Mx3000P and Mx3005P QPCR Systems

Setup and User's Guide

Agilent Technologies

Mx3000P and Mx3005P QPCR Systems

SETUP AND USER'S GUIDE

IN #70225 J.1

For Research Use Only. Not for Use in Diagnostic Procedures.

LIMITED PRODUCT WARRANTY

Agilent warrants to the original purchaser that this product is free from defects in workmanship or materials for a period of one (1) year from the date of purchase, as indicated on the original invoice, under normal use.

The sole and exclusive remedy under this limited warranty is repair or replacement of defective products or parts thereof. Repair or replacement products or parts thereof will be furnished solely on an exchange basis and are obtainable only by the original purchaser. The original purchaser shall return the defective product, or part thereof, properly packaged, postage or shipping costs prepaid to Agilent. Loss or damage during shipment shall be at the risk of the purchaser.

This limited warranty does not include repair or replacement necessitated by accident, neglect, misuse, relocation, unauthorized repair or modification of the product.

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Mx3000P and Mx3005P QPCR Systems
Setup and User's Guide

Mx3000P AND Mx3005P QPCR SYSTEMS
COMPONENTS PROVIDED

Components	Quantity
Mx3000P or Mx3005P QPCR system	1
Power cord	1
Fuse holder kit	1
Notebook or desktop computer with Windows® Vista (or equivalent) and Office 2007 (or equivalent)[a]	1
Serial-to-USB adapter	1
USB driver installation CD	1
Mouse	1
RS-232 serial null modem cable (part #22316)	1
Perfect Fit frame (catalog #401421)	1
Mx3000P/Mx3005P 96-well non-skirted PCR plates (catalog #410088)	5
Mx3000P/Mx3005P optical strip caps (catalog #401425)	60 strips of 8 caps
MxPro QPCR software installation CD	1
Installation qualification test plate[b] and plate setup instructions	1
Setup and User's Guide	1
Installation wall chart	1
Introduction to Quantitative PCR: Methods and Application Guide	1
Warranty card	1

[a] The MxPro QPCR software is compatible with Windows® XP and Windows® Vista operating systems.

[b] Store the qualification test plate at –20°C upon receipt.

SETTING UP THE SYSTEM

Important   *Retain the shipping container and all packaging materials, including the wooden pallet. In the event that the instrument needs to be returned for service, the instrument must be shipped in the original container to maintain warranty protection.*

Locating the Instrument

Select a location for the Mx3000P or Mx3005P instrument according to the following guidelines:

- Select a flat, level surface

- Ensure that the workbench is capable of supporting at least 45 lbs (20.4 kg)

- Allow 4 inches (10.2 cm) airflow on the front and rear of the instrument

- Position the instrument to allow easy access to the instrument power entry module (containing the instrument power switch)

- The instrument should be installed in a location where it will not be exposed to direct sunlight or vibration during use

Environmental Conditions for Use

- Maximum altitude: 2000 m (6562 ft)

- Ambient temperature and relative humidity range for operation: 18–35°C (65–95°F); up to 85% non-condensing humidity

- Installation Category II

    Installation Category ratings establish standardized voltage levels that may occur in the electrical distribution system. Category II is for measurements performed on circuits directly connected to the electrical distribution system (i.e. a wall outlet on normal single-phase supply [115/230 VAC]).

- Pollution Degree 2

    Pollution Degree ratings define the type of environment the equipment is typically installed in. Pollution Degree 2 refers to an office environment with occasional condensation.

- For Indoor Use Only

Unpacking the Instrument

> **Note** *To ensure safety, we recommend that the lifting and unpacking of the system be performed by two people.*

> **Note** *Par mesure de sécurité, nous recommandons d'être deux personnes pour soulever et pour déballer l'instrument.*

1. Remove the instrument from the shipping container. Do not apply force to any part of the instrument directly; instead apply force to the plastic bag or foam supports surrounding the instrument.

   > **Important** *Retain the shipping container and all packaging materials, including the wooden pallet. In the event that the instrument needs to be returned for service, the instrument must be shipped in the original container to maintain warranty protection.*

2. Remove the foam and cardboard supports and the plastic bag, then place the instrument in an appropriate location (see *Locating the Instrument*).

3. Remove the tape securing the instrument door from the instrument front.

4. Open the instrument door by pushing up on the door handle. Remove and retain the foam used to immobilize the scanning-head on the track.

5. Move the scan arm to the left of the thermal block top.

6. To access the thermal block, unlatch the heated lid by pulling forward on the handle and then rotating the lid up and away from the thermal block. Gently push down on the handle to lock the lid into the open position. To close the lid on the thermal block, gently pull up on the handle to release. After rotating the lid back to the closed position, gently push the handle toward the back of the instrument to lock it into the closed position.

7. Surrounding the 96-well thermal block is a plastic Perfect Fit frame. Remove and retain the Perfect Fit frame for later use. See the *Sample Loading* section for more information about the Perfect Fit frame.

Installing the Fuse

Important   *Do not turn on the instrument until you have installed the fuse holder. The fuse is required for proper instrument operation.*

1. Remove the label located over the power entry module on the back of the instrument just above the power switch.

2. Use a small flathead screwdriver or similar tool to open the fuse box.

3. Unpack the fuse holder from its shipping container.

4. The flat edge of the fuse holder has two different voltage labels (**115V** and **230V**) printed in opposite orientation to each other. With the voltage labels facing out, insert the fuse holder into the fuse box so that the label for the desired voltage is in the proper orientation. (If your instrument is 100-120 volts, insert the fuse holder such that the **115V** label is right-side-up. If your instrument is 200-240 volts, insert the fuse holder such that the **230V** label is right-side-up.)

5. Replace the cover on the fuse box. Verify that the correct voltage label is visible through the cover.

Connecting the Instrument to the PC

1. Verify that the power switch located at the rear of the instrument is toggled off (the segment of the switch marked with an O symbol is depressed). Attach the power cord to the power entry module, just below the power switch. Plug the instrument into any properly grounded outlet. The Mx3000P or Mx3005P system must be connected to a power supply with the following specifications: 100–120 volts AC, 50/60 Hz, 10 A or 200–240 volts AC, 50/60 Hz, 5 A.

   Note   *The power supply connected to the instrument should not have any other equipment connected to it which could cause the power available to the instrument to fall below the requirements listed here.*

2. Connect either end of the provided serial cable to the RS-232 serial port at the rear of the instrument. If your PC has a serial port, connect the free end of the cable to the serial port on the back or side of the computer.

   If your PC does not have a serial port, or if you are connecting an additional instrument to the PC using the USB port, connect the free end of the cable to the provided serial-to-USB adapter, and then plug the adapter into the USB port on the back or side of the computer (see Figure 1).

Serial Port   **Serial Cable**   **USB Adapter Cable**
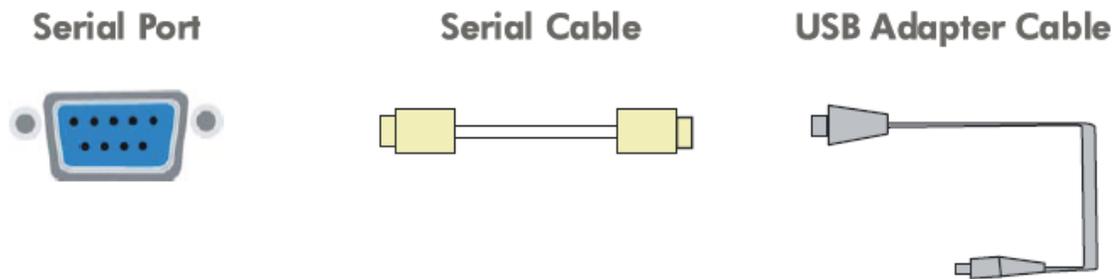
Figure 1   Appearance of the serial port, serial cable and USB adapter used to connect the instrument to the PC. Note that this cable configuration is only required when using a PC that does not have a serial port or when using the PC's USB port to allow Multiple Instrument Control from a single PC.

3. Turn on the PC.

4. Perform any computer setup tasks required by the PC manufacturer.

Configuring the PC and Installing the MxPro QPCR Software

1. Insert the supplied USB driver CD into the CD-ROM drive. The software installation program will start automatically and guide you through the installation process. Complete the hardware setup steps, accepting all defaults. Click **Finish** at the end of the installation program and remove the USB driver CD from the CD drive.

2. Click on the Windows® **Start** menu in the bottom left corner of the screen to expand the menu. Select **Programs** or **All Programs.** Select the Microsoft® Excel® icon to open the Microsoft Excel software. If the Microsoft Excel icon is not visible, select Microsoft Office.

3. An activation Wizard will guide you through the process of activating the Microsoft Office software. Among other items, the activation Wizard will request acceptance of the license agreement, entry of the product key (found on the back of the Microsoft Office installation CD sleeve), and activation of the software either through an encrypted transfer over the Internet or by telephone. Activation is required for exporting the Mx3000P/Mx3005P instrument qualification test data into a Microsoft Excel template, as described in *Performing the Instrument Qualification Test Run*. Following Microsoft Office activation, select the default choices presented by the Wizard. Close Microsoft Excel.

    Note   *The activation Wizard will not be displayed if the information has been previously entered.*

4. Turn on the Mx3000P or Mx3005P instrument, using the power switch located at the rear of the instrument, and wait for both front status indicator lights to turn on and stop blinking.

5. Insert the supplied MxPro QPCR software CD into the CD-ROM drive. In rare cases when the installation process does not start automatically upon insertion of the CD into the drive, select **Run** from the Windows **Start** menu and open the Setup.exe file from the disk drive.

6. The MxPro QPCR software installation program will start automatically. Following the file extraction process, an installation Wizard will guide you through the MxPro QPCR software setup process. Click **Next** to continue with installation.
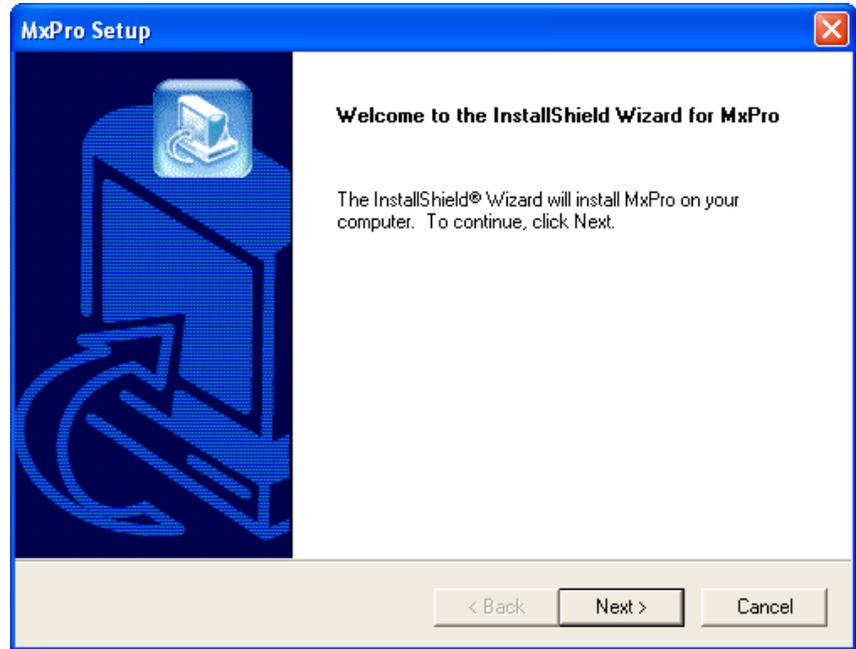
7. A window will open with a copy of the MxPro QPCR software license agreement. If you accept the terms of this agreement, click the **Yes** button to continue loading the software.
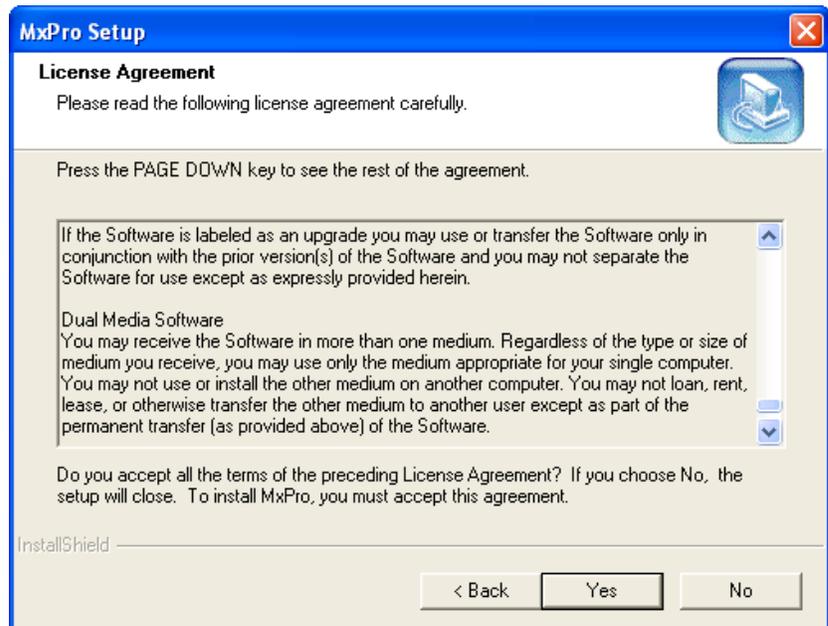
8. When prompted to select an installation type, select **Instrument-Connected System** and then click **Next**.
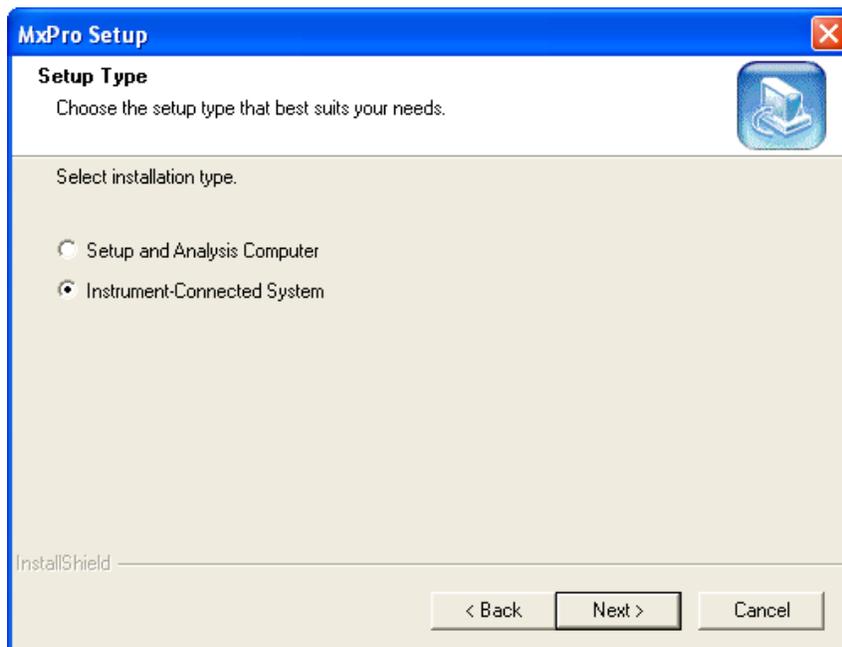
9. When asked to continue in the following dialog box, click **Yes**.
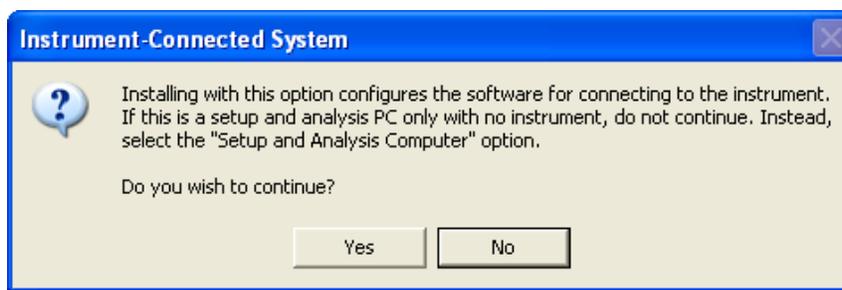
Important   If a **.NET Framework Initialization Error** dialog box appears, please contact Technical Services for assistance before proceeding.

10. When prompted to select additional MxPro installation options, select or deselect the appropriate checkboxes and then click **Next**.
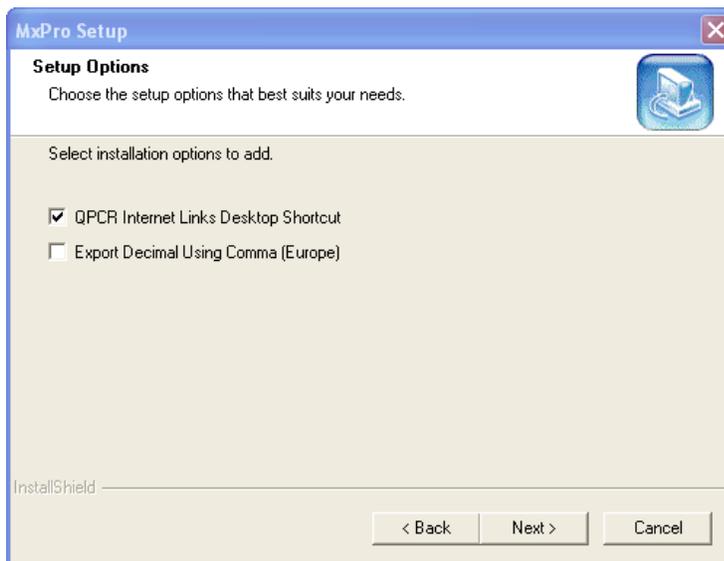
11. If you have purchased the optional 21 CFR Part 11-compatible features which include user authentication, database data storage, and audit trail support, select **21 CFR Part 11 Features** in the installation window below. A sales order number indicating purchase of these features is required to obtain an activation key. If you do not require 21 CFR Part 11-compatible features, click **Non-21 CFR Part 11 Features**. Click **Next** to continue with installation.

   If the **21 CFR Part 11 Features** setup type is selected, continue with step 12. If the **Non-21 CFR Part 11 Features** setup type is selected, proceed to step 15.
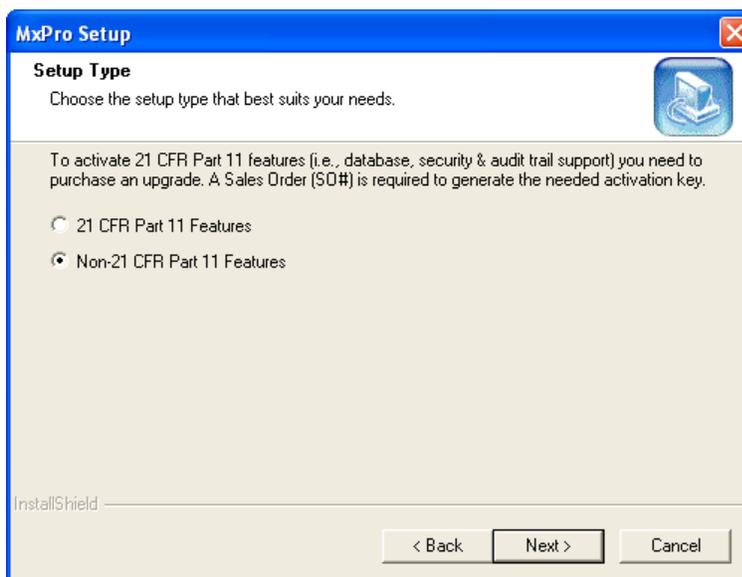
12. If **21 CFR Part 11 Features** was selected, the next installation screen requires that an alphanumeric activation key be entered for 21 CFR Part 11-compatible feature purchase verification and feature activation. The activation key can be obtained by contacting the technical services department by phone, E-mail, or via the Agilent website. The system-generated ID, sales order number, instrument serial number, and purchaser and institution names are required to request and obtain an activation key. After inputting the activation key, click **Next**.
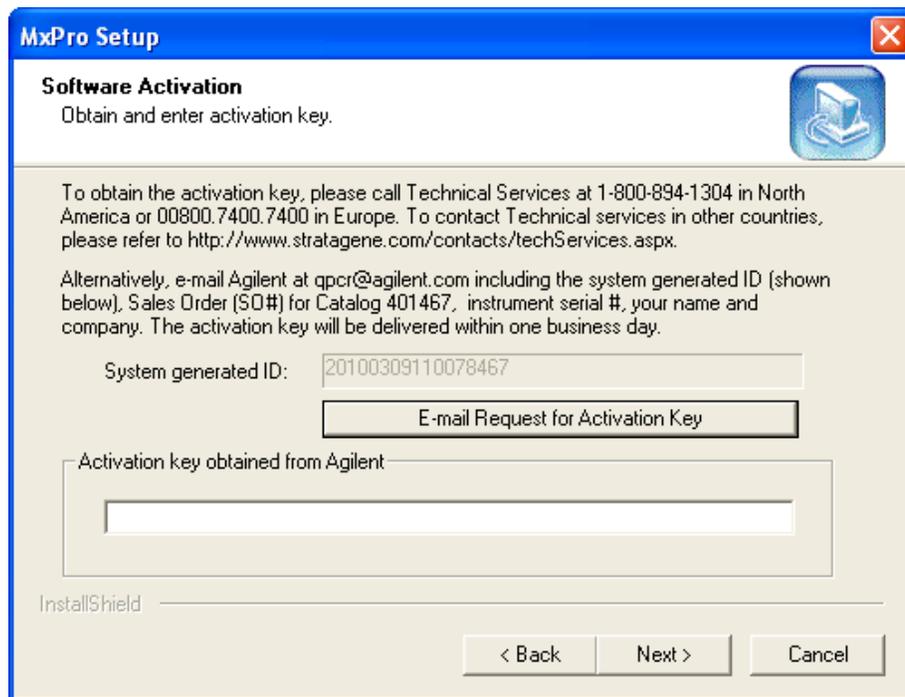
13. When prompted to select a database type for experiment storage, you may connect to a network database, or create and connect to a local database. If the software detects that a local database is already present, a third option will be available to connect to the detected local database. If you choose to create a local database, the Microsoft SQL server desktop engine database will be installed, providing 2 GB of storage. If you are unsure which database type to select, consult with your Information Technology department. Click **Next** once a database type is selected.
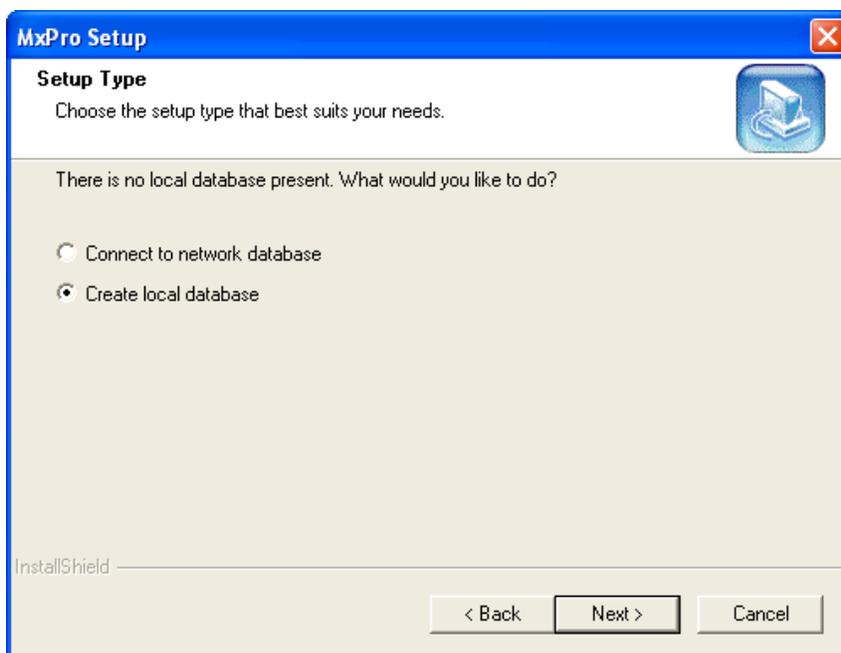
14. Depending on the database option selected, one of the following windows will appear. Complete the appropriate fields, either entering the database user ID and password for the selected network database, or assigning a database password for the local database. If you are unsure about database type, alias, user ID, and/or password fields, consult with your Information Technology department. The database alias field can be left blank when creating a local database if you do not wish to assign an alias name. Click **Next** once the appropriate fields are populated.
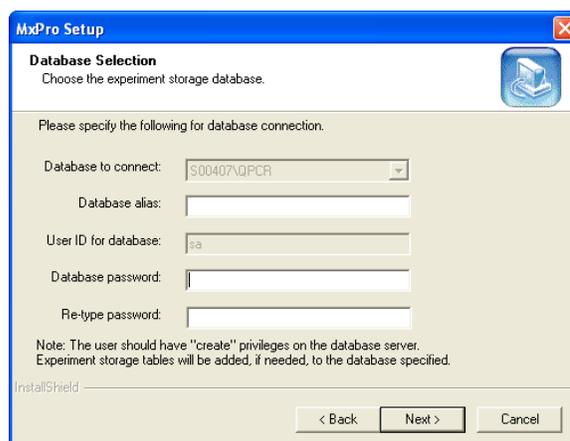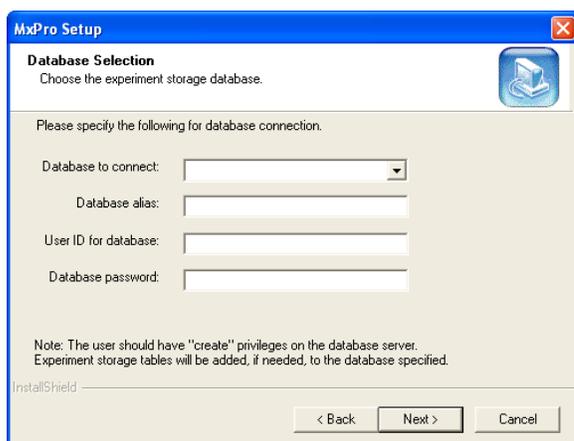
15. When prompted to choose a folder location where MxPro QPCR software files will be stored, either accept the default destination folder (i.e., C:\Program Files\Stratagene\MxPro) or select a different folder location using the **Browse** button. Click **Next** once a folder location is selected.
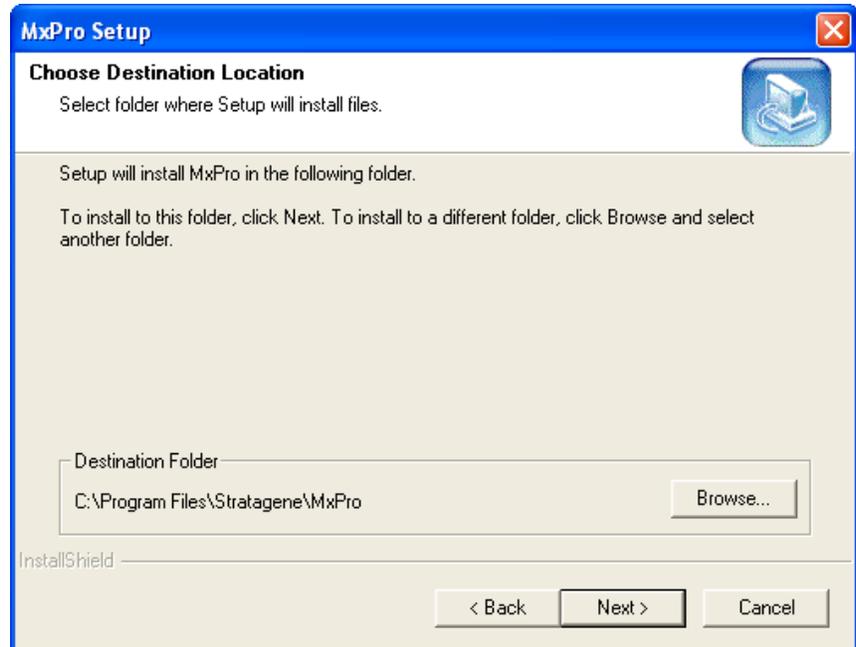
16. Once the MxPro QPCR software files and Microsoft SQL database software files (if applicable), are installed to the designated folder location, select **Yes, I want to restart my computer now**. Click **Finish** from the final installation Wizard window to restart the PC.
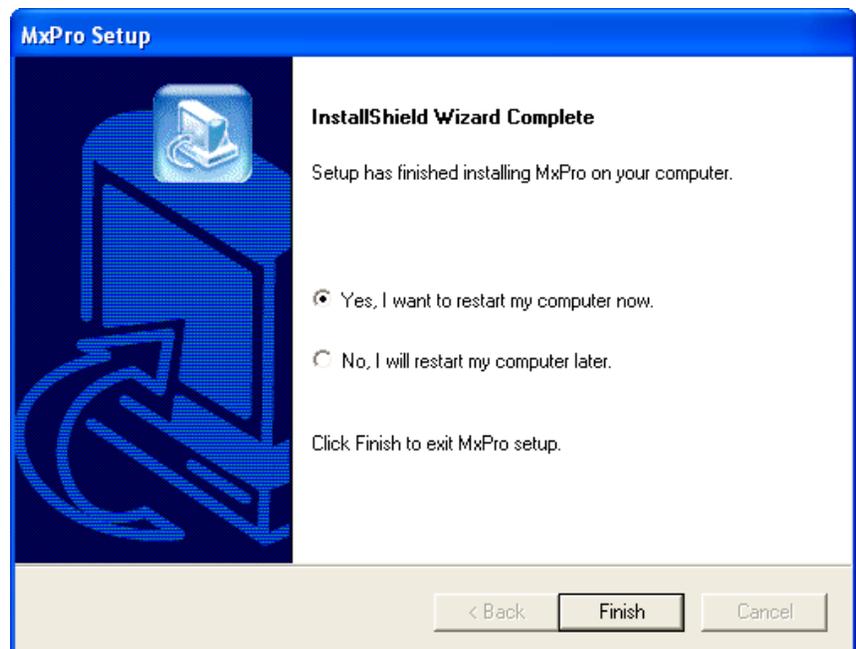
17. Once the PC has restarted, configure the PC Power Options. Click on the Windows **Start** menu in the bottom left corner of the screen and select **Control Panel** or **Settings\Control Panel**. Select **Performance and Maintenance**/**Power Options**. Select the **Always On** Power Scheme and then click **OK**.

18. Click on the MxPro desktop icon to start the MxPro QPCR software application.

19. If the 21 CFR Part 11-compatible features are enabled, a login dialog box will appear when the MxPro QPCR software application is opened. In order to start the application, enter the initial case-sensitive default username **Admin** and case-sensitive default password **password**. Click **Login**.
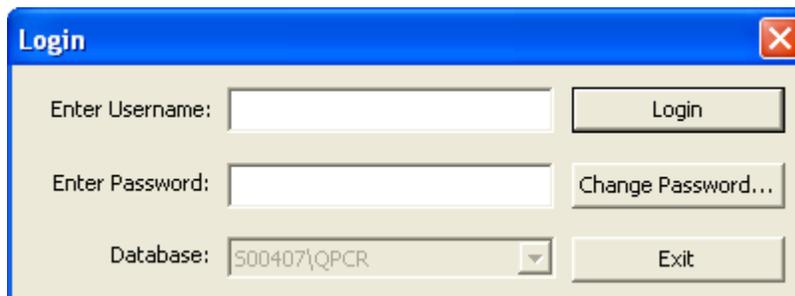
20. The first time you log in, you will be prompted to change your password. Enter the default password **password** in the field corresponding to **Old password**. Enter your new personal password of 6 characters or more, re-type your personal password for verification, and select **OK**.

21. The software will automatically prompt you to begin the Instrument Qualification Test. See the following section for detailed instructions on setting up and running this test.

Performing the Instrument Qualification Test Run

The Instrument Qualification Test verifies that the biological performance of an instrument experimental run is within factory specifications. Completing the run requires approximately 2.5 hours.

The qualification test run uses the installation qualification test plate and a preconfigured plate setup and thermal profile setup. No additional software setup steps are required. A validation protocol Wizard will provide instructions in a series of dialog boxes before, during, and after the run. Since the Qualification Test is a fixed test, only the actions directed by the validation protocol Wizard can be completed.

Do not attempt to modify the Qualification Test experiment or to analyze data during the Qualification Test run.

Note   *Wear chemical-resistant powder-free gloves whenever handling the qualification plate or other sample plates. This will ensure that no finger oils or other debris are transferred to the optical tube seal.*

1.   Prepare the installation qualification test plate just prior to starting the run. Thaw the plate contents at room temperature for 15–30 minutes, leaving the plate sealed in the foil wrapper while thawing.

     After thawing, remove the foil wrapper and then mix the contents of the wells by completing the following actions **five times**:

     •   Invert the plate. Use a sharp, swift, downward motion to move the liquid to the seal on top of each well. Observe the liquid to verify that the liquid has moved from the bottoms to the tops of the tubes.

     •   Re-invert the plate, and use the same motion to return the liquid to the bottom of each well, again verifying liquid movement before proceeding.

     After completing five mixing cycles, collect the liquid at the bottom of the wells. Brief centrifugation of the plate at 1000 rpm is highly recommended, in order to dislodge any bubbles and to collect the liquid.

     If a plate centrifuge is not available, shake the liquid down to the bottom of the tubes (using the same motion described above for mixing). Shake-down the plate until the majority of the liquid is at the bottom of the tubes and only minimal amounts of liquid are retained on the tube seal or sides. It is important to shake-down any noticeable liquid on the tube seal (e.g. visible semi-circles or crescent shapes when viewed from above). Small amounts of liquid spray retained on the tube sides should not affect test results.

Prior to loading the plate, verify that no bubbles are present at the bottoms of the tubes, beneath the liquid. While it is critical to remove any bubbles from the bottom of the tubes, small bubbles near the liquid meniscus should not affect the test results.

Important *Bubbles trapped at the bottom of the tubes will interfere with fluorescence readings, invalidating the test.*

2. The first time that the MxPro QPCR software is opened using an instrument-connected computer, the **Instrumentation Qualification Test-Start** dialog box will appear. (This dialog box may also be opened by selecting **Qualification Test** from the **Instrument** menu.) After the plate is prepared, click **Next**.
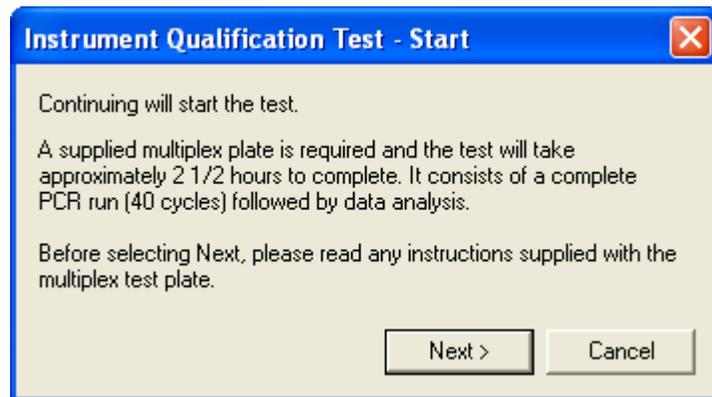
A series of filter set dialog boxes may appear. Accept the default filter set changes and settings.

3. The **Instrumentation Qualification Test-Start Run** dialog box will appear, providing an opportunity to enter any comments for the qualification test.
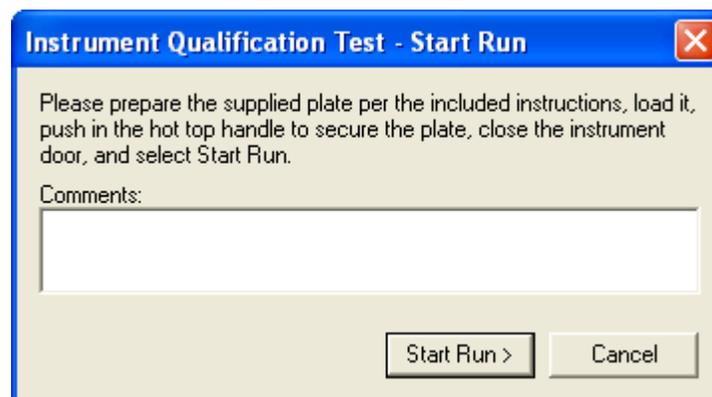
4. Verify that the Perfect Fit frame shipped with the instrument has been removed from the thermal block area. (See the *Sample Loading* section for more information.) Load the plate in the thermal block, verifying the correct orientation (well A1 in the top, left corner), and then close and latch the thermal block lid to secure the plate. Close the instrument door, and then click **Start Run**.

5. If the **Pre-Run Lamp Warm-Up** dialog box appears, select **Run Now**. The system will begin the Instrument Qualification Test run.
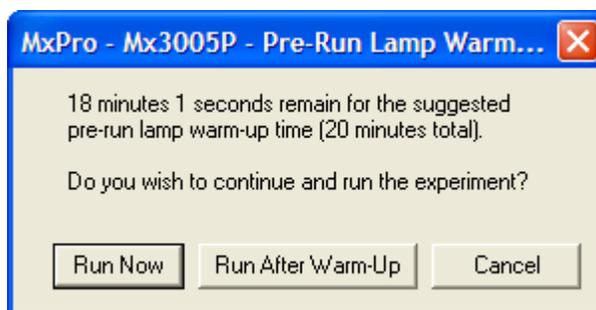
Important   ***Do not open the instrument door during the run.*** *When an experiment is running, the upper LED on the instrument front is in blinking mode. Opening the door while the instrument is running will abort the run.*

If the run is accidentally aborted by opening the instrument door, the following error message will appear: "Do you want to abort or continue the run?" *Close the instrument door and then select* "Continue the run."

Important   ***Ne pas ouvrir la porte de l'instrument lors d'une expérience.*** *Le clignotement de l'indicateur lumineux situé sur le devant de l'instrument indique qu'une expérience est en cours. L'ouverture de la porte lors du fonctionnement de l'instrument entraîne l'arrêt de l'expérience.*

Si une expérience est accidentellement arrêtée par l'ouverture de la porte, le message d'erreur suivant apparaît: "Do you want to abort or continue the run?" *(*"Voulez-vous arrêter ou continuer l'expérience?"*). Refermer la porte de l'instrument et choisir* "Continue the run" *(*"Continuer l'expérience"*).*

6. When the run is complete, the **Instrument Qualification Test-Run Complete** dialog box will appear. Select **Analyze**.
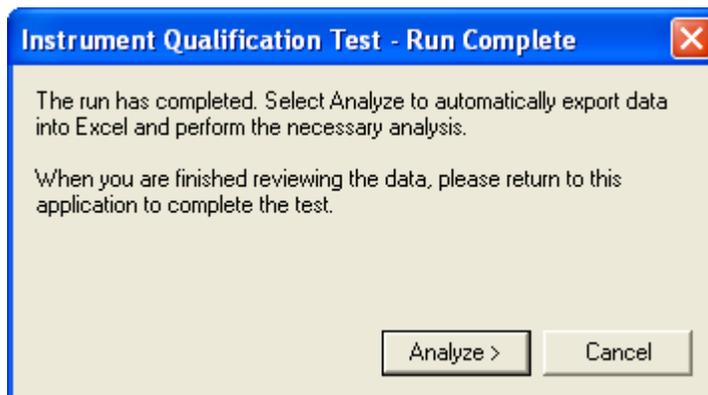
The software will open the Microsoft Excel software Instrument Qualification Validation template and will automatically export the Text Report data from the Instrument Qualification Test run into the Microsoft Excel software template, which has been preconfigured for data analysis.

Notes *If a **Server Busy** error dialog box appears, click **Retry** until the data export succeeds.*

*If any Norton anti-virus dialog boxes appear, select **Notify Again** in **1 Day,** if this option is available, and close the dialog box.*

7. The Microsoft Excel software Validation template will open to the **FAM analysis tab**. First, verify that the Data Count cell reports the result ***Good***, ensuring that all of the data has been correctly imported into Microsoft Excel. Next, verify that the **Result** cell reports a ***Pass*** result. A ***Pass*** result indicates that the instrument is working properly. In this case, it is not necessary to E-mail the data to Technical Services.

If the test reports a ***Check*** result, it is important to call Technical Services and send the three data files, including *Customer Information.txt*, to Technical Services by E-mail for troubleshooting guidance (see step 9).

8. After checking the results displayed on the Microsoft Excel software Validation template, go back into the MxPro QPCR software.

9. For a test report *Check* result only, the **Instrument Qualification Test-E-mail Experiment** dialog box will appear and present the option to automatically E-mail the data or save the data to a disk to later E-mail to Technical Services (QPCR@agilent.com). Include a contact name, the name of your institution and the instrument serial number when sending the data to Technical Services. (See *Mx3000P and Mx3005P QPCR Systems Support* for additional contact information.)

10. The **Instrument Qualification Test-Complete** dialog box appears, indicating that the Instrument Qualification test is complete. Click **Finish** to conclude the test.

HARDWARE INFORMATION AND SPECIFICATIONS

Hardware Specifications

Feature	Specification
Excitation source	Quartz-tungsten halogen lamp
Detector	Photomultiplier tube (PMT)
Filters	Excitation and emission filter wheels with four (Mx3000P system) or five (Mx3005P system) customer-selected filter sets
Thermal system	Peltier-based, 96-well block thermal cycling system
Thermal system temperature range	25–99°C
Operating environment	18–35°C (65–95°F); up to 85% non-condensing humidity
Weight	45 lbs (20.4 kg)
Dimensions	14" W $\times$ 20" D $\times$ 18" H (35.6 cm $\times$ 50.8 cm $\times$ 45.7 cm)
Multiplexing capabilities	Mx3000P system: 4 dyes per well
	Mx3005P system: 5 dyes per well
Sample container configurations	200-μl tube format, configured as 96-well plates, strip tubes, or single tubes
Reaction volume per tube	10–50 μl
Electrical power (input)	100–120 volts AC (+/–10%), 50/60 Hz, 10 A
	200–240 volts AC (+/–10%), 50/60 Hz, 5 A
Notebook computer or desktop computer (minimum requirements for Windows® Vista and Office 2007)	Intel® Core Duo or Intel® Core2 Duo 1.6 GHz processor, 2 GB system RAM, 20 GB hard drive, CD-RW drive, 14.1-inch XGA monitor, 128 MB video memory, internal network card

Cautions and Warnings/Précautions et Avertissements

Safety Symbols/Symboles de Sécurité

On/Off power supply indicator
Indicateur d'alimentation (marche/arrêt)

Caution
Attention

Caution, hot surfaces
Attention, Surface très chaude

Other Warnings

Use caution when loading or unloading samples from the thermal block; the thermal block can be hot.

Never touch the glass areas of the lamp; these surfaces can be hot. If oils from the skin are transferred to the quartz surface of the bulb, it can also result in premature bulb failure. Always handle the bulb assembly by the base.

Use of the equipment in a manner not specified by the manufacturer may impair the protection provided by the equipment.

Important *Do not open the instrument door during a run. When an experiment is running on the instrument the upper LED on the instrument front is in blinking mode. Opening the door while the instrument is running will interrupt the run. A warning dialog box will appear on screen if the door has been opened*

Autres Avertissements

Soyez prudents lorsque vous chargez et déchargez les échantillons du bloc thermique, car ce dernier pourrait être très chaud.

Ne jamais toucher la surfaces en verre des filtres ou de la lampe, car elles pourraient être très chaudes.

Toute utilisation de cet instrument qui n'est pas explicitement prévue par le fabricant peu endommager le dispositif de protection de l'instrument.

Important *Ne pas ouvrir la porte de l'instrument lors d'une expérience. Le clignotement de l'indicateur lumineux situé sur le devant de l'instrument indique que l'expérience est en cours. L'ouverture de la porte lors du fonctionnement de l'instrument entraîne l'arrêt de l'expérience. Suite à l'ouverture de la porte, une fenêtre de dialogue apparaît sur l'écran et l'expérience est intérrompue.*

and the run has been interrupted.

Instrument Status Indicator LED's

The status indicators on the front of the Mx3000P or Mx3005P instrument consist of two LED's.

The lower LED is a power status indicator. When this LED is lit, the instrument is plugged in and powered on.

The upper LED is a **Ready** status indicator for the instrument and has three separate modes, as described in the table below.

Appearance of **Ready** Status Indicator LED	Instrument Status
Continuously lit	The instrument control software is operational and the unit is ready to run an experiment.
Blinking	The unit is not currently available to run an experiment since an experiment is already in process.
Off	The unit is not currently available to run an experiment. Either the power is off, the instrument control software is not operational, or the instrument is still initializing after being powered up.

SAMPLE LOADING

Thermal Block Hardware

Use care when loading and unloading the samples; the thermal block can be hot.

Prior to loading samples, verify that the status LED (upper LED on the instrument front) is solidly lit (glowing green), indicating that the instrument is ready for use. If the LED is not illuminated, the instrument is not powered on or may still be initializing. If the LED is blinking, the instrument is warming up or a run is in progress and the door should not be opened.

Open the door located at the front of the instrument by sliding it all the way to the top.

To expose the thermal block, lift the heated lid by pulling forward on the handle of the lid and then lifting the lid up and away from the thermal block (see Figure 2).

Note   *The lid of the thermal block houses a 96-hole grid (used by the instrument for light path entry) which may be mistaken for a thermal block. Ensure that the lid is raised, exposing the thermal block, prior to loading samples.*

Figure 2 The thermal block assembly, shown with the lid closed (left) and opened (right).

Consumables

The thermal block of the Mx3000P/Mx3005P system is designed to accommodate a variety of 96-well PCR plates, 200-µl PCR strip tubes, or individual 200-µl PCR tubes that have separate, optically clear caps. For best results, use the following Agilent consumables:

96-well PCR plates, 0.2-ml, non-skirted, Catalog #401333
96-well PCR plates, 0.2-ml, semi-skirted, Catalog #401334
8× strip tubes, 0.2-ml, Catalog #401428
8× optical strip caps, Catalog #401425
Single PCR reaction tubes (without caps), 0.2-ml, Catalog #410023

Prior to using a new type of plasticware for a run, test the fit in the thermal block, and attempt to close the heated lid. If you encounter resistance when closing the lid, do not use the plasticware for Mx3000P/Mx3005P system runs. Using plates or tubes that are too tall for the thermal block or using domed sample caps may prevent the heated lid from closing properly and may damage the instrument scanner.

Use only non-skirted or semi-skirted plates with the Mx3000P and Mx3005P systems. Full-skirted plates cannot be properly seated in the thermal block.

Important   *When using individual tubes or strip tubes as sample containers, insert the Perfect Fit frame on the thermal block platform before loading the samples. See* Use of the Perfect Fit Frame with Single Tubes and Strip Tubes *for more information.*

Use of tubes that are not designed for the Mx3000P and Mx3005P instruments can result in poor experimental results or damage to the instrument. The Agilent consumables for use in the Mx4000 instrument are not compatible for use in the Mx3000P or Mx3005P systems, and can result in difficulty closing the heated lid and damage to the heated lid.

Use of the Perfect Fit Frame with Single Tubes and Strip Tubes

Prior to performing a run using individual tube or strip tube sample containers, install the Perfect Fit frame on the thermal block platform. The Perfect Fit frame optimizes the contact between the sample tubes and the heated lid. One Perfect Fit frame is supplied with the Mx3000P/Mx3005P System, and additional frames are available separately (Agilent Catalog #401421). See Figure 3 for an illustration of the Perfect Fit frame and Figure 4 for the appearance of the frame when installed correctly on the thermal block.

Figure 3 The Perfect Fit frame, shown in the proper orientation for installation.

To install the Perfect Fit frame, position the frame around the border of the thermal block. When properly installed, each of the six notches on the bottom of the frame will be positioned in the spaces between wells on the perimeter of the thermal block surface. The Perfect Fit frame should be seated on the thermal block platform with limited freedom of movement. There are no audible sounds associated with the installation. (The frame does not "click" into place.)
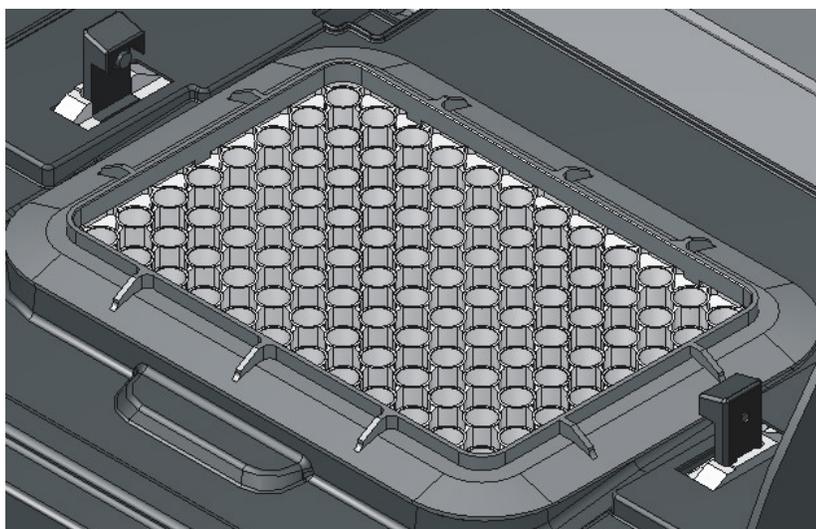
Figure 4 Appearance of the Perfect Fit frame when installed correctly on the thermal block.

Application of Caps

Wear powder-free gloves while applying caps to the sample containers. Caps should be placed on the samples before the samples are loaded into the instrument. For best results, apply the caps while the samples are firmly seated in a 96-well working rack (Agilent Catalog #410094). Apply direct downward force to each cap, and then view the rack from the side to make sure all the caps are seated evenly. Alternatively, caps can be applied using a Storage Mat Applicator (Corning Catalog #3081). Just before loading the samples into the thermal block, centrifuge the samples briefly to collect any liquid that may be on the tube walls or cap.

MAINTAINING THE INSTRUMENT

Replacement Parts

Use only Agilent replacement parts (excluding fuses) in the maintenance of the Mx3000P and Mx3005P systems. Use of non-Agilent parts may void the warranty.

Replacement lamp assemblies are available for purchase (Agilent Catalog #401411). The lamp is the only customer-serviceable part of the system. The system should be returned to Agilent for all other services and repairs.

Changing the Lamp

The lamp of the Mx3000P and Mx3005P systems has an average life expectancy of 2000 hours.

Important *Never touch the glass portion of the quartz-tungsten halogen lamp!*

Before changing the lamp, be sure to power off the instrument.

Warning *The lamp and nearby surfaces may be hot!*

Important *Ne jamais toucher la surface en verre de la lampe quartz-tungstène-halogène!*

Avant de changer la lampe, assurez-vous d'avoir d'avoir éteint l'instrument.

Avertissement *La lampe et les surfaces adjacentes peuvent être très chaudes!*

The lamp is located in the excitation housing, on the left-hand side of the instrument. Before changing the lamp, be sure to turn off the instrument. The lamp is replaced, along with the lamp housing, as follows:

1. Remove the two screws securing the excitation housing cover panel and remove the panel. This panel is located on the left side of the instrument.

2. Disconnect the wire connector for the lamp housing. This is located near the bottom of the module towards the front of the instrument.

3. Loosen the thumbscrew that holds the lamp housing in the excitation module.

4. Remove the old lamp housing and lamp.

5. Insert the new lamp housing and lamp. A keyway on the lamp housing allows the housing to be placed in its holder in only one orientation. Push the housing in as far as it will go.

6. Tighten the thumbscrew.

7. Connect the wire connector.

8. Replace the cover panel and the two cover screws.

9. After the lamp has been changed, reset the MxPro QPCR software lamp timer to 0. To do this, open the **Lamp Reset and Utilities** dialog box (from the software **Instrument** menu) and click the **Reset** button. The timer will be set to 0 and the current date will be entered in the **Last date reset** field.

Replacing the Fuse

The Mx3000P and Mx3005P instruments use a single slow-acting fuse. The fuse housing is located adjacent to the power cord inlet in the power entry module. See *Installing the Fuse* in *Setting Up the System* for pictures of the fuse housing unit.

To replace the fuse, disconnect the instrument from the power source, open the fuse housing cover and remove the fuse holder from the power entry module. Replace the fuse with the appropriate fuse type from the table below and then replace the fuse holder.

Warning   ***High voltage present. Turn off the power switch and remove the power cord from the power entry module prior to replacing the fuse.***

Avertissement   *Présence de haut voltage. Éteigner l'instrument et débrancher le cordon d'alimentation de la prise du bloc d'alimentation avant de remplacer le fusible.*

Instrument Type (Voltage)	Replacement Fuse
110–120 V	T 10 A, 250 V
200–240 V	T 5 A, 250 V

Cleaning the Instrument

Clean the exterior housing of the Mx3000P and Mx3005P systems using only mild soap and warm water on a soft cloth. Do not use alcohol or solvent-based cleaners. The wells of the thermal block should be cleaned with cotton swabs and isopropyl alcohol. Ensure that the block is at room temperature before cleaning.

TROUBLESHOOTING

Observation	Suggestion
Unable to install MxPro software	The software will be unable to install if the account that was used to login to the PC does not have administrative rights. Login under an account with admin rights. If the computer is on a network, you may need to contact your IT department.
During installation of the MxPro software, a **.NET Framework Initialization Error** dialog box appears	Contact Technical Services.
When first connecting or reconnecting the PC to the instrument, "Communication Error" appears in software	Verify that the RS-232 cable between the PC and Mx3000P or Mx3005P instrument is securely connected to the serial-to-USB adapter cable, and that the adapter cable is securely connected to the USB port on the PC. Verify that the USB driver on the CD provided with the system has been loaded on the computer. If the "Communication Error" persists, connect the cable to one of the other USB ports on the PC.
"Communication Error" appears in software	Instrument has not finished warming up. Wait for both front panel LED's to become steady lights and for the instrument communication icon in the bottom right corner of the screen to turn from red to green.
	Verify that the RS-232 cable between the PC and Mx3000P or Mx3005P instrument is attached.
	Verify that the instrument is powered on.
	Exit and reboot the MxPro software. If a run was in progress when communication failed, the software will detect this after communication is reestablished. If a run is still in progress, the software will offer the option to continue displaying data from the current run. If the run completes before communication is reestablished, the software should offer the option to download the data from the last run. If not, you can select the **Download Data From Instrument** option from the Instrument menu.
	If a USB to serial adapter cable is being used, verify that the driver software for the adapter cable has been installed. If the cable has been moved to a different USB port, you may have to use the **Discover Instruments** command under the **Instrument** menu in the MxPro application in order for the instrument to recognize the new COM port location.
Lamp On or other instrument-related software commands are not available	Make sure the running software is not a Standalone version. If using software installed for analysis only or if multiple copies of the software are open, *Standalone* will appear in the upper left corner of the MxPro QPCR software title bar. When communication between the PC and the instrument is established, the communication icon in the lower right corner is green.
Software locks up or shuts down during a run	Turn off the sleep mode for the monitor and disable the screen saver. This can be accessed in the Microsoft Windows software Control Panel. Also verify that the Windows Power Scheme is set to *Always On*.
Lower LED power indicator light never comes on	Verify that the instrument is plugged into the power source or surge protector and that power is getting to the instrument.
	Check fuse on back panel near the power cord. If fuse is burned out, replace the fuse. See *Replacing the Fuse* for more information.

Observation	Suggestion
After switching on the instrument, the upper LED status indicator light does not indicate Ready status (continuously lit)	During instrument power-up, the upper LED should remain off for 2-3 minutes while the instrument warms up. If the LED does not light continuously after the warm up period is completed, reboot the instrument (power off the instrument, wait 30 seconds, and then switch the instrument power on). If this does not address the problem, contact Technical Services.
Error message "A Lamp Error has Occurred" or "Lamp Power Error"	This error is generated when the enabled software detects that there is no current flowing through the bulb. Lack of current could simply indicate that the bulb has failed or is unplugged, or it could indicate a more serious electrical problem that requires repair. If changing the bulb and confirming its electrical connection does not resolve this error, contact Technical Support.
Error message "A Power Supply Error has Occurred"	It is possible that this error can be resolved be upgrading to the most recent version of the MxPro software. If you are running version 3.0 or earlier, contact Technical Services to obtain a software download key. If you are already running version 3.2 or later, contact Technical Services for further troubleshooting.
Very low amplification signal	Low amplification signal could indicate that the lamp is starting to fail. Check lamp, and replace if necessary. See *Changing the Lamp*.
	If the reference dye is showing good signal but the fluorophore is not, there may be a problem with the probe or the stock of dye that was used. If using SYBR Green, verify the concentration. If using a new probe or a new lot of a probe, the problem could be the fluorophore. You can test the probe by performing a nuclease digestion to ensure it is unquenching as expected: incubate 100 nM of probe in 25 µl of $1\times$ buffer with 10 U of DNase or S1 nuclease at room temperature for 30 minutes. This should result in a fluorescence level increase of >5000 RFU. If another probe or a stock of the dye is available, try that stock for comparison.
	If amplification of the signal is seen in spite of the low fluorescence, increasing the gain multiplier may still allow this probe to be used. If the probe or dye stock has been stored in a way that might have exposed it to light, low signal could also be due to photobleaching of the fluorophore. If the samples are still available, run them on a gel to verify the presence of amplified product. If the gel indicates that no amplified product has been formed, assay optimization or new reagents may be necessary.
	If low amplification signal only occurs intermittently and is cleared by rebooting the instrument, it is possible that this problem could be resolved by upgrading to the most recent version of the MxPro software. If you are running version 3.0 or earlier, contact Technical Services to obtain a software download key.
Sudden amplification signal loss or downward drift during run	Check lamp, replace if necessary. See *Changing the Lamp*.
	The bulb may be failing. With a Mx3000P system, unfiltered light can be seen coming through the read head whenever the lamp is on. If no light is seen, or if it looks very dim or yellowish, the bulb has likely failed or is failing. With a Mx3005P system, because the light path is blocked at any time when a scan is not in progress, the only way to check the bulb is to remove it from the instrument for direct inspection. The bulb filament will normally appear as a tight silver spiral. A bulb that appears blackened, deformed or broken should be replaced.

Observation	Suggestion
Signal fluctuates during run	Check lamp, replace if necessary. See *Changing the Lamp*.
	Verify that the raw (multicomponent) fluorescence signal is below the recommended maximum of 35000 and above the recommended minimum of 3000 RFU. If necessary, adjust the **Filter Gain Settings** to bring the signal into the recommended range.
	If ROX is being used as a reference dye, the final ROX concentration in the reaction should be approximately 30 nM. If the dye SYBR® Green I is used as the reporter, ensure that the dye is diluted appropriately before addition to the reaction mix.
	Verify that the instrument is not subjected to vibrations from an external source during the run, such as from another instrument on the same bench. Also verify that the line power provided to the instrument is within the required specifications of 100–120 volts AC (+/−10%), 50/60 Hz, 10 A for a 110V system or 200–240 volts AC (+/−10%), 50/60 Hz, 5 A for a 230V system. If consistent power cannot be maintained by the local power supply, it may be necessary to use a Line Conditioner. An example Line Conditioner that is known to work well is the POWERVAR ABC700-11MED.
	Ensure the instrument is not located near a window where the back of the instrument is exposed to direct sunlight. If this is the case, the window should be blocked or the instrument should be relocated.
Unexpected results in one sample	Check the sample container for contaminating material.
	Check optical clarity of sample container cap.
	Ensure the liquid inside that sample tube did not evaporate during the run, which would indicate a vapor leak in that particular sample. Vapor leaks may occur if the sample was not sealed properly or incorrect plasticware was used.
	Clean the thermal block as outlined in the section *Maintaining the Instrument*.
Decreased volume in sample containers at end of run	Sample containers are not vapor tight. Ensure caps are tightly sealed and containers are not malformed. Also ensure that proper plasticware was used.
Excess condensation in reaction tubes	When using single tubes or strip tubes, install the Perfect Fit frame on the thermal block to optimize contact between the sample tubes and the heated lid.
No amplification plots are visible in the Results screen	The wrong data collection point may have been assigned to the amplification plots (e.g. the dissociation curve collection point). Reset the data collection point for the amplification plots on the **Analysis Selection/Setup** screen.
	Ensure that a data collection point was specified on the **Thermal Profile Setup** screen. Without a data collection point, no data is collected and analysis is impossible.
	If you selected dRn data when a reference dye was not defined for the experiment, you will not see any amplification plots on the Results screen. In this case, there will normally be an error message under the plot stating "None of the wells selected have a reference dye selected." Change the Fluorescence to dR and the amplification plots will be visible.

Observation	Suggestion
Software font difficult to read/Poor resolution of text/Images missing	Microsoft Windows display settings are incorrect. Required minimum settings are 16 bit high color and 1024 x 768 resolution. Do not set DPI higher than 96.
Chart printing is low quality	Check the status of the **High resolution chart printing** option on the **Print Page Setup** dialog box. Chart printing using most printers will be enhanced by selecting the **High resolution chart printing** option. Limitations of certain printer drivers cause incompatibilities with this setting; try deselecting the **High resolution chart printing** checkbox.
When the data are exported to Excel, the charts do not look correct, do not appear, or the data numbers are much higher than they should be	These errors may result if the software was accidentally setup to export data to Excel using the incorrect format for the decimal symbol. In North America, the decimal place holder should be a period (.) while in Europe, it is normally a comma (,). Under the **Options** menu on the toolbar, select **Preferences** to open the **Preferences** dialog box. Make sure the "Export decimal using" selection is set to the correct format for the copy of Microsoft office you are using.
Peaks in dissociation curve are very wide	Make sure the **Moving Average** setting has not been set to more than 3 averaging points (see the **Analysis Term Settings** dialog box under the **Smoothing** tab).
	If the **Moving Average** setting is not the cause of the wide peak, it could be that multiple products are being formed. You may need to optimize the primers or the reaction conditions.
Difficulty in setting baseline or baseline corrected amplification plots do not appear flat in region prior to amplification	When running samples with a broad dynamic range, use the adaptive baseline algorithm. If amplification curves still look abnormal, it may be necessary to manually set the baseline ranges. Baseline ranges may be adjusted in the **Analysis Term Settings** dialog box under the **Baseline Correction** tab. The best way to determine a baseline range is to view the raw amplification plot for each curve (the R fluorescence data) and then set the baseline start and end cycles to span only that region of the curve that is flat (i.e. start the range after any initial tailing and end it before the signal shifts due to amplification).
Signal fluctuations in amplification plots	Use of the moving average algorithm can help correct for moderate levels of signal noise. Consult the MxPro QPCR software **Help** for more information.
	Signal noise can result if the raw signal level is near saturation (>35,000 RFU) or near background (<3000 RFU). In cases where the signal level is running too high, reduce the gain multiplier setting or reduce the concentration of dye/probe used. Low signal levels can indicate the bulb is starting to fail, or indicate a problem with the probe/dye that is used.
	Signal noise may be the result of environmental factors such as vibration of the bench from other instruments, direct sunlight falling on the back of the instrument, or fluctuations in the line power. These problems can normally be resolved by relocating the instrument to a different bench in the lab or, for the case of line power problems, by connecting the instrument to a Power Conditioner or UPS. An Mx3000P or Mx3005P system requires at least 1000 VA of power, so any power conditioner or UPS used must be rated to deliver at least this level of power.
Increased signal noise for run	Use the **Moving Average** algorithm. Consult the MxPro QPCR software **Help** for more information.

Observation	Suggestion
Ct value reported for NTC (no-template control) sample is less than the total number of cycles but the amplification plot curve is horizontal	Examine variation in fluorescence intensity during the run. Review amplification plot and adjust the threshold accordingly.
Low increase in fluorescence with cycling	The probe or beacon is not binding the target efficiently. Lower annealing temperature and verify melting temperature.
	Target PCR product is too long. Redesign primers to yield a PCR product <150 bp in length for probe-based detection, or <400 bp for SYBR Green I detection.
	Magnesium concentration is too low; run a titration to optimize concentration.
	Insufficient or non-specific product. Verify product formation through gel electrophoresis. Re-optimize reaction conditions as necessary.
Increase in fluorescence in control reactions without template	If the amplification plot is showing a strong sigmoid curve, then the reaction has likely been contaminated.
	In SYBR Green reactions, an increase in fluorescence in the absence of template could be due to primer-dimer formation.
	If a probe-based chemistry is used and the amplification plot shows a steady fluorescence increase, rather than a sigmoid curve, the probe may be degrading.

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