GeneProof
Molecular diagnostics for your routine

DEVICE MANUAL

Agilent

AriaMX 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 with the device AriaMX 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 **Agilent AriaMX** software.

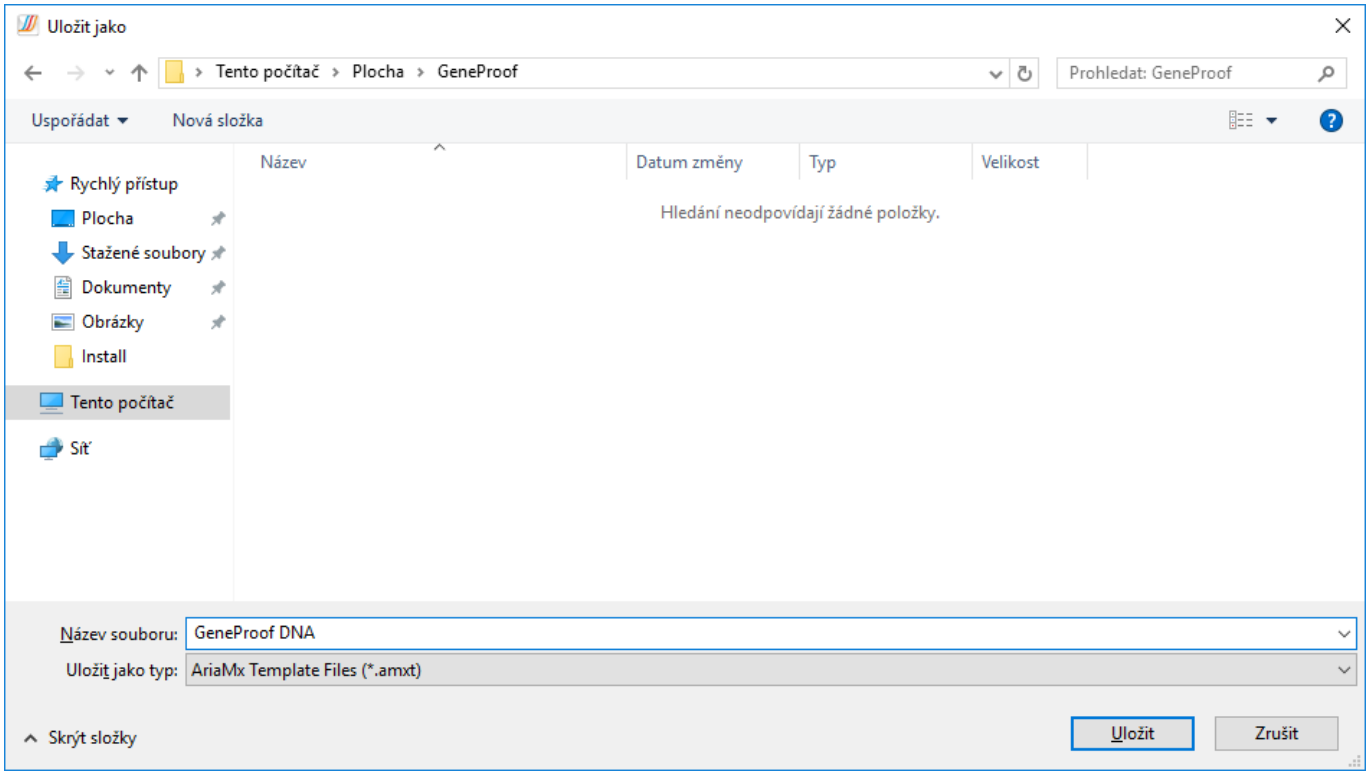

Fig. 1.1 Save template

Save the template into a folder called **GeneProof**, which should be created on your desktop.

1.3. PCR Amplification Start.

1.3.1 Starting the template

1. Start the **Agilent AriaMX software**.
2. *In the **Set Up** column, select **Template** and open template for the given GeneProof PCR kit.*

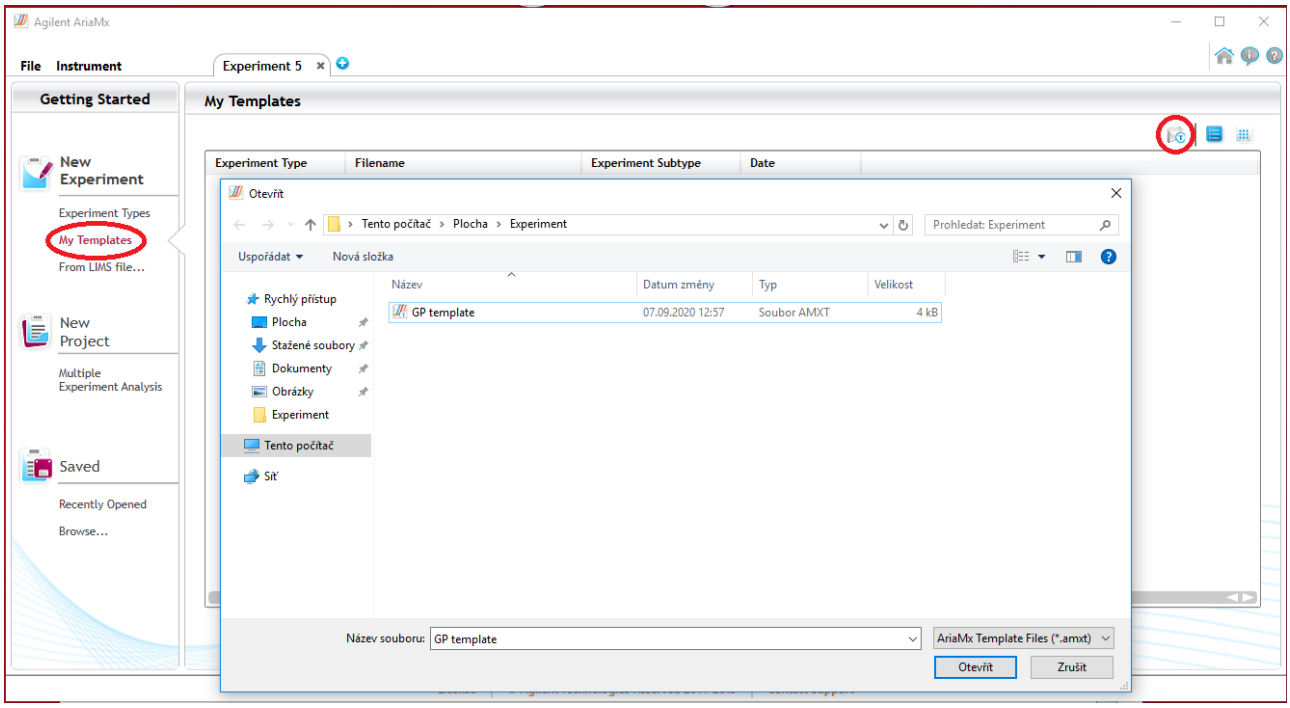

Fig. 1.2 Open template

1.3.2 PCR plate editing

1. In **Plate Setup** choose the position of yout PCR plate with samples and assign them an **Unkown** well type, in the the **Well Type** window.

2. Mark all positions and in the **Add Dyes** check used channels in the chosen positions.

e.g. CNMX detection (4 channels), set:

- Target Name: **CHT**, Dye Name: **FAM**
- Target Name: **CNMX IC**, Dye Name: **HEX**
- Target Name: **NG**, Dye Name: **Cy5**
- Target Name: **MG**, Dye Name: **TexRed**

- e.g. HSV detection (3 channels), set:
- Target Name: **HSV 1**, Dye Name: **FAM**
- Target Name: **HSV IC**, Dye Name: **HEX**
- Target Name: **HSV 2**, Dye Name: **Cy5**

- e.g. MT detection (2 channels), set:
- Target Name: **MT**, Dye Name: **FAM**
- Target Name: **MT IC**, Dye Name: **HEX**

In case of quantitative detection

3. For negative control assign the **NTC** well type and for the positions of standards, assign the **Standard** well type.

4. After uncovering the **Standard Quantities** window, by clicking on the All icon, use **Select Target** to set the standard concentrations for appropriate targets (e.g. FAM and Cy5) in the Starting Amount window, according to the instruction for use of used Geneproof PCR kit, e.g.: 10 000, 1 000, 100 and 10 (keep Copies/µl).

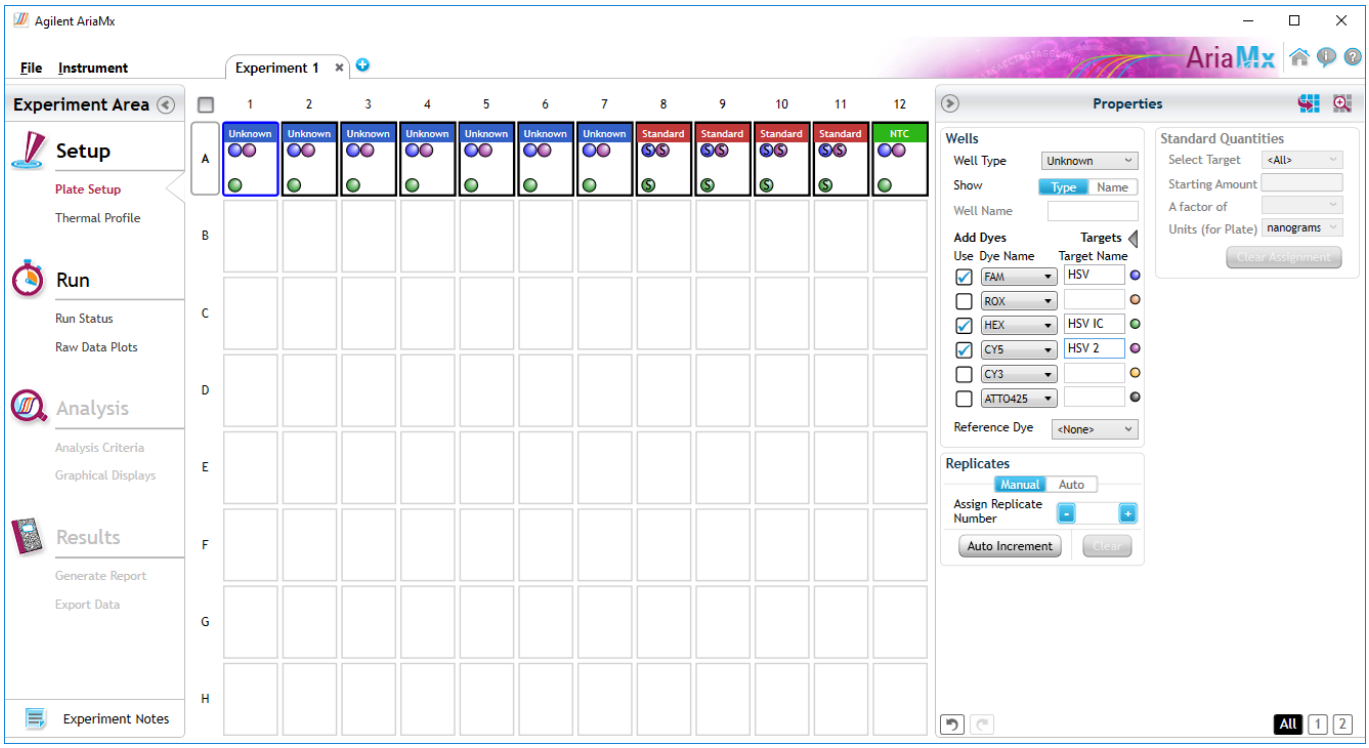

Fig. 1.3 Define samples in case of quantitative detection

1.3.3 Starting the experiment

Save the experiment before starting the instrument.

1. In the top bar, select **Save** and save the created experiment as the **AriaMX Experiment files (*amxd)** type. To make search easier it is recommended to create the **Experiments** folder on your desktop.

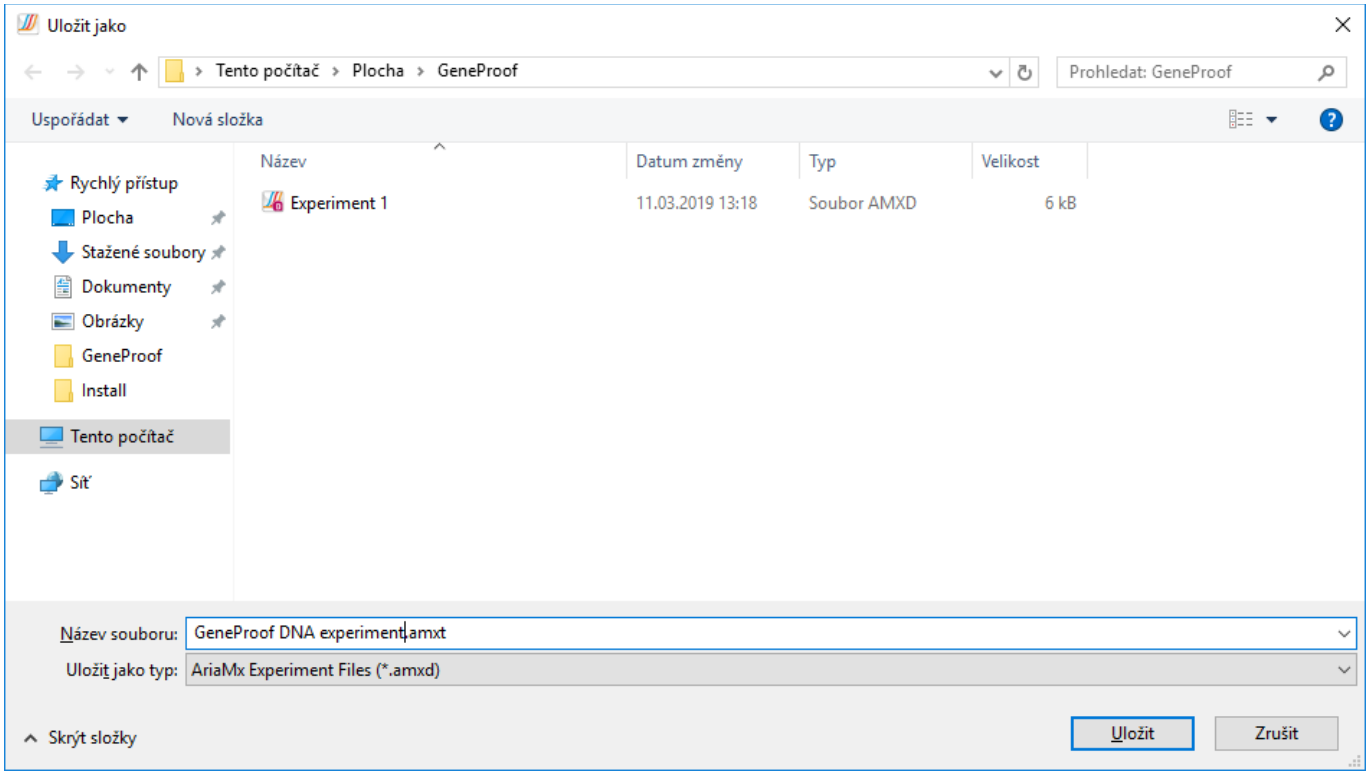

Fig. 1.4 Save experiment

2. In the **Run Status** tab, start the run by clicking the Run button.

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.

After finishing the run, select all the samples for analysis, in the **Analysis Criteria** tab.

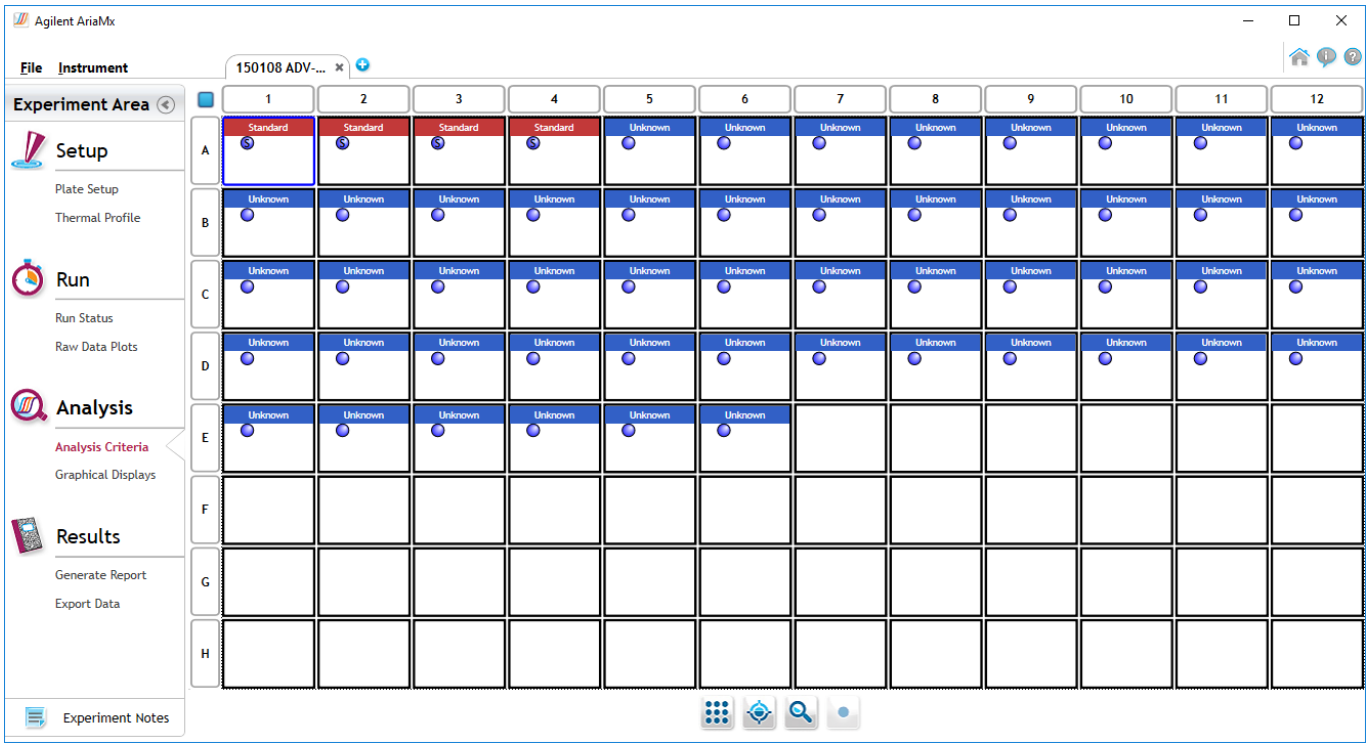

Fig. 1.5 Select the samples for analysis

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 the **Amplification Plots** window click the ▼ icon for uncovering of the advanced options.

2. Select **linear** in the **Graph type** option, if not selected already.

3. Select a desired channel under the **Display Targets b**utton and in the **Threshold Fluorescence** part, after clicking on the 🔒 icon, allow the threshold setting.

4. Set the threshold just above the base noise by adjusting the threshold line in the graph.

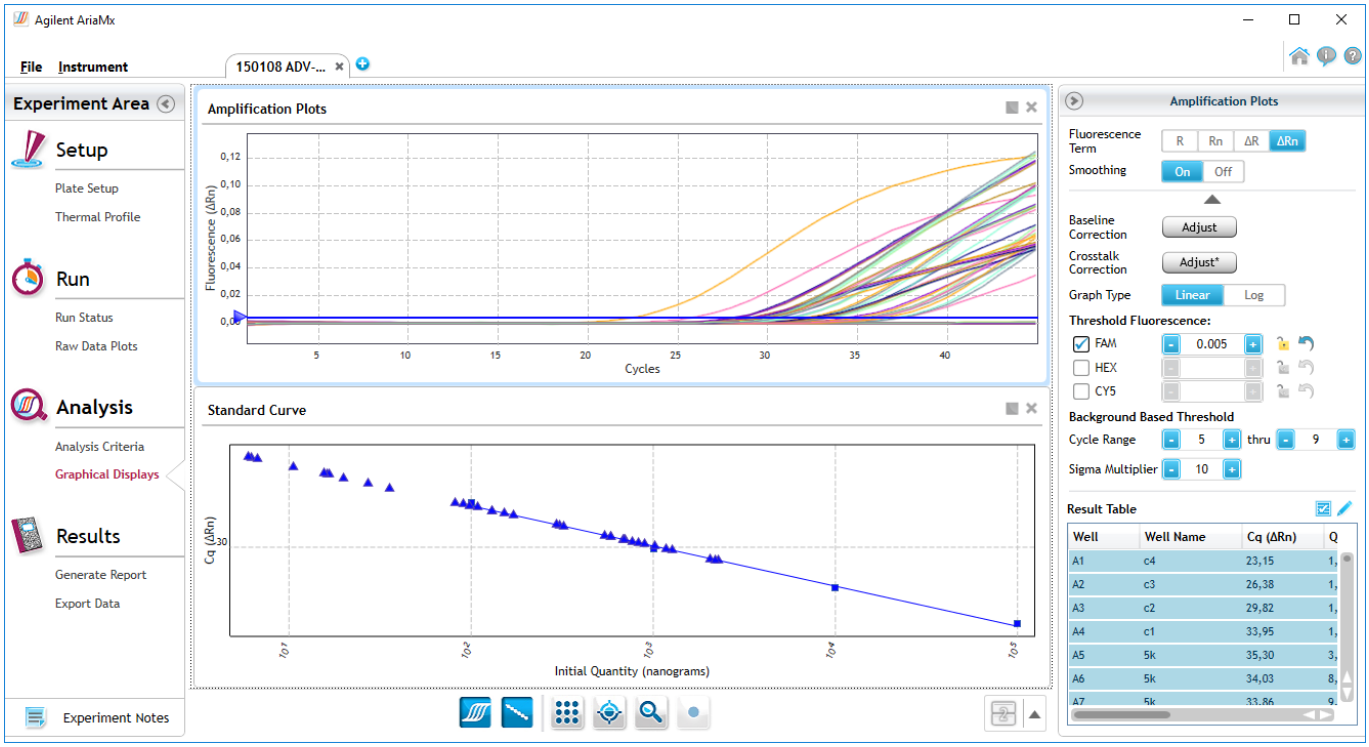

Fig. 1.6 Amplification curves of the monitored microorganism

Cq values are displayed in the **Results Table**. It can be enlarged by clicking the ▲ icon.

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 the **Amplification Plots** window click the ▼ icon for uncovering of the advanced options.

2. Select **log** in the **Graph type** option, if not selected already.

3. Select a desired channel under the **Display Targets b**utton and in the **Threshold Fluorescence** part, after clicking on the 🔒 icon, allow the threshold setting.

4. Set the threshold just above the base noise by adjusting the threshold line in the graph.

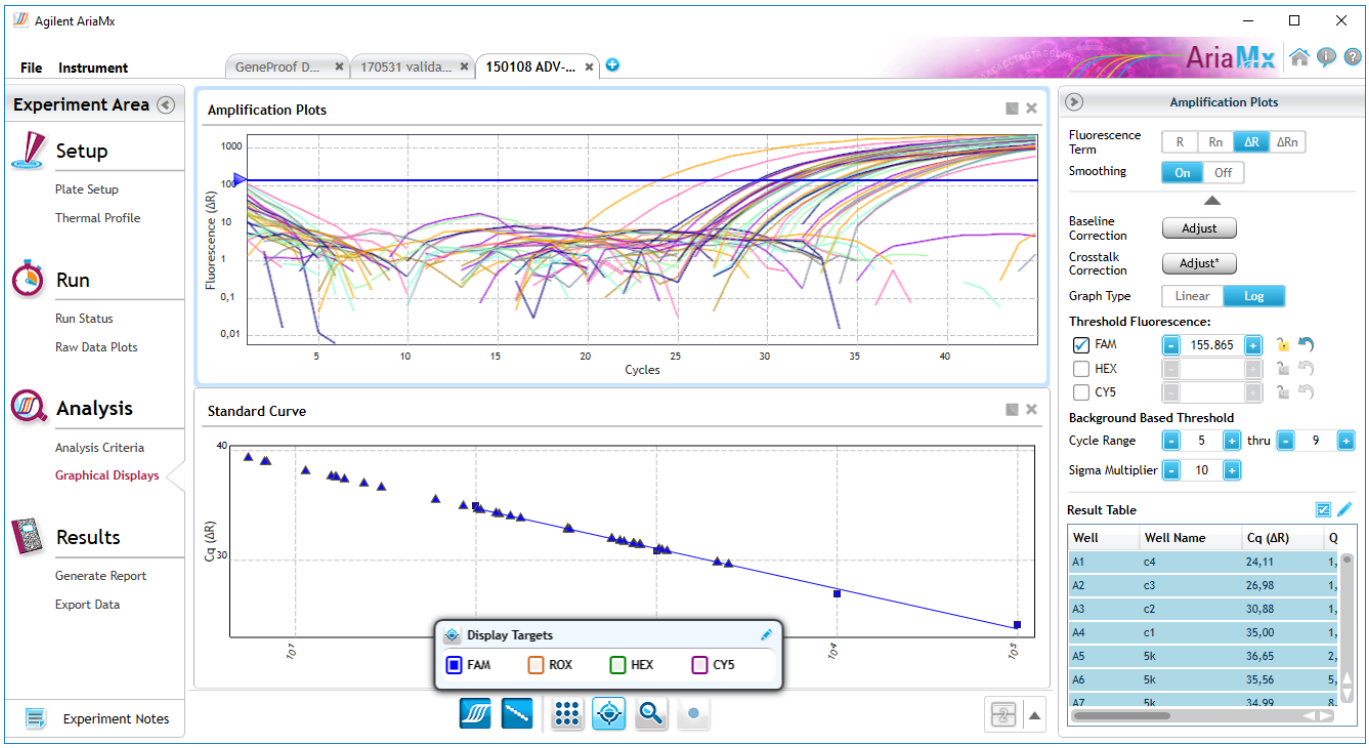

Fig. 1.7 Amplification curves in logarithmic scale

Ct values can be displayed by scrolling in **the Result Table** (as a Cq value), after enlarging it, by clicking on the icon ▲ .
Perform evaluation according to the instruction for use of the used GeneProof PCR kit.

1.5. Result quantitative analysis and detection evaluation

1. Click on the Standard curve graph and evaluate the calibration quality in the **Target information Table**. 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.

2. **Cq** and **Calculated Concentration** values are displayed in the **Results Table.**

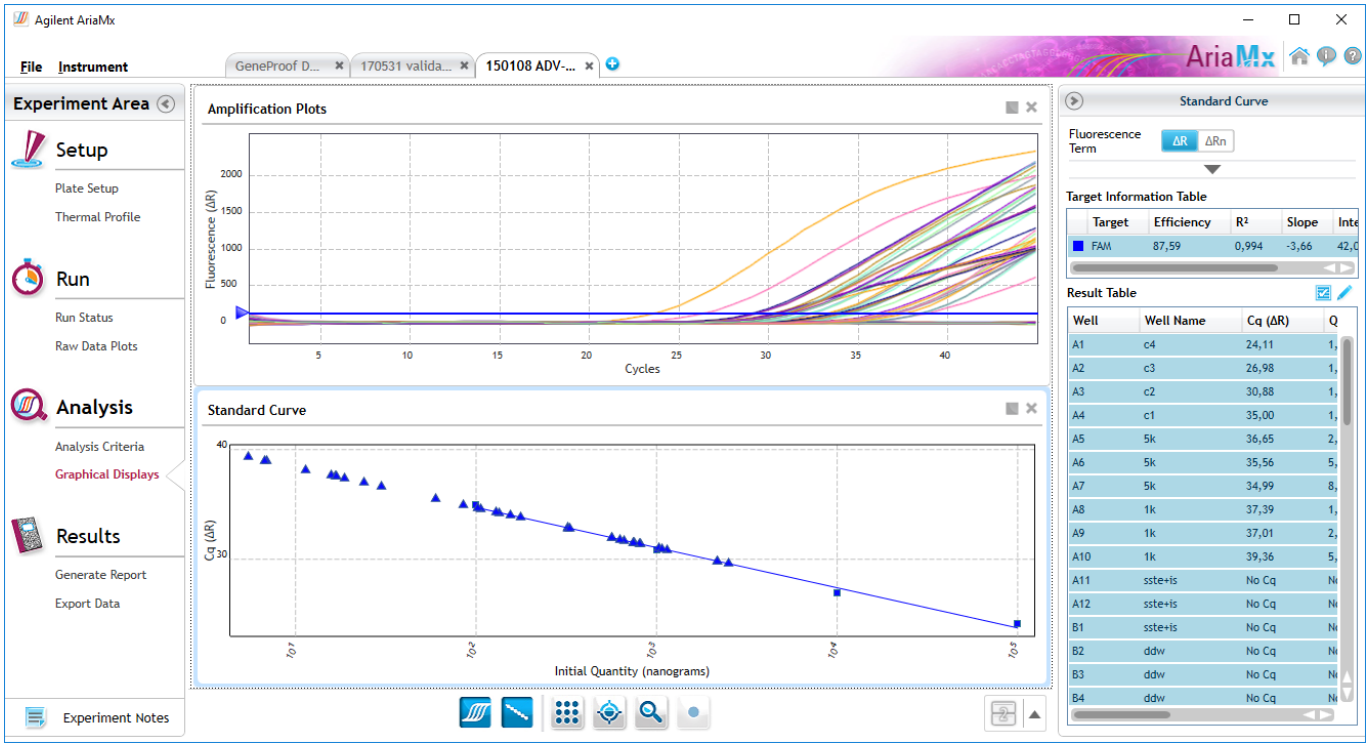

Fig. 1.8 Standard Curve and Sample Results

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

2. Genetic diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the AriaMx 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 Agilent AriaMX software.

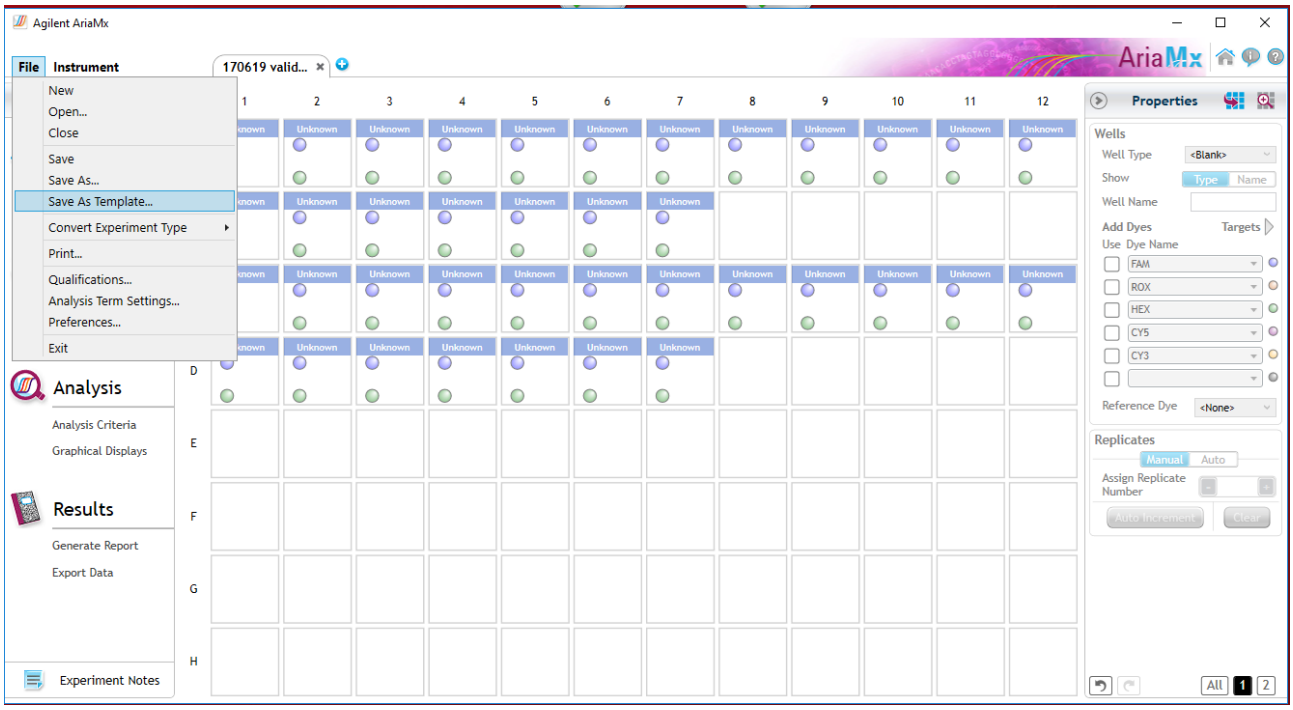

Fig. 2.1 Save template

After saving, the template can be opened from the file **Templates.** 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 Agilent AriaMX software.
2. In the **My Templates** tab, click on **browse to template** and select a template for the given PCR kit.

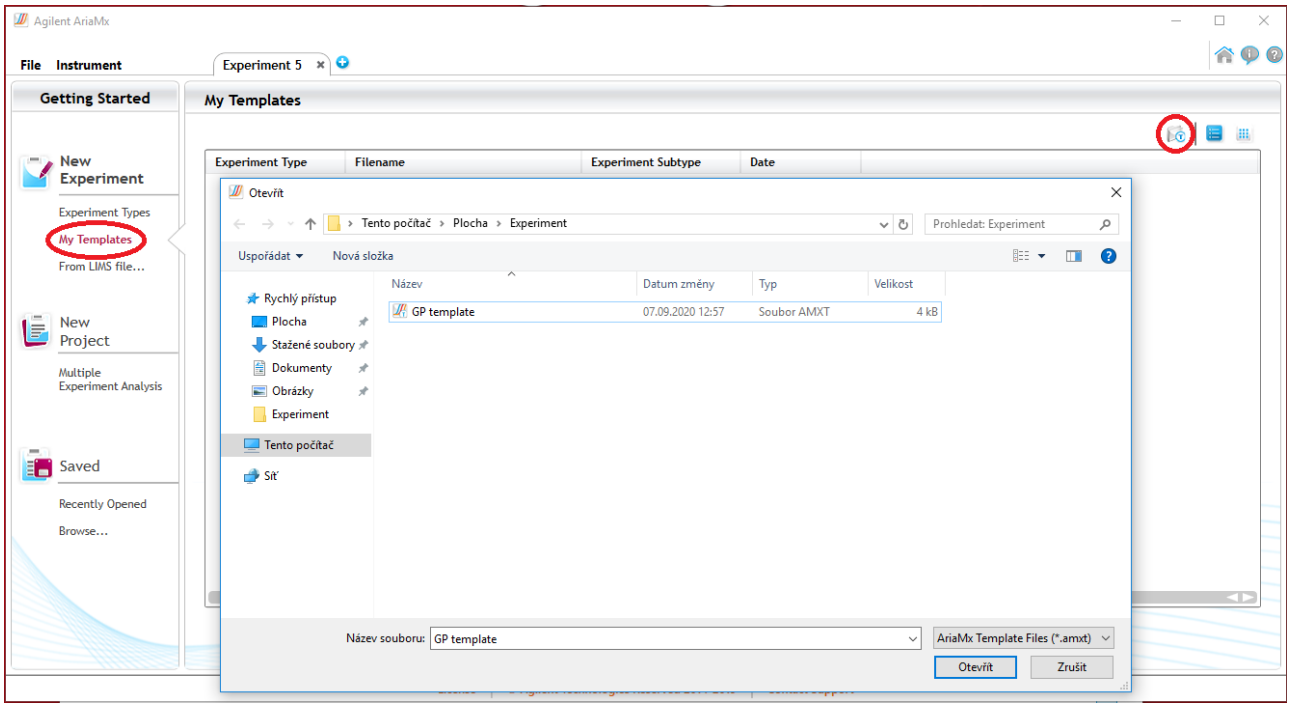

Fig. 1.1 Open template

For each type of examination separately:

1. Assign the appropriate targets, samples, and controls to the used wells of the PCR plate.
2. For each well of the PCR plate, set the appropriate **Well Name** according to the type of test, **Sample name** according to the name of the sample in the well and the **FAM** and **HEX** channels.
3. Set **Allele A**: FAM and **Allele B**: HEX
4. Define positive controls, e.g.:
   FII WT: **Sample name:** FII WT, **well type:** Homo Allele A
   FII MUT: **Sample name:** FII MUT, **well type:** Homo Allele B
   FII HEX: **Sample name:** FII HEX, **well type:** Hetero
5. Define negative controls, e.g.:
   FII NC: **Sample name:** FII NC, **well type:** NTC

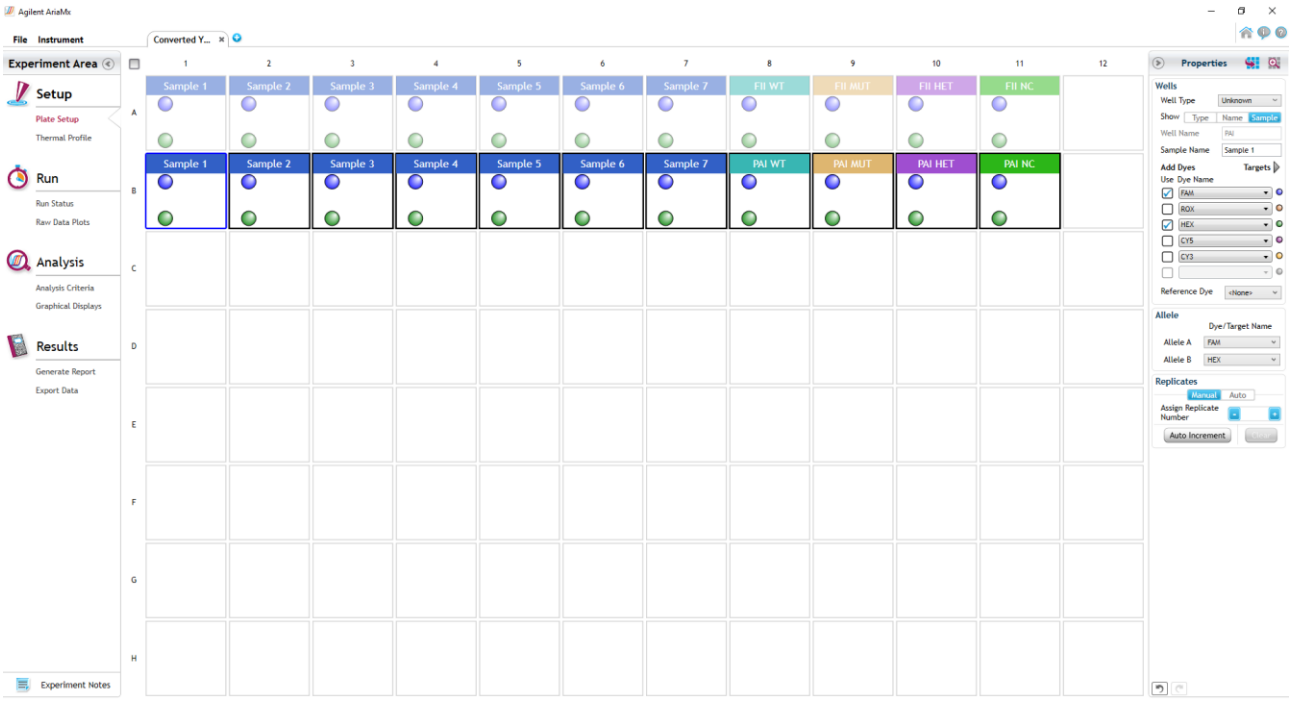

Fig. 2.2 Define targets and samples

2.2.2 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as the **AriaMx Experiment Files (*.amxd).** To make search easier it is recommended to create the Experiments folder.

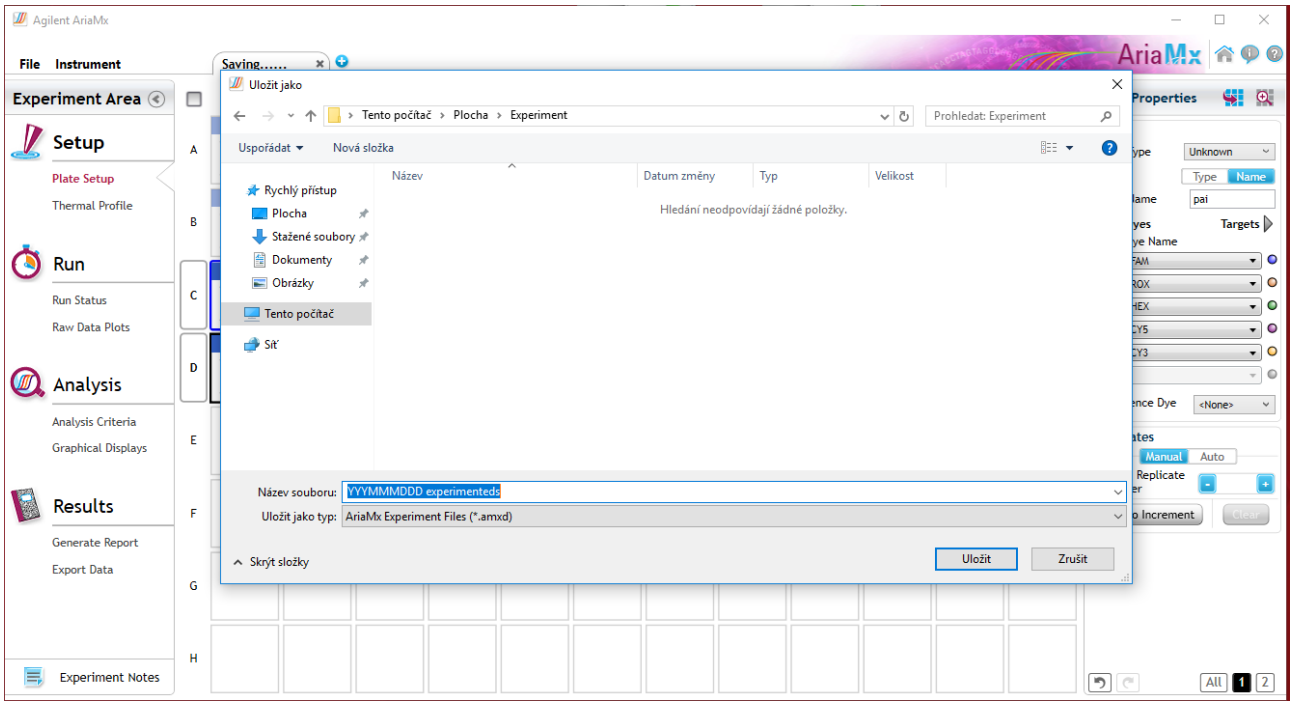

Fig. 2.2 Save experiment

2.3. Analysis of the result and evaluation of detection for genetic detection

In using more MasterMixes in one experiment (e.g. FII and PAI), is necessary to evaluate the result separatelly. (separately FII, separately PAI…).

2.3.3 Setting the Baseline and Treshold parametrs

1. In the **Analysis** column, select **Analysis Crieria**, mark only the positive controls WT, MUT and HET.

2. Switch to **Graphical Displays** and move the mouse Threshold lines in the **Amplifications Plots** graph: only the FAM curves of the WT and HET controls intersect the Threshold line for FAM; and only the HEX curves of the MUT and HET controls intersect the Threshold line for HEX. Lock both thresholds for further analysis.

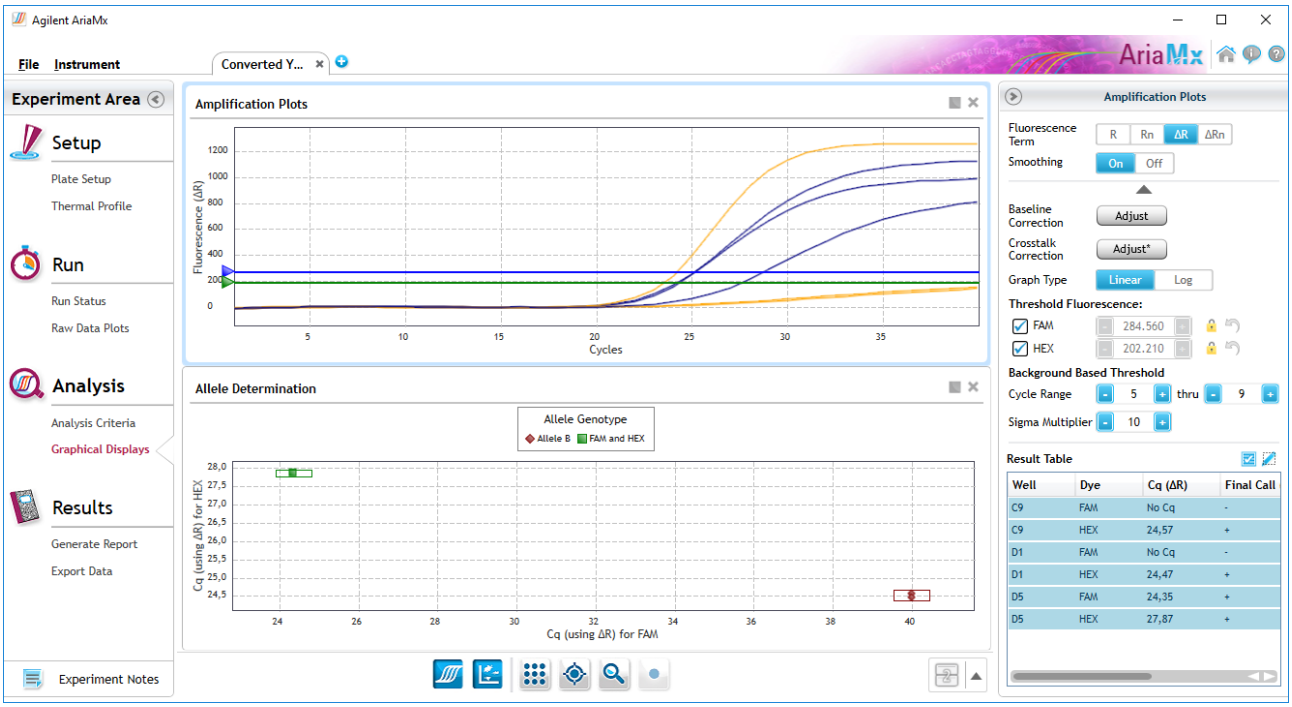

Fig. 2.3 Treshold Settings

2.3.4 *Evaluation*

1. In **Analysis Criteria**, mark samples for evaluation.
2. In **Graphical Displays** click **Allele Determination** graf and set **Genotype Calls: Auto.**

Fig. 2.4 Genotype Settings

3. The **genotype** is displayed in the Result Table in the **Genotype column**.

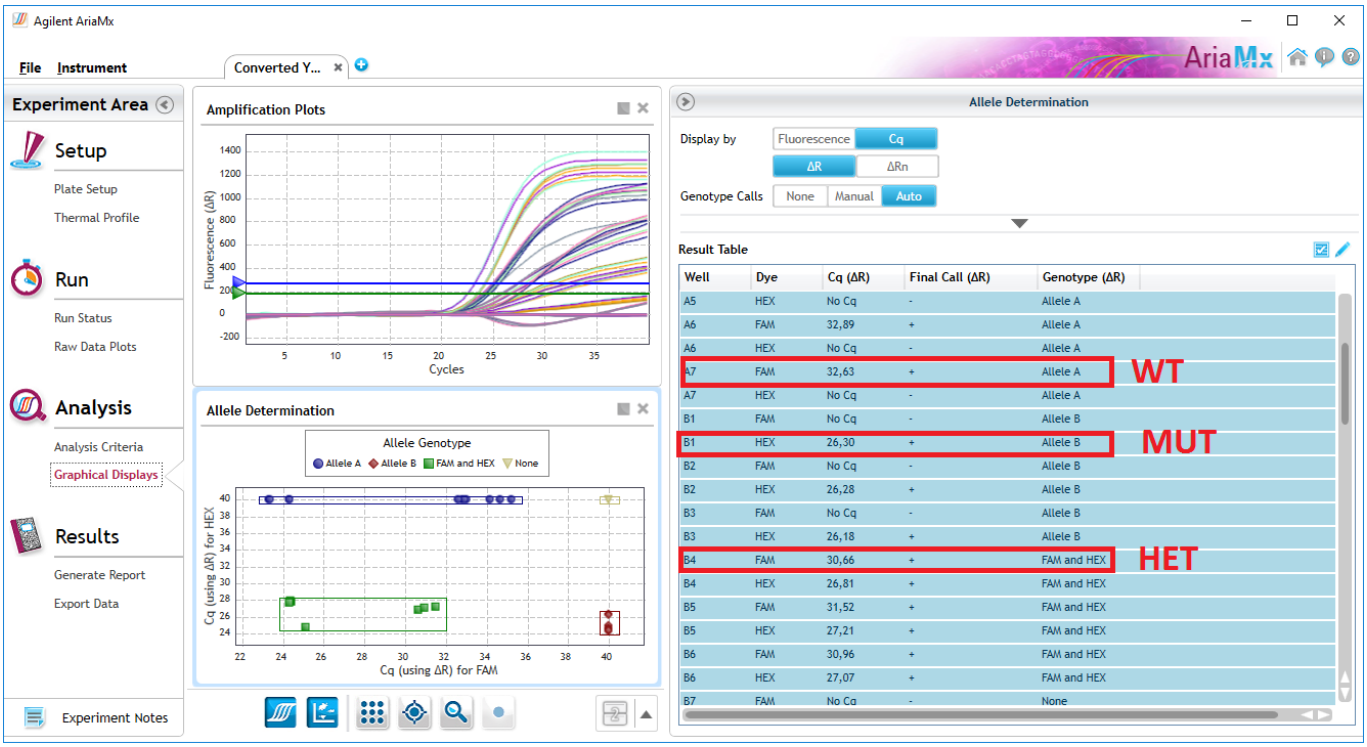

Fig. 2.5 Evaluation

2.3.5 Examples of typical curves

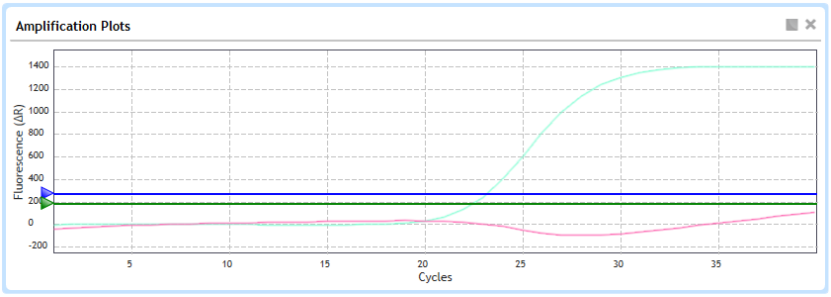

Fig. 2.6 Typical WT curve

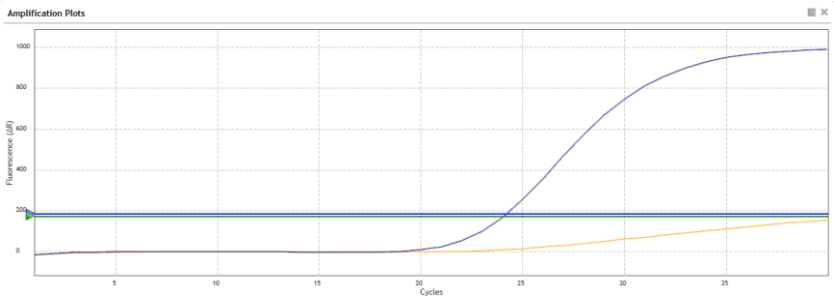

Fig. 2.7 Typical MUT curve

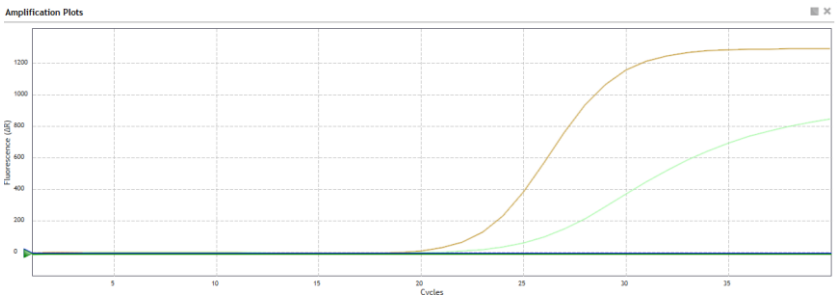

Fig. 2.8 Typical HET curve

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

Orders

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