

USER MANUAL

User manual for CLC MLST Module 25.0.2

Windows, macOS and Linux

August 8, 2025

CLC software is intended for scientific research applications. CLC software is not intended for the diagnosis, prevention or treatment of a disease.

QIAGEN Aarhus AS Kalkværksvej 5, 11. DK - 8000 Aarhus C Denmark



Contents

1	Intro	oductio	n	5
	1.1	What i	s MLST?	5
	1.2	The co	ncept of the QIAGEN CLC MLST Module	5
	1.3	Syster	n requirements	6
	1.4	Contac	t information	6
	1.5	Install	ing modules	7
		1.5.1	Licensing modules	8
		1.5.2	Uninstalling modules	8
	1.6	Acknow	wledgements	10
_				
2	MLS	T quick	t-start	11
3	Han	dling M	LST schemes	12
	3.1	Downle	pading schemes	12
		3.1.1	Download custom schemes	15
	3.2	Creati	ng new schemes	15
	3.3	Mergir	ng schemes	17
	3.4	Viewin	g schemes	18
		3.4.1	Profile table	18
			Extracting alignments	18
			Extracting sequences	19
			Adding and removing sequence information	20
		3.4.2	Allele table	20
			Displaying the sequences	21
			Creating an alignment comparing the allelic types	21

		F	Removing allele sequences	21
4	Isola	ates		22
	4.1	Assemb	le and create isolate	22
	4.2	Working	with isolates	24
		4.2.1 I	solate-scheme dependencies and the sequence type	24
		4.2.2 (Opening contigs	26
		4.2.3	Deleting contigs	26
		4.2.4	Assigning new contigs	26
		4.2.5 A	Align to nearest allele	27
		4.2.6	Assemble to an existing isolate	27
	4.3	View a r	eport of an isolate	27
5	Exte	ending sc	hemes	29
	5.1	Add isol	ates to scheme	29
	5.2	Add seq	juences to scheme	29
~	Inst	all and w	ainstall modules	21
0		an anu ui		эт
0	6.1	Installat	ion of modules	31
0	6.1 6.2	Installat Uninstal	ion of modules	31 33
7	6.1 6.2 Lice	Installat Uninstal nsing rec	ion of modules	31 33 35
7	6.16.2Lice7.1	Installat Uninstal nsing rec Licensir	tion of modules	31 33 35 35
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F	tion of modules	 31 33 35 35 35
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F	tion of modules	31 33 35 35 35 35 36
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F	tion of modules	31 33 35 35 35 35 36 37
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F [(7.1.2 [tion of modules	31 33 35 35 35 36 37 37
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F (7.1.2 [tion of modules	31 33 35 35 35 36 37 37 38
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F (7.1.2 [tion of modules	31 33 35 35 35 36 37 37 38 38
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F (7.1.2 [(7.1.3]	ion of modules	31 33 35 35 35 36 37 37 38 38 38 39
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F (7.1.2 [(7.1.2] (7.1.3] 7.1.4 (ion of modules	31 33 35 35 35 36 37 37 38 38 38 39 40
7	6.1 6.2 Lice 7.1	Installat Uninstal nsing rec Licensin 7.1.1 F (7.1.2 [(7.1.3 7.1.3 7.1.4 (7.1.5 [Initiation of modules	31 33 35 35 35 36 37 37 38 38 38 39 40 42

Bibliography

Chapter 1

Introduction

1.1 What is MLST?

With the use of nucleotide sequencing it is possible to perform a fine-scale typing of microbial variants which can greatly improve our understanding of bacterial species, genera, populations, ecosystems and epidemiology.

In order to compare experiments it is required that DNA sequencing and analysis is performed using a portable standard. One such standard is multilocus sequence typing (MLST) which was first proposed by Maiden et al. [Maiden et al., 1998]. Currently, MLST is used to type isolates of bacterial and fungal species for epidemiological and evolutionary studies. For some recent reviews of MLST technology and its applications, see [Taylor and Fisher, 2003, Urwin and Maiden, 2003, Sullivan et al., 2005, Maiden, 2006].

Briefly described, an MLST scheme for a given organism defines a number of internal fragments to be sequenced. These fragments have a length of approximately 450- to 500-bp and are usually chosen to lie in well conserved regions such as housekeeping genes to ensure that general primers can be designed for PCR amplification and sequencing of all species members.

For each fragment, the scheme also contains a dynamic list of the different known alleles. Each allele is assigned a number and by using this numbering system each new isolate can be assigned a complete allelic profile based on all the defined fragments. Each distinct allelic profile in the scheme is called a sequence type (ST) and given a unique number.

1.2 The concept of the QIAGEN CLC MLST Module

The idea behind the module is to eliminate much of the manual work previously required to do MLST. Once a reference scheme has been downloaded, a standard typing of an isolate is done with a few clicks.

The data structure of the CLC MLST Module can be described with two concepts: scheme and *isolate*. The MLST scheme contains sequence information and allelic profiles of known sequence types. An isolate is a collection of sequences of one bacteria isolate.

The central point of interaction between the scheme and the isolate is the typing of the isolate. When an isolate has been created, it is compared with the MLST scheme to determine the sequence type. This is done dynamically every time the isolate is opened, providing the opportunity to extend the scheme with new types and immediately have an updated sequence type of the isolate.

The MLST schemes are described in further detail in section 3, and isolates are described in further detail in section 4

1.3 System requirements

There are no additional system requirements for the CLC MLST Module beyond those of the software it is installed on.

System requirement information can be found in the relevant platform product user manual:

- CLC Main Workbench system requirements
- CLC Genomics Workbench system requirements

Compatibility

The CLC MLST Module 25.0.2 can be installed on *CLC Main Workbench* 25.0 and *CLC Genomics Workbench* 25.0, and on later versions in the same major release line.

1.4 Contact information

QIAGEN CLC MLST Module is developed by:

QIAGEN Aarhus A/S Kalkværksvej 5, 11. DK - 8000 Aarhus C Denmark

https://digitalinsights.qiagen.com/

Email: ts-bioinformatics@qiagen.com

The QIAGEN Aarhus team continuously improves products with your interests in mind. We welcome feedback and suggestions for new features or improvements. How to contact us is described at: https://resources.giagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Contact_information_citation.html

You can also make use of our online documentation resources, including:

- Core product manuals https://digitalinsights.qiagen.com/technical-support/ manuals/
- Plugin manuals https://digitalinsights.qiagen.com/products-overview/plugins/
- Tutorials https://digitalinsights.qiagen.com/support/tutorials/
- Frequently Asked Questions https://qiagen.my.salesforce-sites.com/KnowledgeBase/ KnowledgeNavigatorPage

1.5 Installing modules

Note: In order to install plugins and modules, the *CLC Workbench* must be run in administrator mode. On Windows, you can do this by right-clicking the program shortcut and choosing "Run as Administrator". On Linux and Mac, it means you must launch the program such that it is run by an administrative user.

Plugins and modules are installed and uninstalled using the Workbench Plugin Manager. To open the Plugin Manager, click on the **Plugins (button** in the top Toolbar, or go to the menu option:

Utilities | Manage Plugins... (💱)

The Plugin Manager has two tabs at the top:

- **Manage Plugins** An overview of your installed plugins and modules is provided under this tab.
- **Download Plugins** Plugins and modules available to download and install are listed in this tab.

To install a plugin, click on the **Download Plugins** tab (figure 6.1). Select a plugin. Information about it will be shown in the right hand panel. Click on the **Download and Install** button to install the plugin.



Figure 1.1: Plugins and modules available for installation are listed in the Plugin Manager under the Download Plugins tab.

Accepting the license agreement

The End User License Agreement (EULA) must be read and accepted as part of the installation process. Please read the EULA text carefully, and if you agree to it, check the box next to the text **I accept these terms**. If further information is requested from you, please fill this in before clicking on the **Finish** button.

Installing a cpa file

If you have a .cpa installer file for QIAGEN CLC MLST Module, you can install it by clicking on the **Install from File** button at the bottom of the Plugin Manager.

If you are working on a system not connected to the internet, plugin and module .cpa files can be downloaded from https://digitalinsights.qiagen.com/products-overview/plugins/using a networked machine, and then transferred to the non-networked machine for installation.

Restart to complete the installation

Newly installed plugins and modules will be available for use after restarting the software. When you close the Plugin Manager, a dialog appears offering the opportunity to restart the *CLC Workbench*.

1.5.1 Licensing modules

When you have installed the QIAGEN CLC MLST Module and start a tool from that module for the first time, the License Assistant will open (figure 6.2).

The License Assistant can also be launched by opening the Workbench Plugin Manager, selecting the installed module from under the Manage Plugins tab, and clicking on the button labeled *Import License*.

To install a license, the *CLC Workbench* must be run in administrator mode. On Windows, you can do this by right-clicking the program shortcut and choosing "Run as Administrator". On Linux and Mac, it means you must launch the program such that it is run by an administrative user.

The following options are available:

- **Request an evaluation license**. Request a fully functional, time-limited license.
- **Download a license**. Use the license order ID received when you purchased the software to download and install a license file.
- **Import a license from a file**. Import an existing license file, for example a file downloaded from the web-based licensing system.
- **Configure license manager connection**. If your organization has a *CLC Network License Manager*, select this option to configure the connection to it.

These options are described in detail in sections under https://resources.giagenbioinformatics. com/manuals/clcgenomicsworkbench/current/index.php?manual=Workbench_Licenses.html.

To download licenses, including evaluation licenses, your machine must have access to the external network. To install licenses on non-networked machines, please see https://resources.giagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Download_static_license_on_non_networked_machine.html.

1.5.2 Uninstalling modules

Plugins and modules are uninstalled using the Workbench Plugin Manager. To open the Plugin Manager, click on the **Plugins** () **button** in the top Toolbar, or go to the menu option:

alid license. plugin. ne plugin for 14 days. will be allowed for each computer. e.
e plugin for 14 days. will be allowed for each computer. e.
e plugin for 14 days. will be allowed for each computer. e.
e.
e.
2.
e Manager that hosts network license(s) for this tion configuration.
e. e Manager that hosts network license(s) for this tion configuration.
e Manager that hosts network license(s) for this
e Manager that hosts network license(s) for this tion configuration.
tion configuration.
ting license file tion letwork License existing connec

Figure 1.2: The License Assistant provides options for licensing modules installed on the Workbench.

Utilities | Manage Plugins... (💱)

This will open the Plugin Manager (figure 6.3). Installed plugins and modules are shown under the Manage Plugins tab of the Plugins Manager.

To uninstall a plugin or module, click on its entry in the list, and click on the **Uninstall** button.

Plugins and modules are not uninstalled until the Workbench is restarted. When you close the Plugin Manager, a dialog appears offering the opportunity to restart the *CLC Workbench*.

Disabling a plugin without uninstalling it

If you do not want a plugin to be loaded the next time you start the Workbench, select it in the list under the Manage Plugins tab and click on the **Disable** button.

Gx Manage Plugins		×
PM Manage Plugins Download Plugins		
P Provider: QIAGEN Aarhus Support contact: ts-bioinformatics@qiagen.com Version: 1.1 (Build: 190328-1503-191404) Provided Councils Association		
biomedical Genomics Analysis	Uninstall Disable	
CLC MLST Module Provider: QIAGEN Aarhus Support contact: ts-bioinformatics@qiagen.com Version: 1.9 (Build: 181115-1337-185442)	Updati availab	e
MLST Module makes it easy and fast to do MultiLocus Se	quence Typing.	-
	Update Import License Uninstall Disable	
CLC Microbial Genomics Module Provider: QIAGEN Aarhus Support contact: ts-bioinformatics@qiagen.com Version: 4.1 (Build: 190129-1433-188333)		
CLC Microbial Genomics Module		
	Import License Uninstall Disable	
Help Proxy Settings Check for Updates	Install from File	Close

Figure 1.3: Installed plugins and modules are listed in the Plugins Manager under the Manage Plugins tab.

1.6 Acknowledgements

The CLC MLST Module provides tools to download MLST schemes from PubMLST (https://pubmlst.org)

Chapter 2

MLST quick-start

This section briefly describes how to get started using the main functionality of the QIAGEN CLC MLST Module.

First, download an MLST Scheme:

Multilocus Sequence Typing () | MLST Schemes () | Download MLST Schemes (PubMLST) ()

In this dialog, select the correct scheme to use as reference for typing. Save the scheme $(\overline{\{\cdot\}})$.

Next, to find a sequence type for your sequencing data, you will have to create an isolate:

Multilocus Sequence Typing (🚔) | Assembly and Create Isolate (🚃)

In this dialog, select all the sequencing data for the isolate that you wish to type. (Note that you will have to **Import** (\square) the data first.)

Click **Next** and select the scheme (\searrow) that you downloaded as reference scheme. Click **Next** through a series of steps where sequences are assigned to each locus in the scheme. If the sequence is not automatically assigned correctly, use the **Add** (\Rightarrow) and **Remove** (\Leftarrow) buttons to make sure the right sequences are assigned.

When you click **Finish**, an isolate is created and a sequence type is assigned. Double-click a row in the upper part of the view to inspect and edit the contig.

Chapter 3

Handling MLST schemes

An MLST scheme (IIII) consists of the following:

- Names of the loci to be sequenced.
- For each of these loci a list of the known alleles is maintained.
- A list of the known allelic profiles or sequence types described by the scheme.

Given this information, sequence data from a new isolate can be used to assign a sequence type.

In the CLC MLST Module, an MLST scheme can be created in two ways:

- It can be downloaded from a database, or
- Created from sequence data imported into the workbench.

The schemes downloaded from a database will already have information about many sequence types, whereas this information needs to be accumulated by the user for self-made schemes.

3.1 Downloading schemes

The tool supports download of 7-gene MLST schemes hosted by PubMLST (https://pubmlst.org/).

Although the list of schemes are hosted by PubMLST, a few of the schemes are located at Institut Pasteur (https://bigsdb.pasteur.fr/). It is mandatory to be registered and logged in when downloading from either database.

To run the tool, go to:

Tools | Multilocus Sequence Typing (🚔) | MLST Schemes (🚘) | Download MLST Schemes (PubMLST) (🏭)

Select the scheme you wish to download in the **Scheme to download** drop-down menu (figure 3.1). To jump to specific schemes, click the drop-down menu once and type the first letters of the desired scheme, e.g., type "es" to reach the first Escherichia spp. scheme.

Download MLST Schemes (PubMLST) Select scheme to download Authentication Result handling Select scheme Scheme to download: Achromoba	ubMLST)	×
 Select scheme to download Authentication Result handling 	Select scheme to download Select scheme Scheme to download: Achromobacter spp.	
Help Reset	Previous Next Finish Cancel	

Figure 3.1: Select the desired MLST Scheme.

Click on **Next** to proceed to the Authorization step.

Authorize access through your account

To download MLST schemes, you must first authorize access to download data on you behalf. For this step, you must have a user account with the relevant MLST scheme provider (PubMLST or Pasteur) depending on which scheme you select. You must also have registered with the specific database in your account settings. How to create an account and register for specific databases is explained at https://pubmlst.org/site-accounts and https://bigsdb.pasteur.fr/register/.

1. Click the **Log in** button (figure 3.2). This will open the relevant login page in an external browser.

🐻 Download MLST Schemes (P	ubMLST) ×
 Select scheme to download Authentication 	Authentication
3. Result handling	Database access BIGSdb login Log in Not logged in
Help Reset	Previous Next Finish Cancel

Figure 3.2: Download MLST Scheme Authorization step.

 If you were not already logged in in the browser, you must now do so. Depending on the scheme you are downloading, log in using your PubMLST or Institut Pasteur account (figure 3.3).

Note: Make sure you are registrered for the specific database you are trying to access. If you have an account, but have not registered for the specific database you are trying to download from, you will not be able to log in.

3. After logging in, you will be asked to authorize *CLC Workbench* to access data on your behalf (figure 3.4). Click "Authorize". This generates an access token and secret for use by



Figure 3.3: Log in to your account. In this case the PubMLST account is needed.

CLC Workbench. No personal data about the account is shared. A verification code will be displayed after authorizing (figure 3.5).

Authorize client sof	tware to	access y	our accour	Logged in
CLC Genomics Workbench You will be able to revoke access for this	application to acconnection acconnection acconnection acconnection acconnection at any	Action CANCEL time.	AUTHORIZE	_

Figure 3.4: You will be asked to allow the Workbench to access your account. Click "Authorize".



Figure 3.5: Following authorization a verification code will appear. Copy the code and return to the Workbench.

4. Copy the code, return to the Workbench, and paste it into the **Verification code** dialog box (figure 3.5). Click **OK**.



Figure 3.6: Paste or type the verification code into the dialog box.

When the verification code has been succesfully entered, you will be logged in and can proceed to the next steps. The browser window can also be closed. For following downloads from the same source, you need not authorize access again. Clicking the **Log in** button should automatically connect to the database on your behalf.

Clicking the **Log out** button will reset the token and secret, allowing you to log in using another account.

3.1.1 Download custom schemes

In addition to the MLST schemes that the CLC MLST Module can automatically download (see section 3), you can also provide a URL to an MLST scheme on another web server. The scheme has to be in the same format as the schemes on PubMLST.

Figure 3.7 shows the dialog where you can enter a URL.

🐻 Download sche	me from an mIstdbNet co	ompatible website	\times
 Specify URL Result handling 	Specify URL URL to mistdbNet com URL http://mistdb.br	patible website mc.uu.se/current.html	
Help	Reset	Previous Next Finish Cancel	

Figure 3.7: Providing a URL for downloading schemes.

3.2 Creating new schemes

A scheme can be created from scratch by defining a number of loci and accumulating sequence information about each loci. The schemes have the same format as the schemes that are downloaded.

To create a new scheme:

```
Multilocus Sequence Typing ( ) | MLST Schemes ( ) | Create MLST Scheme ( )
```

This will bring up a dialog as shown in figure 3.8.

Gx Create MLST Scheme	×
1 Set scheme information	Set scheme information
1. Set scheme information	Name And Profile
2. Result handling	MLST Scheme name: New MLST Scheme
	MLST Scheme Profile Path:
	Number of genes: 3 📩
	Extraction of allelic names Image: Comparison of allelic names Image: Comparison of allelic names
	r Add genes to scheme
0 0 1 0 0 0 1 1 0 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 1 0 1 1 1 0 1	Genes in the scheme gene 1 gene 2 gene 3 gene 3 Remove
Help Reset	Previous Next Finish Cancel

Figure 3.8: Entering basic information for the scheme.

In the top field you can enter a name for the scheme - typically the name of the species in question.

Below, you can specify the location for a text file where all the loci in the scheme are listed. The file must contain a row with the column names 'ST', 'gene_1', 'gene_2', etc. Each subsequent row must contain exactly one loci/sequence type. Note that the file must be in plain text format and the columns must be tab-separated. It is important to update the parameter "Number of genes" to match the number of genes in the selected text file. If such a file does not exist, you can just type, in the "Add genes to scheme" section of the dialog, the name of the locus in the **Name of gene** field, and click the **Add** (i) button.

If you accidentally add a wrong name to the list, select it and click the **Remove** button.

When you are done adding loci for your scheme, click **Next** to start assigning sequences to the loci. Note that the option "Extract allele number from sequence name" assumes that the allele number is part of the allele name (e.g. 'atpA_2'). If this is not the case, disable the option.

Now follows a number of steps - one for each locus in the scheme. In each of these steps, you can assign a number of allelic sequences to each locus.

In figure 3.9 you can see that the locus called *gene1* from figure 3.8 has its own step where sequences can be added. Clicking **Next** will display a similar dialog for *gene2*, and similar for all the loci that were entered in the first dialog.

It is not mandatory to add sequences to the loci. You can also choose to proceed and create the scheme before you add sequences. If you just add individual sequences as shown in figure 3.9, they will not be combined in an allelic profile and the scheme will not yet contain any sequence types. See section section 5 about how to add **Isolates** to a scheme.

Note! If you wish to use automatic assignment of sequences when creating isolates (explained in section 4.1), there has to be at least one sequence per locus in the scheme.

 Set scheme information Add to gene "gene1" Add to gene "gene2" Result handling 	Add to gene "gene1" Sequences from within the workbench Navigation Area Add to gene1_1 	Selected elements (2)
---	---	-----------------------

Figure 3.9: Selecting sequences for gene1.

3.3 Merging schemes

The CLC MLST Module is designed to make it easy to extend a scheme with your own isolates (see chapter 5). This is done be adding sequence information to your local copy of a scheme - either one you created yourself from scratch or a scheme downloaded from a database.

When you extend your own copy of a downloaded scheme, you may find yourself in a situation where there has been updates to the scheme at the database including new alleles and/or sequence types, and you now have two schemes that you wish to combine into one. Just downloading and using the new scheme from the database would mean discarding your own contributions to your local copy of the scheme.

For this reason, the CLC MLST Module provides a merge function that makes it easy to combine two schemes:

Multilocus Sequence Typing (a) | MLST Schemes (a) | Merge MLST Schemes (a)

This will bring up a dialog where you select the two schemes that you wish to merge. The schemes have to have the same loci definitions in order to be merged (i.e. the same number of loci with identical names).

Clicking **Next** displays the dialog shown in figure 3.10.

Gx Merge MLST Schemes	×
Select at least two MLST Schemes Set parameters <i>Result handling</i>	Set parameters Update of links Set new links to merged scheme in all referencing isolates.
Help Reset	Previous Next Finish Cancel

Figure 3.10: This option makes it possible to update all references in isolates previously referring to one of the two old schemes so that they refer to the new scheme.

If you check this option, all the isolates that previously referred to one of the two old schemes in order to determine their sequence type can now be updated to refer to the new scheme instead (see section 4.2.1 for more information). Checking this option means that all isolates are typed according to the new scheme.

Click **Next** if you wish to save the new scheme without opening. If not, click **Finish**, and the new scheme will be opened.

If there are duplicates among the sequence types in the scheme, only one of the types will be included in the new scheme.

3.4 Viewing schemes

There are two ways of viewing the MLST schemes (besides the history view) as described below:

3.4.1 Profile table

The profile table shows all the sequence types in one table (see an example in figure 3.11).

aroC	dnaN	hemD	hisD	purE	sucA	thrA	donal_complex		I Profile Table (I	NGS) Settings	
1	1	1	1	1	1	1	5		Column width		
2	1	1	2	1	1	1	5	=		Automatic -	
3	1	1	2	1	1	1	9		Show column		
4	43	41	16	13	34	13	4		Show column		
5	16	43	45	43	36	39	42			V ST	
6	4	4	4	5	4	4	8			aroC	
7	2	2	3	2	2	2	6				
8	1	1	2	3	1	1	5			dnaN	
9	33	26	30	55	21	87	134			V hemD	
10	5	2	3	6	5	5	10				
11	5	2	3	7	6	6	11	_		V risb	
12	6	5	6	7	5	7	12			V purE	
13	3	3	7	4	3	3	7	_		I aica	
14	7	6	8	8	7	8	13				
15	2	7	9	9	5	9	12	_		👽 thrA	
16	6	/	10	10	8	10	14			✓ clonal complex	
17	8	8	11	11	5	11	15				
18	9	9	6	12	9	12	2			Select Alí	
19	10	/	12	9	5	9	2			Deselect All	
20	11	10	13	13	10	13	4				

Figure 3.11: The profile table.

Each allelic profile is assigned a number - the **Sequence type (ST)**. The following columns displays the allele type for each locus. It is the combination of these allelic types which defines the sequence type (see section 1.1).

The last column displays information about the **Clonal complex** of this sequence type.

The table can be sorted by clicking the column headers. Pressing Ctrl (\Re on Mac) while clicking the column makes a secondary sorting within the primary sorting.

You can search the table by using the **Filter** at the upper right part of the view. E.g. typing *ST-143* will show sequence types within the *ST-143* clonal complex.

Extracting alignments

If you wish to compare the sequences of two or more sequences types in a multilocus sequence analysis, you can easily extract concatenated alignments from the profile table. To extract an alignment displaying the sequences of one or more loci:

Select the sequence types you wish to align | right-click | Extract Alignment | Sequence Types or Alleles

The two options in the alignment right-click menu provide two ways of composing the alignment (figure 3.12):

• Sequence Types (possibly redundant). An alignment is made with alleles from all the selected sequence types, even if the same allele occurs more than once.

aroC		dnaN	hemD		hisD	purE		sucA	thrA		clonal_complex		Profile Table S	ettings	
1	1		1	1		1	1		1	5			Column width		
2	1		1	2		1	1		1	5		1		Automatic 👻	
3	1		1	2		1	1		1	9		-	Chan and and		
			41				34			4			Show column		
5	16		43	Extract A	Alianment	•	Sequenc	e Types (pos	sibly redund	int) 2				V ST	
6	4		4			_				8				20rc	
7	2		2	Extract S	Sequences	•	Alleles (r	nonredundar	nt)	6					
8	1		1	Remove			1		1	5				💟 dnaN	
9	33		26	Kentove	sequence type		21		87	114				V hemD	
10	5		2	3		6	5		5	0				TT hist	
11	5		2	3		7	6		6	11				V HISD	
12	6		5	6		7	5		7	12				🗸 purE	
13	3		3	7		4	3		3	7				V sucA	
14			6	8		8			8	13					
15	2		7	9		9	5		9	12				V thrA	
16	6		2	10	1		8		10	14				clonal_complex	
1/	8		0	11	1	1	5		11	15				Select All	
10	10		7	10	1	2	9		14	4				Jelect All	
13	10	_	10	12	1	2	10		12	2				Deselect All	
	- 4		10	12		4	10		44						o
Show Drofile Ta	h la	1												Help	Save View.

Figure 3.12: Extract alignment from a profile table.

• Alleles (non-redundant). Redundant alleles are excluded from the alignment where possible. If you e.g. select three rows in the table and two of the sequence types have the same allele sequence in all the selected loci, the alignment will only include two sequences (the first option would have included all three sequences in the alignment).

Selecting either option will display a dialog similar to the one shown in figure 3.13 displaying the loci in the scheme.

Gx Select genes
Available genes
🗐 dnaN
hemD
in hisD
m purE
🔲 sucA
🔲 thrA
OK Cancel

Figure 3.13: Selecting genes for alignment.

Before calculating the alignment, the allele sequences for each sequence type will be joined into a "supergene" sequence which is used for the alignment. The checkboxes shown in figure 3.13 let you specify which of the loci that should be used in the joined sequence. Selecting only one locus will create a "non-supergene" alignment with allele sequences for only this particular locus.

When you click **OK**, a dialog with alignment parameters will be shown. To learn more about these parameters, click the dialog's **Help** button.

Extracting sequences

If you wish to extract unaligned concatenated sequences for other analyzes or if you wish to inspect the sequence graphically, you can easily extract the sequences of one or more sequence types to a sequence list:

Select the sequence types you wish to extract | right-click | Extract Sequences | Sequence Types or Alleles (see below) The two options in the right-click menu provide two ways of composing the list (equivalent to extracting alignments (see above)):

- Sequence Types (possibly redundant). A sequence list is made with alleles from all the selected sequence types, even if the same allele occurs more than once.
- Alleles (non-redundant). Redundant alleles are excluded from the list where possible. If you e.g. select three rows in the table and two of the sequence types have the same allele sequence in all the selected loci, the list will only include two sequences (the first option would have included all three sequences in the list).

Selecting either option will display a dialog similar to the one shown in figure 3.13 displaying the loci in the scheme.

The sequences in the sequence list will be created by joining the sequence for each locus that you select in this dialog.

Click **OK** to create the sequence list.

Adding and removing sequence information

For information about *adding* sequence information to a scheme, see chapter 5. To *remove* sequence types from the scheme:

Select the sequence types you wish to remove \mid right-click \mid Remove Sequence Type

This will remove the sequence type from the scheme. The allele sequences will not be removed, since they can be shared among several sequence types. To delete the allele sequences, see chapter 5.

3.4.2 Allele table

The profile table described above provides an overview of the sequence types in a given scheme. The **Allele table** is another way of viewing an MLST scheme which provides an overview of the alleles that are included for each locus (see figure 3.14).

😿 Salmonella e	enterica (2016-01	1-29) ×				
					I Allele Table Se	ettings
Rows: 7		Genes of t	ne scheme	Add Sequences	Column width	-
Gene				Number of alleles		Manuai 👻
aroC					Show column	-
dnaN				544		Allele name
hemD				490		Allelic number
hisD				738		
purE				581		Sequence length
sucA				571		Creation date
thrA				618		
Allele name	Allelic number	Sequence le	Creation date	Sequence types		Sequence types
aroC1	1	501	Fri Jan 29 11:32:43 CET 2016	1; 2; 3; 8; 815; 890; 911; 1856; 1919; 2138; 2160; 2173; 2209; 2230; 2233; 2244; 2254; 2 🔺		Select All
aroC2	2	501	Fri Jan 29 11:32:43 CET 2016	7; 15; 31; 33; 38; 43; 86; 110; 132; 142; 148; 149; 188; 191; 216; 250; 269; 270; 271; 272 =		Deselect All
aroC3	3	501	Fri 1an 29 11:32:43 CET 2016	13: 37: 95: 674: 676: 677: 1215: 1328: 1495: 1668: 2332: 2515: 2990		
<⊓	1			•		
xx 🗙 🖽 🖽	0 1					Help Save View

Figure 3.14: The allele table.

Switching between the two table views of the scheme is done by clicking the buttons at the lower left corner of the view (\overline{k}) / (\overline{k}).

At the top of the view is a master table including all loci of the scheme (in the example shown in figure 3.14 there are seven loci). Selecting a row in the master table updates the allele table

below which displays all the alleles of the selected locus. The columns in the allele table provide information about allele name, allele number, fragment length, creation data and a list of the sequence types in which a given allele is found.

Displaying the sequences

If you wish to open the sequence of one of the allelic types:

Select one or more allelic types | right-click | Extract as Sequence List

This will open a sequence list of the allelic types that were selected.

Creating an alignment comparing the allelic types

If you wish to create an alignment of the sequences of two or more allelic types:

Select the allelic types | right-click | Align Sequences

This will show an alignment parameters dialog. To learn more about these parameters, click the dialog's **Help** button.

Removing allele sequences

To remove one of the allelic types:

Select the allelic type | right-click | Remove

Note! This will also remove all the sequence types containing this allelic type from the scheme. If you remove an allelic type by accident, you can always **Undo** (().

Chapter 4

Isolates

The **Isolate** (②) is the fundamental data object in the CLC MLST Module. An isolate in this context constitutes a collection of sequencing results - one for each locus. A sequencing result is represented as a contig which has been assembled from one or more sequencing reads (usually a forward and a reverse read) and a reference sequence defined by the scheme. The creation of an isolate is always based on a given scheme and consists of the assembly of sequencing data for each loci, assignment of allele type for each loci given the alleles in the scheme, and final assignment of sequence type given the sequence types in the scheme. In the CLC MLST Module all these steps are performed automatically and rapidly.

4.1 Assemble and create isolate

An isolate is created by selecting a number of sequences/sequencing data and an MLST scheme.

To create an isolate:

Multilocus Sequence Typing (🚔) | Assembly and Create Isolate (🚃)

This will bring up a dialog as shown in figure 4.1.



Figure 4.1: Selecting isolate sequences.

In this example, 8 sequences have been selected: a forward and a reverse read for each locus. Even if you do not have sequences for all loci, you can create the isolate and add more sequence later (see section 4.2.6).

Clicking **Next** displays the dialog shown in figure 4.2.

Select nucleotide	Select parameters
sequences	Reference scheme
2. Select parameters	Choose Reference Scheme:
3. Assign to gene "aroC"	Samonena entenca (2010-01-23)
A. Assign to gene "dnalv"	Alignment options
5. Assign to gene "hemD"	Minimum aligned read length: 50 Alignment stringency: Medium 👻
5. Assign to gene "hisD"	
7. Assign to gene "purE"	□ Inmming options
8. Assign to gene "sucA"	
). Assign to gene "thrA"	Information If you press finish in this step all sequences will be assigned automatically. Press next, to assign sequences manually for each gene
0. Result handling	with help of the automatic assignment.

Figure 4.2: Setting a reference scheme for typing the isolate.

At the top of the dialog, you set the MLST scheme to be used for this isolate by clicking the small button (). You have to either create a new scheme or download a scheme and save it in the **Navigation Area** before you can create the isolate (see chapter 3 on how to create and download schemes).

Below there are the options regarding assembling the sequences into contigs:

- Minimum aligned read length. The minimum number of nucleotides in a read which must be successfully aligned to the contig. If this criteria is not met by a read, the read is excluded from the assembly.
- Alignment stringency. Specifies the stringency of the scoring function used by the alignment step in the contig assembly algorithm. A higher stringency level will tend to produce contigs with less ambiguities but will also tend to omit more sequencing reads and to generate more and shorter contigs. Three stringency levels can be set: Low, Medium or High.
- Use existing trim information. If you have previously trimmed (🛟) the sequences, you can select this option to use the trim information. This is generally not necessary since the sequences are assembled to a reference sequence from the scheme. If the contigs in the isolate are not calculated correctly, you can try to trim the sequences first.

When a scheme has been selected, it provides reference sequences for each of the loci. When you click **Next**, the CLC MLST Module automatically compares each of your sequences to these reference sequences and if they are homologous, your sequence is assigned to this locus. This eliminates the need for manually selecting which sequences belong to which loci.

The following steps in the dialog traverse through the loci, showing which sequences have been assigned to each locus. An example is shown in figure 4.3 where two sequences are assigned to the *dnaN* gene (listed to the right).

 Select nucleotide sequences 	Assign to gene "aroC" Assign sequences to gene "aroC" Not assigned		Assigned to gene "aroC"	
2. Select parameters	X dnaN_249_F	A	200 aroC_389_F	
Assign to gene "aroC"	<pre>XX dnaN_249_R XX hemD_464_F</pre>		x aroC_389_R	
4. Assign to gene "dnaN"	XC hemD_464_R XC hisD 9 F			
5. Assign to gene "hemD"	XX hisD_9_R			
5. Assign to gene "hisD"	XC purE_248_F XC purE_248_R			
7. Assign to gene "purE"	XC sucA_154_F XC sucA_154_R			
8. Assign to gene "sucA"	X thrA_1594_F	-		
 Assign to gene "thrA" Result handling 	Information Notice that if you press "Previous"	" all manual assignme	ents are deleted.	

Figure 4.3: Assigning sequences to the dnaN gene.

You can of course manually change which sequences should be assigned to this locus by using the **Add** (\Rightarrow) and **Remove** (\Leftarrow) buttons.

To the left you see the rest of the sequences which have not yet been assigned to a locus. When clicking **Next**, the CLC MLST Module will try to find sequences matching the reference sequence for the next locus, and so on for all the loci. When clicking **Next** at each step, only the sequences listed in the left side of the dialog are considered for the automatic assignment.

When you press Finish, the isolate will be created and opened .

Note! If you press **Finish** while there are sequences listed to the left, they will not be assigned to a locus. Therefore, you should either click **Finish** after the second step when you have selected the scheme, or you should wait until you have stepped through all the assignment steps.

4.2 Working with isolates

A view of an isolate is shown in figure 4.4.

At the top there is a row for each locus. Each row represents a contig which is based on the sequences that have been assigned (either automatically or manually) to this given locus and a reference sequence.

The information in each row is:

- Gene. The name of the locus.
- **Assigned sequence(s)**. The name of the sequences that have been assigned to this locus. In most cases, this will be a reference contig of the selected sequences.
- Allele type. This is the allele type based on comparing the consensus sequence of the contig with the allele sequences in the scheme (this is explained further below).

4.2.1 Isolate-scheme dependencies and the sequence type

An isolate (as shown in figure 4.4) is created by defining a scheme to use as a reference. The scheme is used to:

Rows: 7 Isolate content	Filter to Selection	Filte	er
Gene	Assigned sequence(s)	Allele type	
naN	Reference contig of dnaN	1	
emD	Reference contig of hemD	2	
sD	Reference contig of hisD	New allele	
лЕ	Reference contig of purE	New allele	
JCA	Reference contig of sucA	New allele	
ırA	Reference contig of thrA	New allele	
	Delete Assign Elements	Assemble Align to Nearest Allele	
Sequence Type(s)			
Sequence Type(s) iew Type			
Sequence Type(s) iew Type			
Sequence Type(s) lew Type			
Sequence Type(s) iew Type			
Sequence Type(s)			
Sequence Type(s) lew Type			
Sequence Type(s)			
Sequence Type(s) lew Type			
Sequence Type(s) iew Type			
Sequence Type(s)			
Sequence Type(s) New Type			
Sequence Type(s) New Type MLST Scheme			
Sequence Type(s) lew Type MLST Scheme	11.201		
Sequence Type(s) New Type MLST Scheme Salmonella enterica (2016-0	11-29)		
Sequence Type(s) New Type MLST Scheme Salmonella enterica (2016-(11-29) <i>j</i> ₀		

Figure 4.4: An isolate of Streptococcus Uberis typed as number one.

- Automatically assign sequences to loci (see section 4.1).
- Determine the allele type for the consensus sequence for each locus (at the top of the isolate view).
- Determine the sequence type of the isolate.

For each locus, the CLC MLST Module compares the reference sequence of the isolate's contig with all the allelic types in the scheme. If there is a perfect match, an allelic type is assigned. If not, the allele type will be "New allele" (in this case, the new allele can be compared to the nearest allele in the scheme, see section 4.2.5).

The sequence type is shown in the field at the middle of the view and determined by the CLC MLST Module by comparing the allelic profile (all the allele types) of the isolate with the allelic profiles in the scheme (see section 3.4.1).

If none of the allelic profiles are identical to the isolate's allelic profile, the text field will say: "New type". If there are one or more loci where no sequences have been assigned, there could be a number of possible sequence types because one or more allelic type is unknown. All the possible sequence types will then be listed.

The isolate is dynamically linked to the scheme, meaning that if the scheme is updated, the allelic types and the sequence type of the isolate is calculated again. In this case, a dialog is presented to inform you that the information in the isolate view is about to change.

If you wish to use another scheme as basis for typing the isolate, click the **Browse** button (\overline{k}) next to the scheme name at the bottom of the view.

Clicking **Find in Navigation Area** () will select the scheme that is currently used for typing the isolate.

4.2.2 Opening contigs

The contig of each locus can be opened either by double-clicking the table entry or clicking the **open** (\equiv) button. This lets you inspect and edit the contig for variations and sequencing errors in a separate view (see figure 4.5).



Figure 4.5: A contig opened from an isolate.

If you edit the consensus sequence in the contig, the isolate will automatically update the allelic type and subsequently the sequence type. This means that e.g. correcting a sequencing error will immediately reveal the right allelic type.

All changes to the contig are recorded in the contig's history (
.

4.2.3 Deleting contigs

It may be desirable to delete a contig because of poor quality data or wrong assignment of sequences. To delete a contig:

select the row in the table to delete | Delete

This will remove the contig, and the allelic type will now be: "Unassigned".

4.2.4 Assigning new contigs

If there are no sequences assigned to a locus, you can assign either a sequences or a contig of assembled sequences:

select the relevant row in the table | Assign Elements

This will allow you to assign one sequence or one contig to this locus. Note that if the sequence is not trimmed, you have to do this manually first. Alternatively, use the **Assemble** (A) button

instead. This also allows you to assign several sequences by assembly to the reference sequence in the scheme (see section 4.2.6).

4.2.5 Align to nearest allele

If you have found a new allele, you can compare this with the allele in the scheme which has the highest degree of homology:

select the relevant row in the table | Align to Nearest Allele (

The CLC MLST Module then searches through all the alleles in the scheme and compares them to the new allele. Then an alignment is created with the nearest allele to make it easy to inspect conservation.

4.2.6 Assemble to an existing isolate

CLC MLST Module makes it easy to perform sequence typing in several iterations. If you e.g. find errors in the sequencing data of some of the loci, you can create a preliminary version of the isolate with only sequence data for some of the loci. Then you can re-sequence the missing loci and update the isolate with new sequences.

This is done using the **Assemble to Existing Isolate** tool, available from:

Tools | Multilocus Sequence Typing (a) Assemble to Existing Isolate (

As input for this tool, select exactly one isolate and at least one nucleotide sequence. It is possible to select a nucleotide sequence list (see figure 4.6) or multiple nucleotide sequences.

 Assemble to Existing Isolate Select nucleotide sequences and one isolate Select parameters Result handling 	Select nudeotide sequences and one isolate Navigation Area Q < <enter search="" term=""></enter>
Help Reset	Previous Next Finish Cancel

Figure 4.6: Assembling an isolate.

The rest of the dialogs are identical to the dialogs displayed when creating an isolate *de novo* (see section 4.1).

4.3 View a report of an isolate

An isolate can also be viewed in a graphically more pleasant format. Click the **Show Report** (**M**) to show the isolate report (see figure 4.7).

The view contains the same information as the standard isolate view, but the layout is improved for printing and graphics export.

1. Multilocus sequence typing

1.1 Typing results

This report uses the MLST Scheme Streptococcus uberis

1.1.1 Allelic Profile and Sequence Type

Gene	Allele sequence	Allelic profile
arcC	GCAGCAGCAGCAGATTCGTAAAAAAAC CCTGCAATGCCATTGGATACTTGT GTGGCAATGACCAATTGGATCTATT GGTGCCAGTGACGAAGGATCTATT GATAACGAACTTAAAGAACAAGGC ATTGAAAAAGAAGTGGCTGCAGGT GTGACACAAGTTATTGTTGATAAA AACGACCAAGGATTCACAAAACCAA ACGAACCAAGTGGCCCATCCTTA ATGGAAGAAACTGGAGCAAGCTTT AAAGAAGATTCAGGTCGTGGAGTG GCGTAAAGTGGCATCCAAAACCAA ACCAGTTGGCATCAAAGAAGCTAA ACCAGTTGGCATCAAAGAAGCTAA ACGAGTTGGCATCAAGAAGCTAA GGTGTTGTTGTTGTCACCGCTGGT GGGTGCCGTGTCCCAGTTATTGA AGGACCCAGCGTCCCAGTTATTGA AGGACCCGGTGTCCCAGTTATTGA AGGACCCGGTGTCCCAGTTATTGA AGGACCCCAGTCCCAGTTATTGA AGGACCCGGTGTCCCAGTTATTGA AGGACCCAACAAC	1
ddi	ACAAACCACATGCTCCAATAGCTT TAAAAGCTTGTTCTGCAAAATATCT CATTGTGCAGTGATGTGCCTTCATC AATCGTCGCGGGGAATAGCCATACT TGTCGTCACTGCAGGGCAACATCT TGATGGCTACTATGAGGCAACATCT TTGATGGCTACCATGGCAACATCT TTGATGGCTACTTCACCAGGCAACATCT GATGGCTACTTCAATCACACGTGGTA GGCCAACCTTGTTCAATCACACGTGGTA CGACACCTTGTTCAATCACACGTGCTAATGC GACTATCATATTTTTAAAGCTTAATGC	1

Figure 4.7: An isolate shown as a report.

Chapter 5

Extending schemes

MLST schemes can be extended in two ways: by adding isolates or by adding sequences:

5.1 Add isolates to scheme

If you have an isolate which represents a new sequence type, you can add this isolate to a scheme and hereby expand the scheme with one more sequence type:

Tools | Multilocus Sequence Typing (a) Add Isolates to MLST Scheme (

or Open the isolate you wish to add | Scroll down to the bottom Add Isolate (

You do not have to specify a scheme since an isolate is always based on a scheme when it is created. The isolate will be added to the scheme that is defined at the bottom of the isolate view (see section 4.2.1).

5.2 Add sequences to scheme

You can also add individual sequences to the alleles in the scheme:

```
Tools | Multilocus Sequence Typing (\cong) | MLST Schemes (\cong) | Add Sequences to MLST Scheme (\cong)
```

This will bring up a dialog as shown in figure 5.1.

Gx /	Add Sequences to MLST Sc	eme	
1.	Select one MLST Schem	Select one MLST Scheme Navigation Area Selected elements (1)	
2.	Add to gene "aroC"	Q v <enter search="" term=""></enter>	٦
3.	Add to gene "dnaN"	New MLST Scheme-2	
4 . ∢	Add to gene "hemD"		
	Help Reset	Previous Next Finish Cancel	

Figure 5.1: Define a scheme to add sequences to.

Clicking **Next** takes you through a series of step - one for each locus - where you can assign sequences (see figure 5.2).



Figure 5.2: Adding a sequence to gene abcZ.

The sequences added in this way cannot be combined to an allelic profile and a sequence type, and will thus only contribute to expanding the number of allelic types.

Chapter 6

Install and uninstall modules

6.1 Installation of modules

Note: In order to install plugins and modules, the *CLC Workbench* must be run in administrator mode. On Windows, you can do this by right-clicking the program shortcut and choosing "Run as Administrator". On Linux and Mac, it means you must launch the program such that it is run by an administrative user.

Plugins and modules are installed and uninstalled using the Workbench Plugin Manager. To open the Plugin Manager, click on the **Plugins (button** in the top Toolbar, or go to the menu option:

Utilities | Manage Plugins... (😫)

The Plugin Manager has two tabs at the top:

- **Manage Plugins** An overview of your installed plugins and modules is provided under this tab.
- **Download Plugins** Plugins and modules available to download and install are listed in this tab.

To install a plugin, click on the **Download Plugins** tab (figure 6.1). Select a plugin. Information about it will be shown in the right hand panel. Click on the **Download and Install** button to install the plugin.

Accepting the license agreement

The End User License Agreement (EULA) must be read and accepted as part of the installation process. Please read the EULA text carefully, and if you agree to it, check the box next to the text **I accept these terms**. If further information is requested from you, please fill this in before clicking on the **Finish** button.

Installing a cpa file

If you have a .cpa installer file for QIAGEN CLC MLST Module, you can install it by clicking on the **Install from File** button at the bottom of the Plugin Manager.



Figure 6.1: Plugins and modules available for installation are listed in the Plugin Manager under the Download Plugins tab.

If you are working on a system not connected to the internet, plugin and module .cpa files can be downloaded from https://digitalinsights.qiagen.com/products-overview/ plugins/using a networked machine, and then transferred to the non-networked machine for installation.

Restart to complete the installation

Newly installed plugins and modules will be available for use after restarting the software. When you close the Plugin Manager, a dialog appears offering the opportunity to restart the *CLC Workbench*. When you have installed the QIAGEN CLC MLST Module and start a tool from that module for the first time, the License Assistant will open (figure 6.2).

The License Assistant can also be launched by opening the Workbench Plugin Manager, selecting the installed module from under the Manage Plugins tab, and clicking on the button labeled *Import License*.

To install a license, the *CLC Workbench* must be run in administrator mode. On Windows, you can do this by right-clicking the program shortcut and choosing "Run as Administrator". On Linux and Mac, it means you must launch the program such that it is run by an administrative user.

The following options are available:

- Request an evaluation license. Request a fully functional, time-limited license.
- **Download a license**. Use the license order ID received when you purchased the software to download and install a license file.
- **Import a license from a file**. Import an existing license file, for example a file downloaded from the web-based licensing system.
- **Configure license manager connection**. If your organization has a *CLC Network License Manager*, select this option to configure the connection to it.

ed a valid license. or this plugin. r out the plugin for 14 days. cense will be allowed for each computer. c license.
out the plugin for 14 days. cense will be allowed for each computer. clicense.
out the plugin for 14 days. cense will be allowed for each computer. clicense.
c license.
c license.
nse file.
License Manager that hosts network license(s) for this
connection configuration.
nse file. License Manager that hosts network license(s) fo

Figure 6.2: The License Assistant provides options for licensing modules installed on the Workbench.

These options are described in detail in sections under https://resources.qiagenbioinformatics. com/manuals/clcgenomicsworkbench/current/index.php?manual=Workbench_Licenses.html.

To download licenses, including evaluation licenses, your machine must have access to the external network. To install licenses on non-networked machines, please see https://resources.giagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Download_static_license_on_non_networked_machine.html.

6.2 Uninstalling modules

Plugins and modules are uninstalled using the Workbench Plugin Manager. To open the Plugin Manager, click on the **Plugins (button** in the top Toolbar, or go to the menu option:

Utilities | Manage Plugins... (💱)

This will open the Plugin Manager (figure 6.3). Installed plugins and modules are shown under the Manage Plugins tab of the Plugins Manager.

To uninstall a plugin or module, click on its entry in the list, and click on the **Uninstall** button.

Gx Manage Plugins			X
PM Manage Plugins Download	Plugins		
Provider: QLAGEN Aarhus Support contact: ts-bioinformatics@qiag Version: 1.1 (Build: 190328-1503-19140	jen.com (4)		<u>^</u>
Biomedical Genomics Analysis			Uninstall Disable
CLC MLST Module Provider: QIAGEN Aarhus Support contact: ts-bioinformatics@qiag Version: 1.9 (Build: 181115-1337-18544	jen.com i2)		Update available
MLST Module makes it easy and fast to do MultiL	ocus Sequence Typing.		\smile
		Update Import License	Uninstall Disable
CLC Microbial Genomics Module Provider: QIAGEN Aarhus Support contact: ts-bioinformatics@qiag Version: 4.1 (Build: 190129-1433-18833	jen.com 33)		
CLC Microbial Genomics Module			
		Import License	Uninstall Disable
Hep Proxy Settings Check for Up	dates Install from File		Close

Figure 6.3: Installed plugins and modules are listed in the Plugins Manager under the Manage Plugins tab.

Plugins and modules are not uninstalled until the Workbench is restarted. When you close the Plugin Manager, a dialog appears offering the opportunity to restart the *CLC Workbench*.

Disabling a plugin without uninstalling it

If you do not want a plugin to be loaded the next time you start the Workbench, select it in the list under the Manage Plugins tab and click on the **Disable** button.

Chapter 7

Licensing requirements for the QIAGEN CLC MLST Module

To use tools delivered by the CLC MLST Module, you will need a license. The module can be installed in Viewing Mode on a *CLC Workbench* to access data created using the module's tools without requiring a license.

7.1 Licensing modules on a Workbench

The License Assistant can be launched by opening the Workbench Plugin Manager, selecting the installed module from under the Manage Plugins tab, and clicking on the button labeled *Import License*.

To install a license, the *CLC Workbench* must be run in administrator mode. On Windows, you can do this by right-clicking the program shortcut and choosing "Run as Administrator". On Linux and Mac, it means you must launch the program such that it is run by an administrative user.

7.1.1 Request an evaluation license

We offer a fully functional version of the CLC MLST Module free of charge for a 14 day period for evaluation purposes. The 14 day period commences when the evaluation license is downloaded. If you have questions about the CLC MLST Module features or product licensing options, please send an email to bioinformaticssales@qiagen.com.

When you choose the option **Request an evaluation license**, the dialog shown in figure 7.2 opens.

In this dialog, there are two options:

- **Direct Download**. Download the license directly. This method requires that the Workbench has access to the external network.
- **Go to CLC License Download web page**. The online license download form will be opened in a web browser. This option is suitable for when downloading a license for use on another machine that does not have access to the external network, and thus cannot access the QIAGEN Aarhus servers.

License Assistant		×
	P M Workbench Plugins	
You need a lie	cense	
In order to load the Please choose how	plugin "CLC Cloud Module" you need a valid license. you would like to obtain a license for this plugin.	
Request	an evaluation license	
Choose Please	: this option if you would like to try out the plugin for 14 days. note that only a single evaluation license will be allowed for each computer.	
ODownlo	ad a license	
Use a li	icense order ID to download a static license.	
O Import a	a license from a file	
Import	a static license from an existing license file.	
🔿 Configu	re license manager connection	
Config produc	ure a connection to a CLC Network License Manager that hosts network license(s) for this t, or update or disable an existing connection configuration.	
lf	you experience any problems, please contact <u>QIAGEN Digital Insights Support</u> HostID:	
Proxy Settings	Previous Next	Cancel

Figure 7.1: The License Assistant provides options for licensing modules installed on the Workbench.

Request an evaluation license... Please choose how you would like to request an evaulation license. O Direct Download The workbench will attempt to contact the CLC Licenses Service, and download the license directly.

	This method requires internet access from the workbench.
0	Go to License Download web page
	The workbench will open a Web Browser with the License Download web page. From there you will be able to download your license as a file and import in the next step.

Figure 7.2: Choosing between direct download or going to the license download web page.

After selecting your method of choice, click on the button labeled Next.

Direct download

After choosing the **Direct Download** option and clicking on the button labeled **Next**, a dialog similar to that shown in figure 7.3 will appear if the license is successfully downloaded and installed.



Figure 7.3: A license has been successfully downloaded and installed for use.

When the license has been downloaded and installed, the **Next** button will be enabled.

If there is a problem, a dialog will appear indicating this.

Go to license download web page

After choosing the Go to CLC License Download web page option and clicking on the button labeled **Next**, the license download form will be opened in a web browser, as shown in figure 7.4.

This page can be used to download a license if you are not able to contact the license server directly from your CLC Workbench. You have requested the following license:
Product:
Product Version: 1
Host-ID(s): 05EDE85CEFD , A81AEFF919F , 2A378AC18863:
Host name: laptop-32
To download your license, please click the button below.
Download License
If the request is successful a file containing the license will be downloaded to your computer.
To begin using your license you must import the file into the license assistant wizard. Do this by dicking on the Choose License File button and locate the file on your computer.

Figure 7.4: The license download form opened in a web browser.

Click on the **Download License** button and then save the license file.

Back in the Workbench window, you will now see the dialog shown in 7.5.

Import a license from a file Please click the button below and locate the fil	e containing your license.
	No file selected
I	Choose License File

Figure 7.5: Importing the license file downloaded from the web page.

Click on the Choose License File button, find the saved license file and select it. Then click on the **Next** button.

7.1.2 Download a license using a license order ID

Using a license order ID, you can download a license file via the Workbench or using an online form. When you have chosen this option and clicked Next button, you will see the dialog shown in 7.6. Enter your license order ID into the text field under the title License Order-ID. (The ID can be pasted into the box after copying it and then using menus or key combinations like Ctrl+V on some system or \Re + V on Mac).

In this dialog, there are two options:

• Direct Download. Download the license directly. This method requires that the Workbench has access to the external network.

Download a license	
Please copy-paste your License Order-ID into the field below, and choose how you would like to download your licens When processing your request the license-service will check if the License Order-ID is available for download on this computer.	e.
License Order-ID:	
CLC-LICENSE-2000-200048-600-200000-200000	
Direct Download	
The workbench will attempt to contact the CLC Licenses Service, and download the license directly. This method requires internet access from the workbench.	
Go to License Download web page	
The workbench will open a Web Browser with the License Download web page. From there you will be able to download your license as a file and import in the next step.	

Figure 7.6: Enter a license order ID for the software.

• Go to CLC License Download web page. The online license download form will be opened in a web browser. This option is suitable for when downloading a license for use on another machine that does not have access to the external network, and thus cannot access the **OIAGEN** Aarhus servers.

After selecting your method of choice, click on the button labeled **Next**.

Direct download

After choosing the **Direct Download** option and clicking on the button labeled **Next**, a dialog similar to that shown in figure 7.7 will appear if the license is successfully downloaded and installed.

questing a license lesting and downloading an evaluation license by establishing a direct connection to the CLC bio License
-Service.
An Evaluation License was successfully downloaded
The License is valid until: 2015-04-09

Figure 7.7: A license has been successfully downloaded and installed for use.

When the license has been downloaded and installed, the **Next** button will be enabled.

If there is a problem, a dialog will appear indicating this.

Go to license download web page

After choosing the Go to CLC License Download web page option and clicking on the button labeled **Next**, the license download form will be opened in a web browser, as shown in figure 7.8.

Click on the **Download License** button and then save the license file.

Back in the Workbench window, you will now see the dialog shown in 7.9.

Click on the Choose License File button, find the saved license file and select it. Then click on the **Next** button.



Figure 7.8: The license download form opened in a web browser.

Import a license from a file Please click the button below and locate the fi	e ile containing your license.
	A
	No file selected
	Choose License File

Figure 7.9: Importing the license file downloaded from the web page.

7.1.3 Import a license from a file

If you already have a license file associated with the host ID of your machine, it can be imported using this option.

When you have clicked on the **Next** button, you will see the dialog shown in 7.10.



Figure 7.10: Selecting a license file.

Click the **Choose License File** button and browse to find the license file. When you have selected the file, click on the **Next** button.

7.1.4 Configure license manager connection

If your organization is running a *CLC Network License Manager*, you can configure your Workbench to connect to it to get a license for the module.

To configure a Workbench to connect to a *CLC Network License Manager*, select the **Configure license manager connection** option and click on the **Next** button. A dialog appears, as shown in figure 7.11.

License Manager Settings	X
CLC Network Licens	ing
Enable license manager connection	
○ Automatically detect license manager.	
Manually specify license manager:	
Hostname/IP-address:	
Port: 6200 🚖	
Use custom username when requesting a license	
Username:	
Disable license borrowing	
Help	OK Cancel

Figure 7.11: Configuring the connection to a CLC Network License Manager.

The options in that dialog are:

- **Enable license manager connection**. This box must be checked for the Workbench is to contact the *CLC Network License Manager* to get a license for the *CLC Workbench*.
- Automatically detect license manager. By checking this option the Workbench will look for a *CLC Network License Manager* accessible from the Workbench. Automatic server discovery sends UDP broadcasts from the Workbench on port 6200. Available license servers respond to the broadcast. The Workbench then uses TCP communication for to get a license, if one is available. Automatic server discovery works only on local networks and will not work on WAN or VPN connections. Automatic server discovery is not guaranteed to work on all networks. If you are working on an enterprise network on where local firewalls or routers cut off UDP broadcast traffic, then you may need to configure the details of the *CLC Network License Manager* using the Manually specify license manager option instead.
- **Manually specify license manager**. Select this option to enter the details of the machine the *CLC Network License Manager* is running on, specifically:

- Host name. The address of the machine the CLC Network License Manager is running on.
- Port. The port used by the CLC Network License Manager to receive requests.
- Use custom username when requesting a license. Optional. When unchecked (the default), the username of the account being used to run the Workbench is the username used when contacting the license manager. When this option is checked, a different username can be entered for that purpose. Note that borrowing licenses is not supported with custom usernames.
- **Disable license borrowing on this computer**. Check this box if you do not want users of this Workbench to borrow a license. See section **??** for further details.

Releasing Workbench network licenses

Once a network license for a *CLC Workbench* has been obtained, the Workbench must be shut down to release that license for others to use. While it is possible for a license to be pulled from a running Workbench, in practice, that Workbench will immediately retrieve the license just released.

Modules and network licenses

A relevant license is needed to run tools delivered by modules, and to submit jobs to be run on the cloud via a *CLC Workbench*.

To maximize availability, module licenses are first checked out when a job requiring the module is undertaken. Module licenses are checked back in (returned) when the *CLC Workbench* is closed, or four hours after the most recent job requiring that license was launched, whichever is shortest.

Job completion does not depend on the CLC Workbench having a module license checked out.

A note about borrowing module licenses: If you plan to borrow network module licenses and the *CLC Network License Manager* will not be continuously accessible from the *CLC Workbench*, then while there is still a connection, launch a job that requires the relevant module. If available, the relevant license will be checked out, and can then be borrowed. When a module license is borrowed, the four hour validity period mentioned above is not relevant. The module licenses are borrowed for the time period that you specify.

There may be situations where you wish to use a different license or view information about the license(s) the Workbench is currently using. To do this, open the License Manager using the menu option:

Help | License Manager ()

The license manager is shown in figure 7.12.

This dialog can be used to:

- See information about the license (e.g. what kind of license, when it expires)
- Configure how to connect to a license server (**Configure license manager connection** the button at the lower left corner). Clicking this button will display a dialog similar to figure 7.11.

Network (10.1.10.1)		Status:	Borrow limit:	Borrow:
· · · · · · · · · · · · · · · · · · ·	Never	Valid	7 days	
Network (10.1.10.1)	Never	Valid	7 days	V
Network (10.1.10.1)	Never	Valid	7 days	V
Local Evaluation License	11 Days	Valid	-	
on				
ormation				
ormation aptop-112				
prmation aptop-112 10FF 149FD 726, D8FC 930CB 95C, D4F	C930C8958 DAEC930C8959 F)8EC930CB958.D0	DRE9CE08749	
ormation aptop-112 30FF1A9FD726,D8FC930CB95C,DAF	C930CB958,DAFC930CB959,E	08FC930CB958,D0	DBF9CE08749	
ormation aptop-112 DOFF1A9FD726,D8FC930CB95C,DAF	C930CB958,DAFC930CB959,C	98FC930CB958,D0	DBF9CE08749	
ormation aptop-112 DOFF1A9FD726,D8FC930CB95C,DAF Ei	C930CB958,DAFC930CB959,C	08FC930CB958,D0	DBF9CE08749	
ormation laptop-112 00FF 1A9FD 726,D8FC930C895C,DAF	C930CB958,DAFC930CB959,C xport License Information	98FC930C8958,D0	08F9CE08749	
ormation iaptop-112 00FF1A9FD726,D8FC930CB95C,DAF EE	C930CB958,DAFC930CB959,C xport License Information	98FC930CB958,D0	DBF9CE08749	
	arrow License	aorrow License 11 Days	And the second s	Local Evaluation License 11 Days Valid - 30rrow License 00

Figure 7.12: The license manager.

- Upgrade from an evaluation license by clicking the **Upgrade license** button. This will display the dialog shown in figure 6.2.
- Export license information to a text file.
- Borrow a license

If you wish to switch away from using a network license, click on the button to **Configure license manager connection** and uncheck the box beside the text **Enable license manager connection** in the dialog. When you restart the Workbench, you can set up the new license as described in section 1.5.1.

7.1.5 Download a static license on a non-networked computer

To download a static license for a machine that does not have direct access to the external network, you can follow the steps below:

- Install the CLC MLST Module on the machine you wish to run the software on.
- Start up the software as an administrative user and find the host ID of the machine that you will run the CLC Workbench on. You can see the host ID the machine reported at the bottom of the License Manager window in grey text.
- Make a copy of this host ID such that you can use it on a machine that has internet access.
- Go to a computer with internet access, open a browser window and go to the network license download web page:

https://secure.clcbio.com/LmxWSv3/GetLicenseFile

- Paste in your license order ID and the host ID that you noted down in the relevant boxes on the webpage.
- Click 'download license' and save the resulting .lic file.
- Open the Workbench on your non-networked machine. In the Workbench license manager choose 'Import a license from a file'. In the resulting dialog click 'choose license file' to browse the location of the .lic file you have just downloaded.

If the License Manager does not start up by default, you can start it up by going to the Help menu and choosing License Manager.

• Click on the Next button and go through the remaining steps of the license manager wizard.

Bibliography

- [Maiden et al., 1998] Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., Feavers, I. M., Achtman, M., and Spratt, B. G. (1998). Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A*, 95(6):3140–3145.
- [Maiden, 2006] Maiden, M. C. J. (2006). Multilocus sequence typing of bacteria. Annu Rev Microbiol, 60:561–588.
- [Sullivan et al., 2005] Sullivan, C. B., Diggle, M. A., and Clarke, S. C. (2005). Multilocus sequence typing: Data analysis in clinical microbiology and public health. *Mol Biotechnol*, 29(3):245–254.
- [Taylor and Fisher, 2003] Taylor, J. W. and Fisher, M. C. (2003). Fungal multilocus sequence typing–it's not just for bacteria. *Curr Opin Microbiol*, 6(4):351–356.
- [Urwin and Maiden, 2003] Urwin, R. and Maiden, M. C. J. (2003). Multi-locus sequence typing: a tool for global epidemiology. *Trends Microbiol*, 11(10):479–487.