



# BIONUMERICS®

## version 8 - PLUGINS



*Listeria* functional genotyping plugin



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- Unicycler version 0.5.0, <https://github.com/rrwick/Unicycler/releases> \*
- Velvet for Windows, source code can be downloaded from <https://www.bionumerics.com/download/open-source>
- Bowtie2 version 2.2.5 (<https://bowtie-bio.sourceforge.net/bowtie2/index.shtml>)\*
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- RAxML version 8.2.11, <https://github.com/stamatak/standard-RAxML/releases>

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- sourmash version 4.1.0, <https://github.com/dib-lab/sourmash> \*\*
- SeqSero2 for Windows, source code can be downloaded from <https://www.bionumerics.com/download/open-source>
- Fastp version 0.22.0, <https://github.com/OpenGene/fastp>

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# Chapter 1

## Starting and setting up BIONUMERICS

### 1.1 Introduction

---


The *Listeria functional genotyping plugin* allows you to screen *Listeria* whole genome sequences to predict phenotypic traits such as resistance against antibiotics and detergents, virulence, and serotype. It also allows you to detect phages. The genome sequences can be imported in BIONUMERICS using one of the import routines available in the software or can be the result of a *de novo* assembly performed in BIONUMERICS on a sequence read set.


The *Listeria functional genotyping plugin* is supported in the **BIONUMERICS-SEQ** and **BIONUMERICS-SUITE** configurations.


### 1.2 Startup program

---

Make sure the latest version of BIONUMERICS is installed (<https://www.bionumerics.com/download/software>). The installation manual can be downloaded from <https://www.bionumerics.com/download/manuals>.

When BIONUMERICS is launched from the Windows start panel or when the BIONUMERICS shortcut () on your computer's desktop is double-clicked, the **Startup program** is run. This program shows the *BIONUMERICS Startup* window (see Figure 1.1).


A new BIONUMERICS database is created from the Startup program by pressing the  button.

An existing database is opened in BIONUMERICS with  or by simply double-clicking on a database name in the list.

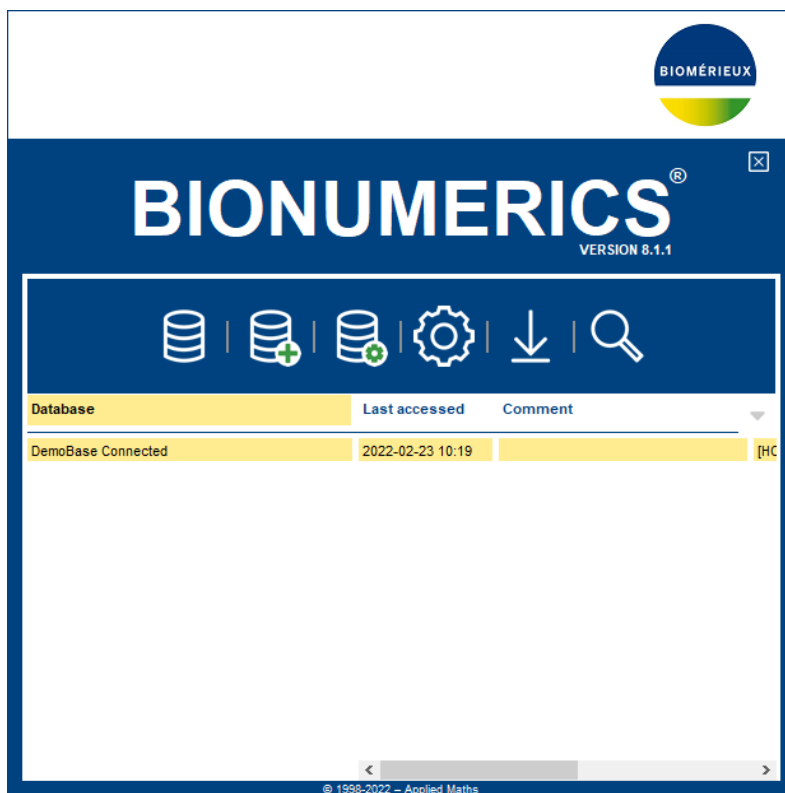
### 1.3 Installation of the *Listeria functional genotyping plugin*

---

Proceed as follows to install the *Listeria functional genotyping plugin*:

3.1 Call the *Plugins and Scripts* dialog box from the *Main* window with **File > Install / remove plugins...** (.

3.2 Select the *Listeria functional genotyping plugin* from the list and press the **<Install>** button.



**Figure 1.1:** The *BIONUMERICS* Startup window.

### 3.3 Confirm the installation of the plugin.

During installation, the plugin downloads online knowledge bases (see 3) from <https://www.bionumerics.com>, which requires a connection to the internet. Depending on the bandwidth of your connection, this process can take up to several minutes. When the download is complete, the question 'Do you want to change the *Listeria* genotyping settings now?' pops up.

### 3.4 Press <Yes> to confirm that you want to change the plugin settings.

The *Listeria genotyping settings* dialog box appears, which will be discussed in detail in 2. For the plugin to work, at the very least we need to specify the sequence experiment containing the (de novo) genome sequences on which the screening will be performed.

### 3.5 If already available, select the sequence experiment in your database that contains genome sequences as **Input sequence experiment** and press <OK>.

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

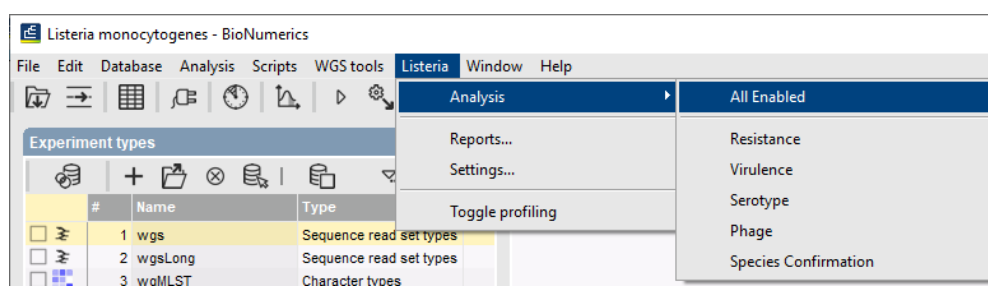
### 3.6 Press <OK> in the confirmation message.

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

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

The *Listeria functional genotyping plugin* installs menu items in the main menu of the software under **Listeria** (see Figure 1.2).

More information regarding the *Listeria functional genotyping plugin* settings is available under 2.



**Figure 1.2:** New menu items, available after installation of the *Listeria* functional genotyping plugin.



## Chapter 2

# Settings of the *Listeria* functional genotyping plugin

### 2.1 Accessing the genotyping settings

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Settings for the *Listeria functional genotyping plugin* can be accessed via **Listeria** > **Settings...** in the *Main* window.

### 2.2 General settings

---

The *General* tab of the *Listeria genotyping settings* dialog box (see Figure 2.1) holds settings for the genotyping reports and for general processing.

Under **Reporting**, the entry information fields that will be displayed in the genotyping reports can be specified in the **Included info fields** list. Simply check the ballot box next to an information field name to include the field in the report.

The **Exports directory** can be specified for all exports from the genotyping reports. By default, the exported files are stored in a subdirectory of the database directory, but a different location can be selected via the <**Browse**> button or entered directly in the text box.

The **Input sequence experiment**, i.e. the sequence experiment containing the whole genome sequences to be screened, should be selected from the corresponding drop-down list. Select the <**Create**> option in case you wish to create a new sequence experiment type. In the latter case, make sure to import whole genome sequences in this experiment type before running the plugin.



It is crucial to specify at least the **Input sequence experiment** in the settings. If not specified, the error message "The input sequence experiment must be set to process entries." will be generated when the plugin is run.

In the **Enabled features** list, all features offered by the plugin are listed and enabled by default. If specific analyses are not required, you can uncheck them here to save on processing time and to omit the corresponding sections from the reports.

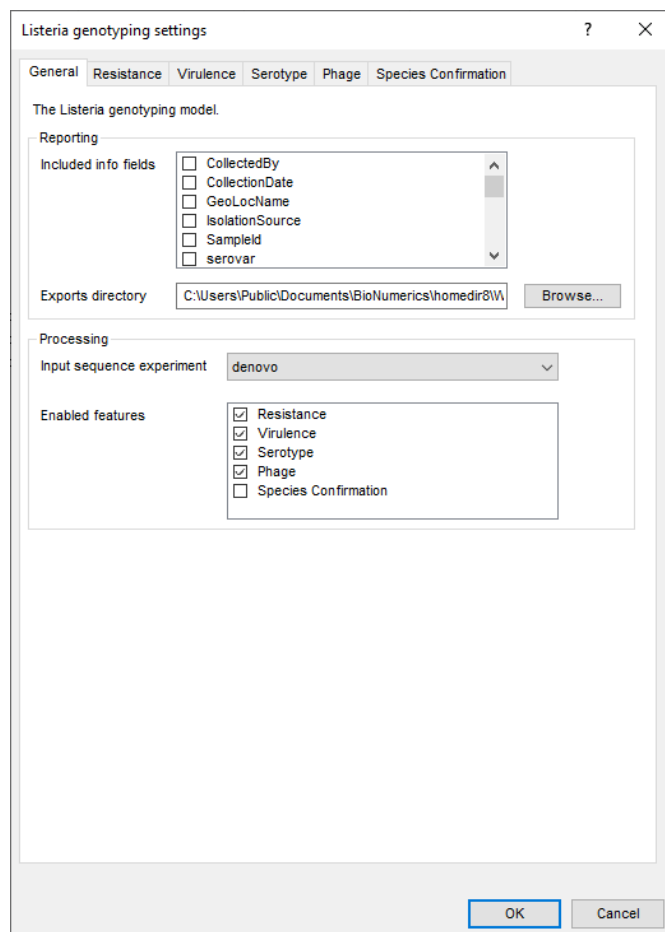


Figure 2.1: The *Listeria* genotyping settings dialog box, General tab.

## 2.3 Resistance settings

The *Resistance* tab in the *Listeria* genotyping settings dialog box (see Figure 2.2) groups all settings for the detection of resistance.

The term resistance for *Listeria* is used to define resistance against anything used for the removal of *Listeria*, either from a host or from a food processing environment. This therefore includes both antibiotics as detergents.

Resistance prediction is based on the absence or presence of certain detergent or antibiotic resistance related genes and alleles in the assembled *Listeria* genome. Presence or absence is detected based on a BLAST approach using the list of antibiotic resistance related genes as query and the de novo assembled genome as target.

Under **Knowledgebase**, the **Name** and **Version** of the specified knowledge base version for this feature is shown. When no knowledge base is specified yet, "<None>" will be indicated in both fields. When a specified knowledge base version cannot be found (e.g. because it is deleted), "<Missing>" is shown in both fields. 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. This requires an internet connection.

See 3 for more information on *Listeria* functional genotyping plugin knowledge bases.

**Figure 2.2:** The *Listeria* genotyping settings dialog box, *Resistance* tab.

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.

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

In the **Results** panel, the experiment types and entry information fields to which the screening results will be written can be dictated. Use the drop-down menu to choose an existing experiment type or information field or select the **<Create>** option to create a new experiment type or information field. A default name is suggested, but you can adjust this if you want to.

Following character experiments can optionally be specified for acquired resistance:

- **Traits experiment:** contains the results for each antibiotic group: 0 = not detected (sensitive), 1 = detected (resistant). The default name is **Resistance\_traits**.
- **Loci experiment:** contains the results for each resistance gene: 0 = not detected (sensitive), when detected (resistant) the % identity of the best BLAST hit is shown. The default name is **Resistance\_loci**.



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. How to rearrange characters in a character type experiment is described in the Reference manual, Chapter Setting up character type experiments.



For the resistance character experiments, it can be convenient to map the character values (-1, 0, 1) to categorical names (indecisive (-), sensitive (S), resistant (R)) by creating a character mapping. How to do this is explained in the Reference manual, Chapter Setting up character type experiments.

Check **Annotate sequence experiment** to annotate the input sequence with the detected genotyping features.

## 2.4 Virulence settings

The *Virulence* tab of the *Listeria* genotyping settings dialog box (see Figure 2.3) groups all settings for the detection of virulence factors and virulence islands.

**Figure 2.3:** The *Listeria* genotyping settings dialog box, *Virulence* tab.

Virulence prediction is based on the absence or presence of certain virulence related genes and alleles in the assembled *Listeria* genome. Presence or absence is detected based on a BLAST approach using the list of virulence related genes as query and the de novo assembled genome as target.



Anything increasing the likelihood of infection is considered to be involved in the virulence, this also includes factors such as cold adaptation proteins which increase the survival of *Listeria* spp. in ready to eat foods, thus making human consumption more likely. Each factor is assigned a trait to provide a quick understanding of how the factor is involved with virulence.

Many of the *Listeria* virulence genes are found on 'virulence islands' (also called 'pathogenicity islands', PAIs) in the chromosome. A BLAST-based tool screens for loci associated to virulence islands and reports the number of found loci, the % of found loci, and the loci spread average minimum inter-loci distance (in bp), for each virulence island.

Under **Knowledgebase**, the **Name** and **Version** of the specified knowledge base version for this feature is shown. When no knowledge base is specified yet, "<None>" will be indicated in both fields. When a specified knowledge base version cannot be found (e.g. because it is deleted), "<Missing>" is shown in both fields. 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. This requires an internet connection.

See 3 for more information on *Listeria functional genotyping plugin* knowledge bases.

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.

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

In the **Results** panel, the experiment types and entry information fields to which the screening results will be written can be dictated. Use the drop-down menu to choose an existing experiment type or information field or select the <**Create**> option to create a new experiment type or information field. A default name is suggested, but you can adjust this if you want to.

Following character experiments can optionally be specified:

- **Traits experiment**: contains the results for each virulence trait: 0 = not detected, 1 = detected. The default name is **Virulence\_traits**.
- **Loci experiment**: contains the results for each virulence gene: 0 = not detected, when detected the % identity of the best BLAST hit is shown. The default name is **Virulence\_loci**.
- **Island counts experiment**: contains the number of detected loci associated to a pathogenicity island. The default name is **Island\_counts**.
- **Island percentages experiment**: contains the percentage of detected loci associated to a pathogenicity island. The default name is **Island\_percentages**.

Following entry information fields can optionally be specified:

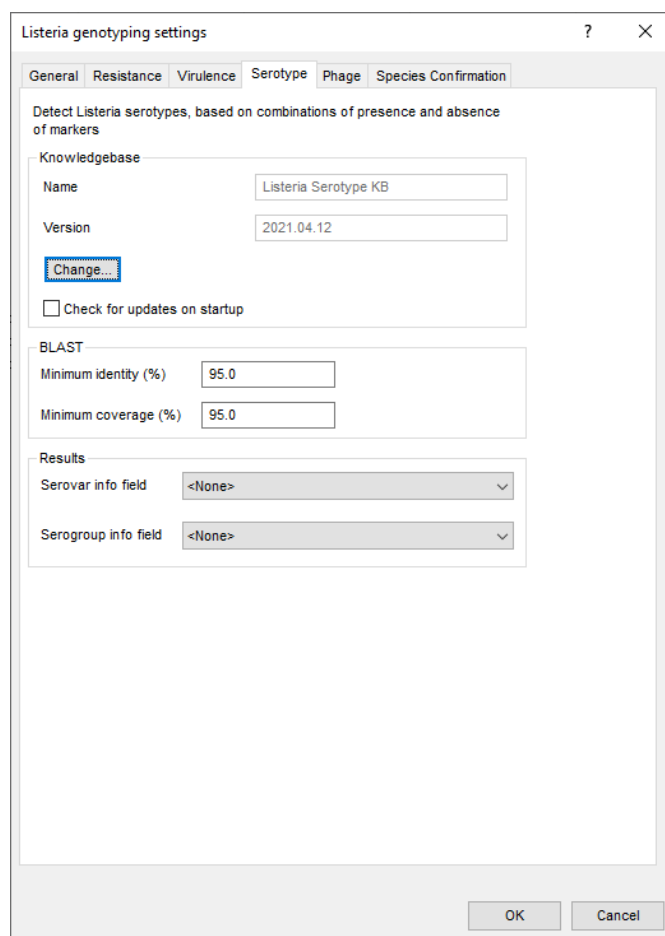
- **Total islands info field**: contains the number of different virulence islands detected. The default field name is **Total islands**.

Check **Annotate sequence experiment** to annotate the input sequence with the detected genotyping features.

Under **Virulence islands**, you can specify the minimum percentage of virulence island loci (**Minimum loci (%)**) that needs to be detected before the presence of the virulence island is shown in the results. Please note that some virulence islands in the BIONUMERICS online knowledge base only contain two loci, for this reason we discourage setting the threshold above 50%.

## 2.5 Serotype settings

The *Serotype* tab in the *Listeria* genotyping settings dialog box (see Figure 2.4) groups all settings for *Listeria* serotype determination.



**Figure 2.4:** The *Listeria* genotyping settings dialog box, *Serotype* tab.

The serotype in *Listeria monocytogenes* is defined based on the O and H antigens. The true serotyping test (binding on specific O and H antigens) is time consuming, difficult to perform and often has ambiguous results. Only a few labs perform the true serotyping, while most replace it with a multiplex PCR test ([2]). This PCR test cannot distinguish between all serovars, but assigns a serogroup to the isolates. Each serogroup can contain several serovars, but the most prevalent serovars in clinical and food isolates (4b and 1/2a respectively) are distributed between two serogroups (IVb and IIa respectively).

The assignment of serovar and serogroup in the plugin is based on these markers, with the addition of the *plcA* gene which is only present in *L. monocytogenes*. Some other *Listeria* spp. do

have an ortholog of this gene, with a similarity below 60%. It is therefore not picked up with the default settings. This gene helps to distinguish between serogroup IVa and other *Listeria* spp. The gene *prs* is specific for all *Listeria* sensu stricto species, the ortholog in the sensu lato group is too far away to be detected with the default settings. As a result, species from the *Listeria* sensu lato group are marked as not *Listeria* by the plugin. Only species from the sensu stricto group, *L. monocytogenes*, *L. marthii*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, and *L. innocua*, are picked up as genus *Listeria* by the plugin.

Under **Knowledgebase**, the **Name** and **Version** of the specified knowledge base version for this feature is shown. When no knowledge base is specified yet, "<None>" will be indicated in both fields. When a specified knowledge base version cannot be found (e.g. because it is deleted), "<Missing>" is shown in both fields. 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. This requires an internet connection.

See 3 for more information on *Listeria* functional genotyping plugin knowledge bases.

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.

In the **Results** panel, the experiment types and entry information fields to which the screening results will be written can be dictated. Use the drop-down menu to choose an existing experiment type or information field or select the <**Create**> option to create a new experiment type or information field. A default name is suggested, but you can adjust this if you want to.

Following entry information fields can optionally be specified:

- **Serovar info field**: contains the possible serovars linked to the serogroup. The default field name is **Serovar**.
- **Serogroup info field**: contains the predicted serogroup based on the identified markers. The default field name is **Serogroup**.

## 2.6 Phage settings

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The *Phage* tab in the *Listeria* genotyping settings dialog box (see Figure 2.5) groups all settings for phage detection.

Phages are detected in the assembled *Listeria* genome by a BLAST-based screening against a collection of known full length phages.

Under **Knowledgebase**, the **Name** and **Version** of the specified knowledge base version for this feature is shown. When no knowledge base is specified yet, "<None>" will be indicated in both fields. When a specified knowledge base version cannot be found (e.g. because it is deleted), "<Missing>" is shown in both fields. A different knowledge base version can be selected by pressing the <**Change...**> button.

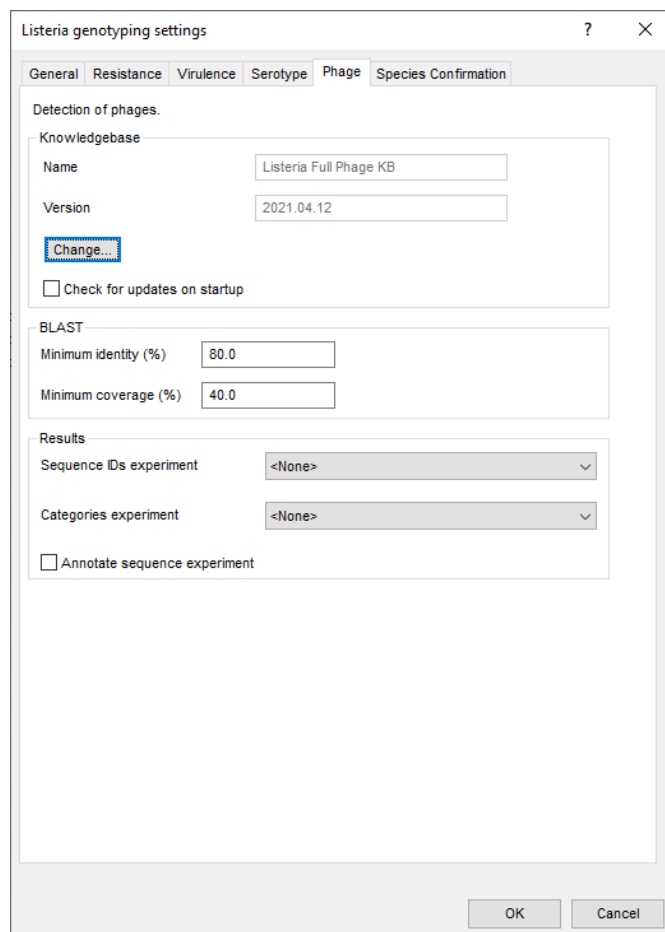


Figure 2.5: The *Listeria* genotyping settings dialog box, *Phage* tab.

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. This requires an internet connection.

See 3 for more information on *Listeria functional genotyping plugin* knowledge bases.

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.

In the **Results** panel, the experiment types and entry information fields to which the screening results will be written can be dictated. Use the drop-down menu to choose an existing experiment type or information field or select the **<Create>** option to create a new experiment type or information field. A default name is suggested, but you can adjust this if you want to.

Following character experiments can optionally be specified:

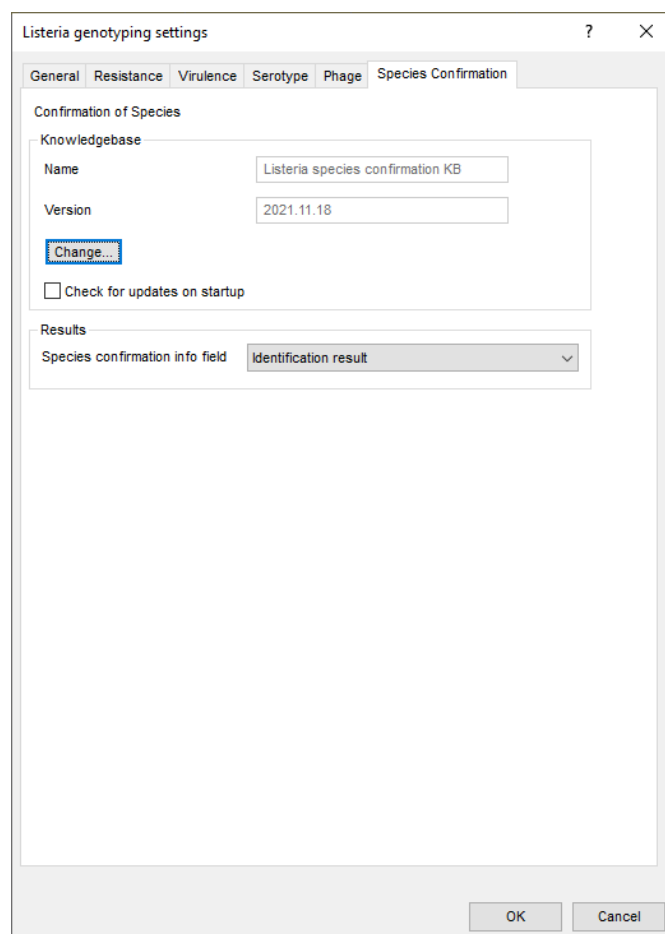
- **Sequence IDs experiment**: contains the results of the phages detection by sequence IDs: 0 = not detected, when detected the % of the detected full phage is shown. The default name is **Phage\_seq\_ids**.

- **Categories experiment:** contains the results of the phages detection by phage categories: 0 = not detected, when detected the % of the detected full phage is shown. The default name is **Phage.categories**.

Check **Annotate sequence experiment** to annotate the input sequence with the detected genotyping features.

## 2.7 Species confirmation settings

The *Species Confirmation* tab in the *Listeria genotyping settings* dialog box (see Figure 2.6) groups all settings for species confirmation.



**Figure 2.6:** The *Listeria genotyping settings* dialog box, *Species Confirmation* tab.

Species confirmation is achieved by comparing the input genome against a set of type strain and other reference genomes from the same genus (or highly related genera) using sourmash [1]. The species confirmation knowledge base specifies ascending thresholds for the hierarchical levels genus, species and subspecies (if subspecies are defined within the species). When the sourmash containment score is above the genus threshold but below the species threshold, only the genus name is returned as identification result. When the species threshold is met but not the subspecies threshold, the binomial name (genus + species) is returned. Finally, when subspecies are defined for the organism and the containment score exceeds the subspecies threshold, the species confirmation result consists of the genus, species and subspecies name.

Under **Knowledgebase**, the **Name** and **Version** of the specified knowledge base version for this feature is shown. When no knowledge base is specified yet, "<None>" will be indicated in both fields. When a specified knowledge base version cannot be found (e.g. because it is deleted), "<Missing>" is shown in both fields. 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. This requires an internet connection.

See the *E. coli* functional genotyping plugin, Chapter Genotyping plugin knowledge bases for more information on *E. coli functional genotyping plugin* knowledge bases.

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## Chapter 3

# Genotyping plugin knowledge bases

### 3.1 Introduction

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Nearly all genotyping features make use of a knowledge base of some kind. Knowledge bases are at the heart of functional genotyping because they literally contain the knowledge on how to interpret genome sequences in function of the feature they were designed for. By providing the knowledge bases online from <https://www.bionumerics.com>, they can easily be updated without the need to install a new plugin version.

All online knowledge bases are based on curated public repositories (e.g. ResFinder <https://cge.cbs.dtu.dk/services/ResFinder/>) and converted to the specific format required by the plugin. Each knowledge base has a change log with detailed information on the changes in each version.



Organism-specific functional genotyping plugins can only use *online* knowledge bases. The downloaded knowledge bases are encrypted and cannot be modified by the user. For using your own, custom knowledge bases we recommend the *Custom genotyping plugin*, which was specifically designed for this purpose.

By default, the plugin uses the most recent knowledge base version available at the time of installation. When more than one knowledge base version is available online, users can specify which version to use.

### 3.2 Specifying a different knowledge base version

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The knowledge base for a genotyping feature is specified in the genotyping settings.

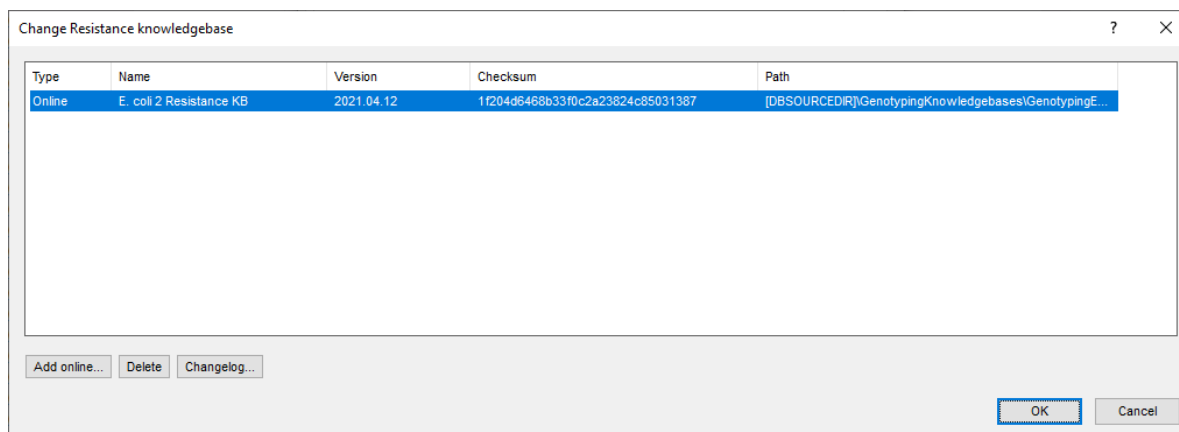
- 2.1 In the *Main* window, select **Listeria** > **Settings...** to open the *Listeria genotyping settings* dialog box.

In all tabs of the *Listeria genotyping settings* dialog box (except the *General tab*) a **Knowledge-base** section is available.

- 2.2 Click on the tab of the feature for which you want to change the knowledge base and press <**Change...**>.

This action opens the *Change knowledge base* dialog box (see Figure 3.1).

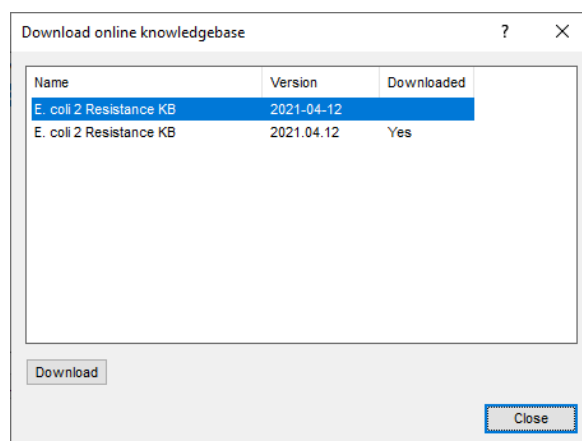
The *Change knowledge base* dialog box shows the downloaded knowledge bases for this feature. The currently used knowledge base (if specified) will be highlighted by default. Following information is displayed about the knowledge bases:



**Figure 3.1:** The *Change knowledge base* dialog box.

- 'Type': either Online or Local. The knowledge base type will always be Online for all organism-specific genotyping plugins and for the *Resistance detection plugin*.
- 'Name': the name of the knowledge base.
- 'Version': the knowledge base version, typically a last modified date formatted as YYYY.MM.DD.
- 'Checksum': MD5 checksum, used to verify the integrity of the downloaded knowledge base.
- 'Path': file path, relative to the source files directory (indicated with the token DBSOURCEDIR), where the knowledge base is downloaded to.

To manually check if other versions (newer or older) of the knowledge base are available online, press <**Add online...**>. This opens the *Download online knowledge base* dialog box (see Figure 3.2).



**Figure 3.2:** The *Download online knowledge base* dialog box.

Opening this dialog will check the BIONUMERICS website (<https://www.bionumerics.com>) for the latest knowledge base version for this feature. When the latest version is already downloaded (i.e. the knowledge base is up to date), the 'Downloaded' field will indicate "Yes". If this is not the case, you can press the <**Download**> button to download the latest version.

Close the dialog with <**Close**>.



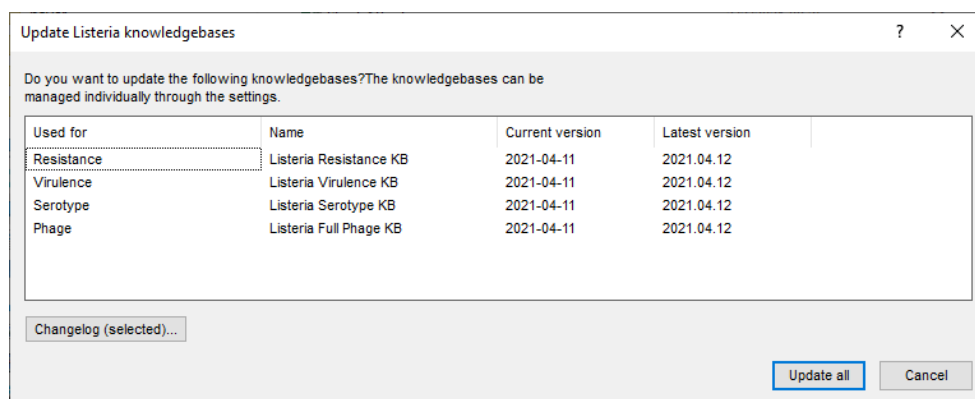
A highlighted knowledge base can be deleted by pressing **<Delete>**. The software will ask for confirmation before actually removing the knowledge base.

By pressing **<Changelog...>** you are referred to the change log page on the BIONUMERICS website of the online knowledge base you have selected. Here you can find information regarding the source(s) used to create the knowledge base and the changes made in respect to prior versions of the knowledge base. This information should help you decide which knowledge base version to use.

To specify a different knowledge base for the feature, click on the preferred knowledge base to highlight it and press **<OK>**. This action will close the *Change knowledge base* dialog box and will show the genotyping settings again with the newly specified knowledge base.

### 3.3 Automated check for knowledge base updates

For each feature that uses an online knowledge base, there is a check box **Check for updates on startup**. When this is checked, BIONUMERICS will automatically connect to <https://www.bionumerics.com> to check for knowledge base updates each time the database is opened. When updates are available, the *Update knowledge bases* dialog box pops up (see Figure 3.3).



**Figure 3.3:** The *Update knowledge bases* dialog box pops up at startup when one or more new knowledge bases are available online.

By pressing **<Changelog (selected)>** you are referred to the change log page on the BIONUMERICS website of the highlighted online knowledge base.

Press **<Update all>** to apply all knowledge base updates. The updated knowledge bases will be downloaded and set as default in the genotyping settings, meaning that the updated knowledge base will be used the next time an analysis is run for the corresponding feature.



Knowledge base updates can only be done in bulk from this dialog box, i.e. for all available updates at once. If you need to update one or more knowledge bases selectively, press **<Cancel>** in the *Update knowledge bases* dialog box and update the knowledge bases one by one via the genotyping settings (see ??).



## Chapter 4

# *Listeria* genotyping analysis

### 4.1 Selecting entries

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Once the plugin is installed and the settings have been specified, the actual screening of the genome sequences of the selected entries is an easy process.

Analyses are performed on the selected entries in the database. For example, to select a single entry, hold the **Ctrl**-key and click on the entry in the *Database entries* panel. Alternatively, use the **space bar** or click the ballot box next to the entry. In order to select a range of entries, hold the **Shift**-key and click on the last entry in the range.

More options for selecting entries can be found in the BIONUMERICS reference manual (see the Reference manual, Chapter Database entries).

### 4.2 Starting an analysis

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

The analysis time increases proportionally with the number of selected entries and the number of enabled genotyping features. It also depends on the type of feature and whether or not additional results are stored in character experiments and/or information fields. A complete analysis may take up to several minutes or even hours.

When the analysis is finished, the progress bar disappears. The screening results are stored in the database experiments and information fields which you have defined in the *Listeria genotyping settings* dialog box. The settings can always be consulted or adjusted using **Listeria > Settings...** (see [2](#) for details).



## Chapter 5

# *Listeria* genotyping reports

### 5.1 Opening functional genotyping reports

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A functional genotyping report (see Figure 5.1) can be opened for the selected entries with **Listeria** > **Reports....**

Clicking on an entry in the *Entries* panel of the *Genotyping report* window (or using the up and down arrow keys on the keyboard) shows the report for the highlighted entry.

At the top of each report the creation date of the report (**Date**), the Key (**Name**), and information fields that were checked in the *General* tab of the genotyping settings are displayed, followed by a summary of the results of all analyzed traits.

Selecting **File** > **Exit** closes the *Genotyping report* window.

### 5.2 Report styles

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In the *Genotyping report* window, three different report styles can be applied from the drop-down list in the panel header or via the menu (**Report** > **Report styles**):

1. **Summary**: only a summary of the results is shown.
2. **Default**: the summarized results and most details are shown in a tabular format. In this report style, all columns of the results tables can be sorted alphabetically or numerically by clicking on their headers.
3. **Complete**: the summarized results and all available details are shown. More exhaustive information is presented in an additional row, for example descriptions of the detected genes, decision trees, etc.. Result tables cannot be sorted in this report style.

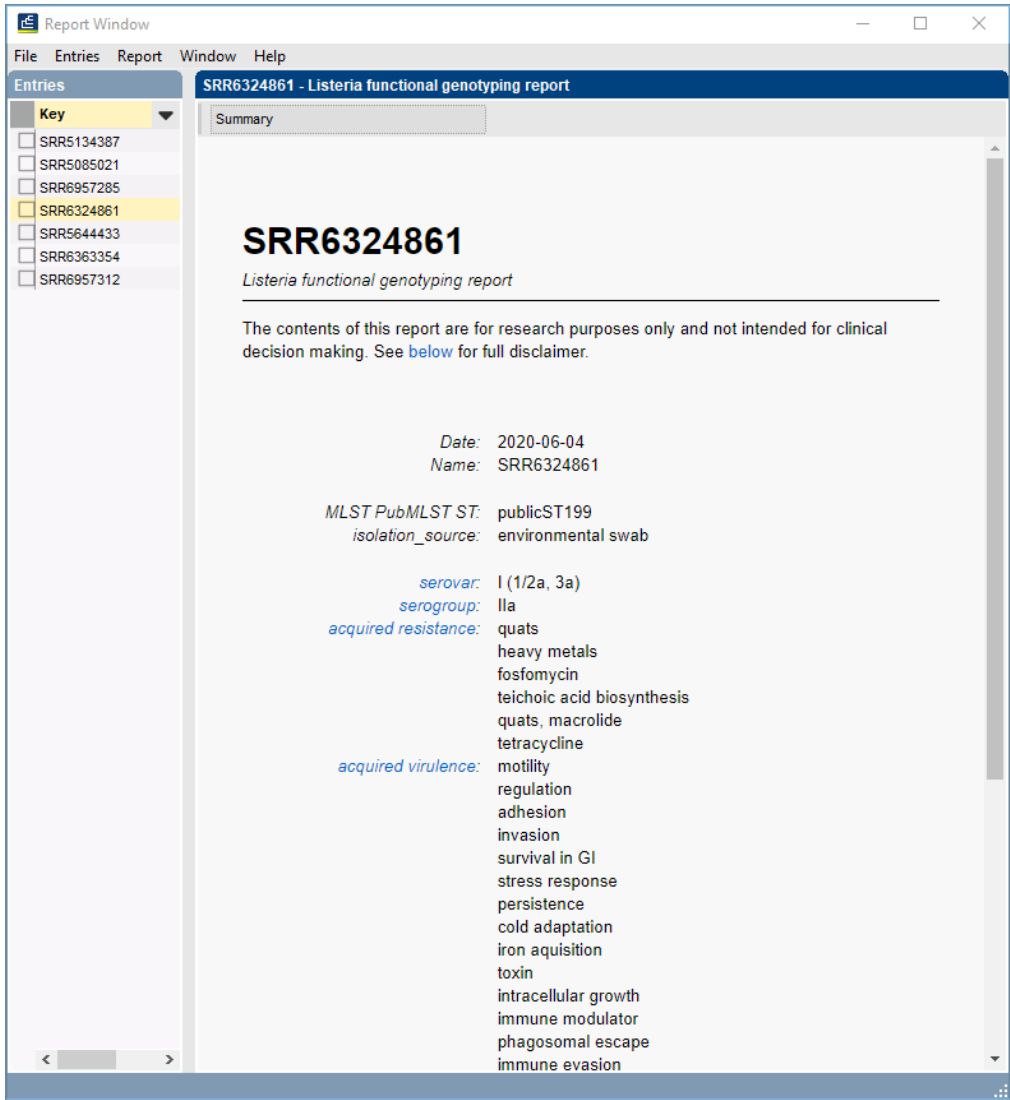
### 5.3 Details section

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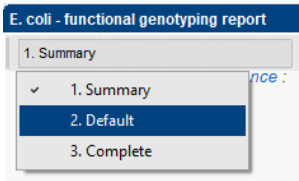
#### 5.3.1 Introduction

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The Details section in the genotyping report contains a detailed result table for each analyzed genotyping feature.



**Figure 5.1:** The *Genotyping report* window, showing a report for sample SRR6324861.



**Figure 5.2:** Drop-down list to select report styles in the *Genotyping report* window.

Some result tables contain hyperlinks. These hyperlinks open in the default web browser, except for the 'Position' field. The latter links open the *Sequence editor* window in BIONUMERICS with the positions highlighted on the sequence.

**5.3.2 Resistance**

All detected acquired resistance traits are listed in the table, with following fields:

- **Trait:** Name of the resistance trait.
- **Locus:** Detected locus that confers the trait.
- **Coverage (%):** Percent overlap between the query and the target sequence.
- **Identity (%):** BLAST identity, expressed as a percentage.
- **Position:** Position(s) of the BLAST match on the input genome. Multiple positions are possible when **Combine fragments** was checked in the genotyping settings.
- **Accession:** Link to the GenBank accession of the detected locus.
- **Description:** Description for the detected locus (in **Complete** template only).
- **Publication:** Link to the publication in PubMed describing the detected locus (in **Complete** template only).

### 5.3.3 Virulence

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All detected acquired virulence traits are listed in the table, with following fields:

- **Trait:** Name of the virulence trait.
- **Locus:** Detected locus that confers the trait.
- **Coverage (%):** Percent overlap between the query and the target sequence.
- **Identity (%):** BLAST identity, expressed as a percentage.
- **Position:** Position(s) of the BLAST match on the input genome. Multiple positions are possible when **Combine fragments** was checked in the genotyping settings.
- **Accession:** Link to the GenBank accession of the detected locus.
- **Description:** Description for the detected locus (in **Complete** template only).
- **Publication:** Link to the publication in PubMed describing the detected locus (in **Complete** template only).

All detected virulence islands are listed in the table, with following fields:

- **Virulence island:** Name of the virulence island.
- **Found:** Number of detected loci for this virulence island.
- **Total:** Total number of loci present in the knowledge base for this virulence island.
- **Percentage:** Percentage of detected loci, i.e.  $100 \frac{Loci_{Found}}{Loci_{Total}}$ .
- **Loci:** Locus names of all detected loci (in **Complete** template only).

### 5.3.4 Serotype

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The *Serotype* section lists the determined **Serovar** and **Serogroup**. Additionally, all detected serotype markers are listed in a table, with following fields:

- **Marker:** Name of the serotype marker.
- **Coverage (%):** Percent overlap between the query and the target sequence.
- **Identity (%):** BLAST identity, expressed as a percentage.
- **Position:** Position of the BLAST match on the input genome.
- **Accession:** Link to the GenBank accession of the detected locus.

### 5.3.5 Phage

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All detected phages are listed in the table, with following fields:

- **Category:** Category to which the detected phage belongs.
- **Sequence ID:** Name of the detected phage.
- **Coverage (%):** Percent overlap between the query and the target sequence.
- **Identity (%):** BLAST identity, expressed as a percentage.
- **Length:** Length in nucleotides of the detected sequence.
- **Accession:** Link to the GenBank accession of the detected phage sequence.

### 5.3.6 Species confirmation

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The best matching identification with one of the reference genomes from the knowledge base is listed in the table, with following fields:

- **Confirmation:** The species confirmation (i.e. identification) result, at genus, species or sub-species level.
- **Containment score (%):** Sourmash containment score, expressed as a percentage.
- **Largest exceeded threshold (%):** The highest containment score threshold (as defined in the knowledge base) that was exceeded.
- **Ref. genome name:** Organism name of the reference genome as obtained from GenBank.
- **Accession:** GenBank accession of the reference genome sequence.
- **Taxid:** Taxonomy ID as used in NCBI's taxonomy browser (<https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>).

## 5.4 Info section

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At the bottom of each report, an *Info section* is shown which contains information regarding the analysis date, plugin version, knowledge base name and version, and settings of each analysis.



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## 5.5 Export report

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The genotype information for all selected entries in the *Genotyping report* window can be exported with **Entries > Export selected**. The results for the currently shown report can be exported with **Report > Export current**.

A Tab Separated Values (\*.tsv) file is created for each functionality and stored in the report export directory as specified in the genotyping settings. The location of the files is opened after the export.

The displayed report can be printed directly to a printer using **Report > Print....**

The dialog box that appears is the standard Windows Print dialog box, allowing you to choose a printer and change the properties. Depending on your system, this also allows printing to a PDF file to create an export of the displayed report. Please note that background colors, such as those in lists and mutational decision trees, may be lost in this step regardless of printer settings.



# Bibliography

- [1] C Titus Brown and Luiz Irber. sourmash: a library for minhash sketching of dna. *Journal of Open Source Software*, 1(5):27, 2016.
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