

BRAF RGQ PCR Kit Handbook



Version 2

For research use only. Not for use in diagnostic procedures.

For use with the Rotor-Gene® Q 5plex HRM instrument

REF 870801



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Intended Use

The BRAF RGQ PCR Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Table 1. List of mutations and COSMIC IDs*

Mutation	Base change	COSMIC ID
V600E	GTG>GAG	476
V600E complex	GTG>GAA	475
V600D	GTG>GAT	473
V600K	GTG>AAG	474
V600R	GTG>AGG	477

* COSMIC IDs are taken from the *Catalog of Somatic Mutations in Cancer* (www.sanger.ac.uk/genetics/CGP/cosmic).

Summary and Explanation

The BRAF RGQ PCR Kit is a ready-to-use kit for the detection of five somatic mutations in the BRAF gene using real-time polymerase chain reaction on the Rotor-Gene Q 5plex HRM instrument.

Using ARMS® (Amplification Refractory Mutation System) and Scorpions® technologies, the BRAF RGQ PCR Kit enables detection of the following mutations at codon 600 of the BRAF oncogene against a background of wild-type genomic DNA.

- V600E
- V600E complex (V600Ec)
- V600D
- V600K
- V600R

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 BRAF 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. *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 fluorescently labeled probe. The fluorophore in this probe is associated 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 a measureable increase in fluorescence from the reaction tube.

Kit format

Five assays are supplied in the BRAF RGQ PCR Kit.

- One control assay (Control Reaction Mix; CTRL)
- Four mutation assays (mutant reaction mixes; V600E/Ec, V600D, V600K, V600R)

The V600E/Ec assay detects both V600E and V600Ec mutations but does not distinguish between them.

All reaction mixes are duplex and contain reagents to detect targets that are labeled with FAM™ and an internal control that is labeled with HEX™. The internal control assay controls for the presence of inhibitors that may lead to false negative results.

Procedure

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

Assays

Control assay

The control assay, labeled with FAM, is used to assess the total amplifiable BRAF DNA in a sample. The control assay amplifies a region of exon 3 of the BRAF gene. The primers and Scorpion probe have been designed to amplify independently of any known BRAF polymorphisms.

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 positive and negative controls.

Positive control

Each run must contain a positive control in tubes 1–5. The BRAF RGQ PCR Kit contains BRAF 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”) in tubes 9–13. The BRAF RGQ PCR Kit contains Water for NTC (NTC) to be used as the “template” for the no template control. The no template control is used to assess any potential contaminations 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 an inaccurate result or an operator setup error has occurred for that tube. If the internal control fails, see “Sample analysis — Sample internal control mutation assays HEX C_T value”, page 40.

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. A tube of Water for Sample Dilution (Dil.) is included with the kit.

Sample assessment

It is strongly recommended to use the Control Reaction Mix (CTRL) supplied with the BRAF RGQ PCR Kit to assess the total amplifiable BRAF DNA in a sample. The control assay amplifies a region of exon 3 of the BRAF gene. It is recommended to set up samples with only the control assay using the BRAF

Positive Control (PC) as a positive control and Water for NTC (NTC) 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 BRAF RGQ PCR Kit.

Materials Provided

Kit contents

BRAF RGQ PCR Kit			(24)
Catalog no.			870801
Number of reactions			24
Control Reaction Mix	Red	1 CTRL	2 x 720 μ l
V600E/Ec Reaction Mix	Purple	2 V600E/Ec	720 μ l
V600D Reaction Mix	Orange	3 V600D	720 μ l
V600K Reaction Mix	Pink	4 V600K	720 μ l
V600R Reaction Mix	Green	5 V600R	720 μ l
BRAF Positive Control	Beige	PC	250 μ l
Taq DNA Polymerase	Mint	Taq	138 μ l
Water for NTC	White	NTC	1.9 ml
Water for Sample Dilution	White	Dil.	1.9 ml
<i>BRAF RGQ PCR Kit Handbook</i>			1

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.

- QIAamp® DNA FFPE Tissue Kit (QIAGEN, cat. no. 56404; see "DNA isolation", page 12)
- 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])†

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

† This is not a complete list of suppliers.

- Dedicated pipets* (adjustable) for sample preparation
- Dedicated pipets* (adjustable) for PCR master mix preparation
- Dedicated pipets* (adjustable) for dispensing of template DNA
- Sterile 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 5plex HRM instrument* with fluorescence channels for Cycling Green and Cycling Yellow (detection of FAM and HEX, respectively)
- Rotor-Gene Q software version 2.2.3.11 or higher (available at www.qiagen.com/products/rotor-geneq.aspx)
- 0.1 ml Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or 981106)
- Sterile 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)

Warnings and Precautions

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.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

General precautions

The user should always pay attention to the following.

- Use sterile 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 at room temperature (15–25°C) for a minimum of 1 hour 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: Reagents for the BRAF 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 µl since this will increase the risk of false negatives.

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

Note: Only use 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: To obtain optimal use of the reagents in the BRAF RGQ PCR Kit, samples should be batched. If samples are assayed individually, this will use up more reagents and reduce the number of samples that can be assayed with the BRAF RGQ PCR Kit.

Note: To ensure correct sample testing and analysis, the user should take particular care when pipetting, placing the PCR strip tubes into the appropriate positions of the 72-well rotor, and entering sample names.

Reagent Storage and Handling

The BRAF RGQ PCR Kit is shipped on dry ice and must still be frozen on arrival. If the BRAF 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).

The BRAF RGQ PCR Kit should be stored immediately upon receipt at –15 to –30°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 thawing and freezing should be avoided. Do not exceed a maximum of 6 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.

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) tissue. 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 mutations detected by the BRAF RGQ PCR Kit.

Interfering substances

Melanin concentrations expected to be encountered in normal use do not influence the performance/sensitivity of the amplification system.

Procedure

DNA isolation

DNA isolation must be carried out using the QIAamp DNA FFPE Tissue Kit (QIAGEN, cat. no. 56404).

Note: The BRAF RGQ PCR Kit has been developed using the QIAamp DNA FFPE Tissue Kit with manual extraction technique. To ensure optimal performance, other extraction products and automated systems are not recommended.

Carry out the DNA purification according to the instructions in the *QIAamp DNA FFPE Tissue 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.
- Purified genomic DNA must be eluted in 120–200 μ l of Buffer ATE (provided in the QIAamp DNA FFPE Tissue Kit). Store purified genomic DNA at –15 to –30°C.

DNA assessment should be based on the Control Reaction Mix (CTRL) supplied with the BRAF RGQ PCR Kit 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 BRAF RGQ PCR Kit.

Note: To ensure sufficient DNA for analysis it is recommended that a minimum of two FFPE slides be co-extracted in the first instance and assessed with the control assay. If insufficient DNA is obtained for PCR, further slides may be extracted and the DNA pooled.

Note: To ensure sufficient DNA for analysis, FFPE sections must be a minimum of 5 μ m thick.

All assays in the BRAF RGQ PCR Kit generate short PCR products. However, the BRAF 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 “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.
- Pipet *Taq* DNA polymerase (*Taq*) by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.
- Up to 24 samples can be assessed using the Control Reaction Mix (CTRL) available.

Things to do before starting

- Before each use, all reagents need to be thawed for a minimum of 1 hour 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* DNA polymerase (*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 Control Reaction Mix (CTRL), Water for No Template Control (NTC), and the Positive Control (PC) at room temperature (15–25°C) for a minimum of 1 hour. 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 (Control Reaction Mix [CTRL] plus Taq DNA polymerase [Taq]) for the DNA samples, one positive control reaction, and one no template control reaction according to the volumes given in Table 2. 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 2. Preparation of control assay master mix*

Component	Volume
Control Reaction Mix (CTRL)	19.5 μ l x (n+1)*
Taq DNA polymerase (Taq)	0.5 μ l x (n+1)*
Total volume	20.0 μl/reaction

* n=number of reactions (samples plus controls). When preparing the master mix, prepare enough for 1 extra sample (n+1) to allow for sufficient overage for the PCR setup. The value n should not exceed 24.

3. Thoroughly mix the master mix by pipetting up and down 10 times. Place the appropriate number of strip tubes in the loading block according to the layout in Figure 1. Immediately add 20 μ l master mix to each PCR strip tube (not provided).

Caps will remain in the plastic container until required. 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.

Assay									
Control	1 (PC)	9	17	25	–	–	–	–	–
Control	2 (NTC)	10	18	26	–	–	–	–	–
Control	3	11	19	–	–	–	–	–	–
Control	4	12	20	–	–	–	–	–	–
Control	5	13	21	–	–	–	–	–	–
Control	6	14	22	–	–	–	–	–	–
Control	7	15	23	–	–	–	–	–	–
Control	8	16	24	–	–	–	–	–	–

Figure 1. Layout of sample assessment assays in the loading block. Numbers denote positions in the loading block and indicate final rotor position.

4. Immediately add 5 μ l of Water for No Template Control (NTC) to the no template control tube (PCR tube number 2) and cap the tube. Add 5 μ l of each sample to the sample tubes (PCR tube numbers 3–26) and cap the tubes. Add 5 μ l of BRAF Positive Control (PC) to the positive control tube (PCR tube number 1) and cap the tube.

Mark the lids of the tubes to show the direction to load the tubes into the Rotor-Gene Q 5plex HRM instrument.

5. After all PCR tubes are capped, perform a visual check on sample tube fill levels to ensure sample has been added to all tubes.
6. Invert all PCR tubes (4 times) to mix samples and reaction mixes.
7. Place the PCR strip tubes into the appropriate positions in the 72-well rotor (Figure 1). If the rotor is not fully occupied, all empty positions on the rotor must be filled with a capped, empty tube.
8. 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.
9. Open the appropriate Rotor-Gene Q series software (2.2.3.11 or higher) and open the appropriate temperature profile.

For instructions on how to create the temperature profile and check the run parameters, see “Protocol: Rotor-Gene Q BRAF setup”, page 26.

10. Ensure that the correct rotor is selected, and check the box to confirm that the locking ring is attached. Click “Next” (Figure 2).

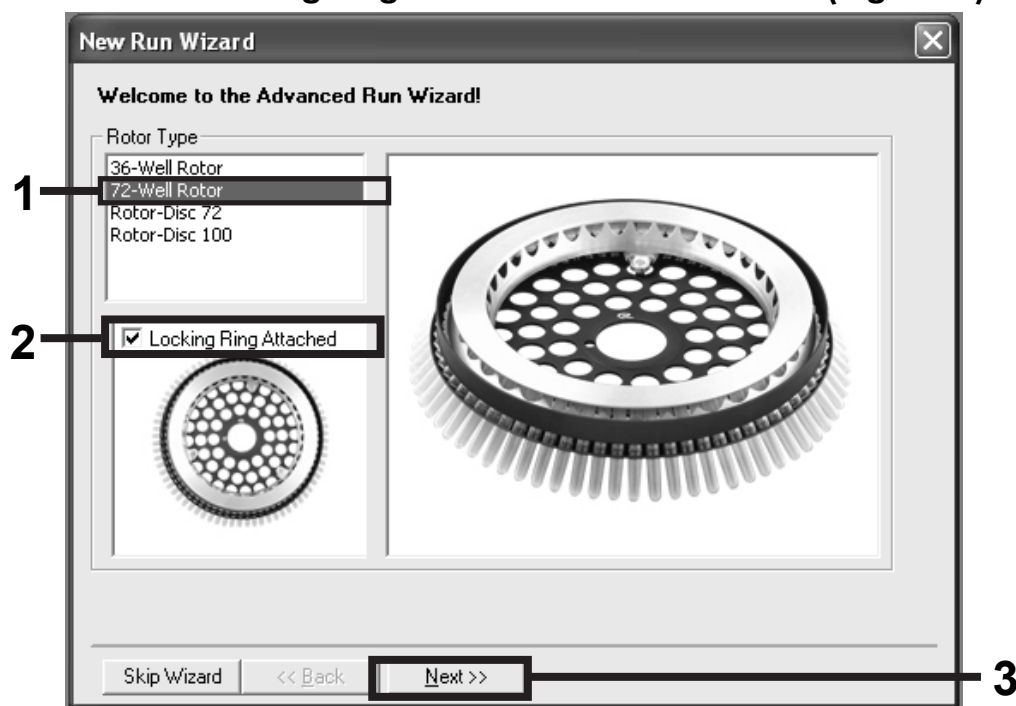


Figure 2. The “New Run Wizard” dialog box.

11. Enter the name of the operator. Add any notes and check that the reaction volume is set to 25 and the "Sample Layout" box reads "1, 2, 3...". Click "Next" (Figure 3).

New Run Wizard

This screen displays miscellaneous options for the run. Complete the fields, clicking Next when you are ready to move to the next page.

Operator :

Notes :

Reaction Volume (µL):

Sample Layout :

This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.

Figure 3. The "New Run Wizard" dialog box.

12. The next window allows editing of the temperature profile. No editing is required if the temperature profile has already been created according to the instructions in "Protocol: Rotor-Gene Q BRAF setup", page 26. Click "Next" (Figure 4).

New Run Wizard

Temperature Profile :

Channel Setup :

Name	Source	Detector	Gain
Green	470nm	510nm	5
Yellow	530nm	555nm	5
Orange	585nm	610nm	5
Red	625nm	660nm	5
Crimson	680nm	710hp	7
HRM	460nm	510nm	7

This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.

Figure 4. The "New Run Wizard" dialog box.

13. Check the summary and click “Start Run” to save the run file and start the run (Figure 5).

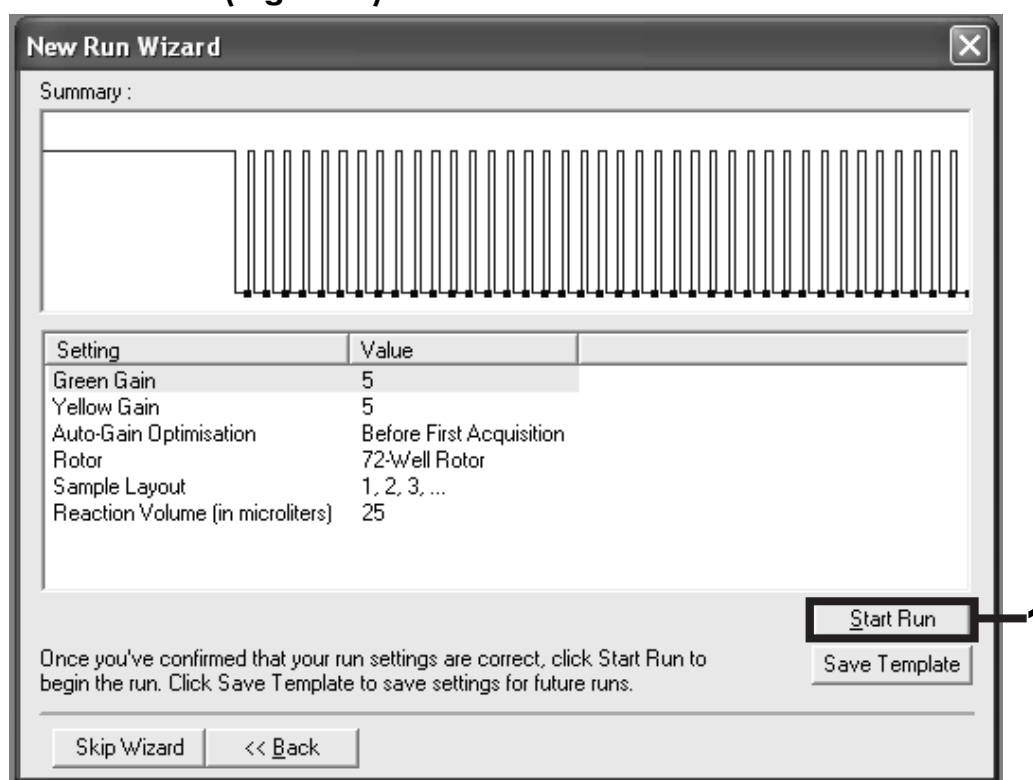


Figure 5. The “New Run Wizard” dialog box.

14. 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.

Clicking “Finish and Lock Samples” will prevent you from editing the sample names. The user should take particular care when entering sample names to ensure correct sample testing and analysis.

15. After the run is complete, analyze the data according to the “Sample assessment data analysis”, page 35.
16. If quantitation reports are required, click the “Reports” icon of the toolbar in the Rotor-Gene Q run file.

17. In the report browser, click “Cycling A Green (page 1)” under “Report Categories” (Figure 6).

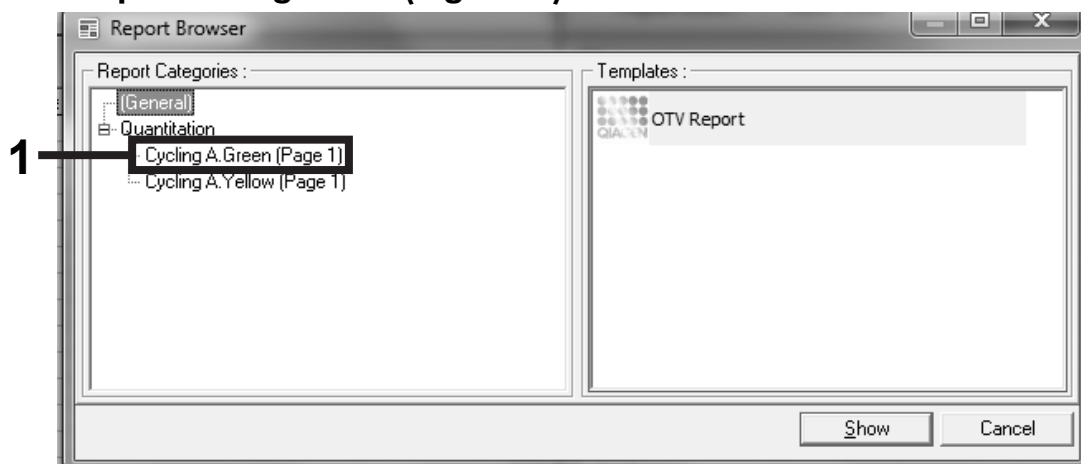


Figure 6. Report browser.

18. Select “Quantitation (Full Report)” under “Templates” (Figure 7).

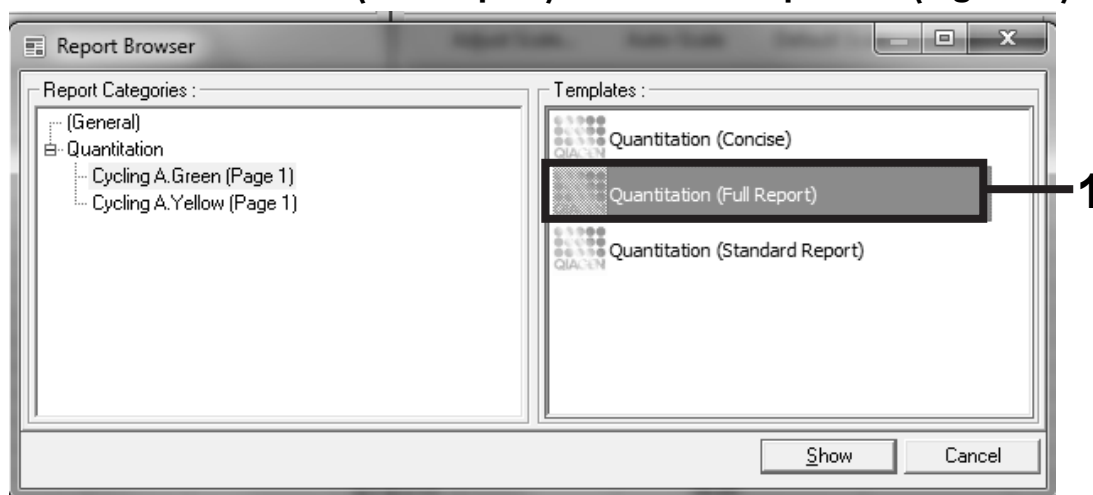


Figure 7. Quantitation report (full report).

19. Click “Show” to generate the report.
20. Click “Save As” to save an electronic version.
21. Repeat for “Cycling A Yellow (Page1)”.

Protocol: BRAF mutation detection

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

Important points before starting

- Before beginning the procedure read “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 BRAF RGQ PCR Kit, samples must be grouped into batches of no less than 6. Smaller batch sizes will mean that fewer samples can be tested with the BRAF RGQ PCR Kit.
- 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 for a minimum of 1 hour 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* DNA polymerase (*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, Water for No Template Control (NTC), and BRAF Positive Control (PC) at room temperature (15–25°C) for a minimum of 1 hour. 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 (reaction mix plus *Taq* DNA polymerase [*Taq*]) for the DNA samples, one positive control reaction, and one no template control reaction according to the volumes given in Table 3. Include reagents for 1 extra sample to allow sufficient overage for the PCR setup.**

The master mixes contain all of the components needed for PCR except the sample.

Table 3. Preparation of assay master mixes*

Assay	Volume of reaction mix	Volume of <i>Taq</i> DNA polymerase (<i>Taq</i>)
Control	19.5 μ l x (n+1)	0.5 μ l x (n+1)
V600E/Ec	19.5 μ l x (n+1)	0.5 μ l x (n+1)
V600D	19.5 μ l x (n+1)	0.5 μ l x (n+1)
V600K	19.5 μ l x (n+1)	0.5 μ l x (n+1)
V600R	19.5 μ l x (n+1)	0.5 μ l x (n+1)

* n=number of reactions (samples plus controls). When preparing the master mix, prepare enough for 1 extra sample (n+1) to allow for sufficient overage for the PCR setup.

3. Thoroughly mix the master mix by pipetting up and down 10 times. Place the appropriate number of strip tubes in the loading block according to the layout in Figure 8. Immediately add 20 μ l master mix to each PCR strip tube (not provided).

Caps will remain in the plastic container until required.

Assay	Controls		Sample number						
	PC	NTC	1	2	3	4	5	6	7
Control	1	9	17	25	33	41	49	57	65
V600E/Ec	2	10	18	26	34	42	50	58	66
V600D	3	11	19	27	35	43	51	59	67
V600K	4	12	20	28	36	44	52	60	68
V600R	5	13	21	29	37	45	53	61	69
–	6	14	22	30	38	46	54	62	70
–	7	15	23	31	39	47	55	63	71
–	8	16	24	32	40	48	56	64	72

Figure 8. Layout of control and mutation assays in the loading block. Numbers denote position in the loading block and indicate final rotor position.

4. Immediately add 5 μ l of Water for No Template Control (NTC) to the no template control PCR strip tubes (PCR tube numbers 9–13) and cap the tubes. Add 5 μ l of each sample to the sample tubes (PCR tube numbers 17–21, 25–29, 33–37, 41–45, 49–53, 57–61, and 65–69) and cap the tubes. Add 5 μ l of BRAF Positive Control (PC) to the positive control tubes (PCR tube numbers 1–5) and cap the tubes. Each DNA sample must be tested with both the control and all of the mutation assays.

Mark the lids of the tubes to show the direction to load the tubes into the Rotor-Gene Q 5plex HRM instrument.

5. After all PCR tubes are capped, perform a visual check on sample tube fill levels to ensure sample has been added to all tubes.
6. Invert all PCR tubes (4 times) to mix samples and reaction mixes.
7. Place the PCR strip tubes into the appropriate positions in the 72-well rotor (Figure 8).

A maximum of 7 samples can be included in each PCR run. If the rotor is not fully occupied, all empty positions on the rotor must be filled with a capped, empty tube.

8. 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.

9. Open the appropriate Rotor-Gene Q series software (2.2.3.11 or higher) and open the appropriate temperature profile.

For instructions on how to create the temperature profile and check the run parameters, see “Protocol: Rotor-Gene Q BRAF setup”, page 26.

10. Ensure that the correct rotor is selected, and check the box to confirm that the locking ring is attached. Click “Next” (Figure 9).

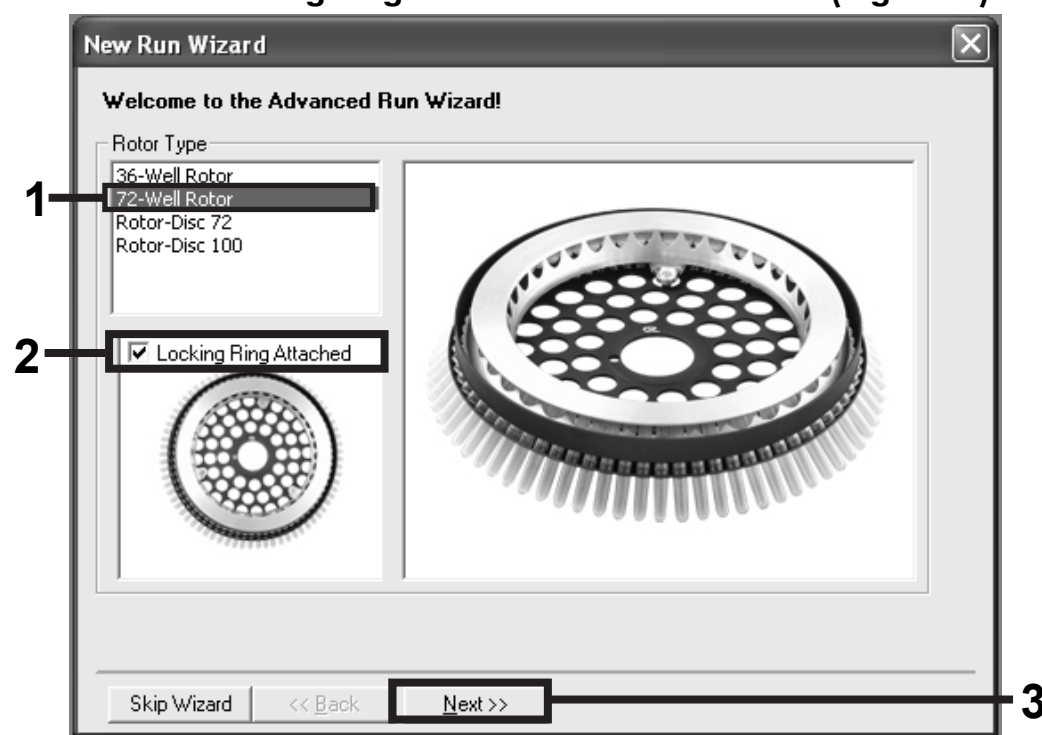


Figure 9. The “New Run Wizard” dialog box.

11. Enter the name of the operator. Add any notes, check that the reaction volume is set to 25 and "Sample Layout" reads "1, 2, 3...". Click "Next" (Figure 10).

New Run Wizard

This screen displays miscellaneous options for the run. Complete the fields, clicking Next when you are ready to move to the next page.

Operator : NAME

Notes :

Reaction Volume (µL): 25

Sample Layout : 1, 2, 3...

Skip Wizard << Back **Next >>**

This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.

Figure 10. The "New Run Wizard" dialog box.

12. The next window allows editing of the temperature profile. No editing is required if the temperature profile has already been created according to the instructions in "Protocol: Rotor-Gene Q BRAF setup", page 26. Click "Next" (Figure 11).

New Run Wizard

Temperature Profile :

Edit Profile ...

Channel Setup :

Name	Source	Detector	Gain	
Green	470nm	510nm	5	
Yellow	530nm	555nm	5	
Orange	585nm	610nm	5	
Red	625nm	660nm	5	
Crimson	680nm	710hp	7	
HRM	460nm	510nm	7	

Gain Optimisation...

Create New... Edit... Edit Gain... Remove Reset Defaults

Skip Wizard << Back **Next >>**

This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.

Figure 11. The "New Run Wizard" dialog box.

13. Check the summary and click “Start Run” to save the run file and start the run (Figure 12).

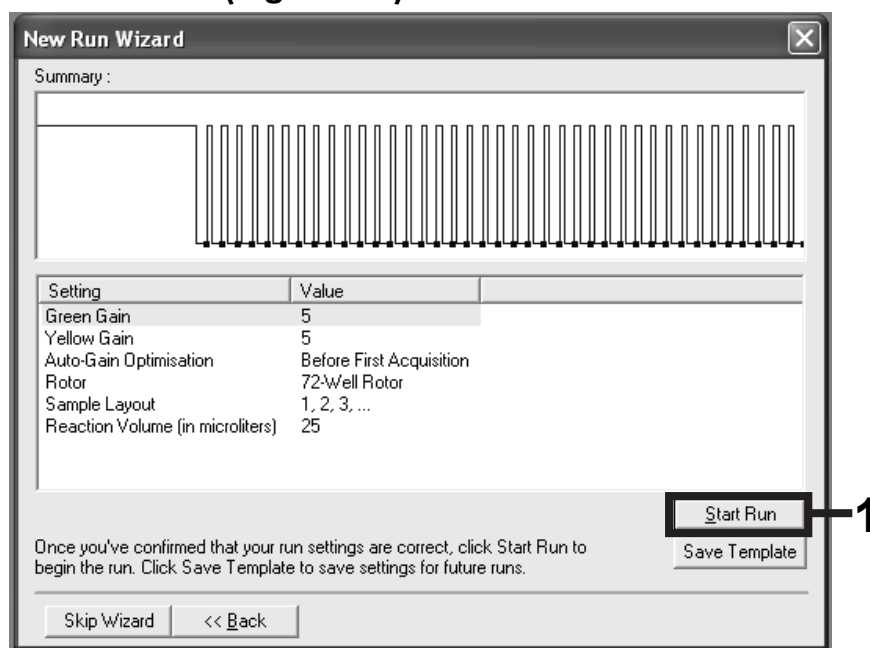


Figure 12. The “New Run Wizard” dialog box.

14. 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.

Clicking “Finish and Lock Samples” will prevent you from editing the sample names. The user should take particular care when entering sample names to ensure correct sample testing and analysis.

15. After the run is complete, analyze the data according to the procedure “BRAF mutation detection data analysis”, page 36.
16. If quantitation reports are required, click the “Reports” icon of the toolbar in the Rotor-Gene Q run file.

17. In the report browser, click “Cycling A Green (page 1)” under “Report Categories” (Figure 13).

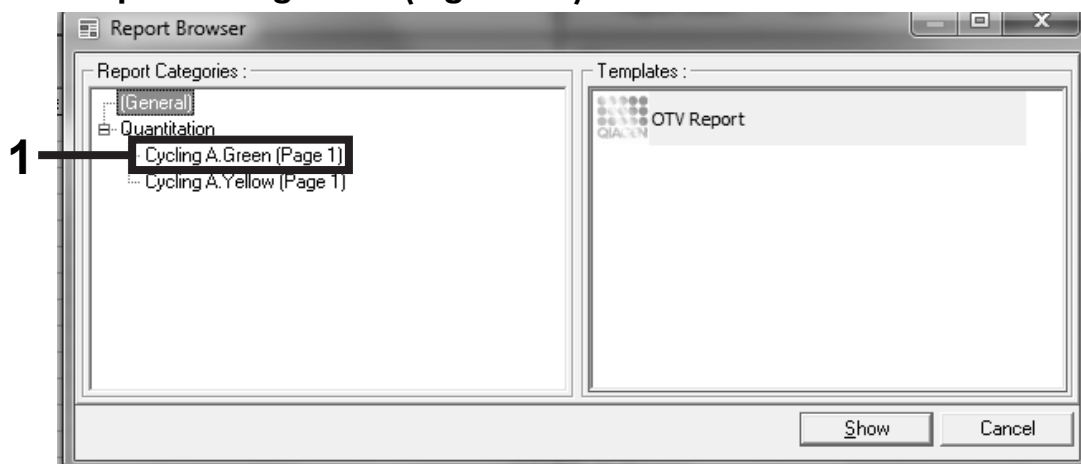


Figure 13. Report browser.

18. Select “Quantitation (Full Report)” under “Templates” (Figure 14).

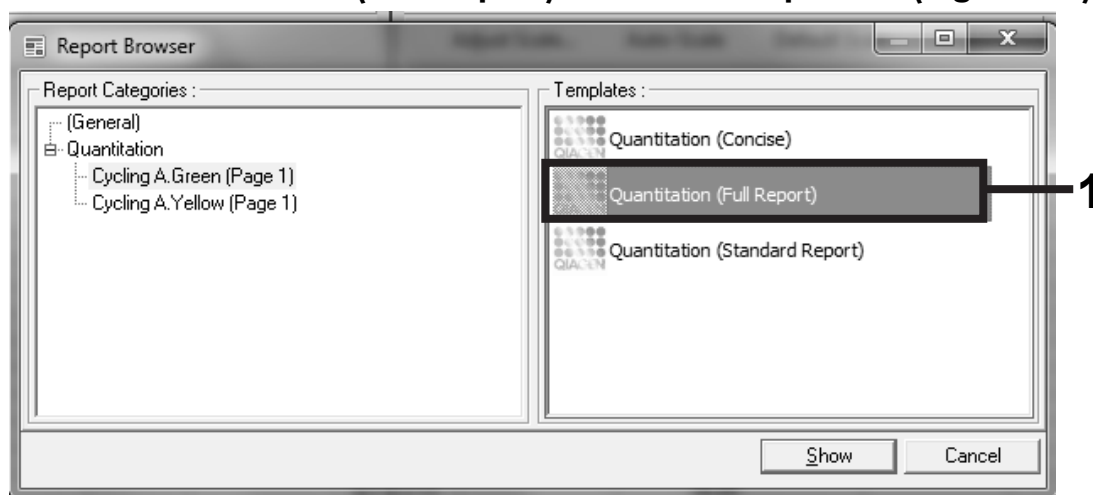


Figure 14. Quantitation report (full report).

19. Click “Show” to generate the report.
20. Click “Save As” to save an electronic version.
21. Repeat for “Cycling A Yellow (Page1)”.

Protocol: Rotor-Gene Q BRAF setup

This protocol is referenced within “Protocol: Sample assessment” (page 13), and “Protocol: BRAF mutation detection” (page 19).

Procedure

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

Setting the general assay parameters	Figures 15–17
Initial activation of the hot-start enzyme	Figures 18–19
Amplification of the DNA	Figures 20–21
Adjusting the fluorescence channels	Figures 22–26

To summarize, the cycling parameters are as follows.

Table 4. 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 2.2.3.11. In the illustrations, these settings are framed in bold black.

2. **Double-click the Rotor-Gene Q Series Software 2.2.3.11 software icon on the desktop of the laptop 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 15).

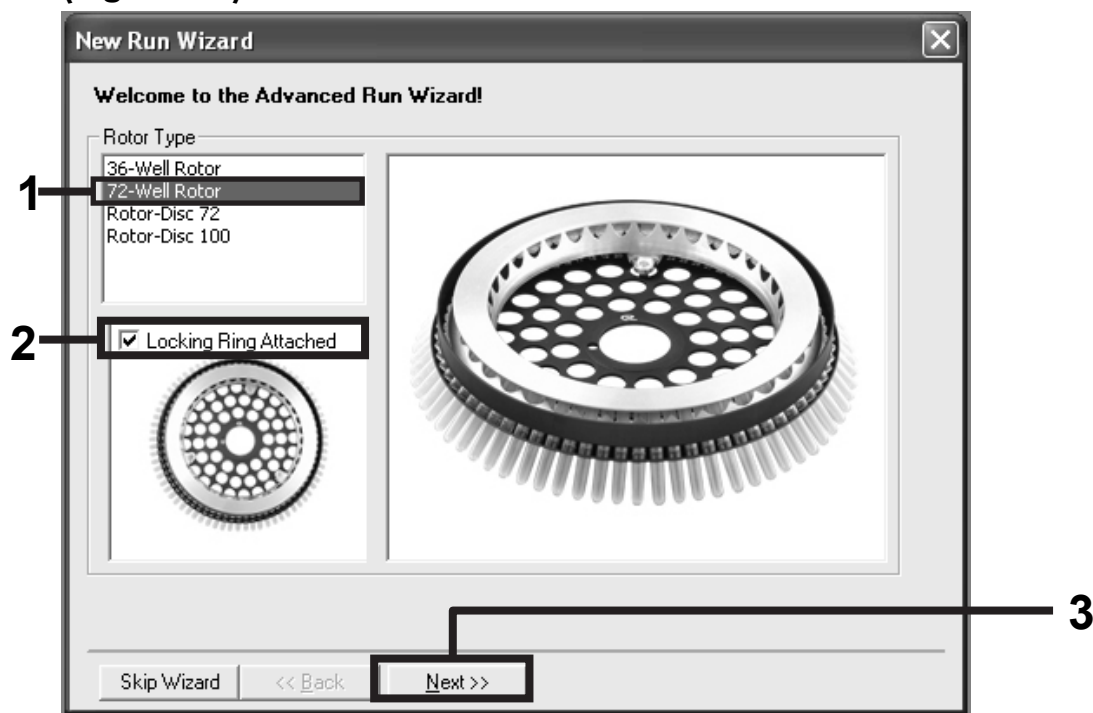


Figure 15. The “New Run Wizard” dialog box.

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 16).

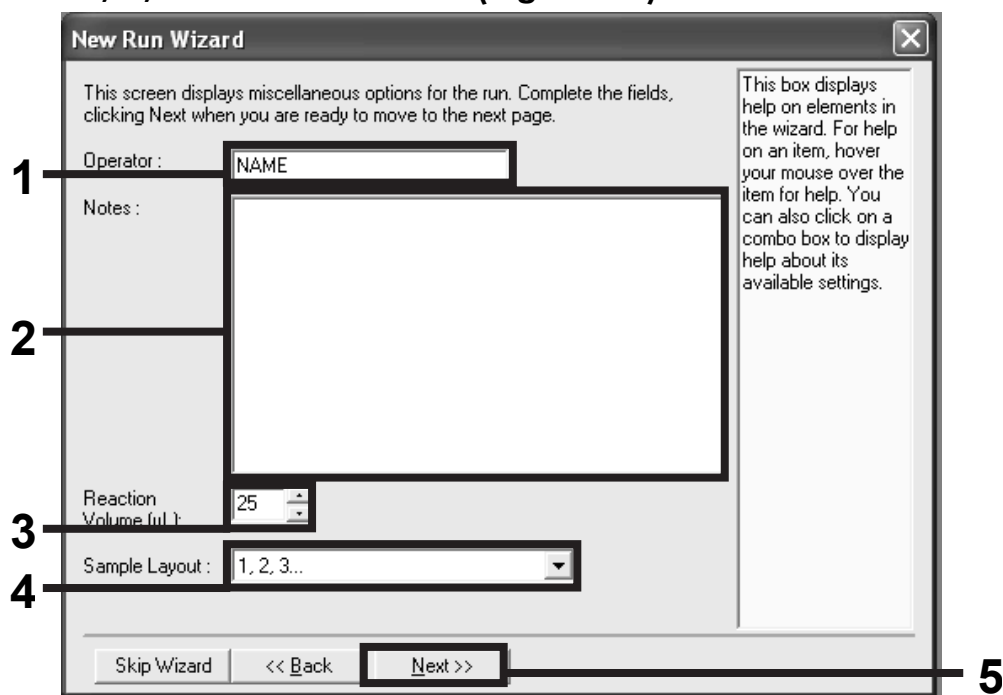


Figure 16. Entering operator name and reaction volumes.

- Click the “Edit Profile” button in the “New Run Wizard” dialog box (Figure 17), and program the run parameters according to the following steps.

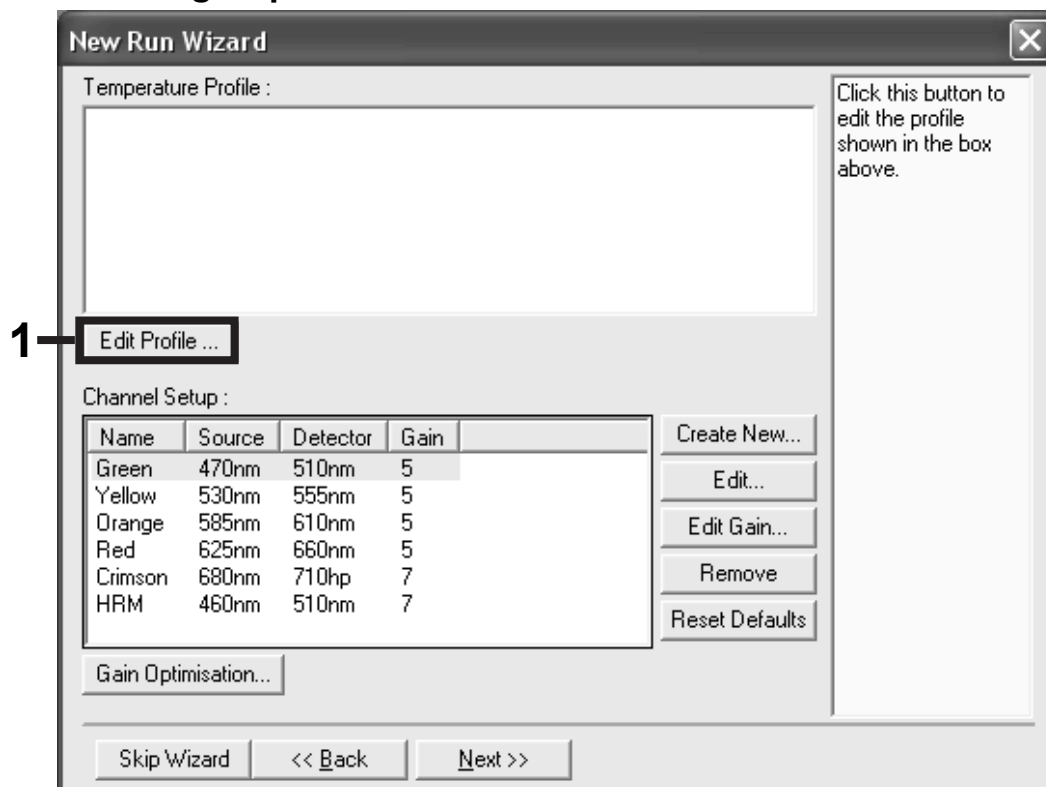


Figure 17. Editing the profile.

- Click the “Insert after” button and select *New Hold at Temperature* (Figure 18).

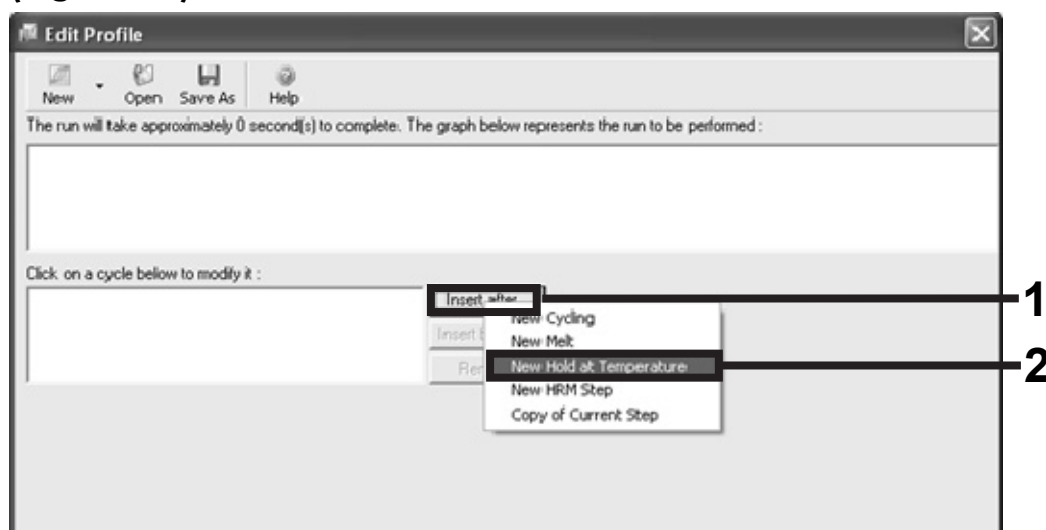


Figure 18. Inserting an initial incubation step.

8. Change "Hold Temperature" to 95°C and "Hold Time" to 15 mins 0 secs. Click the "Insert After" button and then select New Cycling (Figure 19).

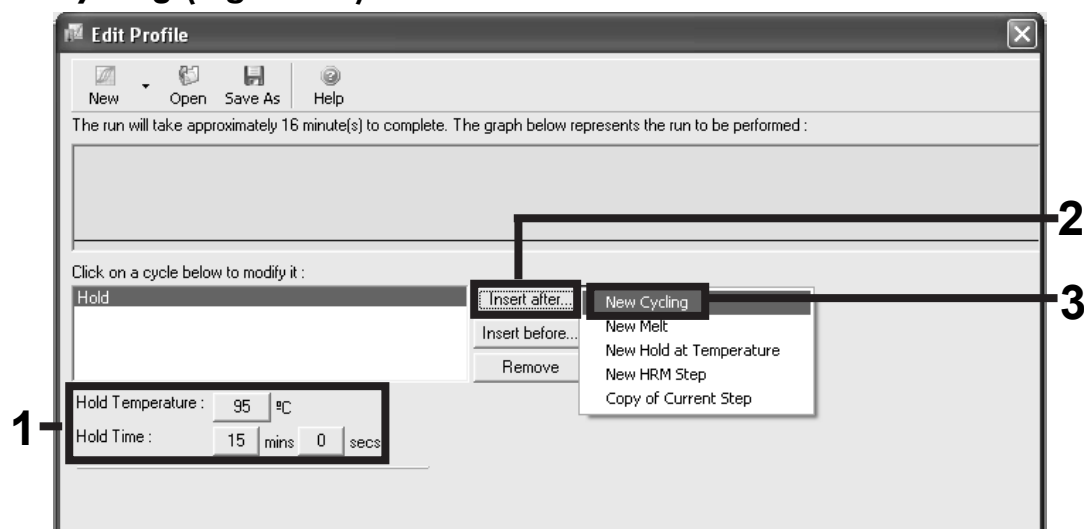


Figure 19. Initial incubation step at 95°C.

9. Change the number of cycle repeats to 40. Select the first step and set to 95°C for 30 secs (Figure 20).

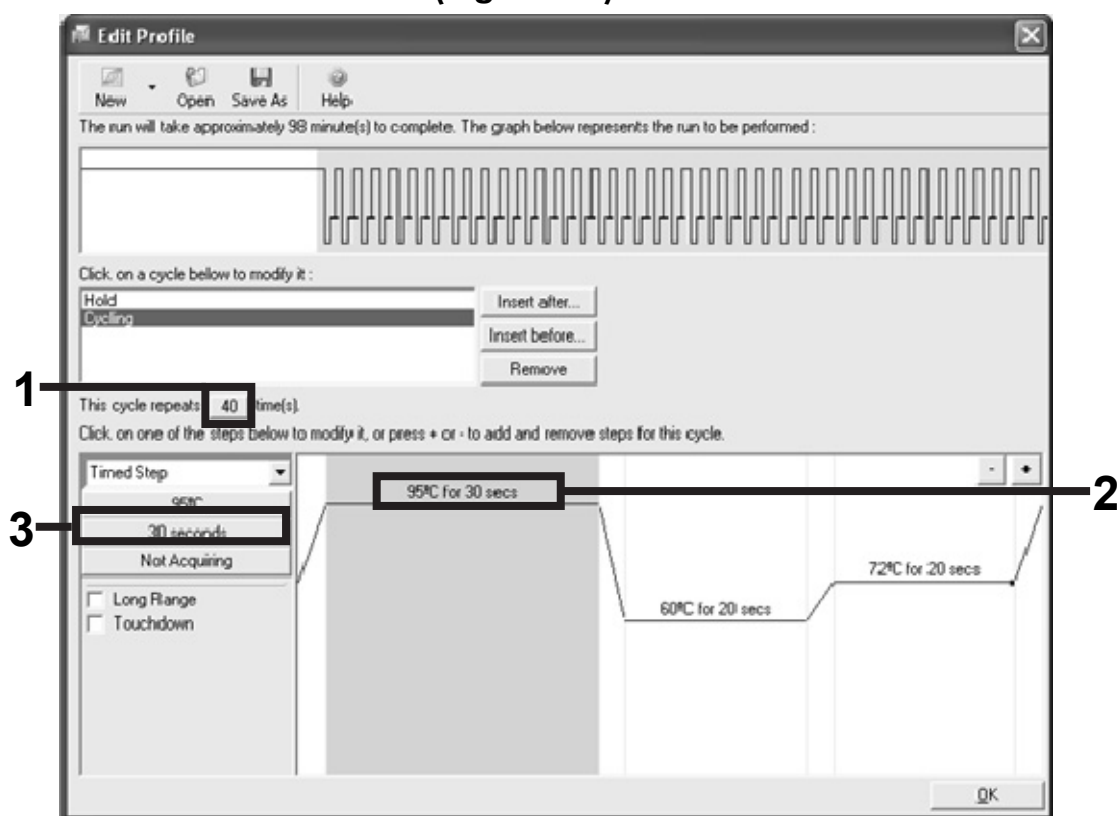


Figure 20. Cycling step at 95°C.

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 21).

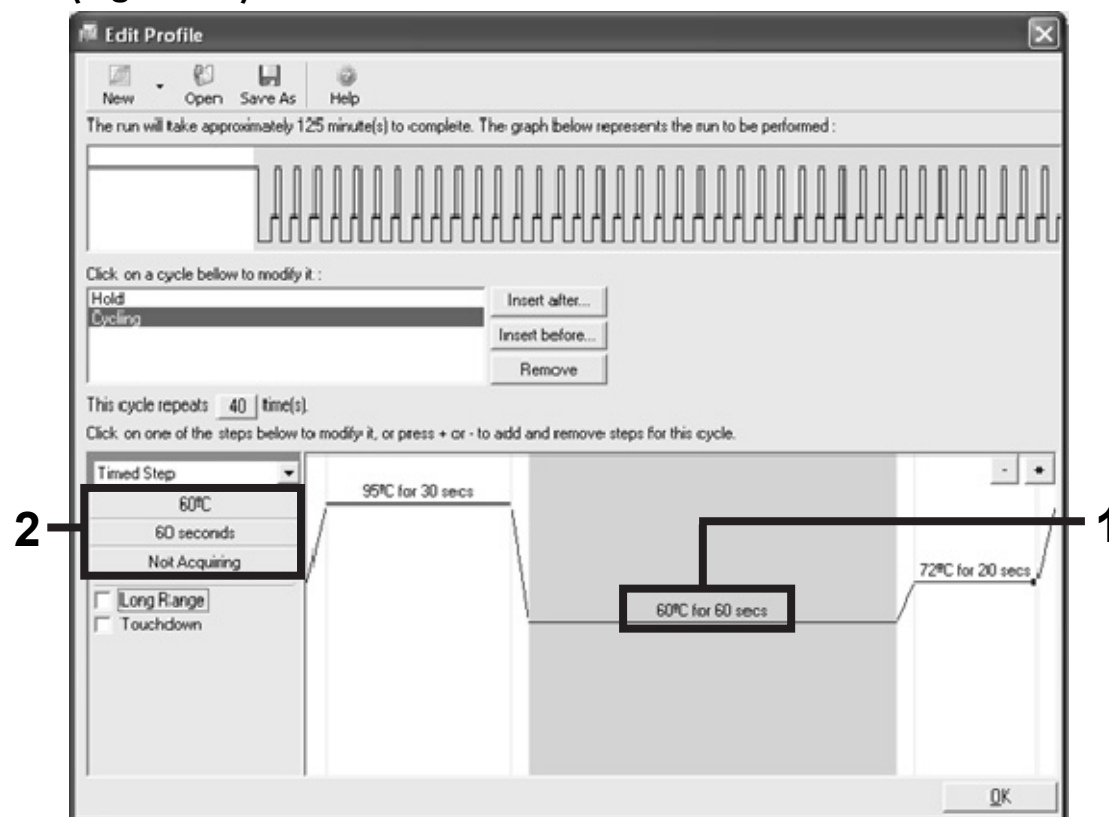


Figure 21. Cycling step at 60°C.

11. Set **Green** and **Yellow** as acquiring channels by selecting the “>” button to transfer these from the “Available Channels” list. Click “OK” (Figure 22).

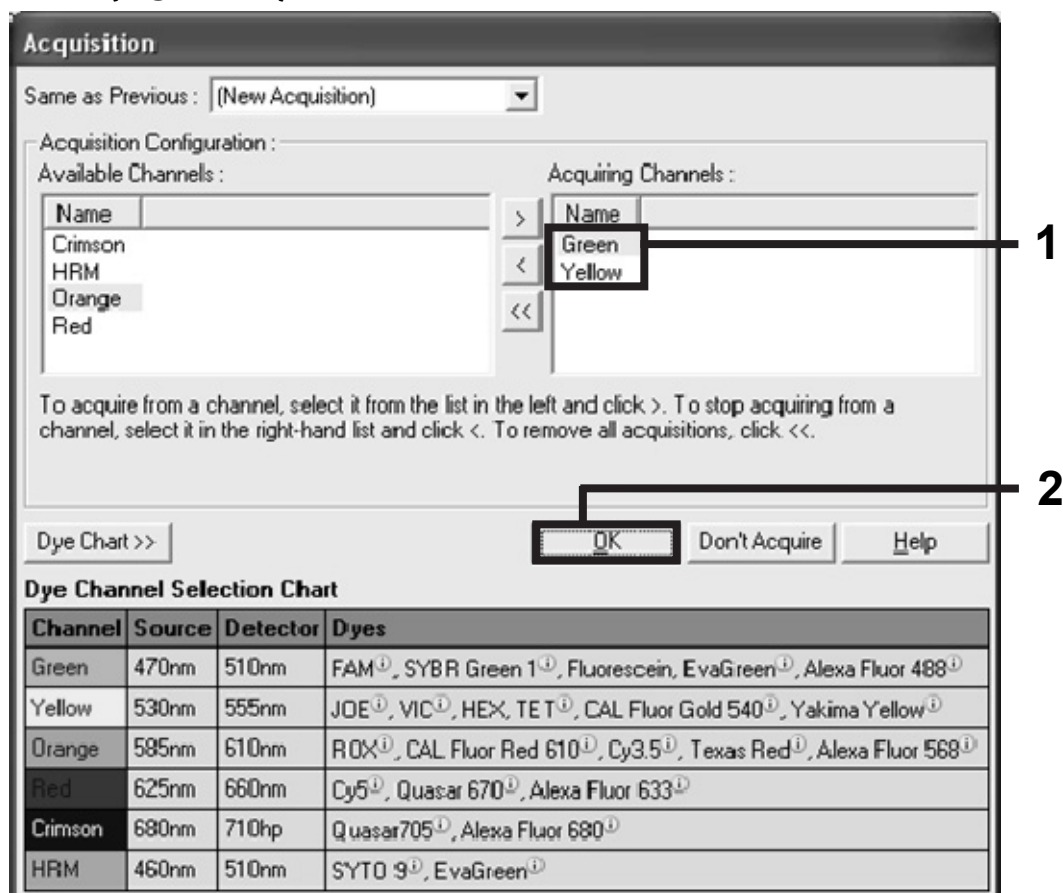


Figure 22. Acquiring at cycling step of 60°C.

12. Highlight the third step and delete by clicking the “-” button. Click “OK” (Figure 23).

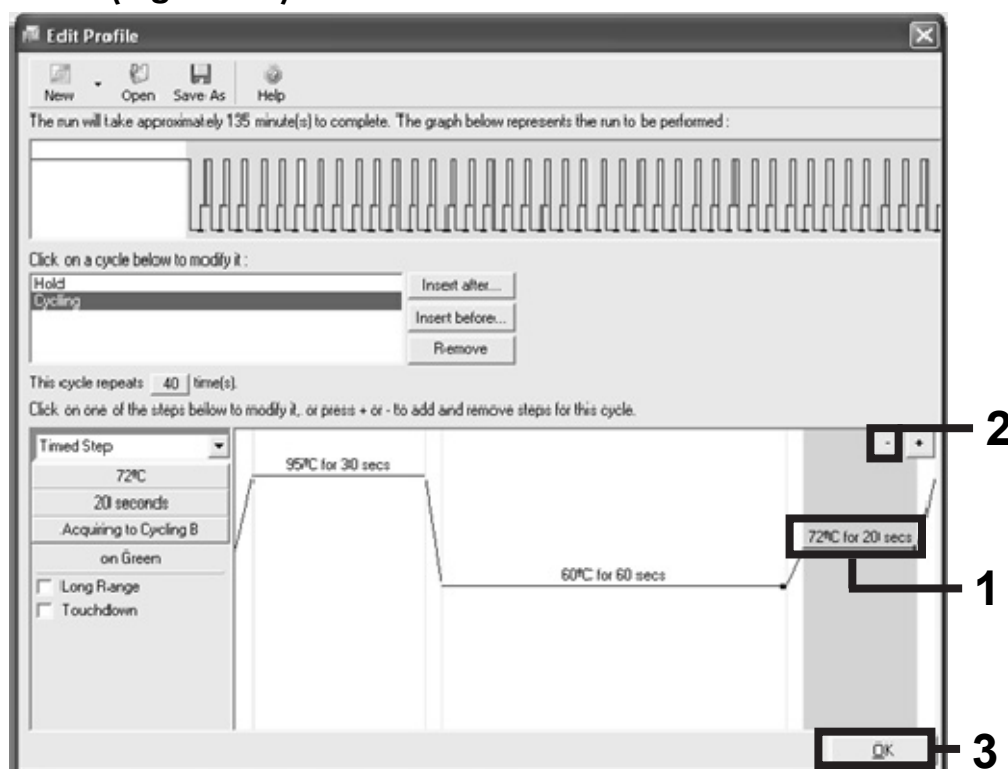


Figure 23. Removal of the extension step.

13. In the next dialog box, click the “Gain Optimisation” button (Figure 24).

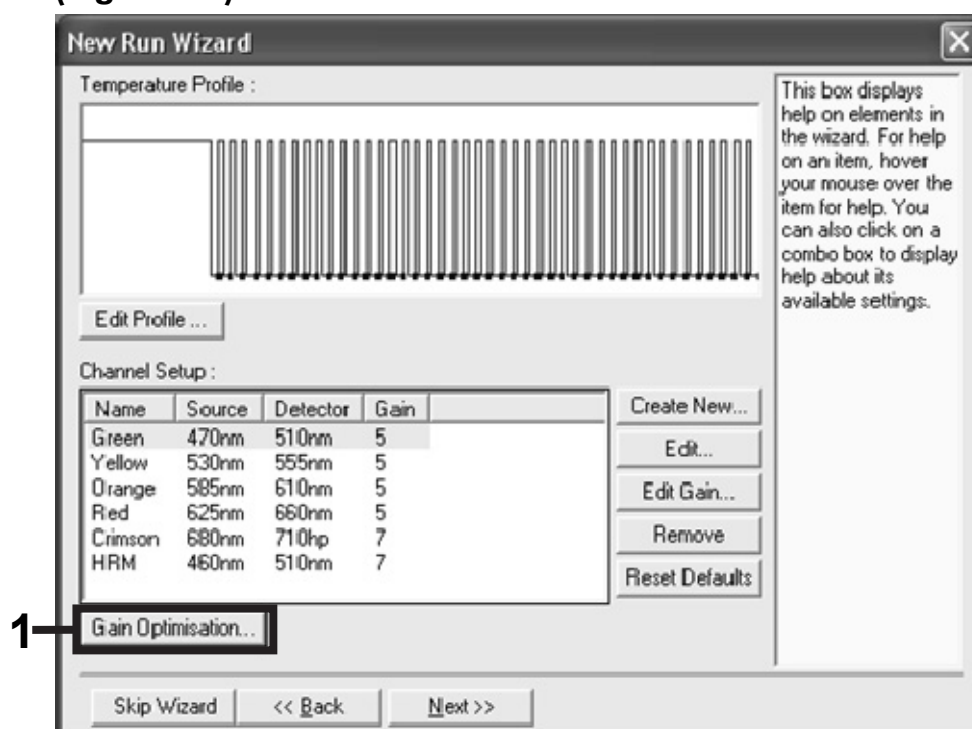


Figure 24. Gain optimization.

14. Click the “Optimise Acquiring” button. Channel settings are displayed for each channel. Accept these default values by clicking “OK” for both channels (Figure 25).

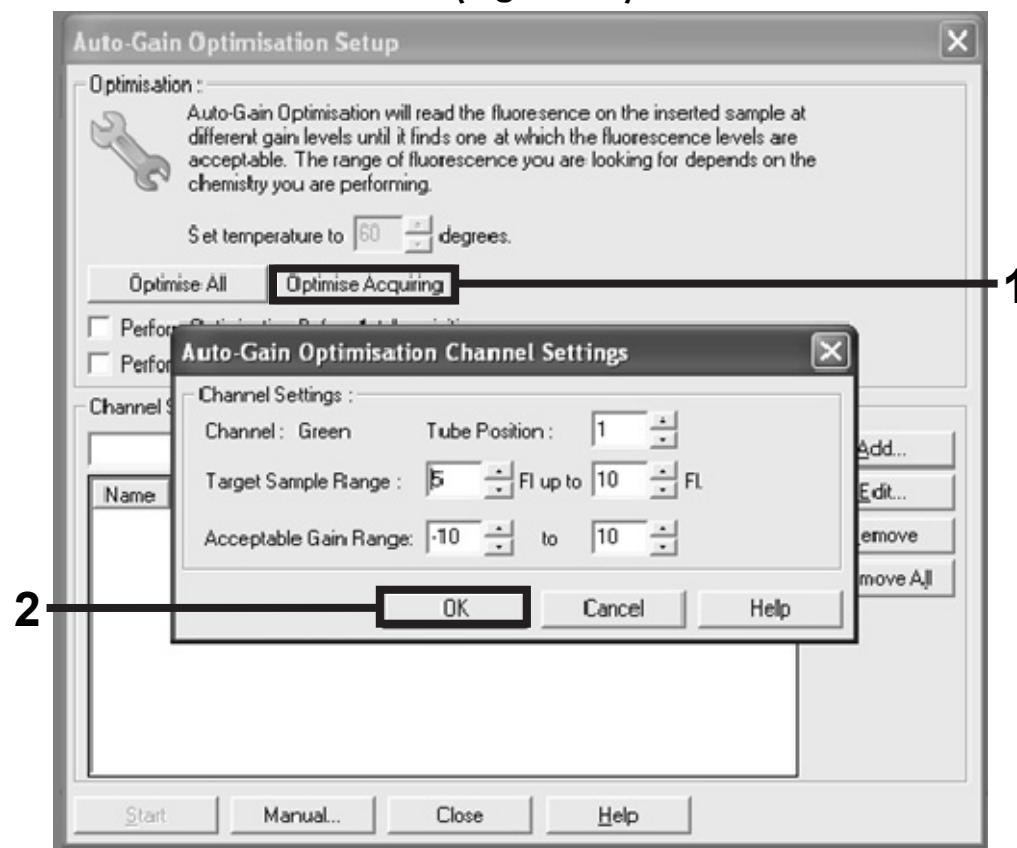


Figure 25. Auto-gain optimization for the green channel.

15. Check the “Perform Optimisation before 1st Acquisition” box, and then click the “Close” button to return to the wizard (Figure 26).

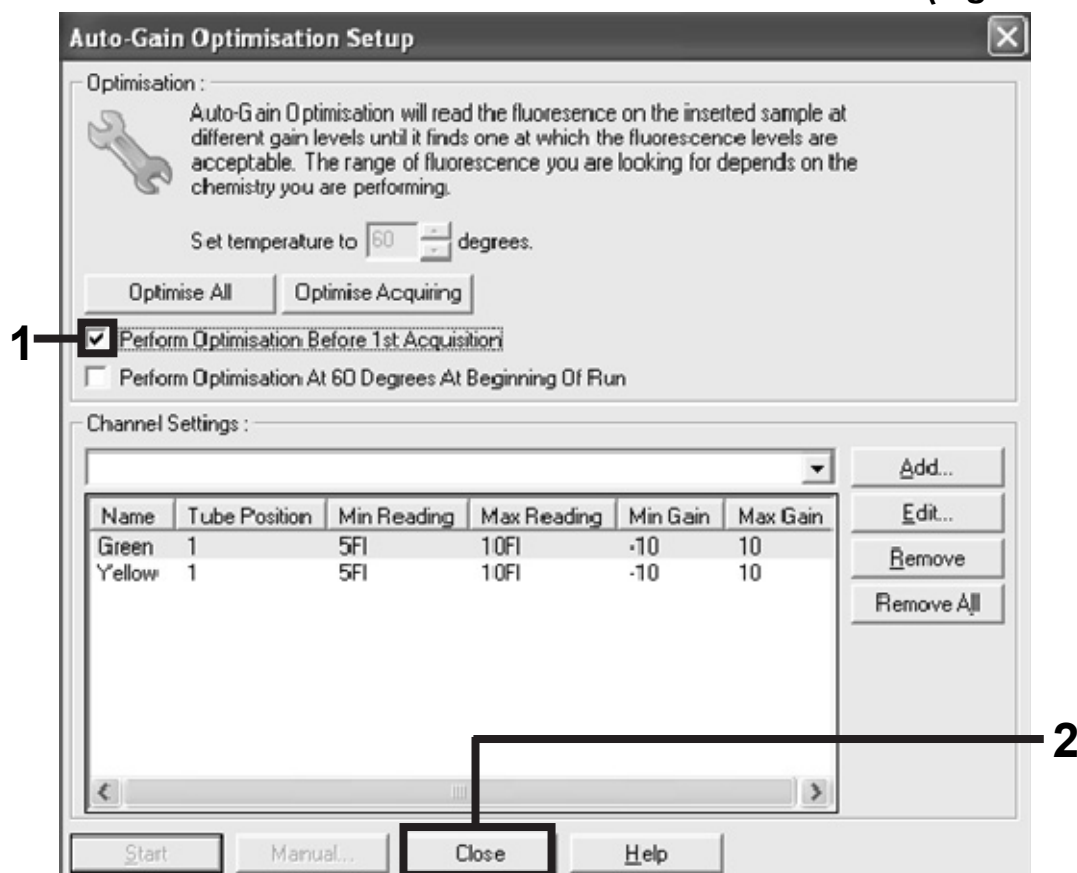


Figure 26. Selection of green and yellow channels.

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

Results

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 (2.2.3.11 or higher).
2. Click "Analysis". On the analysis page, click "Cycling A. Yellow" to view the HEX channel.
3. Check that "dynamic tube" is highlighted.
4. Check that "slope correct" is highlighted.
5. Click "Linear scale".
6. Set the threshold to 0.05.
7. Set the "elimination of cycles" to 15.
8. Check the HEX C_T values.
9. On the analysis page, click "Cycling A. Green" to view the FAM channel.
10. Check that "dynamic tube" is highlighted.
11. Check that "slope correct" is highlighted.
12. Click "Linear scale".
13. Set the threshold to 0.05.
14. Set the "elimination of cycles" to 15.
15. Check the FAM C_T values.

Run control analysis

Refer to the run control analysis flowchart in Figure 27.

- **Negative control:** To ensure that there is no template contamination, the no template control must not generate a C_T value in the green (FAM) channel below 40. To ensure that the plate is set up correctly, the no template control must show amplification in the range of 32.53–38.16 in the yellow (HEX) channel. The values specified are within and including these values.
- **Positive control:** The BRAF Positive Control (PC) must give a control assay C_T (FAM channel) of 27.82–33.85. The values specified are within and including those values. A value outside this range indicates an assay setup problem and is therefore a run failure.

Sample analysis — Control assay

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 of 20.95–33.00 in the green channel. If the sample is out of this range, the following guidance is provided.

- **Sample control assay $C_T < 20.95$:** Samples with a control $C_T < 20.95$ must be diluted since this represents the lower end of the validated assay range. 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. If the sample is close to 20.95, dilution is recommended in order to ensure a result is obtained from the sample test (BRAF mutation detection) run. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).
- **Sample control assay $C_T > 33.00$:** Re-extraction of the sample is recommended since insufficient starting DNA template will be present to detect all mutations at the stated cutoff values for the assay.

BRAF mutation detection 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 (2.2.3.11 or higher).
2. Click “Analysis”. On the analysis page, click “Cycling A. Yellow” to view the HEX channel.
3. Check that “dynamic tube” is highlighted.
4. Check that “slope correct” is highlighted.
5. Click “Linear scale”.
6. Set the threshold to 0.05.
7. Set the “elimination of cycles” to 15.
8. Check the HEX C_T values.
9. On the analysis page, click “Cycling A. Green” to view the FAM channel.
10. Check that “dynamic tube” is highlighted.
11. Check that “slope correct” is highlighted.
12. Click “Linear scale”.
13. Set the threshold to 0.05.
14. Set the “elimination of cycles” to 15.

15. Check the FAM C_T values.

Run control analysis

Refer to the run control analysis flowchart in Figure 27.

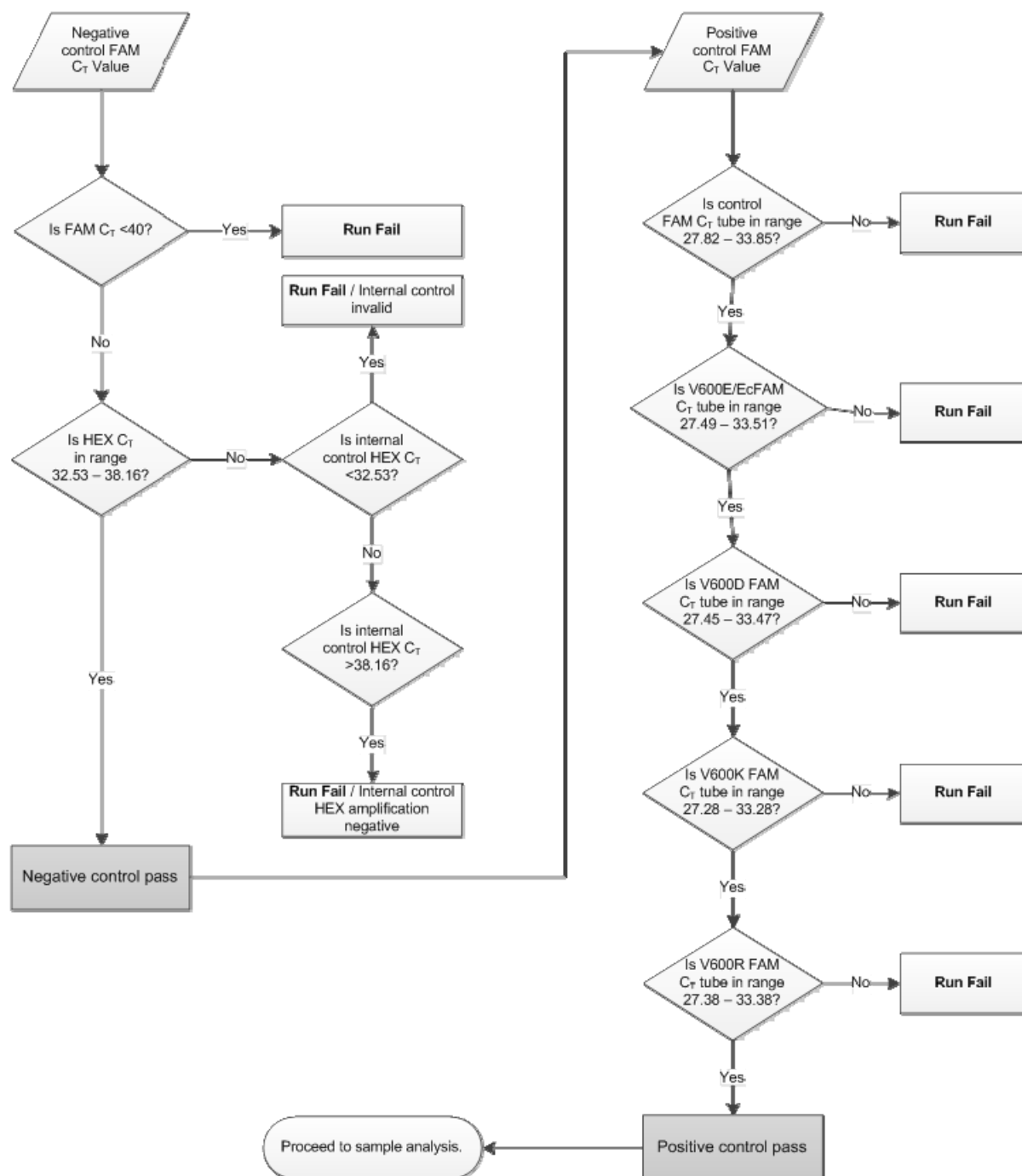


Figure 27. Run control analysis flowchart. For instructions on run control analysis, see pages 36–38.

- **Negative control:** To ensure that there is no contamination of the reaction mix, the no template control must not generate a C_T value in the green channel below 40. To ensure that the plate was set up correctly, the no

template control must show amplification of 32.53–38.16 in the yellow channel. The values specified are within and including those values.

- **Positive control:** The BRAF Positive Control (PC) must give a C_T value for each BRAF assay as shown in Table 5 in the green channel. The values specified are within and including those values. A value outside this range indicates an assay setup problem and is therefore a run failure.

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

Table 5. Acceptable C_T range for reaction controls.

Reaction mix	Sample	Channel	C_T range
Control	PC	FAM	27.82–33.85
V600E/Ec	PC	FAM	27.49–33.51
V600D	PC	FAM	27.45–33.47
V600K	PC	FAM	27.28–33.28
V600R	PC	FAM	27.38–33.38

Sample analysis — Sample control FAM C_T value

Provided that both run controls are valid for the control assay, each sample control C_T value must be within the range of 20.95–33.00 in the green channel. Refer to the sample analysis flowchart in Figure 28.

If the sample is out of this range, the following guidance is provided.

- **Sample control assay $C_T < 20.95$:** Samples with a control $C_T < 20.95$ 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. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).
- **Sample control assay $C_T > 33.00$:** Interpret with caution since very low-level mutations may not be detected.

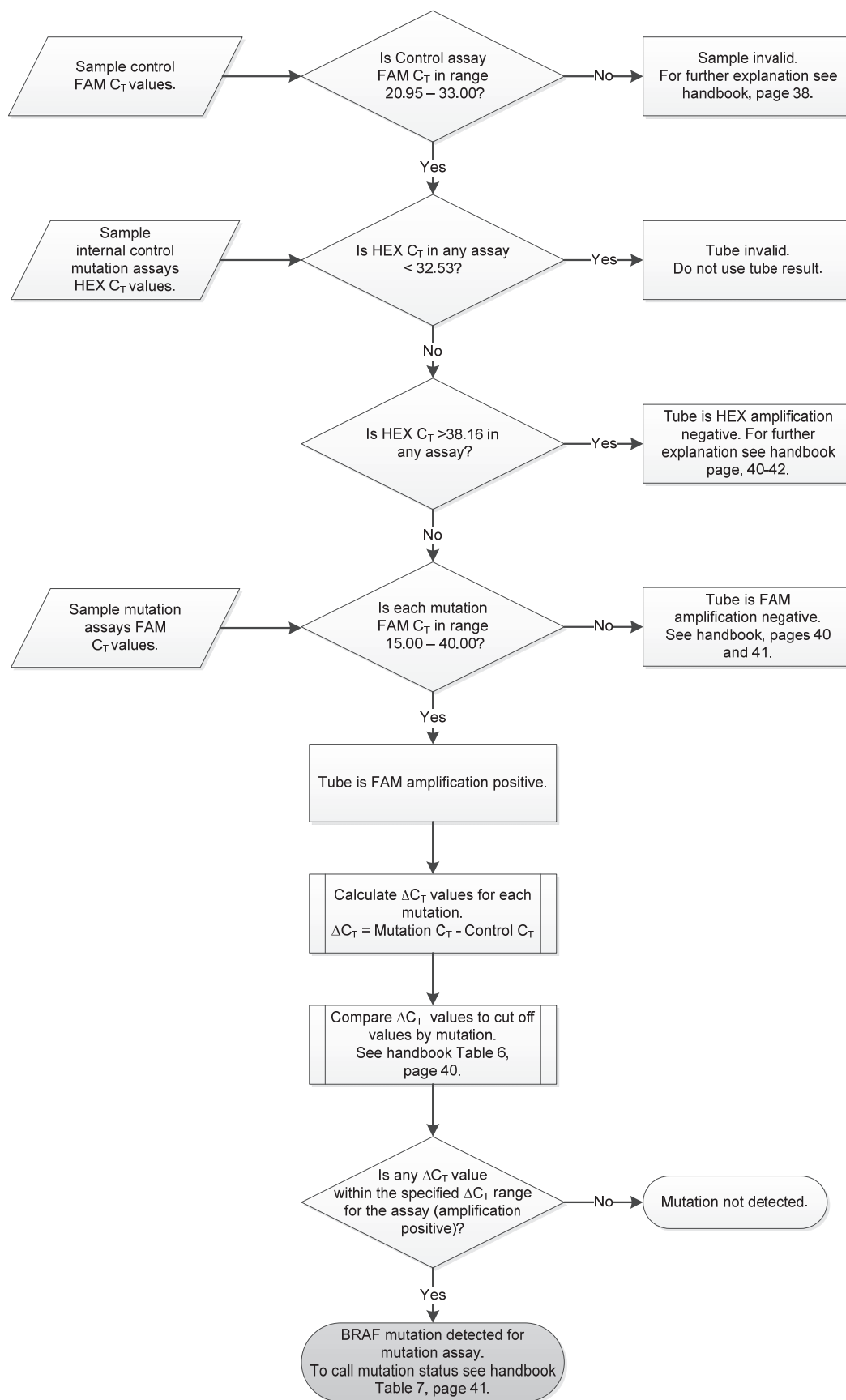


Figure 28. Sample analysis flowchart. For further information, see pages 38–42.

Sample analysis — Sample internal control mutation assays HEX C_T value

Refer to the sample analysis flowchart in Figure 28. All wells of each sample must be analyzed. Check that each well generates a HEX signal from the internal control. There are 3 possible outcomes.

- If the internal control C_T falls within the specified range (32.53–38.16), it is HEX amplification positive and valid.
- If the internal control C_T is above the specified range (>38.16), it is HEX amplification negative. If there is amplification in the FAM channel for that tube, the HEX amplification is valid. If there is no amplification in the FAM channel for that tube, the HEX amplification is invalid.
- If the internal control C_T is below the specified range (<32.53), it is invalid.

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. A tube of Water for Sample Dilution (Dil.) is included with the kit.

Sample analysis — Sample mutation assays FAM C_T value

The FAM values for all 4 reaction mixes should be checked against the values listed in Table 6.

Table 6. Acceptable sample mutation reaction values (FAM)*

Assay	Acceptable C _T range	ΔC _T range
V600E/Ec	15.00–40.00	≤7.0
V600D	15.00–40.00	≤6.9
V600K	15.00–40.00	≤6.0
V600R	15.00–40.00	≤7.0

* Acceptable values are within and including the values shown.

- If the FAM C_T falls within the specified range, it is FAM amplification positive.
- If the FAM C_T is above the specified range or there is no amplification, it is FAM amplification negative.

Calculate the ΔC_T value for each mutation tube that is FAM amplification positive as follows, ensuring that the mutation and control C_T values are from the same sample.

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

Compare the ΔC_T value for the sample with the cutoff point for the assay in question (Table 6), ensuring that the correct cutoff point is applied to each assay.

The cutoff 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_T value is higher than the cutoff point, it is classed as negative or beyond the limits of detection of the kit.

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 ΔC_T are at or below the cutoff value. If multiple mutations are detected, the mutation status should be assigned according to Table 7.

Table 7. Calling sample mutation status

V600E/Ec	V600D	V600K	V600R	Mutation status
Positive	Negative	Negative	Negative	V600E or V600Ec positive
Positive	Negative	Positive	Negative	V600Ec or V600K positive
Positive	Positive	Negative	Negative	V600D positive
Negative	Positive	Negative	Negative	V600D positive
Negative	Negative	Positive	Negative	V600K positive
Negative	Negative	Negative	Positive	V600R positive

■ **Mutation not detected:**

FAM amplification positive and the ΔC_T are above the cutoff value.

FAM amplification negative and HEX (internal control) amplification are positive.

■ **Invalid:**

HEX (internal control) is invalid.

FAM amplification is negative and HEX amplification is negative.

For further explanation, see the flowchart (Figure 28). If a sample is HEX amplification negative in a tube but FAM amplification positive in a different tube, then a “mutation detected” result in the different tube can still be considered valid but the particular mutation identified may not be reliably assigned.

If a sample is HEX amplification negative and FAM amplification positive in the same tube, then the “mutation detected” result should be considered valid.

If a tube is HEX (internal control) invalid, the result of that tube must not be used.

Sample analysis — Sample mutation status

Once all mutation reaction tubes are assessed, the mutation status of the sample is determined as follows.

- **Mutation detected:** One or more of the four mutation reactions are positive. If multiple mutations are detected, the mutation reported should be in accordance with Table 7.
- **Mutation not detected:** All four mutation reactions are negative.
- **Invalid:** No mutation reactions are positive and one or more mutation reactions are invalid.

Note: The BRAF RGQ PCR Kit is intended to detect mutations in the BRAF gene in a DNA sample. When a sample is called BRAF mutation detected, only one specific mutation should be reported. If multiple mutations are detected, the mutation reported should be in accordance with Table 7.

Some cross-reactivity occurs between mutation reactions. For example, the V600E/Ec assay may give a positive result if a V600D mutation is present, the V600E/Ec assay may give a positive result if a V600K mutation is present, and the V600K assay may give a positive result if a V600E complex mutation is present. However, mutation status can be distinguished using Table 7.

Cross-reactivity is due to the ARMS primer detecting other mutations of similar sequence to one another. If a second mutation assay gives a positive result, this is likely to be cross-reactivity. Double mutants have been observed, although these are rare.

Therefore, in rare cases, combinations of positive results may be detected that are not given in Table 7. The sample can still be called BRAF mutation detected. However, due to cross-reactivity, a particular mutation cannot be distinguished. Therefore, the sample should only be called BRAF mutation detected.

If one or more of the mutation reactions are invalid but one or more are positive, the sample can still be called BRAF mutation detected since a mutation is present. However, the specific mutation reported may not be accurate and may be a result of cross-reactivity. Therefore, the sample should only be called BRAF mutation detected.

Notes for data analysis

Linear amplification

Rotor-Gene Q plots from all reactions should be checked. Occasionally, an increase in fluorescence signal is seen in the NTC, negative samples, and empty tubes. 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. Linear amplification within empty tubes is due to a fluorescence artifact. C_T data for the empty tubes should therefore be discarded.

Analysis of the NTC

Figures 29 and 30 show two examples of the behavior of NTC samples. In Figure 29, nonlinear (true) amplification due to sample contamination is seen. This run should be discarded and the samples should be retested. In Figure 30, linear amplification in a NTC is seen. Under these circumstances, the raw fluorescence should be examined. The corresponding raw fluorescence plot is shown in Figure 31, indicating a linear increase in fluorescence rather than a true amplification event. The data from this run can be used, but only if the positive and internal controls have passed. For comparison with Figure 31, Figure 32 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.

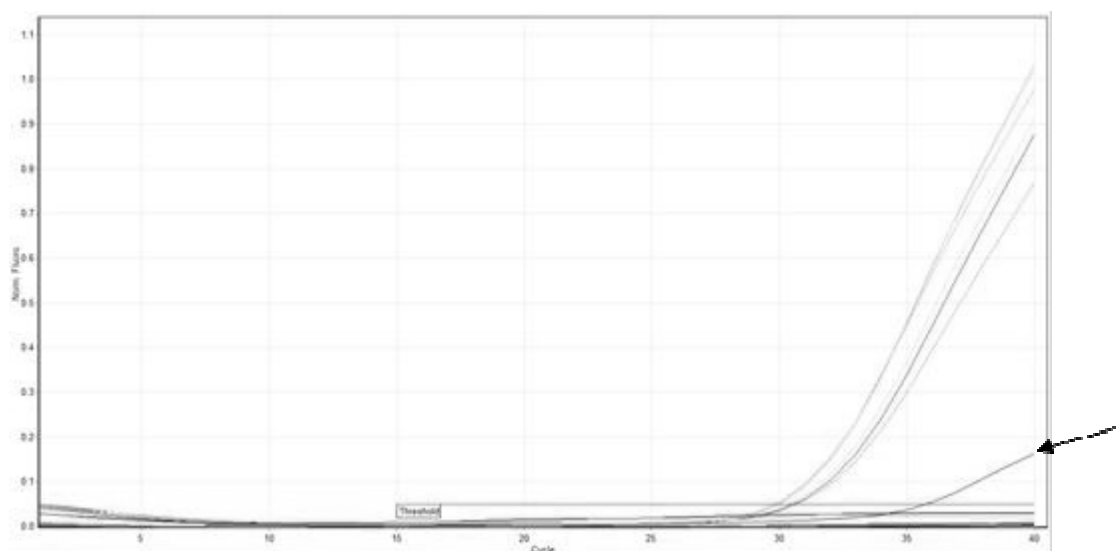


Figure 29. Contamination in a NTC of an assay in an analyzed run. The arrow indicates amplification showing contamination in a NTC tube.

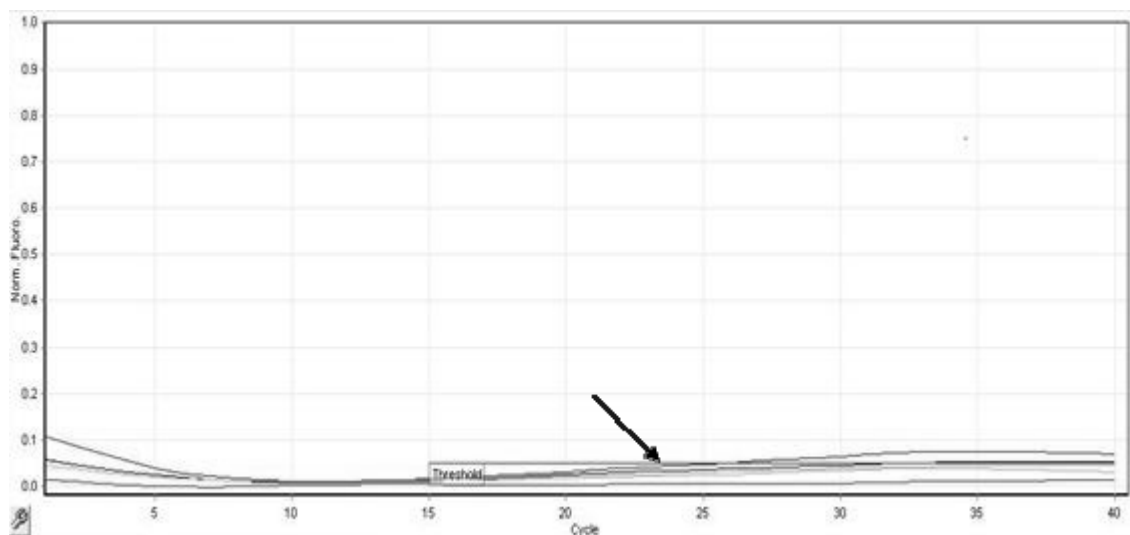


Figure 30. Example of a linear increase in fluorescence in a NTC tube. The arrow indicates linear increase in fluorescence in a NTC tube.

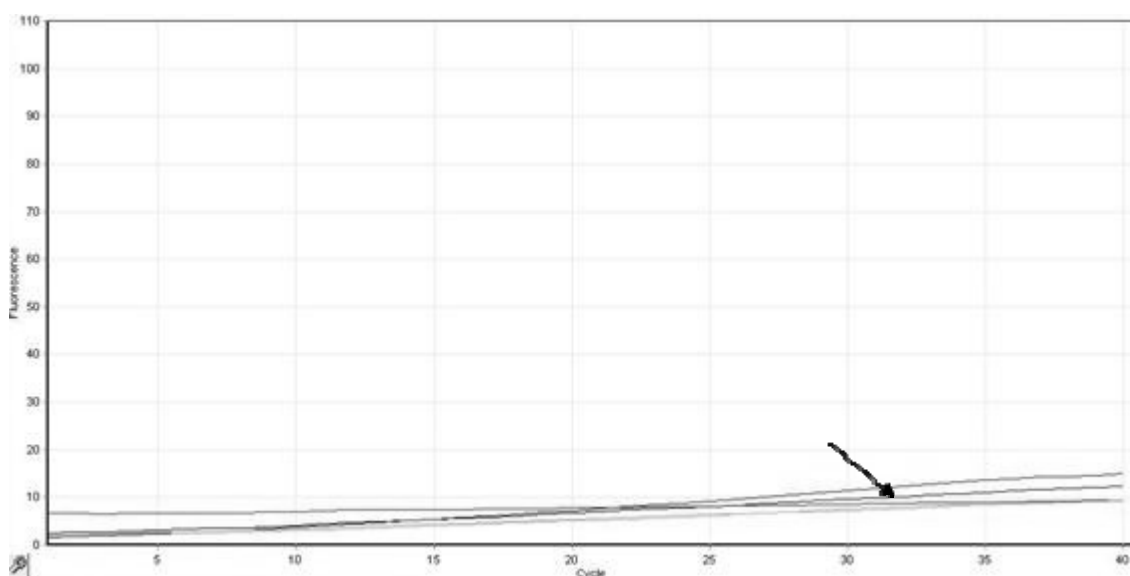


Figure 31. Raw fluorescence of Figure 30. The arrow indicates linear amplification in a NTC well.

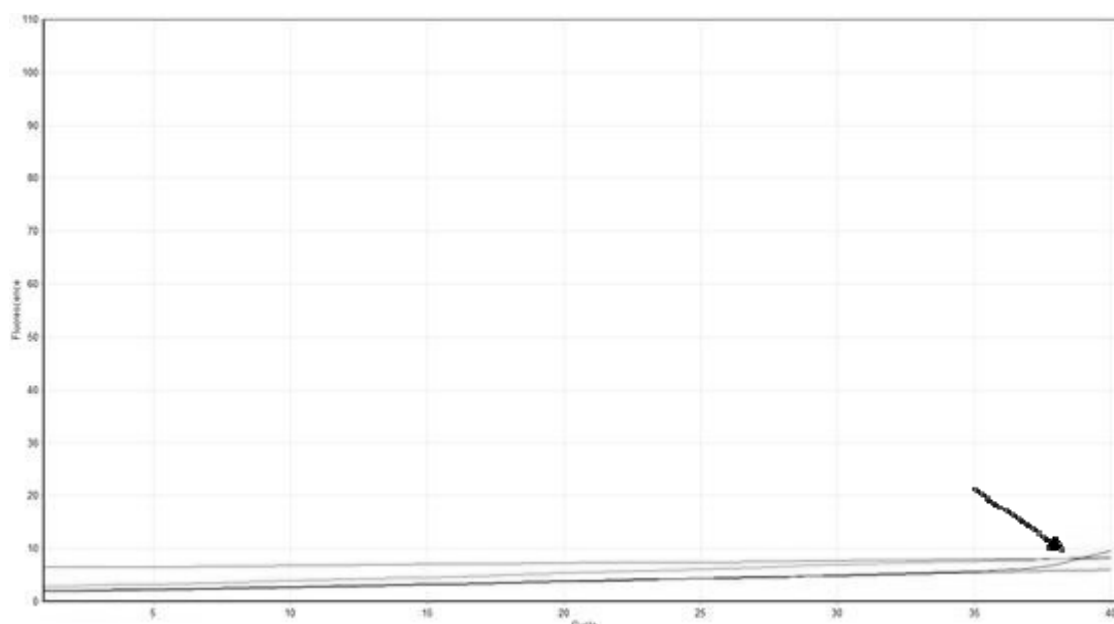


Figure 32. Raw fluorescence data showing a NTC tube with a true amplification event. The arrow indicates contamination in a NTC well. All other wells show no contaminant present.

Analysis of samples

Figures 33 and 34 show two examples of amplification in sample reactions. An example of true amplification in a sample tube in an analyzed run is seen in Figure 33. If a run shows this type of sigmoidal amplification curve, this is true amplification and the data from this run can be used, but only if the positive and internal controls have passed.

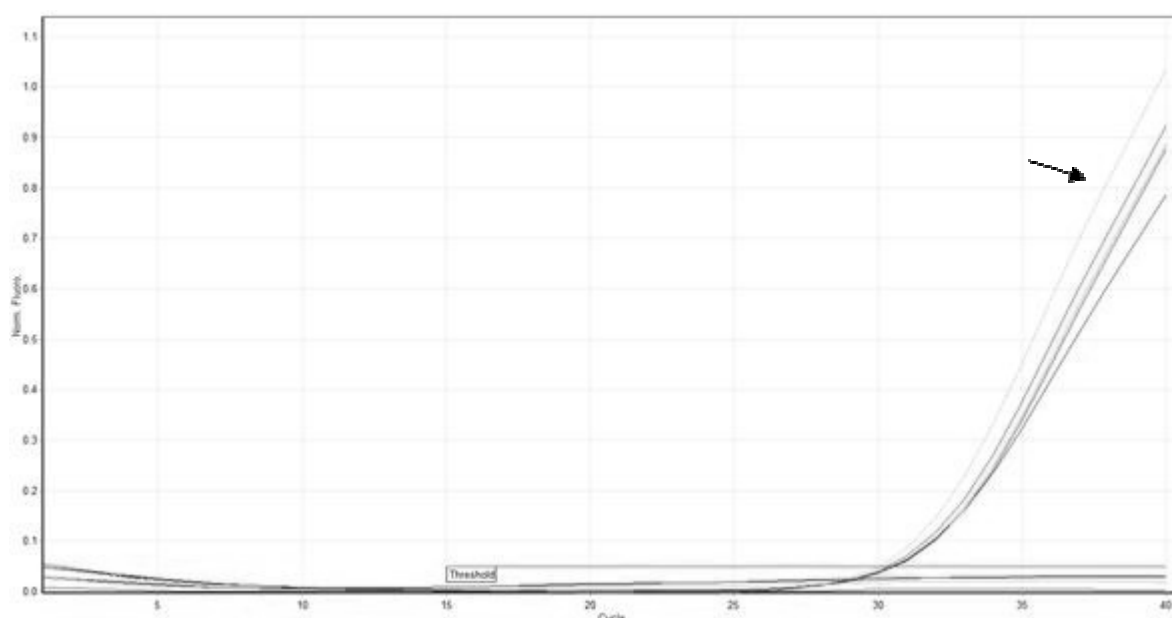


Figure 33. True amplification in a sample tube in an analyzed run. The arrow indicates the plots showing true amplification.

An example of linear amplification in a sample reaction is shown in Figure 34. Under these circumstances, the raw fluorescence data should be examined. The corresponding raw fluorescence plot (Figure 35) indicates that the linear increase observed in Figure 34 corresponds to a linear increase in the raw fluorescence and is not a true amplification. If 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_T ”.

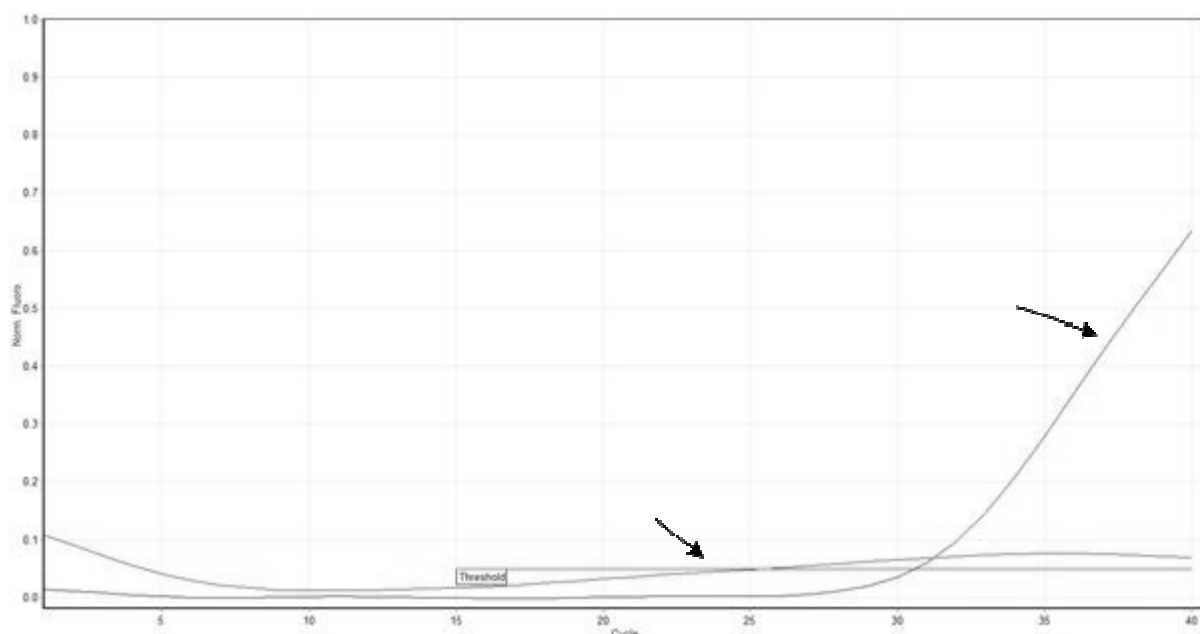


Figure 34. Example of a linear increase in fluorescence in a sample tube. The upper arrow indicates a plot showing true amplification. The lower arrow indicates linear increase in fluorescence in a sample well.

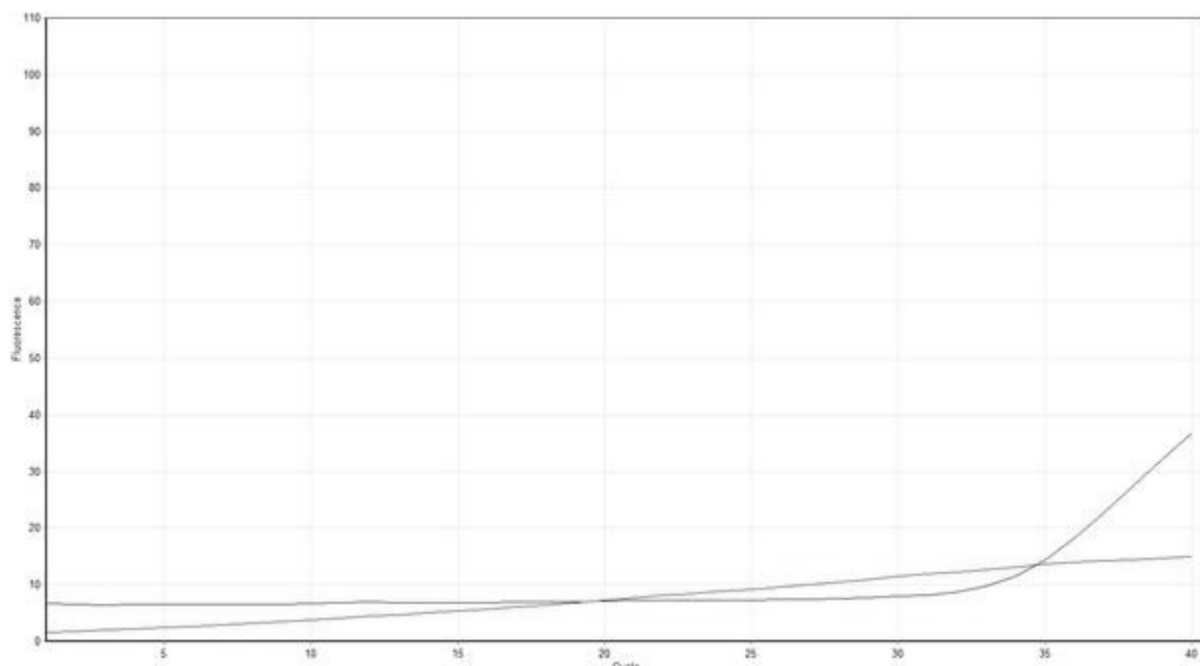


Figure 35. Raw fluorescence of Figure 34.

Detection of low-level mutations

Mutation-negative samples with ΔC_T values only slightly higher than the cutoff values in Table 6 (page 40) could be low-level mutations that are beyond the limit of detection of the assay. See “Limit of detection (LOD)”, below.

Note: Tumors are heterogeneous. Therefore data from a sample of a tumor may not be concordant with data from other sections of the same tumor.

Limit of detection (LOD)

In a study, LOD was defined as the lowest amount of mutant DNA in a background of wild-type DNA at which a mutant sample will provide mutation-positive results in 95% of the test results (C_{95}).

Medium input was defined as a standard giving a control assay C_T of 25.98 (the control assay working range is 20.95–33.00 C_T). The LOD at medium input DNA concentration is given in Table 8. At higher input DNA concentrations, the LOD values are expected to be lower than the values stated in Table 8.

Table 8. LOD values for each mutation assay (medium input)

Assay (mutation)*	LOD C_{95} at medium input DNA (percentage of mutant DNA in wild-type DNA)
V600E (E)	1.82%
V600E (Ec)	4.31%
V600D	3.19%
V600K	4.34%
V600R	4.85%

* Limits of detection for the V600E assay were calculated for both V600E and V600Ec mutations.

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

Increase in fluorescence signal in NTC or negative samples

Fluorescence artifact or contamination

To distinguish between a linear increase in fluorescence, which may have arisen due to a fluorescence artifact, and a true amplification event, which would indicate contamination in the NTC, see “Notes for data analysis”, page 43.

No signal with BRAF 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 <i>BRAF</i> PCR and the fluorescence channel Cycling Yellow for the internal control PCR. |
| b) Incorrect temperature profile of the Rotor-Gene Q 5plex HRM instrument used | Compare the temperature profile with the protocol. If incorrect, set the run parameters again and repeat the run. |
| c) Incorrect configuration of the PCR | Check your 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 BRAF 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. |

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

Contamination occurred during preparation of the PCR

Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. 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 the BRAF RGQ PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

The product is to be used only by personnel specially instructed and trained in laboratory procedures and the Rotor-Gene Q 5plex HRM instrument.

Studies were performed using human DNA extracted from formalin-fixed paraffin-embedded tumor samples and synthetic standards as appropriate for the individual studies.

The product has been tested using the QIAamp DNA FFPE Tissue Kit from QIAGEN.

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

Strict compliance with the *BRAF 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 BRAF RGQ PCR Kit. Additional control mix 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.












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.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Symbols

The following symbols may appear on the packaging and labeling:

 <24 >	Contains reagents sufficient for <24> reactions
	Use by
	Catalog number
	Lot number
	Components
	Contains
	Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Keep away from sunlight

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
BRAF RGQ PCR Kit (24)	For 24 reactions: Control Assay, 4 Mutation Assays, Positive Control, Taq DNA Polymerase, Water for NTC, and Water for Sample Dilution	870801
Rotor-Gene Q and other accessories		
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	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 not included	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
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
QIAamp DNA FFPE Tissue Kit — for purification of genomic DNA from paraffin-embedded tissues		
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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'Not For Use In Determining Risk Of Developing Endometriosis

For U.S.A.:

Not For Use With Thyroid Samples

Not For Use With Peripheral Blood Samples

Not For Use With Cell-Free Bone Marrow Samples

Limited License Agreement for BRAF RGQ PCR Kit

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