GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Thermo Fisher Scientific

QuantStudio™ 3 / QuantStudio™ 5 Real-Time PCR System

Designed for GeneProof diagnostic kits

See www.geneproof.com for the current kits list

CONTENTS

1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological diagnostics the following devices: QuantStudio 3 Real-Time PCR System and QuantStudio 5 Real-Time PCR System.

1.1. PCR Reaction Preparation

Prepare PCR reaction according to the Instruction for use of the used GeneProof PCR kit.

1.2. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits , or download them from the product site of the used GeneProof PCR kits from the website of the company www.geneproof.com. Save the downloaded templates on your local disc and open them in the QuantStudio™ Design & Analysis Software.

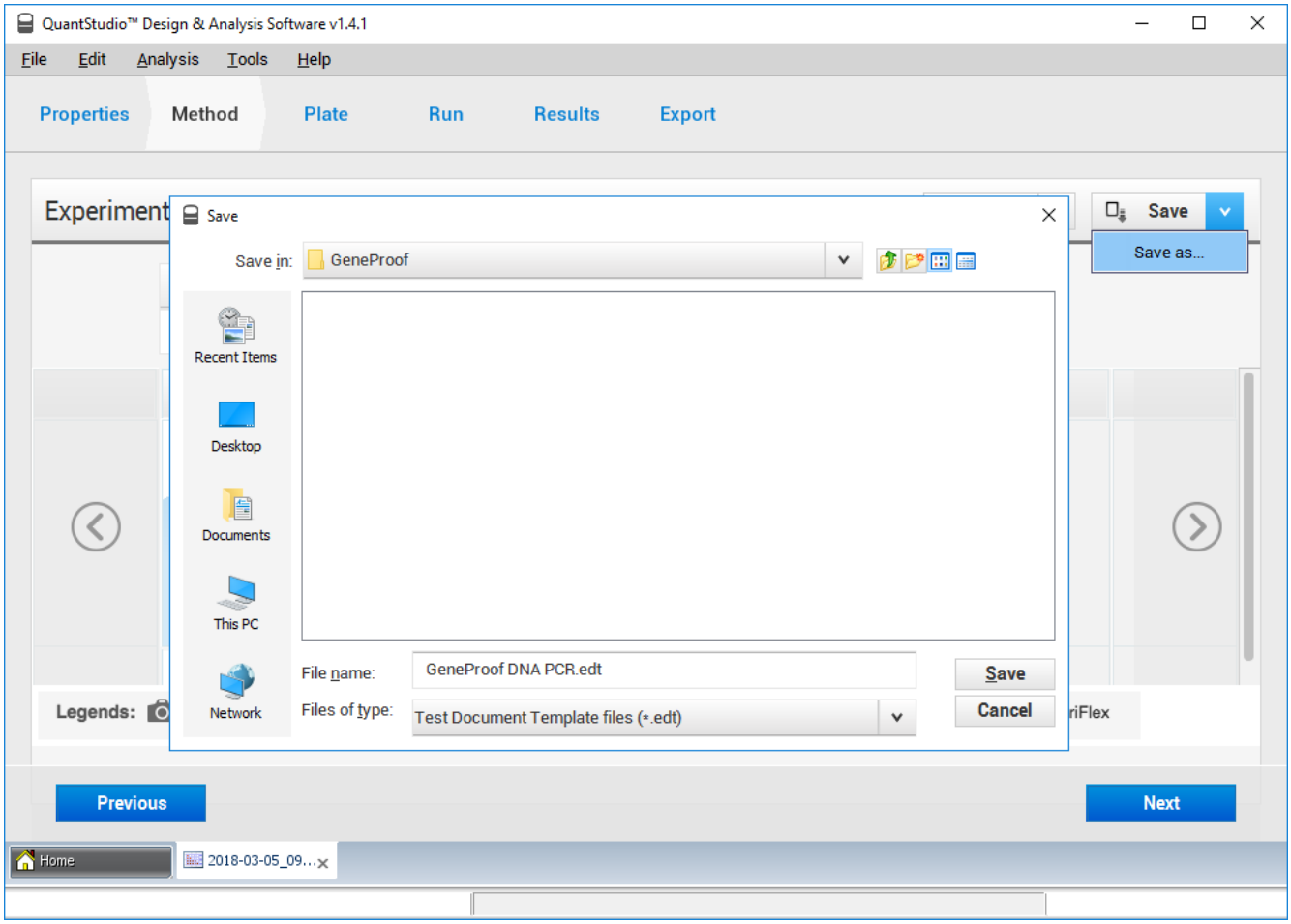


Fig. 1.1 Save template

For easy searching is recommended to create a GeneProof file on the desktop.

1.3. PCR Amplification Start.

1.3.1 Starting the Software

1. Start the **QuantStudio™ Design & Analysis Software**.
2. Click the arrow next to the **Create New Experiment** button and choose **Template**.
3. Open file according to used **GeneProof PCR kit**.

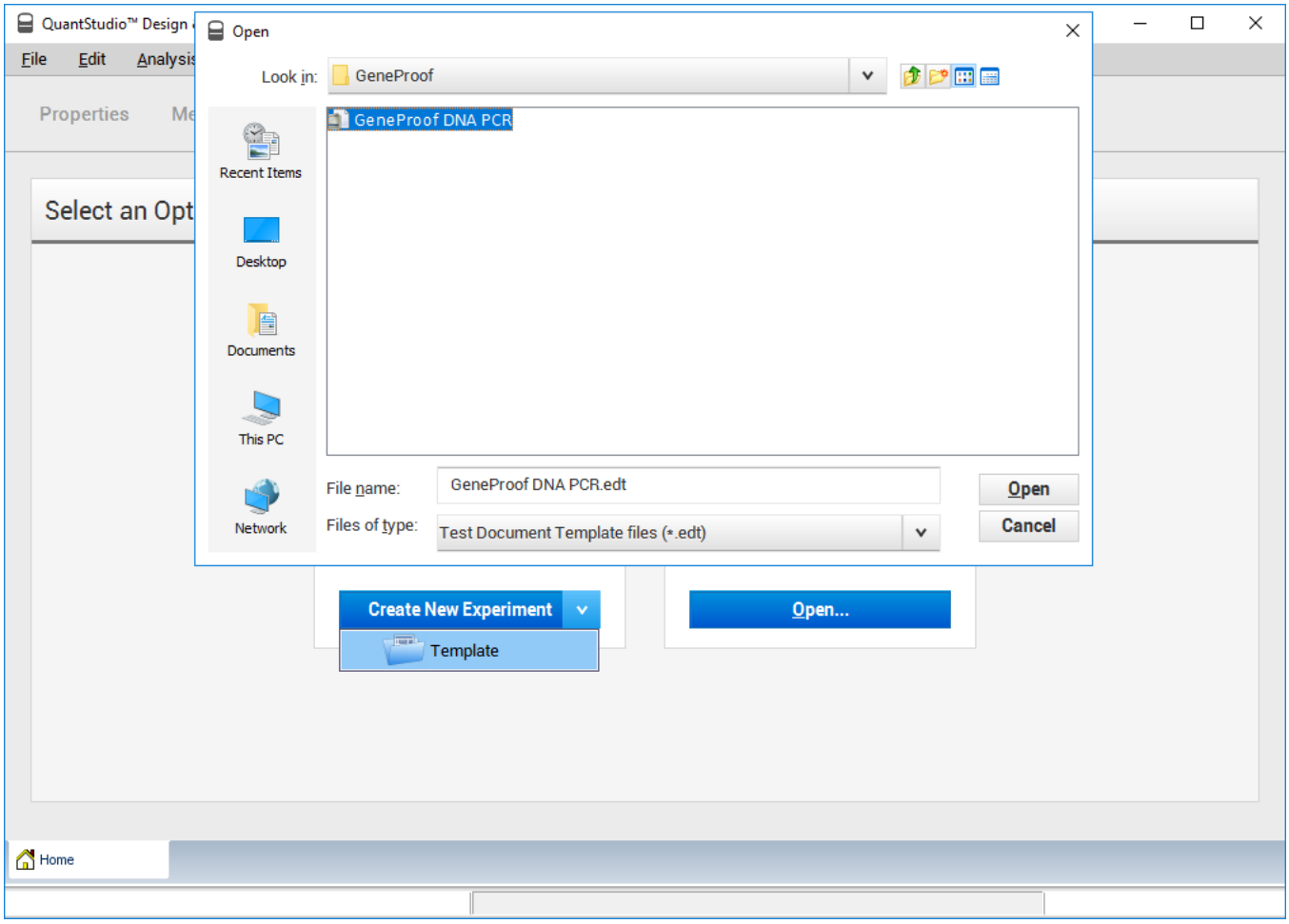


Fig. 1.2 Open template

1.3.2 PCR plate editing

1. In **Properties** tab, enter experiment name into the **Name** row.
2. In **Plate** tab, switch to **Advanced Setup** and use **Add** in **Targets** section to define targets according to the kits used in the experiment.

E.g. for HSV detection (3 channels) set Target Name: **HSV 1**, Reporter: **FAM**, Quencher: **None**; Target Name: **HSV IC**, Reporter: **VIC**, Quencher: **None** and Target Name: **HSV 2**, Reporter: **Cy5**, Quencher: **None**. For MT detection (2 channels) set Target Name: **MT**, Reporter: **FAM**, Quencher: **None** and Target Name: **MT IC**, Reporter: **VIC**, Quencher: **None**. Use **Action -> Save to Library** and **Action -> Import from Library** to save and reuse targets.

3. Assign the appropriate targets for used wells by checking the boxes.
4. For Negative Controls set **N** in the **Task** column of targets.
5. For calibrators (in the case of quantitative detection) set **S** in the **Task** column of pathogen target and enter the corresponding quantity in the **Quantity** column – according to the Package Insert of the used GeneProof PCR kit, e.g.: 10 000, 1 000, 100 a 10.

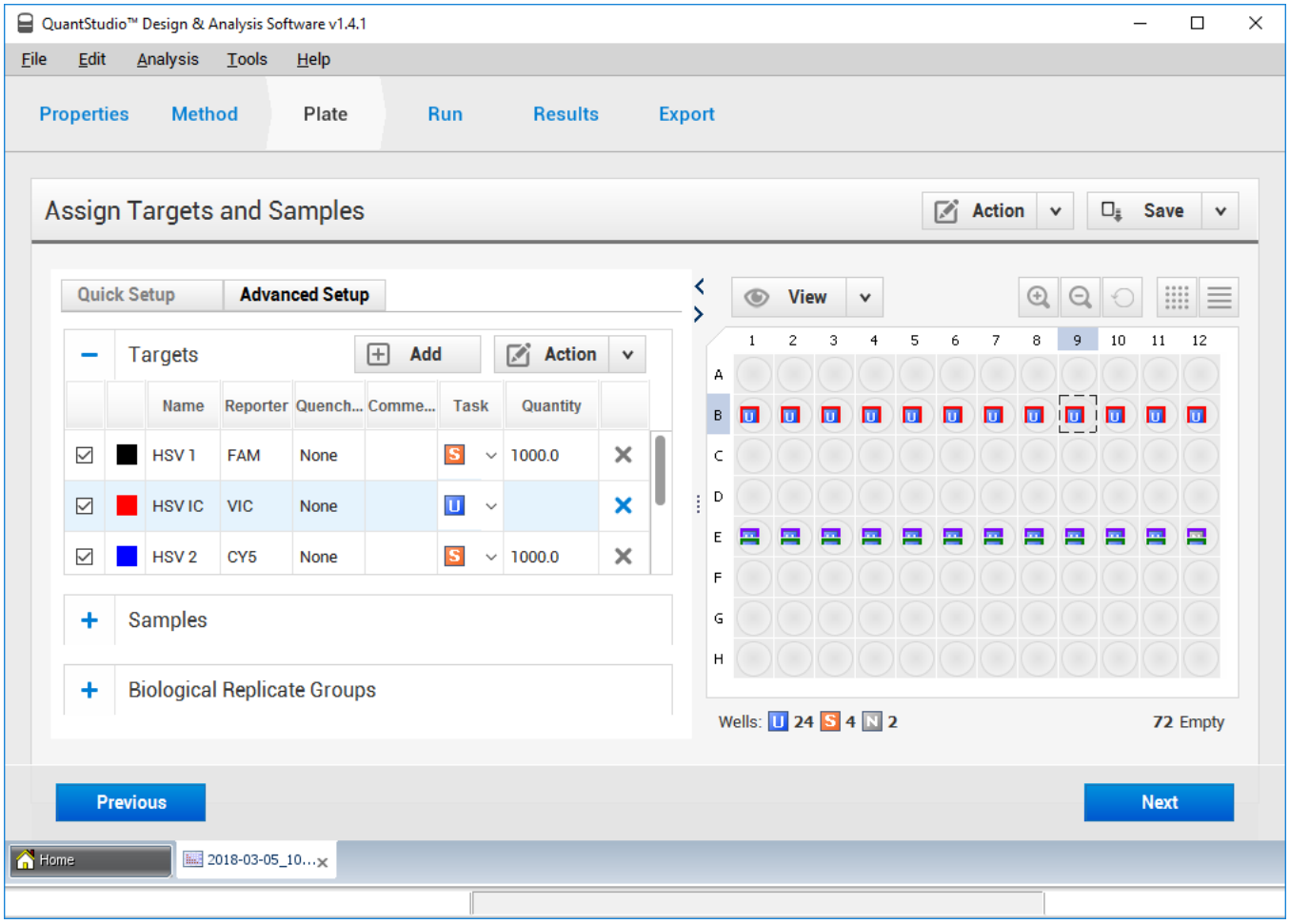


Fig. 1.3 Assign targets

6. Use **Add** in **Samples** section to define the samples used in the experiment.
7. In case of qualitative detection define positive control as the sample, e.g. **Positive Control MT**.
8. Assign the appropriate samples and controls for used wells by checking the boxes.

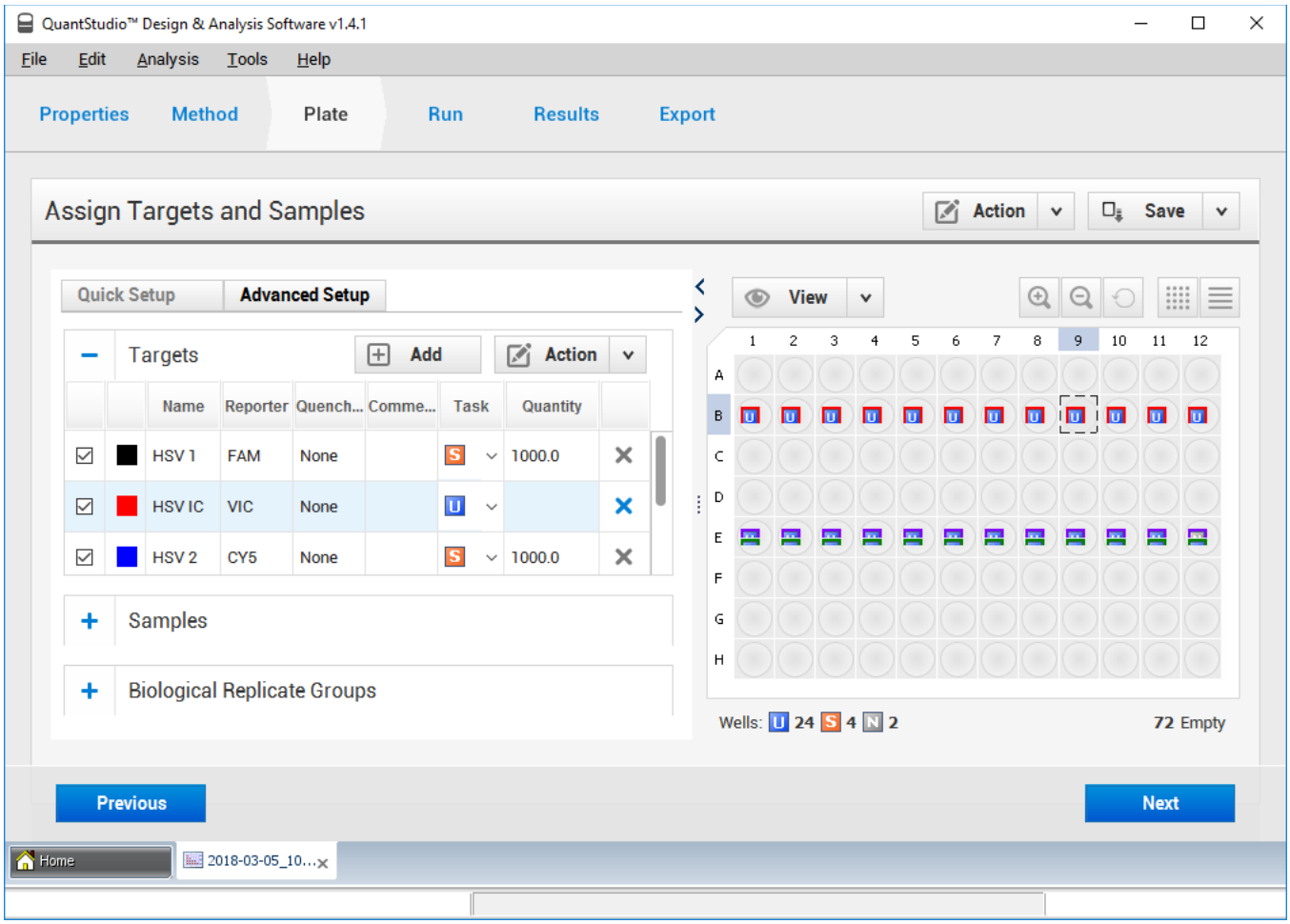


Fig. 1.4 Assign samples

1.3.3 Starting the experiment

Save the experiment before starting the device.

1. Select **File** in the main menu, click **Save** and save the created experiment as the **Test Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments** folder.

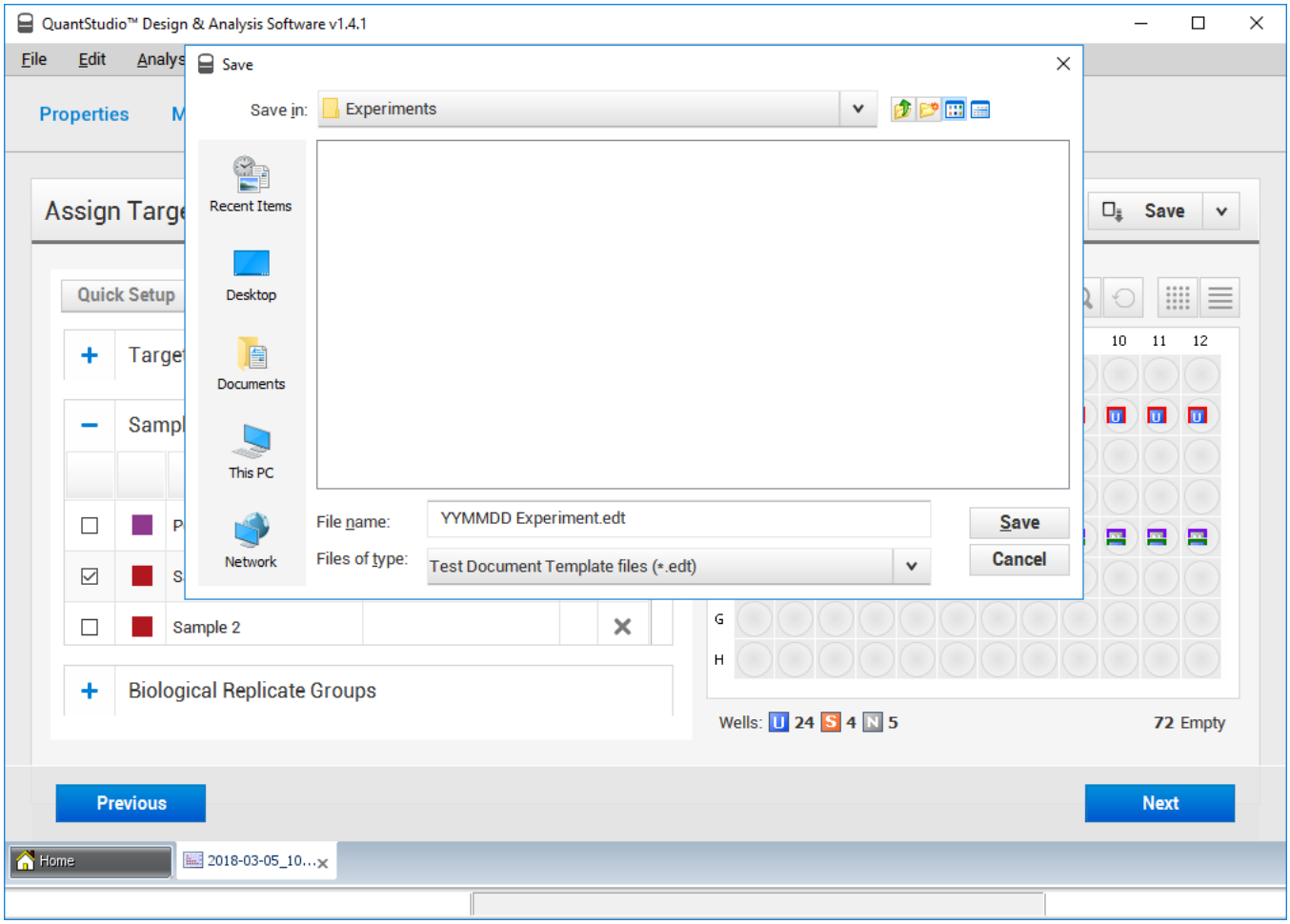


Fig. 1.5 Save experiment

2. In **Run** tab click  button to start the experiment.

1.4. Qualitative analysis of the result and evaluation of detection

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 experiment is finished, **Amplification Plot** is displayed.

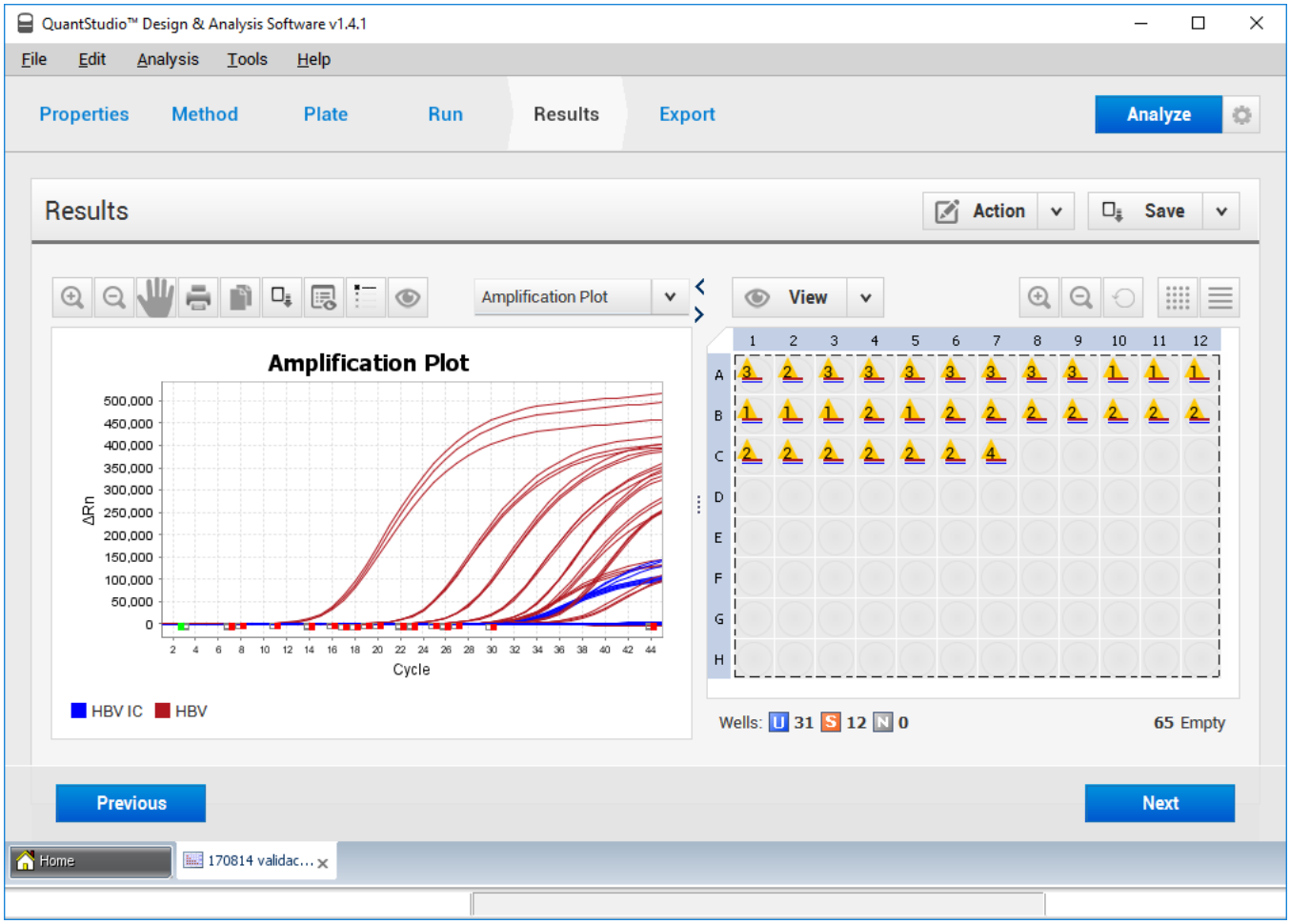


Fig.1.6 Amplification curves

1.4.4 Analysis settings

1. Open  **Analysis Settings**.
2. Uncheck **Default Settings** for all targets.
3. Uncheck **Automatic Threshold** and leave original value.
4. Uncheck **Automatic Baseline** and leave **Start Cycle 3** and **End Cycle 15**.
5. Click **Apply** to confirm.

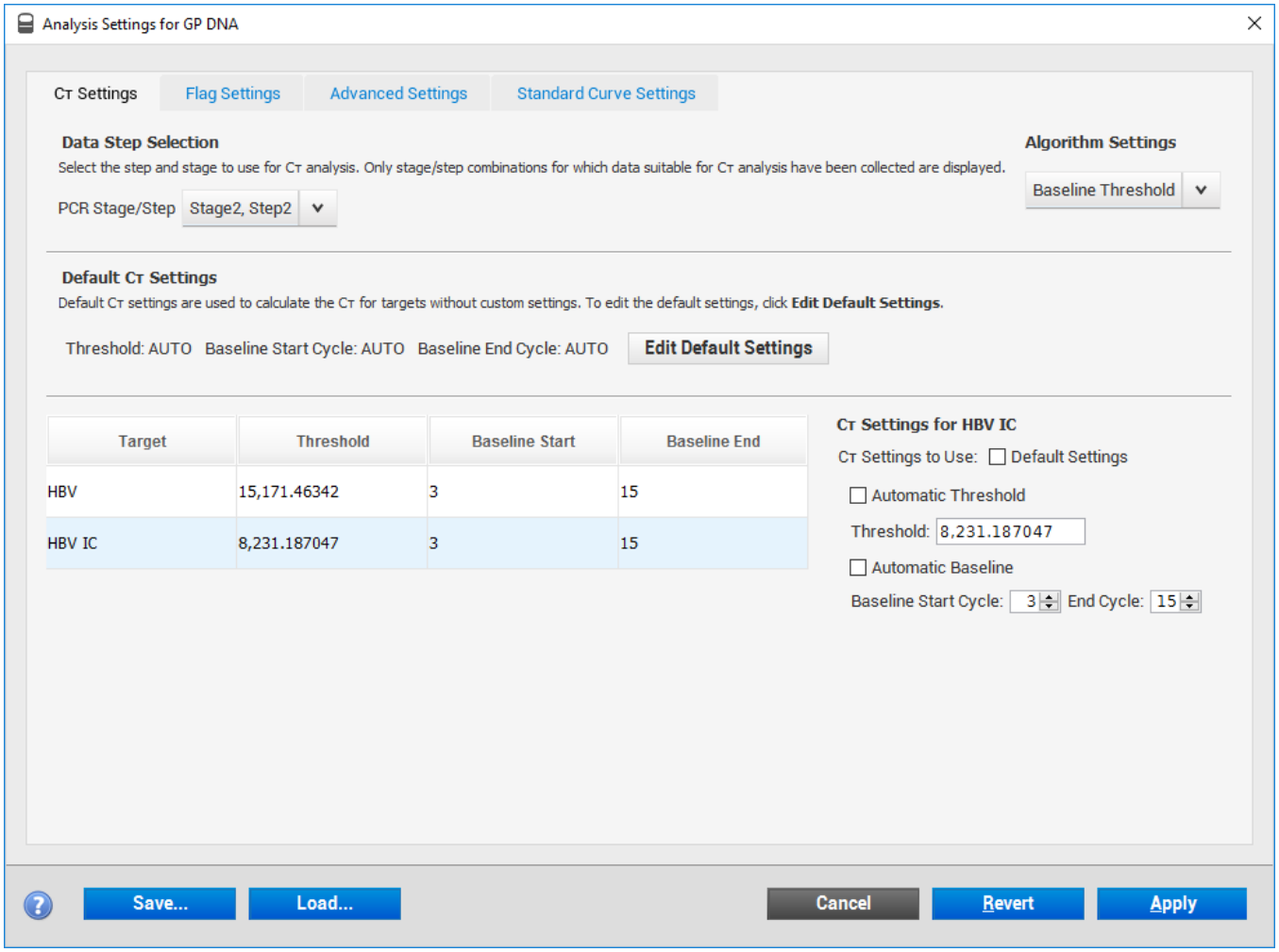


Fig.1.7 Analysis settings

1.4.5 Detection analysis of the studied microorganism

1. In  **Show Plot Settings** select the target microorganism (e.g. HBV) and select Graph Type **Log.**
2. Move the Threshold line just above the reaction basal noise.

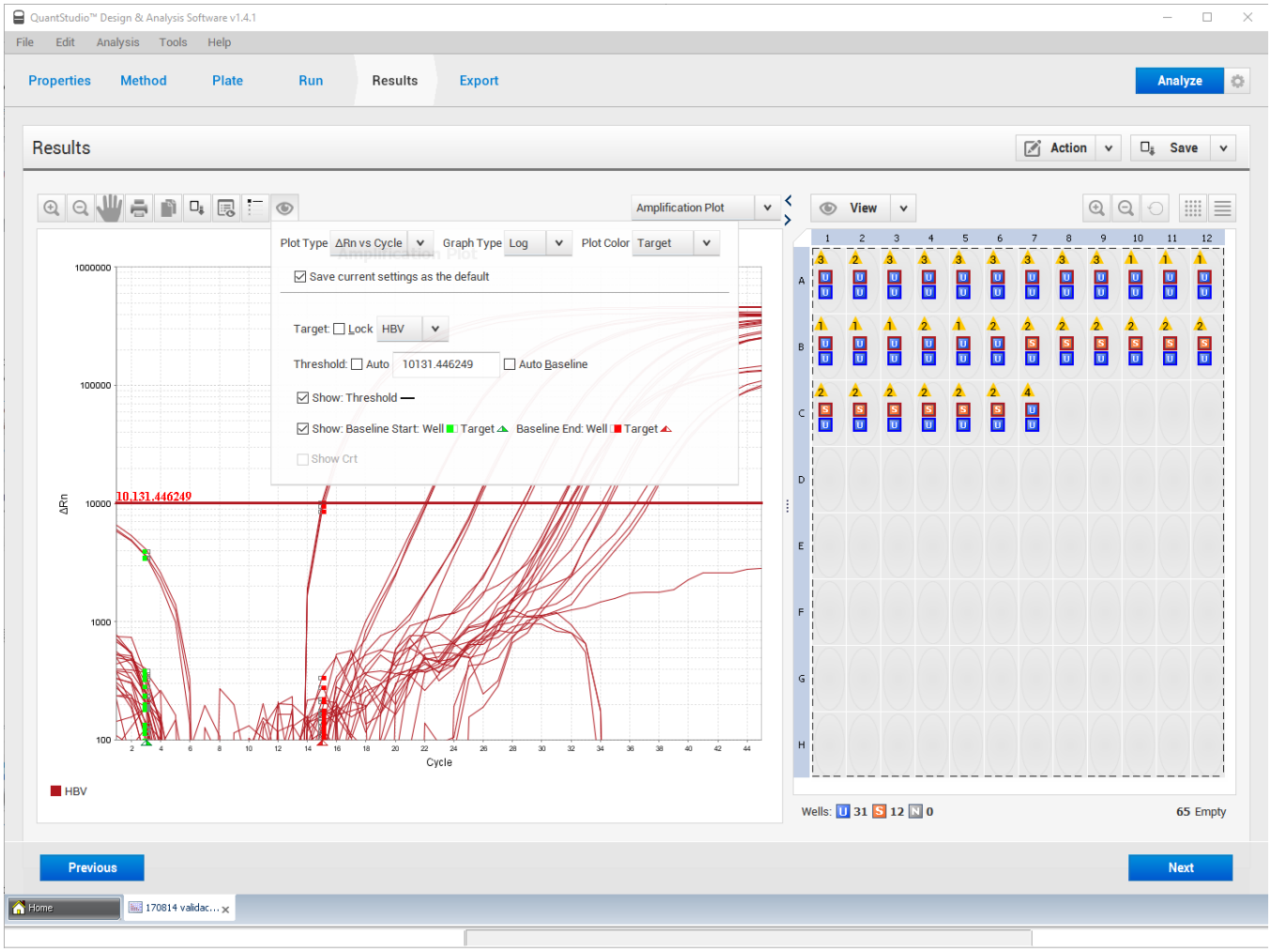


Fig.1.8 Target and threshold set up

3. In  **Show Plot Settings** select Graph Type **Linear.**

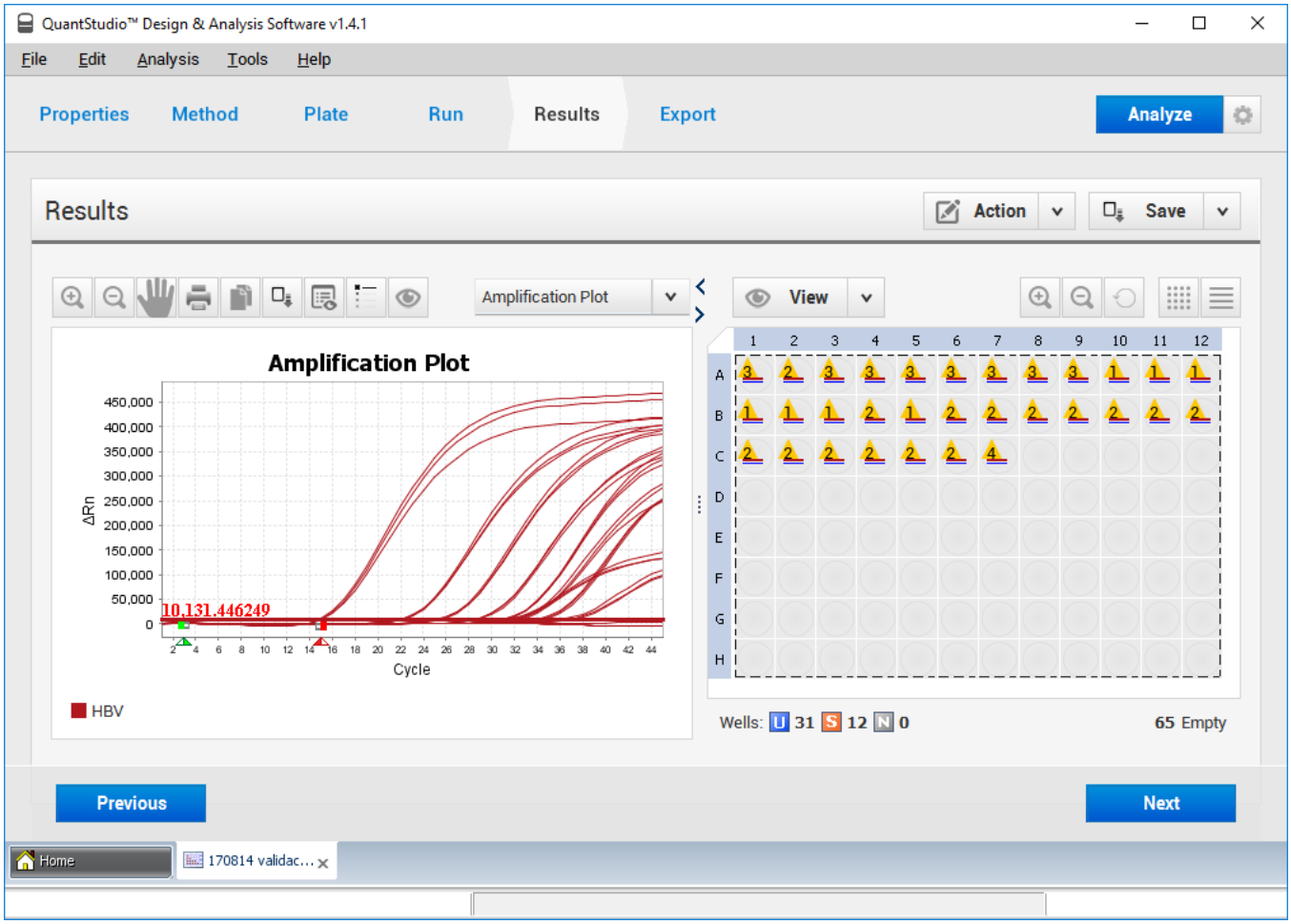


Fig. 1.9 Amplification curves of the studied microorganism in linear scale

In the case of multiplex kit, follow the instructions for all the studied microorganisms.

Use  button to switch to the Results table for **Ct** values.

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.

1.4.6 Internal Standard detection analysis

1. In  **Show Plot Settings** select the internal standard (e.g. HBV IC) and select Graph Type **Log.**
2. Move the Threshold line just above the reaction basal noise.

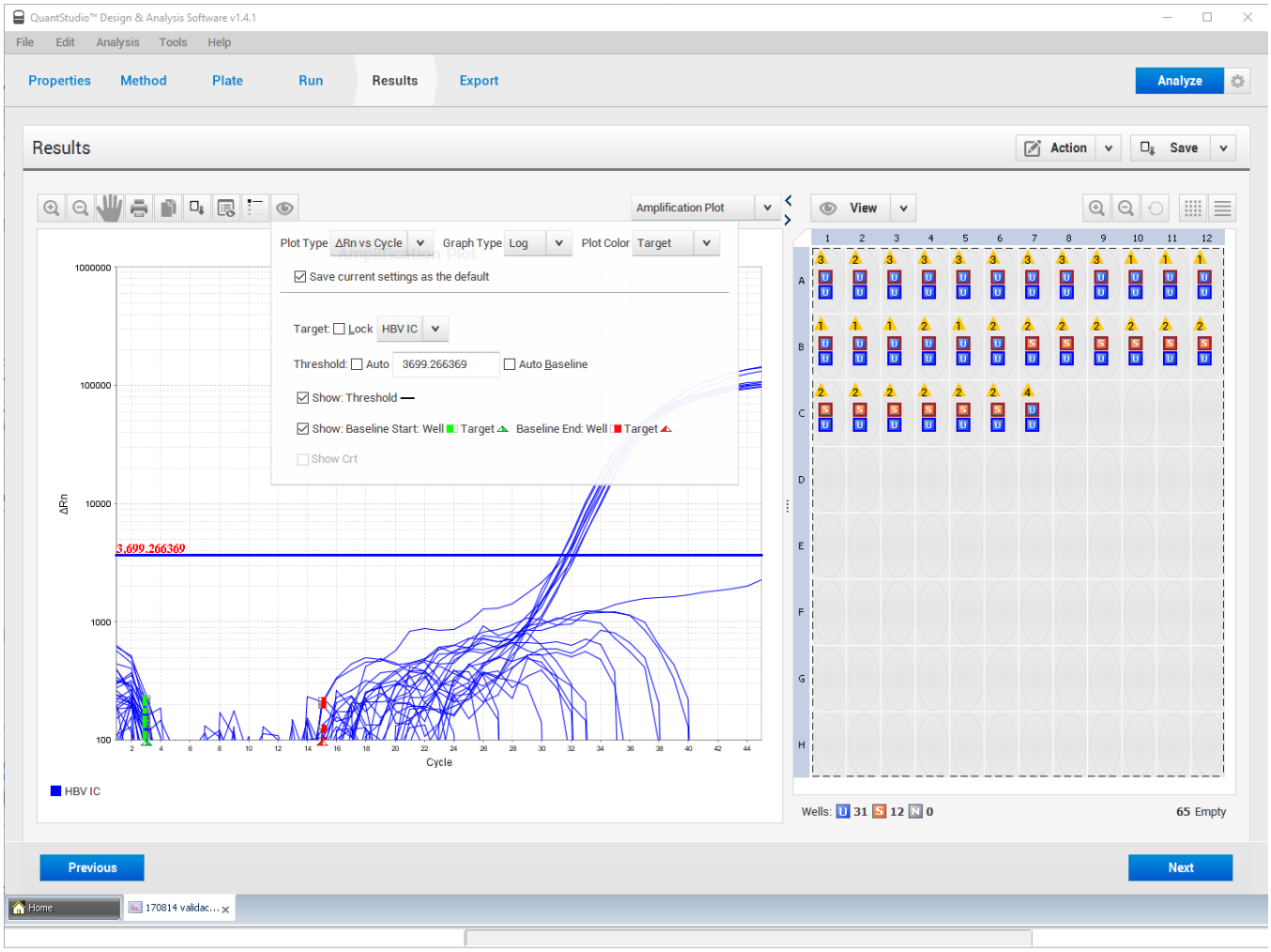


Fig. 1.10 Internal standard and threshold setting

3. In  **Show Plot Settings** select Graph Type **Linear.**

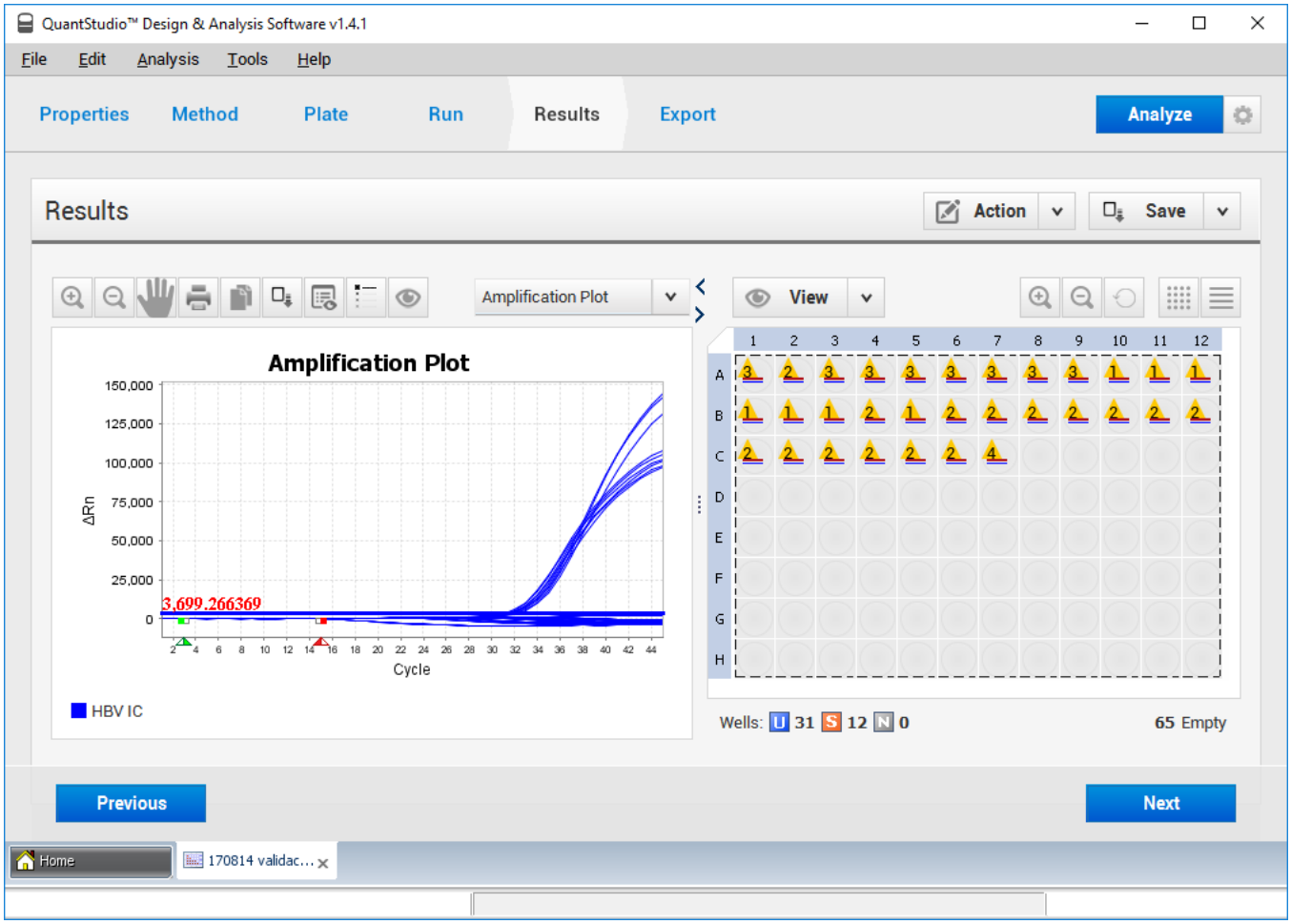


Fig. 1.11 Amplification curves of the internal standard in linear scale

Use  button to switch to the Results table for **Ct** values.

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.

1.5. Result quantitative analysis and detection evaluation

1. In Standard Curve, evaluate the calibration quality. The **R^2** parameter in a well-performed calibration achieves a minimum value of 0.98 or higher. If the **R^2** parameter is lower than 0.98, move the **Threshold** and repeat the analysis.

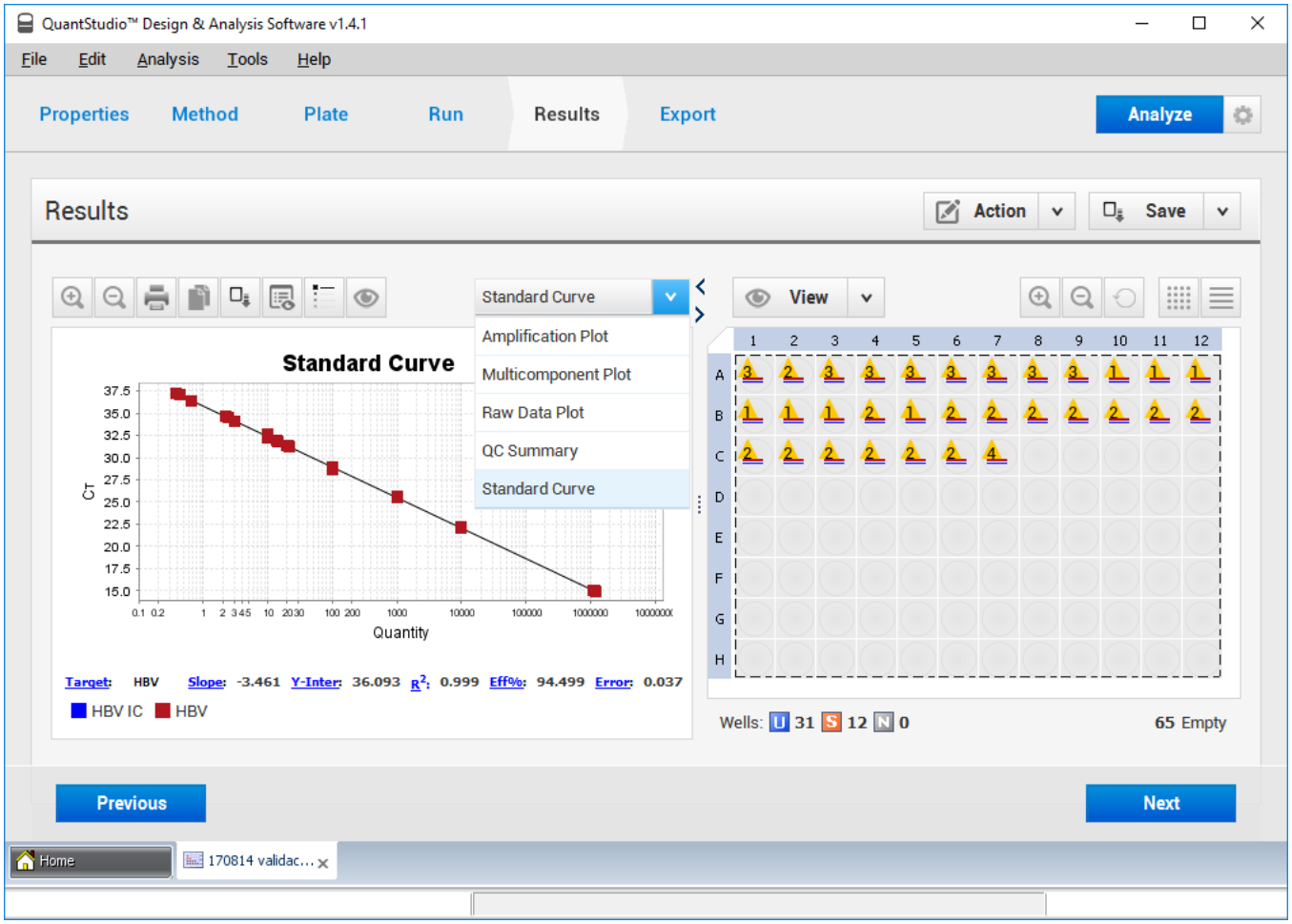


Fig. 1.12 Calibration curve

2. Use  button to switch to the Results table. Concentrations of positive samples are displayed in the **Quantity** column of the table.

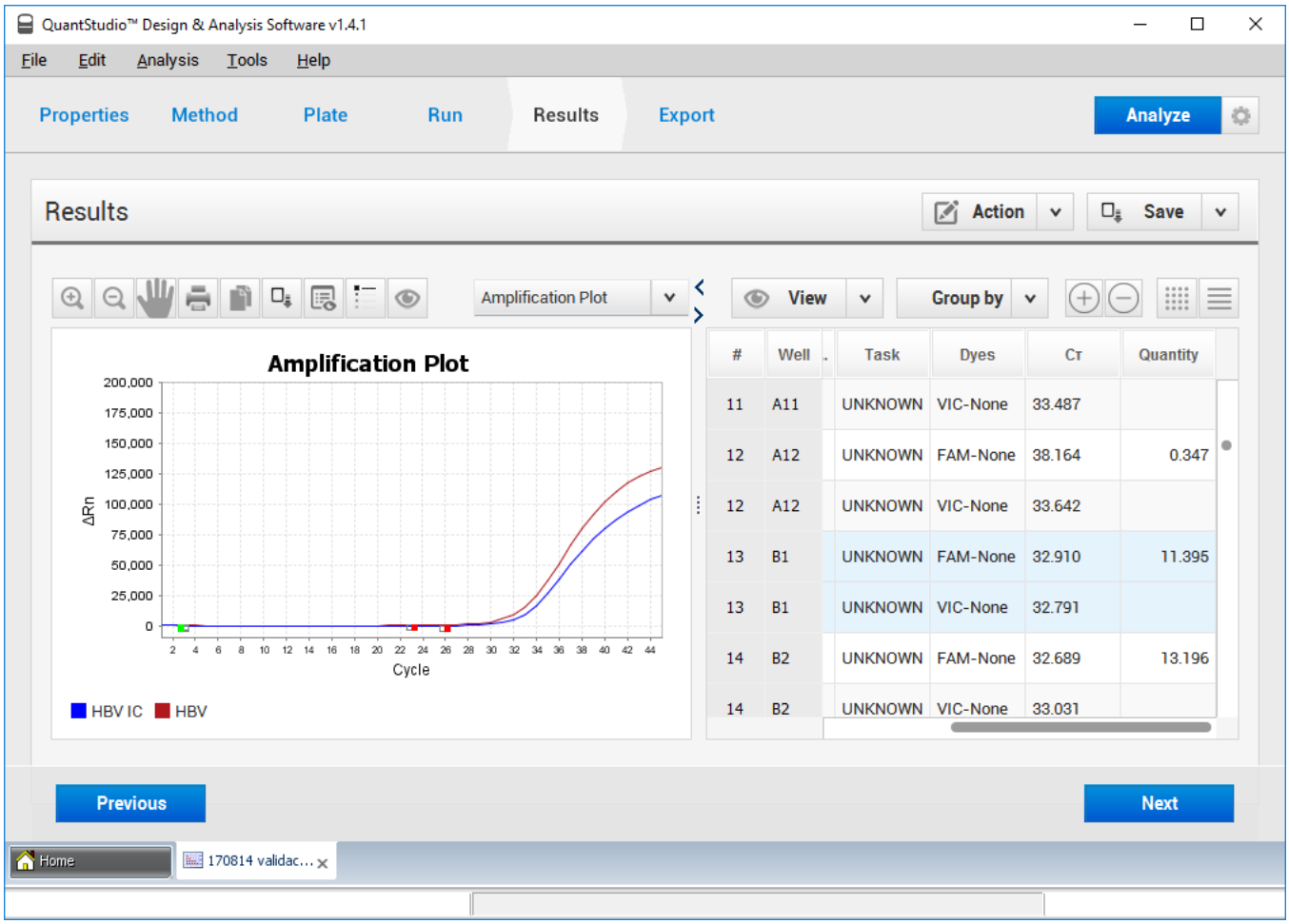


Fig. 1.13 Results table

Perform evaluation, including the virus concentration calculation according to the Instruction for use of the used GeneProof PCR kit.

2. Genetical diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the the following devices: QuantStudio 3 Real-Time PCR System a QuantStudio 5 Real-Time PCR System.

2.1. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits, or download them from the product site of the used GeneProof PCR kits from the website of the company www.geneproof.com.
Save the downloaded templates on your local disc and open them in the QuantStudio™ Design & Analysis Software.

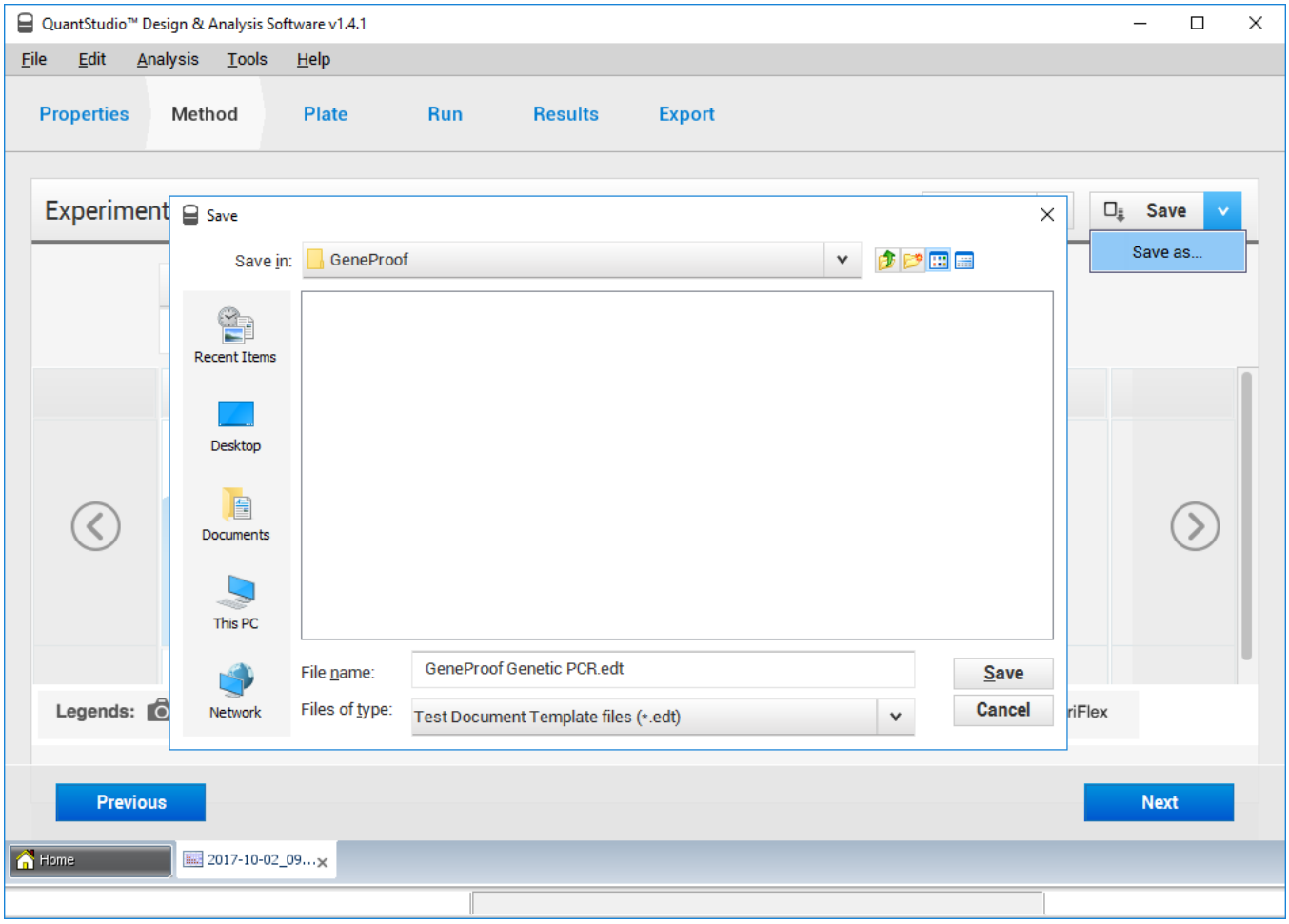


Fig. 2.1 Save template

After saving, the template can be opened from the file GeneProof. With each next usage of GeneProof PCR kits continue from the chapter 2.2 Starting the software.

2.2. Starting the software

2.2.1 Opening of the saved template

1. Start the **QuantStudio™ Design & Analysis Software**.
2. Click the arrow next to the **Create New Experiment** button and choose **Template**.
3. Open file according to used **GeneProof PCR kit**.

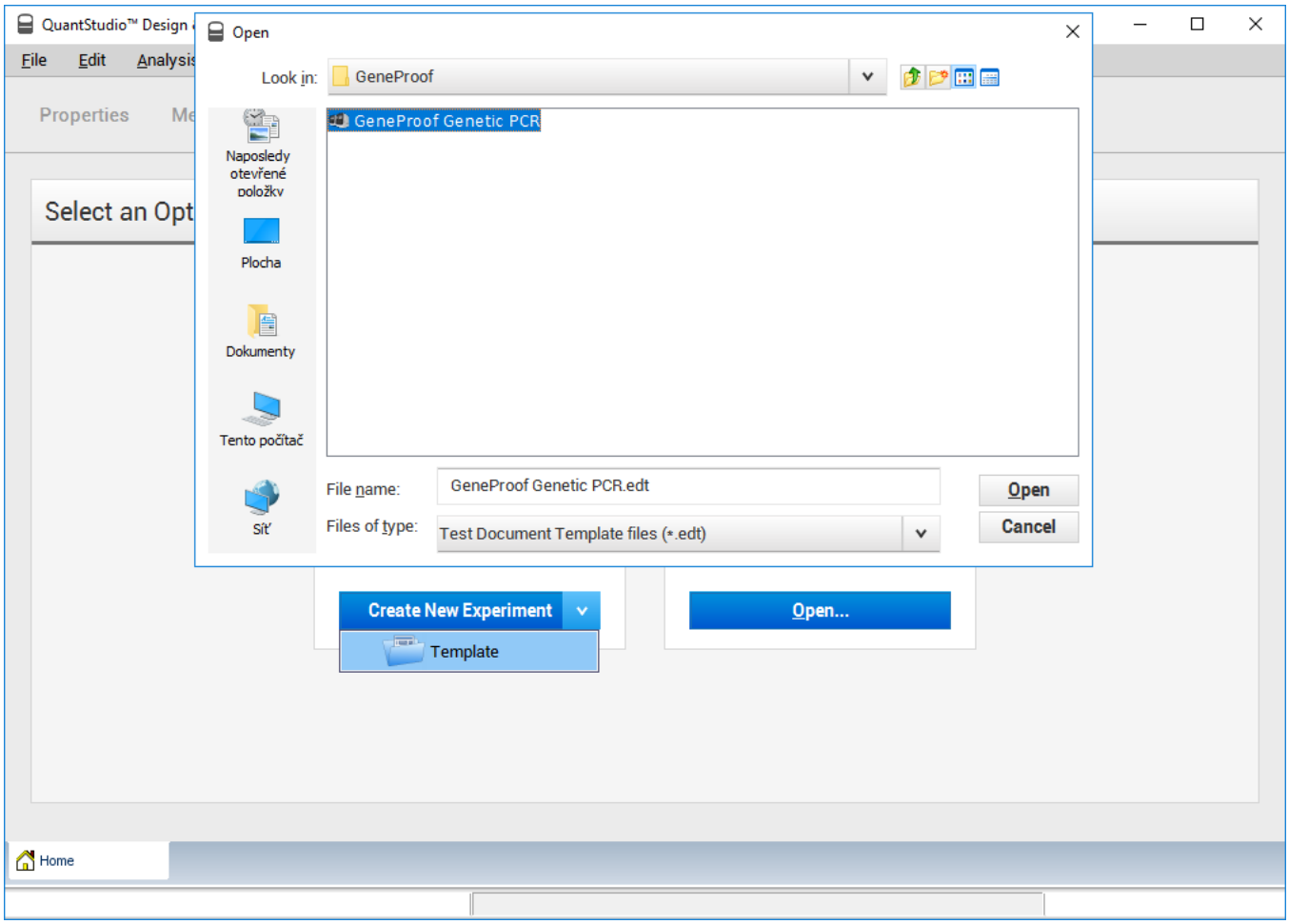


Fig. 2.2 Open template

2.2.2 PCR plate editing

1. In **Properties** tab, enter experiment name into the **Name** row.
2. In **Plate** tab, switch to **Advanced Setup** and use **Add** in **Targets** section to define targets according to the kits used in the experiment.

E.g. for FII detection set Target Name: **FII WT**, Reporter: **FAM**, Quencher: **None** and Target Name: **FII MUT**, Reporter: **VIC**, Quencher: **None**. For FV detection set Target Name: **FV WT**, Reporter: **FAM**, Quencher: **None** and Target Name: **FV MUT**, Reporter: **VIC**, Quencher: **None**. Use **Action -> Save to Library** and **Action -> Import from Library** to save and reuse targets.

3. Assign the appropriate targets for used wells by checking the boxes.
4. For Negative Controls set **N** in the **Task** column of targets.

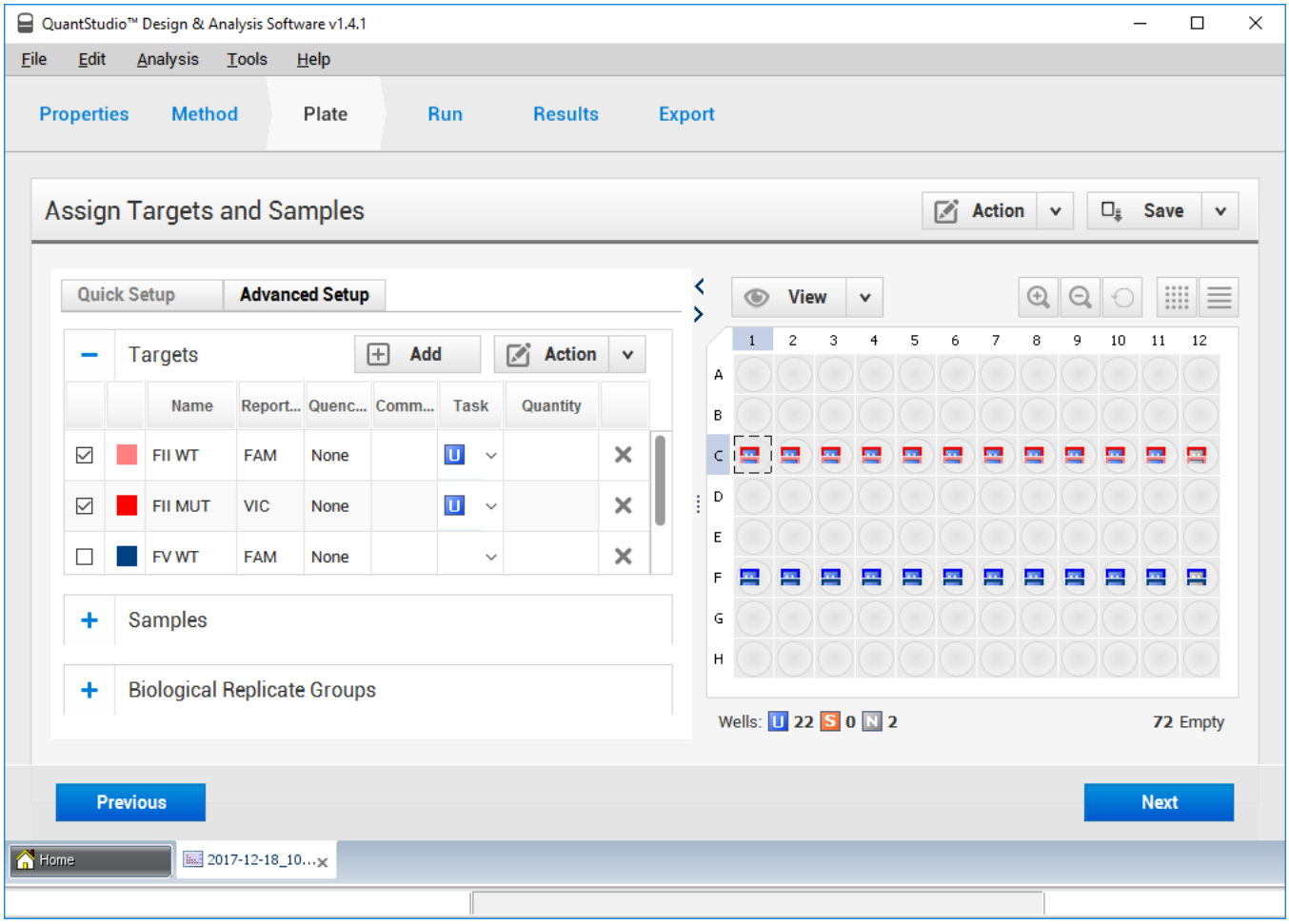


Fig. 2.3 Assign targets

5. Use **Add** in **Samples** section to define the samples used in the experiment.
6. Define positive controls as the samples, e.g. **Positive Control WT**, **Positive Control MUT** and **Positive Control HET**.
7. Assign the appropriate samples and controls for used wells by checking the boxes.

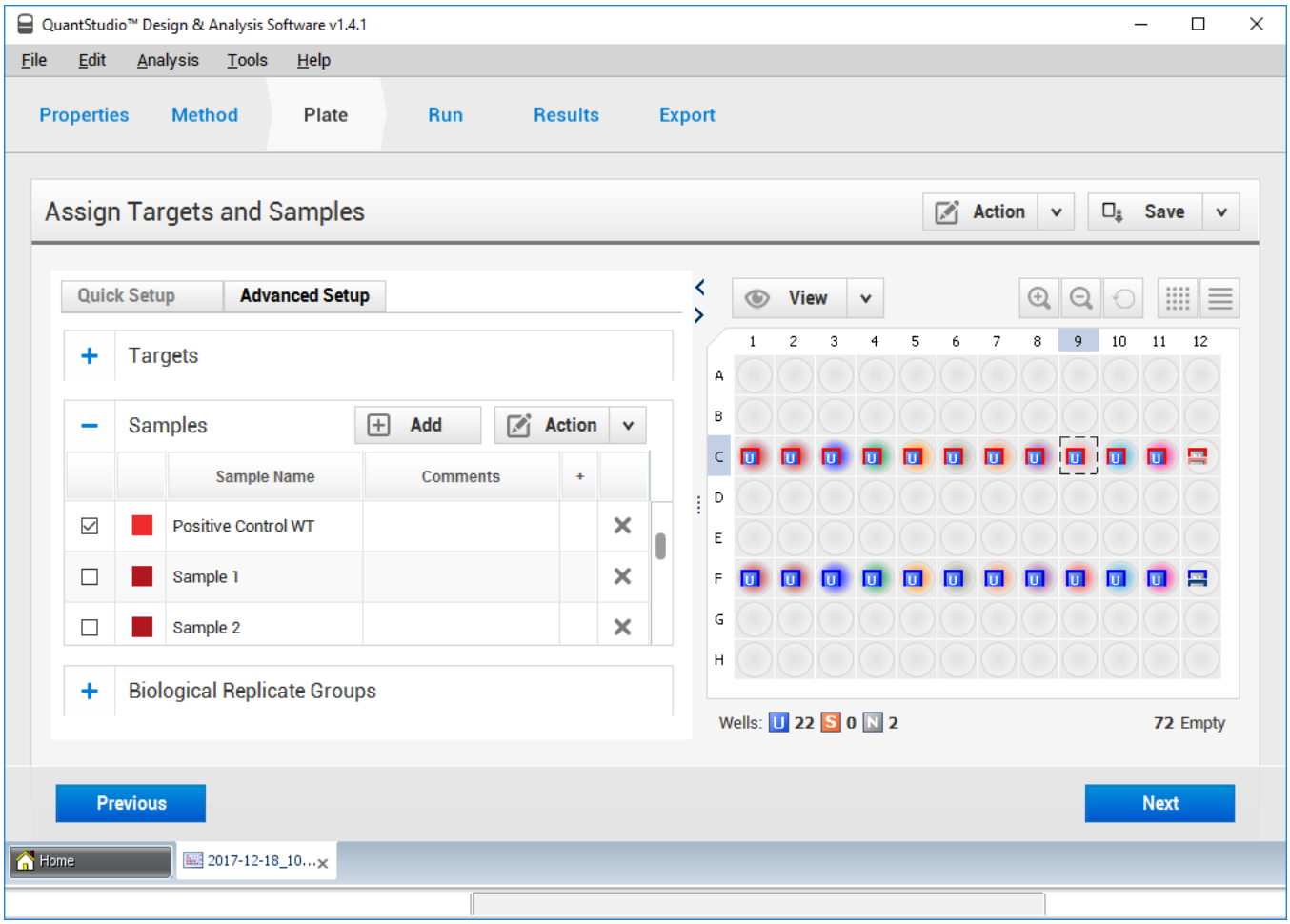


Fig. 2.4 Assign samples

2.2.3 Starting the experiment

Save the experiment before starting the device.

1. Select **File** in the main menu, click **Save** and save the created experiment as the **Test Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments** folder.

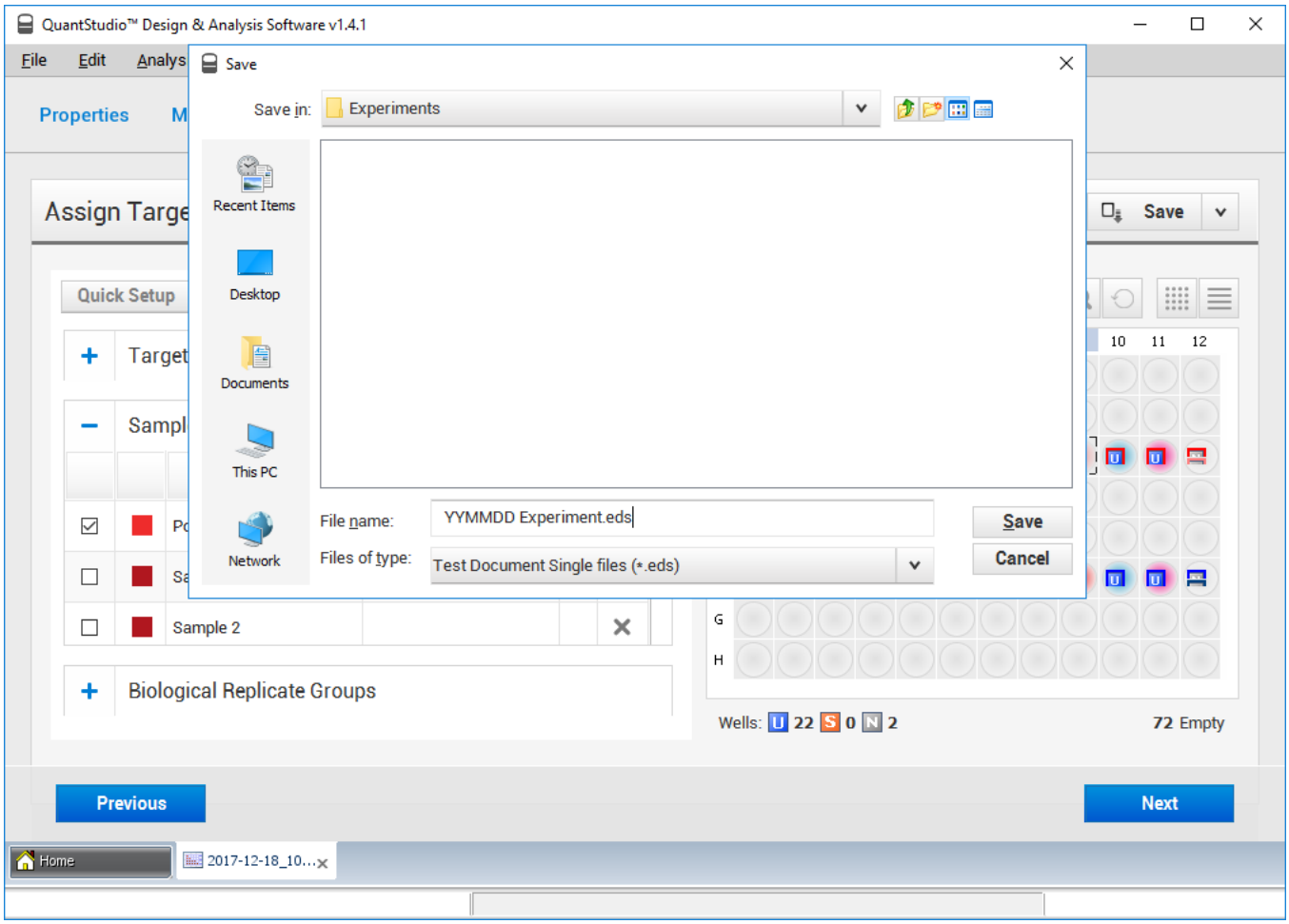


Fig. 2.5 Save experiment

2. In **Run** tab click  button to start the experiment.

2.3. Analysis of the result and evaluation of detection

When the experiment is finished, **Amplification Plot** is displayed.

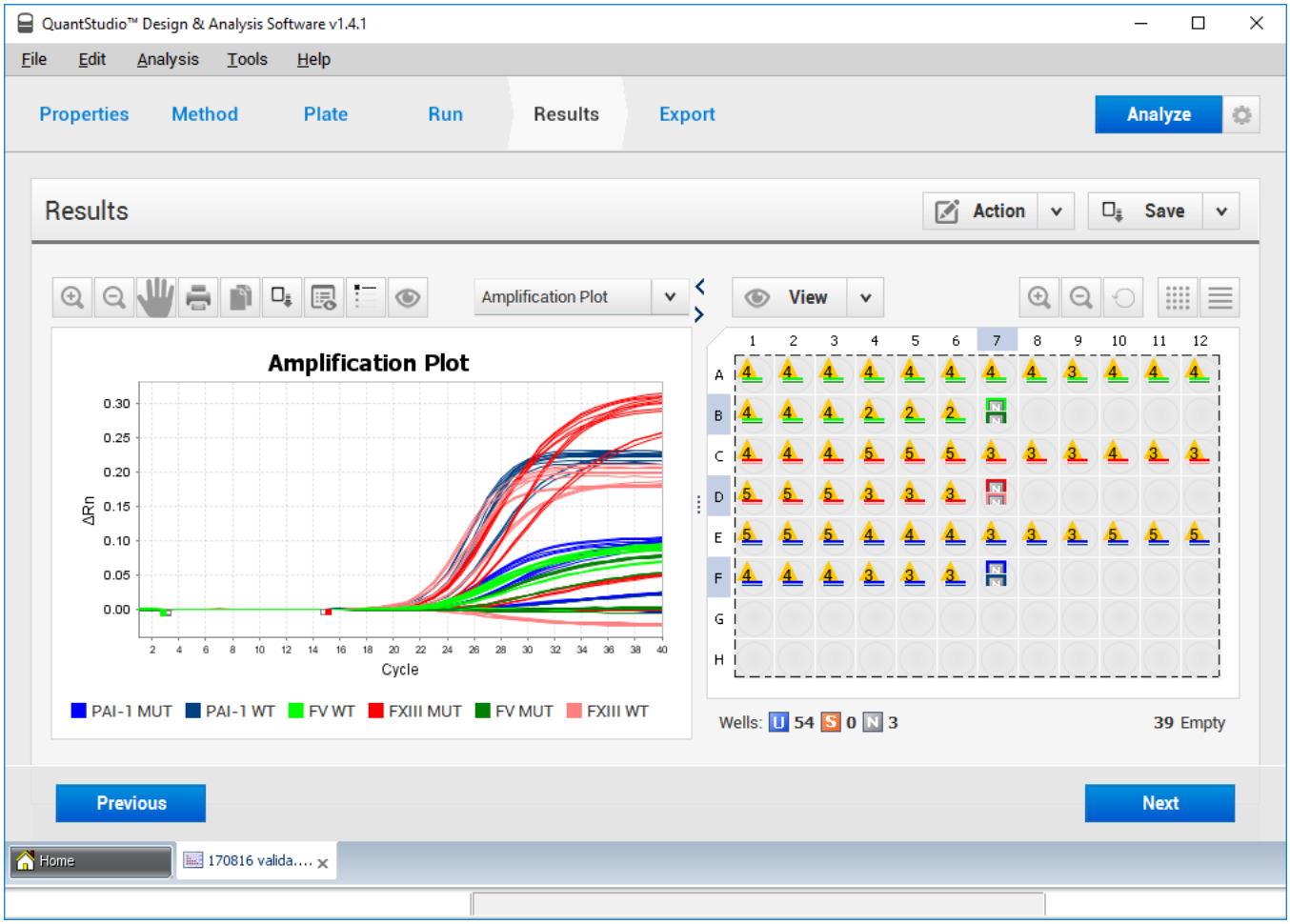


Fig. 2.6 Amplification curves

2.3.4 Analysis settings

1. Open  **Analysis Settings**.
2. Uncheck **Default Settings** for all targets.
3. Uncheck **Automatic Threshold** and leave original value.
4. Uncheck **Automatic Baseline** and leave **Start Cycle 3** and **End Cycle 15**.
5. Click **Apply** to confirm.

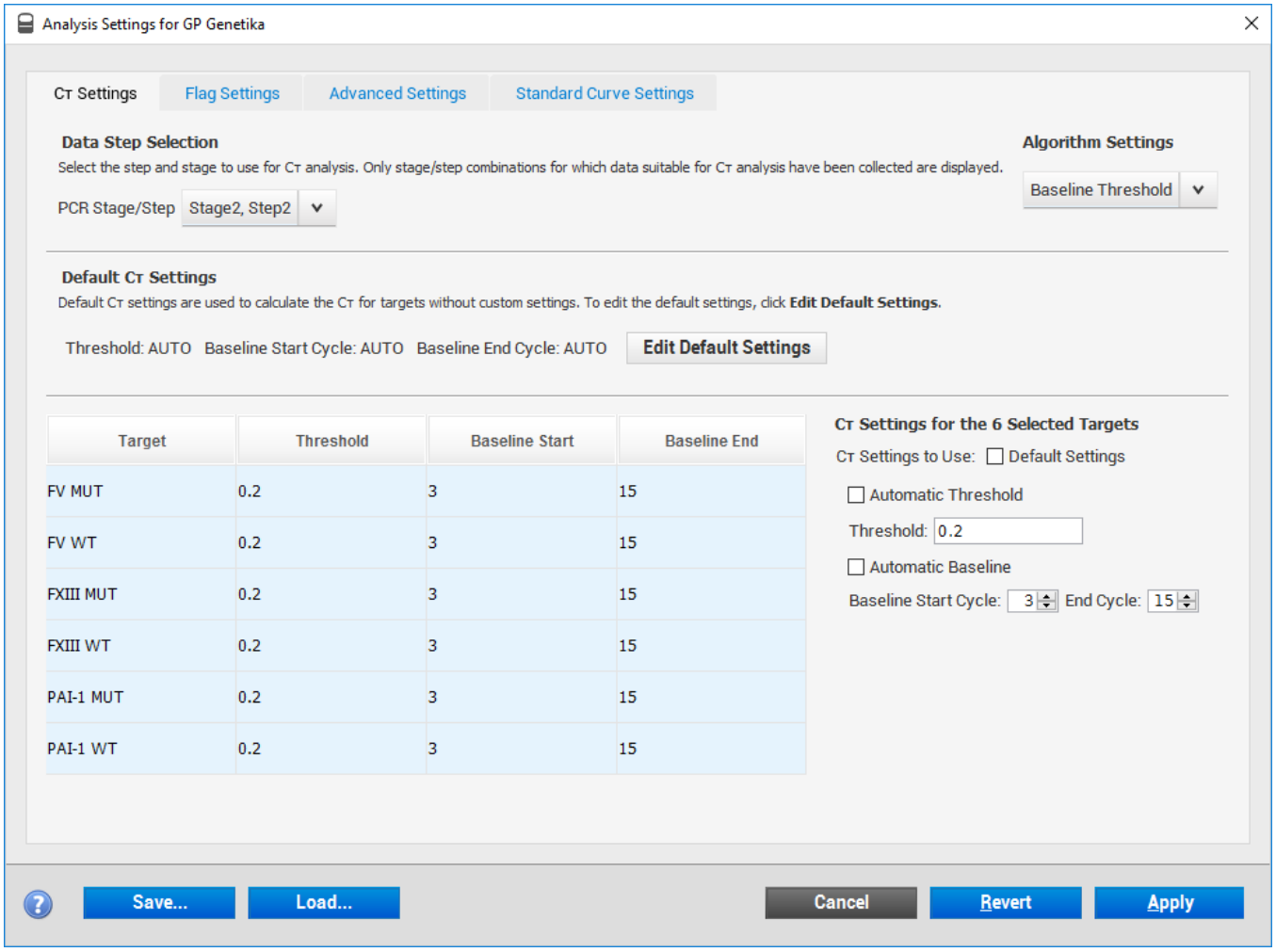


Fig. 2.7 Analysis settings

6. In the plate, select only positive controls WT, MUT and HET of one evaluated kit (e.g. FXIII).

7. In  **Show Plot Settings** select **WT** target of evaluated kit (e.g. FXIII WT).

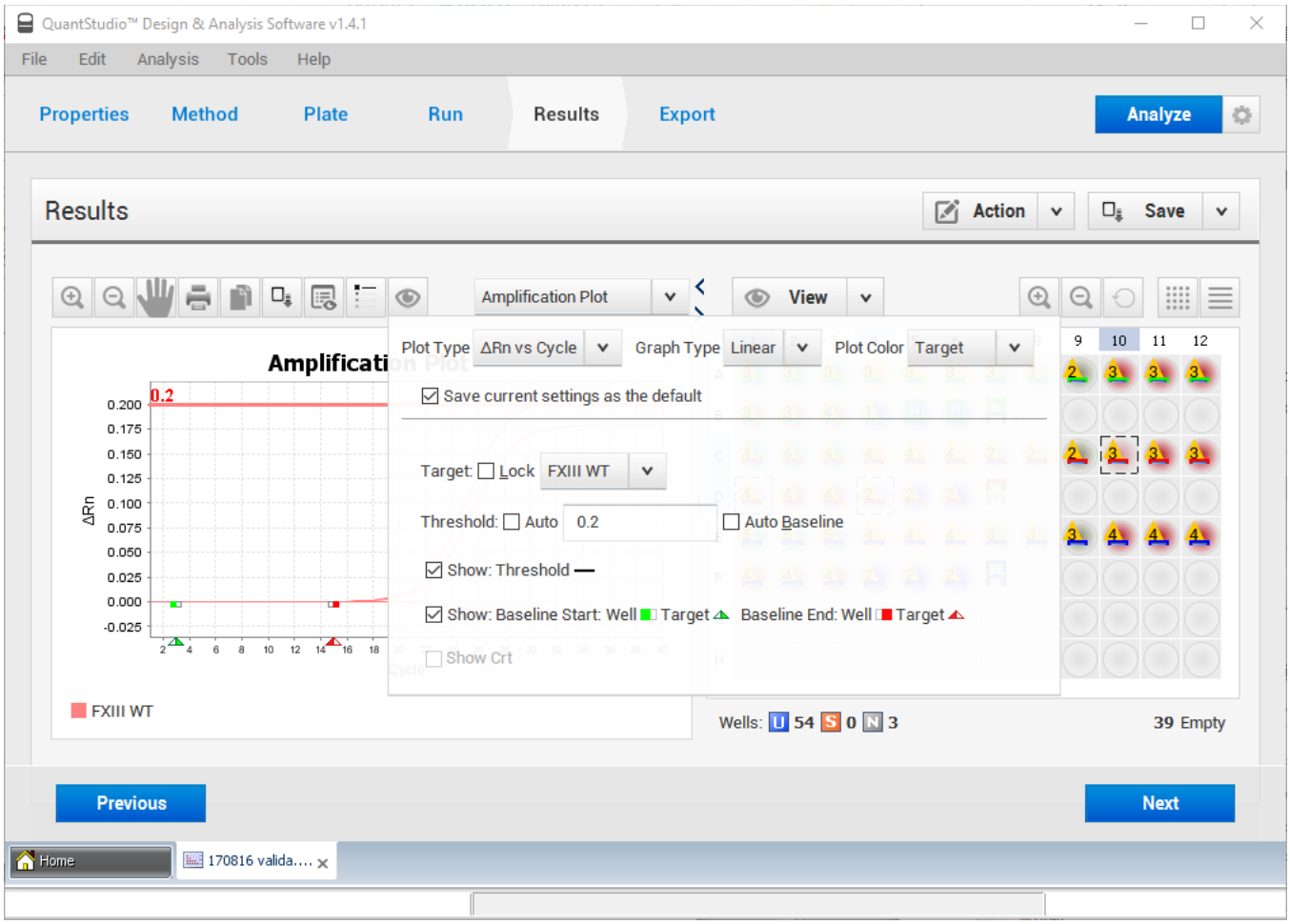


Fig. 2.8 Select WT target

8. Move the Threshold line so that only controls **WT** and **HET** are positive (the Threshold line intersects only the Positive Control WT and Positive Control HET curves).

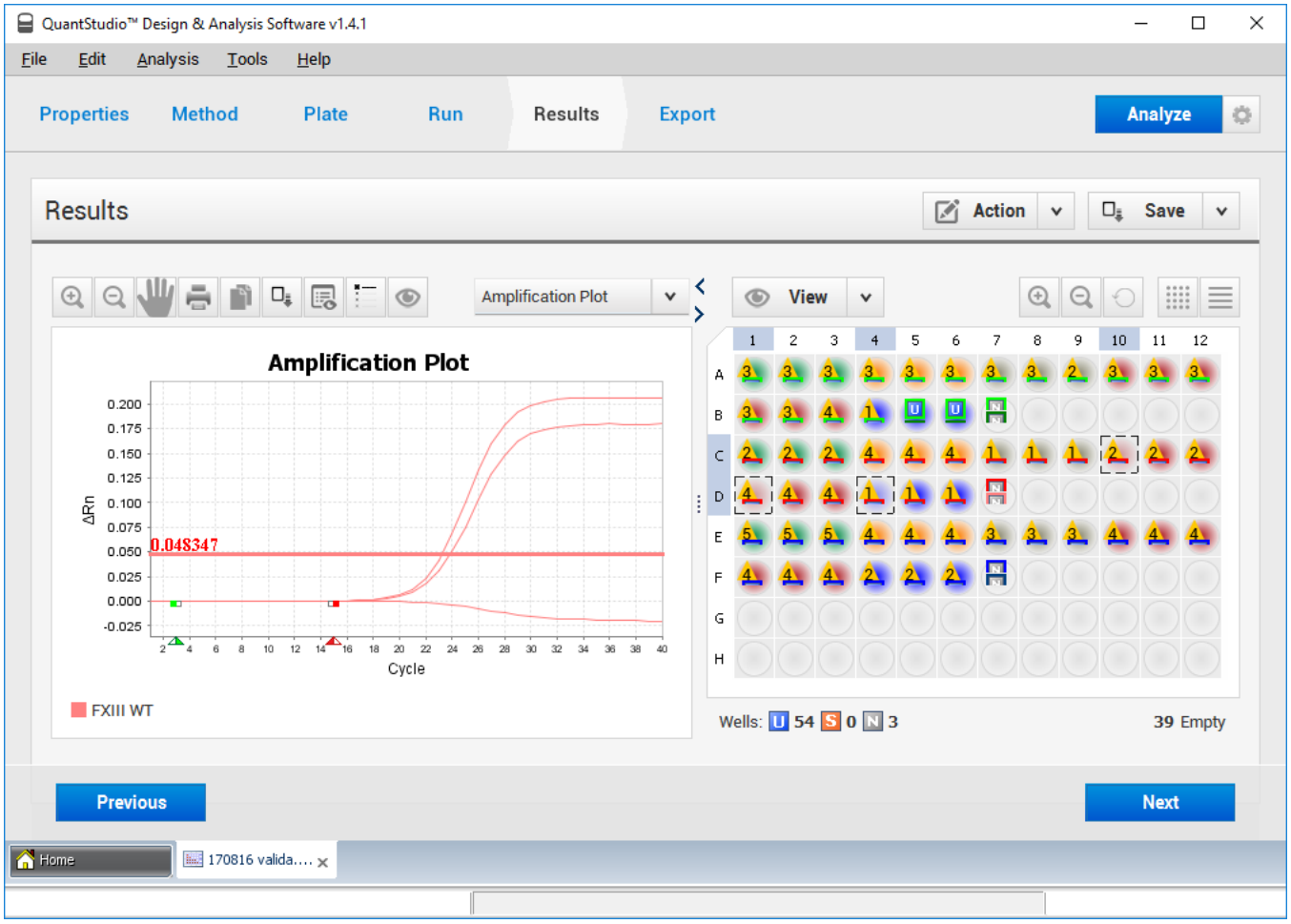


Fig. 2.9 WT Threshold settings

9. In  **Show Plot Settings** select **MUT** target of evaluated kit (e.g. FXIII MUT).

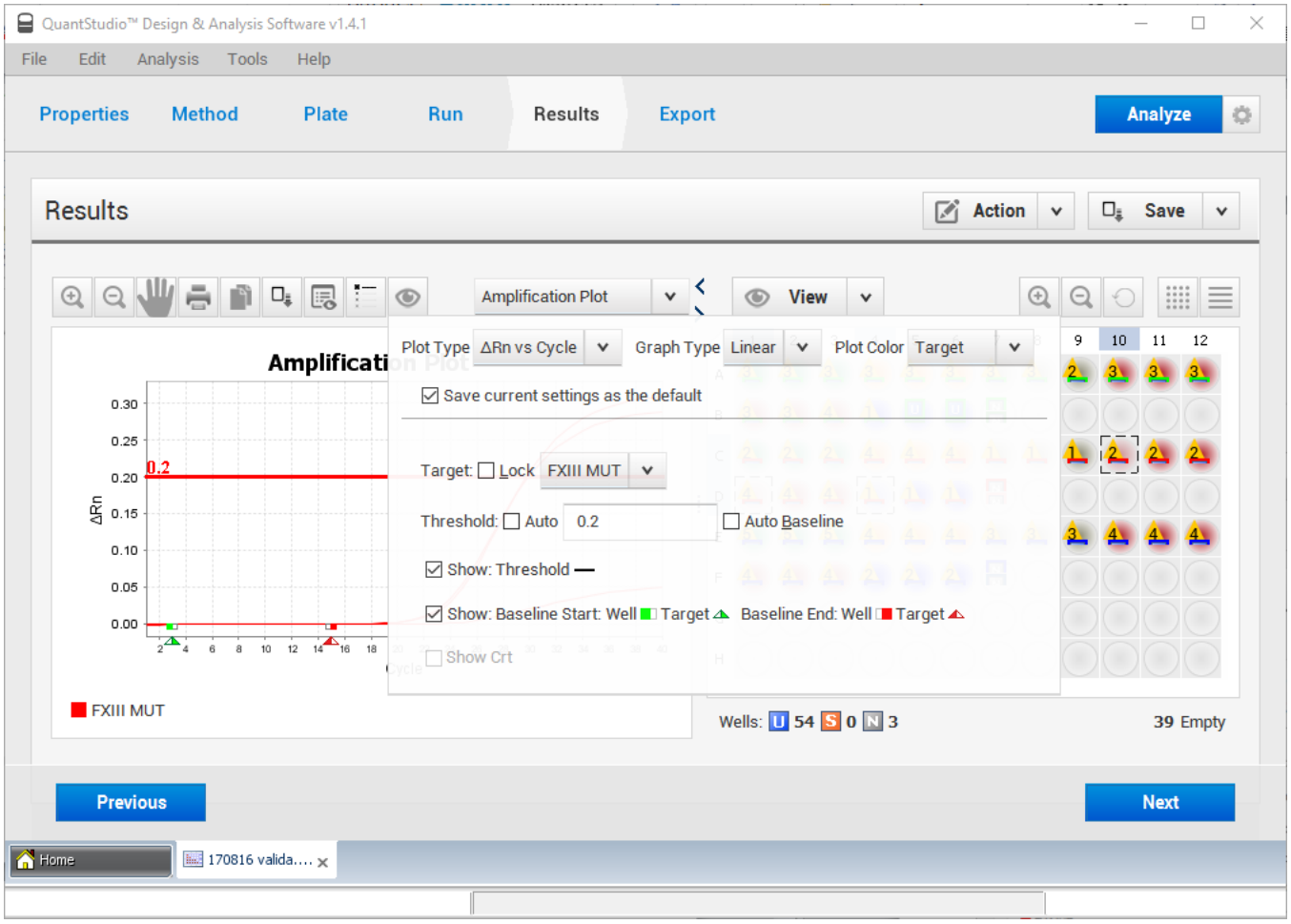


Fig. 2.10 Select MUT target

10. Move the Threshold line so that only controls **MUT** and **HET** are **positive** (the Threshold line intersects only the Positive Control MUT and Positive Control HET curves).

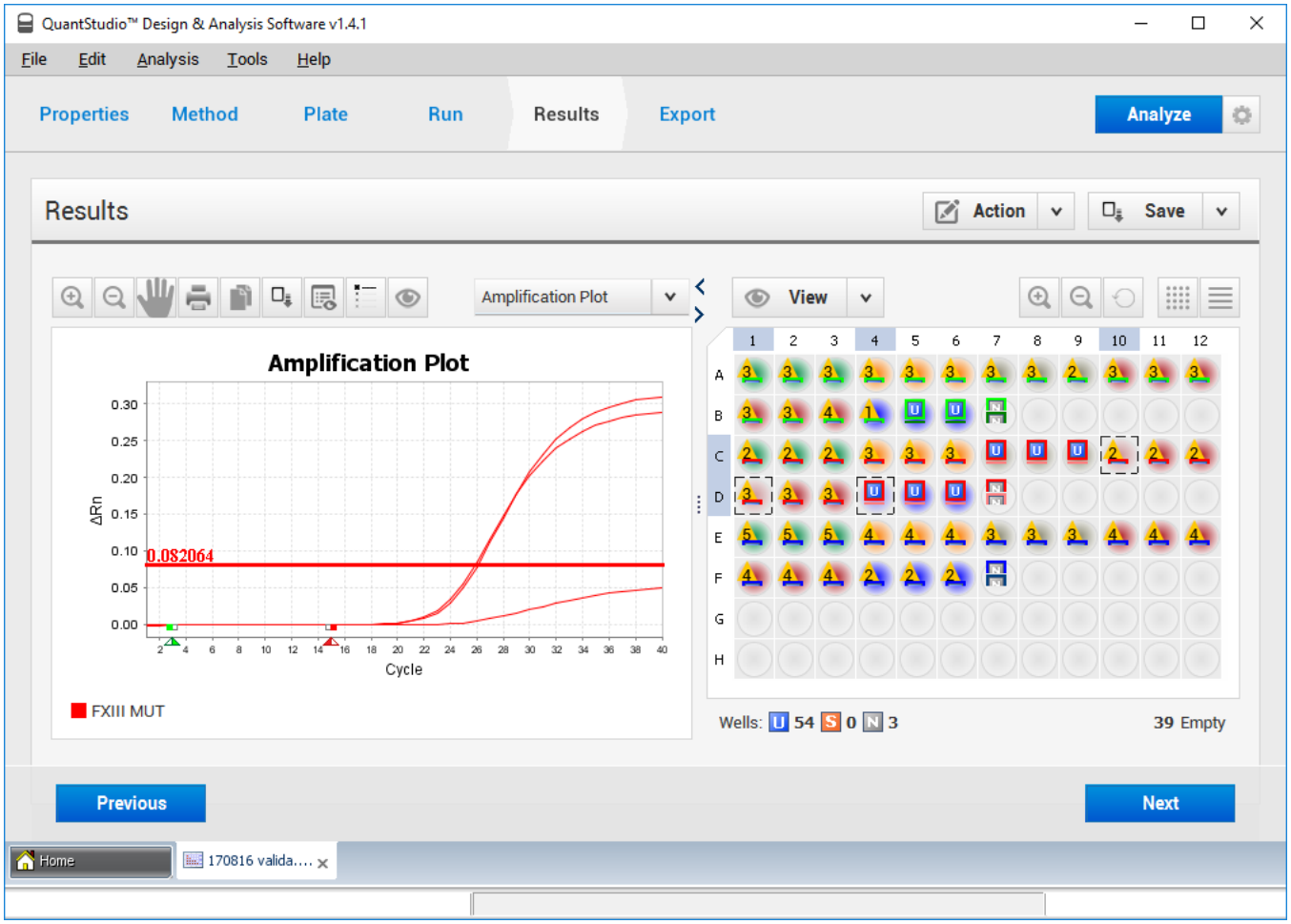


Fig. 2 MUT Threshold settings

11. Repeat steps 6. – 10. for the other kits in the experiment.

2.3.5 Evaluation

1. Use  button to switch to the Results table.

2. If there is a numerical value only for the **WT target** in the **Ct** column – this is a **standard genotype**; numerical value only for the **MUT target** – this is a **mutant genotype**; numerical values for both **targets WT and MUT** – this is a **heterozygote genotype**.

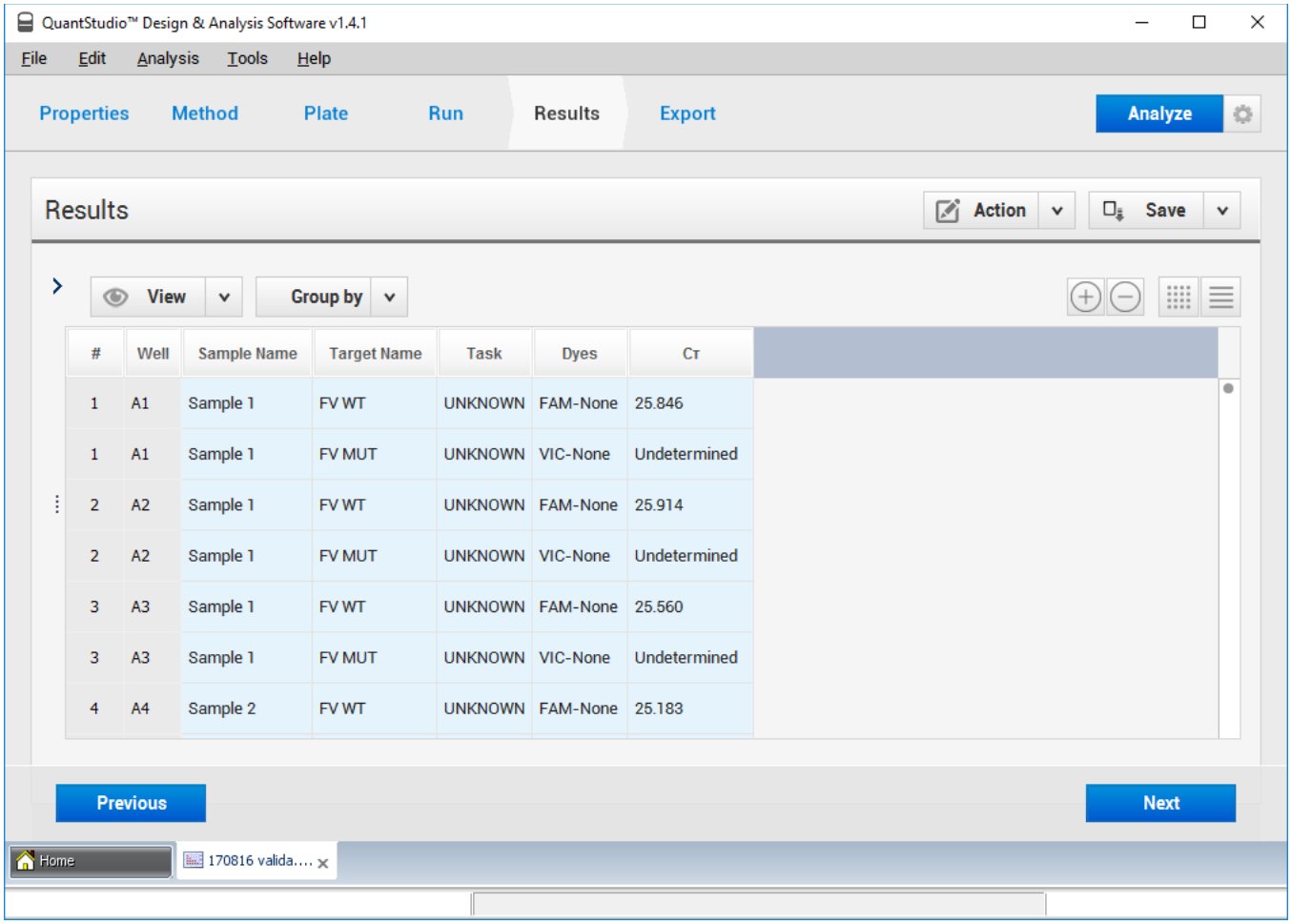
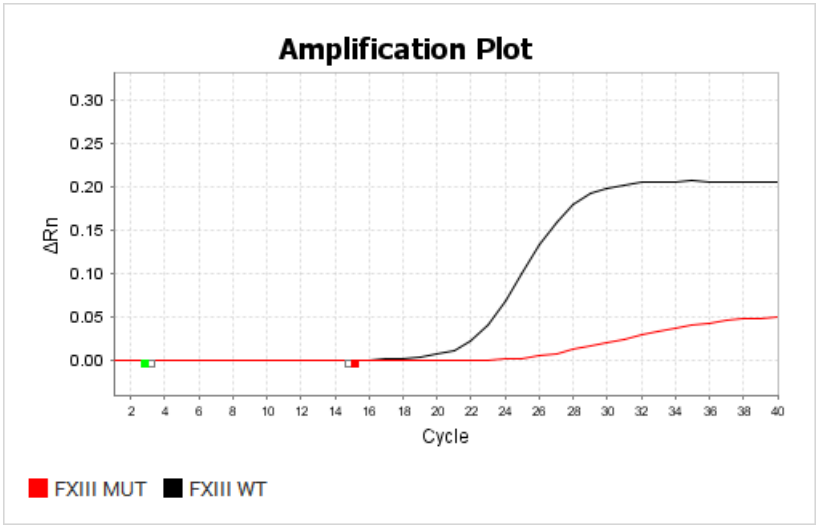
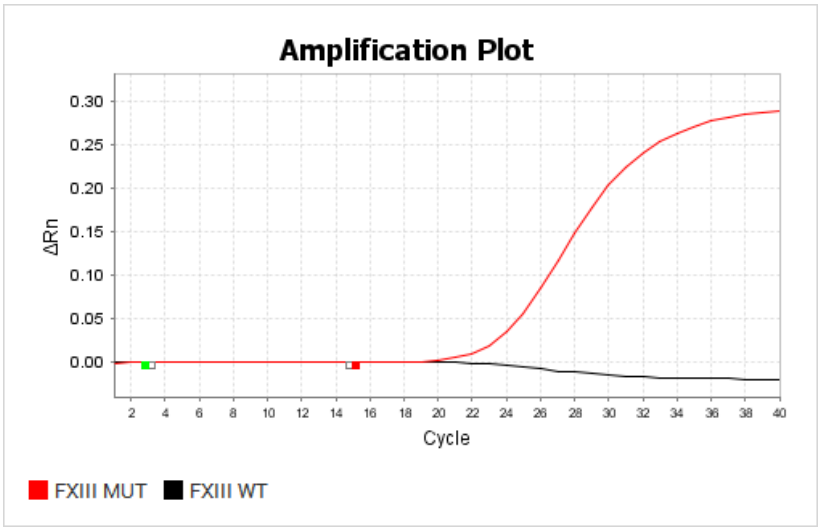


Fig. 2.12 Evaluation

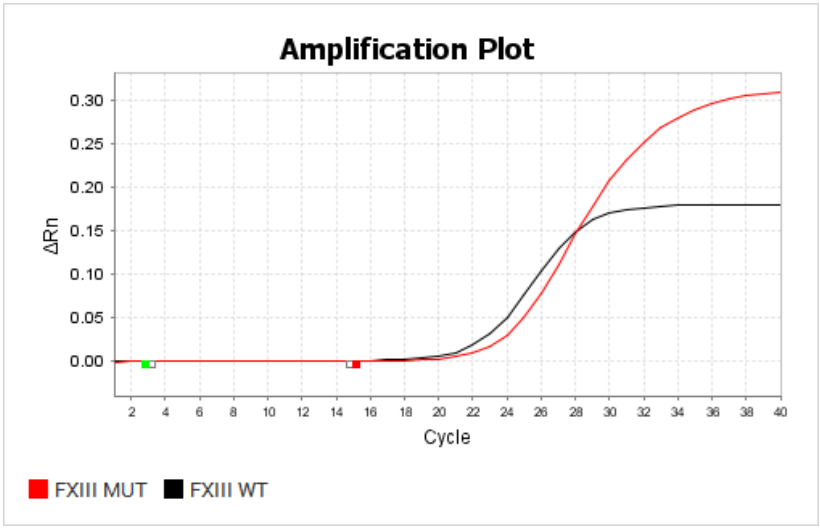
2.3.6 Examples of typical curves



Typical WT curve



Typical MUT curve



Typical HET curve

Fig. 2.13 Typical curves

3. Customer Service

We appreciate all our customers and besides high-quality products we provide, in cooperation with our partners, above-standard customer service including the following:

- Demonstration PCR kits
- Express deliveries
- Quick solution of issues related to the supplied products – service guaranteed within 24 hours from the time of report
- Consultations concerning technological and clinical interpretations

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

- Kit name
- Issue definition
- Kit lot - specified on the kit package
- Used device
- File with the examination log from the used device, if available

4. Contact Information

Support and customer care

Phone: +420 730 176 222
e-mail: support@geneproof.com

Orders

Phone: +420 543 211 679
e-mail: sales@geneproof.com