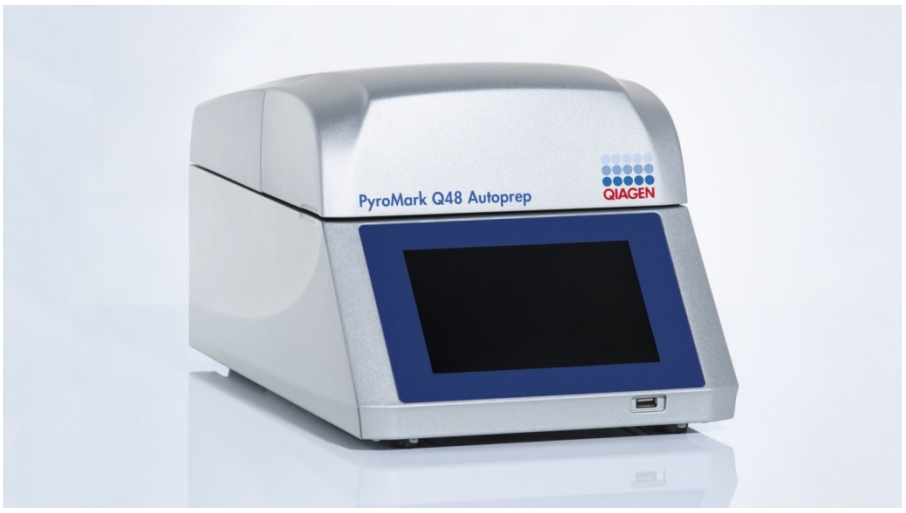
May 2025

PyroMark® Q48 Autoprep System User Manual

Version 1.0



REF 9002470

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Sample to Insight

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1. Introduction

Thank you for choosing the PyroMark Q48 Autoprep System. We are confident it will become an integral part of your laboratory.

Before using the PyroMark Q48 Autoprep System, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

1.1. About this user manual

This user manual provides information about the PyroMark Q48 Autoprep System in the following sections:

1. Introduction
2. Safety Information
3. General Description
4. Installation Procedures
5. Instrument Administration
6. Performing a Run
7. PyroMark Q48 Autoprep Software
8. Maintenance Procedures
9. Troubleshooting
10. Glossary
11. Technical Specifications

The appendices include the following:

- Appendix A – Assay Design and Validation
- Appendix B – Technical Data
- Appendix C – PyroMark Q48 Autoprep Accessories
- Appendix D – Legal Information
- Appendix E – Safety Information (French, FR)
- Appendix F – Safety Information (German, DE)

1.2. General information

1.2.1. Technical assistance

At QIAGEN®, we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the PyroMark Q48 Autoprep System or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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

1.2.2. Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.2.3. Version management

This document is the *PyroMark Q48 Autoprep System User Manual*, version 2.

1.3. Intended use of the PyroMark Q48 Autoprep System

The PyroMark Q48 Autoprep System is designed to detect changes in specified variable positions in, or base-calling of, DNA prepared from biological samples in molecular biology applications.

The PyroMark Q48 Autoprep System is intended to be used only in combination with QIAGEN kits indicated for use with the PyroMark Q48 Autoprep System for the applications described in the kit handbooks.

If the PyroMark Q48 Autoprep System is used with other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The PyroMark Q48 Autoprep System is intended for use by professional users trained in molecular biological techniques and the operation of the PyroMark Q48 Autoprep.

1.4. Requirements for PyroMark Q48 Autoprep System users

This table covers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the PyroMark Q48 Autoprep System.

Table 1. Competence requirement for PyroMark Q48 Autoprep System users

Task	Personnel	Training and experience
Delivery	No special requirements	No special requirements
Installation	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Routine use (running protocols)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Assay design and validation	Scientist or equivalent	Appropriately trained and experienced personnel familiar with molecular biological techniques
Cartridge replacement	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Preventive maintenance	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing	QIAGEN service personnel or service technicians of an authorized agent	Trained and authorized by QIAGEN

2. Safety Information

Before using the PyroMark Q48 Autoprep System, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the PyroMark Q48 Autoprep instrument and to maintain the PyroMark Q48 Autoprep System in a safe condition.

Note: Translations of the Safety Information in French and German are available in Appendix E – Safety Information (French, FR) and Appendix F – Safety Information (German, DE).

The following types of safety information appear in this manual.

WARNING

The term **WARNING** is used to inform you about situations that could result in personal injury to you or others.

Details about these circumstances are given in a box like this one.

CAUTION

The term **CAUTION** is used to inform you about situations that could result in **damage to an instrument** or other equipment.

Details about these circumstances are given in a box like this one.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

2.1. Proper use

WARNING/ CAUTION

Risk of personal injury and material damage

Improper use of the PyroMark Q48 Autoprep System may cause personal injuries or damage to the instrument. The PyroMark Q48 Autoprep System must only be operated by qualified personnel who have been appropriately trained. Servicing of the PyroMark Q48 Autoprep System must only be performed by a QIAGEN field service specialist.

CAUTION

Damage to the instrument

Direct sunlight may bleach parts of the instrument and cartridges and may cause damage to plastic parts.

The PyroMark Q48 Autoprep System and the cartridges must be located out of direct sunlight, away from heat sources and away from sources of vibration and electrical interference.

CAUTION

Damage to the instrument

Avoid spilling water or chemicals onto the PyroMark Q48 Autoprep System. Damage caused by water or chemical spillage will void your warranty.

WARNING/ CAUTION

Risk of personal injury and material damage

Do not attempt to move the PyroMark Q48 Autoprep System during operation.

WARNING/ CAUTION **Explosive atmosphere**

The PyroMark Q48 Autoprep System is not designed for use in an explosive atmosphere.

WARNING **Risk of explosion**

The PyroMark Q48 Autoprep System is intended for use with reagents and substances supplied with QIAGEN kits. Use of other reagents and substances may lead to fire or explosion.

CAUTION **Insertion of absorber strip**

Ensure that the absorber strip is inserted into the PyroMark Q48 Autoprep instrument (as described in Section 6.2.2) to prevent liquid from entering the chamber.

In case of emergency, switch off the PyroMark Q48 Autoprep System at the power switch and unplug the power supply from the power outlet.

2.2. Electrical safety

Note: Disconnect the line power cord from the power outlet before servicing.

WARNING **Electrical hazard**

Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.

Intentional interruption is prohibited.

Lethal voltages inside the instrument

When the instrument is connected to line power, terminals may be live and opening covers or removing parts is likely to expose live parts.

Avoid spilling liquid onto or into the instrument. In case of spilling liquid over the instrument, immediately disconnect the instrument from the mains power.

To ensure satisfactory and safe operation of the PyroMark Q48 Autoprep System, follow these guidelines:

- The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground).
- Keep mains plug easily accessible in case the equipment needs to be disconnected quickly from mains power.
- Use only the power cord delivered by QIAGEN.
- If the instrument becomes electrically unsafe, prevent other personnel from operating it and contact QIAGEN Technical Services. The instrument may be electrically unsafe when:
    - The line power cord appears to be damaged.
    - It has been stored for a prolonged period of time in conditions which are outside of the “Storage conditions”, outlined in Section 11 Technical Specifications.
    - It has been subjected to severe transport stresses.
    - Liquid has entered the instrument.

2.3. Biological safety

When handling biological material, use safe laboratory procedures as outlined in publications such as Biosafety in Microbiological and Biomedical Laboratories, HHS (**www.cdc.gov/labs/BMBL.html**).

WARNING **Biological materials**

Handle biological material with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, 2 pairs of gloves, and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Safety Data Sheets (SDSs) or the OSHA1,* ACGIH,† or COSHH‡ documents.

For more information, visit **www.qiagen.com/safety**.

Venting for fumes and disposal of waste must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

2.4. Chemical safety

WARNING **Hazardous chemicals**

Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.

Always wear safety glasses, 2 pairs of gloves, and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or the OSHA1,* ACGIH,† or COSHH‡ documents.

For more information, visit **www.qiagen.com/safety**.

Venting for fumes and disposal of waste must be in accordance with all national, state, and local health and safety regulations and laws.

Clean any spilled or splashed Denaturation Solution from the instrument immediately. Longer exposure may lead to stains and surface damage.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

2.5. Mechanical hazards

WARNING **Moving parts**

To avoid contact with moving parts during the operation of the PyroMark Q48 Autoprep System, the instrument must be operated with the hood closed.

Do not remove the cover panels since there are no user-serviceable parts inside. If there is a problem with the PyroMark Q48 Autoprep System, contact QIAGEN Technical Services immediately.

WARNING **Moving parts**

The chamber lid of the PyroMark Q48 Autoprep instrument opens and closes automatically during operation to prevent moisture buildup inside the instrument.

CAUTION **Disc insertion**

The PyroMark Q48 Disc must be locked into position to avoid spilling the contents of the disc into the instrument chamber.

Ensure that the disc is locked into position by screwing down the lock nut. Only use the lock nut provided by QIAGEN to prevent damage to the instrument.

CAUTION **Chamber lid operation**

The lid operation is driven by a motor controlled by the software. Insufficient space at the rear of the instrument (less than 20 cm or 7.9 in.) to operate the chamber lid may result in damage to the lid upon opening.

2.6. Heat hazards

WARNING **Hot surface**

The disc heater within the PyroMark Q48 Autoprep instrument can reach temperatures of up to 95°C (203°F). Avoid touching it when it is hot.

CAUTION **Risk of overheating**

To ensure proper ventilation, maintain a minimum clearance of 5 cm (2 in.) at the sides and 20 cm (7.9 in.) at the rear of the PyroMark Q48 Autoprep instrument.

Slits and openings that ensure the ventilation of the PyroMark Q48 Autoprep instrument must not be covered.

2.7. Consumables

CAUTION **Unsupported consumables**

Do not connect or use any consumables, accessories, or external equipment other than that specified.

2.8. Waste disposal

CAUTION **Removal of absorber strip**

Upon completion of the run, the absorber strip will contain Denaturation Solution, which contains sodium hydroxide. This can be irritating to the eyes and skin.

Always wear safety glasses, gloves, and a lab coat when removing the absorber strip.

Disposal of the absorber strip should be in accordance with all national, state and local health and safety regulations and laws.

CAUTION **Disposal of plasticware**

Used plasticware, e.g., PyroMark Q48 Disc, may contain hazardous chemicals, or contagious/biohazardous materials. Such waste must be collected and disposed of properly according to local safety regulations.

2.9. Maintenance safety

Perform the maintenance as described in Section 8. QIAGEN charges for repairs that are required due to incorrect maintenance.

WARNING/ CAUTION **Risk of personal injury and material damage**

Only perform maintenance that is specifically described in this user manual.

WARNING/ CAUTION **Risk of electric shock**

Do not open any panels on the PyroMark Q48 Autoprep System.

Only perform maintenance that is specifically described in this user manual.

CAUTION **Damage to the instrument**

Do not use solvents or reagents containing acids, alkalis, or abrasives to clean the PyroMark Q48 Autoprep instrument.

CAUTION **Damage to the touchscreen and computer**

Do not pour or spray liquids, e.g., cleaning agents, on to PyroMark Q48 Autoprep instrument. Use a tissue moistened with water only for cleaning.

CAUTION **Light detector maintenance**

Use lint-free tissues to carefully clean the window of the photomultiplier (PMT). Do not use paper tissues. See Section 8.2 for further instructions.

CAUTION **Damage to the instrument**

Do not expose the photomultiplier (PMT) to strong light during maintenance.

CAUTION **Injector cleaning**

Injectors must be cleaned within a 12 hour period following the last run to ensure long term injector performance. Failure to comply can result in injectors blocking.

CAUTION **Risk of fire**

When cleaning the PyroMark Q48 Autoprep instrument with alcohol-based disinfectant, leave the instrument lid and the injector cover open to allow flammable vapors to disperse.

2.10. Symbols on the PyroMark Q48 Autoprep products

Symbol	Location	Description
	Type plate on the back of the instrument	CE mark for European Conformity
	Type plate on the back of the instrument	Legal manufacturer
	Type plate on the back of the instrument	Waste Electrical and Electronic Equipment (WEEE) mark for Europe
	Type plate on the back of the instrument	FCC mark of the United States Federal Communications Commission
	Type plate on the back of the instrument	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)
	Type plate on the back of the instrument	RCM mark for Australia/New Zealand
	Type plate on the back of the instrument	CSA-mark for Canada and USA
	Reagents, solutions, disc	Identification of production batch/lot
	Reagents, solutions, disc	Use-by date
	Reagents, solutions, disc	Temperature limitation
	Reagents, cartridge	Read the manual
	Inside the instrument, cartridge	Warning, consult user manual
	Inside the instrument	Warning, hot surface

3. General Description

The PyroMark Q48 Autoprep instrument uses proven real-time sequence-based Pyrosequencing® technology for sequence-based detection and quantification in genetic analysis and epigenetic methylation studies. The system can analyze up to 48 samples simultaneously. An easy-to-use, automated protocol is used to prepare single-stranded DNA samples after PCR. This protocol uses magnetic streptavidin-coated Sepharose® beads (PyroMark Q48 Magnetic Beads), which bind to the biotinylated PCR strand. Annealing of sequencing primers can be automated for up to four different sequencing primers. If more sequencing primers are used, the primers can be manually added to the single-stranded DNA samples.

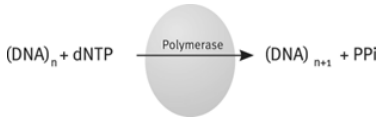
3.1. PyroMark Q48 Autoprep definitions

- PyroMark Q48 Autoprep instrument: Instrument only
- PyroMark Q48 Autoprep software: Software only
- PyroMark Q48 Autoprep System: All of the above, plus any PyroMark Q48 kits

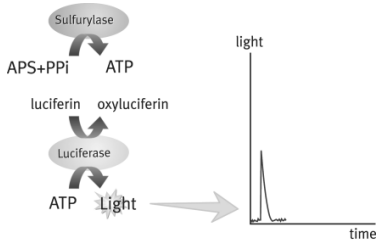
3.2. Pyrosequencing principle

Pyrosequencing uses sequencing by synthesis for accurate and quantitative analysis of DNA sequences.

- A sequencing primer is hybridized to a single-stranded, PCR-amplified DNA template.
- The template is incubated with enzymes and substrates.
- One nucleotide is added to the reaction. If the nucleotide is complementary to one or more bases in the template strand it will be incorporated into the DNA strand by the DNA polymerase.
- Each incorporation event is accompanied by release of pyrophosphate (PPi) in an equimolar quantity to the amount of nucleotides incorporated.

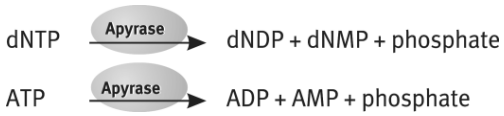


- ATP sulfurylase quantitatively converts PPi to ATP in the presence of adenosine 5′ phosphosulfate.
- This drives the conversion of luciferin to oxyluciferin by luciferase, generating visible light in amounts proportional to the amount of ATP and thus incorporated nucleotides. Light is detected using a photomultiplier tube (PMT) and seen as a peak in the Pyrogram®. Each light signal is proportional to the number of nucleotides incorporated.



nucleotide incorporation generates light seen as a peak in Pyrogram

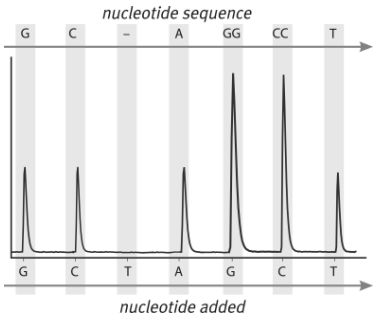
- Apyrase, a nucleotide-degrading enzyme, continuously degrades unincorporated nucleotides and ATP. When degradation is complete, another nucleotide is added.



- Nucleotides are added one at a time.

  Note: Deoxyadenosine alpha-thio triphosphate (dATPaS) is used instead of natural deoxyadenosine triphosphate (dATP) because it is used efficiently by the DNA polymerase, but not recognized by the luciferase.

- As the process continues, the complementary sequence is built up and the nucleotide sequence is determined from the pattern of peaks in the Pyrogram.



3.3. PyroMark Q48 Autoprep principle

- The PyroMark Q48 Autoprep System performs DNA sequencing using Pyrosequencing technology.
- The USB flash drive containing the run file created using PyroMark Q48 Autoprep software is inserted into the USB port at the front of the instrument. Alternatively, the run file can be opened via network drive.
- The PyroMark Q48 Disc is placed into the instrument and the PyroMark Q48 Cartridges are filled with PyroMark Q48 Autoprep Reagents.
- The run is then started by the user.
- Reagents are dispensed into the absorber strip to ensure that the dispensation capillaries are flushed and filled with solution.
- Preparation of single-stranded DNA is performed in an automatic process within the instrument. This process includes a heating step (chamber lid will open for this step) to reduce the volume in the well, a template binding step and a denaturation step to separate the DNA strands. During this process, several wash and centrifugation steps are performed.
- After this process, the samples are prepared for primer annealing, which can be automatically performed by the instrument for up to four different sequencing primers. If more sequencing primers are used, the primers can be manually added to the single-stranded DNA samples.
- Enzyme mixture and then substrate mixture are dispensed into all wells used.
- Nucleotides are dispensed into two waste wells before being dispensed into the wells. Nucleotides are added in a predefined order, and 60 seconds elapses between the additions of each nucleotide to ensure all enzymatic reactions are completed.

- The instrument collects data from all wells using a PMT located above the inserted disc. Data are stored on the instrument.
- After the run, data are automatically transferred to the USB flash drive or the network drive. If the USB flash drive has been removed during a run, data can be retrieved manually from the instrument.

3.4. PyroMark Q48 Autoprep instrument

1. **PyroMark Q48 Autoprep instrument front, open view.**

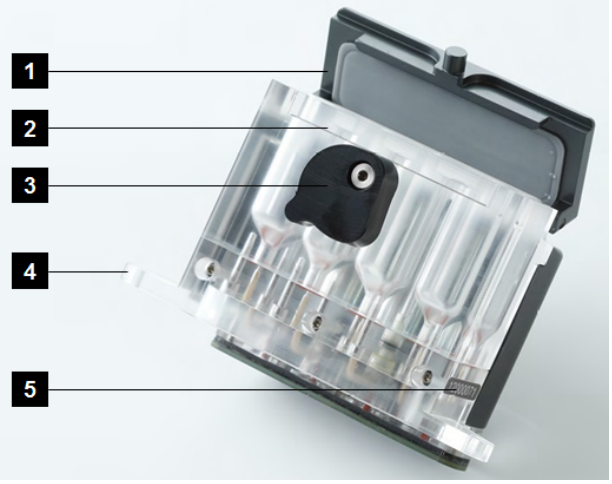
	USB port
1	For import and export of run files.
	User interface
2	Wizard-style touch interface to control the instrument.
	Transport lock screw
3	Prevents the chamber lid from moving during transportation; must be removed during installation.
	Chamber
4	Contains the disc, the optical detection system, the rotor, the disc heater, and an absorber strip cavity.

PyroMark Q48 Autoprep instrument, rear view.

	Chamber lid
5	Software-controlled, motor-operated access to reaction chamber
	Injector cartridges
6ABC	Three cartridges of four injectors each; A: Nucleotide cartridge, B: Primer cartridge, C: Reagent cartridge.
	Injector cover
7	Opens manually, allowing access to the cartridges.
	Ethernet connection
8	For network connection.
	Power switch
9	Instrument on/off switch.

3.4.1. Injector cartridges

The instrument has a total of 12 injectors, divided into three cartridges of four injectors per cartridge. Each cartridge consists of a lid, a lid lock, and four injectors containing a reservoir, filter, injector nozzle, and drop sensor.



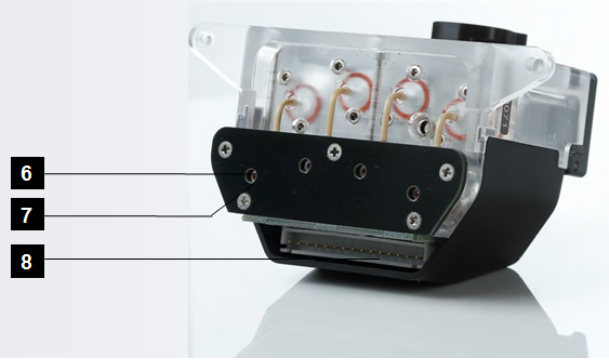
1 **Cartridge lid**
Closes and seals the reagent reservoir.

2 **Reservoir label**
For identification of reagents in each injector.

3 **Cartridge lid lock**
Secures the cartridge lid to seal the reagent reservoir for pressurization.

4 **Hole for thumb screws**
Thumb screws hold cartridges in place. Remove only when cartridges need to be serviced.

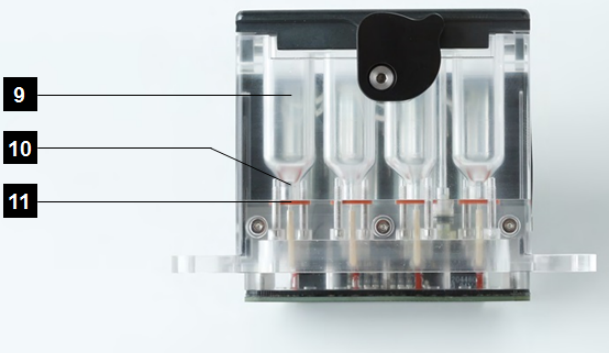
5 **Cartridge serial number**



6 **Injector**
Dispenses reagent into the disc.

7 **Injector drop sensor**
Monitors the quality of each dispensation and delivers feedback during injector priming and injector cleaning operations.

8 **Connector pins**
Connect the cartridge electronics to the instrument.



9 **Reservoir**
Storage of nucleotides/reagents.

10 **Reservoir neck**
Smaller diameter section of the reservoir that holds volumes less than 100 µL.

11 **Filter**
Prevents fine particles from blocking the injector. Be careful not to damage the filter with a pipette tip.

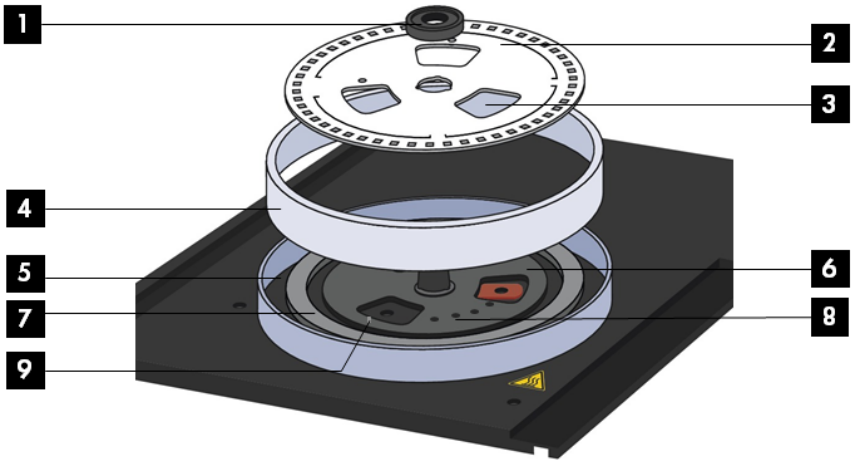
There are three available cartridges described below.

Table 2. Available cartridges

Cartridge name	Storage capacity per injector	Minimum loading volume*	Typical injection volumes	Reagent positions
Nucleotide	3.8 mL	60 µL	100 ± 10% nL	A:dATPaS C: dCTP G: dGTP T: dTTP
Primer	3.8 mL	60 µL	500 ± 10% nL	P1: Primer 1 P2: Primer 2 P3: Primer 3 P4/BB: Primer 4/Binding Buffer
Reagent	3.8 mL	60 µL	500 ± 10% nL	DS: Denaturation Solution E: Enzyme S: Substrate AB: Annealing Buffer

* Includes volume required to complete injector priming.

3.4.2. Chamber



1 **Lock nut**
Screws down to hold disc firmly in place.

2 **Disc**
Well capacity of 20 µL (typical volume of 10 µL). The disc index hole (outer) is used to orient the disc into place, while the disc detection hole (inner) is used as part of the disc detection system.

3 **Disc grip**
Help to hold, insert, and remove the disc.

4 **Absorber strip**
Absorbent strip to collect liquid waste spun out from the disc wells during a wash cycle and priming/cleaning waste.

5 **Absorber strip cavity**
Contains the absorber strip inside the chamber.

6 **Rotor**
The rotor spins at 60 rpm with vibration to facilitate mixing of the reaction. A higher speed is applied during a wash cycle to remove supernatant.

7 **Disc heater ring**
Allows for the heating of wells to achieve sequence primer annealing, and the immobilization of magnetic beads during a wash cycle.

8 **Disc detector**
Optical sensor allows for the determination of the disc type used and if a disc is in place correctly.

9 **Disc index pin**
Ensures correct positioning of the disc into the instrument.

3.5. Analysis software

The PyroMark Q48 Autoprep System is shipped with PyroMark Q48 Autoprep software.

The computer used for setup of runs and data analysis should have the following as minimum specifications:

- Microsoft® Windows® 7 (English version) Operating System
- Pentium® IV processor (3 GHz) or higher
- Minimum 1 GB RAM for 32 bit operating system; minimum 2 GB RAM for 64 bit operating system
- Minimum 100 MB free hard drive capacity
- Display resolution of 1280 x 1024 pixels
- USB port
- CR-ROM
- Pointer device (mouse or similar)

To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe® Reader® can be downloaded at **www.adobe.com**.

4. Installation Procedures

4.1. System delivery

The following items are delivered:

- PyroMark Q48 Autoprep instrument (including USB flash drive)
- Lock nut
- Thumb screws
- Injector cartridges
- Power cords
- User manual (CD)
- PyroMark Q48 Autoprep software (CD)
- Multi-dispense pipette

Reagents and other accessories can be ordered separately. Visit **www.qiagen.com**.

4.2. Requirements

4.2.1. Installation site

The PyroMark Q48 Autoprep System must be located out of direct sunlight, away from heat sources and away from sources of vibration and electrical interference. Refer to Appendix B – Technical Data for the operating conditions (temperature and humidity). The site of installation should be free of excessive drafts, excessive moisture, and excessive dust, and not subject to large temperature fluctuations.

Refer to Appendix B – Technical Data for the weight and dimensions of the PyroMark Q48 Autoprep.

Ensure that the workbench is level, dry, clean, and low vibration, and has additional space for accessories. Approximately 40 cm (15.8 in.) clearance above the workbench is required to accommodate the PyroMark Q48 Autoprep instrument with the chamber lid open. Allow at least 20 cm (8 in.) of free space behind the instrument to allow for opening of the chamber lid. Operation of the PyroMark Q48 Autoprep lid is driven by a motor controlled by the software. Insufficient space to operate the chamber lid may result in damage to the lid on opening.

The PyroMark Q48 Autoprep instrument must be placed within approximately 1.5 m (59 in.) of a properly grounded (earthed) AC power outlet. The power lines to the PyroMark Q48 Autoprep instrument should be voltage regulated and surge protected.

Note: We recommend plugging the instrument directly into its own power outlet and not to share the power outlet with other laboratory equipment. Do not place PyroMark Q48 Autoprep instrument on a vibrating surface or near vibrating objects.

To ensure proper ventilation, maintain a minimum clearance of 5 cm (2 in.) at the sides and 20 cm (8 in.) at the rear of the PyroMark Q48 Autoprep instrument.

Slits and openings that ensure the ventilation of the PyroMark Q48 Autoprep instrument must not be covered.

Note: We recommend using the instrument only in post-PCR environment.

4.2.2. Power requirements

The PyroMark Q48 Autoprep instrument operates at input 100–240 V AC, 50/60 Hz. For details, please refer to Appendix B – Technical Data.

Make sure that the voltage rating of the PyroMark Q48 Autoprep instrument is compatible with the AC voltage available at the installation site. Mains supply voltage fluctuations should not exceed ±10% of nominal supply voltages.

4.2.3. Grounding requirements

To protect operating personnel, the PyroMark Q48 Autoprep instrument must be correctly grounded (earthed). The PyroMark Q48 Autoprep instrument is equipped with a 3-conductor AC power cord (IEC 60320, type C14). To preserve this protection feature, do not operate the PyroMark Q48 Autoprep from AC power outlets that have no ground (earth) connection.

Review the information in Section 2.2 "Electrical safety", in particular the instructions regarding grounding and cable selection.

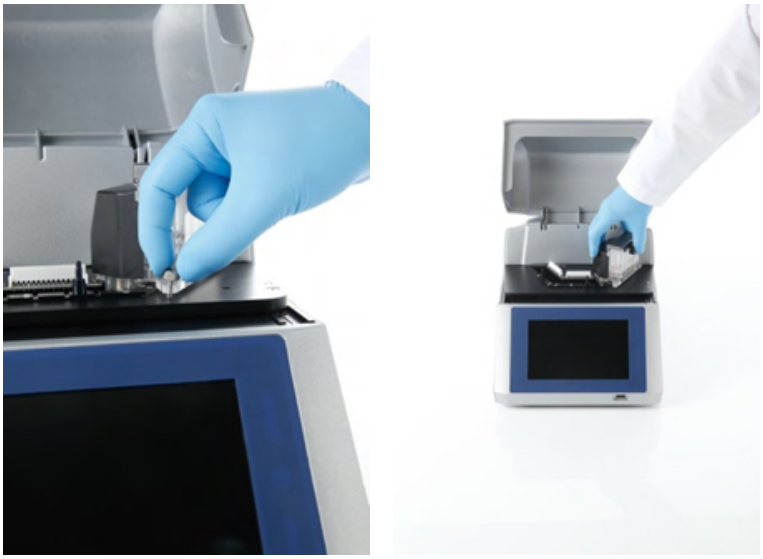
4.3. Unpacking and installation

1. Remove the parts box and foam packing to gain access to the PyroMark Q48 Autoprep instrument. Do not throw away the packaging, as it will be needed if the instrument has to be shipped again.
2. Carefully remove the PyroMark Q48 Autoprep instrument from the box and place it on a surface suitable for installation (see Section 4.2.1).
3. Remove the transport lock screw and store in the screw holder on the back of the instrument, so that it is easily found in case the instrument needs to be transported.

4. Remove the injector cartridges from the boxes and insert them in the following positions as shown in the illustration in Section 3.4:
    - Nucleotide (A, C, G, and T) into the left side
    - Primer (P1, P2, P3, and P4/BB) into the middle
    - Reagent (DS, E, S, and AB) into the right side

Note: We recommend installing the cartridges before switching on the instrument. If no cartridges have been installed, or the cartridges are installed in the wrong order, you will be prompted to install the cartridges correctly upon pressing the **Sequence** button on the Home screen.

5. Slot the cartridges into place ensuring that the air pin and air hole align, and the electrical connectors align. Make sure the cartridges are connected properly by exerting pressure on the rear part of the cartridge (black box).



6. Lock the cartridges into place using the thumb screws.

   Do not insert the silver cartridge thumb screws into the top plate when no cartridges are installed. Without the cartridges in place, the screws can protrude too deep and scratch the lower plate.

WARNING/ CAUTION

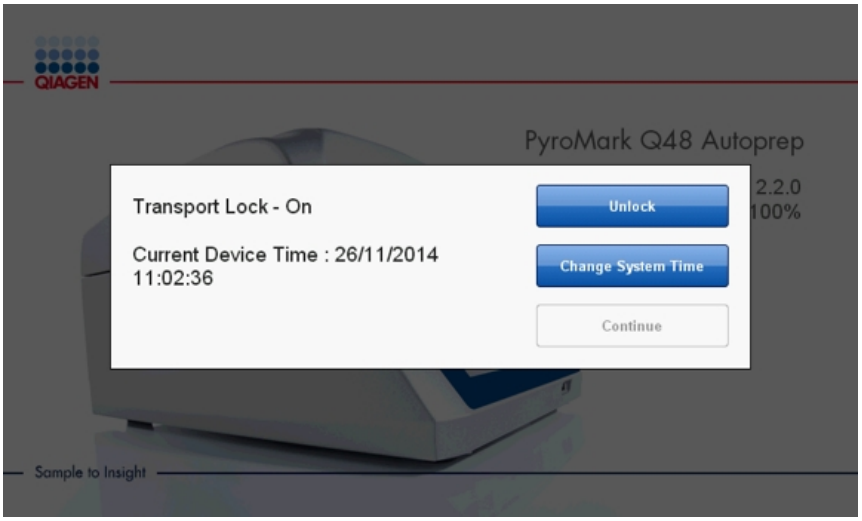
Condensate inside instrument after extreme storage conditions

Condensed humidity inside the instrument may cause personal injuries or damage to the system, when connecting the instrument to the power mains.

To avoid this, let the instrument adjust to the new ambient conditions for at least 2 hours prior to connecting the power cable.

7. Insert the power cable into the back of the PyroMark Q48 Autoprep System and connect it to a power main. Turn on the instrument. The instrument will take a few seconds to initialize.

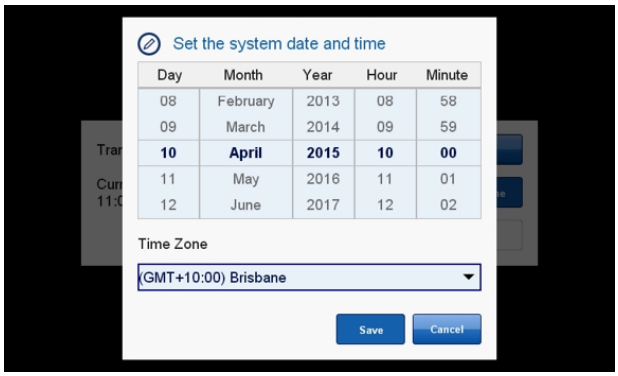
   During the first start up of the PyroMark Q48 Autoprep System, the software will display an installation screen on the instrument touchscreen.



8. Press **Unlock** to open the instrument lid. The lid will automatically slide open.

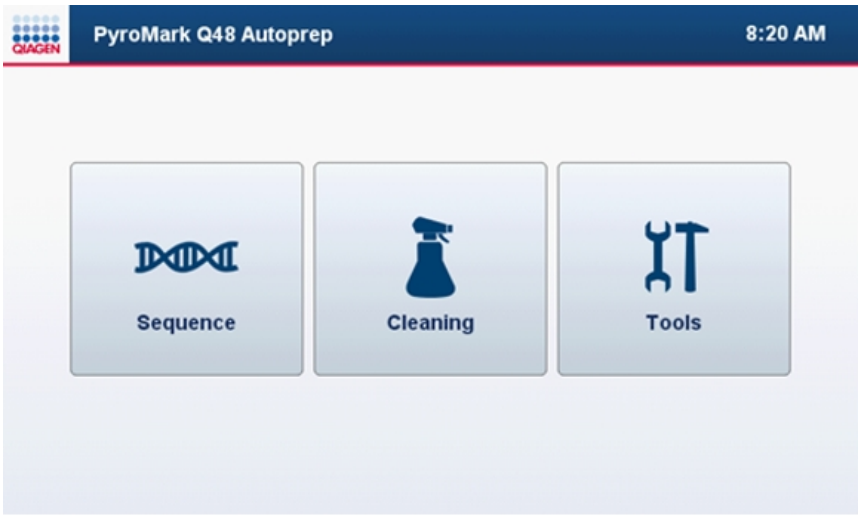
   Note: If the lid is impeded, it will stop and the software will inform the user. Remove any obstacle impeding the lid and press **Retry**.

9. Change the system date, time, and time zone and press **Save**.



10. Press **Continue**.

    The touch screen should now display the Home screen with three available options: **Sequence**, **Cleaning**, and **Tools**. The time will be displayed in the upper right corner of the screen.



    The instrument is now ready for use.

4.4. Repacking the instrument for shipping

When repacking the instrument for shipping, the original packaging materials must be used. If the original packaging materials are not available, please contact QIAGEN Technical Support. Ensure that the instrument is properly decontaminated prior to packing and that it poses no biochemical hazard to others. Follow the unpacking instructions (see Section 4.3) in reverse order. Take special care to carry out the chamber locking program, which must be done while the instrument is powered on. Then add the transport lock screw as described in Section 5.6 “Transport lock”. The cartridges must be packed in the separate boxes and should not be left inside the instrument, as transport vibrations may break the cartridge vessel.

4.5. Installing of the analysis software

4.5.1. Installing PyroMark Q48 Autoprep software

To install or upgrade PyroMark Q48 Autoprep software:

1. Ensure that the computer meets the minimum requirements; see Section 3.5.
2. Close any programs running on the computer.
3. Insert the PyroMark Q48 Autoprep Software CD into the CD drive.
4. In the CD menu, click **Install PyroMark Q48 Autoprep Software**.
5. If the CD menu does not appear automatically, perform the following steps:
    a. Select **(My) Computer** in the Windows **Start** menu.
    b. Right-click the CD drive with the software CD and select **Explore**.
    c. Double-click the file **autorun.exe**.
6. When the software has been successfully installed, click **Exit Setup** in the CD menu.
7. Use Windows Update (**www.update.microsoft.com**) to check for any critical updates to the .NET Framework 4.0.

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your system administrator.

To view reports generated by PyroMark Q48 Autoprep software in PDF format, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

4.5.2. Uninstalling PyroMark Q48 Autoprep software

1. Go to **Start** > **Control Panel** > **Programs**.
2. Click **Programs and Features**.
3. In the list of programs, select **PyroMark Q48 Autoprep**.
4. Click **Uninstall**.
5. Repeat steps 3 and 4 for PyroMark Launcher.

5. Instrument Administration

This section describes how to operate the PyroMark Q48 Autoprep System.

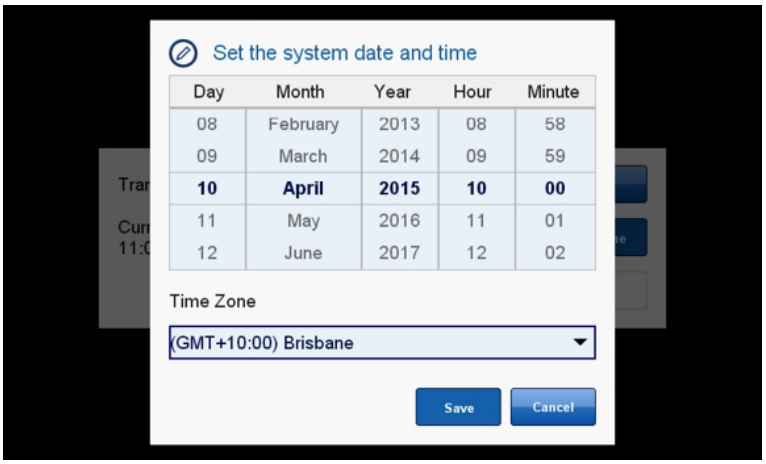
Before proceeding, we recommend reading Section 3.4 to familiarize yourself with features of the instrument.

5.1. Setting date and time

Setting the date and time correctly ensures an accurate date and time stamp in the instrument run logs and the analysis reports. The **System Time** should be updated during instrument installation or after a transport lock.

Set the date and local time by scrolling through the time and date wheels.

Set the time zone using the selections available in the drop-down menu.



5.2. Copying run files

Copies of previous run files are stored on internal memory. Up to 12 previous run files are available with the most recent run files at the top.

Note: When space becomes insufficient, the run files are deleted in chronological order, regardless of whether files have been already copied onto a USB flash drive or not.

Note: We recommend to use the provided USB flash drive that has been verified to work properly with the instrument. Do not connect other device types than USB flash drives to the USB port (e.g., portable USB hard disks) as these are likely to trip the instrument.

Copy run files as follows:

1. Click **Tools**, and select **Run Files** to access run files.
2. Click the run file you want to copy.
3. Click the arrow that appears in the right corner.
4. Alternatively, all run files can be saved at once using the **Copy all** button.

5.3. Viewing software and hardware version

View the PyroMark Q48 Autoprep software and hardware versions by clicking **Tools** and selecting **System**.

5.4. Upgrading the instrument software

If you have received a software upgrade from QIAGEN, upgrade the software as follows:

1. Download the PyroMark Q48 Autoprep–embedded software from the QIAGEN website.
2. Transfer the file to a USB flash drive.

   Note: Ensure that only one version and copy of the PyroMark Q48 Autoprep–embedded software is on the USB flash drive
3. Insert the USB flash drive into the PyroMark Q48 Autoprep instrument.
4. Select the **Tools** option on the Home screen, then select the **System** folder.
5. Press **Update Software** to install the latest software version.

The upgrade version of software will automatically be detected. Select **Yes** to install the software. It will take a few seconds to install the new software. Once installed, select **Yes** to confirm and restart the software. The software will return the user to the Home screen.

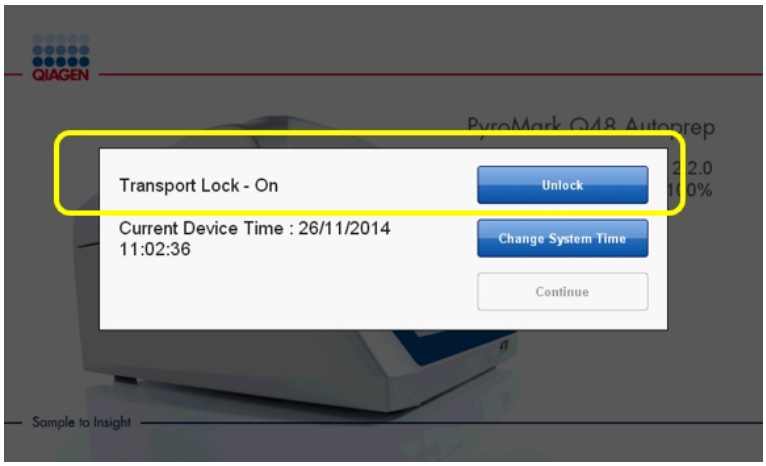
5.5. Reset hardware

In very rare circumstances, the instrument may freeze up with the chamber lid closed and locked, and/or with the heater ring in the up position. Upon restarting the instrument, the **Reset Hardware** button can be used to unlock the lid and return the heater ring to the home position, enabling the instrument for another run.

5.6. Transport lock

Within the **Systems** folder, the user can prepare the instrument for transport by selecting the **Lock** button. Before transporting the device, ensure that the cartridges are empty and removed from the instrument, that there is no disc or lock nut for the disc inside the device, and that the absorber strip has been removed. Once the chamber lid is locked into place, attach the transport lock screw and select **OK** to confirm.

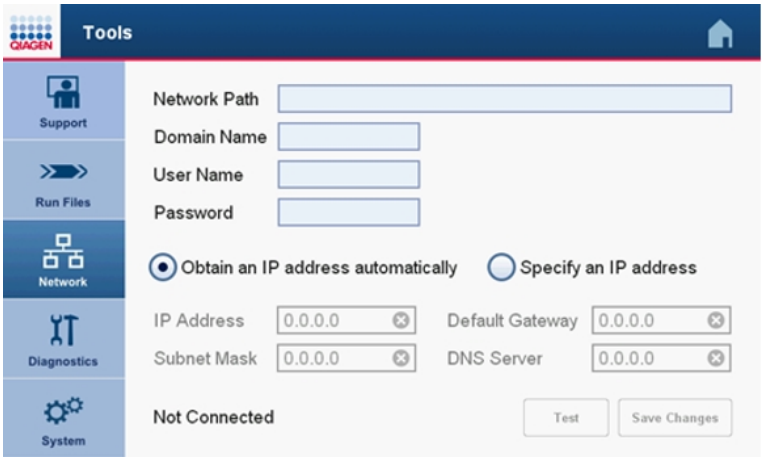
Note: For transporting the instrument short distances, such as to an adjacent lab, we recommend activating the transport lock in the instrument software and attaching the transport lock screw. If possible use a laboratory cart to transport the instrument. If carrying the instrument is unavoidable, one person can carry the instrument by grabbing the base plate from the front and the back of the instrument. Be sure to remove the power cable prior to carrying to avoid a tripping hazard.



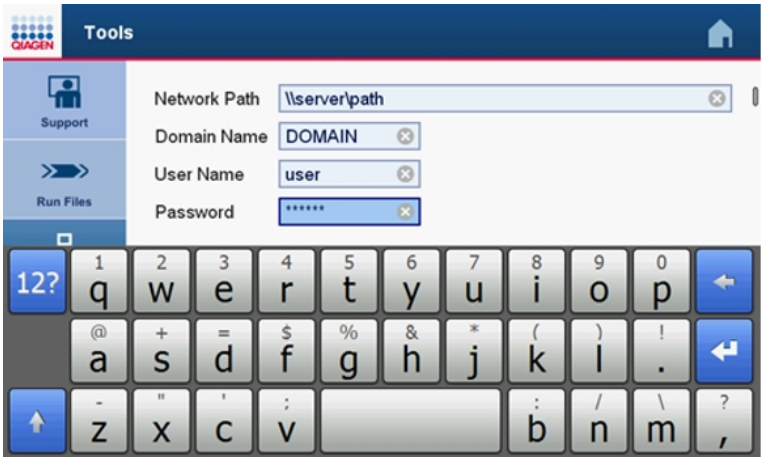
When starting the instrument after it has been transported, press the Unlock button to open the lid. The user must then remove the transport lock screw and confirm by selecting Yes for the chamber lid to open.

5.7. Connecting to a network

The PyroMark Q48 Autoprep System can be connected to a TCP/IP network through the Ethernet port at the rear of the instrument. The Network page in the Tools controls the configuration of this feature. The network administrator should be consulted before configuring the network settings.



Touching any of the input fields will show a virtual keyboard on the screen that can be used for entering the settings. The visible portion of the settings window can be scrolled by touching and dragging the window. The keyboard can be dismissed by touching the blank background of the visible portion of the setting window.



Once the correct network settings have been entered, select the **Save changes** button to save the network settings. It can take up to a minute for the instrument to successfully connect to the network share after changing the settings (see Section 5.7.4).

If the instrument has successfully connected to the network, it will be possible to test the settings entered by touching the **Test** button. If the instrument was able to read and write to the specified network share, a success message will be presented; otherwise, an error message will be displayed. Contact the network administrator for assistance if required.

5.7.1. Network share settings

The PyroMark Q48 Autoprep System can load and save run files to a shared folder that is accessible from the network to which it is connected.

5.7.2. Network path

This defines the location of the network folder used for run files. It must be entered as a UNC path which has the general form:

```
\\<computer-name>\<share-name>\<optional-additional-path>
```

For example, `\\myserver\labfiles\PyroMark Q48 Autoprep-runs` will look for a folder called "PyroMark Q48 Autoprep-runs" in a share called "labfiles" on a computer called "myserver".

Any shared folder that is compatible with Microsoft Windows networking protocols may be used.

5.7.3. User name, domain name, and password

These three fields define the network credentials of the user that will be used to connect to the specified network path.

The domain name should be set to the name of the Microsoft Windows Domain if the target computer is part of a domain. If the target computer is not part of a domain, then enter the name of the target computer here.

The user name and corresponding password should correspond to a user in the domain or target computer. The user must have read-and-write access to the specified network path. This password is stored in the machine and may pose a security risk if the device were to be stolen. For this reason, we recommend that a special user be set up on the target computer or domain that is given read and write access only to the specified network path. Please consult with the network administrator.

5.7.4. IP address settings

The IP address settings control how the PyroMark Q48 Autoprep System connects to the network. By default, the PyroMark Q48 Autoprep System will attempt to obtain the settings from the network automatically. This will be suitable in most circumstances; however, the network administrator may require the instrument to have a fixed IP address .

This can be set by selecting the manual option and entering an IP address (IPv4), subnet mask, default gateway, and DNS address. These settings will be provided by the network administrator.

Note: The network protocol is based on version 1.0 of the SMB protocol. For network security, we recommend using a separate LAN (i.e., a designated lab network). Do not directly connect the instrument to the internet.

A user name and password will likely also be required. Please obtain the proper login settings from your network administrator.

Note: For network security, we recommend using dedicated and limited instrument login credentials instead of personal login credentials to access network drives.

The IP address currently assigned to the instrument will be visible at the bottom left of the screen. If the instrument has been unable to negotiate an address with the network, the Not Connected message will be shown. This may indicate a problem with the IP address settings or a problem with the physical connection to the network. In that case, confirm that the Ethernet cable is correctly connected at the rear of the instrument and that the other end of the cable is connected to the appropriate network equipment. It may take up to a minute to connect to the network after connecting the cable.

6. Performing a Run

This section describes the workflow for how to prepare, run and analyze a run on the PyroMark Q48 Autoprep instrument.

6.1. Preparing a run

Before preparing a run, we recommend reading and familiarizing yourself with the safety information in Section 2.

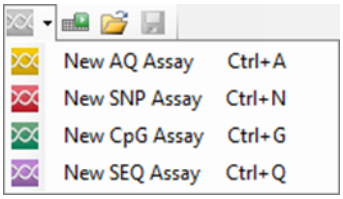
Detailed instructions on setting up a run can be found in Section 7.13 or by using the F1 help function in the PyroMark Q48 Autoprep software.

6.1.1. Starting PyroMark Q48 Autoprep software

In the Windows **Start** menu, select **(All) Programs** > **PyroMark** > **PyroMark Q48 Autoprep**.

6.1.2. Setting up an assay

1. Click  in the toolbar and select the required assay. A new assay file is created.



   For analyzing methylation at CpN sites, select **New CpG Assay** then check the "CpN mode enabled" box to enable the CpN mode.

   Alternatively, you can create a new assay file in the shortcut browser. Right-click the folder you wish to place it in and select **New Assay** followed by the desired assay type from the context menu.

   Note: To add a shortcut to a folder in the shortcut browser, click **Add Folder Shortcut**.

2. Enter the sequence to analyze.

   Note: If creating a CpG assay, we recommend entering the **Sequence Before Bisulfite Treatment**. This enables the software to automatically generate the sequence to analyze and select the most appropriate bisulfite treatment control.

3. Click **Generate Dispensation Order**. If creating an SEQ assay, enter the value in the Dispensation Order field.

4. Click  in the toolbar to save the assay.

   Note: Before running your samples, validate your assay using a reference DNA sample; see Appendix A – Assay Design and Validation.

   Note: When using QIAGEN kits, use the settings stated in the kit handbooks.

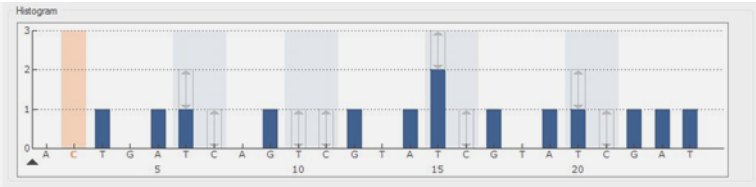
Optional

If desired, enter a note about the assay in the Assay Note field.

Set up the variable positions in the **Variable Positions** tab (AQ, SNP, and CpG assays only).

Click **Lock Assay** at the bottom of the assay setup window to lock the assay for editing.

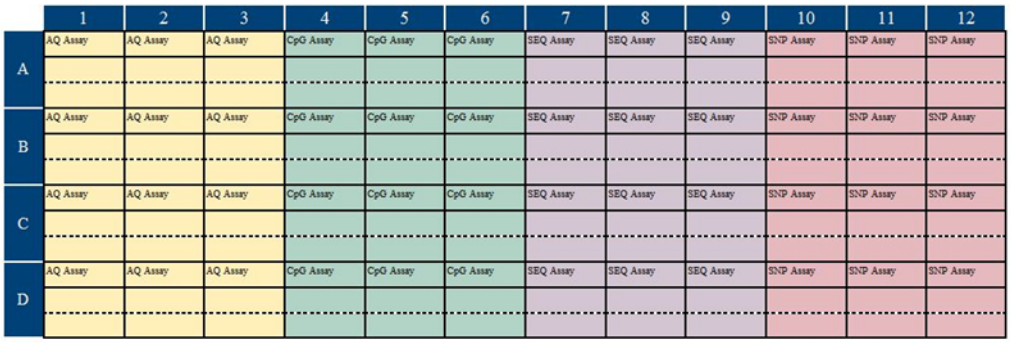
If creating a CpG assay, check that the software has selected a bisulfite treatment control. If no bisulfite treatment control has been automatically selected, add one manually, if possible, preferably at the beginning of the sequence. A bisulfite treatment control can be added manually by adding a **C** before or after a **T** that was a **C** before bisulfite treatment in a forward assay, or by adding a **G** before or after an **A** that was a **G** before bisulfite treatment in a reverse assay, in the dispensation order.



6.1.3. Setting up a run

1. Create a new Run Setup by one of the following methods:
   - Click in the toolbar.
   - Select **New Run** from the **File** menu.
   - Press the R key while holding down the Ctrl key.
   - Right-click a folder in the shortcut browser and select **New Run** from the context menu. Enter a run name and press Enter. To add a shortcut to a folder in the shortcut browser, click **Add Folder Shortcut**.
   - To base your run on a previous run, right-click the processed run file in the shortcut browser and select **Copy and Rerun** from the context menu. Only the run setup, not the run and analysis data, will be copied.
2. If the new run has not yet been saved, click to enter a run name and save the file in the desired folder.
3. Set up the disc in the disc layout of the run file by adding assays to wells and, if desired, entering the sample ID and note for each used well.
4. Add an assay to each well used by performing one of these steps:
   - Dragging an assay from the shortcut browser to a well or a selection of wells.
   - Right-clicking one well and selecting **Load Assay** from the context menu.

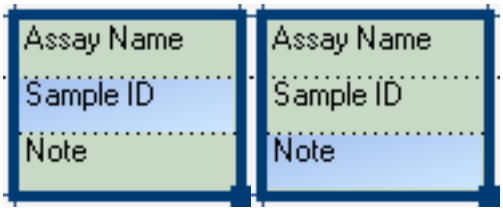
A well is colored according to the assay loaded into the well. Wells with AQ assays are yellow; wells with SNP assays are red; wells with CpG assays are green; wells with SEQ assays are purple.



Disc setup

There are several ways to set up a disc. For example, it is possible to import and paste a sample layout defined in a text file. For more information, see Section 7.13.5.

5. To enter a sample ID or note, select the cell and enter the text. A selected cell is highlighted with a blue background color.



6. In the protocol drop-down list, select the desired protocol for the run.

   Note: There are 2 available protocols for running the PyroMark Q48 Autoprep System, which are selected in the PyroMark Q48 Autoprep software during run setup.

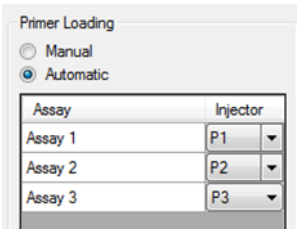
   - Standard protocol

     The **Standard** protocol is used as the default.

   - P4 Extra protocol

     The **P4 Extra** protocol enables the use of the fourth sequence primer injector (P4). When using this protocol, the binding buffer must be loaded manually by the user, because the BB/P4 injector will be used to dispense sequence primer instead of binding buffer.

7. Sequencing primers can be added manually or automatically. Either **Manual** or **Automatic** primer loading must be selected in the Run Setup. In the latter case, one injector can be chosen for each assay.



8. Click  in the toolbar.

9. Press **Pre Run Information** from the **Tools** menu to print the disc setup. When the report opens, click  .

10. Close the run file and copy it to the USB flash drive supplied. The run file can now be processed by inserting the USB flash drive into the USB port at the front of the PyroMark Q48 Autoprep (see Section 3.4).

    Alternatively, the run file can be saved on the network drive and processed on the instrument directly (see Section 5.7).

Optional

If desired, enter the Reagent ID (i.e., the lot number for PyroMark Q48 Autoprep Reagents), a Disc ID of the PyroMark Q48 Disc, and a Run Note in the run file.

6.2. Preparing templates and reagents

At the beginning of the run, the instrument will guide you through run preparation, including absorber strip insertion, injector loading, disc insertion, and bead and template loading.

To start a sequencing run, select **Sequence** on the home screen and load the run either via USB flash drive or via network connection (selectable on the left side of the screen). Start the run setup by clicking on its name and the arrow appearing behind the run name.

Samples to be analyzed using the PyroMark Q48 Autoprep System should be prepared according to the instructions below. The following equipment and reagents are required for template preparation. All reagents and solutions should be at room temperature (15–25°C) before starting. All steps are performed at room temperature unless otherwise stated.

Equipment and reagents to be supplied by the user

- PyroMark Q48 Disc
- Absorber strip
- PyroMark Q48 Magnetic Beads
- Sequencing primer (diluted to 4 µM with PyroMark Annealing Buffer or high-purity water)
- PyroMark Q48 Advanced Reagents or PyroMark Q48 Advanced CpG Reagents (reconstitute the reagents according to the handbook supplied with the reagents)
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Sterile pipette tips with filters for the supplied electronic multi-dispense pipette (please refer to the user manual of the electronic multi-dispense pipette)

6.2.1. DNA amplification

A biotinylated PCR product serves as the template for the Pyrosequencing reaction. Amplify the DNA to be analyzed by PCR with one of the primers biotinylated. To receive valid analysis data, see Appendix A – Assay Design and Validation.

6.2.2. Absorber strip

The absorber strip is an absorbent strip inside the instrument that collects liquid waste spun out from the disc wells during a wash cycle and liquid waste from the priming and cleaning steps.

Important: The absorber strip has a maximum capacity of 8 mL. If a run exceeds this limit in terms of calculated waste disposed, the software will inform the user to replace the strip prior to conducting another run, prime, or clean. Not replacing the absorber strip as recommended could result in some or all of the following issues:

- Oversaturation of the absorber strip and liquid overflow into the instrument chamber
- Condensation within the instrument due to high moisture content in the absorber strip
- Microbial growth

Absorber strip insertion

Insert the absorber strip into the absorber strip cavity by sliding it into place, ensuring that it sits level. Ensure that the ends meet on the left hand side of the absorber strip cavity (9 o'clock position).

Absorber strip removal

CAUTION **Removal of absorber strip**

Upon completion of the run, the absorber strip will contain Denaturation Solution, which contains sodium hydroxide. This can be irritating to the eyes and skin.

Always wear safety glasses, gloves, and a lab coat when removing the absorber strip.

Disposal of the absorber strip should be in accordance with all national, state and local health and safety regulations and laws.

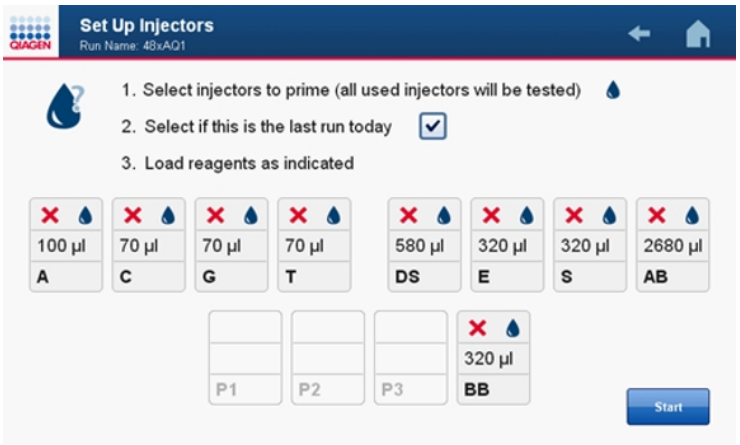
1. Remove the absorber strip by pulling it out. Ensure that excessive amounts of liquid do not drip into the chamber. The chamber cavity can be cleaned with ethanol to remove any excess waste left behind.

2. Insert a clean, dry absorber strip after removing a used absorber strip.

6.2.3. Cartridge loading and injector priming

Injector priming ensures the correct operation of the injectors by loading up the injector system with liquid and removing all the air. During injector priming some of the liquid in the reservoir will be used. Therefore, it is important that the minimum loading volume be adhered to, to ensure that adequate levels of reagent remain to achieve the sequencing run.

1. Open the injector cover and then open each individual cartridge lid.



2. Select injectors to prime by touching on the injector box. Selected injectors will display a droplet symbol.

   All injectors requiring mandatory injector priming will be displayed with a red cross and drop symbol ( ). You cannot override these selected injectors.

   Note: Any injectors not being used in the run will be left blank and cannot be selected for injector priming.

   Injectors with a green tick ( ) indicate that they have been primed and do not require injector priming. However, the user may elect to perform another prime by touching on the injector box to display a drop symbol. Injectors with a question mark suggest discretionary injector priming. These injectors were previously primed, but may require another prime as they have been idle for an extended period of time.

3. Select if this is the last run of the day.

   If there is more than one run per day, the user can enable the software by deselecting this option to calculate and add the extra volume required for the next run without requiring further injector priming.

   Note: If the option "last run of the day" is selected, the software will calculate the minimum volumes required to achieve one run only. A mandatory clean will be required after the last run of the day.

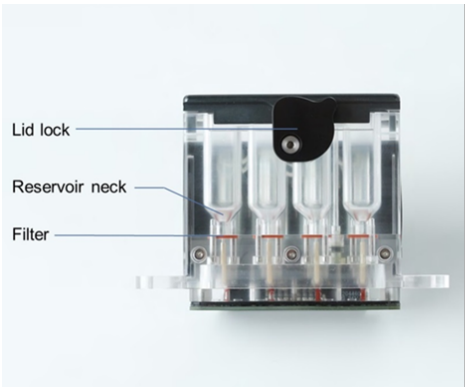
4. Pipet each reagent into the designated injector, according to the volumes shown on the instrument touchscreen. Use the volumes displayed to ensure sufficient reagent is available for the initial injector prime as well as for dispensations during the run. Changing the response in steps 2 or 3 will change the required reagent volumes.

   Important: Ensure that the reservoir capacity of 3.8 mL is not exceeded.

   Important: Ensure that the tip of the pipette does not touch the filter at the bottom. This can remove solid parts from the filter, which can lead to blockage of the cartridge reservoirs.

Important: In case reagents are pipetted into the wrong injector position (e.g., AB into BB injector), we recommend cleaning the affected injector 3 times using the routine cleaning program (see Section 6.5.2 "Cleaning the injectors"). If this occurs, abort the current run, go back to the **Main** menu and start the cleaning program described in Section 6.5.2. Select the affected injector, and run the cleaning program three times in total. After the third cleaning, go back to **Main** menu and reload the run setup file. Continue filling the remaining positions with reagents.

Note: Information about the required sequencing primers can be brought up on screen by selecting the Information button on the sequencing primer injectors.



Note: If loading volumes less than 100 µL, ensure that the solution is dispensed from the top of the reservoir neck.

Note: Slide the chamber lid toward the front of the instrument to make it easier to load into the reservoirs.

Note: When loading injectors try to avoid air bubbles in the liquid as they can influence single dispensations.

Note: In contrast to other PyroMark systems, PyroMark Wash Buffer is not needed and is replaced by PyroMark Annealing Buffer.

5. Once all the reservoirs have been filled with the required reagent, close and lock the cartridge lids using the lid locks.

   Important: Remember to store the reagents at 4°C once all reservoirs are loaded to ensure long reagent shelf life.

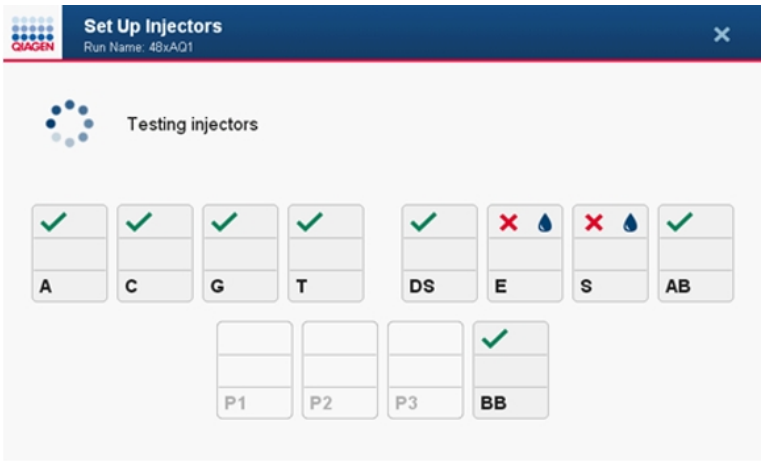
6. Select the **Start** button to begin injector priming and testing.
7. A confirmation message "Start prime and test?" will appear. To confirm, select **Yes**.

All injectors selected for priming will undergo the prime protocol. The chamber will rise to the prime position and the injectors will be pressurized. If the instrument reports a problem with the pump reaching pressure please check that the cartridge lids are not open.

The chamber lid will move the injectors over the front half of the absorber strip and dispense the solution to prime the injectors. All injectors that have undergone a prime will be displayed with a question mark.

Accidentally moving the chamber lid during injector priming will pause injector priming and deliver a warning message indicating that the lid has moved. Select **Retry** to continue with the prime.

An **Abort** icon in the top right corner can be used to stop injector priming. A confirmation message, "Abort prime?", will appear. Select **Yes** to stop injector priming or **No** to continue. Following the abort, the injector status will be updated to denote the current state of each injector.



Following the prime, all required injectors will undergo a number of test shots. The test shots ensure that the injectors are operating correctly by observing the drop sensor data. Injectors that pass the test will be displayed with a green tick (✓).

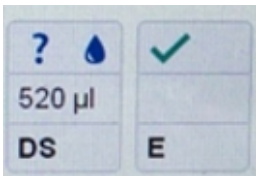
Once injector priming has completed successfully, the software will progress to the next step with the chamber lid automatically opening.

Important: Navigating to Home following injector priming will require performing injector test shots in the following run.

Injectors that fail the test will have additional priming and testing to recheck. Further failure will mean the injectors are displayed with a red cross (✗).

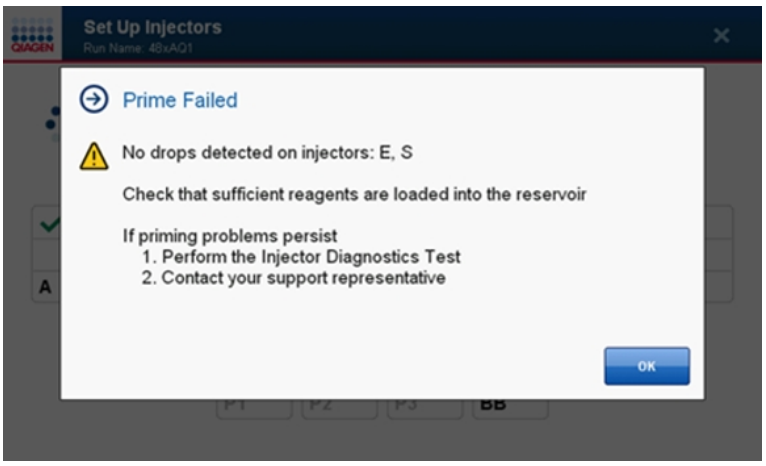
If any of the injectors has failed the prime and test protocol, a Failed Prime warning will appear indicating which injectors have failed. Failure to pass injector priming could be due to insufficient reagent being loaded. The user has the option to Retry the priming and testing or Skip to the next part.

Note: If priming fails and more than one priming step must be performed, the volume in the failed injector is reduced and is not sufficient for dispensations during the run. If this occurs, the display will inform the user to load additional 50 µL into the indicated injector to ensure that it contains the required volume (only the final volumes of the failed injectors are displayed).



If priming problems persist, the user can perform the injector diagnostic test (see Section 8.5).

Important: Using injectors that have failed priming and testing may result in poor data.



6.2.4. Inserting the PyroMark Q48 Disc into the instrument

The PyroMark Q48 Disc may be loaded with template and bead mix after insertion into the instrument. Alternatively, the disc can be loaded outside the instrument and inserted just before the run.

To insert a disc into the instrument, follow these steps:

1. Insert a PyroMark Q48 Disc into the instrument. Hold the disc using the disc grips, position the disc index hole (outer hole near well B1) over the disc index pin on the rotor hub, and insert.

   Note: The orientation of the disc grips should match up to those on the rotor. The disc detection system will recognize and warn the user if a disc is not properly inserted.

2. Lock the disc into position by screwing down the lock nut.

   Important: The disc must be locked into position to avoid spilling the contents of the disc into the chamber.

6.2.5. Loading template and beads into the PyroMark Q48 Disc

The Pyrosequencing template (Biotinylated PCR product) and PyroMark Q48 Magnetic Beads are loaded into a disc manually according to the following instructions.

Note: When running the P4 extra protocol, 5 µL PyroMark Binding Buffer must be manually pipetted into the disc wells along with the Pyrosequencing template and PyroMark Q48 Magnetic Beads.

Note: When running the standard protocol, the PyroMark Binding Buffer is automatically injected into the sample wells by the instrument injector, so there is no need to manually pipet it into the wells.

Note: The two wells on the disc marked by the Ø symbol are waste wells; it is not necessary to load beads into these wells.

PyroMark Q48 Magnetic Beads

PyroMark Q48 Magnetic Beads fall out of suspension very quickly after vortexing/mixing due to their high relative density. Follow these guidelines when pipetting the beads. Failure to do so will result in inconsistent bead amounts and poor sequencing results.

- Use the supplied electronic multi-dispense pipette when dispensing beads into a larger number of wells (>5).

- When using the multi-dispense pipette, never dispense more than five samples at a time, as the beads will clump inside the pipette.
- Always use the reverse pipetting function to ensure sufficient volume for the last dispensation.
- Ensure that the bead slurry is mixed before taking the next aliquot.
- Always centrifuge any residual bead slurry from the top of the stock tube before returning to storage. The consistency of the slurry can change over time if residual solution is left at the top of the stock tube.

Note: See manual of the supplied multi-dispense pipette for detailed information on programming.

1. Vortex the bead slurry to homogeneity before dispensing.
2. Pipet 3 µL PyroMark Q48 Magnetic Beads into the required wells of the PyroMark Q48 Disc immediately after resuspension.

   Note: Position the pipette tip in the middle of the bead slurry to ensure consistency of each aliquot.

   Note: Avoid excess liquid on the outside of the pipette tip. Excess liquid can be removed by gently touching the pipette tip against the top of the bead tube when removing the tip.

3. Use the image below as a visual reference example for required bead amount (image was taken after templates were also loaded into the wells). Well 1 contains too few beads, well 2 contains the correct amount of beads, and well 3 contains too many beads.

Note: It is not necessary to equally distribute the beads within the well. At the beginning of the sequencing run the disc will be shaken, resulting in an equal distribution of beads.

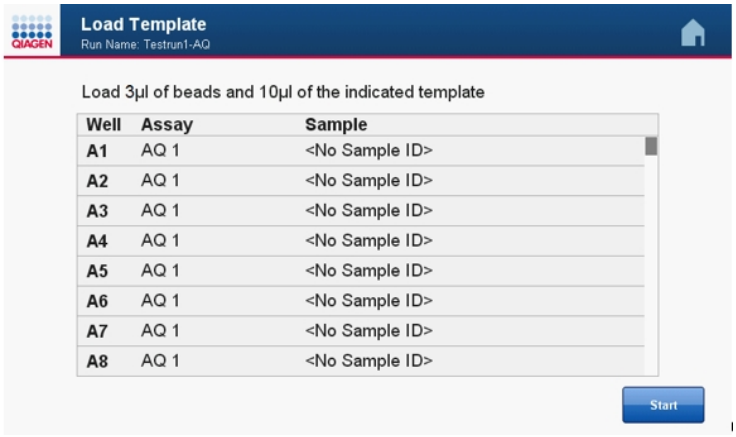
4. Repeat the resuspension step between each pipetting step.
5. After dispensing beads into all required wells, discard the remaining beads in the pipette tip using the purge function.
6. Store the beads immediately after use according to the manufacturer's guidelines to ensure a longer shelf life.

Pyrosequencing template

1. Pipet 10 µL biotinylated PCR product into the correct wells of the PyroMark Q48 Disc. The software indicates the well position for each sample. Alternatively, use the Pre run print out from the PyroMark Q48 Autoprep software as a sample position guide.

   Note: If using less than 10 µL of PCR product, add high-purity water to the sample to give a final volume of 10 µL.

   Samples with no names will be displayed as "No sample ID".



Note: When running the P4 extra protocol, the user will be prompted by the instrument to additionally pipet 5 µL PyroMark Binding Buffer into each well.

2. Ensure that the template thoroughly covers the beads inside the well.
3. Ensure that the disc is correctly inserted into the instrument and locked in place (see Section 6.2.4).

6.3. Starting a run

1. After the template has been loaded, press **Start** to begin the run.
2. A confirmation message will display “Close injector cover. Start run?” To start the run, close the injector cover and select **Yes**.

   To go back and make any adjustments or to confirm any setting, select **No**.

   If the injector cover is not closed at the beginning of the run, the following warning will appear: “Injector cover check. Higher than expected signal detected. Check the injector cover is closed and select retry.”

   Note: During the run, the injector cover must remain closed, such that ambient light does not disturb the highly sensitive PMT measurements.

6.4. Monitoring a run

When a run is started, the instrument will first initialize, which could take a few seconds. The run will then begin depending on the selected protocol.

For both protocols the lid will open during the heating step to avoid condensation build up.

WARNING **Moving parts**

To avoid contact with moving parts during the operation of the PyroMark Q48 Autoprep System, the instrument must be operated with the hood closed.

Do not remove the cover panels since there are no user-serviceable parts inside. If there is a problem with the PyroMark Q48 Autoprep System, contact QIAGEN Technical Services immediately.

WARNING **Moving parts**

The chamber lid of the PyroMark Q48 Autoprep instrument opens and closes automatically during operation to prevent moisture buildup inside the instrument.

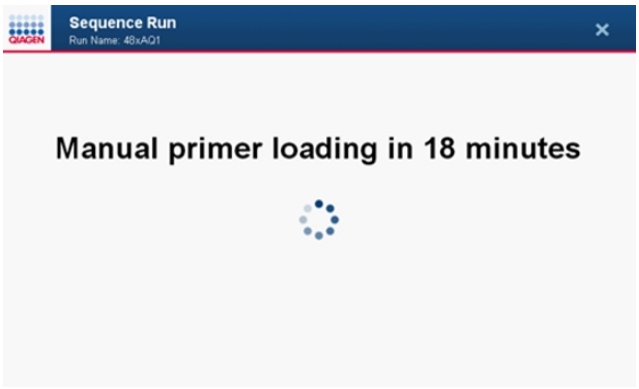
WARNING **Hot surface**

The disc heater within the PyroMark Q48 Autoprep instrument can reach temperatures of up to 95°C (203°F). Avoid touching it when it is hot.

A countdown timer to either manual sequence primer loading or sequencing will appear.

If the user has selected automatic sequence primer addition, no further action is required until the end of the run and the countdown time will display “Sequence starts in x minutes”.

If the user has selected manual sequence primer addition, the software will display the countdown timer as “Manual primer loading in x minutes”.



If a disc has not been inserted, the instrument will warn the user with an audible beep and display the following message: Disc Check – No disc was detected. A 48-well disc is required for this run. Check if sample disc is correctly inserted. Insert the disc and select the **Retry** button.

If the cartridge lid is left open, the instrument will display the following message: “Pressure Failure. Pump failed to reach pressure. Check: (1) All cartridge lids are closed and locked. (2) All cartridges are correctly inserted. If problems persist, contact your support representative.” Close the injector lid and select the **Retry** button.

6.4.1. Manual sequence primer loading

The instrument will notify the user that the sequence primer is required to be loaded, with an audible beep and by displaying the following message: “Select **OK** to open lid and add primers.”

1. Press **OK** to start the manual sequence primer loading.

   The chamber lid will automatically open allowing the user to load the primers. Selecting **Cancel** will bring up a confirmation dialog box: “Do you wish to cancel?” Selecting **Yes** will terminate the run.

2. Manually pipette 2 µL of the required sequence primer (4 µM) into the designated sample.

   The software will display the required sequence primer, as the given assay name, next to the sample well. The final concentration of primer in the 10 µL reaction will be 800 nM.

   Note: Sequence primer concentration can be optimized to suit a particular assay.

3. After all of the sequencing primers are loaded into the samples, press the **Next** button to proceed.

   The chamber lid will close automatically and a countdown timer will display the time until sequencing. No further action is required from the user until the completion of the run.

6.4.2. Automatic sequence primer loading

The instrument will automatically load the required sequencing primer, located in injector positions P1, P2, and/or P3, into the sample well programmed within the PyroMark Q48 Autoprep software. No action is required from the user during this stage or until the completion of the run.

Note: When running the P4 extra protocol, a fourth injector position (P4/BB) can be used for sequencing primers.

Two microliters (2 µL) of sequencing primer (4 µM) will be loaded into an 8 µL volume of annealing buffer per well to give a final concentration of 800 nM.

Multiple primers can be added into one injector to increase assay throughput. Ensure that each of the primers is at the correct concentration (4 µM). Furthermore, ensure that the primers will not cross react.

6.4.3. Automatic gain adjustment

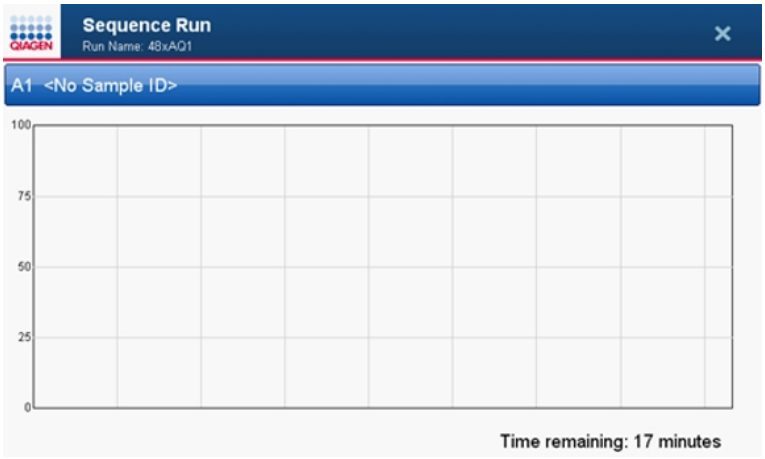
After the addition of enzyme and substrate, the software determines the optimum gain value to provide a similar baseline for all samples within the run to ensure instrument is working in the maximum dynamic range.

Automatic gain adjustment may take a few minutes to complete.

6.4.4. Viewing a Pyrogram during sequencing

During sequencing, the Pyrogram of an individual sample is displayed in real time.

To change the displayed sample, touch the **Sample Table** to bring up the sample list. Scroll to locate the sample of interest and then touch it to display that sample Pyrogram.



A countdown timer is located at the bottom right corner indicating the time left to run completion.

The run name is located in the top left-hand corner.

6.4.5. Aborting a run

A run can be aborted at any time during the sequencing step by selecting the **Abort** button ( ) in the top right corner. The message "Abort Run?" will appear. Select **Yes** to continue the abort. The run will transfer the data to the USB flash drive or network drive. Once transferred, the user will be notified "Run Aborted".

The transferred run file will end with "-Aborted(1)". Multiple aborted runs will be in sequential order to avoid confusion with other aborted runs with the same name. The original run setup will remain available to start again. This negates the need for the user to go back to their PC to setup another run.

Aborting a run during primer annealing

To abort the run during primer annealing, select the **Abort** button in the top right corner. A confirmation message will appear, "Do you wish to abort the run?" If the user selects **Yes**, a warning message may appear informing the user that, "Cooling disc heater to 40°C. Currently: X.X°C".

We highly recommend that the user wait until the temperature is cooled below 40°C before opening the chamber lid. However, the user can **Override** the cool if they want to gain access to the chamber before the temperature is below 40°C.

WARNING **Hot surface**

The disc heater within the PyroMark Q48 Autoprep instrument can reach temperatures of up to 95°C (203°F). Avoid touching it when it is hot.

6.4.6. Run completion

Once the run has completed, the lid will automatically open and the data will be transferred onto the USB flash drive or network drive. It may take a few minutes to transfer larger data files. Once the data are transferred, the user will be notified with “Run Complete”.

Press the **Home** button to return to the home page to begin another run or to clean the injectors.

Remove the USB flash drive and analyze the data on the PyroMark Q48 Autoprep software.

If another run will follow for which other primers are needed, the instrument will inform the user that injectors have been changed since the last run and cleaning of the primer injectors is recommended (only in case of automatic primer loading).

Notification to start the cleaning procedure will automatically appear if the last run of the day was selected.

6.5. Finishing work and shutting down

6.5.1. Removing the PyroMark Q48 Disc

1. Manually open the chamber lid.
2. Unscrew the lock nut.
3. Carefully lift the disc out, using the disc grips.

   This should minimize finger and hand contact with the absorber strip.

6.5.2. Cleaning the injectors

Prior to the first run of the day and upon completion of the final run of the day the injectors must be cleaned with high-purity water.

When an instrument will not be used for a longer time period (approx. 1 month), perform the cleaning more often (2–3 times after last run). During non-usage of the instrument, clean the instrument regularly. Cleaning frequently will not cause any harm.

CAUTION **Injector cleaning**

Injectors must be cleaned within a 12 hour period following the last run to ensure long term injector performance. Failure to comply can result in injectors blocking.

To begin the clean protocol, press the **Cleaning** button on the Home screen.

If the last run of the day was selected, then cleaning of the injectors will be mandatory and the cleaning function will appear automatically.

1. Select injectors to be cleaned by touching on the injector square. A drop symbol ( ) will appear indicating selection. Injectors can be cleaned in between runs if desired or if incorrectly pipetting reagents.

   Injectors with a red cross and drop symbol ( ) require a clean (injectors have been used recently and have not been cleaned). Unlike in injector priming, red cross injectors can be deselected from cleaning.

   We highly recommend, but it is not mandatory, to clean injectors with a question mark (injectors have been cleaned previously but have been idle for over a week). To reduce the potential build-up of material we highly recommend that intermittently used injectors be cleaned regularly.

   Injectors with a green tick ( ) do not require a clean but can be selected if desired.

2. Remove the disc from the chamber.

3. Check that an absorber strip is inserted (refer to Section 6.2.2).

   Important: The absorber strip must be present to avoid liquid being emptied into the chamber during the clean.

4. Pipet 200 µL of high-purity water into the reservoirs of the injectors selected to be cleaned. Ensure that care is taken when pipetting into the cartridge. Pipette tips must not come into contact with the cartridge filter membrane at the bottom of the cartridge.
5. Close and lock the cartridge lids.
6. Select the **Start** button to begin the first clean. Confirm "Empty Injectors" by selecting **Yes**.

   During the first clean, the chamber lid will move each injector into position over the front half of the absorber strip and begin dispensing the liquid out of the injector until the drop sensor detects that there is no more liquid in the injector.

   Injectors that fail to fully empty will be indicated by a warning message describing the issue.

   Note: Depending on the run performed, the residual volume in the injector may be too high to be dispensed within one cycle. In this case a warning message will appear on the touchscreen. Press Retry to start a second round of dispensations to fully empty this injector.

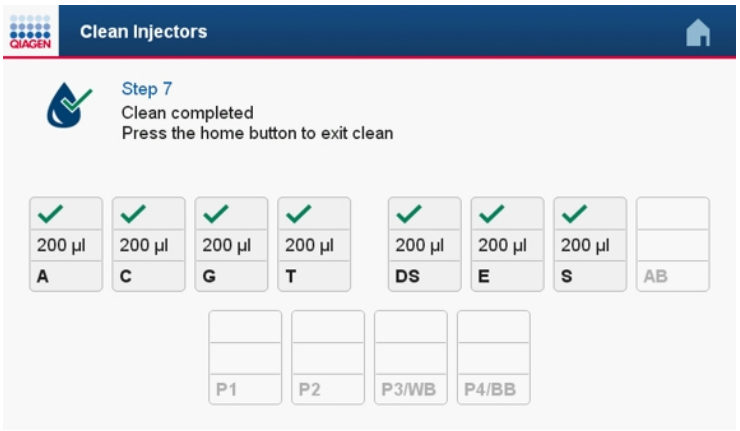
   If the clean is aborted during the purge, the status of each injector that was selected for a clean is updated to a red cross (✖). If the user exits the clean protocol, a message will appear: "Some injectors are not clean; Exit clean?" Selecting **Yes** will return the user to the Home screen. Even though the clean has been aborted, the clean process must be completed in full at another time. Not cleaned injectors will be flagged before any new run begins.
7. After injectors have been emptied, pipet another 200 µL of high-purity water into the reservoirs of the injectors selected to be cleaned.

   Note: Pay attention not to come into contact with the cartridge filter membrane at the bottom of the cartridge.

8. Press the **Start** button to begin the second clean. Confirm "Empty Injectors" by selecting **Yes**.

   During the second clean, the injectors will be dispensed over the back half of the absorber strip. A pause step is introduced after the injectors are loaded to ensure that any leftover reagent is sufficiently dissolved into the water. The injectors are then emptied after the pause.

   Successfully cleaned injectors will be displayed with a green tick (✓). Failed injectors will be displayed with a red cross (✗) and a message indicating what to do next.



9. Unlock the cartridge lids and leave them only slightly open. This helps to prevent condensation and minimizes the amount of dust that might settle into the cartridges.

   Important: Condensation within the reservoirs over time may result in microbial growth and reagent dilution.

10. Select the **Home** button to return back to the Home screen.

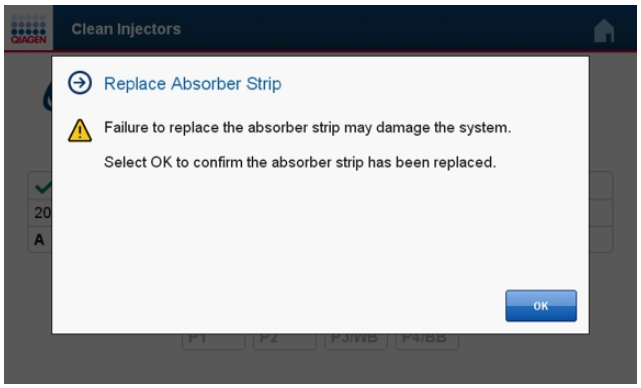
11. Ensure that the injector cover is closed to avoid dust buildup in the cartridges.

    Note: Apply a second wash if it is certain that the instrument will not be used for a period of more than 2 weeks.

6.5.3. Replacing the absorber strip

The user will be notified to replace the absorber strip (refer to Section 6.2.2).

Once the absorber strip is replaced, press **OK** to return to the Home screen.



6.6. Shutting down the instrument

1. To shut down the PyroMark Q48 Autoprep instrument, navigate to the main menu on the instrument touchscreen.
2. Turn off the power using the switch located at the rear of the instrument.

6.7. Analyzing a run

Detailed instructions for analyzing the run are available in Section 7.

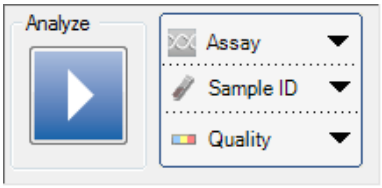
If using a USB flash drive for data transfer, move the processed run file from the USB flash drive to a computer running PyroMark Q48 Autoprep software (see Section 5.2). Alternatively, the run file can be transferred via a network drive (see Section 5.7).

Open the run file by double-clicking the run file in the shortcut browser. If several assay types are included, select analysis mode in the dialog box that opens. The analysis modes displayed in the dialog box are limited to the assay types on the disc.

Note: To update the contents of a folder in the shortcut browser, right-click it and select **Refresh** from the context menu, or press the F5 key.

Note: It is also possible to open the run file by double-clicking it in Windows Explorer.

In the **Overview** tab, analyze selected wells with a valid analysis setup for the current analysis mode.



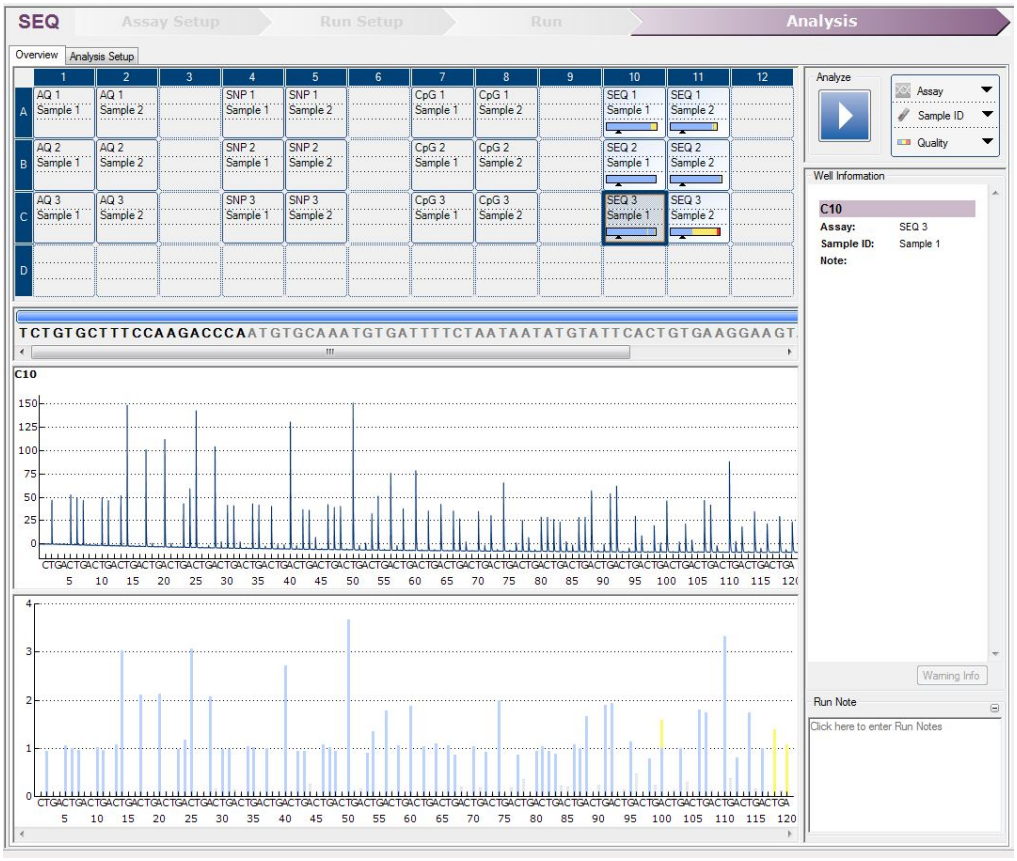
Analysis modes

PyroMark Q48 Autoprep software has four analysis modes: AQ, SNP, CpG, and SEQ. To toggle between the modes, select AQ, SNP, CpG, or SEQ in the toolbar.

Note: How the analysis is performed can be modified using the **Analysis Setup** tab.

6.8. Viewing the analysis results

By selecting an analyzed well in the **Overview** tab, the corresponding Pyrogram is displayed in the Pyrogram area and the well information (including analysis warnings) is listed in the Well Information area.



6.8.1. Quality assessments

The disc overview in the **Overview** tab gives a quick overview of the quality assessments.

The color bar ( ) shows the quality assessment of all variable positions in the well or of all the bases in the base-called sequence.

Quality colors

Blue: Passed

Yellow: Check

Red: Failed

White: Not analyzed*

6.8.2. AQ analysis results

The allele frequencies are displayed in Pyrogram, for example, 

and 

(InDel). The quality assessment is displayed by the background color of the result.

* Either analysis is not supported by the software (e.g., analysis of SNP when in the CpG mode) or the variable position has been deselected by the user (AQ, SNP, and CpG assays only).

6.8.3. CpG analysis results

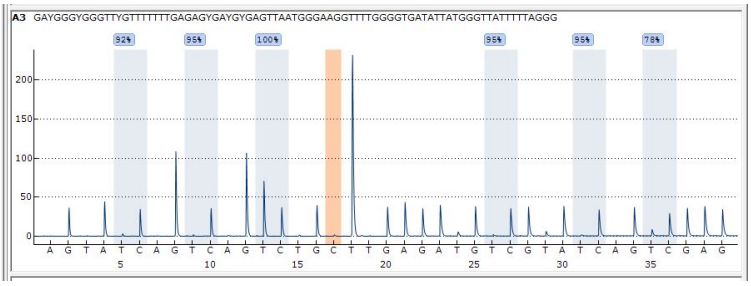
The methylation percentage 96%, or average methylation percentage Average: 25%, are displayed in the Pyrogram. The quality assessment is displayed by the background color of the result.

A methylation bar in the disc overview shows the methylation level for each CpG site in the well.

Light green: Below the expected range

Green: Within the expected range

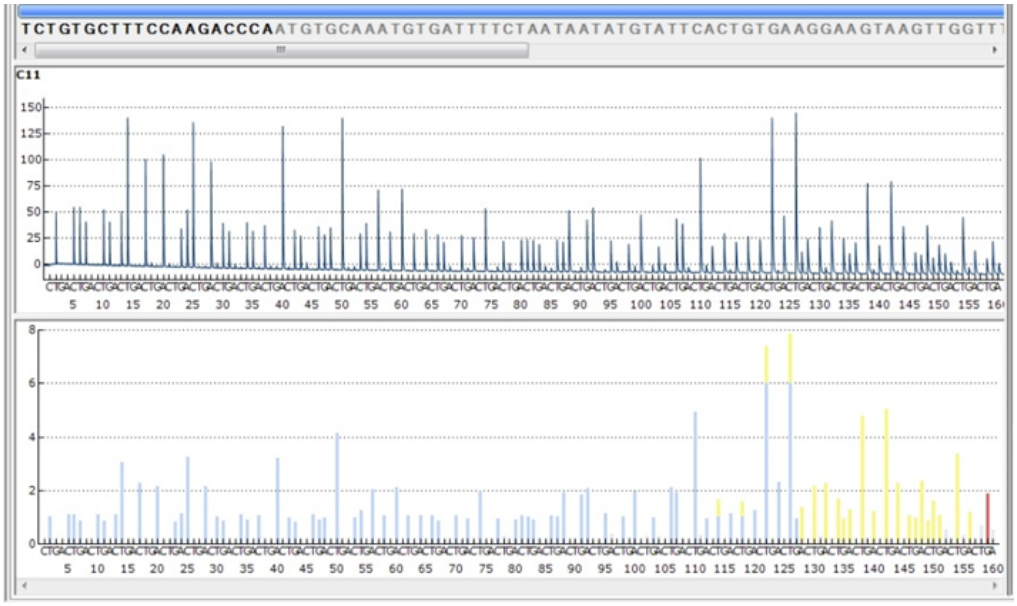
Dark green: Above the expected range



Example Pyrogram for a CpG assay. Variable positions in AQ and CpG assays are highlighted with a blue-gray background color, and bisulfite treatment controls in CpG assays with a light orange background color.

6.8.4. SEQ analysis results

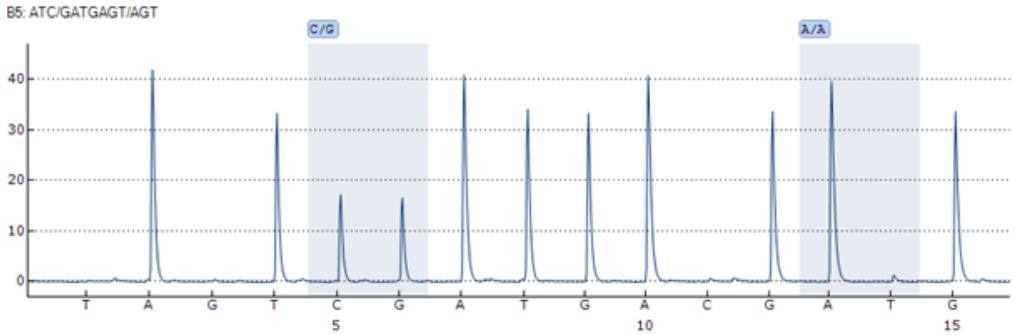
The base-called sequence is displayed in the **Overview** tab. The bases in the base-called sequence and the peaks in the compensated Pyrogram are colored according to their quality assessments.



Example of a base-called sequence and Pyrogram for a SEQ assay.

6.8.5. SNP analysis results

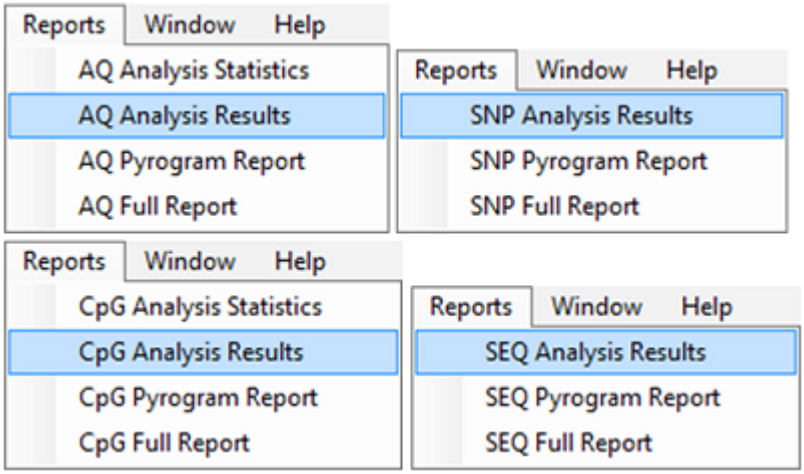
The SNP is displayed in Pyrogram, for example, C/G for heterozygote SNP and A/A for a homozygote SNP. The quality assessment is displayed by the background color of the result.



Example Pyrogram for a SNP assay.

6.8.6. Analysis reports

To generate a report, select the desired report from the **Reports** menu. For more information about the reports, see Section 7.16.



To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

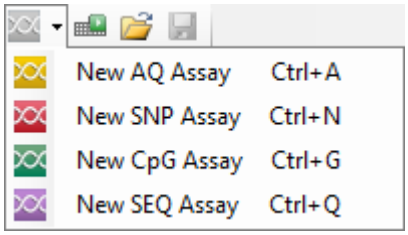
7. PyroMark Q48 Autoprep Software

The PyroMark Q48 Autoprep software enables rapid and straightforward assay and run setup for the PyroMark Q48 Autoprep instrument, as well as accurate analysis of run results. Instrument, software, and other components of the PyroMark Q48 Autoprep System afford:

- High-resolution quantification of di-, tri-, or tetra-allelic mutations
- Genotyping and quantification of InDels
- Built-in quality control for AQ, SNP, and CpG assays using sequence context
- Analysis of methylation at CpN sites
- Analysis of methylation in the presence of SNPs
- Built-in quality control for bisulfite treatment in methylation assays
- Base-calling with quality assessment

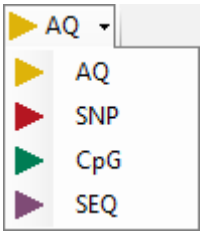
7.1. Analysis modes

PyroMark Q48 Autoprep software has four analysis modes:



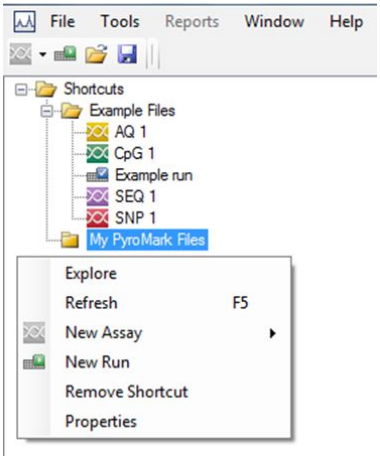
- **AQ**: A variety of quantification studies of mutations such as SNPs and InDels
- **SNP**: Genotype analysis of SNPs and InDels. This mode does not provide quantification of alleles as in AQ mode.
- **CpG**: Methylation analysis of single or multiple CpG sites. For methylation analysis at CpN sites, a CpG assay is used with CpN mode enabled during the assay setup.
- **SEQ**: Base-calling of unknown sequences.

The four different types of analysis can be performed on the same PyroMark Q48 Disc. To toggle between the analysis modes in the Analysis view, select AQ, SNP, CpG, or SEQ in the toolbar.



7.2. Shortcut browser

The shortcut browser provides a quick and easy way to access folder contents and commonly used assay and run files.



The following icons are used to display information about the files:

	AQ assay file
	SNP assay file
	CpG assay file
	SEQ assay file
	A run file that has not been processed
	A run file that has been processed
	Broken shortcut. This may be due to a network server that is temporarily inaccessible or that the file or the folder has been moved, renamed, or deleted outside the software.

The following are instructions to add and remove shortcuts, to update the contents of a folder, and to view file and folder properties:

- Add a shortcut to a folder or drive by clicking **Add Folder Shortcut** or right-clicking the **Shortcuts** folder and select **Add Folder Shortcut** from the context menu.
- Add a shortcut to a file by clicking **Add File Shortcut** or right-clicking the **Shortcuts** folder and select **Add File Shortcut** from the context menu.
- Remove a shortcut by right-clicking the shortcut and selecting **Remove Shortcut** from the context menu. (The files and subfolders in a shortcut folder cannot be removed separately.)
- Update the contents of a folder by right-clicking it and selecting **Refresh** from the context menu or by pressing the F5 key.
- View file or folder properties (e.g., run parameters) by right-clicking the file or folder and selecting **Properties** from the context menu.

Note: If the mouse pointer is positioned over a file or a folder in the shortcut browser, a tooltip displays the file or folder pathway, the file name, the assay note for assay files, and the disc ID and run note for run files (if entered).

The following are instructions to create, open and copy files, and to view the run log for a processed run:

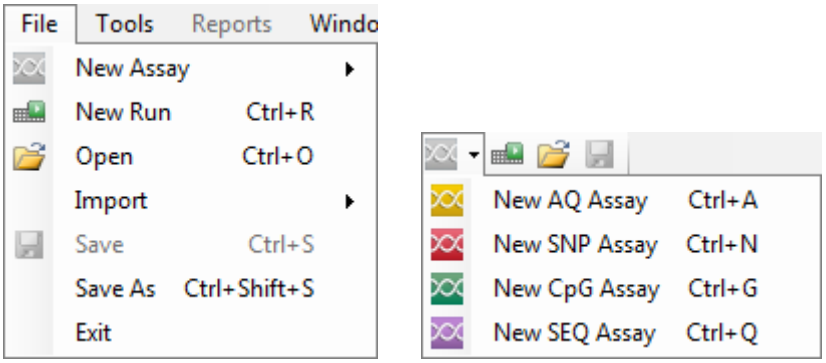
- Create a new assay file by right-clicking the desired folder and selecting **New Assay** and the desired assay type from the context menu. Enter the filename and press **Enter**. To set up the assay, see Section 7.11 for an AQ, SNP, or CpG assay or Section 7.12 for a SEQ Assay.
- Create a new run file by right-clicking the desired folder and selecting **New Run** from the context menu. Enter the filename and press **Enter**. To set up the run, see Section 7.13.
- Copy a processed run file and rerun it by right-clicking the run file and selecting **Copy and Rerun** from the context menu.

  Note: Only the run setup, not the run and analysis data, will be copied.
- Copy a file by right-clicking the folder containing the file and selecting **Explore** from the context menu. Windows Explorer opens. For more information, press the F1 key to open the online help for Windows Explorer.

  Note: To avoid losing data, do not copy a file that is open in PyroMark Q48 Autoprep software.
- Open a file by double-clicking it or right-clicking the file and selecting **Open** from the context menu. To open a processed run file, select **Open** followed by the analysis mode (AQ, SNP, CpG, or SEQ).
- View the run parameters and a run log for a processed run file by right-clicking the file and selecting **Run Information** from the context menu.

7.3. Main menu and toolbars

7.3.1. File menu and toolbars



Select **New Assay** or click in the toolbar and select the desired assay type to create a new assay file. To set up the assay, see Section 7.11 for an AQ, SNP, or CpG assay or Section7.12 for a SEQ Assay.

Select **New Run** or click in the toolbar to create a new run file. To set up the run, see Section 7.13.

Select **Open** or click in the toolbar to open a saved assay or run file.

Select **Create New Run from Sample Layout File** from the **Import** submenu to create a new run using a disc layout for sample IDs and notes (optional) defined in a tab- or comma-delimited text file (*.tsv, *.txt, or *.csv); see "Using the import/insert sample layout file feature" in Section 7.13.5).

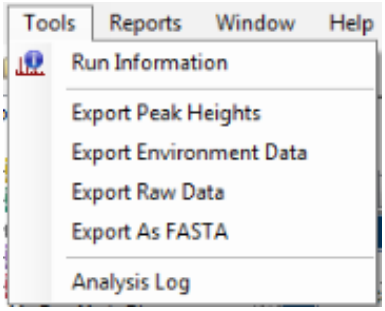
Select **Create New AQ/SNP/CpG Assay from Assay Design File** from the **Import** submenu to create a new AQ, SNP, or CpG assay based on an assay file (*.xml) created with PyroMark Assay Design Software. The software will import the sequence to analyze and the names of the variable positions.

Select **Save** or click in the toolbar to save changes in the current file. If the file has never been saved, select the location and enter the filename in the dialog box that opens.

Select **Save As t**o save a copy of the current file. Select location and enter the filename in the dialog box that opens.

Select **Exit** to shut down the software.

7.3.2. Tools menu for processed run files



Select **Run Information** to view the run parameters and a run log for the current run file. To print the report, click .

Select **Export Peak Heights** to save the peak heights of all used wells as a text file.

Select **Export Environment Data** to save the mixer speed, block temperature, and pressure readings as a text file. The temperatures of the environment, the process chamber lid, and the cooler are also listed.

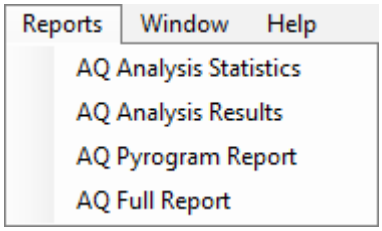
Select **Export Raw Data** to save the intensities and dispensation data as a text file.

Select **Export As FASTA** to save base-called sequences in FASTA format (SEQ assays only). In the dialog that opens, select the wells to be included (all or selected), the sorting order of the wells (row or column), and the bases in the sequences to be included (all, passed, passed + check, or only quality control window).

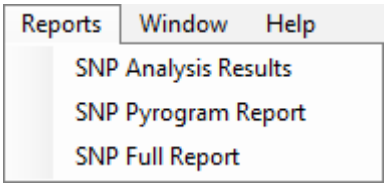
Select **Analysis Log** to view or save the log with all analyses performed on the selected well as an HTML file. Each analysis is logged with the used analysis settings, analysis mode (AQ, SNP, CpG, or SEQ), analysis version, results (including warnings), date and time, and the Windows user account used to perform the analysis (see Section 7.18).

Text files (*.tsv or *.csv) can be imported into Microsoft Excel® or other applications that can handle data that is separated by semicolons (;) or tabs. This is useful when doing further calculations on the data.

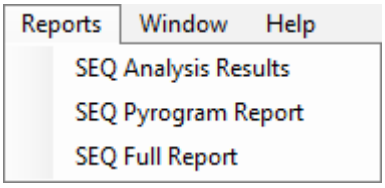
7.3.3. Reports menu



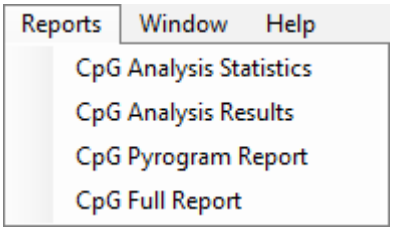
AQ runs.



SNP runs.



SEQ runs.



CpG runs.

The **Analysis Statistics** report includes analysis statistics for all or selected wells.

The **Analysis Results** report includes well information and analysis results for all or selected wells.

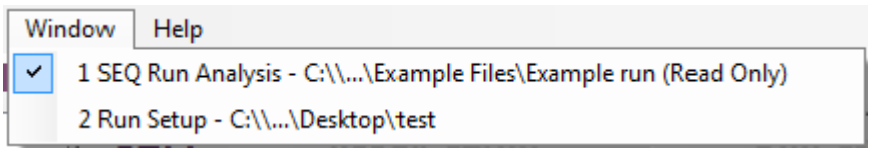
The **Pyrogram Report** includes well information and a Pyrogram for all or selected wells.

The **Full Report** includes run parameters, run log, well information, and analysis results (including Pyrogram) for all or selected wells.

The report options are only available for processed runs. For more information about the reports, see Section 7.16.

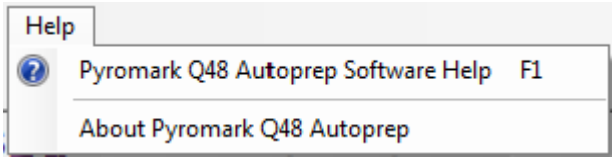
Note: To view reports in PDF format, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

7.3.4. Window menu



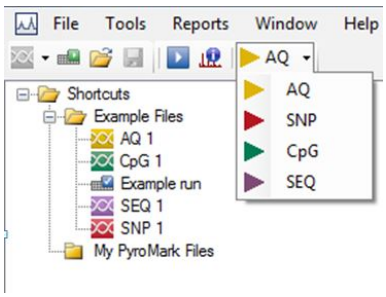
Toggle between open files in the software using the Window menu.

7.3.5. Help menu



Select **PyroMark Q48 Autoprep Software Help** or press the F1 key to open the online help.

7.3.6. Analysis toolbar



Click to analyze selected wells (see Section 7.7) for the current run file.

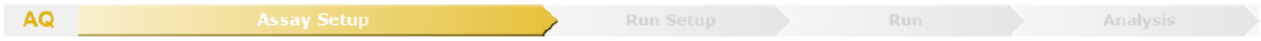
Click to view the run parameters and a run log for the current run file. To print the report, click .

Select **AQ**, **SNP**, **CpG**, or **SEQ** in the toolbar to toggle between the analysis modes.

7.4. Workflow views

The PyroMark Q48 Autoprep software is organized into views that reflect the Pyrosequencing workflow: Assay Setup, Run Setup, and Analysis. The active view is indicated in the status bar at the top of the window.

7.4.1. Assay Setup view



This view becomes active when creating a new assay. The color of the workflow arrow **Assay Setup** in the status bar reflects the type of assay selected. In this view, the user specifies the assay name and the sequence to analyze, and can optionally enter an assay note.

A nucleotide dispensation order is generated by the software. The **Variable Positions** tab displayed upon generating the dispensation order lists the variable positions in the sequence entered and allows the user to name the positions, and indicates which should be analyzed. By default, all variable positions supported in the used analysis mode are selected for analysis. The **Analysis Parameters** tab is used to specify parameters for the data analysis (refer to "Edit analysis parameters in the Analysis Parameters tab" in Section 7.11.6). The **Revert to Default** button resets default assay parameters, and the **Lock Assay** button locks entered assay parameters so they cannot be altered during analysis (see Section 7.15). All changes made to an assay file are recorded in a Change Log, which can be accessed by clicking the **Show Change Log** button.

7.4.2. Run Setup view

A new run file is created in the Run Setup view. This view serves to enter run-specific information such as disc ID, Reagent ID and a note about the run. Using the Disc Setup, assays are added to individual or a group of disc wells.

7.4.3. Analysis view

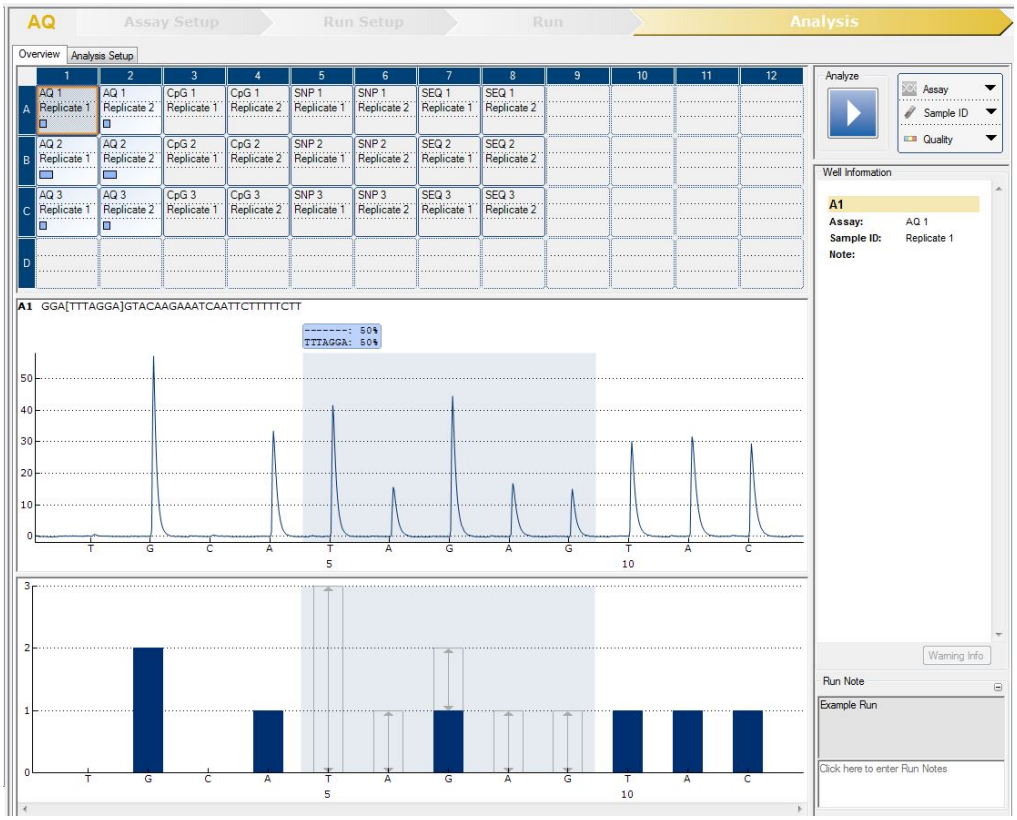
When a run is completed on the PyroMark Q48 Autoprep, the **Analysis** view opens. Alternatively, this view becomes active when any processed run file is opened. The color of the workflow arrow **Analysis** in the status bar reflects the selected analysis mode. This view is used to manage the analysis of individual or groups of disc wells, including making changes to analysis parameters (for unlocked assays). Information for analysis is displayed in two tabs, the **Overview** tab (see Section 7.5) and the **Analysis Setup** tab (see Section 7.6).

Note: A dialog box will request confirmation of any modifications made in the **Overview** or **Analysis Setup** tab prior to switching from one tab to another, or upon selecting (orange outline) another well.

7.5. Overview tab

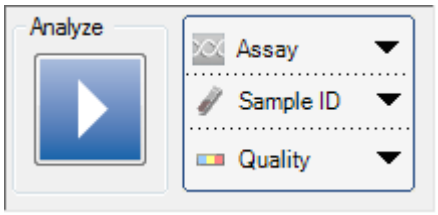
The **Overview** tab in the **Analysis** view displays the disc overview with well-specific information. Directly below, in the Pyrogram pane, is the Pyrogram of the well selected in the disc overview. The corresponding histogram is displayed beneath

the Pyrogram. To the right is the **Well Information** pane and the **Run note**, which display information specific to the well selected in the disc overview.

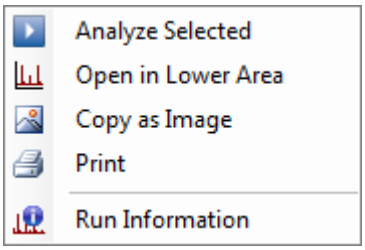


The Pyrogram of the selected well always appears in the upper area of the **Pyrogram** pane and the histogram (for AQ, SNP, and CpG assays) or compensated Pyrogram (for SEQ assays) appears in the lower area. It is also possible to replace the histogram in the lower area with one or more Pyrograms of selected wells (see "Simultaneously view Pyrograms of different wells" in Section 7.15.3).

Above the **Well Information** pane are drop-down menus to specify the type of information to be displayed for each well and an easy access button to initiate analysis of selected wells.



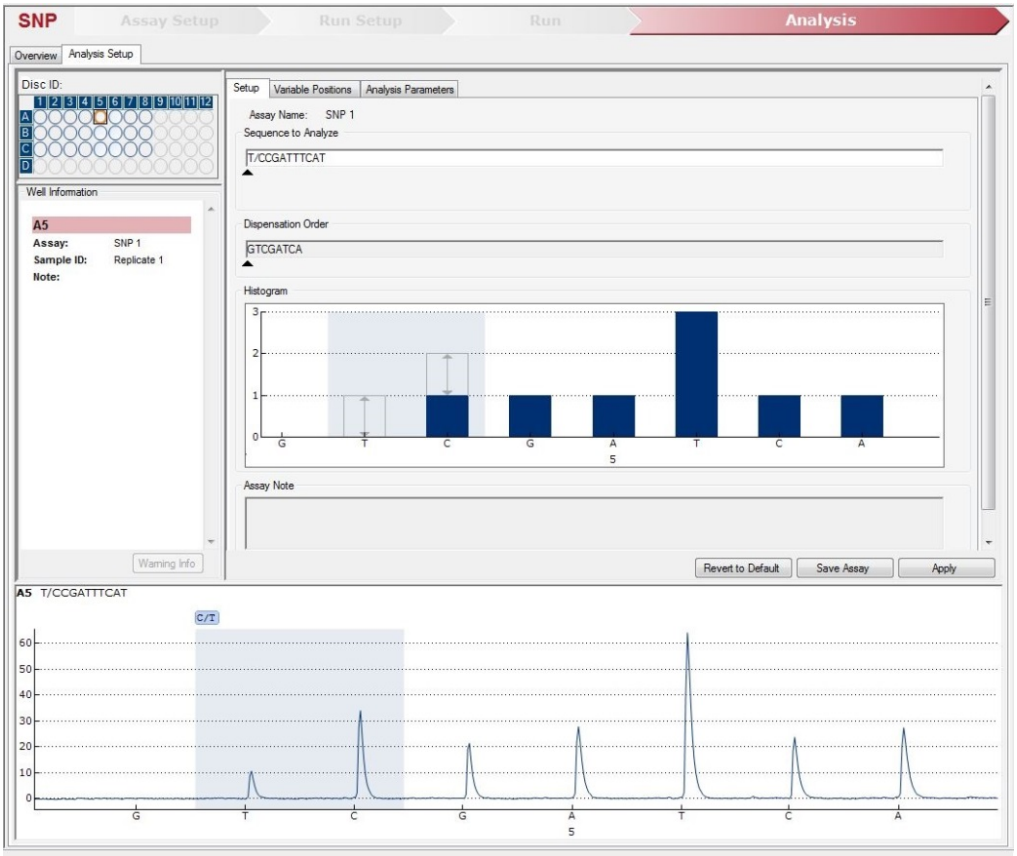
Right-clicking on the disc overview in this tab opens a context menu with the following options:



- **Analyze Selected** initiates analysis of all selected wells.
- **Open in Lower Area** replaces the histogram with one or more Pyrograms of selected wells.
- **Copy as Image**: An image of the full disc layout is copied to the clipboard.
- **Print**: An image of the full disc layout is formatted for printing.
- **Run Information**: the Run Information window is displayed (see Section 7.15.3)

7.6. Analysis Setup tab

The **Analysis Setup** tab displays information specific to the analysis performed on a specific well or selection of wells.



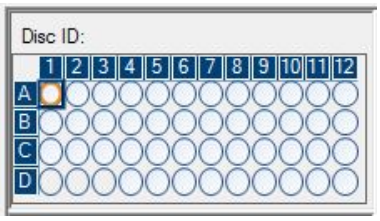
In the upper left pane is a schematic of the disc overview that displays the disc ID and permits the user to select wells (see Section 7.7). The **Well Information** pane summarizes all information associated with the well selected in the disc overview. The Pyrogram of the selected well is displayed at the bottom of the tab.

Details about the assay of a selected well are visible in the large main pane, including assay name, sequence to analyze, nucleotide dispensation order, histogram, and notes entered during assay setup. In addition, three tabs display the analysis setup, variable positions analyzed, and the analysis parameters used.

7.7. Selecting wells

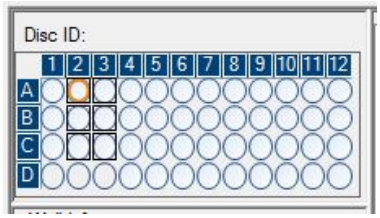
The following methods for selecting wells can be used in the disc overview of both the **Overview** and the **Analysis Setup** tabs. The color schematic for wells applies to both tabs.

To select a single well, simply click it.

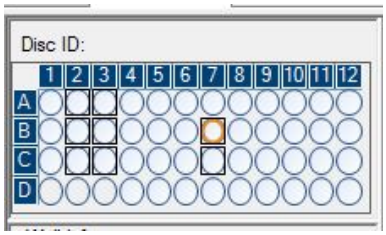


To select a rectangular group of wells, for example, A2–A3, B2–B3, and C2–C3:

- Press and hold down the left mouse button while dragging the mouse pointer from well A2 to C3.
- Select well A2 and press and hold down the **Shift** key while selecting well C3.
- Select well A2 and press and hold down the **Shift** key while pressing the **Right Arrow** key twice and the **Down Arrow** key twice.



To add wells to the selection above, for example, wells B7 and C7, press and hold down the **Ctrl** key while selecting the wells.



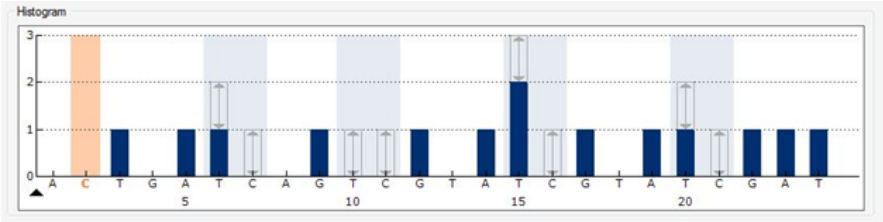
To deselect a well, press and hold down the **Ctrl** key while clicking the well.

Note: If several wells are selected in the disc, information for the well with the orange selection frame (in the Analysis view) is shown in the Well Information area, etc.

Colors of wells in disc overview diagrams

- **Selected well**: a dark blue outline (in the **Overview** tab) or a black outline (in the **Assay Setup** tab)
- **Most recently selected well**: an orange outline
- **Active, analyzed wells**: a light blue outline and pale blue background
- **Active, unanalyzed wells**: a light blue outline and gray background
- **Inactive wells**: a gray outline and background. Inactive wells cannot be selected.
- **Well with an error**: a red cross

7.8. Histogram



Histogram showing a theoretical CpG assay result.

When setting up an AQ, SNP, or CpG assay, the theoretical representation of the expected Pyrosequencing peak pattern is presented in the Histogram area. The following icons and colors are used in the histogram:

Variable regions (which contain one or more variable positions) are highlighted with a blue-gray background color.

When showing reference peaks, blue diamonds are displayed above the reference peaks. A filled blue diamond indicates the reference peak is enabled, and a hollow blue diamond indicates the reference peak is disabled. Reference peaks may be disabled or disabled by left-clicking the peak.

The bisulfite treatment control is highlighted with an orange background color, and the base is displayed in orange. When showing reference peaks, orange diamonds are displayed above the bisulfite treatment controls (CpG assays only).

7.8.1. Zoom histogram

It is possible to zoom in on the histogram by selecting a stretch of it with the left mouse button.

Zoom out either by right-clicking the histogram area and selecting **Zoom Out f**rom the context menu (the zoom is set to the previous level) or by double-clicking the histogram area (the zoom is set to 100%).

It is possible to adjust the heights of histogram bars (see “Adjust heights of histogram bars” in Section 7.11.6).

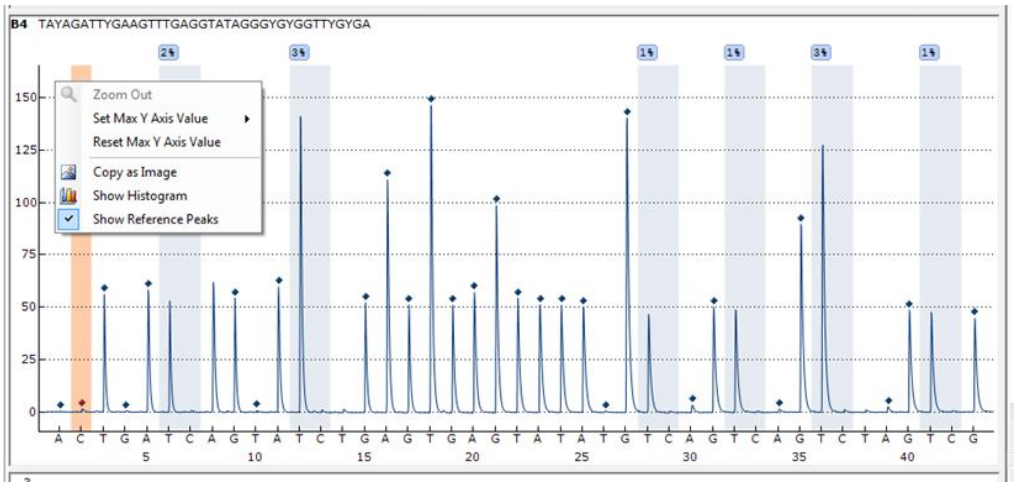
7.8.2. Export the histogram as an image

The histogram can be copied as an image to the clipboard by right-clicking the histogram and selecting **Copy as Image** from the context menu. The image can be pasted into applications that support Enhanced Metafile (EMF) images.

7.9. Pyrogram

The Pyrogram is the graph resulting from a sequencing reaction performed using Pyrosequencing technology. Incorporated nucleotides are shown as peaks in the Pyrogram.

7.9.1. AQ, SNP, and CpG assays



Pyrogram showing a CpG assay result.

The following information, icons and colors are displayed and used in the Pyrogram pane for an AQ, SNP, or CpG assay:

- The well name and the sequence to analyze are shown in the upper left corner.
- The analysis result (allele frequencies in AQ mode A: 4% T: 96%, genotype in SNP mode T/T, or methylation percentage 23% or average methylation percentage Average: 25% in CpG mode or CpN mode) is displayed above each variable position. The background color shows the quality assessment of the analysis result; see "Quality colors" in Section 7.15.3). If a quality assessment has been edited by the user, this is displayed by a border around the analysis result, for example, 44%

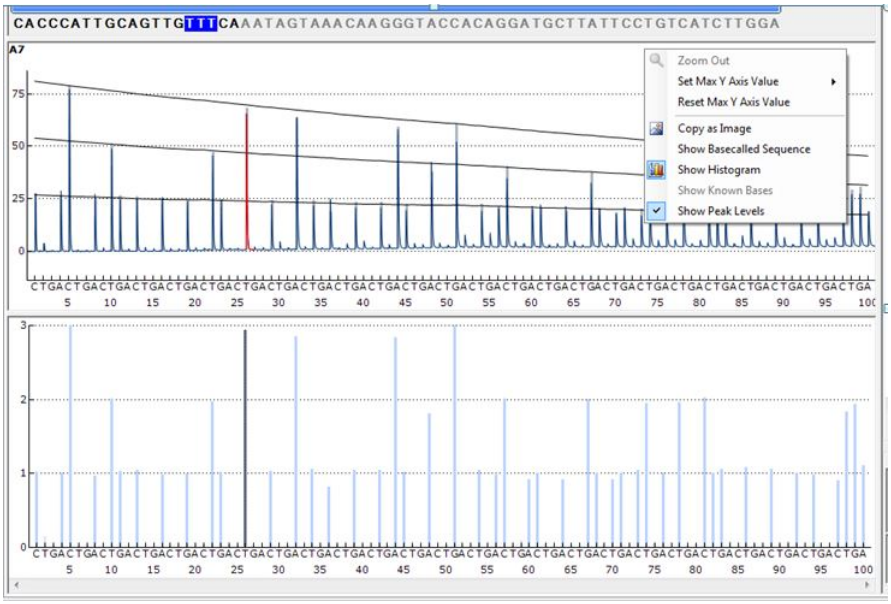
  Note: -- (in white) indicates that a site was deselected by the user. n.a. (in white) indicates that the software does not support the analysis, for example, analysis of SNP in the CpG mode. n.a. (in red) indicates that the analysis was not possible due to lack of data.
- Variable regions are highlighted with a blue-gray background color.
- When showing reference peaks, blue diamonds are displayed above the reference peaks. A filled blue diamond indicates the reference peak is enabled, and a hollow blue diamond indicates the reference peak is disabled.
- Bisulfite treatment controls are highlighted with a light orange background color. When showing reference peaks, orange diamonds are displayed above the bisulfite treatment controls (CpG assays only).
- To view the height of a peak, position the mouse pointer over the top of the peak. A tooltip displays the height.

- When showing the histogram, the histogram is displayed in gray over the peaks. It is best viewed when zoomed in.

  Note: By right-clicking the **Pyrogram** pane, it is possible to toggle between viewing and hiding the histogram and reference peaks.

- The Pyrogram Y axis scale may be manually adjusted by right-clicking on it and selecting **Set Max Y Axis Value**. The value is limited to three digits and must be confirmed by pressing the **Enter** button or by clicking the mouse button.

7.9.2. SEQ assays



When a base is selected in the base-called sequence, the corresponding peak is highlighted in both the upper and lower areas within the Pyrogram pane, and vice versa.

The following information and colors are displayed and used in the Pyrogram pane for an SEQ assay:

- The well name is shown in the upper left corner.
- To view the height of a peak, position the mouse pointer over the top of the peak. A tooltip displays the height.
- When showing the histogram, a compensated Pyrogram is displayed in gray over the peaks in the Pyrogram. It is best viewed when zoomed in.
- When showing known bases, peaks with known bases are marked with blue diamonds in Pyrogram.
- When showing peak levels, calculated peak levels are displayed in the Pyrogram.
- Colors used in the Graph area correspond to quality assessments (see “Quality colors” in Section 7.15.3).
- By positioning the mouse pointer on a base in the base-called sequence, a tooltip displays the position number.

Note: By right-clicking the **Pyrogram** pane, it is possible to toggle between viewing and hiding the histogram, known bases and peak levels.

7.9.3. Zoom Pyrogram

It is possible to zoom in on the Pyrogram by selecting a stretch with the left mouse button. Zoom out either by right-clicking the Pyrogram area and selecting **Zoom Out** from the context menu (the zoom is set to the previous level), or by double-clicking the Pyrogram area (the zoom is set to 100%).

The Pyrogram Y axis scale may also be manually adjusted by right-clicking on it and selecting **Set Max Y Axis Value**. The value is limited to three digits and must be confirmed by pressing the **Enter** button or by clicking the mouse button.

7.9.4. Export Pyrogram as an image

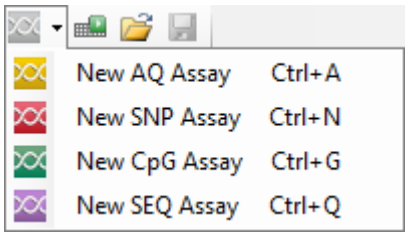
The Pyrogram can be copied as an image to the clipboard by right-clicking the Pyrogram area and selecting **Copy as Image** from the context menu. The image can be pasted into applications that support Enhanced Metafile (EMF) images.

7.10. Starting the software

In the Windows **Start** menu, select **(All) Programs** > **PyroMark** > **PyroMark Q48 Autoprep**. The information in this section on PyroMark Q48 Autoprep software can be accessed at any time by pressing the F1 key when in the software.

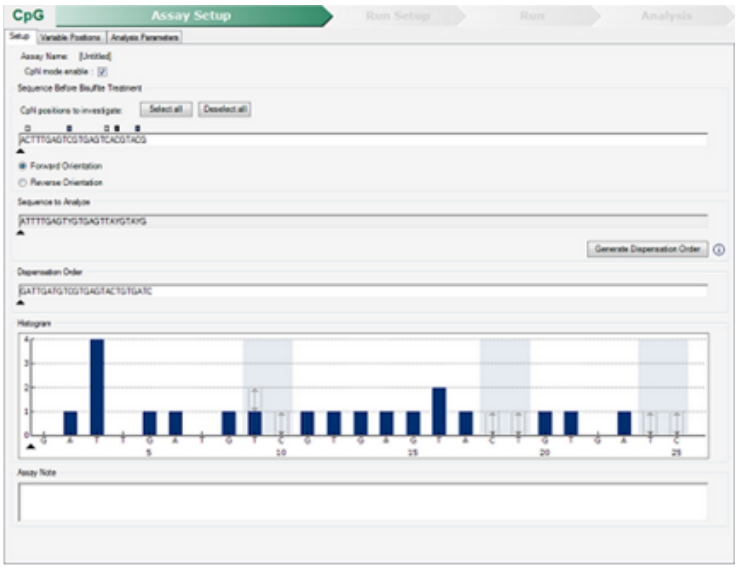
7.11. Setting up an AQ, SNP, or CpG Assay

7.11.1. Workflow to set up an AQ, SNP, or CpG Assay



1. Click  in the toolbar and select **New AQ Assay**, **New SNP Assay**, or **New CpG Assay**. A new assay file is created. For analyzing methylation at CpN sites, create a **New CpG Assay** and then enable the CpN mode by checking the box next to CpN mode enabled.

   Alternatively, you can create a new assay file in the shortcut browser by right-clicking the folder you wish to place it in and selecting **New Assay** followed by **New AQ Assay**, **New SNP Assay**, or **New CpG Assay** from the context menu. Enter the filename and press **Enter**. You cannot use the same name as another file saved in the same folder. To add a shortcut to a folder or drive, click **Add Folder Shortcut**.



2. Enter the sequence to analyze (see Section 7.11.2).

   Note: If creating a CpG assay, we recommend entering the **Sequence Before Bisulfite Treatment** (see "Enter the sequence before bisulfite treatment (CpG assays)" in Section 7.11.2). This enables the software to automatically generate the sequence to analyze and select the most appropriate bisulfite treatment control.

   Note: When CpN mode is enabled, each CpN position in the sequence before bisulfite treatment is indicated by a small check box above the position. The user should select which position(s) to analyze by individually clicking the appropriate check boxes, or by clicking **Select all** or **Deselect all**. Selected boxes are indicated by blue color and deselected boxes are indicated by white color. All of the selected CpN positions will be considered during the analysis as long as they are not deselected in the **Variable Positions** tab.

3. Click the **Generate Dispensation Order** button (see Section 7.11.3).

4. If creating a CpG assay, check that the software has selected a bisulfite treatment control. If no bisulfite treatment control has been automatically selected, add one manually, preferably at the beginning of the sequence (see Section 7.11.4).

5. **Optional**: Enter information about the assay in the Assay Note text box.

   Note: An assay note can be displayed in a tooltip in the shortcut browser by positioning the mouse pointer over the assay file.

6. **Optional**: Set up the variable positions in the **Variable Positions** tab (see Section 7.11.5).

7. Before running your samples, validate your assay using a reference DNA sample (see Appendix A – Assay Design and Validation).

8. **Optional**: If applicable, during the assay validation, edit the analysis parameters.

9. **Optional**: Lock the assay for editing by clicking the **Lock Assay** button at the bottom of the assay setup window. A locked assay ( ) that has been run on the PyroMark Q48 Autoprep instrument cannot be unlocked (i.e., it will not be possible to edit the analysis parameters or results after the assay has been processed).

10. Click in the toolbar to save the file. If the file has never been saved, select location and enter the filename in the dialog box that opens.

7.11.2. Enter the sequence to analyze

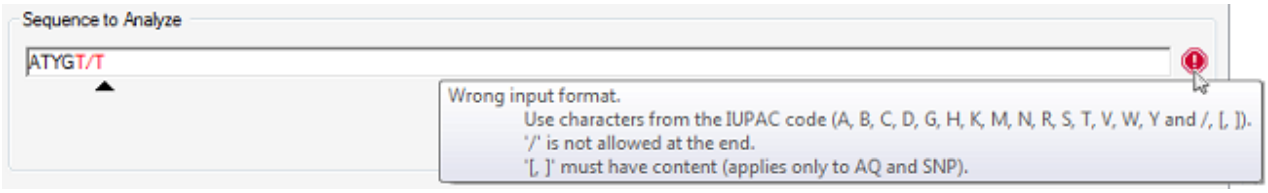
Type or paste the sequence to analyze into the "Sequence to Analyze" text box. If creating a CpG assay, we recommend entering the sequence before bisulfite treatment, if it is known (see "Enter the sequence before bisulfite treatment (CpG assays)").

Note: It is possible to add assays to a well in the disc layout without a sequence to analyze.

The following rules apply when entering the **DNA Sequence to Analyze** in the software:

- The allowed characters for sequence input are A, C, G, and T as well as IUPAC codes.
- Variable positions can be entered using either IUPAC codes or a forward slash (/) as a separator between the two potential bases (e.g., C/T).
- InDels should be entered using square bracket notation [ ] (e.g., [AT]).
- The sequence should not include more than 400 characters or 100 variable positions.
- Variable positions involving a combination of SNPs and InDels should be entered using a combination of / or IUPAC codes and [ ]. For example, [T/A] or [W] represents a tri-allelic polymorphism where the possible alleles are a T, an A, or neither (deletion).
- It is not possible to have a combination of a single nucleotide polymorphism and constant bases within an InDel (e.g., [A/TC]).
- Nested InDels are not supported (e.g., [ATT[C]G]).

If the sequence to analyze contains an error, this is displayed by a red exclamation mark at the end of the text box. Position the mouse pointer over the exclamation mark and a tooltip will display an explanation of the error. The character or characters that caused the error will be marked in red in the sequence to analyze.



As T/T is not a valid variable position, it causes an error.

In SNP assays, a warning message will appear if the sequence to analyze contains multiple variable sites within the same variable region that will generate the same sequence pattern. This will make it difficult to discriminate genotypes.

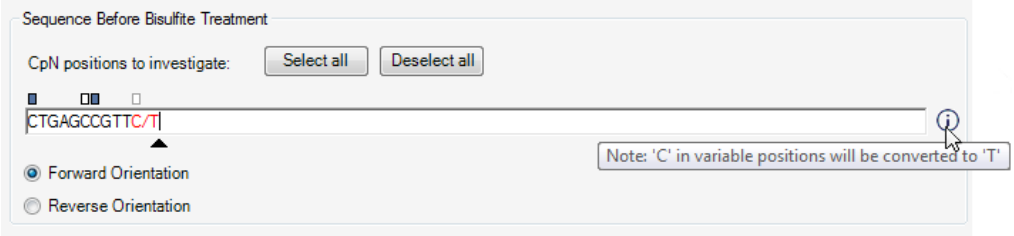
Enter the sequence before bisulfite treatment (CpG assays)

If creating a CpG assay, we recommend entering the sequence before bisulfite treatment into the "Sequence Before Bisulfite Treatment" text box. This enables the software to automatically generate the sequence to analyze and select the most appropriate bisulfite treatment control.

The following rules apply when entering the **DNA Sequence Before Bisulfite Treatment** in the software:

- The allowed characters for sequence input are A, C, G, and T as well as IUPAC codes.
- Variable positions such as SNPs can be entered using either IUPAC codes or a forward slash (/) as a separator between the two potential bases (e.g., C/G).
- The sequence should not include more than 400 characters or 100 variable positions.
- InDels are not supported.

If the software detects inconsistencies in the sequence before bisulfite treatment, the user is informed by an information symbol (ⓘ) next to the "Sequence Before Bisulfite Treatment" text box. The warning text is shown in a tooltip when moving the mouse over the symbol.



If the sequence before bisulfite treatment is edited such that it becomes invalid, the check boxes along with the **Select all** and **Deselect all** buttons will be grayed out, indicating that they are disabled and no longer selectable. The check boxes and buttons become enabled again once the errors in the **Sequence Before Bisulfite Treatment** are corrected.

IUPAC codes

Code	Description	Code	Description
A	Adenine	W	T or A
C	Cytosine	S	C or G
G	Guanine	B	C, T, or G (not A)
T	Thymine	D	A, T, or G (not C)
R	Purine (A or G)	H	A, T, or C (not G)
Y	Pyrimidine (C or T)	V	A, C, or G (not T)
M	C or A	N	Any base (A, C, G, or T)
K	T or G		

Note: S, B, V, and N are not valid after bisulfite treatment.

Valid patterns in a CpG assay

Patterns that cannot exist after bisulfite treatment are not valid in a CpG assay. For example, GC/TGAC/G is not valid because C/TG is a forward CpG site and C/G cannot exist after bisulfite treatment.

The following CpG sites, CpN sites and SNPs can be included in a forward assay:

- **CpG site**: C/TG
- **CpN site**: C/TA, C/TC, C/TG, and C/TT
- **SNPs**: A/T, A/G, G/T, and A/T/G (i.e., C cannot be included)

The following CpG sites, CpN sites, and SNPs can be included in a reverse assay:

- **CpG site**: CG/A
- **CpN site**: AG/A, CG/A, GG/A, and TG/A
- **SNPs**: A/T, A/C, C/T, and A/T/C (i.e., G cannot be included)

Note: The software does not support analysis of CpG sites that include an additional variable position, for example, **A/C/TG**. These kinds of SNPs can be analyzed by typing **C/TG** in the "Sequence to Analyze" text box and **ATCG** in the "Dispensation Order" text box. Proceed with the run as usual. After analysis of the CpG sites, switch to the AQ mode and change **C/TG** to **A/C/TG** (in the "Sequence to Analyze" text box) and analyze the variable position. In the same way, **C/TG/A** can be analyzed by typing **C/TG** in the "Sequence to Analyze" text box and **TCGA** in the "Dispensation Order" text box. After analysis of the CpG sites, switch to the AQ mode and change **C/TG** to **C/TG/A** (in the "Sequence to Analyze" text box) and analyze the variable position.

7.11.3. Generate the dispensation order

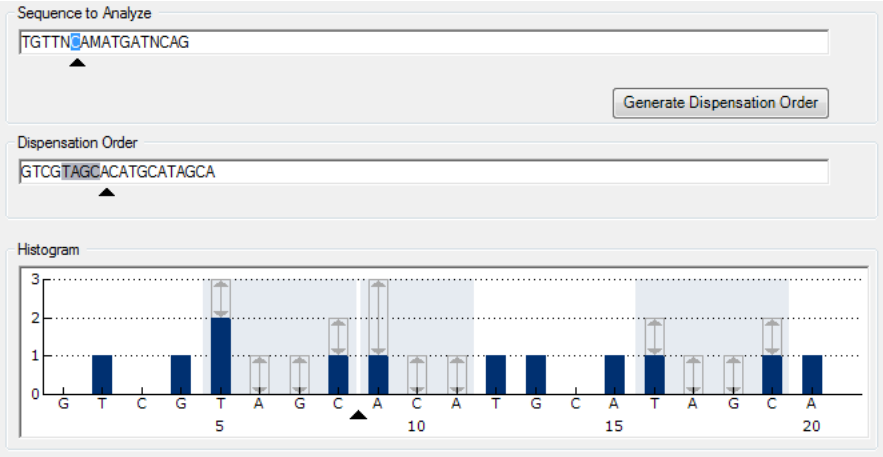
A dispensation order for the entered sequence to analyze is generated by the software by clicking the **Generate Dispensation Order** button. The generated dispensation order includes blank dispensations that are used as negative controls.

When creating CpG assays, the dispensation order should also include bisulfite treatment controls. If the user enters the sequence before bisulfite treatment, the sequence to analyze will be automatically generated, and one bisulfite control will be automatically selected, if possible. If the user directly enters the sequence to analyze, the bisulfite control will need to be added manually by the user, after the dispensation order has been generated (see Section 7.11.4).

If desired, the dispensation order can be entered or adjusted manually.

Note: When clicking **Generate Dispensation Order**, any existing dispensation order will be overwritten.

Note: When a base position is selected in the sequence to analyze, the corresponding dispensation is highlighted with a gray background color, and vice versa.



The arrow in the sequence to analyze, the dispensation order, and the histogram show the position of the cursor.

Note: If the last variable position in the sequence to analyze is a long InDel, dispensation will only be performed until three variable peaks are found, providing the requirement of five reference peaks is fulfilled. To dispense the whole InDel, add a variable position after the InDel or adjust the dispensation order manually.

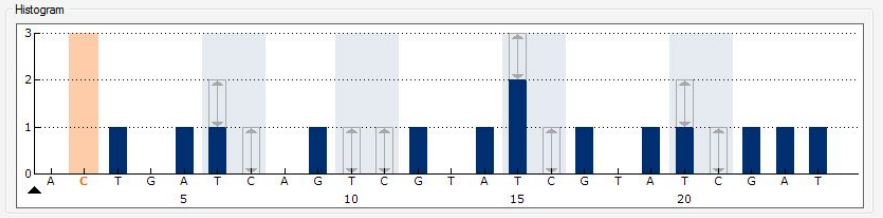
Note: If it is not possible for the sequence to come in phase before 32 alleles are dispensed, the dispensation order will not be completed. For example, the sequence ACTCDDDDG will have the dispensation order ACTC, since the four D polymorphisms will generate an out-of-phase stretch over too many alleles.

Dispensation warnings

If the dispensation order contains a warning, this is displayed by an information symbol ⓘ at the end of the "Dispensation Order" text box. It is possible to run an assay with a dispensation warning, but the warning must be considered when evaluating the analysis result. If you position the mouse pointer over the exclamation mark, a tooltip will display an explanation of the warning.

Warning	Suggested action
Sequence uncertain due to lack of terminal sequence information.	The problem may be resolved by either entering more sequence information or reducing the number of dispensations.
Sequence not in phase at the end of the dispensations.	The problem may be resolved by adjusting the dispensation order (manually or by clicking **Generate Dispensation Order**) or entering more sequence information. **Note**: If the problem is not resolved, the out-of-phase stretch will not be analyzed.
The generated dispensation order contains less reference peaks than required.	If possible, enter more sequence information and increase the number of dispensations. For the best possible quality assessment of the results, five or more reference peaks with the height 1, 2, or 3 are recommended.
Some genotypes will generate the same sequence patterns and will not be distinguishable.	The sequence to analyze contains multiple variable sites within a variable region that will generate sequence patterns that are indistinguishable between genotypes. Review results carefully.

7.11.4. Add or remove bisulfite treatment controls (CpG assays)



CpG assays should contain an internal control to assess successful bisulfite treatment, preferably at the beginning of the sequence. C bases that are not followed by G in the sequence are usually not methylated and should therefore be fully converted to T after bisulfite treatment and PCR. As a result of successful bisulfite treatment, all templates should show only Ts and no Cs in these positions. For reverse assays, all templates should show only As and no Gs in these positions.

We recommend directly entering the sequence before bisulfite treatment, because this enables the software to generate the sequence to analyze and automatically select one appropriate bisulfite treatment control. If the user directly enters the sequence to analyze, or if the software does not find a suitable bisulfite control, one bisulfite control will need to be added manually by the user, after the dispensation order has been generated.

A bisulfite treatment control can be added manually by adding a **C** before or after a **T** in a forward assay, or by adding a **G** before or after an **A** in a reverse assay, in the dispensation order. This is only possible if it is known that in the sequence before bisulfite treatment, the suggested bisulfite treatment controls are **C**s converted to **T**s (read as **G**s and **A**s in a reverse assay).

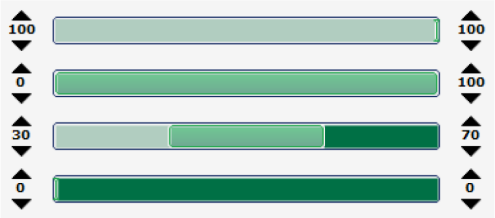
Only one bisulfite control may be included. If additional controls are manually added in the dispensation order, they will be displayed as red **Cs** in the histogram.

Note: For CpG assays with CpN mode enabled, if the sequence before bisulfite treatment is entered, the software will use an unselected CpN site as a bisulfite treatment control.

7.11.5. Set up variable positions

The variable positions can be set up in the **Variable Positions** tab. The available parameters are listed below.

Note: If the sequence to analyze is changed (and a new dispensation order is generated), the variable position parameters are reset to their default values.

Parameter	Description
Position	The location of the variable position in the sequence to analyze, counting from left to right.
Name	The name of the variable position. To change the name, either select the text box (the current contents will be selected) or double-click the text box.
Type	The type of variable position: SNP, InDel, CpG, or CpN site.
Analyze	If this option is checked, the variable position will be analyzed. **Note**: This option is not available for variable positions that cannot be analyzed for the current assay type.
Methylation ranges (CpG assays only)	The expected CpG or CpN methylation. Setting this parameter for all the CpG or CpN sites allows easy identification of sites (in the analysis results) that are outside the user-expected methylation range: • The light green area is below the expected range • The green area is within the expected range • The dark green area is above the expected range Note: The expected methylation cannot be set for CpG sites with the Analyze option unchecked. The expected range area can be moved to the left or to the right by holding down the left mouse button while moving the area with the mouse. The arrows can be used to increase or decrease the expected range. You can also increase or decrease the expected range by: • Positioning the mouse pointer over the left or the right end of the green area, so that the pointer changes from a white arrow to ✢. • Moving the mouse to the left or the right while holding down the left mouse button. To edit all methylation ranges simultaneously, hold down the Shift key while changing one of the ranges. Examples of methylation ranges  1. Expected methylation = 100% 2. Expected methylation = 0–100% 3. Expected methylation = 30–70% (default) 4. Expected methylation = 0%

To reset the parameters in the **Variable Positions** tab and the **Analysis Parameters** tab to their default values, click **Revert to Default**.

7.11.6. Edit analysis parameters

The default analysis settings have been set to give optimal analysis results for most assays. If applicable, during the assay validation, the results may be improved by editing the analysis parameters:

- Edit analysis parameters in the **Analysis Parameters** tab
- Enable or disable reference peaks
- Enable or disable bisulfite treatment controls
- Adjust heights of histogram bars
- Enable or disable variable positions and/or change expected methylation ranges (only CpG assays); see Section 7.11.5

Ensure changes are validated; see Appendix A – Assay Design and Validation.

Note: When using QIAGEN kits, use the settings stated in the kit handbooks.

Note: All saved changes are logged. To view a change log for an assay, open the assay file and click **Show Change Log**.

Edit analysis parameters in the Analysis Parameters tab

The following analysis parameters can be edited in the **Analysis Parameters** tab.

Parameter	Description
Unsuccessful bisulfite treatment (CpG assays only)	These parameters state the highest acceptable percentage of unconverted sequence to achieve "Passed" quality assessment and "Check" quality assessment for the CpG sites. The entered values are compared to the single peak height value that the analysis algorithm determines.
Allowed percentage for passed quality	The highest acceptable percentage of unconverted sequence to achieve "Passed" quality assessment for the CpG sites. Above this value, the warning "Uncertain bisulfite conversion at dispensation: number(s)" is triggered during the analysis and a "Check" quality assessment is assigned. The default value is 5%. **Note**: The value cannot be higher than the allowed percentage value for check quality (see below).
Allowed percentage for check quality	The highest acceptable percentage of unconverted sequence to achieve "Check" quality assessment for the CpG sites. Above this value, the warning "Failed bisulfite conversion at dispensation: number(s)" is triggered during the analysis and a "Failed" quality assessment is assigned. The default value is 7%. **Note**: The value cannot be lower than the allowed percentage value for passed quality (see above).
Peak height threshold	These parameters define the lower intensity limit for the single peak height level of the Pyrogram.
Required peak height for passed quality	The minimum signal value for a peak to achieve "Passed" quality assessment for the variable positions. Below this value, the warning "Uncertain due to low peak height" is triggered during the analysis and a "Check" quality assessment is assigned. The default value is 20. **Note**: The value cannot be lower than the required peak height value for check quality (see below).
Required peak height for check quality	The minimum signal value for a peak to achieve "Check" quality assessment for the variable positions. Below this value, the warning "Failed due to low peak height" is triggered during the analysis and a "Failed" quality assessment is assigned. The default value is 10. **Note**: The value cannot be higher than the required peak height value for passed quality (see above).
Parameters	
A-peak reduction factor	The factor by which the A-peak intensities are multiplied to account for the fact that A-peaks are higher than other peaks. The default value is 0.90.

To reset the parameters in the **Variable Positions** tab and the **Analysis Parameters** tab to their default values, click **Revert to Default**.

Enable or disable reference peaks and bisulfite treatment controls

Nonvariable peaks, that is, peaks that are not a part of a variable position (including blank dispensations), are referred to as reference peaks. Reference peaks are used in the analysis both as references when calculating the single peak height level and as internal controls when assessing the quality. For the best possible quality assessment of the results, we recommend that the reference peaks generated by the software are kept enabled.

By left-clicking a reference peak diamond in the histogram, the peak is either enabled or disabled as a reference peak, depending on the previous status. The diamond displays the status:

- **Filled blue diamond**: Enabled as a reference peak
- **Hollow blue diamond**: Disabled as a reference peak

By left-clicking a bisulfite treatment control diamond (CpG assays only), the control is either enabled or disabled as a control and/or a reference peak, depending on the previous status. The diamond displays the status:

- **Filled orange diamond**: Enabled both as a bisulfite treatment control and a reference peak
- **Filled blue diamond**: Enabled as a reference peak but disabled as a bisulfite treatment control
- **Hollow orange diamond**: Disabled both as a bisulfite treatment control and a reference peak

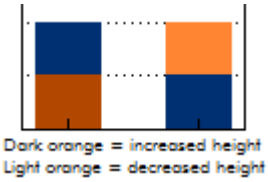
Position the mouse pointer over the diamond and a tooltip will describe the consequence of a click.

Note: To toggle between viewing and hiding reference peaks in the histogram, right-click the histogram and select **Show Reference Peaks** from the context menu.

Adjust heights of histogram bars

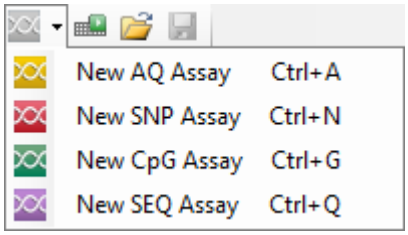
This feature can be used when previous experiences have shown a reproducible deviation in the measured pattern from the theoretical pattern. Use this feature with care.

1. Press and hold down the Ctrl key while left-clicking the top of the histogram bar (left-click when the pointer changes from a white arrow to).
2. Either enter the height in the text box that opens, or increase or decrease the height by using the arrows next to the text box.
3. To apply the new height, press **Enter**.



7.12. Setting up an SEQ Assay

7.12.1. Workflow to set up an SEQ Assay



1. Click in the toolbar and select **New SEQ Assay**. A new assay file is created.

   Alternatively, you can create a new assay file in the shortcut browser by right-clicking the folder you wish to place it in and selecting **New Assay** and **New SEQ Assay** from the context menu. Enter the filename and press **Enter**. To add a shortcut to a folder or drive, click **Add Folder Shortcut**.

2. Enter the dispensation order (see Section 7.12.2).

3. **Optional**: Enter information about the assay in the Assay Note text box.

   Note: An assay note can be displayed in a tooltip in the shortcut browser by positioning the mouse pointer over the assay file.

4. Before running your samples, validate your assay using a reference DNA sample (see Appendix A – Assay Design and Validation).

5. **Optional**: If applicable, during the assay validation, edit the analysis parameters.

6. **Optional**: Click **Lock Assay** to lock at the bottom of the assay setup window to lock the assay for editing. A locked assay ( ) that has been run on the PyroMark Q48 Autoprep instrument cannot be unlocked (i.e., it will not be possible to edit the analysis parameters or results after the assay has been processed).

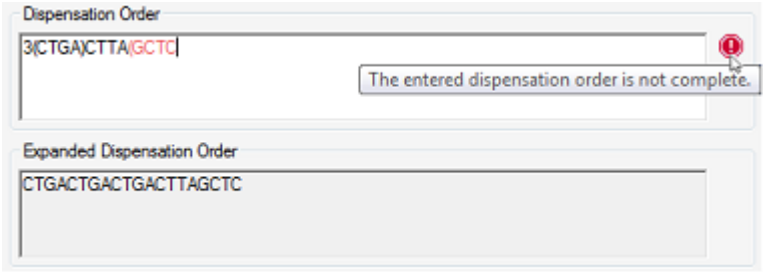
7. Click in the toolbar to save the file. If the file has never been saved, select location and enter the filename in the dialog box that opens.

7.12.2. Enter the dispensation order

Type the dispensation order into the Dispensation Order text box. The following rules apply when entering the dispensation order in the software:

- The allowed characters for input are A, C, G, and T
- To repeat a group of bases, use numbers in combination with parentheses, for example, 3(CTGA) corresponds to CTGACTGACTGA

If the dispensation order contains an error, this is displayed by a red exclamation mark at the end of the text box. Position the mouse pointer over the exclamation mark and a tooltip will display an explanation of the error. The character or characters that caused the error will be marked red in the dispensation order.



The error "The entered dispensation order is not complete" is caused by a missing or incorrect positioned parenthesis. In this example, a closing parenthesis is missing.

7.12.3. Edit analysis parameters

The default analysis settings have been set to give optimal analysis results for most assays. If applicable, during the assay validation, the results may be improved by editing the analysis parameters:

- The Quality Control Window setting in the Settings tab is by default set to 20. If more or less bases are required, change accordingly.
- Edit analysis parameters in the **Analysis Parameters** tab.

Ensure changes are validated; see Appendix A – Assay Design and Validation.

Note: When using QIAGEN kits, use the settings stated in the kit handbook.

Note: All saved changes are logged. To view a change log for an assay, open the assay file and click **Show Change Log** at the bottom of the assay setup window.

Edit analysis parameters in the Analysis Parameters tab

Parameter	Description
Peak height threshold	These parameters define the lower intensity limit for the single peak height level at the beginning of the Pyrogram.
Required peak height for passed quality	The minimum signal value for a peak to achieve "Passed" quality assessment in the base-called sequence. Below this value, the warning "Uncertain due to low peak height" is triggered during the analysis and a "Check" quality assessment is assigned. The default value is 4. **Note**: The value cannot be lower than the required peak height value for check quality (see below).
Required peak height for check quality	The minimum signal value for a peak to achieve "Check" quality assessment in the base-called sequence. Below this value, the warning "Failed due to low peak height" is triggered during the analysis and the "Failed" quality assessment is assigned. The default value is 2. **Note**: The value cannot be higher than the required peak height value for passed quality (see above).
Parameters	
A-peak reduction factor	The factor by which the A-peak intensities are multiplied to account for the fact that A-peaks are higher than other peaks. The default value is 0.90.
Known bases	If there are any known bases in the dispensation order, we recommend that these are entered as this can improve the analysis: 1. Left-click the relevant dispensation and either enter the height in the text box that opens, or increase or decrease the height by using the arrows next to the text box. 2. To apply the height, press Enter.

To reset the parameters in the **Settings** tab and the **Analysis Parameters** tab to their default values, click **Revert to Default**.

7.13. Setting up a run

7.13.1. Workflow to set up a run

1. Create a new Run Setup by one of the following methods:
   - Click  in the toolbar.
   - Select **New Run** from the **File** menu
   - Press the R key while holding down the Ctrl key.
   - Right-click a folder in the shortcut browser and select **New Run** from the context menu. Enter a run name and press **Enter**. To add a shortcut to a folder or drive, click **Add Folder Shortcut**.
   - To base your run on a previous run, right-click the processed run file in the shortcut browser and select **Copy and Rerun** from the context menu. Only the run setup, not the run and analysis data, will be copied.
2. If the new run has not yet been saved, click  to enter a run name and save the file in the desired folder.
3. Select either manual or automatic sequence primer loading.

   If manual primer loading is selected, the user will be instructed to manually load the sequencing primer into the wells during template preparation.

Note: For automatic primer loading, the instrument will dispense the primer from one of the dedicated sequence primer injectors. Allocate each primer used to a primer injector. Multiple primers can be loaded into each injector; however, please validate multiplexing of primers on the instrument.

4. Select a protocol for the run.
5. Enter the remaining run parameters and an optional note (see Section 7.13.2).
6. Set up the disc in the disc layout of the run file by adding assays to wells and, if desired, entering a sample ID and note for each used well (see Section 7.13.5).
7. When the run is set up and ready to run on the PyroMark Q48 Autoprep instrument, click  to save.
8. Press **Pre Run Information** from the **Tools** menu to print the disc setup. When the report opens, click  .

   Note: To print the Pre Run Information report in color, turn on the Print background colors and images option in the Internet Explorer (**Tools** > **Internet Options** > **Advanced** > **Printing**).

7.13.2. Enter run parameters

The following run parameters are available.

Parameter	Description
Run name	The name of the run is given when the file is saved. Renaming the file also changes the name of the run.
Protocol	The Standard protocol is used as the default. The P4 Extra protocol enables the use of the fourth sequence primer injector (P4). When using this protocol, the binding buffer must be loaded manually by the user. The maximum template volume is 10 µL to avoid exceeding the well capacity.
Primer loading	Select either manual or automatic sequence primer loading. For automatic primer loading allocate each primer(s) to a primer injector.
Disc ID	**Optional**: Enter ID of the PyroMark Q48 Disc. **Note**: If you position the mouse pointer over a run file in the shortcut browser, a tooltip displays the entered disc ID.
Bar code	**Optional**: Enter a bar code number for the disc or, if you have a bar code reader connected to your computer, place the mouse cursor in the Bar code text box and scan the bar code.
Reagent lot number	**Optional**: Enter the lot number for the PyroMark Q48 Advanced Reagents or PyroMark Q48 Advanced CpG Reagents to be used. The lot number can be found on the product label. **Note**: We recommend entering the reagent ID so that any unexpected problems with the reagents can be traced.
Run note	**Optional**: Enter a note about the contents or purpose of the run.

7.13.3. Add assay files to the disc

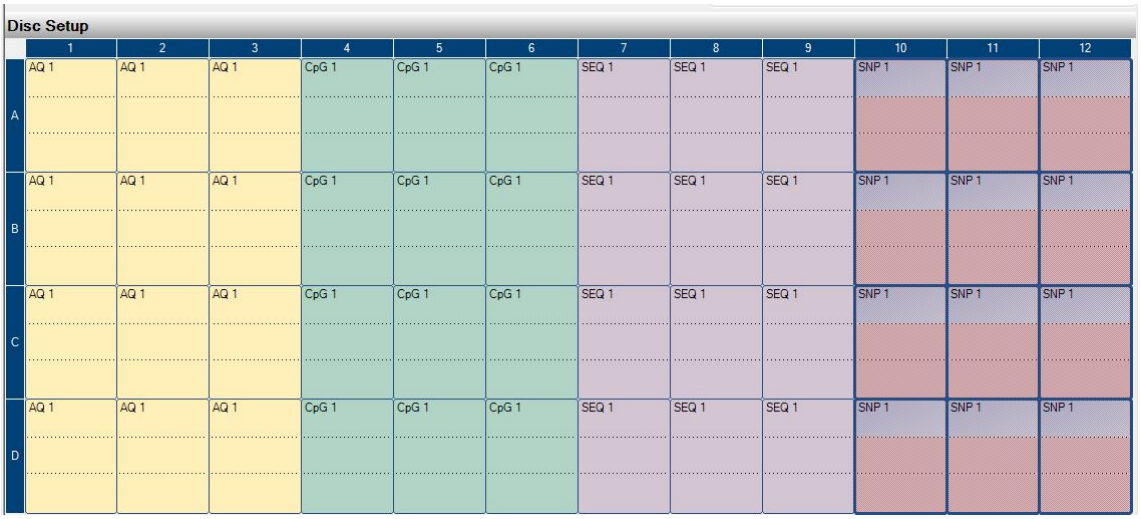
To add assays to wells, you can:

- Select the assay in the shortcut browser and press and hold down the left mouse button while you drag the assay to the well.
- Right-click the well and select **Load Assay** from the context menu (this option is only available when one well is selected).
- Add an assay to several wells by selecting the wells and dragging the assay to the selection. To select several wells, either click and drag the cursor across the wells to be selected, or hold down the **Ctrl** key as you click wells.

Note: It is not possible to add an assay with no dispensation order or add two or more assays with the same assay name but have different dispensation orders.

Note: It is not possible to add assays created with PyroMark Q24 software or PyroMark Q96 software.

Note: If an assay is locked, the well is marked with the icon.



A well is colored according to the assay type loaded to the well. Wells with AQ assays are yellow; wells with SNP assays are red; wells with CpG assays are green; wells with SEQ assays are purple. Different shades of each color indicate wells with different assays of the same type.

7.13.4. Print or export disc setup as image

The Disc Setup can be printed or copied as an image (to the clipboard) by right-clicking the disc and selecting **Print** or **Copy as Image** from the context menu. The image can be pasted into applications that support Enhanced Metafile (EMF) images.

7.13.5. Define sample ID and note externally

By using the **Import** > **Insert Sample Layout File** or **Paste Sample Layout** feature, you can easily use the same layout in several runs and reuse information available in existing documentation.

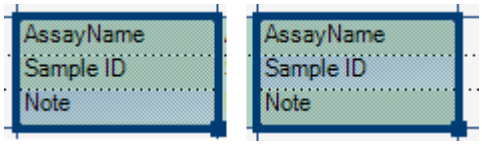
Copy or delete contents from cells

- To cut the contents of a cell to the clipboard, right-click the cell and select **Cut** from the context menu.
- To copy the contents of a cell to the clipboard, either right-click the cell and select **Copy Cell** from the context menu or select the cell and press **Ctrl** + **C**.
- To paste the clipboard to a cell or a selection of cells (see Section 7.7), either right-click the cell or the selection and select **Paste** from the context menu or select the cell(s) and press **Ctrl** + **V**.
- To delete one or more assays, sample IDs, or notes, either right-click the cell or the selection and select Delete from the context menu or select the cell(s) and press **Delete**.

Enter sample IDs and notes

- To enter a sample ID or note, select the corresponding cell (see image below) and enter the text.
- To edit a sample ID or note, double-click the corresponding cell.
- To import a sample and note layout defined in a text file (***.tsv** or ***.csv**), right-click a well and select Insert Sample Layout File from the context menu. For more information, see "Using the import/insert sample layout file feature" in Section 7.13.5.
- To paste a sample layout from the clipboard, right-click a well and select **Paste Sample Layout** from the context menu. For more information, see "Using the paste sample layout feature" in Section 7.13.5.

Note: Commas and semicolon are not supported.

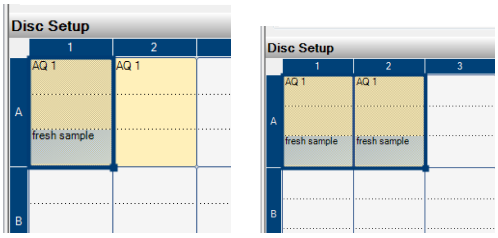


A selected cell is highlighted with a blue background color.

Drag-copy the contents of a cell to other wells

To drag-copy the contents of a cell to other wells:

1. Select the cell that you want to copy.
2. Position the mouse pointer over the lower right square of the selection, and press and hold down the left mouse button while you move the mouse to change the selection.
3. When the left mouse button is released, the contents of the first selected cell are pasted into the selected cells.

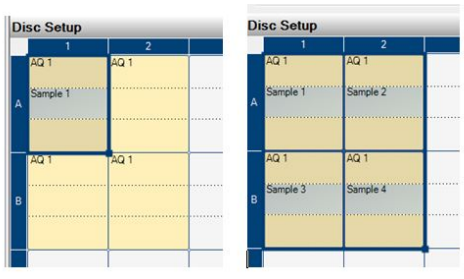


Drag-copy of the note "fresh sample".

Drag-copy and increment sample ID

If the last part of an entered sample ID is a number, the number can be incremented when drag-copying the sample ID:

1. Select the sample ID cell.
2. To increment by row:
   a. Position the mouse pointer over the lower right square of the selection.
   b. Press and hold down the **Ctrl** key + the left mouse button while moving the mouse to change the selection.
   c. First release the left mouse button, then the **Ctrl** key. When the left mouse button is released, the sample ID of the first selected cell is incremented and pasted into the selected cells.
3. To increment by column:
   a. Position the mouse pointer over the lower right square of the selection.
   b. Press and hold down the **Shift and** Ctrl keys + the left mouse button while moving the mouse to change the selection.
   c. First release the left mouse button, then the **Shift** and **Ctrl** keys. When the left mouse button is released, the sample ID of the first selected cell is incremented and pasted into the selected cells.



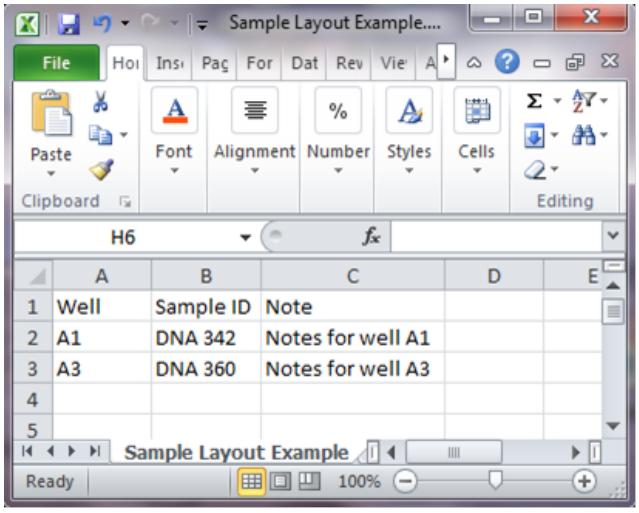
The sample ID "Sample 1" is copied and incremented by column.

Using the import/insert sample layout file feature

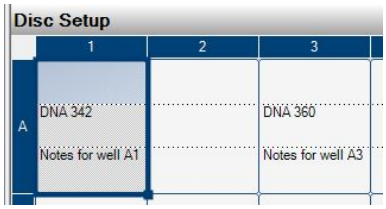
You can, for example, generate layout files from your Laboratory Information Management Systems (LIMS). Sample and note layout files can also be created in Microsoft Excel, Notepad, and similar applications. The layout file must have two or three columns: Well, Sample ID, and Note (optional). Each column must be separated by a tab, comma, or semicolon, and each line must be delimited by a line break. Save the file as a tab- or comma-delimited text file (***.tsv**, ***.txt**, or ***.csv**).

The sample and note layout file can be imported into:

- An existing run file by right-clicking a well in the Disc Setup and selecting **Insert Sample Layout File** from the context menu
- A new run file by selecting **Import** followed by **Create New Run** from **Sample Layout File** from the **File** menu



An example of a sample and note layout file created in Microsoft Excel.



The result when importing the sample and note layout file above.

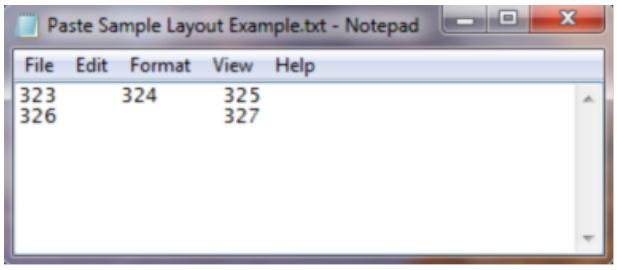
Using the paste sample layout feature

You can, for example, generate and copy layouts from your LIMS. Sample layouts can be copied from Microsoft Excel, Word, Notepad, and other similar applications. In the source file, each column of sample IDs must be delimited by a tab and each row of sample IDs must be delimited by a line break.

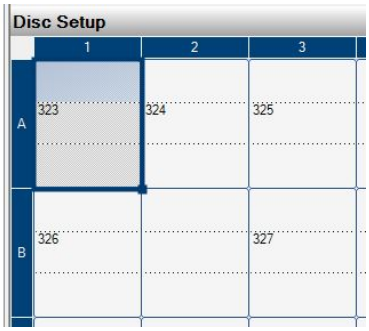
To paste a sample layout into an existing run file:

1. Copy all the information in the source file.
2. Right-click a well in the Disc Setup and select **Paste Sample Layout** from the context menu.

   The software will paste the sample IDs into the disc, starting at well A1. Well notes that have been entered into the wells are kept.



An example of a sample layout created in Microsoft Notepad.



The result when copying and pasting the sample layout created in Microsoft Notepad.

7.13.6. Review the disc setup

The Well Information area shows the following information about a well selected in the Disc Setup:

- Well name
- Type of assay (AQ, CpG, SNP, or SEQ)
- Assay name
- Sample ID (if entered)
- Sequence to analyze, if entered (AQ, CpG, and SNP assays)
- Dispensation order
- Well note (if entered)

If several wells are selected in the Disc Setup, the information for the first selected well is shown. If the wells are selected by clicking while holding down Ctrl, the information for the last well selected will be displayed.

7.14. Processing a run

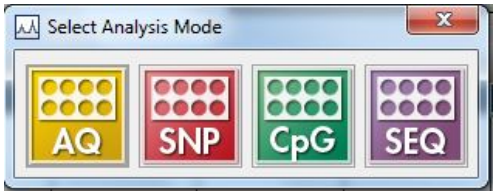
When a run is set up and ready to run on the PyroMark Q48 Autoprep instrument, perform the following steps:

1. Insert the USB flash drive into instrument.
2. Select the run file.
3. Ensure the absorber strip is inserted.
4. Load the injectors with required volumes of reagents, then prime and test the injectors.
5. Load the beads and samples.
6. Start the run.
7. **Optional**: Manually load the sequence primer when instructed.
8. When the run has been completed and data transferred to the USB flash drive, remove the USB flash drive.
9. After the last run of the day, clean the injectors with water and remove the absorber strip.

7.15. Analyzing a run

7.15.1. Workflow to analyze a run

1. Move the processed run file from the USB flash drive to a computer running the PyroMark Q48 Autoprep software. Insert the USB flash drive into the computer's USB port and move the run file to the desired location using Windows Explorer.
2. Press **Open** in the **File** menu or double-clicking the file ( ) in the shortcut browser to open the run file in the PyroMark Q48 Autoprep software. If several assay types are included, select the analysis mode in the dialog box that opens. The analysis modes displayed in the dialog box are limited to the assay types on the disc.



Note: To update the contents of a folder in the shortcut browser, right-click it and select **Refresh** from the context menu, or press the F5 key.

Note: It is also possible to open the run file by double-clicking it in Windows Explorer.

3. All wells on the disc with an Assay Setup corresponding to the selected analysis mode will be automatically analyzed.
4. View the analysis results (see Section 7.15.3).
5. **Optional**: If applicable, modify how the analysis is performed (see Section 7.15.4).
6. **Optional**: Enter an analysis note in the Note text box in the **Overview** tab.

Note: To expand or collapse the Note field, click or .

7. To save the analysis results, click in the toolbar.

Note: A dialog box will request confirmation of any modifications made in the **Overview** and **Analysis Setup** tabs prior to switching from one tab to another, or upon selecting (orange outline) another well.

Note: It is not possible to edit the analysis parameters or enter an analysis note for a locked assay ( ).

7.15.2. Analyze selected wells

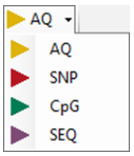
Analysis of selected wells can be initiated by clicking the **Analyze Selected Wells** button ( ) in the **Overview** tab. It is also possible to right-click the selection and select **Analyze Selected** from the context menu (see Section 7.7).

During the analysis, a progress dialog box is shown. This dialog box contains a progress bar, **Stop** button, and the name of the well that is being analyzed. The analysis can be stopped by clicking **Stop**. The progress dialog box closes when the analysis is finished or by clicking **Stop**.

Note: When a well has been analyzed, the well color changes to light blue.

Note: If the analysis of a well resulted in an error, the well is marked with a red cross.

Analysis modes



The PyroMark Q48 Autoprep software has four analysis modes: AQ, SNP, CpG, and SEQ. To toggle between the modes, select **AQ**, **SNP**, **CpG**, or **SEQ** in the toolbar.

A disc can include wells that must be analyzed in different modes. To complete all analyses:

1. With the run file open in the Analysis view, toggle to the desired analysis mode.
2. After selecting the analysis mode, all wells with an Assay Setup corresponding to the analysis mode will be automatically analyzed, and the run will be automatically saved in the background after the analysis is complete.

   If none of the wells has a valid Assay Setup for the chosen analysis mode, the analysis is not performed.

AQ, SNP, and CpG assays can be analyzed in any analysis mode without modifying analysis parameters. To do so, select the relevant wells and click **Analyze selected**. Multiple single nucleotide polymorphisms in a variable region can be analyzed in SNP mode, but not in AQ mode. These variable regions will be automatically disabled in AQ mode. InDels can be analyzed in AQ and SNP mode, but not in CpG mode.

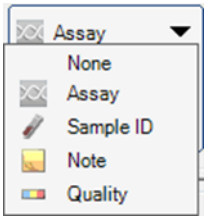
Analyzing assays in CpG mode requires that the sequence to analyze be a valid CpG sequence. If necessary, the appropriate sequence to analyze can be added in the **Analysis Setup** tab. Because the CpG mode does not support automatic analysis of SNPs, methylation percentages and quality assessments are only determined for the CpG sites. SNPs in a CpG assay can be analyzed in the AQ mode using the sequence to analyze used in the CpG setup. To exclude the CpG sites in the SNP reports, select the **Analysis Setup** tab and uncheck the **Analyze** option for these positions in the **Variable Positions** tab.

To analyze SEQ assays in any other analysis mode, a sequence to analyze must be entered in the **Analysis Setup** tab.

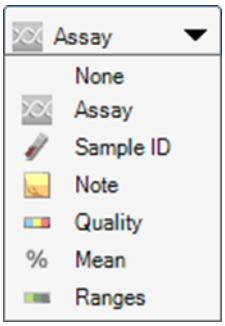
7.15.3. View the analysis results

By selecting an analyzed (light blue) well in the **Overview** tab, the corresponding Pyrogram is displayed in the Pyrogram area and the well information (including analysis warnings) is listed in the Well Information area. If several wells are selected in the disc overview, information for the well with the orange selection frame is shown.

Get an overview of the results



AQ, SNP, and SEQ assays.



CpG assays.

The following well information can be viewed in the disc overview in the **Overview** tab:

	Select to show the assay name.
	Select to show the sample ID.
	Select to show the well note.
	Select to show the quality bar. The quality bar shows the quality assessment of all variable positions in the well or of all the bases in the base-called sequence. See "Quality colors" in Section 7.15.3.
%	Select to show the mean methylation percentage of all CpG sites (or CpN sites if CpN mode was used) in the well.
	Select to show the methylation bar. The methylation bar shows the methylation level for each CpG site (or CpN site if CpN mode was used) in the well. See "Methylation colors" in Section 7.15.3.

Note: Wells with a high substrate peak will be marked with an **Information** icon ( ) in the disc overview. This will not affect the quality assessments.

Note: If analysis parameters, quality assessments or base-called sequence for SEQ results have been edited by the user, the well is marked with a warning icon ( ).

Note: If an assay is locked, the well is marked with the icon.

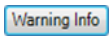
Print or export disc overview as an image

The disc overview can be printed or copied as an image (to the clipboard) by right-clicking the disc overview and selecting **Print** or **Copy as Image** from the context menu. The image can be pasted into applications that support Enhanced Metafile (EMF) images.

Analysis warnings

By selecting an analyzed (light blue) well, the analysis warnings (if any) are listed in the Well Information area. An analysis warning affects the quality assessment in the following way:

- **AQ, SNP, and CpG assays**: Affects the quality assessment for either all variable positions or a single position. If several warnings of the same kind were triggered, only the most serious ones are displayed in the Well Information area.
- **SEQ assays**: Affects the quality assessment for either the whole sequence or from a specific dispensation and forward. All warnings triggered within the quality control window are displayed in the Well Information area.

Clicking the **Warning Info** button (

) provides a short description and recommendation for each shown warning.

For some of the warnings, the criteria for occurrence and the effect on the quality assessment can be modified by the user in the **Analysis Parameters** tab; see Section 7.15.4.

Note: For other warnings, please refer to Section 9 or contact QIAGEN Technical Service.

Quality assessments

The quality assessments of the analysis results are displayed by:

Quality bars ( ) in the disc overview; see “Get an overview of the results” (this section)

- The background color of the analysis results (allele frequencies, the methylation percentages, or genotype in the Pyrogram,e.g., 

, or the base-called sequence)
- The peaks in the compensated Pyrogram are colored according to their quality assessments (SEQ assays only)

Quality colors

- **Blue**: Passed
- **Yellow**: Check
- **Red**: Failed
- **White**: Not analyzed. Either analysis is not supported by the software (e.g., analyzing single nucleotide polymorphisms in CpG mode) or the variable position has been deselected by the user (AQ, SNP, and CpG assays only).

Homopolymers and uncertain bases

For sequences containing homopolymers, quantification of the base at the end of the homopolymer may be uncertain and should be checked by the user. Uncertain bases are indicated in these ways:

- By yellow in the quality bar of the disc overview
- By yellow in the quality bar above the base-called sequence
- By [ ] surrounding the uncertain base in the base-called sequence
- By a yellow bar above the blue bar in the histogram

See Section 7.15.5 to add or remove the uncertain base. If the uncertain base is added to the base-called sequence, the [ ] disappear and the added base is shown in lowercase. If the uncertain base is deleted, the [ ] and the base are both deleted. If an analysis containing uncertain bases is exported to a FASTA-formatted report, uncertain bases with an intensity of 1.5 or higher will be included, while those with an intensity of less than 1.5 will not be included.

Methylation levels

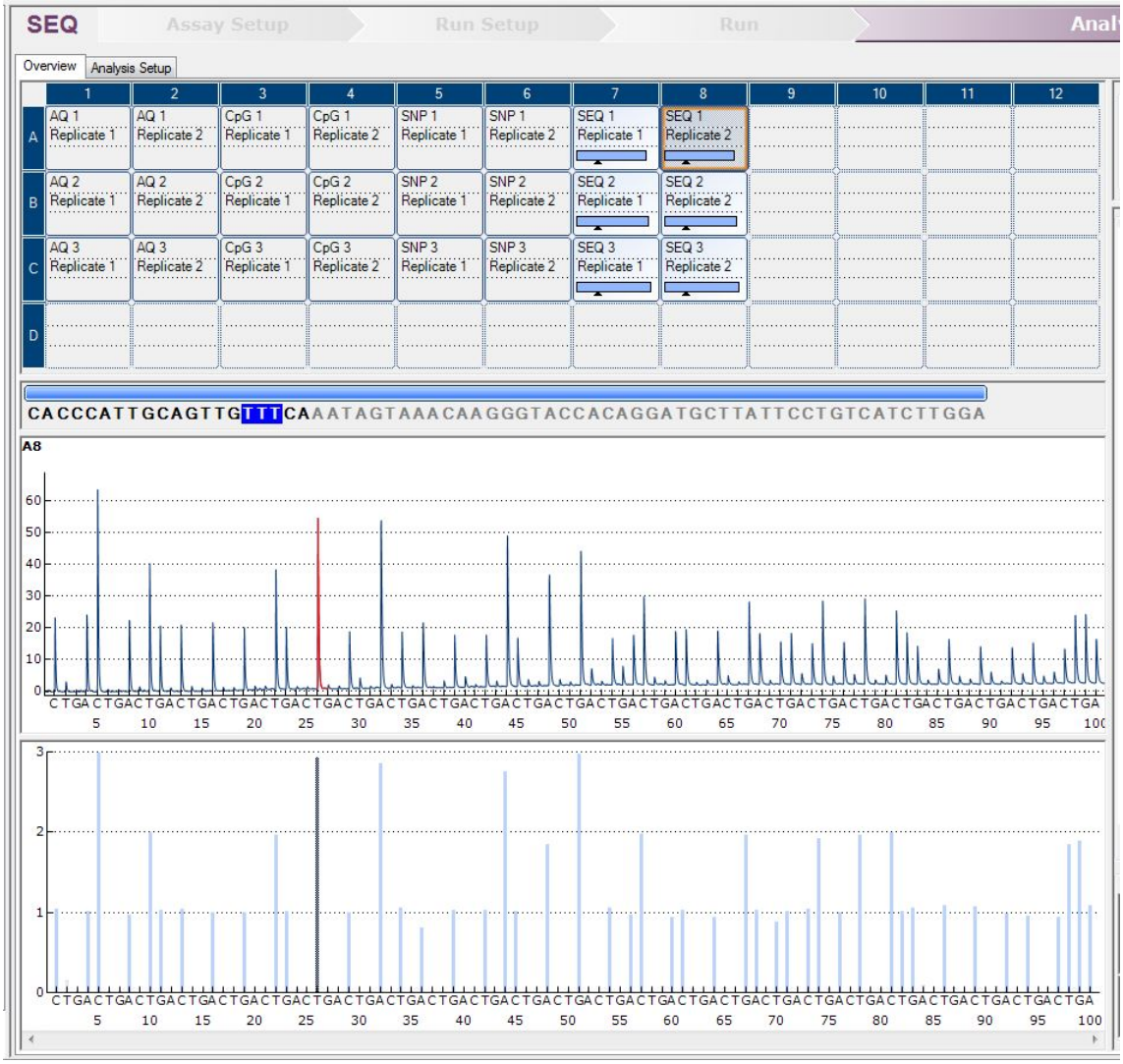
In the CpG mode, a methylation bar in the Overview tab shows the methylation level for each CpG site (or CpN site if CpN mode was used) in the well (see "Get an overview of the results" in this section).

Methylation colors

- **Light green**: Below the expected range
- **Green**: Within the expected range
- **Dark green**: Above the expected range

View and compare Pyrogram

By selecting an analyzed well in the **Overview** tab, the corresponding Pyrogram and theoretical histogram (if an AQ, SNP, or CpG assay) or compensated Pyrogram (if an SEQ assay) are displayed in the Pyrogram pane.



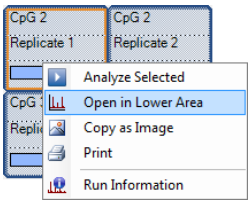
When a base is selected in the base-called sequence, the corresponding peak is highlighted in both Pyrogram areas, and vice versa.

Simultaneously view Pyrograms of different wells

Pyrograms from 2 or more wells can be viewed simultaneously (e.g., if the user wishes to compare Pyrograms) by opening one or more additional Pyrograms of selected wells in the lower area of the Pyrogram pane:

1. Highlight the wells (see Section 7.7) you wish to open in the lower area.
2. Right-click the selection and select **Open** in Lower Area from the context menu.

   Two or more Pyrograms are now displayed in the Pyrogram pane. In the upper area of the pane is the Pyrogram of the well in the disc overview displaying an orange outline. This Pyrogram is fixed. One or more Pyrograms of the additional wells selected for the comparison are presented in the lower area. The Pyrograms are linked, meaning that, if the Y axis value for one of the Pyrograms is set manually, the entered value is used for both Pyrograms. The Pyrograms in the lower area are displayed one at a time.
3. Select the well in the disc overview that should appear as the fixed Pyrogram in the upper area of the Pyrogram pane.
4. Use the scroll bar in the lower area of the Pyrogram pane to change the Pyrogram displayed.



   To close the Pyrogram list in the lower area, click **X** in the upper right corner of the lower area.

Zoom Pyrogram and view description of icons and colors

For information on icons and colors used in the Pyrogram area and how to zoom, see Section 7.9.

7.15.4. Edit analysis parameters

The default analysis settings have been set to give optimal analysis results for most assays. If changing these settings, ensure the changes are validated (see Appendix A – Assay Design and Validation).

Note: When using QIAGEN kits, use the settings stated in the kit handbooks.

Note: It is not possible to edit the analysis parameters for a locked assay ( ).

1. Select the well or wells (see Section 7.7) for which you wish to edit the analysis parameters.

   Note: The changes will only be applied to wells that share the same assay and dispensation order as the displayed well. To edit the analysis parameters for all wells with the same assay and dispensation order, you only have to select one of the wells.

2. Edit analysis parameters in the **Analysis Setup** tab:
   - To enable or disable variable positions and/or change expected methylation ranges (only CpG assays), see Section 7.11.5.
   - To edit other analysis parameters for an AQ, SNP, or CpG assay, see Section 7.11.6.
   - To edit the analysis parameters for an SEQ assay, see Section 7.12.3.

   Note: It is not possible to change the assay name, dispensation, order or assay note.

3. When finished, click **Apply**. In the resulting message box, select if changes should be applied to all wells with the same assay name and dispensation order (**To all**) or to only selected wells with the same assay name and dispensation order (**To selected**).

   Note: It is also possible to enable or disable reference peaks and bisulfite treatment controls (CpG assays only) in the Pyrogram in the **Overview** tab (see "Enable or disable reference peaks and bisulfite treatment controls" in Section 7.11.6). To apply changes made in the Pyrogram, click the green button, which is enabled if a change has been made.

4. In the Apply Analysis Setup dialog box, apply the changes to all or the selected wells:
   - To apply the changes to all wells that share the same assay and dispensation order as the displayed well (i.e., all the white wells in the Apply Analysis Setup dialog box), click **To All**.
   - To apply the changes to the selected wells, (i.e., the white wells that are selected in the Apply Analysis Setup dialog box), click **To Selected**.

   During the analysis, a progress dialog box is shown. The dialog box contains a progress bar, a stop button, and the name of the well that is being analyzed. The analysis can be stopped by clicking **Stop**.

5. To save the changes, click .

   Note: If analysis parameters, quality assessments or base-called sequence for SEQ results have been edited by the user, the well is marked with a warning icon ( ) in the **Overview** tab.

   Note: All changes are logged. To view the analysis log for a selected well, select **Analysis Log** from the **Tools** menu.

Use modified assay in other runs

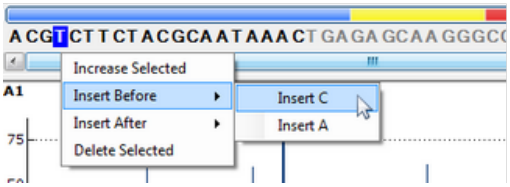
Changes made in the **Analysis Setup** tab will not be saved in the original assay file. To use the modified assay in other runs:

1. Select a well that is using the modified assay and click **Save Assay**. The Save Assay As dialog box opens.
2. Save the changes to the original file or save the modified assay as a new file.

   Select destination (folder) from the Save in drop-down list. Enter filename in the File name text box and click **Save**.

7.15.5. Edit base-called sequences

To edit a base-called sequence, right-click it and select the desired option. It is also possible to copy segments or the complete base-called sequence. Click and drag the mouse across the segment to be copied (selected segment will be highlighted in blue) and press C while holding down the Ctrl key to copy the selection to the clipboard. It is also possible to select a segment of base-called sequence by using the Shift and arrow keys.

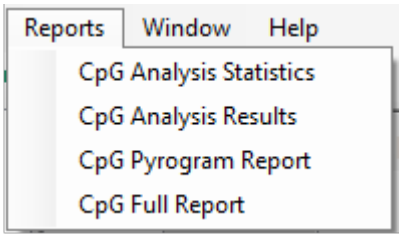


Note: All changes are logged. To view the analysis log for a selected well, select **Analysis Log** from the **Tools** menu.

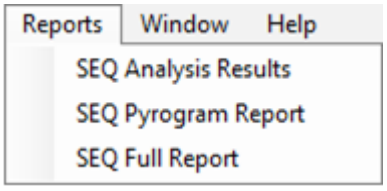
Note: If editing a base-called sequence, note that the quality assessments are still based on the original sequence (the sequence called by the software).

Note: It is not possible to edit the base-called sequences for a locked assay ( ).

7.16. Viewing, printing, and saving Analysis Reports



Reports for AQ and CpG runs.



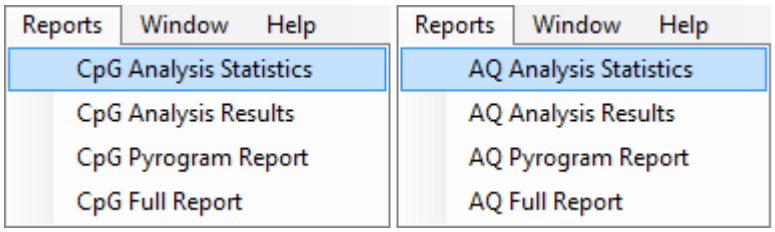
Reports for SNP and SEQ runs.

PyroMark Q48 Autoprep software offers the following analysis reports for processed runs:

- **Analysis Statistics Report**: This includes analysis statistics for all or selected wells.
- **Analysis Results Report**: This includes well information and analysis results for all or selected wells.
- **Pyrogram Report**: This includes well information and Pyrogram for all or selected wells.
- **Full Report**: This includes run parameters, run log, well information, and analysis results (including Pyrogram) for all or selected wells.

Note: To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

7.16.1. Analysis statistics report (only AQ and CpG modes)

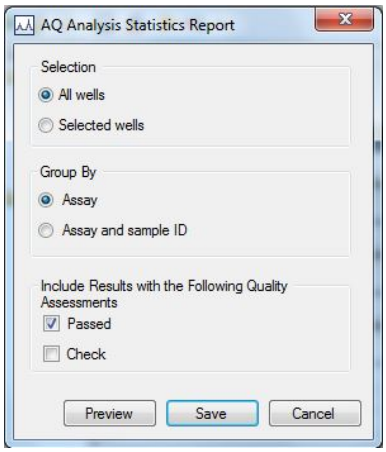


The Analysis Statistics report includes the following information for variable positions in all or selected wells (see Section 7.7):

- The mean allele frequencies (AQ report) or mean methylation percentage (CpG report)
- The highest and the lowest allele frequencies (AQ report) or methylation percentage (CpG report)
- The standard deviation
- The number of values and the wells used in each calculation
- If analysis parameters or quality assessments have been edited by the user, the affected wells are listed at the top of the report

The report can be saved as a text file (***.tsv** or ***.csv**) or an HTML file (***.html**). The report can be imported into Microsoft Excel or other applications that can handle text files (***.tsv** or ***.csv**) with data that is separated by semicolons (;) or tabs. This is useful when doing further calculations on the data.

Report options

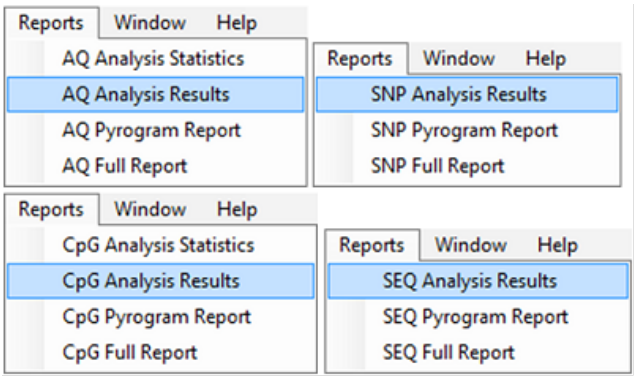


In the Analysis Statistics Report dialog box, there are the following options:

Option	Description
All wells/selected wells	The wells to be included in the report.
Assay/Assay and sample ID	The analysis results statistics can be grouped according to: • Assay Wells with the same assay will be grouped. • Assay and sample ID Wells with the same assay and sample ID will be grouped. Can be useful when experiments with replicates are performed.
Passed/Check	The analysis results to be included. The calculations can be performed on results with "Passed" and/or "Check" quality assessment. **Note**: If all "Passed" and "Check" results are to be included in the report, you can exclude results within this group by turning off the **Analyze** option for these positions in the **Analysis Setup** tab (see Section 7.11.5).

To view the report before saving or printing it, click **Preview**.

7.16.2. Analysis results report



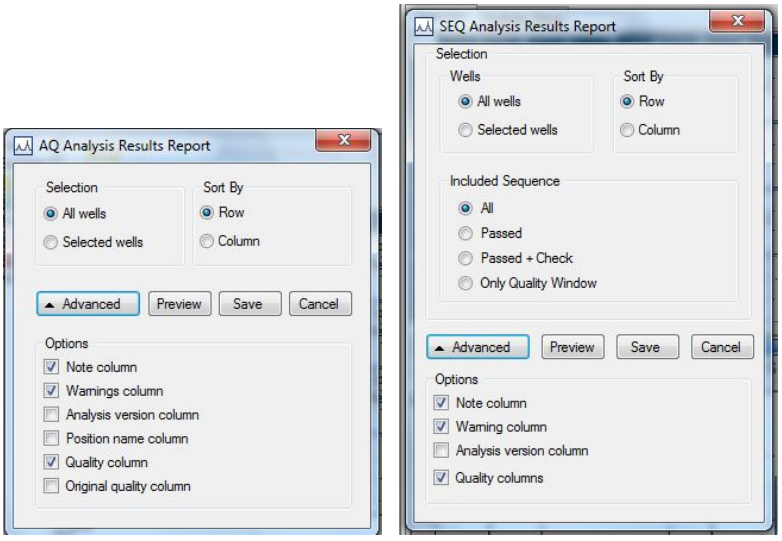
The Analysis Results report includes the following information for all or selected wells (see Section 7.7):

- Well information (well name, assay name, and sample ID)
- The allele frequencies (AQ report), genotypes (SNP report), methylation percentages (CpG report), or base-called sequences (SEQ report), and the quality assessments
- The mean methylation percentage and the standard deviation of all passed CpG sites (or CpN sites if CpN mode was used) in a well (CpG report only)
- The highest and lowest methylation percentage in a well (CpG report only)
- Information on whether the analysis parameters, quality assessments and analysis results (SEQ report only) have been edited by the user or not

Optional: The analysis version, well notes and analysis warnings. In the AQ and CpG reports, it is also possible to include the names and the original and/or the current quality assessments for the variable positions.

The report can be saved as a text file (*.**tsv** or *.**csv**) or an HTML file (*.**html**). The report can be imported into Microsoft Excel or other applications that can handle text files (*.**tsv** or *.**csv**) with data that are separated by semicolons (;) or tabs. This is useful when doing further calculations on the data. The first line in the report states the name of the run. The following two or three lines contain the column headings. Each of the lines following the column headings contains detailed well information and statistics of a specified well.

Report options

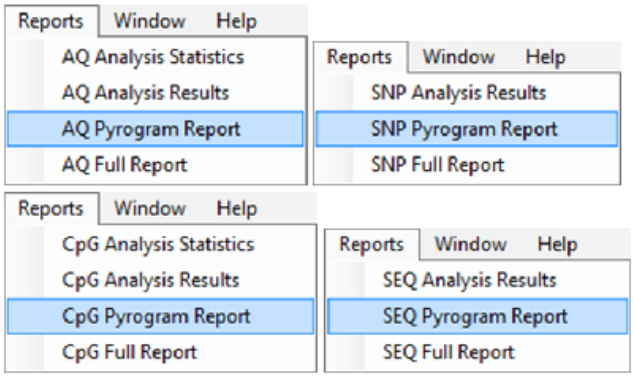


In the Analysis Results Report dialog box, there are the following options:

Option	Description
All wells/selected wells	The wells to be included in the report.
Sort by row/column	The sorting order of the wells.
All/Passed/Passed + Check/Only Quality Window	The bases in the base-called sequences to be included in the report. This option is only available for the SEQ report.
Note column	If this option is checked, a column with well notes is included.
Warnings column	If this option is checked, a column with analysis warnings is included.
Analysis version column	If this option is checked, a column with the analysis version is included.
Position name column	If this option is checked, a column with the names of the variable positions is included. This option is not available for the SEQ report.
Quality column	If this option is checked, a column with the current quality assessments is included.
Original quality columns	If this option is checked, a column with the original quality assessments is included. This option is not available for the SEQ report.

To view the report before saving or printing it, click **Preview**.

7.16.3. Pyrogram report



The Pyrogram report includes well information (well name, assay name, sample ID, and well note) and Pyrograms for all or selected wells (see Section 7.7). If analysis parameters, quality assessments, or base-called sequence for SEQ results have been edited by the user, this is stated in the report.

The following information, icons, and colors are displayed and used in the AQ, SNP, and CpG reports:

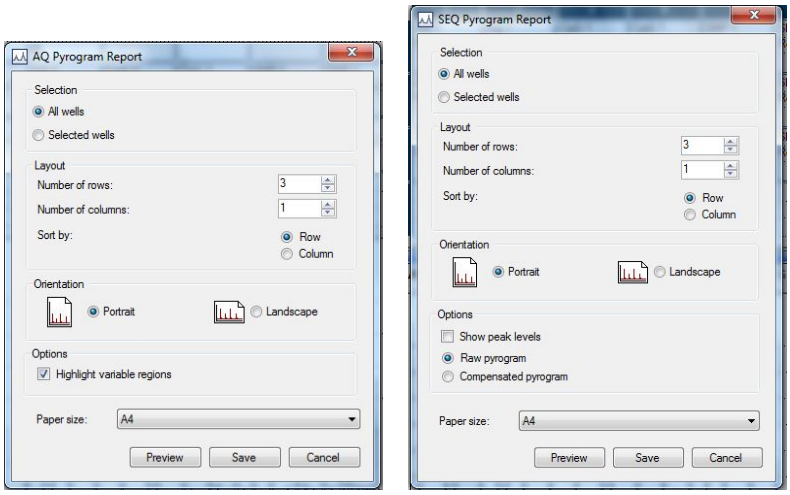
- The well name and the sequence to analyze.
- The analysis result — allele frequencies (AQ report), genotypes (SNP report), or methylation percentages and methylation averages (CpG report) — is displayed above each variable position, for example  (InDel) and  . The background color shows the quality assessment of the analysis result; see "Quality colors" in Section 7.15.3.

  Note:  (in white) indicates that a site was deselected by the user.  (in white) indicates that the software does not support the analysis, for example, analysis of SNP in the CpG mode.  (in red) indicates that the analysis was not possible due to lack of data.
- If desired, the variable positions are highlighted with a blue-gray background color.
- Bisulfite treatment controls are highlighted with a light orange background color (CpG report only).

The following information and colors are displayed and used in the SEQ report:

- The well name
- The base-called sequence. The background color of a base in the sequence is according to its quality assessment; see "Quality colors" in Section 7.15.3.
- If a compensated Pyrogram is included, the peaks are colored according to their quality assessments. Compensated Pyrograms are only shown for analyzed wells.

Report options



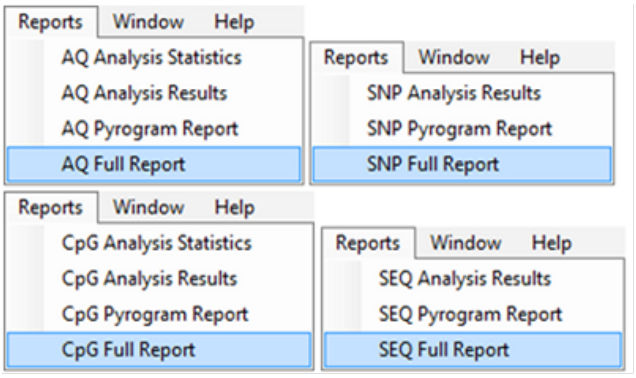
In the Pyrogram Report dialog box, there are the following options:

Option	Description
All wells/selected wells	The wells to be included in the report.
Number of rows/columns	The number of columns and rows of a Pyrogram on each sheet.
Sort by row/column	The sorting order of the wells.
Portrait/Landscape	The paper orientation.
Highlight variable regions	If this option is checked, the variable regions are highlighted with a blue-gray background color. This option is not available for the SEQ report.
Show peak levels	If this option is checked, the calculated peak levels are shown in the Pyrogram. This option is only available for the SEQ report.
Raw Pyrogram/ Compensated Pyrogram	The type of Pyrogram to be included in the report. This option is only available for the SEQ report.
Paper size	The paper size (A4, A3, letter, or tabloid).

To view the report before saving or printing it, click **Preview**.

Note: To view the report, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

7.16.4. Full report

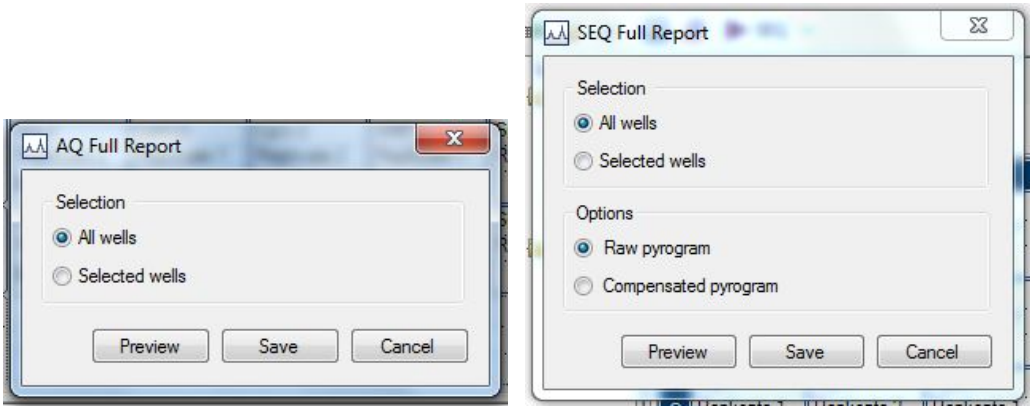


The full report includes the following information for all or selected wells (see Section 7.7):

- Run parameters (run name, run date and time, protocol, instrument name, serial number, operator, disc ID, barcode, reagent ID, and run note) and a run log
- Well information (well name, assay name, sample ID, and well note), analysis version, AQ, SNP, or CpG assay, sequence to analyze
- Pyrogram

  For information on icons and colors used in the Pyrogram area, see Section 7.16.3.
- Allele frequencies (AQ report), genotypes (SNP report), methylation percentages (CpG report), or base-called sequences (SEQ report) and the quality assessments
- Analysis warnings
- If analysis parameters or quality assessments have been edited by the user, the affected wells are listed

Report options



In the Full Report dialog box, there are the following options:

Option	Description
All wells/selected wells	The wells to be included in the report.
Raw Pyrogram/Compensated Pyrogram	The type of Pyrogram to be included in the report. This option is only available for the SEQ report.

To view the report before saving or printing it, click **Preview**.

Note: To view the report, a PDF reader must be installed on the computer. Adobe Reader can be downloaded at **www.adobe.com**.

7.17. Supported files

PyroMark Q48 Autoprep software supports the following file types:

AQ assay	***.qaq**
SNP assay	***.qsnp**
CpG assay	***.qcpg**
SEQ assay	***.qseq**
Disc setup	***.qset**
Processed run	***.qrun**

Files can be opened by either selecting **Open** in the **Files** menu or clicking .

Note: Files created in PyroMark Q24 2.0 software or in PyroMark Q96 2.5 software are not supported.

7.18. General hints and tips

7.18.1. Validation of assays

Validate your assays using reference DNA samples; see Appendix A – Assay Design and Validation

7.18.2. Run log

A log is maintained for each run, detailing events and warnings that occur during a run. This log is available in the Run Information window, which can be accessed from the **Tools** menu.

7.18.3. Analysis log

All analyses performed are logged with analysis settings used, analysis mode (AQ, SNP, CpG, or SEQ), analysis version, results (including analysis warnings), date and time of the analysis, and who performed the analysis. For information on who performed an analysis and who created an assay or run file to be correct, all users must log on to Windows using their own user accounts. For more information about user accounts and logging on and off, see Windows online help or contact your system administrator.

To view the analysis log for a selected well, select **Analysis Log** from the **Tools** menu.

7.18.4. Protection of files

To protect a file from being edited by another user, save the file in a folder that can only be accessed by you. Contact your system administrator for more information.

To protect a file from being accidentally overwritten by you or another user, set the "Read-only" attribute for the file using Windows Explorer:

1. Close the file in the PyroMark Q48 Autoprep software.
2. Open Windows Explorer and locate the file.

   This can be done by right-clicking the folder containing the file in the shortcut browser and selecting Explore from the context menu.
3. In Windows Explorer, right-click the file and select **Properties** from the context menu.
4. When the Properties dialog box opens, turn on () the Read-only attribute and click **OK**.

A backup should be performed frequently.

7.18.5. Protection of analysis results

It is not possible to edit the analysis parameters or results for a locked assay (). To lock an assay, open the assay file and click **Lock Assay** at the bottom of the assay setup window. Lock the assay before adding it to the disc.

8. Maintenance Procedures

The only components requiring general user care during the working life of the instrument are the cartridges and the chamber waste area. Cleaning of both areas is mandatory to ensure proper function of the instrument. We highly recommend exchanging the cartridges annually.

8.1. Cleaning the PyroMark Q48 Autoprep

Important: Switch the instrument off and disconnect the line power cord from the power outlet before cleaning.

WARNING **Hazardous chemicals**

Always wear safety glasses, 2 pairs of gloves, and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or the OSHA1,* ACGIH,† or COSHH‡ documents.

For more information, visit **www.qiagen.com/safety**.

Venting for fumes and disposal of waste must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

WARNING/ CAUTION **Risk of electric shock**

Do not open any panels on the PyroMark Q48 Autoprep instrument.

Only perform maintenance that is specifically described in this user manual.

CAUTION **Damage to the instrument**

Do not use solvents, or reagents containing acids, alkalis, or abrasives to clean the PyroMark Q48 Autoprep instrument.

CAUTION **Damage to the touchscreen and computer**

Do not pour or spray liquids, e.g., cleaning agents, on to the PyroMark Q48 Autoprep instrument. Use a tissue moistened with water only for cleaning.

CAUTION **Risk of fire**

When cleaning PyroMark Q48 Autoprep instrument with alcohol-based disinfectant, leave the instrument lid and the injector cover open to allow flammable vapors to disperse.

Important: We recommend wiping the PyroMark Q48 Autoprep instrument with a damp cloth only.

The following disinfectants and detergents are recommended for cleaning the PyroMark Q48 Autoprep.

Note: If cleaning agents different from those recommended are used, ensure that their compositions are similar to those described below. If in doubt, please contact QIAGEN Technical Services prior to use.

General cleaning of the PyroMark Q48 Autoprep instrument:

- Mild detergents
- 70% ethanol or isopropyl alcohol

8.2. Light detector maintenance

To avoid decreased signal levels, we recommend checking the light detector every 2–4 months and cleaning the photomultiplier tube (PMT) window when dirty. To clean the PMT, follow these steps:

- Manually move the chamber lid to the fully open position (all the way to the back).
- Check that there is no liquid in the cartridges and then turn the instrument onto its side.
- Clean the PMT window with a lint-free tissue suitable for cleaning optical lenses.
- In case of sticky dirt, a few drops of 70% ethanol or isopropyl alcohol may be applied to the tissue. Do not scrub or apply excessive force when cleaning!

CAUTION **Damage to the instrument**

Do not expose the photomultiplier (PMT) to strong light during maintenance.

Important: During this operation, do not expose the PMT to strong light (i.e., do not use a flashlight to check if the window is clean).

8.3. Cartridge care

To ensure injectors are dispensing volumes within specification, it is important to conduct routine cleaning prior to the first run of the day and following a workday. For this cleaning procedure, the cartridges do not need to be removed. Please refer to Section 6.5.2 for details.

The cartridge lifetime is dependent on its usage. All cartridges must be replaced by new ones routinely after 1 year at the latest to ensure proper volume dispensation. When using the instrument and cartridges very often, earlier replacement is needed. If a suspect drop error message affecting one or two drops appears (as highlighted in yellow below), this will not affect the overall run result. However, if this error is seen frequently, it is an indication that the cartridge has reached the end of life and needs to be replaced.

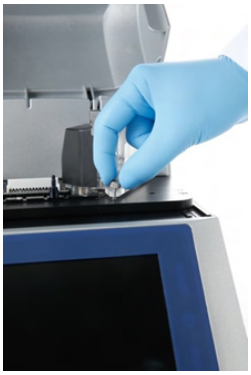
Image of run analysis.

If an individual cartridge shows repeating errors, for example during cartridge priming, it should be replaced separately. Cartridges are available as set of three or can be purchased individually.

8.3.1. Removing cartridges from instrument

1. Ensure that the reservoirs are empty and the cartridge lid is locked, prior to removing the cartridge.
2. Turn the instrument off.
3. Take out the thumb screws holding the cartridge in place. Place the screws in a safe place to avoid losing them.

   Note: Putting back the thumb screws without cartridges installed can damage the instrument.



4. Gently pull the cartridge out from its slot.

5. Always transport the cartridges in the dedicated cartridge boxes.

8.3.2. Installing injector cartridges into instrument

1. To install an injector cartridge, slot the cartridge into place ensuring that the air pin and electrical connectors align.
2. Carefully apply pressure on the rear part of the cartridge (black box). If the pins are aligned correctly, the cartridge will snap into position.

   Important: Do not force the cartridge into position, as this might damage the electrical connectors.
3. Lock the cartridge into place using the previously removed thumb screws.
4. Turn on the instrument.

   The instrument will recognize the type of cartridge inserted along with all the calibrated volumes.

   In case a cartridge is placed into the wrong position or installed incorrectly, the user will be notified.

Do not force the injector cover closed while the cartridge lids are still open, as this may result in damage to the cartridge lids or the injector cover.

Avoid removing the cartridges repeatedly. Continual insertion and removal may result in failure of the sealed connection with the air inlet pin.

8.3.3. Cleaning cartridges

When necessary, clean the cartridges externally and the surrounding with high-purity water and lint-free cloths. After the last run of the day, perform a cleaning using the corresponding function in the instrument software (see Section 6.5.2).

If required, perform a Pyrophosphate clean, as described in Section 8.

8.4. Lid movement test

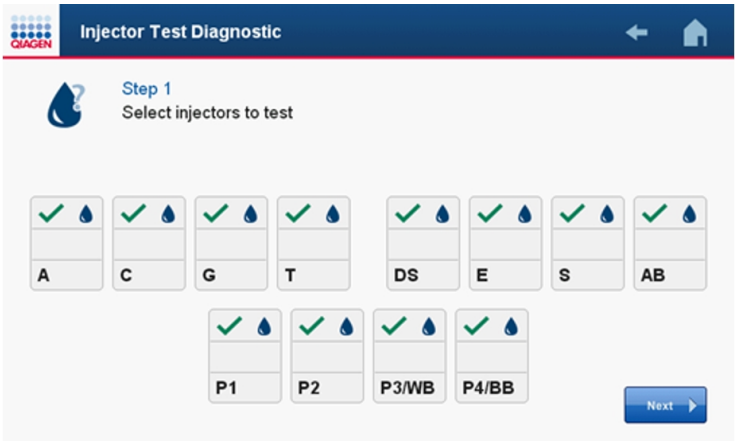
The lid movement test ensures the chamber lid is operating correctly. The lid will be homed and then moved to the open, closed, all forward, and rear injector positions, and then end in the open position.

8.5. Injector test

The injector test performs a diagnostic test of the injector system including the drop sensors.

1. To start the injector test, go to **Tools** > **Diagnostics** > **Injector Test**.
2. Select the injectors to test. The selected injectors will display a drop symbol. To continue, press **Next**.

   Note: The image below shows all injectors to be tested.



3. Add the required volume of purified water to the selected injectors. Ensure that care is taken when pipetting into the cartridge. Pipette tips must not come into contact with the cartridge filter membrane at the bottom of the cartridge. After adding the volumes, press **Next**.
4. Check that an absorber strip is inserted and check that a disc is inserted.

   Note: The absorber strip will be dispensed into during injector priming and at the end of the test to empty the injectors. The disc will be dispensed into as part of the test.
5. Start the test. Press **Yes** to confirm the start of full injector diagnostic test.

   During the prime, the test will determine if the drop sensors are working.

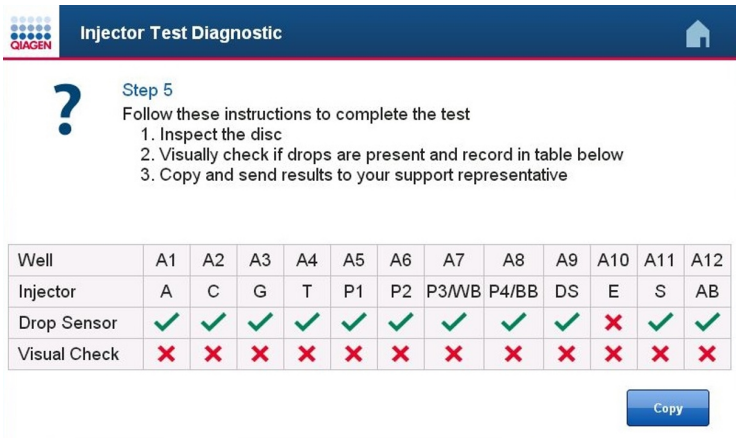
   In the second part of the test, the selected injectors will dispense a number of times into the first 12 corresponding disc wells (e.g., dCTP into well A2).

   At the end of the test the remaining liquid is emptied into the absorber strip.

   Note: As shown in the image, the cartridge dispensing into well A10 failed its injector test. The default data in the Visual Check row is marked with a red cross (✖).

6. After the injector test, perform a virtual assessment of the wells to see if a drop is visible.

   Note: The plate is marked in four sections and each well is numbered. The image below shows the location of well A10.

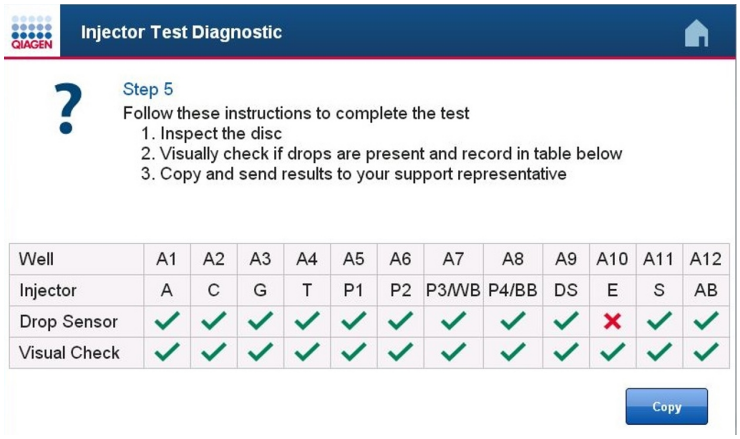


Well	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Injector	A	C	G	T	P1	P2	P3/WB	P4/BB	DS	E	S	AB
Drop Sensor	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓
Visual Check	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

7. Virtually assess all wells displayed on the screen to determine if the drops are present. If the drop is present, press the red x mark(✗) associated with the well. A green check (✓) will appear. Press **Copy** to finish the injector test and copy the injector test data to the USB flash drive.

   Note: If the visual assessment finds that all well contain fluid and the green check (✓) is present for all wells, this will override the sensor data and allow the run to progress.

8. If the well is empty, leave the red x mark (✗) in the box. If the visual check reveals an empty well, this is true injector failure and the red x mark (✗) should remain. Press **Copy** to finish the injector test and copy the injector test data to the USB flash drive.



Well	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Injector	A	C	G	T	P1	P2	P3/WB	P4/BB	DS	E	S	AB
Drop Sensor	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓
Visual Check	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Note: If the injector test fails, refer to Section 6.5.2. If the cartridge has been left idle for more than 12 hours and a flush was not performed, it is possible that more than one cleaning cycle is required to ensure correct functionality of the injector.

9. If any of the injectors have failed, copy the results and send to QIAGEN Technical Services for further help and instructions.

8.6. Pyrophosphate clean

Pyrophosphate contamination may occur and is typically observed as small peaks in control dispensations (where no peaks are expected). Such contamination will have a negative influence on the final analysis result. Pyrophosphate contamination only affects the nucleotide injectors. Removal of the contamination is achieved using the enzyme, pyrophosphatase, and a thorough clean with high-purity water. Use the following table to prepare the pyrophosphatase cleaning solution based on the number of injectors selected to be cleaned. Each injector will require 100 µL.

Table 3. Required volume for Pyrophosphatase cleaning solution

Component	Final concentration	Volume per injector
PyroMark Advanced Annealing buffer	–	95 µL
Pyrophosphatase*	5 U/mL	5 µL (100 U/mL†

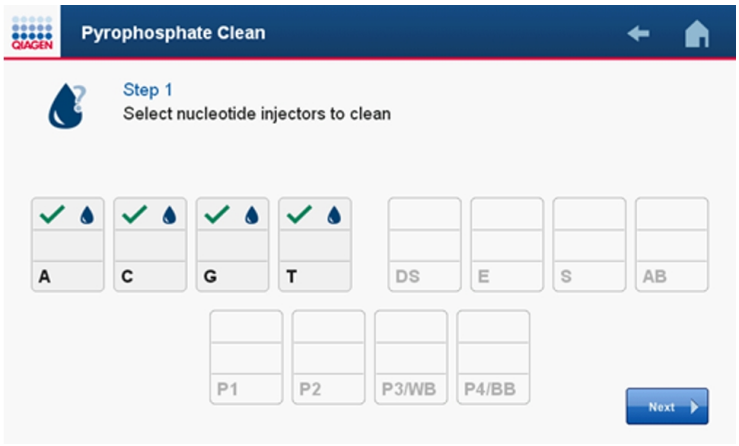
* For example, Pyrophosphatase, Inorganic from New England Biolabs (cat. no. M0361L).

† In case of higher concentrated stock solutions of Pyrophosphatase, dilute Pyrophosphatase solution to 100 U/mL with PyroMark Advanced Annealing Buffer.

Important: During the following pipetting steps, ensure that the tip of the pipette does not touch the filter at the bottom. This can remove solid parts from the filter which can lead to blockage of the cartridge reservoirs.

1. Select **Pyrophosphate Clean** to begin.
2. Ensure that the injectors are empty before beginning. If the injectors are not empty, run the Clean protocol first (refer to Section 6.5.2).

3. Select the nucleotide injectors to clean. Only nucleotide injectors can be selected. All the nucleotide injectors are selected by default.

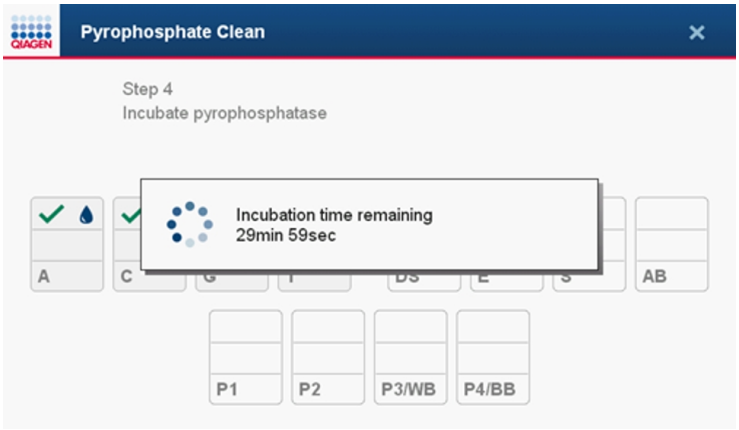


4. Manually pipette 100 µL of pyrophosphatase cleaning solution into selected injectors and then press **Next**.
5. Check that the absorber strip is inserted.
6. Start clean.

   The instrument will automatically prime the injectors to ensure that the cleaning solution is in contact with the entire injector flow channel.

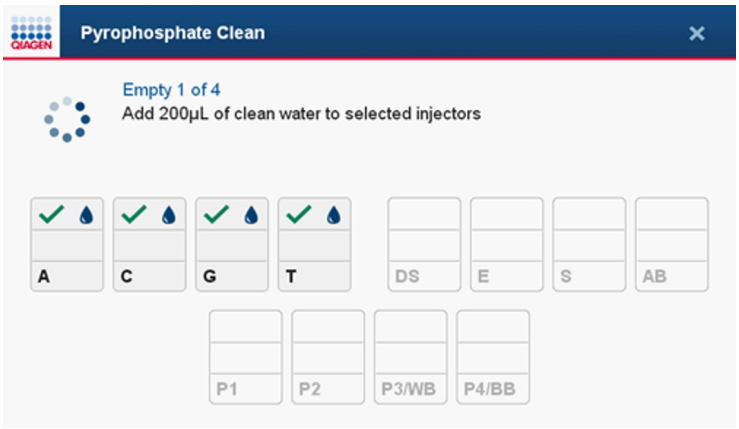
   The software will begin a 30 min countdown timer to allow the solution to incubate. Once started, the pyrophosphate clean procedure cannot be terminated.

   At the completion of the pyrophosphatase incubation, the software will notify the user to continue to the next step with an audible beep.



7. Empty 1 of 4: Manually pipette 200 µL of high-purity water into the selected nucleotide injectors, and then press **Start**.

   The injectors will be emptied into the absorber strip.



8. Empty 2 of 4: Manually pipette 200 µL of high-purity water again, and then press **Start**.
9. Empty 3 of 4: Manually pipette 200 µL of high-purity water again, and then press **Start**.
10. Empty 4 of 4: Manually pipette 200 µL of high-purity water again, and then press **Start**.

    The injectors will be emptied for a last time into the absorber strip.
11. At the end of the clean, the software will instruct the user to open the cartridge lids to prevent condensation.
12. Press **Home** to return to the Home screen.
13. Remove and replace the absorber strip (refer to Section 6.5.3).

8.7. Advanced cleaning

It may be that the injector test still fails after several cleaning cycles. To recover the respective injector, use the **Advanced Cleaning**. Please be aware that only one single injector can be cleaned during each Advanced Cleaning run. The whole process of the Advanced Cleaning can take up to approximately 2 hours. To check if the Advanced Cleaning was successful the injector test is integrated as final step in the Advanced Cleaning.

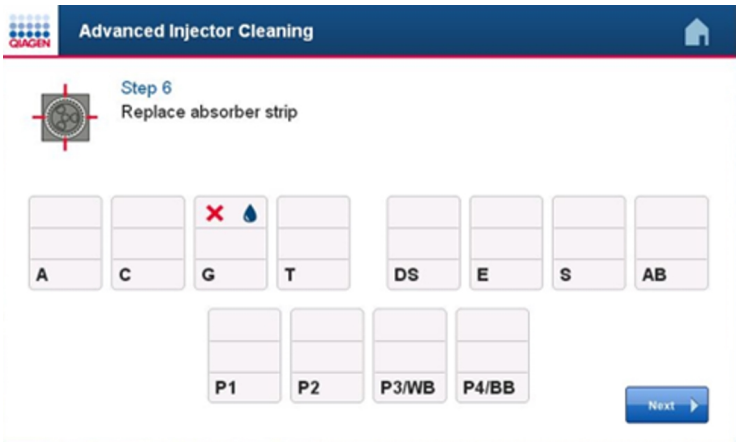
Important: During the following pipetting steps, ensure that the tip of the pipette does not touch the filter at the bottom. This can damage the filter which can lead to a blockage of the cartridge reservoir.

1. Select **Advanced Cleaning** to begin.
2. Ensure that the injectors are empty before beginning. If the injectors are not empty, run the Clean protocol first (refer to Section 6.5.2) and carefully pour the liquid from the reservoir of the blocked injector into the appropriate liquid waste.

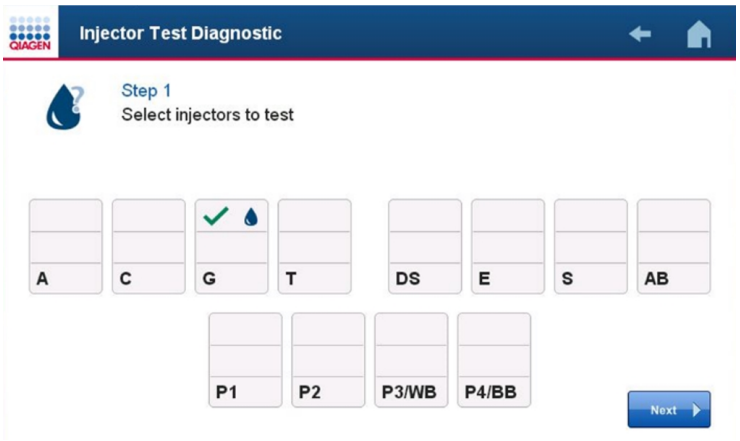
3. Select an injector to clean. Only one injector can be cleaned with the Advanced Cleaning protocol each time.

4. Remove the sample disc from the chamber.
5. Make sure that the absorber strip is inserted.
6. Manually pipette 800 µL high-purity water as the cleaning solution into the selected injector and then press **Start**.
7. The instrument will automatically prime the injectors to ensure that the cleaning solution is in full contact with the entire injector flow channel.
8. The instrument will perform up to three attempts to clear the injector during an Advanced Cleaning run. In case that the clogging is more persistent, an incubation time of 30 min is applied before the instrument starts the next attempt to clear the injector. If the injector is still clogged after the second attempt, this step will be repeated. As soon as the injector is free the process will proceed with step 9. If it is not possible to recover the injector the software will lead you back to the start of the Advanced Cleaning procedure.

   If the software detects that the channel is now dispensing drops, but the channel has not yet been fully emptied, be careful not to overfill the reservoir in the next step. Instead, please ensure that the volume in the next rinse step is approximately 1000 µL.
9. Manually pipette 1000 µL of high-purity water into the selected injector and then press **Start**.
10. After the first rinse replace the absorber strip.



11. Manually pipette 1000 µL of high-purity water into the selected injector and then press **Start**.
12. Wait for the instrument to finish rinsing and repeat step 11.
13. The injector test will now start automatically (refer to Section 8.5). The cleared injector is selected by default.



9. Troubleshooting

If you need to contact QIAGEN Technical Services about an error, note the steps leading to the error and any information given in any dialog boxes. This will help QIAGEN in solving the problem.

When calling QIAGEN Technical Services about errors, please have the following information ready:

- Instrument serial number, type, and version
- Date of last maintenance performed
- Error code (if applicable)
- Time point when the error occurred for the first time
- Frequency of error occurrence (i.e., intermittent or persistent error)
- Photo of error, if possible
- Copy support package

Take the following action before contacting QIAGEN Technical Services.

- Check the run log (in the **Run Information** report) to assess if the system was working properly during the run.
- Consult the Troubleshooting section below.
- **Recommended**: Verify proper installation and operation of your system using PyroMark Control Oligo (see Appendix A – Assay Design and Validation).

9.1. Checking the run log

It is advisable to check the run log to assess if the system was working properly during the run.

1. Open the run file using the PyroMark Q48 Autoprep software.
2. Select **Run Information** from the **Tools** menu or right-click the file in the shortcut browser and select **Run Information** from the context menu. The Run Information report is opened.
3. Check the run log (at the end of the report) for any problems during the run.
4. If deviations from the preset block temperature, pressure and/or mixer speed values are noticed several times during a run and for longer time periods or in repeated runs, please contact QIAGEN Technical Services. If requested, to send an Environment Data file:
    a. Select **Export Environment Data** from the **Tools** menu.
    b. Select the destination folder for the data file from the Save in drop-down list.
    c. Enter the filename in the File name text box and click **Save**.

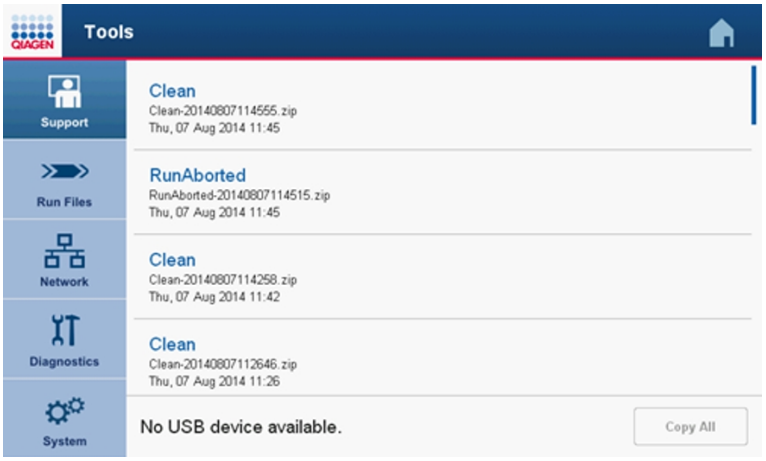
9.2. Copying support packages

Support packages contain information useful to QIAGEN Technical Services. A support package is created for every run, clean, pyrophosphate clean, and injector diagnostic test.

In the event that you need to send a support package to QIAGEN Technical Services, locate and copy the file to a USB flash drive. Support packages will be compressed to allow for easy transfer through email.

Click **Tools** and select **Support** on the instrument screen to access the available support packages.

The last 20 support packages are available and are dated and time stamped with the most recent runs at the top of the list. Alternatively, all the support packages can be saved at once using the **Copy All** button.



9.3. Analysis-related errors

Error or error message	Comments and suggestions
PCR failed due to low DNA quality	Check the PCR samples using an agarose gel to confirm there is one strong specific band. If not, rerun PCR with high-quality DNA. The PyroMark PCR Kit is recommended for highly specific amplification of bisulfite-converted DNA and gDNA from various sources.
Poorly optimized PCR	Check the PCR samples using an agarose gel to confirm there is one strong specific band. If not, reoptimize PCR.
Biotinylation is omitted or not added to the correct PCR primer	Check assay design; see Appendix A – Assay Design and Validation.
Biotinylation is of poor quality	Use a recommended primer supplier. Ensure the biotinylated primer is HPLC-purified or similar.
Insufficient amount of template for immobilization PyroMark Q48 Magnetic Beads	Follow the recommendations for amount of template; see Appendix A – Assay Design and Validation.
Too much PCR product depletes substrate, leading to missing peaks at the end of the sequence	Use less PCR product.
One or several of the compartments in the reagent cartridge were not correctly filled	Be sure to add sufficient reagents (see information shown on the display prior to the run).

Error or error message	Comments and suggestions
One of the injectors in the reagent cartridge is blocked or damaged (noted as missing peaks in Pyrogram)	Perform the injector test (see Section 8.5).
Reagents incorrectly dissolved or stored	Be sure to follow the instructions in the handbook supplied with PyroMark Q48 Autoprep Reagents and PyroMark Q48 Autoprep CpG Reagents. Include an empty well (Annealing Buffer only) in the run setup to check whether background peaks are coming from nucleotides.
Incorrect sequence to analyze	Correct the sequence to analyze and, if necessary, rerun the samples.
Background peaks in Pyrogram	Follow the recommendations in Appendix A – Assay Design and Validation the first time an assay is run. Redesign the assay.
Dispensation error	Perform the injector test (see Section 8.5). If the problem remains, contact QIAGEN Technical Services.
Unknown SNP in sample	Insert the SNP in the sequence to analyze and regenerate the dispensation order. Rerun the sample with the new dispensation order.
Plus shift	Change the dispensation order.
Minus shift	Ensure that homopolymers are followed by an extra dispensation.

9.4. Analysis software-related errors

Error or error message	Comments and suggestions
Red cross over wells in the **Overview** tab during analysis	The analysis of the well resulted in an error. Contact QIAGEN Technical Services.
Exception dialog box appears	Save the error report and send to QIAGEN Technical Services for information. Click **Continue** to proceed with analysis. If the dialog box remains, click **Quit** and restart the software.
Could not create assay from specified PyroMark Assay Design Software file.	Ensure a valid assay file type (AQ, CpG, or SNP) is being imported.
Assay missing dispensation order. Cannot be added to well.	Add a dispensation order to the assay setup.
Failed to save file. Access denied.	The file being saved is open in another application. Save the file with a different name.
The assay setup for well A1 is locked and cannot be transferred to a new analysis mode.	Locked assays on a processed run cannot be unlocked. A new assay and run setup must be created.
No well has a valid assay setup for the current analysis mode. Use **Analyze Selected Wells** or edit the sequence to analyze in the **Analysis Setup** tab.	Warning occurs in AQ, SNP, or CpG mode when Analyze all is clicked in an analysis mode that does not correspond to the assays in the disc. Specific wells can be analyzed in the current analysis mode by selecting the wells and clicking **Analyze Selected Wells**. If the sequence to analyze has not been entered in the assay setup (e.g., in SEQ assays) enter the sequence in the **Analysis Setup** tab.
No well has a valid assay setup for the current analysis mode. Use **Analyze Selected Wells**.	Warning occurs in SEQ mode when Analyze all is clicked and no wells contain SEQ assays. Specific wells can be analyzed in the current analysis mode by selecting the wells and clicking **Analyze Selected Wells**.

9.4.1. Messages of the PyroMark Q48 Autoprep software

The following is a list of use, warning and error messages that may appear in the PyroMark Q48 Autoprep software during assay setup, assay run and data analysis. Messages are organized by analysis mode, though general messages can occur in any mode. For troubleshooting specific to the PyroMark Q48 Autoprep instrument, see Section 9.5.

Message text	Comments and suggestions
General setup messages	
The dispensation order is very long.	The dispensation order is very long (more than 200). Shorten the dispensation order to less than 200.
The InDel position contains a homopolymer.	The dispensations used to analyze the InDel will give a high signal. Quantification may be uncertain. Check results carefully.
Wrong input format.	One of the characters used is not recognized by the software. Use characters from the IUPAC code (A, B, C, D, G, H, K, M, N, R, S, T, V, W, Y and /, [,])
Sequence not in phase at the end of dispensations.	One allele is sequenced ahead of the other(s) and the two (or more) alleles are not in phase with the last dispensation. Add more sequence information, i.e., lengthen the sequence to analyze.
The sequence contains less reference peaks than required.	For reliable results, the software needs at least five dispensations with known results (reference peaks). Do not remove reference peaks.
The generated dispensation order contains less reference peaks than required.	For reliable results the software needs at least five dispensations with known results (reference peaks). Add more sequence information, i.e., lengthen the sequence to analyze.
General analysis messages	
Uncertain due to low peak height. or Failed due to low peak height.	Signal intensity too low. Check PCR yield. Ensure that the correct amount of beads is pipetted into wells of the PyroMark Q48 Disc and correct concentration of sequence primer is used. Verify that the enzyme and substrate mixtures are not expired, are stored correctly, and are completely dissolved.
Uncertain due to wide peaks. or Failed due to very wide peaks.	Wide peaks indicate bad chemistry. Check reagent storage conditions. Check shelf life of reagents. Verify that PyroMark Q48 Autoprep Reagents were used. Redesign the assay (sometimes peak width is assay dependent).
Uncertain due to high peak height deviation in variable position. or Failed due to high peak height deviation in variable position.	Peak heights deviate from expected levels. Even at a variable position, the software expects a certain pattern, e.g., a certain sum of the peak heights. Check sequence to analyze. Check for unknown SNPs.
Uncertain due to high peak height deviation at dispensation: or Failed due to high peak height deviation at dispensation:	Peak height deviates from expected level at indicated dispensation. Check sequence to analyze. Check for unknown SNPs.
Uncertain due to baseline drift. or Failed due to baseline drift.	Too high increase or decrease in baseline. Verify that the environmental temperature did not change (e.g., air-conditioner cycling on or off) and remains under 32°C during the run. Ensure that reagents have been adapted to room temperature prior to the run.
Uncertain due to possible dispensation error at dispensation: or Failed due to possible dispensation error at dispensation:	Desired nucleotide not properly dispensed. Clean cartridge properly (see Section 8.3.3) and rerun. If problem persists, perform injector test (see Section 8.5)
Uncertain due to high peak height deviation at more than 5 dispensations. or Failed due to high peak height deviation at more than 5 dispensations.	Peak heights deviate from expected levels at more than 5 dispensations. Ensure there is no nucleotide degradation/contamination. Check sequence to analyze. Check for unknown SNPs.
Uncertain due to high noise level. or Failed due to high noise level.	The noise of the baseline generated by the instrument is too high. Close injector cover during run. Check instrument lid. Contact QIAGEN Technical Services.

Message text	Comments and suggestions
Missing peaks between dispensations	The software has detected that all four nucleotides have been dispensed, but no signal was detected. Clean the cartridge (see Section 8.3.3) in case there was a dispensation error. If problem persists, perform injector test (see Section 8.5).
Uncertain due to high peak height deviation in surrounding sequence. or Failed due to high peak height deviation in surrounding sequence	Peak heights near the variable position deviate from expected levels. Ensure there is no nucleotide degradation/contamination. Check sequence to analyze. Check for unknown SNPs.
Uncertain reference sequence pattern (overall). or Failed reference sequence pattern (overall).	Peak heights deviate from expected levels. Ensure there is no nucleotide degradation/contamination. Check sequence to analyze. Check for unknown SNPs.
Bad/Suspect drop detected on A/C/G/T at dispensation.	Either no drops or drops with unusual shape have been dispensed from the indicated nucleotide injector. Ensure correct volume of nucleotide was loaded without any air bubbles. If problem persists, run injector diagnostics (see Section 8.5). Ensure injectors are cleaned daily.
"No drops" and drops with unusual shape have been dispensed from the indicated injector.	Either "no drops" or drops with unusual shape have been dispensed from the indicated nucleotide injector. Ensure correct volume of nucleotide was loaded without any air bubbles. If problem persists, run injector diagnostics (see Section 8.5). Ensure injectors are cleaned daily.
A number of "no drops" were detected out of the total number of dispensations of a reagent.	"No drops" have been dispensed from the indicated injector. Ensure correct volume of nucleotide was loaded without any air bubbles. If problem persists, run injector diagnostics (see Section 8.5). Ensure injectors are cleaned daily.
A number of "suspect drops" were detected out of the total number of dispensations of a reagent.	The indicated injector dispensed drops with unusual shape. Ensure correct volume of nucleotide was loaded without any air bubbles. If problem persists, run injector diagnostics (see Section 8.5). Ensure injectors are cleaned daily.
Assay setup messages in AQ mode	
Deselected by user.	Variable position was deselected for analysis by the user.
Sequence uncertain due to lack of terminal sequence information.	The last dispensation is the terminal base of the sequence to analyze. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations are generated. The added base must be different from the base currently at the end of the sequence to analyze.
Last variable position not analyzable due to lack of terminal sequence information.	The last dispensation is included in terminal variable position. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations are generated. The added base must be different from the base currently at the end of the sequence to analyze.
Sequence not in phase at the end of dispensations.	One allele is sequenced ahead of the other(s) and the two (or more) alleles are not in phase with the last dispensation. Add more sequence information, i.e., lengthen the sequence to analyze.
Variable positions with common dispensations cannot be analyzed.	Two or more variable sites share dispensations and can therefore not be distinguished. If possible, use recommended dispensation order.
Analysis messages in AQ mode	
Uncertain due to high peak height deviation in variable position. or Failed due to high peak height deviation in variable position.	Peak heights deviate from expected levels. Even at a variable position, the software expects a certain pattern, e.g., a certain sum of the peak heights. Check sequence to analyze. Check for unknown SNPs.
Missing peaks in variable site.	The software has detected that all four nucleotides have been dispensed, but no signal was detected. Clean the cartridges (see Section 8.3.3) in case there was a dispensation error. If problem persists, perform injector test (see Section 8.5).
Not analyzable due to lack of data.	No peaks detected. Check PCR yield. Check sequencing primer. Clean Cartridges. Check reagents. Check correct usage of buffers.
Assay setup messages in CpG mode	

Message text	Comments and suggestions
Cannot resolve sequence direction.	A forward CpG assay cannot contain a C after bisulfite treatment. A reverse CpG assay cannot contain a G after bisulfite treatment.
Deselected by user.	Variable position was deselected for analysis by the user.
Sequence uncertain due to lack of terminal sequence information.	The last dispensation is the terminal base of the sequence to analyze. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations is generated. The added base must be different from the base currently at the end of the sequence to analyze.
Last variable position not analyzable due to lack of terminal sequence information.	The last dispensation is included in terminal variable position. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations are generated. The added base must be different from the base currently at the end of the sequence to analyze.
Sequence not in phase at the end of dispensations.	One allele is sequenced ahead of the other(s) and the two (or more) alleles are not in phase with the last dispensation. Add more sequence information, i.e., lengthen the sequence to analyze.
Analysis not supported.	This dispensation order will support the analysis of SNPs in a different application mode.
Variable positions with common dispensations cannot be analyzed.	Two or more variable sites share dispensations and can therefore not be distinguished. If possible, use recommended dispensation order.
The position is not valid as bisulfite treatment control.	According to the sequence before bisulfite treatment this is not a possible bisulfite treatment control position.
Analysis messages in CpG mode	
Uncertain bisulfite conversion at dispensation: or Failed bisulfite conversion at dispensation:	Uncertain or failed bisulfite treatment controls are hints for incomplete bisulfite conversion. Review the bisulfite conversion process. Check nucleotide degradation. Normal Pyrosequencing specific background may trigger this warning, although bisulfite treatment was complete.
Uncertain due to high peak height deviation in variable position. or Failed due to high peak height deviation in variable position.	Peak heights deviate from expected levels. Even at a variable position, the software expects a certain pattern, e.g., a certain sum of the peak heights. Check sequence to analyze. Check for unknown SNPs.
Missing peaks in variable site.	The software has detected that all four nucleotides have been dispensed, but no signal was detected. Clean the cartridge (see Section 8.3.3) in case there was a dispensation error. If problem persists, perform injector test (see Section 8.5).
Not analyzable due to lack of data.	No peaks detected. Check PCR yield. Check sequencing primer. Clean cartridges. Check reagents. Check correct usage of buffers.
Assay setup messages in SNP mode	
The InDel position contains a homopolymer.	The dispensations used to analyze the InDel will give a high signal. Quantification may be uncertain. Check results carefully.
Genotyping may be uncertain: the variable position contains a homopolymer.	The dispensations used to analyze the genotype will give a high signal. Genotyping may be uncertain. Check results carefully.
Sequence uncertain due to lack of terminal sequence information.	The last dispensation is the terminal base of the sequence to analyze. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations are generated. The added base must be different from the base currently at the end of the sequence to analyze.
Last variable position not analyzable due to lack of terminal sequence information.	The last dispensation is included in terminal variable position. Add at least one more base at the end of the sequence to analyze, so that one or more additional base dispensations are generated. The added base must be different from the base currently at the end of the sequence to analyze.
Sequence not in phase at the end of dispensations.	One allele is sequenced ahead of the other(s) and the two (or more) alleles are not in phase with the last dispensation. Add more sequence information, i.e., lengthen the sequence to analyze.
Analysis messages in SNP mode	

Message text	Comments and suggestions
Failed genotype determination.	The genotype determination is uncertain, because the pattern of peaks is too similar to another genotype. Check sequence to analyze. Check for unknown SNPs. Check PCR performance (i.e., ensure enough starting material with sufficient quality).
Uncertain due to high peak height deviation in variable position. or Failed due to high peak height deviation in variable position.	Peak heights deviate from expected levels. Even at a variable position, the software expects a certain pattern, e.g., a certain sum of the peak heights. Check sequence to analyze. Check for unknown SNPs.
Missing peaks in variable site.	The software has detected that all four nucleotides have been dispensed, but no signal was detected. Clean the cartridge (see Section 8.3.3) in case there was a dispensation error. If problem persists, perform injector test (see Section 8.5).
Not analyzable due to lack of data.	No peaks detected. Check PCR yield. Check sequencing primer. Clean cartridges. Check reagents. Check correct usage of buffers.
Messages in SEQ mode	
Basecalling not consistent with entered known bases	The known bases entered by the user do not match the result. Remove/change entered known bases.
Low peak height from dispensation:	Signal intensity too low. Check PCR yield. Ensure that the correct amount of beads is pipetted into wells of the PyroMark Q48 disc and correct concentration of sequence primer is used. Verify that the enzyme and substrate mixtures are not expired, are stored correctly, and are completely dissolved.
Very low peak height from dispensation:	Signal intensity too low. Check PCR yield. Ensure that the correct amount of beads is pipetted into wells of the PyroMark Q48 disc and correct concentration of sequence primer is used. Verify that the enzyme and substrate mixtures are not expired, are stored correctly, and are completely dissolved.
Peak height deviates from the expected peak level around dispensation:	Peak heights deviate from expected levels around the indicated dispensation. Check for unknown SNPs.
Peak height deviates from the expected peak levels (overall).	Peaks deviate from expected level. Check annealing procedure. Ensure there is no nucleotide degradation/contamination. Check for unknown SNPs.
Baseline drift	Too high increase or decrease in baseline. Verify that the environmental temperature did not change (e.g., air-conditioner cycling on or off) and remains under 32°C during the run. Ensure that reagents have been adapted to room temperature prior to the run.
Possible dispensation error at dispensation:	Desired nucleotide not properly dispensed. Clean the cartridge properly (see Section Cleaning cartridges) and rerun. If problem persists, perform injector test.
Wide peaks from dispensation: 1.	Wide peaks indicate bad chemistry. Check reagent storage conditions. Check shelf life of reagents. Verify that PyroMark Q48 Autoprep Reagents were used. Redesign the assay (sometimes peak width is assay dependent).
Wide peaks from dispensation:	A certain peak width is needed for correct determination of peak heights. Peaks become wider over time, which is normal. Check results carefully.
Missing peaks in cycle starting at dispensation:	The software has detected that all four nucleotides have been dispensed, but no signal was detected. Clean the cartridge (see Section 8.3.3) in case there was a dispensation error. If problem persists, perform injector test (see Section 8.5).
High noise level.	The noise of the baseline generated by the instrument is too high. Close injector cover during run. Check instrument lid. Contact QIAGEN Technical Services.
Very high noise level.	The noise of the baseline generated by the instrument is too high. Close injector cover during run. Check instrument lid. Contact QIAGEN Technical Services.
Not analyzable due to lack of data	No peaks detected. Check PCR yield. Check sequencing primer. Clean cartridges. Check reagents. Check correct usage of buffers.

9.5. Instrument-related errors

	Comments and suggestions
Temperature	
Chamber not at temperature (reported in PyroMark Q48 Autoprep software)	Ensure that the laboratory is within the operating temperature range (18–30°C). Send support package to QIAGEN Technical Services (see Section Copying support packages).
Fail to reach annealing temperature	Issue with disc heater. Send support package to QIAGEN Technical Services (see Section 9.2).
Optics	
High baseline signal on start acquisition	Ensure that the injector cover is closed.
Higher than expected signal detected.	Check the injector cover is closed and select retry.
Chamber lid	
Lid not moving	Run lid movement test (see Section 8.4).
Lid movement during priming or clean	Press Re-try and avoid moving the lid during injector priming or cleaning.
Lid movement impeded	Ensure that no object is impeding the lid to open or close.
Injectors	
No cartridge detected	Ensure that the cartridges are inserted.
Loaded wrong nucleotide into injector position	Run Injector Clean for that injector (see Section 6.5.2).
Poor drop sensor data on dispensation (Reported in PyroMark Q48 Autoprep software)	Ensure priming was completed successfully. Ensure sufficient liquid was loaded into the cartridge reservoir. Send support package to QIAGEN Technical Services (see Section 9.2).
Pump will not reach pressure	Check the cartridge lids are closed. Check cartridges are firmly seated. If problem persists, contact QIAGEN Technical Services.
Spurious peaks on negative control nucleotide dispensations	Run Pyrophosphate Clean (see Section Maintenance Procedures).
Injector Priming failure	Check to see if there is sufficient liquid in the cartridge reservoir. Potential issue with drop sensor or injector. Run injector test (see Section 8.5). Send support package to QIAGEN Technical Services (see Section 9.2).
Injector Clean failure	Ensure that excess solution in injector reservoirs is removed. Potential issue with drop sensor or injector. Run injector test (see Section 8.5). Send support package to QIAGEN Technical Services (see Section 9.2).
Other	
Liquid or beads outside of the wells	Ensure that the well capacity volume (20 µL) is not exceeded.
<no sample ID>	Input a sample name during PyroMark Q48 Autoprep software run setup.
No disc detected	Ensure that the correct disc type is inserted into the chamber.
No run setup file	Ensure USB flash drive is inserted into the instrument or network connection is working. Ensure that the run file is saved to USB flash drive or in a shared folder of the network.

Note: For all other instrument error messages, please contact QIAGEN Technical Services.

10. Glossary

Glossary terms are listed in alphabetical order.

Term	Description
AQ	Analysis mode used for quantification of different alleles.
Biotin	A molecule that can bind very strongly to streptavidin. PCR primers can be biotinylated to enable the resulting PCR product to bind to streptavidin-coated beads.
Bisulfite	HSO_3^- is referred to as bisulfite (or hydrogen sulfite). In the bisulfite reaction, DNA is treated with sodium bisulfite to convert cytosine residues to uracil, under conditions whereby methylated cytosines remain non-reactive.
Bisulfite treatment control	Pyrosequencing assays can contain an internal control to assess successful bisulfite treatment. C bases that are not followed by G in the sequence are normally not methylated, and should therefore be fully converted to T after bisulfite treatment and PCR. As a result of successful bisulfite treatment, all templates should show only Ts and no Cs in these positions. For reverse assays, all templates should show only As and no Gs in these positions.
CpG	Analysis mode used for analysis of CpG methylation.
CpN	Analysis capability within CpG mode for analysis of methylation at CpN sites
Cyclic dispensation order	A repetitive dispensation order for nucleotide dispensation. Normally used in Pyrosequencing technology for sequencing unknown DNA-sequences. For example, "CTGA" or "TCGA" can be used and repeated for the desired number of times.
Directed dispensation order	Non-cyclic order of dispensation that follows the known sequence. It can be used in Pyrosequencing technology when you know the sequence to be analyzed. For example, the sequence "TCCAGAA" can be analyzed with the dispensation order "TCAGA".
Dispensation order	Defines the nucleotides and the order in which they should be dispensed in Pyrosequencing runs.
Drop off	A continual decrease in peak height normally seen in the Pyrogram.
Enzyme	A protein (or RNA) working as a catalyst, to enhance the speed of a biochemical reaction without altering it. In Pyrosequencing technology, a mixture of Klenow polymerase, sulfurylase, luciferase and apyrase is used in the sequencing reaction.
Histogram	The theoretical representation of the expected Pyrosequencing peak pattern.
Homopolymer	A stretch of identical bases in DNA. In Pyrosequencing technology, a stretch of more than two identical bases is regarded as a homopolymer.
InDels	Insertion and/or deletions.
IUPAC	International Union of Pure and Applied Chemistry. An organization providing recommendations on organic and biochemical nomenclature, symbols, terminology, etc. IUPAC codes: A = Adenine C = Cytosine G = Guanine T = Thymine R = Purine (A or G) Y = Pyrimidine (C or T) M = C or A K = T or G W = T or A S = C or G B = C, T, or G (not A) D = A, T, or G (not C) H = A, T, or C (not G) V = A, C, or G (not T) N = Any base (A, C, G, or T)

Term	Description
PyroMark Q48 Magnetic Beads	Magnetic streptavidin-coated Sepharose beads used for preparation of biotinylated PCR products.
Out of phase	When one of the alleles is sequenced ahead of the other.
Polymorphism	Genetic variations, broadly encompassing any of the many types of variations in DNA sequences that are found within a given population.
Pyrogram	The graph resulting from a sequencing reaction performed using Pyrosequencing technology. Incorporated nucleotides are shown as peaks in the Pyrogram.
Quality control window	Gives an overview of the quality at the end of a defined number of bases in the base-called sequence. A setting in the SEQ analysis mode.
Reference peak	Nonvariable peaks (i.e., peaks that are not a part of a variable position) are referred to as “reference peaks”. Reference peaks are used in the analysis both as references when calculating the single peak height level, and as internal controls when assessing the quality.
RLU	Relative Light Unit (entity used in Pyrosequencing to define peak heights in Pyrogram).
SEQ	Analysis mode used for base-calling of unknown sequences.
Sequence to analyze	A short part of a DNA sequence (in your sample), starting directly after the sequencing primer, which contains one or several variable positions to be analyzed using Pyrosequencing instrument platforms.
Sequencing primer	The sequencing primer is annealed to the template during the template preparation. The 3'-end of the sequencing primer serves as the starting point for the extension by the DNA polymerase.
Shift	Plus shift: A small proportion of the template sequences that incorporates more than one type of nucleotide at a time (if, e.g., there are residues left from the dispensation before) and will be sequenced ahead of the rest of template sequences. Minus shift: A small proportion of the template sequences that fails to incorporate a nucleotide will be sequenced subsequent to the rest of template.
Signal-to-noise ratio	The ratio of the signal height and the noise height. An indication of the clarity of the data. The higher the ratio, the better the data.
Single nucleotide polymorphism (SNP)	SNPs involve the change of one DNA base to another. SNPs and point mutations are structurally identical, differing only in their frequency. Variations that occur in 1% or less of a population are considered point mutations, while those occurring in more than 1% are SNPs.
Streptavidin	A protein that can bind very strongly to biotin.
Substrate	A molecule acted upon by an enzyme. Pyrosequencing technology uses a mixture of the substrates adenosine 5' phosphosulfate (APS) and luciferin in the sequencing reaction.
Variable position	A region in the sequence that varies at one or more variable bases. In the PyroMark Q48 Autoprep software, the variable positions are highlighted with a blue-gray background color in the histogram and Pyrogram.

11. Technical Specifications

QIAGEN reserves the right to change specifications at any time.

11.1. Mechanical data and hardware features

Dimensions	**Width**: 250 mm (9.8 in.) **Depth**: 380 mm (15.0 in.) with chamber lid closed 560 mm (22 in.) with chamber lid open **Height**: 225 mm (8.9 in.) with injector cover closed 390 mm (15.4 in.) with injector cover open
Weight	8.5 kg (18.7 lb.)
Sample disc well capacity	20 µL (typical reaction volume 10 µL)
Rotor speed	30–60 RPM; maximum 2500 RPM during wash cycle
Rotor mixing	Rotor vibration frequency of 50 Hz
Injectors	3 injector cartridges each with 4 x 3.8 mL injectors
Nucleotide injector	100 ± 10% nl/dispensation (dATPαS, dCTP, dGTP, and dTTP)
Primer injector	500 ± 10% nl/dispensation (Primer 1, Primer 2, Primer 3, Primer 4/Binding Buffer)
Reagent injector	500 ± 10% nl/dispensation (Denaturation Solution, Enzyme, Substrate, Annealing Buffer)
Chamber temperature	28 ± 0.5°C
Waste collection	Absorbent waste capture strip with a maximum allowable volume capacity of 8 ml

11.2. Operating conditions

AC Input	100–240V AC, 50/60 Hz Mains supply voltage fluctuations are not to exceed ±10% of the nominal supply voltages
Maximum power consumption	130 VA
Stand-by power consumption	40 VA
Fuse type	F3.15A 250V, 5 mm x 20 mm Fast Acting Fuse, adhering to local regulations
Overvoltage category	II
Air temperature	18–30°C (64–86°F)
Relative humidity	20–80% (noncondensing)
Altitude	Up to 2000 m (6500 ft.)
Place of operation	For indoor use only in a draft-free location No exposure to direct sunlight
Pollution degree	2
Environmental class	3K2 (IEC 60721-3-3)* 3M2 (IEC 60721-3-3)*

* Unless otherwise specified herein.

11.3. Transport conditions

Air temperature	–25°C to 60°C (–13°F to 140°F) in manufacturer's packaging
Relative humidity	Max. 75% (noncondensing)
Environmental class	2K2 (IEC 60721-3-2)* 2M2 (IEC 60721-3-2)*

* Unless otherwise specified herein.

11.4. Storage conditions

Air temperature	15°C to 30°C (59°F to 86°F) in manufacturer's packaging
Relative humidity	Max. 85% (noncondensing)
Environmental class	1K2 (IEC 60721-3-1)* 1M2 (IEC 60721-3-1)*

* Unless otherwise specified herein.

11.5. User software

Instrument computer	Integrated PC with 7 in. touchscreen
Analysis computer	See Section 3.5
File transfer	Via USB storage device or network connection
Interface	Wizard-driven user interface with comprehensive information for loading and running instrument
Compatibility	Linked to PyroMark Q48 Autoprep software

11.6. General

Workflow	Automated template binding, template denaturation, primer annealing, and all associated wash cycles needed for a sequencing reaction
Input template	0.5–2 picomoles of biotinylated PCR product
Sequencing primer	Optional automated sequence primer loading (4 injector positions available)
Applications	Simultaneous running of multiple assays and assay types including SNP, AQ, CpG, and SQA to achieve methylation analysis, de novo sequencing, mutation characterization including In/Dels, speciation, quantitative allele sequencing and SNP genotyping

Appendix A – Assay Design and Validation

Assay design

For design of Pyrosequencing assays, use the latest version of PyroMark Assay Design Software (ADSW). The program automatically generates primer sets that include both PCR and sequencing primers. Each primer set is given a quality score based on several parameters that are specific for Pyrosequencing analysis. Ensure you use the correct assay type in PyroMark ADSW.

PCR

For PCR amplification, we recommend using the PyroMark PCR Kit (QIAGEN), which is specifically optimized for Pyrosequencing analysis and enables highly specific and unbiased amplification of template DNA for various Pyrosequencing applications, such as mutation detection, SNP analysis, methylation analysis, and base calling. The convenient master mix format enables specific amplification of various starting materials such as genomic DNA from a variety of species, as well as bisulfite-converted DNA, using only one protocol.

PCR primers

One of the primers must be biotin labeled to enable immobilization to magnetic streptavidin-coated beads during the preparation of a single-stranded DNA template. The orientation of the assay can either be forward or reverse. The primer that needs to be biotinylated is indicated by the PyroMark ADSW.

The biotinylated primer should be purified by HPLC or an equivalent procedure since free biotin will compete with the biotinylated PCR product for binding sites on PyroMark Q48 Magnetic Beads.

Amplicon length

The optimal amplicon length for Pyrosequencing assays is between 80 and 200 bp, although products up to 500 bp may work well. Amplicons for CpG assays should ideally be shorter than 200 bp.

Sequencing primer

Design sequencing primers using PyroMark ADSW. When designing an InDel assay, we highly recommend that the sequencing primer is located a few bases before the variable position.

PCR setup

PCR reactions of 25 µL are set up using the PyroMark PCR Kit. Ensure that you follow the instructions provided in the *PyroMark PCR Handbook*.

Run the PCR at the optimal annealing temperature for 45 cycles. Using fewer cycles may give insufficient yield and cause background problems in Pyrosequencing reactions due to excess, unused biotinylated primer.

The PCR product should give one strong band with minimal excess of primers when analyzed on an agarose gel.

Starting template

The yield and quality of PCR product is affected by both the quality and quantity of the nucleic acid starting template. This is particularly true for amplification of long regions from DNA that has been fragmented by bisulfite-treatment or extracted from paraffin-embedded material.

Quality of starting template

Because PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR. These include the QIAprep® system for rapid plasmid purification, the QIAamp® and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids, and the RNeasy® system for RNA preparation from a variety of sources. For more information about QIAprep, QIAamp, DNeasy, and RNeasy products, contact QIAGEN Technical Services or visit **www.qiagen.com**.

Quality of starting template when performing CpG assays

Critical parameters for a successful PCR using bisulfite-treated DNA templates include complete bisulfite conversion and DNA fragments that are long enough for PCR. EpiTect® Bisulfite Kit provides a fast and reliable procedure for efficient bisulfite conversion and a unique DNA Protect Buffer prevents DNA fragmentation during the bisulfite conversion reaction. For more information about EpiTect products, contact QIAGEN Technical Services or visit **www.qiagen.com**.

Quantity of starting template

The annealing efficiency of a primer to the template is an important factor in PCR. Owing to the thermodynamic nature of the reaction, the primer:template ratio strongly influences the specificity and efficiency of PCR and should be optimized empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events.

PCR optimization

The PyroMark PCR Kit will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, use of the 25 mM $MgCl_2$ provided in the kit is recommended.

The recommended annealing temperature is 60°C and 56°C for genomic DNA and bisulfite-treated DNA, respectively, when using PyroMark ADSW 2.0.

Addition of Q-Solution® (provided with the PyroMark PCR Kit) can improve PCR yield and specificity for difficult templates that, for example, have a high degree of secondary structure or templates that are GC rich.

For all PCR optimization tests, analyze 5 µL of a 25 µL PCR on an agarose gel and aim for one strong specific band with minimal excess of primers.

Refer to the *PyroMark PCR Handbook* for further troubleshooting.

Equal amplification of both alleles in AQ and CpG assays

Reliable results in quantification assays depend on equal amplification of both alleles and this must be carefully tested.

To ensure equal amplification in a CpG assay, unmethylated DNA can be mixed with increasing proportions of completely methylated DNA. We recommend using EpiTect Control DNAs, which provide bisulfite-treated completely methylated and unmethylated DNA in ready-to-use solutions. Regression analysis of the frequency of one allele measured in the PyroMark Q48 Autoprep as a function of the input (expected) allele, should give an R^2 value greater than 0.9.

For an AQ assay, the allelic variants, including the variable position, can be mixed at different ratios similar to the procedure for a CpG assay. If the variable position in an AQ assay is a SNP, the easiest way to test for equal amplification is to compare the peak heights from a heterozygote. If the SNP is represented by single base incorporations, for example, AAC/TGG, the two alleles (C and T peaks) should give peaks of equal height. An InDel heterozygote should give 50% deletion.

Template preparation

Use 10 µL of a 25 µL PCR for immobilization to PyroMark Q48 Magnetic Beads according to the instructions in Section 6.2.5.

Pyrosequencing analysis

Use default settings in the software for all assay setups if not otherwise stated.

Assay setup

AQ, SNP, and CpG assays

When creating an AQ, SNP, or CpG assay, the sequence to analyze should contain a sufficient number of bases to generate at least five nonvariable reference peaks. For InDels, a few reference peaks should be included before the variable position.

If the sequencing primer is placed adjacent to the position to analyze, include part of the sequence following the variable position in the Sequence to Analyze text box. Ensure that the last base in the text box is not part of the variable position.

Blank dispensations are automatically generated by the software and serve as built-in quality controls for the assay. Never exclude the blank dispensations as they function as excellent indicators of unspecific nucleotide incorporation. When manually generating a dispensation order, include an appropriate number of blank dispensations. If possible, start the dispensation order with one blank dispensation and have at least the same number of blank dispensations as the number of variable positions.

Pay attention to tips and warnings indicated by the red  icon and make suitable modifications when the icon appears.

Bisulfite treatment controls in CpG assays

CpG assays should contain an internal control to assess successful bisulfite treatment, preferably at the beginning of the sequence. Cytosines not followed by a Guanine are usually not methylated and should be fully converted to Thymine after bisulfite treatment and PCR. As a result of successful bisulfite treatment, all templates should show only Ts and no Cs in these positions. For reverse assays, all templates should show As and no Gs in these positions.

We recommend directly entering the sequence before bisulfite treatment, because this enables the software to generate the sequence to analyze and automatically select one appropriate bisulfite treatment control. A bisulfite treatment control can be added manually by adding a C before or after a T that was a C before bisulfite treatment in a forward assay, or by adding a G before or after an A that was a G before bisulfite treatment in a reverse assay, in the dispensation order. Refer to Section 7.

SEQ assays

Experience with sequencing large numbers of templates indicates that the dispensation order n(CTGA) gives, on average, the best sequencing quality. Individual templates may, however, give better results with other dispensation orders.

Where possible, SEQ assays that involve resolution of different sequences should be designed such that resolution does not depend on accurate sequencing of homopolymers. In addition, it may be useful to have a few known bases at the beginning of the sequence, preferably single peaks. These can be used as reference peaks to aid the setting of the peak levels in difficult assays.

Ensure that the initial DNA sample is pure or that the assay is capable of specifically amplifying and/or sequencing only one target sequence in the sample. The assay may otherwise generate mixed-sequence that cannot be analyzed.

Pay attention to tips and warnings indicated by the red  icon and make suitable modifications when the icon appears.

Validation of a new assay

Controls

All new assays have to be validated by the user. Use a reference DNA sample when testing a new assay and ensure that appropriate analysis parameters in the PyroMark Q48 Autoprep software are used. Interactions between primers or loops formed on single-stranded DNA can serve as priming sites for base incorporation by DNA polymerase. The following controls should be included when an assay is analyzed for the first time:

- PCR without template DNA. This will show if the primers interact to give a background signal in Pyrosequencing reactions.
- PCR with template DNA but with no sequencing primer. This will show if the template can loop back on itself and give a background signal in Pyrosequencing reactions.
- Sequencing primer without any PCR product. This will show if the sequencing primer can form duplexes or hairpins and give background signal in Pyrosequencing reactions.
- Biotinylated primer without any PCR product. This will show if the biotinylated primer can form duplexes or hairpins and give background signal in Pyrosequencing reactions.
- Sequencing primer and biotinylated primer together without PCR product. This will show if the sequencing primer and the biotinylated primer can form duplexes and give background signal in Pyrosequencing reactions.

Pyrograms from these controls should not show any significant peak after any nucleotide addition.

Quality assessment

The user will be warned if something in the assay may reduce the quality of the result given by the analysis software. The ultimate goal for a well-optimized assay is that all variable positions in an AQ, SNP, or CpG assay, or the sequence in the quality control window of a SEQ assay, have the quality assessment “Passed” when using default or more stringent analysis parameters. Such results will be shown as blue in the quality bar in the well after analysis. Results of lower quality are indicated as “Check” (yellow) or “Failed” (red) together with error messages.

Analysis results

For samples and positive controls, aim for:

- Sufficient signal intensities. Aim for a single peak height of at least 20 RLU
- No background in blank dispensations
- No background in variable positions (AQ, SNP, and CpG)
- Expected reference sequence pattern (AQ, SNP, and CpG)
- All positions (AQ, SNP, and CpG) and quality control window (SEQ) with quality assessment “Passed”

The quality assessments for AQ, SNP, and CpG assays are based on the sequence context as well as the results in the analyzed positions. Deviations from this built-in quality control are shown as warnings in the Well Information area.

The analysis results for SEQ assays are based on the appearance of the peaks in Pyrogram, related to peak height levels estimated by the software.

The inclusion of known bases in SEQ assays can improve the estimation of peak height level.

Appendix B – Technical Data

Waste Electrical and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users.

The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.

Recycling can be provided by QIAGEN upon request at additional cost. In the European Union, in accordance with the specific WEEE recycling requirements and where a replacement product is being supplied by QIAGEN, free recycling of its WEEE-marked electronic equipment is provided.

To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

FCC Declaration

The ''United States Federal Communications Commission'' (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

This device complies with part 15 of the FCC. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This Class B digital apparatus complies with Canadian ICES-003.

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/ TV technician for help.

QIAGEN GmbH, Germany is not responsible for any radio television interference caused by unauthorized modifications of this equipment or the substitution or attachment of connection cables and equipment other than those specified by QIAGEN GmbH, Germany. The correction of interference caused by such unauthorized modification, substitution or attachment will be the responsibility of the user.

Declaration of conformity

Name and address of the legal manufacturer:

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
Germany

An up-to-date declaration of conformity can be requested from QIAGEN Technical Services.

Appendix C – PyroMark Q48 Autoprep Accessories

For more information and an up-to-date list of available protocols, visit **www.qiagen.com**

Ordering information

Product	Contents	Cat. no.
Instrument, software, and disposables		
PyroMark Q48 Autoprep System	Instrument, software, and pipet	9002470
PyroMark Q48 Discs (50)	50 discs for running PyroMark Q48 Autoprep reactions	974901
PyroMark Q48 Absorber Strips (100)	100 absorber strips for running PyroMark Q48 Autoprep reactions	974912
Software and licenses		
PyroMark Q48 Software License (1)	1 additional license for PyroMark Q48 Software. Only valid together with PyroMark Q48 Autoprep software	9024325
PyroMark Q48 Software License (5)	5 additional licenses for PyroMark Q48 Software. Only valid together with PyroMark Q48 Autoprep software	9024326
PyroMark Assay Design SW 2.0	Software for convenient design of PCR and sequencing primers, optimized for Pyrosequencing analysis	9019077
PyroMark IdentiFire SW 1.0	Software for comparing and matching Pyrosequencing data to a local database	9019087
Reagents and consumables		
PyroMark Q48 Autoprep Starter Kit	PyroMark Q48 Magnetic Beads (300), PyroMark Q48 Advanced CpG Reagents (4 x 48), PyroMark Control Oligo, PyroMark Q48 Discs (50), and PyroMark Q48 Absorber Strips (100)	974230
PyroMark Q48 Advanced Reagents (4 x 48)	Reagents for 4 x 48 PyroMark Q48 Autoprep standard reactions	974002
PyroMark Q48 Advanced CpG Reagents (4 x 48)	Reagents for 4 x 48 PyroMark Q48 Autoprep CpG and long-read reactions	974022
PyroMark Q48 Magnetic Beads (300)	Magnetic streptavidin-coated Sepharose beads for running 300 PyroMark Q48 Autoprep reactions	974203
PyroMark Control Oligo	For installation check of system	979203
PyroMark PCR Kit (200)*	PCR Master Mix for PCR reactions optimized for Pyrosequencing analysis	978703
PyroMark OneStep RT-PCR Kit (50)*	OneStep RT-PCR Enzyme Mix for RT-PCR reactions optimized for Pyrosequencing analysis	978801
EpiTect Fast DNA Bisulfite Kit (50)*†	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59824
EpiTect Fast FFPE Bisulfite Kit (50) *†	For 50 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59844
EpiTect Fast LyseAll Bisulfite Kit (50) *†	For 50 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59864
PyroMark Q24 CpG MLH1	For 96 reactions: Forward primer, Reverse primer, and Sequencing primer for the analysis of MLH1 methylation using the PyroMark Q24, PyroMark Q24 Advanced or PyroMark Q48 Autoprep	970022

Product	Contents	Cat. no.
PyroMark Q24 CpG MGMT	For 96 reactions: Forward primer, Reverse primer, and Sequencing primer for the analysis of MGMT methylation using the PyroMark Q24, PyroMark Q24 Advanced or PyroMark Q48 Autoprep	970032

* Larger kit sizes available.

† 96-well format available.

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.

Appendix D – Legal Information

Warranty statement

Thank you for your purchase of QIAGEN instrumentation. Your instrument has been carefully tested to ensure optimum operating efficiency and reproducibility of results. QIAGEN warrants that all new instrumentation manufactured by QIAGEN will correspond to the product specifications and be free from defects in workmanship and materials for a period of twelve (12) months from the original date of shipment. Repair or replacement of defective parts will be provided to the purchaser during this time period provided the QIAGEN instrumentation is operated under conditions of normal and proper use, but not for damage caused by the customer. If any part or subassembly proves to be defective, it will be repaired or replaced at QIAGEN's sole option, subsequent to inspection at the factory, or in the field by an authorized factory representative, provided that such defect manifested under normal and proper use.

Limitation of warranties and remedies

THE FOREGOING WARRANTY IS QIAGEN'S SOLE AND EXCLUSIVE WARRANTY, AND REPAIR OR REPLACEMENT OF DEFECTIVE PARTS IS THE SOLE AND EXCLUSIVE REMEDY. THERE ARE NO OTHER WARRANTIES OR GUARANTEES, EXPRESS OR IMPLIED. THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE EXPRESSLY EXCLUDED, TO THE FULLEST EXTENT PERMITTED BY LAW. (NOTE: SOME STATES DO NOT PERMIT DISCLAIMERS OF IMPLIED WARRANTIES SO THIS LIMITATION MAY NOT APPLY TO YOU). WITH THE EXCEPTION OF THE ABOVE-REFERENCED REPAIR OR REPLACEMENT REMEDY, QIAGEN SHALL HAVE NO OBLIGATION OR LIABILITY OF ANY NATURE WHATSOEVER WITH RESPECT TO THE QIAGEN INSTRUMENTATION, WHETHER ARISING IN CONTRACT, TORT, STRICT LIABILITY, OR OTHERWISE, INCLUDING BUT NOT LIMITED TO, LIABILITY FOR INDIRECT, CONSEQUENTIAL, INCIDENTAL AND/OR SPECIAL, PUNITIVE, MULTIPLE AND/OR EXEMPLARY DAMAGES AND/OR OTHER LOSSES (INCLUDING LOSS OF USE, LOST REVENUES, LOST PROFITS AND DAMAGE TO REPUTATION), EVEN IF SUCH DAMAGES WERE FORESEEN OR FORSEEABLE, OR WERE BROUGHT TO QIAGEN'S ATTENTION. IN NO EVENT SHALL QIAGEN'S LIABILITY TO YOU EXCEED THE PURCHASE PRICE OF THE PRODUCT.

Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications. All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

Appendix E – Safety Information (French, FR)

Informations de sécurité

Avant d'utiliser le PyroMark Q48 Autoprep System, il est impératif de lire attentivement ce manuel d'utilisation et de porter une attention particulière aux informations de sécurité. Pour garantir un fonctionnement du PyroMark Q48 Autoprep System en toute sécurité et le maintenir en bon état de marche, il est impératif de suivre les instructions et les informations de sécurité fournies dans le manuel d'utilisation.

Les types d'informations de sécurité suivants sont fournis dans ce manuel.

AVERTISSEMENT

Le terme AVERTISSEMENT signale des situations risquant d'entraîner des blessures dont vous ou d'autres personnes pourriez être victimes.

Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

ATTENTION

Le terme ATTENTION est utilisé pour indiquer des situations pouvant entraîner un endommagement de l'instrument ou d'autres équipements.

Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Les conseils dispensés dans ce manuel ont pour but de venir compléter les exigences de sécurité habituelles en vigueur dans le pays de l'utilisateur, et non de s'y substituer.

Utilisation appropriée

AVERTISSEMENT/ ATTENTION

Risque de dommages corporels et matériels

Une utilisation inappropriée du PyroMark Q48 Autoprep System peut entraîner des blessures corporelles ou une détérioration de l'instrument. Le système PyroMark Q48 Autoprep ne doit être utilisé que par du personnel qualifié ayant été convenablement formé.

L'entretien du système PyroMark Q48 Autoprep ne doit être effectué que par un spécialiste de l'entretien sur site QIAGEN.

ATTENTION

Détérioration de l'instrument

La lumière directe du soleil risque de décolorer certaines parties de l'instrument et les cartouches, et d'endommager les pièces en plastique.

Le PyroMark Q48 Autoprep System et les cartouches ne doivent pas être exposés à la lumière directe du soleil et doivent être éloignés des sources de chaleur, des sources de vibration et des interférences électriques.

ATTENTION

Détérioration de l'instrument

Éviter de renverser de l'eau ou des produits chimiques sur le PyroMark Q48 Autoprep System. La détérioration due à la projection d'eau ou de produits chimiques annulera la garantie.

AVERTISSEMENT/ ATTENTION

Risque de dommages corporels et matériels

Ne pas essayer de déplacer le PyroMark Q48 Autoprep System pendant qu'il est en marche.

AVERTISSEMENT/ ATTENTION

Atmosphère explosive

Le PyroMark Q48 Autoprep System n'est pas conçu pour être utilisé dans une atmosphère explosive.

AVERTISSEMENT

Risque d'explosion

Le PyroMark Q48 Autoprep System est conçu pour être utilisé avec les réactifs et substances fournis avec les trousses QIAGEN. L'utilisation d'autres réactifs et substances peut provoquer un incendie ou une explosion.

ATTENTION

Insertion de la bande absorbante

S'assurer que la bande absorbante est insérée dans le PyroMark Q48 Autoprep (comme décrit dans la section 6.2.2) pour empêcher le liquide de pénétrer dans la chambre.

En cas d'urgence, éteindre le PyroMark Q48 Autoprep System via l'interrupteur d'alimentation et débrancher le câble d'alimentation de la prise de courant.

Sécurité électrique

Remarque : avant l'entretien, débrancher le cordon d'alimentation de la prise de courant.

AVERTISSEMENT

Danger électrique

Toute interruption du conducteur de protection (conducteur de terre/de masse) à l'intérieur ou à l'extérieur de l'instrument ou toute déconnexion de la borne du conducteur de protection est susceptible de rendre l'appareil dangereux.

Toute interruption intentionnelle est interdite.

Tensions mortelles à l'intérieur de l'instrument

Lorsque l'instrument est connecté au secteur, les bornes peuvent être sous tension, et l'ouverture des capots ou le retrait d'éléments risque d'exposer des éléments sous tension.

Éviter de renverser du liquide sur ou dans l'instrument. En cas de renversement de liquide sur l'instrument, débrancher immédiatement l'instrument du secteur.

Suivre ces instructions afin que le PyroMark Q48 Autoprep System fonctionne de manière satisfaisante et en toute sécurité :

- Le cordon d'alimentation doit être branché à une prise de courant disposant d'un conducteur de protection (terre/masse).
- Garder la fiche secteur facilement accessible au cas où l'équipement devrait être débranché rapidement du secteur.
- Utiliser uniquement les cordons d'alimentation fournis par QIAGEN.
- Si l'instrument présente un danger électrique, empêcher le reste du personnel de s'en servir et contacter les services techniques de QIAGEN. L'instrument peut présenter un danger électrique dans les cas suivants :
    - Le cordon d'alimentation semble être détérioré.
    - Il a été stocké pendant une période prolongée dans des conditions qui ne correspondent pas aux « Conditions de stockage » décrites à la Section 11 – Caractéristiques techniques.
    - Il a été soumis à des tensions importantes durant le transport.
    - Du liquide a pénétré dans l'instrument.

Sécurité biologique

Lors de la manipulation de matériel biologique, utiliser des procédures de laboratoire sûres, comme décrites dans des publications telles que Biosafety in Microbiological and Biomedical Laboratories, HHS (**www.cdc.gov/labs/BMBL.html**).

AVERTISSEMENT **Matériel biologique**

Manipuler ce matériel biologique avec la plus grande précaution et conformément aux règlementations en matière de sécurité exigées. Toujours porter des lunettes de protection, 2 paires de gants et une blouse de laboratoire.

La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que l'espace de travail environnant est sûr et que les opérateurs de l'instrument sont convenablement formés et ne sont pas exposés à des niveaux dangereux d'agents infectieux comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA,* de l'ACGIH,† or ou du COSHH‡ applicables.

Pour plus d'informations, consulter le site **www.qiagen.com/safety**.

L'évacuation des vapeurs et la mise au rebut des déchets doivent être effectuées conformément à toutes les réglementations et législations nationales, régionales et locales relatives à la santé et à la sécurité.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

Produits chimiques

AVERTISSEMENT/ ATTENTION

Produits chimiques dangereux

Certains produits chimiques utilisés avec cet instrument peuvent être dangereux ou le devenir après l'exécution du cycle d'exécution du protocole.

Toujours porter des lunettes de protection, des gants et une blouse de laboratoire.

La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que l'espace de travail environnant est sûr et que les opérateurs de l'instrument sont convenablement formés et ne sont pas exposés à des niveaux dangereux de substances toxiques (chimiques ou biologiques) comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA,* de l'ACGIH,† or ou du COSHH‡ applicables.

Pour plus d'informations, consulter le site **www.qiagen.com/safety**.

L'évacuation des vapeurs et la mise au rebut des déchets doivent être effectuées conformément à toutes les réglementations et législations nationales, régionales et locales relatives à la santé et à la sécurité.Nettoyer immédiatement toute solution de dénaturation qui s'est renversée sur l'instrument ou qui l'a éclaboussé. Une exposition prolongée peut entraîner des taches et des dommages à la surface

.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

Dangers mécaniques

AVERTISSEMENT

Pièces mobiles

Pour éviter tout contact avec des pièces mobiles pendant le fonctionnement du PyroMark Q48 Autoprep System, l'instrument doit être utilisé avec le capot des injecteurs fermé.

Ne pas retirer les panneaux de recouvrement, car il n'y a aucune pièce pouvant être réparée par l'utilisateur à l'intérieur. En cas de problème avec le PyroMark Q48 Autoprep System, contacter immédiatement les services techniques de QIAGEN.

AVERTISSEMENT

Pièces mobiles

Le couvercle de la chambre du PyroMark Q48 Autoprep instrument s'ouvre et se ferme automatiquement pendant le fonctionnement pour empêcher l'humidité de s'accumuler à l'intérieur de l'instrument.

ATTENTION

Insertion du disque

Le PyroMark Q48 Disc doit être verrouillé en position pour éviter de renverser le contenu du disque dans la chambre de l'instrument.

S'assurer que le disque est verrouillé en position en vissant le contre-écrou. Utiliser uniquement le contre-écrou fourni par QIAGEN pour éviter d'endommager l'instrument.

ATTENTION

Fonctionnement du couvercle de la chambre

Le couvercle est actionné par un moteur contrôlé par le logiciel. Un espace insuffisant à l'arrière de l'instrument (moins de 20 cm) pour actionner le couvercle de la chambre peut endommager ce dernier lors de son ouverture.

Dangers liés à la chaleur

AVERTISSEMENT **Surface brûlante**

Le disque chauffant du PyroMark Q48 Autoprep instrument peut atteindre des températures allant jusqu'à 95 °C. Éviter de le toucher lorsqu'il est chaud.

AVERTISSEMENT **Risque de surchauffe**

Afin de garantir une bonne ventilation, laisser un dégagement d'au moins 5 cm sur les côtés et 20 cm à l'arrière du PyroMark Q48 Autoprep instrument.

Les fentes et les ouvertures qui garantissent la ventilation du PyroMark Q48 Autoprep instrument ne doivent pas être obstruées.

Consommables

ATTENTION **Consommables non pris en charge**

Ne pas raccorder et ne pas utiliser de consommables, d'accessoires ou d'équipements externes autres que ceux spécifiés.

Mise au rebut des déchets

ATTENTION **Retrait de la bandelette absorbante**

À la fin du cycle d'exécution, la bande absorbante contiendra une solution de dénaturation, qui contient de l'hydroxyde de sodium. Cela peut être irritant pour les yeux et la peau.

Toujours porter des lunettes de protection, des gants et une blouse de laboratoire lors du retrait de la bande absorbante.

La mise au rebut de la bande absorbante doit être effectuée conformément à toutes les règlementations et législations nationales, régionales et locales applicables en matière de santé et de sécurité.

ATTENTION **Mise au rebut du matériel en plastique**

Le matériel en plastique usagé, comme le PyroMark Q48 Disc, peut contenir des produits chimiques dangereux ou des matières contagieuses/biologiquement dangereuses. Ces déchets doivent être convenablement collectés et mis au rebut conformément aux règles de sécurité locales.

Sécurité relative à la maintenance

Procéder à la maintenance comme décrit dans la section 8. QIAGEN facture les réparations dues à une maintenance incorrecte.

AVERTISSEMENT/ ATTENTION

Risque d'accident corporel et de détérioration du matériel

Effectuer uniquement la maintenance spécifiquement décrite dans le présent manuel d'utilisation.

AVERTISSEMENT/ ATTENTION

Risque d'accident corporel et de détérioration du matériel

Ne pas ouvrir les panneaux du PyroMark Q48 Autoprep System.

Effectuer uniquement la maintenance spécifiquement décrite dans le présent manuel d'utilisation.

ATTENTION

Détérioration de l'instrument

Ne pas utiliser de solvants ou de réactifs contenant des acides, des agents alcalins ou des produits abrasifs pour nettoyer le PyroMark Q48 Autoprep instrument.

ATTENTION

Détérioration de l'écran tactile et de l'ordinateur

Ne pas verser et ne pas vaporiser de liquides, par exemple des produits de nettoyage, sur le PyroMark Q48 Autoprep instrument. N'utiliser qu'un mouchoir imbibé d'eau pour le nettoyage.

ATTENTION

Maintenance du détecteur de lumière

Utiliser des chiffons non pelucheux pour nettoyer soigneusement la fenêtre du photomultiplicateur (PMT). Ne pas utiliser de mouchoirs en papier. Voir section pour des instructions supplémentaires.

ATTENTION

Détérioration de l'instrument

Ne pas exposer le photomultiplicateur (PMT) à une lumière forte pendant la maintenance.

ATTENTION

Nettoyage de l'injecteur

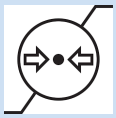
Les injecteurs doivent être nettoyés dans les 12 heures suivant le dernier cycle d'exécution pour garantir leurs performances à long terme. Le non-respect de cette règle peut entraîner le blocage des injecteurs.

ATTENTION

-Risque d'incendie

Lorsque le PyroMark Q48 Autoprep instrument est nettoyé avec un désinfectant à base d'alcool, laisser le couvercle de l'instrument et le capot des injecteurs ouverts pour permettre la dispersion des vapeurs inflammables.

11.7. Symboles sur le PyroMark Q48 Autoprep

Symbole	Emplacement	Description
	Plaque signalétique à l'arrière de l'instrument et étiquette de l'emballage extérieur	Marquage CE pour la conformité européenne
	Plaque signalétique à l'arrière de l'instrument	Fabricant légal
	Plaque signalétique à l'arrière de l'instrument	Marquage de déchets d'équipements électriques et électroniques (DEEE) pour l'Europe
	Plaque signalétique à l'arrière de l'instrument	Marquage FCC de la Federal Communications Commission (USFCC, commission fédérale des communications des États-Unis)
	Plaque signalétique à l'arrière de l'instrument	Marquage RoHS pour la Chine (restriction de l'utilisation de certaines substances dangereuses dans les équipements électriques et électroniques)
	Plaque signalétique à l'arrière de l'instrument	Marquage RCM pour l'Australie et la Nouvelle-Zélande
	Plaque signalétique à l'arrière de l'instrument	Marquage CSA pour le Canada et les États-Unis
	Plaque signalétique à l'arrière de l'instrument	Courant alternatif
	Plaque signalétique à l'arrière de l'instrument	Fusible
	Étiquette de l'emballage extérieur	Conservation
	Étiquette de l'emballage extérieur	Transport
	Étiquette de l'emballage extérieur	Limites de pression atmosphérique
	Étiquette de l'emballage extérieur	Limites d'humidité
	Plaque signalétique à l'arrière de l'instrument et étiquette de l'emballage extérieur	Numéro de série
	Plaque signalétique à l'arrière de l'instrument et étiquette de l'emballage extérieur	Numéro de référence
	Réactifs, solutions, disque	Identification du lot/lot de production

Symbole	Emplacement	Description
	Réactifs, solutions, disque	Date limite d’utilisation
	Réactifs, solutions, disque, étiquette de l’emballage extérieur	Limites de température
	Réactifs, cartouche	Lire le manuel
	À l’intérieur de l’instrument, cartouche	Avertissement, consulter le manuel d’utilisation
	À l’intérieur de l’instrument	Avertissement, surface chaude

Appendix F – Safety Information (German, DE)

Sicherheitshinweise

Vor der Verwendung des PyroMark Q48 Autoprep System sollten Sie dieses Benutzerhandbuch unbedingt sorgfältig durchlesen und insbesondere die Sicherheitshinweise beachten. Die Anweisungen und Sicherheitshinweise in diesem Benutzerhandbuch müssen befolgt werden, um einen sicheren Betrieb des PyroMark Q48 Autoprep System zu gewährleisten und den sicheren Zustand des PyroMark Q48 Autoprep System zu erhalten.

In diesem Handbuch werden die folgenden Typen von Sicherheitshinweise verwendet.

WARNUNG

Der Begriff WARNUNG weist auf Situationen hin, in denen Verletzungsgefahr für Sie oder andere besteht.

Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

VORSICHT

Der Begriff VORSICHT wird verwendet, um Sie über Situationen zu informieren, in denen die Gefahr besteht, dass das System oder andere Geräte beschädigt werden.

Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

Die in diesem Handbuch enthaltenen Hinweise sollen die im Land des Anwenders geltenden normalen Sicherheitsbestimmungen nicht ersetzen, sondern lediglich ergänzen.

Sachgemäße Verwendung

WARNUNG/ VORSICHT

Gefahr von Personen- und Sachschäden

Die unsachgemäße Anwendung des PyroMark Q48 Autoprep System kann zu Verletzungen des Benutzers oder zur Beschädigung des Geräts führen. Das PyroMark Q48 Autoprep-System darf nur durch qualifiziertes, entsprechend geschultes Personal bedient werden.

Die Instandhaltung des PyroMark Q48 Autoprep-Systems darf nur durch einen QIAGEN Außendiensttechniker durchgeführt werden.

VORSICHT

Beschädigung des Geräts

Direktes Sonnenlicht kann zum Ausbleichen von Teilen des Geräts und der Kartuschen führen und Schäden an Kunststoffteilen verursachen.

Das PyroMark Q48 Autoprep System und die Kartuschen dürfen nicht in direktem Sonnenlicht oder in unmittelbarer Nähe zu Wärme- und Vibrationsquellen oder elektrischen Störfeldern aufgestellt werden.

VORSICHT

Beschädigung des Geräts

Verschütten Sie kein Wasser oder keine Chemikalien auf dem PyroMark Q48 Autoprep System. Durch verschüttetes Wasser oder verschüttete Chemikalien verursachte Schäden führen zum Erlöschen der Garantie.

WARNUNG/ VORSICHT

Gefahr von Personen- und Sachschäden

Bewegen Sie das PyroMark Q48 Autoprep System auf keinen Fall während des Betriebs.

WARNUNG/ VORSICHT

Explosionsfähige Atmosphären

Das PyroMark Q48 Autoprep System ist nicht für den Gebrauch in explosionsfähiger Atmosphäre vorgesehen.

VORSICHT

Explosionsgefahr

Das PyroMark Q48 Autoprep System ist für die Verwendung mit Reagenzien und Substanzen bestimmt, die zusammen mit QIAGEN-Kits geliefert werden. Die Verwendung anderer Reagenzien und Substanzen kann zu einem Brand oder zu einer Explosion führen.

VORSICHT

Einlegen des Absorberstreifens

Stellen Sie sicher, dass der Absorberstreifen in den PyroMark Q48 Autoprep eingesetzt ist (wie in Abschnitt 6.2.2beschrieben), um das Eindringen von Flüssigkeit in die Kammer zu verhindern.

Schalten Sie das PyroMark Q48 Autoprep System im Notfall am Netzschalter aus und ziehen Sie den Netzstecker aus der Steckdose.

1.2 Elektrische Sicherheit

Hinweis: Ziehen Sie den Netzstecker aus der Steckdose, bevor Sie Instandhaltungs-/Wartungsarbeiten an einem Gerät vornehmen.

WARNUNG

Stromschlaggefahr

Jede Unterbrechung des Schutzleiters (Erdungs- bzw. Masseleiter) im Gerät oder außerhalb des Geräts und jede Abtrennung des Schutzleiters am Anschluss der Netzleitung erhöht die Gefahr eines Stromschlags.

Eine absichtliche Unterbrechung der Schutzleiterverbindung ist verboten.

Gefährliche Spannung im Gerät

Wenn die Geräte an die Stromversorgung angeschlossen sind, sind die Anschlussstellen spannungsführend, und durch das Öffnen der Abdeckungen oder das Entfernen von Gehäuseteilen können spannungsführende Komponenten freigelegt werden.

Vermeiden Sie das Verschütten von Flüssigkeiten auf oder in das Gerät. Sollten Sie Flüssigkeit über das Gerät verschütten, trennen Sie das Gerät sofort vom Stromnetz.

Um einen zufriedenstellenden und sicheren Betrieb des PyroMark Q48 Autoprep System zu gewährleisten, befolgen Sie bitte die nachstehenden Hinweise:

- Das Netzkabel muss an eine Wechselstrom-Steckdose mit Schutzleiter (Erdungs-/Masseleiter) angeschlossen werden.
- Bewahren Sie den Netzstecker leicht zugänglich auf, falls das Gerät schnell vom Stromnetz getrennt werden muss.
- Es darf nur das von QIAGEN mitgelieferte Netzkabel verwendet werden.

Falls die elektrische Sicherheit bei der Bedienung des Geräts nicht mehr gewährleistet werden kann, muss das Gerät gegen Benutzung durch darüber nicht informiertes Personal gesichert werden. Kontaktieren Sie anschließend den Technischen Service von QIAGEN. Die elektrische Sicherheit des Geräts ist nicht mehr gegeben, wenn:

- das Netzkabel beschädigt erscheint;
- Es über einen längeren Zeitraum unter Bedingungen gelagert wurde, die außerhalb der in Abschnitt 11, Technische Daten beschriebenen „Lagerbedingungen" liegen.
- das Gerät unsachgemäß transportiert worden ist.
- Flüssigkeit in das Gerät eingedrungen ist.

Biologische Sicherheit

Wenden Sie beim Umgang mit biologischen Materialien nur sichere Laborverfahren an, wie sie z. B. in Veröffentlichungen wie Biosafety in Microbiological and Biomedical Laboratories, HHS, **www.cdc.gov/labs/BMBL.html**) beschrieben sind.

WARNUNG **Biologische Materialien**

Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist und die Bediener des Geräts ausreichend geschult sind. Außerdem dürfen die Grenzwerte in Bezug auf Infektionserreger, die in den entsprechenden Sicherheitsdatenblättern (Safety Data Sheets (SDSs)) oder den Vorschriften der OSHA0,* de ACGIH,† oder COSHH‡ festgelegt sind, nicht überschritten werden.

Weiterführende Informationen finden Sie unter **www.qiagen.com/safety**.

Beim Betrieb eines Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze auf Bundes-, Landes- und kommunaler Ebene zu Gesundheitsschutz und Sicherheit am Arbeitsplatz eingehalten werden.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

Chemische Sicherheit

WARNUNG/ VORSICHT

Gefährliche Chemikalien

Einige Chemikalien, die mit diesem Gerät verwendet werden, können gefährlich sein oder nach Beendigung eines Protokolllaufs gefährlich werden.

Tragen Sie immer eine Schutzbrille, Laborhandschuhe und einen Laborkittel.

Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist und die Bediener des Geräts ausreichend geschult sind. Außerdem dürfen die Grenzwerte in Bezug auf toxische (chemische oder biologische) Substanzen, die in den entsprechenden Sicherheitsdatenblättern (Safety Data Sheets, SDS) oder den Vorschriften der OSHA,* de ACGIH,† oder COSHH‡ festgelegt sind, nicht überschritten werden.

Weitere Informationen finden Sie unter **www.qiagen.com/safety**.

Beim Betrieb eines Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze auf Bundes-, Landes- und kommunaler Ebene zu Gesundheitsschutz und Sicherheit am Arbeitsplatz eingehalten werden.

Reinigen Sie verschüttete oder verspritzte Denaturierungslösung sofort vom Gerät. Längere Einwirkung kann zu Flecken und Oberflächenschäden führen.

* OSHA — Occupational Safety and Health Organization (United States of America)
† ACGIH – American Conference of Government Industrial Hygienists (United States of America)
‡ COSHH – Control of Substances Hazardous to Health (United Kingdom)

Gefahren durch mechanische Teile

WARNUNG

Sich bewegende Geräteteile

Um einen Kontakt mit beweglichen Teilen beim Gerätebetrieb des PyroMark Q48 Autoprep System zu vermeiden, darf das Gerät nur mit geschlossener Injektorabdeckung betrieben werden.

Entfernen Sie die Abdeckplatten nicht, da sich im Inneren keine vom Benutzer zu wartenden Teile befinden. Wenn ein Problem mit dem PyroMark Q48 Autoprep-System auftritt, wenden Sie sich umgehend an den technischen Service von QIAGEN.

WARNUNG

Sich bewegende Geräteteile

Der Kammerdeckel des PyroMark Q48 Autoprep-Geräts öffnet und schließt sich während des Betriebs automatisch, um Feuchtigkeitsansammlungen im Geräteinneren zu verhindern.

VORSICHT

Einlegen der Disc

Die PyroMark Q48-Disc muss in ihrer Position verriegelt werden, um zu verhindern, dass der Inhalt der Disc in die Gerätekammer gelangt.

Stellen Sie sicher, dass die Disc durch Festdrehen der Kontermutter in ihrer Position gesichert ist. Um Schäden am Gerät zu vermeiden, verwenden Sie nur die von QIAGEN bereitgestellte Kontermutter.

VORSICHT

Bedienung des Kammerdeckels

Die Bedienung des Deckels wird durch einen softwaregesteuerten Motor gesteuert. Wenn auf der Rückseite des Geräts nicht genügend Platz (weniger als 20 cm bzw. 7,9 Zoll) zum Bedienen des Kammerdeckels vorhanden ist, kann dieser beim Öffnen beschädigt werden.

Gefahr durch Hitze

WARNUNG **Heiße Oberfläche**

Die Disc-Heizung im PyroMark Q48 Autoprep-Gerät kann Temperaturen von bis zu 95 °C (203 °F) erreichen. Berührungen im heißen Zustand sind zu vermeiden.

WARNUNG **Überhitzungsgefahr**

Vergewissern Sie sich, dass ein Mindestabstand von 5 cm (2 Zoll) an den Seiten und 20 cm (7,9 Zoll) an der Rückseite der PyroMark Q48 Autoprep-Geräts und der Wand eingehalten wird, damit eine ausreichende Belüftung der Geräte gewährleistet ist.

Die Lüftungsschlitze und Öffnungen, die die Be- und Entlüftung des PyroMark Q48 Autoprep-Geräts gewährleisten, dürfen nicht verdeckt werden.

Verbrauchsmaterialien

VORSICHT **Nicht unterstützte Verbrauchsmaterialien**

Schließen Sie keine anderen Verbrauchsmaterialien, Zubehörteile oder externe Geräte an und verwenden Sie diese nicht, außer den angegebenen.

Abfallentsorgung

VORSICHT **Entfernen des Absorberstreifens**

Nach Abschluss des Laufs enthält der Absorberstreifen eine Denaturierungslösung, die Natriumhydroxid enthält. Diese kann zu Augen- und Hautreizungen führen.

Tragen Sie beim Entfernen des Absorberstreifens immer eine Schutzbrille, Laborhandschuhe und einen Laborkittel.

Bei der Entsorgung des Absorberstreifens müssen alle Bestimmungen und Gesetze auf Bundes-, Landes- und kommunaler Ebene zu Gesundheitsschutz und Sicherheit am Arbeitsplatz eingehalten werden.

VORSICHT **Entsorgung von Kunststoffartikeln**

Gebrauchte Kunststoffartikel, z. B. PyroMark Q48-Discs, können gefährliche Chemikalien oder ansteckende/biologisch gefährliche Stoffe enthalten. Derartige Abfälle müssen gesammelt und sachgerecht gemäß den geltenden örtlichen Sicherheitsbestimmungen entsorgt werden.

Wartungssicherheit

Führen Sie die Wartungsarbeiten gemäß den Anweisungen in Abschnitt 8 durch. QIAGEN stellt Reparaturen, die auf nicht fachgerecht durchgeführte Wartungsmaßnahmen zurückzuführen sind, in Rechnung.

WARNUNG/ VORSICHT

Gefahr von Personen- und Sachschäden

Es dürfen nur Wartungsarbeiten ausgeführt werden, die in diesem Benutzerhandbuch konkret beschrieben sind.

WARNUNG/ VORSICHT

Gefahr durch Stromschlag

Öffnen Sie keine der Abdeckplatten des PyroMark Q48 Autoprep System.

Es dürfen nur Wartungsarbeiten ausgeführt werden, die in diesem Benutzerhandbuch konkret beschrieben sind.

VORSICHT

Beschädigung des Geräts

Verwenden Sie keine Lösungsmittel oder Reagenzien, die Säuren, Laugen oder Abrasivstoffe enthalten, um das PyroMark Q48 Autoprep-Gerät zu reinigen.

VORSICHT

Beschädigung des Touchscreens und Computers

Gießen oder sprühen Sie keine Flüssigkeiten, z. B. Reinigungsmittel, auf das PyroMark Q48 Autoprep-Gerät. Benutzen Sie zur Reinigung ausschließlich ein mit Wasser angefeuchtetes Tuch.

VORSICHT

Wartung des Lichtdetektors

Reinigen Sie das Fenster des Photomultipliers (PMT) vorsichtig mit fusselfreien Tüchern. Verwenden Sie keine Papiertaschentücher. Siehe Abschnitt 8.2 für weitere Anweisungen.

VORSICHT

Beschädigung des Geräts

Setzen Sie den Photomultiplier (PMT) während der Wartung keinem starken Licht aus.

VORSICHT

Injektorreinigung

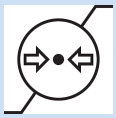
Um eine langfristige Leistung der Injektoren zu gewährleisten, müssen diese innerhalb von 12 Stunden nach dem letzten Betrieb gereinigt werden. Bei Nichtbeachtung kann es zum Verstopfen der Injektoren kommen.

VORSICHT

Brandgefahr

Lassen Sie beim Reinigen des PyroMark Q48 Autoprep-Geräts mit einem alkoholbasierten Desinfektionsmittel den Gerätedeckel und die Injektorabdeckung geöffnet, damit sich brennbare Dämpfe zerstreuen können.

Symbole auf dem PyroMark Q48 Autoprep

Symbol	Ort	Beschreibung
	Typenschild an der Geräterückseite und Etikett der Außenverpackung	CE-Markierung der EU-Konformität
	Typenschild an der Geräterückseite	Hersteller i. S. d. Gesetzes
	Typenschild an der Geräterückseite	WEEE-Markierung (Zertifizierung gemäß Richtlinie über Elektro- und Elektronik-Altgeräte) für Europa
	Typenschild an der Geräterückseite	FCC-Kennzeichnung der Federal Communications Commission der Vereinigten Staaten
	Typenschild an der Geräterückseite	RoHS-Kennzeichen für China (Einschränkungen in Bezug auf den Gebrauch bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)
	Typenschild an der Geräterückseite	RCM-Zeichen für Australien/Neuseeland
	Typenschild an der Geräterückseite	CSA-Zeichen für Kanada und USA
	Typenschild an der Geräterückseite	Wechselstrom
	Typenschild an der Geräterückseite	Sicherung
STORAGE	Etikett der Außenverpackung	Lagerung
TRANSPORT	Etikett der Außenverpackung	Transport
	Etikett der Außenverpackung	Grenzwerte für Atmosphärendruck
	Etikett der Außenverpackung	Grenzwerte für Luftfeuchtigkeit
SN	Typenschild an der Geräterückseite und Etikett der Außenverpackung	Seriennummer
REF	Typenschild an der Geräterückseite und Etikett der Außenverpackung	Katalognummer
LOT	Reagenzien, Lösungen, Disc	Identifizierung der Produktionscharge

Symbol	Ort	Beschreibung
	Reagenzien, Lösungen, Disc	Haltbarkeitsdatum
	Reagenzien, Lösungen, Scheibe, Etikett der Außenverpackung	Temperaturgrenzwerte
	Reagenzien, Kartusche	Lesen Sie das Handbuch
	Im Inneren des Geräts, Kartusche	Warnung, siehe Benutzerhandbuch
	Im Geräteinneren	Warnung, heiße Oberfläche

Document Revision History

Revision	Description
May 2025	Manual is updated with section on the new advanced cleaning protocol. Further minor changes are made to the safety information, so the symbols and description match with the current regulations. Finally, a note was added to the section on networking, warning the user of potential risks with the SMB 1.0 protocol that the instrument uses for connecting to shared network storage.
June 2020	Updated Cartridge loading and injector priming and Pyrophosphate clean Sections to add Important note when pipetting; Important note inserted in several procedures throughout this user manual Updated Cleaning the injectors Section to add information regarding cleaning of injectors when not in use Updated Cartridge care Section to add information regarding cartridge life Revised Injector test procedure Added table titles

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