



BIONUMERICS Tutorial:

***Mycobacterium tuberculosis* complex functional genotyping: predicting phenotypic traits from whole genome sequences**

1 Aim

In this tutorial we will screen whole genome sequences of *Mycobacterium tuberculosis* complex samples for phenotypic traits such as spoligotype, lineage and antibiotic resistance using the *Mycobacterium tuberculosis* complex functional genotyping plugin. The plugin also allows you to perform species confirmation.

The different steps are illustrated using the whole genome demonstration database of the *Mycobacterium tuberculosis* complex. This database is available for download on our website (see [2](#)) and contains 29 publicly available sequence read sets of the *Mycobacterium tuberculosis* complex with already calculated de novo assemblies.

2 Preparing the database

2.1 Introduction to the demonstration database

We provide a **WGS demo database** for the *Mycobacterium tuberculosis* complex containing sequence read set data links for 29 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 the *Mycobacterium tuberculosis* complex 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 *Mycobacterium tuberculosis* complex 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 the *Mycobacterium tuberculosis* complex, 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.

- 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).

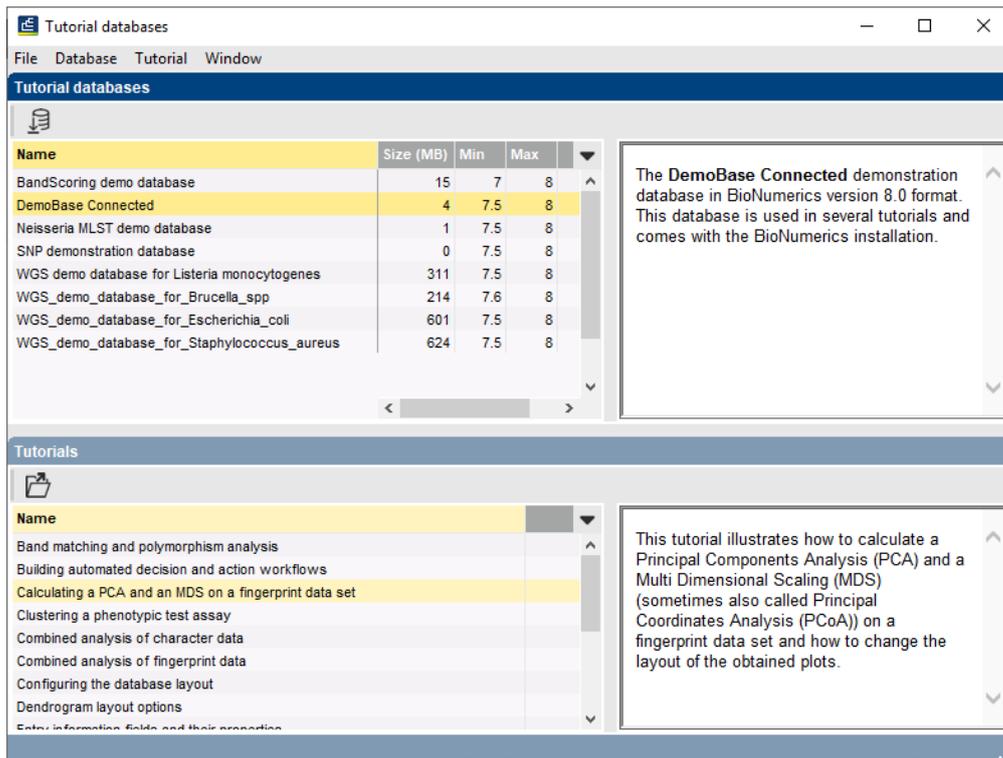


Figure 1: The *Tutorial databases* window, used to download the demonstration database.

- Select **WGS_demo_database_for_MTBC** 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_MTBC** appears in the *BIONUMERICS Startup* window.

- Double-click the **WGS_demo_database_for_MTBC** 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 the *Mycobacterium tuberculosis* complex is also available on our website. This backup can be restored to a functional database in

BIONUMERIC.S.

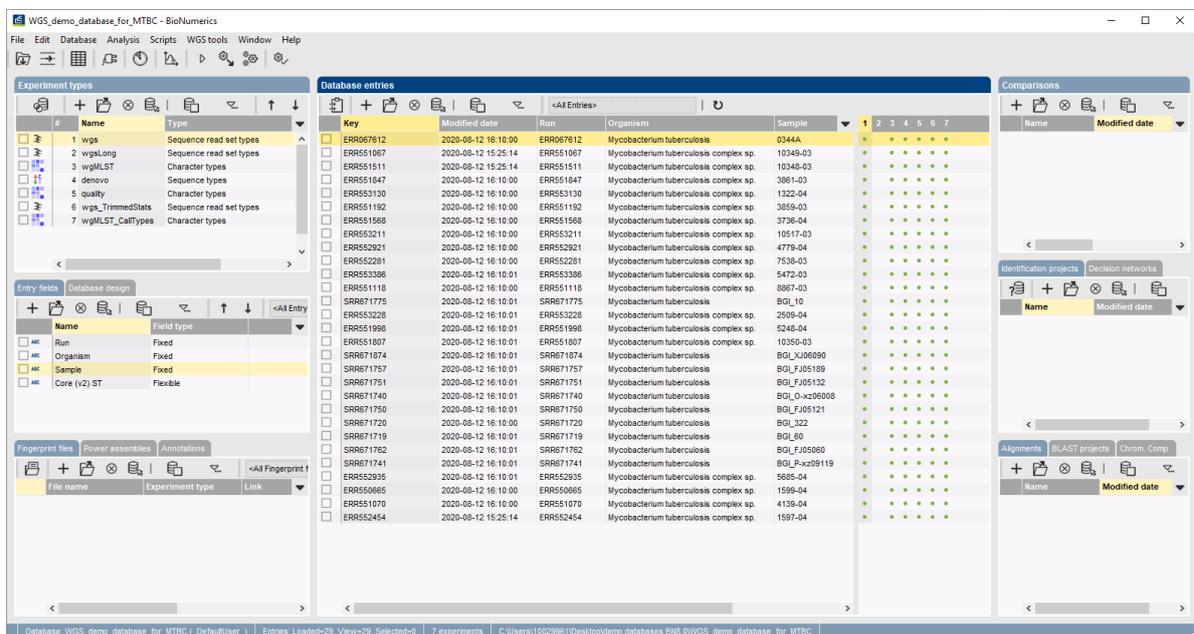
- Download the file WGS_MTBC.bnbk file from <https://www.applied-maths.com/download/sample-data>, under 'WGS_demo_database_for_MTBC'.



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

- In the *BIONUMERIC.S* Startup window, press the  button. From the menu that appears, select **Restore database...**
- Browse for the downloaded file and select **Create copy**. Note that, if **Overwrite** is selected, an existing database will be overwritten.
- Specify a new name for this demonstration database, e.g. "WGS_MTBC_demobase".
- Click **<OK>** to start restoring the database from the backup file.
- Once the process is complete, click **<Yes>** to open the database.

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



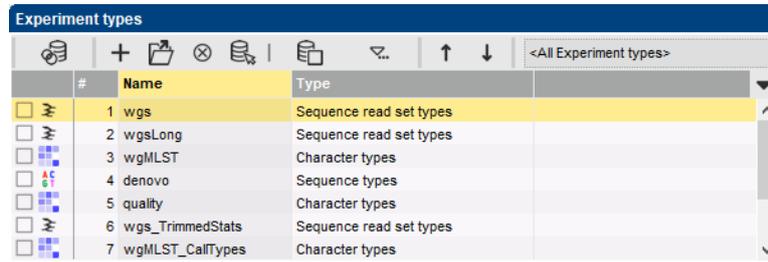
Key	Modified date	Run	Organism	Sample
ERR067612	2020-08-12 16:10:00	ERR067612	Mycobacterium tuberculosis	0344A
ERR51067	2020-08-12 15:25:14	ERR51067	Mycobacterium tuberculosis complex sp.	10348-03
ERR51511	2020-08-12 15:25:14	ERR51511	Mycobacterium tuberculosis complex sp.	10348-03
ERR51167	2020-08-12 16:10:00	ERR51167	Mycobacterium tuberculosis complex sp.	3061-03
ERR55330	2020-08-12 16:10:00	ERR55330	Mycobacterium tuberculosis complex sp.	1322-04
ERR51192	2020-08-12 16:10:00	ERR51192	Mycobacterium tuberculosis complex sp.	3859-03
ERR51568	2020-08-12 16:10:00	ERR51568	Mycobacterium tuberculosis complex sp.	3736-04
ERR53211	2020-08-12 16:10:00	ERR53211	Mycobacterium tuberculosis complex sp.	10517-03
ERR52921	2020-08-12 16:10:00	ERR52921	Mycobacterium tuberculosis complex sp.	4779-04
ERR52281	2020-08-12 16:10:00	ERR52281	Mycobacterium tuberculosis complex sp.	7530-03
ERR53396	2020-08-12 16:10:01	ERR53396	Mycobacterium tuberculosis complex sp.	5472-03
ERR51118	2020-08-12 16:10:01	ERR51118	Mycobacterium tuberculosis complex sp.	8067-03
SR0671775	2020-08-12 16:10:01	SR0671775	Mycobacterium tuberculosis	BGI_10
ERR53228	2020-08-12 16:10:01	ERR53228	Mycobacterium tuberculosis complex sp.	2509-04
ERR51998	2020-08-12 16:10:01	ERR51998	Mycobacterium tuberculosis complex sp.	5248-04
ERR51807	2020-08-12 16:10:01	ERR51807	Mycobacterium tuberculosis complex sp.	10350-03
SR0671874	2020-08-12 16:10:01	SR0671874	Mycobacterium tuberculosis	BGI_X00699
SR0671757	2020-08-12 16:10:01	SR0671757	Mycobacterium tuberculosis	BGI_F105189
SR0671751	2020-08-12 16:10:01	SR0671751	Mycobacterium tuberculosis	BGI_F105132
SR0671740	2020-08-12 16:10:01	SR0671740	Mycobacterium tuberculosis	BGI_O_x206008
SR0671750	2020-08-12 16:10:01	SR0671750	Mycobacterium tuberculosis	BGI_F105121
SR0671720	2020-08-12 16:10:00	SR0671720	Mycobacterium tuberculosis	BGI_322
SR0671719	2020-08-12 16:10:01	SR0671719	Mycobacterium tuberculosis	BGI_00
SR0671762	2020-08-12 16:10:01	SR0671762	Mycobacterium tuberculosis	BGI_F105060
SR0671741	2020-08-12 16:10:01	SR0671741	Mycobacterium tuberculosis	BGI_P_x2059119
ERR52935	2020-08-12 16:10:01	ERR52935	Mycobacterium tuberculosis complex sp.	5685-04
ERR50665	2020-08-12 16:10:00	ERR50665	Mycobacterium tuberculosis complex sp.	1599-04
ERR51070	2020-08-12 16:10:00	ERR51070	Mycobacterium tuberculosis complex sp.	4139-04
ERR52454	2020-08-12 15:25:14	ERR52454	Mycobacterium tuberculosis complex sp.	1597-04

Figure 2: The MTBC 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 29 publicly available sequencing runs. Additional information (in entry info fields Sample and Organism) was collected from NCBI 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).



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

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 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.

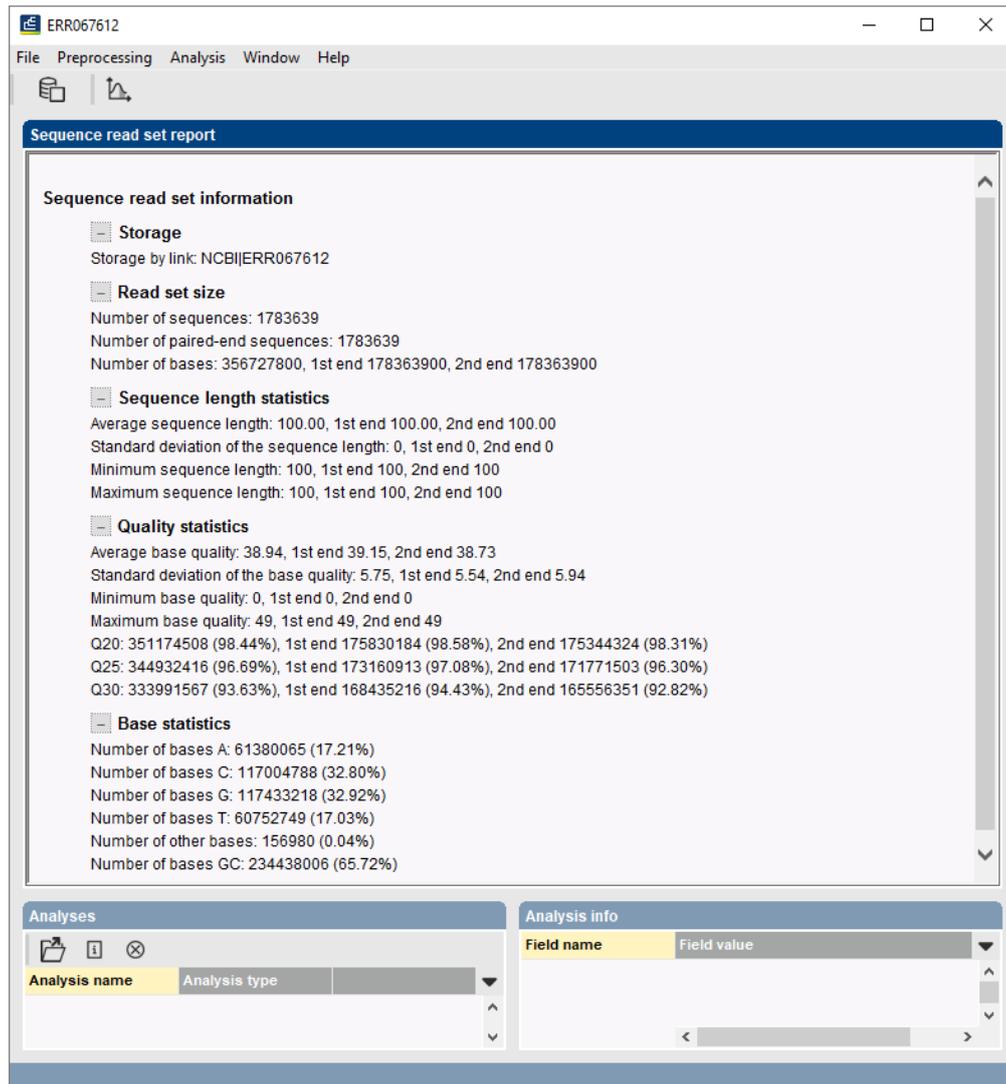


Figure 4: The sequence read set experiment card for an entry.

4 Installing the MTBC functional genotyping plugin

To store the results of your genotyping jobs, you will have to create some new information fields and character/sequence experiments.

1. Make sure the *Database entries* panel is the active panel in the *Main* window and select **Edit** > **Information fields** > **Add information field...**
2. Enter a name, e.g. **Species confirmation** and choose **Space** next to **Optimize storage for** (see Figure 6). The latter is important because otherwise results can get truncated if they contain too much characters. All other settings can be left default.
3. Repeat previous step for following information fields. Make sure you optimize the storage for **Space** each time:

- Unknown genotype
- Resistance summary
- Spoligotype (octal)

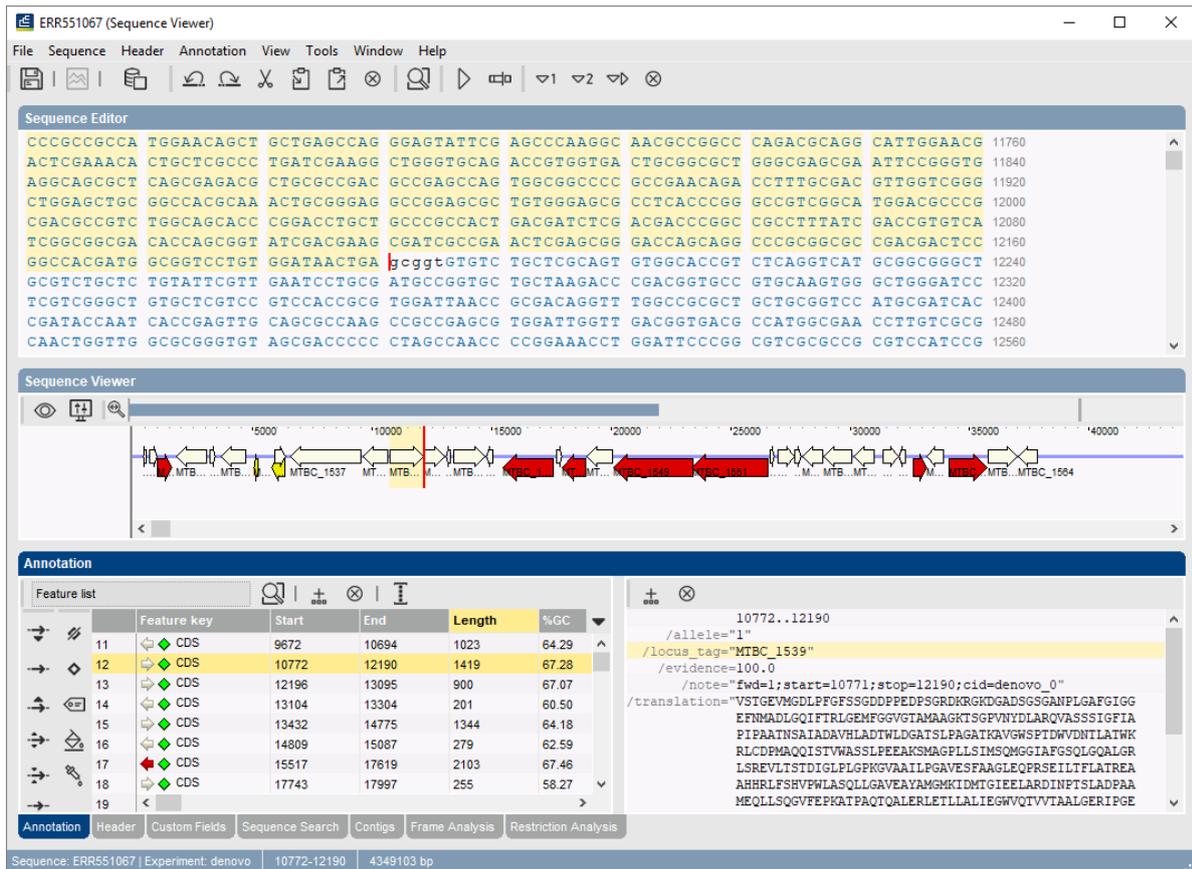


Figure 5: The *Sequence editor* window.

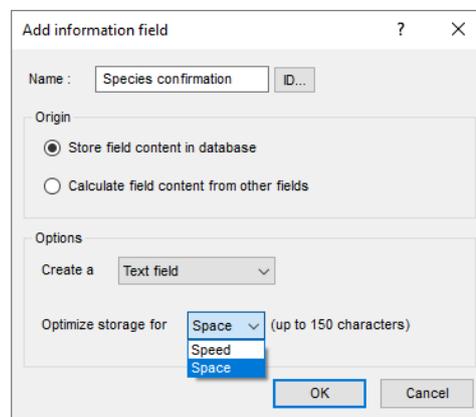


Figure 6: Adding a new information field with the **Space** option.

- Lineage number
- Lineage name

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

4. Call the *Plugins* dialog box from the *Main* window with **File > Install / remove plugins...** (🔧).
5. Select *MTBC functional genotyping* from the list in the *Applications* tab and press the **<Activate>** button.
6. Confirm the installation of the plugin.

7. In the *General tab* of the wizard (see Figure 7) choose **wgs** as **WGS experiment type** and the **Info fields** that will appear in the report. The **Key** and the **Resistance/Lineage** results are default shown in the report. Check for example the **Species confirmation** and **Spoligotype (octal)** information field or any other field that was added. Click **<Next>**.

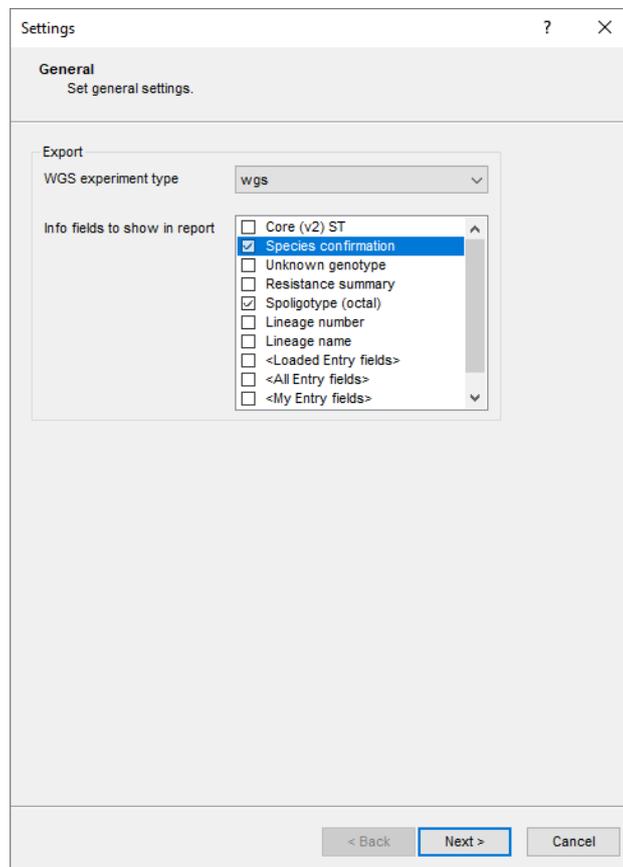


Figure 7: The *General tab*.

8. In the *Species tab* of the wizard choose the **Species field** for the storage of the species confirmation results, change the detection parameters if wanted and click **<Next>** (see Figure 8).
9. In the *Lineage tab* choose the fields for the storage of the **Lineage number** and **Lineage name**, change the detection parameters if wanted and click **<Next>** (see Figure 9).
10. In the *Spoligotyping tab* select **Create New** as **Spoligo presence/absence experiment type**. Choose the field for the storage of the **Octal spoligotype**. Modify the detection parameters if wanted and click **<Next>** (see Figure 10).
11. If **Create new** was selected in the previous step, a dialog box appears prompting for the **Spoligotype absence/present** experiment type name. Enter e.g. **Spoligotype** and click **<OK>**.
12. In the *Resistance tab* (see Figure 11), select the **Resistance database**. Currently, only one database is available: **Curated database (version 6.0)**.
13. Select **Create new** next to the three **Resistance results** experiment types (see Figure 11).
14. Choose the **Resistance summary** information field for the storage of the antibiotics for which known resistance-related mutations (or indels) were found.
15. Choose the **Unknown genotype** information field for the storage of the antibiotics for which unknown mutations or indels (which are not included in the resistance database) were found in known resistance-related genes.

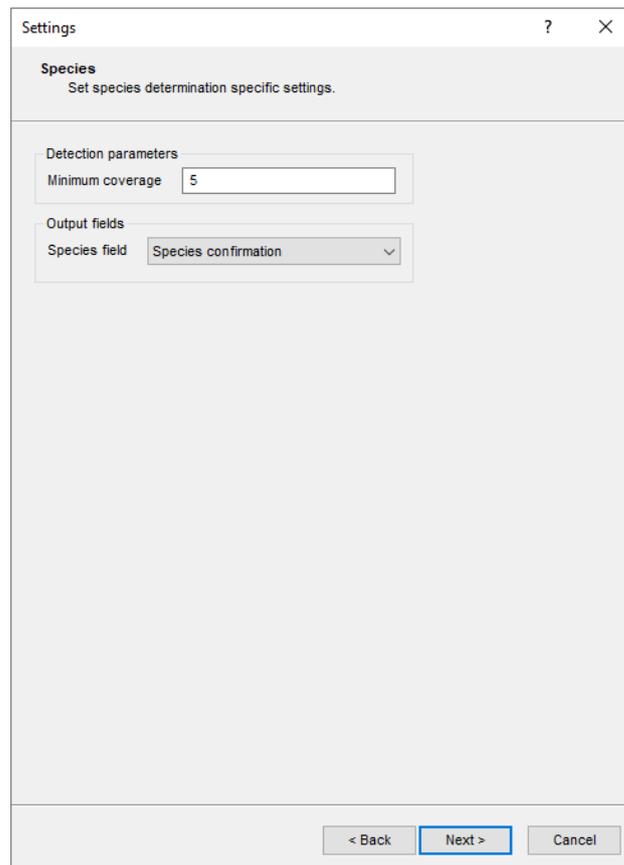


Figure 8: The *Species* tab.

16. Check ***Detect genomic variants*** if you want to store all the mutations in resistance-related genes in an experiment type. Select ***Create new*** or select an existing experiment from the ***Nucleotide variants*** and ***Amino acid variants*** experiment type lists.
17. Click ***<Finish>***.
18. If ***Create new*** was selected in the previous step, a dialog box appears prompting for the experiment type name(s). Enter the name(s) and click ***<OK>***.

When the *MTBC functional genotyping* installation is complete, you will be prompted to restart the database. The *Plugins* dialog box can be closed by pressing the ***<Exit>*** button and the database via ***File > Exit***.

Open the database again from the *BIONUMERICIS Startup* window. A ***MTBC*** menu item is now available in the *Main* window (see Figure 12).

5 Screening of entries

5.1 Submitting jobs

MTBC jobs need to be submitted to the calculation engine. The Calculation engine option requires credits for running jobs on the Applied Maths cloud calculation engine. Credits are linked to credentials that you need to enter when installing the WGS tools plugin. In our demo database, the WGS tools plugin is installed but no credits are assigned to the demo project so no MTBC jobs

Figure 9: The *Lineage* tab.

can be performed on the external calculation engine. Please contact Applied Maths to obtain more information.

Once the *MTBC genotyping plugin* is installed and the settings have been specified, an MTBC genotyping job (spoligotyping, resistance/lineage prediction and species prediction) can be submitted.

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.
2. In order to select a group of entries, hold the **Shift**-key and click on another entry.
3. Select **WGS tools** > **Submit jobs...** (▶) to open the *Submit jobs* dialog box.

From the *Submit jobs* dialog box, one can define which algorithms need to be run on the selected samples and as such, define and launch the related jobs on the calculation engine.

4. Select **Calculation Engine**.
5. Check the box next to **MTBC genotyping** (see Figure 13). Note that this option is only available after successful installation of the plugin and closing and reopening of the database after installation.

At this stage, you can also still change the settings by clicking the **<Settings>** button next to **MTBC genotyping**.

6. Uncheck all other boxes if you do not want to perform any additional analyses (e.g. wgMLST).

Figure 10: The *Spoligotyping* tab.

Jobs that already have been submitted and have been imported successfully, will not be re-launched for analysis, unless the check box in front of ***Re-submit already processed data*** in the ***Jobs*** part is checked.

By default, the *Job overview* window will be opened after submission of the jobs. However, this can be changed by unchecking the option ***Open jobs overview window***.

To analyze one sample with the MTBC genotyping tool, you need 2 credits. The number of credits required to run the selected jobs for the selected entries can be consulted at the bottom of the *Submit jobs* dialog box.

7. Click **<OK>** to launch the MTBC genotyping jobs and open the *Job overview* window for the calculation engine (see Figure 14).

In the *Job overview* window you can see the status of the submitted jobs. The *Job overview* window can be opened from the *Main* window with ***File > Jobs overview...*** (⚙️).

8. Finished jobs can be imported with a manual action (***Jobs > Get results*** (⚙️)) or through an automatic update: select ***File > Settings***, check both options and specify an interval (e.g. 10 min).

The job results can also be imported starting from the entry selection in the *Main* window:

9. Make an entry selection in the *Database entries* panel and select ***WGS tools > Get results*** (⚙️).



The job log files are saved in the *Job log* panel of the *Entry* window. Double-click on an entry in the *Database entries* panel to open the *Entry* window and to consult this information.

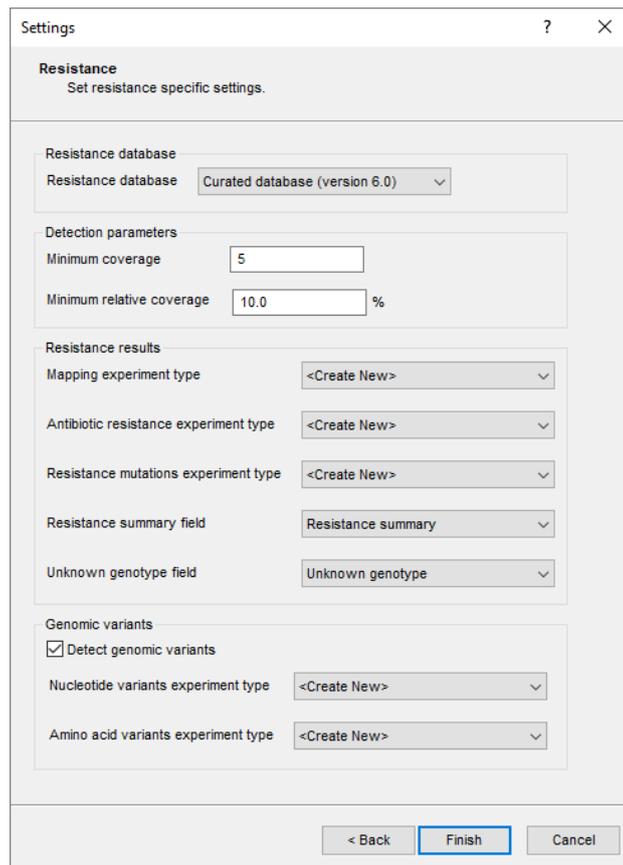


Figure 11: The *Resistance* tab.

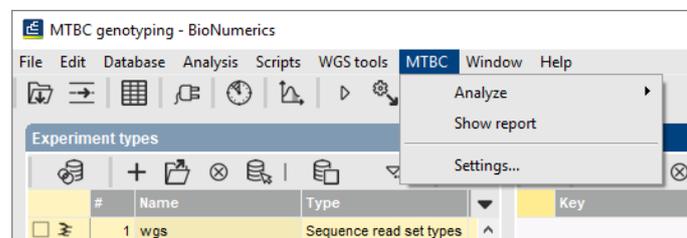


Figure 12: New menu items after installation of the plugin.

Once the results are imported, the corresponding jobs and their underlying data sets are automatically deleted from the calculation engine and as such, from the *Job overview* window.

5.2 Local screening

If you want to redo the lineage or resistance prediction of your samples with other settings, you do not need to submit a new job to the CE. This means that no additional credits will be charged:

1. Make new information fields to store the new lineage or resistance results (optional step).
2. Open the *Settings dialog box* with **MTBC** > **Settings...** in the *Main* window.
3. Select the new information fields and/or create new character experiments to store the new resistance results (optional step).

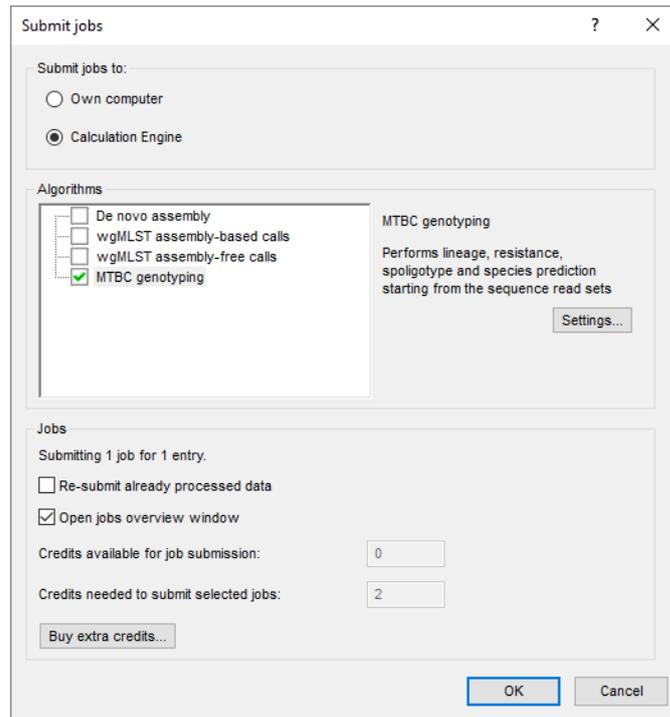


Figure 13: Submitting jobs to the calculation engine.

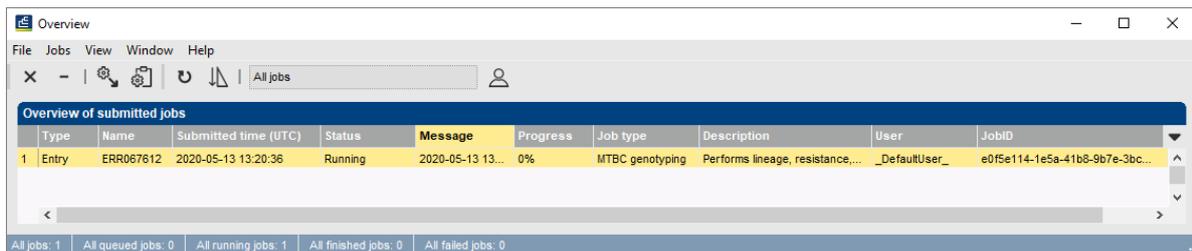


Figure 14: Job overview.

4. Select other settings.
5. Resistance and lineage prediction can be re-analyzed locally by clicking in the *Main* window on **MTBC > Analyze > Resistance** or **MTBC > Analyze > Lineage** respectively. If you want to redo both analysis, select **MTBC > Analyze > All enabled**.



The options **MTBC > Analyze > Resistance/lineage/all enabled** can only be used when there is already a mapping present for the selected entries (this will only be the case if you have already submitted an MTBC genotyping job for this sample to the CE). Please note that only the mapping generated by the MTBC genotyping plugin can be used and not the standard mapping.



If you did not create/select new experiment types in the settings, results in the current experiment types will be overwritten when you re-analyze your samples.



If you click on **MTBC > Analyze > Resistance**, and you also selected new, empty fields for lineage results, the lineage results obtained with the original settings will be used. If you also want to analyze the lineage with new settings, you have to click on **MTBC > Analyze > Lineage** or **MTBC > Analyze > All enabled**.

5.3 Results

The **Species**, **Lineage**, **Spoligotyping** and **Resistance** results are written to the information fields in the *Database entries* panel (see Figure 15). Please note that the shown names of the information fields can be different in your case depending on whether you choose an alternative name during installation.

An additional information field called **MDR_or_XDR** is generated automatically during the first import of results (see Figure 15). MDR: multidrug resistance is defined as predicted resistance to INH and RMP. XDR: extensively drug resistance is defined as predicted resistance to INH, RMP, (CAP, KAN or AMK) and 1 of the FQ (Moxifloxacin, Ofloxacin, Levofloxacin or fluoroquinolone in general). No: not MDR or XDR.

Key	Species confirmation	Lineage number	Lineage name	Spoligotype (octal)	Resistance summary	Unknown genotype	MDR_or_XDR
<input type="checkbox"/> ERR067612	MTBC complex	4 / 4.3 / 4.3.3	Euro-American ...	777777663760771	EMB / PZA / RMP / SM	CAP / FQ / INH / LFX / ...	No
<input type="checkbox"/> ERR551067	MTBC complex	4 / 4.1	Euro-American	70037777760771	EMB / INH / SM	ETH / FQ / LFX / MFL / ...	No

Figure 15: Example output of the Species, Lineage, Spoligotyping and Resistance information fields.

The character experiment types for **Spoligotype**, **Resistance** and **Genomic variants** are created and updated with the predicted traits. Please note that the shown names of the experiment types can be different in your case depending on whether you choose an alternative name during installation.

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

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

Spoligo presence/absence experiment type: character experiment in which the binary code (for each of the 43 spacers: absence or presence) is stored (see Figure 16 and Figure 17).

Antibiotic resistance experiment type: character experiment containing all antibiotics from the selected resistance KB (see Figure 18).

The summary resistance call for an antibiotic can be (see Figure 19):

- Resistant (R; -5):** at least 1 known resistance-related mutation or indel from the selected resistance KB was found with sufficient coverage.
- Unknown (U; -3):** there are no known resistance-related mutations from the selected resistance KB found with sufficient coverage but there is at least 1 unknown mutation or indel found in a resistance-related gene. For positions already included in the resistance KB, every (non-synonymous) mutation or indel with sufficient coverage (as defined in the settings) leads to the 'unknown' status. For positions in resistance-related genes not yet included in the resistance KB, only majority/consensus mutations with more than 75% relative coverage lead to the 'unknown' status. Lastly, if no bases are covered in resistance related genes for a particular antibiotic, the outcome is also 'unknown'.
- Failed (F; -2):** no known or unknown mutations with sufficient coverage were found but also not all positions in the resistance-related genes were covered.
- Susceptible (S; +1):** there are no known or unknown mutations found and all bases of the resistance-related genes were covered sufficiently.

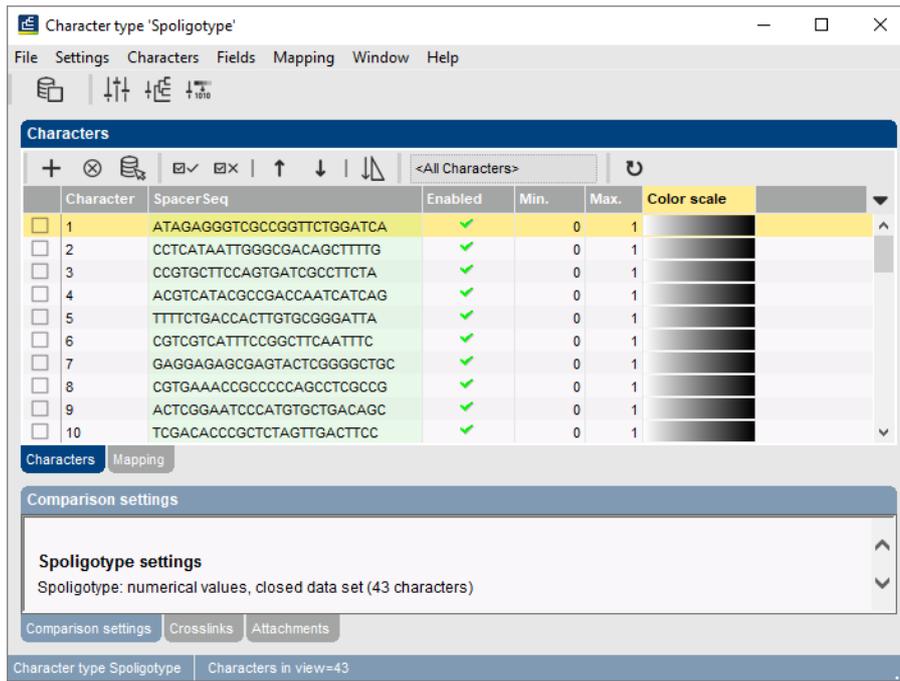


Figure 16: Spoligo presence/absence experiment type. This experiment contains 43 spacer sequences.



Figure 17: Example output of spoligotyping (spoligo presence/absence experiment type) for a sample with spoligotype 00000000003771. White = spacer is absent, black = spacer is present.

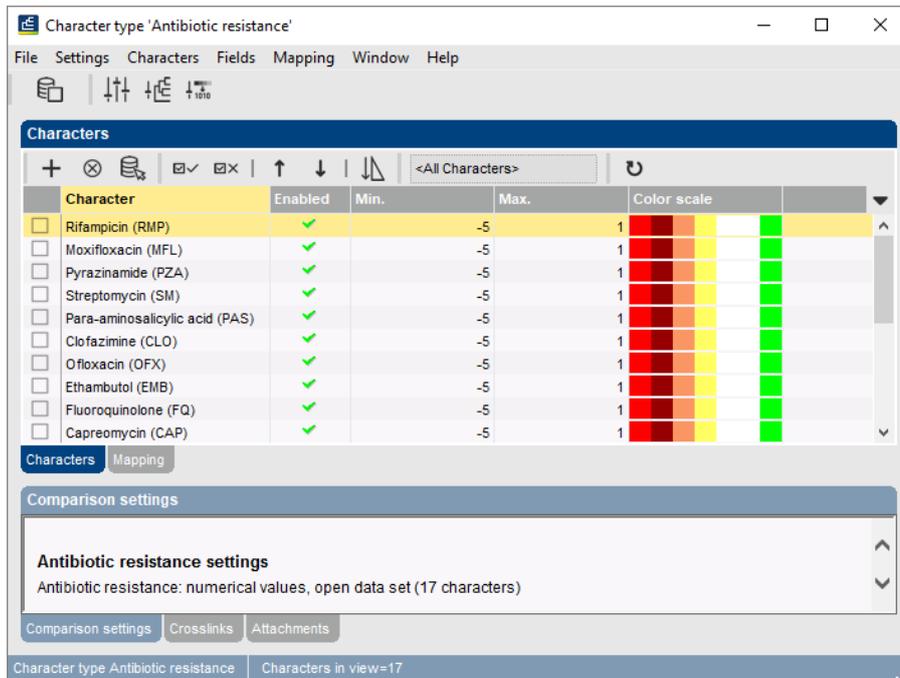


Figure 18: Antibiotic resistance experiment type.

Character	Value	Mapping
Rifampicin (RMP)	-5	R
Moxifloxacin (MFL)	-3	U
Pyrazinamide (PZA)	-5	R
Streptomycin (SM)	-5	R
Para-aminosalicylic ...	-3	U
Clofazimine (CLO)	1	S
Ofloxacin (OFX)	-3	U
Ethambutol (EMB)	-5	R
Fluoroquinolone (FQ)	-3	U
Capreomycin (CAP)	-3	U
Levofloxacin (LFX)	-3	U
Bedaquiline (BED)	1	S
Isoniazid (INH)	-3	U
Amikacin (AMK)	1	S
Kanamycin (KAN)	1	S
Ethionamide (ETH)	1	S
Linezolid (LZD)	1	S

Figure 19: Example output of antibiotic resistance experiment type for sample ERR067612. U = Unknown, R = Resistant, F = Failed, S = Susceptible.

Nucleotide variants experiment type: Character experiment that contains all nucleotide mutations in resistance-related genes that have sufficient coverage. These include (new) mutations which are not in the selected resistance KB (see Figure 20).

Character	Enabled	Min.	Max.	Color scale	LocusName	Gene
2288850	✓	0	100		Rv2043c	pncA
2155802	✓	0	100		Rv1908c	katG
775639	✓	0	100		Rv0676c	mmpL5
759615	✓	0	100		(rpoB_promoter)	rpoB_promoter
4247730	✓	0	100		Rv3795	embB
4242643	✓	0	100		Rv3793	embC
1917972	✓	0	100		Rv1694	tlyA
2518919	✓	0	100		Rv2245	kasA
3073868	✓	0	100		Rv2764c	thyA
764995	✓	0	100		Rv0668	rpoC
761101	✓	0	100		Rv0667	rpoB
762310	✓	0	100		Rv0667	rpoB
576744	✓	0	100		Rv0486	mshA
1834836	✓	0	100		Rv1630	rpsA

Figure 20: Nucleotide variants experiment type. Character: genomic position (H37Rv numbering).

The resistance call for a resistance-related position can be (Figure 21):

- A/C/T/G: nucleotide substitution.
- Multiple variants: there are multiple bases with sufficient coverage.

- Insertion: an insertion is present at this position.
- - : a deletion is present at this position.

Character	Value	Mapping
2288850	2000	Insertion
2155802	5	T
775639	4	G
759615	3	C
4247730	2	A
4242643	5	T
1917972	4	G
2518919	2	A
3073868	4	G
764995	4	G
761101	3	C
762310	4	G
576744	4	G
1834836	3	C
4408156	4	G
4408102	3	C
4407720	4	G
2726350		

Figure 21: Example output of nucleotide variants experiment type for sample ERR067612.

Amino acid variants experiment type: Character experiment that contains all amino acid mutations in resistance-related genes that have sufficient coverage. These include (new) mutations which are not in the selected resistance KB.

Character	Enabled	Min.	Max.	Color scale	LocusName	Gene
2288850	✓	0	100		Rv2043c	pncA
2155802	✓	0	100		Rv1908c	katG
775639	✓	0	100		Rv0676c	mmpL5
759615	✓	0	100		(rpoB_promoter)	rpoB_promoter
4247730	✓	0	100		Rv3795	embB
4242643	✓	0	100		Rv3793	embC
1917972	✓	0	100		Rv1694	tlyA
2518919	✓	0	100		Rv2245	kasA
3073868	✓	0	100		Rv2764c	tlyA
764995	✓	0	100		Rv0668	rpoC
761101	✓	0	100		Rv0667	rpoB
762310	✓	0	100		Rv0667	rpoB
576744	✓	0	100		Rv0486	mshA
1834836	✓	0	100		Rv1630	rpsA

AA_variants settings
AA_variants: numerical values, open data set (11954 characters)

Figure 22: Aminoacid variants experiment type. Character: genomic position (H37Rv numbering).

The resistance call for a resistance-related position can be:

- An amino acid (one letter abbreviation).
- Multiple variants: there are multiple bases with sufficient coverage.
- Insertion: an insertion is present at this position.
- - : a deletion is present at this position.

Character	Value	Mapping
2288850	2000	Insertion
2155802	118	W
775639	120	V
4247730	104	D
4242643	102	R
1917972	111	L
2518919	116	S
3073868	101	A
764995	101	A
761101	115	P
762310	102	R
576744	108	G
1834836	117	T
4408156	102	R
4408102	101	A
4407720	101	A
2726350	111	L
7362		

Figure 23: Example output of amino acid variants experiment type for sample ERR067612.

11. Close the character card(s) and click on the green colored dot corresponding to the Mapping experiment.

Mapping experiment type: Sequence experiment in which the mapped sequence of your sample will be stored. This sequence has the same length as the H37Rv reference genome (4411532 bp) (see Figure 24).

Sequence Editor
KDCCGATG ACCCCGGTTC AGGCTTCACC ACAGTGTGGA ACGCGGTCGT CTCCGAACTT 60
AACGGCGACC CTAAGGTTGA CGACGGACCC AGCAGTGATG CTAATCTCAG CGCTCGGCTG 120
ACCCCTCAGC AAAGGGCTTG GCTCAATCTC GTCCAGCCAT TGACCATCGT CGAGGGGTTT 180
GCTCTGTTAT CCGTGCCGAG CAGCTTTGTC CAAAACGAAA TCGAGCGCCA TCTGCGGGCC 240
CCGATTACCG ACGCTCTCAG CCGCCGACTC GGACATCAGA TCCAACTCGG GGTCCGCATC 300
GCTCCGCCCG CGACCGACGA AGCCGACGAC ACTACCGTGC CGCCTTCCGA AAATCCTGCT 360
ACCACATCGC CAGACACCAC AACCGACAAC GACGAGATTG ATGACAGCGC TCGGGCACGG 420
GGCGATAACC AGCACAGTTG GCCAAGTTAC TTCACCGAGC GCCCGCACAA TACCGATTCC 480
GCTACCGCTG GCGTAACCAG CCTTAACCGT CGGTACACCT TTGATACGTT CGTTATCGGC 540
GCCTCCAACC GGTTCGCGCA CGCCGCCGCC TTGGCGATCG CAGAAGCACC CGCCCGCGCT 600
TACAACCCCG TGTTTCATCTG GGGCGAGTCC GGTCTCGGCA AGACACACCT GCTACACGCG 660

Figure 24: Example output of mapping experiment type for sample ERR067612.

12. Close the Mapping experiment type.

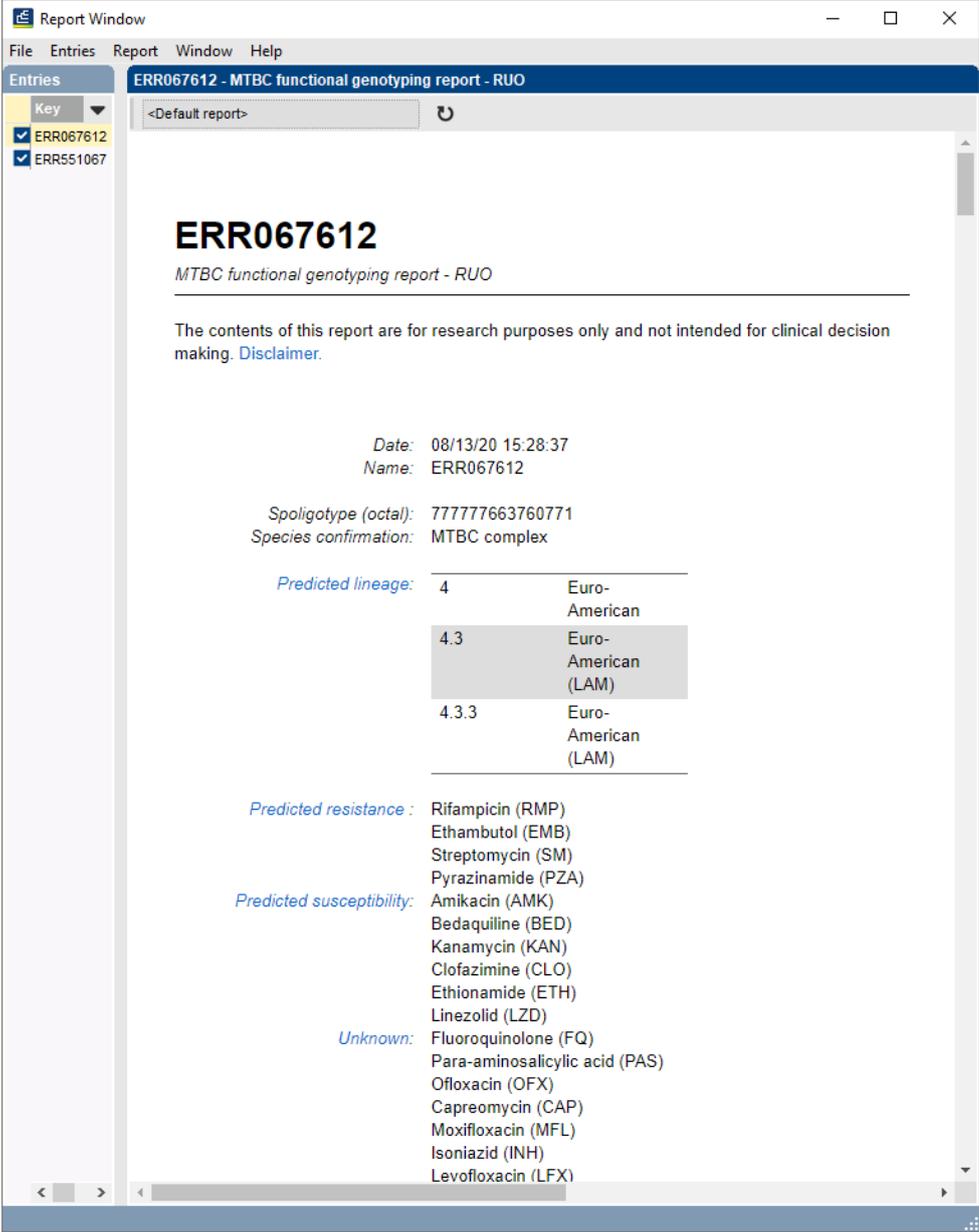
6 Reports

1. To generate a summary report, select the entries of interest and click on **MTBC > Show report**.

The *Report* window contains a genotype report for each of the selected entries (see Figure 25).

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* are displayed in the *Genotype report* panel.



ERR067612
 MTBC functional genotyping report - RUO

The contents of this report are for research purposes only and not intended for clinical decision making. [Disclaimer.](#)

Date: 08/13/20 15:28:37
 Name: ERR067612

Spoligotype (octal): 77777663760771
 Species confirmation: MTBC complex

Predicted lineage:

4	Euro-American
4.3	Euro-American (LAM)
4.3.3	Euro-American (LAM)

Predicted resistance : Rifampicin (RMP)
 Ethambutol (EMB)
 Streptomycin (SM)
 Pyrazinamide (PZA)

Predicted susceptibility: Amikacin (AMK)
 Bedaquiline (BED)
 Kanamycin (KAN)
 Clofazimine (CLO)
 Ethionamide (ETH)
 Linezolid (LZD)

Unknown: Fluoroquinolone (FQ)
 Para-aminosalicylic acid (PAS)
 Ofloxacin (OFX)
 Capreomycin (CAP)
 Moxifloxacin (MFL)
 Isoniazid (INH)
 Levofloxacin (LFX)

Figure 25: Example of a functional genotyping report.

3. To rerun an analysis from the *Report window* (with current MTBC settings) for one entry, click on **Report** > **Reanalyze current report** or click on  at the top of the report.
4. To rerun the analysis for all entries displayed in the *Report window*, click on **Entries** > **Reanalyze all**.
5. Click on a hyperlink of one of the predicted traits to display the detailed results in the *Genotype report* panel.
6. Select **File** > **Exit** to close the *Report* window.