CLC **Sequence** Viewer

User manual

Manual for

CLC Sequence Viewer 6.7

Windows, Mac OS X and Linux

August 8, 2012

This software is for research purposes only.

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Part I

Introduction

Chapter 1

Introduction to *CLC Sequence Viewer*

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Welcome to *CLC Sequence Viewer* — a software package supporting your daily bioinformatics work.

We strongly encourage you to read this user manual in order to get the best possible basis for working with the software package.

This software is for research purposes only.

1.1 Contact information

The *CLC Sequence Viewer* is developed by:

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If you have questions or comments regarding the program, you are welcome to contact our support function:

E-mail: support@clcbio.com

1.2 Download and installation

The *CLC Sequence Viewer* is developed for Windows, Mac OS X and Linux. The software for either platform can be downloaded from http://www.clcbio.com/download.

1.2.1 Program download

The program is available for download on http://www.clcbio.com/download.

Before you download the program you are asked to fill in the **Download** dialog.

In the dialog you must choose:

- Which operating system you use
- Whether you would like to receive information about future releases

Depending on your operating system and your Internet browser, you are taken through some download options.

When the download of the installer (an application which facilitates the installation of the program) is complete, follow the platform specific instructions below to complete the installation procedure.

1.2.2 Installation on Microsoft Windows

Starting the installation process is done in one of the following ways:

If you have downloaded an installer:
Locate the downloaded installer and double-click the icon.

The default location for downloaded files is your desktop.

If you are installing from a CD:
Insert the CD into your CD-ROM drive.

Choose the "Install *CLC Sequence Viewer*" from the menu displayed.

Installing the program is done in the following steps:

- On the welcome screen, click **Next**.
- Read and accept the License agreement and click **Next**.
- Choose where you would like to install the application and click **Next**.
- Choose a name for the Start Menu folder used to launch *CLC Sequence Viewer* and click **Next**.
- Choose if *CLC Sequence Viewer* should be used to open CLC files and click **Next**.
- Choose where you would like to create shortcuts for launching *CLC Sequence Viewer* and click **Next**.
- Choose if you would like to associate .clc files to *CLC Sequence Viewer*. If you check this option, double-clicking a file with a "clc" extension will open the *CLC Sequence Viewer*.
- Wait for the installation process to complete, choose whether you would like to launch *CLC Sequence Viewer* right away, and click **Finish**.

When the installation is complete the program can be launched from the Start Menu or from one of the shortcuts you chose to create.

1.2.3 Installation on Mac OS X

Starting the installation process is done in one of the following ways:

If you have downloaded an installer:
Locate the downloaded installer and double-click the icon.
The default location for downloaded files is your desktop.

If you are installing from a CD:
Insert the CD into your CD-ROM drive and open it by double-clicking on the CD icon on your desktop.

Launch the installer by double-clicking on the "*CLC Sequence Viewer*" icon.

Installing the program is done in the following steps:

- On the welcome screen, click **Next**.
- Read and accept the License agreement and click **Next**.
- Choose where you would like to install the application and click **Next**.

- Choose if *CLC Sequence Viewer* should be used to open CLC files and click **Next**.
- Choose whether you would like to create desktop icon for launching *CLC Sequence Viewer* and click **Next**.
- Choose if you would like to associate .clc files to *CLC Sequence Viewer*. If you check this option, double-clicking a file with a "clc" extension will open the *CLC Sequence Viewer*.
- Wait for the installation process to complete, choose whether you would like to launch *CLC Sequence Viewer* right away, and click **Finish**.

When the installation is complete the program can be launched from your Applications folder, or from the desktop shortcut you chose to create. If you like, you can drag the application icon to the dock for easy access.

1.2.4 Installation on Linux with an installer

Navigate to the directory containing the installer and execute it. This can be done by running a command similar to:

```
# sh CLCSequenceViewer_6_JRE.sh
```

If you are installing from a CD the installers are located in the "linux" directory.

Installing the program is done in the following steps:

- On the welcome screen, click **Next**.
- Read and accept the License agreement and click **Next**.
- Choose where you would like to install the application and click **Next**.
  For a system-wide installation you can choose for example /opt or /usr/local. If you do not have root privileges you can choose to install in your home directory.
- Choose where you would like to create symbolic links to the program
  DO NOT create symbolic links in the same location as the application.
  Symbolic links should be installed in a location which is included in your environment PATH. For a system-wide installation you can choose for example /usr/local/bin. If you do not have root privileges you can create a 'bin' directory in your home directory and install symbolic links there. You can also choose not to create symbolic links.
- Wait for the installation process to complete and click **Finish**.

If you choose to create symbolic links in a location which is included in your PATH, the program can be executed by running the command:

```
# clcseqview6
```

Otherwise you start the application by navigating to the location where you choose to install it and running the command:

```
# ./clcseqview6
```

1.2.5 Installation on Linux with an RPM-package

Navigate to the directory containing the rpm-package and install it using the rpm-tool by running a command similar to:

```
# rpm -ivh CLCSequenceViewer_6_JRE.rpm
```

If you are installing from a CD the rpm-packages are located in the "RPMS" directory.
Installation of RPM-packages usually requires root-privileges.

When the installation process is finished the program can be executed by running the command:

```
# clcseqview6
```

1.3 System requirements

The system requirements of *CLC Sequence Viewer* are these:

- Windows XP, Windows Vista, or Windows 7, Windows Server 2003 or Windows Server 2008
- Mac OS X 10.6 or later. Intel CPU required. However, Mac OS X 10.5.8 is supported on 64-bit Intel systems.
- Linux: RedHat 5 or later. SuSE 10 or later.
- 32 or 64 bit
- 256 MB RAM required
- 512 MB RAM recommended
- 1024 x 768 display recommended

1.4 About CLC Workbenches

In November 2005 CLC bio released two Workbenches: *CLC Free Workbench* and *CLC Protein Workbench*. *CLC Protein Workbench* is developed from the free version, giving it the well-tested user friendliness and look & feel. However, the *CLC Protein Workbench* includes a range of more advanced analyses.

In March 2006, *CLC DNA Workbench* (formerly *CLC Gene Workbench*) and *CLC Main Workbench* were added to the product portfolio of CLC bio. Like *CLC Protein Workbench*, *CLC DNA Workbench* builds on *CLC Free Workbench*. It shares some of the advanced product features of *CLC Protein Workbench*, and it has additional advanced features. *CLC Main Workbench* holds all basic and advanced features of the *CLC Workbenches*.

In June 2007, *CLC RNA Workbench* was released as a sister product of *CLC Protein Workbench* and *CLC DNA Workbench*. *CLC Main Workbench* now also includes all the features of *CLC RNA Workbench*.

In March 2008, the *CLC Free Workbench* changed name to *CLC Sequence Viewer*.

In June 2008, the first version of the *CLC Genomics Workbench* was released due to an extraordinary demand for software capable of handling sequencing data from the new high-throughput sequencing systems like 454, Illumina Genome Analyzer and SOLiD.

For an overview of which features all the applications include, see http://www.clcbio.com/features.

In December 2006, CLC bio released a **Software Developer Kit** which makes it possible for anybody with a knowledge of programming in Java to develop plug-ins. The plug-ins are fully integrated with the CLC Workbenches and the Viewer and provide an easy way to customize and extend their functionalities.

In April 2012, *CLC Protein Workbench*, *CLC DNA Workbench*and *CLC RNA Workbench* were discontinued, and all customers with an valid license were offered to upgrade to *CLC Main Workbench*.

All our software will be improved continuously. If you are interested in receiving news about updates, you should register your e-mail and contact data on http://www.clcbio.com, if you haven't already registered when you downloaded the program.

1.4.1 New program feature request

The CLC team is continuously improving the *CLC Sequence Viewer* with our users' interests in mind. Therefore, we welcome all requests and feedback from users, and hope suggest new features or more general improvements to the program on support@clcbio.com.

1.4.2 Report program errors

CLC bio is doing everything possible to eliminate program errors. Nevertheless, some errors might have escaped our attention. If you discover an error in the program, you can use the **Report a Program Error** function in the **Help** menu of the program to report it. In the **Report a Program Error** dialog you are asked to write your e-mail address (optional). This is because we would like to be able to contact you for further information about the error or for helping you with the problem.

Note! No personal information is sent via the error report. Only the information which can be seen in the **Program Error Submission Dialog** is submitted.

You can also write an e-mail to support@clcbio.com. Remember to specify how the program error can be reproduced.

All errors will be treated seriously and with gratitude.

We appreciate your help.

Start in safe mode

If the program becomes unstable on start-up, you can start it in **Safe mode**. This is done by pressing and holding down the Shift button while the program starts.

When starting in safe mode, the user settings (e.g. the settings in the **Side Panel**) are deleted and cannot be restored. Your data stored in the **Navigation Area** is not deleted. When started in

safe mode, some of the functionalities are missing, and you will have to restart the *CLC Sequence Viewer* again (without pressing Shift).

1.4.3 CLC Sequence Viewer vs. Workbenches

The advanced analyses of the commercial workbenches, *CLC Genomics Workbench* and *CLC Main Workbench* are not present in *CLC Sequence Viewer*. Likewise, some advanced analyses are available in *CLC Genomics Workbench* but not in *CLC Main Workbench*. All types of basic and advanced analyses are available in *CLC Genomics Workbench*.

However, the output of the commercial workbenches can be viewed in all other workbenches. This allows you to share the result of your advanced analyses from e.g. *CLC Main Workbench*, with people working with e.g. *CLC Sequence Viewer*. They will be able to view the results of your analyses, but not redo the analyses.

The CLC Workbenches and the *CLC Sequence Viewer* are developed for Windows, Mac and Linux platforms. Data can be exported/imported between the different platforms in the same easy way as when exporting/importing between two computers with e.g. Windows.

1.5 When the program is installed: Getting started

CLC Sequence Viewer includes an extensive **Help** function, which can be found in the **Help** menu of the program's **Menu bar**. The **Help** can also be shown by pressing F1. The help topics are sorted in a table of contents and the topics can be searched.

We also recommend our **Online presentations** where a product specialist from CLC bio demonstrates our software. This is a very easy way to get started using the program. Read more about online presentations here: http://clcbio.com/presentation.

1.5.1 Quick start

When the program opens for the first time, the background of the workspace is visible. In the background are three quick start shortcuts, which will help you getting started. These can be seen in figure 1.1.

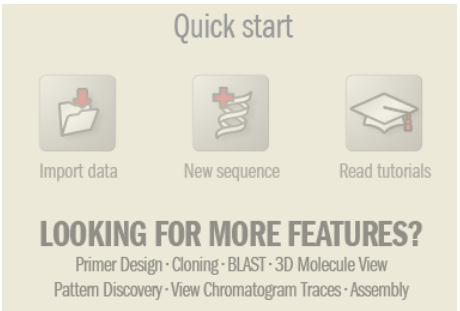

Figure 1.1: *Three available Quick start short cuts, available in the background of the workspace.*

The function of the three quick start shortcuts is explained here:

- **Import data.** Opens the **Import** dialog, which you let you browse for, and import data from your file system.

- **New sequence.** Opens a dialog which allows you to enter your own sequence.
- **Read tutorials.** Opens the tutorials menu with a number of tutorials. These are also available from the **Help** menu in the **Menu bar**.

Below these three quick start shortcuts, you will see a text: "Looking for more features?" Clicking this text will take you to a page on http://www.clcbio.com where you can read more about how to get more functionalities into *CLC Sequence Viewer*.

1.5.2 Import of example data

It might be easier to understand the logic of the program by trying to do simple operations on existing data. Therefore *CLC Sequence Viewer* includes an example data set.

When downloading *CLC Sequence Viewer* you are asked if you would like to import the example data set. If you accept, the data is downloaded automatically and saved in the program. If you didn't download the data, or for some other reason need to download the data again, you have two options:

You can click **Install Example Data** () in the **Help** menu of the program. This installs the data automatically. You can also go to http://www.clcbio.com/download and download the example data from there.

If you download the file from the website, you need to import it into the program. See chapter 6 for more about importing data.

1.6 Plug-ins

When you install *CLC Sequence Viewer*, it has a standard set of features. However, you can upgrade and customize the program using a variety of plug-ins.

As the range of plug-ins is continuously updated and expanded, they will not be listed here. Instead we refer to http://www.clcbio.com/plug-ins for a full list of plug-ins with descriptions of their functionalities.

1.6.1 Installing plug-ins

Plug-ins are installed using the plug-in manager[1]:

Help in the Menu Bar | **Plug-ins and Resources... (**  **)**

or **Plug-ins (**  **) in the Toolbar**

The plug-in manager has four tabs at the top:

- **Manage Plug-ins.** This is an overview of plug-ins that are installed.
- **Download Plug-ins.** This is an overview of available plug-ins on CLC bio's server.
- **Manage Resources.** This is an overview of resources that are installed.

[1] In order to install plug-ins on Windows Vista, the Workbench must be run in administrator mode: Right-click the program shortcut and choose "Run as Administrator". Then follow the procedure described below.

- **Download Resources.** This is an overview of available resources on CLC bio's server.

To install a plug-in, click the **Download Plug-ins** tab. This will display an overview of the plug-ins that are available for download and installation (see figure 1.2).

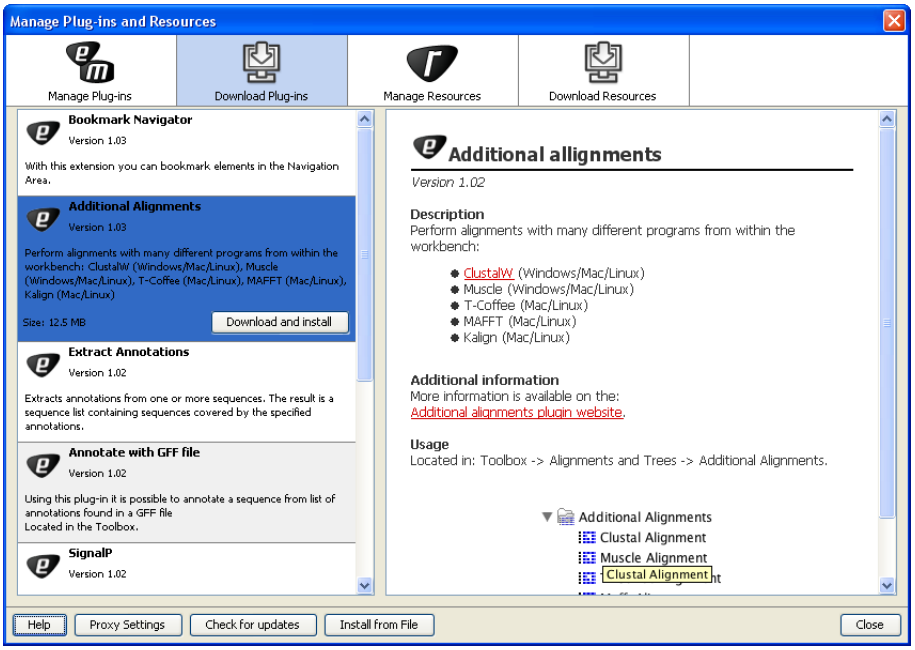

Figure 1.2: *The plug-ins that are available for download.*

Clicking a plug-in will display additional information at the right side of the dialog. This will also display a button: **Download and Install**.

Click the plug-in and press **Download and Install**. A dialog displaying progress is now shown, and the plug-in is downloaded and installed.

If the plug-in is not shown on the server, and you have it on your computer (e.g. if you have downloaded it from our web-site), you can install it by clicking the **Install from File** button at the bottom of the dialog. This will open a dialog where you can browse for the plug-in. The plug-in file should be a file of the type ".cpa".

When you close the dialog, you will be asked whether you wish to restart the *CLC Sequence Viewer*. The plug-in will not be ready for use before you have restarted.

1.6.2 Uninstalling plug-ins

Plug-ins are uninstalled using the plug-in manager:

Help in the Menu Bar | **Plug-ins and Resources... (**  **)**

or **Plug-ins (**  **) in the Toolbar**

This will open the dialog shown in figure 1.3.

The installed plug-ins are shown in this dialog. To uninstall:

Click the plug-in | **Uninstall**

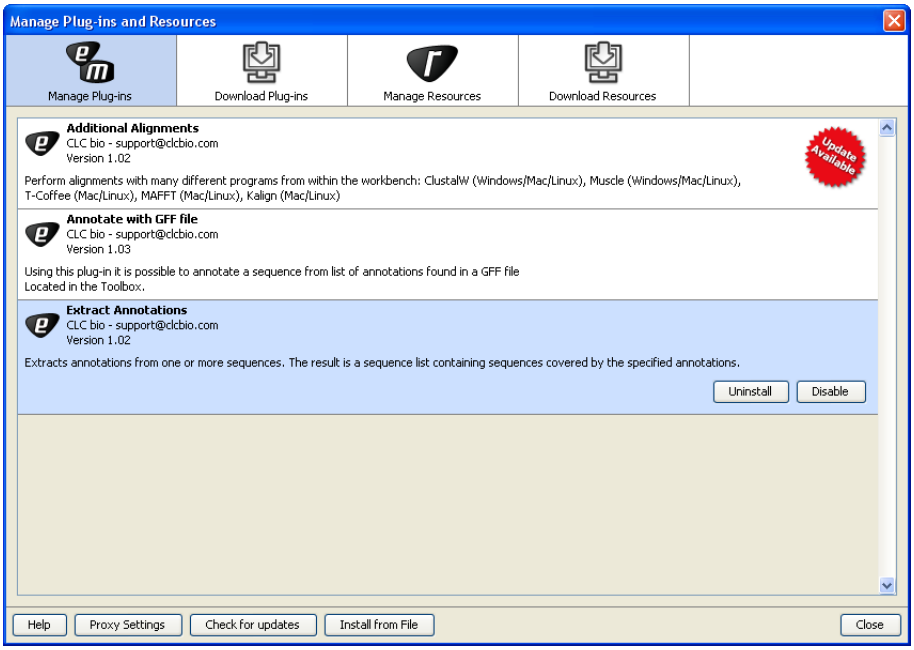

Figure 1.3: *The plug-in manager with plug-ins installed.*

If you do not wish to completely uninstall the plug-in but you don't want it to be used next time you start the Workbench, click the **Disable** button.

When you close the dialog, you will be asked whether you wish to restart the workbench. The plug-in will not be uninstalled before the workbench is restarted.

1.6.3 Updating plug-ins

If a new version of a plug-in is available, you will get a notification during start-up as shown in figure 1.4.

In this list, select which plug-ins you wish to update, and click **Install Updates**. If you press **Cancel** you will be able to install the plug-ins later by clicking **Check for Updates** in the Plug-in manager (see figure 1.3).

1.6.4 Resources

Resources are downloaded, installed, un-installed and updated the same way as plug-ins. Click the **Download Resources** tab at the top of the plug-in manager, and you will see a list of available resources (see figure 1.5).

Currently, the only resources available are PFAM databases (for use with *CLC Genomics Workbench* and *CLC Main Workbench*).

Because procedures for downloading, installation, uninstallation and updating are the same as for plug-ins see section 1.6.1 and section 1.6.2 for more information.

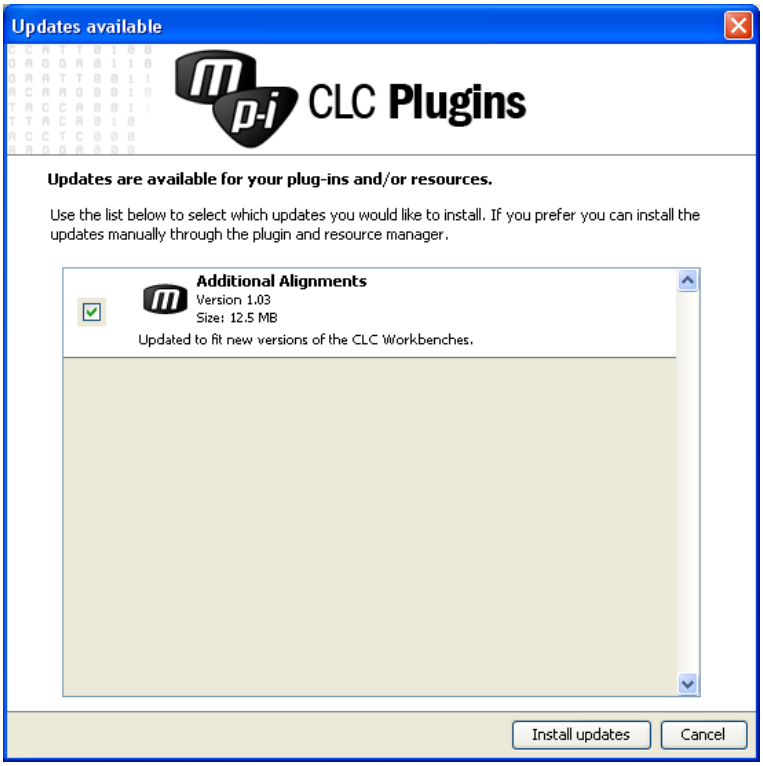

Figure 1.4: *Plug-in updates.*

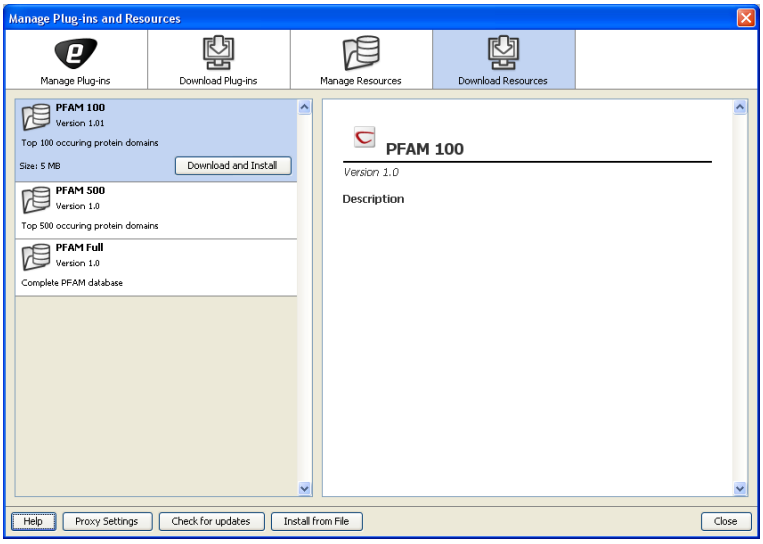

Figure 1.5: *Resources available for download.*

1.7 Network configuration

If you use a proxy server to access the Internet you must configure *CLC Sequence Viewer* to use this. Otherwise you will not be able to perform any online activities (e.g. searching GenBank). *CLC Sequence Viewer* supports the use of a HTTP-proxy and an anonymous SOCKS-proxy.

To configure your proxy settings, open *CLC Sequence Viewer*, and go to the **Advanced**-tab of the **Preferences** dialog (figure 1.6) and enter the appropriate information. The **Preferences** dialog is opened from the **Edit** menu.

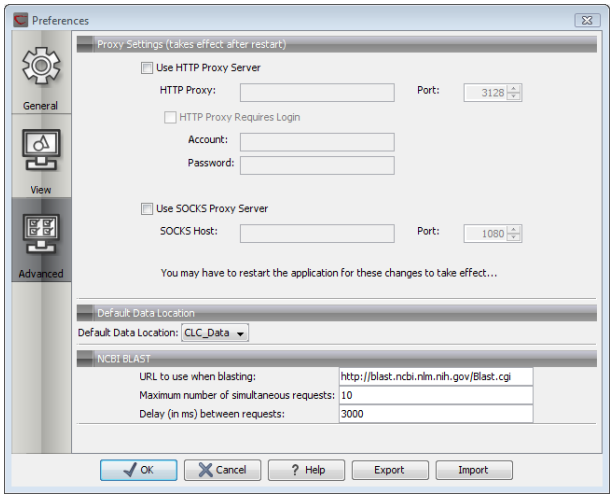

Figure 1.6: *Adjusting proxy preferences.*

You have the choice between a HTTP-proxy and a SOCKS-proxy. *CLC Sequence Viewer* only supports the use of a SOCKS-proxy that does not require authorization.

Exclude hosts can be used if there are some hosts that should be contacted directly and not through the proxy server. The value can be a list of hosts, each separated by a `|`, and in addition a wildcard character `*` can be used for matching. For example: `*.foo.com|localhost`.

If you have any problems with these settings you should contact your systems administrator.

1.8 The format of the user manual

This user manual offers support to Windows, Mac OS X and Linux users. The software is very similar on these operating systems. In areas where differences exist, these will be described separately. However, the term "right-click" is used throughout the manual, but some Mac users may have to use Ctrl+click in order to perform a "right-click" (if they have a single-button mouse).

The most recent version of the user manuals can be downloaded from `http://www.clcbio.com/usermanuals`.

The user manual consists of four parts.

- The **first part** includes the introduction and some tutorials showing how to apply the most significant functionalities of *CLC Sequence Viewer*.
- The **second part** describes in detail how to operate all the program's basic functionalities.
- The **third part** digs deeper into some of the bioinformatic features of the program. In this part, you will also find our "Bioinformatics explained" sections. These sections elaborate on the algorithms and analyses of *CLC Sequence Viewer* and provide more general knowledge of bioinformatic concepts.
- The **fourth part** is the Appendix and Index.

Each chapter includes a short table of contents.

1.8.1 Text formats

In order to produce a clearly laid-out content in this manual, different formats are applied:

- A feature in the program is in bold starting with capital letters. (Example: **Navigation Area**)
- An explanation of how a particular function is activated, is illustrated by "|" and bold. (E.g.: **select the element** | **Edit** | **Rename**)

Chapter 2

Tutorials

Contents

This chapter contains tutorials representing some of the features of *CLC Sequence Viewer*. The first tutorials are meant as a short introduction to operating the program. The last tutorials give examples of how to use some of the main features of *CLC Sequence Viewer*. **Watch video tutorials at `http://www.clcbio.com/tutorials`.**

2.1 Tutorial: Getting started

This brief tutorial will take you through the most basic steps of working with *CLC Sequence Viewer*. The tutorial introduces the user interface, shows how to create a folder, and demonstrates how to import your own existing data into the program.

When you open *CLC Sequence Viewer* for the first time, the user interface looks like figure 2.1.

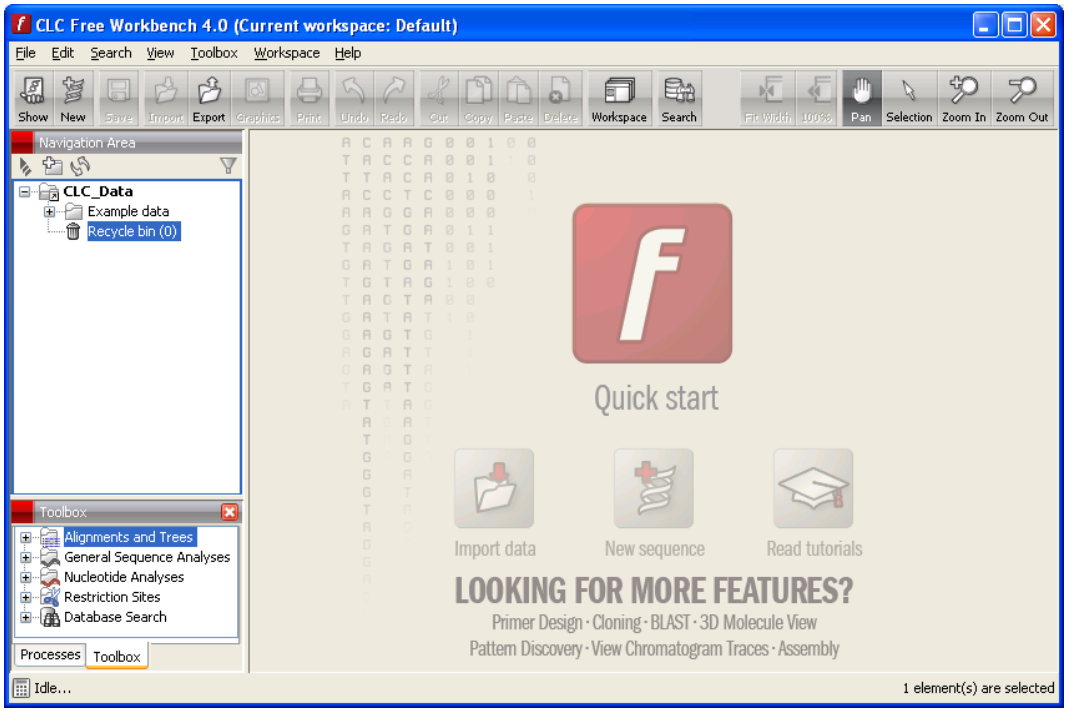

Figure 2.1: *The user interface as it looks when you start the program for the first time. (Windows version of* **CLC Sequence Viewer***. The interface is similar for Mac and Linux.)*

At this stage, the important issues are the **Navigation Area** and the **View Area**.

The **Navigation Area** to the left is where you keep all your data for use in the program. Most analyses of *CLC Sequence Viewer* require that the data is saved in the **Navigation Area**. There are several ways to get data into the **Navigation Area**, and this tutorial describes how to import existing data.

The **View Area** is the main area to the right. This is where the data can be 'viewed'. In general, a **View** is a display of a piece of data, and the **View Area** can include several **Views**. The **Views** are represented by tabs, and can be organized e.g. by using 'drag and drop'.

2.1.1 Creating a a folder

When *CLC Sequence Viewer* is started there is one element in the **Navigation Area** called **CLC_Data**[1]. This element is a **Location**. A location points to a folder on your computer where your data for use with *CLC Sequence Viewer* is stored.

The data in the location can be organized into folders. Create a folder:

File | **New** | **Folder (**  **)**
or **Ctrl + Shift + N (⌘ + Shift + N on Mac)**

Name the folder 'My folder' and press **Enter**.

[1] If you have downloaded the example data, this will be placed as a folder in *CLC_Data*

2.1.2 Import data

Next, we want to import a sequence called HUMDINUC.fsa (FASTA format) from our own Desktop into the new 'My folder'. (This file is chosen for demonstration purposes only - you may have another file on your desktop, which you can use to follow this tutorial. You can import all kinds of files.)

In order to import the HUMDINUC.fsa file:

> **Select 'My folder' | Import ( ) in the Toolbar | navigate to HUMDINUC.fsa on the desktop | Select**

The sequence is imported into the folder that was selected in the **Navigation Area**, before you clicked **Import**. Double-click the sequence in the **Navigation Area** to view it. The final result looks like figure 2.2.

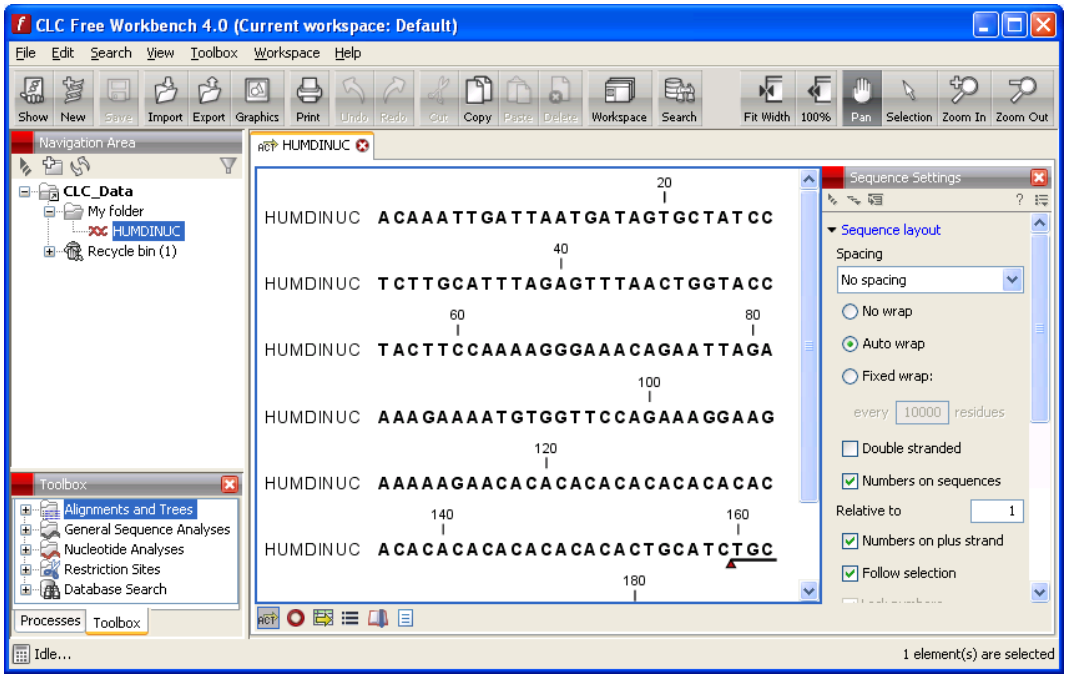

Figure 2.2: *The HUMDINUC file is imported and opened.*

2.2 Tutorial: View sequence

This brief tutorial will take you through some different ways to display a sequence in the program. The tutorial introduces zooming on a sequence, dragging tabs, and opening selection in new view.

We will be working with the sequence called *pcDNA3-atp8a1* located in the 'Cloning' folder in the Example data. Double-click the sequence in the **Navigation Area** to open it. The sequence is displayed with annotations above it. (See figure 2.3).

As default, *CLC Sequence Viewer* displays a sequence with annotations (colored arrows on the sequence like the green promoter region annotation in figure 2.3) and zoomed to see the residues.

In this tutorial we want to have an overview of the whole sequence. Hence;

Figure 2.3: *Sequence pcDNA3-atp8a1 opened in a view.*

click Zoom Out ( ) in the Toolbar | click the sequence until you can see the whole sequence

This sequence is circular, which is indicated by << and >> at the beginning and the end of the sequence.

In the following we will show how the same sequence can be displayed in two different views - one linear view and one circular view. First, zoom in to see the residues again by using the **Zoom In** ( ) or the **100%** ( ). Then we make a split view by:

press and hold the Ctrl-button on the keyboard (⌘ on Mac) | click Show as Circular ( ) at the bottom of the view

This opens an additional view of the vector with a circular display, as can be seen in figure 2.4.

Make a selection on the circular sequence (remember to switch to the **Selection** ( ) tool in the tool bar) and note that this selection is also reflected in the linear view above.

2.3 Tutorial: Side Panel Settings

This brief tutorial will show you how to use the **Side Panel** to change the way your sequences, alignments and other data are shown. You will also see how to save the changes that you made in the **Side Panel** .

Open the protein alignment located under *Protein orthologs* in the **Example data**. The initial view of the alignment has colored the residues according to the Rasmol color scheme, and the alignment is automatically wrapped to fit the width of the view (shown in figure 2.5).

Now, we are going to modify how this alignment is displayed. For this, we use the settings in the **Side Panel** to the right. All the settings are organized into groups, which can be expanded / collapsed by clicking the name of the group. The first group is **Sequence Layout** which is

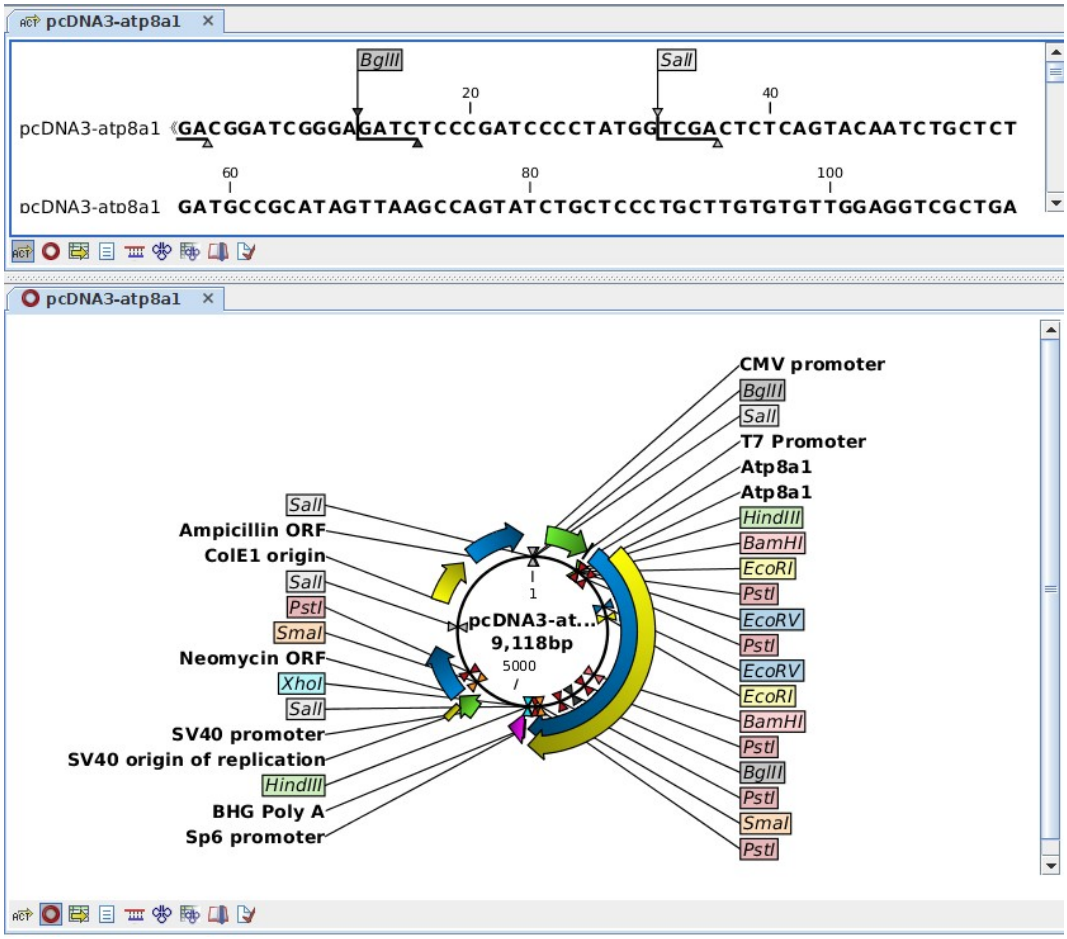

Figure 2.4: *The resulting two views which are split horizontally.*

expanded by default.

First, select **No wrap** in the **Sequence Layout**. This means that each sequence in the alignment is kept on the same line. To see more of the alignment, you now have to scroll horizontally.

Next, expand the **Annotation Layout** group and select **Show Annotations**. Set the **Offset** to "More offset" and set the **Label** to "Stacked".

Expand the **Annotation Types** group. Here you will see a list of the types annotation that are carried by the sequences in the alignment (see figure 2.6).

Check the "Region" annotation type, and you will see the regions as red annotations on the sequences.

Next, we will change the way the residues are colored. Click the **Alignment Info** group and under **Conservation**, check "Background color". This will use a gradient as background color for the residues. You can adjust the coloring by dragging the small arrows above the color box.

2.3.1 Saving the settings in the Side Panel

Now the alignment should look similar to figure 2.7.

At this point, if you just close the view, the changes made to the **Side Panel** will not be saved.

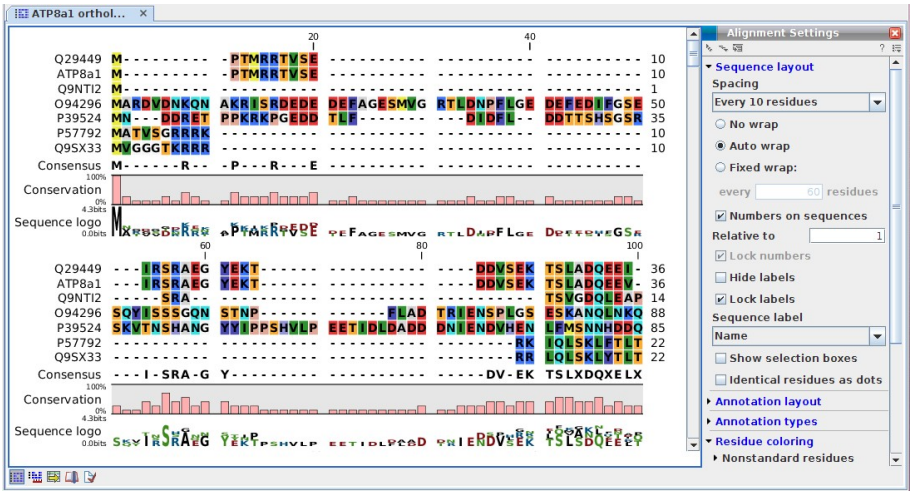

Figure 2.5: *The protein alignment as it looks when you open it with background color according to the Rasmol color scheme and automatically wrapped.*

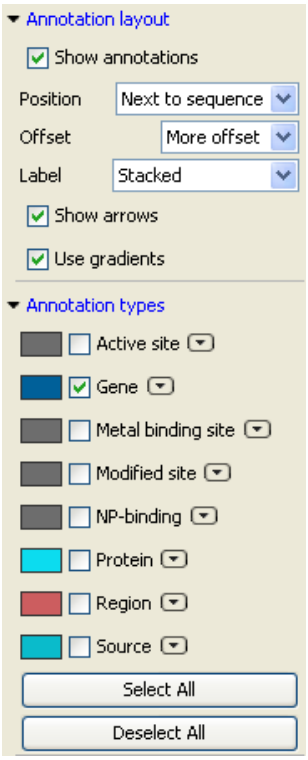

Figure 2.6: *The Annotation Layout and the Annotation Types in the Side Panel.*

This means that you would have to perform the changes again next time you open the alignment. To save the changes to the **Side Panel**, click the **Save/Restore Settings** button ( ) at the top of the **Side Panel** and click **Save Settings** (see figure 2.8).

This will open the dialog shown in figure 2.9.

In this way you can save the current state of the settings in the **Side Panel** so that you can apply them to alignments later on. If you check **Always apply these settings**, these settings will be applied every time you open a view of the alignment.

Type "My settings" in the dialog and click **Save**.

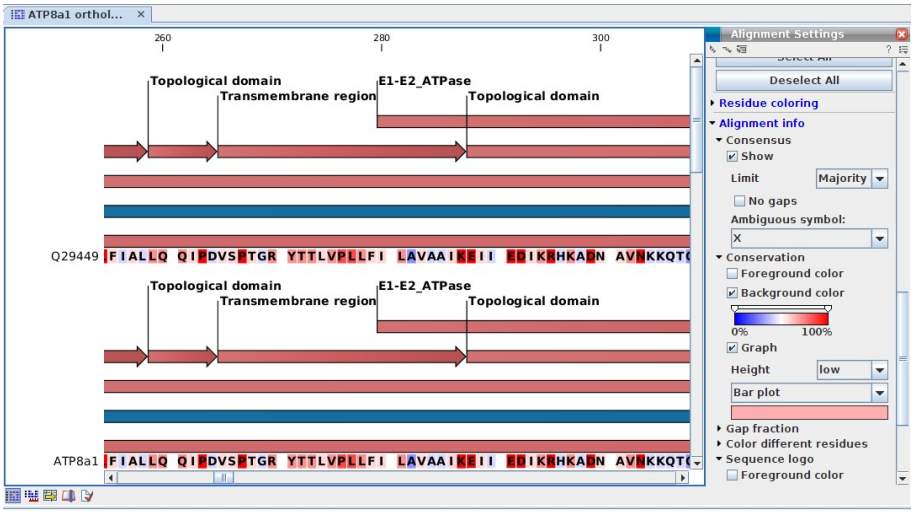

Figure 2.7: *The alignment when all the above settings have been changed.*

Figure 2.8: *Saving the settings of the Side Panel.*

Figure 2.9: *Dialog for saving the settings of the Side Panel.*

2.3.2 Applying saved settings

When you click the **Save/Restore Settings** button ( ) again and select **Apply Saved Settings**, you will see "My settings" in the menu together with some pre-defined settings that the *CLC Sequence Viewer* has created for you (see figure 2.10).

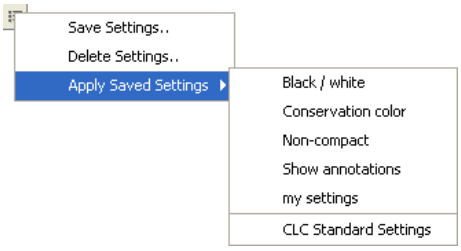

Figure 2.10: *Menu for applying saved settings.*

Whenever you open an alignment, you will be able to apply these settings. Each kind of view has its own list of settings that can be applied.

At the bottom of the list you will see the "CLC Standard Settings" which are the default settings for the view.

2.4 Tutorial: GenBank search and download

The *CLC Sequence Viewer* allows you to search the NCBI GenBank database directly from the program, giving you the opportunity to both open, view, analyze and save the search results without using any other applications. To conduct a search in NCBI GenBank from *CLC Sequence Viewer* you must be connected to the Internet.

This tutorial shows how to find a complete human hemoglobin DNA sequence in a situation where you do not know the accession number of the sequence.

To start the search:

Download | Search for Sequences at NCBI ( )

This opens the search view. We are searching for a DNA sequence, hence:

Nucleotide

Now we are going to adjust parameters for the search. By clicking **Add search parameters** you activate an additional set of fields where you can enter search criteria. Each search criterion consists of a drop down menu and a text field. In the drop down menu you choose which part of the NCBI database to search, and in the text field you enter what to search for:

Click Add search parameters until three search criteria are available | choose Organism in the first drop down menu | write 'human' in the adjoining text field | choose All Fields in the second drop down menu | write 'hemoglobin' in the adjoining text field | choose All Fields in the third drop down menu | write 'complete' in the adjoining text field

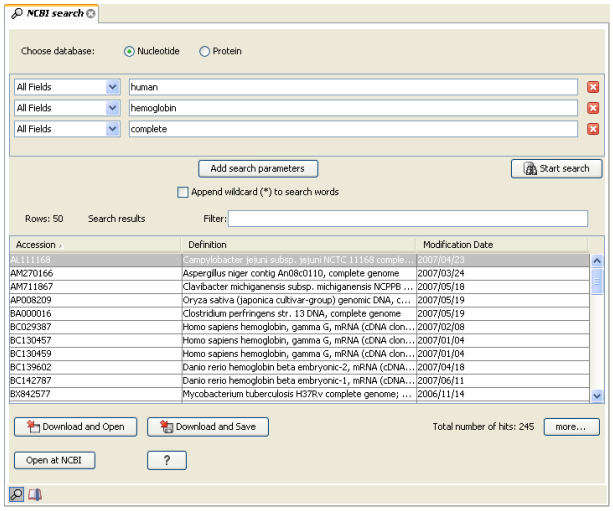

Figure 2.11: *NCBI search view.*

Click **Start search ( )** to commence the search in NCBI.

2.4.1 Searching for matching objects

When the search is complete, the list of hits is shown. If the desired complete human hemoglobin DNA sequence is found, the sequence can be viewed by double-clicking it in the list of hits from the search. If the desired sequence is not shown, you can click the 'More' button below the list to see more hits.

2.4.2 Saving the sequence

The sequences which are found during the search can be displayed by double-clicking in the list of hits. However, this does not save the sequence. You can save one or more sequence by selecting them and:

click Download and Save

or **drag the sequences into the Navigation Area**

2.5 Tutorial: Align protein sequences

This tutorial outlines some of the alignment functionality of the *CLC Sequence Viewer*. In addition to creating alignments of nucleotide or peptide sequences, the software offers several ways to view alignments. The alignments can then be used for building phylogenetic trees.

Sequences must be available via the **Navigation Area** to be included in an alignment. If you have sequences open in a View that you have not saved, then you just need to select the view tab and press Ctrl + S (or ⌘ + S on Mac) to save them.

In this tutorial six protein sequences from the Example data folder will be aligned. (See figure 2.12).

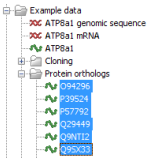

Figure 2.12: *Six protein sequences in 'Sequences' from the 'Protein orthologs' folder of the Example data.*

To align the sequences:

select the sequences from the 'Protein' folder under 'Sequences' | **Toolbox** | **Alignments and Trees** ( )| **Create Alignment** ( )

2.5.1 The alignment dialog

This opens the dialog shown in figure 2.13.

It is possible to add and remove sequences from **Selected Elements** list. Since we had already selected the eight proteins, just click **Next** to adjust parameters for the alignment.

Clicking **Next** opens the dialog shown in figure 2.14.

Leave the parameters at their default settings. An explanation of the parameters can be found by clicking the help button (?). Alternatively, a tooltip is displayed by holding the mouse cursor on the parameters.

Click **Finish** to start the alignment process which is shown in the **Toolbox** under the **Processes** tab. When the program is finished calculating it displays the alignment (see fig. 2.15):

Note! The new alignment is not saved automatically.

To save the alignment, drag the tab of the alignment view into the **Navigation Area**.

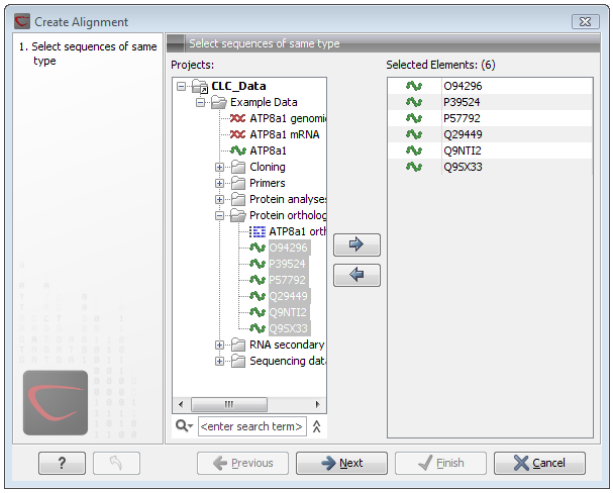

Figure 2.13: *The alignment dialog displaying the six protein sequences.*

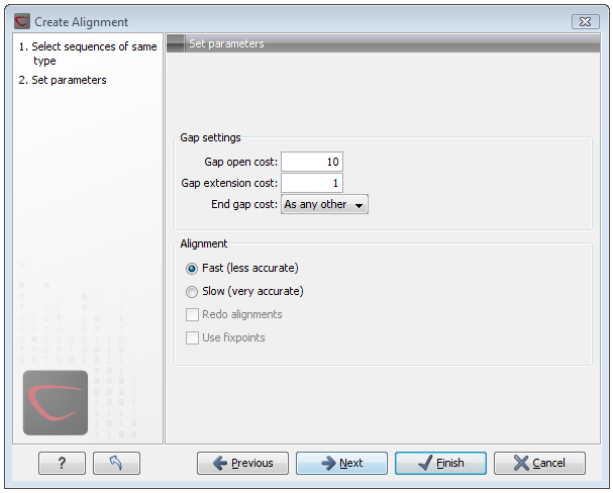

Figure 2.14: *The alignment dialog displaying the available parameters which can be adjusted.*

Installing the Additional Alignments plugin gives you access to other alignment algorithms: ClustalW (Windows/Mac/Linux), Muscle (Windows/Mac/Linux), T-Coffee (Mac/Linux), MAFFT (Mac/Linux), and Kalign (Mac/Linux). The Additional Alignments Module can be downloaded from `http://www.clcbio.com/plugins`. Note that you will need administrative privileges on your system to install it.

2.6 Tutorial: Create and modify a phylogenetic tree

You can make a phylogenetic tree from an existing alignment. (See how to create an alignment in the tutorial: "Align protein sequences").

We use the 'ATPase protein alignment' located in 'Protein orthologs' in the Example data. To create a phylogenetic tree:

> **click the 'ATPase protein alignment' in the Navigation Area | Toolbox | Alignments and Trees ( )| Create Tree ( )**

A dialog opens where you can confirm your selection of the alignment. Click **Next** to move to the next step in the dialog where you can choose between the neighbor joining and the UPGMA

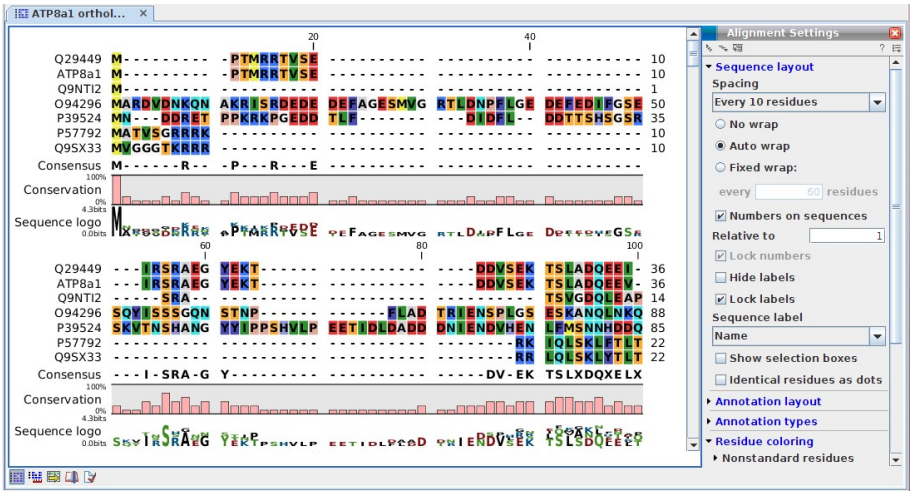

Figure 2.15: *The resulting alignment.*

algorithms for making trees. You also have the option of including a bootstrap analysis of the result. Leave the parameters at their default, and click **Finish** to start the calculation, which can be seen in the **Toolbox** under the **Processes** tab. After a short while a tree appears in the **View Area** (figure 2.16).

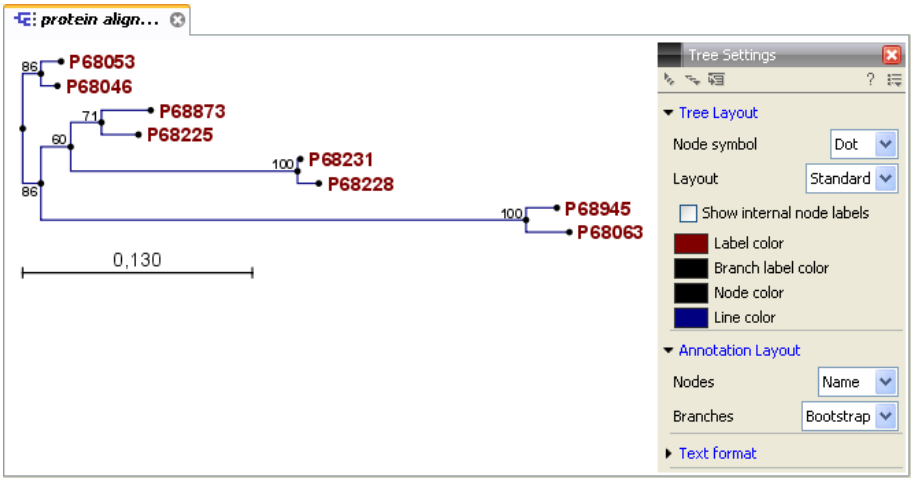

Figure 2.16: *After choosing which algorithm should be used, the tree appears in the View Area. The Side panel in the right side of the view allows you to adjust the way the tree is displayed.*

2.6.1 Tree layout

Using the **Side Panel** (in the right side of the view), you can change the way the tree is displayed.

Click **Tree Layout** and open the **Layout** drop down menu. Here you can choose between standard and topology layout. The topology layout can help to give an overview of the tree if some of the branches are very short.

When the sequences include the appropriate annotation, it is possible to choose between the accession number and the species names at the leaves of the tree. Sequences downloaded from GenBank, for example, have this information. The **Labels** preferences allows these different node annotations as well as different annotation on the branches.

The branch annotation includes the bootstrap value, if this was selected when the tree was calculated. It is also possible to annotate the branches with their lengths.

2.7 Tutorial: Find restriction sites

This tutorial will show you how to find restriction sites and annotate them on a sequence.

There are two ways of finding and showing restriction sites. In many cases, the dynamic restriction sites found in the **Side Panel** of sequence views will be useful, since it is a quick and easy way of showing restriction sites. In the **Toolbox** you will find the other way of doing restriction site analyses. This way provides more control of the analysis and gives you more output options, e.g. a table of restriction sites and a list of restriction enzymes that can be saved for later use. In this tutorial, the first section describes how to use the Side Panel to show restriction sites, whereas the second section describes the restriction map analysis performed from the **Toolbox**.

2.7.1 The Side Panel way of finding restriction sites

When you open a sequence, there is a **Restriction sites** setting in the **Side Panel**. By default, 10 of the most popular restriction enzymes are shown (see figure 2.17).

Figure 2.17: *Showing restriction sites of ten restriction enzymes.*

The restriction sites are shown on the sequence with an indication of cut site and recognition sequence. In the list of enzymes in the **Side Panel**, the number of cut sites is shown in parentheses for each enzyme (e.g. *SalI* cuts three times). If you wish to see the recognition sequence of the enzyme, place your mouse cursor on the enzyme in the list for a short moment, and a tool tip will appear.

You can add or remove enzymes from the list by clicking the **Manage enzymes** button.

2.7.2 The Toolbox way of finding restriction sites

Suppose you are working with sequence 'ATP8a1 mRNA' from the example data, and you wish to know which restriction enzymes will cut this sequence exactly once and create a 3' overhang. Do the following:

> **select the ATP8a1 mRNA | Toolbox in the Menu Bar | Restriction Sites ( ) | Restriction Site Analysis ( )**

Click **Next** to set parameters for the restriction map analysis.

In this step first select **Use existing enzyme list** and click the **Browse for enzyme list** button ( ). Select the 'Popular enzymes' in the Cloning folder under Enzyme lists.

Then write 3' into the filter below to the left. Select all the enzymes and click the **Add** button ( ). The result should be like in figure 2.18.

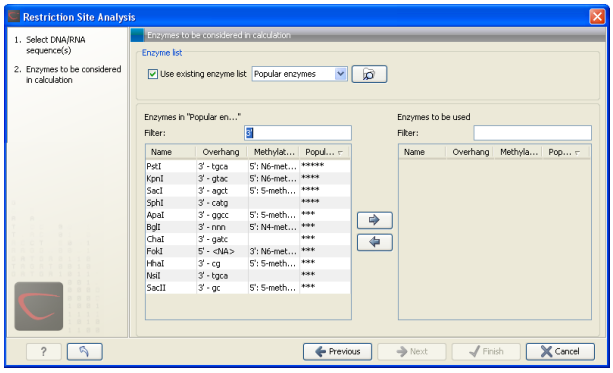

Figure 2.18: *Selecting enzymes.*

Click **Next**. In this step you specify that you want to show enzymes that cut the sequence only once. This means that you should de-select the **Two restriction sites** checkbox.

Click **Next** and select that you want to **Add restriction sites as annotations on sequence** and **Create restriction map**. (See figure 2.19).

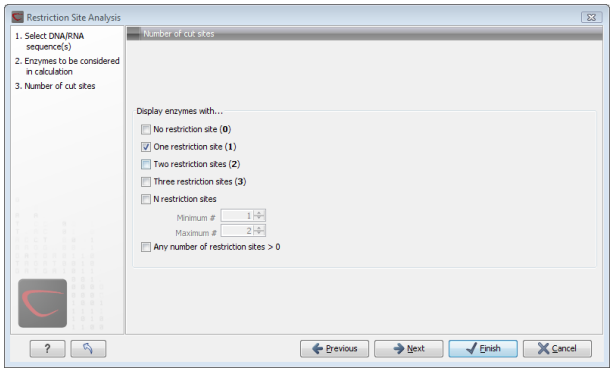

Figure 2.19: *Selecting output for restriction map analysis.*

Click **Finish** to start the restriction map analysis.

View restriction site

The restriction sites are shown in two views: one view is in a tabular format and the other view displays the sites as annotations on the sequence.

The result is shown in figure 2.20.

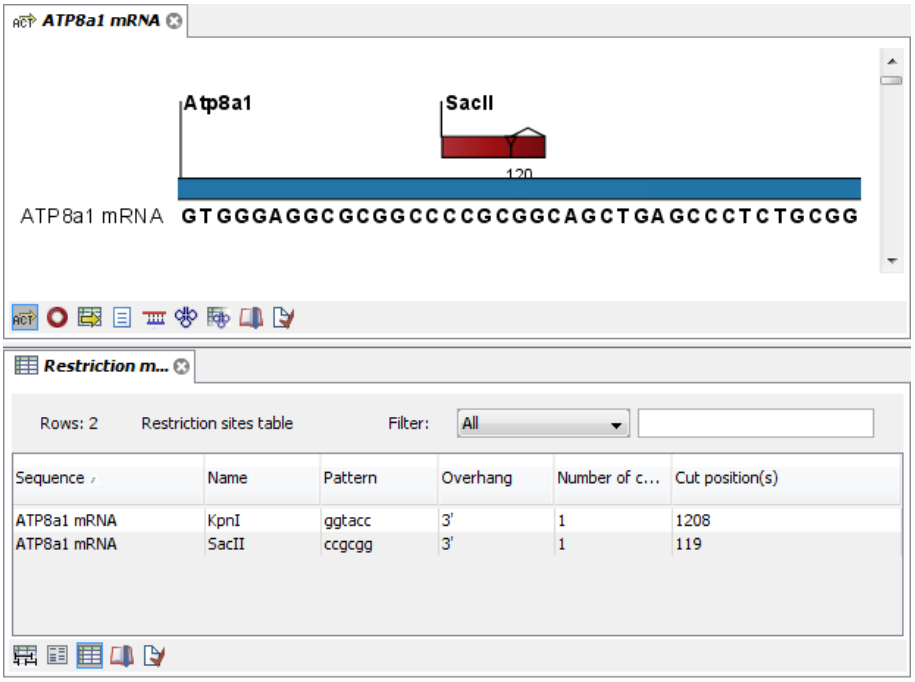

Figure 2.20: *The result of the restriction map analysis is displayed in a table at the bottom and as annotations on the sequence in the view at the top.*

Part II

Core Functionalities

Chapter 3

User interface

Contents

This chapter provides an overview of the different areas in the user interface of *CLC Sequence Viewer*. As can be seen from figure 3.1 this includes a **Navigation Area**, **View Area**, **Menu Bar**, **Toolbar**, **Status Bar** and **Toolbox**.

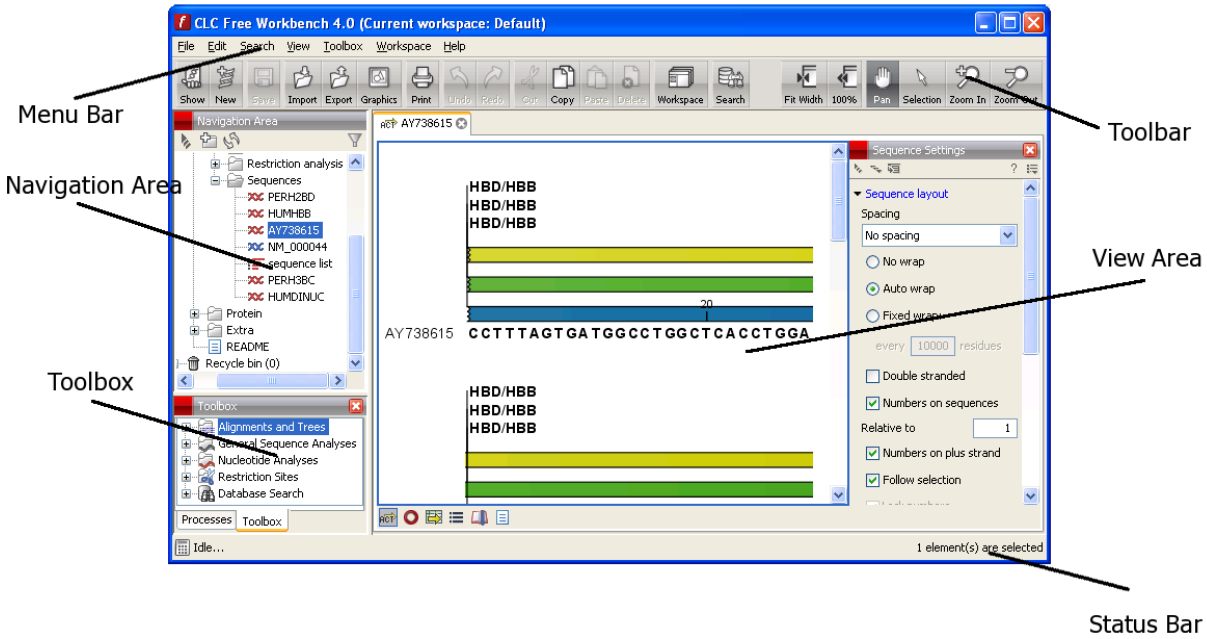

Figure 3.1: *The user interface consists of the Menu Bar, Toolbar, Status Bar, Navigation Area, Toolbox, and View Area.*

3.1 Navigation Area

The **Navigation Area** is located in the left side of the screen, under the **Toolbar** (see figure 3.2). It is used for organizing and navigating data. Its behavior is similar to the way files and folders are usually displayed on your computer.

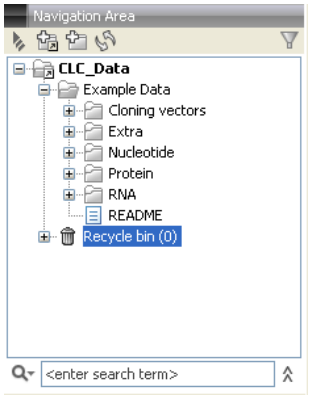

Figure 3.2: *The Navigation Area.*

3.1.1 Data structure

The data in the **Navigation Area** is organized into a number of **Locations**. When the *CLC Sequence Viewer* is started for the first time, there is one location called *CLC_Data* (unless your computer administrator has configured the installation otherwise).

A location represents a folder on the computer: The data shown under a location in the **Navigation Area** is stored on the computer in the folder which the location points to.

This is explained visually in figure 3.3.

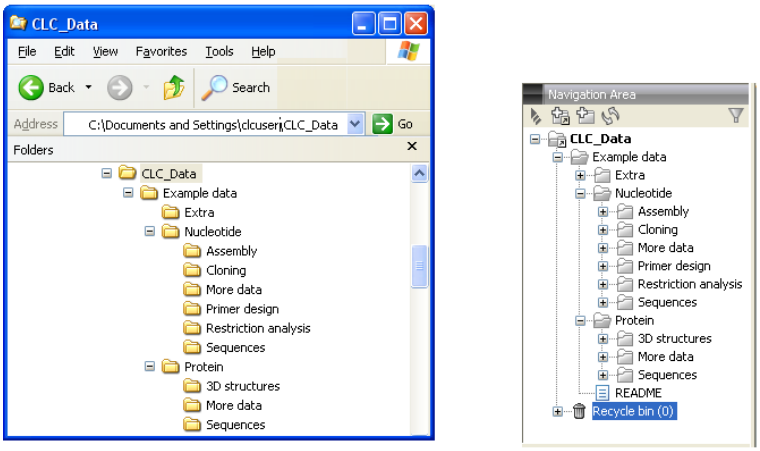

Figure 3.3: *In this example the location called 'CLC_Data' points to the folder at C:\Documents and settings\clcuser\CLC_Data.*

Opening data

The elements in the **Navigation Area** are opened by :

Double-click the element

or **Click the element | Show ( ) in the Toolbar | Select the desired way to view the element**

This will open a view in the **View Area**, which is described in section 3.2.

Adding data

Data can be added to the **Navigation Area** in a number of ways. Files can be imported from the file system (see chapter 6). Furthermore, an element can be added by dragging it into the **Navigation Area**. This could be views that are open, elements on lists, e.g. search hits or sequence lists, and files located on your computer.

If a file or another element is dropped on a folder, it is placed at the bottom of the folder. If it is dropped on another element, it will be placed just below that element.

If the element already exists in the **Navigation Area**, you will be asked whether you wish to create a copy.

3.1.2 Create new folders

In order to organize your files, they can be placed in folders. Creating a new folder can be done in two ways:

right-click an element in the Navigation Area | **New** | **Folder ()**

or **File** | **New** | **Folder ()**

If a folder is selected in the **Navigation Area** when adding a new folder, the new folder is added at the bottom of this folder. If an element is selected, the new folder is added right above that element.

You can move the folder manually by selecting it and dragging it to the desired destination.

3.1.3 Sorting folders

You can sort the elements in a folder alphabetically:

right-click the folder | **Sort Folder**

On Windows, subfolders will be placed at the top of the folder, and the rest of the elements will be listed below in alphabetical order. On Mac, both subfolders and other elements are listed together in alphabetical order.

3.1.4 Multiselecting elements

Multiselecting elements means that you select more than one element at the same time. This can be done in the following ways:

- Holding down the <Ctrl> key (⌘ on Mac) while clicking on multiple elements selects the elements that have been clicked.
- Selecting one element, and selecting another element while holding down the <Shift> key selects all the elements listed between the two locations (the two end locations included).
- Selecting one element, and moving the curser with the arrow-keys while holding down the <Shift> key, enables you to increase the number of elements selected.

3.1.5 Moving and copying elements

Elements can be moved and copied in several ways:

- Using **Copy** (), **Cut** () and **Paste** () from the **Edit** menu.
- Using Ctrl + C (⌘ + C on Mac), Ctrl + X (⌘ + X on Mac) and Ctrl + V (⌘ + V on Mac).
- Using **Copy** (), **Cut** () and **Paste** () in the **Toolbar**.
- Using drag and drop to move elements.
- Using drag and drop while pressing Ctrl / Command to copy elements.

In the following, all of these possibilities for moving and copying elements are described in further detail.

Copy, cut and paste functions

Copies of elements and folders can be made with the copy/paste function which can be applied in a number of ways:

> **select the files to copy** | **right-click one of the selected files** | **Copy ()** | **right-click the location to insert files into** | **Paste ()**

or **select the files to copy** | **Ctrl + C (⌘ + C on Mac)** | **select where to insert files** | **Ctrl + P (⌘ + P on Mac)**

or **select the files to copy** | **Edit in the Menu Bar** | **Copy ()** | **select where to insert files** | **Edit in the Menu Bar** | **Paste ()**

If there is already an element of that name, the pasted element will be renamed by appending a number at the end of the name.

Elements can also be moved instead of copied. This is done with the cut/paste function:

> **select the files to cut** | **right-click one of the selected files** | **Cut ()** | **right-click the location to insert files into** | **Paste ()**

or **select the files to cut** | **Ctrl + X (⌘ + X on Mac)** | **select where to insert files** | **Ctrl + V (⌘ + V on Mac)**

When you have cut the element, it is "greyed out" until you activate the paste function. If you change your mind, you can revert the cut command by copying another element.

Note that if you move data between locations, the original data is kept. This means that you are essentially doing a copy instead of a move operation.

Move using drag and drop

Using drag and drop in the **Navigation Area**, as well as in general, is a four-step process:

> **click the element** | **click on the element again, and hold left mouse button** | **drag the element to the desired location** | **let go of mouse button**

This allows you to:

- Move elements between different folders in the **Navigation Area**
- Drag from the **Navigation Area** to the **View Area**: A new view is opened in an existing **View Area** if the element is dragged from the **Navigation Area** and dropped next to the tab(s) in that **View Area**.
- Drag from the **View Area** to the **Navigation Area**: The element, e.g. a sequence, alignment, search report etc. is saved where it is dropped. If the element already exists, you are asked whether you want to save a copy. You drag from the **View Area** by dragging the tab of the desired element.

Use of drag and drop is supported throughout the program, also to open and re-arrange views (see section 3.2.6).

Note that if you move data between locations, the original data is kept. This means that you are essentially doing a copy instead of a move operation.

Copy using drag and drop

To copy instead of move using drag and drop, hold the Ctrl (⌘ on Mac) key while dragging:

> **click the element** | **click on the element again, and hold left mouse button** | **drag the element to the desired location** | **press Ctrl (⌘ on Mac) while you let go of mouse button release the Ctrl/⌘ button**

3.1.6 Change element names

This section describes two ways of changing the names of sequences in the **Navigation Area**. In the first part, the sequences themselves are not changed - it's their representation that changes. The second part describes how to change the name of the element.

Change how sequences are displayed

Sequence elements can be displayed in the **Navigation Area** with different types of information:

- Name (this is the default information to be shown).
- Accession (sequences downloaded from databases like GenBank have an accession number).
- Latin name.
- Latin name (accession).
- Common name.
- Common name (accession).

Whether sequences can be displayed with this information depends on their origin. Sequences that you have created yourself or imported might not include this information, and you will only be able to see them represented by their name. However, sequences downloaded from databases like GenBank will include this information. To change how sequences are displayed:

> **right-click any element or folder in the Navigation Area** | **Sequence Representation** | **select format**

This will only affect sequence elements, and the display of other types of elements, e.g. alignments, trees and external files, will be not be changed. If a sequence does not have this information, there will be no text next to the sequence icon.

Rename element

Renaming a folder or an element in the **Navigation Area** can be done in three different ways:

> **select the element** | **Edit in the Menu Bar** | **Rename**

or **select the element** | **F2**

> **click the element once** | **wait one second** | **click the element again**

When you can rename the element, you can see that the text is selected and you can move the cursor back and forth in the text. When the editing of the name has finished; press **Enter** or select another element in the **Navigation Area**. If you want to discard the changes instead, press the **Esc**-key.

3.1.7 Delete elements

Deleting a folder or an element can be done in two ways:

right-click the element | **Delete (**  **)**

or **select the element** | **press Delete key**

This will cause the element to be moved to the **Recycle Bin** () where it is kept until the recycle bin is emptied. This means that you can recover deleted elements later on.

For deleting annotations instead of folders or elements, see section 9.3.2.

Restore Deleted Elements

The elements in the **Recycle Bin** ( ) can be restored by dragging the elements with the mouse into the folder where they used to be.

If you have deleted large amounts of data taking up very much disk space, you can free this disk space by emptying the **Recycle Bin** ( ):

Edit in the Menu Bar | **Empty Recycle Bin (**  **)**

Note! This cannot be undone, and you will therefore not be able to recover the data present in the recycle bin when it was emptied.

3.1.8 Show folder elements in a table

A location or a folder might contain large amounts of elements. It is possible to view their elements in the **View Area**:

select a folder or location | **Show (**  **) in the Toolbar** | **Contents (**  **)**

An example is shown in figure 3.4.

When the elements are shown in the view, they can be sorted by clicking the heading of each of the columns. You can further refine the sorting by pressing Ctrl (⌘ on Mac) while clicking the heading of another column.

Sorting the elements in a view does not affect the ordering of the elements in the **Navigation Area**.

Note! The view only displays one "layer" at a time: the content of subfolders is not visible in this view. Also note that only sequences have the full span of information like organism etc.

Batch edit folder elements

You can select a number of elements in the table, right-click and choose **Edit** to batch edit the elements. In this way, you can change the e.g. the description or common name of several

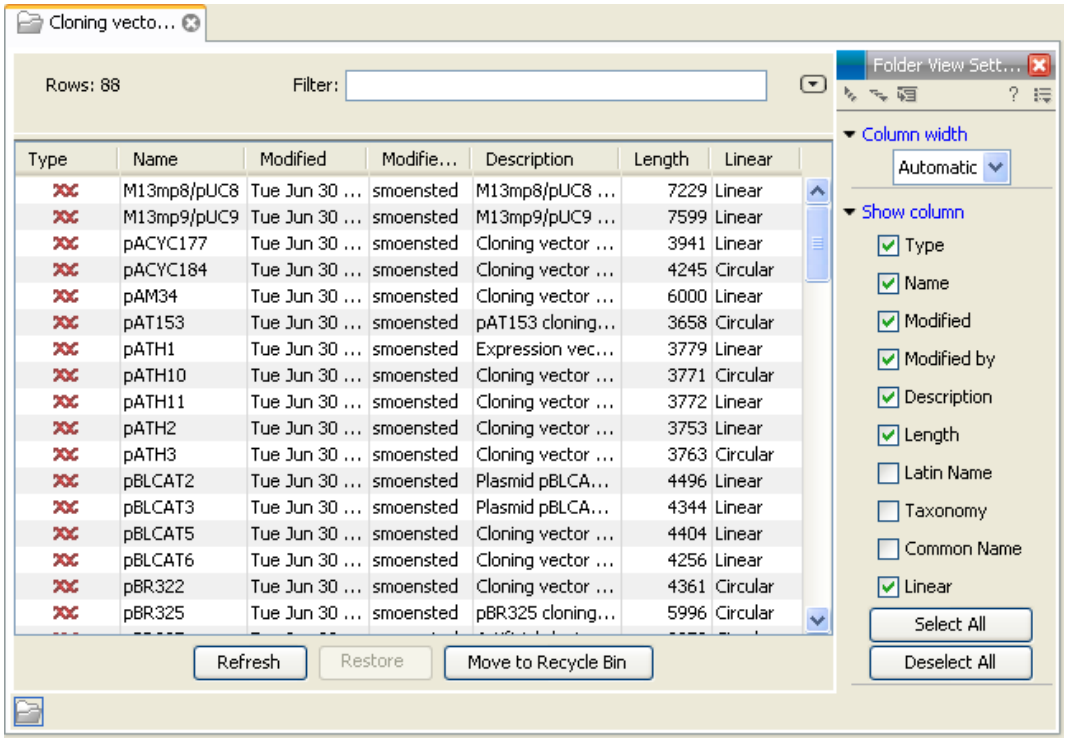

Figure 3.4: *Viewing the elements in a folder.*

elements in one go.

In figure 3.5 you can see an example where the common name of five sequence are renamed in one go. In this example, a dialog with a text field will be shown, letting you enter a new common name for these five sequences. **Note!** This information is directly saved and you cannot

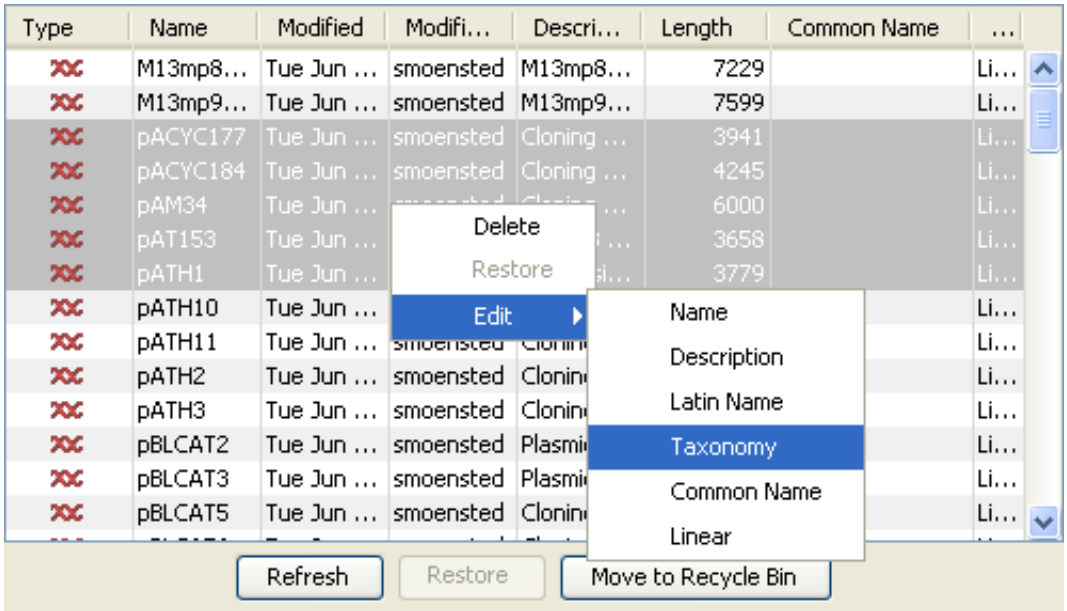

Figure 3.5: *Changing the common name of five sequences.*

undo.

3.2 View Area

The **View Area** is the right-hand part of the screen, displaying your current work. The **View Area** may consist of one or more **Views**, represented by tabs at the top of the **View Area**.

This is illustrated in figure 3.6.

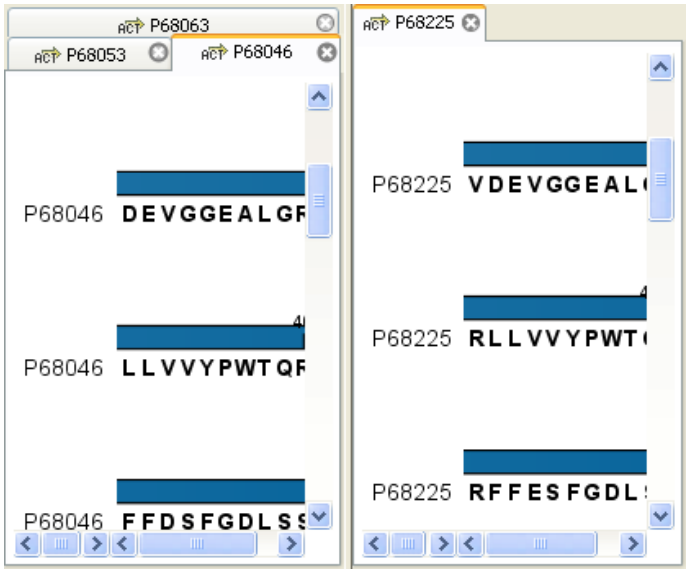

Figure 3.6: *A View Area can enclose several views, each view is indicated with a tab (see right view, which shows protein P68225). Furthermore, several views can be shown at the same time (in this example, four views are displayed).*

The tab concept is central to working with *CLC Sequence Viewer*, because several operations can be performed by dragging the tab of a view, and extended right-click menus can be activated from the tabs.

This chapter deals with the handling of views inside a **View Area**. Furthermore, it deals with rearranging the views.

Section 3.3 deals with the zooming and selecting functions.

3.2.1 Open view

Opening a view can be done in a number of ways:

double-click an element in the Navigation Area

or **select an element in the Navigation Area** | **File** | **Show** | **Select the desired way to view the element**

or **select an element in the Navigation Area** | **Ctrl + O (⌘ + B on Mac)**

Opening a view while another view is already open, will show the new view in front of the other view. The view that was already open can be brought to front by clicking its tab.

Note! If you right-click an open tab of any element, click **Show**, and then choose a different view of the same element, this new view is automatically opened in a split-view, allowing you to see both views.

See section 3.1.5 for instructions on how to open a view using drag and drop.

3.2.2 Show element in another view

Each element can be shown in different ways. A sequence, for example, can be shown as linear, circular, text etc.

In the following example, you want to see a sequence in a circular view. If the sequence is already open in a view, you can change the view to a circular view:

Click Show As Circular ( ) at the lower left part of the view

The buttons used for switching views are shown in figure 3.7).

Figure 3.7: *The buttons shown at the bottom of a view of a nucleotide sequence. You can click the buttons to change the view to e.g. a circular view or a history view.*

If the sequence is already open in a linear view ( ), and you wish to see both a circular and a linear view, you can split the views very easily:

Press Ctrl (⌘ on Mac) while you | Click Show As Circular ( ) at the lower left part of the view

This will open a split view with a linear view at the bottom and a circular view at the top (see 9.5).

You can also show a circular view of a sequence without opening the sequence first:

Select the sequence in the Navigation Area | Show ( ) | As Circular ( )

3.2.3 Close views

When a view is closed, the **View Area** remains open as long as there is at least one open view.

A view is closed by:

right-click the tab of the View | Close

or **select the view | Ctrl + W**

or **hold down the Ctrl-button | Click the tab of the view while the button is pressed**

By right-clicking a tab, the following close options exist. See figure 3.8

- **Close.** See above.
- **Close Tab Area.** Closes all tabs in the tab area.
- **Close All Views.** Closes all tabs, in all tab areas. Leaves an empty workspace.
- **Close Other Tabs.** Closes all other tabs, in all tab areas, except the one that is selected.

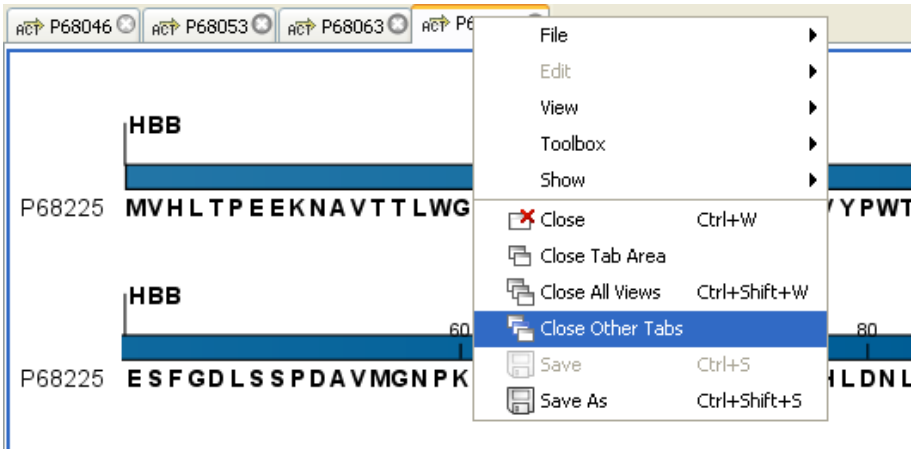

Figure 3.8: *By right-clicking a tab, several close options are available.*

3.2.4 Save changes in a view

When changes are made in a view, the text on the tab appears *bold and italic* (on Mac it is indicated by an * before the name of the tab). This indicates that the changes are not saved. The **Save** function may be activated in two ways:

Click the tab of the view you want to save | **Save (**  **) in the toolbar.**

or **Click the tab of the view you want to save** | **Ctrl + S (⌘ + S on Mac)**

If you close a view containing an element that has been changed since you opened it, you are asked if you want to save.

When saving a new view that has not been opened from the Navigation Area (e.g. when opening a sequence from a list of search hits), a save dialog appears (figure 3.9).

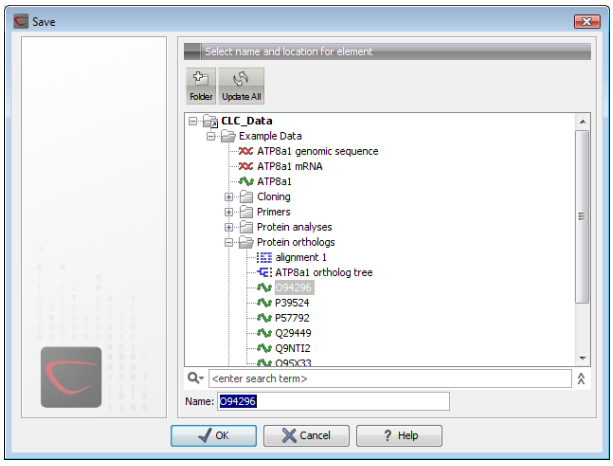

Figure 3.9: *Save dialog.*

In the dialog you select the folder in which you want to save the element.

After naming the element, press **OK**

3.2.5 Undo/Redo

If you make a change in a view, e.g. remove an annotation in a sequence or modify a tree, you can undo the action. In general, **Undo** applies to all changes you can make when right-clicking in a view. **Undo** is done by:

Click undo ( ) in the Toolbar

or **Edit | Undo ( )**

or **Ctrl + Z**

If you want to undo several actions, just repeat the steps above. To reverse the undo action:

Click the redo icon in the Toolbar

or **Edit | Redo ( )**

or **Ctrl + Y**

Note! Actions in the **Navigation Area**, e.g. renaming and moving elements, cannot be undone. However, you can restore deleted elements (see section 3.1.7).

You can set the number of possible undo actions in the Preferences dialog (see section 4).

3.2.6 Arrange views in View Area

Views are arranged in the **View Area** by their tabs. The order of the **views** can be changed using drag and drop. E.g. drag the tab of one view onto the tab of a another. The tab of the first view is now placed at the right side of the other tab.

If a tab is dragged into a view, an area of the view is made gray (see fig. 3.10) illustrating that the view will be placed in this part of the **View Area**.

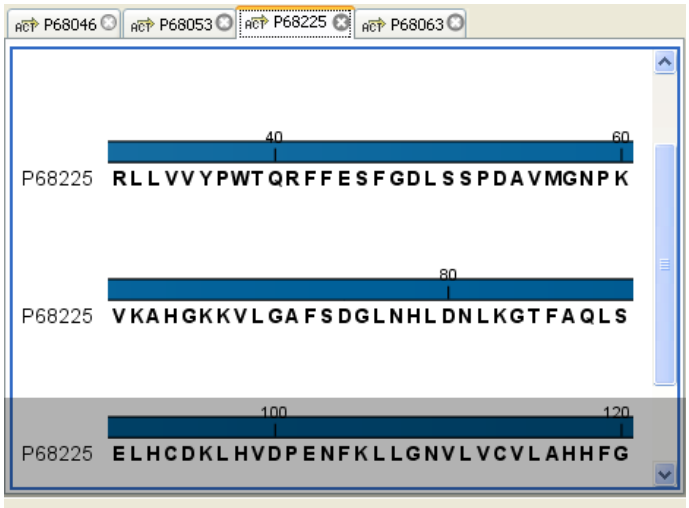

Figure 3.10: *When dragging a view, a gray area indicates where the view will be shown.*

The results of this action is illustrated in figure 3.11.

You can also split a **View Area** horizontally or vertically using the menus.

Splitting horisontally may be done this way:

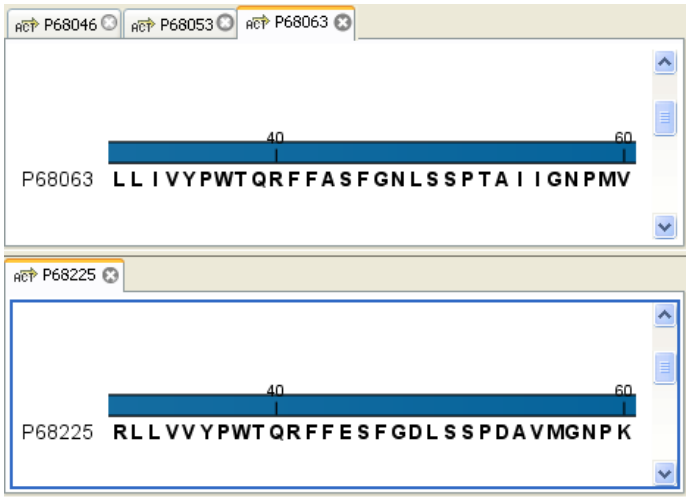

Figure 3.11: *A horizontal split-screen. The two views split the View Area.*

right-click a tab of the view | View | Split Horizontally ( )

This action opens the chosen view below the existing view. (See figure 3.12). When the split is made vertically, the new view opens to the right of the existing view.

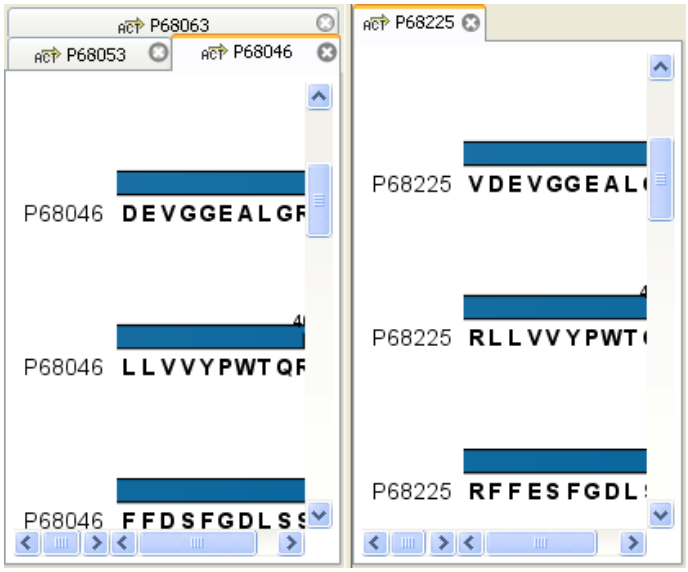

Figure 3.12: *A vertical split-screen.*

Splitting the **View Area** can be undone by dragging e.g. the tab of the bottom view to the tab of the top view. This is marked by a gray area on the top of the view.

Maximize/Restore size of view

The **Maximize/Restore View** function allows you to see a view in maximized mode, meaning a mode where no other **views** nor the **Navigation Area** is shown.

Maximizing a view can be done in the following ways:

select view | Ctrl + M

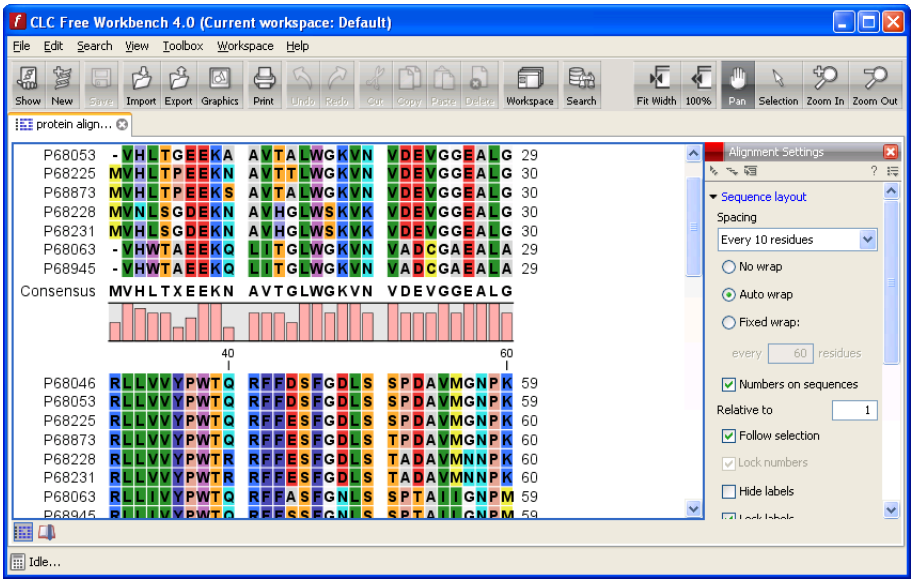

Figure 3.13: *A maximized view. The function hides the Navigation Area and the Toolbox.*

or **select view** | **View** | **Maximize/restore View ( )**

or **select view** | **right-click the tab** | **View** | **Maximize/restore View ( )**

or **double-click the tab of view**

The following restores the size of the view:

Ctrl + M

or **View** | **Maximize/restore View ( )**

or **double-click title of view**

3.2.7 Side Panel

The **Side Panel** allows you to change the way the contents of a view are displayed. The options in the **Side Panel** depend on the kind of data in the view, and they are described in the relevant sections about sequences, alignments, trees etc.

Side Panel are activated in this way:

select the view | **Ctrl + U (⌘ + U on Mac)**

or **right-click the tab of the view** | **View** | **Show/Hide Side Panel ( )**

Note! Changes made to the **Side Panel** will not be saved when you save the view. See how to save the changes in the **Side Panel** in chapter 4 .

The **Side Panel** consists of a number of groups of preferences (depending on the kind of data being viewed), which can be expanded and collapsed by clicking the header of the group. You can also expand or collapse all the groups by clicking the icons ( )/ ( ) at the top.

3.3 Zoom and selection in View Area

The mode toolbar items in the right side of the **Toolbar** apply to the function of the mouse pointer. When e.g. **Zoom Out** is selected, you zoom out each time you click in a view where zooming is relevant (texts, tables and lists cannot be zoomed). The chosen mode is active until another mode toolbar item is selected. (**Fit Width** and **Zoom to 100%** do not apply to the mouse pointer.)

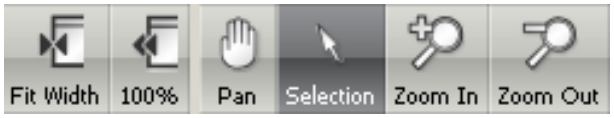

Figure 3.14: *The mode toolbar items.*

3.3.1 Zoom In

There are four ways of **Zooming In**:

Click Zoom In () in the toolbar | **click the location in the view that you want to zoom in on**.

or **Click Zoom In () in the toolbar** | **click-and-drag a box around a part of the view** | **the view now zooms in on the part you selected**

or **Press '+' on your keyboard**

The last option for zooming in is only available if you have a mouse with a scroll wheel:

or **Press and hold Ctrl (⌘ on Mac)** | **Move the scroll wheel on your mouse forward**

When you choose the Zoom In mode, the mouse pointer changes to a magnifying glass to reflect the mouse mode.

Note! You might have to click in the view before you can use the keyboard or the scroll wheel to zoom.

If you press the **Shift** button on your keyboard while clicking in a **View**, the zoom function is reversed. Hence, clicking on a sequence in this way while the **Zoom In** mode toolbar item is selected, zooms out instead of zooming in.

3.3.2 Zoom Out

It is possible to zoom out, step by step, on a sequence:

Click Zoom Out () in the toolbar | **click in the view until you reach a satisfying zoomlevel**.

or **Press '-' on your keyboard**

The last option for zooming out is only available if you have a mouse with a scroll wheel:

or **Press and hold Ctrl (⌘ on Mac)** | **Move the scroll wheel on your mouse backwards**

When you choose the Zoom Out mode, the mouse pointer changes to a magnifying glass to reflect the mouse mode.

Note! You might have to click in the view before you can use the keyboard or the scroll wheel to

zoom.

If you want to get a quick overview of a sequence or a tree, use the **Fit Width** function instead of the **Zoom Out** function.

If you press **Shift** while clicking in a **View**, the zoom function is reversed. Hence, clicking on a sequence in this way while the **Zoom Out** mode toolbar item is selected, zooms in instead of zooming out.

3.3.3 Fit Width

The **Fit Width** ( ) function adjusts the content of the **View** so that both ends of the sequence, alignment, or tree is visible in the **View** in question. (This function does not change the mode of the mouse pointer.)

3.3.4 Zoom to 100%

The **Zoom to 100%** ( ) function zooms the content of the **View** so that it is displayed with the highest degree of detail. (This function does not change the mode of the mouse pointer.)

3.3.5 Move

The Move mode allows you to drag the content of a **View**. E.g. if you are studying a sequence, you can click anywhere in the sequence and hold the mouse button. By moving the mouse you move the sequence in the **View**.

3.3.6 Selection

The Selection mode ( ) is used for selecting in a **View** (selecting a part of a sequence, selecting nodes in a tree etc.). It is also used for moving e.g. branches in a tree or sequences in an alignment.

When you make a selection on a sequence or in an alignment, the location is shown in the bottom right corner of the screen. E.g. '23^24' means that the selection is between two residues. '23' means that the residue at position 23 is selected, and finally '23..25' means that 23, 24 and 25 are selected. By holding ctrl / ⌘ you can make multiple selections.

3.3.7 Changing compactness

There is a shortcut way of changing the compactness setting for read mappings:

or **Press and hold Alt key** | **Scroll using your mouse wheel or touchpad**

3.4 Toolbox and Status Bar

The **Toolbox** is placed in the left side of the user interface of *CLC Sequence Viewer* below the **Navigation Area**.

The **Toolbox** shows a **Processes tab** and a **Toolbox tab**.

3.4.1 Processes

By clicking the **Processes** tab, the **Toolbox** displays previous and running processes, e.g. an NCBI search or a calculation of an alignment. The running processes can be stopped, paused, and resumed by clicking the small icon ( ) next to the process (see figure 3.15).

Running and paused processes are not deleted.

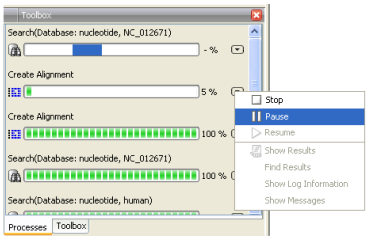

Figure 3.15: *A database search and an alignment calculation are running. Clicking the small icon next to the process allow you to stop, pause and resume processes.*

Besides the options to stop, pause and resume processes, there are some extra options for *a selected number* of the tools running from the Toolbox:

- **Show results**. If you have chosen to save the results (see section 8.1), you will be able to open the results directly from the process by clicking this option.
- **Find results**. If you have chosen to save the results (see section 8.1), you will be able to high-light the results in the Navigation Area.
- **Show Log Information**. This will display a log file showing progress of the process. The log file can also be shown by clicking **Show Log** in the "handle results" dialog where you choose between saving and opening the results.
- **Show Messages**. Some analyses will give you a message when processing your data. The messages are the black dialogs shown in the lower left corner of the Workbench that disappear after a few seconds. You can reiterate the messages that have been shown by clicking this option.

The terminated processes can be removed by:

View | **Remove Terminated Processes ( )**

If you close the program while there are running processes, a dialog will ask if you are sure that you want to close the program. Closing the program will stop the process, and it cannot be restarted when you open the program again.

3.4.2 Toolbox

The content of the **Toolbox** tab in the **Toolbox** corresponds to **Toolbox** in the **Menu Bar**.

The **Toolbox** can be hidden, so that the **Navigation Area** is enlarged and thereby displays more elements:

View | **Show/Hide Toolbox**

The tools in the toolbox can be accessed by double-clicking or by dragging elements from the **Navigation Area** to an item in the **Toolbox**.

3.4.3 Status Bar

As can be seen from figure 3.1, the **Status Bar** is located at the bottom of the window. In the left side of the bar is an indication of whether the computer is making calculations or whether it is idle. The right side of the **Status Bar** indicates the range of the selection of a sequence. (See chapter 3.3.6 for more about the Selection mode button.)

3.5 Workspace

If you are working on a project and have arranged the views for this project, you can save this arrangement using **Workspaces**. A Workspace remembers the way you have arranged the views, and you can switch between different workspaces.

The **Navigation Area** always contains the same data across **Workspaces**. It is, however, possible to open different folders in the different **Workspaces**. Consequently, the program allows you to display different clusters of the data in separate **Workspaces**.

All **Workspaces** are automatically saved when closing down *CLC Sequence Viewer*. The next time you run the program, the **Workspaces** are reopened exactly as you left them.

Note! It is not possible to run more than one version of *CLC Sequence Viewer* at a time. Use two or more **Workspaces** instead.

3.5.1 Create Workspace

When working with large amounts of data, it might be a good idea to split the work into two or more **Workspaces**. As default the *CLC Sequence Viewer* opens one **Workspace**. Additional **Workspaces** are created in the following way:

Workspace in the Menu Bar) | **Create Workspace** | **enter name of Workspace** | **OK**

When the new **Workspace** is created, the heading of the program frame displays the name of the new **Workspace**. Initially, the selected elements in the **Navigation Area** is collapsed and the **View Area** is empty and ready to work with. (See figure 3.16).

3.5.2 Select Workspace

When there is more than one **Workspace** in the *CLC Sequence Viewer*, there are two ways to switch between them:

Workspace () in the Toolbar | **Select the Workspace to activate**

or **Workspace in the Menu Bar** | **Select Workspace ()** | **choose which Workspace to activate** | **OK**

The name of the selected **Workspace** is shown after "*CLC Sequence Viewer*" at the top left corner of the main window, in figure 3.16 it says: (default).

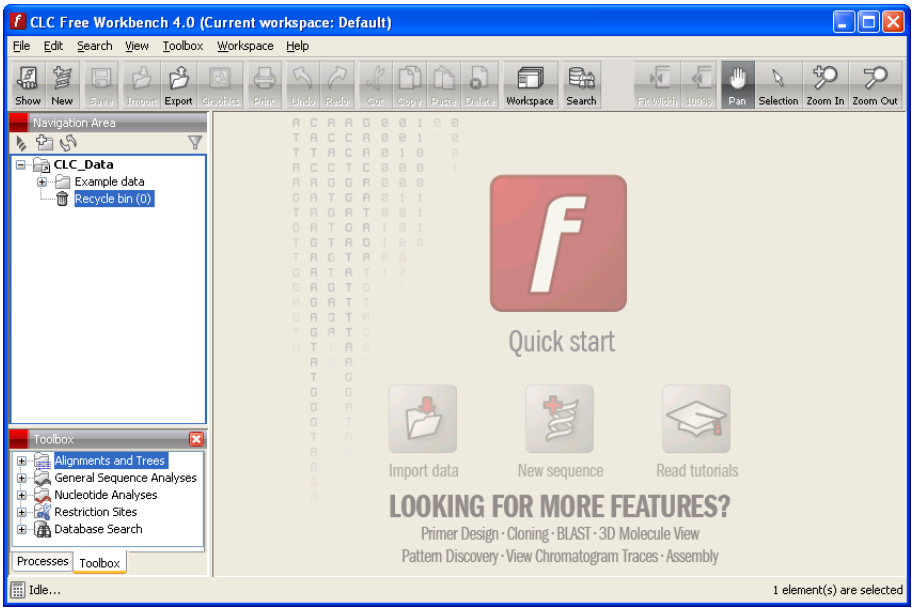

Figure 3.16: *An empty Workspace.*

3.5.3 Delete Workspace

Deleting a **Workspace** can be done in the following way:

> **Workspace in the Menu Bar** | **Delete Workspace** | **choose which Workspace to delete** | **OK**

Note! Be careful to select the right **Workspace** when deleting. The delete action cannot be undone. (However, no data is lost, because a workspace is only a representation of data.)

It is not possible to delete the default workspace.

3.6 List of shortcuts

The keyboard shortcuts in *CLC Sequence Viewer* are listed below.

Action	Windows/Linux	Mac OS X
Adjust selection	Shift + arrow keys	Shift + arrow keys
Change between tabs[1]	Ctrl + tab	Ctrl + Page Up/Down
Close	Ctrl + W	⌘ + W
Close all views	Ctrl + Shift + W	⌘ + Shift + W
Copy	Ctrl + C	⌘ + C
Cut	Ctrl + X	⌘ + X
Delete	Delete	Delete or ⌘ + Backspace
Exit	Alt + F4	⌘ + Q
Export	Ctrl + E	⌘ + E
Export graphics	Ctrl + G	⌘ + G
Find Next Conflict	Space or .	Space or .
Find Previous Conflict	,	,
Help	F1	F1
Import	Ctrl + I	⌘ + I
Maximize/restore size of View	Ctrl + M	⌘ + M
Move gaps in alignment	Ctrl + arrow keys	⌘ + arrow keys
Navigate sequence views	arrow keys	arrow keys
New Folder	Ctrl + Shift + N	⌘ + Shift + N
New Sequence	Ctrl + N	⌘ + N
View	Ctrl + O	⌘ + O
Paste	Ctrl + V	⌘ + V
Print	Ctrl + P	⌘ + P
Redo	Ctrl + Y	⌘ + Y
Rename	F2	F2
Reverse Complement	Ctrl + R	⌘ + R
Save	Ctrl + S	⌘ + S
Search local data	Ctrl + F	⌘ + F
Search within a sequence	Ctrl + Shift + F	⌘ + Shift + F
Search NCBI	Ctrl + B	⌘ + B
Search UniProt	Ctrl + Shift + U	⌘ + Shift + U
Select All	Ctrl + A	⌘ + A
Selection Mode	Ctrl + 2	⌘ + 2
Show/hide Side Panel	Ctrl + U	⌘ + U
Sort folder	Ctrl + Shift + R	⌘ + Shift + R
Split Horizontally	Ctrl + T	⌘ + T
Split Vertically	Ctrl + J	⌘ + J
Translate to Protein	Ctrl + Shift + T	⌘ + Shift + T
Undo	Ctrl + Z	⌘ + Z
User Preferences	Ctrl + K	⌘ + ;
Zoom In Mode	Ctrl + + (plus)	⌘ + 3
Zoom In (without clicking)	+ (plus)	+ (plus)
Zoom Out Mode	Ctrl + - (minus)	⌘ + 4
Zoom Out (without clicking)	- (minus)	- (minus)
Inverse zoom mode	press and hold Shift	press and hold Shift

Combinations of keys and mouse movements are listed below.

[1] On Linux changing tabs is accomplished using Ctrl + Page Up/Page Down

Action	Windows/Linux	Mac OS X	Mouse movement
Maximize View			Double-click the tab of the View
Restore View			Double-click the View title
Reverse zoom function	Shift	Shift	Click in view
Select multiple elements	Ctrl	⌘	Click elements
Select multiple elements	Shift	Shift	Click elements

"Elements" in this context refers to elements and folders in the **Navigation Area** selections on sequences, and rows in tables.

Chapter 4

User preferences and settings

Contents

The first three sections in this chapter deal with the general preferences that can be set for *CLC Sequence Viewer* using the **Preferences** dialog. The next section explains how the settings in the **Side Panel** can be saved and applied to other views. Finally, you can learn how to import and export the preferences.

The **Preferences** dialog offers opportunities for changing the default settings for different features of the program.

The **Preferences** dialog is opened in one of the following ways and can be seen in figure 4.1:

Edit | Preferences ()

or **Ctrl + K (⌘ + ; on Mac)**

4.1 General preferences

The **General** preferences include:

- **Undo Limit.** As default the undo limit is set to 500. By writing a higher number in this field, more actions can be undone. Undo applies to all changes made on sequences, alignments or trees. See section 3.2.5 for more on this topic.

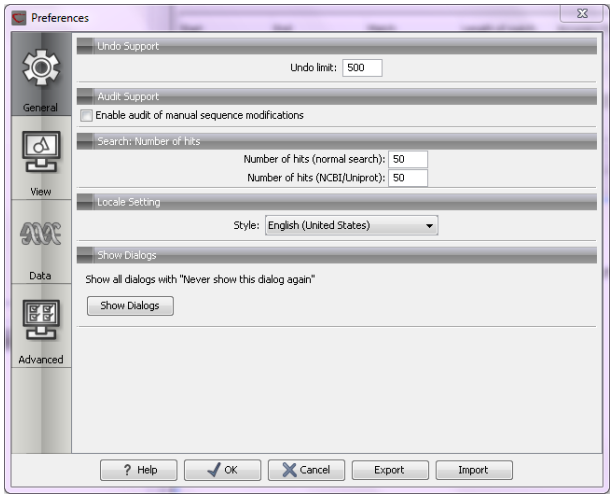

Figure 4.1: *Preferences include General preferences, View preferences, Colors preferences, and Advanced settings.*

- **Audit Support.** If this option is checked, all manual editing of sequences will be marked with an annotation on the sequence (see figure 4.2). Placing the mouse on the annotation will reveal additional details about the change made to the sequence (see figure 4.3). Note that no matter whether **Audit Support** is checked or not, all changes are also recorded in the **History** ( ) (see section 7).
- **Number of hits.** The number of hits shown in *CLC Sequence Viewer*, when e.g. searching NCBI. (The sequences shown in the program are not downloaded, until they are opened or dragged/saved into the Navigation Area.
- **Locale Setting.** Specify which country you are located in. This determines how punctation is used in numbers all over the program.
- **Show Dialogs.** A lot of information dialogs have a checkbox: "Never show this dialog again". When you see a dialog and check this box in the dialog, the dialog will not be shown again. If you regret and wish to have the dialog displayed again, click the button in the General Preferences: **Show Dialogs**. Then all the dialogs will be shown again.

Figure 4.2: *Annotations added when the sequence is edited.*

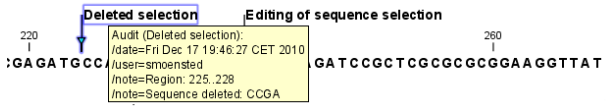

Figure 4.3: *Details of the editing.*

4.2 Default view preferences

There are five groups of default **View** settings:

1. **Toolbar**
2. **Side Panel Location**
3. **New View**
4. **View Format**
5. **User Defined View Settings**.

In general, these are default settings for the user interface.

The **Toolbar preferences** let you choose the size of the toolbar icons, and you can choose whether to display names below the icons.

The **Side Panel Location** setting lets you choose between **Dock in views** and **Float in window**. When docked in view, view preferences will be located in the right side of the view of e.g. an alignment. When floating in window, the side panel can be placed everywhere in your screen, also outside the workspace, e.g. on a different screen. See section 4.5 for more about floating side panels.

The **New view** setting allows you to choose whether the **View preferences** are to be shown automatically when opening a new view. If this option is not chosen, you can press (Ctrl + U (⌘ + U on Mac)) to see the preferences panels of an open view.

The **View Format** allows you to change the way the elements appear in the **Navigation Area**. The following text can be used to describe the element:

- Name (this is the default information to be shown).
- Accession (sequences downloaded from databases like GenBank have an accession number).
- Latin name.
- Latin name (accession).
- Common name.
- Common name (accession).

The **User Defined View Settings** gives you an overview of the different **Side Panel** settings that are saved for each view. See section 4.5 for more about how to create and save style sheets.

If there are other settings beside **CLC Standard Settings**, you can use this overview to choose which of the settings should be used per default when you open a view (see an example in figure 4.4).

In this example, the **CLC Standard Settings** is chosen as default.

4.2.1 Number formatting in tables

In the preferences, you can specify how the numbers should be formatted in tables (see figure 4.5).

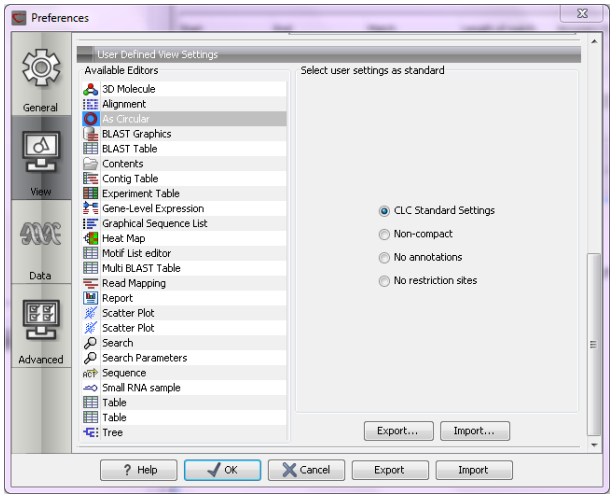

Figure 4.4: *Selecting the default view setting.*

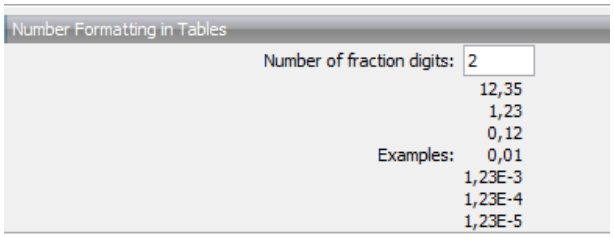

Figure 4.5: *Number formatting of tables.*

The examples below the text field are updated when you change the value so that you can see the effect. After you have changed the preference, you have to re-open your tables to see the effect.

4.2.2 Import and export Side Panel settings

If you have created a special set of settings in the **Side Panel** that you wish to share with other CLC users, you can export the settings in a file. The other user can then import the settings.

To export the **Side Panel** settings, first select the views that you wish to export settings for. Use Ctrl+click (⌘ + click on Mac) or Shift+click to select multiple views. Next click the **Export...**button. Note that there is also another export button at the very bottom of the dialog, but this will export the other settings of the **Preferences** dialog (see section 4.4).

A dialog will be shown (see figure 4.6) that allows you to select which of the settings you wish to export.

When multiple views are selected for export, all the view settings for the views will be shown in the dialog. Click **Export** and you will now be able to define a save folder and name for the exported file. The settings are saved in a file with a .vsf extension (View Settings File).

To import a **Side Panel** settings file, make sure you are at the bottom of the **View** panel of the **Preferences dialog**, and click the **Import...** button. Note that there is also another import button at the very bottom of the dialog, but this will import the other settings of the **Preferences** dialog (see section 4.4).

The dialog asks if you wish to overwrite existing **Side Panel** settings, or if you wish to merge the

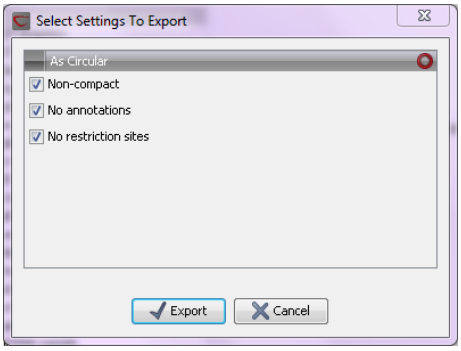

Figure 4.6: *Exporting all settings for circular views.*

imported settings into the existing ones (see figure 4.7).

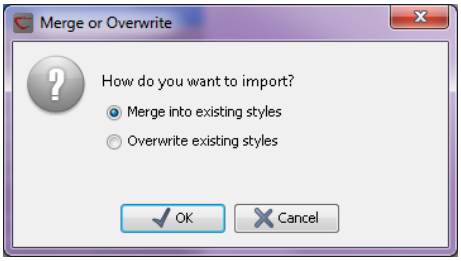

Figure 4.7: *When you import settings, you are asked if you wish to overwrite existing settings or if you wish to merge the new settings into the old ones.*

Note! If you choose to overwrite the existing settings, you will loose all the **Side Panel** settings that you have previously saved.

To avoid confusion of the different import and export options, here is an overview:

- Import and export of **bioinformatics data** such as sequences, alignments etc. (described in section 6.1).
- **Graphics** export of the views which creates image files in various formats (described in section 6.3).
- Import and export of **Side Panel Settings** as described above.
- Import and export of all the **Preferences** except the Side Panel settings. This is described in the previous section.

4.3 Advanced preferences

The **Advanced** settings include the possibility to set up a proxy server. This is described in section 1.7.

4.4 Export/import of preferences

The user preferences of the *CLC Sequence Viewer* can be exported to other users of the program, allowing other users to display data with the same preferences as yours. You can also use the export/import preferences function to backup your preferences.

To export preferences, open the **Preferences** dialog (Ctrl + K (⌘ + ; on Mac)) and do the following:

> **Export** | **Select the relevant preferences** | **Export** | **Choose location for the exported file** | **Enter name of file** | **Save**

Note! The format of exported preferences is .cpf. This notation must be submitted to the name of the exported file in order for the exported file to work.

Before exporting, you are asked about which of the different settings you want to include in the exported file. One of the items in the list is "User Defined View Settings". If you export this, only the information about which of the settings is the default setting for each view is exported. If you wish to export the **Side Panel Settings** themselves, see section 4.2.2.

The process of importing preferences is similar to exporting:

> **Press Ctrl + K (⌘ + ; on Mac) to open Preferences** | **Import** | **Browse to and select the .cpf file** | **Import and apply preferences**

4.4.1 The different options for export and importing

To avoid confusion of the different import and export options, here is an overview:

- Import and export of **bioinformatics data** such as sequences, alignments etc. (described in section 6.1).
- **Graphics** export of the views which creates image files in various formats (described in section 6.3).
- Import and export of **Side Panel Settings** as described in the next section.
- Import and export of all the **Preferences** except the Side Panel settings. This is described above.

4.5 View settings for the Side Panel

The **Side Panel** is shown to the right of all views that are opened in *CLC Sequence Viewer*. By using the settings in the **Side Panel** you can specify how the layout and contents of the view. Figure 4.8 is an example of the **Side Panel** of a sequence view.

By clicking the black triangles or the corresponding headings, the groups can be expanded or collapsed. An example is shown in figure 4.9 where the **Sequence layout** is expanded.

The content of the groups is described in the sections where the functionality is explained. E.g. **Sequence Layout** for sequences is described in chapter 9.1.1.

When you have adjusted a view of e.g. a sequence, your settings in the **Side Panel** can be saved. When you open other sequences, which you want to display in a similar way, the saved settings can be applied. The options for saving and applying are available in the top of the **Side Panel** (see figure 4.10).

To save and apply the saved settings, click ( ) seen in figure 4.10. This opens a menu, where the following options are available:

Figure 4.8: *The Side Panel of a sequence contains several groups: Sequence layout, Annotation types, Annotation layout, etc. Several of these groups are present in more views. E.g. Sequence layout is also in the Side Panel of alignment views.*

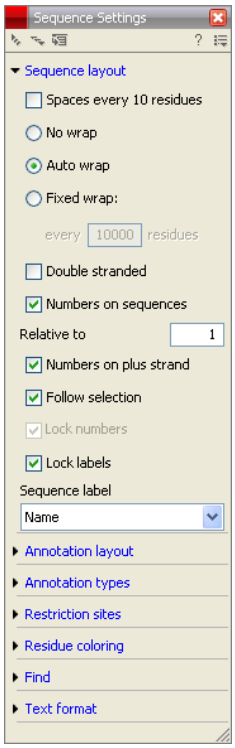

Figure 4.9: *The Sequence layout is expanded.*

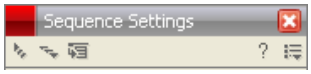

Figure 4.10: *At the top of the Side Panel you can: Expand all groups, Collapse all preferences, Dock/Undock preferences, Help, and Save/Restore preferences.*

- **Save Settings.** This brings up a dialog as shown in figure 4.11 where you can enter a name for your settings. Furthermore, by clicking the checkbox **Always apply these settings**, you can choose to use these settings every time you open a new view of this type. If you wish to change which settings should be used per default, open the **Preferences** dialog (see section 4.2).

- **Delete Settings.** Opens a dialog to select which of the saved settings to delete.
- **Apply Saved Settings.** This is a submenu containing the settings that you have previously saved. By clicking one of the settings, they will be applied to the current view. You will also see a number of pre-defined view settings in this submenu. They are meant to be examples of how to use the **Side Panel** and provide quick ways of adjusting the view to common usages. At the bottom of the list of settings you will see **CLC Standard Settings** which represent the way the program was set up, when you first launched it.

Figure 4.11: *The save settings dialog.*

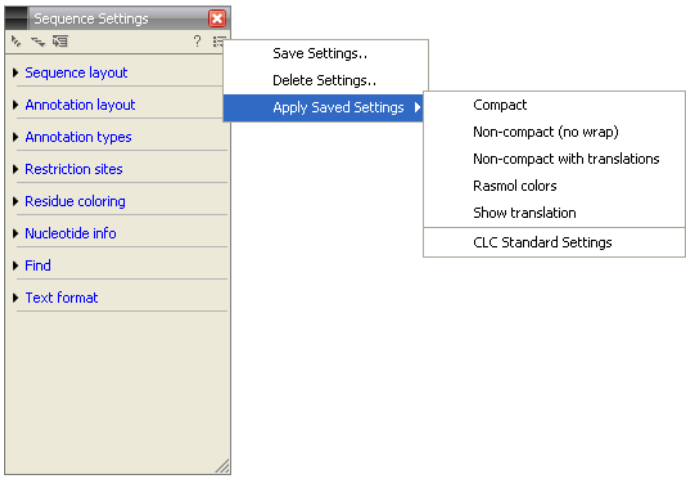

Figure 4.12: *Applying saved settings.*

The settings are specific to the type of view. Hence, when you save settings of a circular view, they will not be available if you open the sequence in a linear view.

If you wish to export the settings that you have saved, this can be done in the **Preferences** dialog under the **View** tab (see section 4.2.2).

The remaining icons of figure 4.10 are used to; **Expand all groups**, **Collapse all groups**, and **Dock/Undock Side Panel**. **Dock/Undock Side Panel** is to make the **Side Panel** "floating" (see below).

4.5.1 Floating Side Panel

The Side Panel of the views can be placed in the right side of a view, or it can be floating (see figure 4.13).

By clicking the Dock icon ( ) the floating Side Panel reappear in the right side of the view. The size of the floating Side Panel can be adjusted by dragging the hatched area in the bottom right.

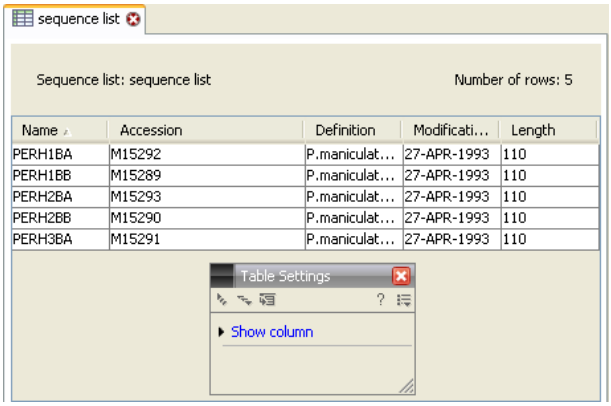

Figure 4.13: *The floating Side Panel can be moved out of the way, e.g. to allow for a wider view of a table.*

Chapter 5

Printing

Contents

CLC Sequence Viewer offers different choices of printing the result of your work.

This chapter deals with printing directly from *CLC Sequence Viewer*. Another option for using the graphical output of your work, is to export graphics (see chapter 6.3) in a graphic format, and then import it into a document or a presentation.

All the kinds of data that you can view in the **View Area** can be printed. The *CLC Sequence Viewer* uses a WYSIWYG principle: What You See Is What You Get. This means that you should use the options in the Side Panel to change how your data, e.g. a sequence, looks on the screen. When you print it, it will look exactly the same way on print as on the screen.

For some of the views, the layout will be slightly changed in order to be printer-friendly.

It is not possible to print elements directly from the **Navigation Area**. They must first be opened in a view in order to be printed. To print the contents of a view:

select relevant view | Print ( ) in the toolbar

This will show a print dialog (see figure 5.1).

In this dialog, you can:

- Select which part of the view you want to print.
- Adjust **Page Setup**.
- See a print **Preview** window.

These three options are described in the three following sections.

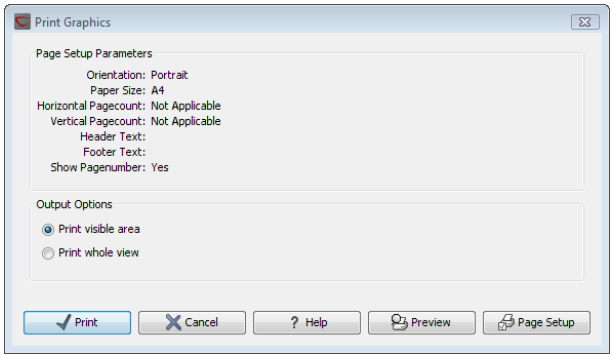

Figure 5.1: *The Print dialog.*

5.1 Selecting which part of the view to print

In the print dialog you can choose to:

- **Print visible area**, or
- **Print whole view**

These options are available for all views that can be zoomed in and out. In figure 5.2 is a view of a circular sequence which is zoomed in so that you can only see a part of it.

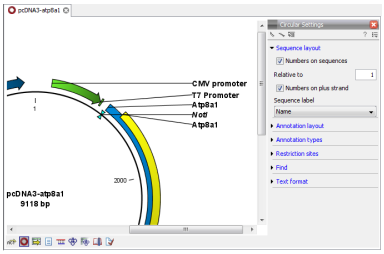

Figure 5.2: *A circular sequence as it looks on the screen.*

When selecting **Print visible area**, your print will reflect the part of the sequence that is *visible* in the view. The result from printing the view from figure 5.2 and choosing **Print visible area** can be seen in figure 5.3.

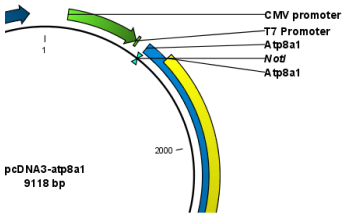

Figure 5.3: *A print of the sequence selecting Print visible area.*

On the other hand, if you select **Print whole view**, you will get a result that looks like figure 5.4. This means that you also print the part of the sequence which is not visible when you have zoomed in.

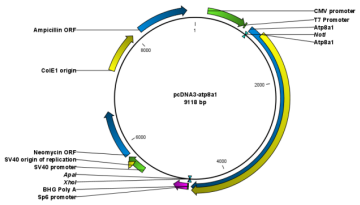

Figure 5.4: *A print of the sequence selecting Print whole view. The whole sequence is shown, even though the view is zoomed in on a part of the sequence.*

5.2 Page setup

No matter whether you have chosen to print the visible area or the whole view, you can adjust page setup of the print. An example of this can be seen in figure 5.5

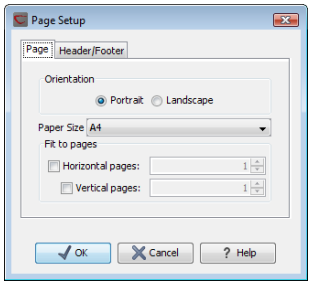

Figure 5.5: *Page Setup.*

In this dialog you can adjust both the setup of the pages and specify a header and a footer by clicking the tab at the top of the dialog.

You can modify the layout of the page using the following options:

- **Orientation**.
  - **Portrait**. Will print with the paper oriented vertically.
  - **Landscape**. Will print with the paper oriented horizontally.
- **Paper size**. Adjust the size to match the paper in your printer.
- **Fit to pages**. Can be used to control how the graphics should be split across pages (see figure 5.6 for an example).
  - **Horizontal pages**. If you set the value to e.g. 2, the printed content will be broken up horizontally and split across 2 pages. This is useful for sequences that are not wrapped
  - **Vertical pages**. If you set the value to e.g. 2, the printed content will be broken up vertically and split across 2 pages.

Note! It is a good idea to consider adjusting view settings (e.g. **Wrap** for sequences), in the **Side Panel** before printing. As explained in the beginning of this chapter, the printed material will look like the view on the screen, and therefore these settings should also be considered when adjusting **Page Setup**.

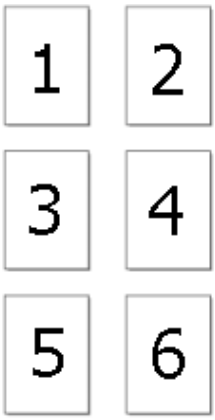

Figure 5.6: *An example where Fit to pages horizontally is set to 2, and Fit to pages vertically is set to 3.*

5.2.1 Header and footer

Click the **Header/Footer** tab to edit the header and footer text. By clicking in the text field for either **Custom header text** or **Custom footer text** you can access the auto formats for header/footer text in **Insert a caret position**. Click either **Date**, **View name**, or **User name** to include the auto format in the header/footer text.

Click **OK** when you have adjusted the **Page Setup**. The settings are saved so that you do not have to adjust them again next time you print. You can also change the **Page Setup** from the **File** menu.

5.3 Print preview

The preview is shown in figure 5.7.

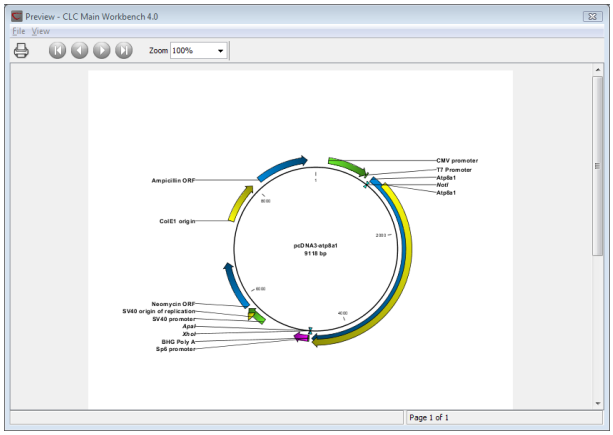

Figure 5.7: *Print preview.*

The **Print preview** window lets you see the layout of the pages that are printed. Use the arrows in the toolbar to navigate between the pages. Click Print ( ) to show the print dialog, which lets you choose e.g. which pages to print.

The **Print preview** window is for preview only - the layout of the pages must be adjusted in the **Page setup**.

Chapter 6

Import/export of data and graphics

Contents

CLC Sequence Viewer handles a large number of different data formats. In order to work with data in the Workbench, it has to be imported (). Data types that are not recognized by the Workbench are imported as "external files" which means that when you open these, they will open in the default application for that file type on your computer (e.g. Word documents will open in Word).

This chapter first deals with importing and exporting data in bioinformatic data formats and as external files. Next comes an explanation of how to export graph data points to a file, and how export graphics.

6.1 Standard import

CLC Sequence Viewer has support for a wide range of bioinformatic data such as sequences, alignments etc. See a full list of the data formats in section D.1.

These data can be imported through the Import dialog, using drag/drop or copy/paste as explained below.

Import using the import dialog

To start the import using the import dialog:

click Import () in the Toolbar

This will show a dialog similar to figure 6.1. You can change which kind of file types that should be shown by selecting a file format in the **Files of type** box.

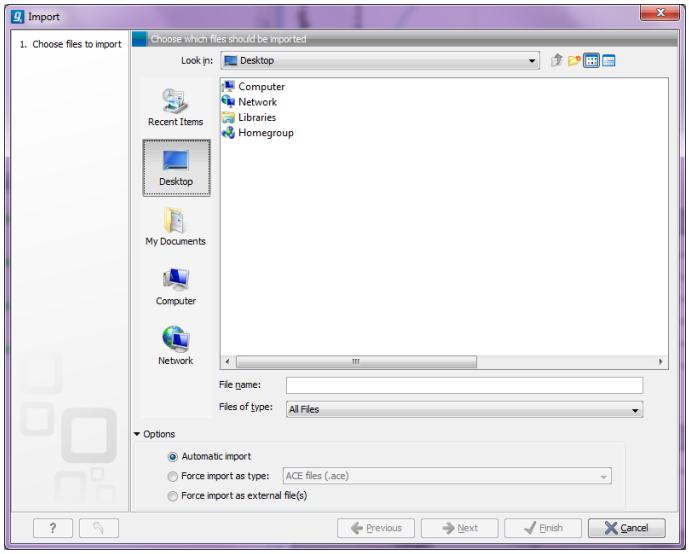

Figure 6.1: *The import dialog.*

Next, select one or more files or folders to import and click **Next**.

This allows you to select a place for saving the result files.

If you import one or more folders, the contents of the folder is automatically imported and placed in that folder in the **Navigation Area**. If the folder contains subfolders, the whole folder structure is imported.

In the import dialog (figure 6.1), there are three import options:

Automatic import This will import the file and *CLC Sequence Viewer* will try to determine the format of the file. The format is determined based on the file extension (e.g. SwissProt files have .swp at the end of the file name) in combination with a detection of elements in the file that are specific to the individual file formats. If the file type is not recognized, it will be imported as an external file. In most cases, automatic import will yield a successful result, but if the import goes wrong, the next option can be helpful:

Force import as type This option should be used if *CLC Sequence Viewer* cannot successfully determine the file format. By forcing the import as a specific type, the automatic determination of the file format is bypassed, and the file is imported as the type specified.

Force import as external file This option should be used if a file is imported as a bioinformatics file when it should just have been external file. It could be an ordinary text file which is imported as a sequence.

Import using drag and drop

It is also possible to drag a file from e.g. the desktop into the **Navigation Area** of *CLC Sequence Viewer*. This is equivalent to importing the file using the **Automatic import** option described above. If the file type is not recognized, it will be imported as an external file.

Import using copy/paste of text

If you have e.g. a text file or a browser displaying a sequence in one of the formats that can be imported by *CLC Sequence Viewer*, there is a very easy way to get this sequence into the **Navigation Area**:

> **Copy the text from the text file or browser** | **Select a folder in the Navigation Area** | **Paste ()**

This will create a new sequence based on the text copied. This operation is equivalent to saving the text in a text file and importing it into the *CLC Sequence Viewer*.

If the sequence is not formatted, i.e. if you just have a text like this: "ATGACGAATAGGAGTTC-TAGCTA" you can also paste this into the **Navigation Area**.

Note! Make sure you copy all the relevant text - otherwise *CLC Sequence Viewer* might not be able to interpret the text.

6.1.1 External files

In order to help you organize your research projects, *CLC Sequence Viewer* lets you import all kinds of files. E.g. if you have Word, Excel or pdf-files related to your project, you can import them into the **Navigation Area** of *CLC Sequence Viewer*. Importing an external file creates a copy of the file which is stored at the location you have chosen for import. The file can now be opened by double-clicking the file in the **Navigation Area**. The file is opened using the default application for this file type (e.g. Microsoft Word for .doc-files and Adobe Reader for .pdf).

External files are imported and exported in the same way as bioinformatics files (see section 6.1). Bioinformatics files not recognized by *CLC Sequence Viewer* are also treated as external files.

6.1.2 Import Vector NTI data

There are several ways of importing your Vector NTI data into the CLC Workbench. The best way to go depends on how your data is currently stored in Vector NTI:

- Your data is stored in the Vector NTI Local Database which can be accessed through Vector NTI Explorer. This is described in the first section below.
- Your data is stored as single files on your computer (just like Word documents etc.). This is described in the second section below.

Import from the Vector NTI Local Database

If your Vector NTI data are stored in a Vector NTI Local Database (as the one shown in figure 6.2), you can import all the data in one step, or you can import selected parts of it.

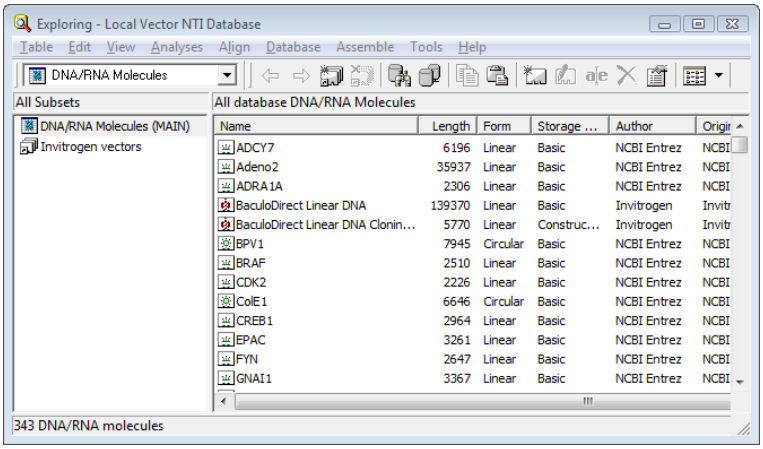

Figure 6.2: *Data stored in the Vector NTI Local Database accessed through Vector NTI Explorer.*

Importing the entire database in one step

From the Workbench, there is a direct import of the whole database (see figure 6.3):

File | Import Vector NTI Database

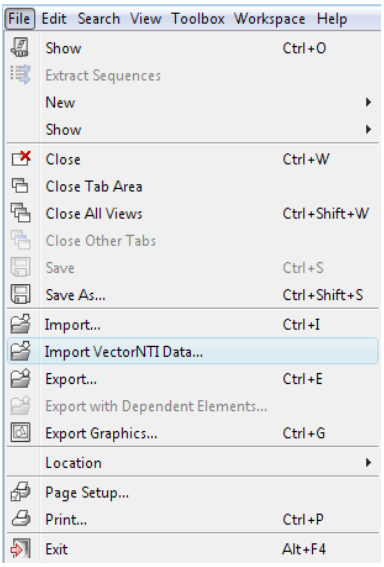

Figure 6.3: *Import the whole Vector NTI Database.*

This will bring up a dialog letting you choose to import from the default location of the database, or you can specify another location. If the database is installed in the default folder, like e.g. *C:\VNTI Database*, press **Yes**. If not, click **No** and specify the database folder manually.

When the import has finished, the data will be listed in the **Navigation Area** of the Workbench as shown in figure 6.4.

If something goes wrong during the import process, please report the problem to support@clcbio.com. To circumvent the problem, see the following section on how to import parts of the database. It will take a few more steps, but you will most likely be able to import this way.

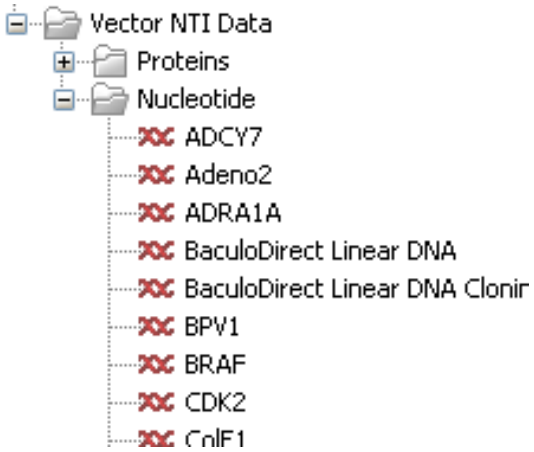

Figure 6.4: *The Vector NTI Data folder containing all imported sequences of the Vector NTI Database.*

Importing parts of the database

Instead of importing the whole database automatically, you can export parts of the database from Vector NTI Explorer and subsequently import into the Workbench. First, export a selection of files as an archive as shown in figure 6.5.

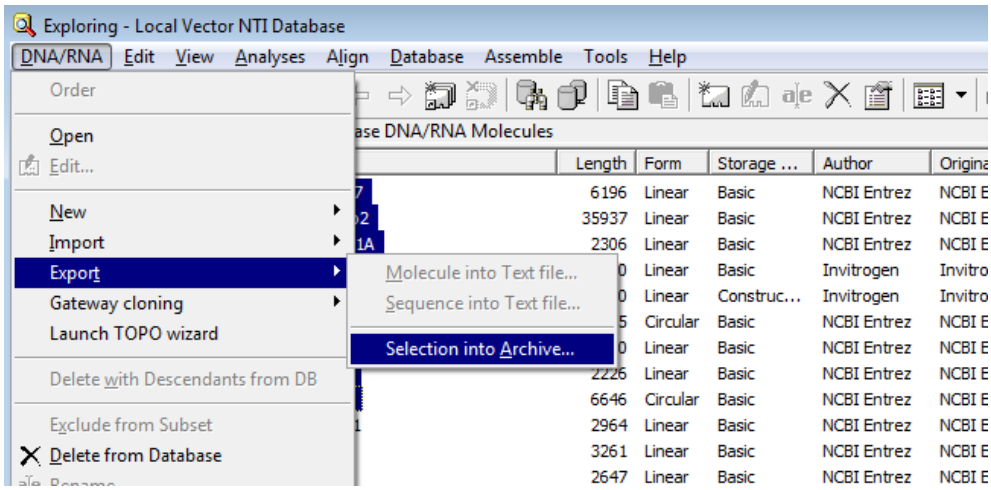

Figure 6.5: *Select the relevant files and export them as an archive through the File menu.*

This will produce a file with a ma4-, pa4- or oa4-extension. Back in the CLC Workbench, click **Import** ( ) and select the file.

Importing single files

In Vector NTI, you can save a sequence in a file instead of in the database (see figure 6.6).

This will give you file with a .gb extension. This file can be easily imported into the CLC Workbench:

Import ( ) | select the file | Select

You don't have to import one file at a time. You can simply select a bunch of files or an entire folder, and the CLC Workbench will take care of the rest. Even if the files are in different formats.

You can also simply drag and drop the files into the **Navigation Area** of the CLC Workbench.

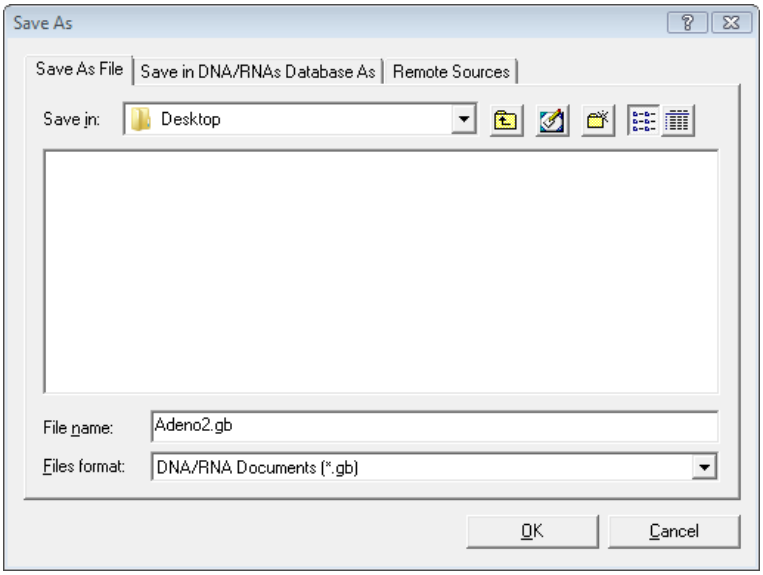

Figure 6.6: *Saving a sequence as a file in Vector NTI.*

The Vector NTI import is a plug-in which is pre-installed in the Workbench. It can be uninstalled and updated using the plug-in manager (see section 1.6).

6.2 Data export

CLC Sequence Viewer can export bioinformatic data in most of the formats that can be imported. There are a few exceptions. See section 6.1.

To export a file:

> **select the element to export** | **Export** () | **choose where to export to** | **select 'File of type'** | **enter name of file** | **Save**

When exporting to CSV and tab delimited files, decimal numbers are formatted according to the Locale setting of the Workbench (see section 4.1). If you open the CSV or tab delimited file with spreadsheet software like Excel, you should make sure that both the Workbench and the spreadsheet software are using the same Locale.

Note! The **Export** dialog decides which types of files you are allowed to export into, depending on what type of data you want to export. E.g. protein sequences can be exported into GenBank, Fasta, Swiss-Prot and CLC-formats.

Export of folders and multiple elements

The .zip file type can be used to export all kinds of files and is therefore especially useful in these situations:

- Export of one or more folders including all underlying elements and folders.
- If you want to export two or more elements into one file.

Export of folders is similar to export of single files. Exporting multiple files (of different formats) is done in .zip-format. This is how you export a folder:

select the folder to export | **Export ()** | **choose where to export to** | **enter name** | **Save**

You can export multiple files of the same type into formats other than ZIP (.zip). E.g. two DNA sequences can be exported in GenBank format:

select the two sequences by <Ctrl>-click (⌘-click on Mac) or <Shift>-click | **Export ()** | **choose where to export to** | **choose GenBank (.gbk) format** | **enter name the new file** | **Save**

Export of dependent elements

When exporting e.g. an alignment, *CLC Sequence Viewer* can export the alignment including all the sequences that were used to create it. This way, when sending your alignment (with the dependent sequences), your colleagues can reproduce your findings with adjusted parameters, if desired. To export with dependent files:

select the element in Navigation Area | **File in Menu Bar** | **Export with Dependent Elements** | **enter name of of the new file** | **choose where to export to** | **Save**

The result is a folder containing the exported file with dependent elements, stored automatically in a folder on the desired location of your desk.

Export history

To export an element's history:

select the element in Navigation Area Export () | **select History PDF(.pdf)** | **choose where to export to** | **Save**

The entire history of the element is then exported in pdf format.

The CLC format

CLC Sequence Viewer keeps all bioinformatic data in the CLC format. Compared to other formats, the CLC format contains more information about the object, like its history and comments. The CLC format is also able to hold several elements of different types (e.g. an alignment, a graph and a phylogenetic tree). This means that if you are exporting your data to another CLC Workbench, you can use the CLC format to export several elements in one file, and you will preserve all the information.

Note! CLC files can be exported from and imported into all the different CLC Workbenches.

Backup

If you wish to secure your data from computer breakdowns, it is advisable to perform regular backups of your data. Backing up data in the *CLC Sequence Viewer* is done in two ways:

- Making a backup of each of the folders represented by the locations in the **Navigation Area**.
- Selecting all locations in the **Navigation Area** and export () in .zip format. The resulting file will contain all the data stored in the **Navigation Area** and can be imported into *CLC*

Sequence Viewer if you wish to restore from the back-up at some point.

No matter which method is used for backup, you may have to re-define the locations in the **Navigation Area** if you restore your data from a computer breakdown.

6.3 Export graphics to files

CLC Sequence Viewer supports export of graphics into a number of formats. This way, the visible output of your work can easily be saved and used in presentations, reports etc. The **Export Graphics** function ( ) is found in the **Toolbar**.

CLC Sequence Viewer uses a WYSIWYG principle for graphics export: What You See Is What You Get. This means that you should use the options in the Side Panel to change how your data, e.g. a sequence, looks in the program. When you export it, the graphics file will look exactly the same way.

It is not possible to export graphics of elements directly from the **Navigation Area**. They must first be opened in a view in order to be exported. To export graphics of the contents of a view:

select tab of View | Graphics ( ) on Toolbar

This will display the dialog shown in figure 6.7.

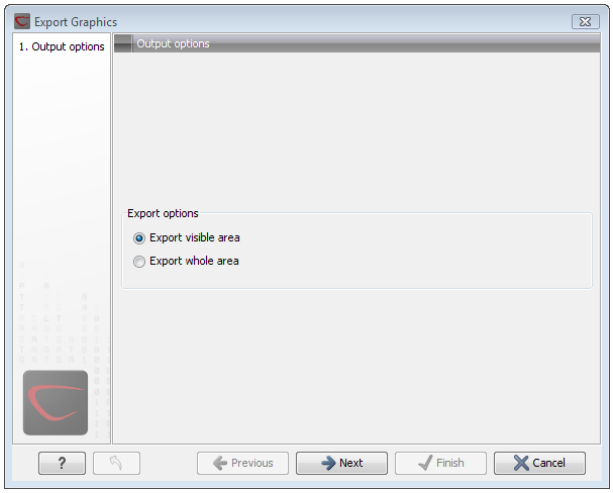

Figure 6.7: *Selecting to export whole view or to export only the visible area.*

6.3.1 Which part of the view to export

In this dialog you can choose to:

- **Export visible area**, or
- **Export whole view**

These options are available for all views that can be zoomed in and out. In figure 6.8 is a view of a circular sequence which is zoomed in so that you can only see a part of it.

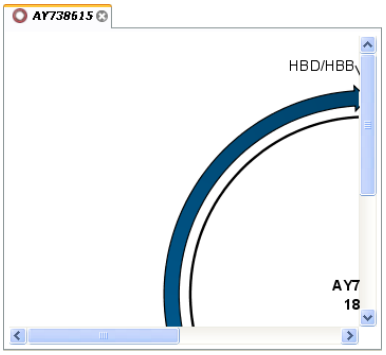

Figure 6.8: *A circular sequence as it looks on the screen.*

When selecting **Export visible area**, the exported file will only contain the part of the sequence that is *visible* in the view. The result from exporting the view from figure 6.8 and choosing **Export visible area** can be seen in figure 6.9.

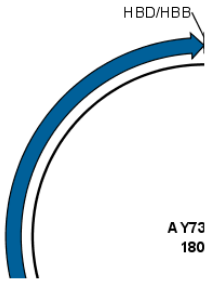

Figure 6.9: *The exported graphics file when selecting Export visible area.*

On the other hand, if you select **Export whole view**, you will get a result that looks like figure 6.10. This means that the graphics file will also include the part of the sequence which is not visible when you have zoomed in.

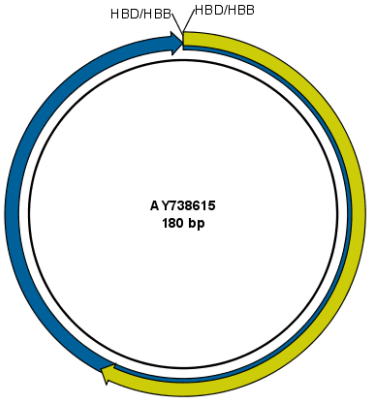

Figure 6.10: *The exported graphics file when selecting Export whole view. The whole sequence is shown, even though the view is zoomed in on a part of the sequence.*

Click **Next** when you have chosen which part of the view to export.

6.3.2 Save location and file formats

In this step, you can choose name and save location for the graphics file (see figure 6.11).

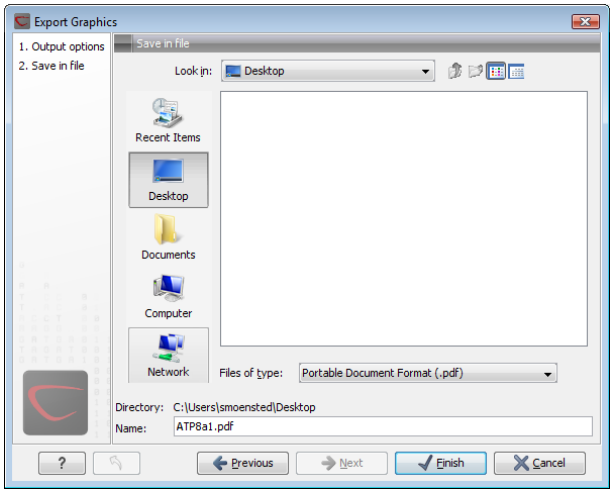

Figure 6.11: *Location and name for the graphics file.*

CLC Sequence Viewer supports the following file formats for graphics export:

Format	Suffix	Type
Portable Network Graphics	.png	bitmap
JPEG	.jpg	bitmap
Tagged Image File	.tif	bitmap
PostScript	.ps	vector graphics
Encapsulated PostScript	.eps	vector graphics
Portable Document Format	.pdf	vector graphics
Scalable Vector Graphics	.svg	vector graphics

These formats can be divided into bitmap and vector graphics. The difference between these two categories is described below:

Bitmap images

In a bitmap image, each dot in the image has a specified color. This implies, that if you zoom in on the image there will not be enough dots, and if you zoom out there will be too many. In these cases the image viewer has to interpolate the colors to fit what is actually looked at. A bitmap image needs to have a high resolution if you want to zoom in. This format is a good choice for storing images without large shapes (e.g. dot plots). It is also appropriate if you don't have the need for resizing and editing the image after export.

Vector graphics

Vector graphic is a collection of shapes. Thus what is stored is e.g. information about where a line starts and ends, and the color of the line and its width. This enables a given viewer to decide how to draw the line, no matter what the zoom factor is, thereby always giving a correct image. This format is good for e.g. graphs and reports, but less usable for e.g. dot plots. If the image is to be resized or edited, vector graphics are by far the best format to store graphics. If you open

a vector graphics file in an application like e.g. Adobe Illustrator, you will be able to manipulate the image in great detail.

Graphics files can also be imported into the **Navigation Area**. However, no kinds of graphics files can be displayed in *CLC Sequence Viewer*. See section 6.1.1 for more about importing external files into *CLC Sequence Viewer*.

6.3.3 Graphics export parameters

When you have specified the name and location to save the graphics file, you can either click **Next** or **Finish**. Clicking **Next** allows you to set further parameters for the graphics export, whereas clicking **Finish** will export using the parameters that you have set last time you made a graphics export in that file format (if it is the first time, it will use default parameters).

Parameters for bitmap formats

For bitmap files, clicking **Next** will display the dialog shown in figure 6.12.

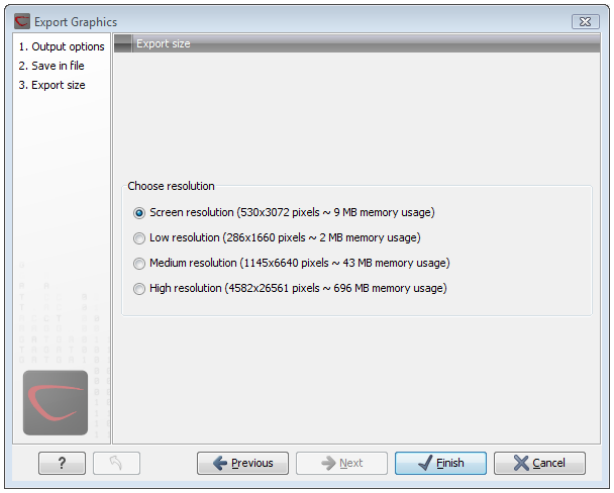

Figure 6.12: *Parameters for bitmap formats: size of the graphics file.*

You can adjust the size (the resolution) of the file to four standard sizes:

- Screen resolution
- Low resolution
- Medium resolution
- High resolution

The actual size in pixels is displayed in parentheses. An estimate of the memory usage for exporting the file is also shown. If the image is to be used on computer screens only, a low resolution is sufficient. If the image is going to be used on printed material, a higher resolution is necessary to produce a good result.

Parameters for vector formats

For pdf format, clicking **Next** will display the dialog shown in figure 6.13 (this is only the case if the graphics is using more than one page).

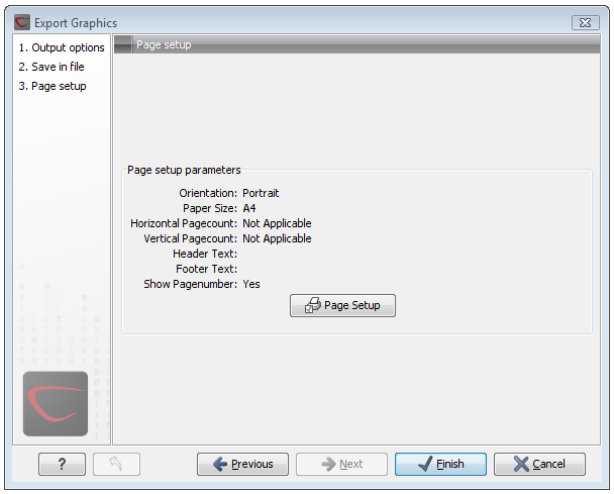

Figure 6.13: *Page setup parameters for vector formats.*

The settings for the page setup are shown, and clicking the **Page Setup** button will display a dialog where these settings can ba adjusted. This dialog is described in section 5.2.

The page setup is only available if you have selected to export the whole view - if you have chosen to export the visible area only, the graphics file will be on one page with no headers or footers.

6.3.4 Exporting protein reports

It is possible to export a protein report using the normal **Export** function ( ) which will generate a pdf file with a table of contents:

Click the report in the Navigation Area | Export ( ) in the Toolbar | select pdf

You can also choose to export a protein report using the **Export graphics** function ( ), but in this way you will not get the table of contents.

6.4 Export graph data points to a file

Data points for graphs displayed along the sequence or along an alignment, mapping or BLAST result, can be exported to a semicolon-separated text file (csv format). An example of such a graph is shown in figure 6.14. This graph shows the coverage of reads of a read mapping (produced with *CLC Genomics Workbench*).

To export the data points for the graph, right-click the graph and choose **Export Graph to Comma-separated File**. Depending on what kind of graph you have selected, different options will be shown: If the graph is covering a set of aligned sequences with a main sequence, such as read mappings and BLAST results, the dialog shown in figure 6.15 will be displayed. These kinds of graphs are located under **Alignment info** in the Side Panel. In all other cases, a normal file dialog will be shown letting you specify name and location for the file.

In this dialog, select whether you wish to include positions where the main sequence (the

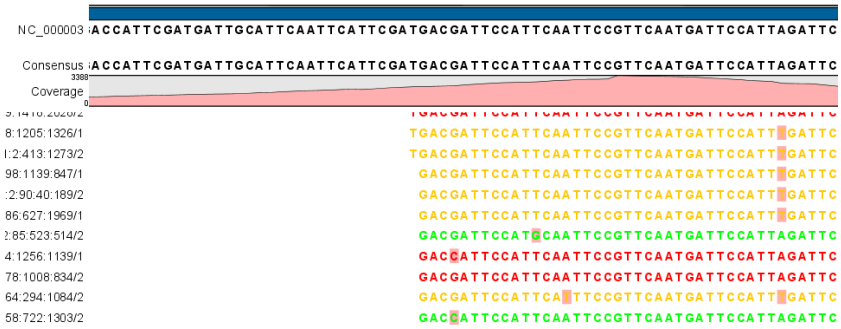

Figure 6.14: *A graph displayed along the mapped reads. Right-click the graph to export the data points to a file.*

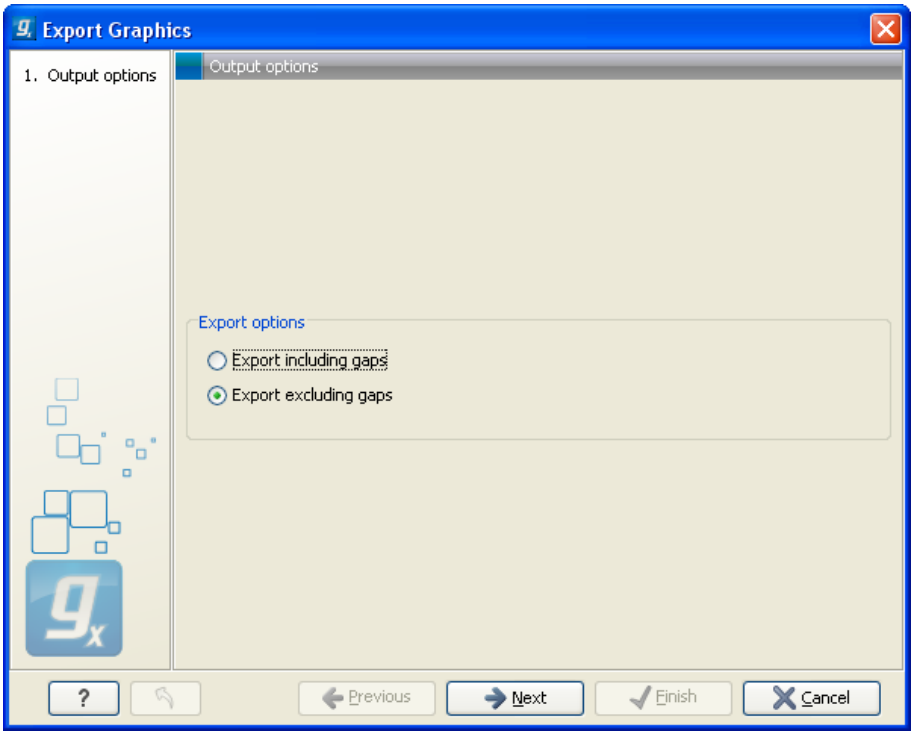

Figure 6.15: *Choosing to include data points with gaps*

reference sequence for read mappings and the query sequence for BLAST results) has gaps. If you are exporting e.g. coverage information from a read mapping, you would probably want to exclude gaps, if you want the positions in the exported file to match the reference (i.e. chromosome) coordinates. If you export including gaps, the data points in the file no longer corresponds to the reference coordinates, because each gap will shift the coordinates.

Clicking **Next** will present a file dialog letting you specify name and location for the file.

The output format of the file is like this:

```
"Position";"Value";
"1";"13";
"2";"16";
"3";"23";
"4";"17";
```

...

6.5 Copy/paste view output

The content of tables, e.g. in reports, folder lists, and sequence lists can be copy/pasted into different programs, where it can be edited. *CLC Sequence Viewer* pastes the data in tabulator separated format which is useful if you use programs like Microsoft Word and Excel. There is a huge number of programs in which the copy/paste can be applied. For simplicity, we include one example of the copy/paste function from a **Folder Content** view to Microsoft Excel.

First step is to select the desired elements in the view:

> **click a line in the Folder Content view** | **hold Shift-button** | **press arrow down/up key**

See figure 6.16.

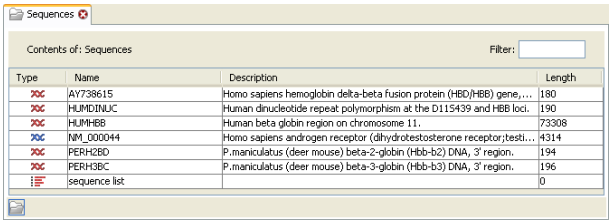

Figure 6.16: *Selected elements in a Folder Content view.*

When the elements are selected, do the following to copy the selected elements:

> **right-click one of the selected elements** | **Edit** | **Copy (** **)**

Then:

> **right-click in the cell A1** | **Paste (** **)**

The outcome might appear unorganized, but with a few operations the structure of the view in *CLC Sequence Viewer* can be produced. (Except the icons which are replaced by file references in Excel.)

Note that all tables can also be **Exported** ( ) directly in Excel format.

Chapter 7

History log

Contents

CLC Sequence Viewer keeps a log of all operations you make in the program. If e.g. you rename a sequence, align sequences, create a phylogenetic tree or translate a sequence, you can always go back and check what you have done. In this way, you are able to document and reproduce previous operations.

This can be useful in several situations: It can be used for documentation purposes, where you can specify exactly how your data has been created and modified. It can also be useful if you return to a project after some time and want to refresh your memory on how the data was created. Also, if you have performed an analysis and you want to reproduce the analysis on another element, you can check the history of the analysis which will give you all parameters you set.

This chapter will describe how to use the **History** functionality of *CLC Sequence Viewer*.

7.1 Element history

You can view the history of all elements in the **Navigation Area** except files that are opened in other programs (e.g. Word and pdf-files). The history starts when the element appears for the first time in *CLC Sequence Viewer*. To view the history of an element:

Select the element in the Navigation Area | **Show ( ) in the Toolbar** | **History ( )**

or **If the element is already open** | **History ( ) at the bottom left part of the view**

This opens a view that looks like the one in figure 7.1.

When opening an element's history is opened, the newest change is submitted in the top of the view. The following information is available:

- **Title**. The action that the user performed.
- **Date and time**. Date and time for the operation. The date and time are displayed according

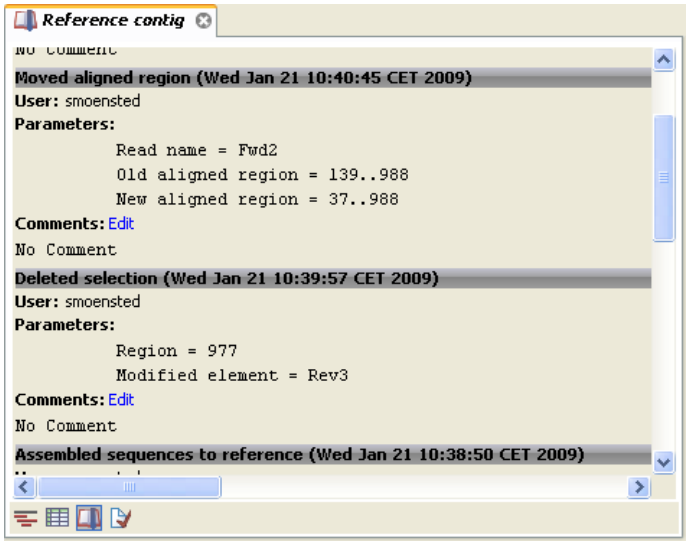

Figure 7.1: *An element's history.*

to your locale settings (see section 4.1).

- **User**. The user who performed the operation. If you import some data created by another person in a CLC Workbench, that persons name will be shown.
- **Parameters**. Details about the action performed. This could be the parameters that was chosen for an analysis.
- **Origins from**. This information is usually shown at the bottom of an element's history. Here, you can see which elements the current element origins from. If you have e.g. created an alignment of three sequences, the three sequences are shown here. Clicking the element selects it in the **Navigation Area**, and clicking the 'history' link opens the element's own history.
- **Comments**. By clicking **Edit** you can enter your own comments regarding this entry in the history. These comments are saved.

7.1.1 Sharing data with history

The history of an element is attached to that element, which means that exporting an element in CLC format (*.clc) will export the history too. In this way, you can share folders and files with others while preserving the history. If an element's history includes source elements (i.e. if there are elements listed in 'Origins from'), they must also be exported in order to see the full history. Otherwise, the history will have entries named "Element deleted". An easy way to export an element with all its source elements is to use the **Export Dependent Elements** function described in section 6.2.

The history view can be printed. To do so, click the **Print** icon ( ). The history can also be exported as a pdf file:

Select the element in the Navigation Area | Export ( ) | in "File of type" choose History PDF | Save

Chapter 8

Batching and result handling

Contents

8.1 How to handle results of analyses

This section will explain how results generated from tools in the Toolbox are handled by *CLC Sequence Viewer*. Note that this also applies to tools not running in batch mode (see above). All the analyses in the **Toolbox** are performed in a step-by-step procedure. First, you select elements for analyses, and then there are a number of steps where you can specify parameters (some of the analyses have no parameters, e.g. when translating DNA to RNA). The final step concerns the handling of the results of the analysis, and it is almost identical for all the analyses so we explain it in this section in general.

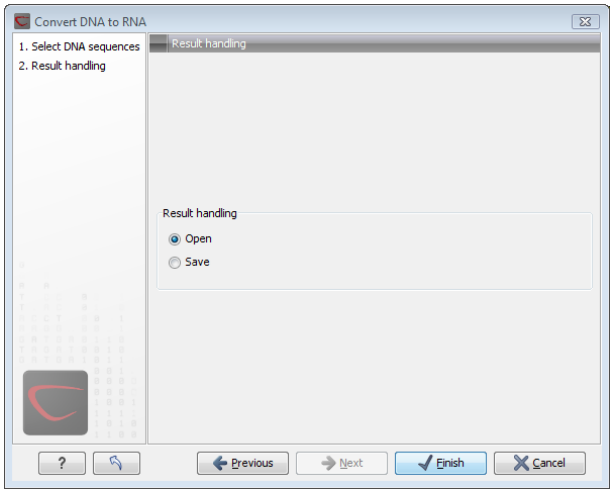

Figure 8.1: *The last step of the analyses exemplified by Translate DNA to RNA.*

In this step, shown in figure 8.1, you have two options:

- **Open.** This will open the result of the analysis in a view. This is the default setting.
- **Save.** This means that the result will not be opened but saved to a folder in the **Navigation Area**. If you select this option, click **Next** and you will see one more step where you can specify where to save the results (see figure 8.2). In this step, you also have the option of creating a new folder or adding a location by clicking the buttons ( )/ ( ) at the top of the dialog.

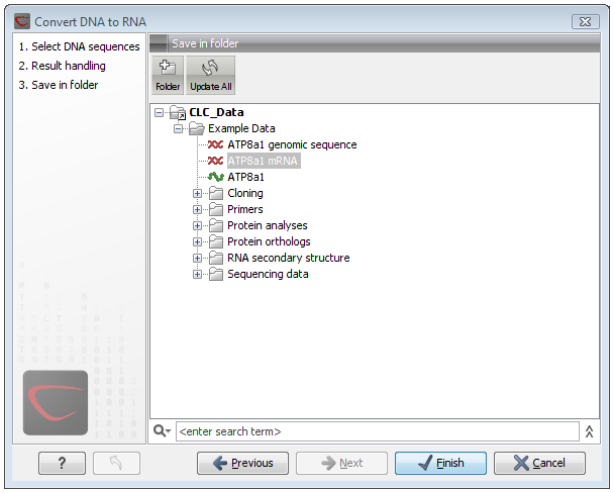

Figure 8.2: *Specify a folder for the results of the analysis.*

8.1.1 Table outputs

Some analyses also generate a table with results, and for these analyses the last step looks like figure 8.3.

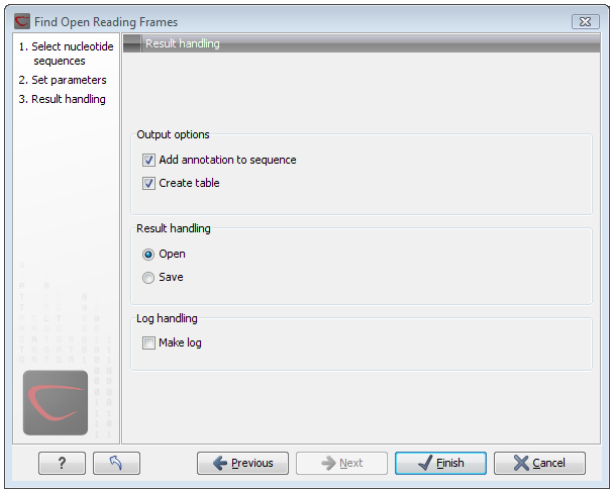

Figure 8.3: *Analyses which also generate tables.*

In addition to the **Open** and **Save** options you can also choose whether the result of the analysis should be added as annotations on the sequence or shown on a table. If both options are selected, you will be able to click the results in the table and the corresponding region on the sequence will be selected.

If you choose to add annotations to the sequence, they can be removed afterwards by clicking **Undo** ( ) in the **Toolbar**.

8.1.2 Batch log

For some analyses, there is an extra option in the final step to create a log of the batch process (see e.g. figure 8.3). This log will be created in the beginning of the process and continually updated with information about the results. See an example of a log in figure 8.4. In this example, the log displays information about how many open reading frames were found.

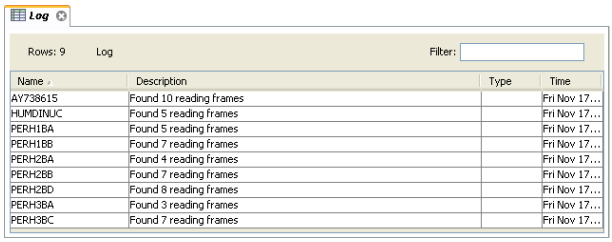

Figure 8.4: *An example of a batch log when finding open reading frames.*

The log will either be saved with the results of the analysis or opened in a view with the results, depending on how you chose to handle the results.

Part III

Bioinformatics

Chapter 9

Viewing and editing sequences

Contents

CLC Sequence Viewer offers five different ways of viewing and editing single sequences as described in the first five sections of this chapter. Furthermore, this chapter also explains how to create a new sequence and how to gather several sequences in a sequence list.

9.1 View sequence

When you double-click a sequence in the **Navigation Area**, the sequence will open automatically, and you will see the nucleotides or amino acids. The zoom options described in section 3.3 allow you to e.g. zoom out in order to see more of the sequence in one view. There are a number of options for viewing and editing the sequence which are all described in this section. All the options described in this section also apply to alignments (further described in section 14.2).

9.1.1 Sequence settings in Side Panel

Each view of a sequence has a **Side Panel** located at the right side of the view (see figure 9.1.

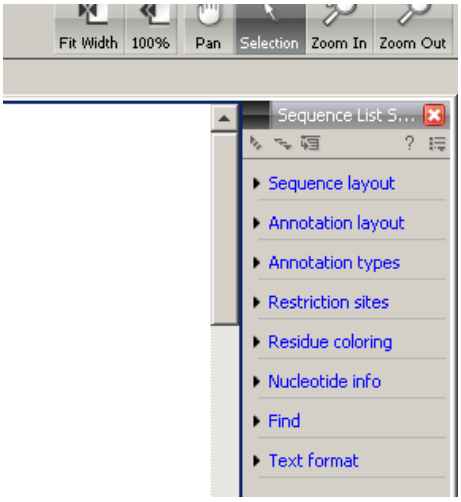

Figure 9.1: *Overview of the Side Panel which is always shown to the right of a view.*

When you make changes in the **Side Panel** the view of the sequence is instantly updated. To show or hide the **Side Panel**:

select the View | Ctrl + U

or **Click the ( ) at the top right corner of the Side Panel to hide | Click the gray Side Panel button to the right to show**

Below, each group of settings will be explained. Some of the preferences are not the same for nucleotide and protein sequences, but the differences will be explained for each group of settings.

Note! When you make changes to the settings in the **Side Panel**, they are not automatically saved when you save the sequence. Click **Save/restore Settings** ( ) to save the settings (see section 4.5 for more information).

Sequence Layout

These preferences determine the overall layout of the sequence:

- **Spacing.** Inserts a space at a specified interval:
  - **No spacing.** The sequence is shown with no spaces.
  - **Every 10 residues.** There is a space every 10 residues, starting from the beginning of the sequence.
  - **Every 3 residues, frame 1.** There is a space every 3 residues, corresponding to the reading frame starting at the first residue.
  - **Every 3 residues, frame 2.** There is a space every 3 residues, corresponding to the reading frame starting at the second residue.
  - **Every 3 residues, frame 3.** There is a space every 3 residues, corresponding to the reading frame starting at the third residue.

- **Wrap sequences.** Shows the sequence on more than one line.
  - **No wrap.** The sequence is displayed on one line.
  - **Auto wrap.** Wraps the sequence to fit the width of the view, not matter if it is zoomed in our out (displays minimum 10 nucleotides on each line).
  - **Fixed wrap.** Makes it possible to specify when the sequence should be wrapped. In the text field below, you can choose the number of residues to display on each line.
- **Double stranded.** Shows both strands of a sequence (only applies to DNA sequences).
- **Numbers on sequences.** Shows residue positions along the sequence. The starting point can be changed by setting the number in the field below. If you set it to e.g. 101, the first residue will have the position of -100. This can also be done by right-clicking an annotation and choosing **Set Numbers Relative to This Annotation**.
- **Numbers on plus strand.** Whether to set the numbers relative to the positive or the negative strand in a nucleotide sequence (only applies to DNA sequences).
- **Follow selection.** When viewing the same sequence in two separate views, "Follow selection" will automatically scroll the view in order to follow a selection made in the other view.
- **Lock numbers.** When you scroll vertically, the position numbers remain visible. (Only possible when the sequence is not wrapped.)
- **Lock labels.** When you scroll horizontally, the label of the sequence remains visible.
- **Sequence label.** Defines the label to the left of the sequence.
  - Name (this is the default information to be shown).
  - Accession (sequences downloaded from databases like GenBank have an accession number).
  - Latin name.
  - Latin name (accession).
  - Common name.
  - Common name (accession).

Annotation Layout and Annotation Types

See section 9.3.1.

Restriction sites

See section 9.1.2.

Motifs

See section **??**.

Residue coloring

These preferences make it possible to color both the residue letter and set a background color for the residue.

- **Non-standard residues.** For nucleotide sequences this will color the residues that are not C, G, A, T or U. For amino acids only B, Z, and X are colored as non-standard residues.
  - **Foreground color.** Sets the color of the letter. Click the color box to change the color.
  - **Background color.** Sets the background color of the residues. Click the color box to change the color.
- **Rasmol colors.** Colors the residues according to the Rasmol color scheme. See http://www.openrasmol.org/doc/rasmol.html
  - **Foreground color.** Sets the color of the letter. Click the color box to change the color.
  - **Background color.** Sets the background color of the residues. Click the color box to change the color.
- **Polarity colors (only protein).** Colors the residues according to the polarity of amino acids.
  - **Foreground color.** Sets the color of the letter. Click the color box to change the color.
  - **Background color.** Sets the background color of the residues. Click the color box to change the color.
- **Trace colors (only DNA).** Colors the residues according to the color conventions of chromatogram traces: A=green, C=blue, G=black, and T=red.
  - **Foreground color.** Sets the color of the letter.
  - **Background color.** Sets the background color of the residues.

Find

The Find function can also be invoked by pressing Ctrl + Shift + F (⌘ + Shift + F on Mac).

The Find function can be used for searching the sequence. Clicking the find button will search for the first occurrence of the search term. Clicking the find button again will find the next occurrence and so on. If the search string is found, the corresponding part of the sequence will be selected.

- **Search term.** Enter the text to search for. The search function does not discriminate between lower and upper case characters.
- **Sequence search.** Search the nucleotides or amino acids. For amino acids, the single letter abbreviations should be used for searching. The sequence search also has a set of advanced search parameters:
  - Include negative strand. This will search on the negative strand as well.
  - Treat ambiguous characters as wildcards in search term. If you search for e.g. ATN, you will find both ATG and ATC. If you wish to find literally exact matches for ATN (i.e. only find ATN - not ATG), this option should not be selected.

  - Treat ambiguous characters as wildcards in sequence. If you search for e.g. ATG, you will find both ATG and ATN. If you have large regions of Ns, this option should not be selected.

  Note that if you enter a position instead of a sequence, it will automatically switch to position search.

- **Annotation search.** Searches the annotations on the sequence. The search is performed both on the labels of the annotations, but also on the text appearing in the tooltip that you see when you keep the mouse cursor fixed. If the search term is found, the part of the sequence corresponding to the matching annotation is selected. Below this option you can choose to search for translations as well. Sequences annotated with coding regions often have the translation specified which can lead to undesired results.

- **Position search.** Finds a specific position on the sequence. In order to find an interval, e.g. from position 500 to 570, enter "500..570" in the search field. This will make a selection from position 500 to 570 (both included). Notice the two periods (..) between the start an end number (see section **??**). If you enter positions including thousands separators like 123,345, the comma will just be ignored and it would be equivalent to entering 123345.

- **Include negative strand.** When searching the sequence for nucleotides or amino acids, you can search on both strands.

- **Name search.** Searches for sequence names. This is useful for searching sequence lists, mapping results and BLAST results.

This concludes the description of the **View Preferences**. Next, the options for selecting and editing sequences are described.

Text format

These preferences allow you to adjust the format of all the text in the view (both residue letters, sequence name and translations if they are shown).

- **Text size.** Five different sizes.

- **Font.** Shows a list of Fonts available on your computer.

- **Bold residues.** Makes the residues bold.

9.1.2 Restriction sites in the Side Panel

Please see section 13.1.

9.1.3 Selecting parts of the sequence

You can select parts of a sequence:

> **Click Selection ( ) in Toolbar | Press and hold down the mouse button on the sequence where you want the selection to start | move the mouse to the end of the selection while holding the button | release the mouse button**

Alternatively, you can search for a specific interval using the find function described above.

If you have made a selection and wish to adjust it:

drag the edge of the selection (you can see the mouse cursor change to a horizontal arrow

or **press and hold the Shift key while using the right and left arrow keys to adjust the right side of the selection.**

If you wish to select the entire sequence:

double-click the sequence name to the left

Selecting several parts at the same time (multiselect)

You can select several parts of sequence by holding down the **Ctrl** button while making selections. Holding down the **Shift** button lets you extend or reduce an existing selection to the position you clicked.

To select a part of a sequence covered by an annotation:

right-click the annotation | Select annotation

or **double-click the annotation**

To select a fragment between two restriction sites that are shown on the sequence:

double-click the sequence between the two restriction sites

(Read more about restriction sites in section 9.1.2.)

Open a selection in a new view

A selection can be opened in a new view and saved as a new sequence:

right-click the selection | Open selection in New View ()

This opens the annotated part of the sequence in a new view. The new sequence can be saved by dragging the tab of the sequence view into the **Navigation Area**.

The process described above is also the way to manually translate coding parts of sequences (CDS) into protein. You simply translate the new sequence into protein. This is done by:

right-click the tab of the new sequence | Toolbox | Nucleotide Analysis ()| Translate to Protein ()

A selection can also be copied to the clipboard and pasted into another program:

make a selection | Ctrl + C (⌘ + C on Mac)

Note! The annotations covering the selection will not be copied.

A selection of a sequence can be edited as described in the following section.

9.1.4 Editing the sequence

When you make a selection, it can be edited by:

right-click the selection | Edit Selection ( )

A dialog appears displaying the sequence. You can add, remove or change the text and click **OK**. The original selected part of the sequence is now replaced by the sequence entered in the dialog. This dialog also allows you to paste text into the sequence using Ctrl + V (⌘ + V on Mac).

If you delete the text in the dialog and press **OK**, the selected text on the sequence will also be deleted. Another way to delete a part of the sequence is to:

right-click the selection | Delete Selection ( )

If you wish to only correct only one residue, this is possible by simply making the selection only cover one residue and then type the new residue.

9.1.5 Sequence region types

The various annotations on sequences cover parts of the sequence. Some cover an interval, some cover intervals with unknown endpoints, some cover more than one interval etc. In the following, all of these will be referred to as *regions*. Regions are generally illustrated by markings (often arrows) on the sequences. An arrow pointing to the right indicates that the corresponding region is located on the positive strand of the sequence. Figure 9.2 is an example of three regions with separate colors.

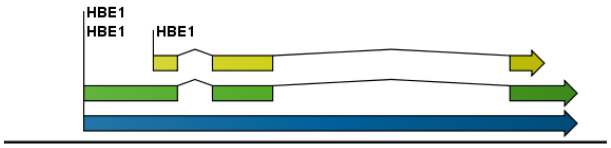

Figure 9.2: *Three regions on a human beta globin DNA sequence (HUMHBB).*

Figure 9.3 shows an artificial sequence with all the different kinds of regions.

9.2 Circular DNA

A sequence can be shown as a circular molecule:

select a sequence in the Navigation Area | Show in the Toolbar | As Circular ( )

or **If the sequence is already open | Click Show As Circular ( ) at the lower left part of the view**

This will open a view of the molecule similar to the one in figure 9.4.

This view of the sequence shares some of the properties of the linear view of sequences as described in section 9.1, but there are some differences. The similarities and differences are listed below:

- **Similarities**:
  - The editing options.
  - Options for adding, editing and removing annotations.
  - **Restriction Sites**, **Annotation Types**, **Find** and **Text Format** preferences groups.

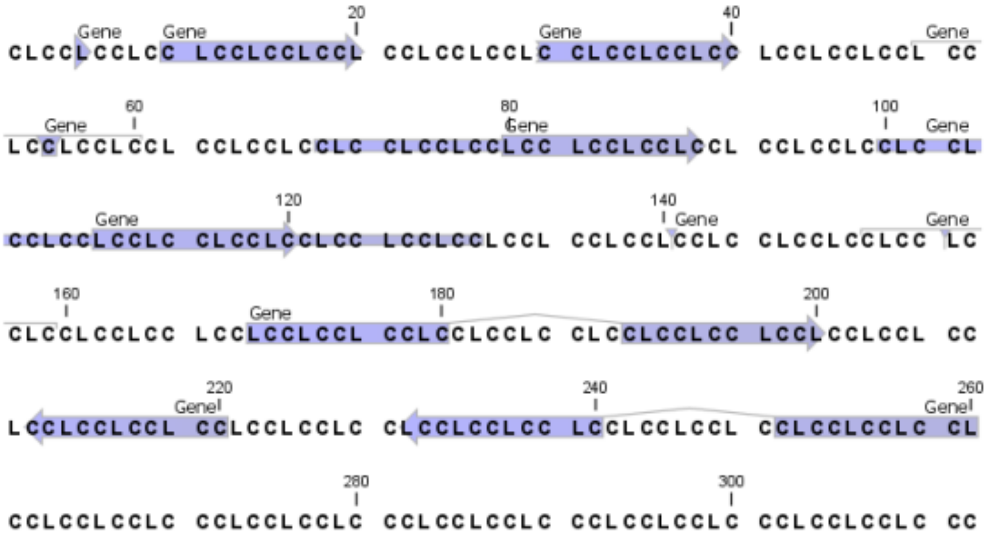

Figure 9.3: *Region #1: A single residue, Region #2: A range of residues including both endpoints, Region #3: A range of residues starting somewhere before 30 and continuing up to and including 40, Region #4: A single residue somewhere between 50 and 60 inclusive, Region #5: A range of residues beginning somewhere between 70 and 80 inclusive and ending at 90 inclusive, Region #6: A range of residues beginning somewhere between 100 and 110 inclusive and ending somewhere between 120 and 130 inclusive, Region #7: A site between residues 140 and 141, Region #8: A site between two residues somewhere between 150 and 160 inclusive, Region #9: A region that covers ranges from 170 to 180 inclusive and 190 to 200 inclusive, Region #10: A region on negative strand that covers ranges from 210 to 220 inclusive, Region #11: A region on negative strand that covers ranges from 230 to 240 inclusive and 250 to 260 inclusive.*

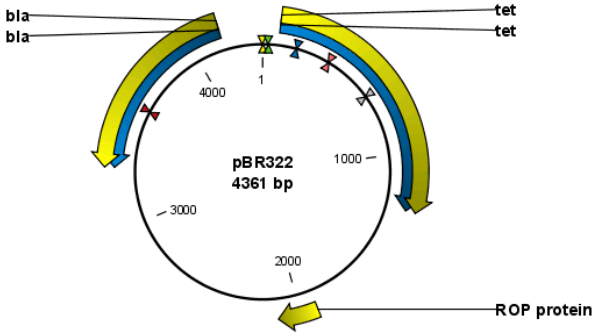

Figure 9.4: *A molecule shown in a circular view.*

- **Differences**:
  - In the **Sequence Layout** preferences, only the following options are available in the circular view: **Numbers on plus strand**, **Numbers on sequence** and **Sequence label**.
  - You cannot zoom in to see the residues in the circular molecule. If you wish to see these details, split the view with a linear view of the sequence
  - In the **Annotation Layout**, you also have the option of showing the labels as **Stacked**. This means that there are no overlapping labels and that all labels of both annotations and restriction sites are adjusted along the left and right edges of the view.

9.2.1 Using split views to see details of the circular molecule

In order to see the nucleotides of a circular molecule you can open a new view displaying a circular view of the molecule:

> **Press and hold the Ctrl button (⌘ on Mac) | click Show Sequence (ACT) at the bottom of the view**

This will open a linear view of the sequence below the circular view. When you zoom in on the linear view you can see the residues as shown in figure 9.5.

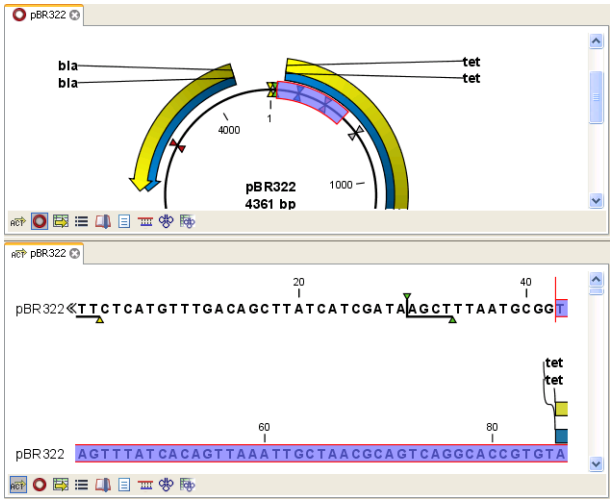

Figure 9.5: *Two views showing the same sequence. The bottom view is zoomed in.*

Note! If you make a selection in one of the views, the other view will also make the corresponding selection, providing an easy way for you to focus on the same region in both views.

9.2.2 Mark molecule as circular and specify starting point

You can mark a DNA molecule as circular by right-clicking its name in either the sequence view or the circular view. In the right-click menu you can also make a circular molecule linear. A circular molecule displayed in the normal sequence view, will have the sequence ends marked with a ».

The starting point of a circular sequence can be changed by:

> **make a selection starting at the position that you want to be the new starting point | right-click the selection | Move Starting Point to Selection Start**

Note! This can only be done for sequence that have been marked as circular.

9.3 Working with annotations

Annotations provide information about specific regions of a sequence. A typical example is the annotation of a gene on a genomic DNA sequence.

Annotations derive from different sources:

- Sequences downloaded from databases like GenBank are annotated.

- In some of the data formats that can be imported into *CLC Sequence Viewer*, sequences can have annotations (GenBank, EMBL and Swiss-Prot format).
- The result of a number of analyses in *CLC Sequence Viewer* are annotations on the sequence (e.g. finding open reading frames and restriction map analysis).

Note! Annotations are included if you export the sequence in GenBank, Swiss-Prot, EMBL or CLC format. When exporting in other formats, annotations are not preserved in the exported file.

9.3.1 Viewing annotations

Annotations can be viewed in a number of different ways:

- As arrows or boxes in the sequence views:
  - Linear and circular view of sequences ( ) / ( ).
  - Alignments ( ).
  - Graphical view of sequence lists ( ).
- In the table of annotations ( ).
- In the text view of sequences ( )

In the following sections, these view options will be described in more detail.

In all the views except the text view ( ), annotations can be deleted. This is described in the following sections.

View Annotations in sequence views

Figure 9.6 shows an annotation displayed on a sequence.

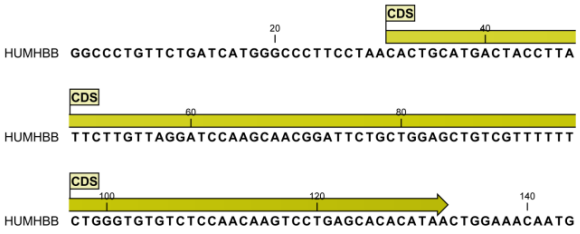

Figure 9.6: *An annotation showing a coding region on a genomic dna sequence.*

The various sequence views listed in section 9.3.1 have different default settings for showing annotations. However, they all have two groups in the **Side Panel** in common:

- **Annotation Layout**
- **Annotation Types**

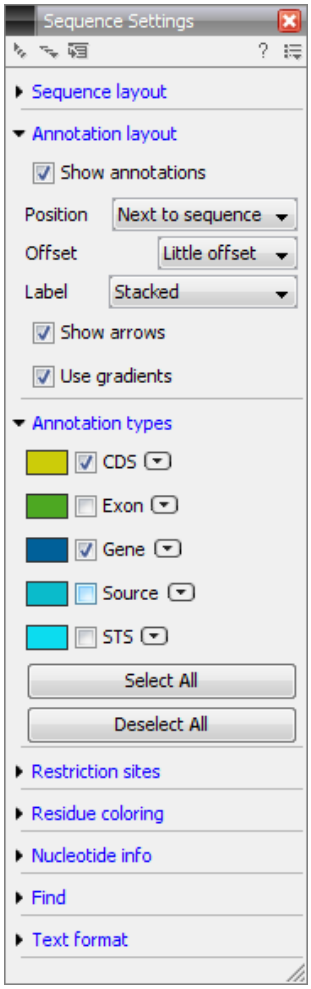

Figure 9.7: *Changing the layout of annotations in the Side Panel.*

The two groups are shown in figure 9.7.

In the **Annotation layout** group, you can specify how the annotations should be displayed (notice that there are some minor differences between the different sequence views):

- **Show annotations.** Determines whether the annotations are shown.
- **Position.**
  - **On sequence.** The annotations are placed on the sequence. The residues are visible through the annotations (if you have zoomed in to 100%).
  - **Next to sequence.** The annotations are placed above the sequence.
  - **Separate layer.** The annotations are placed above the sequence and above restriction sites (only applicable for nucleotide sequences).
- **Offset.** If several annotations cover the same part of a sequence, they can be spread out.
  - **Piled.** The annotations are piled on top of each other. Only the one at front is visible.
  - **Little offset.** The annotations are piled on top of each other, but they have been offset a little.

  - **More offset.** Same as above, but with more spreading.
  - **Most offset.** The annotations are placed above each other with a little space between. This can take up a lot of space on the screen.

- **Label.** The name of the annotation can shown as a label. Additional information about the sequence is shown if you place the mouse cursor on the annotation and keep it still.
  - **No labels.** No labels are displayed.
  - **On annotation.** The labels are displayed in the annotation's box.
  - **Over annotation.** The labels are displayed above the annotations.
  - **Before annotation.** The labels are placed just to the left of the annotation.
  - **Flag.** The labels are displayed as flags at the beginning of the annotation.
  - **Stacked.** The labels are offset so that the text of all labels is visible. This means that there is varying distance between each sequence line to make room for the labels.
- **Show arrows.** Displays the end of the annotation as an arrow. This can be useful to see the orientation of the annotation (for DNA sequences). Annotations on the negative strand will have an arrow pointing to the left.
- **Use gradients.** Fills the boxes with gradient color.

In the **Annotation Types** group, you can choose which kinds of annotations that should be displayed. This group lists all the types of annotations that are attached to the sequence(s) in the view. For sequences with many annotations, it can be easier to get an overview if you deselect the annotation types that are not relevant.

Unchecking the checkboxes in the **Annotation Layout** will not remove this type of annotations them from the sequence - it will just hide them from the view.

Besides selecting which types of annotations that should be displayed, the **Annotation Types** group is also used to change the color of the annotations on the sequence. Click the colored square next to the relevant annotation type to change the color.

This will display a dialog with three tabs: Swatches, HSB, and RGB. They represent three different ways of specifying colors. Apply your settings and click **OK**. When you click **OK**, the color settings cannot be reset. The **Reset** function only works for changes made before pressing **OK**.

Furthermore, the **Annotation Types** can be used to easily browse the annotations by clicking the small button () next to the type. This will display a list of the annotations of that type (see figure 9.8).

Clicking an annotation in the list will select this region on the sequence. In this way, you can quickly find a specific annotation on a long sequence.

View Annotations in a table

Annotations can also be viewed in a table:

select the sequence in the Navigation Area | Show () | Annotation Table ()

or **If the sequence is already open | Click Show Annotation Table () at the lower left part of the view**

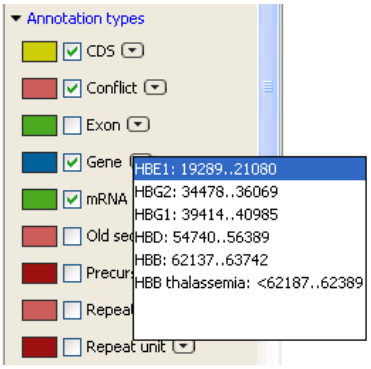

Figure 9.8: *Browsing the gene annotations on a sequence.*

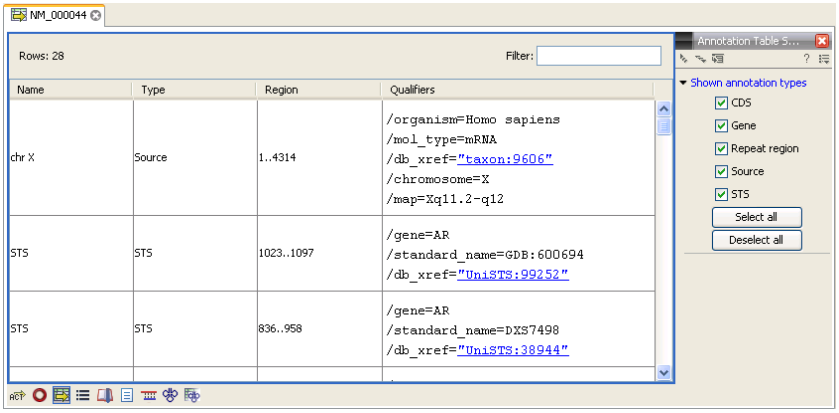

Figure 9.9: *A table showing annotations on the sequence.*

This will open a view similar to the one in figure 9.9).

In the **Side Panel** you can show or hide individual annotation types in the table. E.g. if you only wish to see "gene" annotations, de-select the other annotation types so that only "gene" is selected.

Each row in the table is an annotation which is represented with the following information:

- **Name.**
- **Type.**
- **Region.**
- **Qualifiers.**

9.3.2 Removing annotations

Annotations can be hidden using the **Annotation Types** preferences in the **Side Panel** to the right of the view (see section 9.3.1). In order to completely remove the annotation:

> **right-click the annotation | Delete | Delete Annotation ( )**

If you want to remove all annotations of one type:

> **right-click an annotation of the type you want to remove | Delete | Delete Annotations of Type "type"**

If you want to remove all annotations from a sequence:

right-click an annotation | Delete | Delete All Annotations

The removal of annotations can be undone using Ctrl + Z or Undo ( ) in the Toolbar.

If you have more sequences (e.g. in a sequence list, alignment or contig), you have two additional options:

right-click an annotation | Delete | Delete All Annotations from All Sequences

right-click an annotation | Delete | Delete Annotations of Type "type" from All Sequences

9.4 Element information

The normal view of a sequence (by double-clicking) shows the annotations as boxes along the sequence, but often there is more information available about sequences. This information is available through the **Element info** view.

To view the sequence information:

select a sequence in the Navigation Area | Show ( ) in the Toolbar | Element info ( )

This will display a view similar to fig 9.10.

Figure 9.10: *The initial display of sequence info for the HUMHBB DNA sequence from the Example data.*

All the lines in the view are headings, and the corresponding text can be shown by clicking the text.

- **Name.** The name of the sequence which is also shown in sequence views and in the **Navigation Area**.
- **Description.** A description of the sequence.
- **Comments.** The author's comments about the sequence.
- **Keywords.** Keywords describing the sequence.

- **Db source.** Accession numbers in other databases concerning the same sequence.
- **Gb Division.** Abbreviation of GenBank divisions. See section 3.3 in the GenBank release notes for a full list of GenBank divisions.
- **Length.** The length of the sequence.
- **Modification date.** Modification date from the database. This means that this date does not reflect your own changes to the sequence. See the history (section 7) for information about the latest changes to the sequence after it was downloaded from the database.
- **Organism.** Scientific name of the organism (first line) and taxonomic classification levels (second and subsequent lines).

The information available depends on the origin of the sequence. Sequences downloaded from database like NCBI and UniProt (see section 10) have this information. On the other hand, some sequence formats like fasta format do not contain this information.

Some of the information can be edited by clicking the blue **Edit** text. This means that you can add your own information to sequences that do not derive from databases.

Note that for other kinds of data, the **Element info** will only have **Name** and **Description**.

9.5 View as text

A sequence can be viewed as text without any layout and text formatting. This displays all the information about the sequence in the GenBank file format. To view a sequence as text:

select a sequence in the Navigation Area | Show in the Toolbar | As text

This way it is possible to see background information about e.g. the authors and the origin of DNA and protein sequences. Selections or the entire text of the **Sequence Text View** can be copied and pasted into other programs:

Much of the information is also displayed in the **Sequence info**, where it is easier to get an overview (see section 9.4.)

In the **Side Panel**, you find a search field for searching the text in the view.

9.6 Creating a new sequence

A sequence can either be imported, downloaded from an online database or created in the *CLC Sequence Viewer*. This section explains how to create a new sequence:

New ( ) in the toolbar

The **Create Sequence** dialog (figure 9.11) reflects the information needed in the GenBank format, but you are free to enter anything into the fields. The following description is a guideline for entering information about a sequence:

- **Name.** The name of the sequence. This is used for saving the sequence.
- **Common name.** A common name for the species.

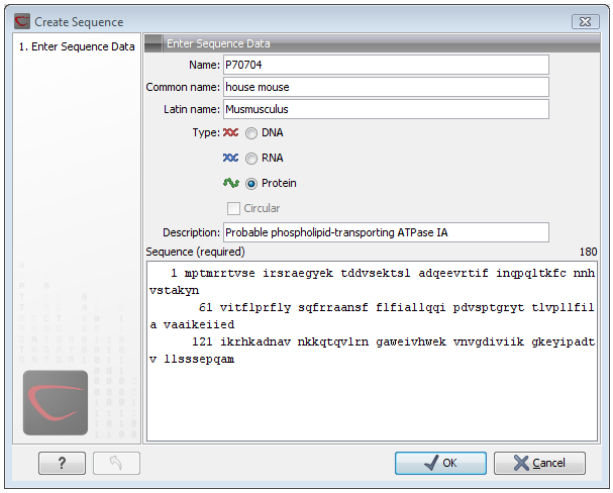

Figure 9.11: *Creating a sequence.*

- **Latin name.** The Latin name for the species.
- **Type.** Select between DNA, RNA and protein.
- **Circular.** Specifies whether the sequence is circular. This will open the sequence in a circular view as default. (applies only to nucleotide sequences).
- **Description.** A description of the sequence.
- **Keywords.** A set of keywords separated by semicolons (;).
- **Comments.** Your own comments to the sequence.
- **Sequence.** Depending on the type chosen, this field accepts nucleotides or amino acids. Spaces and numbers can be entered, but they are ignored when the sequence is created. This allows you to paste (Ctrl + V on Windows and ⌘ + V on Mac) in a sequence directly from a different source, even if the residue numbers are included. Characters that are not part of the IUPAC codes cannot be entered. At the top right corner of the field, the number of residues are counted. The counter does not count spaces or numbers.

Clicking **Finish** opens the sequence. It can be saved by clicking **Save** ( ) or by dragging the tab of the sequence view into the **Navigation Area**.

9.7 Sequence Lists

The **Sequence List** shows a number of sequences in a tabular format or it can show the sequences together in a normal sequence view.

Having sequences in a sequence list can help organizing sequence data. The sequence list may originate from an NCBI search (chapter 10.1). Moreover, if a multiple sequence fasta file is imported, it is possible to store the data in a sequences list. A **Sequence List** can also be generated using a dialog, which is described here:

select two or more sequences | **right-click the elements** | **New** | **Sequence List ( )**

This action opens a **Sequence List** dialog:

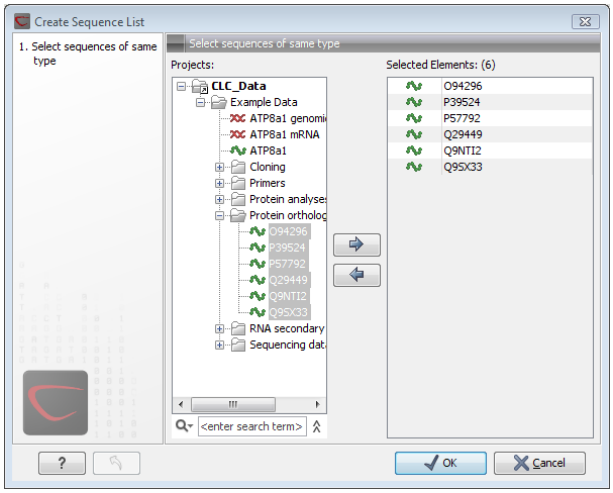

Figure 9.12: *A Sequence List dialog.*

The dialog allows you to select more sequences to include in the list, or to remove already chosen sequences from the list.

Clicking **Finish** opens the sequence list. It can be saved by clicking **Save** ( ) or by dragging the tab of the view into the **Navigation Area**.

Opening a Sequence list is done by:

> **right-click the sequence list in the Navigation Area | Show ( ) | Graphical Sequence List ( ) OR Table ( )**

The two different views of the same sequence list are shown in split screen in figure 9.13.

9.7.1 Graphical view of sequence lists

The graphical view of sequence lists is almost identical to the view of single sequences (see section 9.1). The main difference is that you now can see more than one sequence in the same view.

However, you also have a few extra options for sorting, deleting and adding sequences:

- To add extra sequences to the list, right-click an empty (white) space in the view, and select **Add Sequences**.
- To delete a sequence from the list, right-click the sequence's name and select **Delete Sequence**.
- To sort the sequences in the list, right-click the name of one of the sequences and select **Sort Sequence List by Name** or **Sort Sequence List by Length**.
- To rename a sequence, right-click the name of the sequence and select **Rename Sequence**.

9.7.2 Sequence list table

Each sequence in the table sequence list is displayed with:

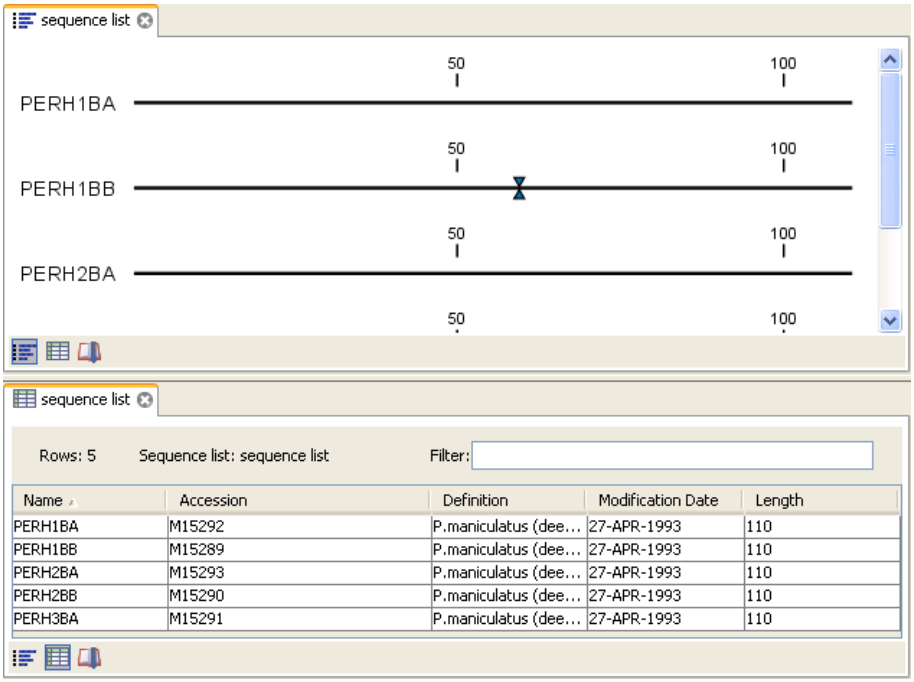

Figure 9.13: *A sequence list containing multiple sequences can be viewed in either a table or in a graphical sequence list. The graphical view is useful for viewing annotations and the sequence itself, while the table view provides other information like sequence lengths, and the number of sequences in the list (number of Rows reported).*

- Name.
- Accession.
- Description.
- Modification date.
- Length.

The number of sequences in the list is reported as the number of Rows at the top of the table view.

Learn more about tables in section C.

Adding and removing sequences from the list is easy: adding is done by dragging the sequence from another list or from the **Navigation Area** and drop it in the table. To delete sequences, simply select them and press **Delete** ( ).

You can also create a subset of the sequence list:

select the relevant sequences | **right-click** | **Create New Sequence List**

This will create a new sequence list which only includes the selected sequences.

9.7.3 Extract sequences

It is possible to extract individual sequences from a sequence list in two ways. If the sequence list is opened in the tabular view, it is possible to drag (with the mouse) one or more sequences

into the **Navigation Area**. This allows you to extract specific sequences from the entire list. Another option is to extract all sequences found in the list. This can also be done for:

- Alignments ()
- Contigs and read mappings ()
- Read mapping tables ()
- BLAST result ()
- BLAST overview tables ()
- RNA-Seq samples ()
- and of course sequence lists ()

For mappings and BLAST results, the main sequences (i.e. reference/consensus and query sequence) will not be extracted.

To extract the sequences:

Toolbox | **General Sequence Analysis ()**| **Extract Sequences** ()

This will allow you to select the elements that you want to extract sequences from (see the list above). Clicking **Next** displays the dialog shown in 9.14.

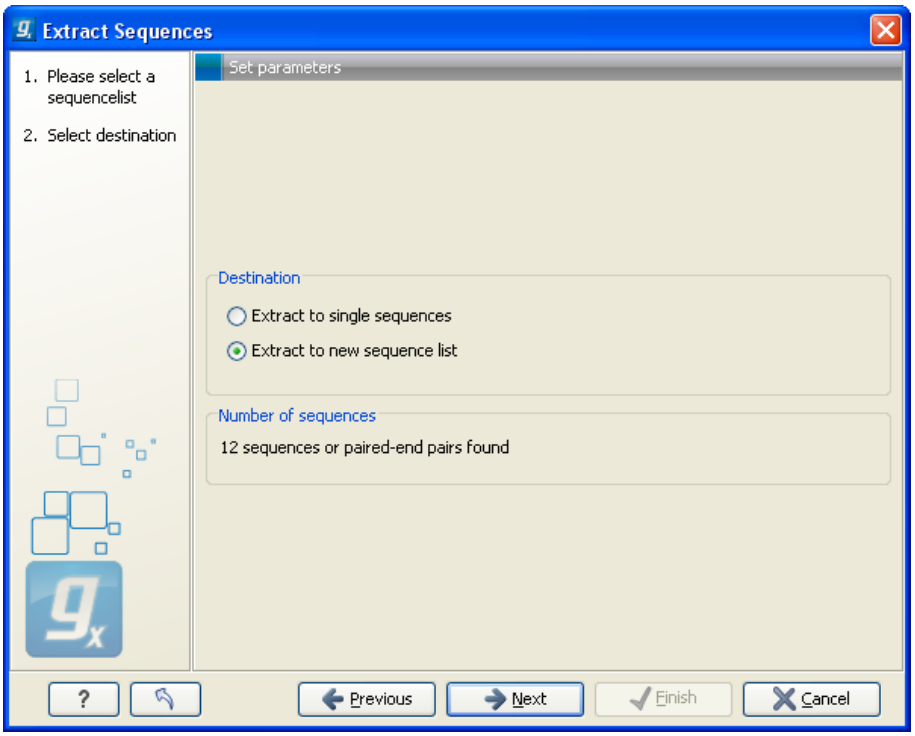

Figure 9.14: *Choosing whether the extracted sequences should be placed in a new list or as single sequences.*

Here you can choose whether the extracted sequences should be placed in a new list or extracted as single sequences. For sequence lists, only the last option makes sense, but for alignments, mappings and BLAST results, it would make sense to place the sequences in a list.

Below these options you can see the number of sequences that will be extracted.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

Chapter 10

Data download

Contents

CLC Sequence Viewer allows you to search the for sequences on the Internet. You must be online when initiating and performing searches in NCBI.

10.1 GenBank search

This section describes searches for sequences in GenBank - the **NCBI Entrez** database. The NCBI search view is opened in this way (figure 10.1):

Download | Search for Sequences at NCBI ()

or **Ctrl + B (⌘ + B on Mac)**

This opens the following view:

10.1.1 GenBank search options

Conducting a search in the **NCBI Database** from *CLC Sequence Viewer* corresponds to conducting the search on NCBI's website. When conducting the search from *CLC Sequence Viewer*, the results are available and ready to work with straight away.

You can choose whether you want to search for nucleotide sequences or protein sequences.

As default, *CLC Sequence Viewer* offers one text field where the search parameters can be entered. Click **Add search parameters** to add more parameters to your search.
Note! The search is a "and" search, meaning that when adding search parameters to your search, you search for both (or all) text strings rather than "any" of the text strings.

You can append a wildcard character by checking the checkbox at the bottom. This means that you only have to enter the first part of the search text, e.g. searching for "genom" will find both

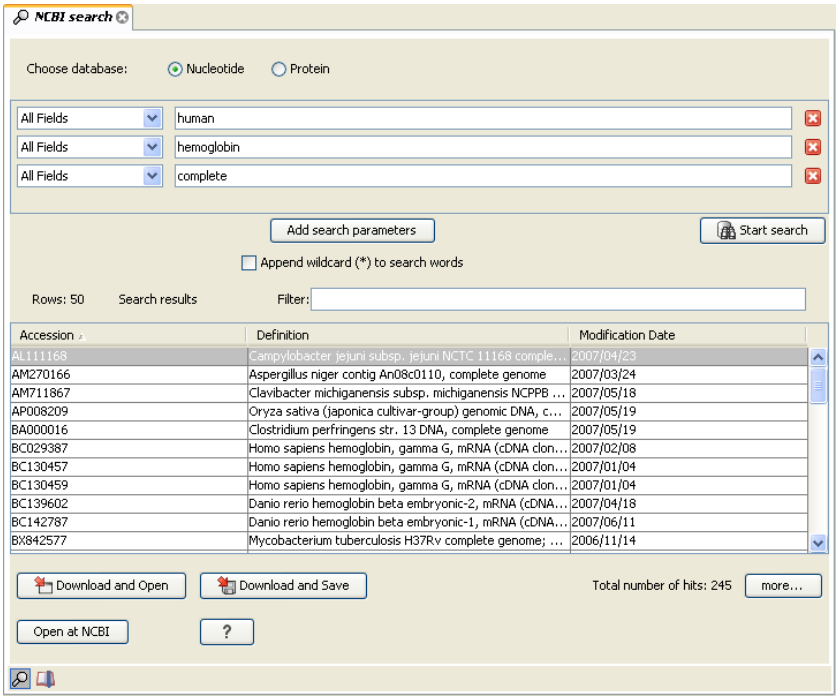

Figure 10.1: *The GenBank search view.*

"genomic" and "genome".

The following parameters can be added to the search:

- **All fields**. Text, searches in all parameters in the NCBI database at the same time.
- **Organism**. Text.
- **Description**. Text.
- **Modified Since**. Between 30 days and 10 years.
- **Gene Location**. Genomic DNA/RNA, Mitochondrion, or Chloroplast.
- **Molecule**. Genomic DNA/RNA, mRNA or rRNA.
- **Sequence Length**. Number for maximum or minimum length of the sequence.
- **Gene Name**. Text.

The search parameters are the most recently used. The **All fields** allows searches in all parameters in the NCBI database at the same time. **All fields** also provide an opportunity to restrict a search to parameters which are not listed in the dialog. E.g. writing `gene[Feature key] AND mouse` in **All fields** generates hits in the GenBank database which contains one or more genes and where 'mouse' appears somewhere in GenBank file. You can also write e.g. `CD9 NOT homo sapiens` in **All fields**.

Note! The 'Feature Key' option is only available in GenBank when searching for nucleotide sequences. For more information about how to use this syntax, see `http://www.ncbi.nlm.nih.gov/books/NBK3837/`

When you are satisfied with the parameters you have entered, click **Start search**.

Note! When conducting a search, no files are downloaded. Instead, the program produces a list of links to the files in the NCBI database. This ensures a much faster search.

10.1.2 Handling of GenBank search results

The search result is presented as a list of links to the files in the NCBI database. The **View** displays 50 hits at a time. This can be changed in the **Preferences** (see chapter 4). More hits can be displayed by clicking the **More...** button at the bottom right of the **View**.

Each sequence hit is represented by text in three columns:

- Accession.
- Description.
- Modification date.
- Length.

It is possible to exclude one or more of these columns by adjust the View preferences for the database search view. Furthermore, your changes in the View preferences can be saved. See section 4.5.

Several sequences can be selected, and by clicking the buttons in the bottom of the search view, you can do the following:

- Download and open, doesn't save the sequence.
- Download and save, lets you choose location for saving sequence.
- Open at NCBI, searches the sequence at NCBI's web page.

Double-clicking a hit will download and open the sequence. The hits can also be copied into the **View Area** or the **Navigation Area** from the search results by drag and drop, copy/paste or by using the right-click menu as described below.

Drag and drop from GenBank search results

The sequences from the search results can be opened by dragging them into a position in the **View Area**.

Note! A sequence is not saved until the **View** displaying the sequence is closed. When that happens, a dialog opens: Save changes of sequence x? (Yes or No).

The sequence can also be saved by dragging it into the **Navigation Area**. It is possible to select more sequences and drag all of them into the **Navigation Area** at the same time.

Download GenBank search results using right-click menu

You may also select one or more sequences from the list and download using the right-click menu (see figure 10.2). Choosing **Download and Save** lets you select a folder where the sequences are saved when they are downloaded. Choosing **Download and Open** opens a new view for each of the selected sequences.

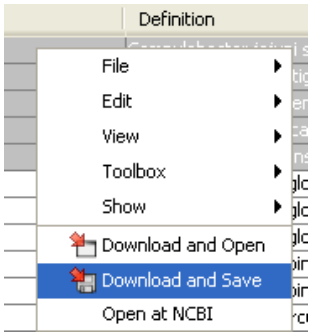

Figure 10.2: *By right-clicking a search result, it is possible to choose how to handle the relevant sequence.*

Copy/paste from GenBank search results

When using copy/paste to bring the search results into the **Navigation Area**, the actual files are downloaded from GenBank.

To copy/paste files into the **Navigation Area**:

> **select one or more of the search results | Ctrl + C (⌘ + C on Mac) | select a folder in the Navigation Area | Ctrl + V**

Note! Search results are downloaded before they are saved. Downloading and saving several files may take some time. However, since the process runs in the background (displayed in the **Status bar**) it is possible to continue other tasks in the program. Like the search process, the download process can be stopped. This is done in the **Toolbox** in the **Processes** tab.

10.1.3 Save GenBank search parameters

The search view can be saved either using dragging the search tab and and dropping it in the **Navigation Area** or by clicking **Save** (). When saving the search, only the parameters are saved - not the results of the search. This is useful if you have a special search that you perform from time to time.

Even if you don't save the search, the next time you open the search view, it will remember the parameters from the last time you did a search.

Chapter 11

General sequence analyses

Contents

CLC Sequence Viewer offers different kinds of sequence analyses, which apply to both protein and DNA.

11.1 Shuffle sequence

In some cases, it is beneficial to shuffle a sequence. This is an option in the **Toolbox** menu under **General Sequence Analyses**. It is normally used for statistical analyses, e.g. when comparing an alignment score with the distribution of scores of shuffled sequences.

Shuffling a sequence removes all annotations that relate to the residues.

select sequence | **Toolbox in the Menu Bar** | **General Sequence Analysis (**  **)**| **Shuffle Sequence (**  **)**

or **right-click a sequence** | **Toolbox** | **General Sequence Analysis (**  **)**| **Shuffle Sequence (**  **)**

This opens the dialog displayed in figure 11.1:

If a sequence was selected before choosing the Toolbox action, this sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

Click **Next** to determine how the shuffling should be performed.

In this step, shown in figure 11.2: For nucleotides, the following parameters can be set:

- **Mononucleotide shuffling.** Shuffle method generating a sequence of the exact same mononucleotide frequency

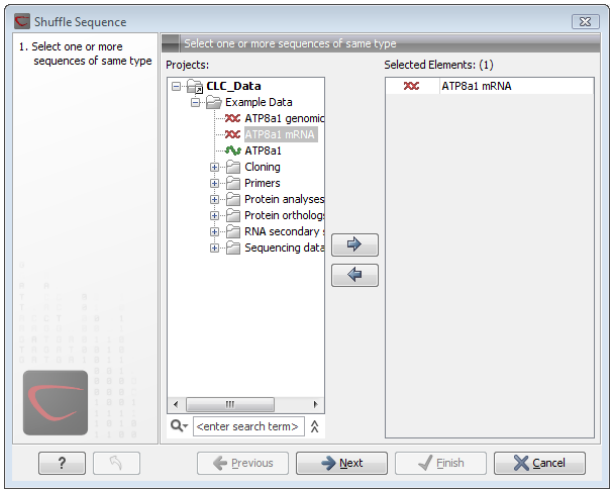

Figure 11.1: *Choosing sequence for shuffling.*

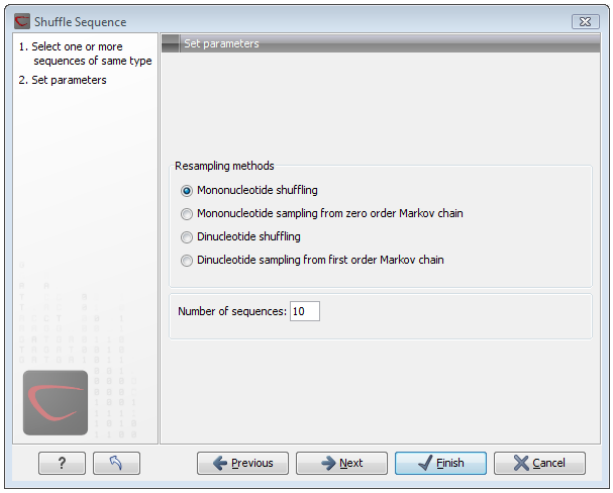

Figure 11.2: *Parameters for shuffling.*

- **Dinucleotide shuffling.** Shuffle method generating a sequence of the exact same dinucleotide frequency
- **Mononucleotide sampling from zero order Markov chain.** Resampling method generating a sequence of the same expected mononucleotide frequency.
- **Dinucleotide sampling from first order Markov chain.** Resampling method generating a sequence of the same expected dinucleotide frequency.

For proteins, the following parameters can be set:

- **Single amino acid shuffling.** Shuffle method generating a sequence of the exact same amino acid frequency.
- **Single amino acid sampling from zero order Markov chain.** Resampling method generating a sequence of the same expected single amino acid frequency.
- **Dipeptide shuffling.** Shuffle method generating a sequence of the exact same dipeptide frequency.

- **Dipeptide sampling from first order Markov chain.** Resampling method generating a sequence of the same expected dipeptide frequency.

For further details of these algorithms, see [Clote et al., 2005]. In addition to the shuffle method, you can specify the number of randomized sequences to output.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

This will open a new view in the **View Area** displaying the shuffled sequence. The new sequence is not saved automatically. To save the sequence, drag it into the **Navigation Area** or press ctrl + S (⌘ + S on Mac) to activate a save dialog.

11.2 Sequence statistics

CLC Sequence Viewer can produce an output with many relevant statistics for protein sequences. Some of the statistics are also relevant to produce for DNA sequences. Therefore, this section deals with both types of statistics. The required steps for producing the statistics are the same.

To create a statistic for the sequence, do the following:

select sequence(s) | Toolbox in the Menu Bar | General Sequence Analysis ( )| Create Sequence Statistics ( )

This opens a dialog where you can alter your choice of sequences which you want to create statistics for. You can also add sequence lists.

Note! You cannot create statistics for DNA and protein sequences at the same time.

When the sequences are selected, click **Next**.

This opens the dialog displayed in figure 11.3.

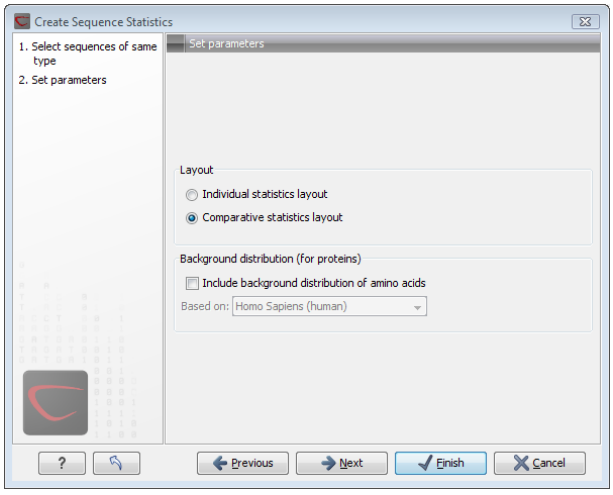

Figure 11.3: *Setting parameters for the sequence statistics.*

The dialog offers to adjust the following parameters:

- **Individual statistics layout.** If more sequences were selected in **Step 1**, this function generates separate statistics for each sequence.

- **Comparative statistics layout.** If more sequences were selected in **Step 1**, this function generates statistics with comparisons between the sequences.

You can also choose to include Background distribution of amino acids. If this box is ticked, an extra column with amino acid distribution of the chosen species, is included in the table output. (The distributions are calculated from UniProt `www.uniprot.org` version 6.0, dated September 13 2005.)

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**. An example of protein sequence statistics is shown in figure 11.4.

1 Protein statistics

1.1 Sequence information

Sequence type	Protein
Length	147
Organism	Mus musculus
Name	CAA32220
Description	haemoglobin beta-h0 chain [Mus musculus].
Modification Date	18-APR-2005
Weight	16,412 kDa

1.2 Half-life

N-terminal aa	Half-life mammals	Half-life yeast	Half-life E.Coli

Figure 11.4: *Comparative sequence statistics.*

Nucleotide sequence statistics are generated using the same dialog as used for protein sequence statistics. However, the output of Nucleotide sequence statistics is less extensive than that of the protein sequence statistics.

Note! The headings of the tables change depending on whether you calculate 'individual' or 'comparative' sequence statistics.

The output of comparative protein sequence statistics include:

- Sequence information:
  - Sequence type
  - Length
  - Organism
  - Name
  - Description
  - Modification Date
  - Weight. This is calculated like this: $sum_{unitsinsequence}(weight(unit)) - links * weight(H2O)$ where `links` is the sequence length minus one and `units` are amino acids. The atomic composition is defined the same way.
  - Isoelectric point
  - Aliphatic index

- Sequence Information:

  - Sequence type
  - Length
  - Organism
  - Name
  - Description
  - Modification Date
  - Weight
  - Isoelectric point
  - Aliphatic index
- Amino acid distribution
- Annotation table

The output of nucleotide sequence statistics include:

- General statistics:
  - Sequence type
  - Length
  - Organism
  - Name
  - Description
  - Modification Date
  - Weight (calculated as single-stranded DNA)
- Nucleotide distribution table
- Annotation table

11.2.1 Bioinformatics explained: Protein statistics

Every protein holds specific and individual features which are unique to that particular protein. Features such as isoelectric point or amino acid composition can reveal important information of a novel protein. Many of the features described below are calculated in a simple way.

Molecular weight

The molecular weight is the mass of a protein or molecule. The molecular weight is simply calculated as the sum of the atomic mass of all the atoms in the molecule.

The weight of a protein is usually represented in Daltons (Da).

A calculation of the molecular weight of a protein does not usually include additional posttranslational modifications. For native and unknown proteins it tends to be difficult to assess whether posttranslational modifications such as glycosylations are present on the protein, making a calculation based solely on the amino acid sequence inaccurate. The molecular weight can be determined very accurately by mass-spectrometry in a laboratory.

Isoelectric point

The isoelectric point (pI) of a protein is the pH where the proteins has no net charge. The pI is calculated from the pKa values for 20 different amino acids. At a pH below the pI, the protein carries a positive charge, whereas if the pH is above pI the proteins carry a negative charge. In other words, pI is high for basic proteins and low for acidic proteins. This information can be used in the laboratory when running electrophoretic gels. Here the proteins can be separated, based on their isoelectric point.

Aliphatic index

The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of the following amino acids: alanine, valine, leucine and isoleucine. An increase in the aliphatic index increases the thermostability of globular proteins. The index is calculated by the following formula.

$$Aliphaticindex = X(Ala) + a * X(Val) + b * X(Leu) + b * (X)Ile$$

X(Ala), *X(Val)*, *X(Ile)* and *X(Leu)* are the amino acid compositional fractions. The constants a and b are the relative volume of valine (a=2.9) and leucine/isoleucine (b=3.9) side chains compared to the side chain of alanine [Ikai, 1980].

Estimated half-life

The half life of a protein is the time it takes for the protein pool of that particular protein to be reduced to the half. The half life of proteins is highly dependent on the presence of the N-terminal amino acid, thus overall protein stability [Bachmair et al., 1986, Gonda et al., 1989, Tobias et al., 1991]. The importance of the N-terminal residues is generally known as the 'N-end rule'. The N-end rule and consequently the N-terminal amino acid, simply determines the half-life of proteins. The estimated half-life of proteins have been investigated in mammals, yeast and *E. coli* (see Table 11.1). If leucine is found N-terminally in mammalian proteins the estimated half-life is 5.5 hours.

Extinction coefficient

This measure indicates how much light is absorbed by a protein at a particular wavelength. The extinction coefficient is measured by UV spectrophotometry, but can also be calculated. The amino acid composition is important when calculating the extinction coefficient. The extinction coefficient is calculated from the absorbance of cysteine, tyrosine and tryptophan using the following equation:

$$Ext(Protein) = count(Cystine) * Ext(Cystine) + count(Tyr) * Ext(Tyr) + count(Trp) * Ext(Trp)$$

where Ext is the extinction coefficient of amino acid in question. At 280nm the extinction coefficients are: Cys=120, Tyr=1280 and Trp=5690.

This equation is only valid under the following conditions:

- pH 6.5
- 6.0 M guanidium hydrochloride

Amino acid	Mammalian	Yeast	E. coli
Ala (A)	4.4 hour	>20 hours	>10 hours
Cys (C)	1.2 hours	>20 hours	>10 hours
Asp (D)	1.1 hours	3 min	>10 hours
Glu (E)	1 hour	30 min	>10 hours
Phe (F)	1.1 hours	3 min	2 min
Gly (G)	30 hours	>20 hours	>10 hours
His (H)	3.5 hours	10 min	>10 hours
Ile (I)	20 hours	30 min	>10 hours
Lys (K)	1.3 hours	3 min	2 min
Leu (L)	5.5 hours	3 min	2 min
Met (M)	30 hours	>20 hours	>10 hours
Asn (N)	1.4 hours	3 min	>10 hours
Pro (P)	>20 hours	>20 hours	?
Gln (Q)	0.8 hour	10 min	>10 hours
Arg (R)	1 hour	2 min	2 min
Ser (S)	1.9 hours	>20 hours	>10 hours
Thr (T)	7.2 hours	>20 hours	>10 hours
Val (V)	100 hours	>20 hours	>10 hours
Trp (W)	2.8 hours	3 min	2 min
Tyr (Y)	2.8 hours	10 min	2 min

Table 11.1: **Estimated half life**. Half life of proteins where the N-terminal residue is listed in the first column and the half-life in the subsequent columns for mammals, yeast and *E. coli*.

- 0.02 M phosphate buffer

The extinction coefficient values of the three important amino acids at different wavelengths are found in [Gill and von Hippel, 1989].

Knowing the extinction coefficient, the absorbance (optical density) can be calculated using the following formula:

$$Absorbance(Protein) = \frac{Ext(Protein)}{Molecular\ weight}$$

Two values are reported. The first value is computed assuming that all cysteine residues appear as half cystines, meaning they form di-sulfide bridges to other cysteines. The second number assumes that no di-sulfide bonds are formed.

Atomic composition

Amino acids are indeed very simple compounds. All 20 amino acids consist of combinations of only five different atoms. The atoms which can be found in these simple structures are: Carbon, Nitrogen, Hydrogen, Sulfur, Oxygen. The atomic composition of a protein can for example be used to calculate the precise molecular weight of the entire protein.

Total number of negatively charged residues (Asp+Glu)

At neutral pH, the fraction of negatively charged residues provides information about the location of the protein. Intracellular proteins tend to have a higher fraction of negatively charged residues than extracellular proteins.

Total number of positively charged residues (Arg+Lys)

At neutral pH, nuclear proteins have a high relative percentage of positively charged amino acids. Nuclear proteins often bind to the negatively charged DNA, which may regulate gene expression or help to fold the DNA. Nuclear proteins often have a low percentage of aromatic residues [Andrade et al., 1998].

Amino acid distribution

Amino acids are the basic components of proteins. The amino acid distribution in a protein is simply the percentage of the different amino acids represented in a particular protein of interest. Amino acid composition is generally conserved through family-classes in different organisms which can be useful when studying a particular protein or enzymes across species borders. Another interesting observation is that amino acid composition variate slightly between proteins from different subcellular localizations. This fact has been used in several computational methods, used for prediction of subcellular localization.

Annotation table

This table provides an overview of all the different annotations associated with the sequence and their incidence.

Dipeptide distribution

This measure is simply a count, or frequency, of all the observed adjacent pairs of amino acids (dipeptides) found in the protein. It is only possible to report neighboring amino acids. Knowledge on dipeptide composition have previously been used for prediction of subcellular localization.

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11.3 Join sequences

CLC Sequence Viewer can join several nucleotide or protein sequences into one sequence. This feature can for example be used to construct "supergenes" for phylogenetic inference by joining several disjoint genes into one. Note, that when sequences are joined, all their annotations are carried over to the new spliced sequence.

Two (or more) sequences can be joined by:

select sequences to join | **Toolbox in the Menu Bar** | **General Sequence Analyses** | **Join sequences** ()

or **select sequences to join** | **right-click any selected sequence** | **Toolbox** | **General Sequence Analyses** | **Join sequences** ()

This opens the dialog shown in figure 11.5.

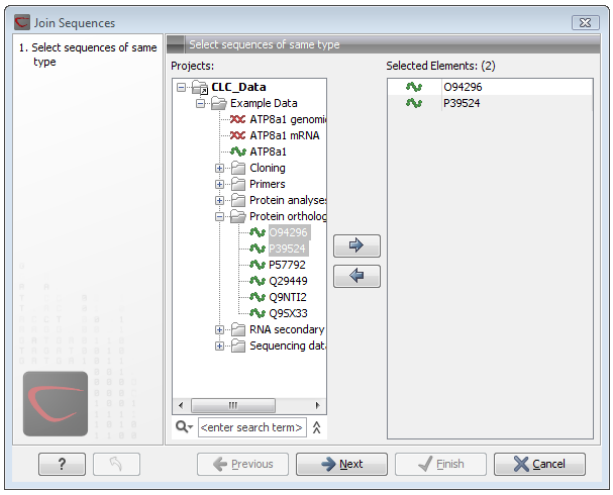


Figure 11.5: *Selecting two sequences to be joined.*

If you have selected some sequences before choosing the Toolbox action, they are now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences from the selected elements. Click **Next** opens the dialog shown in figure 11.6.

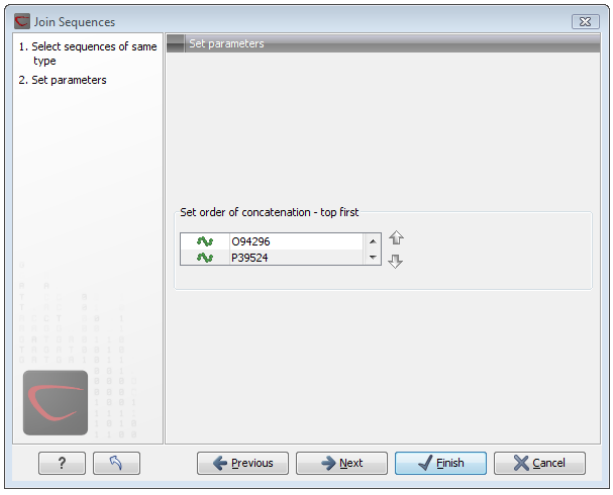


Figure 11.6: *Setting the order in which sequences are joined.*

In step 2 you can change the order in which the sequences will be joined. Select a sequence and use the arrows to move the selected sequence up or down.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

The result is shown in figure 11.7.

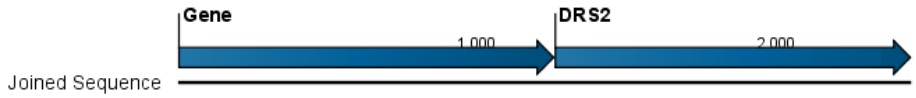


Figure 11.7: *The result of joining sequences is a new sequence containing the annotations of the joined sequences (they each had a HBB annotation).*

Chapter 12

Nucleotide analyses

Contents

CLC Sequence Viewer offers different kinds of sequence analyses, which only apply to DNA and RNA.

12.1 Convert DNA to RNA

CLC Sequence Viewer lets you convert a DNA sequence into RNA, substituting the T residues (Thymine) for U residues (Urasil):

select a DNA sequence in the Navigation Area | **Toolbox in the Menu Bar** | **Nucleotide Analysis** ( )| **Convert DNA to RNA** ( )

or **right-click a sequence in Navigation Area** | **Toolbox** | **Nucleotide Analysis** ( )| **Convert DNA to RNA** ( )

This opens the dialog displayed in figure 12.1:

If a sequence was selected before choosing the Toolbox action, this sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

Note! You can select multiple DNA sequences and sequence lists at a time. If the sequence list contains RNA sequences as well, they will not be converted.

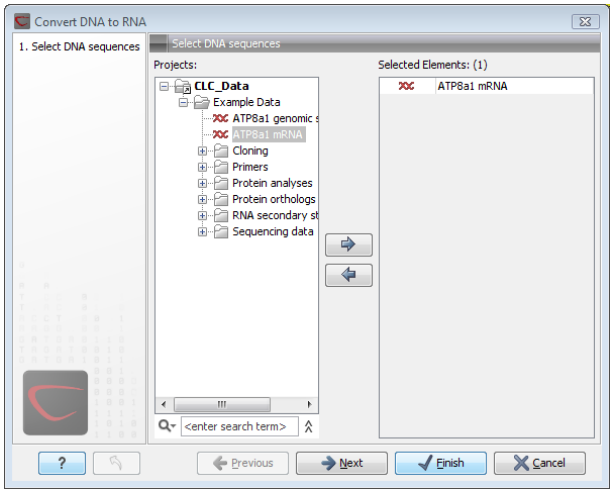


Figure 12.1: *Translating DNA to RNA.*

12.2 Convert RNA to DNA

CLC Sequence Viewer lets you convert an RNA sequence into DNA, substituting the U residues (Urasil) for T residues (Thymine):

select an RNA sequence in the Navigation Area | Toolbox in the Menu Bar | Nucleotide Analysis ( )| Convert RNA to DNA ( )

or **right-click a sequence in Navigation Area | Toolbox | Nucleotide Analysis ( )| Convert RNA to DNA ( )**

This opens the dialog displayed in figure 12.2:

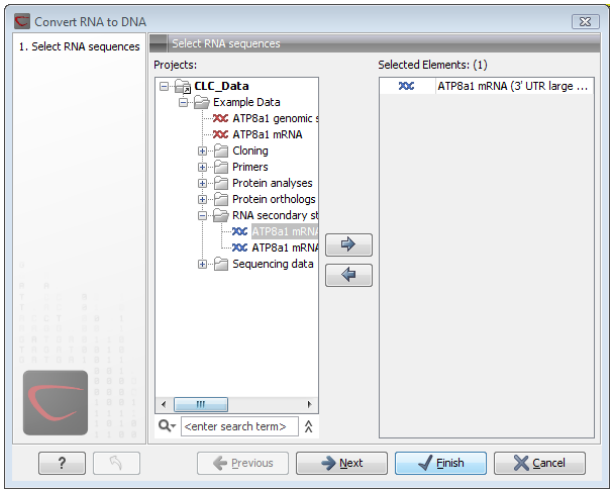


Figure 12.2: *Translating RNA to DNA.*

If a sequence was selected before choosing the Toolbox action, this sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

This will open a new view in the **View Area** displaying the new DNA sequence. The new sequence is not saved automatically. To save the sequence, drag it into the **Navigation Area** or press Ctrl

+ S (⌘ + S on Mac) to activate a save dialog.

Note! You can select multiple RNA sequences and sequence lists at a time. If the sequence list contains DNA sequences as well, they will not be converted.

12.3 Reverse complements of sequences

CLC Sequence Viewer is able to create the reverse complement of a nucleotide sequence. By doing that, a new sequence is created which also has all the annotations reversed since they now occupy the opposite strand of their previous location.

To quickly obtain the reverse complement of a sequence or part of a sequence, you may select a region on the negative strand and open it in a new view:

right-click a selection on the negative strand | **Open selection in New View** ( )

By doing that, the sequence will be reversed. This is only possible when the double stranded view option is enabled. It is possible to copy the selection and paste it in a word processing program or an e-mail. To obtain a reverse complement of an entire sequence:

select a sequence in the Navigation Area | **Toolbox in the Menu Bar** | **Nucleotide Analysis** ( )| **Reverse Complement** ( )

or **right-click a sequence in Navigation Area** | **Toolbox** | **Nucleotide Analysis** ( )| **Reverse Complement** ( )

This opens the dialog displayed in figure 12.3:

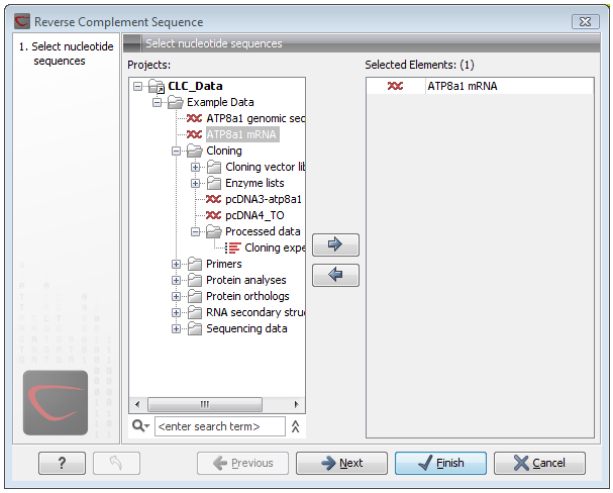


Figure 12.3: *Creating a reverse complement sequence.*

If a sequence was selected before choosing the Toolbox action, the sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

This will open a new view in the **View Area** displaying the reverse complement of the selected sequence. The new sequence is not saved automatically. To save the sequence, drag it into the **Navigation Area** or press Ctrl + S (⌘ + S on Mac) to activate a save dialog.

12.4 Translation of DNA or RNA to protein

In *CLC Sequence Viewer* you can translate a nucleotide sequence into a protein sequence using the **Toolbox** tools. Usually, you use the +1 reading frame which means that the translation starts from the first nucleotide. Stop codons result in an asterisk being inserted in the protein sequence at the corresponding position. It is possible to translate in any combination of the six reading frames in one analysis. To translate:

select a nucleotide sequence | **Toolbox in the Menu Bar** | **Nucleotide Analysis ()**| **Translate to Protein ()**

or **right-click a nucleotide sequence** | **Toolbox** | **Nucleotide Analysis ()**| **Translate to Protein ()**

This opens the dialog displayed in figure 12.4:

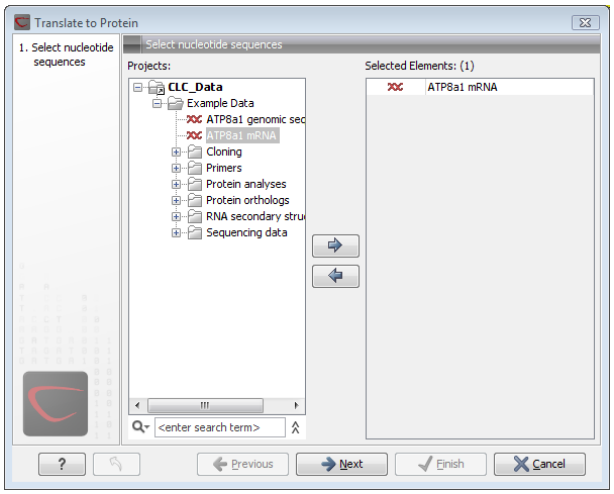


Figure 12.4: *Choosing sequences for translation.*

If a sequence was selected before choosing the Toolbox action, the sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

Clicking **Next** generates the dialog seen in figure 12.5:

Here you have the following options:

Reading frames If you wish to translate the whole sequence, you must specify the reading frame for the translation. If you select e.g. two reading frames, two protein sequences are generated.

Translate coding regions You can choose to translate regions marked by and CDS or ORF annotation. This will generate a protein sequence for each CDS or ORF annotation on the sequence.

Genetic code translation table Lets you specify the genetic code for the translation. The translation tables are occasionally updated from NCBI. The tables are not available in this printable version of the user manual. Instead, the tables are included in the **Help**-menu in the **Menu Bar** (in the appendix).

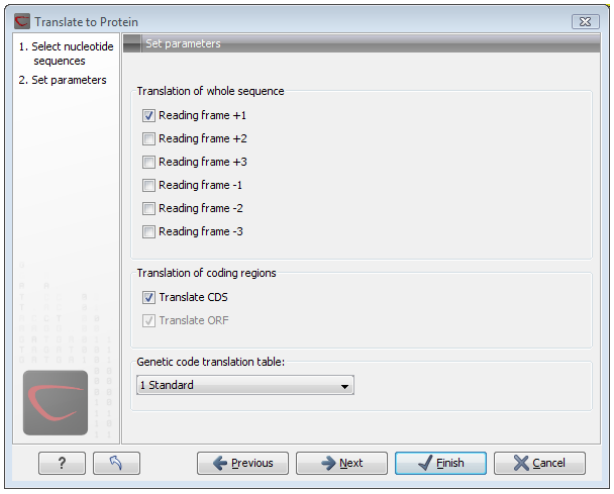


Figure 12.5: *Choosing +1 and +3 reading frames, and the standard translation table.*

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**. The newly created protein is shown, but is not saved automatically.

To save a protein sequence, drag it into the **Navigation Area** or press Ctrl + S (⌘ + S on Mac) to activate a save dialog.

12.5 Find open reading frames

The *CLC Sequence Viewer* **Find Open Reading Frames** function can be used to find all open reading frames (ORF) in a sequence, or, by choosing particular start codons to use, it can be used as a rudimentary gene finder. ORFs identified will be shown as annotations on the sequence. You have the option of choosing a translation table, the start codons to use, minimum ORF length as well as a few other parameters. These choices are explained in this section.

To find open reading frames:

select a nucleotide sequence | **Toolbox in the Menu Bar** | **Nucleotide Analysis ()**| **Find Open Reading Frames ()**

or **right-click a nucleotide sequence** | **Toolbox** | **Nucleotide Analysis ()**| **Find Open Reading Frames ()**

This opens the dialog displayed in figure 12.6:

If a sequence was selected before choosing the Toolbox action, the sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

If you want to adjust the parameters for finding open reading frames click **Next**.

12.5.1 Open reading frame parameters

This opens the dialog displayed in figure 12.7:

The adjustable parameters for the search are:

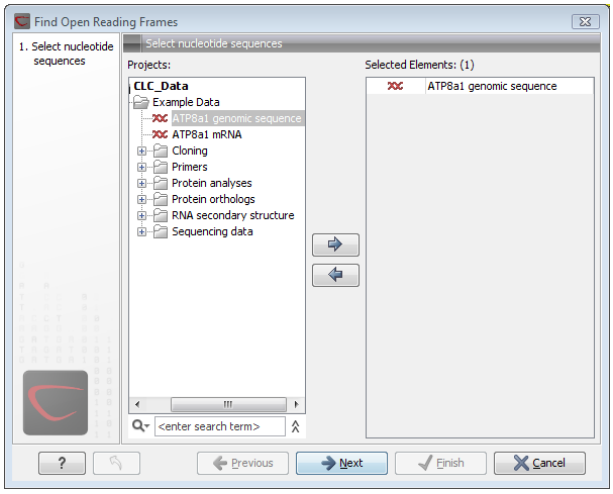


Figure 12.6: *Create Reading Frame dialog.*

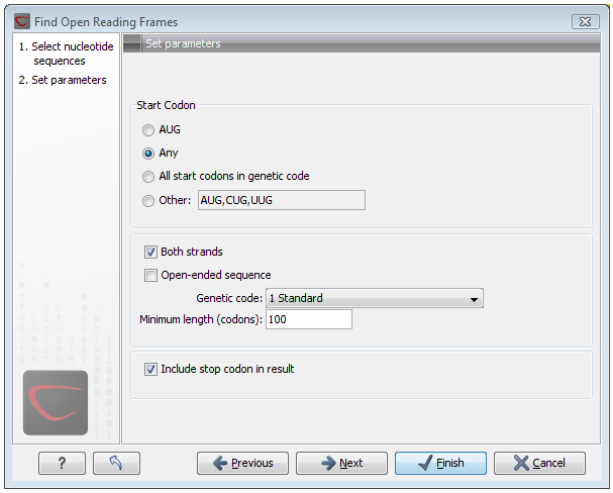


Figure 12.7: *Create Reading Frame dialog.*

- **Start codon**:
  - **AUG**. Most commonly used start codon.
  - **Any**. Find all open reading frames.
  - **All start codons in genetic code**.
  - **Other**. Here you can specify a number of start codons separated by commas.
- **Both strands**. Finds reading frames on both strands.
- **Open-ended Sequence**. Allows the ORF to start or end outside the sequence. If the sequence studied is a part of a larger sequence, it may be advantageous to allow the ORF to start or end outside the sequence.
- **Genetic code translation table**.
- **Include stop codon in result** The ORFs will be shown as annotations which can include the stop codon if this option is checked. The translation tables are occasionally updated from NCBI. The tables are not available in this printable version of the user manual. Instead, the tables are included in the **Help**-menu in the **Menu Bar** (in the appendix).

- **Minimum Length**. Specifies the minimum length for the ORFs to be found. The length is specified as number of codons.

Using open reading frames for gene finding is a fairly simple approach which is likely to predict genes which are not real. Setting a relatively high minimum length of the ORFs will reduce the number of false positive predictions, but at the same time short genes may be missed (see figure 12.8).

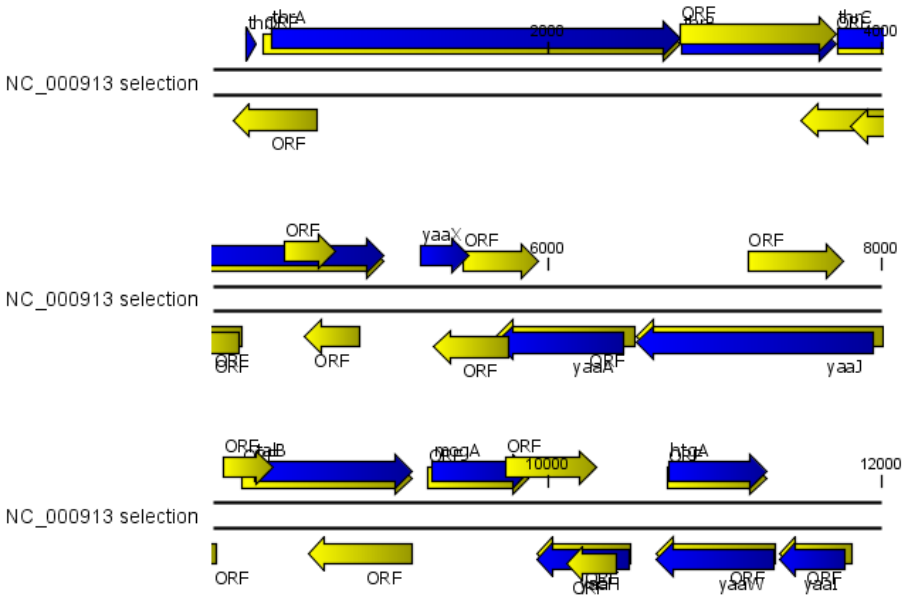


Figure 12.8: *The first 12,000 positions of the* E. coli *sequence NC_000913 downloaded from GenBank. The blue (dark) annotations are the genes while the yellow (brighter) annotations are the ORFs with a length of at least 100 amino acids. On the positive strand around position 11,000, a gene starts before the ORF. This is due to the use of the standard genetic code rather than the bacterial code. This particular gene starts with CTG, which is a start codon in bacteria. Two short genes are entirely missing, while a handful of open reading frames do not correspond to any of the annotated genes.*

Click **Next** if you wish to adjust how to handle the results (see section 8.1). If not, click **Finish**.

Finding open reading frames is often a good first step in annotating sequences such as cloning vectors or bacterial genomes. For eukaryotic genes, ORF determination may not always be very helpful since the intron/exon structure is not part of the algorithm.

Chapter 13

Restriction site analyses

Contents

There are two ways of finding and showing restriction sites:

- In many cases, the dynamic restriction sites found in the **Side Panel** of sequence views will be useful, since it is a quick and easy way of showing restriction sites.
- In the **Toolbox** you will find the other way of doing restriction site analyses. This way provides more control of the analysis and gives you more output options, e.g. a table of restriction sites and you can perform the same restriction map analysis on several sequences in one step.

This chapter first describes the dynamic restriction sites, followed by "the toolbox way". The final section in this chapter focuses on enzyme lists which represent an easy way of managing restriction enzymes.

13.1 Dynamic restriction sites

If you open a sequence, a sequence list etc, you will find the **Restriction Sites** group in the **Side Panel**.

As shown in figure 13.1 you can display restriction sites as colored triangles and lines on the sequence. The **Restriction sites** group in the side panel shows a list of enzymes, represented by different colors corresponding to the colors of the triangles on the sequence. By selecting or deselecting the enzymes in the list, you can specify which enzymes' restriction sites should be displayed.

Figure 13.1: *Showing restriction sites of ten restriction enzymes.*

The color of the restriction enzyme can be changed by clicking the colored box next to the enzyme's name. The name of the enzyme can also be shown next to the restriction site by selecting **Show name flags** above the list of restriction enzymes.

There is also an option to specify how the **Labels** shown be shown:

- **No labels**. This will just display the cut site with no information about the name of the enzyme. Placing the mouse button on the cut site will reveal this information as a tool tip.
- **Flag**. This will place a flag just above the sequence with the enzyme name (see an example in figure 13.2). Note that this option will make it hard to see when several cut sites are located close to each other. In the circular view, this option is replaced by the Radial option:
- **Radial**. This option is only available in the circular view. It will place the restriction site labels as close to the cut site as possible (see an example in figure 13.4).
- **Stacked**. This is similar to the flag option for linear sequence views, but it will stack the labels so that all enzymes are shown. For circular views, it will align all the labels on each side of the circle. This can be useful for clearly seeing the order of the cut sites when they are located closely together (see an example in figure 13.3).

Note that in a circular view, the **Stacked** and **Radial** options also affect the layout of annotations.

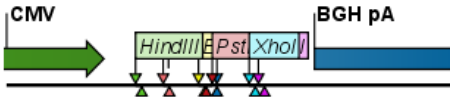


Figure 13.2: *Restriction site labels shown as flags.*

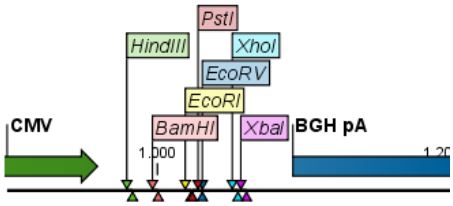


Figure 13.3: *Restriction site labels stacked.*

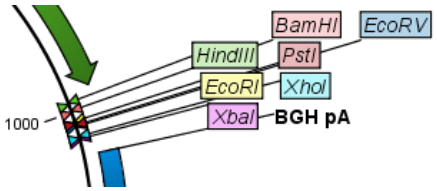


Figure 13.4: *Restriction site labels in radial layout.*

13.1.1 Sort enzymes

Just above the list of enzymes there are three buttons to be used for sorting the list (see figure 13.5):

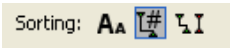


Figure 13.5: *Buttons to sort restriction enzymes.*

- **Sort enzymes alphabetically** (A$_A$). Clicking this button will sort the list of enzymes alphabetically.
- **Sort enzymes by number of restriction sites** (#). This will divide the enzymes into four groups:
  - Non-cutters.
  - Single cutters.
  - Double cutters.
  - Multiple cutters.

  There is a checkbox for each group which can be used to hide / show all the enzymes in a group.
-
- **Sort enzymes by overhang** (I). This will divide the enzymes into three groups:
  - Blunt. Enzymes cutting both strands at the same position.
  - 3'. Enzymes producing an overhang at the 3' end.
  - 5'. Enzymes producing an overhang at the 5' end.

  There is a checkbox for each group which can be used to hide / show all the enzymes in a group.

13.1.2 Manage enzymes

The list of restriction enzymes contains per default 20 of the most popular enzymes, but you can easily modify this list and add more enzymes by clicking the **Manage enzymes button.** This will display the dialog shown in figure 13.6.

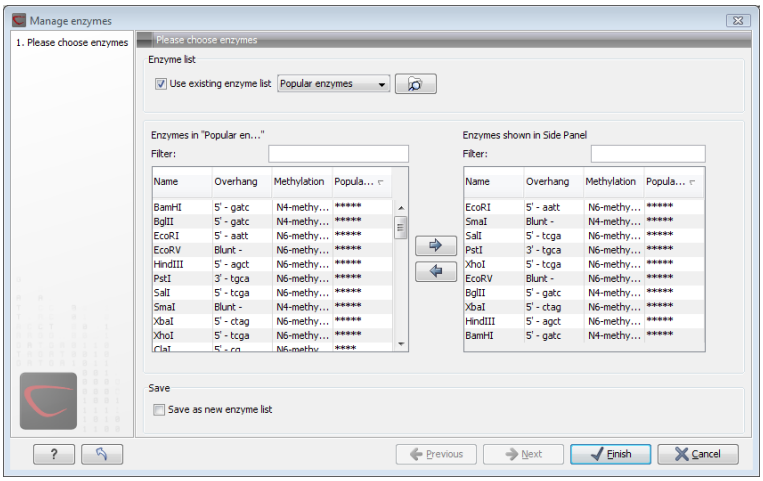


Figure 13.6: *Adding or removing enzymes from the Side Panel.*

At the top, you can choose to **Use existing enzyme list**. Clicking this option lets you select an enzyme list which is stored in the **Navigation Area**. See section 13.3 for more about creating and modifying enzyme lists.

Below there are two panels:

- To the **left**, you see all the enzymes that are in the list select above. If you have not chosen to use an existing enzyme list, this panel shows all the enzymes available [1].
- To the **right**, there is a list of the enzymes that will be used.

Select enzymes in the left side panel and add them to the right panel by double-clicking or clicking the **Add** button (➡). If you e.g. wish to use EcoRV and BamHI, select these two enzymes and add them to the right side panel.

If you wish to use all the enzymes in the list:

Click in the panel to the left | press Ctrl + A (⌘ + A on Mac) | Add (➡)

The enzymes can be sorted by clicking the column headings, i.e. Name, Overhang, Methylation or Popularity. This is particularly useful if you wish to use enzymes which produce e.g. a 3' overhang. In this case, you can sort the list by clicking the Overhang column heading, and all the enzymes producing 3' overhangs will be listed together for easy selection.

When looking for a specific enzyme, it is easier to use the Filter. If you wish to find e.g. HindIII sites, simply type HindIII into the filter, and the list of enzymes will shrink automatically to only include the HindIII enzyme. This can also be used to only show enzymes producing e.g. a 3' overhang as shown in figure 13.17.

[1] The *CLC Sequence Viewer* comes with a standard set of enzymes based on http://www.rebase.neb.com. You can customize the enzyme database for your installation, see section **??**

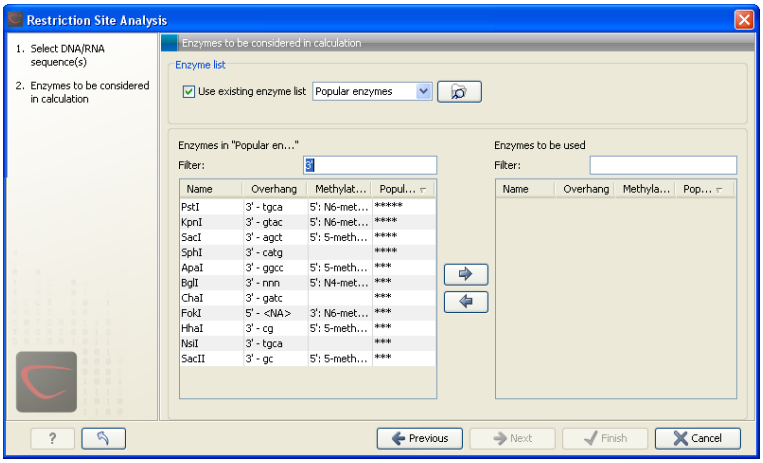


Figure 13.7: *Selecting enzymes.*

If you need more detailed information and filtering of the enzymes, either place your mouse cursor on an enzyme for one second to display additional information (see figure 13.18), or use the view of enzyme lists (see 13.3).

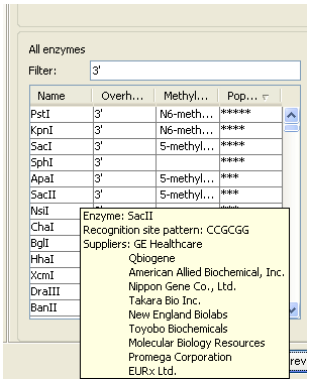


Figure 13.8: *Showing additional information about an enzyme like recognition sequence or a list of commercial vendors.*

At the bottom of the dialog, you can select to save this list of enzymes as a new file. In this way, you can save the selection of enzymes for later use.

When you click **Finish**, the enzymes are added to the Side Panel and the cut sites are shown on the sequence.

If you have specified a set of enzymes which you always use, it will probably be a good idea to save the settings in the Side Panel (see section 3.2.7) for future use.

13.2 Restriction site analysis from the Toolbox

Besides the dynamic restriction sites, you can do a more elaborate restriction map analysis with more output format using the Toolbox:

Toolbox | **Restriction Sites (**  **)** | **Restriction Site Analysis (**  **)**

This will display the dialog shown in figure 13.9.

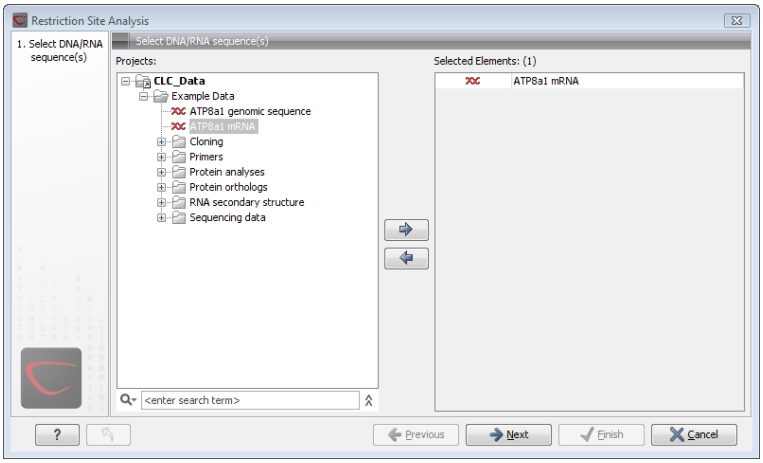


Figure 13.9: *Choosing sequence ATP8a1 mRNA for restriction map analysis.*

If a sequence was selected before choosing the Toolbox action, this sequence is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences or sequence lists from the selected elements.

13.2.1 Selecting, sorting and filtering enzymes

Clicking **Next** lets you define which enzymes to use as basis for finding restriction sites on the sequence. At the top, you can choose to **Use existing enzyme list**. Clicking this option lets you select an enzyme list which is stored in the **Navigation Area**. See section 13.3 for more about creating and modifying enzyme lists.

Below there are two panels:

- To the **left**, you see all the enzymes that are in the list select above. If you have not chosen to use an existing enzyme list, this panel shows all the enzymes available [2].
- To the **right**, there is a list of the enzymes that will be used.

Select enzymes in the left side panel and add them to the right panel by double-clicking or clicking the **Add** button (➡). If you e.g. wish to use EcoRV and BamHI, select these two enzymes and add them to the right side panel.

If you wish to use all the enzymes in the list:

Click in the panel to the left | press Ctrl + A (⌘ + A on Mac) | Add (➡)

The enzymes can be sorted by clicking the column headings, i.e. Name, Overhang, Methylation or Popularity. This is particularly useful if you wish to use enzymes which produce e.g. a 3' overhang. In this case, you can sort the list by clicking the Overhang column heading, and all the enzymes producing 3' overhangs will be listed together for easy selection.

When looking for a specific enzyme, it is easier to use the Filter. If you wish to find e.g. HindIII sites, simply type HindIII into the filter, and the list of enzymes will shrink automatically to only include the HindIII enzyme. This can also be used to only show enzymes producing e.g. a 3' overhang as shown in figure 13.17.

[2]The *CLC Sequence Viewer* comes with a standard set of enzymes based on http://www.rebase.neb.com.

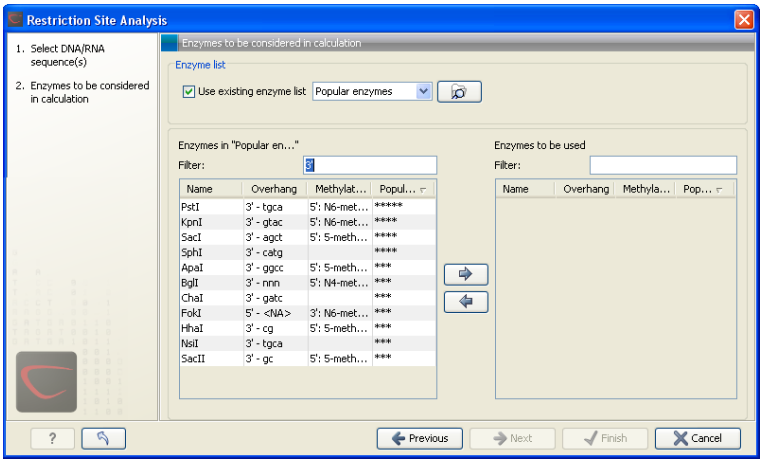


Figure 13.10: *Selecting enzymes.*

If you need more detailed information and filtering of the enzymes, either place your mouse cursor on an enzyme for one second to display additional information (see figure 13.18), or use the view of enzyme lists (see 13.3).

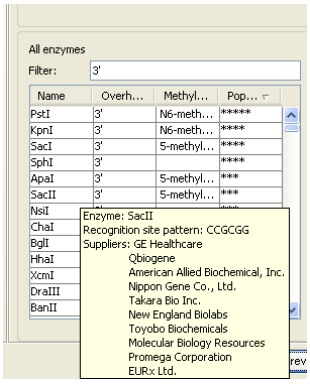


Figure 13.11: *Showing additional information about an enzyme like recognition sequence or a list of commercial vendors.*

13.2.2 Number of cut sites

Clicking **Next** confirms the list of enzymes which will be included in the analysis, and takes you to the dialog shown in figure 13.12.

If you wish the output of the restriction map analysis only to include restriction enzymes which cut the sequence a specific number of times, use the checkboxes in this dialog:

- No restriction site (**0**)
- One restriction site (**1**)
- Two restriction sites (**2**)
- Three restriction site (**3**)
- N restriction sites

You can customize the enzyme database for your installation, see section **??**

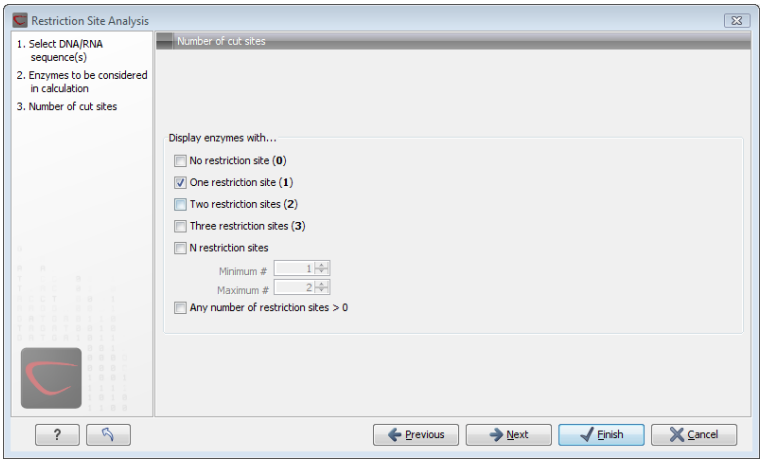


Figure 13.12: *Selecting number of cut sites.*

  - Minimum
  - Maximum

- Any number of restriction sites > 0

The default setting is to include the enzymes which cut the sequence one or two times.

You can use the checkboxes to perform very specific searches for restriction sites: e.g. if you wish to find enzymes which do not cut the sequence, or enzymes cutting exactly twice.

13.2.3 Output of restriction map analysis

Clicking next shows the dialog in figure 13.13.

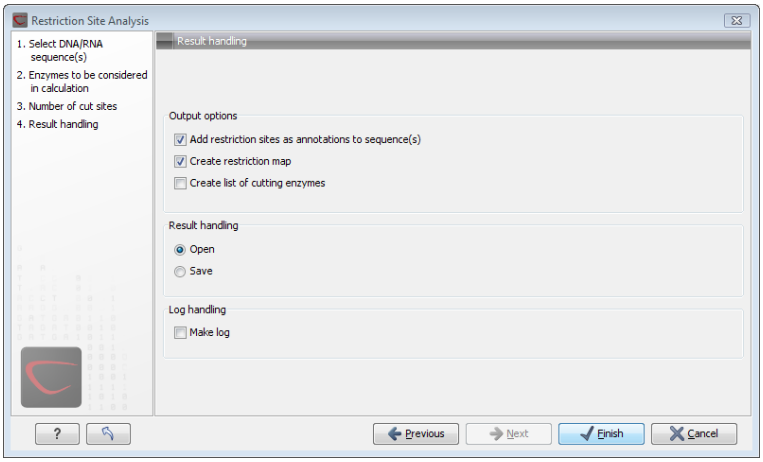


Figure 13.13: *Choosing to add restriction sites as annotations or creating a restriction map.*

This dialog lets you specify how the result of the restriction map analysis should be presented:

- **Add restriction sites as annotations to sequence(s)**. This option makes it possible to see the restriction sites on the sequence (see figure 13.14) and save the annotations for later use.

- **Create restriction map**. The restriction map is a table of restriction sites as shown in figure 13.15. If more than one sequence were selected, the table will include the restriction sites of all the sequences. This makes it easy to compare the result of the restriction map analysis for two sequences (or more).

The following sections will describe these output formats in more detail.

In order to complete the analysis click **Finish** (see section 8.1 for information about the Save and Open options).

13.2.4 Restriction sites as annotation on the sequence

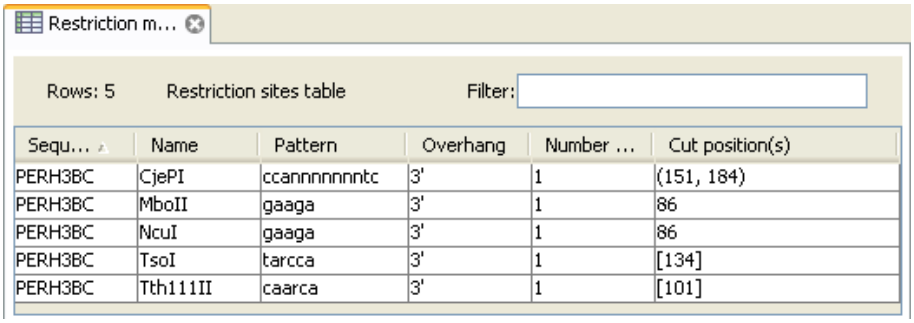
If you chose to add the restriction sites as annotation to the sequence, the result will be similar to the sequence shown in figure 13.14. See section 9.3 for more information about viewing

Figure 13.14: *The result of the restriction analysis shown as annotations.*

annotations.

13.2.5 Table of restriction sites

The restriction map can be shown as a table of restriction sites (see figure 13.15).



Sequ...	Name	Pattern	Overhang	Number ...	Cut position(s)
PERH3BC	CjePI	ccannnnnnntc	3'	1	(151, 184)
PERH3BC	MboII	gaaga	3'	1	86
PERH3BC	NcuI	gaaga	3'	1	86
PERH3BC	TsoI	tarcca	3'	1	[134]
PERH3BC	Tth111II	caarca	3'	1	[101]

Figure 13.15: *The result of the restriction analysis shown as annotations.*

Each row in the table represents a restriction enzyme. The following information is available for each enzyme:

- **Sequence**. The name of the sequence which is relevant if you have performed restriction map analysis on more than one sequence.
- **Name**. The name of the enzyme.
- **Pattern**. The recognition sequence of the enzyme.
- **Overhang**. The overhang produced by cutting with the enzyme (3', 5' or Blunt).
- **Number of cut sites**.

- **Cut position(s)**. The position of each cut.
  - **,** If the enzyme cuts more than once, the positions are separated by commas.
  - **[]** If the enzyme's recognition sequence is on the negative strand, the cut position is put in brackets (as the enzyme TsoI in figure 13.15 whose cut position is [134]).
  - **()** Some enzymes cut the sequence twice for each recognition site, and in this case the two cut positions are surrounded by parentheses.

13.3 Restriction enzyme lists

CLC Sequence Viewer includes all the restriction enzymes available in the **REBASE** database[3]. However, when performing restriction site analyses, it is often an advantage to use a customized list of enzymes. In this case, the user can create special lists containing e.g. all enzymes available in the laboratory freezer, all enzymes used to create a given restriction map or all enzymes that are available form the preferred vendor.

In the example data (see section 1.5.2) under Nucleotide->Restriction analysis, there are two enzyme lists: one with the 50 most popular enzymes, and another with all enzymes that are included in the *CLC Sequence Viewer*.

This section describes how you can create an enzyme list, and how you can modify it.

13.3.1 Create enzyme list

CLC Sequence Viewer uses enzymes from the **REBASE** restriction enzyme database at `http://rebase.neb.com`[4].

To create an enzyme list of a subset of these enzymes:

File | **New** | **Enzyme list (**  **)**

This opens the dialog shown in figure 13.16

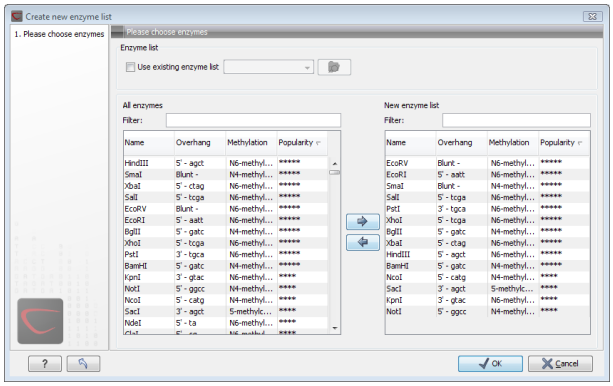


Figure 13.16: *Choosing enzymes for the new enzyme list.*

At the top, you can choose to **Use existing enzyme list**. Clicking this option lets you select an enzyme list which is stored in the **Navigation Area**. See section 13.3 for more about creating and modifying enzyme lists.

[3] You can customize the enzyme database for your installation, see section **??**
[4] You can customize the enzyme database for your installation, see section **??**

Below there are two panels:

- To the **left**, you see all the enzymes that are in the list select above. If you have not chosen to use an existing enzyme list, this panel shows all the enzymes available [5].
- To the **right**, there is a list of the enzymes that will be used.

Select enzymes in the left side panel and add them to the right panel by double-clicking or clicking the **Add** button (). If you e.g. wish to use EcoRV and BamHI, select these two enzymes and add them to the right side panel.

If you wish to use all the enzymes in the list:

Click in the panel to the left | press Ctrl + A (⌘ + A on Mac) | Add ()

The enzymes can be sorted by clicking the column headings, i.e. Name, Overhang, Methylation or Popularity. This is particularly useful if you wish to use enzymes which produce e.g. a 3' overhang. In this case, you can sort the list by clicking the Overhang column heading, and all the enzymes producing 3' overhangs will be listed together for easy selection.

When looking for a specific enzyme, it is easier to use the Filter. If you wish to find e.g. HindIII sites, simply type HindIII into the filter, and the list of enzymes will shrink automatically to only include the HindIII enzyme. This can also be used to only show enzymes producing e.g. a 3' overhang as shown in figure 13.17.

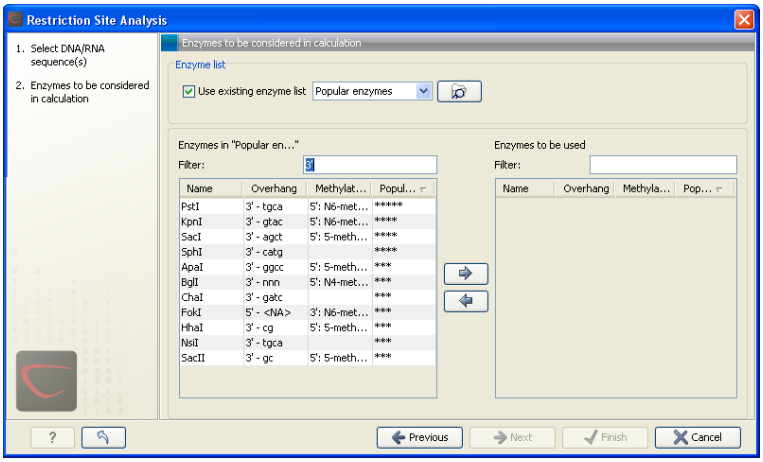


Figure 13.17: *Selecting enzymes.*

If you need more detailed information and filtering of the enzymes, either place your mouse cursor on an enzyme for one second to display additional information (see figure 13.18), or use the view of enzyme lists (see 13.3).

Click **Finish** to open the enzyme list.

13.3.2 View and modify enzyme list

An enzyme list is shown in figure 13.19. The list can be sorted by clicking the columns,

[5] The *CLC Sequence Viewer* comes with a standard set of enzymes based on http://www.rebase.neb.com. You can customize the enzyme database for your installation, see section **??**

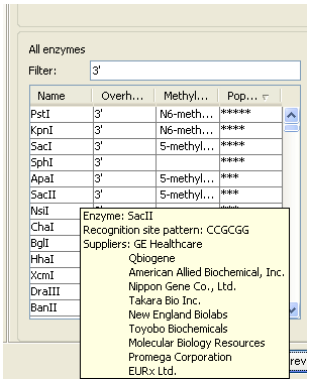


Figure 13.18: *Showing additional information about an enzyme like recognition sequence or a list of commercial vendors.*

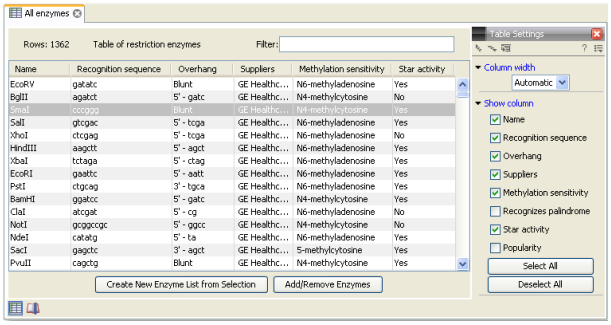


Figure 13.19: *An enzyme list.*

and you can use the filter at the top right corner to search for specific enzymes, recognition sequences etc.

If you wish to remove or add enzymes, click the **Add/Remove Enzymes** button at the bottom of the view. This will present the same dialog as shown in figure 13.16 with the enzyme list shown to the right.

If you wish to extract a subset of an enzyme list:

> **open the list** | **select the relevant enzymes** | **right-click** | **Create New Enzyme List from Selection** ()

If you combined this method with the filter located at the top of the view, you can extract a very specific set of enzymes. E.g. if you wish to create a list of enzymes sold by a particular distributor, type the name of the distributor into the filter, and select and create a new enzyme list from the selection.

Chapter 14

Sequence alignment

Contents

CLC Sequence Viewer can align nucleotides and proteins using a *progressive alignment* algorithm (see section 14.4 or read the White paper on alignments in the **Science** section of http://www.clcbio.com).
This chapter describes how to use the program to align sequences. The chapter also describes alignment algorithms in more general terms.

14.1 Create an alignment

To create an alignment in *CLC Sequence Viewer*:

select sequences to align | **Toolbox in the Menu Bar** | **Alignments and Trees** ( )| **Create Alignment** ( )

or **select sequences to align** | **right-click any selected sequence** | **Toolbox** | **Alignments and Trees** ( )| **Create Alignment** ( )

This opens the dialog shown in figure 14.1.

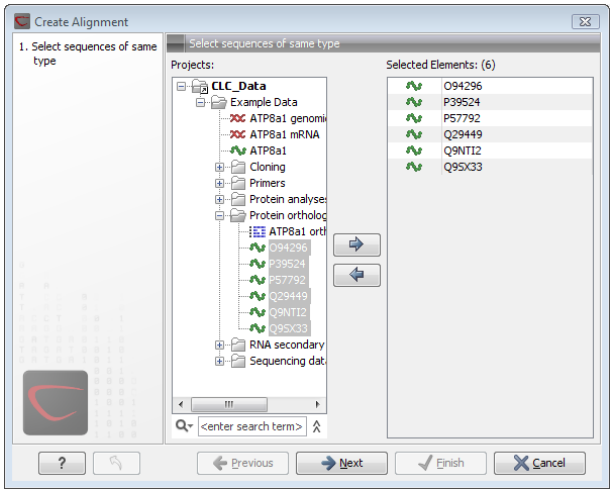


Figure 14.1: *Creating an alignment.*

If you have selected some elements before choosing the Toolbox action, they are now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove sequences, sequence lists or alignments from the selected elements. Click **Next** to adjust alignment algorithm parameters. Clicking **Next** opens the dialog shown in figure 14.2.

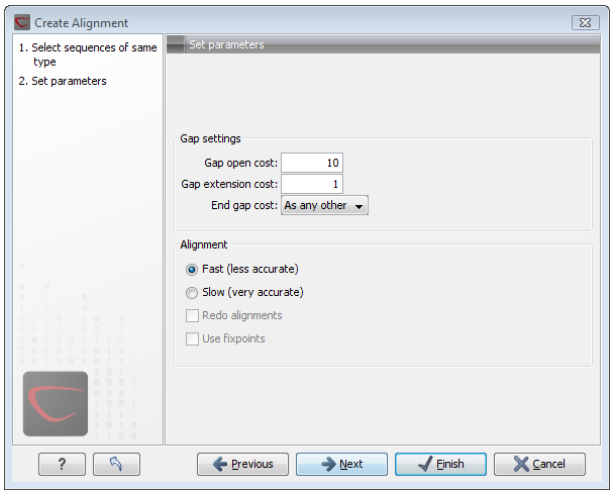


Figure 14.2: *Adjusting alignment algorithm parameters.*

14.1.1 Gap costs

The alignment algorithm has three parameters concerning gap costs: Gap open cost, Gap extension cost and End gap cost. The precision of these parameters is to one place of decimal.

- **Gap open cost**. The price for introducing gaps in an alignment.
- **Gap extension cost**. The price for every extension past the initial gap.

If you expect a lot of small gaps in your alignment, the Gap open cost should equal the Gap extension cost. On the other hand, if you expect few but large gaps, the Gap open cost should be set significantly higher than the Gap extension cost.

However, for most alignments it is a good idea to make the Gap open cost quite a bit higher than the Gap extension cost. The default values are 10.0 and 1.0 for the two parameters, respectively.

- **End gap cost**. The price of gaps at the beginning or the end of the alignment. One of the advantages of the *CLC Sequence Viewer* alignment method is that it provides flexibility in the treatment of gaps at the ends of the sequences. There are three possibilities:
  - **Free end gaps**. Any number of gaps can be inserted in the ends of the sequences without any cost.
  - **Cheap end gaps**. All end gaps are treated as gap extensions and any gaps past 10 are free.
  - **End gaps as any other**. Gaps at the ends of sequences are treated like gaps in any other place in the sequences.

When aligning a long sequence with a short partial sequence, it is ideal to use free end gaps, since this will be the best approximation to the situation. The many gaps inserted at the ends are not due to evolutionary events, but rather to partial data.

Many homologous proteins have quite different ends, often with large insertions or deletions. This confuses alignment algorithms, but using the **Cheap end gaps** option, large gaps will generally be tolerated at the sequence ends, improving the overall alignment. This is the default setting of the algorithm.

Finally, treating end gaps like any other gaps is the best option when you know that there are no biologically distinct effects at the ends of the sequences.

Figures 14.3 and 14.4 illustrate the differences between the different gap scores at the sequence ends.

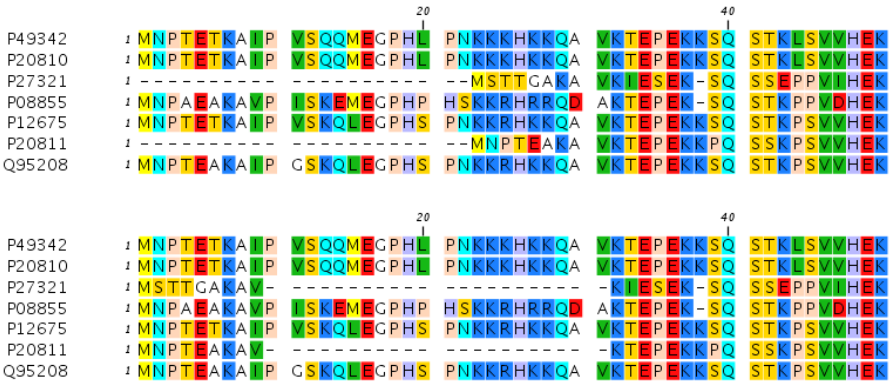


Figure 14.3: *The first 50 positions of two different alignments of seven calpastatin sequences. The top alignment is made with cheap end gaps, while the bottom alignment is made with end gaps having the same price as any other gaps. In this case it seems that the latter scoring scheme gives the best result.*

14.1.2 Fast or accurate alignment algorithm

CLC Sequence Viewer has two algorithms for calculating alignments:

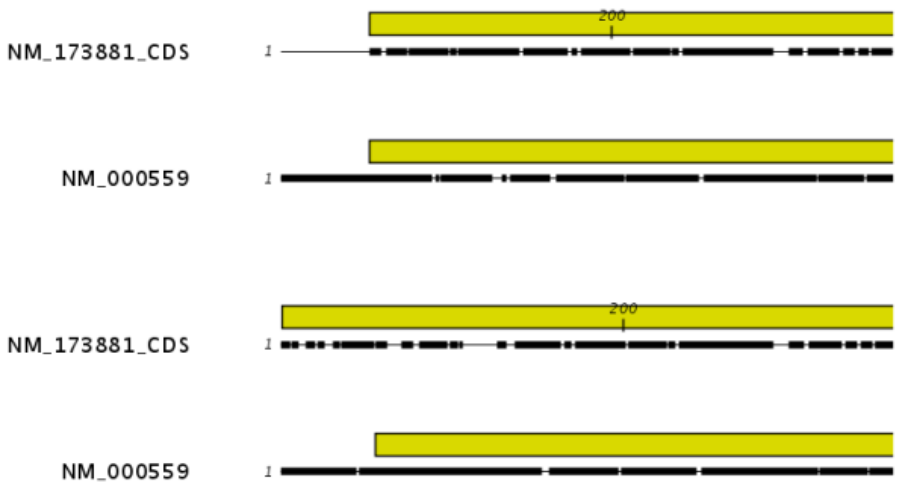


Figure 14.4: *The alignment of the coding sequence of bovine myoglobin with the full mRNA of human gamma globin. The top alignment is made with free end gaps, while the bottom alignment is made with end gaps treated as any other. The yellow annotation is the coding sequence in both sequences. It is evident that free end gaps are ideal in this situation as the start codons are aligned correctly in the top alignment. Treating end gaps as any other gaps in the case of aligning distant homologs where one sequence is partial leads to a spreading out of the short sequence as in the bottom alignment.*

- **Fast (less accurate).** This allows for use of an optimized alignment algorithm which is very fast. The fast option is particularly useful for data sets with very long sequences.
- **Slow (very accurate).** This is the recommended choice unless you find the processing time too long.

Both algorithms use progressive alignment. The faster algorithm builds the initial tree by doing more approximate pairwise alignments than the slower option.

14.2 View alignments

Since an alignment is a display of several sequences arranged in rows, the basic options for viewing alignments are the same as for viewing sequences. Therefore we refer to section 9.1 for an explanation of these basic options.

However, there are a number of alignment-specific view options in the **Alignment info** and the **Nucleotide info** in the **Side Panel** to the right of the view. Below is more information on these view options.

Under **Translation** in the **Nucleotide info**, there is an extra checkbox: **Relative to top sequence**. Checking this box will make the reading frames for the translation align with the top sequence so that you can compare the effect of nucleotide differences on the protein level.

The options in the **Alignment info** relate to each column in the alignment:

- **Consensus.** Shows a consensus sequence at the bottom of the alignment. The consensus sequence is based on every single position in the alignment and reflects an artificial sequence which resembles the sequence information of the alignment, but only as one

single sequence. If all sequences of the alignment is 100% identical the consensus sequence will be identical to all sequences found in the alignment. If the sequences of the alignment differ the consensus sequence will reflect the most common sequences in the alignment. Parameters for adjusting the consensus sequences are described below.

- **Limit.** This option determines how conserved the sequences must be in order to agree on a consensus. Here you can also choose **IUPAC** which will display the ambiguity code when there are differences between the sequences. E.g. an alignment with **A** and a **G** at the same position will display an **R** in the consensus line if the **IUPAC** option is selected. (The IUPAC codes can be found in section F and E.)
- **No gaps.** Checking this option will not show gaps in the consensus.
- **Ambiguous symbol.** Select how ambiguities should be displayed in the consensus line (as **N**, **?**, *****, **.** or **-**). This option has now effect if **IUPAC** is selected in the **Limit** list above.

The **Consensus Sequence** can be opened in a new view, simply by right-clicking the **Consensus Sequence** and click **Open Consensus in New View**.

- **Conservation.** Displays the level of conservation at each position in the alignment. The conservation shows the conservation of all sequence positions. The height of the bar, or the gradient of the color reflect how conserved that particular position is in the alignment. If one position is 100% conserved the bar will be shown in full height, and it is colored in the color specified at the right side of the gradient slider.
  - **Foreground color.** Colors the letters using a gradient, where the right side color is used for highly conserved positions and the left side color is used for positions that are less conserved.
  - **Background color.** Sets a background color of the residues using a gradient in the same way as described above.
  - **Graph.** Displays the conservation level as a graph at the bottom of the alignment. The bar (default view) show the conservation of all sequence positions. The height of the graph reflects how conserved that particular position is in the alignment. If one position is 100% conserved the graph will be shown in full height. Learn how to export the data behind the graph in section 6.4.
    - **Height.** Specifies the height of the graph.
    - **Type.** The type of the graph.
      - **Line plot.** Displays the graph as a line plot.
      - **Bar plot.** Displays the graph as a bar plot.
      - **Colors.** Displays the graph as a color bar using a gradient like the foreground and background colors.
    - **Color box.** Specifies the color of the graph for line and bar plots, and specifies a gradient for colors.
- **Gap fraction.** Which fraction of the sequences in the alignment that have gaps. The gap fraction is only relevant if there are gaps in the alignment.
  - **Foreground color.** Colors the letter using a gradient, where the left side color is used if there are relatively few gaps, and the right side color is used if there are relatively many gaps.

  - **Background color.** Sets a background color of the residues using a gradient in the same way as described above.
  - **Graph.** Displays the gap fraction as a graph at the bottom of the alignment (Learn how to export the data behind the graph in section 6.4).
    * **Height.** Specifies the height of the graph.
    * **Type.** The type of the graph.
      · **Line plot.** Displays the graph as a line plot.
      · **Bar plot.** Displays the graph as a line plot.
      · **Colors.** Displays the graph as a color bar using a gradient like the foreground and background colors.
    * **Color box.** Specifies the color of the graph for line and bar plots, and specifies a gradient for colors.

- **Color different residues.** Indicates differences in aligned residues.
  - **Foreground color.** Colors the letter.
  - **Background color.** Sets a background color of the residues.

14.3 Edit alignments

14.3.1 Move residues and gaps

The placement of gaps in the alignment can be changed by modifying the parameters when creating the alignment (see section 14.1). However, gaps and residues can also be moved after the alignment is created:

select one or more gaps or residues in the alignment | drag the selection to move

This can be done both for single sequences, but also for multiple sequences by making a selection covering more than one sequence. When you have made the selection, the mouse pointer turns into a horizontal arrow indicating that the selection can be moved (see figure 14.5).

Note! Residues can only be moved when they are next to a gap.

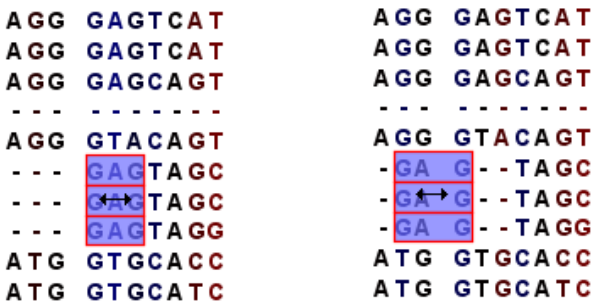


Figure 14.5: *Moving a part of an alignment. Notice the change of mouse pointer to a horizontal arrow.*

14.3.2 Insert gaps

The placement of gaps in the alignment can be changed by modifying the parameters when creating the alignment. However, gaps can also be added manually after the alignment is

created.

To insert extra gaps:

select a part of the alignment | **right-click the selection** | **Add gaps before/after**

If you have made a selection covering e.g. five residues, a gap of five will be inserted. In this way you can easily control the number of gaps to insert. Gaps will be inserted in the sequences that you selected. If you make a selection in two sequences in an alignment, gaps will be inserted into these two sequences. This means that these two sequences will be displaced compared to the other sequences in the alignment.

14.3.3 Delete residues and gaps

Residues or gaps can be deleted for individual sequences or for the whole alignment. For individual sequences:

select the part of the sequence you want to delete | **right-click the selection** | **Edit Selection (**  **)** | **Delete the text in the dialog** | **Replace**

The selection shown in the dialog will be replaced by the text you enter. If you delete the text, the selection will be replaced by an empty text, i.e. deleted.

To delete entire columns:

select the part of the alignment you want to delete | **right-click the selection** | **Delete columns**

The selection may cover one or more sequences, but the **Delete columns** function will always apply to the entire alignment.

14.3.4 Move sequences up and down

Sequences can be moved up and down in the alignment:

drag the name of the sequence up or down

When you move the mouse pointer over the label, the pointer will turn into a vertical arrow indicating that the sequence can be moved.

The sequences can also be sorted automatically to let you save time moving the sequences around. To sort the sequences alphabetically:

Right-click the name of a sequence | **Sort Sequences Alphabetically**

If you change the Sequence name (in the **Sequence Layout** view preferences), you will have to ask the program to sort the sequences again.

14.3.5 Delete and rename sequences

Sequences can be removed from the alignment by right-clicking the label of a sequence:

right-click label | **Delete Sequence**

This can be undone by clicking **Undo** () in the Toolbar.

If you wish to delete several sequences, you can check all the sequences, right-click and choose **Delete Marked Sequences**. To show the checkboxes, you first have to click the **Show Selection Boxes** in the **Side Panel**.

A sequence can also be renamed:

right-click label | **Rename Sequence**

This will show a dialog, letting you rename the sequence. This will not affect the sequence that the alignment is based on.

14.4 Bioinformatics explained: Multiple alignments

Multiple alignments are at the core of bioinformatical analysis. Often the first step in a chain of bioinformatical analyses is to construct a multiple alignment of a number of homologs DNA or protein sequences. However, despite their frequent use, the development of multiple alignment algorithms remains one of the algorithmically most challenging areas in bioinformatical research.

Constructing a multiple alignment corresponds to developing a hypothesis of how a number of sequences have evolved through the processes of character substitution, insertion and deletion. The input to multiple alignment algorithms is a number of homologous sequences i.e. sequences that share a common ancestor and most often also share molecular function. The generated alignment is a table (see figure 14.6) where each row corresponds to an input sequence and each column corresponds to a position in the alignment. An individual column in this table represents residues that have all diverged from a common ancestral residue. Gaps in the table (commonly represented by a '-') represent positions where residues have been inserted or deleted and thus do not have ancestral counterparts in all sequences.

14.4.1 Use of multiple alignments

Once a multiple alignment is constructed it can form the basis for a number of analyses:

- The phylogenetic relationship of the sequences can be investigated by tree-building methods based on the alignment.
- Annotation of functional domains, which may only be known for a subset of the sequences, can be transferred to aligned positions in other un-annotated sequences.
- Conserved regions in the alignment can be found which are prime candidates for holding functionally important sites.
- Comparative bioinformatical analysis can be performed to identify functionally important regions.

14.4.2 Constructing multiple alignments

Whereas the optimal solution to the pairwise alignment problem can be found in reasonable time, the problem of constructing a multiple alignment is much harder.

The first major challenge in the multiple alignment procedure is how to rank different alignments i.e. which *scoring function* to use. Since the sequences have a shared history they are correlated through their *phylogeny* and the scoring function should ideally take this into account. Doing so

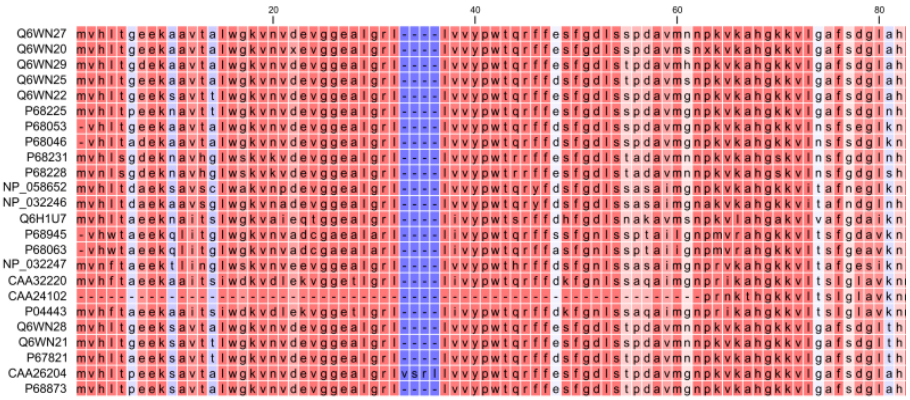


Figure 14.6: *The tabular format of a multiple alignment of 24 Hemoglobin protein sequences. Sequence names appear at the beginning of each row and the residue position is indicated by the numbers at the top of the alignment columns. The level of sequence conservation is shown on a color scale with blue residues being the least conserved and red residues being the most conserved.*

is, however, not straightforward as it increases the number of model parameters considerably. It is therefore commonplace to either ignore this complication and assume sequences to be unrelated, or to use heuristic corrections for shared ancestry.

The second challenge is to find the optimal alignment given a scoring function. For pairs of sequences this can be done by *dynamic programming* algorithms, but for more than three sequences this approach demands too much computer time and memory to be feasible.

A commonly used approach is therefore to do *progressive alignment* [Feng and Doolittle, 1987] where multiple alignments are built through the successive construction of pairwise alignments. These algorithms provide a good compromise between time spent and the quality of the resulting alignment

Presently, the most exciting development in multiple alignment methodology is the construction of *statistical alignment* algorithms [Hein, 2001], [Hein et al., 2000]. These algorithms employ a scoring function which incorporates the underlying phylogeny and use an explicit stochastic model of molecular evolution which makes it possible to compare different solutions in a statistically rigorous way. The optimization step, however, still relies on dynamic programming and practical use of these algorithms thus awaits further developments.

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Chapter 15

Phylogenetic trees

Contents

CLC Sequence Viewer offers different ways of inferring phylogenetic trees. The first part of this chapter will briefly explain the different ways of inferring trees in *CLC Sequence Viewer*. The second part, "Bioinformatics explained", will give a more general introduction to the concept of phylogeny and the associated bioinformatics methods.

15.1 Inferring phylogenetic trees

For a given set of aligned sequences (see chapter 14) it is possible to infer their evolutionary relationships. In *CLC Sequence Viewer* this is done by creating a phylogenetic tree:

Toolbox in the Menu Bar | **Alignments and Trees (**  **)**| **Create Tree (**  **)**

or **right-click alignment in Navigation Area** | **Toolbox** | **Alignments and Trees (****)**| **Create Tree (****)**

This opens the dialog displayed in figure 15.1:

If an alignment was selected before choosing the Toolbox action, this alignment is now listed in the **Selected Elements** window of the dialog. Use the arrows to add or remove elements from the **Navigation Area**. Click **Next** to adjust parameters.

15.1.1 Phylogenetic tree parameters

Figure 15.2 shows the parameters that can be set:

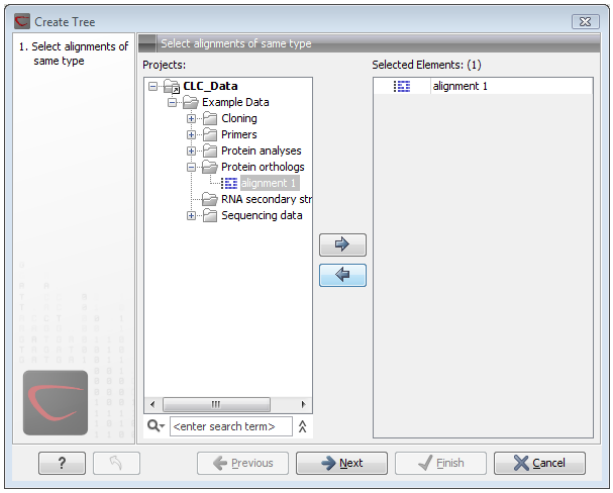

Figure 15.1: *Creating a Tree.*

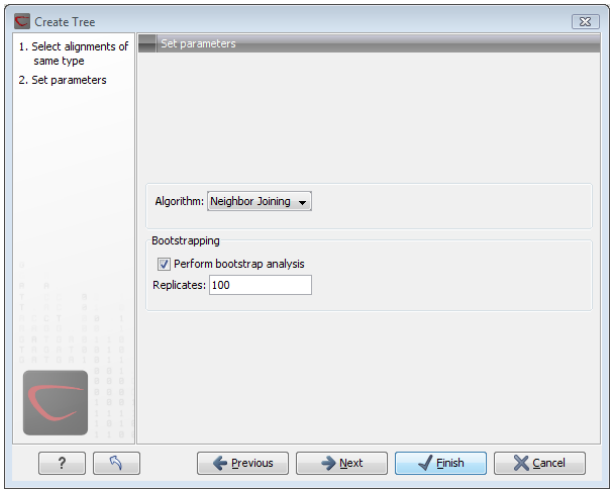

Figure 15.2: *Adjusting parameters.*

- Algorithms
  - The **UPGMA** method assumes that evolution has occured at a constant rate in the different lineages. This means that a root of the tree is also estimated.
  - The **neighbor joining** method builds a tree where the evolutionary rates are free to differ in different lineages. *CLC Sequence Viewer* always draws trees with roots for practical reasons, but with the neighbor joining method, no particular biological hypothesis is postulated by the placement of the root. Figure 15.3 shows the difference between the two methods.
- To evaluate the reliability of the inferred trees, *CLC Sequence Viewer* allows the option of doing a **bootstrap** analysis. A bootstrap value will be attached to each branch, and this value is a measure of the confidence in this branch. The number of replicates in the bootstrap analysis can be adjusted in the wizard. The default value is 100.

For a more detailed explanation, see "Bioinformatics explained" in section 15.2.

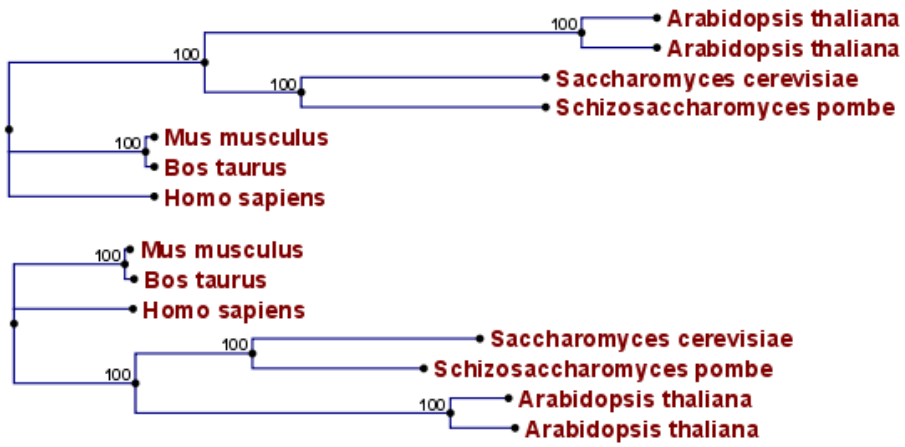

Figure 15.3: *Method choices for phylogenetic inference. The top shows a tree found by neighbor joining, while the bottom shows a tree found by UPGMA. The latter method assumes that the evolution occurs at a constant rate in different lineages.*

15.1.2 Tree View Preferences

The **Tree View** preferences are these:

- **Text format.** Changes the text format for all of the nodes the tree contains.
  - **Text size.** The size of the text representing the nodes can be modified in tiny, small, medium, large or huge.
  - **Font.** Sets the font of the text of all nodes
  - **Bold.** Sets the text bold if enabled.

- **Tree Layout.** Different layouts for the tree.
  - **Node symbol.** Changes the symbol of nodes into box, dot, circle or none if you don‘t want a node symbol.
  - **Layout.** Displays the tree layout as standard or topology.
  - **Show internal node labels**. This allows you to see labels for the internal nodes. Initially, there are no labels, but right-clicking a node allows you to type a label.
  - **Label color.** Changes the color of the labels on the tree nodes.
  - **Branch label color.** Modifies the color of the labels on the branches.
  - **Node color.** Sets the color of all nodes.
  - **Line color.** Alters the color of all lines in the tree.

- **Annotation Layout.** Specifies the annotation in the tree.
  - **Nodes.** Sets the annotation of all nodes either to name or to species.
  - **Branches.** Changes the annotation of the branches to bootstrap, length or none if you don‘t want annotation on branches.

Note! Dragging in a tree will change it. You are therefore asked if you want to save this tree when the **Tree Viewer** is closed.

You may select part of a **Tree** by clicking on the nodes that you want to select.

Right-click a selected node opens a menu with the following options:

- Set root above node (defines the root of the tree to be just above the selected node).
- Set root at this node (defines the root of the tree to be at the selected node).
- Toggle collapse (collapses or expands the branches below the node).
- Change label (allows you to label or to change the existing label of a node).
- Change branch label (allows you to change the existing label of a branch).

You can also relocate leaves and branches in a tree or change the length. It is possible to modify the text on the unit measurement at the bottom of the tree view by right-clicking the text. In this way you can specify a unit, e.g. "years".

Note! To drag branches of a tree, you must first click the node one time, and then click the node again, and this time hold the mouse button.

In order to change the representation:

- Rearrange leaves and branches by
  Select a leaf or branch | **Move it up and down (Hint: The mouse turns into an arrow pointing up and down)**
- Change the length of a branch by
  Select a leaf or branch | **Press Ctrl** | **Move left and right (Hint: The mouse turns into an arrow pointing left and right)**

Alter the preferences in **Side Panel** for changing the presentation of the tree.

Note! The preferences will not be saved. Viewing a tree in different viewers gives you the opportunity to change into different preferences in all of the viewers. For example if you select the **Annotation Layout** species for a node then you will only see the change in the specified view. If you now move leaves, the leaves in all views are moved. The options of the right-click pop up menu are changing the tree and therefore they change all views.

15.2 Bioinformatics explained: phylogenetics

Phylogenetics describes the taxonomical classification of organisms based on their evolutionary history i.e. their *phylogeny*. Phylogenetics is therefore an integral part of the science of *systematics* that aims to establish the phylogeny of organisms based on their characteristics. Furthermore, phylogenetics is central to evolutionary biology as a whole as it is the condensation of the overall paradigm of how life arose and developed on earth.

15.2.1 The phylogenetic tree

The evolutionary hypothesis of a phylogeny can be graphically represented by a phylogenetic tree.

Figure 15.4 shows a proposed phylogeny for the great apes, *Hominidae*, taken in part from Purvis [Purvis, 1995]. The tree consists of a number of nodes (also termed vertices) and branches (also termed edges). These nodes can represent either an individual, a species, or a higher grouping and are thus broadly termed taxonomical units. In this case, the terminal nodes (also called leaves or tips of the tree) represent extant species of *Hominidae* and are the *operational taxonomical units* (OTUs). The internal nodes, which here represent extinct common ancestors of the great apes, are termed *hypothetical taxonomical units* since they are not directly observable.

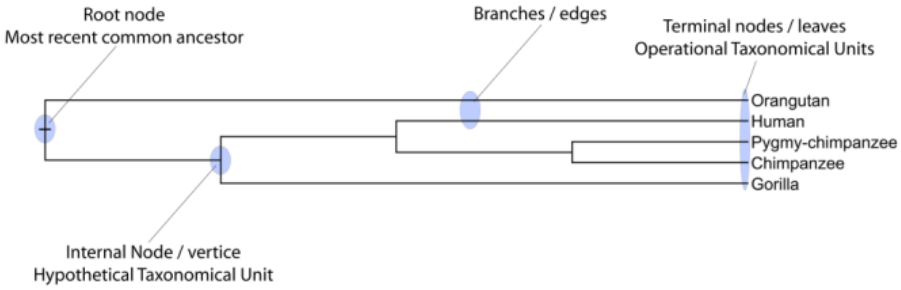

Figure 15.4: *A proposed phylogeny of the great apes (Hominidae). Different components of the tree are marked, see text for description.*

The ordering of the nodes determine the tree *topology* and describes how lineages have diverged over the course of evolution. The branches of the tree represent the amount of evolutionary divergence between two nodes in the tree and can be based on different measurements. A tree is completely specified by its topology and the set of all edge lengths.

The phylogenetic tree in figure 15.4 is rooted at the most recent common ancestor of all *Hominidae* species, and therefore represents a hypothesis of the direction of evolution e.g. that the common ancestor of gorilla, chimpanzee and man existed before the common ancestor of chimpanzee and man. In contrast, an unrooted tree would represent relationships without assumptions about ancestry.

15.2.2 Modern usage of phylogenies

Besides evolutionary biology and systematics the inference of phylogenies is central to other areas of research.

As more and more genetic diversity is being revealed through the completion of multiple genomes, an active area of research within bioinformatics is the development of comparative machine learning algorithms that can simultaneously process data from multiple species [Siepel and Haussler, 2004]. Through the comparative approach, valuable evolutionary information can be obtained about which amino acid substitutions are functionally tolerant to the organism and which are not. This information can be used to identify substitutions that affect protein function and stability, and is of major importance to the study of proteins [Knudsen and Miyamoto, 2001]. Knowledge of the underlying phylogeny is, however, paramount to comparative methods of inference as the phylogeny describes the underlying correlation from shared history that exists between data from different species.

In molecular epidemiology of infectious diseases, phylogenetic inference is also an important tool. The very fast substitution rate of microorganisms, especially the RNA viruses, means that these show substantial genetic divergence over the time-scale of months and years. Therefore, the phylogenetic relationship between the pathogens from individuals in an epidemic can be resolved and contribute valuable epidemiological information about transmission chains and epidemiologically significant events [Leitner and Albert, 1999], [Forsberg et al., 2001].

15.2.3 Reconstructing phylogenies from molecular data

Traditionally, phylogenies have been constructed from morphological data, but following the growth of genetic information it has become common practice to construct phylogenies based on molecular data, known as *molecular phylogeny*. The data is most commonly represented in the form of DNA or protein sequences, but can also be in the form of e.g. restriction fragment length polymorphism (RFLP).

Methods for constructing molecular phylogenies can be distance based or character based.

Distance based methods
Two common algorithms, both based on pairwise distances, are the UPGMA and the Neighbor Joining algorithms. Thus, the first step in these analyses is to compute a matrix of pairwise distances between OTUs from their sequence differences. To correct for multiple substitutions it is common to use distances corrected by a model of molecular evolution such as the Jukes-Cantor model [Jukes and Cantor, 1969].

UPGMA. A simple but popular clustering algorithm for distance data is Unweighted Pair Group Method using Arithmetic averages (UPGMA) ([Michener and Sokal, 1957], [Sneath and Sokal, 1973]). This method works by initially having all sequences in separate clusters and continuously joining these. The tree is constructed by considering all initial clusters as leaf nodes in the tree, and each time two clusters are joined, a node is added to the tree as the parent of the two chosen nodes. The clusters to be joined are chosen as those with minimal pairwise distance. The branch lengths are set corresponding to the distance between clusters, which is calculated as the average distance between pairs of sequences in each cluster.

The algorithm assumes that the distance data has the so-called *molecular clock* property i.e. the divergence of sequences occur at the same constant rate at all parts of the tree. This means that the leaves of UPGMA trees all line up at the extant sequences and that a root is estimated as part of the procedure.

Neighbor Joining. The neighbor joining algorithm, [Saitou and Nei, 1987], on the other hand, builds a tree where the evolutionary rates are free to differ in different lineages, i.e., the tree does not have a particular root. Some programs always draw trees with roots for practical reasons, but for neighbor joining trees, no particular biological hypothesis is postulated by the placement of the root. The method works very much like UPGMA. The main difference is that instead of using pairwise distance, this method subtracts the distance to all other nodes from the pairwise distance. This is done to take care of situations where the two closest nodes are not neighbors in the "real" tree. The neighbor join algorithm is generally considered to be fairly good and is widely used. Algorithms that improves its cubic time performance exist. The improvement is only significant for quite large datasets.

Character based methods. Whereas the distance based methods compress all sequence information into a single number, the character based methods attempt to infer the phylogeny

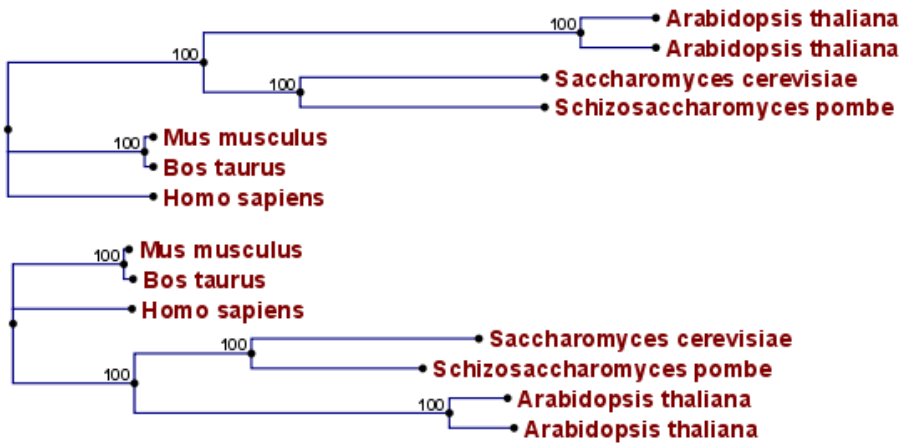

Figure 15.5: *Algorithm choices for phylogenetic inference. The bottom shows a tree found by the neighbor joining algorithm, while the top shows a tree found by the UPGMA algorithm. The latter algorithm assumes that the evolution occurs at a constant rate in different lineages.*

based on all the individual characters (nucleotides or amino acids).

Parsimony. In parsimony based methods a number of sites are defined which are informative about the topology of the tree. Based on these, the best topology is found by minimizing the number of substitutions needed to explain the informative sites. Parsimony methods are not based on explicit evolutionary models.

Maximum Likelihood. Maximum likelihood and Bayesian methods (see below) are probabilistic methods of inference. Both have the pleasing properties of using explicit models of molecular evolution and allowing for rigorous statistical inference. However, both approaches are very computer intensive.

A stochastic model of molecular evolution is used to assign a probability (likelihood) to each phylogeny, given the sequence data of the OTUs. Maximum likelihood inference [Felsenstein, 1981] then consists of finding the tree which assign the highest probability to the data.

Bayesian inference. The objective of Bayesian phylogenetic inference is not to infer a single "correct" phylogeny, but rather to obtain the full posterior probability distribution of all possible phylogenies. This is obtained by combining the likelihood and the prior probability distribution of evolutionary parameters. The vast number of possible trees means that bayesian phylogenetics must be performed by approximative Monte Carlo based methods. [Larget and Simon, 1999], [Yang and Rannala, 1997].

15.2.4 Interpreting phylogenies

Bootstrap values

A popular way of evaluating the reliability of an inferred phylogenetic tree is bootstrap analysis. The first step in a bootstrap analysis is to re-sample the alignment columns with replacement. I.e., in the re-sampled alignment, a given column in the original alignment may occur two or more times, while some columns may not be represented in the new alignment at all. The re-sampled alignment represents an estimate of how a different set of sequences from the same genes and the same species may have evolved on the same tree.

If a new tree reconstruction on the re-sampled alignment results in a tree similar to the original

one, this increases the confidence in the original tree. If, on the other hand, the new tree looks very different, it means that the inferred tree is unreliable. By re-sampling a number of times it is possibly to put reliability weights on each internal branch of the inferred tree. If the data was bootstrapped a 100 times, a bootstrap score of 100 means that the corresponding branch occurs in all 100 trees made from re-sampled alignments. Thus, a high bootstrap score is a sign of greater reliability.

Other useful resources

The Tree of Life web-project
http://tolweb.org

Joseph Felsensteins list of phylogeny software
http://evolution.genetics.washington.edu/phylip/software.html

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Part IV

Appendix

Appendix A

More features

You are currently using CLC bio's Sequence Viewer. If you want more features, try one of our commercial workbenches. You can download a one-month demo at http://www.clcbio.com/software.

See a list of all the features available below.

- CLC Sequence Viewer (■)
- CLC Main Workbench (■)
- CLC Genomics Workbench (■)

Data handling	Viewer	Main	Genomics
Add multiple locations to Navigation Area		■	■
Share data on network drive		■	■
Search all your data		■	■

Assembly of sequencing data	Viewer	Main	Genomics
Advanced contig assembly		■	■
Importing and viewing trace data		■	■
Trim sequences		■	■
Assemble without use of reference sequence		■	■
Map to reference sequence		■	■
Assemble to existing contig		■	■
Viewing and edit contigs		■	■
Tabular view of an assembled contig (easy data overview)		■	■
Secondary peak calling		■	■
Multiplexing based on barcode or name		■	■

Next-generation Sequencing Data Analysis	Viewer	Main	Genomics
Import of 454, Illumina Genome Analyzer, SOLiD and Helicos data			■
Reference assembly of human-size genomes			■
De novo assembly			■
SNP/DIP detection			■
Graphical display of large contigs			■
Support for mixed-data assembly			■
Paired data support			■
RNA-Seq analysis			■
Expression profiling by tags			■
ChIP-Seq analysis			■

Expression Analysis	Viewer	Main	Genomics
Import of Illumina BeadChip, Affymetrix, GEO data		■	■
Import of Gene Ontology annotation files		■	■
Import of Custom expression data table and Custom annotation files		■	■
Multigroup comparisons		■	■
Advanced plots: scatter plot, volcano plot, box plot and MA plot		■	■
Hierarchical clustering		■	■
Statistical analysis on count-based and gaussian data		■	■
Annotation tests		■	■
Principal component analysis (PCA)		■	■
Hierarchical clustering and heat maps		■	■
Analysis of RNA-Seq/Tag profiling samples		■	■

Molecular cloning	Viewer	Main	Genomics
Advanced molecular cloning		■	■
Graphical display of in silico cloning		■	■
Advanced sequence manipulation		■	■

Database searches	Viewer	Main	Genomics
GenBank Entrez searches	■	■	■
UniProt searches (Swiss-Prot/TrEMBL)		■	■
Web-based sequence search using BLAST		■	■
BLAST on local database		■	■
Creation of local BLAST database		■	■
PubMed lookup		■	■
Web-based lookup of sequence data		■	■
Search for structures (at NCBI)		■	■

General sequence analyses	Viewer	Main	Genomics
Linear sequence view	■	■	■
Circular sequence view	■	■	■
Text based sequence view		■	■
Editing sequences	■	■	■
Adding and editing sequence annotations		■	■
Advanced annotation table		■	■
Join multiple sequences into one	■	■	■
Sequence statistics	■	■	■
Shuffle sequence	■	■	■
Local complexity region analyses		■	■
Advanced protein statistics		■	■
Comprehensive protein characteristics report		■	■

Nucleotide analyses	Viewer	Main	Genomics
Basic gene finding	■	■	■
Reverse complement without loss of annotation	■	■	■
Restriction site analysis	■	■	■
Advanced interactive restriction site analysis		■	■
Translation of sequences from DNA to proteins	■	■	■
Interactive translations of sequences and alignments		■	■
G/C content analyses and graphs		■	■

Protein analyses	Viewer	Main	Genomics
3D molecule view		■	■
Hydrophobicity analyses		■	■
Antigenicity analysis		■	■
Protein charge analysis		■	■
Reverse translation from protein to DNA		■	■
Proteolytic cleavage detection		■	■
Prediction of signal peptides (SignalP)		■	■
Transmembrane helix prediction (TMHMM)		■	■
Secondary protein structure prediction		■	■
PFAM domain search		■	■

Sequence alignment	Viewer	Main	Genomics
Multiple sequence alignments (Two algorithms)	■	■	■
Advanced re-alignment and fix-point alignment options		■	■
Advanced alignment editing options	■	■	■
Join multiple alignments into one		■	■
Consensus sequence determination and management	■	■	■
Conservation score along sequences	■	■	■
Sequence logo graphs along alignments		■	■
Gap fraction graphs		■	■
Copy annotations between sequences in alignments		■	■
Pairwise comparison	■	■	■

RNA secondary structure	Viewer	Main	Genomics
Advanced prediction of RNA secondary structure		■	■
Integrated use of base pairing constraints		■	■
Graphical view and editing of secondary structure		■	■
Info about energy contributions of structure elements		■	■
Prediction of multiple sub-optimal structures		■	■
Evaluate structure hypothesis		■	■
Structure scanning		■	■
Partition function		■	■

Dot plots	Viewer	Main	Genomics
Dot plot based analyses		■	■

Phylogenetic trees	Viewer	Main	Genomics
Neighbor-joining and UPGMA phylogenies	■	■	■
Maximum likelihood phylogeny of nucleotides		■	■

Pattern discovery	Viewer	Main	Genomics
Search for sequence match	■	■	■
Motif search for basic patterns		■	■
Motif search with regular expressions		■	■
Motif search with ProSite patterns		■	■
Pattern discovery		■	■

Primer design	Viewer	Main	Genomics
Advanced primer design tools		■	■
Detailed primer and probe parameters		■	■
Graphical display of primers		■	■
Generation of primer design output		■	■
Support for Standard PCR		■	■
Support for Nested PCR		■	■
Support for TaqMan PCR		■	■
Support for Sequencing primers		■	■
Alignment based primer design		■	■
Alignment based TaqMan probe design		■	■
Match primer with sequence		■	■
Ordering of primers		■	■
Advanced analysis of primer properties		■	■

Molecular cloning	Viewer	Main	Genomics
Advanced molecular cloning		■	■
Graphical display of in silico cloning		■	■
Advanced sequence manipulation		■	■

Virtual gel view	Viewer	Main	Genomics
Fully integrated virtual 1D DNA gel simulator		■	■

For a more detailed comparison, we refer to http://www.clcbio.com/compare.

Appendix B

Graph preferences

This section explains the view settings of graphs. The **Graph preferences** at the top of the **Side Panel** includes the following settings:

- **Lock axes.** This will always show the axes even though the plot is zoomed to a detailed level.
- **Frame.** Shows a frame around the graph.
- **Show legends.** Shows the data legends.
- **Tick type**. Determine whether tick lines should be shown outside or inside the frame.
  - Outside
  - Inside
- **Tick lines at.** Choosing Major ticks will show a grid behind the graph.
  - None
  - Major ticks
- **Horizontal axis range**. Sets the range of the horizontal axis (x axis). Enter a value in **Min** and **Max**, and press Enter. This will update the view. If you wait a few seconds without pressing Enter, the view will also be updated.
- **Vertical axis range**. Sets the range of the vertical axis (y axis). Enter a value in **Min** and **Max**, and press Enter. This will update the view. If you wait a few seconds without pressing Enter, the view will also be updated.
- **X-axis at zero**. This will draw the x axis at y = 0. Note that the axis range will not be changed.
- **Y-axis at zero**. This will draw the y axis at x = 0. Note that the axis range will not be changed.
- **Show as histogram**. For some data-series it is possible to see the graph as a histogram rather than a line plot.

The **Lines and plots** below contains the following settings:

- **Dot type**
  - None
  - Cross
  - Plus
  - Square
  - Diamond
  - Circle
  - Triangle
  - Reverse triangle
  - Dot
- **Dot color.** Allows you to choose between many different colors. Click the color box to select a color.
- **Line width**
  - Thin
  - Medium
  - Wide
- **Line type**
  - None
  - Line
  - Long dash
  - Short dash
- **Line color.** Allows you to choose between many different colors. Click the color box to select a color.

For graphs with multiple data series, you can select which curve the dot and line preferences should apply to. This setting is at the top of the **Side Panel** group.

Note that the graph title and the axes titles can be edited simply by clicking with the mouse. These changes will be saved when you **Save** () the graph - whereas the changes in the **Side Panel** need to be saved explicitly (see section 4.5).

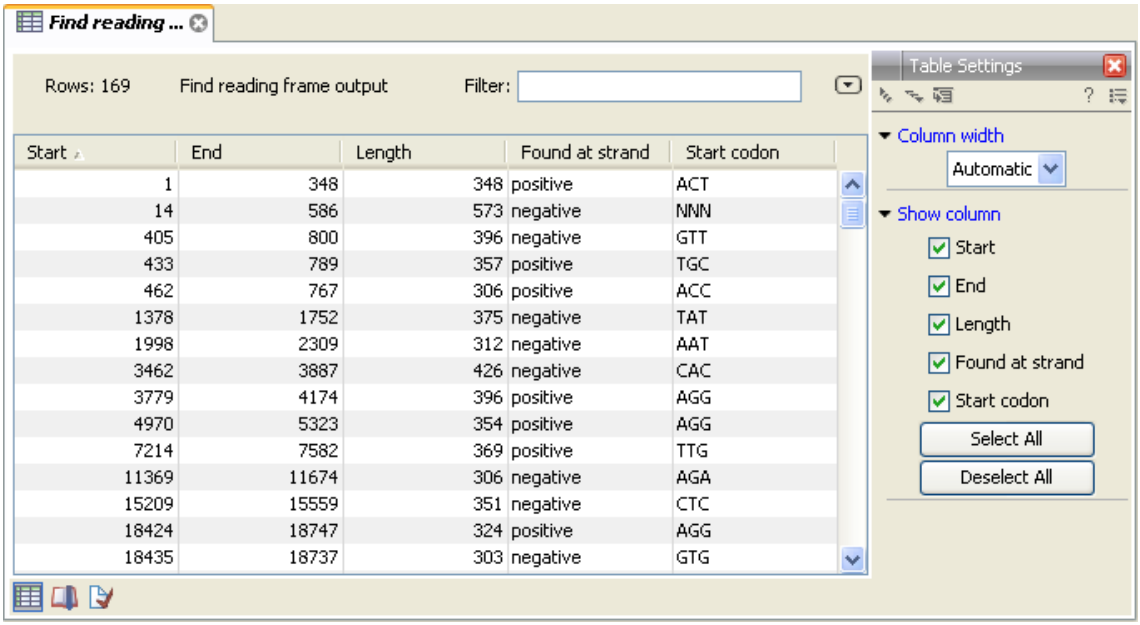
For more information about the graph view, please see section B.

Appendix C

Working with tables

Tables are used in a lot of places in the *CLC Sequence Viewer*. The contents of the tables are of course different depending on the context, but there are some general features for all tables that will be explained in the following.

Figure C.1 shows an example of a typical table. This is the table result of **Find Open Reading Frames** (). We will use this table as an example in the following to illustrate the concepts that are relevant for all kinds of tables.



Start	End	Length	Found at strand	Start codon
1	348	348	positive	ACT
14	586	573	negative	NNN
405	800	396	negative	GTT
433	789	357	positive	TGC
462	767	306	positive	ACC
1378	1752	375	negative	TAT
1998	2309	312	negative	AAT
3462	3887	426	negative	CAC
3779	4174	396	positive	AGG
4970	5323	354	positive	AGG
7214	7582	369	positive	TTG
11369	11674	306	negative	AGA
15209	15559	351	negative	CTC
18424	18747	324	positive	AGG
18435	18737	303	negative	GTG

Figure C.1: *A table showing open reading frames.*

First of all, the columns of the table are listed in the **Side Panel** to the right of the table. By clicking the checkboxes you can hide/show the columns in the table.

Furthermore, you can **sort** the table by clicking on the column headers. (Pressing Ctrl - ⌘ on Mac - while you click will refine the existing sorting).

C.1 Filtering tables

The final concept to introduce is **Filtering**. The table filter as an advanced and a simple mode. The simple mode is the default and is applied simply by typing text or numbers (see an example in figure C.2).

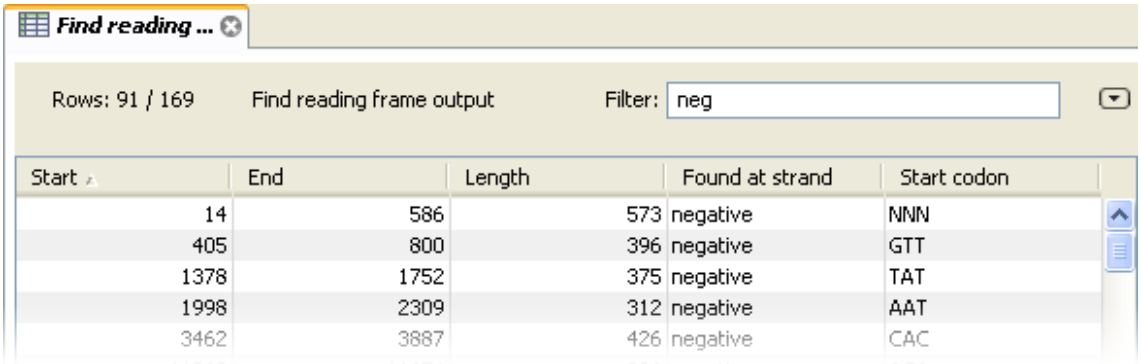


Figure C.2: *Typing "neg" in the filter in simple mode.*

Typing "neg" in the filter will only show the rows where "neg" is part of the text in any of the columns (also the ones that are not shown). The text does not have to be in the beginning, thus "ega" would give the same result. This simple filter works fine for fast, textual and non-complicated filtering and searching.

However, if you wish to make use of numerical information or make more complex filters, you can switch to the advanced mode by clicking the **Advanced filter** ( ) button. The advanced filter is structure in a different way: First of all, you can have more than one criterion in the filter. Criteria can be added or removed by clicking the **Add** ( ) or **Remove** ( ) buttons. At the top, you can choose whether all the criteria should be fulfilled (**Match all**), or if just one of the needs to be fulfilled (**Match any**).

For each filter criterion, you first have to select which column it should apply to. Next, you choose an operator. For numbers, you can choose between:

- **=** (equal to)
- **<** (smaller than)
- **>** (greater than)
- **<>** (not equal to)
- **abs. value <** (absolute value smaller than. This is useful if it doesn't matter whether the number is negative or positive)
- **abs. value >** (absolute value greater than. This is useful if it doesn't matter whether the number is negative or positive)

For text-based columns, you can choose between:

- **contains** (the text does not have to be in the beginning)
- **doesn't contain**

- = (the whole text in the table cell has to match, also lower/upper case)

Once you have chosen an operator, you can enter the text or numerical value to use.

If you wish to reset the filter, simply remove () all the search criteria. Note that the last one will not disappear - it will be reset and allow you to start over.

Figure C.3 shows an example of an advanced filter which displays the open reading frames larger than 400 that are placed on the negative strand.

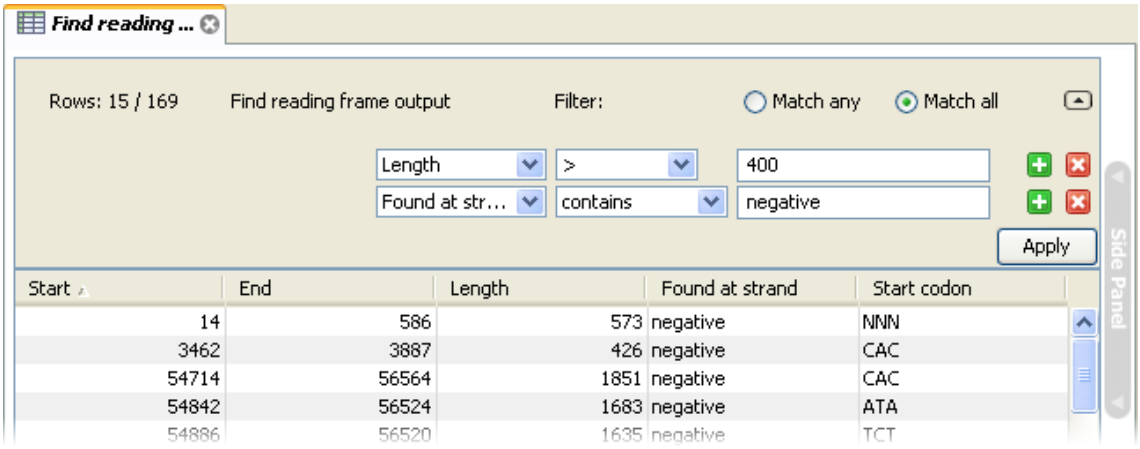


Figure C.3: *The advanced filter showing open reading frames larger than 400 that are placed on the negative strand.*

Both for the simple and the advanced filter, there is a counter at the upper left corner which tells you the number of rows that pass the filter (91 in figure C.2 and 15 in figure C.3).

Appendix D

Formats for import and export

D.1 List of bioinformatic data formats

Below is a list of bioinformatic data formats, i.e. formats for importing and exporting sequences, alignments and trees.

D.1.1 Sequence data formats

File type	Suffix	Import	Export	Description
FASTA	.fsa/.fasta	X	X	Simple format, name & description
AB1	.ab1	X		Including chromatograms
ABI	.abi	X		Including chromatograms
CLC	.clc	X	X	Rich format including all information
Clone Manager	.cm5	X		
CSV export	.csv		X	Annotations in csv format
CSV import	.csv	X		One sequence per line: name; description(optional); sequence
DNAstrider	.str/.strider	X	X	
DS Gene	.bsml	X		
Embl	.embl	X	X	Only nucleotide sequence
GCG sequence	.gcg	X		Rich information incl. annotations
GenBank	.gbk/.gb/.gp	X	X	Rich information incl. annotations
Gene Construction Kit	.gck	X		
Lasergene	.pro/.seq	X		
Nexus	.nxs/.nexus	X	X	
Phred	.phd	X		Including chromatograms
PIR (NBRF)	.pir	X		Simple format, name & description
Raw sequence	any	X		Only sequence (no name)
SCF2	.scf	X		Including chromatograms
SCF3	.scf	X	X	Including chromatograms
Staden	.sdn	X		
Swiss-Prot	.swp	X	X	Rich information (only proteins)
Tab delimited text	.txt		X	Annotations in tab delimited text format
Vector NTI archives	.ma4/.pa4/.oa4	X		Archives in rich format
Vector NTI Database		X		Special import full database
Zip export	.zip		X	Selected files in CLC format
Zip import	.zip/.gzip./.tar	X		Contained files/folder structure

D.1.2 Alignment formats

File type	Suffix	Import	Export	Description
Aligned fasta	.fa	X	X	Simple fasta-based format with – for gaps
CLC	.clc	X	X	Rich format including all information
Clustal Alignment	.aln	X	X	
GCG Alignment	.msf	X	X	
Nexus	.nxs/.nexus	X	X	
Phylip Alignment	.phy	X	X	
Zip export	.zip		X	Selected files in CLC format
Zip import	.zip/.gzip./.tar	X		Contained files/folder structure

D.1.3 Tree formats

File type	Suffix	Import	Export	Description
CLC	.clc	X	X	Rich format including all information
Newick	.nwk	X	X	
Nexus	.nxs/.nexus	X	X	
Zip export	.zip		X	Selected files in CLC format
Zip import	.zip/.gzip./.tar	X		Contained files/folder structure

D.1.4 Miscellaneous formats

File type	Suffix	Import	Export	Description
BLAST Database	.phr/.nhr	X		Link to database imported
CLC	.clc	X	X	Rich format including all information
CSV	.csv		X	All tables
Excel	.xls/.xlsx		X	All tables and reports
GFF	.gff	X	X	See http://www.clcbio.com/annotate-with-gff
mmCIF	.cif	X		3D structure
PDB	.pdb	X		3D structure
Tab delimited	.txt		X	All tables
Text	.txt	X	X	All data in a textual format
Zip export	.zip		X	Selected files in CLC format
Zip import	.zip/.gzip./.tar	X		Contained files/folder structure

Note! The Workbench can import 'external' files, too. This means that all kinds of files can be imported and displayed in the **Navigation Area**, but the above mentioned formats are the only ones whose *contents* can be shown in the Workbench.

D.2 List of graphics data formats

Below is a list of formats for exporting graphics. All data displayed in a graphical format can be exported using these formats. Data represented in lists and tables can only be exported in .pdf format (see section 6.3 for further details).

Format	Suffix	Type
Portable Network Graphics	.png	bitmap
JPEG	.jpg	bitmap
Tagged Image File	.tif	bitmap
PostScript	.ps	vector graphics
Encapsulated PostScript	.eps	vector graphics
Portable Document Format	.pdf	vector graphics
Scalable Vector Graphics	.svg	vector graphics

Appendix E

IUPAC codes for amino acids

(Single-letter codes based on International Union of Pure and Applied Chemistry)

The information is gathered from: http://www.ebi.ac.uk/2can/tutorials/aa.html

One-letter abbreviation	Three-letter abbreviation	Description
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
J	Xle	Leucine or Isoleucineucine
L	Leu	Leucine
I	ILe	Isoleucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
O	Pyl	Pyrrolysine
U	Sec	Selenocysteine
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine
B	Asx	Aspartic acid or Asparagine Asparagine
Z	Glx	Glutamic acid or Glutamine Glutamine
X	Xaa	Any amino acid

Appendix F

IUPAC codes for nucleotides

(Single-letter codes based on International Union of Pure and Applied Chemistry)

The information is gathered from: http://www.iupac.org and http://www.ebi.ac.uk/2can/tutorials/aa.html.

Code	Description
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
U	Uracil
R	Purine (A or G)
Y	Pyrimidine (C, T, or U)
M	C or A
K	T, U, or G
W	T, U, or A
S	C or G
B	C, T, U, or G (not A)
D	A, T, U, or G (not C)
H	A, T, U, or C (not G)
V	A, C, or G (not T, not U)
N	Any base (A, C, G, T, or U)

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Part V

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