BioNumerics Tutorial:

E. coli functional genotyping: predicting phenotypic traits from whole genome sequences

1 Aim

In this tutorial we will screen genome sequences of *Escherichia coli* samples for phenotypic traits using the *E. coli functional genotyping plugin*. This plugin contains public databases for serotype, virulence and resistance prediction, as well as plasmid and prophage detection. An in silico PCR tool is also implemented, making it possible to detect Shiga toxin gene subtypes and virulence genes, mimicking the wet lab PCR.

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 denovo 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 2.3).

2.2 Option 1: Download demo database from the Startup Screen

1. Click the ***Download example databases*** link, located in the lower right corner of the *BioNumerics Startup* window.

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

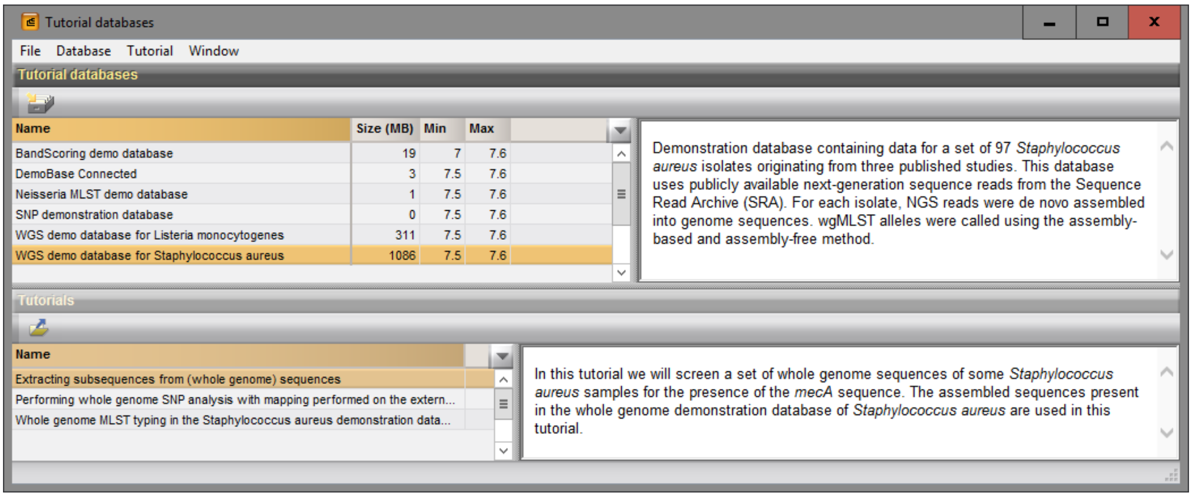
2. Select the **WGS demo database for Escherichia coli** from the list and select ***Database*** > ***Download*** ( ).

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

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

The **WGS demo database for Escherichia coli** appears in the *BioNumerics Startup* window.

5. Double-click the **WGS demo database for Escherichia coli** in the *BioNumerics Startup* window to open the database.



Name	Size (MB)	Min	Max
BandScoring demo database	19	7	7.6
DemoBase Connected	3	7.5	7.6
Neisseria MLST demo database	1	7.5	7.6
SNP demonstration database	0	7.5	7.6
WGS demo database for Listeria monocytogenes	311	7.5	7.6
WGS demo database for Staphylococcus aureus	1086	7.5	7.6

Demonstration database containing data for a set of 97 *Staphylococcus aureus* isolates originating from three published studies. This database uses publicly available next-generation sequence reads from the Sequence Read Archive (SRA). For each isolate, NGS reads were de novo assembled into genome sequences. wgMLST alleles were called using the assembly-based and assembly-free method.

Name
Extracting subsequences from (whole genome) sequences
Performing whole genome SNP analysis with mapping performed on the extern...
Whole genome MLST typing in the Staphylococcus aureus demonstration data...

In this tutorial we will screen a set of whole genome sequences of some *Staphylococcus aureus* samples for the presence of the *mecA* sequence. The assembled sequences present in the whole genome demonstration database of *Staphylococcus aureus* are used in this tutorial.

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

6. Download the file `WGS_EC.bnbk` file from `http://www.applied-maths.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.bnbk` database backup file into `WGS_EC.zip`. If this happens, you should manually remove the `.zip` file extension and replace with `.bnbk`. A warning will appear (”If you change a file name extension, the file might become unusable.”), but you can safely confirm this action. Keep in mind that Windows might not display the `.zip` file extension if the option ”Hide extensions for known file types” is checked in your Windows folder options.

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

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

9. Specify a new name for this demonstration database, e.g. “WGS Ecoli demobase”.

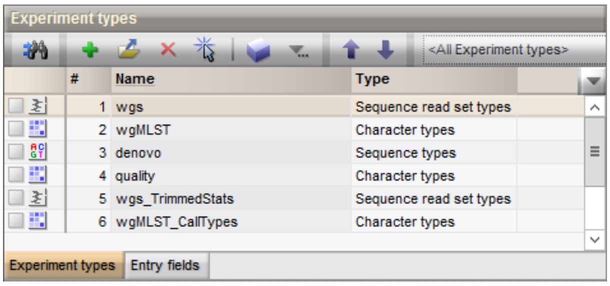
10. Click <***OK***> to start restoring the database from the backup file.

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

The *Main* window is displayed.

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. Six experiments are present in the demo database and are listed in the *Experiment types* panel (see Figure 2).

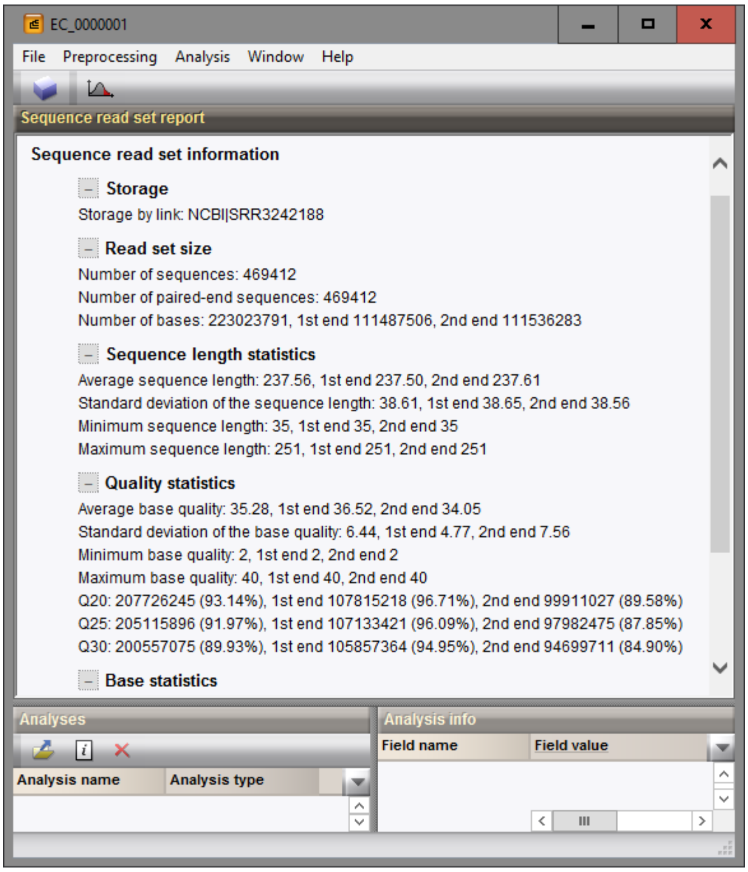


#	Name	Type
1	wgs	Sequence read set types
2	wgMLST	Character types
3	denovo	Sequence types
4	quality	Character types
5	wgs_TrimmedStats	Sequence read set types
6	wgMLST_CallTypes	Character types

Figure 2: 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 3).



Sequence read set information

Storage

Storage by link: NCBI|SRR3242188

Read set size

Number of sequences: 469412
Number of paired-end sequences: 469412
Number of bases: 223023791, 1st end 111487506, 2nd end 111536283

Sequence length statistics

Average sequence length: 237.56, 1st end 237.50, 2nd end 237.61
Standard deviation of the sequence length: 38.61, 1st end 38.65, 2nd end 38.56
Minimum sequence length: 35, 1st end 35, 2nd end 35
Maximum sequence length: 251, 1st end 251, 2nd end 251

Quality statistics

Average base quality: 35.28, 1st end 36.52, 2nd end 34.05
Standard deviation of the base quality: 6.44, 1st end 4.77, 2nd end 7.56
Minimum base quality: 2, 1st end 2, 2nd end 2
Maximum base quality: 40, 1st end 40, 2nd end 40
Q20: 207726245 (93.14%), 1st end 107815218 (96.71%), 2nd end 99911027 (89.58%)
Q25: 205115896 (91.97%), 1st end 107133421 (96.09%), 2nd end 97982475 (87.85%)
Q30: 200557075 (89.93%), 1st end 105857364 (94.95%), 2nd end 94699711 (84.90%)

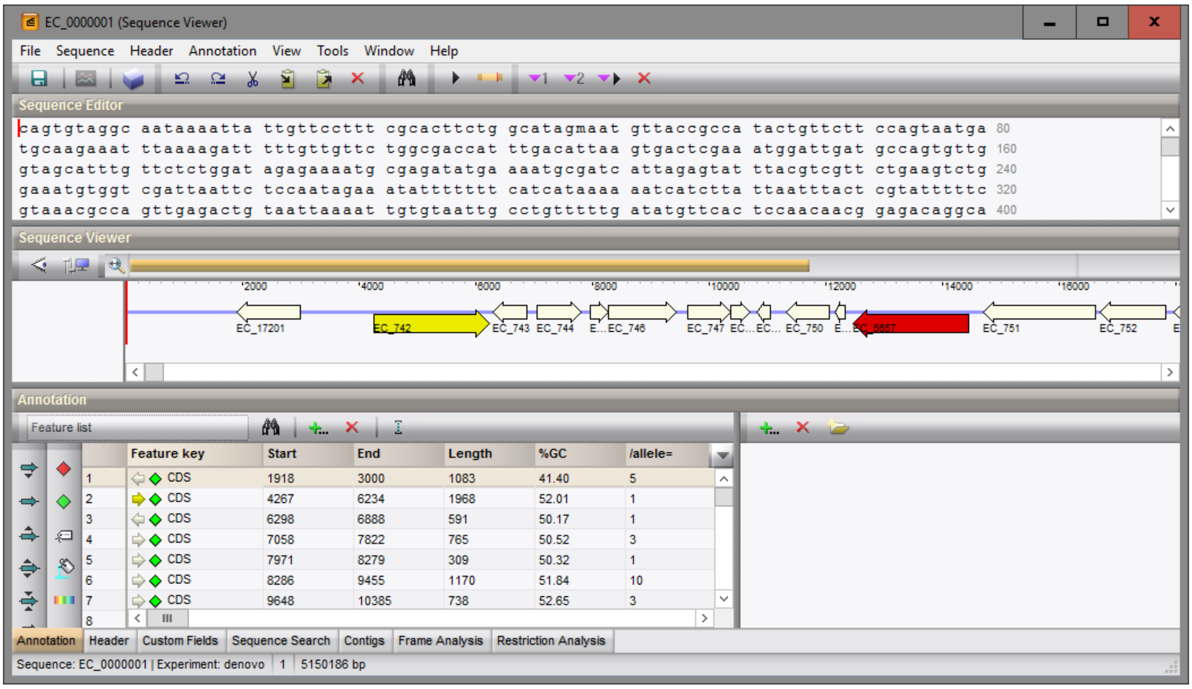
Base statistics

Figure 3: The sequence read set experiment card for an entry.

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

3. Click on the green colored dot for one of the entries in the third column in the *Experiment presence* panel. Column 3 corresponds to the third 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 4).


EC_0000001 (Sequence Viewer)
File Sequence Header Annotation View Tools Window Help
Sequence Editor
Sequence Viewer
2000 4000 6000 8000 10000 12000 14000 16000
EC_17201 EC_742 EC_743 EC_744 E... EC_746 EC_747 EC... EC... EC_750 E... EC_5667 EC_751 EC_752 E
Annotation
Feature list
Annotation Header Custom Fields Sequence Search Contigs Frame Analysis Restriction Analysis

```
cagtgtaggc aataaaatta ttgttccttt cgcacttctg gcatagmaat gttaccgcca tactgttctt ccagtaatga 80
tgcaagaaat ttaaaagatt tttgttgttc tggcgaccat ttgacattaa gtgactcgaa atggattgat gccagtgttg 160
gtagcatttg ttctctggat agagaaaatg cgagatatga aaatgcgatc attagagtat ttacgtcgtt ctgaagtctg 240
gaaatgtggt cgattaattc tccaatagaa atattttttt catcataaaa aatcatctta ttaatttact cgtatttttc 320
gtaaacgcca gttgagactg taattaaaat tgtgtaattg cctgtttttg atatgttcac tccaacaacg gagacaggca 400
```

	Feature key	Start	End	Length	%GC	/allele=
1	CDS	1918	3000	1083	41.40	5
2	CDS	4267	6234	1968	52.01	1
3	CDS	6298	6888	591	50.17	1
4	CDS	7058	7822	765	50.52	3
5	CDS	7971	8279	309	50.32	1
6	CDS	8286	9455	1170	51.84	10
7	CDS	9648	10385	738	52.65	3
8						

Sequence: EC_0000001 | Experiment: denovo | 1 | 5150186 bp

Figure 4: The *Sequence editor* window.

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 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 E. coli functional genotyping plugin

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

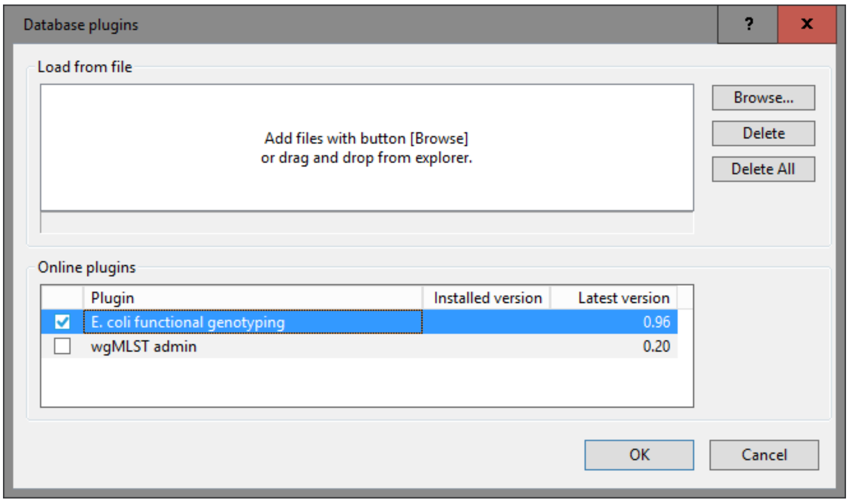
The *E. coli functional genotyping plugin* is provided as an *online plugin*. Online plugins are available from the Applied Maths website, from which they can be downloaded and installed in the database in just a few mouse clicks. Since administrator rights are not required for installation of a plugin in the database, online plugins can be easily updated to take advantage of the latest improvements in program code and search data.

2. Select the *Database Functionality tab* in the *Plugins* dialog box and press the <***Add / Update...***> button.

3. Check the box that corresponds to the *E. coli functional genotyping plugin* (see Figure 5) and press <***OK***>.

The actual download of the plugin file can take a couple of minutes, depending on the speed of your internet connection.

The first page of the *E. coli genotyping settings* wizard prompts for some general settings (see Figure 6):



Database plugins

Load from file

Add files with button [Browse]
or drag and drop from explorer.

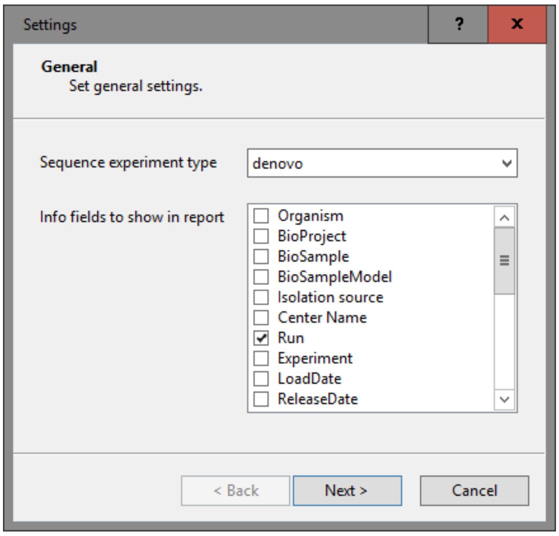
Online plugins

	Plugin	Installed version	Latest version
☑	E. coli functional genotyping		0.96
□	wgMLST admin		0.20

Figure 5: Adding an online plugin.

- The ***Sequence experiment type*** that holds the (whole) genome sequences that will be screened.
- The ***Information fields*** that will appear in the report (see 6).

4. 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 check the ***Run*** number to include in the report (see Figure 6).



Settings

General
Set general settings.

Sequence experiment type: denovo

Info fields to show in report:
□ Organism
□ BioProject
□ BioSample
□ BioSampleModel
□ Isolation source
□ Center Name
☑ Run
□ Experiment
□ LoadDate
□ ReleaseDate

Figure 6: The *General settings* wizard page.

5. Press <***Next***>.

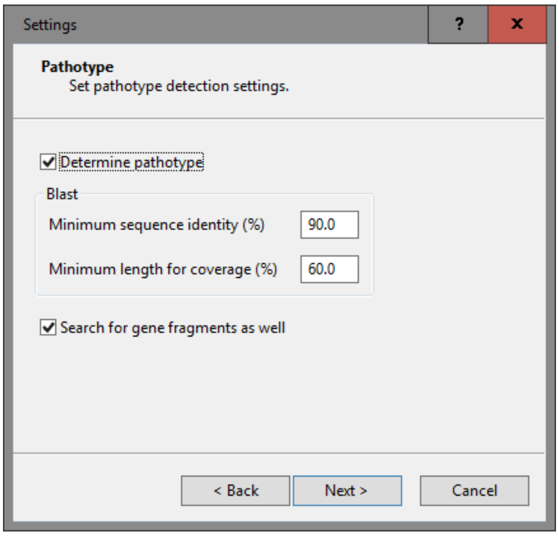
The next steps group the settings for each possible search: serotype, pathotype, resistance, virulence, plasmid, prophage, complete plasmid, and in silico PCR search.

The ***BLAST*** search settings become available when checking the ***Determine*** (or ***Find***) check box in each step (see Figure 7 for an example). The ***BLAST settings*** include two thresholds that a BLAST hit should fulfill:

- A ***Minimum sequence identity (%)*** between the subsequence found in the (whole genome) sequence and the sequence in the reference database, expressed as a percentage.

- A ***Minimum length for coverage (%)***, i.e. a minimum overlap between the subsequence found in the (whole genome) sequence and the sequence in the reference database, expressed as a percentage.

With the check box ***Search for gene fragments as well*** enabled in the ***Pathotype***, ***Resistance*** and ***Virulence*** dialogs, sequence fragments (with a maximum of 3 fragments) are also considered.



Pathotype
Set pathotype detection settings.

☑ Determine pathotype

Blast

Minimum sequence identity (%) 90.0

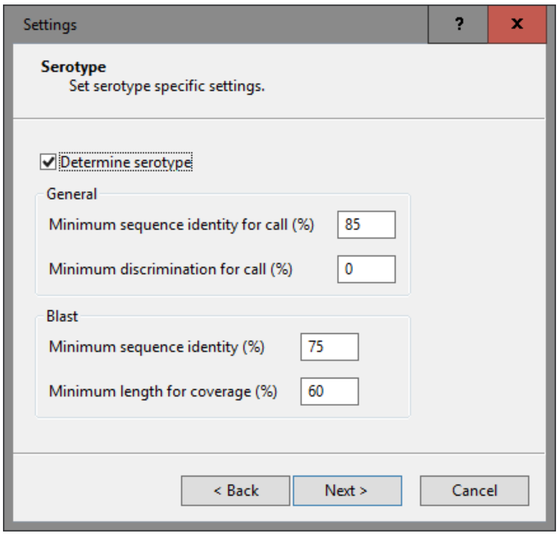
Minimum length for coverage (%) 60.0

☑ Search for gene fragments as well

Figure 7: Pathotype settings.

In the *Serotype settings dialog* two additional settings are listed to check the discrimination between the two best hits that passed the BLAST criteria (see Figure 8). Only if the best hit passes these discrimination criteria, the best hit is used for serotype prediction.

- ***Minimum sequence identity for call (%)***: This is the minimum sequence identity the best BLAST hit should have to be considered for serotype prediction.
- ***Minimum discrimination for call (%)***: This is minimum required discrimination between the two best hits in order to predict the serotype based on the best BLAST hit.



Serotype
Set serotype specific settings.

☑ Determine serotype

General

Minimum sequence identity for call (%) 85

Minimum discrimination for call (%) 0

Blast

Minimum sequence identity (%) 75

Minimum length for coverage (%) 60

Figure 8: The *Serotype settings* wizard page.

The discrimination D is calculated as:

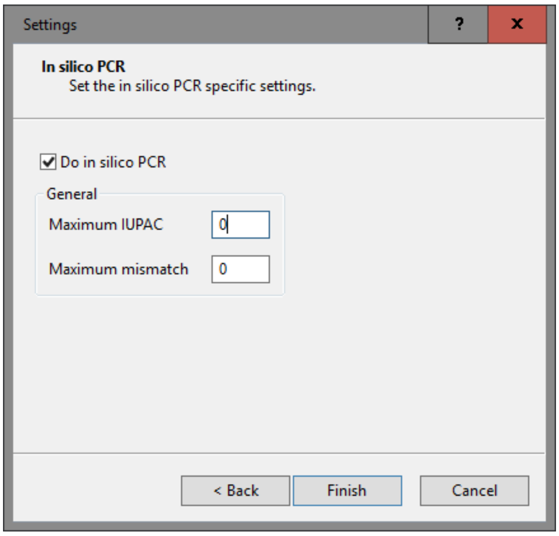
$$D = \frac{P_1 - P_2}{100 - P_{min}}$$

with P_{min} = minimum sequence identity percentage specified for BLAST, P_1 = sequence identity percentage of best BLAST hit, P_2 = sequence identity percentage of second best BLAST hit. When there is only one best hit, $P_2 = P_{min}$.

The last step prompts for the in silico PCR specific settings (see Figure 9):

- ***Maximum IUPAC***: Maximum number of allowed IUPAC codes in the subsequence of the (whole genome) sequence. The different possibilities for the ambiguous positions are considered when performing the matching against the sequences in the reference database.

- ***Maximum mismatch***: Maximum number of allowed mismatches between the subsequence of the (whole genome) sequence and the sequence in the reference database.



☑ Do in silico PCR

Maximum IUPAC: 0

Maximum mismatch: 0

Figure 9: The *In silico PCR settings* wizard page.

6. In this tutorial, check all search options and use the default settings in each step.

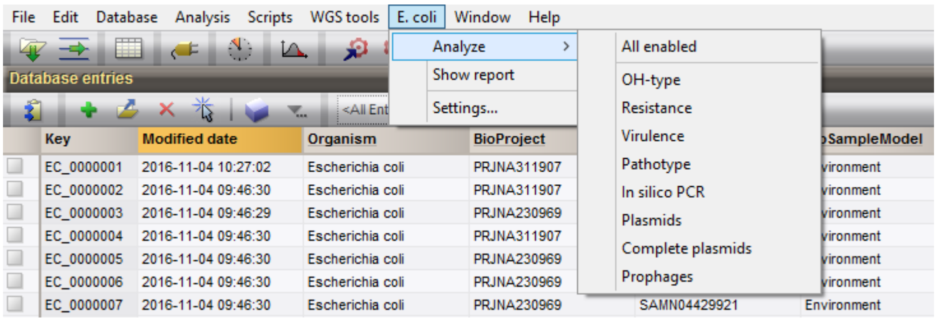
7. In the last step press <***Finish***> to complete the installation of the plugin.

8. Press <***Exit***> to close the *Plugins* dialog box.

The *E. coli functional genotyping plugin* installs menu items in the main menu of the software under ***E. coli*** (see Figure 10).

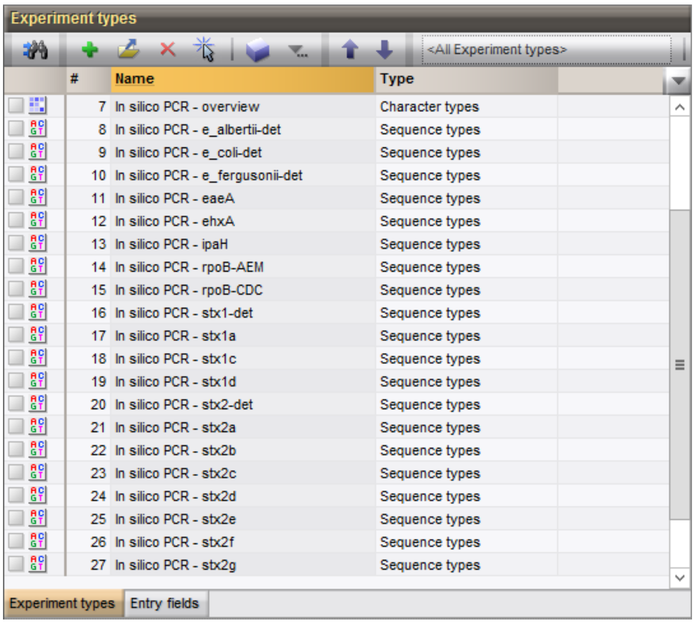
An **In silico PCR overview** character type is added to the *Experiment types* panel. This experiment will summarize the in silico PCR search results. The predicted **In silico PCR** sequences will be stored in the corresponding sequence type experiments (see Figure 11).

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



Key	Modified date	Organism	BioProject		SampleModel
EC_0000001	2016-11-04 10:27:02	Escherichia coli	PRJNA311907		vironment
EC_0000002	2016-11-04 09:46:30	Escherichia coli	PRJNA311907		vironment
EC_0000003	2016-11-04 09:46:29	Escherichia coli	PRJNA230969		vironment
EC_0000004	2016-11-04 09:46:30	Escherichia coli	PRJNA311907		vironment
EC_0000005	2016-11-04 09:46:30	Escherichia coli	PRJNA230969		vironment
EC_0000006	2016-11-04 09:46:30	Escherichia coli	PRJNA230969		vironment
EC_0000007	2016-11-04 09:46:30	Escherichia coli	PRJNA230969	SAMN04429921	Environment

Figure 10: New menu-items after installation of the plugin.



#	Name	Type
7	In silico PCR - overview	Character types
8	In silico PCR - e_albertii-det	Sequence types
9	In silico PCR - e_coli-det	Sequence types
10	In silico PCR - e_fergusonii-det	Sequence types
11	In silico PCR - eaeA	Sequence types
12	In silico PCR - ehxA	Sequence types
13	In silico PCR - ipaH	Sequence types
14	In silico PCR - rpoB-AEM	Sequence types
15	In silico PCR - rpoB-CDC	Sequence types
16	In silico PCR - stx1-det	Sequence types
17	In silico PCR - stx1a	Sequence types
18	In silico PCR - stx1c	Sequence types
19	In silico PCR - stx1d	Sequence types
20	In silico PCR - stx2-det	Sequence types
21	In silico PCR - stx2a	Sequence types
22	In silico PCR - stx2b	Sequence types
23	In silico PCR - stx2c	Sequence types
24	In silico PCR - stx2d	Sequence types
25	In silico PCR - stx2e	Sequence types
26	In silico PCR - stx2f	Sequence types
27	In silico PCR - stx2g	Sequence types

Figure 11: In silico PCR experiments.

5 Screening of entries

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

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

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

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

A group of entries can be unselected the same way.

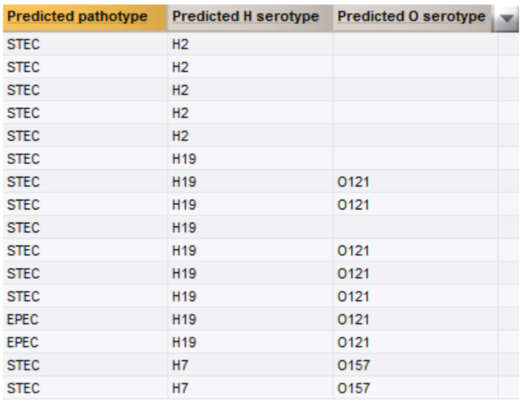
3. Make sure a few entries are selected in the *Database entries* panel of the demonstration database.

Screening for the phenotypic traits can be done for all tools checked in the *Settings* dialog box (***E. coli*** > ***Analyze*** > ***All enabled***) or for each tool separately (***E. coli*** > ***Analyze*** > **...**).

4. Select ***E. coli*** > ***Analyze*** > ***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 **H and O serotypes** that passed the specified call criteria (see Figure 8) and the **pathotypes** that passed the BLAST criteria (see Figure 7) are displayed in the corresponding information fields in the *Database entries* panel (see Figure 12).



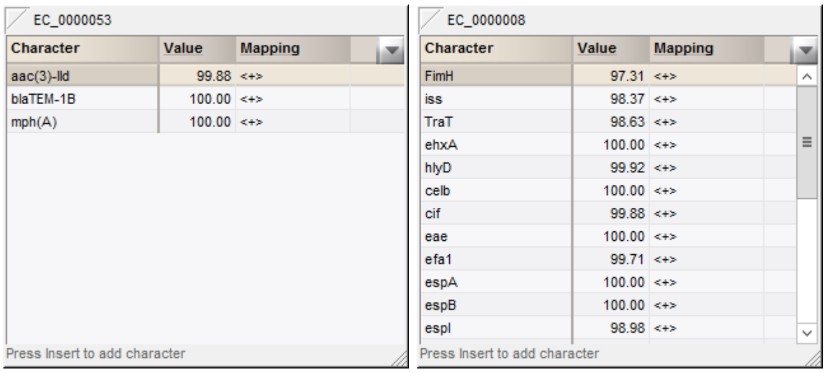
Predicted pathotype	Predicted H serotype	Predicted O serotype
STEC	H2	
STEC	H2	
STEC	H2	
STEC	H2	
STEC	H2	
STEC	H19	
STEC	H19	O121
STEC	H19	O121
STEC	H19	
STEC	H19	O121
STEC	H19	O121
STEC	H19	O121
EPEC	H19	O121
EPEC	H19	O121
STEC	H7	O157
STEC	H7	O157

Figure 12: Updated information fields.

The **Resistance**, (**Complete**) **Plasmids**, **Virulence**, **Prophages** character types are created and updated with the predicted traits that passed the BLAST settings.

5. Open a character card (**Resistance**, (**Complete**) **Plasmids**, **Virulence** or **Prophages**) for one of the analyzed entries by clicking on the corresponding green colored dot in the *Experiment presence* panel.

The traits that passed the BLAST criteria are displayed with their identity percentages (see Figure 13 for a few sample character cards).



EC_0000053

Character	Value	Mapping
aac(3)-IId	99.88	<+>
blaTEM-1B	100.00	<+>
mph(A)	100.00	<+>

Press Insert to add character

EC_0000008

Character	Value	Mapping
FimH	97.31	<+>
iss	98.37	<+>
TraT	98.63	<+>
ehxA	100.00	<+>
hlyD	99.92	<+>
celb	100.00	<+>
cif	99.88	<+>
eae	100.00	<+>
efa1	99.71	<+>
espA	100.00	<+>
espB	100.00	<+>
espI	98.98	<+>

Press Insert to add character

Figure 13: Resistance and virulence experiments.

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

7. Open the **In silico PCR - overview** character card for one of the analyzed entries by clicking on the corresponding green colored dot in the *Experiment presence* panel.

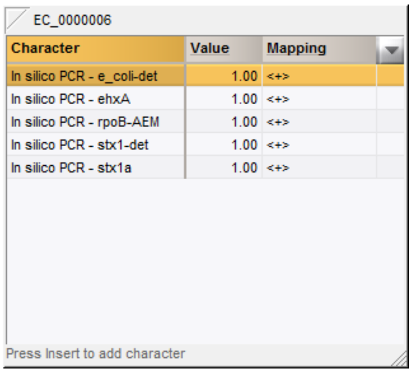
The **In silico PCR - overview** character card (see Figure 14) lists all in silico PCR sequences that passed the search criteria (see Figure 9).

8. Close the character card by clicking in the top left corner of the card.

The predicted **In silico PCR** sequences are stored in the corresponding sequence type experiments.

9. Clicking on a green colored dot for an in silico experiment opens the *Sequence editor* window displaying the sequence (see Figure 15).

10. Close the *Sequence editor* window.

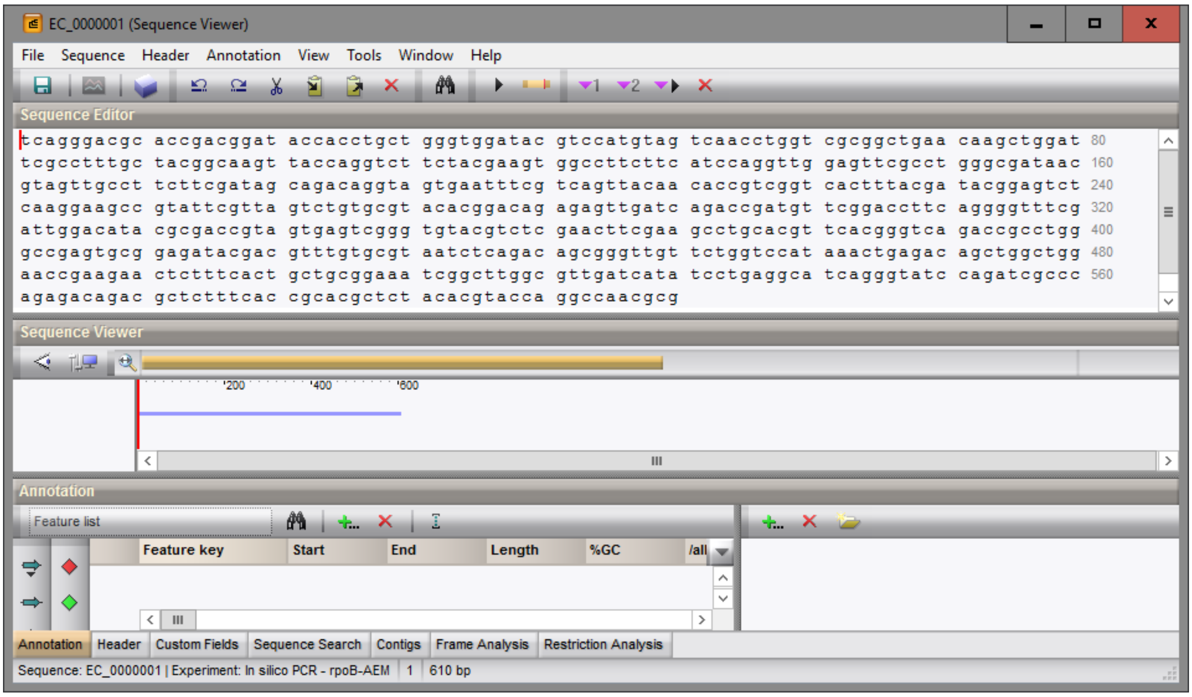


EC_0000006

Character	Value	Mapping
In silico PCR - e_coli-det	1.00	<+>
In silico PCR - ehxA	1.00	<+>
In silico PCR - rpoB-AEM	1.00	<+>
In silico PCR - stx1-det	1.00	<+>
In silico PCR - stx1a	1.00	<+>

Press Insert to add character

Figure 14: Overview of the in silico PCR search.



```
tcagggacgc accgacggat accacctgct gggtggatac gtccatgtag tcaacctggt cgcggctgaa caagctggat 80
tcgcctttgc tacggcaagt taccaggtct tctacgaagt ggccttcttc atccaggttg gagttcgcct gggcgataac 160
gtagttgcct tcttcgatag cagacaggta gtgaatttcg tcagttacaa caccgtcggt cactttacga tacggagtct 240
caaggaagcc gtattcgtta gtctgtgcgt acacggacag agagttgatc agaccgatgt tcggaccttc aggggtttcg 320
attggacata cgcgaccgta gtgagtcggg tgtacgtctc gaacttcgaa gcctgcacgt tcacgggtca gaccgcctgg 400
gccgagtgcg gagatacgac gtttgtgcgt aatctcagac agcgggttgt tctggtccat aaactgagac agctggctgg 480
aaccgaagaa ctctttcact gctgcggaaa tcggcttggc gttgatcata tcctgaggca tcagggtatc cagatcgccc 560
agagacagac gctctttcac cgcacgctct acacgtacca ggccaacgcg
```

Sequence: EC_0000001 | Experiment: In silico PCR - rpoB-AEM | 1 | 610 bp

Figure 15: In silico PCR sequence.

6 Reports

1. Open the genotype report for the selected entries with ***E. coli*** > ***Show report***.

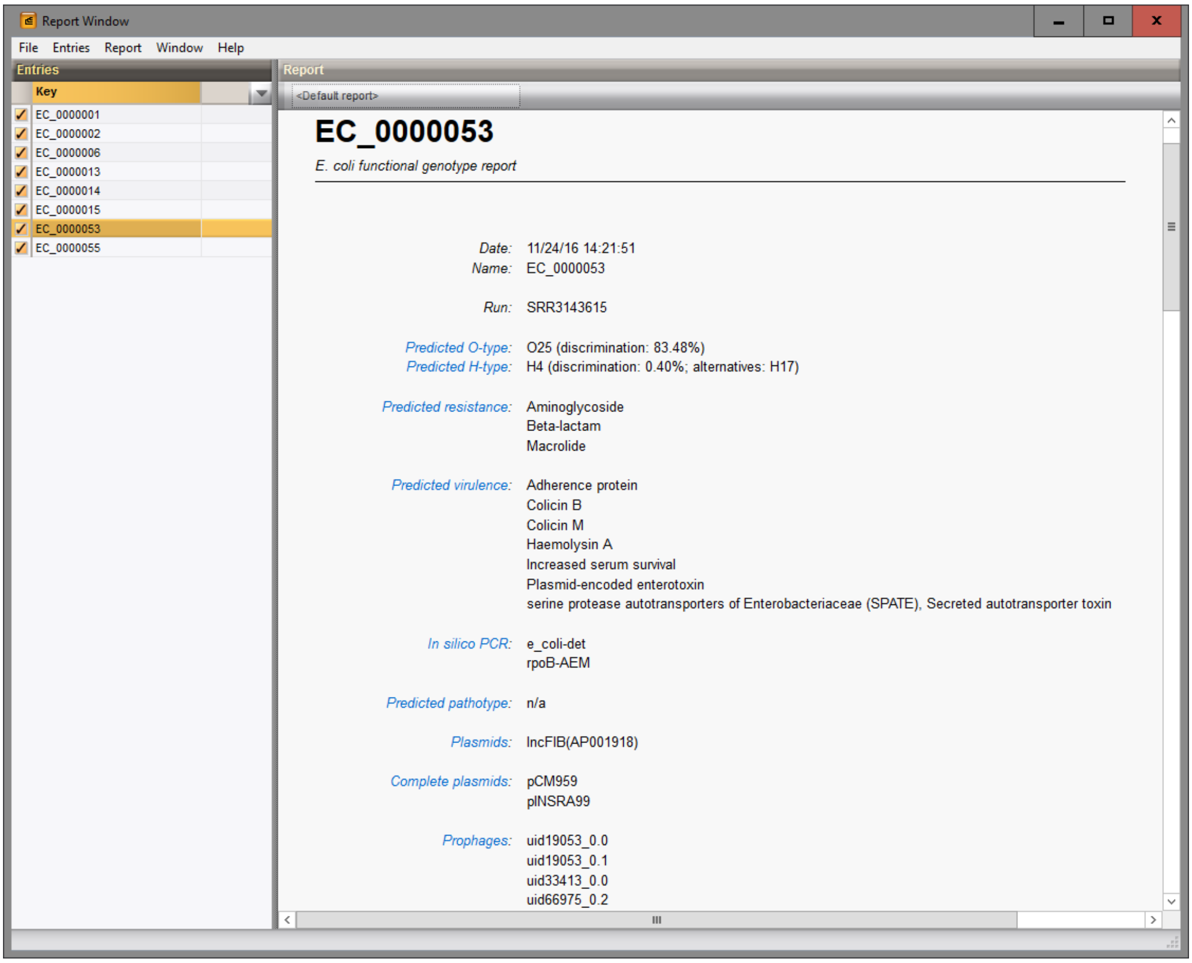
The *Report* window contains a genotype report for each of the selected entries (see Figure 16).

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

The data the report was run (***Date***), the Key (***Name***), and information fields checked in the *Settings* dialog box (see Figure 6) are displayed in the *Genotype report* panel, followed by a summary of the results of all analyzed traits.

The discrimination calculated for serotype prediction is displayed next to the predicted serotypes, optionally followed by the second best BLAST hit that passed the BLAST criteria (***alternatives***).

All hits that passed the settings for **Resistance**, **Virulence**, **In silico PCR**, **Pathotype**, (**Complete**) **Plasmids** and **Prophages** screening are listed.



Key
✓ EC_0000001
✓ EC_0000002
✓ EC_0000006
✓ EC_0000013
✓ EC_0000014
✓ EC_0000015
✓ EC_0000053
✓ EC_0000055

EC_0000053

E. coli functional genotype report

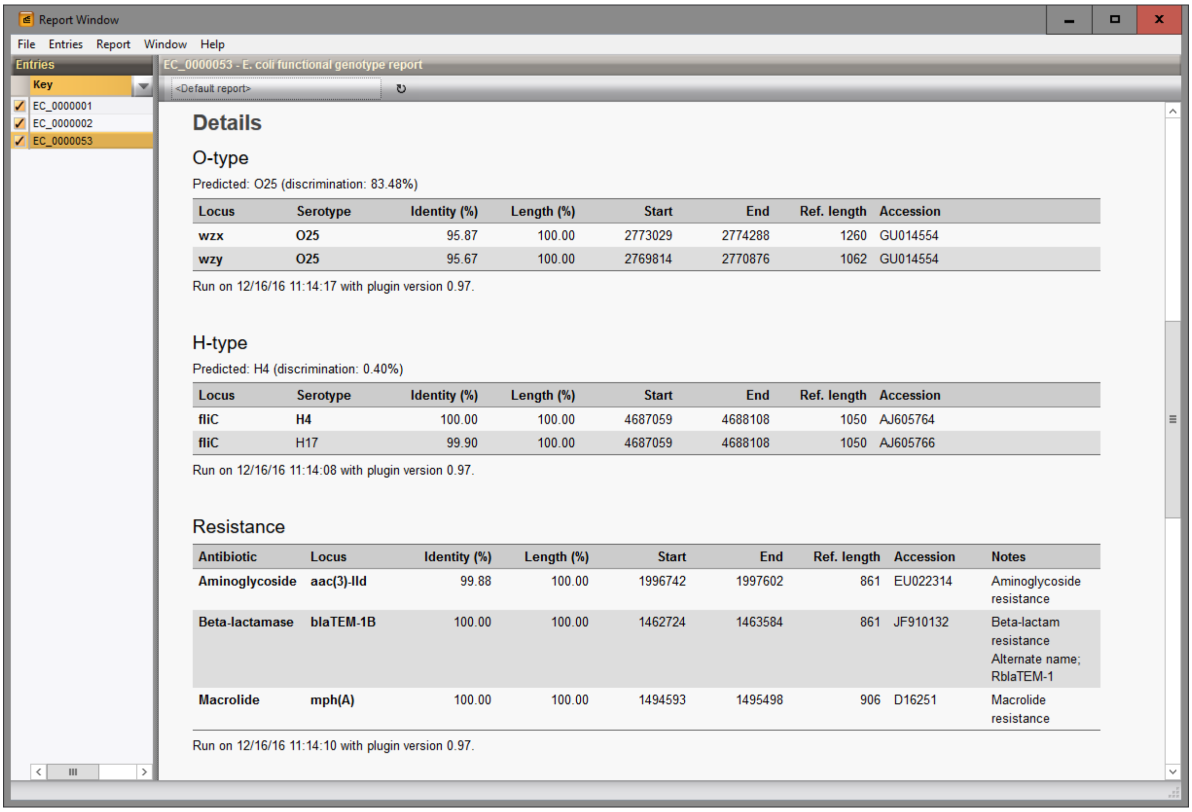
Date:	11/24/16 14:21:51
Name:	EC_0000053
Run:	SRR3143615
Predicted O-type:	O25 (discrimination: 83.48%)
Predicted H-type:	H4 (discrimination: 0.40%; alternatives: H17)
Predicted resistance:	Aminoglycoside Beta-lactam Macrolide
Predicted virulence:	Adherence protein Colicin B Colicin M Haemolysin A Increased serum survival Plasmid-encoded enterotoxin serine protease autotransporters of Enterobacteriaceae (SPATE), Secreted autotransporter toxin
In silico PCR:	e_coli-det rpoB-AEM
Predicted pathotype:	n/a
Plasmids:	IncFIB(AP001918)
Complete plasmids:	pCM959 pINSRA99
Prophages:	uid19053_0.0 uid19053_0.1 uid33413_0.0 uid66975_0.2

Figure 16: Genotype report.

3. Click on a hyperlink of one of the predicted traits to display the detailed BLAST results in the *Genotype report* panel.

Detailed BLAST results include locus identifiers, BLAST similarity scores and descriptive information on the detected genes (see Figure 17). The date the analysis was launched and the version number of the *E. coli functional genotyping plugin* that was used to perform the analysis are indicated.

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



Report Window
File Entries Report Window Help
Entries
Key
EC_0000001
EC_0000002
EC_0000053
EC_0000053 - E. coli functional genotype report
<Default report>

Details

O-type

Predicted: O25 (discrimination: 83.48%)

Locus	Serotype	Identity (%)	Length (%)	Start	End	Ref. length	Accession
wzx	**O25**	95.87	100.00	2773029	2774288	1260	GU014554
wzy	**O25**	95.67	100.00	2769814	2770876	1062	GU014554

Run on 12/16/16 11:14:17 with plugin version 0.97.

H-type

Predicted: H4 (discrimination: 0.40%)

Locus	Serotype	Identity (%)	Length (%)	Start	End	Ref. length	Accession
fliC	**H4**	100.00	100.00	4687059	4688108	1050	AJ605764
fliC	H17	99.90	100.00	4687059	4688108	1050	AJ605766

Run on 12/16/16 11:14:08 with plugin version 0.97.

Resistance

Antibiotic	Locus	Identity (%)	Length (%)	Start	End	Ref. length	Accession	Notes
Aminoglycoside	**aac(3)-IId**	99.88	100.00	1996742	1997602	861	EU022314	Aminoglycoside resistance
Beta-lactamase	**blaTEM-1B**	100.00	100.00	1462724	1463584	861	JF910132	Beta-lactam resistance Alternate name; RblaTEM-1
Macrolide	**mph(A)**	100.00	100.00	1494593	1495498	906	D16251	Macrolide resistance

Run on 12/16/16 11:14:10 with plugin version 0.97.

Figure 17: Report details.