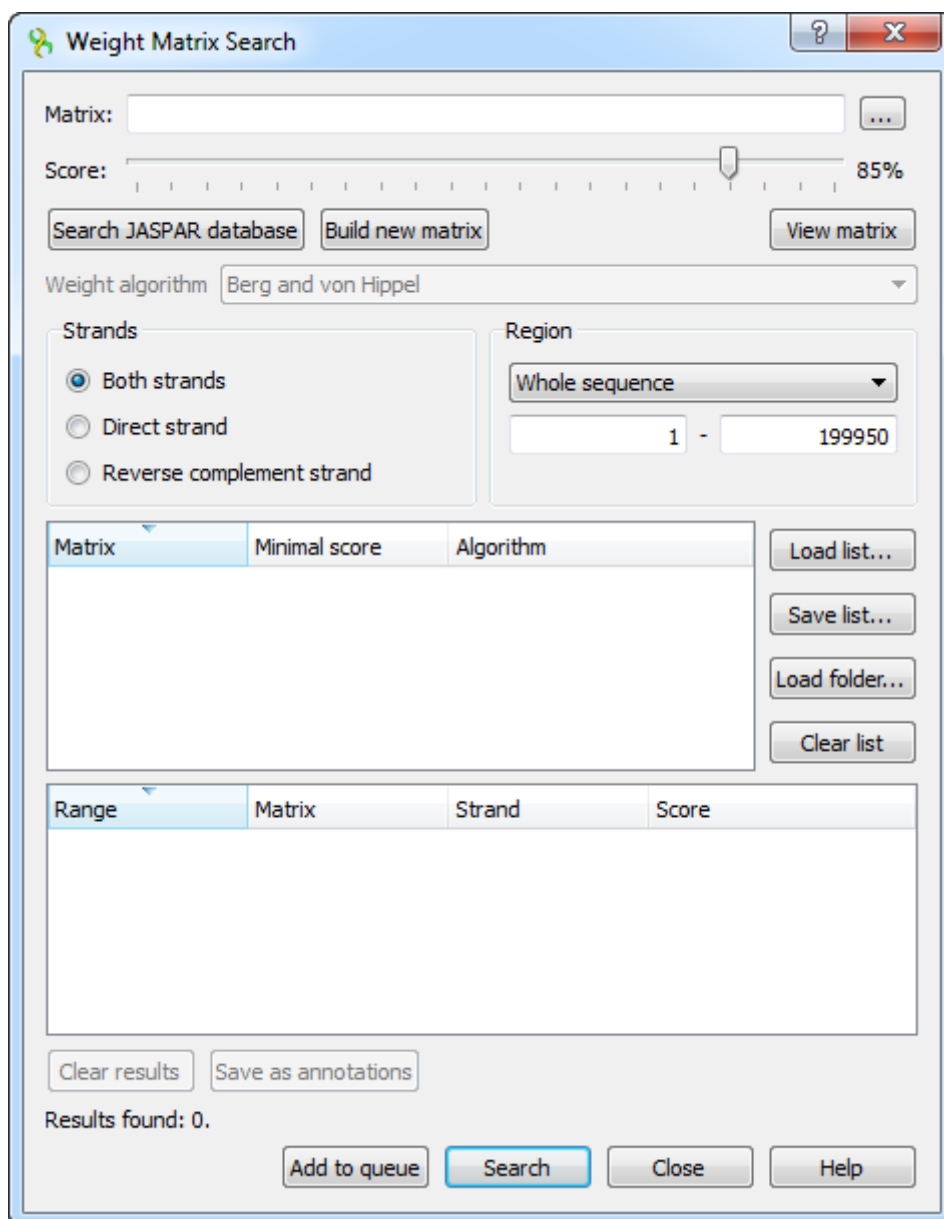
The *Weight Matrix* plugin is a tool for solving the problem of a sequence annotating. As well as for the *SITECON*, the main use case of the plugin is recognition of potential transcription factor binding sites on basis of the data about conservative conformational and physicochemical properties revealed with the binding sites sets analysis.

The *Weight Matrix* contains a lot of *position frequency matrices* (PFM 's) and *position weight matrices* (PWM 's, also known as *position specific score matrices* — PSSM 's). The matrices came from two wide-known open archives: *JASPAR*, which contains frequency matrices,

and UniPROBE containing weight matrices.

Also the *Weight Matrix* plugin provides a tool for creating specific position frequency and weight matrices from an existing alignment or from a file with several sequences. The created matrix can be used as a profile for the search as well as the JASPAR and UNIPROBE ones.

To search for transcription factor binding sites in a DNA sequence select the *Analyze Search TFBS with matrices* context menu item. The *Weight matrix search* dialog will appear:



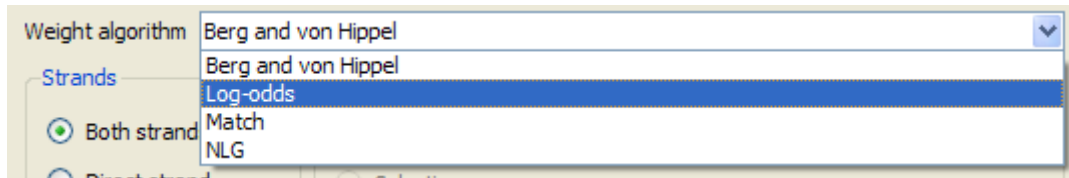
In the search dialog you must specify a file with PWM or PFM. You can do so by pressing the browse button and selecting the file.

Also you can use the *special interface to choose a JASPAR matrix* by pressing the *Search JASPAR database* button.

Alternative way to specify the position weight/frequency matrix is to create a specific one from an alignment or a file with several sequences with the *build a new matrix* tool.

After the profile (the matrix) is loaded, you can adjust the threshold value. The threshold sets the minimal identity score for a result to pass. The more the result score is, the more it is homologically related to the aligned region. By changing the threshold you can filter low-scoring results.

If the loaded matrix is a position frequency matrix, you must also specify the algorithm to build the corresponding position weight matrix which will represent the transcription factor. There are four algorithms available.



Also you can add a selected matrix with the specified *Minimal score* and the *Algorithm* to the matrices list. To do it, select the matrix and other options and press the *Add to queue* button. The plugin will search with all matrices specified in the list.

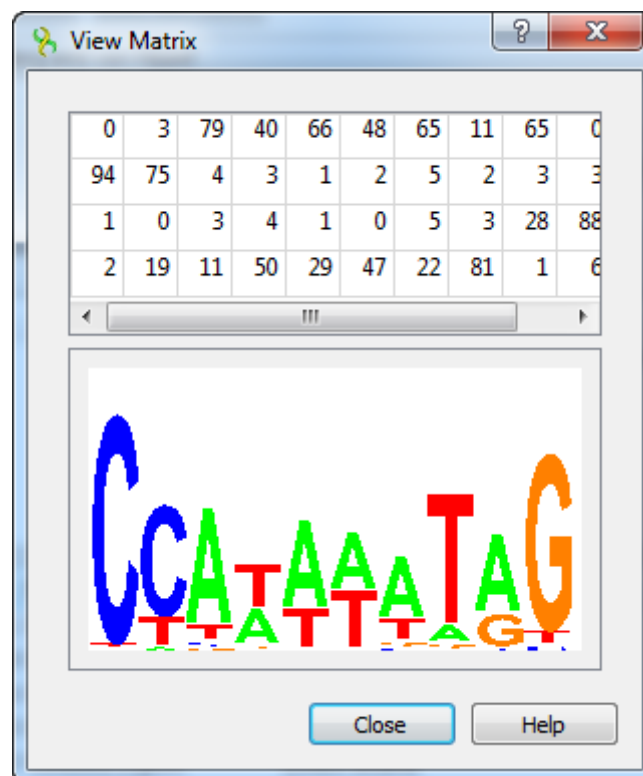
You can use the *Save list* button to export the list of matrices to a *.csv file. Later the list can be loaded from the file using the *Load list* button.

The rest options are standard sequence search options: the strand and the sequence region where to search for matches.

After specifying the necessary options press the *Search* button. The found results will appear in the dialog table. The corresponding results identity scores are in the *Score* column.

Range	Matrix	Strand	Score
199944..199949	MA0271.1.pfm	Direct strand	31.26%
199943..199948	MA0271.1.pfm	Direct strand	62.39%
199942..199947	MA0271.1.pfm	Direct strand	53.92%
199941..199946	MA0271.1.pfm	Direct strand	26.86%
199940..199945	MA0271.1.pfm	Direct strand	26.86%
199939..199944	MA0271.1.pfm	Direct strand	14.07%
199938..199943	MA0271.1.pfm	Direct strand	57.69%

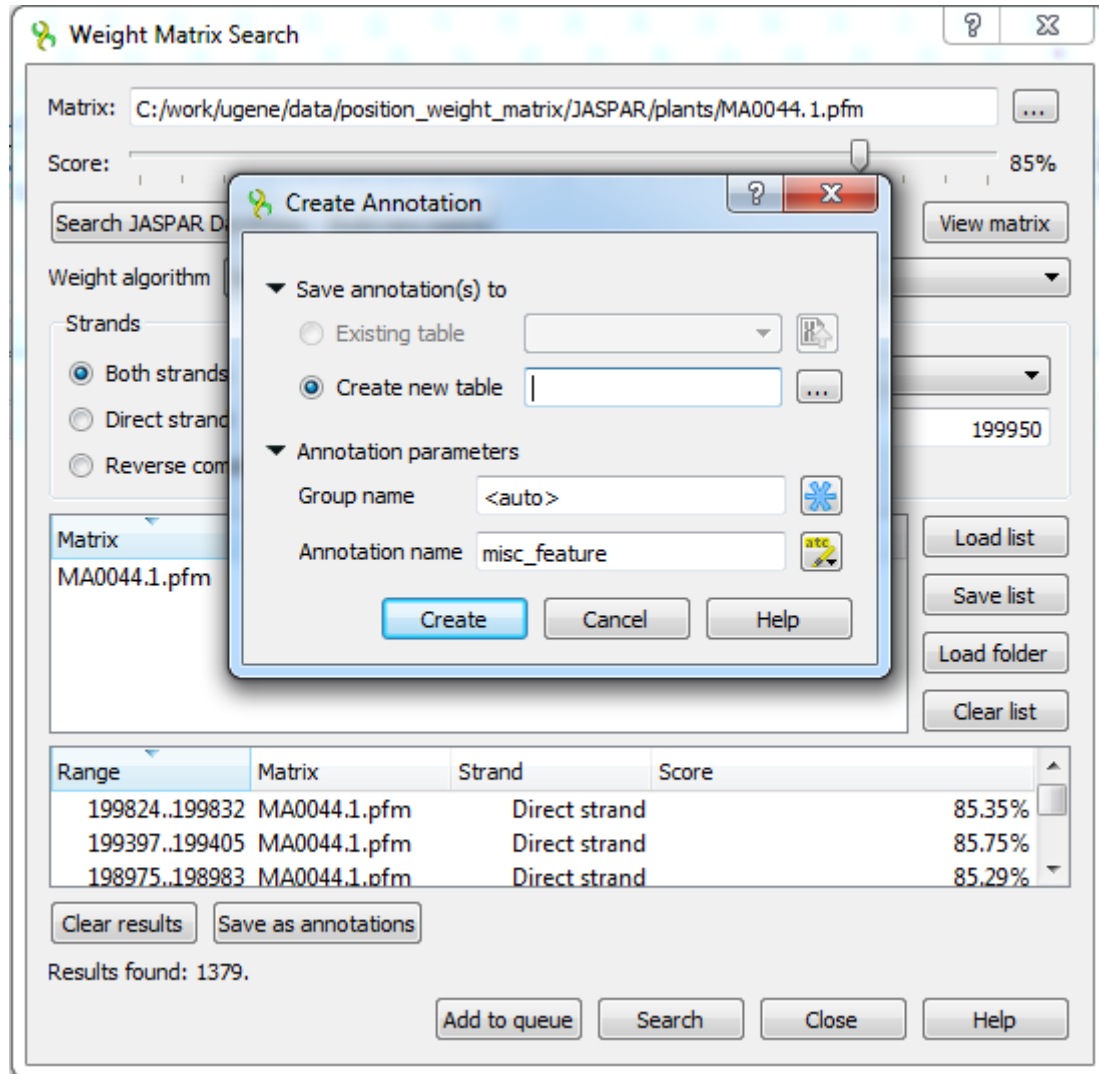
Also you can see the matrix by using the *View matrix* button:



The regions found by the weight matrix algorithm can be saved as annotations to the DNA sequence in the Genbank format by pressing the *Save as annotations* button.

After saving, the file with resulting annotations will be automatically added to the current project, and the annotations will be added to the original sequence.

Note that in case of selecting JASPAR or UNIPROBE matrix, the resulting annotations will contain the given matrix properties.

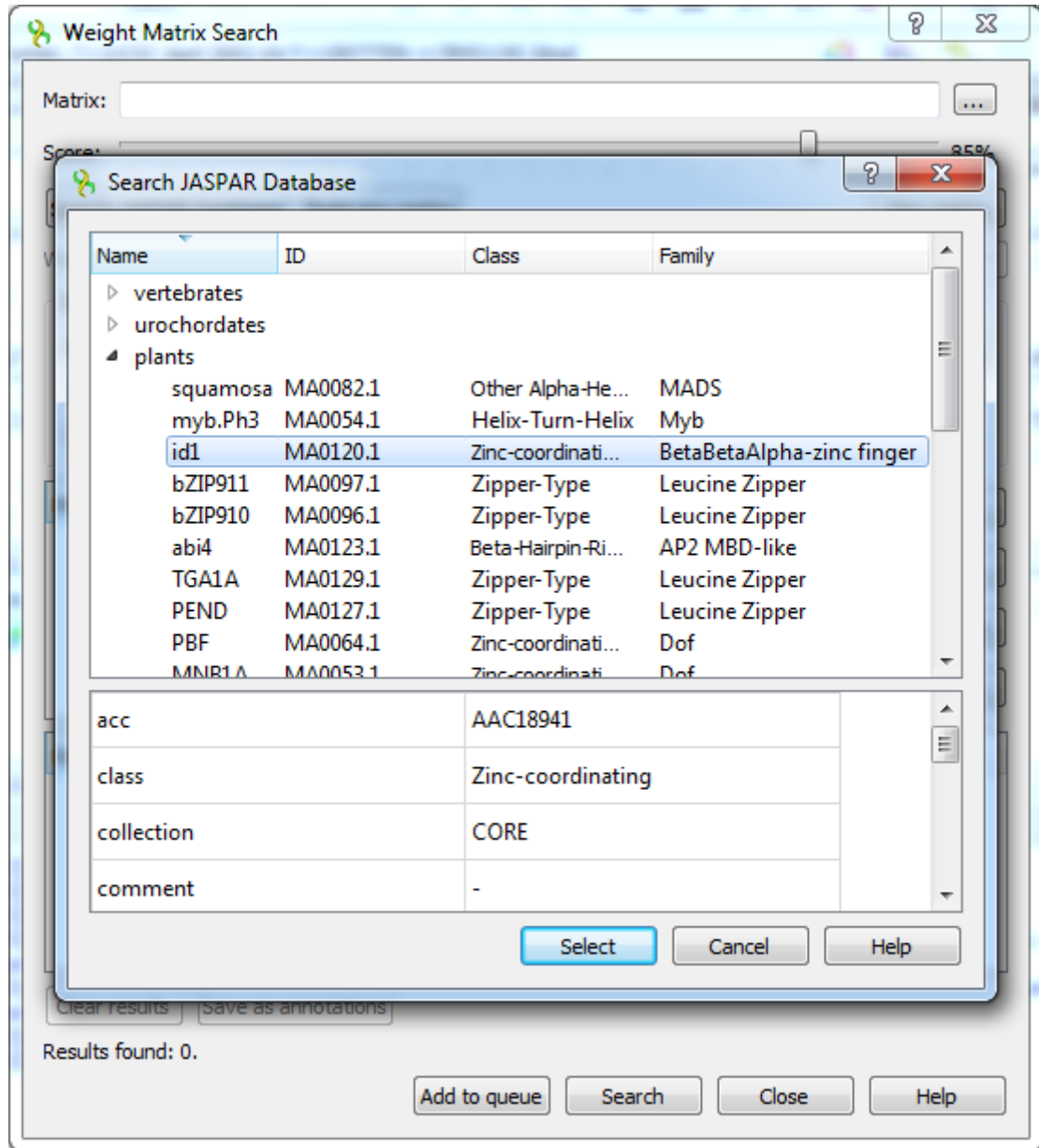


See also:

- Searching JASPAR Database
- Building New Matrix

Searching JASPAR Database

Press the *Search JASPAR database* button in the *Weight matrix search* dialog. The following dialog will appear:



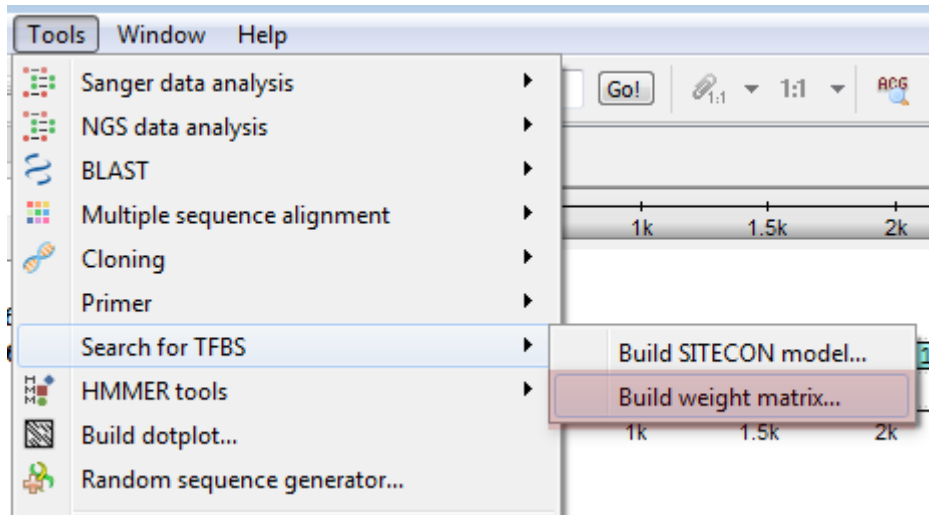
Here the matrices are divided into categories and you can read detailed information of a matrix which is represented by its properties. It could help you to choose the matrix properly.



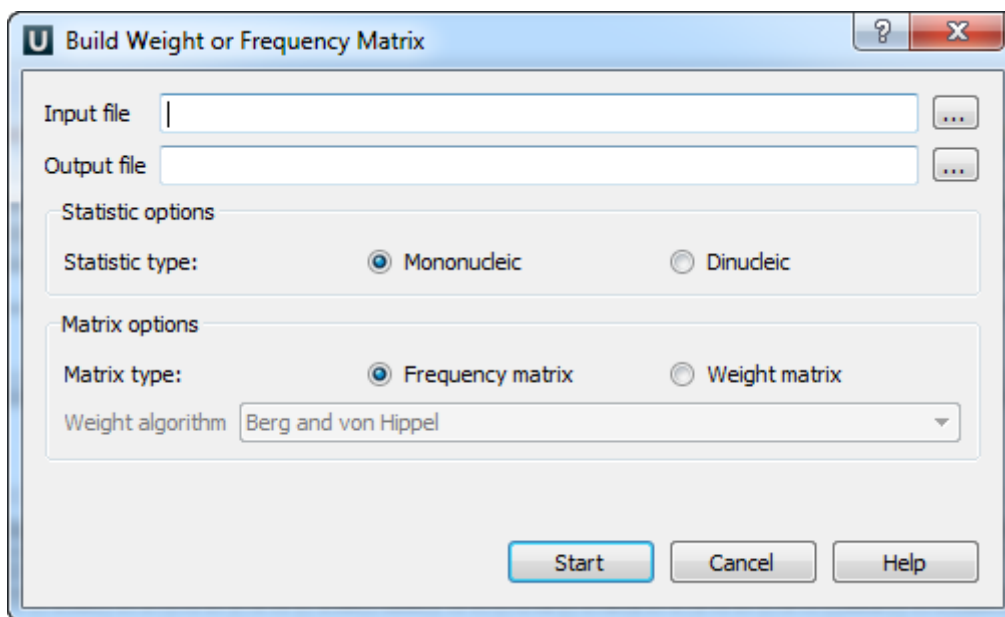
The matrices provided with UGENE are located in the \$UGENE/data/position_weight_matrix folder.

Building New Matrix

To create a position weight or frequency matrix from an alignment or a file with several sequences, press the *Build new matrix* button in the *Weight matrix search* dialog, or select the *Tools Search for TFBS Build weight matrix* main menu item:



The *Build weight or frequency matrix* dialog will appear:



The following parameters are available:

Input file — an alignment or a file with several sequences to build the matrix from. The parameter is mandatory.

Output file — the resulting matrix will be saved in this file. The parameter is mandatory.

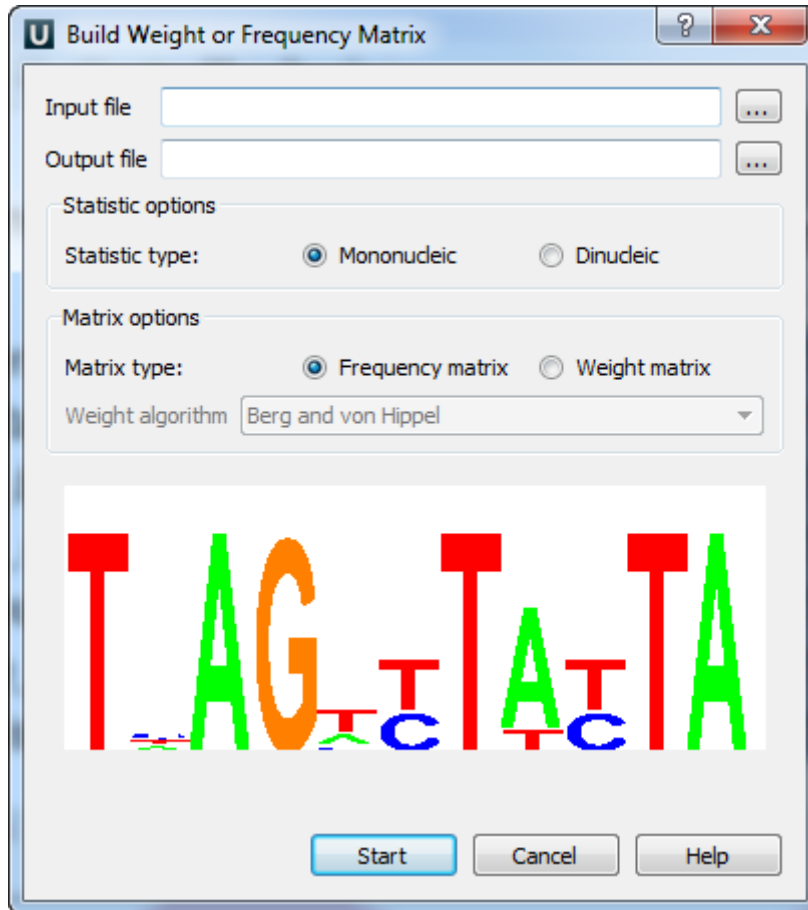
Statistic type — defines the way in which the statistics will be collected. The *Mononucleic* option is basically good for small alignments, and the *Dinucleic* option must give more appropriate results for big alignments.

Matrix type — defines the type of the resulting matrix.

If the *Frequency matrix* option is selected then the frequency matrix will be created and saved into the resulting file.

If the *Weight matrix* option is selected then the intermediate frequency matrix will be created and then transformed into a weight matrix on basis of the selected *Weight algorithm*. Then the weight matrix will be saved into the resulting file.

For some input files the colored “Alignment Logo” appears at the bottom of the dialog. It gives the representation of the selected alignment.



The "Alignment logo" appears when:

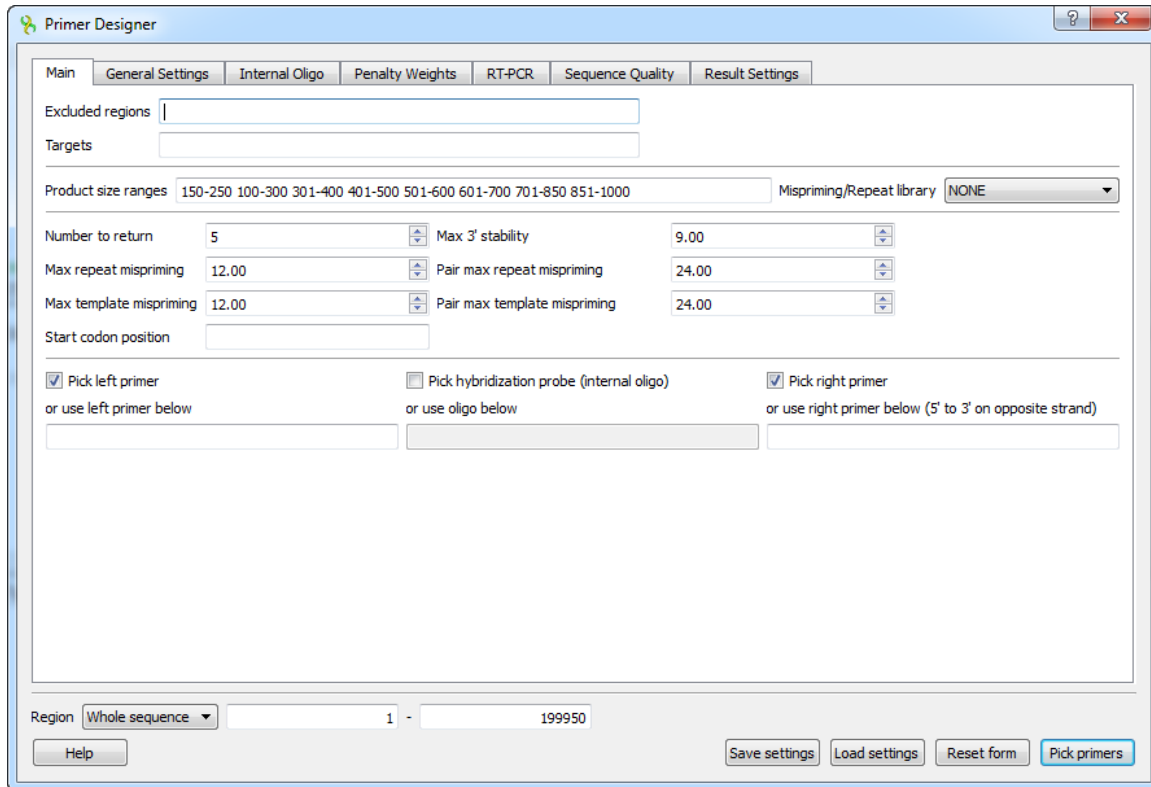
- The input file format is *.pfm, *.aln or it is a file with several sequences;
- The size of the input file is small enough.

To start the operation, press the *Start* button. The matrix will be created and saved. If the *Build weight or frequency matrix* dialog was invoked from the *Weight matrix search* dialog, then the matrix also will be chosen as the current profile.

Primer3

The *Primer3* plugin is a part of the *Primer3* tool. It is intended to pick primers from a DNA sequence.

To use the *Primer3*, open a DNA sequence and select the *Analyze Primer3* context menu item. The dialog will appear:



All available parameters are the same as in the original Primer3.

However there is one additional feature available which is not originally a part of [Primer3 tool](#). It allows user design primers for RT-PCR experiments by choosing which exons/introns to span with the primer product. This feature is described in detailed below. When you select the parameters you can save and load settings with a help of the corresponding buttons in the right corner of the dialog.

- [RT-PCR Primer Design](#)

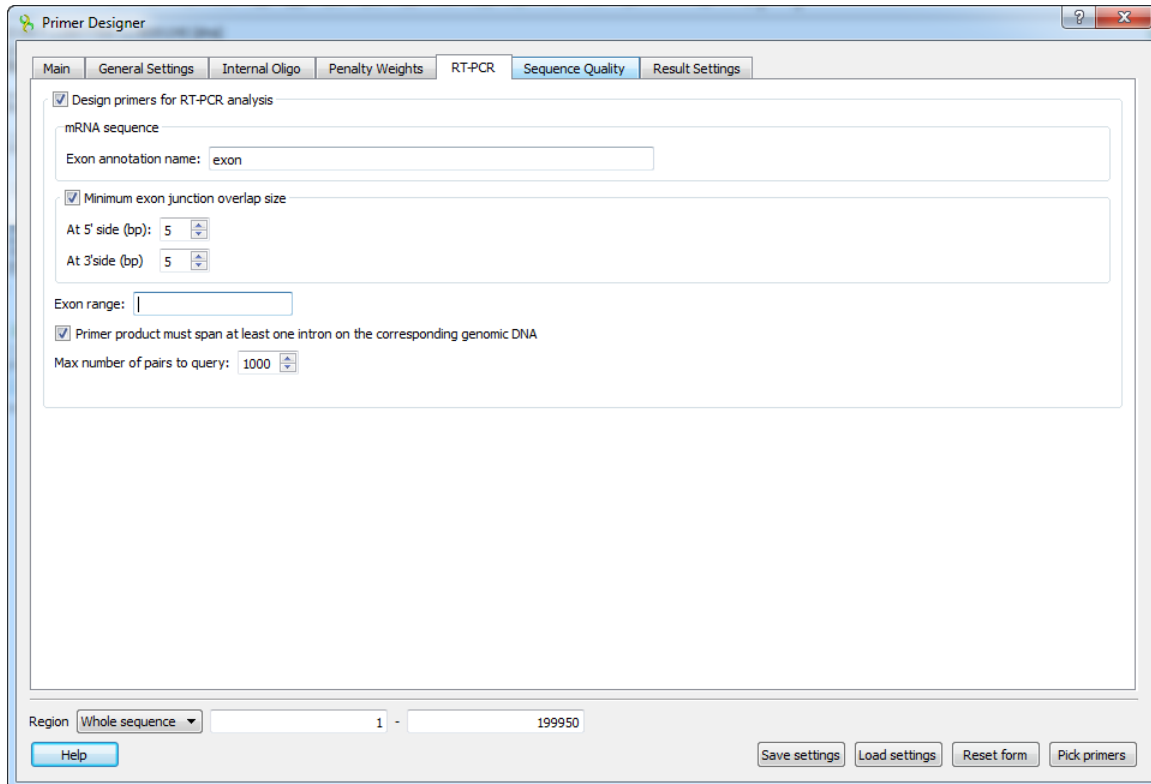
RT-PCR Primer Design

This feature allows to search for primer pairs that span introns on the genomic sequence or exon junctions on the mRNA sequence.

Note that RT-PCR design is only available for mRNA/cDNA sequences with annotated exons. There are several ways to obtain the cDNA for a corresponding DNA sequence.

- From NCBI or ENSEMBL database.
For example, one can download the ***TMPRSS2 transcript variant 1*** from NCBI Genbank using identifier [NM_001135099.1](#). This can be also done from UGENE using option [Access remote database](#) or [Search NCBI Genbank](#).
- Align the genomic and cDNA sequences using [spliced aligner](#).
For this option one must have both genomic and cDNA sequences.
In UGENE the spliced alignment can be performed using the [Spidey tool](#).
To run the alignment open the genomic sequence and select action *Align Align to mRNA sequence*.
The generated exon annotations can be then exported using action *Export Export sequence of selected annotations*

To design primers for your mRNA sequence and go to the RT-PCR tab of the *Primer Designer* dilaog:



The following parameters are available:

Exon annotation name

To detect exon boundaries UGENE searches for exonic annotations. This option allows to set custom name for annotations denoting exons. Default value is "exon"

Minimum exon junction overlap size

If checked, then only the pairs with at least one of the primers overlapping exon junction in the mRNA sequence will be selected.

At 5' side (bp)

Minimum overlap size on the 5' side of the exon junction. Default is 5 bp.

At 3' side (bp)

Minimum overlap size on the 3' side of the exon junction. Default is 5 bp.

Exon range

This option allows to limit the sequence region, where the primers are searched for. For example, setting value "3-5" will limit the search to a sequence region consisting of exons 3,4,5 of the transcript, as defined by the order in the sequence. Default value is an empty string, which means that there are no limitations.

Span at least one intron

This option makes sure that primer product should span an intron on the genomic sequence i.e the forward and reverse primers must be located in different exons. The option is enabled by default.

Max numbers of pairs to query

The algorithm applied in RT-PCR primer design first searches for all available primers in a given sequence. Then it filters the detected pairs to make sure that they satisfy the selected configuration. This option allows to set the maximum number of pairs for the initial search query. Larger number will result in increased sensitivity, but also in a longer running time. Default value is 1000.

Important: using the **RT-PCR** primer design tab will reset the values set in the *Excluded regions* and *Targets* of the **Main** configuration tab. Additionally if the *Exon range* option is set, the defined sequence region will be ignored.

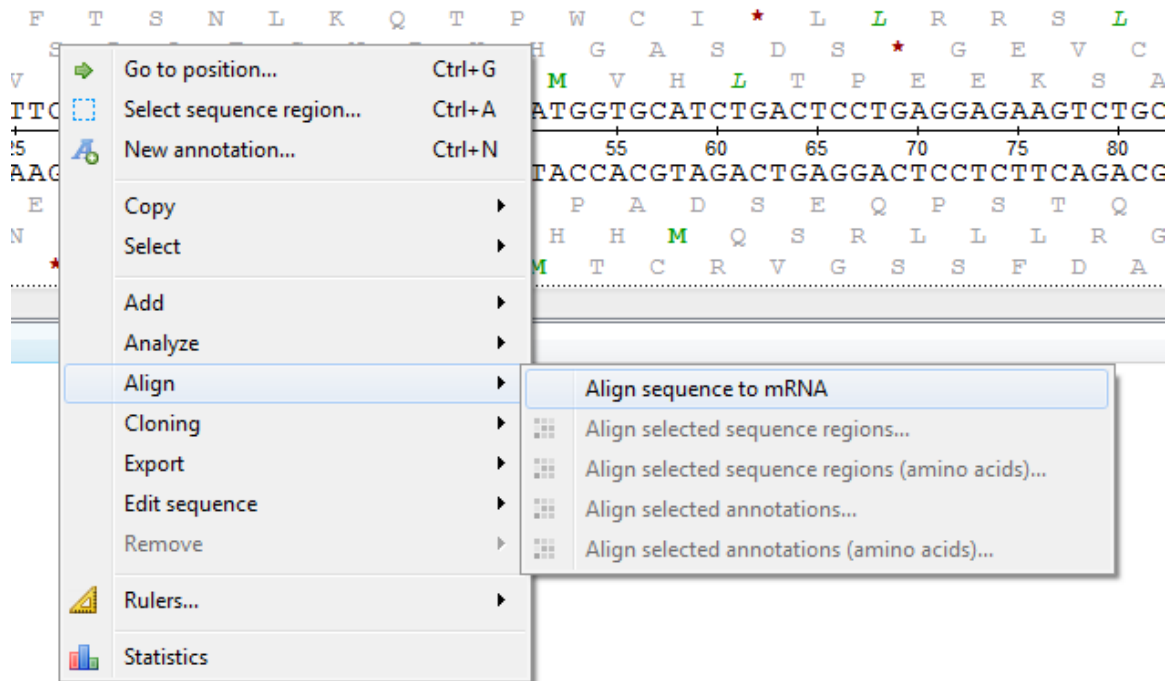
Spliced Alignment mRNA and cDNA

UGENE allows to align spliced mRNA/cDNA sequence to genomic sequences.

The default underlying algorithm which is used for the alignment is an external tool called *Spidey*.

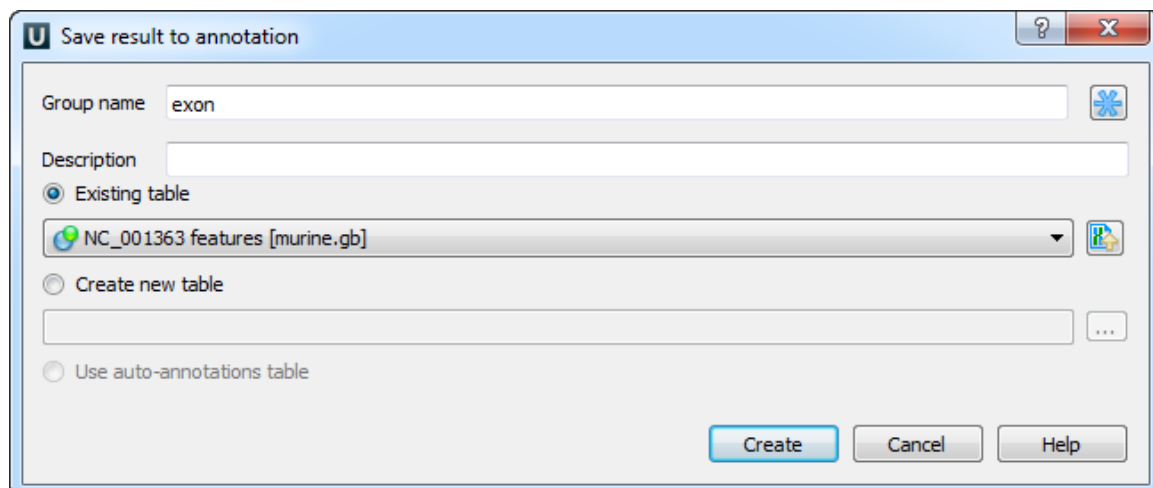
Before running the alignment make sure that *Spidey* is available and validated in the list of *External Tools*.

To perform the alignment of a mRNA sequence to a genomic sequence open the the genomic sequence in the *Sequence View*. Next activate context menu item *Align -> Align to sequence to mRNA*.



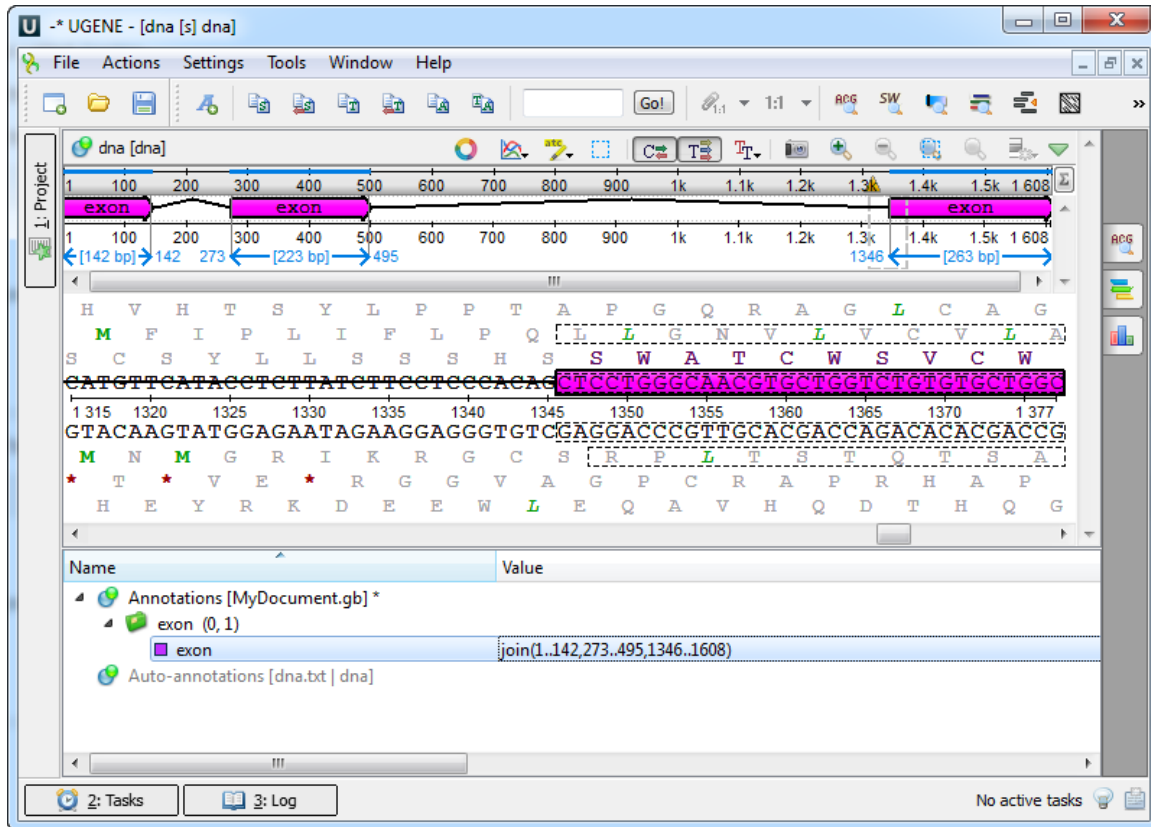
In the list of sequences select the corresponding mRNA sequence and click OK.

The following dialog will appear:



Here you can set up a file to store annotations. It could be either an existing annotation table object or a new annotation table or auto-annotations table (if it is possible). Also you can modify the group name parameter and add a description.

The resulting alignment will be saved as an annotation with the corresponding name:



External Tools Plugin

The *External Tools* plugin allows one to launch an external tool from UGENE.

To use an external tool from UGENE, the tool needs to be installed on the system and the path to it should be properly configured. However, there is no need in the additional configuration, if you've installed the UGENE Full Package, as it already contains all the tools by default.

Otherwise, if you've installed the UGENE Standard Package, you would need to configure an external tool in order to use it. Note that in this case you can download the package with all the external tools from [this page](#).

To learn how to configure an external tool, read below.

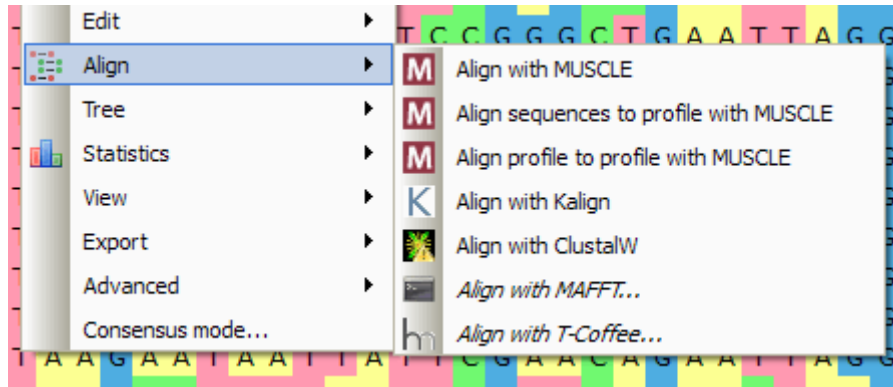
- [Configuring External Tool](#)

Configuring External Tool

To configure an external tool:

1. Make sure the tool is installed on your system.
2. Set a path to the tool executable file in UGENE. It can be set on the *External Tools* tab of the *Application Settings* dialog.

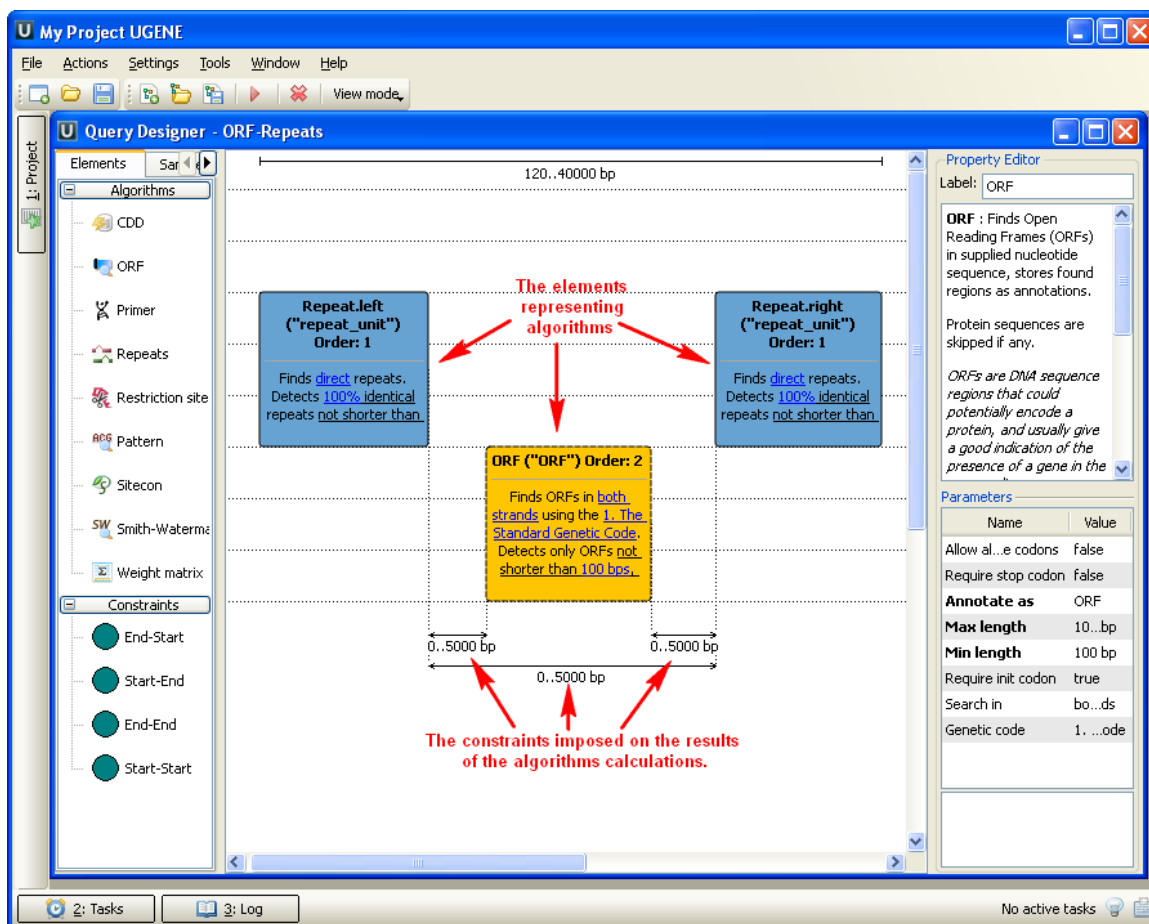
If the path hasn't been set for a tool, UGENE menu items that launch the tool are displayed in italic. For example, on the image below a path for the ClustalW external tool has been set, and paths for MAFFT and T-Coffee has not:



Query Designer

The *Query Designer* allows a molecular biologist to analyze a nucleotide sequence using different algorithms (Repeats finder, ORF finder, Weight matrix matching, etc.) at the same time imposing constraints on the positional relationship of the results obtained from the algorithms.

A user-friendly interface is used to create a schema of the algorithms and constraints.



Alternatively, you can create / edit a schema using a text editor.

When the schema has been created and all its parameters have been set you can run it for a nucleotide sequence. The results are saved as a set of annotations to the specified file in the Genbank format. Also when you have query designer scheme you can analyze a nucleotide sequence from the sequence view with a help of this schema. Call the *Analyze->Analyze with query schema* context menu item for this.

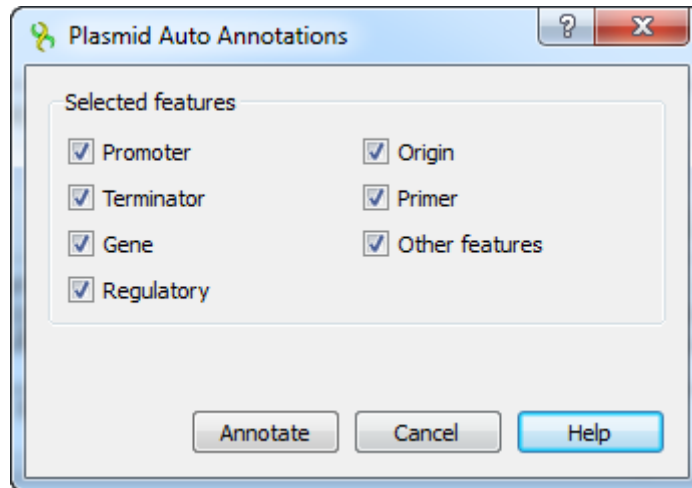
To learn more about the *Query Designer* read the *Query Designer Manual*.

Plasmid Auto Annotation

Plasmid Auto Annotation feature allows to automatically annotate possible functional elements of the given sequence such as promoters, terminators, origin of replication, known genes, common primers and other features. Conceptually this functionality is similar to the one offered by *PlasMapper* software. The database for plasmid auto-annotation is based on the following [resource](#).

To activate Plasmid Auto Annotation upon your sequence use the menu item *Analyze Annotate plasmid and custom features*. In the appear

d dialog one can selected the features to search in sequence.



The detected plasmid features are stored as automatic annotations and can be controlled through corresponding menu. Refer *Automatic Annotations Highlighting* to learn more.

The database containing features and their sequences is located in a subfolder of UGENE data folder: ***data/custom_annotations/plasmid_features.txt***.

ClustalO

Clustal is a widely used multiple sequence alignment program. It is used for both nucleotide and protein sequences. Clustal Omega is the latest addition to the Clustal family. It offers a significant increase in scalability over previous versions, allowing hundreds of thousands of sequences to be aligned in only a few hours. It will also make use of multiple processors, where present.

Clustal home page: <http://www.clustal.org>

If you are using Windows OS, there are no additional configuration steps required, as *ClustalO* executable file is included to the UGENE distribution package. Otherwise:

- Install the *Clustal* program on your system.
- Set the path to the *ClustalW* executable on the *External tools* tab of UGENE *Application Settings* dialog.

Now you are able to use *ClustalO* from UGENE.

Open a multiple sequence alignment file and select the *Align with ClustalO* item in the context menu or in the *Actions* main menu. The *Align with ClustalO* dialog will appear (see below), where you can adjust the following parameters:

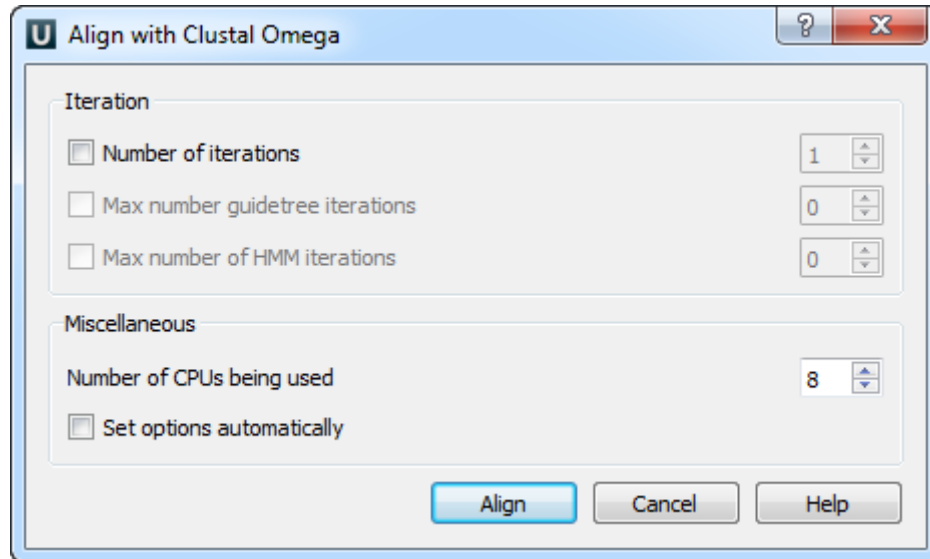
Number of iterations — number of (combined guide tree/HMM) iterations.

Max number guidetree iterations — maximum guide tree iterations.

Max number of HMM iterations — maximum number of HMM iterations.

Number of CPUs being used - number of processors to use.

Set options automatically - set options automatically (might overwrite some of your options).

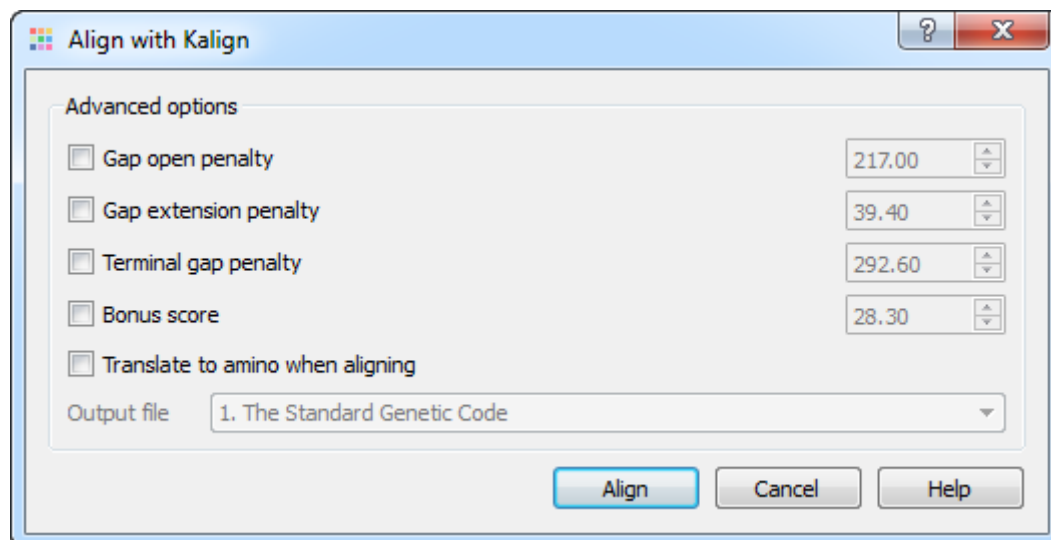


Kalign Aligning

Kalign is a fast and accurate multiple sequence package designed to align large numbers of protein sequences.

Kalign home page: [KAlign](#)

To use *Kalign* open a multiple sequence alignment file and select the *Align with Kalign* item in the context menu or in the *Actions* main menu. The following dialog appears:



The following parameters are available:

Gap opening penalty — indicates the penalty applied for opening a gap. The penalty must be negative.

Gap extension penalty — indicates the penalty applied for extending a gap.

Terminal gap penalty — the penalty to extend gaps from the N/C terminal of protein or 5'/3' terminal of nucleotide sequences.

Bonus score - a bonus score that is added to each pair of aligned residues.

Translate to amino when aligning - translates an alignment to amino when aligning.

Shared Database

The rational storage of biological data is an ever-present issue. It is not only about large data sizes, but also about the requirement of simultaneous access to them by several scientists. For instance, a few researchers from a lab may need to work on the same data, like a set of primers or data produced by sequencing. That information has to be updated and synchronized between different users and kept in a common storage. That is what UGENE Shared Database is intended for.



To start sharing data via UGENE you need to deploy a public database server. MySQL servers are currently supported. See [this paragraph](#) for details about the required server configuration.

After that any UGENE user (who knows the correct login/password, however) can [connect to the database](#). The connected database is shown in the [Project View](#) as a document exactly the same way as if the data were located on the local computer.

As described in [this paragraph](#) the users can have a read-only access to the database or be able to modify its content. A user with a read-only access can:

- Browse the data in the database
- Open the data in the UGENE views
- [Export the data to the local computer](#)

Users with write access, in addition, can:

- [Add new objects to the database](#)
- Create new folders to order the data in the database
- Modify the folders hierarchy inside the database (using [drag'n'drop](#))
- Rename objects and folders
- Delete existed objects
- Delete folders

All UGENE instances connected to a database constantly monitors the state of the database and shows changes, made by other users.



UGENE accesses large remote data, such as NGS assemblies, so that only a viewed part of them is loaded to a client computer. So, if you store the assembly data on a server, the data can be browsed in the UGENE Assembly Browser on a local computer almost instantly, without the need to copy the data on the computer or use the hard disk space.

For details see the documentation below:

- [Configuring Database](#)
- [Connecting to a Shared Database](#)
- [Adding Data to the Database](#)
- [Database in the Project](#)
- [Deleting Data](#)
- [Drag'n'drop in the Database](#)
- [Exporting Objects from the Database](#)

Configuring Database

To make use of a shared database follow the steps below:

1. *Deploy a MySQL database server*

We recommend you to download MySQL binaries from the [official site](#). Note that UGENE supports MySQL versions from 5.5 to 5.7. [Here](#) you can also find instructions on how to install and launch a MySQL server instance for each platform.

2. *Create an empty database*

Log in to the MySQL server as a user with administrative privileges (you must be able to create databases and users, and to grant privileges to the created users). In the MySQL console or in your favorite SQL browser execute the following command:
> CREATE DATABASE `your_database_name`;

3. *Create database users*

You may probably want to limit possible influence on the shared database by the UGENE users who will use it. In this case create a distinct MySQL user for each UGENE user (or a group of users). In order to do this, execute the following commands:

> CREATE USER `user_nickname` IDENTIFIED BY `user_password`;

Decide whether the created user is allowed to modify the database content or only to view it. In the first case execute the command below:

> GRANT ALL PRIVILEGES ON your_database_name.* TO `user_nickname`@`%` IDENTIFIED BY `user_password`;

and in the second case execute:

> GRANT SELECT ON your_database_name.* TO `user_nickname`@`%` IDENTIFIED BY `user_password`;

4. *Use the database from a UGENE instance*

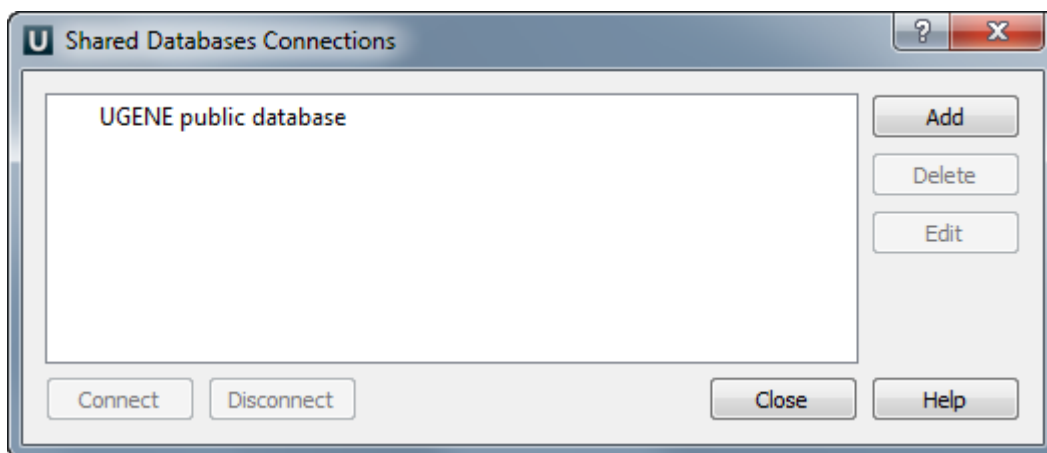
The database with "your_database_name" is now available from a UGENE instance (version 1.14 or higher). It's time to try it out and fill it with some initial data. To do it, open UGENE and [connect to the database](#). As we need to add the data to the database, use "user_nickname" and "user_password" of a user with privileges to modify the database. As soon as connection is established, [add the required data to the database](#).

From now on the data will be available for all users from this and other UGENE instances who connected to the same database.

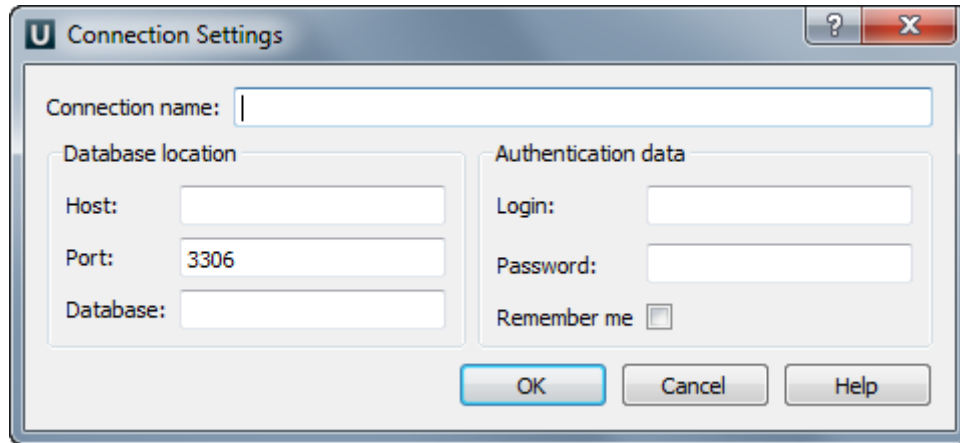
Connecting to a Shared Database

To start using the shared database you need to have a running public MySQL database server. Usually the system administrator of your department does it. You should ask him or her to give you the access to a MySQL database. Particularly, you need a few parameters to connect to the database: the IP-address of the server (the computer where a MySQL server is running), a user name for the MySQL database and a password. You can also install a MySQL server by yourself on any public computer you have an access to (even on your workstation), following the steps described in the [Configuring Database](#) section.

To connect to the database use the *File->Connect to shared database* main menu item. The following dialog appears:



To add new connection click on the *Add* button. The following dialog appears:



Here you need to specify *Host* (IP-address of the server), *Port* (number of the port used by the MySQL server) and *Database* (name of the database). You may also fill *Login* and *Password* fields. Otherwise, you are asked to input them every time you are establishing this connection until you check the *Remember me* box. Click on the *OK* button, then the connection is created and the appropriate item appears in the previous *Shared Database Connections* dialog.

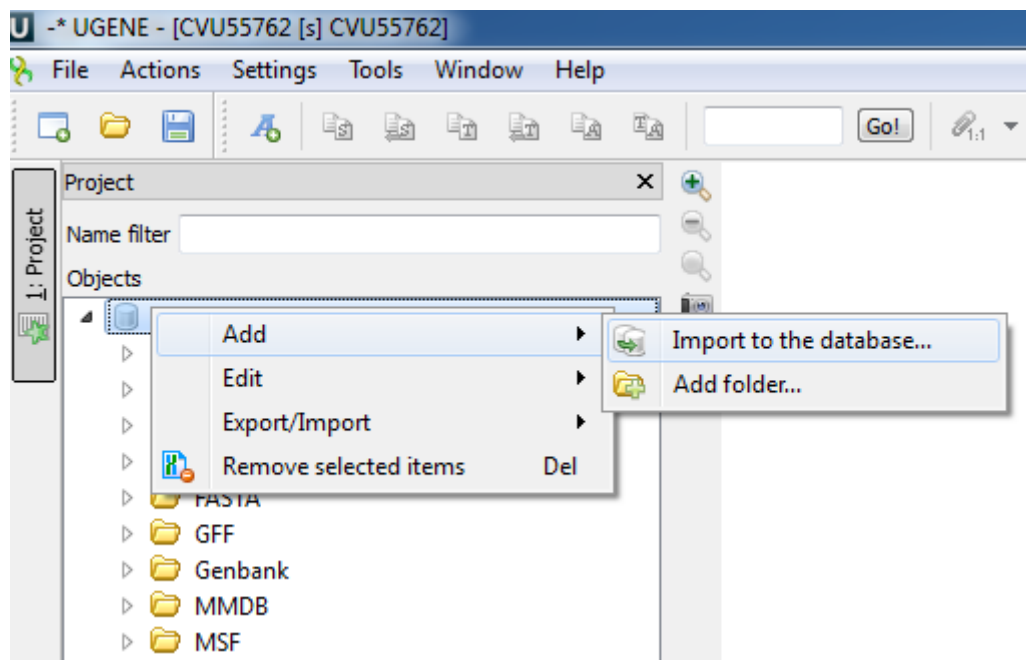
If you want to use already existing connection choose the appropriate item in the *Shared Database Connections* dialog and press the *Connect* button. This can also be done by double clicking the item. If the specified database is empty, UGENE has to initialize it. This routine is done only once. In this case you get an appropriate message box, asking whether to initialize the database or not. If you choose *Yes* the database is populated with UGENE data structures, if *No* it remains empty and UGENE does not connect to it.

If you want to delete some connection select it in the *Shared Database Connections* dialog and click on the *Delete* button. You may also edit connection parameters using the *Edit* button.

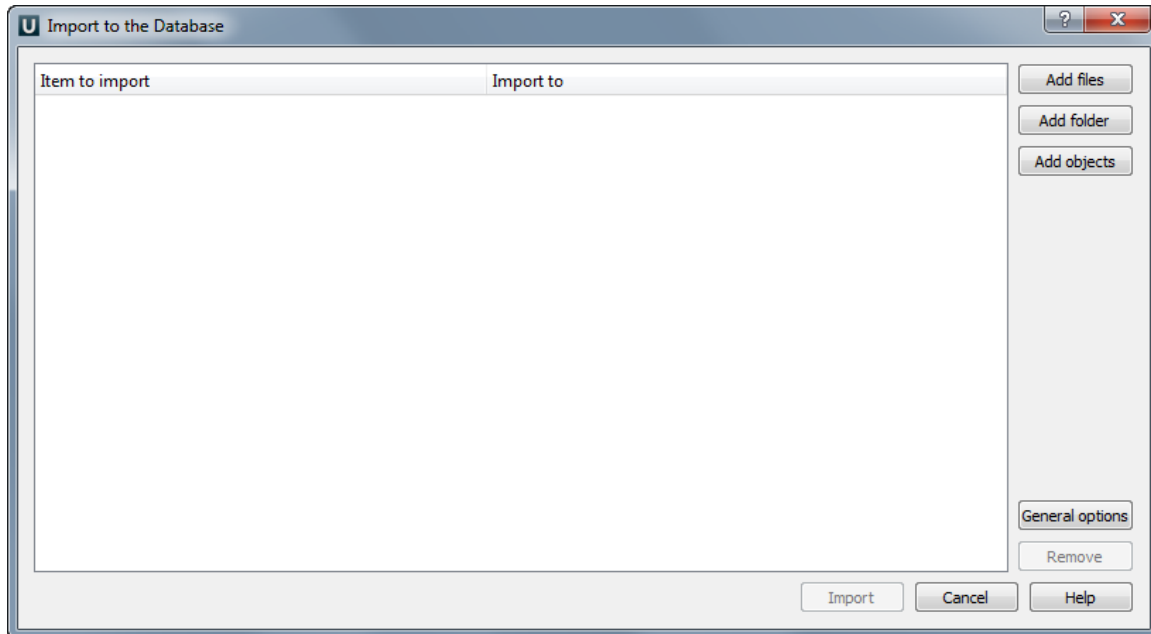
An established connection can be terminated by pressing the *Delete* button. The same effect is produced by removing the database document item from *Project View*.

Adding Data to the Database

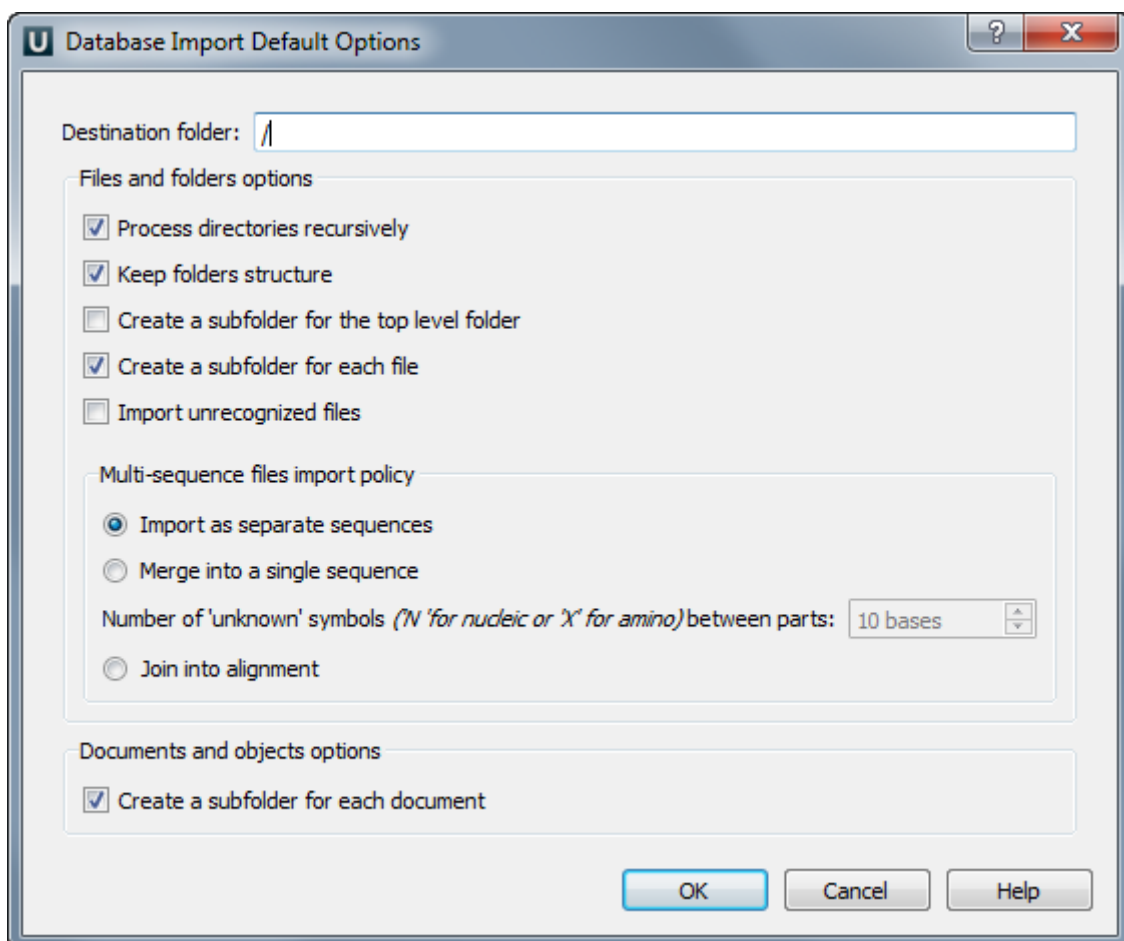
To add data to the database use the *Add->Import to the database* context menu item of the database in the project tree view. Also you can drag'n'drop it to a shared database folder.



The following dialog will appear:



Here you can add to the database files, folders or other objects from the current [Project View](#). To do this use corresponding buttons. After specifying your data click on the *Import* button. The data will be imported and appear in the database data tree. Also you can change import settings. To do this click on the *General options* button. The following dialog will appear:



Available parameters are described below:

Process directories recursively - if this option is checked, the import procedure recreates the hierarchy of the imported directories and all their sub-directories in the database. Otherwise, only the content of the directories, specified for import, is uploaded to the *Destination folder* without taking into account any sub-directories.

Create a subfolder for each file - if this option is checked, for each file uploaded to the database a new folder is created, having the same name as the file, and the file content is placed in the folder. Otherwise, the file data are imported into the *Destination folder*.

Import as separate sequences - if this option is selected and an uploaded file contains several sequences, they are represented by distinct sequence objects in the database after the import is done.

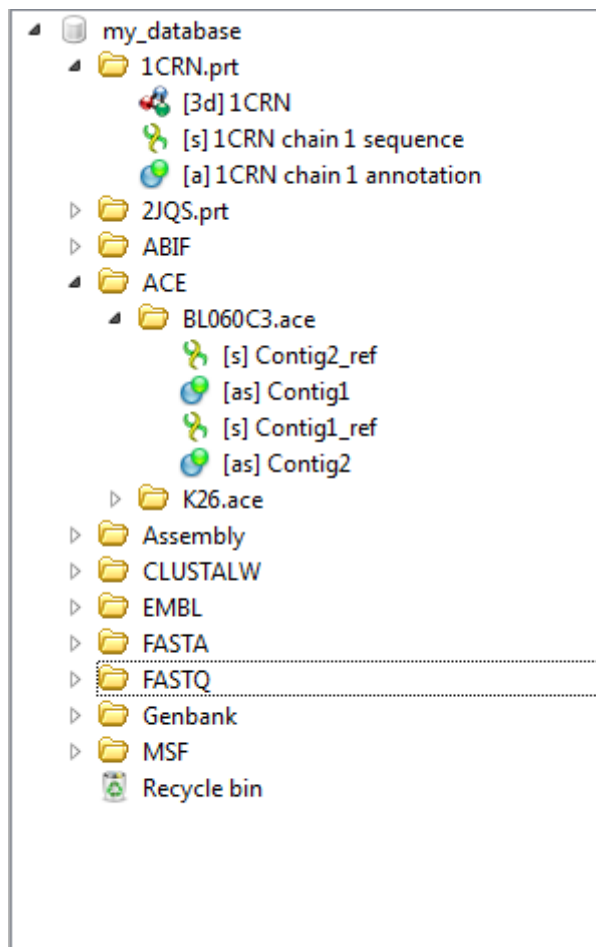
Merge into a single sequence - if this option is selected and an uploaded file contains several sequences, they are merged into a single sequence object in the database after the import is done.

Join into alignment - if this option is selected and an uploaded file contains several sequences, they are joined into a multiple sequence alignment object in the database after the import is done.

Create a subfolder for each document - if this option is checked, for each document or object uploaded to the database a new folder is created, having the same name as the file, and the data are placed in the folder. Otherwise, the data are imported into the *Destination folder*.

Database in the Project

The database in UGENE [Project View](#) looks like as a tree with folders and objects:



You can add a new folder to the database tree. To do that use the *Add->Add folder* database context menu item. To add a subfolder to some existing folder use the *Add->Add folder* folder context menu item. To delete an object or a folder press the *Delete* button or drag'n'drop it to the *Recycle bin*.

In this version of UGENE objects in the database are read-only. Nevertheless, there is a workaround to edit them. First, you need export the objects to files on your computer using the *Export/Import* object context menu. Then you can change that files locally, upload them to database and, finally, delete the originals.

If new data are added to the database by another user or removed from it, UGENE detects this and shows updates automatically in [Project View](#).

Deleting Data

To remove an object or a folder select it and press the *Delete* button or drag it to the *Recycle bin* folder.

All removed items are located in the *Recycle bin* folder.

To delete all files from *Recycle bin* click on the *Empty recycle bin* context menu item of the *Recycle bin*.

To restore objects from the *Recycle bin* select them and call the *Restore selected items* context menu item.

When the database is updated outside, UGENE shows these changes on your computer automatically.



You cannot delete any object from *Recycle bin* if it is opened on the other computer. This situation can appear if the object was being viewed by another user when you moved it to *Recycle Bin*.

Drag'n'drop in the Database

In the database tree you can drag'n'drop objects between folders, folders between folders. Also you can drag'n'drop other objects and documents from project to the database.

Exporting Objects from the Database

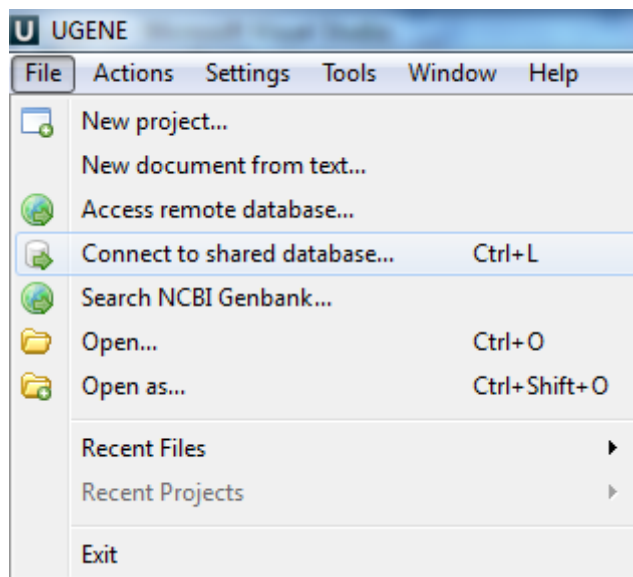
The objects in the database can not be altered though they can be deleted. To edit the objects you need to export them to the project, then make you modifications locally and replace existing originals. More detailed information about exporting you can find [here](#).

UGENE Public Storage

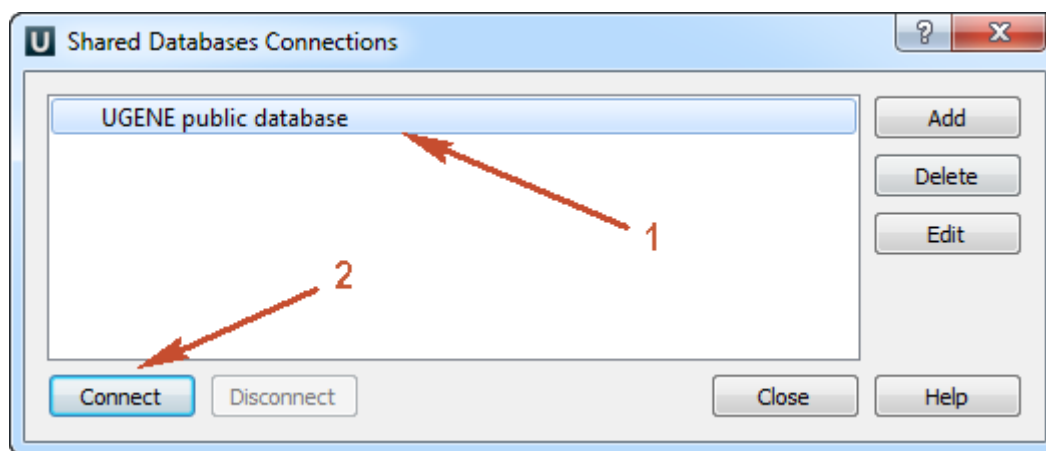
UGENE provides the free-to-use public bioinformatics data storage. This storage keeps DNA sequences of several popular genomes such as human, mouse, drosophila melanogaster, etc. and hundreds of plasmid sequences.

Follow the instructions for accessing the storage:

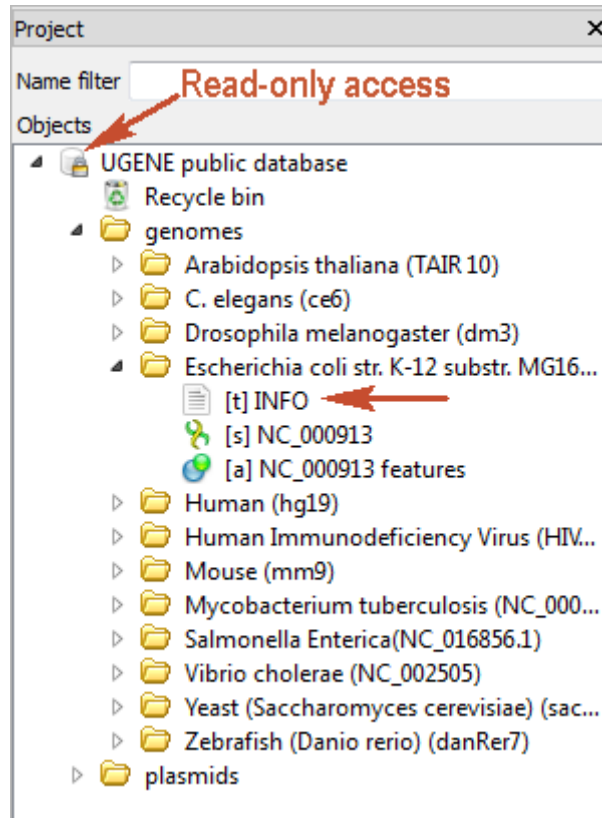
1. Use the menu *File* -> *Connect to shared database* (or press the *Ctrl+L* shortcut).




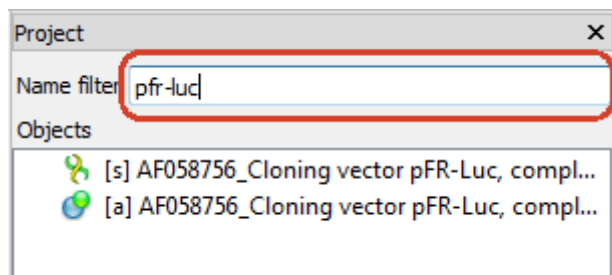
2. Choose the predefined "UGENE public database" item and click the *Connect* button.



3. Browse the storage content.



- The storage document is marked with the lock icon . It means that the storage provides the read-only access. Any data modifications are unavailable for such type of access (importing, removing or replacing of data).
- Each genome folder contains the *INFO* text object. It is the information about the genome or its source.
- You can [export](#) the data to your computer for working with the data locally.
- There are hundreds of plasmids in the storage. Use the name filter for fast navigating and searching an interesting plasmid:



The list of available genomes:

- Human (hg19)
- Mouse (mm9)
- Arabidopsis thaliana (TAIR 10)
- C. elegans (ce6)
- Drosophila melanogaster (dm3)
- Escherichia coli str. K-12 substr. MG1655 K12(NC_000913.3)
- Human Immunodeficiency Virus (HIV-2)
- Mycobacterium tuberculosis (NC_000962.3)
- Salmonella Enterica(NC_016856.1)
- Vibrio cholerae (NC_002505)
- Yeast (Saccharomyces cerevisiae)(sacCer3)
- Zebrafish (Danio rerio) (danRer7)

UGENE Command Line Interface

UGENE command line interface (CLI) was developed keeping in mind the following principles:

- To make it as easy as popular shell commands.
- To include all significant UGENE features.
- To allow users to add their own commands.

To use UGENE CLI make sure to add the path to the UGENE executable to your **%PATH%** environment variable.

The general syntax is the following:

```
ugene [--task=]task_name] [--task_parameter=value ...] [-task_parameter value ...]
[--option[=value]] [-option[ value]]
```

Here:

task_name — task to execute, it can be one of the *predefined tasks* or *a task you have created*.

task_parameter — parameter of the specified task. Some parameters of a task are required, like *in* and *out* parameters of some tasks.

option — one of the *CLI options*.

See the example below:

```
ugene align --in=COI.aln -out result.aln -log-level-details
```

- CLI Options
- CLI Predefined Tasks
 - Format Converting Sequences
 - Converting MSA
 - Extracting Sequence
 - Finding ORFs
 - Finding Repeats
 - Finding Pattern Using Smith-Waterman Algorithm
 - Adding Phred Quality Scores to Sequence
 - Local BLAST Search
 - Local BLAST+ Search
 - Remote NCBI BLAST and CDD Requests
 - Annotating Sequence with UQL Schema
 - Building Profile HMM Using HMMER2
 - Searching HMM Signals Using HMMER2
 - Aligning with MUSCLE
 - Aligning with ClustalW
 - Aligning with ClustalO
 - Aligning with Kalign
 - Aligning with MAFFT
 - Aligning with T-Coffee
 - Building PFM
 - Searching for TFBS with PFM
 - Building PWM
 - Searching for TFBS with Weight Matrices
 - Building Statistical Profile for SITECON
 - Searching for TFBS with SITECON
 - Fetching Sequence from Remote Database
 - Gene-by-Gene Report
 - Reverse-Complement Converting Sequences
 - Variants Calling
 - Generating DNA Sequence
- Creating Custom CLI Tasks

CLI Options

`--help | -h [<option_name> | <task_name>]`

Shows help information. For example:

```

ugene --help          ## Shows general UGENE CLI help.
ugene -h

ugene --help=<option_name>  ## Shows help for the <option_name> option.
ugene -h <option_name>

ugene --help=<task_name>    ## Shows help for the <task_name> task.
ugene -h <task_name>

```

`--task=<task_name> [<task_parameter>=value ...]`

Specifies the task to run. A user-defined UGENE workflow schema can be used as a task name. For example:

```

ugene --task=align --in=COI.aln -out result.aln

ugene --task=C:\myschema.uwl --in=COI.aln --out=res.aln

```

`--log-no-task-progress`

A task progress is shown by default when a task is running. This option specifies not to show the progress.

`--log-level="[<category1>=<level1> [, ...]]"`

Sets the log level per category. If a category is not specified, the log level is applied to all categories.

The following categories are available:

- "Algorithms"
- "Console"
- "Core Services"
- "Input/Output"
- "Performance"
- "Remote Service"
- "Scripts"
- "Tasks".

The following log levels are available: TRACE, DETAILS, INFO, ERROR or NONE.

By default, loglevel=ERROR.

For example:

```

ugene --log-level=NONE

ugene --log-level="Tasks=DETAILS, Console=DETAILS"

```

`--log-format="<format_string>"`

Specifies the format of a log line.

Use the following notations: L - level, C - category, YYYY or YY - year, MM - month, dd - day, hh - hour, mm - minutes, ss - seconds, zzz - milliseconds.

By default, logformat="[L][hh:mm]".

`--license`

Shows license information.

`--lang=language_code`

Specifies the language to use (e.g. for the log output). The following values are available:

- CS (Czech)

- *EN* (English)
- *RU* (Russian)

--log-color-output

If log output is enabled, this option make it colored: *ERROR* messages are displayed in red, *DETAILS* messages are displayed in green, *TRACE* messages are displayed in blue.

--session-db

Session database is stored in the temporary file that is created for every UGENE run. But it can supplied with the command line argument. If the supplied file does not exist it will be created. The session database file is removed after closing of UGENE.

For example:

```
ugene --session-db=D:/session.ugenedb
```

--version

Shows version information.

--tmp-dir=<path_to_file>

Path to temporary folder.

--ini-file=<path_to_file>

Loads configuration from the specified .ini file. By default the UGENE.ini file is used.

--genome-aligner

UGENE Genome Aligner is an efficient and fast tool for short read alignment. It has 2 work modes: build index and align short reads (default mode).

If there is no index available for reference sequence it will be built on the fly.

Usage: *ugene --genome-aligner { --option[=argument] }*

The following options are available:

--build-index Use this flag to only build index for reference sequence.

--reference Path to reference genome sequence

--short-reads Path to short-reads data in FASTA or FASTQ format

--index Path to prebuilt index (base file name or with .idx extension). If not set, index is searched in system temporary directory. If **--build-index** option is applied, index will be saved to specified path.

--result Path to output alignment in UGENEDB or SAM format (see **--sam**)

--memsize Memory size (in Mbs) reserved for short-reads. The bigger value the faster algorithm works. Default value depends on available system memory.

--ref-size Index fragmentation size (in Mbs). Small fragments better fit into RAM, allowing to load more short reads. Default value is 10.

--n-mis Absolute amount of allowed mismatches per every short-read (mutually exclusive with **--pt-mis**). Default value is 0.

--pt-mis Percentage amount of allowed mismatches per every short-read (mutually exclusive with **--n-mis**). Default value is 0.

--rev-comp Use both the read and its reverse complement during the aligning.

--best Report only about best alignments (in terms of mismatches).

--omit-size Omit reads with qualities lower than the specified value. Reads which have no qualities are not omitted. Default value is 0.

--sam Output aligned reads in SAM format. Default value is false.

For example:

```
Build index for reference sequence:
ugene --genome-aligner --build-index --reference=/path/to/ref
```

```
Align short reads using existing index:
ugene --genome-aligner --reference=/path/to/ref --short-reads=/path/to/reads
--result=/path/to/result
```

CLI Predefined Tasks

Using current version of UGENE you can perform the following tasks by running a simple command:

- Format Converting Sequences
- Converting MSA
- Extracting Sequence
- Finding ORFs
- Finding Repeats
- Finding Pattern Using Smith-Waterman Algorithm
- Adding Phred Quality Scores to Sequence
- Local BLAST Search
- Local BLAST+ Search
- Remote NCBI BLAST and CDD Requests
- Annotating Sequence with UQL Schema
- Building Profile HMM Using HMMER2
- Searching HMM Signals Using HMMER2
- Aligning with MUSCLE
- Aligning with ClustalW
- Aligning with ClustalO
- Aligning with Kalign
- Aligning with MAFFT
- Aligning with T-Coffee
- Building PFM
- Searching for TFBS with PFM
- Building PWM
- Searching for TFBS with Weight Matrices
- Building Statistical Profile for SITECON
- Searching for TFBS with SITECON
- Fetching Sequence from Remote Database
- Gene-by-Gene Report
- Reverse-Complement Converting Sequences
- Variants Calling
- Generating DNA Sequence

Format Converting Sequences

Task Name: convert-seq

Converts a sequence from one format to another.

Parameters:

in — input sequence file. [String, Required]

out — name of the output file. [String, Required]

format — format of the output file. [String, Optional]

The following values are available:

- fasta
- fastq
- genbank
- gff
- raw

Example:

```
ugene convert-seq --in=human_T1.fa --out=human_T1.gbk --format=genbank
```

Converting MSA

Task Name: convert-msa

Converts a multiple sequence alignment file from one format to another.

Parameters:

in — input multiple sequence alignment file. [String, Required]

out — name of the output file. [String, Required]

format — format of the output file. [String, Optional]

The following values are available:

- clustal (default)
- fasta
- mega
- msf
- nexus
- phylip-interleaved
- phylip-sequential
- stockholm

Example:

```
ugene convert-msa --in=CBS.sto --out=CBS --format=msf
```

Extracting Sequence

Task Name: extract-sequence

Extracts annotated regions from an input sequence.

Parameters:

in — semicolon-separated list of input files. [String, Required]

out — output file. [String, Required]

annotation-names — list of annotations names which will be accepted or filtered. [String, Optional]

annotation-names-file - file with annotation names, separated with whitespaces which will be accepted or filtered. [String, Optional]

accumulate - *accumulate all incoming data in one file or create separate files for each input. In the latter case, an incremental numerical suffix is added to the file name (using 'True' by default).* [Boolean]

accept-or-filter — if set to *true*, accepts only the specified annotations, if set to *false*, accepts all annotations except the specified ones. [Boolean, Optional]

complement — complements the annotated regions if the corresponding annotation is located on the complement strand. [Boolean, Optional]

extend-left — extends the resulting regions to the left for the specified number of base symbols. [Number, Optional]

extend-right — extends the resulting regions to the right for the specified number of base symbols. [Number, Optional]

gap-length — inserts a gap of the specified length between the merged annotations.

transl - translates the annotated regions. [Boolean, Optional]

Example:

```
ugene extract-sequence --in=sars.gb --out=res.fa --annotation-names=gene
```

Finding ORFs

Task Name: find-orfs

Searches for Open Reading Frames (ORFs) in nucleotide sequences and saves the regions found as annotations.

Parameters:

in — semicolon-separated list of input files. [String, Required]

out — output file with the annotations. [String, Required]

name — name of the annotated regions. [String, Optional, Default: "ORF"]

min-length — ignores ORFs shorter than the specified length. [String, Optional, Default: 100]

require-stop-codon — ignores boundary ORFs that last beyond the search region (i.e. have no stop codon within the range). [Boolean, Optional, Default: false]

require-init-codon — allows ORFs starting with any codon other than terminator. [Boolean, Optional, Default: true]

allow-alternative-codons — allows ORFs starting with alternative initiation codons, accordingly to the current translation table. [Boolean, Optional, Default: false]

Example:

```
ugene find-orfs --in=human_T1.fa --out=result.gb --require-init-codon=false
```

Finding Repeats

Task Name: find-repeats

Searches for repeats in sequences and saves the regions found as annotations.

Parameters:

in — semicolon-separated list of input files. [String, Required]

out — output file with the annotations. [String, Required]

name — name of the annotated regions. [String, Optional, Default: "repeat_unit"]

min-length — minimum length of the repeats. [Number, Optional, Default: 5]

identity — percent identity between repeats. [Number, Optional, Default: 100]

min-distance — minimum distance between the repeats. [Number, Optional, Default: 0]

max-distance — maximum distance between the repeats. [Number, Optional, Default: 5000]

inverted — if *true*, searches for the inverted repeats. [Boolean, Optional, Default: false]

Example:

```
ugene find-repeats --in=murine.gb --out=murine_repeats.gb --identity=99
```

Finding Pattern Using Smith-Waterman Algorithm

Task Name: find-sw

Searches for a pattern in a nucleotide or protein sequence using the Smith-Waterman algorithm and saves the regions found as annotations.

Parameters:

in — input sequence file. [String, Required]

out — output file with the annotations. [String, Required]

name — name of the annotated regions. [String, Optional, Default: "misc_feature"]

ptrn — subsequence pattern to search for (e.g. AGGCC7). [String, Required]

score — percent identity between the pattern and a subsequence. [Number, Optional, Default: 90]

matrix — scoring matrix. [String, Optional, Default: "Auto"]

Among others the following values are available:

- blosum62
- dna
- rna
- dayhoff
- gonnet
- pam250
- etc.

The matrices available are stored in the \$UGENE\data\weight_matrix directory.

filter — results filtering strategy. [String, Optional, Default: "filter-intersections"]

The following values are available:

- filter-intersections
- none

Example:

```
ugene find-sw --in=human_T1.fa --out=sw.gb --ptrn=TGCT --filter=none
```

Adding Phred Quality Scores to Sequence

Task Name: join-quality

Adds Phread quality scores to a sequence and saves the result to the output FASTQ file.

Parameters:

in — input sequence file. [String, Required]

quality — input Phred quality scores file. [String, Required]

out — output FASTQ file. [String, Required]

Example:

```
ugene join-quality --in=e_coli.fa --quality=e_coli.qual --out=res.fastq
```

Local BLAST Search

Task Name: local-blast

Performs a search on a local BLAST database using old version of the NCBI BLAST.



BLAST is used as an *external tool* and must be installed on your system.

Parameters:

toolpath — path to the blastall executable. By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

tmpdir — directory for temporary files. By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

in — semicolon-separated list of input sequence files. [String, Required]

dbpath — path to the BLAST database files. [String, Required]

dbname — base name of the BLAST database files. [String, Required]

out — output Genbank file, the results of the search are stored as annotations. [String, Required]

name — name of the annotations. [String, Optional, Default: "blast result"]

p — type of the BLAST search. [String, Optional, Default: "blastn"]

The following values are available:

- blastn
- blastp
- blastx
- tblastn
- tblastx

e — expectation value threshold. [Number, Optional, Default: 10]


Example:

```
ugene local-blast --in=input.fa --dbpath=. --dbname=mydb --out=output.gb
```

Local BLAST+ Search

Task Name: local-blast+

Performs a search on a local BLAST database using BLAST+.

 BLAST+ is used as an *external tool* and must be installed on your system.

Parameters:

toolpath — path to an appropriate BLAST executable (e.g. blastn, blastp, etc.). By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

tmpdir — directory for temporary files. By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

in — semicolon-separated list of input sequence files. [String, Required]

dbpath — path to the BLAST database files. [String, Required]

dbname — base name of the BLAST+ database files. [String, Required]

out — output Genbank file, the results of the search are stored as annotations. [String, Required]

name — name of the annotations. [String, Optional, Default: "blast result"]

p — type of the BLAST search. [String, Optional, Default: "blastn"]

The following values are available:

- blastn
- blastp
- blastx
- tblastn
- tblastx

e — expectation value threshold. [Number, Optional, Default: 10]

Example:


```
ugene local-blast+ --in=input.fa --dbpath=. --dbname=mydb --out=output.gb
```

Remote NCBI BLAST and CDD Requests

Task Name: remote-request

Performs remote requests to the NCBI. Saves the results as annotations.

Parameters:

in — semicolon-separated list of input files. A file can be of any format containing sequences or alignments. [String, Required]

db — database to search in. [String, Optional, Default: "ncbi-blastn"]

The following databases are available:

- "ncbi-blastn" for nucleotide sequences
- "ncbi-cdd" for amino acid sequences
- "ncbi-blastp" for amino acid sequences

out — output Genbank file. [String, Required]

eval — specifies the statistical significance threshold for reporting matches against database sequences. [Number, Optional, Default: 10]

hits — maximum number of hits, that will be shown. [Number, Optional, Default: 10]

name — name of the result annotations. If not set, name will be specified with the "cdd" result or the "blast" result. [String, Optional, Default: "cdd" or "blast"]

short — optimizes search for short sequences. [Boolean, Optional, Default: false]

blast-output — path to the file with the NCBI-BLAST output (only for the "ncbi-blastp" and "ncbi-blastn" databases). [Boolean, Optional, Default: the file is not saved]

Example:

```
ugene remote-request --in=seq.fa --db=ncbi-blastp --out=res.gb
```

Annotating Sequence with UQL Schema

Task Name: query

Annotates a sequence in compliance with a UGENE Query Language (UQL) schema. This allows to analyze a sequence using different algorithms at the same time imposing constraints on the positional relationship of the results.

To learn more about the UQL schemas read the [Query Designer Manual](#).

Parameters:

in — semicolon-separated list of input sequence files. [String, Required]

out — output Genbank file with the annotations. [String, Required]

schema — UQL schema. [String, Required]

merge — if true, merges regions of each result into a single annotation. [Boolean, Optional, Default: false]

offset — if *merge* is set to true, specified left and right offsets for merged annotations. [Number, Optional, Default: 0]

Example:

```
ugene query --in=input.fa --out=result.gb --schema=RepeatsWithORF.uql
```

Building Profile HMM Using HMMER2

Task Name: hmm2-build

Builds a profile HMM using the HMMER2 tools.

Parameters:

in — semicolon-separated list of input multiple sequence alignment files. [String, Required]

out — output HMM file. [String, Required]

name — name of the profile HMM. [String, Optional, Default: "hmm_profile"]

calibrate — enables/disables calibration. [Boolean, Optional, Default: true]

seed — random seed, a non-negative integer. [Number, Optional, Default: 0]

Example:

```
ugene hmm2-build --in=CBS.sto --out=CBS.hmm
```

Searching HMM Signals Using HMMER2

Task Name: hmm2-search

Searches each input sequence for the significantly similar sequence that matches to all specified profile HMM using the HMMER2 tool.

Parameters:

seq — semicolon-separated list of the input sequence files. [String, Required]

hmm — semicolon-separated list of the input HMM files. [String, Required]

out — output file with annotations. [String, Required]

name — name of the result annotations. [String, Optional, Default: "hmm_signal"]

e-val — e-value that can be used to exclude low-probability hits from the result. [Number, Optional, Default: 1e-1]

score — score based filtering which is an alternative to e-value filtering to exclude low-probability hits from the result. [Number, Optional, Default: -1000000000]

Example:

```
ugene hmm2-search --seq=CBS_seq.fa --hmm=CBS.hmm --out=CBS_hmm.gb
```

Aligning with MUSCLE

Task Name: align

Performs multiple sequence alignment with MUSCLE algorithm and saves the resulting alignment to file. Source data can be of any format containing sequences or alignments.

Parameters:

in - Input alignment [Url datasets]
max-iterations - Maximum number of iterations (using '2' by default) [Number]
mode - Selector of preset configurations, that give you the choice of optimizing accuracy, speed, or some compromise between the two. The default favors accuracy (using 'MUSCLE default' by default) [Number]
range - Whole alignment or column range e.g. 1..100 (using 'Whole alignment' by default) [String]
stable - Do not rearrange aligned sequences (using 'True' by default) [Boolean]
format - Document format of output alignment (using 'clustal' by default) [String]
out - Output alignment [String]


Example:

```
ugene align --in=test.aln --out=test_out.aln --format=clustal
```

Aligning with ClustalW

Task Name: align-clustalw

Multiple sequence alignment with ClustalW.

 ClustalW is used as an *external tool* and must be installed on your system.

Parameters:

toolpath — path to the ClustalW executable. By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

tmpdir — directory for temporary files. [String, Optional]

in — semicolon-separated list of input files. [String, Required]

out — output file. [String, Required]

format — format of the output file. [String, Optional]

Example:

```
ugene align-clustalw --in=COI.aln --out=COI.sto --format=stockholm
```

Aligning with ClustalO

Task Name: align-clustalo

Create alignment with ClustalO. ClustalO is a general purpose multiple sequence alignment program for proteins.

 ClustalO is used as an *external tool* and must be installed on your system.

Parameters:

in - Input alignment [Url datasets]

format - Document format of output alignment (using 'clustal' by default) [String]

out - Output alignment [String]

max-guidetree-iterations - Maximum number guidetree iterations (using '0' by default) [Number]

max-hmm-iterations - Maximum number of HMM iterations (using '0' by default) [Number]

iter - Number of (combined guide-tree/HMM) iterations (using '1' by default) [Number]

toolpath - ClustalO location (using the path specified in UGENE by default) [String]

auto - Set options automatically (might overwrite some of your options) (using 'False' by default) [Boolean]

tmpdir - Directory to store temporary files (using UGENE temporary directory by default) [String]

Example:

```
ugene align-clustalw --in=test.aln --out=test_out.aln --format=clustal
```

Aligning with Kalign

Task Name: align-kalign

Multiple sequence alignment with Kalign.

Parameters:

in — semicolon-separated list of input files. [String, Required]

out — output file in the ClustalW format. [String, Required]


Example:

```
ugene align-kalign --in=COI.aln --out=COI_aligned.aln
```

Aligning with MAFFT

Task Name: align-mafft

Multiple sequence alignment with MAFFT.

 MAFFT is used as an *external tool* and must be installed on your system.

Parameters:

toolpath — path to the MAFFT executable. By default, the path specified in the *Application Settings* is applied. [String, Optional, Default: "default"]

tmpdir — directory for temporary files. [String, Optional]

in — semicolon-separated list of input files. [String, Required]

out — output file. [String, Required]

format — format of the output file. [String, Required]

op — penalty for opening a gap. [Number, Optional]

ep — penalty for extending a gap. [Number, Optional]

maxiterate — maximum number of cycles of iterative refinement. [Number, Optional]

Example:

```
ugene align-mafft --in=COI.aln --out=COI_aligned.aln
```

Aligning with T-Coffee

Task Name: align-tcoffee

Create alignment with T-Coffee. T-Coffee is a collection of tools for computing, evaluating and manipulating multiple alignments of DNA, RNA, Protein Sequences.

 T-Coffee is used as an *external tool* and must be installed on your system.

Parameters:

gap-ext-penalty - Gap Extension Penalty. Positive values give rewards to gaps and prevent the alignment of unrelated segments (using '0' by default) [Number]

gap-open-penalty - Gap Open Penalty. Must be negative, best matches get a score of 1000 (using '-50' by default) [Number]

iter-max - Number of iteration on the progressive alignment: 0 - no iteration (default), -1 - Nseq iterations (using '0' by default) [Number]

toolpath - T-Coffee location (using the path specified in UGENE by default) [String]

tmpdir - Directory to store temporary files (using UGENE temporary directory by default) [String]

in - Input alignment [Url datasets]

format - Document format of output alignment (using 'clustal' by default) [String]

out - Output alignment [String]

Example:

```
ugene align-tcoffee --in=test.aln --out=test_out.aln --format=clustal
```

Building PFM

Task Name: pfm-build

Builds a position frequency matrix from a multiple sequence alignment file.

Parameters:

in — semicolon-separated list of input MSA files. [String, Required]

out — output file. [String, Required]

type — type of the matrix. [Boolean, Optional, Default: false]

The following values are available:

- true (dinucleic type)
- false (mononucleic type)

Dinucleic matrices are more detailed, while mononucleic ones are more useful for small input data sets.

Example:

```
ugene pfm-build --in=COI.aln --out=result.pfm
```

Searching for TFBS with PFM

Task Name: pfm-search

Searches for transcription factor binding sites (TFBS) with position weight matrices (PWM) converted from input position frequency matrices (PFM) and saves the regions found as annotations.

Parameters:

seq — semicolon-separated list of input sequence files to search TFBS in. [String, Required]

matrix — semicolon-separated list of the input PFM. [String, Required]

out — output Genbank file.

name — name of the annotated regions. [String, Optional, Default: "misc_feature"]

type — type of the matrix. [Boolean, Optional, Default: false]

The following values are available:

- true (dinucleic type)
- false (mononucleic type)

Dinucleic matrices are more detailed, while mononucleic ones are more useful for small input data sets.

algo — algorithm used to convert a PFM to a PWM. [String, Optional, Default: "Berg and von Hippel"]

The following values are available:

- Berg and von Hippel
- Log-odds
- Match
- NLG

score — minimum percentage score to detect TFBS. [Number, Optional, Default: 85]

strand — strands to search in. [Number, Optional, Default: 0]

The following values are available:

- 0 (both strands)
- 1 (direct strand)
- -1 (complement strand)

Example:

```
ugene pfm-search --seq=in.fa --matrix=MA0265.1.pfm;MA0266.1.pfm --out=res.gb
```

Building PWM

Task Name: pwm-build

Builds a position weight matrix from a multiple sequence alignment file.

Parameters:

in — semicolon-separated list of input MSA files. [String, Required]

out — output file. [String, Required]

type — type of the matrix. [Boolean, Optional, Default: false]

The following values are available:

- true (dinucleic type)
- false (mononucleic type)

Dinucleic matrices are more detailed, while mononucleic ones are more useful for small input data sets.

algo — algorithm used to build the matrix. [String, Optional, Default: "Berg and von Hippel"]

The following values are available:

- Berg and von Hippel
- Log-odds
- Match
- NLG

Example:

```
ugene pwm-build --in=COI.aln --out=result.pwm
```

Searching for TFBS with Weight Matrices

Task Name: pwm-search

Searches for transcription factor binding sites (TFBS) with position weight matrices (PWM) and saves the regions found as annotations.

Parameters:

seq — semicolon-separated list of input sequence files to search TFBS in. [String, Required]

matrix — semicolon-separated list of the input PWM. [String, Required]

out — output Genbank file.

name — name of the annotated regions. [String, Optional, Default: "misc_feature"]

min-score — minimum percentage score to detect TFBS. [Number, Optional, Default: 85]

strand — strands to search in. [Number, Optional, Default: 0]

The following values are available:

- 0 (both strands)
- 1 (direct strand)
- -1 (complement strand)

Example:

```
ugene pwm-search --seq=input.fa --matrix=Aro80.pwm;Aft1.pwm --out=res.gb
```

Building Statistical Profile for SITECON**Task Name:** sitecon-build

Builds a statistical profile for SITECON. It can be later used to search for TFBS.

Parameters:

in — semicolon-separated list of input DNA multiple sequence alignment files. An input file must not contain gaps. [String, Required]

out — output file. If several input files have been supplied, then a sitecon profile is built for each input file, i.e. several output files (with different indexes) are generated. [String, Required]

*wsiz*e — window size. The window is a region of the alignment used to build the profile. It is picked up from the center of the alignment and occupies the specified length. The edges of the alignment beyond the window are not taken into account. The recommended length is a bit less than the alignment length, but not more than 50 bp. [Number, Optional, Default: 40]

*clen*gth — length of a random synthetic sequence used to calibrate the profile. [Number, Optional, Default: 1000000]

rseed — random seed used to calibrate the profile, e.g. to generate the random synthetic sequence. Use the same value to get the same calibration results twice on the same data. By default, new random seed is generated each time a calibration occurs. [Number, Optional, Default: 0]

walg — specifies to use the *Algorithm 2* weight algorithm. In most cases it is not required, but in some cases it can increase the recognition quality. [Boolean, Optional, Default: false]

Example:

```
ugene sitecon-build --in=COI.aln --out=result.sitecon
```

Searching for TFBS with SITECON**Task Name:** sitecon-search

Searches for transcription factor binding sites (TFBS) with SITECON and saves the regions found as annotations.

Parameters:

in — semicolon-separated list of input sequence files to search TFBS in. [String, Required]

inmodel — input SITECON profile(s). If several profiles have been supplied, searches with all profiles one by one and outputs merged set of annotations for each input sequence. [String, Required]

out — output Genbank file. [String, Required]

annotation-name — name of the annotated regions. [String, Optional, Default: "misc_feature"]

min-score — recognition quality threshold. The value must be between 60 and 100. Choosing too low threshold will lead to recognition of too many TFBS recognised with too low trustworthiness. Choosing too high threshold may result in no TFBS recognised. [Number, Optional, Default: 85]

min-err1 — setting for filtering results, minimal value of Error type I. [Number, Optional, Default: 0]

max-err2 — setting for filtering results, maximum value of Error type II. [Number, Optional, Default: 0.001]

strand — strands to search in. [Number, Optional, Default: 0]

The following values are available:

- 0 (both strands)
- 1 (direct strand)
- -1 (complement strand)

Example:

```
ugene sitecon-search --in=input.fa --inmodel=profile.sitecon --out=res.gb
```

Fetching Sequence from Remote Database

Task Name: fetch-sequence

Fetches a sequence from a remote database. The supported databases are accessed via alias.

Database	Alias
NCBI Genbank (DNA)	genbank
NCBI Genbank (protein)	genbank-protein
Protein Data Bank	pdb
SwissProt	swissprot
Uniprot	uniprot

Parameters:

db — database alias to read from. [String, Required]

id — semicolon-separated list of resource IDs in the database. [String, Required]

save-dir — directory to store sequence files loaded from the database. [String, Optional]

Example:

```
ugene fetch-sequence --db=PDB --id=3INS;1CRN
```

Gene-by-Gene Report

Task Name: gene-by-gene

Suppose you have genomes and you want to characterize them. One of the ways to do that is to build a table of what genes are in each genome and what are not there.

1. Create a local BLAST db of your genome sequence/contigs. One db per one genome.
2. Create a file with sequences of genes you want to explore. This file will be the input file for the scheme
3. Setup location and name of BLAST db you created for the first genome.
4. Setup output files: report location and output file with annotated (with BLAST) sequence. You might want to delete the "Write Sequence" element if you do not need output sequences.
5. Run the scheme
- 5*. Run the scheme on the same input and output files changing BLAST db for each genome that you have.

As the result you will get the report file. With "Yes" and "No" field. "Yes" answer means that the gene is in the genome. "No" answer MIGHT mean that there is no gene in the genome. It is a good idea to analyze al

l the "No" sequences using annotated files. Just open a file and find a sequence with a name of a gene that has "No" result.

Parameters:

in - Input sequence file [Url datasets]

final-name - Annotation name used to compare genes and reference genomes (using 'blast_result' by default) [String]

exist-file - If a target report already exists you should specify how to handle that. Merge two table in one. Overwrite or Rename existing file (using 'Merge' by default) [String]

ident - Identity between gene sequence length and annotation length in per cent. BLAST identity (if specified) is checked after (using '90.0' percents by default) [Number]

out - Output report file [String]

blast-out - Location of BLAST output file [String]

search-type - Type of BLAST searches (using 'blastn' by default) [String]

db-name - Name of BLAST DB [String]

blast-path - Path to BLAST DB [String]

expected-value - This setting specifies the statistical significance threshold for reporting matches against database sequences (using '10.0' by default) [Number]

gapped-aln - Perform gapped alignment (using 'use' by default) [Boolean]

blast-name - Name for annotations (using 'blast_result' by default) [String]

tmpdir - Directory for temporary files (using UGENE temporary directory by default) [String]

toolpath - External tool path (using the path specified in UGENE by default) [String]

out-type - Type of BLAST output file (using 'XML (-m 7)' by default) [String]

Example:

```
ugene gene-by-gene --in=human_T1.fa --out=human_T1_report
```

Reverse-Complement Converting Sequences

Task Name: revcompl

Convert input sequence into its reverse, complement or reverse-complement counterpart and write result sequence to file

Parameters:

type - Type of operation. Available are 'Reverse Complement', 'Complement' and 'Reverse' (using 'Reverse Complement' by default) [String]

in - Input file [Url datasets]

accumulate - Accumulate all incoming data in one file or create separate files for each input. In the latter case, an incremental numerical suffix is added to the file name (using 'True' by default) [Boolean]

format - Output file format (using 'fasta' by default) [String]

split - Split each incoming sequence on several parts (using '1' by default) [Number]

out - Output file [String]

Example:

```
ugene revcompl --in=human_T1.fa --out=human_T1_result.fa --format=fasta
--type=reverse
```

Variants Calling

Task Name: snp

Call variants for an input assembly and a reference sequence using SAMtools mpileup and bcftool

Parameters:

bam - Input sorted BAM file(s) [Url datasets]

ref - Input reference sequence [Url datasets]

wout - Out file with variations [String]

bN - A/C/G/T only [Boolean]

bl - List of sites [String]
ml - BED or position list file [String]
bg - Per-sample genotypes [Boolean]
mC - Mapping quality downgrading coefficient [Number]
bT - Pair/trio calling [String]
mB - Disable BAQ computation [Boolean]
me - Gap extension error [Number]
mE - Extended BAQ computation [Boolean]
bF - Indicate PL [Boolean]
vw - Gap size [Number]
m6 - Illumina-1.3+ encoding [Boolean]
bi - INDEL-to-SNP Ratio [Number]
bA - Retain all possible alternate [Boolean]
vD - Max number of reads per input BAM [Number]
md - Max number of reads per input BAM [Number]
mL - Max INDEL depth [Number]
va - Alternate bases [Number]
v2 - BaseQ bias [String]
vd - Minimum read depth [Number]
v4 - End distance bias [Number]
v3 - MapQ bias [Number]
Q - Minimum RMS quality [Number]
v1 - Strand bias [Number]
mQ - Minimum base quality [Number]
mq - Minimum mapping quality [Number]
bd - Min samples fraction [Number]
b1 - N group-1 samples [Number]
bU - N permutations [Number]
bG - No genotype information [Boolean]
ml - No INDELS [Boolean]
mo - Gap open error [Number]
mP - List of platforms for indels [String]
vp - Log filtered [Boolean]
bP - Prior allele frequency spectrum. [String]
bQ - QCALL likelihood [Boolean]
mr - Pileup region [String]
bs - List of samples [String]
mh - Homopolymer errors coefficient [Number]
bt - Mutation rate [Number]

mA - Count anomalous read pairs [Boolean]

vW - A/C/G/T only [Number]

Example:

```
ugene snp --bam=test.bam --ref=test_ref.fa --wout=test_out.vcf
```

Generating DNA Sequence

Task Name: generate-dna

Generates a random DNA sequence with specified nucleotide content

Parameters:

algo - Algorithm for generating (using 'GC Content' by default) [String]

content - Specifies if the nucleotide content of generated sequence(s) will be taken from reference or specified manually (A, G, C, T parameters) (using 'manual' by default) [String]

count - Number of sequences to generate (using '1' by default) [Number]

length - Length of the resulted sequence(s) (using '1000' bp by default) [Number]

a - Adenine content (using '25' percents by default) [Number]

c - Cytosine content (using '25' percents by default) [Number]

g - Guanine content (using '25' percents by default) [Number]

t - Thymine content (using '25' percents by default) [Number]

ref - Path to the reference file (could be a sequence or an alignment) [String]

seed - Value to initialize the random generator. By default (seed = -1) the generator is initialized with the system time (using '-1' by default) [Number]

wnd-size - Size of window where set content (using '1000' by default) [Number]

accumulate - Accumulate all incoming data in one file or create separate files for each input. In the latter case, an incremental numerical suffix is added to the file name (using 'True' by default) [Boolean]

format - Output file format (using 'fasta' by default) [String]

split - Split each incoming sequence on several parts (using '1' by default) [Number]

out - Output file [String]

Example:

```
ugene generate-dna --length=2000 --a=45 --out=test.fa
```

Creating Custom CLI Tasks

The *predefined tasks* are actually the *Workflow Designer* schemas stored in the \$UGENE/data/cmdline directory.

Follow the instructions in the *Workflow Designer Manual* on how to create a schema and to run it from the command line.

You may also find useful the following video tutorial devoted to the creating of a custom console command:

- [Creating custom console command \(MUSCLE alignment with various output format\)](#)

APPENDIXES

- Appendix A. Supported File Formats
 - Specific File Formats
 - UGENE Native File Formats
 - Other File Formats

Appendix A. Supported File Formats



UGENE is able to read and write files compressed with Unix/Linux *gzip* utility. You don't have to unpack the files.

- Specific File Formats
- UGENE Native File Formats
- Other File Formats

Specific File Formats

File format	File extension	Read	Write	Comment
ABIF	*.ab1, *.abi, *.abif	+	-	A chromatogram file format. See also: <i>Chromatogram Viewer</i>
ACE	*.ace	+	-	A file format for storing data about genomic contigs. See also: <i>Alignment Editor</i>
APR	*.apr	+	-	Vector NTI / AlignX Alignment format. See also: <i>Alignment Editor</i>
Bairoch	*.bairoch	+	+	A file format to store enzymes. See also: <i>Restriction Analysis</i>
BAM	*.bam	+	-	Binary compressed SAM format. See also: <i>Assembly Browser</i>
ClustalW	*.aln	+	+	A multiple sequence alignments (MSA) file format. See also: <i>Alignment Editor</i>
EBWT	*.ebwt	+	+	A Bowtie prebuilt index file. See also: <i>Bowtie</i>
EMBL	*.em, *.emb, *.embl	+	-	A rich format for storing sequences and their annotations. See also: <i>Sequence View</i>

FASTA	*.fa, *.mpfa, *.fna, *.fsa, *.fas, *.fasta, *.sef *.seqs	+	+	One of the oldest and simplest sequence file format. See also: Sequence View
FASTQ	*.fastq	+	+	A file format used to store a sequence and its corresponding quality scores. It was originally developed at the "Wellcome Trust Sanger Institute". See also: Sequence View
Genbank	*.gb, *.gbk, *.gen, *.genbank	+	+	A rich format for storing sequences and associated annotations. See also: Sequence View
GFF	*.gff	+	+	The Gene Finding Format (GFF) format is used to store features and annotations. See also: Sequence View
HMM	*.hmm	+	+	A file format to store HMM profiles. See also: HMM2 , HMM 3
MMDB	*.prt	+	-	ASN.1 format used by the Molecular Modeling Database (MMDB). See also: 3D Structure Viewer
MSF	*.msf	+	+	A multiple sequence alignments file format. See also: Alignment Editor
Mega	*.meg, *.meg.gz	+		A multiple sequence alignments file format. See also: Alignment Editor
Newick	*.nwk, *.newick	+	+	A tree file format. See also: Building Phylogenetic Tree , Phylogenetic Tree Viewer

Nexus	*.nex *.nxs	+	+	A multiple alignment and phylogenetic trees file format. See also: Alignment Editor , Building Phylogenetic Tree , Phylogenetic Tree Viewer
PDB	*.pdb	+	-	The Protein Data Bank (PDB) format allows to view the 3D structure of the sequence. See also: 3D Structure Viewer
pDRAW32	*.pdw	+	-	A sequence file format used by pDRAW32 software. See also: Sequence View
PFM	*.pfm	+	+	A file format for a position frequency matrix. See also: Weight Matrix
Phylip	*.phy	+	+	A multiple alignment file format. See also: Alignment Editor
PWM	*.pwm	+	+	A file format for a position weight matrix. See also: Weight Matrix
Raw	*.seq	+	+	A raw sequence format. See also: Sequence View
SAM	*.sam	+	+	The Sequence Alignment/Map (SAM) format is a generic alignment format for storing read alignments against reference sequences. See also: Assembly Browser , Bowtie , UGEN E Genome Aligner
SCF	*.scf	+	-	It is a Standard Chromatogram Format. See also: Chromatogram Viewer
SITECON	*.sitecon	+	-	A file format to store TFBS profile. See also: SITECON

Stockholm	*.sto	+	+	A multiple sequence alignments file format. See also: Alignment Editor
Swiss-Prot	*.txt *.sw	+	-	An annotated protein sequence in format of the UniProtKB/Swiss-Prot database. See also: Sequence View
Vector NTI Sequence	*.gb *.gp	+	+	A rich format for storing sequences and associated annotations, produced by Vector NTI software. See also: Sequence View
VCF	*.vcf	+	+	The VCF specifies the format of a text file used for storing gene sequence variations. See also: Assembly Browser

UGENE Native File Formats

File format	File extension	Read	Write	Comment
Dotplot	*.dpt	+	+	Stores a dotplot of a sequence. See also: Dotplot
UGENE database file	*.ugenedb	+	+	UGENE database files stores information for imported BAM or SAM files and can be used for converting this information into a SAM file. See also: Import BAM/SAM File
Short Reads FASTA	*.srfa, *.srfasta	+	+	A multiple sequence alignments file format. See also: Alignment Editor
UGENE Workflow Language	*.uwl	+	+	Human-readable format to store workflows, created in UGENE Workflow Designer . See also: Workflow Designer

UGENE Query Language	*.uql	+	+	Human-readable format to store schemas, created in UGENE <i>Query Designer</i> . See also: Query Designer
Workflow element for command line tool	*.etc	+	+	Format for storing workflow elements that can launch an external command line tool. See also: Workflow Designer

Other File Formats

File format / extension	Comment
*.csv	Example of usage: annotations can be exported to this format; the <i>Weight Matrix</i> matrices list can also be saved to this format.
*.html	For example it is used to store reports.
image formats: *.bmp, *.jpg, *.png, *.tiff, *.svg, etc.; *.pdf	These formats are used throughout the program to save screenshots, etc.
*.txt	It is possible to view and modify plain text files in UGENE.

Tutorials

- Using BioMart with UGENE
 - Environment requirements
 - Installing UGENE extension on Mozilla Firefox
 - Opening data found using BioMart in UGENE
 - Opening BioMart data in UGENE by ID
 - Opening selected data in UGENE

Using BioMart with UGENE

The [BioMart](#) system enables scientists to perform advanced querying of a wide range of biological data sources through a single web interface, regardless of the data sources geographical locations.

This tutorial describes how data found through the *BioMart* web interface can be easily opened for further analysis in UGENE by a couple of mouse-clicks.

- Environment requirements
- Installing UGENE extension on Mozilla Firefox
- Opening data found using BioMart in UGENE
- Opening BioMart data in UGENE by ID
- Opening selected data in UGENE

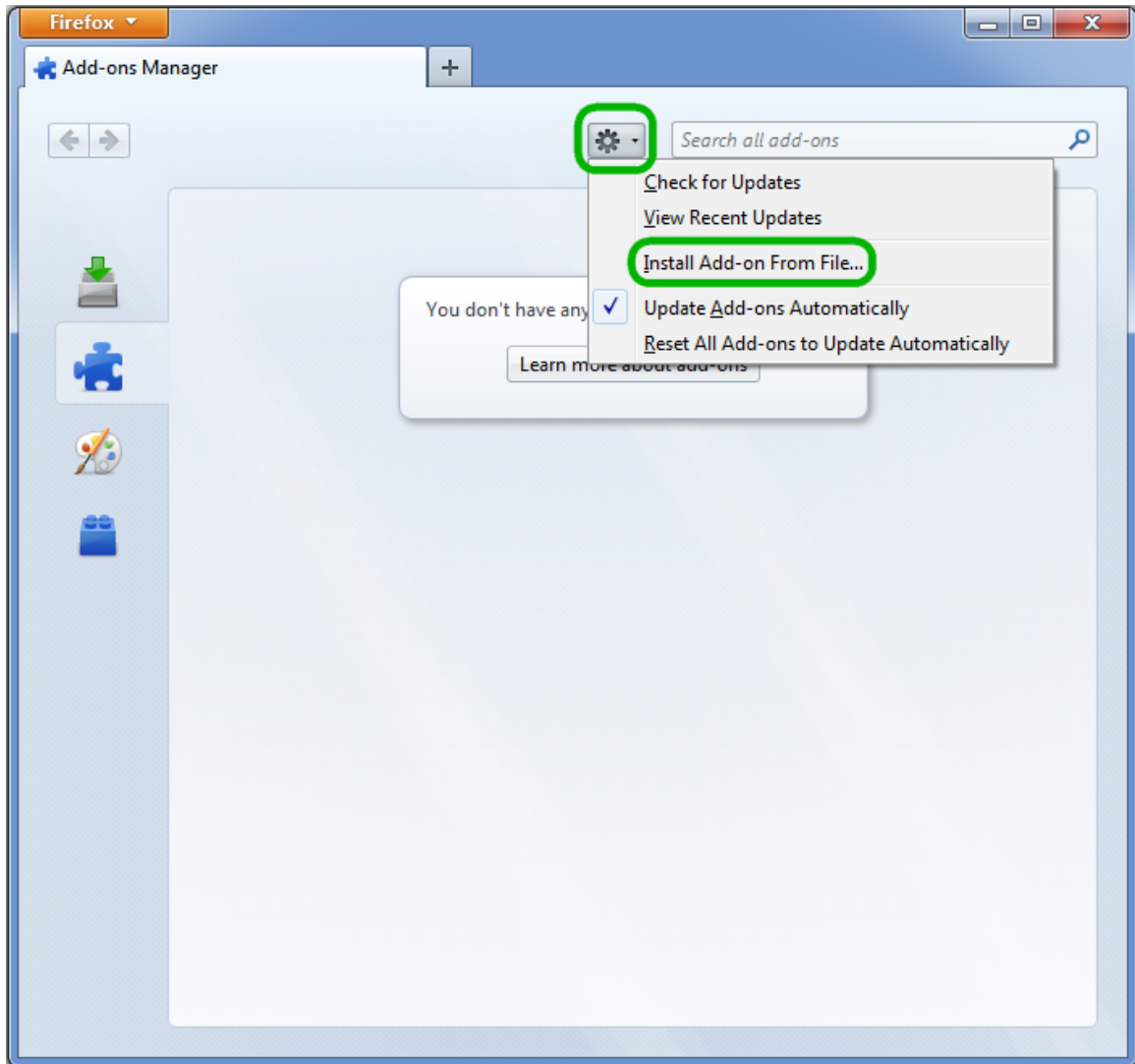
Environment requirements

Currently UGENE extension is available for Mozilla Firefox web browser only. Please make sure to launch UGENE before using the extension!

Follow the instructions [below](#) to install the extension.

Installing UGENE extension on Mozilla Firefox

To install UGENE extension on Mozilla Firefox open *Add-ons Manager* and select *Install Add-on From File* item in the settings menu:



In the browse dialog select *ugene.xpi* file that you can find in the *Firefox* directory of the UGENE Web Browsers Extensions Package that there is on the [Download page](#).

Opening data found using BioMart in UGENE

For now there are two options to open data found using BioMart in UGENE:

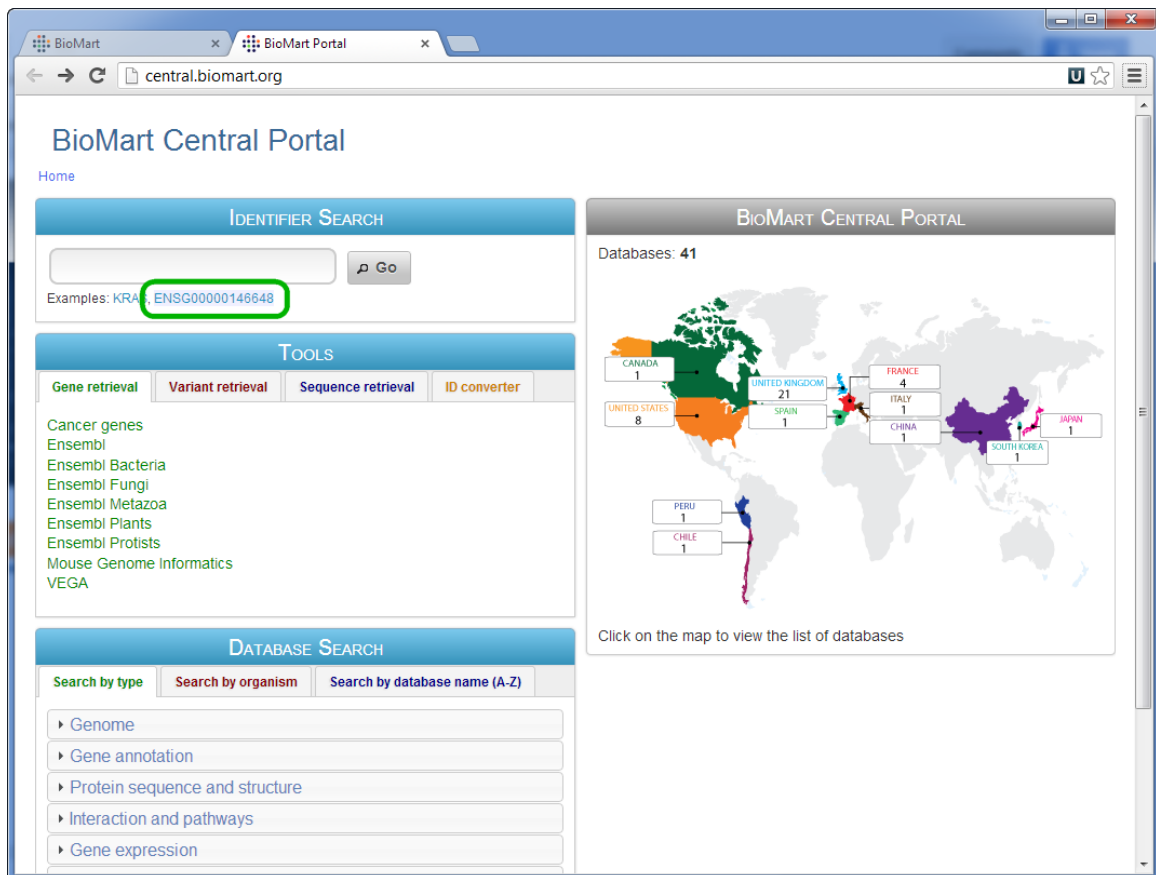
- Open data by ID, for example, by an Ensembl ID.
- Open selected data.

Opening BioMart data in UGENE by ID

Let's open [web site](#):



Click, for example, on the *Proceed to Bio Portal* link. The following page will appear:

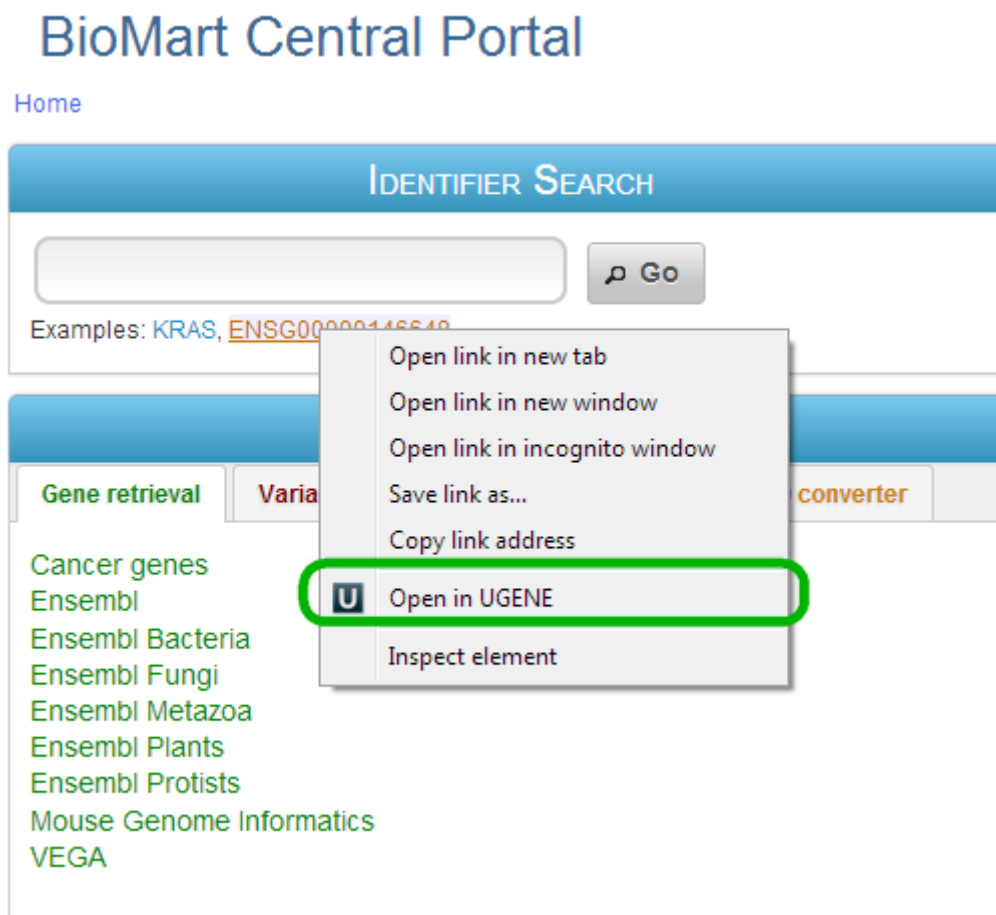


Notice that an example Ensembl ID below the search bar is highlighted (it has a light blue background).

Current version of the UGENE extension allows detecting the following types of identification numbers:

1. Ensembl Gene ID
2. Ensembl Protein ID
3. PDB ID

Right-click on the ID and select *Open in UGENE* item in the context menu:



The sequence with the selected ID will be opened in UGENE.

Opening selected data in UGENE

Imagine that you have browsed for required data (e.g. a sequence with annotations) and opened, for example, an html view for the data in a web browser. Now you would like to open the data in UGENE to analyze them in more detail. Or, alternatively, maybe you would like to analyze a certain sequence part.

In this case you select the required data in the web browser window. the *Open selected in UGENE* item should now appear in the context menu:

The screenshot shows the Ensembl genome browser interface. The browser address bar displays the URL: `www.ensembl.org/Homo_sapiens/Export/Output/Gene?db=core;flank3_display=0;flank5_disp`. The page title is "Export Gene Data". The left sidebar shows a navigation menu with categories like "Gene-based displays", "Comparative Genomics", "Genetic Variation", "External data", and "ID History". The main content area displays a list of genomic features with their corresponding DNA sequences. A context menu is open over a selected line of DNA sequence, with the option "Open selection in UGENE" highlighted in green. The context menu also includes options like "Copy", "Print...", "Search Google for...", and "Inspect element".

Gene-based displays

- Gene summary
- Splice variants (1)
- Supporting evidence
- Sequence
- External references
- Regulation
- Comparative Genomics
 - Genomic alignments
 - Gene tree (image)
 - Gene tree (text)
 - Gene tree (alignment)
 - Gene gain/loss tree
 - Orthologues
 - Paralogues
 - Protein families
- Phenotype
- Genetic Variation
 - Variation table
 - Variation image
 - Structural variation
- External data
 - Personal annotation
- ID History
 - Gene history

Configure this page

Add your data

Export data

Bookmark this page

Share this page

Export Gene Data

```
>ENSG00000232606:ENST00000413525 cdna:KNOWN_lincRNA
AGCTTCACATGTGAGATAAATGCACTCAAAGATTCTCACAAAGTAGCTCTTTGGAGCTTC
AGATGTGAAATGGATCATTCTCTCAATCTGTAATAGACCCCTTCTGTGAAGCTCTTCAATCA
AACCAGAGAATTCAAGAGTTTCCAACACCTAAGAGTGGTATTTGGCAAATGGTGGGCCAA
AGGAATAAAGAAGGCATGCAAACTCTTGACAGAAGACATTGAGAAATGATTTGATATC
AGATACAAGGAGAAAATATGCCAGTAAGAAAATGCATTTTTCAAGATTAATTCGGCATT
TGTTACTTAATAGCATTGTGCATATTCCAATTTTTTCATATGTAGTAAATTCATTTCAAAT
CA
>ENSG00000232606:ENST00000413525 cds:KNOWN_lincRNA
>ENSG00000232606:ENST00000413525 ENSE00001765550 exon:KNOWN_lincRNA
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AGATGTGAAATGGATCATTCTCTCAATCTGTAATAGACCCCTTCTGTGAAGCTCTTCAATCA
AACCAGAGAATTCAAG
>ENSG00000232606:ENST00000413525 ENSE00001709530 exon:KNOWN_lincRNA
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TGCAAACTCTTGACAGAAGACATTGAGAAATGATTTGATATCAGATACAAGGAGAAAA
TATGCCAGTAAGAAAATGCATTTTTCAAGATTAATTCGGCATTGTACTTAATAGCAT
TTGCATATTCCAATTTTTTCATATGTAGTAAATTCATTTCAAATCA
>ENSG00000232606:ENST00000413525 ENSE00001709530 exon:KNOWN_lincRNA
GTGAAATTGAGGGG
AGGAACCTGAGGTA
AAGGCTGTCAATCA
AGACRAGCTGTAA
TGACAACCCTTAG
CTCTCTATTGAATT
GAAAAACATTAGT
AAGAATTGATATATTTTATAAAGTAATGATCCTCATAGTTACATCTTATTTAGGGATTAT
GTTTGTAGATCATGTAATGAGTTTTAATAAGTTTGTTCAGTAAGCAGTAAGACAGAG
TTACCTCTGGTAAGGAAAAATAAAGAAGATCTATTCTGAAATAGTTTTTGTGTCACAT
TTTTAATTGATTTGGTGCCACATTTCCAGTTGGACATTCTAGGTTAATTTTTTTTATT
ATACTTGAAGTTTTAGGGTACACGTGCACAACGTGCAGGTACATATGTATACATGTGCC
ATGTTGGTGTGCTGCACCCATTAACTCGTCATTTAACATTAGGTATATCTCTCTAGTTTA
ATTTTTAAGAACTCTAGAATTTTCAATGGGCTATTTAGTTTGGAGAAGCAGTGAATCCT
GACCCBACCAGTATCBBBCTGTCTTTBGBATTTATGCTCBBATBGGATATGT
```

Copy Ctrl+C

Print...

Search Google for '>ENSG00000232606:ENST00000413525...'

U Open selection in UGENE

Inspect element

The selected data will be opened in UGENE.