

GeneProof®

PCR Kit

This manual is designed for the following kits:

Mycobacterium tuberculosis PCR kit, *Borrelia burgdorferi* PCR kit, *Chlamydia trachomatis* PCR kit, *Chlamydia pneumoniae* PCR kit, *Neisseria gonorrhoeae* PCR kit, *Cytomegalovirus* (CMV) PCR kit, *Epstein-Barr virus* (EBV) PCR kit, *Herpes Simplex virus* (HSV) PCR kit, *Varicella-Zoster virus* (VZV) PCR kit, *Hepatitis B virus* (HBV) PCR kit and *Legionella pneumophila* PCR kit

CE
in vitro Diagnostics

Manuals for use with the following devices

Rotor-Gene 3000

Rotor-Gene 6000

Rotor-Gene Q

Contents

GENEPROOF PCR KIT	3
ISIN AND ISEX VERSIONS OF THE GENEPROOF PCR KIT	4
USER MANUAL FOR ROTOR-GENE 3000.....	5
Reaction preparation and device programming	5
Detection evaluation.....	10
Qualitative analysis of the detection results.....	10
Quantitative analysis of the detection results	14
INSTRUCTIONS FOR USE OF THE ROTOR-GENE 6000/ROTOR-GENE Q	16
Reaction preparation and device programming	16
Detection evaluation.....	21
Qualitative analysis of the detection results.....	21
Quantitative analysis of the detection results	25
TROUBLESHOOTING.....	27
NOTES:	29

GeneProof PCR kit

GeneProof PCR kits, designed for the detection and quantification of pathogen DNA, are based on the principle of amplifying specific target sequences of microorganisms and measuring the amplification product concentration growth in the course of the polymerase chain reaction by means of fluorescence-marked probes (the probe designated for pathogen detection is marked by the FAM or Cy5 fluorophore). The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process. Amplification of IS results in positive signal in JOE channel. The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity and contains the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products.

GeneProof PCR kits

- Use the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity.
- Contain uracil-DNA-glycosylase (UDG), controlling possible contamination of the PCR reaction by amplification products.
- All PCR kits for pathogen DNA detection can be amplified by means of a universal amplification program.
- Are easy to use; the kits always contain one tube with MasterMix and one tube with Positive Control (or with an Internal Standard) or a set of calibration controls.
- Are designed for in vitro diagnostics (CE IVD certification)

ISIN and ISEX versions of the GeneProof PCR Kit

All GeneProof PCR kits include an Internal Standard providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods. **GeneProof develops and sells two basic versions of PCR kits with various compositions of the Internal Standard:**

PCR kit ISIN (Cat. No. *PCR kit/ISIN/*)

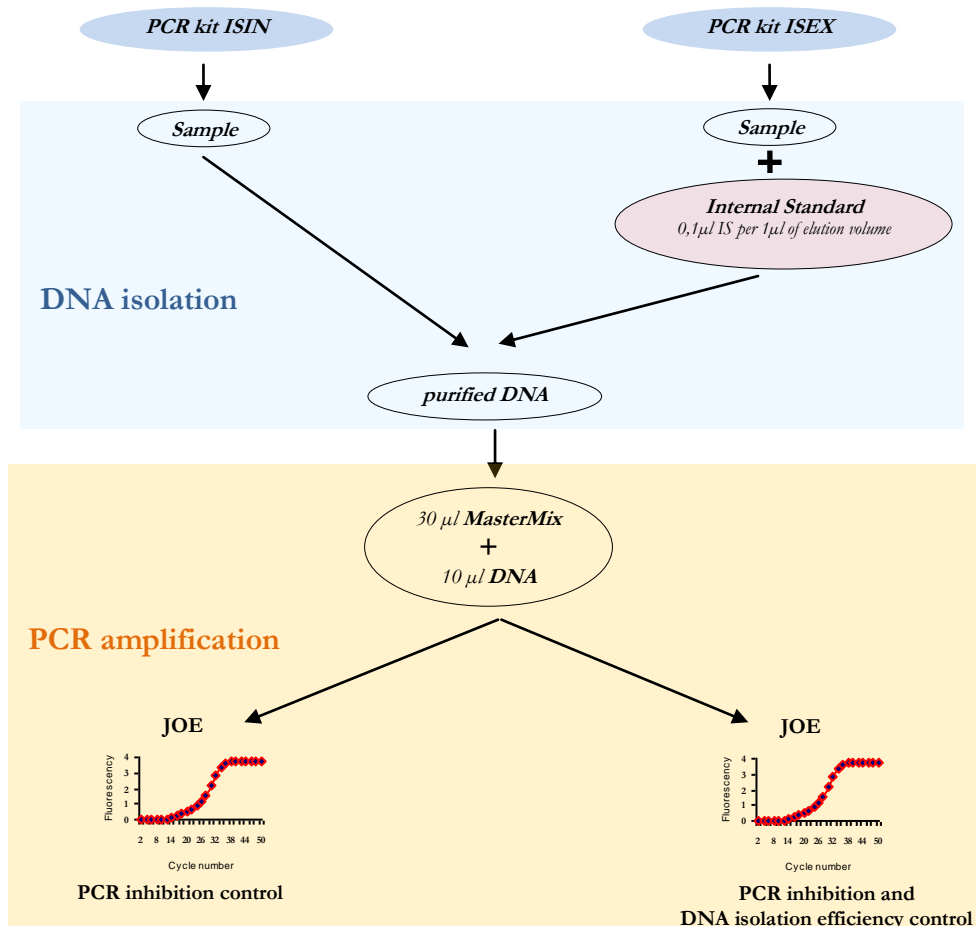
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

PCR kit ISEX (Cat. No. *PCR kit/ISEX/*)

In this PCR kit version the Internal Standard is included as an independent item within the package. This PCR kit enables both, PCR inhibition control and DNA isolation process efficiency control.

The Internal Standard should be added into the sample at the beginning of the isolation process as follows: 0.1 µl of the Internal Standard per 1 µl of elution volume:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



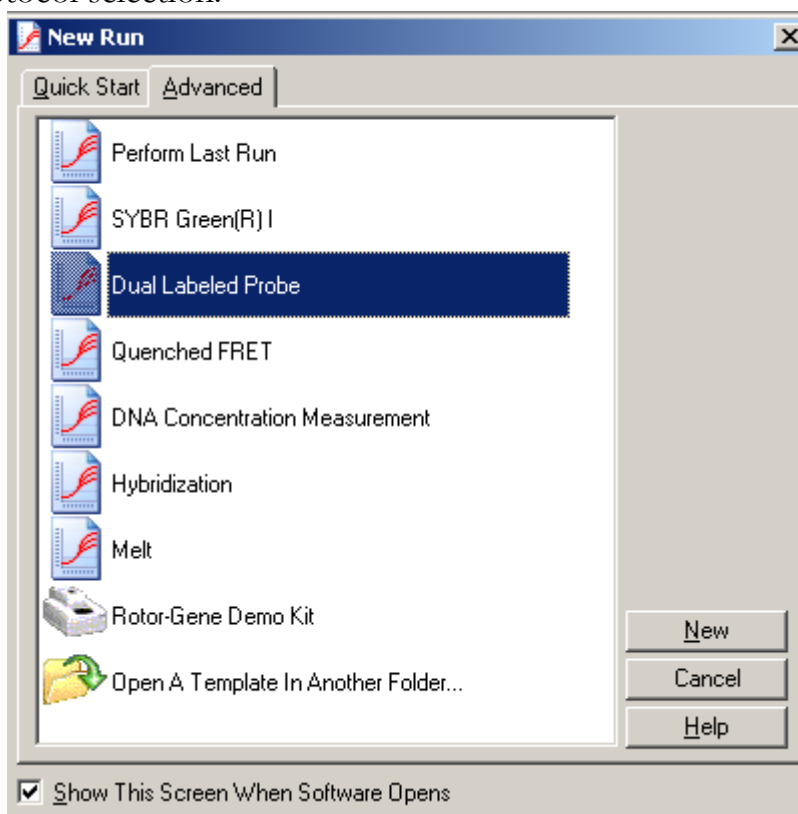
User manual for Rotor-Gene 3000

Reaction preparation and device programming

Add **30 µl** of the **MasterMix** and **10 µl** of the **DNA isolate** or **10 µl** of the **Positive Control** into a tube. The final reaction mix volume will be 40 µl. Close the tubes and centrifuge them shortly. Insert them into the device and program according to the following procedure:

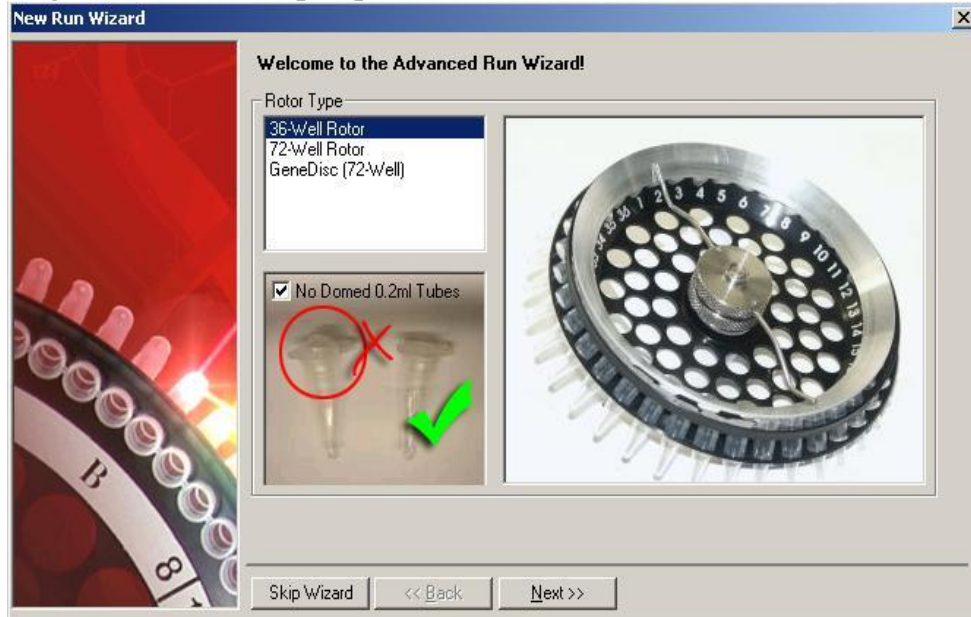
1. Start the software, select the **Advanced** tab in the main window and then select the **Dual Labeled Probe** protocol. Click the **New** button to open the PCR protocol (Fig. 1).

Fig. 1. PCR protocol selection.



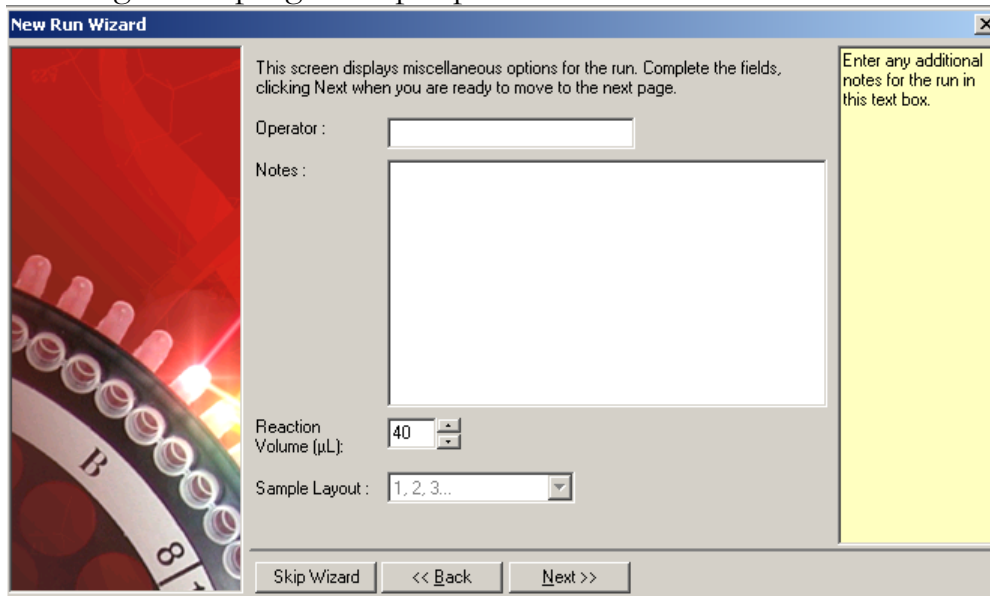
2. Enter device input parameters in the **New Run Wizard** screen. Select items **36-Well Rotor** and **No Domed 0.2ml Tubes**. Continue programming by clicking the **Next** button (Fig. 2).

Fig. 2. Setting of the device input parameters:



3. Fill out the fields **Operator** and **Notes** according to the lab requirements. Enter **40** into the **Reaction Volume (μL)** field. (Fig. 3). Continue programming by clicking the **Next** button.

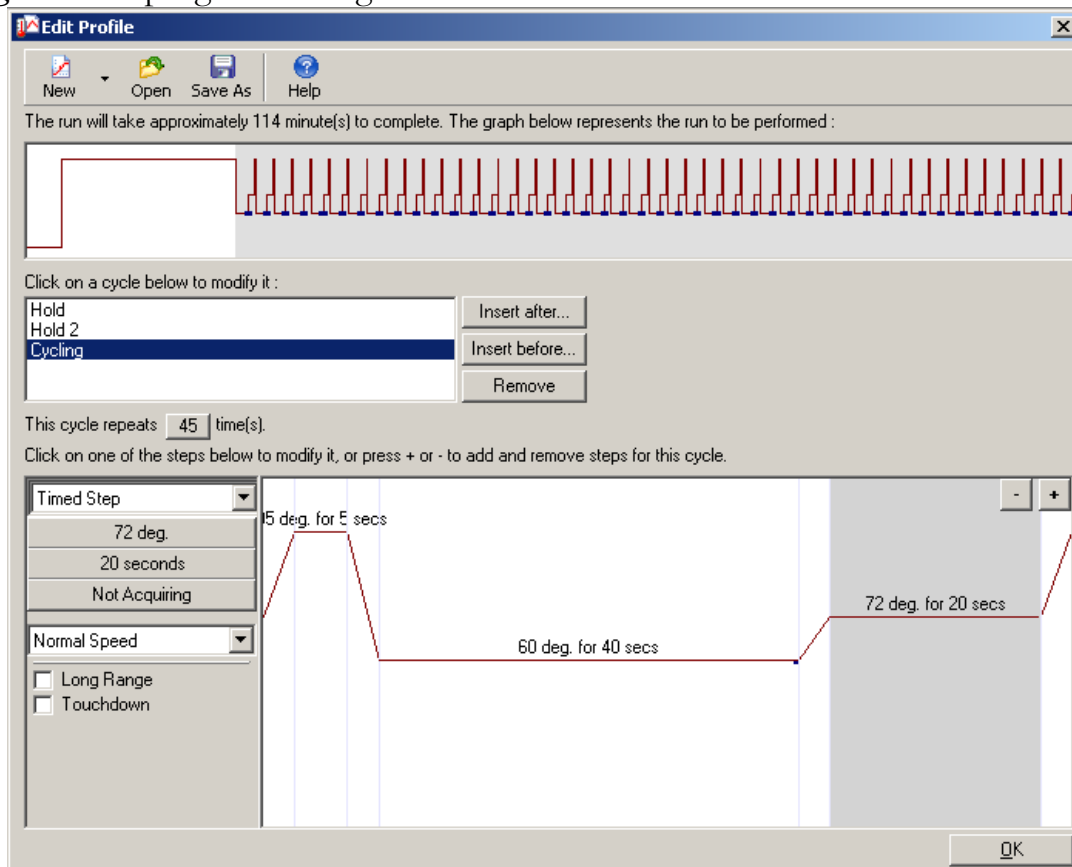
Fig. 3. Setting of the program input parameters:



4. Program the PCR detection profile (Fig. 4.). Click the **Edit Profile** button to modify the PCR program in the **New Run Wizard** screen.

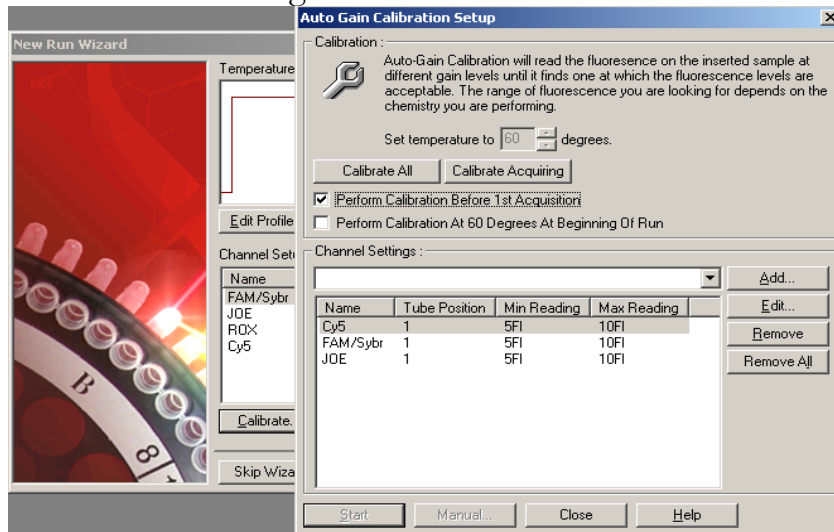
- Step 1: Select the **Hold** profile and set temperature to **37°C** and time to **2 minutes**.
- Step 2: Click the **Insert after (New Hold at Temperature)** button to select the **Hold** profile. Set temperature to **95°C** and time to **10 minutes**.
- Step 3: Click the **Insert after** button to select the **Cycling** profile. Click the button in **This cycle repeats** row and enter **45**. Enter the **Time Step** parameter.
- Set the 1st step of the program to **95°C** and time to **5 seconds**, select **Not Acquiring**.
 - Set the 2nd step of the program to **60°C** and time to **40 seconds**, click the **Acquiring to Cycling A** button and then use the arrows to select the **Acquisition Channels FAM/Sybr** and **JOE**.
 - Set the 3rd step of the program to **72°C** and time to **20 seconds**, select **Not Acquiring**.
 - Click **OK** to finish the PCR profile programming.

Fig. 4. PCR program setting.



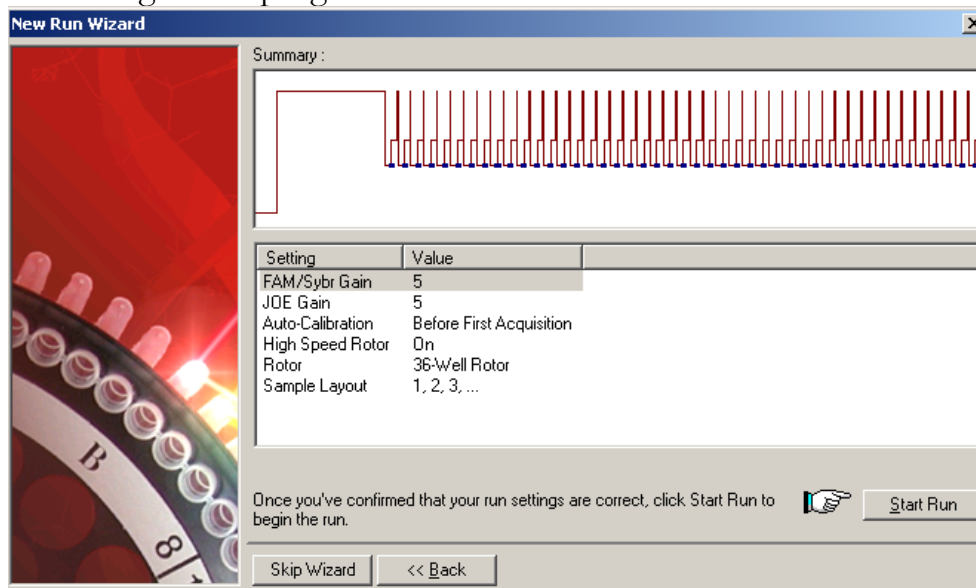
- Set the Gain calibration. Click **Calibrate** in the **New Run Wizard** screen. Click **Calibrate Acquiring** in the open **Auto Gain Calibration Setup** screen and then check **Perform Calibration Before 1st Acquisition**. Click **Close** to close the settings (Fig. 5).

Fig 5. Gain calibration setting.



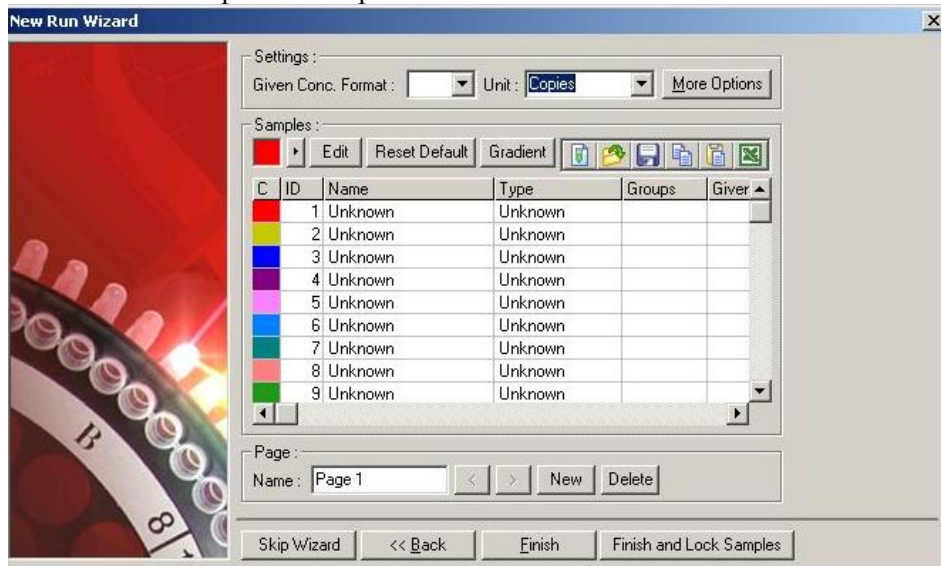
- Start the PCR program. Click **Next** in the **New Run Wizard** screen. Check the parameters set in the table and then click the **Start Run** button to start the program. Save the protocol into the relevant folder in your computer. (Fig. 6).

Fig. 6. Starting of the program.



- Describe the inserted samples (Fig. 7.). After the protocol has been saved and the program started the window for sample editing will pop up. Fill in the required sample data depending on the used qualitative or quantitative detection method (see Qualitative/quantitative detection analysis).

Fig. 7. Inserted sample description.



- Click the **Finish** button.

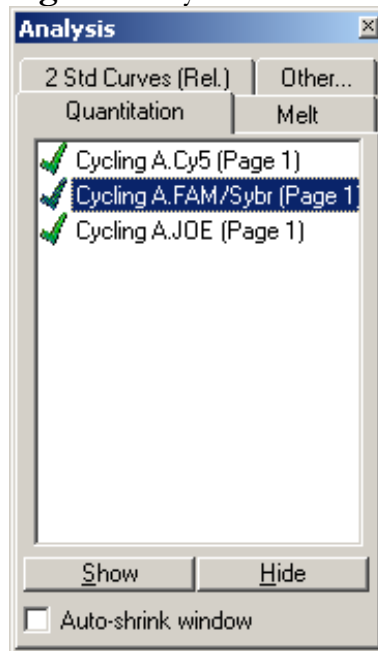
Detection evaluation

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.

Qualitative analysis of the detection results

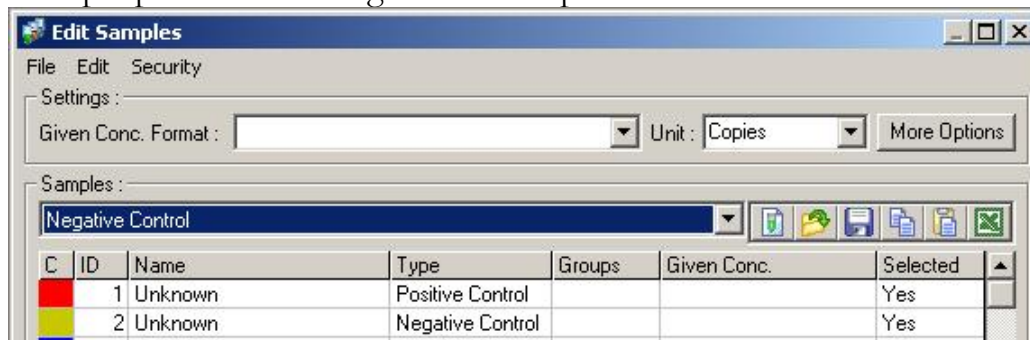
1. When the program finishes, select **Analysis** in the top toolbar. Select the **Quantification** tab (Fig. 8.).

Fig. 8. Analysis selection.



2. Select values for the detection qualitative analysis in the **Samples** tab. **Positive Control** and **Negative Control** are minimum required samples for a valid assessment of a PCR reaction (Fig. 9.).

Fig. 9. Sample parameter settings in the Samples tab.



3. Positive sample assessment in the Linear scale (Fig. 10.).

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 than 40 it is recommended to use the Log. Scale assessment (see Chapter 4).

- a) Select **Cycling A.FAM/Sybr and Cy5** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.JOE** for Internal Standard detection assessment. Press **Show**.
- b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
- c) Enter **NTC 10** in the **More Settings** tab.
- d) Select **Linear scale**.
- e) Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

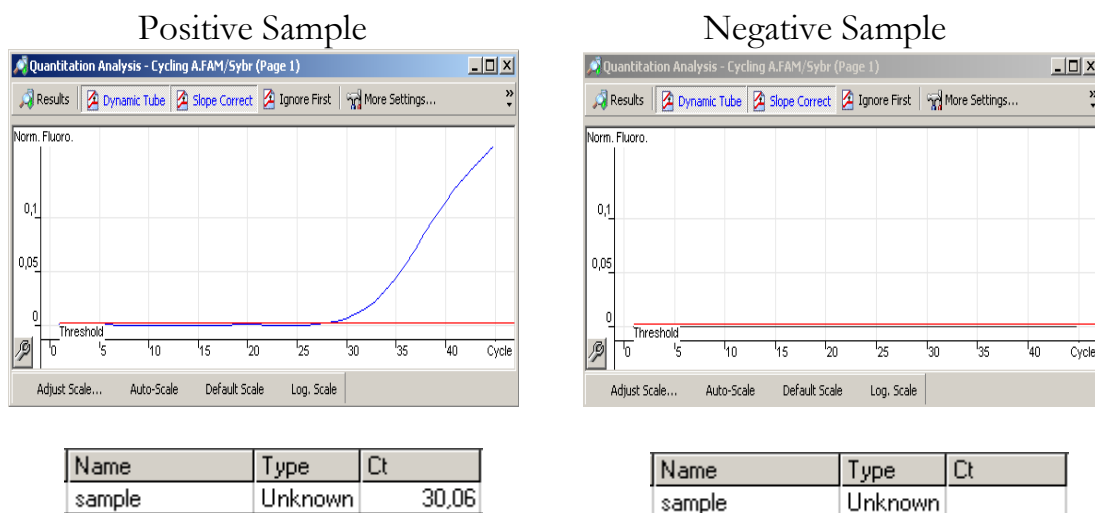
Positive sample amplification

In the graphic display for **FAM/Sybr and Cy5** (if available) you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table.

Internal Standard Amplification

In the graphic display for **JOE** you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table.

Fig. 10. Positive sample assessment in the Linear scale.



4. Assessment of weakly positive samples in the Log. scale (Fig. 11).

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”!

- a) Select **Cycling A.FAM/Sybr and Cy5** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.JOE** for Internal Standard detection assessment. Press **Show**.
- b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
- c) Enter **NTC 10** in the **More Settings** tab.
- d) Select **Log. scale**.
- e) Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

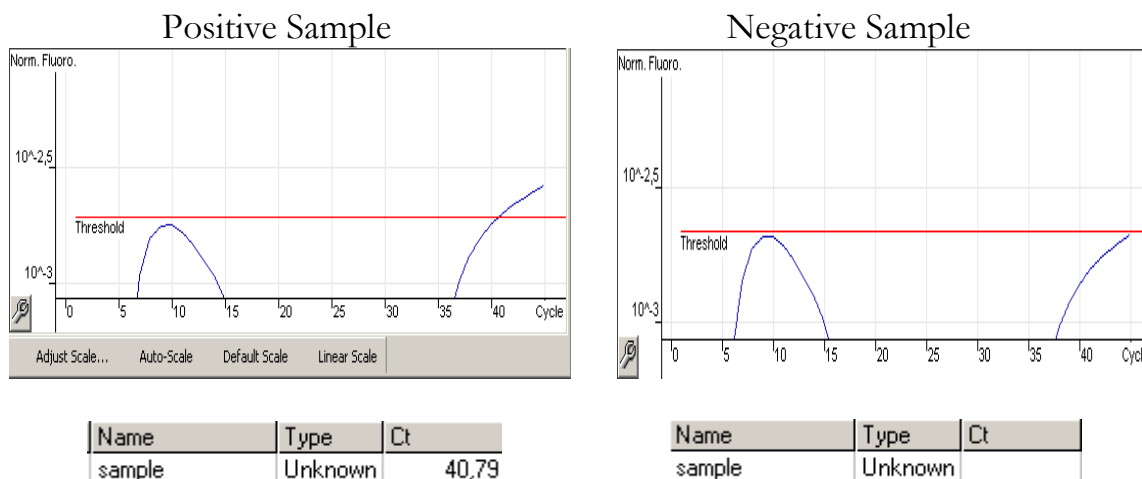
Amplification of a weakly positive sample

In case of sample positivity you can see an ascending curve intersecting the manually set threshold in the graphic display for **FAM/Sybr and Cy5** (if available). A numeric value **Ct** is assigned to this curve in the assessment table.

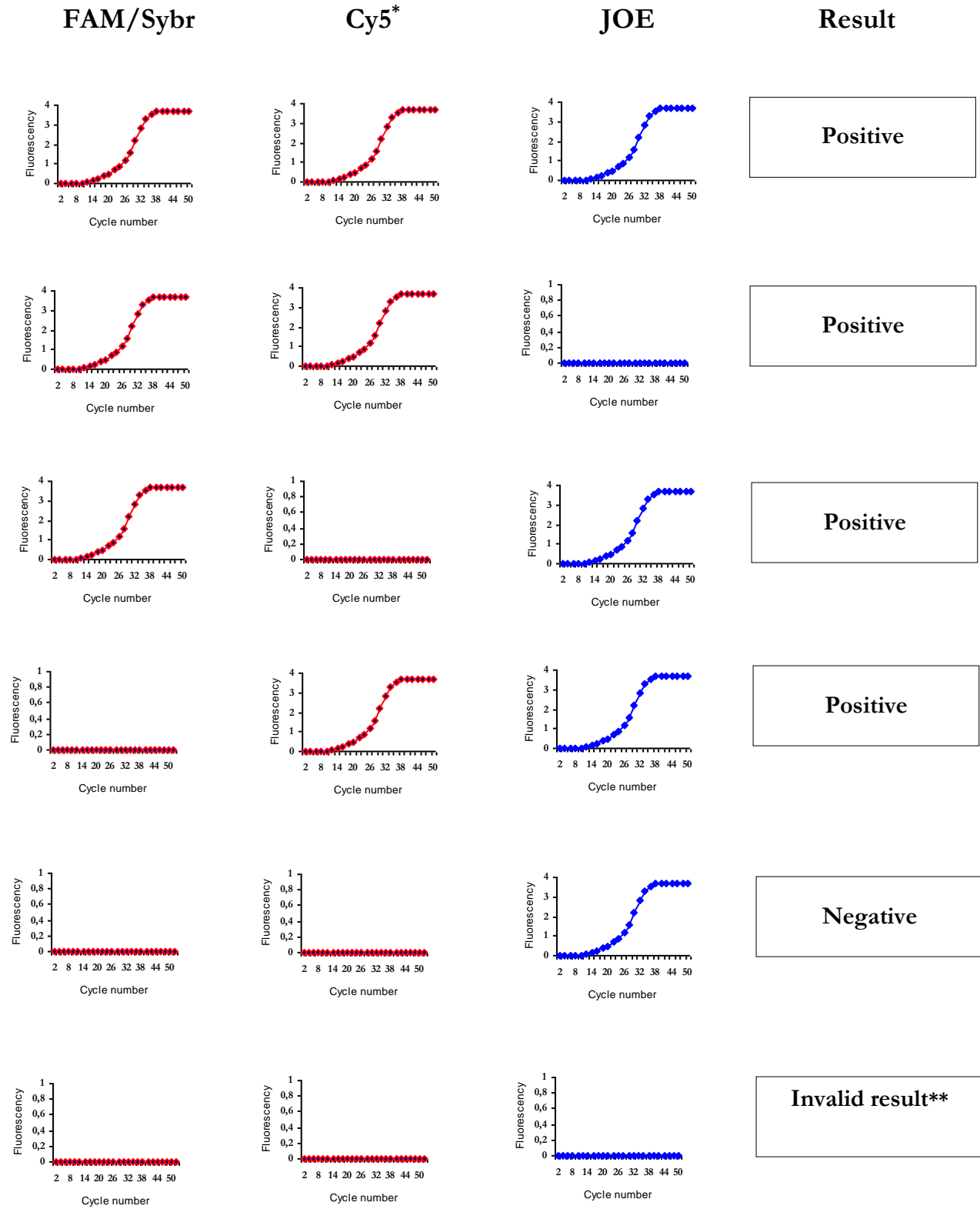
Internal Standard Amplification

Internal Standard assessment is governed by the same rules as described in Section 3 of this manual.

Fig. 11. Assessment of weakly positive samples in the Log. scale



5. Analysis evaluation:



* if available
 ** see Detection Troubleshooting, page 27

Quantitative analysis of the detection results

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

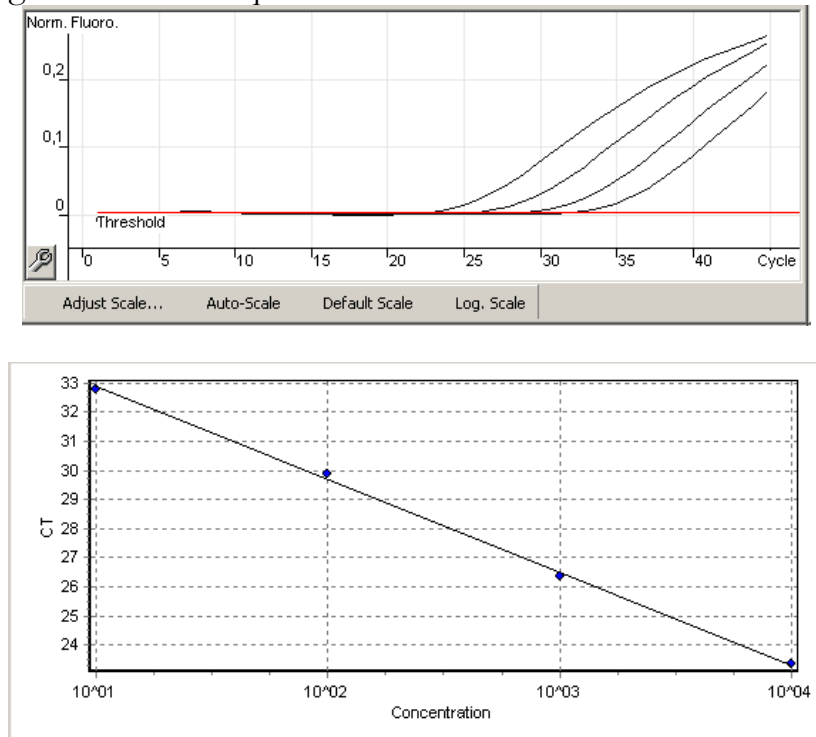
1. When the program finishes, select **Analysis** in the top toolbar. Select the **Quantification** tab (see Fig. 8.).
2. Select values for the detection quantitative analysis in the **Samples** tab. **Calibration Control** (enter **Standard** into the **Type** column and then enter the concentration of the inserted calibration control into the **Given Conc.** column) and **Negative Control** are the minimum required samples for a valid quantitative assessment of a PCR reaction.

Fig. 12. Sample description for a quantitative assessment.

C	ID	Name	Type	Groups	Given Conc.	Selected
Red	1	K1	Standard		1,00E+04	Yes
Yellow	2	K2	Standard		1,00E+03	Yes
Blue	3	K3	Standard		1,00E+02	Yes
Purple	4	K4	Standard		1,00E+01	Yes
Pink	5	Negative control	Negative Control			Yes

3. Setting of a detection quantitative assessment (Fig. 13.).
 - a) Select **Cycling A.FAM/Sybr and Cy5** (if available) for the qualitative assessment of the positive signal detection. Press **Show**.
 - b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
 - c) Enter **NTC 10** in the **More Settings** tab.
 - d) Select **Linear scale**.
 - e) Click **Auto-Find Threshold** for an automatic detection assessment.

Fig. 13. Setting of a detection quantitative assessment.



Only concentrations in the range specified by the calibration curve may be measured for a quantitative evaluation of the results.

- a) Quantification of samples out of calibration curve should be considered to be not very precise.
- b) Samples upper the highest concentrated calibrator could be diluted to achieve more precise quantification.
- c) Samples with lower concentrations then the lowest concentrated calibrator can be quantified approximately only.
- d) The following formula can be used to convert sample concentrations to *units/ml* taking into account the isolation procedure:

$$\text{Concentration/ml} = \frac{cVZ \times EO}{I}$$

cVZ = sample concentration in units/ μ l
 EO = selected retention volume in μ l
 I = volume of material used for isolation in ml

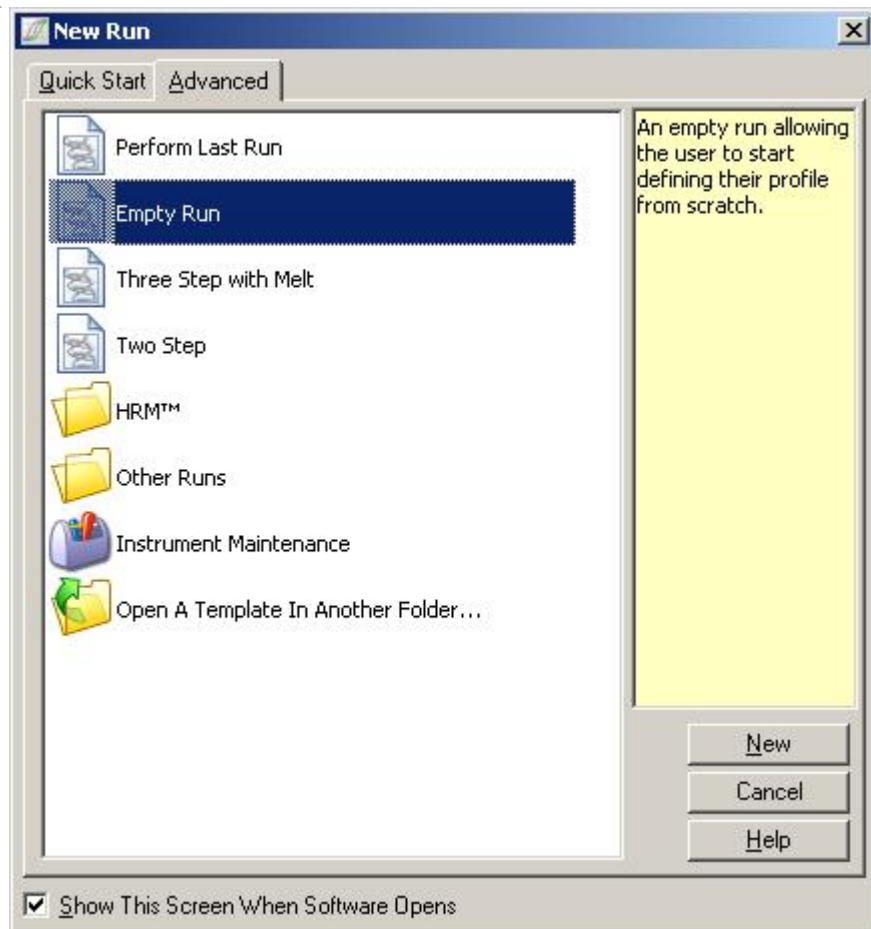
Instructions for use of the Rotor-Gene 6000/Rotor-Gene Q

Reaction preparation and device programming

Add 30 μl of the MasterMix and 10 μl of the DNA isolate or 10 μl of the Positive Control into a tube. The final reaction mix volume will be 40 μl . Close the tubes and centrifuge them shortly. Insert them into the device and program according to the following procedure:

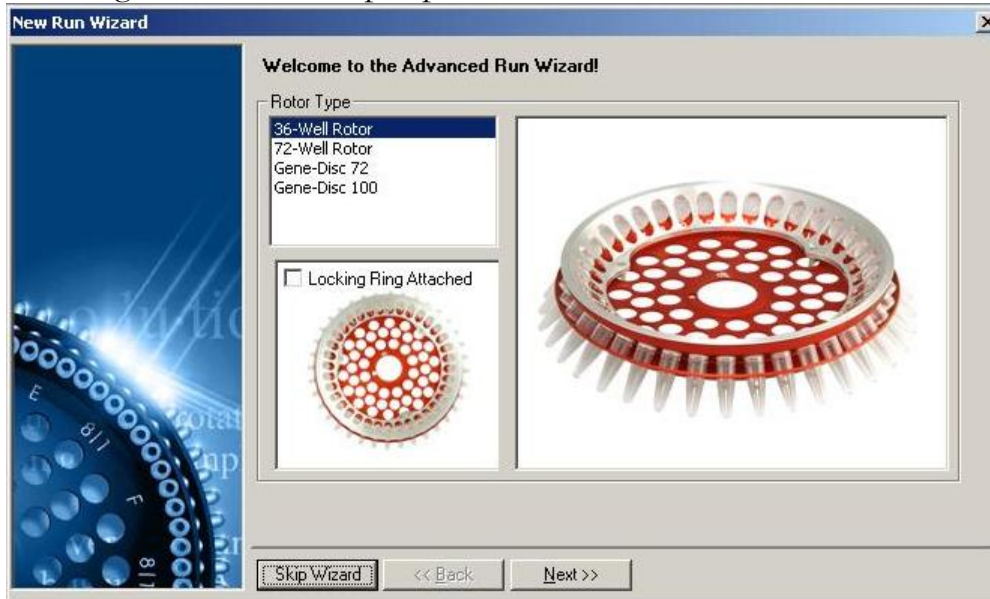
1. Start the software, select the **Advanced** tab in the main window and then select the **Empty Run** protocol. Click the **New** button to open the PCR protocol (Fig. 1.).

Fig. 1. PCR protocol selection.



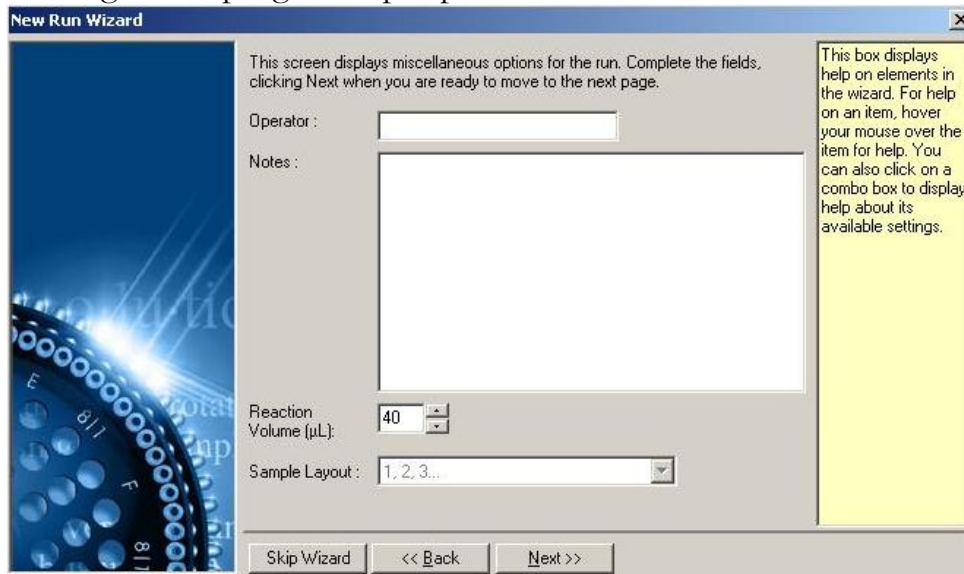
2. Enter device input parameters in the **New Run Wizard** screen. Select options **36-Well Rotor** and **Locking Ring Attached**. Continue programming by clicking the **Next** button (Fig. 2).

Fig. 2. Setting of the device input parameters:



3. Fill out the fields **Operator** and **Notes** according to the lab requirements. Enter **40** into the **Reaction Volume (μL)** field. (Fig. 3). Continue programming by clicking the **Next** button.

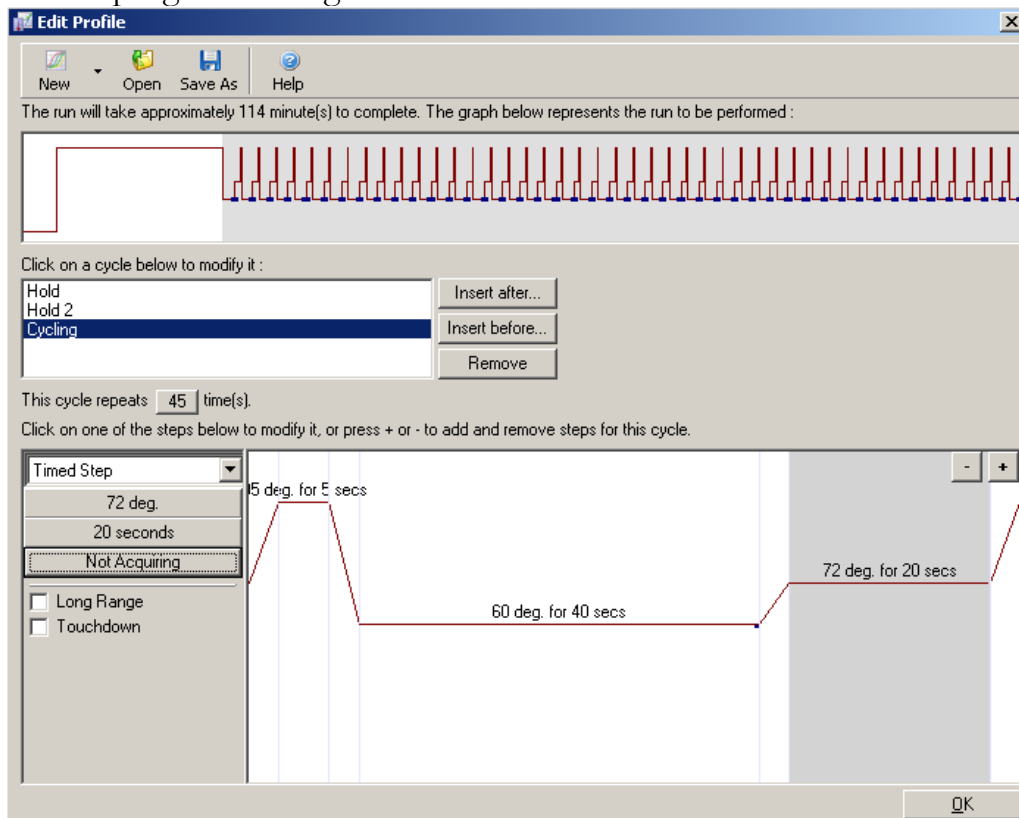
Fig. 3. Setting of the program input parameters:



4. Program the PCR detection profile (Fig. 4). Click the **Edit Profile** button to modify the PCR program in the **New Run Wizard** screen.

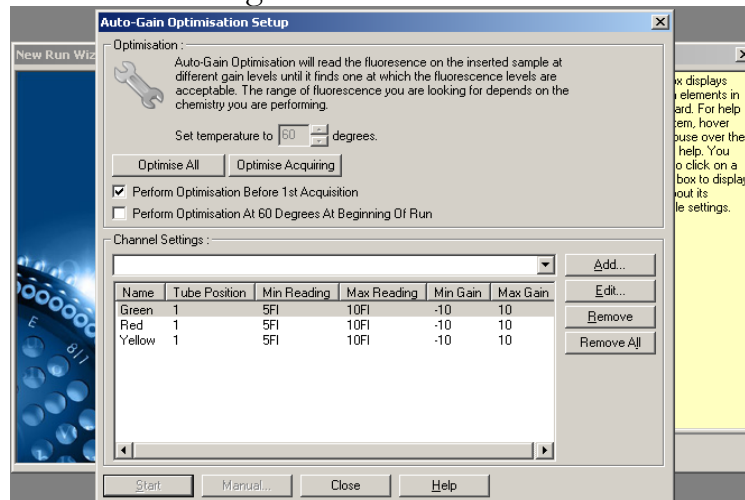
- Step 1: Select the **Hold** profile and set temperature to **37°C** and time to **2 minutes**.
- Step 2: Click the **Insert after (New Hold at Temperature)** button to select the **Hold** profile. Set temperature to **95°C** and time to **10 minutes**.
- Step 3: Click the **Insert after** button to select the **Cycling** profile. Click the button in **This cycle repeats** row and enter **45**. Enter the **Time Step** parameter.
- Set the 1st step of the program to **95°C** and time to **5 seconds**, select **Not Acquiring**.
 - Set the 2nd step of the program to **60°C** and time to **40 seconds**, click the **Acquiring to Cycling A** button and then use the arrows to select the **Acquisition Channels Green and Red** (if available) and **Yellow**.
 - Set the 3rd step of the program to **72°C** and time to **20 seconds**, select **Not Acquiring**.
 - Click **OK** to finish the PCR profile programming.

Fig. 4. PCR program setting.



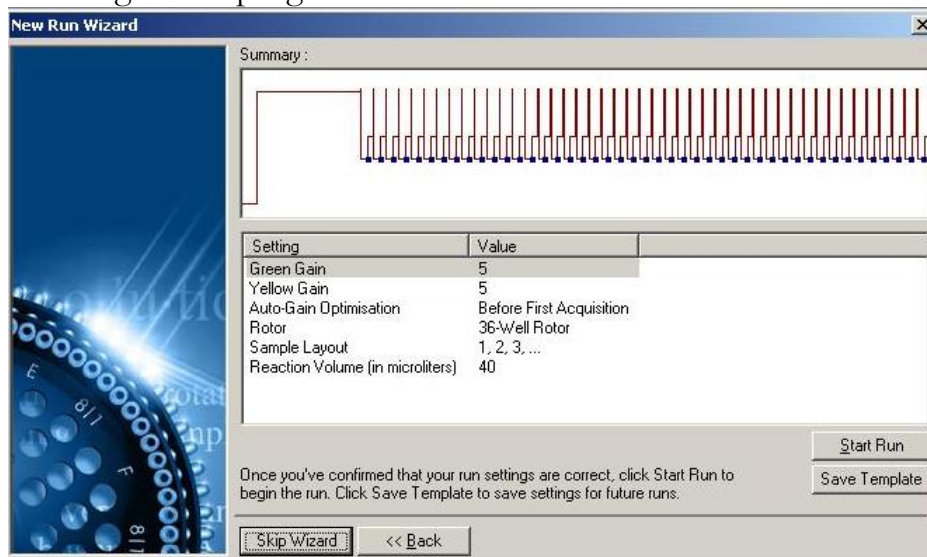
- Set the Gain calibration. Click **Gain Optimisation** in the **New Run Wizard** screen. Click **Calibrate Acquiring** in the open **Auto Gain Calibration Setup** screen and then check **Perform Calibration Before 1st Acquisition**. Click **Close** to close the settings (Fig. 5).

Fig 5. Gain calibration setting.



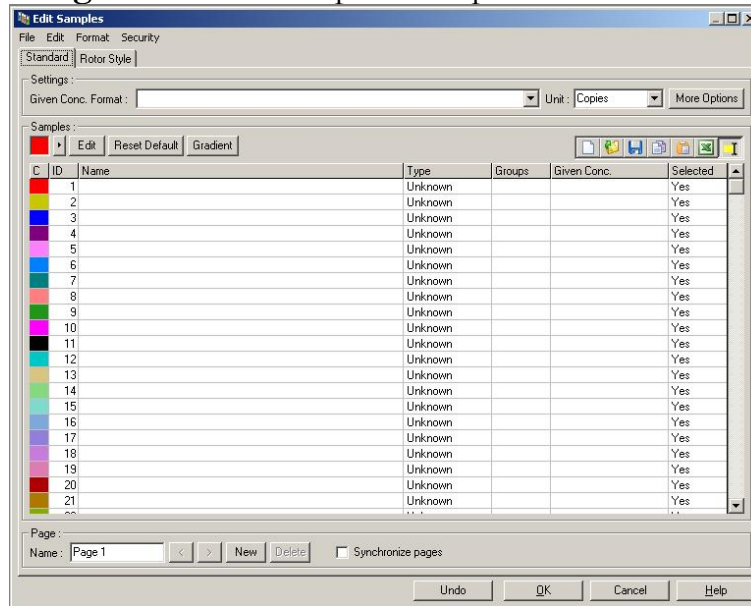
- Start the PCR program. Click **Next** in the **New Run Wizard** screen. Check the parameters set in the table and then click the **Start Run** button to start the program. Save the protocol into the relevant folder in your computer. (Fig. 6).

Fig. 6. Starting of the program.



- Describe the inserted samples. After the protocol has been saved and the program started the window for sample editing will pop up. Fill in the required sample data depending on the used qualitative or quantitative detection method (see Qualitative/quantitative detection analysis) (Fig. 7.).

Fig. 7. Inserted sample description.



- Click the **Finish** button.

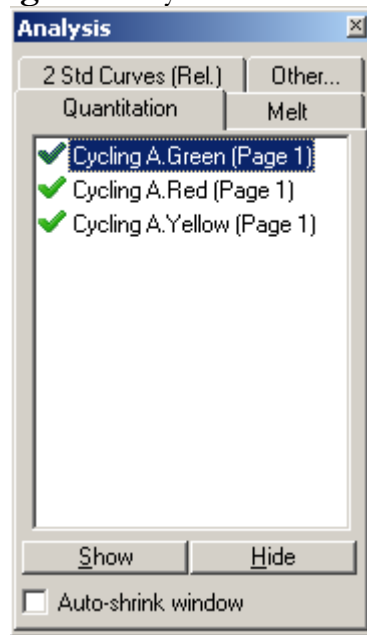
Detection evaluation

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.

Qualitative analysis of the detection results

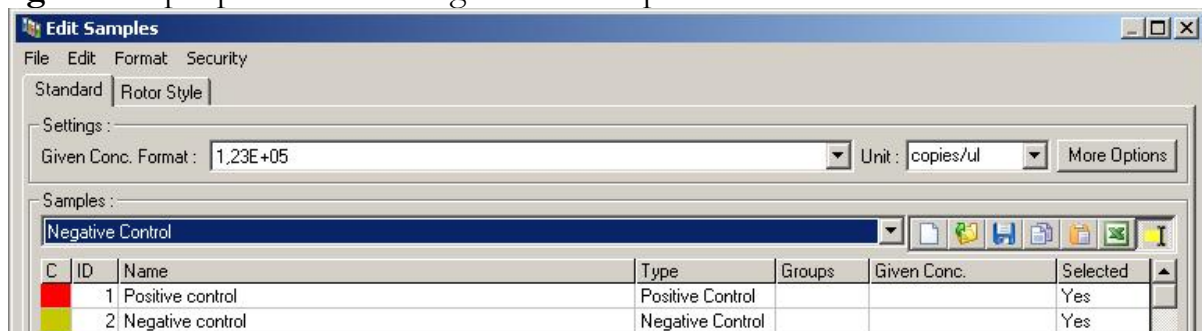
1. When the program finishes, select **Analysis** in the top toolbar. Select the **Quantification** tab (Fig. 8).

Fig. 8. Analysis selection.



2. Select values for the detection qualitative analysis in the **Samples** tab. **Positive Control** and **Negative Control** are minimum required samples for a valid assessment of a PCR reaction (Fig. 9).

Fig. 9. Sample parameter settings in the Samples tab.



3. Positive sample assessment in the Linear scale (Fig. 10.).

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 than 40 it is recommended to use the Log. Scale assessment (see Chapter 4).

- a) Select **Cycling A.Green and Red** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.Yellow** for Internal Standard detection assessment. Press **Show**.
- b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
- c) Enter **NTC 10** in the **Outlier Removal** tab.
- d) Select **Linear scale**.
- e) Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

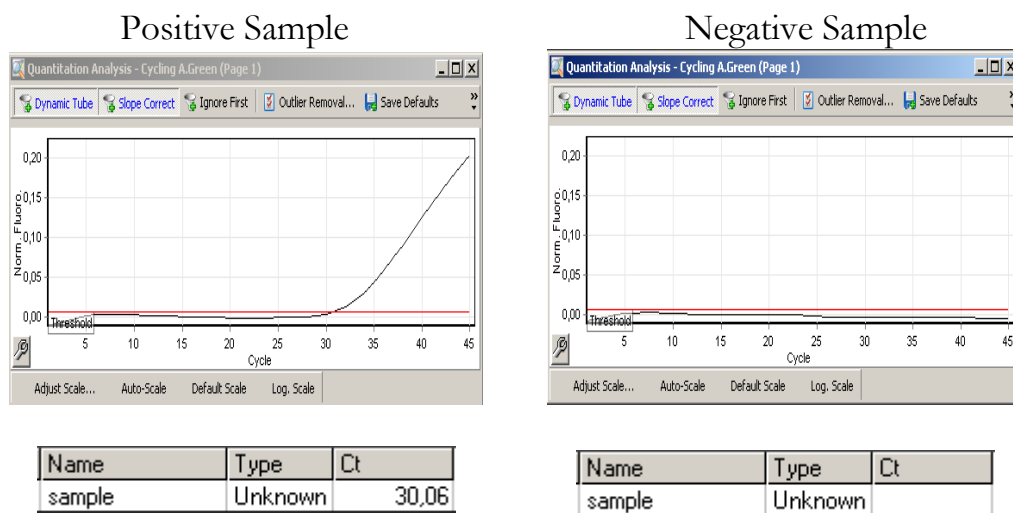
Positive sample amplification

In the graphic display for **Green and Red** (if available) you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table.

Internal Standard Amplification

In the graphic display for **Yellow** you can see an ascending curve intersecting the manually set threshold. A numeric value **Ct** is assigned to this curve in the assessment table.

Fig. 10. Positive sample assessment in the Linear scale.



4. Assessment of weakly positive samples in the Log. scale (Fig. 12.).

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”!

- a) Select **Cycling A.Green and Red** (if available) for the qualitative assessment of the positive signal detection and **Cycling A.Yellow** for Internal Standard detection assessment. Press **Show**.
- b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
- c) Enter **NTC 10** in the **Outlier Removal** tab.
- d) Select **Log. scale**.
- e) Manually set the threshold above the basic fluorescent reaction noise in the left part of the graphic display.

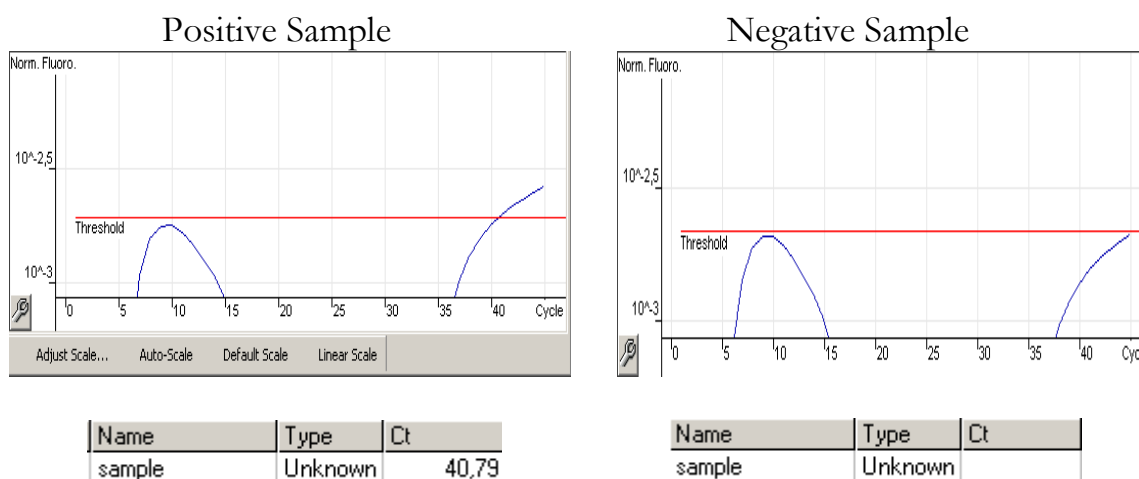
Amplification of a weakly positive sample

In case of sample positivity you can see an ascending curve intersecting the manually set threshold in the graphic display for **Green a Red** (if available). A numeric value **Ct** is assigned to this curve in the assessment table.

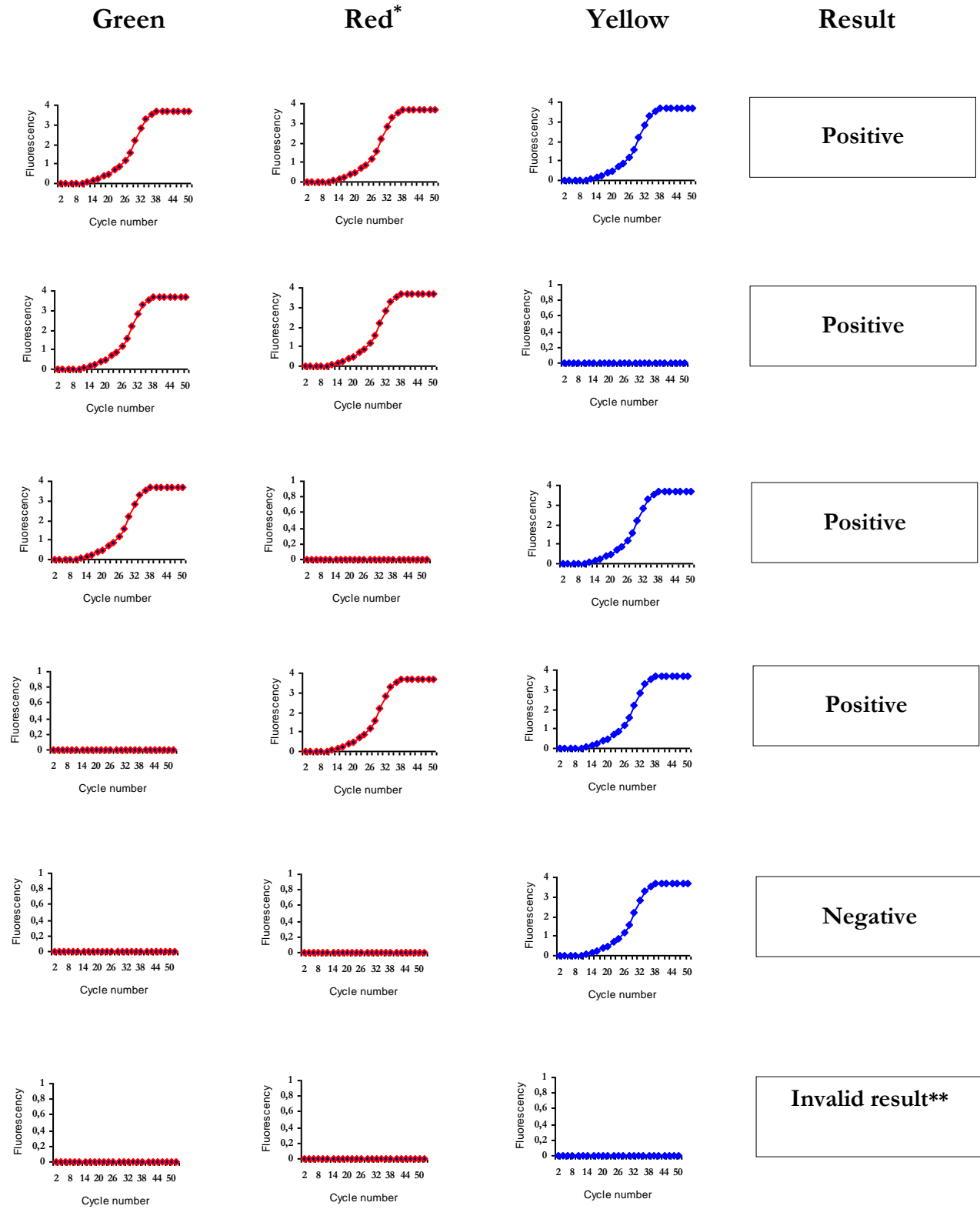
Internal Standard Amplification

Internal Standard assessment is governed by the same rules as described in Section 3 of this manual.

Fig. 11. Assessment of weakly positive samples in the Log. scale



1. Analysis evaluation:



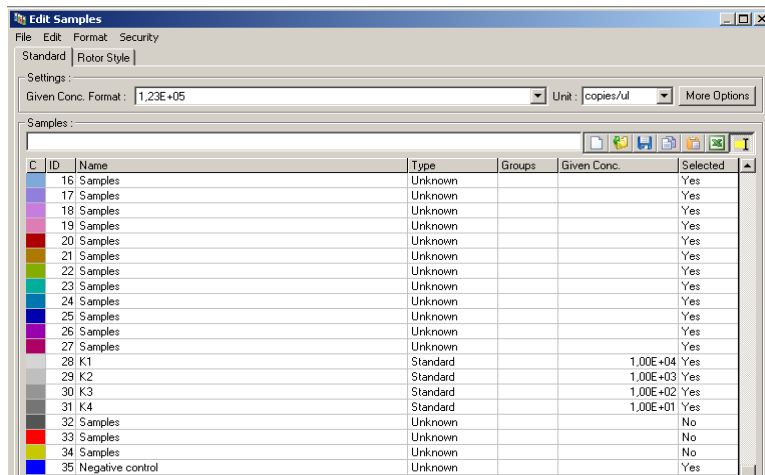
* if available
 ** see Detection Troubleshooting, page 27

Quantitative analysis of the detection results

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

1. When the program finishes, select **Analysis** in the top toolbar. Select the **Quantification** tab (see Fig. 8).
2. 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 quantitative assessment of a PCR reaction (Fig. 12).

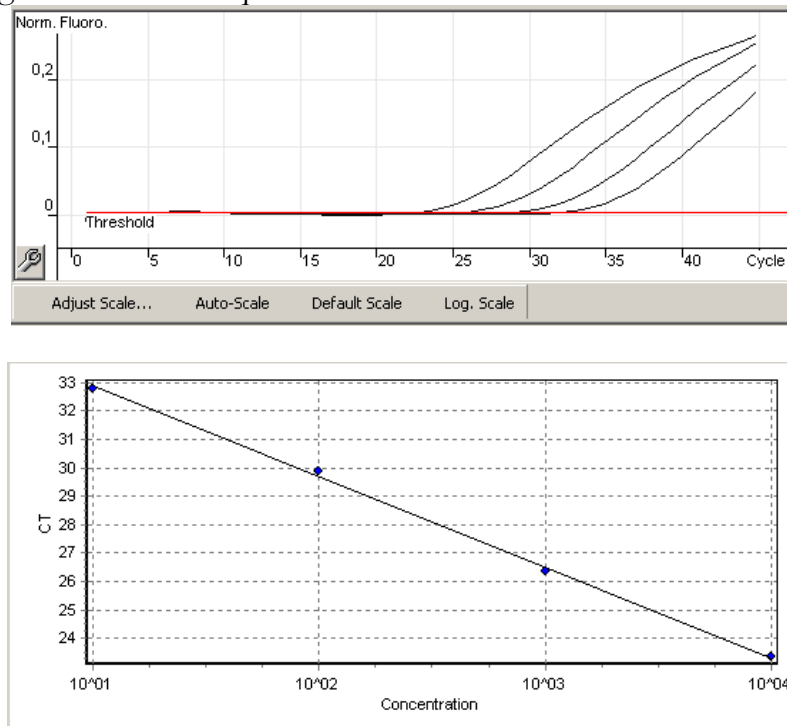
Fig. 12. Main screen of the Absolute Quantification analysis.



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

3. Setting of a detection quantitative assessment (Fig. 13.).
 - a) Select **Cycling A.Green** and **Cycling A.Red** (if available) for the qualitative assessment of the positive signal detection. Press **Show**.
 - b) Fill the checkbox next to the **Dynamic Tube** and **Slope Correct** parameters. Select **Ignore None** in the **Ignore First** tab.
 - c) Enter **NTC 10** in the **Outlier Removal** tab.
 - d) Select **Linear scale**.
 - e) Click **Auto-Find Threshold** for an automatic detection assessment.

Fig. 13. Setting of a detection quantitative assessment.



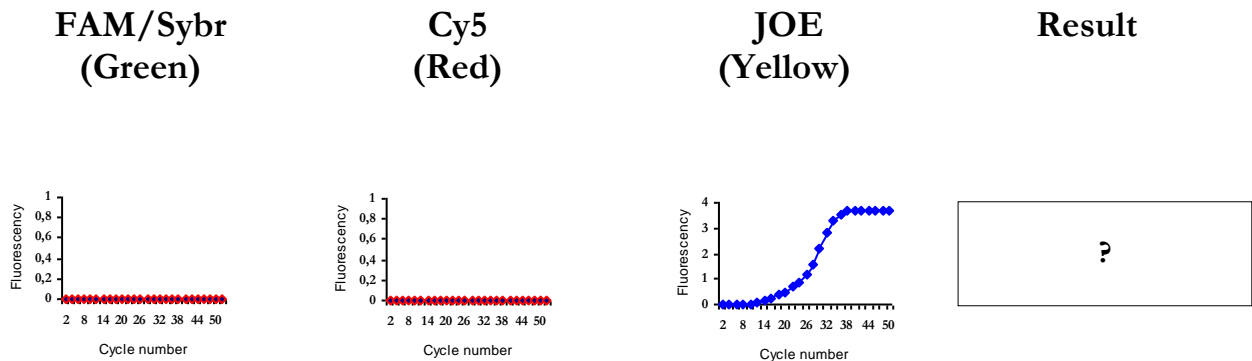
Only concentrations in the range specified by the calibration curve may be measured for a quantitative evaluation of the results.

- a) Quantification of samples out of calibration curve should be considered to be not very precise.
- b) Samples upper the highest concentrated calibrator could be diluted to achieve more precise quantification.
- c) Samples with lower concentrations than the lowest concentrated calibrator can be quantified approximately only.
- d) The following formula can be used to convert sample concentrations to *units/ml* taking into account the isolation procedure:

$\text{Concentration/ml} = \frac{cVZ \times EO}{I}$	
cVZ	= sample concentration in units/ μ l
EO	= selected retention volume in μ l
I	= volume of material used for isolation in ml

Troubleshooting

Invalid result of a Positive Control analysis



❖ Problem: *Incorrect programming of the PCR amplification*

Problem resolution:

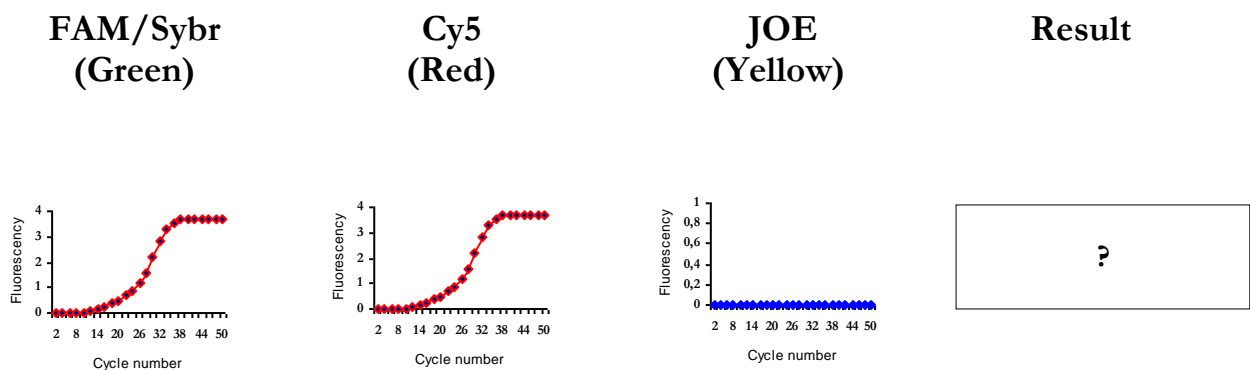
1. Check device programming according to the manual
2. Check correct temperature settings in the individual program blocks

❖ Problem: *Positive control incorrectly held in storage* (see Storage and transportation conditions)

Problem resolution:

1. Check whether kit component storage is in harmony with manufacturer's recommendations
2. Submultiple the Positive control and do not freeze and thaw it

Invalid result of a Negative Control analysis

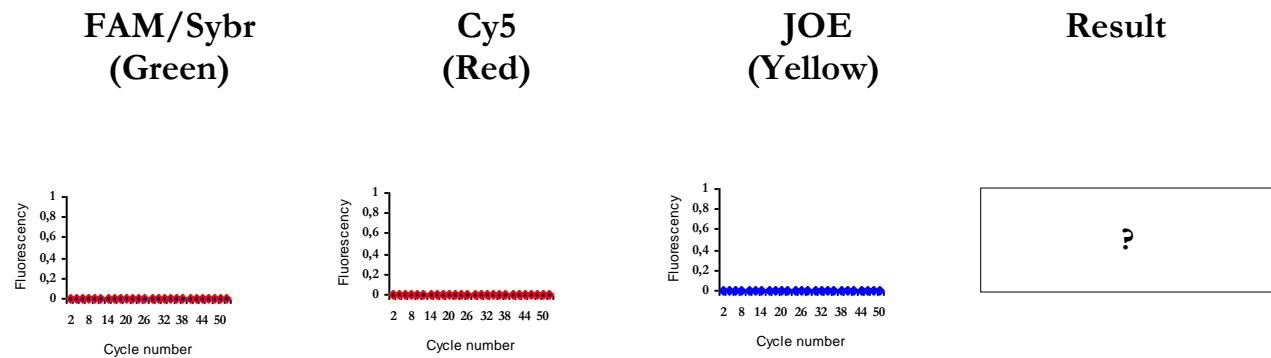


❖ Problem: *PCR reaction contamination*

Problem resolution:

1. Check the process of preparation and pipetting of the PCR mix into tubes
2. Check the handling of sterile plastics and filtered tips
3. Clean the PCR box
4. Add uracil-DNA-glycosylase (UDG) into the reaction

Invalid result of an Unknown Sample analysis



- ❖ Problem: *PCR reaction inhibition* (PCR kit ISIN and ISEX)
Problem resolution:
 1. Repeat DNA isolation
 2. Check the process of preparation and pipetting of the PCR mix into tubes
- ❖ Problem: *Invalid process of DNA isolation* (PCR kit ISEX)
Problem resolution:
 3. Repeat DNA isolation
 4. Check the process of preparation and pipetting of the Internal Standard at the beginning of the isolation process.
- ❖ Problem: *Incorrect storage of the MasterMix* (see Storage and transportation conditions)
Problem resolution:
 1. Check whether MasterMix storage is in harmony with manufacturer's recommendations
 2. Submultiple the MasterMix and do not freeze and thaw it

If you have any questions please contact our Product Support Department at:
support@geneproof.com

Notes:

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