DTMASTER

User Guide

Version 1.1

Document R004-01

DNA-Technology, LLC

CONTENTS

Introduction 4
Chapter 1 Purpose of DTmaster Software 5
Chapter 2 Software and Hardware Requirements 6
Chapter 3 Setting Up 7
DTmaster Software Installation 7
Software Update 7
Running DTmaster 7
Chapter 4 Working in DTmaster 10
Description of the DTmaster Menu 10
Protocol Mode 13
Creating and Editing Tests 13
Creating a Temperature Program 20
Creating a Protocol 27
Additional Features of Protocol Mode 41
Run Mode 43
Stages of Work in the Run Mode 44
Upload of Protocol 44
View Details about the Selected Protocol 45
Connecting the Real-Time PCR Instrument 46
Positioning the Tubes in the Real-Time PCR Instrument 49
Running the Analysis 49
Viewing the Analysis Results in Real Time 51
Additional Features of Run Mode 51
Analysis Mode 52
Selecting a Protocol to View the Analysis Results 54
Viewing the Analysis Results 55
Fluorescence Level Graphs 57
Viewing the Results 62
Generation of the Report 80
Saving a Protocol to File 83
Exporting Data 83
Additional Features of the Analysis Mode 84
Accessibility of Users with Administrator Rights 88
Setting up User Accounts 88
Basic Protocol Settings 91
Configuring DTmaster Interface 91
Configuring Protocol Name Template 91
Viewing Video Archive 92
Interaction with LIS 93
Using a Scenario 94
Viewing Module Details 94
Exiting DTmaster 94
Chapter 5 Real-time PCR Instrument Setup and Diagnostics 95
Checking the Geometric Settings of the Optical System 95
Checking the Purity of the Wells 97

Selecting exposure correction factors 97
Measuring the Height of the Tubes 98
Chapter 6 Emergencies 99
Chapter 7 Technical Support 102
Appendix A. Test Parameters 103
A.1. Preliminary Analysis 103
A.2. Qualitative Test Parameters 105
A.3. Quantitative Test Parameters 106
A.4. Relative Test Parameters 107
A.5. HRM Test Parameters 107
Appendix B. User Permissions 109
Contact Information 111
Glossary 112

INTRODUCTION

DTmaster software is intended for the analysis of data obtained from DT series detection thermal cyclers manufactured by DNA-Technology Research&Production, LLC and thermal cyclers of other manufacturers that support results upload in RDML (Real-time PCR Data Markup Language) format, in order to obtain reports with the results of the test.

The software is intended for use in clinical diagnostic laboratories of medical institutions and research practice

CHAPTER 1 PURPOSE OF DTMASTER SOFTWARE

The software is designed to control the operation of DT series real-time PCR instruments manufactured by "DNA-Technology", LLC.

The following operations can be performed using DTmaster software:

1. Protocol creation.
2. Creating and editing tests.
3. Creating, launching, and monitoring of the temperature program.
4. Analysis of optical measurements data.
5. Data analysis report.
6. RDML data exchange support.
7. Checking geometrical and optical settings of the real-time PCR instrument.
8. Settings and diagnostics of the real-time PCR instrument.
9. Interaction with laboratory information systems (LIS).

CHAPTER 2 SOFTWARE AND HARDWARE REQUIREMENTS

Correct functioning of the DTmaster software is possible provided that the minimum hardware and system software requirements for the personal computer (PC) are met.

Operating system requirements: Windows 10 and above.

The minimum hardware requirements are shown in table 1.

Table 1 – Minimum hardware requirements for the user's work PC

Parameter	Requirement
Processor	Intel Core i3 2100 / AMD Ryzen 3 1200 or higher
RAM capacity	4 GB
Free disk space	From 64 GB
Video adapter	Operation with a resolution no lower than 1024x768
Video monitor	LCD monitor with a working resolution of at least 1024x768
Keyboard	A keyboard compatible with the user's PC is required
Mouse	A two-button mouse is required

CHAPTER 3 SETTING UP

DTMASTER SOFTWARE INSTALLATION

To install DTmaster, run the version 1.1 installation file.

The installation file that is supplied on a USB flash drive, can also be obtained from the Internet.

The DTmaster Setup Wizard window appears on the screen.

Complete the software installation following the wizard's prompts. To go to the next step of the installation, use the **Next** button, to go to the previous step - use the **Back** button. Click the **Cancel** button to close the installation wizard (without installing the software on your computer).

Upon completion of the installation process, a message will be displayed in the wizard window.

Click the **Finish** button to exit the installation wizard.

As a result of installing DTmaster on the user's PC:

1. A DTmaster shortcut will appear on the Desktop to launch the program.
2. The "DTmaster" directory will be displayed on the Start menu (unless otherwise was selected during installation) containing a shortcut to launch the DTmaster program.

SOFTWARE UPDATE

The latest version of the DTmaster software is available on the official website of "DNA-Technology", LLC.

For installation and updating of the DTmaster software, contact the Customer Support:

+7 (495) 640-16-93 (for CIS countries and abroad, the charge applies),

8-800-200-75-15 (free call from within Russia).

Email: hotline@dna-technology.ru

RUNNING DTMASTER

DTmaster can be ran in two ways:

1. Use the Windows Start menu:

   Start → All Programs → DTmaster → DTmaster

2. Use the 

icon on the Desktop.

After running the software, the user authorization window will appear on the screen. By default, for the first time the program is launched as "admin1" (fig. 1).

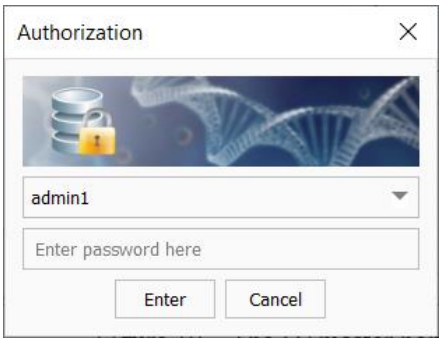


Figure 1 – User authorization window

Enter the password to continue.

The preset password for the first run of the program is specified in the documentation included in the delivery real-time PCR instrument set. Subsequently, this password can be changed or canceled (see par. Setting up User Accounts).

After entering the password, the DTmaster home screen will be displayed (fig. 2).

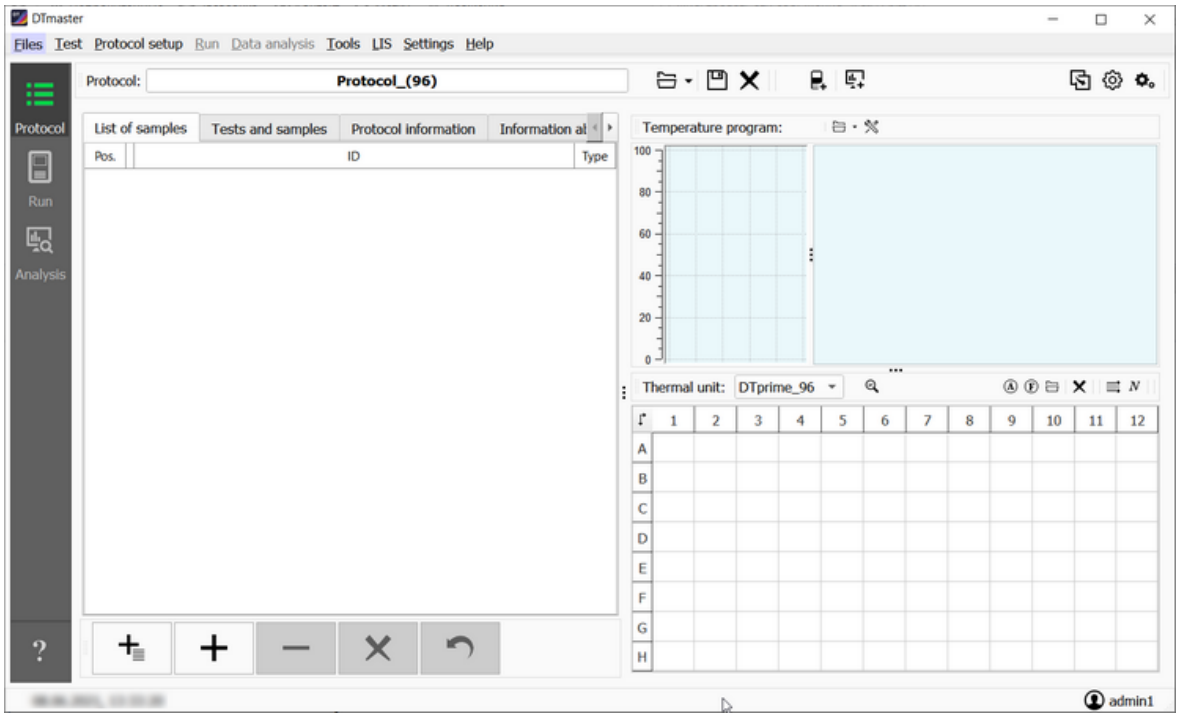


Figure 2 – The DTmaster home screen

If the password set for "admin1" was not entered, but the **Login** button was clicked, a warning will appear on the screen: "Please input correct password".

If the password is not entered, then when you click the **Cancel** button, a message about the possibility of work under the Guest account will appear on the screen.

To run the program as "Guest", select a "Guest" in the authorization window from the drop-down list box, and then click the **Login** button. DTmaster software will be launched, but with limited access rights to different functions. By default, for this user, the protocol can be loaded into the **Protocol** and **Analysis** modes for viewing. In the future, the set of permissions for the account can be changed by the administrator. For more details on creating and configuring user accounts, see par. Setting up User Accounts.

Click the **Login** button to start using the program.

CHAPTER 4 WORKING IN DTMASTER

DESCRIPTION OF THE DTMASTER MENU

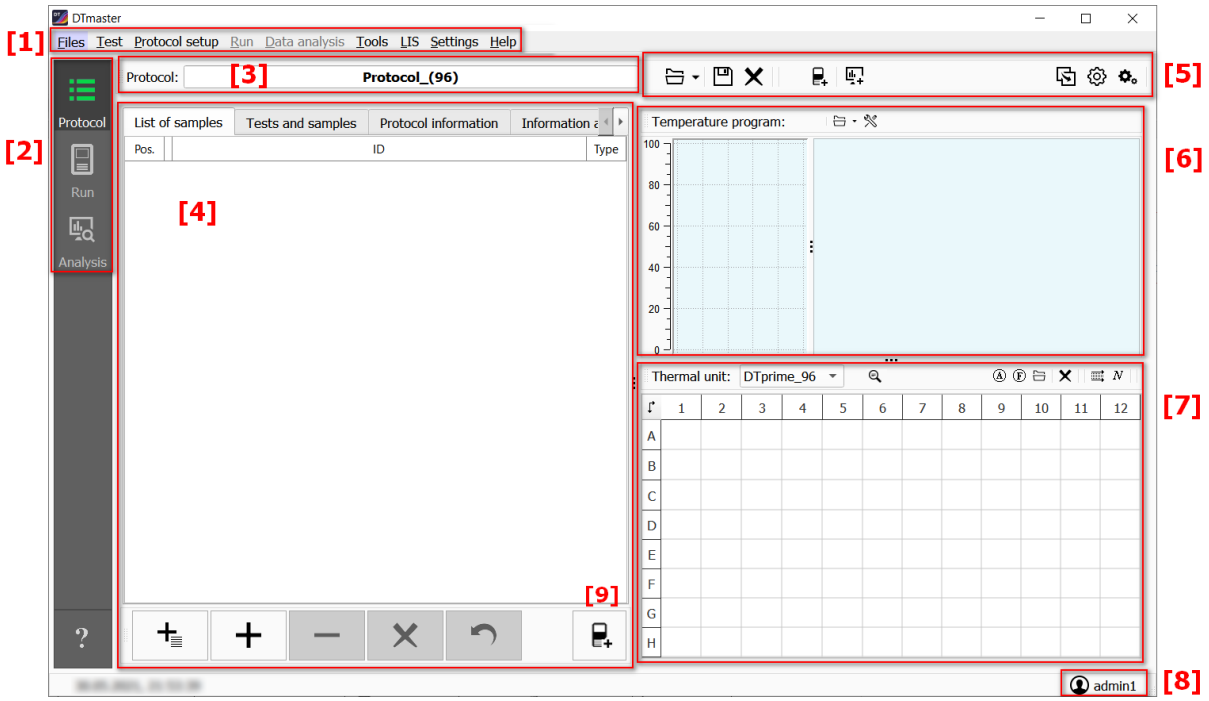


Figure 3 – DTmaster welcome page

The DTmaster welcome page includes (fig. 3):

[1] – programs menu (table 2);

[2] – buttons for switching between different modes;

[3] – field for entering/displaying the name of the protocol;

[4] – workspace for forming a list of samples;

[5] – toolbar;

[6] – temperature program workspace;

[7] – workspace for selecting a thermal unit and placing test tubes;

[8] – the account of the authorized user;

[9] – the button to load the protocol into the Run mode

Table 2 – DTmaster menu

Section	Structure	Legend	Use	More details
Files – selection of the current operating mode in DTmaster	Protocol		Switching to the Protocol mode	par. Creating a Protocol
	Run		Switching to the Run mode	par. Upload of Protocol
	Analysis		Switching to the Analysis mode	par. Analysis Mode
	Temperature		Switching to the window for	par. Temperature

Section	Structure	Legend	Use	More details
	program editor		creating and editing the temperature program	Program Editor
	View videoarchive		Switching to the window "Image Viewing"	par. Viewing Video Archive
	Exit		Exiting from DTmaster	par. Exiting DTmaster
Test – formation of a set of tests used in everyday work	Test block copying		Loading a group of tests into the program	par. Copy Block Tests/Containers
	Edit test		Creating, editing and deleting tests	par. Edit Tests
Protocol setup – formation and configuration of the Run file	Open protocol		Uploading a previously saved protocol	par. Loading Protocol into DTmaster
	Open XML protocol		Downloading the protocol generated in the DTmaster version 7	par. Loading Protocol into DTmaster
	Save as template		Saving the prepared protocol to .rt file	par. Saving a Template
	Clear protocol		Deleting the protocol data from the program window	par. Deleting Protocol
	Upload to the Run window		Uploading the prepared protocol to the Run mode	par. Upload of Protocol
	Upload to the Analysis window		Uploading the prepared protocol to the Analysis mode	–
	Edit protocol settings		Editing the advanced settings of the protocol	par. Basic Protocol Settings
Run – connecting and configuring the real-time PCR instrument, run the test according to the selected protocol	Select protocol		Loading the protocol into the program in .rt format	par. Upload of Protocol
	Select device		Choosing a real-time PCR instrument to connect to DTmaster	par. Connecting the Real-Time PCR Instrument
Data analysis – viewing the results of the test, generating a report with	Open protocol		Loading the protocol into the program in .rt, .r96, .r48, *.192 or .384 format	par. Selecting a Protocol to View the Analysis Results
	Save as		Saving the results of the test to a .rt file	par. Saving a Protocol to File

Section	Structure	Legend	Use	More details
the results	Protocol information		Viewing general information about the protocol and temperature program	par. Selecting a Protocol to View the Analysis Results
	Preliminary analysis report		Loading the preliminary analysis report window	par. Generation of the Report with Preliminary Analysis Results
	Specific report		Loading the report viewing window	par. Generation of the Basic Analysis Report
	Export results		Exporting results of the test to XML file	par. Exporting Data
	Email		Sending a message to Customer Support	par. Technical Support
	Load to protocol		Loading data about the temperature program, about the samples and their placement into the Protocol mode	–
	Reboot optical data in another protocol		Loading optical data from the current protocol into the protocol with the correct placement of tubes and the required test	par. Loading Optical Data on a Different Protocol
	Edit tests in the protocol		Editing test parameters for a loaded protocol	par. Editing Test Parameters
	Edit protocol settings		Editing loaded protocol settings	par. Basic Protocol Settings
	RDML import		Uploading the protocol to DTmaster in RDML format	par. Selecting a Protocol to View the Analysis Results
	RDML export		Export of amplification results in RDML format	par. Exporting Data
Tools	Script	–	Conducting research according to the planned scenario	–
LIS – setting up a connection to a remote server and interaction with it (receiving	Get the Protocol for execution	–	Loading a protocol into DTmaster program for carrying out amplification from LIS	par. Obtaining a Protocol for Amplification
	Get the Protocol with results		Obtaining a protocol with the results of PCR test in order to analyze the data obtained from the device	par. Obtaining a Protocol with the Analysis Results

Section	Structure	Legend	Use	More details
protocols, sending results)	Send the Protocol with results		Sending a protocol with the results of the test to LIS	par. Sending a Protocol with the Analysis Results
Setting	General settings	–	General DTmaster settings: language and font, protocol name template	par. Configuring DTmaster Interface, Configuring Protocol Name Template
	Log in		Create and configure user accounts, change the current authorized user	par. Setting up User Accounts
	LIS export settings	–	Editing settings for interaction with LIS	par. Exporting Data
Help – help on working with software, data on the current version of the software	DTmaster Help	–	Display CHM file with User manual	–
	About application		Data on the creation of the program and the date of the last update	–
	About modules		Information about installed modules, their version and size	par. Viewing Module Details

PROTOCOL MODE

CREATING AND EDITING TESTS

Test – a set of all Run parameters that are necessary for PCR and subsequent analysis of the results.

Test can be:

1. Created by the user using the basic test types, pre-installed in DTmaster.
2. Added by copying tests previously created by other users.

Creation and addition of tests is implemented in the **Protocol** mode.

Test creation and editing is carried out in the "Test editor" window (see par. Edit Tests), which can be opened in one of the following ways:

- click the **Edit test** button  , on the toolbar;
- on the **Test** menu, click **Edit test**.

Test is added in the "Copy a group of tests" window (see par. Copy Block Tests/Containers), which can be opened in one of the following ways:

- click the **Test block coping** button  , on the toolbar;
- on the **Test** menu, click **Test block coping**.

Attention! The **Edit** test and the **Test block coping** menu options are available to users who have the appropriate permission settings: "Edit test data" and "Copy block tests" (see par. Creating a Permission Set for User).

Tests are saved in the **Tests list** available for a specific user or group of users in the "Test editor" window.

Basic tests include:

- qualitative;
- quantitative;
- relative;
- HRM.

Test settings are described in Appendix A.

The user can group several tests with the same temperature program and analysis type into a **test container**. When you create a protocol, tests that are combined in a container are added together.

Edit Tests

The test editor is intended for creating, copying, editing and saving tests.

The "Test editor" window contains (fig. 4):

[1] – Tests list area;

[2] – toolbar;

[3] – area for viewing and editing the selected test.

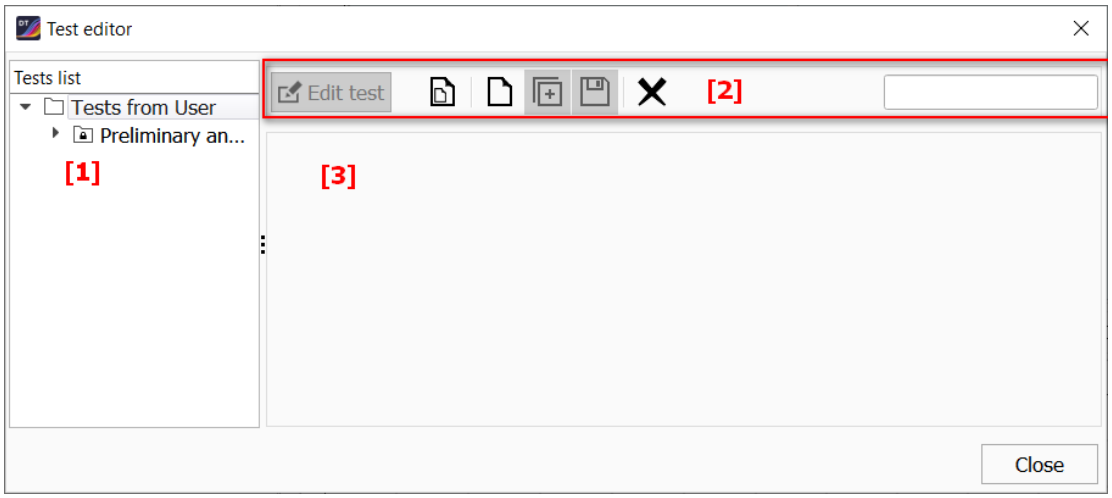


Figure 4 – "Test editor" window

The list of tests can include two groups of tests:

1. Basic type tests.
2. Containers.

The toolbar of the "Test editor" contains buttons, which are described in table 3.

Table 3 – Buttons on the toolbar of the "Test Editor" and their purpose

Name	Symbol	Use
New container		Creation of test containers - combining several tests into a group with the same temperature program and type of analysis for subsequent joint loading into the protocol
New test		Creation of new tests using basic (predefined) test types or test types of additional analysis modules
Copy test		1. Copying a previously created test for: • creating a test with a new name and settings of the original test; • creating a new test by editing the settings of the original test; 2. Copying a previously created container for: • creating a container with a new name, but fully preserved set of tests of the original container; • creating a new container by changing the composition of the tests included in the original container.
Save test		• saving created or edited test; • saving created or edited container
Edit test		Changing the parameters of a previously created test. Changing the composition of the container
Clear draft		Removing information from the "Test Editor" to select a new action

When working with the selected test, the test parameters are displayed in the Test Editor window – a set of tabs containing information about test settings. The number and composition of tabs depends on the type of test.

To view test parameters, select the required test in the Tests list and click the Edit test button. The parameters of the selected test will be displayed in the workspace of the "Test editor" window (fig. 5).

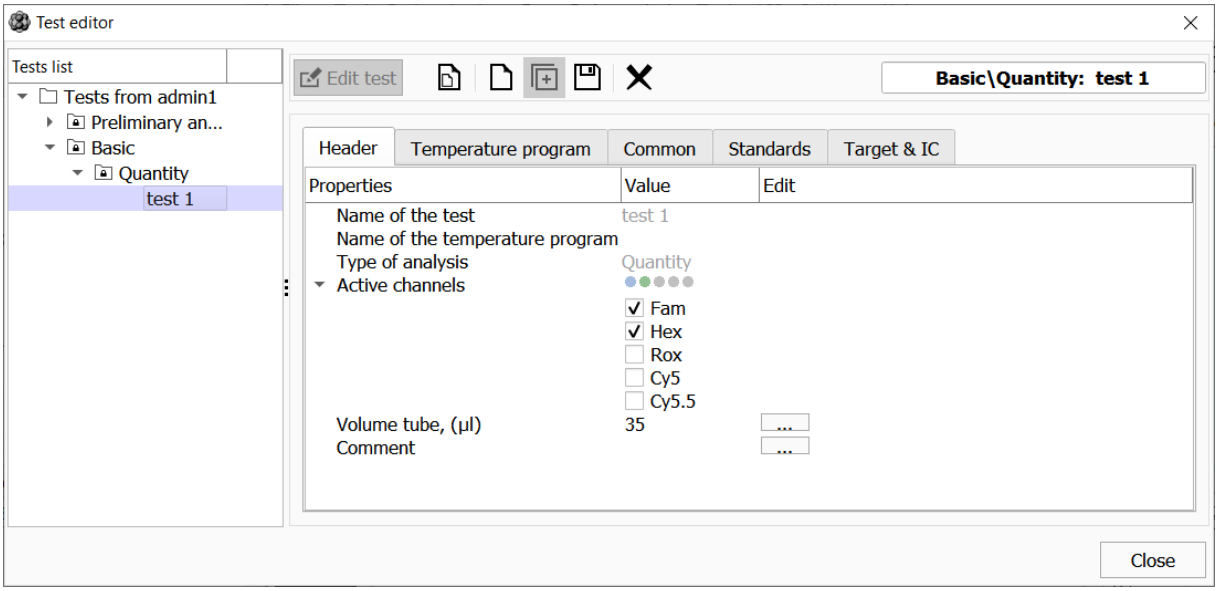


Figure 5 – View test parameters

Parameters for test are described in Appendix A.

Creating a Test

To create a test:

1. Click the **New test** button . The window for selecting the analysis type of the created test will be displayed.

By default, the list of tests includes basic test types. When the user installs the Library of Analysis Modules for DTmaster, the list will also include test types corresponding to the installed additional analysis modules.

2. Select the test type from the drop-down list box and click the **OK** button.

The window for entering the name of the created test will be displayed.

3. Enter the name of the test in the appropriate field and click the **OK** button. The tabs for setting the parameters of the new test will be displayed in the editor.
4. Specify the test parameters in the appropriate tabs (see Appendix A).
5. Click the **Save test** button located on the editor toolbar. Confirm your choice in the window displayed on the screen.

If the created settings for the new test should be saved, click the **Yes** button. In this case, the new test will be saved and will be automatically added to the **Tests list**.

If the created settings for the new test should not be saved, click the **No** button. In this case, the new test will not be saved and will not be automatically added to the **Tests list**.

Creating a Container with Tests

Container is an option that allows you to combine several tests with the same temperature program and type of analysis in one study, creating batch studies.

To create a container with tests:

1. Click the **New container** button . The window for entering the container name will be displayed.
2. Enter the container name in the **Name of the container field** and click the **OK** button.

The Test Editor workspace will display a list of tests available for merging into a container.

3. Select the tests to be included in the container (fig. 6).

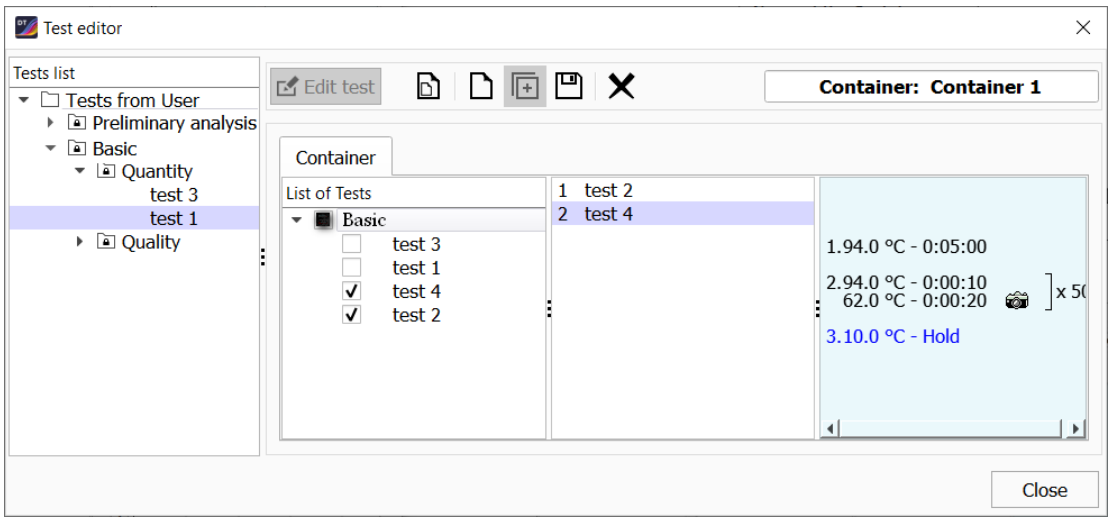


Figure 6 – Selecting tests in the container

When a test is selected, the temperature program is automatically displayed in the right part of the working field. This allows you to choose tests with matching temperature programs.

4. Click the **Save test** button on the toolbar to complete a container creation.

If tests with different temperature programs are selected for one container, message about the mismatch of temperature programs appears at the stage of saving the container.

5. Click the **OK** button, to continue working with the container in the Test Editor.

If all tests for the container are selected correctly, then after clicking the **Save test** button, a confirmation dialog box for saving the container will appear on the screen.

Click the **Yes** button. The container will be saved and automatically added to the **Tests list**.

If the created container should not be saved, click the **No** button. In this case, the new container will not be saved and will not be automatically added to the **Tests list**.

Clearing the Workspace of Test Editor

Using the **Clear draft** button, you can close a record about a test or container opened for editing in the workspace of the "Test editor".

In this case, if the parameters of a test or container were edited and not saved, then when you click the **Clear draft** button, a confirmation dialog box for saving the changes will be displayed on the screen.

Click:

the **Yes** button – the changes will be saved; you can continue working in the "Test Editor" window.

the **No** button – the changes will not be saved; the workspace of the editor will be cleared; the procedure for editing test/container parameters must be repeated or new actions must be started in the "Test editor" window.

the **Cancel** button – will be saved; the filling of the workspace of the editor will be saved; you can continue making changes.

Editing Test/Container

The **Edit test** button allows you to change test setup settings or container composition. This button becomes available after selecting a test or container in the **Tests list**.

There are two ways to edit a test or container in the Test Editor (fig. 7):

[1] – select the required test/container in the **Tests list**, then click the **Edit test** button;

[2] – select the required test/container in the **Tests list**, then right-click on the name of the test/container to call the context menu. Click the **Edit test** option.

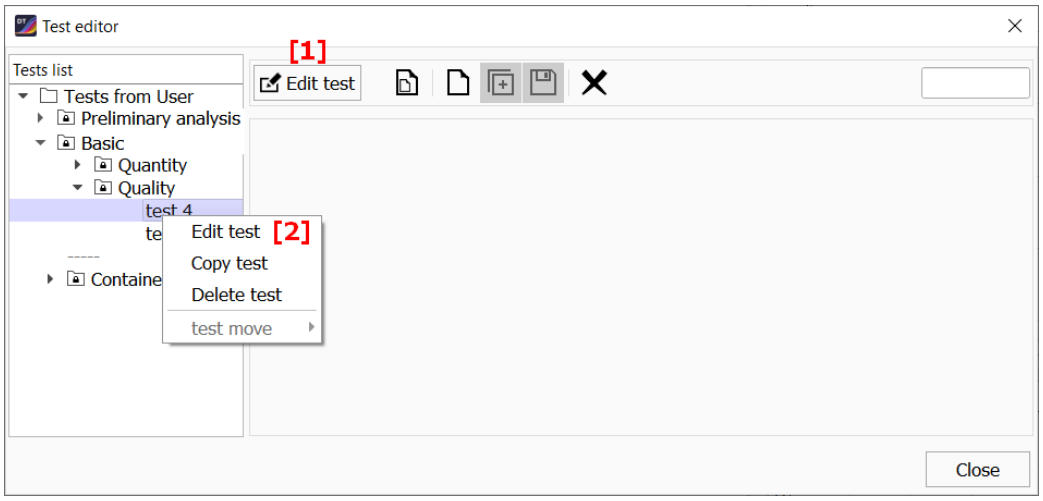


Figure 7 – Methods for editing test/container parameters

Test/container settings are displayed in the workspace of the "Test editor" window.

Copy Test/Container

The **Copy test** button allows you to copy tests or containers previously created and saved in the **Tests List**.

This button becomes active after the required test/container is opened for editing.

There are two ways to copy a test or container in the Test Editor (fig. 8):

[1] – select the required test/container in the **Tests list**, click the **Edit test** and then click the **Copy test button** will become active, click on it.

[2] – select the required test/container in the **Tests list**, and then right-click on the test/container name to bring up the context menu. Click the **Copy test option**.

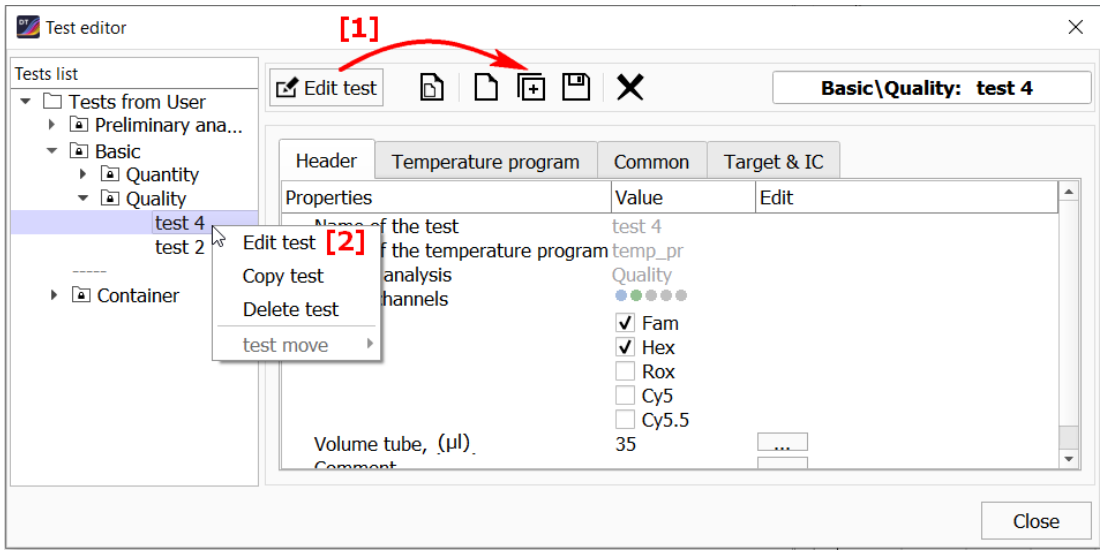


Figure 8 – Ways to copy a test or container

A window for entering the name of a new test/container will appear on the screen.

Specify test name in the **Test Name** field and click the **OK** button.

If the specified name coincides with the name of a previously created test, a warning message will appear on the screen.

Click the **OK** and repeat the test/container copy procedure.

Further, two options are possible:

1. To save a new test with the settings of the original test (for a container – with the original **Tests list**), click the **Save test** button on the toolbar.
2. To save a new test with edited settings (for a container – with a modified **Tests list**), make the necessary changes to the test parameters (edit the contents of the container), and then click the **Save test** button on the toolbar.

A confirmation window for saving the test/container will appear on the screen.

Click the **Yes** button. The copied test/container will be saved and automatically added to the **Tests list**.

Click the **No** button, to continue working with the copied test/container settings.

Removing Test/Container

To remove a test or container from the **Tests list**:

1. Right-click the name of the test/container that should be deleted from the **Tests list**. The context menu will be displayed on the screen.
2. Click the **Delete test** option. The screen will display the confirmation window for deleting the test/container.

If the test/container should be deleted, click the **Yes** button. After that the test/container will be removed from the **Tests list**.

If the test/container should not be deleted, click the **No** button and continue working in the Test Editor.

Copy Block Tests/Containers

The function **Test block copying** is intended for copying one or more tests or containers with tests from the **Test source** to the selected **Test receiver**.

The following can be used as a source of the test:

- another user 's (DTmaster user) test;
- files in XML format;
- protocols saved in .rt and .trt formats.

The directory for copies can be:

- user accounts;
- files in XML format.

Test block copying is implemented in the **Protocol** mode and is carried out in a separate window "Copy a group of tests", which can be opened in one of the following ways (fig. 9):

[1] – click the **Test block copying** button on the toolbar.

[2] – on the **Test** menu, click **Test block copying**.

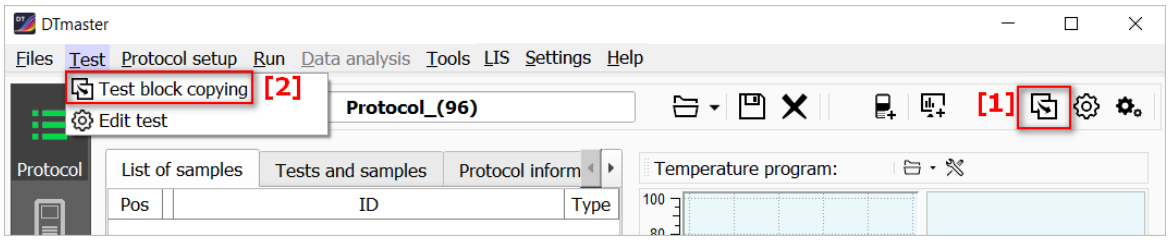


Figure 9 – The Test block copying option in the Protocol mode

The window "Copy a group of tests" will appear on the screen.

To copy a block of tests/containers:

1. Select the source using the drop-down list box on the left side of the window. A source will determine a list of tests and containers available for moving to the Directory.
2. Select the required tests and containers from the list. At the bottom of the window, the number of selected tests/containers will be displayed relative to the total number of records in the source.

   If you need to copy all tests from the selected source, choose "All tests".
3. Using the drop-down list in the right part of the window, select the directory for copying. After selecting a directory, the **Copy** button located at the bottom of the window will become active.
4. Click the **Copy** button. Further actions depend on the selected directory:

If the directory is a user account, then after clicking the **Copy** button, a message about the successful completion of file copying will appear on the screen.

If the directory is "to the XML file", then to successfully copy the selected tests and containers, you must enter the file name and select the folder in which it will be saved.

The copied files will be displayed in the workspace of the Directory.

CREATING A TEMPERATURE PROGRAM

Temperature program – data on the conditions of thermal cycling (temperature and duration of temperature shelves, number of cycles, availability of optical measurements).

The temperature program is displayed in the Temperature program workspace of the **Protocol** and **Run** modes (fig. 10).

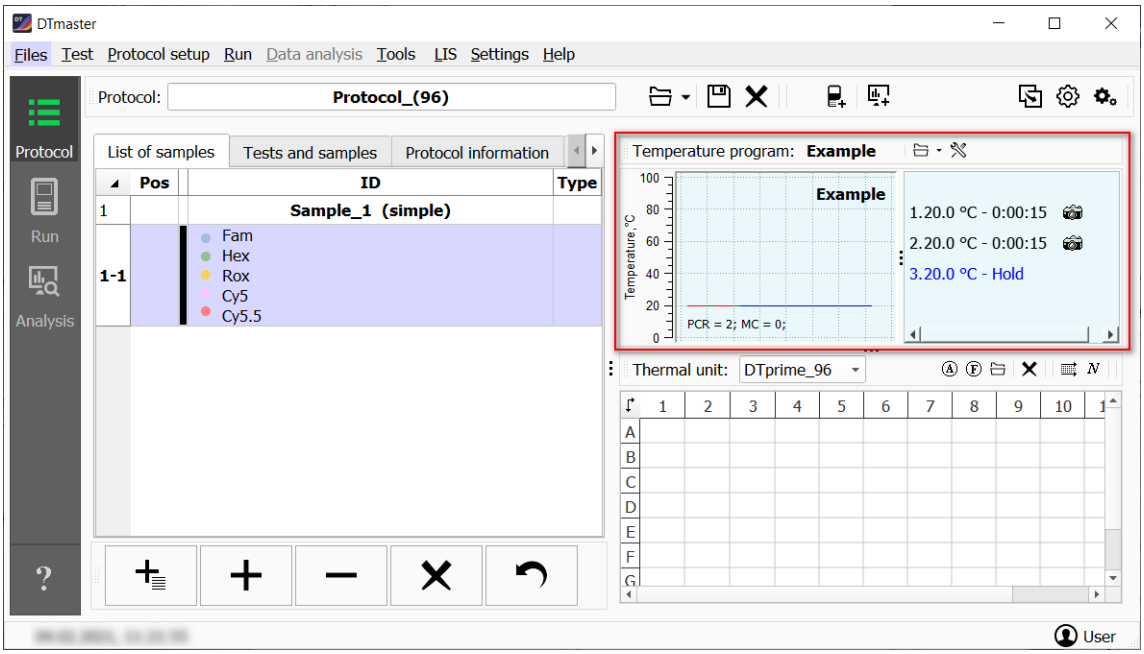


Figure 10 – **Temperature program** workspace in the **Protocol** mode

This workspace provides the following information about the temperature program:

- program name;
- temperature program;
- stages of the temperature program.

When creating a test using the **Test editor**, the temperature program is entered by the user only once (see par. Edit Tests).

When using the **Test block copying** function, the temperature program is loaded into the software automatically (see par. Copy Block Tests/Containers).

In addition, the temperature program can be:

- loaded from a file with a previously prepared temperature program (.rta file) or from a file with a protocol;
- created manually in the Temperature program editor.

Temperature Program Editor

The Temperature Program Editor window can be opened in one of the following ways (fig. 11):

[1] – click the **Edit temperature program** button in the **Temperature program** workspace;

[2] – on the **Files** menu, click **Temperature programs editor**.

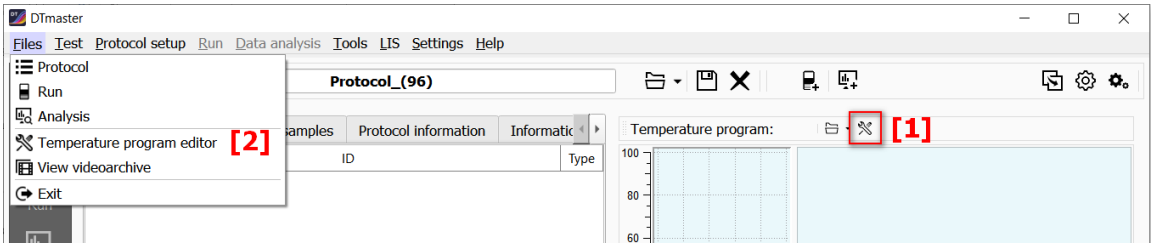


Figure 11 – Launching the Temperature program editor in the **Protocol mode**

The Temperature Program Editor window will appear on the screen (fig. 12), which includes:

[1] – control elements (table 4);

[2] – area of presentation of the temperature program in the form of a table;

[3] – area of presentation of the temperature program in the form of a graph.

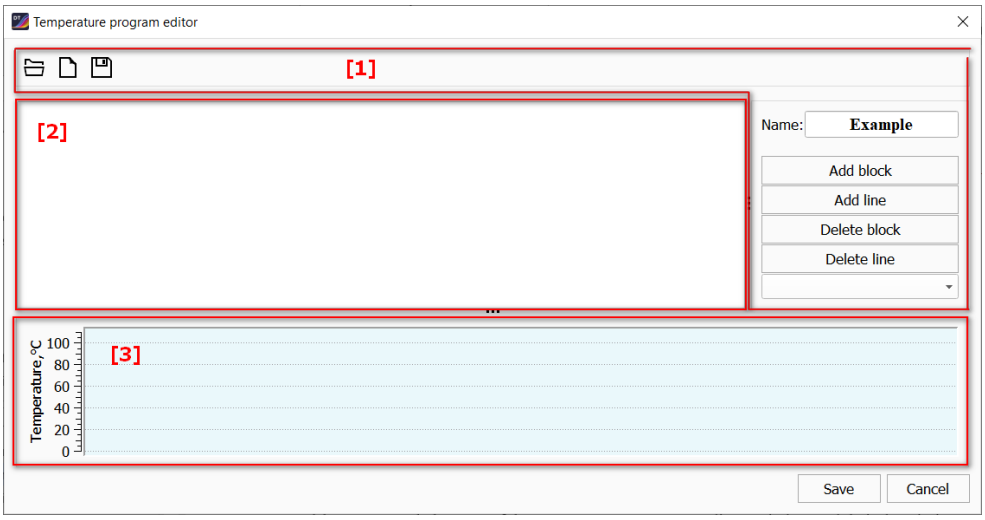


Figure 12 – Window «Temperature Program Editor»

Table 4 – Control elements of the «Temperature Program Editor» window and their description

Name	Symbol	Description
Open Temperature Program		Selection and loading into the Editor of an existing temperature program (from the protocol file or from the file with the temperature program)
New Temperature Program		Manual creation of a temperature program by forming a sequence of temperature shelves
Save Temperature Program		Saving the temperature program to .rta file
Name	–	The field for entering the name of the temperature program. The name of the program should not exceed 15 characters, the use of special characters, spaces and any alphabet other than English is not allowed
Add block	Add block	Adding a stage to the program. A stage can be one of the following: • cycle; • melting curve; • pause; • standby
Add line	Add line	Adding a temperature shelf within a block
Delete block	Delete block	Delete stage from a temperature program
Delete line	Delete line	Deleting the temperature shelf from the block
Block type	Cycle	Sets the repetition mode of temperature shelves and optical measurements at this stage of the temperature program until the specified number

Name	Symbol	Description
		of cycles is reached. Added by default when creating a block
	Melting Curve	Allows you to assign a range of temperature change in the block with setting the temperature increment. Used to register/obtain melting curves.
	Pause	Sets a pause in the program execution mode
	Standby	Sets the storage temperature after the program is done. **Attention!** This type of block can only be at the end of the program.

The choice of the block type is carried out using the drop-down list box (fig. 13).

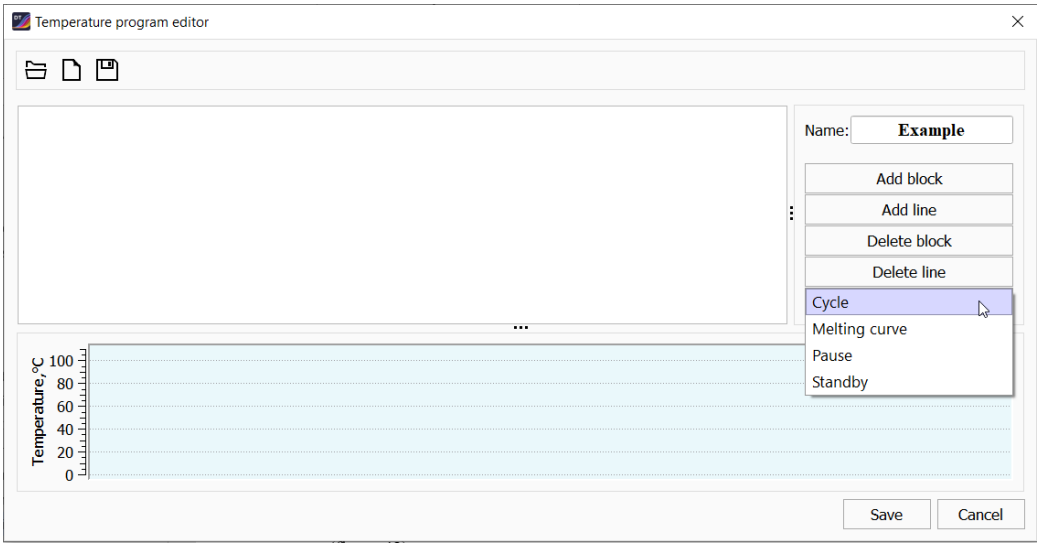


Figure 13 – The choice of the block type of the temperature program

Creating and Editing a Temperature Program

To create a temperature program:

1. Click the **New temperature program** button on the toolbar. In the area of the table representation of the temperature program, a row with the program parameters will appear.
2. In the **Name** field, enter the name of the temperature program.

By default, the field displays the name "Example".

3. Form the stages of the program. At the first stage, you need to add a block using the **Add block** button (fig. 14). After that, you can add lines (the **Add line** button) and subsequent blocks if necessary.

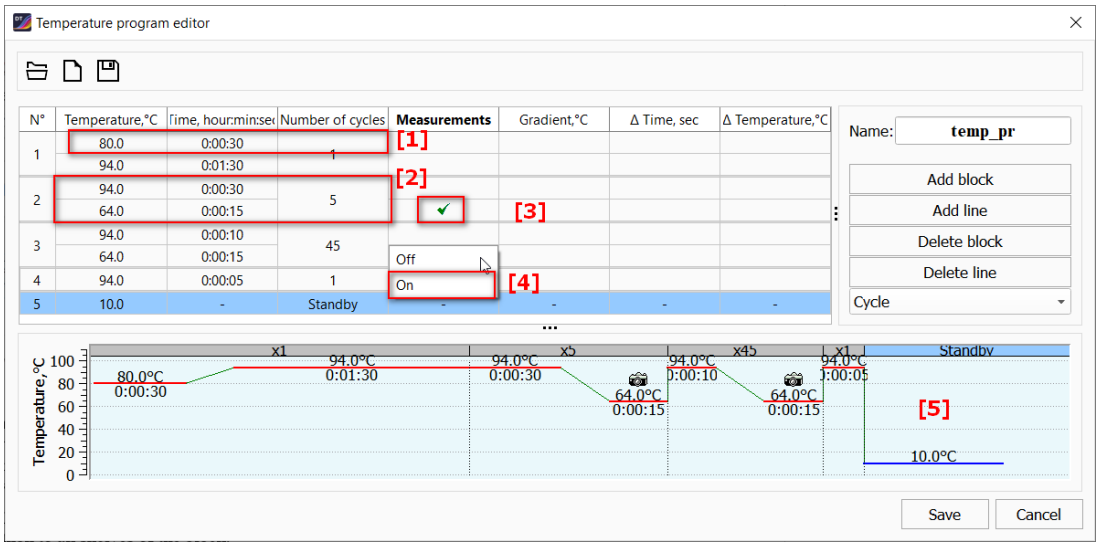


Figure 14 – Formation of the temperature program

Each line in the table corresponds to one temperature shelf of the temperature program [1]. The temperature shelf contains data on the temperature of the thermal plate heating per unit of time, the actual time of the plate heating at a given temperature and the required number of cycles (the set minimum is 1).

Temperature shelves (rows) can be combined into blocks [2]. The number of cycles is common to all shelves of the block.

4. Edit the numerical parameters of the program (temperature, time, number of cycles). Values are entered directly in the table.

Only one optical measurement per block is allowed. Do not leave the fields for temperature and time values blank, except for the **Pause** and **Standby** blocks.

Time is not set for **Pause** and **Standby** blocks, however, temperature is a mandatory parameter.

5. Set the mode of optical measurements [3] in the corresponding lines of the temperature program. The introduction of this parameter is carried out in the cells of the "Measurements" column [4].

Attention! Only one optical measurement is allowed within one block.

6. If necessary, add the Standby or Pause modes to the temperature program.

The **Standby** mode can be set only at the end of the temperature program.

All steps for creating a temperature program are displayed graphically in the lower part of the editor window [5].

To save the temperature program in the .rta file:

1. On the toolbar, click the **Save temperature program** button .
2. Select the directory for saving the file, specify the name of the saved file and then click the **Save** button.

The temperature program will be saved in the selected directory. After saving, you can continue working in the Editor window on the current version of the temperature program.

To save the temperature program in the current protocol, click the **Save** button of the Editor window. The Editor window will be closed and the created temperature program will be displayed in the Program window of the **Setup** mode.

If saving the temperature program is not required, click the **Cancel** button – the Editor window will be closed without saving the actions performed to create or edit the temperature program.

Additional Features of the Temperature Program Editor

Additional features of the Temperature Program Editor:

- Gradient function;
- Time increment function;
- Temperature increment function.

Gradient function

Gradient is a function that allows you to create different temperature conditions across the thermal plate per unit of time.

The function is available only for DTprime real-time PCR instrument.

There are two options:

1. Temperature Gradient (for device with solid matrix):
   a. vertical – eight temperature conditions in twelve replicates each;
   b. horizontal – twelve temperature conditions in eight replicates each.

   The maximum value of the gradient across the thermal plate is ±8°C.
2. Temperature drop (for instruments with isolated pseudo-units) – create independent temperature conditions with equal duration for each pseudo-unit. The maximum value of the gradient across the units is ± 8 ° C.

To specify a gradient:

1. Click the **New temperature program** button  . Add as many blocks and lines as needed.
2. Select the line with the temperature that will be determined as the starting temperature for creating the gradient, and click on it. A button  will appear to open the "Gradient" window (fig. 15).

N°	Temperature,°C	Time, hour:min:sec	Number of cycles	Measurements	**Gradient,°C**	Δ Time, sec
1	80.0	0:00:30	1			
	94.0	0:01:30				
2	94.0	0:00:30	5			
	64.0	0:00:15		✓		
3	94.0	0:00:10	45		0.0 ...	
	64.0	0:00:15		✓		

Figure 15 – Switching to "Gradient" window

3. Select the gradient type (vertical/horizontal) from the drop-down list box.
4. Set the temperature of the front edge of the thermal plate to select a vertical gradient (fig. 16, a) or of the right edge of the thermal plate to select a horizontal gradient (fig.16, b).

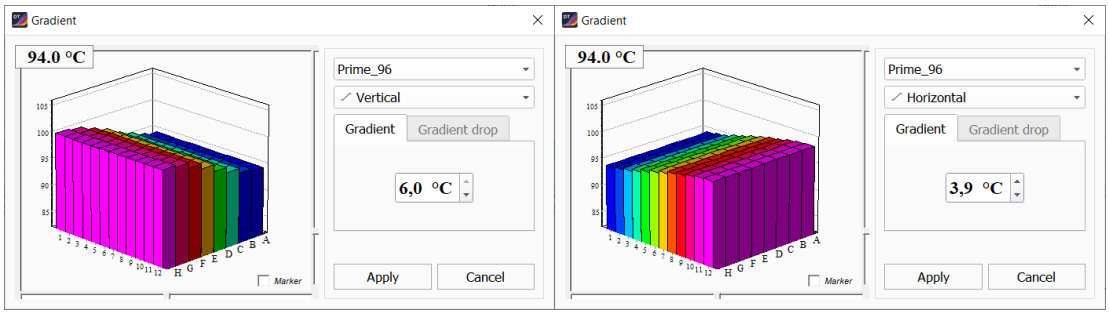


Figure 16 – "Gradient" window: a – vertical; b – horizontal

To create a temperature drop across the thermal plate:

1. Click the **New temperature program** button . Add as many blocks and lines as needed.
2. Using the ... button, open the "Gradient" window.
3. In the "Gradient" window, select the **Gradient drop** tab (active when the "DTprime" real-time PCR instrument in M3 or M6 modification is on) or the Gradient drop in the drop-down list box.
4. In the opened 4x4 matrix, enter the corresponding values of the temperature drop by blocks (colored area in fig. 17). Click the **Apply** button to run the program.

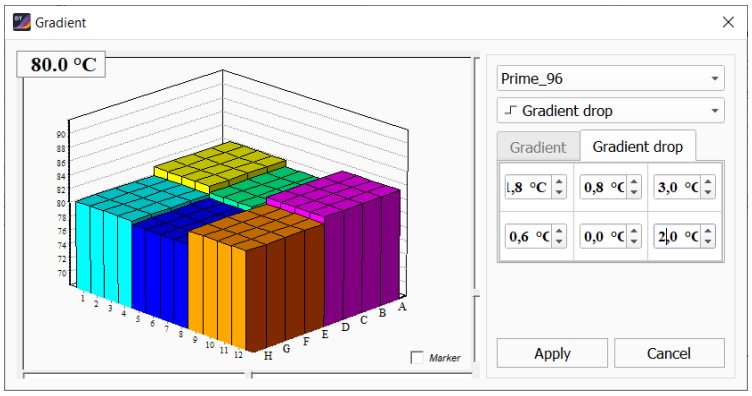


Figure 17 – Gradient drop

Increment in time function

The Increment in time function determines a sequential increase in the duration of the temperature shelf by a given value for each subsequent amplification cycle.

To set the value of time increment, it is necessary to enter the value of the increment (in seconds) in the column **Δ time, sec** in the corresponding line of the table (fig. 18).

N°	Temperature,°C	Time, hour:min:sec	Number of cycles	Measurements	Gradient,°C	**Δ Time, sec**	Δ Temperature,°C
1	80.0	0:00:05	15				
	94.0	0:00:05				0 ...	
2	94.0	0:05:00	1				
3	94.0	0:00:30	5				
	64.0	0:00:15		✓			
4	94.0	0:00:10	45				
	64.0	0:00:15		✓			
5	94.0	0:00:05	1				

Figure 18 – Creating an increment in time

Increment in temperature function

The Increment in temperature function determines a sequential change in the temperature of the thermal plate by a given value when performing a specific temperature shelf for each subsequent amplification cycle.

The function can be used to create **Cycle** and **Melting curve** blocks.

To create a temperature increment:

1. Click the **New temperature program** button . Add as many blocks and lines as needed.
2. Select the line with the temperature that will be determined as the starting temperature for creating the increment, and click on it. A button ... will appear to open the "Temperatures increment" window.
3. To implement the function, three of the four parameters displayed in the window must be set (for example: initial temperature, increment value and number of cycles). The fourth parameter will be calculated automatically.
4. Click the **Apply** button to run the program.

Loading the Temperature Program

Loading the previously saved temperature program is carried out at the stage of forming the protocol in the **Protocol** mode using the **Open temperature program** button .

Select the file with the required program and click the **Open** button.

The selected temperature program will be loaded into the **Temperature program** workspace of the **Protocol** mode.

CREATING A PROTOCOL

A protocol is a set of specified parameters for conducting a study: the number of samplest to be tested and controls, standards/calibrators and their arrangement on the thermal unit plate; temperature program. The protocol can be saved as .rt file.

The protocol contains the following information:

1. Detectable analytes.
2. Sample parameters:
   a. name;
   b. quantity;
   c. type (unknowm sample, positive control (C+), negative control (C-), standard (calibration sample);
   d. layout on the thermal unit plate.
3. Temperature program.
4. Type of analysis.
5. Fluorophores.
6. Active channels:
   a. quantity;
   b. purpose (internal control (VC), specificity).
7. Additional settings, if necessary.

After the end of the Run, in addition to the above-mentioned parameters, the protocol contains information on optical measurement, and it is saved as a Run file in the user's folder, unless otherwise was specified. After the Run is complete, you can view the protocol by double-clicking on the saved file.

The protocol can be created in several ways:

1. Manually.
2. Using the template (.trt file).
3. Using the protocol (.rt file).
4. Using an XML file.

Manual protocol creation includes:

1. Entering the name of the protocol.
2. Forming a list of samples and used tests.
3. Location of the tubes in the thermal block.

if necessary:

4. Creation, editing of the temperature program.
5. Editing protocol settings.

The prepared protocol is loaded to the Run mode for setting up on a real-time PCR instrument (see par. Upload of Protocol).

Protocol Name

The name of the protocol is specified in the **Protocol** field (fig. 19).

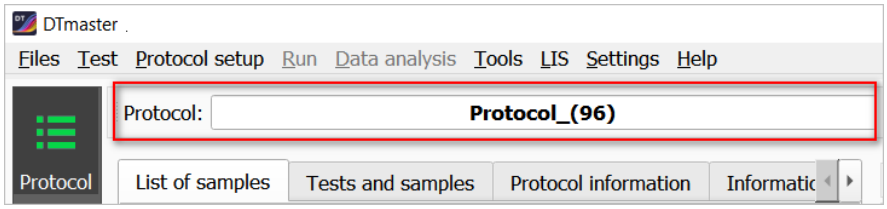


Figure 19 – Entering the protocol name

The name of the protocol can be entered in any form or using a template (see par. Configuring Protocol Name Template).

Forming a List of Samples

The list of samples is formed in the workspace of the **Protocol** mode, highlighted in fig. 20.

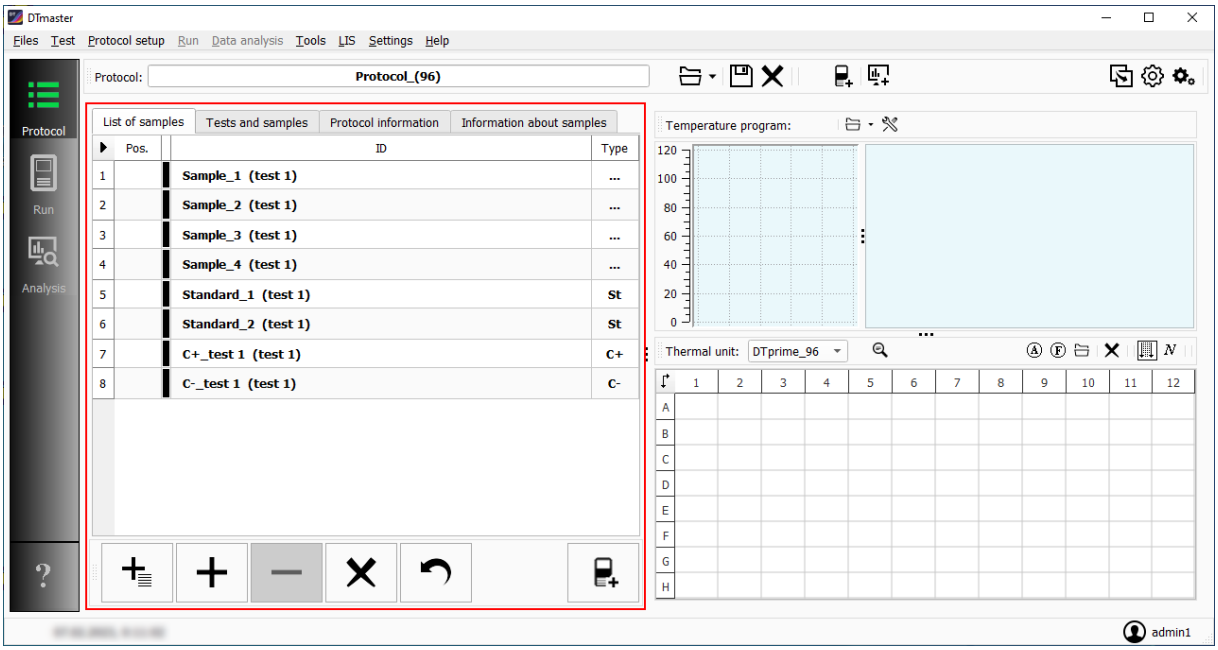


Figure 20 – Workspace for generating a list of samples

This workspace contains the following tabs:

- **List of samples**;
- **Tests and samples**;
- **Protocol information**;
- **Information about samples**.

The list of samples is formed using the buttons on the List of samples tab (table 5).

Table 5 – Buttons for forming a list of samples and their purpose

Name	Symbol	Purpose
Add Samples/Tests		Adding sample records to the protocol
Add sample		Adding to the protocol: • sample for preliminary analysis (simple, see par. 3.2.1); • if a sample for any test has already been added to the protocol, then pressing this button will add another sample of this test
Delete sample		Deleting a highlighted sample
Delete all samples		Deleting all sample records
Undo		Allows to undo up to five of the previous actions
Upload to the Run window		Loading the prepared protocol into the Run mode

For each sample the following information is indicated:

- serial number;
- name;
- type (C+, C-, St) if necessary.

For each test tube the following information is indicated:

- number in the m-n format, where m is the sample number, and n is the ordinal number of the test tube in the list;
- location on the thermal unit plate;
- color;
- fluorescence channel.

Options for editing the names of a group of samples are available in the context menu of this tab:

1. "Paste sample names from clipboard";
2. "Copy samples names with digits increment";
3. "Rename as first selected sample".

The **Tests and Samples** tab contains information about tests and samples (fig. 21).

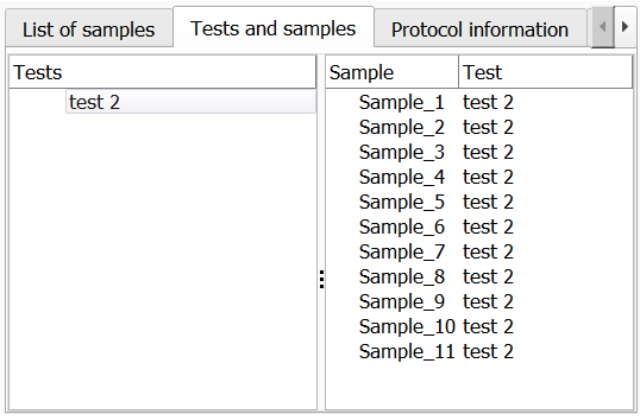


Figure 21 – The **Tests and Samples** tab

The Protocol information tab (fig. 22) provides general information about the protocol.

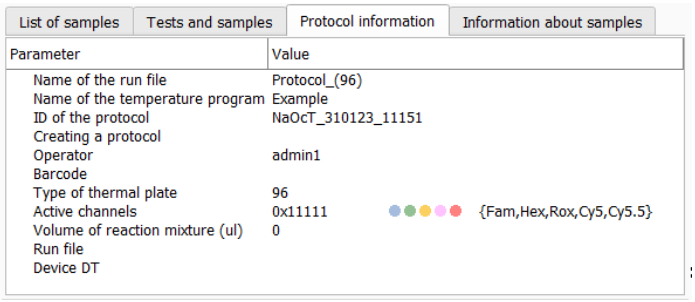


Figure 22 – The **Protocol Information** tab

The **Information about samples** tab contains additional data about samples [1], which may be required to form a report (fig. 23). Filling in the data is carried out by entering the required information into the appropriate cells of the **Value** column [2].

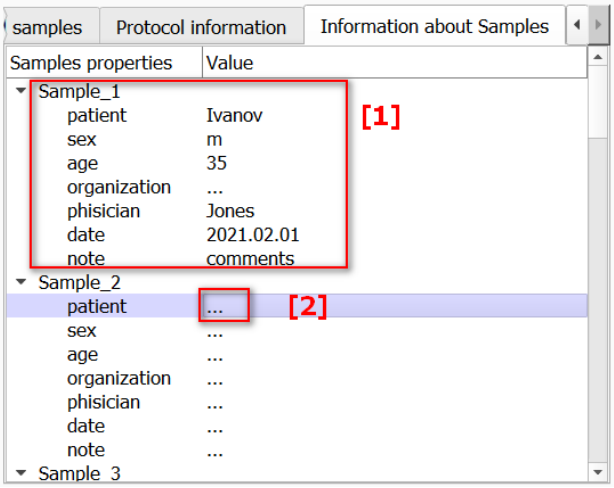


Figure 23 – The Information about samples tab

When forming the protocol, you can add a new sample using "Add Samples/Tests" window in one of the three ways described below. Click the **Add Samples/Tests** button  to open this window.

"Add Samples/Tests" window contains the following tabs:

1. **Add samples to test** – allows you to select a specific test and determine the required number of samples for it, which will be automatically added to the List of samples.
2. **Add tests to sample** – allows you to select the required number of tests for **one sample** (multi-test mode) and add it to the **List of samples**. The same temperature program for all tests is the condition for the correct choice.
3. **Container** – allows you to select a group of tests combined into a container for **one or several samples**, or select certain tests from this group.

Attention! The number of samples that can be added to the protocol is limited by the capacity of the thermal unit plate and characteristics of the test (fig. 24).

For example, the capacity of the thermal unit plate is 96 wells [1], the feature of the selected test is that one study for one sample is carried out in 16 tubes [2], so one sample occupies 16 wells of the thermal unit plate [3]. Thus, 6 samples are the maximum of samples for a given test that can be placed in a 96-well plate.

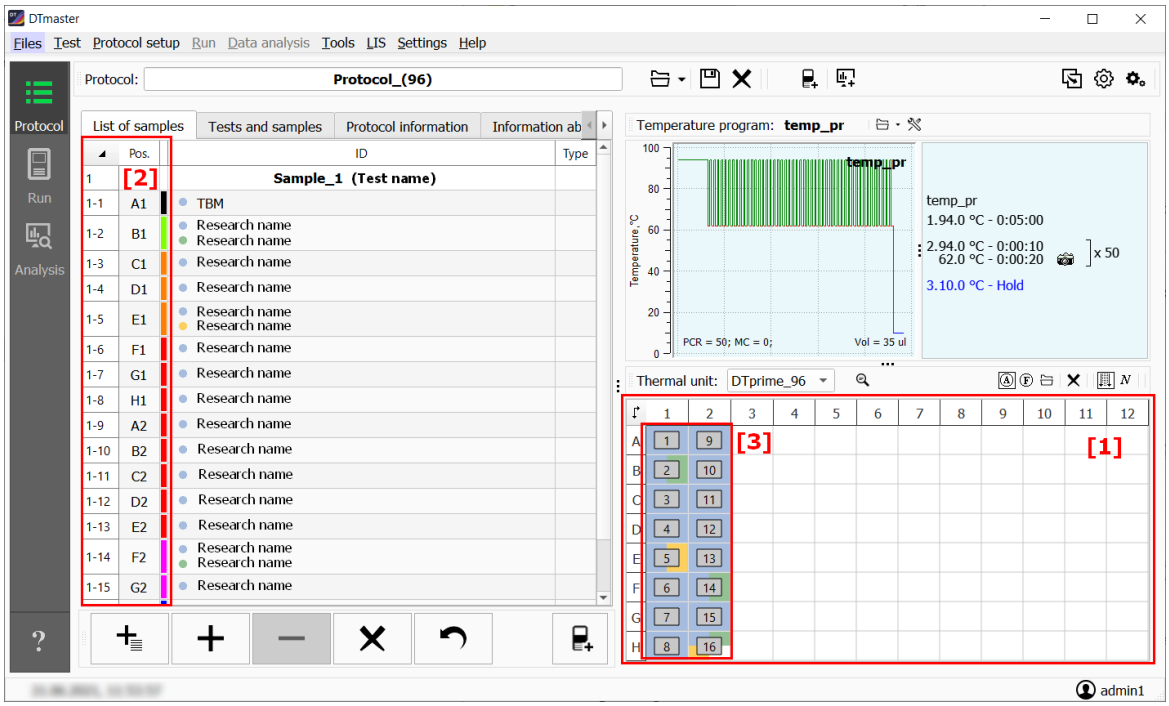


Figure 24 – Correspondence of the number of samples to the capacity of the thermal plate

If the number of added tubes exceeds the capacity of the thermal unit plate, a warning message will appear on the screen.

Adding a Sample in the Test Tab

To add a sample in the **Add samples to test** tab:

1 Click the **Add Samples/Tests** button . The "Add Samples/Tests" window will appear on the screen (fig. 25).

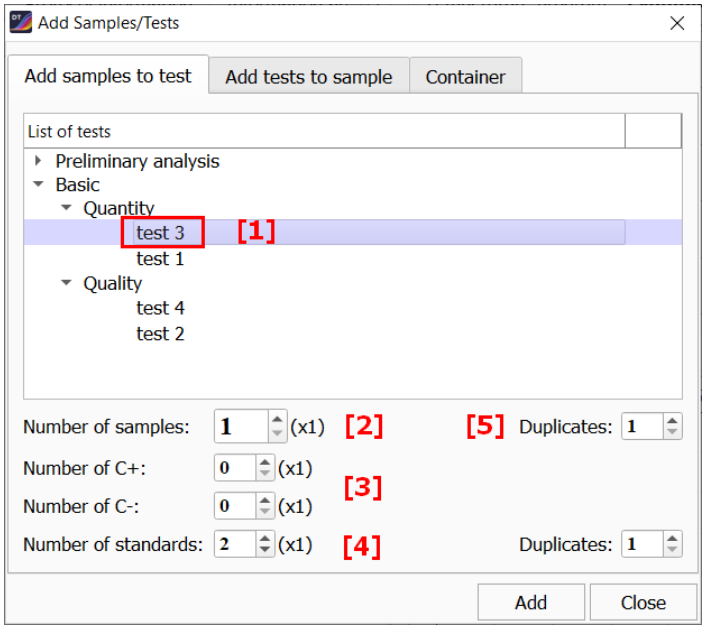


Figure 25 – "Add Samples/Tests" window, the **Add samples to test** tab

2 Select a test from the **List of tests** [1], indicate the number of samples [2], positive and negative control samples [3], standards (if provided by the kit) [4]. If necessary, indicate the number of duplicates for the samples [5].

3 Click the **Add** button – the sample record will be added to the List of samples.

The "Add Samples/Tests" window remains on the screen after adding a test and its corresponding list of samples to the protocol. This allows you to continue the selection of tests and samples when it is necessary to perform several tests in one protocol.

To close the "Add Samples/Tests" window, click the **Close** button.

Adding a Sample in the Add tests to sample tab

To add a sample in the **Add tests to sample** tab:

1. Click the **Add Samples/Tests** button . The "Add Samples/Tests" window will appear on the screen.
2. Click the **Add tests to sample** tab. The **Sample** field automatically displays the name of the sample for which you want to select the test or tests.
3. Write the sample name.
4. Select the required number of tests.
5. Select type: sample, C+, C- (fig. 26).

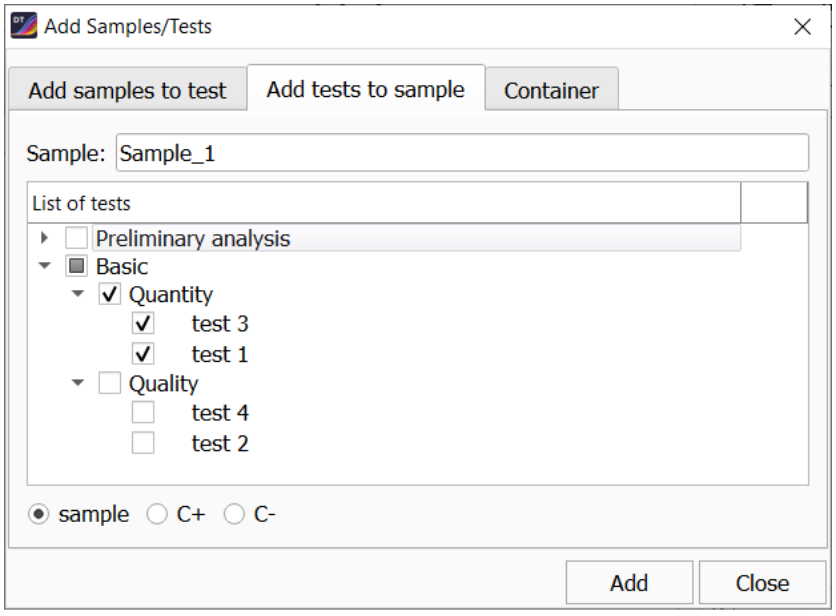


Figure 26 – Selecting tests in the **Add tests to sample** tab

6. Click the **Add** button. The sample record will be added to the **List of samples**.
7. To close the "Add Samples/Tests" window, click the **Close** button.

Adding a Sample in the Container tab

To add a sample in the **Container** tab:

1. Click the **Add Samples/Tests** button . The "Add Samples/Tests" window will appear on the screen.

2. Click the **Container** tab. The Sample field automatically displays the name of the sample for which you want to select the container (or containers) with tests or separate tests from container (or containers).
3. Write the sample name.
4. Select the required number of tests (fig. 27). If necessary, specify the number of samples for which the marked set of containers or tests will be used.
5. Select type: sample, C+, C-.

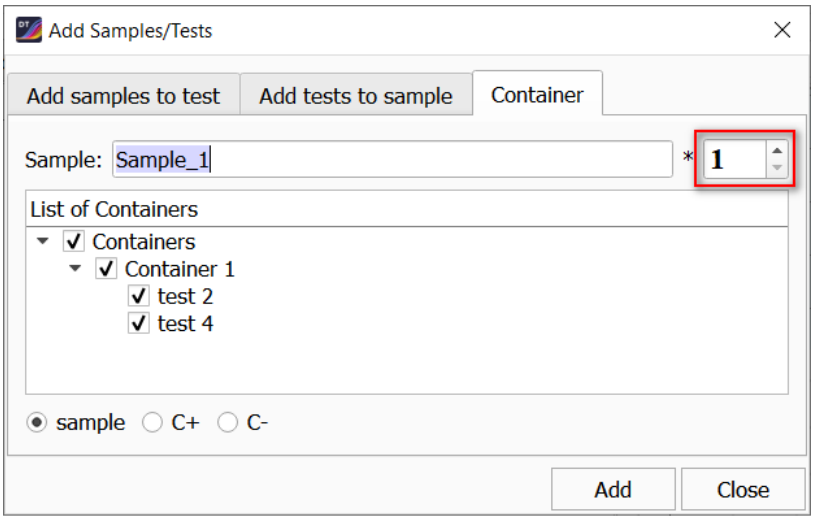


Figure 27 – Selecting tests and the number of samples in the **Container** tab

6. Click the **Add** button, the sample record will be added to the **List of samples**.
7. To close the "Add Samples/Tests" window, click the **Close** button.

Editing Sample Name

1. In the list of samples, switch to viewing headers (see par. Changing the Format of the List of Samples).
2. Highlight the entry about the desired sample and double-click the left mouse button. The data in the row will become available for editing (fig. 28).

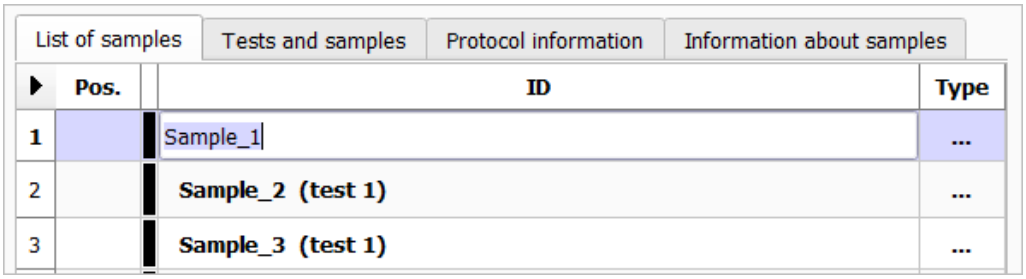


Figure 28 – Editing the sample name

3. Make the necessary changes and press the <Enter> key. The new name will be displayed in the list of samples. Pressing the <Enter> key again will automatically switch to editing the name of the next sample.

Editing the Sample Type

1. Double-click with the left mouse button on cell with ... in the row with the entry about the sample (see fig. 28). A drop-down list with types will become available:
   - "C+" – positive control sample;

- "C-" – negative control sample;
- "St" – standard;
- "-" – sample;

2. Select the desired type from the list. The changes will be automatically applied to the protocol.

Layout of Tubes in the Thermal Unit Plate

Layout of tubes in the thermal unit plate is carried out in the **Thermal unit** workspace of the **Protocol** mode (fig. 29).

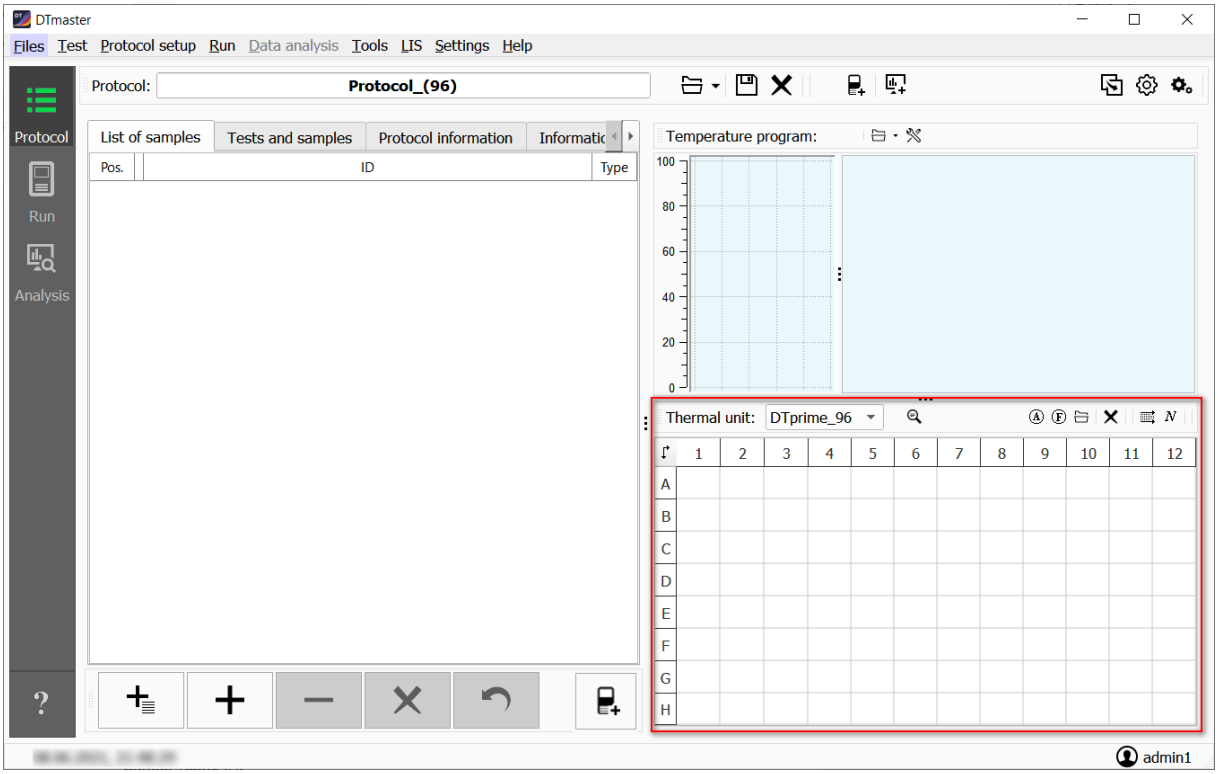


Figure 29 – Layout of tubes in the **Thermal unit** workspace

This section of the program contains the following data:

- drop-down list box defining the capacity of the thermal unit plate;
- toolbar (table 6);
- a table showing the layout of tubes in the thermal unit plate.

Table 6 – Buttons for placing tubes in the thermal unit plate and their purpose

Name	Symbol	Purpose
Zoom plate		View detailed tube placement information in a separate window
Auto layout		Automatic placement of tubes
Free layout		Manual placement of tubes
User layout		User placement of tubes
Clear template		Deleting tubes placement data

Name	Symbol	Purpose
Layout direction		Changing the layout direction
Numbering/ Color mode	*N*	Switching the "Numbering/Color" mode

Determining the Capacity of the Thermal Unit

The software provides the possibility to select the following modifications of the thermal unit plate: 48, 96, 192 and 384-wells.

To select the necessary capacity, use the drop-down list box located in the **Thermal unit** row.

Automatic Mode of Tube Layout

To activate the **Auto** mode, click the **Auto layout** button Ⓐ on the toolbar [1] (fig. 30).

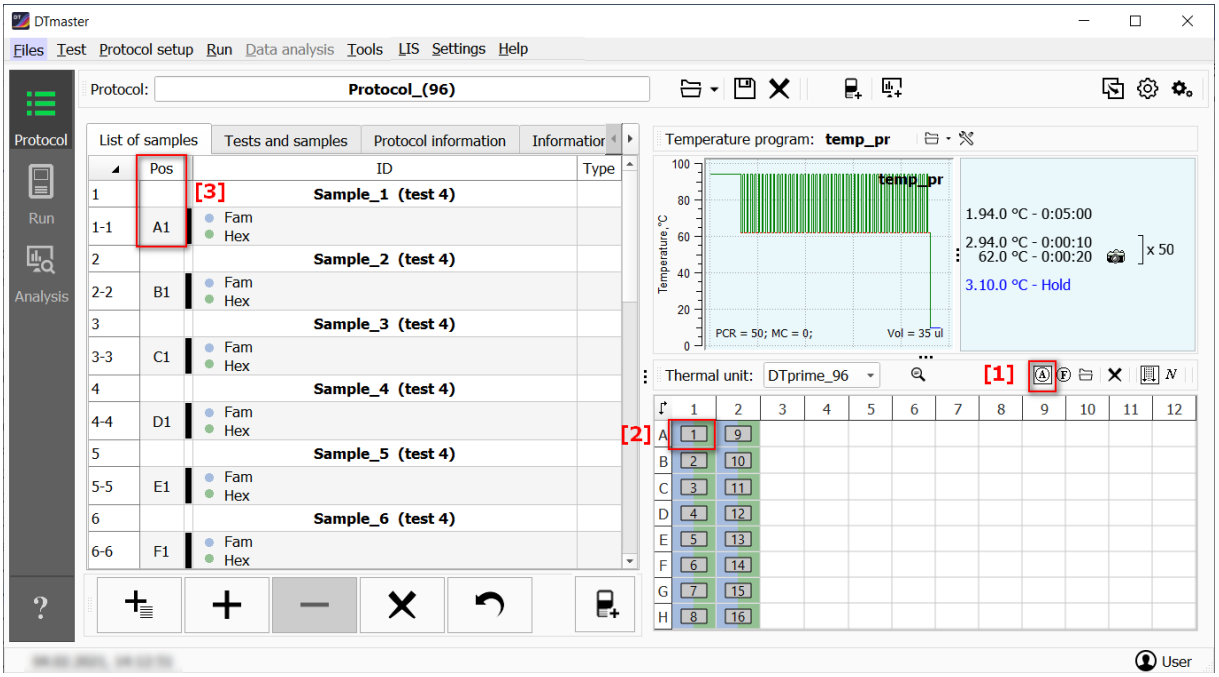


Figure 30 – Switching to the automatic layout of tubes

Tubes will be placed sequentially, and every tube's position [2] on the thermal unit plate [3] will be indicated in the list of samples.

Deleting Data on the Layout of Tubes

To delete the data on the layout of tubes, click the **Clear template** button ✕ located on the toolbar.

The layout data will be removed from the protocol.

Free Mode of the Tube Layout

The **Free** mode allows the user to choose the location of each tube on the thermal unit plate.

To place the tubes in the Manual mode, follow these steps:

1 Click the **Free layout** button Ⓕ on the toolbar. An information massage about switching to the **Free** mode will appear on the screen.

2 Move the mouse pointer to the cell in the table where you want to place the tube, and press the left button. The mouse pointer on the layout diagram is displayed as .

3 The corresponding records will be displayed on the layout diagram and in the column of the sample position [2] (fig. 31).

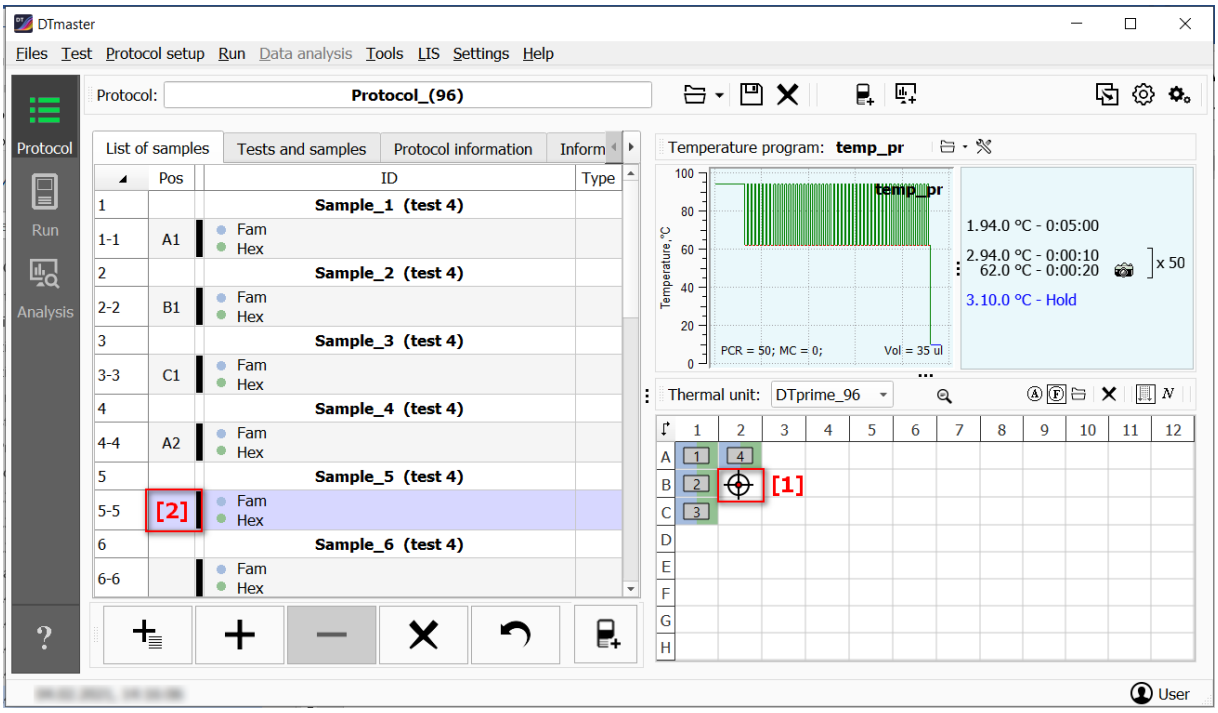


Figure 31 – Manual layout of tubes

In this way, by sequentially clicking on the required cells, fill in the entire matrix.

User Mode of Tube Layout

User mode allows loading the layout of tubes from a previously saved Protocol with the ability to edit it.

To place the tubes in the **User** mode:

1 Click the **User Layout** button on the toolbar. The "Open protocol (template)" window will appear on the screen.

By default, this window displays the contents of the Placement_Templates folder, containing templates (protocols) with standard layouts for "DTprime_384" thermal unit.

2 Select the required protocol (.rt file) or template (.trt file) and click **Open** button. The layout from this protocol (template) will be displayed in the thermal plate matrix.

If the protocol or template does not correspond to the selected capacity of the thermal unit plate, a warning about an incorrect protocol format will appear on the screen.

Changing the Direction of the Layout

To change a direction of the layout, click the **Layout direction** button on the toolbar. The layout will change direction and the button will change to (fig. 32).

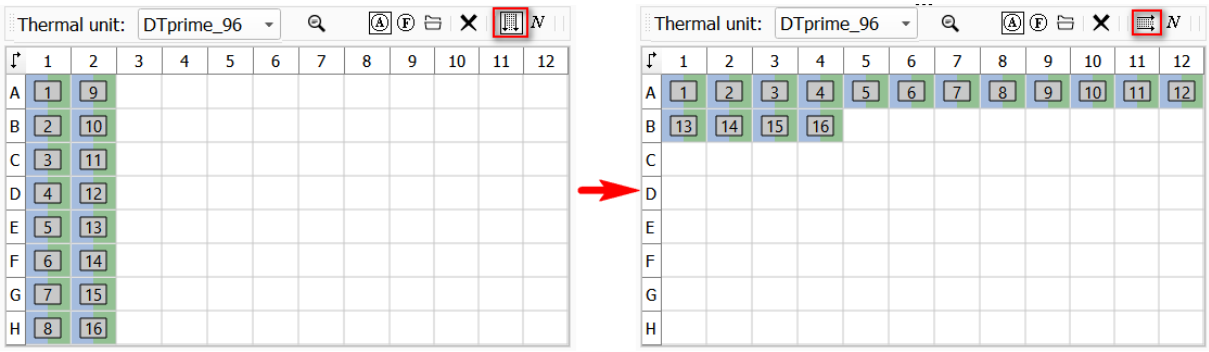


Figure 32 – Changing the layout

When choosing a direction of the layout the thermal plate is filled line by line from left to right, when choosing a layout – in columns from top to bottom.

Numbering/Color Mode

The software has two modes of displaying the layout of tubes (fig. 33):

1. **Numbering** [1], by default. In this mode, the order number of a tube is displayed for each occupied cell, as well as the colors of the active channels used in the test for this tube.

To switch from the "Numbering" mode to the "Color" mode, click the **Numbering/Color** mode button on the toolbar

2. **Color** [2] – editing mode for each tube or group of tubes, in which the fluorescence curve will be stained during data analysis.

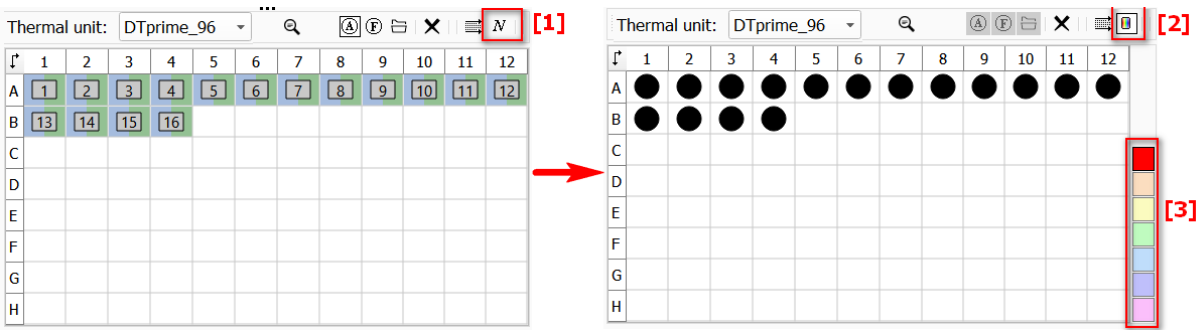


Figure 33 – Selecting the mode of displaying the tubes

To change the color of a test tube, select the desired color on the panel [3], move the cursor over the required test tube and press the left mouse button.

If it is necessary to change the color for a group of tubes, select the area of their placement with the cursor [1], and they will automatically change the color to the selected [2] (fig. 34).

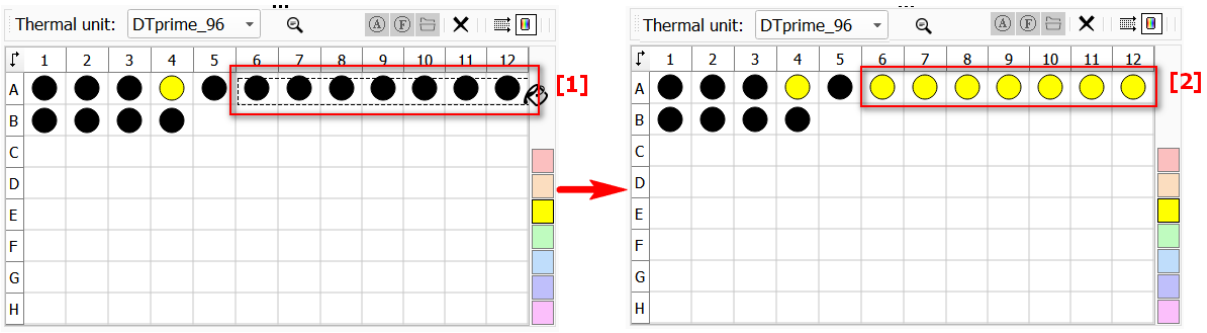


Figure 34 – Editing color for a group of tubes

To switch from the **Color** mode to the **Numbering** mode, click the Numbering/Color mode button .

Note. The mouse pointer in the Color mode is displayed as .

Inverting records in a placement table

To display tubes in reverse order in the selected row or column, select any cell in the required row/column and press the right mouse button. A context menu with available inversion options will be displayed on the screen (fig. 35, a). Select the required option, after which the applied changes will be automatically displayed in the placement table (fig. 35, b, c).

To invert the entire table, select the Invert all Plate option from the context menu. An example of the inversion of the entire table is illustrated in fig. 35, d.

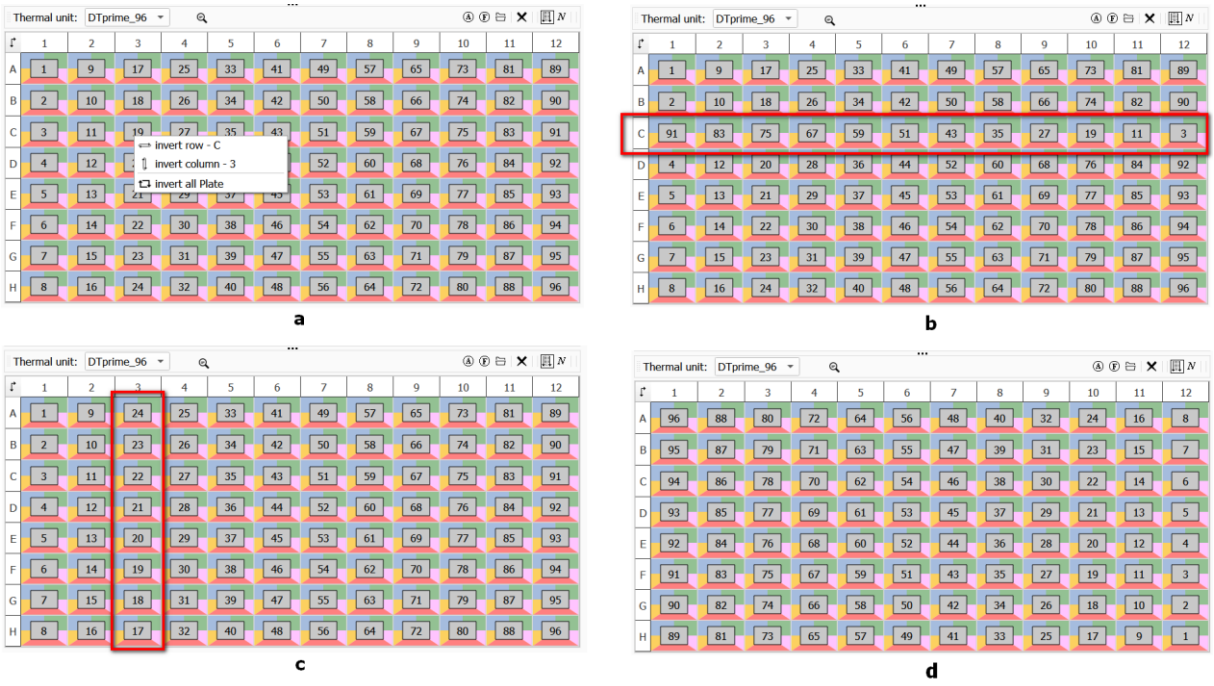


Figure 35 – Using inversion: a - options for inverting records available in the context menu; b - line inversion; c - column inversion; d - inverse of the entire placement table

Grouping tubes by test

If a multi-test study is planned for the samples, then to automatically group the tubes by test:

1. Perform automatic placement of tubes (click the **Auto layout** button on the toolbar).
2. Select the Test layout option from the context menu in the **Thermal unit** workspace (fig. 36).

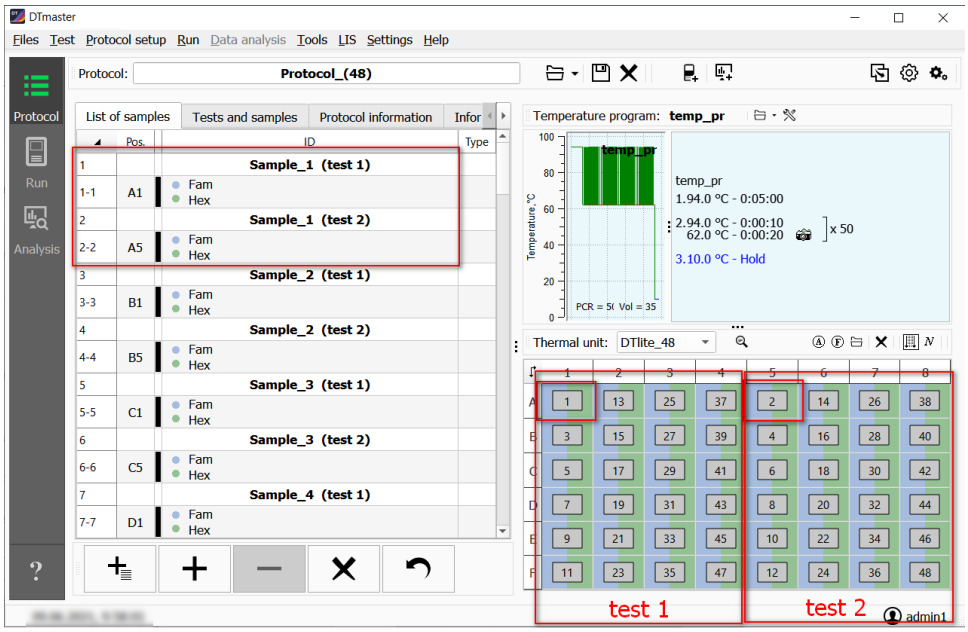


Figure 36 – Grouping tubes by test

View detailed tube placement information

Click the Zoom plate button on the toolbar to display a window with detailed information on the location of the tubes (fig. 37).

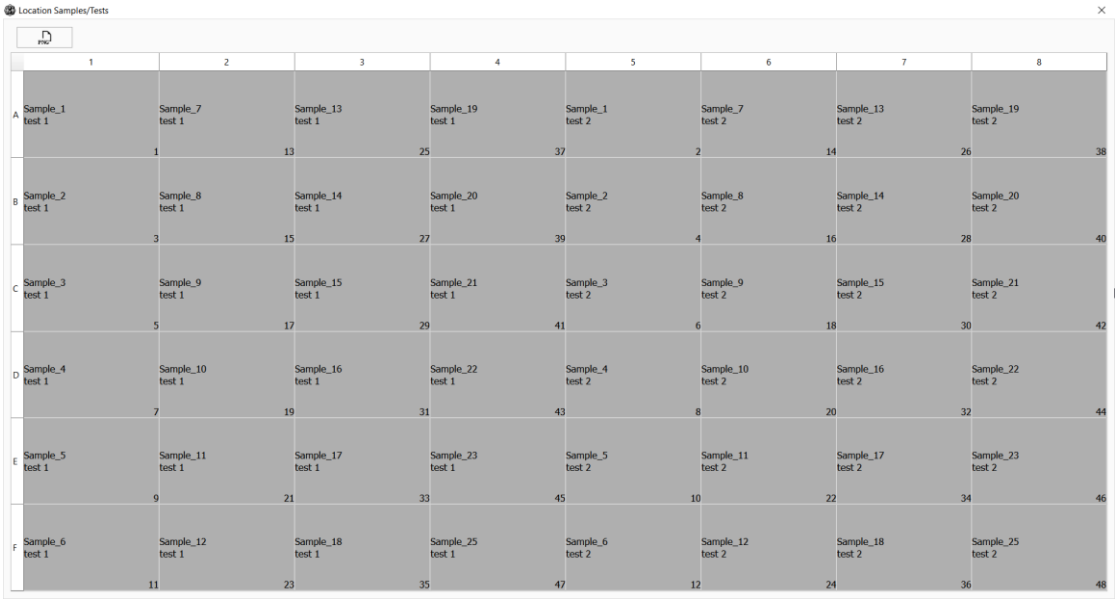


Figure 37 – The "Location Samples/Tests" window

This window shows a graphical image of the thermal unit plate, where each cell indicates:

- name of the sample;
- test;
- serial number of the tube in the list of samples.

This image can be saved as a PNG file using the PNG button at the top of the window.

Saving a Template

A protocol created in DTmaster in the **Protocol** mode can be saved as a Template.

The template contains all the information inherent in any protocol, but it does not go through the stage of running the temperature program and subsequent analysis of the results.

To create a template:

1. Create a protocol. Click the Save as Template button .
2. In the "Save as template" window, click the **Save** button.

The protocol will be saved in the user folder (unless otherwise selected) as a .trt file.

The saved template can be reused and edited for the current Run conditions.

Loading Protocol into DTmaster

The protocol can be created using previously created files: template .trt, protocol .rt and XML file.

Loading Protocol from .trt, .rt files

To create a protocol using a template .trt or a previously used protocol .rt:

1. Click the **Open protocol** button , on the toolbar.

   The "Open Protocol (Template)" window will appear on the screen.
2. Select the required file and click the **Open** button.

The data from the selected file will be displayed in the **Protocol** mode.

If the protocol has been already loaded into the software, it will be in the list of quick access downloads. To view the list, click the **Last protocols** button next to the **Open protocol** button . A list of the recently uploaded protocols with the system path to each of them will be displayed on the screen.

Loading Protocol from XML File

To upload a protocol from XML file:

1. On **Protocol setup** menu, click **Open XML protocol**.
2. Select the required file and click the **Open** button.

The loaded protocol will be displayed in the program.

ADDITIONAL FEATURES OF PROTOCOL MODE

Creating a Preliminary Analysis Protocol (Simple)

To add a record about a new assay, click the **Add sample** button + [1]. The record will be displayed in the list [2] (fig. 38).

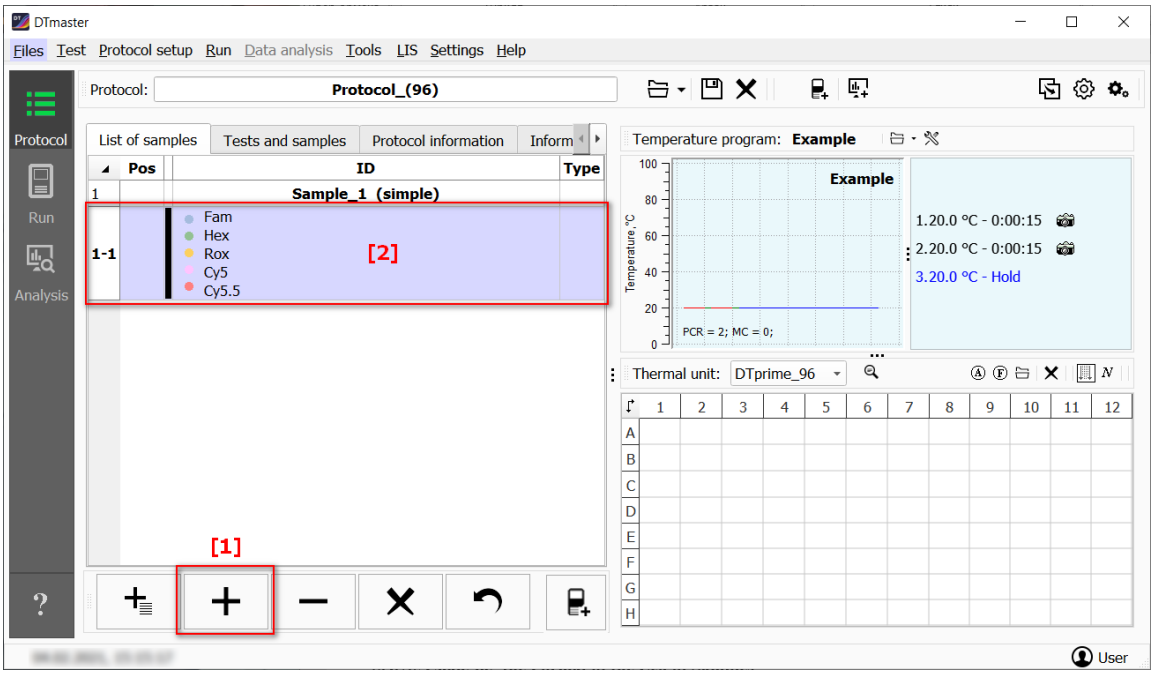


Figure 38 – An example of adding a test without using the **Test** option

You cannot add more records to the list of samples than is specified in **Thermal unit** workspace. If you try to do this, the warning message about the filling of the thermal unit will appear on the screen.

Changing the Format of the List of Samples

To switch the view of the list of samples to the mode of displaying only the names of samples, click the ◢ button on the **List of samples** tab (fig. 39).

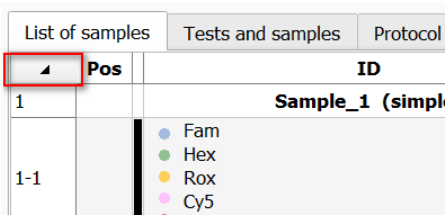


Figure 39 – Button for changing the format of the list of samples

Only the names of samples will be displayed in the list (fig. 40).

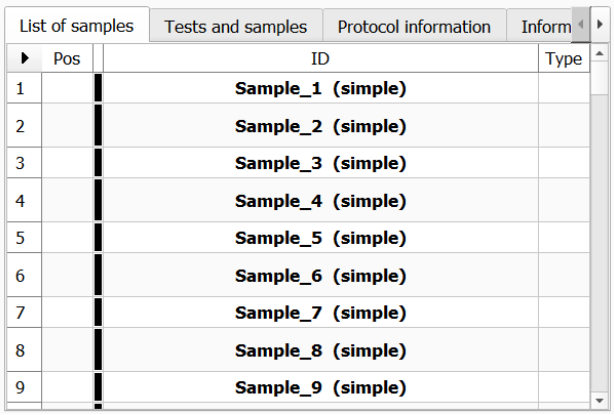


Figure 40 – List of samples

Deleting Protocol

To delete protocol data, click the **Delete all samples** button ✕ located in the line for entering the protocol name.

Protocol data will be deleted.

RUN MODE

Initialization and configuration of the protocol run are carried out in the **Run** mode (fig. 41).

The **Run** mode window includes:

[1] – fluorescence graph area;

[2] – temperature graph;

[3] – **Protocol** workspace

[4] – **Device** workspace;

[5] – a field with description and indicator of the current operation in DTmaster.

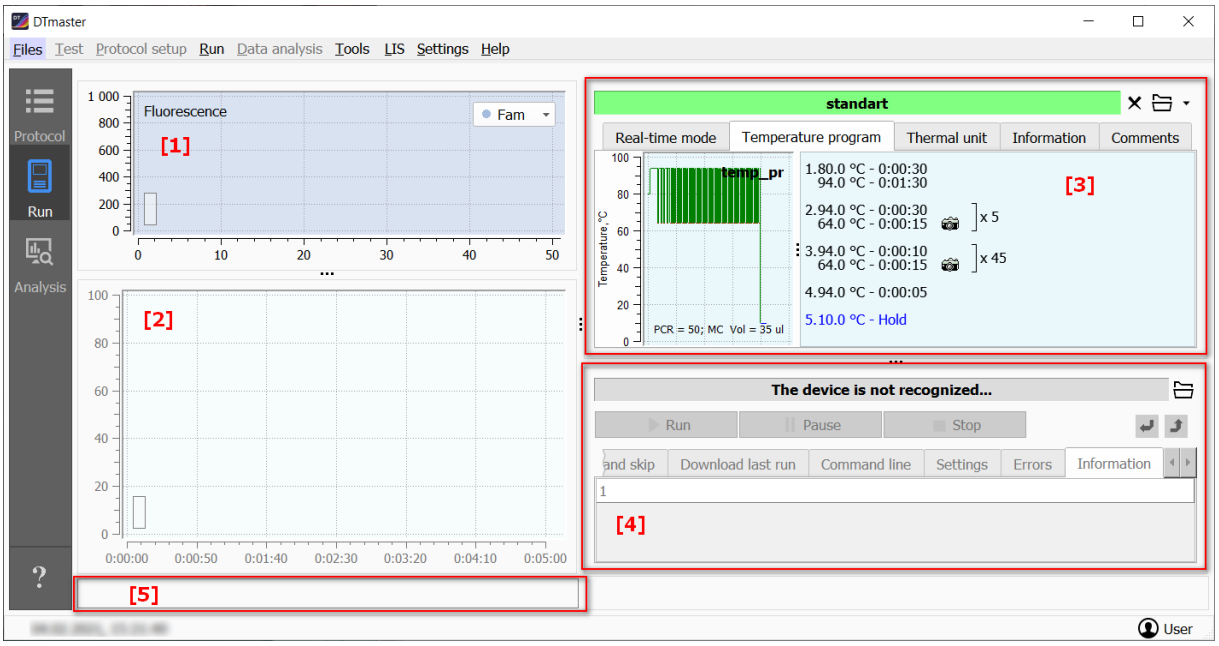


Figure 41 – Information about the protocol in the **Run** mode

In the area of fluorescence graphs, real time graphs of exponential growth of the fluorescence level are displayed during the Run (fig. 42).

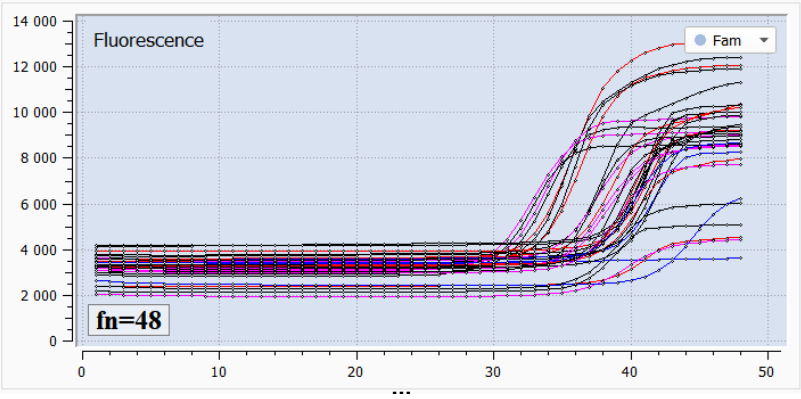


Figure 42 – Graph of exponential growth of the fluorescence level

The temperature graph (fig. 43) displays a real time graph of the temperature change of the thermal unit, indicating the current temperature [1].

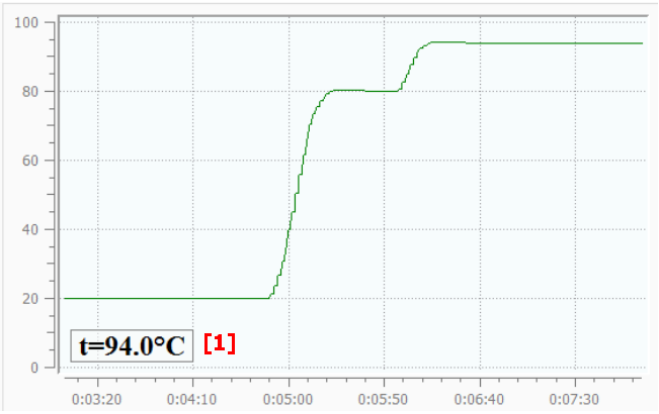


Figure 43 – Temperature graph

In the **Protocol** workspace, the protocol loading functionality (see par. Upload of Protocol) as well as viewing information about the selected protocol are implemented.

The **Device** workspace is designed to connect the real-time PCR instrument and run the protocol (see par. Running the Analysis).

STAGES OF WORK IN THE RUN MODE

The analysis is performed in several stages.

1. Choice of protocol.
2. Connecting and configuring the real-time PCR instrument.
3. Placement of tubes in the real-time PCR instrument.
4. Running the analysis.
5. Viewing the results of the analysis in real time.

UPLOAD OF PROTOCOL

There are several ways to add a Protocol to the **Run** mode:

1. If the required protocol is already open in the **Protocol** mode [1], then click the **Upload to the run window** button [2] to load it into the **Run** mode or on the **Protocol setup** menu, click **Upload to the run window** [3] (fig. 44).

Attention! Protocols in the "Protocol" and "Run" modes are not automatically synchronized. If, after loading the protocol into the "Run" window, you have made any changes to the original protocol (in the "Protocol" mode), click the **Upload to the run window** button [2] again. This will ensure that the correct protocol is sent to Run mode. Otherwise, the "outdated" protocol will remain in the "Run" mode, and when you try to run it for execution, an informational message about the protocol mismatch in the "Protocol" and "Run" modes will be displayed on the screen.

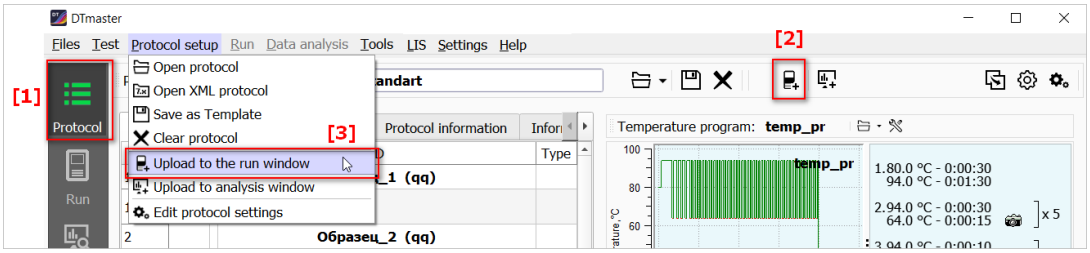


Figure 44 – Loading the protocol from the Protocol mode

2. If the protocol (template) is saved on the user's computer as .rt, .trt file, open the **Run** mode [1], click the **Open protocol** button [2] and select the file with the required protocol (template) in the displayed window. Or on the **Run** menu, click **Select protocol** [3] (fig. 45)

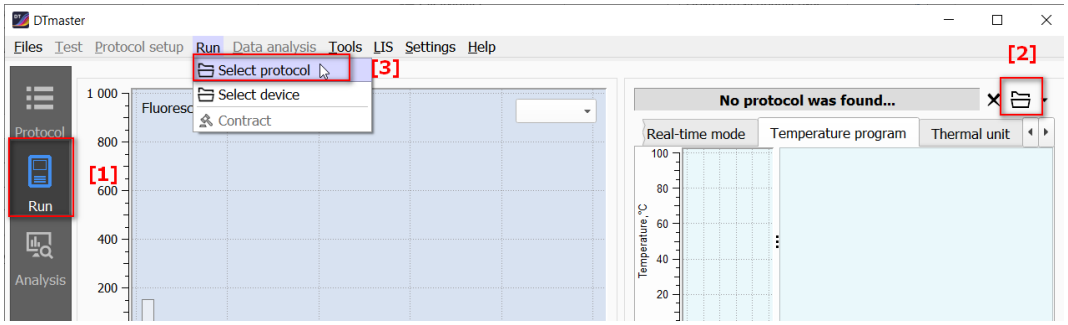


Figure 45 – Loading a previously generated protocol in the **Run** mode window

The selected protocol will be displayed in the **Protocol** workspace in the **Run** mode (fig. 46).

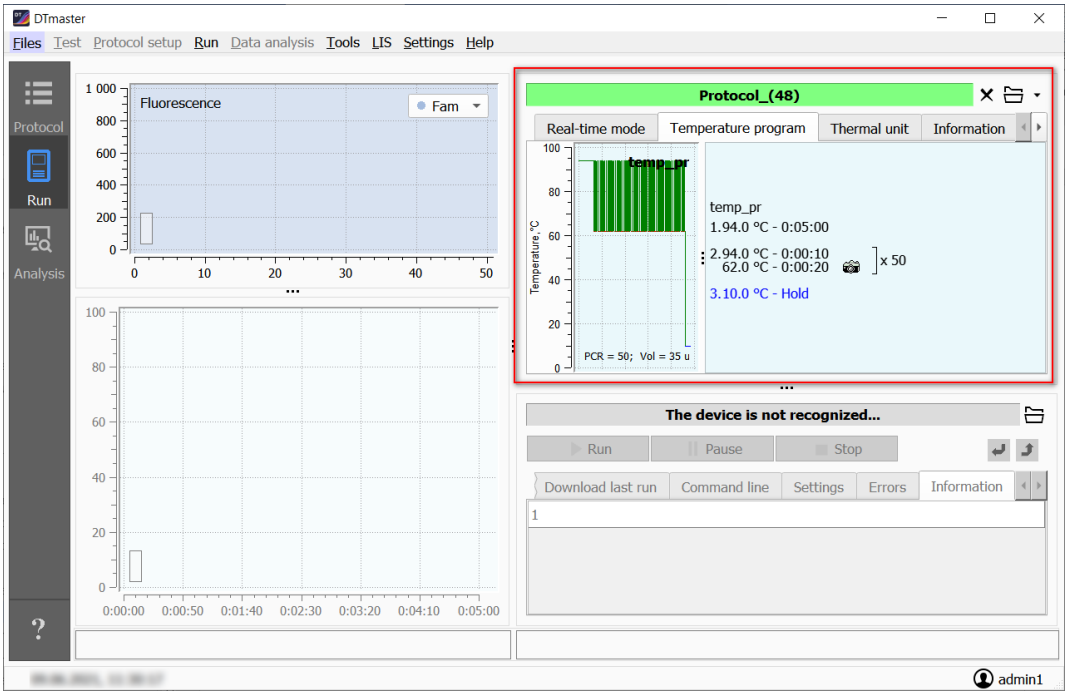


Figure 46 – Protocol workspace

VIEW DETAILS ABOUT THE SELECTED PROTOCOL

Information about the selected protocol is in the **Protocol** workspace, which includes the following tabs:

- **Real-time mode**;

- **Temperature program**;
- **Thermal unit**;
- **Information**;
- **Comments**.

The following data is displayed on the **Real-time mode** tab (fig. 47):

[1] – the sequence of blocks of the temperature program;

[2] – the name of the program;

[3] – execution time of the program;

[4] – number of the executed cycle and the number of remaining cycles;

[5] – indicator of the passage of the temperature shelf.

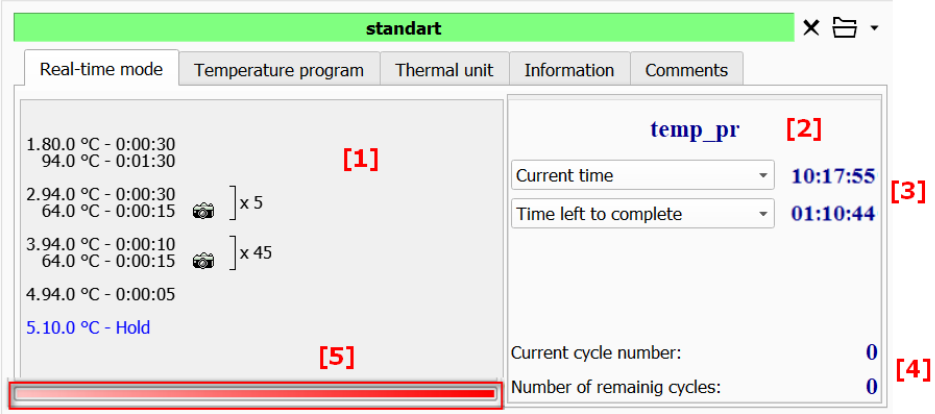


Figure 47 – The **Real-time mode** tab

The **Temperature program** tab contains a temperature program with a temperature graph and program.

The **Thermal unit** tab contains:

- toolbar;
- information about the scheduled tests and samples (fig. 48, a);
- color scheme of tubes (fig. 48, b).

The toolbar of this tab contains buttons described and illustrated in par. Viewing the Analysis Results.

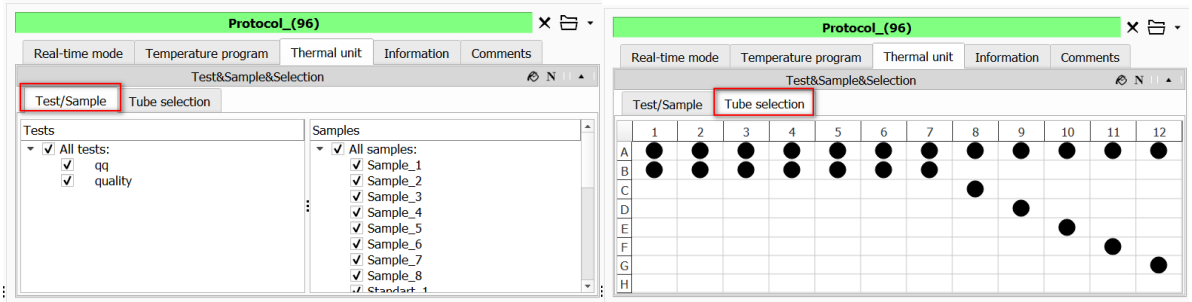


Figure 48 – The **Thermal unit** tab

The **Information** tab contains general information about the protocol.

The **Comments** tab contains note to the run file.

CONNECTING THE REAL-TIME PCR INSTRUMENT

ATTENTION! Preparing the instrument for operation

Before the first launch of the instrument, it is necessary to check the geometric settings of the optical system of the instrument, check the purity of wells, configure the height of tubes, set the exposure of optical measurements.

- When using PCR kits, the optimal exposure values are requested from the manufacturer of the PCR kit.
- The optimal exposure values are determined by the manufacturer, setup by default in the settings of each test, and are presented in conventional exposure units (c.u.e.). If necessary, exposure compensation factors can be entered for all active channels (see Appendix A of this manual). This allows you to obtain correct optical measurements without changing the preset (factory) exposure values of the device. For test-specific exposure correction values, contact the manufacturer of the kit or select in the "Exposure" window (see par. Selecting exposure correction factors).
- When using kits with different types of plastic (low-, medium- and high-profile plastic), tubes with convex or flat lids, strips, it is necessary to measure the height of the tubes (see par. Measuring the Height of the Tubes).
- Checking the purity of the wells of the thermal unit is carried out in the laboratory every 20 runs (but at least once a week) to eliminate the possibility of invalid results due to increased background fluorescence.
- Checking of geometric settings of the optical system (see par. Checking the Geometric Settings of the Optical System) and of the exposure (see par. Selecting exposure correction factors) of the real-time PCR instrument is carried out once during the installation procedure. It may be necessary to change these parameters when changing the manufacturer of PCR kits, as well as if it is specified in the instructions for the PCR kits or if there are doubts about the correctness of the instrument's settings.

The real-time PCR instrument is connected in the **Device** workspace.

To connect the real-time PCR instrument:

1. Click the **Select device** button in the row with "The device is not recognized..." entry.

   A window with a list of available devices will appear on the screen (fig. 49).

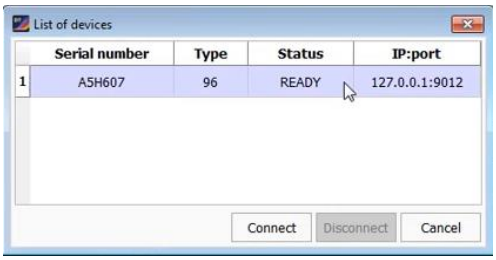


Figure 49 – List of available instruments

   Serial number, type (thermal plate format), WinID (status) and IP-port to which it is connected are specified for real-time PCR every instrument.

   Possible statuses:

   - READY – the device is ready to be connected;
   - BUSY – the device is busy with another application;
   - CONNECTED – the device is connected to this application.

2. Select the required real-time PCR instrument and click the **Connect** button.

Information about the selected real-time PCR instrument will be displayed in the **Device** workspace on the **Information** tab (figure 55).

If the selected device does not comply with the protocol (for example, by the plate format), error message will be displayed on the screen. In addition, the name of the protocol and device will be highlighted in yellow.

The **Device** workspace contains the following tabs:

- **Turn off**;
- **Download last run**;
- **Command line**;
- **Settings**;
- **Errors**;
- **Information**.

On the **Turn off** tab, the device automatically goes into the sleep mode after the completion of the temperature program (fig. 50). To do this, checkbox the line with the corresponding option.

Figure 50 – The **Sleep mode** tab

On the **Download last run** tab there is a possibility to download the results of optical measurements for viewing as a result of the last run of the instrument.

Click the **Last Protocol** button (fig. 51).

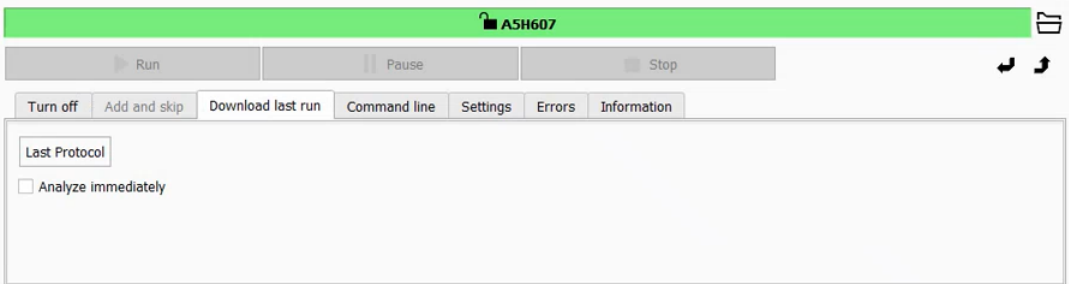


Figure 51 – The **Download last run** tab

The program will display data about the last run on this real-time PCR instrument.

When the **Analyze immediately** checkbox is selected, the program will automatically open the **Analysis** mode, which will provide information about the results of the last run.

On the **Command line** tab, the ability to interact with four microcontrollers of the device using the command line is implemented.

Attention! Working with the command line is allowed only for specialists who have undergone appropriate training or under the direct supervision of a representative of "DNA-Technology" company.

The **Settings** tab (fig. 52) provides access to viewing and changing the device settings, such as:

- checking the geometric settings of the optical system (creating a video);

- checking exposure;
- measuring the height of the test tube.

For details on these settings, see par. Real-time PCR Instrument Setup and Diagnostics.

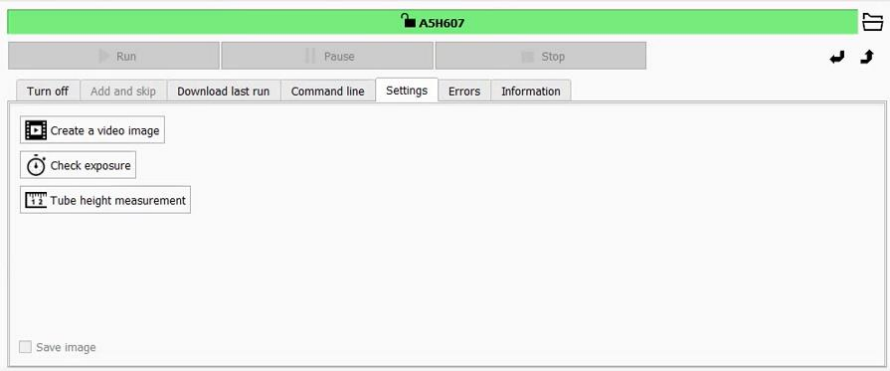


Figure 52 – Setting up the instrument

The list of errors that occurred during device operation can be viewed on the **Errors** tab.

The "Information" tab contains data about the connected device.

Multi-window Mode

To control several simultaneously connected DTprime or DTlite real-time PCR instruments, it is necessary to start several DTmaster programs (the number of launches corresponds to the number of devices connected simultaneously). Each DTmaster must be connected to its own instrument.

POSITIONING THE TUBES IN THE REAL-TIME PCR INSTRUMENT

To position the tubes in the thermal unit:

1. Open the thermal unit by clicking the **Open thermal unit** button .
2. Place the tubes into the thermal unit in accordance with the previously made layout at the stage of protocol creation.
3. Close the thermal unit by clicking the **Close thermal unit** button .

You can use the control buttons of the device to open and close the thermal unit.

RUNNING THE ANALYSIS

To run the analysis, click the **Run** button on the toolbar of the **Device** workspace.

The "Prelaunch start" message will be displayed on the screen, after that the analysis will begin according to the specified temperature program (fig. 53).

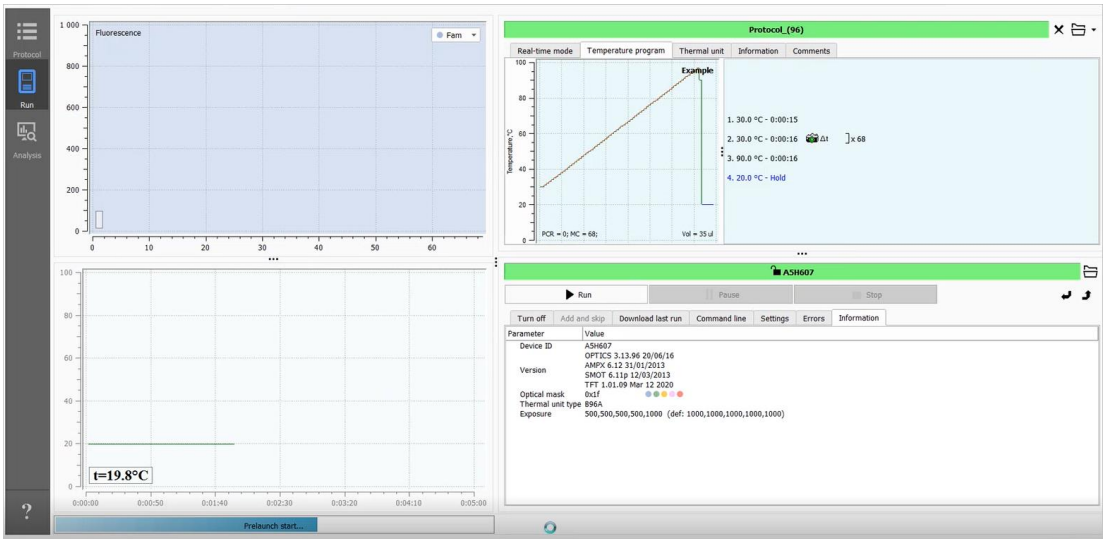


Figure 53 – Running the analysis

Attention! If exposure compensation factors are specified in the test parameters of the protocol (fig. 54), then these coefficients will be automatically applied to the current exposure value (fig. 55) after starting the analysis.

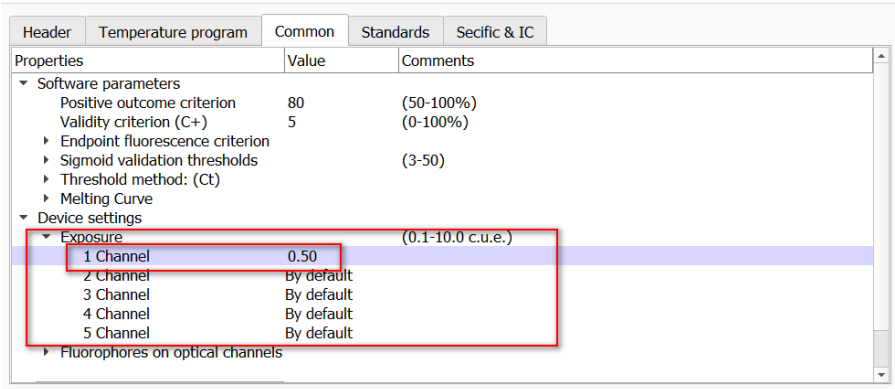


Figure 54 – Test editor, setting the exposure of optical measurements

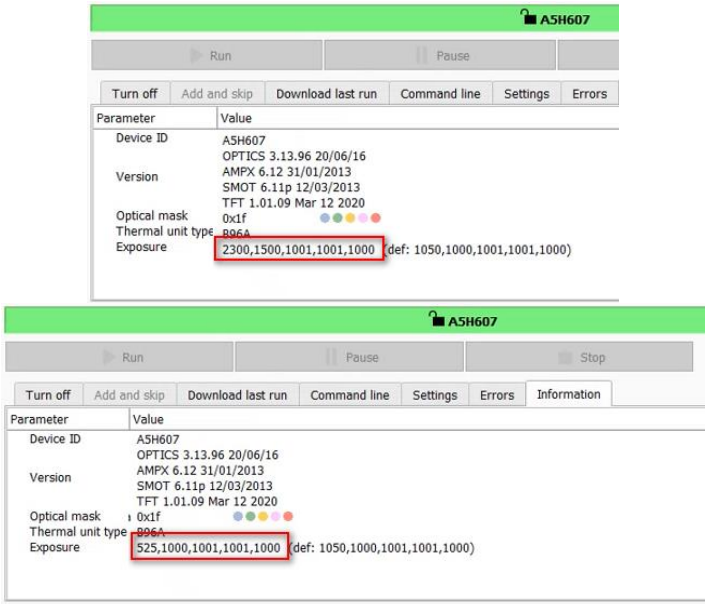


Figure 55 – Values of optical measurements exposure: a – before starting the analysis; b – after starting the analysis and applying the coefficients from the test settings

VIEWING THE ANALYSIS RESULTS IN REAL TIME

The software allows you to monitor the progress of the analysis in real time (fig. 56). To do this, the following data blocks are presented in the **Run** section:

- fluorescence graph showing the current cycle [1];
- temperature graph showing current temperature [2];
- the Real-time mode tab, containing data about:
- program operation time intervals [3];
- the cycle number [4];
- number of remaining program cycles [5];
- the current stage of the temperature program [6].

Figure 56 – Viewing the analysis progress in real time

After the end of the temperature program, DTmaster automatically switches to **Analysis** mode (see par. Analysis Mode).

ADDITIONAL FEATURES OF RUN MODE

Pausing the Analysis

The **Pause** function allows you to pause the protocol execution. In this case, the instrument continues to maintain the temperature of the current temperature shelf.

To pause the process, click the **Pause** button on the toolbar of the **Device** workspace.

To resume optical measurements, click the **Continue** button (fig. 57).

Figure 57 – Resuming program execution

Stopping the Analysis

To stop the temperature program, click the **Stop** button located on the toolbar of the **Device** workspace. The confirmation window for stopping the analysis will appear on the screen.

Click the **Yes** button to stop the analysis.

Click the **Cancel** button, if stopping the analysis is not required.

It is strongly recommended not to stop the program before it is completed.

ANALYSIS MODE

Analysis of test results is carried out in the **Analysis** mode (fig. 58).

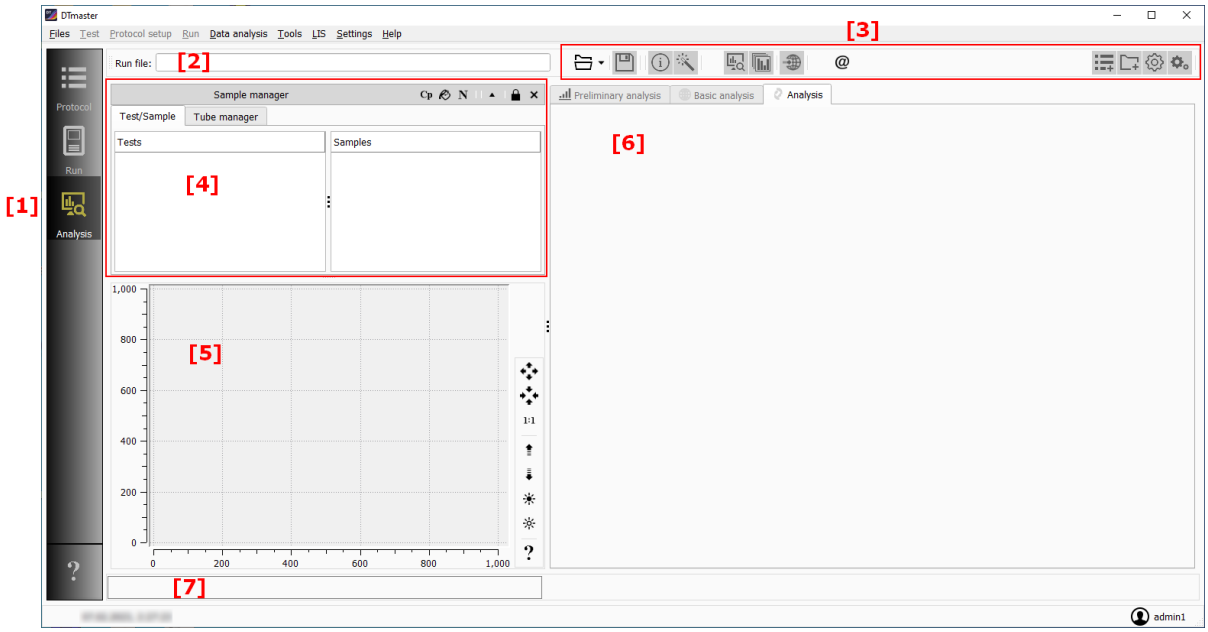


Figure 58 – Analysis mode

The **Analysis** mode window [1] includes:

- protocol name [2];
- toolbar (table 7) [3];
- **Sample manager** workspace, which contains general information about samples and tests performed on them, as well as the **Tube Manager** [4];
- graph of exponential growth of fluorescence level [5];
- workspace with analysis results [6];
- a field with a description and indicator of the current operation in the **Analysis** mode [7].

Table 7 – Buttons on the toolbar in the **Analysis** mode and their purpose

Name	Symbol	Purpose	More details
Open Protocol		Uploading a file with a protocol to the **Analysis** mode	par. Selecting a Protocol to View the Analysis Results
Save as		Saving analysis results to a .rt file	par. Saving a Protocol to File
Protocol Information		Viewing information about the protocol and temperature program	par. Selecting a Protocol to View the Analysis Results
Highlight		Displaying of the **Sample Manager** workspace	par. Setting the Workspace of the Analysis Mode
Preliminary analysis report		Viewing the preliminary analysis report	par. Generation of the Report with Preliminary Analysis Results
Specific report		Formation of an answer form for basic analysis	par. Generation of the Basic Analysis Report
Export results		Exporting analysis results to XML file	par. Exporting Data

Name	Symbol	Purpose	More details
Email		Sending a message to Customer Support	par. Technical Support
Load to Protocol		Loading the protocol into the **Protocol** mode	–
Reboot optical data in another protocol		Reloading optical data on a different protocol	par. Loading Optical Data on a Different Protocol
Edit tests in the protocol		Editing test parameters	par. Editing Test Parameters
Edit protocol settings		Editing protocol settings	par. Basic Protocol Settings

SELECTING A PROTOCOL TO VIEW THE ANALYSIS RESULTS

There are two ways to select a protocol for viewing the analysis results:

1. In the **Analysis** mode, click the **Open protocol** button and select the required file in the "Open Protocol" window (.rt, .r48, .r96, .192, .384 files);
2. In Explorer, go to the file with the required protocol and open it by double-clicking the left mouse button. While the selected protocol will be open only for viewing, its editing will be prohibited.

To upload the RDML protocol:

1. Select **RDML \ RDML import** from the **Analysis** menu. A window to select the protocol file will be displayed.
2. Select the desired file and click the **Open** button.

The selected protocol will be displayed in the **Analysis** mode (fig. 59).

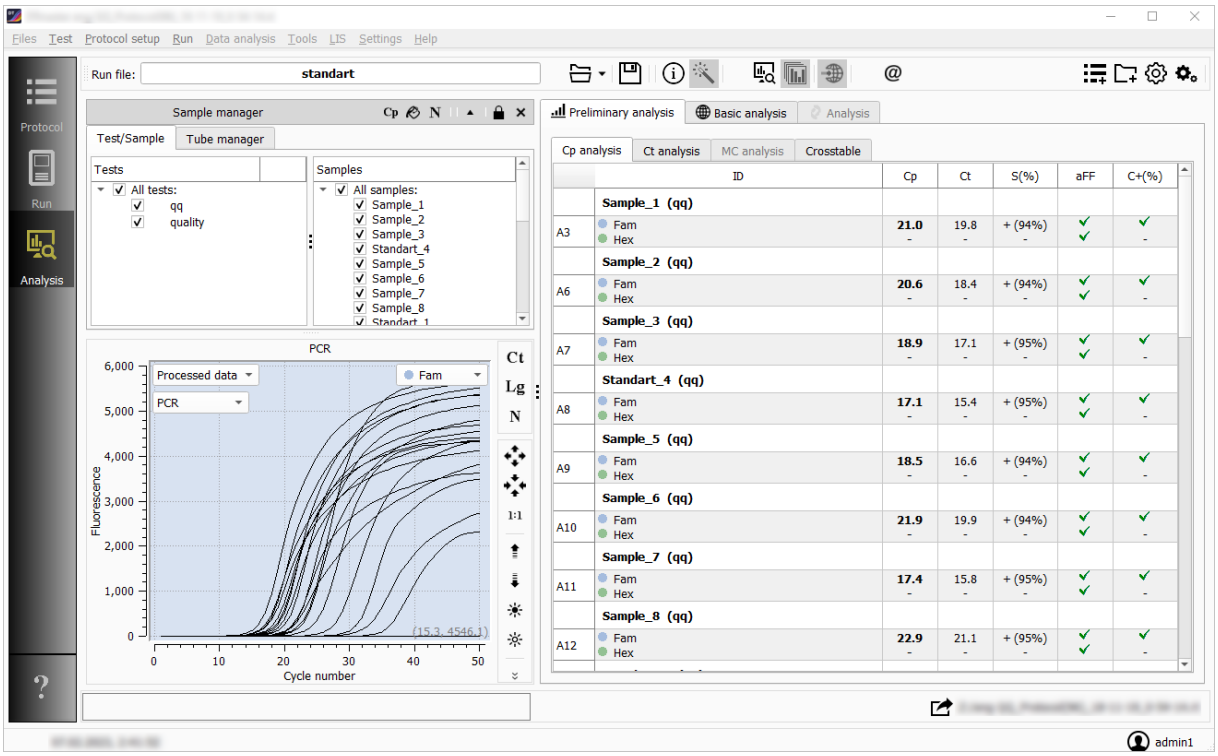


Figure 59 – Example of a protocol for data analysis

To view general information about the selected protocol and its temperature program, click the **Protocol information** button ⓘ on the toolbar. The screen will display a window containing the **Protocol**, **Temperature program** and **Comments** tabs.

VIEWING THE ANALYSIS RESULTS

By default, the workspace with analysis results displays data for all tests, samples and tubes from the protocol.

In the **Sample manager**, you can now edit a set of analysis results for viewing in the following ways:

1. Changing the list of tests and samples for viewing (**Test/Sample** tab, fig. 60, a).
2. Changing the selection of tubes (**Tube manager** tab, fig. 60, b).

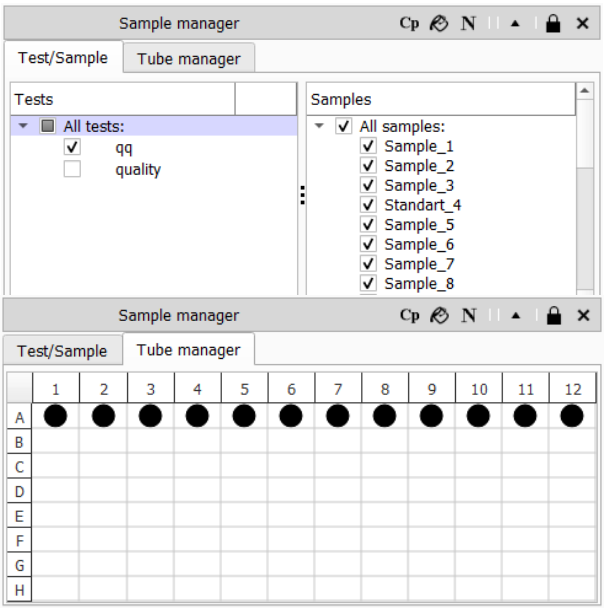


Figure 60 – The **Sample manager** workspace: a – **Test/Sample** tab; b – **Tube manager** tab

Information on the **Test/Sample** tab is presented in the form of two tables, the left table displays a list of tests from the protocol, and the right one – a list of samples. The checkbox, located in the rows of the tables, allows you to include and exclude tests/samples from the workspace with a graph of exponential growth of the fluorescence level and workspace with the analysis results.

Information on the **Tube manager** tab is presented in the form of a table showing the placement of tubes in the thermal unit plate.

To remove a tube from the table, hover the cursor over the cell with the required tube and press the left mouse button. The tube will be removed from the table (fig. 61), and its data will not be presented in the analysis results.

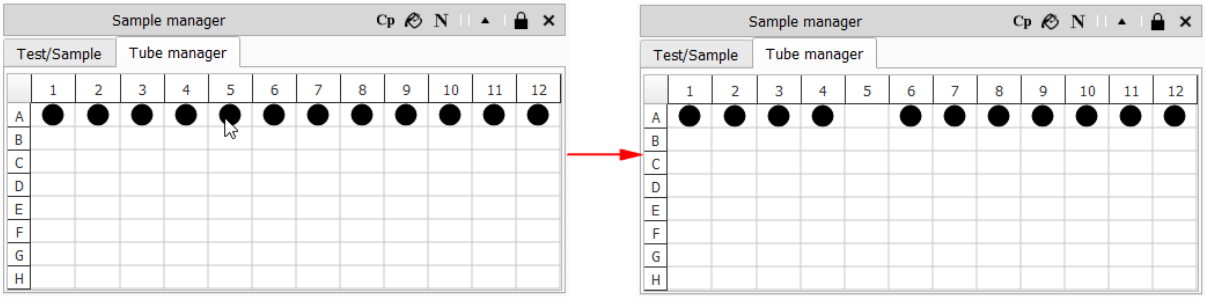


Figure 61 – Example of removing a tube in the **Sample manager**

To cancel a tube removal from the table, place the cursor in empty cell and press the left mouse button. The tube will be displayed in the table again, and the analysis result for this tube will be displayed on the graph and in the analysis results.

To change the color of the tube, follow these steps (fig. 62):

1. Click the **Color Select** button [1]. The panel for editing the color of tubes [2] will appear in the right part of the workspace.
2. Select the desired color on this panel [3]. The mouse pointer changes to .
3. Place the pointer over the cell with the required tube [4] and press the left mouse button.

The color of the tube will be changed to the selected one [5]. The color of the curve for a given tube on the graph of exponential growth of the fluorescence level will also change [6].

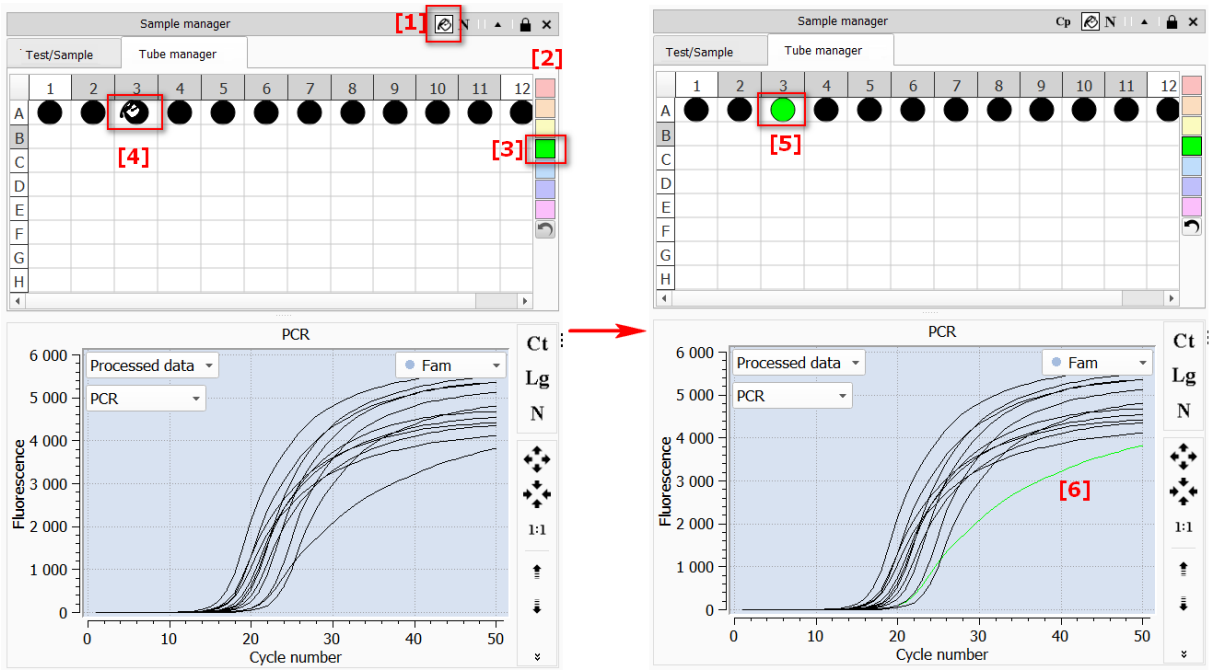


Figure 62 – Panel for editing the color of tubes

If you need to change the color for a group of tubes, select the area for placing the required tubes in the table; after that all tubes from this area will change their color to the selected one.

To view the numbering of tubes (fig. 63) click the **Tubes numeration** button [1]. The numbers of the samples will be displayed in the cells of the table, according to their placement in the thermal unit plate [2]. In this case, the tubes excluded from the results will also be displayed in the table [3].

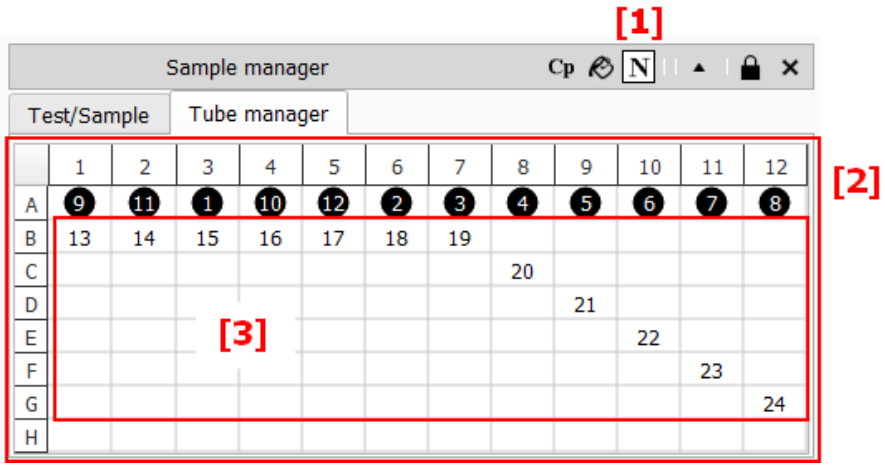


Figure 63 – Viewing the numbering of tubes

To **view the qualitative result** of amplification by plate (Cp), depending on the selected channel on the fluorescence graph, press the **Cp result select** button Cp.

FLUORESCENCE LEVEL GRAPHS

The **Fluorescence graph** workspace contains graphs of the exponential growth of the fluorescence level when the temperature program is running for each tube (fig. 64), as well as the following controls:

[1] – toolbar (table 8);

[2] – drop-down list box for selecting the data type;

[3] – drop-down list box for selecting the analysis type;

[4] – drop-down list box for selecting the active fluorescence channel.

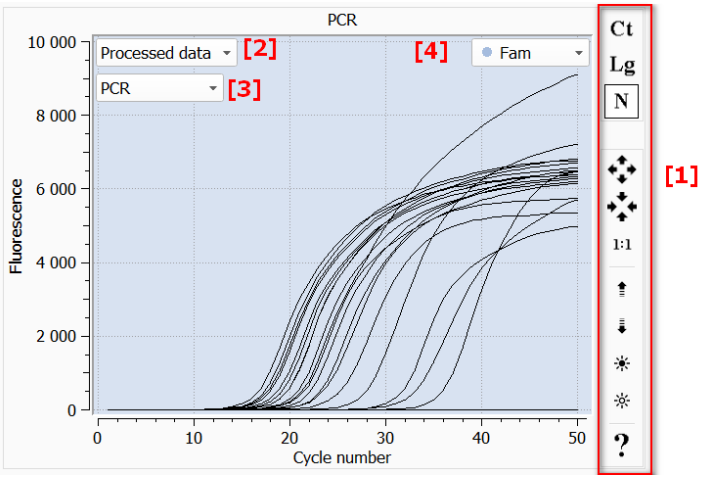


Figure 64 – The **Fluorescence graph** workspace

Table 8 – Buttons on the toolbar of the **Fluorescence graph** workspace and their purpose

Name	Symbol	Purpose	More details
Ct mode	Ct	Ct mode is an alternative approach to Cp for quantifying the exponential growth of fluorescence (threshold method)	Available only for PCR
Lg mode	Lg	Semi-logarithmic graph of the PCR curve in the case of processed data	
Normalization Mode	N	Normalization of the graph of exponential growth of the fluorescence level relative to the value: for Cp - the value along the "y" axis at the Cp point, for Ct - by the averaged value at the end of PCR	
Melting Marker	Mt°	"Mt°" – show temperature marker - this option allows you to determine the temperature at the point of intersection of the marker line with the melting curve;	Shown only for melting curves
Color result	Rt°	"Rt°" – color the curves based on the analysis results - the option is available for special DNA-Technology kits from the section of SNP genotyping (color marking of genotypes);	
-(dF)/(dT)	-dF/dT	Melting curve inversion	
Scale up		Changing the scale of graphs (this function is also performed by the mouse scroll wheel)	Provides configuration and scaling of graphs
Scale down			
Auto scaling	1:1	Switch to the default graph scale	
Increase marker size		Increase/decrease the size of markers on curves without changing the size of the graphs	

Name	Symbol	Purpose	More details
Reduce marker size			
Thickness up		Increase / decrease the brightness of the graph lines	
Thickness down			
Help		Displaying a hint for navigation rules	

When the mouse pointer is pressed on a certain graph (fig. 65), it is highlighted by a thickened line, the number of the tube is next to it [1]. To remove the selection, click on an empty area of the graph [2].

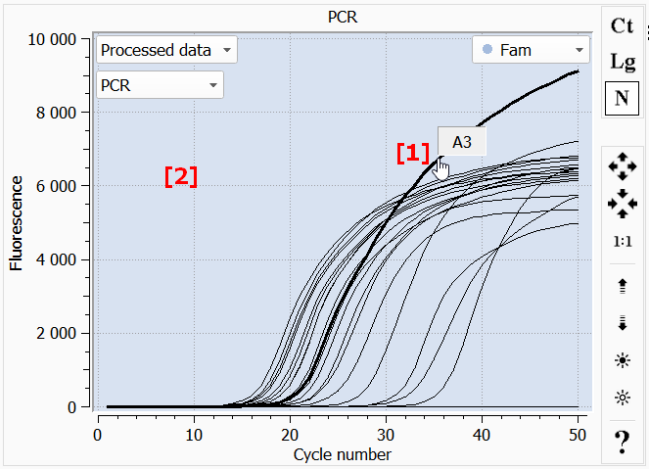


Figure 65 – An example of highlighting the graph of exponential growth of the fluorescence level

Select the data type to be displayed in the graph

Data types for displaying in the **Fluorescence graph**:

- raw data – data obtained from the device;
- filtered data – original data with the application of a digital filter and smoothing;
- processed data:
- for PCR analysis: filtered data minus baseline (in the initial section);
- for the "Melting curve" analysis: the first derivative of the original curve with filters and smoothing.

To change the type of displayed data, use the drop-down list box located in the top part of the graph (fig. 66).

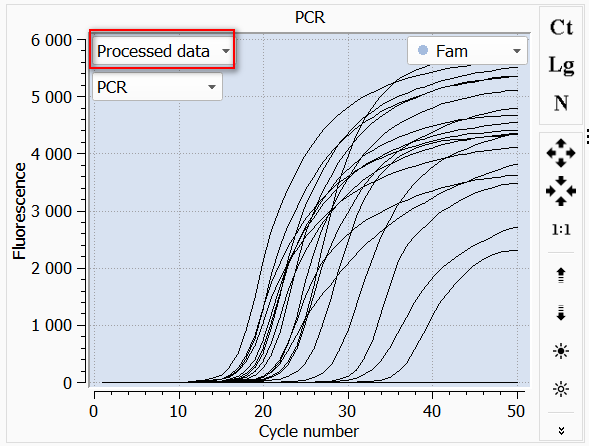


Figure 66 – Changing the type of data displayed in the graph

Selecting Analysis Type

DTmaster implements two analysis types:

1. PCR;
2. Melting curve.

To select the analysis type, use the appropriate drop-down list box (fig. 67).

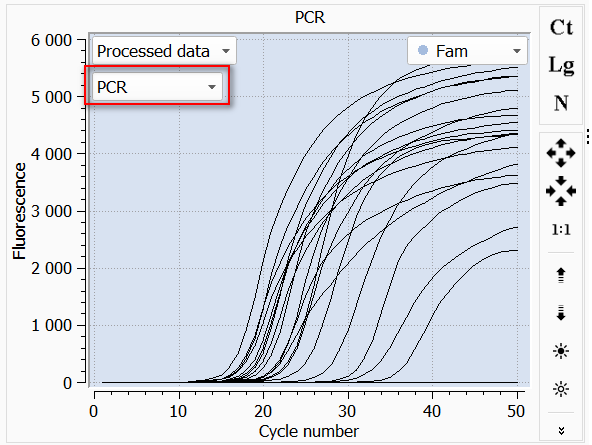


Figure 67 – Changing the analysis typ

Selecting the Active Fluorescence Channel

The software provides the ability to view the analysis graph on each of the active fluorescence channels used in tests, as well as on all channels simultaneously.

To select the necessary active channel, use the appropriate drop-down list box located in the top part of the graph. Channels not involved in the analysis are marked with gray in this list and are not available for selection.

Viewing modes

The program provides three additional viewing modes for the analysis graph and additional functionality for PCR (fig. 68):

1. **Ct** mode [Ct] – an alternative to "Cp" approach for quantifying the exponential growth of fluorescence level (threshold method). When this mode is enabled, the "threshold" is displayed as a horizontal black line at the level of 10 rms of the noise term at the initial PCR site. The intersection of this line with the processed PCR curve on the "x" axis provides the Ct value.

2. **Logarithm** mode [Lg] – semi-logarithmic graph of the PCR curve in the case of processed data (after the break-off point there is a straight-line section, which corresponds to the exponential growth on the PCR curve).
3. **Normalization** mode [N] – normalization of the graph of exponential growth of the fluorescence level relative to the value: for Cp - the value along the "y" axis at the Cp point, for Ct - by the averaged value at the end of PCR. For each curve, this value is determined, the maximum value is selected, and then the normalization coefficient for each curve is calculated relative to it, and the entire curve is multiplied by this coefficient (idealization of the PCR process throughout the plate).

These modes are enabled using the toolbar in the graph area.

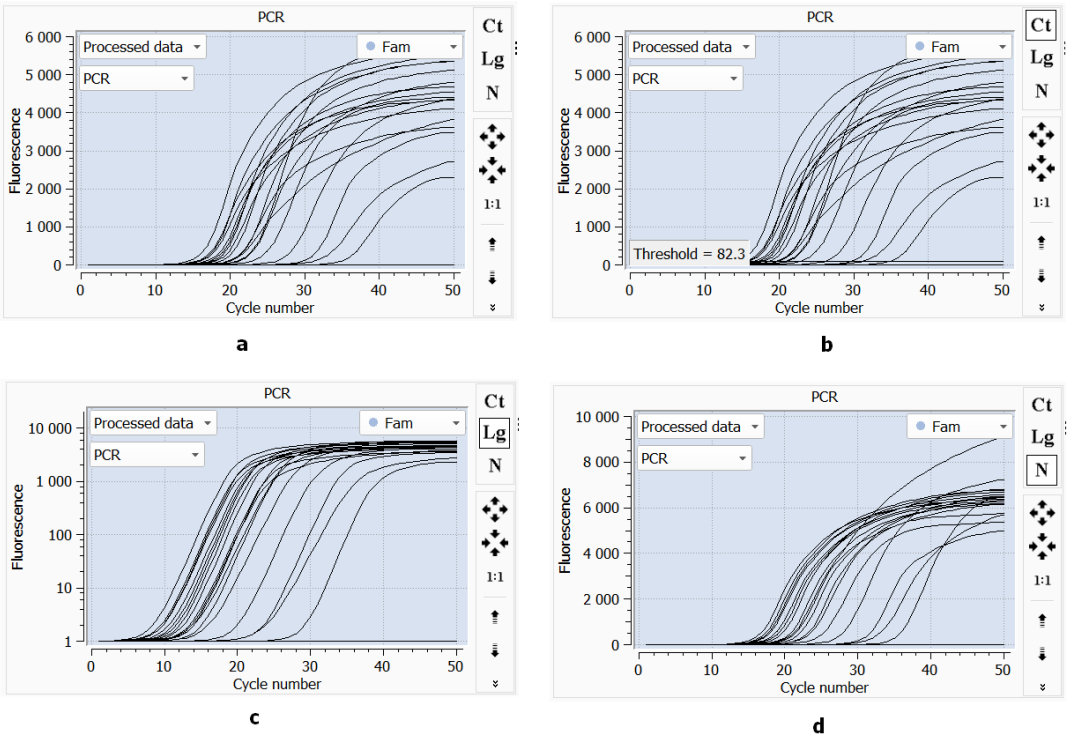


Figure 68 – Modes of viewing the graphs: a – with additional modes turned off; b – in the Ct mode; c – in the **Logarithm** mode; d – in the **Normalization** mode

For the melting curves, the following modes of viewing the analysis graph and additional functionality are implemented:

- **Melting Marker** [Mt°] – show temperature marker – this option allows you to determine the temperature at the point of intersection of the marker line with the melting curve (fig. 69);
- **Color result** [Rt°] – color the curves based on the analysis results (fig. 70);
- **- (dF)/(dT)** [-dF/dT] – invert the melting curves (fig. 71).

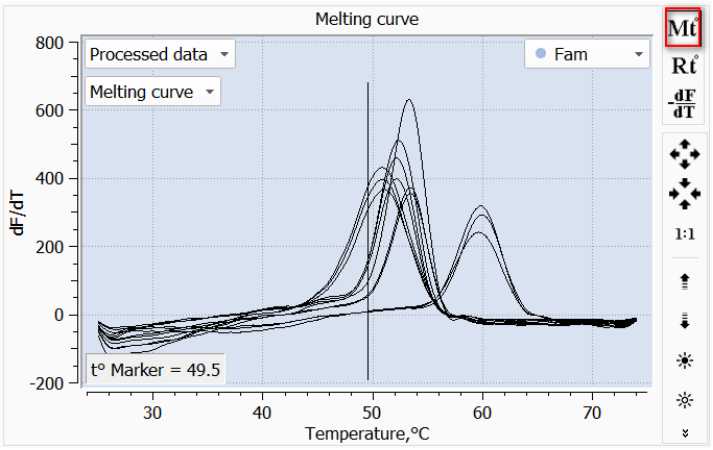


Figure 69 – An example of displaying a temperature marker in the graph area

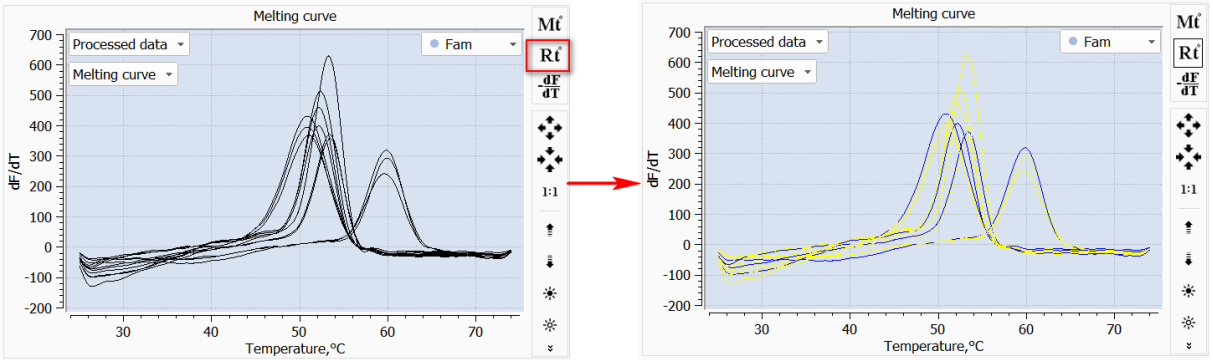


Figure 70 – An example of using the **Color result** mode

The **Color result** [Rt°] option is available for special DNA-Technology kits from the section of SNP genotyping (color marking of genotypes)

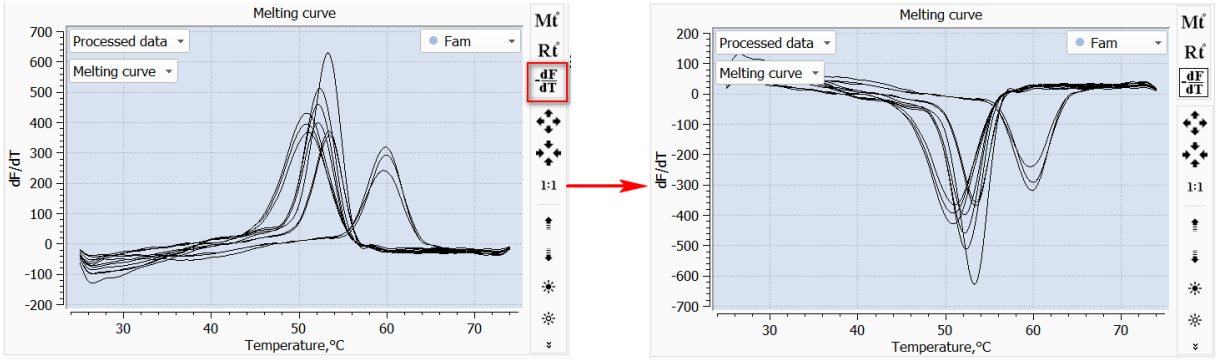


Figure 71 – An example of using the mode of melting curves inversion

VIEWING THE RESULTS

The analysis results are presented in the area on the right side of the **Analysis** mode. The data is grouped into several tabs:

- **Preliminary analysis** – contains information about the results of PCR analysis up to the Cp/Ct level;
- **Basic analysis** – this tab is available if basic types of tests used in the analysis;

- **Analysis** – this tab is available if the protocol used special tests developed by “DNA-Technology” company.

Viewing the Results of the Preliminary Analysis

The results of the preliminary analysis are presented on the tab of the same name, containing the following subsections (fig. 72):

- **Cp analysis**;
- **Ct analysis**;
- **MC analysis** (if this analysis was performed);
- **CrossTable**.

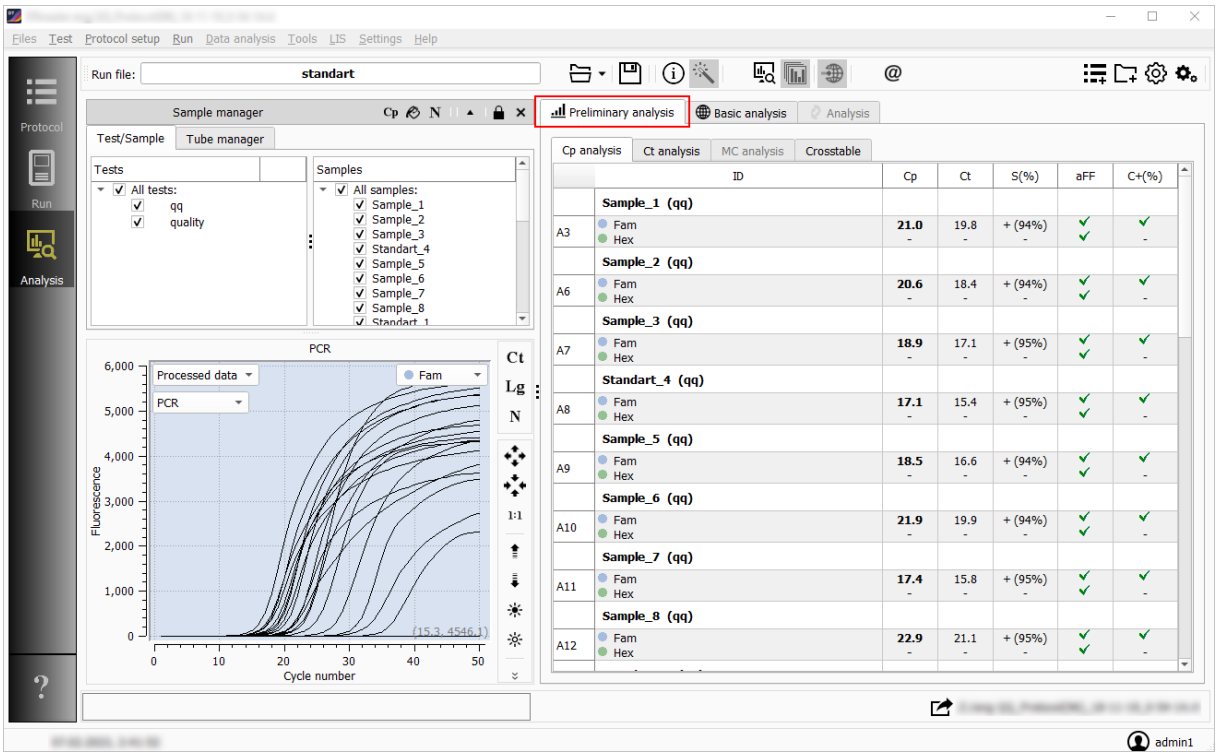


Figure 72 – The **Preliminary analysis** tab

The **Ct analysis** and **CrossTable** tabs are available for viewing if the appropriate permissions are selected in the current user account settings (see Appendix B).

Cp results are viewed on the **CP analysis** tab.

The data on the tab are presented in the form of a table with a list of samples and Cp results for each of them. The Cp value is indicated for all active channels (fig. 73).

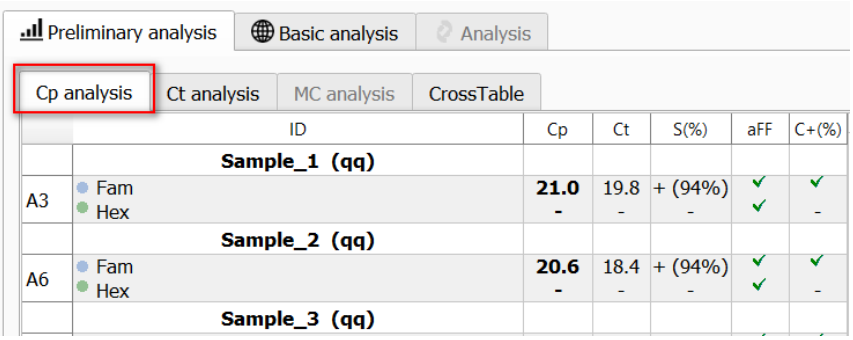


Figure 73 – The **Cp analysis** tab

Additionally, the following parameters are indicated for each sample:

1. **Ct** – the intersection of the Threshold with the melting curve (quantitative threshold method);

The Threshold value allows you to set the "threshold" level of fluorescence when determining the indicator cycle (taken into account in the threshold method of curve analysis).

2. **S** – robustness of sigmoidal fitting (in percentage) – the main step in determining Cp.

In the test parameters (see par. Creating and Editing Tests) on the **Common** tab there is the **Sigmoid validation thresholds: min. and max. value** parameter (default values are 7 and 20). If the calculated validity parameter is:

- less than minimum border – the result is positive "+" (there is a Cp value);
- greater than maximum limit – the result is negative "–" (Cp not found);
- between two borders – the result is considered as positive for subsequent calculations, but the table will contain the "?" symbol and a corresponding warning will be indicated on the **Attention** tab for the corresponding tube;

3. **aFF** – endpoint flare up filter (for positive and negative curves).

This filter is configured in the Test Editor (see par. Creating and Editing Tests) on the **Common** tab in the **Endpoint fluorescence criterion** block (fig. 74). The following filter settings are available:

- checkbox **Apply this method in analysis**, which determines whether this filter will be used in the analysis of results;
- baseline fluorescence (relative to baseline exposure);
- minimum and maximum thresholds.

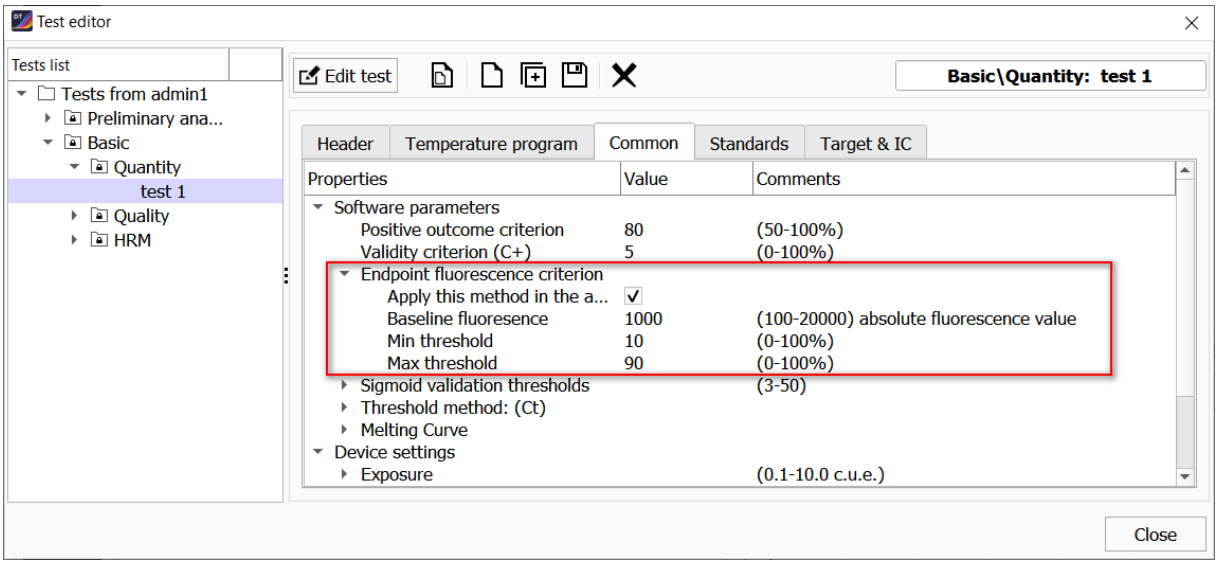


Figure 74 – Parameters of the filter on endpoint flare up in the test settings

This method calculates for positive curves:

- true flare up of the curve;
- minimum flare up level: multiply the base line by the % of the minimum threshold.

Next, a comparison is carried out: if the actual flare up is greater than the minimum threshold, then the positive result is confirmed – ✓ icon in the table. If the actual flare up is less than the minimum threshold, then the positive result is refuted – ✗ icon (the curve becomes negative, Cp is not detected). If the flare up is higher than the threshold up to 5% – ? icon (the corresponding entry on the **Attention** tab), but the result is considered positive.

For negative curves (not passing according to the sigmoidal fitting criterion), the following is calculated:

- true flare up of the curve;
- maximum flare up level: base line multiply by % of the maximum threshold.

If the actual flare up is greater than the maximum threshold, then the curve remains negative, but the filter's result is an ? icon in the table (the corresponding entry on the **Attention** tab).

4. **C+** – filter of the validity of a positive result relative to C+ (only for positive curves).

The filter is configured in the Test Editor (see par. Creating and Editing Tests) on the **General Settings** tab. The only filter parameter is **Validity criterion (C+)** (fig. 75). If a non-zero value is specified for this parameter, then this filter is applied in the analysis of results.

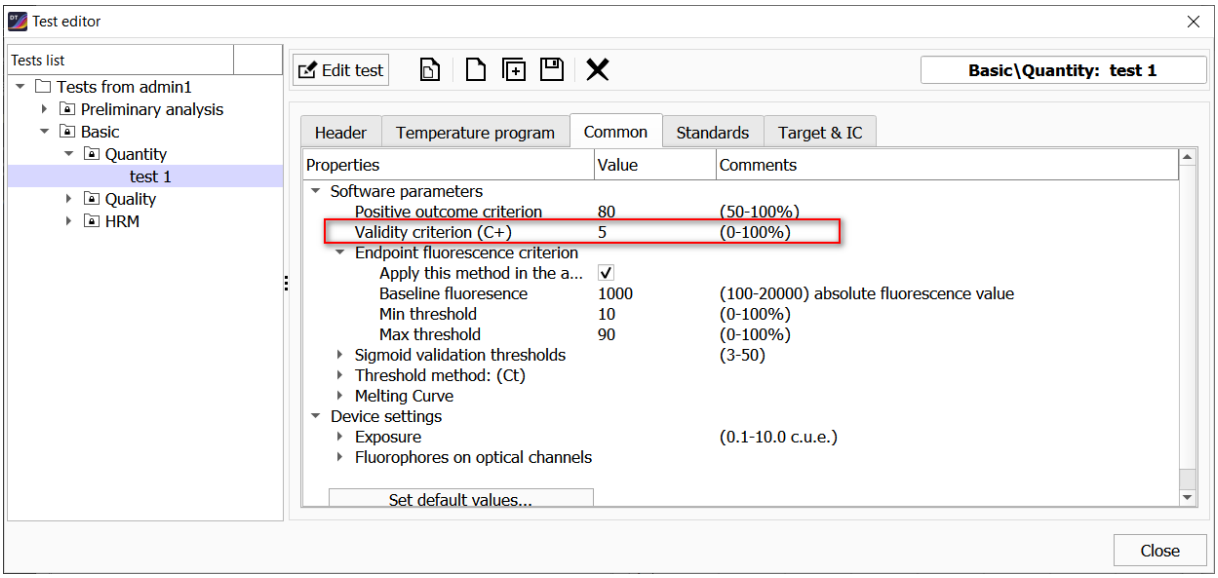


Figure 75 – Filter parameters by the criterion of the validity of a positive result

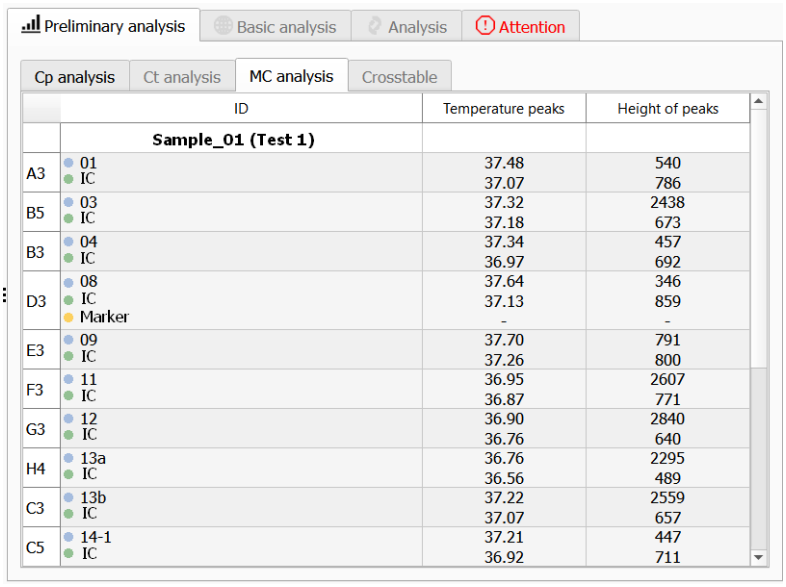
The filter of the validity of a positive result relative to C+ works if there are two or more positive results in the same test. In this situation, the fluorescence values are calculated at the Cp point for curves of the same type, then the maximum value (C+) is found. In other cases: if the fluorescence values are more than % (C+ validity criterion) of the maximum, then a positive result is confirmed (icon in the table).

If less, then the positive result is refuted (icon), and the curve becomes negative (Cp not found). If the fluorescence values are higher than the threshold up to 5% – icon (the corresponding entry on the **Attention** tab), but the result is considered positive.

5. **Cp_s** – an alternative approach in determining Cp. It is used in a situation where the curve shapes in the protocol differ significantly from each other.

For the protocols in recording melting curves, the magnitude of peaks of melting temperature can be estimated.

Results are viewed on the **MC analysis** tab. The data are presented in the form of a list of samples indicating the temperature peaks in ℃and the peak heights in arbitrary units for each tube (fig. 76).

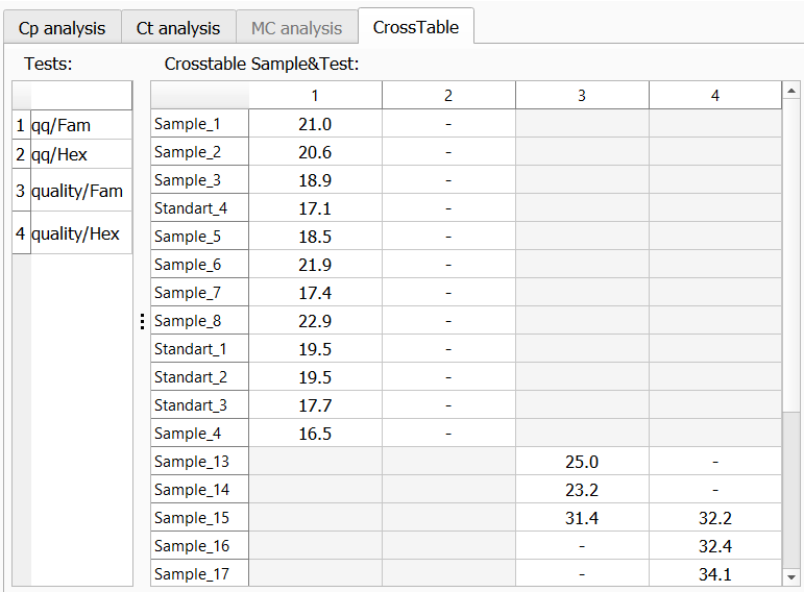


	ID	Temperature peaks	Height of peaks
	Sample_01 (Test 1)		
A3	01 IC	37.48 37.07	540 786
B5	03 IC	37.32 37.18	2438 673
B3	04 IC	37.34 36.97	457 692
D3	08 IC Marker	37.64 37.13 -	346 859 -
E3	09 IC	37.70 37.26	791 800
F3	11 IC	36.95 36.87	2607 771
G3	12 IC	36.90 36.76	2840 640
H4	13a IC	36.76 36.56	2295 489
C3	13b IC	37.22 37.07	2559 657
C5	14-1 IC	37.21 36.92	447 711

Figure 76 – Results of the **MC analysis**

The **CrossTable** tab contains a list of tests carried out within this protocol, as well as a cross-table (fig. 77).

A cross-table is a summary table of Cp results (or Ct, or temperature peaks) where the row headings are samples and the column headings are tests.



1	qq/Fam
2	qq/Hex
3	quality/Fam
4	quality/Hex

	1	2	3	4
Sample_1	21.0	-		
Sample_2	20.6	-		
Sample_3	18.9	-		
Standart_4	17.1	-		
Sample_5	18.5	-		
Sample_6	21.9	-		
Sample_7	17.4	-		
Sample_8	22.9	-		
Standart_1	19.5	-		
Standart_2	19.5	-		
Standart_3	17.7	-		
Sample_4	16.5	-		
Sample_13			25.0	-
Sample_14			23.2	-
Sample_15			31.4	32.2
Sample_16			-	32.4
Sample_17			-	34.1

Figure 77 – The **CrossTable** tab

Switching between Cp, Ct and temperature peaks results is carried out using the context menu available in the area highlighted in fig. 78.

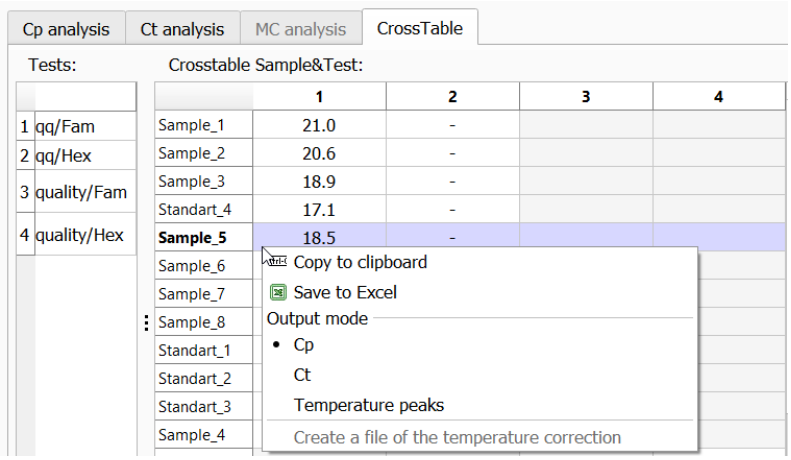


Figure 78 – Switching between Cp, Ct and temperature peaks results

Viewing Basic Analysis Results

Data with the results of basic analyzes carried out in the program are viewed on the **Basic Analysis** tab (fig. 79).

The basic tests include tests based on the qualitative and quantitative type of tests.

If the protocol did not contain these test types, the tab will be unavailable for viewing.

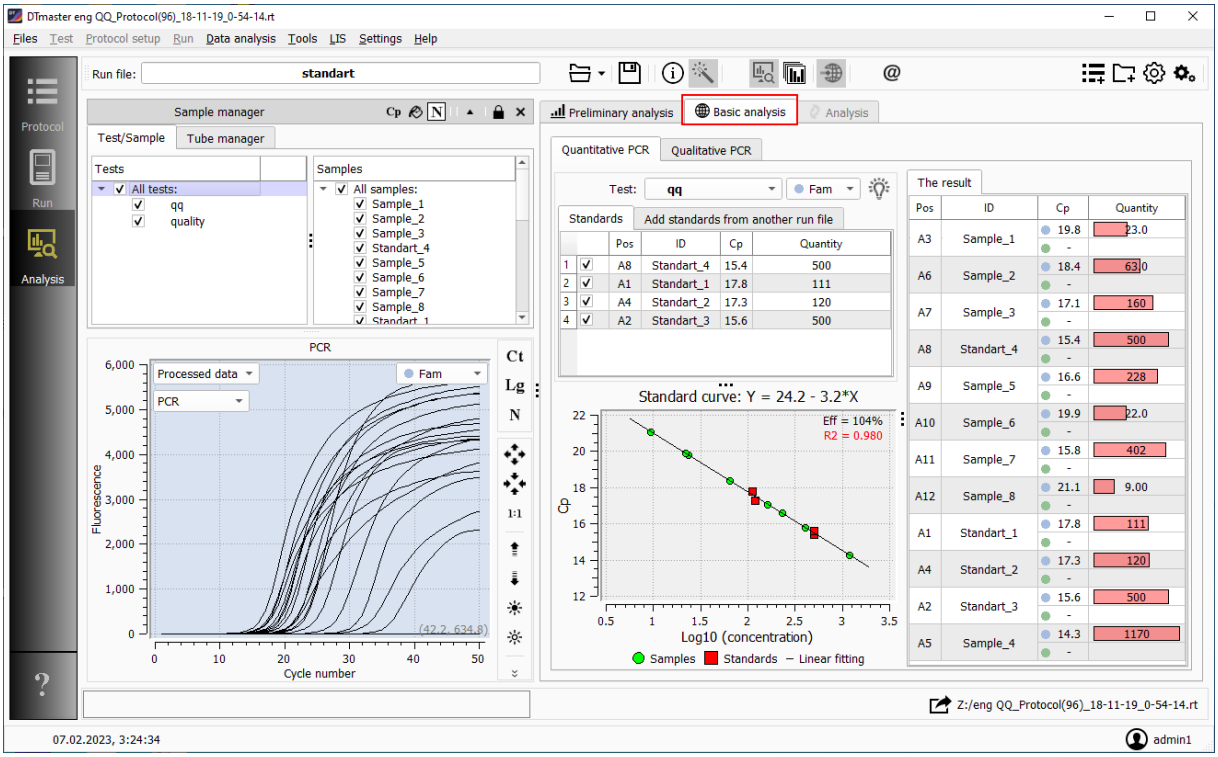


Figure 79 – Tab with the results of basic analysis

To view the results of the **Quantitative PCR** type of analysis (fig. 80), go to the tab of the same name [1] in the **Basic analysis** section.

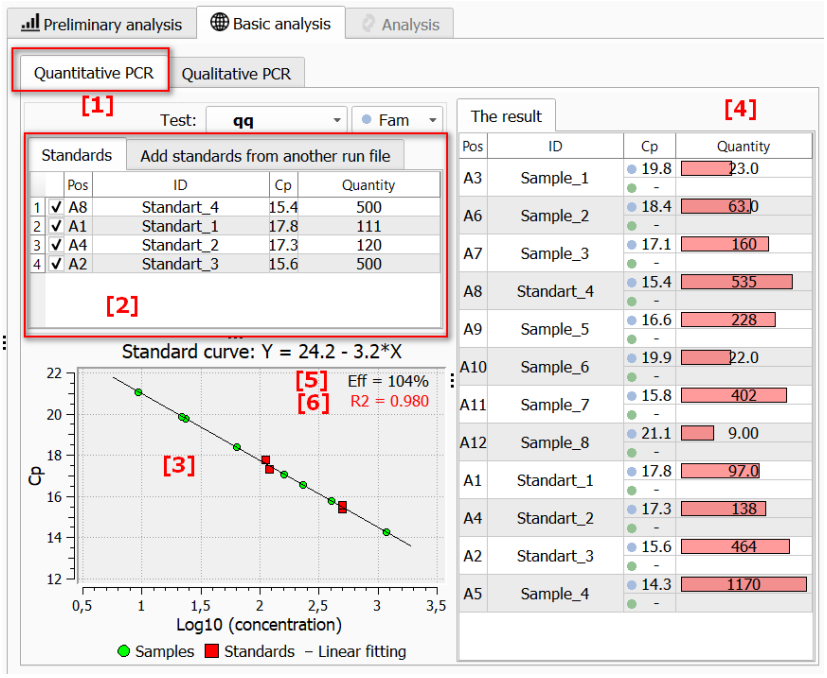


Figure 80 – The **Quantitative PCR** tab

Quantitative analysis using calibration samples (standards) allows you to determine the amount of the desired DNA fragment in the sample. For quantitative analysis, it is necessary to include samples with a known amount of the desired DNA (calibration samples, standards) in the protocol [2].

After carrying out the study with calibration samples, the program will automatically build a calibration graph [3] and determine the concentration of the desired DNA in unknown samples [4].

The **Standard Curve** graph displays the following data:

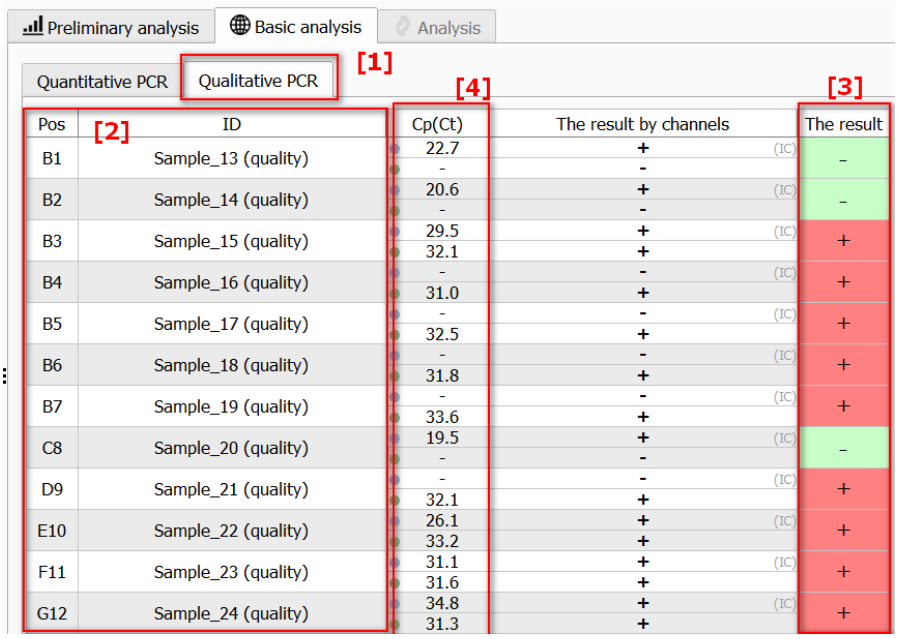
- coordinate system (axis "x" – decimal logarithm of sample concentration Log10 (Concentration), axis "y" – Ct or Cp value);
- calibration samples (icon);
- samples under study (icon);
- standard curve;
- PCR efficiency values (Eff, [5]), accuracy of approximation (R2, [6]).

The program draws a straight line of the form y = A* x + B from the points of the calibration samples for which the concentration and Ct (or Cp) value are known. The equation of the resulting straight line is indicated in the name of the Standard Curve graph.

On the plotted curve, the samples under study are displayed as icons, in accordance with the obtained Ct (Cp) value. Based on the found decimal logarithm of concentration, the program calculates the concentration of the samples under study.

The approximation quality of the "Standard Curve" is represented by the value of Approximation Confidence.

To view the results of the **Qualitative PCR** study (fig. 81), go to the tab of the same name [1] in the **Basic analysis** section.



Pos	ID	Cp(Ct)	The result by channels	The result
B1	Sample_13 (quality)	22.7 -	+ - (IC)	-
B2	Sample_14 (quality)	20.6 -	+ - (IC)	-
B3	Sample_15 (quality)	29.5 32.1	+ + (IC)	+
B4	Sample_16 (quality)	- 31.0	- + (IC)	+
B5	Sample_17 (quality)	- 32.5	- + (IC)	+
B6	Sample_18 (quality)	- 31.8	- + (IC)	+
B7	Sample_19 (quality)	- 33.6	- + (IC)	+
C8	Sample_20 (quality)	19.5 -	+ - (IC)	-
D9	Sample_21 (quality)	- 32.1	- + (IC)	+
E10	Sample_22 (quality)	26.1 33.2	+ + (IC)	+
F11	Sample_23 (quality)	31.1 31.6	+ + (IC)	+
G12	Sample_24 (quality)	34.8 31.3	+ + (IC)	+

Figure 81 – The **Qualitative PCR** tab

Qualitative analysis allows you to determine the presence or absence of the desired DNA fragment in the sample.

The first and second columns of the **Quality PCR** tab contain the numbers of the tubes and their identifiers [2].

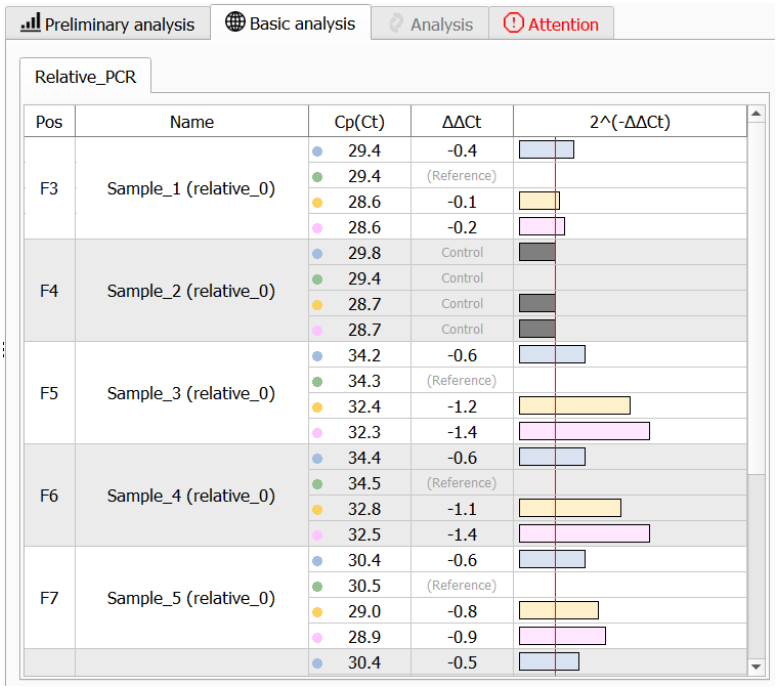
The results of the qualitative analysis are displayed in the **Result** column as the following values [3]:

- "+" – the sample contains fragments of the desired DNA (an exponential growth of the signal for the Specificity channel was registered);
- "-" – there are no fragments of the desired DNA in the sample (there is no exponential growth of the signal for the Specificity channel in the case of using an internal control sample (IC) - an exponential growth of the signal for the IC channel is registered);
- "?" – unreliable result (when using IC – there is no exponential growth of the signal for Specificity and IC channel).

The "Cp (Ct)" column [4] indicates the values of the exit cycle for the used fluorophore for each sample (Ct or Cp – depending on the selected analysis method).

Relative analysis ($2^{-\Delta\Delta CT}$) is a relative quantification technique that uses information about the threshold cycle from an experiment to calculate the relative gene expression in target and control samples. Reference genes are used to normalize PCR to correct for differences in the amount of added template for each sample and to reduce variations caused by errors in PCR setup and inaccuracies in thermal cycling conditions.

To view the research results, go to the **Relative PCR** tab (fig. 82).



Pos	Name	Cp(Ct)	ΔΔCt
F3	Sample_1 (relative_0)	29.4	-0.4
		29.4	(Reference)
		28.6	-0.1
		28.6	-0.2
F4	Sample_2 (relative_0)	29.8	Control
		29.4	Control
		28.7	Control
		28.7	Control
F5	Sample_3 (relative_0)	34.2	-0.6
		34.3	(Reference)
		32.4	-1.2
		32.3	-1.4
F6	Sample_4 (relative_0)	34.4	-0.6
		34.5	(Reference)
		32.8	-1.1
		32.5	-1.4
F7	Sample_5 (relative_0)	30.4	-0.6
		30.5	(Reference)
		29.0	-0.8
		28.9	-0.9
		30.4	-0.5

Figure 82 – The **Relative PCR** tab

ΔΔCt is the difference in ΔCT between target and control samples, which is

ΔΔCt = ΔCt (target sample) - ΔCt (control sample)

The end result of this method is presented in the form of a change in the expression of the target gene in the target sample relative to the control sample, normalized to the reference gene. For control samples, the relative gene expression is set to 1 because ΔΔCt is 0 and therefore 2° = 1.

Viewing HRM Analysis Results

HRM is a high-resolution melting test used to detect differences in nucleotide sequences.

The method is based on PCR technique using dyes capable of fluorescence upon binding to double-stranded DNA, registration of fluorescence during the melting of amplification products, analysis of melting data using special algorithms. Due to the high specificity and sensitivity of the method, it is possible to determine the minimum differences in the nucleotide composition. The HRM method is applicable for SNP genotyping, analysis of methylation and GC sequence, search for unknown mutations (gene scan).

View data

Preliminary data. Preliminary analysis results are viewed on the **Preliminary analysis** tab. For details on viewing the results of the preliminary analysis, see par. Viewing the Results of the Preliminary Analysis.

HRM analysis data. Viewing and analysis of HRM data is carried out on the **Basic analysis** tab of the **Analysis** mode (fig. 83).

This tab displays:

[1] – workspace with graphs of raw and processed data;

[2] – workspace with analysis parameters and sampling;

[3] – graph of cluster analysis (in the form of a scatter diagram);

[4] – table with results.

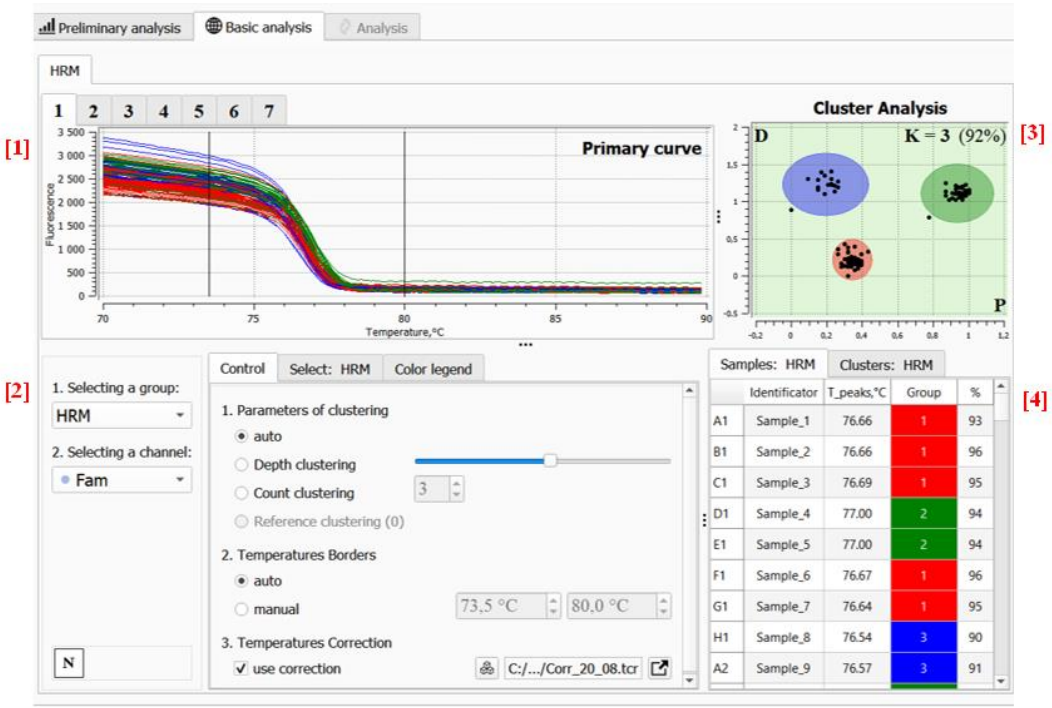


Figure 83 – Viewing HRM analysis data on the **Basic analysis** tab

The workspace with graphs of raw and processed data contains 7 tabs that allow viewing melting curves in various formats (fig. 84).

To view the graph, select the corresponding tab (1 – 7).

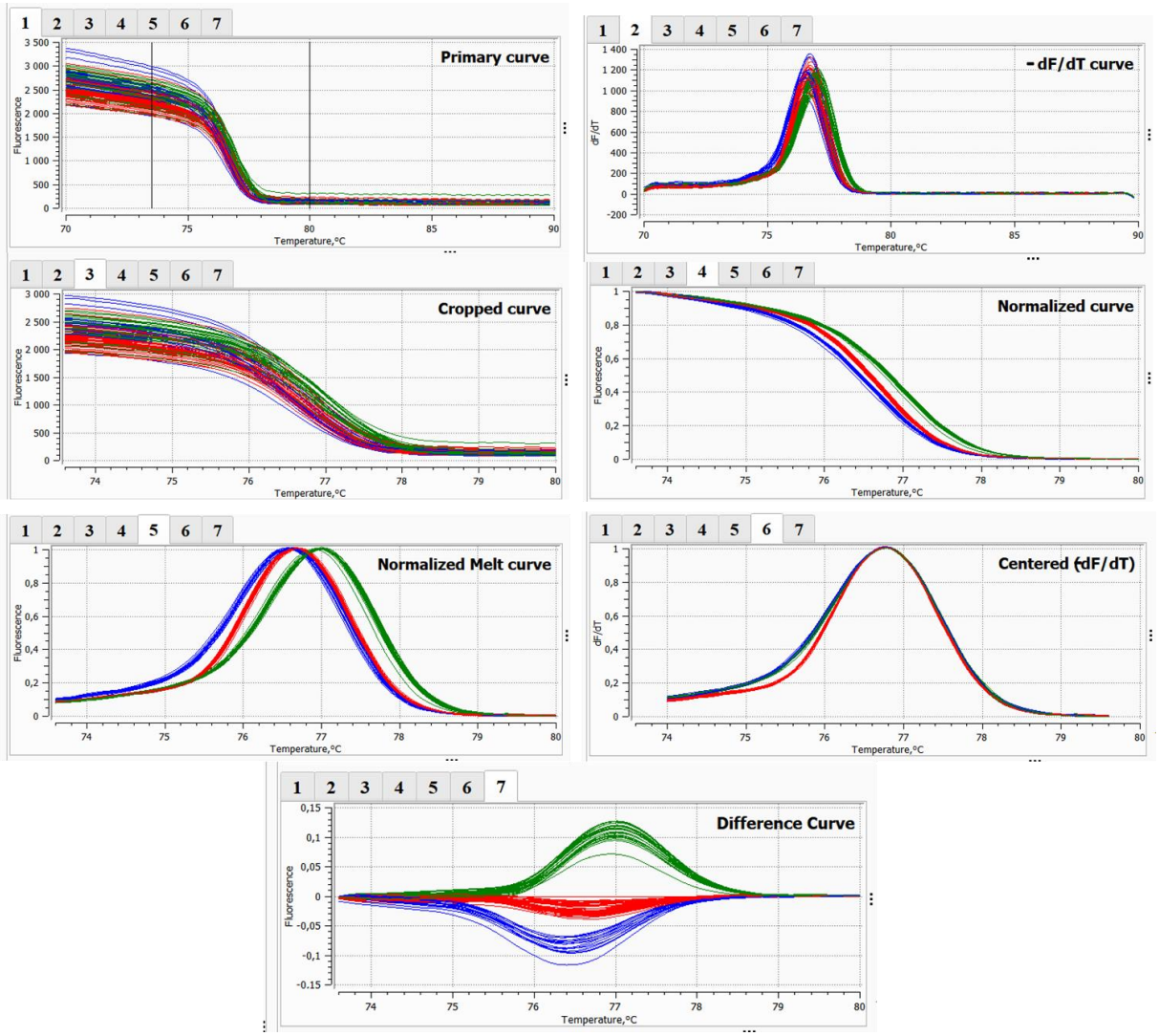


Figure 84 – Graphs of HRM analysis data

Tab 1 contains raw melting data (melting (dissociation) curves of PCR products in the form of plots of fluorescence change versus temperature (Tm, °C)).

Tab 2 contains melting curves in the form of -dF / dT (differential assessment of the rate of fluorescence change).

Tab 3 contains raw data about melting over a specified temperature range.

Tab 4 contains normalized graphs of melting curves over a specified temperature range.

Tab 5 contains normalized data for melting in the form -dF / dT over a specified temperature range.

Tab 6 contains centered data for melting in the form of -dF/dT over a specified temperature range.

Tab 7 contains a **Difference data** graph showing the differences in the dynamics of fluorescence changes between samples and the reference curve (the choice of the reference curve is carried out automatically by the program) over a specified temperature range.

The analysis results are presented on the **cluster analysis** graph and at the **table of results**.

The **clustering analysis graph** shows the result of the cluster analysis in the form of scatterplots (fig. 85). Clustering objects (samples) are displayed as dots. Clusters of points on the graph, united by colored outlines, with the specified analysis parameters, represent the required clusters. The number of clusters – K and value of the confidence indicator in percent (%) are displayed in the upper right corner of the coordinate plane of the graph.

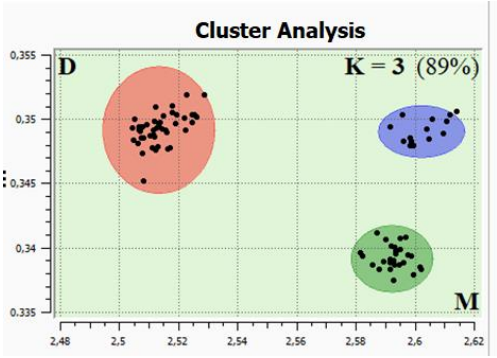


Figure 85 – Graph of cluster analysis

The information is grouped in two tabs of the result summary table: **Samples: HRM** and **Clusters: HRM**.

The **Samples: HRM** tab contains a table (fig. 86) containing the following data:

- sample positions on the thermal unit;
- sample identifiers;
- **T peak, °C** – value of the maximum temperature of the melting-curves peak;
- **Cluster** – the result of clustering - the melting curve (sample) belongs to a particular cluster (the software automatically assigns a number and a color marker to the cluster);
- **%** is an indicator of the reliability of the curve belonging to the cluster.

Clustering results with a confidence indicator below the value set by the test settings are highlighted with the "*" symbol.

Samples: HRM | Clusters: HRM

	Identificator	T_peaks,°C	Group	%
A1	Sample_1	76.71	1	61 *
B1	Sample_2	76.72	1	54 *
C1	Sample_3	76.74	1	45 *
D1	Sample_4	76.94	2	74 *
E1	Sample_5	76.85	2	93
F1	Sample_6	76.59	1	66 *
G1	Sample_7	76.51	3	56 *
H1	Sample_8	76.34	3	23 *
A2	Sample_9	76.50	3	81
B2	Sample_10	76.92	2	79

Figure 86 – The **Samples: HRM** tab

The **Clusters: HRM** tab (fig. 87) displays the following information:

- the number of differentiated clusters (the first and second columns of the table);
- color legend of clusters;
- distribution of samples by clusters.

Column **G_type** is used to assign a name to the cluster. To enter a record, double-click on the required cell and enter the name of the genetic variant. After assigning a name, the clusters will be renamed in the sample analysis table.

For example, when determining a replacement of the G> A substitution, genotype variants corresponding to three clusters – GG, GA, AA – can be indicated in the cells. To enter a record, double-click on the desired cell and enter the name of the genetic variant.

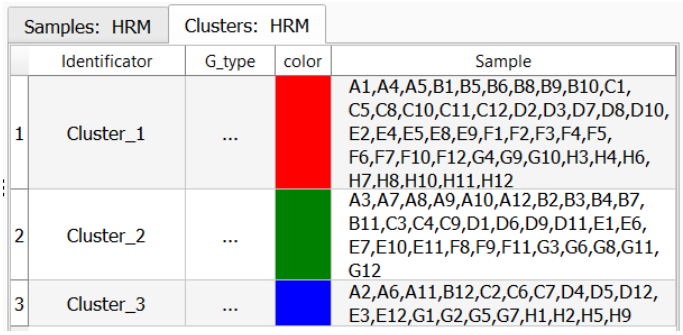


Figure 87 – The **Clusters: HRM** tab

Analysis parameters

The analysis parameters are presented in the area shown in fig. 88.

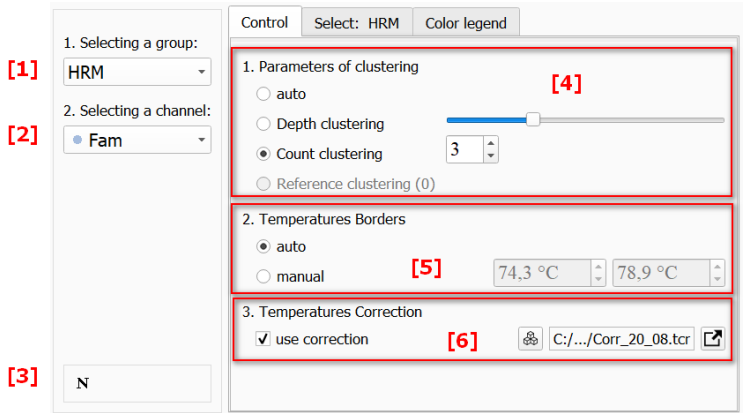


Figure 88 – Workspace with analysis parameters

On the left side of the workspace there are:

[1] – **Selecting a group** setting (used to switch analysis in a multi-test protocol format);

[2] – channel indicator;

[3] – button for enabling/disabling the numbering of tubes.

In the center of the workspace are located:

[4] – clustering parameters (allow you to select the method of clustering samples, including the automatic format, clustering by specifying the number of clusters, reference clustering);

[5] – temperature limits of melting analysis (if necessary, the limits of the melting analysis area can be set manually);

[6] – temperature correction (applied optionally, allows reading and applying the data of the **Temperatures correction** in the analysis).

The **Sampling: HRM** tab contains information on the number of samples and their placement in the cells of the thermal unit. The color identifiers of the samples reflect their belonging to the cluster (fig. 89).

To view the numbering of tubes, click the **Numerate tubes** button N in the lower left part of the settings field. Second clicking cancels the numbering.

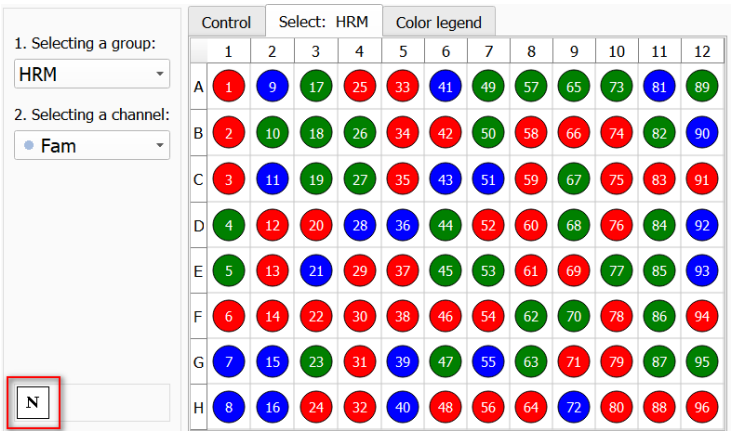


Figure 89 – The **Sample: HRM** tab, viewing the tubes numbering

The color legend tab is to be used for changing the color legends of the clusters (fig. 90)

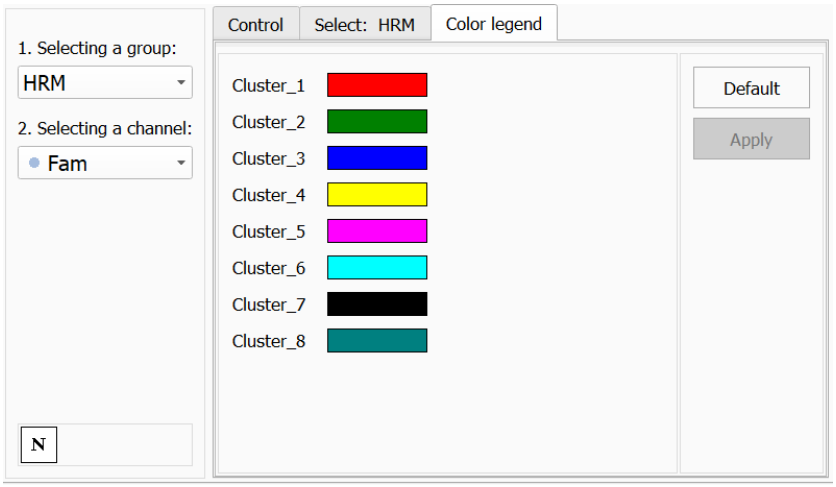


Figure 90 – The **Color legend** tab

Data analysis. Upon completion of the optical data analysis, the program performs the analysis automatically, including clustering and plotting graphs of raw and processed data. Samples marked as negative ("C-") are excluded from clustering by the program.

Configuring clustering parameters. The automatic clustering results are available for viewing after the protocol analysis is complete. If you need to optimize the analysis, you should change its settings. Changing these settings leads to a revision of the analysis results:

- Depth clustering – an analysis setting that allows you to change the number of distinguished clusters by setting the sensitivity threshold of cluster analysis. To apply the setting, move the cursor and left-click on the **Depth clustering** switch. Clustering depth is changed by moving the slider (fig. 91).

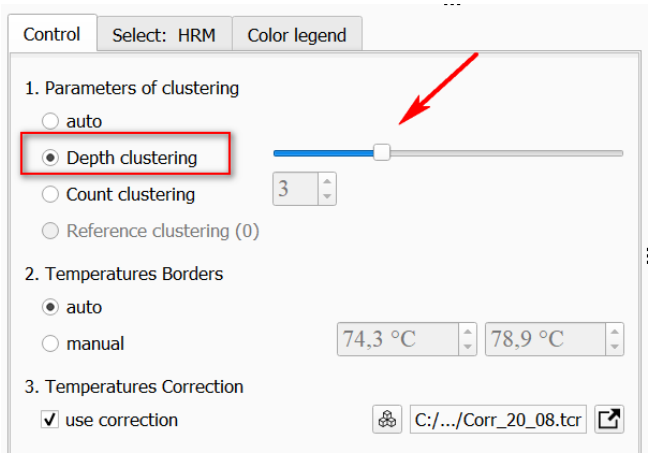


Figure 91 – Changing the **Depth clustering** parameter in the settings

In the extreme left position of the slider, the minimum differences in the melting curves are taken into account as significant, which makes it possible to obtain the maximum number of clusters. Moving the slider to the right allows you to reduce the sensitivity to differences in curves, and thus leads to decrease in the number of clusters.

- **Count clustering** – this setting allows you to analyze the protocol for a specified number of clusters. To apply the setting, move the cursor and left-click on the **Count clustering** switch, set the required number of clusters in the settings window (fig. 92).

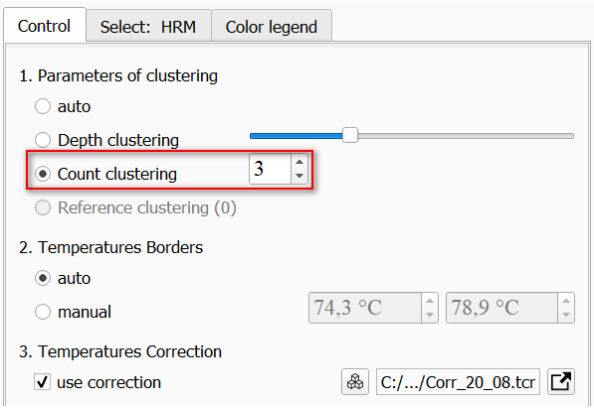


Figure 92 – Changing the number of clusters in the settings

- **Reference Clustering** – allows you to perform analysis based on reference samples (or assignment of reference melting curves). The number of standards determines the number of clusters. The analysis takes into account the data on the melting of reference samples as reference parameters for the corresponding clusters.

To assign standards, go to the **Select: HRM** tab, move the cursor over the required sample and press the right mouse button to open the context menu, choose "standard" - this sample will be selected as a reference (fig. 93). Assign all reference samples in the same way. On the graph, reference samples are marked with colored squares with the R identifier (abbreviation for reference sample); other samples will be as colored circles.

For reference analysis, go to the **Control** tab, select **Reference clustering** (fig. 94).

To deselect the reference samples, choose “delete all reference samples” option in the context menu of the **Select: HRM** tab.

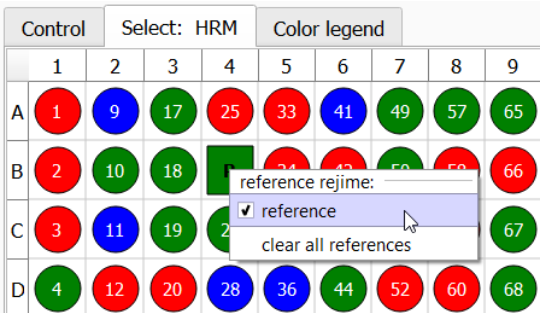


Figure 93 – Choosing reference samples

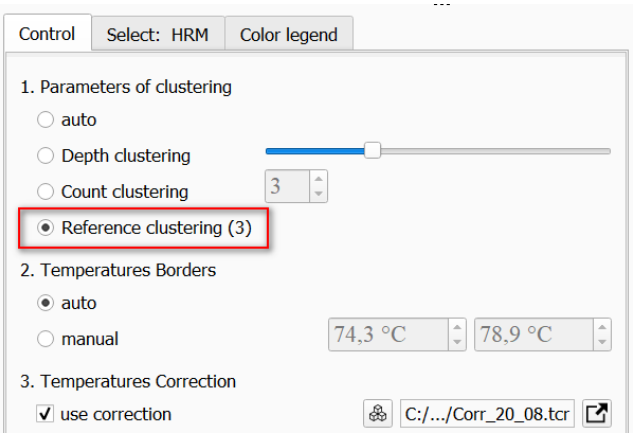


Figure 94 – Choosing Reference Clustering

- **Temperatures Borders** – a setting that allows you to set the limits of the melting analysis area. Select manual entry of temperature limits. To change the limits of the melting region, enter the required numerical values in the temperature limit entry windows (fig. 95).

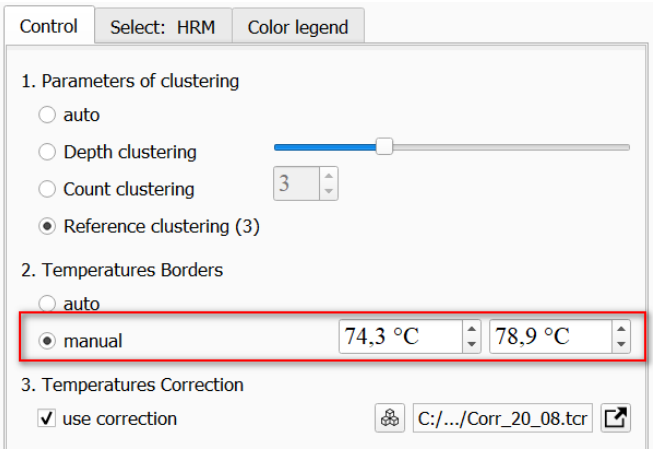


Figure 95 – Manual setting of the temperature limits

Select the **auto** format to revert to the automatic clustering result.

Exclusion of samples from analysis. By default, all samples, except for negative control samples (C-), are taken into account by the program in automatic mode. Analysis settings allow you to exclude samples from the analysis. In this case, the program revises the clustering result.

To exclude samples, go to the **Select: HRM** tab. Move the cursor over the cell with the required sample and press the left mouse button – the color of the sample will change to white (fig. 96). The selected sample will be excluded from the analysis; its data will not be presented in the graphs and tables of clustering results.

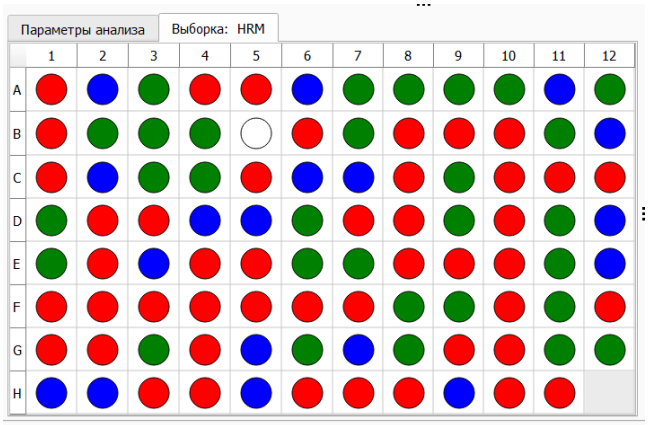


Figure 96 – Excluding a sample from clustering

– excluded sample;

– "C-".

To include samples in the analysis, move the cursor over the cell with the required sample and press the left mouse button – the sample will be colored.

Change the color of the cluster legend. Customization is performed using the context menu of the **Color Legend** tab. To call the color menu, select the cursor and left-click on the cluster marker using the color palette, select the required color and click **OK** button. To apply, click the **Apply** button in the upper corner of the Color Legend tab. After application, the color of the cluster legend and the system of images belonging to the cluster will change. To undo the assigned changes to the cluster legend, click the **Default** button.

Temperature correction. This option allows taking into account the non-uniformity heating of the thermal unit cells, corrects the analysis results based on the coefficients of the **Temperature correction** file. The **Temperature Correction** file can be obtained by sharing the melt correction reagent kit.

Click the button to apply the temperature correction option. The "Open file correction" window will appear on the screen, select the required .tcr file with correction), click the button – the data will be loaded into the analysis, the name of the selected file will be in the analysis settings field (fig. 97).

Figure 97 – Loading a file with temperature correction data

To apply temperature correction in the analysis, set the checkbox “use correction” - the analysis results will be automatically revised (fig. 98).

Figure 98 – Using the temperature correction option in the analysis

To cancel the temperature correction, uncheck the use correction checkbox.

To visualize the data of the **Temperature correction file**, press the button, the visualization window will appear on the screen. Click the **Open** button, the window for selecting the temperature

correction file will open, then select the necessary file and click the **Open** button in the file selection window. A graph of the temperature field of the thermal unit will be displayed in the window (fig. 99).

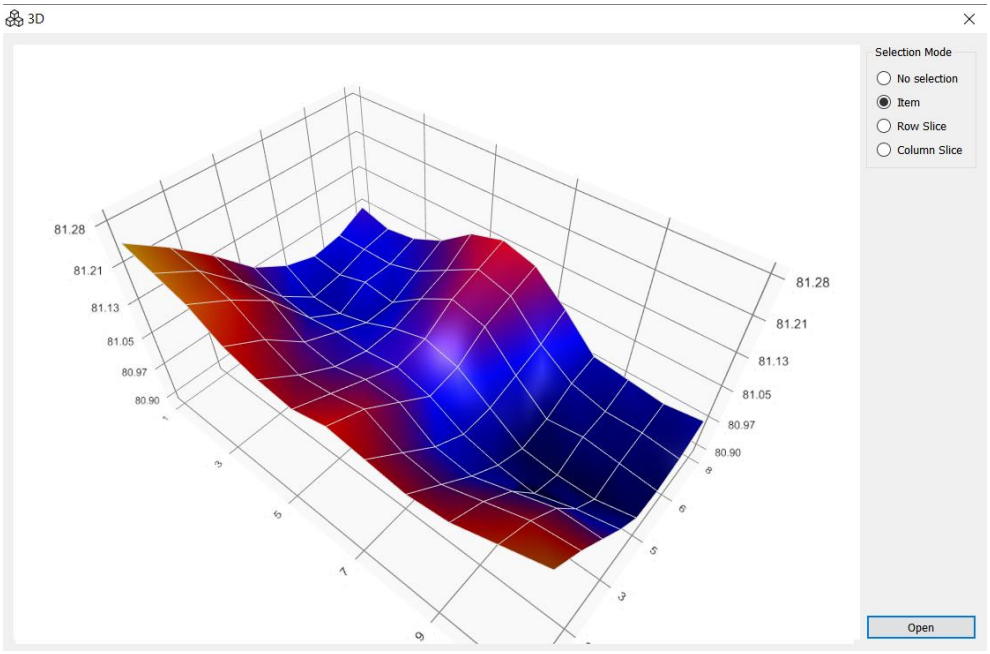


Figure 99 – 3D-graph of the temperature field of the thermal unit

The graph is rotated by moving the cursor while holding down the right mouse button. The temperature values of the thermal unit cells are displayed when you hover the cursor over the corresponding nodal points of the graph by pressing the left mouse button. The graph view can be changed using the format selection option: "No selection", "Item", "Row Slice", "Column Slice".

Data export. Graphs and tabular analysis data can be saved or copied to the PC clipboard (see par. Exporting Data).

GENERATION OF THE REPORT

Generation of the Report with Preliminary Analysis Results

To generate a report with the results of the preliminary analysis, click the **Preliminary analysis report** button  located on the toolbar of the **Analysis** mode.

This button is available when the **Preliminary analysis** tab is active (selected).

A window with a report on the preliminary analysis results will be displayed on the screen (fig. 100).

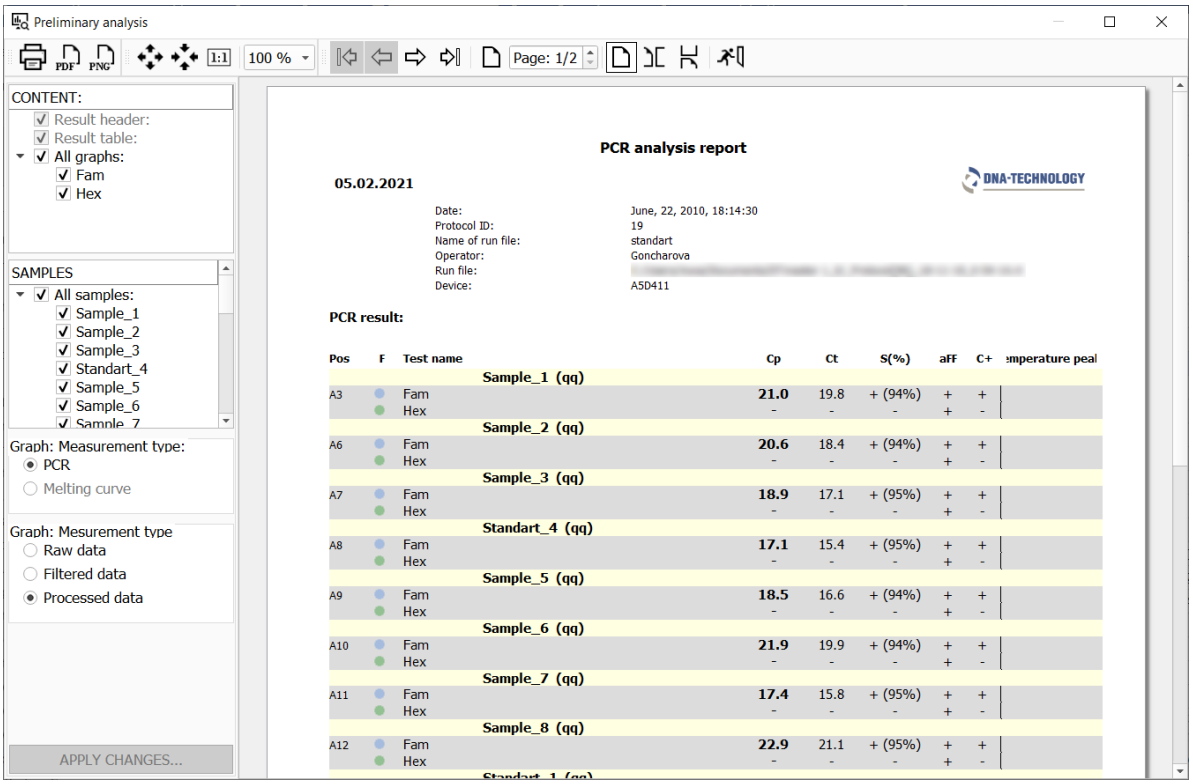


Figure 100 – Example of a report

The toolbar of the Preliminary Analysis window contains controls from table 9.

Table 9 – Control elements on the toolbar of the Preliminary analysis window and their purpose

Name	Symbol	Purpose
Print		Send the report to print
Print to PDF file	PDF	Save report in PDF format
Print to PNG file	PNG	Save report in PNG format
Increase page		Zoom in
Decrease page		Zoom out
Size 1: 1	1:1	Return to default scale (100%)
–	100 %	Specify scale using the drop-down list;
Go to the first page		Go to the beginning of the document
Go to the previous page		Go to the previous page
Go to the next page		Go to the next page

Name	Symbol	Purpose
Go to the final page		Go to the last page
Go to the specified page		Entering the page number
–	Страница:1/10	Sequential transition between pages
One page mode		Set up the viewing mode of report pages on the screen
Two page mode		
Countinous mode		
Exit Preview		Close preview window

In the left part of the **Preliminary analysis** window (fig. 101) there is a block of settings that allows you to change the contents of the report (include or exclude certain data from it) [1].

For example, to exclude the Hex graph from the report, you must uncheck the corresponding checkbox in the **Contents** section [2], and then click the **Apply changes** button [3].

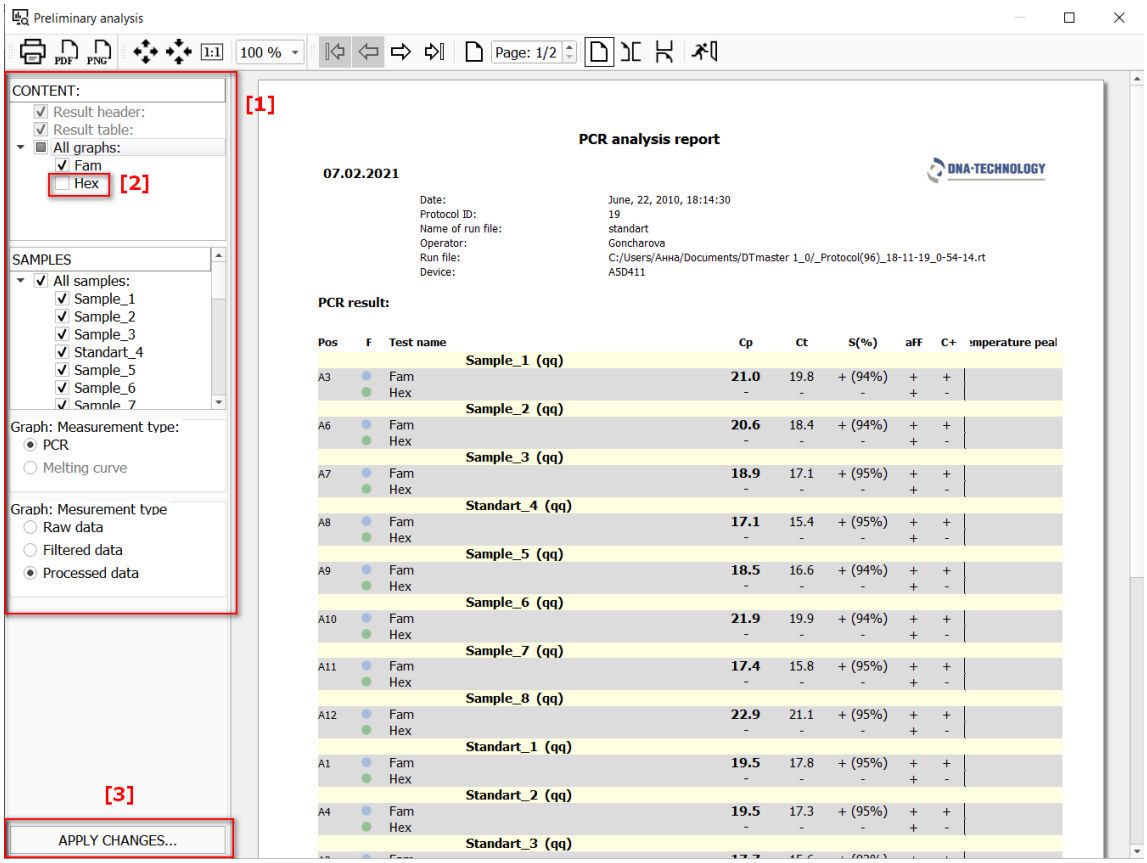


Figure 101 – Block of report settings

Generation of the Basic Analysis Report

To generate a basic analysis report, click the **Report** button  on the toolbar.

The report preview window will appear on the screen.

Switching between qualitative and quantitative analysis is carried out using the drop-down list in the upper right corner of the report form.

The upper part of the preview window contains the controls described in table 8.

In the left part of the preview window, there is a sample selection block that allows you to change the contents of the report (include or exclude data for certain samples).

For example, in order to exclude the results for one of the samples from the report, you must uncheck the checkbox for the required sample, and then click the **Apply changes** button. The selected sample data will be excluded from the report.

SAVING A PROTOCOL TO FILE

To save the protocol with the analysis results to .rt file, click the **Save as** button  on the toolbar of the **Analysis** mode. Next, in the **Save As** window that appears on the screen, specify the name of the file and the folder in which it will be saved. Click the **Save** button. The protocol will be saved in the specified folder.

EXPORTING DATA

RDML export

RDML is a real-time PCR data markup language (official standard website - rdml.org).

Data export in RDML format provides amplification results upload in the international standard of PCR data exchange. The data are saved in a file with the *.xml extension.

To export:

1. Select **RDML \ RDML export** from the **Analysis** menu. The **Save As** window will be displayed.
2. If necessary, change the name of the file to be saved. By default, the name is formed by the template: <RDML_Protocol_*_time*_date*.xml>.
3. Select the directory for saving the file.
4. Click the **Save** button.

The file will be saved in the specified directory.

Exporting to an XML file

You can upload data for export to LIS using the **Export results** button  on the toolbar of the **Analysis** mode.

Clicking this button will generate a report in XML format, containing the calculated results in the numerical form. The user in the **Export and Report Settings** window specifies the folder with the saved XML file.

To open this window, on the **Setting** menu, click the **LIS export settings**.

Additional data export options

Measurements can also be exported as an Excel table, as a file with an image, or copied to the clipboard.

These options are implemented in the context menu of the graphs and tables area:

1. Copy to clipboard – the data will be saved to the clipboard, after which it can be loaded into any third-party program, such as "Notepad". To do this, just open the required program and press the

key combination <Ctrl + V> on the keyboard. The copied data will be presented as a matrix of numeric values.

2. Save to Excel – allows you to save the graph values in a CSV file. After selecting this option, a window for entering a file name and choosing a destination will appear on the screen. The data in the file will be represented as a matrix of numeric values.

The data is saved for each channel separately and depends on the choice of the data type: processed, raw.

3. Copy to clipboard as image – the current graph image will be saved to the clipboard in PNG format. Then the copied data can be loaded into any third-party program, for example "MS Word". To do this, just open the necessary program (if necessary, create a new document) and press the key combination <Ctrl + V> on the keyboard. The copied image will be displayed in the program.
4. Save as PNG image – allows you to save the current graph image as a PNG file. After selecting this option, a window for entering a file name and choosing a destination will appear on the screen.

The **Copy** option is not available for tables and graphs presented on the **Ct analysis** tab.

ADDITIONAL FEATURES OF THE ANALYSIS MODE

Setting the Workspace of the Analysis Mode

The borders between the workspaces, as well as the borders between elements within the workspace with the analysis results are marked with ⋮ and ⋯ items (fig. 102).

To change the size of the workspaces, move the mouse pointer over the border between the required areas – the cursor will change to the icon [1] or , then press the left mouse button and, without releasing it, move the border to the desired position.

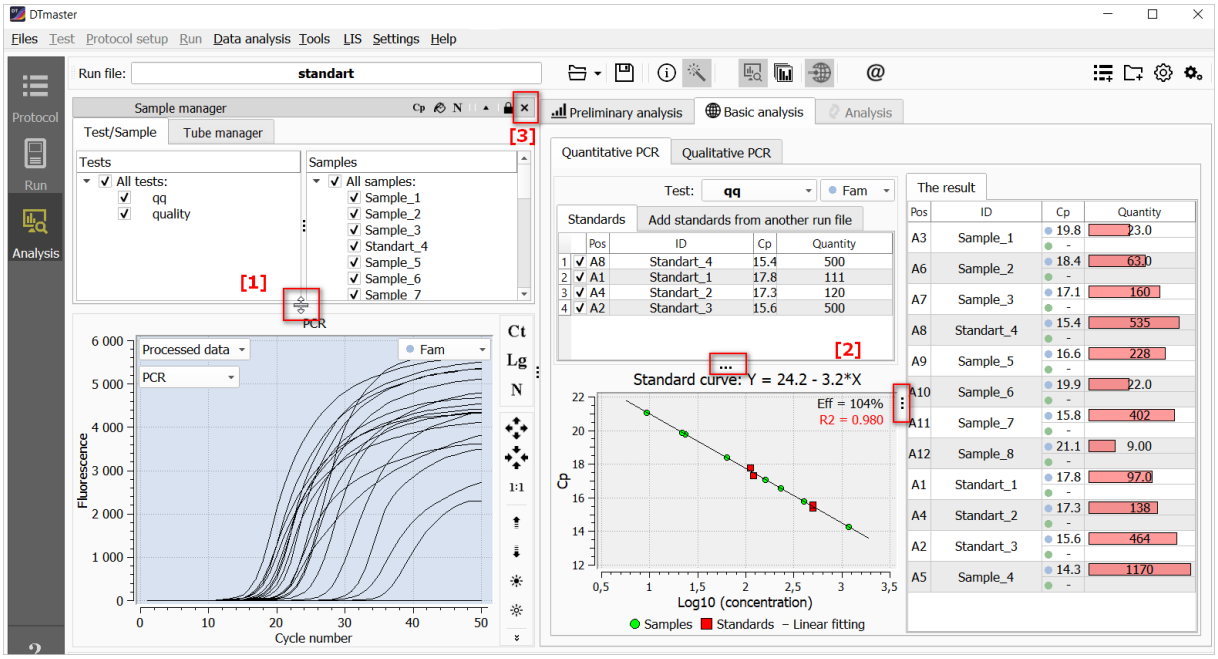


Figure 102 – Tools for customizing the workspaces

In a similar way, you can move the borders within the workspace with the analysis results [3].

Additionally, DTmaster has the ability to hide the **Sample manager** workspace. To do this, click the **Close** button [3].

To restore the display of the **Sample manager** workspace in the **Analysis** mode, click the **Highlight** button on the toolbar. The workspace will be displayed in the program window.

You can also move the **Sample manager** into a separate window by clicking the **Attach\Detach** button located in the title bar of the workspace (fig. 103). You can cancel this action by clicking the **Attach\Detach** button.

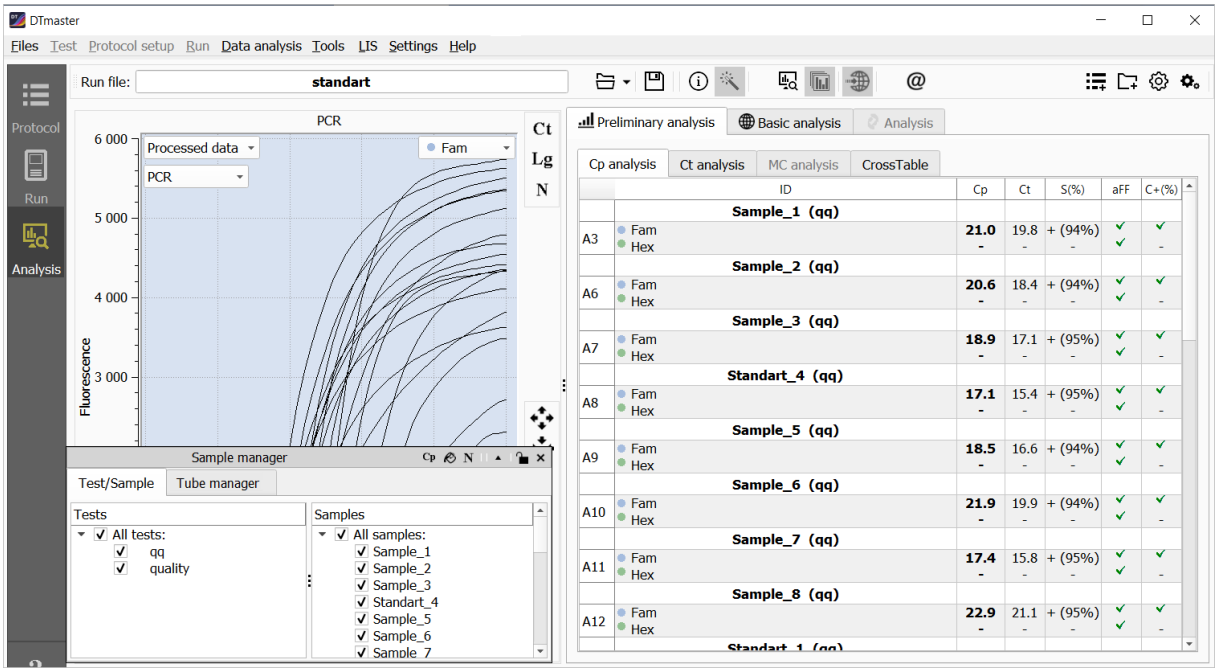


Figure 103 – The **Sample manager** workspace in a separate window

Loading Optical Data on a Different Protocol

The optical data load function allows you to import data (all except optical) of a previously run protocol or template data into the current protocol (a protocol opened in the **Analysis** mode). This allows you to correct information on samples in the current protocol: tubes placement on the thermal unit plate, sample type, name.

To load optical data into the current protocol, two options are available:

1. Using data from a previously run protocol or template.
2. Creation of a new protocol for subsequent import of data on samples to the selected protocol.

To use the data of a previously run protocol or template:

1. In the Analysis mode, open the protocol for which the data on the samples of the previously run protocol or template will be imported.
2. Click the Reboot optical data in another protocol button located on the toolbar. The "Reopen the run file" window will appear on the screen to select a protocol file or template.
3. Select the required protocol or template and click the Open button.
4. The software will transfer the data to the optical data of the current protocol and correct the results of optical measurements in accordance with the new configuration.

5. Save the changes by clicking the **Save As** button on the toolbar, if necessary.

To use the new protocol data:

1. In the Analysis mode, open the protocol for which the data will be imported.
2. In the Protocol mode, create a list of samples (see par. Forming a List of Samples), for which the results of optical measurements of the current protocol will be applied, and place the tubes on the plate.
3. Click the Upload to analysis window button on the Analysis mode toolbar. Sample data from the new protocol will automatically match the optical measurements of the current protocol
4. Save the changes by clicking the **Save As** button on the toolbar, if necessary.

The Optical data load function on a different protocol cannot be used for protocols with different thermal unit modification. When trying to combine these protocols, a window with a warning about the wrong size of the thermal unit appears.

Editing Test Parameters

The software implements the ability to adjust the test parameters at the stage of the results analysis.

The default values are optimal for day-to-day lab work. When conducting scientific research and for a more detailed analysis of the data, you can change the specified parameters for processing the results of optical measurements and data analysis (fig. 104).

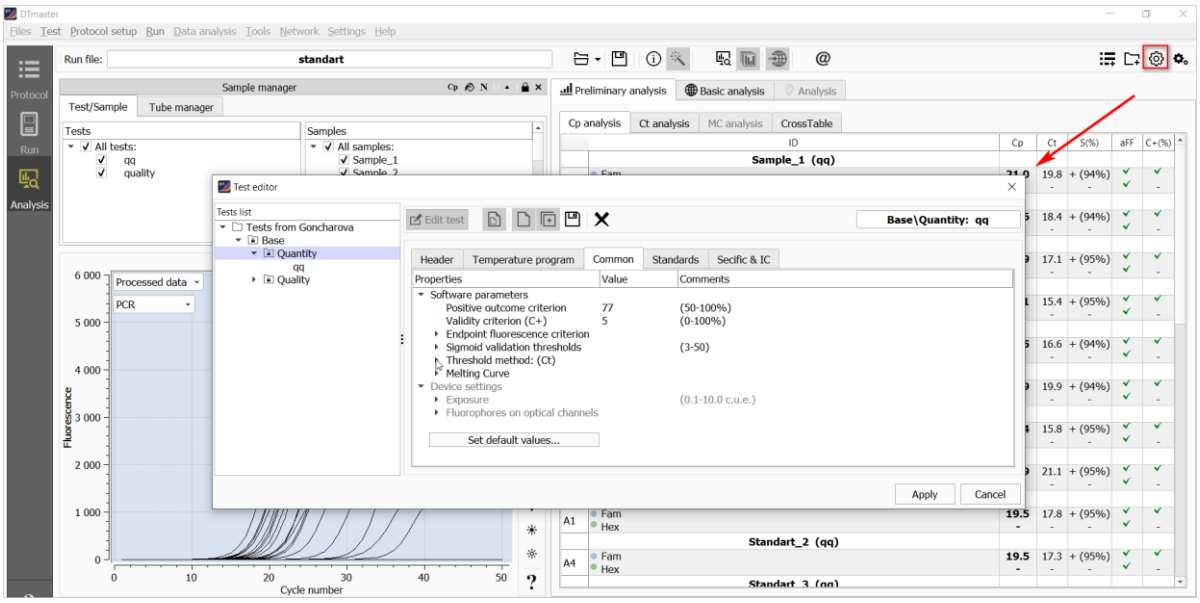


Figure 104 – Analysis parameters

Changing test parameters is valid only within the opened protocol.

Recalculation of results is carried out automatically after saving the edited test parameters.

Ct Analysis

Results of Ct analysis are viewed on the **Ct analysis** tab (fig. 105).

The data on the tab is presented in the form of the following information blocks:

1. Graph of the performed PCR (positive) with a set of potential breakaway points.
2. Graph of the experimental curve (marked with green), sigmoids (fitting, blue) with the Cp point.

3. Table with PCR results for each channel (positive – red, negative – green).
4. Table with the results of fitting:
   - Cp – X value, where the Y value of (the second derivative – acceleration) is the maximum + additional algorithm;
   - F(Cp) – Y value at the Cp point;
   - chi^2 – Chi-square, a statistical criterion representing the relation between model and implementation;
   - sigma – root-mean-square deviation (rms) of the sigmoid fit;
   - eff – theoretical value of eff. PCR according to the sigmoid;
   - Min – overall, generalized parameter of the fitting quality (comprehensive estimation of relative error).

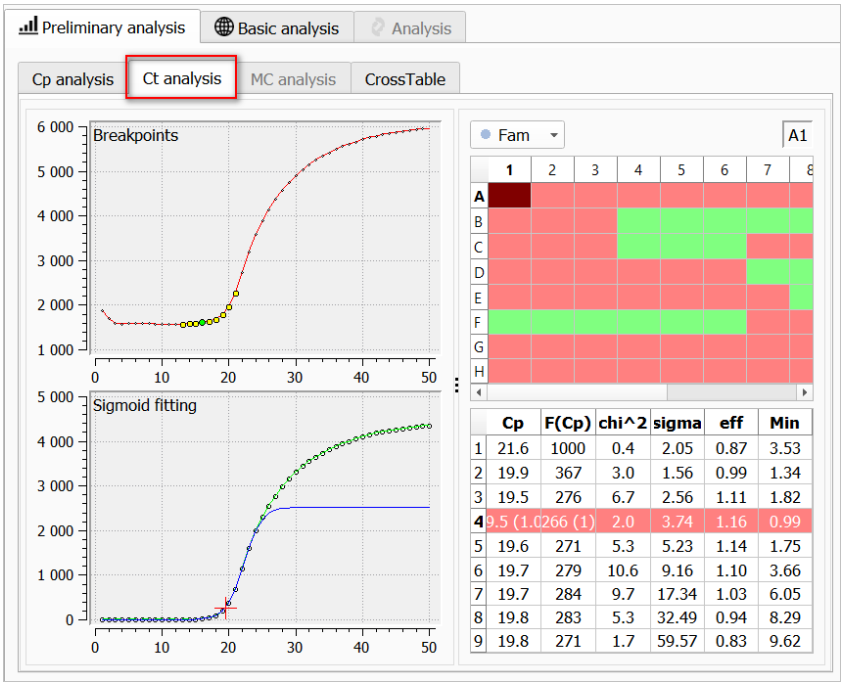


Figure 105 – **Ct analysis** tab

Viewing the List of Analysis Errors

The list of errors that occurred during the analysis is displayed on the **Attention** tab (fig. 106).

Table 10 – Error messages and their description

Error message	Description
Marker position mismatch (or lack thereof) in tubes	The marker was not found. Check the correct positioning of the strip in the thermal unit plate
The optical signal has exceeded the permissible maximum in channels	The optical signal has reached saturation (overflow). It is recommended to adjust the exposure.
Protocol settings changed!	In the current protocol, the basic settings have been changed (see par. Basic Protocol Settings). The default settings are optimal for data analysis, so changing them may lead to incorrect results

Error message	Description
Uncertain test tube results found	Problems (uncertainty) in calculating Cp. The user should pay attention to the preliminary analysis results obtained for the specified tubes.

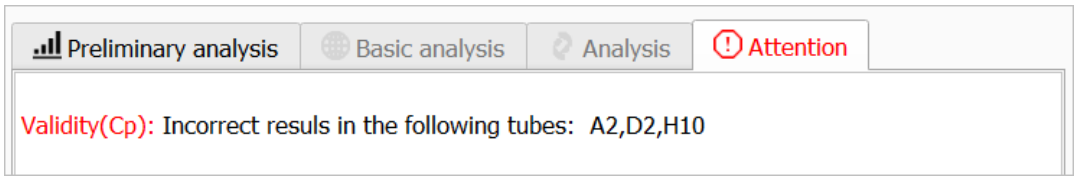


Figure 106 – An example of displaying a record of discrepancy

The **Attention** tab is displayed in the workspace only if there are warnings for the loaded protocol.

ACCESSIBILITY OF USERS WITH ADMINISTRATOR RIGHTS

SETTING UP USER ACCOUNTS

To configure user accounts, on the **Settings** menu, click the **Log in**, then click the **Edit user properties**.

The "Authorization - Settings" window will appear on the screen (fig. 107).

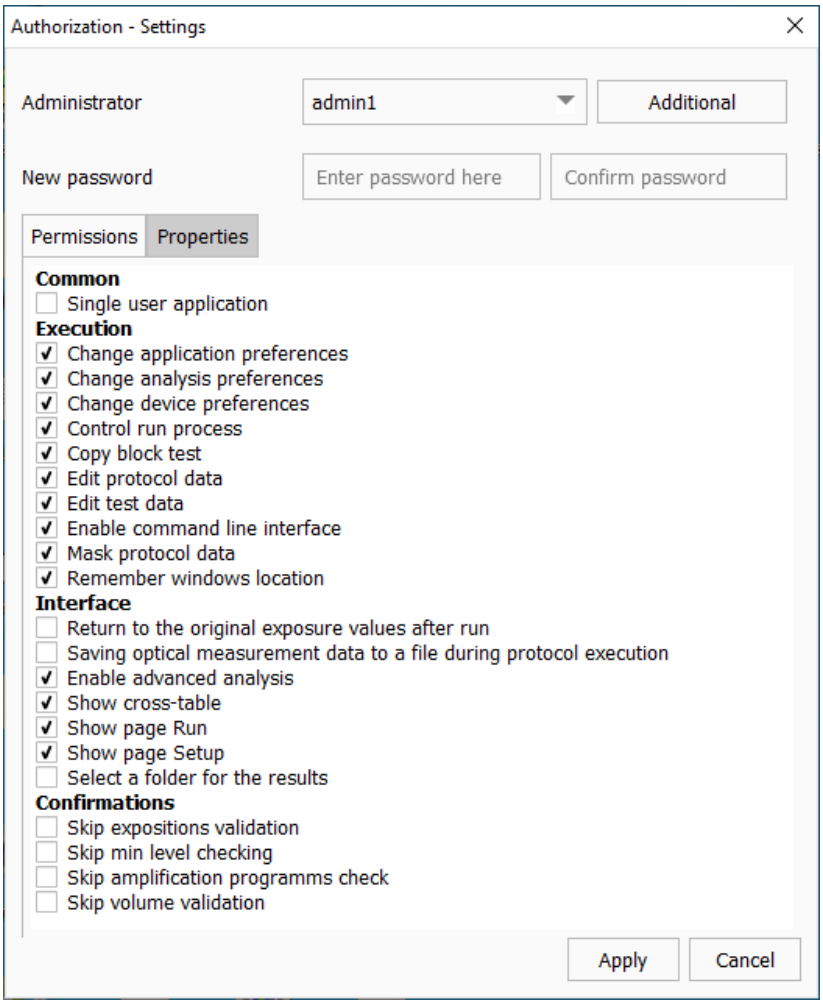


Figure 107 – "Authorization - Settings" window

Editing Password

To edit the password, enter a new password in the corresponding fields of the DTmaster settings window and click Apply button.

The new password must contain numbers, upper and lower case letters of the English alphabet. If the new password does not meet this requirement, a window with a corresponding message will be displayed on the screen.

If the password has been successfully changed, the screen will display the "Completed" entry.

Creating User Account

DTmaster implements the ability to form a list of users and configure their permissions (actions that they can perform in the software).

To create a user:

1. Click the **Additional** button in the DTmaster settings window.
2. Select **Add new user** from the drop-down list box. The user creation window will be displayed on the screen.
3. Enter your login (user name) in the appropriate field of the window and click **Create** button.

A window will appear on the screen with a message about the successful creation of the user. This message also contains:

- password for authentication of the user RealTime_PCR;
- user's home directory set by default (it can be changed later, see par. Filing in Account Properties);
- e-mail address - by default it is not filled in, it can be entered on the “Properties” tab (see section Filing in Account Properties).

Assigning Administrator Privileges

Administrator is a software user who can change the list of permissions for himself or herself and other users.

In order to assign administrator rights to a user:

1. Select the necessary user from the drop-down list box.
2. Click the **Additional** button In the DTmaster settings window and select "Admin privileges".

When you click the **Additional** button again, a check box will appear next to the "Administrator privileges" item, which means that the administrator rights have been successfully assigned.

Creating a Permission Set for User

To generate a permission set for user:

1. Select the necessary user from the drop-down list box.
2. On the Permissions tab check the boxes with the tasks that should be available to the selected user.

A description of the permissions is provided in Appendix B of this manual.

3. Click the **Apply** button.

The selected permissions will be saved for the user.

Filing in Account Properties

User account properties include:

- user's email address;
- home directory.

The user's home directory is a directory in the OS that stores the analysis results carried out by this user.

To enter the user's email address:

1. From the general list, select the user for whom you want to change the email address.
2. Open the Properties tab.
3. Click "Email address". A window for entering the address will appear on the screen.
4. Click the **Apply** button. The entered address will be displayed in the account properties.
5. Click the **Apply** button to save your changes.

The user's home directory is created and registered in the properties automatically when the account is created. Follow the steps below to change the directory:

1. From the general list, select the user for whom you want to change the home directory.
2. Open the **Properties** tab.
3. Double-click the left mouse button on the line with the current user's home directory. A standard OS window for selecting a directory will appear on the screen.
4. Specify the desired directory and click the **Select folder** button. The line with the entry for the home directory will display the system path to the selected directory.
5. Click the **Apply** button.

The screen will display "Completed" and the selected home directory will be saved for the user.

Loading a Profile from a File

Loading a profile from a file is convenient when several users need to be assigned the same rights (select the same permissions).

To load a profile from .upf file:

1. Select the user from the list.
2. Click the **Additional** button and select **Apply preset**. The screen will display a standard OS window for searching and selecting a file to download. By default, there are four profiles available in the software: doctor, laboratory assistant and researcher.
3. Select the necessary file and click the **Open** button.

The user account will be automatically assigned permissions from the selected profile.

Deleting a User

To delete a user:

1. Select the necessary entry from the list of users.
2. Click the **Additional** button and select **Delete operator**.
3. Click the **Apply** button in the displayed dialog box to confirm that you want to delete the user.

A window with a message that the user has been successfully removed from DTmaster will appear on the screen.

Saving a Profile to a File

A profile is a set of configured permissions for a user account.

To save a user profile to a file:

1. Select the required user from the list.
2. Click the **Additional** button and select **Save preset**. The screen will display a standard OS window for entering the name of the file and selecting the directory for saving.
3. Enter a filename and specify the directory in which the file should be saved.
4. Click the **Save** button.

The file will be saved in the specified directory in .upf file.

BASIC PROTOCOL SETTINGS

The following test parameters are configured for each protocol in the software:

- apply spectral correction – excludes overlapping of a part of the signal between channels;
- apply optical irregularity correction – used to adjust the optical characteristic associated with the reduction of sharpness from center to edge;
- applying digital filter – used to adjust the signal from the noise component and individual emissions.

To edit protocol settings, click the **Edit protocol settings** button  . The settings window will be displayed on the screen.

Make the necessary changes to the settings and click the **Apply** button. Your changes will be saved in the software.

Only users with the appropriate privileges can edit protocol settings.

CONFIGURING DTMASTER INTERFACE

DTmaster interface settings include:

- program language (Russian / English)
- the font used in the program.

To edit these settings:

1. On the **Settings** menu, click the **General settings**. The "General settings" window will appear on the screen.
2. Make the necessary changes on the **Interface Settings** tab.
3. Click the **Apply** button.

Restart DTmaster to display the new settings in the interface.

CONFIGURING PROTOCOL NAME TEMPLATE

The protocol name template is used to make it easier to enter the protocol name when it is created.

You can add to the protocol name template:

- name – constant variable that will be present in the protocol name;
- plate layout – will be specified after the protocol name in parentheses;
- date and time of protocol creation in the required format (by default dd-mm-yyyy_hh-mm-ss).

To configure the protocol name template:

1. On the **Settings** menu, click the **General settings**. The "General settings" window will appear on the screen.
2. Click the **Protocol name template** tab.
3. Select the **User template** checkbox (fig. 108) and form the desired template using the proposed parameters.

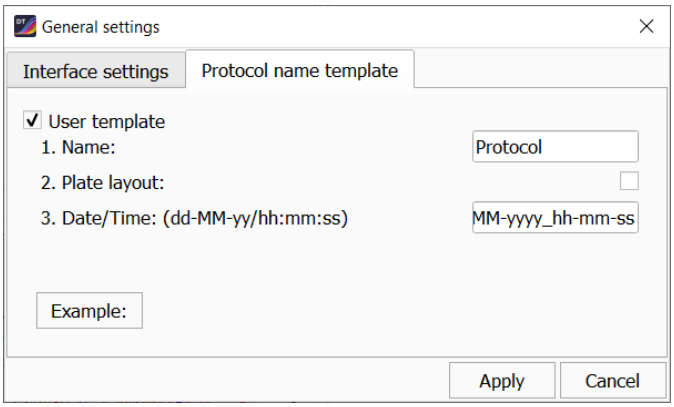


Figure 108 – Configuring the protocol name template

4. Click the **Example**: button to view the template according to the specified parameters.
5. Save the template by clicking the **Apply** button.

Restart DTmaster to display the new settings in the interface.

VIEWING VIDEO ARCHIVE

Viewing the video archive is carried out in the "View videoarchive" window (on menu **File**, click the **View VideoArchive**).

To view a video of the plate with tubes for each measurement, press the button located in the upper part of the window. Then select the file with the archive (in .dat file) and click the **Open** button.

A plate with tubes will be displayed in the "View videoarchive" window. To view images, use the and buttons located in the lower part of the window (fig. 109).

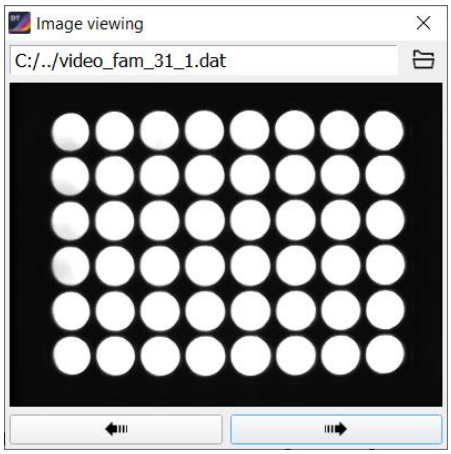


Figure 109 – Viewing the image

To create a video archive, after running the temperature program, choose **Save image** in the **Settings** tab.

INTERACTION WITH LIS

In DTmaster, interaction with LIS is carried out using the following options located in the **LIS** menu:

- Get the protocol for execution – download protocols from LIS for analysis;
- Get the protocol with results – load the results into DTmaster (for example, received from a remote device);
- Send the protocol withresults – send a protocol with the results to LIS.

A successful connection to the server is indicated by the identifier at the bottom of the DTmaster window.

Obtaining a Protocol for Amplification

To load the protocol for amplification from LIS into DTmaster, on the **LIS** menu, click the **Get the protocol for execution**.

The screen will display the "List of Web Protocols" window containing the list of protocols available for downloading from LIS.

To update the list, click the button.

To load a protocol into DTmaster, highlight the required entry in the list and click the Select button, after which the selected protocol will be displayed in DTmaster in the Protocol mode.

Obtaining a Protocol with the Analysis Results

To obtain a protocol with the analysis results in order to analyze the data obtained from the device, execute on the **LIS** menu, click the **Get the protocol with the results**.

The "List of Web Protocols" window will appear on the screen, containing the list of protocols available for download.

Press the button to update the list.

To load a protocol into DTmaster, highlight the required entry in the list and the **Select** button, after which the selected protocol will be displayed in DTmaster.

Sending a Protocol with the Analysis Results

To send a protocol with the analysis results to LIS, on the **LIS** menu, click the **Send the protocol with results**.

The screen will display a standard OS window for searching and selecting the desired file. Select the file and click the **Open** button. The selected protocol will be sent to LIS.

USING A SCENARIO

There is an ability to automate basic processes in the software. For example, loading a specific protocol (or template), connecting the instrument, launching it, analyzing and sending the result to LIS.

For this, previously prepared XML files are used – scenarios. Work with scripts is carried out in the "Activity scenario" window.

The scenario files are stored in the DTmaster directory: DNA-Technology\ DTmaster\scenario

Scenarios are edited using a third-party XML file editor.

Click the **Open** button to view a list of available scenarios. The screen will display a standard OS window with available scenarios.

Next, select the scenario sce-file and click the **Open** button. In the "Activity Scenario" window, the program stages will be displayed, according to the selected scenario.

Click the **Start** button to start the scenario. The sequential execution of the scenario stages will begin.

If an error occurs at some stage of the scenario execution, the ✕ icon will be displayed in the **Status** column for this stage, and an error message will appear at the bottom of the window.

The scenario can be stopped using the **Stop** button.

To delete a scenario from the "Activity Scenario" window, click the **Clear** button.

VIEWING MODULE DETAILS

The list of modules included in DTmaster is presented in the "About Modules" window. To open this window: on the **Help** menu, click the **About Modules**. For each module, the list indicates its version, size in kilobytes and the date of the last update.

EXITING DTMASTER

Finish work with DTmaster in one of the following ways (figure 137):

- on the **File** menu, click **Exit**;
- use standard OS tools to close the program.

The program will be terminated.

CHAPTER 5 REAL-TIME PCR INSTRUMENT SETUP AND DIAGNOSTICS

The instrument is configured in the **Run** mode on the **Settings** tab (fig. 110).

The real-time PCR instrument settings include:

- checking the geometric settings of the optical system (creating a video);
- checking the purity of wells;
- checking the exposure;
- measuring the height of tubes.

Figure 110 – The **Settings** tab of the **Device** workspace

CHECKING THE GEOMETRIC SETTINGS OF THE OPTICAL SYSTEM

When you turn on the instrument for the first time after transportation or any movement, it is recommended to check the geometric settings of the optical system.

To check the geometric settings, it is necessary to place tubes with fluorophore (for example, reaction tubes) into all corner wells of the thermal unit. For incitements of version X (384 wells), a plate with fluorophores must be placed.

Next, you need to do the following:

1. Click the **Create a video image** on the **Settings** tab.

The video creation window will appear on the screen (fig. 111).

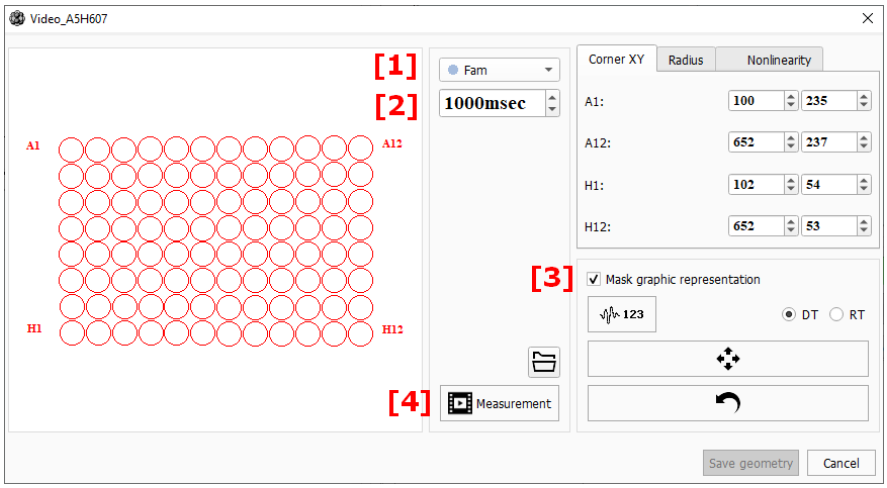


Figure 111 – "Video" window

2. Select the Fam channel using the corresponding drop-down list box at the top of the window [1].
3. Set the required exposure value so that the signals are in the range of 1000 – 3000 msec [2].
4. Check the **Mask graphic representation** checkbox [3].
5. Click **Measurement** button [4].
6. Analyze the resulting image (fig. 112).

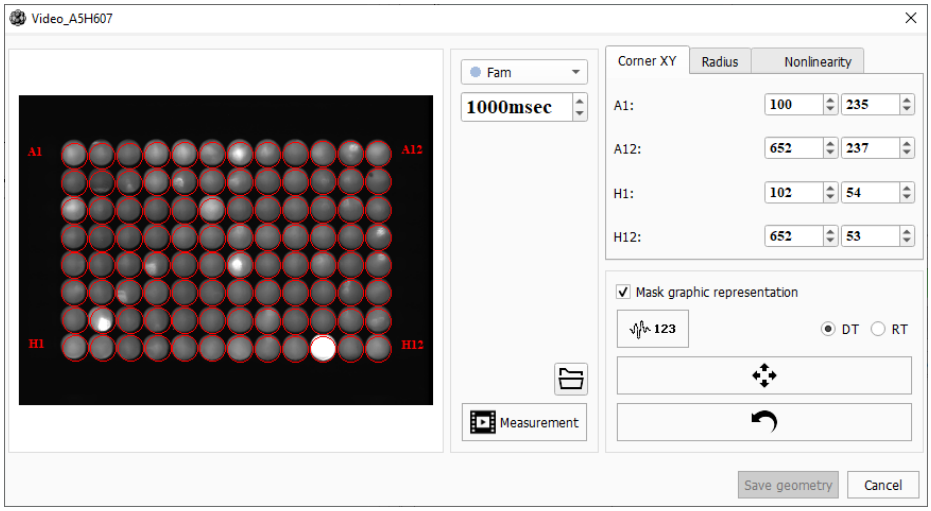


Figure 112 – Measurement result

In the resulting image, the contours denoting the boundaries of the measurement area (red circles) should not go beyond the limits of the light spot.

If the center of the pixel that makes up the light spot is inside the red circle, it is taken into account during the measurement. Pixels whose centers are outside the red circle will not be taken into account.

If the circles are displaced relative to the light spots, it is necessary to correct the geometry of the optical image.

You can correct the coordinates of the corner points and the size of the light spot by changing the values in the corresponding boxes.

The switch **DT** and **RT** allows you to compare the digitization in a test tube by the device ("DT") and the program ("RT").

You can return to the initial values using the button.

You can also load a pre-formed mask from MRT file (this file is prepared in the DTcheck program). To do this, click the **Load mask** button, select the required file and click the **Open** button. The mask from the file will be loaded into the "Video" window.

To save the changes, click the **Save geometry** button.

CHECKING THE PURITY OF THE WELLS

The purity of the wells of the thermal unit is evidenced by the absence of bright spots on the image of empty wells when checking the geometric settings of the optical unit.

To check the cleanliness of wells and the of stray reflected optical signals, do the following:

1. Close the thermal unit, make sure there are no tubes in the wells.
2. Open the "Video" window, set the exposure to twice the working exposure value.
3. Click the **Measurement** button, start the process of optical measurement in FAM channel (selected by default).
4. Make sure that the optical signal levels for empty wells in the "Digitization of video signal" window does not exceed 1500 conventional units, otherwise clean luminous wells and repeat the measurement.
5. Successively choose the rest channels of the instrument and set for the exposure values (twice bigger than the working exposure values), repeat these measurements for all channels (by clicking the **View** button and controlling the levels of optical signals in the "Digitization of video signal" window).

It is recommended to check the purity of the wells immediately before placing tubes with PCR samples if there is a possibility of contamination.

The regular check of the purity of wells should be carried out by the user in accordance with the device maintenance recommendations (see par. Real-time PCR Instrument Setup and Diagnostics of the first part of this manual).

SELECTING EXPOSURE CORRECTION FACTORS

The optimal exposure values are determined by the manufacturer for each type of device (basic exposure) and are presented in conventional exposure units (c.u.e.). If necessary, for each test separately, you can enter the exposure correction factors for all active channels (see Appendix A). To select exposure correction factors, click the **Check exposure** button. The "Exposure" window will appear on the screen.

Place the test tubes with the reaction mixture into the device, for which it is necessary to select the coefficients. Enter the optical metering exposure for all channels by selecting them sequentially using the appropriate drop-down list. The exposure compensation factor corresponding to the entered exposure value is automatically indicated below in parentheses. Perform a test measurement by pressing the **Measurement** button, as a result of which a graphical display of the current values of optical measurements in three-dimensional coordinates will be displayed in the left part of the window (fig. 113).

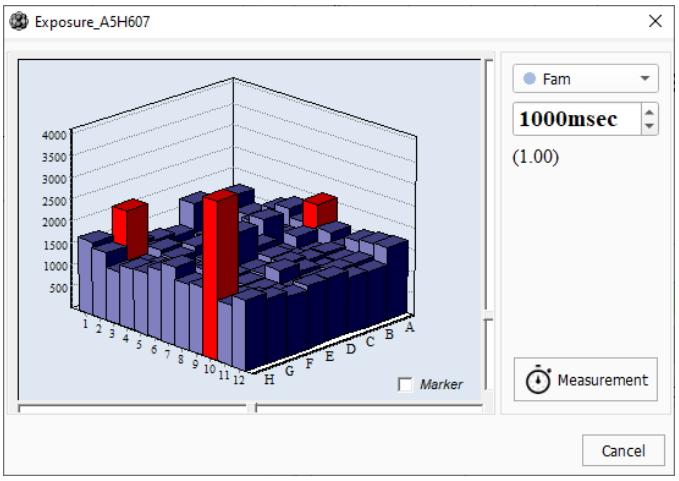


Figure 113 – Current values of optical measurements in three-dimensional coordinates for a given exposure

The Marker option allows displaying numerical values for each tube.

The red color of the column warns that the fluorescence value in this thermal unit well is above the linear measuring range.

Select the correct exposure value for each channel, then enter the appropriate exposure correction factors (indicated in parentheses) in the test settings (see Appendix A).

MEASURING THE HEIGHT OF THE TUBES

Attention! When measuring the height of the tube, place at least 32 test tubes or 4 strips evenly over the thermal unit plate.

Measurement of the height of the tube is obligated when changing to a different type of plastic consumables (tube, strips, plates) or when there is a doubt about the correct pressure on the tubes by the "hot lid".

To measure the height of the tube:

- click the **Open thermal unit** button (or button on the front panel of the instrument) and place the tubes or strips evenly over the thermal unit plate;
- click the **Tube height measurement** on the **Settings** tab, and wait for the message about the successful measurement;
- save the measured height of the tube by clicking the **Yes** button.

CHAPTER 6 EMERGENCIES

Types of emergencies:

- low level – occurs during interaction between Server_Dev and the connected instrument; the alarm message is displayed in the “Errors” tab of the “Instrument” workspace in the “Run” mode (see par. Connecting the Real-Time PCR Instrument);
- high level – occurs during interaction of DTmaster and Server_Dev; in this case the alarm message is displayed as a separate dialog box.

Server_Dev is an instrument server software that ensures the quality of communication between DTmaster and the connected instrument.

Each type of emergency with indication of further user actions are presented in tables 11 and 12.

Table 11 – Low level emergencies and recommended user actions

Error message	Error description	User action
Instrument initialization error	Instrument initialization error occurred when connecting to the instrument	It is necessary to repeat the connection procedure to the instrument
USB error	Error during data transfer via USB/CAN	Repeat the read/record procedure
CAN error		
Error when reading/writing data block via USB channel		
The instrument is not ready, waiting for the end of initialization...	Instrument is being initialized	Wait until instrument initialization is complete
Error in the instrument! Instrument needs to be turned off and on...	Error during operation	Restart the instrument
Opening the instrument! The program is running...	Instrument cannot be opened during the amplification program	Wait for amplification program to complete
Drive error!	Instrument drive jammed	Repeat the command to open/close the instrument. If repeating the command does not solve the problem, turn the instrument off and on
Error – instrument is open!	Amplification program cannot be started with the instrument open	Close the instrument and start amplification program
Error at startup!	Error starting the amplification program	Restart the amplification program
Error receiving a data block!	Error when receiving data block with optical measurements	Wait until the amplification program is finished. Read the data from the last run. If there was also an error when reading the data, restart the

Error message	Error description	User action
		instrument and read the last run again
Error positioning the filter wheel	Failure when changing the optical channel in the instrument	Contact customer support
Unknown error!	Instrument failure	Contact customer support
No response to request to the instrument	Server_Dev does not respond to DTmaster command(s)	Restart Server_Dev by removing the USB cable from the instrument (for 5 seconds)

Table 12 – High level emergencies and recommended user actions

Error indicator	Error message	Error description	User action
⚠	Warning! The instrument cannot work with the current version of the program! Please contact customer support...	DTmaster is connected to an outdated instrument model (pre-2015)	Contact customer support
⚠	Warning! According to the conditions of the contract the time of use of the instrument has expired. We recommend to contact a representative of DNA-Technology!	The contract time of the instrument has expired	Contact a representative of DNA-Technology
⛔	Unable to read INFO_INSTRUMENT... Please restart the instrument!	Failed to identify the instrument during connection to the instrument	Repeat the connection procedure to the instrument
⛔	It is not possible to change the exposure values in the instrument...	Error at protocol startup when applying the exposure values specified in the test	Check the exposures specified in the test. If incorrect values of exposures are specified in the test, it is necessary to edit them. If they are correct, restart the instrument.
⚠	Warning! No basic exposure values detected in the instrument! Please contact customer service...	Basic exposure values not found on the instrument	Contact customer support
⚠	Warning! An error occurred during the measurement of the tube height!	Error occurred when performing the tube height measurement operation	Repeat the test tube height measurement
⚠	Warning! Different volumes of the working	Protocol validation revealed discrepancies in the amplification	Check and edit the test amplification programs,

Error indicator	Error message	Error description	User action
	mixture in the test tubes have been detected! The current protocol cannot be started!	programs of the tests involved in the protocol	namely the specified volume of the working mixture
	Warning! Different exposure values for the tests are detected! The current protocol cannot be started!	Protocol validation revealed a discrepancy in the amplification programs of the tests involved in the protocol	Check and edit the test amplification programs, namely the exposure parameters
	Warning! The amplification program of the protocol is different from the corresponding test program! The current protocol cannot be run!	Protocol validation revealed a discrepancy between the amplification programs of the protocol and the test(s)	Check and edit the protocol and test(s) amplification programs
	Warning! Unacceptable exposure value detected... (Instrument, Protocol)	Protocol validation revealed incorrect exposure settings for the test used	Exposure parameters in the test need to be corrected
	Instrument does not support gradient...	The temperature gradient/drop of the amplification program loaded into the instrument does not correspond to the temperature block of the instrument, or MAY not correspond (in the case of older instruments, no information about the temperature block is available)	Appropriate changes need to be made in the amplification software

CHAPTER 7 TECHNICAL SUPPORT

If you have encountered any problems with DTmaster and you have not found a solution in this manual, the manufacturer's Customer support service will assist you.

To send a message about an error to the Customer support service:

1. Click the **Email** button  in the Analysis mode or on the **Data analysis** menu, click the **Email**.
2. Fill in the form with the following information:

   - sender's Email;
   - subject and text of the message.

   You can also attach to the message:

   - file – to do this, click the [+] button, then in the standard OS window, select the necessary file and click the **Open** button. An entry about the selected file will be displayed in the **Attachment** field;
   - protocol – click the **Run file** button, and then the path to the file with the protocol currently open in the program will be displayed in the **Attachment** field. The copy of the protocol sent to the Customer Support Service is automatically deleted from the patient information, and the sample names are replaced with "Sample_1" and so on in ascending order.
   - screenshot – to do this, click the **Screenshot** button, the program will automatically take a screenshot of the current program window (without the message creation form) and save it in the local directory. The list of files attached to the letter will display an entry of the system path to this file.

3. Click the **Send** button, the message will be sent to the Customer Support Service.

APPENDIX A. TEST PARAMETERS

A.1. PRELIMINARY ANALYSIS

The **preliminary analysis** is intended for PCR analysis to the Cp / Ct level.

Proceeded for qualitative and quantitative tests.

Contains one test – simple (figure A.1).

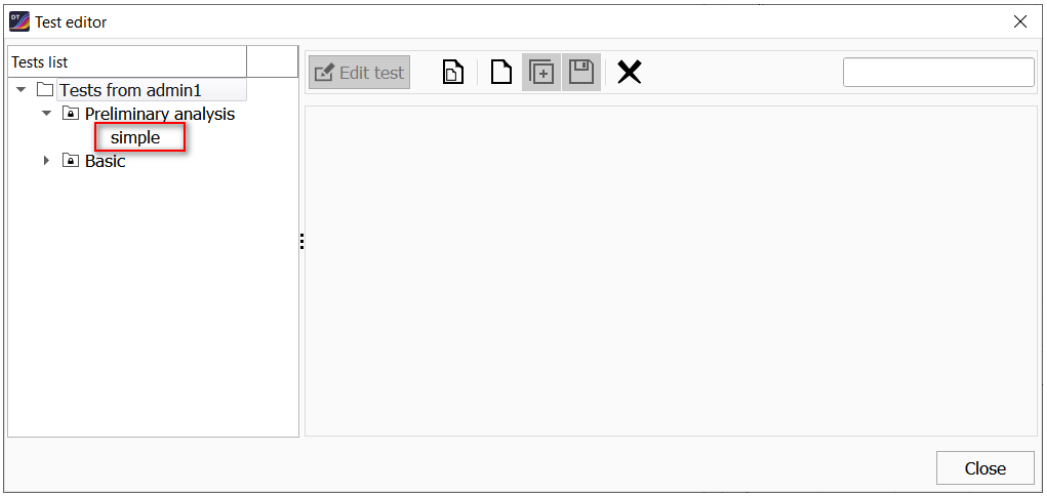


Figure A.1 – The simple test

To view test parameters, select "Simple" in the **Tests list** and click the **Edit test** button. The parameters of the selected test will be displayed in the workspace of the "Test editor" window (figure A.2).

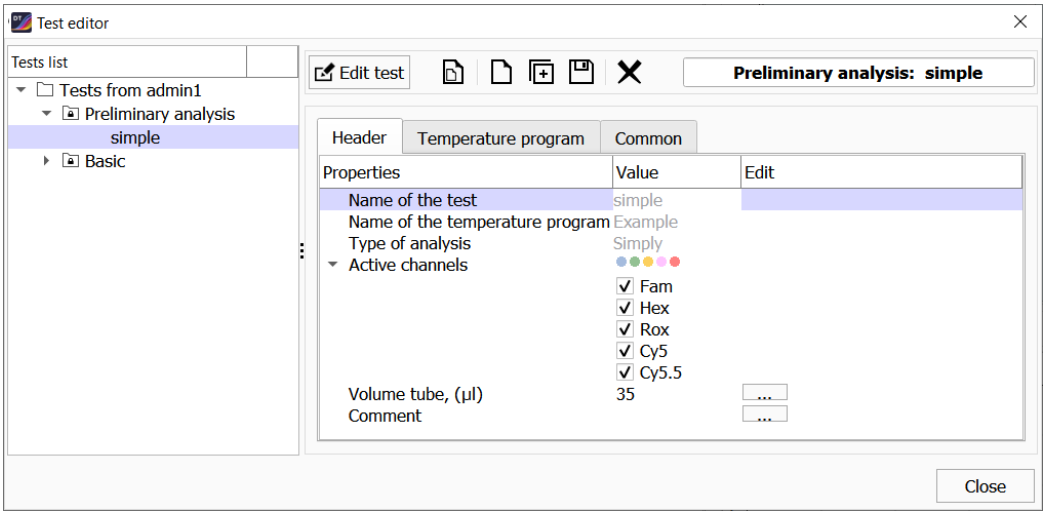


Figure A.2 – View parameters of the Simple test

The parameters of the Simple test are presented on the following tabs:

1. **Header** – general properties of the test (name, active channels, etc.);
2. **Temperature program** – contains the temperature program;
3. **Common** – general parameters of the program and the real-time PCR instrument;

On the **Header** tab (see figure 3) the following parameters are available for editing:

- active channels;

- tube volume in µL.

On this tab you can add a comment to the test (using the ... button in the **Comment** line).

On the **Temperature program** tab, a temperature program is formed that determines the order of the analysis (figure A.3).

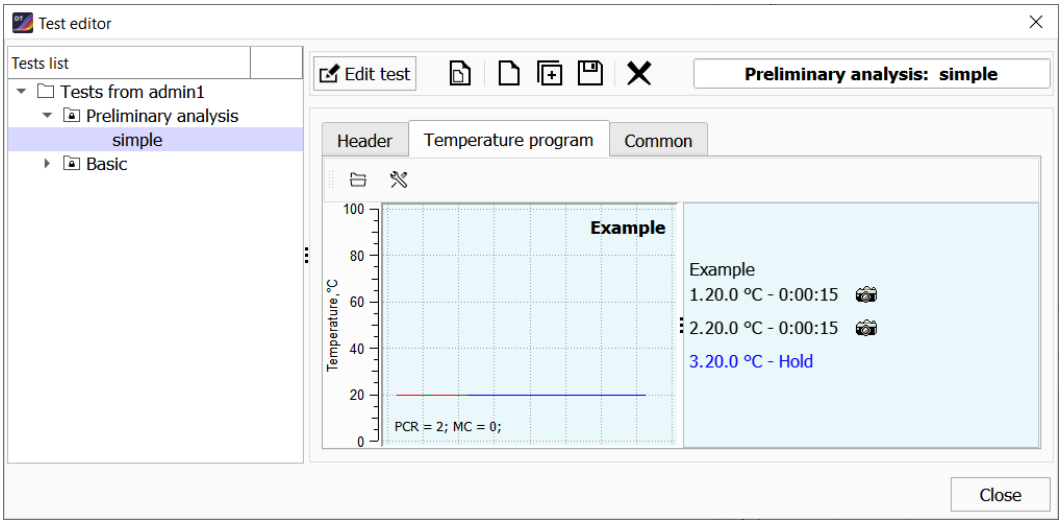


Figure A.3 – The **Temperature program** tab

The **Common** tab contains software and instrument settings (figure A.4). To edit the settings, use the ... button located in the line with the necessary setting.

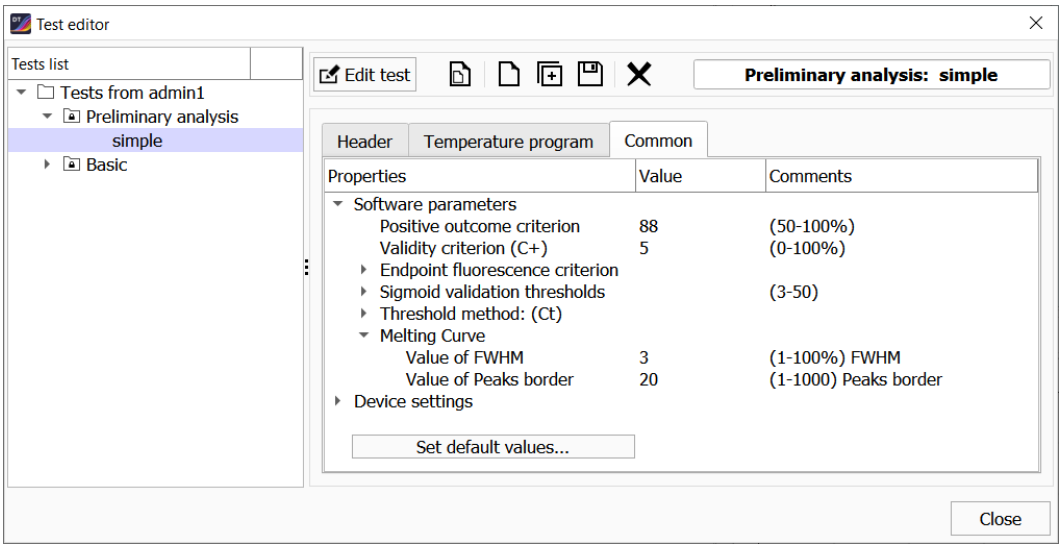


Figure A.4 – The **Common** tab

The default values are optimal for day-to-day lab work. When conducting scientific research and for a more detailed analysis of the data, you can change the specified parameters for processing the results of optical measurements and data analysis.

Criterion of the PCR positive result (in the range from 50 to 100%) allows changing the sensitivity to the rate of growth of PCR products. By decreasing the value of this parameter, visualization and calculation of more gently sloping curves can be achieved.

C+ – filter of the validity of a positive result relative to C+ (only for positive curves). If a non-zero value is specified for this parameter, then this filter is applied in the analysis of results.

Endpoint flare up filter (for positive and negative curves). The following filter settings are available:

- checkbox Apply this method in analysis, which determines whether this filter will be used in the analysis of results;
- baseline fluorescence (relative to baseline exposure);
- minimum and maximum thresholds.

Threshold (Ct) and Geometric (Cp) methods for analyzing the DNA accumulation curve during PCR are based on different approaches to determining the indicator cycle of amplification.

The Threshold (Ct) method is based on conducting a threshold line that is parallel to X line and determining the cycle number (threshold cycle), at which the amplification curve for this tube crosses the threshold line. Threshold cycle is one of the variants of indicator cycles.

Geometric method (Cp) is based on a mathematical analysis of the shape of the DNA accumulation curve during PCR.

By default, when analyzing optical measurements, DTmaster uses the Geometric method (Cp). In case of non-standard situations, when the amplification curve differs significantly from the classical DNA accumulation curve in the course of PCR (sigmoid), it is possible to analyze the results using the Threshold method (Ct), which, in such cases, gives a better estimate.

Along with the processed results (deduction of the baseline, fitting), when analyzing the results of optical measurements, it is possible to view raw data, selecting the appropriate position in the dropdown list of analysis methods.

In the instrument parameters, you must specify the coefficients for correcting the exposure value for each channel. If coefficients for exposure correction are specified in the test parameters of the protocol, then these coefficients will be automatically applied to the current exposure value after starting the analysis.

The default value of the coefficient is 1.0 c.u.e.

The coefficient equal to 0.5 c.u.e. halves the exposure.

The coefficient equal to 2.0 c.u.e. doubles the exposure.

You must select a fluorophore for each channel using the corresponding drop-down lists. If R6G dye is used in the kit, then while creating (editing) a Test for the "2 channel" select the "R6G" value.

A.2. QUALITATIVE TEST PARAMETERS

The parameters of the Qualitative type of analysis are presented on the following tabs:

1. **Header** – general properties of the test (name, active channels, etc.);
2. **Temperature program** – contains the temperature program;
3. **Common** – general parameters of the program and the real-time PCR instrument;
4. **Target & IC** – the purpose of each optical channel is determined when analyzing the results.

Tabs 1-3 are similar to tabs for the preliminary analysis.

On the **Target & IC** tab, the purpose of each optical channel is determined when analyzing the results (figure A.5):

- **Target** – the main, specific signal;
- **IC** – signal from the internal control sample.

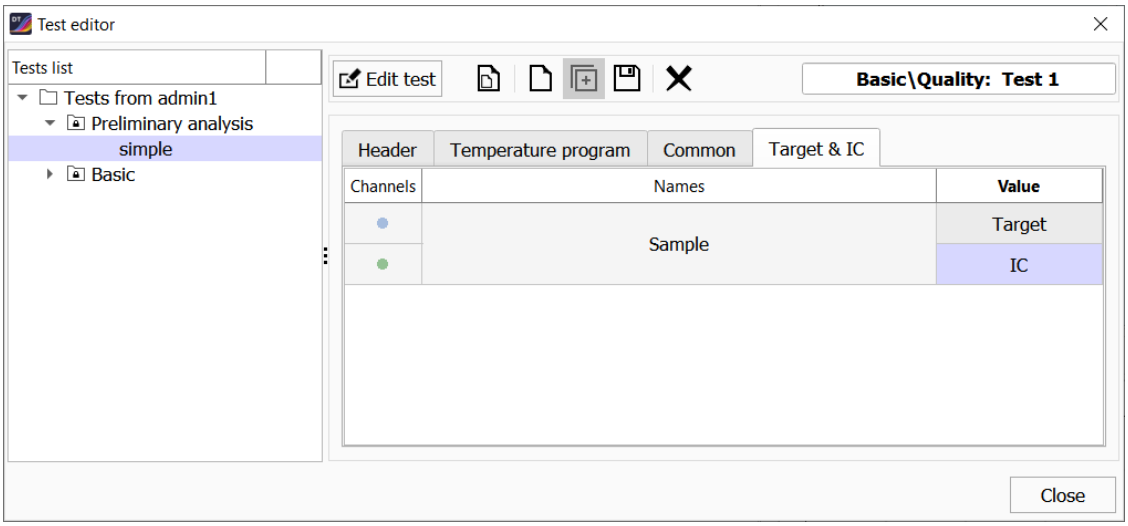


Figure A.5 – The **Target & IC** tab

A.3. QUANTITATIVE TEST PARAMETERS

The parameters of the Quantitative type of analysis are presented on the following tabs (figure A.6):

1. **Header** – general properties of the test (name, active channels, etc.);
2. **Temperature program** – contains the temperature program;
3. **Common** – general parameters of the program and the real-time PCR instrument;
4. **Standards** – setting up calibration samples necessary for analysis;
5. **Target & IC** – the purpose of each optical channel is determined when analyzing the results.

Tabs 1-3 are similar to tabs for the preliminary analysis.

Tab 5 is similar to tab for the Qualitative test.

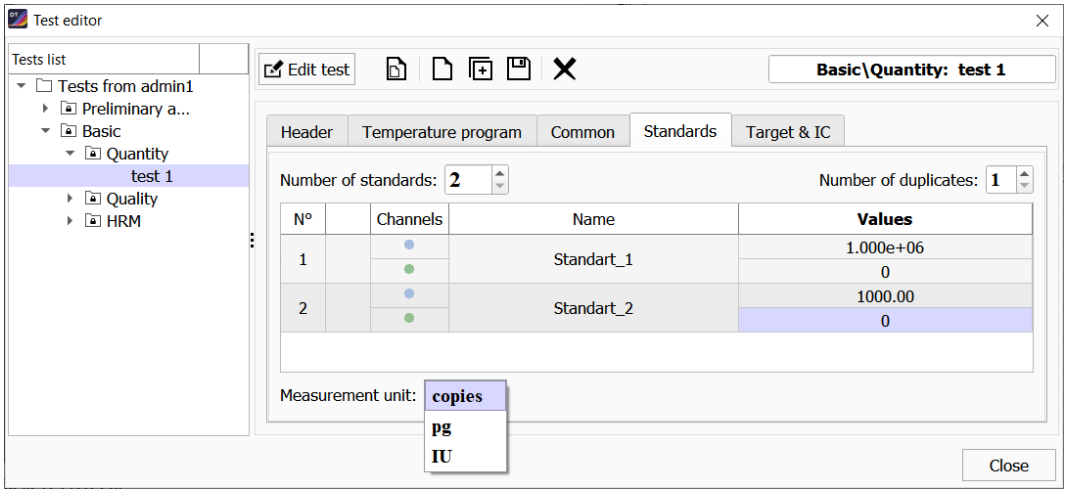


Figure A.6 – The **Standards** tab

On the **Standards** tab a list of calibration samples (standards) used in this test is formed.

To do this, fill in the following data:

- number of standards – number of different concentrations of calibration samples ("standards");
- number of duplicates – number of doubles of each concentration of calibration samples ("standards");

- table of "standards" values (specify the name and concentration of each "standard").

The number of rows in the table corresponds to the number of variants of "standards". For each "standard" you need to specify the name (by default this is "Standard_1", "Standard_2", etc.) and specify the value (concentration of the "standard").

Units (copies, picograms, IU) can be selected at the bottom of the tab.

A.4. RELATIVE TEST PARAMETERS

The parameters of the Relative type of analysis are presented on the following tabs:

- **Header** – general properties of the test (name, active channels, etc.);
- **Temperature program** – contains the temperature program;
- **Common** – general parameters of the program and the real-time PCR instrument;
- **Target & Reference** – the purpose of each optical channel is determined when analyzing the results.

Tabs 1-3 are similar to tabs for the preliminary analysis.

The **Target & Reference** tab defines the purpose of each optical channel when analyzing the results (figure A.7):

- "Target gene" – the main, specific signal;
- "Reference gene" – a signal from a control sample.

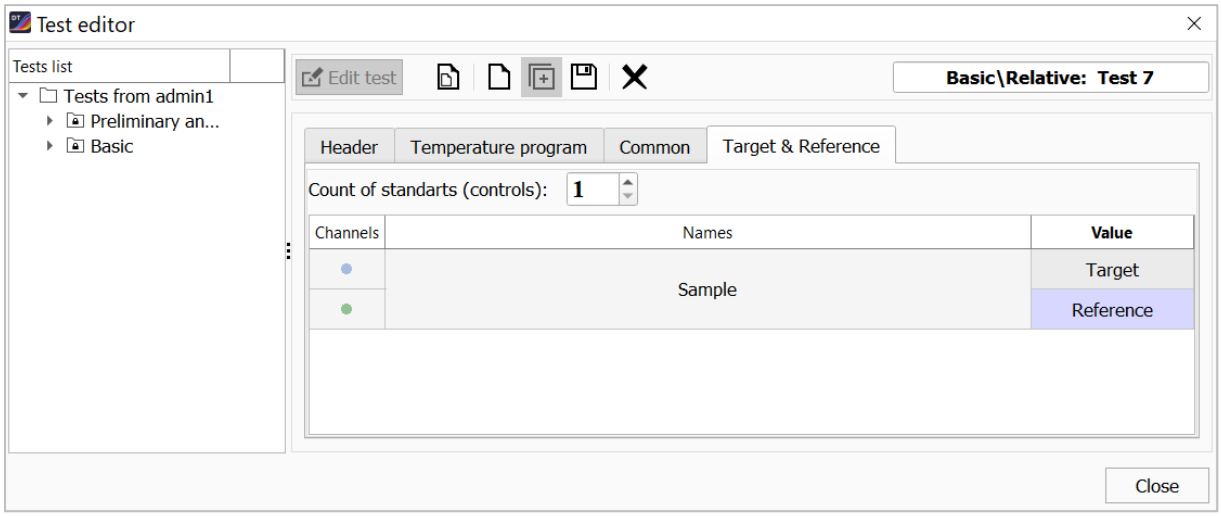


Figure A.7 – The **Target & Reference** tab

A.5. HRM TEST PARAMETERS

Filling in the **Header**, **Temperature program**, **Common** tabs is similar to filling in the Quantitative analysis parameters (see A.1. Preliminary Analysis).

In the **Common** tab, the default values for the confidence parameters of the melting peak can be changed.

In the **Other** tab (figure A.8); a special setting of the clustering quality indicator is available. The setting allows you to set the value of the quality indicator of assigning a sample to a cluster from 0 to 100%. The default value for the parameter is 75% (the recommended value for the parameter). Clustering results with a confidence indicator below the value set by the test settings are highlighted with the "*" symbol.

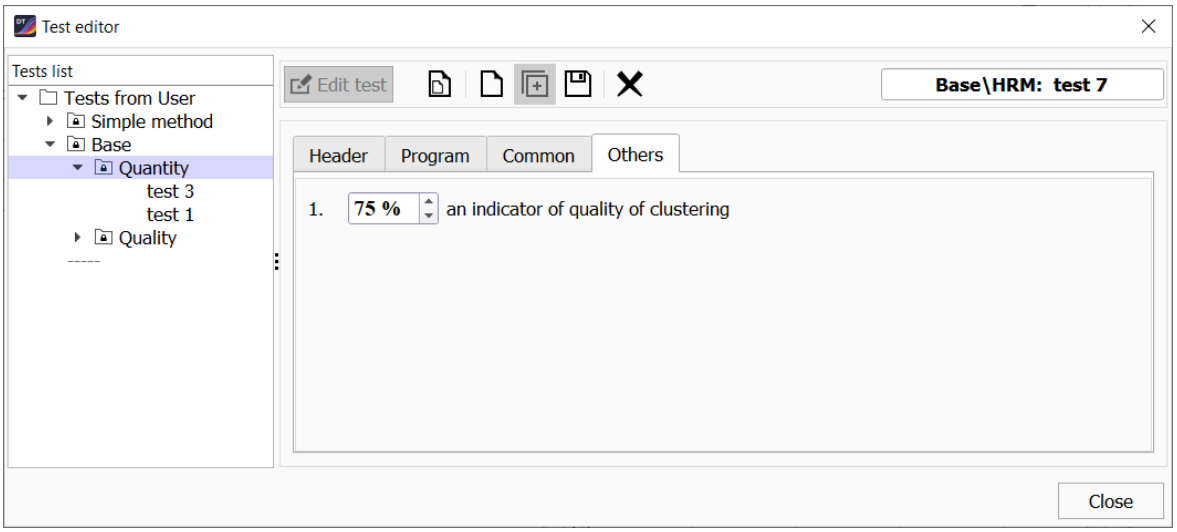


Figure A.8 – Configuring the clustering quality indicator

Special recommendations for HRM analysis on DT real-time PCR instruments

Attention! It is not recommended to conduct HRM-studies using Real-time PCR instruments manufactured before 2017.

Attention! Incorrect results may be related to the technical condition of the Real-time PCR instrument being used; to obtain reliable results, preliminary diagnostics are recommended (checking the geometric settings of the optical unit, diagnostics of photometric characteristics, checking the state of the temperature system). Diagnostics of the main functional units of the Real-time PCR instrument is carried out by engineers of the instrument service department, specialists of the Customer support service or authorized partners.

- use the manufacture exposure settings of the Real-time PCR instrument in the start protocols;
- use the types and names of PCR-plastic, tested for work with the Real-time PCR instrument;
- measure the height of tubes before running the protocol;
- to fill the thermal unit with samples as much as possible, use empty tubes of the same type for filling the thermal unit. If the thermal unit is not completely filled with samples, it is recommended not to place test tubes in the edge rows of thermal unit wells;
- when creating a test program, set the mode of optical measurements of melting curves in the range of ±5-10 °C from the expected value of the peak maximum temperature in 0.2 °C increments;
- establish the registration of the accumulation of PCR products with a complete assessment of the post-amplification characteristics of the samples (data are available in the "Preliminary analysis" section (PCR graphs, Cp \ Ct-result)). It is recommended to exclude from the analysis samples that are characterized by an atypical shape of the accumulation curve, have not reached the amplification plateau, samples with late addition Cp\Ct (Cp\Ct ≥ 30\27);
- when running an HRM analysis of the protocol, preliminarily exclude samples with a low % quality of clustering;
- carry out a protocol for analyzing the uniformity of heating of the cells of the thermal unit of the Real-time PCR instrument using a set of reagents to correct the HRM analysis data before starting a new series of HRM protocols (using the temperature correction settings in the analysis).

APPENDIX B. USER PERMISSIONS

Attention! Only a user with administrator rights can edit the permissions of any user.

The description of permissions is presented in table 13.

Table 13 – DTmaster User Account Permissions List and their Description

Permission name	Permission description
Single user application	Possibility of log in the program without entering a password (for all users)
Change application preferences	Access to edit DTmaster settings (interface, protocol name template)
Change analysis preferences	Access to editing settings common for all tests: spectral correction, optical unevenness, digital filters (see par. 4.1 of this manual)
Change device preferences	Access to change the device settings (viewing the video with the ability to change the optical mask, checking the exposure with the ability to change and measure the tube height)
Control run process	Ability to start, pause and stop protocol execution
Copy block test	Access to copy a group of tests
Edit protocol data	Access to the functionality of editing downloaded protocols
Edit test data	Access to Test editor
Enable command line interface	Ability to use the command line to control the device
Mask protocol data	Ability to edit the analyzed protocol
Remember windows location	When the user finishes working with DTmaster, information about the current size of the program window and all internal windows is saved
Return to the original exposure values after run	Used when DTmaster and RealTime PCR software version 7.9 work together. The Real-time PCR instrument after the completion of the start of the DTmaster protocol automatically restores the exposure value transmitted at the last start of the RealTime PCR protocol
Saving optical measurement data to a file during protocol execution	Saving optical data after each measurement in .rt file
Enable advanced analysis	Displaying the Ct analysis tab in the preliminary analysis results, as well as additional information about the reliability of the sigmoidal fitting as a result of the filters operation on the Cp analysis tab
Show cross-table	Display in the Analysis mode of the CrossTable tab with the results of preliminary analysis

Permission name	Permission description
Show page Run	By default, these sections are prohibited for the Guest account.
Show page Setup	
Select a folder for the results	Displaying a window for entering the name of the protocol and selecting the folder in which the obtained results will be saved after running the protocol in the Run mode
Skip expositions validation	Removes the ban on running a protocol with tests, in the settings of which different exposures by channels are specified. In this case, the exposure from the test of the last added sample will be used in the protocol. This permission is only recommended for advanced users in scientific research and is prohibited for clinical laboratory diagnostics.
Skip min level checking	Removes the prohibition on running a protocol for which the calculated minimum measurement time (measurement, processing, recording to SD, etc.) for all active channels for the minimum shelf is greater than the time specified for this temperature shelf in the temperature program. This permission is only recommended for advanced users in scientific research and is prohibited for clinical laboratory diagnostics.
Skip amplification programs check	Removes the ban on running the protocol with tests, the settings of which indicate different temperature programs. In this case, the amplification programs from the test of the last added sample will be used in the protocol. This permission is only recommended for advanced users in scientific research and is prohibited for clinical laboratory diagnostics.
Skip volume validation	Removes the prohibition on running the protocol with tests, in the settings of which a different volume of the reaction mixture is specified

CONTACT INFORMATION

Manufacturer: DNA-Technology, Research & Production, LLC, Russia.

Manufacturer's address: DNA-Technology, Research & Production, LLC

20 Zheleznodorozhnaya Street, Protvino, Moscow Region, Russia, 142281

Place of manufacture: DNA-Technology, Research & Production, LLC

20 Zheleznodorozhnaya Street, Protvino, Moscow Region, Russia, 142281

Complaints regarding the operation of the DTmaster software should be addressed to: "DNA-Technology", LLC, Varshavskoye Hwy, 125Zh, Bld. 6, Room 14, Moscow 117587,

Phone/Fax +7 (495) 640-17-71,

www.dna-technology.com

Customer support service:

8 (800) 200-75-15 (free for Russia),

+7 (495) 640-16-93 (for the CIS countries and abroad, calls are charged),

Email: hotline@dna-technology.ru

GLOSSARY

Amplification – the accumulation of multiple copies of a specific DNA fragment during the polymerase chain reaction (PCR).

Command line – an interface built into the software interface that allows you to control the real-time PCR instrument microcontrollers using text commands.

Duplicate, duplicates – parallel run of two or several identical reactions. It is used to average the influence of random factors on the result.

Fitting – smoothing of experimental data.

Fluorescent dye – a molecule that has the ability to glow by absorbing the light energy. Used to visualize amplification products.

Hot lid – a module as a part of real-time PCR instrument, which maintains the temperature of the caps of the tubes at 105 ± 1 °C. Designed to prevent condensation on the tube lids.

Internal control sample (internal control, internal amplification control) is an artificially created sample of DNA or RNA, which has an oligonucleotide sequence that is fundamentally different from the detected one. For internal control (IC), strictly complementary primers are introduced into the reaction mixture. IC is created to control all stages of PCR (can be used at the stage of nucleic acid extraction and/or amplification).

Kit – components that are packaged together to perform a specific in vitro diagnostic study.

Matrix – a graphic representation of the thermal unit part in which the test tubes are located. It is presented in the form of a table containing the number of cells that corresponds to a specific modification of the real-time PCR instrument: 48-, 96-, 192- or 384-well.

Measurement exposure – duration of optical measurements.

Melting curve – graph of the fluorescence intensity of PCR products (amplicons) with a stepwise change in temperature.

Optical measurement histogram – graphic display of the current optical measurements in three-dimensional coordinates.

Protocol – a set of preset parameters: the number of samples and controls, standards/calibrators and their layout on the thermal unit plate; temperature program. The protocol can be saved as a .rt file.

Qualitative analysis – determining of the presence or absence of nucleic acids of the detected microorganism in the sample.

Quantitative analysis – determining the concentration of nucleic acids of the detected microorganism in the test sample.

Real-time PCR instrument – a device designed for real-time polymerase chain reaction (PCR). Provides cyclic cooling and heating of test tubes with a reaction mixture in accordance with the specified temperature program and simultaneous accounting of fluorescence results.

Run file – file with analysis data in .r48, .r96, .192, .384 or .rt formats containing information on the location of tubes in the thermal plate, the temperature program and the results of optical measurements.

Sample – specimen or environmental samples intended for analysis.

Temperature program – data on thermal cycling conditions (temperature and duration of temperature shelves, number of cycles, availability of optical measurements). It is saved as a .rta file.

Template – contains all the information specific to the protocol, but it does not pass the stage of running the temperature program and the subsequent analysis of the obtained results.

Test – a set of software settings containing data on the type of analysis, dyes, controls, the temperature program, the volume of the reaction mixture, the algorithm for analyzing the analysis results and the type of report form (automatic conclusion, report form).

Thermal unit – an element of a high-speed thermal control system, which includes a heating plate (matrix), Peltier thermoelectric elements, a radiator and a protective case.

User – person operating the device.

XML file – file with analysis results in XML format.