GeneProof
Molecular diagnostics for your routine

# DEVICE MANUAL

Bioer

# LineGene 9600 Real-Time PCR Detection System
# LineGene 9600 Plus Real-Time PCR Detection System
# LineGene K Plus Real-Time PCR Detection System

**Designed for GeneProof diagnostic kits**

**Microbiological DNA diagnostics**

**Current list of kits at www.geneproof.com**

# CONTENTS

# 1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological DNA diagnostics with the following devices: LineGene 9600 Real-Time PCR Detection System, LineGene 9600 Plus Real-Time PCR Detection System and LineGene K Plus Real-Time PCR Detection System.

# 2. PCR Reaction Preparation

1. Add **30 µl of MasterMix** and **10 µl of DNA isolate** or **10 µl of Positive Control** into the tube in case of qualitative detection or **10 µl of calibrators** in case of quantitative detection. The final reaction mix volume will be **40 µl**.
2. Close the tubes, centrifuge shortly, insert into the device and start the PCR test.

# 3. Device Programming

When using the GeneProof PCR kits for the first time it is necessary to program the detectors and the amplification profile and save it as a template.
During subsequent uses of the GeneProof PCR kits start from chapter **4. PCR Amplification Start**. The software remembers the saved settings.

## 3.1. Starting the software

1. Start the **LineGene 9600** software (LineGene 4800 with LineGene K Plus).
2. Click the **Absolute** button in the main window.

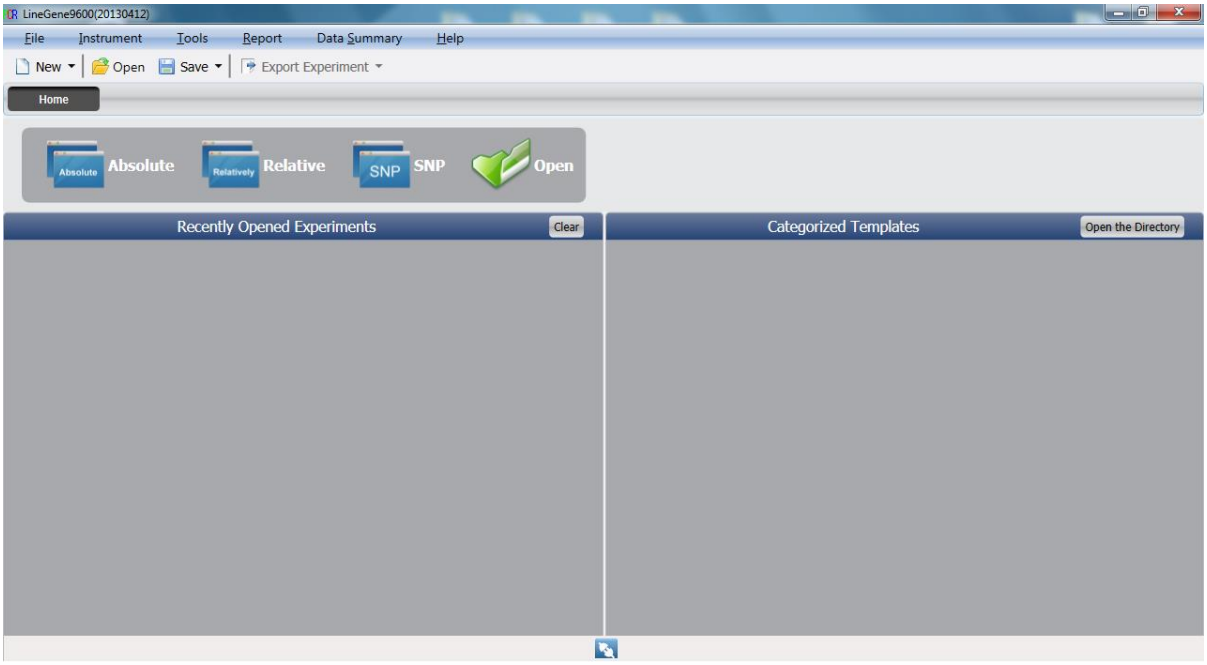


*Fig. 3.1 Starting the software*

## 3.2. Detector programming

1. Click twice the **Add Assay** button in the **Detector** tab of the **Setup** menu and select **FAM**, **HEX** and **Cy5** in the **Reporter** column.

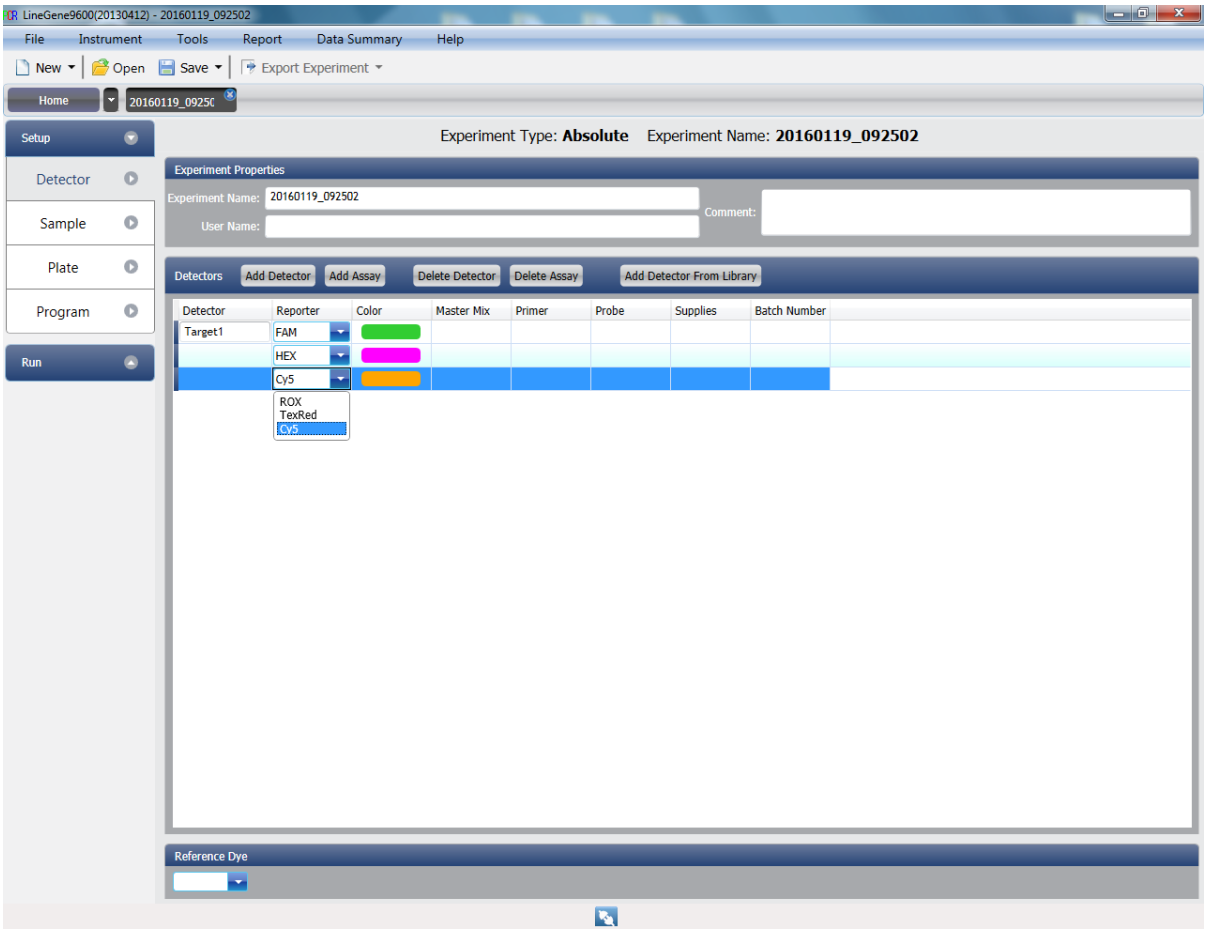


*Fig. 3.2 Detector programming*

## 3.3. Plate programming

1. Move to the **Plate** tab, select the complete plate and check all the **Assay Items** in the **Detectors** table.

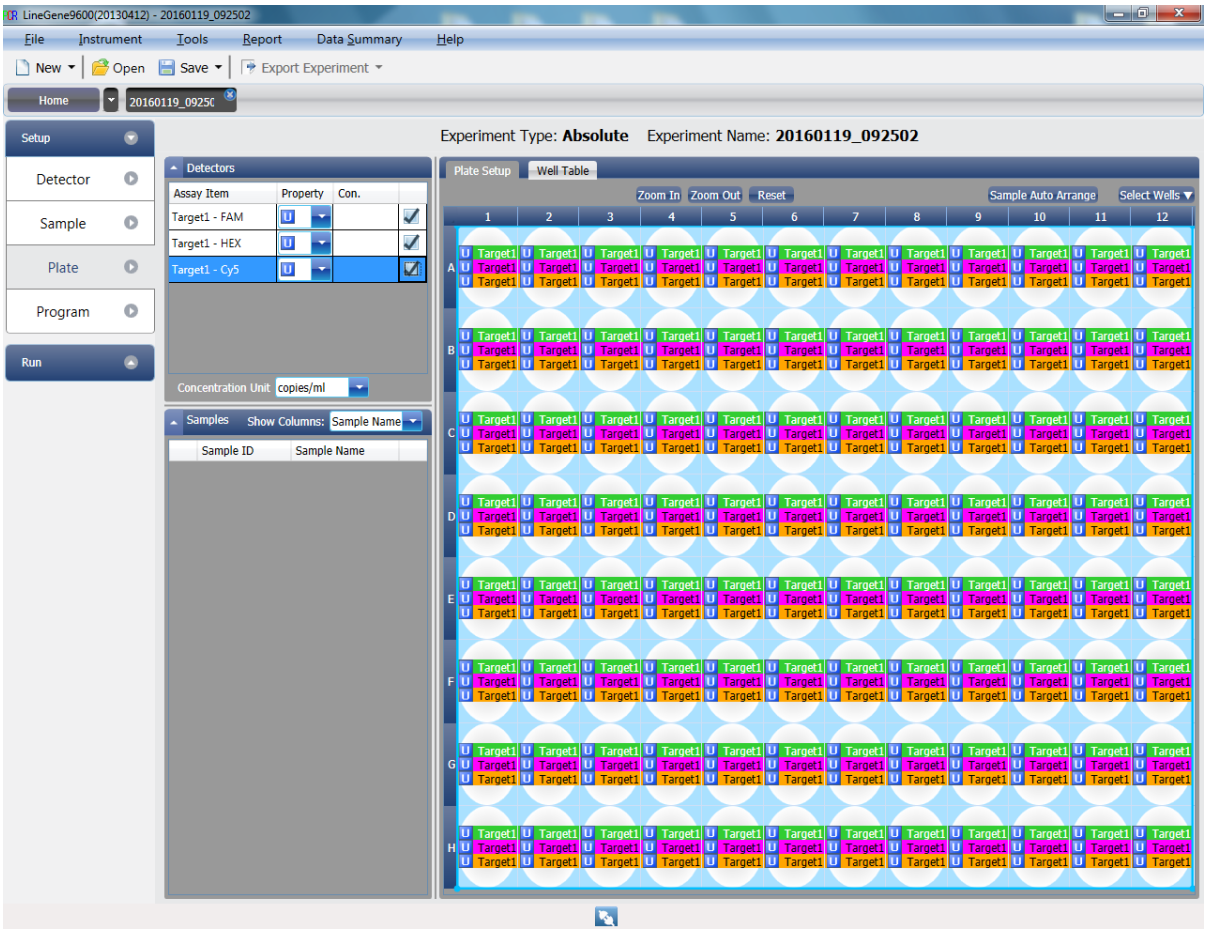


*Fig. 3.3 Plate programming*

## 3.4. Amplification profile programming

1. Move to the **Program** tab and program the amplification profile according to the following instructions. Set the **Cycles 45** in the **PCR Stage**.

*Tab. 3.1 Amplification profile*

| **Stage** | **Hold Stage** | | **PCR Stage (45 cycles)** | | |
|---|---|---|---|---|---|
| **Temperature** | **37 °C** | **95 °C** | **95 °C** | **60 °C** | **72 °C** |
| **Time** | 2:00 min. | 10:00 min. | 0:05 sec. | 0:40 sec. | 0:20 sec. |
| | | | | **Check Sampling** | |

2. Check the **Hotlid(C)** box and enter **105**.
3. Enter **40** in the **Liquid Quant.(ul)** box.

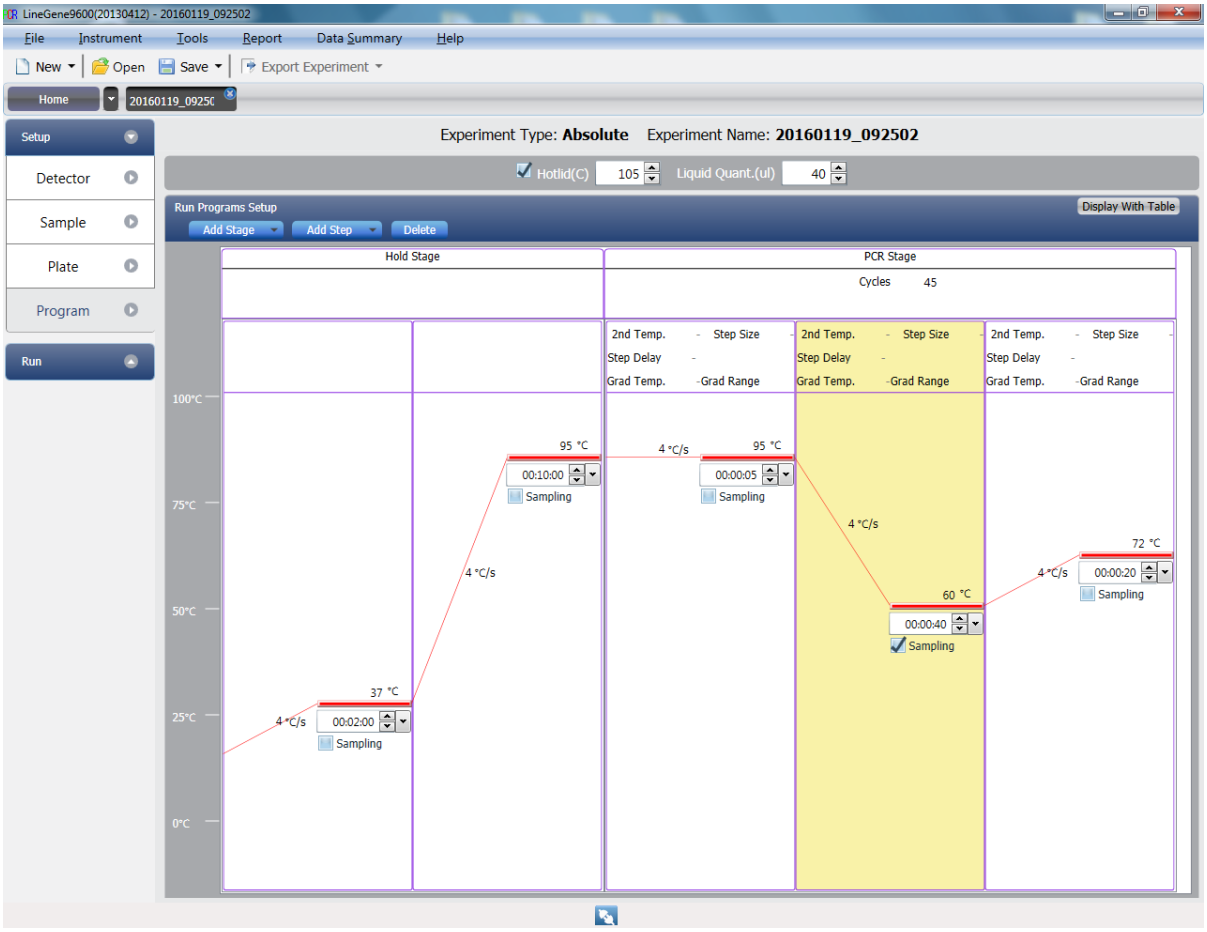


*Fig. 3.4 Amplification profile programming*

### 3.5. Saving the template

1. Click the arrow symbol near the **Save** button in the upper bar and select **Save As Template**.

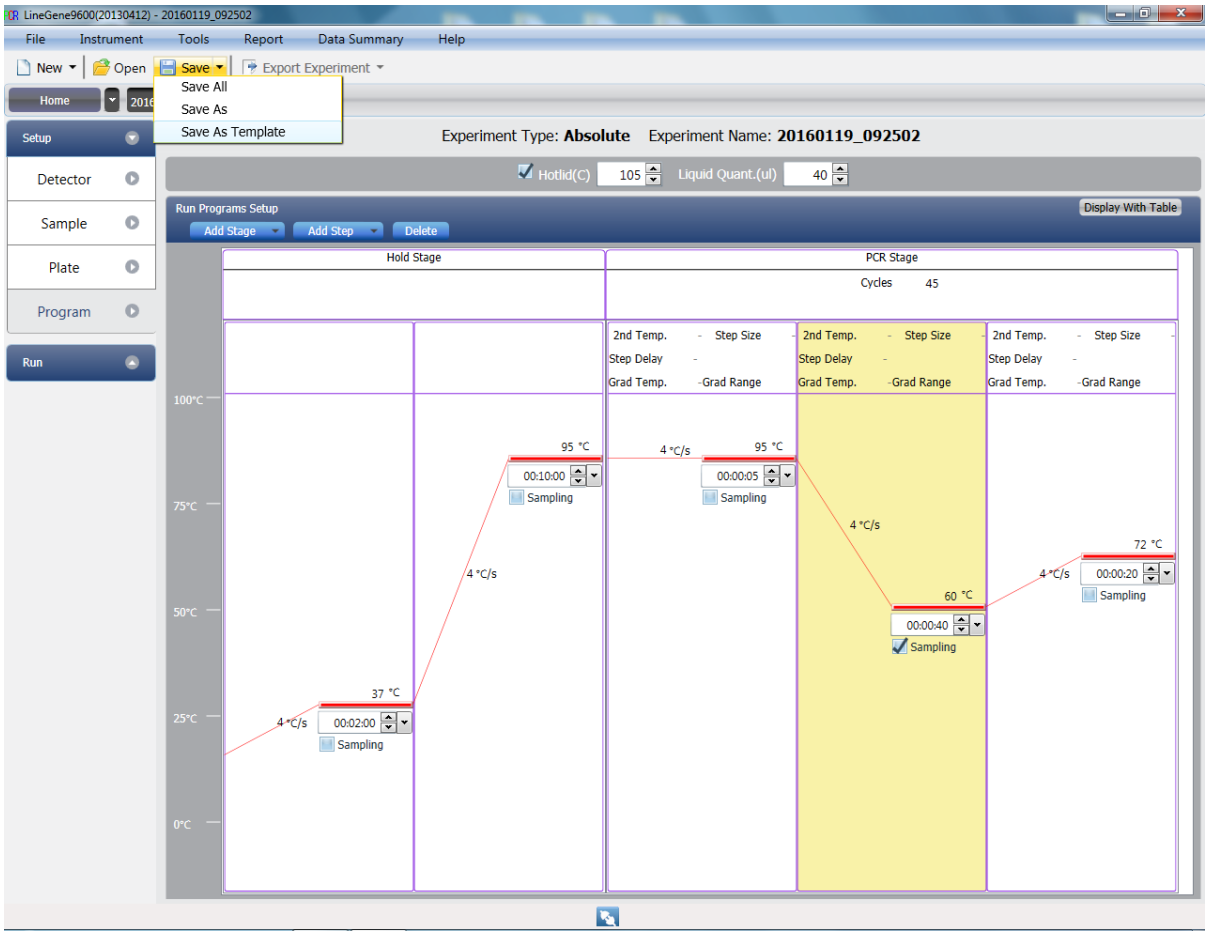


*Fig. 3.5 Save template*

2. Enter/select **GeneProof** in **Template Type** box and enter **GeneProof DNA PCR** in **Template Name** box, check all **Template Content** items.

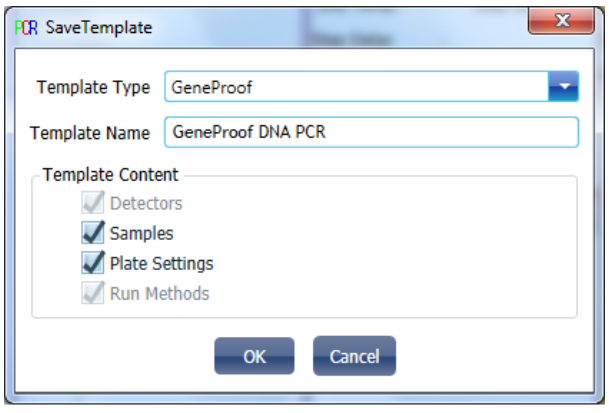


*Fig. 3.6 Template name*

# 4. PCR Amplification Start

When using the GeneProof PCR kits for the first time it is necessary to program the detectors and the amplification profile and save them as a template (see chapter **3. Device Programming**). The software will remember the saved settings for subsequent GeneProof PCR kit uses.

## 4.1. Opening of the saved template

1. Start the **LineGene 9600** software (LineGene 4800 with LineGene K Plus).
2. Click the **+ GeneProof** button and select the **GeneProof DNA PCR** in the right section of main window.

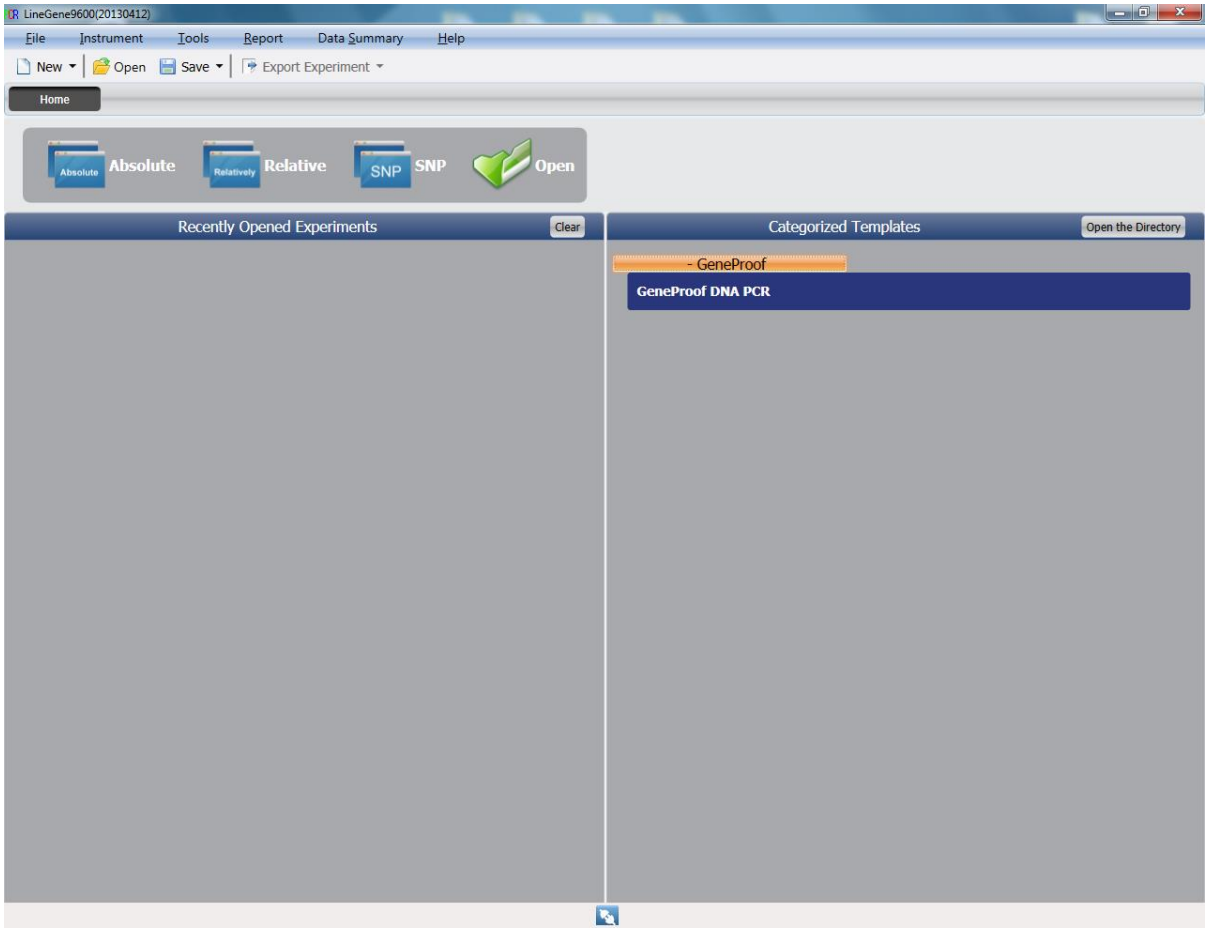


*Fig. 4.1 Template opening*

## 4.2. Detector editing

1. Enter experiment name in the **Experiment Name** box.
2. Rename Target1 detector in the **Detector** column according to the **studied microorganism** (e. g. HSV).

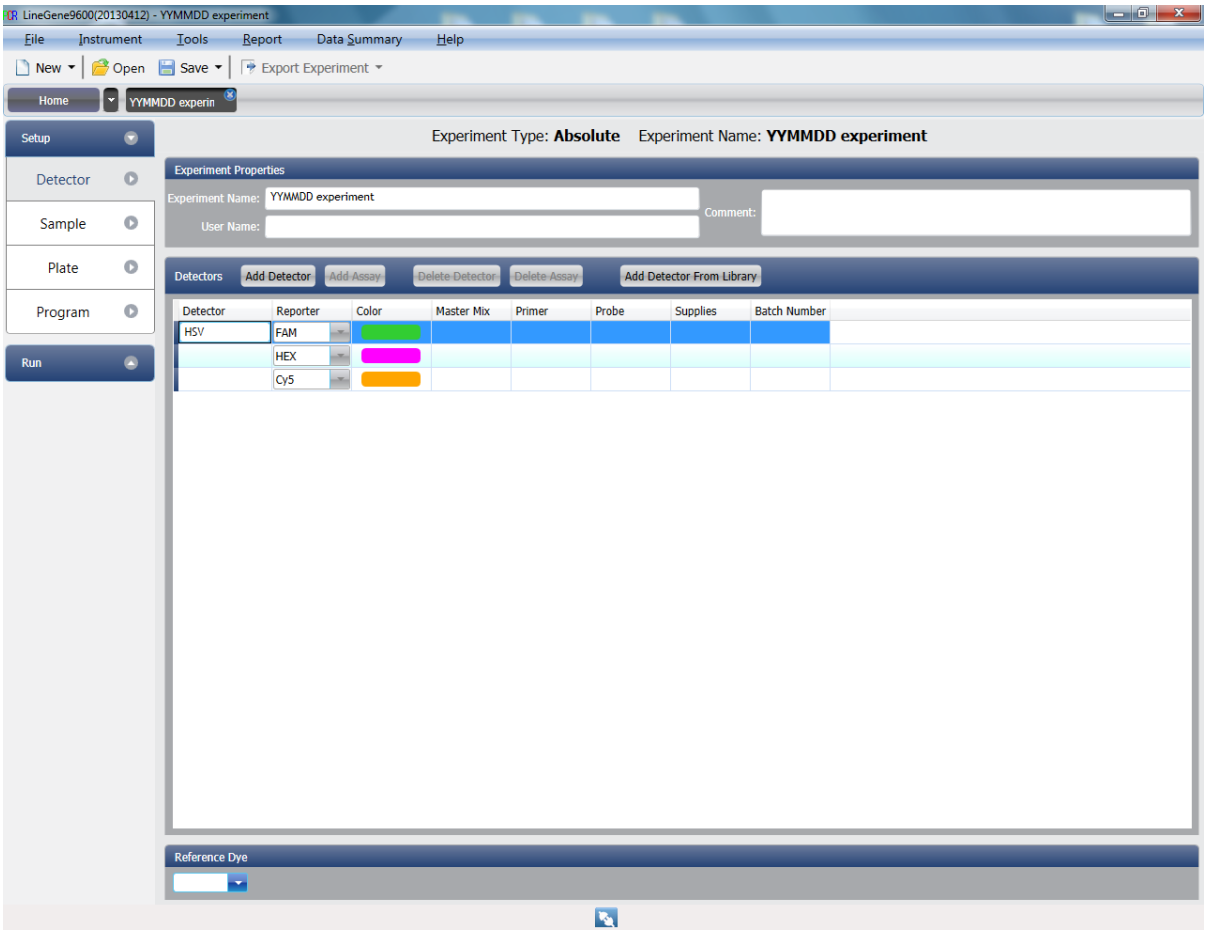


*Fig. 4.2 Detector editing*

3. In case of studying more microorganism in the experiment use **Add Detector** and **Add Assay** buttons to add more detectors and relevant reporters.

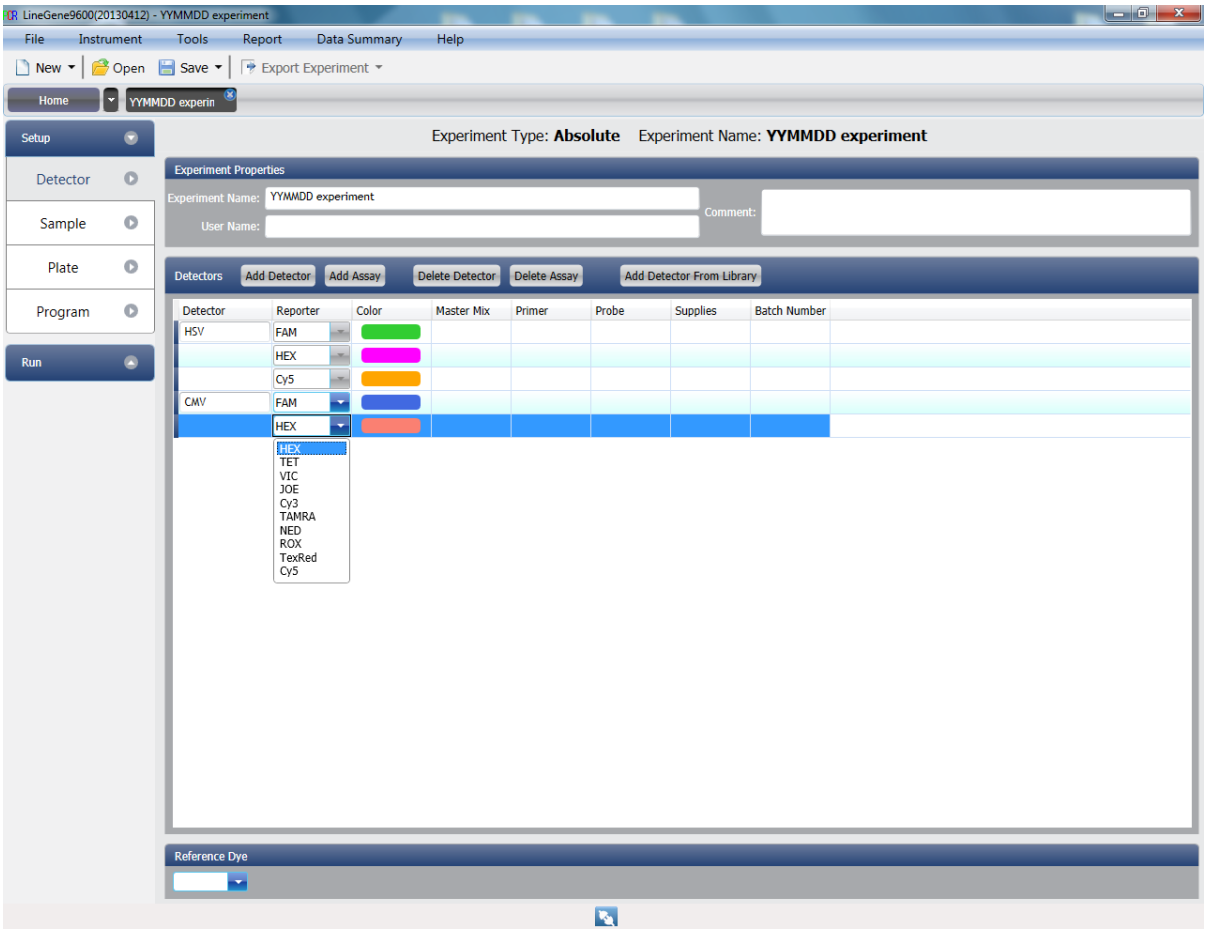


*Fig. 4.3 Add detector*

## 4.3. Sample editing

1. Move to the **Sample** tab to add samples. Enter sample ID in the **Sample ID** box and press the **Enter** key. The **Batch Add** button can be used to create batch of samples 01-XX.

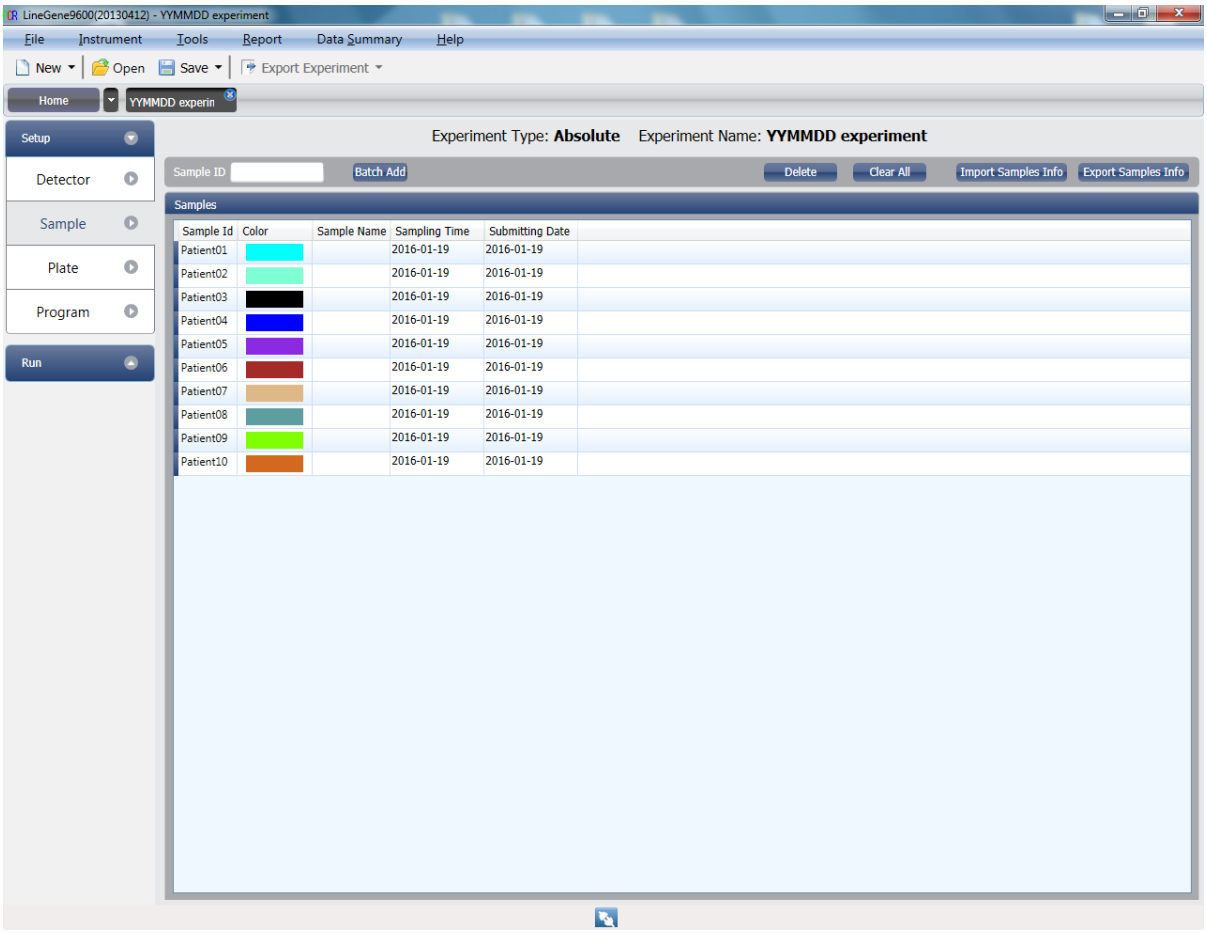


*Fig. 4.4 Sample editing*

## 4.4. Plate editing

1. Select positions for the individual examinations and then check the corresponding **Assay Items** in the **Detectors** table to assign detectors, uncheck empty positions.
2. Check the relevant samples in the **Samples** table to assign them to the positions with samples for examination (select positions and click **Sample Auto Arrange** to apply either horizontal or vertical automatic sample assignment).
3. In positions designated for negative control, positive control or calibrators navigate to the **Detectors** table and in the **FAM** and **Cy5** channels change the value in the **Property** column to **N** in case of **negative** control, **P** in case of **positive** control and **S** in case of a **calibrator**. For calibrators you also have to enter the particular calibrator **concentration** in the **Con.** column.

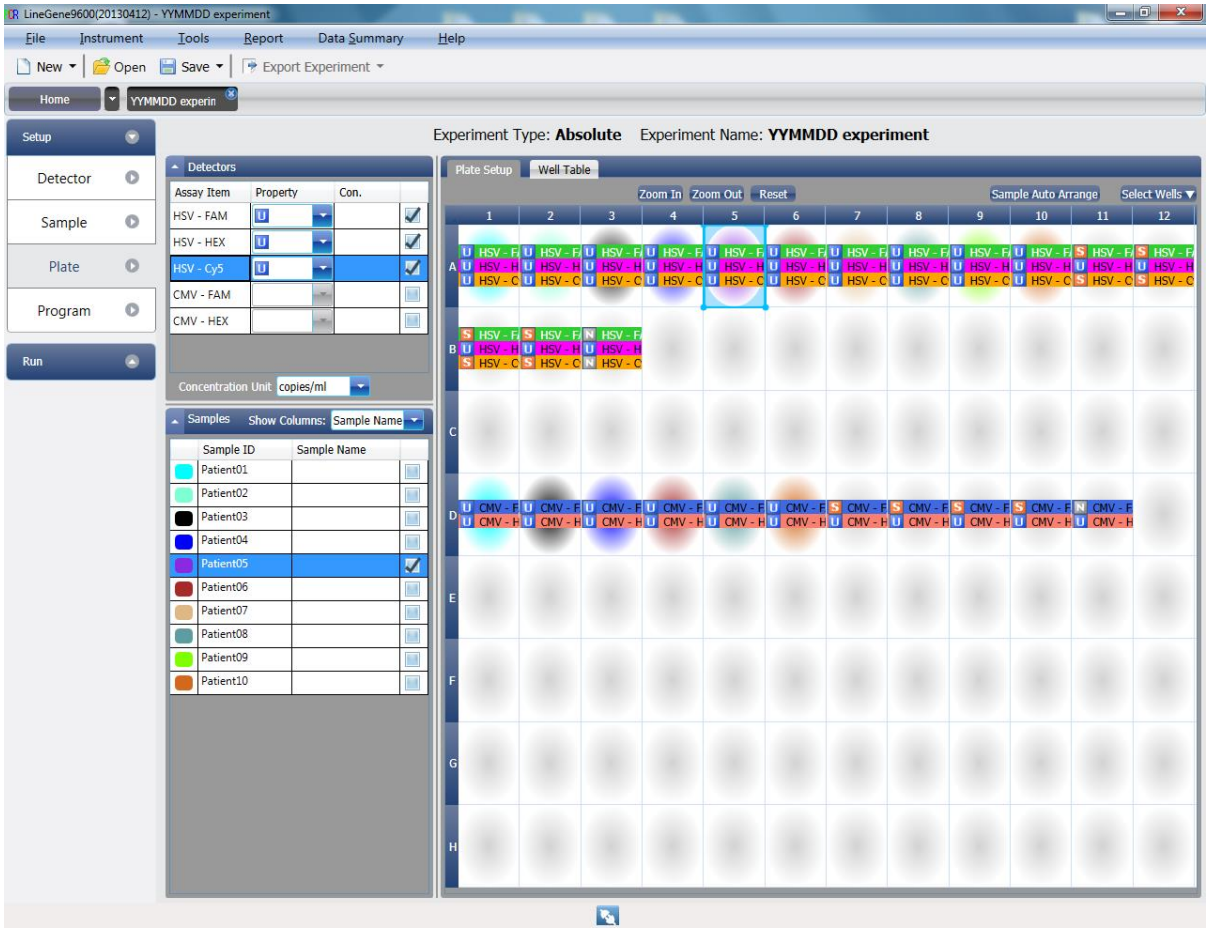


*Fig. 4.5 Plate editing*

## 4.5. Starting the PCR test

1. Click the **Save** button in the upper bar to save the experiment.
2. Move to the **Fluorescence Curve** tab of the **Run** menu and click the **Start Run** button.

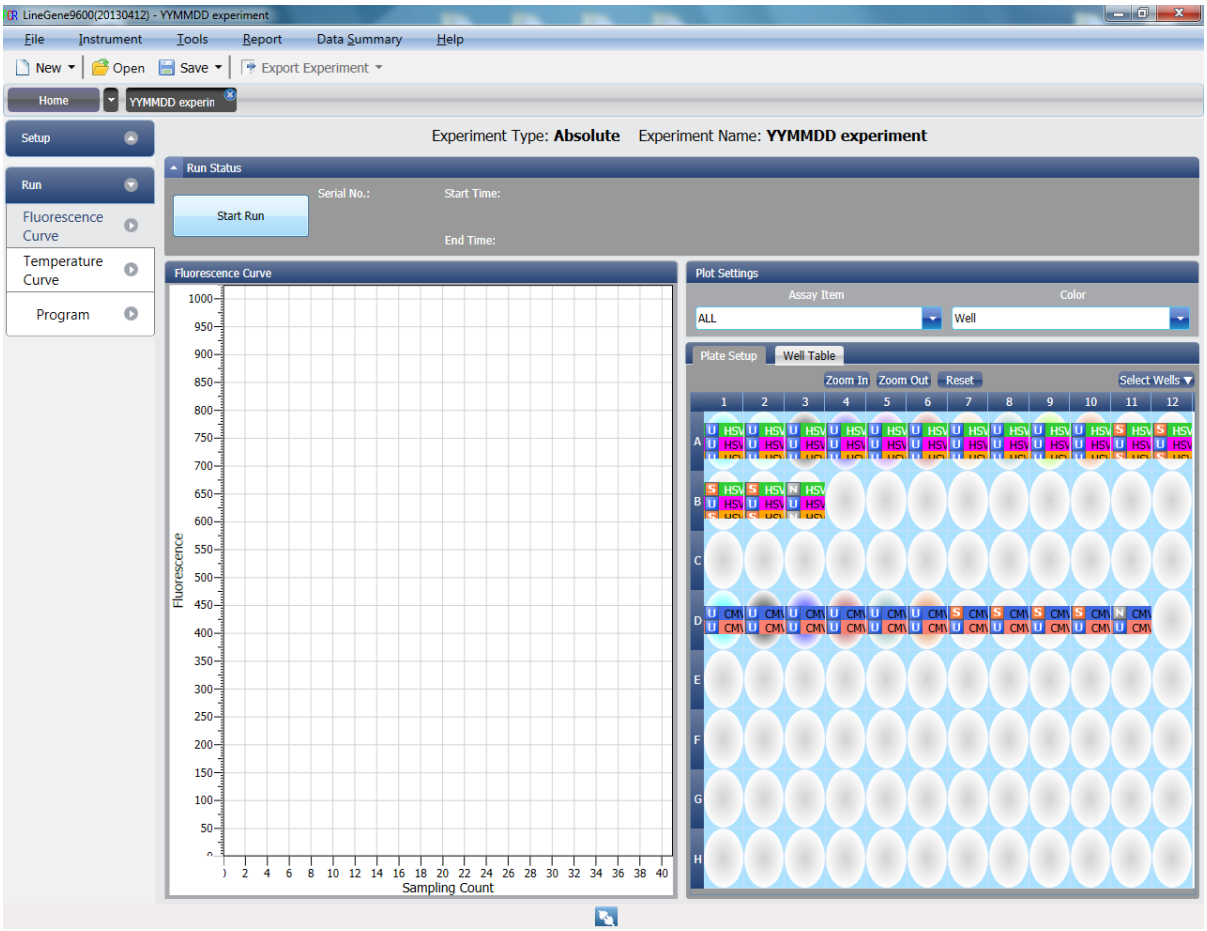


*Fig. 4.6 Start run*

3. The **Confirm** window is displayed. Check the **Auto-Gain** and click **OK** button to confirm.

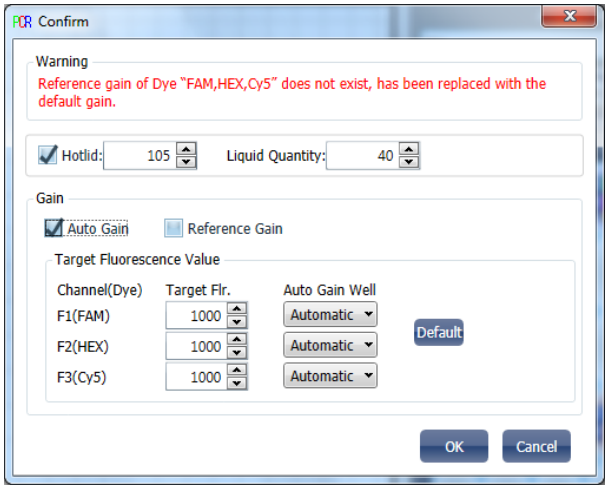


*Fig. 4.7 Gain setting*

4. The **Experiment is completed** notice will be displayed in the lower left corner of the screen. Click the **Yes** button to save the data.

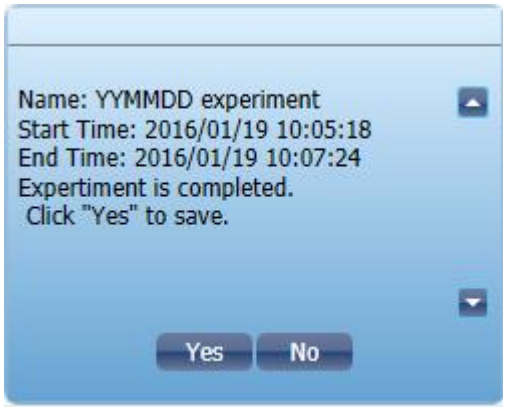


*Fig. 4.8 Save experiment*

# 5. Result qualitative analysis and detection evaluation

PCR detection result evaluation must be **always** performed qualitatively first; if you use the PCR kit for quantitative assessment, continue to quantify positive samples in the second step.

When the notice of completed experiment is confirmed, the **Amplification Plot** tab of the **Analysis** menu is displayed.

## 5.1. Detection analysis of the studied microorganism

1. Select the **FAM** channel of the studied microorganism (e. g. CMV-FAM) in the **Assay** box of **Plot Settings** section.

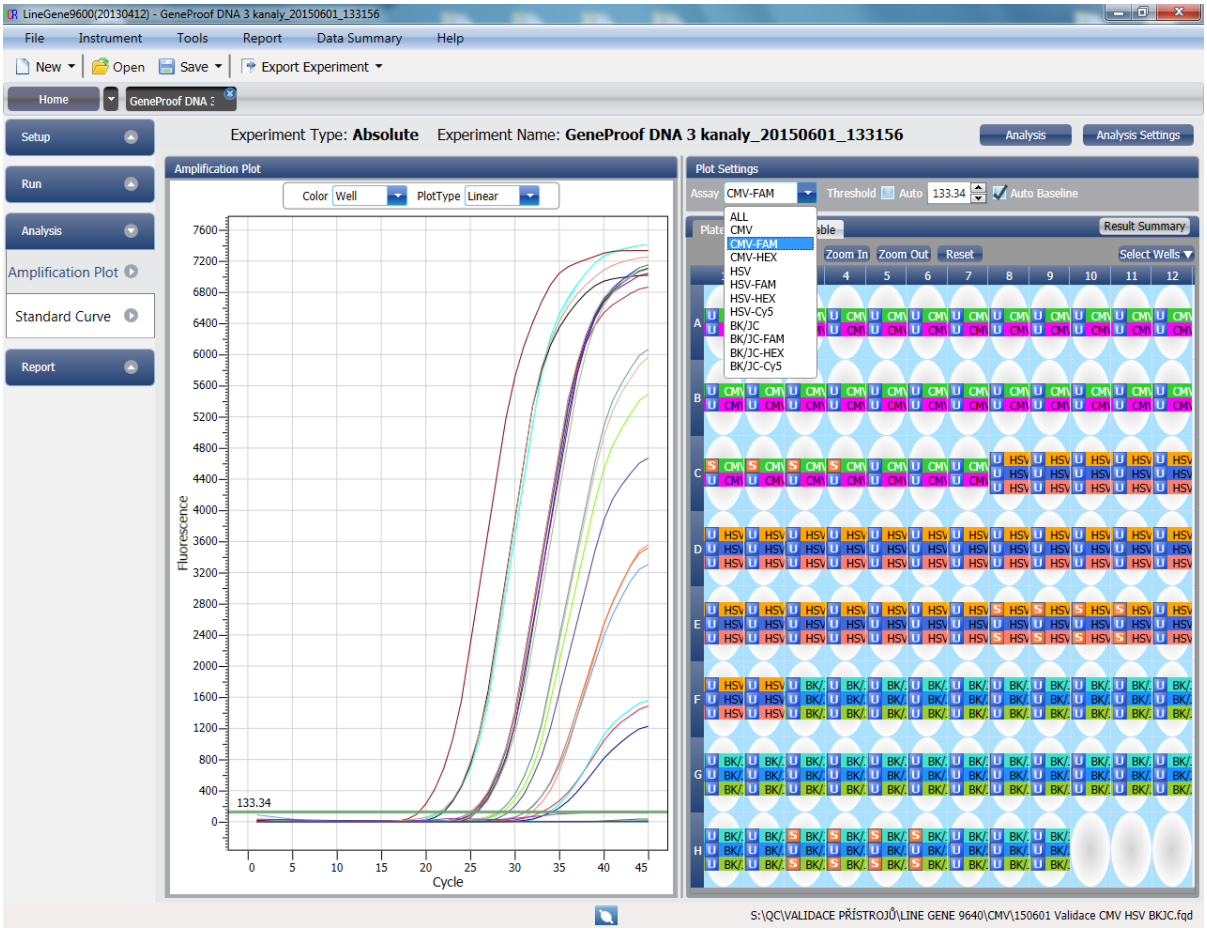


*Fig. 5.1 Detection analysis of the studied microorganism*

2. Parameters **Threshold** and **Baseline** are determined automatically. When needed you can adjust the **Threshold** by moving the threshold line in the chart – for example if the Threshold is automatically set above the weakly positive curve. For easier identification of weakly positive samples use the logarithmic scale by selecting **Log** in the **PlotType** box.

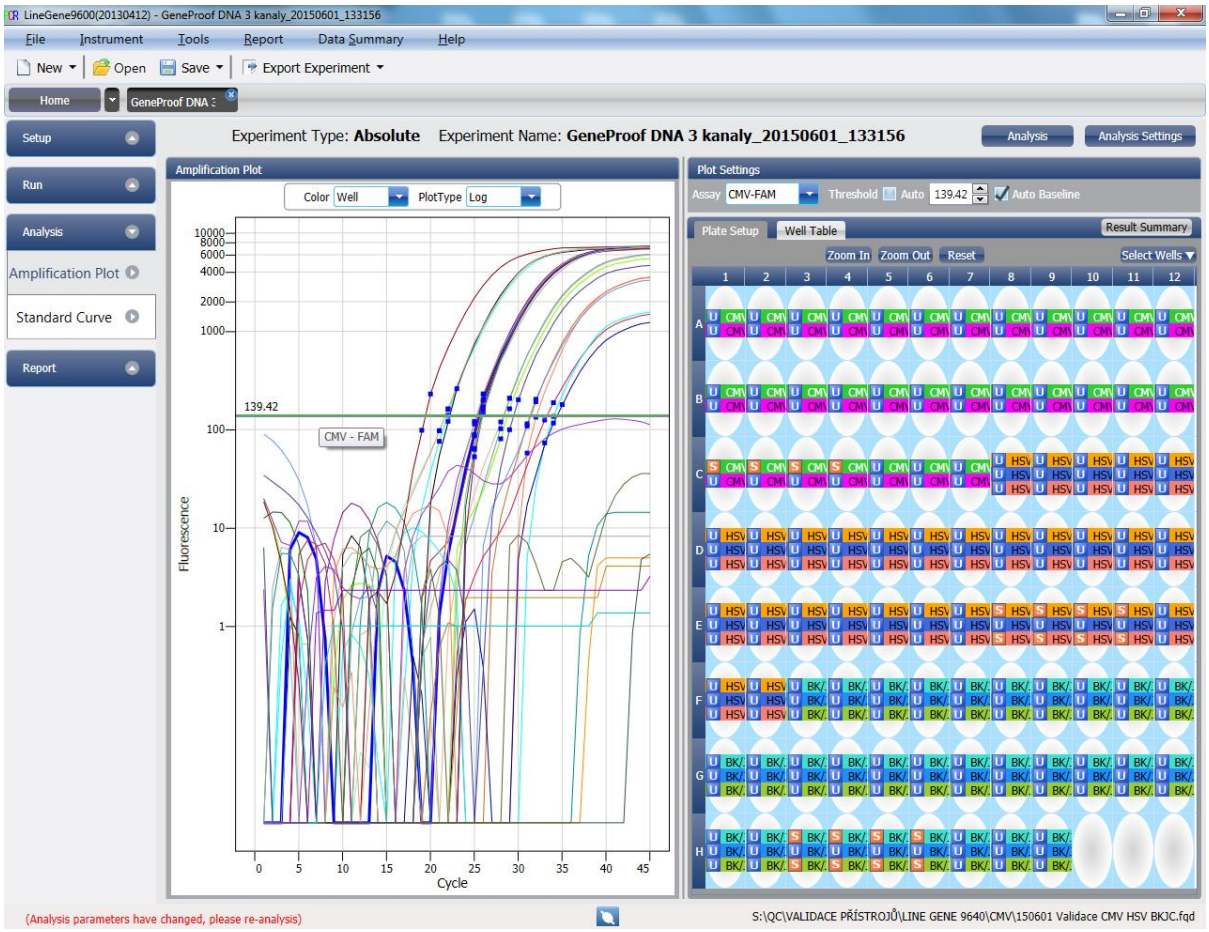


*Fig. 5.2 Adjusting Threshold*

3. Always click the **Analysis** button after a **Threshold** modification to recalculate the analysis. For **Ct** values for the individual positive samples see the **Well Table**.

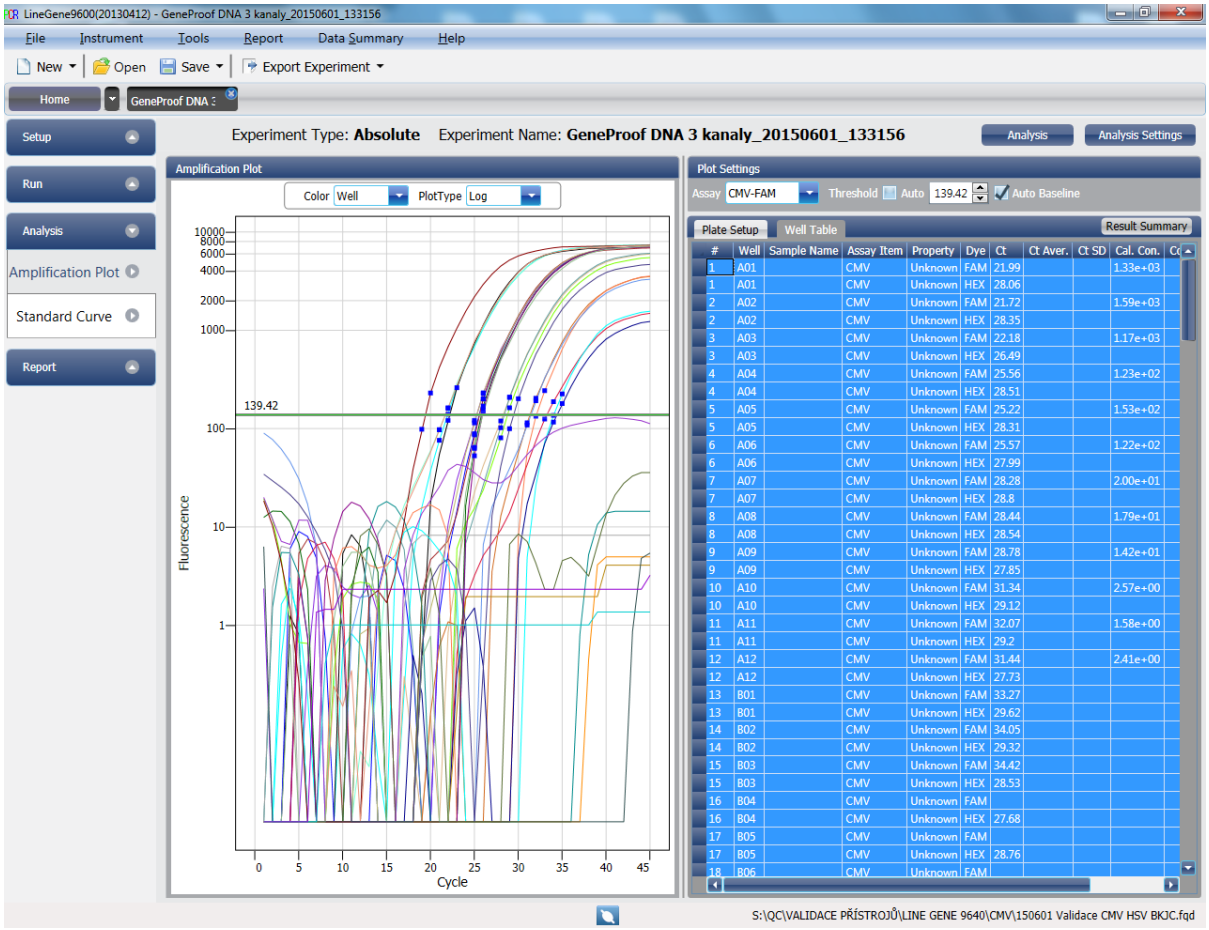


*Fig. 5.3 Ct values*

Perform the same for the **Cy5** channel when using a PCR kit performing detection in this channel.

Perform evaluation according to the Package Insert of the used GeneProof PCR kit.

## 5.2. Internal Standard detection analysis

1. Select the **HEX** channel of the studied microorganism (e. g. CMV-HEX) in the **Assay** box of **Plot Settings** section.

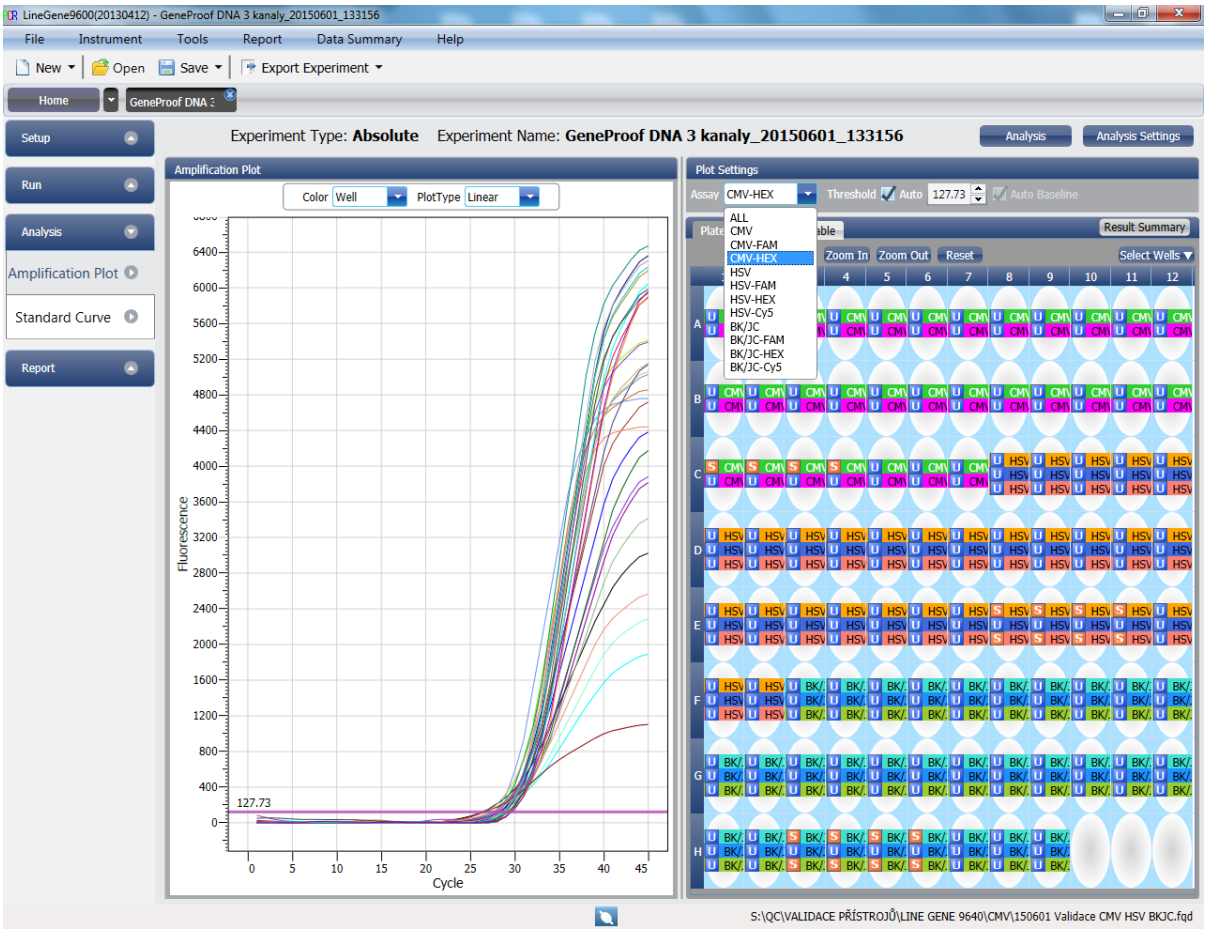


*Fig. 5.4 Internal Standard detection analysis*

2. When needed you can adjust the **Threshold** by moving the threshold line in the chart – for example if the Threshold is automatically set above the weakly positive curve.

Perform evaluation according to the Package Insert of the used GeneProof PCR kit.

# 6. Result quantitative analysis and detection evaluation

1. Move to the **Standard Curve** tab and evaluate the calibration quality. Calibration parameters are located under the **Standard Curve** chart. The absolute value of the **Correlation** parameter in a well-performed calibration achieves a minimum value of **0.99**. If the absolute value of the **Correlation** parameter is lower than **0.99**, move the **Threshold** and repeat the analysis.

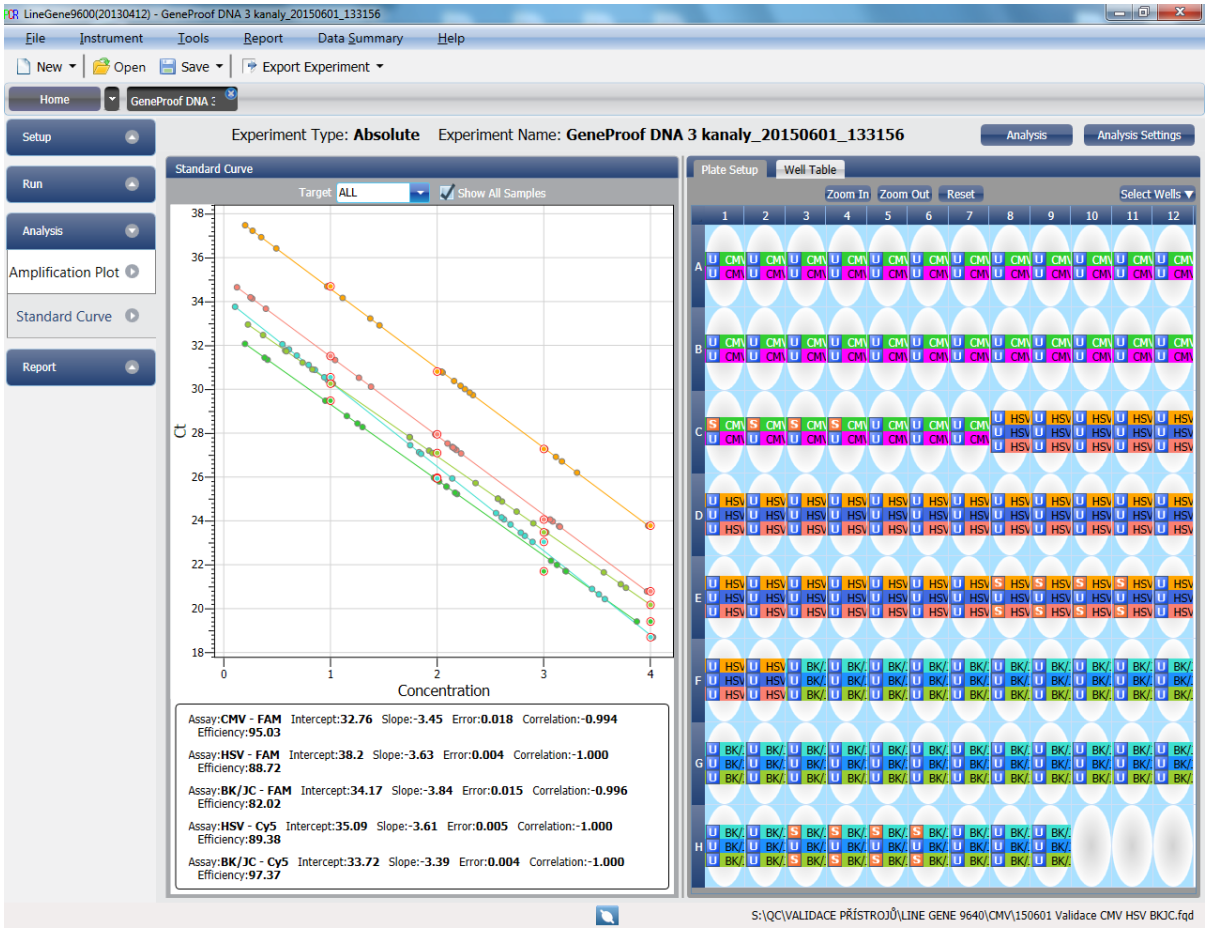


*Fig. 6.1 Standard curve*

2. For details of the quantitative evaluation see the **Well Table**. Concentration is displayed in **Cal. Con.** column.

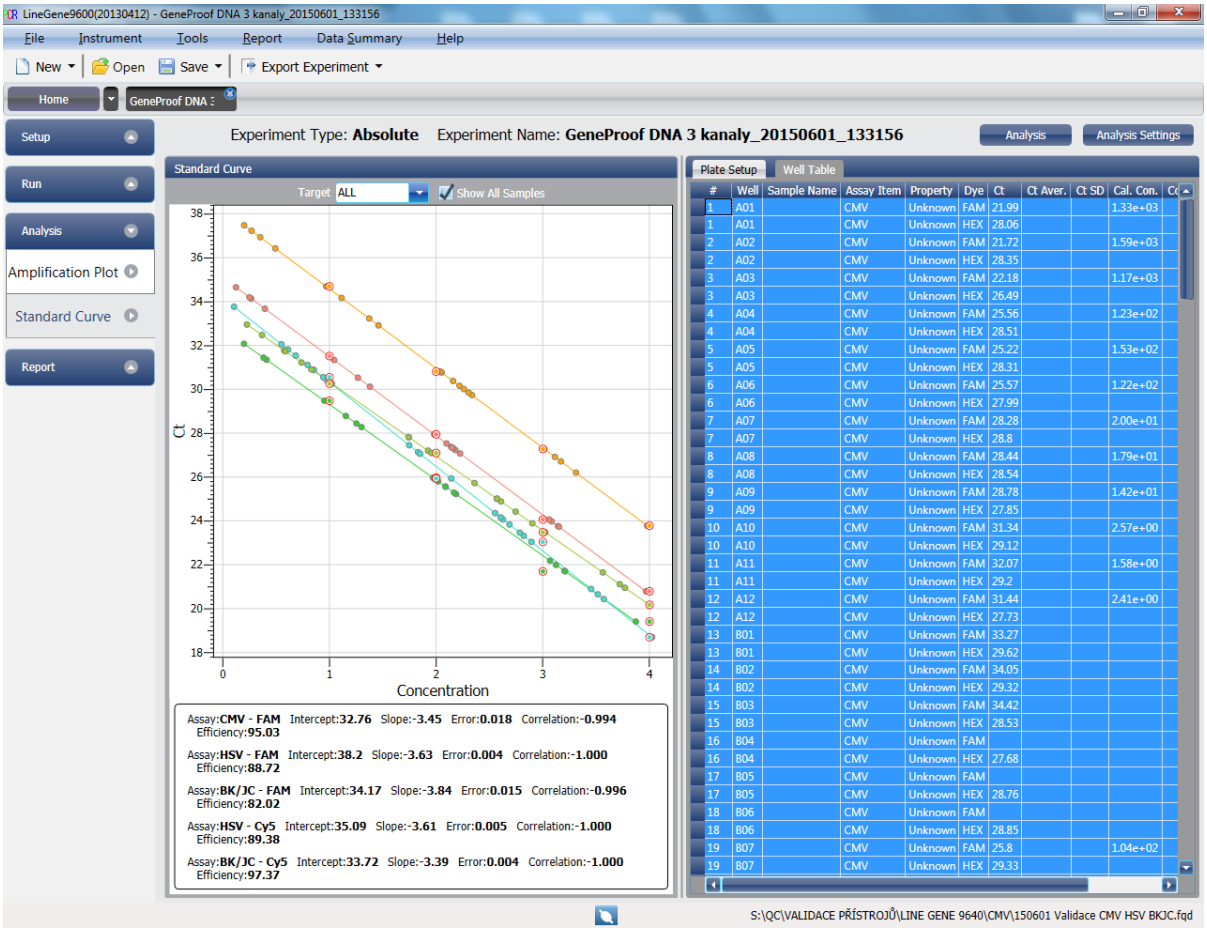


*Fig. 6.2 Quantitative evaluation details*

Click **Result Summary** in the **Amplification Plot** tab to display a summary of results (Ct values and concentrations) displayed in positions corresponding to the PCR plate.

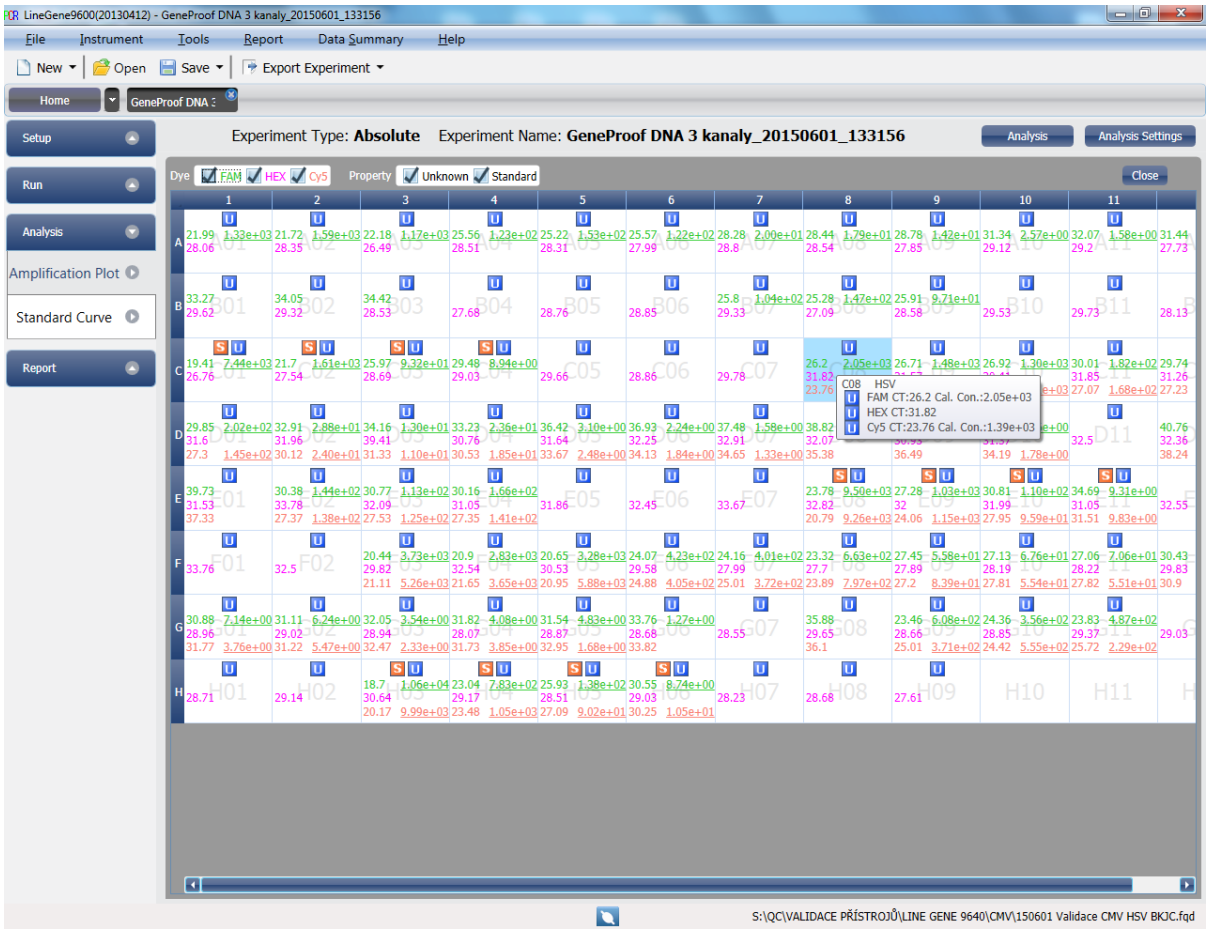


*Fig. 6.3 Result summary*

Perform evaluation, including the virus concentration calculation in copies/ml (IU/ml) according to the Package Insert of the used GeneProof PCR kit.

## 7. Customer Service

**We appreciate all our customers and besides high-quality products we provide superior customer service including the following:**

- provision of free PCR kits samples
- express deliveries
- quick solution of problems related to the supplied products – service guaranteed within 24 hours from the time of announcement
- consultations concerning technological and clinical interpretations

**To assure the quickest possible solution of any problem we always require the GeneProof PCR Kit users to provide the following information:**

- kit name
- problem definition
- kit lot - specified on the kit package
- used device
- file with the examination log from the used device

## 8. Contacts

Support and customer care

Phone: +420 543 211 679
Fax: +420 516 770 824
E-mail: support@geneproof.com

Orders

Phone: +420 543 211 679
Fax: +420 516 770 824
E-mail: sales@geneproof.com