BioNumerics®

MICROBIAL DATA ANALYSIS SOFTWARE

SmartFinder plugin

PLUGINS

VERSION 7.6

www.applied-maths.com

Contents

NOTES

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- The Python® 2.7.4 release from the Python Software Foundation (http://www.python.org/).
- A library for XML input and output from the Apache Software Foundation (http://www.apache.org).
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- Gecko engine version 21 (https://developer.mozilla.org/en-US/docs/Mozilla/Gecko).
- pymzML Python® module for high throughput bioinformatics on mass spectrometry data (https://github.com/pymzml/pymzML).
- Numpy Python® library version 1.8.1 (http://www.numpy.org/).
- BioPython Python® library version 1.64 (http://www.biopython.org/).
- PIL Python library® version 1.1.7 (http://www.pythonware.com/products/pil/).
- The SPAdes genome assembler version 3.7.1 (http://bioinf.spbau.ru/spades).

Chapter 1

Starting and setting up BioNumerics

1.1 Introduction

This guide is designed as a tutorial for the *SmartFinder plugin*. The minimal configuration for the installation of this plugin includes the Trend data module (for the storage of the melt and peak curves) and the Character data module (storage of the calls). For more information about the different modules of the BioNumerics software, see the Reference manual, Chapter The concepts of BioNumerics.

For more information about the SmartFinder technology, see `http://www.pathofinder.com/technology`.

1.2 Startup program

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

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

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

1.3 Creating a new database

3.1 Press the  button in the BioNumerics *BioNumerics Startup* window to enter the *New database* wizard.

3.2 Enter a name for the database, and press <***Next***>.

A new dialog box pops up, prompting for the type of database (see Figure 1.2).

3.3 Since we want to create a new database to demonstrate the features of the plugin, leave the default option selected and press <***Next***>.

A new dialog box pops up, prompting for the database engine (see Figure 1.3).

3.4 Leave the default option selected and press <***Next***>.

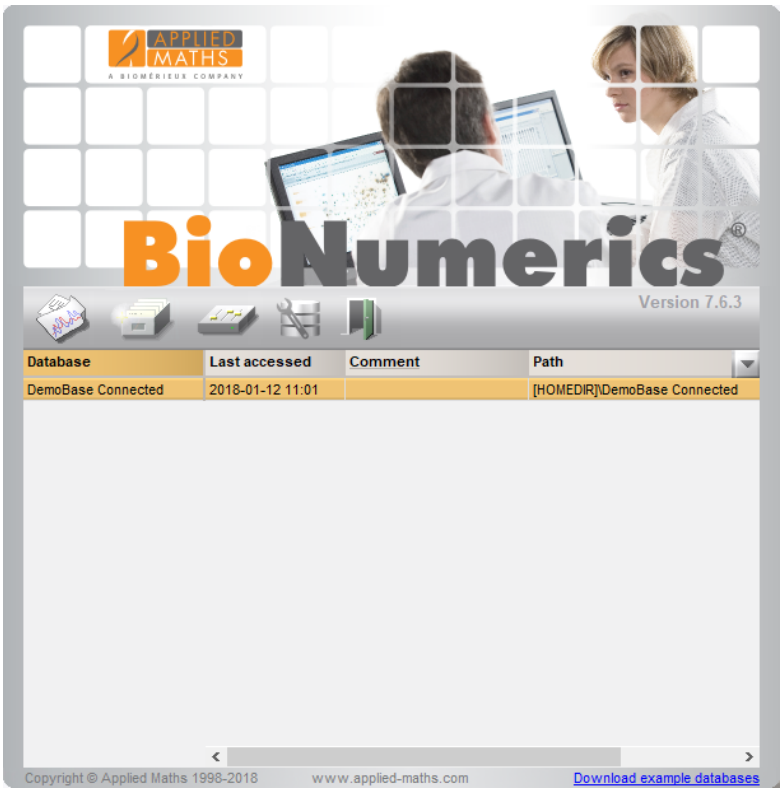


Figure 1.1: The *BioNumerics Startup* window.

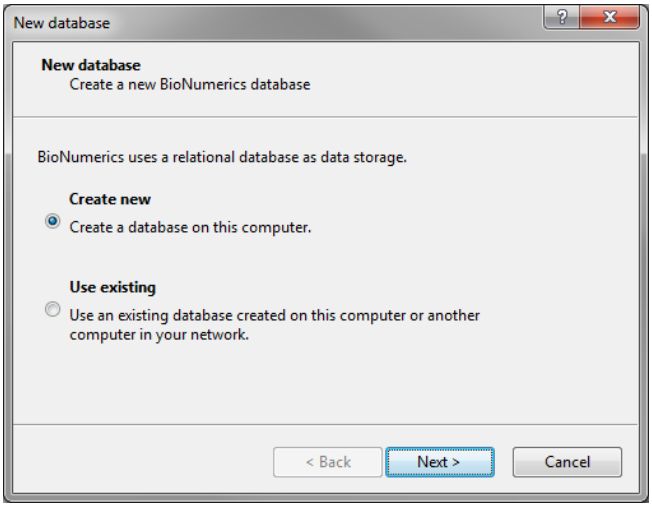


Figure 1.2: The *New database* wizard page.

3.5 Press <***Finish***> to complete the setup of the new database.

The *Plugins* dialog box appears.

1.4 Installing the SmartFinder plugin

If a database is opened for the first time, the *Plugins* dialog box will appear by default (see Figure 1.4).

If the database has already been opened previously, the *Plugins* dialog box can be called from the *Main*

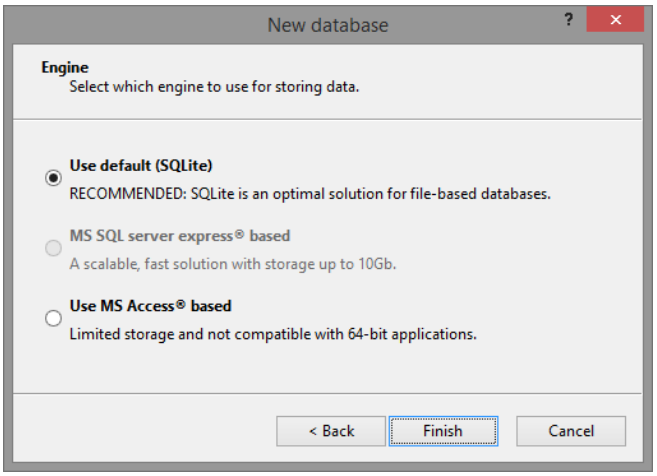


Figure 1.3: The *Database engine* wizard page.

window by selecting ***File*** > ***Install / remove plugins...*** ( ).

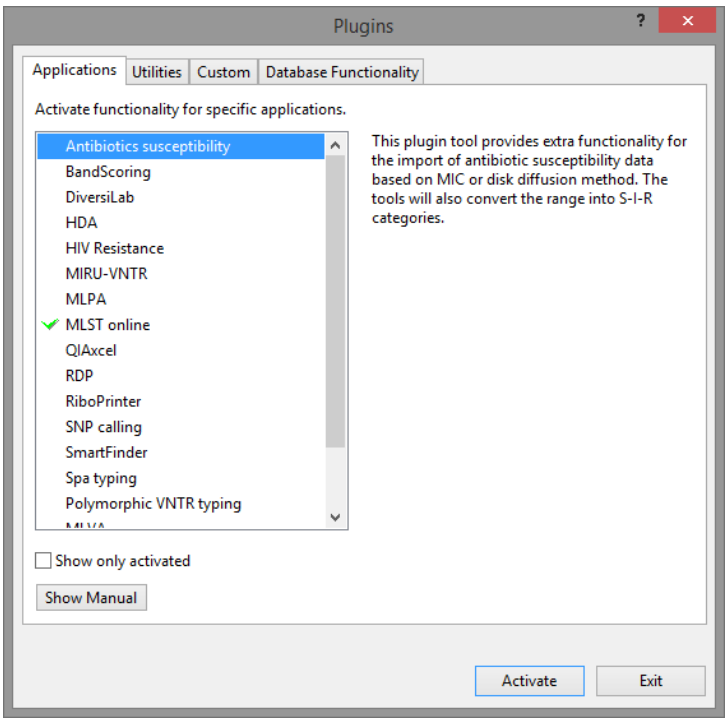


Figure 1.4: The *Plugins* dialog box.

When a particular plugin is selected from the list of plugins, a short description appears in the right panel.

A selected plugin can be installed with the <***Activate***> button. The software will ask for confirmation before installation. Some plugins depend on functionality offered by specific BioNumerics modules. If a required module is missing, the plugin cannot be installed and an error message will be generated.

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.

4.1 Select the *SmartFinder plugin* from the list in the *Applications tab* and press the <***Activate***> button.

The installation of the *SmartFinder plugin* requires administrator privileges.

4.2 The program will ask to confirm the installation of the plugin. Press <***Yes***> to confirm the installation.

The *License string* dialog box pops up (see Figure 1.5). The *SmartFinder plugin* can only be installed and activated with a valid *plugin license string*, which needs to be purchased from Applied Maths.

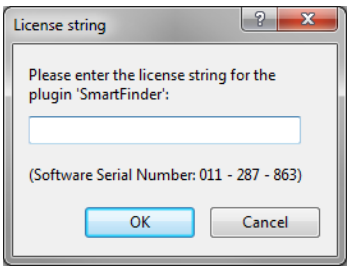


Figure 1.5: The *License string* dialog box.

The *License string* dialog box prompts for the license string that is compatible with the ***Software Serial Number*** listed in the dialog box.

4.3 Enter the six digits license string and press <***OK***>.

4.4 Press <***Proceed***> (or <***Exit***>) to close the *Plugins* dialog box and to continue to the *Main* window.

4.5 Close and reopen the database to activate the features of the *SmartFinder plugin*.

The *SmartFinder plugin* installs menu items in the *Main* window under the menu ***SmartFinder*** (see Figure 1.6).

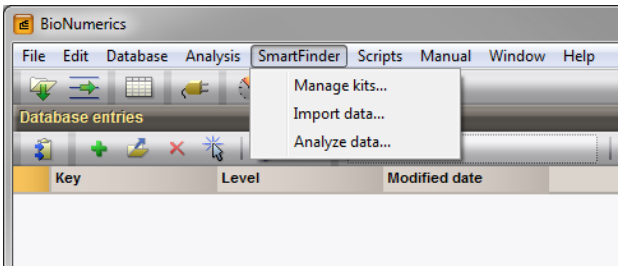


Figure 1.6: SmartFinder menu items in the *Main* window.

1.5 Managing kit definitions

Since the *SmartFinder plugin* is compatible with several kits developed by PathoFinder B.V., the first step after installation of the plugin will be the import of the correct kit definitions.

Selecting ***SmartFinder*** > ***Manage kits*** in the *Main* window calls the *Manage SmartFinder kits* dialog box as depicted in Figure 1.7.

On the left side of the dialog box, a list with ***Imported SmartFinder kits*** is displayed (initially empty).

To remove the highlighted kit from the list press <***Remove***>. A confirmation message will appear.

When pressing the <***Export***> button, the highlighted kit definition will be exported as a text file and opened in the default text editor, e.g. Notepad.

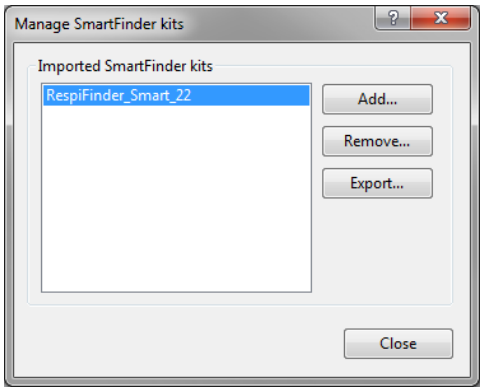


Figure 1.7: The *Manage SmartFinder kits* dialog box.

An exported kit definition can be edited and then imported again to make changes to the probe mix definition, e.g. in case new control probes were added to the mix.

Pressing <***Add***> will display the *Select SmartFinder kit* dialog box (see Figure 1.8).

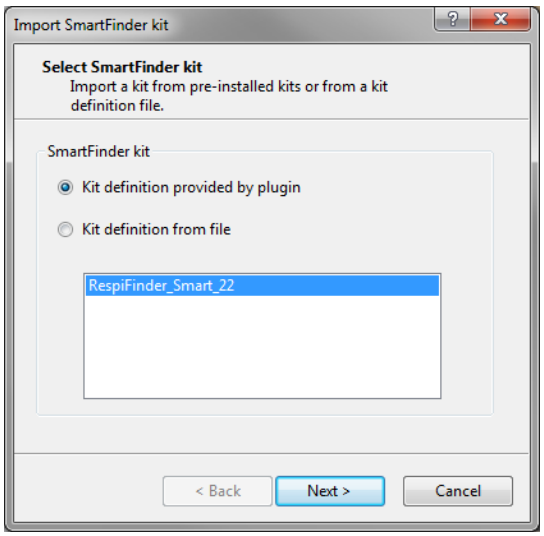


Figure 1.8: The *Select SmartFinder kit* dialog box.

Two options are available for importing a SmartFinder kit definition:

- ***Kit definition provided by plugin***: One of the kit definitions that were developed by PathoFinder B.V. and which come with the plugin can be selected from the list below.
- ***Kit definition from file***: A custom kit definition that is contained in an external file can be imported by browsing for the kit definition file.

Pressing <***Next***> displays the *SmartFinder kit experiments* dialog box.

The *SmartFinder kit experiments* dialog box asks for the ***Kit experiment name***, as it will be used in the name of the corresponding experiment types in the BioNumerics database. The name can be edited if desired.

In case that the same SmartFinder kit is run on different real-time PCR machines (e.g. on Rotor-Gene and LC480), it is recommended to create a different experiment type per machine, for example by using the machine name as suffix.

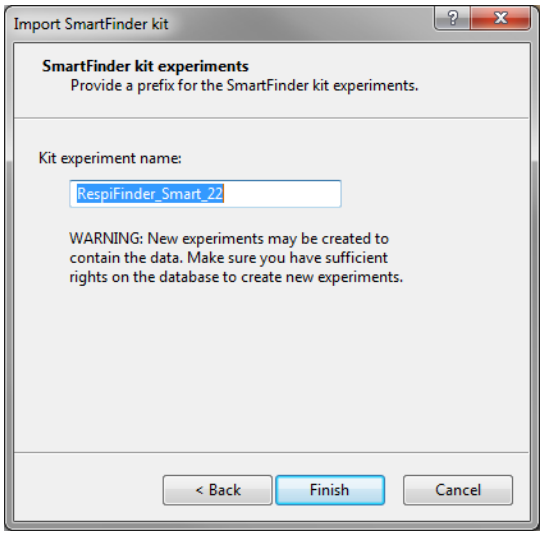


Figure 1.9: The *SmartFinder kit experiments* dialog box.

Pressing <***Finish***> creates the corresponding experiment types in the BioNumerics database and adds the kit to the *Manage SmartFinder kits* dialog box.

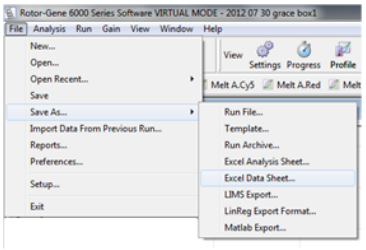
Selecting <***Close***> in the *Manage SmartFinder kits* dialog box closes the dialog box.

Chapter 2

SmartFinder data import

The *SmartFinder plugin* is compatible with data generated with Rotor-Gene and LightCycler 480 real-time PCR instruments. After finishing the analysis of the run, an export file has to be generated by the Rotor-Gene or LC 480 software, respectively. This export file can be imported by the *SmartFinder plugin*.

- **Rotor-Gene:** An export file can be generated by saving the run as an Excel Data Sheet using the Rotor-Gene software. Select ***File*** > ***Save as...*** > ***Excel data sheet*** to save the run data.



- **LightCycler 480:** When using an LC480, both the run file and the corresponding Color Compensation run file have to be exported.
  - Open the Navigator in the LC480 software by clicking .
  - Select the run file you want to export.
  - By clicking , a new window opens.
  - Select the appropriate location and the correct file format: Experiment Text File (*.txt). Please make sure that you export the Color Compensation run file and **NOT** the Color Compensation file which is generated by the LC 480 software.

Before importing data, one needs to decide where to store the sample numbers: they can be stored in the 'Key' field (the unique identifier in a BioNumerics database) or in a dedicated information field. In the latter case, a new information field should be created before importing any SmartFinder data. Sample numbers should be unique and it is good practice to employ consistent naming conventions. A new information field can be added to the database with ***Edit*** > ***Information fields*** > ***Add information field...***.

Selecting ***SmartFinder*** > ***Import data*** opens the first page of the import wizard (see Figure 2.1), which will guide the user through the import process.

With the <***Browse***> button, one can browse for a data file on the local computer or on a network drive. The full path will then be displayed next to ***File name***.

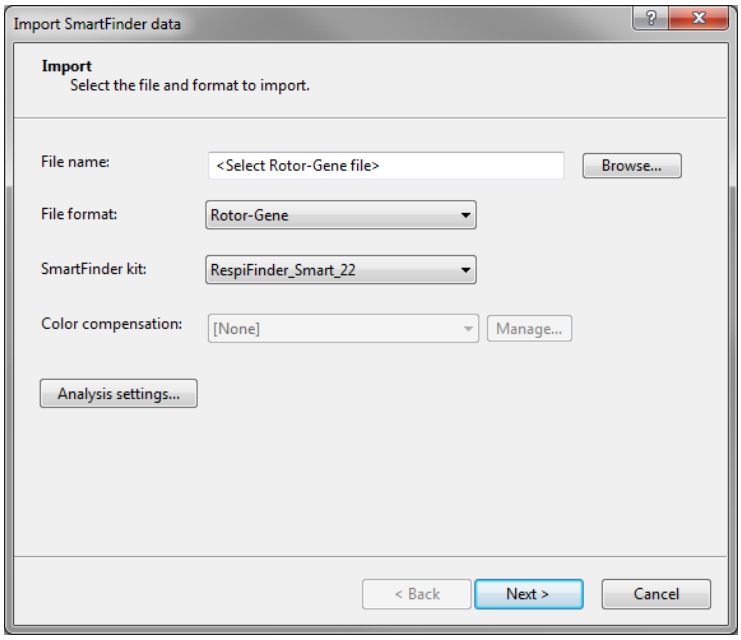


Figure 2.1: The *Import* dialog box.

From the ***File format*** drop-down list, the file format that corresponds to the data file at hand can be selected. Options are ***LightCycler 480*** and ***Rotor-Gene***. For ***LightCycler 480***, *.txt and *.xml files are supported; for ***Rotor-Gene***, *.txt and *.csv files can be used.

With the ***SmartFinder kit*** drop-down list, any of the installed SmartFinder kits (see 1.5) can be selected. The kit that was used to generated the data, should be selected here.

A ***Color compensation*** step is only needed for LightCycler data. This option is inactive for Rotor-Gene, since these data are already compensated for cross-talk between the different channels by the software of the real-time PCR apparatus. When ***LightCycler 480*** is selected from the ***File format*** drop-down list, pressing <***Manage***> will open the *Manage LC480 color compensation* dialog box (see Figure 2.2).

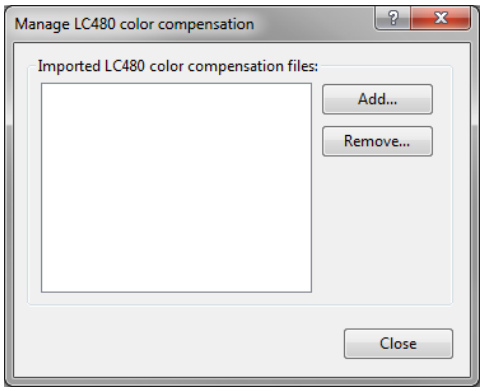


Figure 2.2: The *Manage LC480 color compensation* dialog box.

From this dialog box, color compensation runs can be added and removed:

- Pressing <***Add***> will prompt you to <***Browse***> for a ***File name*** of a color compensation file. The name that will be used to refer to this color compensation run file can be entered next to ***CC name***.
- A highlighted color compensation run file can be deleted with <***Remove***>. The software will ask for confirmation before actually removing the file.

Once a color compensation file is added via the *Manage LC480 color compensation* dialog box, it can be selected from the ***Color compensation*** drop-down list in the *Import* dialog box to correct the LightCycler data during import.

BioNumerics only stores the color-compensated data, not the raw, uncorrected data. Therefore, if you want to use a different color compensation run to correct data for, the corresponding LightCycler files need to be imported again.

The settings for the melt curve analysis can be accessed by pressing <***Analysis settings***> and are further discussed in 3.3.

Pressing <***Next***> will display the second page of the wizard (see Figure 2.3).

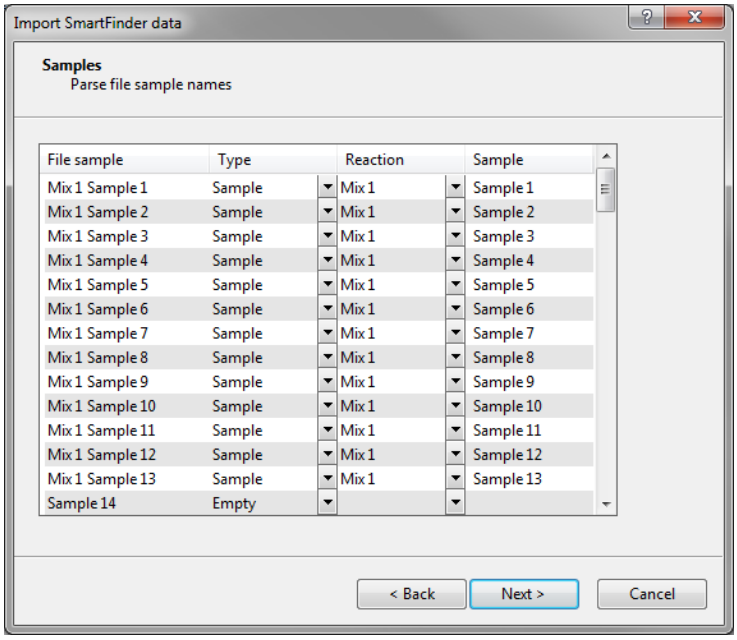


Figure 2.3: The *Samples* dialog box.

This page provides an editable preview of how the data will be parsed from the data file. A grid is displayed with following fields:

- ***File sample***: The sample name as used in the data file.
- ***Type***: The type of sample, which can be one of following values:
  - ”Sample” (a normal (patient) sample to be analyzed),
  - ”Negative control” (a no template control (NTC) or blank), or
  - ”Empty” (an empty well that should be excluded from the import).
- ***Reaction***: The reaction mix (”Mix 1” or ”Mix 2”) that the sample belongs to.
- ***Sample***: The name of the sample as it will be used in the database.

The ***Type*** and ***Reaction*** can be edited for samples individually by selecting one of the predefined values from the drop-down list. The ***Sample*** field can be edited directly via the keyboard.

Parsing of the sample names will occur automatically if following **naming convention** was followed: <SampleName><Delimiter><MixName>. The <Delimiter> can be a space, underscore, etc..

Samples that are labeled ”Blanco” will be automatically recognized as negative controls.

A warning message will appear when attempting to import samples without sufficient negative control samples (at least one negative control sample per reaction mix should be present) or when non-unique sample numbers were encountered.

Pressing <***Next***> will display the final page of the import wizard (see Figure 2.4).

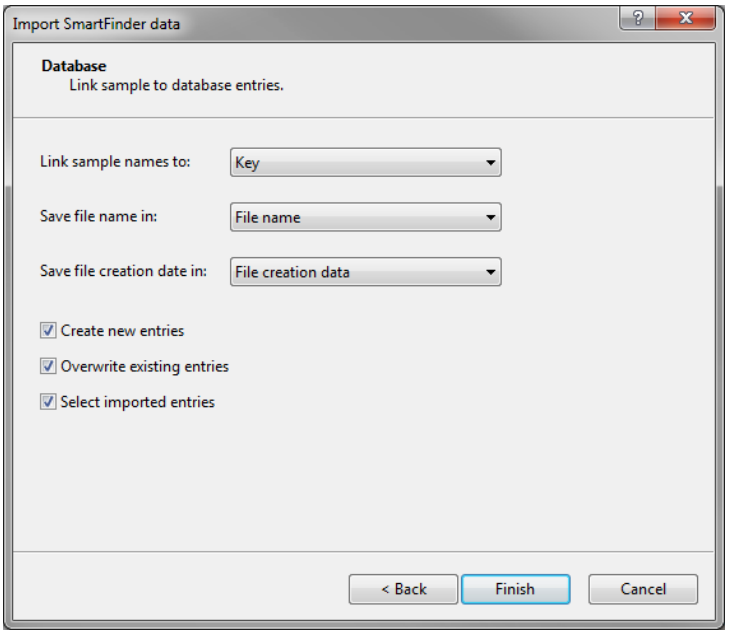


Figure 2.4: The *Database* dialog box.

From the drop-down list next to ***Link sample names to***, the database field to hold the sample number needs to be selected.

Optionally, the ***File name*** and ***File creation date*** information can be linked to non-default information fields (if defined in the database).

The option ***Create new entries*** should normally be checked during import. It can be unchecked to make sure that no new entries are being added during an import of previously analyzed data.

If ***Overwrite existing entries*** is checked, samples with the same name as in the data file can be overwritten.

Checking ***Select imported entries*** will select imported samples. This feature makes it easier to retrieve the last imported samples in large databases.

Pressing <***Finish***> will start the import.

Chapter 3

Melting curve analysis

3.1 The SmartFinder analysis window

After an import, the *SmartFinder analysis* window (see Figure 3.2) appears for the imported samples.

Alternatively, the *SmartFinder analysis* window can be opened for any selection of entries with ***SmartFinder*** > ***Analyze data***. If more than one kit is defined in the database, the *Select SmartFinder kit* dialog box is displayed (see Figure 3.1).

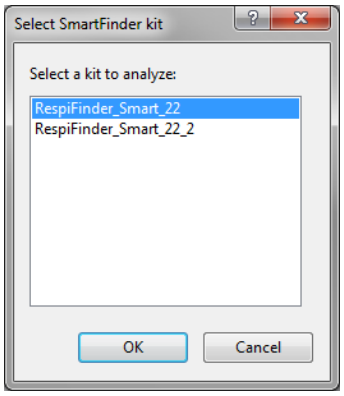


Figure 3.1: The *Select SmartFinder kit* dialog box.

Select a kit to analyze from the list and press <***OK***> to open the *SmartFinder analysis* window.

The *SmartFinder analysis* window consists of three panels:

- The *Entries panel* displays for each of the samples the same information fields as in the *Main* window and a colored cell per probe in the probe mix. The colors are determined in the character type experiment that corresponds to the probe mix. By default, they are green for a negative reaction and red for a positive reaction.
- The *Analysis panel* displays the curves for the highlighted entry in the *Entries panel*. They are organized in columns by reaction (Mix 1 and Mix 2) and in rows by channel (Rox, Cy5 and BHQ). See 3.2 for the display options of this panel.
- The *Report panel* shows a detailed report for the highlighted entry in the *Entries panel*. The report contains information fields, the results (positive or negative) for each of the probes, the analysis settings used and miniatures of the curves. For more information about reports, see 3.6.

With ***File*** > ***Exit***, the *SmartFinder analysis* window can be closed again.

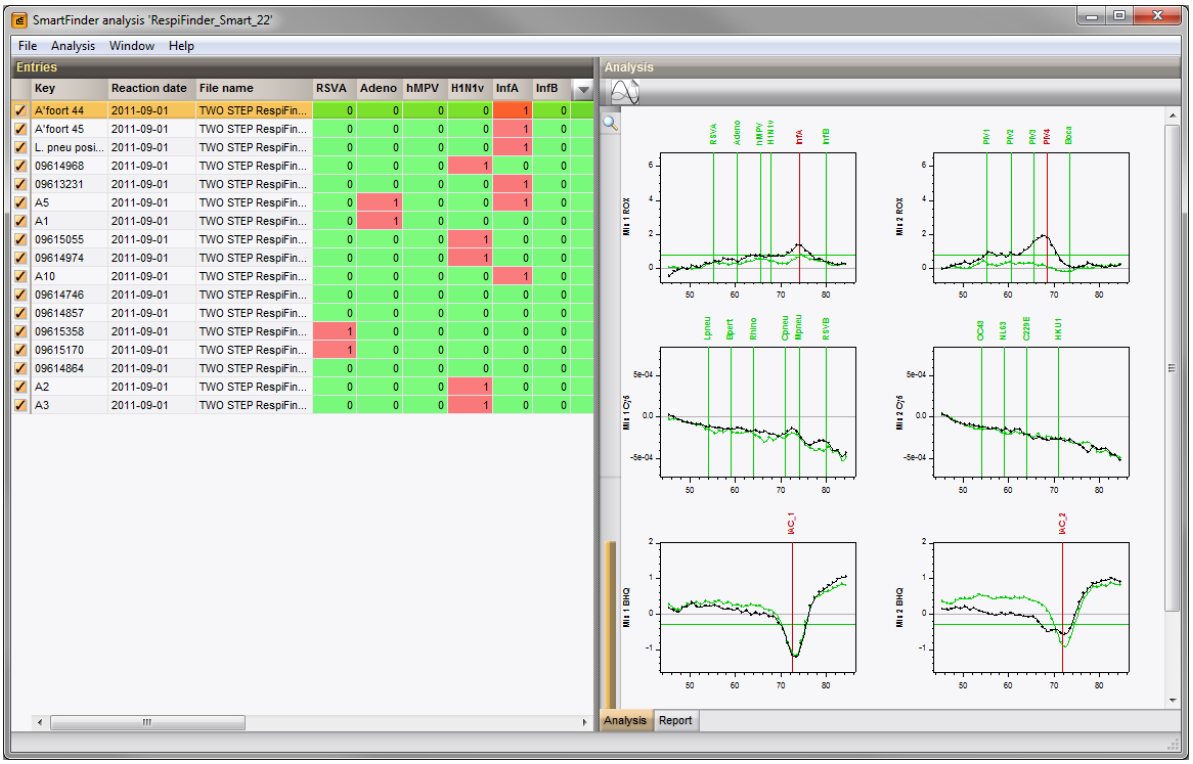


Figure 3.2: The *SmartFinder analysis* window.

3.2 Display settings

A number of display settings for the *Analysis panel* can be specified via ***Analysis*** > ***Display settings***. This pops up the *SmartFinder display settings* dialog box (see Figure 3.3).

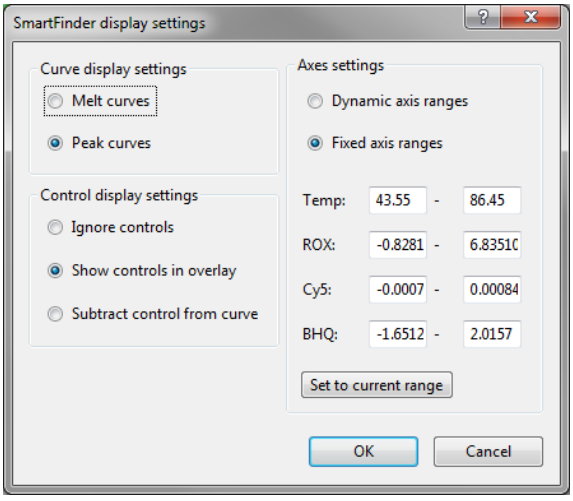


Figure 3.3: The *SmartFinder display settings* dialog box.

Under ***Curve display settings*** either the raw data (***Melt curves***) or the derived ***Peak curves*** can be selected for display.

The ***Control display settings*** deal with the way that the negative controls are being treated. Three different options are available:

- ***Ignore controls***: Negative controls are displayed nor subtracted.
- ***Show controls in overlay***: The negative control for each sample is displayed as a green curve on the plot.
- ***Subtract control from curve***: The negative control curve is subtracted from the sample curve before the sample curve is plotted.

The ***Axes settings*** determine the ranges for the X-axis (Temperature) and Y-axes (signal from the ROX, Cy5 or BHQ channels) used on the plots. The default setting is ***Dynamic axis ranges***, which uses the minimum and maximum values of the currently plotted samples as respectively the lower and upper limits for the axis ranges. When ***Fixed axis ranges*** is checked, lower and upper limits can be entered for ***Temp*** (= X-axis), ***ROX***, ***Cy5***, and ***BHQ***. By pressing the <***Set to current range***>, the minimum and maximum values of the currently plotted samples are automatically filled in.

To set a sensible fixed axis range for a complete data file, first select all samples from the file and display the corresponding curves on the plot as an inactive overlay with ***Analysis*** > ***Toggle overlay selected*** (). Next, call the display settings again, check ***Fixed axis ranges*** and press <***Set to current range***>.

In addition to the negative controls, it can be useful to display other samples in overlay of the currently active sample. This makes it easier to discriminate ”real” peaks from background noise in case of low signals. Selected samples can be shown in an inactive overlay with ***Analyze*** > ***Toggle overlay selected*** or by pressing the  button. Executing the command again removes the overlay from display.

3.3 Automatic calling

During import (see 2), samples are automatically called. In the *SmartFinder analysis* window, an automatic calling can be performed via ***Analysis*** > ***Automatic calling***. The *SmartFinder analysis settings* dialog box (see Figure 3.4) will pop up.

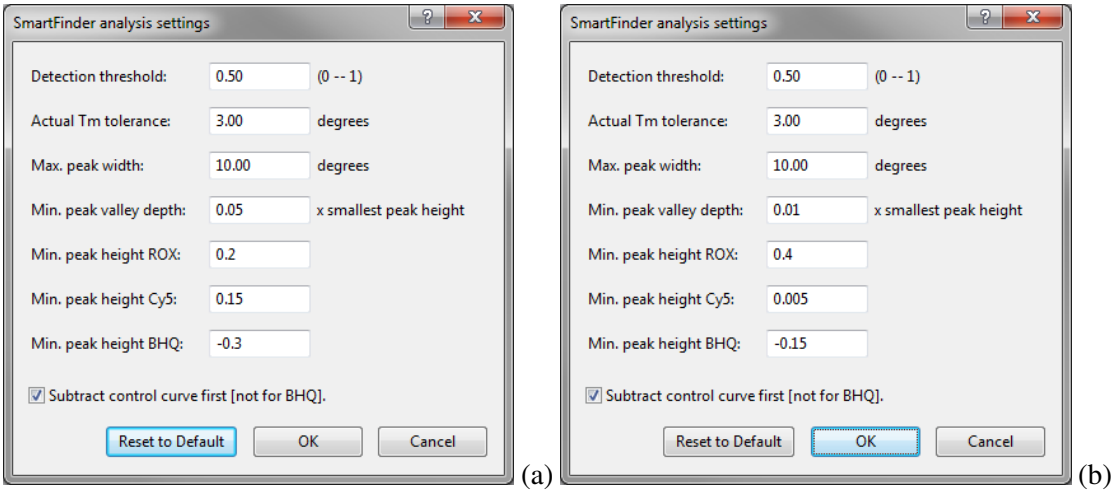


Figure 3.4: The *SmartFinder analysis settings* dialog box, showing the optimized settings for LightCycler 480 (a) and Rotor-Gene data (b).

The ***Detection threshold*** can vary from 0 to 1 and is a parameter for peak shape. The lower this value, the more sensitive the algorithm becomes for peaks, i.e. even shapes that differ only slightly from a straight line will be considered as peaks.

The ***Actual Tm tolerance*** determines how much the peak position can vary from the Actual T_m as set during melt temperature calibration (see 3.5). For example, the default setting of 3° Celsius means that peaks are searched in the range $ActualT_m - 1.5°C \leq T_m \leq ActualT_m + 1.5°C$.

With the ***Max. peak width*** setting, probe peaks can be discriminated from background elevations. The ***Max. peak width*** is measured as the full peak width at half height. Any peak that is wider than this value, will be disregarded.

The ***Min. peak valley depth*** is used to discriminate overlapping peaks, originating from two neighboring probes. This parameter is expressed as a fraction of the smallest peak height.

Using ***Min. peak height ROX***, ***Min. peak height Cy5*** and ***Min. peak height BHQ***, absolute thresholds can be entered for peak detection in the respective channels. Peak heights are measured as the distance of the top of the peak to the local background signal.

When the option ***Subtract control curve first*** is checked, the negative control curve is subtracted from the sample curve prior to peak detection. When negative control curves are available, it is highly recommended to check this option as it will improve automatic peak detection. For detection of the IAC peak, the negative control is never subtracted.

With <***Reset to Default***>, all settings will be reset to their default values, i.e. the settings as displayed in Figure 3.4 (a).

3.4 Manual calling

Since an automatic calling is prone to errors, the result should be verified visually and – if needed – the calls should be manually adjusted.

By clicking on a sample in the *Entries panel* or by using the up or down arrows on the keyboard, the *Analysis panel* is updated with the curves of the highlighted sample.

A call can be modified manually by clicking on the probe position in the corresponding curve to highlight it and selecting ***Analysis*** > ***Change call (selected pathogen)***. Alternatively, simply hold the **Ctrl**-key on the keyboard and click the probe position to toggle the call.

When a call is changed, the changes are immediately propagated to the database.

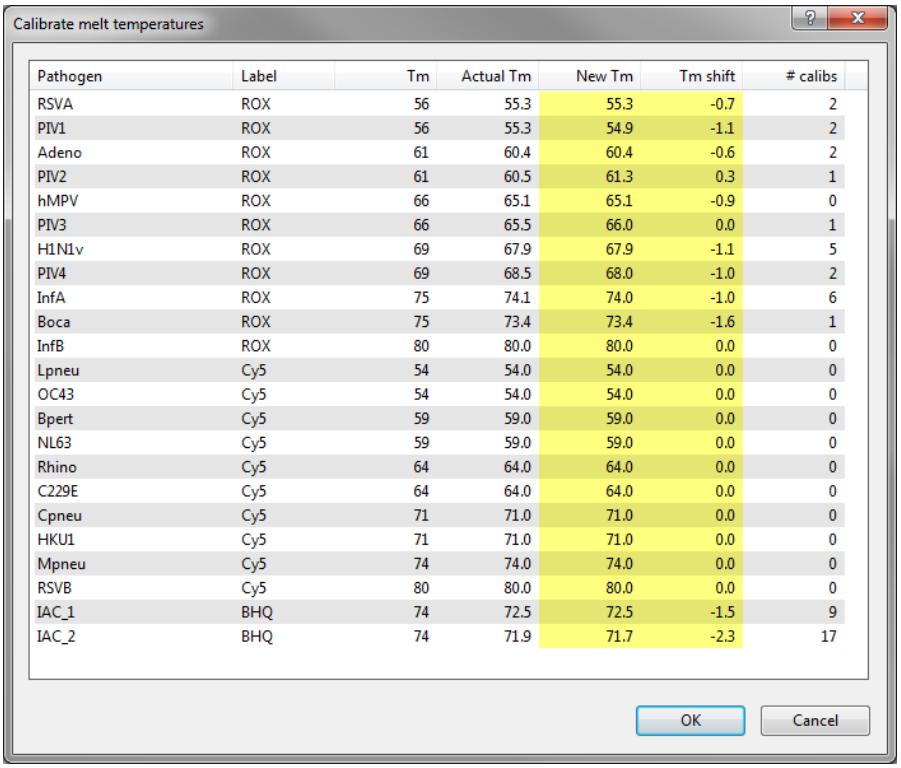
3.5 Calibrating melting temperatures

The observed melting temperatures of the probes in the mix varies from the theoretical melting temperatures by an extent that is dependent on the apparatus used. Therefore, the first time a positive control sample is run, the melting temperatures need to be calibrated. After an overhaul of the PCR apparatus, it might be necessary to repeat this calibration.

First, a set of positive control samples needs to be imported (see 2 for instructions).

Next, select ***Analysis*** > ***Calibrate melting temperatures*** to pop up the *Calibrate melt temperatures* dialog box (see Figure 3.5).

The dialog box displays for each probe in the probe mix its name ('Pathogen'), the fluorophore used ('Label', i.e. ROX, Cy5 or BHQ), the theoretical melting temperature or T_m ('Tm'), the observed T_m ('Actual Tm') that was used to determine the probe positions, the T_m that was calculated on the currently loaded samples ('New Tm'), the difference between the theoretical T_m and the new T_m ('Tm shift'), and the number of measurements on which the new T_m is calculated ('# calibs'). The columns 'New Tm' and 'Tm shift' (highlighted in yellow) can be edited and are synchronized: editing either field will dynamically update the other. Manual editing allows fine-tuning of the probe positions and/or adjusting the melting temperature of



Pathogen	Label	Tm	Actual Tm	New Tm	Tm shift	# calibs
RSVA	ROX	56	55.3	55.3	-0.7	2
PIV1	ROX	56	55.3	54.9	-1.1	2
Adeno	ROX	61	60.4	60.4	-0.6	2
PIV2	ROX	61	60.5	61.3	0.3	1
hMPV	ROX	66	65.1	65.1	-0.9	0
PIV3	ROX	66	65.5	66.0	0.0	1
H1N1v	ROX	69	67.9	67.9	-1.1	5
PIV4	ROX	69	68.5	68.0	-1.0	2
InfA	ROX	75	74.1	74.0	-1.0	6
Boca	ROX	75	73.4	73.4	-1.6	1
InfB	ROX	80	80.0	80.0	0.0	0
Lpneu	Cy5	54	54.0	54.0	0.0	0
OC43	Cy5	54	54.0	54.0	0.0	0
Bpert	Cy5	59	59.0	59.0	0.0	0
NL63	Cy5	59	59.0	59.0	0.0	0
Rhino	Cy5	64	64.0	64.0	0.0	0
C229E	Cy5	64	64.0	64.0	0.0	0
Cpneu	Cy5	71	71.0	71.0	0.0	0
HKU1	Cy5	71	71.0	71.0	0.0	0
Mpneu	Cy5	74	74.0	74.0	0.0	0
RSVB	Cy5	80	80.0	80.0	0.0	0
IAC_1	BHQ	74	72.5	72.5	-1.5	9
IAC_2	BHQ	74	71.9	71.7	-2.3	17

Figure 3.5: The *Calibrate melt temperatures* dialog box.

probes for which there is no positive control sample available.

Pressing <***OK***> will update the 'Actual Tm' values and therefore the probes positions (green and red vertical lines) in the melting curves.

To exit the *Calibrate melt temperatures* dialog box without making any changes, press the <***Cancel***> button.

The actual T_m can also be set in the 'Actual Tm' character information field in the *Character type* window of the character type experiment that is named after the SmartFinder kit. If the T_m of a single probe needs to be adjusted, this editing method is actually more convenient than via the *Calibrate melt temperatures* dialog box.

3.6 Reporting options

The *Report panel* shows a detailed report for an individual sample, in casu the highlighted entry in the *Entries panel* (see Figure 3.6). When the active entry in the latter panel is changed, the *Report panel* is updated to reflect this change.

The **Patient info** in the report consists of active information fields in the *Entries panel* of the *SmartFinder analysis* window. Please note that setting the active fields for the *Entries panel* will only result in an updated *Report panel* after highlighting a different entry.

The **SmartFinder results** section summarizes the test by listing all pathogens for which the sample was found positive.

More detailed results are reported in the **SmartFinder analysis** section:

- **Automatic calling settings** lists the version of the calling algorithm and all settings used (see 3.3) at the moment the automatic call was performed.

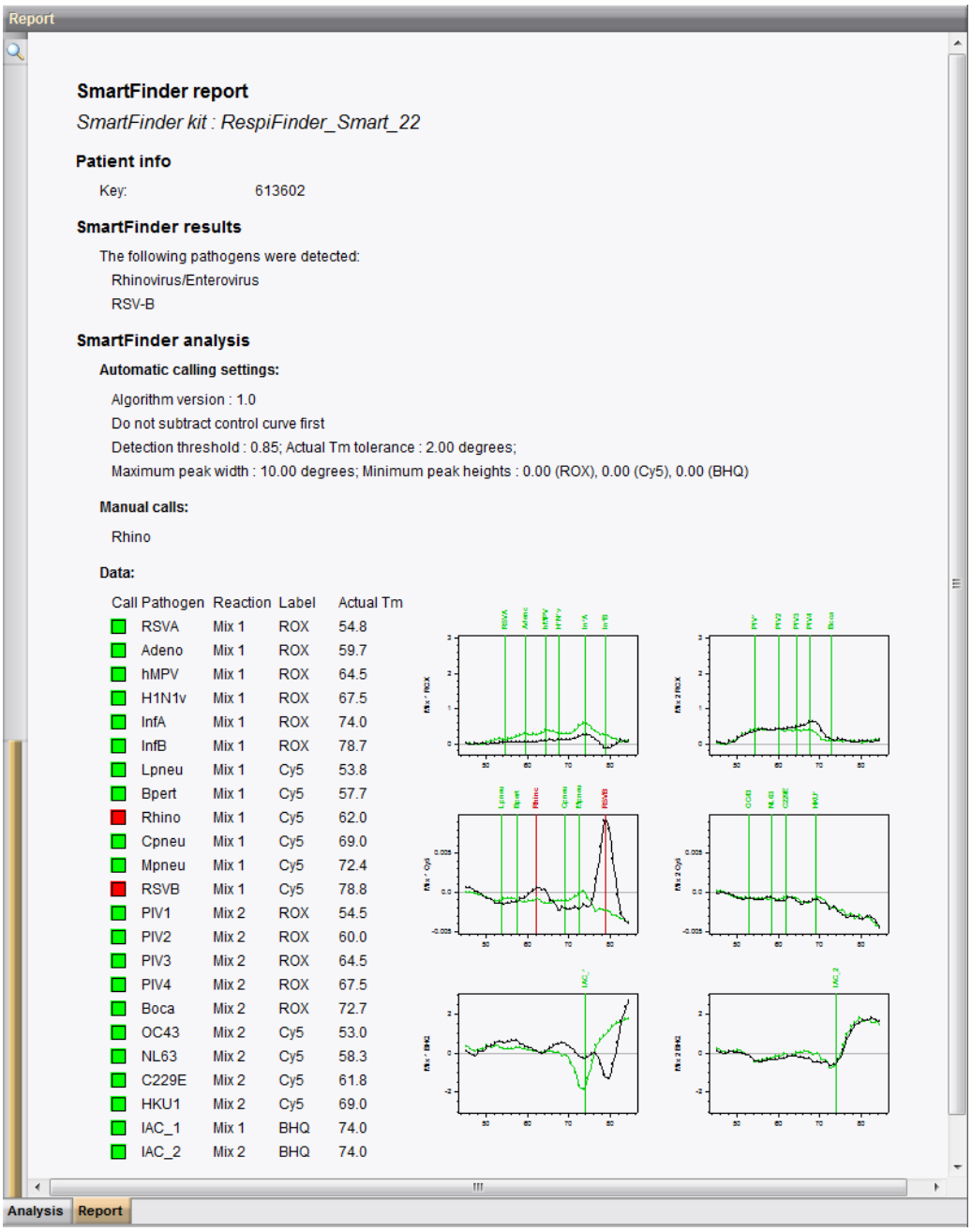


Figure 3.6: The *Report panel* in the *SmartFinder analysis* window.

- **Manual calls** will list each manual override of a call (see 3.4).
- **Data** indicates the outcome (positive or negative) for each of the tests using colors. Furthermore, the name of the detected pathogen, reaction (Mix 1 or Mix 2), fluorescent label and actual T_m used for automatic calling are indicated for each test. As a visual check for the calling, miniatures of the plots from the *Analysis panel* (see 3.1) are included.

Positive tests are red and negative tests are green. However, these default colors can be changed in the character type settings, if required.

The content from the *Analysis panel* (i.e. the plots for the currently highlighted sample) can be exported as an enhanced metafile to the Windows clipboard with ***File*** $>$ ***Copy analysis to clipboard***. From the clipboard, it can then be pasted into Microsoft programs such as e.g. Word or PowerPoint.

Similarly, the content from the *Report panel* can be exported with ***File*** $>$ ***Copy report to clipboard***. This can be useful to produce customized reports with specific headers and footers, i.e. containing the logo of the hospital, contact information of the lab, etc..

A report can also be printed directly with ***File*** $>$ ***Print report***, which will display a dialog box with the printer settings.

APPLIED MATHS

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