



BIONUMERICS Tutorial:

Importing and processing AATI Fragment Analyzer curve files

1 Aim

The Fragment Analyzer is a capillary electrophoresis instrument from the company Advanced Analytical Technologies (<http://www.aati-us.com>). The generated electropherograms can be exported from the AATI PROSize[®] data analysis software in csv or txt format and imported in BIONUMERICS after installation of the *Import fingerprint tools plugin*.

In this tutorial the installation of the *Import fingerprint tools plugin* and the import and processing of the AATI Fragment Analyzer curve files is covered.

2 Preparing the database

1. Create a new database (see tutorial "Creating a new database") or open an existing database.

Installing a plugin in a BIONUMERICS database is done from the *Plugins* dialog box (see Figure 1), which can be called from the *Main* window by selecting **File** > **Install / remove plugins...** (⌘P).

Once a plugin is installed, it is marked with a green V-sign. It can be removed again with the **<Deactivate>** button.

If the selected plugin is documented, pressing **<Show Manual>** will open its manual in the *Help* window.

Proceed as follows to install the *Import fingerprint tools plugin*, starting from the *Plugins* dialog box:

2. Select the *Utilities tab* in the *Plugins* dialog box.
3. Select the *Import fingerprint tools plugin* in the list and press the **<Activate>** button.
4. Confirm the installation of the plugin.
5. Confirm the installation of the plugin and press **<OK>**.

Once the plugin is successfully installed, it is marked with a green V-sign in the *Plugins* dialog box (see Figure 3).

6. Close the *Plugins* dialog box.

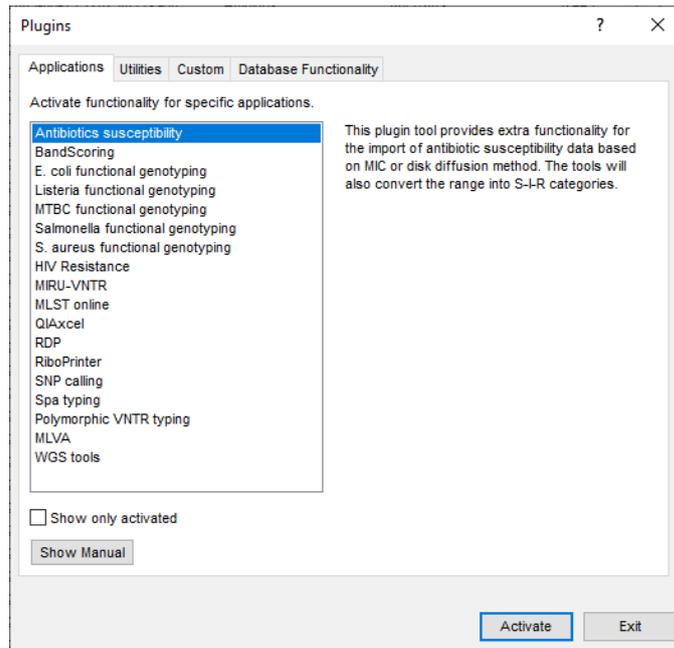


Figure 1: The *Plugins* dialog box.

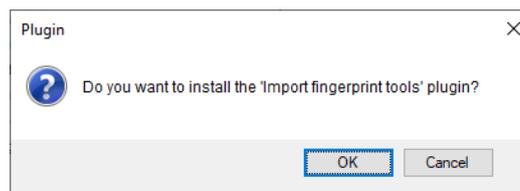


Figure 2: Confirm installation of the *Import fingerprint tools* plugin.

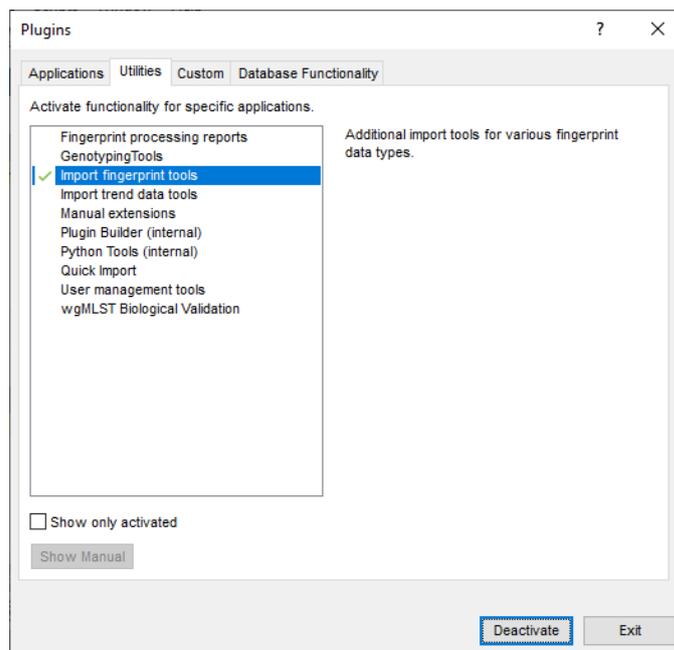


Figure 3: Installed plugin.

7. Close and reopen the database to activate the features of the *Import fingerprint tools plugin*.

Next a new fingerprint type experiment will be created that will hold the imported Fragment Analyzer data.

8. In the *Main* window, click on **+** in the toolbar of the *Experiment types* panel and select **Fingerprint type** from the list (see Figure 4).

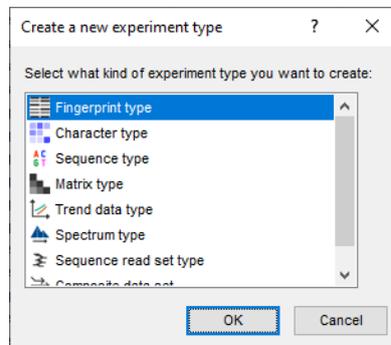


Figure 4: The *Create a new experiment type* dialog box.

9. Press **<OK>**, enter a name, for example “FragmentAnalyzer” and press **<Next>** (see Figure 5).

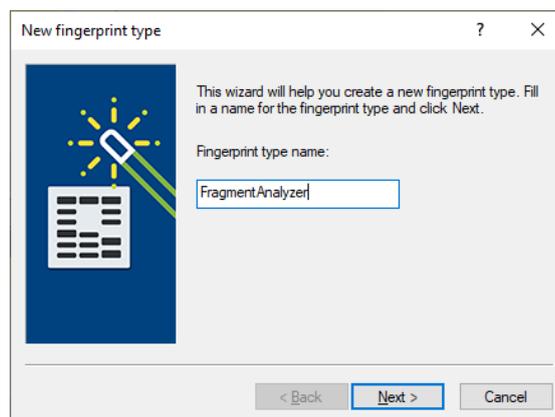


Figure 5: Specify a name.

10. In the next window, make sure that **Two-dimensional TIFF files** is selected and select the dynamic range of your data (e.g. **12-bit**). Press **<Next>**.
11. Press **<Next>** twice without altering the settings and press **<Finish>** to complete the creation of the new fingerprint type.

The *Experiment types* panel now lists the fingerprint type **FragmentAnalyzer**.

3 Importing Fragment Analyzer data

1. Select **File > Import...** (📁, **Ctrl+I**) to call the *Import* dialog box.
2. Select **Import FragmentAnalyzer curves** under **Fingerprint type data** (see Figure 7) and press **<Import>** button to start with the import of the data.

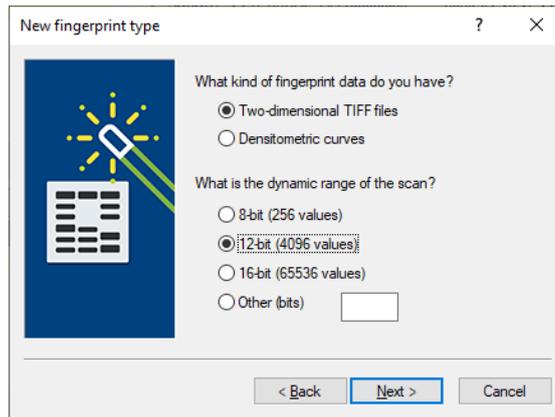


Figure 6: Fingerprint settings.

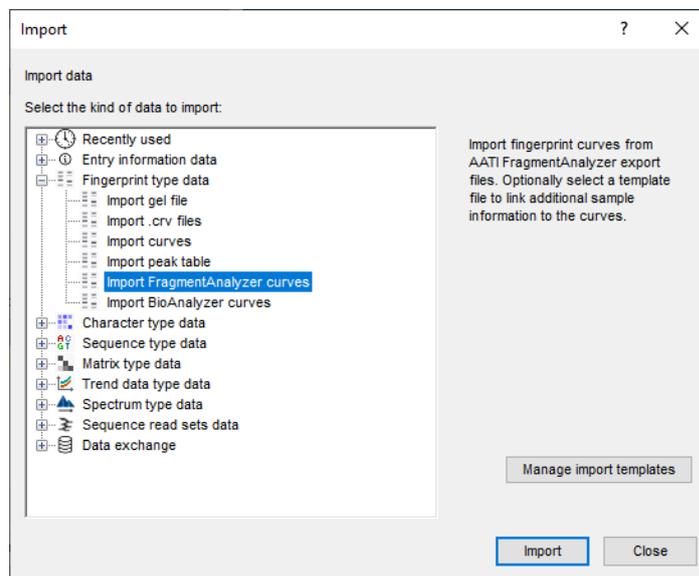


Figure 7: The *Import* dialog box.

The *Import FragmentAnalyzer data* wizard page appears (see Figure 8).

The FragmentAnalyzer import routine accepts FragmentAnalyzer *csv files* and *txt files*.

Pressing the **<Browse>** button allows you to select the csv or text file(s) that you want to import, located on your computer, external drive or on a network location. Alternatively, files can be added to the import list through drag and drop. The number of files and total size is displayed below the list. With the **<Delete>** button all selected files are removed from the import list. All files are deleted at once from the import list when pressing **<Delete All>**.

Using a *template file* sample information can be imported together with the curves. A *template file* is a simple tab- or comma delimited file with two columns (no headers). The first column should contain the *column names* present in the curve files and the second column should contain the sample information (see Figure 9 for an example). In the last step of the wizard, the corresponding BIONUMERICS information field can be selected.

3. Browse for the Fragment Analyzer file(s), optionally select a template file and press **<Next>**.

The *Import rules* dialog box is displayed (see Figure 10).

The content present in the first row in the csv/txt file corresponds to the first row in the grid. The

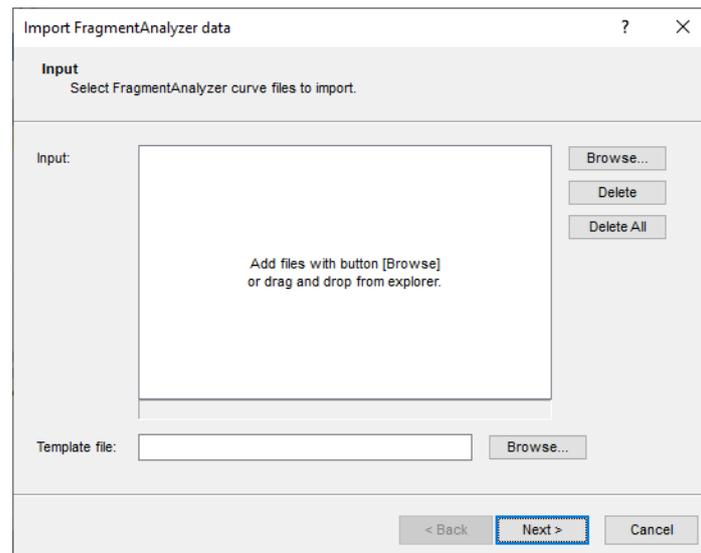


Figure 8: The *Import FragmentAnalyzer data* wizard page.

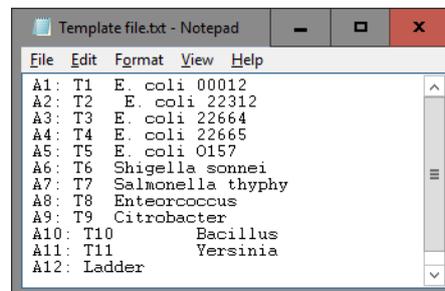


Figure 9: A template file: column names (left) and sample information (right).

text **Column ID** is specified in the **Source** column.

Double-clicking on the row opens a new dialog, where the data destination can be selected (see Figure 11). Typically, the information in the header row is linked to a new or existing **Lane info field** or **Entry info field**.

Using the last row in the grid, the (parsed) file name of the selected file(s) can be stored in the database. The text **File** is specified in the **Source type** column and the text **Name** is displayed in the **Source** column.

4. Specify a *destination* for one or more selected rows by pressing the **<Edit destination>** button or by double-clicking (see Figure 12 for an example).
5. Press **<Preview>** to check the defined rules (see Figure 13 for an example). Close the preview.
6. Press **<Next>** to go to the next step.

In the *Import curves* wizard page, following settings are available:

- The **Curve start** and **Curve stop** positions are by default set to 0% and 100% respectively. Based on these settings, the complete curves are imported. By entering other start and stop positions as percentages, specific parts of the fingerprint curves can be imported.
- Any peak with a height exceeding the OD range of the fingerprint experiments will appear truncated. To avoid this, a **Height factor** can be applied. The **Height factor** is by default

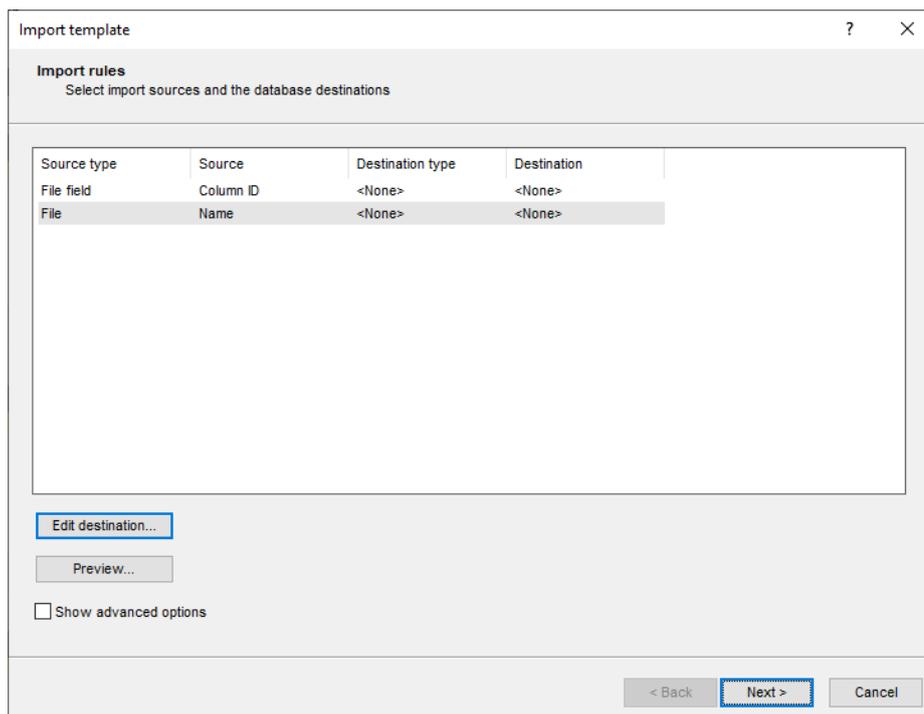


Figure 10: The *Import rules* dialog box.

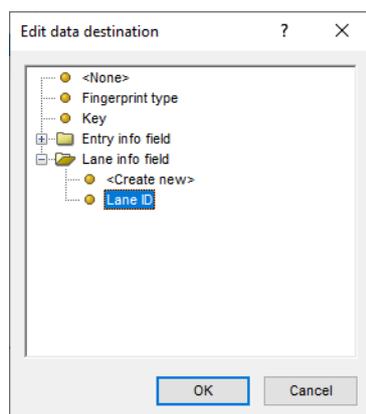


Figure 11: Edit data destination.

set to “1.0” (= no height reduction). If the **Height factor** is set to e.g. “2”, the heights are reduced by a factor two.

- Checking the option **Invert curve X-axis** will invert the curves (fragments at the top of the curve will appear at the bottom and vice-versa) right before they are added to the database.
- With the option **Do not link standard (ladder) lane** checked, the ladder lane will not be linked to an entry in the database.

7. Press **<Next>** to go to the next step.

The last step prompts for some final settings.

- If a row in the grid is linked to the **Key** field in the database, **Key** is automatically selected as the entry link field. If entries are already present in the database with the same (parsed)

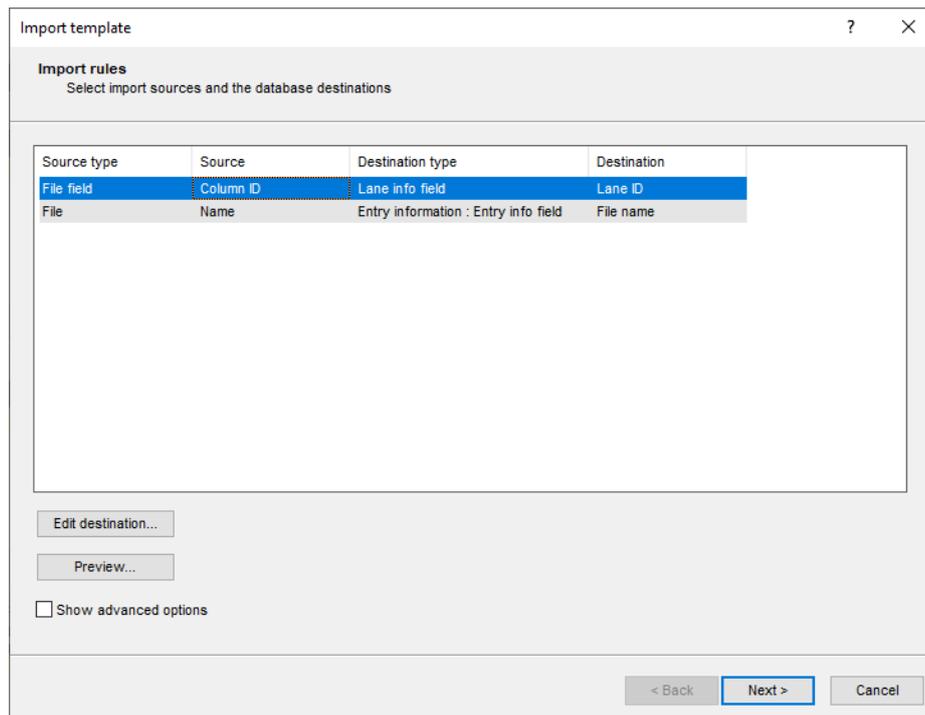


Figure 12: Import template.

| Nr. | File name | Lane ID |
|-----|---------------|----------|
| 1 | 20200325_0025 | a1: t1 |
| 2 | 20200325_0025 | a2: t2 |
| 3 | 20200325_0025 | a3: t3 |
| 4 | 20200325_0025 | a4: t4 |
| 5 | 20200325_0025 | a5: t5 |
| 6 | 20200325_0025 | a6: t6 |
| 7 | 20200325_0025 | a7: t7 |
| 8 | 20200325_0025 | a8: t8 |
| 9 | 20200325_0025 | a9: t9 |
| 10 | 20200325_0025 | a10: t10 |
| 11 | 20200325_0025 | a11: t11 |

Figure 13: Preview.

key information, the import tool will link the data to these entries.

- If no row entry in the grid is linked to the **Key** field, but one or more rows are linked to an entry information field in the database, these fields can be selected from the list. If entries are already present in the database with this linked information, the import tool will link the data to these entries. If the entries are not yet present in the database, the data will be linked to new entries in the database (if the option **Create x entries** is checked in the last step of the wizard).
- If no fields are selected from the list, no check for existing entries will be performed, and all

The screenshot shows a dialog box titled "Import template" with a close button (X) and a help button (?). The main heading is "Import curves" with the instruction "Choose how to import the curves". Below this, there are three input fields: "Curve start:" with a value of "0.0" and a percentage sign, "Curve end:" with a value of "100.0" and a percentage sign, and "Height factor:" with a value of "1.0" and an "x" multiplier. There are two checkboxes: "Invert curve X-axis" which is unchecked, and "Do not link standard (ladder) lane" which is checked. At the bottom right, there are three buttons: "< Back", "Next >" (highlighted with a blue border), and "Cancel".

Figure 14: The *Import curves* wizard page.

The screenshot shows a dialog box titled "Import template" with a close button (X) and a help button (?). The main heading is "Import links" with the instruction "Choose how to link records to database entries". Below this, there is a text block: "If the entry Key is not imported, linked information fields will be used to look up the database entries. The linked information field content of existing entries will not be modified." There is a checkbox labeled "File name" which is unchecked, followed by a large empty rectangular box. At the bottom right, there are three buttons: "< Back", "Finish" (highlighted with a blue border), and "Cancel".

Figure 15: The *Import links* dialog box.

data will be linked to new entries in the database (if the option **Create x entries** is checked in the last step of the wizard). New keys are automatically generated during import.

8. Press <**Finish**> to go to the final step.

Each import template has its own unique **Name**. Optionally, a descriptive text string can be entered

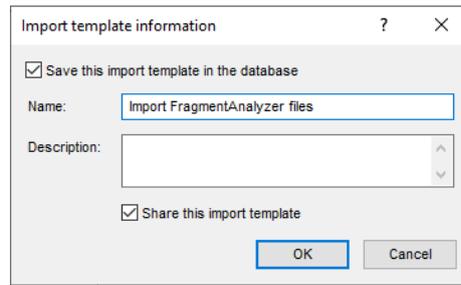


Figure 16: The *Import template information* dialog box.

in the **Description** input field.

9. Specify a template name (e.g. **Import FragmentAnalyzer files**) and press **<OK>** to save all template settings to the database.

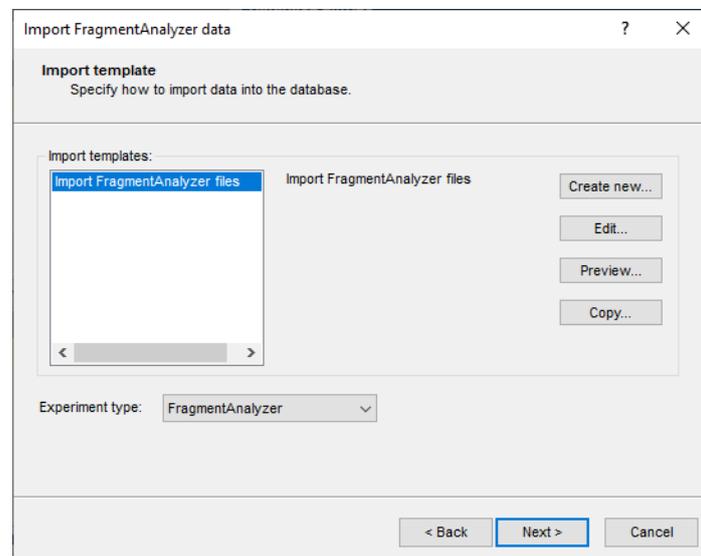


Figure 17: Import template

When a template has been created and saved, the template **Name** is shown in the *Import templates panel* and is automatically selected (see Figure 17). The template **Description** is shown in panel on the right.

The patterns can be linked to an existing fingerprint type experiment or to a new fingerprint type experiment (**<Create New>**). When the fingerprints are linked to a new fingerprint type experiment, the next dialog will prompt for the fingerprint type name (see Figure 18). The creation of the new experiment needs to be confirmed (twice).

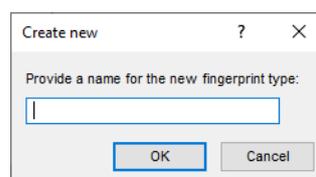


Figure 18: Create a new fingerprint type experiment.

10. Press **<Next>** to go to the next step.

The last step prompts for some final settings (see Figure 19).

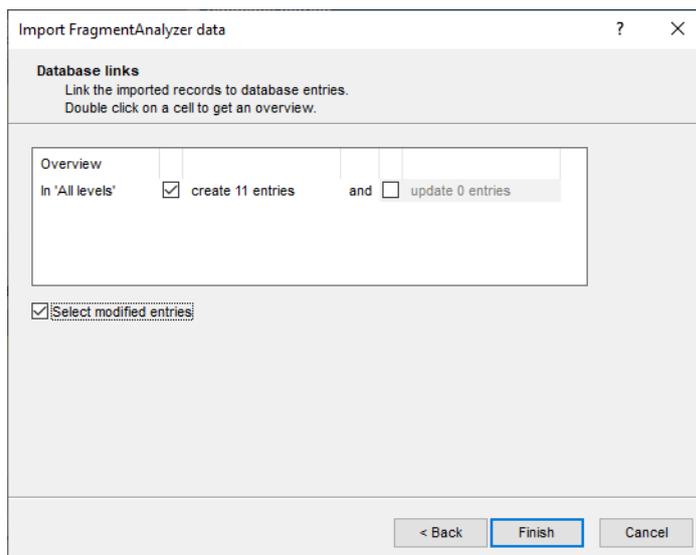


Figure 19: Database links.

- When **Create x entries** is checked, the import tool is allowed to create the new entries in the database.
- Check the option **Update x entries** if you want the software to be able to update the information for existing entries.
- If the option **Select modified entries** is checked, entries in the database that were modified during the import routine will be selected after import.

11. Press **<Finish>** to start the import.

Entries are created/updated and are displayed in the *Database entries* panel of the *Main* window. Linked sample information - if defined - is stored in the corresponding entry fields. When the option **Select modified entries** was checked, the new/updated entries are marked by a checked ballot box (☑).

The fingerprint files are displayed in the *Fingerprint files* panel and contain the corresponding fingerprint patterns. The patterns are linked to the appointed fingerprint type experiment in the database. The presence of a pattern for an entry/experiment combination is indicated with a green colored dot in the *Experiment presence* panel.

4 Processing Fragment Analyzer data

1. In the *Fingerprint files* panel, double-click on one of the imported files to open the *Fingerprint* window.
2. In the *Fingerprint* window, select **File > Edit fingerprint data...** (📄) to open the *Fingerprint processing* window.

The OD range defined for the linked fingerprint type experiment is applied on every imported file.

3. To check (and optionally update) the OD range, select **Edit > Edit settings...** (⚙️) and select the **Raw data** tab.

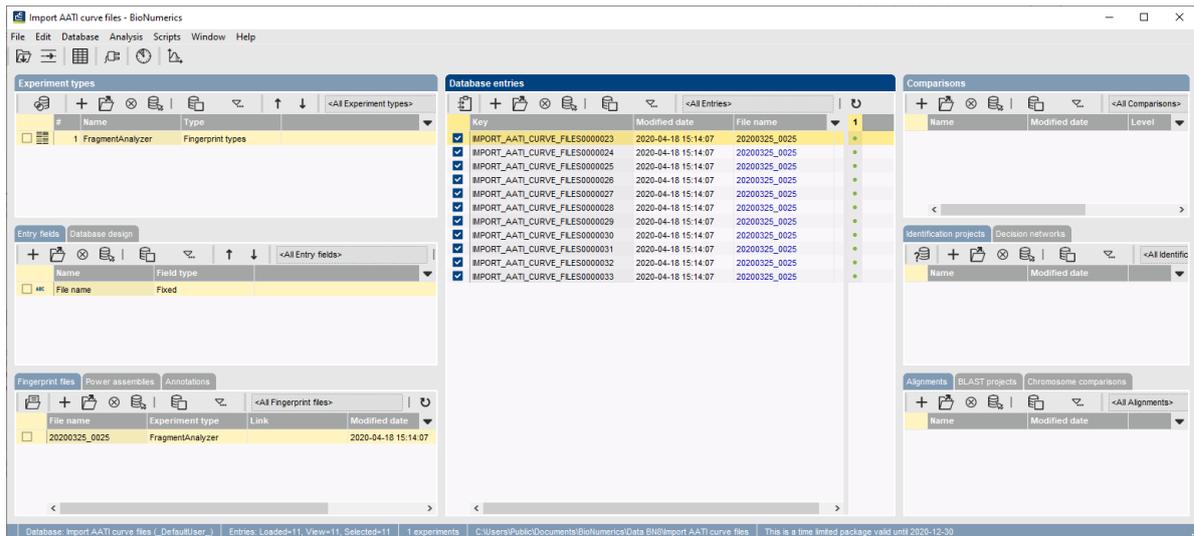


Figure 20: The *Main* window after import of FragmentAnalyzer data.

The OD (or intensity) range can be update to any other value if needed: e.g. **12-bit (4096 values)**, **16-bit (65536 values)**,

4. Close the *Fingerprint processing settings* dialog box.
5. Make sure the **Normalization** tab is selected in the *Fingerprint processing* window.
6. Press  to enter the normalized view.

For now, the "normalized view" looks the same as the original view.

7. Select the reference lane and press  to assign it as a reference package lane or select **References > Use as reference lane**.
8. Right-click on a band in the reference lane and choose **Add external reference position**.
9. Enter the size (in bp) of the reference band. Make sure to only add numerical values (so without bp or kb).
10. Repeat this action for all other reference bands in the reference lane.
11. Press .
12. The program may prompt with the following question: "The resolution of this gel differs considerably from the normalized track resolution. Do you wish to update the normalized track resolution?" If the question appears, answer < **Yes** >.

The reference system is now defined and saved with the fingerprint type experiment. When processing new fingerprint files, run with the same reference system, the assignment of the reference positions can be skipped: just assign the correct lane as reference lane (**References > Use as reference lane**), execute **Normalization > Auto assign...** () and inspect the assignments made.

13. Select the **Bands** tab in the *Fingerprint processing* window.

If you want to use the curves to compare the patterns, no bands need to be assigned in the sample lanes. If you want to compare the patterns using bands, you will need to assign bands in the sample lanes in the last step. Usually, assigning bands in the sample lanes is done first with

the software's automatic band search, followed by manual corrections. Some trial and error might be required to find the best settings for the automatic band search.

14. To automatically search for bands, press  or select **Bands > Auto search bands**.

In the *Band search* dialog box, the currently selected lane is shown along the bottom.

15. To scroll through other lanes, press the < and > buttons on the left and right sides of the curve.
16. Press <**Search on all lanes**> to execute the band search with the specified settings.

Bands that are found are marked with a green horizontal line. Incorrect band assignments can be edited manually. For more information we refer to the reference manual.

17. After you are satisfied with the band assignments, press  to save the file.
18. Exit the *Fingerprint processing* window by selecting **File > Exit**.

When a reference system is added to a fingerprint type experiment, a calibration curve needs to be created for this reference system that translates all band positions into metrics.

19. Open the fingerprint experiment type (**FragmentAnalyzer** in this workflow) by double-clicking on the experiment type in the *Experiment types* panel.
20. In the *Fingerprint type* window, select **Settings > Edit reference system** or double-click on **R01**.

The *Fingerprint Reference system* window appears with the message: "Could not calculate calibration curve. Not enough markers."

21. When the molecular weights were entered as names for the reference positions, the molecular weights can be copied by selecting **Metrics > Copy markers from reference system....** Confirm the action.
22. Designate a metric unit with **Metrics > Assign units...** enter e.g. **kb** and press <**OK**>.
23. Close the *Fingerprint Reference system* window, and close the *Fingerprint type* window.

More information about the follow-up analysis tools that can be applied on the patterns can be found in the tutorials on the Applied Maths website or in the reference manual.