BIONUMERICS Tutorial:

Custom genotyping plugin: predicting phenotypic traits from whole genome sequences

1 Aim

The *Custom genotyping plugin* allows you to detect and extract sequences from genome sequences using a BLAST or in silico PCR approach. Additionally, it allows the detection of mutations using a BLAST approach and the confirmation of species identity using sourmash.

In this tutorial we will use the custom knowledgebases which have been created in the "Creation of custom knowledgebases" tutorial to screen whole genome sequences of *Salmonella* with the *Custom genotyping* plugin for acquired and mutational resistance. We will also show how these custom knowledgebases can be used to perform an in-silico PCR analysis and to perform species confirmation.

2 Preparing the database

2.1 Introduction to the demonstration database

We provide a **WGS demo database** for *Salmonella* containing sequence read set data links for 62 samples, calculated de novo assemblies and wgMLST results (allele calls and quality information).

The **WGS demo database** for *Salmonella* can be downloaded directly from the *BIONUMERICS Startup* window (see 2.2), or restored from the back-up file available on our website (see 2.3).

2.2 Option 1: Download demo database from the Startup Screen

1. Click the  button, located in the toolbar in the *BIONUMERICS Startup* window.

This calls the *Tutorial databases* window (see Figure 1).

2. Select **WGS_demo_database_for_Salmonella_enterica** from the list and select ***Database*** > ***Download*** ().

3. Confirm the installation of the database and press <***OK***> after successful installation of the database.

4. Close the *Tutorial databases* window with ***File*** > ***Exit***.

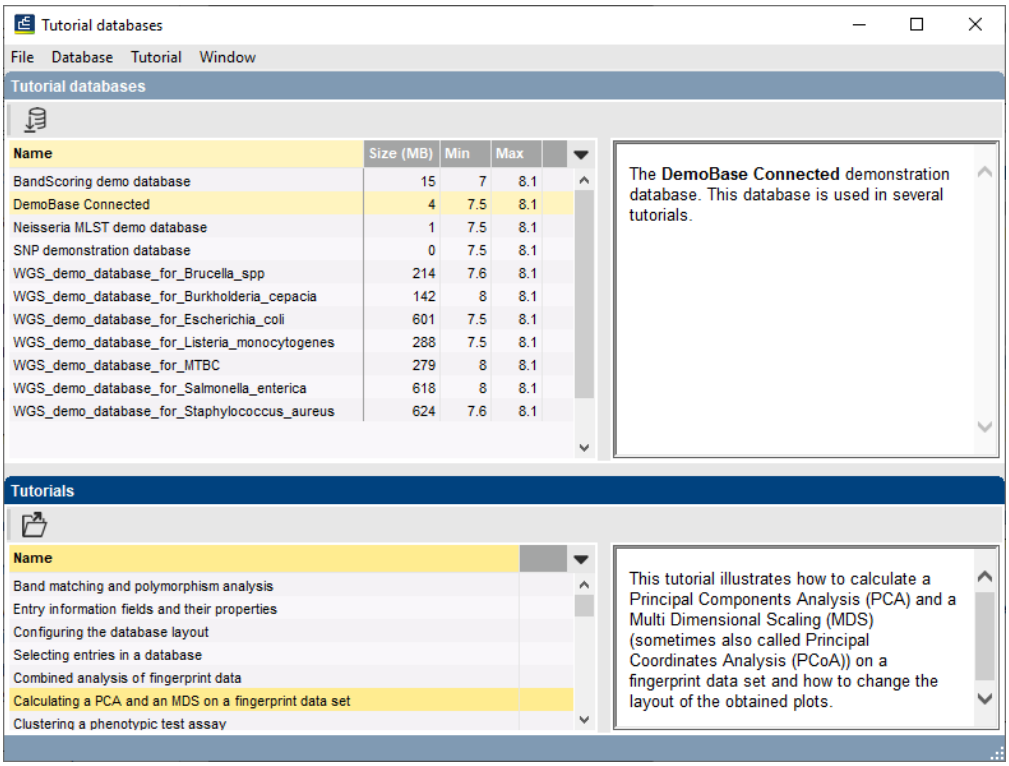


Figure 1: The *Tutorial databases* window, used to download the demonstration database.

The **WGS_demo_database_for_Salmonella_enterica** appears in the *BIONUMERICS Startup* window.

5. Double-click the **WGS_demo_database_for_Salmonella_enterica** in the *BIONUMERICS Startup* window to open the database.

2.3 Option 2: Restore demo database from back-up file

A BIONUMERICS back-up file of the demo database for *Salmonella enterica* is also available on our website. This backup can be restored to a functional database in BIONUMERICS.

6. Download the file `WGS_Salm.bnbk` file from https://www.bionumerics.com/download/sample-data, under 'WGS_demo_database_for_Salmonella_enterica'.

In contrast to other browsers, some versions of Internet Explorer rename the `WGS_Salm.bnbk` database backup file into `WGS_Salm.zip`. If this happens, you should manually remove the `.zip` file extension and replace with `.bnbk`. A warning will appear ("If you change a file name extension, the file might become unusable."), but you can safely confirm this action. Keep in mind that Windows might not display the `.zip` file extension if the option "Hide extensions for known file types" is checked in your Windows folder options.

7. In the *BIONUMERICS Startup* window, press the  button. From the menu that appears, select ***Restore database...***.

8. Browse for the downloaded file and select ***Create copy***. Note that, if ***Overwrite*** is selected, an existing database will be overwritten.

9. Specify a new name for this demonstration database, e.g. “WGS_Salmonella_demobase”.

10. Click <***OK***> to start restoring the database from the backup file.

11. Once the process is complete, click <***Yes***> to open the database.

The *Main* window is displayed (see Figure 2).

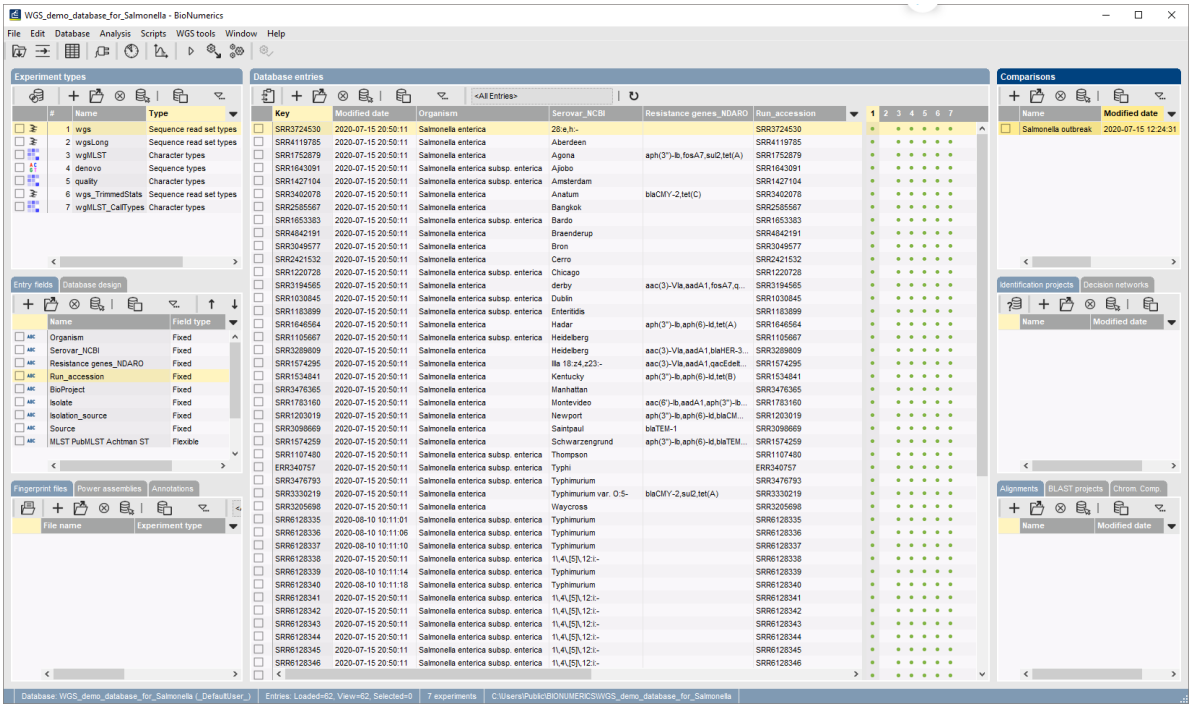


Figure 2: The *Salmonella* demonstration database: the *Main* window.

3 About the demonstration database

The WGS demo database contains links to sequence read set data on NCBI’s sequence read archive (SRA) for 62 publicly available sequencing runs. Additional information (in entry info fields Organism, Serovar etc.) was collected from the corresponding publications and added to the demonstration database.

Seven experiments are present in the demo database and are listed in the *Experiment types* panel (see Figure 3).

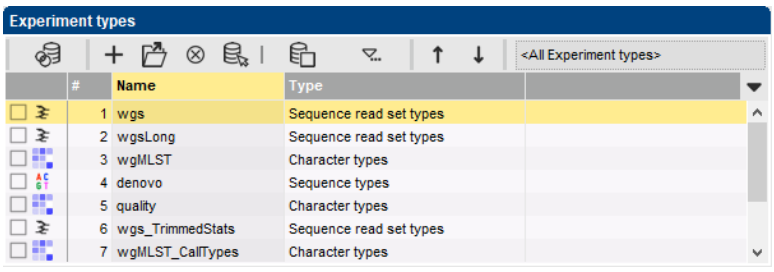


Figure 3: The *Experiment types* panel in the *Main* window.

1. Click on the green colored dot for one of the entries in the first column in the *Experiment presence* panel. Column 1 corresponds to the first experiment type listed in the *Experiment types* panel, which is **wgs** in the default configuration.

In the *Sequence read set experiment* window, the link to the sequence read set data on NCBI (SRA) with a summary of the characteristics of the sequence read set is displayed: *Read set size*, *Sequence length statistics*, *Quality statistics*, *Base statistics* (see Figure 4).

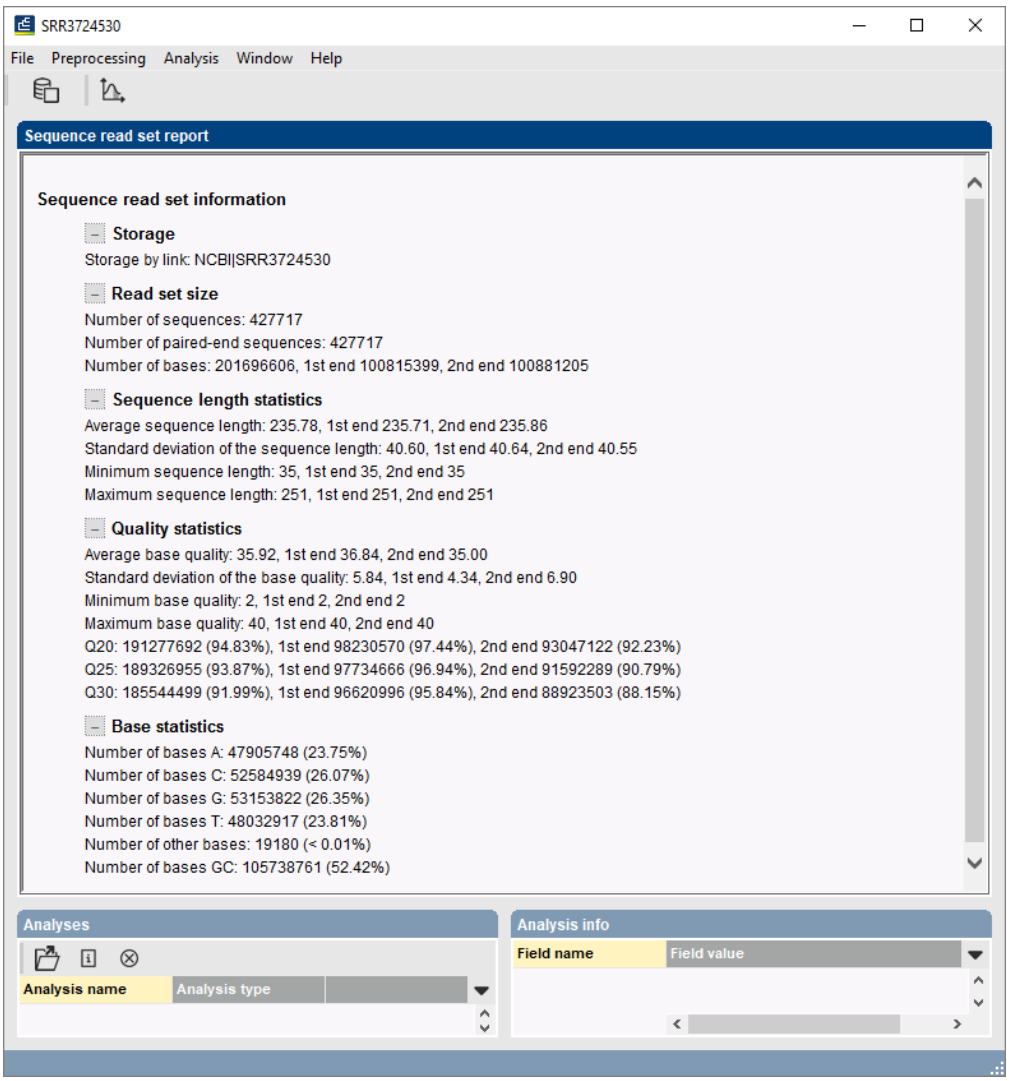


Figure 4: The sequence read set experiment card for an entry.

2. Close the *Sequence read set experiment* window.

3. Click on the green colored dot for one of the entries in the fourth column in the *Experiment presence* panel. Column 4 corresponds to the fourth experiment type listed in the *Experiment types* panel, which is **denovo** in the default configuration.

The *Sequence editor* window opens, containing the results from the de novo assembly algorithm, i.e. concatenated de novo contig sequences (see Figure 5).

4. Close the *Sequence editor* window.

The sequence read set experiment type **wgs_TrimmedStats** contains some data statistics about the reads retained after trimming, used for the de novo assembly.

The sequence read set experiment type **wgsLong** contains the links to long read sequence read

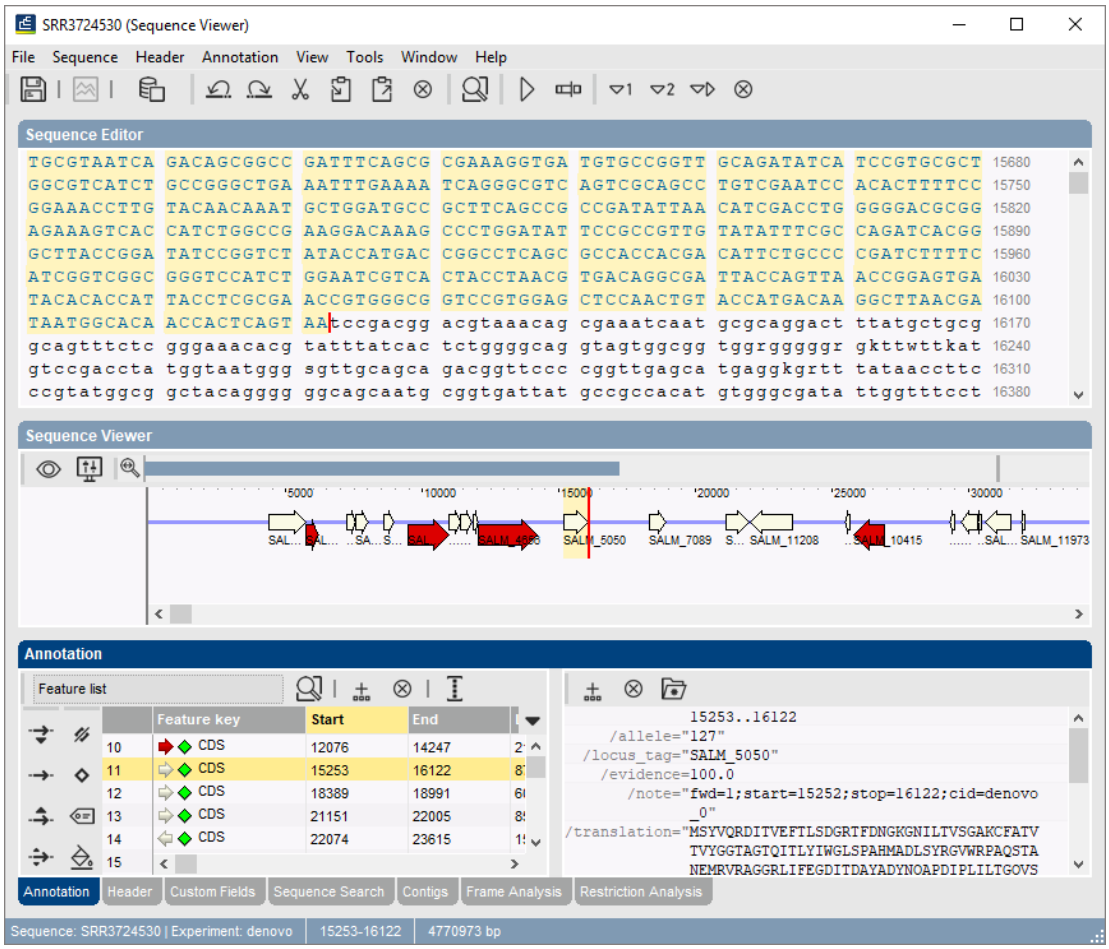


Figure 5: The *Sequence editor* window.

data (typically PacBio or MinION datasets). In this demo database, no links are defined for this experiment.

The other three experiments contain data related to the wgMLST analysis performed on the samples:

- Character experiment type **wgMLST** contains the allele calls for detected loci in each sample, where the consensus from assembly-based and assembly-free calling resulted in a single allele ID.
- Character experiment type **quality** contains quality statistics for the raw data, the de novo assembly and the different allele identification algorithms.
- Character experiment type **wgMLST_CallTypes**: contains details on the call types.

4 Installing the custom genotyping plugin

Proceed as follows to install the *Custom genotyping plugin*:

1. Call the *Plugins and Scripts* dialog box from the *Main* window with ***File*** > ***Install / remove plugins...*** ( ).
2. Select the *Custom genotyping plugin* from the list and press the <***Install***> button.

3. Confirm the installation of the plugin.

A message appears, confirming the installation of the plugin and prompting you to restart BIONUMERICS.

4. Press <***OK***> in the confirmation message.

5. Press <***Close***> to close the *Plugins and Scripts* dialog box.

6. Close and re-open the database to complete the installation of the plugin.

The *Custom genotyping plugin* installs menu items in the main menu of the software under ***Genotyping*** (see Figure 6).

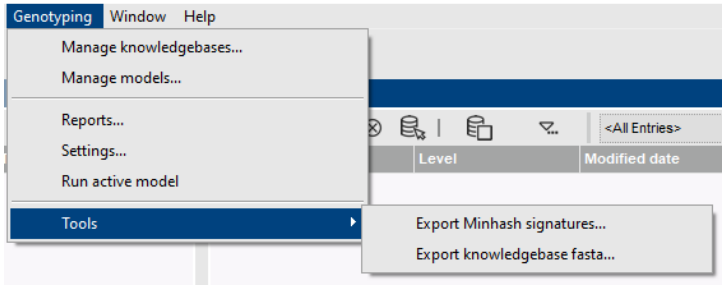


Figure 6: New menu items, available after installation of the *Custom genotyping plugin*.

5 Managing knowledgebases

Before a genotyping model can be created, at least one knowledgebase should be available. The following custom knowledgebases have been created in the ”Creation of custom knowledgebases” tutorial:

- Disinfectant_resistance: A BLAST-based knowledgebase for the detection and extraction of disinfectant resistance genes.
- In-silico PCR: An in-silico PCR-based knowledgebase for the detection and extraction of markers for the identification of *Salmonella enterica* serovars.
- Mutational_resistance_Salmonella: A BLAST-based knowledgebase for the detection of mutational antibiotic resistance.
- Species_confirmation_Salmonella: A minhash-based knowledgebase for *Salmonella* species confirmation.

These four custom knowledgebases can be downloaded from the BIONUMERICS website (https://www.bionumerics.com/download/sample-data, click on ”Custom knowledgebases”).

1. In the *Main* window of the BIONUMERICS database select ***Genotyping*** > ***Manage knowledgebases...*** to open the *Manage knowledge bases* dialog box (see Figure 7).

The *Manage knowledge bases* dialog box shows all currently available genotyping knowledgebases in the BIONUMERICS database. Initially, this dialog is empty.

2. Press <***Add local...***> to open the *Register local knowledge base* dialog box.

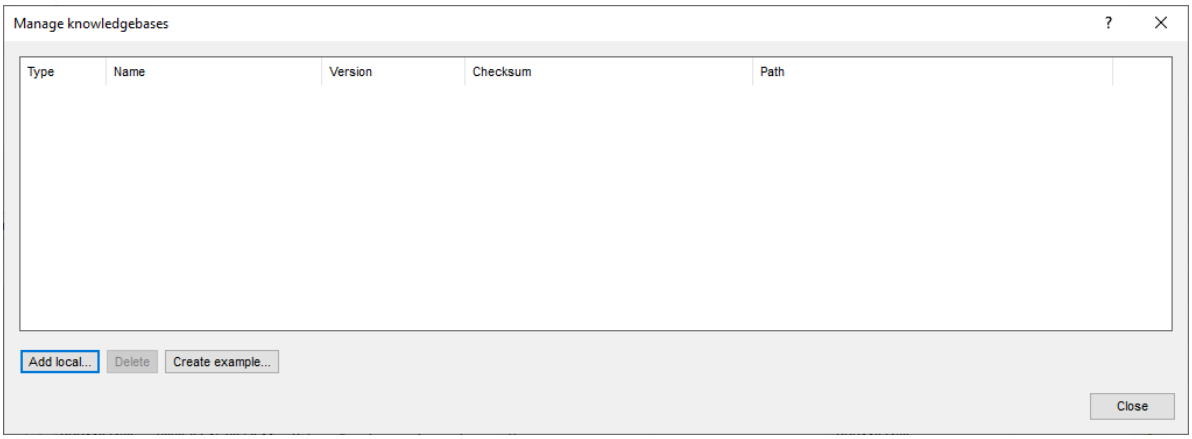


Figure 7: The *Manage knowledge bases* dialog box.

3. Press <***Browse...***> and browse for the `Disinfectant_resistance` knowledgebase folder in the downloaded `Custom knowledgebases` folder (see Figure 8).

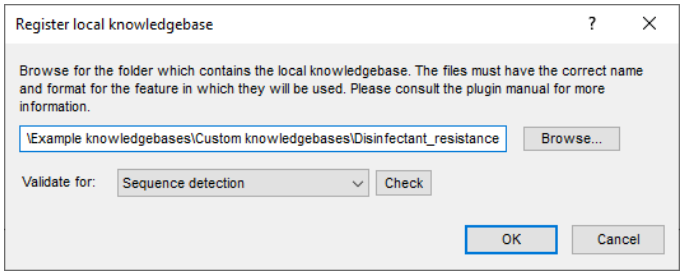


Figure 8: The *Register local knowledge base* dialog box.

Optionally, the knowledgebase can be validated before it is added.

4. Select the type of features for which the knowledgebase is intended for (i.e. Sequence detection, Sequence extraction and Acquired traits detection) and press <***Check***>.

Any detected issues will be reported in the *Knowledge base validation issues* dialog box. An error means that the knowledge base is not usable for the selected feature type: a different knowledgebase should be specified or the knowledgebase's files should be corrected according to the error description. It will not be possible to add a knowledgebase with a validation error.

A knowledge base for which only warning messages are raised might be usable for the selected feature, but not all options of the feature are applicable.

5. Press <***OK***> in the *Register local knowledge base* dialog box to add the validated knowledgebase to the list in the *Manage knowledge bases* dialog box.

6. Repeat the previous steps to add the other three downloaded knowledgebases as well (i.e. "In-silico PCR", "Mutational_resistance_Salmonella" and "Species_confirmation_Salmonella"). The "In-silico PCR" knowledgebase can be validated for the in-silico PCR detection and extraction features, the "Mutational_resistance_Salmonella" knowledgebase for the mutational traits detection and mutation scanning features and the "Species_confirmation_Salmonella" knowledgebase for the Species Confirmation feature.

The *Manage knowledge bases* dialog box should now look like Figure 9.

7. Press <***Close***> to close the *Manage knowledge bases* dialog box.

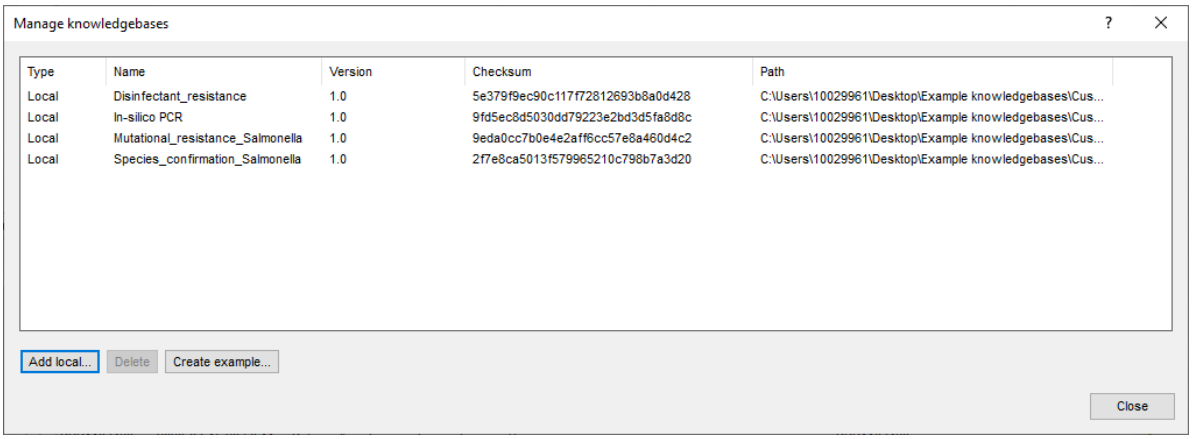


Figure 9: The *Manage knowledge bases* dialog box.

6 Creating a genotyping model

A genotyping model defines which genotyping analyses (i.e. features) will be executed as well as which knowledgebases and other feature settings will be used during the execution of the model. A user can create several models and switch between these models to change the active model. Only one model can be active, and hence be executed, at the same time.

1. Select ***Genotyping*** > ***Manage models...*** to open the *Manage genotyping models* dialog box (see Figure 10).

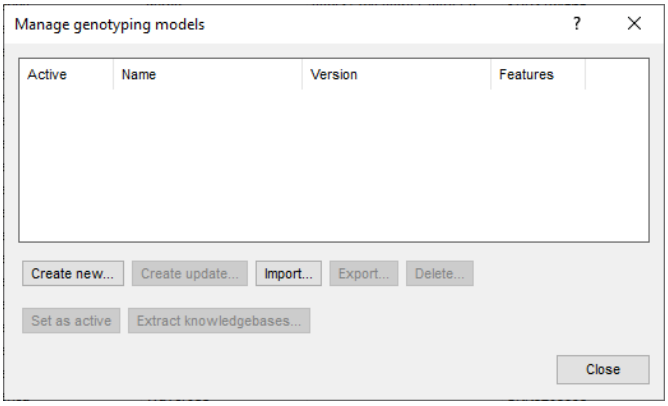


Figure 10: The *Manage genotyping models* dialog box.

The *Manage genotyping models* dialog box lists all genotyping models available in the BIONUMERICS database, with their 'Name', 'Version' and the number of features present in the model ('Features'). Only one of the available models is the *active model*, i.e. the model that can be executed. Initially, this list shows up empty.

2. Press <***Create new...***> to start the *Create model* wizard (see Figure 11).

3. Specify a ***Model name*** e.g. "MySalmonellaModel" and ***Version*** e.g. ""1.0". This combination should be unique for each model. Optionally, enter a ***Description*** for the model. Keep the default ***Translation table*** i.e.'Bacterial' (see Figure 12).

Do not use spaces in the model name or in the feature names as this interferes with the functionality of the BLAST-based features!

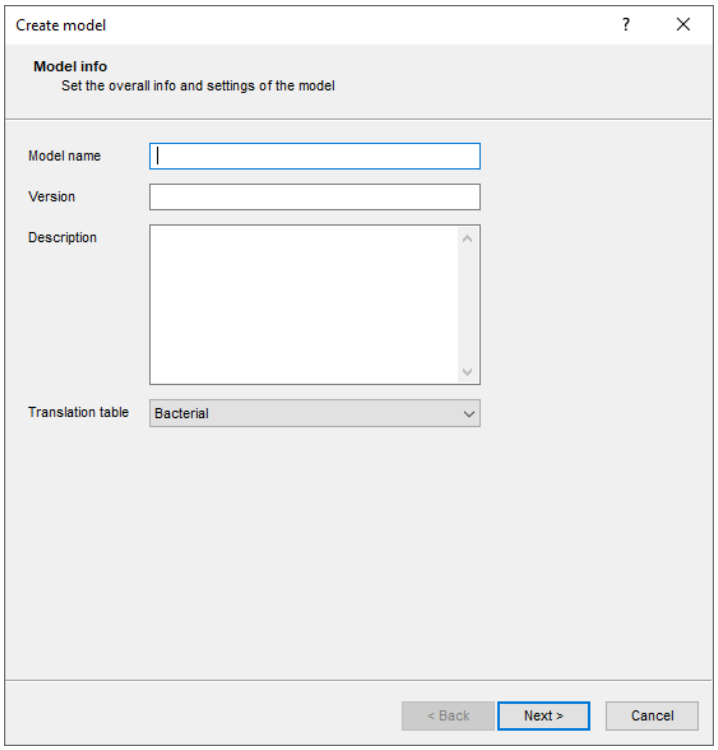


Figure 11: The *Create model* wizard.

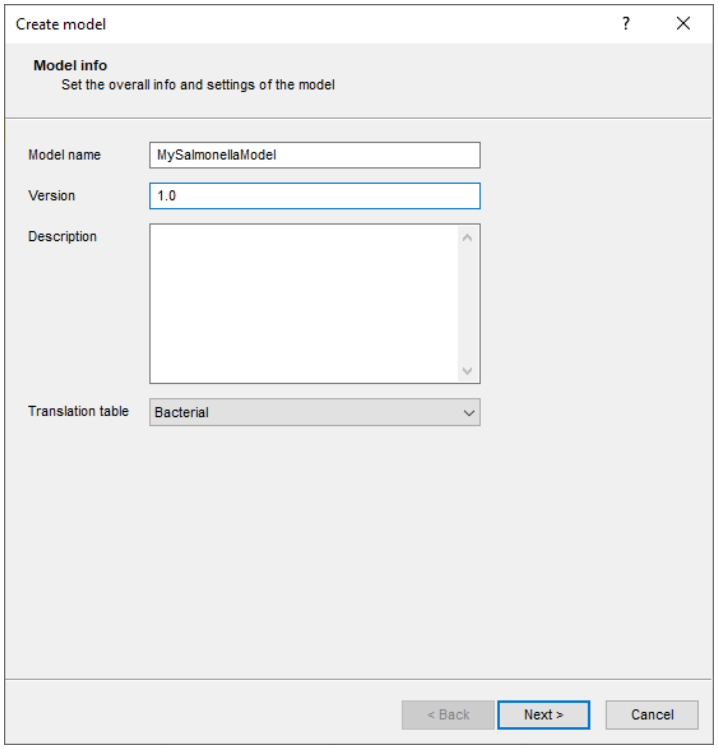


Figure 12: The *Create model* wizard.

4. Press <***Next***> to proceed to the second page of the *Create model* wizard.

In the second page of the *Create model* wizard, one or more features can be added to the genotyping model. Note that a model should contain at least one feature.

We will add all features to our model except for the ”sequence detection” feature as this functionality (i.e. the detection of sequences) is also included in the ”Acquired traits detection” feature.

5. In the tree control, highlight the ”Sequence extraction” feature and press the <***Add...***> button.

The *New genotyping feature name* dialog box prompts you to enter a name for the new genotyping feature.

6. Enter a name (do not use spaces!) and press the <***OK***> button.

7. Repeat the previous actions to add the ”Acquired traits detection”, ”Mutational traits detection”, ”Mutation scanning”, ”In-Silico PCR detection”, ”In-Silico PCR extraction” and ”Species confirmation” features to the model.

The new features will be listed in the bottom part of the *Create model* wizard (see Figure 13).

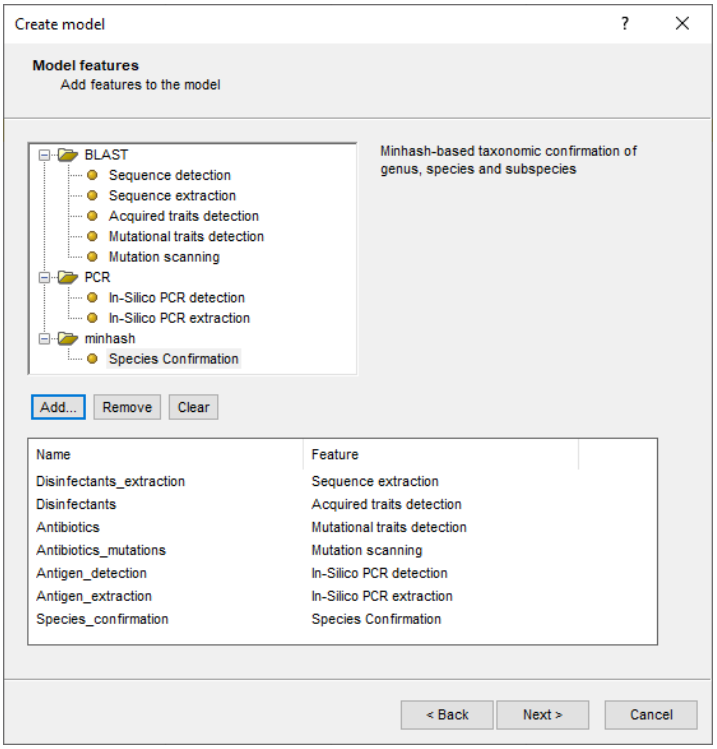


Figure 13: The *Create model* wizard.

8. Press the <***Next...***> button to start the *Create feature* wizard for each feature in the model (see Figure 14).

The ***Knowledgebases*** section is available for all genotyping features.

The ***BLAST*** section is available for all features using BLAST, i.e. sequence detection, sequence extraction, acquired traits detection, mutational traits detection and mutation scanning features. In the ***BLAST*** panel, two settings for the BLAST algorithm can be specified:

- ***Minimum identity (%)*** is the minimum sequence identity (as percentage) of the query sequence against the knowledge base's reference sequences.
- ***Minimum length for coverage*** specifies the minimum overlap (as percentage) between the subsequence found in the target assembly sequence and the reference sequence from the knowledge base.

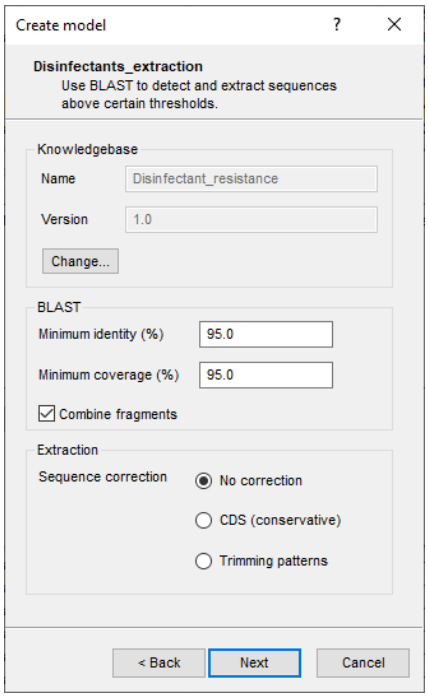


Figure 14: The *Create feature* wizard.

If the option ***Combine fragments*** is checked, genes that occur fragmented in the genome (i.e. split over two contigs) can still be detected.

The ***Extraction*** section is only available for BLAST-based sequence extraction features. When mismatches occur at the edges of a query sequence, BLAST may return a truncated sequence to optimize the similarity score. Therefore, when the knowledgebase contains only a single allele or a very limited set of alleles for a certain gene, a ***Sequence correction*** might be needed. For the latter, one out of three options should be selected:

- ***No correction***: The BLAST hit is taken as-is. This is the default option since no correction is needed when the knowledgebase covers sufficient diversity.
- ***CDS (conservative)***: The BLAST hit is extended to retrieve a full protein coding sequence (CDS), i.e. starting from the first encountered start codon upstream and ending at the first encountered stop codon downstream.
- ***Trimming patterns***: The BLAST hit is extended and trimmed to length using the trimming patterns, present in the knowledgebase.

9. Press <***Change...***> to display the *Manage knowledge bases* dialog box, from which a knowledgebase can be specified.
10. For the ”sequence extraction” feature select the ”Disinfectant_resistance” knowledgebase from the list of knowledgebases and press the <***OK***> button.

When a knowledgebase is selected, its ***Name*** and ***Version*** are indicated (see Figure 14).

11. Press the <***Next...***> button to open the *Create feature* wizard of the next feature.

12. Repeat the previous steps to add the appropriate knowledgebase to each feature i.e. the ”Disinfectant_resistance” knowledgebase for the ”Acquired traits detection” feature, the ”Mutational_resistance_Salmonella” knowledgebase for the ”Mutational traits detection” and ”Mutation scanning” features, the ”In-Silico PCR” knowledgebase for the ”In-Silico PCR detection” and ”In-Silico PCR extraction” features and the ”Species_Confirmation_Salmonella” knowledgebase for the ”Species confirmation” feature.

13. Press <***Finish***> to complete the creation of the features in the model.

The *Create feature* wizard runs for each feature in the model. When the model creation is complete, the question ”Do you want to set the new model as active?” pops up. In the *Custom genotyping plugin*, only the active model can be executed, so typically you will want to answer <***Yes***> to this question.

14. Press <***Yes***>.

Next, the software asks ”Do you want to modify the new model settings now?”.

15. Press <***Yes***> to open the *Settings* dialog box.

The *Settings* dialog box pops up and consists of a general tab and a tab for each feature that was added to the model.

The settings for the active genotyping model in the *Custom genotyping plugin* can be accessed at any time via ***Genotyping*** > ***Settings...*** in the *Main* window.

7 Managing genotyping model settings

In the *General tab* the following general settings need to be specified:

- ***Included info fields***: In this list the entry information fields that will be displayed in the genotyping report can be specified.
- ***Exports directory***: With <***Browse...***> you can specify an export directory to store all exports from the genotyping reports.
- ***Input Sequence experiment***: From the drop-down list you can specify the sequence experiment that holds the (whole) genome sequences that will be screened.
- ***Enabled features***: This list contains all offered features of the genotyping plugin. Features which are not required can be disabled in this list to save on processing time and omit the corresponding sections from the report. By default, all features are enabled.

1. In our demonstration database, the assembled sequences are stored in the ***denovo*** sequence experiment. Make sure this experiment is selected from the drop-down list and optionally check the ***Isolate*** to include in the report (see Figure 15).

The other tabs group the settings for each possible search: Resistance (Acquired and mutational resistance), in-silico PCR detection and extraction and Species confirmation.

All tabs contain a *Knowledgebase* and *Results* panel and the BLAST-based features also include a *BLAST* panel:

1. *Knowledgebase*: in this panel the ***Version*** and ***Name*** of the knowledge base that is being used for this feature is shown.

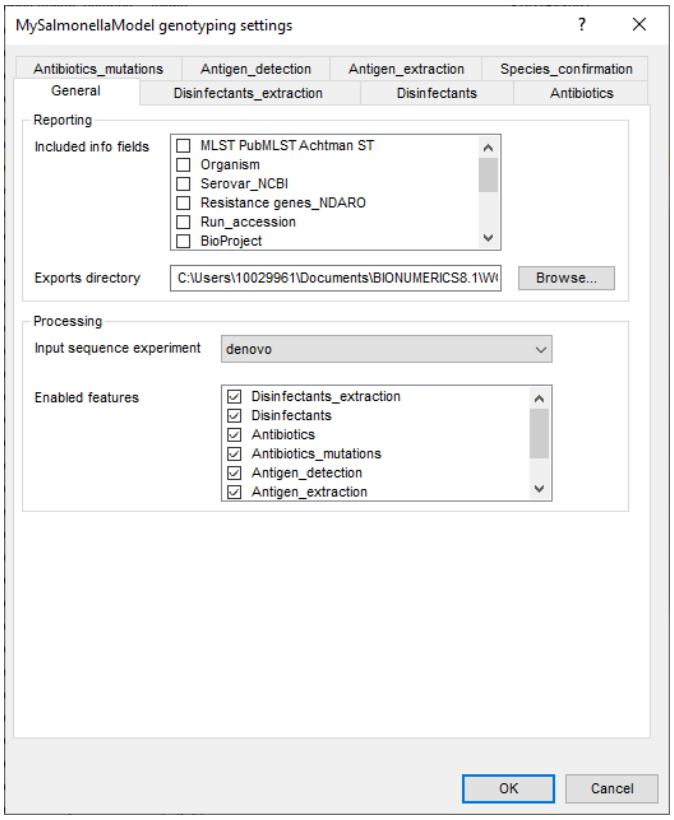


Figure 15: The *Settings* dialog box: *General* tab.

2. *BLAST*: in this panel the two settings for the BLAST algorithm are specified; the ***Minimum percent identity (%)*** and the ***Minimum coverage (%)*** of your query sequence against the knowledge base's reference sequences.

3. *Results*: in this panel the output database information fields and experiments to which the screening results will be written can be dictated. Use the drop-down list to choose an existing experiment type or field, or the <***Create***> option to create new experiments and fields. A default name for the experiment or information field is suggested, but you can adjust this if you want to. Check ***Annotate sequence experiment*** to annotate the input sequence with the detected genotyping features.

2. In this tutorial, specify the experiment types and information fields in all tabs by selecting the <***Create***> option in the drop-down lists and accepting the default names. Leave the other settings unaltered.

3. In the *Results* panel of the *Antigen_extraction* tab click on <***Change...***> next to *Sequence extraction* to open the *Change sequence experiment* dialog box. Click on <***Auto configure...***> to set the sequence experiments automatically and click on <***OK***>.

4. In the *Results* panel of the *Disinfectants_extraction* tab click on <***Change...***> next to *Sequence extraction* to open the *Change sequence experiment* dialog box. In the drop-down list of the **formA** PCR target select the <***Create***> option and accept the default name. Click on <***OK***> to close the *Change sequence experiment* dialog box.

5. Click on <***OK***> in the *Settings* dialog box to close the dialog box.

6. Press <***Close***> to close the *Manage genotyping models* dialog box.

8 Running the active genotyping model

The screening can be done on any selection of entries in the database.

1. Select a single entry in the *Database entries* panel by holding the **Ctrl**-key and left-clicking on the entry. Alternatively, use the **space bar** to select a highlighted entry or click the ballot box next to the entry.

Selected entries are marked by a checked ballot box ( ) and can be unselected in the same way.

2. In order to select a group of entries, hold the **Shift**-key and click on another entry.

A group of entries can be unselected the same way.

3. Make sure a few entries are selected in the *Database entries* panel of the demonstration database.

Screening selected entries with the active genotyping model can be done using ***Genotyping*** > ***Run active***.

The analysis time increases proportionally with the number of selected entries and the number of genotyping features in the model. A complete analysis may take up to several minutes or even hours. The progress bar disappears when the analysis is finished.

4. Select ***Genotyping*** > ***Run active*** to start the screening of the selected entries.

The species confirmation results (***Species confirmation***) are written to the information field in the *Database entries* panel (see Figure 16). Please note that the shown name of the information field is the one that was created per default, but can be different in your case depending on whether you have chosen an alternative name.

Database entries

<All Entries>

Key	Modified date	Organism	Serovar_NCBI	Species confirmation
SRR3194565	2022-04-13 17:22:57	Salmonella enterica	derby	Salmonella enterica enterica
SRR1030845	2022-04-13 17:23:08	Salmonella enterica subsp. enterica	Dublin	Salmonella enterica enterica
SRR1183899	2022-04-13 17:23:18	Salmonella enterica subsp. enterica	Enteritidis	Salmonella enterica enterica
SRR1646564	2022-04-13 17:23:29	Salmonella enterica	Hadar	Salmonella enterica enterica
SRR1105667	2022-04-13 17:23:40	Salmonella enterica subsp. enterica	Heidelberg	Salmonella enterica enterica
SRR3289809	2022-04-13 17:23:53	Salmonella enterica	Heidelberg	Salmonella enterica enterica
SRR1574295	2022-04-13 17:24:04	Salmonella enterica	IIIa 18:z4,z23:-	Salmonella enterica arizonae
SRR1534841	2022-04-13 17:24:15	Salmonella enterica	Kentucky	Salmonella enterica enterica
SRR3476365	2022-04-13 17:24:25	Salmonella enterica	Manhattan	Salmonella enterica enterica

Figure 16: Example output of the Species confirmation information field.

The character experiment types for Resistance (Acquired and mutational resistance) and in-silico PCR screening are created and updated with the predicted traits. Please note that the shown names of the experiment types are those created per default, but can be different in your case depending on whether you have chosen an alternative name.

5. Open a character card for one of the analyzed entries by clicking on the corresponding green colored dot in the *Experiment presence* panel.

The characters in the characters experiments are displayed in the same order they are listed in their knowledge base. However, it might be more convenient for interpretation to have them displayed alphabetically. This can be done in the *Character type* window with the option ***Characters*** > ***Arrange characters by field...*** ( ).

Below, the interpretation of the results gathered in the character experiment types is given.

Acquired traits detection (see Figure 17):

- ***Disinfectants_traits***: contains the results for each disinfectant: 0 = not detected (sensitive), 1 = detected (resistant).
- ***Disinfectants_detections***: contains the results for each resistance gene: 0 = not detected (sensitive), when detected (resistant) the % identity of the best hit is shown.

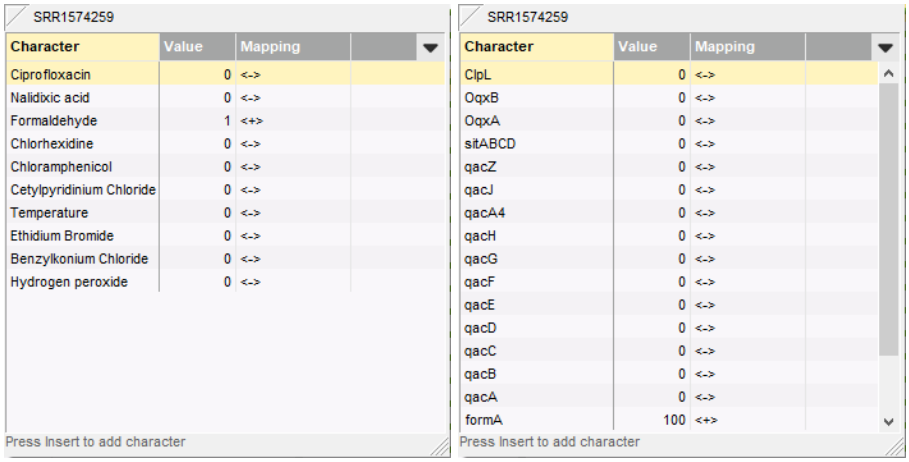


Figure 17: Example output of the ***Disinfectants_traits*** and the ***Disinfectants_detections*** experiment types for sample SRR1574259.

Sequence extraction (see Figure 18):

If sequence experiments have been created in the settings tab of the ”sequence extraction” feature, the detected sequences are stored in the corresponding sequence type experiments.

6. Click on the green colored dot of the **formA** sequence experiment for the entry with Key SRR1574259. The *Sequence editor* window opens and displays the extracted sequence (see Figure 18).

7. Close the *Sequence editor* window.

Mutational traits detection (see Figure 19):

- ***Antibiotics_traits***: contains the results for each antibiotic: 0 = not detected (sensitive), 1 = detected (resistant).
- ***Antibiotics_mutations***: contains the results for each known resistance mutation: -2 = partially indecisive, -1 = fully indecisive, 0 = not detected (sensitive), 1 = detected (resistant).

Mutation scanning (see Figure 20):

- ***Antibiotics_mutations_mutations_all***: contains the results for each mutation: 1 = detected.

In-silico PCR detection (see Figure 21):

- ***Antigen_detection_amplicons***: contains the result for the in-silico PCR: 0 = no amplicon, 1 = amplicon generated.

In-silico PCR extraction (see Figure 22):

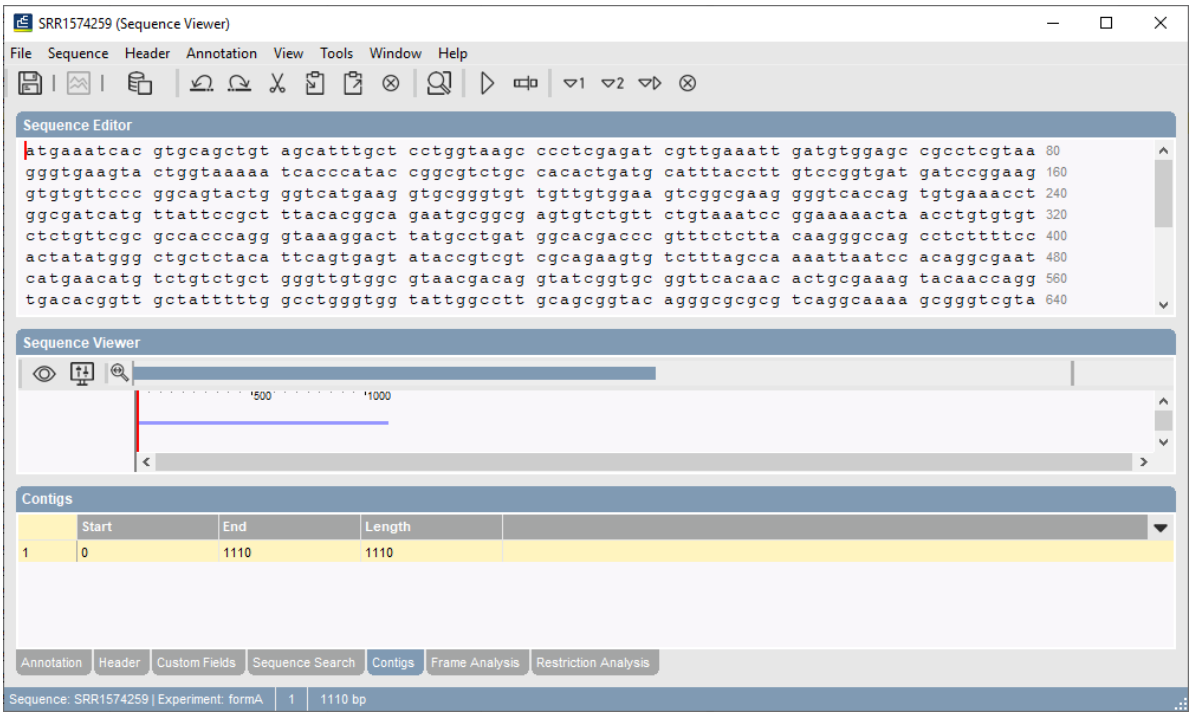


Figure 18: Example output of the **formA** experiment type for the entry with Key SRR1574259.

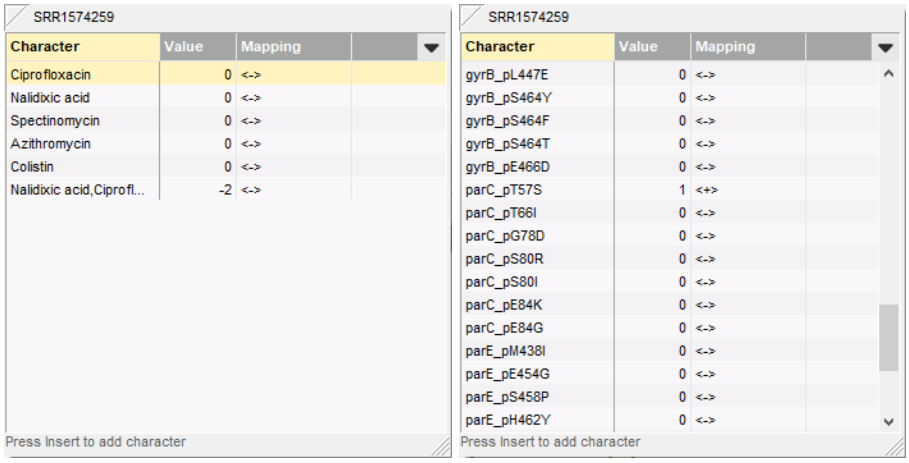
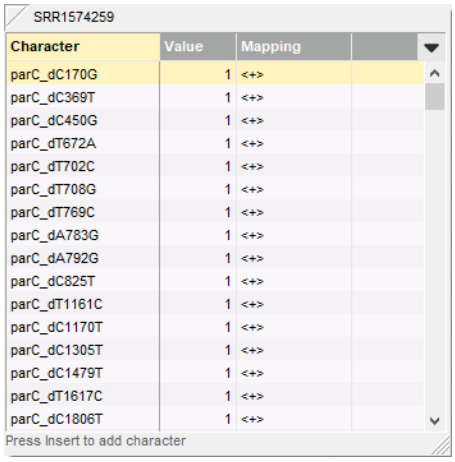


Figure 19: Example output of the ***Antibiotics_traits*** and the ***Antibiotics_mutations*** experiment type for the entry with Key SRR1574259.

If sequence experiments have been created in the settings tab of the ”in-silico PCR extraction” feature, the detected sequences are stored in the corresponding sequence type experiments.

8. Click on the green colored dot of the **fliCcom-fliCd** sequence experiment for the entry with Key SRR1574259. The *Sequence editor* window opens and displays the extracted sequence (see Figure 22).

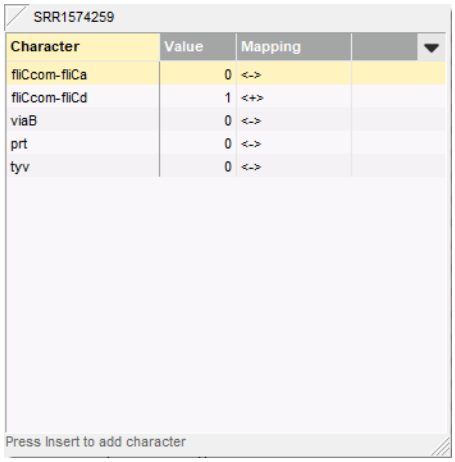
9. Close the character and sequence card(s).


SRR1574259

Character	Value	Mapping
parC_dC170G	1	<+>
parC_dC369T	1	<+>
parC_dC450G	1	<+>
parC_dT672A	1	<+>
parC_dT702C	1	<+>
parC_dT708G	1	<+>
parC_dT769C	1	<+>
parC_dA783G	1	<+>
parC_dA792G	1	<+>
parC_dC825T	1	<+>
parC_dT1161C	1	<+>
parC_dC1170T	1	<+>
parC_dC1305T	1	<+>
parC_dC1479T	1	<+>
parC_dT1617C	1	<+>
parC_dC1806T	1	<+>

Press Insert to add character

Figure 20: Example output of the ***Antibiotics_mutations_mutations_all*** experiment type for the entry with Key SRR1574259.


SRR1574259

Character	Value	Mapping
fliCcom-fliCa	0	<->
fliCcom-fliCd	1	<+>
viaB	0	<->
prt	0	<->
tyv	0	<->

Press Insert to add character

Figure 21: Example output of the ***Antigen_detection_amplicons*** experiment type for the entry with Key SRR1574259.

9 Reports

1. Open the genotype report for the selected entries with ***Genotyping*** > ***Reports...***.

The *Report* window contains a genotype report for each of the selected entries (see Figure 23)).

2. Select another entry in the *Entries* panel to update the results in the *Genotype report* panel.

The creation date of the report (***Date***), the Key (***Name***), and information fields checked in the *Settings* dialog box are displayed in the *Genotype report* panel.

3. Select ***Report*** > ***Report styles*** in the *Report* window and make sure the option ***Summary*** is selected.

A summary of the results of all analyzed traits is displayed in the *Report* window.

4. Select ***Report*** > ***Report styles*** in the *Report* window (see Figure 24) and select the option ***Complete***.

In the ***Complete*** view, the summarized results as well as all available details are shown. All hits that passed the settings for resistance (Acquired and mutational resistance), in-silico PCR

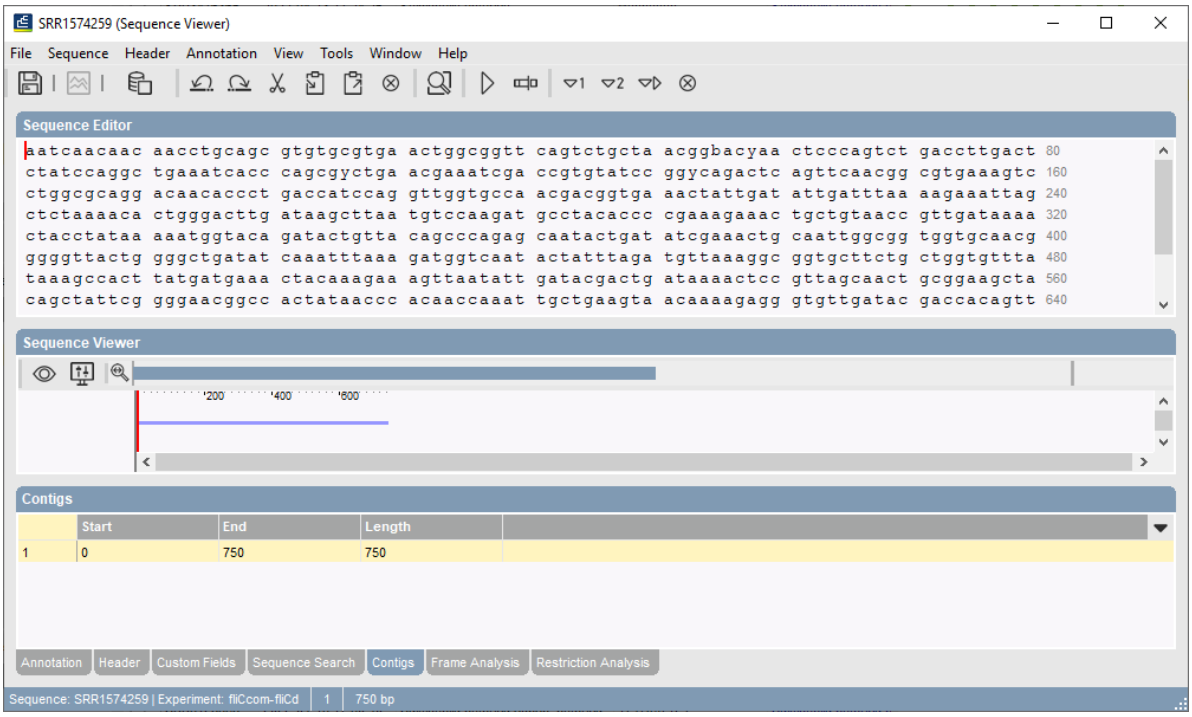


Figure 22: Example output of the **fliCcom-fliCd** experiment type for the entry with Key SRR1574259.

screening and species confirmation are listed and described.

5. Click on a hyperlink of one of the predicted traits to display the detailed results in the *Genotype report* panel (see Figure 25).

6. Select ***File*** > ***Exit*** to close the *Report* window.

For more detailed information on the genotyping analyses and interpretation of the reported results, please check the genotyping plugin manual.

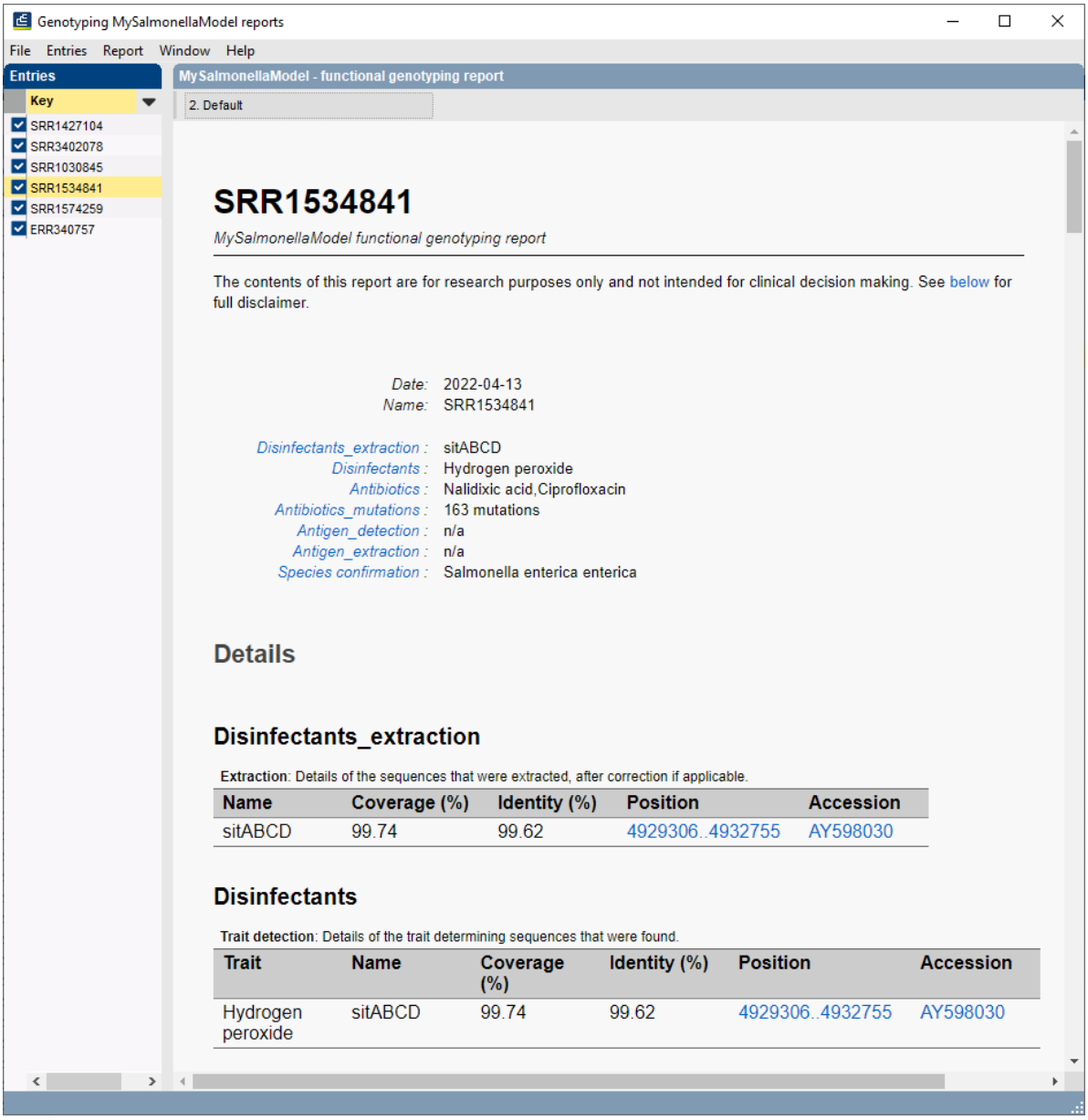


Figure 23: Example of a functional genotyping report.

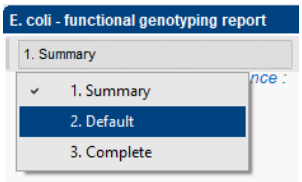


Figure 24: Report styles in the *Report window*.

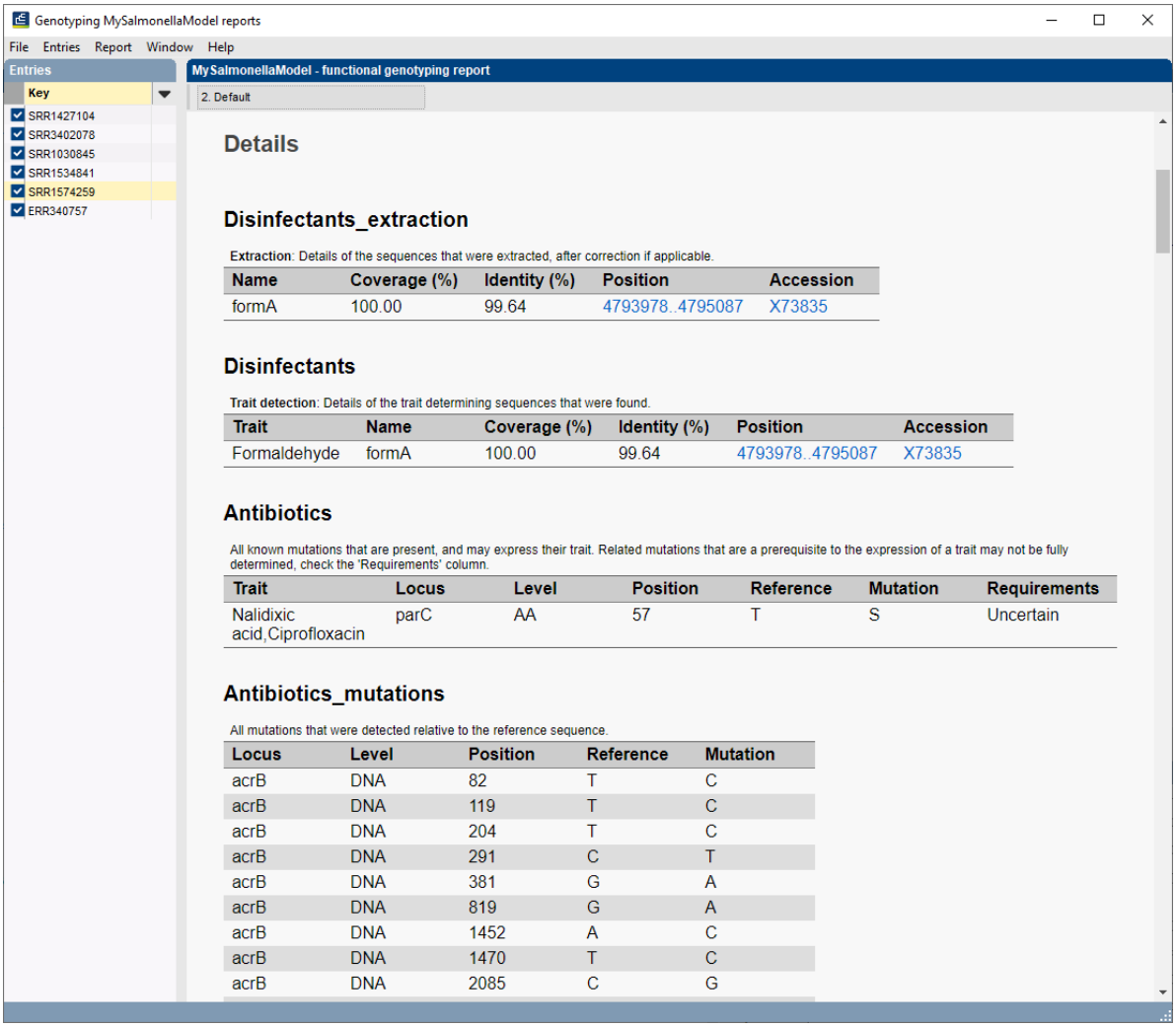


Figure 25: Report details.