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PyroMark® Q96 MD User Manual

QIAGEN®

Sample & Assay Technologies

Trademarks

QIAGEN®, PyroMark®, Pyrosequencing®, Pyrogram® (QIAGEN Group); Intel®, Core™ (Intel Corporation); Microsoft®, Windows® (Microsoft Corporation); Milli-Q® (Merck KGaA); Oracle® (Oracle International Corporation); Sepharose® (GE Healthcare).

Contents

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1 Safety Information

Before using the PyroMark Q96 MD, 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.

The following types of safety information appear throughout this manual.

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

1.1 Proper use

WARNING 	**Risk of personal injury and material damage** [W1] Improper use of the PyroMark Q96 MD may cause personal injuries or damage to the instrument. The PyroMark Q96 MD must only be operated by qualified personnel who have been appropriately trained. Servicing of the PyroMark Q96 MD must only be performed by QIAGEN Field Service Specialists.

WARNING	**Risk of personal injury and material damage** [W2] The PyroMark Q96 MD is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

Perform the maintenance as described in Section 7. QIAGEN charges for repairs that are required due to incorrect maintenance.

1.2 Electrical safety

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

WARNING	**Electrical hazard** [W3] 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.

To ensure satisfactory and safe operation of the PyroMark Q96 MD and PyroMark Q96 Vacuum Workstation, follow the advice below:

- The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground)
- Keep mains plugs easily accessible in case the equipment needs to be disconnected quickly from mains power
- Use only power supplies and cords supplied with the instrument
- When replacing the mains fuse, replace only with the type and current rating specified on the rating label

1.3 Chemical hazards

WARNING 	**Hazardous chemicals** [W4] The Denaturation Solution used with the Vacuum Workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, 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 not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

1.4 Mechanical hazards

The lid of the PyroMark Q96 MD must remain closed during operation of the instrument.

WARNING 	**Moving parts** [W5] To avoid contact with moving parts during operation of the PyroMark Q96 MD, the instrument must be operated with the lid closed. Do not remove the cover panels since there are no user-serviceable parts inside. If there is a problem with the PyroMark Q96 MD, contact QIAGEN Technical Services immediately.

WARNING 	**Pinch and impact hazards** [W6] The dispensing unit and process chamber lid may move at any time.

WARNING	**Sharp needles** [W7] Do not touch the sharp needles on the Reagent Dispensing Tips and Capillary Dispensing Tips.

- The PyroMark Q96 MD has an interlock system. This system has sensors in three places: The instrument lid above the process chamber, the robot compartment lid, and the stacker. These sensors check that components are correctly inserted. An audible warning sounds if components are incorrectly positioned and the run cannot start.
- If a lid is opened or the stacker withdrawn during a run, an audible warning will be heard and the run will stop when the test or sequencing has been completed.

1.5 Heat hazards

WARNING	**Hot surface** [W8] The external heating block can reach temperatures of up to 80°C (176°F). Avoid touching them when they are hot.

CAUTION	**Risk of overheating** [C1] To ensure proper ventilation, maintain a minimum clearance of 15 cm (5.9 in.) at the sides and rear of the PyroMark Q96 MD. Slits and openings that ensure the ventilation of the PyroMark Q96 MD must not be covered.

1.6 Symbols used on the PyroMark Q96 MD

Symbol	Location	Language	Description
	Type plate on the back of the instrument	EN	CE mark
	All products	EN	Catalog or recorder number
	All products	EN	Temperature limitation
	Type plate on the back of the instrument and all other products	EN	Legal manufacturer
	Type plate on the back of the instrument	EN	Warning, consult user manual
	Type plate on the back of the instrument and box	EN	Serial number
	Type plate on the back of the instrument	EN	Waste Electrical and Electronic Equipment (WEEE)
	Type plate on the back of the instrument	EN	FCC mark of the United States Federal Communications Commission
	Type plate on the back of the instrument	EN	C-Tick mark for Australia (supplier identification N17965/N15128)

Symbol	Location	Language	Description
	Type plate on the back of the instrument	EN	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)

2 Introduction

Thank you for choosing the PyroMark Q96 MD. We are confident it will become an integral part of your laboratory.

Before using the PyroMark Q96 MD, 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. A French and German translation of the safety information can be found in Appendices C and D, respectively.

2.1 About this user manual

This user manual provides information about the PyroMark Q96 MD and PyroMark Q96 MD Automated in the following sections:

1. Safety Information
2. Introduction
3. General Description
4. Installation Procedures
5. Operating Procedures
6. PyroMark Q96 MD Software
7. Maintenance
8. Troubleshooting
9. Glossary

   Appendices

The appendices include:

- Technical data
- Warranty terms
- French and German translations of safety information

2.2 General information

2.2.1 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 Q96 MD 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/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

2.2.2 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.

2.2.3 Version management

This document is the *PyroMark Q96 MD User Manual*, version 2.0.

2.3 Intended use of the PyroMark Q96 MD

The PyroMark Q96 MD System detects changes in specified variable positions in DNA prepared from biological samples.

The PyroMark Q96 MD Instrument and PyroMark Q96 Vacuum Workstation are intended to be used only in combination with QIAGEN kits indicated for use with the PyroMark Q96 MD Instrument for the applications described in the kit handbooks.

If the PyroMark Q96 MD Instrument and PyroMark Q96 Vacuum Workstation are used with kits other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The PyroMark Q96 MD System is intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the PyroMark Q96 MD System.

2.3.1 Requirements for PyroMark Q96 MD users

The table below covers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the PyroMark Q96 MD.

Task	Personnel	Training and experience
Delivery	No special requirements	No special requirements
Installation	**QIAGEN Field Service Specialists only**	
Routine use (running protocols)	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 and annual preventive maintenance	**QIAGEN Field Service Specialists only**	

3 General Description

The PyroMark Q96 MD System is a multi-application sequence analysis system which uses Pyrosequencing® technology for the real-time analysis of specific DNA sequences including single nucleotide polymorphisms (SNPs), mutation, and methylation analysis. The instrument can analyze 96 samples simultaneously. An easy-to-use protocol is used to prepare samples.

Enclosed in the light-proof instrument housing, a light detection system with cooled charged coupled device (CCD) camera detects light emitted from reactions in the wells of the PyroMark Q96 HS Plate. The instrument includes an embedded computer module with hard disk and network interface.

3.1 PyroMark Q96 MD definitions

- PyroMark Q96 MD Instrument: Instrument only
- PyroMark Q96 MD Software: Software only
- PyroMark Q96 Vacuum Workstation: Vacuum Workstation only
- PyroMark Q96 MD: Instrument, software, and installation
- PyroMark Q96 MD System: All of the above, plus any PyroMark kits

3.2 PyroMark Q96 MD principle

The PyroMark Q96 MD performs DNA sequencing using Pyrosequencing technology.

1. After setting up a run in the PyroMark Q96 MD software, the PyroMark Q96 HS Plate containing the samples is put onto the heating block in the instrument and the dispensing tip holder containing the reagents is placed in the dispensing unit.
2. The run is started when the user clicks the “Run” button in the “Run Setup” dialog. The operator’s computer sends all information regarding the analysis to PyroMark Q96 MD.

Note: During a run, data are continuously stored in the database and on the instrument embedded computer. If the communication between the instrument and the operator's computer is interrupted during the run, data from the interrupted run will automatically be restored once communication is re-established.

3. The temperature of the heating block adjusts to the preset level.
4. The mixer speed adjusts to the preset level.
5. The pressure in the pneumatic dispensing system rises to the preset level.
6. When the temperature, mixer speed and pressure have reached the preset levels (may take several minutes), the predispensation and dispensation of reagents begins.
7. Enzyme and substrate mixtures are predispensed into the rectangular well in the PyroMark Q96 HS Plate to ensure that the dispensation capillaries are flushed and filled with solutions.
8. The dispensing tips are positioned over each well in turn and the enzyme mixture is dispensed (the process takes 65 s). This process is then repeated for the substrate mixture.

   Note: Dispensation of all reagents into the PyroMark Q96 HS Plate is in a zigzag fashion. The mixing system (vibrating process chamber) is operational during the whole sequencing run.
9. The dispensation pressure is increased.
10. The nucleotides are predispensed into the rectangular well in the PyroMark Q96 HS Plate.
11. The dispensing tips are positioned over each well in turn and the programmed nucleotide is dispensed.
12. The CCD camera collects data from all the wells simultaneously during the whole process. The CCD camera is cooled to approximately 0°C to optimize performance and reduce background noise.

13. In wells where there is a positive reaction with the added nucleotide, light is emitted, giving rise to a peak in the Pyrogram®.
14. A period of 65 s (cycle time) is allowed between the addition of each nucleotide to ensure that all enzymatic reactions are completed.
15. The programmed sequence of nucleotide addition continues until the end of the program has been reached.

3.3 External features of the PyroMark Q96 MD and PyroMark Q96 MD Automated

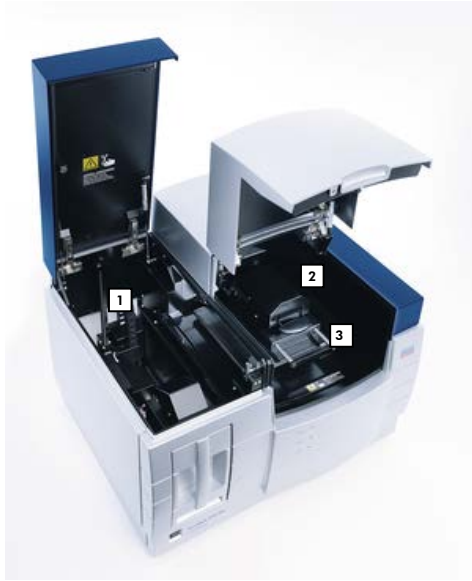
The PyroMark Q96 MD and PyroMark Q96 MD Automated (PyroMark Q96 MDA) are similar instruments in dimension and location of most instrument parts, but differ in the robot module, which is a feature of the PyroMark Q96 MDA only. The robot module occupies the left-side chamber of the PyroMark Q96 MDA and consists of a plate stacker that holds up to ten 96-well plates, a bar-code reader to automatically track plates, and a robotic arm that feeds plates into the process chamber of the instrument. Analyzed plates are deposited into the PyroMark Q96 HS Plates Bin through the waste slot. The PyroMark Q96 MD has the left-side chamber, but the robotic arm, stacker, bar-code reader, and openings for stacker and waste slot are absent.

The PyroMark Q96 MD/MDA.

1. Robot module (PyroMark Q96 MDA only)
2. Plate stacker (PyroMark Q96 MDA only)
3. Standby switch
4. Instrument lid
5. Indicator lamps
6. Waste slot (PyroMark Q96 MDA only)

Note: The PyroMark Q96 MD and PyroMark Q96 MDA are delivered with a laptop to serve as the operator's computer (see Section 3.12).

3.4 Internal features of the PyroMark Q96 MD and PyroMark Q96 MD Automated



Internal view of the PyroMark Q96 MD/MDA.

1 Robotic arm (PyroMark Q96 MDA only)

2 Dispensing unit

3 Process chamber

3.5 Process chamber

Samples are processed in the process chamber using PyroMark Q96 HS Plates. Plates are loaded manually. If using the automated PyroMark Q96 MDA, up to 10 PyroMark Q96 HS Plates can be loaded in the stacker for automated batch runs.

The process chamber includes a heating block with a Peltier cooler that maintains the correct temperature of the PyroMark Q96 HS Plate and its contents.

A mixer located beneath the process chamber enables fast mixing of samples and reagents in the PyroMark Q96 HS Plate.

The plate is secured using a mechanism that opens up automatically when the process chamber lid is opened. For the non-automated instrument, the plate holding mechanism is opened and closed manually.

3.6 Dispensing unit

Reagents are added to the dispensing tips in the dispensing tip holder which is inserted into the dispensing unit on top of the process chamber lid.

During a run, the dispensing unit is positioned over each well in the PyroMark Q96 HS Plate and reagents are dispensed by a pneumatic system.

3.7 Robot module

The robot module is located on the left-hand side of the instrument (PyroMark Q96 MDA only). It consists of an integrated robotic arm that fetches the PyroMark Q96 HS Plate scans the bar-code, and loads the plate into the process chamber.

After analysis, the robot discards the PyroMark Q96 HS Plate through the waste slot at the front of the stacker. The PyroMark Q96 HS Plates Bin is placed below the waste slot to collect the analyzed plates.

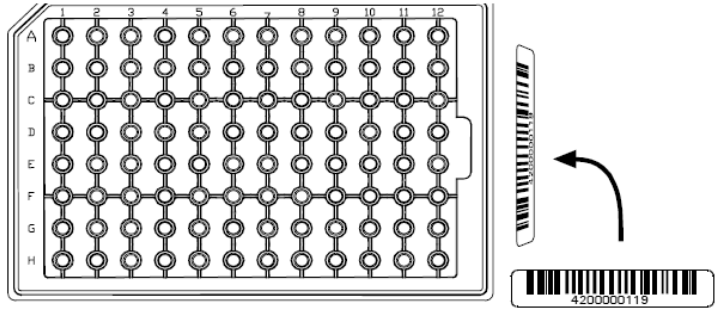
3.8 Bar-code reader and bar-codes

The bar-code reader is located in the robot compartment above the stacker (PyroMark Q96 MDA only).

An external bar-code reader can be used, but is not included with the PyroMark Q96 MD.

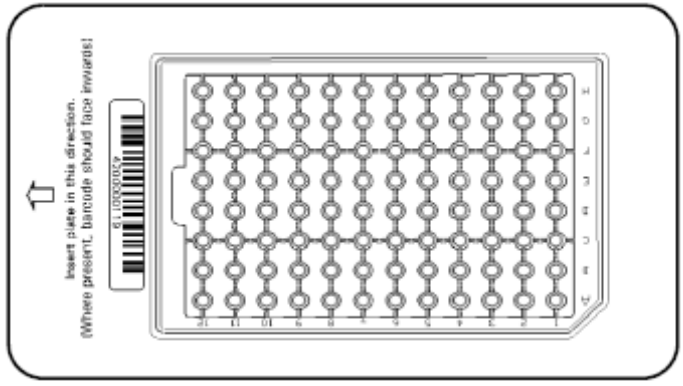
It is recommended to use bar-codes of type 128 with a bar height ≥4 mm and a bar width <40 mm. Bar-codes should

be placed on the side of the PyroMark Q96 HS Plates as shown.



3.9 Stacker

The stacker can hold up to 10 PyroMark Q96 HS Plates (PyroMark Q96 MDA only). Plates should be inserted into the stacker with the bar-codes facing inwards (see below). A metal stop keeps the plates in place while moving the stacker. Analyzed plates are discarded through the waste slot at the front of the stacker.



3.10 PyroMark Q96 HS Plates Bin

The PyroMark Q96 HS Plates Bin is placed under the instrument to the left and is secured with a screw (PyroMark Q96 MDA only).

The PyroMark Q96 HS Plates Bin collects analyzed plates that are discarded via the waste slot in the stacker.

It is recommended that a plastic bag is placed in the PyroMark Q96 HS Plates Bin for easy removal of analyzed plates after a run is completed.

3.11 Controls and indicators

3.11.1 Switches and indicators on the front panel

The standby switch is located on the lower left-hand side of the front panel.

Note: The standby switch does not disconnect the instrument completely from mains power. Always disconnect the mains power cable before any maintenance is performed.

Three indicator lights can be found in the middle of the front panel:

- Top light: Power (green). When illuminated, this indicates the instrument has been connected to the mains electricity supply and is receiving power
- Middle light: Busy (yellow). When this light is illuminated or flashing, a run is in progress and the lid should not be opened
- Bottom light: Information (yellow). If this light is illuminated during a run, the operator's computer has lost contact with the instrument

During the start-up sequence:

- All the lights illuminate
- The instrument software loads (takes 1–3 minutes) and the "Busy" light goes off and the "Information" light starts flashing. This indicates that the computer is ready to receive instructions from the operator's computer

- When an application is selected and “Run” is clicked in the “Setup Run” window, the flashing “Information light will turn off and the “Busy” light will illuminate

3.11.2 Rear panel

The following connectors are located on the rear panel:

- Mains power input connector: Should be connected to a grounded mains power outlet
- Keyboard: Keyboard interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist
- Mouse: Mouse interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist
- Net: Ethernet network connector. In a stand-alone installation, the cable marked “Cross-connected TP” should be connected here.
- Monitor: Monitor interface for service purposes. This should only be used by a certified QIAGEN Field Service Specialist

3.12 Computer and software

The PyroMark Q96 MD is shipped with a laptop that serves as the operator’s computer.

The computer used for data analysis should have the following as minimum specifications:

- Microsoft® Windows® XP (English version) Operating System
- Intel® Core™ Duo processor (2 GHz) or higher
- 200 MB free hard drive capacity
- 512 MB RAM
- Ethernet ports (or external USB Ethernet adaptor)
- Monitor with 1280 x 800 pixels, True Color (32 bit)
- Graphics card supporting the resolution of the monitor
- Pointer device (mouse or similar)

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4 Installation Procedures

4.1 Instrument delivery and installation

The unpacking and installation of the PyroMark Q96 MD is carried out by a certified QIAGEN Field Service Specialist. A member of your group who is familiar with laboratory and computer equipment should be present during the installation.

The following items are delivered:

- PyroMark Q96 MD instrument
- Operator's computer
- PyroMark Q96 MD Software
- PyroMark Q96 Vacuum Workstation (must be ordered separately)
- One PyroMark 96 HS Capillary Tip Holder
- One PyroMark 96 HS Dispensing Tip Holder
- PyroMark Q96 HS Thermoplate
- *PyroMark Q96 MD User Manual* and other technical documentation in PDF format on accompanying CD-ROM

4.2 Requirements

Site

The PyroMark Q96 MD and PyroMark Q96 Vacuum Workstation must be located out of direct sunlight, away from heat sources, and away from sources of vibration and electrical interference. Refer to Appendix A for the operating conditions (temperature and humidity). The site of installation should be free of excessive drafts, excessive moisture, excessive dust, and not subject to large temperature fluctuations.

Refer to Appendix A for the weight and dimensions of the PyroMark Q96 MD. Ensure that the workbench is level, dry, clean, vibration-proof, and has additional space for accessories. The workbench should be stable and able to support the weight of the instrument (100 kg). Approximately

110 cm (43.3 in.) clearance above the workbench is required to accommodate the PyroMark Q96 MD with the lid open and to allow ventilation. Allow at least 15 cm (5.9 in.) of free space behind the instrument for cabling. Allow at least 25 cm (9.8 in.) of free space to the right-hand side of the instrument for simple disconnection of the mains cable during maintenance and service.

The operator's computer requires at least 50 cm (19.7 in.) clearance above it if placed on a shelf below the bench.

The PyroMark Q96 MD must be placed within approximately 1.5 m (59 in.) of a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.

Note: It is recommended to plug the instrument directly into its own wall socket and not to share the socket with other lab equipment.

CAUTION 	**Risk of overheating** [C1] To ensure proper ventilation, maintain a minimum clearance of 15 cm (5.9 in.) at the sides and rear of the PyroMark Q96 MD. Slits and openings that ensure the ventilation of the PyroMark Q96 MD must not be covered.

Power requirements

The PyroMark Q96 MD operates at:

- 100–240 V AC, 50–60 Hz

The PyroMark Q96 Vacuum Workstation operates at:

- 100 V AC, 50/60 Hz, 1.7/1.4 A
- 115 V AC, 60 Hz, 1.5 A
- 230 V AC, 50 Hz, 0.6 A

Ensure that the voltage rating of the PyroMark Q96 MD and PyroMark Q96 Vacuum Workstation is compatible with the AC voltage available at the installation site. Mains supply voltage fluctuations are not to exceed 10% of nominal supply voltages.

Grounding requirements

To protect operating personnel, the PyroMark Q96 MD must be correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

4.3 Moving the PyroMark Q96 MD

WARNING	**Risk of personal injury and material damage** [W2] The PyroMark Q96 MD is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

If you need to transport the PyroMark Q96 MD, package the instrument as follows:

1. When the instrument is not processing, shut down PyroMark Q96 MD software and exit from Microsoft Windows.
2. Switch off the operator's computer and PyroMark Q96 MD and disconnect the mains power cable from the instrument and the computer.
3. To move the instrument to a new location within the same laboratory, go to step 4. To move the instrument to another site, go to step 7.
4. Place the instrument onto a trolley and move to the new location.

   Note: The instrument weighs 100 kg. Two persons are required.
5. Position the PyroMark Q96 MD on the bench. See Section 4.2 for site requirements.
6. Follow the reinstallation procedures in Section 4.4 for stand alone installation or Section 4.5 for network installation.

7. To move the instrument to another site: First, secure the process chamber lid to the flat surface using package tape and mount the pink transport protection block in the robot compartment.
8. Pack the PyroMark Q96 MD and other components using the original packaging supplied with the instrument, if available.

   Note: The total weight of the package including the instrument is 160 kg. The instrument alone weighs 100 kg. Use suitable lifting equipment when moving the package.
9. At the new site, unpack the instrument.
10. Remove the transport protection block from the robot compartment and the package tape from the process chamber lid.
11. Follow the reinstallation procedures in Section 4.4 for stand-alone installation or Section 4.5 for network installation.

4.4 Reinstalling the PyroMark Q96 MD: Stand alone

When delivered, the PyroMark Q96 MD System is configured as a peer-to-peer network. The operator's computer acts as the host and PyroMark Q96 MD as the guest.

Install a stand-alone system as follows:

1. Identify the cable marked "Cross-connected TP" provided with PyroMark Q96 MD. Connect one end of this cable to the network connector on PyroMark Q96 MD ("net").

   Note: The "keyboard", "mouse", and "monitor" connectors are for the use of QIAGEN Field Service Specialists only.
2. Connect the other end of the Ethernet cable to the network card connector on the operator's computer.
3. Connect mains power as per Section 4.6.

4.5 Reinstalling the PyroMark Q96 MD: Network installation

The operator's computer is delivered with two network cards, one for connection to PyroMark Q96 MD and one for connection to a Local Area Network (LAN).

The main advantages of connecting PyroMark Q96 MD to a LAN are:

- Easier access to run data files
- Assays can be set up and analyzed on office computers
- The database where runs are stored can be shared and the results analyzed on office computers connected to the LAN
- Simpler back-up procedures
- Access to LAN printing resources

Install a LAN system as follows:

1. Connect one end of the cross-connected Ethernet network cable provided to the "NET" connector on the rear panel of PyroMark Q96 MD.
2. Connect the other end of the network cable to the LAN outlet on the operator's computer.
3. Connect the second Ethernet network cable between the second network card in the operator's computer and the nearest LAN connection point.
4. Connect mains power as per Section 4.6.
5. Start up the operator's computer and assign a unique IP address to the second network adapter for communication with the LAN.

4.6 Connecting mains power

1. Ensure the standby switch on the front of the PyroMark Q96 MD is in position ⏻ (press the lower part of the switch).
2. Connect the mains cable to the inlet on the rear of the PyroMark Q96 MD. Connect the mains cable to a grounded mains power outlet. The PyroMark Q96 MD will automatically adjust to the correct voltage.

4.7 Setting up network properties

4.7.1 Used IP addresses for instrument and computer

The instrument IP address and the host IP address (operator's computer) are fixed and do not need to be changed if the PyroMark Q96 MD is connected to a LAN.

- Operator's computer IP address: 192.168.255.200
- PyroMark Q96 MD IP address: 192.168.255.201

4.7.2 Assigning an IP address to the second network adapter

If you plan to connect the PyroMark Q96 MD System to a LAN, contact your LAN administrator regarding set-up of the second network adapter. A unique IP address has to be assigned to the second network adapter for communication with the LAN.

Note: The standard PyroMark Q96 MD software license allows up to four office installations.

5 Operating Procedures

This section describes how to operate the PyroMark Q96 MD System.

Before proceeding, it is recommended that you familiarize yourself with the features of the PyroMark Q96 MD by referring to Sections 3.3 and 3.4.

5.1 Required items

The following items are needed to perform a run:

- Operator's computer loaded with PyroMark Q96 MD Software
- PyroMark Q96 Vacuum Workstation
- Sample preparation buffers
- PyroMark Q96 Gold Reagents
- PyroMark Q96 HS Plate Stacker (PyroMark Q96 MDA only)
- PyroMark Q96 HS Reagent Dispensing Tips (RDTs)
- PyroMark Q96 HS Nucleotide Dispensing Tips (NDTs) or PyroMark Q96 HS Capillary Dispensing Tips (CDTs)
- PyroMark Q96 HS Dispensing Tip Holder or PyroMark Q96 HS Capillary Tip Holder
- PyroMark Q96 HS Plate, a 96-well microtiter plate specially designed for use with the PyroMark Q96 MD
- PyroMark Q96 HS Plates Bin, to collect analyzed plates (PyroMark Q96 MDA only)
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Powder-free gloves
- Lint-free tissues

5.2 Starting the system

The PyroMark Q96 MD Instrument requires a warm-up time of 90 minutes for the CCD camera output to stabilize. To save time, switch on the instrument before starting to prepare samples.

5.2.1 Starting the PyroMark Q96 MD

Before starting the PyroMark Q96 MD, ensure that the mains power cable is correctly inserted into the mains power connector on the rear panel and connected to a grounded mains power socket.

Make sure that the instrument is connected from the "Net" connector on the rear of the instrument to the connector on the operator's computer.

Turn the power switch to **I** (On). All indicator lights turn on when power is connected. After 1–3 minutes, the "Busy" light will turn off and the "Information" light will start flashing. The instrument is now ready for a run.

When a run is started, the flashing "Information" light will turn off and the "Busy" light will illuminate. Keep the lid closed during a run.

If the "Information" light illuminates during a run, the operator's computer has lost contact with the instrument.

5.2.2 Starting the computer and software

1. Start the computer installed with PyroMark Q96 MD software and log on to Microsoft Windows.
2. In the Windows Start menu, choose Programs/Biotage/PyroMark Q96 MD. You can also double-click the PyroMark Q96 MD icon on the Windows desktop.
3. Enter your username and password in the "Login" dialog box and click "OK".

   Note: When you log in for the first time, you can write "user" as login name and "user" as password.

   Please refer to the Online Help, available under the "Help" menu in the software, for instructions on how to add a new user.
4. The PyroMark Q96 MD software start screen opens. The PyroMark Q96 MD start screen is divided into six areas.

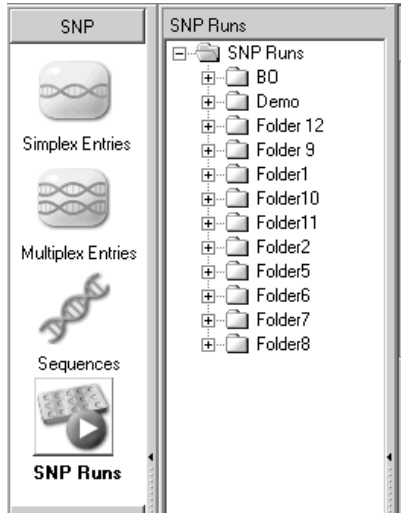
Start screen area	Content
Main menu	Gives access to software modules
Tree view area	Displays contents of active selection
Work area	Where parameters are entered and run progress is viewed
Title bar	Shows the current position in the application
Menu bar	Provides general functions for the file handling and window settings
Status bar	Displays instrument status and run progress

5.3 Preparing a run

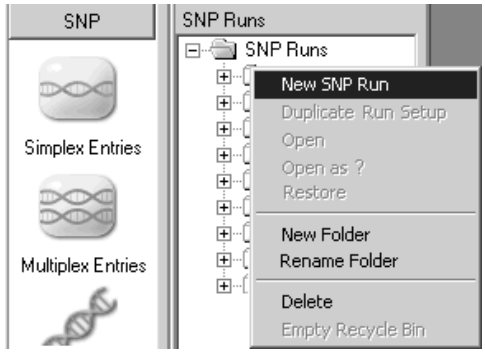
5.3.1 Set up a SNP run

Set up of a SNP run using the following procedure:

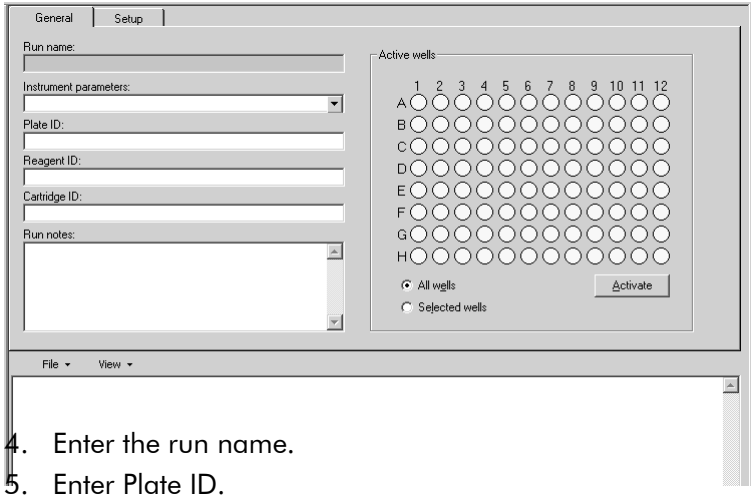
1. Click “SNP Runs” in the SNP main menu.



2. Right-click in the tree-view area on the folder where you want to store the setup and select “New SNP Run”.



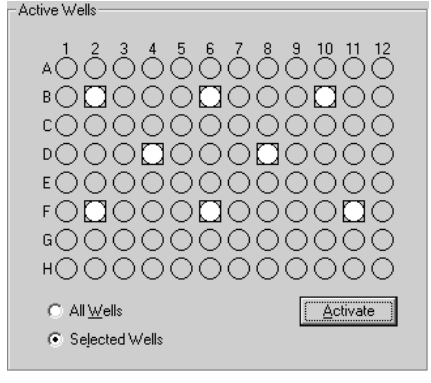
3. The “Run Setup” window opens, showing the “General” tab.



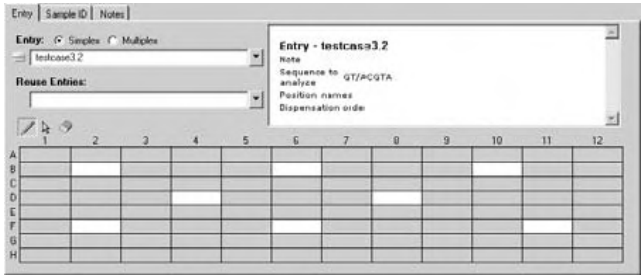
4. Enter the run name.
5. Enter Plate ID.
6. Select the needed “Instrument parameters” file from the drop-down list under the arrow. If you are going to include the run in a batch, the “Instrument parameters” selected in the “Batch” window will overrule “Instrument parameters” selected in the “SNP Run Setup” dialog box.

   Note: To create new instrument parameter files, see Section 5.3.4.

7. Click in the bar-code area and read the bar-code on the plate with an external bar-code reader. Alternatively, type in the bar-code.
8. Select the wells to be used in the run. All wells are selected by default. If this is the case, continue to step 9. Otherwise:
   - To select wells in a rectangular pattern, drag the mouse pointer to include all the wells to be selected and then release the mouse button. Selected wells appear as white surrounded by a square. Click "Activate". The selected wells stay white while unselected wells turn gray.
   - To select wells one-by-one, hold down the "Ctrl" or "Shift" key and click in the wells. Selected wells appear as light beige surrounded by a square. Click "Activate". The selected wells stay light beige while unselected wells turn gray.

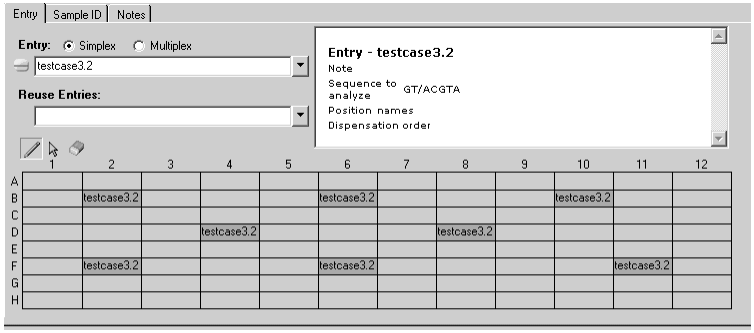


9. Click the "Setup" tab followed by the "Entry" tab. Selected wells are shown in white. Click "Simplex" or "Multiplex".



10. Select a "SNP entry" from the drop-down list. The selected SNP entry appears in the window to the right.

    Note: To define new entries, click "Simplex Entries" or "Multiplex Entries" in the SNP main menu or right-click in the drop-down list area. Entries can also be imported from Excel or as an XML-file. For further help, refer to the Online Help, available under the "Help" menu.



11. Use the pencil  to place the entry in the desired wells. Click each well or complete several wells by holding down the left mouse button and dragging the mouse over the wells. Repeat from step 10 if more than one entry is used.

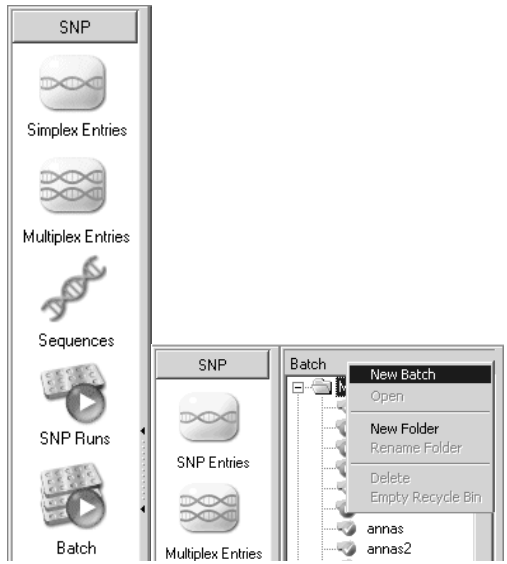
12. Click the "Sample ID" tab and select samples for each well in the same manner as you chose "Entry". Alternatively, type directly in the wells (click the desired well and type).

    To define new samples, click "Samples" in the main menu.

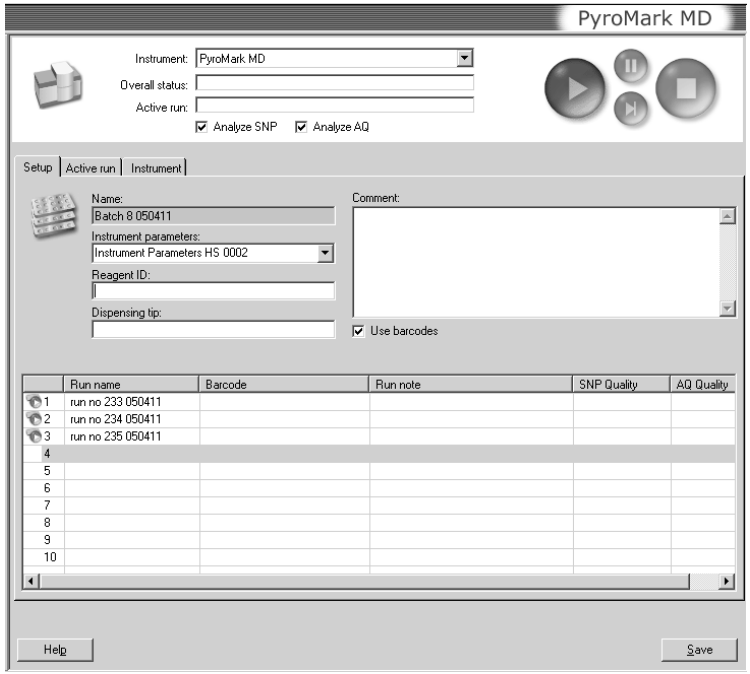
13. Notes about individual wells can be added under the "Notes" tab.
14. If setting up a run on a non-automated instrument, continue as per Section 5.3.3.
15. Click "Save".

5.3.2 Batch run setup (PyroMark Q96 MDA only)

1. Repeat the SNP setup procedure described in Section 5.3.1 for each of the PyroMark Q96 HS Plates in the batch.
2. Once all SNP runs are set up, click "Batch" in the SNP main menu. Right-click in the tree view area on the folder where you want to store the setup and select "New Batch".



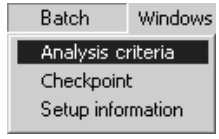
3. The “Batch” window opens, showing the “Setup” tab.



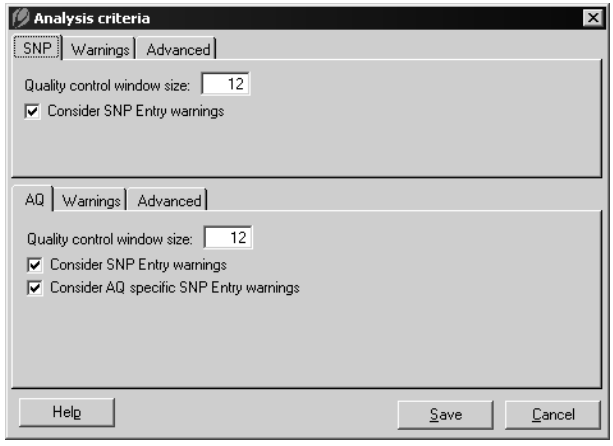
4. Enter the following information:
   - “Name” for batch
   - “Instrument”, choose from the list under the arrow
   - “Instrument parameters”, choose from the list under the arrow. The “Instrument parameters” selected in the “Batch” window are used for all plates in the batch. If other “Instrument parameters” have been selected in the individual SNP runs, these will be overruled.
   - “Comments”, “Reagent ID”, and “Dispensing tip” are all optional
5. If performance of automatic SNP analysis and/or AQ analysis is not desired, uncheck the “Analyze SNP” and/or “Analyze AQ” boxes.
6. In the main menu, click “SNP runs”.

7. Enter the "Run name" for each plate by dragging the SNP run from the SNP runs tree view and dropping it into the "Run name" column in the run list.

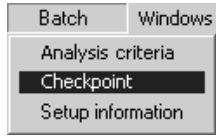
   Alternatively, click in the bar-code column in the run list and type in the bar-code for the run to include in the batch and press "Enter". The run appears in the run list. If several runs match the bar-code, a pop-up window displaying the possible runs appears. Select the desired run and click "Select".
8. Enter a "Description" (optional).
9. If the box "Use bar-codes" is checked, the bar-coded plates will be combined with the correct "Run name" irrespective of what order they are placed in the stacker. Otherwise, the plates must be placed in the stacker in the same order as in the run list, starting with plate number one at the top of the stacker.
10. After each plate has been run, it will be analyzed using default analysis criteria. These criteria can be changed in "Analysis criteria" in the "Batch" menu.



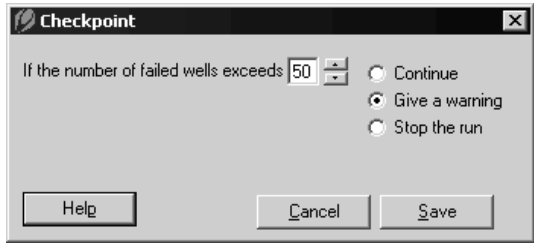
11. Make the changes required in the three tabs and click "Save".



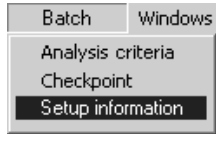
12. If too many wells have failed in the SNP analysis of the first plate, the instrument can provide a warning or stop the run. To utilize this feature, click “Checkpoint” in the “Batch menu”.



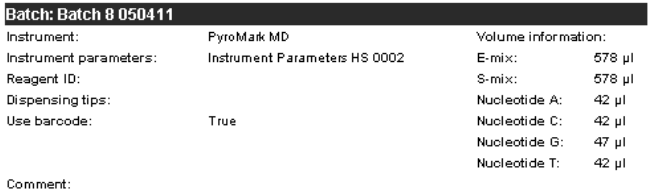
13. Choose a failure rate and the action you want the instrument to take if this rate is exceeded and click “Save”.



14. In the “Batch menu”, choose “Setup information” to view the volume information for the batch in the Internet browser.



15. Note the recommended volumes for enzyme mix, substrate mix, and each dNTP in order to calculate how much of each reagent to pipet into the dispensing tips later.

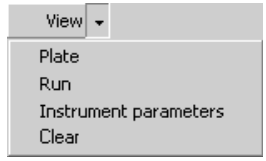


Batch: Batch 8 050411			
Instrument:	PyroMark MD	Volume information:	
Instrument parameters:	Instrument Parameters HS 0002	E-mix:	578 µl
Reagent ID:		S-mix:	578 µl
Dispensing tips:		Nucleotide A:	42 µl
Use barcode:	True	Nucleotide C:	42 µl
		Nucleotide G:	47 µl
		Nucleotide T:	42 µl
Comment:			

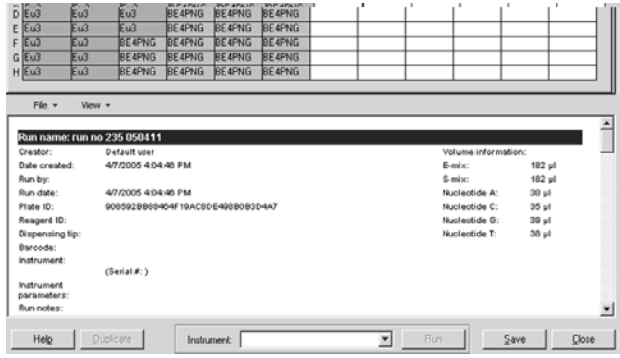
16. If setting up the run on an office computer, click "Save" in the "Batch" window.

5.3.3 Non-automated instrument (PyroMark Q96 MD): Finishing run setup

1. In the Browser area, click "View" and select "Run".



2. Note the recommended volumes for enzyme mix, substrate mix, and each dNTP in order to calculate how much of each reagent to pipet into the dispensing tips later.



3. If setting up the run on an office computer, click "Save".

5.3.4 Managing instrument parameters

Instrument parameters should be selected according to the reagents and dispensing tips that will be used for the run. The lot number printed on the PyroMark Q96 HS Nucleotide Tips, PyroMark Q96 HS Reagent Tips, and PyroMark Q96 Capillary Tips corresponds to specific instrument parameter settings provided at www.qiagen.com/Products/PyroMarkQ96MD.aspx.

Note: We recommend that only instrument parameters supplied by QIAGEN are used.

Managing instrument parameters in PyroMark Q96 MD Software:

1. Click "Instrument" and then "Parameters" to reveal the list of installed instrument parameter files.
2. Right-click an existing instrument parameter file from the list and click "Duplicate".
3. Enter a new file name. To easily identify the new instrument parameter file, name the file with the lot numbers of the dispensing tips to be used (e.g., "Instrument parameters 630088/450009" for NDTs and RDTs or "Instrument parameters 740001/450009" for CDTs and RDTs).
4. Go to the Web page cited above and open the file "Managing instrument parameters for the PyroMark Q96 MD", found under the User Support tab. In the provided tables, find the dispensing pressure and pulse time settings that correspond to the lot number printed on the dispensing tips to be used.
5. Enter the dispensing pressures and pulse times into the corresponding fields of the "Instrument Parameters" dialog box.
6. Save the new instrument parameter file.
7. When creating a new Run Setup, select this new instrument parameter file from the list of instrument parameter files.

Managing instrument parameters in PyroMark CpG Software:

1. Go the User Support tab of the Web page www.qiagen.com/Products/PyroMarkQ96MD.aspx.
2. Double-click the instrument parameter file that corresponds to the combination of dispensing tips to be used.
3. Unzip the downloaded file and save the instrument parameter file to the operator's computer:

- If using PyroMark CpG SW Version 1.0.9 or lower, save the file to:
  C:\Program Files\Biotage\Pyro Q-CpG 1.0.X\Instrument Parameters
  (where "X" represents the installed version of PyroMark CpG Software)
- If using PyroMark CpG SW Version 1.0.11 or higher, save the file to:
  C:\Program Files\QIAGEN\PyroMark CpG Software 1.0.11.14\Instrument Parameters

4. The downloaded file will now appear in the list of available instrument parameter files and can be selected with creating a new Run Setup.

Instrument parameters

In the “Instrument Parameters” dialog box, the following parameters are available.

Reagent pressure	Pressure (millibar) for dispensation of the enzyme mix and substrate mix.
Enzyme pulse time	Dispensation time (milliseconds) for the enzyme mix.
Substrate pulse time	Dispensation time (milliseconds) for the substrate mix.
Nucleotide pressure	Pressure (millibar) from the dispensation of nucleotides.
Nucleotide pulse time	Dispensation time (milliseconds) for nucleotides.
Note	Note about the instrument method (optional).

5.3.5 Sample preparation

Equipment and reagents to be supplied by the user

- PyroMark Q96 Vacuum Workstation
- PyroMark Q96 HS Plate (cat. no. 979101)
- PyroMark Q96 HS Sample Prep Thermoplate (supplied with the PyroMark Q96 MD)
- Mixer for immobilization to beads
- Heating block capable of attaining 80°C
- 96-well PCR plate and adhesive foil or strip caps
- Biotinylated PCR template
- Streptavidin Sepharose® HP (GE Healthcare, cat. no. 17-5113-01)
- Sequencing primer
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Ethanol (70%)

- PyroMark Binding Buffer (cat. no. 979006)
- PyroMark Denaturation Solution (cat. no. 979007)
- PyroMark Wash Buffer concentrate (cat. no. 979008)
- PyroMark Annealing Buffer (cat. no. 979009)

Assembly of PyroMark Q96 Vacuum Workstation

Assemble the PyroMark Q96 Vacuum Workstation as follows:

1. Place the PyroMark Q96 Vacuum Workstation on a flat surface.
2. Connect the vacuum tubing to the connector on the back of the PyroMark Q96 Vacuum Workstation. Attach the other end of the tubing to the liquid waste container, which should be connected to the vacuum pump via the vacuum tubing as shown below.
3. Attach the Vacuum Prep Tool to the vacuum tubing on top of the PyroMark Q96 Vacuum Workstation as shown in the image below.

   Note: The liquid waste container must be able to withstand absolute vacuum.

Immobilization of the PCR product to beads for use on the PyroMark Q96 MD

Biotinylated PCR products are immobilized on streptavidin-coated Sepharose beads (Streptavidin Sepharose High Performance, GE Healthcare).For each sample the final immobilization volume is 80 µl. The reaction setup is shown in the table below.

Component	Volume per sample	
	For lot number 10057037 or higher	For lot numbers lower than 10057037
Biotinylated PCR product	5–20 µl	5–20 µl
Streptavidin Sepharose HP*	1 µl	2 µl
PyroMark Binding Buffer	40 µl	40 µl
High-purity water†	Variable†	Variable†
Total volume	**80 µl**	**80 µl**

* Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher use 1 µl. For lot numbers lower than 10057037, use 2 µl.

† The volume of water depends on the amount of PCR product used.

Allow all reagents and solutions to equilibrate to room temperature (15–25°C) before starting. All steps are performed at room temperature, unless stated.

1. Gently shake the bottle with streptavidin-coated Sepharose beads from side to side until a homogenous solution is obtained.
2. Prepare a mixture of Streptavidin Sepharose beads, Binding Buffer, and high-purity water sufficient for all samples to be analyzed.
3. Add 60 or 75 µl of the mixture prepared in step 2 to the wells of a 96-well PCR plate according to the plate setup (see Section 5.3.1).

4. Add 5–20 µl of a well-optimized, biotinylated PCR product to each well used.

   Note: The total volume per well should be 80 µl.

   Note: If performing multiplex analysis of pooled PCR products, use 5–10 µl of each product and reduce water volume to maintain the total volume.
5. Seal the PCR plate using adhesive foil or strip caps. Ensure that no leakage is possible between the wells.
6. Agitate the PCR plate constantly for at least 5–10 min using a mixer (1400 rpm).

   Note: Sepharose beads sediment quickly and capturing of beads must take place immediately once the agitation is complete. If more than 1 minute has elapsed, agitate the plate again before capturing the beads.

   Note: During immobilization, prepare the Vacuum Workstation for the sample preparation.
7. Proceed with strand separation, see next section.

Strand separation

WARNING 	**Hazardous chemicals** [W4] The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, 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 not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Procedure:

1. Ensure that the PyroMark Q96 Vacuum Workstation has been assembled correctly and securely, see 'Assembly of PyroMark Q96 Vacuum Workstation', page 5-15. The mains plug should be easily accessible in case the vacuum pump needs to be disconnected quickly from the mains power.

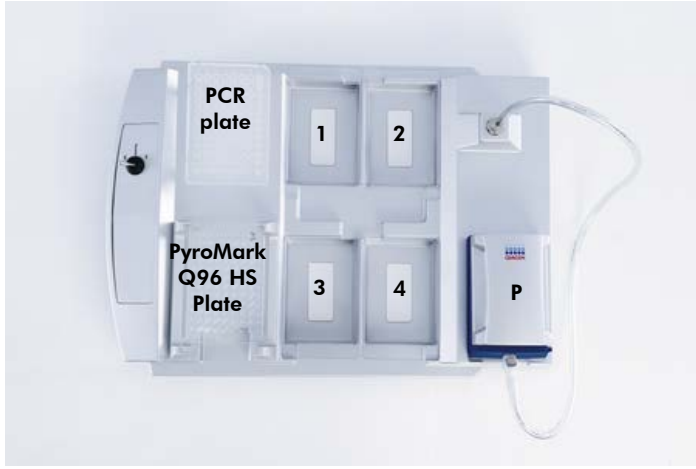
   Note: Perform the function test to ensure that the filter probes are working properly (see Section 7.12.1). All probes should be replaced after preparation of approximately 100 plates, or when needed.

   Note: Check and empty the waste container, if necessary (see Section 5.6.5).

2. Fill five separate troughs (supplied with the PyroMark Q96 Vacuum Workstation) as follows:
   - Approximately 110 ml ethanol (70%) (1)
   - Approximately 90 ml Denaturation Solution (2)
   - Approximately 110 ml Wash Buffer (3)
   - Approximately 110 ml high-purity water (4)
   - Approximately 180 ml high-purity water (Parking Position, P).

   A suggested setup is shown below. Refill the troughs to these levels whenever necessary.

   The total volume that can be added to each trough is 180 ml. There is a mark on each trough indicating 90 ml.



PyroMark Q96 Vacuum Workstation. Vacuum tool is in the trough of the "Parking" position (**P**).

3. Switch on the vacuum pump.
4. Apply vacuum to the tool by opening the vacuum switch.
5. Wash the filter probes by lowering the probes into high-ml high-purity water.
   Ensure that the water is being transferred to the waste container. If it is not, ensure the tubing is connected correctly and is not broken. Broken tubing should be replaced, see Section 7.13.
   Ensure that the tubing is connected properly and there is no leakage.
6. Refill Parking Position with 180 ml high-purity water.
7. Close the vacuum switch on the worktable (Off) and place the tool in the Parking Position.
8. Add 12 µl of 0.3 µM sequencing primer in Annealing Buffer to each well of a PyroMark Q96 HS Plate that is to be used.
9. After shaking, place the PCR plate and PyroMark Q96 HS Plate on the worktable. Ensure that both plates are in the same orientation as when samples were loaded.
   Note: Sepharose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was

agitated, agitate again for 1 min before capturing the beads.

10. Apply vacuum to the tool by opening the vacuum switch.
11. Carefully lower the filter probes into the PCR plate to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.
12. Ensure that all liquid is aspirated from the wells and that all beads have been captured onto the filter probe tips.
    Note: If the wells still contain liquid or white beads remain, the filter probes may need replacing, see Section 7.12.2.
13. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.
14. Transfer the tool to the trough containing Denaturation Solution (trough 2). Flush the filter probes for 5 s.
15. Transfer the tool to the trough containing Wash Buffer (trough 3). Flush the filter probes for 10 s.
16. Raise the tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes (see image, next page).

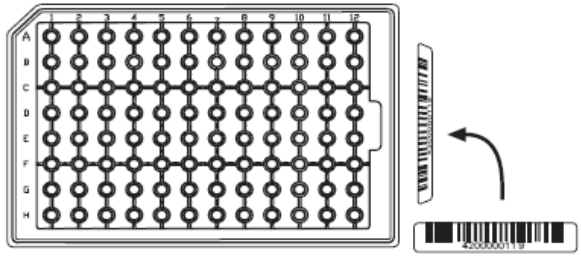
17. While holding the tool over the PyroMark Q96 HS Plate, close the vacuum switch on the worktable (Off).
18. Release the beads in the plate containing 0.4 µM sequencing primer in 0.3 µM sequencing primer in 12 µl Annealing Buffer, by shaking the tool gently from side to side in the plate.

19. Transfer the tool to the trough containing high-purity water (trough 4) and agitate the tool for 10 s.
20. Wash the filter probes by lowering the probes into high-purity water (Parking Position) and applying vacuum. Flush the probes with 180 ml high-purity water.
21. Raise the tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes.
22. Close the vacuum switch on the worktable (Off) and place the tool in the Parking Position.
23. If more than one plate is prepared at once, refill the troughs (step 2) and repeat the procedure from step 8.
24. Turn off the vacuum pump.

Note: The filter probes should be left to air dry when not in use, i.e., empty the trough and ensure there is no water in the Parking Position when the tool is left there.

Bar-codes

If bar-codes are to be used on the PyroMark Q96 HS Plate, ensure that they are placed on the side of the plate.



5.3.6 Annealing of sequencing primer to samples

WARNING	**Hot surface** [W8] The thermoplate and the heating block (for annealing) can reach temperatures of up to 80°C (176°F). Avoid touching them when they are hot.

1. Place the PyroMark Q96 HS Plate in a prewarmed PyroMark Q96 HS Sample Prep Thermoplate, and heat the samples on a heating block at 80°C for 2 min.
2. Remove the plate from the thermoplate and allow the samples to cool to room temperature (15–25°C) for at least 5 min. The plate can now be processed in the PyroMark Q96 MD.

5.3.7 Reagent preparation

Prepare reagents according to instructions in the *PyroMark Gold Q96 Reagents Handbook* provided with PyroMark Gold Q96 Reagents.

Filling dispensing tips and assembling the dispensing tip holder

- Wear a lab coat and powder-free gloves when handling the dispensing tips to prevent dust particles from clogging the tip heads of the nucleotide tips (NDT) or capillary tips (CDT)
- Store the assembled dispensing tip holder in the dispensing tip holder box when not placed in the instrument

The following items are required (see images below):

- PyroMark HS Dispensing Tip Holder or PyroMark HS Capillary Tip Holder
- 2 x PyroMark Q96 HS Reagent Tips (RDT)
- 4 x PyroMark Q96 HS Nucleotide Tips (NDT) or 4 x PyroMark Q96 HS Capillary Tips (CDT)

PyroMark Q96 HS Dispensing Tip Holder.

PyroMark Q96 HS Capillary Tip Holder.

Procedure:

1. Pull apart the springs on the dispensing tip holder and insert the two RDTs and the four NDTs/CDTs. Use the PyroMark Q96 HS Dispensing Tip Holder with NDTs and the PyroMark Q96 HS Capillary Tip Holder with CDTs. The dispensing tips should be held in place when the springs are released.

   When filling dispensing tips, point the pipet tip head towards the narrowest corner of the dispensing tip to avoid getting air bubbles in the solution. If any air bubbles are visible near the tip head (NDTs/CDTs), gently tap on the side of the tip to remove the bubbles.

Note: It is important that the reagents are dispensed into the correct dispensing tips.

2. Place the dispensing tip holder with the four NDTs/CDTs closest to you. Pipet the recommended volume of enzyme solution into the narrowest corner of the **E** dispensing tip.
3. Pipet the recommended volume of substrate solution into the narrowest corner of the **S** dispensing tip.
4. Pipet the recommended volume of each dNTP into the narrowest corners of the A, C, G, and T dispensing tips.
5. Place the filled dispensing tip holder in the dispensing tip holder box when transporting it to the instrument to avoid getting dust particles in the NDT/CDT tip heads.
   Note: Do not use filter tips for pipetting nucleotides or reagents.

5.4 Run

5.4.1 Loading the PyroMark Q96 HS Plates using the plate stacker

Up to 10 PyroMark Q96 HS Plates can be loaded into the stacker.

Allow plates containing samples to reach room temperature (15–25°C) before loading them into the instrument.

1. Insert a plastic bag into the PyroMark Q96 HS Plates Bin.
2. Take out the stacker.

   Note: Do not insert hands into the robotic compartment.
3. Pull out the metal stop on the rear of the stacker.
4. Insert PyroMark Q96 HS Plates into the stacker with the bar-codes facing inwards (see label on the stacker).
5. Replace the metal stop to keep the PyroMark Q96 HS Plates in place.
6. Insert the stacker into the instrument. Push the stacker until it is fully inserted.
7. Load the dispensing tip holder according to the instructions in Section 5.4.3.

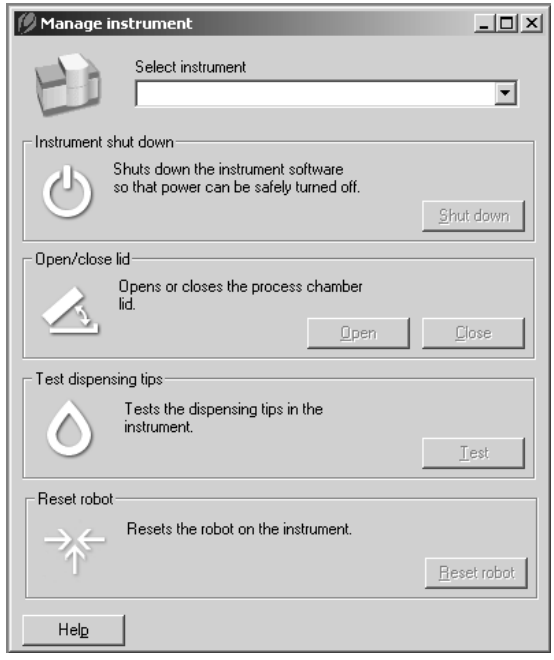
5.4.2 Loading a single PyroMark Q96 HS Plate manually

Allow plates containing samples to reach room temperature (15–25°C) before loading them into the instrument.

1. Open the instrument lid.

   Note: the dispensing unit and the process chamber will move if a run is accidentally started.

2. Open the process chamber lid using the software. In the main menu, click "Instrument" and then click "Manage".
3. In the "Manage Instruments" window, select the instrument and click "Open".



4. Place the PyroMark Q96 HS Plate on the heating block.
5. Close the process chamber lid by clicking "Close" in the "Instrument" window.

   It is also possible to open the process chamber lid manually and place the plate on the heating block.

- Open the process chamber lid manually and hold it open.
- Place the PyroMark Q96 HS Plate on the heating block and press to open the latch. When the plate is in position, release the latch.
- Close the process chamber lid.

6. Load the dispensing tip holder according to the instructions in Section 5.4.3.

Loading a PyroMark Q96 HS Plate manually

5.4.3 Loading the dispensing tip holder

If reusing a dispensing tip holder within 2 hours, ensure that it has been stored in the instrument with the dispensing unit cover closed. If needed, add more reagents before the run. It is advisable to run a dispensing test before the next run to check the function of the dispensing tips (Section 7.9). If more than 2 hours has elapsed, clean the whole dispensing tip holder directly after use and before using the dispensing tip holder again (Section 7.8).

1. Open the instrument lid (if not already opened).
2. Open the dispensing tip holder box and lift out the dispensing tip holder.
3. Open the dispensing unit cover by releasing the latch.
4. With the two RDTs furthest away from you, insert the filled dispensing tip holder into position (see image, next page).
5. Close the dispensing unit cover. Make sure that the latch snaps into its locked position.
6. Close the instrument lid.

Loading the dispensing tip holder.

5.4.4 Starting the run (automated instrument)

For a non-automated run, see Section 5.4.7.

The run can be started from the "Batch" window once all the required information has been entered. To open a previously entered batch, double-click the "Batch Name" in the "Batch" tree view area.

Check that the instrument name at the top of the window is correct. If not, choose the correct instrument from the dropdown list.

Check that the "Analyze SNP" and "Analyze AQ" boxes are checked, as desired.

Click the "Run" button at the top of the "Batch" window to start the run.

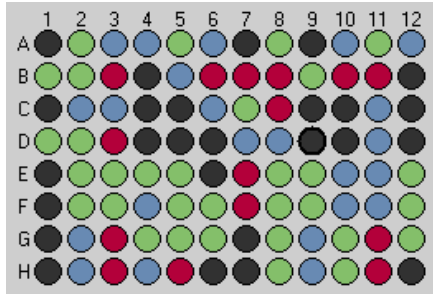
5.4.5 Monitoring the run (automated; PyroMark Q96 MDA)

When the run is underway, the status bars "Overall status" and "Active" run at the top of the "Batch window" indicate the status of the batch and of the current plate. Texts in the status bars describe the instrument status as well as robot actions during the run.

Active run tab

Well overview

A graphical representation of the wells of the current plate is shown indicating the current dispensation in each well. Click on a well to display the Pyrogram in real-time in the graph area.



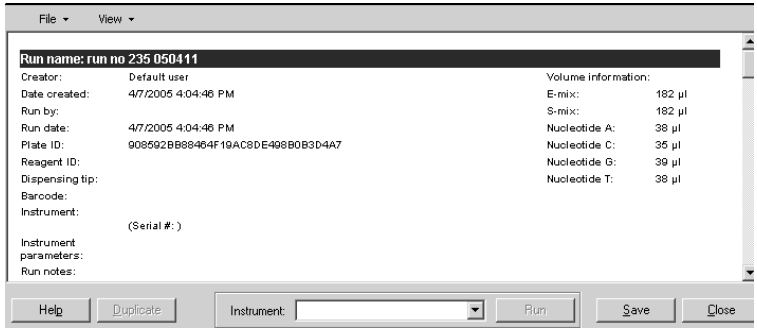
- Beige: Enzyme
- Purple: Substrate
- Black: G
- Blue: C
- Red: T
- Green: A

Graph area

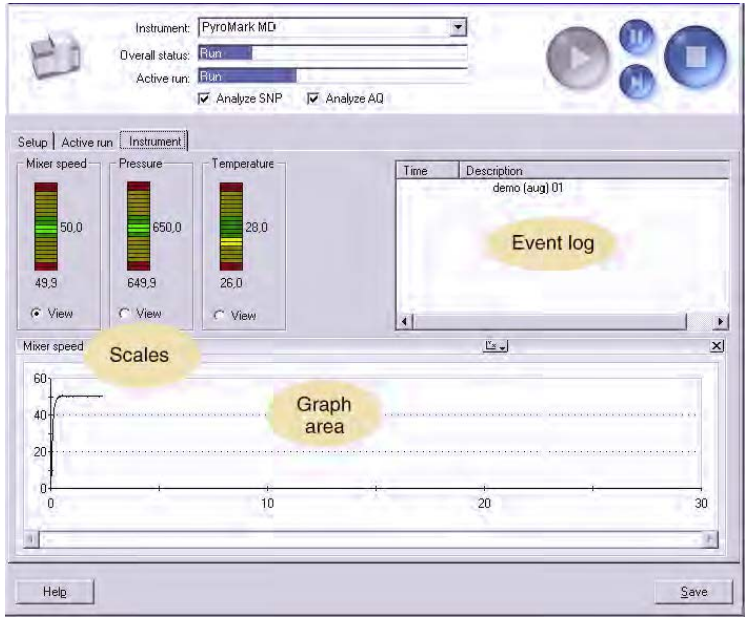
The Pyrogram is shown in a graphical display. Dispensed nucleotides are indicated below the graph.

Browser area

Click the "View" dropdown list in the Browser area to display information about the run.



Instrument tab



Scales

The bar scales display the instrument parameters for the run. The current values are shown under the scales. The optimum range for each parameter is illustrated in the middle of each scale.

Graph area

To view graphs for the mixer speed, pressure or block temperature, click View under the parameter to be shown.

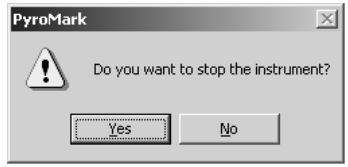
Event log

Messages appearing during the run are shown in the event log.

5.4.6 Change plates in the stacker

If you want to make any changes to the plates in the stacker:

1. Click the "Stop" button at the top of the "Batch" window.
   This warning appears:



2. Click "Yes" to stop the run immediately, or click "No" to stop when the current plate is finished.
3. Proceed with the post-run procedures, see Section 5.5.

5.4.7 Starting the run (non-automated; PyroMark Q96 MD)

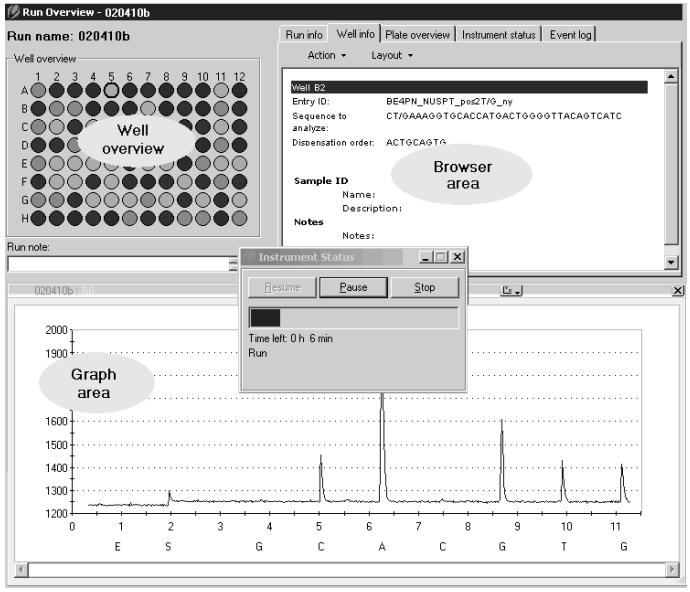
The run can be started from the "Run Setup" window once all the required information has been entered. To open a previously entered run setup, double-click the "Run Name" in the "SNP Runs" tree view area.

Check that the instrument name at the top of the window is correct. If not, choose the correct instrument from the dropdown list.

Click the "Run" button at the top of the "Run Setup" window. The "Instrument Status" window is shown on top of the "Run overview" window and a humming noise can be heard from the instrument.

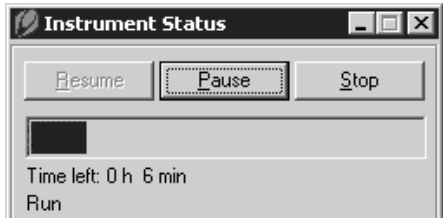
5.4.8 Monitoring the run (non-automated; PyroMark Q96 MD)

When the run is underway, the "Run Overview" is displayed on the screen.



Instrument status window

The “Instrument status” window is displayed on top of the “Run Overview” during the run.

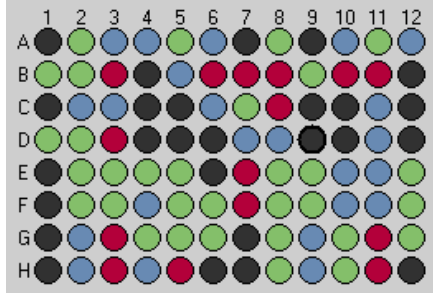


A progress bar indicates the elapsed and remaining time. Time remaining and current instrument status are shown. The following texts describing the instrument status are normally shown in the following order during the run:

- Preparing for run
- Adding reagent
- Run
- Prepare Stop
- Stop
- Run finished

Well overview

A graphical representation of the wells of the current plate is shown indicating the current dispensation in each well. Click on a well to display the Pyrogram in real-time in the graph area.

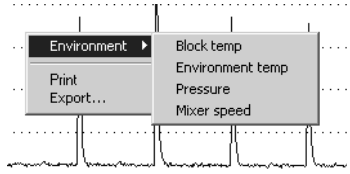


- Beige: Enzyme
- Purple: Substrate
- Black: G
- Blue: C
- Red: T
- Green: A

Graph area

The Pyrogram is shown in a graphical display. Dispensed nucleotides are indicated below the graph.

Right-click in the graph area to display the following pop-up menu:

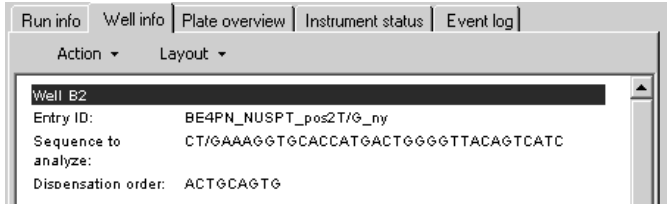


To view graphs for block temperature, environment temperature, pressure, or mixer speed, select "Environment" and choose the desired option. To return to the Pyrogram display, click the well again.

The current values for the parameters (except for environment temperature are displayed on the "Instrument Status" tab. Current values are displayed below each scale, whilst set values are displayed to the right of the scales.

Browser area

Click the tabs in the Browser area to display information about the run.



5.5 Post-run procedures

5.5.1 View the results (automated; PyroMark Q96 MDA)

When a batch run is finished, the status message "Batch complete" is displayed in the "Overall status" bar in the "Batch" window.

To view results for a batch run containing analyzed runs:

1. In the SNP main menu, choose "Result Browser" to open the "Result Browser" window.
2. Choose "Batch" in the SNP main menu. Drag and drop the completed batch run in the "Result Browser" window. The analyzed runs appear in the "Run" column.
3. Select the format for result presentation in the "Layout" dropdown list and click "Go" to display the results.

5.5.2 Analysis of results (non-automated; PyroMark Q96 MD)

When a run is finished, a "Close" button appears in the "Instrument Status" window.

1. Click "Close".
2. Double-click the "Run name" in the tree view area. The "SNP Analysis" window opens.
   Note: SNP analysis can be performed in either "AQ" or "SNP" mode.

3. Click “Analyze:All” or mark desired wells and click “Analyze:Selected”. The program will analyze the samples with default analysis criteria.

5.6 Finishing work

5.6.1 Removing and cleaning the dispensing tip holder

1. Open the instrument lid.
2. Open the dispensing unit cover.
3. For reuse within 2 hours, store the dispensing tip holder in the instrument with the dispensing unit cover closed. If needed, add more reagents before the next run.
   Note: It is advisable to run a dispensing test before the next run to check the function of the dispensing tips (Section 7.9).
   For reuse more than 2 hours later, clean the whole dispensing tip holder directly after use.
   See Section 7.8 for detailed cleaning instructions.
4. Remove the dispensing tip holder and immediately place it in the dispensing tip holder box.
5. Close the dispensing unit cover. Make sure that the latch snaps into its locked position.

5.6.2 Removing the PyroMark Q96 HS Plate

1. Open the process chamber lid and hold it open.
2. Open the latch and remove the PyroMark Q96 HS Plate from the heating block.
3. Release the latch and close the process chamber lid.

5.6.3 Disposing of dispensing tip holder/tips and plate

PyroMark Q96 HS tip holders, PyroMark Q96 HS dispensing tips, and PyroMark Q96 HS Plates should be disposed of according to local regulations for plasticware containing potentially hazardous materials. To avoid injury, put the tips in a plastic bag prior to disposal.

Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

WARNING 	**Sharp needles** [W7] Do not touch the sharp needles on the Reagent Dispensing Tips and Capillary Dispensing Tips.

5.6.4 Cleaning the PyroMark Q96 MD

If necessary, clean the instrument, see Section 7.3.

The instrument may be shut down if it is not to be used for 3 hours or longer. Remember that the instrument must be switched on a minimum of 90 minutes before the next run to ensure stable operating conditions.

For detailed instructions on instrument shutdown, see Section 7.1.

5.6.5 Emptying the waste container and troughs

WARNING	**Hazardous chemicals** [W4]
	The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, 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 not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

The following item is required:

- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e., the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Discard any solutions left in the troughs.
3. Rinse the troughs with high-purity water, or replace them, if necessary.
4. Empty the waste container.
   Note: The cap can be removed without disconnecting the tubing.
5. If the vacuum workstation must be cleaned (e.g., dust or spillage), follow the instructions in Section 7.11.

6 PyroMark Q96 MD Software

The operator's computer is delivered with the PyroMark Q96 MD Software and an Oracle® database preinstalled.

The computer is configured for communication with the PyroMark Q96 MD. An office version of the PyroMark Q96 MD Software can be installed on up to four computers (office computers), not supplied. Additional licenses can be purchased from QIAGEN.

6.1 System requirements

6.1.1 Operator's and office computers

See Section 3.12 for minimum specifications.

An Oracle client must be installed on the office computer for communication with the database. This is automatically performed during the installation of the PyroMark Q96 MD Software. If an earlier version of an Oracle client is installed, it will automatically be updated.

6.1.2 Printer

All printers supported by Windows 2000 are suitable. QIAGEN recommends the use of a laser printer.

6.1.3 Data storage device

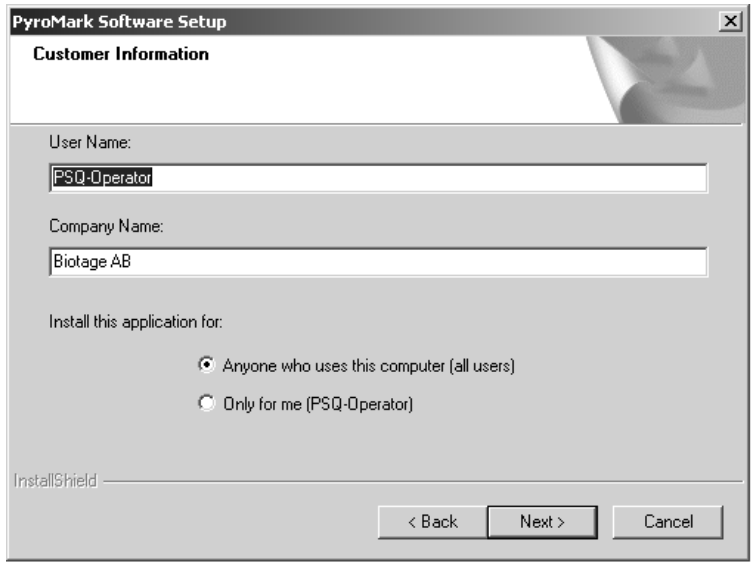
A removable hard drive or similar should be used for backing up the result files and the database. This is required if the computer used to analyze the data is not connected to a LAN.

6.2 Installing PyroMark Q96 MD Software

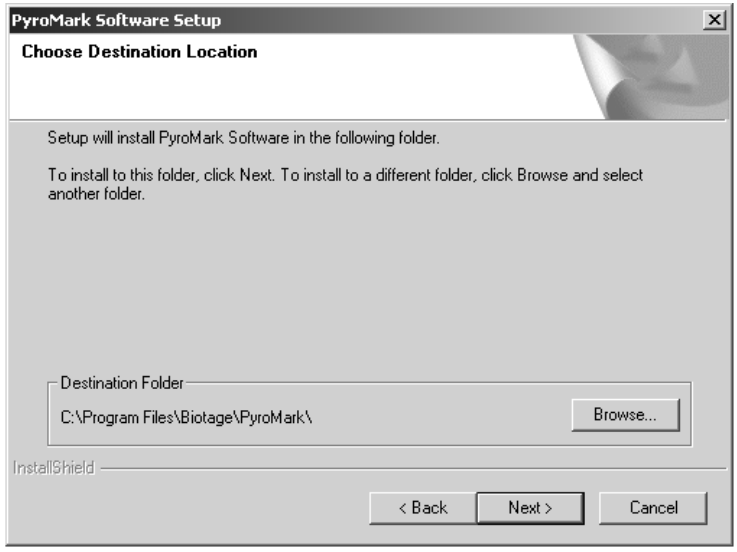
Use of PyroMark Q96 MD Software is restricted via a license key. The installation procedures are the same for the operator's computer and the office computer.

Procedure:

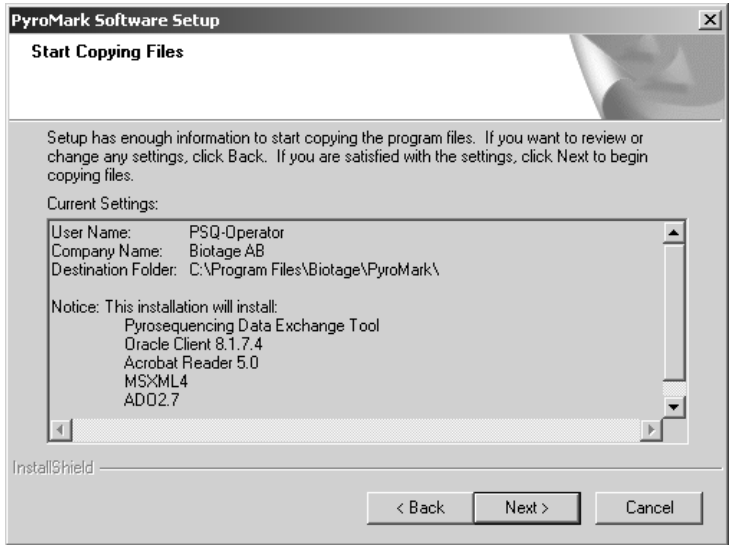
6. Ensure that the operator's computer is logged on using an Administrator account.
7. Insert the QIAGEN software CD into the CD-drive of the computer.
8. Follow the instructions on the installation wizard that will start automatically. Should the wizard fail to start, click "Run" in the Windows "Start" menu, specify the path to the CD-drive and type "autorun.exe".
9. When the installation wizard has started, click "Next" to proceed with the installation. Click "Yes" to accept the license agreement.
10. Enter "User Name" and "Company Name". Using the radio buttons, select who the application should be installed for.



11. Select the location for the files. The default location is C:\Program Files\Biotage\PyroMark. Click "Next" to proceed.

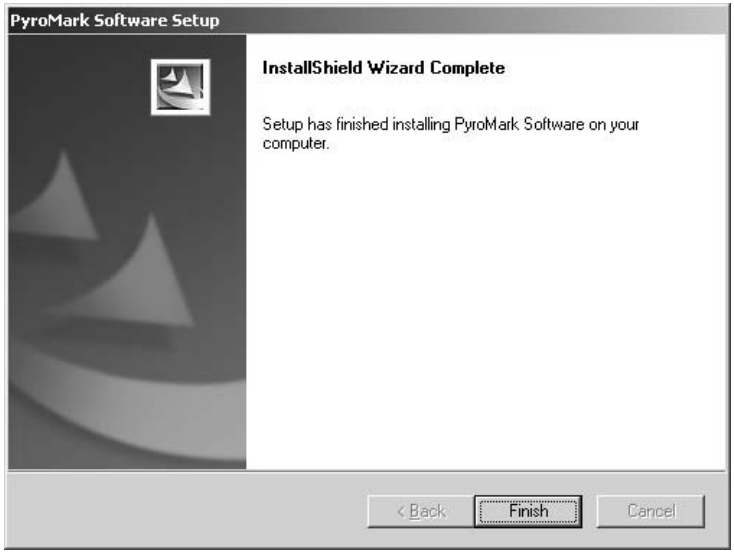


12. Review the installation settings, then click "Next" to start the installation.

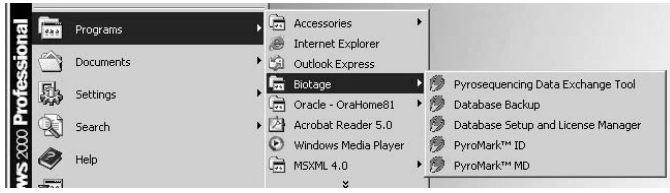


13. The installation wizard shows the progress of the installation. If the dialog box "Shared File Detection" is shown, check the box "Don't display this message again" and click "Yes" to continue the installation.

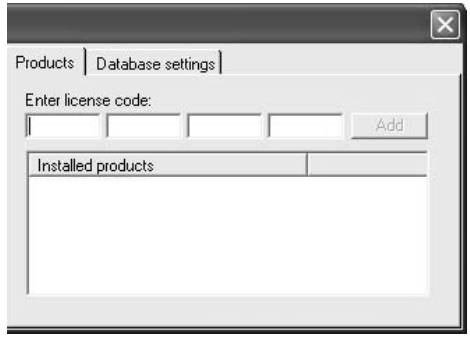
14. Click “Finish” to close the wizard.



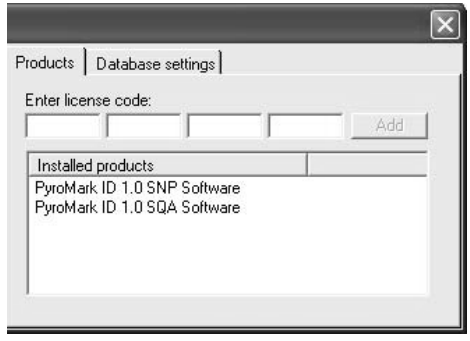
15. The Pyrosequencing software should appear in the Windows “Start” menu.



16. Run “Database Setup and License Manager”. On the “Products” tab, enter the license key(s) enclosed with the CD. Click “Add” for each license.



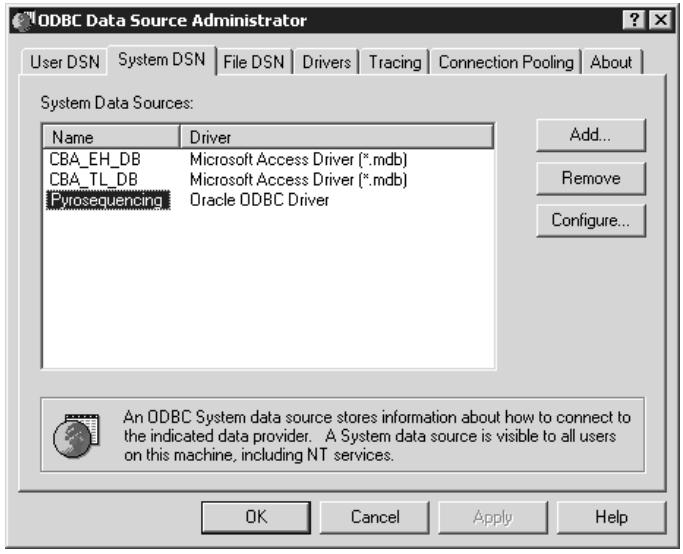
17. When the license keys have been entered, the PyroMark Q96 MD Software is enabled. To remove the software, click on the software name.



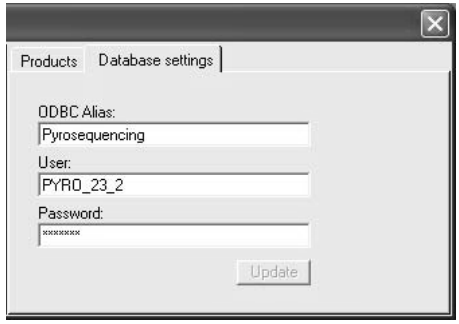
18. Setup the database, see Section 6.3.

6.3 Setting up the database

1. Configure the database access using Oracle. See the instructions included in the documentation from Oracle.
2. Open the "ODBC Control Panel (Administrator Tools/Data Source)" and add the Oracle ODBC Driver (which can be found under "Oracle" in "OraHome81"). Configure the data source by entering the name that was defined at the original installation ("Pyrosequencing" in the illustration, next page).



3. Run “Database Setup and License Manager” from the “Pyrosequencing” menu. Click on the “Database Settings” tab.



4. On the “Database Settings” tab, enter the “ODBC Alias”, defined in the “ODBC Control Panel” (see step 2).
5. The default “User” name and “Password” are already entered. Update these parameters only if they were changed during database setup.
6. Click “Update”.

6.4 Data backup

All data are saved in the Oracle database located on the operator's computer or on a server on the LAN. Good data management requires that data backups are made on a regular basis. The Oracle software includes a backup tool.

Note: QIAGEN is not responsible for the setup and administration of the user's backup routine.

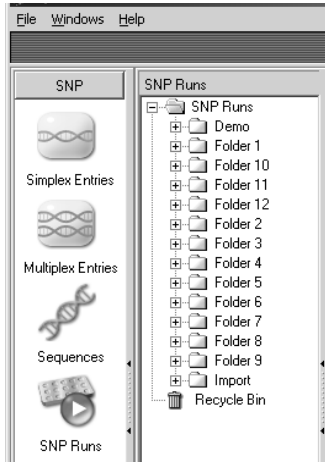
6.5 Installation testing

Stand-alone systems and LAN systems should be tested after installation using the appropriate test method (see Sections 6.5.1 and 6.5.2). This test will check the main functions of the hardware and software. If the test functions correctly, the PyroMark Q96 MD can be used for running samples.

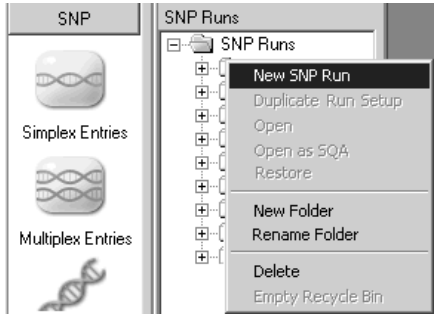
6.5.1 Installation testing (automated instrument)

Procedure:

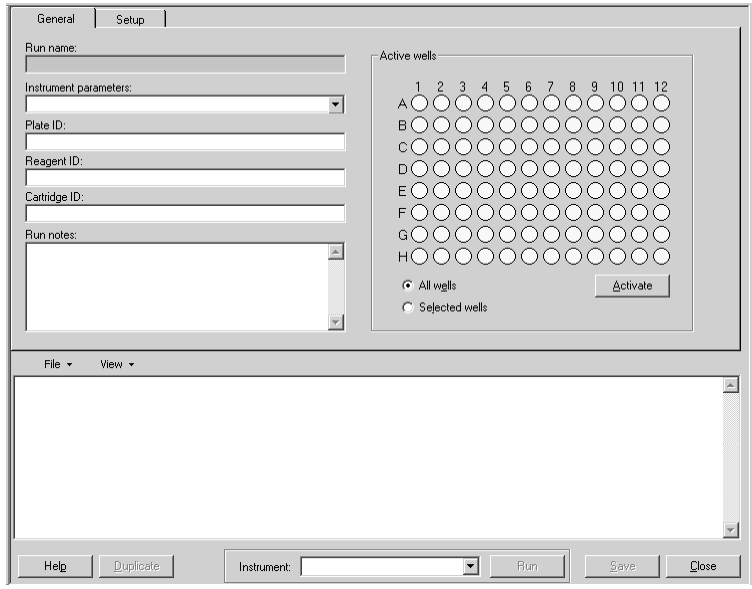
1. Start the operator's computer. Microsoft Windows starts and displays the welcome screen.
2. Press "Ctrl" + "Alt" + "Delete" and log on as the Administrator. No password is required. The Windows desktop will appear.
3. Start the PyroMark Q96 MD with the standby switch on the front panel. All lights on the front panel will illuminate. The instrument software is loaded onto the PyroMark Q96 MD. This takes 1–3 min. The yellow "Busy" light on the instrument will then go out and the yellow "Information" light will start to flash. The instrument is now ready to receive instructions from the operator's computer.
4. Start the PyroMark Q96 MD Software: Choose Programs/Biotage/PyroMark Q96 MD from the Windows "Start" menu.
5. Log on as user "system" with the password "manager". The main application window is displayed.
6. Choose "SNP/SNP Runs" from the main menu.



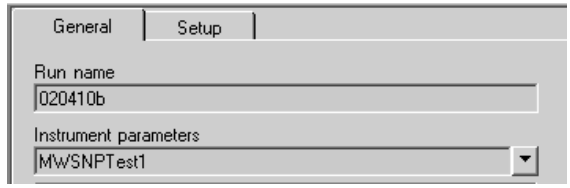
7. Right-click in a folder in the tree-view and select "New SNP Run".



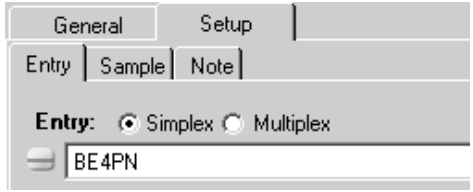
The "Run Setup" window will open.



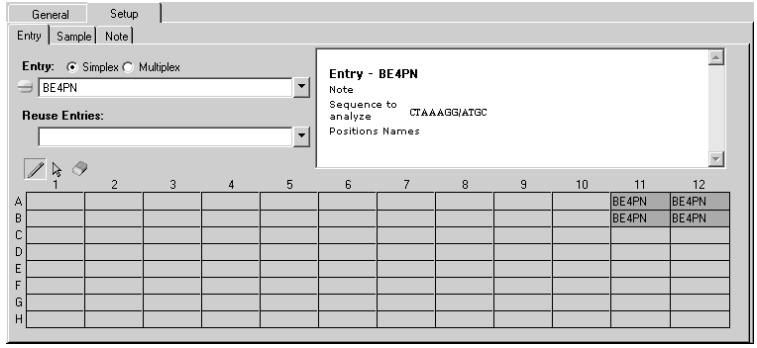
8. Enter a “Run Name”.



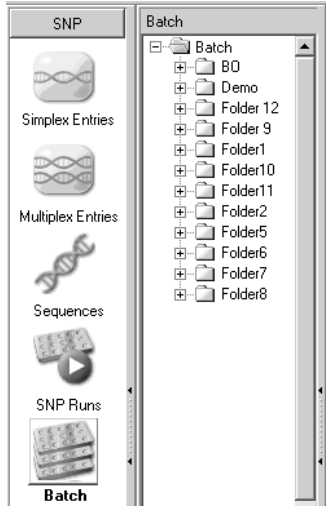
9. Click on the “Setup” tab. Under “Entry”, select the SNP entry “ BE4PN” from the list.



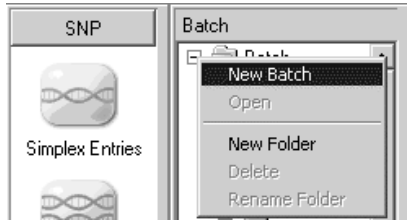
Use the pen tool and "paint" the SNP entry over the four wells A11, A12, B11, and B12.

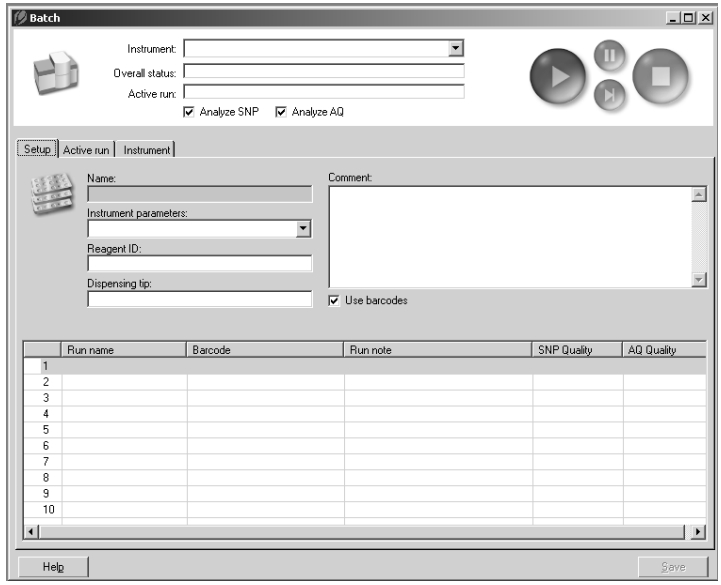


10. Click "Save" at the bottom of the "Setup Run" dialog box to save the run setup in the database. Click "Close" to close the window.
11. Choose "SNP/Batch" from the main menu.



12. Right-click in a folder in the tree view and click “New Batch”. The “Batch” window will open.

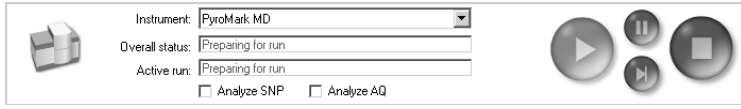




13. Select the instrument connected to the operator’s computer from the “Instrument” list.
14. Enter a “Name” and select “Test Parameters HS” from the “Instrument parameters” list.
15. Choose “SNP/SNP Runs” from the main menu.
16. Drag the “SNP Run” made previously to the “Run name” list in the “Batch” window.
17. If using bar-coded plates, check the box “Use barcodes”.
18. Choose “Batch/Checkpoint”, complete 1 and check “Give a warning”.
19. Click “Save” at the bottom of the “Batch” window.

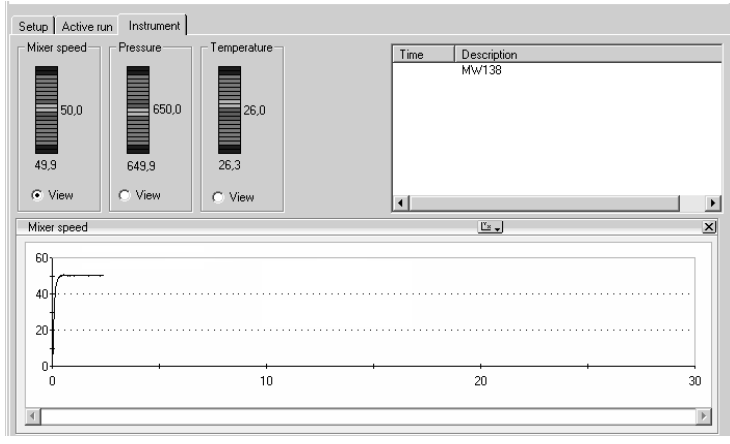
20. Take out the stacker. Pull out the metal stop and insert an empty PyroMark Q96 HS Plate. Replace the metal stop and reinsert the stacker into the instrument.
21. Open the instrument lid.
22. Open the dispensing unit cover and insert an empty dispensing tip holder.
23. Close the dispensing unit cover and press the latch down into its locked position.
24. Close the instrument lid.
25. Click “Run” and check that the run starts. The flashing “Information” light will go out and the “Busy” light will illuminate. If the text, “No connection” appears in the “Status Bar” and the “Information” light is still flashing, check that the Ethernet cable is properly connected to the instrument and the operator’s computer.
26. Check robot actions in the “Overall status” field in the “Batch” window.

    The robot should count the number of PyroMark Q96 HS Plates, fetch a plate and move it to the process chamber.
27. Check that the run continues with dispensing activities. Text in the “Active run” bar will show the status of the run.

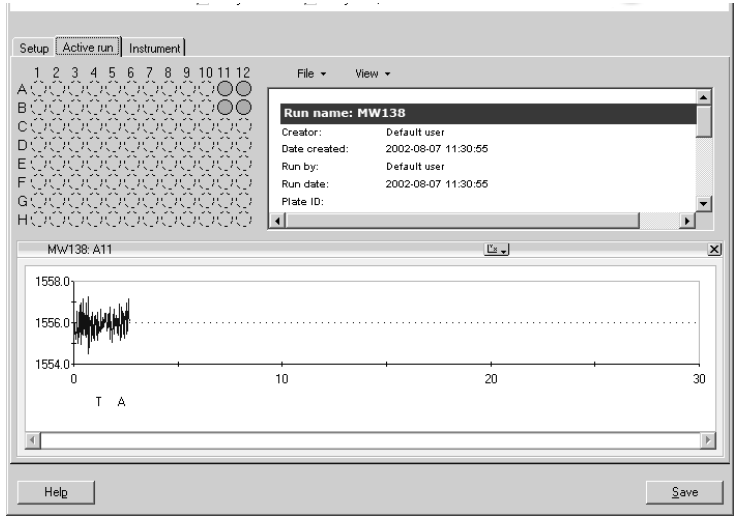


28. Click the “Instrument” tab to see parameters.

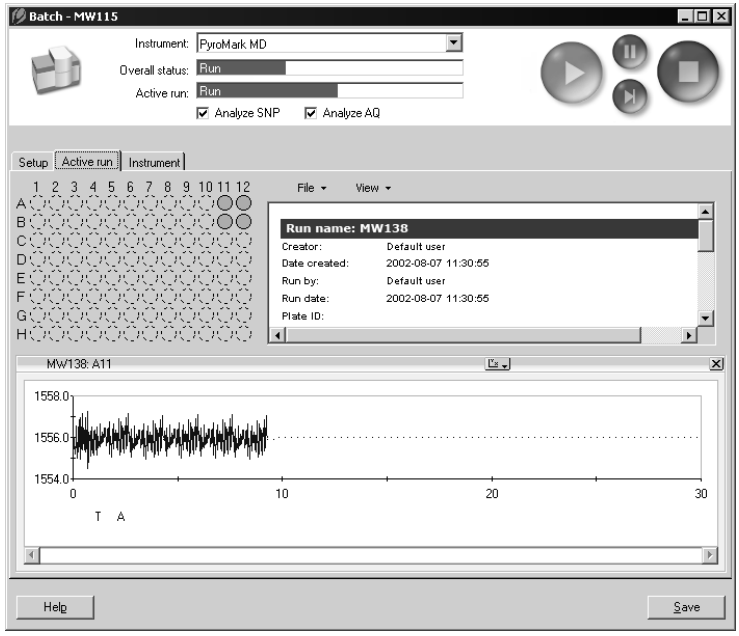
    The heating block temperature, mixer, and pressure will stabilize to preset values.



29. Click the “Active run” to see the wells.



30. When the dispensation of enzyme and substrate is complete, the pressure will increase to the nucleotide pressure value defined in “Instrument Parameters” and dispensation of the nucleotides begins.



31. Function test: Open the instrument lid.
32. There will be an audible warning signal and the yellow “Information” light will illuminate. A message box is displayed.
33. Take out the stacker.
34. Reinsert the stacker.
35. Open the process chamber lid.
36. The X-Y table should move at a rate of about 1 well every 0.6 s; the pneumatic dispensation system should deliver and air pulse every 0.6 s; the heating block should oscillate (vibrate).
37. Close the process chamber lid and the instrument lid. The “Information” light on the instrument will go out and the audible warning signal will cease.

38. Open the instrument lid.

    There will be an audible warning signal and the yellow "Information" light will illuminate. A message box is displayed.

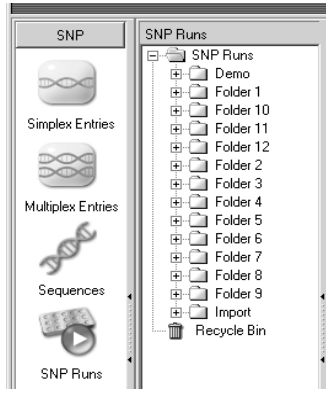
39. Open the process chamber lid using the software. Choose "Instrument/PyroMark Q96 MD" from the main menu and click "Open".
40. Click "Close". The process chamber lid will close.
41. Click the "Stop" button at the top of the "Batch" window. Click "Stop now" to confirm termination of the run. The run will be stopped immediately and data will be saved.
42. A "Batch complete" message is displayed in the "Overall status" field in the "Batch" window.

6.5.2 Installation testing (non-automated; PyroMark Q96 MD)

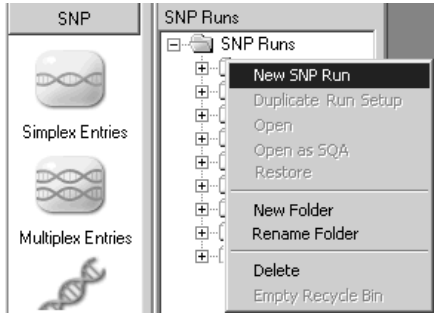
Procedure:

1. Start the operator's computer. Microsoft Windows starts and displays the welcome screen.
2. Press "Ctrl" + "Alt" + "Delete" and log on as the Administrator. No password is required. The Windows desktop will appear.
3. Start the non-automated PyroMark Q96 MD with the electric switch on the front panel. All lights on the front panel will illuminate. The instrument software is loaded onto the PyroMark Q96 MD. This takes 1–3 min. The yellow "Busy" light on the instrument will then go out and the yellow "Information" light will start to flash. The instrument is now ready to receive instructions from the operator's computer.
4. Start the PyroMark Q96 MD Software: Choose Programs/Biotage/PyroMark Q96 MD from the Windows "Start" menu.
5. Log on as user "system" with the password "manager". The main application window is displayed.

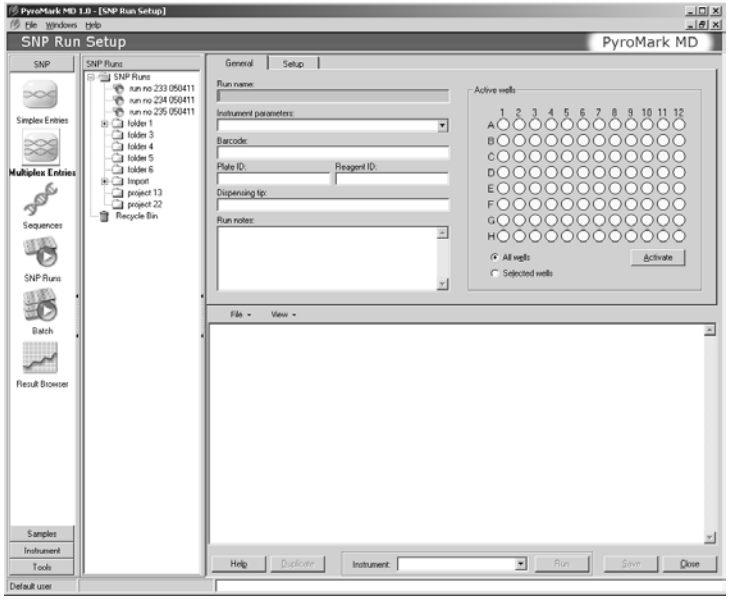
6. Choose “SNP/SNP Runs” from the main menu.



7. Right-click in a folder in the tree-view and select “New SNP Run”.

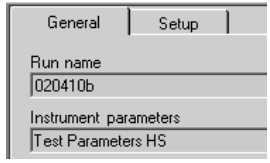


The “Run Setup” window will open.

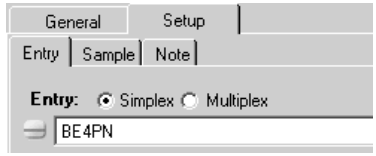


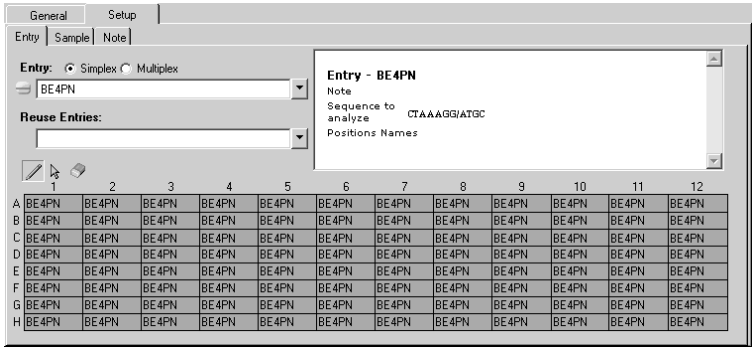
8. Select the instrument connected to the operator's computer from the "Instrument" list at the bottom of the "Run Setup" window.

9. Enter a "Run name" and select "Test Parameters HS" from the "Instrument Parameters" box.



10. Click on the "Setup" tab. Under "Entry", select the SNP entry "BE4PN" from the list. Use the pen tool to "paint" the SNP entry over all wells.

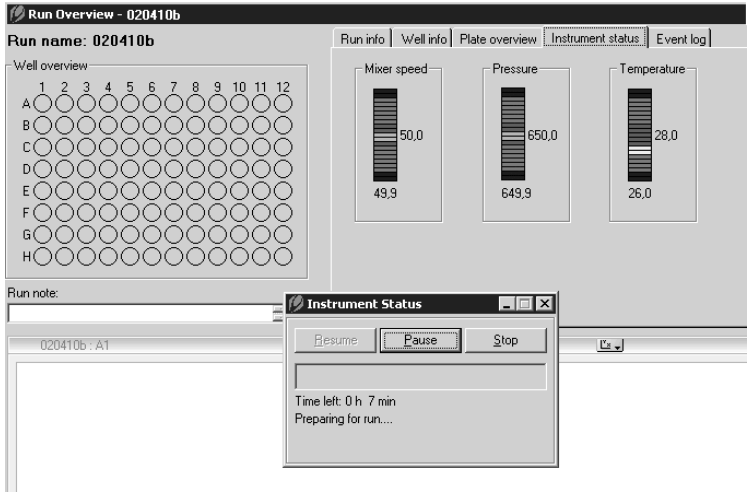




11. Click “Save” at the bottom of the “Setup Run” dialog box. The run setup is saved in the database.
12. Open the instrument lid.
13. Open the process chamber lid and hold it open.
14. Place an empty PyroMark Q96 HS Plate on the heating block and press to open the latch. When the plate is in position, release the latch.
15. Close the process chamber lid.
16. Open the dispensing unit cover and insert an empty dispensing tip holder.
17. Close the dispensing unit cover and press the latch down into its locked position.
18. Close the instrument lid.
19. Click “Run” and check that the run starts. The flashing “Information” light will go out and the “Busy” light will illuminate. If the text, “No connection” appears in the “Status Bar” and the “Information” light is still flashing, check that the Ethernet cable is properly connected to the instrument and the operator’s computer.
20. Check that the run continues with dispensing activities. The “Run Overview” window opens showing the “Instrument Status” dialog on top of it.

    The heating block temperature, mixer, and pressure will stabilize to preset values.

    When these values are reached, dispensation of the enzyme (beige) and substrate (purple) begins.

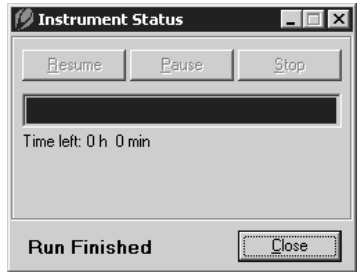


21. When the dispensation of enzyme and substrate is completed, the pressure will increase to the nucleotide pressure value defined in Instrument Parameters. The "Instrument Status" changes to "Run" and dispensation of the nucleotides begins.
22. Function test: Open the instrument lid.

    There will be an audible warning signal and the yellow "Information" light will illuminate. A message box is displayed.
23. Open the process chamber lid.
24. The X-Y table should move at a rate of about 1 well every 0.6 s; the pneumatic dispensation system should deliver an air pulse every 0.6 s; the heating block should oscillate (vibrate).
25. Close the process chamber lid and the instrument lid. The "Information" light on the instrument will go out and the audible warning signal will cease.
26. Click on each well ion the "Well Overview". The intensity level in the Pyrogram should be approximately 32,700 for all wells.
27. Click the "Stop" button in the "Instrument Status" window. Click "Yes" to confirm termination of the run.

    The run will be stopped and data saved immediately.

28. “Instrument Status” changes to “Run Finished”. Click “Close” to close the “Instrument Status” window.



7 Maintenance

The following maintenance procedures must be carried out to ensure reliable operation of the PyroMark Q96 MD:

- Regular performance checks
- Cleaning of the instrument

Following these procedures ensures that the PyroMark Q96 MD is free of dust and liquid spills.

Before undertaking maintenance procedures, it is recommended that you familiarize yourself with the safety information by referring to Section 1.

Important: Disconnect the instrument from mains power before cleaning.

Servicing

The PyroMark Q96 MD is supplied with a warranty that lasts for 1 year, beginning on the date of shipment. The warranty includes all repairs due to mechanical breakdown. Application development, software upgrades, accessories, and disposable items are not included in the warranty.

QIAGEN offers comprehensive Service Support Agreements, including Warranty Extensions, Full Cover Support Agreements, and instrument/application training, including on-site installation and annual preventative maintenance. Service Support Agreements maximize productivity and ensure high performance from your instrument. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Field Service Specialist or your local distributor for more information about flexible Service Support Agreements from QIAGEN.

7.1 Shutdown procedure

Before carrying out maintenance, ensure the PyroMark Q96 MD is shutdown using the following procedure:

1. Choose “Instrument” from the main menu.

2. Click on the “Manage” icon. The “Manage Instruments” dialog appears.
3. Select the instrument, which is connected to the operator’s computer and click “Shut down”.
4. Click “Yes” to confirm shutdown of the selected instrument. Error messages will appear if the selected instrument is not connected or the selected instrument is busy.
5. All indicators on the front panel illuminate and the shutdown procedure begins. A message stating that the network connection is closed will be shown at the lower right-hand side of the screen. Shutdown is complete when the message “Now it is safe to turn off instrument” is displayed.
6. Turn off the standby switch on the front of PyroMark Q96 MD and check that the Power indicator lamp is turned off.

7.2 Resetting the robot

If the robot is not at its correct home base, it can be reset using the following procedure:

1. Choose “Instrument” from the main menu.
2. Click on the “Manage” icon. The “Manage Instruments” dialog appears.
3. Select the instrument, which is connected to the operator’s computer and click “Reset robot”.

7.3 Routine maintenance of the PyroMark Q96 MD

The PyroMark Q96 MD should be checked and cleaned regularly to remove dust and spillages.

Important points before starting:

- Avoid harsh cleaners and chemicals, and getting moisture inside the instrument
- The cleaning liquid must be applied to the cloth only

The following items are required:

- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Ethanol (70%)
- Powder-free gloves
- Clean, non-abrasive, lint-free cloths
- Flat-head screwdriver

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Open the instrument lid.
3. Clean the area around the dispensing unit and process chamber using a lint-free cloth moistened with 70% ethanol.
4. Open the process chamber lid. Clean the area around the heating block.

7.4 Cleaning the area beneath the heating block

The following items are required for cleaning the area beneath the heating block:

- Ethanol (70%)
- Clean, non-abrasive, lint-free cloths

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Loosen the bayonet screw behind the heating block by turning it ¼ turn counter-clockwise until a click is heard.
3. Lift out the heating block. If the heating block appears to be stuck, press on the surface behind the heating block and turn or move the heating block slightly.
4. Clean the area underneath the heating block carefully using a lint-free cloth moistened with 70% ethanol.
5. If necessary, clean the lens array, as described in Section 7.5.
6. Allow the area to dry before mounting the heating block.

7. Replace the heating block. Ensure the rear part of the heating block is properly seated over the contact pins. Do not force the heating block into place.
8. Turn the bayonet screw ¼ turn clockwise.

7.5 Cleaning the lens array

The following items are required for cleaning the lens array beneath the heating block:

- Ethanol (70%)
- Clean, non-abrasive, lint-free cloths

Procedure:

1. Shut down the instrument, see Section 7.1.
2. Open the instrument lid.
3. Loosen the bayonet screw behind the heating block by turning it ¼ turn counter-clockwise until a click is heard.
4. Lift out the heating block. If it is stuck, press lightly on the surface behind the heating block and turn or move the heating block slightly.
5. Loosen the four screws on the underside of the heating block and remove them.
6. Carefully insert the screwdriver into the slot, without damaging the metal parts, and lift up the lens array.
7. Clean the flat side of the lens array carefully using a lint-free cloth moistened with 70% ethanol.
8. Ensure that the four washers are placed over the holes for the screws.
9. Replace the lens array with the flat side inwards and replace and tighten the four screws.
10. Place the heating block in the instrument. Ensure that the rear part of the heating block is properly seated over the contact pins. Do not force the heating block into place.
11. Turn the bayonet screw ¼ turn clockwise.

7.6 Cleaning in the robot compartment

The robot compartment should only be cleaned if something has been spilled in the compartment.

Important: Do not use liquid in the robot compartment, as the electronics may be damaged. If absolutely necessary, moisten a lint-free cloth with high-purity water.

Procedure:

1. If the robot is not at its correct home base, reset it according to Section 7.2.
2. Shut down the instrument, see Section 7.1.
3. Turn the electric switch off and disconnect the mains cable.
4. Open the instrument lid.
5. Press the button on the robot compartment and open the lid.
6. Take out the stacker.
7. Remove any dropped plates, as necessary.
8. If the robot stand or robot gripper arms require drying, this should be performed with great care as the calibration settings of the robot may be altered.
9. Clean the area using a dry lint-free tissue. If absolutely necessary, moisten the lint-free tissue with high-purity water.

7.7 Removing dispensing tips from the dispensing tip holder

Remove dispensing tips from the dispensing tip holder by pulling apart the springs on the dispensing tip holder and taking out the two RDTs and the four NDTs/CDTs.

7.8 Cleaning the dispensing tips

The following items are required:

- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Powder-free gloves
- Lint-free cleanroom tissues (cleanroom class 100)

7.8.1 Re-using and cleaning RDTs and CDTs

Re-using RDTs and CDTs

If the RDTs and/or CDTs are to be reused, they must be cleaned immediately after a run. There are two cleaning procedures:

- Standard cleaning, see page 7-6
- Thorough cleaning, see page 7-7

It is recommended that RDTs and CDTs are used a maximum of 20 times.

To ensure that RDTs and CDTs are reusable, perform an RDT/CDT dispensing test (Section 7.9.1) or a PyroMark Q96 HS Dispensing Tips dispensing test (Section 7.9.2) before the run.

Standard cleaning of RDTs and CDTs

1. Remove the RDTs/CDTs from the dispensing tip holder (Section 7.7), mark them for reuse in the same position and place in an empty dispensing tip holder.
2. Discard any remaining solutions.
3. Fill the RDTs/CDTs with high-purity water. Discard the water. Repeat this step 3 times.
4. Fill RDTs and CDTs to the top with high-purity water. Check for obstruction by pressing on the top of the compartment with a finger (wear gloves). Ensure tip heads are clear. A spray or droplet should be visible at the edge of the tip head.

   If there is a blockage, wet the tip heads with a drop of high-purity water placed on a finger and repeat the test.
5. Press several droplets through each tip head.

6. Discard the water.
7. Disassemble the dispensing tip holder according to the instructions in Section 7.7.
8. Leave the dispensing tip holder to dry.

   Note: Ensure that the tip heads are dry on the outside before using the RDTs/CDTs.

Thorough cleaning of RDTs and CDTs

If solutions have been left in the RDTs/CDTs, a more thorough cleaning process may be required to clear the tips.

1. Remove the RDTs/CDTs from the dispensing tip holder (Section 7.7), mark them for reuse in the same position and place in an empty dispensing tip holder.
2. Discard any remaining solutions.
3. Fill the RDTs/CDTs with high-purity water. Discard the water. Repeat this step 3 times.
4. Fill the RDTs/CDTs with high-purity water and immerse them in a beaker containing enough high-purity water to cover the tips. Leave them for 1 h.
5. Follow the standard cleaning procedure above from step 3.

7.8.2 Re-using and cleaning NDTs

Re-using NDTs

NDTs with solutions can be stored in the dispensing tip holder in the PyroMark Q96 HS Dispensing Tip Holder Box for up to 24 hours at 4°C.

For re-use within 2 hours, leave the Dispensing Tip Holder in the instrument with the dispensing unit cover closed. If needed, add more reagents before the next run. Perform a dispensing test before the next run to check the dispensing tips.

For re-use more than 2 hours later, remove and clean both the RDTs and NDTs/CDTs directly after use.

It is recommended that NDTs are used a maximum of 10 times.

To ensure that NDTs are reusable, perform a tip holder dispensing test (Section 7.9.2) before the run.

Important: Always wear a lab coat and powder-free gloves when handling NDTs to avoid particles in the tip heads. Handle the dispensing tips with care and store in the boxes as much as possible.

Cleaning NDTs

Important: During the cleaning procedure, do not bring tip heads into contact with water (often a result of over-filling the NDTs during cleaning). Water contact may degrade the hydrophobic coating.

1. Remove the RDTs from the dispensing tip holder (Section 7.7), mark them, and place in an empty dispensing tip holder.
2. Discard any remaining solutions from the NDTs.
3. Fill the NDTs carefully with high-purity water. Discard the water. Repeat this step 2 times.
4. Remove the NDTs from the dispensing tip holder and mark them for re-use in the same positions.
5. If a tip head came into contact with water, press the tip head gently against the lint-free tissue at the bottom of the PyroMark Q96 HS Dispensing Tip Storage Box.
6. Leave the dispensing tip holder to dry. To avoid particle contamination, leave the NDTs to dry lying on a lint-free cleanroom tissue in the PyroMark Q96 HS Dispensing Tip Storage Box for at least 3 h before the next run.

7.9 Dispensing tests for the dispensing tips

7.9.1 RDT and CDT dispensing test

Perform the following test to check whether RDTs and CDTs can be used for analysis.

Important: Do not use this test for NDTs.

1. In the assembled dispensing tip holder, completely fill the RDTs/CDTs with high-purity water. Take care not to allow the water to run over the edge of the tips and wet the tip heads on the outside.
2. Press firmly on top of each RDT/CDT with a finger (wear gloves). A small jet of water should squirt from the RDT/CDT. If no water comes out, fill the RDT/CDT with water and repeat the process.
3. Check that the water jet is straight in the direction of the RDT/CDT and not bent. If not, repeat the test, but press more firmly on the top of the RDT/CDT.
4. If the test fails, discard the RDT/CDT.

7.9.2 Dispensing test for PyroMark Q96 HS Dispensing Tips

This test can also be performed in order to test re-used tips before a new run.

See “Filling dispensing tips and assembling the dispensing tip holder”, page 5-22 for instructions on filling dispensing tips.

1. Place two RDTs and four NDTs/CDTs in a dispensing tip holder.
2. Fill the NDTs/CDTs with at least 30 µl of each nucleotide solution or according to the displayed volume information.
3. Fill the RDTs with at least 50 µl of each reagent or according to the displayed volume.
4. Load the dispensing tip holder into the instrument.
5. Cover an empty plate with transparent tape and load into the instrument.

6. In the software main menu, choose “Instrument” and then “Manage”.
7. Click “Test”
8. Click “OK”. The instrument will dispense from each dispensing tip. After a minute, a window will appear stating “Test of dispensing tips ready”.
9. Click “OK”.
10. Open the lid and remove the test plate.
11. Check for liquid on the wells D5, D8, E5, E6, E7, and E8. If one or several droplets are missing, replace the corresponding dispensing tip and redo the test.

Well	Solution
D5	Enzyme
D8	Substrate
E5	A
E6	C
E7	G
E8	T

12. After the test procedure, remove the dispensing tip holder from the instrument and check if there is liquid on the outside of any of the NDT/CDT tip heads. Any NDTs/CDTs with liquid on the outside must be discarded.

7.10 Changing the fuses

Ensure that the PyroMark Q96 MD is disconnected from the mains power before removing fuses from the instrument.

The following item is required:

- Small flat-head screwdriver

If the power indicator is not on, even though the power cable is connected and the power is switched on, proceed as follows:

1. Shut down the instrument, see Section 7.1.
2. Disconnect the mains power cable.
3. Check that the mains power outlet is live by connecting a desk lamp or other apparatus to the power outlet and checking that it functions as normal. If the outlet is live, the fuses in the PyroMark Q96 MD should be checked as described in steps 4–9.
4. If necessary, carefully turn the instrument, so that the rear panel can be easily accessed.
5. Use a small screwdriver to release the securing lugs on each side of the fuse holder.
6. Pull out the fuse holder using your fingers. The fuse holder contains two fuses (type F 6.3 A H, 250 V). Check both fuses and replace as necessary.

   Note: Replace fuses only with fuse of the specified type and current rating.
7. Insert the fuse holder (it is keyed and can only be replaced in one way) and push it into place using your fingers.
8. Reposition the instrument, reconnect the power cable and turn the standby switch on.
9. If the instrument still does not work, please contact QIAGEN Technical Services.

7.11 Cleaning the PyroMark Q96 Vacuum Workstation

WARNING	**Hazardous chemicals** [W4]
	The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin. Always wear safety glasses, 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 not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents. For more information, visit www.qiagen.com/safety. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

If the Vacuum Workstation needs to be cleaned to remove dust and spillage, follow the instructions below.

The following items are required:

- Powder-free gloves
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- A mild detergent (if necessary)
- Clean, lint-free cloths.

Procedure:

1. Ensure that no vacuum is applied to the vacuum prep tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Clean the worktable and the tool, except for the filter probes, using a clean, lint-free cloth moistened with water or a mild detergent.

Note: Do not touch the tips of the filter probes.

4. Wipe the worktable and the tool, except for the filter probes, dry using a clean, lint-free cloth.
5. Reconnect the vacuum pump to the mains power.

7.12 Testing and replacing the filter probes

7.12.1 Function test for filter probes

Before using the PyroMark Q96 Vacuum Workstation, check that the filter probes are working by performing the function test as follows:

1. Add 100 µl high-purity water to each well of a 96-well PCR plate.
2. Fill a trough with 70 ml high-purity water.
3. Start the vacuum pump
4. Apply vacuum to the vacuum prep tool by opening the vacuum switch.
5. Lower the filter probes into the trough. Keep them in position for approximately 20 s. Ensure that the water is transferred to the waste container, i.e., that vacuum has been applied. If this has not happened, check the connections.
6. Lower the filter probes into the PCR plate and check that the water is aspirated evenly across all wells and that all wells are empty after a maximum of 10 s.
7. If the wells are not empty after 10 s, repeat the procedure from step 1. If the function test fails twice, replace the filter probes (see Section 7.12.2).

7.12.2 Replacing filter probes

Each filter probe can be replaced individually. To ensure proper flow rate trough the filter probes, all probes should be replaced after preparation of approximately 100 plates.

Note: Use gloves (powder-free) to avoid contaminating the filter probes.

The following items are required:

- Powder-free gloves
- Allen key
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- New filter probes (cat. no. 979010)

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Remove the tool from the tubing.
4. Loosen the four screws using an Allen key.
5. Pull out the old filter probes.
6. Gently insert new filter probes without pressing on the filter tips.
7. Replace and fasten the four screws and reconnect the vacuum pump to the mains power.

7.13 Replacing the tubing

If the tubing is broken or distorted, replace it.

Be sure to observe all federal, state, and local environmental regulations for the disposal of laboratory waste.

The following items are required:

- New tubing (contact QIAGEN Technical Services)
- Beaker

Procedure:

1. Ensure that no vacuum is applied to the Vacuum Prep Tool, i.e. the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Disconnect the vacuum pump from the mains power.
3. Remove the broken tubing at one end and empty any liquid waste into an empty beaker.
4. Disconnect the other end of the tubing and discard the tubing and any liquid waste.
5. Cut the new vacuum tubing into three pieces and assemble it. Ensure that the tubing is connected to the pump's vacuum fitting.
6. Reconnect the vacuum pump to the mains power.

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8 Troubleshooting

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

When calling QIAGEN Technical Service 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

Take the following action before contacting QIAGEN Technical Services.

1. Check important parameters in the “Run Overview” in the “Instrument Status” tab to assess if the system was working properly during the run.
2. Consult the Troubleshooting sections below.

8.1 Environmental parameters

8.1.1 Reading parameter values

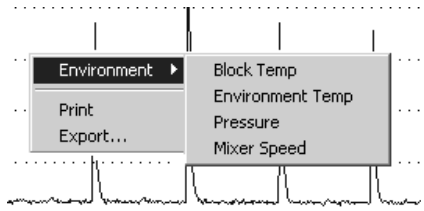
Click the “Instrument” tab to view the environment parameters. For the non-automated instrument, click “Instrument Status” tab. Current values are displayed on the middle of the scales, while the optimum values are displayed to the right of the scales.

The parameters are described below:

Parameter	Description
Mixer speed (35 Hz)	The mixer shakes the PyroMark Q96 HS Plate continuously during dispensation to ensure rapid mixing of the well contents. The number of movements per second is displayed in Hz.
Temperature (28°C)	The temperature of the heating block.
Pressure (400 mbar/enzyme and substrate; 650 mbar/dNTP	The current dispensing (air pulse) pressure.

8.1.2 Viewing and printing environmental parameters

1. For the automated PyroMark Q96 MD, click "View" below the scale to view the parameter as a graph.
2. For the automated PyroMark Q96 MD, right-click in the Pyrogram view to display the following pop-up menu:



3. For the automated PyroMark Q96 MD, select "Environment" and choose the desired parameter to view as a graph.

   For both automated and non-automated instruments:

4. To print a graph, right-click and select "Print". The graph will be printed on the printer which is selected as default in Windows.

5. To export the graph, right-click and select “Export”. You can export the graph in different image formats.

8.2 Unexpected results

		Comments and suggestions
a)	No result in any wells of the plate	Dispensing tip holder is not correctly filled with reagent and/or nucleotides. Check the dispensing tip holder and ensure the dispensing unit cover is closed.
		Reagents dissolved in an incorrect solution.
		Obstructed or damaged RDTs and/or NDTs/CDTs.
		Insufficient sample or incorrectly conditioned sample (e.g., too much dsDNA or salt present). Follow sample preparation instructions carefully, see Section 5.3.5.
		Check the PCR samples using a gel technique to confirm there is only one specific band.
b)	Results only in some wells of the plate	Insufficient sample amounts. Follow sample preparation instructions carefully, see Section 5.3.5.
		Wells marked in the software do not correspond to the sample placement in the PyroMark Q96 HS Plate. Check the plate was loaded according to the information under the “Entry” and “Sample” tabs in the “Run Setup” dialog.
		Insufficient enzyme or substrate for all the wells. Check that the dispensing tips were filled according to the “Volume Information”.
		One of the RDTs and/or NDTs or CDTs in the dispensing tip holder is blocked or damaged. Replace or rinse the dispensing tip, see Section 7.8.

8.3 Sequence errors

	Comments and suggestions
a) Some peaks appear, but the sequence stops	One of the NDTs or CDTs in the dispensing tip holder is not filled with nucleotide. One of the NDTs or CDTs in the dispensing tip holder is blocked or damaged. Replace or rinse the dispensing tip, see Section 7.8. Check the sample and sample preparation. Contaminated samples will lead to an unusually high consumption of substrate at substrate addition, giving a high presequencing signal and no light produced at nucleotide dispensation.
b) Faulty or poor sequence	Contaminated or nonhomogeneous samples. Follow sample preparation instructions carefully, see Section 5.3.5. Reagents incorrectly stored or dissolved. Too much or too little template. Wrong sequencing primer added. Wrong sequence to analyze entered. Wrong dispensation order chosen.

8.4 Peak errors

	Comments and suggestions
a) Wide peaks	Assay-specific problem due to secondary structure.
	Contaminated or nonhomogeneous samples. Follow sample preparation instructions carefully, see Section 5.3.5.
	Reagents incorrectly stored or dissolved.
	Too much template DNA. Check amount of DNA template used.
	Reaction volume in wells was too high. Use lower volume.
b) Nonspecific peaks	Sequence signals from the self-annealed sequencing primer and/or biotinylated PCR primer and DNA template or unspecific annealing of the sequencing primer.
	Run DNA template, sequencing primer, and biotinylated PCR primer in individual wells. If significant peaks appear, consider redesigning the PCR and/or sequencing primer(s).
	Contaminated nucleotides.
	Background of contaminating sequence from PCR. Consider redesigning sequencing primer.
	Contact QIAGEN Technical Services if this problem persists.

		Comments and suggestions
c)	Low signal	Too little template. Reagents incorrectly stored or dissolved. Too much salt in the sample. Follow sample preparation instructions carefully (see Section 5.3.5.) and optimize or redesign the PCR. Dirty lens array. Clean the lens array, see Section 7.5.
d)	Drifting baseline	CCD camera temperature is unstable. Contact QIAGEN Technical Services.
e)	Occasional peaks between dispensations	Cross-talk or dispensation errors. Avoid placing assays with high signals close to assays with low signals. Check and replace the dispensing tip holder, if necessary. Light leaking into the instrument. Do not place the instrument in strong sunlight. Contact QIAGEN Technical Services for camera calibration.

8.5 Instrument-related errors

		Comments and suggestions
a)	No contact between the operator's computer and the PyroMark Q96 MD	Check mains power supply to instrument and ensure the "Power" light is illuminated. Check the network cables are properly connected at the rear of the instrument and the wall outlets. If contact was broken during a run, a restore of the run may be necessary. No match between IP numbers for the instrument and the operator's computer or the IP number conflicts with other equipment on the network. Contact your system administrator.
b)	All lights on the PyroMark Q96 MD remain on 5 minutes after it has been switched on	Instrument software problem. Contact QIAGEN Technical Services.
c)	Heating block temperature control is not working correctly	Remove and reinsert the heating block, see Section 7.4. If there is liquid between the heating block and the instrument, clean the lens array, see Section 7.5. If the problem persists, the contact pins may need replacing. Contact QIAGEN Technical Services.

8.6 Stacker errors

		Comments and suggestions
a)	The robotic arm gets stuck and cannot fetch a plate from the stacker	The plate stacker has come loose and prevents the robot from moving forward. Close the plate stacker lock. A plate has been dropped in the robot compartment. Remove the plate and clean the robot compartment, see Section 7.6.
b)	The robotic arm drops a plate in the robot compartment when fetching plates from the stacker	Defective plate. Use a new plate. Stacker incorrectly inserted into the instrument. Damage to the gripper arms and/or plate sensors or the stacker or plate stacker lock. Insert the stacker fully until the stop. Error in calibration of the robotic arm movement. Contact QIAGEN Technical Services. A lid has been placed on the plate. Remove lid and retry.
c)	The plate is incorrectly placed on the heating block and/or gets stuck in the heating block	Defective plate. Use a new plate. Check the heating block is correctly mounted in the instrument. Damage to the gripper arms and/or plate sensors. Error in calibration of the robotic arm movement. Contact QIAGEN Technical Services.
d)	The robotic arm is not at the correct home base at the start	Power failure or mechanical error. When the instrument is started, the robotic arm will automatically find its home base. The robotic arm can be manually reset, see Section 7.2. Contact QIAGEN Technical Services if this problem persists.

		Comments and suggestions
e)	The bar-code is read incorrectly	The bar-code is wrong, or is incorrectly placed or sized. See Section 3.8 for bar-code requirements.

8.7 Vacuum Workstation-related errors

		Comments and suggestions
a)	No vacuum is received	Turn off the vacuum pump and open the cap to the waste container to release any pressure. Close the cap and start the pump again. Empty the waste container if full. Ensure that the tubing is connected correctly and that there is no leakage.
b)	Vacuum lost during sample preparation	Ensure that the tubing is connected correctly and there is no leakage.
c)	Filter probes not working properly	Ensure filter probes are working properly; see Section 7.12.1.
d)	Liquid left in some wells in the immobilization plate	Replace the corresponding filter probes; see Section 7.12.2
e)	White remains (Sepharose beads) in the immobilization plate	If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.

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9 Glossary

Term	Description
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 Sepharose beads.
Dispensation order	Defines the nucleotides and the order in which they should be dispensed in Pyrosequencing runs.
Drop off	A continual decrease in signal 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.
Instrument methods	A method that describes physical settings for the instrument, such as mixer frequency, block temperature, and pulse time settings.

Term	Description
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) H = A, T, or C (not G) V = A, C, or G (not T) N = Any base (A, C, G, or T)
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.
Presequencing signal	As the substrate is dispensed into a well (indicated by an "S"), contaminants (such as ATP or PPi) will be converted to light. Too high a substrate peak indicates that high levels of a contaminant might be present in, for example, buffers.
Pyrogram	The graph resulting from a sequencing reaction performed using Pyrosequencing technology. Each incorporated nucleotide is shown as a peak in Pyrogram.
Reference peak	Nonvariable peaks, i.e. peaks that are not a part of an SNP site, are referred to as "reference peaks". Reference peaks are used in the analysis both as references when calculating the single peak height, and as internal controls when assessing the quality.

Term	Description
RLU	Relative Light Unit (entity used in Pyrosequencing to define peak heights in Pyrogram).
Sepharose beads	Streptavidin-coated beads can be used for preparation of biotinylated PCR products.
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 sample preparation. The 3'-end of the sequencing primer serves as the starting point for the extension by the polymerase.
Shift	Plus shift: A small proportion of the template sequences that incorporates more than one type of nucleotide at a time (if, for example, 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.

Term	Description
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.
Theoretical outcome	The possible genotypes for a specific polymorphic position are calculated on the basis of the sequence to analyze and the dispensation order.

Appendix A

Technical data

QIAGEN reserves the right to change specifications at any time.

Environmental conditions

Operating conditions: PyroMark Q96 MD

Power	100–120 V, 220–240 V; 50–60 Hz Power consumption: 280 VA max.
Overvoltage category	II
Air temperature	18–28°C (64–82°F)
Relative humidity	30–90% relative humidity
Altitude	Up to 2000 m (6500 ft.)
Place of operation	For indoor use only Draft-free location, not close to window. Keep instrument out of direct sunlight
Pollution level	2
Environmental class	3K2 (IEC 60721-3-3)

Transportation conditions

Air temperature	–25°C to 60°C (–13°F to 140°F)
Relative humidity	Max. 75% (noncondensing)

Storage conditions

Air temperature 10°C to 40°C (50°F to 104°F)

Relative humidity Max. 75% (noncondensing)

Mechanical data and hardware features: PyroMark Q96 MD

Dimensions (closed)	Width: 760 mm (29.94 in.) Height: 620 mm (24.43 in.) Depth: 650 mm (25.61 in.)
Clearance space	Width: 1010 mm (39.79 in.) Height: 730 mm (28.76 in.) Depth: 800 mm (31.52 in.)
Mass	100 kg (220.46 lb.)
Capacity	Up to 96 samples per plate, 10 plates
Chemical resistance	Instrument covers: pH 4 to pH 9, common detergents, 70% ethanol
	User accessible areas Inside the instrument: water and 70% ethanol. Liquids should not be used in the robot compartment unless absolutely necessary due to sensitive electronics

PyroMark Q96 MD Software

Operating system	Microsoft Windows XP, English version
Processor	Intel Core Duo processor (2 GHz) or higher
RAM	512 MB
Free hard disk space	200 MB
Graphics card	Supporting the resolution of the monitor
Monitor	1280 x 800 pixels, True Color (32 bit) or higher
Pointer device	Mouse or similar
Interfaces	Ethernet ports (or external USB Ethernet adaptor).

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 applicable local laws and regulations.

In the European Union the European Directive 2002/96/EC on WEEE requires proper disposal of electrical and electronic equipment when it reaches its end of life.

The separate collection and recycling of waste electrical and 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

Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to 47 CFR, 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's FCC Compliance Tests were conducted using QIAGEN-supported peripheral devices and shielded cables, such as those you receive with your system. Changes or modifications not expressly approved by QIAGEN could void the user's authority to operate the equipment.

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Appendix B

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.

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Appendix C

1 Informations de sécurité

Avant d'utiliser le PyroMark Q96 MD, il est impératif de lire attentivement ce manuel et de porter une attention particulière aux informations de sécurité. Afin de garantir un fonctionnement du système en toute sécurité et de maintenir le système en bon état de marche, il est impératif de suivre les instructions et les informations de sécurité fournies dans le présent manuel d'utilisation.

Les types d'informations de sécurité suivants sont fournis tout au long du manuel.

AVERTISSEMENT 	Le terme AVERTISSEMENT signale des situations risquant d'entraîner des **accidents corporels** dont l'utilisateur, ou d'autres personnes, pourraient être victime. Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

ATTENTION 	Le terme ATTENTION signale des situations risquant d'entraîner des **détériorations de l'appareil** ou de tout autre matériel. Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Les conseils donnés 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.

1.1 Utilisation appropriée

AVERTISSEMENT 	**Risque d'accident corporel et de détérioration du matériel** L'utilisation inappropriée du PyroMark Q96 MD peut entraîner des accidents corporels ou une détérioration de l'appareil. L'utilisation du PyroMark Q96 MD est réservée exclusivement au personnel qualifié ayant été convenablement formé. L'entretien du PyroMark Q96 MD doit être effectué uniquement par des spécialistes de l'entretien sur site QIAGEN.

Procéder à la maintenance comme décrit à la Section 7. QIAGEN® facture les réparations rendues nécessaires suite à une maintenance inappropriée.

AVERTISSEMENT 	**Risque d'accident corporel et de détérioration du matériel** L'appareil PyroMark Q96 MD est trop lourd pour être soulevé par une seule personne. Afin d'éviter tout accident corporel et toute détérioration du matériel, ne pas soulever l'appareil seul.

1.2 Sécurité électrique

Avant l'entretien, débrancher les cordons d'alimentation des prises 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'appareil 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'équipement** Lorsque l'équipement est relié à l'alimentation, les bornes peuvent être sous tension et l'ouverture de capots ou le retrait de pièces risque d'exposer des éléments sous tension.

Afin que le PyroMark Q96 MD fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants :

- Les cordons d'alimentation de l'appareil doivent être branchés sur des prises d'alimentation munies d'un conducteur de protection (terre/masse).
- Les fiches d'alimentation reliées au secteur doivent être facilement accessibles s'il est nécessaire de débrancher rapidement l'équipement du secteur.
- Utiliser uniquement les fiches et cordons d'alimentation fournis avec le système.
- Si vous remplacez les fusibles, ne les remplacer qu'avec des fusibles du même type et équivalent en voltage, comme indiqué sur le fusible

1.3 Produits chimiques

AVERTISSEMENT	**Produits chimiques dangereux**
	La solution de dénaturation utilisée avec le poste de travail sous vide contient de l'hydroxyde de sodium qui est irritant pour les yeux et la peau. 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 le lieu de travail environnant est sûr et que les opérateurs de l'appareil ne sont pas exposés à des niveaux dangereux de substances (chimiques ou biologiques) toxiques comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA *, de l'ACGIH † ou du COSHH ‡ applicables. Pour plus d'informations, voir le site www.qiagen.com/safety. L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

* OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).

† ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).

‡ COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

1.4 Dangers mécaniques

Quand l'appareil PyroMark Q 96 MD est en marche, son couvercle doit rester fermé.

AVERTISSEMENT	**Pièces mobiles** Afin d'éviter le contact avec les pièces en mouvement lorsque l'appareil PyroMark Q96 MD est en marche, son couvercle doit rester fermé. Ne pas démonter les panneaux du capot. Ils ne renferment aucune pièce réparable par l'utilisateur. En cas de problème avec l'appareil PyroMark Q96 MD, contacter immédiatement les Services techniques de QIAGEN.

AVERTISSEMENT	**Risque de pincement et d'impact** L'unité de distribution, le couvercle de la chambre de réaction et l'instrument peuvent bouger à tout moment.

AVERTISSEMENT	**Aiguilles pointues** Veuillez ne pas toucher aux aiguilles pointues situées sur les réservoirs de distribution des réactifs et sur les capillaires de distribution des réactifs.

- Le PyroMark Q96 MD a un système de verrouillage. Ce système possède des senseurs à trois endroits : le couvercle au dessus de la chambre de réaction, le couvercle principal de l'instrument, et l'unité de stockage. Ces senseurs vérifient que les composants sont correctement insérés. Un avertissement sonore se fait entendre si les composants ne sont pas correctement mis en place et le test ne peut pas démarré.
- Si un couvercle est ouvert ou si l'unité de stockage est retirée durant un test, un avertissement sonore se fait entendre et le test en cours s'arrête après la fin du séquençage.

1.5 Dangers liés à la chaleur

WARNING 	**Surface chaude** Le bloc chauffant peut atteindre des temperatures jusqu'à 80°C (176°F). Eviter de le toucher quand il est chaud.

ATTENTION 	**Risque de surchauffe** Afin de garantir une bonne ventilation, laisser un dégagement d'au moins 10 cm sur les côtés et à l'arrière de l'appareil PyroMark Q96 MD. Les fentes et les ouvertures qui assurent la ventilation de l'appareil ne doivent pas être obstruées.

1.6 Symboles sur le PyroMark Q96 MD

Symbole	Emplacement	Langue	Description
CE	Plaque signalétique à l'arrière de l'appareil	EN	Marquage CE
REF	Réactifs, solutions, cartouche, plaque	EN	Référence
	Réactifs, solutions, cartouche, plaque	EN	Limitations de température
	Plaque signalétique à l'arrière de l'appareil et de tous les autres produits	EN	Fabricant légal

Symbole	Emplacement	Langue	Description
	À l'intérieur de l'appareil	EN	Avertissement, se reporter au manuel d'utilisation.
	Type de plaque à l'arrière de l'instrument et de la boite	EN	Numéro de série
	Plaque signalétique à l'arrière de l'appareil	EN	Symbole DEEE pour l'Europe
	Plaque signalétique à l'arrière de l'appareil	EN	Label FCC de la Federal Communications Commission des États-Unis
	Plaque signalétique à l'arrière de l'appareil	EN	Label C-Tick pour l'Australie (identifiant du fournisseur N17965)
	Plaque signalétique à l'arrière de l'appareil	EN	Label RoHS pour la Chine (restriction de l'utilisation de certaines substances dangereuses dans l'équipement électrique et électronique)

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Appendix D

1 Sicherheitshinweise

Vor der Inbetriebnahme des PyroMark Q96 MD sollten Sie dieses Handbuch sorgfältig durchlesen – beachten Sie insbesondere die Sicherheitshinweise. Die Anweisungen und Sicherheitsinformationen in diesem Handbuch müssen vom Anwender befolgt werden, um einen sicheren Betrieb des PyroMark Q96 MD zu gewährleisten und das Gerät in einem sicheren Zustand zu erhalten.

In diesem Handbuch werden die folgenden beiden Kategorien von Sicherheitshinweisen verwendet:

WARNUNG 	Der Begriff „WARNUNG“ (“WARNING”) weist Sie auf Situationen hin, in denen eine **Verletzungsgefahr** für Sie selbst oder andere Personen besteht. Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

ACHTUNG 	Der Begriff „ACHTUNG“ (“CAUTION”) weist Sie auf Situationen hin, in denen das **Gerät oder andere Geräte beschädigt** werden könnten. Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.

Die in diesem Handbuch enthaltenen Hinweise stellen eine Ergänzung und keinen Ersatz der üblichen Sicherheits-anforderungen dar, die im jeweiligen Land gelten.

1.1 Sachgemäße Handhabung

WARNUNG	**Verletzungsgefahr und Beschädigung des Geräts** Die unsachgemäße Bedienung des PyroMark Q96 MD kann zu einer Verletzung des Benutzers oder zur Beschädigung des Geräts führen. Die Bedienung des PyroMark Q96 MD darf nur durch qualifiziertes, entsprechend geschultes Personal erfolgen. Die Instandhaltung des PyroMark Q96 MD darf nur durch einen Servicespezialisten des QIAGEN Außendiensts durchgeführt werden.

Führen Sie alle Wartungsarbeiten gemäß den Anweisungen in Abschnitt 7 dieses Handbuchs durch. QIAGEN® stellt alle Reparaturen in Rechnung, die nachweislich auf eine inkorrekte Wartung zurückzuführen sind.

WARNUNG	**Verletzungsgefahr und Beschädigung des Geräts** Der PyroMark Q96 MD ist sehr schwer und sollte nicht von einer Person angehoben werden. Heben Sie das Gerät nicht allein an, um eine Verletzung und/oder Beschädigung des Geräts zu vermeiden.

1.2 Schutz vor Stromschlag

Ziehen Sie die Netzanschlusskabel aus den Steckdosen, bevor Sie Wartungsarbeiten am Gerät vornehmen.

WARNUNG	**Gefahr durch Stromschlag** 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 Schutzleiter-verbindung ist verboten. **Gefährliche Spannung im Gerät** Wenn das Gerät an die Stromversorgung angeschlossen ist, sind die Anschlussstellen spannungsführend. Durch das Öffnen der Abdeckungen oder das Entfernen von Gehäuseteilen können spannungsführende Komponenten freigelegt werden.

Um einen zufriedenstellenden und sicheren Betrieb des PyroMark Q96 MD zu gewährleisten, befolgen Sie bitte die nachstehenden Hinweise:

- Die Geräte-Netzkabel müssen an Wechselstrom-Steckdosen mit Schutzleiter (Erdungs-/Masseleiter) angeschlossen werden.
- Sorgen Sie dafür, dass die Netzstecker jederzeit frei zugänglich sind, falls das Gerät einmal schnell vom Stromnetz getrennt werden muss.
- Benutzen Sie nur Netzanschlüsse und -kabel, die mit dem Gerät geliefert werden.
- Beim Austausch der Netzsicherung ersetzen Sie diese nur durch eine desselben Typs und der Stromstärke, die auf dem Etikett/Typenschild angegeben ist.

1.3 Chemikalien

WARNUNG	**Gefährliche Chemikalien**
	Die Denaturierungslösung, die zusammen mit der Vakuum-Arbeitsstation verwendet wird, enthält Natriumhydroxid, das auf Augen und Haut reizend wirkt. 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. Auch dürfen die Grenzwerte in Bezug auf infektiöse Erreger, die in den entsprechenden Sicherheitsdatenblättern (SDS) oder den Vorschriften der OSHA*, 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 zu Gesundheitsschutz und Sicherheit am Arbeitsplatz auf übernationaler, nationaler und regionaler Ebene eingehalten werden.

* OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)

† ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika)

‡ COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

1.4 Gefahren durch mechanische Teile

Der Deckel des PyroMark Q96 MD muss während des Betriebs geschlossen sein.

WARNUNG	**Sich bewegende Geräteteile** Um einen Kontakt mit sich bewegenden Teilen beim Betrieb des PyroMark Q96 MD zu vermeiden, darf das Gerät nur mit geschlossenem Deckel betrieben werden. Entfernen Sie nicht die Abdeckplatten; im Geräteinneren befinden sich keine Bauteile, die vom Anwender gewartet werden müssen. Setzen Sie sich umgehend mit dem Technischen Service von QIAGEN in Verbindung, falls einmal ein Problem mit dem PyroMark Q96 MD auftreten sollte.

WARNUNG	**Quetsch- und Stoßgefahr** Die Dispensiereinheit, der Prozesskammer-Deckel und/oder Teile des Automationsmoduls könnten jederzeit in Bewegung gesetzt werden.

WARNUNG	**Scharfe Nadeln** Berühren Sie nicht die scharfen Nadeln an den Reagenzien-Dispensierspitzen und die Kapillar-Dispensierspitzen.

- Der PyroMark Q96 MD ist mit einem Verriegelungssystem ausgestattet. Dieses System hat an drei Stellen einen Sensor: am Gerätedeckel oberhalb der Prozesskammer, am Deckel des Automationsmoduls und am Stapler. Diese Sensoren prüfen, ob die Komponenten korrekt eingesetzt wurden. Es ertönt ein hörbares Warnsignal, wenn Komponenten falsch positioniert wurden und der Lauf nicht gestartet werden kann.
- Falls ein Deckel geöffnet oder ein Stapler während eines Laufs entnommen wird, ertönt ein hörbares Warnsignal und der Lauf wird abgebrochen, wenn der Test oder die Sequenzierung abgeschlossen ist.

1.5 Überhitzungsgefahr

WARNUNG 	**Heiße Oberflächen** [W8] Der externe Heizblock kann Temperaturen von bis zu 80 °C erreichen. Berühren Sie diese Teile nicht, wenn sie aufgeheizt sind.

ACHTUNG 	**Überhitzungsgefahr** Vergewissern Sie sich, dass ein Mindestabstand von 10 cm zwischen Seitenwänden und Rückseite des PyroMark Q96 MD und der Wand eingehalten wird, damit eine ausreichende Belüftung des Geräts gewährleistet ist. Die Lüftungsschlitze und Öffnungen, die für die Be- und Entlüftung des PyroMark Q96 MD bestimmt sind, dürfen nicht verdeckt werden.

1.6 Symbole auf dem PyroMark Q96 MD

Symbol	Fundstelle	Sprache	Beschreibung
	Typenschild auf der Geräterückseite	EN	CE-Kennzeichnung
	Reagenzien, Lösungen, Kartusche, Platte	EN	Katalognummer
	Reagenzien, Lösungen, Kartusche, Platte	EN	Zulässiger Temperaturbereich
	Typenschild auf der Rückseite des Geräts und allen anderen Produkten	EN	Hersteller i. S. d. Gesetzes

Symbol	Fundstelle	Sprache	Beschreibung
	Im Geräteinneren	EN	Warnhinweis; lesen Sie das Handbuch
	Typenschild auf Geräterückseite und Verpackungskarton	EN	Seriennummer
	Typenschild auf der Geräterückseite	EN	WEEE-Markierung (Zertifizierung gemäß europäischer Richtlinien bzw. Elektro- und Elektronik-Altgeräte-Verordnung)
	Typenschild auf der Geräterückseite	EN	FCC-Kennzeichen der Federal Communications Commission der Vereinigten Staaten
	Typenschild auf der Geräterückseite	EN	C-Tick-Mark-Zeichen für Australien (Herstellerkennung: N17965)
	Typenschild auf der Geräterückseite	EN	Markierung gemäß RoHS-Richtlinie für China (Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)

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