BioNumerics Tutorial:

Setup and analysis using a publicly available MLST scheme

1 Introduction

In this tutorial, we will illustrate the most common usage scenario of the *MLST online plugin*, i.e. when you want to perform MLST using an already published MLST schema, made available via http://pubmlst.org or http://www.mlst.net or any other online repository. For many clinically relevant organisms, an MLST schema is already available and using this schema ensures a consistent nomenclature.

2 Creating a new database

To illustrate the complete setup of the *MLST online plugin* for using a publicly available MLST schema, we will start by creating a new, empty database.

1. Double-click on the BioNumerics icon () on the desktop.

2. In the *BioNumerics Startup* window, press the button to enter the *New database* wizard.

3. Enter a database name, e.g. “Neisseria database”.

4. Click <***Next***> and then <***Finish***>.

A new dialog box pops up, asking whether to create a new relational database for data storage or to use an existing one.

5. Leave the default option ***Create new*** enabled and press <***Next***>.

The next dialog asks which database engine should be used for storing data.

6. Select the default option and press <***Finish***>.

The *Plugins* dialog box pops up which allows you to install additional functionality.

7. Press <***Proceed***> to start BioNumerics.

The *Main* window opens with an empty database.

3 Installing the MLST online plugin

In this section we will install the *MLST online plugin* in our database and we will set it up to use the publicly available *Neisseria* MLST schema.

1. The *Plugins* dialog box is called from the *Main* window by selecting ***File*** > ***Install / remove plugins...*** ().

2. Select the *MLST online plugin* from the list in the *Applications tab* and press the <***Activate***> button.

The next dialog asks to confirm the installation of the *MLST online plugin*. Installation of the plugin requires administrator privileges on the relational database.

3. Press <***Yes***> to confirm the installation of the *MLST online plugin*.

We are asked to select the organism source. In the next section we will import sequences from the organism *Neisseria* in our sample database. Since this organism has an MLST repository online we will use this online database to retrieve the allele, sequence type and clonal complex information for our sample data set.

4. Choose the option ***Select organism from on-line list*** and press <***Next***>.

Any organism for which an MLST repository is available online, is listed in the next step.

5. Select ”Neisseria spp.” from the list and press <***Next***>.

It is possible to specify a location for the profile and allele definition files, which can be located on your own computer, local area network or on the internet. However, the default location should point to the correct files already. The option ***Update profiles and alleles at database startup*** can also be checked, to avoid having to do a manual update.

6. Press <***Next***> to continue.

7. In the next step, leave ***Calculate trimming patterns automatically*** checked and press <***Next***>.

In the final step, the program prompts for database information fields to store the ***Sequence types*** and ***Clonal complexes*** information.

8. For this exercise, use the default ”MLST ST” and ”MLST CC” fields and press <***Next***>.

9. Pressing <***Finish***> starts with the installation of the *MLST online plugin*.

All remotely stored MLST information (allele numbering, sequence types and clonal complexes) for the selected organism will be downloaded in the BioNumerics database during the installation of the plugin. This might take several minutes. When querying for allele numbers, sequence types and clonal complexes in BioNumerics, this locally stored information will be used.

When the *MLST online plugin* is successfully installed, a confirmation message is displayed.

10. Press <***OK***> to close this message.

11. Press <***Exit***> to close the *Plugins* dialog box.

12. Close and reopen the database to activate the features of the *MLST online plugin*.

The *MLST online plugin* installs menu items in the main menu of the software under ***MLST*** (see Figure 1). In the *Main* window, the *MLST online plugin* has installed following items:

- Extra information fields in the *Database entries* panel (default names: **MLST ST**; **MLST CC**).
- One character type called **MLST**, one composite dataset called **MLST_CMP**, and seven sequence types, each named after a housekeeping gene.

Upon installation of the plugin, the trimming patterns of *Neisseria* were automatically calculated. Since a lot of sequences exist for each housekeeping gene in the online repository, the trimming patterns of the *Neisseria* housekeeping genes contain a lot of degenerated positions. The presence of these degenerated positions might result in the assignment of the wrong trimming positions on our sample sequences. Less

© 2018 Applied Maths NV. All rights reserved.

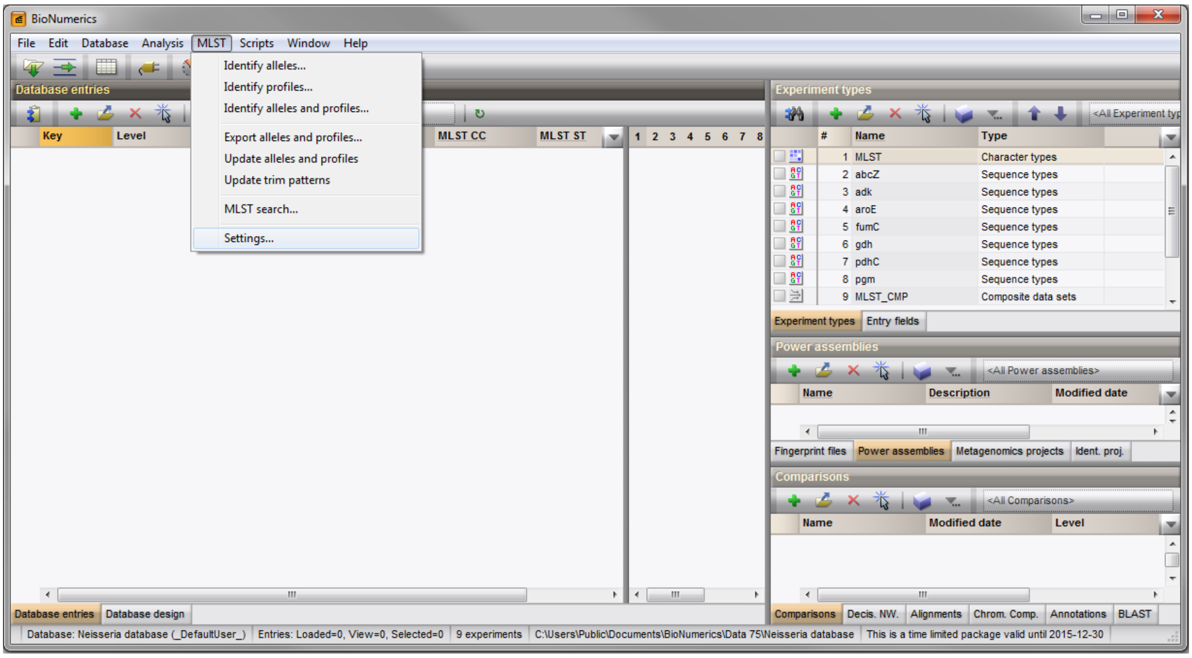


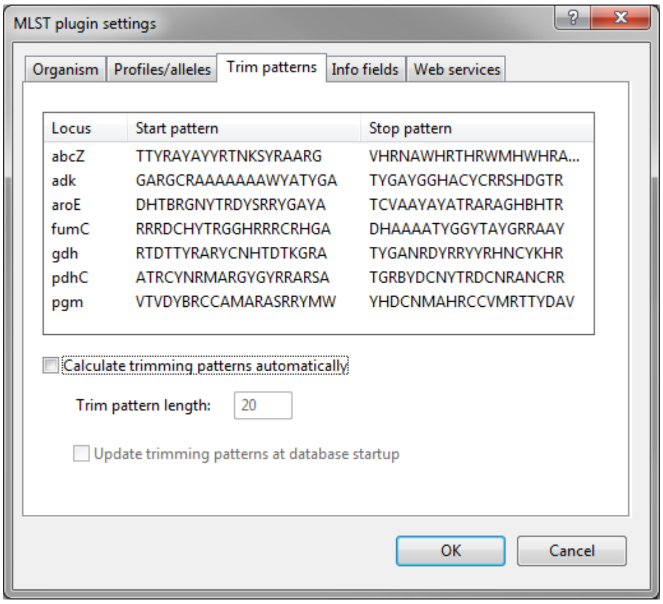
Figure 1: The *Main* window after installation of the MLST online plugin.

degenerated patterns can be found in the `MLST trimming patterns.txt` file that can be downloaded from the Applied Maths website (`http://www.applied-maths.com/download/sample-data`, click on ”MLST sample SCF trace files”).

13. Select ***MLST*** > ***Settings*** to call the *MLST plugin settings* dialog box.

14. Click on the *Trim patterns tab*.

15. Uncheck ***Calculate trimming patterns automatically*** and copy the patterns from the `MLST trimming patterns.txt` file to the correct genes in the grid (see Figure 2).



Locus	Start pattern	Stop pattern
abcZ	TTYRAYAYYRTNKSYRAARG	VHRNAWHRTHRWMHWHRA...
adk	GARGCRAAAAAAAWYATYGA	TYGAYGGHACYCRRSHDGTR
aroE	DHTBRGNYTRDYSRRYGAYA	TCVAAYAYATRARAGHBHTR
fumC	RRRDCHYTRGGHRRRCRHGA	DHAAAATYGGYTAYGRRAAY
gdh	RTDTTYRARYCNHTDTKGRA	TYGANRDYRRYYRHNCYKHR
pdhC	ATRCYNRMARGYGYRRARSA	TGRBYDCNYTRDCNRANCRR
pgm	VTVDYBRCCAMARASRRYMW	YHDCNMAHRCCVMRTTYDAV

☐ Calculate trimming patterns automatically

Trim pattern length: 20

☐ Update trimming patterns at database startup

Figure 2: Trimming patterns after correction.

16. Close the *MLST plugin settings* dialog box.

© 2018 Applied Maths NV. All rights reserved.

The plugin is now set up and we are ready to start assembling allele sequences.

4 Importing and assembling trace files in batch

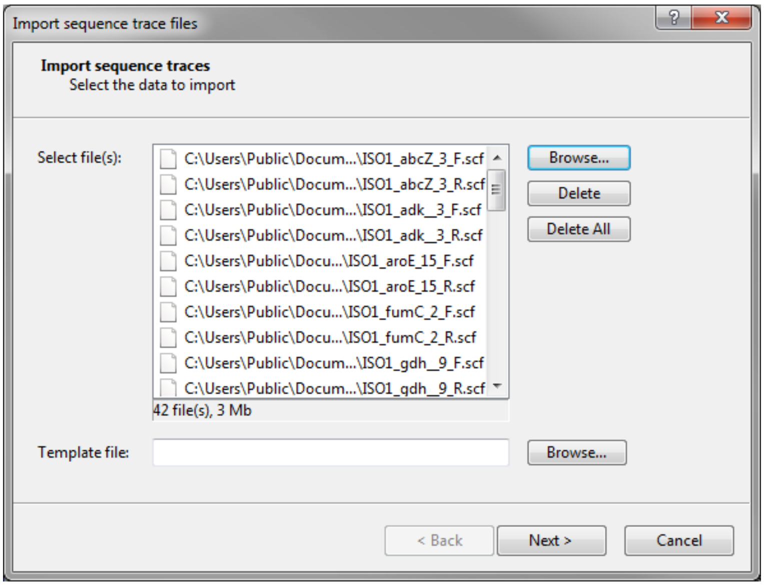
A set of *Neisseria* trace files can be downloaded from the Applied Maths website (http://www.applied-maths.com/download/sample-data, click on ”MLST sample SCF trace files”) and are used in this guide to explain the work flow of the *MLST online plugin*.

1. Select ***File*** > ***Import...*** (, **Ctrl+I**) to call the *Import* dialog box.

2. Select ***Import and assemble trace files*** under ***Sequence type data*** and press <***Import***>.

3. Select the <***Browse***> button, navigate to the correct path, select all the sequence trace files and press <***Open***>.

The *Import sequence traces* wizard page is updated (see Figure 3).



Import sequence trace files

Import sequence traces
Select the data to import

Select file(s):

C:\Users\Public\Docum...\ISO1_abcZ_3_F.scf
C:\Users\Public\Docum...\ISO1_abcZ_3_R.scf
C:\Users\Public\Docum...\ISO1_adk_3_F.scf
C:\Users\Public\Docum...\ISO1_adk_3_R.scf
C:\Users\Public\Docu...\ISO1_aroE_15_F.scf
C:\Users\Public\Docu...\ISO1_aroE_15_R.scf
C:\Users\Public\Docu...\ISO1_fumC_2_F.scf
C:\Users\Public\Docu...\ISO1_fumC_2_R.scf
C:\Users\Public\Docum...\ISO1_gdh_9_F.scf
C:\Users\Public\Docum...\ISO1_gdh_9_R.scf

42 file(s), 3 Mb

Template file:

Figure 3: Select trace files.

4. Press <***Next***> to go the next step.

The way the information should be imported in the database can be specified with an import template. In the example data set, the ***Key*** and ***Sequence experiment name*** are provided in the trace file name. A new import template needs to be defined:

5. Press the <***Create new***> button to call the *Import rules* dialog box.

The only source of information available in the newly created import template is the file name.

6. Double-click on the ***Name*** row or select the row and press <***Edit Destination***>. Select ***Key*** as destination and press <***OK***> (see Figure 4).

The import rule in the *Import rules* dialog box is updated.

7. Check the option ***Show advanced options*** and press the <***Edit parsing***> button.

8. In the *Data parsing* dialog box, fill in following data parsing string: “[DATA]_*”.

© 2018 Applied Maths NV. All rights reserved.

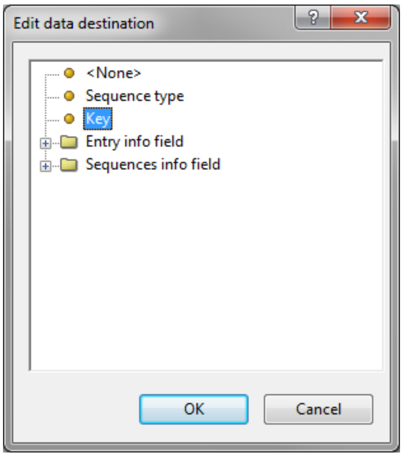
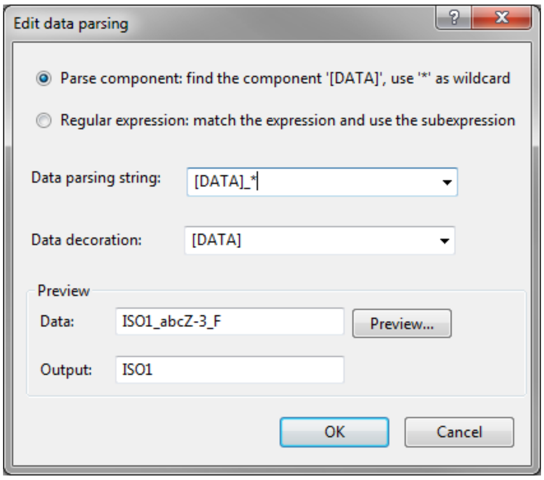


Figure 4: The *Edit data destination* dialog box.

This parsing string will only take into account the text occurring before the first underscore (_). The asterisk (*) serves as a wildcard, meaning that all characters after the first underscore will be ignored.

9. Press the <***Preview***> button and press <***OK***> when the parsing is correct (see Figure 5).



● Parse component: find the component '[DATA]', use '*' as wildcard

○ Regular expression: match the expression and use the subexpression

Data parsing string: [DATA]_*

Data decoration: [DATA]

Preview

Data: ISO1_abcZ-3_F

Output: ISO1

Figure 5: Data parsing string.

Next, we will specify a new rule that links the part of the file name appearing between the first underscore (_) and the hyphen (-) to the ***Sequence type name***.

10. Press <***Add rule***> and select the file <***Name***> as data source and press <***Next***> (see Figure 6).

11. Choose ***Sequence type*** as destination and press <***Next***> (see Figure 7).

12. In the *Data parsing* dialog box, fill in following data parsing string: "*_[DATA]-*".

This parsing string will only take into account the text occurring between the first underscore (_) and the hyphen (-). The asterisk (*) serves as a wildcard, meaning that all characters before the first underscore and after the hyphen will be ignored.

13. Press the <***Preview***> button and press <***Next***> when the parsing is correct (see Figure 8). Press <***Finish***> to add the rule to the *Import rules* dialog box.

The *Import rules* dialog box should now look like Figure 9.

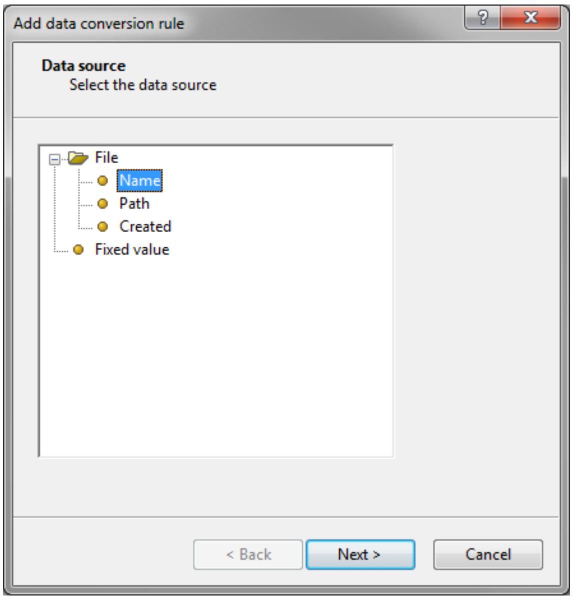


Figure 6: Data source.

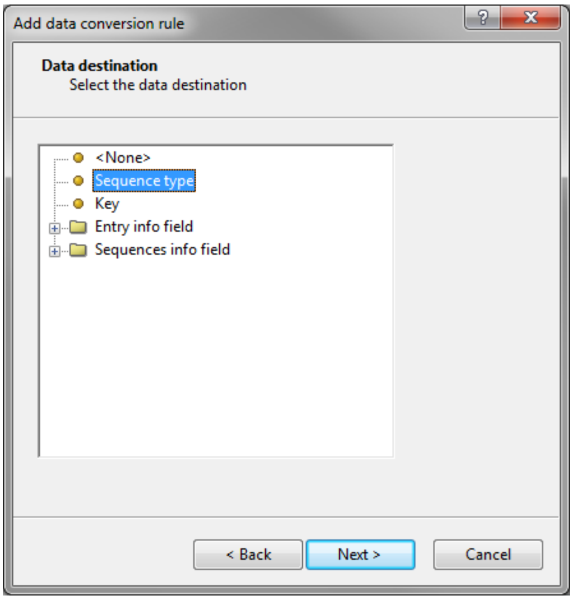


Figure 7: Data destination.

14. Press <***Next***> and <***Finish***>.

15. Specify a template name, e.g. **Import MLST SCF trace files** and press <***OK***>.

16. Make sure the newly created template is selected and press the <***Preview***> button.

The preview should now look like Figure 10.

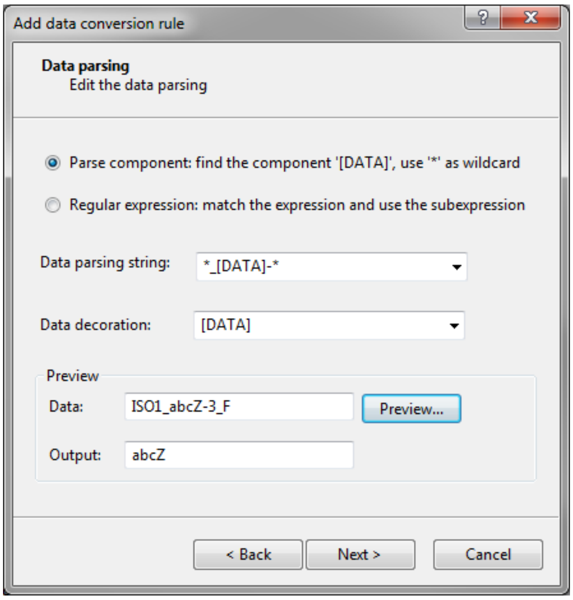
17. Close the preview.

18. Make sure the newly created template is selected and press <***Next***>.

19. Press <***Next***> to confirm the creation of 3 new entries (see Figure 11).

The *Processing* wizard page opens (see Figure 12).

© 2018 Applied Maths NV. All rights reserved.


Add data conversion rule

Data parsing
Edit the data parsing

● Parse component: find the component '[DATA]', use '*' as wildcard

○ Regular expression: match the expression and use the subexpression

Data parsing string: *_[DATA]-*

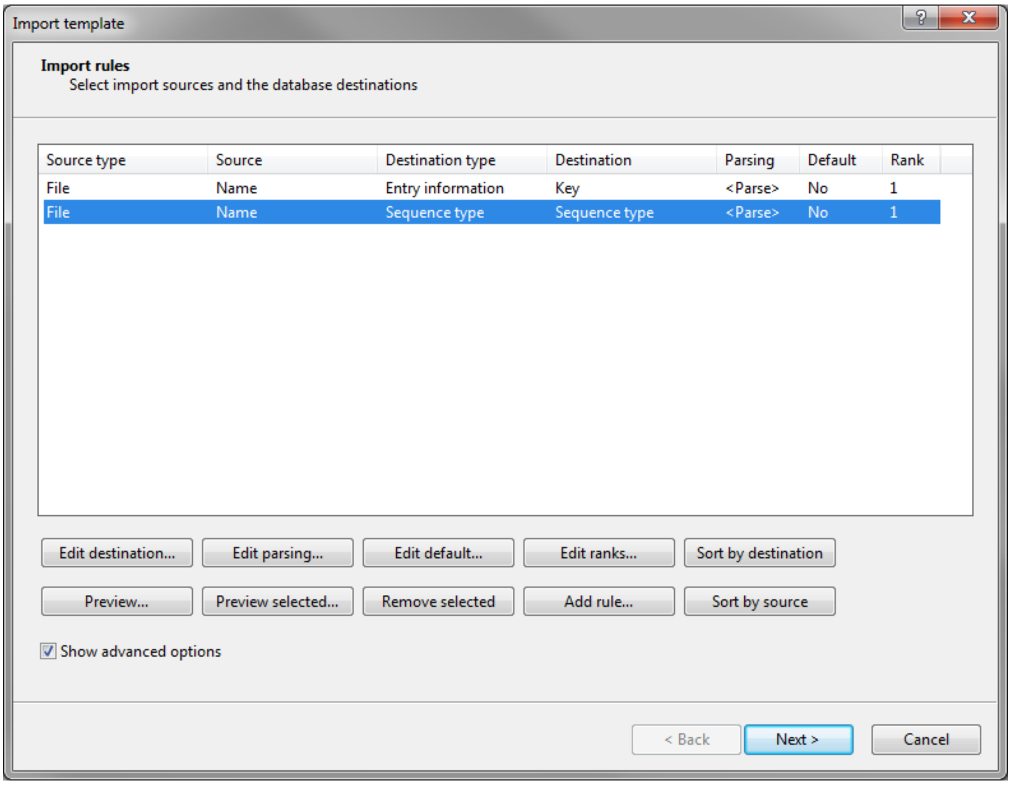
Data decoration: [DATA]

Preview

Data: ISO1_abcZ-3_F

Output: abcZ

Figure 8: Data parsing string.


Import template

Import rules
Select import sources and the database destinations

Source type	Source	Destination type	Destination	Parsing	Default	Rank
File	Name	Entry information	Key	<Parse>	No	1
File	Name	Sequence type	Sequence type	<Parse>	No	1

☑ Show advanced options

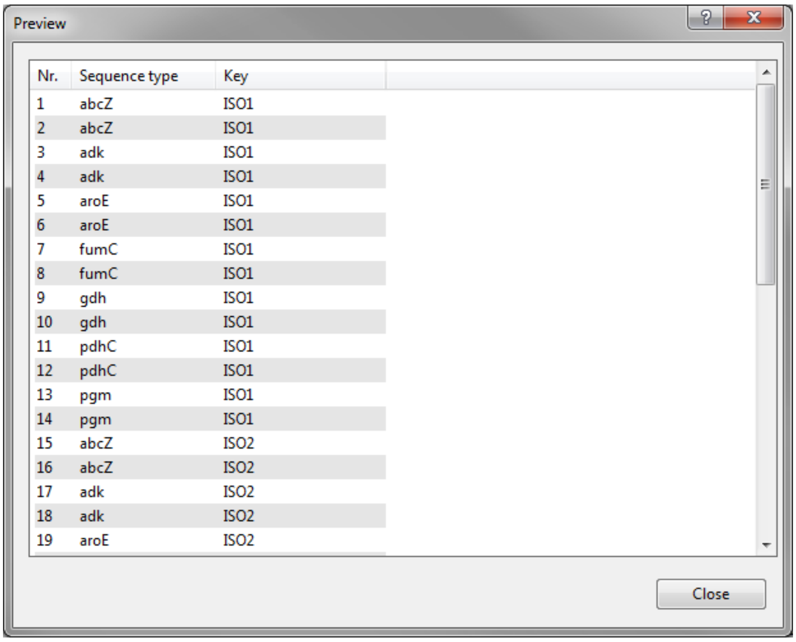
Figure 9: Import rules.

In the *Reports panel*, the ***Maximum# of unresolved bases reported*** can be specified (default value 20). Likewise, the ***Maximum # of align inconsistencies reported*** can be entered (default value 20). Align inconsistencies are positions where the consensus is resolved, but where one or more sequences are different from the consensus.

20. Press <***Trimming settings***> to pop up the *Assembly trimming settings* dialog box.

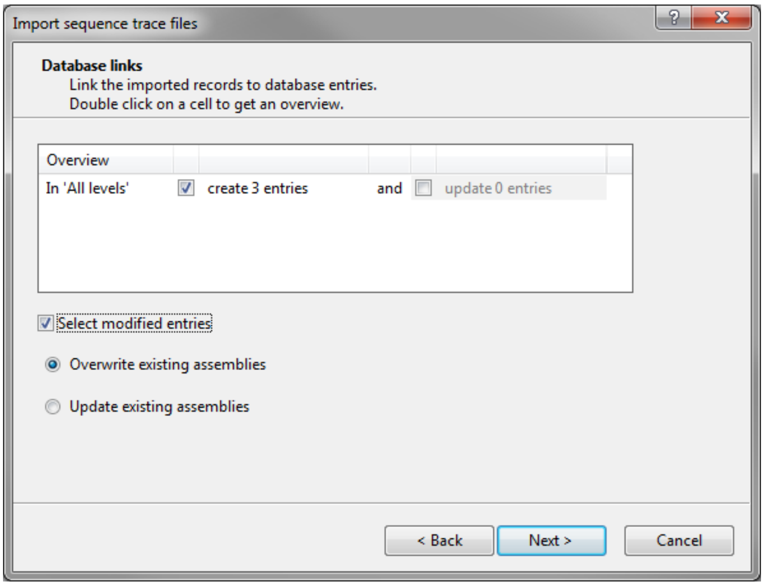
The trimming patterns entered in the *Trim patterns tab* (see Figure 2) are shown in the ***Start pattern*** and

© 2018 Applied Maths NV. All rights reserved.



Nr.	Sequence type	Key
1	abcZ	ISO1
2	abcZ	ISO1
3	adk	ISO1
4	adk	ISO1
5	aroE	ISO1
6	aroE	ISO1
7	fumC	ISO1
8	fumC	ISO1
9	gdh	ISO1
10	gdh	ISO1
11	pdhC	ISO1
12	pdhC	ISO1
13	pgm	ISO1
14	pgm	ISO1
15	abcZ	ISO2
16	abcZ	ISO2
17	adk	ISO2
18	adk	ISO2
19	aroE	ISO2

Figure 10: Preview of the parsing.



Database links

Link the imported records to database entries.
Double click on a cell to get an overview.

Overview				
In 'All levels'	☑	create 3 entries	and	□ update 0 entries

☑ Select modified entries

● Overwrite existing assemblies

○ Update existing assemblies

Figure 11: Database links.

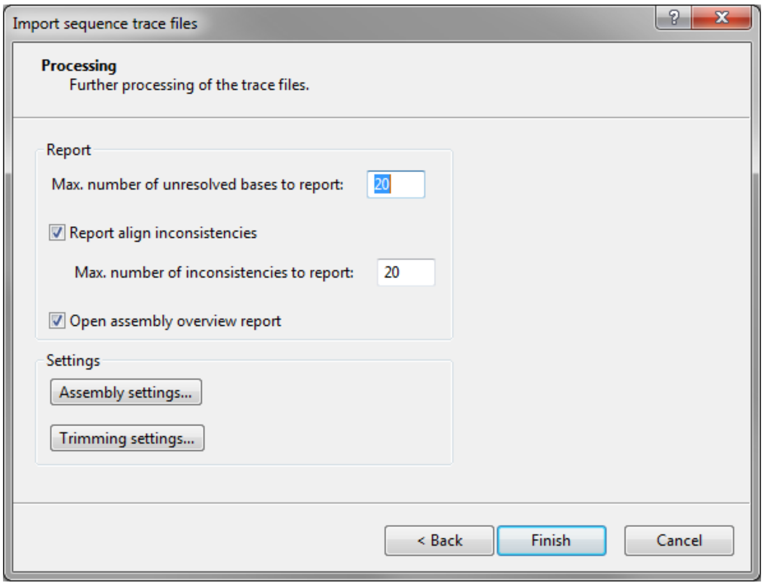
Stop pattern columns (see Figure 13).

21. Double-click on the <*Edit*> button for experiment **abdZ** to call the *Assembly trimming settings* dialog box (see Figure 14).

In the *Assembly trimming settings* dialog box, a number of additional settings can be specified for each individual sequence experiment:

- ***Minimum # of sequences*** specifies the minimum number of trace sequences that should contribute to the subsequence on the consensus that matches the trimming targets. For example, if “2” is entered, a trimming target will only be set if the matching region on the consensus is *fully* defined by at least 2 sequences.

© 2018 Applied Maths NV. All rights reserved.



Import sequence trace files

Processing
Further processing of the trace files.

Report

Max. number of unresolved bases to report: 20

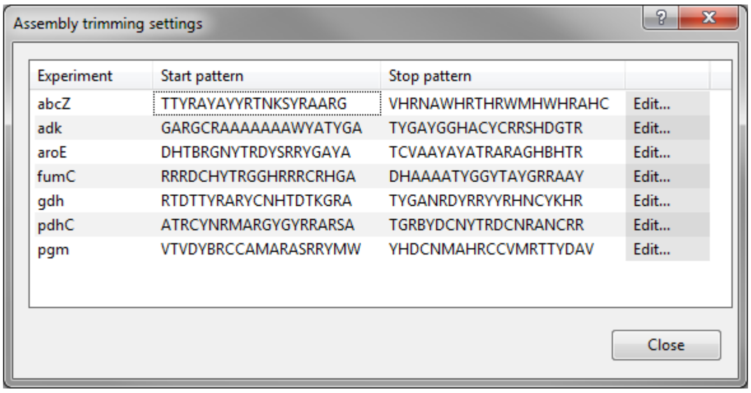
☑ Report align inconsistencies

Max. number of inconsistencies to report: 20

☑ Open assembly overview report

Settings

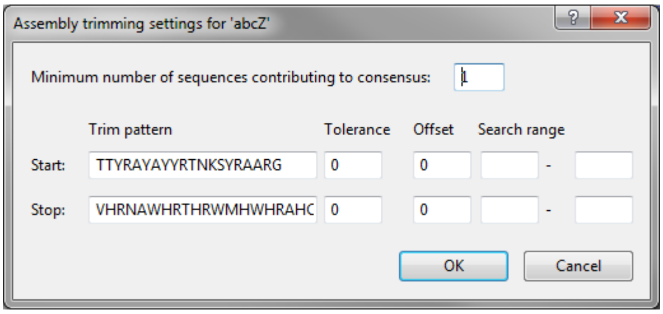
Figure 12: The *Processing* wizard page.



Assembly trimming settings

Experiment	Start pattern	Stop pattern	
abcZ	TTYRAYAYYRTNKSYRAARG	VHRNAWHRTHRWMHWHRAHC	Edit...
adk	GARGCRAAAAAAAWYATYGA	TYGAYGGHACYCRRSHDGTR	Edit...
aroE	DHTBRGNYTRDYSRRYGAYA	TCVAAYAYATRARAGHBHTR	Edit...
fumC	RRRDCHYTRGGHRRRCRHGA	DHAAAATYGGYTAYGRRAAY	Edit...
gdh	RTDTTYRARYCNHTDTKGRA	TYGANRDYRRYYRHNCYKHR	Edit...
pdhC	ATRCYNRMARGYGYRRARSA	TGRBYDCNYTRDCNRANCRR	Edit...
pgm	VTVDYBRCCAMARASRRYMW	YHDCNMAHRCCVMRTTYDAV	Edit...

Figure 13: The *Assembly trimming settings* dialog box.



Assembly trimming settings for 'abcZ'

Minimum number of sequences contributing to consensus: 1

	Trim pattern	Tolerance	Offset	Search range		
Start:	TTYRAYAYYRTNKSYRAARG	0	0		-	
Stop:	VHRNAWHRTHRWMHWHRAHC	0	0		-	

Figure 14: The *Assembly trimming settings* dialog box.

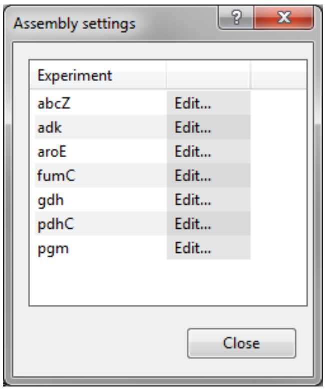
- For both the ***Start position*** and ***Stop position***, a ***Trim pattern*** is displayed. The use of IUPAC code for ambiguous positions is supported. The ***Tolerance*** defines the number of mismatches allowed for a sequence to be recognized as a trim pattern. With the ***Offset***, one can specify that the consensus is trimmed at a certain offset from the start and end trimming targets positions. If no offset is specified (zero), the trimming targets are included in the trimmed consensus. With the ***Search range*** one can restrict the search to certain regions on the consensus, e.g. to prevent incidental matches inside the targeted consensus sequence.

© 2018 Applied Maths NV. All rights reserved.

The entered trim patterns will be searched on the consensus sequence in both directions, i.e. on the consensus as it appears as well as on its complementary strand. In case the trim patterns match the complementary strand of the consensus, it will be automatically invert-complemented. If the ***Trim pattern*** text boxes are left empty, no preference sense is available.

22. Leave the predefined settings unaltered and press <***OK***> and <***Close***> to close dialog boxes.

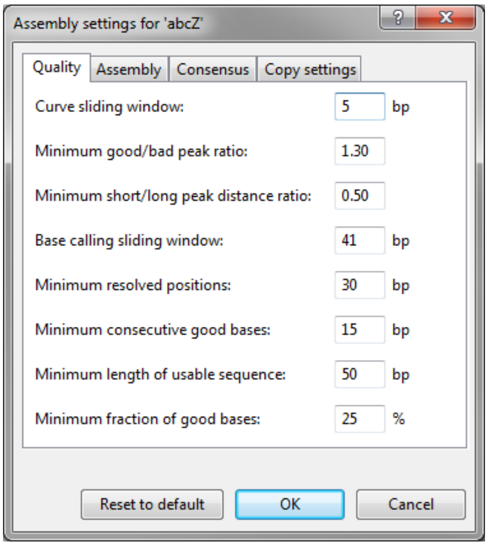
23. Press the <***Assembly settings***> button to call the *Assembly settings* dialog box (see Figure 15).



Experiment	
abcZ	Edit...
adk	Edit...
aroE	Edit...
fumC	Edit...
gdh	Edit...
pdhC	Edit...
pgm	Edit...

Figure 15: The *Assembly settings* dialog box.

24. Double-click on the <***Edit***> button for experiment **abcZ** to call the *Assembly settings* dialog box (see Figure 16).



Curve sliding window:	5	bp
Minimum good/bad peak ratio:	1.30	
Minimum short/long peak distance ratio:	0.50	
Base calling sliding window:	41	bp
Minimum resolved positions:	30	bp
Minimum consecutive good bases:	15	bp
Minimum length of usable sequence:	50	bp
Minimum fraction of good bases:	25	%

Figure 16: The *Assembly settings* dialog box.

The Assembly settings are grouped in tabs per settings dialog box in *Assembler*: ***Quality*** assignment, ***Assembly*** and ***Consensus*** determination. For a detailed description of the Assembler program settings, see the BioNumerics manual. In the last tab the Assembly settings can be copied from or to another sequence type experiment.

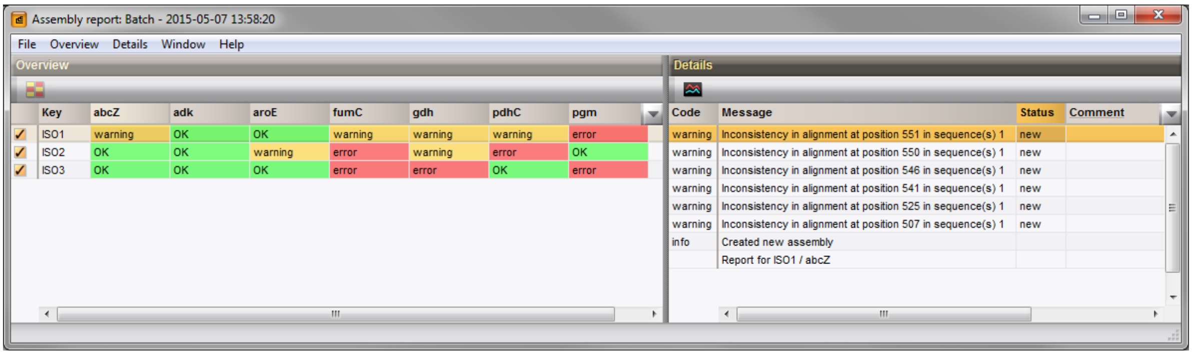
25. For this exercise, do not change the settings and press <***OK***> and <***Close***>.

26. Make sure the option ***Open assembly overview report*** is checked and press <***Finish***> to assemble the selected trace files from the example dataset into separate contig projects.

© 2018 Applied Maths NV. All rights reserved.

5 Checking the sequence assemblies

When the assemblies are processed, an interactive report window appears (see Figure 17). This window can also be displayed from the *Main* window with ***Analysis*** > ***Sequence types*** > ***Batch assembly reports...***.



	Key	abcZ	adk	aroE	fumC	gdh	pdhC	pgm
✓	ISO1	warning	OK	OK	warning	warning	warning	error
✓	ISO2	OK	OK	warning	error	warning	error	OK
✓	ISO3	OK	OK	OK	error	error	OK	error

Code	Message	Status	Comment
warning	Inconsistency in alignment at position 551 in sequence(s) 1	new	
warning	Inconsistency in alignment at position 550 in sequence(s) 1	new	
warning	Inconsistency in alignment at position 546 in sequence(s) 1	new	
warning	Inconsistency in alignment at position 541 in sequence(s) 1	new	
warning	Inconsistency in alignment at position 525 in sequence(s) 1	new	
warning	Inconsistency in alignment at position 507 in sequence(s) 1	new	
info	Created new assembly		
	Report for ISO1 / abcZ		

Figure 17: The *Batch sequence assembly report* window.

The *Overview* panel displays the entries (keys) as rows and the experiments as columns. Each cell in the grid, corresponding to a key/experiment pair, provides information about the current status of the contig project. Only for those entries that have a green (= **OK** or **Solved**) or orange (= **Warning**) status, the allele IDs can be assigned.

1. Click a cell, e.g. ***ISO1/pgm*** to update the *Details* panel on the right-hand side.

2. In the *Details* panel double-click on the first message.

This will open the sequence in the *Contig assembly* window, with the corresponding position in focus. The position can now be examined and - if needed - the base calling can be changed manually. In the Assembler project of the pgm of ISO1, three unresolved bases are present in the consensus sequence. For each of these unresolved bases, an error is listed in the *Details* panel.

3. To obtain an optimal view of the curves, use the zoom sliders in the *Traces* panel or use the zoom buttons.

The three unresolved bases in the consensus sequences are displayed in pink. The consensus sequence can be screened for (nearest) allele matches:

4. In the *Assembly view*, select ***MLST*** > ***Identify allele***.

The consensus sequence is screened against the downloaded allele information of the selected organism.

The *MLST identification plot window* pops up. The sequence type of the sequence that is shown in Assembler is displayed in white. The other sequence types are shown in gray (see Figure 19). The identification result is shown below the sequence type boxes.

5. In our example, four mismatches are reported with allele ID 9 (see Figure 19).

6. Select the first mismatch from the list.

The focus in Assembler is updated.

7. Change the `G` at position 820 in the reverse sequence to a `T`. The consensus sequence at position 820 is automatically updated.

8. Update the consensus sequence according to other editing suggestions: enter `G` at position 818, enter `A` at position 817, and remove `A` at position 806. Save the contig project with ***File*** > ***Save*** (💾, **Ctrl+S**).

9. Select ***MLST*** > ***Identify allele***. The consensus should now have a perfect match with allele ID 9.

© 2018 Applied Maths NV. All rights reserved.

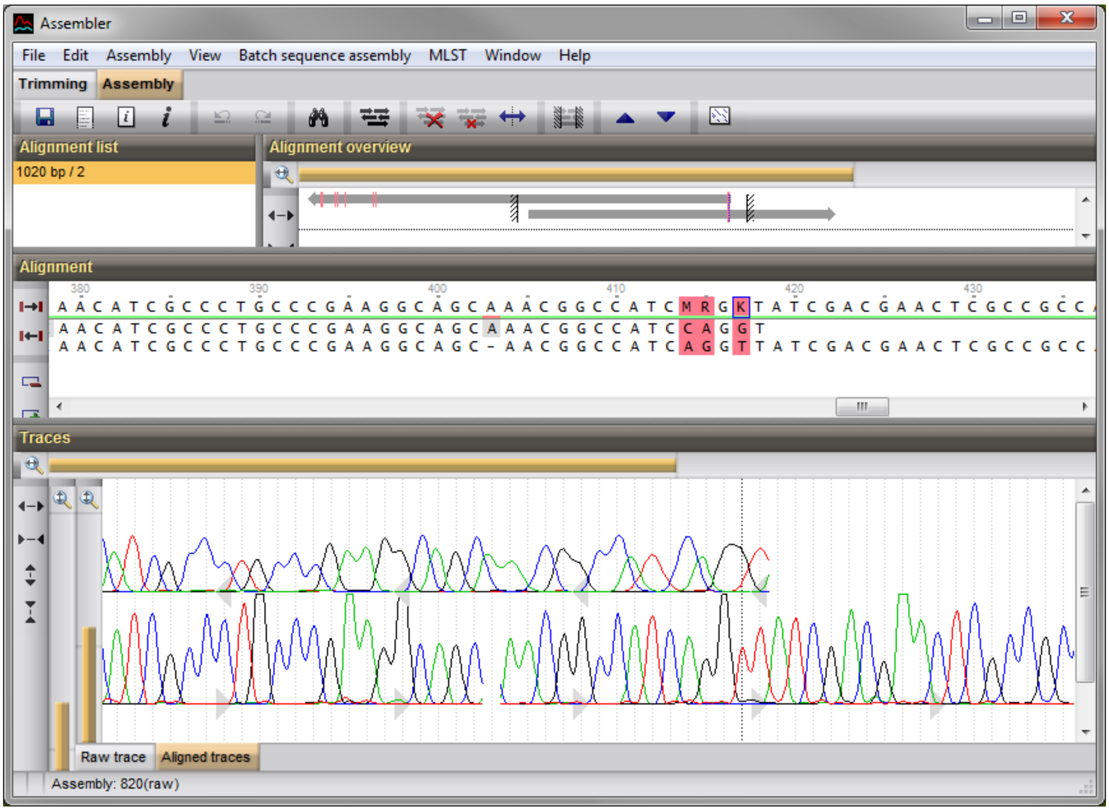
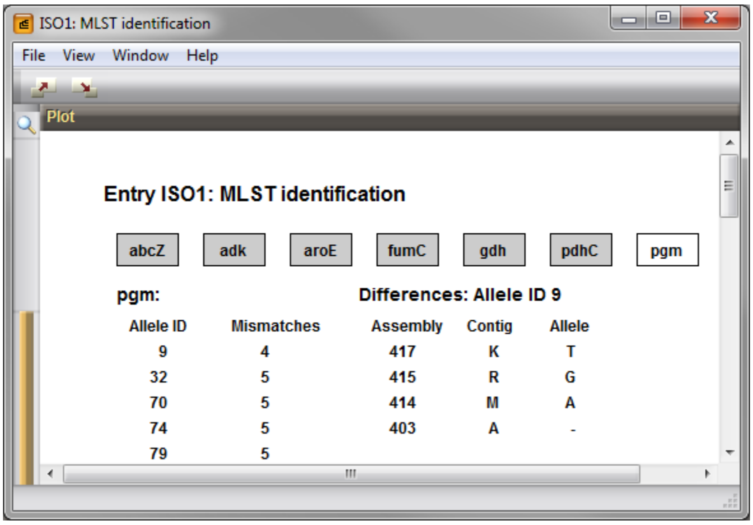


Figure 18: The *Aligned traces* panel.



Entry ISO1: MLST identification

abcZ adk aroE fumC gdh pdhC pgm

pgm:

Differences: Allele ID 9

Allele ID	Mismatches	Assembly	Contig	Allele
9	4	417	K	T
32	5	415	R	G
70	5	414	M	A
74	5	403	A	-
79	5			

Figure 19: The *MLST identification plot window*: Best match with allele ID 9.

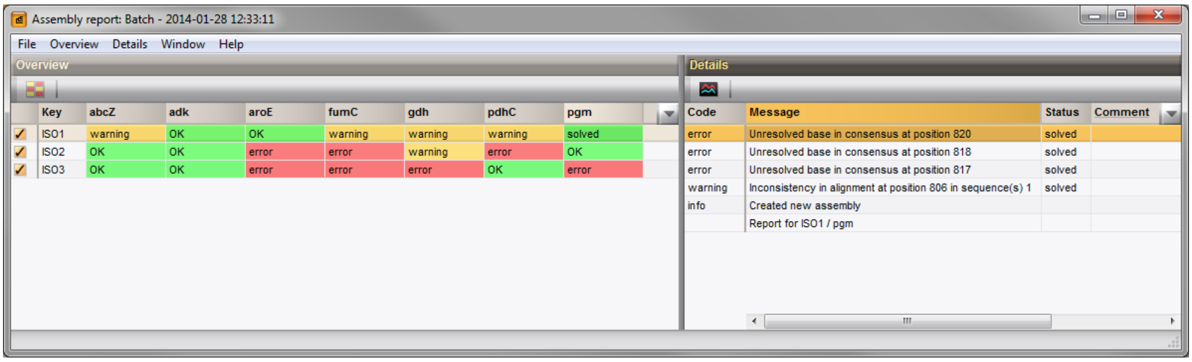
10. Select ***Batch sequence assembly*** > ***Set report to solved, save and close*** (**Ctrl+Shift+S**) in the *Contig assembly* window.

The corresponding key/experiment cell in the *Overview* panel is updated and displayed in green. The status ”solved” is displayed in the cell and in the ***Status*** column of the *Details* panel.

11. Click on the **ISO1/abcZ** warning cell in the *Overview* panel to update the *Details* panel on the right-hand side.

12. In the *Details* panel double-click on the first warning message to open the *Contig assembly* window.

© 2018 Applied Maths NV. All rights reserved.


Assembly report: Batch - 2014-01-28 12:33:11

	Key	abcZ	adk	aroE	fumC	gdh	pdhC	pgm
✓	ISO1	warning	OK	OK	warning	warning	warning	solved
✓	ISO2	OK	OK	error	error	warning	error	OK
✓	ISO3	OK	OK	error	error	error	OK	error

Code	Message	Status	Comment
error	Unresolved base in consensus at position 820	solved	
error	Unresolved base in consensus at position 818	solved	
error	Unresolved base in consensus at position 817	solved	
warning	Inconsistency in alignment at position 806 in sequence(s) 1	solved	
info	Created new assembly		
	Report for ISO1 / pgm		

Figure 20: Solved status.

Six alignment inconsistencies are reported. False peaks in the trace files result in the insertion of six false bases in the consensus sequence. These bases can be deleted from the consensus sequence as follows:

13. Click on the first alignment inconsistency in the *Details* panel. The focus is automatically updated.

14. Press the **Del**-key on the keyboard to delete the base from the consensus sequence.

15. Repeat this step for the other alignment inconsistencies.

16. Save the contig project with ***File*** > ***Save*** (💾, **Ctrl+S**).

17. Select ***MLST*** > ***Identify allele***. The consensus should now have a perfect match with allele ID 3.

18. Select ***Batch sequence assembly*** > ***Set report to solved, save and close*** (**Ctrl+Shift+S**) in the *Contig assembly* window.

19. Check all other warnings for ISO 1. In the *Assembly view*, select ***MLST*** > ***Identify allele*** to get an idea of the (closest) matches.

20. As an extra exercise, check the errors and warnings of the ISO 2 and ISO 3 samples.

6 Identifying alleles and profiles

After all sequence assemblies are checked, we can use the *MLST online plugin* for the identification of our sequences.

1. Make sure all three entries in the database are selected.

2. In the *Main* window, select ***MLST*** > ***Identify alleles***.

The matched allele IDs are stored as character values in the **MLST** character type, as can be seen in the *Experiment card* window:

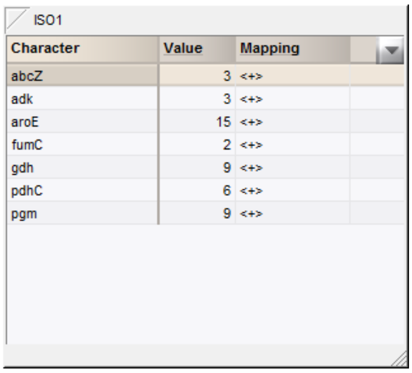
3. Click on the green dot in the **MLST** column of the *Experiment presence* panel to open the character *Experiment card* window for an entry (see Figure 21).

4. Close the *Experiment card* window by clicking in the small triangle-shaped button in the left upper corner.

Next we will screen the allelic profiles of the entries against the sequence type and clonal complex information of *Neisseria*.

5. In the *Main* window, select ***MLST*** > ***Identify profiles***.

© 2018 Applied Maths NV. All rights reserved.



ISO1

Character	Value	Mapping
abcZ	3	<+>
adk	3	<+>
aroE	15	<+>
fumC	2	<+>
gdh	9	<+>
pdhC	6	<+>
pgm	9	<+>

Figure 21: Character card.

The matched sequences types and clonal complexes are displayed in the MLST information fields ”MLST ST” and ”MLST CC”, respectively.

© 2018 Applied Maths NV. All rights reserved.