GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Bio Molecular Systems

Mic qPCR Cycler

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 DNA diagnostics with the device Mic qPCR Cycler.

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 assays, 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 assays on your local disc and open them in the software micPCR.

Click the arrow next to the **Save As** button and save the created Assay under the name **of used PCR kit** as the **micPCR Files** type.

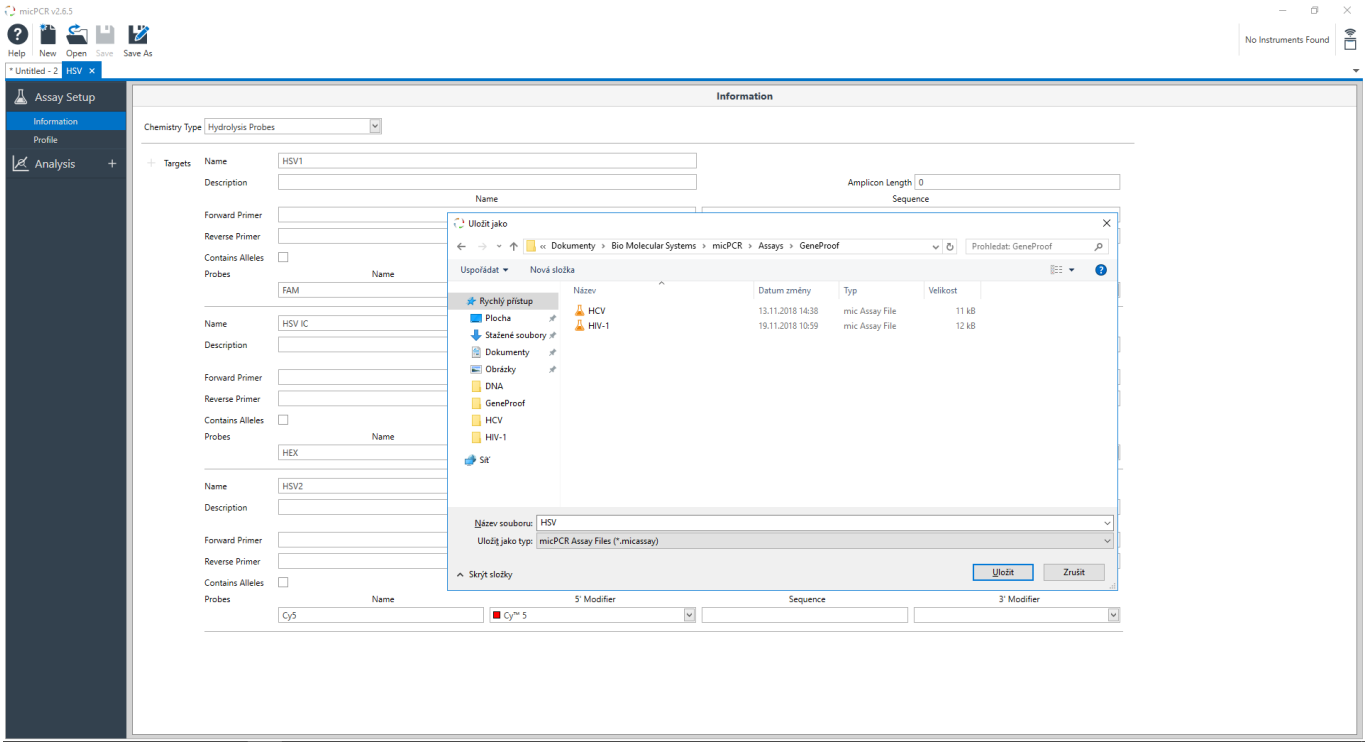

Fig. 1.1 Save Assay

Save the Assay into a folder called **GeneProof**, which should be created in the default **Assays** folder. Default path is Documents\Bio Molecular Systems \micPCR\Assays.

1.3. PCR Amplification Start

1.3.1 Saved Assay opening

1. Start the **micPCR** software and select **New -> Run.**

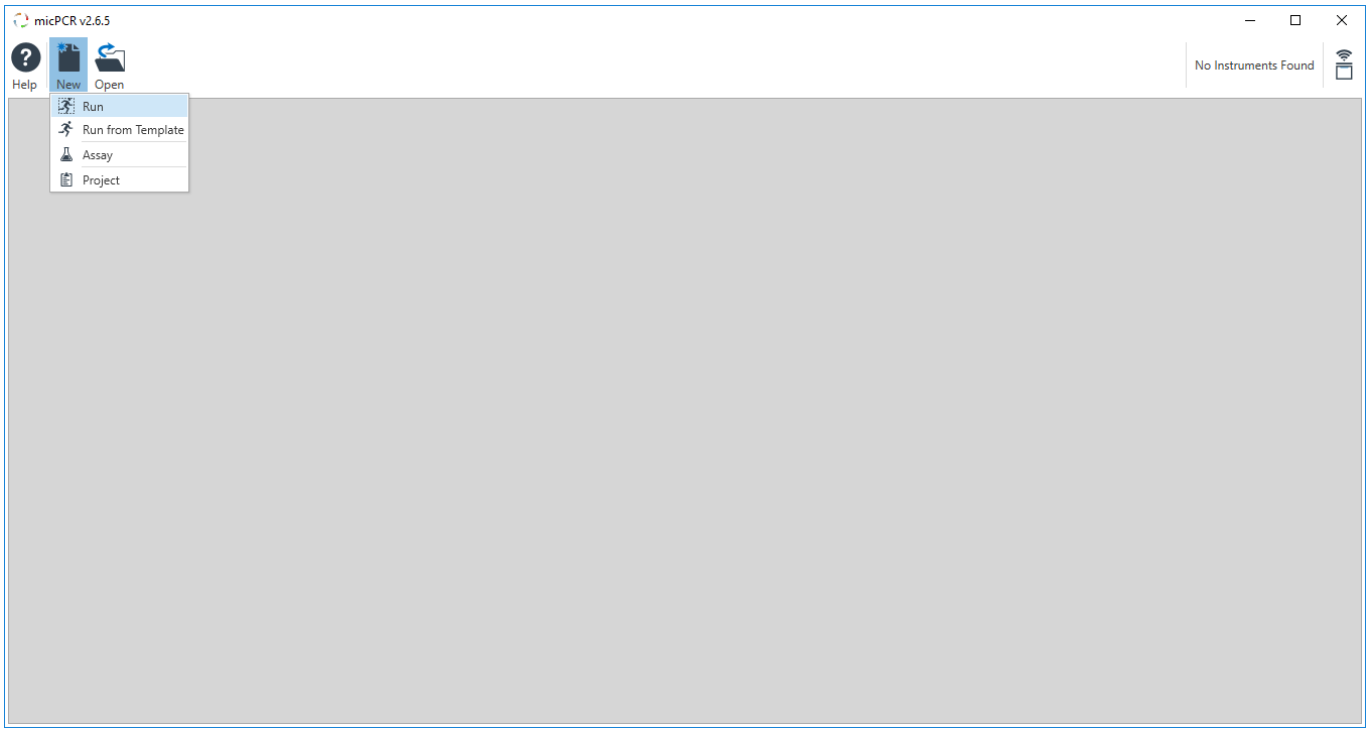

Fig. 1.2 New experiment

2. In the Assays field click on the **+** symbol and choose the assay of your choice from the **GeneProof** folder in the **My assays** tab.

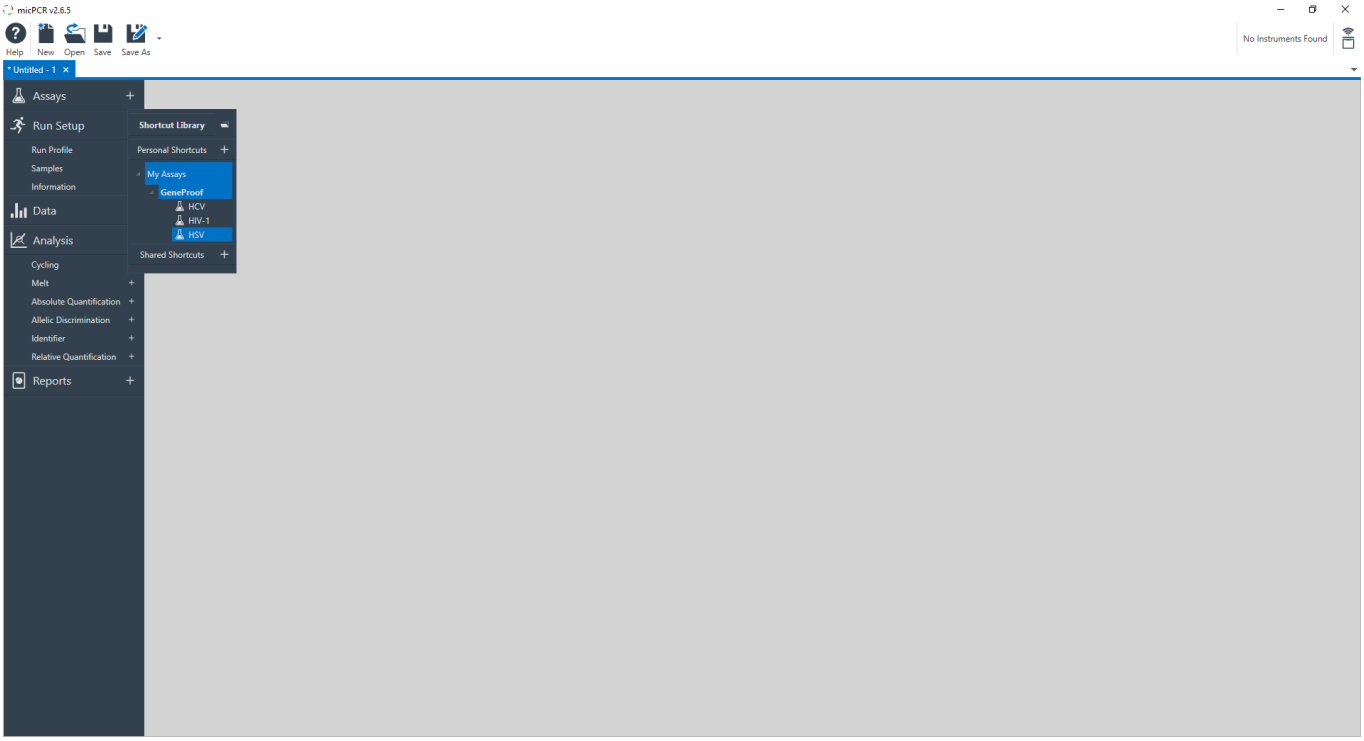

Fig. 1.3 Loading of a saved Assay

1.3.2 Samples editing

1. In **Samples** tab, define the samples used in the experiment.
2. Add an assay to all of the samples in the **Assay** column.

In case of quantitative detection

3. For negative controls set **Negative control** in the **Type** column.
4. For calibrators set **Standard** in the **Type** column and enter the corresponding quantity in the **Standard Concentration** column: 10 000, 1 000, 100 a 10 (keep Copies/µl).

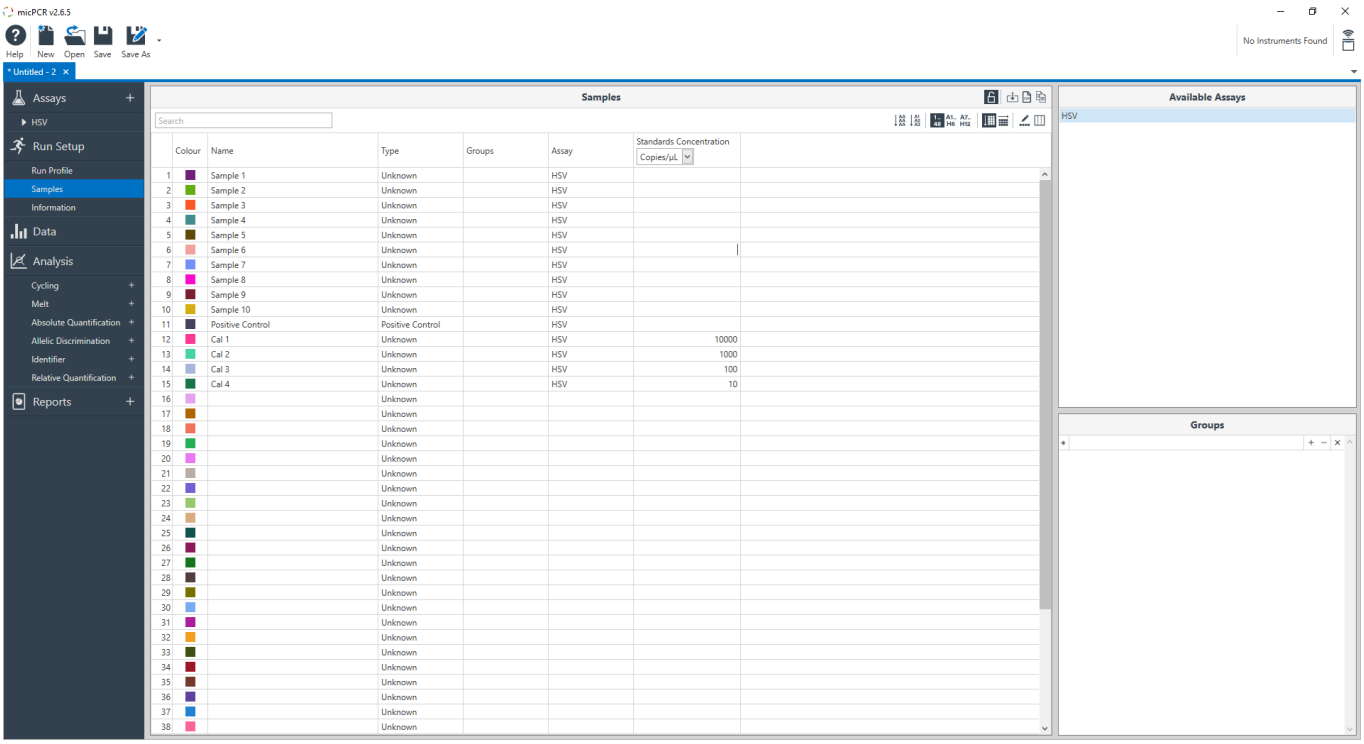

Fig. 1.4 Define samples in case of quantitative detection

In case of qualitative detection

3. For positive controls set **Positive control** in the **Type** column.
4. For negative controls set **Negative control** in the **Type** column.

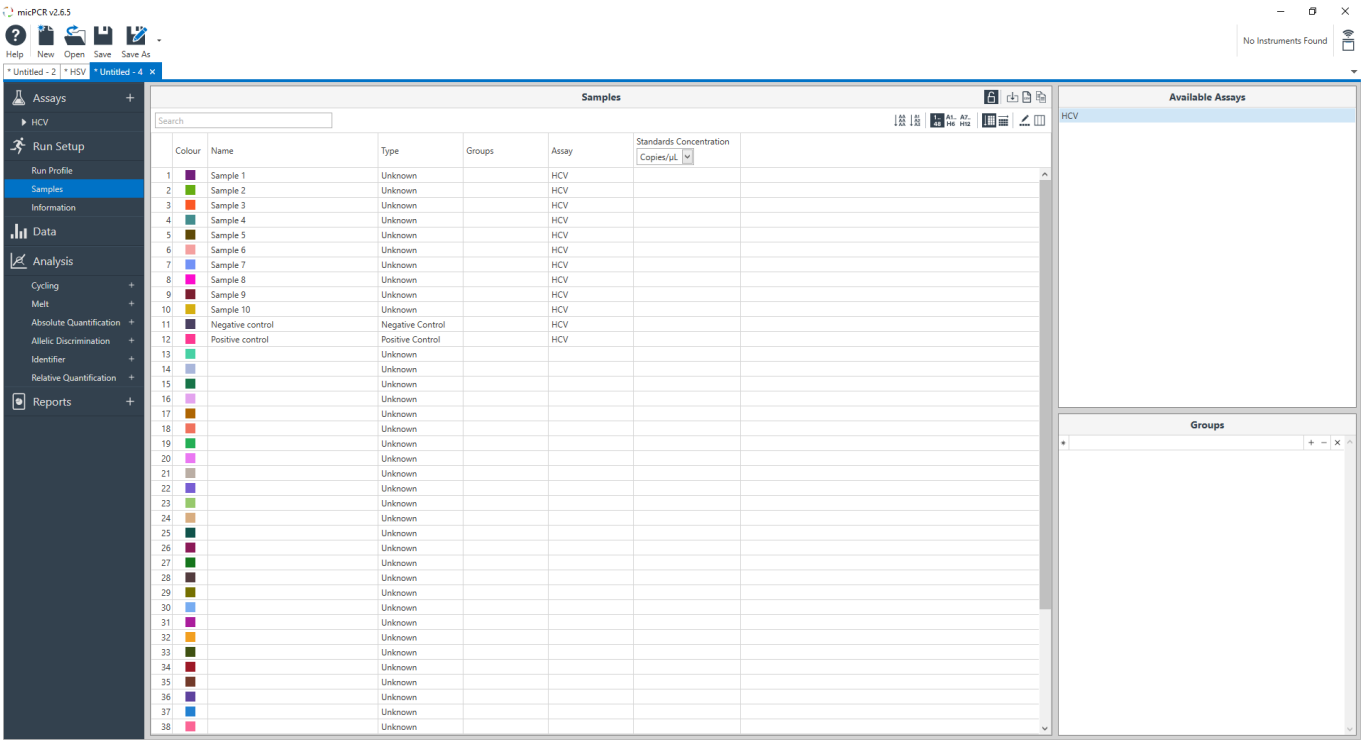

Fig. 1.5 Define samples in case of qualitative detection

1.3.3 Experiment starting

Save the experiment before starting the device.

1. In the top bar, select **Save** and save the created experiment as the **micPCR Run Files** type. To make search easier it is recommended to create the **Experiments** folder.

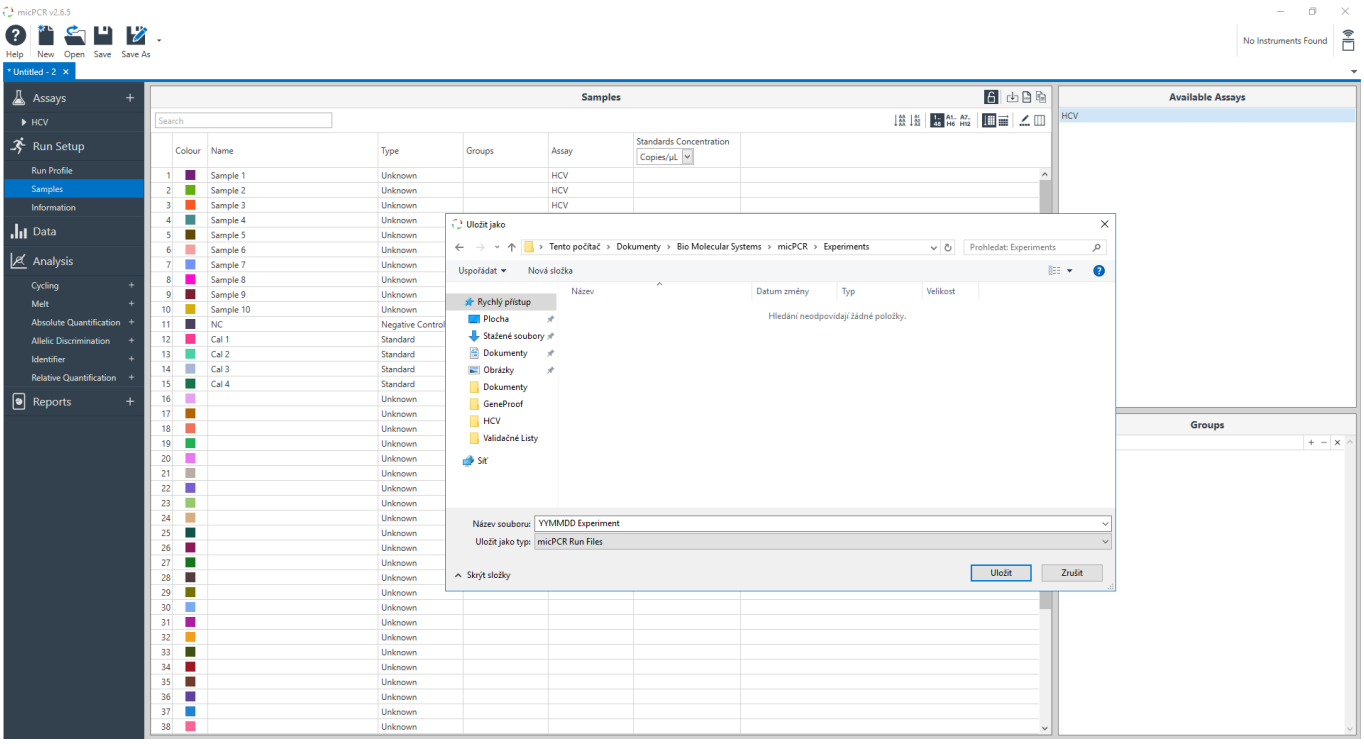

Fig. 1.6 Save experiment

2. In the upper right corner of the window, **select the device** and click the **Start Run** button.

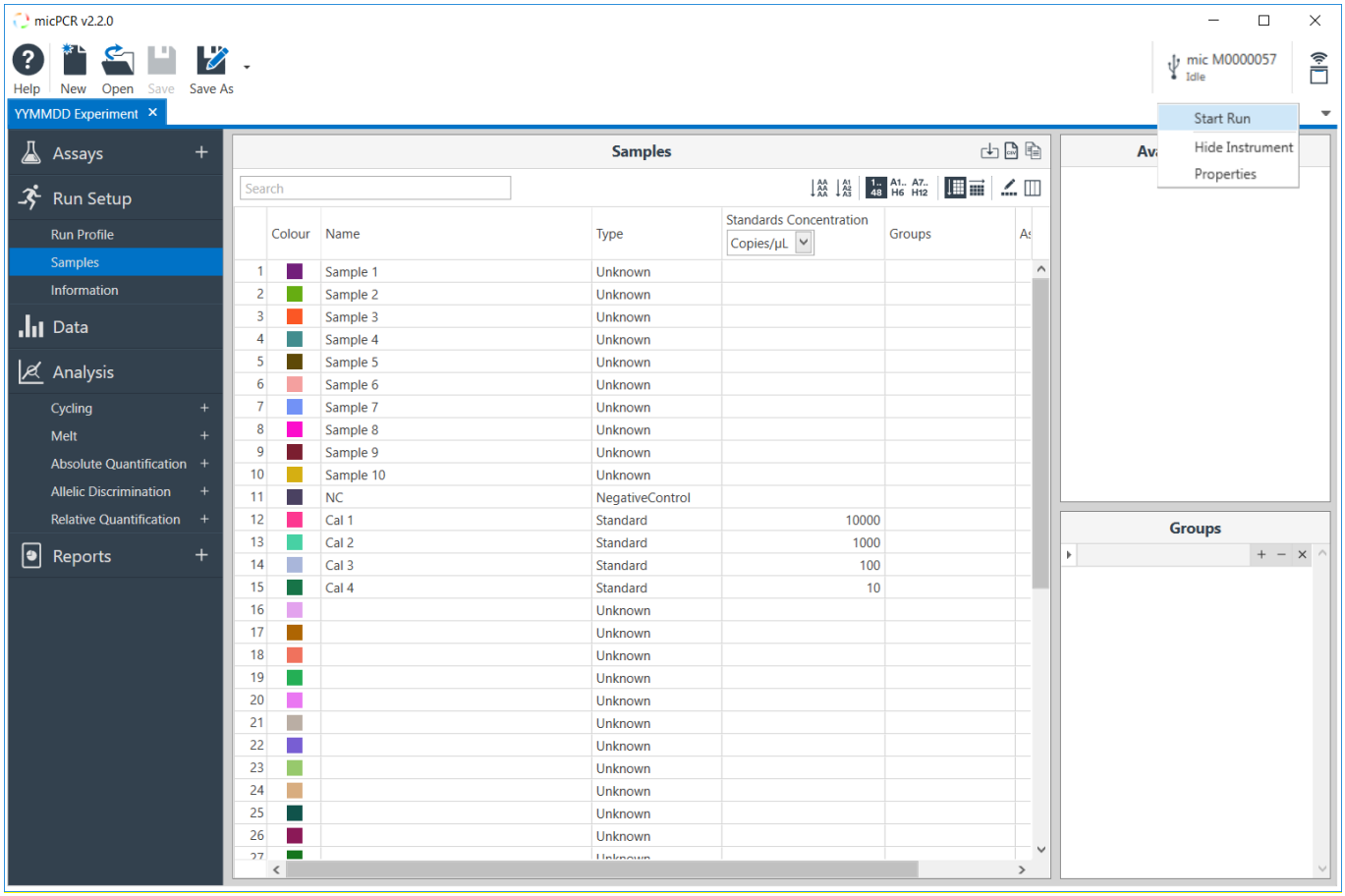

Fig. 1.7 Start experiment

1.4. 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.

1.4.4 Detection analysis in linear scale

The manufacturer recommends using this method for evaluation of the detection result as a first choice and also in the presence of clearly positive samples with a Ct value lower than 40. For the evaluation of samples with a Ct value greater than 40, it is recommended to use logarithmic evaluation (see **1.4.5 Detection analysis in logarithmic scale**).

1. In **Analysis** tab select **Cycling -> Name of the target of your detection** to evaluate **studied microorganism**, or **Cycling -> Name of your detection with IS sufix** to evaluate **internal standard**.
2. Select **linear scale** with the  button in the upper right corner of **Cycling Analysis** window.
3. In **Parameters** window uncheck **Auto Set Threshold** and move the threshold line just above the reaction basal noise.

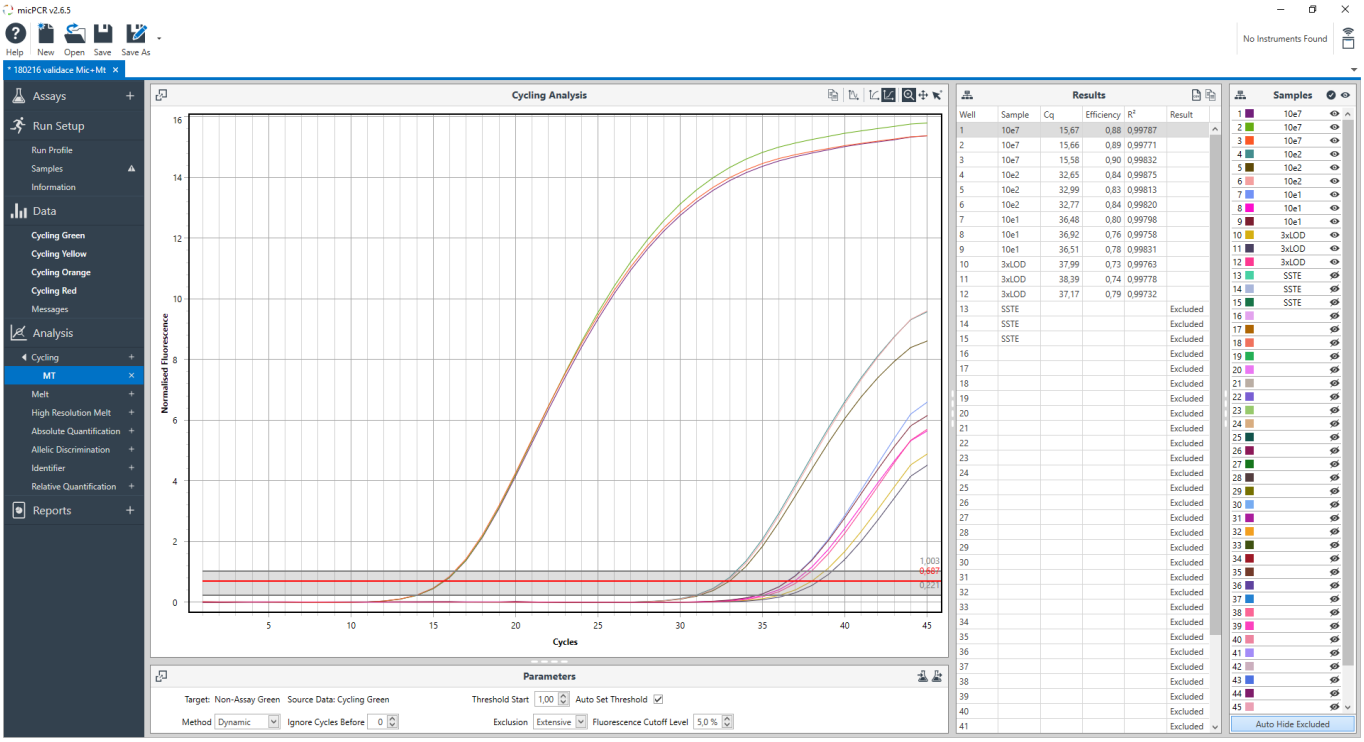

Fig. 1.8 Linear scale analysis

Cq values are displayed in the **Results** window.

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

1.4.5 Detection analysis in logarithmic scale

In this method for evaluation, it is possible to detect weakly positive samples which, when evaluated using linear scale, could escape detection. Any sample with a numerical Cq value greater than 40 is considered to be a weakly positive sample, requiring this method of evaluation. Only one sample should be viewed at the same time for this evaluation method.

1. In **Analysis** tab select **Cycling -> Name of the target of your detection** to evaluate **studied microorganism**, or **Cycling -> Name of your detection with IS sufix** to evaluate **internal standard**.
2. Select **logarithmic scale** with the  button in the upper right corner of **Cycling Analysis** window.
3. In **Parameters** window uncheck **Auto Set Threshold** and move the threshold line just above the reaction basal noise.

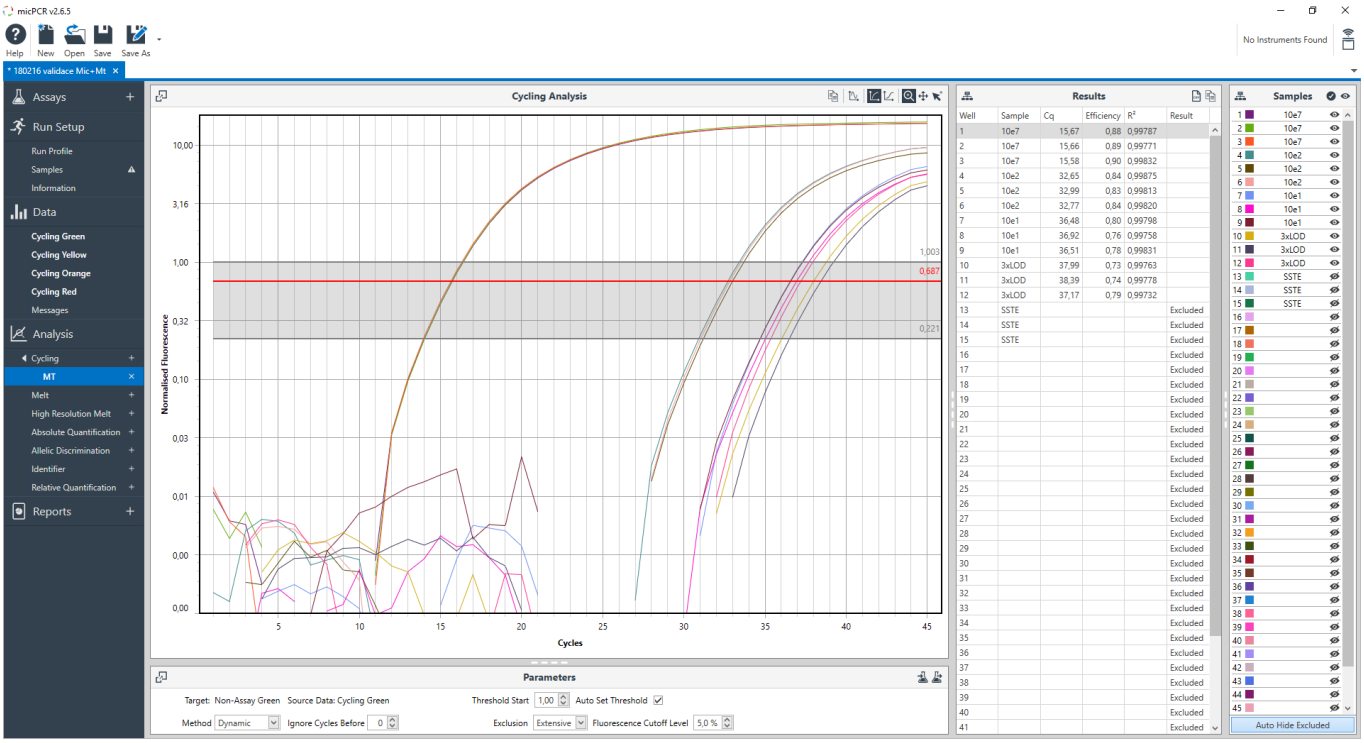

Fig. 1.9 Logarithmic scale analysis

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

1.5. Result quantitative analysis and detection evaluation

1. In **Analysis** tab select **Absolute Quantification -> Name of the target of your detection -> Cycling**.
2. On the bottom of the **Samples** tab uncheck the **Auto Hide Excluded** button, so that it turns grey.
3. In **Parameters** window uncheck **Auto Set Threshold** and move the threshold line just above the reaction basal noise.

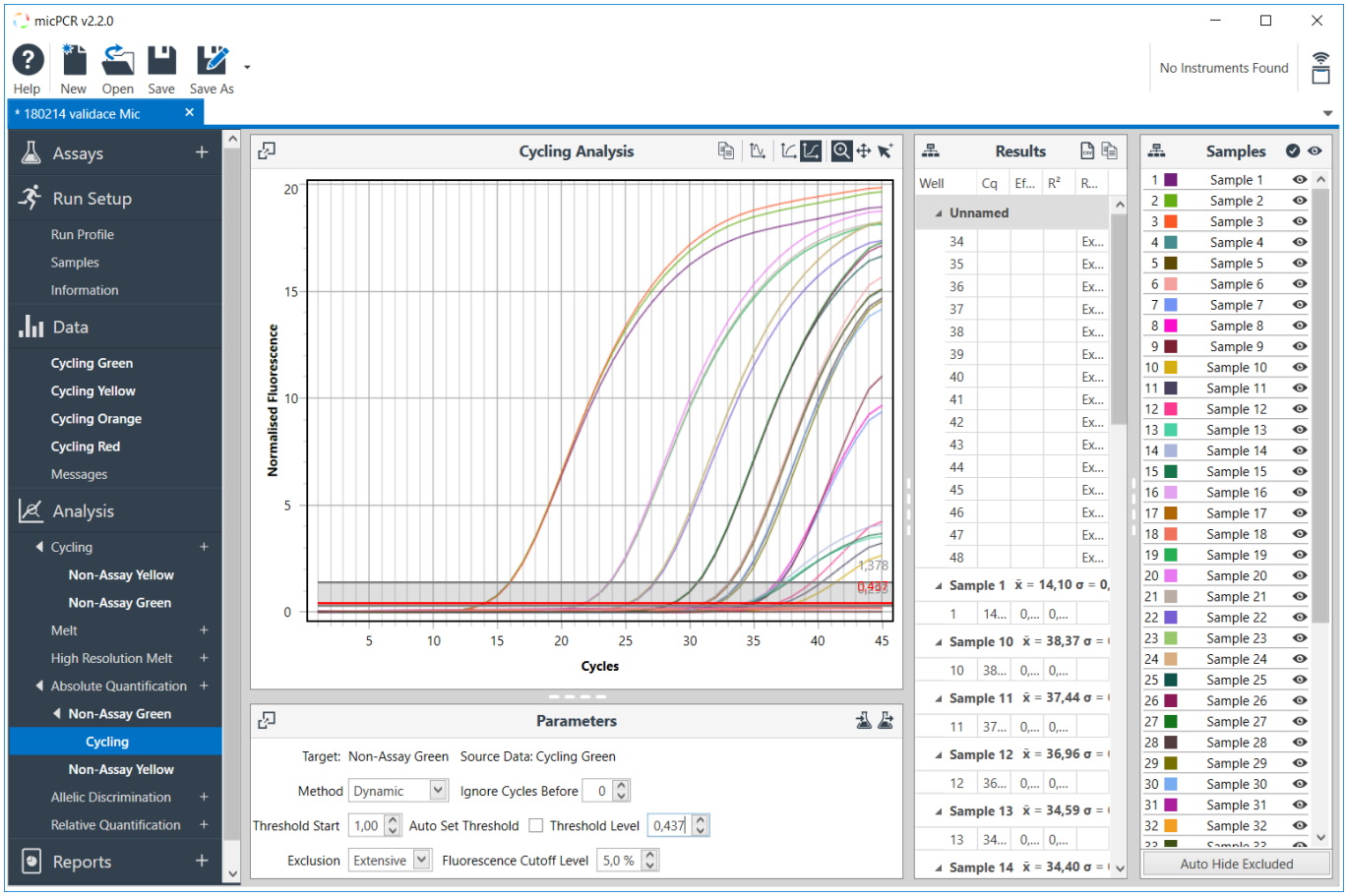

Fig. 1.10 Threshold settings

4. Switch up to **Absolute Quantification -> Name of the target of your detection** and evaluate the calibration quality in **Standard Curve Characteristics** window. 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.

5. **Cq** and **Calculated Concentration** values are displayed in the **Sample Results** window.

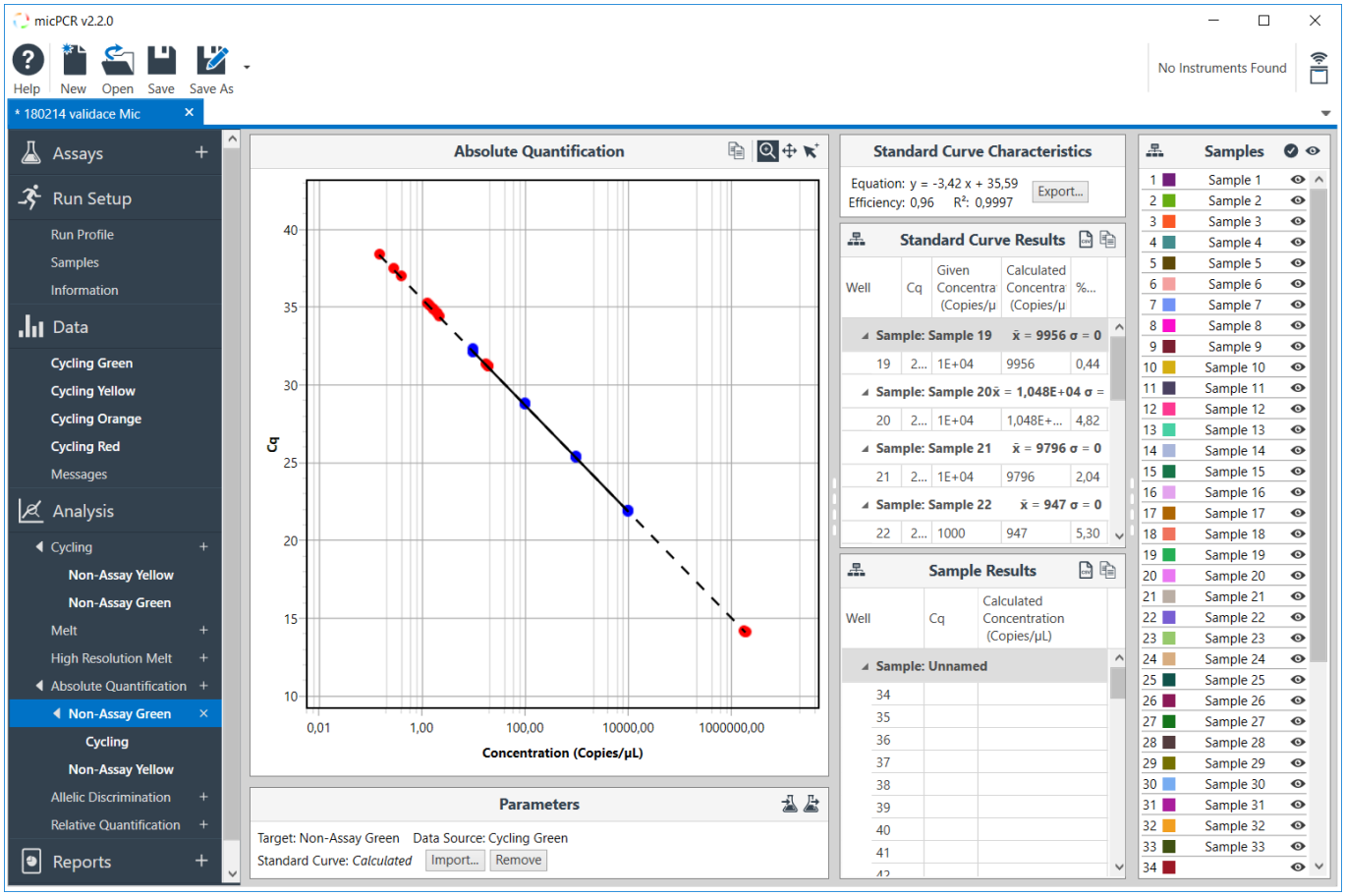

Fig. 1.11 Standard Curve and Sample Results

Perform evaluation, including the pathogen concentration calculation in copies/ml (or IU/ml) according to the Instruction for use of the used GeneProof PCR kit.

1.6. Troubleshooting

1.6.6 Invalid positive curves

Some positive curves have an high initial fluorescence and are therefore tilted. In the **Parameters** window for **Method Dynamic**, increase the value in the **Ignore Cycles Before** field so that the curves are aligned.

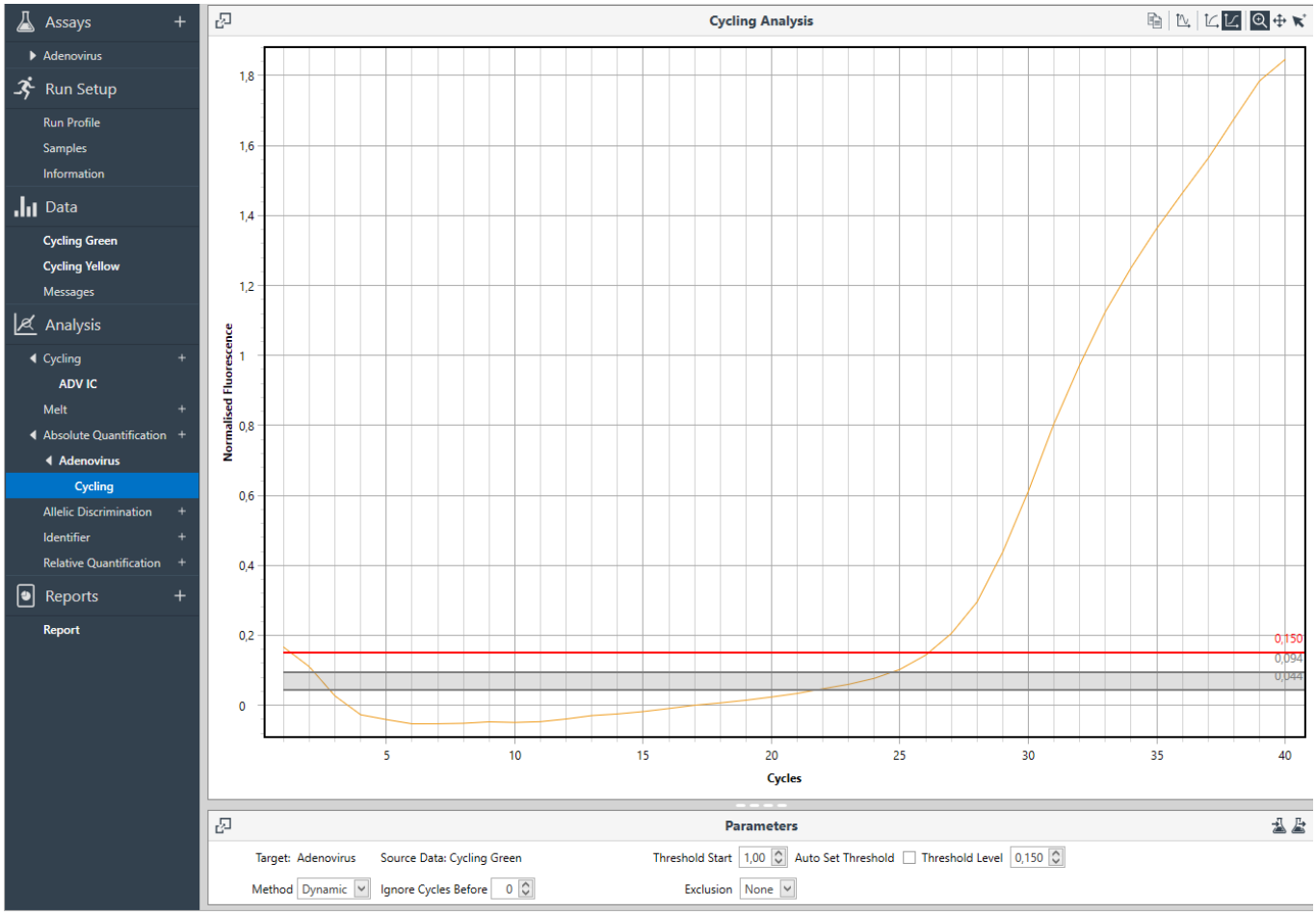

Fig. 1.12 Tilted curve in linear scale before correction

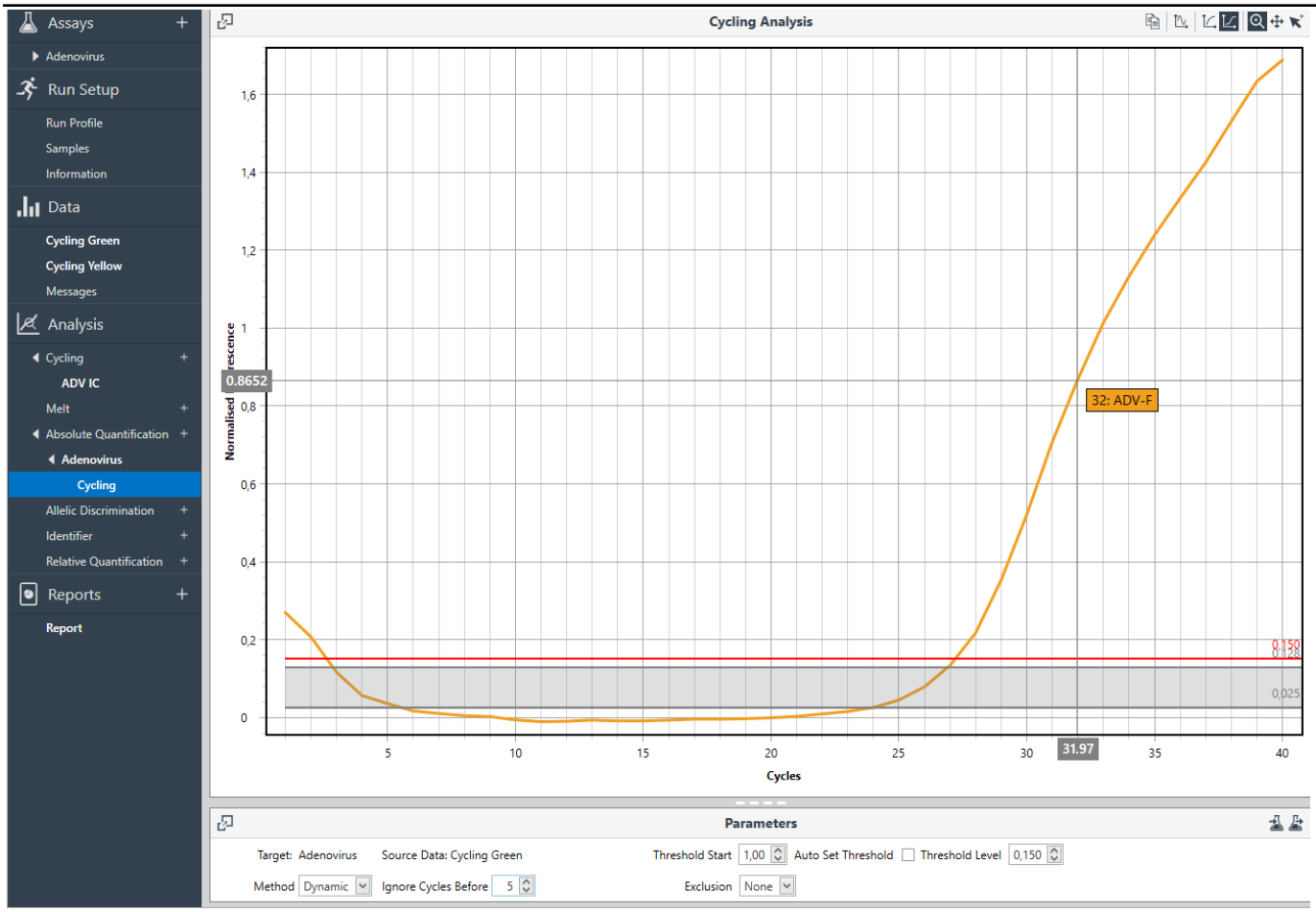

Fig. 1.13 Tilted curve after correction

1.6.7 Invalid negative curves

Due to lower fluorescence the negative samples may feature curves with linear growth of fluorescence and without the logarithmic shape that is so typical for amplification curves:

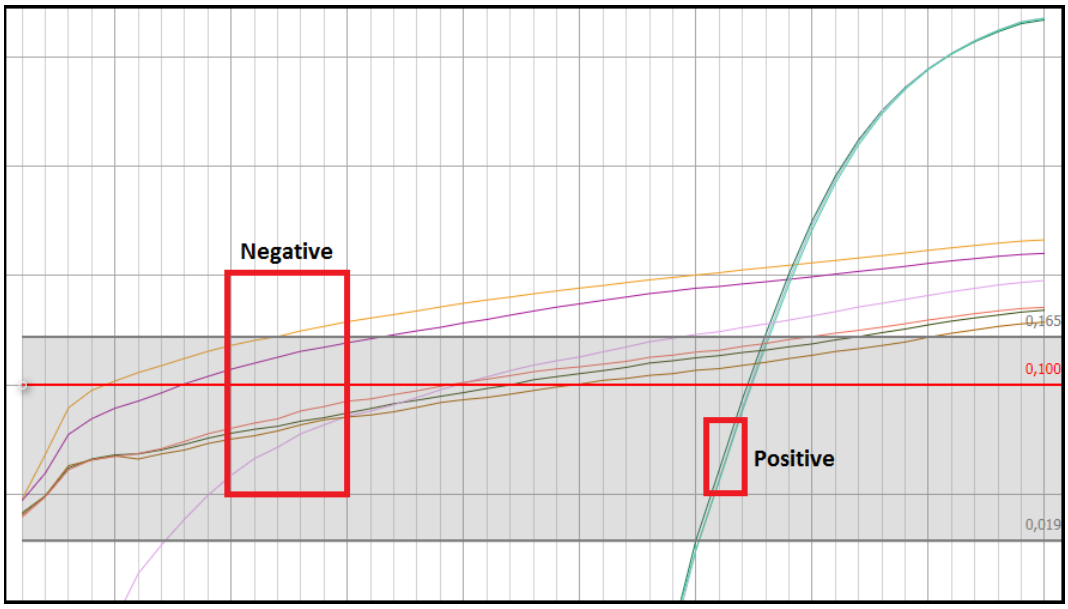

Fig. 1.14 Invalid curves in logarithmic scale

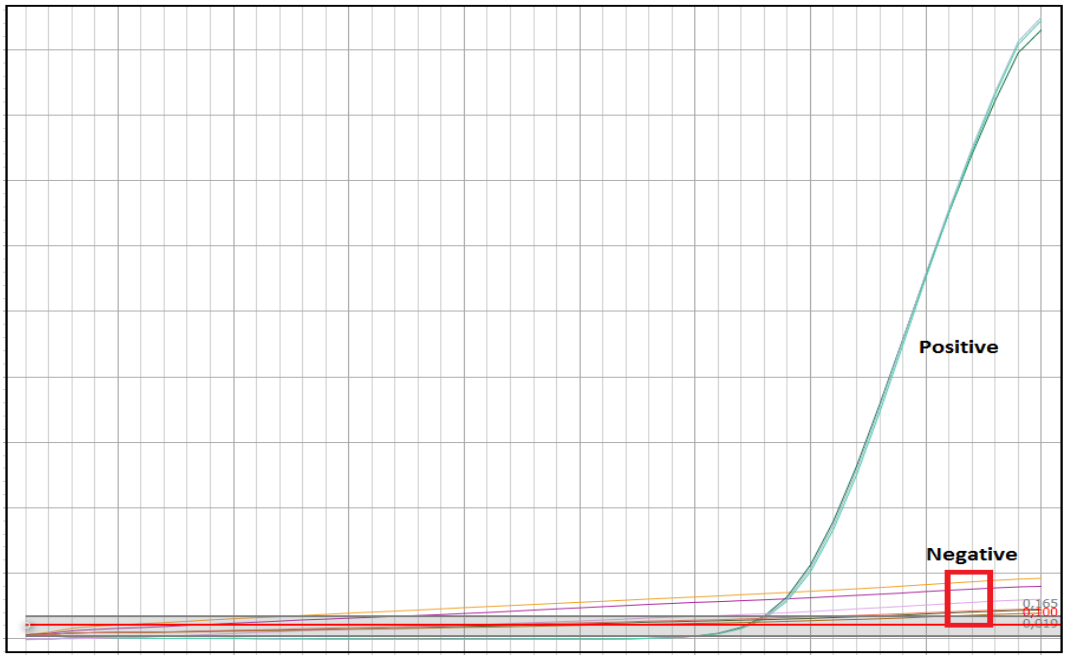

Fig. 15 Invalid curves in linear scale

These curves are considered negative. Turn them off before setting the threshold.

2. GENETIC DIAGNOSTICS

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the instruments Mic qPCR Cycler.

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 micPCR software.

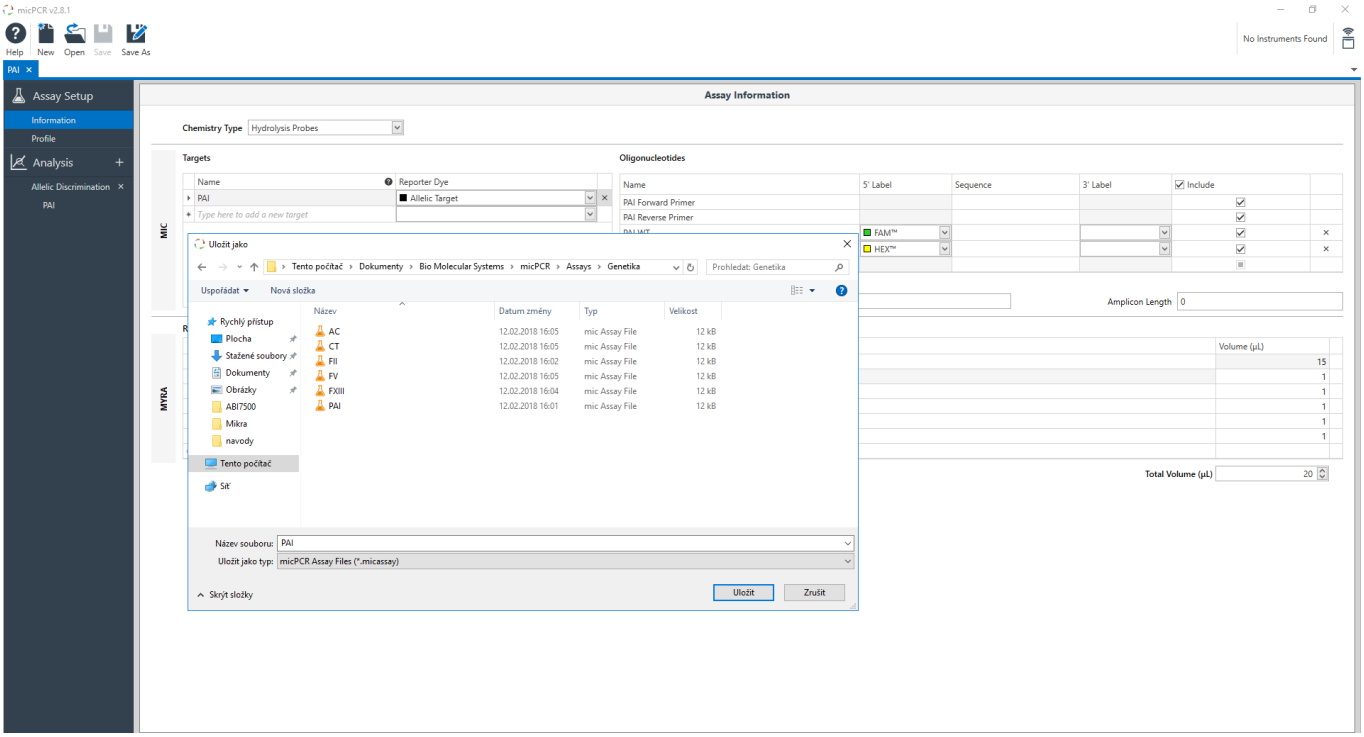

Fig. 2.1 Save template

Save the **Assay** to **GeneProof** file. Create an Assay folder, pathway as usual: Documents\Bio Molecular Systems\micPCR\Assays.

2.2. PCR amplification start

2.2.1 Opening of the saved template

1. Start the micPCR software, select **New -> Run.**

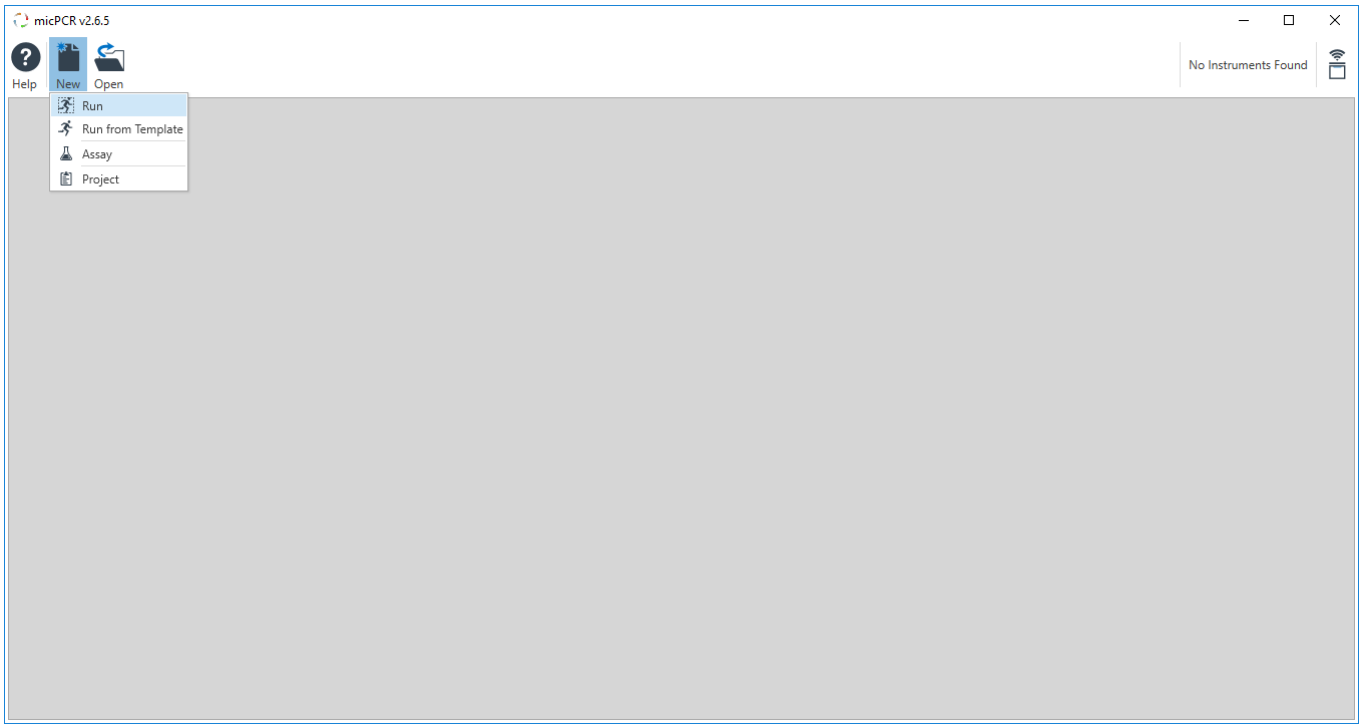

Fig. 2.2 New experiment

2. In the Assays field click on the **+** symbol and choose the assay of your choice from the **GeneProof** folder in the **My assays** tab.

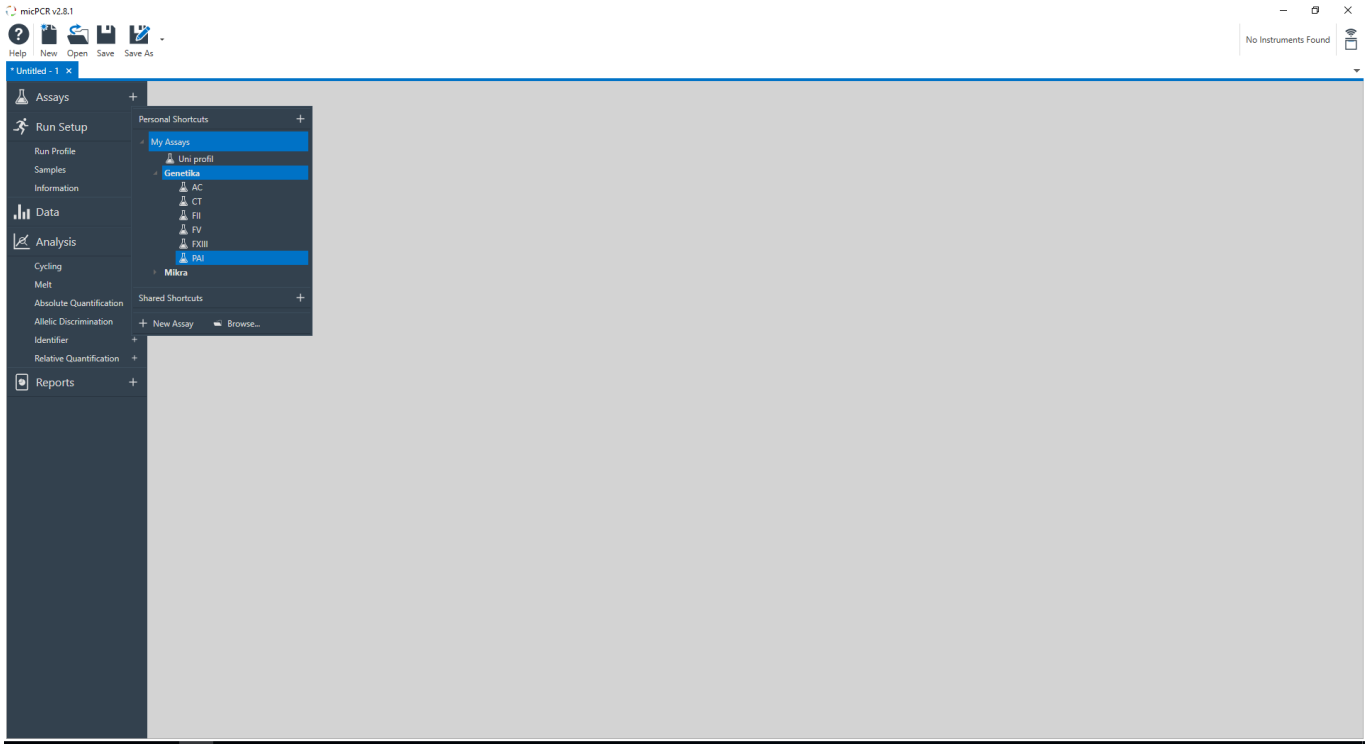

Fig. 2.3 Loading of a saved Assay

2.2.2 Sample editing

1. On the **Samples tab**, define the samples used in the experiment.
2. Assign an Assay to the **Assay** column for each sample.
3. For Negative controls in columnt **Type** set **Negative Control**.
4. For Positive controls in columnt **Type** set **Positive Control**.

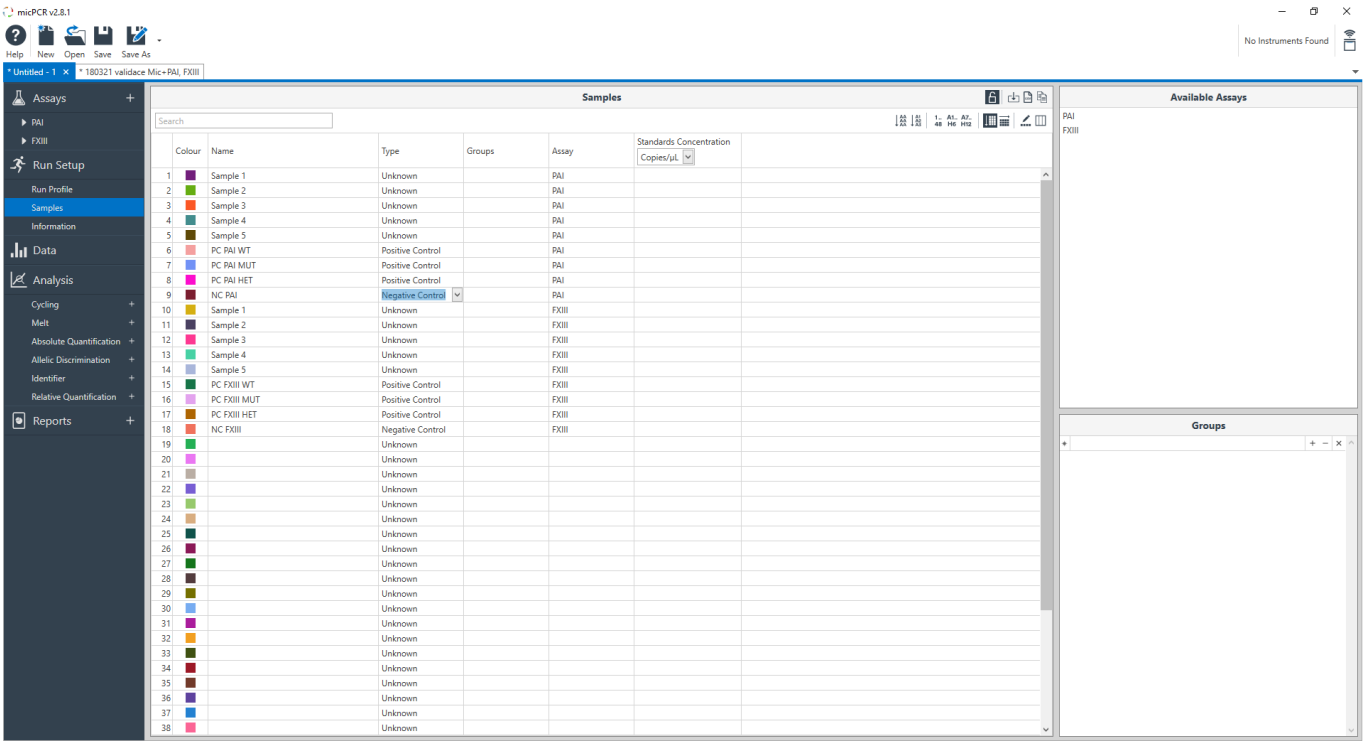

Fig. 2.4 Sample editing

2.2.3 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as **micPCR Run Files** type. To make search easier it is recommended to create the **Experiments folder**.

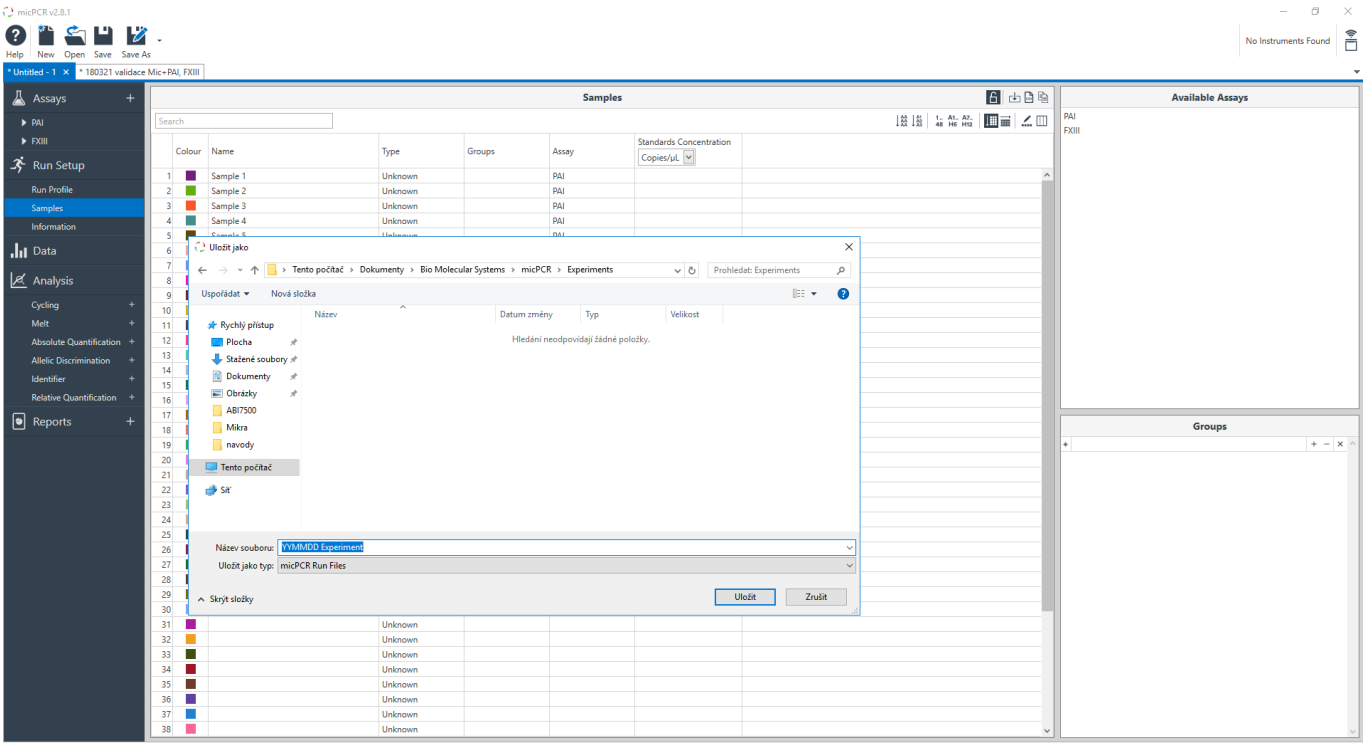

Fig. 2.5 Save experiment

2. Click **Start Run** button in upper right corner of **select device** window.

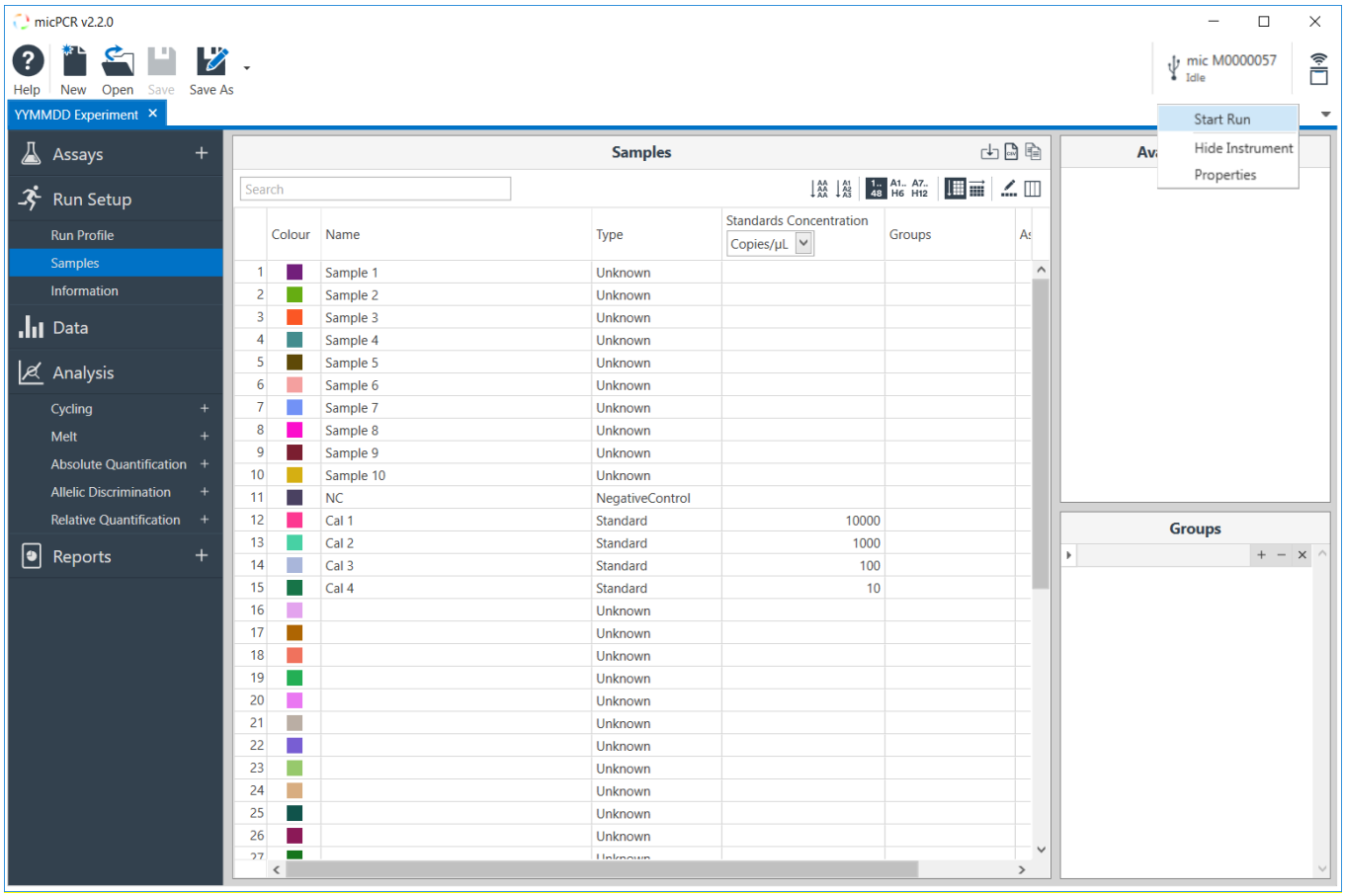

Fig. 2.6 Start experiment

2.3. Analysis of the result and evaluation of detection

2.3.4 Detection Analysis

1. *In the **Analysis** tab select* **Allelic Discrimination-> mutation name** for **Genotype** evaluation**.**
2. Set the threshold line manually so that in the **Results table** be: positive control WT evaluated as WT genotype, positive control MUT evaluated as MUT genotype and positive control HET evaluated as HET genotype.

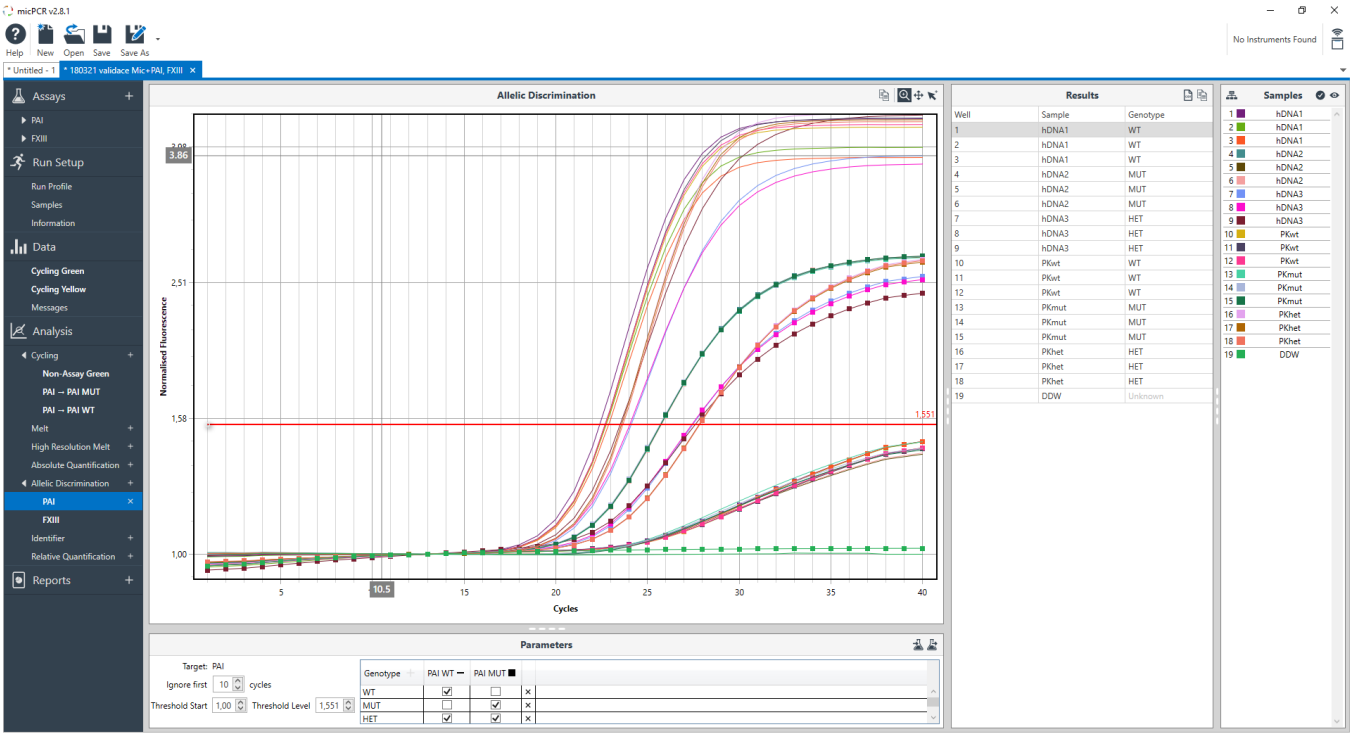

Fig. 2.7 Genotype Analysis

The genotype results are displayed in the **Results window**.

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

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Orders

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