GeneProof
Molecular diagnostics for your routine

# DEVICE MANUAL

QIAGEN

# Rotor-Gene™ 3000
# Rotor-Gene™ 6000
# Rotor-Gene™ Q

**Designed for GeneProof diagnostic kits**

**See www.geneproof.com for the current kits list**

# CONTENTS

# 1. Microbiologic diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for microbiological diagnostics using the instruments Rotor-Gene 3000, Rotor-Gene 6000 and Rotor-Gene Q.

## 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 software Rotor-Gene Q series Software.exe.

**Please note: In case of two channel kit, leave all Green(FAM), Yellow(HEX) and Orange(Tex) detection channels on.**

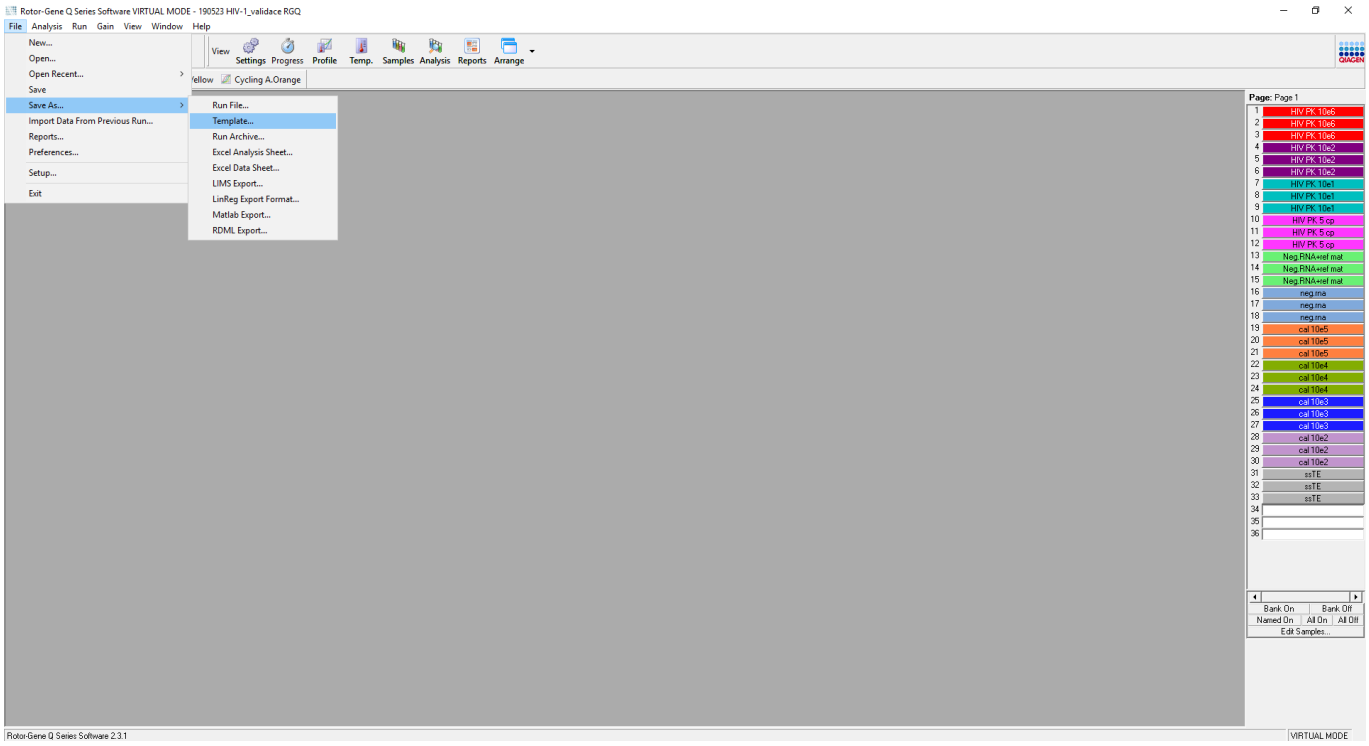


*Fig 1.1 Save template*

After saving, the template can be opened from the file **Templates.**

With each next usage of GeneProof PCR kits continue from the chapter **1.3 Starting the PCR amplification**

## 1.3. Starting the PCR amplification

1. Open **Rotor-Gene Q series Software.exe** software.
2. In the left part of the upper bar click on the button **New** and choose the template for the used GeneProof PCR kit.

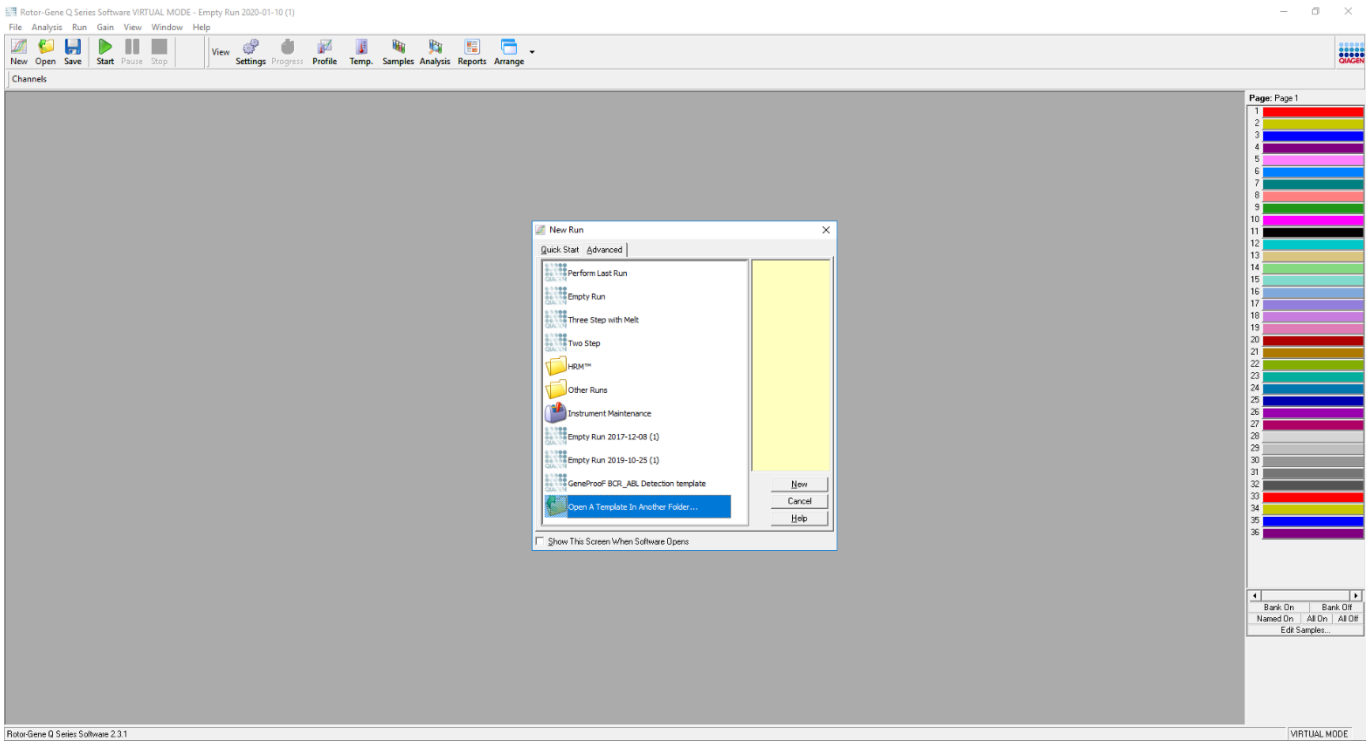



*Fig 1.2 Open saved template*

3. Select **36-Well Rotor** and check **Locking Ring Attached** (for RG 3000 check **No Domed 0.2 ml Tubes**). Click **Next** to continue.

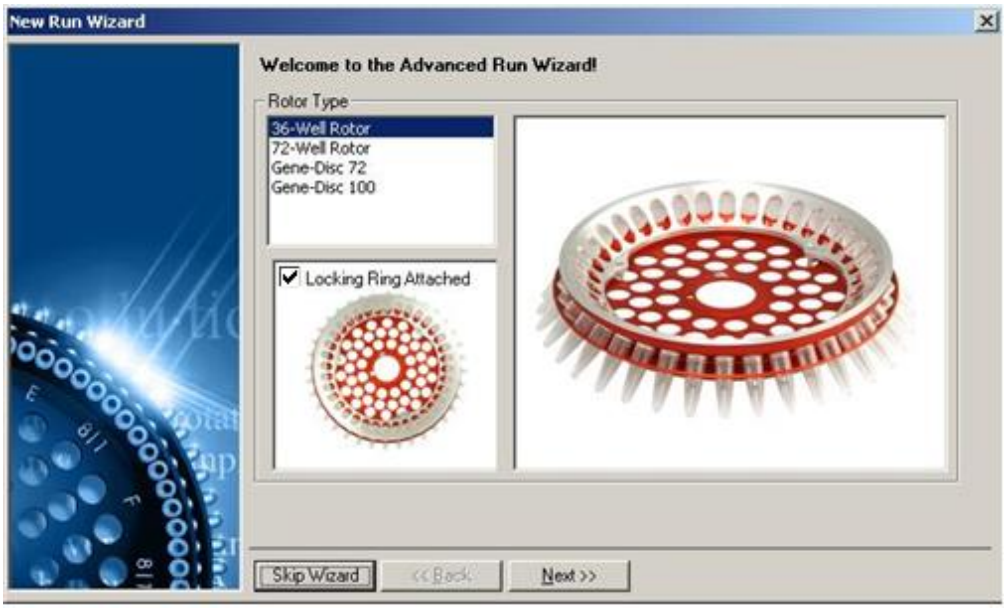



*Fig. 1.3 Set the device input parameters*

4. Fill out the fields **Operator** and **Notes** according to the lab requirements. In the **Reaction Volume (µL)** field enter the correct volume according to the instruction for use of the used GeneProof PCR kit. Click **Next** to continue.

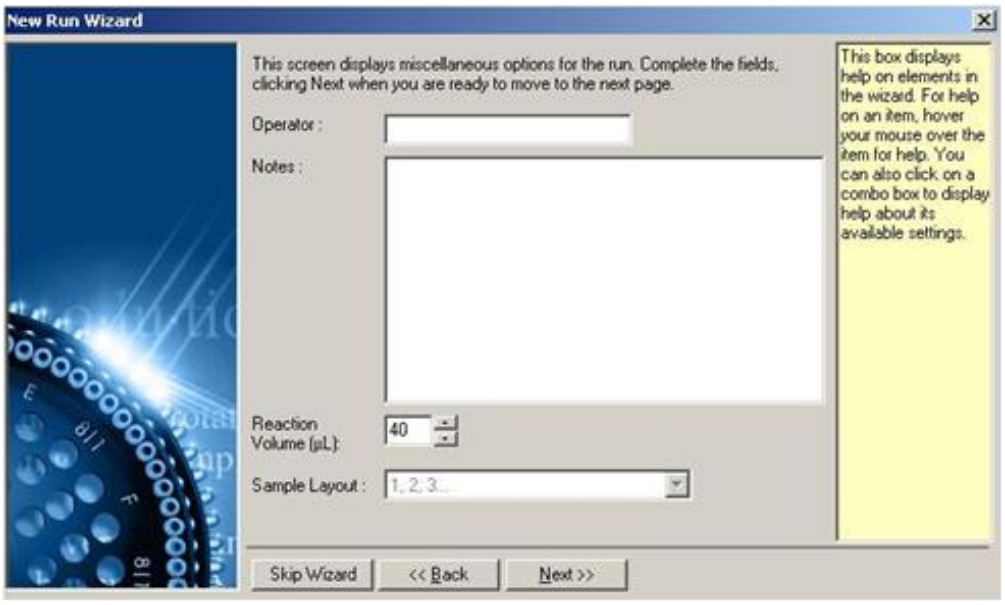



*Fig. 1.4 Set the program input parameters*

## 1.4. Gain calibration settings

1. Click **Gain Optimisation** (RG 6000/RG Q) or **Calibrate**(RG 3000) in the **New Run Wizard** box.

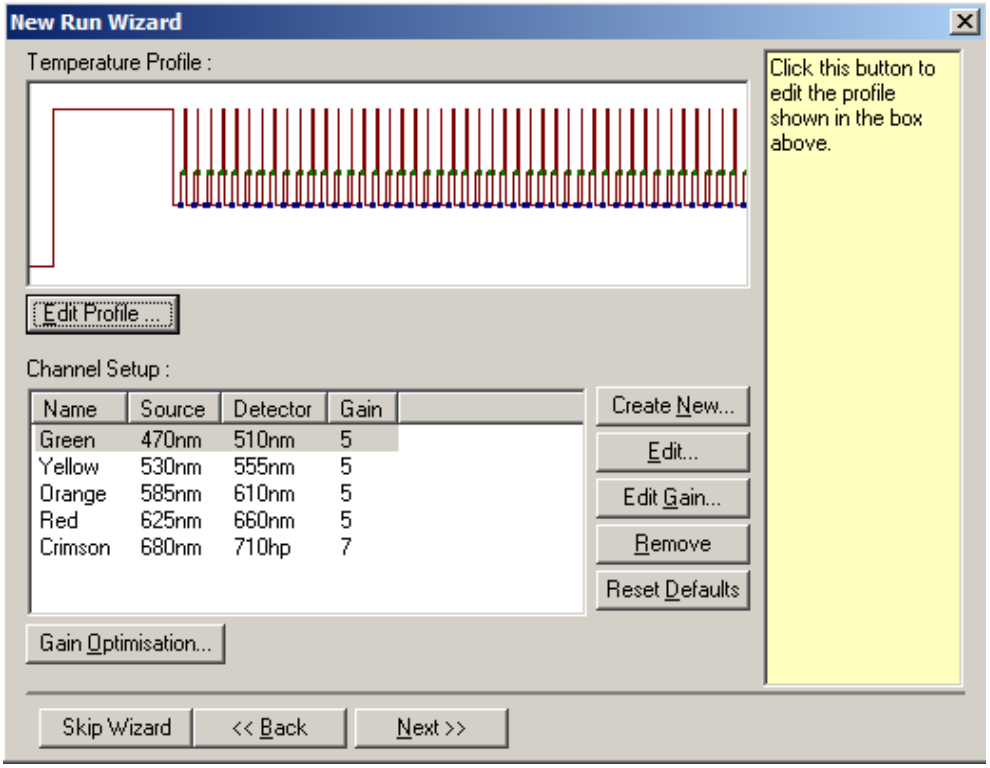



*Fig. 1.5 Edit Gain RG 6000/RG Q*

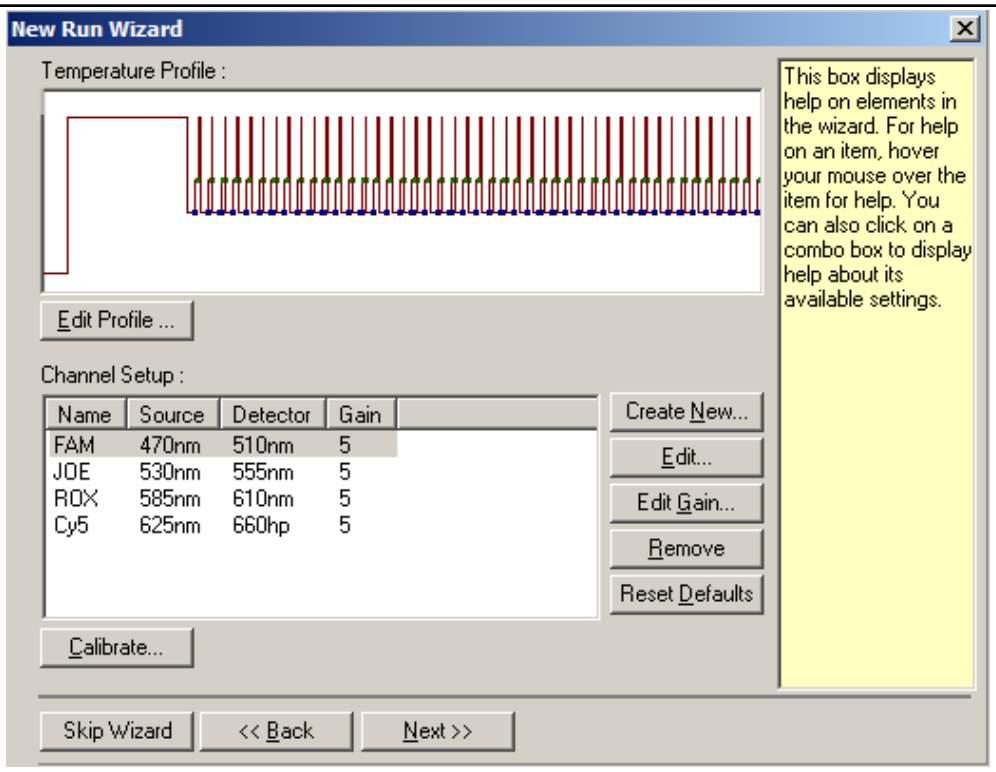



*Fig. 1.6 Edit Gain RG 3000*

2. Click **Optimise Acquiring**, check the individual channels by clicking the **OK** button and then check **Perform Optimisation Before 1st Acquisition** in the **Auto Gain Optimisation Setup** box. Click **Close** to close the box.

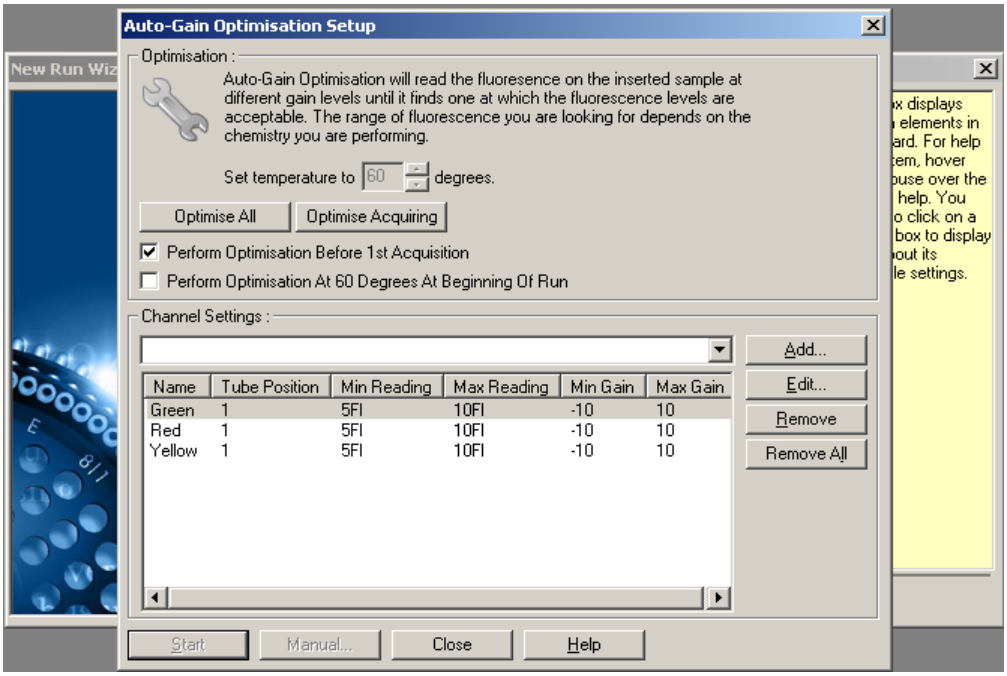



*Fig. 1.7 Set calibration for Gain RG 6000/RG Q*

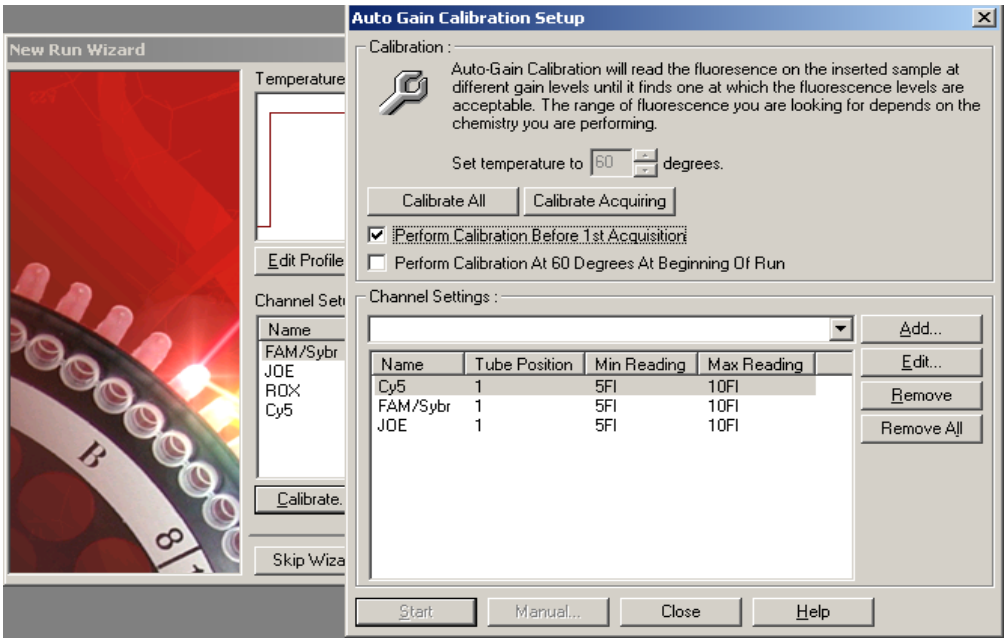



*Fig. 1.8 Set calibration for Gain RG 3000*

## 1.5. Amplification profile programming

When opening a run from a template, the thermal profile of the ampification is preprogrammed for the given GeneProof PCR kit, so there is no need for editing it.

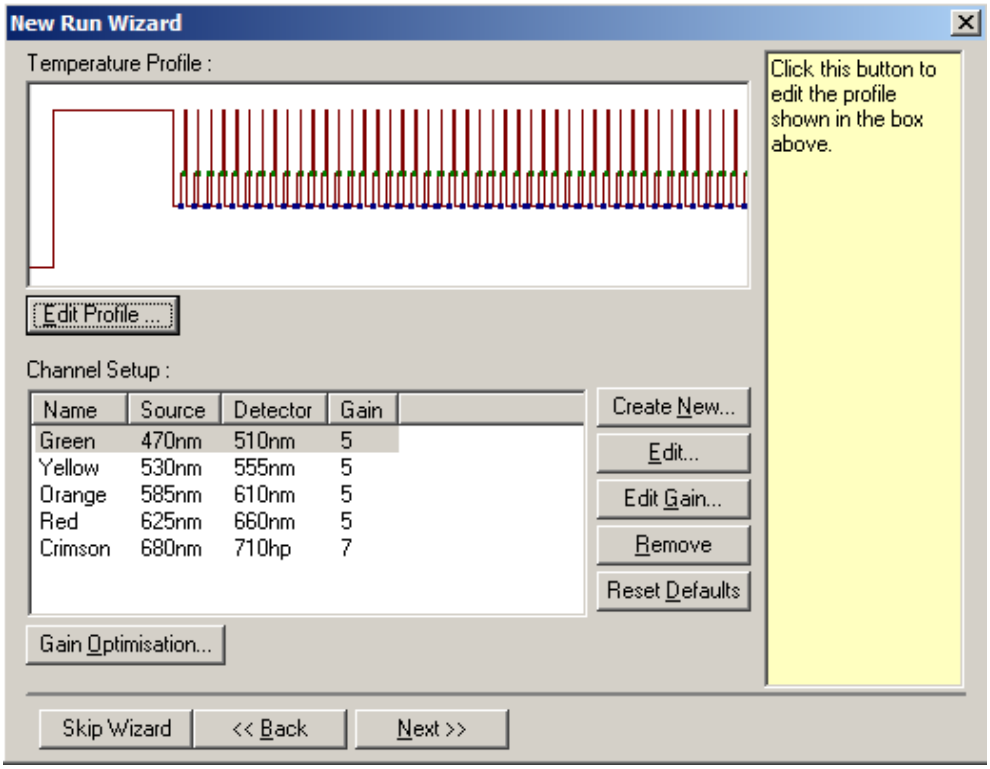



*Fig. 1.9 No need for editing the thermal profile of amplification*

## 1.6. PCR Amplification Start

1. Click **Next** in the **New Run Wizard** screen.
2. Click **Start Run** to run the program and save the protocol into a PC folder.

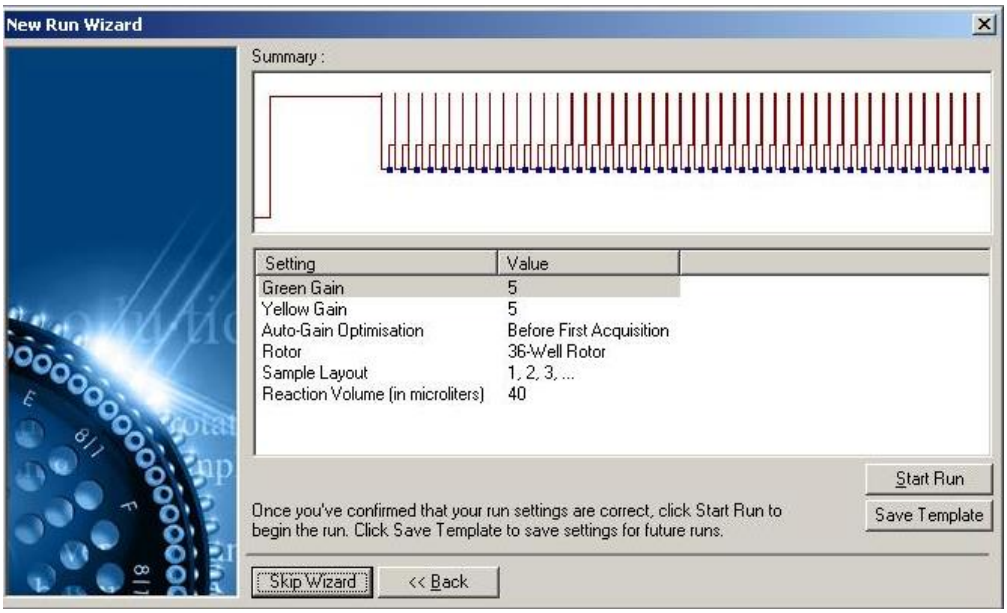



*Fig. 1.10 Start amplification*

4. After the protocol has been saved and the program started the **Edit Samples** box pops up. Fill in the required sample data depending on the used qualitative or quantitative detection method (see chapters **2.7** and **2.8** – **Result qualitative/quantitative analysis and detection evaluation**).

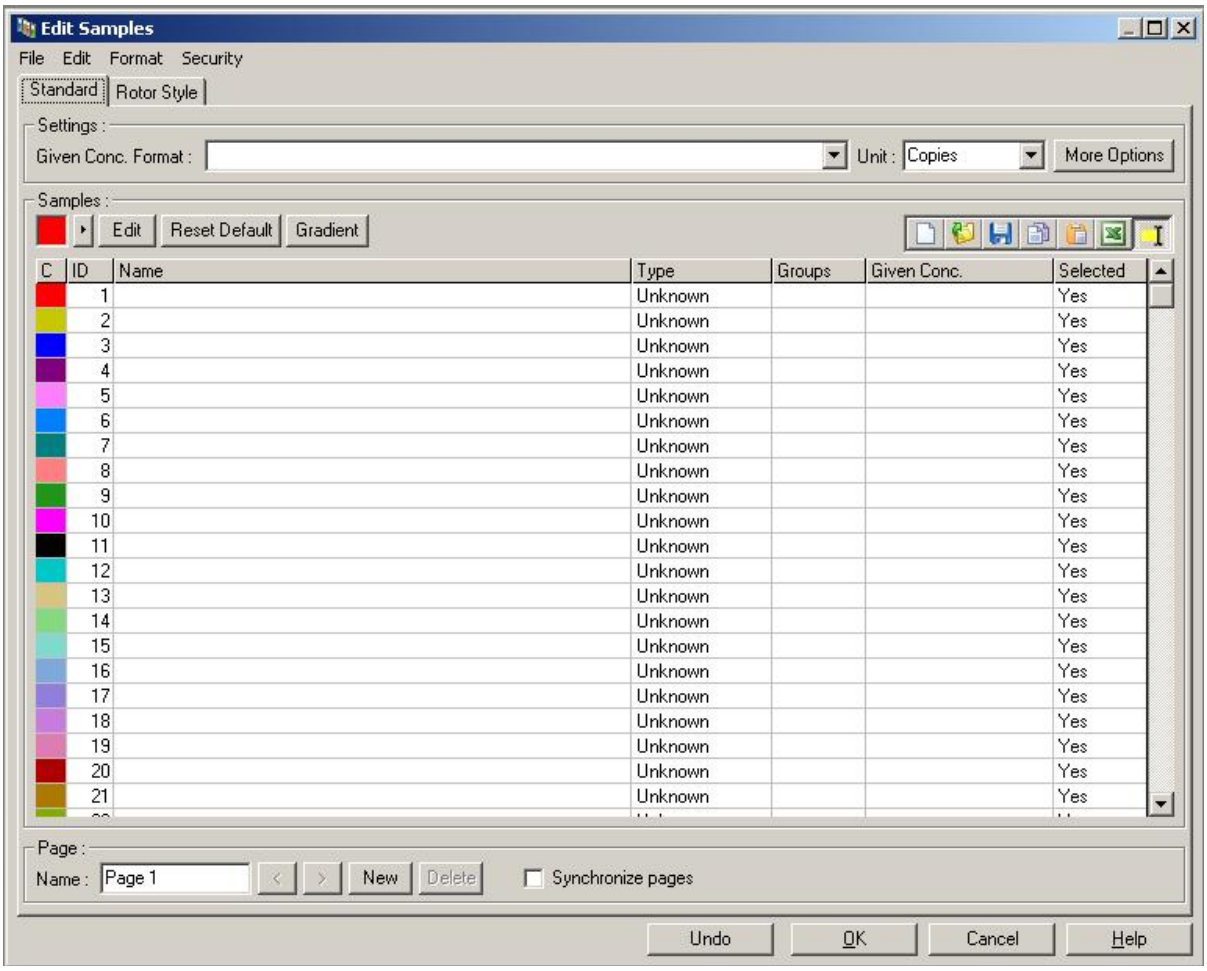



*Fig. 1.11 Inserted samples description*

5. Click **OK**.

## 1.7. Result qualitative analysis and detection evaluation in microbiologic diagnostics

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

1. Select values for the detection qualitative analysis in the **Edit Samples** box of the **Samples** tab. **Positive Control** and **Negative Control** are the minimum required samples for a valid assessment of a PCR reaction.

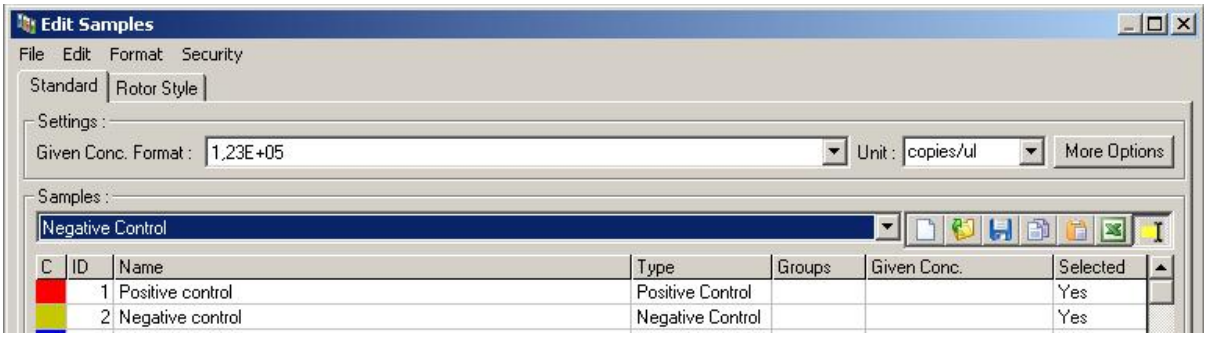



*Fig. 1.12 Sample parameter settings in the Samples tab*

2. When the program is finished, click **Analysis** in the main menu and select **Quantification**.

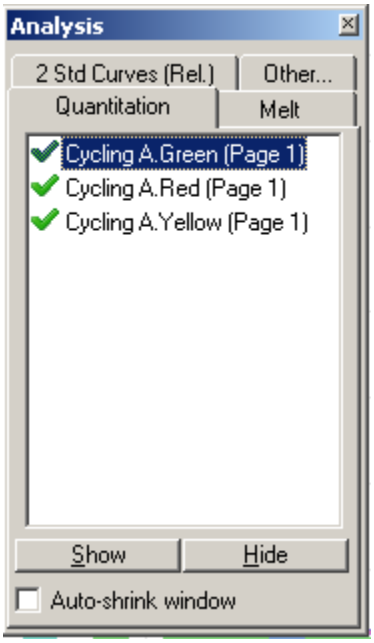



*Fig. 1.13 Analysis selection*

### *1.7.1Positive sample assessment in the Linear Scale*

The manufacturer recommends using this method of the detection result assessment as the first option and also if clearly positive samples with the Ct value lower than 40 are present. For assessing samples with Ct values higher then 40 it is recommended to use the Log. Scale (see **1.7.2 Positive sample assessment in the Log. Scale**).

1. Select **Cycling A.Green and Red** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.Yellow** for Internal Standard/Internal Control detection assessment. Press **Show**.
2. Check the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
3. Enter **NTC 0** in the **Outlier Removal** tab. Select **Linear Scale**.
4. Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

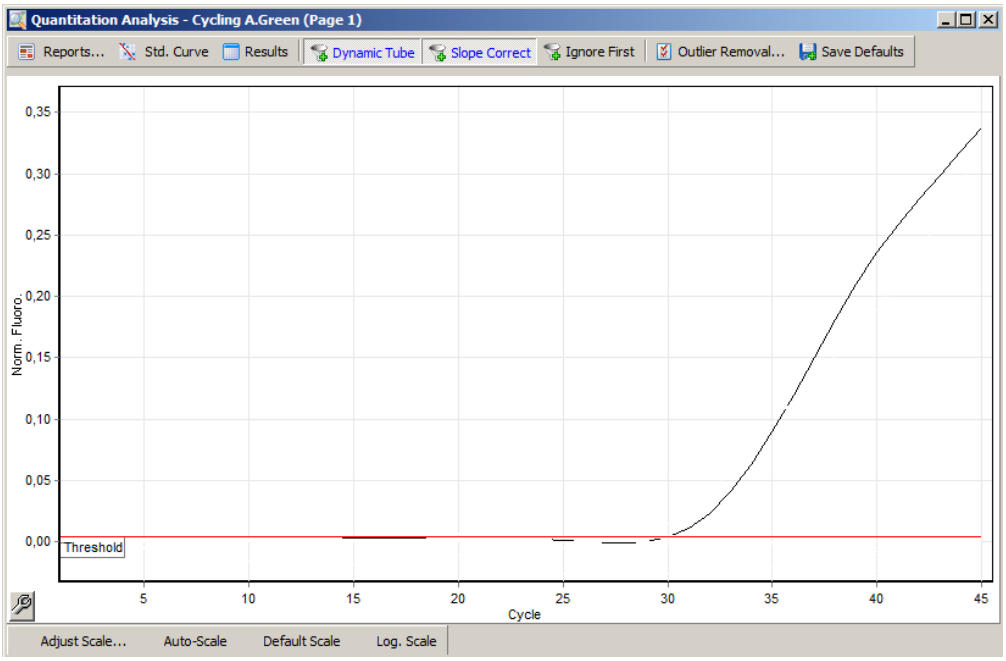



*Fig. 1.14 Positive sample assessment in the Linear Scale*

### *1.7.2 Positive sample assessment in the Log. Scale*

This assessment method can reveal even weakly positive samples, which could be missed out when using the Linear Scale assessment. Any sample with Ct value exceeding 40 should be considered a weakly positive sample requiring this method of assessment. When using this assessment method you should only work with the sample under assessment, all other samples must be “turned off”!

1. Select **Cycling A.Green and Red** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.Yellow** for Internal Standard/Internal Control detection assessment. Press **Show**.
2. Check the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
3. Enter **NTC 0** in the **Outlier Removal** tab. Select **Log. Scale**.
4. Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

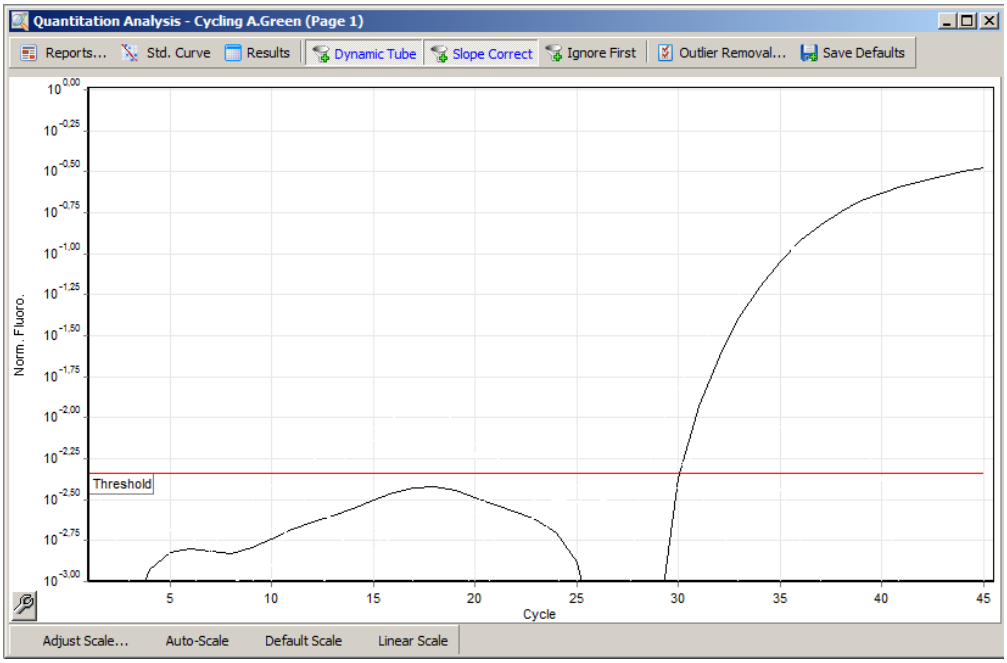


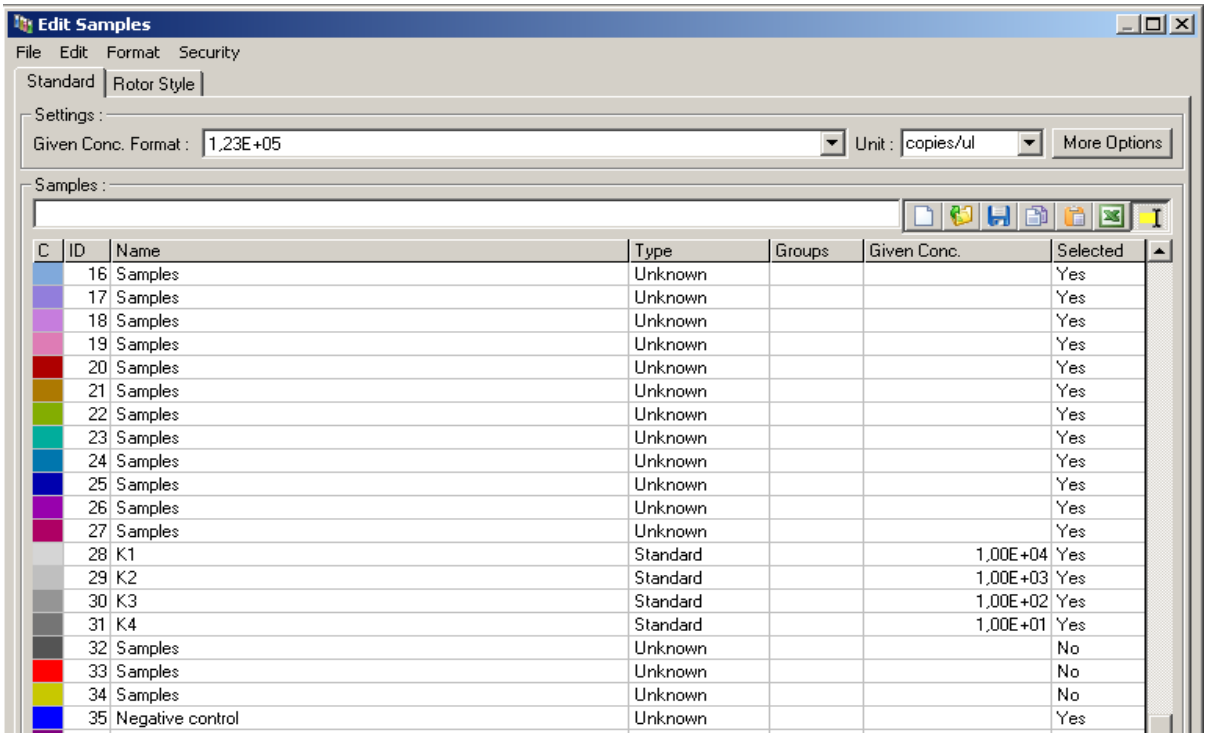

*Fig. 1.15 Assessment of weakly positive samples in the Log. Scale*

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

## 1.8. Result quantitative analysis and detection evaluation in microbiological diagnostics

Quantitative analysis should be performed for samples evaluated as positive in the course of the qualitative analysis procedure.

1. Select values for the detection quantitative analysis in the **Samples** tab. **Calibration Control** (enter **Standard** into the **Type** column and then enter the concentration of the inserted calibration control into the **Given Conc.** column) and **Negative Control** are the minimum required samples for a valid assessment of a PCR reaction.




| C | ID | Name | Type | Groups | Given Conc. | Selected |
|---|---|---|---|---|---|---|
| | 16 | Samples | Unknown | | | Yes |
| | 17 | Samples | Unknown | | | Yes |
| | 18 | Samples | Unknown | | | Yes |
| | 19 | Samples | Unknown | | | Yes |
| | 20 | Samples | Unknown | | | Yes |
| | 21 | Samples | Unknown | | | Yes |
| | 22 | Samples | Unknown | | | Yes |
| | 23 | Samples | Unknown | | | Yes |
| | 24 | Samples | Unknown | | | Yes |
| | 25 | Samples | Unknown | | | Yes |
| | 26 | Samples | Unknown | | | Yes |
| | 27 | Samples | Unknown | | | Yes |
| | 28 | K1 | Standard | | 1,00E+04 | Yes |
| | 29 | K2 | Standard | | 1,00E+03 | Yes |
| | 30 | K3 | Standard | | 1,00E+02 | Yes |
| | 31 | K4 | Standard | | 1,00E+01 | Yes |
| | 32 | Samples | Unknown | | | No |
| | 33 | Samples | Unknown | | | No |
| | 34 | Samples | Unknown | | | No |
| | 35 | Negative control | Unknown | | | Yes |

*Fig. 1.16 Inserted samples description*

2. When the program is finished, click **Analysis** in the main menu and select **Quantification** (see Fig. 1.13).

*1.8.1Setting of a detection quantitative assessment*

1. Select **Cycling A.Green and Red** (if available) for the quantitative assessment of the **positive signal** detection.
2. Click **Show**.
3. Check the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
4. Enter **NTC 0** in the **Outlier Removal** tab. Select **Log. Scale**.
5. Click **Auto-Find Threshold** for an automatic detection assessment. Threshold can be set manually. For valid evaluation **R^2** value of standard curve must be **≥ 0,98.**

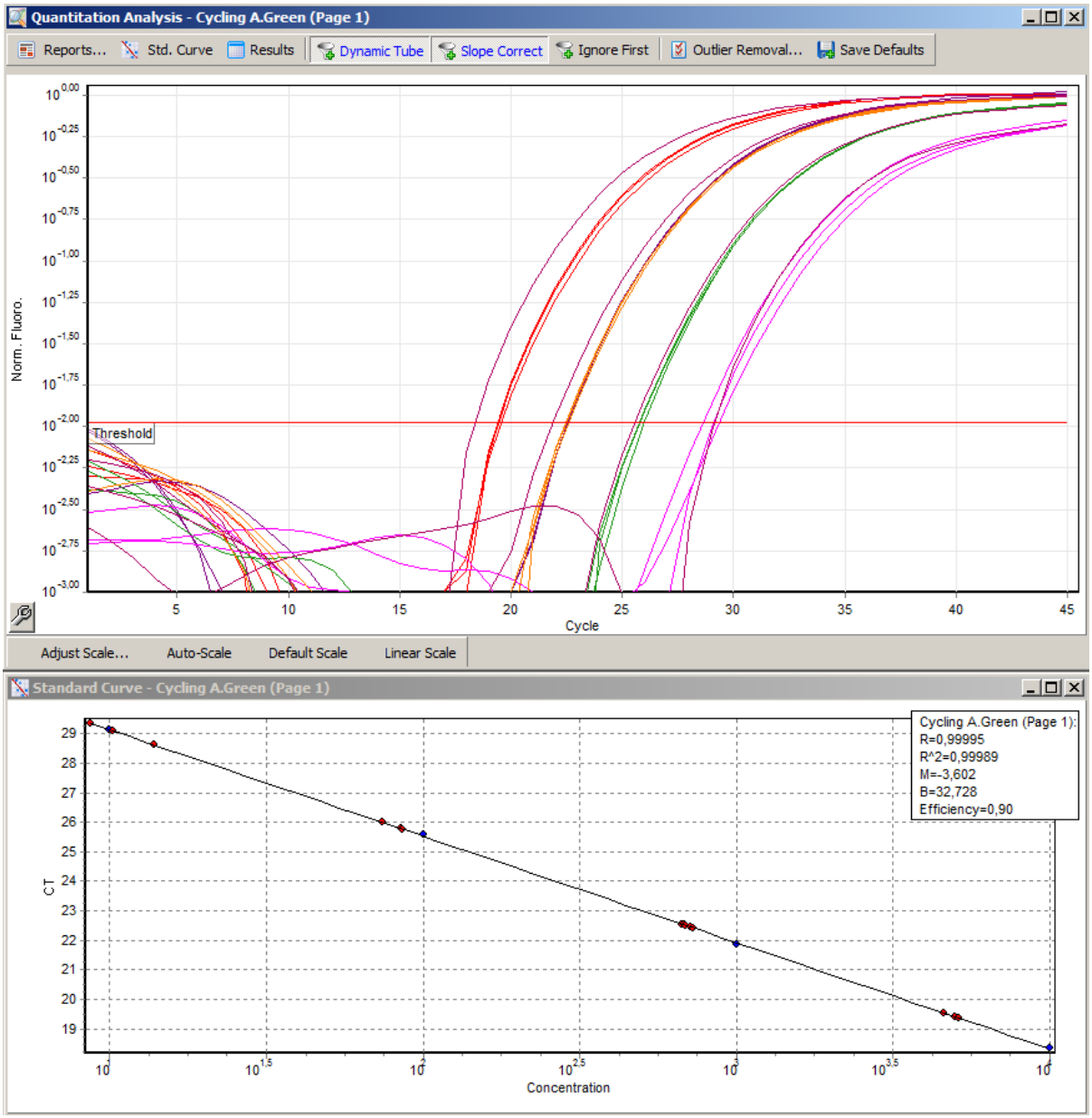



*Fig. 1.17 Quantitative evaluation*

The table with results is shown below the chart. 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.

## 1.9. Troubleshooting

Some specific Rotor-Gene devices demonstrate overvaluation of input temperatures by 2 – 4 °C (even immediately after the device validation by its manufacturer). This overvaluation, especially of the annealing temperature, could impact sensitivity, total fluorescence and the amplification curve shapes:

### *1.9.1Low sensitivity*

We recommend verifying sensitivity using a simple test.

1. Prepare control (calibrator) dilution with the concentration of $10^2$ according to the following table:

*Table 1.1 Control dilution*

| Kit version | ISIN | | ISEX | |
|---|---|---|---|---|
| Resulting concentration | $10^1$ copies | 5 copies | $10^1$ copies | 5 copies |
| Control (calibrator) | 10µl $10^2$ | 50µl $10^1$ | 10µl $10^2$ | 50µl $10^1$ |
| Internal Standard | - | - | 10µl | 10µl |
| NFW | 90µl | 50µl | 80µl | 40µl |
| Total volume | 100µl | 100µl | 100µl | 100µl |

2. Prepare 3 samples of negative DNA (note for the ISEX version - mix 90µl of DNA with 10µl of Internal Standard).
3. Pipette 30 µl of MasterMix and add 10 µl of the sample into the PCR tubes according to the following table:

*Table 1.2 PCR preparation*

| Tube sequence | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Sample | $10^2$ copies | $10^1$ copies | 5 copies | 5 copies | 5 copies | Neg. DNA 1 | Neg. DNA 2 | Neg. DNA 3 |
| Assumed result of the pathogen detection (FAM) | + | + | + | + | + | - | - | - |
| Assumed result of the Internal Standard detection (HEX) | + | + | + | + | + | + | + | + |

Samples with the concentration of 5 copies (tubes 3-5) should be positive in all 3 repetitions and Internal Standard should be detected in all samples. If this is not true, lower the annealing temperature to **58°C** and repeat the test.

If this problem persists, please contact Technical Support.

### *1.9.2Invalid 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:

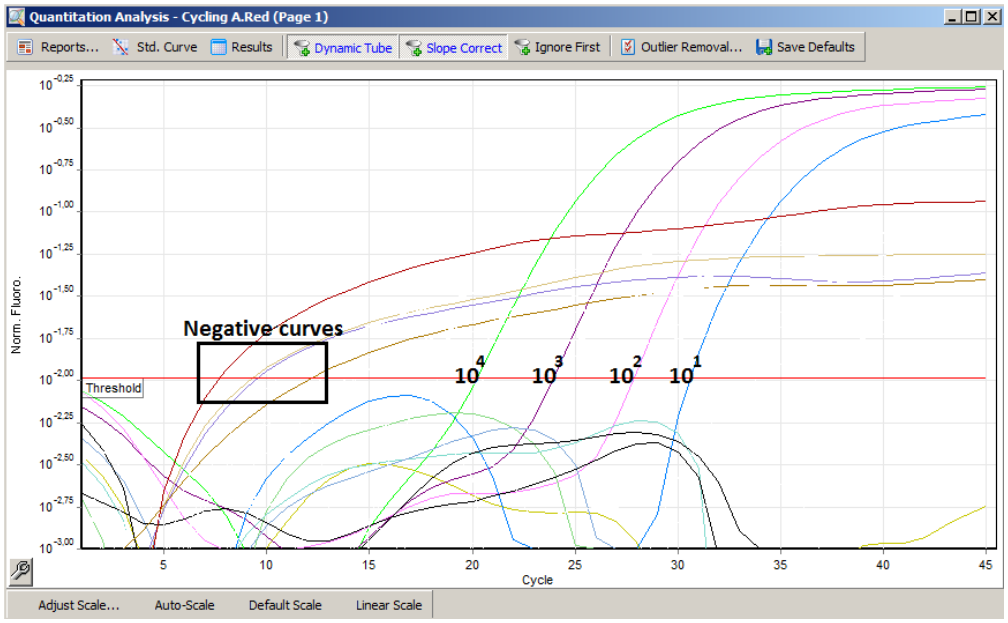



*Fig. 1.18 Negative curves in Log. Scale*

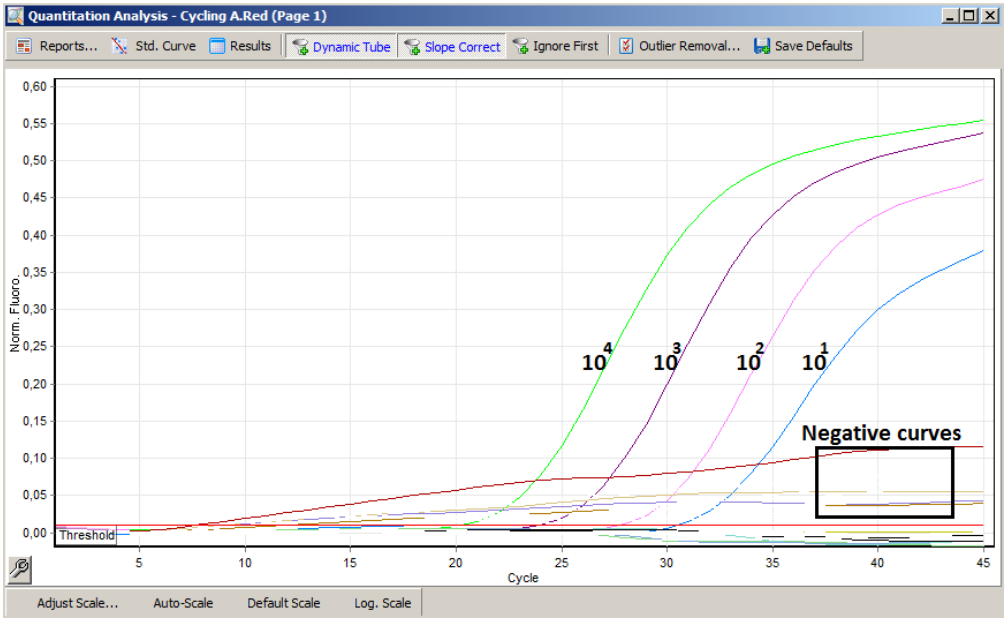



*Fig 1.19 Negative curves in Linear Scale*

**These curves have to be considered invalid and should be turned 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 Rotor-Gene 3000, Rotor-Gene 6000 and Rotor-Gene Q.

## 2.1. PCR Reaction Preparation

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

## 2.2. 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 software Rotor-Gene Q series Software.exe.

**Please note: In case of two channel kit, leave all Green(FAM), Yellow(HEX) and Orange(Tex) detection channels on.**

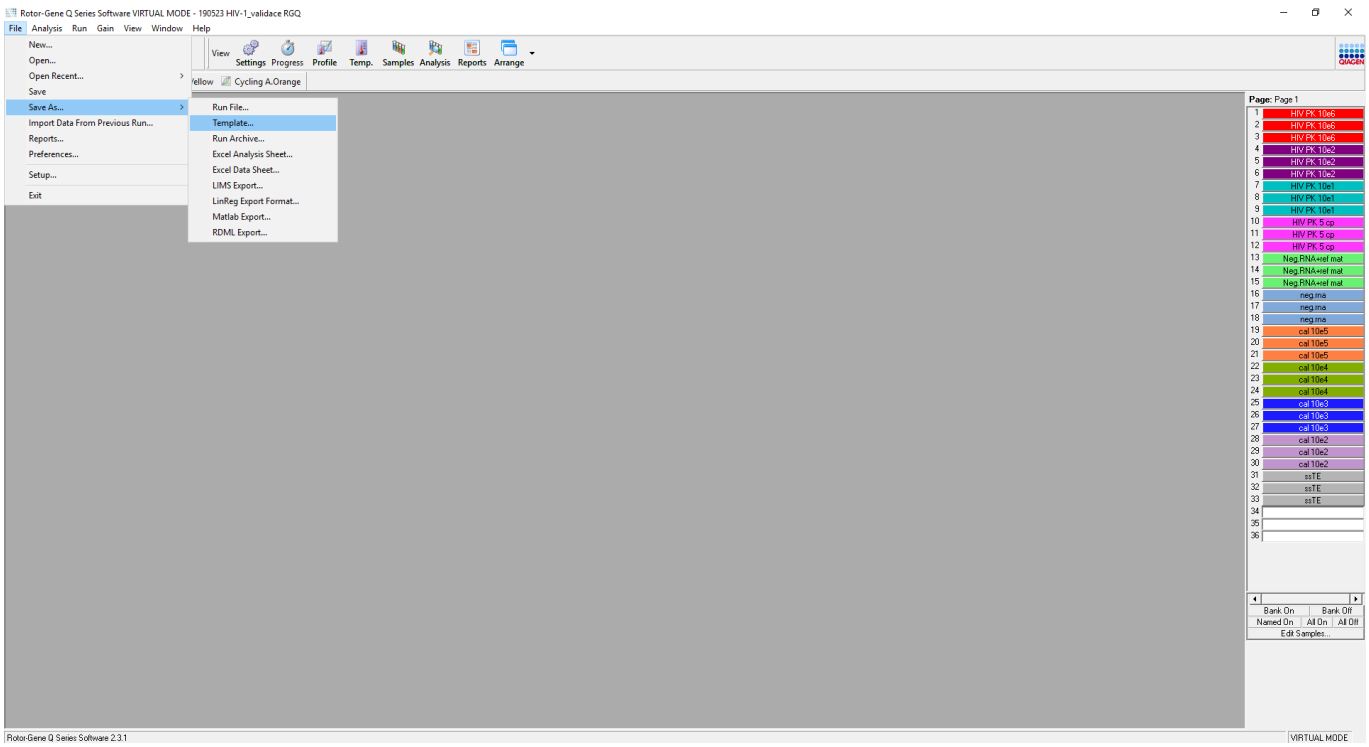


*Fig 2.1 Save template*

After saving, the template can be opened from the file **Templates.**

With each next usage of GeneProof PCR kits continue from the chapter **2.3 Starting the PCR amplification**

## 2.3. Starting the PCR amplification

1. Open **Rotor-Gene Q series Software.exe** software.
2. In the left part of the upper bar click on the button **New** and choose the template for the used GeneProof PCR kit.

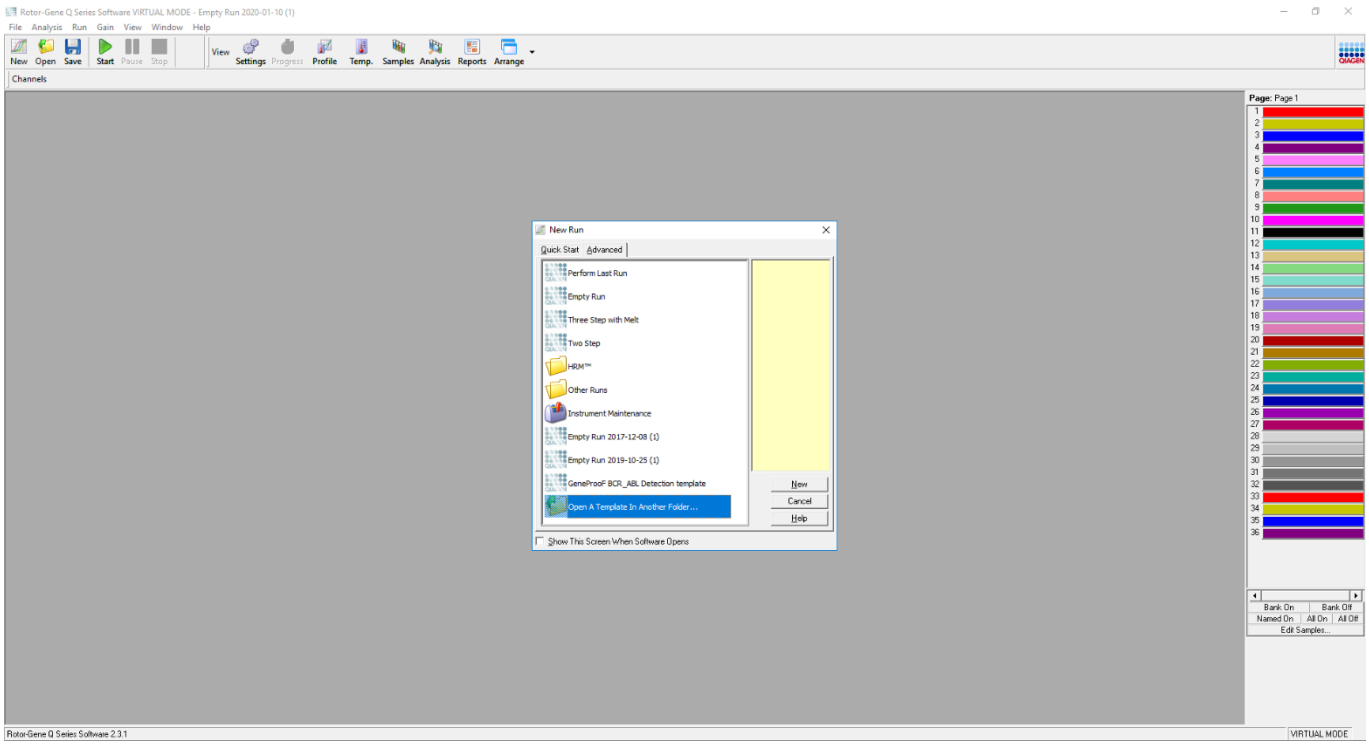



*Fig 2.3 Open saved template*

3. Select **36-Well Rotor** and check **Locking Ring Attached** (for RG 3000 check **No Domed 0.2 ml Tubes**). Click **Next** to continue.

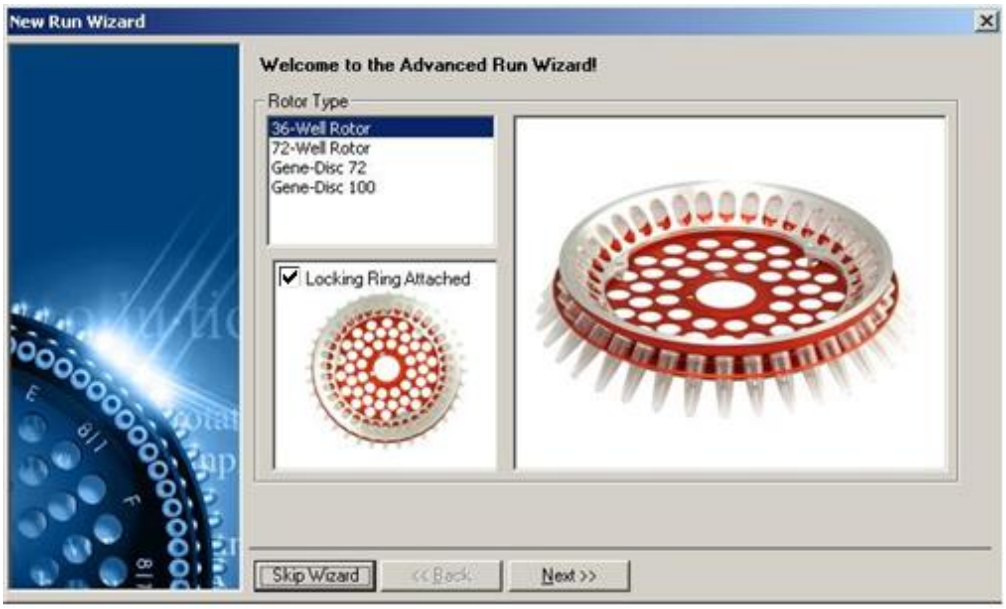



*Fig. 2.3 Set the device input parameters*

4. Fill out the fields **Operator** and **Notes** according to the lab requirements. In the **Reaction Volume (µL)** field enter the correct volume according to the instruction for use of the used GeneProof PCR kit. Click **Next** to continue.

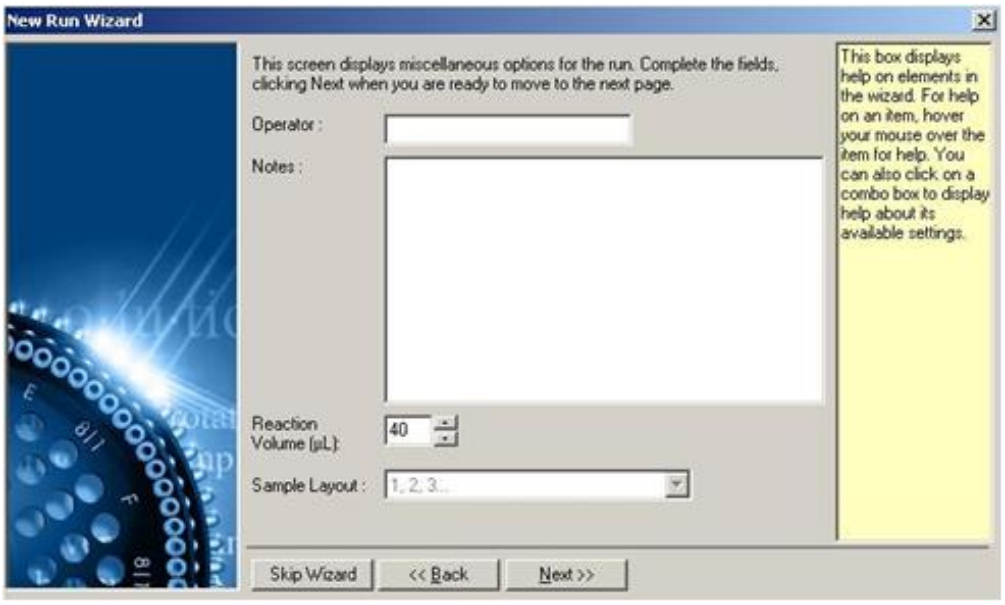



*Fig. 2.4 Set the program input parameters*

## 2.4. Gain calibration settings

1. Click **Gain Optimisation** (RG 6000/RG Q) or **Calibrate**(RG 3000) in the **New Run Wizard** box.

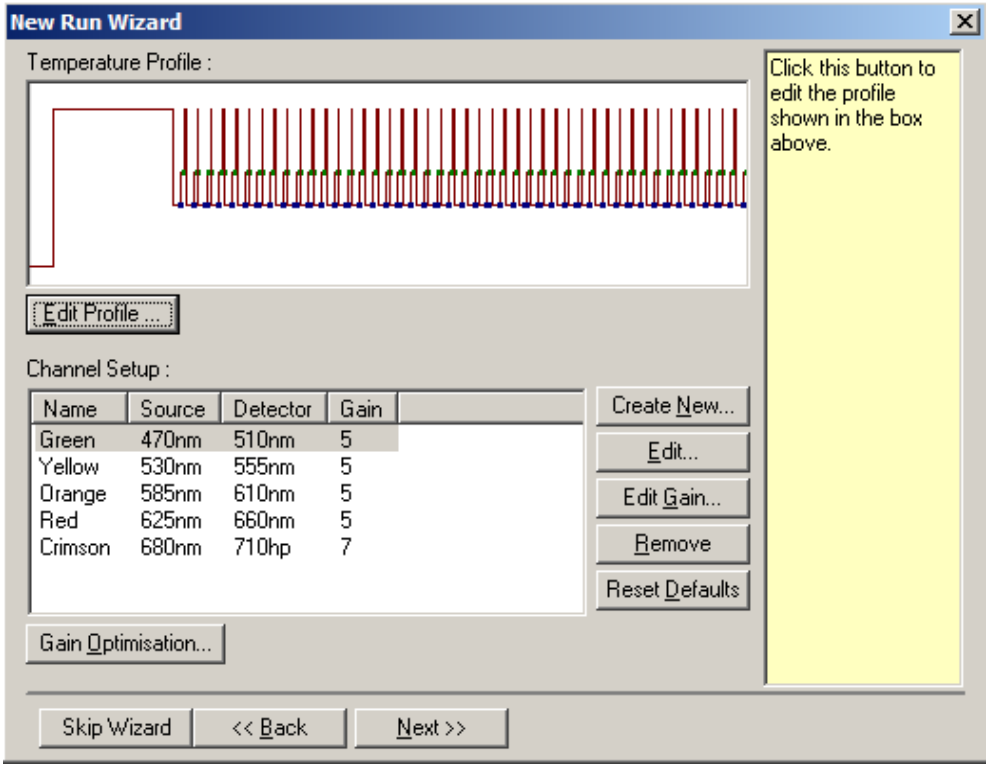



*Fig. 2.5 Edit Gain RG 6000/RG Q*

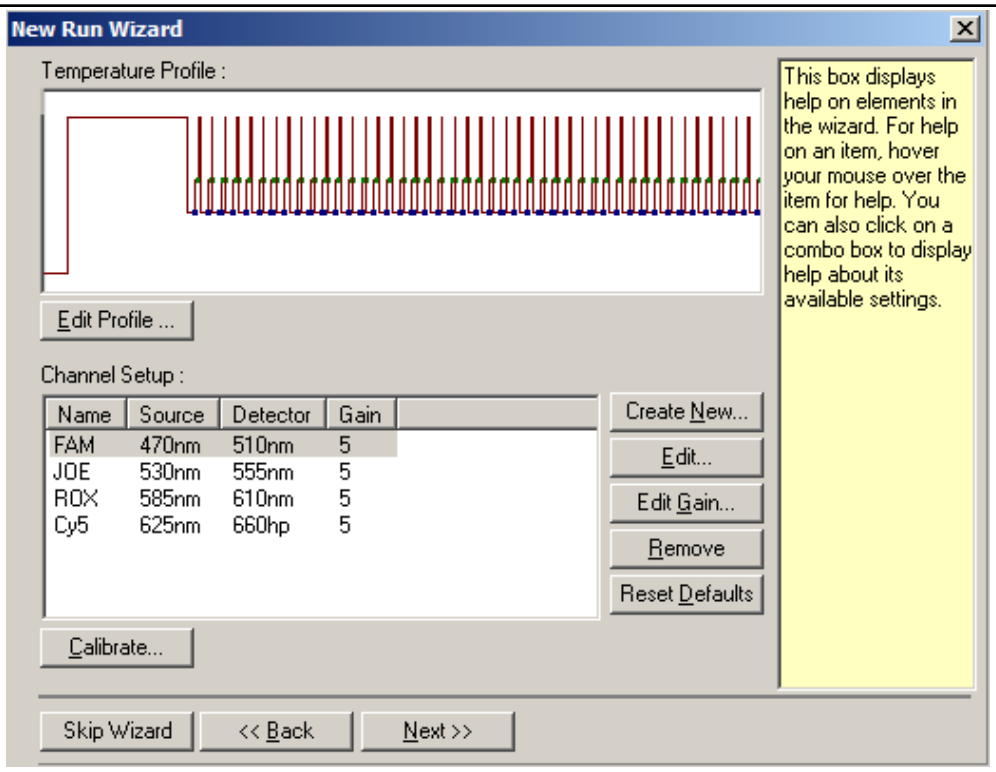



*Fig. 2.6 Edit Gain RG 3000*

2. Click **Optimise Acquiring**, check the individual channels by clicking the **OK** button and then check **Perform Optimisation Before 1st Acquisition** in the **Auto Gain Optimisation Setup** box. Click **Close** to close the box.

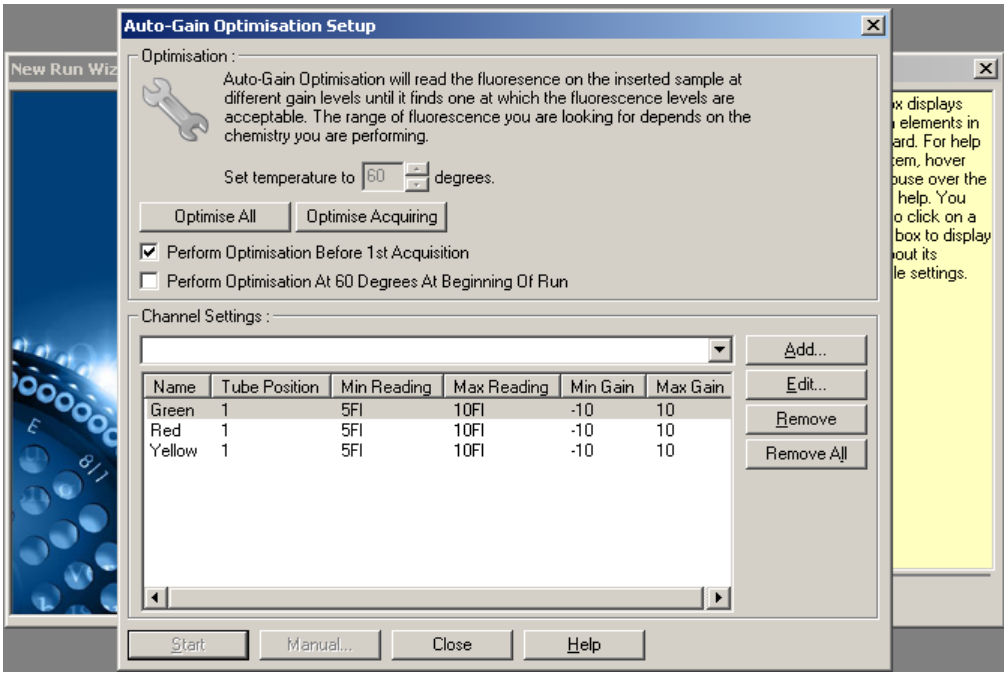



*Fig. 2.7 Set calibration for Gain RG 6000/RG Q*

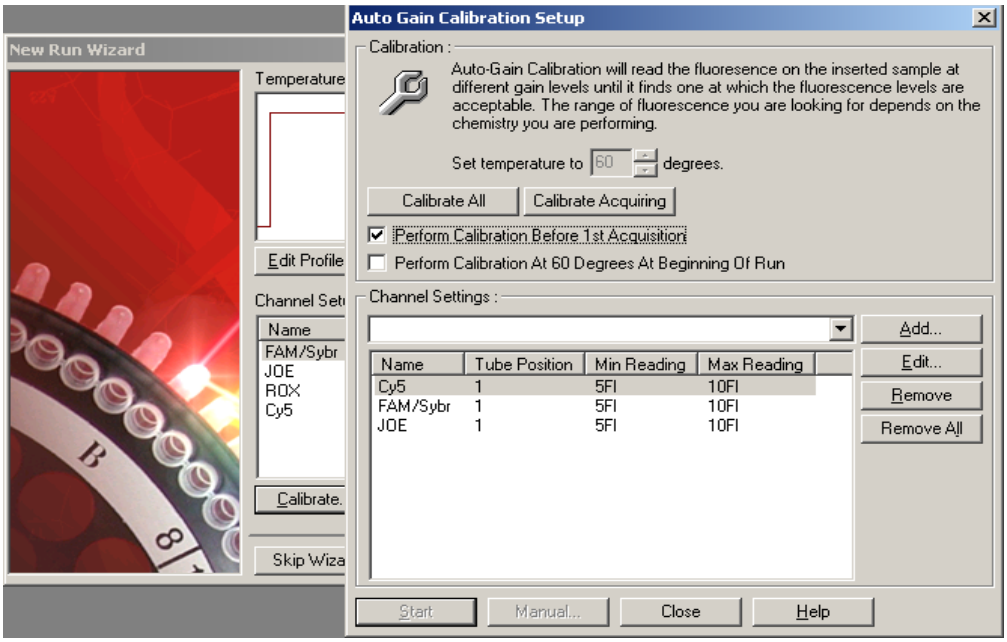



*Fig. 2.8 Set calibration for Gain RG 3000*

## 2.5. Amplification profile programming

When opening a run from a template, the thermal profile of the ampification is preprogrammed for the given GeneProof PCR kit, so there is no need for editing it.

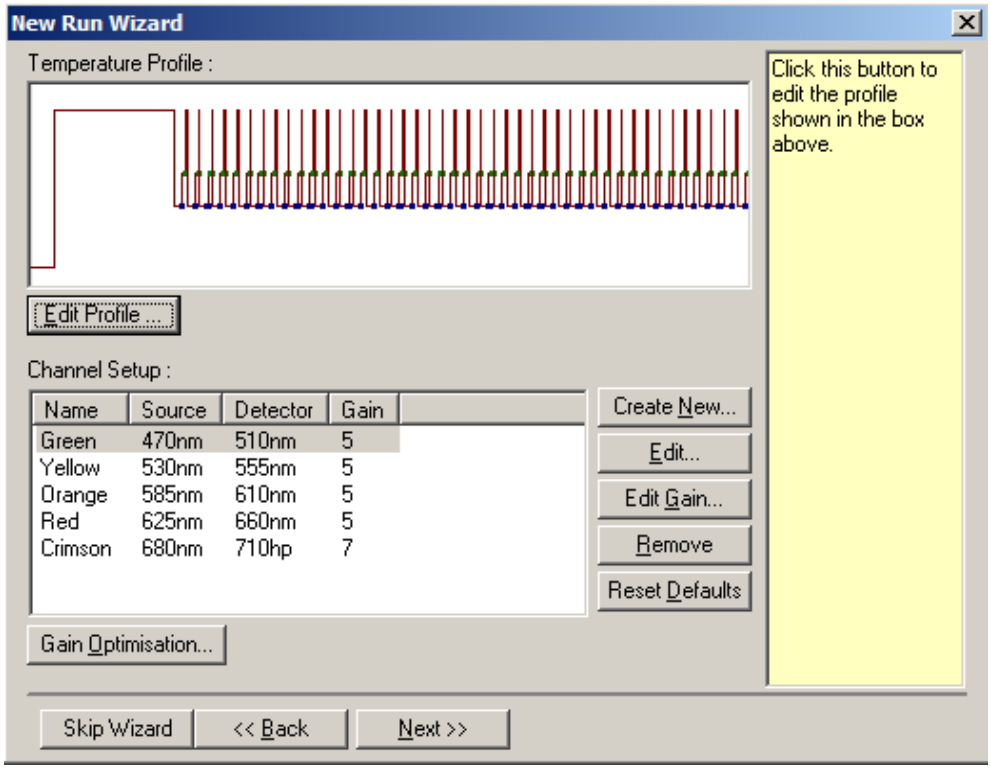



*Fig. 2.9 No need for editing the thermal profile of amplification*

## 2.6. PCR Amplification Start

1. Click **Next** in the **New Run Wizard** screen.
3. Click **Start Run** to run the program and save the protocol into a PC folder.

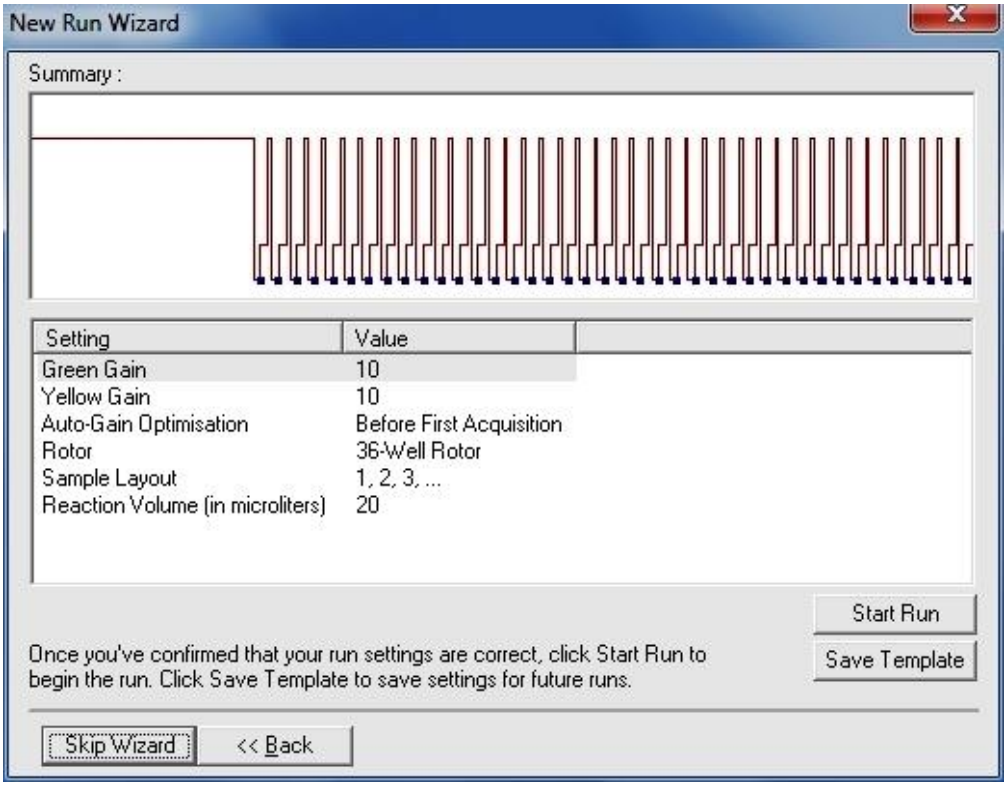



*Fig. 2.10 Start amplification*

4. After the protocol has been saved and the program started the **Edit Samples** box pops up. In the **Name** column fill in the names of all of the samples and controls, in the column **Type** choose **Unknown** for samples, **Positive Control** and **NTC** for controls.

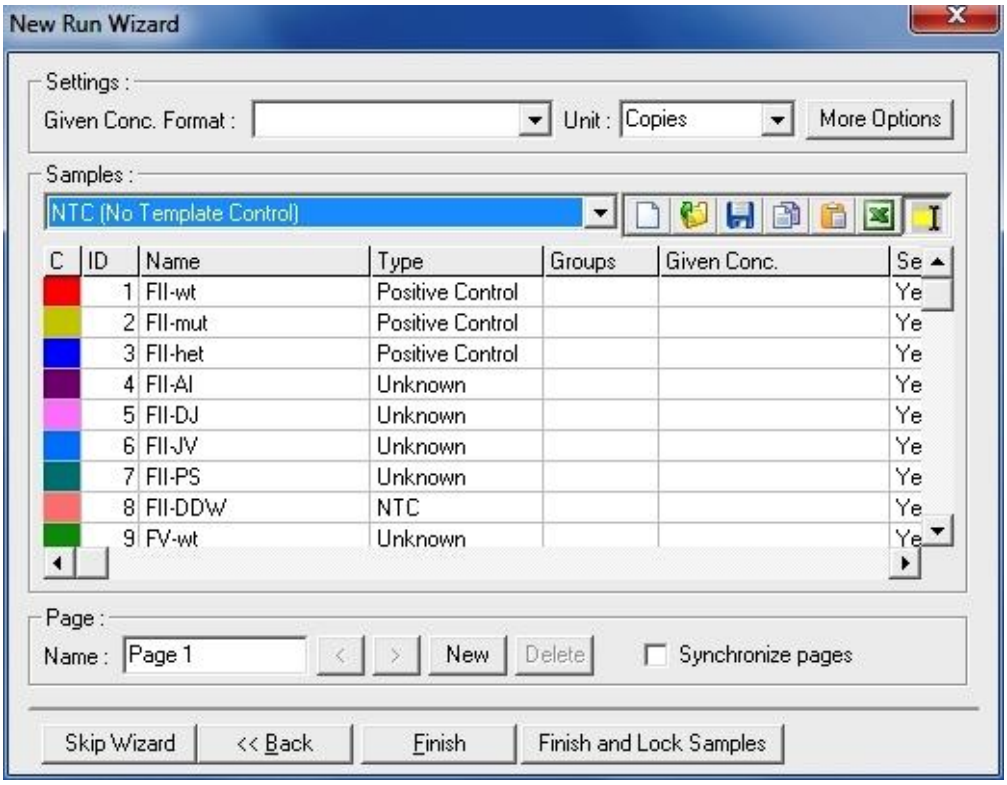



*Fig. 2.11 Inserted samples description*

5. Click **Finish**.

## 2.7. Result analysis and detection evaluation in genetic diagnostics

1. When the program is finished, click **Analysis** in the main menu and select **Allelic Discrimination**.


*Fig. 2.12 Analysis selection*

2. Select channels **Green** and **Yellow** and click **Show**. **Dynamic Tube** and **Slope Correct** filters must be active.

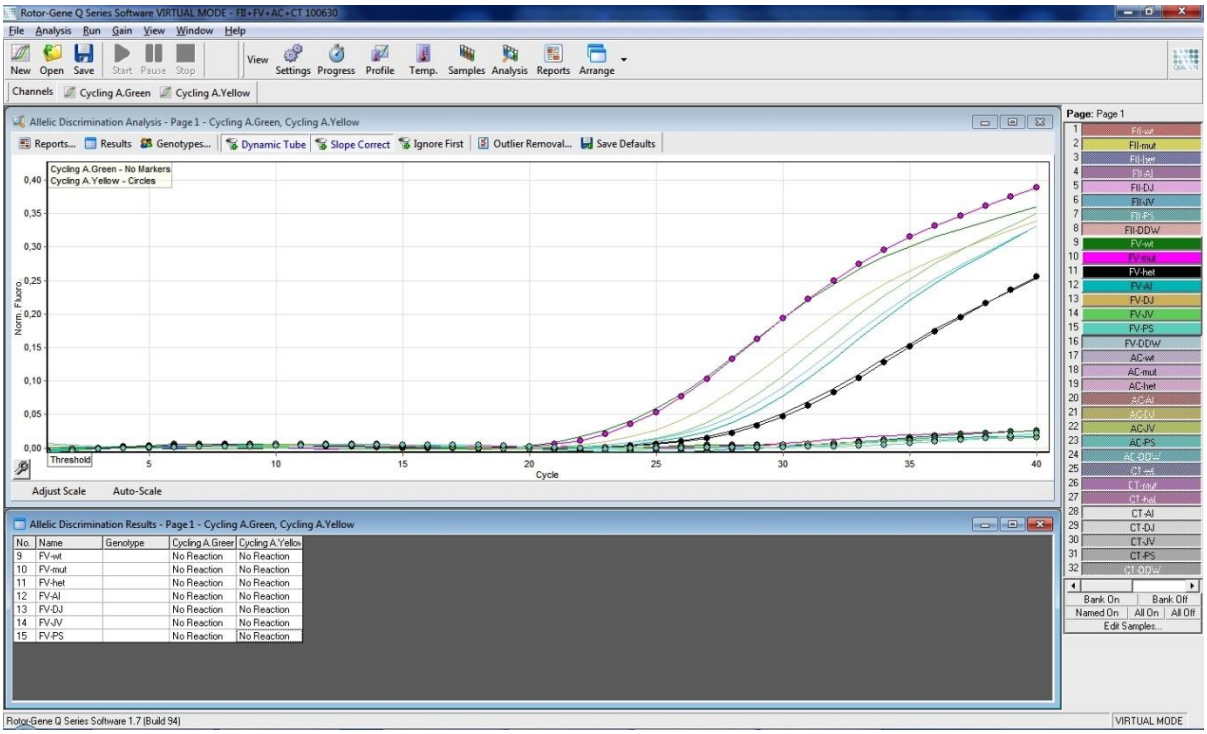



*Fig. 2.13 Analysis*

3. Click **Outlier Removal** and set parameter **Percentage of largest FI change** to 0%.

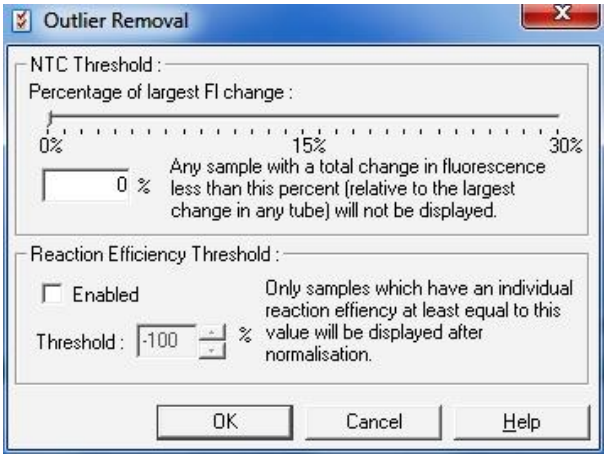



*Fig. 2.14 Outlier Removal*

4. Click **Genotypes**. Assign **Green** channel to **Wild Type** genotype, **Yellow** channel to **Mutant** genotype and both **Green** and **Yellow** channels to **Heterozygous** genotype.


*Fig. 2.15 Genotyping*

5. Select only **positive controls WT** and **MUT**. Click Auto-Scale in **Allelic Discrimination** window. Move **Threshold** manually so that the positive control **WT** is evaluated as **Wild Type** genotype and positive control **MUT** is evaluated as **Mutant** genotype (**Allelic Dicrimination Results** table).

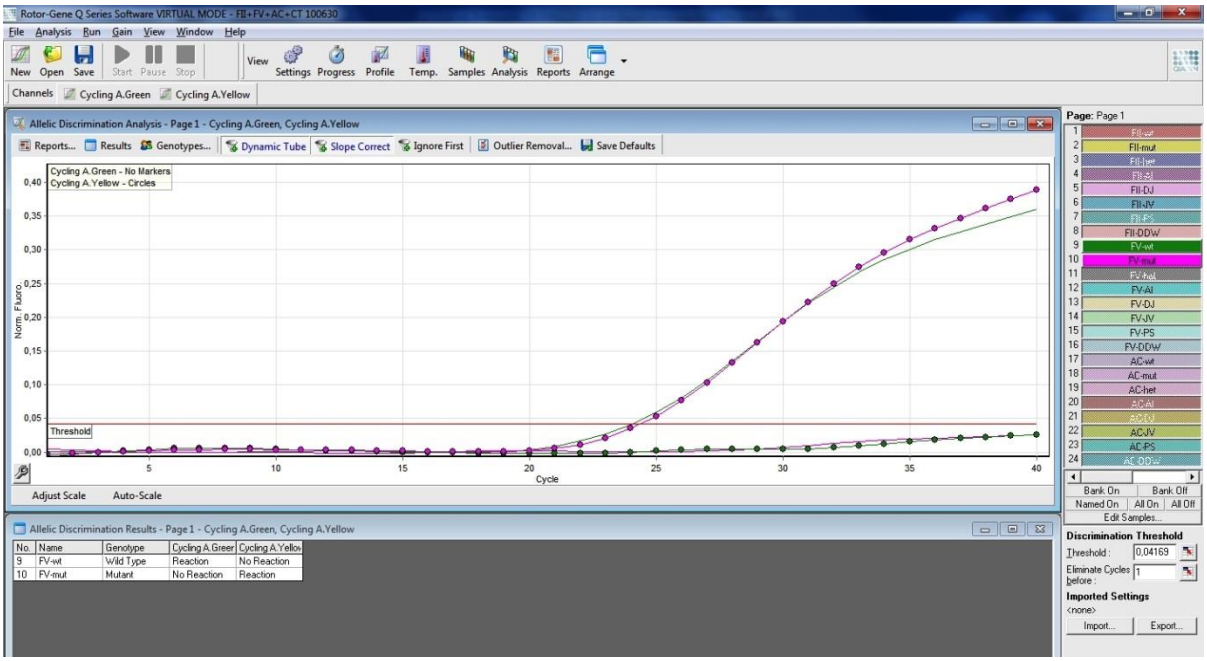
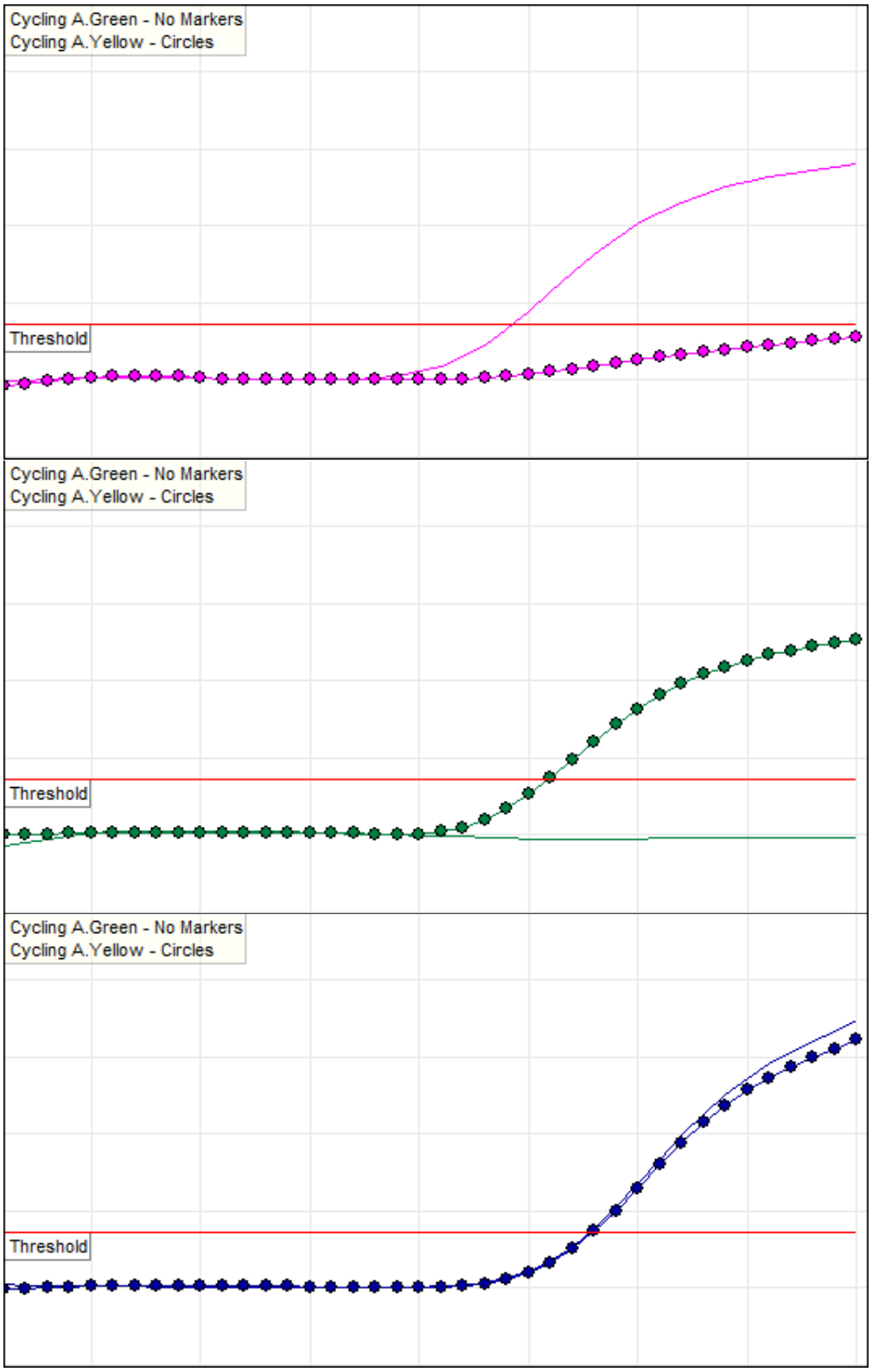



*Fig. 2.16 Threshold setting*

6. Select all samples and click **Results** to generate genotyping report.

### *2.7.1Typical curve examples*




*Typical WT curve*

*Typical MUT curve*

*Typical HET curve*

*Fig. 2.17 Typical curves*

## 2.8. Troubleshooting

Some specific Rotor-Gene devices demonstrate overvaluation of the annealing temperature by up to 4°C (even immediately after the device validation by its manufacturer). It can happen in individual cases, that under the recommended annealing temperature of 64°C the patient examination results are negative and there is detected no or very weak fluorescence signal in the positive controls or results of positive controls and patient examination results show non-specific background.

In these cases we recommend to optimize the annealing temperature in range of 66°C to 60°C. Validity of the annealing temperature can be verified by using positive controls, which are included in the used GeneProof PCR kit. When evaluating the experiment, Cp values of positive controls should be in range of 20 to 30 and genotypes of positive controls must be identified correctly and unambiguously.

# 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