



BIONUMERICS Tutorial:

Resistance detection genotyping: predicting acquired resistance for Gram-negative bacteria

1 Aim

In this tutorial we will screen whole genome sequences of any *Gram-negative* bacterial samples for phenotypic antibiotic resistance traits using the *Resistance detection plugin*.

The different steps are illustrated using the whole genome demonstration database of *Escherichia coli*. This database is available for download on our website (see [2](#)) and contains 60 publicly available sequence read sets of *Escherichia coli* with already calculated de novo assemblies.

2 Preparing the database

2.1 Introduction to the demonstration database

We provide a **WGS demo database** for *Escherichia coli* containing sequence read set data links for 60 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_Escherichia_coli** can be downloaded directly from the *BIONUMERICS Startup* window (see [2.2](#)), or restored from the back-up file available on our website (see [2.2](#)).

Installation of the *Resistance detection 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 *Escherichia coli*, please check if your BIONUMERICS home directory does not contain any spaces:

1. Click on 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

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

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

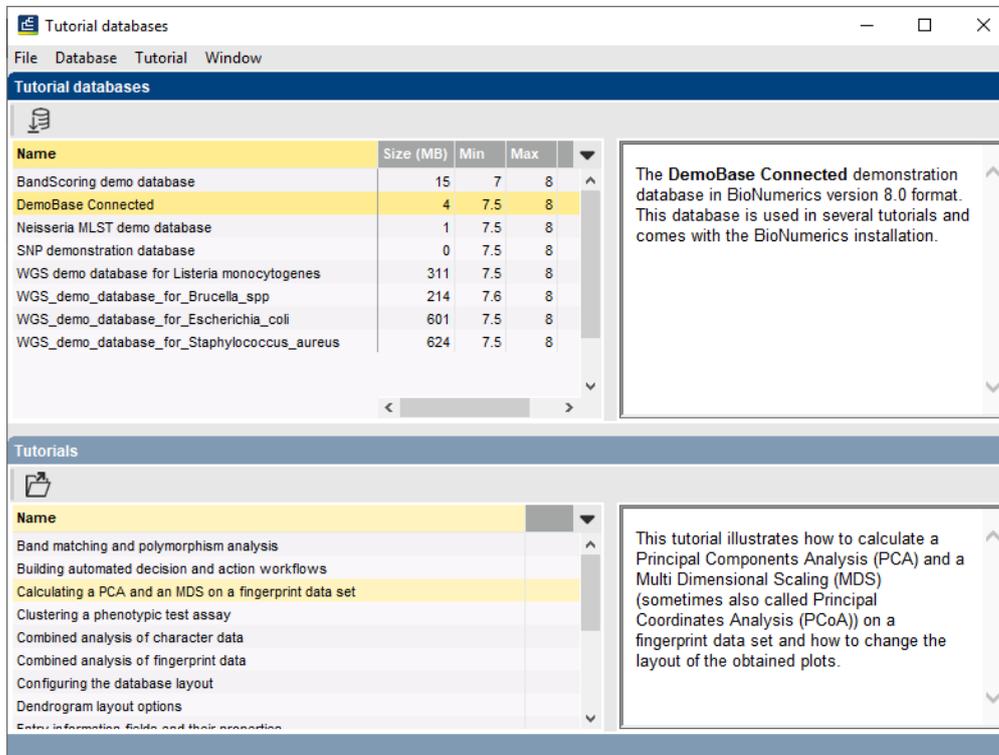


Figure 1: The *Tutorial databases* window, used to download the demonstration database.

- Select **WGS_demo_database_for_Escherichia_coli** from the list and select **Database** > **Download** (.
- Confirm the installation of the database and press <OK> after successful installation of the database.
- Close the *Tutorial databases* window with **File** > **Exit**.

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

- Double-click the **WGS_demo_database_for_Escherichia_coli** 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 *Escherichia coli* is also available on our website. This backup can be restored to a functional database in BIONUMERICS.

- Download the file WGS_EC.bnbk file from <https://www.bionumerics.com/download/sample-data>, under 'WGS_demo_database_for_Escherichia_coli'.



In contrast to other browsers, some versions of Internet Explorer rename the `WGS_EC.bmbk` database backup file into `WGS_EC.zip`. If this happens, you should manually remove the `.zip` file extension and replace with `.bmbk`. 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  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, e.g. “WGS_Escherichia_coli_demobase”.
12. Click **<OK>** to start restoring the database from the backup file.
13. Once the process is complete, click **<Yes>** to open the database.

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

Key	Organism	BioProject	BioSample	BioSampleModel	Isolation source	Center Name	Flags
EC_0000001	Escherichia coli	PRJNA11907	SAMN0455946	Environment	Pizza flour mix	CFSAN	SRF
EC_0000002	Escherichia coli	PRJNA11907	SAMN0448541	Environment	Food - Pizza Dough	CFSAN	SRF
EC_0000003	Escherichia coli	PRJNA11907	SAMN0448546	Environment	dry powder	CFSAN	SRF
EC_0000004	Escherichia coli	PRJNA11907	SAMN0455944	Environment	Pizza flour mix	CFSAN	SRF
EC_0000005	Escherichia coli	PRJNA23969	SAMN0445847	Environment	dry powder	CFSAN	SRF
EC_0000006	Escherichia coli	PRJNA23969	SAMN0426020	Environment	Flour	CFSAN	SRF
EC_0000007	Escherichia coli	PRJNA23969	SAMN0426921	Environment	Flour	CFSAN	SRF
EC_0000008	Escherichia coli	PRJNA28275	SAMN0517142	Environment	Beef Cubed SteakUSA	CFSAN	SRF
EC_0000009	Escherichia coli	PRJNA28275	SAMN0492707	Environment	Product-Raw-Intact-Beef	USDA-FSS	SRF
EC_0000010	Escherichia coli	PRJNA28206	SAMN0492766	Environment	Product-Raw-Intact-Beef	USDA-FSS	SRF
EC_0000011	Escherichia coli	PRJNA28206	SAMN0490488	Environment	Product-Raw-Intact-Beef	USDA-FSS	SRF
EC_0000012	Escherichia coli	PRJNA218110	SAMN0421199	Clinical	Unbleached White Flour	EBL-B-CDC	SRF
EC_0000013	Escherichia coli	PRJNA23969	SAMN0528973	Environment	Enriched White Flour	CFSAN	SRF
EC_0000014	Escherichia coli	PRJNA23969	SAMN0528404	Environment	Enriched White Flour	CFSAN	SRF
EC_0000015	Escherichia coli	PRJNA218110	SAMN0421116	Clinical	Enriched White Flour	EBL-B-CDC	SRF
EC_0000016	Escherichia coli	PRJNA23969	SAMN0524302	Environment	Unbleached White Flour	CFSAN	SRF
EC_0000017	Escherichia coli	PRJNA23969	SAMN0528977	Environment	Unbleached White Flour	CFSAN	SRF
EC_0000018	Escherichia coli	PRJNA23969	SAMN0521990	Environment	Unbleached White Flour	CFSAN	SRF
EC_0000019	Escherichia coli	PRJNA23969	SAMN0426924	Environment	Flour	CFSAN	SRF
EC_0000020	Escherichia coli	PRJNA23969	SAMN0426924	Environment	Flour	CFSAN	SRF
EC_0000021	Escherichia coli	PRJEB11514	SAMEA363259	Cattle	Cattle	UNIVERSITY OF ABE...	ERR
EC_0000022	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000023	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000024	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000025	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000026	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000027	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000028	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000029	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR
EC_0000030	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR
EC_0000031	Escherichia coli	PRJNA27984	SAMN04519374	Environment	Bos taurus-raw milk	CFSAN	SRF
EC_0000032	Escherichia coli	PRJNA27984	SAMN04519400	Environment	soil	CFSAN	SRF
EC_0000033	Escherichia coli	PRJNA27984	SAMN04519406	Environment	soil	CFSAN	SRF
EC_0000034	Escherichia coli	PRJNA27984	SAMN04519394	Environment	water	CFSAN	SRF
EC_0000035	Escherichia coli	PRJNA27984	SAMN04519376	Environment	Bos taurus-raw milk	CFSAN	SRF
EC_0000036	Escherichia coli	PRJNA29490	SAMN0449312	Clinical	Bovine feces	CFSAN	SRF
EC_0000037	Escherichia coli	PRJNA29490	SAMN0449312	Clinical	Bovine feces	CFSAN	SRF
EC_0000038	Escherichia coli	PRJNA23969	SAMN0449950	Environment	Bulk Flour	CFSAN	SRF
EC_0000039	Escherichia coli	PRJNA218110	SAMN0454544	Clinical	Shoal	EBL-B-CDC	SRF
EC_0000040	Escherichia coli	PRJNA28206	SAMN0492588	Environment	Comminuted Beef	USDA-FSS	SRF
EC_0000041	Escherichia coli	PRJNA28206	SAMN04621601	Environment	Comminuted Beef	USDA-FSS	SRF
EC_0000042	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR
EC_0000043	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR
EC_0000044	Escherichia coli	PRJEB11514	SAMEA363259	Clinical	Clinical	UNIVERSITY OF ABE...	ERR
EC_0000045	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR
EC_0000046	Escherichia coli	PRJEB11514	SAMEA363259	FoodEnvironment	FoodEnvironment	UNIVERSITY OF ABE...	ERR

Figure 2: The *Escherichia coli* demonstration database: the *Main* window.

3 About the demonstration database

The `WGS_demo_database_for_Escherichia_coli` contains data for a set of 60 samples. The sample information, stored in entry info fields (Isolation source, Center Name, etc.) was collected from the publications.

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

#	Name	Type
1	wgs	Sequence read set types
2	wgMLST	Character types
3	denovo	Sequence types
4	quality	Character types
5	wgs_TrimmedStats	Sequence read set types
6	wgMLST_CallTypes	Character types
7	wgsLong	Sequence read set types

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 *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).

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 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 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 resistance detection plugin

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

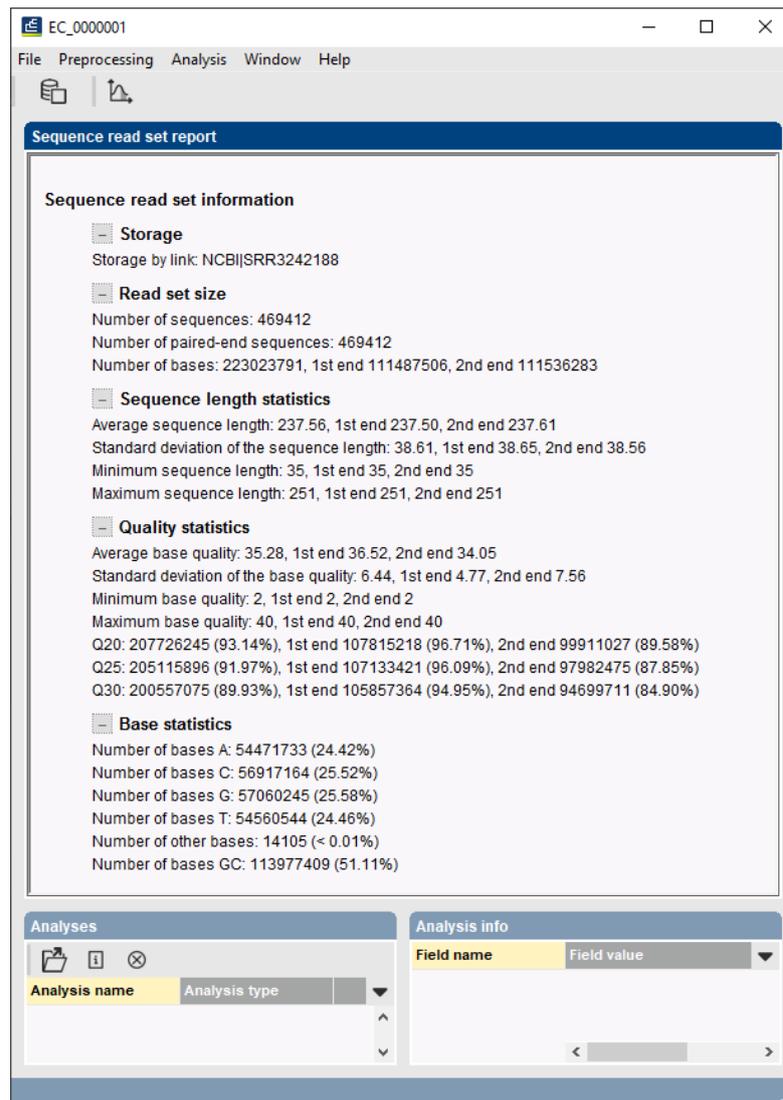


Figure 4: The sequence read set experiment card for an entry.

2. Select the *Resistance detection plugin* in the *Applications tab* and press the **<Activate>** button (see Figure 6).
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.

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

The *Resistance detection settings* dialog box pops up, consisting of 2 tabs (see Figure 7). 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.

The screenshot shows the 'Sequence Editor' window for 'EC_0000001'. The top section displays a DNA sequence with coordinates from 8550 to 9630. The middle section shows a genomic map with features like EC_17201, EC_746, EC_751, EC_752, EC_753, EC_754, EC_755, EC_756, EC_757, EC_758, EC_759, EC_760, EC_761, EC_762, EC_763, EC_764, EC_765, EC_766, EC_767, EC_768, EC_769, EC_770, EC_771, EC_772, EC_773, EC_774, EC_775, EC_776, EC_777, EC_778, EC_779, EC_780, EC_781, EC_782, EC_783, EC_784, EC_785, EC_786, EC_787, EC_788, EC_789, EC_790, EC_791, EC_792, EC_793, EC_794, EC_795, EC_796, EC_797, EC_798, EC_799, EC_800, EC_801, EC_802, EC_803, EC_804, EC_805, EC_806, EC_807, EC_808, EC_809, EC_810, EC_811, EC_812, EC_813, EC_814, EC_815, EC_816, EC_817, EC_818, EC_819, EC_820, EC_821, EC_822, EC_823, EC_824, EC_825, EC_826, EC_827, EC_828, EC_829, EC_830, EC_831, EC_832, EC_833, EC_834, EC_835, EC_836, EC_837, EC_838, EC_839, EC_840, EC_841, EC_842, EC_843, EC_844, EC_845, EC_846, EC_847, EC_848, EC_849, EC_850, EC_851, EC_852, EC_853, EC_854, EC_855, EC_856, EC_857, EC_858, EC_859, EC_860, EC_861, EC_862, EC_863, EC_864, EC_865, EC_866, EC_867, EC_868, EC_869, EC_870, EC_871, EC_872, EC_873, EC_874, EC_875, EC_876, EC_877, EC_878, EC_879, EC_880, EC_881, EC_882, EC_883, EC_884, EC_885, EC_886, EC_887, EC_888, EC_889, EC_890, EC_891, EC_892, EC_893, EC_894, EC_895, EC_896, EC_897, EC_898, EC_899, EC_900, EC_901, EC_902, EC_903, EC_904, EC_905, EC_906, EC_907, EC_908, EC_909, EC_910, EC_911, EC_912, EC_913, EC_914, EC_915, EC_916, EC_917, EC_918, EC_919, EC_920, EC_921, EC_922, EC_923, EC_924, EC_925, EC_926, EC_927, EC_928, EC_929, EC_930, EC_931, EC_932, EC_933, EC_934, EC_935, EC_936, EC_937, EC_938, EC_939, EC_940, EC_941, EC_942, EC_943, EC_944, EC_945, EC_946, EC_947, EC_948, EC_949, EC_950, EC_951, EC_952, EC_953, EC_954, EC_955, EC_956, EC_957, EC_958, EC_959, EC_960, EC_961, EC_962, EC_963, EC_964, EC_965, EC_966, EC_967, EC_968, EC_969, EC_970, EC_971, EC_972, EC_973, EC_974, EC_975, EC_976, EC_977, EC_978, EC_979, EC_980, EC_981, EC_982, EC_983, EC_984, EC_985, EC_986, EC_987, EC_988, EC_989, EC_990, EC_991, EC_992, EC_993, EC_994, EC_995, EC_996, EC_997, EC_998, EC_999, EC_1000.

The bottom section shows an 'Annotation' table with the following data:

Feature key	Start	End	Length	%GC
4	4267	6234	1968	52.01
5	6298	6888	591	50.17
6	7058	7822	765	50.52
7	7971	8279	309	50.32
8	8286	9455	1170	51.84
9	9648	10385	738	52.65
10	10385	10711	327	50.00
11	10837	11055	219	52.29
12				

The right side of the annotation window shows a translation of the selected feature (8286..9455):

```

/allele="10"
/locus_tag="EC_746"
/evidence=100.0
/notes="fwd=1;start=4050;stop=5220;cid=denovo_1"
/translation="MLELFLFLFPVAAAYGNMGRSRRAQNKODEANRLSRDYVAGWVFLLSNQDQKAVDLFLD
MLKEDTGTVEARHLTGNLFRSRGEVDRAIRIHQTMESASLTYEORLLAIQQLGRDYMAA
GLYDRAEDMFNQLTDEDFRIGALQQLLQIYQATSEWQKADVAERLVKLGDKORVEIA
HFYCELALQHMASDDLORAMLLKKGAAADKNSARVSIIMGRVFMKGEVAKVESLQV
ISQDRELVSLEMLQTCYQQLGHTAEWAEIFQRAVEENTGADAEMLLADIIEARDGSEA
AQVYITRQLQRHPTMRFVFKMLMDYHLEAEGRAKESLVLRDMVGEKVRSPRYRCQK
GFTAYTLWHPCFSCRAWSTIKPIRGLDGL"

```

Figure 5: The Sequence editor window.

The screenshot shows the 'Plugins' window with the 'Database Functionality' tab selected. The window contains a list of applications to activate, with 'Resistance detection' highlighted. The list includes:

- Antibiotics susceptibility
- BandScoring
- E. coli functional genotyping
- Custom Genotyping
- Listeria functional genotyping
- MTBC functional genotyping
- Resistance detection
- Salmonella functional genotyping
- S. aureus functional genotyping
- HIV Resistance
- MIRU-VNTR
- MLST online
- QIAxcel
- RDP

There is a checkbox for 'Show only activated' and a 'Show Manual' button. At the bottom, there are 'Activate' and 'Exit' buttons.

Figure 6: Install plugin.

- **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.

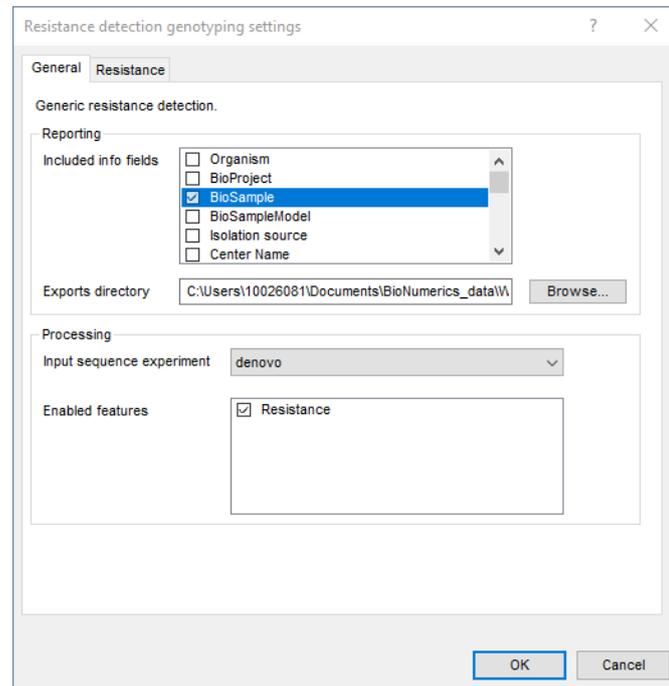


Figure 7: The *Resistance detection settings* dialog box: *General* tab.

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 **BioSample** to include in the report (see Figure 7).

The other tab groups the settings for the search of acquired resistance traits. It contains 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.
6. In this tutorial, specify the experiment types and information fields in the *Resistance* tab by selecting the <**Create**> option in the drop-down lists and accepting the default names. Leave the other settings unaltered.

7. Click on <**OK**> in the *Resistance detection settings* dialog box.
8. When the *Resistance Detection plugin* is successfully installed, a confirmation message pops up. Press <**OK**>.
9. Press <**Exit**> to close the *Plugins* dialog box.
10. Close and reopen the database to activate the features of the *Resistance Detection plugin*.

The *Resistance Detection genotyping plugin* installs menu items in the main menu of the software under **Resistance detection** (see Figure 8).

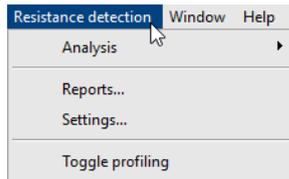


Figure 8: New menu-items after installation of the *Resistance Detection plugin*.



The settings specified during installation of the plugin can be called again at any time with **Resistance detection > 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 acquired resistance can either be done for all tools checked in the *Resistance detection settings* dialog box (using **Resistance detection > Analysis > All Enabled**) or by **Resistance detection > Analysis > Resistance**.

4. Select **Resistance detection > 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 character experiment types for **Resistance** are created and updated with the predicted acquired resistance 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 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 9):

- **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.

Character	Value	Mapping
Unknown Beta-lactam	0	<->
linezolid	0	<->
Netilmicin	0	<->
Telithromycin	0	<->
tetracycline	0	<->
neomycin	0	<->
Lincomycin	0	<->
Streptomycin	0	<->
Unknown Fluoroquin...	1	<->
Butirosin	0	<->
Ampicillin	0	<->
clindamycin	0	<->

Character	Value	Mapping
vgbA	0	<->
vgb(B)	0	<->
vgb(A)	0	<->
vgaB	0	<->
vgaA	0	<->
vga(E)	0	<->
vga(D)	0	<->
vga(C)	0	<->
vga(B)	0	<->
vga(A)V	0	<->
vga(A)LC	0	<->
vga(A)	0	<->

Figure 9: Example output of the **Resistance_traits** and the **Resistance_loci** experiment types for sample EC_0000012.

6. Close the character card(s).

6 Reports

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

The *Report* window contains a genotype report for each of the selected entries (see Figure 10).

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 *Resistance detection 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 11) 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** screening 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 12).

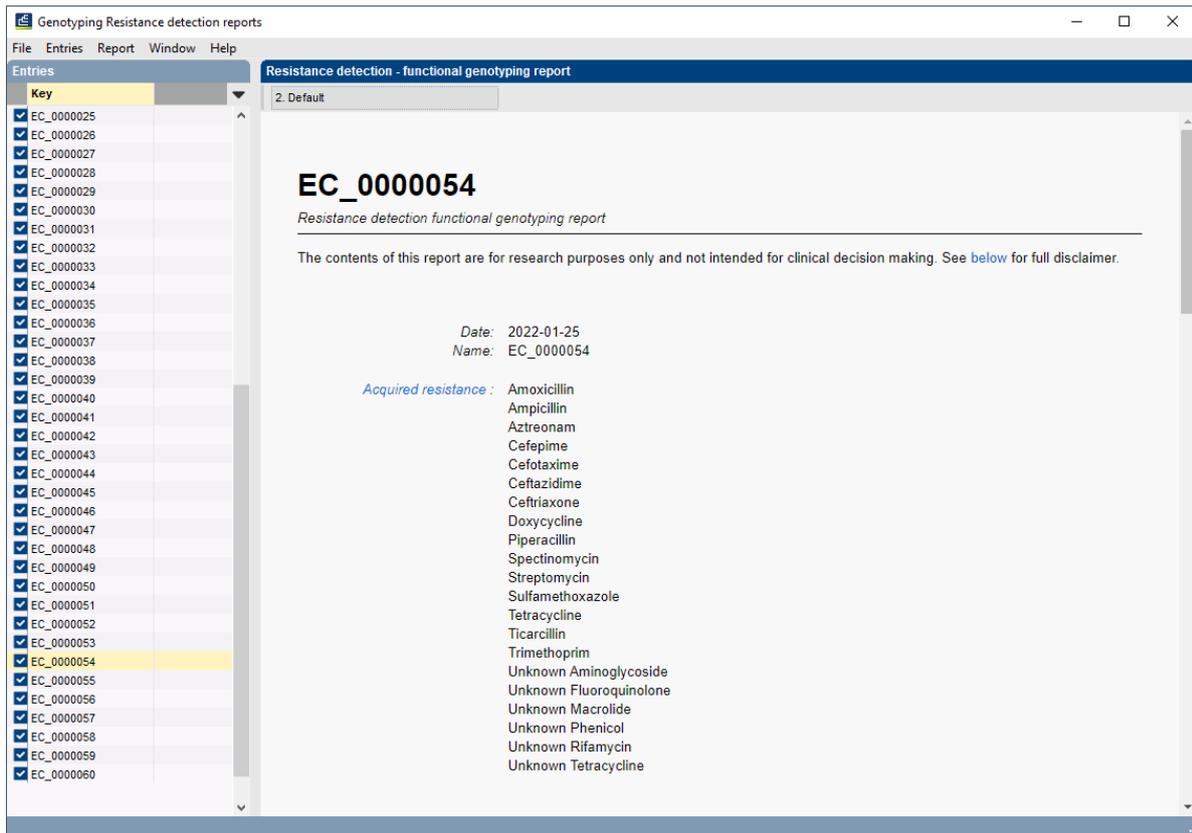


Figure 10: Example of a resistance detection report.

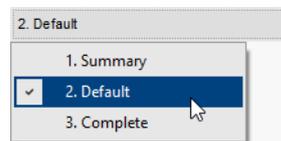


Figure 11: Report styles in the *Report window*.

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

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

Resistance

Acquired resistance results

Trait	Locus	Coverage (%)	Identity (%)	Position	Accession
Unknown Macrolide	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Unknown Aminoglycoside	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Unknown Tetracycline	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Unknown Fluoroquinolone	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Unknown Phenicol	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Unknown Rifamycin	mdf(A)	100.00	97.65	2391302..2390070	Y08743
Trimethoprim	dfrA17	100.00	100.00	255479..255952	FJ460238
Spectinomycin	aadA5	100.00	100.00	256083..256871	AF137361
Streptomycin	aadA5	100.00	100.00	256083..256871	AF137361
Streptomycin	aph(3")-Ib	100.00	100.00	3414705..3415508	AF321551
Streptomycin	aph(6)-IId	100.00	100.00	3415514..3416344	CP000971
Sulfamethoxazole	sul1	100.00	100.00	257418..258257	U12338
Sulfamethoxazole	sul2	100.00	100.00	3413829..3414642, 3414681..3414718	FJ197818
Amoxicillin	blaCTX-M-27	100.00	100.00	3686247..3685372	AY156923
Ampicillin	blaCTX-M-27	100.00	100.00	3686247..3685372	AY156923
Aztreonam	blaCTX-M-27	100.00	100.00	3686247..3685372	AY156923
Cefepime	blaCTX-M-27	100.00	100.00	3686247..3685372	AY156923

Figure 12: Report details.