February 2018

Investigator® Quantiplex® Pro RGQ Kit Handbook

For quantification of human and male DNA in forensic samples

Sample to Insight

QIAGEN

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Kit Contents

Investigator Quantiplex Pro RGQ Kit **Catalog no.** **Number of 20 µl reactions**	**(200)** **387316** **200**
Quantiplex Pro RGQ Reaction Mix	1 x 1.9 ml
Quantiplex Pro RGQ Primer Mix	1 x 1.9 ml
Male Control DNA M1 (50 ng/µl)	0.2 ml
QuantiTect® Nucleic Acid Dilution Buffer	1 vial

Storage

Kit reagents should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. After first use, store the kit components at 2–8°C. Avoid freezing the kit components. The QuantiTect Nucleic Acid Dilution Buffer may also be stored at –30°C to –15°C, if desired. Quantiplex Pro RGQ Primer Mix must be stored protected from the light. DNA samples should be stored separately from PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Intended Use

The Investigator Quantiplex Pro RGQ Kit is intended for molecular biology applications in forensic, human identity and paternity testing. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend that all users of QIAGEN® products adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Investigator Quantiplex Pro RGQ kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Human identification is commonly based on the analysis of short tandem repeats (STRs), single nucleotide polymorphisms (SNPs) or deletion insertion polymorphisms (DIPs), depending on the demands of an examination or on the sample quality. These multiplex assays used for human identification are complex systems that require a defined range of template input.

The Investigator Quantiplex Pro RGQ Kit provides quantification of human genomic DNA, male DNA and the integrity of DNA in a sample using quantitative real-time PCR. The kit is designed to confirm whether a sample contains sufficient DNA to enable DNA fingerprinting analysis (such as STR, DIP or SNP analysis). Furthermore, the kit may help in establishing whether a sample contains inhibitors that may interfere with such applications, thus

necessitating further sample purification. In addition, the newly developed DNA degradation systems allows for a more precise assessment of the degradation status of the DNA.

The Investigator Quantiplex Pro RGQ Kit uses QuantiNova® DNA Polymerase, a novel hot-start enzyme, and QuantiNova Guard, a novel additive. These unique components further improve the stringency of the antibody-mediated hot-start.

The kit also features a built-in control for visual identification for correct pipetting, and Q-Bond®, an additive in the buffer that enables short cycling steps without loss of PCR sensitivity and efficiency.

Principle and Procedure

The Investigator Quantiplex Pro RGQ Kit is a ready-to-use system for the detection of human and male DNA, and parallel assessment of DNA degradation using quantitative real-time PCR. The kit provides fast and accurate quantification of human DNA in forensic database and casework samples.

The kit contains reagents and a DNA polymerase for specific amplification of 4NS1C®, which is a 91 bp proprietary region present on several autosomes of the human genome, and for detection of the specific PCR products on the Rotor-Gene® Q System. The human quantification target region was selected to achieve high sensitivity. The human quantification target region is detected using the yellow channel on the Rotor-Gene Q.

The target region for male DNA quantification was selected to achieve high sensitivity in the presence of mixed female/male DNA samples. The male quantification target region is detected as an 81 bp fragment using the green channel on the Rotor-Gene Q.

In addition, the Investigator Quantiplex Pro RGQ Kit contains a balanced internal amplification control that is used to test successful amplification, and identify the presence of

PCR inhibitors. This heterologous amplification system is detected as a 434 bp internal control (IC), in the crimson channel, on the Rotor-Gene Q.

Furthermore, the kit detects a longer autosomal amplification product (353 bp) targeting the same locus (4NS1C) as the 91 bp autosomal target. Due to the differently sized autosomal targets, the longer autosomal target is more susceptible to DNA degradation, allowing for a precise assessment of the degradation status of the DNA. The larger autosomal quantification target region is detected as a 353 bp fragment, using the red channel on the Rotor-Gene Q.

A unique feature of the kit is that it detects a longer gonosomal amplification product (359 bp) targeting the same locus as the smaller 81 bp gonosomal male target. Due to the differently sized gonosomal targets, the longer gonosomal target is more susceptible to DNA degradation, allowing for a precise assessment of the degradation status of male DNA. The larger gonosomal quantification target region is detected as a 359 bp fragment, using the orange channel on the Rotor-Gene Q.

Detection of amplification is performed using TaqMan® probes and a novel, fast PCR chemistry. Dual-labeled probes, such as TaqMan probes, contain a fluorescent reporter and a quencher at their 5' and 3' ends, respectively. During the extension phase of PCR, the 5' and 3' exonuclease activity of QuantiNova DNA Polymerase cleaves the fluorophore from the quencher. This results in detectable fluorescence that is proportional to the amount of accumulated PCR product.

Internal control

The Internal Control (IC) is amplified and detected in the crimson channel on the Rotor-Gene Q system. The IC is designed to be more sensitive to inhibitors than the human and male quantification targets. The comparison of the Cq values of the IC system for DNA standards with the Cq values of the IC system for unknown samples may provide an indication of potential inhibition of the reaction in the unknown samples. Therefore, even if the IC system

reports the presence of inhibitors in the sample, the DNA quantification will typically provide a reliable result. The presence of inhibitors in the sample may affect the downstream application and must be considered.

Laboratory validation with relevant inhibitors should be performed to determine criteria for detecting inhibition.

Quantiplex Pro RGQ Reaction Mix

The Quantiplex Pro RGQ Reaction Mix contains the novel hot start QuantiNova DNA Polymerase and Quantiplex Pro RGQ reaction buffer. QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient or higher temperatures. The antibody-mediated hot-start mechanism prevents the formation and extension of nonspecific PCR products and primer-dimers during reaction setup and the first denaturation step. Therefore, this mechanism allows higher PCR specificity and accurate quantification. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and Guard, which stabilize the complex and improve the stringency of the hot start. After raising the temperature for 2 minutes to 95°C, the QuantiNova Antibody and Guard are denatured and the QuantiNova DNA Polymerase is activated, enabling PCR amplification. The hot start enables rapid and convenient room-temperature setup.

Furthermore, the specially developed Quantiplex Pro reaction buffer contains the additive Q-Bond, which allows short cycling times on the Rotor-Gene Q. Q-Bond increases the affinity of the DNA polymerase for short, single-stranded DNA, reducing the time required for primer/probe annealing to a few seconds. In addition, the unique composition of the buffer supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

The Quantiplex Pro RGQ Reaction Mix is also based on the unique QIAGEN PCR buffer system. The buffer contains a balanced combination of KCl and NH_4Cl, which promotes a

high ratio of specific-to-nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity.

Male Control DNA M1 and standard curve

DNA quantification standards are critical for accurate analysis. We strongly recommend a 27-fold dilution series with 4 concentration points in the standard curve, for each assay. The Control DNA contains pooled male DNA at a concentration of 50 ng/µl. To ensure pipetting accuracy, the minimum input volume of DNA for dilutions should be 5 µl. The standard curve is designed to be easily set up using a convenient 1:27 dilution series. If using QuantiTect Nucleic Acid Dilution Buffer to dilute the Control DNA, the dilutions are stable for at least 1 week at 2–8°C.

Important: Male Control DNA M1 is optimized for use with the Investigator Quantiplex Pro RGQ kits only.

Templates for routine work

In order to streamline the instrument setup and the analysis of the results on the Rotor-Gene Q system, QIAGEN has developed a set of template files. Download the template files from the product resources page at **www.qiagen.com/QPpro-rgq-template-files**.

Description of protocols

Protocols for the Rotor-Gene Q system are provided in this handbook.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment

- Cooling device or ice
- Real-time thermal cycler
  - Rotor-Gene Q system (5plex/6plex) with 72-Well Rotor/Rotor-Disc® 72 Rotor/Rotor-Disc 100 Rotor
  - Q-Rex Software
  - Q-Rex Absolute Quantification HID Plug-in
  - Q-Rex QIAgility® Wizard Plug-in
  - QIAGEN Quantification Assay Data Handling and STR Setup Tool

Material

- Pipettes and pipette tips
- Strip Tubes and Caps, 0.1 ml (cat. no./ID: 981103 or cat. no./ID: 981106)
- Rotor-Disc 72; (cat. no./ID: 981301 or cat. no./ID: 981303)
- Rotor-Disc 100; (cat. no./ID: 981311 or cat. no./ID: 981313)

Reagents

- Nuclease-free (RNase/DNase-free) consumables: special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive detection of human DNA.

Important Notes

Selecting kits and protocols

This handbook contains protocols and recommendations for DNA quantification using the Rotor-Gene Q (5plex/6plex), with protocols listed in Table 1. Real-time cyclers other than the Rotor-Gene Q (5plex/6plex) have not been validated by QIAGEN for DNA quantification, using the Investigator Quantiplex Pro RGQ Kit.

Table 1. Protocols for the Investigator Quantiplex Pro RGQ Kit with Rotor-Gene Q

Real-time thermal cycler	Protocol
Rotor-Gene Q, manual setup using Template Files in Q-Rex	Page 13
Rotor-Gene Q, setup with the QIAGEN Quantification Assay Data Handling and STR Setup Tool	Page 24

Contamination risks

Do not remove the seal or caps of the reaction tubes once the amplification is complete. Removing the seal or caps increases the risk of contaminating subsequent reactions with amplified product.

All reaction mixtures should be set up in an area separate from that used for DNA isolation and PCR product analysis (post-PCR), to minimize the potential for cross-contamination. Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Controls

No-template control (NTC)

Replicates of NTC reactions should be included in each quantification run to detect contamination. NTCs should contain all the components of the reaction, except for the

template. Quantification using the Investigator Quantiplex Pro RGQ Kit is highly sensitive; despite the fact that the reagents contained in the Quantiplex Kit undergo strict quality controls to assess that they are free of human DNA contamination, background DNA may be detected in rare cases due to the high sensitivity of the assay. Take great care to avoid contamination when pipetting the NTC.

We recommend performing at least duplicate NTC reactions.

Internal positive control

An internal positive control (detected using a TaqMan probe) is used to test for successful amplification, and for the presence of PCR inhibitors. The primers, TaqMan probe and template for the internal control are all contained in the Quantiplex Pro RGQ Primer Mix.

Protocol: Quantification of DNA Using Manual Setup and Template Files in Q-Rex and Rotor-Gene Q

This protocol is optimized for assay setup on the Rotor-Gene Q using Q-Rex Software 1.0 and template files for manual setup.

For general instructions on instrument setup and Q-Rex software, refer to the Rotor-Gene Q User Manual and Q-Rex Software User Manual available on **www.qiagen.com**.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Use the cycling conditions specified in the protocol. The cycling is optimized for this assay.
- Use the template volume specified in the protocol. The reaction is optimized for use with 2 µl template DNA. Do not use more or less than 2 µl per 20 µl reaction.
- Dilutions of DNA quantification standards in QuantiTect Nucleic Acid Dilution Buffer can be stored between 2 and 8°C for at least 1 week.
- Optimal analysis settings are a prerequisite for accurate quantification data. Readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.
- We recommend using the provided template files for manual setup in Q-Rex to streamline the instrument setup and the analysis of the results on the Rotor-Gene Q.

Procedure:

1. Mix all solutions thoroughly before use to avoid localized concentrations of salt.
2. Prepare fresh serial dilutions of the Male Control DNA M1 according to Table 2. Vortex for at least 5 s, and centrifuge each dilution briefly before removing an aliquot for the next dilution. Use a new pipette tip for each dilution.

Make sure not to introduce cross-contamination.

Table 2. Serial dilutions of Male Control DNA M1

Serial dilution of Control DNA (ng/µl)	Control DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
50	Undiluted DNA	–
1.8519	5	130
0.0686	5	130
0.0025	5	130

Note: Alternative standard curves are listed in "Appendix: Alternative Standard Curves", page 70.

3. Thaw the template nucleic acids.

   Mix all solutions thoroughly before use to avoid localized concentrations of salt.

4. Prepare a master mix according to Table 3.

   The master mix contains all of the components needed for PCR, besides the template (sample) DNA and nuclease-free water.

   Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.

   Reaction setup can usually be performed at room temperature (15–25°C). However, we recommend keeping the reagents, samples and controls on ice or in a cooling device.

Table 3. Master mix for DNA quantification

Component	Volume per 20 µl reaction	Final concentration
Quantiplex Pro RGQ Reaction Mix	9 µl	1x
Quantiplex Pro RGQ Primer Mix	9 µl	1x
Total volume of master mix	**18 µl**	**–**

5. Mix the master mix thoroughly, and dispense 18 µl into Rotor-Gene Q Tubes or into the Rotor-Disc.
6. Add 2 µl QuantiTect Nucleic Acid Dilution Buffer to the NTC tubes.

   Make sure that the NTC wells do not come into contact with human DNA.
7. Add 2 µl control DNA dilutions, or 2 µl unknown sample DNA, to the individual tubes and mix thoroughly. Close the tubes.

   Mix carefully to avoid localized concentrations of salt and DNA.

   Table 4 shows a possible tube setup. Make sure that the master mix and template are thoroughly mixed. It is required to run duplicates of the control DNA dilutions for each assay and on each reaction plate.

Table 4. Possible setup of reactions on the Rotor-Gene Q using Strip Tubes

Tube contents																	
1	50	9	NTC	17	UNK	25	UNK	33	UNK	41	UNK	49	UNK	57	UNK	65	UNK
2	50	10	NTC	18	UNK	26	UNK	34	UNK	42	UNK	50	UNK	58	UNK	66	UNK
3	1.8519	11	UNK	19	UNK	27	UNK	35	UNK	43	UNK	51	UNK	59	UNK	67	UNK
4	1.8519	12	UNK	20	UNK	28	UNK	36	UNK	44	UNK	52	UNK	60	UNK	68	UNK
5	0.0686	13	UNK	21	UNK	29	UNK	37	UNK	45	UNK	53	UNK	61	UNK	69	UNK
6	0.0686	14	UNK	22	UNK	30	UNK	38	UNK	46	UNK	54	UNK	62	UNK	70	UNK
7	0.0025	15	UNK	23	UNK	31	UNK	39	UNK	47	UNK	55	UNK	63	UNK	71	UNK
8	0.0025	16	UNK	24	UNK	32	UNK	40	UNK	48	UNK	56	UNK	64	UNK	72	UNK

All amounts in ng/µl. UNK: Unknown sample.

8. Place closed PCR tubes in the appropriate rotor in the Rotor-Gene Q cycler and attach the locking ring.

   If you are using tubes, empty positions in the rotor should be filled with empty PCR tubes.

9. Open Q-Rex Software 1.0 and login as a user or as an administrator (for further details on how to do a first-time login on Q-Rex, about user roles or how to add new users, see Q-Rex Software User Manual available on **www.qiagen.com**).

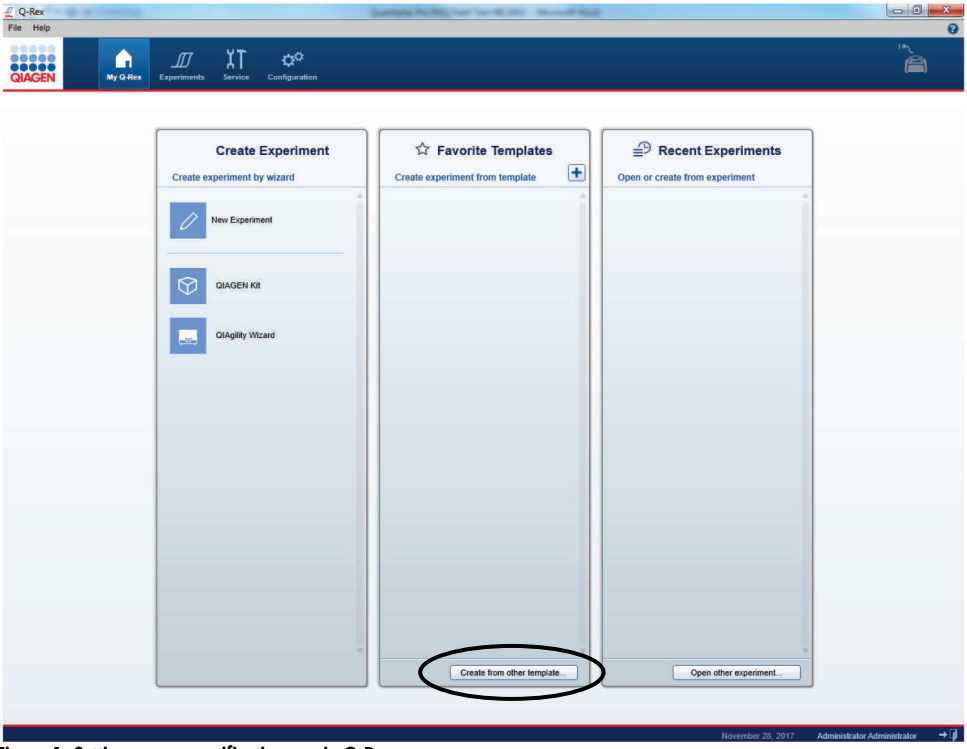

Figure 1. Setting up a quantification run in Q-Rex.

10. Select Create from other template (Figure 1).

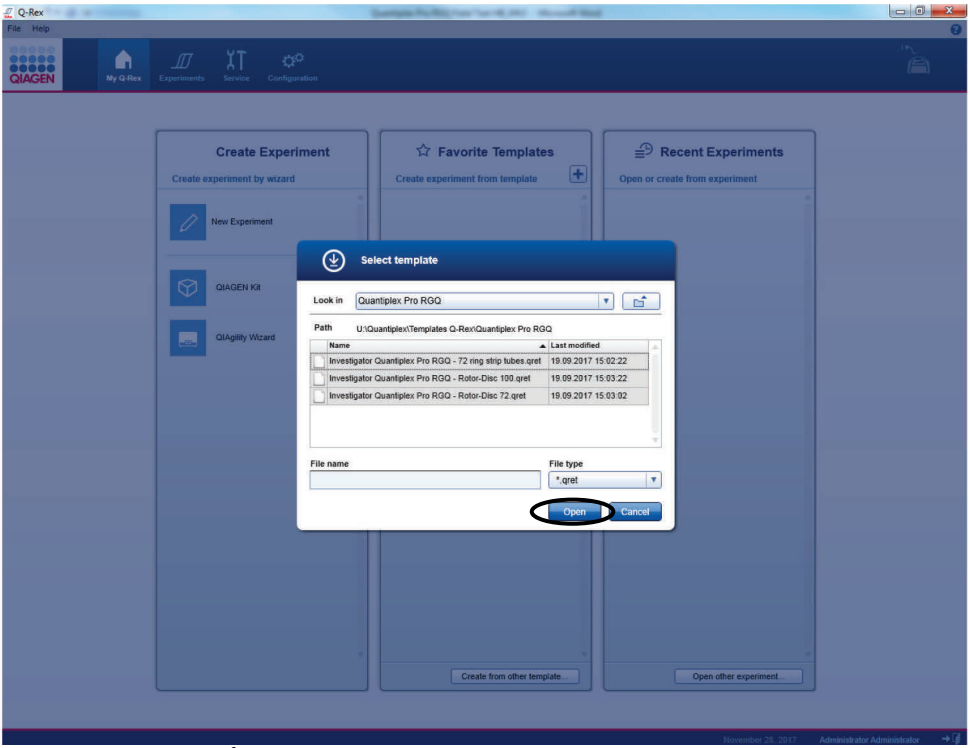

Figure 2. Setting up a quantification run in Q-Rex.

11. Browse to locate the saved Quantiplex Pro RGQ template files for manual setup and select the correct Quantiplex Pro RGQ template file for 0.1 ml strip tubes, Rotor-Disc 72 or Rotor-Disc 100 (Figure 2).
12. Click on open (Figure 2).

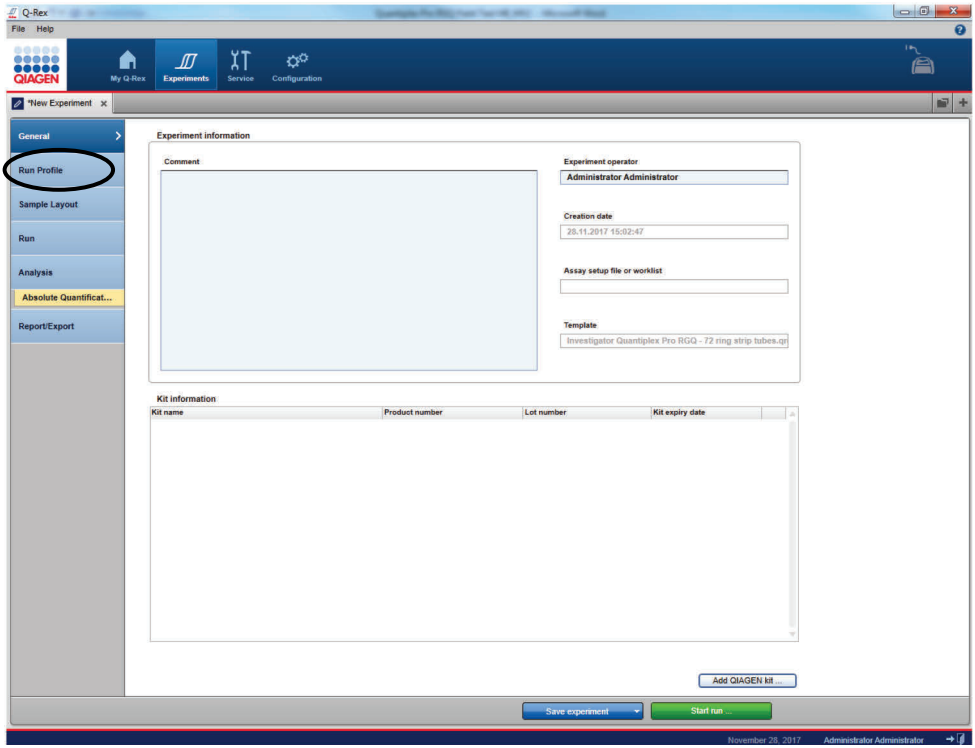

Figure 3. General settings in Q-Rex.

13. In the comments field add any additional information needed (e.g. kit lot number, kit expiry date, etc.) to be entered for the run (Figure 3).
14. Click on left side in the step marker on "Run Profile" (Figure 3).

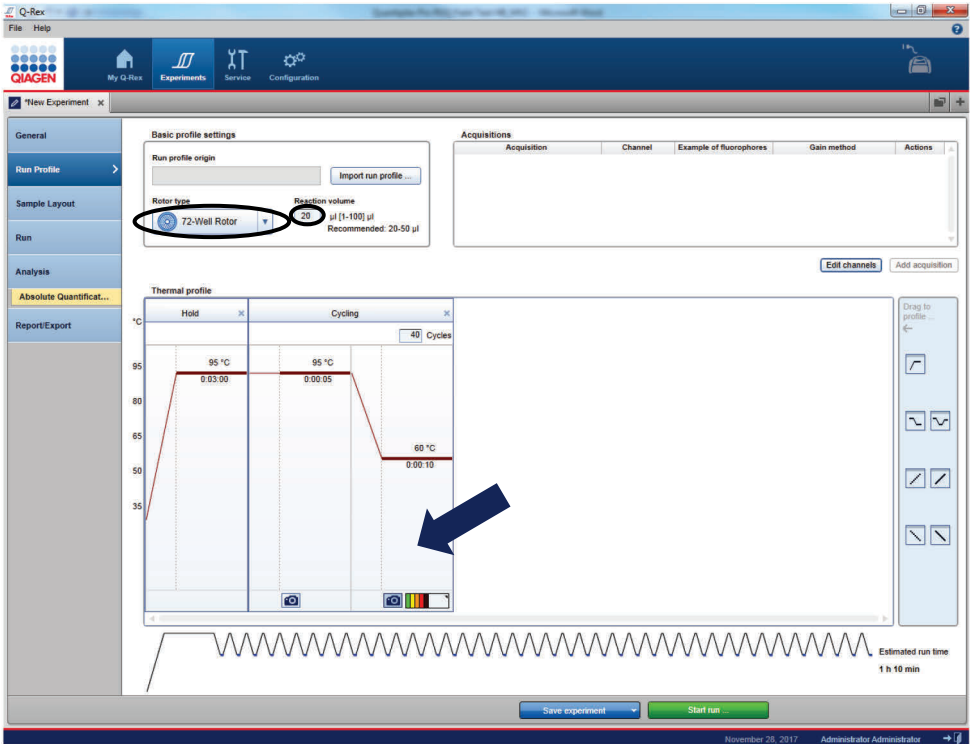

Figure 4. Run profile settings in Q-Rex.

15. Confirm Rotor type and Reaction volumes" (Figure 4).
16. Confirm that the cycling conditions preset in the template file are the same as outlined in Table 5.
17. Click in the thermal profile in the area above the colored camera symbol (indicated by arrow in Figure 4). Acquisition settings will be displayed (Figure 5).
18. Click on the "Auto-gain" field for the green channel (Figure 5).

Table 5. Cycling conditions for the Rotor-Gene Q

Step	Time	Temperature	Number of cycles	Comment
Initial activation step	3 min	95°C	–	PCR requires an initial incubation at 95°C to activate the DNA polymerase.
Two-step cycling:			**40**	
Denaturation	5 s	95°C		
Combined annealing/extension	10 s	60°C		Perform fluorescence data collection using the green, yellow, orange, red and crimson channels with auto-gain optimization, specified in table 6.

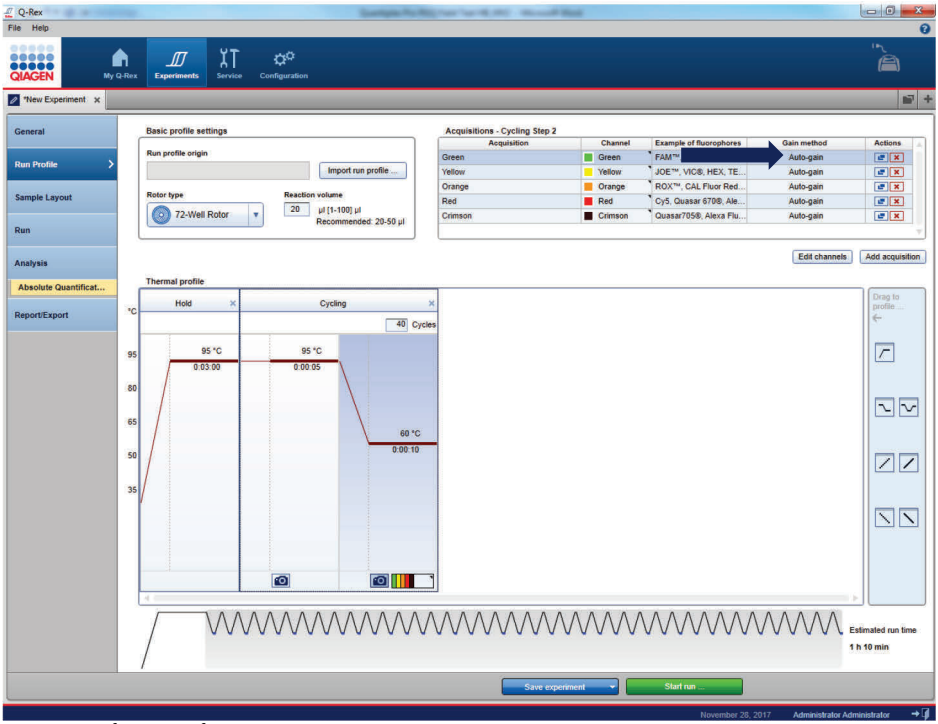

Figure 5. Confirmation of auto-gain settings in Q-Rex.

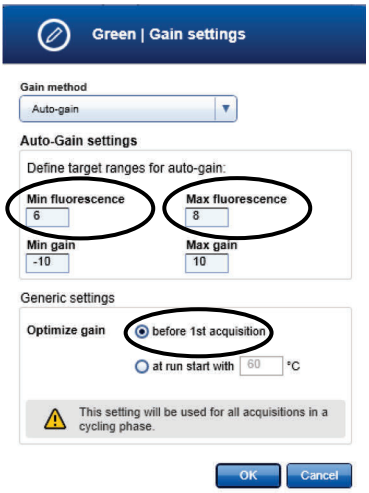

Figure 6. Confirm auto-gain settings on Q-rex.

19. Confirm that ''Auto-gain'' is selected (see Figure 6), and settings for the green channel preset in the template are the same as outlined in Table 6.'' Optimize gain'' should be selected before 1st acquisition. Confirm auto-gain settings also for the yellow, orange, red and crimson channels, according to Table 6.

Table 6. Auto-gain settings for the different channels.

Channel	Min. fluorescence	Max. fluorescence	Optimize gain
Green	6	8	Before 1st acquisition
Yellow	6	8	Before 1st acquisition
Orange	2	4	Before 1st acquisition
Red	6	8	Before 1st acquisition
Crimson	5	7	Before 1st acquisition

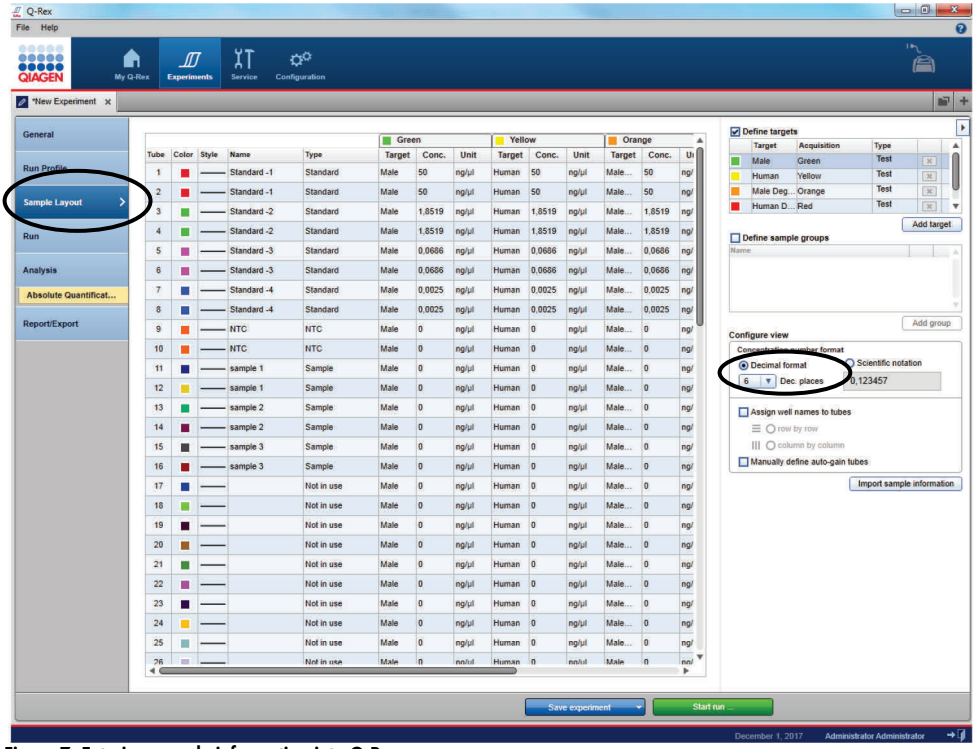

Figure 7. Entering sample information into Q-Rex.

20. Click on the left side in the step marker on "Sample Layout" (Figure 7). Confirm or edit sample layout for Standards and NTCs.
21. Enter samples IDs and set sample type. Sample type can be set/changed by right clicking on the "Type" field. Tubes that are not being used have to be marked as "Not in use". Select appropriate decimal format (Figure 7).
22. Start the Rotor-Gene cycler by clicking "Start run".
23. Proceed to data analysis on page 46.

Protocol: Quantification of DNA Using the QIAGEN Quantification Assay Data Handling Tool and Rotor-Gene Q

This protocol is optimized for assay setup with the QIAGEN Quantification Assay Data Handling Tool on the Rotor-Gene Q using Q-Rex Software 1.0 and template files for use with the Data Handling Tool.

For general instructions on instrument setup and Q-Rex software, refer to the Rotor-Gene Q User Manual and Q-Rex Software User Manual available on **www.qiagen.com**.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Use the cycling conditions specified in the protocol. The cycling is optimized for this assay.
- Use the template volume specified in the protocol. The reaction is optimized for use with 2 µl template DNA. Do not use more or less than 2 µl per 20 µl reaction.
- Dilutions of DNA quantification standards in QuantiTect Nucleic Acid Dilution Buffer can be stored between 2 and 8°C for at least 1 week.
- Optimal analysis settings are a prerequisite for accurate quantification data. Readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.
- **Note:** This protocol requires the Q-Rex QIAgility Wizard Plug-in to be installed. If the Q-Rex QIAgility Plug-in is not installed proceed to protocol on page 13.

Procedure:

1. Mix all solutions thoroughly before use to avoid localized concentration of salt.
2. Prepare fresh serial dilutions of the Male Control DNA M1 according to Table 7. Vortex for at least 5 s, and centrifuge each dilution briefly before removing an aliquot for the next dilution. Use a new pipette tip for each dilution.

   Make sure to not introduce cross-contamination.

Table 7. Serial dilutions of Male Control DNA M1

Serial dilution of Control DNA (ng/µl)	Control DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
50	Undiluted DNA	–
1.8519	5	130
0.0686	5	130
0.0025	5	130

   Note: Alternative standard curves are listed in "Appendix: Alternative Standard Curves", page 70.

3. Thaw the template nucleic acids.

   Mix all solutions thoroughly before use to avoid localized concentration of salt.

4. Prepare a master mix according to Table 8.

   The master mix contains all of the components needed for PCR besides the template (sample) DNA and nuclease-free water.

   Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.

   Reaction setup can usually be done at room temperature (15–25°C). However, we recommend keeping the reagents, samples and controls on ice, or in a cooling device.

Table 8. Master mix for DNA quantification

Component	Volume per 20 µl reaction	Final concentration
Quantiplex Pro RGQ Reaction Mix	9 µl	1x
Quantiplex Pro RGQ Primer Mix	9 µl	1x
Total volume of master mix	**18 µl**	–

5. Mix the master mix thoroughly, and dispense 18 µl into Rotor-Gene Q Tubes or into the Rotor-Disc.
6. Add 2 µl QuantiTect Nucleic Acid Dilution Buffer to the NTC tubes.

   Make sure that the NTC tubes do not come in contact with human DNA.
7. Add 2 µl control DNA dilutions, or 2 µl unknown sample DNA, to the individual tubes and mix thoroughly. Close the tubes.

   Mix carefully in order to avoid localized concentration of salt.

   Table 9 shows a possible tube setup. Make sure that the master mix and template are thoroughly mixed.

   It is required to run duplicates of the control DNA dilutions for each assay and for each run.

Table 9. Possible setup of reactions on the Rotor-Gene Q using Strip Tubes.

Tube contents																	
1	50	9	NTC	17	UNK	25	UNK	33	UNK	41	UNK	49	UNK	57	UNK	65	UNK
2	50	10	NTC	18	UNK	26	UNK	34	UNK	42	UNK	50	UNK	58	UNK	66	UNK
3	1.8519	11	UNK	19	UNK	27	UNK	35	UNK	43	UNK	51	UNK	59	UNK	67	UNK
4	1.8519	12	UNK	20	UNK	28	UNK	36	UNK	44	UNK	52	UNK	60	UNK	68	UNK
5	0.0686	13	UNK	21	UNK	29	UNK	37	UNK	45	UNK	53	UNK	61	UNK	69	UNK
6	0.0686	14	UNK	22	UNK	30	UNK	38	UNK	46	UNK	54	UNK	62	UNK	70	UNK
7	0.0025	15	UNK	23	UNK	31	UNK	39	UNK	47	UNK	55	UNK	63	UNK	71	UNK
8	0.0025	16	UNK	24	UNK	32	UNK	40	UNK	48	UNK	56	UNK	64	UNK	72	UNK

All amounts in ng/µl. UNK: Unknown sample.

8. Place closed PCR tubes in the appropriate rotor in the Rotor-Gene Q cycler, and attach the locking ring.

   If you are using tubes, empty positions in the rotor should be filled with empty PCR tubes.

9. Open the QIAGEN Quantification Assay Data Handling and STR Setup Tool.

10. Click the ''Configuration'' worksheet tab (Figure 8).

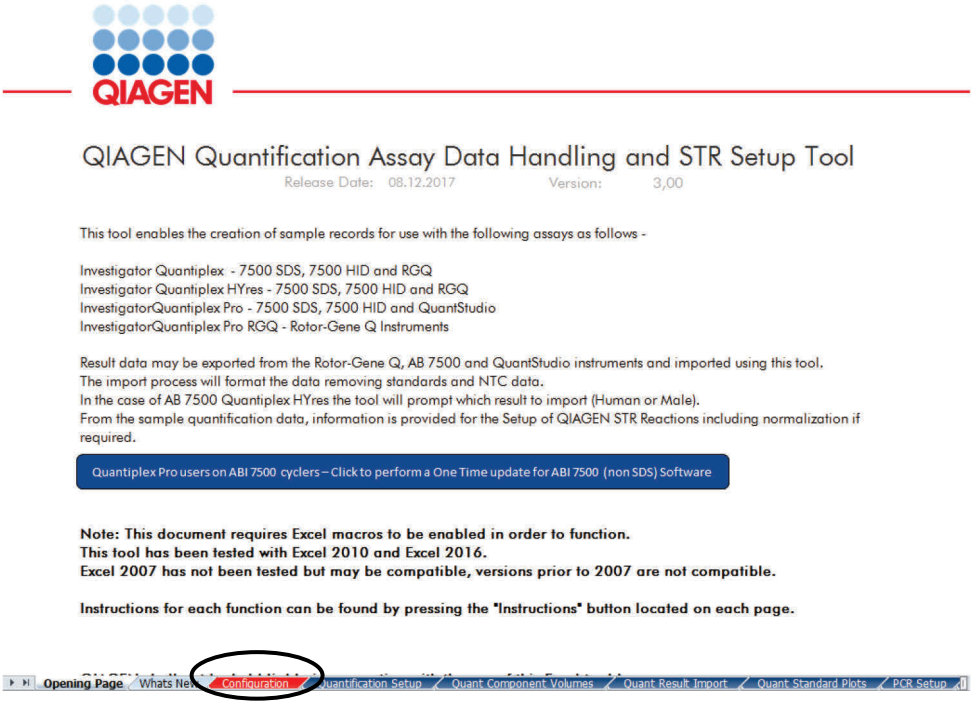

Figure 8. Opening the "Configuration" worksheet.

11. Set the root/home directory to save Quant batch files (Figure 9).
12. Set the root/home directory to import Quant result files (Figure 9).
13. Set cycler dependent default target naming and tube setup for RGQ (Figure 10).

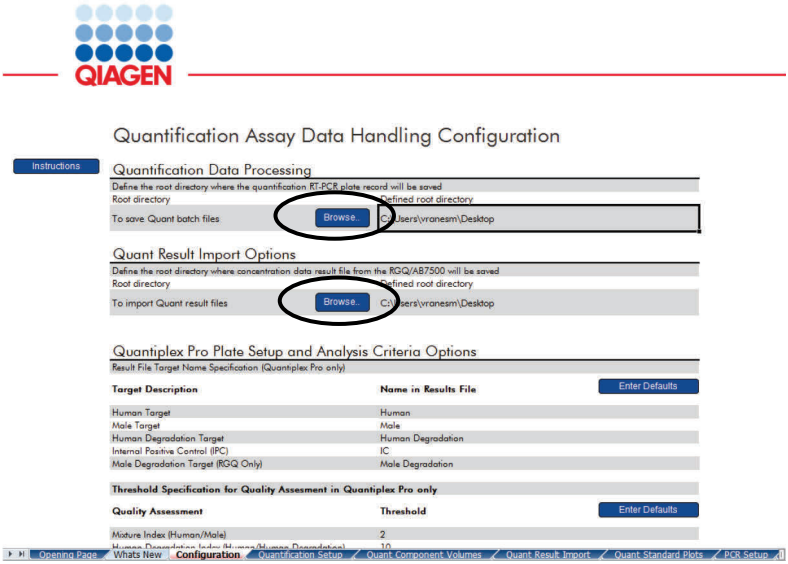

Figure 9. Setting the Quant batch, Quant result files, tube setup and analysis criteria.

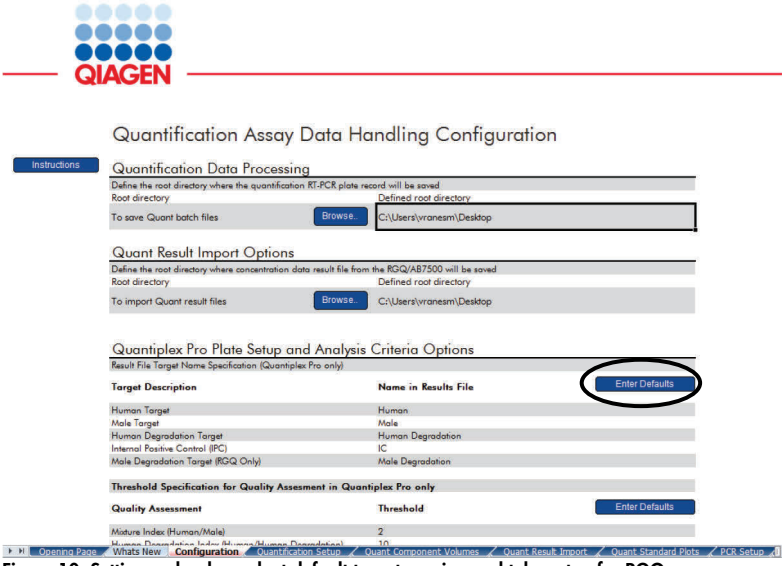

Figure 10. Setting cycler dependent default target naming and tube setup for RGQ.

14. Click the ''Quantification Setup'' worksheet tab (Figure 11).

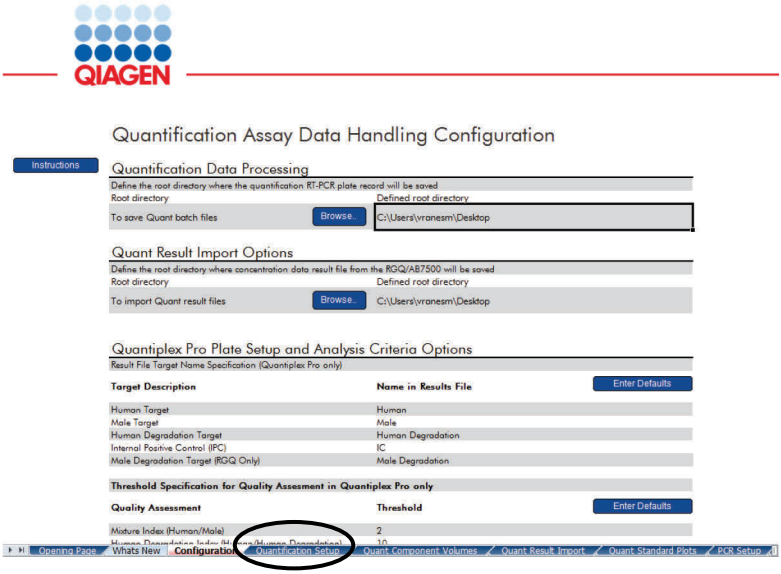

Figure 11. Setting the ''Quantification Setup''.

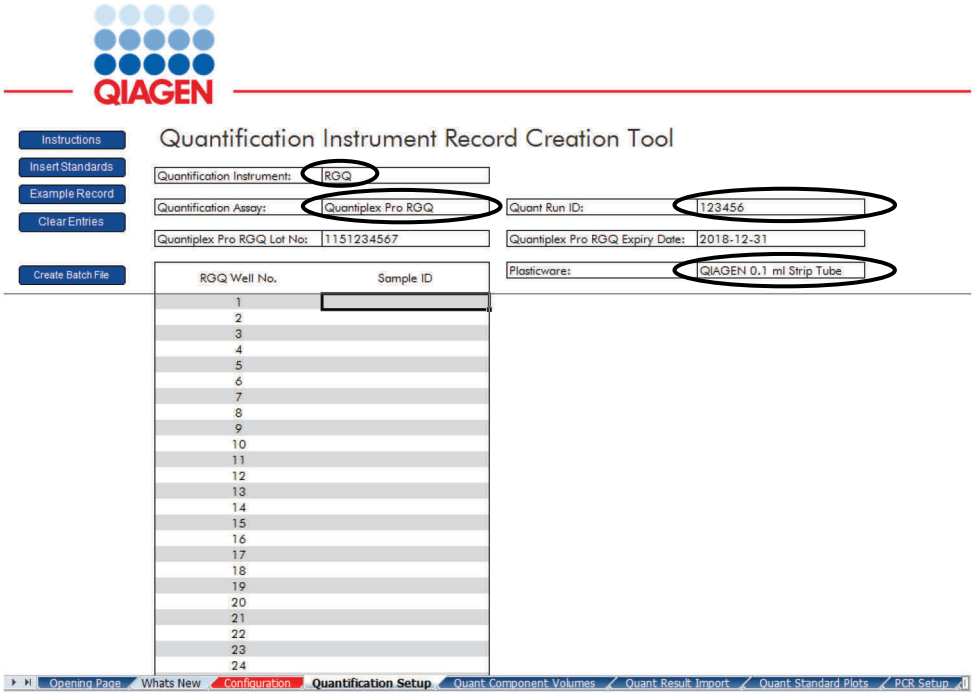

Figure 12. Setting the "Quantification Setup".

15. Select RGQ instrument in the drop down menu (Figure 12).
16. Select Assay – Quantiplex Pro RGQ (Figure 12).
17. Enter your Run ID for the run (Figure 12).
18. Select plasticware: Strip Tubes and Caps
    Rotor-Disc 72
    Rotor-Disc 100
19. Optionally enter kit lot number and kit expiry date (Figure 12).

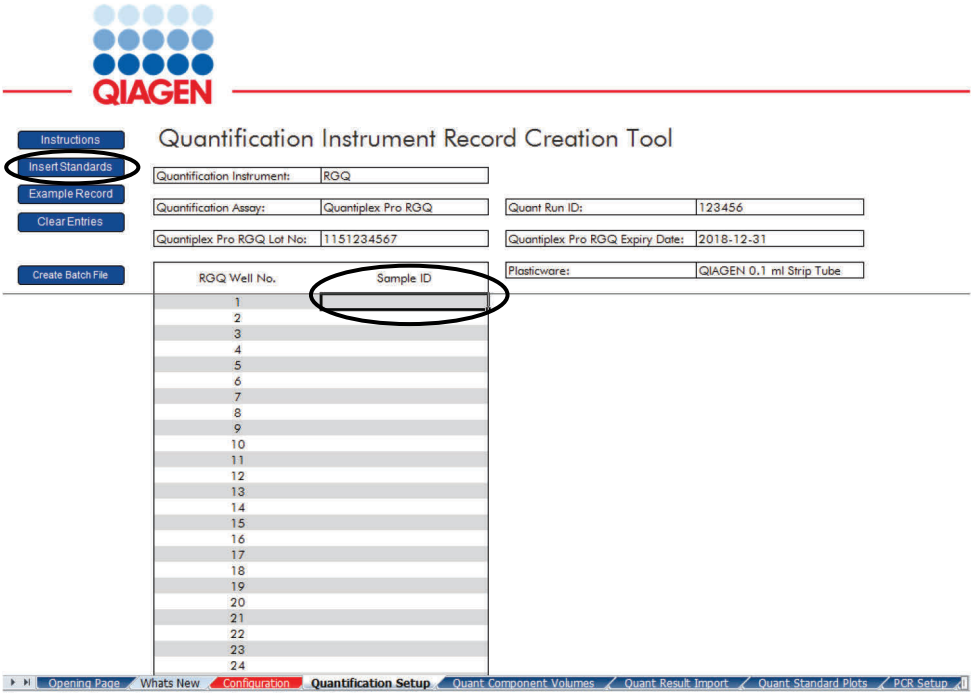

Figure 13. Inserting standards and sample IDs.

20. Click in the sample ID field (e. g. tube 1) to enter the DNA standards (Figure 13).
21. Then, click on the button "Insert Standards" and choose the layout for the standards (Figure 13).
22. Enter further sample IDs for each tube used (Figure 14).
23. Finish ''Quantification Setup'' by clicking "Create Batch file" (Figure 15). The Quant Batch file will be created in the root directory specified in step 11 (Figure 9). Use this Quant Batch file for simple run setup in Q-Rex. Proceed to step 24.

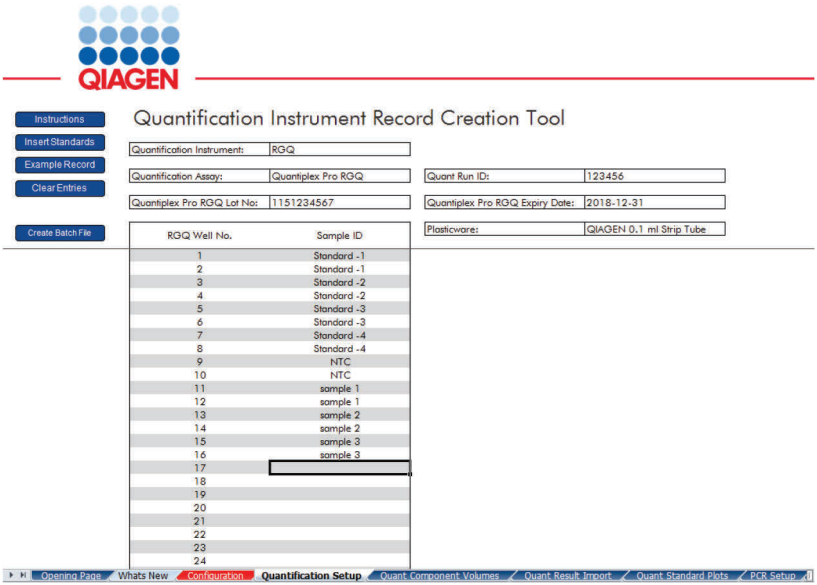

Figure 14. Inserting standards and sample IDs.

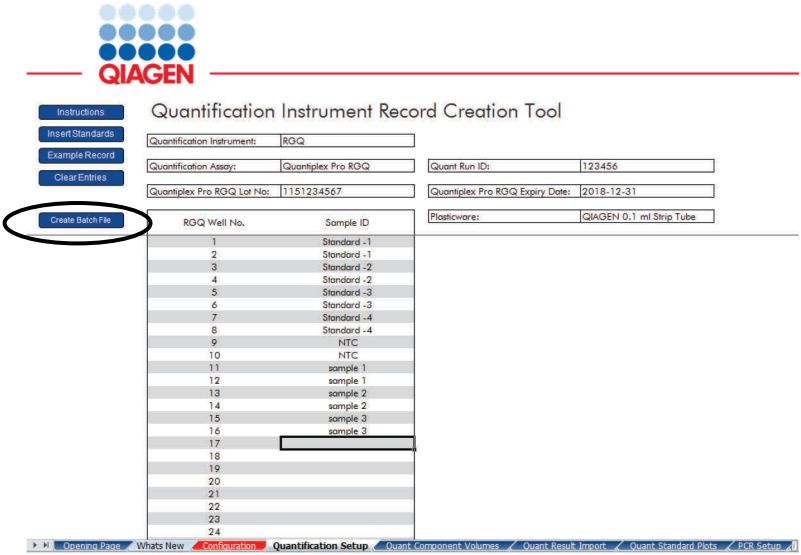

Figure 15. Creating a Quant Batch file.

24. Open Q-Rex Software 1.0 and login as a user or as an administrator (for further details on how to do a first time login in Q-Rex, about user roles or how to add new users, see Q-Rex Software User Manual available on **www.qiagen.com**).

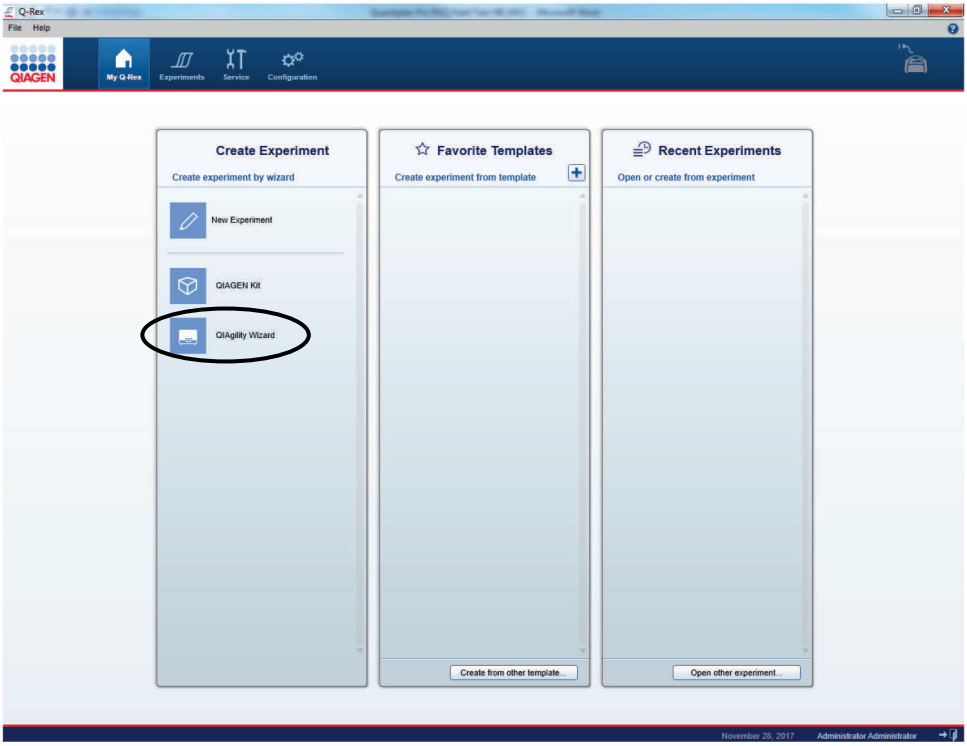

Figure 16. Setting up a quantification run in Q-Rex.

25. Select QIAgility Wizard (Figure 16).

Note: To import the Quant Batch file into Q-Rex the Q-Rex QIAgility Wizard Plug-in 1.0.1 needs to be installed. The plugin is available on **www.qiagen.com**.

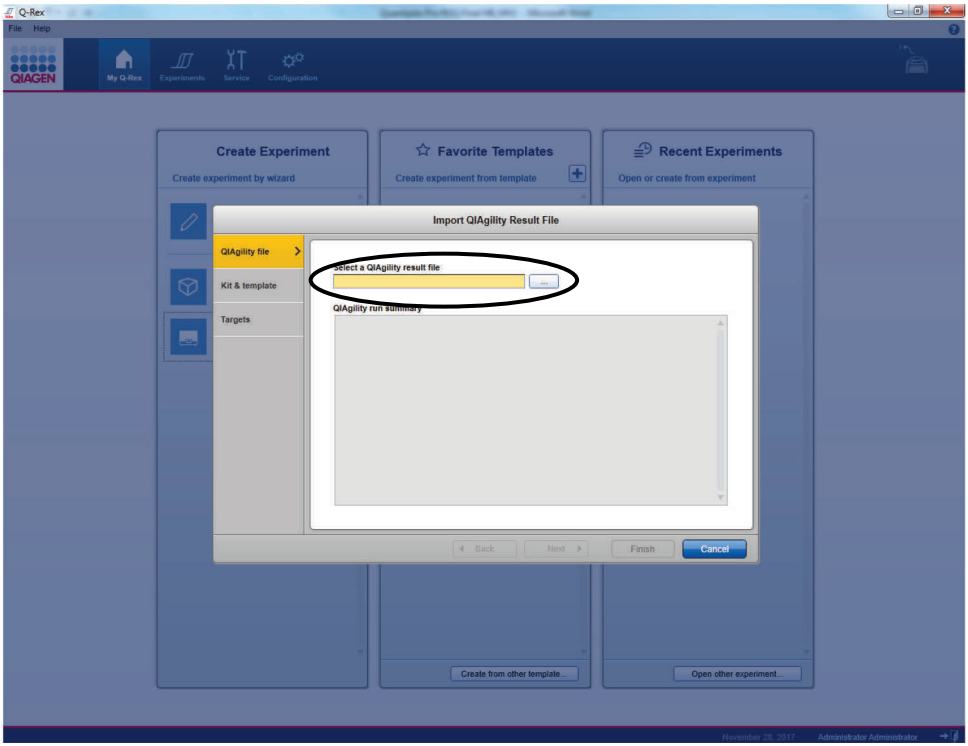

Figure 17. Selecting the Quant Batch file.

26. Select the Quant Batch file created in step 23 (Figure 17).

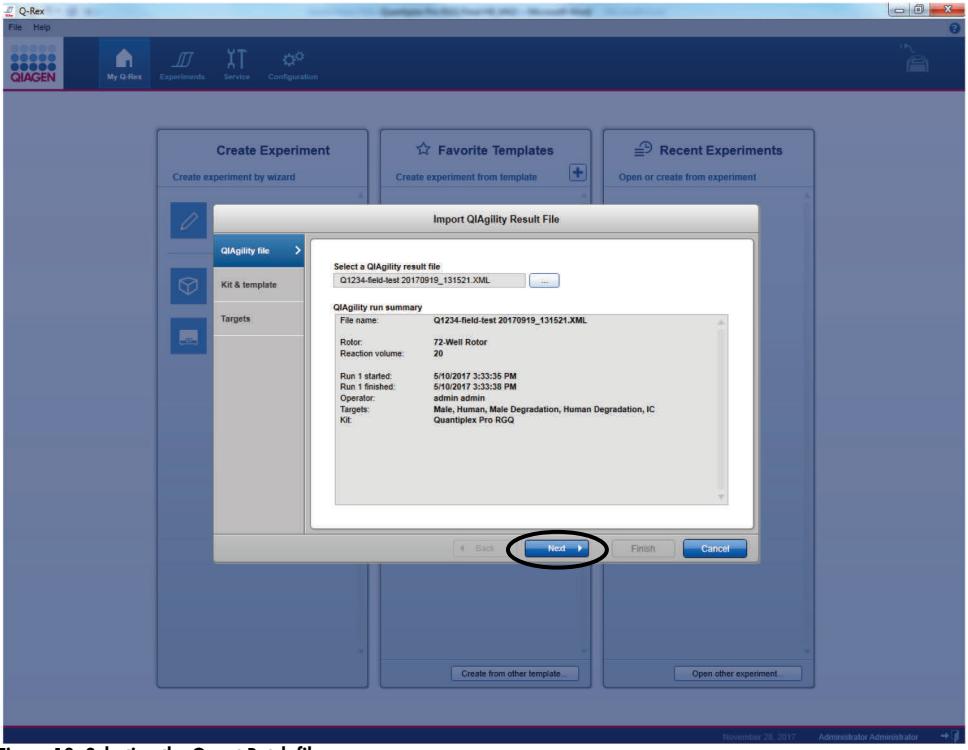

Figure 18. Selecting the Quant Batch file.

27. Click on next (Figure 18) and browse to locate the saved Quantiplex Pro RGQ Template files for use with the Data Handling Tool and select the correct Quantiplex Pro RGQ Template file for 0.1 ml strip tubes, Rotor-Disc 72 or Rotor-Disc 100 (Figure 19).

28. Click on open (Figure 19).

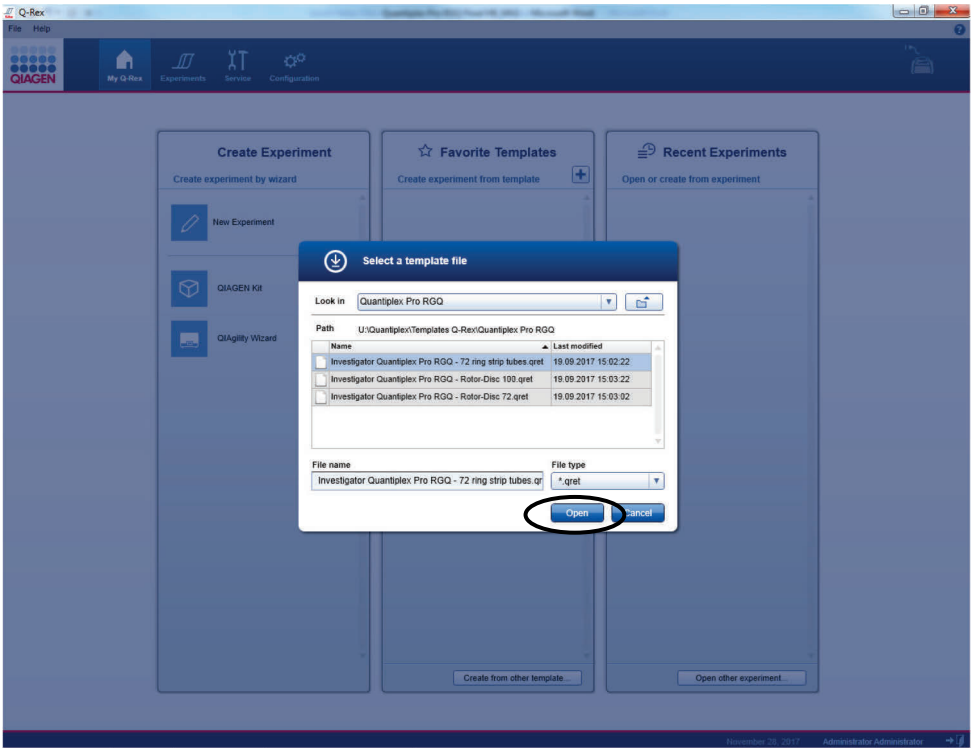

Figure 19. Selecting the Quantiplex Pro RGQ Template Q-Rex.

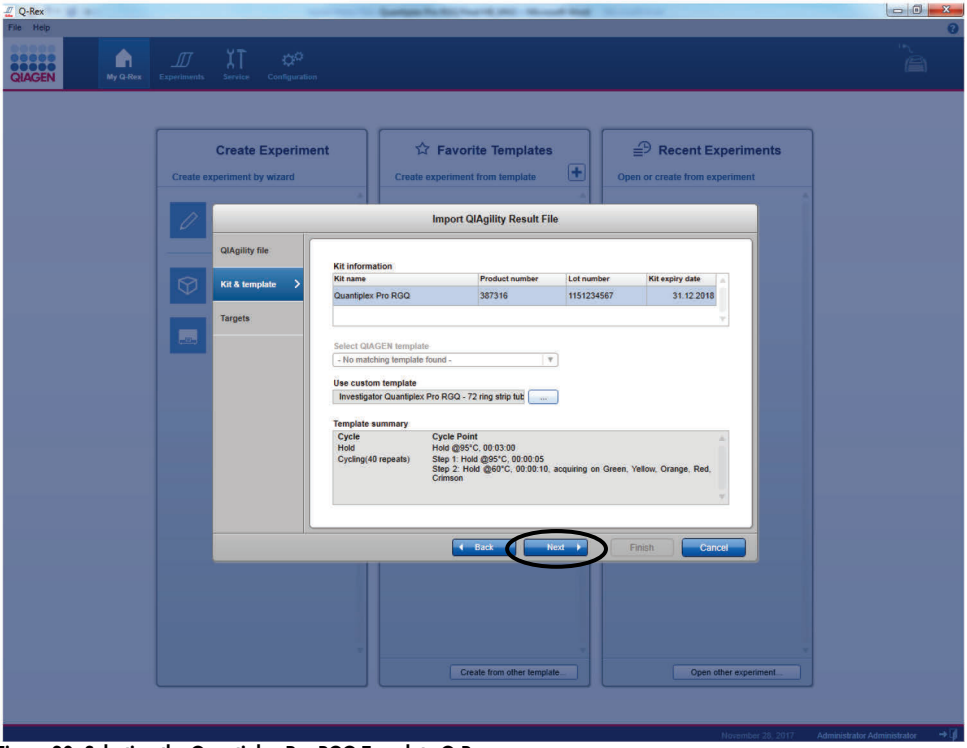

Figure 20. Selecting the Quantiplex Pro RGQ Template Q-Rex.

29. Confirm kit information, Q-Rex Template and click next (Figure 20).

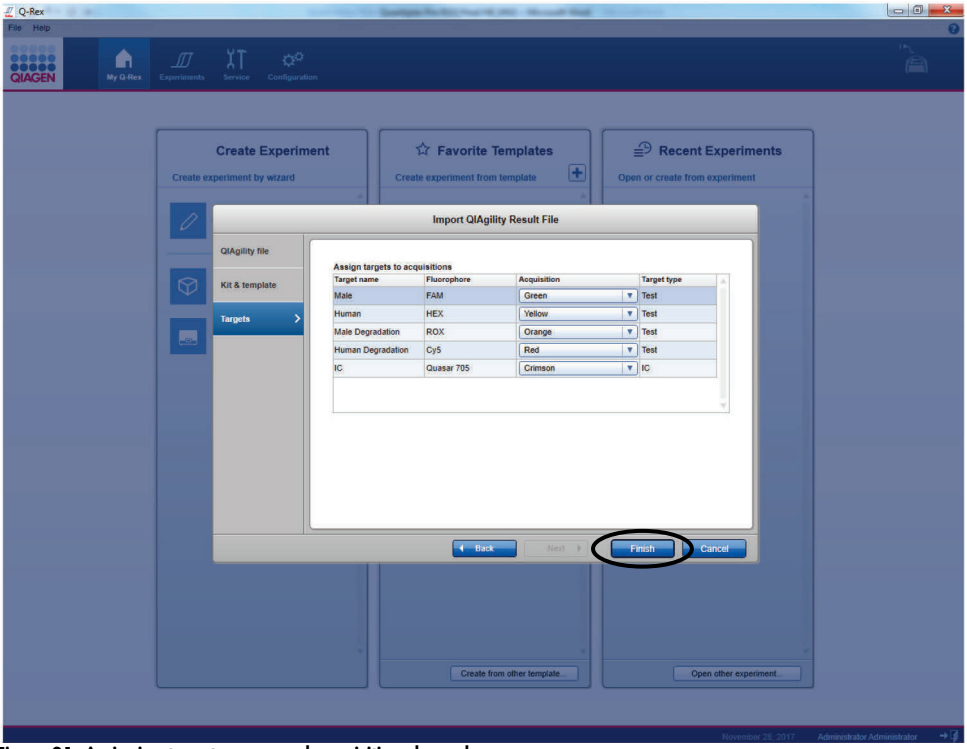

Figure 21. Assigning target names and acquisition channels.

30. Target names are automatically assigned to acquisition channels; click on finish (Figure 21).

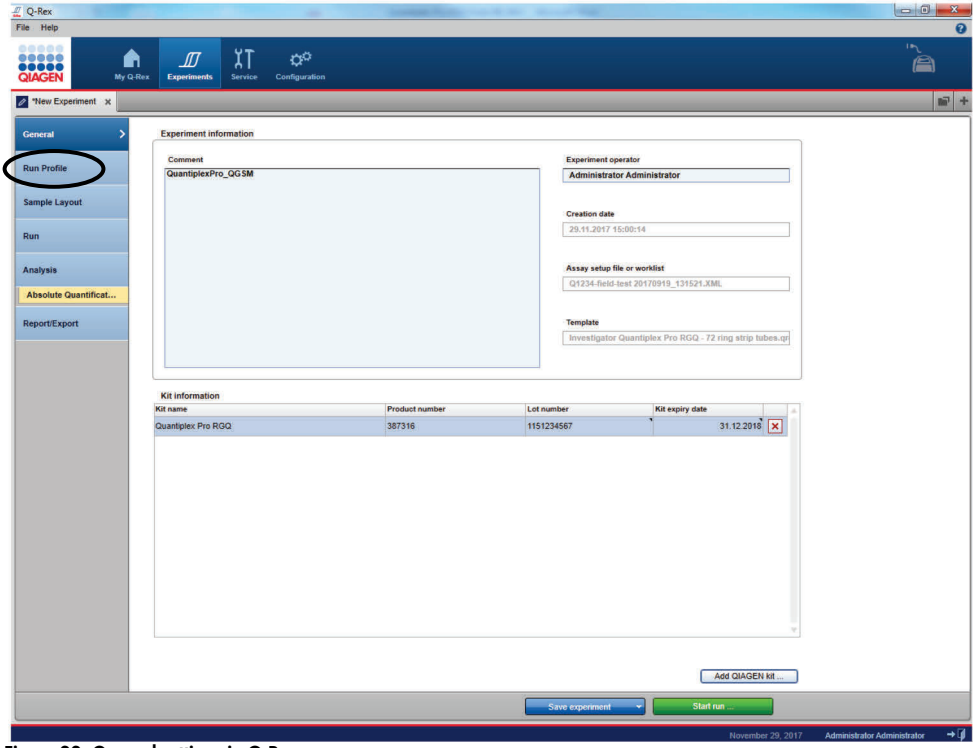

Figure 22. General settings in Q-Rex.

31. In the comments field add any additional information needed to be entered for the run (Figure 22).
32. Click on left side in the step marker on “Run Profile” (Figure 22).

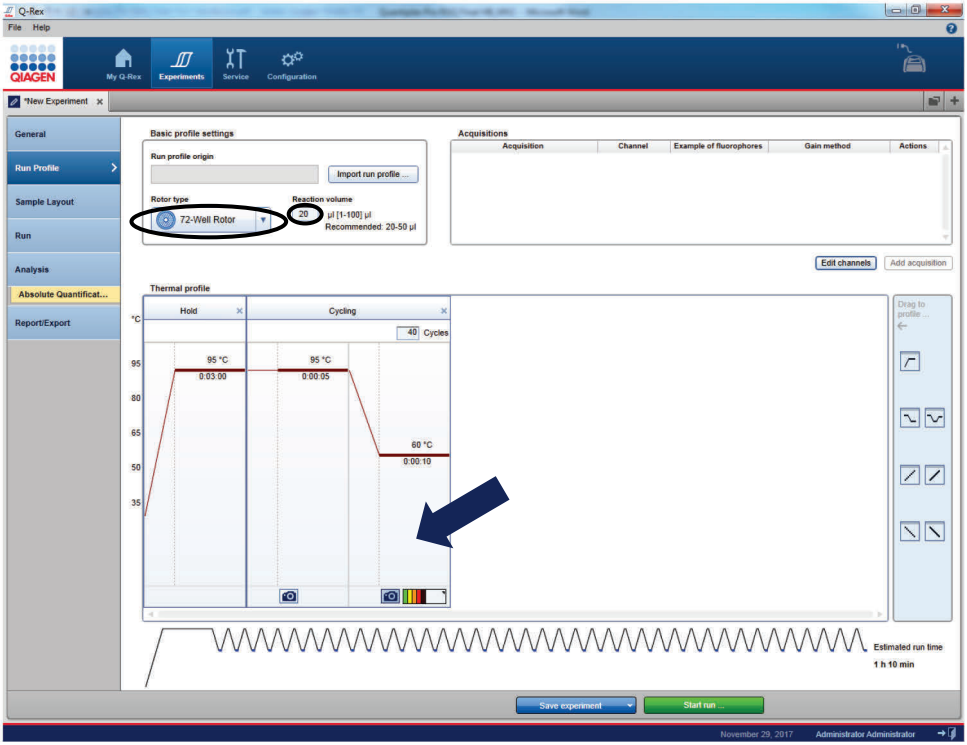

Figure 23. Run profile settings in Q-Rex.

33. Confirm Rotor type and Reaction volumes" (Figure 23).
34. Confirm that the cycling conditions preset in the template file are the same as outlined in Table 10.
35. Click in the thermal profile in the area above the colored camera symbol (indicated by arrow in Figure 23). Acquisition settings will be displayed (Figure 24).
36. Click on the "Auto-gain" field for the green channel (Figure 24).

Table 10. Cycling conditions for the Rotor-Gene Q

Step	Time	Temperature	Number of cycles	Comment
Initial activation step	3 min	95°C	–	PCR requires an initial incubation at 95°C to activate the DNA polymerase.
Two-step cycling:			**40**	
Denaturation	5 s	95°C		
Combined annealing/extension	10 s	60°C		Perform fluorescence data collection using the green, yellow, orange, red and crimson channels with auto-gain optimization specified in table 11.

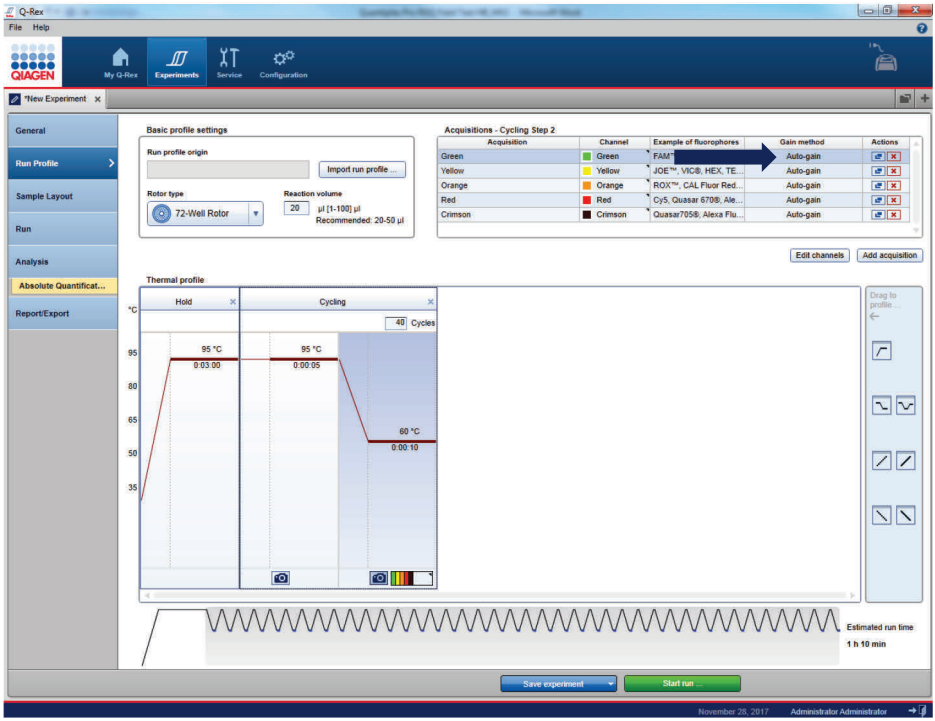

Figure 24. Confirmation of Auto-gain settings in Q-Rex.

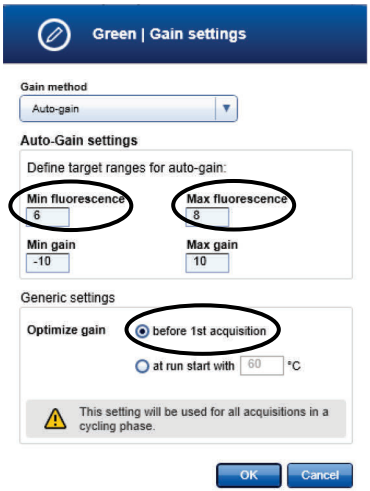

Figure 25. Confirm auto-gain settings in Q-Rex.

37. Confirm that ''Auto-gain'' is selected, and settings for the green channel preset in the template are the same as outlined in Table 11. Optimize gain should be selected before 1st acquisition. Also confirm auto-gain settings for the yellow, orange, red and crimson channels according to Table 11.

Table 11. Auto-gain Settings for the different channels.

Channel	Min fluorescence	Max fluorescence	Optimize gain
Green	6	8	Before 1st acquisition
Yellow	6	8	Before 1st acquisition
Orange	2	4	Before 1st acquisition
Red	6	8	Before 1st acquisition
Crimson	5	7	Before 1st acquisition

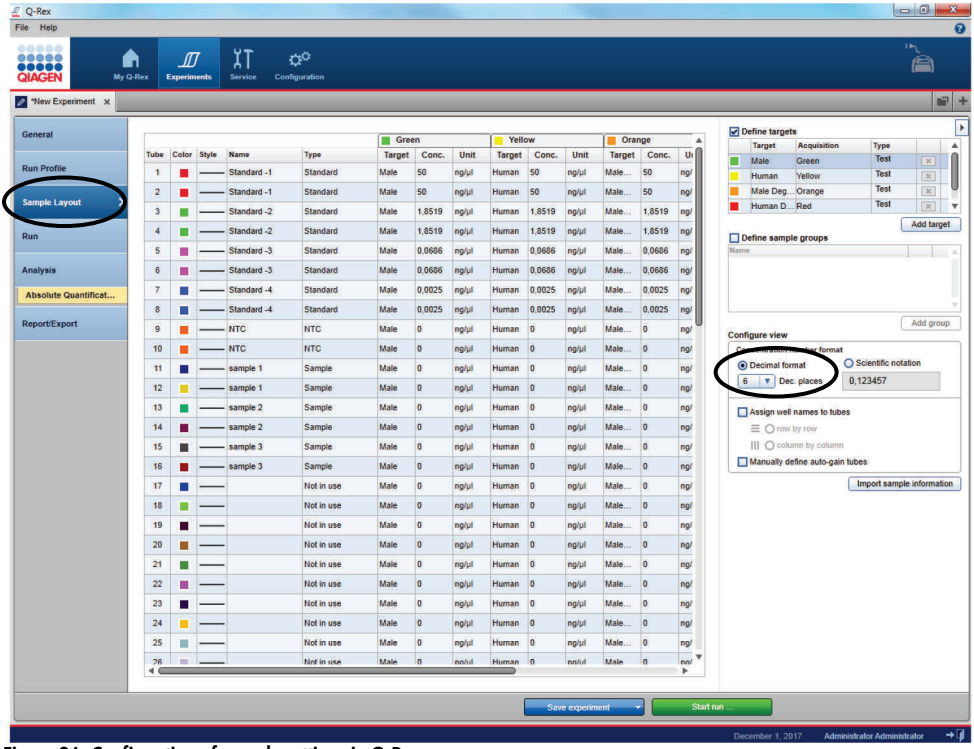

Figure 26. Confirmation of sample settings in Q-Rex.

38. Click on left side in the step marker on "Sample Layout" (Figure 26). Confirm sample layout (Standards, NTCs and Samples). Tubes that are not being used should be marked as "Not in use". Sample type can be changed by right clicking on the "Type" field. Select appropriate decimal format (Figure 26).

Note: For tubes marked as "Not in use" a standard acquisition target (green, yellow, orange, red and crimson) can be added in addition to Male, Human, Human Degradation, Male Degradation and IC. Please check if additional targets have been added and remove the additional targets for "Not in use" tubes, as described in step 39, otherwise proceed to step 40.

39. Scroll down in the ''Define targets" windows and remove the additional targets (green, yellow, orange, red and crimson) assigned for ''Not in use" tubes by clicking on the red delete field (Figure 27). Take care not to remove the imported targets Male, Human, Male Degradation, Human Degradation and IC.

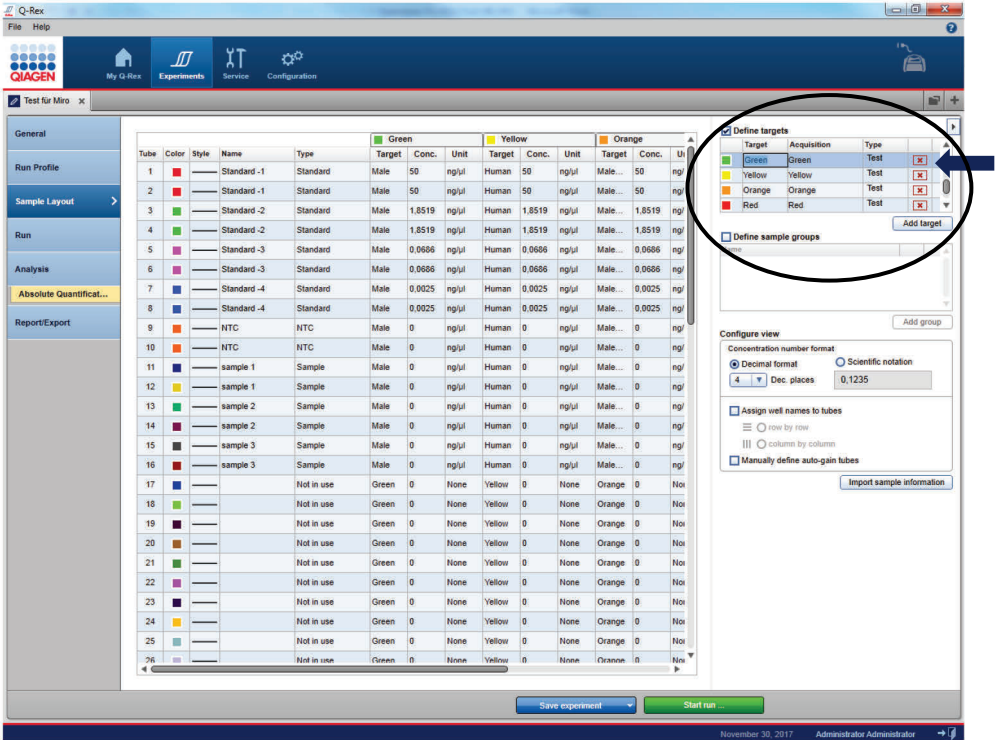

Figure 27. Remove additional targets for "Not in use" tubes.

40. Start the Rotor-Gene cycler by clicking "Start run".

41. Proceed to data analysis on page 46.

Data analysis

Optimal analysis settings are a prerequisite for accurate quantification data. Readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.

Procedure

1. Open the run file using the Q-Rex software. Go to "File", followed by "Open Experiment and then browse to locate the saved run file.
2. Standards must be defined before a standard curve can be created. If the standards were defined before the run was started, proceed to step 3, otherwise edit sample layout as described on page 44, Figure 26.

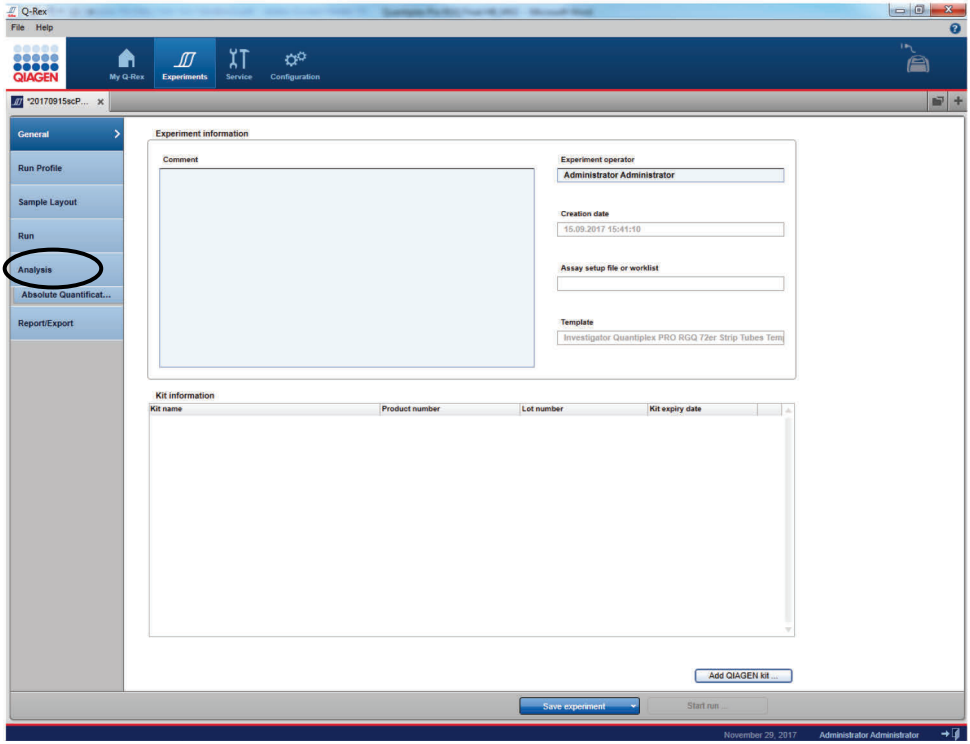

Figure 28. Setting analysis parameters in Q-Rex.

3. Click on the left side in the step marker on "Analysis" (Figure 28).

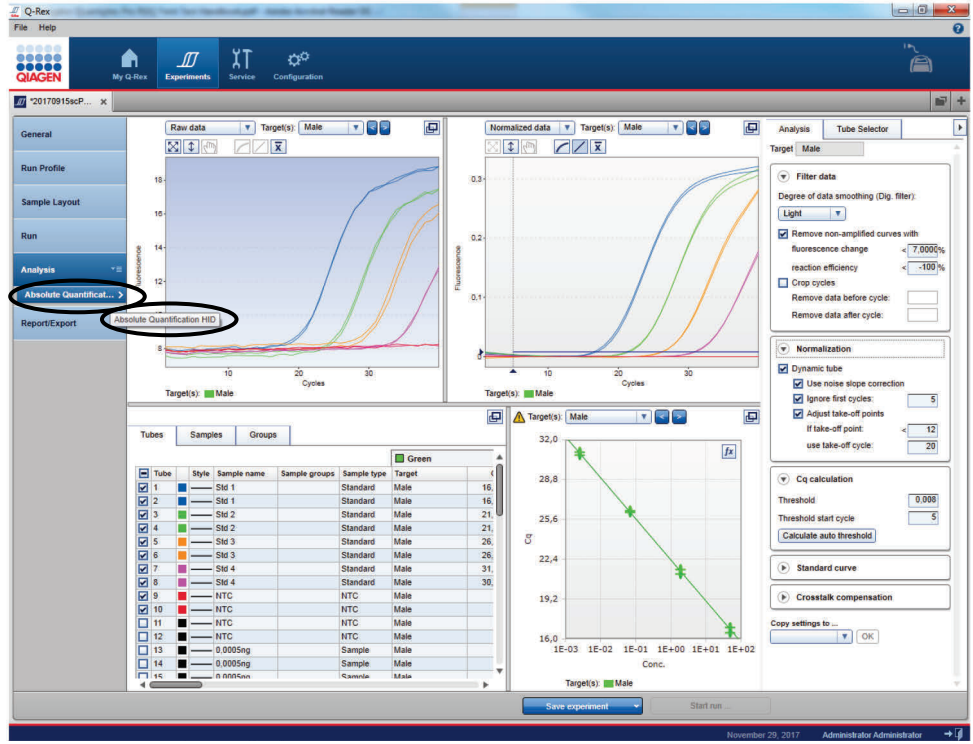

Figure 29. Setting analysis method in Q-Rex.

4. Confirm that the correct analysis method is selected (Absolute Quantification HID; Figure 29). If yes proceed to step 6, if not proceed to next step.
5. Add analysis method by clicking on the selection menu within the "Analysis" step marker on the left side, followed by "Add analysis" and then selecting ''Absolute Quantification HID'' (Figure 30).

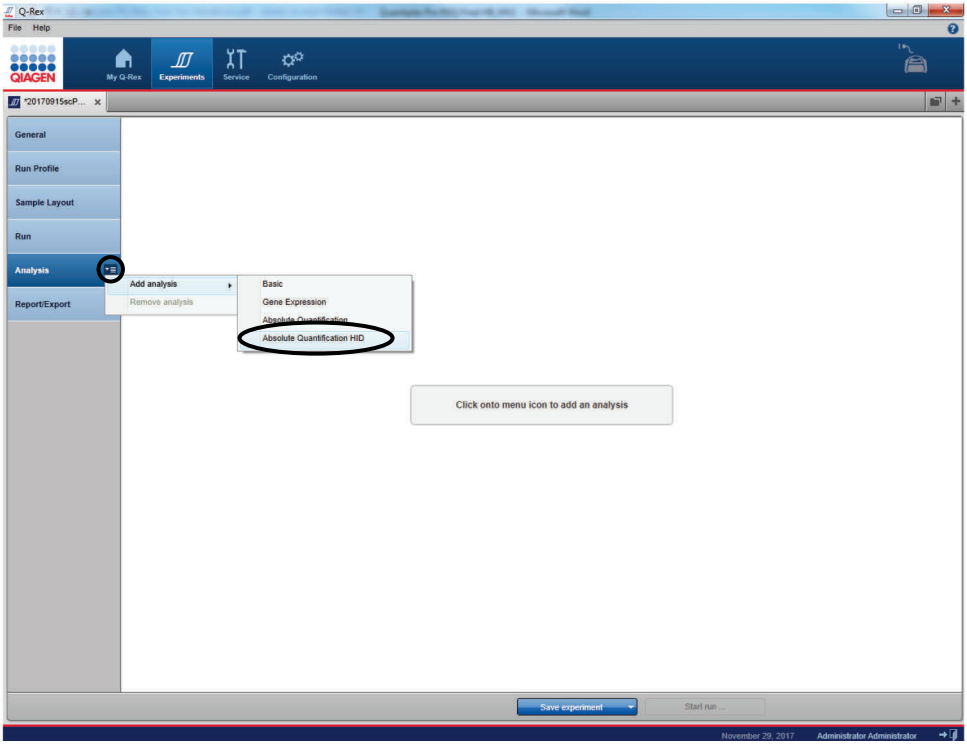

Figure 30. Selecting "Absolute Quantification HID" in Q-Rex.

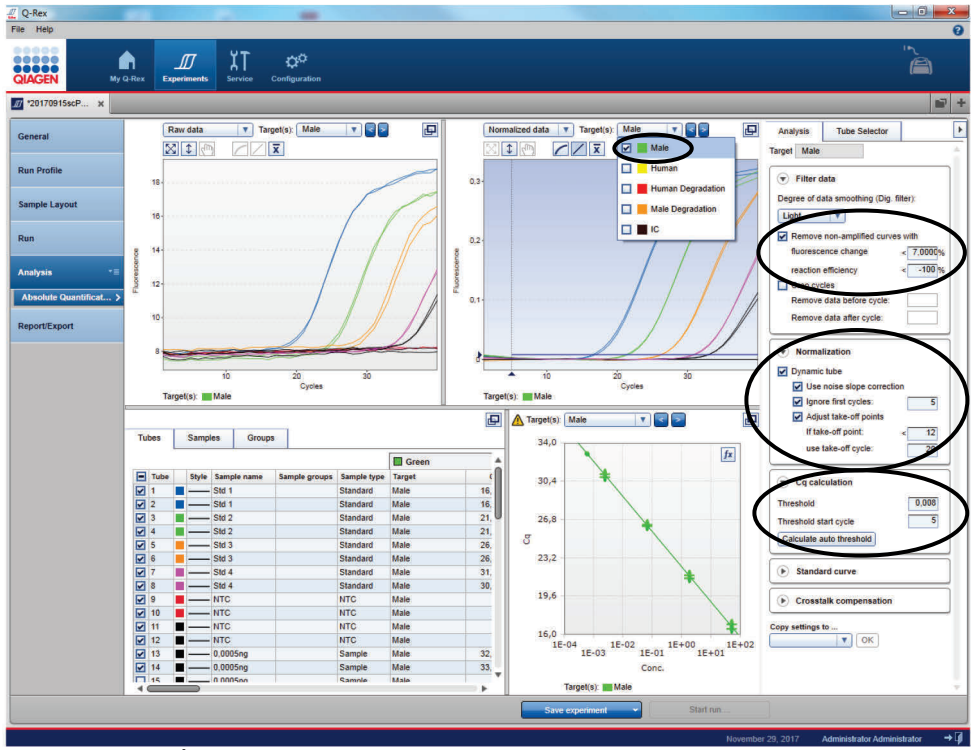

Figure 31. Setting analysis parameters in Q-Rex.

6. Select the green channel (Male target) within the normalized data section (Figure 31).
7. Select "Filter data" on the right panel (Figure 31).
8. Confirm selection of "Remove non-amplified curves with" and enter following values:

for "fluorescence change" <7%

for "reaction efficiency" < -100%

9. Select "Normalization'' (Figure 31).
10. Confirm that "Dynamic tube", "Use noise slope correction", "Ignore first cycles = set to 5" are selected and set.

11. Confirm "Adjust take-off points" is enabled and set to: if take-off point <12 use take-off cycle 20.
12. Select "Cq calculation" on the right panel (Figure 31).
13. Confirm ''Threshold'' is set to 0.008 and ''Threshold start cycle'' is set to 5.
14. Repeat step 7 to 14 for the yellow (Human target), orange (Male Degradation target), red (Human Degradation target) and crimson (IC) channel, and confirm analysis settings according to table 12.

Table 12. Analysis settings for the different channels on Rotor-Gene Q

Parameters	Green	Yellow	Orange	Red	Crimson
Filter data "Remove non-amplified curves''					
fluorescence change	<7%	<4%	<2%	<5%	NA
reaction efficiency	< -100%	< -100%	< -100%	< -100%	NA
Normalization					
Dynamic tube	Enable	Enable	Enable	Enable	Enable
Use noise slope correction	Enable	Enable	Enable	Enable	Enable*
Ignore first cycles	5	5	5	5	2
Adjust take-off points	Enable	Enable	Enable	Enable	Enable
If take-off point	12	12	12	12	10
Use take-off cycle	20	20	20	20	20
Cq calculation					
Threshold	0.008	0.02	0.03	0.02	0.03
Threshold start cycle	5	5	5	5	5

*For older Corbett Rotor Gene 6000 5plex Instruments disable "Use noise slope correction" for the IC in the crimson channel.

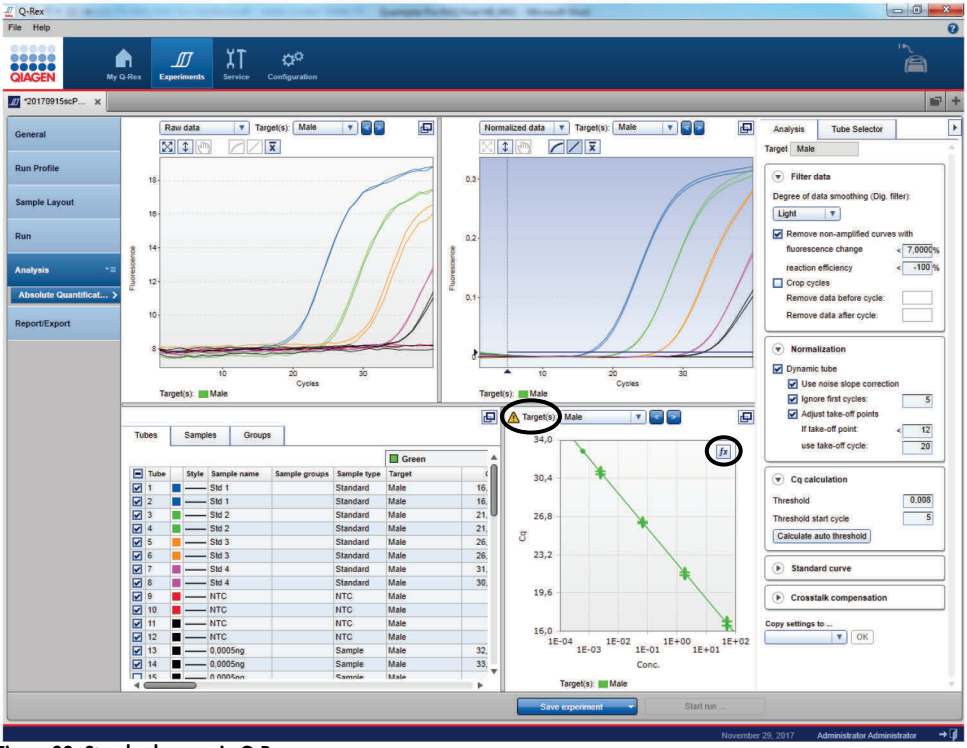

Figure 32. Standard curves in Q-Rex.

15. The Standard Curves are shown in the "Targets" windows. Select one or multiple targets. View the calculated regression line, slope (M), y-intercept (B), R^2 and efficiency values by clicking on the *fx*-symbol (Figure 32).

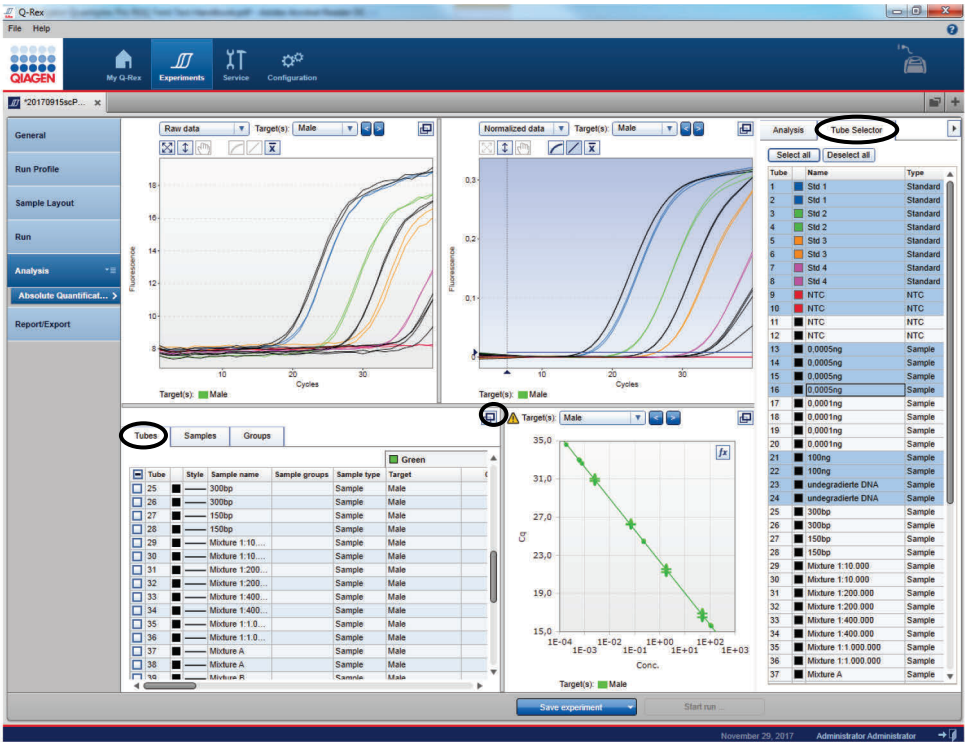

Figure 33. Tube selector in Q-Rex.

16. Individual tubes can be selected or deselected for further analysis by using the "Tube selector" (Figure 33). View the concentration of the unknown samples in the tube table section on the left panel (Figure 33). All windows can be enlarged by clicking on the enlarge button (Figure 33).

17. To export the results in .csv format for further analysis with the QIAGEN Quantification Assay Data Handling Tool, click on the left side in the step marker on "Report/Export" (Figure 34).

    Note: A standard curve needs to be set and defined in order to export calculated concentrations for unknown samples. Only selected tubes will be exported. All channels/targets need to have a valid threshold set.

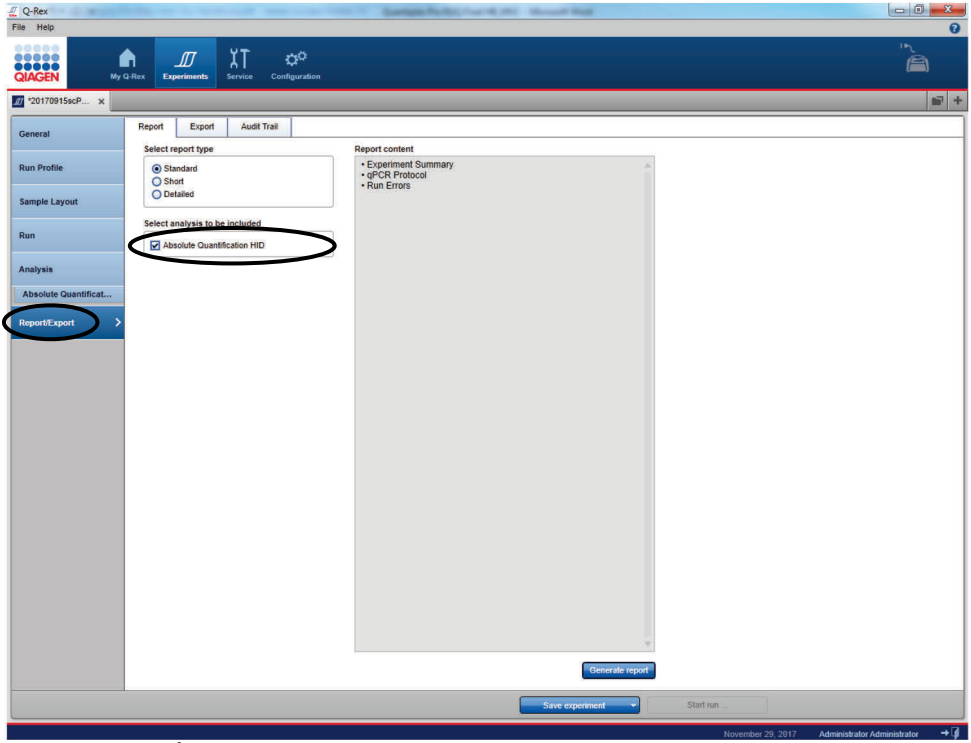

Figure 34. Exporting data in Q-Rex.

18. Confirm that the analysis method "Absolute Quantification HID" has been selected (Figure 34).
19. Select ''Export sheet'' (Figure 35).

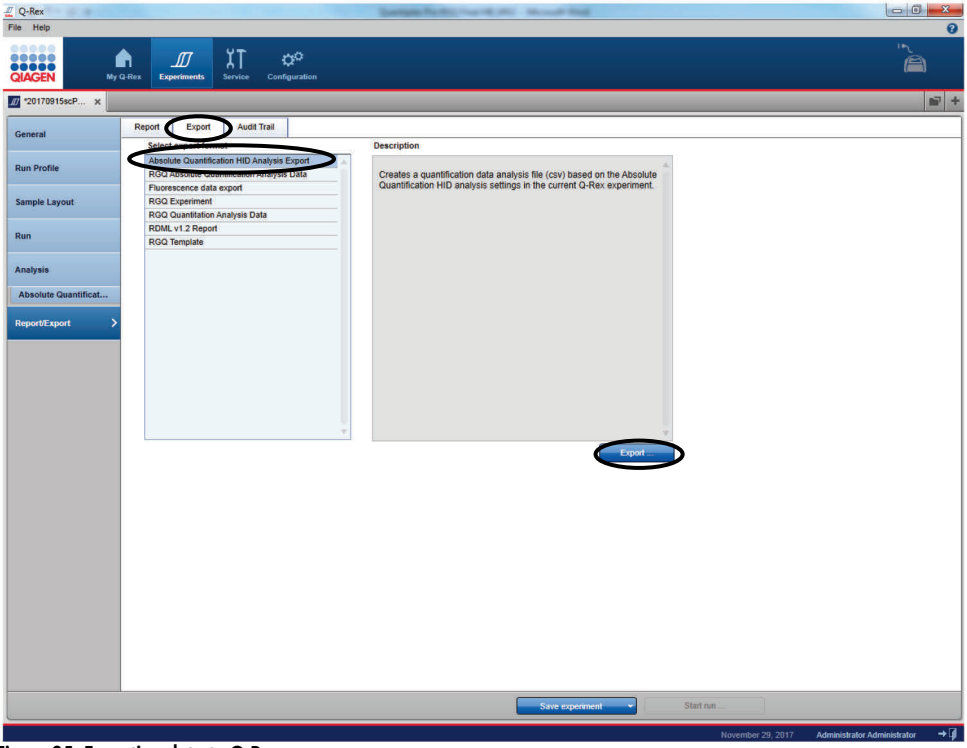

Figure 35. Exporting data to Q-Rex.

20. Confirm export format as "Absolute Quantification HID Analysis Export'' (Figure 35).

21. Click ''Export'' (Figure 35).

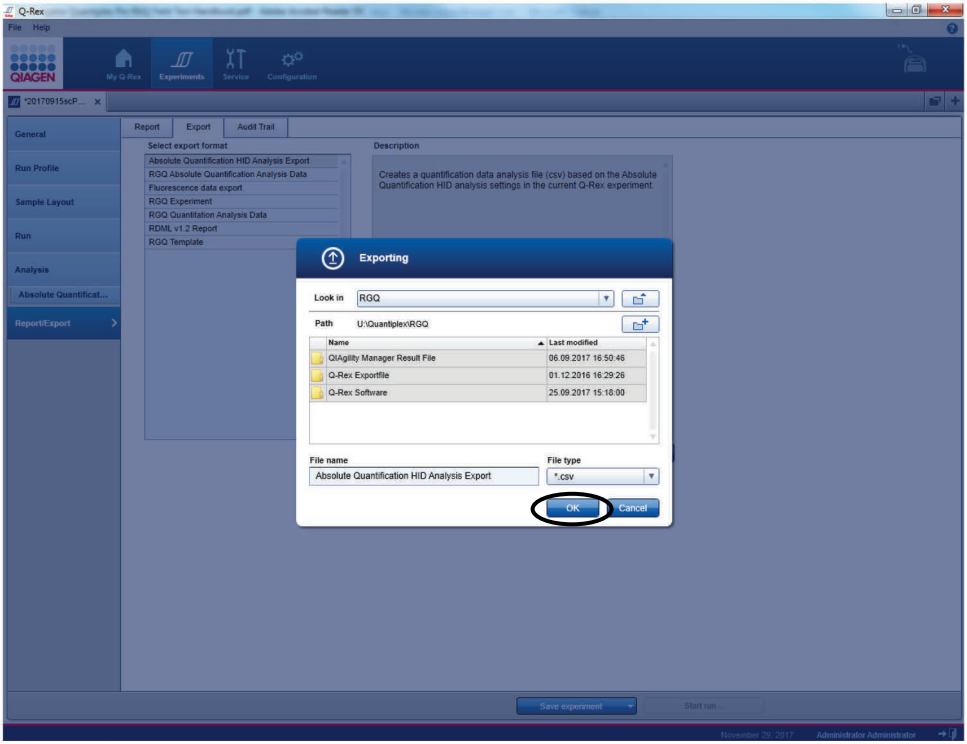

Figure 36. Selecting export directory in Q-Rex.

22. Choose directory for data export and click ok (Figure 36).
23. To interpret the results, see “Interpreting Data Using the QIAGEN Quantification Assay Data Handling Tool” on page 57.

Interpreting Data Using the QIAGEN Quantification Assay Data Handling Tool

The QIAGEN Quantification Assay Data Handling Tool is designed for accurate quantification, data analysis and interpretation. The Opening Page worksheet contains information on version number and software requirements/compatibility. On the Configuration worksheet, the root directories for data processing, result import options and default values for analysis criteria and thresholds can be set. Each worksheet contains an instruction button, which, when pressed, provides detailed instructions on using the functions of the specific worksheet.

Procedure:

1. Open the QIAGEN Quantification Assay Data Handling Tool. Click the ''Configuration'' worksheet tab (Figure 37).

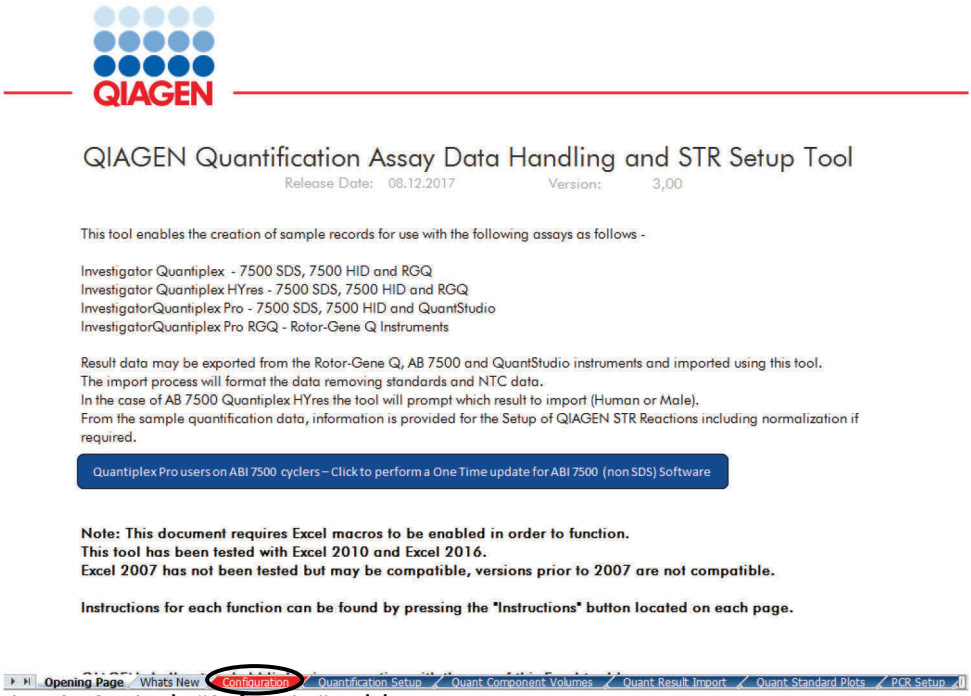

Figure 37. Opening the ''Configuration'' worksheet.

2. Set the root directory to save Quant batch files (Figure 38).
3. Set the root directory to import Quant result files (Figure 38).

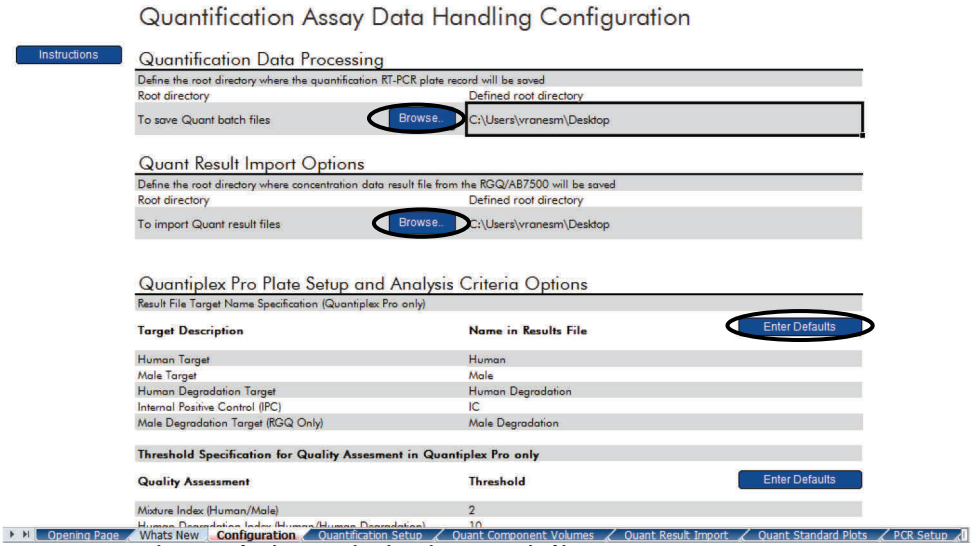

Figure 38. Setting directories for the Quant batch and Quant results files.

4. Target names should be assigned for the Rotor-Gene Q. Default names for the targets are "Human" (Human Target), "Male" (Male Target), "Human Degradation "(Human Degradation Target), Male Degradation (Male Degradation Target) and "IC" (Internal Positive Control). Defaults can be restored for Rotor-Gene Q by clicking the "Enter defaults" button (Figure 38).
5. Threshold setting for the Quality Assessment can be changed/adjusted as needed. The default threshold settings are
   - Mixture index (Human/Male): **2**
   - Degradation Index (Human/Degradation): **10**
   - Inhibition Index (IC Shift): **1**
   - Male Degradation Index (Male/Male Degradation): **10**

Note: Setting the appropriate threshold values may require further internal validation at your facility. 9948 will be filtered from the import providing it is included in the section "Quantification QC Control Specification". Removing it allows it to be kept in the final data set.

Defaults can be restored by clicking the ''Enter defaults'' button.

6. To import quantification results click the ''Quant Result Import'' worksheet tab (Figure 39).

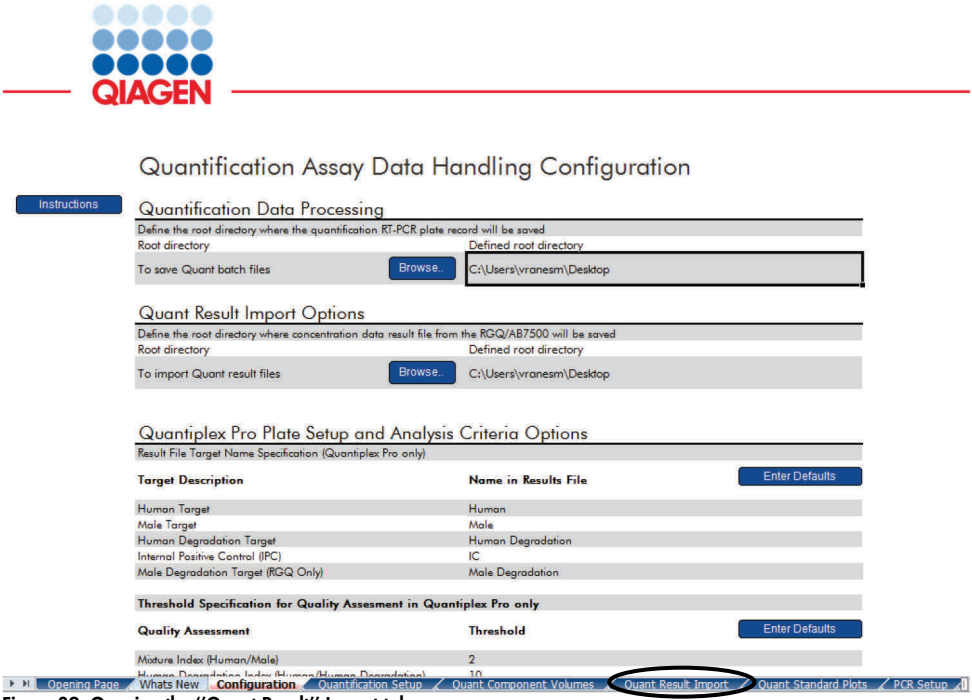

Figure 39. Opening the ''Quant Result'' Import tab.

7. To import your quantification results, click the ''Import QuantData'' button (Figure 40).

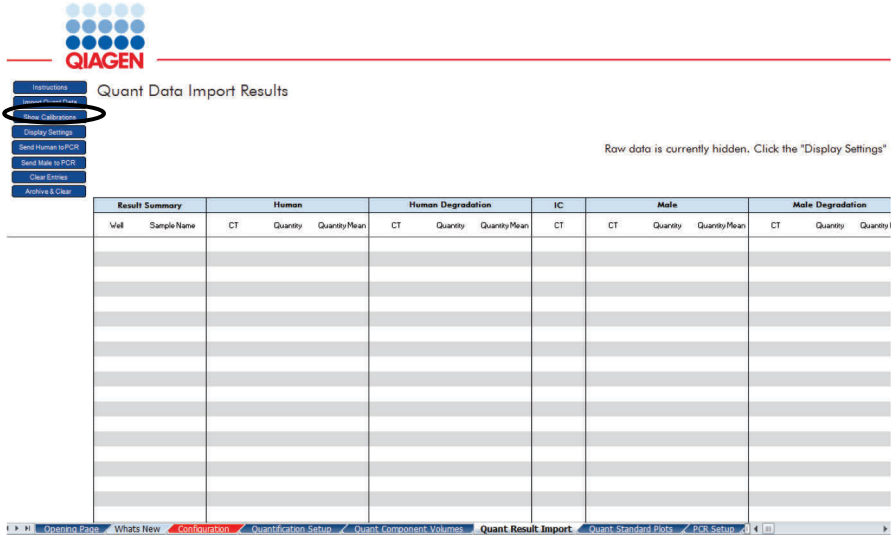

Figure 40. Quant Result Import worksheet: Importing quantification results.

8. Confirm that your data are in the necessary format (Figure 41).

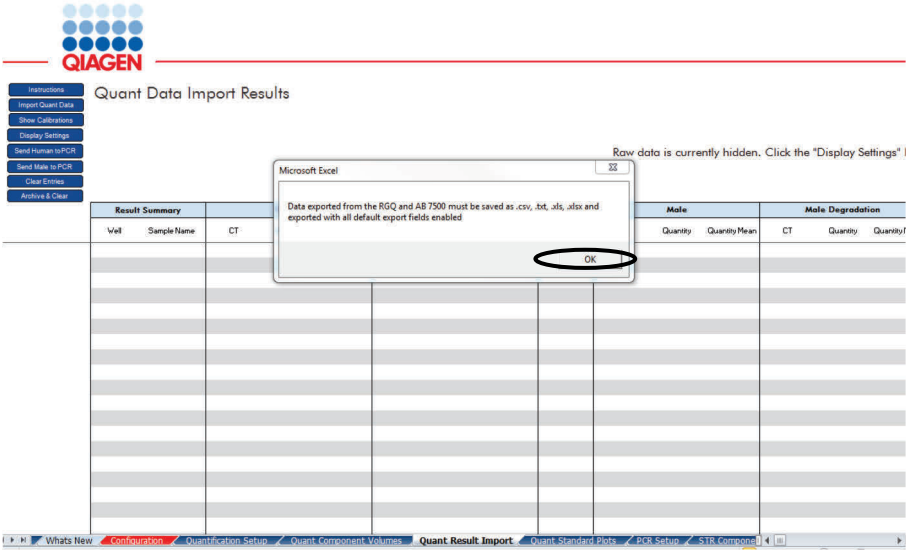

Figure 41. "Quant Result Import" worksheet: Confirming your data format.

9. Your quantification data are now imported and the data is analyzed. The Mixture Index, Degradation Indices and Inhibition Index are calculated and tagged as “Below Threshold”, “Possible Mixture”, “Possible Degradation” or “Possible Inhibition” (Figure 42).

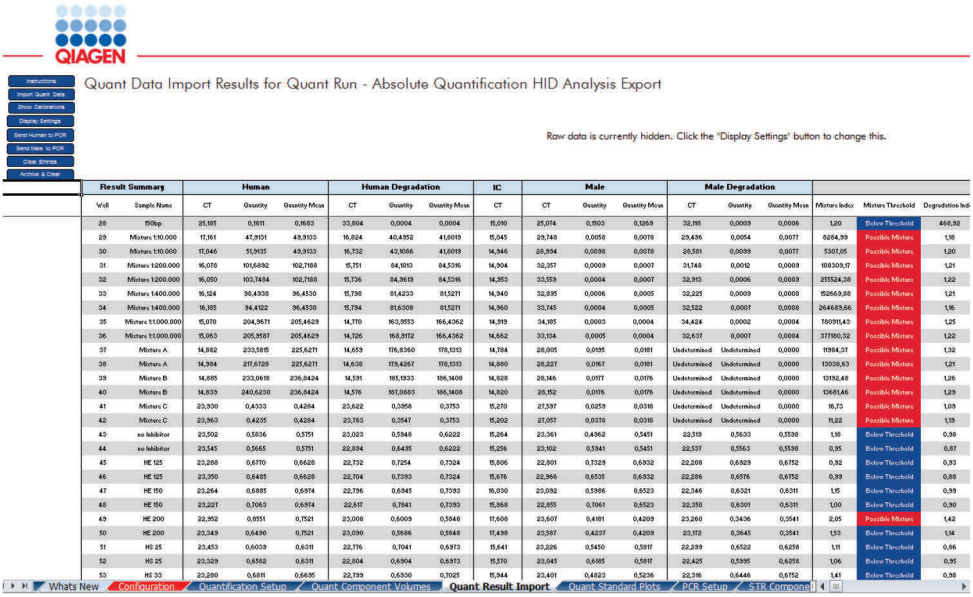

Figure 42. ‘‘Quant Result Import’’ worksheet: data analyzed.

10. Display options can be adjusted by clicking on ‘‘Display settings’’:

    - **Show Raw Data**
    - **Show Quantity Mean Values**
    - **Show Cq Values**

    Note: The Degradation Indices for Human and Male are set to 10, as a default. Full STR profiles can be obtained with DNA fragmented to an average fragment size of approximately 300 bp. The default Human Degradation and Male Degradation Indices of 10 should allow differentiation between DNA fragments larger or smaller than 300 bp.

Note: The default Inhibition Index is set to 1, as a default. The IC acts as a quality sensor and reports the presence of inhibitors with a Cq shift while quantification remains reliable. The default value can be changed and adjusted for relevant degrees of inhibition. Hence laboratory validation should be performed to determine criteria for detecting inhibition.

General Interpretation of Results

General considerations for data analysis

Real-time PCR data are produced as sigmoidal-shaped amplification plots (when using a linear scale), in which fluorescence is plotted against the number of cycles.

The threshold cycle (Cq value) serves as a tool for calculation of the starting template amount in each sample. This is the cycle in which there is the first detectable significant increase in fluorescence.

The optimal threshold setting depends on the reaction chemistries used for PCR. Therefore, an optimal threshold setting that has been established for another kit may not be suitable for the Investigator Quantiplex Pro RGQ Kit and may need to be adjusted.

For DNA quantification using the Investigator Quantiplex Pro RGQ Kit, the analysis settings must be adjusted for both reporter dyes.

Standard curve

The standard curve is the best fit for a linear regression to the standard dilution series data. The equation is in the form

$$y = mx + b$$

where x = log concentration and y = Cq.

The slope

The slope (m) describes the PCR efficiency. A slope of –3.3 indicates 100% PCR efficiency (i.e., the number of copies of amplification product is doubled in each cycle). Typically, the slope ranges between –3.0 and –3.6. If the values fall outside of this range, see the Troubleshooting Guide, page 67, for more information.

The Y-intercept

The Y-intercept (b) indicates the expected Cq value for a sample with a quantity value of 1 (for example, 1 ng/µl).

The R^2 value

The R^2 value is a measure of the fit of the data points to the regressed line. In general, the standard curve has an R^2 value of ≥0.990. Low R^2 values (R^2 ≤0.98) may occur for many different reasons. In case of low R^2 values, see the Troubleshooting Guide, page 67, for more information.

Internal control

The internal control (IC)is intended to report chemistry or instrument failure, errors in assay setup and the presence of inhibition in the sample. The IC system is designed to be more sensitive to inhibition than the specific target for human DNA. Therefore, the quantification will be valid even if some inhibition is present in the sample. In this case, the operator will get information both about the concentration of DNA in the sample and about the presence of inhibitors. Comparison of the Cq value of the IC system for DNA standards with the Cq values of the IC system for unknown samples can provide an indication of potential inhibition. At higher concentrations of inhibitor, the quantification data may be affected, and this must be considered for downstream applications. In general, the internal control can be interpreted in the following manner.

a)	IC system shows normal amplification No IC shift greater than specified is observed No amplification of the Human, Degradation and Male Targets is detectable	No or insufficient DNA was present.
b)	IC shift is greater than specified Degradation Index is below threshold	Sample contains inhibitors. DNA is not degraded.
c)	IC shift is greater than specified Degradation Index is above threshold	Sample contains inhibitors. DNA is possibly degraded. **Note**: Extremely high concentrations of inhibitors can inhibit amplification of the degradation target and trigger the Degradation Index.

Important: Internal laboratory validation with relevant inhibitors should be performed to determine criteria for detecting inhibition.

Quantification of unknowns

The Investigator Quantiplex Pro RGQ Kit can quantify a broad range of DNA amounts in a sample, from 200 ng/µl to approximately 0.5 pg/µl of human genomic DNA. When 2 µl of a sample with very low concentrations is loaded in a reaction, the well probably contains less than one diploid human genome equivalent. In the low DNA concentration range, statistical effects, known as stochastic variations, can significantly affect the assay result. When using samples with low concentrations of DNA, make sure that as many replicates as possible are assayed in order to confirm the result.

Quantification of female/male mixtures

The Investigator Quantiplex Pro RGQ Kit provides high sensitivity to detect low amounts of male DNA, even in a very high background of female DNA. The Mixture Index provides information on whether a sample is a female/male mixture. In general, the Mixture Index can be interpreted in the following manner.

a)	The sample has a Mixture Threshold below the index specified	The sample contains only male DNA or only low levels of female DNA.
b)	The sample has a Mixture Threshold above the index specified	The sample contains a possible male DNA/female DNA mixture.

Degradation status assessment

Environmental degradation may occur with forensic casework samples and is a typical challenge in routine genetic fingerprinting. The Investigator Quantiplex Pro RGQ Kit contains a newly developed system for detection of human DNA degradation in general and male specific DNA degradation in mixture samples. In general, the Degradation Indices can be interpreted in the following manner.

a)	The sample has a Degradation Threshold below the index specified No IC shift is detected	DNA is most likely not degraded. The sample most likely contains no inhibitors.
b)	The sample has a Degradation Threshold below the index specified IC shift is detectable above the threshold	DNA is most likely not degraded. The sample may contain inhibitors.
c)	The sample has a Degradation Threshold above the index specified No IC shift is detected	DNA is most likely degraded. The sample most likely contains no inhibitors.
d)	The sample has a Degradation Threshold above the index specified IC shift is detectable above the threshold	DNA may or may not be degraded. The sample contains inhibitors.

Note: When 2 µl of a sample with very low concentrations is loaded in a reaction, the well probably contains less than one diploid human genome equivalent. In the low DNA concentration range, statistical effects, known as stochastic variations, can affect the assay. In case of degraded DNA with a very low DNA concentration, the Degradation targets can be affected. If the Degradation targets have an undetermined value, the sample will be tagged with "Possible Degradation". Extremely high inhibitor concentrations can also affect the Degradation targets and lead to a "Possible Degradation" flag.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx.** The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

		Comments and suggestions
No signal or one or more signals detected late in PCR		
a)	Incorrect cycling conditions	Always use the optimized cycling conditions specified in the protocols. Make sure to select ROX as the passive dye on Applied Biosystems® instruments.
b)	Pipetting error, missing or degraded reagent	Check the storage conditions of the reagents. Repeat the assay.
c)	Incorrect or no detection step	Make sure that fluorescence detection takes place during the combined annealing/extension step.
d)	Insufficient amount of starting template	Increase the amount of template, if possible. Ensure that sufficient copies of the template DNA are present in the sample.
e)	Problems with starting template	Check the storage conditions of the starting template DNA. Efficient removal of PCR inhibitors is essential for optimal results. Purify nucleic acids from your sample using an appropriate purification method. Ensure that all reagents, buffers and solutions used for isolating and diluting template nucleic acids are free from nucleases.
f)	Wrong detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for each reporter dye. Ensure that the chosen combination of reporter dyes is compatible with the selected detection channels or filter sets.
g)	Degraded control DNA	Make new serial dilutions of the control DNA from the stock solution. Repeat the assay using the new dilutions.

		Comments and suggestions
Differences in Cq values or in PCR efficiencies between runs		
a)	Incorrect cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Ensure that the cycling conditions include the initial step for activation of the DNA polymerase and the specified times for denaturation and annealing/extension.
b)	Analysis settings (e.g., threshold and baseline settings) not optimal	Check the analysis settings (threshold and baseline settings) for each reporter dye. Repeat analysis using optimal settings for each reporter dye.
No linearity in ratio of Cq value/crossing point to log of the template amount		
	Amount of template in unknown sample too high	Linearity is guaranteed within the range of the standard curve. If signals appear at very early Cq values, dilute the sample and repeat the reaction.
Increased fluorescence or Cq value for no-template control		
a)	Contamination of reagents	Discard all the components of the assay (e.g., master mix). Repeat the assay using new components.
b)	Minimal probe degradation, leading to sliding increase in fluorescence	Check the amplification plots, and adjust the threshold settings.
c)	Crosstalk problems	Depending on the instrument, different techniques are used to avoid spectral crosstalk when using multiple fluorophores for multiplex assays. However, minimal crosstalk, as a result of residual spectral overlap, may be observed in the NTC wells, especially if the instrument is in need of calibration.
Varying fluorescence intensity		
a)	Contamination of real-time cycler	Reactions were contaminated with target DNA. Decontaminate the real-time workstations and the cycler according to the manufacturer's instructions. Use new reagents and solutions.
b)	Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.
c)	Wavy curve at high template amounts for highly concentrated targets	In the analysis settings, reduce the number of cycles used for background calculation (if the real-time cycler allows this) or reduce the amount of template.

		Comments and suggestions
Slope for the standard curve differs significantly from –3.33 or R^2 value is significantly less than 0.98–0.99		
a)	Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
b)	Real-time cycler and/or pipettes no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions. Calibrate pipettes to minimize pipetting variability.
c)	Wavy curve at high template amounts for highly concentrated targets	In the analysis settings, reduce the number of cycles used for background calculation or reduce the amount of template.
d)	Problem with dilution of standards	Ensure that the DNA standard is completely thawed and mixed thoroughly before use. Ensure that dilutions of the DNA standard are mixed thoroughly before removing each aliquot for the serial dilution. Always use a sample volume of 2 µl. Change pipette tips between each dilution step.
e)	Plate not sealed	Carefully seal the plates to avoid evaporation.
f)	Error made during dilution of the DNA standard	Verify all calculations, and repeat dilution of the DNA standard.
g)	Incorrect concentration values entered in the software	Verify the concentrations for all samples used to generate the standard curve.
h)	Abnormal fluorescence	Do not write on the plate. Use caution when handling plates. Wear gloves.
i)	Statistical variation	Some variation in the reaction is normal, particularly when the DNA target is present at a low copy number. Perform at least duplicates for the standard curve to minimize the effect of this variation.

Appendix: Alternative Standard Curves

Table 13. Alternative 5-point standard curve (10x dilution)

Serial dilution of control DNA (ng/µl)	Amount of control DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
50	Undiluted DNA	–
5	5	45
0.5	5	45
0.05	5	45
0.005	5	45

Table 14. Alternative 6-point standard curve (9x dilution)

Serial dilution of control DNA (ng/µl)	Amount of control DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
50	Undiluted DNA	–
7.1429	5	40
1.0204	5	40
0.0686	5	40
0.0076	5	40
0.0030	5	40

Table 15. Alternative 7-point standard curve (5x dilution)

Serial dilution of control DNA (ng/µl)	Amount of control DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
50	Undiluted DNA	–
10	10	40
2	10	40
0.4	10	40
0.08	10	40
0.016	10	40
0.0032	10	40

Ordering Information

Product	Contents	Cat. no.
Investigator Quantiplex Pro RGQ Kit (200)	Reaction Mix, Primer Mix, Control DNA, QuantiTect Nucleic Acid Dilution Buffer	387316
Related products		
Investigator human identification PCR kits		
Investigator Quantiplex Kit (200)	Reaction Mix FQ, Primer Mix IC FQ, Control DNA Z1, QuantiTect Nucleic Acid Dilution Buffer	387016
Investigator Quantiplex HYres Kit (200)	Reaction Mix YQ, Primer Mix IC YQ, Control DNA Z1, QuantiTect Nucleic Acid Dilution Buffer	387116
Investigator 24plex QS Kit (100)*	Primer mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder 24plex QS, DNA size standard 550 (BTO), and nuclease free water	382415
Investigator ESSplex SE QS Kit (100)*	Primer Mix, Fast Reaction Mix including *Taq* DNA Polymerase, Control DNA, Allelic Ladder ESSplex SE QS, DNA size standard 550 (BTO) and Nuclease-Free Water	381575
Investigator IDplex Plus Kit*	Primer mix, Fast Reaction Mix including HotStarTaq Plus DNA Polymerase, Control DNA, allelic ladder IDplex Plus, DNA size standard 550 (BTO), and nuclease-free water	381625

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Investigator HDplex Kit (100)	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381215
Investigator Triplex AFS QS Kit (400)	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	380317
Investigator Triplex DSF Kit (400)	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	380327
Investigator Argus X 12 Kit (25)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	383213
Investigator Argus Y 12 QS Kit (100)	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	383615
Investigator DIPplex Kit (100)	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	384015
DNA extraction and purification		
QIAamp® DNA Investigator Kit (50)	50 QIAamp MinElute® Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
MinElute Reaction Cleanup Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Rotor-Gene Q consumables		
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
Rotor-Disc 72 (24)	24 individually wrapped discs for 1728 reactions	981301
Rotor-Disc 72 (240)	10 x 24 individually wrapped discs for 17,280 reactions	981303
Rotor-Disc 100 (30)	30 individually wrapped discs for 3000 reactions	981311
Rotor-Disc 100 (300)	10 x 30 individually wrapped discs for 30,000 reactions	981313
Rotor-Disc Heat Sealing Film (60)	60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981601
Rotor-Disc Heat Sealing Film (600)	10 x 60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981604
Rotor-Disc Heat Sealer 110 V	Heat sealing instrument for use with Rotor-Discs; requires Rotor-Disc 72 or 100 Loading Block	9018898
Rotor-Disc Heat Sealer 230 V	Heat sealing instrument for use with Rotor-Discs; requires Rotor-Disc 72 or 100 Loading Block	9019725

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