March 2015

PyroMark® Q24 Control Oligo Handbook

Version 1

IVD

For installation check of PyroMark Q24 MDx system.

For in vitro diagnostic use

CE

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Contents

Kit Contents

PyroMark Q24 Control Oligo	
Catalog no.	**979303**
Control Oligo 20 µM	50 µl
10x Dilution Buffer	2 x 1.7 ml
Handbook 	1

Symbols

 Use by

 In vitro diagnostic medical device

 Catalog number

 Lot number

 Material number

 Components

 Contains

 Number

 Sodium hydroxide

 Global Trade Item Number

 Temperature limitations

 Legal manufacturer

Refer to information given in the handbook

Important note

Storage

The PyroMark Q24 Control Oligo should be stored at –30°C to –15°C upon arrival. Repeated thawing and freezing (>5 x per year) should be avoided. The PyroMark Q24 Control Oligo is stable until the expiration date when stored under these conditions.

Intended Use

The PyroMark Q24 Control Oligo is intended to provide a means to verify proper installation of PyroMark Q24 MDx system in in vitro diagnostic Pyrosequencing® applications.

Product Use Limitations

For in vitro diagnostic medical use, the PyroMark Q24 MDx System may only be operated by

- personnel who have received special education and training with regard to procedures utilizing in vitro diagnostic medical devices, and
- accredited medical testing laboratories.

All operations must be performed according to PyroMark Q24 MDx system instructions, as provided through dialog messages appearing on the screen of the PyroMark Q24 MDx, the associated user manuals, handbooks, and technical support from QIAGEN, and within the limits set by the technical specifications.

Materials for sample preparation before Pyrosequencing analysis are not included in the product.

The product is intended solely for use on the PyroMark Q24 MDx system.

Strict compliance with the instrument user manual and this handbook is required for optimal results. Dilution of the reagents, other than as described in this handbook, is not recommended and will result in a loss of performance.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of PyroMark Q24 Control Oligo is tested against predetermined specifications to ensure consistent product quality.

Results from the PyroMark Q24 MDx system must be interpreted within the context of all relevant clinical and laboratory findings.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service 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 Q24 Control Oligo or QIAGEN products in general, please do not hesitate to contact us.

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

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

Warnings and precautions

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

Introduction

The PyroMark Q24 Control Oligo provides a means to verify proper installation of the PyroMark Q24 MDx System. In addition, the PyroMark Q24 Control Oligo can be used in troubleshooting to determine if an unexpected result is related to the instrument, to the PyroMark Q24 MDx Vacuum Workstation, or to the assay.

Principle and procedure

The PyroMark Q24 Control Oligo is a biotinylated oligonucleotide, which allows the user to verify that both the PyroMark Q24 MDx and PyroMark Q24 MDx Vacuum Workstation are functioning properly.

Under defined conditions, the oligonucleotide can form an internal stem–loop structure. This structure enables self-priming of the oligonucleotide for extension by the DNA polymerase and eliminates the need for a sequencing primer in the Pyrosequencing reaction. The sequenced region includes single bases of all nucleotides, homopolymers of 2 and 3 bases, and a wobbled/degenerated base. This variable position is automatically analyzed by the software, and results are presented as %C and %T. Figure 1 shows the structure of the oligonucleotide.

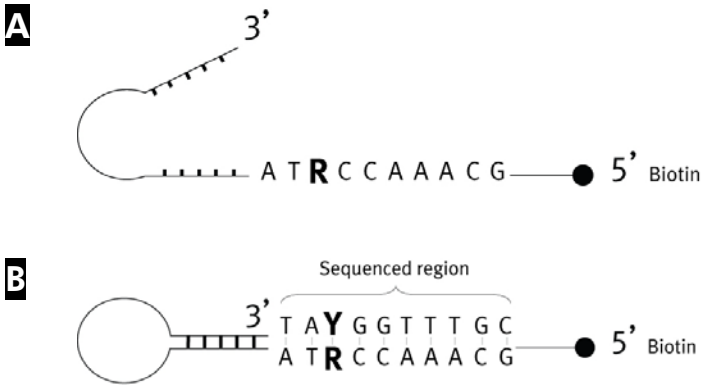

Figure 1. Structure of the PyroMark Q24 Control Oligo. A The open structure of the oligonucleotide. B The self-primed structure of the oligonucleotide, with the analyzed sequence indicated.

Description of protocols

It is recommended that 2 runs be performed to verify proper installation of the PyroMark Q24 MDx System.

Function of the PyroMark Q24 MDx

To verify correct function of the PyroMark Q24 MDx, follow "Protocol: Verifying the Function of PyroMark Q24 MDx", page 10. The PyroMark Q24 Control

Oligo is added directly to PyroMark Q24 Plate **without** prior preparation on the PyroMark Q24 MDx Vacuum Workstation.

Function of the PyroMark Q24 MDx system

To verify correct function of the whole PyroMark Q24 MDx system, follow "Protocol: Verifying the Function of the PyroMark Q24 MDx System", page 14. The PyroMark Q24 Control Oligo is prepared using the PyroMark Q24 MDx Vacuum Workstation before analysis on the PyroMark Q24 MDx.

Troubleshooting the PyroMark Q24 MDx system

To perform a troubleshooting of the whole system, follow "Protocol: Troubleshooting Procedure", page 21. A Pyrosequencing reaction is performed with 8 wells containing the PyroMark Q24 Control Oligo and 8 wells containing the PyroMark Q24 Control Oligo prepared on the PyroMark Q24 MDx Vacuum Workstation.

Equipment and Reagents to Be Supplied by User

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

- Pipets (adjustable)*
- Sterile pipet tips with filters
- Streptavidin Sepharose® High Performance (GE Healthcare, cat. no. 17-5113-01; www.gelifesciences.com)
- PyroMark Q24 MDx (cat. no. 9001513)*†
- PyroMark Q24 MDx Software (cat. no. 9019063)†
- PyroMark Q24 Plate (cat. no. 979301)†
- PyroMark Q24 Cartridge (cat. no. 979302)†
- PyroMark Q24 MDx Vacuum Workstation (cat. no. 9001515 or 9001517)*†
- PyroMark Gold Q24 Reagents (cat. no. 971802)†
- PyroMark Binding Buffer (cat. no. 979306)†
- PyroMark Denaturation Solution (cat. no. 979307)†
- PyroMark Wash Buffer, concentrate (cat. no. 979308)†
- PyroMark Annealing Buffer (cat. no. 979309)†
- Plate mixer* for immobilization to beads
- Heating block* capable of attaining 80°C
- 24-well PCR plate or strips
- Strip caps
- 1.5 ml or 2 ml microcentrifuge tubes for dilution of the PyroMark Q24 Control Oligo
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Ethanol (70%)

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

† CE-IVD-marked in accordance with EU Directive 98/79/EC. All other products listed are not CE-IVD-marked based on EU Directive 98/79/EC.

Protocol: Verifying the Function of PyroMark Q24 MDx

This protocol describes how to use the PyroMark Q24 Control Oligo to verify the function of PyroMark Q24 MDx only. To verify the function of the whole PyroMark Q24 MDx system, including the PyroMark Q24 MDx Vacuum Workstation, see "Protocol: Verifying the Function of the PyroMark Q24 MDx System", page 14.

Important point before starting

- For further information on how to create an Assay Setup and a Run Setup, see the *PyroMark Q24 Analysis Software User Guide*.

Things to do before starting

- Follow the instructions in *PyroMark Q24 User Manual* to install the PyroMark Q24 MDx system.
- The dilution buffer provided with the PyroMark Q24 Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Place the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 11.

Procedure

1. **Set up an assay for the PyroMark Q24 Control Oligo by using the PyroMark Q24 MDx Software.**
2. **Click in the toolbar and select "New AQ Assay".**
3. **Type the following sequence in "Sequence to Analyze".**
   TAYGGTTTGC

   For more information on how to create an Assay Setup file, see the *PyroMark Q24 Analysis Software User Guide*.
4. **Click the "Generate Dispensation Order" icon to get the following nucleotide dispensation order:**
   CTGACTGTG

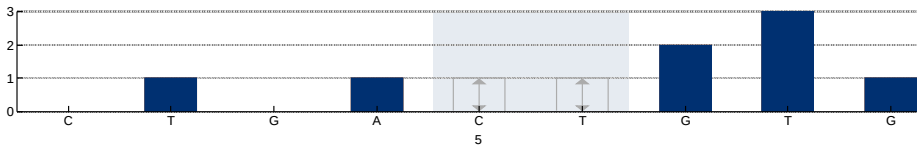

Figure 2. Histogram for AQ mode. Nucleotide additions 1 and 3 are blank dispensations and serve as negative controls. The fifth and the sixth dispensations analyze the variable position (wobbled/degenerated base).

5. **Click in the toolbar to save the assay.**
6. **Create a Run Setup by importing the assay parameters to all 24 wells.**

   To add an assay to a well, you can either:

   - Right-click the well and select "Load Assay" from the context menu
   - Select the assay in the shortcut browser, and click and drag the assay to the well.

   A well is color-coded according to the assay loaded to the well.

   For more information on how to create a Run Setup file, see the *PyroMark Q24 Analysis Software User Guide*.
7. **Save the Run Setup to a USB memory stick (supplied with the PyroMark Q24 MDx system).**
8. **Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select "Pre Run Information" from the "Tools" menu and, when the report appears, click .**
9. **Dilute the PyroMark Q24 Control Oligo to 0.04 µM as shown in Table 1.**

Table 1. Dilution of the PyroMark Q24 Control Oligo

Component	Volume	Concentration
PyroMark Q24 Control Oligo	10 µl	20 µM
Dilution buffer 1x*	90 µl	–
First serial dilution	**100 µl**	**2 µM**
First serial dilution (from above)	30 µl	2 µM
Dilution buffer 1x*	1470 µl	–
Final dilution	**1500 µl**	**0.04 µM**

* Make sure that the 10x dilution buffer supplied with the PyroMark Q24 Control Oligo is diluted with high-purity water before use. See "Things to do before starting", page 10.

10. **Add 25 µl of the diluted (0.04 µM) PyroMark Q24 Control Oligo to each well of a PyroMark Q24 Plate.**
11. **Heat the PyroMark Q24 Plate with the PyroMark Q24 Oligos at 80°C for 2 min using a heating block and the prewarmed PyroMark Q24 Plate Holder.**

12. **Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min.**
13. **Load the PyroMark Q24 Cartridge with the appropriate volumes of PyroMark Gold Q24 Reagents, as given in the Pre Run Information report from step 8.**

    The Pre Run Information report, found in the "Tools" menu at run setup (see the *PyroMark Q24 Analysis Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.
14. **Open the cartridge gate and insert the filled PyroMark Q24 Cartridge with the label facing out. Push the cartridge in fully and then push it down.**
15. **Ensure that the line is visible in front of the cartridge and close the gate.**
16. **Open the plate-holding frame and place the plate on the heating block.**
17. **Close the plate-holding frame and the instrument lid.**
18. **Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.**

    ⓘ Do not remove the USB port before the run is finished.
19. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
20. **Select the run file using the ▲ and ▼ screen buttons.**

    ⓘ To view the contents of a folder, select the folder and press "Select". To go back to the previous view, press "Back".
21. **When the run file is selected, press "Select" to start the run.**
22. **When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press "Close".**
23. **Remove the USB memory stick.**
24. **Open the instrument lid.**
25. **Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.**
26. **Close the gate.**
27. **Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.**
28. **Close the plate-holding frame and the instrument lid.**
29. **Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the *PyroMark Gold Q24 Reagents Handbook*).**

30. Open the run in the PyroMark Q24 MDx Software and analyze all wells. The peak pattern for Run 1 should look like the one in Figure 3.

To obtain peak height values, select "Export Peak Heights" from the "Tools" menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft® Excel (Delimited), and calculate the mean single peak height for each well as described below.

- **Perform a quality assessment.**
  All wells should give "Passed" quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram®. If the quality assessment is "Check" or "Failed", look in "Well Information" for explanations.
- **Evaluate peak heights.**
  The peak height should ideally be 75 ± 20 RLU.

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see "Troubleshooting Guide", page 29, for possible reasons for the failure and rerun Run 1. If the repeat of Run 1 fails, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

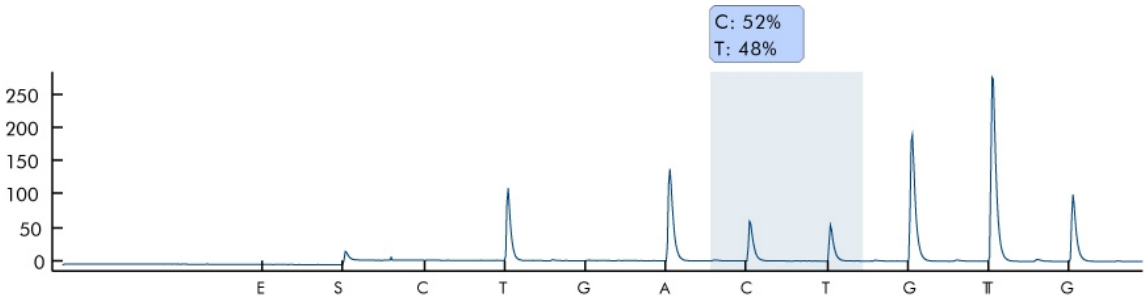

Figure 3. Pyrogram of Run 1.

Protocol: Verifying the Function of the PyroMark Q24 MDx System

This protocol describes how to use the PyroMark Q24 Control Oligo to verify the function of the PyroMark Q24 MDx system, including the PyroMark Q24 MDx Vacuum Workstation. To verify the function of the PyroMark Q24 MDx only, see "Protocol: Verifying the Function of PyroMark Q24 MDx", page 10.

Important point before starting

- For further information on how to create an Assay Setup and a Run Setup, see the *PyroMark Q24 Analysis Software User Guide*.

Things to do before starting

- Follow the instructions in *PyroMark Q24 User Manual* to install the PyroMark Q24 MDx system.
- The dilution buffer provided with the PyroMark Q24 Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Place the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 30.
- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. **Set up an assay for the PyroMark Q24 Control Oligo by using the PyroMark Q24 MDx Software.**
2. **Click in the toolbar and select "New AQ Assay".**
3. **Type the following sequence in "Sequence to Analyze".**
   TAYGGTTTGCA

   For more information on how to create an Assay Setup file, see the *PyroMark Q24 Analysis Software User Guide*.
4. **Manually enter the following "Dispensation Order".**
   ACGTTATCGTTGC

   For more information on how to create an Assay Setup file, see the *PyroMark Q24 Analysis Software User Guide*.

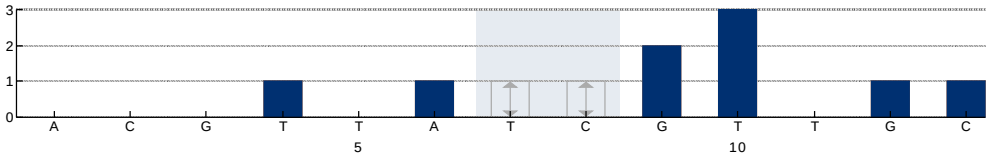

Figure 4. Histogram for AQ mode. Nucleotide additions 1, 2, 3, 5, and 11 are blank and serve as negative controls. The seventh and the eight dispensations analyze the variable position.

5. **Click in the toolbar to save the assay.**
6. **Create a Run Setup by importing the assay parameters to all 24 wells.**

   To add an assay to a well, you can either:

   - Right-click the well and select "Load Assay" from the context menu
   - Select the assay in the shortcut browser, and click and drag the assay to the well.

   A well is color-coded according to the assay loaded to the well.

   For more information on how to create a Run Setup file, see the *PyroMark Q24 Analysis Software User Guide*.
7. **Save the Run Setup to a USB memory stick (supplied with the PyroMark Q24 MDx system).**
8. **Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select "Pre Run Information" from the "Tools" menu and, when the report appears, click .**
9. **Gently shake the bottle containing Streptavidin Sepharose High Performance until it is a homogeneous solution.**
10. **Prepare a master mix for DNA immobilization according to Table 2. Prepare a volume 10% greater than that required for the total number of reactions to be performed.**

Table 2. Master mix for DNA immobilization

Number of samples	**1**	**26***
Streptavidin Sepharose High Performance	2 µl	52 µl
PyroMark Binding Buffer	40 µl	1040 µl
High-purity water	13 µl	338 µl
Total volume	**55 µl**	**1430 µl**

* Provides a sufficient amount for the 24 samples required.

11. Dilute the PyroMark Q24 Control Oligo to 0.04 µM as shown in Table 3.

Table 3. Dilution of the PyroMark Q24 Control Oligo

Component	Volume	Concentration
PyroMark Q24 Control Oligo	10 µl	20 µM
Dilution buffer 1x*	90 µl	–
First serial dilution	**100 µl**	**2 µM**
First serial dilution (from above)	30 µl	2 µM
Dilution buffer 1x*	1470 µl	–
Final dilution	**1500 µl**	**0.04 µM**

* Make sure that the 10x dilution buffer supplied with the PyroMark Q24 Control Oligo is diluted with high-purity water before use. See Things to do before starting", page 14.

12. Shake the tube containing the master mix, and add 55 µl of the master mix and 25 µl of the diluted (0.04 µM) PyroMark Q24 Control Oligo to all 24 wells of a 24-well PCR plate or strips.

13. Seal the PCR plate (or strips) using strip caps.

14. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

ⓘ Sepharose beads sediment quickly. Capturing of beads must take place immediately following agitation.

ⓘ During this step, prepare the PyroMark Q24 MDx Vacuum Workstation for sample preparation (see Appendix A, page 39).

15. Add 25 µl of PyroMark Annealing Buffer to each well of a PyroMark Q24 Plate.

ⓘ Keep one of the PyroMark Q24 Plate Holders (supplied with the PyroMark Q24 MDx Vacuum Workstation) at room temperature (15–25°C), and use it as support when preparing and moving the plate.

16. Place the PCR plate (or strips) and the PyroMark Q24 Plate on the worktable of the PyroMark Q24 MDx Vacuum Workstation (see Figure 5).

ⓘ Ensure that the plate is in the same orientation as when samples were loaded.

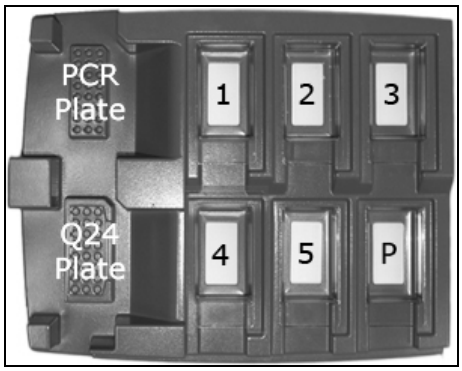

Figure 5. Placement of PCR plate (or strips) and PyroMark Q24 Plate on the PyroMark Q24 MDx Vacuum Workstation. The marked positions contain 70% ethanol (**1**), PyroMark Denaturation Solution (**2**), PyroMark Wash Buffer (**3**), and high-purity water (**4**, **5**). **P**: Parking position.

17. **Apply vacuum to the vacuum tool by opening the vacuum switch.**
18. **Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.**

    ⓘ 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.

19. **Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.**
20. **Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.**
21. **Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.**
22. **Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 6).**

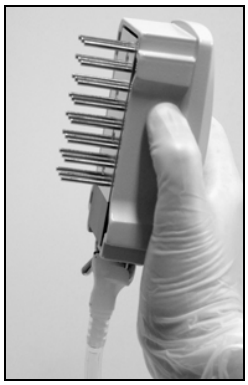

Figure 6. Illustration of the vacuum tool raised to beyond 90° vertical.

23. **While the tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).**

24. **Release the beads in the plate containing 25 µl PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.**
25. **Transfer the tool to the first trough containing high-purity water (trough 4) and agitate the tool for 10 s.**
26. **Wash the filter probes by lowering the probes into the second trough with high-purity water (trough 5) and applying vacuum. Flush the probes with 70 ml high-purity water.**
27. **Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 6).**
28. **Close the vacuum switch on the tool (Off), and place the tool in the Parking (P) position.**
29. **Turn off the vacuum pump.**

    ⓘ At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q24 MDx Vacuum Workstation should be checked for dust and spillage, see Appendix B, page 40.
30. **Heat the PyroMark Q24 Plate with the samples at 80°C for 2 min using a heating block and the prewarmed PyroMark Q24 Plate Holder.**
31. **Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min.**
32. **Load the PyroMark Q24 Cartridge with the appropriate volumes of PyroMark Gold Q24 Reagents, as given in the Pre Run Information report from step 8.**

    The Pre Run Information report, found in the "Tools" menu at run setup (see the *PyroMark Q24 Analysis Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.
33. **Open the cartridge gate and insert the filled PyroMark Q24 Cartridge with the label facing out. Push the cartridge in fully and then push it down.**
34. **Ensure that the line is visible in front of the cartridge and close the gate.**
35. **Open the plate-holding frame and place the plate on the heating block.**
36. **Close the plate-holding frame and the instrument lid.**
37. **Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.**

    ⓘ Do not remove the USB port before the run is finished.

38. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
39. **Select the run file using the ▲ and ▼ screen buttons.**

    To view the contents of a folder, select the folder and press "Select". To go back to the previous view, press "Back".
40. **When the run file is selected, press "Select" to start the run.**
41. **When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press "Close".**
42. **Remove the USB memory stick.**
43. **Open the instrument lid.**
44. **Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.**
45. **Close the gate.**
46. **Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.**
47. **Close the plate-holding frame and the instrument lid.**
48. **Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the *PyroMark Gold Q24 Reagents Handbook*).**
49. **Open the run in the PyroMark Q24 MDx Software and analyze all wells. The peak pattern for Run 2 should look like the one in Figure 7.**

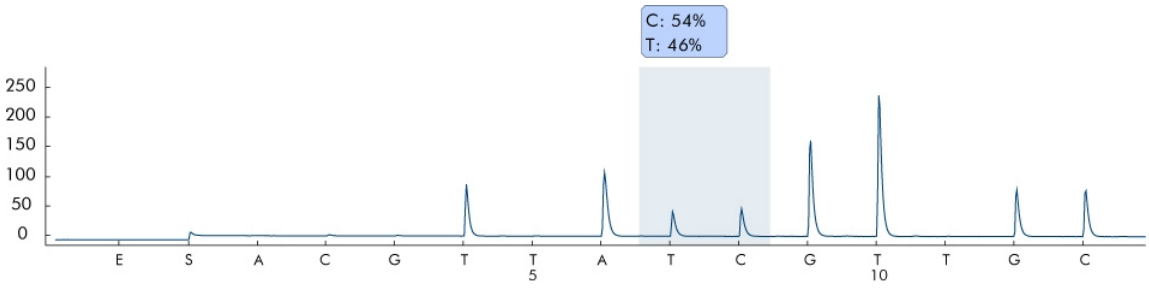

Figure 7. Pyrogram of Run 2.

50. **Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, single peak heights, and background.**

    To obtain peak height values, select "Export Peak Heights" from the "Tools" menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height and background for each well as described below.

- **Perform a quality assessment.**
  All wells should give "Passed" quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram. If the quality assessment is "Check" or "Failed", look in "Well Information" for explanations.

- **Evaluate the quantification results.**
  Select the "AQ Analysis Statistics Report" from the "Reports" menu. Quantification results are given in the report with standard deviation. The % C should be in the range 40–60%. The standard deviation should not exceed 2 % units.

- **Evaluate single peak heights.**
  The mean single peak height should ideally be 75 ± 20 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**
  Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

ⓘ If the values are within the set limits, the system is properly installed. If the results are not as stated above, see "Troubleshooting Guide", page 29, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Protocol: Troubleshooting Procedure

If an unexpected result has been obtained it is crucial to determine whether this is related to the PyroMark Q24 MDx, the PyroMark Q24 MDx Vacuum Workstation, or the assay. This protocol describes how to use the PyroMark Q24 Control Oligo to verify the function of the PyroMark Q24 MDx system, comparing results with or without the PyroMark Q24 MDx Vacuum Workstation.

Important point before starting

- For further information on how to create an Assay Setup and a Run Setup, see the *PyroMark Q24 Analysis Software User Guide*.

Things to do before starting

- Follow the instructions in *PyroMark Q24 User Manual* to install the PyroMark Q24 MDx system.
- The dilution buffer provided with the PyroMark Q24 Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Place the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 30.
- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. **Set up an assay for the PyroMark Q24 Control Oligo by using the PyroMark Q24 MDx Software.**
2. **Click in the toolbar and select "New AQ Assay".**
3. **Type the following sequence in "Sequence to Analyze".**
   TAYGGTTTGCA

   For more information on how to create an Assay Setup file, see the *PyroMark Q24 Analysis Software User Guide*.
4. **Manually enter the following "Dispensation Order".**
   ACGTTATCGTTGC

   For more information on how to create an Assay Setup file, see the *PyroMark Q24 Analysis Software User Guide*.

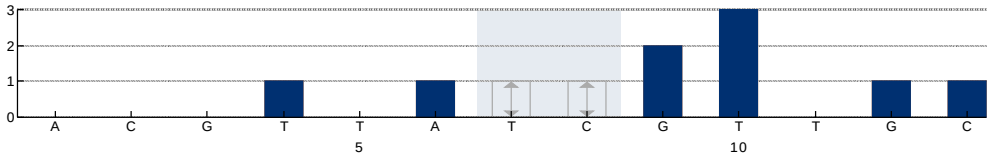

Figure 8. Histogram for AQ mode. Nucleotide additions 1, 2, 3, 5, and 11 are blank and serve as negative controls. The seventh and the eight dispensations analyze the variable position.

5. **Click in the toolbar to save the assay.**
6. **Create a Run Setup by importing the assay parameters to the appropriate wells.**

   We recommend using 16 wells: 8 wells for samples prepared using the PyroMark Q24 MDx Vacuum Workstation and 8 samples added directly to PyroMark Q24 Plate.

   To add an assay to a well, you can either:

   - Right-click the well and select "Load Assay" from the context menu
   - Select the assay in the shortcut browser, and click and drag the assay to the well.
   - Recommended: Fill in Sample ID, Plate ID, Barcode, Reagent ID, and Run Note.

   A well is color-coded according to the assay loaded to the well.

   For more information on how to create a Run Setup file, see the *PyroMark Q24 Analysis Software User Guide*.

7. **Save the Run Setup to a USB memory stick (supplied with the PyroMark Q24 MDx system).**
8. **Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select "Pre Run Information" from the "Tools" menu and, when the report appears, click .**
9. **Gently shake the bottle containing Streptavidin Sepharose High Performance until it is a homogeneous solution.**
10. **Prepare a master mix for DNA immobilization according to Table 4. Prepare a volume 10% greater than that required for the total number of reactions to be performed.**

Table 4. Master mix for DNA immobilization

Number of samples	1	9*
Streptavidin Sepharose High Performance	2 µl	18 µl
PyroMark Binding Buffer	40 µl	360 µl
High-purity water	13 µl	117 µl
Total volume	**55 µl**	**495 µl**

* Provides a sufficient amount for the 8 samples required.

11. Dilute the PyroMark Q24 Control Oligo to 0.04 µM as shown in Table 5.

Table 5. Dilution of the PyroMark Q24 Control Oligo

Component	Volume	Concentration
PyroMark Q24 Control Oligo	10 µl	20 µM
Dilution buffer 1x*	90 µl	–
First serial dilution	**100 µl**	**2 µM**
First serial dilution (from above)	30 µl	2 µM
Dilution buffer 1x*	1470 µl	–
Final dilution	**1500 µl**	**0.04 µM**

* Make sure that the 10x dilution buffer supplied with the PyroMark Q24 Control Oligo is diluted with high-purity water before use. See "Things to do before starting", page 21.

12. Shake the tube containing the master mix, and add 55 µl of the master mix and 25 µl of the diluted (0.04 µM) PyroMark Q24 Control Oligo to 8 wells of a 24-well PCR plate or strips.

13. Seal the PCR plate (or strips) using strip caps.

14. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

ⓘ Sepharose beads sediment quickly. Capturing of beads must take place immediately following agitation.

ⓘ During this step, prepare the PyroMark Q24 MDx Vacuum Workstation for sample preparation (see Appendix A, page 39).

15. **Add 25 µl of PyroMark Annealing Buffer to each well of the PyroMark Q24 Plate that will be used with the immobilized PyroMark Q24 Control Oligo to be processed with the PyroMark Q24 MDx Vacuum Workstation. Add 25 µl of the diluted (0.04 µM) PyroMark Q24 Control Oligo to 8 additional wells according to the Run Setup.**

ⓘ Keep one of the PyroMark Q24 Plate Holders (supplied with the PyroMark Q24 MDx Vacuum Workstation) at room temperature (15–25°C), and use it as support when preparing and moving the plate.

16. **Place the PCR plate (or strips) and the PyroMark Q24 Plate on the worktable of the PyroMark Q24 MDx Vacuum Workstation (see Figure 9).**

ⓘ Ensure that the plate is in the same orientation as when samples were loaded.

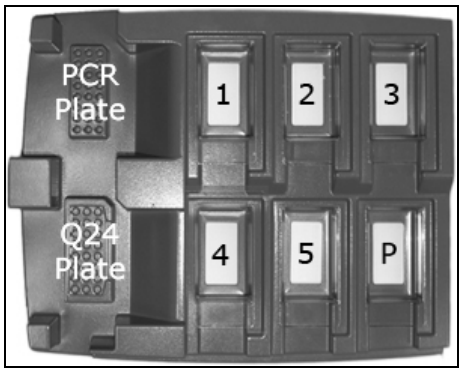

Figure 9. Placement of PCR plate (or strips) and PyroMark Q24 Plate on the PyroMark Q24 MDx Vacuum Workstation. The marked positions contain 70% ethanol (**1**), PyroMark Denaturation Solution (**2**), PyroMark Wash Buffer (**3**), and high-purity water (**4**, **5**). **P**: Parking position.

17. **Apply vacuum to the tool by opening the vacuum switch.**
18. **Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.**

ⓘ 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.

19. **Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.**
20. **Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.**
21. **Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.**

22. **Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 10).**

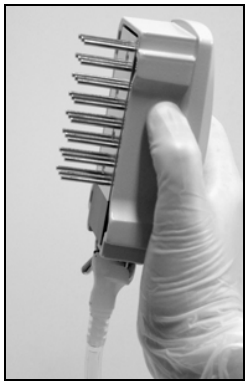

Figure 10. Illustration of the vacuum tool raised to beyond 90° vertical.

23. **While the tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).**
24. **Release the beads in the plate containing 25 µl PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.**
25. **Transfer the tool to the first trough containing high-purity water (trough 4) and agitate the tool for 10 s.**
26. **Wash the filter probes by lowering the probes into the second trough with high-purity water (trough 5) and applying vacuum. Flush the probes with 70 ml high-purity water.**
27. **Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 10).**
28. **Close the vacuum switch on the tool (Off), and place the tool in the Parking (P) position.**
29. **Turn off the vacuum pump.**

ⓘ At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q24 MDx Vacuum Workstation should be checked for dust and spillage, see Appendix B, page 40.

30. **Heat the PyroMark Q24 Plate with the samples at 80°C for 2 min using a heating block and the prewarmed PyroMark Q24 Plate Holder.**
31. **Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min.**

32. **Load the PyroMark Q24 Cartridge with the appropriate volumes of PyroMark Gold Q24 Reagents, as given in the Pre Run Information report from step 8.**

    The Pre Run Information report, found in the "Tools" menu at run setup (see the *PyroMark Q24 Analysis Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.

33. **Open the cartridge gate and insert the filled PyroMark Q24 Cartridge with the label facing out. Push the cartridge in fully and then push it down.**
34. **Ensure that the line is visible in front of the cartridge and close the gate.**
35. **Open the plate-holding frame and place the plate on the heating block.**
36. **Close the plate-holding frame and the instrument lid.**
37. **Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.**

    ⓘ Do not remove the USB port before the run is finished.

38. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
39. **Select the run file using the ▲ and ▼ screen buttons.**

    ⓘ To view the contents of a folder, select the folder and press "Select". To go back to the previous view, press "Back".

40. **When the run file is selected, press "Select" to start the run.**
41. **When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press "Close".**
42. **Remove the USB memory stick.**
43. **Open the instrument lid.**
44. **Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.**
45. **Close the gate.**
46. **Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.**
47. **Close the plate-holding frame and the instrument lid.**
48. **Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the *PyroMark Gold Q24 Reagents Handbook*).**
49. **Open the run in the PyroMark Q24 MDx Software and analyze all wells. The peak pattern should look like the one in Figure 11.**

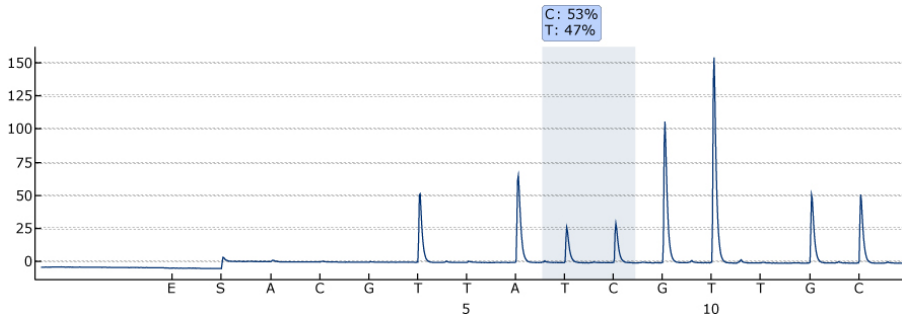

Figure 11. Pyrogram of Run 3.

50. Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, single peak heights, and background.

To obtain peak height values, select "Export Peak Heights" from the "Tools" menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height and background for each well as described below.

- **Perform a quality assessment.**
  All wells should give "Passed" quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram. If the quality assessment is "Check" or "Failed", look in "Well Information" for explanations.
- **Evaluate the quantification results.**
  Select the "AQ Analysis Statistics Report" from the "Reports" menu. Quantification results are given in the report with standard deviation. The % C should be in the range 40–60%. The standard deviation should not exceed 2 % units.
- **Evaluate single peak heights.**
  The mean single peak height should ideally be 75 ± 20 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**
  Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

51. Evaluate the difference in peak heights with and without sample preparation. The reduction in peak height between samples prepared using the PyroMark Q24 MDx Vacuum Workstation compared with PyroMark Q24 Control Oligo added directly to the PyroMark Q24 Plate should not be more than 20%.

ⓘ If the values are within the set limits, the system is properly installed. If the results are not as stated above, see "Troubleshooting Guide", page 29, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Troubleshooting Guide

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

Sections are included for each evaluation performed:

- Quality assessment, below
- Quantification results, page 33
- Single peak heights, page 34
- Background, page 37
- Difference in peak height with and without sample preparation, page 37

ⓘ Refer to the *PyroMark Q24 User Manual* for general troubleshooting of the instrument.

Quality assessment

	Comments and suggestions
Warning from software about broad peaks	
Concentration of PyroMark Q24 Control Oligo too high	ⓘ Follow the relevant protocol. Make sure to dilute the PyroMark Q24 Control Oligo in dilution buffer as described in the protocols.
High substrate peak	
Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal)	ⓘ Change buffers. Only use buffers that are supplied by QIAGEN or QIAGEN authorized distributors. ⓘ Use the zoom in function to check if any peaks have been generated (select a section of Pyrogram with the left mouse button).

	Comments and suggestions
Poor or incorrect sequence	
a) PyroMark Q24 Control Oligo not correctly prepared	ⓘ Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
b) Incorrect dispensation order	ⓘ Check that the correct sequence was typed in the Assay Setup.
c) Buffers or reagents incorrectly diluted or incorrectly stored	ⓘ Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides.
d) Dispensation error (seen, for example, as split peaks)	ⓘ Clean or replace the PyroMark Q24 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com).
e) Blocked PyroMark Q24 Cartridge	ⓘ Nucleotides are not dispensed correctly due to a blocked needle in the PyroMark Q24 Cartridge. Clean the PyroMark Q24 Cartridge and check that it is working properly.
f) Damaged PyroMark Q24 Cartridge	ⓘ Discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
g) Annealing time too long	ⓘ Carry out annealing for the correct time and at the temperatures described in the protocols.

	Comments and suggestions
Small or missing peaks	
a) Insufficient amount of template for immobilization	ⓘ Make sure to dilute the PyroMark Q24 Control Oligo correctly and use the amounts specified in the protocols.
b) Not enough enzyme or substrate for all wells	ⓘ Fill the PyroMark Q24 Cartridge according to the instructions in the Pre Run Information report.
c) Wells marked in the Run Setup do not agree with sample placement in the plate	ⓘ Check that you loaded the PyroMark Q24 Plate correctly, according to the Run Setup.
d) One or more of the nucleotide compartments in the PyroMark Q24 Cartridge not correctly filled with reagents or nucleotides	ⓘ Make sure that sufficient reagents are added to the PyroMark Q24 Cartridge. Follow the instructions for use supplied with the products.
e) Dispensation error (seen, for example, as split peaks)	ⓘ Clean or replace the PyroMark Q24 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com).
f) Blocked PyroMark Q24 Cartridge	ⓘ Nucleotides are not dispensed correctly due to a blocked needle in the PyroMark Q24 Cartridge. Clean the PyroMark Q24 Cartridge and check that it is working properly. ⓘ Enzymes or substrates are not dispensed correctly due to a blocked PyroMark Q24 Cartridge (as indicated by a missing presequencing signal and no peaks in the Pyrogram). Clean the PyroMark Q24 Cartridge and check that it is working properly.

	Comments and suggestions
g) Damaged PyroMark Q24 Cartridge	ⓘ Discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
h) Buffers or reagents incorrectly diluted or incorrectly stored	ⓘ Follow the instructions supplied with the reagents.
i) PyroMark Q24 MDx started without a plate inserted	ⓘ Clean the heating block and the light guides/lens array according to instructions in the *PyroMark Q24 User Manual*.
j) PyroMark Q24 Control Oligo not correctly prepared	ⓘ Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
k) Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal)	ⓘ Change buffers. Only use buffers that are supplied by QIAGEN or QIAGEN authorized distributors. ⓘ Use the zoom in function to check if any peaks have been generated (select a section of Pyrogram with the left mouse button).
Warning regarding signal to noise	
Various	ⓘ See points a) to k) in “Small or missing peaks”, above.

Quantification results

	Comments and suggestions
Poor or incorrect sequence	
a) PyroMark Q24 Control Oligo not correctly prepared	ⓘ Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
b) Incorrect sequence to analyze or dispensation order	ⓘ Check that the correct sequence was typed in the Assay Setup.
c) Buffers or reagents incorrectly diluted or incorrectly stored	ⓘ Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides.
d) Dispensation error (seen, for example, as split peaks)	ⓘ Clean or replace the PyroMark Q24 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com).
e) Blocked PyroMark Q24 Cartridge	ⓘ Nucleotides are not dispensed correctly due to a blocked needle in the PyroMark Q24 Cartridge. Clean the PyroMark Q24 Cartridge and check that it is working properly.
f) Damaged PyroMark Q24 Cartridge	ⓘ Discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
g) Annealing time too long	ⓘ Carry out annealing for the correct time and at the temperatures described in the protocols.

	Comments and suggestions
High background	
a) The storage conditions for one or more reagent did not comply with the instructions given in "Storage", page 5	Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary. Nucleotides must never be frozen!
b) Reagents have expired	Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary.

Single peak heights

	Comments and suggestions
Poor or incorrect sequence	
a) PyroMark Q24 Control Oligo not correctly prepared	Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
b) Incorrect sequence to analyze or dispensation order	Check that the correct sequence was typed in the Assay Setup.
c) Buffers or reagents incorrectly diluted or incorrectly stored	Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides.
d) Dispensation error (seen, for example, as split peaks)	Clean or replace the PyroMark Q24 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
e) Blocked PyroMark Q24 Cartridge	ⓘ Nucleotides are not dispensed correctly due to a blocked needle in the PyroMark Q24 Cartridge. Clean the PyroMark Q24 Cartridge and check that it is working properly.
f) Damaged PyroMark Q24 Cartridge	ⓘ Discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
g) Annealing time too long	ⓘ Carry out annealing for the correct time and at the temperatures described in the protocols.
Small or missing peaks	
a) Insufficient amount of template for immobilization	ⓘ Make sure to dilute the PyroMark Q24 Control Oligo correctly and use the amounts specified in the protocols.
b) Not enough enzyme or substrate for all wells	ⓘ Fill the PyroMark Q24 Cartridge according to the instructions in the Pre Run Information report.
c) Wells marked in the Run Setup do not agree with sample placement in the plate	ⓘ Check that you loaded the PyroMark Q24 Plate correctly, according to the Run Setup.
d) One or more of the nucleotide compartments in the PyroMark Q24 Cartridge not correctly filled with reagents or nucleotides	ⓘ Make sure that sufficient reagents are added to the PyroMark Q24 Cartridge. Follow the instructions for use supplied with the products.
e) Dispensation error (seen, for example, as split peaks)	ⓘ Clean or replace the PyroMark Q24 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
f) Blocked PyroMark Q24 Cartridge	ⓘ Nucleotides are not dispensed correctly due to a blocked needle in the PyroMark Q24 Cartridge. Clean the PyroMark Q24 Cartridge and check that it is working properly. ⓘ Enzymes or substrates are not dispensed correctly due to a blocked PyroMark Q24 Cartridge (as indicated by a missing presequencing signal and no peaks in the Pyrogram). Clean the PyroMark Q24 Cartridge and check that it is working properly.
g) Damaged PyroMark Q24 Cartridge	ⓘ Discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
h) Buffers or reagents incorrectly diluted or incorrectly stored	ⓘ Follow the instructions supplied with the reagents.
i) PyroMark Q24 Control Oligo not correctly prepared	ⓘ Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
j) Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal)	ⓘ Change buffers. Only use buffers that are supplied by QIAGEN or QIAGEN authorized distributors. ⓘ Use the zoom in function to check if any peaks have been generated (select a section of Pyrogram with the left mouse button).

	Comments and suggestions
Very high peaks	
PyroMark Q24 Control Oligo not correctly prepared	Follow the instruction in the protocols for preparing the PyroMark Q24 Control Oligo. Make sure to dilute the PyroMark Q24 Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.

Background

	Comments and suggestions
High background	
a) The storage conditions for one or more reagent did not comply with the instructions given in "Storage", page 5	Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary. Nucleotides must never be frozen!
b) Reagents have expired	Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary.

Difference in peak height with and without sample preparation

	Comments and suggestions
Incorrect sample preparation	
a) Liquid left in some wells or tubes when capturing the beads containing immobilized template to the filter probes	Replace corresponding filter probe in the vacuum tool of the PyroMark Q24 MDx Vacuum Workstation. See the sample preparation guidelines available at our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

	Comments and suggestions
b) Filter probes not working properly	Check the filter probes. Add 80 µl of high-purity water to each well of a PCR plate. Start the vacuum pump and apply vacuum by opening the vacuum switch (On). Lower the vacuum tool into the PCR plate and wait 10 s. Check that all wells of the PCR plate are empty. If not, replace the failed filter probes and repeat the test.
c) White debris (Streptavidin Sepharose High Performance beads) in some wells or tubes when capturing the beads containing immobilized template to the filter probes	Do not leave the PCR plate, used in the immobilization step, for longer than 1 min after mixing is finished. If necessary, mix for an extra minute before capturing the beads.
d) Leakage of the PyroMark Q24 MDx Vacuum Workstation	Ensure that the tubing is connected properly and that there is no leakage. The waste filter might be wet and need to be replaced.

Appendix A: Preparation of the PyroMark Q24 MDx Vacuum Workstation

This protocol is a description of how to prepare the PyroMark Q24 MDx Vacuum Workstation before using it for preparation of single-stranded DNA.

Procedure

1. **Fill 5 separate troughs (supplied with the PyroMark Q24 MDx Vacuum Workstation) as follows.**
   - **Approximately 50 ml ethanol (70%) (1)**
   - **Approximately 40 ml PyroMark Denaturation Solution (2)**
   - **Approximately 50 ml PyroMark Wash Buffer (3)**
   - **Approximately 50 ml high-purity water (4)**
   - **Approximately 70 ml high-purity water (5)**

   A suggested setup is shown in Figure 12. Refill the troughs to these levels whenever necessary.

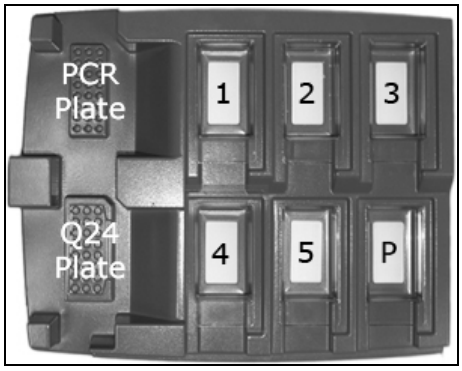

Figure 12. Positions on the PyroMark Q24 MDx Vacuum Workstation.

2. **Switch on the vacuum pump.**
3. **Apply vacuum to the tool by opening the vacuum switch.**
4. **Wash the filter probes by lowering the probes into high-purity water (trough 5). Flush the probes with 70 ml high-purity water. Make sure that the water is being transferred to the waste container. If it is not, then make sure that the tubing is connected correctly and is not broken. Broken tubing should be replaced, see "Replacing the tubing" in the *PyroMark Q24 User Manual*.**
5. **Make sure that the waste filter is dry. If the filter is wet, it should be replaced, see "Replacing the waste filter" in the *PyroMark Q24 User Manual*.**
6. **Refill trough 5 with 70 ml high-purity water.**
7. **Close the vacuum switch on the tool (Off) and place the tool in the Parking (P) position.**

Appendix B: Emptying the Waste Container and Troughs

WARNING	Hazardous chemicals
	The PyroMark Denaturation Solution used with the PyroMark Q24 MDx 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 (SDs) or OSHA,*ACGIH,† or COSHH‡ documents. 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 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, www.millipore.com, or equivalent).

Procedure

1. **Ensure that no vacuum is applied to the vacuum tool, 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.**

   The cap can be removed without disconnecting the tubing.

5. **If the PyroMark Q24 MDx Vacuum Workstation must be cleaned (for dust or spillage), follow the instructions in "Cleaning the PyroMark Q24 Vacuum Workstation" in the *PyroMark Q24 User Manual*.**

References

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

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

Ordering Information

Product	Contents	Cat. no.
PyroMark Q24 Control Oligo	For installation check of system	979303
Accessories		
PyroMark Gold Q24 Reagents (5 x 24)	For 5 x 24 samples for use on the PyroMark Q24 MDx: Enzyme Mixture, Substrate Mixture, and Nucleotides	971802
PyroMark Annealing Buffer (250 ml)	For annealing sequencing primer to single-stranded PCR product and for Pyrosequencing reaction	979309
PyroMark Binding Buffer (200 ml)	For binding of biotinylated PCR product to Sepharose beads	979306
PyroMark Denaturation Solution (500 ml)	For denaturation of double-stranded PCR product into single-stranded template DNA	979307
PyroMark Wash Buffer, concentrate (200 ml)	For washing of single-stranded DNA	979308
PyroMark Q24 Plate (100)	24-well sequencing reaction plate	979301
PyroMark Q24 Cartridge (3)	Cartridges for dispensing nucleotides and reagents	979302
Related products		
PyroMark Q24 MDx	Sequence based detection platform for Pyrosequencing of 24 samples in parallel	9001513
PyroMark Q24 MDx Software	Application software	9019063
PyroMark Q24 MDx Vacuum Workstation	Vacuum Workstation (220 V) for preparing 24 samples in parallel, from PCR product to single-stranded template	9001515* 9001517†

* For rest of world (not UK).

† For the UK.

Product	Contents	Cat. no.
PyroMark Q24 Validation Oligo	For performance check of system	979304

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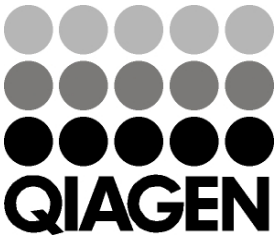

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