

# *therascreen*<sup>®</sup> EGFR RGQ PCR Kit Handbook

Version 1



**IVD**

For in vitro diagnostic use

For use with the Rotor-Gene<sup>®</sup> Q MDx 5plex HRM instrument



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## Intended Use

The *therascreen* EGFR RGQ PCR Kit is an in vitro diagnostic test for the detection of 29 somatic mutations in the EGFR cancer-related gene and will provide a qualitative assessment of mutation status.

The *therascreen* EGFR RGQ PCR Kit is to be used by trained personnel in a professional laboratory environment, with DNA samples extracted from formalin-fixed paraffin-embedded, non-small cell lung cancer (NSCLC) tissue. Results are intended to aid the clinician in identifying patients with NSCLC who may benefit from treatment with tyrosine kinase inhibitors.

The *therascreen* EGFR RGQ PCR Kit is intended for in-vitro diagnostic use.

## Summary and Explanation

The *therascreen* EGFR RGQ PCR Kit is a ready-to-use kit for the detection of 29 somatic mutations in the EGFR cancer-related gene using polymerase chain reaction (PCR) on the Rotor-Gene Q instrument.

Using Scorpions<sup>®</sup> and ARMS<sup>®</sup> technologies, the *therascreen* EGFR RGQ PCR Kit enables detection of the following mutations against a background of wild-type genomic DNA.

- 19 deletions in exon 19 (detects the presence of any of 19 deletions but does not distinguish between them)
- T790M
- L858R
- L861Q
- G719X (detects the presence of G719S, G719A, or G719C, but does not distinguish between them)
- S768I
- 3 insertions in exon 20 (detects the presence of any of 3 insertions, but does not distinguish between them)

The methods used are highly selective and, depending on the total amount of DNA present, enable detection of a low percentage of mutant in a background of wild-type genomic DNA. These selectivity and detection limits are superior to technologies such as dye terminator sequencing.

## Principle of the Procedure

The *therascreen* EGFR RGQ PCR Kit utilizes two technologies — ARMS and Scorpions — for detection of mutations in real-time PCR.

### ARMS

Allele- or mutation-specific amplification is achieved using ARMS (Amplification Refractory Mutation System). *Taq* DNA polymerase (*Taq*) is effective at distinguishing between a match and a mismatch at the 3' end of a PCR primer. Specific mutated sequences are selectively amplified, even in samples where the majority of the sequences do not carry the mutation. When the primer is fully matched, the amplification proceeds with full efficiency. When the 3' base is mismatched, only low-level background amplification occurs.

### Scorpions

Detection of amplification is performed using Scorpions. Scorpions are bifunctional molecules containing a PCR primer covalently linked to a probe. The fluorophore in this probe interacts with a quencher, also incorporated into the probe, which reduces fluorescence. During PCR, when the probe binds to the amplicon, the fluorophore and quencher become separated. This leads to an increase in fluorescence from the reaction tube.

### Kit format

Eight assays are supplied in the *therascreen* EGFR RGQ PCR Kit:

- One control assay (Ctrl)
- Seven mutation assays

All reaction mixes contain reagents to detect targets which are labeled with FAM™, and an internal control assay labeled with HEX™. The internal control assay can detect the presence of inhibitors that may lead to false negative results. FAM amplification can out-compete the internal control amplification and the purpose of the internal control is simply to show that where there is no FAM amplification this is a true negative result and not a failed PCR reaction.

### Procedure

The *therascreen* EGFR RGQ PCR Kit comprises a two-step procedure. In the first step, the control assay is performed to assess the total DNA in a sample. In the second step, both the mutation and control assays are performed to determine the presence or absence of mutated DNA.

## **Assays:**

### **Control assay**

The control assay, labeled with FAM, is used to assess the total DNA in a sample. This assay amplifies a region of exon 2 of the EGFR gene. The primer and probe have been designed to avoid any known EGFR polymorphisms.

We strongly recommend using the Control Reaction Mix (Ctrl) supplied with the *therascreen* EGFR RGQ PCR Kit to assess the total DNA in a sample. The control assay amplifies a region of exon 2 of the EGFR gene. We recommend setting samples up with only the control assay using the EGFR Positive Control (PC) as a positive control and Nuclease-Free Water (H<sub>2</sub>O) as the no template control.

**Note:** DNA assessment should be based on PCR and may differ from quantification based on absorbance readings. Additional Control Reaction Mix (Ctrl) is supplied to enable assessment of quality and quantity of the DNA in samples before analysis with the *therascreen* EGFR RGQ PCR Kit.

### **Mutation assays**

Each mutation assay contains a FAM-labeled Scorpion probe and an ARMS primer for discrimination between the wild-type DNA and a specific mutant DNA.

### **Controls**

**Note:** All experimental runs must contain the following controls.

#### **Positive control**

Each run must contain a positive control in tubes 1–8. The *therascreen* EGFR RGQ PCR Kit contains EGFR Positive Control (PC) to be used as the template in the positive control reaction. The positive control results will be assessed to ensure that the kit performs within the stated acceptance criteria.

#### **Negative control**

Each run must contain a negative control (“no template control”, NTC) in tubes 9–16. The NTC consists of Nuclease-Free Water (H<sub>2</sub>O) to be used as the “template” for the no template control. The no template control is used to assess any potential contamination during the run setup and to assess the performance of the internal control reaction.

#### **Internal control reaction assessment**

Each reaction mix contains an internal control in addition to the target reaction. A failure indicates that either there may be inhibitors present that could lead to false negative results or that an operator setup error has occurred for that tube.

If the internal control failure is due to PCR inhibition, diluting the sample may reduce the effect of inhibitors but it should be noted that this would also dilute

the target DNA. FAM amplification can out-compete the internal control amplification so that the IC C<sub>T</sub> (HEX) value generated may fall outside the specified range. The FAM results are still valid for these samples.

## Materials Provided

### Kit contents

<b><i>therascreen</i> EGFR RGQ PCR Kit</b>				<b>(24)</b>
<b>Catalog no.</b>				<b>870111</b>
<b>Number of reactions</b>				<b>24</b>
Red	Control Reaction Mix	Ctrl		2 x 600 µl
Purple	T790M Reaction Mix	T790M		600 µl
Orange	Deletions Reaction Mix	Del		600 µl
Pink	L858R Reaction Mix	L858R		600 µl
Green	L861Q Reaction Mix	L861Q		600 µl
Yellow	G719X Reaction Mix	G719X		600 µl
Gray	S768I Reaction Mix	S768I		600 µl
Blue	Insertions Reaction Mix	Ins		600 µl
Brown	EGFR Positive Control	PC		300 µl
Turquoise	Taq DNA Polymerase	Taq		138 µl
White	Nuclease-Free Water	H <sub>2</sub> O		2 x 1.9 ml
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## Materials Required but Not Provided

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.

- DNA isolation kit (see “DNA isolation”, page 13)
- Xylene
- Ethanol (96–100%)\*
- 1.5 ml or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann [Safe-Lock, cat. no. 022363204], Eppendorf [Safe-Lock, cat. no. 0030 120.086], or Sarstedt [Safety Cap, cat. no. 72.690])†
- Dedicated pipets‡ (adjustable) for sample preparation
- Dedicated pipets‡ (adjustable) for PCR master mix preparation
- Dedicated pipets‡ (adjustable) for dispensing of template DNA\*
- DNase, RNase and DNA-free pipet tips with filters (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 90°C‡
- Benchtop centrifuge‡ with rotor for 2 ml reaction tubes
- Vortex
- Rotor-Gene Q MDx 5plex HRM instrument‡§ with fluorescence channels for Cycling Green and Cycling Yellow (detection of FAM and HEX, respectively)
- Rotor-Gene Q software, version 2.0.2 or higher
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- DNase, RNase, and DNA-free microcentrifuge tubes for preparing master mixes
- Loading Block 72 x 0.1 ml Tubes, aluminum block for manual reaction setup with a single-channel pipet (QIAGEN, cat. no. 9018901)

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers.

‡ Ensure that instruments have been checked and calibrated according to the manufacturer’s recommendations.

§ Rotor-Gene Q 5plex HRM instrument, if applicable.  
Also known as Rotor-Gene Q MDx in some countries.

# Warnings and Precautions

For In Vitro Diagnostic Use

## 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](http://www.qiagen.com/safety), where you can find, view, and print the SDS for each QIAGEN kit and kit component.

## 24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:  
CHEMTREC

**USA & Canada** ■ Tel: 1-800-424-9300

**Outside USA & Canada** ■ Tel: +1-703-527-3887 (collect calls accepted)

## General precautions

The user should always pay attention to the following.

- Use DNase, RNase, and DNA-free pipet tips with filters and make sure that pipets have been calibrated according to the manufacturer's instructions.
- Store and extract positive materials (specimens and positive controls) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components by inverting each tube 10 times and centrifuge briefly.

**Note:** Use extreme caution to prevent contamination of PCRs with synthetic control material. We recommend using separate, dedicated pipets for setting up reaction mixes and adding DNA template. Preparation and dispensing of reaction mixes must be carried out in a separate area to the addition of template. Rotor-Gene Q tubes must not be opened after the PCR run has finished. This is to prevent laboratory contamination with post-PCR products.

**Note:** The reagents are validated for manual setup. If an automated method is used, this may reduce the number of possible reactions due to reagent required to fill "dead volumes" on these instruments.

**Note:** All reagents in the *therascreen* EGFR RGQ PCR Kit are formulated specifically for use with the stated tests. All reagents supplied in the *therascreen* EGFR RGQ PCR Kit are intended to be used solely with the other reagents in the same *therascreen* EGFR RGQ PCR kit. Substitutions to the reagents in the kit must not be made if optimal performance is to be maintained.

**Note:** Use only the *Taq* DNA polymerase (*Taq*) provided in the kit. Do not substitute with *Taq* DNA polymerase from other kits of the same or any other type, or with *Taq* DNA polymerase from another supplier.

**Note:** Reagents for the *therascreen* EGFR RGQ PCR Kit have been optimally diluted. We do not recommend further dilution of reagents, as this may result in a loss of performance. We do not recommend using reaction volumes of less than 25  $\mu$ l, since this will increase the risk of false negatives.

## Reagent Storage and Handling

The *therascreen* EGFR RGQ PCR Kit is shipped on dry ice and must still be frozen on arrival. If the *therascreen* EGFR RGQ PCR Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note, handbook or the reagents, please contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

The *therascreen* EGFR RGQ PCR Kit should be stored immediately upon receipt at  $-15$  to  $-25^{\circ}\text{C}$  in a constant-temperature freezer and protected from light. When stored under the recommended storage conditions in the original packaging, the kit is stable until the expiration date stated on the label. Repeated freezing and thawing should be avoided. We recommend a maximum of 7 freeze-thaw cycles.

**Note:** To ensure optimal activity and performance, Scorpions (as with all fluorescently-labeled molecules) must be protected from light to avoid photo bleaching.

**Note:** To obtain optimal use of the reagents in the *therascreen* EGFR RGQ PCR Kit, samples should be batched. If samples are tested individually, this will use more reagents and reduce the number of samples that can be tested with the *therascreen* EGFR RGQ PCR Kit.

## Specimen Handling and Storage

**Note:** All samples must be treated as potentially infectious material.

Sample material must be human genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE), non-small cell lung tumor samples. Specimens must be transported according to standard pathology methodology to ensure specimen quality.

Tumor samples are non-homogeneous and data from a sample of tumor may not be concordant with other sections from the same tumor. Tumor samples may also contain non-tumor tissue. DNA from non-tumor tissue would not be expected to contain the EGFR mutations detected by the *therascreen* EGFR RGQ PCR Kit.

## Procedure

### Determination of the level of tumor cells required for EGFR analysis

The tissue used for EGFR analysis is formalin-fixed paraffin-embedded (FFPE), non-small cell lung cancer samples (NSCLC) tissue. DNA extracted from cells within this tumor tissue may be wild type with respect to EGFR mutations or may carry one or more mutations.

The FFPE NSCLC tissue used for the extraction may also contain normal, non-tumor tissue, which will be wild type with respect to EGFR mutations. The wild type DNA from this tissue can dilute the mutant DNA, potentially to a level where it is no longer detectable by the kit. However, it is recommended that even samples with low levels of tumor be tested as there is potential for high-level mutations to be detected and a treatment decision made for the patient.

To maximize the chances of detecting mutations proceed as follows.

- Hematoxylin and eosin (H&E) stain at least one slide from each patient sample.
- Ensure a pathologist reviews the stained slide for the presence of tumor.
- If possible the pathologist should review several slides from throughout the FFPE block.
- All samples with tumor present can be tested with the *therascreen* EGFR RGQ PCR kit.

### DNA isolation

DNA isolation must be carried out using the QIAamp® DNA FFPE Tissue Kit.

Carry out the DNA purification according to the instructions in the *QIAamp DNA FFPE Tissue Kit Handbook* with the following changes.

- Collect FFPE sections on glass slides.
- Scrape excess paraffin away from around the tissue sections using a fresh, sterile scalpel.
- Scrape tissue sections into microcentrifuge tubes using a fresh scalpel for each sample to be extracted.
- Proteinase K digestion should be performed for 1 hour.
- Purified genomic DNA must be eluted in 200 µl of Buffer ATE (provided in the QIAamp DNA FFPE Tissue Kit).
- Store purified genomic DNA at –15 to –30°C.

- Where information is available, slides adjacent to the H&E stained slide with the most tumor content should be used.

**Note:** All assays in the *therascreen* EGFR RGQ PCR Kit generate short PCR products. However, the *therascreen* EGFR RGQ PCR Kit will not work with heavily fragmented DNA.

## Protocol: Sample assessment

This protocol is used to assess the total amplifiable DNA in samples.

### Important points before starting

#### Before beginning the procedure read "24-hour emergency information

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CHEMTREC

**USA & Canada** ■ Tel: 1-800-424-9300

**Outside USA & Canada** ■ Tel: +1-703-527-3887 (collect calls accepted)

- General precautions", page 10.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Do not vortex *Taq* DNA polymerase (*Taq*) or any mix containing *Taq* DNA polymerase (*Taq*) as this may inactivate the enzyme.
- Pipet *Taq* DNA polymerase (*Taq*) by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.

### Things to do before starting

- Before each use, all reagents need to be thawed completely at room temperature (15–25°C), mixed by inverting 10 times, and centrifuged briefly to collect the contents at the bottom of the tube.
- Allow *Taq* DNA Polymerase (*Taq*) to reach room temperature (15–25°C) before each use. Centrifuge the tube briefly to collect the enzyme at the bottom of the tube.

### Procedure

1. **Thaw the Control Reaction Mix (Ctrl), Nuclease-Free Water for No Template Control (NTC), and EGFR Positive Control (PC) at room temperature (15–25°C). When the reagents have thawed, mix them by inverting each tube 10 times to avoid localized concentrations of salts and then centrifuge briefly to collect contents at the bottom of the tube.**
2. **Prepare sufficient master mixes for the DNA samples, one positive control reaction, and one no template control reaction according to the volumes in Table 1. Include reagents for 1 extra sample to allow sufficient overage for the PCR setup.**

The master mix contains all of the components needed for PCR, except the sample.

**Table 1. Preparation of control assay master mix\***

<b>Component</b>	<b>Volume/reaction (<math>\mu</math>l)</b>
Control Reaction Mix (Ctrl)	19.5
<i>Taq</i> DNA polymerase ( <i>Taq</i> )	0.5
<b>Total volume</b>	<b>20.0</b>

\* When preparing the master mix, prepare enough for one extra sample.

- 3. Thoroughly mix the master mix by gently pipetting up and down 10 times. Immediately add 20  $\mu$ l master mix to PCR strip tube (not provided).**

**Note:** For sample assessment, control assay master mix should be added to one positive control well, one negative control well, and one well for each sample.

- 4. Immediately add 5  $\mu$ l sample of Nuclease-Free Water (H<sub>2</sub>O) to the no template control tube (PCR tube number 9) and cap the tube. Add 5  $\mu$ l of sample DNA to the sample tubes and cap the tubes. Add 5  $\mu$ l of EGFR Positive Control (PC) to the positive control tube (PCR tube 1) and cap the tube.**
- 5. Place the PCR strip tubes into the appropriate positions in the rotor and visually check that all tubes contain an equal volume.**

**Note:** Ensure that strips of tubes are not reversed when transferring them to the rotor.
- 6. If the rotor is not full, fill remaining spaces with capped empty tubes.**
- 7. Immediately place the 72-well rotor into the Rotor-Gene Q 5plex HRM instrument. Make sure that the locking ring (accessory of the Rotor-Gene Q instrument) is placed on top of the rotor to secure the tubes during the run.**
- 8. Refer to Rotor-Gene Q instrument setup (see "Protocol: Rotor-Gene Q EGFR setup", page 21) to create the temperature profile and start the run.**



**Table 2. Cycling parameters**

<b>Cycles</b>	<b>Temperature</b>	<b>Time</b>	<b>Data acquisition</b>
1	95°C	15 minutes	None
40	95°C	30 seconds	None
	60°C	60 seconds	Green and yellow

- 9. After the run is finished, analyze the data according to “Sample assessment data analysis”, page 30.**

## Protocol: Detection of EGFR mutations

This protocol is for detection of EGFR mutations. Once a sample has passed the sample assessment, it can be tested using the EGFR mutation assays.

### Important points before starting

#### Before beginning the procedure read "24-hour emergency information

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**USA & Canada** ■ Tel: 1-800-424-9300

**Outside USA & Canada** ■ Tel: +1-703-527-3887 (collect calls accepted)

- General precautions", page 10.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Do not vortex *Taq* DNA Polymerase (*Taq*), or any mix containing *Taq* DNA polymerase, as this may inactivate the enzyme.
- For efficient use of the *therascreen* EGFR RGQ PCR Kit, samples must be grouped into batches of 7 to fill the 72-well rotor. Smaller batch sizes will mean that fewer samples can be tested with the *therascreen* EGFR RGQ PCR Kit.
- Pipet *Taq* by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.
- For each DNA sample, the control and mutation assays must be analyzed in the same PCR run to avoid run-to-run variations.

### Things to do before starting

- Before each use, all reagents need to be thawed completely at room temperature (15–25°C), mixed by inverting 10 times, and centrifuged briefly to collect the contents at the bottom of the tube.
- Ensure that *Taq* is at room temperature (15–25°C) before each use. Centrifuge the tube briefly to collect the enzyme at the bottom of the tube.

### Procedure

1. **Thaw the reaction mixes, Nuclease-Free Water for No Template Control (NTC), and EGFR Positive Control (PC) at room temperature (15–25°C). When the reagents have thawed, mix them by inverting each tube 10 times to avoid localized concentrations of salts and**

then centrifuge briefly to collect the contents at the bottom of the tube.

2. Prepare sufficient master mixes for the DNA samples, one positive control reaction, and one no template control reaction according to the volumes in Table 3. Include reagents for 1 extra sample to allow sufficient overage for the PCR setup.

The master mix contains all of the components needed for PCR, except the sample.

**Table 3. Preparation of master mixes\***

<b>Component</b>	<b>Volume/reaction (<math>\mu</math>l)</b>
Reaction mix	19.5
<i>Taq</i> DNA polymerase ( <i>Taq</i> )	0.5
<b>Total volume</b>	<b>20.0</b>

\* When preparing the master mix, prepare enough for one extra sample.

3. Thoroughly mix each master mix by gently pipetting up and down 10 times. Immediately add 20  $\mu$ l master mix to each PCR strip tube (not provided).
4. Immediately add 5  $\mu$ l of Nuclease-Free Water ( $H_2O$ ) to the no template control PCR strip tubes (PCR tube numbers 9–16) and cap the tubes. Add 5  $\mu$ l of each sample to the sample tubes (PCR tubes 17–72) and cap the tubes. Add 5  $\mu$ l of EGFR Positive Control (PC) to the positive control tubes (PCR tube numbers 1–8). Each DNA sample must be tested with both the control and all mutation assays. The layout is shown in Table 4.

**Table 4. Layout of control and mutation assays**

Assay	Controls		Sample number						
	PC	NTC	1	2	3	4	5	6	7
Ctrl	1	9	17	25	33	41	49	57	65
T790M	2	10	18	26	34	42	50	58	66
Deletions	3	11	19	27	35	43	51	59	67
L858R	4	12	20	28	36	44	52	60	68
L861Q	5	13	21	29	37	45	53	61	69
G719X	6	14	22	30	38	46	54	62	70
S768I	7	15	23	31	39	47	55	63	71
Insertions	8	16	24	32	40	48	56	64	72

- Place the PCR strip tubes into the appropriate positions in the rotor and visually check that all tubes contain an equal volume.  
**Note:** Ensure that strips of tubes are not reversed when transferring them to the rotor.
- If the rotor is not full, fill remaining spaces with capped empty tubes.
- Immediately place the rotor into the Rotor-Gene Q 5plex HRM instrument. Make sure that the locking ring (accessory of the Rotor-Gene Q instrument) is placed on top of the rotor to secure the tubes during the run.
- Refer to Rotor-Gene Q instrument setup (see "Protocol: Rotor-Gene Q EGFR setup", page 21) to create the temperature profile and start the run.

**Table 5. Cycling parameters**

Cycles	Temperature	Time	Data acquisition
1	95°C	15 minutes	None
40	95°C	30 seconds	None
	60°C	60 seconds	Green and yellow

- After the run is finished, analyze the data according to "EGFR mutation data analysis", page 33.

## Protocol: Rotor-Gene Q EGFR setup

This protocol is referenced within "Protocol: Sample assessment", page 15, and "Protocol: Detection of EGFR mutations", page 18.

### Procedure

#### 1. Create a temperature profile according to the following steps.

Setting the general assay parameters	Figures 1–3
Initial activation of the hot-start enzyme	Figure 4
Amplification of the DNA	Figures 5–7
Adjusting the fluorescence channels	Figures 8–12
Starting the run	Figure 13

To summarize, the cycling parameters are as follows.

**Table 6. Cycling parameters**

Cycles	Temperature	Time	Data acquisition
1	95°C	15 minutes	None
40	95°C	30 seconds	None
	60°C	60 seconds	Green and yellow

All specifications refer to the Rotor-Gene Q software version 2.0.2. Please find further information on programming Rotor-Gene instruments in the instrument user manual. In the illustrations, these settings are framed in bold black.

- 2. Double-click the Rotor-Gene Q Series Software 2.0.2 software icon on the desktop of the PC connected to the Rotor-Gene Q 5plex HRM instrument. Select the "Advanced" tab in the "New Run" dialog box that appears.**
- 3. To create a new template, select "Empty Run" and then click "New" to enter the "New Run Wizard".**

4. Select **72-Well Rotor** as the rotor type. Confirm that the locking ring is attached and check the “**Locking Ring Attached**” box. Click “**Next**” (Figure 1).

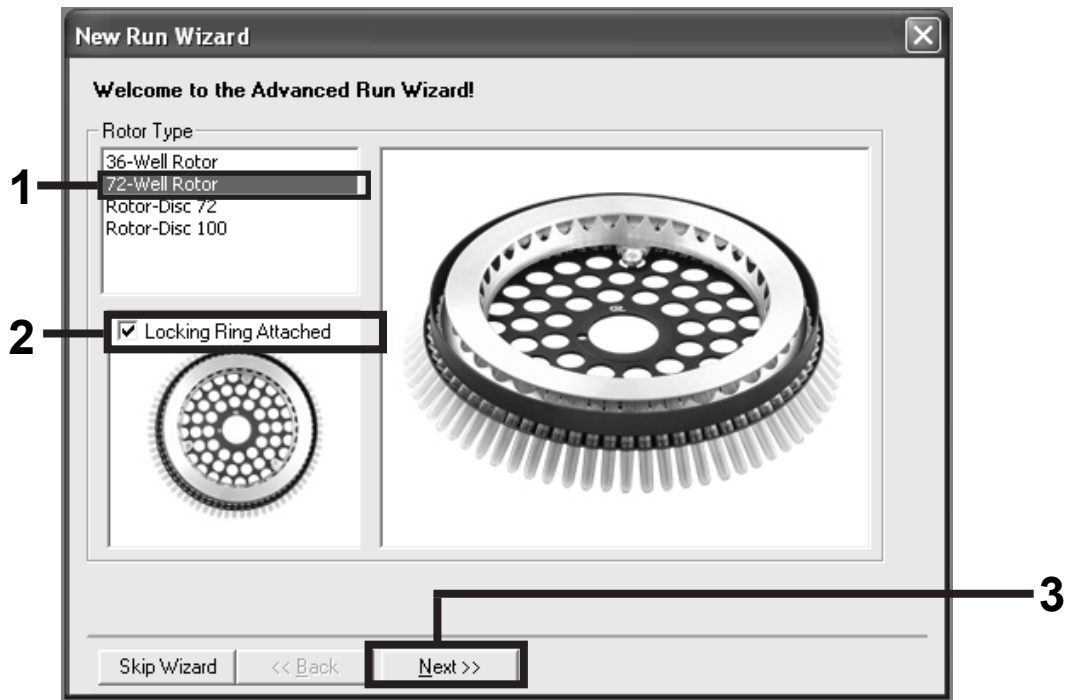


Figure 1. The “New Run Wizard” dialog box.

5. Enter the name of the operator. Add any notes and enter the reaction volume as **25**. Ensure that “**Sample Layout**” reads “**1,2,3...**”. Click “**Next**” (Figure 2).

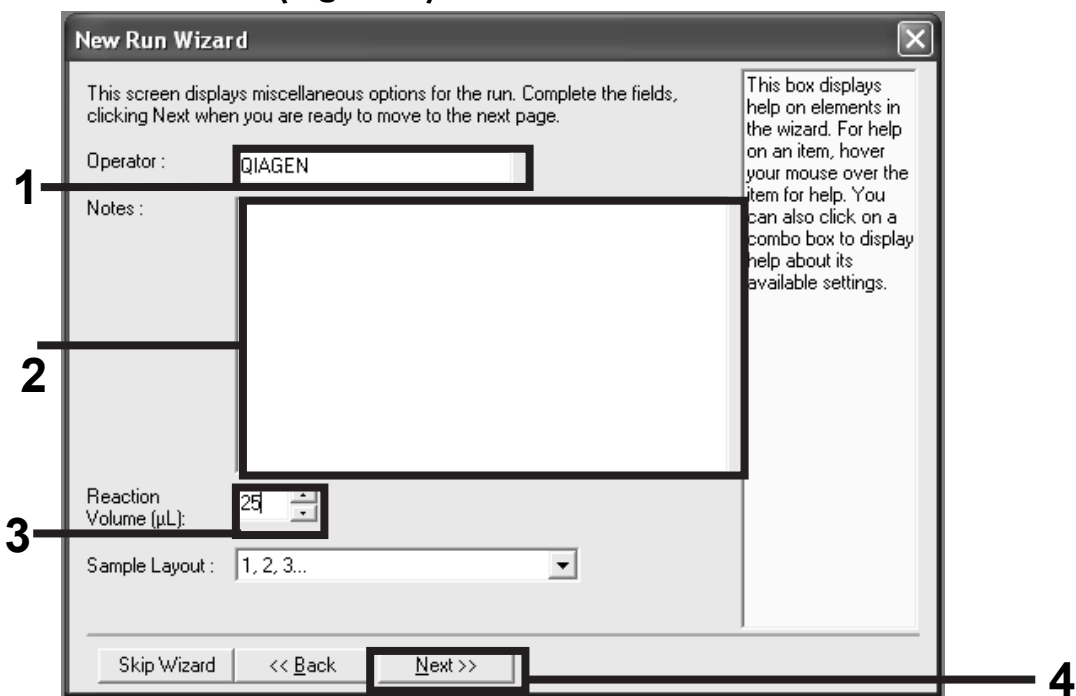


Figure 2. Setting the general assay parameters.

- Click the “Edit Profile” button in the next “New Run Wizard” dialog box (Figure 3) and program the temperature profile according to the information in the following steps.

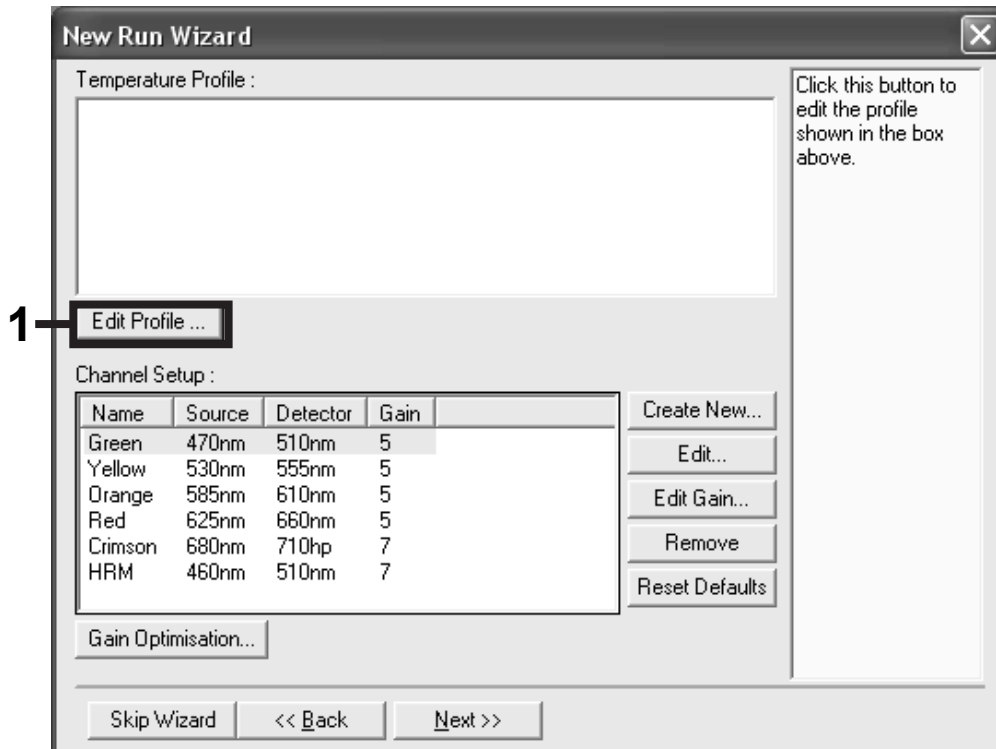


Figure 3. Editing the profile.

- Click the “Insert after” button and select *New Hold at Temperature* (Figure 4).

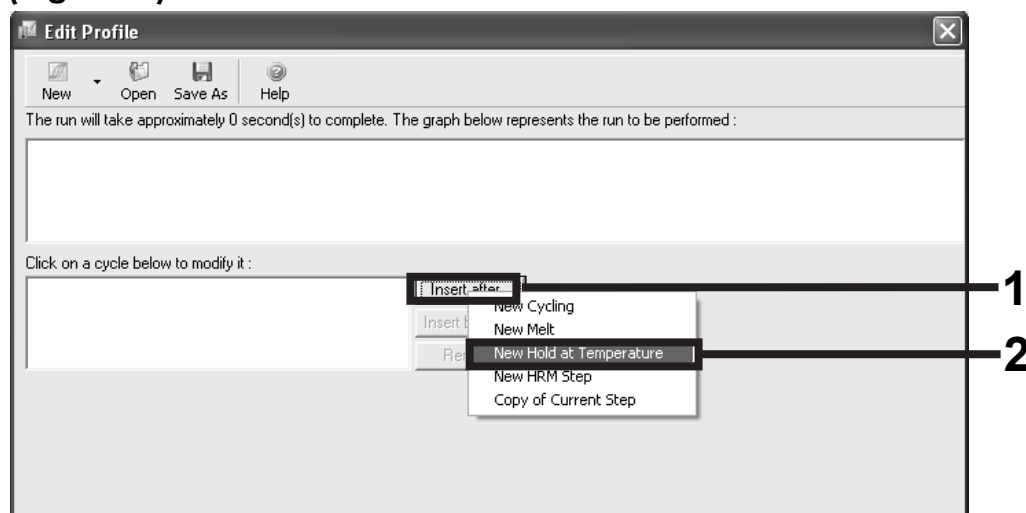


Figure 4. Initial incubation step at 95°C.

- Change the “Hold Temperature” to 95°C and the “Hold Time” to 15 mins. Click the “Insert After” button and then select New Cycling (Figure 5).

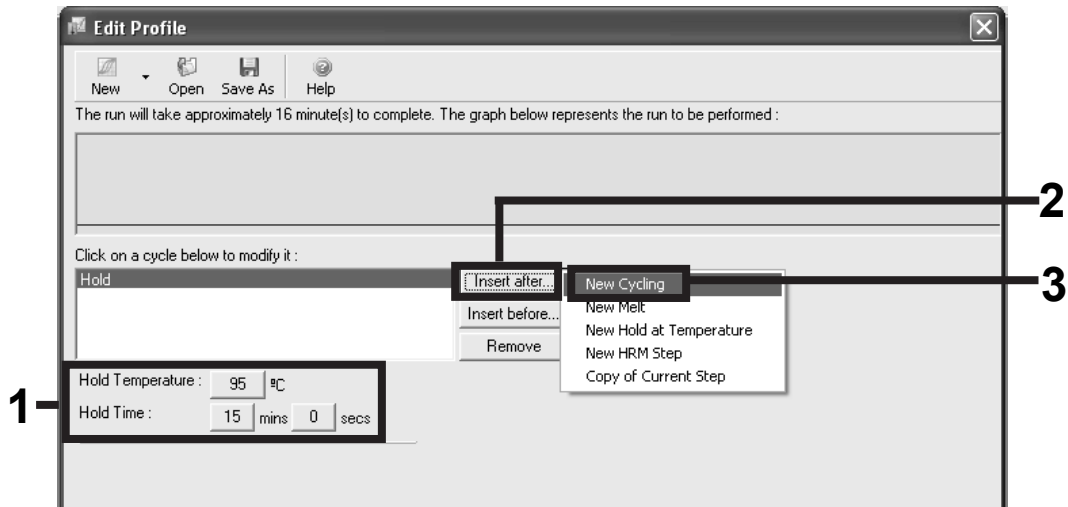


Figure 5. Initial incubation step at 95°C.

- Change the number of cycle repeats to 40. Select the first step and set to 95°C for 30 secs (Figure 6).

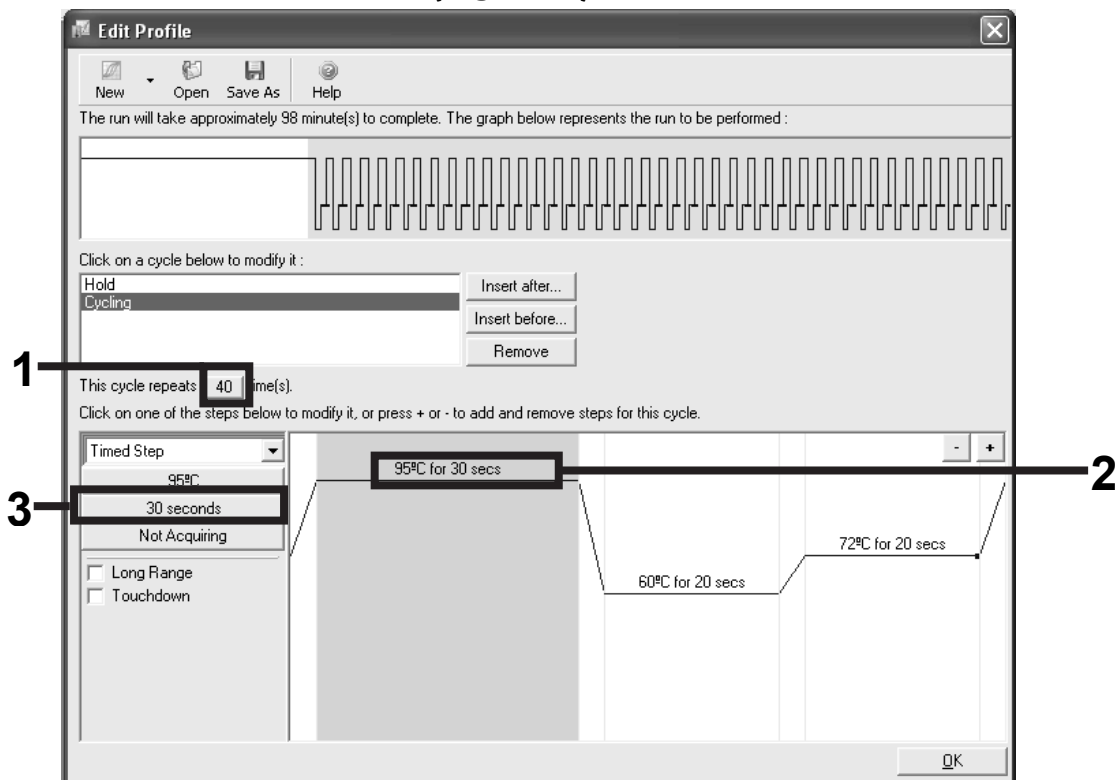


Figure 6. Cycling step at 95°C.



10. Highlight the second step and set to 60°C for 60 secs. Enable data acquisition during this step by selecting the “Not Acquiring” button (Figure 7).

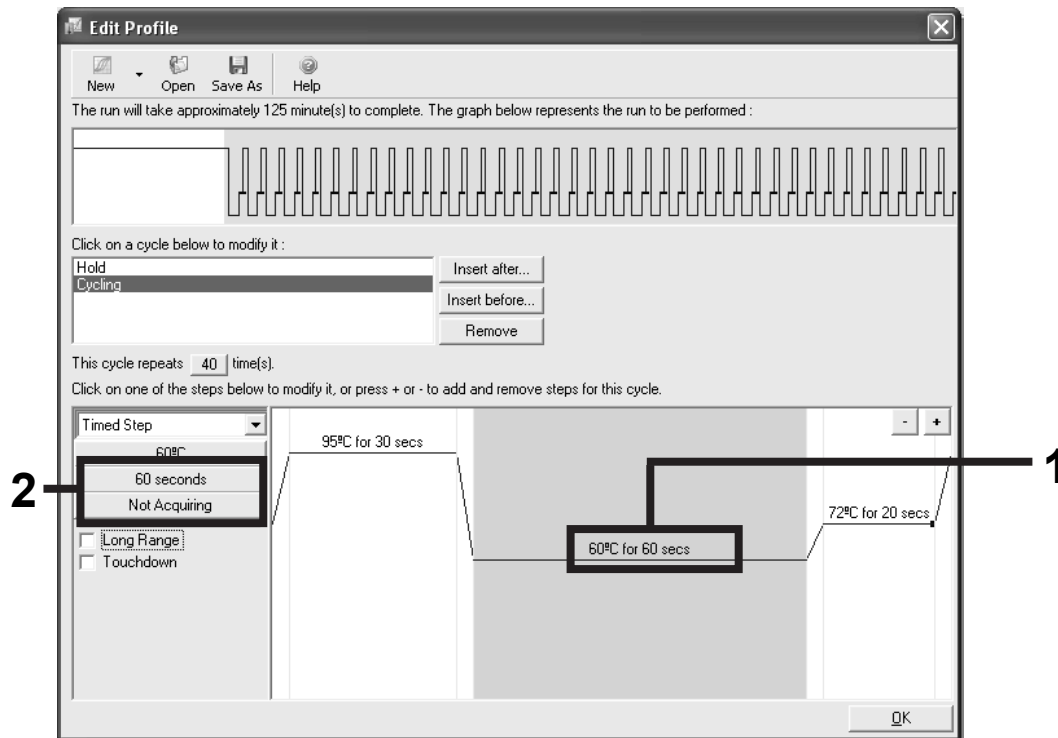


Figure 7. Cycling step at 60°C.

11. Set Green and Yellow as acquiring channels by selecting the ">" button to transfer these from the "Available Channels" list. Click "OK" (Figure 8).

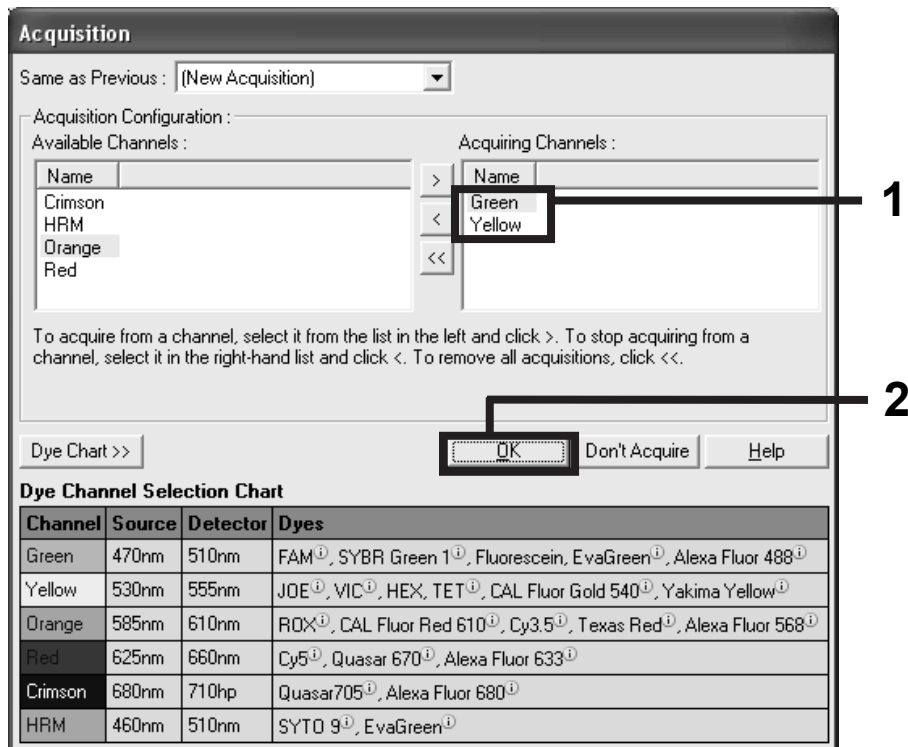


Figure 8. Acquiring at cycling step of 60°C.

12. Highlight the third step and delete by clicking the "-" button. Click "OK" (Figure 9).

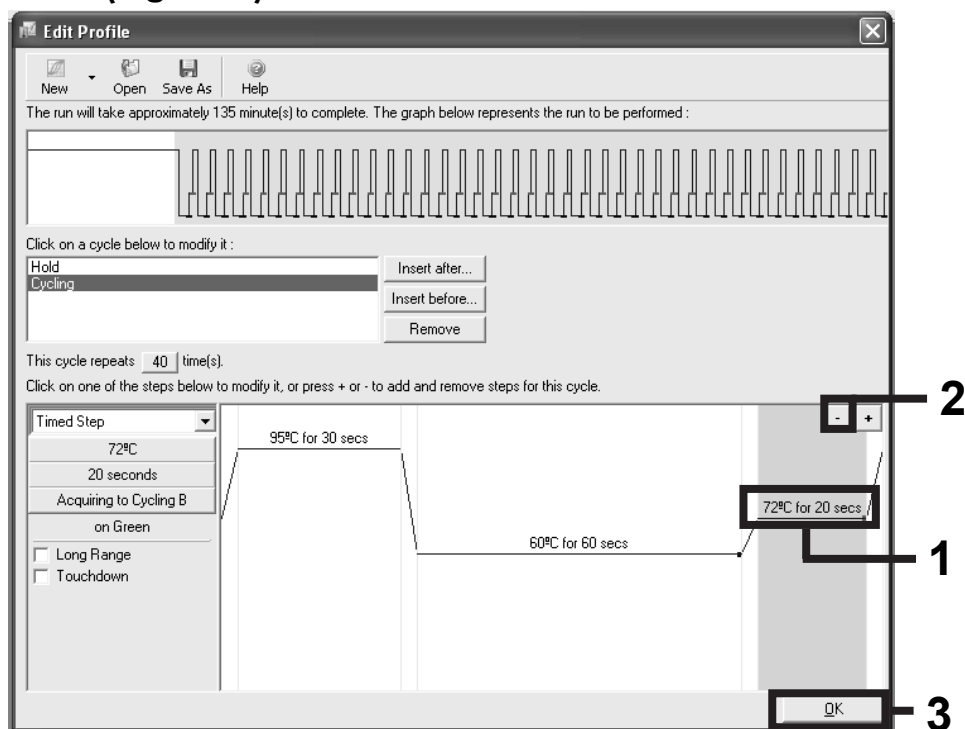


Figure 9. Removal of extension step.

13. In the next dialog box, click the “Gain Optimisation” button (Figure 10).

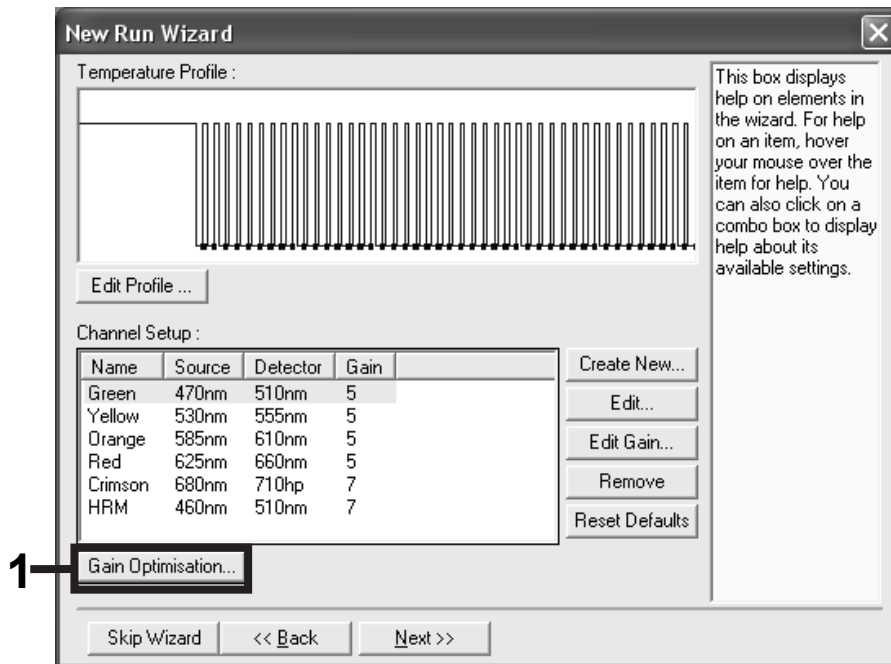


Figure 10. Gain optimization.

14. Click the “Optimise Acquiring” button. Channel settings are then displayed for each channel. Accept these default values by clicking “OK” for both channels (Figure 11).

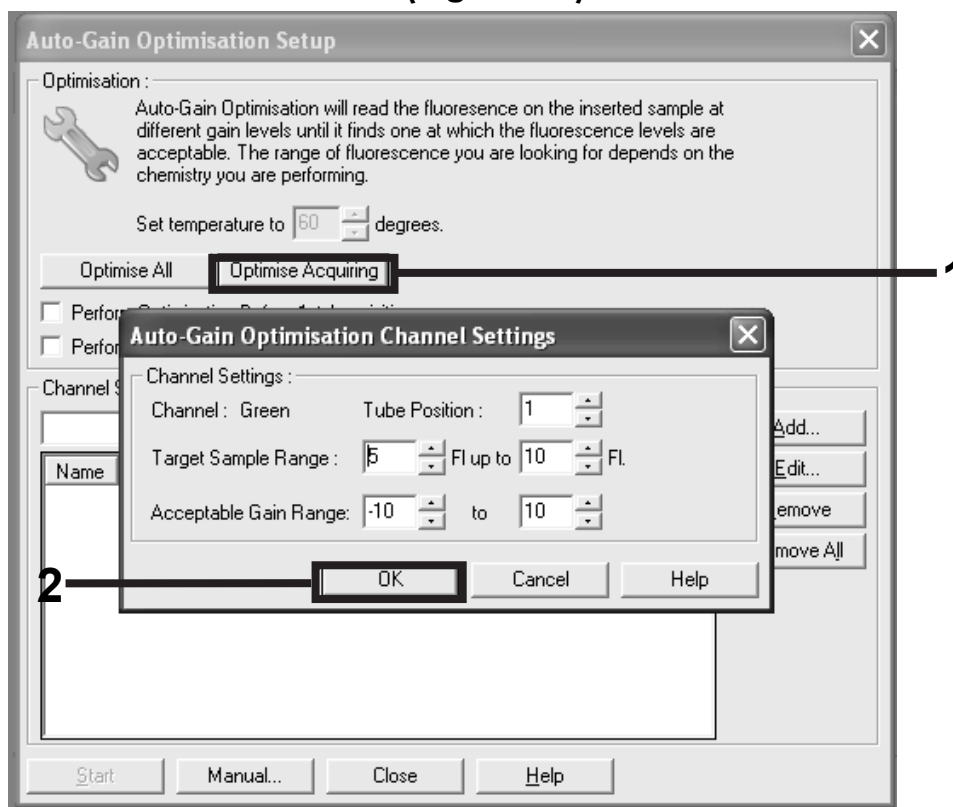


Figure 11. Auto-gain optimization for the green channel.

15. Check the “Perform Optimisation before 1st Acquisition” box, and then click the “Close” button to return to the wizard (Figure 12).

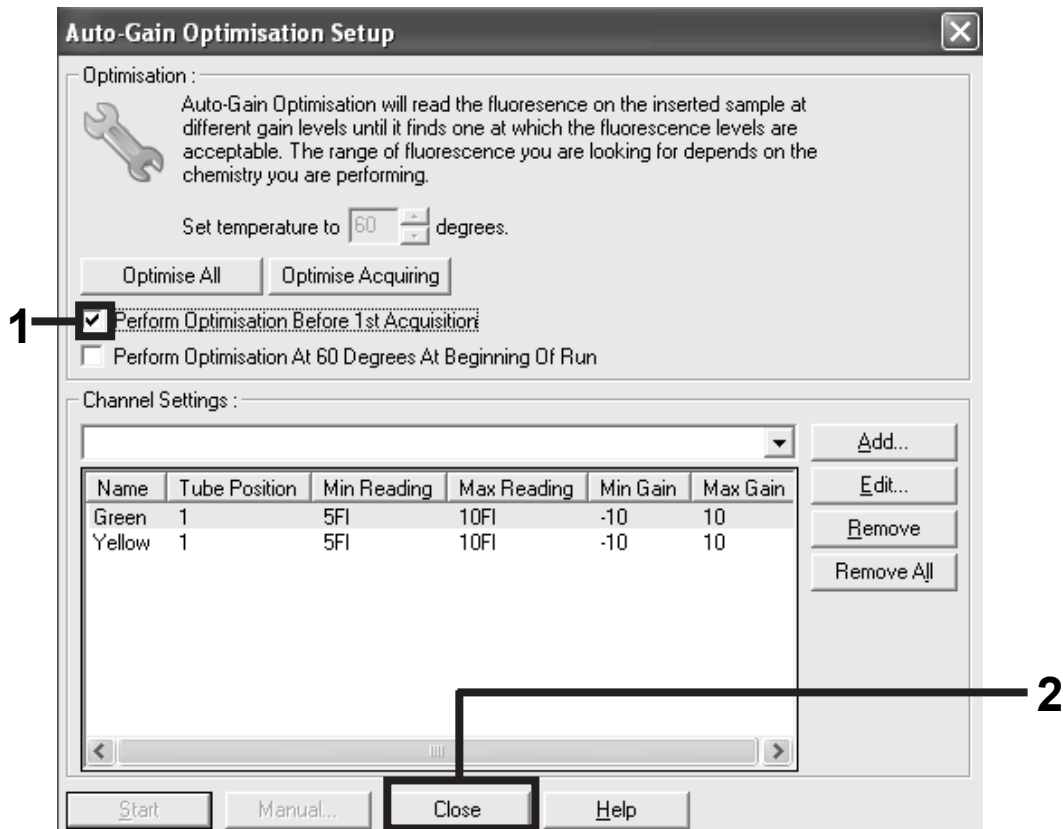
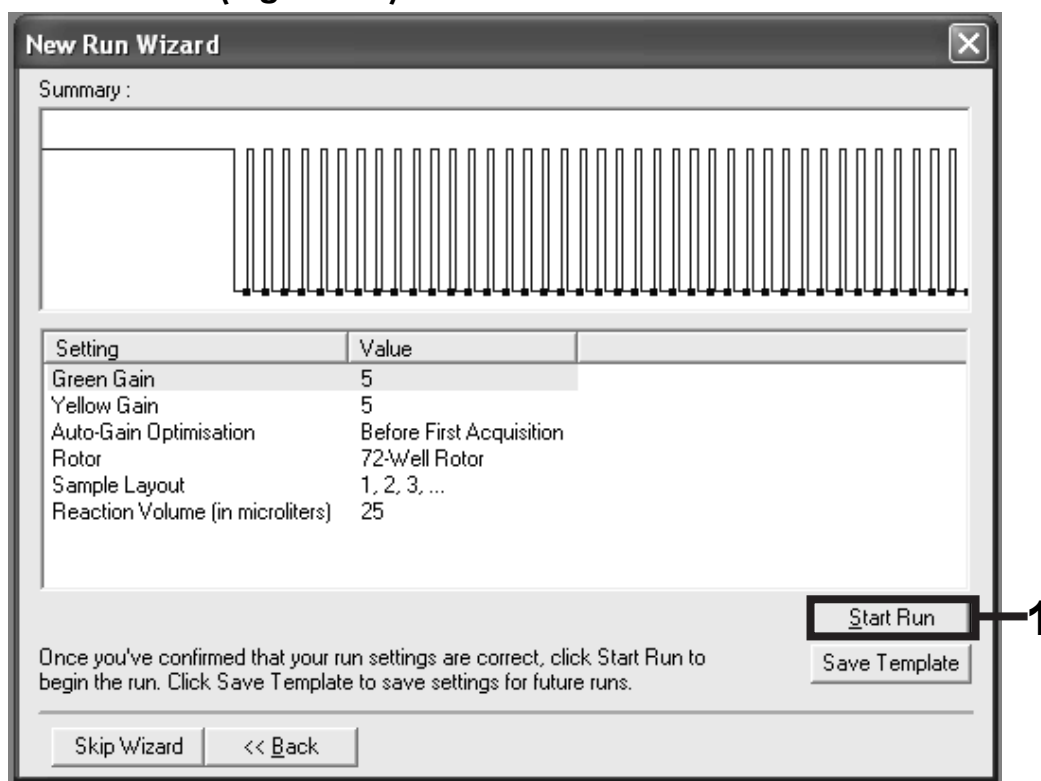


Figure 12. Selection of green and yellow channels.

16. Click “Next” to save the template in an appropriate location by selecting “Save Template”.

**17. Check the summary and click “Start Run” to save the run file and start the run (Figure 13).**



**Figure 13. Starting the run.**

**18. After the run starts, a new window appears in which you can either enter sample names now or click “Finish” and enter them later by selecting the “Sample” button during the run, or once the run is complete.**

**19. After the run is finished, analyze the data according to the appropriate protocol:**

- For sample assessment, see “Sample assessment data analysis”, page 30.
- For mutation analysis, see “EGFR mutation data analysis”, page 33.

# Interpretation of Results

## $\Delta C_T$ analysis method

Scorpions real-time assays use the number of PCR cycles necessary to detect a fluorescent signal above a background signal as a measure of the target molecules present at the beginning of the reaction. The point at which the signal is detected above background fluorescence is called the cycle threshold ( $C_T$ ).

Sample  $\Delta C_T$  values are calculated as the difference between the mutation assay  $C_T$  and control assay  $C_T$  from the same sample:

$$\Delta C_T = \text{mutation } C_T - \text{control } C_T$$

**Note:** Samples are classed as mutation positive if they give a  $\Delta C_T$  less than the cut-off  $\Delta C_T$  value for that assay. Above this value, the sample may either contain less than the percentage of mutation able to be detected by the kit (beyond the limit of the assays), or the sample is mutation-negative.

**Note:** Mutation  $C_T$  values of 40 or above will be scored as negative or beyond the limits of the kit.

When using ARMS primers, some inefficient priming may occur giving a very late background  $C_T$  from DNA not containing a mutation. All  $\Delta C_T$  values calculated from background amplification will be greater than the cut-off  $\Delta C_T$  values and the sample will be classed as mutation-negative.

## Sample assessment data analysis

After the run is complete, analyze the data according to the following procedure.

### Software analysis settings

1. Open the appropriate file using the Rotor-Gene Q series software version (2.0.2) and above.
2. Check samples are labeled.
3. When on the raw channel page for each detector/channel, click "Options" and enter *Crop start cycles*. On the page with "Remove data before cycle", enter 15 and click "OK".
4. Click "Analysis". On the analysis page, click "Cycling A (from 15), Yellow" to check the HEX channel.
5. Check that "dynamic tube" is highlighted. Click "Slope correct" and "Linear scale".
6. Set the threshold at 0.02 and check the HEX  $C_T$  values.

7. On the analysis page, click “Cycling A (from 15), Green” to view the FAM channel.
8. The dynamic tube should be highlighted. Click “Slope correct” and “Linear scale”.
9. Set the threshold at 0.075 and check the FAM  $C_T$  values.

After the run has finished, analyze the data as follows.

- **Negative control:** To ensure that no template contamination is present, the NTC must not generate a  $C_T$  value in the green (FAM) channel below 40. Refer to “Notes for data interpretation” on page 39 for important information on the analysis of no template control (NTC) plots. To ensure that the run was set up correctly, the NTC must show amplification of 31–37 in the yellow (HEX) channel.

If there is positive amplification in the green channel and/or amplification outside the range 31–37 in the yellow signal, the sample results must be discarded.

- **Positive control:** The EGFR Positive Control (PC) must give a control assay  $C_T$  (FAM channel) of 26.26–30.95. A run with a  $C_T$  value outside this range indicates an assay setup problem and the run should be designated as a failure. If the positive control assay  $C_T$  is 26.26–30.95 (exon 2, FAM) but the internal control  $C_T$  (HEX) is outside the range 31–37, continue with the analysis.

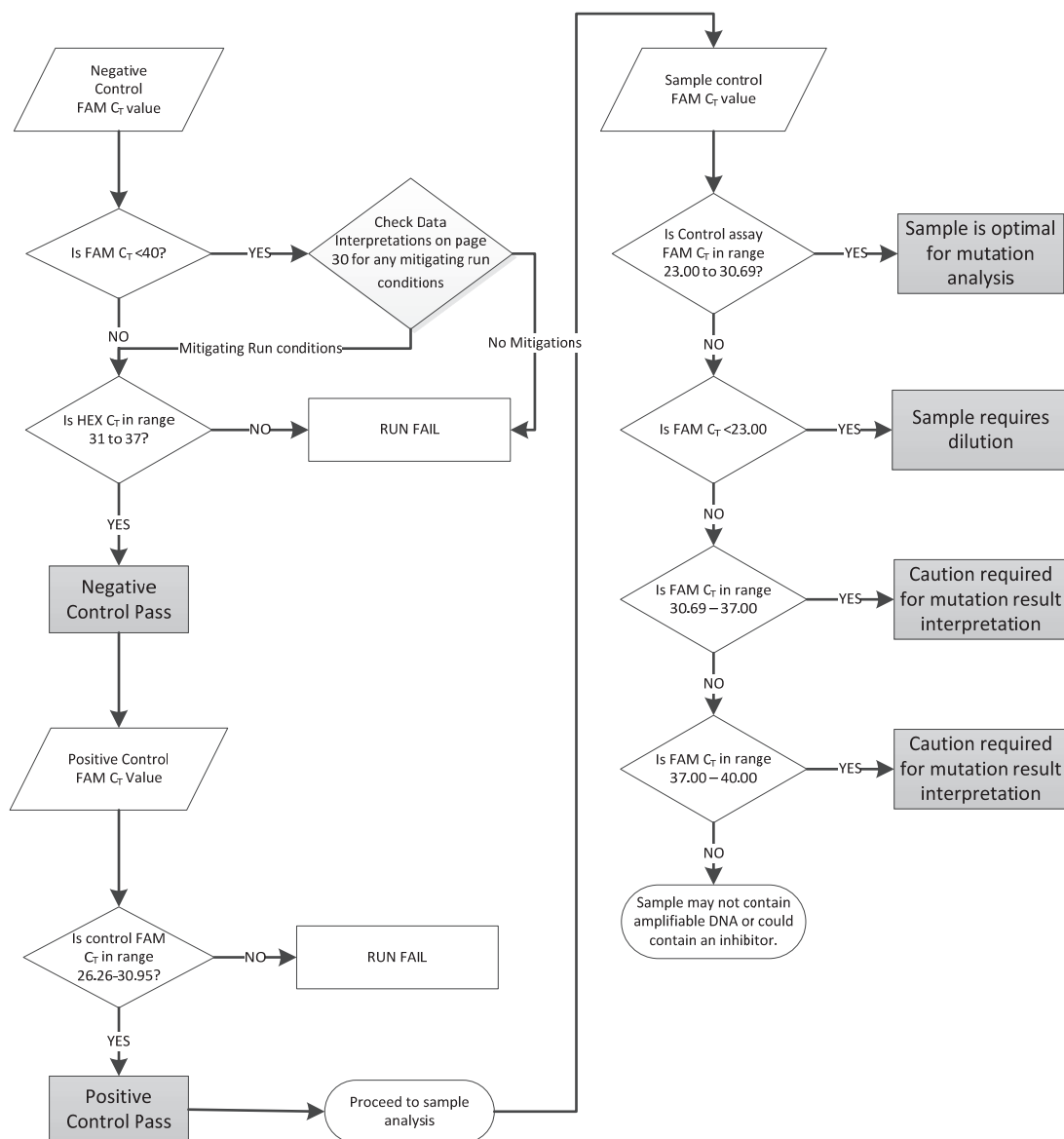
**Note:** Sample data must not be used if either of these two run controls has failed.

Provided that both run controls are valid, each sample  $C_T$  value must be within the range 23–30.69 in the green (FAM) channel. If the sample is out of this range, the following guidance is provided.

- **Sample control assay  $C_T$  of <23:** Samples with a control  $C_T$  of <23 will overload the mutation assays and must be diluted. To detect each mutation at a low level, over-concentrated samples must be diluted to fall within the above range on the basis that diluting by half will increase the  $C_T$  by 1.
- **Sample control assay  $C_T$  of 30.69–37:** Interpret with caution as very low level mutations may not be detected.
- **Sample control assay  $C_T$  of 37–40:** Interpret with caution as only very high level mutations will be detected.
- **Sample control assay  $C_T$  >40:** Sample does not contain sufficient DNA to permit analysis.

**Note:** If a sample does not generate a  $C_T$  (i.e.,  $C_T > 40$ ), it may be due to the presence of an inhibitor, an error in assay setup, or there is no amplifiable EGFR DNA.

- **Internal control  $C_T$  value of 31–37:** There is no amplifiable EGFR DNA.
- **Internal control  $C_T$  value is not within the range of 31–37:** This could indicate an assay setup error or the presence of an inhibitor. It is possible to reduce the effect of an inhibitor by diluting the sample, although this will also dilute the DNA.



**Figure 14. Sample assessment analysis workflow.**



## EGFR mutation data analysis

After the run is complete, analyze the data according to the following procedure.

### Software analysis settings

1. Open the appropriate file using the Rotor-Gene Q series software version (2.0.2) and above.
2. Check that the samples are labeled.
3. When on the raw channel page for each detector/channel, click "Options" and enter *Crop start cycles*. On the page with "Remove data before cycle", enter 15 and click "OK".
4. Click "Analysis". On the analysis page, click "Cycling A (from 15), Yellow" to view the HEX channel.
5. Check that "dynamic tube" is highlighted. Click "Slope correct" and "Linear scale".
6. Set the threshold at 0.02 and check the HEX  $C_T$  values.
7. On the analysis page, click "Cycling A (from 15), Green" to view the FAM channel.
8. Check that "dynamic tube" is highlighted. Click "Slope correct" and "Linear scale".
9. Set the threshold at 0.075 and check the FAM  $C_T$  values.

### Run control analysis:

Refer to the "Run Control Analysis" flowchart in Figure 15.

- **Negative control:** To ensure that no template contamination is present, the NTC must not generate a  $C_T$  value in the green (FAM) channel below 40. Refer to "Notes for data interpretation" page 39 for important information on the analysis of no template control (NTC) plots. To ensure that the run was set up correctly, the NTC must show amplification of  $C_T$  31–37 in the yellow (HEX) channel.

If there is positive amplification in the green channel and/or amplification outside the range 31–37 in the yellow signal, the sample results must be discarded.

- **Positive control:** The EGFR Positive Control (PC) must give a control assay  $C_T$  of 26.26–30.95 in the green channel. A run with a  $C_T$  value outside this range indicates an assay setup problem and the run should be designated as failure. If the positive control assay  $C_T$  is 26.26–30.95 (exon 2, FAM) but the internal control  $C_T$  (HEX) is outside the range 31–37, continue with the analysis.

Calculate the  $\Delta C_T$  value for each mutation assay as follows, ensuring that the mutation and control  $C_T$  values are from the positive control.

$$\Delta C_T = \text{mutation } C_T - \text{control } C_T$$

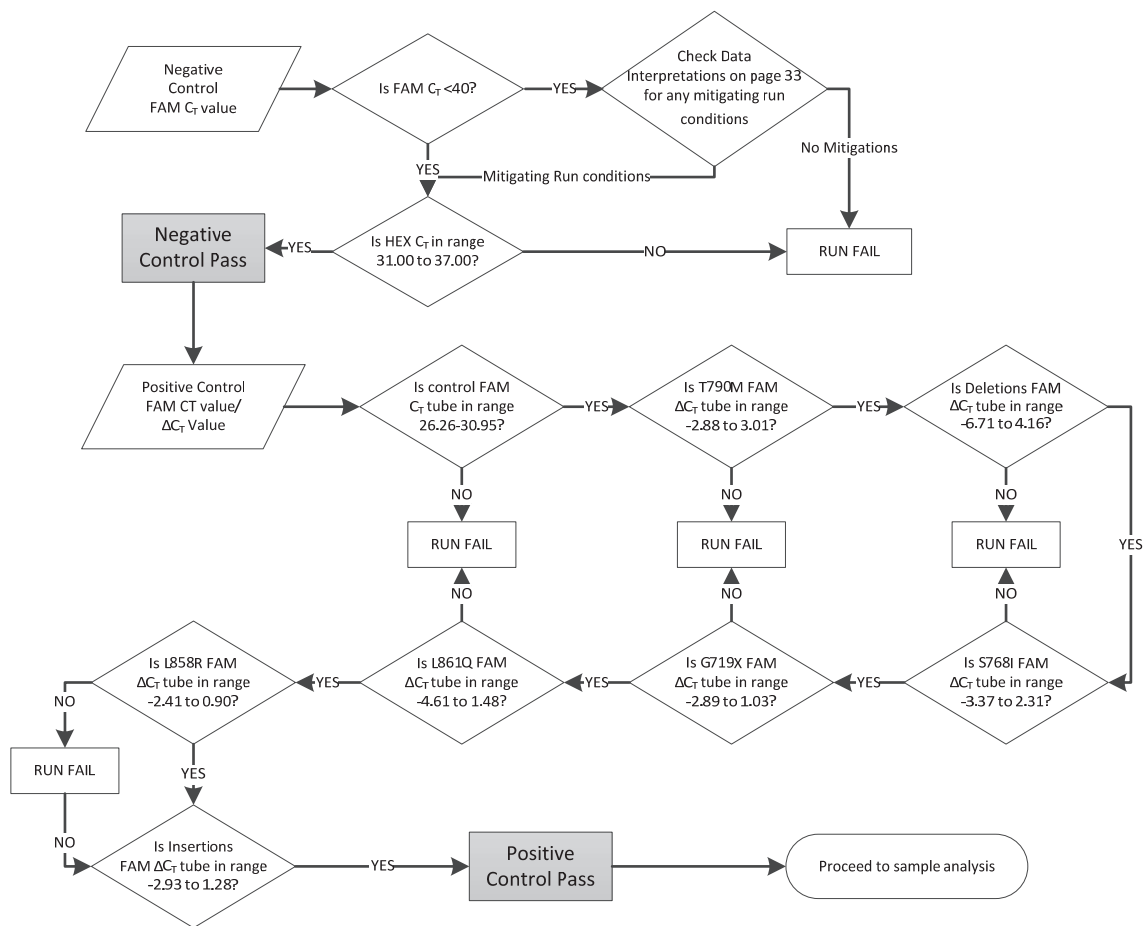
The EGFR Positive Control (PC)  $\Delta C_T$  values should fall within the values given in Table 7.

**Table 7. Expected positive control  $\Delta C_T$  values\***

<b>Assay</b>	<b>Positive control <math>\Delta C_T</math> value</b>
T790M	-2.88 to 3.01
Deletions	-6.71 to 4.16
L858R	-2.41 to 0.90
L861Q	-4.61 to 1.48
G719X	-2.89 to 1.03
S768I	-3.37 to 2.31
Insertions	-2.93 to 1.28

\* Rotor-Gene Q software (2.0.2)

**Note:** Sample data must not be used if either of the negative or positive run controls has failed.



**Figure 15. Run control analysis workflow.**

## Sample analysis:

### Sample control FAM $C_T$ value

Provided that both run controls are valid, each sample control  $C_T$  value must be within the range of 23–30.69 in the green channel. Refer to “Sample Analysis” flowchart in Figure 16.

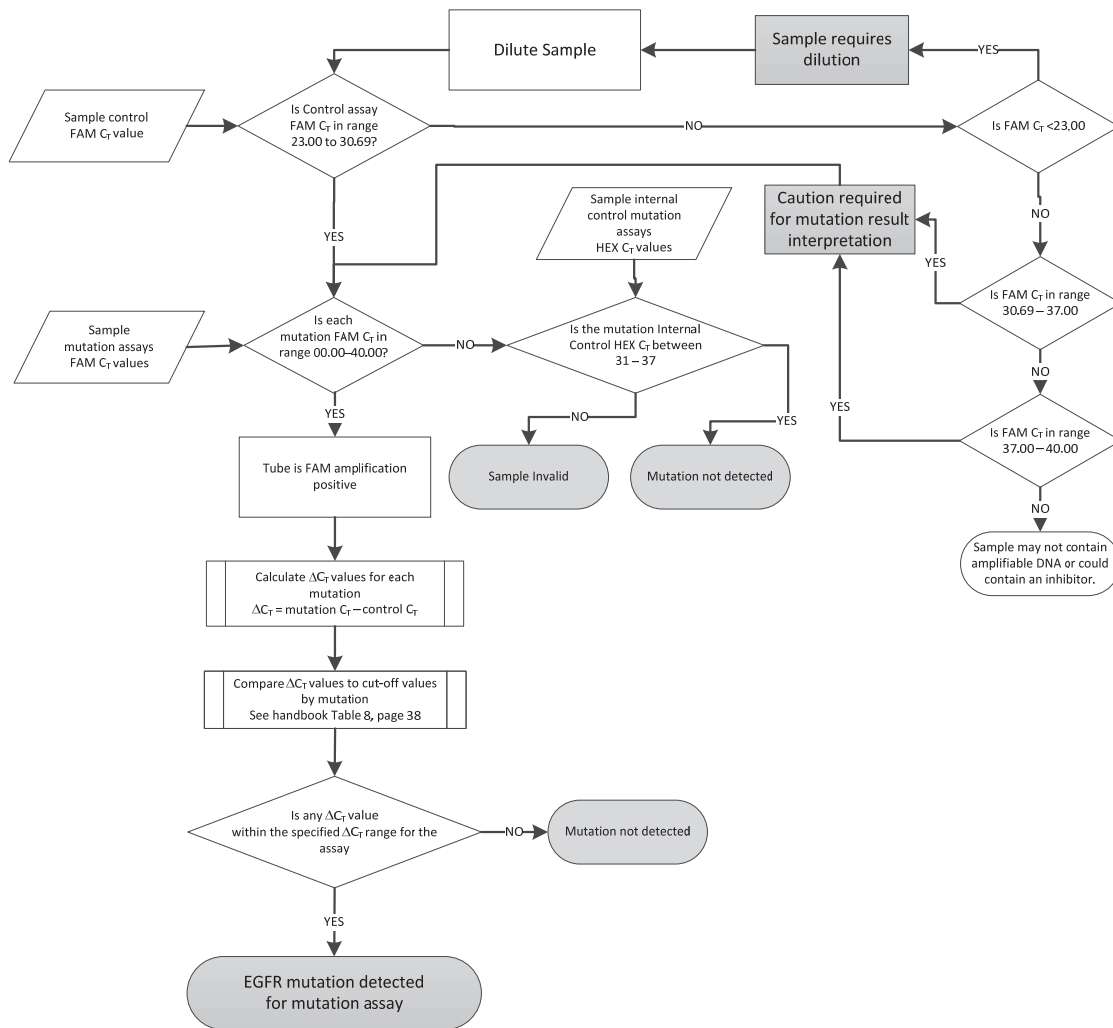
- **Sample control assay  $C_T < 23$ :** Samples with a control  $C_T$  of  $< 23$  will overload the mutation assays and must be diluted. To detect each mutation at a low level, over-concentrated samples must be diluted to fall within the above range on the basis that diluting by half will increase the  $C_T$  by 1.
- **Sample control assay  $C_T$  in the range of 30.69–37:** Interpret with caution, as very low level mutations may not be detected.
- **Sample control assay  $C_T$  in the range of 37–40:** Interpret with caution, as only very high level mutations will be detected.
- **Sample control assay  $C_T > 40$ :** Sample does not contain sufficient DNA to permit analysis.

**Note:** If the sample FAM  $C_T$  value is between 23 and  $< 37$ , there is no need to evaluate the internal control.

**Note:** If a sample does not generate a  $C_T$  (i.e.,  $C_T > 40$ ), it may be due to the presence of an inhibitor, an error in assay setup, or there is no amplifiable EGFR DNA.

- **Internal control  $C_T$  value of 31–37:** The assay is functioning correctly, but there is no amplifiable EGFR DNA.
- **Internal control  $C_T$  value is not within the range of 31–37:** This could indicate an assay setup error or the presence of an inhibitor. It is possible to reduce the effect of an inhibitor by diluting the sample, although this will also dilute the DNA.

**Note:** If the mutation assay FAM reaction does not generate a  $C_T$  value and the internal control reactions generate a  $C_T$  value outside the range of 31–37, the data must be discarded as there may be inhibitors present which could lead to false negative results. Diluting the sample may reduce the effect of inhibitors but it should be noted that this would also dilute the DNA.



**Figure 16. Mutation analysis flowchart.**

## Sample mutation assays FAM C<sub>T</sub> value

The FAM values for all seven mutation reaction mixes should be checked against the values listed in Table 8.

**Table 8. Acceptable sample mutation reaction values (FAM)\***

Assay	Acceptable C <sub>T</sub> range	Cut-off ΔC <sub>T</sub> value
T790M	15.00–40.00	6.38
Deletions	15.00–40.00	9.06
L858R	15.00–40.00	8.58
L861Q	15.00–40.00	9.26
G719X	15.00–40.00	9.31
S768I	15.00–40.00	9.26
Insertions	15.00–40.00	7.91

\*Acceptable values are within and including the values shown.

- If the FAM C<sub>T</sub> falls within the specified range of 15.00–40.00, it is FAM amplification positive.
- If the FAM C<sub>T</sub> falls above the specified range, or there is no amplification, it is FAM amplification negative.

Calculate the ΔC<sub>T</sub> value for each mutation sample showing positive amplification as follows, ensuring that the mutation and control C<sub>T</sub> values are from the same sample.

$$\Delta C_T = \text{mutation } C_T - \text{control } C_T$$

Compare the ΔC<sub>T</sub> value for the sample with the cut-off point for the assay in question (Table 8), ensuring that the correct cut-off point is applied to each assay.

The cut-off point is the point above which a positive signal could potentially be due to background signal of the ARMS primer on wild type DNA. If the sample ΔC<sub>T</sub> value is higher than the cut-off point, it is classed as “mutation not detected” or beyond the limits of detection of the kit. If the sample value is at or below the cut-off point, the sample is considered positive for a mutation detected by that assay.

**Note:** For samples showing no FAM mutation C<sub>T</sub>, an evaluation of the internal control (HEX) C<sub>T</sub> is required to determine whether the mutation is not detected

or whether the assay is invalid. If the HEX  $C_T$  value is between 31 and 37 then the mutation is not detected. If the HEX  $C_T$  value is outside of the range 31–37, then the sample is invalid.

In summary, for every sample, each mutation reaction will be given a status of mutation detected; mutation not detected, or invalid using the following criteria.

- **Mutation detected:** FAM amplification positive and the  $\Delta C_T$  are at or below the cut-off value. If multiple mutations are detected, all can be reported.
- **Mutation not detected:**  
FAM amplification positive and the  $\Delta C_T$  are above the cut-off value.  
FAM amplification negative and HEX (internal control) amplification positive.
- **Invalid:**  
FAM amplification negative and HEX amplification outside specifications.

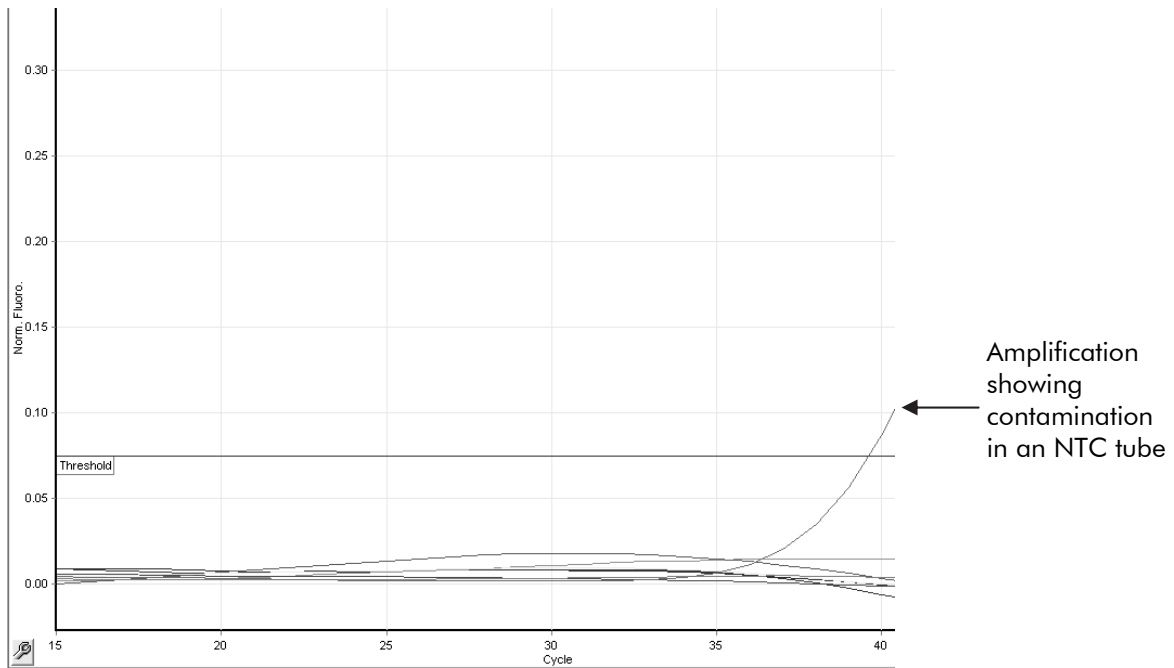
## Notes for data interpretation

### Linear amplification

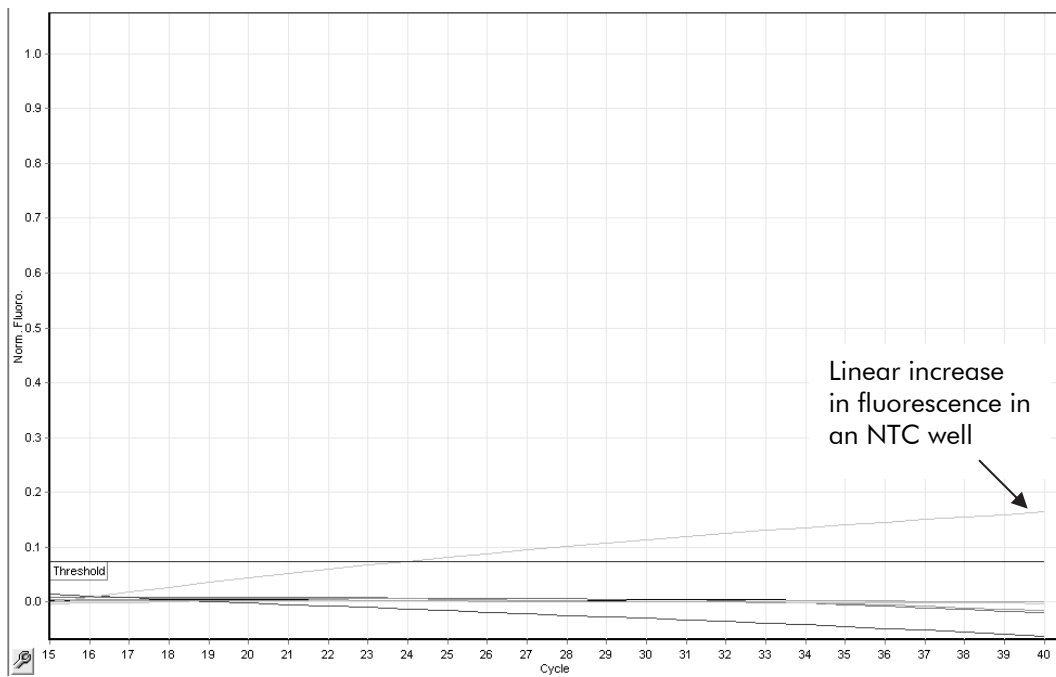
Rotor-Gene Q plots from all reactions should be checked. Occasionally, an increase in fluorescence signal is seen in the NTC and negative samples. If this is the case and a  $C_T$  value is obtained, the user is required to distinguish between a true amplification event, which would indicate contamination in the NTC, and a linear increase in fluorescence, which may have arisen due to a fluorescence artifact.

### Analysis of the NTC

Figures 17–18 show two examples of the behavior of NTC samples. In Figure 17, nonlinear (true amplification) due to sample contamination is seen. This run should be discarded and the samples should be retested. In Figure 18, linear amplification in an NTC is seen. Under these circumstances, the raw fluorescence should be examined. The corresponding raw fluorescence plot is shown in Figure 19, indicating a linear increase in fluorescence rather than a true amplification event. The data from this run can be used, providing the positive and internal controls have passed. For comparison with Figure 19, Figure 20 shows raw fluorescence data where true amplification has taken place. Under these circumstances, the data should be discarded and the samples retested as this indicates that contamination is present.

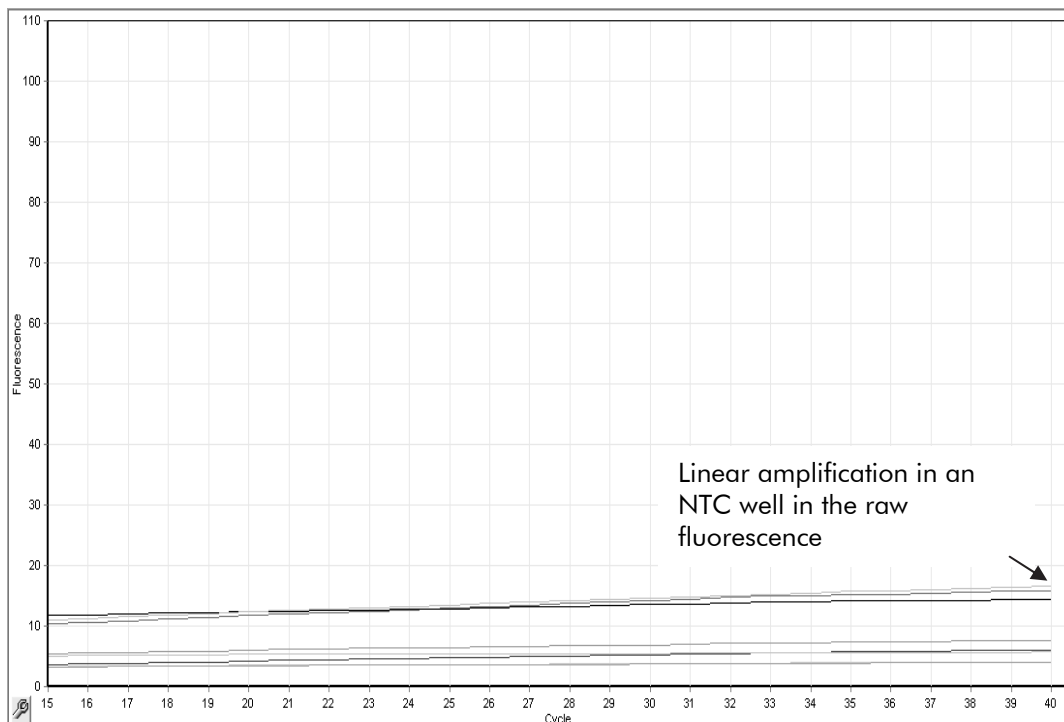


**Figure 17. Contamination in an NTC of an assay in an analyzed run.**

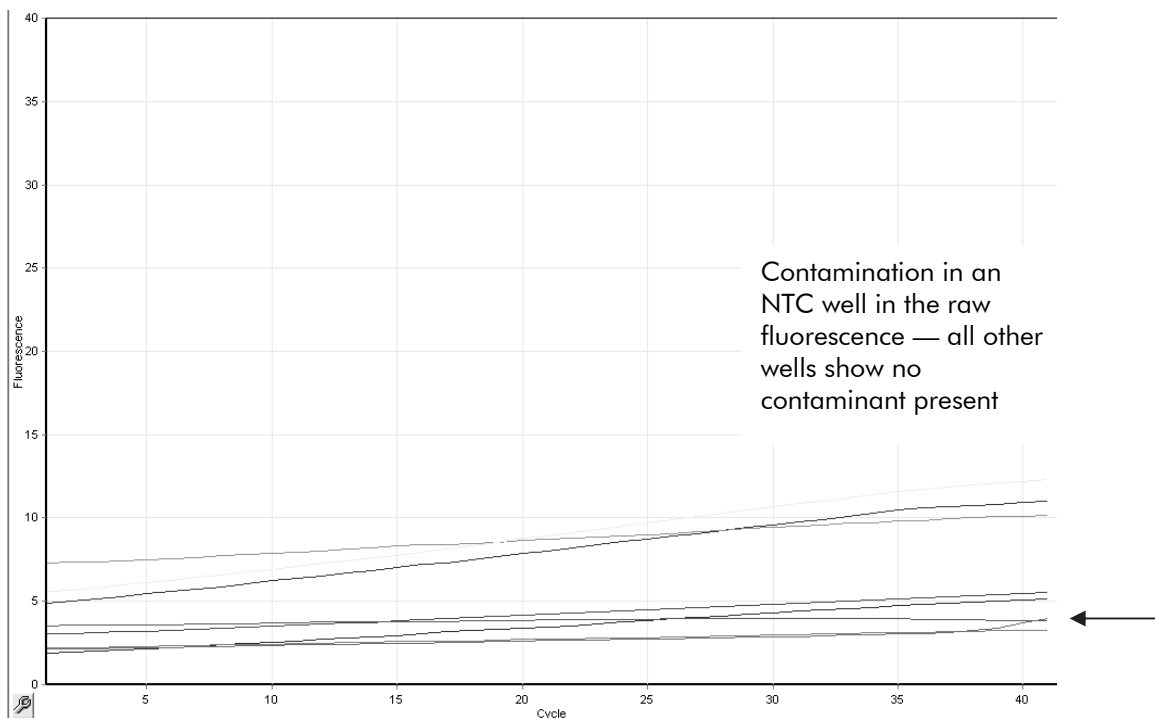


**Figure 18. Example of a linear increase in fluorescence in an NTC well.**





**Figure 19. Raw fluorescence of Figure 18.**

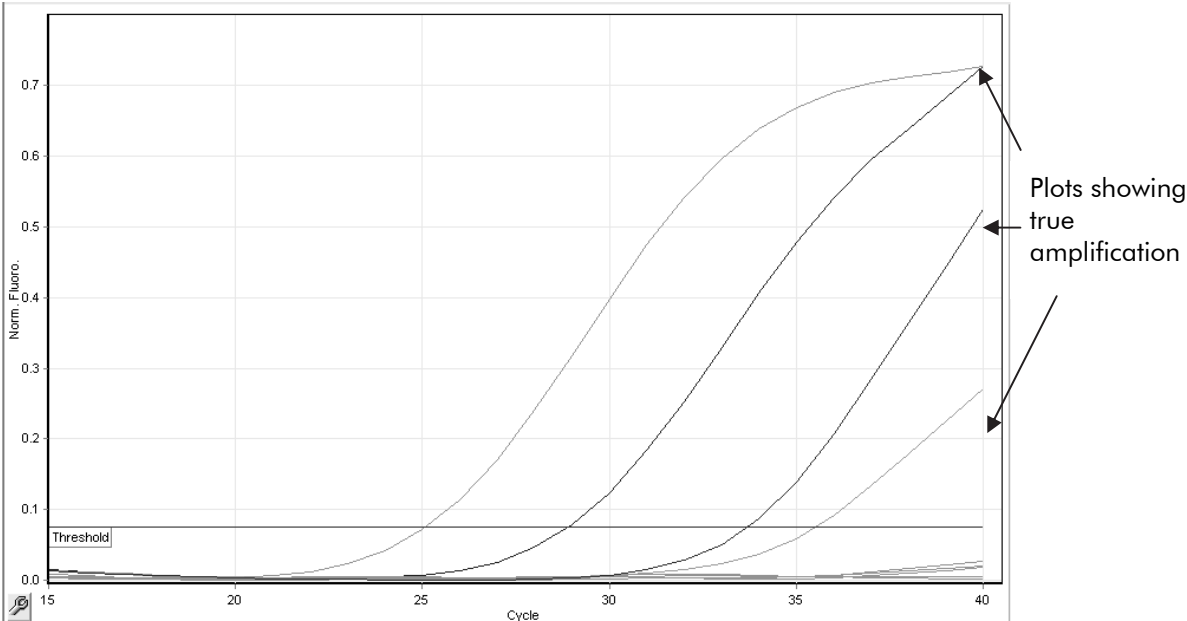


**Figure 20. Raw fluorescence data showing an NTC well with a true amplification event.**

### Analysis of samples

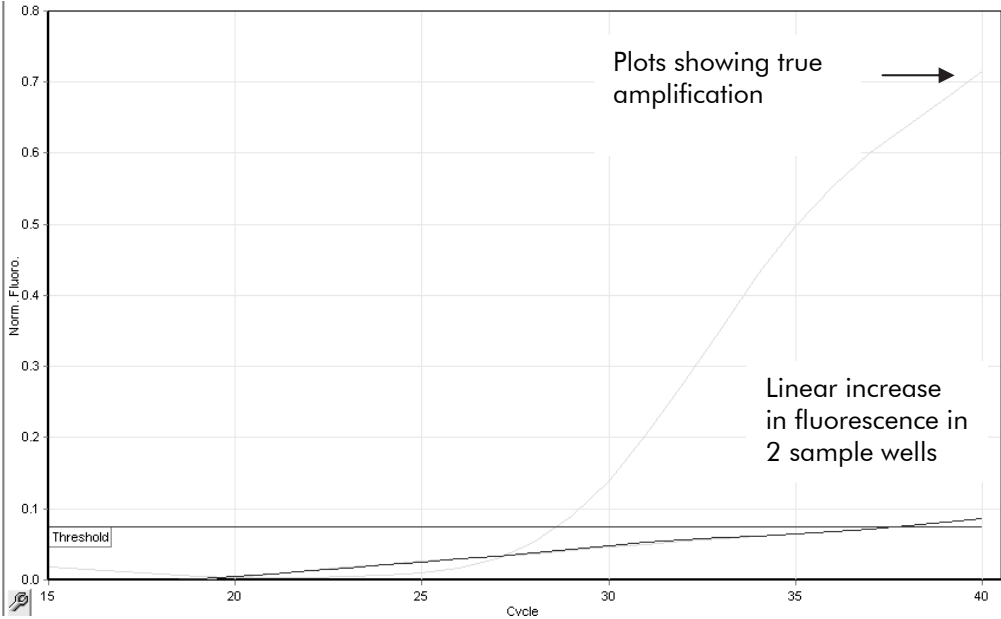
Figures 21–22 show two examples of amplification in sample reactions. An example of true amplification in a sample well in an analyzed run is seen in Figure 21. If a run shows this type of sigmoidal amplification curve, this is true

amplification and the data from this run can be used, providing the positive and internal controls have passed.

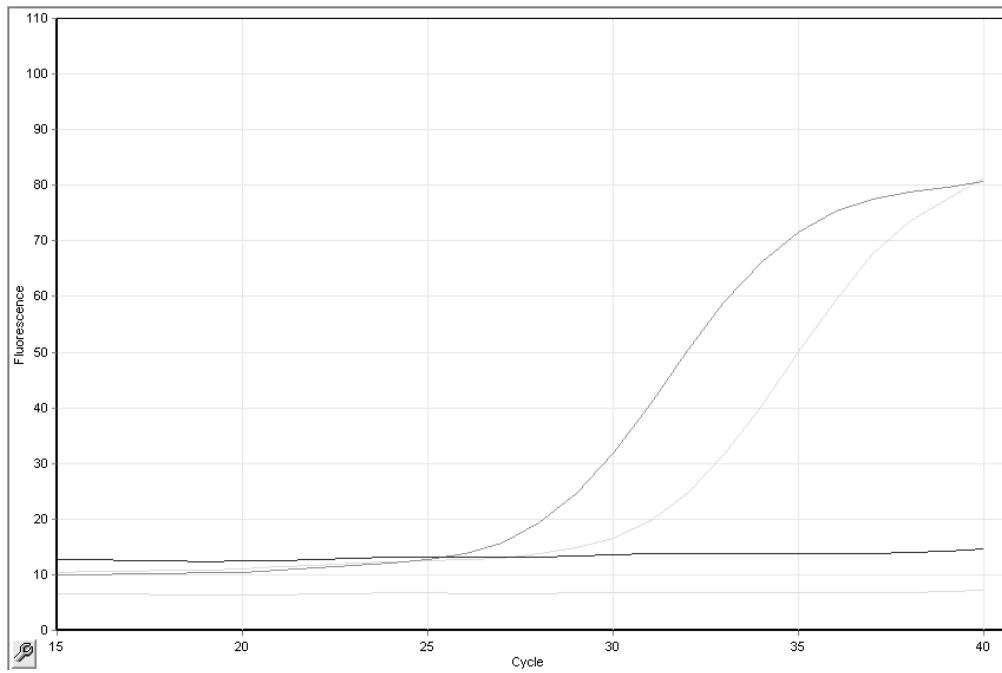


**Figure 21. True amplification in a sample well in an analyzed run.**

An example of linear amplification in a sample reaction is shown in Figure 22. Under these circumstances, the raw fluorescence data should be examined. The corresponding raw fluorescence plot (Figure 23) indicates that the linear increase observed in Figure 22 corresponds to a linear increase in the raw fluorescence and is not a true amplification. Providing the positive and internal control checks have passed, sample results can be used from these runs with caution, such that linear amplification is called as “no C<sub>T</sub>”.



**Figure 22. Example of a linear increase in fluorescence in two sample wells.**



**Figure 23. Raw fluorescence of Figure 22.**

## 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](http://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](http://www.qiagen.com)).

### Comments and suggestions

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#### **No signal with EGFR Positive Control (PC) in fluorescence channel Cycling Green**

- |                                                                                                                                                 |                                                                                                                                                                        |
|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) The selected fluorescence channel for PCR data analysis does not comply with the protocol                                                    | For data analysis, select the fluorescence channel Cycling Green for the analytical EGFR PCR and the fluorescence channel Cycling Yellow for the internal control PCR. |
| b) Incorrect programming of the temperature profile of the Rotor-Gene instrument                                                                | Compare the temperature profile with the protocol and if incorrect then repeat the run.                                                                                |
| c) Incorrect configuration of the PCR                                                                                                           | Check your work steps by means of the pipetting scheme and repeat the PCR, if necessary.                                                                               |
| d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 11) | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.                                              |
| e) The <i>therascreen</i> EGFR RGQ PCR Kit has expired                                                                                          | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.                                              |

## Comments and suggestions

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### Signals with the negative controls in fluorescence channel Cycling Green of the analytical PCR

- |                                                         |                                                                                                                                                                                                                               |
|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Contamination occurred during preparation of the PCR | Repeat the PCR with new reagents in replicates.<br>If possible, close the PCR tubes directly after addition of the sample to be tested.<br>Make sure that work space and instruments are decontaminated at regular intervals. |
| b) Contamination occurred during extraction             | Repeat the extraction and PCR of the sample to be tested using new reagents.<br>Make sure that work space and instruments are decontaminated at regular intervals.                                                            |

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *therascreen* EGFR RGQ PCR Kit is tested against predetermined specifications to ensure consistent product quality.

## Limitations

Results from the product must be interpreted within the context of all relevant clinical and laboratory findings and are not to be used alone for diagnosis.

The product is to be used only by personnel specially instructed and trained in in vitro diagnostics procedures and the Rotor-Gene Q.

Analytical validation studies included human DNA extracted from formalin-fixed paraffin-embedded tumor samples.

The product is intended for use only on the Rotor-Gene Q real-time PCR cycler, 5plex HRM series.

Strict compliance with the *therascreen* EGFR RGQ PCR Kit Handbook is required for optimal results. Dilution of the reagents, other than as described in this handbook, is not recommended and will result in a loss of performance.

It is important that the amount and quality of DNA in the sample is assessed prior to performing sample analysis using the *therascreen* EGFR RGQ PCR Kit. Additional Control Reaction Mix (Ctrl) is provided to determine that the  $C_T$  value is acceptable for the assay. Absorbance readings must not be used as they do not correlate with the  $C_T$  values in fragmented DNA samples.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

## Performance Characteristics

### Cut-offs

171 FFPE samples were tested using a method following guidance in NCCLS EP17-A (2004). Data from 159 samples were used in establishing cut-offs for the kit. The control reaction  $C_T$  range was established as 23.00 to 30.69  $C_T$ . The cut-off values were established and are shown in Table 8.

### Limit of detection (LOD)

To determine the LOD for the *therascreen* EGFR RGQ PCR Kit, a sample set was developed by mixing synthetic mutant DNA with wild-type genomic DNA to simulate a range of mutation percentages for each of the 29 mutations. The LOD of each assay is defined as the percent mutation at which 95% of replicates were determined positive by the *therascreen* EGFR PCR RGQ Kit. The LOD values are given in Table 9. For the multiplex assays, which detect multiple mutations (G719X, the deletions and the insertions), the value for the reaction that gave the highest LOD is given.

**Table 9. LODs for each of the seven EGFR mutation assays**

Mutation	Percentage mutation detectable (%)
T790M	7.02
Deletions	1.64
L858R	1.26
L861Q	0.50
G719X	5.43
S768I	1.37
Insertions	2.03

### Precision

To determine precision of the *therascreen* EGFR RGQ PCR Kit, a sample set was developed by mixing synthetic mutant DNA with wild-type genomic DNA to

simulate a low level of mutation percentage for each of the seven mutation assays. Precision was assessed by testing samples at one testing site, using multiple kit batches, operators and runs over different days, with two replicates of each sample. The variation seen, in terms of estimated standard deviation from Variance Component Analysis, was less than 1  $\Delta C_T$  and can be used as an estimate of precision (Table 10).

**Table 10. Results of within-laboratory tests\***

<b>Assay</b>	<b>Percentage testing mutation positive</b>	<b>Estimate of standard deviation (<math>\Delta C_T</math>)</b>
T790M	100%	0.33
Deletions	100%	0.40
L858R	100%	0.45
L861Q	100%	0.49
G719X	97.9%	0.59
S768I	97.9%	0.31
Insertions	97.9%	0.38

\* 93 replicates were tested for each mutation.

## Reproducibility

Reproducibility was assessed by testing high mutation level samples in a background of wild-type genomic DNA at three testing sites, using multiple kit batches, operators and runs over different days, with two replicates of each sample. For all seven mutation assays 96.1–100% of mutant DNA samples tested mutation-positive. Wild type samples tested mutation-negative in all assays at all sites.

## Effect of input DNA concentration

To determine the effect of changing input DNA concentration on the results produced by the *therascreen* EGFR RGQ PCR Kit close to the LOD, a sample set for all 29 mutations was developed by mixing synthetic mutant DNA with wild-type genomic DNA to produce samples at low, medium, and high total input DNA levels

The high and low levels of input DNA were targeted to represent the control assay  $C_T$  value range (23.50 to 29.50).

An assessment of the input DNA data set (29 mutations, at concentrations close to the LOD and at three different input DNA levels) revealed a 95.44% mutation positive rate.

These data indicate that varying the level of input DNA, within the working range of the assay, does not impact the  $\Delta C_T$  or mutation call of a sample.

## Interfering substances

The effect on kit performance of components that could potentially carry over from the QIAGEN® QIAamp DNA FFPE Tissue Kit during the processing of FFPE samples was assessed.

Formalin, paraffin wax, xylene, ethanol, buffer ATL, proteinase K, buffer AL, wash buffer AW1, and wash buffer AW2 were used at the highest (“worst case”) concentrations expected (assuming that each washing or purification step in the extraction kit protocol resulted in a reduction in the concentration component by 1 log).

The study used three times LOD samples rather than a much higher level of mutation to ensure that potential interference could be detected.

A difference in  $\Delta C_T$  of  $\geq 3$  standard deviations (taken from the precision study) between the “test” and the “control” (i.e., no interfering substance) was considered to indicate a potential interference.

None of the potential interfering substances evaluated had a  $\Delta C_T$  change of  $\geq 1$  standard deviation when compared to controls.

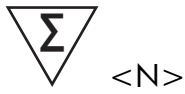
## References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

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## Symbols



Contains reagents sufficient for <N> tests



Use by



In vitro diagnostic medical device



Catalog number



Lot number



Material number



Components



Contains



Number



Temperature limitation



Manufacturer



Consult instructions for use

## Contact Information

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## Appendix: Mutation Details

COSMIC IDs are taken from the *Catalogue of Somatic Mutations in Cancer* ([www.sanger.ac.uk/genetics/CGP/cosmic](http://www.sanger.ac.uk/genetics/CGP/cosmic)).

**Table 11. List of mutations and COSMIC IDs.**

Mutation	Exon	Base change	COSMIC ID
<b>T790M</b>	20	2369C>T	6240
<b>L858R</b>	21	2573T>G	6224
<b>L861Q</b>	21	2582T>A	6213
<b>S768I</b>	20	2303G>T	6241
<b>G719A</b>	18	2156G>C	6239
<b>G719S</b>	18	2155G>A	6252
<b>G719C</b>	18	2155G>T	6253
<b>Insertions</b>	20	2307_2308ins9	12376
		2319_2320insCAC	12377
		2310_2311insGGT	12378
<b>Deletions</b>	19	2235_2249del15	6223
		2235_2252>AAT (complex)	13551
		2236_2253del18	12728
		2237_2251del15	12678
		2237_2254del18	12367
		2237_2255>T (complex)	12384
		2236_2250del15	6225
		2238_2255del18	6220
		2238_2248>GC (complex)	12422
		2238_2252>GCA (complex)	12419
		2239_2247del9	6218
2239_2253del15	6254		

**Table 11. List of mutations and COSMIC IDs (continued)**

<b>Mutation</b>	<b>Exon</b>	<b>Base change</b>	<b>COSMIC ID</b>
		2239_2256del18	6255
		2239_2248TTAAGAGAAG>C (complex)	12382
<b>Deletions</b>	19	2239_2258>CA (complex)	12387
		2240_2251del12	6210
		2240_2257del18	12370
		2240_2254del15	12369
		2239_2251>C (complex)	12383

## Ordering Information

Product	Contents	Cat. no.
<i>therascreen</i> EGFR RGQ PCR Kit (24)	For 24 reactions: 1 Control Assay, 7 Mutation Assays, Positive Control, Taq DNA Polymerase	870111
<b>Rotor-Gene Q and accessories</b>		
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002033
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002032
Rotor-Gene Q 5plex HRM System	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001650
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001580
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
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

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