BIONUMERICS Tutorial:

Listeria functional genotyping: predicting phenotypic traits from whole genome sequences

1 Aim

In this tutorial we will screen whole genome sequences of *Listeria* samples for phenotypic traits using the *Listeria functional genotyping plugin*. Phenotypic traits include: serotype, virulence, and detergent and antibiotic resistance. The plugin also allows you to detect phages.

The different steps are illustrated using the whole genome demonstration database of *Listeria monocytogenes*. This database is available for download on our website (see 2) and contains 51 publicly available sequence read sets of *Listeria monocytogenes* with already calculated de novo assemblies.

2 Preparing the database

2.1 Introduction to the demonstration database

We provide a **WGS demo database** for *Listeria monocytogenes* containing sequence read set data links for 51 samples, calculated de novo assemblies and wgMLST results (allele calls and quality information).

The wgMLST workflow and results will not be discussed in this tutorial.

The **WGS demo database** for *Listeria monocytogenes* 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).
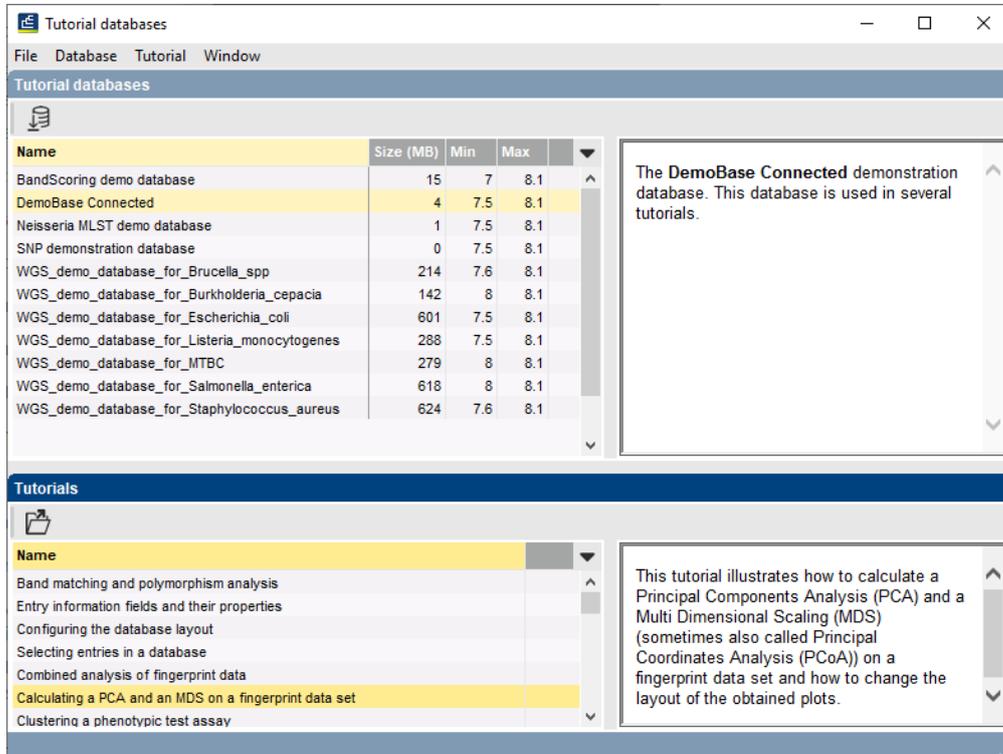
Installation of the *Listeria functional genotyping plugin* is only possible when no spaces are present in the BIONUMERICS home directory and in the name of the database. Before downloading or restoring the **WGS demo database** for *Listeria monocytogenes*, please check if your BIONUMERICS home directory does not contain any spaces:

1. Click the ⚙ button, located in the toolbar in the *BIONUMERICS Startup* window and select **Change home directory...** to call the *Home directory* dialog box.

2. In case the currently specified home directory contains spaces, update the path to a path containing no spaces and close the *Home directory* dialog box.

2.2 Option 1: Download demo database from the Startup Screen

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

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

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

4. Select the **WGS_demo_database_for_Listeria_monocytogenes** from the list and select *Database > Download* (⬚).

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

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

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

7. Double-click the **WGS_demo_database_for_Listeria_monocytogenes** in the *BIONUMERICS Startup* window to open the database.

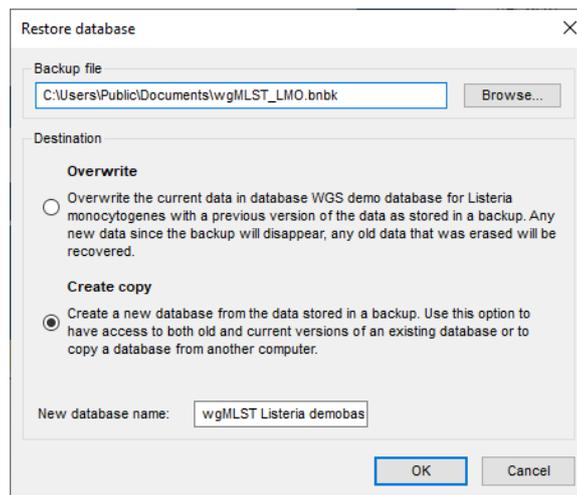## 2.3 Option 2: Restore demo database from back-up file

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

8. Download the file WGS_LM01.bnbk file from https://www.bionumerics.com/download/sample-data, under 'WGS_demo_database_for_Listeria_monocytogenes'.

In contrast to other browsers, some versions of Internet Explorer rename the `WGS_LM01.bnbk` database backup file into `WGS_LM01.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.

9. In the *BIONUMERICS Startup* window, press the ![icon] button. From the menu that appears, select **Restore database...**.

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

11. Specify a new name for this demonstration database and make sure the name does not contain any spaces to ensure the successful installation of the *Listeria functional genotyping plugin*. Specify for example: "WGS_Listeria_demobase".

12. Click <**OK**> to start restoring the database from the backup file (see Figure 2).

Figure 2: Restoring the **WGS demonstration database** from the backup file `WGS_LM01.bnbk`.

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

The *Main* window is displayed (see Figure 3).

3   About the demonstration database

The WGS demo database contains links to sequence read set data on NCBI's sequence read archive (SRA) for 51 publicly available sequencing runs. Additional information, stored in entry info fields (CollectionDate, CollectedBy, serovar, etc.) was collected from the corresponding publications and added to the demo database.

Seven experiments are present in the demo database and are listed in the *Experiment types* panel (see Figure 4).
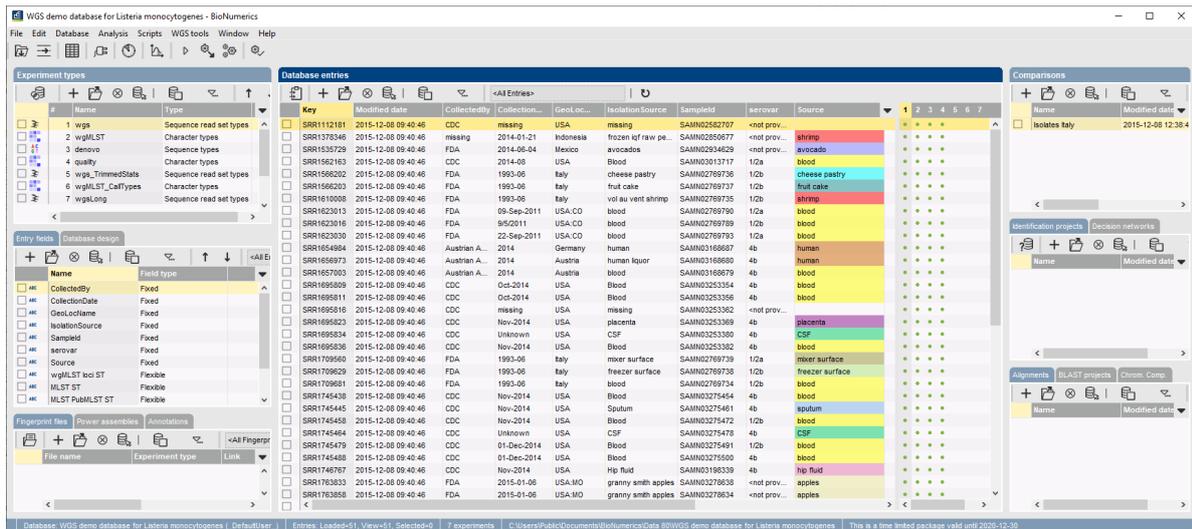
Figure 3: The *Listeria monocytogenes* demonstration database: the *Main* window.
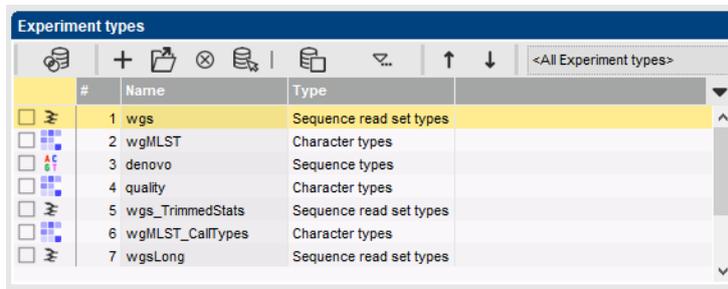

Figure 4: The *Experiment types* panel in the *Main* window.

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 *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 5).

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

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

The *Sequence editor* window opens, containing the results from the de novo assembly algorithm, i.e. concatenated de novo contig sequences (see Figure 6).

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 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 sam-
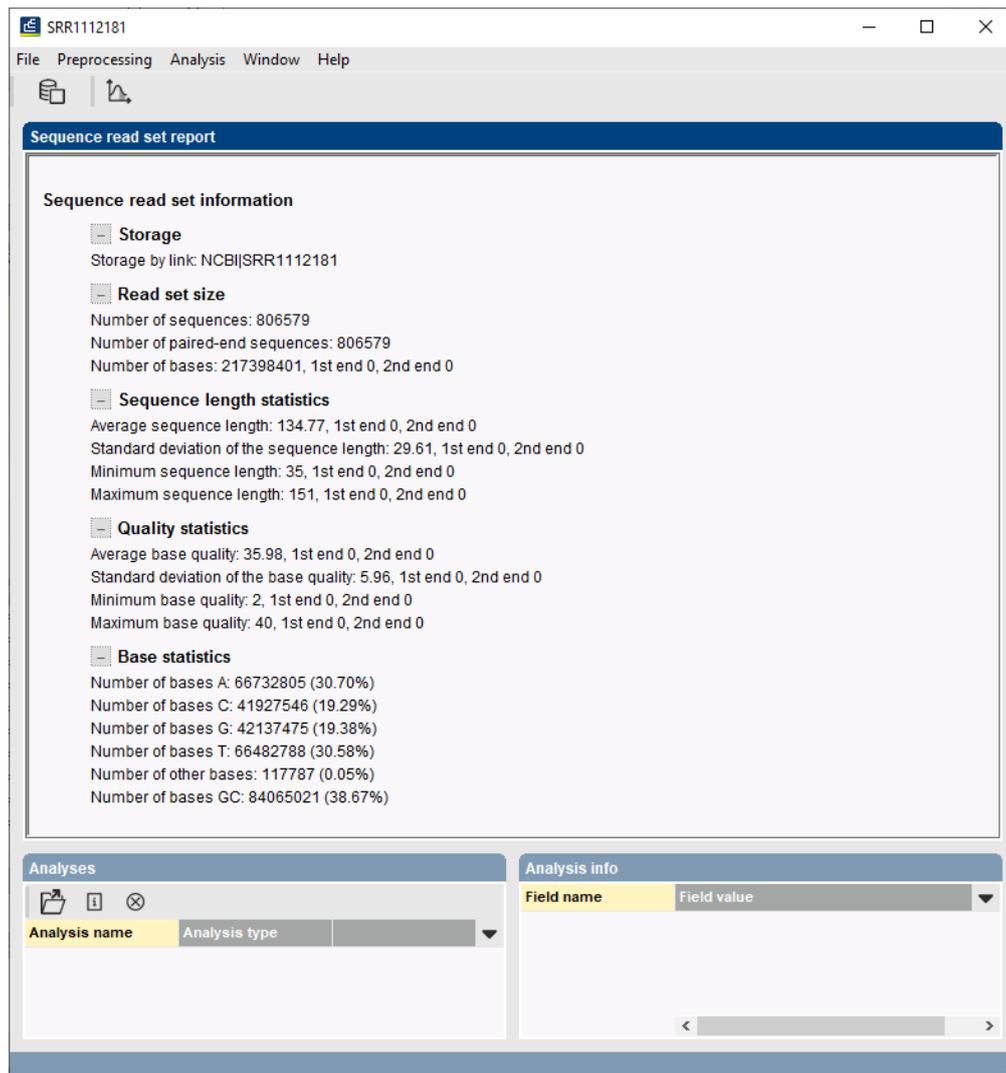
Figure 5: The sequence read set experiment card for an entry.

ples:

- 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 *Listeria* functional genotyping plugin

1. Call the *Plugins and Scripts* dialog box from the *Main* window by selecting **File** > **Install** / **remove plugins...** ( ).

2. Select the *Listeria functional genotyping plugin* and press the <**Install**> button (see Figure 7).
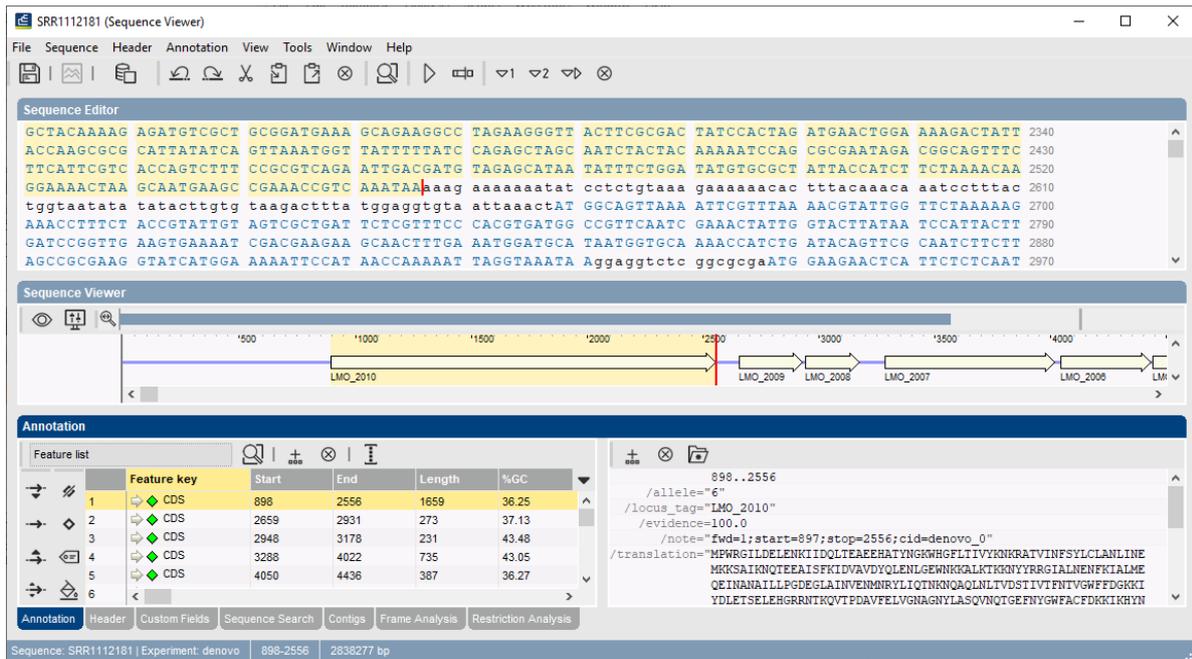
Figure 6: The *Sequence editor* window.

3. Confirm the installation of the plugin.

During installation, the plugin downloads online knowledge bases from https://www.bionumerics.com, which requires a connection to the internet.
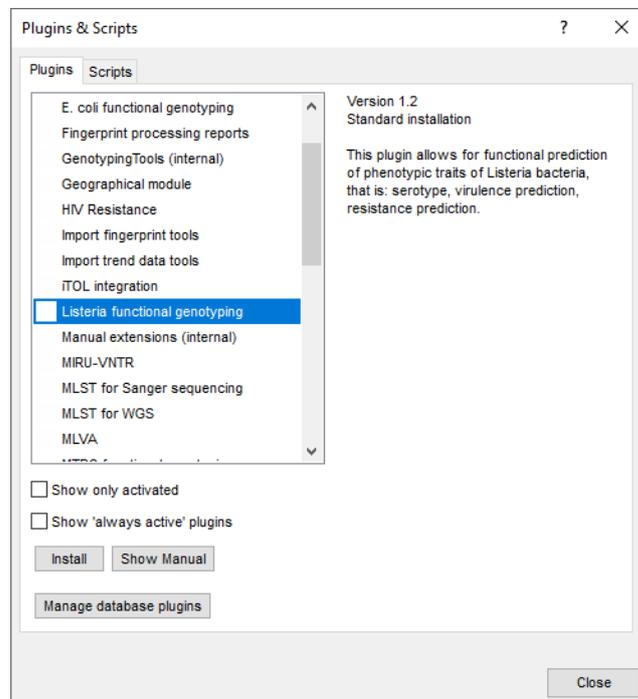

Figure 7: Install the plugin.

4. Click on <***Yes***> to review the settings.

The *Listeria genotyping settings* dialog box pops up, consisting of 6 tabs (see Figure 8). 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.
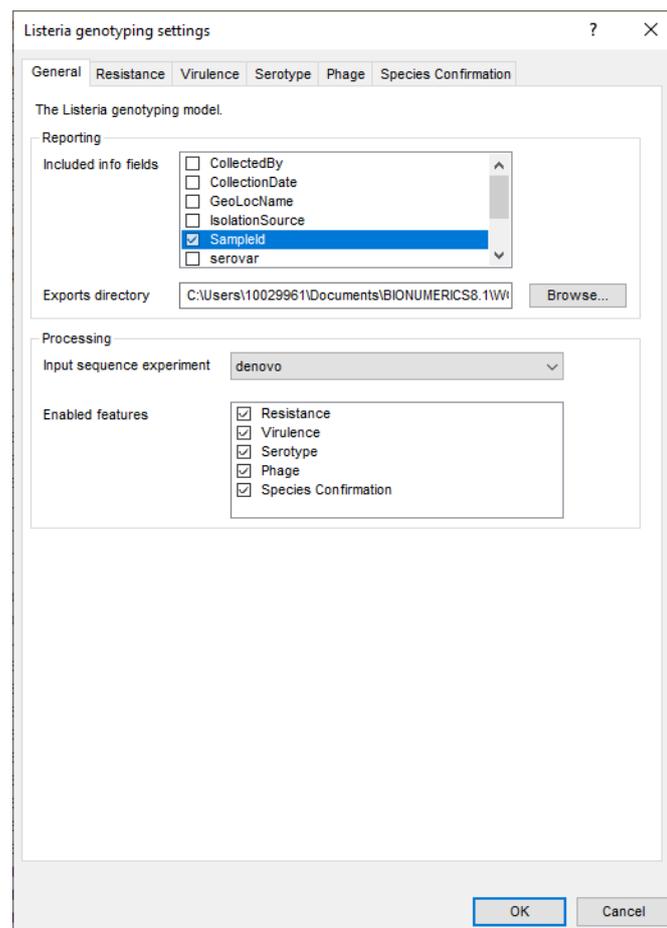
Figure 8: The *Settings dialog*: *General* tab.

5. 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 *SampleId* to include in the report.

The other tabs group the settings for each possible search: Resistance (Acquired resistance), Virulence (Acquired virulence and Virulence islands), Serotype, Phage and Species confirmation.

Except for the Species confirmation feature which is based on sourmash instead of BLAST, all feature tabs contain a *Knowledgebase*, *BLAST* and *Results* panel:

1. *Knowledgebase*: in this panel the **Version** and **Name** of the knowledge base that is being used for this feature is shown. A different knowledge base version can be selected by pressing the <*Change...*> button. With **Check for updates on startup** checked, BIONUMERICS

will check if a newer knowledge base version is available online for this feature each time the database is opened.

2. *BLAST*: in this panel two settings for the BLAST algorithm can be specified; the **Minimum percent identity (%)** and the **Minimum coverage (%)** of your query sequence against the knowledge base's reference sequences. If the option **Combine fragments** is checked, genes that occur fragmented in the genome (i.e. split over two contigs) can still be detected.

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.

Note that the default BLAST settings have been optimized for *L. monocytogenes*, for other species in the genus *Listeria*, it is advised to lower these parameters.

In the *Virulence* tab there is an additional panel (*Virulence islands*) where you can specify the minimum percentage of virulence island loci that needs to be detected (**Minimum loci (%)**) before the presence of the virulence island is shown in the results.

6. 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, except for the **Serovar** field: specify **Serovar2** since the field **Serovar** is already in use in the demo database. Leave the other settings unaltered.

7. Click on <**OK**> in the *Listeria genotyping settings* dialog box.

8. When the *Listeria functional genotyping plugin* is successfully installed, a confirmation message pops up. Press <**OK**>.

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

10. Close and reopen the database to activate the features of the *Listeria functional genotyping plugin*.

The *Listeria functional genotyping plugin* installs menu items in the main menu of the software under **Listeria** (see Figure 9).
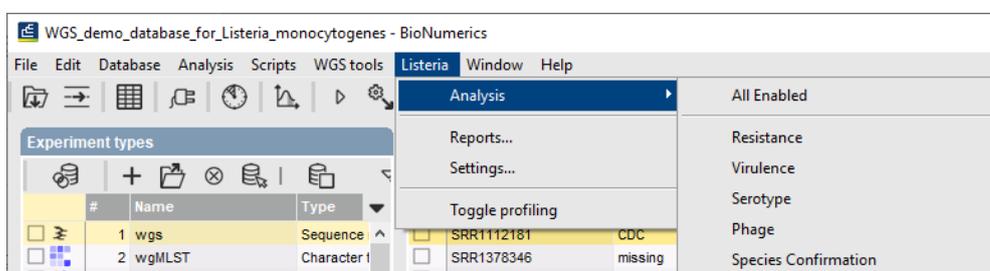
Figure 9: New menu-items after installation of the *Listeria functional genotyping plugin*.

> The settings specified during installation of the plugin can be called again at any time with **Listeria** > **Settings...**.

5 Screening of entries

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 for the phenotypic traits can be done for all tools checked in the *Listeria genotyping settings* dialog box (**Listeria** > **Analysis** > **All Enabled**) or for each tool separately (**Listeria** > **Analysis** > **...**).

4. Select **Listeria** > **Analysis** > **All Enabled** to screen the selected entries for all enabled traits.

A progress bar appears. The analysis time depends on the number of selected entries. When the analysis is finished, the progress bar disappears. The detected traits for the screened entries are stored in the database.

The Serotype prediction (**Serovar** and **Serogroup**), Virulence islands results (**Total Islands**) and Species confirmation (**Species confirmation**) are written to the information fields in the *Database entries* panel (see Figure 10). Please note that the shown names of the information fields are those created per default, but can be different in your case depending on whether you have chosen an alternative name during installation.

Figure 10: Example output of information fields.

The character experiment types for **Resistance**, **Virulence** and **Phage** 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 whether you have chosen an alternative name during installation.

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 Resistance (see Figure 11):

- **Resistance_traits**: contains the results for each antibiotic: 0 = not detected (sensitive), 1 = detected (resistant).

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

Figure 11: Example output of the **Resistance_traits** and the **Resistance_loci** experiment types.

Acquired Virulence (see Figure 12):

- **Virulence_loci**: contains the results for each virulence gene: 0 = not detected, when detected the % identity of the best hit is shown.

- **Virulence_traits**: contains the results for each virulence type: 0 = not detected, 1 = detected.

Figure 12: Example output of the **Virulence_traits** and the **Virulence_loci** experiment types.

Virulence Islands (see Figure 13):

- **island_counts**: contains the number of detected loci associated to a pathogenicity island.

- **island_percentages**: contains the percentage of detected loci associated to a pathogenicity island.

Phage detection (see Figure 14):

- **Phage_seq_ids**: contains the results of the phages detection by sequence IDs: 0 = not detected, when detected the % of the detected full phage is shown.

Figure 13: Example output of the *island_counts* and the *island_percentages* experiment types.

- ***Phage_categories***: contains the results of the phages detection by phage categories: 0 = not detected, when detected the % of the detected full phage is shown.

Figure 14: Example output of the ***Phage_seq_ids*** and ***Phage_categories*** experiment types.

6. Close the character card(s) by clicking in the top left corner of the card.

6 Reports

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

The *Report* window contains a genotype report for each of the selected entries (see Figure 15).

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 *Listeria genotyping 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.
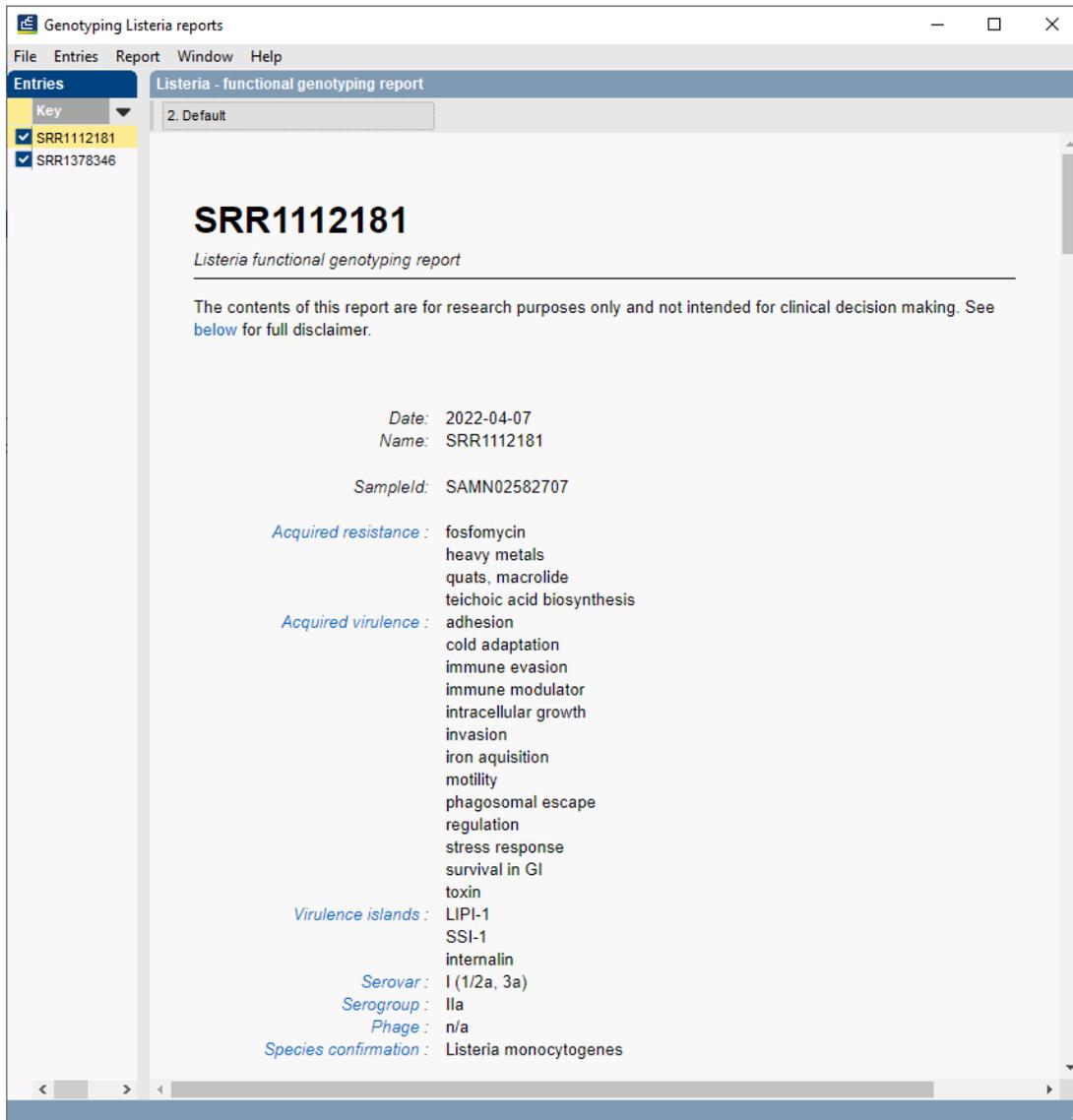
Figure 15: Example of a functional genotyping report.

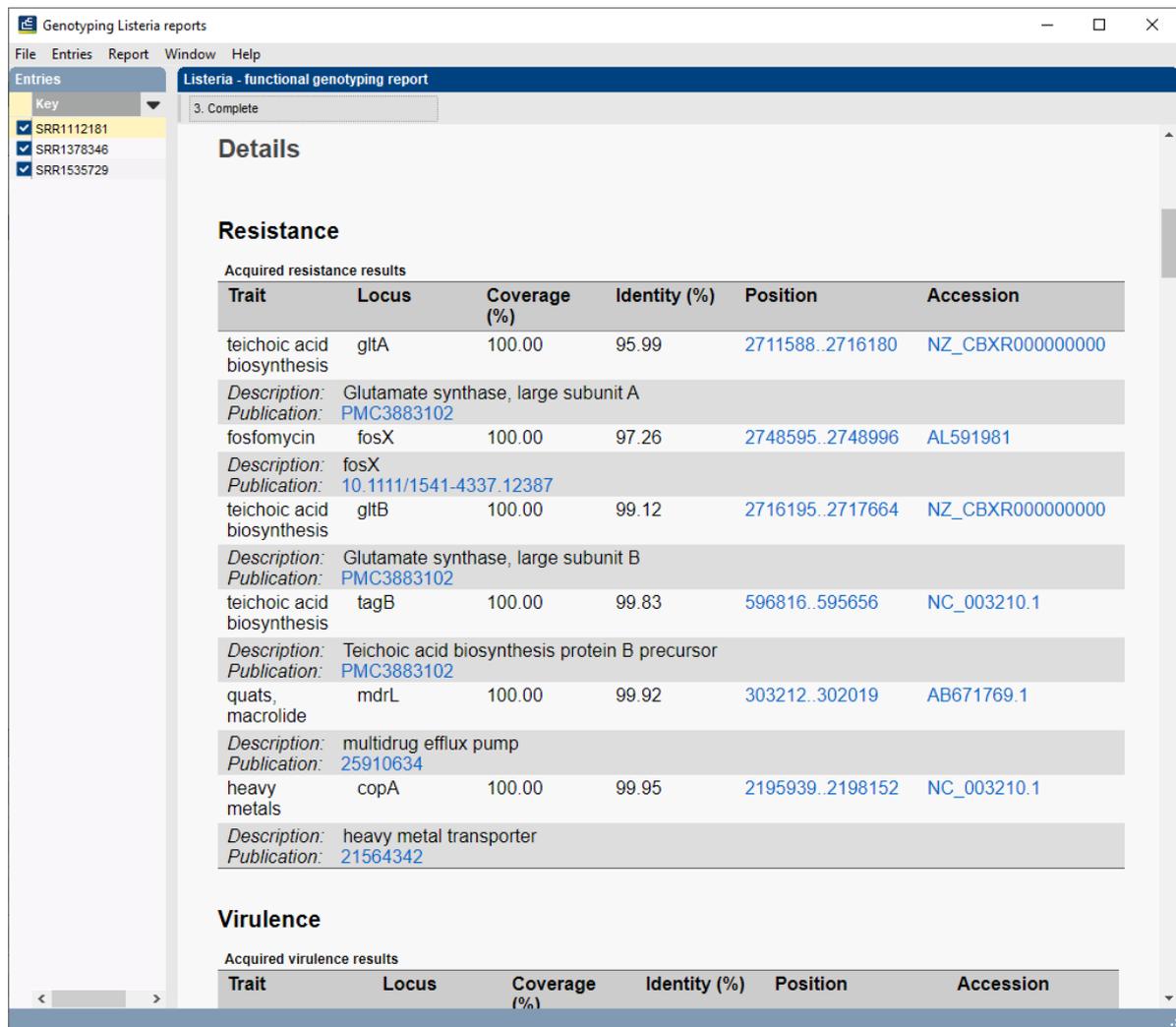A summary of the results of all analyzed traits is displayed in the *Report* window.

4. Select **Report** > **Report styles** in the *Report* window 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**, **Virulence**, **Serotype**, **Phage** and **Species confirmation** screening are listed and described.
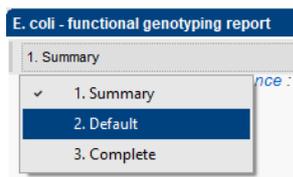
Figure 16: Report templates in the *Report window*.

5. Click on a hyperlink of one of the predicted traits to display the detailed results in the *Genotype report* panel (see Figure 17).

Figure 17: Report details.

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.